GENERAL METHODS FOR THE PREPARATION OF α AND/OR β DEUTERIUM LABELLED 6-HYDROXYDOPAMINE DERIVATIVES

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SUMMARY

A convenient synthetic method for the preparation of α and/or β deuterium-labelled 6-hydroxydopamines has been developed. Nitromethane was condensed with 2,4,5-trimethoxybenzaldehyde to give 2,4,5-trimethoxy-α-nitrostyrene (2). The nitroethylene side chain of 2 was reduced with sodium borodeuteride to afford β-[^{2}H]-β-(2,4,5-trimethoxyphenyl)-α-nitroethane (3). A reduction with sodium borohydride in the presence of a deuterium source (e.g. EtOD) afforded mono and dideuterated α-[^{2}H]-β-(2,4,5-trimethoxyphenyl)-α-nitroethanes (9, 10). A reduction of the nitrostyrene 2 with sodium borodeuteride in EtOD resulted in the isolation of α,β-[^{2}H]-β-(2,4,5-trimethoxyphenyl)-α-nitroethanes (15 and 16). Subsequent reduction of the deuterated α-nitroethanes 3, 9, 10, 15 and 16 gave the appropriately labelled phenethylamine derivatives 5, 11, 12, 17 and 18. Removal of the phenol protecting groups afforded the deuterium labelled 2,4,5-trihydroxyphenethylamines (6-hydroxydopamines).

Key Words: 6-Hydroxydopamine, Catecholamines, Deuterium Labelling

INTRODUCTION

Phenethylamines such as 6-hydroxydopamine (2,4,5-trihydroxyphenethylamine) are important tools for the study of adrenergic and dopaminergic function. For example 6-hydroxydopamine is used as an

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agent to produce chemical sympathectomy, that being destruction of monoaminergic nerve terminals (1). Earlier work in our laboratories has involved the synthesis and biological evaluation of several 6-hydroxydopamine analogs (2-4). In this paper we examine the various means available to specifically label the phenethylamine side chain of 6-hydroxydopamine. The availability of specifically labelled 6-hydroxydopamines should facilitate the in vivo study of uptake, binding, storage and metabolism of this molecule.

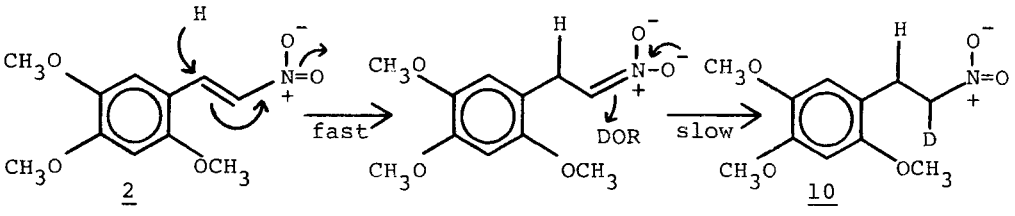
Isotopic labelling of the ethylamine side chain of physiologically important phenethylamines has been reported by several groups (5-10). The reduction of nitrostyrene derivatives 2 with sodium borodeuteride or borotritiide resulted in nearly quantitative incorporation of an isotope at the β or benzylic position of the phenethylamine side chain (5). This procedure was used to label 6-hydroxydopamine (5). The incorporation of deuterium or tritium at the β-position of 6-hydroxydopamine has also been accomplished by quenching the anion of 2,4,5-trimethoxybenzylcyanide with D_2O or T_2O (6). Battersby and coworkers (7,8) successfully prepared the separate α- and β-labelled dopamines through sodium borodeuteride or borotritiide reduction of the appropriate aldehyde precursors. Perel et al. (9) synthesized α,α-dideuterodopamine by reducing a benzylcyanide with lithium aluminum deuteride. An α,β-dideuterophenethylamine was prepared by one step reduction of the corresponding nitrostyrene with lithium aluminum deuteride (10).

The general methods we used to synthesize the deuterated 6-hydroxydopamines are illustrated in Scheme 1. Nitromethane was condensed with 2,4,5-trimethoxybenzaldehyde (1) using the method of Benigni and Minnis (11) to give the key nitrostyrene 2. The work of Rotman et al. (5) was repeated and the incorporation of deuterium at the β-position (3) was seen, however, it was not quantitative.

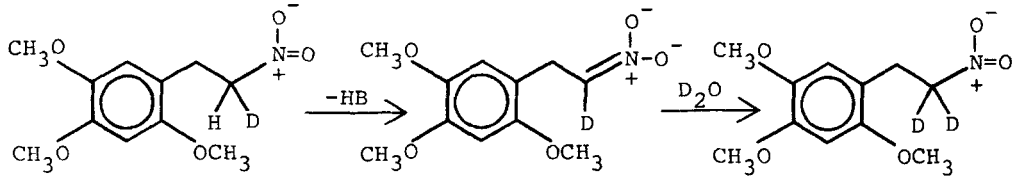
We found approximately 13% unlabelled material 4 as determined by mass spectral analysis. The reduction of 3 to 5 was accomplished using Red-al (sodium bis(2-methoxy-ethoxy) aluminum hydride) or catalytic hydrogenation. The Red-al reduction provided the highest yields, shortest reaction times and least loss of label. Catalytic hydrogenation resulted in a small loss of label at the benzylic position (see Table 1). Demethylation of 5 was accomplished using boron tribromide (4).

The incorporation of a label at the α-position of 6-hydroxydopamine (13, 14) was achieved by quenching the borohydride reduction of nitrostyrene 2 with EtOD or D_2O. This approach was suggested by Rotman *et al.* (5), but shown to be effective in our laboratories. When the sodium borohydride reduction of 2 was carried out in EtOD alone for 24 hours, 22% of the isolated material was the unlabelled reduction product 4 with the remaining material being mono or dideuterated. When the reduction was run in EtOD and quenched with D_2O, only 3% of the product was unlabelled, 55% dideuterated (9) and 37% monodeuterated (10). These results indicate that the reduction of the conjugated double bond is rapid compared to the quenching of the resulting anion (see equation 1). Further, if the quenching species possesses a very labile deuterium

(1)



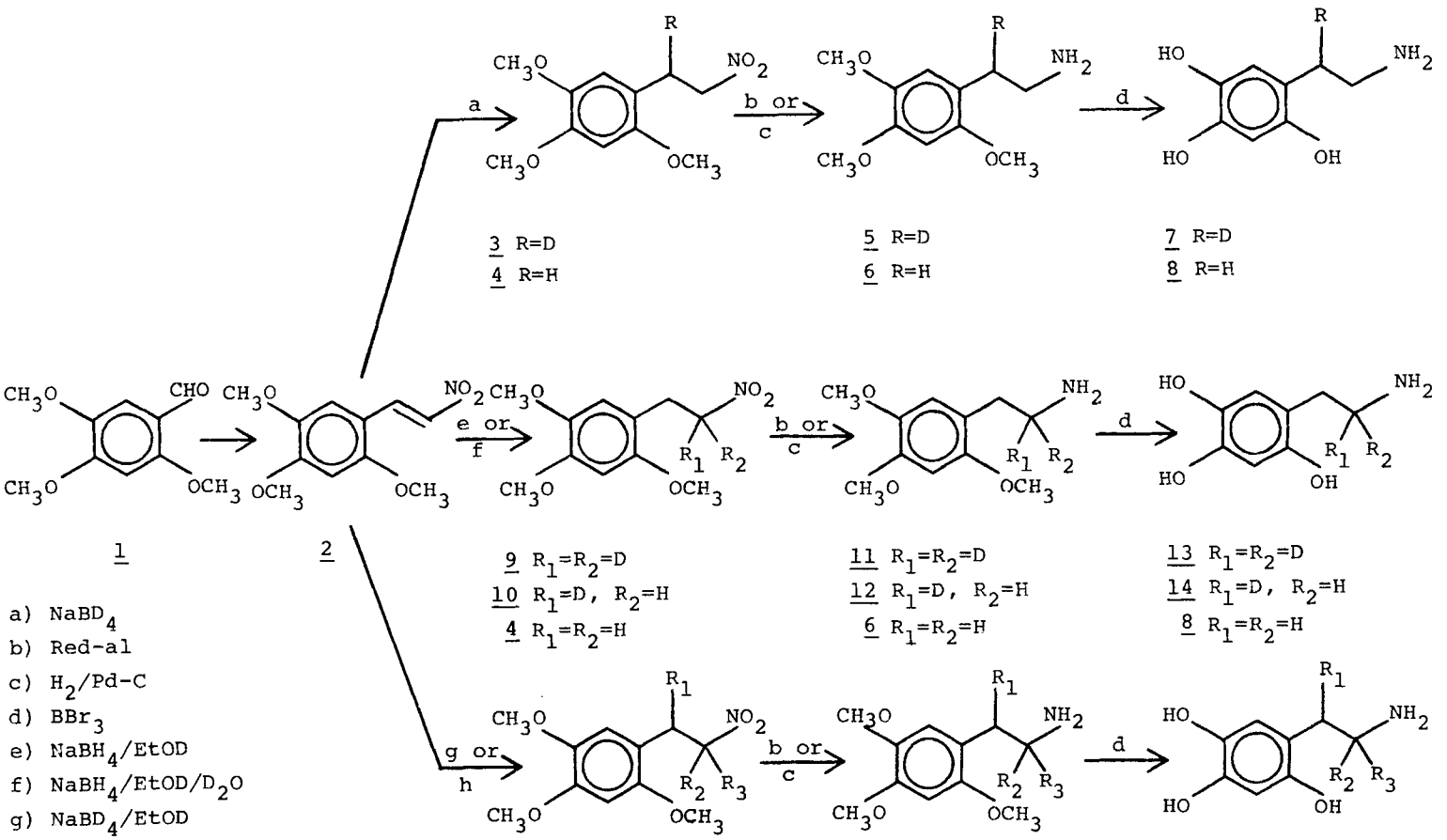
such as D_2O, the basicity which develops throughout the course of the reaction catalyzes further exchange as pictured in equation 2.

(2) 

10 9

The reduction of 9 and 10 with Red-al resulted in a significant loss of α-label producing a change in the ratio of mono- to dideuterated compounds (Table 1).

The α,β-dideutero-β-(2,4,5-trimethoxyphenyl)-α-nitroethanes 15 and 16 were generated by the reduction of nitrostyrene 2 with sodium borodeuteride in EtOD followed by a D_2O quench. The isolated materials showed a 91% incorporation of at least two deuterium atoms. Further reduction of 15 and 16 followed by deprotection afforded the α,β-deuterated 6-hydroxydopamines 19 and 20.


a) $NaBD_4$
b) Red-al
c) H_2/Pd-C
d) BBr_3
e) $NaBH_4$/EtOD
f) $NaBH_4$/EtOD/D_2O
g) $NaBD_4$/EtOD
h) $NaBD_4$/EtOD/D_2O
1
2
3 R=D
4 R=H
5 R=D
6 R=H
7 R=D
8 R=H
9 $R_1=R_2=D$
10 $R_1=D$, $R_2=H$
4 $R_1=R_2=H$
11 $R_1=R_2=D$
12 $R_1=D$, $R_2=H$
6 $R_1=R_2=H$
13 $R_1=R_2=D$
14 $R_1=D$, $R_2=H$
8 $R_1=R_2=H$
15 $R_1=R_2=R_3=D$
16 $R_1=R_2=D$, $R_3=H$
3 $R_1=D$, $R_2=R_3=H$
17 $R_1=R_2=R_3=D$
18 $R_1=R_2=D$, $R_3=H$
5 $R_1=D$, $R_2=R_3=H$
19 $R_1=R_2=R_3=D$
20 $R_1=R_2=D$, $R_3=H$
7 $R_1=D$, $R_2=R_3=H$

Scheme 1

Table 1

Reduction Products of 2,4,5-Trimethoxynitrostyrene

Reagents	Starting Materials	Products	% Composition[a]
$NaBD_4$/EtOH	2	3	80
		4	14
$NaBH_4$/EtOD	2	4	22
		9	29
		10	44
$NaBH_4$/EtOD/D_2O	2	4	3
		9	55
		10	37
$NaBD_4$/EtOD	2	3	27
		15	21
		16	49
$NaBD_4$/EtOD/D_2O	2	3	9
		15	50
		16	41
$NaAlH_2(OCH_2CH_2OCH_3)_2$	3,4	5	78
		6	12
	4,9,10	6	28
		11	10
		12	61
H_2/Cat.	3,4	5	73
		6	22
	4,9,10	6	0
		11	42
		12	47

a) percent of total purified material as determined by mass spectra. Totals may be less than 100% in each case due to minor impurities.

Table 2

NMR SPECTRAL DATA

Chemical Shifts (δ)[a]

Compound	
2	8.15, 1H, d(J=14 Hz), $CH{=}C\underline{H}NO_2$; 7.73, 1H, d(J=14 Hz), $C\underline{H}{=}CHNO_2$; 6.91, 1H, s, Ar$\underline{H}$; 6.58, 1H, s, Ar$\underline{H}$; 3.97, 3H, s, $OC\underline{H}_3$; 3.94, 3H, s, $OC\underline{H}_3$; 3.88, 3H, s, $OC\underline{H}_3$.
4	6.68, 1H, s, Ar$\underline{H}$; 6.51, 1H, s, Ar$\underline{H}$; 4.56, 2H, t, (J=7.40 Hz), $C\underline{H}_2NO_2$; 3.87, 3H, s, $OC\underline{H}_3$; 3.82, 6H, s, $OC\underline{H}_3$; 3.23, 2H, t (J=7.40 Hz), $C\underline{H}_2CH_2NO_2$.
3	6.69, 1H, s, Ar$\underline{H}$; 6.51, 1H, s, Ar$\underline{H}$; 4.56, 2H, d (J=7.6 Hz), $C\underline{H}_2NO_2$; 3.88, 3H, s, $OC\underline{H}_3$, 3.82, 3H, s, $OC\underline{H}_3$; 3.24, 1H, t (J=7.6 Hz), $CD\underline{H}CH_2NO_2$.
10	6.69, 1H, s, Ar$\underline{H}$; 6.51, 1H, s, Ar$\underline{H}$; 4.56, 1H, t (J=7.5 Hz), $CDHNO_2$; 3.88, 3H, s, $OC\underline{H}_3$; 3.82, 6H, s, $OC\underline{H}_3$; 3.22, 2H, d (J=7.5 Hz), $C\underline{H}_2CDHNO_2$.
15	6.68, 1H, s, Ar$\underline{H}$; 6.51, 1H, s, Ar$\underline{H}$; 3.87, 3H, s, $OC\underline{H}_3$; 3.81, 6H, s, $OC\underline{H}_3$.
6	6.82, 1H, s, Ar$\underline{H}$; 6.51, 1H, s, Ar$\underline{H}$; 3.87, 3H, s, $OC\underline{H}_3$; 3.83, 3H, s, $OC\underline{H}_3$; 3.80, 3H, s, $OC\underline{H}_3$; 3.05, 4H, m, $C\underline{H}_2C\underline{H}_2NH_2$.

a) in parts per million downfield from tetramethylsilane internal standard. All spectra are in $CDCl_3$.

EXPERIMENTAL

Electron impact mass spectra were recorded on a Varian-MAT CH-5 mass spectrometer with an RDS Data system for computer analysis of spectra. N.M.R. spectra were obtained with either a Varian FT-80A or a Brucker WP-80 spectrometer. The $NaBD_4$ was 98 atom % deuterium from Sigma Chemical Co., the EtOD and D_2O were 99.5 and 99.8 atom % deuterium respectively from Aldrich Chemical Co.

2,4,5-Trimethoxy-α-nitrostyrene (2). To 10 mL of acetic acid were added 1.0 g (5.1 mmol) 2,4,5-trimethoxybenzaldehyde (1), 0.79 g (10 mmol) ammonium acetate and 0.83 mL (15 mmol) distilled nitromethane. The mixture was brought to a reflux for three hours, cooled to room temperature and stirred an additional twelve hours. The acetic acid was removed under vacuum, 100 mL of 95% ethanol added and the hot solution filtered to remove insoluble ammonium salts. The ethanol filtrate was reduced to approximately 5 mL and allowed to cool on the bench overnight. The brilliant orange crystals were isolated by suction filtration, washed with a small amount of ethanol and vacuum dried. The 0.81 g (67%) of dried solid had a m.p. of 126-128° (lit. (12) m.p. = 132°).

β-[^{2}H]-β-(2,4,5-trimethoxyphenyl)-α-nitroethane (3). In a clean dry flask were placed 0.75 g (3.1 mmol) of 2 and 0.26 g (6.3 mmol) $NaBD_4$ as dry powders. The flask was sealed with a septum, purged with dry nitrogen and chilled to 0°. A 10 mL portion of absolute ethanol was added with stirring. As the initial frothing subsided, the flask was allowed to warm to room temperature and stirring was continued. The orange color discharged completely after approximately 4 hours, however, stirring was continued overnight. The reaction was reduced *in vacuo* and the resulting residue shaken with 20 mL each of chloroform and water. The organic layer was washed with brine and dried over sodium sulfate. Evaporation *in vacuo* resulted in 0.56 g (74%) of viscous brown oil. The coloration was removed via column chromatography

(2 x 10" silica gel) eluted with acetone/dichloromethane (1:1). The colorless fractions were combined and reduced to 0.29 g (39%) of highly purified product. The nmr indicated a doublet at δ 4.56 (J=7.58) corresponding to the α-protons and a triplet at δ 3.24 (J=7.58) for the β-protons (see Table 2). Mass spectral data indicated approximately 14% of unlabelled material (M=241) 4.

β-[^{2}H]-2,4,5-Trimethoxyphenethylamine (5). Method a): A 0.75 g (0.31 mmol) portion of 3 and 4 was dissolved in 5 mL dry benzene, 3.0 mL Red-al (70% sodium bis(2-methoxy-ethoxy) aluminum hydride in toluene) was added, the flask sealed under nitrogen and stirred approximately twelve hours. The excess Red-al was destroyed by adding 3x1 mL portions of water to the chilled reaction mixture with vigorous stirring. The organic phase was decanted from the clumped aluminate paste. The paste was washed with a few small portions of benzene. The benzene washes were combined, dried over sodium sulfate and flashed to a light yellow oil - 67 mg (92% free base). The free amine was converted to its hydrochloride salt in ethanolic HCl and crystallized from dichloromethane to give colorless needles, m.p. 190-191° (lit. (13) m.p. = 193-195°). The nmr showed both the α- and β-protons collapsed to an undecipherable group of signals centered at δ 3.05 (see Table 2). The results of the mass spectral analysis are listed in Table 1.

Method b): A 0.076 g (0.31 mmol) portion of 3 and 4 was dissolved in 25 mL of absolute EtOH and 0.5 mL $CHCl_3$ (in situ HCl source) (14). The reaction mixture was shaken for 4.5 hours over 50 mg of platinum oxide in a 50 lb. in.^{2} hydrogen atmosphere. The mixture was suction filtered through a celite pad to remove catalyst and the filtrate was reduced to a tacky colorless solid. The residue was dissolved in 10 mL 0.2 M HCl and extracted with 3x3 mL portions of methylene chloride. The aqueous layer was basified to pH 11-12 with sodium carbonate and re-extracted with 3x5 mL portions of methylene chloride.

The methylene chloride extracts of the aqueous basic layer were combined, dried and flashed to a colorless residue 28.5 mg (43% free base). The free amine was converted to the hydrochloride salt as outlined in Method a. Mass spectral analysis (Table 1) showed some loss of label at the benzylic position.

β-[^{2}H]-2,4,5-Trihydroxyphenethylamine (7). A 0.50 g (0.24 mmol) portion of 5 and 6 along with 10 mL of dry dichloromethane were placed in a flask under dry nitrogen. A 1.5 mL portion of 1M boron tribromide in dichloromethane was added slowly and the reaction stirred for 12 hours at room temperature. The mixture was cooled to 0° and 10 mL of methanol added dropwise. The resulting precipitate was collected by filtration to give 0.040 g (68%) of the hydrobromide salt, m.p. 218-220°(lit. (13) m.p. = 218-220°).

α-[^{2}H]-β-(2,4,5-Trimethoxyphenyl)-α-nitroethane (9, 10). Method a): The preparation of α-deuterated nitroethanes 9 and 10 was carried out using the same proportions and identical procedure as that employed in the synthesis of 3 with the following exceptions: $NaBH_4$ was used in place of $NaBD_4$ and EtOD was used in place of EtOH as the reaction solvent. In this manner 0.584 g (77%) of crude product afforded 0.310 g (41%) of material following chromatographic purification (see synthesis of 3). The nmr indicated a mixture of products 4, 9 and 10 - unlabelled, mono- and dideuterated material, respectively. The mass spectral data supported the nmr results and indicated 22% unlabelled nitroethane 4 (M=241).

Method b): A flask containing 0.075 g (0.31 mmol) of 2 and 0.024 g (0.62 mmol) $NaBH_4$ was sealed with a septum and purged with nitrogen. The reaction vessel was cooled in an ice bath as 4 mL of EtOD was added via syringe. After the initial frothing subsided the reaction was allowed to warm to room temperature while stirring. The reaction proceeded for 2 hours then a 2 mL portion of D_2O was added via syringe

and stirring was continued for an additional 30 minutes. The volume was reduced to approximately 2 mL on the flash evaporator, then 5 mL of ethylacetate and 2 mL of water were added. The organic layer was separated and the aqueous layer washed with a second portion of ethylacetate. The organic extracts were combined, dried over Na_2SO_4, filtered and the solvent removed in vacuo to yield a light tan oil, 0.039 g (51%), which was chromatographically pure. The nmr spectrum showed almost complete disappearance of the downfield α-nitro protons at δ 4.56 (see Table 2). The mass spectrum supported the nmr data showing 97% of the material had at least one deuterium on the α-carbon (see Table 1).

α-[^{2}H]-2,4,5-Trimethoxyphenethylamine (11, 12). Method a): A 0.076 g (0.31 mmol) portion of the mixture of 9 and 10 was reduced using Red-al and the identical procedure employed in the synthesis of 5 (Method a). The free amine was isolated as a faint yellow oil (65 mg, 67%) and was converted to the hydrochloride salt in the same manner as 5 (Method a). The hydrochloride salts of 11 and 12 were isolated as colorless needles m.p. 190-191° (lit. (13) m.p. 193-195°). Mass spectral data showed 61% of the desired monodeuterated product 12, 10% of the α,α-dideutero material 11 and 28% unlabelled material 6 (versus 22% before reduction).

Method b): Catalytic reduction of a 0.030 g (0.124 mmol) portion of 9 and 10 over 10 mg 10% Pd-C was carried out using the identical procedure employed in the synthesis of 5 (Method b). Following workup 5 mg (19% free base) of colorless oily free amine was isolated and converted to its hydrochloride salt which was identical to previous samples. The mass spectrum showed an almost 1:1 ratio of dideutero material 11 (M=213) to the desired monodeuterated product 12 (M=212).

α-[^{2}H]-2,4,5-Trihydroxyphenethylamine (13, 14). The preparation of this compound followed the procedure described above for β-[^{2}H]-6-hydroxydopamine 7.

α,β-[^{2}H]-β-(2,4,5-Trimethoxyphenyl)-α-nitroethane (15, 16). Method a): The preparation of 15 and 16 was carried out using the same proportions and procedures employed in the synthesis of 3 and 10 (Method a). In this instance both $NaBD_4$ and EtOD were required for α- and β-labelling. Following isolation and purification, mass spectral analysis indicated only 41% of the desired product 16 and 27% of 3.

Method b): The preparation of 15 and 16 was carried out using the same proportions and procedure employed in the synthesis of 10 (Method b). $NaBD_4$, EtOD and D_2O were used to achieve maximum labelling. Of the purified material isolated, 91% contained at least two deuterium atoms (see Table 1). The nmr spectrum showed a complete disappearance of both α- and β-proton signals (see Table 2).

α,β-[^{2}H]-2,4,5-Trimethoxyphenethylamine (17, 18). The reduction of 15 and 16 was carried out according to the procedure outlined in the synthesis of 5 (Method a) using Red-al. Mass spectral examination of the purified product indicated the Red-al reduction resulted in some loss of α-label.

α,β-[^{2}H]-2,4,5-Trihydroxyphenethylamine (19, 20). The preparation of this compound followed the procedure described earlier for β-[^{2}H]-6-hydroxydopamine 7.

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