SYNTHESIS OF [^{11}C](-)-α,α-DIDEUTERO-PHENYLEPHRINE FOR *IN VIVO* KINETIC ISOTOPE STUDIES

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SUMMARY

(-)-[^{11}C]Phenylephrine and positron emission tomography could potentially be used to assess neuronal monoamine oxidase activity in the heart. Previous data for (-)-[^{11}C]phenylephrine indicate that, although its retention and neuronal selectivity parallel that of the neuronal mapping agent (-)-[^{11}C]hydroxyephedrine, its neuronal storage and clearance properties are quite different. In order to study the *in vivo* kinetics of (-)-[^{11}C]phenylephrine in greater detail, the dideutero analog [^{11}C]-(-)-α,α-dideutero-phenylephrine, 1, was synthesized by [^{11}C]methylation of the precursor (-)-α,α-dideutero-*m*-octopamine. The key step in the procedure was BD_3 reduction of the cyanohydrin derived from 3-hydroxybenzaldehyde. Deuterium incorporation at the alpha positions of *m*-octopamine was confirmed by NMR and mass spectroscopy of the deuterated product and by comparison of spectral data with undeuterated *m*-octopamine. (-)-α,α-Dideutero-*m*-octopamine was methylated with $CF_3SO_3{}^{11}CH_3$ to give 1 suitable for animal and clinical studies.

Key words: phenylephrine, deuterium, carbon-11, monoamine oxidase, myocardium

INTRODUCTION

Monoamine oxidase (MAO, EC 1.4.3.4) is a flavoprotein known to deaminate amines in the brain, heart, liver and other tissues (1). A number of radiotracers that are MAO substrates have been prepared for cardiac imaging by positron emission tomography (PET) including [^{13}N]-β-phenethylamine (2), [^{18}F]-6-fluoronorepinephrine (3) and [^{18}F]-6-fluorodopamine (4). Recently, we reported the radiosynthesis of (-)-[^{11}C]phenylephrine and its promising characteristics as a PET tracer for assessing neuronal MAO activity in the heart (5).

The detailed mechanistic aspects of MAO deamination are still controversial (6) although it is widely accepted that the rate-determining step involves removal of an alpha hydrogen (7,8). The use of the deuterium isotope effect as a mechanistic tool in radiotracer development has been applied in brain (9) and heart (10,11). The synthesis of [^{18}F]-dideutero-6-fluorodopamine (10) and the effect of deuterium substitution on heart tracer

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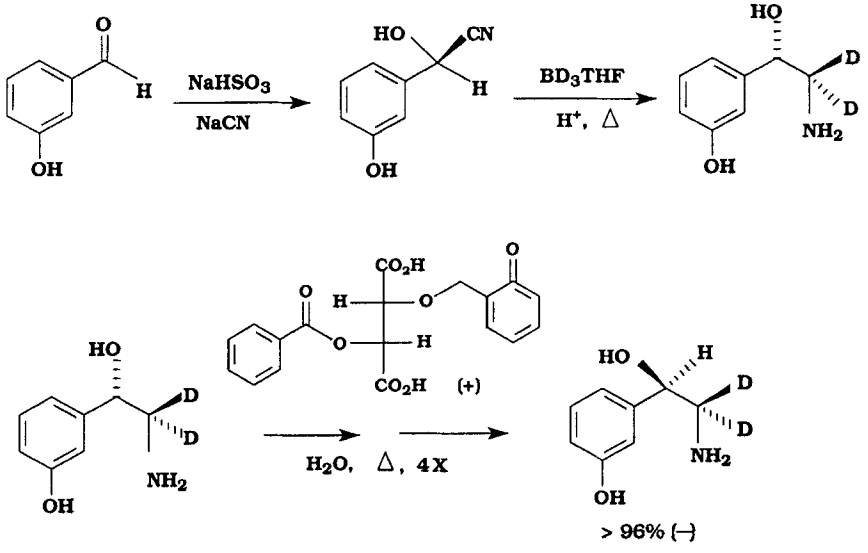
effux rate have been reported by Ding and coworkers (11). We felt that a similar strategy employing deuterated phenylephrine would be helpful in understanding the myocardial kinetics of (-)-[^{11}C]phenylephrine. This information would provide evidence for the direct involvement of MAO in modulating the rate of efflux of radioactivity from the heart. We describe here the stereospecific synthesis of (-)-[^{11}C]-α,α-dideutero-phenylephrine, **1**, achieved by N-[^{11}C]methylation of the normethyl precursor, (-)-α,α–dideutero-*m*-octopamine.

RESULTS AND DISCUSSION

The synthesis and resolution of the critical intermediate (-)-α,α-dideutero-*m*-octopamine is shown in Scheme **1**. The key step in the synthesis of **1** is the large scale synthesis of (±)-α,α-dideutero-*m*-octopamine. This reaction sequence involves borane reduction of the intermediate cyanohydrin derived from 3-hydroxybenzaldehyde (12,13,14).



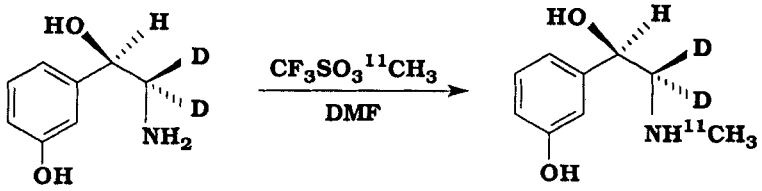
Scheme 1

We have also successfully used this procedure in the synthesis of 2-amino-1-(3-methoxyphenyl)ethanol which provided an HPLC standard in our earlier work on [^{11}C]phenylephrine (5). In pilot experiments, it was found by HPLC sampling that the isolation of (±)-α,α-dideutero-*m*-octopamine was most efficiently achieved by strong cation exchange chromatography provided the pH of the crude reaction mixture was carefully adjusted prior to chromatography. The scale-up of this synthetic protocol was an important consideration in the synthesis of **1**. In our earlier work with [^{11}C]phenylephrine, it was found crucial to isolate (-)-*m*-octopamine as the free base form from the resolution procedure in order to maintain reproducible radiomethylations and good radiochemical yields. This required large quantities of (±)-*m*-octopamine for the resolution. The optimized large scale

synthesis of (±)-α,α-dideutero-*m*-octopamine made possible the resolution and isolation of the desired enantiomer as a tractable solid using our previously described crystallization methods (6).

Deuterium incorporation at the alpha positions of *m*-octopamine was confirmed by NMR and mass spectroscopic data of the deuterated product and comparison of spectral data with undeuterated *m*-octopamine. Details are given in the experimental section. The -CH(OH)-CH$_2$- second order splitting pattern in the ^{1}H NMR of *m*-octopamine is simplified to a singlet at 4.32 ppm for the deuterated compound. In *m*-octopamine, this resonance appears as a doublet of doublets due to coupling to the two vicinal nonequivalent methylene protons. No corresponding resonances are observed in this region for the two diastereotopic methylene protons (≈ 2.4-3.0 ppm) alpha to nitrogen in the undeuterated compound. Furthermore, the ^{13}C NMR spectrum of the deuterated compound shows a complex multiplet for the carbon bearing the two diastereotopic deuteriums. Similarly, both the low resolution and high resolution mass spectroscopic data of the two compounds show a homologous fragmentation pattern and clear substitution of two deuteriums for two protons.

The resolution of (-)-α,α-dideutero-*m*-octopamine was accomplished by a procedure identical to that employed for resolving and isolating (-)-*m*-octopamine (5,15). (-)-α,α-Dideutero-*m*-octopamine reacted smoothly with [^{11}C]methyl triflate(16) at room temperature to yield the target compound, **1** (Scheme **2**) (5).



α,α-d$_2$-(-)-*m*-Octopamine **1**

Scheme 2

HPLC-purified **1** is suitable for subsequent animal and clinical studies. In conclusion, the current work above illustrates a useful method for the synthesis of deuterium-labeled chiral radiopharmaceuticals. The availability of **1** provides a promising tool for directly measuring the relative *in vivo* kinetics of MAO metabolism in the human heart by PET.

EXPERIMENTAL

Materials

3-Hydroxybenzaldehyde, O,O-dibenzoyl (+)-tartaric acid, Dowex X8-50 and dimethylformamide were purchased from Aldrich Chemical Co. and used without further purification. Trideuteroborane:THF (>98% deuterium labeled) was purchased as a 0.25 M tetrahydrofuran solution from Alfa, Johnson Matthey Co.

(±)-α,α-Dideutero-*m*-Octopamine[1,1-Dideutero-2-amino-1-(3-hydroxyphenyl)ethanol]

A solution of 12.21 g (0.10 mol) 3-hydroxybenzaldehyde and 4.90 g (0.10 mole) of sodium cyanide in 16 mL water was treated dropwise with a prefiltered solution (37.5 g/50 mL water) of sodium bisulfite (12). After addition of 14 mL of the sodium bisulfite solution to the above solution, 30 g of ice was added, followed by a second portion (14 mL) of sodium bisulfite. The reaction mixture was vigorously stirred for one min and transferred to a separatory funnel. The cyanohydrin was extracted with anhydrous ether (2 x 100 mL), dried over calcium chloride and rotoevaporated to give a yellow-orange syrup.

The oil was dissolved in anhydrous ether (50 mL) and added dropwise by an addition funnel without delay to a 1000 mL Schlenk flask equipped with pressure equalization adaptors containing trideuteroborane solution in THF (0.25 mole) under an argon atmosphere. During the addition of the last portion of the cyanohydrin, the solution turned cloudy and eventually coagulated to a white solid with concurrent evolution of gas. The resulting slurry was refluxed for three hours and allowed to cool to ambient temperature overnight.

The following day, the major portion of the white solid was treated with ≈10 drops of concentrated hydrochloric acid, rotoevaporated and azeotroped with absolute ethanol to give a gelatinous precipitate. The precipitate was dissolved in a solution of 21 mL concentrated HCl in 200 mL water and refluxed for one hour. The mixture was placed in a refrigerator at 4°C overnight. The resulting crystalline solid was filtered and discarded. The filtrate was rotoevaporated to dryness and azeotroped with ethanol. The residue was redissolved in 100 mL water and cooled at 4°C overnight. The solution was re-filtered and the pH of the filtrate was adjusted to ≈4.7 by careful dropwise addition of concentrated ammonium hydroxide and concentrated hydrochloric acid. The resulting solution was cooled at 4°C overnight and filtered the next day. The total volume of the solution was adjusted to 300 mL with water. The solution was then passed through a pre-washed column of Dowex X8-50 (60 g) after which the column was washed with water (1L). The desired product was eluted with ethanol/ammonium hydroxide (65:35) and obtained as a yellow-brown oil from rotoevaporation [(yield 11.2 g, 73.1%), ^{1}H NMR(d_6-DMSO, 360 MHz): 4.32(s, 1H), 6.60 (m, 1H), 6.72(m, 2H), 7.08 (t, 1H, J=7.76); ^{13}C NMR(d_6-DMSO, 360 MHz): 49.4 (m), 74.3, 112.9, 113.7, 116.5, 128.9, 148.0, 157.3; MS (low resolution, m/e): 155 (M^+, 18.7%), 136 (1.6%), 124 (100%), 105 (6.3%), 95 (52.8%); MS (high resolution): calculated for $C_8D_2H_9NO_2$: 155.0915, found: 155.0913]. An authentic sample of (±)-*m*-octopamine was also prepared according to the above procedure for comparison [^{1}H NMR(d_6-DMSO, 360 MHz): 2.40-2.66 (m, 2H), 4.32(d,d, 1H, J=4.2Hz), 6.59 (m, 1H), 6.72(m, 2H), 7.08 (t, 1H, J=7.75); MS (low resolution, m/e): 153 (M^+, 16.7%), 134 (2.6%), 124 (100%), 105 (6.2%), 95 (51.4%); MS (high resolution): calculated for $C_8H_{11}NO_2$: 153.0790, found: 153.0791].

(-)-α,α-Dideutero-*m*-octopamine

The (-)-isomer was resolved as the tartrate salt using O,O-dibenzoyl-(+)-tartaric acid (53.8 g) and (±)-α,α-dideutero-*m*-octopamine (23.31 g)(5,15). Repeated recrystallization (4x) from hot water(5) afforded 1,1-dideutero-2-amino-1-(3-hydroxyphenyl)ethanol as the (+)(-)-tartrate salt (4.71 g, 12.2% yield) with >96% enantiomeric purity as determined by copper Schiff base chiral HPLC(18); [R_t(-)=24.8 min, R_t(+)=29.7 min; column: Sumitomo Sumichiral OA-5500; flow: 0.25 mL/min; 0.001 M copper acetate]. A portion of the tartrate salt (1.67 g) was converted to the free base using strong cation-exchange chromatography (Dowex X8-50W, 4 g); subsequent elution with ammonium hydroxide/ethanol (35:65) solution (yield: 0.42 g, 81.3%) (5,15).

(-)-[^{11}C]-α,α-Dideutero-phenylephrine, 1

α,α-Dideutero-*m*-octopamine was methylated at room temperature with $CF_3SO_3{}^{11}CH_3$ (16) as previously described for *m*-octopamine **(5)**. Gaseous methyl triflate, $CF_3SO_3{}^{11}CH_3$ was trapped at -40°C in a solution of the precursor (≈ 1 mg in 300 μL) in anhydrous dimethylformamide. The desired product was isolated and purified by strong cation-exchange HPLC to give **1** [200-300 mCi, >97% (by HPLC) radiochemical purity, 30-60% radiochemical yield (EOS), >1000 Ci/mmol] as previously described **(5)**.

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