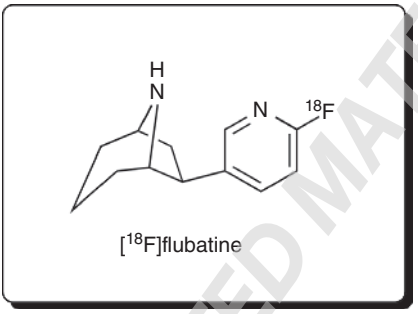
CHAPTER 1

SYNTHESIS OF (–)-[^{18}F]FLUBATINE ([^{18}F]FLBT)

MEGAN N. STEWART, BRIAN G. HOCKLEY, AND PETER J. H. SCOTT

Department of Radiology, University of Michigan School of Medicine, Ann Arbor, Michigan, USA



1 INTRODUCTION

Cognitive or depressive disorders and neurodegenerative diseases such as Alzheimer's disease (AD), dementia, and Parkinson's disease (PD) may be related to dysfunctional signaling through α4β2-nicotinic acetylcholine receptors (α4β2-nAChRs) [1, 2]. Alterations in the cholinergic system are also implicated in the progression of cognitive decline in the aforementioned neurodegenerative diseases, particularly AD [2–4]. The development of (–)-[^{18}F]flubatine as a high affinity and selective PET radiotracer with improved kinetics over the earlier developed ligands allows for noninvasive quantification of nAChRs [2, 5].

The first reported radiosynthesis of (–)-[^{18}F]flubatine, a derivative of epibatidine, utilized a norchloro-bromo-homoepibatidine (NCBrHEB) precursor that underwent a nucleophilic substitution with the bromine leaving group, then the enantiomers separated, and the product purified appropriately via HPLC [4–7]. However, due to low radiochemical yields, other candidate precursors were explored for radiolabeling and the trimethylammonium iodide-Boc-protected compound ((5-((1*R*,5*S*,6*S*)-8-*tert*-butoxycarbonyl)-8-azabicyclo[3.2.1]-octan-6-yl)-*N,N,N*-trimethylpyridin-2-aminium iodide, Boc-trimethylammonium homoepibatidine, **1**) was shown to give the best yields of

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approximately 60% and further adapted for fully automated synthesis [2, 8]. This precursor has since become commercially available, making [^{18}F]flubatine more accessible for clinicians and has been validated for clinical use in nonhuman primates [4].

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 [^{18}F]Fluoride

[^{18}O]H_2O (1.5 ml) [9] was loaded into the [^{18}F]fluoride target [10] of a General Electric Medical Systems (GEMS) PETtrace cyclotron [11]. The target was bombarded (60 μA beam for 30 min) to generate approximately 1.5 Ci (55.5 GBq) of [^{18}F]fluoride by the ^{18}O(p,n)^{18}F nuclear reaction.

2.2 Azeotropic Drying of [^{18}F]Fluoride

The [^{18}F]fluoride was delivered to a GEMS TRACERlab FX_{FN} synthesis module [11] as a solution in [^{18}O]H_2O (1.5 ml). This solution was passed through a Sep-Pak® QMA-Light cartridge [12] to trap the [^{18}F]fluoride and recycle the [^{18}O]H_2O. The [^{18}F]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) [13]. A solution of Kryptofix 222 (5 mg in 1 ml MeCN) [14] 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]FLBT

A solution of (–)-FLBT precursor [15] (**1**, 0.5–1.0 mg) in anhydrous dimethyl sulfoxide (DMSO) [16] (0.6 ml) was added to the dried [^{18}F]fluoride, and the reaction was heated to 120°C with stirring for 10 min (Fig. 1). After this time, the reaction was cooled to 40°C, and 1.0 M aqueous hydrochloric acid (1 ml) was added. The reaction was stirred for 5 min at 80°C to hydrolyze the Boc protecting groups. The reaction mixture was neutralized with 0.5 M aqueous sodium hydroxide (2 ml) [17].

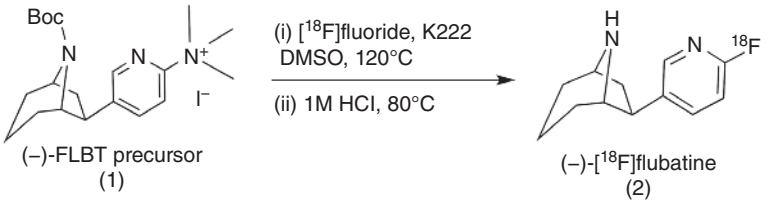

FIGURE 1 Synthesis of (–)-[^{18}F]FLBT.

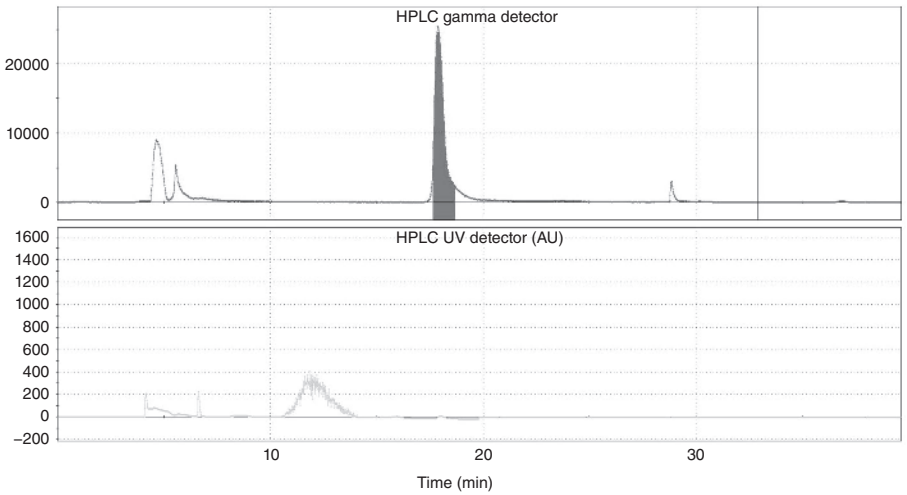

FIGURE 2 Semipreparative UV and radioactive HPLC traces for [^{18}F]FLBT.

2.4 Purification and Formulation of (−)-[^{18}F]FLBT

After hydrolysis, the crude reaction mixture was purified by semipreparative HPLC (Luna 10u C18(2) 250 × 10 mm column [18], flow rate = 4 ml/min), and a representative HPLC trace is shown in Fig. 2.

The fraction corresponding to (−)-[^{18}F]FLBT (typically eluting between 20 and 25 min) was collected for 1 min into a vial precharged with 0.9% sodium chloride, USP [19] (6 ml). The final formulation (10 ml) was then passed through a sterile filter [20] into a sterile vial [21] to provide (−)-[^{18}F]FLBT (typically 50–115 mCi (1.85–4.3 GBq)) in an isotonic solution released for quality control. After synthesis was complete, the semipreparative HPLC column was flushed with 70% ethanol.

3 QUALITY CONTROL PROCEDURES

CAUTION: All radiochemicals produced for clinical use must have local regulatory approval (e.g., FDA, EMEA, MHRA, PFSB, etc.) prior to human use. Quality control procedures must be carried out by trained personnel, and each dose must meet all established QC criteria before release to the clinic.

Quality control (QC) procedures for (−)-[^{18}F]FLBT, based upon the current requirements for radiopharmaceuticals laid out in the US Pharmacopeia (USP) [22], are summarized in the following text. Complete QC data for three repeat batches of (−)-[^{18}F]FLBT produced using the method disclosed herein are summarized in Table 1. Each of the three doses met all of the established QC criteria

TABLE 1 QC Data for three Repeat Runs of (−)-[18F]FLBT

QC Test	Release Criteria	Run 1	Run 2	Run 3
Yield/mCi (GBq)	N/A	79.7 (2.9)	101 (3.7)	65 (2.4)
Visual inspection	Clear, colorless	Clear, colorless	Clear, colorless	Clear, colorless
Radiochemical identity	RRT = 0.9–1.1	1.01	1.01	1.01
FLBT concentration	No limit established	0.84 µg/ml	0.66 µg/ml	0.33 µg/ml
Radiochemical purity	≥95%	99.9	100	100
Specific activity	No limit established	1.5 Ci/µmol	2.7 Ci/µmol	4.0 Ci/µmol
Residual solvent analysis	DMSO <5000 ppm	Pass	Pass	Pass
	Acetone <5000 ppm	Pass	Pass	Pass
	Acetonitrile <410 ppm	Pass	Pass	Pass
Dose pH	4.5–7.5	4.5	4.5	4.5
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	112	109	109
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

3.1 Visual Inspection

The (−)-[18F]FLBT dose is examined behind a PET L-block and must be clear, colorless, and free of particulate matter.

3.2 Radiochemical Identity

HPLC analysis of radiochemical identity was conducted using a Shimadzu LC-2010A$_{HT}$ Liquid Chromatograph [23] fitted with UV detectors and Bioscan γ-detectors [24] (column, Phenomenex Synergi Polar RP 150×4.6 mm [25]; mobile phase, 50% acetonitrile 50% water +0.1% acetic acid [26]; flow rate, 1 ml/min, λ=254 nm). The retention time of [18F]FLBT is compared to that of the [19F]FLBT reference standard [15] and must be ±10% (relative retention time (RRT) must be 0.9–1.1).

3.3 Radiochemical Purity

HPLC analysis of radiochemical purity was conducted using a Shimadzu LC-2010A$_{HT}$ Liquid Chromatograph [23] fitted with a UV detector and a Bioscan γ-detector [24] (column, Phenomenex Synergi Polar RP 150×4.6 mm [25]; mobile phase, 50% acetonitrile 50% water +0.1% acetic acid [26]; flow rate, 1 ml/min,

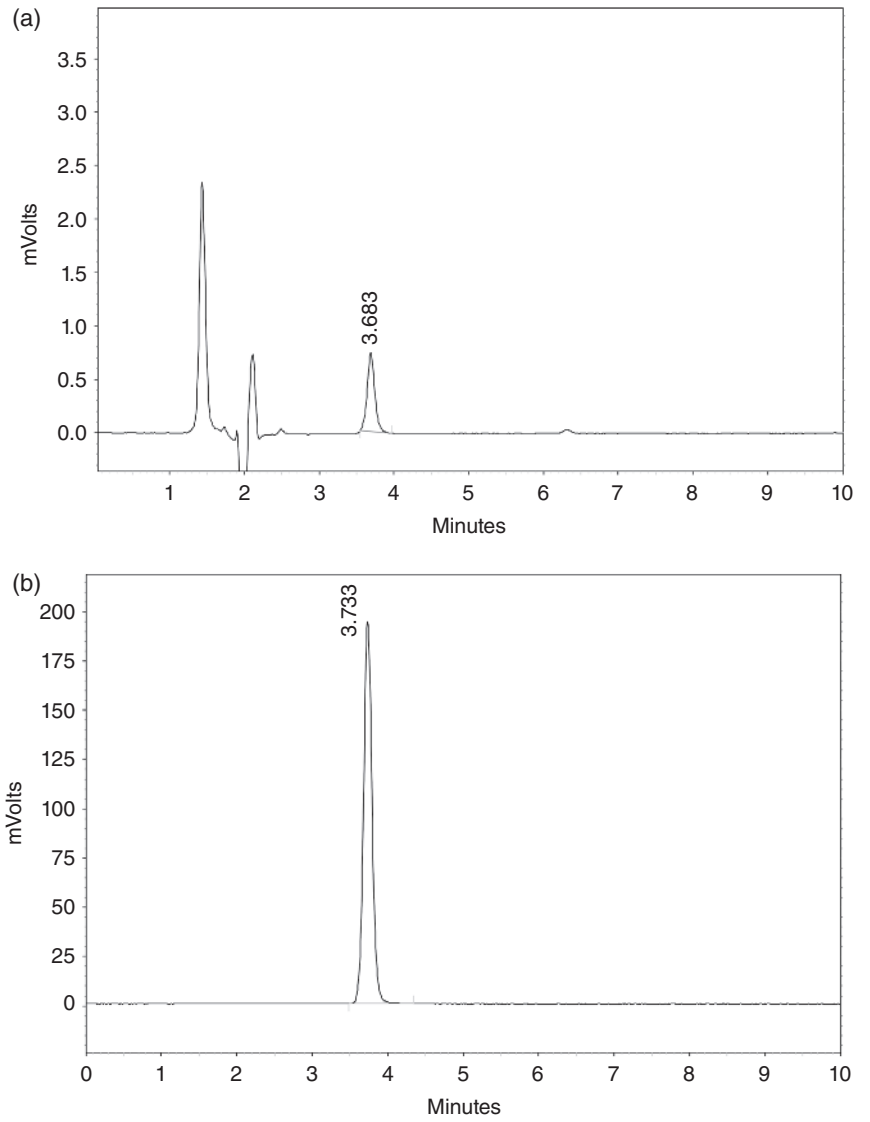

FIGURE 3 Analytical UV (a) and radioactive (b) HPLC trace for (−)-[^{18}F]FLBT.

$\lambda = 254$ nm). Radiochemical purity must be greater than 95%. Representative analytical HPLC traces are displayed in Fig. 3.

3.4 Specific Activity

There is no specific activity release limit for (−)-[^{18}F]FLBT in place at our institution. The injection volume is based on the injectable mass limit for (−)-FLBT and is calculated on a dose-by-dose basis by the administering study team.

3.5 Residual Solvent Analysis

Analysis of residual solvent levels (acetone (from TRACERlab FX_{FN} drying cycle), DMSO, and acetonitrile) in [18F]FLBT doses was performed using a Shimadzu GC-2010 gas chromatograph (GC) equipped with a split/splitless inlet and flame ionization detector and a Restek Stabilwax GC column (35 m × 0.25 mm) using conditions outlined in the following text [23]. Limits imposed by the ICH Harmonised Tripartite Guidelines are <410 ppm/day for acetonitrile and <5000 ppm/day for class 3 solvents (DMSO, acetone) [27].

GC Conditions

Oven temperature profile:

1. Hold at 30°C for 3 min.
2. Increase 30–180°C at 60°C/min.
3. Hold at 180°C for 5 min.

Inlet temperature: 180°C
Column linear velocity: 18.8 cm/s
FID temperature: 250°C

3.6 Dose pH

The pH of the [18F]FLBT dose was analyzed by applying a small amount of the dose to colorpHast® pH 2.0–9.0 nonbleeding pH-indicator strips [28] and determined by visual comparison to the scale provided. Dose pH must be 4.5–7.5.

3.7 Residual Kryptofix 222 Analysis

Residual Kryptofix 222 levels in (−)-[18F]FLBT doses were analyzed using the established spot test [29]. Strips of plastic-backed silica gel TLC plates saturated with iodoplatinate reagent [29] were spotted with water (negative control), 50 μg/ml Kryptofix 222 standard (positive control) and (−)-[18F]FLBT dose. If Kryptofix 222 is present in a sample, a blue-black spot appears. Spots for the three samples were compared, and a visual determination of residual Kryptofix 222 in the (−)-[18F]FLBT dose was made. Less than 50 μg/ml is acceptable.

3.8 Sterile Filter Integrity Test

The sterile filter from the (−)-[18F]FLBT (with needle still attached) was connected to a nitrogen supply via a regulator. The needle was submerged in water and the nitrogen pressure was gradually increased. If the pressure was raised above the filter acceptance pressure (50 psi) without seeing a stream of bubbles, the filter was considered intact. If a stream of bubble occurs at less than 50 psi, the test fails.

3.9 Radionuclidic Identity

Activities were measured using a Capintec CRC®-15R Radioisotope Dose Calibrator [30], and half-life was calculated using Equation 1. Calculated half-life must be 105–115 min:

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

3.10 Endotoxin Analysis

Endotoxin content in doses of (−)-[^{18}F]FLBT was analyzed by a Charles River Laboratories Endosafe® Portable Testing System [31] and according to the USP. Doses must contain less than or equal to 17.5 endotoxin units (EU)/ml.

3.11 Sterility Testing

Samples of (−)-[^{18}F]FLBT were placed into fluid thioglycolate media (FTM) plates and soybean-casein digest agar media (SCDM) tubes. FTM tubes are used to test for anaerobes, aerobes, and microaerophiles, while SCDM tubes are used to test for nonfastidious and fastidious microorganisms [32]. (−)-[^{18}F]FLBT tubes were 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]. Tubes were visually inspected on the 3rd, 8th, and 14th days of the test period and compared to the positive and negative standards. Positive standards must show growth (turbidity) in the tubes, and (−)-[^{18}F]FLBT/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, DC: National Academy Press, 1995).

CHEMICAL ABSTRACTS NOMENCLATURE (REGISTRY NUMBER)

Acetonitrile (75-05-8)
5-(((1*R*, 5*S*, 6*S*)-8-*tert*-butoxycarbonyl)-8-azabicyclo[3.2.1]octan-6-yl)-*N,N,N*-trimethylpyridin-2-aminium iodide (CAS-RN not assigned)
Carbonic acid, potassium salt (1:2) (584-08-7)
DMSO (67-68-5)
Ethanol (64-17-5)
(1*R*,5*S*,6*S*)-6-(6-Fluoropyridin-3-yl)-8-azabicyclo[3.2.1]octane (CAS-RN not assigned)
4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (23978-09-8)
Hydrochloric acid (7647-01-0)
Sodium hydroxide (1310-73-2)

REFERENCES AND NOTES

For detailed supplier information, see Appendix 1.

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9. Virgin [^{18}O]H_2O purchased from ABX, Amic, Rotem, or Medical Isotopes, and used as received.
10. GEMS silver high-yield [^{18}F]fluoride target.
11. GE Healthcare, United States.
12. 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.
13. Potassium carbonate purchased from Sigma-Aldrich (part no. 209619) and used as received. Sterile water purchased from Hospira (part no. 0409-4887-50) and used as received.
14. 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.
15. (–)-[^{18}F]FLBT precursor and reference standard were purchased from ABX and used as received.
16. Anhydrous DMSO was purchased from Acros (part no. 61042-10000) and used as received. We notice a gradual decline in yield throughout the lifetime of a bottle of DMSO and stress the importance of using anhydrous solvents in this synthesis.
17. Sodium hydroxide 97% was purchased from Sigma-Aldrich (part no. 221465) and used as received.
18. Semi-preparative HPLC column: Phenomenex Luna C18(2), 10 µ, 250 × 10 mm (part no. 00G-4253-NO); flow rate = 4 ml/min; λ = 254 nm; semipreparative column was equilibrated with 200–300 ml of mobile phase prior to synthesis.
19. 0.9% Sodium Chloride, USP, was purchased from Hospira (part no. 0409-4888-50) and used as received.
20. Millex-GV sterile 0.22 µm filters were purchased from Millipore (part no. SLGV013SL) and used as received.
21. 10 ml sterile vials were purchased from Hollister-Stier (part no. 7515ZA) and vented with a sterile Millex-FG vent filter (part no. SLFG025LS).
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23. Shimadzu Corporation, United States.
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25. Analytical HPLC column was purchased from Phenomenex (part no. 00F-4336-E0); flow rate = 1.0 ml/min; λ = 254 nm; analytical column was equilibrated with mobile phase for 30–60 min prior to use.
26. Analytical mobile phase: 50% MeCN, 50% water + 0.1%acetic acid.
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33. US Pharmacopeia <71>. Sterility tests. US Patent 32–NF 27. 2009.