SYNTHESIS OF 20-[^{18}F]FLUOROARACHIDONIC ACID: A POTENTIAL PHOSPHOLIPID METABOLIC AGENT

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SUMMARY

A fluorine-18 labelled analog of arachidonic acid, 20-[^{18}F]fluoroarachidonic acid, was prepared for evaluation as a potential phospholipid metabolic agent. The radiosynthesis was accomplished by [^{18}F]fluoride ion displacement on the tosylate precursor followed by basic hydrolysis of the ester group, in isolated radiochemical yield (not decay corrected) of 10 % in overall synthesis time (including HPLC purification) of 110 min.

Key words: 20-[^{18}F]fluoroarachidonic acid, [^{18}F]fluoride ion, tosylate displacement, ^{18}F-labeling

INTRODUCTION

Arachidonic acid (all cis-eicosa-5,8,11,14-tetraenoic acid) is an important constituent of membrane phospholipids in brain tissue which can be liberated through several enzymatic pathways. Recently it has been shown that [1-^{14}C] arachidonic acid is a useful fatty acid for monitoring the dynamics of regional brain phospholipid metabolism by autoradiographic and biochemical analysis[1]. This procedure is based on the biochemical observation that intravenously injected [1-^{14}C] arachidonate is rapidly cleared from rat plasma and radiolabels mainly the sn-2 position of phosphatidyl choline and phosphatidyl inositol in brain membranes[2]. The extention of this method to PET requires the synthesis of a radiotracer labelled with a positron emitting isotope. Recently, arachidonic acid has been labelled with carbon-11 at the carboxylic carbon, and preliminary experiments using rhesus monkey suggest that polyunsaturated fatty acids such as arachidonic acid hold strong potential for imaging brain phospholipid turnover in humans[3].

Due to the short half-life of carbon-11 (20.4 min), the development of a fluorine-18 ($T_{1/2}$ 110 min) labelled arachidonic acid that behaves in the same manner as the parent molecule is more attractive because ^{18}F-analog radiotracers would extend the time available for uptake and PET imaging, although the necessity for a longer uptake tissue has not been investigated. In addition, this

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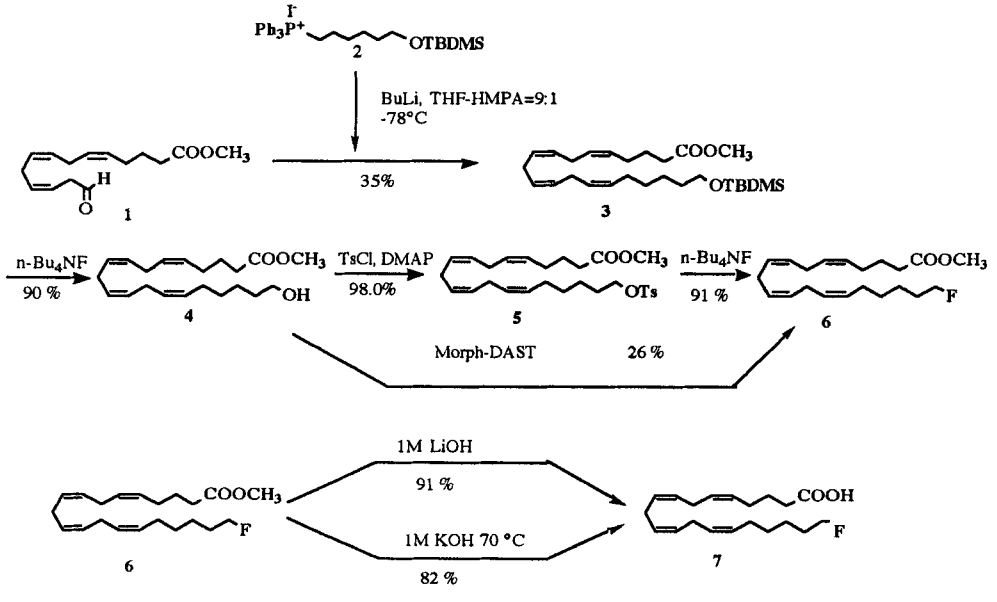
may provide a more convenient radiosynthesis, analysis and allow time to transport the radiopharmaceutical to sites remote from a cyclotron facility.

Our investigation has been directed toward the development of [^{18}F]-labelled analogs of arachidonic acid as a longer-lived alternative to the ^{11}C-labelled form. Several monofluorinated and difluorinated analogs of arachidonic acid have been described in the literature[4]. In the present work, we have chosen to study, aliphatic fluorination at the ω-position of arachidonic acid, based upon the relative ease of synthesis. We also expect this analog to show minimal influence on uptake and metabolic behavior, compared to arachidonic acid. This paper describes the preparation of 20-[^{18}F]fluoroarachidonic acid using [^{18}F]fluoride ion displacement of a tosylate precursor.

RESULTS AND DISCUSSION

The synthetic route used to prepare 20-fluoroarachidonic acid (**7**) is showed in Scheme 1. Of the various procedures available for the preparation of polyunsaturated fatty acids[5], the construction of the polyenic skeleton via Wittig condensation of the two synthons shown, seemed most convenient, as this would allow for introduction of fluorine-18 via tosylate displacement at the end of synthesis. Thus, our strategy is convergent with the key step being Wittig coupling of the known ω-oxo ester (**1**) derived from arachidonic acid[6] with the phosphonium salt (**2**). The phosphonium salt was obtained from *t*-butyldimethylsilyl (TBDMS) protected 6-chlorohexanol according to a literature procedure[7].

Generation of the ylide from **2** and condensation with the ω-oxo ester (**1**) at low temperature in THF-HMPA (9:1) furnished the TBDMS protected methyl ester (**3**) in moderate yield (35 %).



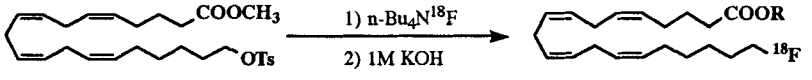
Scheme 1

Deprotection of the TBDMS group of **3** with n-butylammonium fluoride gave the ω-hydroxy ester (**4**). The alcohol (**4**) thus obtained was converted to tosylate (**5**) and displacement of the tosylate by n-butylammonium fluoride yielded the desired methyl 20-fluoroarachidonate (**6**) in 91% yield. This fluoro compound (**6**) was also obtained by direct fluorination of **4** with Morph-DAST but only in moderate yield. Finally, the hydrolysis of fluoro methyl ester (**6**) with either LiOH[8] (24 hr, room temperature) or KOH (15 min, 80°C) led to fluoro acid (**7**) in a good yield. The structure was confirmed by ^{1}H-NMR analysis. The stereoisomeric purity was evaluated by ^{13}C-NMR spectroscopy. The spectrum of **7** contained all the resonances expected for the arachidonic skeleton, including a signal at δ 84.14 due to the CH_2F group. In addition, signals of very low intensity in the region 127~130 ppm (olefinic carbon) were observed, indicating that **7** was contaminated with ca 5 % of the corresponding trans-isomer. This degree of isomeric purity is tolerable for the biological evaluation in animals. Chromatographic separation using TLC and HPLC could not be achieved. In conclusion, we have been able to prepare 20-fluoroarachidonic acid (**7**) with reasonable stereoisomeric and chemical purity.

The radiosynthesis was achieved by [^{18}F]fluoride ion displacement on the tosylate precursor in the same manner as described above (Scheme 2). Various conditions were explored for this exchange reaction. Radiolabelling using K^+/Kryptofix 2.2.2 as a supported cation for the [^{18}F]fluoride only allowed for a low incorporaion. The use of tetra-n-butyl ammonium hydroxide as the base was more successful. Optimal reaction conditions were found to be a 20 min reaction time at 90°C in acetonitrile, using the tosylate and n-$Bu_4N^{18}F$. The yields (isolated by reverse phase HPLC) for the [^{18}F]fluoride were typically 20~30 %. Base hydrolysis of the radiolabelled intermediate was achieved in the following manner. The crude fluoroester was quickly purified through Sep-Pak(silica gel) and hydrolyzed in 1M KOH solution at 80°C for 15 min. Final purification by reverse phase HPLC afforded 20-[^{18}F]fluoroarachidonic acid (**9**) in 10 % overall radiochemical yield (not corrected for decay) (Scheme 2). Total synthetic time including HPLC purification was 110 min. The radiochemical purity was greater than 98 % and the product showed identical behavior to the non-radioactive compound on HPLC and TLC.

During the purification by HPLC, mass was detected by refractive index co-eluting with the desired radioactive product, and this small contaminant could not be removed completely from the product. Assuming that chemical impurity has similar extinction coefficient at 215 nm as 20-fluoroarachidonic acid, the specific activity of 20-[^{18}F]fluoroarachidonic acid obtained was in the range of 20~30 Ci/mmol at the end-of-synthesis, starting from 3~4 mCi of the [^{18}F]fluoride. We also assume that the isomeric purity is identical to that determined for the product from non-radioactive synthesis.

In summary, we have synthesized 20-[^{18}F]fluoroarachidonic acid in reasonable yield by [^{18}F]fluoride ion displacement of a tosylate precursor followed by base hydrolysis. The compound exhibited high radiochemical purity. This synthetic approach is general enough to allow for the



5

8: R=Me
9: R=H

Scheme 2

preparation of ω-^{18}F-labelled eicosapentaenoic acid as well as other functionalized arachidonic acid analogs. The biological evaluation of 20-[^{18}F]fluoroarachidonic acid as an analog of [1-^{11}C] arachidonic acid will be reported elsewhere.

EXPERIMENTAL

The ^{1}H NMR spectra were measured in $CDCl_3$ using a JEOL GX-270 (270MHz) spectrometer and Varian Unity plus 400 (400MHz) spectrometer. The ^{13}C NMR spectra were measured in $CDCl_3$ using a Varian Unity plus 400. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane. Low resolution field desorption mass spectra were obtained on a JEOL JMS-D300 spectrometer. High resolution fast atom bombardment mass spectra (HRFABMS) were taken on a JEOL NMS-SX 102 spectrometer. Ultraviolet spectra were obtained on a Hitachi 220A spectrometer. Infrared (IR) spectra were taken on a JASCO IR Report-100 spectrometer. Analytical thin-layer chromatography (TLC) was carried out on a TLC plate (Kieselgel 60 F_{254}, 0.2 mm, Merck) and visualization was achieved by heating after spraying with a 5% ethanol solution of sodium phosphomolybdate. Column chromatography was undertaken using Fuji devision BW-200. High performance liquid chromatography (HPLC) was done using a Waters model M-45 fitted with a YMC-Pack AQ 324 S-5 ODS (10 x 250 mm) with monitoring the radioactivity as well as the refractive index. The radioactivity was also quantified with a Capintec radioisotope calibrator (CRC-30). The identification of radiolabelled compounds was supported by HPLC co-injection studies.

Fluorine-18 was produced from 8 or 16% enriched [^{18}O]H_2O by the ^{18}O(p,n)^{18}F reaction. Tetrabutylammonium [^{18}F]fluoride was prepared by the addition of 1M tetrabutylammonium hydroxide in methanol solution (4.5 μl) to the irradiated water in a TPX (polymethylpentene) vessel and subsequent removal of the water under a stream of argon at 110 °C by co-evaporation with dry acetonitrile. Radiochemical yields were expressed at the end-of-synthesis (not corrected for decay) relative to the amount of the [^{18}F]fluorinating agent measured as total radioactivity present in the reaction vessel.

Methyl 20-(tert-butyldimethylsilyloxy)-5,8,11,14-eicosatetraenoate **3**

The phosphonium salt (**2**) was prepared from *t*-butyldimethylsilyl (TBDMS) protected 6-chlorohexanol by displacement with sodium iodide in refluxing acetone for 8 hr, followed by treatment with triphenylphosphine/potassium carbonate in refluxing acetonitrile for 10 hr[7]. To a solution of the phosphonium salt (**2**) in anhydrous THF was added dropwise n-butyllithium (1.56 M-hexane solution) under argon at -78°C. The temperature was gradually raised from -78°C to -40°C. HMPA (0.28 ml) was then added to the mixture, which was again cooled to -78°C. A solution of the aldehyde (**1**) obtained according to a modified literature[6] in THF (1.5 ml) was added dropwise. The mixture thus obtained was allowed to warm from -78°C to 0°C over 2 hr and a solution of 50% ammonium acetate (5 ml) was added. The mixture was extracted with ethyl acetate (10 ml x 3). The combined extracts were dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using hexane:ethyl acetate=4:1 to give **3** (8.9 mg, 35 %) as a pale yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 5.43-5.30 (m, 8H), 3.67 (s, 3H),

3.60 (t, J=6.6 Hz, 2H), 2.86-2.78 (m, 6H), 2.32 (t, J=7.6 Hz 2H), 2.27-2.03 (m, 4H), 1.76-1.66 (m, 2H), 1.50-1.21 (m, 6H), 0.89 (s, 9H), 0.05 (s, 6H). IR (neat) 3000, 2925, 2850, 1740 cm^{-1}. FDMS m/z: 448(M^+), 433(M^+-Me), 391(M^+-*tert*-Bu).

Methyl 20-hydroxy-5,8,11,14-eicosatetraenoate **4**

To a solution of the silyl ether (**3**) (80 mg, 0.179 mmol) in anhydrous THF (5 ml) was added n-tetrabutylammonium fluoride (1M-THF solution, 0.36 ml) under argon. The mixture was stirred at room temperature for 3 hr, and diluted with ether (30 ml), washed with water (5 ml) and brine (5 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel using hexane:ethyl acetate=4:1 to give the alcohol (**4**) (54 mg, 90 %) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 5.45-5.30 (m, 8H), 3.67 (s, 3H), 3.64 (t, J=5.9 Hz, 2H), 2.86-2.79 (m, 6H), 2.33 (t, J=7.6 Hz, 2H), 2.15-1.68 (m, 2H), 1.60-1.36 (m, 6H), 1.27 (br, 1H). IR (neat) 3400, 3000, 2925, 2850, 1740 cm^{-1} FDMS m/z: 334(M^+).

Methyl 20-(p-toluenesulfonyloxy)-5,8,11,14-eicosatetraenoate **5**

To a solution of the alcohol **3** (10 mg, 0.03 mmol) in dry methylene chloride (1 ml) was added 4-dimethylaminopyridine (11 mg, 0.09 mmol), p-toluenesulfonyl chloride (12 mg, 0.06 mmol) under argon at 0°C. The reaction mixture was stirred at room temperature for 6 hr, diluted with ether (10 ml), washed with water (5 ml) and brine (5 ml). The organic layer was dried over anhydrous sodium sulfate, evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using hexane:ethyl acetate=4:1 to give the tosylate (**5**) (14.4 mg, 98%) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.79 (d, J=8.3 Hz, 2H), 7.34 (d, J=8.3 Hz, 2H), 5.24-5.29 (m, 8H), 4.02 (t, J=6.4 Hz, 2H), 3.67 (s, 3H), 2.84-2.76 (m, 6H), 2.45 (s, 3H), 2.32 (t, J=7.6 Hz, 2H), 2.15-2.01 (m, 4H), 1.76-1.62 (m, 4H), 1.36-1.26 (m, 4H). IR (neat) 3000, 2925, 2850, 1730, 1360, 1180 cm^{-1}. HRFABMS m/z :489.2675 calcd. for $C_{28}H_{41}O_5S$. Found:489.2688.

Methyl 20-fluoroarachidonate **6**

(A) To a solution of the alcohol **4** (6 mg, 0.018 mmol) in dry methylene chloride (0.5 ml) was added Morph-DAST (6.3 mg, 0.036 mmol) under argon at -78°C. The reaction mixture was allowed to warm from -78°C to 0°C over a period of 3 hr, then quenched by the addition of aqueous saturated Na_2CO_3 (5 ml), and extracted with methylene chloride (10 ml x 3). The combined organic layer was dried over anhydrous sodium sulfate, evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using hexane:ethyl acetate=4:1 to give fluoro arachidonic acid methylester (**7**) (1.6 mg, 26 %) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 5.45-5.32 (m, 8H), 4.44 (dt, J=47.2, 6.2 Hz, 2H), 3.67 (s, 3H), 2.86-2.81 (m, 6H), 2.33 (t, J=7.6 Hz, 2H), 2.15-2.07 (m, 4H), 1.76-1.62 (m, 4H), 1.44-1.39 (m, 4H). IR (neat) 3000, 2925, 2850, 1740 cm^{-1}. HRFABMS m/z : 337.2544 calcd. for $C_{21}H_{34}O_2F$. Found:337.2524.

(B) A mixture of n-tetrabutylammonium fluoride (1M-THF solution, 0.22 ml, 0.22 mmol) and the tosylate (**5**) (26.9 mg, 0.056 mmol) was stirred under argon at room temperature for 2 hr, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using hexane:ethyl acetate=4:1 to give fluoro arachidonic acid methylester **6** (17 mg, 91%) as a pale yellow oil.

20-Fluoroarachidonic acid **7**

(A) A mixture of the ester (**6**) (5 mg, 0.024 mmol) in THF:water=5:1 (0.4 ml) and 1M aqueous LiOH (48 μl) was stirred at room temperature for 24 hr. The solution was acidified with 1M aqueous oxalic acid and the THF was removed under reduced pressure. The residue was diluted with water (5 ml) and extracted with ethyl acetate (10 ml x 3). The organic layer was dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using hexane:ethyl acetate=3:1 to give **7** (4.6 mg, 96%) as a colorless oil. ^{13}C NMR (400MHz, $CDCl_3$) δ: 178.79, 129.97, 129.05, 128.44, 128.20, 128.16, 127.96, 84.14, 33.20, 30.33, 29.23, 27.10, 26.46, 25.63, 24.84, 24.51. ^1H NMR (270 MHz, $CDCl_3$) δ: 5.47-5.31 (m, 8H), 4.44 (dt, J=45.7, 6.2 Hz, 2H), 2.86-2.76 (m, 6H), 2.37 (t, J=7.4 Hz, 2H), 2.17-2.07 (m, 4H), 1.77-1.60 (m, 4H), 1.47-1.35 (m, 4H). IR (neat) 3400-2450, 1700 cm^{-1}. HRFABMS m/z: 323.2386. calcd. for $C_{20}H_{32}O_2F$. Found: 323.2378.

(B) To a solution of the ester (**6**) (8 mg, 0.0238 mmol) in methanol (500 μl) was added 1M aqueous KOH (500 μl), and the mixture was stirred at 70 °C for 10 min. The reaction mixture was run over resin column (1.2 x 4.0 cm, Dowex 50-X8), eluted with methanol, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using hexane:ethyl acetate=3:1 to give pure **7** (6.3 mg, 82%) as a pale yellow oil.

Methyl 20-[^{18}F]fluoroarachidonate **8**

The tosylate (**5**) (1.5 mg) dissolved in dry acetonitrile (300 μl) was added to a TPX vessel containing the n-$Bu_4N^{18}F$ (3-4 mCi). The vessel was closed and heated in a oil bath at 80°C for 20 min, and the mixture was run over silica gel Sep-Pack (Waters). The Sep-Pak was washed with 15 ml of chloroform:methanol=9:1 and the solvent was removed under reduced pressure. The crude mixture was purified by reversed phase HPLC (RCM 8x10 C18, eluent: CH_3CN:H_2O=7:3, flow rate: 3 ml/min)to give radiochemically pure methyl 20-[^{18}F]fluoroarachidonate (**8**) (t_R=16 min) in 20~30 % radiochemical yields (total synthetic time: 90 min).

20-[^{18}F]Fluoroarachidonic acid **9**

Following the reaction of **5** with n-$Bu_4N^{18}F$ (3-4 mCi) as described above, the fraction of crude methyl 20-[^{18}F]fluoroarachidonate obtained through the Sep-Pak procedure was evaporated, and to the residue was added MeOH (30 μl) and 1M aqueous KOH (30 μl). The reaction mixture was heated at 80°C for 15 min. After cooling to room temperature, the mixture was passed through resin column (1.2 x 4.0 cm, Dowex 50-X8), and eluted with MeOH (10 ml). The solvent was removed under reduced pressure and the crude product was purified by reversed phase HPLC (YMC-Pack ODS, 10 x 250 mm, CH_3CN:H_2O:CH_3COOH=90:10:0.1, flow rate: 3 ml/min). The radioactive fraction eluting at t_R=11.5 min corresponding to that of authentic 20-fluoroarachidonic acid (**7**) was corrected. Total synthetic time was 110 min. The radiochemical yields (not corrected for decay) were around 10%. No radiochemical contaminations were detected by HPLC. The specific activity of the product was estimated by u.v. spectroscopy to be in the range of 20~30 Ci/mmol at the end-of-synthesis.

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