

SYNTHESIS OF ^3H -LABELED SYMPATHOMIMETIC AMINES FOR NEURONAL MAPPING*

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Summary

The synthesis of two tritium labeled sympathomimetic amines, (R)-(-)-phenylephrine and (1R,2S)-(-)-meta-hydroxyephedrine, is described. The tritium label was introduced into the aromatic ring in the final step by catalytic reductive dehalogenation of the corresponding iodinated precursor. (1R,2S)-(-)-meta-hydroxyephedrine was synthesized in four steps from commercially available metaraminol. A novel HPLC purification method provided the labeled compounds in a medium ready for direct animal evaluation. Chemical and radiochemical purity was >98%; specific activity was > 22 Ci/mmol.

Keywords: meta-hydroxyephedrine, phenylephrine, tritium-labeled sympathomimetic amines, heart neuronal markers, adrenergic nerves.

INTRODUCTION

Our laboratory has actively pursued the synthesis of radiolabeled sympathomimetic amines for use in neuronal imaging of the heart. We recently reported the marked accumulation of 6-[F-18]fluorometaraminol in the adrenergic neurons of the heart. (1) This agent has successfully undergone preclinical evaluation as a myocardial imaging agent but its low specific activity precluded clinical studies (2). This development has prompted us to investigate the neuronal specificity of the structurally related sympathomimetic amines phenylephrine and meta-hydroxyephedrine. Both of these amines bear an N-methyl group and are thus amenable to labeling in high specific activity with [C-11]methyl iodide for positron emission tomography studies. Our initial strategy, therefore, was to tritium label these amines for evaluation in in vivo biological screening tests that would hopefully identify the optimum agent for subsequent ^{11}C -labeling.

Since previous reports have demonstrated the importance of the R(-)configuration at the benzylic position for intraneuronal granular storage (3,4), we focused our attention on the 1R and 1R,2S isomers of phenylephrine and meta-hydroxyephedrine, respectively. Literature evidence suggests that biogenic amines labeled with tritium at position 8 of the side chain (ie. on the alpha

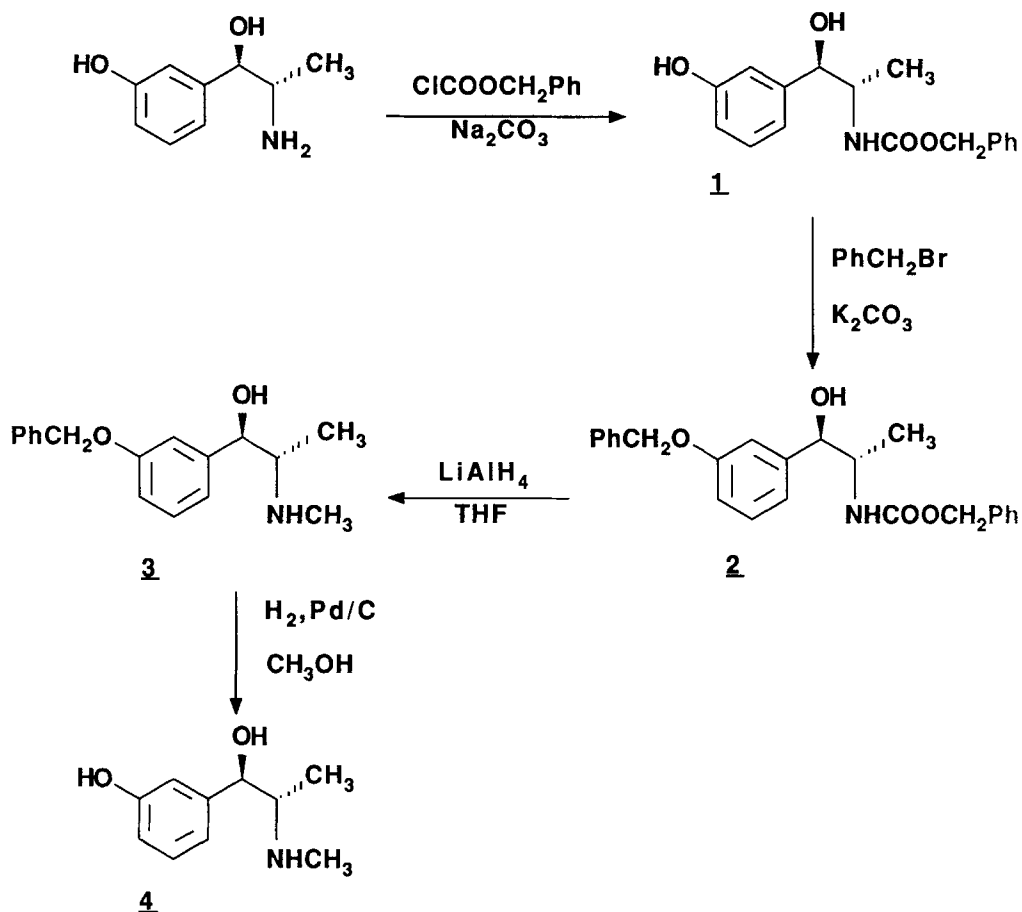
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carbon) are susceptible to metabolic loss of label (5). Our objective was therefore to achieve a regiospecific incorporation of tritium into phenylephrine and meta-hydroxyephedrine in a final synthetic step by catalytic reductive dehalogenation of the appropriate aromatic ring-iodinated precursors. In this report we detail the synthesis of these two aromatic ring ^3H -labeled sympathomimetic amines and their iodinated precursors.

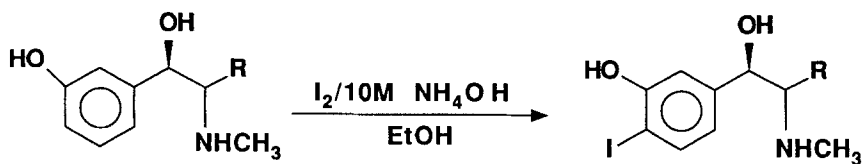
RESULTS AND DISCUSSION

Since (1R,2S)-(-)-meta-hydroxyephedrine (**4**) was not available from commercial sources and its synthesis was not reported in the literature, we chose to synthesize it from commercially available (1R,2S)-(-)-3-hydroxy- α -[1-(aminoethyl)]benzenemethanol (metaraminol). Our initial attempts to synthesize **4** by direct reductive N-methylation of metaraminol with $\text{HCHO}/\text{NaH}_2\text{PO}_3$ by the procedure of Loibner et al (6) afforded a product identified by NMR as a 1,2,3,4 tetrahydroisoquinoline analog. There is literature precedence for a similar pathway in the reaction of phenylephrine with aldehydes (7). A multistep approach was therefore devised to prepare **4** as shown in Scheme I. Utilizing a procedure adapted from Boger and coworkers (8), metaraminol bitartrate was treated with benzylchloroformate and sodium carbonate to afford the carbamate **1** in 90% yield. The use of the carbamate functionality which served later as the precursor to the N-methyl group, also allowed easy purification of the intermediates by normal phase column chromatography. Protection of the phenol as its benzyl ether **2** was accomplished by treatment of **1** with benzylbromide and potassium carbonate in dry DMF. Subsequent reduction of **2** with lithium aluminum hydride provided **3** in good yield (86%). The benzyl protecting group was removed by catalytic hydrogenolysis of **3** with 5% Pd-C to afford meta-hydroxyephedrine **4** in 95% yield. The physical properties of **4** (i.e. mp, ^1H and ^{13}C NMR, and optical rotation) matched those of a small analytical sample generously provided by Sterling Winthrop Research Institute, Rensselaer, New York. Iodinations of meta-hydroxyephedrine and phenylephrine were performed in 10 M aqueous NH_4OH with equimolar amounts of ethanolic I_2 using a procedure adapted from Michel and coworkers (9) to give the corresponding 4-iodinated analogs (Scheme II). This is consistent with our previous findings in the iodination of metaraminol (**2**) and meta-octopamine by this method which in the latter case afforded the known 4-iodo analog (**10**).

Scheme I. SYNTHESIS OF (1R,2S)-(-)-meta-HYDROXYEPHEDRINE



Scheme II. SYNTHESIS OF IODINATED PRECURSORS



R = H; Phenylephrine **6**

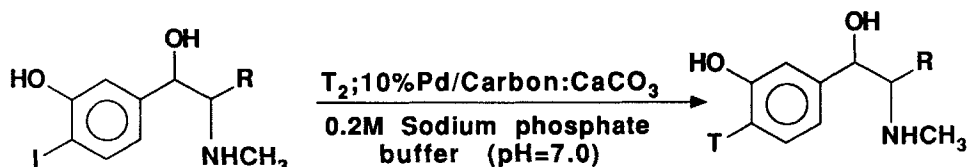
7 R = H 30% yield

R = CH_3 ; meta-Hydroxyephedrine **4**

5 R = CH_3 12% yield

[^3H]-**4** and [^3H]-**6** were prepared by catalytic reductive deiodination of **5** and **7**, respectively, in the presence of tritium gas using a 1:1 mixture of 10% Pd-C and 10% Pd-CaCO₃ (Scheme III). Although the regiospecificity of tritium incorporation in the 4 position was not confirmed by tritium NMR, reductive tritiodahalogenation of similar biogenic amines such as

Scheme III: TRITIUM LABELING OF SYMPATHOMIMETIC AMINES



R = H; PHENYLEPHRINE

R = CH₃; m-HYDROXYEPHEDRINE

octopamine is known to give regiospecific incorporation (11). Confirmation that racemization at the β -hydroxy carbon did not take place during the tritiation procedure is based on the following evidence: a) hydrogenation of **5** and **7** conducted under identical conditions used for the tritiation procedure afforded **4** and **6**, respectively, with optical rotation values and spectroscopic properties that matched those of authentic samples; b) HPLC comparison of **4** with a diastereomeric mixture obtained by partially racemizing **4** in refluxing 6N HCl (12). Radiochemical purity of [^3H]-**4** and [^3H]-**6** was 88% and 99%, respectively, as assessed by radio-TLC. Further purification, which afforded 98% radiochemical and chemical purity, was carried out by preparative reverse phase HPLC in which the labeled compounds were completely separated from their iodinated precursors. An attractive aspect of this purification method was the use of 0.2 M HOAc:EtOH (9:1) as the HPLC eluant which eliminated the need for solvent evaporation prior to formulation for animal studies. A simple dilution of the labeled compound to the required specific concentration of radioactivity with 0.15 M sodium acetate buffer (pH=4.5) was sufficient for i.v. administration to rats.

EXPERIMENTAL

Metaraminol bitartrate was obtained from Sigma Chemical Company, St. Louis, MO. The 10% Pd/CaCO₃ was purchased from BHD Chemicals Ltd, Poole, England. An analytical sample of (1R,2S)-(-)-meta-hydroxyephedrine as the hydrochloride salt was obtained as a gift from Sterling-Winthrop Research Institute, Rensselaer, N.Y. R-(-)-Phenylephrine·HCl and all other

chemical reagents were obtained from Aldrich Chemical Company, Milwaukee, WI. Flash chromatography was performed by the method of Still *et al* (13). Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 727B spectrometer. ^1H -NMR spectra were obtained on a Bruker WM-360 (360 MHz) instrument. ^{13}C -NMR spectra were recorded on a Bruker WM-360 instrument at 90.5 MHz and are completely decoupled. Mass spectra were obtained on a Finnigan 4021 GCMS/DS (low resolution) or a UG70-250-S (high resolution) instrument. Optical rotations were recorded on a Perkin-Elmer EM-241 polarimeter. Elemental analyses were performed by Spang Microanalytical Laboratories, Eagle Harbor, MI.

Thin layer chromatography of the radioactive products (Radio-TLC) were performed on either Whatman K6F silica gel glass-backed plates (20 cm, 250 μ) or Whatman KC ^{18}F reversed phase glass-backed plates (20 cm, 200 μ). Radio-TLC chromatograms were scanned on a Berthold Model LB 2832 TLC-linear analyzer equipped with a Model LB 500 data acquisition system.

HPLC was carried out on either a Waters system consisting of a Model 680 automated gradient controller, two Model 510 pumps, a Model U6K injection and Kratos Model SF773 UV detector or Beckman Instrument Model 344 gradient liquid chromatograph equipped with a Beckman Model 155-00 UV detector. Radioactivity was monitored with a Radiomatic Instruments Model DR/IC Flo-One radioactive flow detector with a Model CU data acquisition system upgrade containing a 340 μL solid scintillant cell. UV absorbance was monitored in all cases at 280 nm.

Specific activity determinations were estimated from a standard curve relating mass to UV absorbance peak area as described previously (14).

(1R,2S)-3-Hydroxy- α -[1-(benzyloxycarbonylamino)ethyl]benzenemethanol (1).

Metaraminol bitartrate (10.0 g, 31.5 mmol) in a two-phase mixture of $\text{H}_2\text{O}:\text{Et}_2\text{O}$ (200 mL:160 mL) was treated successively with sodium carbonate (10.0 g, 94.5 mmol) and benzyl chloroformate (5.38 g, 31.5 mmol) and stirred at room temperature for 18 h. The ether layer was removed, the aqueous layer extracted further with Et_2O (2 x 100 mL) and the combined organic layers washed with saturated brine (200 mL) and dried (Na_2SO_4). Removal of the solvent *in vacuo* and recrystallization of the crude product from $\text{EtOAc}:\text{hexanes}$ (1:4) gave 8.6 g (90%) of analytically pure material: mp 119-120 $^\circ\text{C}$; IR (KBr) 3420, 3260, 1680, 1665 cm^{-1} ; ^1H NMR (CDCl_3) δ 7.37-7.33 (m, 5, Ar-H), 7.19 (t, 1, Ar-H), 6.87-6.74 (m, 3, Ar-H) 5.11 (s, 2, $\text{O}-\text{CH}_2\text{-Ar}$), 4.99 (d, 1, $J=9.08$ Hz, CHOH), 4.83 (s, 1, NH), 4.07-4.01 (m, 1, CHCH_3), 1.63 (s, 1, OH), 0.99 (d, 3, $J=6.91$ Hz, CH_3).

Anal. Calcd. for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.35; N, 4.65. Found: C, 67.78;

H, 6.37; N, 4.56.

(1R,2S)-3-Benzoyloxy- α -[1-(benzyloxycarbonylamino)ethyl]benzenemethanol (2)

A mixture of **1** (8.20 g, 27.2 mmol) and anhydrous K_2CO_3 (7.52 g, 54.4 mmol) in dry DMF (45 mL) was treated with benzyl bromide (4.65 g, 27.2 mmol) and tetrabutylammonium iodide (1.0 g, 2.7 mmol) and stirred under an argon atmosphere at ambient temperature for 6 h. The reaction mixture was filtered and the residue washed with dry DMF (5 mL). The combined filtrates were treated with H_2O (50 mL) and extracted with EtOAc (3x100 mL). The organic extract was washed with saturated brine (100 mL), dried (Na_2SO_4), and the solvent removed *in vacuo*. Flash chromatography on silica with CH_2Cl_2 :EtOAc (7:3) afforded 9.58 g (90%) of **2**: mp 109-110°C (EtOAc:hexanes; 1:3); 1R (KBr) 3320, 1688 cm^{-1} ; 1H NMR ($CDCl_3$) δ 7.43-6.87 (m, 14, Ar-H), 5.12 (s, 2, OCH_2Ar), 5.04 (s, 2, OCH_2Ar), 4.96 (d, 1, $J=7.88$ Hz, $CHOH$), 4.87 (s, 1, NH), 4.07-4.02 (m, 1, $CHCH_3$), 2.74 (s, 1, OH), 0.98 (d, 3, $J=6.91$ Hz, CH_3).

Anal. Calcd. for $C_{24}H_{25}NO_4$: C, 73.63; H, 6.44; N, 3.58. Found: C, 73.68;

H, 6.49; N, 3.47.

(1R,2S)-3-Benzoyloxy- α -[1-(N-methylamino)ethyl]benzenemethanol (3)

A vigorously stirred mixture of lithium aluminum hydride (2.29 g, 60.3 mmol) in dry THF (60 mL) at 0°C under argon was treated dropwise with a solution of **2** (3.94 g, 10.1 mmol) in dry THF (60 mL) over a period of 30 min. The reaction was warmed to room temperature and then refluxed for 6 h. The reaction mixture was cooled to 0°C and treated cautiously with 60 mL of saturated $(NH_4)_2SO_4$, filtered, and the solid residue washed with hot EtOAc (100 mL). The filtrate was poured into saturated brine (100 mL) extracted with EtOAc (3 x 100 mL) and dried (Na_2SO_4). Removal of volatiles *in vacuo* gave a pale yellow oil which was flash chromatographed on silica with $CHCl_3$: CH_3OH : NH_4OH (8:2:1) to afford 2.28 g (84%) of a clear oil that gave white crystals on standing. A small portion of this material was converted to the hydrochloride salt by treatment of an Et_2O solution with dry HCl gas to give white fluffy crystals mp 166-167°C ($EtOH$: Et_2O 1:1); 1H NMR ($CDCl_3$): δ 7.45-6.86 (m, 9, Ar-H), 5.07 (s, 2, OCH_2Ar), 4.75 (d, 1, $J=3.80$ Hz, $CHOH$), 2.82-2.76 (m, 1, $CHCH_3$), 2.47 (s, 3, NCH_3), 0.83 (d, 3, $J=6.51$ Hz, $CHCH_3$).

HRMS m/e 272.1641 ($C_{17}H_{22}NO_2$ (MH^+) requires 272.1650). Anal. Calcd. for $C_{17}H_{22}ClNO_2$ (HCl salt): C, 66.33; H, 7.20; N, 4.55. Found: C, 66.27; H, 7.08; N, 4.36.

(1R,2S)-(-)-3-Hydroxy- α -[1-(N-methylamino)ethyl]benzenemethanol (4)

Five percent Pd/C (200 mg) was added to a solution of **3** (2.12 g, 7.8 mmol) in CH_3OH (50 mL) and hydrogenated at 50 psi for 6 h. The catalyst was removed by filtration through Celite and solvent was removed under reduced pressure to afford a pale yellow oil which upon trituration with Et_2O gave 1.35g (95%) of tan solid which was homogeneous by TLC. This was converted to the hydrochloride salt by passing HCl gas through an ethanol solution of the above; mp 218-220°C ($\text{EtOH}:\text{Et}_2\text{O}, 1:1$); $[\alpha]_{\text{D}}^{25} -39.0(\text{C } 5, \text{CH}_3\text{OH})$; $[\alpha]_{\text{D}}^{25} -38.0(\text{C } 5, \text{CH}_3\text{OH})$ for authentic sample from Sterling Winthrop (IR (KBr) 3470, 3160, 2970, 2760, cm^{-1} . ^1H NMR ($\text{CD}_3\text{OD}:\text{CDCl}_3, 1:1$) δ 6.62 (t, 1, Ar-H), 6.26-6.13 (m, 3, Ar-H), 4.46 (d, 1, $J=3.02$ Hz, CHOH), 4.16 (s, 3, NH, OH), 2.76-2.70 (m, 1, CHCH_3), 2.15 (s, 1, NCH_3), 0.50 (d, 3, CHCH_3).

Anal Calcd. for $\text{C}_{10}\text{H}_{16}\text{ClNO}_2$ (HCl salt): C, 55.17; H, 7.41; N, 6.43. Found: C, 55.17; H, 7.44; N, 6.39.

(1R,2S)-3-Hydroxy-4-iodo- α -[1-(N-methylamino)ethyl]benzenemethanol (5)

A solution of **4** (0.75g, 4.1 mmol) in aqueous 10M NH_4OH (143 mL) was treated dropwise at room temperature under an argon atmosphere with a solution of I_2 (1.09g, 4.3 mmol) in 95% EtOH (72 mL). The reaction mixture was stirred in the dark for 18h and concentrated under reduced pressure to give 2.36g of crystalline solid, which was treated with saturated brine (10 mL) and extracted with EtOAc (3x50 mL). The combined organic layers were washed with aqueous 10% $\text{Na}_2\text{S}_2\text{O}_3$ (10 mL), dried (Na_2SO_4), and evaporated to give 0.91g of pale yellow oil. Flash chromatography on silica with $\text{CHCl}_3:\text{CH}_3\text{OH}:\text{NH}_4\text{OH}$ (7:3:0.1) provided 240 mg of cream colored crystals which were washed with ice-cold EtOAc (3 mL) followed by ice-cold CH_3CN (3 mL) to afford 156 mg (12%) of white crystals homogeneous by TLC (silica; $\text{CHCl}_3:\text{CH}_3\text{OH}:\text{NH}_4\text{OH}$; 7:3:0.1; $R_f=0.47$; R_f of **4** = 0.10). Reverse phase HPLC analysis [Beckman Ultrasphere ODS, 4.6 x 150 mm, 5- μm particle size, C-18 CH_3CN : 0.2 M HCOONH_4 ; (1:3) 1 mL min^{-1}] retention time of **5** = 9.7 min, retention time of **4** = 4.7 min; mp 206-208°C (d); ^1H NMR (CD_3COCD_3) δ 7.64 (d, 1, $J=8.52$ Hz, Ar-H) 7.21 (d, 1, $J=2.93$ Hz, Ar-H), 6.70 (dd, 1, $J_1=3.02$ Hz, $J_2=8.52$ Hz, Ar-H), 5.51 (brs, 1, NH or OH), 5.43 (d, 1, $J=1.91$ Hz, CHOH); 3.77-3.71 (m, 1, CHCH_3) 3.02 (s, 3, NHCCH_3), 2.94 (br s, 2, NH, OH), 1.26 (d, 3, $J=6.77$ Hz, CHCH_3). HRMS m/e 308.0146 ($\text{C}_{10}\text{H}_{15}\text{INO}_2$ requires 308.0147) CIMS (isobutane), m/e (relative intensity) 308 (MH^+ , base), 290 (35), 254 (12), 182 (43), 164 (20), 142 (16), 123 (15), 91 (7), 81 (9).

(1R)-3-Hydroxy-4-iodo- α -(N-methylamino)methylbenzenemethanol (7).

A rapidly stirred suspension of phenylephrine hydrochloride **6** (2.0g, 9.8 mmol) in aqueous 10M NH_4OH (340 mL) was treated dropwise with a solution of I_2 (2.59g, 10.2 mmol) in 95% EtOH (170 mL) under argon. Upon completion of the addition, the clear pale yellow solution was stirred at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure and the resulting crystalline solid was dissolved in aqueous 5% NaHCO_3 (100 mL). Following filtration to remove a little solid residue, the clear aqueous solution gave fine white needles on standing overnight at 5°C . Recrystallization from CH_3OH afforded 0.86g (30%), mp $164\text{--}166^\circ\text{C}$ (d). ^1H NMR ($\text{DMSO}-d_6$): δ 7.55 (d, 1, $J=8.03$ Hz, Ar-H); 6.89 (d, 1, $J=1.81$ Hz, Ar-H); 6.54 (dd, 1, $J_1=1.81$ Hz, $J_2=8.06$ Hz, Ar-H); 4.51 (t, 1, $J=5.9$ Hz, CHOH); 2.53 (d, 2, $J=6.79$ Hz, CH_2); 2.28 (s, 3, NHCH_3).

Anal. Calcd. for $\text{C}_9\text{H}_{12}\text{INO}_2$: C 36.88; H 4.13; N 4.78. Found: C 36.93; H 4.18; N 4.67.

(1R,2S)-(-)-3-Hydroxy-4- $[\text{}^3\text{H}]$ - α -[1-(N-methylamino)ethyl]benzene-methanol.

$[\text{}^3\text{H}]$ **4** was prepared by the Amersham Corporation, Arlington Heights, IL., by catalytic deiodination as follows: To **5** (17 mg) dissolved in 0.2 M sodium phosphate (2 mL, pH 7) was added 10% Pd on charcoal (10 mg) and 10% Pd on CaCO_3 (10 mg) and the mixture stirred with tritium gas (10 Ci) at atmospheric pressure and room temperature for 16h (when uptake of tritium had ceased). The reaction mixture was filtered through a Millipore-HA filter and the filter washed with H_2O (10 mL) followed by EtOH (10 mL). Following removal of the labile tritium by repeated evaporation with EtOH (3×5 mL); the residue was formulated in EtOH (25 mL) to give 170 mCi of $[\text{}^3\text{H}]$ **4**. The radiochemical purity was 88% as assessed by the following TLC systems.

- 1) Silica; $\text{CHCl}_3\text{:CH}_3\text{OH:NH}_4\text{OH}$ (7:3:0.1) Rf of **4** = 0.14 Rf of **5** = 0.35.
- 2) C-18; CH_3CN : 0.2 M $\text{HCOO}^-\text{NH}_4^+$ (1:1) Rf of **4** = 0.50.

The labeled product was stored at -20°C in EtOH at a concentration of 1 mCi/mL. Further purification of a portion of this material was achieved by reverse phase preparative HPLC as follows: A 10 mL EtOH solution of the above (10 mCi) was concentrated under reduced pressure at 30°C to a volume of approximately 0.5 mL and then diluted with 10 mM acetic acid (1 mL). Residual EtOH was removed by further evaporation under reduced pressure and the crude mixture (approximately 1 mL) purified by preparative HPLC [Beckman Ultrasphere ODS, 4.6×250 mm with guard column, $5\text{-}\mu\text{m}$ particle size, C-18 0.2 M HOAc in 95% EtOH: H_2O (1:9) 1 mL min^{-1} ,

with radioactivity detection (Flo-one)] to afford 8.5 mCi of product in 98% radiochemical and chemical purity. The retention times for [^3H] **4** and **5** was 7.3 min and 36.3 min respectively under these conditions and the specific activity of the purified product was 22 Ci mmol $^{-1}$.

(1R)-(-)-3-Hydroxy-4-[^3H]- α -[(N-methylamino)methyl]benzenemethanol.

A procedure analogous to that described for the synthesis of [^3H] **4** utilizing 15 mg of **Z** provided 670 mCi of [^3H] **6**. The radiochemical purity was 99% as assessed by the following TLC systems.

- 1) Silica; n-BuOH:HOAc:H₂O (4:1:5) R_f of **6** = 0.40
- 2) C-18; THF: 0.2 M NH₄H₂PO₄ (3:7) R_f of **6** = 0.60.

Further purification was conducted by reverse phase HPLC (same system as described for that of [^3H] **4**) to ensure that the product was chemically pure. The retention times for [^3H] **6** and **Z** were 6.70 and 30 min, respectively, and the specific activity of the product was 29 Ci mmol $^{-1}$.

Acid catalyzed isomerization of (1R,2S)-(-)-meta-hydroxyephedrine.

A solution of **4** (8.0 mg, 0.037 mmoles) in 6N HCl (2 mL) was refluxed with stirring for 48 h. The reaction mixture was evaporated to dryness *in vacuo* and the residue dried under high vacuum at 80°C. Analysis by ^1H NMR (CDCl₃:CD₃OD; 1:1 by volume with TMS as internal standard) showed the relative concentration of the erythro and threo isomers to be 7:3 by integration of the erythro-and threo-H₁ doublets, respectively. The erythro-H₁ doublet (1R, 2S configuration) was centered at 4.43 ppm, J=2.74 Hz and the threo-H₁ doublet (1S, 2S configuration) was centered at 3.84 ppm, J=9.06 Hz. The racemized mixture was also analysed by HPLC (Waters Associates μ Bondapak, 3.9 x 300 mm, 5- μm particle size, C-18; 0.2 M NH₄H₂PO₄; 1 mL min $^{-1}$). Retention times for the erythro and threo isomers were 11.24 and 15.52 min, respectively.

Confirmation of retention of optical activity during catalytic hydrogenation.

Catalytic hydrogenation of **5** conducted as described for the synthesis of [^3H] **4** gave *meta*-hydroxyephedrine **4** [α]_D²⁵ -34.6 (C 5, CH₃OH); {[α]_D²⁵ -35.2 (C 5, CH₃OH) for the authentic free base form of **4**}. Similarly hydrogenation of **Z** gave phenylephrine **6** [α]_D²⁵ -32.6 (C 5, CH₃OH); {[α]_D²⁵ -31.6 (C 5, CH₃OH) for the authentic free base form of **6**}.

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REFERENCES

1. Mislankar S.G., Gildersleeve D.L., Wieland D.M., Massin C.M., Mulholland G.K., and Toorongian S.A.: *J. Med. Chem.* **31**:362(1988).
2. Wieland D.M., Rosenspire K.C., Hutchins G.D., Van Dort M.E., Rothley J.M., Mislankar S.G., Lee H.T., Massin C.M., Gildersleeve D.G., Sherman P.S., and Schwaiger M.: *J. Med. Chem.* (In press).
3. Shore P.A., Busfield D., and Alpers H.S.: *J. Pharmacol. Exp. Therap.* **146**:194 (1964).
4. Sugrue M.F., and Shore P.A.: *J. Pharmacol. Exp. Therap.* **177**:389 (1971).
5. Trendelenburg U., Stefano F.J.E., and Grohmann M.: *Naunyn-Schmiedeberg's Arch. Pharmacol.* **323**:128 (1983).
6. Loibner H., Pruckner A., and Stutz, A.: *Tetrahedron Lett.* **25**:2535 (1984).
7. Fourneau J.P., Gagnault G., Jacquier R., Stoven O., and Davy M.: *Chim. Therap.* **4**:67 (1969).
8. Boger D.L., and Yohannes D.: *J. Org. Chem.* **52**:5283 (1987).
9. Michel R., Klepping J., Tron-Loisel H., and Truchot R.: *Biochem. Pharmacol.* **12**:717 (1963).
10. Leclerc G., Bizec J.C., Bieth N., and Schwartz J.: *J. Med. Chem.* **23**:738 (1980).
11. Bloxsidge J.P., Elvidge J.A., Gower M., Junes J.R., Evans E.A., Kitcher J.P. and Warrell D.C.: *J. Labelled Compds. Radiopharm.* **18**:1141 (1981).
12. Saari, W.S., Raab, A.W., and Engelhardt, E.L.: *J. Med. Chem.* **11**:1115 (1968).
13. Clark Still, W., Kahn, M., and Mitra, A.: *J. Org. Chem.* **43**:2923 (1978).
14. Van Dort M.E., Ciliax B.J., Gildersleeve D.L., Sherman P.S., Rosenspire K.C., Young A.B., Junck L., and Wieland D.M.: *J. Med. Chem.* **31**:2081 (1988).