Supporting Information for

Synthesis of enantiopure $^{18}$F-trifluoromethyl cysteine as a structure-mimetic amino acid tracer for glioma imaging

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1. Material and general methods

All chemical reagents and solvents used in the experiments were obtained from commercial sources without further purification. K$_2$CO$_3$, Kryptofix 2.2.2 (K$_{2.2.2}$), anhydrous CH$_3$CN and DMF used for radiosynthesis were purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. Sep-Pak light QMA, Sep-Pak Alumina N Plus Light, and Sep-Pak C18 Plus Short cartridges were purchased from Waters (Milford, USA), and Biotechnology grade AG® 1 1 A resin was obtained from Bio-Rad (Hercules, California, USA). Radioactivity was measured by a gamma counter (γ-counter) (SN-6105, Shanghai Nuclear Rihuan Photoelectric Instrument LLC, China). The analytical high-performance liquid chromatography (HPLC) (1260 series, Agilent, USA) and radioactive thin layer chromatography scanner (radio-TLC) (miniGita Star, Raytest, Straubenhardt, Germany) were applied to monitor the reaction extent and analysis of the product purity. The chiral HPLC column (Astec® CHIROBIOTIC® TAG, 25 cm × 4.6 mm, 5 μm) was purchased from Sigma-Aldrich (Shanghai) Trading Co. Ltd. The nuclear magnetic resonance (NMR) spectra including $^1$H, $^{13}$C, and $^{19}$F NMR were recorded on a Bruker 400 MHz or 600 MHz spectrometer. All chemical shifts are reported in ppm relative to tetramethylsilane and peak multiplicities are given as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m).
2. Positron-labelled sulfur-containing amino acid (SAA) tracers

### A. $^{11}C$-labelled SAA tracers
- L-methyl-$^{[11}C$]methionine 1980s
- S-$^{[11}C$]-methyl-L-cysteine 1L *Tang et al.* (2011)
- S-$^{[11}C$]-methyl-D-cysteine 1D *Tang et al.* (2015)

### B. $^{18}F$-labelled SAA tracers
- S-$^{[2\times^{18}F}$fluoroethyl]-L-homocysteine *Tang et al.* (2003)
- S-$^{[3\times^{18}F}$fluoroethyl]-L-homocysteine *Bourrier et al.* (2011)
- S-$^{[3\times^{18}F}$fluoroethyl]-D-homocysteine *Bourrier et al.* (2011)
- $^{[18}F$]-B-MET *Chen et al.* (2017)
- S-$^{[18}F]$CF$_2$-L-CYS 2L *This study*
- S-$^{[18}F]$CF$_2$-D-CYS 2D *This study*

**Figure S1.** Positron-labelled sulfur-containing amino acid (SAA) tracers. (A) $^{11}C$-labelled SAA tracers; (B) $^{18}F$-labelled SAA tracers.

3. Synthetic procedures and spectroscopic data for the compounds
**Scheme S1.** Synthesis of 3L, 3D, S-CF3-L-CYS 10L, and S-CF3-D-CYS 10D. Reagents and conditions: a. (Boc)2O, 1M NaOH aq., Dioxane, r.t.; b. 2-tert-Butyl-1,3-diisopropylisourea, THF, r.t., 4 h; c. SOCl2, imidazole, Et3N, CH2Cl2, -20 °C - r.t., 10 h; d. NaIO4, RuCl3 · H2O, CH3CN, H2O, 0 °C, 2 h; e. PDFA, S8, CsF, DMF, 70 °C, 0.5 h; f. 10 % NaH2PO4 aq., EtOAc, 50 °C, 2 h; g. TFA, CH2Cl2, r.t., 24 h.

As shown in scheme S1, firstly, the amino protection reaction of L-serine or D-serine with di-tert-butyl carbonate in a mixture of 1M NaOH aq. and dioxane gave compounds 6L,D.[1] Then the carboxyl group was protected by esterification with 2-tert-Butyl-1,3-diisopropylisourea in THF at room temperature to give compounds 7L,D.[2, 3] Next, the obtained amino β-alcohols were reacted with thionyl chloride in the presence of triethylamine under the catalysis of imidazole to afford the intermediate cyclic-sulfamidites which were subsequently oxidized by sodium periodate with catalytic ruthenium(III) chloride to give cyclic-sulfamidates 3L,D.[4]

With the desired cyclic-sulfamidates in hand, we immediately set out to investigate whether these compounds were applicable substrates for the exogenous-fluoride-mediated nucleophilic trifluoromethylthiolation reaction. Much to our delight, the ring-opening reaction of these cyclic-sulfamidates 3L,D proceeded smoothly in the presence of PDFA (Ph3P+CF2CO2-) and S8 (elemental sulfur) by the addition of external fluoride ion (CsF) under a mild heating condition (70 °C), leading to the formation of sulfamic acids 8L,D as the intermediates which were selectively hydrolyzed by a 10% aqueous NaH2PO4 solution (pH 4.5) in EtOAc (1:1, v/v) to afford 9L,D. Finally, simultaneous cleavages of the tert-butyl and tert-butyloxycarbonyl protecting groups were carried out
in a mixture of TFA and CH₂Cl₂ (3:1, v/v) at room temperature to afford S-CF₃-L-CYS 10L and S-CF₃-D-CYS 10D.

1) Potassium 2-bromo-2,2-difluoroacetate

![Chemical Structure of Potassium 2-Bromo-2,2-Difluoroacetate]

To a solution of potassium hydroxide (2.80 g, 50.00 mmol) in methanol (50 mL) was slowly dropped ethyl 2-bromo-2,2-difluoroacetate (10.25 g, 50.50 mmol) at 0 °C. Afterward, the mixture was naturally warmed to room temperature and stirred for 18 h. The organic solvent was removed, and the residue was further dried under vacuum to obtain a white powder (16.90 g, 95%), which was directly used in the next reaction without further purification.

*Note*: The white powder is easy to absorb moisture.

2) 2,2-difluoro-2-(triphenylphosphonio)acetate (PDFA)

![Chemical Structure of PDFA]

Triphenylphosphine (12.46 g, 47.50 mmol) was added to a solution of potassium bromodifluoroacetate (16.90 g, 47.50 mmol) in anhydrous DMF (45 mL). The reaction mixture was stirred at room temperature for 21 h. After that, the reaction mixture was filtered with a Buchner funnel, and the residue was thoroughly washed with DMF (3 × 20 mL), H₂O (3 × 20 mL), Et₂O (3 × 20 mL), then dried under reduced pressure to give a white powder (14.04 g, 81%). ¹H NMR (600 MHz, DMSO-d₆) δ 7.95 – 7.91 (m, 2H),
7.83 – 7.75 (m, 9H), 7.65 – 7.54 (m, 1H), 7.41 (dd, $J = 10.2, 5.9$ Hz, 3H), 7.27 – 7.22 (m, 2H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 136.67, 136.59, 135.33, 135.31, 134.50, 134.43, 133.31, 133.18, 130.29, 130.20, 128.99, 128.80, 128.75, 124.19, 116.45, 115.89; $^{19}$F NMR (565 MHz, DMSO-$d_6$) δ -93.37 (s), -93.55 (s).

3) (tert-butoxycarbonyl)-L-serine 6L

To a solution of L-serine (10.5 g, 100 mmol) in 1M NaOH aq. (200 mL) and dioxane (100 mL) was slowly added ditertbutylidicarbonate (21.8 g, 100 mmol). The mixture was stirred for overnight at room temperature. The reaction solution was washed with diethyl ether (3 × 100 mL). Then the aqueous phase was acidified to pH = 3 with 1N HCl aq., extracted with ethyl acetate (3 × 200 mL). The combined organic phase was washed with water (300 mL), brine (300 mL) and dried over Na$_2$SO$_4$, then evaporated the solvent to afford a colorless oil (18.46 g, 90%). [α]$_D^{22}$ 6.58 (c 1.0, CHCl$_3$).

4) tert-butyl (tert-butoxycarbonyl)-L-serinate 7L

To a solution of (tert-butoxycarbonyl)-L-serine (8.20 g, 40 mmol) in anhydrous THF
(100 mL) was slowly added O-tert-Butyl-N, N’-diisopropylisourea (20.03 g, 100 mmol). Then the mixture was stirred for 4 h at room temperature. The mixture was filtered through Celite® and the organic solvent was evaporated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:5) to give the title compound as a colorless oil which turned to a white solid on standing (6.79 g, 65%). Rf 0.50 (ethyl acetate/petroleum ether 1:2), mp 78-80 °C, [α]D22 +9.73 (c 1.0, CHCl3). 1H NMR (400 MHz, CDCl3) δ 5.42 (s), 4.26 (s), 3.90 (dd, J = 6.0, 4.0 Hz), 2.39 (s), 1.49 (s), 1.46 (s). 13C NMR (101 MHz, CDCl3) δ 169.95, 156.03, 82.70, 80.22, 64.10, 56.47, 28.42, 28.11.

5) di-tert-butyl (4S)-1,2,3-oxathiazolidine-3,4-dicarboxylate 2,2-dioxide 3L

To a solution of imidazole (2.73 g, 40.00 mmol) and triethylamine (3.06 ml, 22.00 mmol) in anhydrous dichloromethane (100 mL) was slowly added thionyl chloride (0.81 mL, 11.00 mmol) during 10 min at about 0 °C. The reaction mixture was stirred at this temperature for 0.5 h and then cooled to -20 °C. A solution of tert-butyl (tert-butoxycarbonyl)-L-serinate (2.61 g, 10.00 mmol) in dry dichloromethane (50 mL) was slowly added to the resulting mixture for 30 min. Afterward, the mixture was naturally warmed to room temperature and stirred for 10 h. The reaction mixture was diluted with water (100 mL), extracted with dichloromethane (2 × 100 mL), combined the organic
phase, washed with 10% citrate solution (200 mL), water (200 mL), brine (200 mL),
dried over Na₂SO₄, and concentrated the solvent to afford the crude cyclic-sulfinamide
as a yellow oil which was directly used in the next reaction without further purification.

Ruthenium (III) chloride hydrate (23 mg, 0.11 mmol) and sodium periodate (2.34 g,
11.00 mmol) were added to a solution of the crude cyclic-sulfinamide in a mixture
acetonitrile/water (7: 5, 120 ml) at 0 °C. After stirring at 0 °C for 2 h, the mixture was
diluted with water (100 mL), extracted with ethyl acetate (3 × 100 mL), combined the
organic phase, washed with water (200 mL), brine (200 mL) dried over Na₂SO₄, and
concentrated the solvent in vacuo. The residue was purified by column chromatography
on silica gel (ethyl acetate/petroleum ether 1: 6) to give the title compound (2.59 g,
80%) as a white powder. R₇ 0.40 (ethyl acetate/petroleum ether 1:4), mp 115-117 °C;
[α]D²² -38.77 (c 0.5, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 4.74 (t, J = 8.6 Hz, 1H),
4.63 (d, J = 8.5 Hz, 2H), 1.56 (s, 9H), 1.51 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ
166.16, 148.25, 86.00, 84.62, 67.88, 58.27, 28.04, 27.96. MS (EI) Calcd. for
C₁₂H₂₁NO₇S [M+H]⁺: 323.10; found 323.11.

6) tert-butyl N-(tert-butoxycarbonyl)-S-(trifluoromethyl)-L-cysteinate 9L

To a 20 mL sealed tube were added di-tert-butyl (4S)-1,2,3-oxathiazolidine-3,4-
dicarboxylate 2,2-dioxide (194 mg, 0.6 mmol), S8 (115.2 mg, 0.45 mmol), PDFA
(427.6 mg, 1.2 mmol), CsF (455.7 mg, 3.0 mmol) and anhydrous dimethyl formamide (4.5 mL) under inert atmosphere (Ar). The reaction mixture was stirred at 70 °C for 30 min. After being cooled to room temperature, the solution was transferred to a 100 mL round bottom flask containing a mixture of ethyl acetate (20 mL) and 10% NaH$_2$PO$_4$ aq. (20 mL) and then stirred at 50 °C for 2 h. The resulting mixture was taken up with ethyl acetate (80 mL), washed with water (2 × 100 mL), brine (100 mL), dried over Na$_2$SO$_4$, and concentrated the solvent under vacuum. The resulting solid residue was then washed with cold petroleum ether (20 mL), filtrated and concentrated to obtain the crude product which was purified by short column chromatography (diameter 3 cm × length 6 cm) on silica gel (gradually from petroleum ether to petroleum ether/ethyl acetate 100: 1) to give the title compound (106 mg, 51%) as a yellow oil. R$_f$ 0.3 (ethyl acetate/petroleum ether 30: 1). $[\alpha]_D^{22} +18.19$ (c 0.5, CHCl$_3$). $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 5.37 (d, $J = 3.8$ Hz), 4.49 (d, $J = 3.3$ Hz), 3.48 (dd, $J = 9.0, 2.9$ Hz), 3.29 (dd, $J = 9.0, 2.8$ Hz), 1.49 (s), 1.45 (s); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 168.72, 155.08, 133.82, 131.79, 129.76, 127.73, 83.67, 80.46, 53.46, 32.31, 29.83, 29.77, 28.36, 28.02; $^{19}$F NMR (565 MHz, CDCl$_3$) $\delta$ -40.92 (s). HRMS (EI) Calcd. for C$_{13}$H$_{22}$F$_3$NO$_4$S [M+H]$^+$: 345.1222; found 345.1219.
Analysis of hydrolysis reaction mixture by using TLC (ethyl acetate/petroleum ether 1:30 as the developing solvent).

**Note 1:** To isolate the CF₃-product (easily soluble in petroleum ether) from Ph₃P=S (triphenylphosphorus sulfide, slightly soluble in petroleum ether). It is necessary for the crude product to wash with cold petroleum ether.

**Note 2:** The short column chromatography was gradually eluted from petroleum ether to petroleum ether/ethyl acetate 100:1. It's necessary to use a large amount of petroleum ether (about 5~6 L) throughout the purification process.

**Note 3:** Redistilled petroleum ether could effectively decrease the residual solvents (high-boiling point) in the final product.

### 8) S-(trifluoromethyl)-L-cysteine 10L

To a solution of tert-butyl N-(tert-butoxycarbonyl)-S-(trifluoromethyl)-L-cysteinate (69 mg, 0.2 mmol) in anhydrous dichloromethane (1.0 mL) was added Trifluoroacetic
acid (TFA, 3 mL), and the reaction mixture was stirred at room temperature. After stirring for 6 h, the solvent was removed under high vacuum, anhydrous diethyl ether (10 mL) was added to the residue. The crude S-CF₃-L-CYS was filtered (syringe filter, PTFE, 25 mm diameter, 0.45 μm), then washed with diethyl ether (2 × 10 mL), and finally dissolved with methanol (2 × 10 mL). The organic solvent was concentrated in vacuo to give the title product as a white powder (36 mg, 95%). Mp 232-234 °C; [α]D²² +12.93 (c 0.1, MeOH). ¹H NMR (600 MHz, D₂O) δ 4.04 (dd, J = 7.4, 4.4 Hz, 1H), 3.56 (dd, J = 15.3, 4.2 Hz, 1H), 3.40 (dd, J = 15.2, 7.7 Hz, 1H). ¹³C NMR (151 MHz, CD₃OD) δ 169.48, 134.92, 132.89, 130.86, 128.84, 53.69, 30.42. ¹⁹F NMR (565 MHz, CD₃OD) δ -42.85 (s), -76.55 (s) (trifluoroacetic acid). MS (EI) Calcd. for C₄H₆F₃NO₂S [M+H]⁺: 189.01; found 189.00.

**Workup of the deprotection reaction by using ethyl ether**

Diethyl ether

White power: S-(trifluoromethyl)-L-cysteine 10L

9) (tert-butoxycarbonyl)-D-serine 6D
6D was synthesized from D-serine as starting material. The synthetic procedure of D-6 was the same with that of 6L. White solid, mp 58-60 °C, [α]D22 -12.75 (c 1.0, CHCl3).

10) tert-butyl (tert-butoxycarbonyl)-D-serinate 7D

The synthetic procedure of 7D was the same with that of 7L. White solid, mp 76-78 °C, [α]D22 -22.54 (c 1.0, CHCl3). 1H NMR (400 MHz, CDCl3) δ 5.43 (s, 1H), 4.26 (s, 1H), 3.90 (d, J = 3.6 Hz, 2H), 1.49 (s, 9H), 1.46 (s, 9H). 13C NMR (101 MHz, CDCl3) δ 170.00, 155.98, 82.51, 80.09, 63.80, 56.40, 28.37, 28.05.

11) di-tert-butyl (4R)-1,2,3-oxathiazolidine-3,4-dicarboxylate 2,2-dioxide 3D

The synthetic procedure of 3D was the same with that of 3L. White solid, mp 123-125 °C, [α]D22 +21.45 (c 1.0, CHCl3). 1H NMR (400 MHz, CDCl3) δ 4.76 – 4.71 (m), 4.67 – 4.60 (m), 1.56 (s), 1.51 (s). 13C NMR (101 MHz, CDCl3) δ 166.13, 148.21, 85.98, 82.97.
13) S-(trifluoromethyl)-D-cysteine 10D

The synthetic procedure of 10D was the same with that of 10L. White solid, mp 217-219 °C, [α]D22 -14.01 (c 0.1, MeOH). 1H NMR (600 MHz, D2O) δ 4.00 (dd, J = 7.4, 4.4 Hz, 1H), 3.55 (dd, J = 15.2, 4.3 Hz, 1H), 3.39 (dd, J = 15.2, 7.7 Hz, 1H). 13C NMR (151 MHz, CD3OD) δ 169.86, 135.08, 133.06, 131.03, 129.00, 53.94, 30.63. 19F NMR (565 MHz, CD3OD) δ -42.85 (S), -76.55 (S) (trifluoroacetic acid). HRMS (EI) Calcd. for
C₄H₆F₃NO₂S [M+H]⁺: 189.0071; found 189.0067.

4. Radiosynthesis of S-[¹⁸F]CF₃-L-CYS and S-[¹⁸F]CF₃-D-CYS

1) Optimization of the reaction conditions (¹⁸F-trifluoromethylthiolation)

We started our initial research on the ¹⁸F-trifluoromethylthiolation using the conditions reported in the literature.[⁵] We selected the cyclic-sulfamidate 3L as a model substrate and used the heating-block-dried K₂₂₂/K¹⁸F instead of CsF to react with PDFA and S₈. However, the intermediate sulfamidite 4L resulting from the ring-opening ¹⁸F-trifluoromethylthiolation of cyclic-sulfamidates 3L was obtained in low radiochemical yields (RCY) in 2 minutes, when the reaction was carried out at 70 °C in CH₃CN without additives (Table S1, entries 1-2). Subsequent attempts found that the RCY of 4L increased with the reaction time extending, but no apparent increases beyond 5 minutes (entries 3-4). The use of additives to further optimize the reaction conditions was of no obvious positive impact on the reaction yields (entries 5-7). After that, the investigation of solvent effect indicated that using DMF couldn’t give higher RCY (entries 8-9), mainly because the polar intermediate 4L wasn’t easily trapped by the C18 cartridge in the presence of DMF, even if be diluted by water at least 20-fold. Thus, it may not be so helpful to use solvents with a higher polarity in the preparation of 2L and 2D. Moreover, we knew that the increase of stoichiometric ratio might be favorable for the improvement of RCY, but this was not optimal because the insoluble substances
would rise accordingly; and as such, we had been committed to exploring this reaction under low stoichiometry conditions.

**Table S1** Optimization of the reaction conditions

| Entry | additive* | Solvent | Time (min) | RCYb (%) |
|-------|-----------|---------|------------|----------|
| 1     | Non       | CH₃CN   | 1          | 6%       |
| 2     | Non       | CH₃CN   | 2          | 10%      |
| 3     | Non       | CH₃CN   | 5          | 23%      |
| 4     | Non       | CH₃CN   | 10         | 26%      |
| 5     | CuBr      | CH₃CN   | 5          | 15%      |
| 6     | CuI       | CH₃CN   | 5          | 20%      |
| 7     | TBNI      | CH₃CN   | 5          | 22%      |
| 8     | Non       | DMF     | 5          | 13%      |
| 9     | Non       | DMF     | 10         | 16%      |

*aMolar ratio of 3L : PDFA : S₈ : additive (1:1:2:1)

bRCY of 4L isolated after Sep-Pak C18 plus short cartridge purification (n = 3)

2) Apparatus and Sep Pak cartridges used for the radiosynthesis of 2L and 2D

Equipment for the nucleophilic [¹⁸F]trifluoromethylthiolation reaction

The solid phase extraction (SPE) cartridges used for the final product (2L and 2D) purification
3) The nucleophilic $^{18}\text{F}$-trifluoromethylthiolation protocol

Scheme S2. The nucleophilic $^{18}\text{F}$-trifluoromethylthiolation protocol for synthesizing enantiopure $^{18}\text{F}$-trifluoromethylated cysteines.

As illustrated in Scheme S2, serine-derived cyclic sulfamidates (3L and 3D) were used as chiral synthons for the synthesis of $^{18}\text{F}$-trifluoromethylated cysteines (2L and 2D).

2L and 2D were synthesized stereoselectively from the serine-derived cyclic sulfamidates 3L and 3D via a nucleophilic $^{18}\text{F}$-trifluoromethylthiolation reaction followed by a deprotection reaction.

5. Radio-TLC analysis of the radiochemical purity

The radiochemical purity of S-$^{[18}\text{F}]\text{CF}_3\text{-L-CYS}$ or S-$^{[18}\text{F}]\text{CF}_3\text{-D-CYS}$ was determined by radio-Thin Layer Chromatography (radio-TLC). Radio-TLC was carried out using aluminum sheets pre-coated with silica gel 60 F254 (E. Merck, Germany).

Solvent system: acetonitrile/methanol/water (40:10:10)
Figure S2. Radio-TLC analysis of the radiochemical purity of S-[18F]CF3-L-CYS 2L dose

Figure S3. Radio-TLC analysis of the radiochemical purity of S-[18F]CF3-D-CYS 2D dose

6. HPLC analysis of S-[18F]CF3-L-CYS and S-[18F]CF3-D-CYS enantiomers

As shown in the figure below, S-[18F]CF3-L-CYS (Rt = 7.646 min) and S-[18F]CF3-D-CYS (Rt = 10.640 min) enantiomers could be well separated, when the pH value of the product solutions was neutralized to pH 7 (AG® 11 A8 Resin-treated). Almost no
racemization was detected. The relative retention times were compared with $S$-CF$_3$-CYS enantiomers (L/D, 4:6) injected under similar conditions to determine $S$-[${}^{18}$F]CF$_3$-L-CYS and $S$-[${}^{18}$F]CF$_3$-D-CYS, respectively.

| Apparatus          | Agilent 1260 |
|--------------------|--------------|
| Column             | Astec® CHIROBIOTIC® TAG, 25 cm × 4.6 mm, 5 μm |
| Mobile phase       | [A] MeOH; [B] H$_2$O (75:25, A:B) |
| Flow rate          | 1 mL/min |
| Sample 1           | 0.4 mg $S$-CF$_3$-L-CYS and 0.6 mg $S$-CF$_3$-D-CYS in 1 mL H$_2$O |
| Sample 2           | $S$-[${}^{18}$F]CF$_3$-L-CYS aq. (pH = 7) |
| Sample 3           | $S$-[${}^{18}$F]CF$_3$-D-CYS aq. (pH = 7) |
| Injection          | 10 μL |
| Detector 1         | UV, 205 nm (Model 35900E, Agilent, USA) |
| Detector 2         | Radioactive Detector (Model BFC-3200, Bioscan. Inc., USA) |

**Figure S4.** Chiral-HPLC analysis of $S$-[${}^{18}$F]CF$_3$-CYS enantiomers

*Note:* There was a difference in retention times between UV signal and radiation signal due to the distance between the series-connection detectors (UV detector preceding the radiodetector in a line-setting).
2) HPLC analysis of \( S-[^{18}\text{F}]\text{CF}_3\)-CYS enantiomers using the test tube method

To further improve the detection sensitivity (radioactive signal), the test tube method\(^6\) was used for HPLC analysis of \( S-[^{18}\text{F}]\text{CF}_3\)-CYS enantiomers. As shown in the figure below, HPLC samples were collected in 30-second fractions by using the test tube (10 mL) for 20 min. All the samples were counted using a \( \gamma \)-counter for 20 s. The counts were decay-corrected to the initial injection time and added together. The radioactivity counts were expressed as the plotted intensity (cpm) versus fractions to show the profile of the sample. The data were plotted to reconstruct the HPLC spectrum by using Origin\(^\circledast\) 9 (a complete graphing and data analysis software). The results further confirmed that almost no racemization was detected.

| Apparatus | Agilent 1260 |
|-----------|--------------|
| Column    | Astec\(^\circledast\) CHIROBIOTIC\(^\circledast\) TAG, 25 cm × 4.6 mm, 5 \( \mu \)m |
| Mobile phase | [A] MeOH; [B] H\(_2\)O (75:25, A:B) |
| Flow rate | 1 mL/min |
| Sample 1  | \( S-[^{18}\text{F}]\text{CF}_3\)-L-CYS aq. (pH = 7) |
| Sample 2  | \( S-[^{18}\text{F}]\text{CF}_3\)-D-CYS aq. (pH = 7) |
| Injection | 10 \( \mu \)L |
| Detector  | \( \gamma \)-counter (model SN-6105) |
7. *In vitro* stability assay

A solution of \( S-{^{18}F}\text{CF}_3\)-L-CYS or \( S-{^{18}F}\text{CF}_3\)-D-CYS (2.0 MBq, 20 μL) in sterile saline was added to PBS (pH = 7.4, 200 μL) and was incubated at 37 °C for 2 h. \( S-{^{18}F}\text{CF}_3\)-L-CYS and \( S-{^{18}F}\text{CF}_3\)-D-CYS exhibited good stability in PBS, as determined by chiral-HPLC analysis. Almost no racemization was found, and the intact tracer was more than 95%.

| Apparatus    | Agilent 1260                        |
|--------------|-------------------------------------|
| Column       | Astec® CHIROBIOTIC® TAG, 25 cm × 4.6 mm, 5 μm |
| Mobile phase | [A] MeOH; [B] H2O (75:25, A:B)       |
| Flow rate    | 1 mL/min                            |
| Sample 1     | \( S-{^{18}F}\text{CF}_3\)-L-CYS in PBS |
| Sample 2     | \( S-{^{18}F}\text{CF}_3\)-D-CYS in PBS |
| Injection    | 20 μL                               |
| Detector     | Radioactive Detector (Model BFC-3200, Bioscan. Inc., USA) |

![HPLC analysis of the stability of \( S-{^{18}F}\text{CF}_3\)-CYS enantiomers in PBS](image)

**Figure S5.** HPLC analysis of the stability of \( S-{^{18}F}\text{CF}_3\)-CYS enantiomers in PBS
8. Determination of partition coefficients (logP)

The partition coefficients for each enantiomers (2L or 2D) were determined by adding radiotracer solution (20 μL, 5 μCi; 0.19 MBq) to an equal volume (n-octanol/water; 5 mL/5 mL) mixture. The mixture was vortexed vigorously for 5 min and then centrifuged at 3000 rpm for 15 min. Aliquot of 300 μL n-octanol or 300 μL water was taken out from each phase, then respectively counted by the γ-counter, taking care to avoid cross-contamination and interaction effect between two phases. The partition coefficients were calculated according to the formula: logP = log_{10} (counts of n-octanol/counts of water) and reported by averaging nine measurements.

9. Cell culture

The C6 glioma cell line was kindly provided by the Cell Bank of the Chinese Academy of Sciences in Shanghai. All cells were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% fetal bovine serum (FBS, GIBCO), 1% 100 μg/ml streptomycin and 100 IU/ml penicillin (MRC) at 37 °C in the humidified circumstance containing 5% CO₂. When cells reached 90% confluence in the logarithmic growth phase, they were harvested or passaged.

10. C6 tumor-bearing mice models

All the animal experiments were approved by the Committee on the Ethics of Animal Experiments of the First Affiliated Hospital, Sun Yat-sen University and performed
strictly according to the Guide for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of the People's Republic of China.

Four- to six-week-old male nude mice of BALB/c origin were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. To establish C6 tumor models, they were subcutaneously implanted $5 \times 10^6$ C6 cells in 0.1 ml of PBS into the right shoulder flanks. When the tumor size reached 0.6-1.0 cm in diameter (about 2-3 weeks after inoculation), animals were subjected to studies.

11. Turpentine-induced inflammation

Eight Kunming mice (6–8 weeks old, 25–30 g weight) were used to induce aseptic inflammatory models. Turpentine (0.2 mL) was injected intramuscularly into the thigh of the left hind leg. The mass of the inflammatory tissue grew to 10–15 mm on the 4th day after inoculation of turpentine oil. The mice were randomly assigned to two groups and were anesthetized with 2% pentobarbital (35 mg/Kg). Then, the mice were intravenously administered 0.74 MBq (20 μCi) [18F]FDG or 2D through the tail vein, respectively. Sixty minutes post-injection, the mice were sacrificed by cervical dislocation. The organs of interest (blood, brain, inflammatory muscle and normal muscle) were rapidly dissected and weighed, then the radioactivity was counted with a $\gamma$-counter. All measurements were background-subtracted and decay-corrected to the time of injection, and the values were expressed as percent of injected dose per gram.
of wet tissue (% ID/g). The uptake ratios of inflammation-to-muscle, and inflammation-
to-blood were also calculated.

Table S2. Uptake of [18F]FDG and S-[18F]CF3-D-CYS 2D in mice model of inflammation.

| Parameter                  | [18F]FDG          | S-[18F]CF3-D-CYS |
|----------------------------|-------------------|------------------|
| Brain                      | 4.37 ± 0.25       | 0.91 ± 0.08      |
| Inflammation               | 6.93 ± 0.90       | 1.63 ± 0.11      |
| Muscle                     | 0.95 ± 0.07       | 1.32 ± 0.17      |
| Blood                      | 1.02 ± 0.15       | 1.44 ± 0.24      |
| Inflammation-to-muscle ratio| 7.25 ± 0.67       | 1.25 ± 0.16      |
| Inflammation-to-Blood ratio| 6.82 ± 0.74       | 1.15 ± 0.16      |

Data (organ uptake) are expressed as average %ID/g ± SD (n = 4, 60 min post-injection).

12. Time-dependent cell uptake assay

C6 cells at a density of 1×10^5 cells/well were seeded into 24-well plates and incubated at 37 °C for overnight. After washing with warm phosphate-buffered saline (PBS), the cells were incubated with S-[18F]CF3-L-CYS 2L or S-[18F]CF3-D-CYS 2D (0.187 MBq; 5 μCi in 200 μl of PBS) at 37 °C for 5, 10, 20, 30, 60, 90 and 120 min. The cells were washed three times with cold PBS, lysed by 0.5 ml of 0.5 M NaOH aq., collected into test tubes and measured by a γ-counter (SN-6105). The protein content was calculated by the method of the bicinchoninic acid (BCA) assay. Cell uptake was expressed as the percentage of the injected dose per 100 microgram of protein (%ID/100 μg protein). Experiments were performed three times with quadruplicate wells.

13. Competitive cell uptake assay

C6 cells at a density of 1×10^5 cells/well were seeded into 24-well plates 24 h prior to experiments, which were repeated three times with quadruplicate wells for each group.
The inhibition experiments were performed with 137 mmol/L NaCl medium or Choline Chloride medium in sterile water for incubation to induce the condition with or without the presence of Na⁺ (+Na⁺ or -Na⁺). The solution in +Na⁺ or -Na⁺ medium was at a concentration of 15 mmol/L inhibitors or S-methyl-L-cysteine (L-MCY), such as α-(methylamino)isobutyric acid (MeAIB) targeting at system A, serine (Ser) system ASC, 2-amino-2-norbornane-carboxylic acid (BCH) system B⁰⁺ in +Na⁺ condition and L in -Na⁺ condition.

After washing with warm +Na⁺ or -Na⁺ medium, cells were incubated with 200 μL of S-[¹⁸F]CF₃-L-CYS 2L or S-[¹⁸F]CF₃-D-CYS 2L (0.187 MBq; 5 μCi in +Na⁺ or -Na⁺ medium) at 37 °C for 15 min, with co-incubation of 200 μL of each inhibitor or 200 μL of pure NaCl medium or Choline Chloride medium for control. The cells were washed three times with cold PBS, lysed by 0.5 mL of 0.5 M NaOH aq., collected into test tubes and measured by a γ-counter. The results of each well are normalized by the equation: the uptake data (expressed as a percentage of the injected dose per 100 microgramm of protein, % ID/100 μg protein) = (the final measured radioactivity/the decay-corrected radioactivity originally added)/protein content. And the final uptake values of every group were averaged and expressed as the percentage of the uptake of the Na⁺-containing control group, which was supposed to be 100%.

14. Protein incorporation assay
C6 cells at a density of 2×10^6 cells/well were seeded into 6-well plates 24 h before experiments. After washing with warm PBS, cells per well were added 400 μL of S-[^18F]CF3-L-CYS 2L or S-[^18F]CF3-D-CYS 2D (0.925 MBq; 25 μCi in PBS) and incubated at 37 °C for 30 and 120 min. The cells were washed three times with cold PBS, separated by 0.5 ml of 0.25% trypsin, and resuspended in PBS. The samples were centrifuged (13,000 rpm, 5 min), the supernatant removed, and the cells suspended in 200 μL of 1% Triton-X 100. After vortex mixing, the samples were added 0.5 mL of 20% trichloroacetic acid (TCA), and put into ice for 10 min. After that, the samples were centrifuged (13000 rpm, 15 min) and the supernatant was collected into test tubes. The TCA-precipitate was washed by PBS and centrifuged for three cycles. Every time the supernatant was collected into corresponding test tubes mentioned above. The pellets were re-suspended in PBS and transferred into new test tubes. The radioactivity in TCA-solution fraction or TCA-precipitation was measured by a gamma counter. Protein incorporation was reported as the percentage of TCA-perceptible activity. Experiments were performed twice times with triplicate wells.

**15. Biodistribution studies by dissection**

Normal healthy Kunming mice were used instead of tumor-bearing mice to reduce the costs and numbers of animal models used in the biodistribution study. Mice (6–8 weeks old, 25–30 g weight) were injected with 0.74 MBq (20 μCi) of 2L or 2D in 100 μL of saline via the tail vein, respectively. The groups of mice (four mice per group at each
time point) were sacrificed by cervical dislocation at various times (5, 30, 60 and 90 min after injection of 2L and 2D). The organs of interest (blood, brain, heart, lung, liver, spleen, pancreas, kidney, stomach, intestine, bone and muscle) were rapidly dissected and weighed, and the radioactivity was counted with a γ-counter. All measurements were background-subtracted and decay-corrected to the time of injection, and the values were expressed as percent of injected dose per gram of wet tissue (% ID/g).

16. Small-animal PET imaging studies

*In vivo* PET/CT imaging was conducted by the Inveon small-animal PET/CT scanner (Siemens, Germany) equipped with the Inveon Research Workplace 4.1 software.

1) The static micro-PET imaging studies were conducted with 3.7 MBq of 2D (injection via the tail vein) on the C6 tumor-bearing BALB/c mice (n = 3) at 45, 60, 75 min post-injection. All mice (male) were kept fasting for 4 h and were anesthetized with 2% pentobarbital (35 mg/Kg) *before the imaging experiments*.

2) The static micro-PET imaging studies were conducted with 3.7 MBq of $[^{18}\text{F}]$FDG (injection via the tail vein) on the same C6 tumor-bearing BALB/c mice (n = 3) at 60 min post-injection. The mice (male) were kept fasting for 4 h and were anesthetized with 2% pentobarbital (35 mg/Kg) *before the injection of $[^{18}\text{F}]$FDG*.

3) The dynamic micro-PET imaging studies were conducted with 3.7 MBq of 2D (injection via the tail vein) on other C6 tumor-bearing BALB/c mice (n = 3) over a
period of 90 min (dynamic, 5 min/frame). All mice (male) were kept fasting for 4 h and were anesthetized with 2% pentobarbital (35 mg/Kg) before the injection of 2D.

The images were reconstructed, and regions of interest (ROIs) were drawn over the tumor and organs on decay-corrected whole-body coronal images using vendor software (Iveon Research Workplace). The radioactivity in each volume of interest was obtained from mean pixel values, which were converted to MBq/mL using a conversion factor. Assuming a tissue density of 1 g/mL, the ROIs were converted to MBq/g and then divided by the administered activity to obtain an imaging ROI-derived %ID/g.

Figure S6. Dynamic micro-PET images of C6 glioma-bearing mice scanned after injection of S-[18F]CF3-D-CYS 2D (from 0 to 90 min).

Note: Although no systematic studies were performed, pentobarbital might interfere with the distribution patterns of 2D by slowing down the excretion of the tracer from the liver.
17. H&E staining of subcutaneous-induced C6 tumor in BALB/c nude mice

Mice were sacrificed by mercy killing. C6 tumor tissues were removed, fixed in 4% paraformaldehyde, and embedded in paraffin for hematoxylin and eosin (H&E) staining. Subsequently, samples were observed under an optical microscopy.

18. H&E staining of turpentine-induced inflammation in Kunming mice

Mice were sacrificed by mercy killing. Inflammatory tissues and normal striated muscle tissues were removed, fixed in 4% paraformaldehyde, and embedded in paraffin for hematoxylin and eosin (H&E) staining. Subsequently, samples were observed under an optical microscopy.
19. References

1. Pave G, Usse-Versluis S, Viaud-Massuard MC, Guillaumet G. Synthesis of 3-aminochroman derivatives by radical cyclization. Org lett. 2003; 5: 4253-6.

2. Ducrot P-H, Allais F, Martinet S. Straightforward Total Synthesis of 2-O-Feruloyl-l-malate, 2-O-Sinapoyl-l-malate and 2-O-5-Hydroxyferuloyl-l-malate. Synthesis. 2009; 2009: 3571-8.

3. Cambeiro XC, Martin-Rapun R, Miranda PO, Sayalero S, Alza E, Llanes P, et al. Continuous-flow enantioselective alpha-aminoxylation of aldehydes catalyzed by a polystyrene-immobilized hydroxyproline. Beilstein J Org Chem. 2011; 7: 1486-93.

4. McConathy J, Martarello L, Malveaux EJ, Camp VM, Simpson NE, Simpson CP, et al. Radiolabeled Amino Acids for Tumor Imaging with PET: Radiosynthesis and Biological Evaluation of 2-Amino-3-[18F]fluoro-2-methylpropanoic Acid and 3-[18F]Fluoro-2-methyl-2-(methylamino)propanoic Acid. J Med Chem. 2002; 45: 2240-9.

5. Zheng JL, Wang L, Lin JH, Xiao JC, Liang SH. Difluorocarbene-Derived Trifluoromethylthiolation and [18F]Trifluoromethylthiolation of Aliphatic Electrophiles. Angew Chem. 2015; 54: 13236-40.

6. Lieberman BP, Ploessl K, Wang L, Qu WC, Zha ZH, Wise DR, et al. PET imaging of glutaminolysis in tumors by (2S,4R)-4-[18F]fluoroglutamine. J Nucl Med. 2011; 52: 1947-55.
20. $^1$H, $^{13}$C and $^{19}$F NMR spectra for the compounds
