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A Fully-automated One-pot Synthesis of [¹⁸F]Fluoromethylcholine with Reduced Dimethylaminoethanol Contamination via [¹⁸F]Fluoromethyl Tosylate

Melissa E. Rodnick¹, Allen F. Brooks¹, Brian G. Hockley¹, Bradford D. Henderson¹, and Peter J. H. Scott^{1,2}

¹Division of Nuclear Medicine, Department of Radiology, The University of Michigan Medical School, Ann Arbor, MI 48109, USA

²The Interdepartmental Program in Medicinal Chemistry, The University of Michigan, Ann Arbor, MI 48109, USA

Abstract

Introduction—A novel one-pot method for preparing [¹⁸F]fluoromethylcholine ([¹⁸F]FCH) via *in situ* generation of [¹⁸F]fluoromethyl tosylate ([¹⁸F]FCH₂OTs), and subsequent [¹⁸F]fluoromethylation of dimethylaminoethanol (DMAE), has been developed.

Methods—[¹⁸F]FCH was prepared using a GE TRACERlab FX_{FN}, although the method should be readily adaptable to any other fluorine-18 synthesis module. Initially ditosylmethane was fluorinated to generate [¹⁸F]FCH₂OTs. DMAE was then added and the reaction was heated at 120°C for 10 min to generate [¹⁸F]FCH. After this time, reaction solvent was evaporated, and the crude reaction mixture was purified by solid-phase extraction using C18-Plus and CM-Light Sep-Pak cartridges to provide [¹⁸F]FCH formulated in USP saline. The formulated product was passed through a 0.22 μm filter into a sterile dose vial, and submitted for quality control testing. Total synthesis time was 1.25 hours from end-of-bombardment.

Results—Typical non-decay-corrected yields of [¹⁸F]FCH prepared using this method were 91 mCi (7% non-decay corrected based upon ~1.3 Ci [¹⁸F]fluoride), and doses passed all other quality control (QC) tests.

Conclusion—A one-pot liquid-phase synthesis of [¹⁸F]FCH has been developed. Doses contain extremely low levels of residual DMAE (31.6 μg / 10 mL dose or ~3 ppm) and passed all other requisite QC testing, confirming their suitability for use in clinical imaging studies.

Keywords

[¹⁸F]fluoromethylcholine; prostate cancer imaging; positron emission tomography; fluorine-18

1. Introduction

Therapeutic strategies for patients with prostate cancer are dependent upon what stage the disease is diagnosed at, and range from curative therapy for patients with localized prostate cancer, to life prolonging treatment and palliation for patients with disseminated prostate

cancer (Poulsen et al., 2012). Accurate staging of prostate cancer is therefore critical for the choice of treatment, course of the disease, and patient prognosis. The current gold standard for staging prostate cancer is pelvic lymph node dissection (LND) (Heidenreich et al., 2010). However this method is invasive and metastases outside the region of the LND can still be missed (Poulsen et al., 2012; Weckermann et al., 2007). Reflecting this, it is estimated that 30–40% of patients treated with curative therapy relapse (Poulsen et al., 2012), and it is estimated that half of these relapses are due to metastatic disease (Bott, 2004), mainly caused by metastases missed during primary staging of the cancer (Giovacchini et al., 2010).

This has created an urgent need for accurate and non-invasive methods for staging of prostate cancer, and PET-CT, which offers the synergistic combination of anatomical imaging from the CT scan, and functional data from the corresponding PET scan, is one such method. PET-CT is enticing because it promises more accurate staging of the disease in a non-invasive fashion (Bouchelouche et al., 2010). One approach utilizes radioactive choline, and related analogs. Primary prostate cancer cells, along with its metastases, are known to up-regulate 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 radiolabeled choline can readily detect this trapping, and differentiate prostate cancer cells from neighboring non-malignant tissue (DeGrado et al., 2001a; DeGrado et al., 2001c). Imaging has been accomplished using both [^{11}C]choline and [^{18}F]fluoromethylcholine ([^{18}F]FCH). [^{11}C]Choline is attractive as it is a radiolabeled version of endogenous choline, which greatly simplifies pharmacology and toxicology testing requirements to translate into clinical use. The main drawback is that the short half-life of carbon-11 (20 min) restricts [^{11}C]choline-PET to those PET imaging suites afforded the luxury of an on-site cyclotron capable of producing carbon-11, and scanning a single patient per batch of radiopharmaceutical. To address this issue, DeGrado and co-workers reported a synthesis of [^{18}F]fluoromethylcholine in 2001 (DeGrado et al., 2001a; DeGrado et al., 2001c), and the radiopharmaceutical has seen widespread clinical use, mostly in Europe, since its introduction (for recent reviews of clinical use see (Bauman et al., 2012; Mertens et al., 2010)). The favorable half-life of fluorine-18 (109 min), when compared to that of carbon-11, facilitates distribution of radiopharmaceuticals from radiochemistry production facilities to satellite PET centers that do not own a cyclotron, and permits scanning multiple patients from a single batch. Reflecting this, demand for [^{18}F]FCH continues to increase at the University of Michigan, creating a need to optimize the synthesis for clinical production at our facility.

The original method for production of [^{18}F]FCH, reported by DeGrado and coworkers, involves [^{18}F]fluoromethylation of dimethylaminoethanol (DMAE) via the gaseous reactive intermediate [^{18}F]fluorobromomethane (DeGrado et al., 2001a; DeGrado et al., 2001b; DeGrado et al., 2001c). This remains the most widely used method to date, having been adapted for SPE purification, allowing automated production with cassette-based synthesis modules such as the TRACERLab MX_{FDG} (Kryza et al., 2008; Nader and Hoepping, 2005), a TRACERlab FX_{FDG} (Sperandeo et al., 2009), and, in our laboratory, a TRACERLab FX_{FN} (Shao et al., 2011b). However, in our hands and on our synthesis modules this method has proven problematic, plagued by low yields (4%) and, despite recently published purification improvements (Slaets et al., 2010), high DMAE contamination (1 mg/mL) (Shao et al., 2011a; Shao et al., 2011b). These low yields are an issue in our lab because it makes it difficult to make enough product to complete QC testing, and have sufficient dose remaining to complete the patient scan, as well as rendering it impossible to scan multiple patients from a single batch or distribute doses to satellite PET centers. Utilizing the same synthesis methods and CM Sep-Pak purification methods, other labs have reported this reaction to be higher yielding (>15% non-decay corrected), although residual DMAE levels were also frequently very high (>300 µg/mL) (Kryza et al., 2008), or not reported

(Sperandeo et al., 2009). The low yields obtained in our lab can, we believe, be attributed to performing gas phase reactions on a synthesis module optimized for liquid phase chemistry. In contrast, Kryza was able to modify his synthesis module to render it compatible with gas phase reactions by incorporation of closed pinch valves at the entrance and exit of the reactor. These modifications sealed the reaction vessel during the synthesis, and permitted high yielding reaction conditions. Such modifications are not readily achievable on the TRACERlab FX series due to the number and complexity of the reactor connections.

High levels of DMAE contamination are an even larger issue, because DMAE has been shown to be an inhibitor of [^{11}C]choline and [^{18}F]FCH transport (Slaets et al., 2010). Mintz, *et. al.* showed that uptake of DMAE in a variety of tumor cell lines was significantly better (2–7 times higher) than that of the clinically utilized radiolabeled choline tracer (Mintz et al., 2008). Additionally, DMAE has been shown to have inhibitory effects on choline transport across the blood-brain barrier (Cornford et al., 1978), in alveolar type II epithelial cells (Dodia et al., 1992), fetal rat cerebral hemispheres (Yavin, 1980), as well as inhibiting uptake of [^{18}F]FCH in prostate cancer cells (Nader et al., 2011a; Slaets et al., 2010; Sperandeo et al., 2009). An additional study showed that residual DMAE levels ($< 2.5 \mu\text{M}$) that were lower than plasma choline levels ($7\text{--}10 \mu\text{M}$) were still significant enough to compete with [^{18}F]FCH for tumor uptake (Slaets et al., 2010). Despite the abundance of information supporting the inhibitory effects of DMAE on [^{18}F]FCH uptake, PET scans have been routinely conducted globally with doses containing residual DMAE levels in excess of $300 \mu\text{g/mL}$. To address this issue, injection limits of DMAE are $20 \mu\text{g} / \text{mL}$ at the University of Michigan. However, this makes the synthesis challenging, as little work has been done to circumvent the problem and lower the DMAE levels in [^{18}F]FCH patient doses. Slaets and co-workers did recently report a novel purification method that permitted reduction of residual DMAE in doses to 3 ppm without compromising [^{18}F]FCH yields (Slaets et al., 2010). However, this procedure requires that the Sep-Paks undergo 5 separate washing steps followed by product elution with saline. Standard automated synthesis modules do not have the capacity to accommodate 6 successive washing steps, making the method difficult to translate to our existing systems.

Therefore, to promote use of [^{18}F]FCH in clinical imaging, we and others have explored development of improved strategies for preparing, purifying and analyzing [^{18}F]FCH (Nader et al., 2011b; Shao et al., 2011b; Slaets et al., 2010). For example, [^{18}F]FCH has also been prepared using a range of other alkylating agents, many of which are the subject of patent claims (Chi et al., 2006; DeGrado et al., 2001b; Lim, 2005), but include [^{18}F]fluoroiodomethane (Zhang et al., 2004), [^{18}F]fluoromethyl triflate (Iwata et al., 2002), and [^{18}F]fluoromethyl tosylate ([^{18}F]FCH₂OTs) (Neal et al., 2005). We saw potential for adaptation and optimization of this latter chemistry to prepare [^{18}F]FCH via *in situ* formation of [^{18}F]fluoromethyl tosylate, and herein we report our fully automated one-pot liquid-phase method for preparing [^{18}F]FCH. Radiochemical yields (RCY) are almost double those obtained using the gas phase [^{18}F]fluorobromomethane method in our laboratory. Moreover, doses contain extremely low levels of residual DMAE (3 ppm) and passed all other requisite QC testing, confirming their suitability for use in clinical imaging studies. Concurrent with our efforts, a similar synthesis of related [^{18}F]FCH analogs has been successfully developed using a cassette-based method in a two-pot approach (Aboagye et al., 2012).

2. Materials and Methods

2.1 General considerations

Chemicals and solvents were purchased from Sigma-Aldrich (Milwaukee, WI) or Fisher Scientific (Fair Lawn, NJ) and used without further purification. Unlabeled

fluoromethylcholine reference standard was purchased from ABX Advanced Biochemicals (Radeberg, Germany). Ditosylmethane was prepared in house as described in Section 2.2, or purchased from ABX Advanced Biochemicals. HPLC column: cation-exchange IC-PAK 150 x 3.9 mm (Waters). For preparation of [^{18}F]fluoromethylcholine, Sep-Pak C18-Plus cartridges were purchased from Waters and conditioned with ethanol (10 mL) and water (10 mL) prior to use. QMA-light Sep-Pak cartridges were purchased from Waters and conditioned with ethanol (10 mL), aq. potassium carbonate (10 mL) and water (10 mL) prior to use. Sep-Pak Accell Plus CM-Light cation-exchange cartridges were purchased from Waters and conditioned with ethanol (10 mL) and water (20 mL) prior to use.

2.2 Chemistry

Ditosylmethane—To an oven dried flask charged with a stir bar, silver p-toluenesulfonate (0.5210 g, 1.867 mmol) was suspended in acetonitrile (4 mL). To this suspension, diiodomethane (0.06 mL, 0.747 mmol) was added dropwise. The reaction mixture was stirred at reflux for 16 h. When complete, the reaction was cooled to room temperature, and the silver salt side product was collected on a sintered glass frit. The filtrate was collected and reduced *in vacuo* to give an off white solid. The filtrate was purified by SiO_2 column chromatography (eluting with hexane: ethyl acetate, 4:1) to give 0.2662 g (83 % yield) of ditosylmethane as a white crystalline product: R_f 0.3 (4:1, hexane: ethyl acetate); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.58 (d, J = 8, 4H), 7.38 (d, J = 8, 4H), 5.90 (s, 2H), 2.40 (s, 6H); MS (ESI): m/z 379 ($\text{M}+\text{Na}$) $^+$.

2.3 Radiochemistry

[^{18}F]Fluoromethylcholine was prepared using an automated GE TRACERLab FX $_{\text{FN}}$ synthesis module. The TRACERLab was configured as shown in Figure 1 and the reagent vials were loaded as follows: Vial 1: potassium carbonate (3.5 mg in 0.5 mL water); Vial 2: kryptofix-2.2.2 (15 mg in 1 mL MeCN); Vial 3: ditosylmethane (7–8 mg in 750 μL MeCN, 10 μL water); Vial 4: DMAE (40 μL in 350 μL MeCN); Vial 5: sterile water (5.5 mL); Vial 6: sterile water (5.5 mL); Vial 7: sterile water for injection, USP (20 mL); Vial 8: 0.9% sodium chloride for injection, USP (3 mL); Vial 9: ethanol (15 mL); Round bottom flask: ethanol (10 mL); product vial: 0.9% sodium chloride for injection, USP (7 mL).

Fluoride-18 (~1.3 Ci) was produced via the $^{18}\text{O}(p,n)^{18}\text{F}$ nuclear reaction using a GE PETTrace cyclotron equipped with a high yield fluorine-18 target. The [^{18}F]Fluoride was delivered from the cyclotron (in a 1.5 mL bolus of [^{18}O]H $_2$ O and trapped on a QMA-Light Sep-Pak to remove [^{18}O]H $_2$ O. [^{18}F]Fluoride was then eluted into the reaction vessel using aqueous potassium carbonate (3.5 mg in 0.5 mL of water). A solution of kryptofix-2.2.2 (15 mg in 1 mL of acetonitrile) was then added to the reaction vessel and the [^{18}F]fluoride was dried by evaporating the water–acetonitrile azeotrope. Evaporation of the azeotrope was achieved by heating the reaction vessel to 80 $^\circ\text{C}$ and drawing full vacuum for 4 min. After this time, the reaction vessel was cooled to 60 $^\circ\text{C}$ and subjected to both an argon (TRACERlab FX $_{\text{FN}}$ synthesis modules in our laboratory are equipped with argon rather than helium) stream and vacuum draw simultaneously for another 4 min. A solution of ditosylmethane (7–8 mg) 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 $^\circ\text{C}$ with stirring for 10 min. Subsequently, the reaction mixture was cooled to 50 $^\circ\text{C}$, followed by the addition of DMAE (40 μL in 350 μL MeCN) which was heated to 120 $^\circ\text{C}$ with stirring for an additional 10 min. The reaction mixture was then cooled to 60 $^\circ\text{C}$, and underwent evaporation of the reaction solvent by maintaining 60 $^\circ\text{C}$ and subjecting the reaction to both a continuous argon stream and vacuum draw for 5 min. Sterile water (5.5 mL) was added to the dried reaction mixture and passed through the C18-Plus Sep-Pak into the round bottom flask containing ethanol (10 mL) to trap unreacted ditosylmethane and [^{18}F]fluoromethyltosylate, as well as any

tosylmethylcholine generated as a by-product. This was repeated with an additional lot of sterile water (5.5 mL). The water/ethanol mixture was transferred through the CM-Light Sep-Pak to trap the desired [^{18}F]fluoromethylcholine. The CM-Light Sep-Pak 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 into a sterile dose vial to provide doses of [^{18}F]fluoromethylcholine as an isotonic solution submitted for quality control testing according to Section 2.4 and Table 1. Total synthesis time was 1.25 hours from end-of-bombardment, and typical non-decay corrected yields were 91 mCi (7% based upon 1.3 Ci of starting fluoride).

2.4 Quality Control

Quality control of radiopharmaceuticals prepared at the University of Michigan is carried out, in accordance with the U.S. Pharmacopoeia, as described below.

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

2.4.2. Dose pH—The pH of the doses was analyzed by applying a small amount of the dose to colorpHasts pH 2.0–9.0 non-bleeding pH-indicator strips and determined by visual comparison with the scale provided. Dose pH must be 4.5–7.5.

2.4.3. Radionuclidic identity—Radionuclidic identity was confirmed by determining the half-life of the dose and comparing it with the known half-life of fluorine-18 (110 min).

2.4.4 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 and Bioscan FC3300 flow count radioactivity detector. The system was not equipped with an ion suppressor. Column: Waters SCX column, IC-PakTM Cation M/D, 3.9x150 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 Figure 2. Radiochemical purity must be > 95%, and residual DMAE must be <20 $\mu\text{g/mL}$.

2.4.5 Residual kryptofix-222—Residual kryptofix-222 (K222) levels in [^{18}F]FCH doses were analyzed using the established spot test. Strips of plastic-backed silica gel TLC plates saturated with iodoplatinate reagent were spotted with water (negative control), 50 mg/mL kryptofix-2.2.2 standard (positive control) and with the [^{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. <50 $\mu\text{g/mL}$ is acceptable and all doses of fluorocholine prepared in this study were found to contain residual K222 below this level.

2.4.6 Residual Solvent Analysis—Levels of residual solvents in doses were analyzed using a Shimadzu GC-2010 with an AOC-20 autoinjector, split/splitless inlet, a flame ionization detector (FID) and a Restek column (Stabilwax 30m x 0.25mm, 0.25m G16 stationary phase). Limits of residual solvents are based upon the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines.

2.4.7 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.

2.4.8 Bacterial endotoxins—Endotoxin content in radiopharmaceutical doses was analyzed using a Charles River Laboratories EndoSafe® Portable Testing System and according to the US Pharmacopeia. Doses must contain <175 endotoxin units.

2.4.9 Sterility—Culture tubes of fluid thioglycolate media (FTM) and soybean casein digest agar media (SCDM) were inoculated with samples of [^{18}F]FCH doses and incubated (along with positive and negative controls) for 14 days. 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) on the plates and dose/negative controls must have no culture growth after 14 days to be indicative of sterility.

3. Results and Discussion

3.1 Radiosynthesis

Initial attempts to develop alternative synthetic approaches to [^{18}F]FCH focused upon a direct one-step fluorination of tosylmethylcholine (Scheme 1). However, synthesis of the requisite precursor proved elusive and, despite careful efforts to control reaction stoichiometry, treatment of DMAE with ditosylmethane (prepared in house from diiodomethane and AgOTs as previously described (Lim, 2005)) resulted in complex mixtures of by-products. Therefore, it quickly became apparent that *in situ* generation of [^{18}F]fluoromethyl tosylate (Scheme 2), and subsequent [^{18}F]fluoromethylation of DMAE would be a more suitable approach for generating [^{18}F]FCH.

[^{18}F]FCH was prepared using a GE TRACERlab FX_{FN} automated synthesis module (Figure 1), although the method should be readily adaptable to any other fluorine-18 synthesis module. [^{18}F]Fluoride was trapped on a QMA cartridge and eluted with K_2CO_3 . Kryptofix-222 was added and the azeotropic water/acetonitrile mixture was evaporated to dryness. To this was added a solution of ditosylmethane (7 mg) in MeCN (750 μL) and water (10 μL), and the reaction heated at 120°C for 10 min to generate [^{18}F]FCH₂OTs. Analysis of the crude reaction mixture at this point confirmed 38% non-corrected yield of [^{18}F]FCH₂OTs, (50% decay-corrected yield which is comparable to yields reported in the literature (Neal et al., 2005)). The reaction mixture was then cooled to 60°C and DMAE (40 μL) in MeCN (350 μL) was added. The reactor was heated at 120°C for an additional 10 min. After this time, the reaction was cooled to 60 °C and analysis of the crude reaction mixture by analytical HPLC revealed 45% conversion to [^{18}F]FCH (based upon [^{18}F]FCH₂OTs). Confident that this method would allow us to prepare larger quantities of [^{18}F]FCH than we have managed previously (Shao et al., 2011b), we focused next upon strategies for purification of [^{18}F]FCH and removal of residual DMAE.

3.2 Purification

HPLC analysis of the crude reaction mixture revealed a 45:55 ratio of [^{18}F]FCH : [^{18}F]FCH₂OTs in the radiochemical trace, and [^{19}F]FCH (cold mass), unreacted ditosylmethane, in addition to residual DMAE and an unidentified impurity (presumably tosylmethylcholine generated as a by-product but, as described above, we have been unable

to prepare the corresponding unlabeled reference compound to confirm this) in the UV and IC traces. Thus, a purification strategy had to be developed to remove each of these impurities, in addition to residual K_2CO_3 , and K222, which do not show up in HPLC traces. No residual $[^{18}F]$ fluoride was detected in the crude reaction mixture, suggesting that the initial conversion to $[^{18}F]FCH_2OTs$ was very efficient. However, $[^{18}F]$ fluoride can stick to HPLC columns, and as a result may not be detected by HPLC. Therefore we also wished to account for this during purification optimization.

Initially we diluted the crude reaction mixture in water and passed the resulting solution through a C18-Plus Sep-Pak cartridge. Careful reanalysis of the resulting solution confirmed that the C18-Plus Sep-Pak removed all residual ditosylmethane (confirmed by HPLC; chromatograms not shown), $[^{18}F]FCH_2OTs$, K222, and the impurity suspected to be tosylmethylcholine. Thus the solution contained $[^{18}F]FCH$ in >99% radiochemical purity, contaminated with residual DMAE, and presumably potassium carbonate. This solution was diluted with ethanol and passed through a CM-Light cation capture Sep-Pak. $[^{18}F]FCH$ was trapped on the Sep-Pak, and the cartridge was then washed with ethanol (15 mL) to remove any residual DMAE. An advantage of this method over our previous work using the $[^{18}F]$ fluorobromomethane method (Shao et al., 2011b), is that it requires 10-fold less DMAE (40 μ L vs. 400 μ L), making its eventual removal operationally simpler. The cartridge was then rinsed with sterile water for injection to remove residual ethanol, and final elution with saline, USP provided $[^{18}F]FCH$. Analysis of the saline elution by ion chromatography (equipped with a radiochemical detector) revealed $[^{18}F]FCH$ in >99% RCP, and residual DMAE of ~3 ppm (3.16 μ g/mL or 31.6 μ g/dose), a factor of 30–300 less than we have previously been able to achieve (Shao et al., 2011b), and well below the 20 μ g/mL limit specified in the release criteria for the University of Michigan. Residual DMAE can also be analyzed by GC, but in our experience this has been less reliable than ion chromatography because standards tend to leach from the column and reproducibility has historically been problematic per our earlier paper (Shao et al., 2011b).

With a suitable purification method in hand, attention was turned to automation of the purification process using the module configuration illustrated in Figure 1. Thus, after automated $[^{18}F]$ fluoromethylation of DMAE as described above, the reactor was cooled to 60 °C and the reaction solvent (MeCN) was evaporated (vacuum + argon stream for 5 min). The reaction mixture was then diluted with water (5.5 mL) and passed through a C18-Plus Sep-Pak into the round-bottom dilution flask charged with ethanol (10 mL). The procedure was repeated with additional water (5.5 mL) to rinse the reactor, and the resulting water-ethanol mixture was passed through a CM-Light cartridge. $[^{18}F]FCH$ was trapped on the CM-Light cartridge, and the cartridge was flushed with ethanol (15 mL) to remove residual DMAE. The CM-Light cartridge was subsequently rinsed with water (20 mL) to remove residual ethanol, before final elution of $[^{18}F]FCH$ with USP saline (3 mL) into the product collection flask containing additional saline (7 mL). The formulated product (10 mL) was passed through a 0.22 μ m filter into a sterile dose vial, and submitted for QC testing as described.

After automation of the entire process, 3 process verification runs were conducted and non-decay corrected yields of $[^{18}F]FCH$ were 91 mCi (7% based upon 1.3 Ci of starting fluoride). Doses passed all other requisite QC testing (Table 1), confirming their suitability for use in $[^{18}F]FCH$ clinical PET imaging studies.

4. Conclusion

A one-pot synthesis of $[^{18}F]FCH$ has been developed. $[^{18}F]FCH_2OTs$ is prepared *in situ* from ditosylmethane, and reacted with DMAE to generate $[^{18}F]FCH$. Yields are

significantly higher than previously obtained in our lab, and doses contained extremely low levels of residual DMAE and passed all other requisite QC testing, confirming their suitability for use in clinical imaging studies.

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Highlights

- A novel fully automated synthesis of [^{18}F]fluoromethylcholine has been developed
- Typical non-decay-corrected yields were 91 mCi (7% non-decay corrected, n = 3)
- QC testing confirmed suitability for clinical use (RCP >99%; SA 16,147 Ci/mmol)
- Doses contain amongst the lowest residual DMAE ever reported

Figure 1.
Synthesis Module Configuration

Figure 2.
Typical Analytical HPLC Trace of Formulated $[^{18}\text{F}]$ Fluoromethylcholine

Scheme 1.
Proposed One-step Fluorination of Tosylmethylcholine

Scheme 2.
Synthesis of $[^{18}\text{F}]$ Fluoromethylcholine

Table 1Quality Control Data for [^{18}F]FCH (n=3)

Visual Inspection	pH	$t_{1/2}$	RCP	SA	Residual DMAE	RSA MeCN-EtOH	K222	Bubble point	Endo-toxins	Sterility
Pass	5.0	110 min	99.7%	16,147 Ci/mmol	3.16 $\mu\text{g/mL}$ *	Pass	<50 $\mu\text{g/mL}$	Pass	<2.0 EU/mL	Pass

* corresponding to 31.6 μg / 10 mL dose or ~ 3ppm