JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 389–394.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.677

Research Article

Synthesis of deuterium-labeled 3-*O*-methyldopa and 4-*O*-methyldopa

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Summary

Concise methods for the synthesis of 4-hydroxy-3-[$^2H_3$]-methoxyphenylalanine (3-*O*-[$^2H_3$]-methydopa) and 3-hydroxy-4-[$^2H_3$]-methoxyphenylalanine (4-*O*-[$^2H_3$]-methydopa) are described. The 3-*O*-[$^2H_3$]-methydopa is a valuable internal standard for the tandem MS quantification of 3-*O*-methyldopa, a metabolite of value in the diagnosis of aromatic L-amino acid decarboxylase (AADC) deficiency. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: 3-*O*-methyldopa; aromatic L-amino acid decarboxylase; tyrosine

Introduction

Aromatic L-amino acid decarboxylase (AADC) is the enzyme that converts 3,4-dihydroxyphenylalanine (L-dopa) to dopamine and 5-hydroxytryptophan to serotonin. Inherited deficiency of this enzyme leads to decreased levels of these two important neurotransmitters resulting in a severe early onset neurological disorder.[1] In the absence of AADC activity, L-dopa is methylated to 4-hydroxy-3-methoxyphenylalanine (3-*O*-methyldopa), which then accumulates in blood, urine and

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Received 13 August 2002
Revised 27 September 2002
Accepted 17 October 2002

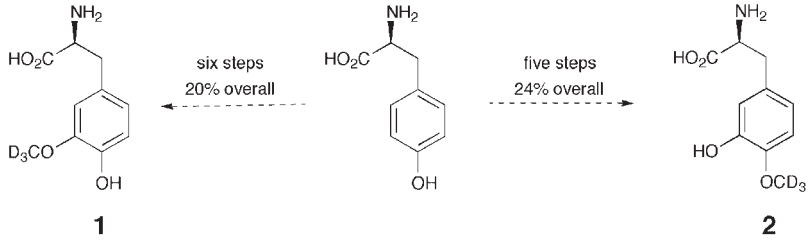
cerebrospinal fluid in infants and children with a deficiency of this enzyme.[2] 3-*O*-methyldopa therefore provides a biochemical marker that can be used to screen for this disease.

The analysis of multiple compounds eluted from dried blood spots collected from neonates using tandem mass spectrometry (MS) can be used to screen for over 30 inherited diseases.[3] The tandem MS analysis includes amino acids, and 3-*O*-methyldopa can be incorporated into the screening process to help identify patients with AADC deficiency. Accurate quantification of metabolites by tandem MS, however, requires incorporation of stable isotope internal standards for each of the compounds analyzed. We have therefore developed syntheses of stably labeled 3-*O*-[$^2H_3$]-methyldopa (**1**) and its isomer 4-*O*-[$^2H_3$]-methyldopa (**2**).

Initial attempts at the synthesis of these compounds involved various methods for the direct methylation of 3,4-dihydroxyphenylalanine (dopa). This 'simple' approach was problematic, and instead the target compounds were synthesized starting with the selectively protected dopa precursors **3** and **5**. These compounds are readily available from tyrosine by a sequence of reactions described by Jung and Lazarova involving a Reimer–Tiemann formylation and a Dakin oxidation.[4] Similar strategies for the synthesis of selectively protected dopa derivatives had previously been described by the Boger group,[5] and more recent modifications of this general strategy have also appeared in the literature.[6] We now report that 3-*O*-[$^2H_3$]-methyldopa (**1**) and its isomer 4-*O*-[$^2H_3$]-methyldopa (**2**) have been synthesized from tyrosine in overall yields of 20% (6 steps) and 24% (5 steps) (Scheme 1).

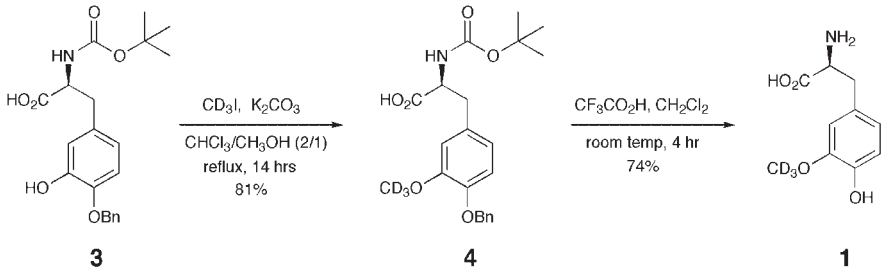
Discussion

The straightforward synthesis of labeled 3-*O*-methyldopa **1** began with the selectively protected dopa analogue **3** (Scheme 2), which is available



1 **2**

Scheme 1.



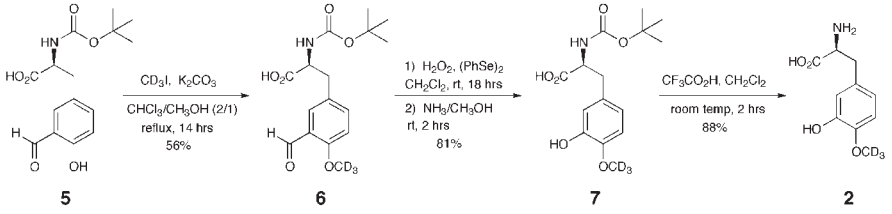
Scheme 2.

in 4 steps from tyrosine in 33% yield.[4] The deuterium label was incorporated by alkylation of the monoprotected catechol **3** with excess CD_3I, which was followed by removal of both protecting groups (*N*-*t*BOC and *O*-benzyl) by treatment with trifluoroacetic acid in CH_2Cl_2. Crude **1** was purified by preparative reverse phase TLC (water/methanol/sodium octylsulfonate 9:1:1 mM), and the ion-pairing reagent was subsequently removed by filtration through H^+-form Dowex-50 × 4 (NH_4OH eluant).

Preparation of the labeled 4-*O*-methyldopa **2** began with hydroxybenzaldehyde **5**, which can be synthesized in two steps from tyrosine (59% overall).[4] Introduction of the label was again accomplished by alkylation with excess CD_3I. Diphenyl diselenide-mediated oxidation of the alkylated benzaldehyde **6**, followed by aminolysis of the resulting formate ester, afforded the protected dopa derivative **7**, which was deprotected and purified in analogy with the 3-*O*-methyldopa derivative.

Conclusion

We have found that selectively protected l-dopa derivatives **3** and **5**, (Scheme 3) which are reliably available in good yield, are useful intermediates for the synthesis of deuterium-labeled methyldopa



Scheme 3.

derivatives. The deuterium label incorporation is accomplished by a phenol alkylation, which is performed using a large excess of deuterated methyl iodide in order to minimize reaction time and avoid racemization.

Experimental

*N-[(1,1- Dimethylethoxy)carbonyl 4-benzyloxy-3-[$^2H_3$]-methoxyphenyl-alanine (**4**)*

A suspension of anhydrous potassium carbonate (0.5 g, 3.6 mmol, 7 eq) in chloroform/methanol (2/1; 6 ml) was refluxed under N_2 for 15 min and then cooled. Compound **3** (0.20 g, 0.52 mmol, 1 eq) and CD_3I (1 ml, 4.5 mmol, 8.7 eq) were added and the mixture was refluxed under N_2 for an additional 14 h. The reaction mixture was diluted with water and ethyl acetate, and the aqueous layer was acidified to pH 1 with 1 N HCl, the layers separated, and the aqueous layer back extracted with ethyl acetate (3 $\times$ 100 ml). The combined organic extracts were washed with brine and water, dried over $MgSO_4$, and concentrated under vacuum. Flash column chromatography (75 g silica gel, 22:1 CH_2Cl_2/MeOH 1% acetic acid) afforded the desired product (0.17 g, 0.42 mmol, 81%) as a brown oil.

4-*hydroxy-3-[$^2H_3$]-methoxyphenylalanine (**1**)*

Compound **4** (0.2 g, 0.50 mmol, 1 eq) was dissolved in 2 ml of dichloromethane and 2 ml of triflouroacetic acid was carefully added. The reaction was stirred under N_2 for 4 h at room temperature. The reaction mixture was then concentrated under vacuum and the product dissolved in 1 ml water. Preparative reverse phase TLC (1 mM 1-octanesulfonic acid [sodium salt] in water/methanol [9/1], pH 2) afforded an oil that was dissolved in 10% acetic acid (3 ml), loaded on an ion exchange column (Dowex 50 $\times$ 4-400, acid form, 5 g) that was then washed extensively with water. Elution of the resin with concentrated ammonium hydroxide followed by concentration under vacuum afforded the desired product (0.08 g, 0.37 mmol, 74%) as a light brown power.

*N-[(1,1-dimethylethoxy) carbonyl] 3-formyl-4-[$^2H_3$]-methoxyphenyl-alanine (**6**)*

A suspension of anhydrous potassium carbonate (0.5 g, 3.6 mmol, 4.4 eq) in chloroform/methanol (2/1; 6 ml) was refluxed under N_2 for

15 min and then cooled. Compound **5** (0.25 g, 0.81 mmol, 1 eq) and CD_3I (1 ml, 4.5 mmol, 5.5 eq) were added and the mixture was refluxed under N_2 for an additional 14 hrs. The reaction mixture was diluted with water and ethyl acetate, and the aqueous layer was acidified to pH 1 with 1 N HCl, the layers separated, and the aqueous layer back extracted with ethyl acetate (3 × 50 ml). The combined organic extracts were washed with brine and water, dried over $MgSO_4$, and concentrated under vacuum. Flash column chromatography (75 g silica gel, 1% acetic acid in 22:1 CH_2Cl_2/MeOH) afforded the desired product (0.17 g, 0.45 mmol, 56%) as a brown oil.

*N-[(1,1-dimethylethoxy)carbonyl] 3-hydroxy-4-[$^2H_3$]-methoxyphenylalanine (***7***)*

Diphenyl diselenide (0.003 g, 0.01 mmol, 0.03 eq) and aqueous hydrogen peroxide (0.5 ml of a 35% aqueous solution, 4.6 mmol, ~15 eq) was added to a solution of compound **6** (0.1 g, 0.31 mmol, 1 eq) in dichloromethane (3 ml). The reaction mixture was stirred under N_2 at room temperature for 24 h and then diluted with water (5 ml) and ethyl acetate (20 ml). The layers were separated and the organic layer washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude residue was redissolved in 1 ml methanol, 3 ml of 2 M ammonia in methanol was added, and the reaction stirred at room temperature for 2 h. The mixture was then concentrated and diluted with water (5 ml) and ethyl acetate (10 ml). The layers were separated and then the aqueous layer was acidified to pH 1 with 1 N HCl and back extracted with ethyl acetate (3 × 25 ml). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated to afford 0.08 g (0.25 mmol, 81%) of reasonably pure **7**.

3-*hydroxy-4-[$^2H_3$]-methoxyphenylalanine (***2***)*

Compound **7** (0.1 g, 0.32 mmol, 1 eq) was dissolved in 2 ml of dichloromethane and then 2 ml of triflouroacetic acid was carefully added. The reaction was stirred for 2 h at room temperature and then concentrated under vacuum. Preparative reverse phase TLC (water/methanol [9/1] with 1 mM 1-octanesulfonic acid sodium salt, pH 2) afforded an oil that was dissolved in 10% acetic acid (3 ml), loaded on an ion exchange column (Dowex 50 × 4-400, acid form, 5 g) that was then washed extensively with water. Elution of the resin with concentrated ammonium hydroxide followed by concentration under

vacuum afforded the desired product (0.06 g, 0.28 mmol, 88%) as a light brown power.

Acknowledgements

This research was supported by a grant from the Robert A. Welch Foundation (Grant #AA-1355).

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7. All new compounds were thoroughly characterized. Complete experimental and characterization details (NMR spectra, mass spectra, and HPLC traces) are available from the authors upon request.

