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SYNTHESIS OF L-6-[^{123}I]IODO-*m*-TYROSINE A POTENTIAL SPECT BRAIN IMAGING AGENT

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Abstract

L-6-[^{123}I]Iodo-*m*-tyrosine was synthesized by the direct iodination of L-*m*-tyrosine with [^{123}I]-NaI and Chloramine-T. The product was purified by reverse phase HPLC to give the final product in 57% radiochemical yield with a radiochemical purity of greater than 97%.

Key Words: L-6-[^{123}I]iodo-*m*-tyrosine, dopamine metabolism, SPECT,

Introduction

L-6-[^{18}F]Fluorodopa (6-FD) is rapidly becoming one of the most useful imaging agents used in PET to study dopamine metabolism in vivo (1,2,3). However, the metabolic formation of 3-*O*-methyl-6-fluoro-L-dopa has complicated the biochemical interpretation of the PET data. Recently, workers at UCLA (4,5) and at the University of Chicago (6,7) have investigated the synthesis and use of fluorinated and brominated derivatives of *m*-tyrosine to study striatal dopaminergic function. It has been shown that 4-[^{18}F]-fluoro-L-*m*-tyrosine accumulates in the striatum in monkeys with a striatum/cerebellum ratio of 4 at 180 min (4,5) and that 6-[^{75}Br]-bromo-L-*m*-tyrosine selectively accumulated in the striatum with a ratio of approximately 3 after 30 min (6,7). It has also been shown that the fluorinated compound is not a substrate for catechol-*O*-methyl transferase but is a substrate for aromatic amino acid decarboxylase (5).

As part of our ongoing study to prepare ^{123}I-labelled SPECT brain imaging agents (8) we now report the synthesis and of L-6-[^{123}I]iodo-*m*-tyrosine.

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Experimental

General

D,L-*m*-Tyrosine was purchased from Sigma, L-*m*-Tyrosine was obtained by resolution of the D,L mixture by the action of α-chymotrypsin on their ethyl esters (9). Chloramine-T hydrate was purchased from Aldrich and no carrier added $Na^{123}I$ was obtained from Nordion International. All other chemicals were purchased commercially and were used with no further purification.

NMR spectroscopy was performed on a 300 MHz spectrometer, DCI mass spectroscopy was done at the B.C. Regional Mass Spectroscopy Center, UBC, Vancouver. Elemental Analyses were performed by Canadian Microanalytical Service, Ltd., Delta, B.C. All melting points were determined on a capillary oil bath instrument and are uncorrected. High Performance Liquid Chromatography (HPLC) was carried out on a Spectra Physics System using a 90:10 (0.02 M KOAc/MeOH or a 0.02 M KOAc/Ethanol) eluant adjusted to pH 3.9 with glacial Acetic acid with a flow rate of 3 ml/min for an analytical C-18 Waters RCM column (10 cm x 0.8 cm, 4 μm, 8NV C-18, λ 280 nm) and a flow rate of 4 ml/min for a semi-preparative C-18 Waters column (25 cm x 0.78 cm, P/N 84176, λ 280 nm).

Confirmation of the optical purity of L-*m*-Tyrosine and [^{123}I]-iodo-*m*-Tyrosine (6-IMT) was achieved using a chiral HPLC system as described previously (10) (eluant pH 3.65, flow rate 3 ml/min, λ 280 nm).

The direct radioiodination of L-*m*-Tyrosine was accomplished by a modification of the Chloramine-T method for iodinating proteins(11). 6-Bromo-D,L-*m*-Tyrosine was synthesized by the method described for the synthesis of 6-bromo-L-dopa (12). Attempts to iodinate L-*m*-Tyrosine by the exchange of Br for I failed. 6-BrMT was used together with *m*-Tyrosine as a reference for the assignment of ^{13}C nmr chemical shifts.

6-Iodo-D,L-*m*-Tyrosine

To a stirred solution of 0.5 g (2.76 mmol) of D,L-*m*-Tyrosine in 5N NH_4OH (150 ml) at room temperature was added dropwise a solution of 0.765 g (3.01 mmol) of I_2 in anhydrous ether (180 ml) aliquots of the aqueous layer were taken at certain intervals for HPLC analysis. After all the I_2 solution was added, the reaction mixture was stirred for 2 hr at room temperature and the ether layer was separated. The aqueous layer was evaporated to dryness under reduced pressure. The crude product

was dissolved in 1*N* HCl, treated with activated carbon, filtered through a sintered funnel to remove carbon and then through a Millipore membrane filter (Millex SR 0.5 μm).

An aliquot of the solution was analyzed by HPLC to show two structural isomers [analytical column, (90:10) 0.02M KOAc/CH_3OH, flow rate 3 mL/min, retention times: 10.5 min, (minor isomer) 12.08 min (main isomer) ratio 1:6 respectively; semi-preparative column, (90:10) 0.02 M KOAc/ethanol eluant, flow rate 4 mL/min, retention times 9.39 min and 11.22 min]. The pH of the rest of the solution was adjusted to 3.5 with saturated $NaHCO_3$ solution and cooled in the refrigerator. The solid precipitated was filtered and air dried. A second crop was obtained after concentrating the filtrate by rotoevaporation and adjusting the pH.

The combined crops were dissolved in 1N HCl, treated twice with activated carbon, as above, and the clear solution filtered through a membrane filter followed by pH adjustment to 3.5 with saturated $NaHCO_3$. The mixture was cooled overnight, the white solid filtered and dried on the pump. The yield of the structural isomer mixture was 0.525 g, (62%). Some of this product (30 mg dissolved in 1 mL H_2O/4N HCl) was purified by semi-preparative chromatography using a CH_3OH/H_2O (1:2) eluant at a flow rate of 3 mL/min. (4-5 injections of 200-300 μL each). Inversion of retention times with this eluant was obtained (main isomer 6.05 min, minor isomer 8.29 min). Both structural isomers were collected together since complete separation was difficult. After rotoevaporation of the eluant and drying under vacuum, a white powder (24 mg) was obtained; m.p. 217-218°, mass spec. calcd. 307; found: 308 (M+1). Anal. calcd. for $C_9H_{10}NO_3I.\frac{1}{2}H_2O$: C 34.20, H 3.51, N 4.43, I 40.15; found: C 34.21, H 3.40, N 4.42, I 39.33.

^{1}H-nmr, ^{13}C-nmr (Table 1), NOE difference, carbon-proton heterocorrelation map and comparison against 6-bromo-D,L-*m*-tyrosine confirmed the major product of this reaction being 6-Iodo-D,L-*m*-tyrosine. These nmr spectra were obtained for the 6:1 isomeric mixture. We were able, in the ^{13}C nmr spectrum, to distinguish between the main and secondary isomers but could not see the secondary isomer in the proton nmr spectrum. Chiral HPLC analysis showed the L- and D- enantiomers for the major isomer with retention times 2.34 min and 6.36 min respectively and for the minor isomer 1.5 min and 4.52 min respectively.

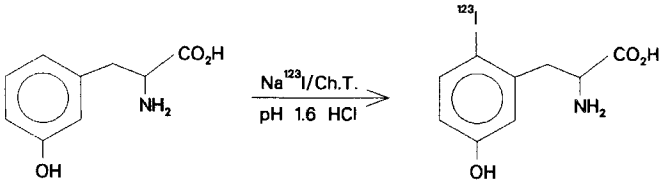
6-Bromo-D,L-*m*-Tyrosine

To a stirred solution of D,L-m-Tyrosine (0.89 g, 4.95 mmol) in glacial acetic acid (75 ml) plus a few drops of conc. HCl for dissolution was added dropwise a solution of Br_2 (0.79 g, 4.95 mmol) in glacial acetic acid (40 ml). The reaction was followed by HPLC [C-18 analytical column, (90:10) 0.02M KOAc/CH_3OH/glacial acetic acid pH 3.9, flow rate 3 mL/min, λ 280 nm, retention times: *m*-tyrosine 2 min, 6-bromo-*m*-tyrosine 6.2 min].

When the reaction was complete, the solvent was evaporated under reduced pressure (with successive additions of H_2O to help evaporate the residual acid). The oily-solid was suspended in H_2O and the solid-liquid mixture cooled overnight. The white solid was filtered off, washed with anhydrous ether and air dried.

The aqueous filtrate was concentrated under reduced pressure and cooled again to afford a second crop. This process was repeated twice. The collected samples were combined and purified by dissolving in H_2O/HCl, adjusting the pH to 3.5 with saturated $NaHCO_3$ and cooling to re-precipitate. Yield: 0.995 g (77% yield). Some of this product (32 mg dissolved in 1 mL H_2O/4N HCl) was purified by semipreparative chromatography using a methanol/water (1:2) eluant at a flow rate of 2 mL/min, detection at 280 nm by making 12-13 injections of 50-100 μL each and collecting the fraction at 7.4

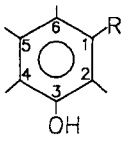
Synthesis of L-6-[^{123}I]Iodo-*m*-Tyrosine



L-*m*-Tyrosine L-6-[^{123}I]Iodo-*m*-Tyrosine

TABLE 1

^{1}H and ^{13}C-nmr chemical shifts (ppm)[a]



	^{1}H-nmr			^{13}C-nmr				
	Ar–H	–CH–	–CH_2–	–CO_2H–	Ar(–C–)	Ar(CH)	–CH[b]	–CH_2–
				main isomer				
IMT[c]	d 6.53 d 5.77 dd 5.50	m 3.30	dd 2.14 dd 2.02	171.2	155.7 C_3 136.1 C_1 83.8 C_6	140.3 C_5 123.7 C_4 116.5 C_2	54.1	35.5
				minor isomer				
				171.0	156.6 C_3 138.1 C_1 88.9 C_4	141.2 C_5 118.6 C_2 117.8 C_6	53.0	40.8
BrMT[c]	d 6.15 d 5.6 dd 5.48	m 3.15	dd 2.13 dd 1.9	171.2	155.6 C_3 134.7 C_5 114.6 C_4	134.5 C_1 119.0 C_2 117.4 C_6	52.9	36.5
MT[d,e]	t 6.97 m 6.55	m 4.05	dd 2.95 dd 2.81	171.6	156.4 C_3 136.1 C_1	131.2 C_5 122.1 C_6 116.8 C_2 115.5 C_4	54.4	35.9

[a] center of multiplet, [b] aliphatic CH, [c] D_2O/DCl, peaks of main isomer, [d] D,L isomer, [e] for comparative purposes, L–isomer

min. After evaporation of the collected eluant and drying under vacuum a white powder (21 mg) was obtained, m.p. 254-255°(dec.). Anal. Calc. for $C_9H_{10}NO_3Br.\frac{1}{3}H_2O$: C 40.63, H 4.04, N 5.27, Br 30.04; found: C 40.77, H 3.77, N 5.26, Br 31.07. M.S. calcd. 261; found: 262 [M+1, ($^{79}Br/^{81}Br$) 260/262 (1:1)].

^1H-nmr, ^{13}C-nmr (Table 1), NOE difference, and carbon-proton heterocorrelation map, identified the product as 6-bromo-*m*-tyrosine. The assignment of all aromatic ring carbon atoms are also shown in Table 1. Chiral HPLC analysis of the product was done as described above. The observed retention times for its enantiomers were 1.7 min (L) and 2.18 min (D).

6-[^{123}I]-Iodo-L-*m*-Tyrosine

To a 1 mL v-vial with teflon septum, screw cap and magnetic stirring bar containing L-*m*-tyrosine [0.5 mg/600μL, HCl (pH 1.6, approx. 0.05N)] was added 150 μL $Na^{123}I$ solution (36.1 mCi). After stirring for 2 minutes, a solution of freshly prepared Chloramine-T (75 μL, 10 mg/mL in borate buffer, pH 8.3) was added. After 10 minutes of stirring at room temperature, the reaction was quenched by the addition of a solution of $NaHSO_3$ containing $Na_2S_2O_5$ (100 μl, 40 mg/ml in borate buffer, pH 8.3).

The reaction mixture was purified by HPLC using a C-18 semi-preparative Waters column (25 cm x 0.78 cm) using 0.02M KOAc/EtOH (90:10), pH adjusted to 3.9 with glacial acetic acid as eluant, with a flow rate of 4 ml/min, and uv detection at 280 nm. The radioactive product with retention time 11.22 min corresponding to L-6-^{123}I-iodo-*m*-tyrosine (6-IMT) was collected, [retention time, L-*m*-tyrosine, 3.46 min, minor radioactive products; 5.05 (unincorporated $Na^{123}I$, 10%), 9.39 (4-IMT,<5%)and 15.3 min (<5%)]. The radiochemical yield of 6-IMT was 57% (decay corrected) and the radiochemical purity was greater than 97% (no other radioactive components were detected upon reinjection of the HPLC purified product). Since no carrier L-6-iodo-*m*-tyrosine was detected, the estimated specific activity was calculated to be greater than 30,000 Ci/mmol at EOS (based on limits of U.V. detection).

Results and Discussion

Initial attempts to synthesize 6-IMT by the exchange reaction on 6-bromo-*m*-tyrosine as accomplished in the case of 6-iododopa (8) were unsuccessful. The synthesis of 6-IMT was achieved by the direct

iodination of L-*m*-tyrosine with Na^{123}I/chloramine-T. This direct labelling in pH 7 phosphate buffer lead to two radioactive products with retention times corresponding to those for the 4- and 6-iodo isomers of the cold iodo-*m*-tyrosine. Regioselectivity was achieved by adjustment of the pH of the reaction mixture. At pH 1.6 (HCl soln.) the 6-isomer predominates while at pH 8.3 (borate buffer) the 4-iodo isomer is preferentially formed. The pure 6-isomer was obtained by semi-preparative HPLC and only this isomer was studied for its biodistribution in rats because it was expected that the 4-iodo isomer (ortho to the hydroxyl group) would be much less stable in-vivo. No deiodination of the 6-iodo product was detected after 24 h in saline as determined by HPLC. The retention time of the product on both reverse phase and chiral HPLC was identical to that of the cold standard.

The 4- and 6- ring isomers of iodo-*m*-tyrosine could not be distinguished by proton nmr alone since their nmr spectra are very similar to each other. Therefore, a combination of NOE, ^{13}C, and ^{1}H-^{13}C correlation map experiments were used to determine the structure of the product.

Animal studies on 6-IMT are currently underway and will be reported elsewhere.

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References

1. Calne D.B., Langston J.W., Martin W.R.W., Stoessl A.J., Ruth T.J., Adam M.J. and Pate B.D. *Nature*, 317:246(1985).

2. Garnett E.S., Firnau G. and Nahmias C. *Nature*, 305:137(1983).

3. Martin W.R.W., Adam M.J., Bergstrom W.A., Harrop R., Laihinen A., Rogers J., Ruth T.J., Sayre C., Stoessl J., Pate B.D., and Calne D.B. *Recent Developments in Parkinson's Disease, ed. S.Fahn*, Raven Press, New York, 1986.

4. Barrio J.R., Perlmutter M.M., Luxen A., Melega W.P., Grafton S.T., Huang S.C., Hoffman J.M., Van Moffaert G., Mazziotta J.C., and Phelps M.E. *J. Nucl. Med.*, 30:752(1989).

5. Melega W.P., Perlmutter M.M., Luxen A., Nissenson C.H.K., Grafton S.T., Huang S-C., Phelps M.E., and Barrio J.R. *J. Neurochem.*, 53:311(1989).

6. DeJesus O.T., Chen C-A., Mukherjee J., and Khalifah R.G. *J. Nucl. Med.*, 30:924(1989).

7. DeJesus O.T. and Mukherjee J. *Biochem. and Biophys. Res. Commun.*, 150:1027(1987).

8. Adam M.J., Zea Ponce Y., Berry J.M., Hoy K. *J. Lab. Compd. Radiopharm*, 28:155(1990).

9. Tong J.H., Petitclerc C., D'Iorio A., and Benoiton N.L. *Can. J. Biochem.*, 49:878 (1971).

10. Grierson J.R., and Adam M.J. *J. Chromatog.*, 325:103 (1985).

11. Hadi U.A.M., Malcolme-Lawes D.J., and Oldham G. *Int. J. Appl. Radiat. Isot.*, 29:621 (1978).

12. Wong M., and De Jesus O.T. *J. Lab. Compd. Radiopharm.*, 24:1373 (1987).