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Metal-free ^{18}F labeling of aryl-CF_2H via nucleophilic radiofluorination and oxidative C-H activation

Gengyang Yuan,[a, b] Feng Wang,[c] Nickeisha Stephenson,[a] Lu Wang,[a] Benjamin H. Rotstein,[a] Neil Vasdev,[a] Pingping Tang[c] and Steven H. Liang*[, a]

[a] Gordon Center for Medical Imaging & Division of Nuclear Medicine and Molecular Imaging, Massachusetts General Hospital and Department of Radiology, Harvard Medical School, 55 Fruit St., Boston, MA, 02114 (USA)

[b] Department of Chemistry & Chemical Biology, Northeastern University, 360 Huntington Ave., Boston, MA, 02115 (USA)

[c] State Key Laboratory and Institute of Elemento-Organic Chemistry, Nankai University, Collaborative Innovation Center of Chemical Science and Engineering, Tianjin, 300071 (China)

Email: liang.steven@mgh.harvard.edu

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GENERAL INFORMATION

All solvents were of reagent or anhydrous grade quality and purchased from Sigma-Aldrich, Alfa Aesar, or Fisher Scientific. All reagents were purchased from Sigma-Aldrich, Alfa Aesar, Fisher Scientific, or Oakwood Chemical, unless otherwise stated. All deuterated solvents were purchased from Cambridge isotopes. Analytical thin-layer chromatography (TLC) was performed on pre-coated glass-backed plates (EMD TLC Silica gel 60 F_{254}) and visualized using a UV lamp (254 nm), potassium permanganate stain. Flash column chromatography was performed using a Biotage Isolera One system and preloaded Biotage Zip silica gel columns. Silica gel for flash chromatography was high purity grade 40-63 μm pore size and purchased from Sigma-Aldrich. Yields refer to purified and spectroscopically pure compounds.

^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker 300 MHz spectrometer, and resonances are given in parts per million (ppm) relative residual solvent. Peak multiplicities are designated by the following abbreviations: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets; br, broad; and *J*, coupling constant in Hz.

HRMS spectra were recorded on a Bruker microTOFII ESI or Advion Expression CMS compact mass spectrometer.

ORGANIC CHEMISTRY

1) General procedure for the synthesis of standard aryl-CFH_2 compounds:

Compounds **2a-2i** were synthesized from **1a-1i** via *tetra-n*-butylammonium fluoride (TBAF) fluorination. Briefly, in a dried 3-neck round bottom flask, 0.36 mmol **1a-1i** were dissolved in 1.0 mL tetrahydrofuran (THF), and then 1.08 mL TBAF (1.0 M in THF solution) was added under nitrogen atmosphere. The reaction mixture was heated to reflux for 2 h or overnight. The resulting mixture was quenched with water, extracted with ethyl acetate (3 x 10 mL), and dried with anhydrous sodium sulfate. Solvent was removed under vacuum, and the crude product was purified via silica gel chromatography.

2) General procedure for the synthesis of standard aryl-CF_2H compounds:

The aryl-CF_2H products were synthesized followed by our previous method with the aryl-CH_3 substrates[1-2]: In general, under nitrogen atmosphere, to the corresponding aryl-CH_3 (1.00 mmol, 1.00 equiv), $Na_2S_2O_8$ (5.00 mmol, 5.00 equiv) and 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(hexafluorophosph-ate) (Selectfluor, 3.00 mmol, 3.00 equiv) in Schlenk tube were added MeCN (4.0 mL) and H_2O (4.0 mL). The mixture was cooled by liquid nitrogen, then $AgNO_3$ (0.100 mmol, 0.100 equiv) was added. The reaction mixture was degassed three times by Freeze-Pump-Thaw cycles and then was stirred for 3.0 h or overnight at 80 °C. After cooling to room temperature, the mixture was filtered through a pad of celite, eluting with ethyl acetate. The filtrate was washed with saturated $NaHCO_3$ solution (10 mL), sat. NaCl, and dried over Na_2SO_4. The filtrate was concentrated in vacuo and the residue was purified by chromatography on silica gel, eluting with hexane/ethyl acetate to afford the **3a-3h.**

*Synthesis of methyl 5-bromo-2-(fluoromethyl)benzoate (**2e**).* ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (t, *J* = 1.7 Hz, 1H), 7.72 (dd, *J* = 8.5Hz, 2.1 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 5.78 (d, *J* = 48.3 Hz, 2H), 3.90 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6, 138.9 (d, *J* = 17.7Hz), 135.8 (d, *J* = 2.1Hz), 133.4, 128.1 (d, *J* = 3.5Hz), 127.5 (d, *J* = 17.0Hz), 121.3 (d, *J* = 2.1Hz), 82.3 (d, *J* = 169.1Hz), 52.4; ^{19}F NMR (282 MHz, $CDCl_3$) δ -214.9 (t, *J* = 48.2Hz). Mass-

Spectrometry: HRMS-ESI (m/z): Calcd for $C_9H_8BrFO_2H$ $[M+H]^+$, 246.9764. Found, 246.9759.

Synthesis of 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo [2.2.2] octane bis(hexafluorophosphate) (F-TEDA-PF_6) [3] was achieved by using a reported method. See reference 3.

Synthesis of 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo [2.2.2] octane bis(trifluoromethane sulfonate) (F-TEDA-OTf. In a 15 mL round bottom flask, Selectfluor (0.1 g, 0.28 mmol), LiOTf (0.45 g, 2.8 mmol) and 2.5 mL H_2O were added. The resulting mixture was stored in fridge (2-5 °C) overnight. The product precipitated out as white crystal, which was washed with 2*3 mL water to get the pure product (0.12 g, 90%). ^{1}H NMR (300 MHz, D_2O) δ 5.47 (s, 2H), 4.94 (dd, J = 14.7 Hz, 7.0 Hz, 6H), 4.48 (t, J = 7.3 Hz, 6H); ^{13}C NMR (75 MHz, D_2O) δ 112.9, 121.7, 117.5, 113.3, 69.0, 57.4, 57.2, 53.7 (t, J = 3.3 Hz); ^{19}F NMR (282 MHz, D_2O) δ -74.9. LC-MS found 329.2 $[M\text{-}OTf]^+$.

RADIOCHEMISTRY

General methods for radioisotope preparation

A GE PETtrace 16.5 MeV cyclotron was used for [^{18}F]fluoride production by the ^{18}O(p,n)^{18}F nuclear reaction to irradiate ^{18}O-enriched water. [^{18}F]fluoride was delivered to a lead-shielded hot cell in ^{18}O-enriched water by nitrogen gas pressure. [^{18}F]Fluoride was prepared for radiofluorination of aromatics by the following method: a solution of base (tetraethylammonium bicarbonate (TEAB)) or potassium carbonate/2,2,2-crypt (Kryptofix®) in acetonitrile and water (1 mL, v/v 7 : 3) was added to an aliquot of target water (≤ 1 mL) containing the appropriate amount of [^{18}F]fluoride in a V-shaped vial sealed with a teflon-lined septum. The vial was heated to 110 °C (for TEAB) or 95 °C (for K_2CO_3/Kryptofix®) while nitrogen gas was passed through a P_2O_5-Drierite™ column followed by the vented vial. When no liquid was visible in the vial, it was removed from heat, anhydrous acetonitrile (1 mL) was added, and the heating was resumed until dryness. This step was repeated an additional three times. The vial was then cooled at room temperature under nitrogen pressure. The contents were resolubilized in the suitable solvents.

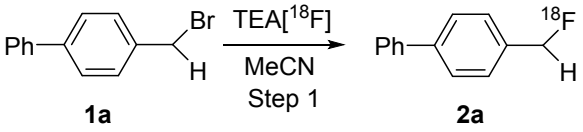
General methods for analysis of radiofluorination reactions

Radiochemical incorporation yields were determined by radioTLC. EMD TLC Silica gel 60 plates (10 x 2 cm) were spotted with an aliquot (1-5 μL) of crude reaction mixture approximately 1.5 cm from the bottom of the plate (baseline). TLC plates were developed in a chamber containing ethyl acetate (EtOAc) until within 2 cm of the top of the plate (front). Analysis was performed using a Bioscan AR-2000 radio-TLC imaging scanner and WinScan software. Radiochemical identity and purity were determined by radioHPLC. A Phenomenex Luna C18, 250 x 4.6 mm, 5 μm HPLC column was used for the analytical analysis with a Waters 1515 Isocratic HPLC Pump equipped with a Waters 2487 Dual λ Absorbance Detector, a Bioscan Flow-Count equipped with a NaI crystal, and Breeze software. The

mobile phase for analytical HPLC analysis was MeCN in 0.1 M $NH_4 \cdot HCO_2$ (aq); The flow rate was 1mL/min. The semi-preparative purifications were performed on a Phenomenex Luna C18, 250 x 100 mm, 5 μm HPLC column with MeCN in 0.1 M $NH_4 \cdot HCO_2$ (aq) and the flow rate was 5 mL/min. All radiochemical yields are non-decay corrected. Each radiochemical labeling was conducted at least two times ($n \geq 2$).

Optimization of radiofluorination conditions

Optimizations of the 1st step radiosynthesis of [^{18}F]**2a**



(1) Method:

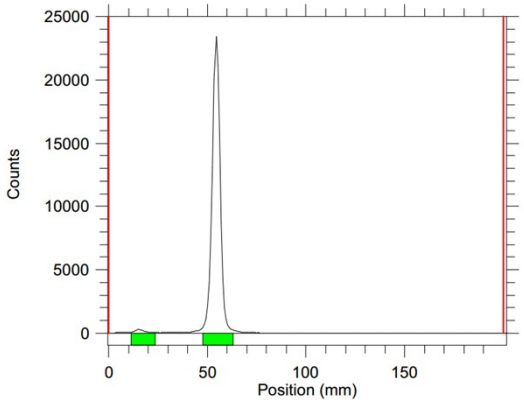
5 mg tetraethyl ammonium bicarbonate (TEAB) was used as a base to dry [^{18}F]fluoride. 0.5 mL MeCN was used to solubilize the TEA^{18}F; 2.0 mg precursor (**1a**) was added into the reaction vial, to which the indicated amount of TEA^{18}F in MeCN was added. The resulting reaction mixture was tested at different reaction conditions, and the resulting radiochemical conversions (RCCs) were determined by RadioTLC chromatography.

*(2) 1st step radiosynthesis of [18F]**2a** optimization results*

Entry	**1a** (mg)	Base (mg)	Time (min)	Temp. (°C)	[18F]**2a** RCC (%)
1	2.0	TBAH (1.56)	5	130	40 ± 2 (n = 2)
2	2.0	TBAH (3.12)	5	130	43 ± 1 (n = 2)
3	2.0	TBAH (4.69)	5	130	39 ± 1 (n = 2)
4	2.0	TBAH (3.12)	10	130	49 ± 2 (n = 2)
4	2.0	TEAB (0.5)	10	130	50 ± 2 (n = 2)
5	2.0	TEAB (1.0)	10	130	85 ± 1 (n = 2)
6	2.0	TEAB (2.0)	10	130	98 ± 2% (n > 10)
7	2.0	TEAB (2.5)	10	130	88 ± 1 (n = 2)
8	2.0	TEAB (2.0)	10	100	90 ± 2 (n = 3)
9	2.0	TEAB (2.0)	10	80	85 ± 3 (n = 3)

Table S1: Optimizations of the 1st step radiosynthesis of [18F]**2a.**

(3) RadioTLC chromatogram of 1st step reaction mixture:



	1	2	3	4	5	mean	standard deviation
RCC (%)	99	98	95	96	99	98	2

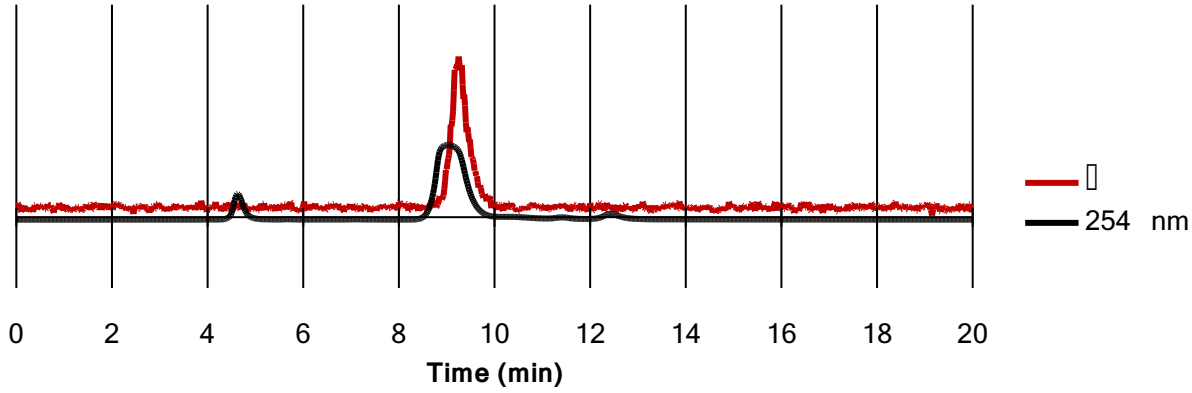
(4) RadioHPLC chromatogram:

a) Analytical analysis:

Column: Phenomenex Luna, C18, 250 x 4.6 mm, 5 µm

Mobile phase: 70% MeCN, 30% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



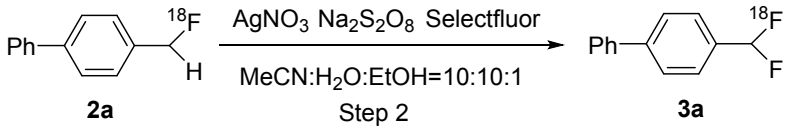
b) Preparative HPLC purification:

Column: Phenomenex Luna C18, 250 x 100 mm, 5 µm

Mobile phase: 65% MeCN, 35% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 5 mL/min;

Optimizations of the 2nd step radiosynthesis of [^{18}F]**3a**



2a **3a**

(1) Method:

The isolated [^{18}F]**2a** was diluted with 30 mL H_2O, concentrated with t-C18 plus Sep-Pak® cartridge (Waters; pre-activated with 10 mL EtOH, 10 mL air, followed by 10 mL H_2O), and then eluted with 1 mL MeCN. This solution was divided into several aliquots (0.2 mL each) into

separate vials, containing the indicated amounts of $AgNO_3$, $Na_2S_2O_8$ and Selectfluor. Then, 0.2 mLH_2O and EtOH was added to each vial, and the reaction was allowed to react at specified conditions. At the end, each reaction was quenched with 0.4 mL mobile, and the resulting RCCs were determined by RadioHPLC chromatography.

*(2) 2nd step radiosynthesis of [18F]**3a** optimization results*

Entry	Degas	EtOH (µL)	$AgNO_3$ (mg)	$Na_2S_2O_8$ (mg)	Selectfluor (mg)	Temp. (°C)	Time (min)	**[18F]3a RCC (%)**
1	Y	N	2	4	20	80	10	0 (n = 1)
2	Y	N	2	4	20	100	10	0 (n = 1)
3	Y	N	2	4	20	120	10	4 ± 1 (n = 1)
4	Y	20	2	4	20	120	10	20 (n = 1)
5	Y	20	4	4	20	120	10	44 ± 7 (n = 2)
6	N	N	2	4	20	120	10	0 (n = 1)
7	N	20	2	4	20	120	10	14 ± 2 (n = 2)
8	N	100	2	4	20	120	10	0 (n = 2)
9	N	20	4	4	20	120	10	38 ± 5 (n = 2)
10	N	20	8	4	20	120	10	20 ± 2 (n = 2)
11	N	20	12	4	20	120	10	22 ± 3 (n = 2)
12	N	20	4	8	20	120	10	40 ± 3 (n = 2)
13	N	20	4	12	20	120	10	23 ± 3 (n = 2)
14	N	20	4	16	20	120	10	20 ± 4 (n = 2)
15	N	20	4	8	5	120	10	0 (n = 2)
16	N	20	4	8	10	120	10	9 ± 1 (n = 2)
17	N	20	4	8	15	120	10	33 ± 3 (n = 2)
18	**N**	**20**	**0**	**8**	**20**	**120**	**10**	**50 ± 5 (n = 3)**
19	N	N	0	8	20	120	10	0 (n = 3)
20[a]	N	N	4	8	20	120	10	41 ± 6 (n = 3)
21[a]	N	N	0	8	20	120	10	25 ± 5 (n = 2)

[a] one-pot synthesis

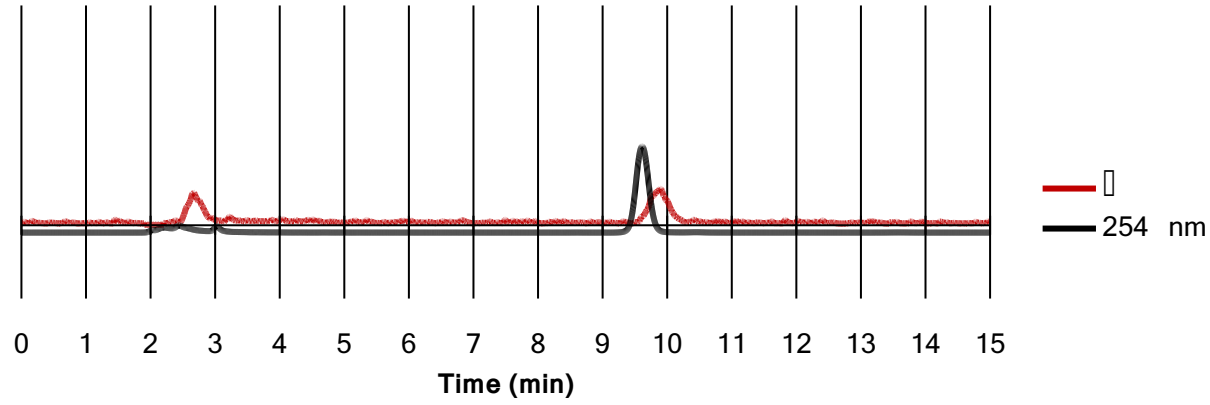
Table S2: Optimizations of the 2nd step radiosynthesis of [18F]**3a.**

(3) RadioHPLC chromatogram:

Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 µm

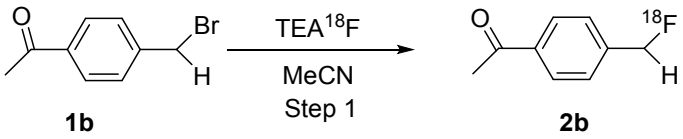
Mobile phase: 70% MeCN, 30% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



Application of the optimal reaction condition of [^{18}F]3a toward [^{18}F]3b-3i.

1st step radiosynthesis of [^{18}F]**2b**

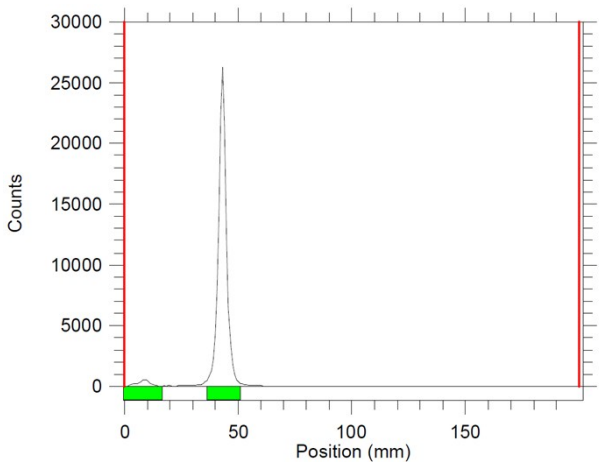


1b **2b**

(1) Method:

The same optimal reaction conditions as that of [^{18}F]**2a** were used with 2.0 mg compound **1b**.

*(2) RadioTLC chromatogram of [^{18}F]**2b**:*



	1	2	3	4	5	mean	standard deviation
RCC (%)	99	98	95	96	99	98	2

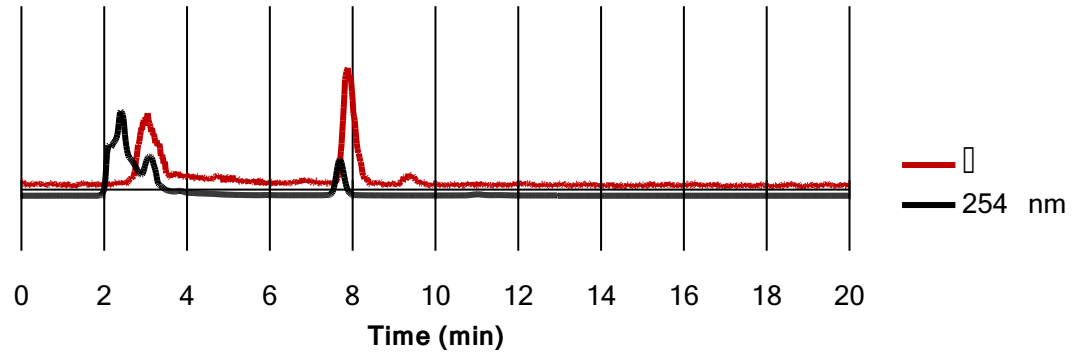
(3) RadioHPLC chromatogram:

a) Analytical analysis:

Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 µm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



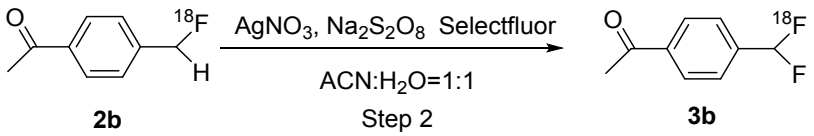
b) Preparative HPLC purification:

Column: Phenomenex Luna C18, 250 x 100 mm, 5 µm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 5 mL/min;

2nd step radiosynthesis of [^{18}F]**3b**



2b **3b**

(1) Method:

The same optimal reaction conditions as that of [^{18}F]**3a** were used for the synthesis of [^{18}F]**3b**, but no EtOH was added.

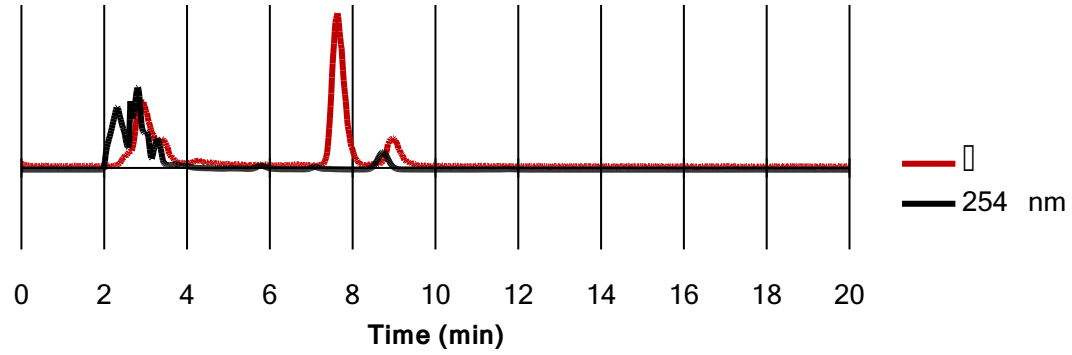
Entry	EtOH (μL)	$AgNO_3$ (mg)	$Na_2S_2O_8$ (mg)	Selectfluor (mg)	**3b RCC (%)**
1	20.0	0.0	8.0	20.0	0 (n = 2)
2	0.0	4.0	8.0	20.0	10 ± 2% (n = 2)
3	0.0	0.0	8.0	20.0	10 ± 2% (n = 3)

(2) RadioHPLC chromatogram:

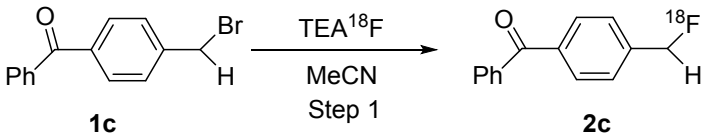
Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 μm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



1st step radiosynthesis of [^{18}F]**2c**

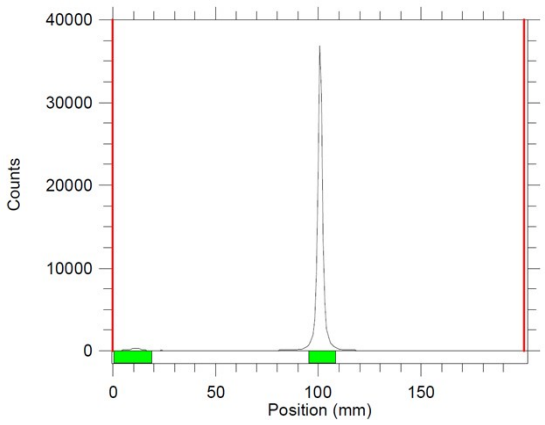


1c **2c**

(1) Method:

The same optimal reaction conditions as that of [^{18}F]**2a** were used with 2.0 mg compound **1c**.

*(2) RadioTLC chromatogram of [^{18}F]**2c**:*



	1	2	3	4	5	mean	standard deviation
RCC (%)	99	98	97	94	99	98	2

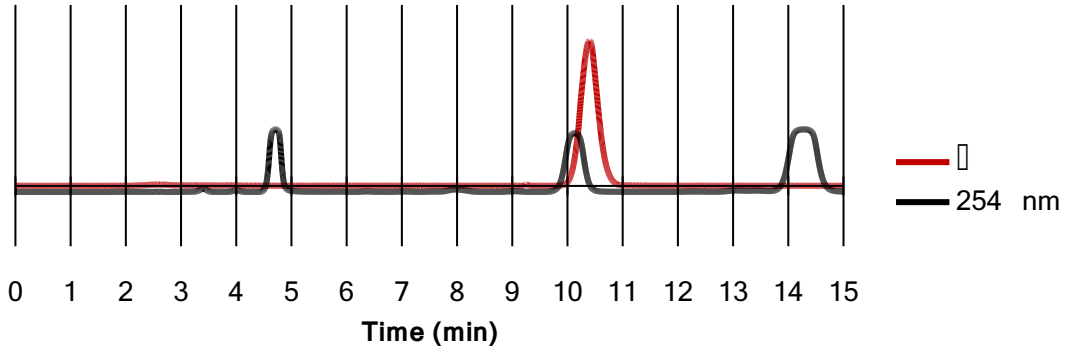
(3) RadioHPLC chromatogram:

a) Analytical analysis:

Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 µm

Mobile phase: 60% MeCN, 40% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



b)

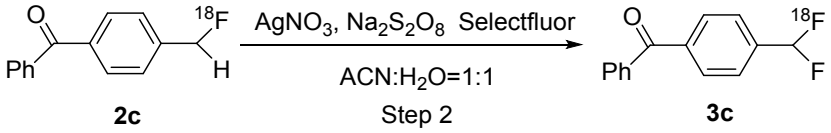
Preparative HPLC purification:

Column: Phenomenex Luna C18, 250 x 100 mm, 5 µm

Mobile phase: 60% MeCN, 40% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 5 mL/min;

2nd step radiosynthesis of [^{18}F]**3c**



2c **3c**

(1) Method:

The same optimal reaction conditions as that of [^{18}F]**3a** were used for the synthesis of [^{18}F]**3c**, but no EtOH was added.

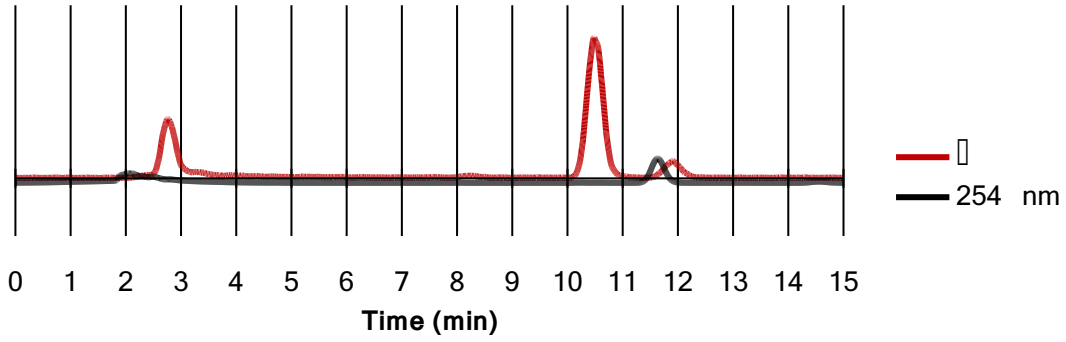
Entry	EtOH (μL)	AgNO$_3$ (mg)	Na$_2$S$_2$O$_8$ (mg)	Selectfluor (mg)	**3c RCC (%)**
1	20.0	0.0	8.0	20.0	0 (n = 2)
2	0.0	4.0	8.0	20.0	12 ± 2% (n = 2)
3	0.0	0.0	8.0	20.0	15 ± 3% (n = 3)

(2) RadioHPLC chromatogram:

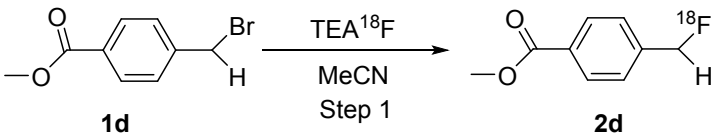
Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 μm

Mobile phase: 60% MeCN, 40% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



1st step radiosynthesis of [^{18}F]**2d**

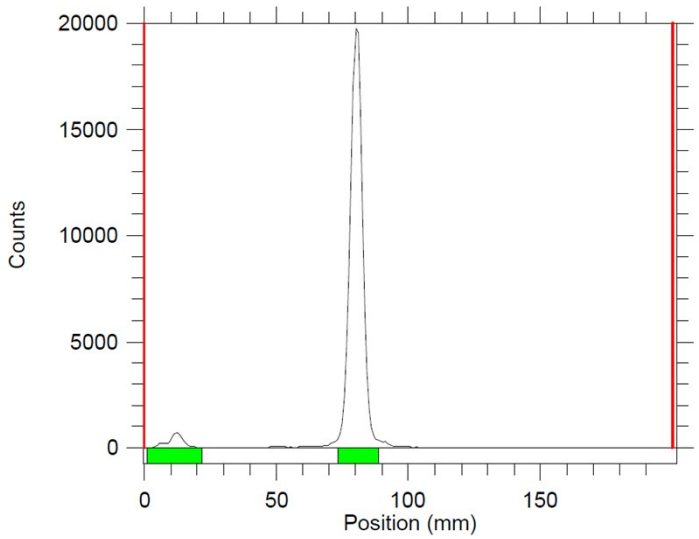


1d **2d**

(1) Method:

The same optimal reaction conditions as that of [^{18}F]**2a** were used with 2.0 mg compound **1d**.

*(2) RadioTLC chromatogram of [^{18}F]**2d**:*



	1	2	3	4	5	mean	standard deviation
RCC (%)	99	98	97	94	98	98	2

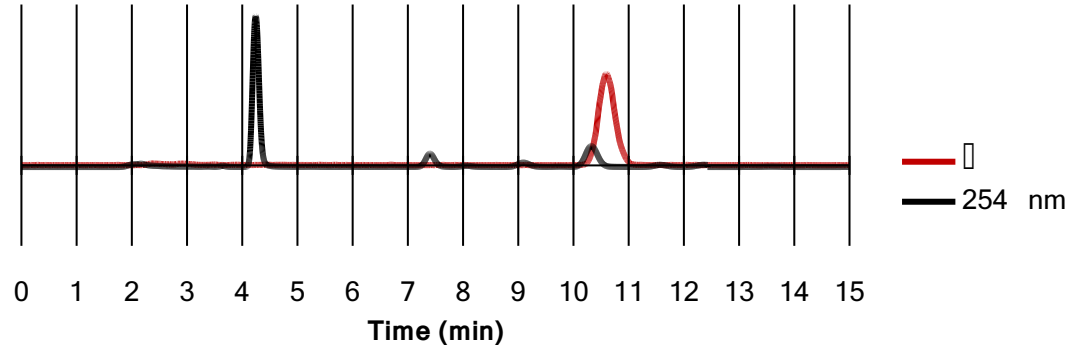
(3) RadioHPLC chromatogram:

a) Analytical analysis:

Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 µm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



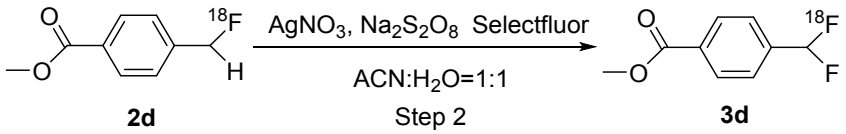
b) Preparative HPLC purification:

Column: Phenomenex Luna C18, 250 x 100 mm, 5 µm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4{\cdot}HCO_2$(aq);

Flow rate: 5 mL/min;

2nd step radiosynthesis of [^{18}F]**3d**



(1) Method:

The same optimal reaction conditions as that of [^{18}F]**3a** were used for the synthesis of [^{18}F]**3d**, but no EtOH was added.

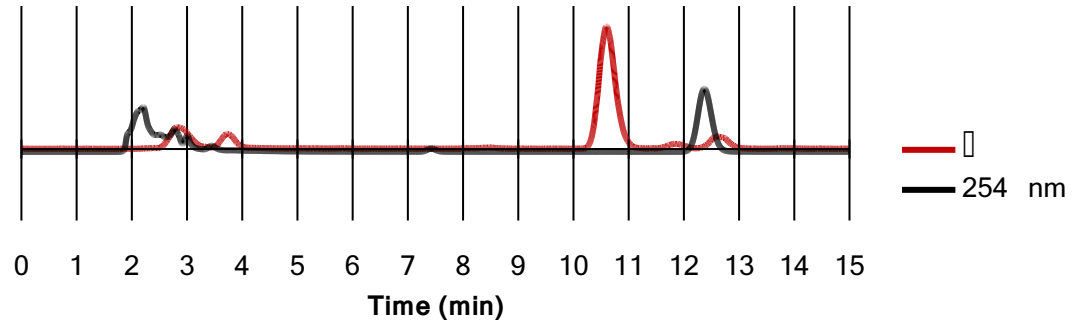
Entry	EtOH (µL)	$AgNO_3$ (mg)	$Na_2S_2O_8$ (mg)	Selectfluor (mg)	**3d RCC (%)**
1	20.0	0.0	8.0	20.0	0 (n = 2)
2	0.0	4.0	8.0	20.0	8 ± 1% (n = 2)
3	0.0	0.0	8.0	20.0	12 ± 2% (n = 3)
4	0.0	4.0	8.0	40.0	8 ± 1% (n = 2)
5	0.0	4.0	16.0	20.0	5 ± 2% (n = 2)
6	0.0	8.0	16.0	20.0	5 ± 2% (n = 2)

(2) RadioHPLC chromatogram:

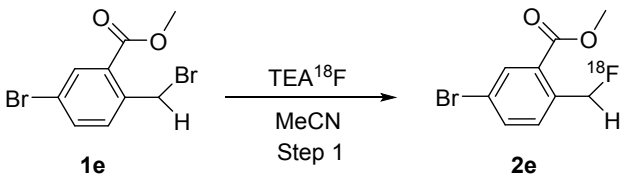
Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 µm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4{\cdot}HCO_2$(aq);

Flow rate: 1 mL/min;



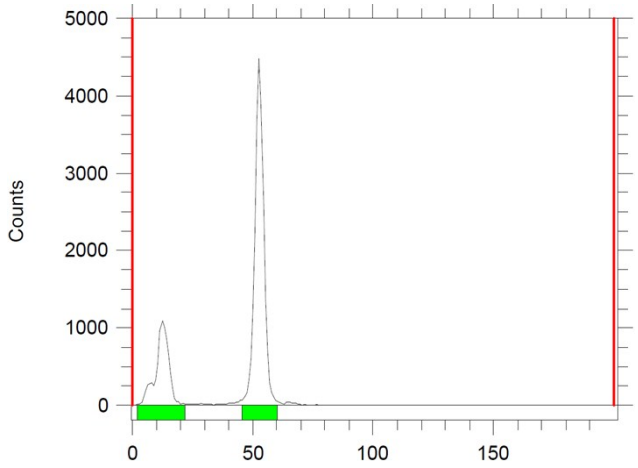
1st step radiosynthesis of [^{18}F]**2e**



(1) Method:

The same optimal reaction conditions as that of [^{18}F]**2a** were used with 2.0 mg compound **1e**.

*(2) RadioTLC chromatogram of [^{18}F]**2e**:*



	1	2	3	4	5	mean	standard deviation
RCC (%)	80	92	85	82	86	85	5

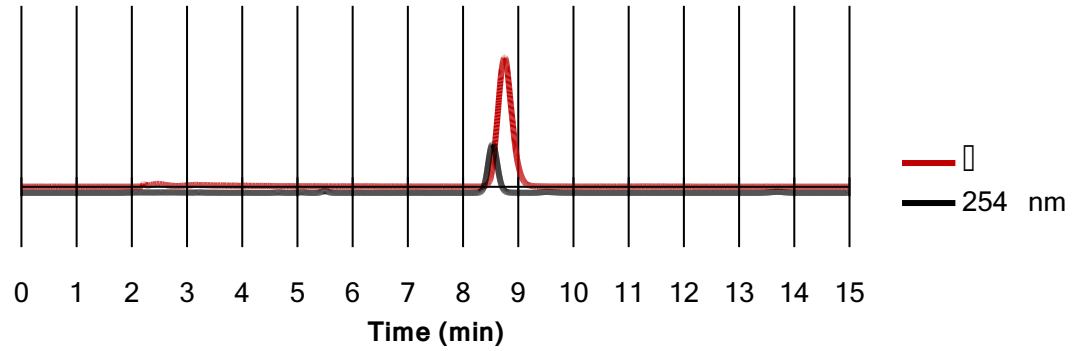
(3) RadioHPLC chromatogram:

a) Analytical analysis:

Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 µm

Mobile phase: 60% MeCN, 40% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



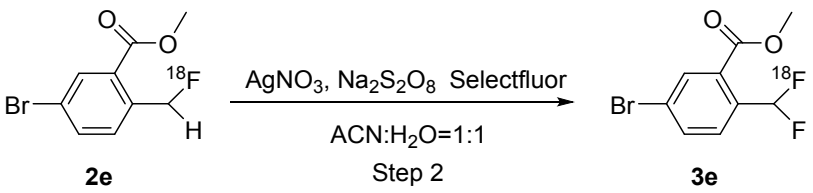
b) Preparative HPLC purification:

Column: Phenomenex Luna C18, 250 x 100 mm, 5 µm

Mobile phase: 60% MeCN, 40% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 5 mL/min;

2nd step radiosynthesis of [^{18}F]**3c**



2e **3e**

(1) Method:

The same optimal reaction conditions as that of [^{18}F]**3a** were used for the synthesis of [^{18}F]**3e**, but no EtOH was added.

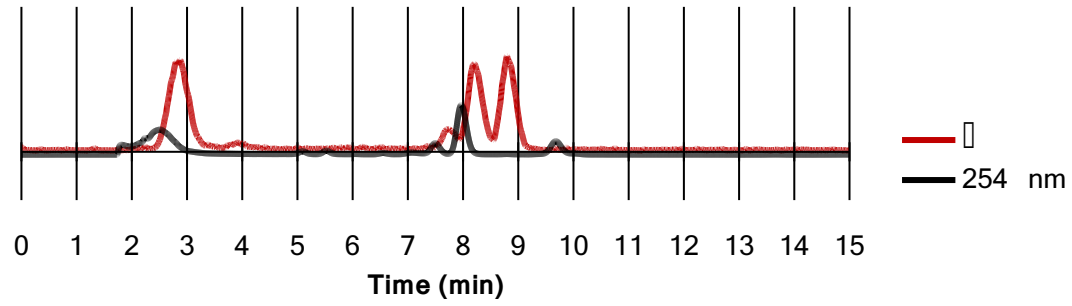
Entry	EtOH (μL)	$AgNO_3$ (mg)	$Na_2S_2O_8$ (mg)	Selectfluor (mg)	**3c RCC (%)**
1	20.0	0.0	8.0	20.0	0 (n = 2)
2	0.0	4.0	8.0	20.0	12 ± 2% (n = 2)
3	0.0	0.0	8.0	20.0	28 ± 3% (n = 3)

(2) RadioHPLC chromatogram:

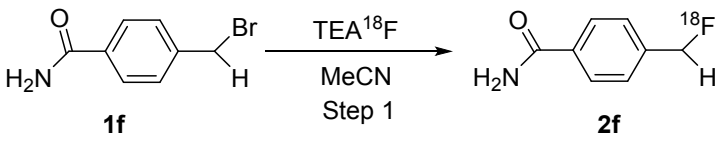
Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 μm

Mobile phase: 60% MeCN, 40% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



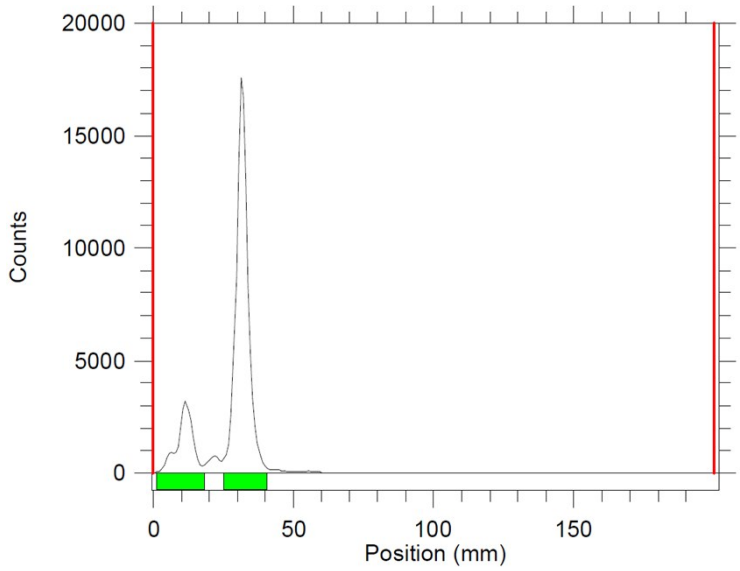
1st step radiosynthesis of [^{18}F]**2f**



(1) Method:

The same optimal reaction conditions as that of [^{18}F]**2a** were used with 2.0 mg compound **1f**.

*(2) RadioTLC chromatogram of [^{18}F]**2f**:*



	1	2	3	4	5	mean	standard deviation
RCC (%)	67	75	73	68	70	70	3

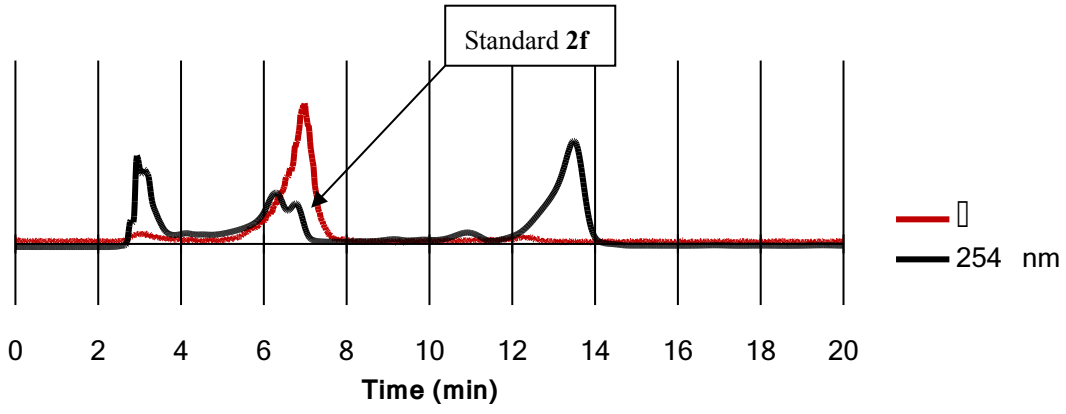
(3) RadioHPLC chromatogram:

a) Analytical analysis:

Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 µm

Mobile phase: 25% MeCN, 75% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



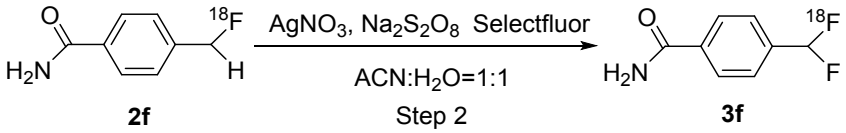
b) Preparative HPLC purification:

Column: Phenomenex Luna C18, 250 x 100 mm, 5 µm

Mobile phase: 10% MeCN, 90% 0.1 M $NH_4{\cdot}HCO_2$(aq);

Flow rate: 5 mL/min;

2nd step radiosynthesis of [^{18}F]**3c**



2f **3f**

(1) Method:

The same optimal reaction conditions as that of [^{18}F]**3a** were used for the synthesis of [^{18}F]**3f**, but no EtOH was added, and 40.0 mg Selectfluor was used.

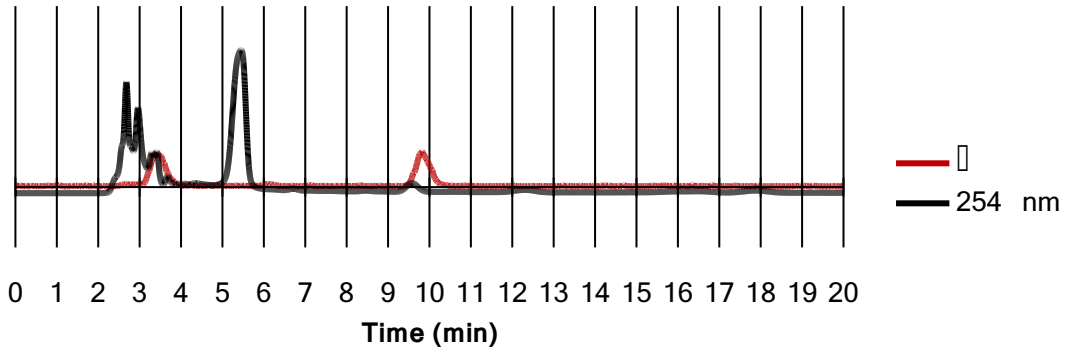
Entry	EtOH (μL)	$AgNO_3$ (mg)	$Na_2S_2O_8$ (mg)	Selectfluor (mg)	**3c RCC (%)**
1	20.0	0.0	8.0	20.0	0 (n = 2)
2	0.0	0.0	8.0	20.0	18 ± 3% (n = 2)
3	0.0	4.0	8.0	40.0	20 ± 2% (n = 2)
4	0.0	0.0	8.0	40.0	45 ± 5% (n = 3)

(2) RadioHPLC chromatogram:

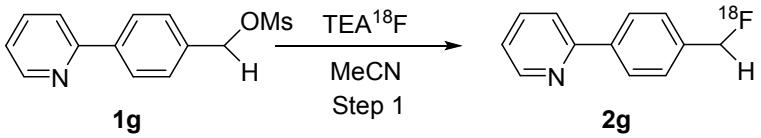
Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 μm

Mobile phase: 25% MeCN, 75% 0.1 M $NH_4{\cdot}HCO_2$(aq);

Flow rate: 1 mL/min;



1st step radiosynthesis of [^{18}F]**2g**



1g **2g**

(1) Method:

The same optimal reaction conditions as that of [^{18}F]**2a** were used with 2.0 mg compound **1g**.

*(2) RadioTLC chromatogram of [^{18}F]**2g**:*

	1	2	3	4	5	mean	standard deviation
RCC (%)	98	98	97	96	99	98	2

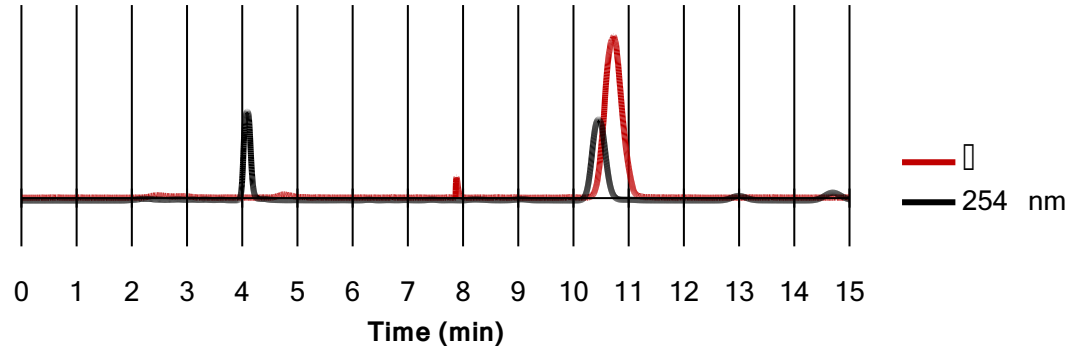
(3) RadioHPLC chromatogram:

a) Analytical analysis:

Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 µm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



b)

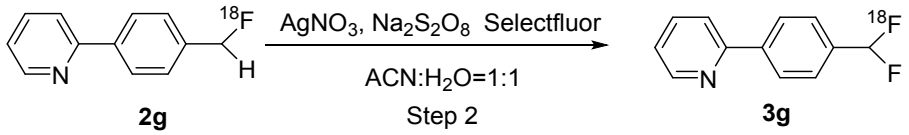
Preparative HPLC purification:

Column: Phenomenex Luna C18, 250 x 100 mm, 5 µm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 5 mL/min;

2nd step radiosynthesis of [^{18}F]**3g**



2g **3g**

(1) Method:

The same optimal reaction conditions as that of [^{18}F]**3a** were used for the synthesis of [^{18}F]**3g**, but no EtOH was added.

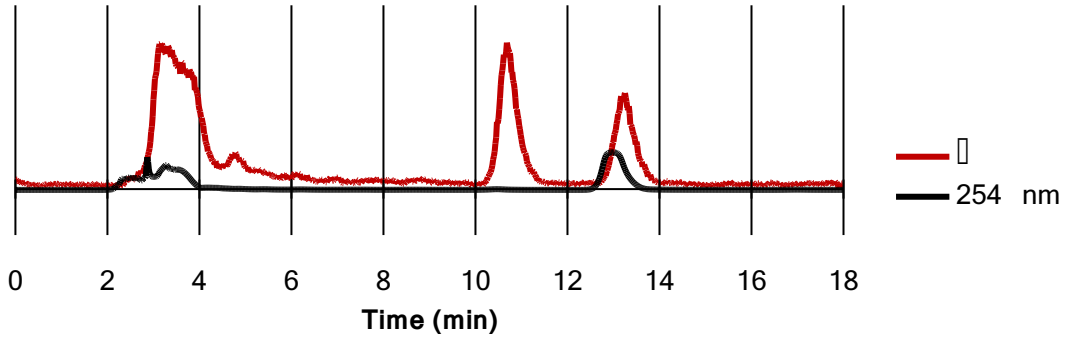
Entry	EtOH (µL)	$AgNO_3$ (mg)	$Na_2S_2O_8$ (mg)	Selectfluor (mg)	**3c RCC (%)**
1	20.0	0.0	8.0	20.0	0 (n = 2)
2	0.0	4.0	8.0	20.0	18 ± 2% (n = 3)
3	0.0	0.0	8.0	20.0	20 ± 3% (n = 3)

(2) RadioHPLC chromatogram:

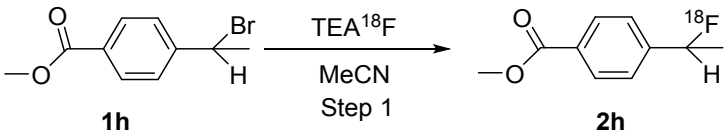
Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 µm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



1st step radiosynthesis of [^{18}F]**2h**



(1) Method:

The same optimal reaction conditions as that of [^{18}F]**2a** were used with 2.0 mg compound **1h**.

*(2) RadioTLC chromatogram of [^{18}F]**2h**:*

	1	2	3	4	5	mean	standard deviation
RCC (%)	99	98	97	94	99	98	2

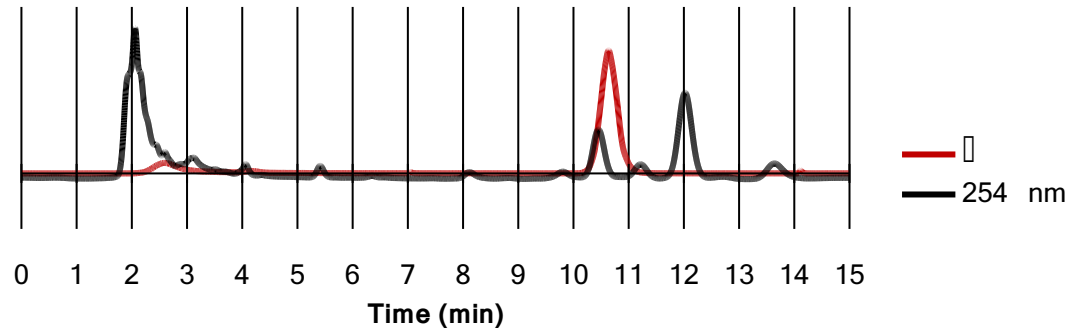
(3) RadioHPLC chromatogram:

a) Analytical analysis:

Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 µm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



b)

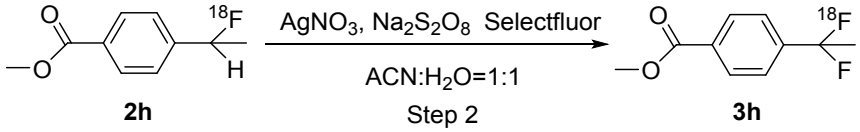
Preparative HPLC purification:

Column: Phenomenex Luna C18, 250 x 100 mm, 10 µm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 5 mL/min;

2nd step radiosynthesis of [^{18}F]**3h**



(1) Method:

The same optimal reaction conditions as that of [^{18}F]**3a** were used for the synthesis of [^{18}F]**3h**, but no EtOH was added.

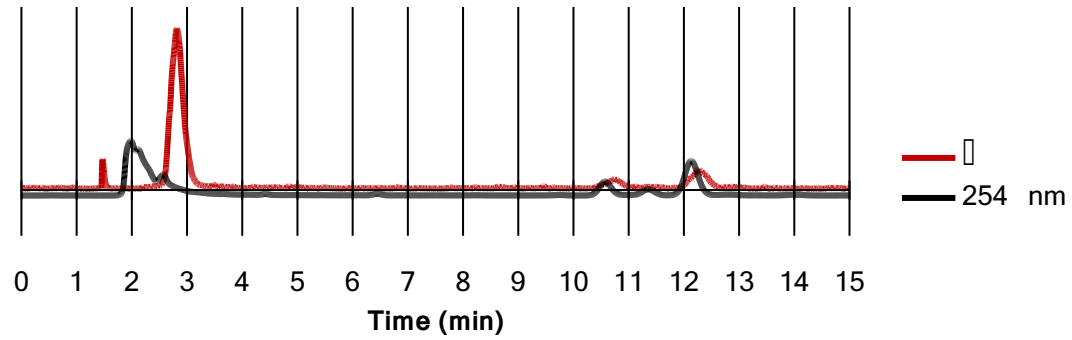
Entry	EtOH (µL)	$AgNO_3$ (mg)	$Na_2S_2O_8$ (mg)	Selectfluor (mg)	**3c RCC (%)**
1	20.0	0.0	8.0	20.0	0 (n = 2)
2	0.0	4.0	8.0	20.0	10 ± 3% (n = 2)
3	0.0	0.0	8.0	20.0	15 ± 2% (n = 3)

(2) RadioHPLC chromatogram:

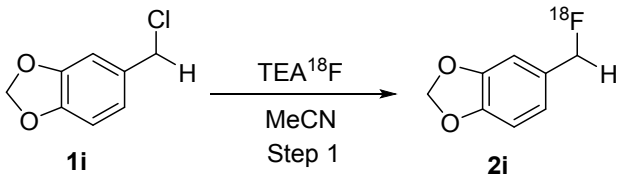
Column: Phenomenex Luna C18, 250 x 4.6 mm, 5 µm

Mobile phase: 50% MeCN, 50% 0.1 M $NH_4 \cdot HCO_2$(aq);

Flow rate: 1 mL/min;



1st step radiosynthesis of [^{18}F]**2i**



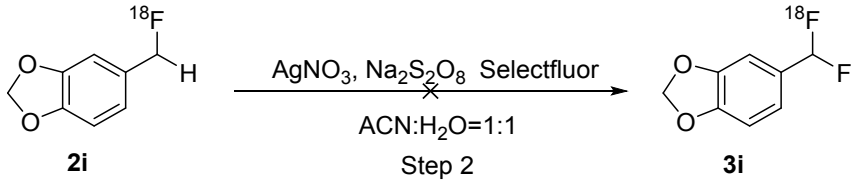
(1) Method:

The same optimal reaction conditions as that of [^{18}F]**2a** were used with 2.0 mg compound **1i**.

*(2) RadioTLC chromatogram of [^{18}F]**2i**:*

	1	2	3	4	5	mean	standard deviation
RCC (%)	98	97	97	94	99	98	2

2nd step radiosynthesis of [^{18}F]**3c**



(1) Method:

The same optimal reaction conditions as that of [^{18}F]**3a** were used for the synthesis of [^{18}F]**3c**, but no EtOH was added.

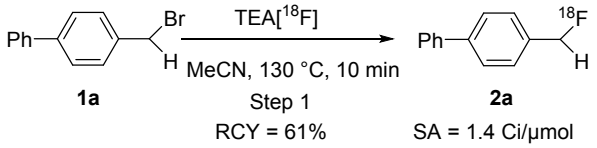
Entry	EtOH (μL)	$AgNO_3$ (mg)	$Na_2S_2O_8$ (mg)	Selectfluor (mg)	**3c RCC (%)**
1	20.0	0.0	8.0	20.0	0 (n = 2)
2	0.0	4.0	8.0	20.0	0 (n = 2)
3	0.0	0.0	8.0	20.0	0 (n = 2)
4	0.0	0.0	0.0	4.0	0 (n = 2)
5	0.0	0.0	8.0	20.0 (F-TEDA-PF6)	0 (n = 2)
6	0.0	0.0	0.0	4.0 (F-TEDA-PF6)	0 (n = 2)

Result: the analytic HPLC showed only a peak at 2-3min, indicating the desired product [^{18}F]**3i** was not formed and the intermediate [^{18}F]**2i** was decomposed.

SA determinations of the 4-([^{18}F]fluoromethyl)-1,1'-biphenyl(2a) and 4-([^{18}F]difluoromethyl)-1,1'-biphenyl(3a)

Step 1.

(1) Automated synthesis of [^{18}F]2a by GE TracerLab FX$_{FN}$ method



Following completion of bombardment, the [^{18}F]fluoride was transferred to the GE TRACERlab™ FX$_{FN}$ radiosynthesis module via helium gas overpressure. A schematic diagram of the GE medical systems commercial TRACERlab™ FX$_{FN}$ radiosynthesis module used for the synthesis of **[^{18}F]2a** is shown in Figure S1.

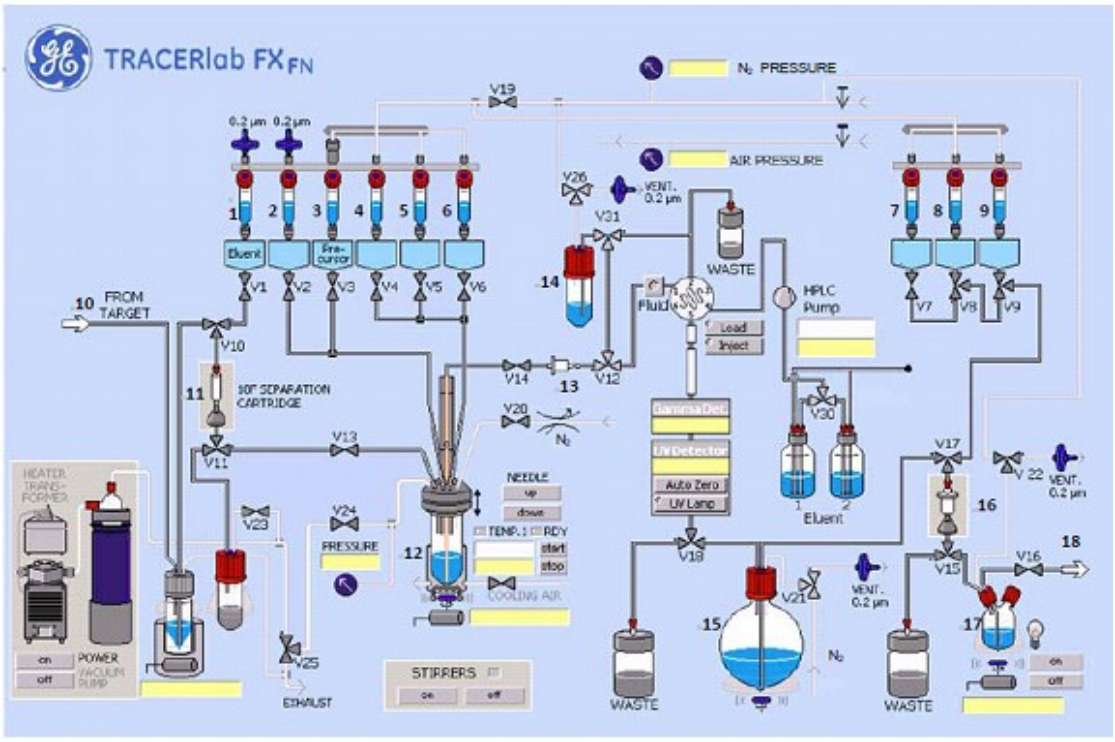


Figure S1. Schematic of the GE TRACERlab™ FX$_{FN}$ radiosynthesis module automated synthesis manifold for **[^{18}F]2a**.

Automated synthesis involves the following: (1) azeotropic drying of [^{18}F]fluoride; (2) [^{18}F]fluorination; and (3) HPLC purification, followed by solid-phase formulation of the final

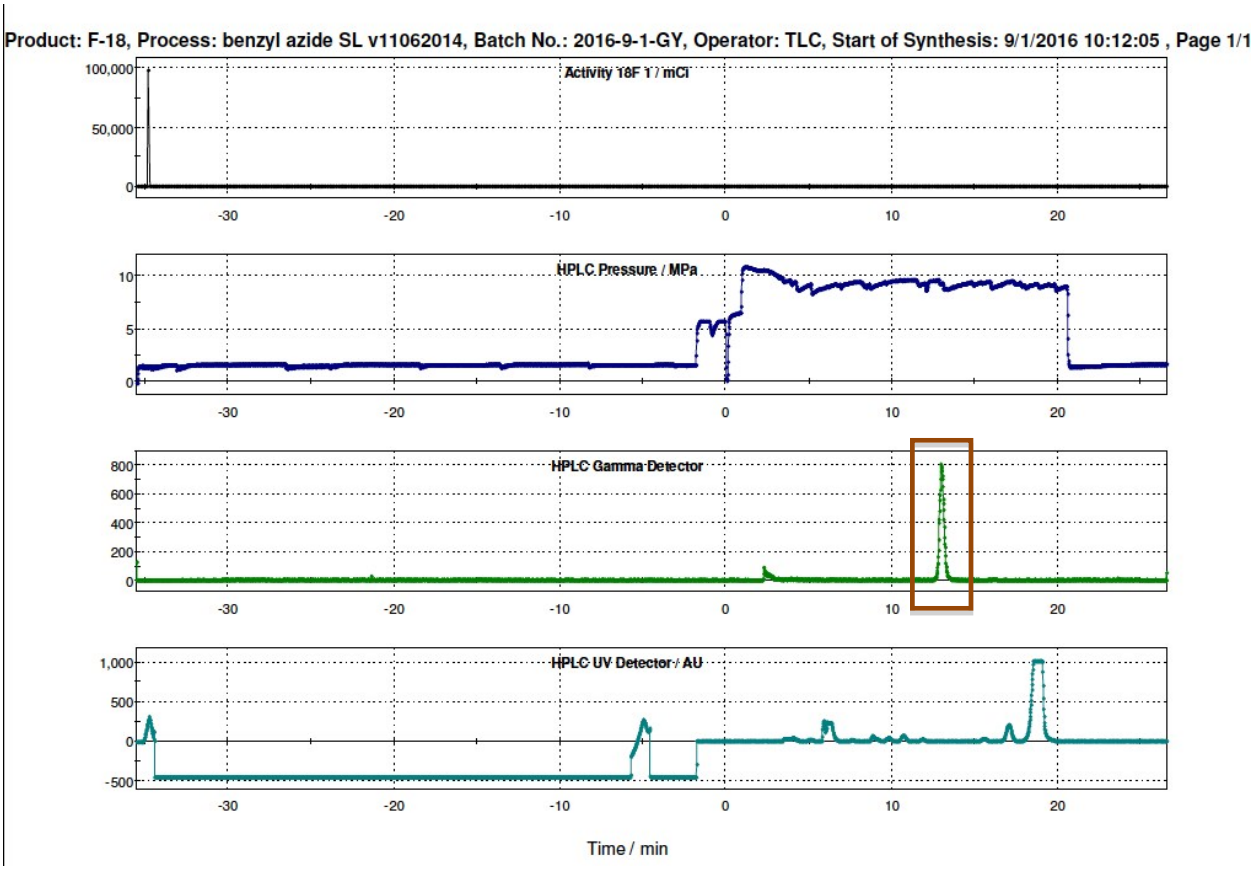
product. The synthesis module was operated in the following sequences with numerical references to Figure S1.

- [^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction using a GE cyclotron and delivered to the radiosynthesis module via 10. The [^{18}F]fluoride was quantitatively trapped on a QMA carbonate ion exchange solid phase extraction (SPE) light cartridge (Waters; activated with 6 mL of trace grade H_2O).
- Automated synthesis began with the elution of resin-bound [^{18}F]fluoride using a solution of tetraethylammonium bicarbonate (6 mg in 300μL H_2O and 700μL CH_3CN), pre-loaded into 1 and delivered to the reactor (12).
- The reaction mixture (12) was dried azeotropically by addition of 1 mL anhydrous CH_3CN, preloaded into 5, at 85 °C under N_2 flow and vacuum over 8 min, then at 110 °C under N_2 flow and vacuum for 4 min.
- Precursor **1a** (6 mg in 0.6 mL CH_3CN) pre-loaded into 3 was added to 12. The reactor was sealed via the closure of valve V13, V20 and V24 and the reaction mixture was heated to 130 ºC and this temperature was maintained for 10 min.
- The reaction mixture was then cooled to 40 °C, vented via valves V24 and V25, and diluted with 1:1 CH_3CN/ H_2O (3 mL), pre-loaded into 6.
- The crude reaction mixture was eluted into 14 and the contents of 14 were transferred to the HPLC loop via N_2 pressure via a fluid detector, injected onto a semi-preparative column (Luna C18 semi-preparative, 250 × 10.00 mm, 5μ), and eluted with 65:35 CH_3CN/ 0.1M ammonium formate by volume at a flow rate of 5 mL/min. The eluent was monitored by UV (λ = 254 nm) and radiochemical detectors connected in series.
- The fraction containing the major radiochemical product was collected, via valve 18, into a large dilution vessel (15), which was preloaded with 23 mL of sterile water for injection (United States Pharmacopeia (USP); Hospira).
- The diluted HPLC fraction was then loaded onto a C18 SPE cartridge (16) (Waters; preactivated with 5 mL EtOH followed by 10 mL H_2O).
- Cartridge 16 was washed with 10 mL sterile water for injection, USP, preloaded into 7, to remove traces of salts, HPLC mobile phase, and [^{18}F]fluoride. Then 16 was eluted with 2.0 mL CH_3CN preloaded into 8, into collection vial 17. We next took 0.4 mL of product

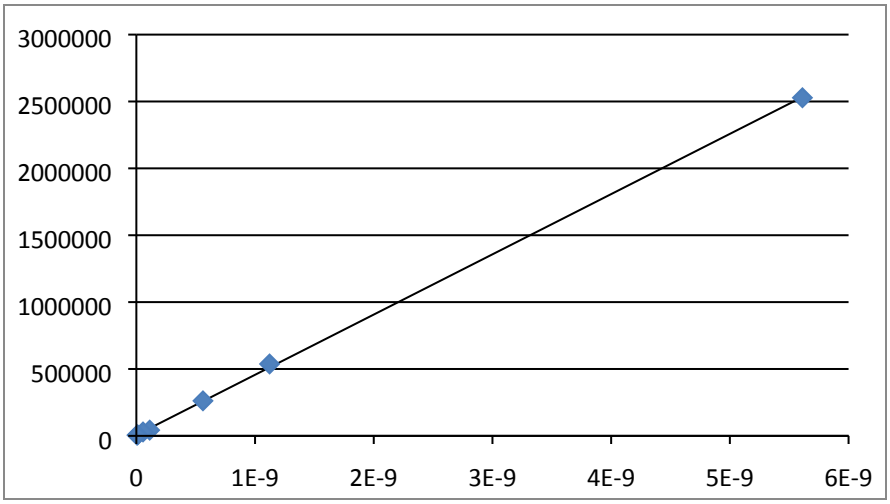
solution and carried out the step 2.

Analyses of radioactive mixtures were performed by HPLC with an in-line UV (λ = 254 nm) detector in series with a CsI PIN diode radioactivity detector. Uncorrected radiochemical yields of **[^{18}F]2a** were 61% (non-decay corrected) relative to starting [^{18}F]fluoride.

(2) RadioHPLC chromatogram from GE TRACERlab™ FX_{FN} radiosynthesis module

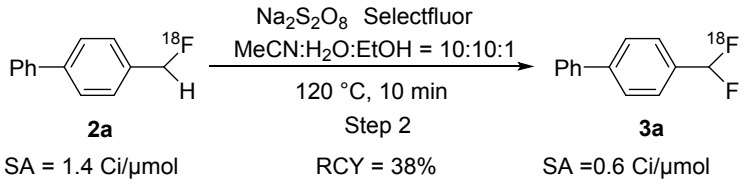


(3) Specific Activity (SA) determination for [^{18}F]2a:



Standard curve for the specific activity determination of [^{18}F]**2a**.

Step 2. Manual Labeling of [^{18}F]3a

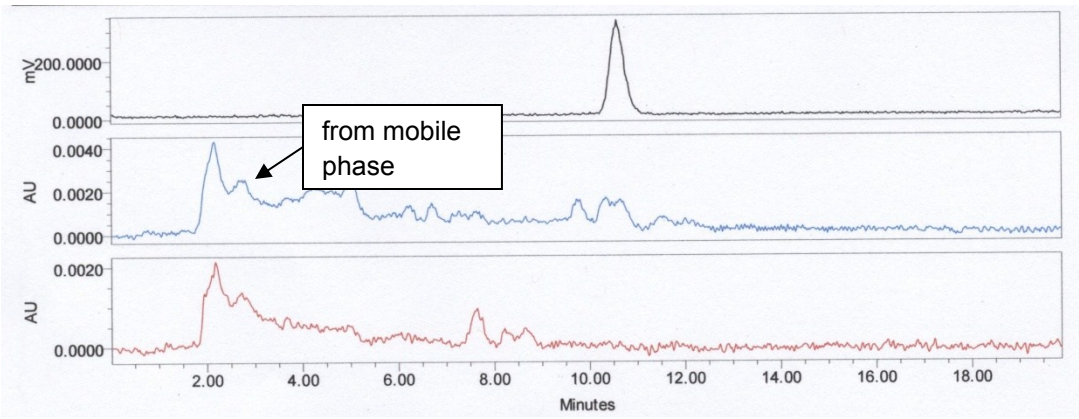


(1) Method:

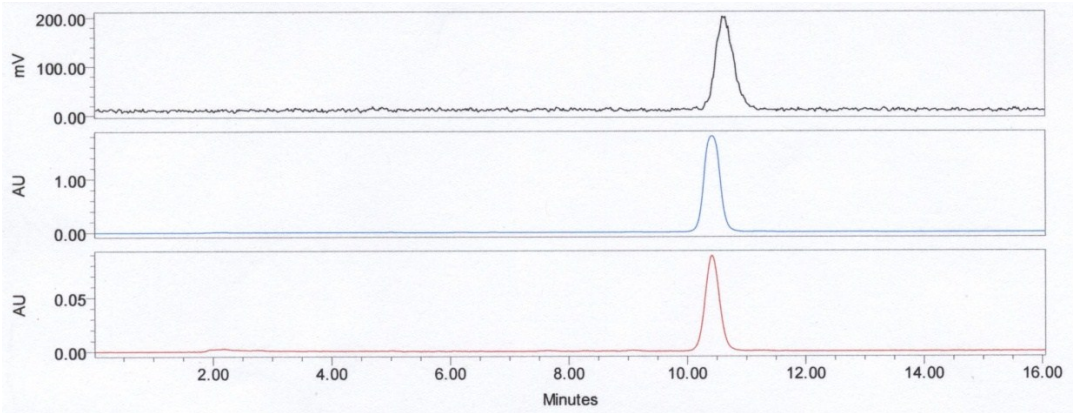
The isolated [^{18}F]**2a** in 2.0 mL CH_3CN from FX_{FN} was diluted with 2.0 mL sterile water, then 0.4 mL above solution was added to a reaction vial containing 8.0 mg $Na_2S_2O_8$ and 20.0 mg Selectfluor and 20 μL EtOH was added (Attention: the vial cap should be closed tightly to avoid solvent evaporation.) The reaction was allowed to react at 120 °C for 10 min, after which it was cooled immediately by an ice-water bath, and quenched with 1.6 mL mobile phase (MeCN/ 0.1 M $NH_4{\cdot}HCO_2$(aq), v/v 6.5:3.5). Preparative HPLC purification (Phenomenex Luna C18, 250 x 100 mm, 5 μm, 5 mL/min) led to the desired [^{18}F]**3a** (1.5 mL mobile phase). The chemical & radiochemical purities and SA of [^{18}F]**3a** was determined via analytical HPLC (Phenomenex Luna C18, 250 x 4.6 mm, 5 μm, 70% MeCN, 30% 0.1 M $NH_4{\cdot}HCO_2$(aq), 1 mL/min).

*(2) Quality Control of [^{18}F]**3a**:*

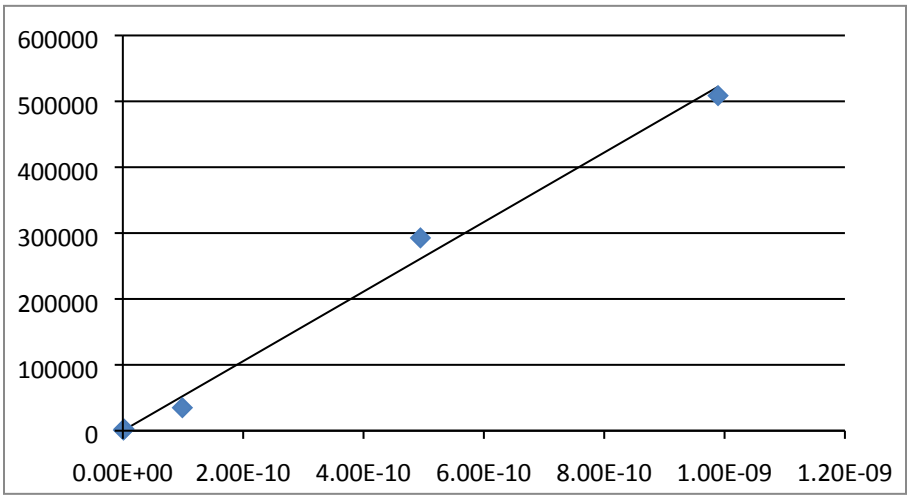
a) Chemical & radiochemical purity



b) Co-injection with standard **3a**



*(3) Specific Activity (SA) determination for [^{18}F]**3a**:*

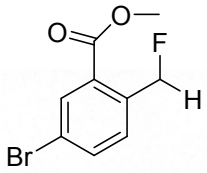


Standard curve for the specific activity determination of [^{18}F]**3a**.

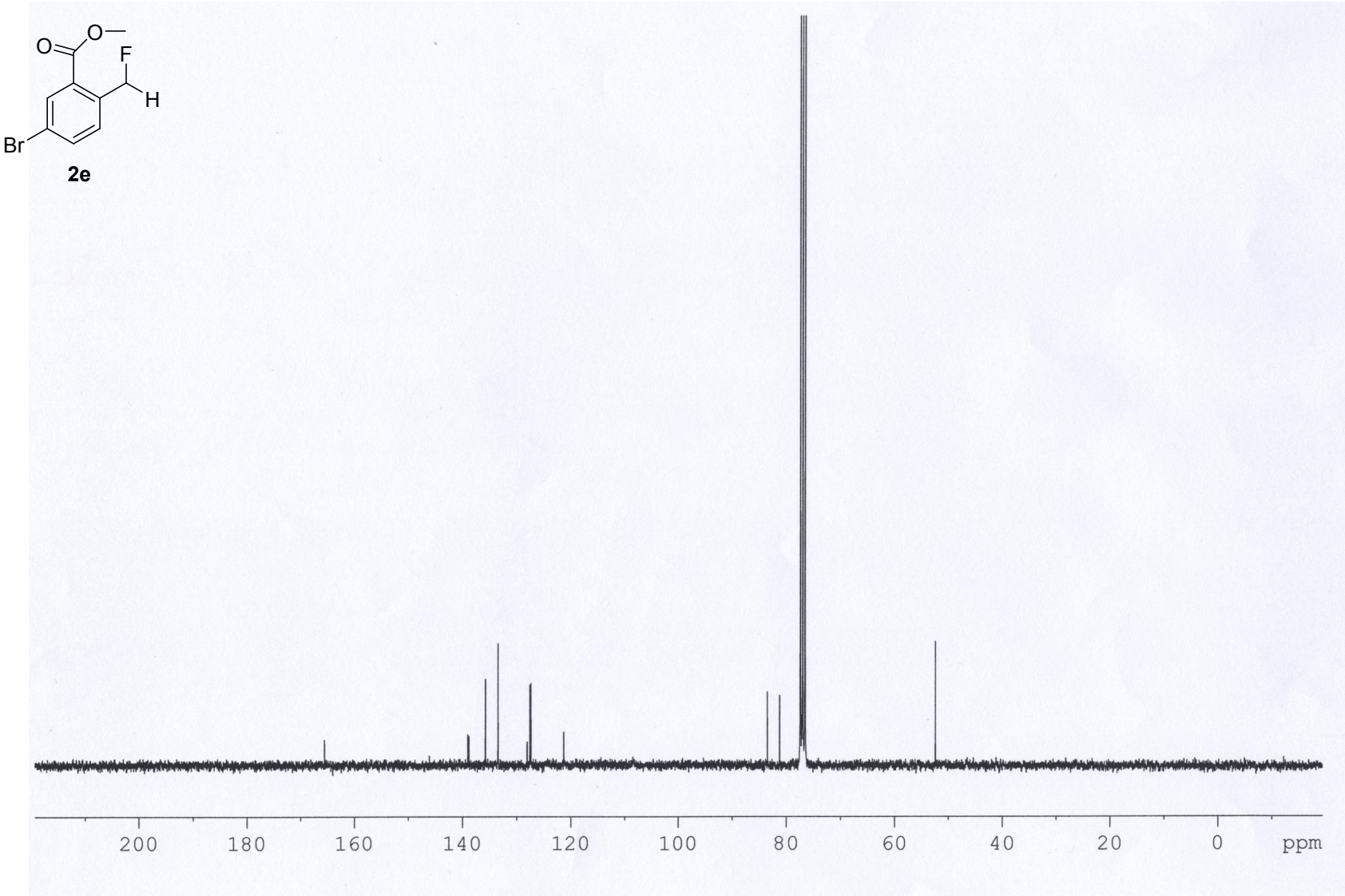
Reference and notes

1. Xu, P.; Guo, S.; Wang, L.; Tang, P. *Angewandte Chemie* **2014,** *53*, 5955.
2. Ma, J. J.; Yi, W. B.; Lu, G. P.; Cai, C. *Organic & biomolecular chemistry* **2015,** *13*, 2890.
3. Furuya, T.; Strom, A. E.; Ritter, T. *Journal of the American Chemical Society* **2009,** *131*, 1662.

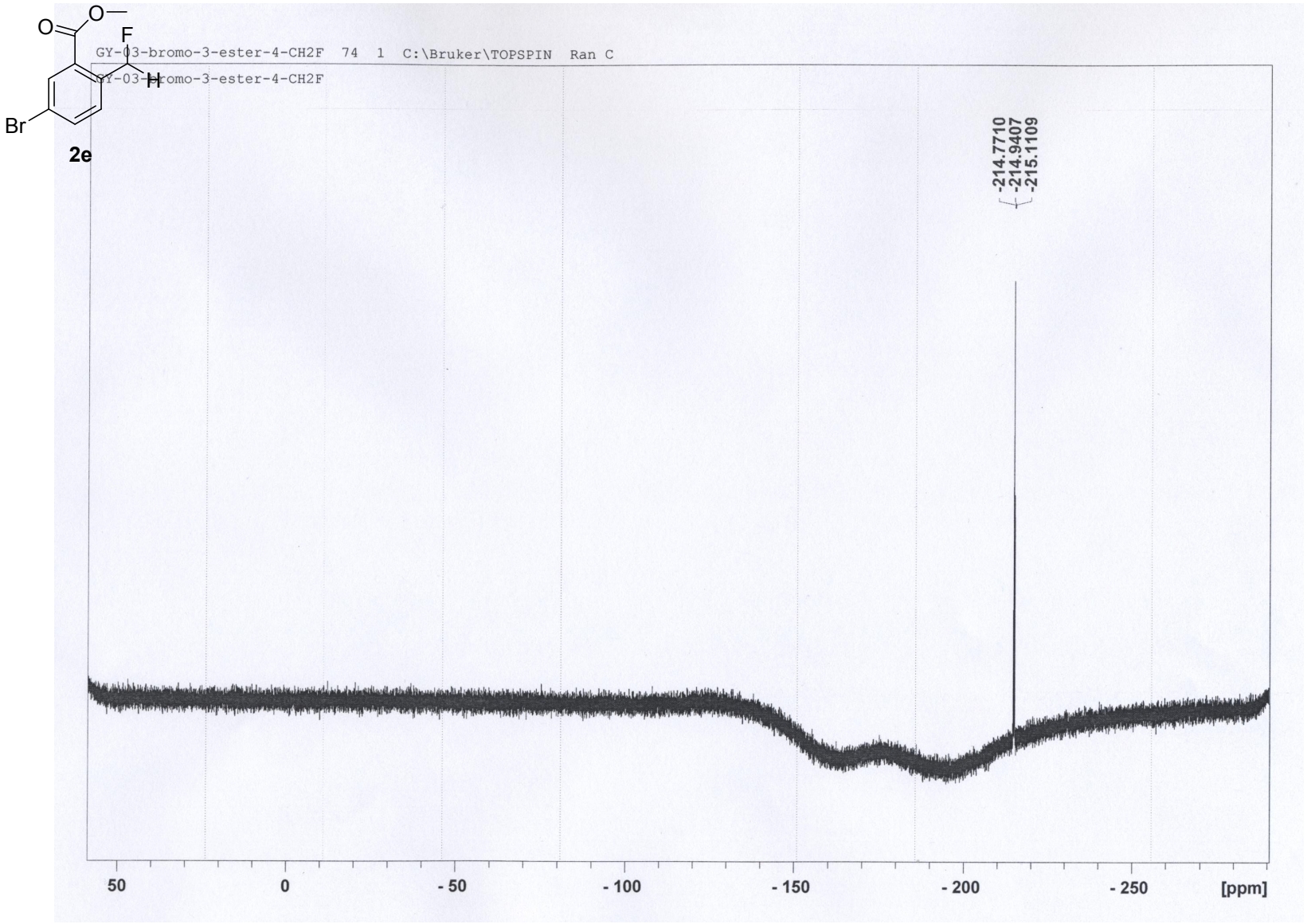
^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra of new compound 2e:

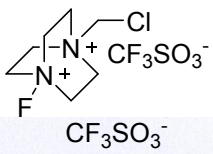


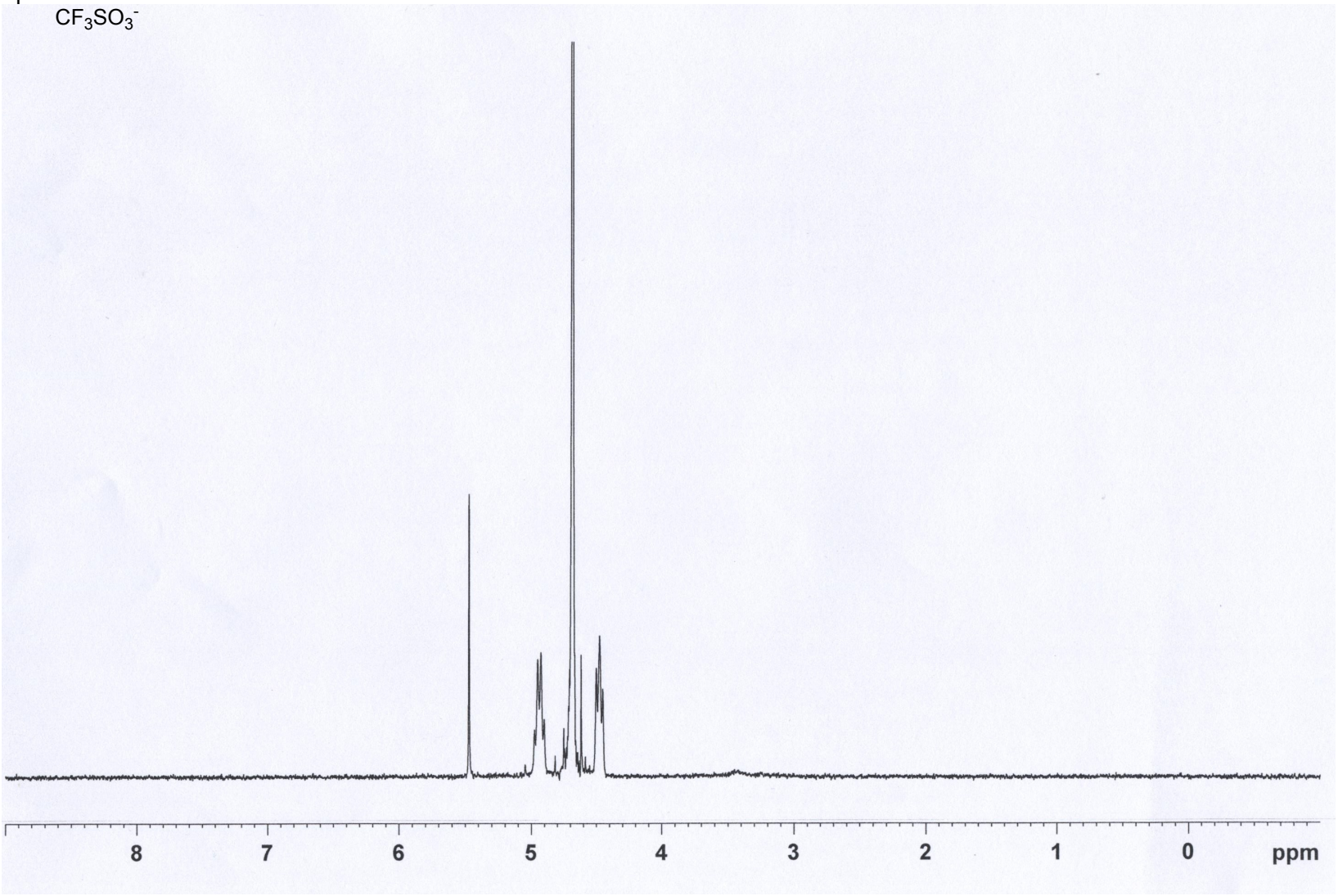
2e

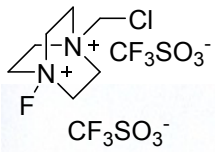


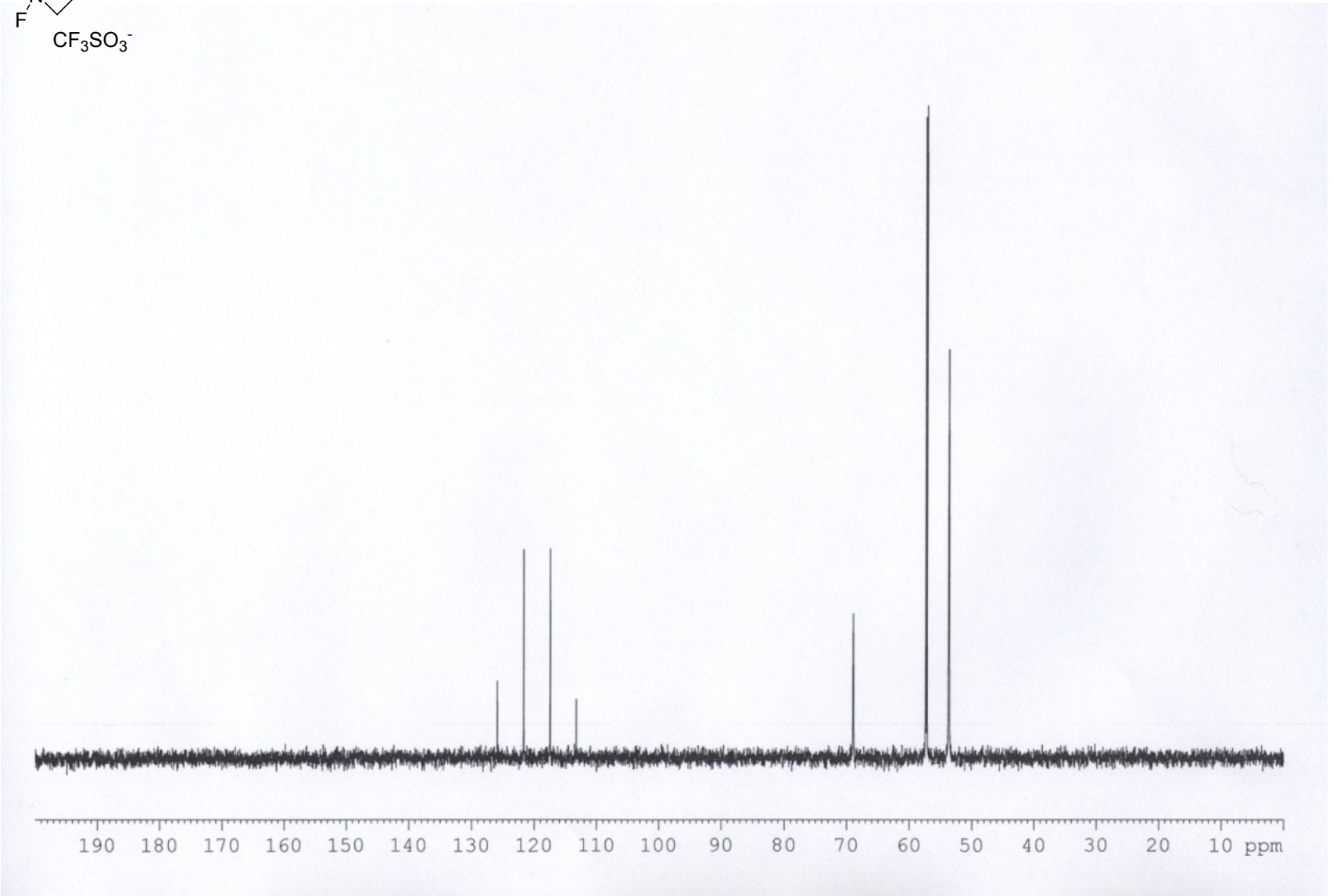
2e

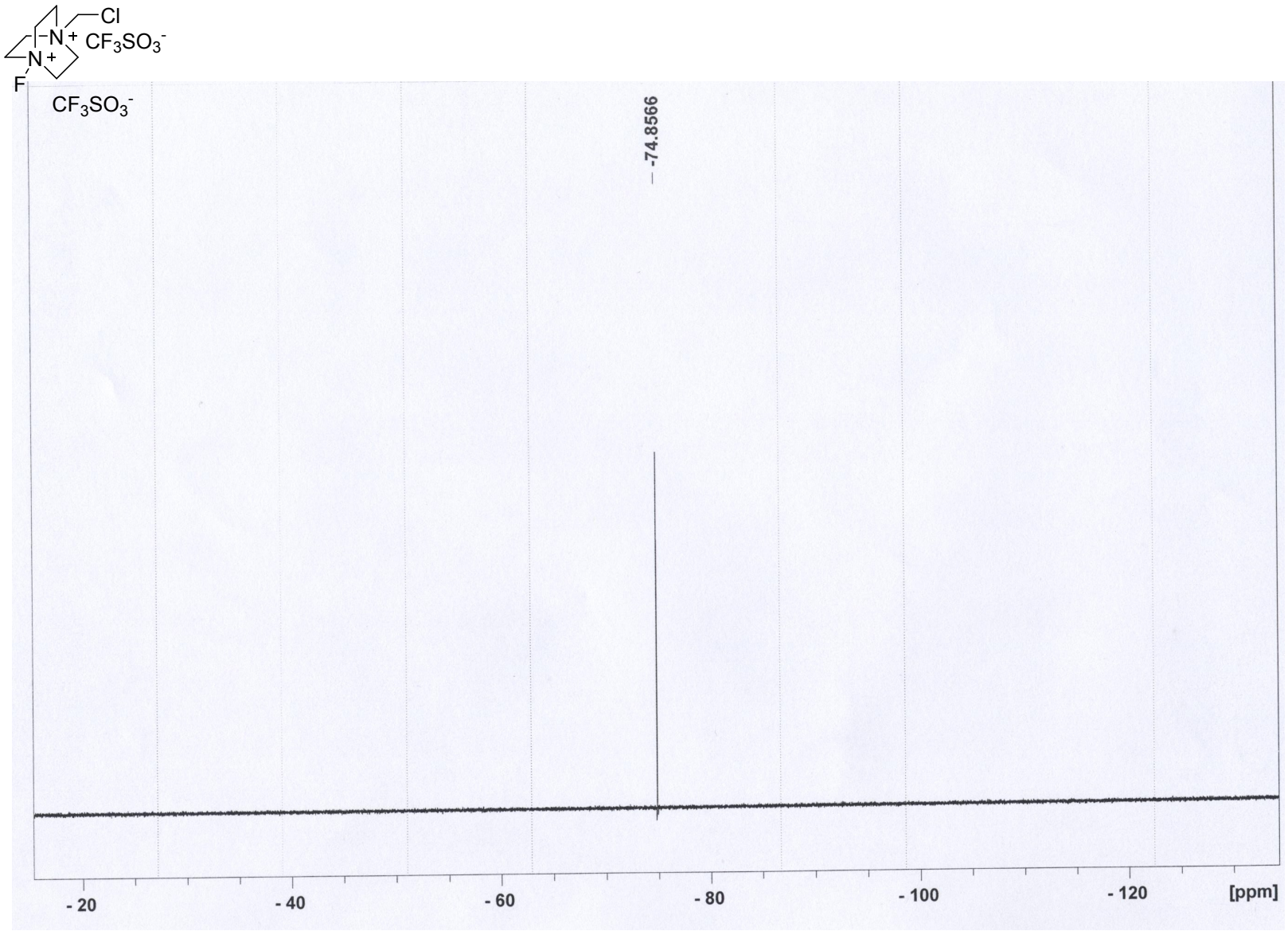

O
O
F
H
Br
2e
GY-03-bromo-3-ester-4-CH2F 74 1 C:\Bruker\TOPSPIN Ran C
GY-03-bromo-3-ester-4-CH2F
-214.7710
-214.9407
-215.1109
50
0
- 50
- 100
- 150
- 200
- 250
[ppm]


Cl
N+
CF3SO3-
N+
F
CF3SO3-


8
7
6
5
4
3
2
1
0
ppm


Cl
N+
CF3SO3-
N+
F
CF3SO3-


190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


Cl
N+
CF3SO3-
N+
F
CF3SO3-
-74.8566
- 20
- 40
- 60
- 80
- 100
- 120
[ppm]