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# An improved synthesis of [$^{11}$C]MENET via Suzuki coupling with [$^{11}$C]methyl iodide

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**[$^{11}$C]MENET, a promising norepinephrine transporter imaging agent, was prepared by Suzuki cross coupling of 1 mg *N-t*-Boc pinacolborate precursor with [$^{11}$C]$CH_3I$ in DMF using palladium complex generated *in situ* from $Pd_2(dba)_3$ and ($o$-$CH_3C_6H_4)_3P$ together with $K_2CO_3$ as the co-catalyst, followed by deprotection with trifluoroacetic acid. This improved radiolabeling method provided [$^{11}$C]MENET in high radiochemical yield at end of synthesis (EOS, 51 ± 3%, decay-corrected from end of $^{11}CH_3I$ synthesis, $n = 6$), moderate specific activity (1.5–1.9 Ci/μmol at EOS), and high radiochemical (>98%) and chemical purity (>98%) in a synthesis time of 60 ± 5 min from the end of bombardment. Copyright © 2013 John Wiley & Sons, Ltd.**

**Keywords:** [$^{11}$C]MENET; Suzuki coupling; carbon-11; pinacolborate

## Introduction

[$^{11}$C]MENET ([$^{11}$C](2*S*,3*S*)-2-[α-(2-methylphenoxy)phenylmethyl] morpholine), a carbon-11 labeled reboxetine analogue, is a promising norepinephrine transporter (NET) imaging agent.[1] *In vivo* microPET imaging studies in rhesus monkeys showed that [$^{11}$C]MENET possesses appropriate regional cerebral activity distributions correlated to known NET density profiles with the ratio of uptake of [$^{11}$C]MENET in midbrain to that in caudate peaked at 1.45 at 45 min, and a specific binding peak equilibrium was achieved in less than 1 h after injection, a highly favorable pharmacokinetic property for mapping NET occupancy in the human brain. [$^{11}$C]MENET was initially prepared by Stille coupling of the corresponding aryltrimethyl stannane with [$^{11}$C]$CH_3I$. In the process of preparation of [$^{11}$C]MENET for initial clinical studies, unfortunately, the specific activity of [$^{11}$C]MENET was determined to be 140–200 mCi/μmol at EOS, which was far below the criteria (must be >300 mCi/μmol at time of patient administration) suitable for use in clinical PET imaging studies. Although the mechanism for the formation of low specific activity has not been fully clarified, transfer of one of the methyl groups in the trimethyl stannane was suggested to contribute to the amount of unlabeled product and consequently resulted in low specific activity.[2–4] One possible alternative approach is to use tributyl stannane as the precursor. However, probably because of steric hindrance, none or only trace amount of tributyltin precursor was obtained in spite of numerous attempts (data not presented). Recent advances in palladium-catalyzed methylation of organoboron compounds with [$^{11}$C]$CH_3I$ based on Suzuki–Miyaura coupling prompted us to pursue a boron precursor as an alternative approach. [$^{11}$C]MENET was prepared by cross coupling of *N-t*-Boc arylboronate ester with [$^{11}$C]$CH_3I$ under palladium catalysis followed by deprotection with TFA in high radiochemical yield and moderate specific activity.

## Experimental

### General

(2*S*,3*S*)-*N-tert*-Butoxycarbonyl-2-[α-(2-iodophenoxy)phenylmethyl] morpholine (**1**) was synthesized according to the recently reported procedure.[1] All reagents used were obtained from commercially available sources and were used without further purification. Solvents were anhydrous grade purchased from Aldrich and used as received. $^1$H NMR spectra were recorded on a Varian spectrometer at 300 MHz and referenced to the NMR solvent (chemical shifts in ppm values, *J* values in Hz). High-resolution mass spectra were acquired on a VG 70-S double focusing mass spectrometer using electron ionization. Flash chromatography was carried out using Merck silica gel 60 (40–63 μm particle size). Glassware used in the radiolabeling was foil-wrapped and depyrogenated.

### Synthesis of the aryl boronate precursor (2)

To a solution of **1** (81 mg, 0.16 mmol) in dioxane (2 mL) was added Pd $(OAc)_2$ (1.8 mg, 8 μmol), $Et_3N$ (92 μL, 0.66 mmol), and (2-biphenyl) dicyclohexyl phosphine (11.2 mg, 0.032 mmol). The mixture was purged under Ar for 15 min, and then pinacolborane (70 μL, 0.48 mmol) was added dropwise. The reaction mixture was heated at 75 °C for 1 h. After cooling to room temperature, the reaction was quenched by adding a sat. solution of $NH_4Cl$, and the aqueous phase was extracted with $CH_2Cl_2$. The extracts were combined and dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica (deactivated with 5% $Et_3N$) eluted with hexane/EtOAc (80:20) to afford **2** as colorless oil (64 mg, 79%). $^1$H NMR ($CDCl_3$, 300 MHz), δ 7.62 (dd, $J = 7.3$, 1.7 Hz, 1H), 7.45 (d, $J = 6.7$ Hz, 2H), 7.17–7.33 (m, 4H), 6.86 (m, 1H), 6.68 (d, $J = 8.2$ Hz, 1H), 5.22 (d, $J = 5.0$ Hz,

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1H), 3.82–3.95 (m, 4H), 3.52 (dt, $J = 2.6$, 11.7 Hz, 1H), 2.89 (m, 1H), 2.71 (m, 1H), 1.39 (m, 21H). HRMS $[MH]^+$ Calcd for $C_{28}H_{39}BNO_6$: 496.2865, Found: 496.2862.

### Radiosynthesis of [$^{11}$C]MENET

No carrier-added [$^{11}$C]$CO_2$ was produced through the bombardment of $^{14}N_2$ gas containing 1% $^{16}O_2$ (dual targets, 55 μA, 80 min) by a Siemens 11 MeV RDS 111 cyclotron (Knoxville, TN, USA) at Emory University Center for Systems Imaging through the $^{14}N[p,\alpha]^{11}C$ reaction. A GE TracerLab FX MeI system was employed for the conversion of [$^{11}$C]$CO_2$ to [$^{11}$C]$CH_3I$. $Pd_2(dba)_3$ (1.8 mg, 2.0 μmol), (*o*-Tol)$_3$P (2.4 mg, 7.7 μmol), and $K_2CO_3$ (2.8 mg, 20 μmol) were placed in a 3-mL dry conical vial, and then the vial was sealed and purged with Ar for 15 min. A solution of **2** (1.0 mg, 2.0 μmol) in DMF (300 μL) was added to the vial, and then $^{11}CH_3I$ was bubbled through the solution by a stream of helium (12 mL/min) at room temperature. When the maximum delivery of [$^{11}$C]MeI was reached, the vial was heated at 90 °C for 4 min, TFA (0.22 mL, ~750 equiv) was added, and the solution was heated at 90 °C for another 6 min, cooled in an ice bath for 40 s, and neutralized by addition of 5 M $NH_4OH$(aq) (0.6 mL, ~800 equiv.). The reaction mixture was pressure filtered through a 0.45-μm Acrodisc filter into a conical vial to remove salts and palladium residues before loading on a semipreparative HPLC (Water XTerra Prep $RP_{18}$ 5 μm, 19 × 100 mm) for purification. The column was eluted with buffered mobile phase consisting 45:55:0.1 v/v/v EtOH/$H_2O$/$Et_3N$ at a 9-mL/min flow rate. Under these conditions, the [$^{11}$C]MENET product had a retention time of approximately 11 min. The desired fractions eluting at this time were collected, diluted with a double volume of water, and loaded onto a Waters $tC_{18}$ SepPak cartridge, which was then washed with saline (0.9% NaCl, 40 mL). The radioactive product was eluted from the cartridge by absolute ethanol (1.0 mL) into a sealed sterile vial containing 9.0 mL of saline. The resulting solution was then filtered under pressure through a 0.2-μm Millex-FG filter to give a sterile aqueous solution of [$^{11}$C]MENET, which was submitted for quality control testing.

Radiochemical and chemical purities were assessed using a Waters Alliance 2695 HPLC equipped with a Bioscan flow-count radioactivity detector (Waters Nova-Pak $C_{18}$ 4 μm, 3.9 × 300 mm; mobile phase: MeOH/$H_2O$/$Et_3N$ = 75:25:0.1; flow rate: 1.0 mL/min; $t_R$ = 7.39 min). A representative chromatogram is shown in Figure 1. Specific activity was determined by using the same analytical HPLC system against a calibration curve prepared with the [$^{12}$C]MENET standard. Sterility testing was performed in-house per US Pharmacopeial guidelines. Briefly, culture tubes of soybean casein and fluid thioglycollate mediums were inoculated with samples of [$^{11}$C]MENET and incubated, along with positive and negative controls, for 14 days with daily visual inspect (excluding weekends and holidays). All [$^{11}$C]MENET-doped cultures were clear of any growth of microorganisms ($n = 6$). Bacterial endotoxin pyrogen testing was performed using the Charles River Laboratories Endosafe Portable Testing System and in accordance with US Pharmacopeia. All results showed an endotoxin concentration of $<5.0$ EU/mL ($n = 6$).

## Results and discussion

The *N*-*t*-Boc pinacolboranate precursor (**2**) was prepared by palladium-catalyzed borylation of iodo compound (**1**)[1] with pinacolborane.

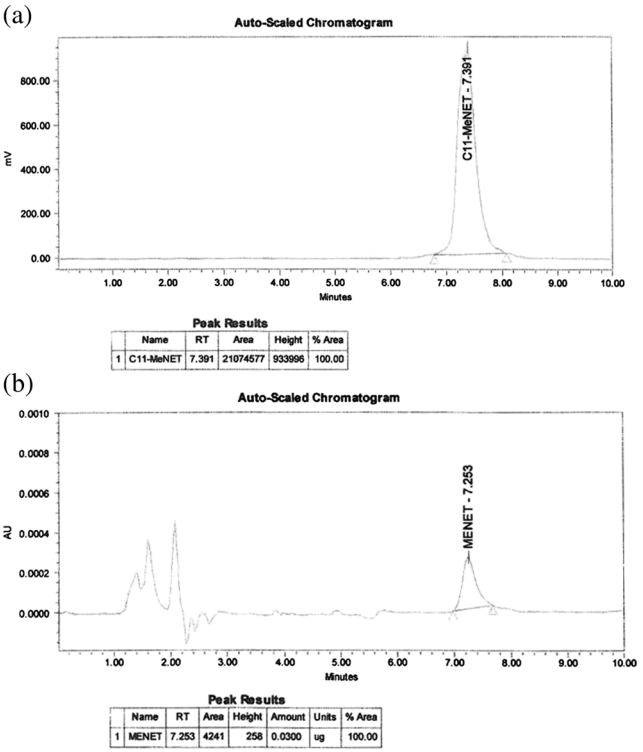
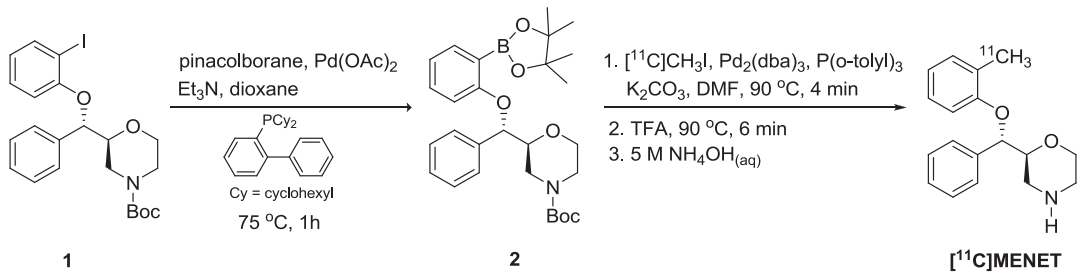



**Figure 1.** Chromatograms of analytical HPLC of [$^{11}$C]MENET: (a) radioactivity and (b) UV absorbance at 280 nm.

Although several methods were reported for the synthesis of sterically hindered ortho-substituted arylboronic acid or arylbornate, in our hands, **2** was efficiently synthesized using the procedure developed by Baudoin *et al.*[5–9] As shown in Scheme 1, under the catalytic system of $Pd(OAc)_2$ and sterically hindered phosphine ligand with a ratio of 1:4, pinacolboranate precursor **2** was obtained in a high isolated yield of 79% by reaction of iodo compound **1** with pinacolborane in dioxane using $Et_3N$ as the base under relatively mild condition.

[$^{11}$C]MENET was prepared from *N*-*t*-Boc pinacolboranate (**2**) in a two-step, one-pot radiosynthesis. The first step was a palladium-catalyzed cross coupling reaction between **2** and [$^{11}$C]$CH_3I$ using a modified procedure developed by Suzuki *et al.*[10] Palladium-catalyzed cross coupling reactions utilizing organoboron compounds, commonly referred to as Suzuki couplings, have recently been shown to be an effective labeling method alternative to the Stille coupling for the synthesis of compounds containing a [$^{11}$C]methylphenyl moiety.[10–14]

Reacting **2** (1 mg) with [$^{11}$C]$CH_3I$ at 90 °C using palladium complex generated *in situ* from $Pd_2(dba)_3$ and (*o*-$CH_3C_6H_4)_3P$ (1:4) together with $K_2CO_3$ as the co-catalyst gave us a high




**Scheme 1.** Synthesis of *N*-*t*-Boc pinacolborane precursor (**2**) and radiosynthesis of [$^{11}$C]MENET.

coupling yield. The *N*-Boc group was cleaved under acidic conditions, and the reaction mixture was then neutralized before HPLC purification.

The total synthesis time (including purification and formulation) of [$^{11}$C]MENET was 60 ± 5 min from the end of bombardment. The dose of [$^{11}$C]MENET was a clear, colorless, and particulate-free solution with pH = 5.

Starting with 950–1100 mCi of [$^{11}$C]methyl iodide, typical syntheses provided 104–140 mCi (uncorrected) of [$^{11}$C]MENET at EOS in an average radiochemical yield of 51 ± 3% ($n$ = 6, decay-corrected from end of $^{11}CH_3I$ synthesis). The radiochemical and chemical purity of [$^{11}$C]MENET were consistently >98%, and the specific activities were in the range of 1.5–1.9 Ci/μmol at EOS ($n$ = 6).

It is worthy to point out that, to the best of our knowledge, this is the first report that showed successful radiolabeling using only 1 mg precursor in the Suzuki coupling or Stille coupling with [$^{11}$C]$CH_3I$, whereas all other previous reports used 2 mg or more precursor in the coupling reaction.

Radiosynthesis of [$^{11}$C]MENET reported herein using pinacolborate as the precursor considerably improved the previously reported results in terms of radiochemical yield and specific activity. Under the current reaction conditions, a dose of 104–140 mCi of [$^{11}$C]MENET with moderate specific activity of 1.5–1.9 Ci/μmol at EOS could be produced, whereas only 25–40 mCi of [$^{11}$C]MENET with low specific activity of 140–200 mCi/μmol could be synthesized from the previous method.

## Conclusion

In this study, an improved method for the preparation of the NET imaging agent [$^{11}$C]MENET has been reported. The method based on the Suzuki cross-coupling of *N*-*t*-Boc pinacolboranate precursor with [$^{11}$C]$CH_3I$ under palladium catalysis followed by deprotection provided [$^{11}$C]MENET in high radiochemical yield and improved specific activity. Imaging studies using [$^{11}$C]MENET in humans are now in progress.

## Conflict of Interest

The authors did not report any conflict of interest.

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