Sulfo-click chemistry with ^{18}F-labeled thio acids

SUPPLEMENTARY INFORMATION

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Material and methods. All chemicals used obtained from Sigma-Aldrich®, with exception of thiolane-2,5-dione which was purchased from BOCSCI Inc.. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on an Agilent/Varian VNMRS three-channel 600 MHz spectrometer. Chemical shifts are given in ppm referenced to internal standards (s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, m = multiplet). Mass spectra were recorded using a Micromass ZabSpec Hybrid Sector-TOF by positive mode electrospray ionization. Thin-layer chromatography (TLC) was monitored using HF254 silica gel. HPLC analyses were performed on a semi-preparative Luna C18 column (100 Å, 10 μm, 250 x 10 mm) or Jupiter C12 ((100 Å, 10 μm, 250 x 10 mm). Both columns were connected to their corresponding guard columns (Phenomenex Nucleosil LUNA (II) RP C18 pre-column (5 μm, 50x10mm) and Jupiter C12 pre-column (5 μm, 50x10mm)). UV detection was performed at 210 nm and 254 nm. Radioactivity detection was achieved using a well-scintillation NaI (Tl) detector. [^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction through proton irradiation of enriched (98%) ^{18}O water (3.0 ml, Rotem, Germany) using a TR19/9 cyclotron (Advanced Cyclotron Systems, Inc., Richmond, BC, Canada). [^{18}F]SFB and 4-[^{18}F]fluorobenzyl amine were prepared according to procedures published by our group.[18,19]

4-Fluorobenzenecarbothioic *S*-acid (1). A total of 0.71 mL (5.99 mmol) of 4-fluorobenzoyl chloride was added to a stirred solution of 300 mg (3.99 mmol) of thioacetamide in 3.0 mL of benzene. The mixture was heated to 30 °C and stirred for 3.5 h. After cooling to room temperature, 5 mL of 10% NaOH was added. The mixture was stirred for 30 min at room temperature. The mixture was then acidified using 10% HCl. The mixture was extracted twice with ethyl acetate. The organic layer was dried on Na_2SO_4 and evaporated to give a yellow, crystal solid. The solid was washed with diethyl ether to extract the yellow impurity, giving a white solid. Yield: 73%; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.98-8.01 (m, 2H), 7.10-7.14 (m, 2H); *m/z* (ESI) C_7H_4FOS $(M-H)^-$ calcd. 155.2, found 154.8.

4-[(4-Fluorobenzyl)amino]-4-oxobutanethioic *S*-acid (2). 91 µL (0.080 mmol) of 4-fluoro-benzylamine was added to 3 mL of CH_2Cl_2. To this solution, 111 µL (0.080 mmol) of triethylamine was added. In a separate vial, 278 mg (0.240 mg) of thiolane-2,5-dione was dissolved in 1 mL of CH_2Cl_2 and was then added to the reaction flask. The reaction was then set to reflux at 40 ˚C for 3 h. It was cooled to room temperature, and 20 mL of CH_2Cl_2 was added. The mixture was then washed twice with 0.5 M citric acid and twice with water.
The organic layer was dried on Na_2SO_4 and evaporated to dryness. The residue was dissolved in methanol, and filtered by vacuum filtration. The filtrate was evaporated, leaving a brown oil.
The oil was purified using column chromatography (50% EtOAc/hexane). Yield: 18%; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.30-7.16 (m, 2H), 6.96-6.91 (m, 2H), 4.34 (d, 2H, J=5.4 Hz), 2.96 (t, 2H, J=6.6 Hz), 2.52 (t, 2H, J=6.6 Hz); *m/z* (ESI) $C_{11}H_{11}FNO_2S$ $(M-H)^-$ calcd. 240.2, found 240.1.

General procedure for sulfo-click reaction with 4-fluorobenzenecarbothioic *S*-acid (1) for the synthesis of reference compounds 3-6 and 12. To a stirred solution of sulfonyl azide (1.0 equiv.) in methanol was added 2,6-lutidine (2.0 equiv.) and 4-fluorobenzenecarbothioic *S*-acid **1** (2.0 equiv.). The mixture was stirred at ambient temperature for 2-6 h. The solvent was removed in vacuo and purification of the residue by flash column chromatography gave the corresponding *N*-acylsulfonamide products.

4-Fluoro-*N*-((2,4,6-triisopropylphenyl)sulfonyl)benzamide (3). White solid; Yield: 41%; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.65 (s, 1H), 7.69-7.72 (m, 2H), 7.11 (s, 2H), 7.03-7.05 (m, 2H), 4.21 (sept, 2H, J=6.6 Hz), 2.81 (sept, 1H, J=6.6 Hz), 1.20 (d, 12H, J=7.2 Hz), 1.16 (d, 6H, J=7.2 Hz); ^{13}C-NMR (150.9 MHz, $CDCl_3$): δ 165.8 (d, J=255.0 Hz), 163.5, 154.2, 151.5, 131.1, 130.2 (d, J=10.56), 127.6, 124.2, 116.3 (d, J=22.6 Hz), 34.2, 29.7, 24.6, 23.5; *m/z* (ESI) $C_{22}H_{29}FNO_3S$ $(M+H)^+$ calcd. 406.4, found 406.2.

4-(*N*-(4-Fluorobenzoyl)sulfamoyl)benzoic acid (4). White solid. Yield: 60%; ^{1}H-NMR (600 MHz, CD_3OD): δ 8.10-8.05 (m, 4H), 7.85-7.82 (m, 2H), 7.09-7.06 (m, 2H); ^{13}C-NMR (150.9 MHz, CD_3OD): δ 167.2, 166.9, 165.5 (d, J=252.00 Hz), 154.7, 144.6, 134.8, 130.8 (d, J=9.05 Hz), 129.5, 127.8, 115.0 (d, J=21.13 Hz); *m/z* (ESI) $C_{14}H_9FNO_5S$ $(M-H)^-$ calcd. 322.3, found 322.0.

***N*-((4-Acetamidophenyl)sulfonyl)-4-fluorobenzamide (5)**. White solid. Yield: 76%; ^{1}H-NMR (600 MHz, $DMSO_{d6}$): δ 12.48 (s, 1H), 10.40 (s, 1H), 7.92-7.96 (m, 4H), 7.79-7.81 (m, 2H), 7.31-7.34 (m, 2H), 2.09 (s, 3H); ^{13}C-NMR (150.9 MHz, $DMSO_{d6}$): δ 169.6 165.4 (d, J=252.00 Hz), 164.7, 144.3, 133.2, 131.8 (d, J=10.56 Hz), 129.6, 128.6, 118.8, 116.2 (d, J=22.6 Hz), 24.6; *m/z* (ESI) $C_{15}H_{14}FN_2O_4S$ $(M+H)^+$ calcd. 337.3, found 337.2.

4-Fluoro-*N*-tosylbenzamide (6). White solid. Yield: 76%; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 9.01 (s, 1H), 7.95 (d, 2H, J=8.4 Hz), 7.73-7.75 (m, 2H), 7.27 (d, 2H, J=8.4 Hz), 7.00-7.03 (m, 2H), 2.35 (s, 3H); ^{13}C-NMR (150.9 MHz, $CDCl_3$): δ 165.9 (d, J=255.02 Hz), 163.0, 145.4, 135.3, 130.4 (d, J=22.56 Hz), 129.7, 128.7, 127.4, 116.2 (d, J=22.64 Hz), 21.7; *m/z* (ESI) $C_{14}H_{13}FNO_3S$ $(M+H)^+$ calcd. 294.3, found 294.1.

4-Fluoro-*N*-((4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)sulfonyl)benzamide (12). White solid; Yield: 85%; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.52 (s, 1H), 8.04 (d, 2H, J=12.0 Hz), 7.67-7.70 (m, 2H), 7.42 (d, 2H, J=12.0 Hz), 7.03-7.08 (m, 6H), 6.65 (s, 1H), 2.30 (s, 3H); ^{13}C-NMR (150.9 MHz, $CDCl_3$): ∂ 166.0 (d, J=256.53 Hz), 163.0, 145.4, 144.3 (q, J=39.23 Hz), 143.6, 139.9, 137.6, 130.5 (d, J=10.56 Hz), 129.9, 129.8, 128.7, 127.2 (d, J=3.02 Hz), 125.6, 125.1, 121.0 (q, J=268.60 Hz), 116.2 (d, J=22.5 Hz), 106.6, 21.4; *m/z* (ESI) $C_{24}H_{18}F_4N_3O_3S$ $(M+H)^+$ calcd. 504.5, found 504.3.

General procedure for sulfo-click reaction with 4-[(4-Fluorobenzyl)amino]-4-oxobutanethioic *S*-acid (2) for the synthesis of reference compounds 7-10 and 13. To a stirred solution of sulfonyl azide (1.0 equiv.) in methanol was added 2,6-lutidine (2.0 equiv.) and 4-[(4-fluorobenzyl)amino]-4-oxobutanethioic *S*-acid **(2)** (2.0 equiv.). The mixture was stirred at ambient temperature for 2-6 h. The solvent was removed in vacuo and purification of the residue by flash column chromatography gave the corresponding *N*-acylsulfonamide products.

***N*^{1}-(4-Fluorobenzyl)-*N*^{4}-((2,4,6-triisopropylphenyl)sulfonyl)succinamide (7).** Yellow-brown oil. Yield: 43%; ^{1}H-NMR (600 MHz, CD_3OD): δ 7.20-7.16 (m, 2H) 7.08 (s, 2H), 6.91-6.87 (m, 2H), 4.31 (s, 2H), 4.21-4.27 (sep, 2H, J=7.2 Hz), 3.38 (t, 2H, J=6.6 Hz), 2.8 (sep, 1H, J=7.2 Hz), 2.67 (t, 2H, 6.6 Hz), 1.15 (d, 6H, J=7.2 Hz), 1.09 (d, 12H, J=7.2 Hz); ^{13}C-NMR (150.9 MHz, CD_3OD): δ 168.6, 151.9, 148.9, 136.8, 129.6 (d, J=9.05 Hz), 123.0, 114.8 (d, J=22.6 Hz), 44.1, 34.0, 30.3, 29.2, 28.4, 23.6, 22.7; *m/z* (ESI) $C_{26}H_{36}FN_2O_4S$ $(M+H)^+$ calcd. 491.6, found 491.2.

4-(*N*-(4-((4-Fluorobenzyl)amino)-4-oxobutanoyl)sulfamoyl)benzoic acid (8). Yellow-brown oil. Yield 43%; ^{1}H-NMR (600 MHz, CD_3OD): ∂ 8.17 (m, 4H) 7.94 (m, 2H), 7.18 (m, 2H), 4.28 (d, 2H, J=6.0 Hz), 3.52 (t, 2H, J=6.6 Hz), 2.74 (t, 2H, J=6.6 Hz); ^{13}C-NMR (150.9 MHz, CD_3OD): ∂ 174.2, 168.2, 167.5, 163.2 (d, J=247.06), 143.9, 139.6, 130.8, 129.8, 129.0, 125.8, 115.3 (d, J=22.6), 46.3, 34.1, 33.7; *m/z* (ESI) $C_{18}H_{18}FN_2O_6S$ $(M+H)^+$ calcd. 409.4, found 409.1.

***N*^{1}-((4-Acetamidophenyl)sulfonyl)-*N*^{4}-(4-fluorobenzyl)succinamide (9)**. Yellow-brown oil. Yield 55%; ^{1}H-NMR (600 MHz, CD_3OD): δ 7.72 (m, 4H) 7.32 (m, 2H), 7.05 (m, 2H), 4.35 (d, 2H, J=6.0 Hz), 3.58 (t, 2H, J=6.6 Hz), 2.79 (t, 2H, J=6.6 Hz), 2.56 (s, 3H); ^{13}C-NMR (150.9 MHz, CD_3OD): δ 170.6, 166.4, 164.2, 163.4 (d, J=252.06), 144.4, 139.1, 135.4, 129.4, 128.8, 119.2, 115.0 (d, J=22.6), 43.8, 34.1, 33.9, 24.0, *m/z* (ESI) $C_{19}H_{21}FN_3O_5S$ $(M+H)^+$ calcd. 422.4, found 422.2.

***N*¹-(4-Fluorobenzyl)-*N*⁴-tosylsuccinamide (10)**. Yellow oil. Yield 55%; ^{1}H-NMR (600 MHz, CD_3OD): δ 7.55 (d, 2H, J=8.4 Hz) 7.19 (d, 2H, J=8.4 Hz), 7.13-7.15 (m, 2H), 6.87- 6.90 (m, 2H), 4.29 (s, 2H), 3.45 (t, 2H, J=6.6 Hz), 2.68 (t, 2H, J=6.6 Hz), 2.31 (s, 3H); ^{13}C-NMR (150.9 MHz, CD_3OD): δ 175.5, 169.1, 162.2 (d, J=244.46), 142.4, 140.9, 129.5, 129.0 (d, J=9.05 Hz), 125.7, 114.7 (d, J=22.5 Hz), 44.3, 30.5, 30.2, 20.0; *m/z* (ESI) $C_{18}H_{20}FN_2O_4S$ $(M+H)^+$ calcd. 379.4, found 379.3.

***N*¹-(4-Fluorobenzyl)-*N*⁴-((4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)sulfonyl)-succinamide (13).** Yellow-brown oil. Yield: 60%; ^{1}H-NMR (600 MHz, $CDCl_3$): ð 7.82 (m, 2H), 7.37-7.33 (m, 2H), 7.12-6.90 (m, 8H), 6.66 (s, 1H), 4.32 (d, 2H, J=5.4 Hz) 3.66 (t, 2H, J=5.4 Hz), 2.96 (t, 2H, J=6.6 Hz), 2.30 (s, 3H); ^{13}C-NMR (150.9 MHz, $CDCl_3$): ð 174.4, 168.4, 163.3, 145.2, 143.9, 143.1, 141.7, 139.6, 129.8 (d, J=9.05 Hz), 129.7, 128.7, 127.4, 125.8, 125.2, 115.8 (d, J=21.13 Hz), 106.0, 45.5, 31.1, 29.3, 21.3; *m/z* (ESI) $C_{28}H_{23}F_4N_4O_4S$ $(M-H)^-$ calcd. 587.6, found 587.2.

General procedure for one pot/three-component procedure for radiosynthesis of compounds [^{18}F]3-[^{18}F]6 and [^{18}F]12. In an eppendorf tube, 2.5 mg of NaSH was dissolved in 200 µL of deionized water. In a separate eppendorf tube, 5 mg of sulfonyl azide was dissolved in 200 µL of CH_3CN. Next, 20-30 MBq of the [^{18}F]SFB was added to the NaSH solution, and the sulfonyl azide solution was added to the reaction mixture. This was then stirred at 50 °C for 30 min. Product formation was monitored with radio-TLC analysis. Radiochemical conversions (RCCs) refer to the percentage of radiolabeled products **[^{18}F]3**-**[^{18}F]6** **[^{18}F]12** and present in the reaction mixture as determined by radio-TLC analysis (10% MeOH/CH_2Cl_2).

4-[^{18}F]Fluoro-*N*-((2,4,6-triisopropylphenyl)sulfonyl)benzamide ([^{18}F]3). RCC: 77%. Radio-TLC (10% MeOH/CH_2Cl_2): R_f = 0.75.

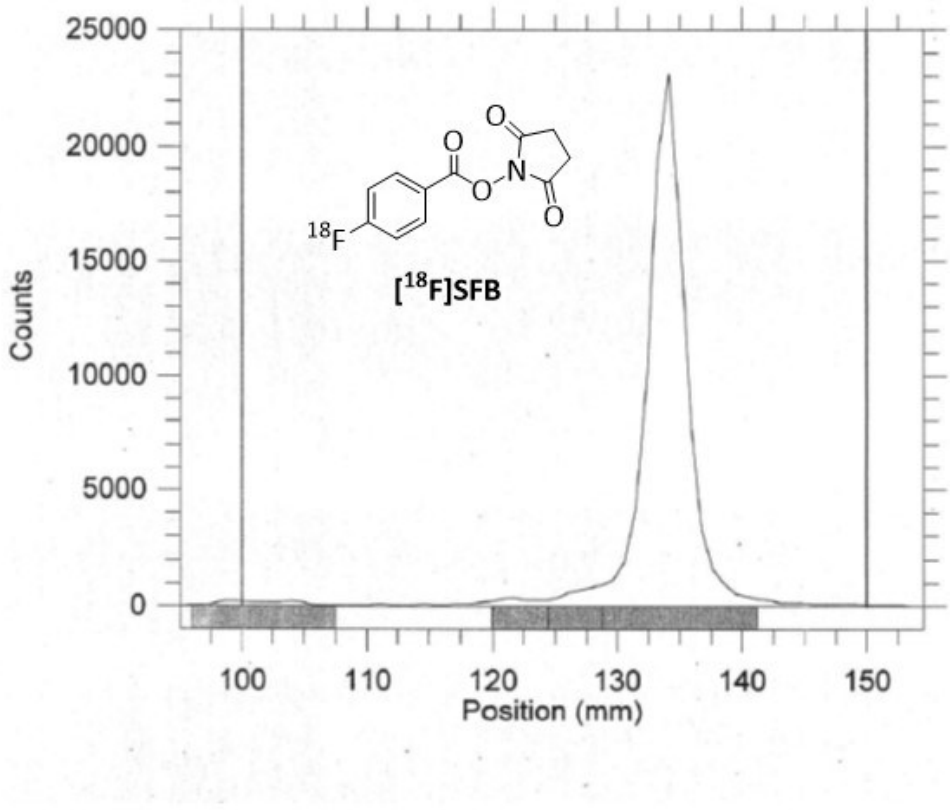
4-(*N*-(4-[^{18}F]Fluorobenzoyl)sulfamoyl)benzoic acid ([^{18}F]4). RCC: 76%. Radio-TLC (10% MeOH/CH_2Cl_2): R_f = 0.5.

***N*-((4-Acetamidophenyl)sulfonyl)-4-[^{18}F]fluorobenzamide ([^{18}F]5)**. RCC: 70%. Radio-TLC (10% MeOH/CH_2Cl_2): R_f = 0.6.

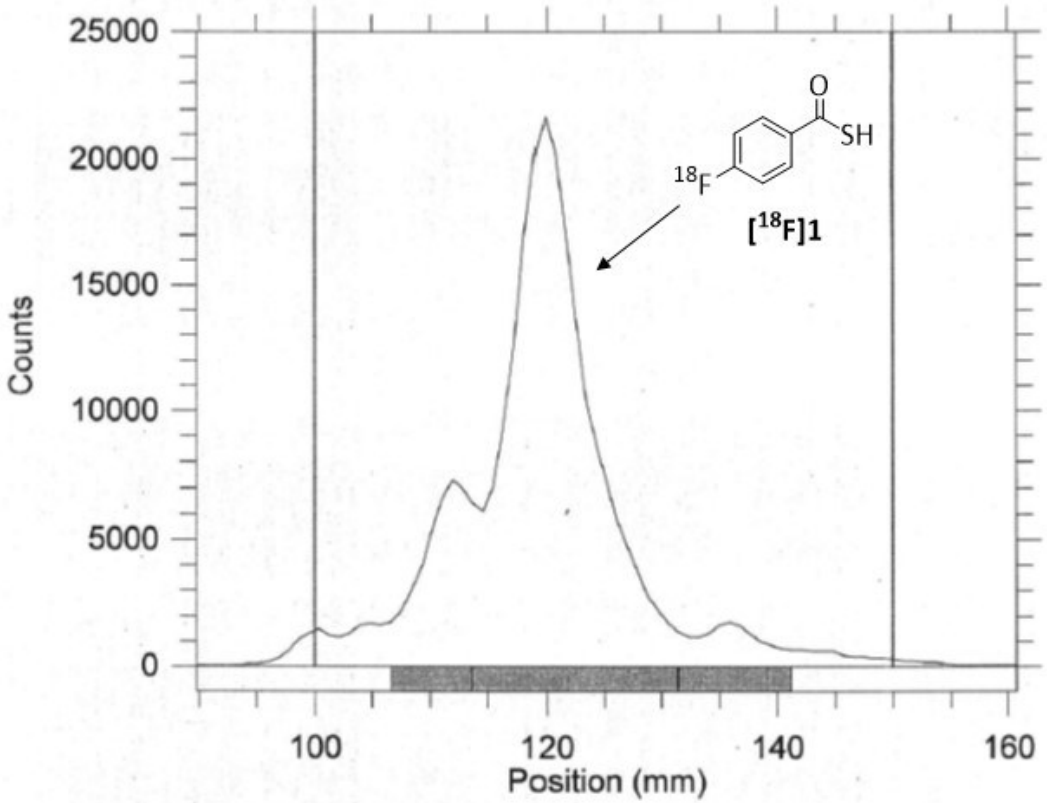
4-[^{18}F]Fluoro-*N*-tosylbenzamide ([^{18}F]6). RCC: 99%. Radio-TLC (10% MeOH/CH_2Cl_2): R_f = 0.7.

4-[^{18}F]Fluoro-*N*-((4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)sulfonyl)benzamide ([^{18}F]12). RCC: 67%. Radio-TLC (10% MeOH/CH_2Cl_2): R_f = 0.3.

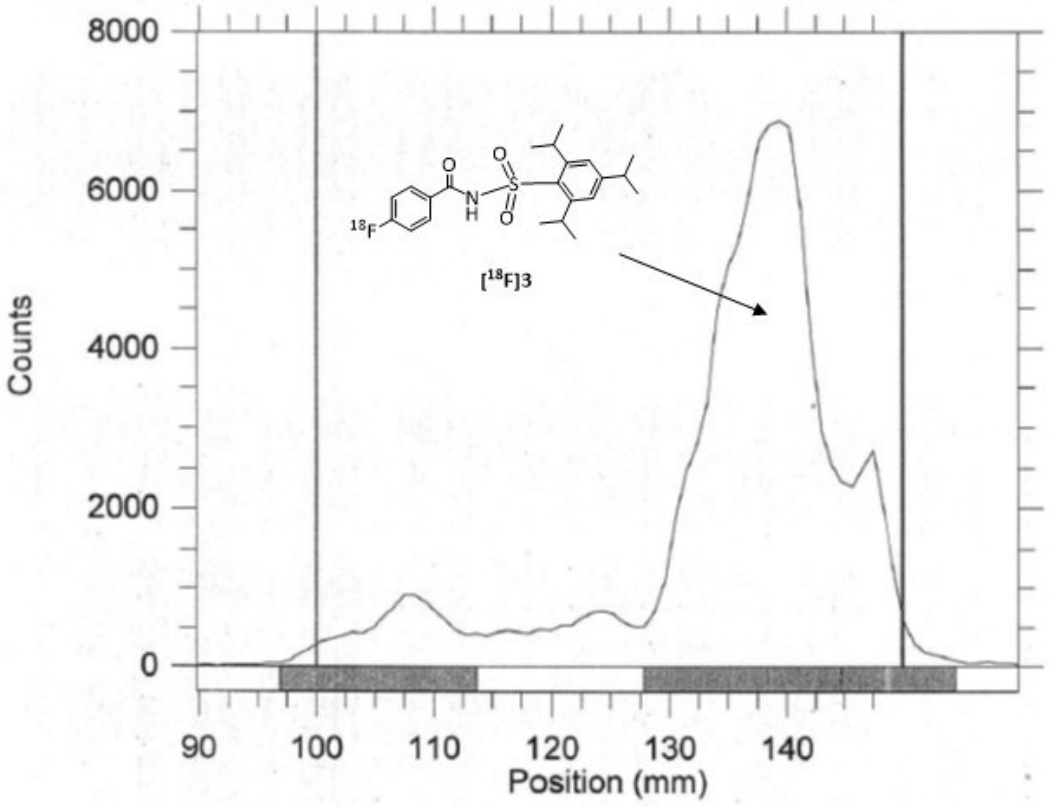
Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	96.0	107.5	101.3	0.027	1805.0	1805.0	1.79	1.82
Rgn 2	119.9	125.2	122.2	0.444	1795.0	1795.0	1.78	1.81
Rgn 3	124.4	129.7	127.1	0.542	3788.0	3788.0	3.76	3.82
Rgn 4	128.8	141.2	134.0	0.680	91801.0	91801.0	91.05	92.55
4 Peaks					99189.0	99189.0	98.37	100.00



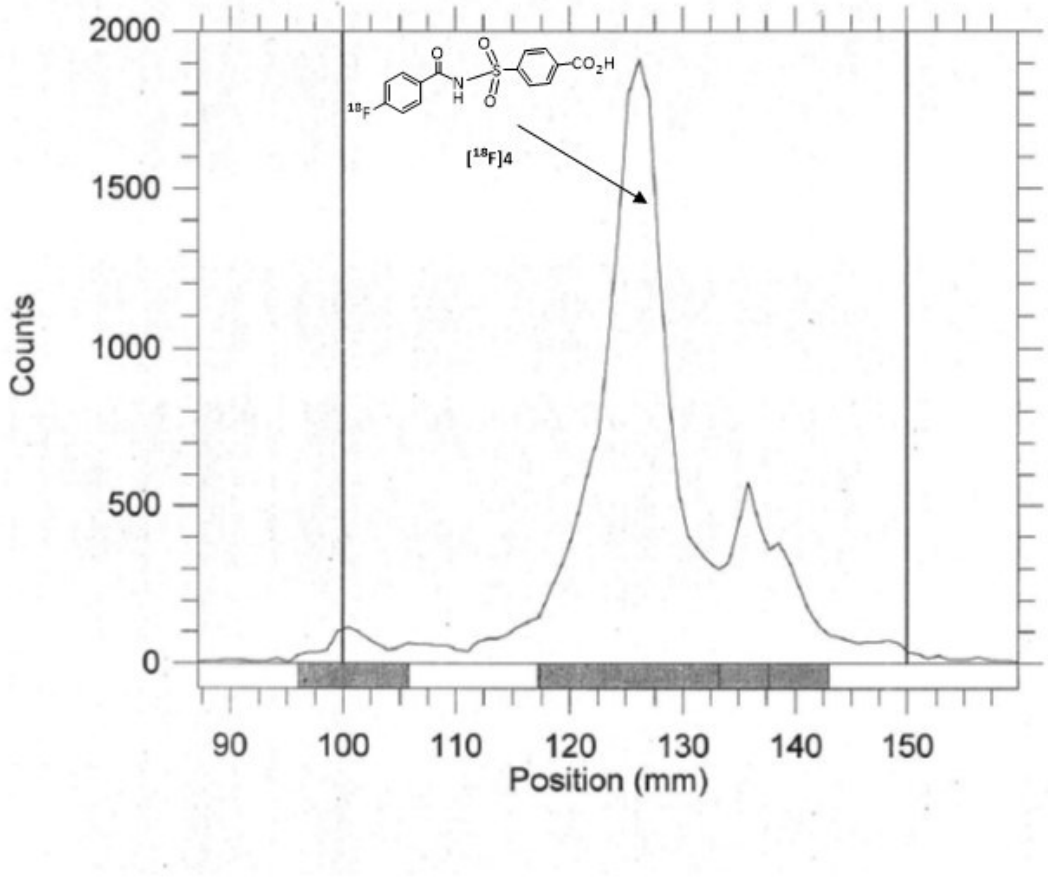
Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	106.6	114.6	111.1	0.221	43475.0	43475.0	16.28	17.05
Rgn 2	113.7	131.5	120.6	0.413	197787.0	197787.0	74.07	77.57
Rgn 3	131.5	141.2	135.5	0.711	13715.0	13715.0	5.14	5.38
3 Peaks					254977.0	254977.0	95.48	100.00



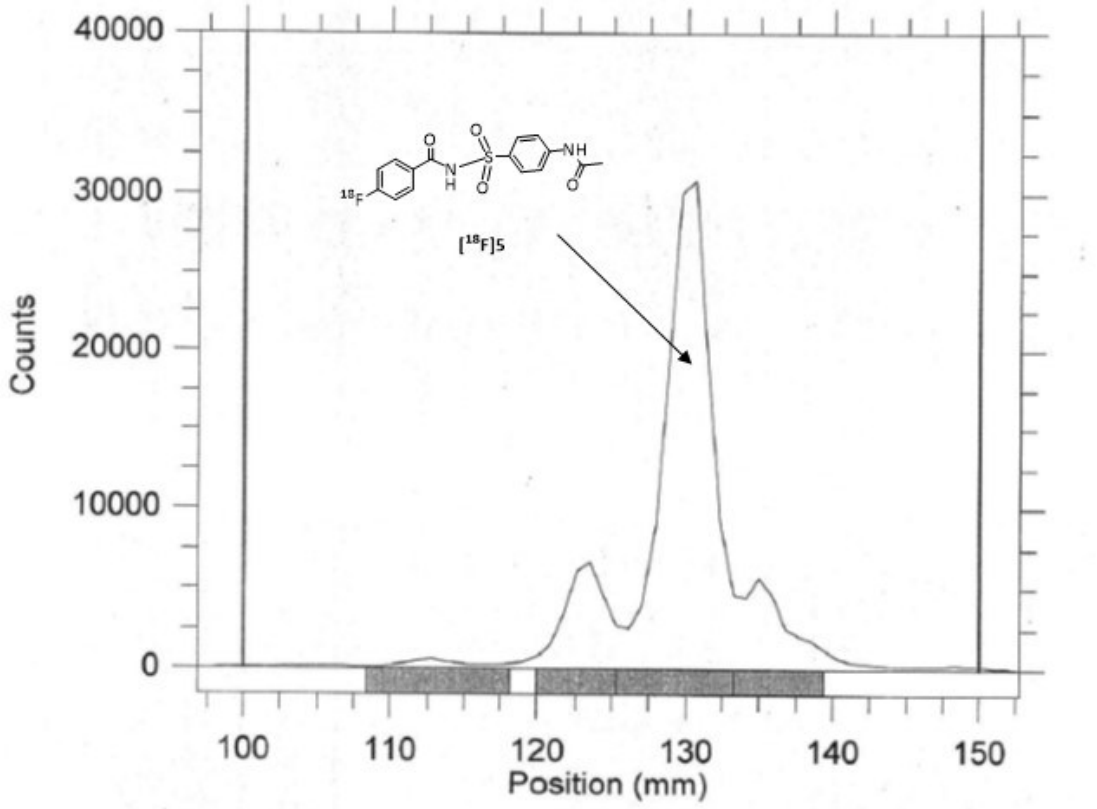
Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	96.9	113.7	106.8	0.135	9079.0	9079.0	8.36	8.92
Rgn 2	127.9	145.6	137.7	0.754	78326.0	78326.0	72.12	76.99
Rgn 3	144.8	154.5	147.1	0.943	14325.0	14325.0	13.19	14.08
3 Peaks					101730.0	101730.0	93.67	100.00



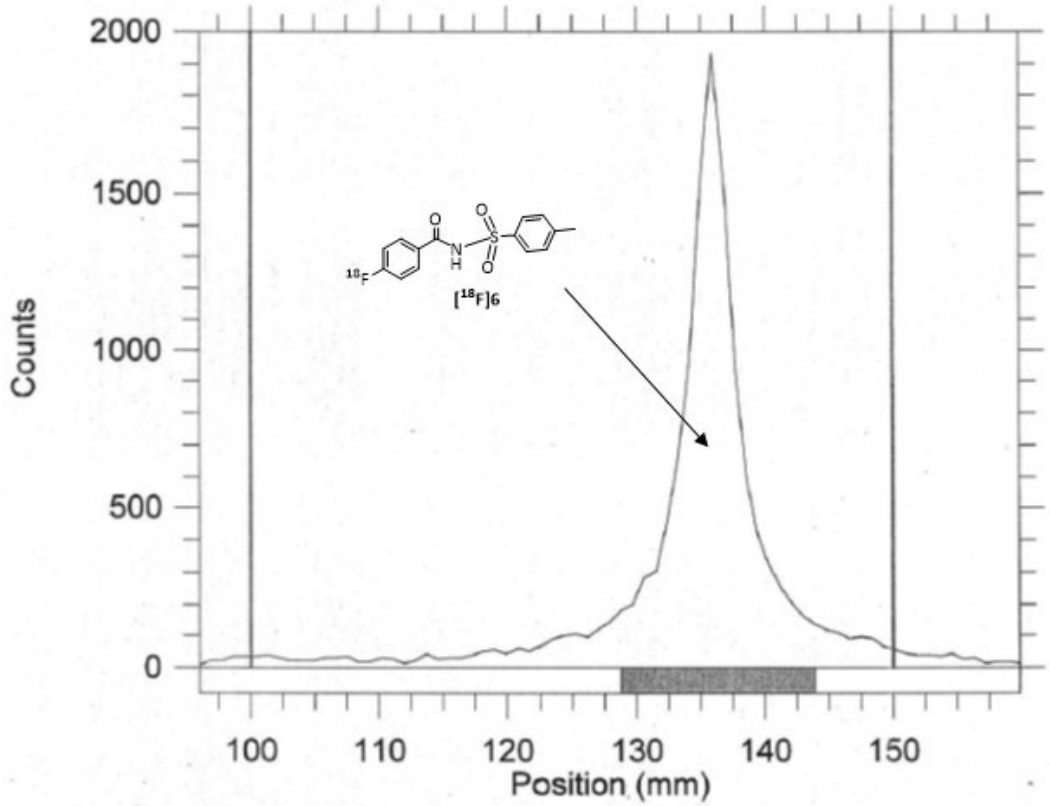
Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	96.0	105.7	100.8	0.016	687.0	687.0	3.25	3.51
Rgn 2	117.3	134.1	125.7	0.514	14844.0	14844.0	70.20	75.81
Rgn 3	133.2	138.5	135.6	0.712	2439.0	2439.0	11.53	12.46
Rgn 4	137.7	143.0	139.3	0.787	1610.0	1610.0	7.61	8.22
4 Peaks					19580.0	19580.0	92.59	100.00



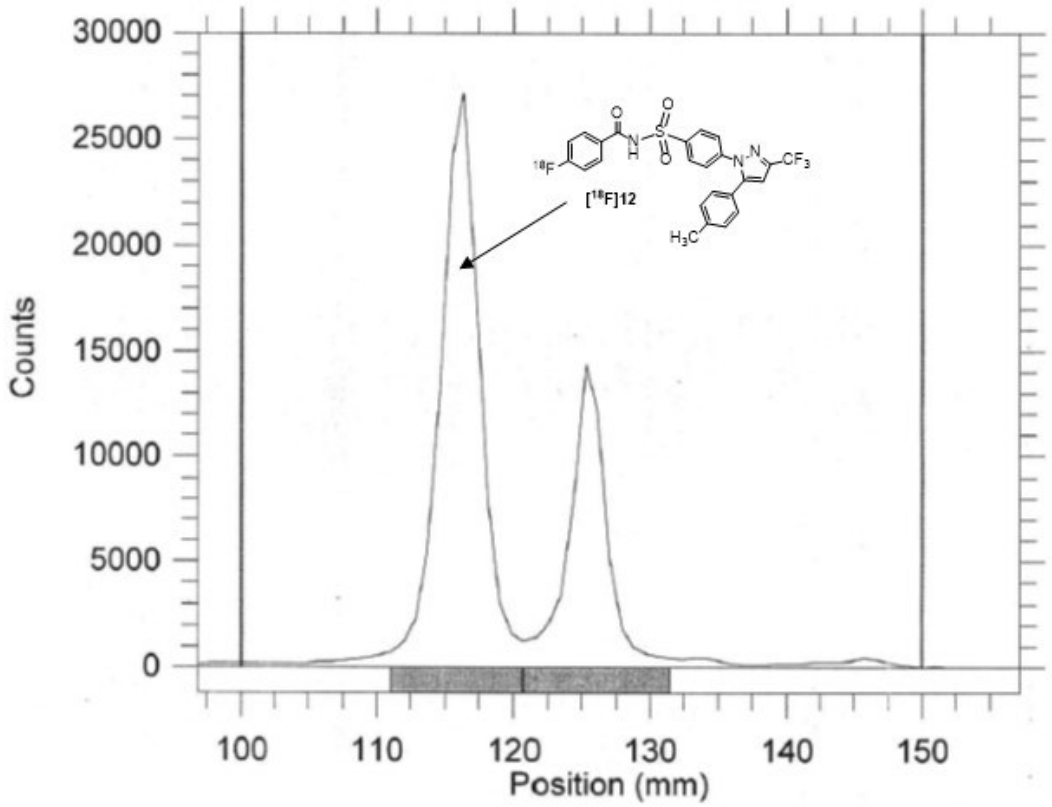
Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	108.4	118.1	113.2	0.263	3627.0	3627.0	1.94	1.94
Rgn 2	119.9	126.1	123.1	0.462	26199.0	26199.0	13.98	14.00
Rgn 3	125.2	134.1	130.0	0.599	131548.0	131548.0	70.19	70.28
Rgn 4	133.2	139.4	135.3	0.706	25799.0	25799.0	13.77	13.78
4 Peaks					187173.0	187173.0	99.88	100.00



Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	128.8	143.9	135.8	0.717	10933.0	10933.0	76.74	100.00
1 Peaks					10933.0	10933.0	76.74	100.00



Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	111.1	120.8	116.1	0.321	104523.0	104523.0	62.86	66.80
Rgn 2	120.8	131.5	125.3	0.507	51939.0	51939.0	31.24	33.20
2 Peaks					156462.0	156462.0	94.10	100.00



General procedure for one pot/three-component procedure for radiosynthesis of compounds [^{18}F]7-[^{18}F]10 and [^{18}F]13. In an eppendorf tube, 2 mg of thiolane-2,5-dione was dissolved in 250 µL of DMF and 30-40 MBq of 4-[^{18}F]fluorobenzylamine in THF was added. The reaction was heated to 80 °C for 15 min. Then, 3-5 mg of sulfonyl dissolved in 200 µL of THF was added. The reaction mixture was stirred at 50 °C for 30 min. Product formation was monitored with radio-TLC analysis. Radiochemical conversions (RCCs) refer to the percentage of radiolabeled products **[^{18}F]7**-**[^{18}F]10** and **[^{18}F]13** present in the reaction mixture as determined by radio-TLC analysis (10% MeOH/CH_2Cl_2).

***N*^{1}-(4-[^{18}F]Fluorobenzyl)-*N*^{4}-((2,4,6-triisopropylphenyl)sulfonyl)succinamide ([^{18}F]7).** RCC: 38%. Radio-TLC (50% EtOAc/hexane): R_f = 0.6.

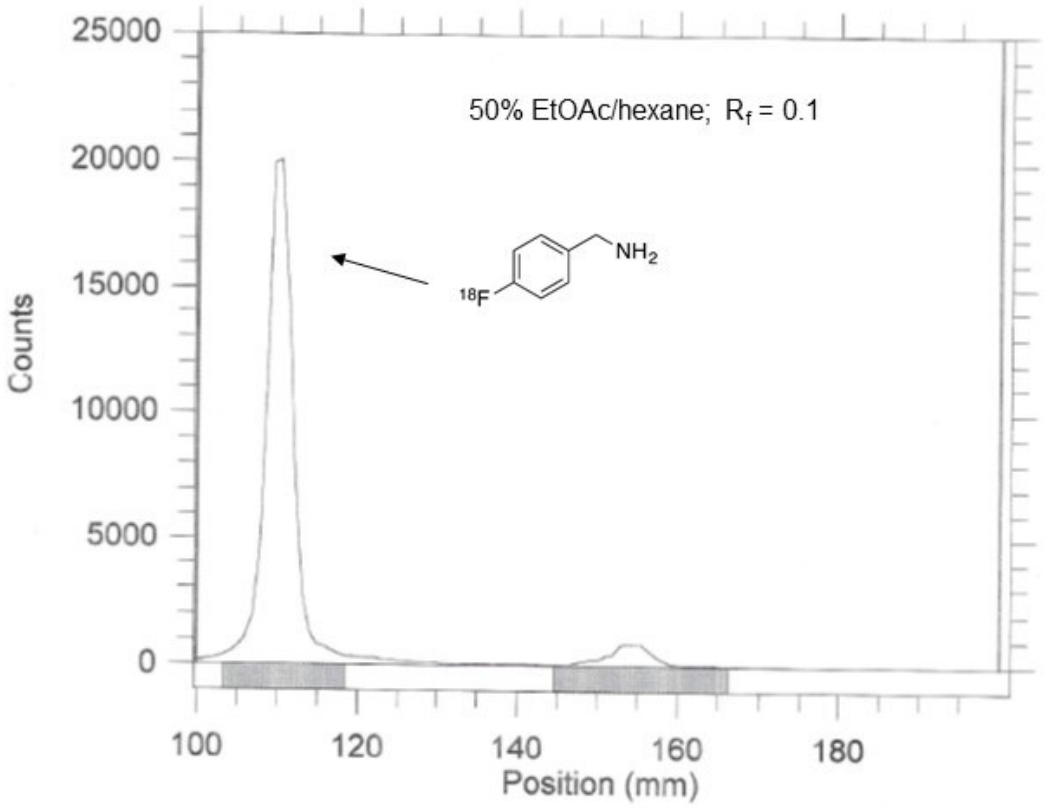
4-(*N*-(4-((4-[^{18}F]Fluorobenzyl)amino)-4-oxobutanoyl)sulfamoyl)benzoic acid ([^{18}F]8). RCC: 78%. Radio-TLC (50% EtOAc/hexane): R_f = 0.16.

***N*^{1}-((4-Acetamidophenyl)sulfonyl)-*N*^{4}-(4-[^{18}F]fluorobenzyl)succinamide ([^{18}F]9)**. RCC: 70%. Radio-TLC (50% EtOAc/hexane): R_f = 0.54.

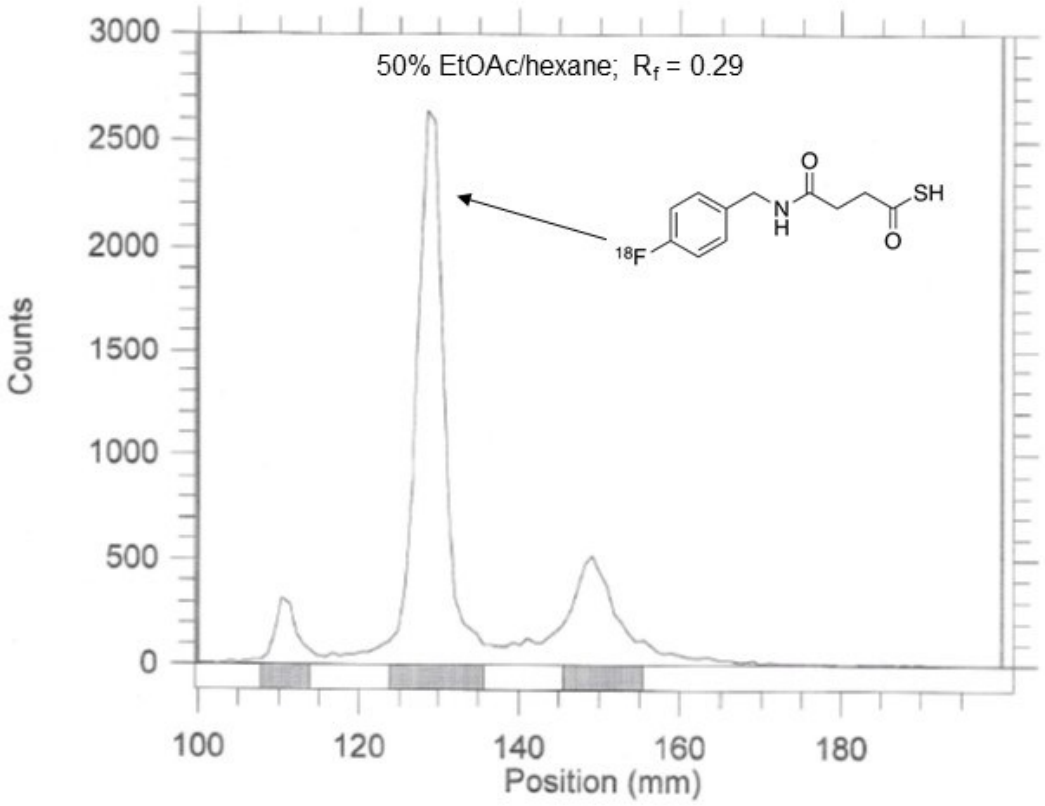
***N*^{1}-(4-[^{18}F]Fluorobenzyl)-*N*^{4}-tosylsuccinamide ([^{18}F]10)**. RCC: 30%. Radio-TLC (50% EtOAc/hexane): R_f = 0.46.

***N*^{1}-(4-[^{18}F]Fluorobenzyl)-*N*^{4}-((4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)-sulfonyl)succinamide ([^{18}F]13).** RCC: 55%. Radio-TLC (50% EtOAc/hexane): R_f = 0.37.

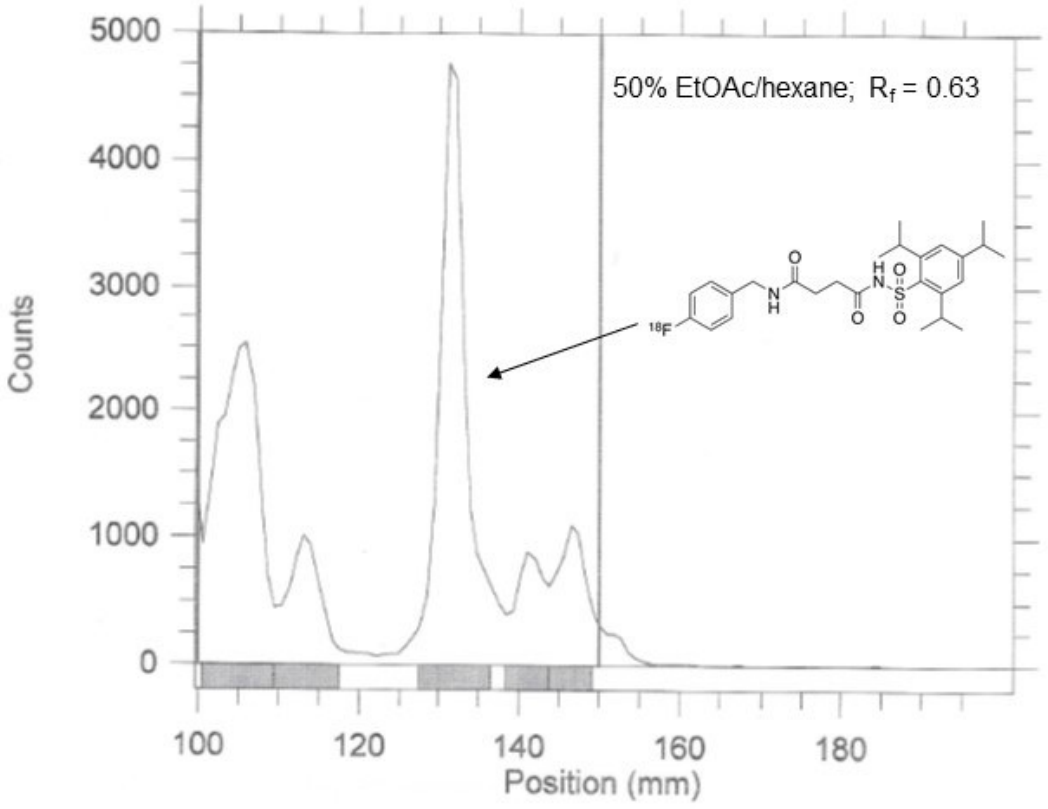
Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	103.2	118.5	110.2	0.102	87850.0	87850.0	88.71	92.97
Rgn 2	144.7	166.3	153.9	0.539	6646.0	6646.0	6.71	7.03
2 Peaks					94496.0	94496.0	95.42	100.00



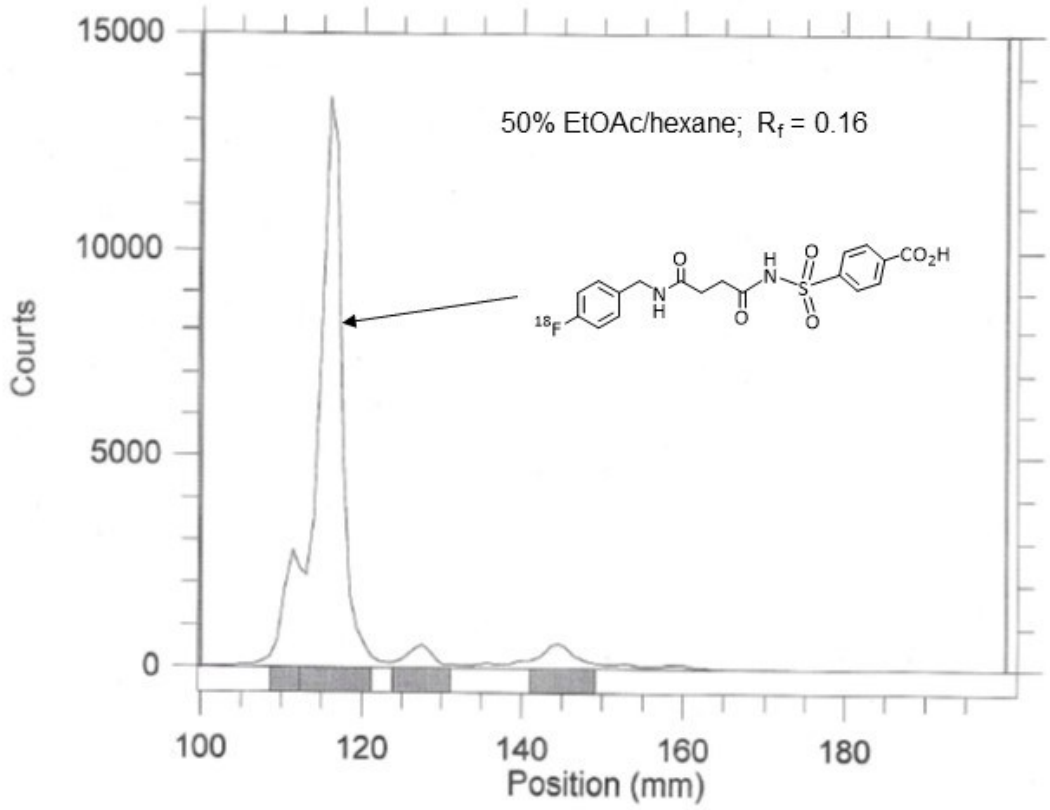
Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	107.7	114.0	110.9	0.109	1048.0	1048.0	5.54	6.46
Rgn 2	123.9	135.7	129.0	0.290	11814.0	11814.0	62.46	72.84
Rgn 3	145.6	155.5	149.5	0.495	3358.0	3358.0	17.75	20.70
3 Peaks					16220.0	16220.0	85.75	100.00



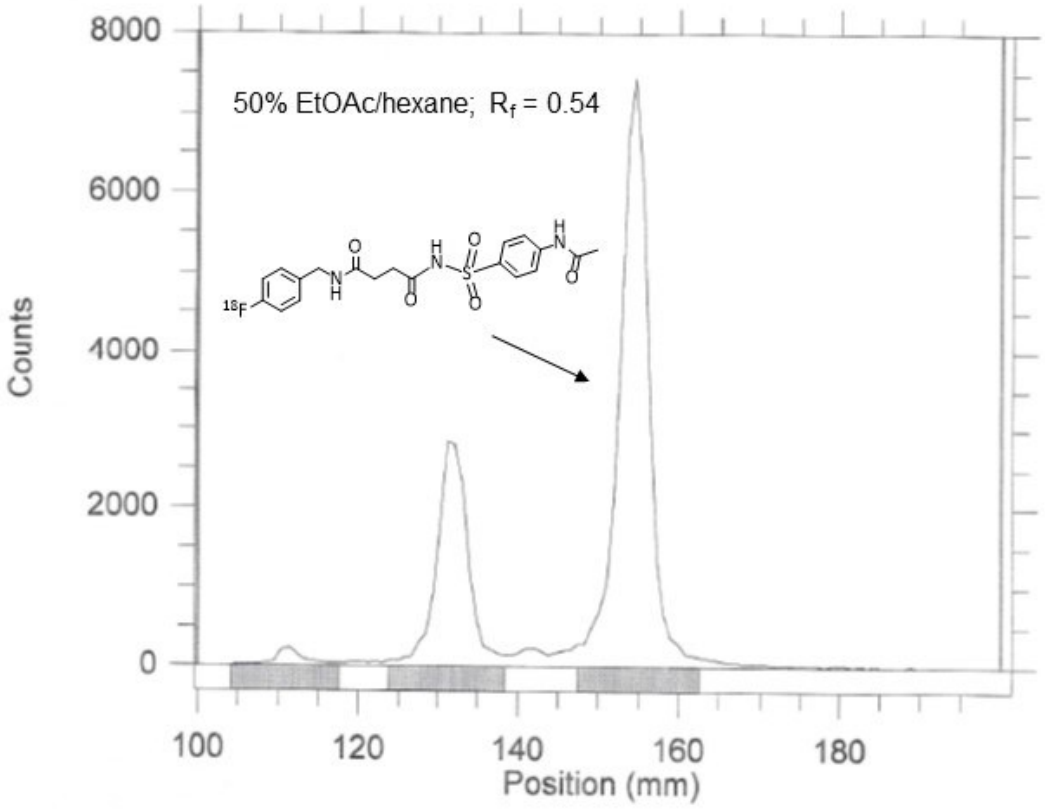
Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	100.5	109.5	104.6	0.093	17700.0	17700.0	30.64	34.04
Rgn 2	109.5	117.6	113.0	0.260	5605.0	5605.0	9.70	10.78
Rgn 3	127.5	136.6	131.7	0.634	19731.0	19731.0	34.15	37.94
Rgn 4	138.4	143.8	140.9	0.818	3953.0	3953.0	6.84	7.60
Rgn 5	143.8	149.2	146.1	0.923	5016.0	5016.0	8.68	9.65
5 Peaks					52005.0	52005.0	90.02	100.00



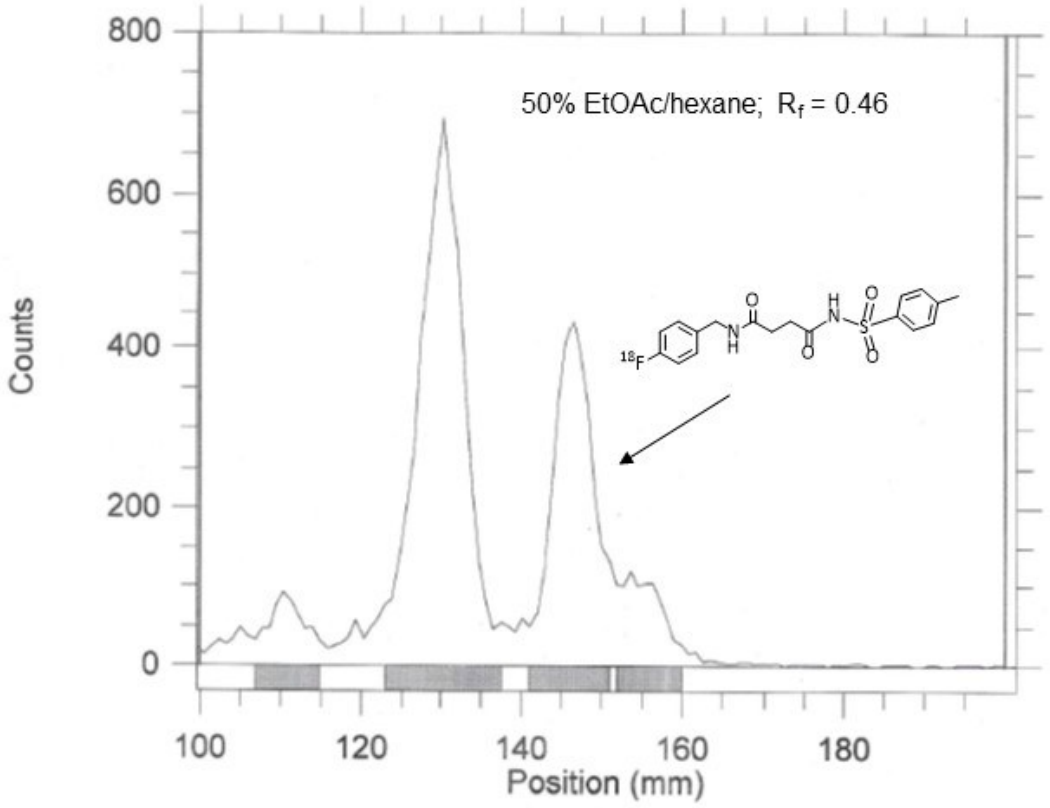
Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	108.6	113.1	111.2	0.112	7883.0	7883.0	12.21	12.48
Rgn 2	112.2	121.2	115.9	0.159	49655.0	49655.0	76.92	78.60
Rgn 3	123.9	131.2	127.1	0.271	2393.0	2393.0	3.71	3.79
Rgn 4	141.1	149.2	144.5	0.445	3240.0	3240.0	5.02	5.13
4 Peaks					63171.0	63171.0	97.86	100.00



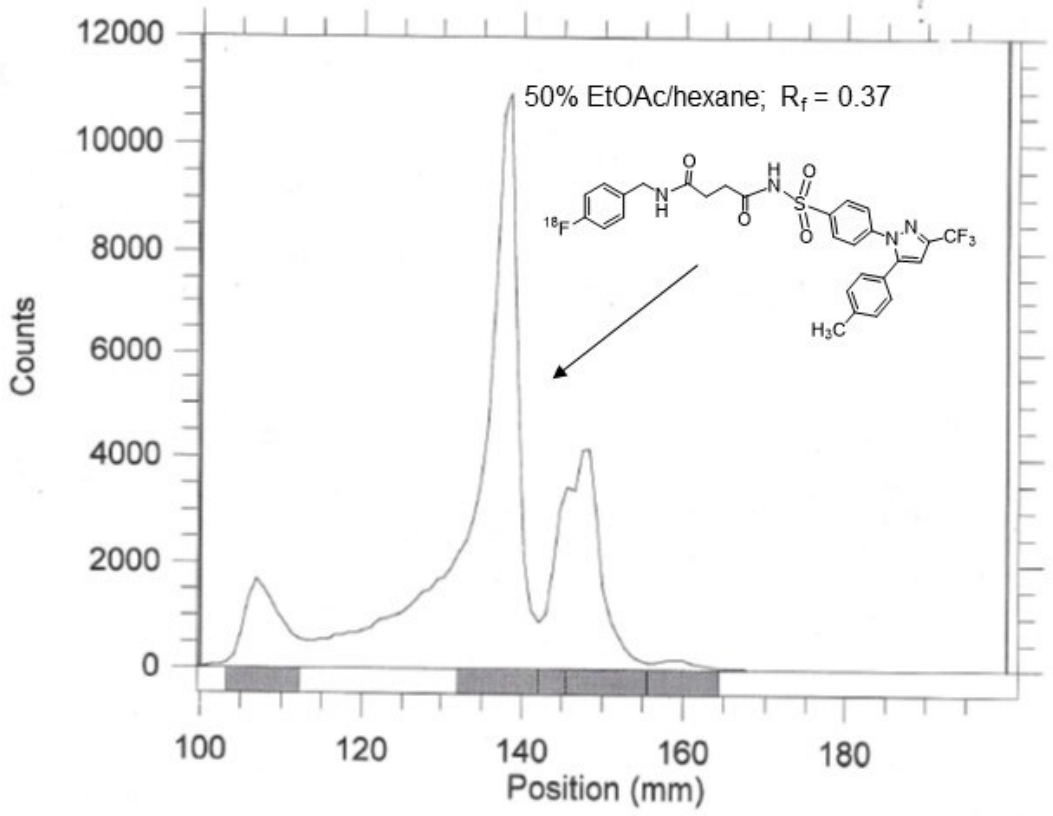
Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	104.1	117.6	111.7	0.117	1032.0	1032.0	1.92	2.03
Rgn 2	123.9	138.4	131.7	0.317	14256.0	14256.0	26.51	28.10
Rgn 3	147.4	162.7	154.3	0.543	35437.0	35437.0	65.90	69.86
3 Peaks					50725.0	50725.0	94.33	100.00



Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	106.8	114.9	110.6	0.106	523.0	523.0	5.17	5.76
Rgn 2	123.0	137.5	129.9	0.299	5007.0	5007.0	49.47	55.19
Rgn 3	141.1	151.0	146.3	0.463	2737.0	2737.0	27.04	30.17
Rgn 4	151.9	160.0	154.9	0.549	805.0	805.0	7.95	8.87
4 Peaks					9072.0	9072.0	89.63	100.00



Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	103.2	112.2	107.8	0.078	9393.0	9393.0	8.18	9.76
Rgn 2	132.1	142.0	136.9	0.369	52972.0	52972.0	46.15	55.04
Rgn 3	142.0	146.5	144.4	0.444	10338.0	10338.0	9.01	10.74
Rgn 4	145.6	155.5	148.1	0.481	22168.0	22168.0	19.31	23.03
Rgn 5	155.5	164.5	159.1	0.591	1370.0	1370.0	1.19	1.42
5 Peaks					96241.0	96241.0	83.84	100.00



Peptide synthesis and radiolabeling. Sulfonyl azide-decorated tetrapeptide Gly-Leu-Ser-Phe **14** was prepared using a 4-benzyloxybenzyl alcohol (Wang) resin, preloaded with a C-terminal Fmoc-protected phenylalanine (loading 0.67 mmol/g), provided the solid support for the peptide synthesis. The peptide was synthesised by solid phase peptide synthesis (SPPS) using a fully automated peptide synthesizer (SyroI, Multisyntech/Biotage). The method used consisted of swelling the pre-loaded Wang resin in 2 mL of *N,N*-dimethylformamide (DMF) for 15 min. Fmoc-deprotection was achieved by treatment with 40% piperidine/DMF for 5 min and followed by a second incubation with 20% piperidine/DMF for 15 min. Fmoc-protected amino acids and the 4-(azidosulfonyl)benzoic acid (5 equiv.) were activated and coupled using 5 eq. of O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU), 5 equiv. of ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma), and 10 equiv. *N,N*-diisopropylethylamine (DIPEA) over a 60 min time period followed by wash steps with DMF. The cleavage of the protection groups from the peptide and the peptide from the resin was done in 95% trifluoroacetic acid (TFA) and 5% water for 2 hours at room temperature. Due to the sensitivity of the sulfonyl azide group to reduction, no scavengers were added. The peptide was separated from the resin through a syringe filter and precipitated by the addition of ice-cold diethyl ether. The crude peptides were obtained by removing the residual ether using a syringe filter and dried under vacuum. Purification by HPLC with semi-preparative HPLC at 2 mL/min using a gradient of water/0.2% TFA (A) and MeCN (B): 10% eluent B for 5 min, increased to 30% eluent B over an additional 5 min, then to 50% eluent B over 15 min, then 90% eluent B over 5 minutes, holding at that point for a final 10 min. Subsequent lyophilization gave sufficiently pure (>98% purity by HPLC) peptide as a white powder (10.9 mg, 34.4% yield). Analysis: t_R = 34.6 min. MW $C_{27}H_{33}N_7O_9S$ calculated 631.21 g/mol, found LC-MS (ESI, positive) m/z 632.2 $[M + H]^+$, HR-MS (ESI, negative) m/z 630.2 $[M - H]^-$.

Synthesis of reference compound 15. 2 mg (3.17 µmol) of peptide **14** was mixed with 1 mg (6.4 µmol) of 4-fluorobenzothioic *S*-acid **1** in the presence of 0.74 µL (6.4 µmol) of 2,6-lutidine in 1 mL of methanol. The resulting mixture was allowed to react for 4 hours at room temperature. Purification by HPLC with semi-preparative HPLC at 2 mL/min using a gradient of water/0.2% TFA (A) and MeCN (B): 10% eluent B for 5 min, increased to 30% eluent B over an additional 5 min, then to 50% eluent B over 15 min, then 90% eluent B over 5 min, holding at that point for a final 10 min. Subsequent lyophilization gave sufficiently pure (>98% purity by HPLC) peptide as a white powder (1.6 mg, 70.0% yield). Analysis: t_R = 35.0 min; MW $C_{34}H_{38}FN_5O_{10}S$ calculated 727.23 g/mol, found LC-MS (ESI, positive) m/z 728.2 $[M + H]^+$.

Radioynthesis of [^{18}F]15. 100-150 MBq of [^{18}F]SFB in 250 µL CH_3CN was added to 1 mg (17.8 µmol) of NaSH and left to react at 50 °C for 5 min. 1.1 mg (1.7 µmol) of peptide **14** and 0.2 µL (2.0 µmol) of 2,6-lutidine was added and reacted at 50 °C for 30 min. Purification by HPLC with semi-preparative HPLC at 2 mL/min using a gradient of water/0.2% TFA (A) and MeCN (B): 20%

eluent B for 5 min, increased to 50% eluent B over an additional 10 min, then to 90% eluent B over 10 min, holding at that point for a final 10 min. The desired peak was isolated and had a decay-corrected radiochemicalyield of 25% and a final radiochemical purity >99%. Analysis: t_R = 23.2 min (radioactivity trace), 23.6 min (UV trace) (**Figure S1**).

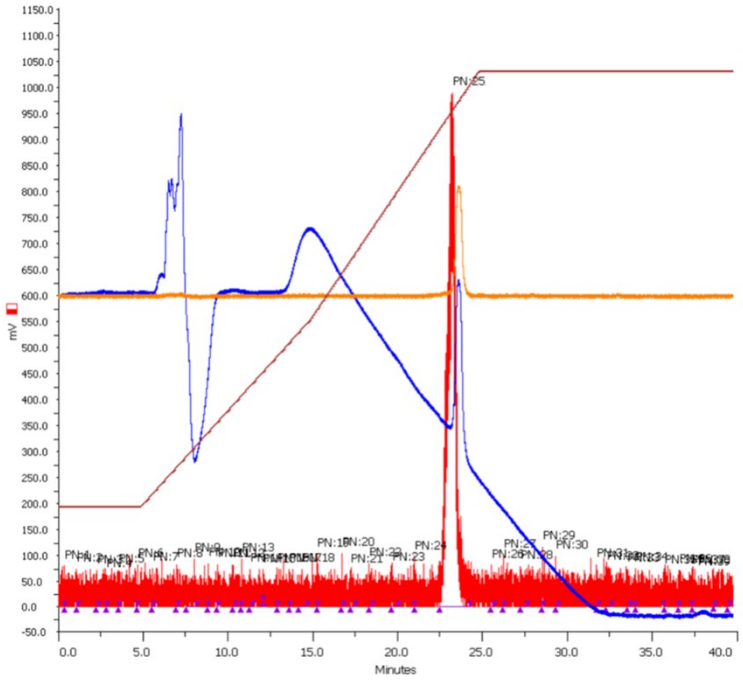


Figure S1. Radio-HPLC trace (red) of purified **[^{18}F]15** co-injected with reference compound **15** (blue; 210 nm; orange 254 nm).

Synthesis of reference compound 16. 0.34 µL (2.5 µmol) of 4-fluorobenzylamine was added to 0.28 µL (1.6 µmol) of 2,6-lutidine and 0.34 mg (3.0 µmol) of thiolane-2,5-dione in 0.5 mL of DMF and reacted at room temperature for 30 min yielding a clear, lightly yellow liquid. 1.6 mg (2.5 µmol) of peptide **14** was added and incubated at 50 °C for 30 min. Purification by HPLC with semi-preparative HPLC at 2 mL/min using a gradient of water/0.2% TFA (A) and MeCN (B): 10% eluent B for 5 min, increased to 30% eluent B over an additional 5 min, then to 50% eluent B over 15 min, then 90% eluent B over 5 min, holding at that point for a final 10 min. Subsequent lyophilization gave sufficiently pure (>99% purity by HPLC) peptide as a white powder (1.6 mg, 74.5% yield). Analysis: t_R = 35.0 min; MW $C_{38}H_{45}FN_6O_{11}S$ calculated 812.29 g/mol, found LC-MS (ESI, positive) m/z 813.2 $[M + H]^+$.

Radioynthesis of [^{18}F]16. 100-150 MBq of 4-[^{18}F]fluorobenzyl amine in 500 µL THF was added to 0.2 mg (1.7 µmol) of thiolane-2,5-dione and allowed to react for 10 min at 50 °C. Then, 1.1 mg (1.7 µmol) of peptide **14** and 0.2 µL (1.8 µmol) of 2,6-lutidine was added and allowed to

react for 30 min at 50 °C. Purification by HPLC with semi-preparative HPLC at 2 mL/min using a gradient of water/0.2% TFA (A) and MeCN (B): 20% eluent B for 5 min, increased to 50% eluent B over an additional 10 min, then to 90% eluent B over 10 min, holding at that point for a final 10 min. The desired peak was isolated and had a decay-corrected radiochemical yield of 20% and a final radiochemical purity >99%. Analysis: t_R = 23.2 min (radioactivity trace), 23.6 min (UV trace) (**Figure S2**).

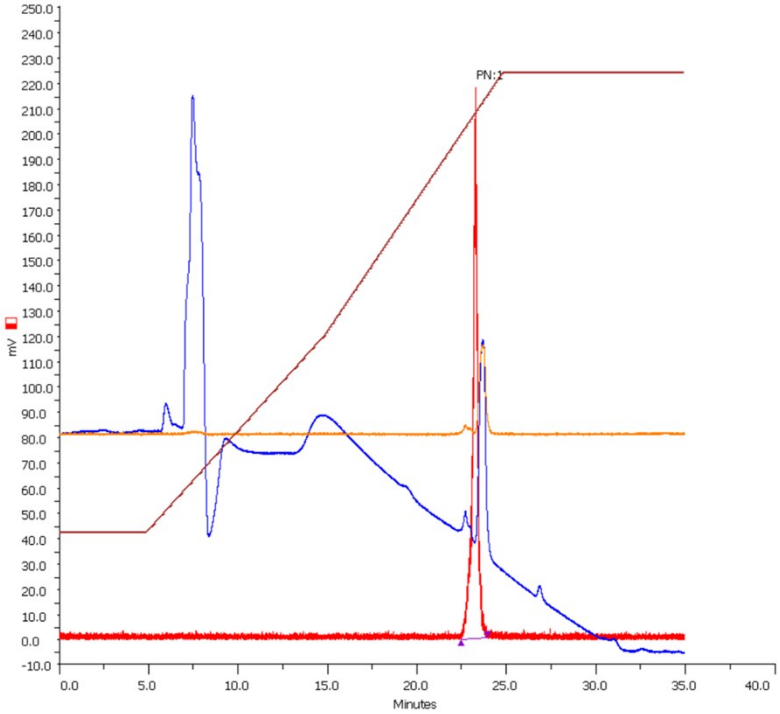


Figure S2. Radio-HPLC trace (red) of purified **[^{18}F]16** co-injected with reference compound **16** (blue; 210 nm; orange 254 nm).