CHAPTER 3

SYNTHESIS OF [^{18}F]FLUOROMETHYLCHOLINE ([^{18}F]FCH) VIA [^{18}F]FLUOROMETHYL TOSYLATE

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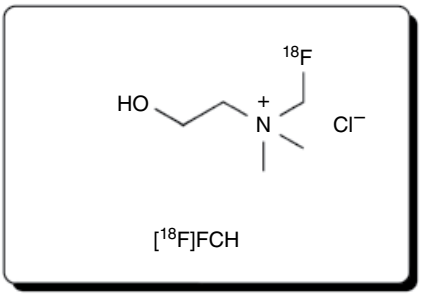

1 INTRODUCTION

Prostate cancer therapy ranges from curative therapy for localized prostate cancer to life-prolonging treatment and palliation for in the case of disseminated prostate cancer [1]. Accurate staging of prostate cancer is essential to in selecting the most appropriate treatment for a given patient. This has created an urgent need for accurate and noninvasive methods for staging of prostate cancer [2], and [^{18}F]fluoromethylcholine-PET is one such method. Primary prostate cancer cells, along with its metastases, are known to upregulate choline kinase, which, in turn, leads to an elevated uptake of choline for the biosynthesis of phospholipids. Intracellular phosphorylation traps choline inside the cell, and therefore, PET imaging with [^{18}F]fluoromethylcholine can readily detect this trapping, and differentiate prostate cancer cells from neighboring nonmalignant tissue [3–5].

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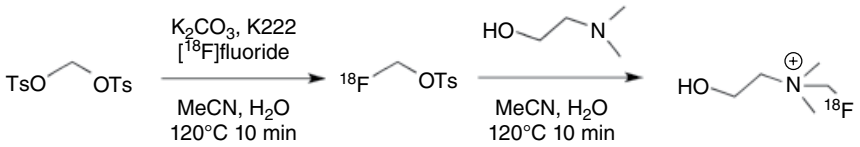

FIGURE 1 Synthesis of [18F]fluoromethylcholine.

Historically, [18F]fluoromethylcholine has been prepared by fluoromethylation of dimethylaminoethanol (DMAE) with [18F]fluorobromomethane [3–10]. However, we have only obtained low yields of product with this method in our laboratory because of compatibility issues between gaseous [18F]fluorobromomethane and the liquid phase synthesis modules we employ for most routine radiosyntheses of fluorine-18-labeled radiotracers. We therefore desired a liquid-phase method of preparing [18F]fluoromethylcholine that is complementary to the gas-phase method reported in Volume 1 of this series [7], so that radiochemists can select the method that is most compatible with their own synthesis equipment. We recently developed a fully automated one-pot liquid-phase synthesis of [18F]fluoromethylcholine (Fig. 1) via *in situ* formation of [18F]fluoromethyl tosylate [11] and present an expanded discussion of the synthesis herein.

2 SYNTHESIS PROCEDURES

CAUTION: All radiochemical syntheses must be carried out using appropriate equipment in a facility authorized for the use of radioactive materials. Personal protective equipment must be worn, and all local radiation safety laws followed.

2.1 Production of [18F]Fluoride

[18O]H_2O (2 ml) [12] was loaded into the [18F]fluoride target [13] of a General Electric Medical Systems (GEMS) PETtrace cyclotron [14]. The target was bombarded (60 μA beam for 30 min) to generate approximately 1.5 Ci (55.5 GBq) of [18F]fluoride by the $^{18}O(p,n)^{18}F$ nuclear reaction.

2.2 Azeotropic Drying of [18F]Fluoride

The [18F]fluoride was delivered to a GEMS TRACERlab FX_{FN} synthesis module [14] as a solution in [18O]H_2O (2 ml). This solution was passed through a Sep-Pak® QMA-Light cartridge [15] to trap the [18F]fluoride and recycle the [18O]H_2O. The [18F]fluoride was then eluted into the TRACERlab FX_{FN} glassy carbon reaction vessel using a solution of aqueous potassium carbonate (3.5 mg in 0.5 ml H_2O) [16]. A solution of Kryptofix 222 (5 mg in 1 ml MeCN) [17] was added and the reaction mixture was azeotropically dried, initially at 80°C under vacuum for 4 min and subsequently at 60°C with both vacuum and argon flow for an additional 4 min.

2.3 Synthesis of [^{18}F]Fluoromethylcholine

A solution of methylene ditosylate (7–8 mg) [18] in anhydrous MeCN (750 µl) and sterile water (10 µl) was added to the dried [^{18}F]fluoride, and the reaction was heated to 120°C with stirring for 10 min. Subsequently, the reaction mixture was cooled to 50°C, followed by the addition of DMAE (40 µl in 350 µl MeCN), which was heated to 120°C with stirring for an additional 10 min. The reaction mixture was then cooled to 60°C and underwent evaporation of the reaction solvent by maintaining 60°C and subjecting the reaction to both a continuous argon stream and vacuum draw for 5 min.

2.4 Purification and Formulation of [^{18}F]Fluoromethylcholine

Sterile water (5.5 ml) was added to the dried reaction mixture and passed through the Sep-Pak C18 Plus [19] into the round bottom flask containing ethanol (10 ml) to trap unreacted ditosylmethane and [^{18}F]fluoromethyl tosylate, as well as any tosylmethylcholine generated as a by-product. This was repeated with an additional aliquot of sterile water (5.5 ml). The water/ethanol mixture was transferred through the Sep-Pak CM-Light [20] to trap the desired [^{18}F]fluoromethylcholine. The Sep-Pak CM-Light was washed with ethanol (15 ml) to remove unreacted DMAE and water (20 ml) to remove residual ethanol to waste. Subsequently, [^{18}F]fluoromethylcholine was eluted off into a collection vial containing 0.9% sodium chloride for injection, USP (7 ml) with 0.9% sodium chloride for injection, USP (3 ml). The final formulation (10 ml) was then passed through a 0.22 µm sterile filter [21] into a sterile dose vial [22] to provide doses of [^{18}F] fluoromethylcholine as an isotonic solution submitted for quality control (QC) testing.

3 QUALITY CONTROL

CAUTION: All radiopharmaceuticals produced for clinical use must have local regulatory approval before use in humans. Trained personnel must carry out QC procedures, and each dose must meet all established QC criteria before release to the clinic.

The QC of radiopharmaceuticals prepared at the University of Michigan is carried out in accordance with the US Pharmacopeia (USP) [23], which is summarized in the following text. QC data for three repeat batches of [^{18}F]fluoromethylcholine produced using the method disclosed herein are summarized in Table 1. Each of the three doses met all of the established QC criteria.

3.1 Visual Inspection

Doses were examined visually and had to be clear, colorless, and free of particulate matter.

TABLE 1 Quality Control Data for [18F]FCH

QC Test	Release Criteria	Run 1	Run 2	Run 3
Yield/mCi (GBq)	N/A	102 (3.8)	81 (3.0)	86 (3.2)
Visual inspection	Clear, colorless	Clear, colorless	Clear, colorless	Clear, colorless
Radiochemical identity	RRT = 0.9–1.1	1.06	1.03	1.03
FCH concentration	No limit established	0.10 μg/ml	0.10 μg/ml	0.07 μg/ml
Radiochemical purity	≥95%	100	99.9	99.2
Specific activity	No limit established	15.5 Ci/μmol	13.2 Ci/μmol	19.7 Ci/μmol
Residual solvent analysis	DMAE <20 ppm	Pass	Pass	Pass
	Ethanol <5000 ppm	Pass	Pass	Pass
	Acetonitrile <410 ppm	Pass	Pass	Pass
	Acetone <5000 ppm	Pass	Pass	Pass
Dose pH	4.5–7.5	5.0	5.0	5.0
Residual Kryptofix 222	≤50 μg/ml	≤50 μg/ml	≤50 μg/ml	≤50 μg/ml
Sterile filter integrity test	>50 psi	>50 psi	>50 psi	>50 psi
Radionuclidic identity ($t_{1/2}$)	105–115 min	115	106	110
Endotoxin analysis	≤17.5 EU/ml	≤2 EU/ml	≤2 EU/ml	≤2 EU/ml
Sterility testing	No colony growth out to 14 days	Pass	Pass	Pass

3.2 Dose pH

The pH of the doses was analyzed by applying a small amount of the dose to colorpHast pH 2.0–9.0 nonbleeding pH-indicator strips [24] and determined by visual comparison with the scale provided.

3.3 Radionuclidic Identity

Activities were measured using a Capintec CRC®-15R Radioisotope Dose Calibrator [25], and half-life was calculated using Equation 1. Calculated half-life must be 105–115 min:

$$T_{1/2} = -\ln 2\left(\frac{\text{time difference}}{\ln(\text{ending activity/starting activity})}\right) \quad (1)$$

3.4 Chemical and Radiochemical Purity

The radiochemical purity (RCP) and concentration of fluoromethylcholine and DMAE in each batch were determined using a Shimadzu HPLC system with the following components: SCL-10Avp system controller, DGU-14A in-line degassing unit, LC-10ADvp pump, CDD-10Avp conductivity detector with temperature-controlled cell, CTO-20A oven [26], and Bioscan FC3300 flow count radioactivity detector [27]. The system was not equipped with an ion suppressor: Column, Waters SCX column, IC-Pak™ Cation M/D, 3.9 × 150 mm, pn WAT036570; mobile phase, 5 mM HCl (Fisher Scientific); flow rate, 1.25 ml/min; RT ~ 6.5 min; a representative HPLC trace is shown in Fig. 2. RCP must be >95%, and residual DMAE must be <20 μg/ml.

3.5 Radiochemical Identity

Using the HPLC system described in Section 3.4, the retention time of [^{18}F]fluorocholine is compared to that of the [^{19}F]fluorocholine-unlabeled reference standard [28] and must be ±10% (relative retention time (RRT) must be 0.9–1.1):

$$\mathrm{RRT} = \frac{t_r\ ([^{18}\mathrm{F}]\mathrm{FCH})}{t_r\ ([^{19}\mathrm{F}]\mathrm{FCH})}$$

3.6 Residual Kryptofix 222

Residual Kryptofix 222 (K222) levels in [^{18}F]FCH doses were analyzed using the established spot test [29]. Strips of plastic-backed silica gel TLC plates saturated with iodoplatinate reagent were spotted with water (negative control), 50 mg/ml Kryptofix 222 standard (positive control), and [^{18}F]FCH dose. If K222 was present in a sample, a blue-black spot appeared. Spots for the three samples were compared and a visual determination of residual K222 in the dose was made. Less than 50 μg/ml is acceptable, and all doses of fluorocholine prepared in this study were found to contain residual K222 below this level.

3.7 Sterile Filter Integrity Test

Sterile filters from doses (with needle still attached) were connected to a nitrogen supply via a regulator. The needle was then submerged in water and the nitrogen pressure gradually increased. If the pressure was raised above the filter acceptance pressure without seeing a stream of bubbles, the filter was considered intact.

3.8 Bacterial Endotoxins

Endotoxin content in radiopharmaceutical doses was analyzed using a Charles River Laboratories Endosafe® Portable Testing System [30] and according to the USP [31]. Doses must contain <175 endotoxin units.

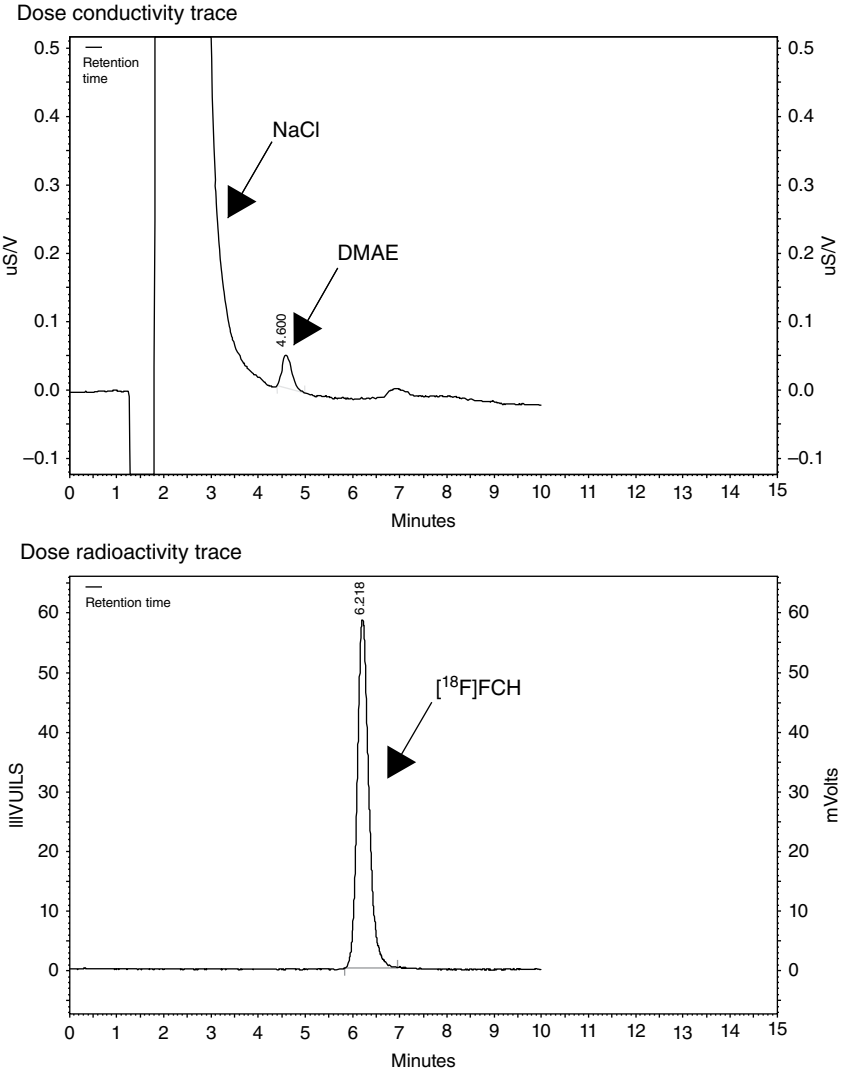

FIGURE 2 Analytical HPLC tracers for [18F]fluoromethylcholine.

3.9 Sterility

Culture tubes of fluid thioglycolate media (FTM) and soybean-casein digest agar media (SCDM) [32] were inoculated with samples of [18F]FCH doses and incubated (along with positive and negative controls) for 14 days. FTM tubes were incubated at 32°C and SCDM tubes were incubated at 22°C according to current USP guidelines [33]. FTM is used to test for anaerobes, aerobes, and microaerophiles, while SCDM is used to test for nonfastidious and fastidious microorganisms. Culture tubes were visually inspected on the 3rd, 8th, and

14th days of the test period and compared with the positive and negative standards. Positive standards must show growth (turbidity) in the tubes, and dose/negative controls must have no culture growth after 14 days to be indicative of sterility.

WASTE DISPOSAL INFORMATION

All hazardous chemicals and toxic materials were disposed of according to Prudent Practices in the Laboratory (Washington, D.C.: National Academy Press, 1995).

CHEMICAL ABSTRACTS NOMENCLATURE (REGISTRY NUMBER)

Acetonitrile (75-05-8)
Carbonic acid, potassium salt (1:2) (584-08-7)
2-Dimethylaminoethanol (108-01-0)
Ethanaminium, *N*-(fluoromethyl)-2-hydroxy-*N,N*-dimethyl-, chloride (459424-38-5)
Ethanol (64-17-5)
4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (23978-09-8)
Hydrochloric acid (7647-01-0)
Methanol, 1-(fluoro-^{18}F)-,1-(4-methylbenzenesulfonate) (113426-16-7)
Methylene ditosylate (24124-59-2)

REFERENCES AND NOTES

For detailed supplier information, see Appendix 1.

1. M. H. Poulsen, K. Bouchelouche, P. F. Høilund-Carlsen, H. Petersen, O. Gerke, S. I. Steffansen, N. Marcussen, N. Svolgaard, W. Vach, U. Geertsen, S. Walter, *BJU Int*, 2012, 110, 1666.
2. A. Heidenreich, J. Bellmunt, M. Bolla, S. Joniau, M. Mason, V. Matveev, N. Mottet, H. P. Schmid, T. van der Kwast, T. Wiegel, F. Zattoni, *Eur Urol*, 2011, 59, 61.
3. T. R. DeGrado, S. W. Baldwin, S. Wang, M. D. Orr, R. P. Liao, H. S. Friedman, R. Reiman, D. T. Price, R. E. Coleman, *J Nucl Med*, 2001, 42, 1805–1814.
4. T. R. DeGrado, R. E. Coleman, S. W. Baldwin, D. T. Price, M. D. Orr, S. Wang. ^{18}F-Labeled Choline Analogs, WO 2001/82864 A2, 2001. 74 pp.
5. T. R. DeGrado, R. E. Coleman, S. Wang, S. W. Baldwin, M. D. Orr, C. N. Robertson, T. J. Polascik, D. T. Price, *Cancer Res*, 2001, 61, 110.
6. D. Kryza, V. Tadino, M. A. Filannino, G. Villeret, L. Lemoucheux, *Nucl Med Biol*, 2008, 35, 255.
7. D. Kryza, Synthesis of [^{18}F]fluorocholine ([^{18}F]FCH), in *Radiochemical Syntheses Volume 1: Radiopharmaceuticals for Positron Emission Tomography* P. J. H. Scott and B. G. Hockley, editors, John Wiley and Sons, Hoboken, NJ, 2012.
8. M. Nader, A. Hoepping, *Nuklearmedizin*, 2005, 44 (A192), 7.
9. A. Sperandeo, A. Cistaro, N. Paligoric, D. Busetta, R. Mangiapane, U. Ficola, *J Nucl Med*, 2009*,* 50 (Suppl. 2), 28.
10. X. Shao, B. G. Hockley, R. Hoareau, P. L. Schnau, P. J. H. Scott, *Appl Rad Isotop* 2011, 69, 403.
11. M. E. Rodnick, A. F. Brooks, B. G. Hockley, B. D. Henderson, P. J. H. Scott, *Appl Rad Isotop*, 2013*,* 78, 26.
12. Virgin [^{18}O]H_2O purchased from ABX or Rotem and used as received.
13. GEMS silver high-yield [^{18}F]fluoride target.

14. GE Healthcare, United States.
15. Sep-Pak® QMA-Light cartridges were purchased from Waters (part no. WAT023525) and conditioned with 10 ml ethanol, 10 ml water, 10 ml 0.5 M sodium bicarbonate, and a further 10 ml water prior to use.
16. Potassium carbonate purchased from Aldrich (part no. 209619) and used as received. Sterile water purchased from Hospira (part no. 0409-4887-50) and used as received.
17. Kryptofix 222 was purchased from Acros (part no. 29195-0010) and used as received. Anhydrous acetonitrile was purchased from Acros (part no. 61096-1000) and used as received.
18. Ditosylmethane can either be purchased from ABX advanced biochemicals (part no. 6177.0010) or prepared in-house as follows: silver *p*-toluenesulfonate (0.52 g, 1.87 mmol) was suspended in MeCN (4 ml) in an oven-dried flask. To this suspension, diiodomethane (0.06 ml, 0.75 mmol) was added dropwise, and the reaction was stirred at reflux for 16 h. After this time, the reaction was cooled to room temperature and passed through a sintered glass frit to remove the silver salt side product. The filtrate was collected and concentrated under vacuum, and the resulting residue was purified by flash chromatography (eluting with hexane/ethyl acetate, 4:1) to give 0.27 g (83% yield) of ditosylmethane as a crystalline white solid: R_f 0.3 (4:1, hexane/ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58 (d, J 1⁄4 8, 4H), 7.38 (d, J 1⁄4 8, 4H), 5.90 (s, 2H), 2.40 (s, 6H); MS (ESI): m/z 379 $(M+Na)^+$.
19. Sep-Pak C18 Plus was purchased from Waters (part no. WAT020515).
20. Sep-Pak CM-Light was purchased from Waters (part no. WAT023531).
21. Millex-GV sterile 0.22 μm filters were purchased from Millipore (part no. SLGV013SL) and used as received.
22. 10 ml sterile vials were purchased from Hollister-Stier (part no. 7515ZA) and vented with a sterile Millex-FG vent filter (part no. SLFG025LS).
23. US Pharmacopeia <823>. Radiopharmaceuticals for positron emission tomography-compounding. US Patent 32–NF 27. 2009.
24. EMD Chemicals, Inc., United States (part no. 9578-3).
25. Capintec, Inc., United States.
26. Shimadzu Corporation, United States.
27. Bioscan, Inc., United States.
28. Unlabeled reference standard was purchased from ABX advanced biochemicals (part no. 6130).
29. B. H. Mock, W. Winkle, M. T. Vavrek, *Nucl Med Biol*, 1997, 24, 193.
30. Charles River Laboratories, USA.
31. US Pharmacopeia <85>. Bacterial endotoxins test. US Patent 32–NF 27. 2009.
32. Becton, Dickinson and Company, USA.
33. US Pharmacopeia <71>. Sterility tests. US Patent 32–NF 27. 2009.