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Improved Syntheses of the PET Radioligands, [^{11}C]FLB 457, [^{11}C]MDL 100907 and [^{11}C]ß-CIT-FE, by the Use of [^{11}C]Methyl Triflate

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Summary

The highly reactive labelling agent, [^{11}C]methyl triflate, was used to synthesise three recently developed PET radioligands. The labelling reactions were ^{11}C-methylations of phenols for the preparations of the D_2 receptor radioligand, [^{11}C]FLB 457, and the 5-HT_{2A} receptor radioligand, [^{11}C]MDL 100907, and ^{11}C-methylation of a carboxylic acid for the preparation of the dopamine transporter radioligand, [^{11}C]ß-CIT-FE. The synthesis of the 5-HT_{1A} receptor radioligand, [*O-methyl*-^{11}C]WAY-100635, was used to establish general reaction conditions for the methylation of phenols with [^{11}C]methyl triflate. Compared to the previous use of [^{11}C]methyl iodide in these radiosyntheses, [^{11}C]methyl triflate demanded less precursor, allowed faster reactions and improved radiochemical yields. Normal-phase HPLC was used to speed up product purifications (except for [^{11}C]ß-CIT-FE). Hence, preparation times were shorter, resulting in radioligands with higher specific radioactivity.

Key words: [^{11}C]Methyl triflate, FLB 457, MDL 100907, ß-CIT-FE.

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INTRODUCTION

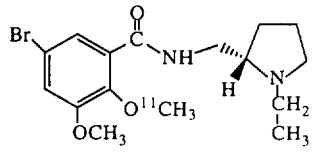
Methylation is widely used for labelling compounds with carbon-11 ($t_{1/2}$ = 20.4 min) for investigations in animals and human subjects with positron emission tomography (PET). [^{11}C]Methyl iodide was developed as a ^{11}C-methylating agent two decades ago (1, 2) and continues to be widely used in the preparation of PET radiopharmaceuticals. Quite recently [^{11}C]methyl triflate has been introduced as a more powerful methylating agent (3). The use of [^{11}C]methyl triflate generally gives higher yields from smaller amounts of precursor, shorter reaction times and lower reaction temperatures (4-9). These factors allow PET radiopharmaceuticals to be produced efficiently and reliably by conserving costly or rare precursors, reducing the duration and complexity of labelling reactions and easing product purification. Several ^{11}C-methylations of amines with [^{11}C]methyl triflate have been reported (4, 5, 7-9) and only a few of thiols, phenols, amides (6) and carboxylic acids (10)

In this study, we describe the use of [^{11}C]methyl triflate for improved syntheses of three recently developed PET radioligands. Two of these are methyl ethers, [^{11}C]FLB 457 ([*O-methyl*-^{11}C]-(*S*)-5-bromo-*N*-((1-ethyl-2-pyrrolidinyl)-methyl)-2,3-dimethoxybenzamide) and [^{11}C]MDL 100907 ([3-*O-methyl*-^{11}C]-(*R*)-(+)-4-(1-hydroxy-1-(2,3-dimethoxy-phenyl)methyl)-*N*-2-(4-fluoro-phenylethyl)piperidine) (Figure 1). [^{11}C]FLB 457 is used to image D_2 receptors (11), for which it is an antagonist with very high affinity (K_i = 20 pM). [^{11}C]MDL 100907 has been developed recently for imaging 5-HT_{2A} receptors (12) for which it is a potent selective antagonist.

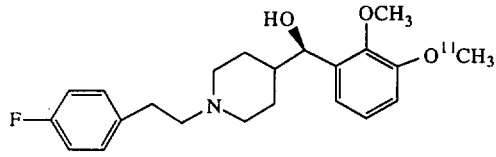
WAY-100635 (*N*-(2-(4-(2-methoxy-phenyl)-1-piperazin-1-yl)ethyl)-*N*-(2-pyridinyl)-cyclohexanecarboxamide) is a 5-HT_{1A} receptor antagonist, which has been labelled in its methoxy group for use as PET radioligand (Figure 1) (13-15). Recently, it has been shown that the labelling of WAY-100635 in the carbonyl position gives a more favourable radioligand (16). However, our good availability of the desmethyl precursor made it a useful model substance for initial optimization of the reaction of phenols with [^{11}C]methyl triflate.

The dopamine/serotonin transporter radioligand, [*O-methyl*-^{11}C]ß-CIT (2ß-carbomethoxy-3ß-(4-iodophenyl)tropane), has been prepared using [^{11}C]methyl iodide and/or [^{11}C]methyl triflate (17, 10). The newer 2ß-fluoroethyl analogue, [^{11}C]ß-CIT-FE (Figure 1), is more selective and is suitable for quantitation of the dopamine transporter *in vivo* with PET (18, 19).

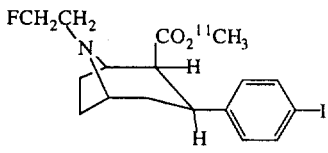
Here we also present a new method for preparing [^{11}C]ß-CIT-FE, based on esterification of the corresponding carboxylic acid precursor with [^{11}C]methyl triflate.



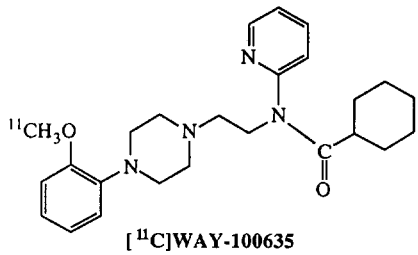
[^{11}C]FLB 457



[^{11}C]MDL 100907



[^{11}C]ß-CIT-FE



[^{11}C]WAY-100635

Figure 1. Structures of the ^{11}C-labelled radioligands synthesized from [^{11}C]methyl triflate

EXPERIMENTAL

Materials & General Methods

Materials

Silver triflate was purchased from Aldrich and Graphpac GC (80–100 mesh) from Alltech. Silver triflate-impregnated graphitized carbon was prepared according to Jewett (3). Desmethyl-WAY-100635 and WAY-100635 were synthesized as described elsewhere (14), as were ß-CIT-FE and the corresponding acid (*N*-(2'-fluoroethyl)-2ß-carboxyl-3ß-(4-iodophenyl)nortropane) (20). FLB 457 and the desmethyl analogue, FLB 604, were kindly supplied by Astra (Södertälje, Sweden). MDL 100907 and its 3-desmethyl analogue, MDL 105725, were kindly supplied by Dr A.A. Carr (Hoechst Marion Roussel; Fehraltorf, Switzerland). Dimethylformamide (DMF) was obtained from Merck, distilled under vacuum and dried over molecular sieves (4 Å). Other chemicals were obtained from commercial sources and were of analytical grade.

Production of [^{11}C]carbon dioxide

[^{11}C]Carbon dioxide was produced at the Karolinska Hospital with a Scanditronix RNP 16 cyclotron using 16 MeV protons in the $^{14}N(p,\alpha)^{11}C$ nuclear reaction on nitrogen. The [^{11}C]carbon dioxide produced was trapped in a stainless steel coil cooled with liquid nitrogen before being transferred to the [^{11}C]methyl iodide/[^{11}C]methyl triflate system (21, 7)

Preparation of [^{11}C]methyl triflate

[^{11}C]Methyl iodide was prepared from trapped cyclotron-produced [^{11}C]carbon dioxide as described previously (21) and passed through a heated soda glass column (oven temperature, 170 °C) containing silver triflate-impregnated graphitized carbon (Figure 2). [^{11}C]Methyl iodide was converted to [^{11}C]methyl triflate without any significant breakthrough of [^{11}C]methyl iodide (3).

$$[^{11}C]CH_3I \xrightarrow[170\ °C]{AgOSO_2CF_3/Graphpac\ GC} [^{11}C]CH_3OSO_2CF_3$$

$$R\text{-}OH \xrightarrow{[^{11}C]CH_3OSO_2CF_3} R\text{-}O[^{11}C]CH_3$$

(R = aryl, alkyl-CO-)

Figure 2. Preparation of [^{11}C]methyl triflate from [^{11}C]methyl iodide and use in *O*-^{11}C-methylations

Separation of radioligands, and measurement of radiochemical yield

Each radioligand was separated to >99% radiochemical purity by HPLC on a system comprising the specified column, a pump (type 420; Kontron), automatic sample injector (type VICI with 1 mL loop), absorbance detector (type 432 set at 254 nm; Kontron) and radioactivity detector (GM-tube). Elution conditions are described in the preparative descriptions below. Each radioligand was formulated for intravenous administration by evaporation of mobile phase and dissolution in sterile physiological phosphate buffer.

Radiochemical yields were estimated by comparing the radioactivity in the product peak with the total radioactivity. They are decay-corrected from the initial [^{11}C]methyl triflate, or when cited from the initial [^{11}C]carbon dioxide.

HPLC analysis of radioligands

Each radioligand was analyzed for radiochemical purity by reverse phase HPLC on a μ-Bondapak C-18 column (300 mm x 7.8 mm, 10 μm; Waters) using a PC-controlled system with Intelligent pump (type L-6200A; Merck-Hitachi), injector (type 7125 with 50 μL loop; Rheodyne) and UV absorbance detector (L-4000A, 254-270 nm; Merck-Hitachi) in series with a radioactivity detector (Flo-one; Radiomatic) equipped with a PET flow-cell (600 μL; Packard). The column was eluted with acetonitrile:0.01M phosphoric acid (30:70 v/v) at 6 mL/min. The response of the absorbance detector for each ligand was calibrated for mass by injection of known amounts of authentic compounds.

Preparation of [^{11}C]WAY-100635

[^{11}C]Methyl triflate was trapped over 3-4 min at room temperature in a sealed reaction vial (1.0 mL mini-vial, Alltech), containing desmethyl-WAY-100635, aqueous sodium hydroxide solution (0.5 M) and acetone (400 μL). The radiochemical yield of [^{11}C]WAY-100635 was investigated as a dependency against several parameters. The amount of precursor was varied between 0.04–0.5 mg (0.1–1.23 μmol) and the kinetics during heating at 60°C was followed from 0-60 min. Different molar ratios (1–25) of sodium hydroxide to precursor (0.3 mg; 0.74 μmol) were used. DMF was tested as solvent with 0.08 mg precursor and compared to the normally used solvent, acetone. All reactions were quenched by adding HPLC mobile phase (600 μL).

Preparation of [^{11}C]FLB 457

[^{11}C]Methyl triflate was trapped over 3-4 min at room temperature in a sealed reaction vial (1.0 mL mini-vial, Alltech), containing FLB 604 (0.3 mg), acetone (400 μL) and sodium hydroxide solution (2 μL; 0.5 M). HPLC mobile phase (600 μL) was added to the reaction mixture immediately after the trapping of [^{11}C]methyl triflate was complete. [^{11}C]FLB 457 was separated from the reaction mixture by HPLC on a μ-Porasil column (300 mm x 7.8 mm, 10 μm; Waters) eluted with dichlormethane-methanol-triethylamine (96:4:0.04 by vol.) at 2 mL/min (retention time, 11-12 min).

Preparation of [^{11}C]MDL 100907

The labelling procedure and HPLC separation were the same as in the preparation of [^{11}C]FLB 457, except that MDL 105725 (0.3 mg) was used as precursor. The retention time of [^{11}C]MDL 100907 was 8-10 min.

Preparation of [^{11}C]ß-CIT-FE

[^{11}C]Methyl triflate was trapped in a sealed vial (1.0 mL mini-vial, Alltech), containing *N*-(2'-fluoroethyl)-2ß-carboxyl-3ß-(4-iodophenyl)nortropane (0.4–0.7 mg) in acetone (400 µL) and freshly prepared aqueous tetrabutylammonium hydroxide solution (0.4 M; 2–4 µL). After completion of reagent trapping (3-4 min), HPLC mobile phase (600 µL) was added and the [^{11}C]ß-CIT-FE separated on a µ-Bondapak C18-column (300 mm x 7.8 mm, 10 µm; Waters) eluted with acetonitrile-water-triethylamine (60: 40: 0.1 by vol.) at 6 mL/min (retention time, 9-10 min).

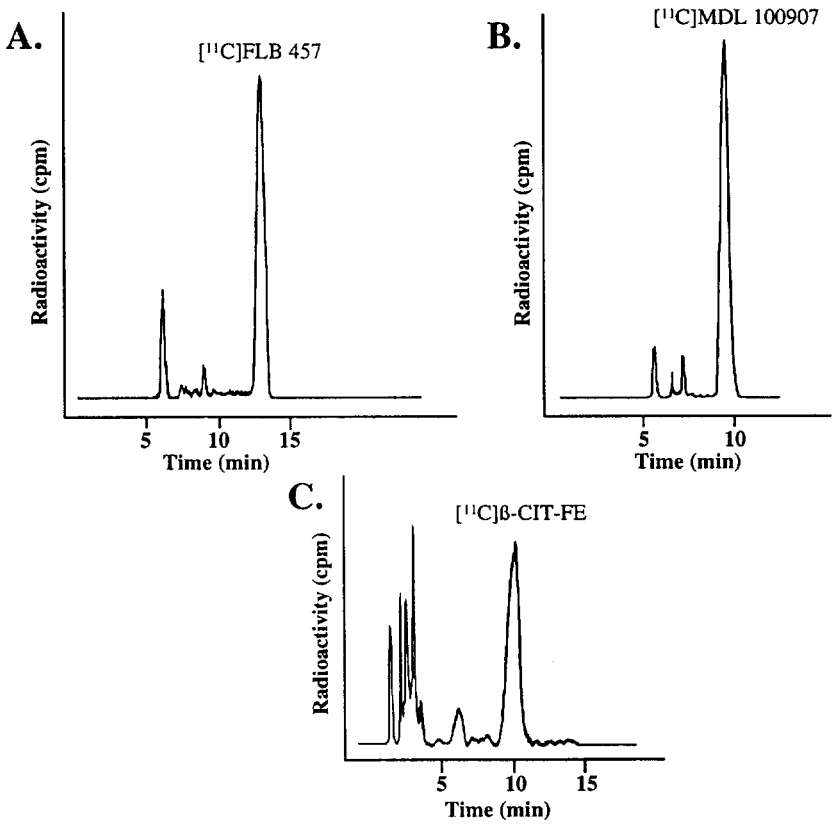


Figure 3. Semi-preparative HPLC chromatograms from the purification of **A:** [^{11}C]FLB 457, **B:** [^{11}C]MDL 100907, and **C:** [^{11}C]ß-CIT-FE.

RESULTS & DISCUSSION

Phenol methylations

Desmethyl-WAY-100635 was used as the precursor for establishing general labelling conditions for the methylation of phenols with [^{11}C]methyl triflate. The best conditions obtained were then applied directly to the other two ligands containing methoxy groups, namely [^{11}C]FLB 457 and [^{11}C]MDL 100907.

Reactions of desmethyl-WAY-100635 (0.3 mg) with [^{11}C]methyl triflate in acetone with sodium hydroxide as base at room temperature , over the short period (3-4 min) needed to trap the labelling agent, gave [^{11}C]WAY-100635 in 90–95% radiochemical yield (Figure 4). Heating the reaction mixture for an extra 5 min in an oil bath at 60 °C after trapping did not significantly increase the yield. Lower amounts of precursor (0.04–0.16 mg) gave lower radiochemical yields (20–70%), whether the reaction was heated or not (Figure 4).

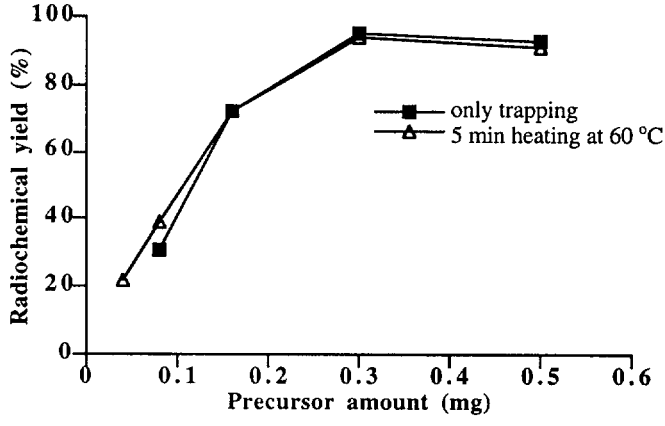


Figure 4. The dependence of radiochemical yield of [^{11}C]WAY-100635 on the amount of precursor (trapping only and with further heating for 5 min at 60 °C)

Radiochemical yields of [^{11}C]WAY-100635 depended little on the molar ratio of base (sodium hydroxide) to precursor. Reactions performed with 0.3 mg of precursor and 0.8–6 molar equivalents of base gave similarly high radiochemical yields (90–95%) (Figure 5).

Raising the molar ratio to 25, by using 3.8 μL of 5 M sodium hydroxide solution, reduced the radiochemical yield slightly to 85%. Thus, [^{11}C]methyl triflate is not rapidly destroyed by the strong base, sodium hydroxide, under these conditions. However, when the same amount of base is added in a greater amount of water (38 μL) the radiochemical yield is reduced to 66% (Figure 5). Hence, it appears important not to have too much water present in these reactions.

It is interesting to note that a reaction or [^{11}C]methyl triflate with desmethyl-WAY-100635 (0.3 mg) in acetone at room temperature without base gave a 15% radiochemical yield of [^{11}C]WAY-100635 (Figure 5). A combination of effects may explain this observation. First, the phenolic hydroxy group may itself act as a weak nucleophile for reaction with the [^{11}C]methyl triflate. Secondly, the amino group may act as a weak base to generate the more reactive phenoxide ion.

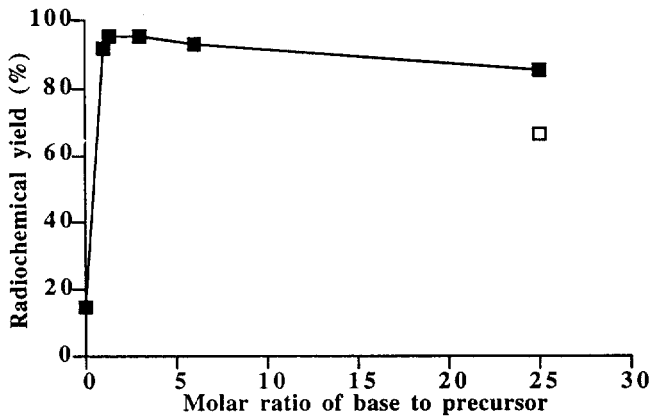


Figure 5. The dependence of the radiochemical yield of [^{11}C]WAY-100635 on the molar ratio of sodium hydroxide to precursor (last point:-■-NaOH ,5M in 3.8 μL water, -□-NaOH ,0.5M in 38 μL water).

DMF is a more polar solvent than acetone and usually gives higher yields in nucleophilic substitution reactions. This was true in reactions of [^{11}C]methyl triflate with 0.08 mg precursor, which gave 32% radiochemical yield of [^{11}C]WAY-100635 in acetone and 83% in DMF. However, the use of a higher amount of precursor (0.3 mg) in acetone gives excellent

radiochemical yield (95%). Also acetone is easily removed and therefore allows purification by normal-phase HPLC. This reduces the total synthesis time. It also has the advantage that the radioactive product elutes ahead of the much greater mass of precursor, and so contamination of the radioactive product by precursor is avoided.

It was concluded that [^{11}C]WAY-100635 was best prepared by using 0.3 mg precursor and 2 μL of 0.5 M sodium hydroxide solution in 300 μL acetone at room temperature for the period needed to trap the [^{11}C]methyl triflate (3-4 min). These conditions use 70% less precursor than the reported radiosynthesis from [^{11}C]methyl iodide giving a lower radiochemical yield (85%) (22).

Application of the optimal conditions for [^{11}C]WAY-100635 synthesis to the ^{11}C-methylation of other phenols gave [^{11}C]FLB 457 in greater than 90% radiochemical yield and [^{11}C]MDL 100907 in greater than 98% radiochemical yield. These results indicate the general applicability of these 'standard conditions' for effective ^{11}C-methylation of phenols. The radiochemical yields of [^{11}C]FLB 457 and [^{11}C]MDL 100907 are substantially greater than those earlier obtained using [^{11}C]methyl iodide (Table 1).

Typical radioligand productions

The 'standard conditions' for ^{11}C-methylation with [^{11}C]methyl triflate (0.3 mg precursor, 1 μmol sodium hydroxide, 400 μL acetone) were applied to the production of [^{11}C]FLB 457 and [^{11}C]MDL 100907. Each radioligand was purified by normal-phase HPLC.

[^{11}C]FLB 457 was typically obtained, using [^{11}C]methyl triflate, in 40–50% radiochemical yield from [^{11}C]carbon dioxide. Increasing the amount of starting material to 0.7 mg gave a slightly higher radiochemical yield. [^{11}C]FLB 457 eluted from the HPLC system after 12-13 min with good separation from the precursor. The radiosynthesis time was about 30 min. At present, a typical production of [^{11}C]FLB 457, using [^{11}C]methyl triflate as labelling agent, gives an overall radiochemical yield of 60–70%. The specific radioactivity of the radioligand is 2000–2500 Ci/mmol (74–93 GBq/μmol) at the time of administration, significantly higher than that obtained by using [^{11}C]methyl iodide as labelling agent in our laboratory (1000 Ci/mmol; 37 GBq/μmol). The normal amount of radioligand administered in a PET experiment on a human subject is 300 MBq and hence only about 1.5 μg of carrier is coinjected under these conditions. The specific radioactivity obtained for [^{11}C]MDL 100907 was comparable to that obtained for [^{11}C]FLB 457.

Typically, [^{11}C]MDL 100907 was obtained in 50–60% radiochemical yield from [^{11}C]carbon dioxide. [^{11}C]MDL 100907 eluted from the HPLC between 13–15 min and the radiosynthesis time was 25–30 min.

Table 1. Incorporation yields of PET radioligands prepared from [^{11}C]methyl iodide and [^{11}C]methyl triflate

	[^{11}C]Methyl iodide (n=3)			[^{11}C]Methyl triflate (n=3)	
Radioligand	Precursor (mg)	Reported yield (%)*	Reference	Precursor (mg)	Yield (%)*
[^{11}C]FLB 457	2.2	40-50	(11)	0.3	80-85
[^{11}C]MDL 100907	0.5	60-80	(12)	0.3	>95
[^{11}C]WAY-100635	1.0	85	(22)	0.3	90-95
[^{11}C]ß-CIT-FE	0.5-0.7	50-60	(18)	0.4	60-70

*% incorporation of radioactivity analyzed by HPLC.

Esterification

[^{11}C]ß-CIT-FE was labelled by esterification of the corresponding carboxylic acid with [^{11}C]methyl triflate. The reaction proceeded rapidly in acetone at room temperature with tetrabutyl ammonium hydroxide in a molar ratio of 0.8–1.2 to precursor (0.4-0.7 mg). The radiochemical yield from [^{11}C]methyl triflate was 60–70% at the end of reagent trapping. Further heating did not increase the radiochemical yield. Normal-phase HPLC failed to separate [^{11}C]ß-CIT-FE adequately from byproducts. [^{11}C]ß-CIT-FE was successfully purified by reverse-phase HPLC. Radioactive byproducts eluted with the void volume and were well-separated from [^{11}C]ß-CIT-FE, which eluted between 9 and 10 min. The radiosynthesis time was 25–30 min and the radiochemical yield was 40–50% from [^{11}C]carbon dioxide.

CONCLUSION

The use of [^{11}C]methyl triflate improved the radiochemical yields of the prepared radioligands compared to radiochemical yields from [^{11}C]methyl iodide. Reduced amounts of precursor could be used and no further reaction after trapping of the [^{11}C]methyl triflate was needed to obtain a high radiochemical yield. Generally, the use of acetone as solvent enables normal-phase HPLC to be used, which speeds up the purification. These improvements result in an overall shorter synthesis time and higher specific radioactivity. The results further show the versatility of [^{11}C]methyl triflate for methylation reactions, even in the presence of strong base.

Acknowledgements

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