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Synthesis of the dopamine D_2/D_3 receptor agonist (+)-PHNO via supercritical fluid chromatography: preliminary PET imaging study with [3-^{11}C]-(+)PHNO

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ABSTRACT

Carbon-11 labeled (+)-4-[1-^{11}C]propyl-3,4,4*a*,5,6,10*b*-hexahydro-2*H*-naphtho[1,2-*b*][1,4]oxazin-9-ol ([1-^{11}C]-(+)-PHNO) is a dopamine D_3-preferring agonist radiopharmaceutical used for medical imaging by positron emission tomography (PET). We report the synthesis of (+)-PHNO using supercritical fluid chromatography for enantiomeric resolution of its norpropyl derivative, HNO, followed by propylation. (+)-HNO was used to prepare the radiolabeling precursor, (+)-*trans*-4-acetyl-9-triisopropylsilyloxy-2,3,4*a*,5,6,10*b*-hexahydro-4*H*-naphth[1,2*b*][1,4]oxazine, in 12 steps. Modifications to the labeling procedure were made to ensure consistent preparation of [3-^{11}C]-(+)-PHNO via [^{11}C]CH_3I. A preliminary PET imaging study was carried out with this tracer in an attempt to image dopamine receptors in brown adipose tissue (brown fat) in vivo.

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(+)-4-Propyl-3,4,4*a*,5,6,10*b*-hexahydro-2*H*-naphtho[1,2-*b*][1,4]oxazin-9-ol ((+)-PHNO) is a naphthoxazine that was synthesized nearly 30 years ago and was considered to be the most potent dopamine D_2 receptor agonist.[1] Subsequent synthesis of its carbon-11 ($t_{½}$ = 20.4 min) labeled isotopologue, [1-^{11}C]-(+)-PHNO,[2,3] referred to as a D_3-preferring agonist radiotracer, represents a major advance in the field of medical imaging by positron emission tomography (PET)[4] and is the most suitable D_3 imaging agent available. [1-^{11}C]-(+)-PHNO and other dopamine receptor agonist radiotracers are more sensitive to the effects of dopamine displacement than antagonists and have provided important insights toward high-affinity states of D_2 and D_3 receptors in vivo.[5]

The original published method for the preparation of [1-^{11}C]-(+)-PHNO uses a four-step labeling procedure (Scheme 1A).[2,3] This approach involves ^{11}C-carboxylation of the Grignard reagent ethylmagnesium bromide and conversion to [^{11}C]propionyl chloride that must be purified by distillation. The latter is reacted with the amine precursor ((+)-HNO) affording the ^{11}C-amide intermediate which is reduced to give [1-^{11}C]-(+)-PHNO. Recent efforts have been made to optimize and automate this process from [^{11}C]propionyl chloride.[6–8] An alternative methodology for radiolabeling an *N*-propyl chain is via [^{11}C]iodopropane,[9] however, attempts to synthesize [^{11}C]-(+)-PHNO via alkylation of (+)-HNO with [^{11}C]iodopropane were unsuccessful primarily because of the formation of large amounts of the nonradiolabeled *N*-ethyl congener which complicated the HPLC purification.[3]

Compared with labeling agents synthesized from [^{11}C]CO_2, [^{11}C]CH_3I is routinely prepared in significantly higher specific activities by iodination of [^{11}C]CH_4 using commercially available synthesis modules. Recently [3-^{11}C]-(+)-PHNO was labeled at the terminal carbon of the *N*-propyl chain by a one-pot reaction of [^{11}C]CH_3I with an *O*-TIPS-protected acetyl amide precursor, (+)-*trans*-4-acetyl-9-triisopropylsilyloxy-2,3,4*a*,5,6,10*b*-hexahydro-4*H*-naphth[1,2*b*][1,4]oxazine (**1**), followed by simultaneous reduction of the carbonyl group and deprotection (Scheme 1B).[10] [3-^{11}C]-(+)-PHNO was found to be comparable to [1-^{11}C]-(+)-PHNO in preclinical imaging studies of the dopaminergic pathway.[11]

We here report an improved synthesis of (+)-PHNO, as well as the synthesis of two key precursors for radiolabeling, (+)-HNO and **1**, as preparations of these latter compounds have not been reported. The resolution of the HNO enantiomers was successfully achieved by supercritical fluid chromatography (SFC). Modifications to the labeling procedure were made to ensure consistent preparation of [3-^{11}C]-(+)-PHNO, and a preliminary PET imaging study was performed with this tracer in an attempt to image

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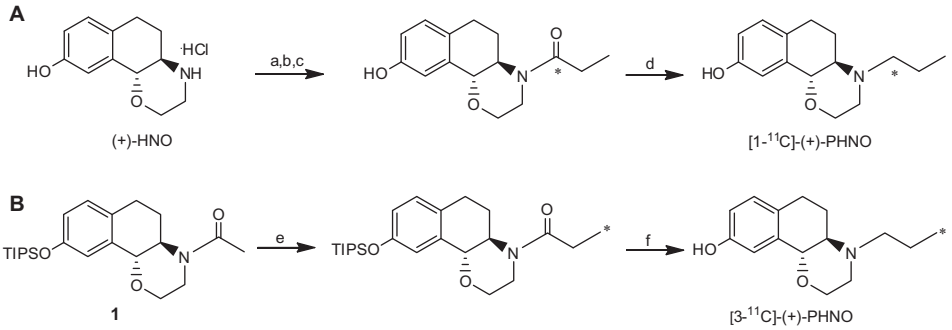


Scheme 1. (A) Radiosynthesis of [1-^{11}C]-(+)-PHNO[3]: (a) EtMgBr, [^{11}C]CO_2, (b) phthaloyl dichloride/2,6-di-*tert*-butylpyridine, (c) distill; THF, *N*,*N*-diisopropylethylamine, 60 °C, (d) 1 M $LiAlH_4$, then HCl; (B) [3-^{11}C]-(+)-PHNO[10]: (e) lithium hexamethyldisilazide (LHMDS), [^{11}C]CH_3I, THF, 0 °C; (f) 1 M $LiAlH_4$, 60 °C, then AcOH, 60 °C. Asterisk denotes site of ^{11}C.

dopamine receptors in brown adipose tissue (BAT; aka brown fat) in rat.

Synthesis of labeling precursor **1** involves 12 steps (see ESI for details) and uses modifications of the method originally described by Jones et al.[1] for the preparation of PHNO and subsequent procedures of Perone et al.[12] and Delgado et al.[13] Starting with 7-methoxy-1-tetralone, demethylation using aluminum trichloride in toluene gave 7-hydroxy-1-tetralone (**2**) in 90% yield which was converted to 7-benzyloxy-1-tetralone (**3**) using benzyl bromide in 83% yield. It was worthy of note that the benzyloxy derivative provided superior yield in the subsequent deprotection reactions on compound **10** compared with alternative protecting groups, including methyl ether. Compound **3** was treated with hydroxylamine hydrochloride in dry pyridine which afforded the desired hyroxyiminotetralone (**4**) in high yield. Neber rearrangement[12] was used to synthesize 2-amino-1-tetralone **6** starting from the tosyloxy derivative **5**. Stereoselective reduction of **6** via sodium borohydride gave the corresponding *trans*-2-amino-1-tetraol **7** as the major product.[14] The *trans*-amino tetralone **7** was acylated with chloroacetyl chloride and cyclized with NaH in THF. Reduction of the benzoxazine-3-one **9** with $LiAlH_4$ afforded the desired *trans*-benzoxyoxazine **10**. Debenzylation by catalytic hydrogenation gave racemic HNO (see Scheme 2).

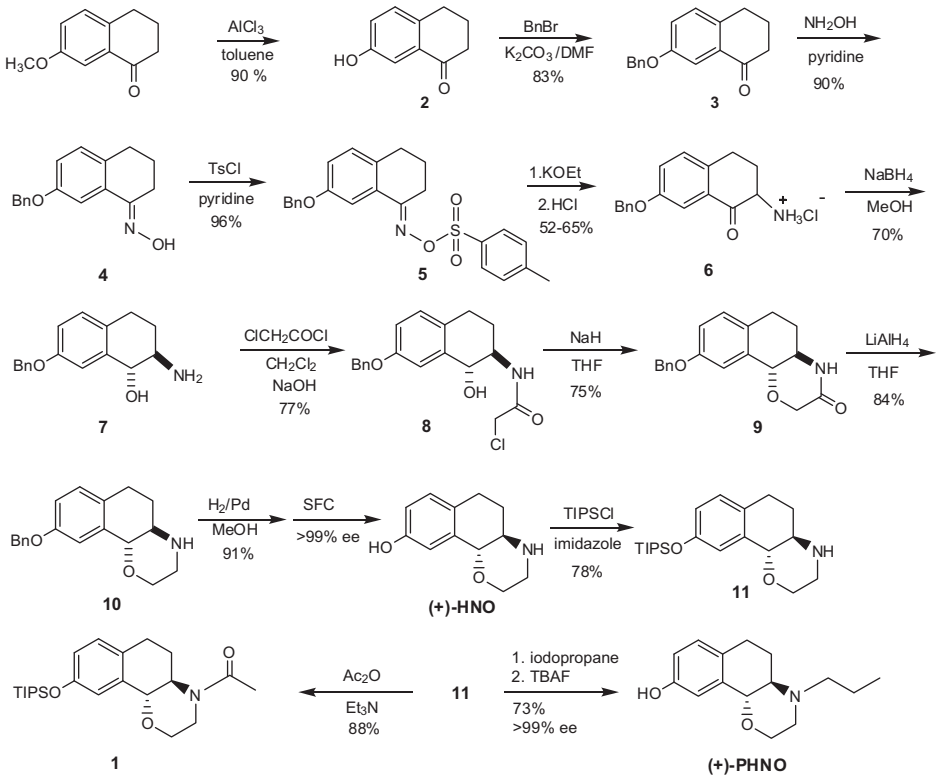
Resolution of HNO enantiomers was performed on an AD-H column by supercritical fluid chromatography (SFC). Products were analyzed by UltraPerformance Convergence Chromatography (UPC^2, Waters; see ESI for details). An extensive screening of chiral stationary phases indicated that the CHIRALPAK AD-H column offered a desirable separation of the HNO racemate in less than 6 min, as shown in Figure 1A. Compared to the separation by HPLC, the SFC method presented here was three times faster, resulting in a three-fold productivity improvement when scaled up to the preparative scale. The productivity was further improved by employing stacked injections; performed under isocratic conditions, injections are made during the course of chromatography such that the first peak from a subsequent injection elutes off the column adjacent to the last peak of the preceding injection, without compromising chromatographic efficiency. A total of 1 g of the racemate was purified in less than 2 h. Post-purification analysis showed that the (+)-isomer had an enantiomeric excess (ee) >99% (Fig. 1B). A white solid of (+)-HNO was obtained after drying followed by trituration with hexanes.

The (+)-HNO isomer was O-silylated using 2.5 equiv of triisopropylsilyl chloride (TIPSCl) and imidazole in THF to yield **11**. Acetylation with acetic anhydride and triethylamine gave the protected acetyl amide **1**. (+)-PHNO was also prepared from (+)-HNO using iodopropane in 73% yield and >99% ee.

The one-pot radiosynthesis for [3-^{11}C]-(+)PHNO was optimized from the variable literature procedure (2–30% uncorrected radiochemical yield)[10] for consistent preparation starting from **1**. Carbon-11 labeling was achieved by ^{11}C-methylation of the corresponding enolate of **1** generated by hindered base lithium hexamethyldisilazide (LHMDS; 1 M in THF).[10] We have found that commercial bottles of this reagent contain unsuitable amounts of base impurity, most likely formed from LHMDS induced decomposition of THF that reacts with [^{11}C]CH_3I to give a polar ^{11}C-labeled side-product in 40–99% radiochemical yield. In contrast, use of commercial solutions of 1 M LHMDS in hexanes, which are clear and colorless, consistently reduces variability by decreasing side-product formation to 15–40%.

Using our modified manual radiosynthesis procedure for [3-^{11}C]-(+)PHNO, [^{11}C]CH_3I (prepared via [^{11}C]CO_2 using a GE Tracerlab FX_M module) was trapped in a vial cooled to 0 °C containing **1** (3 mg) in THF (200 μL). Subsequently, a solution of 1 M LHMDS in hexanes (20 μL) was then added to the vial. The reaction mixture was held at 0 °C for 3 min and then allowed to warm to 25 °C over 3 min, at which time the mixture was quenched using 0.2 M anhydrous methanol in THF (80 μL). Analytical HPLC analysis[10] of aliquots from reaction mixtures showed conversion to the ^{11}C-labeled amide was between 60% and 85%. Amide reduction was achieved with 1 M $LiAlH_4$ in THF (100 μL) followed by heating at 60 °C for 7 min. The mixture was quenched at 60 °C with 0.5 mL of 50% acetic acid for dissolving salts and removing the silyl protecting group concurrently. [3-^{11}C]-(+)PHNO was isolated by reverse-phase HPLC (Phenomenex Luna 10μ C18, 250 × 10 mm, 20% acetonitrile 80% 0.1 M ammonium formate (pH 4.5 adjusted with acetic acid). The desired fraction (6 mL/min, t_R = 7 min) was collected and diluted with 30 mL of water containing 60 mg of sodium carbonate. This solution was passed through a solid phase extraction cartridge (SPE; C18 SepPak®; Waters) which was followed by washing with 10 mL of water. [^{11}C]-(+)PHNO was eluted from the SPE with 1 mL of ethanol into a vial containing 10 mL of 0.9% saline and the resulting solution was filtered through a 0.22 μm sterile syringe filter (Millex®GV; Millipore). Synthesis was complete within 40 min with more stable radiochemical yields (10–20% uncorrected relative to [^{11}C]CH_3I, n = 3) and consistent specific activities suitable for clinical use (38–84 GBq/μmol; 1.0–2.3 Ci/mol at end of synthesis) were achieved with high radiochemical purities (>99%). In future, an automated radiosynthesis with this revised procedure would enable higher starting activities of [^{11}C]CO_2 to be used and consequently higher specific activities to be achieved. This will ensure that pharmacological effects caused by this agonist radiotracer are avoided.

A preliminary PET imaging study with [3-^{11}C]-(+)-PHNO was conducted in rat in order to determine whether this tracer is suitable for imaging BAT, (brown fat). Dopamine receptors are known to be expressed in BAT.[15,16] The thermogenic activity of BAT is modulated by dopaminergic compounds[15,17] and may play a role



Scheme 2. Synthesis of (+)-PHNO, (+)-HNO and **1**.

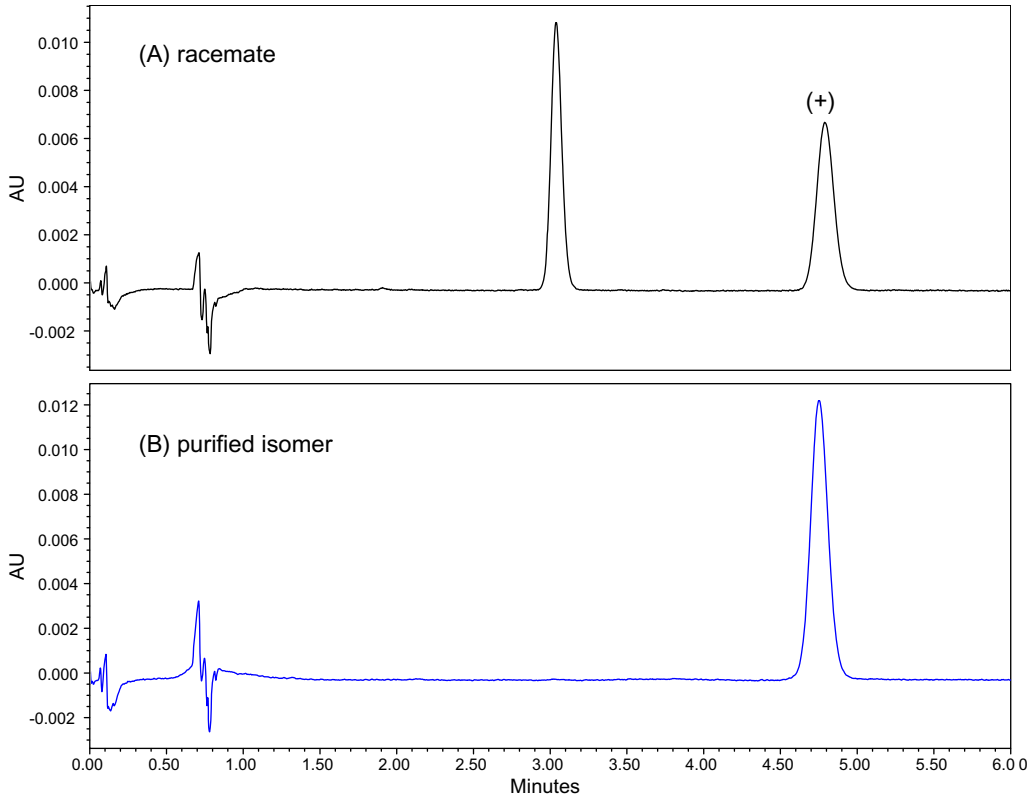


Figure 1. UltraPerformance Convergence Chromatography (UPC^2; Waters) UV chromatograms of (A) (±)-HNO and (B) (+)-HNO, as purified by preparative SFC.

in weight gain associated with treatment by antipsychotic drugs.[18] A recent study provided preliminary evidence that two other D_2-like receptor radioligands, namely, the antagonist tracer [^{18}F]fallypride and the agonist [^{18}F]FHXPAT, have enhanced uptake in BAT that enable imaging with PET.[19] Thus, our imaging session focused on the shoulders and upper thorax of the animal where

BAT deposits are located. However no enhanced uptake was observed (see ESI for image).

The present work describes a new synthetic route to (+)-PHNO and the first reported syntheses of the two radiolabeling precursors, (+)-HNO and **1**, via supercritical fluid chromatography. Efforts to optimize the radiosynthesis of [3-^{11}C]-(+)-PHNO described herein should be broadly useful for the radiosyntheses of carbon-11 labeled *N*-propyl moieties from [^{11}C]CH_3I.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.11.113.

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