(±)-N-[^{11}C]METHYL-*PARA*-HYDROXYTRANYLCYPROMINE: SYNTHESIS AND EVALUATION AS A PET RADIOTRACER FOR QUANTIFYING CARDIAC SYMPATHETIC NERVE DENSITY

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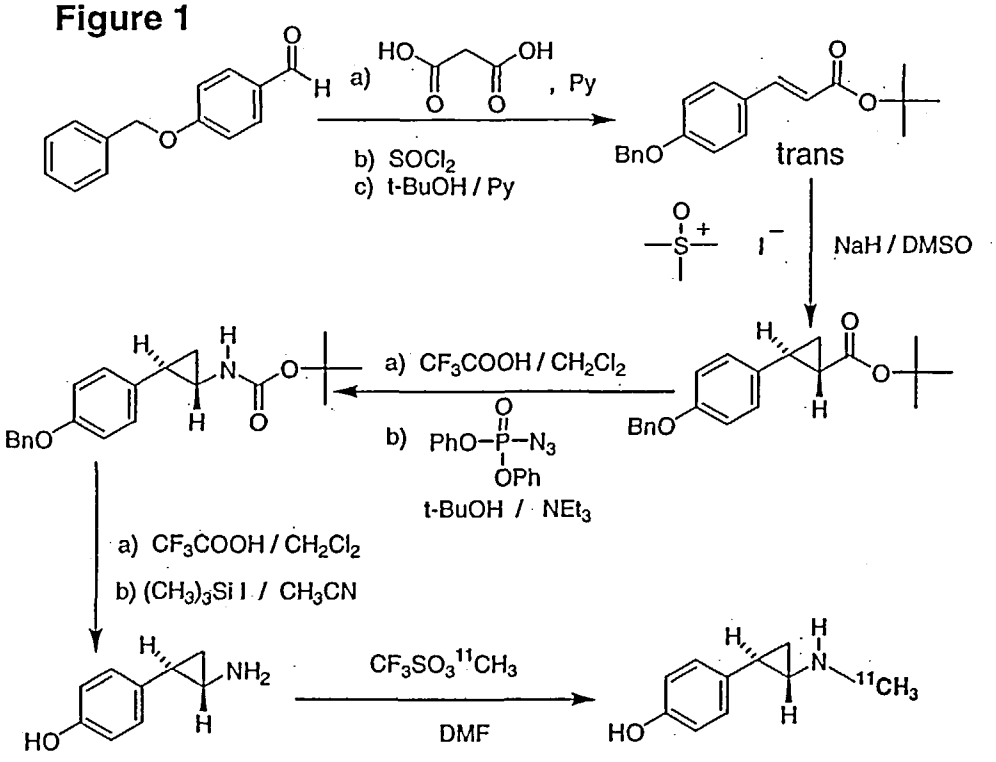
Our laboratory has previously developed several radiotracers that can be used to noninvasively assess regional cardiac sympathetic nerve integrity in the living human, including radioiodinated *meta*-iodobenzylguanidine (MIBG) for single-photon imaging studies[1] and [^{11}C]*meta*-hydroxyephedrine (HED) for PET imaging studies[2]. However, the kinetic properties of the current generation of neuronal imaging agents are not conducive to quantitative analysis with compartmental modeling techniques, which limits our ability to obtain sensitive measures of neuronal density. In an effort to develop a sympathetic nerve tracer with more favorable kinetic properties for compartmental modeling, we synthesized a ^{11}C-labeled analog of tranylcypromine[3] (TCP), a suicide inhibitor of monoamine oxidase (MAO) that is also a substrate for neuronal uptake by the norepinephrine transporter (NET). In principle, a NET substrate that is transported into sympathetic neurons more slowly than HED and is subsequently trapped intraneuronally by a rapid and irreversible binding process would possess ideal kinetic properties for absolute quantification of neuronal density.

The synthesis of (±)-*para*-hydroxytranylcypromine was accomplished in an 8-step reaction sequence as shown in **Figure 1.** *Para*-benzyloxybenzaldehyde was reacted with malonic acid in pyridine to afford *para*-benzyloxycinnamic acid. The acid was converted to an acid chloride by refluxing with thionyl chloride in benzene and followed by treatment with dry *t*-butyl alcohol in the presence of pyridine to give the *t*-butyl ester. Cyclopropanation was conducted by 1,4-addition of dimethyl-oxosulfonium methylide generated from a mixture of trimethylsulfonium iodide and sodium hydride in DMSO. The *t*-butyl ester moiety was cleaved by trifluoroacetic acid in CH_2Cl_2 to afford cyclopropanecarboxylic acid. A conversion of carboxylic acid to carbamate was achieved by treatment of diphenylphosphorazidate (DPPA) in the presence of *t*-butyl alcohol and triethylamine. The *t*-butyl carbamate was hydrolyzed with trifluoroacetic acid in CH_2Cl_2, followed by treatment with excess iodotrimethylsilane to furnish the desired (±)-*para*-hydroxytranylcypromine.

(±)-N-[^{11}C]methyl-*para*-hydroxytranylcypromine was synthesized from (±)-*para*-hydroxytranylcypromine freebase and $CF_3SO_3{}^{11}CH_3$ in DMF at ambient temperature, followed by purification by SCX HPLC. Collected fractions containing the material of interest were analyzed by reversed-phase ion-pair radio-HPLC. The desired

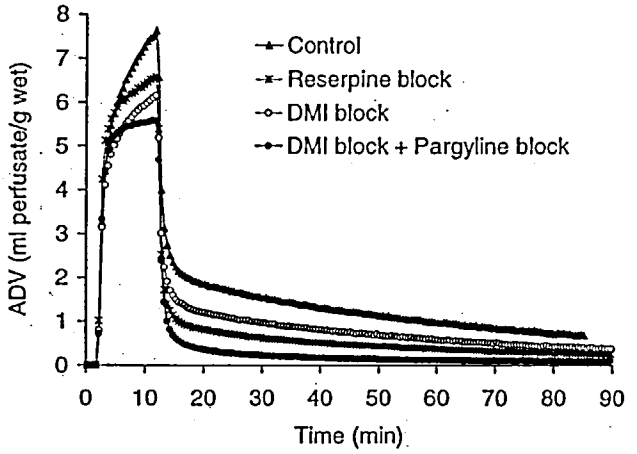
product was produced with an estimated specific activity of 500-1000 Ci/mmol, a radiochemical yield of >40% (EOS) and radiochemical purity >87%.

Figure 1



The neuronal uptake and retention of the compound was investigated using an isolated working rat heart system[4-6]. Hearts were infused with tracer concentrations of (±)-N-[^{11}C]methyl-*para*-hydroxytranylcypromine for 10 min, then switched to tracer-free perfusate for 80 min to study tracer clearance (**Figure 2**). Under control conditions, heart uptake of the compound proceeded at 0.28 ml/min/g wet, considerably slower than HED (2.4 ml/min/g wet). Following rapid clearance of tracer from capillaries and interstitium, the remaining tracer in tissue cleared with a half-time of 46 min, suggesting that the compound does not bind irreversibly to MAO. Pretreatment with reserpine decreased the peak uptake at the end of tracer infusion and reduced the amount retained in the heart, indicating that vesicular storage of the tracer occurs. Blocking neuronal uptake with desipramine (DMI) reduced but did not eliminate uptake and retention, indicating that extraneuronal uptake occurs. Since most MAO in the rat heart is localized extraneuronally, binding to extraneuronal MAO is likely to be the main retention mechanism in this case. Consistent with this hypothesis, the combination of MAO inhibition with pargyline and DMI block of neuronal uptake largely eliminated the uptake and retention of the tracer.

Figure 2. N-[^{11}C]methyl-*para*-hydroxytranylcypromine kinetics in isolated rat heart.



Biodistribution studies were performed in rats and the results are given in **Table 1.**

Table 1. Biodistribution data in rats (% dose/g tissue, $n = 4$, mean ± SD).

Tissue	5 min	30 min	60 min
Left atrium	0.45 ± 0.02	0.28 ± 0.04	0.21 ± 0.02
Right atrium	0.48 ± 0.03	0.28 ± 0.02	0.17 ± 0.01
Left ventricle	0.43 ± 0.02	0.27 ± 0.03	0.17 ± 0.02
Right ventricle	0.45 ± 0.02	0.29 ± 0.03	0.16 ± 0.02
Liver	1.22 ± 0.15	1.44 ± 0.22	1.18 ± 0.33
Lung	0.89 ± 0.13	0.48 ± 0.02	0.32 ± 0.06
Spleen	0.88 ± 0.07	0.51 ± 0.03	0.34 ± 0.05
Brain	0.62 ± 0.05	0.45 ± 0.02	0.36 ± 0.02
Blood	0.28 ± 0.01	0.23 ± 0.03	0.14 ± 0.01

In conclusion, (±)-N-[^{11}C]methyl-*para*-hydroxytranylcypromine has been synthesized as a potential PET tracer for quantifying cardiac sympathetic nerve density. Neuronal uptake, storage, and binding to MAO have been demonstrated in isolated rat hearts. Biodistribution data for the compound suggest that its uptake rate may be too slow and/or it may not be trapped well enough for it to be a viable neuronal imaging agent.

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