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# SYNTHESIS AND CARBON-11 LABELING OF THE STEREOISOMERS OF *meta*-HYDROXYEPHEDRINE (HED) AND *meta*-HYDROXYPSEUDOEPHEDRINE (HPED)

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## SUMMARY

The synthesis of the four stereoisomers of carbon-11 labeled *meta*-hydroxyephedrine (HED) and *meta*-hydroxypseudoephedrine (HPED) was undertaken for evaluation of their *in vivo* kinetic behavior. The stereoisomers of HED and HPED were synthesized by conversion of their respective enantiomerically-pure, normethyl precursors (*meta*-hydroxyphenylpropanolamine stereoisomers) to the carbamate derivatives and subsequent reduction with lithium aluminum hydride. Direct *N*-[$^{11}$C]methylation of the appropriate normethyl precursor with [$^{11}$C]methyl triflate and HPLC purification provided the radiotracers in 26-42% (average = 36%; n = 12) decay-corrected radiochemical yields in a 40 min synthesis time from end-of-bombardment. The specific activity of the radiotracers was 1260-1625 Ci/mmol (average = 1368 Ci/mmol; n = 8) at end-of-synthesis and the radiochemical purity >98%.

**Key Words:** [$^{11}$C]methyl triflate, cardiac imaging, metaraminol, positron emission tomography, sympathomimetic amines, sympathetic neuronal marker

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## INTRODUCTION

We previously reported the synthesis of carbon-11 labeled (-)-(1R,2S)-*meta*-hydroxyephedrine, (-)-[$^{11}$C]HED, for use as an *in vivo* marker of noradrenergic neurons (1). PET studies with (-)-[$^{11}$C]HED have permitted noninvasive assessment of the integrity of the human cardiac sympathetic nervous system in the normal and transplanted heart, and in disease states such as acute myocardial infarction, diabetic neuropathy and dilated cardiomyopathy (2-6).

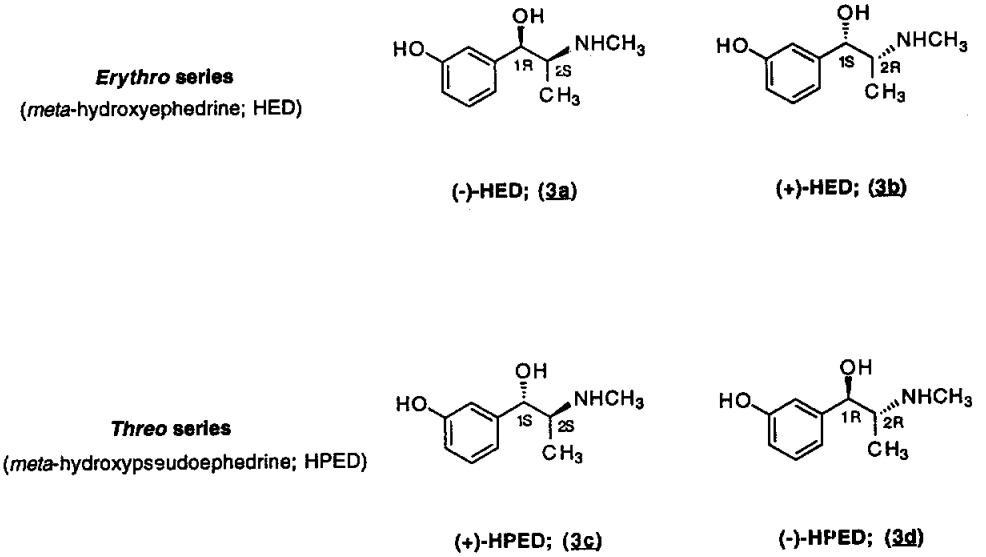



**Figure 1.** Stereoisomers of HED and HPED

(-)-HED, which has the 1R,2S configuration, is one of four possible stereoisomers (Figure 1). These consist of two enantiomeric pairs, configurationally related to either ephedrine (*erythro* configuration) or pseudoephedrine (*threo* configuration). By analogy, these stereoisomers are denoted as follows: (-)(1R,2S)- and (+)(1S,2R)-*meta*-hydroxyephedrine (HED) and (-)(1R,2R)- and (+)(1S,2S)-*meta*-hydroxypseudoephedrine (HPED), respectively.

Studies conducted by Shore (7,8) and others (9) have demonstrated a stereochemical dependency for the neuronal retention of the optical isomers of metaraminol, which are the respective normethyl analogs of HED, in the mouse heart following i.v. administration. Specifically, although both stereoisomers demonstrated

high initial accumulation in this tissue, the (+)-stereoisomer showed rapid efflux over 2-3 hours, whereas the (-)-stereoisomer was retained (efflux $T_{1/2}$ = 2 - 3 days). These findings prompted us to investigate the heart neuronal retention of the optical isomers of HED and HPED. A radiotracer with more favorable kinetics of neuronal accumulation than (-)-[$^{11}$C]HED, (i.e. slower uptake and/or faster clearance) would likely allow improved kinetic modeling of cardiac sympathetic innervation in human PET studies (10). Accordingly, we report here the synthesis and characterization of the optical isomers of HED and HPED and their carbon-11 labeled derivatives.

## EXPERIMENTAL

**Chemistry:** All chemical reagents were obtained from Aldrich Chemical Co., Milwaukee, WI. The optical isomers of the normethyl derivatives of HED and HPED were synthesized and purified by semipreparative chiral chromatography as previously described (11). Melting points were determined in open capillary tubes using a Thomas Hoover melting point apparatus and are uncorrected. $^1$H NMR spectra were obtained in either $CDCl_3$ or $CD_3OD$ with a Bruker WM-360 (360 MHz) instrument using tetramethylsilane (TMS) as internal standard. Chemical shifts ($\delta$) are reported in parts per million (ppm) downfield from TMS. Splitting patterns are designated as follows: s, singlet; d, doublet; dd, doublet doublet; t, triplet; m, multiplet. Mass spectra were obtained with a Finnigan 4021 GCMS/DS (low resolution) or a UG70-250-S (high resolution) instrument. Optical rotations were measured at 589 nm in a 1 ml quartz microcell of 1 dm optical pathlength using a Perkin-Elmer (Norwalk, CT) 241 polarimeter and *c* is expressed in g/100 mL. Flash chromatography was performed using E. Merck Kieselgel 60 (230 - 400 mesh) by the method of Still *et al.*(12).

**Radiochemistry:** Synthetic reactions with carbon-11 were conducted using a remotely controlled apparatus. [$^{11}$C]$CO_2$ was produced with a biomedical cyclotron (CS-30 accelerator; Cyclotron Corporation) by the $^{14}N(p,\alpha)^{11}C$ reaction using high purity nitrogen gas in an aluminum target. [$^{11}$C]$CO_2$ was converted to [$^{11}$C]$CH_3OH$ by $LiAlH_4$ reduction and treated with aqueous HI at reflux to generate [$^{11}$C]$CH_3I$ according to the method of Crouzel and coworkers (13). Conversion of [$^{11}$C]$CH_3I$

to [$^{11}$C]methyl trifluoromethanesulfonate ([$^{11}$C]methyl triflate) was accomplished as previously described (14).

Radiochemical yields were calculated based on the initial [$^{11}$C]$CO_2$ produced at end-of-bombardment (EOB) and are corrected for decay. [$^{11}$C]$CO_2$ produced at EOB was estimated from a previously determined calibration curve of [$^{11}$C] activity produced versus irradiation times in the same target. Radioactivity measurements of the radiolabeled products were obtained with a Capintec CRC-12 radioisotope calibrator.

Specific activity estimates were obtained by quantitation of the HPLC peak area corresponding to the radiolabeled product using a previously generated calibration curve with authentic standards. Specific activity is reported at end-of-synthesis (EOS).

Carbon-11 methylation was conducted by trapping [$^{11}$C]methyl triflate (carried by a nitrogen stream) in a solution of **1** (0.6 mg, 3.6 μmol) in anhydrous DMF (0.15 mL) at room temperature. After maximum radioactivity had accumulated (approximately 3 - 4 min), the reaction mixture was transferred to the HPLC system for purification using a remotely controlled sample injection valve. HPLC purification was performed on a semi-preparative cation exchange column [Phenomenex Optisil 10 SCX; 10 μm particle size; 250 x 9.4 mm I.D. with guard 50 x 9.4 mm I.D; (Torrance, CA)] eluted with 0.1 M $NaH_2PO_4$ (pH = 4.0) at 6 mL per minute. The column effluent was monitored with a flow-through radiation detector. The radioactive peak containing [$^{11}$C]**3** (elution time 9.5 - 11 min), was diverted using a two-way valve and filtered through an in-line 0.22 μm sterile filter (Anotop) into a sterile multi-dose vial. The radioactive product was diluted with sterile normal saline for animal studies. The synthesis time from [$^{11}$C]$CO_2$ production was 40 min. The isolated radiochemical yield ranged from 26-42% (n = 12) at EOB and the average specific activity was 1368 Ci/mmol at EOS.

**Analytical Chromatography:** HPLC analysis of the radiotracers was carried out on a Phenomenex Partisil 10 SCX cation exchange column ( 10 μm particle size, 250 x 4.6 mm I.D. with guard 30 x 4.6 mm I.D.) eluted with 0.04 M $NaH_2PO_4$ (pH = 5.0) at a flow rate of 2 mL/min. UV absorbance was monitored at 280 nm. The retention times for (±)HED and its normethyl precursor (**1a**,**1b**) were 7.24 min ($k'$ = 2.30) and

4.26 min ($k'$ = 0.94), respectively, and the corresponding values for (±)HPED and its normethyl precursor (**1c,1d**) were 7.91 min ($k'$ = 2.61) and 4.52 min ($k'$ = 1.06), respectively, under these conditions. All chromatographic procedures were conducted at ambient temperatures.

Radio-TLC analyses were performed on Analtech silica glass-backed TLC plates (10 cm, 250 μ) using $CH_3OH$:conc. $NH_4OH$ (10:0.1) as the eluting solvent. The labeled compound was co-spotted with the authentic unlabeled compound prior to plate development. TLC plates were scanned for radioactivity using a Berthold Model LB 2832 TLC-Linear Analyzer equipped with a Model LB 500 Data Acquisition System. The $R_f$ values for (±)HED and its normethyl precursor (**1a,1b**) were 0.27 and 0.50, respectively, and the corresponding values for (±)HPED and its normethyl precursor (**1c,1d**) were 0.32 and 0.43, respectively.

**General Procedure for synthesis of carbamates (2a-2d)**

The appropriate normethyl precursor (**1a-1d**) (0.20 g; 1.2 mmol) in 20 mL of a two-phase mixture of $H_2O$:$Et_2O$ (1:1) was treated successively with $Na_2CO_3$ (0.15 g, 1.4 mmol) and benzyl chloroformate (0.24 g, 1.4 mmol) and stirred at ambient temperature for 18 h. The $Et_2O$ layer was removed, the aqueous layer extracted further with $Et_2O$ (2 x 25 mL) and the combined organic layers washed with saturated brine (50 mL), $H_2O$ (50 mL) and dried ($Na_2SO_4$). Removal of volatiles and flash chromatography of the crude product ($CH_2Cl_2$:EtOAc; 7:3) provided the pure product in 80 - 90% yield.

**(1R,2S)-1-(*meta*-hydroxyphenyl)-2-benzyloxycarbonylamino-1-propanol (2a)**

Prepared from (-)-(1R,2S)-*meta*-hydroxyphenylpropanolamine (**1a**) in 90% yield: mp 119-120 °C; $^1$H NMR ($CDCl_3$): δ 7.37-7.33 (m, 5H, ArH), 7.19 (t, 1H, J = 7.82 Hz, ArH), 6.86 (d, 1H, J = 7.07 Hz, ArH), 6.81 (s, 1H, ArH), 6.75 (dd, 1H, J = 7.87 Hz, J = 2.42 Hz, ArH), 5.11 (s, 2H, $OC\underline{H}_2Ar$), 4.99 (d, 1H, J = 9.08 Hz, $C\underline{H}OH$), 4.07-4.01 (m, 1H, $C\underline{H}CH_3$), 0.99 (d, 3H, J = 6.91 Hz, $CHC\underline{H}_3$); High resolution MS (EI at 70 eV): m/z 301.1318 ($C_{17}H_{19}NO_4$ [$M^+$] requires 301.1314).

**(1S,2R)-1-(*meta*-hydroxyphenyl)-2-benzyloxycarbonylamino-1-propanol (2b)**

Prepared from (+)-(1S,2R)-*meta*-hydroxyphenylpropanolamine (**1b**) in 85% yield as a viscous oil. The $^1$H NMR and mass spectral data corresponded with that reported for the (1R,2S) isomer above.

**(1S,2S)-1-(*meta*-hydroxyphenyl)-2-benzyloxycarbonylamino-1-propanol (2c)**

Prepared from (+)-(1S,2S)-*meta*-hydroxyphenylpropanolamine (**1c**) in 80% yield as a viscous oil: $^1$H NMR ($CDCl_3$): δ 7.27 (s, 5H, ArH), 7.08 (t, 1H, J = 7.79 Hz, ArH), 6.79 (s, 1H, ArH), 6.75-6.69 (m, 2H, ArH), 5.20 (d, 1H, J = 7.16 Hz, C<u>H</u>OH), 5.09-4.93 (m, 2H, OC<u>H$_2$</u>Ar), 3.88 (m, 1H, C<u>H</u>CH$_3$), 0.99 (d, 3H, J = 6.25 Hz, CHC<u>H$_3$</u>); High resolution MS (EI at 70 eV): m/z 301.1318 ($C_{17}H_{19}NO_4$ [$M^+$] requires 301.1314).

**(1R,2R)-1-(*meta*-hydroxyphenyl)-2-benzyloxycarbonylamino-1-propanol (2d)**

Prepared from (-)-(1R,2R)-*meta*-hydroxyphenylpropanolamine (**1d**) in 84% yield as a viscous oil. The $^1$H NMR and mass spectral data corresponded with that reported for the (1S,2S) isomer above.

**General Procedure for synthesis of HED and HPED stereoisomers (3a-3d)**

A solution of the appropriate carbamate derivative (**2a-2d**) (0.150 g, 0.5 mmol) in dry THF (5 mL) was added dropwise to a stirred suspension of $LiAlH_4$ ( 0.113 g, 3.0 mmol) in dry THF (10 mL) at 5 °C under argon. The reaction was allowed to warm to ambient temperature and then refluxed for 4 to 6 h until TLC ($CH_2Cl_2$:EtOAc; 7:3) indicated completion of reaction. The reaction mixture was cooled to 5 °C, treated dropwise with saturated $(NH_4)_2SO_4$:THF (1:1), filtered and the solid residue rinsed with $CH_3OH$. The filtrate was evaporated *in vacuo* and the residue subjected to gradient flash chromatography [$CHCl_3$:$CH_3OH$; 7:3 (100 mL) followed by $CHCl_3$:$CH_3OH$:$NH_4OH$; 7:3:0.1)] which provided the pure products as amorphous solids in 61-80% yield. Treatment of the free base with a 1:1 mixture of absolute EtOH:ethereal HCl (1.0 M) gave the HCl salt which was recrystallized from EtOH:$Et_2O$ (1:4).

**(-)-(1R,2S)-1-(*meta*-hydroxyphenyl)-2-methylamino-1-propanol [(-)HED, 3a]**
Prepared from 2a in 80% yield: mp (HCl salt): 217-219 °C (dec); $[\alpha]^{25}_D$ = - 26 ° (*c* = 0.1, $H_2O$); $^1$H NMR (HCl salt in $CD_3OD$): δ 7.19 (t, 1H, J = 7.76 Hz, Ar H-5), 6.87 - 6.84 (m, 2H, Ar H-4, Ar H-6), 6.72 (dd, 1H, J = 2.49 Hz, 8.19 Hz, Ar H-2), 5.02 (d, 1H, J = 3.11 Hz, CHOH), 3.40 - 3.37 (m, 1H, $CHCH_3$), 2.75 (s, 3H, $NCH_3$), 1.07 (d, 3H, J = 6.75 Hz, $CHCH_3$); High resolution MS (DCI with $NH_3$): m/z 168.1020 ($C_9H_{14}NO_2$ [M+H$^+$] requires 168.1024).

**(+)-(1S,2R)-1-(*meta*-hydroxyphenyl)-2-methylamino-1-propanol [(+)HED, 3b]**
Prepared from 2b in 71% yield: mp (HCl salt): 216-218 °C (dec); $[\alpha]^{25}_D$ = + 28.3 ° (*c* = 0.1, $H_2O$). The $^1$H NMR and mass spectral data corresponded with that reported for the (1R,2S) isomer above.

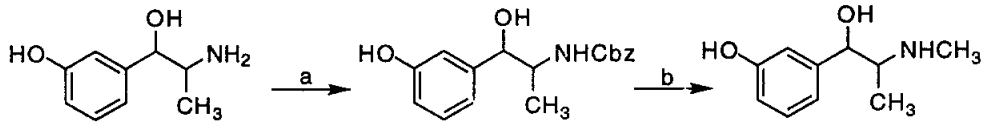
**(+)-(1S,2S)-1-(*meta*-hydroxyphenyl)-2-methylamino-1-propanol [(+)HPED, 3c]**
Prepared from 2c in 61% yield: mp (HCl salt): mp 165 °C (dec; softens at 115 °C); $[\alpha]^{25}_D$ = + 44.9 ° (*c* = 0.15, $H_2O$); $^1$H NMR (HCl salt in $CD_3OD$): δ 7.20 (t, 1H, J = 8.03 Hz, Ar H-5), 6.88 - 6.86 (m, 2H, Ar H-4, Ar H-6), 6.77 (dd, 1H, J = 1.90 Hz, 7.99 Hz, Ar H-2), 4.48 (d, 1H, J = 9.0 Hz, CHOH), 3.35 - 3.30 (m, 1H, $CHCH_3$), 2.72 (s, 3H, $NCH_3$), 1.11 (d, 3H, J = 6.52 Hz, $CHCH_3$); High resolution MS (DCI with $NH_3$): m/z 168.1022 ($C_9H_{14}NO_2$ [M+H$^+$] requires 168.1024).

**(-)-(1R,2R)-1-(*meta*-hydroxyphenyl)-2-methylamino-1-propanol [(-)HPED, 3d]**
Prepared from 2d in 64% yield: mp (HCl salt): mp 164 °C (dec; softens at 115 °C); $[\alpha]^{25}_D$ = - 44 ° (*c* = 0.15, $H_2O$). The $^1$H NMR and mass spectral data corresponded with that reported for the (1S,2S) isomer above.

## RESULTS AND DISCUSSION

The four stereoisomers of *meta*-hydroxyphenylpropanolamine (1a-1d) were synthesized and purified by chiral chromatography as previously reported (11).

Conversion of each stereoisomer to its carbamate derivative (2a-2d) was achieved in 80-90% yield by treatment with benzylchloroformate and sodium carbonate in a biphasic solvent system (Figure 2). The use of the carbamate

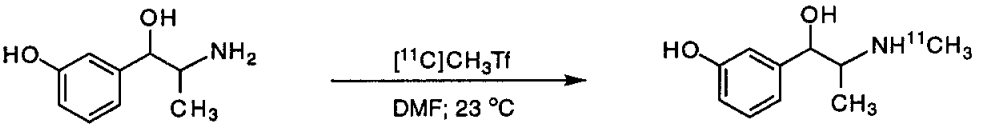



1a - 1d 2a - 2d 3a - 3d

Reagents and conditions: (a) CbzCl/$Na_2CO_3$, $H_2O$:$Et_2O$ (1:1) ; (b) $LiAlH_4$, THF, reflux

**Figure 2.** Synthesis of HED (3a,3b) and HPED (3c,3d)

functionality (which served as the precursor to the *N*-methyl group in the following reaction step) allowed facile purification of these intermediates by normal phase chromatography. Subsequent reduction of 2a-2d with lithium aluminum hydride followed by chromatographic purification provided the HED and HPED stereoisomers (3a-3d) in their free base forms as amorphous solids. These were converted to their crystalline HCl salts by treatment with ethanol:ethereal HCl. $^1$H NMR analysis showed distinctive benzylic proton coupling constants of 3.1 and 9.0 Hz for HED and HPED, respectively, indicative of their *erythro* and *threo* configurations (15,16). The presence of the other diastereomer, which would result from racemization at the benzylic carbon, was not detected in the final products by $^1$H NMR analysis. The final products were fully characterized with respect to their physical (melting point, optical rotation) and spectral ($^1$H NMR, mass spectra) properties.




1a - 1d 3a - 3d

**Figure 3.** Radiosynthesis of [$^{11}$C]HED and [$^{11}$C]HPED

The stereoisomers of [$^{11}$C]HED and [$^{11}$C]HPED were synthesized by direct *N*-[$^{11}$C]methylation of their respective normethyl precursors (1a-1d) with [$^{11}$C]methyl

triflate as shown in Figure 3. The radiotracers were purified by semipreparative cation-exchange chromatography using 0.1 M $NaH_2PO_4$ (pH = 4.0) as eluant. Typically, the radiolabeled product was collected in a 10 mL elution volume which was then diluted with 0.9% normal saline to provide a directly injectable solution. The synthesis time was approximately 40 min from EOB. The radiochemical yields were 26-42% (average = 36%; n = 12) at EOB and the specific activity was 1260-1625 Ci/mmol (average = 1368 Ci/mmol; n = 8) at EOS. The radiochemical purity of the final products was greater than 98% as determined by analytical HPLC and radio-TLC.

The stereoisomeric purity of the radiolabeled products was determined using analytical radio-HPLC by coinjection with the authentic nonradioactive standards. Racemization, if it were to occur, is most likely at the hydroxyl-bearing carbon producing a diastereomer which is detectable under our radioanalytical HPLC conditions. No evidence of racemization during radiolabeling was observed by radio-HPLC analysis of the final labeled products.

## CONCLUSION

The four stereoisomers of HED and HPED have been synthesized and radiolabeled with carbon-11 by direct *N*-[$^{11}$C]methylation of their respective normethyl precursors with [$^{11}$C]methyl triflate. The radiotracers were obtained in high radiochemical purity and specific activity after HPLC purification suitable for conducting *in vivo* PET studies. The biological evaluation of these radiotracers as sympathetic neuronal markers is currently underway and will be reported elsewhere.

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