# Synthesis of [$^{18}$F]-N-3-fluoropropyl-2$\beta$-carbomethoxy-3$\beta$-(4-iodophenyl) nortropane([$^{18}$F]-FP-$\beta$-CIT)

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**Abstract** The ligand of N-(3-fluoropropyl)-2$\beta$-carbomethoxy-3$\beta$-(4'-iodophenyl) nortropane (FP-$\beta$-CIT) and mesylate precursor were synthesized by hydrolysis of cocaine, followed by dehydration, esterification, Grignard reaction, N-demethylation, iodination, N-alkylation with 3-bromopropanol and methylsulfonylation. Finally, $^{18}$F-FP-$\beta$-CIT was prepared by nucleophilic fluorination of the mesylate with $K^{18}F/K_{2.2.2}$ (Kryptofix). The labeling yield of $^{18}$F-FP-$\beta$-CIT is 25%~30%. The total radiochemical yield of this compound, calculated from the end of bombardment (EOB) with decay correction, is 10%~12% with a synthesis time of 100~110 min. The radiochemical purity of $^{18}$F-FP-$\beta$-CIT is greater than 90%, and this compound in aqueous solution is also stable for more than 4 hours at room temperature. It is stable enough for clinical study.

**Keywords** Cocaine derivatives, Tropane derivatives, Dopamine Transporter, FP-$\beta$-CIT, Imaging agent

**CLC numbers** O621.3$^+$5, O628.5$^+$1, O629.7, R817.9

## 1 INTRODUCTION

Single photon emission computed tomography (SPECT) and positron emission tomography (PET) provide sensitive and powerful means for detecting specific molecular targets in brain. In neuron abnormality, molecular targets for SPECT or PET brain imaging agents may be used to reveal the status of associated neurons. The dopamine (DA) transporter (DAT) is a protein complex localized almost exclusively presynaptically at the dopaminergic nerve terminal. Increasing evidence suggests that the DAT is an important marker for physiological and pathological changes in DA neurons. In living brain or in postmortem tissue, DA neurons are severely depleted in patients with Parkinson's disease, and probes targeted to the DAT can visualize the depletion.

Several useful ligands, such as $^{11}$C-CIT (2$\beta$-carbomethoxy-3$\beta$-(4'-iodophenyl)tropane)[1], $^{11}$C-CFT (2$\beta$-carbomethoxy-3$\beta$-(4'-fluorophenyl)tropane)[2], $^{18}$F-FP-$\beta$-CIT(N-(3-fluoropropyl)-2$\beta$-carbomethoxy-3 $\beta$-(4'-iodophenyl)nortropane)[3,4], and so on for PET imaging, and

Supported by the National Natural Science Foundation (39770230); Natural Science Foundation of Jiangsu Province (BK99163) and Department of Health of Jiangsu Province (H200006)

Manuscript received date:2001-01-02

$^{123}$I-$\beta$-CIT (2$\beta$-carbomethoxy-3$\beta$-(4'-iodophenyl) tropane)[5], $^{123}$I-FP-$\beta$-CIT[6], $^{123}$I-IPT (N-(3-iodopropen-2-yl)-2$\beta$-carbomethoxy-3$\beta$-(4'-chlorophenyl)nortropane)[7], and so on for SPECT imaging, have displayed high binding affinity and excellent imaging characteristics for DAT.

In this work, the synthesis of mesylate precursor and preparation of [F-18]FP-$\beta$-CIT by nucleophilic fluorination of the mesylate are reported.

## 2 MATHERIAL AND METHODS

### 2.1 Synthetic scheme

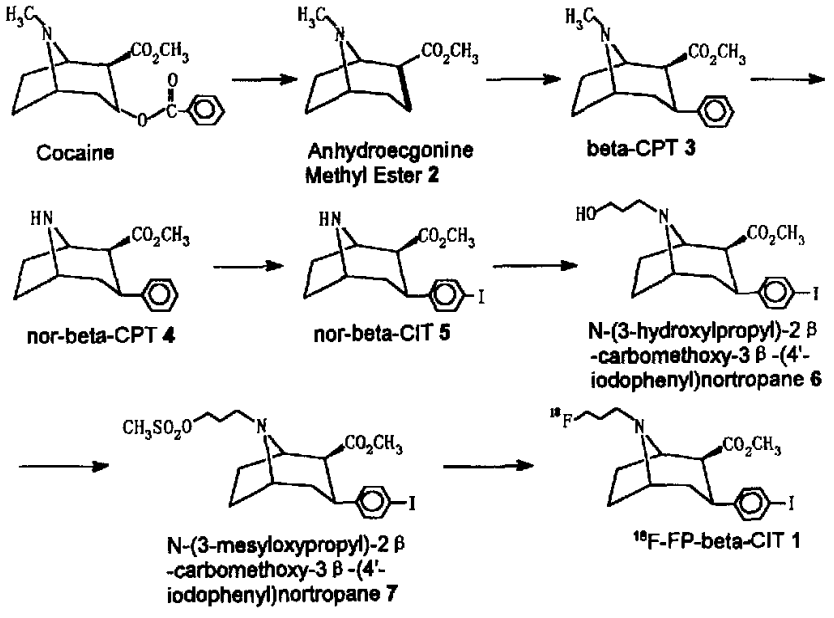


Fig.1 Synthetic Scheme of $^{18}$F-FP-$\beta$-CIT

Mesylate precursor was synthesized from cocaine, and $^{18}$F-FP-$\beta$-CIT was prepared by nucleophilic fluorination of the mesylate.

$[^{18}F]^-$ was produced by a RDS 111 cyclotron. A reverse-phase semi-preparative column (Waters Delta Pak C-18 15 micro spherical, 120 angstroms pore size, $\phi$7.8mm×300mm) was employed for the purification of the product, using methanol/water/triethylamine (75/25/0.2) as eluent. Analytical HPLC was performed using C18 reverse phase column (5 micro spherical, $\phi$4.6mm×150 mm).

### 2.2 Experimental procedures

**(R)-(-)-Anhydroecgonine methyl ester (2).** The procedure of Meltzer *et al*[8] was followed and **2** (85%) was obtained as an oil: bp 113~114°C (0.35 kPa).

**2$\beta$-carbomethoxy-3 $\beta$-phenyltropane (3, $\beta$-CPT).** The procedures of Clarke *et al*[9] and Carroll *et al*[10] were followed with minor modifications. A mixture of phenylmagnesium bromide (100 mmol) in anhydrous ether (300 mL) was cooled to −40°C. Anhydroecgonine methyl ester (3.62 g 20 mmol) in anhydrous ether (30 mL) was added dropwise. The reaction mixture was stirred at −40°C for 90 min, then cooled to −78°C. TFA (100 mmol) in ether (20 mL) was added, and the reaction mixture after being stirred for 5 min at −78°C was allowed to warm to −5°C. Then water (120 g) was added. The reaction mixture was stirred for 5 min, acidified to pH 1.0 with concentrated HCl, and the ether layer was discarded. The aqueous layer was basified to pH 10∼11 with $NH_4OH$, and extracted with ether (3×150 mL). The combined ether layer was dried over $Na_2SO_4$, filtered and concentrated to dryness. The residue was purified by chromatography (hexane/ether/triethylamine, 70/30/1, V/V). 2.1 g (41%) of **3** was obtained, recrystallization in hexane, gave a colorless crystalline product of **3**, mp 58∼61°C, $[\alpha]_D^{27}$ −49.6° (1% in $CH_3OH$), $[\alpha]_D^{27}$ −8.2° (0.5% in $CHCl_3$), (Ref.[9]: mp 62∼64.5°, $[\alpha]_D^{25}$ −5.3° (1% in $CHCl_3$)). IR(KBr): 1748 (C=O), 2845 ($OCH_3$), 2798 ($NCH_3$), 1499 1602 (Ar), 1173 (C-O), 748 703 ($C_6H_5$) $cm^{-1}$. MS(m/e): 259 ($M^+$, 46%), 228 ($M^+$-$OCH_3$, 5%), 200 ($M^+$-$COOCH_3$, 11%), 182 ($M^+$-$C_6H_5$, 5%), 82 ($C_5H_8N$, 100%).

**2$\beta$-carbomethoxy-3$\beta$-phenylnortropane (4, nor-$\beta$-CPT).** $\beta$-CPT (3, 3.0 g, 11.6 mmol) and $\alpha$-chloroethyl chloroformate (ACE-Cl) (5 mL, 46.3 mmol) were heated at 80°C for 1 h. Excess ACE-Cl was then removed under reduced pressure, and methanol (50 mL) was added to the residue. The mixture was then refluxed for 30 min and then concentrated to dryness. The obtained residue was dissolved in $CH_2Cl_2$ (75 mL), washed with saturated $NaHCO_3$ solution, dried over sodium sulfate, filtered, and concentrated. Purification of the crude demethylated product by flash chromatography ($Et_2O$/$Et_3N$ 90/10) gave 1.6 g, (56%) of **4** as a white-solid, mp 87.5∼88.5°C, $[\alpha]_D^{27}$ = −124.1° (0.1% in $CH_3OH$), (Ref.[9] $[\alpha]_D^{25}$ =−110.0° (1% in $H_2O$)). IR (KBr): 1706(C=O), 2874($OCH_3$), 1500 1602(Ar), 1175 (C-O), 776 701(-$C_6H_5$) $cm^{-1}$. MS(m/e): 245($M^+$, 37%), 214($M^+$-$OCH_3$, 8%), 186 ($M^+$-$COOCH_3$, 10%), 83($C_5H_9N$, 100%).

**2$\beta$-carbomethoxy-3$\beta$-(4'-iodophenyl)nortropane (5, nor-$\beta$-CIT).** A mixture of nor-$\beta$-CPT (**4**, 1.5 g, 6.1 mmol) and $I_2$ (1.6 g, 6.3 mmol) in 25 mL of glacial acetic acid was stirred and treated dropwise with a mixture of 2.5 mL concentrated nitric acid and 2.5 mL concentrated sulfuric acid. The reaction mixture was heated to 55°C, and stirred for 2 h, then cooled to room temperature and poured onto ice (60 g) and filtered. The pH of the filtrate was adjusted to 9.5 by the addition of concentrated ammonium hydroxide at 0∼5°C. The resulting precipitate was removed by filtration and dissolved in $CH_2Cl_2$ (250 mL). The filtrate was extracted with two 50 mL portion of $CH_2Cl_2$. The extracts and solution of precipitate were combined, washed with brine (50 mL) and dried over magnesium sulfate. After the removal of the solvent, the residue was recrystallized in petroleum ether (60∼ 90°C), 1.1 g (71%) of the free base **5** as a straw yellow solid was obtained, mp: 118∼120°C. $[\alpha]_D^{28}$ = −88.3° (0.25% in $CHCl_3$), (Ref. [11]: $[\alpha]_D^{25}$ = −67.4° (1% in $CHCl_3$)). IR (KBr): 1719(C=O), 3310(NH), 2876($OCH_3$), 1170(C-O),825(-$C_6H_4$-) $cm^{-1}$. $^1$HNMR($CDCl_3$) (400 MHz): 1.57∼1.82 (4H, m, $CH_2CH_2$), 1.95∼2.20 (1H, m,

CH), 2.38 (1H, t, J=12.20, CH), 2.50~2.79 (2H, m, CH), 3.18(1H, m, CH), 3.40 (3H, s, $OCH_3$), 3.73 (2H, m, NH, CH), 6.94 (2H, d, J=8.43, ArH), 7.59 (2H, d, J=8.42, ArH) ppm. MS(m/e): 371 (M+, 56%), 340 ($M^+$-$OCH_3$, 8%), 312($M^+$-COOCH3, 10%), 83($C_5H_9N$, 100%). Anal. calcd. for $C_{15}H_{18}NIO_2$: C, 48.52. H, 4.85. N, 3.77. Found: C, 47.66. H, 4.73. N, 3.56.

**N-(3-hydroxylpropyl)-2$\beta$-carbomethoxy-3$\beta$-(4'-iodophenyl)nortropane (6).** The procedure of Neumeyer *et al*[12] was followed and **6** (73%) was obtained as a colorless liquid. IR (film): 1744(C=O), 3380(OH), 2950 2852($OCH_3$), 1173(C-O), 818(-$C_6H_4$-) $cm^{-1}$. MS(m/e): 429 ($M^+$, 60%), 398 ($M^+$-$OCH_3$, 20%), 384 ($M^+$-$HOCH_2CH_2$, 45%), 370 ($M^+$-$HOCH_2CH_2CH_2$, 20%), 83($C_5H_9N$, 100%).

**N-(3-mesyloxypropyl)-2$\beta$-carbomethoxy-3$\beta$-(4'-iodophenyl)nortropane (7).** The procedure of Neumeyer *et al*[12] was followed and **7** (70%) was obtained as white semisolid at room temperature. IR (KBr): 1704(C=O), 2953($OCH_3$), 1349($SO_2$), 784(-$C_6H_4$-) $cm^{-1}$. MS(m/e): 507 ($M^+$, 37%), 476 ($M^+$-$OCH_3$, 4%), 448 ($M^+$-$COOCH_3$, 12%), 428 ($M^+$-$CH_3SO_2$, 8%), 412 ($M^+$-$CH_3SO_3$, 12%), 398 ($M^+$-$CH_3SO_3CH_2$, 5%), 384 ($M^+$-$CH_3SO_3CH_2CH_2$, 38%), 124 ($CH_3SO_3CH_2CH_3$, 100%).

**N-(3-fluoropropyl)-2$\beta$-carbomethoxy-3$\beta$-(4'-iodophenyl)nortropane (FP-$\beta$-CIT).** A solution of nor-$\beta$-CIT (**5**) ( 250 mg, 0.67 mmol), 1-bromo-3-fluoropropane (300 mg, 2.13 mmol), and triethylamine (0.5 mL) in toluene (20 mL) was refluxed under dry nitrogen atmosphere for 4 h, cooled, and filtered. The separated residue was washed twice with toluene (2 mL); the combined filtrate and washings were concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel and eluted with hexane/ether/triethylamine (10/7/0.1 V/V) to give 225.6 mg (78%) of FP-$\beta$-CIT as a white solid: mp 78~80°C; $[\alpha]_D^{20} = -36.6°$ (0.1% in $CH_3OH$). UV($CH_3CN$)$\lambda_{max}$=230 nm, $\varepsilon$=1.77×$10^4$. IR (KBr):1736(C=O), 2980(F-$CH_2$), 2955 2834 ($OCH_3$), 1196(C-O), 817(-$C_6H_4$-) $cm^{-1}$. $^1$HNMR($CDCl_3$) (400 MHz): 1.58~1.93 (5H, m, CH, $CH_2$), 1.96~2.18 (2H, m, $CH_2$), 2.30~2.50 (2H, m, $CH_2$), 2.54 (1H, t d, J1=2.56, $J_2$=6.23, CH), 2.89 (1H, t, J=4.03, COCH), 2.96 (1H, ddd, $J_1$=5.49, $J_2$=12.08, Ar-CH), 3.42(1H, m, CH), 3.49 (3H, s, $OCH_3$), 3.70 (1H, m, CH), 4.52 (2H, t d, $J_{HH}$=5.86, $J_{FH}$=47.23, $FCH_2$), 7.03 (2H, d, J=8.42, ArH), 7.58 (2H, d, J=8.05, ArH) ppm. MS(m/e): 431 ($M^+$, 88%), 400($M^+$-$OCH_3$, 12%), 384 ($M^+$-$FCH_2CH_2$, 42%), 372($M^+$-$COOCH_3$, 27%), 128 ($C_7H_{11}FN$, 100%), 83 ($C_5H_9N$, 92%). Anal. calcd. for $C_{18}H_{23}NFIO_2$: C, 50.12. H, 5.34. N, 3.25. Found: C, 50.03. H, 5.77. N, 2.96.

**$^{18}$F-N-(3-fluoropropyl)-2$\beta$-carbomethoxy-3$\beta$-(4'-iodophenyl)nortropane (1, $^{18}$F-FP-$\beta$-CIT).** 1 mL of stock solution containing 56 mCi $^{18}$F-fluoride, 10 mg K-222 (Kryptofix), 3 mg potassium carbonate, 0.05 mL water and 0.95 mL acetonitrile was evaporated to dryness with nitrogen stream while heating at 110°C. 2 mL of acetonitrile was added and evaporation was conducted to remove the residual water azeotropically. Mesylate precursor (3.5 mg, 6.9$\mu$mol) of FP-$\beta$-CIT in MeCN (1 mL) was then added, and the resulting mixture was heated at 100° for 10 min. The mixture was cooled in a water bath briefly (in the meantime, the labeling yield was determined), then passed through a short Sep-Pak column of silica gel (about 25 mm) in a 5 mL microvial. The reaction

tube was rinsed with 1 mL of ethyl acetate and the solution added to the column. The liquid was pushed through the column with air and then the column was eluted with an additional 1 mL of EtOAc. The eluate was evaporated with a stream of nitrogen while being warmed at 60°C. The residue was dissolved in a minimum solution of HPLC eluent (methanol/water/triethylamine 75/25/0.2) and loaded onto a reverse-phase semi-preparative column (Waters Delta Pak C-18 15 micro spherical, 120 angstroms pore size, 7.8×300 mm), A flow rate of 3 ml/min was used and $^{18}$F-FP-$\beta$-CIT was eluted after 16.8 min. The FP-$\beta$-CIT was detected by ultraviolet (UV) detector (254 nm) at the same HPLC condition. In the meantime, the radiochemical purity of $^{18}$F-FP-$\beta$-CIT was determined by radioactivity detector.

### 2.3 Determination of radiochemical purity

High performance liquid chromatography (HPLC) was used to evaluate the radiochemical purity of $^{18}$F-FP-$\beta$-CIT. Waters 515 HPLC system, Waters 486 Tunable Absorbance Detector and Waters$^{TM}$ 600 Controller $\gamma$-RAM IN/US system (USA) were used in combination with a RP-C18 column ($\phi$4.6mm×150 mm), using methanol/water/triethylamine (75/25/0.2) as mobile phase, with a flow rate of 2.0ml/min at room temperature.

### 2.4 Determination of in vitro stability

$^{18}$F-FP-$\beta$-CIT was allowed to stand at 25° for 4 hours, in which radiochemical purity was determined at regular intervals.

## 3 RESULTS AND DISCUSSIONS

### 3.1 Chemistry

CPT was obtained as a mixture of $\alpha$, $\beta$-epimer, $\beta$-epimer being the main product at low temperature. Two routes for the preparation of nor-$\beta$-CIT were tried: (1) the N-demethylation (56%) of $\beta$-CPT and then iodination (71%), and (2) the iodination (74%) of $\beta$-CPT and then N-demethylation (24%). It is obvious that the method reported in this paper provides a good yield of the final product that can be purified easily and satisfactorily.

If the radiochemical purity of $^{18}$F-FP-$\beta$-CIT was over 90% after passing through a short Sep-Pak column of silica gel, the labeled compound could be used as such without any further purification. FP-$\beta$-CIT over 99% of purity was also prepared for confirmation of the $^{18}$F-FP-$\beta$-CIT.

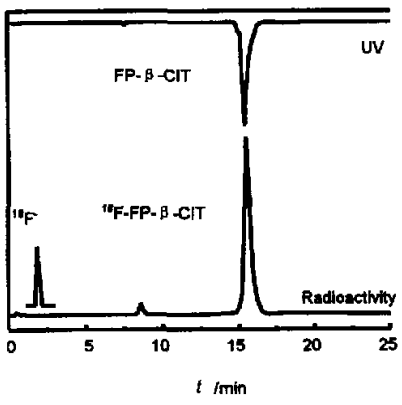


Fig.2 HPLC Chromatography of $^{18}$F-FP-$\beta$-CIT (detected by radioactivity) and FP-$\beta$-CIT (detected by UV) for preparation

### 3.2 Preparation and Radiochemical purity

The HPLC retention time ($t_R$) of $^{18}$F-FP-$\beta$-CIT and $^{18}F^-$ were 16.8 min and 2.4 min, respectively. The labeling yield of $^{18}$F-FP-$\beta$-CIT was 25%~32%, and the total radiochemical yield calculated from end of bombardment (EOB) with decay correction, was 10%~12% with a synthesis time of 100~110 min. The $t_R$ of FP-$\beta$-CIT, detected by a UV detector (254 nm), was also 16.8 min (Fig.2). The radiochemical purity of $^{18}$F-FP-$\beta$-CIT in analyzed HPLC was greater than 90% with $t_R$ of 8.4 and 0.5 min for $^{18}$F-FP-$\beta$-CIT and $^{18}F^-$, respectively.

### 3.3 Stability

The radiochemical purity of $^{18}$F-FP-$\beta$-CIT in aqueous solution had no obvious change after standing for 4 h at room temperature. It is stable enough for clinical study.

In conclusion, the fluorinated PET ligand was prepared with relatively high yield and stability. The imaging properties in vivo will be reported elsewhere.

### Acknowledgements

The authors thank Prof. Chan CHOU, Dr. Zhao-hui ZHU and Dr. Rui-xue CUI (PET Center, PUMC Hospital, CAMS and PUMC, Beijing, China) for their help.

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