Syntheses of 5-fluoro-D/L-dopa and [^{18}F]5-fluoro-L-dopa

Mario Argentini, Claudia Wiese* and Regin Weinreich†
Institute of Medical Radiobiology, University of Zurich and Paul Scherrer-Institut, CH-5232 Villigen-PSI (Switzerland)

(Received May 28, 1993; accepted October 6, 1993)

Abstract

This paper describes the synthesis of 5-fluoro-D/L-dopa and the corresponding [^{18}F]5-fluoro-L-dopa starting from 5-nitrovanillin via malonic ester synthesis, the Balz–Schiemann reaction and the separation of the racemic mixture [^{18}F]5-fluoro-D/L-dopa with a chiral HPLC system. The inactive 5-fluoro-D/L-dopa was obtained in an eight-step synthesis with an overall yield of 10%. For a reliable synthesis, the nitro group was reduced with hydrazine hydrate and ruthenium on charcoal.

Introduction

Dopa (3,4-dihydroxyphenylalanine) is an intermediate product in the biological synthesis of catecholamines in animals. Catecholamines can function as neurotransmitters in the central nerve system (dopamine, norepinephrine) and in the peripheral nerve system (norepinephrine).

The radioactive [^{18}F]-labelled substitute is used *in vivo* for investigation of neurotransmission by positron emission tomography (PET) in humans [1]. It is known, however, that fluorine substitution changes the biochemical behaviour of dopa in some way, and that the 5-substituted form shows a different metabolic behaviour than 6-[^{18}F]fluoro-dopa. Both 5-[^{18}F]fluoro-dopa and 6-[^{18}F]fluoro-dopa can be used *in vivo* in connection with a drug-inhibiting COMT (catechol-O-methyl-transferase). In order to clarify this effect, the metabolism of 5- and 6-fluoro-dopa has been investigated in aggregating cell cultures [2, 3]. These experiments require large amounts of both isomers, and we have therefore developed an improved synthesis of 5- and 6-fluoro-dopa in the inactive form.

The synthesis of 5-fluoro-D/L-dopa used here is a revised and upgraded version of the synthesis developed by Firnau *et al.* in 1973 [4]. It has been extended to produce enantiomerically pure [^{18}F]5-fluoro-L-dopa for medical applications. The main improvements are the use of diazomethane for alkylation of a phenolic group, the reduction of the nitro group with hydrazine hydrate and ruthenium on charcoal, and the malonic ester synthesis using potassium t-butoxide as the strong base. 6-D/L-Fluoro-dopa was synthesized as described by Grierson and Adam [5] with improvement of some details, mostly taken from ref. 6.

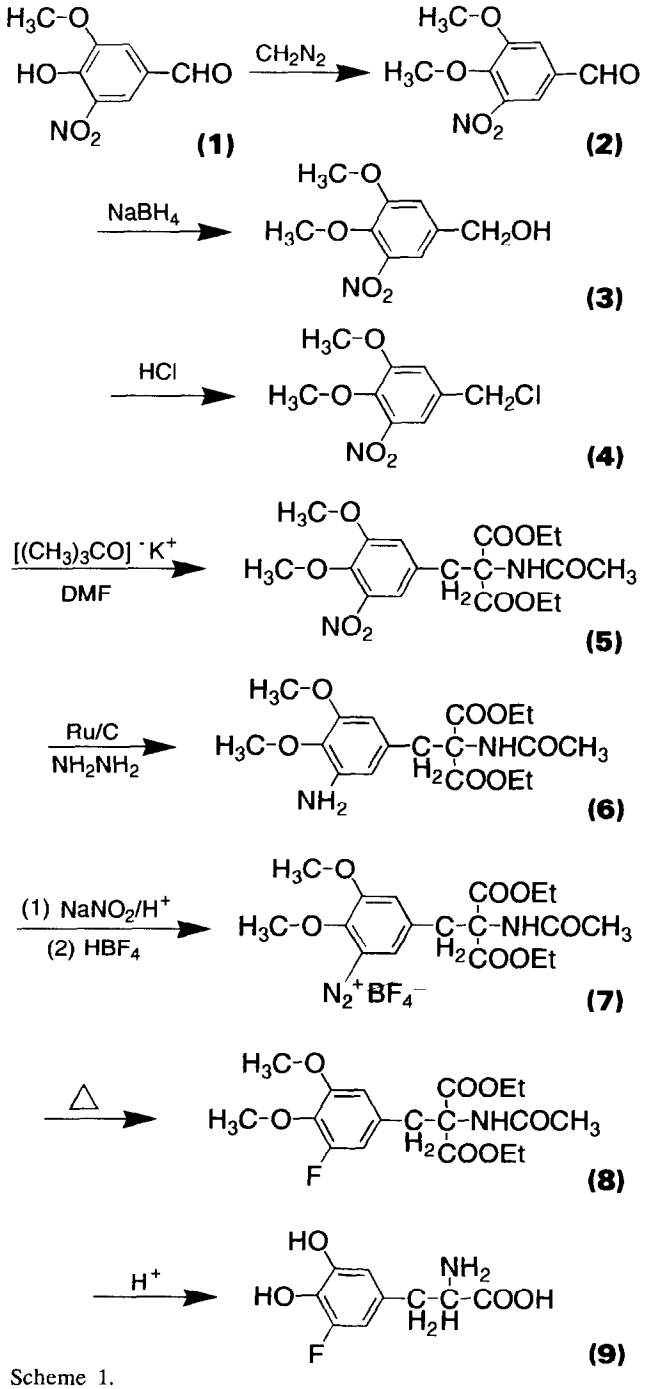
Results and discussion

The starting material was commercially available 5-nitrovanillin (4-hydroxy-3-methoxy-5-nitrobenzaldehyde (**1**). The crude material was purified via the corresponding potassium salt [7] to obtain a very pure substance characterized by a sharp melting point. The synthesis was then carried out according the following route.

The phenolic OH group at the 4-position of 5-nitrovanillin (**1**) was methylated with diazomethane under very mild reaction conditions (a drastic methylation process should be avoided, since 5-nitrovanillin tends to polymerize easily). The benzaldehyde **2** was reduced to the benzyl alcohol **3** with sodium borohydride. The corresponding benzyl chloride **4** was formed by boiling with concentrated hydrochloric acid. As the next step, the malonic ester synthesis to **5** was carried out with potassium t-butoxide in dimethylformamide [8]. The nitro group of this malonic ester derivative **5** was reduced to the corresponding amino compound **6** with hydrazine hydrate and ruthenium on charcoal, as the catalyst, in ethanol [9]. The catalyst must be used in sufficient amounts (in our case 20 wt.%) to avoid the formation of hydrazo derivatives which cannot be reduced further under these conditions [10]. The best results were achieved with ruthenium on charcoal from

*Present address: Laboratory of Viral and Molecular Pathogenesis, NINDS, National Institutes of Health, Bethesda, MD 20282, USA.
†Author to whom correspondence should be addressed.

SSDI 0022-1139(93)03032-H



Scheme 1.

Aldrich. Catalysts from other manufacturers gave the same reaction product **6**; however, less pure and in remarkably lower yield. As a free base, the amino derivative **6** tends to polymerize rather quickly. However, when converted into the corresponding hydrochloride, it is very stable over a long period at room temperature. The diazonium tetrafluoroborate **7** was then synthesized from **6** with fluoroboric acid and sodium nitrite. The diazonium tetrafluoroborate **7** was pyrolyzed to give the fluoro compound **8** via a Balz–Schiemann reaction in solution at temperatures above 120 °C [11]. Finally, the hydrolysis of the fluoro compound **8** to racemic 5-fluoro-D/L-dopa (**9**) was carried out with hydrobromic acid.

To produce [^{18}F]5-fluoro-L-dopa, the diazonium tetrafluoroborate **7** was allowed to exchange isotopically with ^{18}F as hydrofluoric acid in the target water immediately after cyclotron production of this nuclide via the nuclear reaction ^{18}O(p,n)^{18}F [12]. The degree of this isotopic exchange was less than 1.0. Pyrolysis of the labelled diazonium tetrafluoroborate **7** was carried out in xylene at 120 °C. Hydrolysis to [^{18}F]5-fluoro-D/L-dopa was performed in boiling hydrobromic acid. Separation of this racemic mixture was achieved via the chiral column CHIRALPAK WH (Daicel Chem. Comp.) with 0.05 M sodium dihydrogen phosphate as the eluent [13].

Experimental

Preparation of the starting compounds

The commercially available 5-nitrovanillin (**1**) was purified by dissolution in water with concentrated potassium hydroxide solution followed by precipitation by neutralization with hydrochloric acid [7]. All solvents were purified by distillation. Dimethylformamide was distilled *in vacuo*. The melting points reported are not corrected.

3,4-Dimethoxy-5-nitrobenzaldehyde (2)

To a suspension consisting of 25 g of 5-nitrovanillin (3-methoxy-4-hydroxy-5-nitrobenzaldehyde) in 500 ml of tetrahydrofuran was added an excess of diazomethane in diethyl ether with stirring at room temperature, until no starting material was detectable by TLC (SiO_2 and ethyl acetate/ethanol 7:3). The solution obtained was stirred for another hour at room temperature, then evaporated to dryness. The crude material was crystallized from 60% aqueous ethanol to give light yellow needles: 26 g, m.p. 88–89 °C, yield 95%.

3,4-Dimethoxy-5-nitrobenzyl alcohol (3)

To a solution consisting of 5 g of 3,4-dimethoxy-5-nitrobenzaldehyde in 10 ml of tetrahydrofuran was added 5 g of sodium borohydride, and the reaction was allowed to proceed for 1 h at 40–60 °C. After cooling, 30 ml of water was added and the reaction mixture allowed to stand overnight, then the solution was extracted in ether. The ether extracts were collected, dried over sodium sulphate and evaporated to dryness. The crude substance was crystallized from 60% aqueous ethanol to give pale yellow crystals: 4.54 g, m.p. 79–81 °C, yield 90%. Analysis: $C_9H_{11}NO_5$ requires: C, 50.70; H, 5.16; N, 6.57%. Found: C, 51.05; H, 5.00; N, 6.70%.

3,4-Dimethoxy-5-nitrobenzyl chloride (4)

3,4-Dimethoxy-5-nitrobenzyl alcohol (**3**) (5 g) was boiled with 50 ml of concentrated hydrochloric acid for 40 min. After cooling, the reaction mixture was diluted with 50 ml of water and extracted with 4×100 ml portions of benzene. The benzene extracts were collected, washed with 1 N sodium carbonate solution and dried over sodium sulphate. After evaporation to dryness, the remaining oil solidified in a refrigerator and was then recrystallized from 50% aqueous ethanol. Yellow needles of **4** were obtained: 5 g, m.p. 66–67 °C, yield 93%. Analysis: $C_9H_{10}ClNO_4$ requires: C, 46.75; H, 4.33; Cl, 15.15; N, 6.06%. Found: C, 46.51; H, 4.60; Cl, 15.20; N, 5.91%.

5-(2',2'-Dicarbethoxy-2'-acetamidoethyl)-2,3-dimethoxynitrobenzene (5)

In a 500 ml three-necked flask, equipped with a reflux condenser, thermometer, dropping funnel and magnetic stirrer, were placed 14 g (60 mmol) of diethylacetaminomalonate, 7.5 g (65 mmol) potassium t-butoxide and 100 ml of dry dimethylformamide. The mixture was heated to 120 °C in an oil bath and a solution consisting of 11.5 g (50 mmol) of 3,4-dimethoxy-5-nitrobenzyl chloride in 100 ml of dry dimethylformamide was added dropwise under stirring, at a rate such that the temperature remained constant at 120 °C. After the addition was completed, the reaction was continued for a further 2 h at 120 °C. After cooling to room temperature, the dimethylformamide was distilled under vacuum. The crude material was crystallized from 50% aqueous ethanol to give pale yellow crystals: 17.6 g, m.p. 128–130 °C, yield 85%. Analysis: $C_{18}H_{24}N_2O_9$ requires: C, 52.43; H, 5.82; N, 6.80%. Found: C, 52.15; H, 6.00; N, 6.92%.

5-(2',2'-Dicarbethoxy-2'-acetamidoethyl)-2,3-dimethoxyaniline hydrochloride (6)

5-(2',2'-Dicarbethoxy-2'-acetamidoethyl)-2,3-dimethoxynitrobenzene (5.5 g, 13 mmol) was reduced with 5 ml of 99% hydrazine hydrate and 1 g of 5% ruthenium on charcoal in 200 ml of 95% aqueous ethanol for 24 h under reflux conditions. The catalyst was then filtered and washed with ethanol. The ethanol solution was concentrated under vacuum to approx. 50 ml, diluted with 100 ml of water and extracted with 4×100 ml portions of ether. The ether extracts were dried over sodium sulphate and evaporated to dryness. The remaining pale yellow oil was dissolved in 100 ml of acetic acid and added to 10 ml of concentrated hydrochloric acid. After standing for 2 h at room temperature, the solution was evaporated under vacuum to dryness. The crude oil was dissolved in a very small amount of tetrahydrofuran and precipitated with diethyl ether. This purification was repeated twice to give white very hygroscopic crystals, which were dried carefully under vacuum: 3.9 g, yield 70%. Analysis: $C_{18}H_{27}ClN_2O_7$ requires: C, 51.67; H, 6.46; Cl, 8.37; N, 6.70%. Found: C, 51.53; H, 6.91; Cl, 9.12; N, 7.05%.

5-(2',2'-Dicarbethoxy-2'-acetamidoethyl)-2,3-dimethoxyphenyldiazonium tetrafluoroborate (7)

The solution of 1 g of 5-(2',2'-dicarbethoxy-2'-acetamidoethyl)-2,3-dimethoxyaniline hydrochloride in 8 ml of 0.5 N hydrochloric acid was cooled to −5 °C in an ice/salt bath. A solution of 0.2 g of sodium nitrite in 1 ml of water was added dropwise, keeping the temperature below 0–5 °C. After stirring for 10 min, 54% tetrafluoroboric acid in ether (1.5 ml) was added, and the reaction was continued for 30 min at −5 °C to 0 °C. The oily precipitate became solid on stirring, and was filtered and washed with ether. The diazonium tetrafluoroborate **7** was dissolved in acetone and precipitated with ether, filtered, washed with ether again and dried under vacuum. A yellow powder was obtained: 0.98 g, yield 85%. Analysis: $C_{18}H_{24}BF_4N_3O_7$ requires: F, 15.80%. Found: F, 16.07%.

5-(2',2'-Dicarbethoxy-2'-acetamidoethyl)-2,3-dimethoxyfluorobenzene (8)

5-(2',2'-Dicarbethoxy-2'-acetamidoethyl)-2,3-dimethoxyphenyldiazonium tetrafluoroborate (2 g) was pyrolyzed in 30 ml of xylene by heating for 2 h at the boiling point. After cooling, the xylene was distilled under vacuum. The remaining orange oil was dissolved in ethyl acetate and chromatographed on an SiO_2 column (length, 15 cm; i.d., 4 cm) and eluted with ethyl acetate. The first fraction was collected and evaporated to dryness. The crude product was crystallized from diisopropyl ether to give white crystals: 416 mg, m.p. 114–116 °C, yield 26%. Analysis: $C_{18}H_{24}FNO_7$ requires: C, 56.10; H, 6.23; F, 4.94; N, 3.64%. Found: C, 56.21; H, 6.02; F, 5.51; N, 3.73%.

5-Fluoro-D/L-dopa hydrobromide (9)

5-(2',2'-Dicarbethoxy-2'-acetamidoethyl)-2,3-dimethoxyfluorobenzene (**8**) (1 g) was hydrolyzed in 10 ml of 48% aqueous hydrobromic acid for 2 h under reflux conditions. After cooling, the reaction mixture was evaporated under vacuum to dryness. The residue was dissolved in 20 ml of water and evaporated again under vacuum. This crude product was carefully dried under vacuum, then crystallized from diisopropanol/diisopropylether to give white crystals: 655 mg, yield 85%. Analysis: $C_9H_{11}BrFNO_4$ requires: C, 36.49; H, 3.72; Br,

27.03; F, 6.42; N, 4.73%. Found: C, 36.40; H, 3.80; Br, 26.01; F, 7.41; N, 4.81%.

[^{18}F]5-Fluoro-L-dopa

To a solution of $H^{18}F$ in $D_2{}^{18}O$ (5 ml) after bombardment in the cyclotron and without any intermediate manipulation, 50 mg of the diazonium tetrafluoroborate 7 were added and isotope exchange was carried out for 20 min at 60 °C. The $D_2{}^{18}O$ was recovered by vacuum distillation. The residue was dissolved in 3 ml of xylene, the temperature raised to 120–125 °C and the solution left for 20 min to react. Xylene was removed under vacuum and the residue was hydrolyzed using 1 ml of 48% hydrobromic acid and 3 ml of water for 20 min at the boiling point, and then evaporated under vacuum. After cooling, the residue was dissolved in 2 ml of 0.05 M sodium dihydrogen phosphate buffer and chromatographed on a chiral column CHIRALPAK WH (length, 250 mm; i.d., 4.5 mm) eluting with the same phosphate buffer. The retention time for [^{18}F]5-fluoro-L-dopa at a flow rate of 1.5 ml min^{-1} was 17.4 min (D-enantiomer, 5.6 min).

The overall reaction time, including the HPLC separation, was *c.* 100 min. The overall labelling yield was 54%, time-corrected. The corresponding yield of [^{18}F]5-fluoro-L-dopa was 24% since the enantiomers were obtained nearly in the ratio of 1:1.

Acknowledgements

The authors are grateful to Dr A. Gut for many valuable suggestions and to Dr C. Hodgkinson, NIH, for critical comments on the manuscript.

References

1 D.J. Brooks and R.S.J. Frackowiak, *J. Neurol., Neurosurg., Psychiatry, Spec. Suppl.,* (1989) 68.
2 C. Wiese, M. Cogoli-Greuter, R. Weinreich and K.H. Winterhalter, *J. Neurochem., 58* (1992) 219.
3 C. Wiese, M. Cogoli-Greuter, M. Argentini, T. Mäder, R. Weinreich and K.H. Winterhalter, *Biochem. Pharmacol., 44* (1992) 99.
4 G. Firnau, C. Nahmias and S. Garnett, *J. Med. Chem., 16* (1973) 416.
5 J.R. Grierson and M.J. Adam, *J. Labelled Compd. Radiopharm., 23* (1986) 1019.
6 J. Kollonitsch, *Eur. Pat. 80 106 408.0*(1980); [*Chem. Abs., 95* (1981) 169 781].
7 E. Späth and H. Röder, *Monatsh. Chem., 43* (1922) 93.
8 C. Kaiser and A. Burger, *J. Am. Chem. Soc., 79* (1957) 4365.
9 S. Pietra, *Ann. Chim. (Rome), 52* (1962) 727.
10 S. Pietra, *Ann. Chim. (Rome), 47* (1957) 410.
11 R.A. Bartsch, H. Chen, N.F. Haddock and P.N. Juri, *J. Am. Chem. Soc., 98* (1976) 6753.
12 M. Vogt, I. Huszar, M. Argentini, H. Oehninger and R. Weinreich, *Appl. Radiat. Isotopes, 37* (1986) 448.
13 T. Mäder, M. Argentini, C. Wiese and R. Weinreich, *Fresenius J. Anal. Chem., 343* (1992) 324.