Supporting information

Nucleophilic radiosynthesis of boron neutron capture therapy-oriented PET probe [^{18}F]FBPA using aryldiboron precursors

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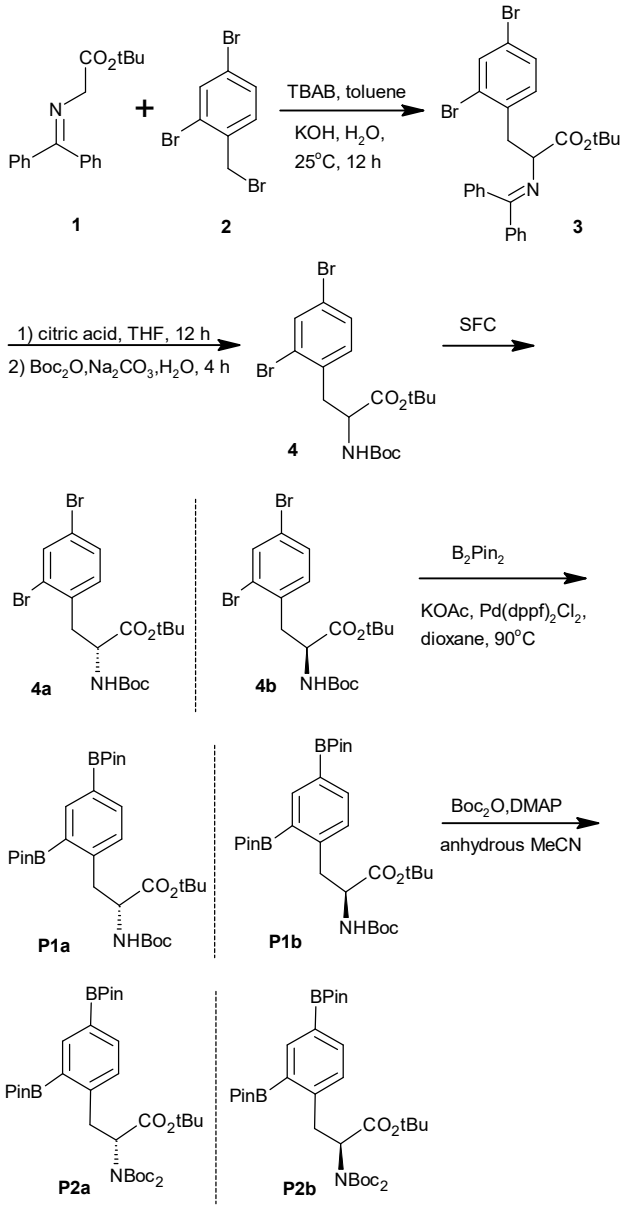
Materials

Kryptofix 222 (K_{222}), $Cu(OTf)_2Py_4$, pyridine (Py), anhydrous N,N-dimethylacetamide (DMA), anhydrous nBuOH and anhydrous MeOH for the radiolabeling experiment were purchased from Sigma-Aldrich. EtOH, HOAc, water and $NaHCO_3$ for the preparation of mobile phase of preparative radio-HPLC and formulation of 2-[^{18}F]FBPA preparation were of pharmaceutical grade.

Synthesis of the precursors and characterisation



Scheme S1 Synthesis of the precursors

The N-tert-Butyloxycarbonyl (N-Boc) precursors and N,N-diBoc precursors were synthesized according to the following procedures (Scheme S1).

*Procedure of preparation of intermediate **3***

To a solution of N-(diphenylmethylene)glycerine tert-butyl ester **1** (40 g, 135.4 mmol), 2,4-dibromo-1-(bromomethyl)benzene **2** (44.53 g, 135.4 mmol) and tetrabutylammonium bromide (TBAB) (436.6 mg, 1.35 mmol) in 300 mL toluene was added KOH (100 g, 1.78 mol) in 80 mL H_2O. Then the mixture was stirred at room temperature for 12 h. Thin layer chromatography (TLC) (Petroleum ether: EtOAc=20:1) showed compound **1** was consumed completely, and the desired spot formed. The reaction mixture was diluted with 100 mL EtOAc and extracted with 400 mL EtOAc (200 mL×2). The combined organic layers were washed with 600 mL brine (300 mL×2), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc=100:1 to 20:1). Compound **3** was obtained as white solid (15.0 g, 20.4% yield). ^{1}H NMR of compound **3** (Fig. S1): δ (ppm) 7.56 - 7.46 (m, 3H), 7.35 - 7.16 (m, 7H), 7.01 (d, J=8.2 Hz, 1H), 6.59 (br d, J=6.8 Hz, 2H), 4.23 (dd, J=4.2, 9.5 Hz, 1H), 3.31 (dd, J=4.0, 13.4 Hz, 1H), 3.11 (dd, J=9.6, 13.5 Hz, 1H), 1.41 - 1.28 (m, 9H). ESI-MS measurement (Agilent 6110 MSD) found M/Z$^+$ 544.0 $[M+H]^+$.

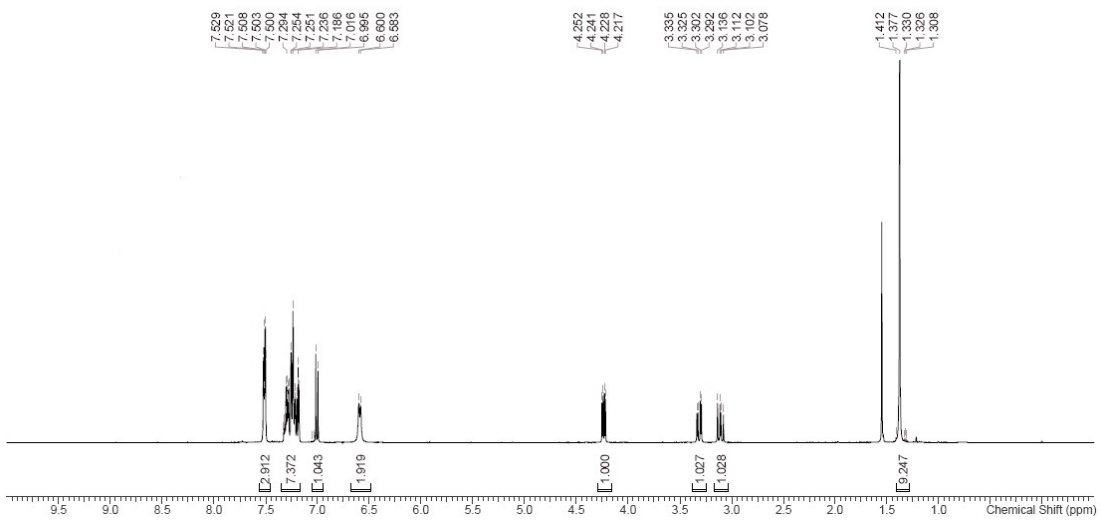


Fig. S1 ^{1}HNMR spectrum of compound **3** (400 MHz, $CDCl_3$)

*Procedure of preparation of intermediate **4***

To a solution of compound **3** (30 g, 55.2 mmol) in 70 mL THF was added citric acid (31.83 g, 165.7 mmol) in 120 mL H_2O and stirred for 12 h. Then Na_2CO_3 (29.26 g, 276.1 mmol) in 150 mL H_2O and Boc_2O (13.26 g, 60.7 mmol) were added to the mixture and stirred for 4 h. TLC (petroleum ether: EtOAc=10:1) showed compound **3** was consumed completely. The reaction mixture was extracted with 400 mL EtOAc (200 mL×2). The combined organic layers were washed with 400 mL brine (200 mL×2), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc=100:1 to 10:1). Compound **4** was obtained as white solid (19.6 g, 40.6 mmol, 74% yield). ^{1}H NMR of compound **4** (Fig. S2): δ (ppm) 7.72 (d, J=1.5 Hz, 1H), 7.37 (dd, J=1.8, 8.1 Hz, 1H), 7.13 (br d, J=8.1 Hz, 1H), 5.06 (br d, J=8.3 Hz, 1H), 4.59 - 4.38 (m, 1H), 3.23 (dd, J=5.9, 13.9 Hz, 1H), 3.00 (br dd, J=8.6, 13.8 Hz, 1H), 1.40 (br d, J=16.6 Hz, 17H). ESI-MS measurement (Agilent 6110 MSD) found M/Z^+ 323.9 [M-

155]$^+$.

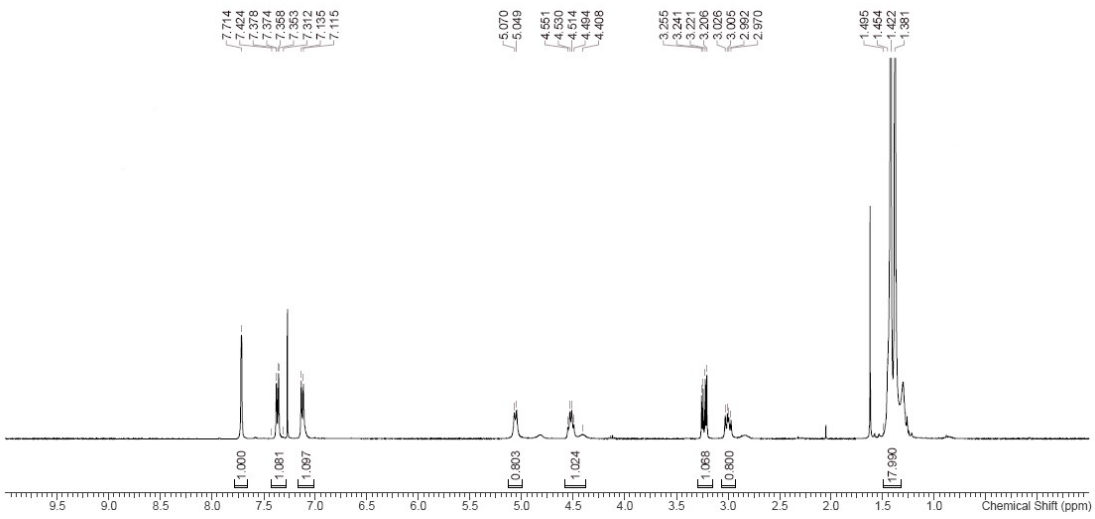


Fig. S2 ^{1}HNMR spectrum of compound **4** (400 MHz, $CDCl_3$)

*Procedure of preparation of intermediate **4a** and **4b***

The compound **4** (19.35 g) was separated by chiral supercritical fluid chromatography (SFC). The preparative column was DAICEL CHIRALPAK AY (250 mm×50 mm I.D., 10 μm). CO_2 (phase A) and 0.1% $NH_3{\cdot}H_2O$/MeOH (phase B) were used as mobile phases. Compound **4a** (9.1 g, 47.0% yield) and compound **4b** (9.2 g, 47.5% yield) were each obtained as white solid, confirmed by ^{1}H NMR.

^{1}H NMR of compound **4a** (Fig. S3): δ (ppm) 7.72 (d, J=1.8 Hz, 1H), 7.37 (dd, J=1.8, 8.2 Hz, 1H), 7.13 (br d, J=8.1 Hz, 1H), 5.05 (br d, J=8.1 Hz, 1H), 4.58 - 4.46 (m, 1H), 3.23 (dd, J=5.9, 13.9 Hz, 1H), 3.00 (br dd, J=8.6, 13.6 Hz, 1H), 1.40 (br d, J=16.9 Hz, 18H).

^{1}H NMR of compound **4b** (Fig. S4): δ (ppm) 7.72 (d, J=1.7 Hz, 1H), 7.37 (dd, J=2.0, 8.2 Hz, 1H), 7.13 (br d, J=8.2 Hz, 1H), 5.06 (br d, J=7.9 Hz, 1H), 4.59 - 4.43 (m, 1H),

3.23 (dd, J=6.0, 13.8 Hz, 1H), 3.00 (br dd, J=8.5, 13.6 Hz, 1H), 1.40 (br d, J=17.0 Hz, 18H).

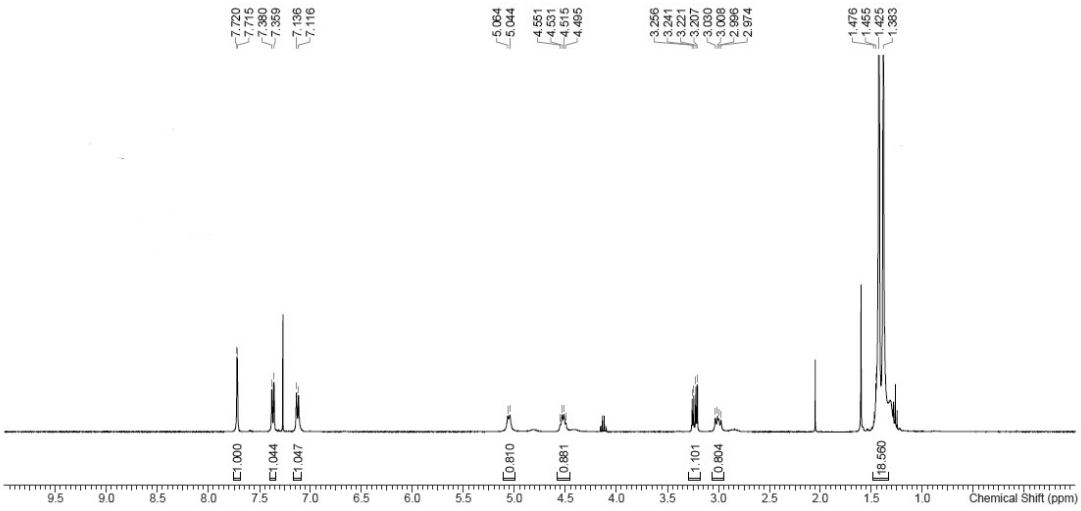


Fig. S3 ^{1}HNMR spectrum of compound **4a** (400 MHz, $CDCl_3$)

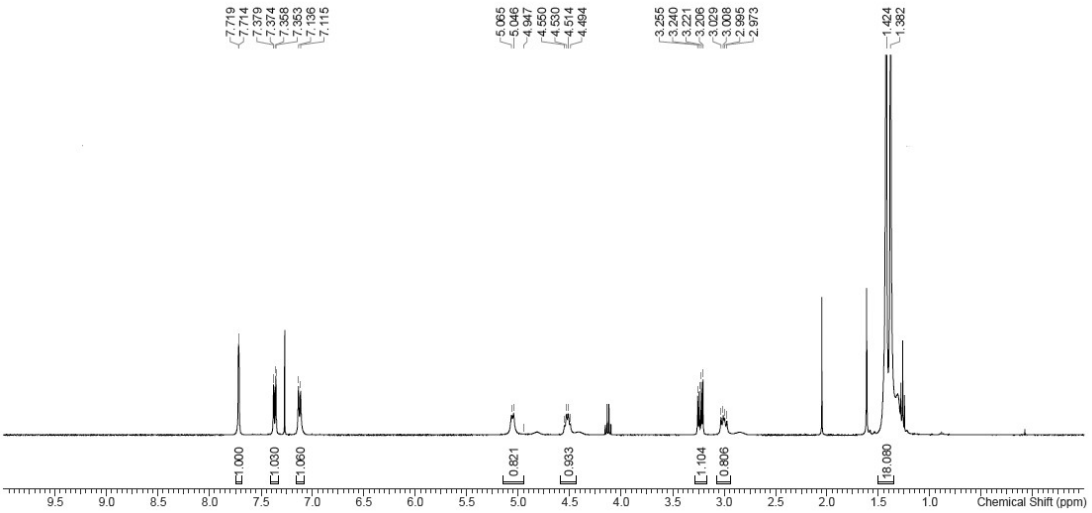


Fig. S4 ^{1}HNMR spectrum of compound **4b** (400 MHz, $CDCl_3$)

The compounds **4a** and **4b** were also analysed by chiral SFC under the following condition. Analytical chiral SFC column: Chiralpak AY-3 (50×4.6 mm I.D., 3 μm); mobile phase: CO_2 (phase A)/0.05% diethylamine (DEA) in MeOH (phase B); gradient

elution: phase B in phase A from 5% to 40%; flow rate: 3mL/min; UV wavelength: 220 nm; column tempature: 35°C; back pressure: 100 Bar. The retention times of compounds **4a** and **4b** were 0.76 min and 1.54 min (Figs. S5 and S6), respectively.

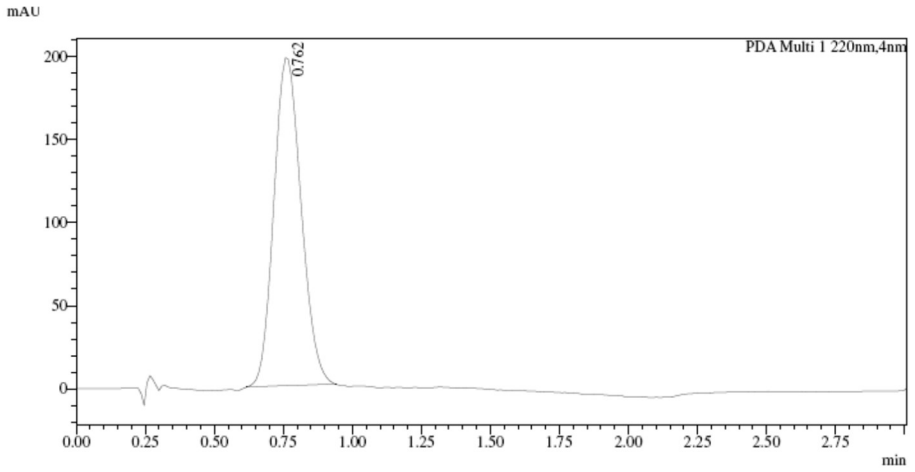


Fig. S5 Chiral SFC chromatogram of compound **4a**

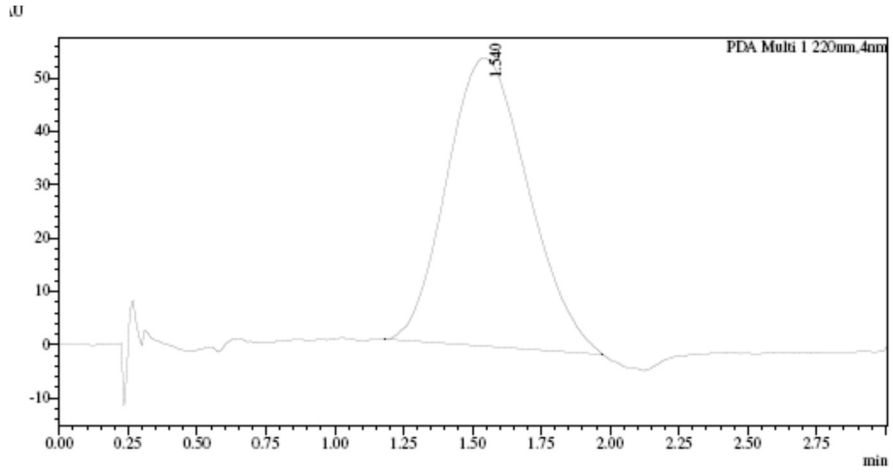


Fig. S6 Chiral SFC chromatogram of compound **4b**

*Procedure of preparation of precursors **P1a** and **P1b***

A mixture of compound **4a** (7.5 g, 15.7 mmol, 1 eq), B_2Pin_2 (19.8 g, 78.0 mmol, 5 eq), AcOK (6.14 g, 62.6 mmol, 4 eq), $Pd(dppf)Cl_2$ (1.15 g, 1.57 μmol, 0.1 eq) in 75 mL dioxane was degassed and purged with N_2 gas for 3 times. Then the mixture was stirred

at 90 °C for 1 h under N_2 gas atmosphere. The crude reaction mixture was filtered and diluted with 200 mL H_2O and extracted with 300 mL EtOAc (100 mL×3). The organic was washed with 200 mL brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The combined crude product was purified by preparative RP C18 HPLC with water and MeCN mixture as mobile phase for gradient elution to obtain precursor **P1a** as light yellow solid (6.20 g, 69% yield, 96.3% purity). The precursor **P1b** (5.37 g, 97.0% purity) was also prepared using the same precedure from compound **4b**.

^{1}H NMR of compound **P1a** (Fig. S7): δ (ppm) 8.24 (s, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.30 (d, J = 7.6 Hz, 1H), 5.90 (d, J = 8.4 Hz, 1H), 4.19 - 4.24 (m, 1H), 3.24 - 3.19 (m, 2H), 1.47 (s, 9H), 1.39 (s, 12H), 1.34 (s, 12H), 1.32 (s, 9H).

^{1}H NMR of compound **P1b** (Fig. S8): δ (ppm) 8.24 (s, 1 H), 7.84 (dd, J = 1.47, 7.60 Hz, 1 H), 7.30 (d, J = 8.00 Hz, 1 H), 5.89 (br d, J = 8.40 Hz, 1 H), 4.19 - 4.26 (m, 1 H), 3.16 - 3.29 (m, 2 H), 1.47 (s, 9 H), 1.39 (s, 12 H), 1.31 - 1.35 (m, 21 H).

The MS spectra of **P1a** and **P1b** analysed by LC-MS (Agilent 1200 LC & Agilent 6110 MSD) (Table S1) found M/Z$^+$ 418.3 $[M-156+H]^+$, 596.4 $[M+Na]^+$ and 1169.7 $[2M+Na]^+$ (Figs. S9 and S10).

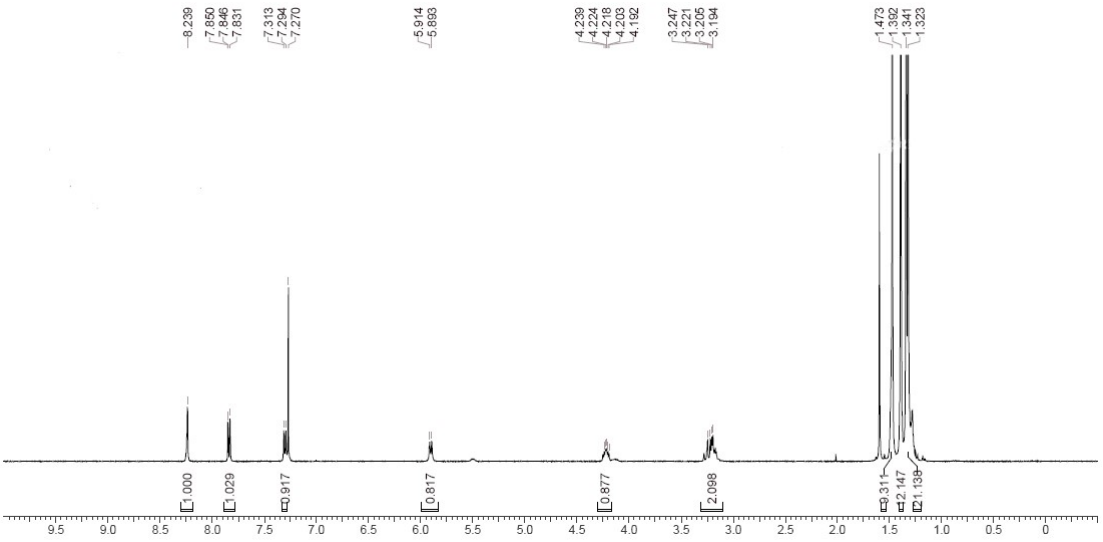


Fig S7 ^{1}HNMR spectrum of compound **P1a (**400 MHz, $CDCl_3$)

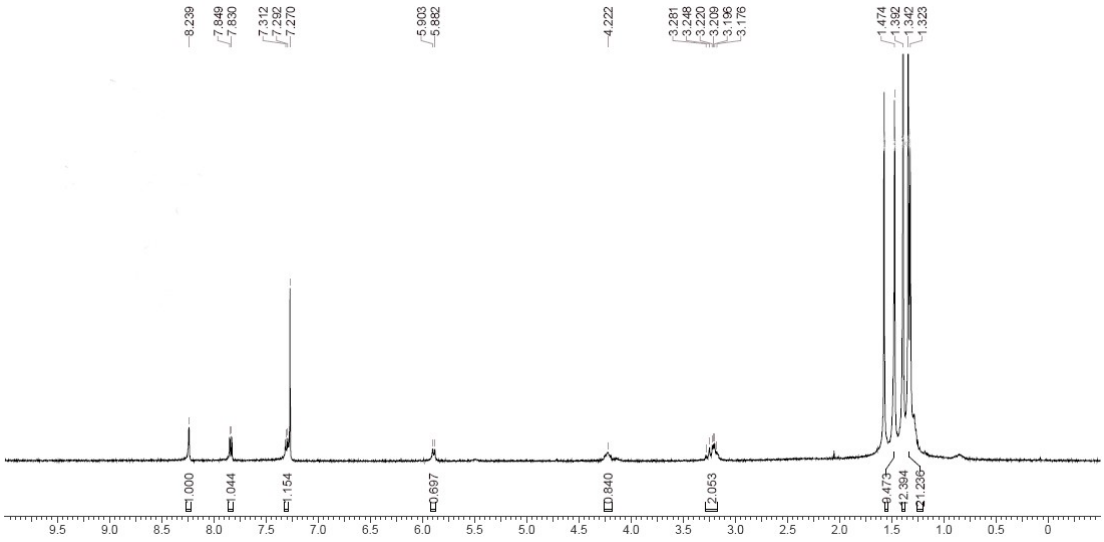


Fig S8 ^{1}HNMR spectrum of compound **P1b (**400 MHz, $CDCl_3$)

Table S1 The LC-MS conditions for compounds **P1a** and **P1b**

HPLC	Column	Agilent ZORBAX 5μm SB-Aq, 2.1×50 mm		
	Mobile Phase	A: 0.0375% TFA in water (v/v)		
		B: 0.02% TFA in MeCN (v/v)		
	Gradient	Time (min)	B (%)	Flow rate(mL/min)
		0.00	25	0.8
		0.40	25	0.8
		3.40	100	0.8
		3.90	100	0.8

		3.91	25	0.8
		4.00	25	1.0
		4.50	25	1.0
	Column Temperature	50℃		
	Detector	DAD（220nm, 254nm）		
MS	Ionization source	ESI		
	Drying Gas	N_2		
	Drying Gas Flow	10 L/min		
	Nebulizer Pressure	40 psi		
	Drying Gas Temperature	350 ℃		
	Capillary Voltage	2500(V) Positive		
	MS Polarity	Positive		
	MS Mode	Scan		
	Mass Range	100-1500		

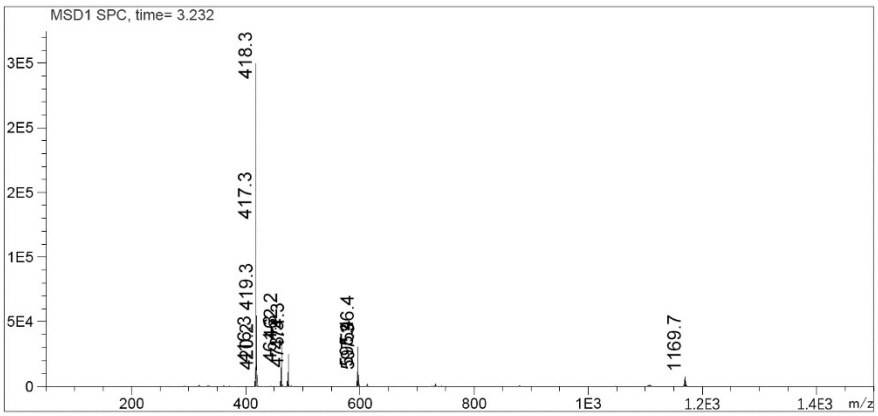


Fig S9 MS spectrum of compound **P1a**

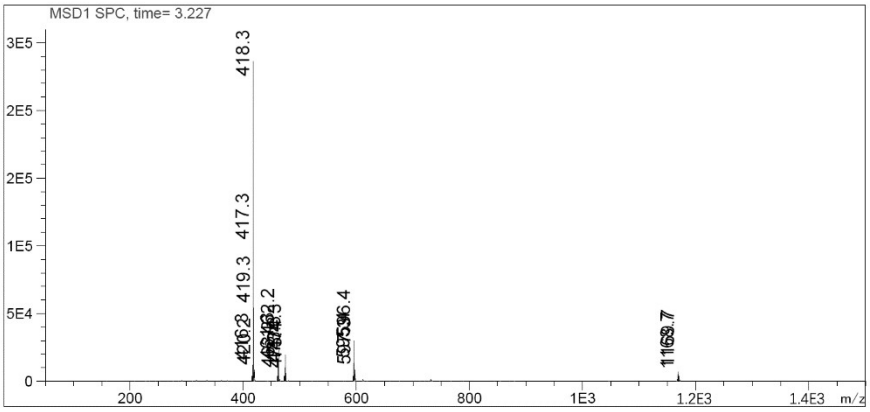


Fig S10 MS spectrum of compound **P1b**

The compounds **P1a** and **P1b** were also measured with chiral SFC using Chiralcel IC-3 column (100×4.6 mm, 3 μm). CO_2 (A) and 0.05% Diethylamine (DEA) in isopropanol (B) were used as mobile phases for gradient elution (B in A: 5%, 0.01 min; 5%-20%, 0.01-2 min; 20%, 2-3 min). The retention times of **P1a** and **P1b** were 1.7 min and 1.911 min, respectively (Figs. S11 and S12).

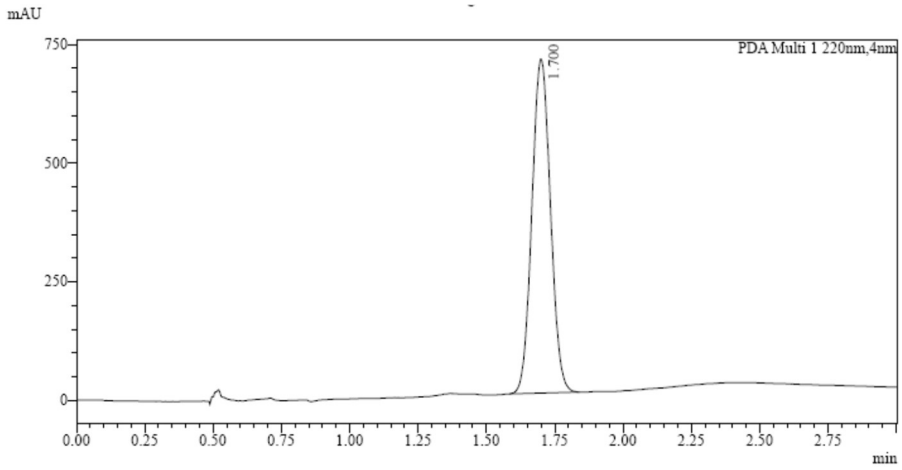


Fig S11 Chiral SFC chromatogram of compound **P1a**

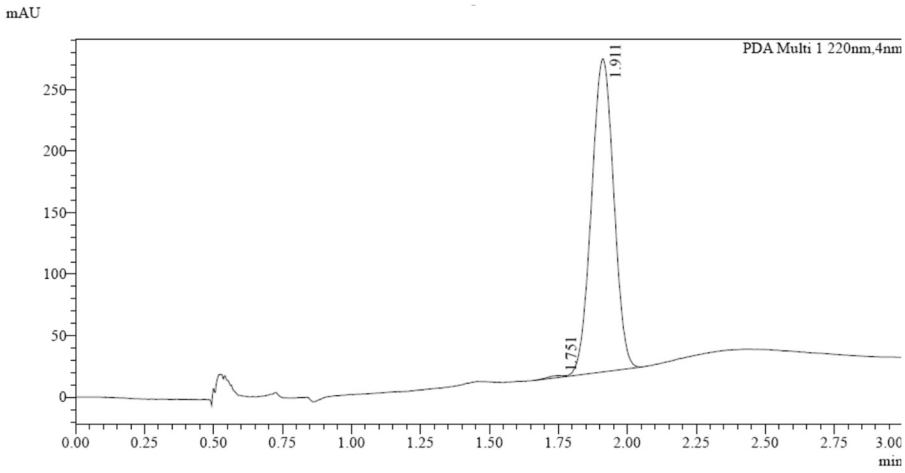


Fig S12 Chiral SFC chromatogram of compound **P1b**

The optical rotation of compounds **P1a** and **P1b** were also measured with an Autopol IV polarimeter (Rudolph). The results showed that **P1a** and **P1b** were D-form and L-form, respectively.

*Procedure of preparation of the precursors **P2a** and **P2b***

A mixture of compound **P1a** (2.00 g, 3.49 mmol, 1 eq), Boc_2O (2.52 g, 11.6 mmol, 3.3 eq) and DMAP (467 mg, 3.82 mmol, 1.09 eq) were dissolved in 30 mL anhydrous MeCN in a round-bottom flask and stirred at room temperature for 24 h. The consumption of most of comound **P1a** was monitored by TLC assay (EtOAc/hexane=1:4). The solvent of mixture was distilled under reduced pressure and purified with RP C18 HPLC. The fraction of compound **P2a** was collected, concentrated by distillation under reduced pressure and dried in vacco at 60 °C to give product **P2a** (740 mg, 31.5% yield, 97% purity). The compound **P2b** was also prepared using the same procedure from **P1b**.

^{1}H NMR of compound **P2a** (Fig. S13): δ (ppm) 8.21 (d, J = 0.8 Hz, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.04 (d, J = 7.6 Hz, 1H), 5.21 (dd, J = 3.6, 11.6 Hz, 1H), 3.97 (dd, J = 3.8, 13.2 Hz), 3.13 (dd, J = 11.2, 13.2 Hz, 1H), 1.49 (s, 9H), 1.35 (s, 12H), 1.34 (s, 12H), 1.33 (s, 9H), 1.32 (s, 9H).

^{1}H NMR of compound **P2b** (Fig. S14): δ (ppm) 8.13 (d, J = 0.8 Hz, 1H), 7.66 (d, J = 7.6 Hz, 1H), 6.95 (d, J = 7.6 Hz, 1H), 5.14 (dd, J = 4.0, 11.2 Hz, 1H), 3.90 (dd, J = 4.0, 13.2 Hz), 3.09 – 3.03 (m, 1H), 1.42 (s, 9H), 1.27 (s, 12H), 1.26 (s, 12H), 1.25 (m, 18H).

The MS spectra of **P2a** and **P2b** analysed with LC-MS found M/Z^+ 418.2 $[M-256+H]^+$, 696.3 $[M+Na]^+$ and 1369.7 $[2M+Na]^+$ (Figs. S15 and S16).

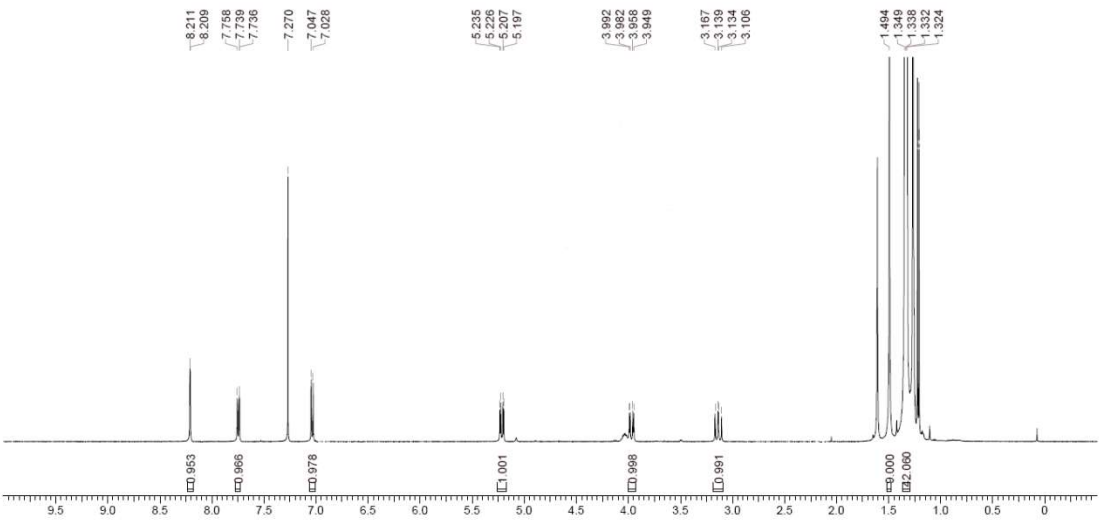


Fig S13 [1]HNMR spectrum of compounds **P2a** (400 MHz, $CDCl_3$)

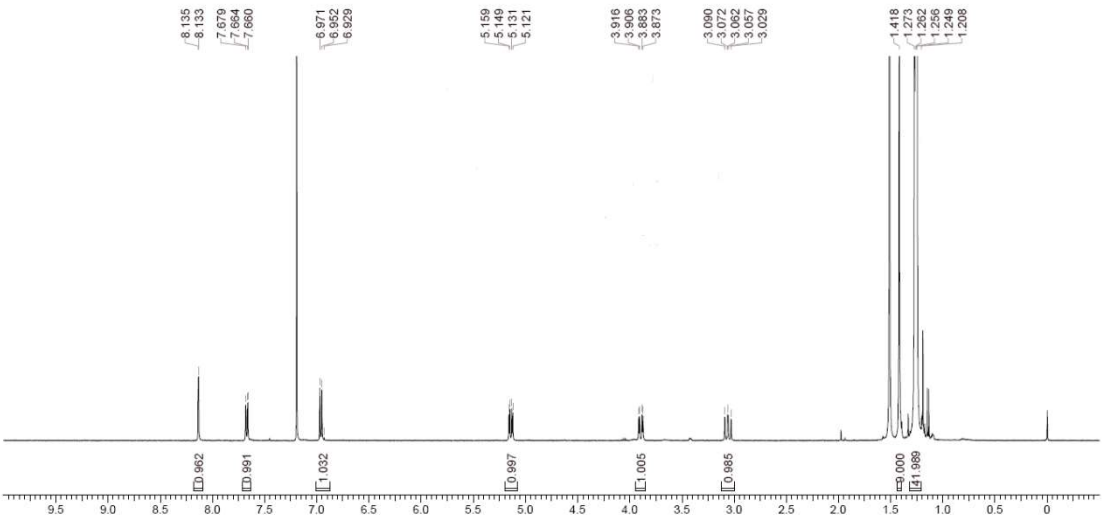


Fig S14 [1]HNMR spectrum of compounds **P2b** (400 MHz, $CDCl_3$)

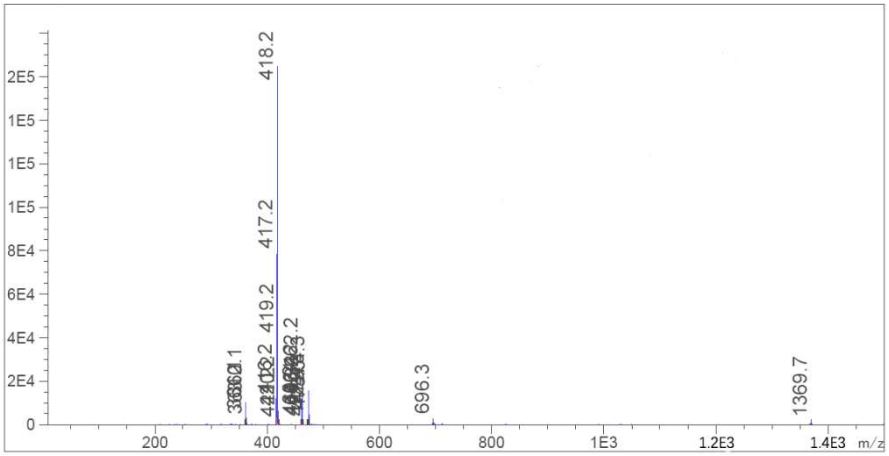


Fig S15 MS spectrum of compound **P2a**

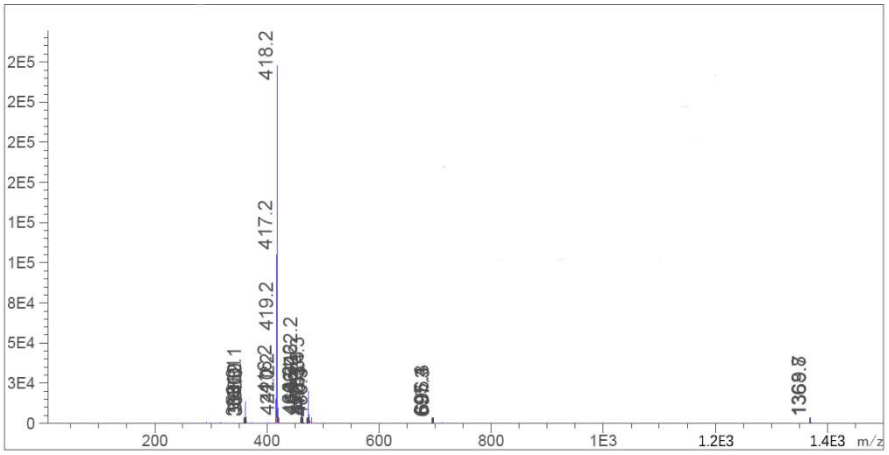


Fig S16 MS spectrum of compound **P2b**

The compounds **P2a** and **P2b** were also measured with analytical chiral SFC using a (R,R)Whelk-O1 column (50×4.6 mm, 1.8 μm). CO_2 (A) and 0.05% DEA/IPA (B) were used as mobile phases for gradient elution (B in A from 5% to 40%; flow rate: 3 mL/min; column temperature: 35°C). The retention times of **P2a** and **P2b** were 0.910 min and 0.985 min, respectively (Figs. S17 and S18).

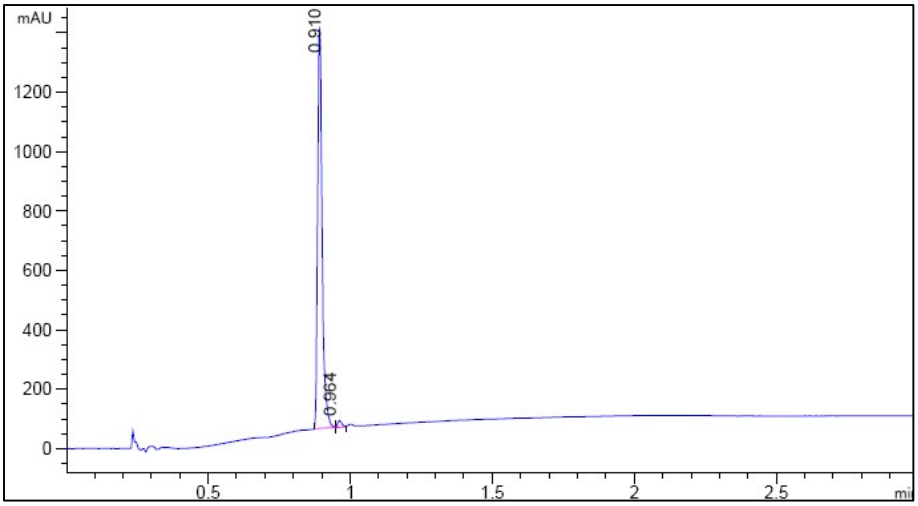


Fig. S17 Chiral SFC chromatogram of **P2a**

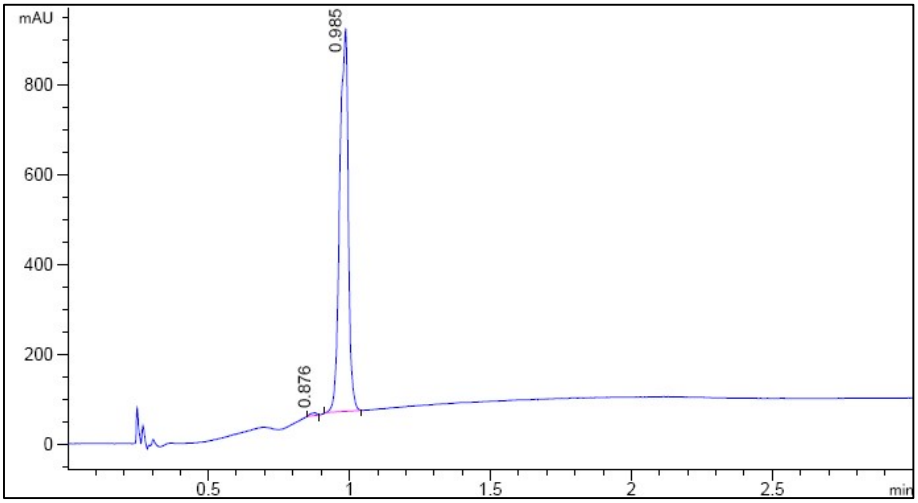


Fig. S18 Chiral SFC chromatogram of **P2b**

Manual radiosynthesis of 2-[^{18}F]FBPA with the aryldiboron precursors

The aryldiboron precursors **P1a** and **P1b** were radiolabelled with [^{18}F]fluoride using the copper-mediated method.[1-6] Firstly, [^{18}F]fluoride ion was produced from a HM20S medical cyclotron (Sumitomo Heavy Industries, Ltd.) via ^{18}O(p, n)^{18}F nuclear reaction by irradiation of [^{18}O]H_2O with proton beam. The [^{18}F]fluoride carried by [^{18}O]H_2O was captured by a Waters SepPak QMA light cartridge pre-conditioned with 2 mL 200 mM $KHCO_3$ and 10 mL H_2O, and then eluted from the QMA cartridge with K_{222}/ K_2CO_3 mixture in MeOH. An aliquot of the eluted [^{18}F]fluoride (37–370 MBq) was dried by heating at 95 °C, then the precursor and $Cu(OTf)_2Py_4$ dissolved in DMA/nBuOH/pyridine mixture were added under air atmosphere and heated at 100–130 °C. Radio-TLC (Silica gel plate, 95% MeCN/H_2O) was used to monitor the radiochemical conversion (RCC). The RCC values under different ratios of precursor **P1b**/copper salt/pyridine were shown in Table S2.

Table S2 RCC of the radiolabeling of aryldiboron precursor **P1b** with [^{18}F]fluoride

Entry	K_{222}/K_2CO_3/**P1b** (weight ratio, mg/mg/mg)	**P1b**/$Cu(OTf)_2Py_4$/Pyridine (molar ratio)	RCC (%)
1	0.3: 0.03: 6	1: 0.5: 0	83
2	0.36: 0.007: 6	1: 0.5: 7.5	74
3	0.36: 0.007: 6	1: 0.75: 10	84
4	0.3: 0.003: 6	1: 1: 0	90
5	2: 0.13: 6	1: 1: 10	83
6	1.5: 0.13: 6	1: 1: 10	81
7	0.3: 0.003: 6	1: 1: 15	93
8	1.5: 0.18: 6	1: 1: 15	80
9	3: 0.3: 6	1: 1: 15	82
10	1: 0.3: 6	1: 2: 20	90
11	1: 0.3: 6	1: 2: 100	61
12	1: 0.3: 6	1: 2: 200	37

To investigate the production of 2-[^{18}F]FBPA by hydrolysis, the radiolabelled reaction mixture was cooled down to room temperature, diluted with 1.2 mL MeOH and passed through a Alltech Max-Clean IC-H-0.5ml cartridge pre-washed with MeOH, then dried by heating under reduced pressure, and further hydrolysed with 6 M HCl at 110 °C. The hydrolysed product was dried under reduced pressure, dissolved with 0.1% HOAc in water and analysed by radio-HPLC with an Agilent 1260 Infinity Quaternary HPLC system equipped with UV detector and online radio-detector (Flow-Count B-FC-3500-

A diode detector, Eckert&Ziegler, Germany) and YMC-Pack ODS-A C18 column (250×4.6 mm, S-5 μm, 12 nm). The mobile phases were 0.1% HOAc/1% EtOH (phase A) and 0.1% HOAc/10% EtOH (phase B). The injection volume was 20 μl and the flow rate of mobile phase was 1 mL/min. The column temperature was set at 30 °C. The mobile phase composition of gradient elution condition was shown in Table S3.

Table S3 Mobile phase composition of gradient elution

Time (min)	Mobile phase (A: 0.1% HOAc/1% EtOH; B: 0.1% HOAc/10% EtOH)
0	100% A
13	100% A
13-15	100% A → 100% B
25	100% B
25.01	100% A
30	100% A

The typical radio-HPLC chromatograms of hydrolysed products of ^{18}F radiolabelled precursor were shown in Fig S19. By comparing to the standards, The radioactive peaks at retention time of 11.5 min and 23.2 min were identified as 2-[^{18}F]FBPA and 4-[^{18}]FBPA, respectively. Several other radioactive peaks were also found in the hydrolysed mixture. Among them 2-[^{18}F]F-tyrosine, 2-[^{18}F]F-phenylalanine and 4-[^{18}F]F-phenylalanine were identified by comparing their retention times to the standard compounds.

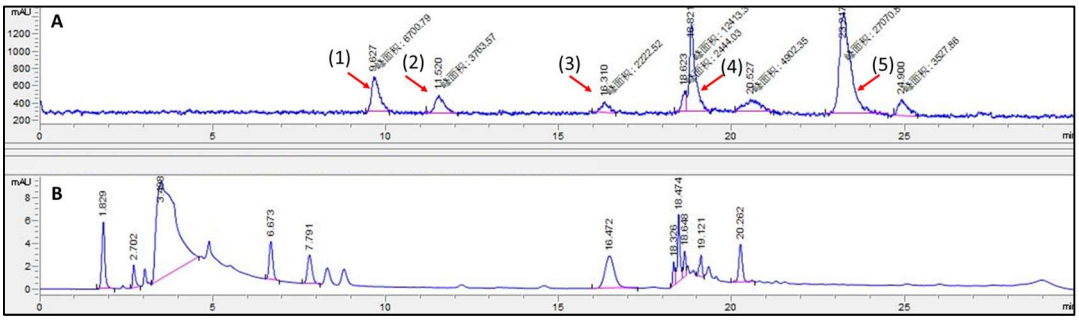


Fig. S19 Typical HPLC chromatograms of hydrolysed products of ^{18}F radiolabelled procursor **P1b. A**) Radioactive chromatogram. Peaks **1**, **2** , **3**, **4** and **5** were identified as 2-[^{18}F]F-tyrosine, 2-[^{18}F]FBPA, 2-[^{18}F]F-phenylalanine, 4-[^{18}F]F-phenylalanine and 4-[^{18}]FBPA, respectively; **B**) UV absorbance chromatogram at 260 nm.

Some ^{18}F radiolablled consititutes of the hydrolysed sample could not be eluted from the C18 column by the HPLC condition in Table S3. An alternative gradient method with improved ratio of organic solvent was set up enabling complete washout of the hydrolysed products (Table S4).

Table S4 Mobile phase composition of gradient elution

Time (min)	Mobile phase (A: 0.1% HOAc/1% EtOH; B: 0.1% HOAc/98% MeOH)
0	100% A
13	100% A
13-15	100% A → 100% B
35	100% B
35.01	100% A
40	100% A

The typical radio-HPLC chromatograms of hydrolysed products of ^{18}F radiolabelled precursor **P2b** using the alternative gradient method were shown in Fig. S20.

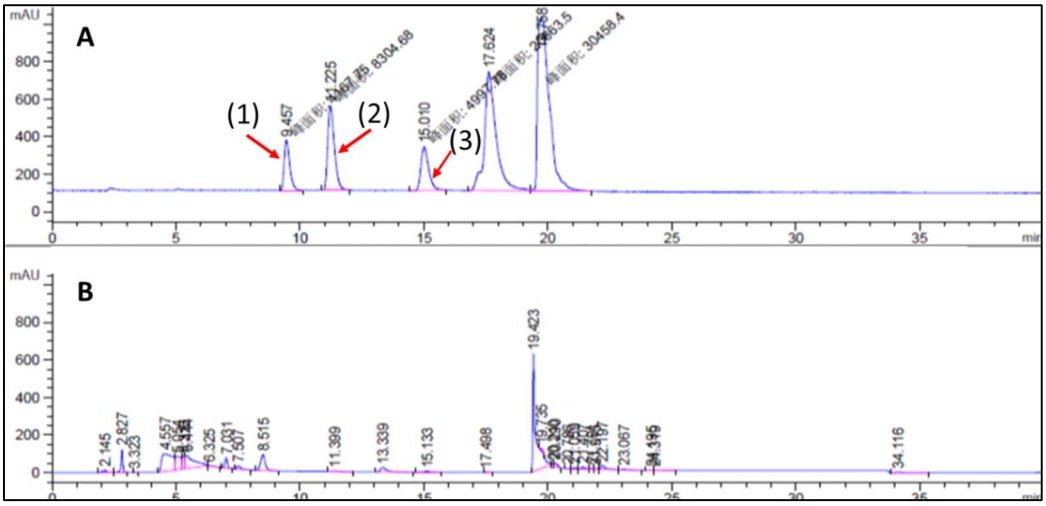


Fig. S20 Typical HPLC chromatograms of hydrolysed products of ^{18}F radilabelled procursor **P2b. A**) Radioactive chromatogram. Peaks **1**, **2** and **3** were identified as 2-[^{18}F]F-tyrosine, 2-[^{18}F]FBPA and 2-[^{18}F]F-phenylalanine, respectively; **B**) UV absorbance chromatogram at 260 nm.

Radiosynthesis of 2-[^{18}F]FBPA with automatic module

The automatic radiosynthesis of 2-[^{18}F]FBPA with the N-Boc precursor and N,N-diBoc precursor was investigated on a cassette-type CFN-MPS200 module controlled by the Cupid System (Sumitomo Heavy Industries, Ltd.). The schematic diagram of the procedure was shown in Fig. S21. The automatic radiosynthesis used two reaction vials, one was for nucleophilic radiolabeling of the precursor, the other was for acidic hydrolysis of the radiolabelled intermediate.

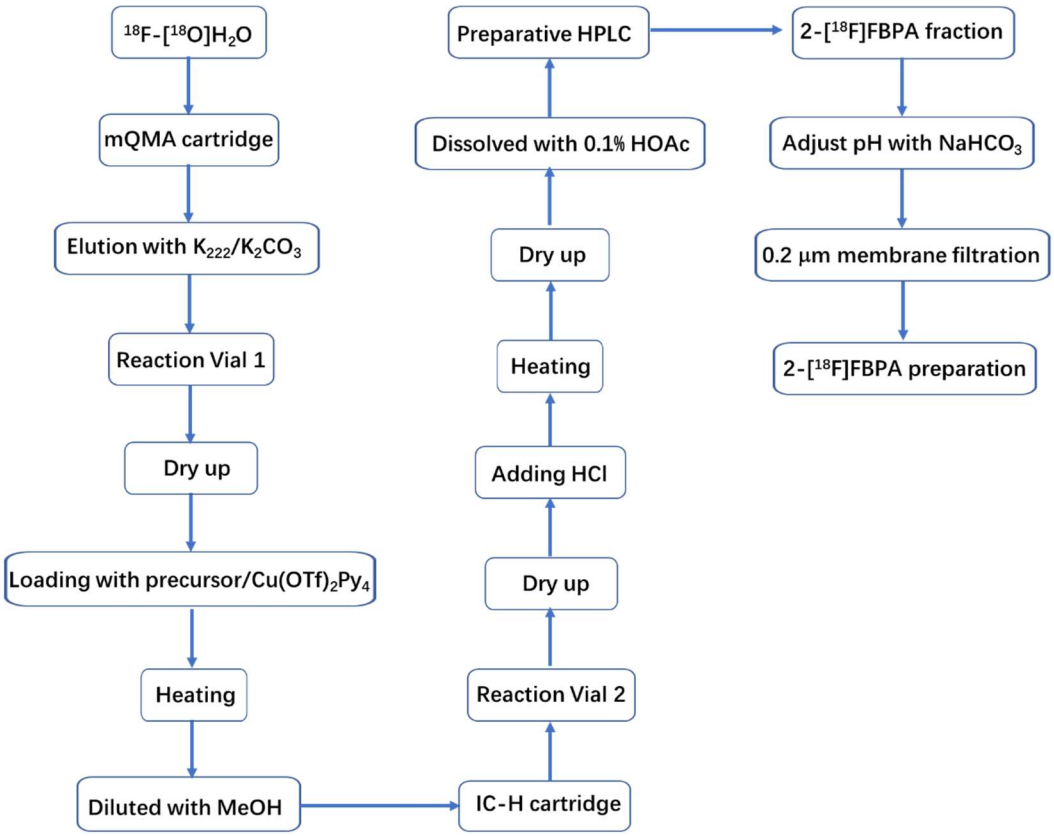


Fig. S21 Schematic diagram of the radiosynthesis of 2-[^{18}F]FBPA with the automatic module

The [^{18}F]fluoride produced by the cyclotron was captured on a small cartridge filled with 23 mg of SepPak QMA exchanger (mQMA cartridge) pre-conditioned with 1 mL 200 mM $KHCO_3$ and 5 mL H_2O, and then eluted from the mQMA cartridge with methanolic K_{222}/K_2CO_3. After drying the K_{222}/[^{18}F]KF solution by heating at 100 °C in vacuo, the precursor and $Cu(OTf)_2Py_4$ dissolved in DMA/nBuOH/pyridine mixture (precursor: 6–15 mg, 10 mg/mL) were added to the first reaction vial and heated at 120 °C under air atmosphere for 25 minutes. Then the vial was cooled down to 40 °C and was added 2.6 mL of MeOH. After bubbled with nitrogen gas for 2 minutes, the solution was transferred into the other reaction vial through an Alltech Max-Clean IC-H-0.5ml

cartridge pre-washed with MeOH. The solution was heated at 90 °C to dry in vacuo. To the vial was added 0.6 mL 6 M HCl and heated at 130 °C for 5 minutes. Then the acid was vapored in vacuo. 1.95 mL of 0.1% HOAc was added into the vial and transferred through an online 0.45 μm membrane filter to the preparative radio-HPLC equipped with a YMC-Pack ODS-A C18 column (250×10 mm, S-5 μm, 12 nm). The isocratic elution used 0.1% HOAc/1% MeOH as mobile phase. The flow rate was 4 mL/min. 2-[^{18}F]FBPA fraction was collected in according to the retention time of FBPA standard, added with 200 mM $NaHCO_3$ to adjust pH 4.5-7.5 and filtered with 0.22 μm sterile membrane filter to give the [^{18}F]FBPA preparation. Radiochemical purity (RCP) of the [^{18}F]FBPA preparation were determined by the analytical radio-HPLC. The HPLC chromatograms of 2-[^{18}F]FBPA preparation (Fig. S22) showed the RCP was 100% and no peak of 2-FBPA carrier and other chemical impurity was detected.

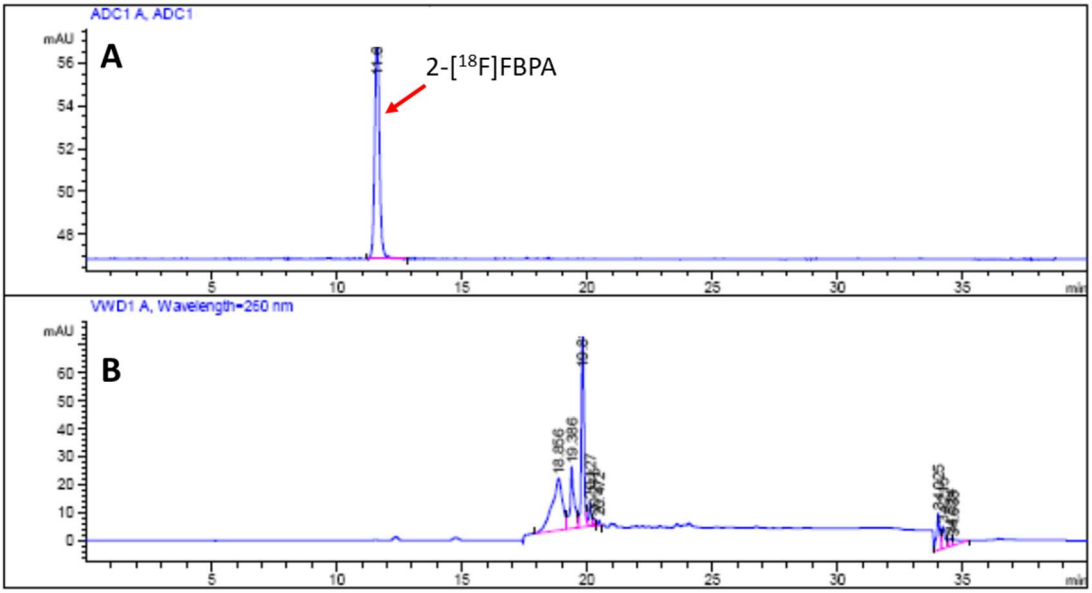


Fig. S22 HPLC chromatograms of 2-[^{18}F]FBPA preparation. **A**) Radioactive chromatogram; **B**) UV absorbance chromatogram at 260 nm.

HPLC analysis of 2-FBPA standards

A 0.5 mg/mL stock solution of 2-FBPA in saline was prepared in the 0.1% HOAc/1% EtOH solvent and diluted serially to make standard solutions of 0.25, 0.125, 0.0625, 0.03125 and 0.01563 mg/mL. These standard solutions and the solvent as blank were measured by HPLC using the same method as that for 2-[^{18}F]FBPA preparation. The peak area of 2-FBPA had good linear response to the concentration (Fig. S23). The detection limit and quantitation limit were estimated to be 5 μg/mL and 14 μg/mL, respectively, from the standard deviation of intercept and slope of the fitting curve.

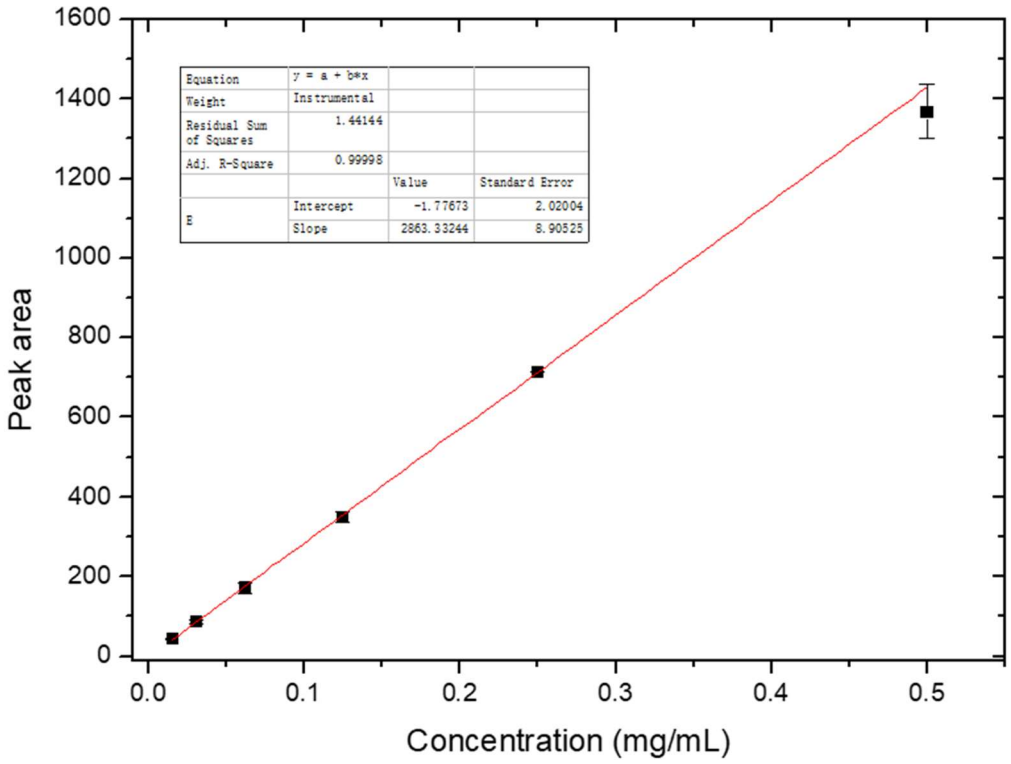


Fig. S23 The linear relationship of UV absorbance peak areas of 2-FBPA standard solutions with their concentrations

The 2-[^{18}F]FBPA preparations were also analysed by chiral radio-HPLC with a Daicel Crownpak CR(+) analytical column (4×150 mm, 5 μm) protected with a Daicel Crownpak CR(+) guard column (4×10 mm, 5 μm). $HClO_4$, pH 2.0 was used as mobile

phase for isocratic elution at a flow rate of 1 mL/min. The column temperature was 20 °C. The injection volume of sample was 20 µl. The retention times of 4-borono-2-fluoro-L-phenylalanine (L-2-FBPA) and 4-borono-2-fluoro-D-phenylalanine (D-2-FBPA) were about 13.0 min and 8.0 min, respectively, showing good separation of these isomers. The analytical results indicated that the chiral purities of L-2-[^{18}F]FBPA and D-2-[^{18}F]FBPA preparations were almost 100% (Fig. S24).

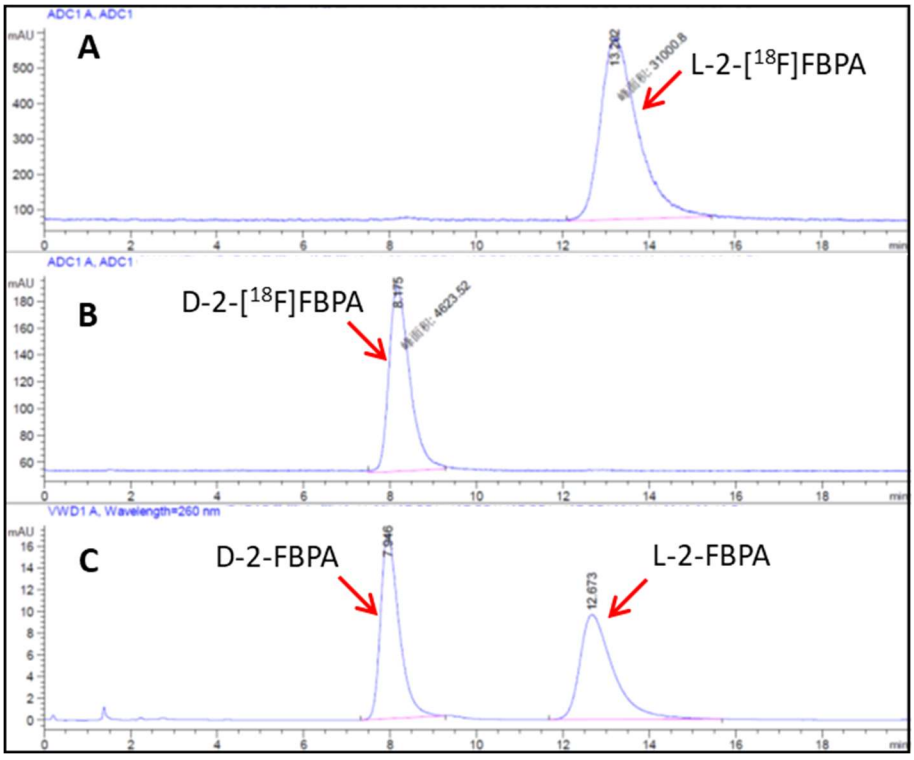


Fig. S24 Chiral analytical HPLC chromatograms. **A**) Radioactive chromatogram of L-2-[^{18}F]FBPA radiosynthesized from precursor **P2b**; **B**) Radioactive chromatogram of D-2-[^{18}F]FBPA radiosynthesized from precursor **P2a**; **C**) UV absorbance chromatogram at 260 nm of D,L-2-FBPA standard sample.

Quality control of the 2-[^{18}F]FBPA preparation

The 2-[^{18}F]FBPA preparation was clear during 6 hours of storage in borosilicate glass vial. The pH value was in a range of 4.5–7 as determined by precise pH strips. Nuclear

purity, radionuclide half-life, sterilization and endotoxin of the 2-[^{18}F]FBPA preparations were determined using the recommended methods for PET pharmaceuticals by Chinese Pharmacopeia.[7] The RCP determined by the analytical radio-HPLC. EtOH, MeOH, nBuOH, DMA and pyridine were determined by GC (Agilent 6850 GC). The GC was equipped with an automatic injector, a FID and a Restek Stabilwax column (Carbowax^{TM} polyethylene glocol, 30 m, 0.53 mm I.D., 1 μm). Initial oven temperature: 55 °C, holding 4 min; temperature range: 55 °C to 200 °C, 40 °C/min, holding 3 min; FID temperature: 240 °C, hydrogen: 30 mL/min, air: 400 mL/min; makeup nitrogen: 25 mL/min; sample injection: 1 μl. The presence of EtOH was no more than 1%. nBuOH and pyridine in the 2-[^{18}F]FBPA preparations were below the detection limit of QC. The detected concentrations of residual MeOH and DMA in the [^{18}F]FBPA preparations (6 batches) were 260±233.9 μg/mL and 193±140.3 μg/mL, respectively, which were much lower than the concentration limits of ICH Q3C guideline for these two solvents (MeOH: 3000 ppm, DMA: 1090 ppm).[8]

The copper ion in 2-[^{18}F]FBPA preparation was detected by a convenient visual inspection after V/V 1:1 mixing the sample with 0.1 mM 4-(2-pyridylazo)resorcinol (PAR) in 25 mM acetate buffer, pH 6.0. Cu^{2+} concentration ≥ 10 μM could significantly change the colour of PAR solution from light yellow to orange - reddish orange. No colour change caused by the 2-[^{18}F]FBPA preparation was visualized, indicating the concentration of Cu^{2+} was smaller than 10 μM , Therefore, the copper content in the 2-[^{18}F]FBPA preparation for a single dose (supposing a 10 mL of maximum injection volume) was <6.35 μg, much lower than the 300 μg/day limit of ICH Q3D guideline.[9]

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