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**Research Article**

# Microwave-assisted synthesis and chiral HPLC separation of $^{18}$F-labeled MaxiPost$^{TM}$. An agent for post-stroke neuroprotection

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## Summary

The syntheses of (*S*)-3-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-[$^{18}$F]-fluoro-6-(trifluoromethyl)-1H-indol-2-one was accomplished by the microwave assisted carrier-added $^{18}$F fluorination of (*R*, *S*)-3-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-chloro-6-(trifluoromethyl)-1H-indol-2-one, followed by chiral HPLC separation to afford the desired $^{18}$F-labeled enantiomer in radiochemical yields of 5–15% (EOS) and synthesis and purification times of 60–67 min. Biodistribution studies in rodents were consistent with previously reported studies using racemic $^{18}$F-radiolabeled material. Copyright © 2003 John Wiley & Sons, Ltd.

**Key Words:** neuroprotection; stroke; Maxipost$^{TM}$; $^{18}$F; microwave

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## Introduction

Stroke represents one of the largest disease states for which there remains significant unmet medical need. In the United States alone, there are more than 700 000 new cases each year.[1] Stroke can be classified as being either hemorrhagic in origin, resulting from vessel rupture, or ischemic in origin resulting from vessel occlusion. Approximately 85% of all strokes are ischemic in origin. In acute ischemic stroke, a core area of severely damaged tissue located distal to an occluded vessel, is surrounded by a penumbra of tissue at risk because of proximity to the core and as a result of the low vascular perfusion to the tissue.[2]

At the location of insult, the resulting hypoxia results in release of an extraordinary level of excitatory neurotransmitters resulting in pathologically high intracellular levels of calcium ($Ca^{2+}$). Our approach to neuroprotective therapy, has been to augment the opening of a particular type of potassium channel, the large-conductance maxi-K $Ca^{2+}$-dependent potassium channel. Previous publications have addressed the chemistry, and biology leading to the identification and subsequent development of BMS-204352, (*S*)-3-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2H]indol-2-one, **(1)** Maxipost™ as a clinical candidate for post-stroke neuroprotection (Figure 1).[3–6]

Kiesewetter *et al*. reported the first radiosynthesis of the $^{18}F$ labeled racemate, (*R, S*)-3-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-[$^{18}F$]-fluoro-6-(tri-fluoromethyl)-2H]indol-2-one, **(2)** via the silver assisted nucleophilic substitution of [$^{18}F$] labeled fluoride on the corresponding chloro precursor **(3)**.[7] In their carrier-added radiosynthesis they obtained HPLC purified material in 67 min from EOB, with routine radiochemical yields of 15% at the end of the synthesis (EOS). Yields of

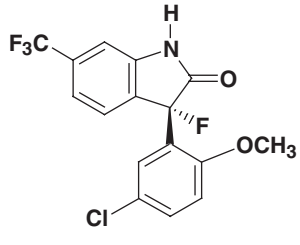
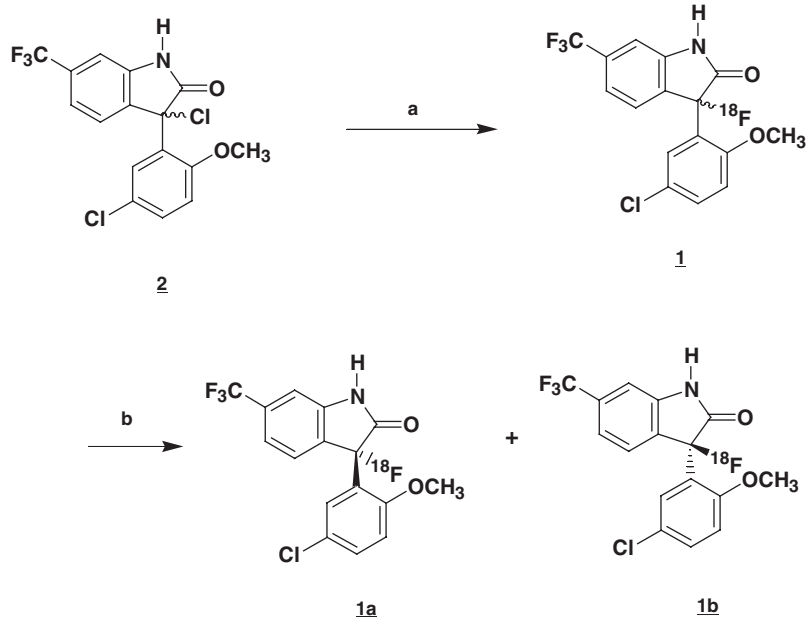



**Figure 1. Maxipost™**

the no carrier-added radiosynthesis were significantly lower (~1%). Biodistribution studies in rodents with **(2)** confirmed that uptake of this agent was rapid and evenly distributed throughout the brain. Biodistribution studies conducted with **(2)** and increasing amounts of non-radiolabeled drug showed no difference in uptake of the compound in any brain region and indicating that no saturable specific uptake mechanism was involved in the brain uptake of this compound.[7]

The objective of this study was to radiolabel the actual clinical candidate, (*S*)-3-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-[$^{18}$F]-fluoro-6-(trifluoromethyl)-2H]indol-2-one, **(1a)** for use in subsequent biological studies in animals and ultimately PET studies in humans (Scheme 1). Our approach for this carrier-added synthesis involved investigating the use of microwave to improve the radiochemical yield and reduce the overall synthesis time while also incorporating a chiral HPLC purification to resolve both of the radiolabeled enantiomers **(1a, 1b)** from each other as well as the corresponding enantiomers of the chloro precursor **(2)**.




**Scheme 1. Synthesis of (*S*)-3-(5-Chloro-2-Methoxyphenyl)-1,3-Dihydro-3-[$^{18}$F]-Fluoro-6-(Trifluoromethyl)-2H-Indol-2-one, 1a Reagents: a, [$^{18}$F[F$^-$, $(Bu)_4NF$, AgOTf, $CH_3CN$, microwave; b, Chiral HPLC**

## Materials and methods

[$^{18}$F]-Fluoride was obtained from P.E.T.Net® Pharmaceuticals, Inc. All reagents were obtained from Aldrich Chemical Company and were either ACS grade of the highest quality material commercially available. Chiralcel OJ and OD and Chiralpak AD and AS HPLC columns were obtained from Chiral Technologies, Inc. Micro vials (5 ml) were obtained from Kontes. The microwave heater model RI 520A was obtained from Resonance Instruments, Inc. The identity of intermediates and final product was established by co-elution of the radiolabeled material with authentic unlabeled compound on HPLC.[4,6] HPLC purification and analysis was performed on a Rainin Dynamax HPLC system consisting of two SD-200 pumps, a Rainin UV-1 detector and an *INUS* $\gamma$-RAM radioactive flow through detector. Radiochemical purity and specific activity were determined by HPLC.

### *Microwave cavity modification*

An aluminum collar containing an air line inlet (1/8″ orifice) connected to pressure regulated house air (29 psi) was attached to the top of the microwave cavity via strong adhesive (see Figure 2). The inside walls of the collar have a 30° lead to aid in inserting the vial into the cavity. There is also a cut away window in the collar to allow for visual inspection of the vial during the reaction as well as maximizing efficient airflow to aid in cooling. Using this system we have achieved cooling of a 0.5 ml sample of water from 79 to 53°C in 1 min, 38°C in 2 min and 31°C in 3 min. This system also permits immediate cooling of boiling reaction mixtures during microwave heating.

*(S-)-3-(5-Chloro-2-methoxyphenyl)-1,3-dihydro-3-[$^{18}$F]fluoro-6-(trifluoromethyl)-2H]indol-2-one,* **1c**. Into a 5 ml micro reaction vial containing 20 µl of water containing 3 µmol of $Me_4NF$ was added [$^{18}$F]-fluoride (6–20 mCi) dissolved in 0.3–0.5 ml of $H_2O$. The vial was placed in an oil bath (105°C) and the water evaporated with a stream of argon. Three portions of $CH_3CN$ (200–400 µl) were sequentially added and then evaporated to aid in removal of residual water. Following the last addition of $CH_3CN$, the solution was evaporated down to a volume of $\sim$20 µl and the vial allowed to cool by placing it in a small crystallizing dish containing $H_2O$ for $\sim$1 min. To the vial was then added $CH_3CN$ (100 µl), a solution

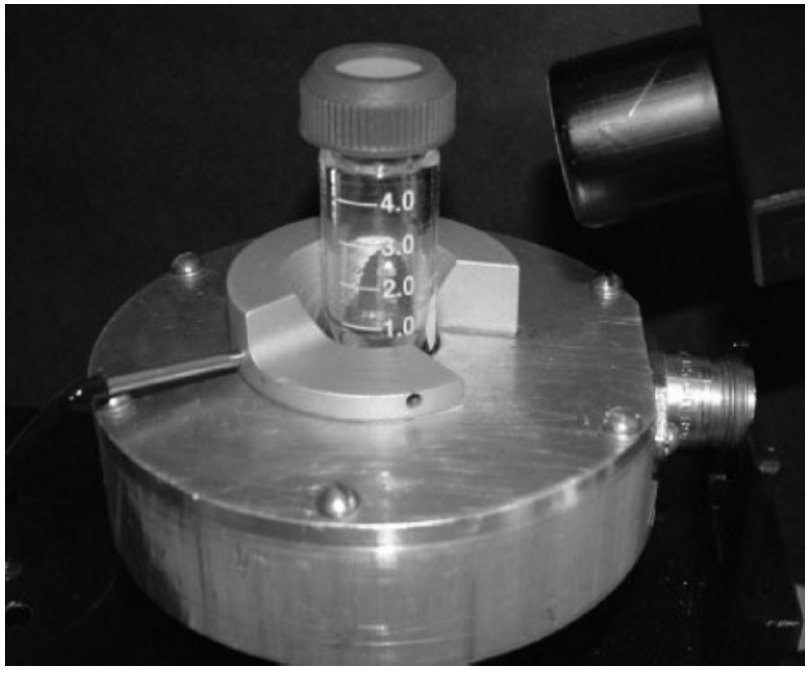



**Figure 2. Modified microwave cavity cell**

of **(2)** (1.9 mg in 200 µl of $CH_3CN$) and a solution of silver trifluoromethanesulfonate (3 µmol dissolved in 30 µl of $CH_3CN$). The vial was capped with a teflon septum and open top screw cap and placed in the microwave cavity. The vial was then treated with 100 W of microwave power for 2 min and then air-cooled by using a source of high pressure house air. The solution was then cannulated into a glass pipet containing silica gel (5 mm) and collected in a second 5 ml micro vial. (Following incorporation of the radiolabel into **(2)**, the use of glass vessels, tubing, syringes, etc., instead of plastic, is recommended to avoid loss of the radiolabeled material). The original reaction vial was rinsed with $CH_3CN$ (0.3–0.5 ml) and the solvent also passed through the silica gel column and collected. The solvent was then evaporated from the second vial with a stream of nitrogen. Evaporation was aided by placing the vial intermittently in an oil bath (105°C). Prior to the vial being taken to dryness, EtOH (0.2–0.4 ml) was added and then evaporated to $\sim$100 µl, and then diluted with hexane (1.5 ml). This solution was then applied to a Chiralpak AS column and purified via HPLC (Method A, Figure 3). The sample was then collected and

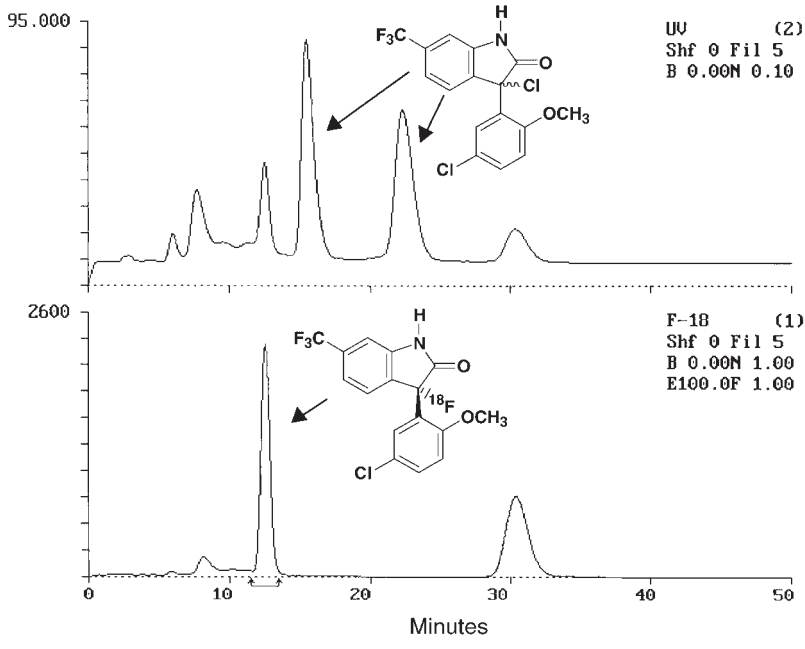



**Figure 3. Radio-HPLC purification of $^{18}$F-Maxipost. Chiralpak AS column (10 mm × 250 mm) with a mobile phase of 97% hexane/3% absolute EtOH, flow rate of 6.0 ml/min (Method A)**

concentrated in a rotary evaporator prior to formulating the material as described below. The specific activity of 1a from a typical radiolabeling experiment was determined to be 0.9 mCi/μmol.

*High performance liquid chromatography*

*Method A.* In this method samples were loaded onto a Chiralpak AS column (10 × 250 mm) with a mobile phase of 97% hexane and 3% absolute EtOH at a flow rate of 6.0 ml/min. The UV-1 detector was set at 220 nm. In this system, the desired enantiomer $^{18}$F labeled **(1a)** had a $R_t$ of 10.5–12 min while the undesired $^{18}$F labeled enantiomer had a $R_t$ of 16–18 min.

*Method B.* In this method samples were loaded onto two Chiralpak AS column (10 × 250 mm) run in series with a mobile phase of 97% hexane and 3% absolute EtOH at a flow rate of 6.0 ml/min. The UV-1 detector was set at 220 nm. In this system, the desired enantiomer $^{18}$F labeled **(1a)** had a $R_t$ of 23.5–26.0 min while the undesired $^{18}$F labeled

enantiomer had a $R_t$ of 59.0–65.4 min. The enantiomers of the chloro precursor **(2)** had a retention times of 28.4–33.0 min and 43.5–49.4 min, respectively.

*Method C*. In this method samples were loaded onto a Chiralpak AS column (4.6 × 250 mm) with a mobile phase of 97% hexane and 3% absolute EtOH at a flow rate of 1.0 ml/min. The UV-1 detector was set at 220 nm. In this system, the desired enantiomer $^{18}$F labeled **(1a)** had a $R_t$ of 16.5 min while the undesired $^{18}$F labeled enantiomer had a $R_t$ of 49.3 min. The enantiomers of the chloro precursor **(2)** had a retention times of 20.5 and 36.0 min, respectively.

*Biodistribution studies in rat*

All animal studies were performed under a protocol approved by the Bristol-Myers Squibb Animal Care and Use Committee (Wallingford, CT). Rats were obtained from Hilltop Lab Animals, Scottsdale, PA with a surgically placed polyurethane jugular vein cannula. The cannula was flushed with saline just prior to use to verify patency. [$^{18}$F]-**1a** was formulated in a cocktail consisting of PEG 300 (40% w/w), Tween 80 (15% w/w), absolute ethanol (10% w/v), and deionized water (35% w/v) using Hamilton glass syringes. This cocktail was selected to be consistent with previous studies done in-house. Rats were injected intravenously via jugular cannula with [$^{18}$F]-**1a**, 12–17 μCi (30–50 μl). The cannula was flushed with saline following injection. A minimum number of four rats were used per each time point. Rats were humanely euthanized ($CO_2$ asphyxiation) at designated time points. Blood was collected and selected tissues were dissected from each animal and weighed. Corrected data refers to the activity injected into the animal (activity in syringe minus activity remaining in cannula following injection). A Wallac Wizard 3″ 1480 Automatic Gamma Counter was used to determine the radioactivity in each tissue and the percent injected dose per gram tissue was calculated.

## Results and discussion

This radiosynthesis builds upon that of Kiesewetter *et al*. who reported that substitution of the tertiary chloride of **(2)** with [$^{18}$F]-fluoride was

achieved only following the addition of silver triflate to increase the $S_N1$ character of the reaction.[7] In our procedure, we utilized microwaves at powers of 65–200 W to accelerate the fluorination reaction. Using capped micro reaction vials, we were concerned about the vigorous refluxing achieved with 200 W of power and decided upon a microwave power 100 W which provided us with a more controlled reflux. At this power setting, no increase in radioactive yields was realized in increasing the refluxing time from 2 to 4 min. During the course of this work, we modified the microwave cavity to include a custom built remote controlled air-cooling collar which permits visualization of the reaction vessel while also providing a mechanism for immediate cooling.

Efforts were made to prepare carrier-free $^{18}$F-labeled **1a** using the microwave assisted method. Reaction conditions were identical to that of the carrier-added procedure except that $Bu_4NF$ was replaced with an equimolar amount of $Me_4NOH$. Microwave powers of 100 or 200 W (*air cooling of the outside of the reaction vessel was required during the 200 W experiment*) were utilized with reactions times as long as 5 min. In all experiments only trace amounts ($<0.5\%$) of the radiolabeled product was obtained. These results are also comparable to that previously reported using thermal heating.[7]

Three chiral analytical HPLC columns were evaluated (Chiralcel OJ, Chiralpak AD and Chiralpak AS) using a mobile phase of 95–97% hexane/ethanol to purify Maxipost™ **(1a)** (Method C, Figure 4). Of these three systems, the Chiralpak AS HPLC column provided us with the best separation (Figure 4, Panel A). In this system the desired radiolabeled enantiomer **(1a)** elutes first off the column and is well resolved from the undesired radiolabeled enantiomer **(1b)** and one of the two enantiomers of **(2)**. In some radiolabeling experiments, it was found that depending upon the solvent composition of the injectate and the care with which the **(1a)** was collected off of the column, some samples contained the earlier eluting undesired non-radioactive enantiomer of **(2)**. Complete separation of this non-radiolabeled enantiomer from **(1a)** was achieved by using two Chiralpak AS columns run in series.

Biodistribution studies of **(1a)** were in excellent agreement with that observed with the racemic material (Table 1).[7] Uptake of the activity in femur as a function of time (indicating defluorination) was also similar to that observed with the racemic material.[7]

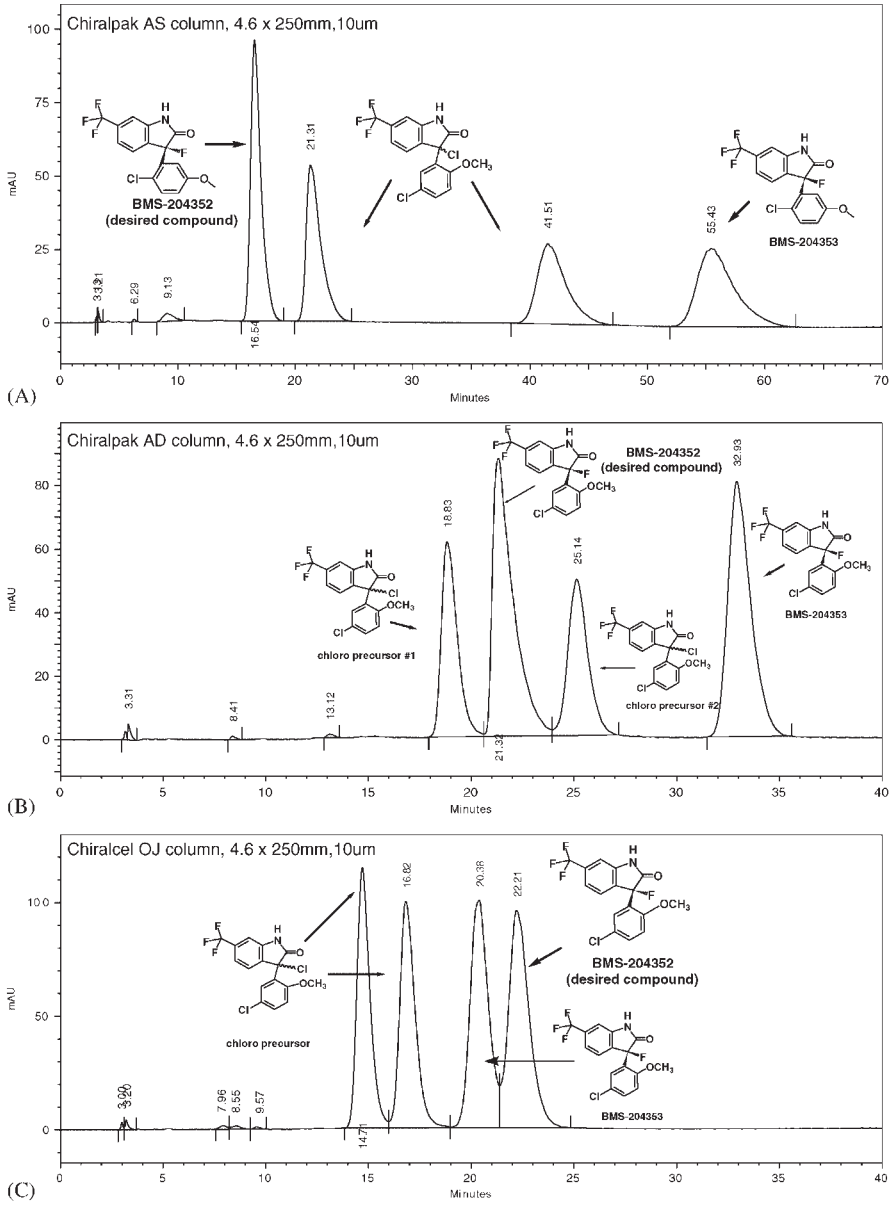



**Figure 4. Each of the above HPLC columns were run under the following conditions: 97% hexane/3% ethanol at 1.0 ml/min. Samples were prepared with 0.5 mg/ml in ethanol. Injection volumes were 5 ul. UV absorbance was monitored at 220 nm**

**Table 1. Time course of the biodistribution of $^{18}$F-labeled MaxiPost™ in rat ($n \geqslant 4$)**

| Time | Data | Blood | Brain | Femur | Heart | Kidney | Liver |
|---|---|---|---|---|---|---|---|
| 15 min | Average of %ID/g | 0.176 | 0.510 | 0.394 | 0.566 | 0.720 | 1.627 |
| | (s.d.) | (0.021) | (0.006) | (0.010) | (0.004) | (0.037) | (0.033) |
| 30 min | Average of %ID/g | 0.138 | 0.345 | 0.711 | 0.346 | 0.479 | 0.956 |
| | (s.d.) | (0.016) | (0.059) | (0.121) | (0.061) | (0.066) | (0.170) |
| 60 min | Average of %ID/g | 0.082 | 0.204 | 1.292 | 0.235 | 0.316 | 0.629 |
| | (s.d.) | (0.010) | (0.029) | (0.229) | (0.029) | (0.028) | (0.084) |

## Conclusions

The syntheses of (*S*)-3-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-[$^{18}$F]-fluoro-6-(trifluoromethyl)-1H-indol-2-one was accomplished by the microwave assisted carrier-added $^{18}$F fluorination of (*R*,*S*)-3-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-chloro-6-(trifluoromethyl)-1H-indol-2-one, followed by chiral HPLC separation. The specific activity of this material was 0.9 mCi/μmol which was acceptable for its intended use in biodistribution studies. The results of rat biodistribution studies using the $^{18}$F radiolabeled enantiomer were in excellent agreement to that previous reported for the racemic material.

### Acknowledgements

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