[^{18}F] FLUORINE LABELED ALIPHATIC AMINO ACIDS

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Summary

The synthesis of 4-[^{18}F] fluoroproline is described, but attempts to prepare 3-[^{18}F] fluoroalanine failed. In the synthetic pathway to this compound different routes are investigated to make a labeled precursor, *viz* [^{18}F] fluoromethyliodide, which, however, were unsuccessful.

Keywords: 4-[^{18}F] fluoroproline, 3-[^{18}F] fluoroalanine and [^{18}F] fluoromethyliodide

Introduction

[^{18}F] Fluorine chemistry has received a lot of attention. Regarding its long life time compared with other positron emitting nuclides and its high bond-strength with carbon ^{18}F is an attractive isotope for the labeling of radiopharmaceuticals [1]. However, the introduction of fluorine into organic compounds is not simple and often the yields are low while extreme reaction conditions are required. Then, special procedures must be developed for the preparation of [^{18}F] labeled compounds. Introduction of ^{18}F has been accomplished nucleophilically with reagents such as $Cs^{18}F$ [2], $K^{18}F$ [3], $Et_4N^{18}F$ [4-7], $Rb^{18}F$ [8], $Ag^{18}F$ [9] or with $^{18}F^{\ominus}$ in the Schiemann reaction [10] and the triazene reaction [11]. Electrophilic introduction was realized with [^{18}F] F_2 [12], [^{18}F] $FOOCCH_3$ [13] and [^{18}F] F_2Xe [14]. We became interested in the preparation of the ^{18}F-labeled aliphatic amino acids 4-[^{18}F] fluoroproline and 3-[^{18}F] fluoroalanine, because both compounds are known in their non-radioactive form [15,16]. In our present production method of ^{18}F (reactor irradiation of Li_2CO_3) we are limited

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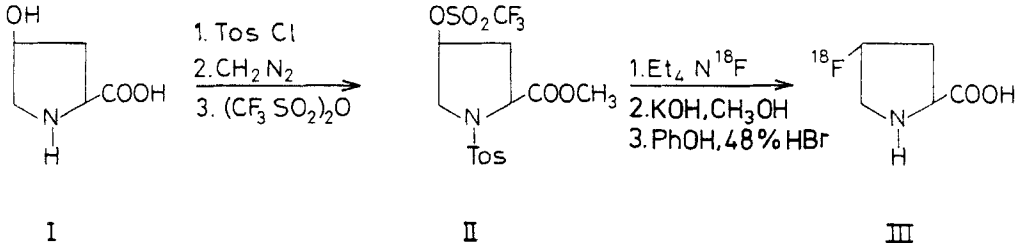
to its nucleophilic introduction. So a number of reaction paths to the fluorinated amino acids are excluded.
A promising pathway to the preparation of 4-[^{18}F]fluoroproline is given in Scheme A. Protection of the nitrogen with *p*-toluenesulfonylchloride followed by esterification

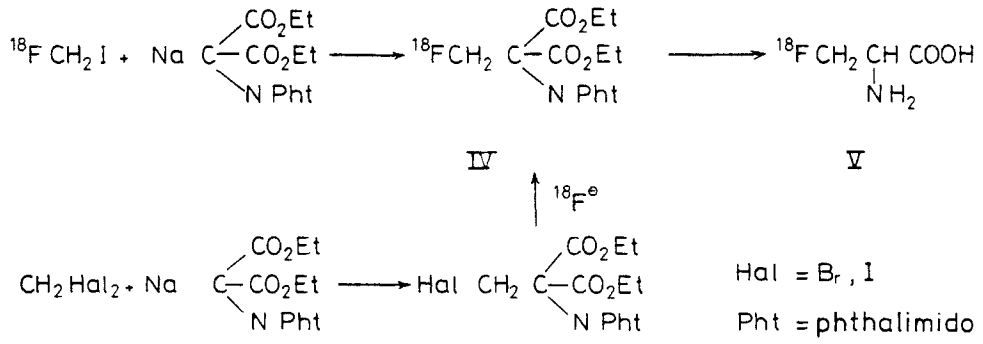
Scheme A



I II III

with diazomethane gives N-tosyl-4-hydroxy-L-proline methyl ester [17]. Treatment with trifluoromethanesulfonyl anhydride then gives triflate (II) [18]. Nucleophilic introduction of $^{18}F^{\ominus}$ with $Et_4N^{18}F$ and successive deprotection then leads to 4-[^{18}F]fluoroproline (III).
Two proposals for the synthesis of 3-[^{18}F]fluoroalanine are given in Scheme B. The first approach makes use of the unknown reagent [^{18}F]fluoromethyliodide. Alkylation with sodium diethyl phthalimidomalonate followed by deprotection should give 3-[^{18}F]fluoroalanine (V).

Scheme B



[^{18}F]CH_2I could possibly also be used for nucleophilic introduction of a [^{18}F]CH_2- moiety by means of Cu [19]. Therefore firstly attention was given to the preparation of this compound while in this way an interesting extension of the

^{18}F-labeling techniques might be realized.
In the second approach the halogen methylation step is followed by nucleophilic substitution of the halogen with $^{18}F^{\ominus}$.

Experimental

Tetraethyl ammoniumfluoride and diethylphthalimidomalonate refer to Fluka; trifluoromethanesulfonyl anhydride and 4-hydroxy-L-proline were obtained from Aldrich. Lithiumcarbonate refers to B.D.H. T.L.C.- and P.L.C.-plates were purchased from Merck (Si-gel 60 F-254). Distilled DMF was stored over mol sieves. ^1H NMR spectra were obtained with a Varian EM 360 spectrometer using $CDCl_3$ as solvent and tetramethylsilane as internal standard. Mass spectra were recorded on a Varian MAT 711 double focussing mass spectrometer with a combined EI/FI/FD ion source. Reactions with $[^{18}F]$ fluoride were carried out in 10 ml reacti-flasks sealed with teflon septa.
N-tosyl-4-hydroxy-L-proline methyl ester was prepared according to Fujita *et al.* [17].

N-tosyl-O-trifluoromethanesulfonyl-L-proline methyl ester was synthesized following the procedure of Tewson *et al.* [18] from N-tosyl-4-hydroxy -L-proline methyl ester and trifluoromethanesulfonyl anhydride in 33% yield (addition of the anhydride at - 78 °C). After the reaction was complete the product was purified by chromatography on Si-gel (Chloroform-ethylacetate 80-20). ^1H NMR ($CDCl_3$): δ 2.5 - 2.8 (m, 2H, CH_2); δ 2.6 (s, 3H, CH_3); δ 4.0-4.3 (m, 2H, CH_2); δ 4.1 (s,3H,$COOCH_3$); δ 4.6 (t, 1H,CH); δ 5.6-5.9 (m, 1H, CH); δ 8.1 (q, aromprot , 4H) ppm.Ms : m/e 431 (100%); m.p. 44 °C.

Diethyl bromomethylphthalimidomalonate
A solution of 0.92 g (3 mmol) diethyl phthalimidomalonate in 4 ml DMF was added dropwise to 110 mg of sodium hydride (3.7 mmol) dispersion in mineral oil.After stirring for 15 min this solution was added dropwise to a solution of 650 µl methylenebromide (5.6 mmol)

in 3 ml DMF at 80°. The reaction mixture was kept overnight at this temperature. Then water was added and this solution was extracted twice with ether. The organic layer was dried and concentrated *in vacuo*. Purification was accomplished by preparative chromatography on silica-gel (chloroform). ^{1}H NMR ($CDCl_3$): δ 1.4 (t, J 7Hz, 6 H, CH_3); δ 4.5 (s, 2H, $BrCH_2$); δ 4.6 (q, J, 7Hz, 4 H, $C\underline{H}_2CH_3$); δ 8.3 (s, 4H, arom prot) ppm. Ms: m/e 397, 399 (100%). An analytical sample was obtained after recrystallisation from petroleumether 60-80/chloroform at -30°, m.p. 83-84°. El. Anal. (calc.): C 48.22 (48.26); H 4.17 (4.05); N 3.43 (3.51); Br 19.97 (20.06)%.
The corresponding iodomethyl compound was prepared and purified in an analogous way, m.p. 99 °C (yield 33%). ^{1}H NMR ($CDCl_3$): δ 1.4 (t, J 7Hz, 6 H, CH_3); δ 4.4 (s, 2H, CH_2I), δ 4.6 (q, J 7Hz, 4H, $C\underline{H}_2CH_3$); δ 8.3 (s, 4H, aromprot) ppm. Ms: m/e 445 (100%). El. Anal. (calc): C 43.12 (43.17); H 3.70 (3.63); N 3.02 (3.14); I 28.50 (28.48)%.

Preparation of $Et_4N^{18}F$

$[^{18}F]$ fluoride was produced by irradiation of 25 mg of Li_2CO_3 in quartz ampoules for 20 min in the nuclear reactor of ECN at Petten (thermal neutron flux: 5.10^{13} n $cm^{-2}s^{-1}$). Following the irradiation, ion exchange technique (Dowex 50W-X4) was employed for the preparation and the details were described by the Kleyn *et al.* [4].

4-$[^{18}F]$ fluoroproline

A solution of 25.5 mg (59 μMol) of triflate (II) and 20 mg of $Et_4N^{18}F$ (108 μMol) in 1 ml acetone was stirred at 40° for 10 min. Thin layer chromatographic analysis (silicagel, chloroform - ethylacetate 70 - 30) of the protected fluoroproline-derivative showed that 55% of the total activity was retained with a Rf-value of 0.78 and the radiochemical yield ranged from 38 to 80%. The Rf-value corresponded to that of an authentic sample prepared in a non-radioactive synthesis.

When the solvent system was changed from chloroform-ethylacetate to toluene-ether 1-2, the Rf-value decreased to 0.56 but the same yield was obtained. The rest of the activity remained at the origin. The reaction mixture was concentrated *in vacuo* followed by the addition of 50 µl 5N KOH and 1 ml of methanol. After refluxing for 30 min the solvent was removed under reduced pressure and the residue partitioned between 3 ml of 2 N HCl and 3 ml of ethylacetate. N-protected acid was obtained by concentrating the organic layer *in vacuo*. Then 20 mg of phenol and 300 µl of 48% HBr were added and the reaction mixture was refluxed at 110 °C for 50 min. After addition of 1 ml of water and 1 ml of ethylacetate, the aqueous layer was separated and passed through a column of Dowex 1-X8 (acetate form). 4-[^{18}F]fluoroproline was obtained with a radiochemical yield of 34% and a specific activity of 28 K Bq/mg. About 8% of the activity in the aqueous phase remained on the column. The product was identified by paper chromatography and the Rf-value in the solvent system 1-butanol-acetic acid-pyridine-water 4-1-1-2 was determined. In agreement with the literature[15], this value was 0.2. The radiochemical purity was better than 95%. A mass spectrum of the recrystallized (ethanol) sample showed a base peak on m/e 88 (M-COOH).
In the attempted synthesis of 3-[^{18}F] fluoroalanine normal literature procedures were followed.

Results and discussion

Gottlieb *et al.* [15] synthesized trans-4-fluoroproline *via* the tosyloxy-L-proline by nucleophilic displacement with fluoride ion. However, this method is not applicable in this case, because of the seven equivalents potassium fluoride used and the long reaction time. Therefore the triflate group was chosen as leaving group [20].
Triflate (II) was prepared in reasonable yield from N-tosyl-4-hydroxy-L-proline methyl ester. Highest yields were obtained when the sulfonylanhydride was added at -78 °C and triflate (II) was purified directly after its formation. It had to be stored at - 30 °C. Attempts to prepare in an analogous way, the corresponding N-benzyloxycarbonyl triflate were, however, not successful.

As shown in Scheme A the crucial step to the [^{18}F] fluorinated proline forms the reaction between this triflate and $Et_4N^{18}F$. Gatley and Shaughnessy [6] described an analogous reaction for the synthesis of [^{18}F]3-deoxy-3-fluoro-D-glucose while recently Levy *et al.* [2b] reported about the preparation of [^{18}F]-2-deoxy-2-fluoro-D-glucose starting from the protected triflate. These reactions were carried out in DMF at high temperature. When acetone was used as the solvent [^{18}F]-fluorination was effected in 55% yield. This yield ranged from 38% to 80%. Deprotection of the acid group was accomplished with 5 N KOH in refluxing methanol. Without purification the N-tosyl group was removed, successively in 48% HBr in the presence of phenol [25].

For the preparation of 3-[^{18}F] fluoroalanine attention was given to the synthesis of a possible precursor [^{18}F]-fluoromethyl iodide. Two general modes of preparation are found in the literature: the Hunsdiecker type reaction of silver fluoroacetate with iodine [21] and the reaction between HgF_2 and methylene iodide [22,23]. However, the first route mentioned could not be repeated under the reaction conditions given, while the second route is inattractive because of its low chemical yield and from the radiochemical point of view.

Efforts to introduce fluoride into methylene iodide by means of Et_4NF, KF (crown ether) or AgF failed, while nucleophilic substitution of iodide in iodomethyl triphenylphosphonium iodide ($ICH_2PPh_3^{\oplus}I^{\ominus}$) with Et_4NF did not give the desired fluoro-derivative, but the corresponding phosphorane (see also refs. 23, 24). Interestingly Gatley [26] recently reported about the reaction between [^{18}F]-fluoride and methylene iodide; the only identificable compound appeared to be [^{18}F]-fluoromethane.

The second approach to the 3-[^{18}F] labeled fluoroalanine involves alkylation of sodium diethylphthalimidomalonate with methylene bromide (or iodide) followed by nucleophilic displacement of halogen with fluoride (Scheme B).

The compounds (VI) (a = Br, b = I) were readily prepared from the starting materials and completely analyzed. However, substitution of the halogen by means of Et_4NF, KF, HgF_2 or AgF did not result in the desired compound (IV). Replacement of the halogen by the trifluoromethanesulphonate group with the help of silver trifluoromethanesulphonate also did not succeed [20]. So both proposed routes to 3-[18F] fluoroalanine failed. However, 3-fluoroalanine has been prepared by using CF_3OF [16b]. Another possible pathway to the synthesis of the labeled analogue may be through the use of [18F]-$F_3CO^{18}F$ [27].

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