REGIOSELECTIVE SYNTHESES OF DEUTERIUM LABELLED 6-HYDROXYDOPAMINES

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SUMMARY

Convenient syntheses of 2,4-,α,α- and β,β-deuterium labelled 6-hydroxydopamines have been developed. 2,4,5-Trimethoxybenzaldehyde (1) was reductively aminated to give 2,4,5-trimethoxybenzylamine (2). Quaternization of the amine with methyliodide followed by displacement with cyanide gave 2,4,5-trimethoxybenzylcyanide (4). A $LiAlD_4$ reduction of 4 gave α,α-[^{2}H]-β-(2,4,5-trimethoxyphenyl)-ethylamine (5). Treatment of benzylcyanide 4 with n-butyllithium/D_2O gave α,α-[^{2}H]-α-cyano-2,4,5-trimethoxytoluene (7) which upon reduction afforded β,β-[^{2}H]--β-(2,4,5-trimethoxyphenyl)-ethylamine (8). Treatment of 2,4,5-trimethoxydimethylbenzylamine 2 with n-butyllithium/D_2O gave 3,6-[^{2}H]-2,4,5-trimethoxydimethylbenzylamine (10). The ring deuterium atoms were retained through subsequent steps to afford β-(3,6-[^{2}H]-2,4,5-trimethoxyphenyl)-ethylamine (13). Removal of the phenol protecting groups afforded the deuterium labelled 2,4,5-trihydroxyphenethylamines (6-hydroxydopamines).
Key Words: 6-Hydroxydopamines, Catecholamines, Deuterium labelling

INTRODUCTION

6-Hydroxydopamine (2,4,5-trihydroxyphenethylamine) has become an important tool in the study of biogenic amine function, since it produces chemical 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) including 6-hydroxydopamines with deuterium-labelled ethylamine side chains (5). In this study we have examined other methods to label 6-hydroxydopamine regioselectively on the phenethylamine side chain, as well as methods to label the aromatic ring regioselectively. The availability of specifically hydrogen labelled 6-hydroxydopamines,

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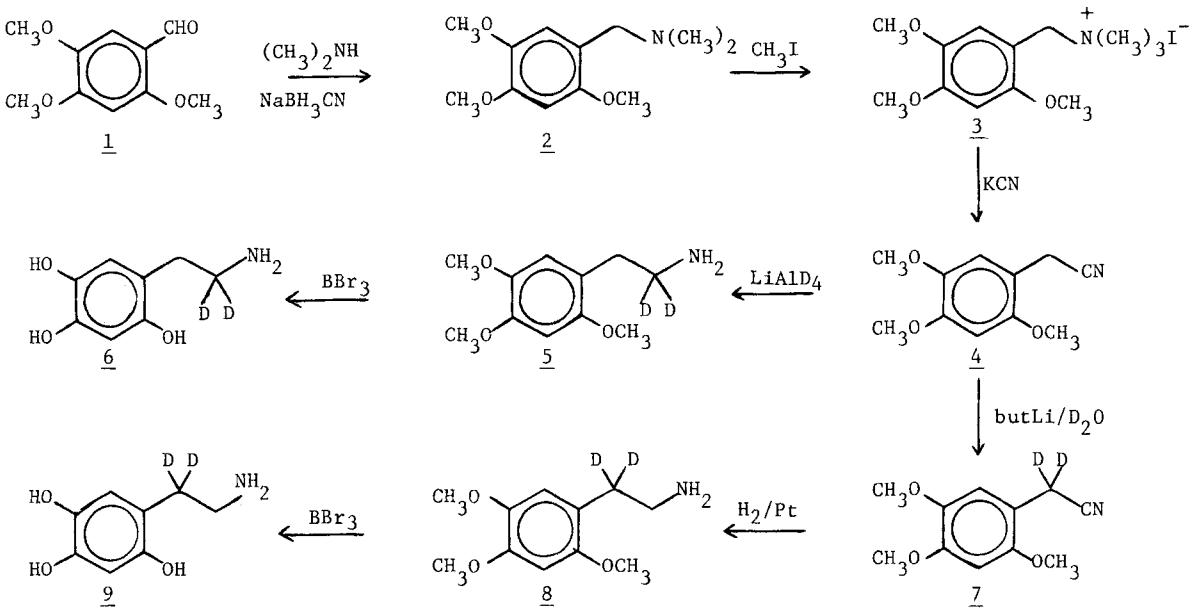
particularly the ring labelled analogs, should facilitate the elucidation of its mode of action.

Several groups have reported isotopic hydrogen labelling of physiologically important phenethylamines (5-11). Rotman and coworkers (6) employed $NaBD_4/NaBT_4$ reductions of an α-nitrostyrene to incorporate an isotope at the β- or benzylic position enroute to 6-hydroxydopamine. Jacob et al (7) were successful in preparing β-[^{3}H]-6-hydroxydopamine via benzylic exchange on a benzylcyanide intermediate. Perel et al (10) have prepared α-[^{2}H]-dopamine by a $LiAlD_4$ reduction of a benzylcyanide intermediate. A method for the synthesis of either α- or β-[^{2}H]-6-hydroxydopamine starting from 2,4,5-trimethoxy-α-nitrostyrene and $NaBD_4$ has been reported recently (5).

The general methods used to synthesize the deuterated 6-hydroxydopamines are illustrated in Scheme 1. A modification of the method of Borch et al (12) was used to aminate 2,4,5-trimethoxybenzaldehyde (1) to give dimethylbenzylamine 2. Alkylation of 2 with methyliodide gave the quaternary ammonium salt 3 which upon treatment with KCN and molecular sieves in refluxing DMF resulted in 2,4,5-trimethoxybenzylcyanide (4) in moderate yields (40-70%); which was an improvement over that reported by Short et al (13).

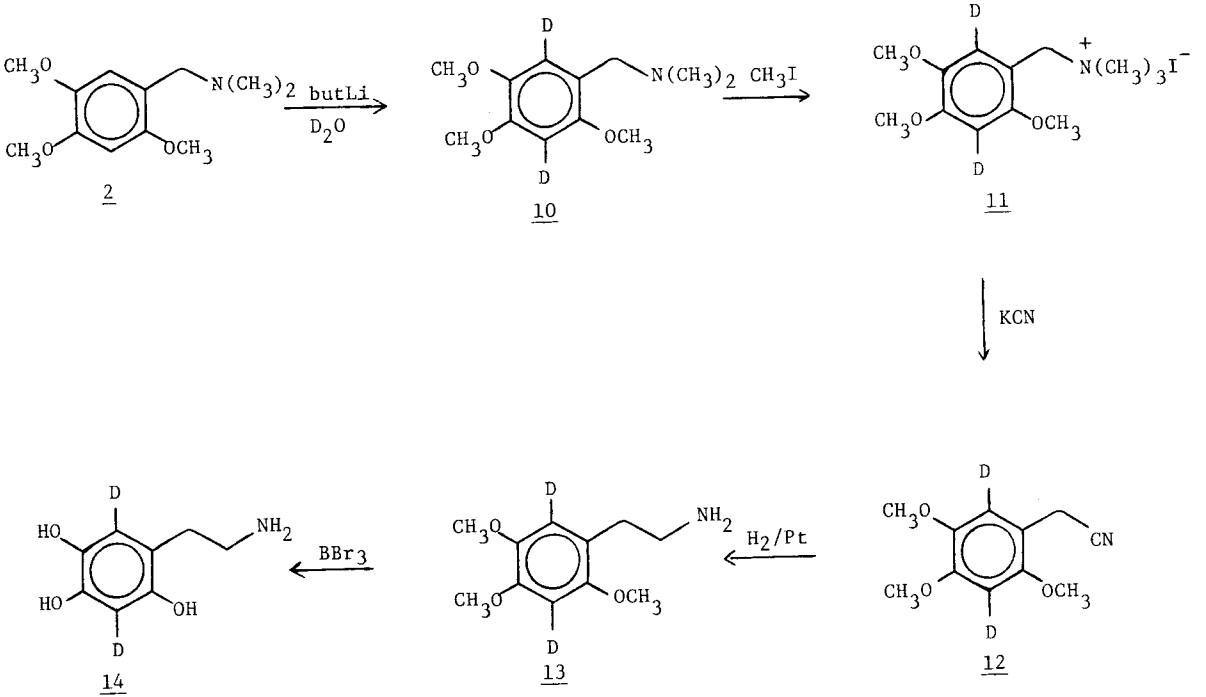
Incorporation of deuterium at the α-position was achieved by reduction of benzylcyanide 4 with $LiAlD_4$ to yield 5. The reaction as reported by Perel et al (10) suffered from poor yields; however, there was good overall


CH3O
CHO
CH3O
OCH3
1
(CH3)2NH
NaBH3CN
N(CH3)2
2
CH3I
+
N(CH3)3I−
3
KCN
CN
4
butLi/D2O
D D
5
LiAlD4
NH2
6
BBr3
HO
OH
7
H2/Pt
8
9

Scheme 1

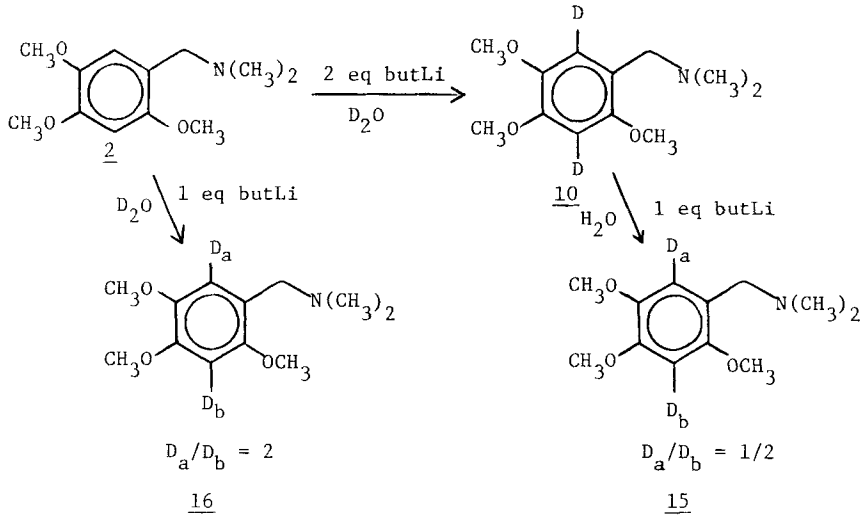
incorporation of label into 5. Using a modification of the procedure of Jacob and coworkers (7) benzylcyanide 4 was treated with as little as one equivalent of butyllithium followed by a D_2O quench which resulted in the isolation of β,β-dideutero-compound 7 in good yields. Catalytic hydrogenation of the cyano group of 7 to yield 8 was carried out according to the procedure of Short et al (13).

Labelling the aromatic ring of 6-hydroxydopamine was accomplished as shown in Scheme 2. Based on the observations of Jones et al (14) and Slocum and Jennings (15), benzylamine 2 was treated with butyllithium /D_2O to give the deuterium labelled benzylamine 10. In an attempt to label selectively either the 3 or 6 position of benzylamine 2, the compound was treated with one equivalent of butyllithium and quenched with D_2O (see equation 1). Approximately 66% of the deuterium label was incorporated at the 3-position. Conversely when the dideuterobenzylamine 10 was treated with one equivalent of n-butyllithium and quenched with H_2O, the deuterium at the 3-position exchanged more rapidly than at the 5-position as determined by NMR. The labelled amine 10 could be quaternized to 11 and treated with KCN to give the benzylcyanide 12 with no loss of label. Catalytic reduction of 12 afforded 13, but again with disappointing yields. Demethylation of 5, 8 and 13 was accomplished using boron tribromide (4).


CH3O
CH3O
N(CH3)2
OCH3
2
butLi
D2O
D
10
CH3I
+
N(CH3)3I−
11
KCN
CN
12
H2/Pt
NH2
13
BBr3
HO
OH
14

Scheme 2

Equation 1



Schemes 1 and 2 represent versatile syntheses of 6-hydroxydopamines which allow selective deuterium labelling of the ring (intermediate 2) or either carbon atom of the side chain (intermediate 4). The regioselectivity as outlined in these schemes is as good as that demonstrated in our earlier work with nitrostyrenes (5). The basic disadvantage of the nitrostyrene route lies in the inability to label the ring selectively. The syntheses outlined in Schemes 1 and 2 provides a potentially more convenient and less expensive way of incorporating a carbon isotope at the α-position of 6-hydroxydopamine than does the nitrostyrene route (5).

EXPERIMENTAL

Electron impact mass spectra were recorded on either a Varian-MAT CH-5 or Riber R-10-10 mass spectrometer with a RDS data system for computer analysis of spectra. NMR

spectra were obtained with either a Varian T-60 or Varian FT-80a spectrometer and were run in 1% TMS/$CDCl_3$ unless otherwise noted. The $LiAlD_4$ was 99 atom% deuterium from Merck, Sharp & Dohme and the D_2O was 99.8 atom % deuterium from Aldrich Chemical Co.

2,4,5-Trimethoxybenzylamine (2)

A modification of the method of Borch et al (12) was used to prepare 2. To 50ml of absolute methanol were added 1.0 g (5.1 mmol) 2,4,5-trimethoxybenzaldehyde (1), 2.50 g (30.6 mmol) dry dimethylamine hydrochloride and 1.0 g of 3A° molecular sieve pellets. The mixture was stirred at room temperature for 15 minutes then 0.34 g (5.1 mmol) $NaBH_3CN$ was added in one portion. The reaction was flushed with nitrogen, stoppered and stirred at room temperature overnight. After approximately 18 hours at room temperature the mixture was suction filtered to remove insoluble material. The insoluble solids were washed with 2 x 5 ml portions of methanol and the filtrate reduced in vacuo. To the residue was added 2N HCl (10 ml) and the resulting solution was stirred vigorously at ambient temperature for one hour, then washed with 3 x 5 ml portions of methylene chloride. The washed acidic aqueous layer was basified to pH 12 with NaOH and extracted with 3 x 10 ml portions of methylene chloride. The organic extracts were washed with one 20 ml portion of water and one 20 ml portion of brine. The organic layer was filtered through a cotton plug, flashed to a residue and dried overnite in vacuo. The light

tan oil solidified on standing to yield 0.795g (69%) of a waxy solid, m.p. 59-61°C (lit (16) bp_{10} 154-155°C), which was homogeneous to thin layer chromatography (silica gel, 16% CH_3OH/CH_2Cl_2 trace of NH_4OH).

NMR: 6.94,1H, s, ArH; 6.41, 1H, s, ArH; 3.70, 3H, s, OCH_3; 3.68, 3H, s, OCH_3; 3.64, 3H, s, OCH_3; 3.34, 2H, s, $CH_2N(CH_3)_2$; 2.20, 6H, s, $N(CH_3)_2$.

2,4,5-Trimethoxytrimethylbenzylammonium Iodide (3)

To 15 ml of absolute ethanol were added 1.31 g (5.8 mmol) of 2 and 0.72 ml (11.6 mmol) of methyliodide. The reaction proceeded under nitrogen at ambient temperature for 24 hrs at which time the solution was diluted with 15 ml of dry acetone and the insoluble solid isolated by suction filtration. The filtrate was concentrated in vacuo, the residue redissolved in warm dry acetone and diluted with Skellysolve B. A second crop of insoluble solid was isolated by suction filtration and the two crops of light yellow solid were combined and vacuum dried - 1.94 g (92%), mp 275°dec.

NMR: 7.46, 1H, s, ArH; 6.59, 1H, s, ArH; 4.84, 2H, s, $CH_2N^+(CH_3)_3$; 3.92, 9H, brd s, OCH_3; 9H, s, $N^+(CH_3)_3$; 1% TMS/$CDCl_3$/DMSO-d_6.

2,4,5-Trimethoxybenzylcyanide (4)

A modification of the method of Short et al (13) was used to prepare 4. A 0.10 g (0.27 mmol) portion of 3, 0.037 g (0.54 mmol) of KCN and approximately 0.5 g of 3A molecular sieves were brought to 160° C in 5 ml of dry dimethylformamide. After 36 hours the reaction was cooled and the

solvent gently removed in vacuo. The residue was extracted with 4 x 5 ml portions of ethylacetate. The ethylacetate extracts were washed with 3 x 5 ml of water, 1 x 10 ml of brine and dried over Na_2SO_4. The extracts were treated with a small amount of Norit, filtered through a celite pad and concentrated in vacuo. Vacuum drying to a constant weight resulted in 0.038 g (68%) of 4 - m.p. 81-83°C (lit. (17) 83-86°C).

NMR: 6.88, 1H, s, ArH; 6.53, 1H, s, ArH; 3.85, 9H, brd s, OCH_3; 3.63, 2H, s, CH_2CN.

α,α-[^{2}H]-2,4,5-Trimethoxyphenethylamine (5)

The $LiAlD_4$ reduction of 4 to 5 was carried out according to the method employed by Perel et al (10) to synthesize α,α-deuterodopamine. The only differences were in the use of methylene chloride as an extracting solvent and the use of ethanolic HCl to generate the amine hydrochloride salt. A 0.038 g (29%) portion of 5 was isolated as the amine hydrochloride - m.p. 188-190°C (lit. (13) 193-195°C).

NMR: 6.82, 1H, s, ArH; 6.51, 1H, s, ArH; 3.87, 3H, s, OCH_3; 3.83, 3H, s, OCH_3;3.80, 3H, s, OCH_3; 3.3, 2H, m, $CH_2CD_2NH_2$; 1% TMS/$CDCl_3$/DMSO-d_6.

α,α-[^{2}H]-2,4,5-Trihydroxyphenethylamine (6)

The BBr_3 demethylation procedure of Borchardt et al (4) was used to prepare 6. The procedure afforded 0.040 g (66%) of 6 as the hydrobromide salt - mp 218-220°C (lit. (18) 218-219°C).

α,α-[^{2}H]-2,4,5-Trimethoxybenzylcyanide (7)

A modification of the method of Jacob et al (7) was employed to prepare 7. A 0.3 ml (0.49 mmol, 1.55 M in hexane) portion of n-butyllithium was added dropwise at ambient temperature to 0.069 g (0.33 mmol) of 4 in 1.5 ml dry tetrahydrofuran under nitrogen. The solution turned deep red and a precipitate formed over one hour. The reaction was quenched with 50 µl of D_2O and stirred vigorously overnight. Benzene (5 ml) and water (2 ml) were added to the reaction with stirring. The organic layer was separated and the aqueous layer extracted with a second 5 ml portion of benzene. The benzene extracts were washed with 10 ml of brine, dried over Na_2SO_4, filtered and reduced to a residue in vacuo. The faint red-orange residue solidified on standing - 0.078 g (quantitative). The material could be purified by chromatography (silica gel, 50% acetone/methylene chloride) or sublimation (120°C, 0.05 mm) with approximately 65% recovery in each case.
NMR: 6.88, 1H, s, ArH; 6.53, 1H, s, ArH; 3.85, 9H, brd s, OCH_3.

β,β-[^{2}H]-2,4,5-Trimethoxyphenethylamine (8)

The catalytic reduction employed was essentially that of Short et al (13) with the only exception being the choice of PtO_2 catalyst. Colorless needles of the hydrochloride salt of 8 were isolated - 0.054 g (57%), m.p. 188-190°C (lit. (13) 193-195°C).

NMR: 6.82, 1H, s, ArH; 6.51, 1H, s ArH; 3,87, 3H, s, OCH_3; 3.83, 3H, s, OCH_3; 3.80, 3H, s, OCH_3; 3.05, 2H, m, CH_2NH_2; 1% TMS/$CDCl_3$/DMSO-d_6.

β,β-[^{2}H]-2,4,5-Trihydroxyphenethylamine (9)

The preparation of this compound followed the procedure described above for α,α-[^{2}H]-6-hydroxydopamine 6.

3,6-[^{2}H]-2,4,5-Trimethoxydimethylbenzylamine (10)

To a stirred solution of 0.147 g (0.65 mmol) of 2 in 1.5 ml of dry tetrahydrofuran under nitrogen was added 1.0 ml (1.5 mmol, 1.5 M in hexane) butyllithium over 3 minutes at ambient temperature. The reaction became deep red and developed a heavy precipitate over one hour. The reaction was quenched with 150 µl of D_2O and stirred vigorously overnight. Benzene (3 ml) and water (2 ml) were added with stirring. The benzene layer was removed and the aqueous layer extracted with a second portion of benzene. The extracts were washed with water, brine, dried over Na_2SO_4, filtered and reduced to a residue in vacuo. The faint yellow oil solidified after vacuum drying - 0.134 g (94%). A microdistillation (120-125°C, 0.2 mm) afforded 0.055 g (37%) of light yellow solid, m.p. 38-39°C.

NMR: 3.70, 3H, s, OCH_3; 3.68, 3H, s, OCH_3, 3.64, 3H, s, OCH_3; 3.34, 2H, s, $CH_2N(CH_3)_2$; 2.20, 6H, s, $N(CH_3)_2$.

3,6-[^{2}H]-2,4,5-Trimethoxytrimethylbenzylammonium Iodide (11)

The alkylation of 10 was carried out according to the procedure outlined in the synthesis of 3. The desired

material 11, 0.352 g (81%), was isolated without loss of deuterium label.

NMR: 4.84, 2H, s, $CH_2N^+(CH_3)_3$; 3.90, 9H, brd s, OCH_3; 3.39, 9H, s, $N(CH_3)_3$; 1% TMS/$CDCl_3$/DMSO-d_6.

3,6-[^{2}H]-2,4,5-Trimethoxybenzylcyanide (12)

The procedure employed was identical to that outlined in the synthesis of 4. A 0.109 g (55%) portion of the desired benzylcyanide 12 was isolated without loss of deuterium label.

NMR: 3.87, 9H, brd s, OCH_3; 3.63, 2H, s, CH_2CN.

3,6-[^{2}H]-2,4,5-Trimethoxyphenethylamine (13)

The catalytic reduction was carried out according to the procedure used to prepare 5. The colorless needles, 0.027 g (48%), were isolated as the hydrochloride salt.

NMR: 3.87, 3H, s, OCH_3; 3.83, 3H, s, OCH_3; 3.80, 3H, s, OCH_3; 3.05, 4H, m, $CH_2CH_2NH_2$; 1% TMS/$CDCl_3$/DMSO-d_6.

3,6-[^{2}H]-2,4,5-Trihydroxyphenethylamine (14)

The preparation of 14 followed the procedure used in the preparation of α,α-[^{2}H]-6-hydroxydopamine (6).

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