Electronic Supplementary Material (ESI) for ChemComm.

Electronic supplementary material (ESI) for

Rapid ^{18}F-Labeling via Pd-Catalyzed *S*-Arylation in Aqueous Medium †

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Contents:

1. Material and methods

1.1. General

Chemicals and solvents were purchased from VWR International (Radnor, PA, USA), Sigma-Aldrich (Steinheim, Germany), ChemPUR (Karlsruhe, Germany), ABCR GmbH (Karlsruhe, Germany), Fluka AG (Buchs, Switzerland) and other commercial vendors, and used without further purification

1.2. Nuclear magnetic resonance (NMR) spectroscopy

NMR-Spectra were measured at ambient temperature in deuterium oxide (D_2O), deuterochloroform ($CDCl_3$), deuteromethanol (CD_3OD) or deuterodimethylsulfoxide (DMSO-d_6) as indicated using a Bruker Avance 200 (^{1}H: 200 MHz; ^{13}C: 50 MHz; ^{19}F: 188 MHz), a Varian Inova (^{1}H: 400 MHz; ^{13}C: 101 MHz; ^{19}F: 376 MHz) or a Bruker Avance III HD (^{1}H: 600 MHz; ^{13}C: 151 MHz; ^{19}F: 565 MHz). The measured chemical shifts are reported in δ (ppm) relative to residual peaks of deuterated solvents. The observed signal multiplicities are characterized as follows: s = singlet, d = doublet, t = triplet, m = multiplet and dd = doublet of doublet. Coupling constants *J* are reported in Hertz (Hz).

1.3. Mass spectroscopy (MS)

Low resolution mass spectra (LRMS) were measured with a MSQ PlusTM mass spectrometer (Thermo Electron Corporation, San Jose, USA).

High resolution mass spectra were measured with a FTICR “LTQ FT Ultra” (Thermo Fisher Scientific Inc., Bremen, Germany).

MALDI mass spectra were measured with a LTQ-Orbitrap XL Hybrid with a MALDI ionisation source. Samples were prepared with α-cyano-4-hydroxycinnamic acid (5 mg/mL in MeCN/water/TFA 50:50:0.1) (Thermo Fisher Scientific Inc., Bremen, Germany).

1.4. Elemental analysis

Elemental analysis of 5-iodopyridin-2-yl trifluoromethanesulfonate was performed by HEKAtech GmbH (Wegberg, Germany).

1.5. Column chromatography

Manual column chromatography was performed with silica gel, 60Å, 230–400 mesh particle size from Fluka AG (Buchs, Switzerland), silica gel (w/Ca, 0.1%), 60Å, 230–400 mesh particle size from Sigma-Aldrich GmbH (Steinheim, Germany). Automated column chromatography was performed either on a Grace Reverelis X1 (Columbia, Maryland, USA) or a Büchi Pure C-

815 Flash system using Si60 FlashPure cartridges or Reveleris™ C18 reversed phase cartridges.

1.6. Thin layer chromatography (TLC)

TLC was performed using aluminum sheets coated with silica gel 0.25 mm SIL G/UV 254 (Merck KGaA, Darmstadt, Germany). Chromatograms were inspected under UV light ($\lambda = 254$ nm) and/or stained with phosphomolybdic acid (20% in EtOH).

1.7. High pressure liquid chromatography (HPLC)

HPLC analyses were carried out on a HPLC system consisting of a Knauer Smartline 1000 pump, a Knauer Smartline 2020 variable wavelength UV detector and a Harschaw 3 inch NaI(Tl) borehole detector connected to an Ortec Model 276 photomultiplier and an ACE mate 925 spectroscopy System. For the data acquisition Gina Star (Raytest Elysia) was used. Two Rheodyne 6 port injections valves equipped with equal sample loops were installed before and behind the chromatographic column. The UV and radioactivity detectors were connected in sequence, giving a time delay of 0.1–0.2 min between the corresponding responses, depending on the flow rate. Semipreparative HPLC was performed on a dedicated semipreparative system consisting of a Knauer K-100 pump, a Knauer K-2501 UV Detector, a Rheodyne 6 port injection valve equipped with a 2 mL injection loop and a custom-made Geiger counter. All HPLC columns used were purchased from Phenomenex (Aschaffenburg, Germany).

1.8. Determination of pH values

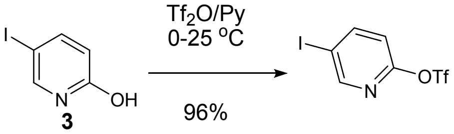
The pH values of aqueous solutions were measured with a 766 pH meter (Knick, Berlin, Germany) and an Inlab® Micro electrode (Mettler Toledo, Columbus, USA). The pH values of aqueous-organic mixtures were determined by spotting a small aliquot on pH 1–14 universal indicator paper (Merck KGaA, Darmstadt, Germany).

1.9. Chemistry

All reactions were carried out with magnetic stirring. Organic extracts were dried over anhydrous $MgSO_4$. Air or moisture sensitive reagents were handled under argon (>99.999%, Air Liquide GmbH). Solutions were concentrated under reduced pressure (1–900 mbar) at 40 °C using a rotary evaporator (Büchi Labortechnik, Essen, Germany). Solvent proportions are indicated in a volume/volume ratio. RD2-Cys peptide (H-ptlhthnr$_5$-NH$_2$, **12**) was supplied by peptides & elephants GmbH (Germany). PSMA I&S (**16**) was prepared according to the literature.[1]

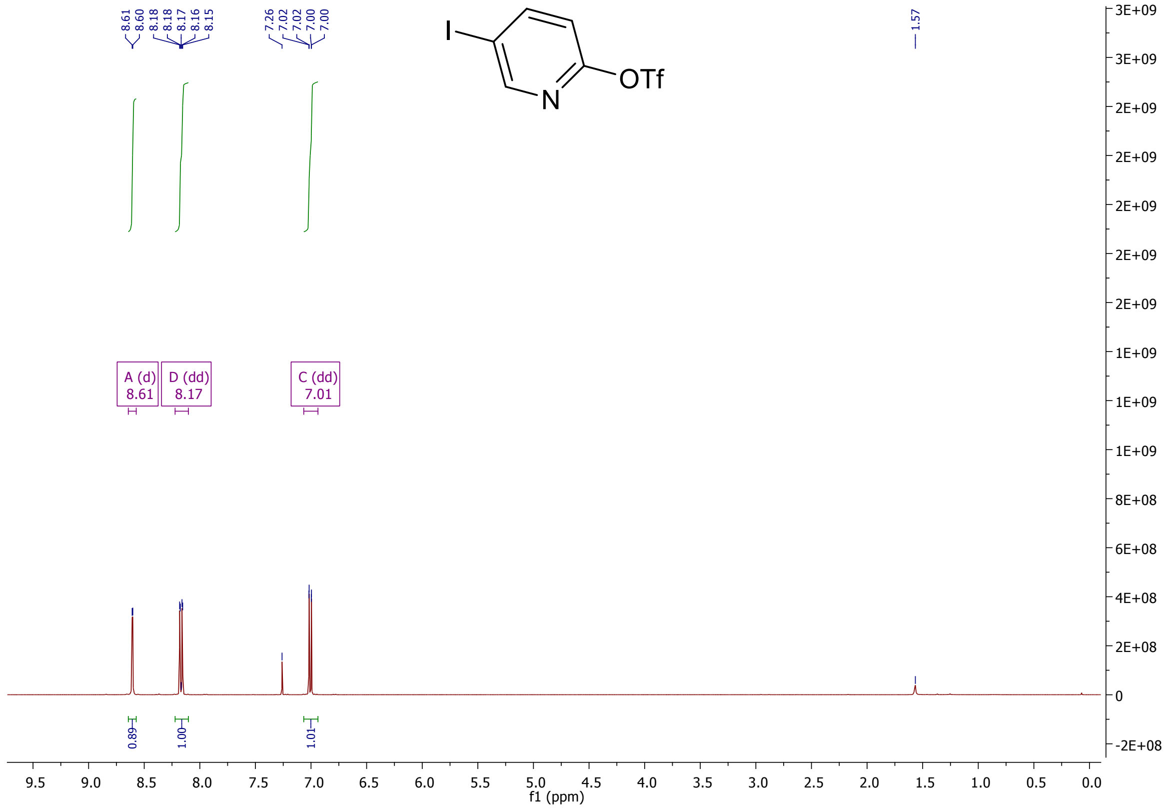
2. Organic chemistry. Synthesis of precursors and reference compounds

2.1. 5-Iodopyridin-2-yl trifluoromethanesulfonate



3

Triflic anhydride (3.78 mL, 6.50 g, 23.04 mmol) was added dropwise to an ice-cold solution of 2-hydroxy-5-iodopyridine (**3**) (3 g, 13.57 mmol) in anhydrous pyridine (40 mL). The resulting black solution was allowed to warm to ambient temperature and stirred for further 16 h. The reaction mixture was concentrated under reduced pressure, the residue was taken up into H_2O and pentane (100 mL of each), the organic fraction was separated and washed with H_2O (3×50 mL), 1 M $NaHSO_4$ (3×50 mL), H_2O (3×50 mL), brine (2×50 mL), dried and concentrated under reduced pressure. The faint yellow residual liquid was purified by column chromatography (EtOAc:hexane 1:7) furnishing the title compound[2] (4.6 g, 96%) as a colorless liquid. R_f=0.56 (EtOAc). ^{1}H-NMR (400 MHz, $CDCl_3$): 8.60 (d, J = 2.3 Hz, 1H), 8.17 (dd, J = 8.5 Hz, J = 2.3 Hz, 1H), 7.01 (dd, J = 8.5 Hz, J=0.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): 155.4, 154.8, 149.1, 118.6 (q, J =320.6 Hz), 117.2, 92.1. ^{19}F NMR (376 MHz, $CDCl_3$): –72.9. HR-MS (ESI) *m/z:* $[M + Na]^+$ calcd for $C_6H_3F_3INO_3SNa^+$: 375.8723; found: 375.8721; $[M + NH_4]^+$ calcd for $C_6H_7F_3IN_2O_3S^+$: 370.9169; found: 370.9165; $[M + H]^+$ calcd for $C_6H_4F_3INO_3S^+$: 353.8903; found: 353.8904. Elementary analysis: calcd for $C_6H_3F_3INO_3S$: C 20.41%, H: 0.86 %, N: 3.97 %, S: 9.08 %; found: C 20.04%, H: 0.86%, N: 3.68%, S: 9.01%.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8.18
8.17
8.16
8.15
7.26
7.02
7.02
7.00
7.00
1.57
I
N
OTf
A (d) 8.61
D (dd) 8.17
C (dd) 7.01
0.89
1.00
1.01
3E+09
3E+09
2E+09
2E+09
2E+09
2E+09
2E+09
1E+09
1E+09
1E+09
8E+08
6E+08
4E+08
2E+08
0
-2E+08
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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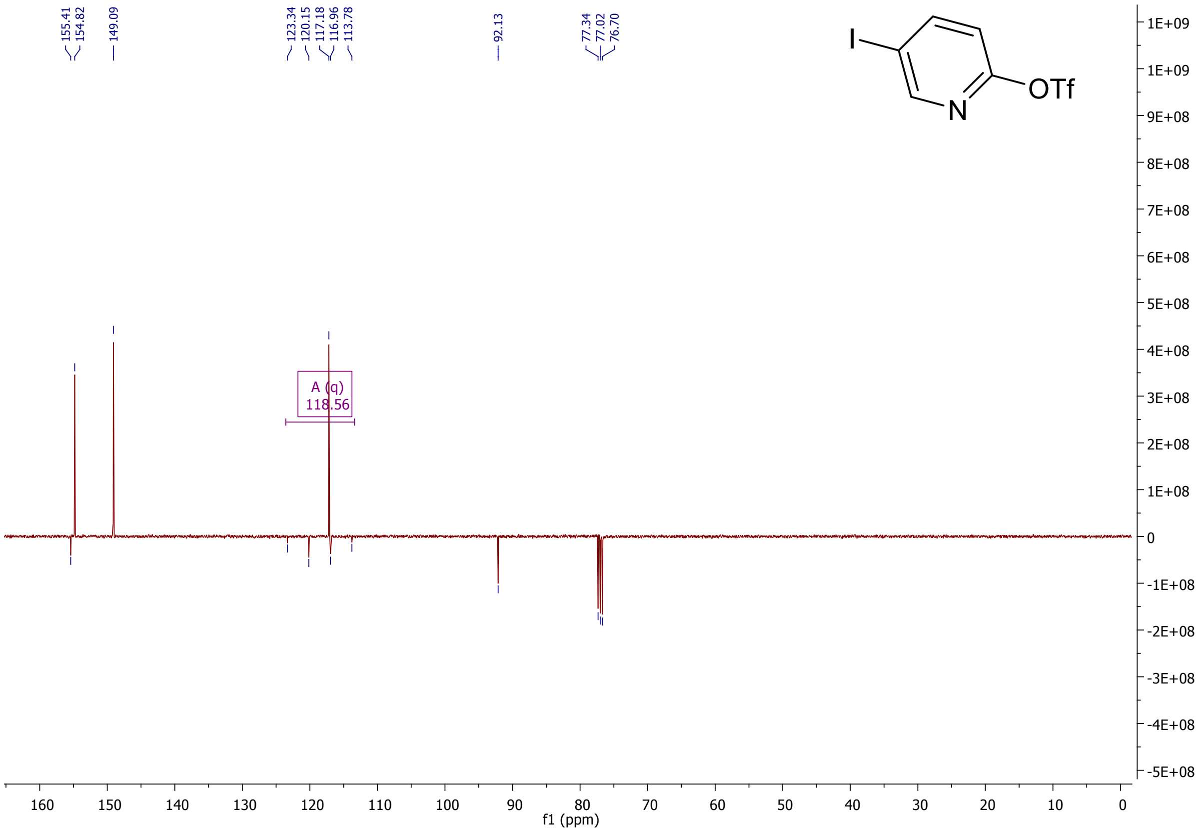
f1 (ppm)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120.15
117.18
116.96
113.78
92.13
77.34
77.02
76.70
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N
OTf
A (q) 118.56
1E+09
1E+09
9E+08
8E+08
7E+08
6E+08
5E+08
4E+08
3E+08
2E+08
1E+08
0
-1E+08
-2E+08
-3E+08
-4E+08
-5E+08
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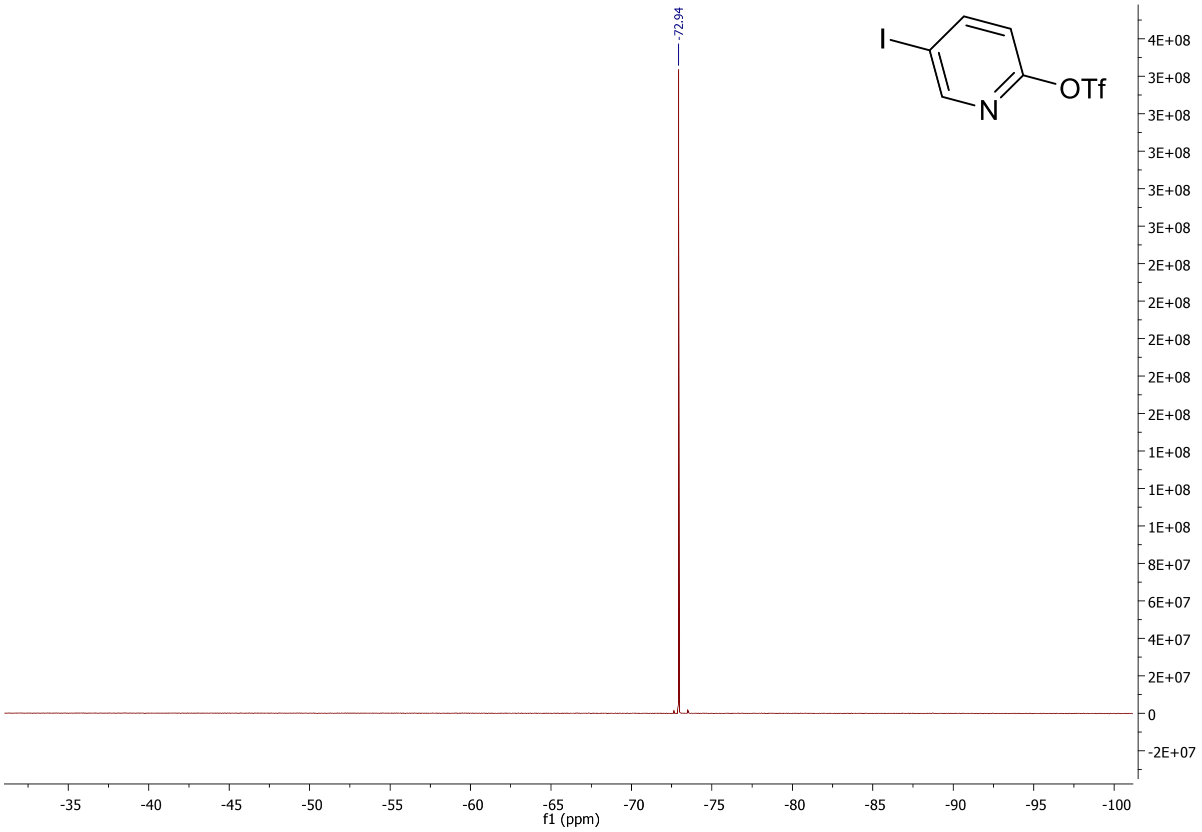
f1 (ppm)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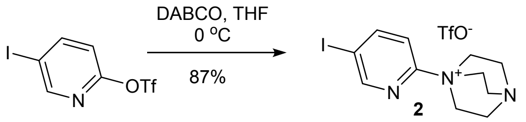
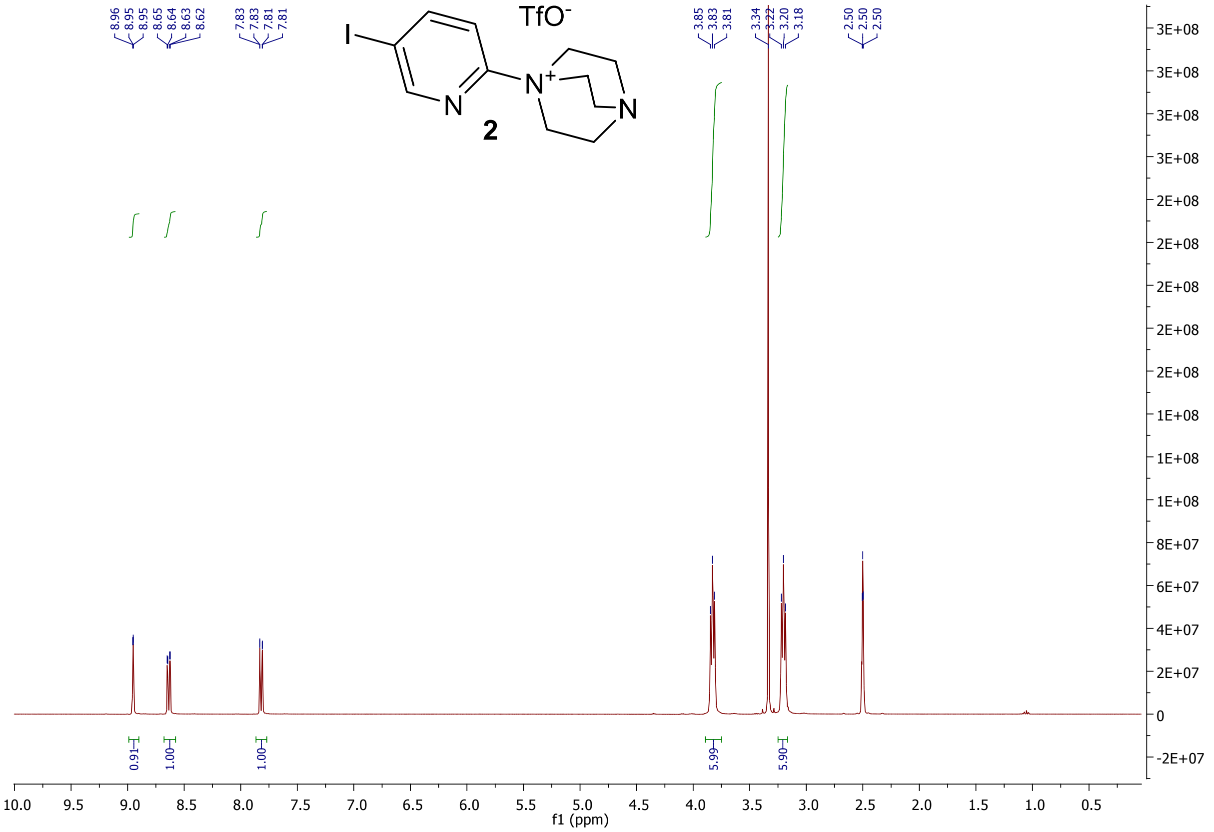
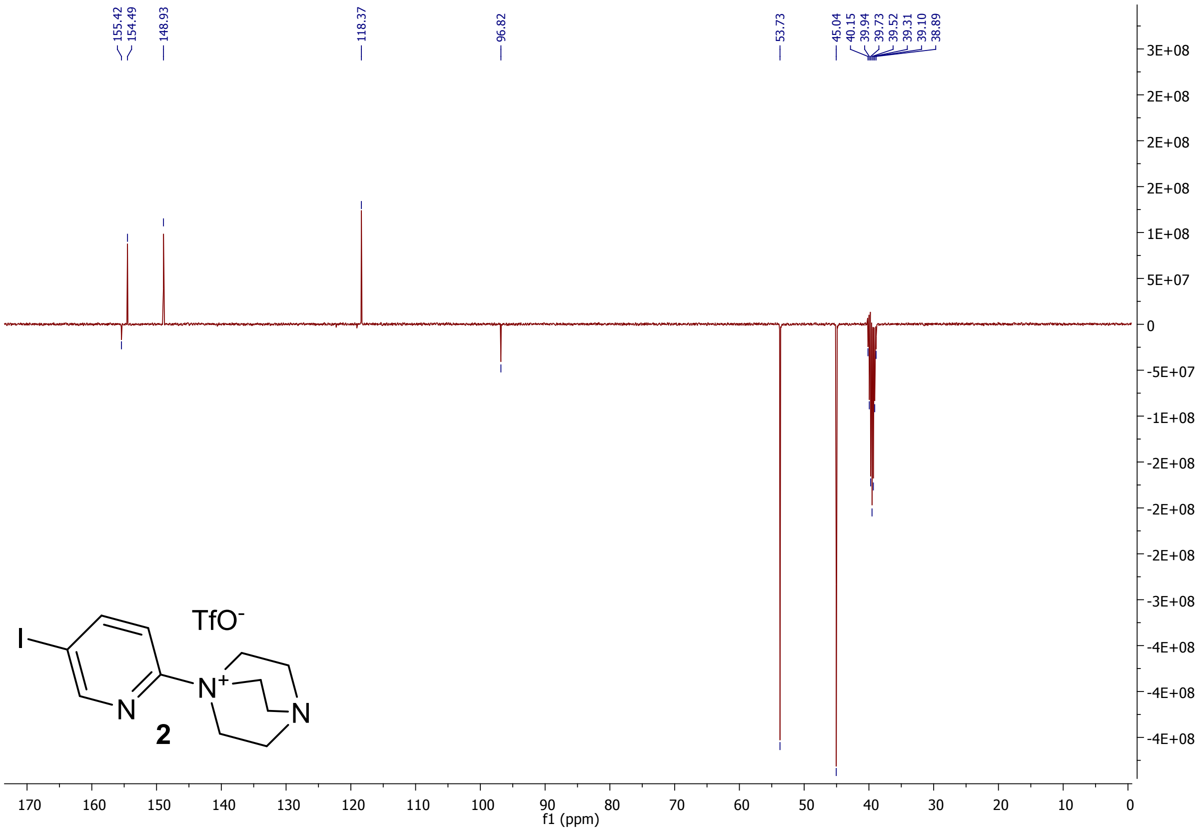
2E+08
1E+08
1E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
-35
-40
-45
-50
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
f1 (ppm)

2.2. 1-(5-Iodopyridin-2-yl)-1,4-diazabicyclo[2.2.2]octan-1-ium trifluoromethanesulfonate (2)



A solution of DABCO (293 mg, 2.61 mmol, 1 eq.) in anhydrous THF (2 mL) was added dropwise via the rubber septum within 5 min to an ice-cold solution of 5-iodopyridin-2-yltriflate (920 mg, 2.61 mmol, 1 eq.) in anhydrous THF (3.5 mL). The cooling bath was removed and the reaction mixture was stirred for 4 days whereupon the solvent gradually evaporated. The dry residue was triturated with Et_2O, the precipitate was filtered off and the crude product was recrystallized from MeOH/Et_2O affording **2** as a colorless solid (0.98 g, 2.11 mmol, 81%). ^{1}H-NMR [400 MHz, $(CD_3)_2SO$]: 8.95 (dd, *J* = 2.2 Hz, *J* = 0.4 Hz 1H), 8.64 (dd, *J* = 8.7 Hz, *J* = 2.2 Hz, 1H), 7.82 (dd, *J* = 8.7 Hz, *J* = 0.4 Hz, 1H), 3.83 (t, *J* = 7.3 Hz 6H), 3.20 (t, *J* = 7.5 Hz 6H). ^{13}C-NMR [100 MHz, $(CD_3)_2SO$]: 155.42, 154.49, 148.93, 120.67 (q, *J* = 322.4 Hz), 118.37, 96.82, 53.73, 45.05. ^{19}F-NMR [376 MHz, $(CD_3)_2SO$]: –77.74. HR-MS (ESI) *m/z*: $[M]^+$ calcd for $C_{11}H_{15}IN_3^+$: 316.0305; found: 316.0305.

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154.49
148.93
118.37
96.82
53.73
45.04
40.15
39.94
39.73
39.52
39.31
39.10
38.89
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
-5E+07
-1E+08
-2E+08
-2E+08
-2E+08
-3E+08
-4E+08
-4E+08
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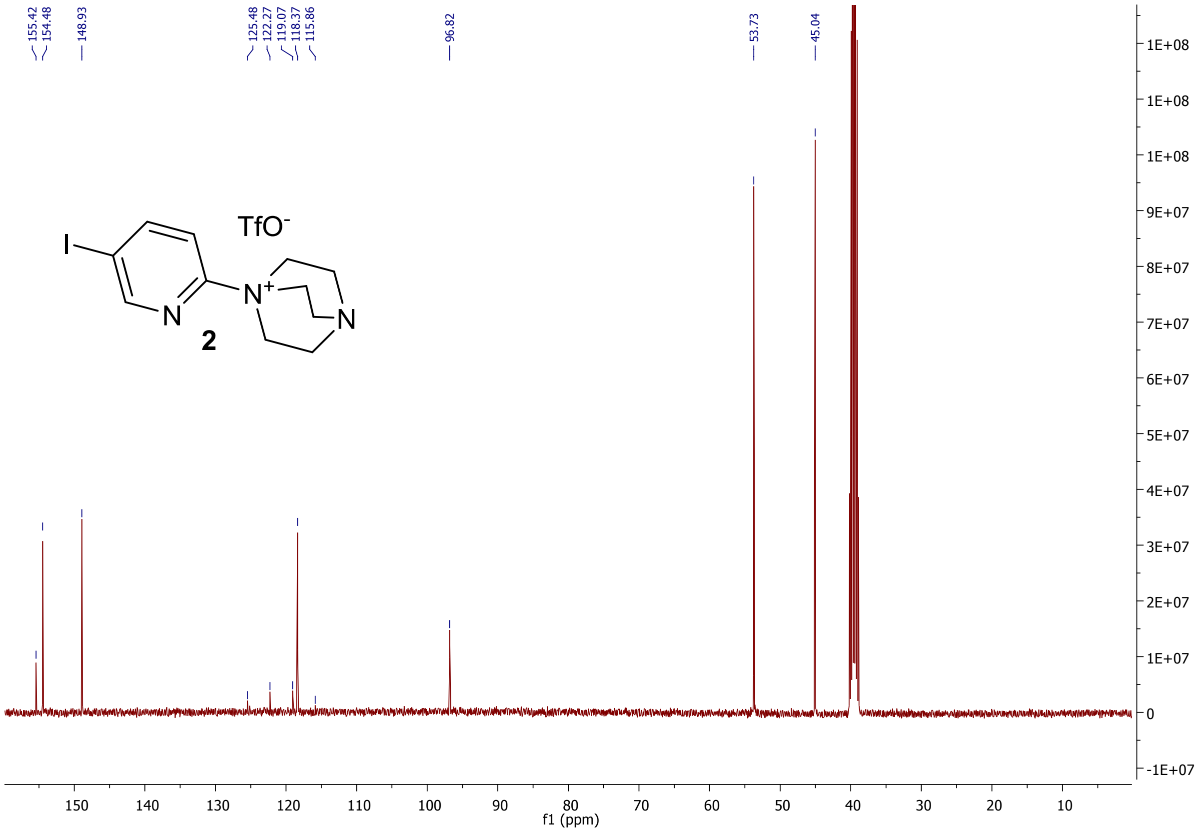
I
N
N+
N
TfO-
2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119.07
118.37
115.86
96.82
53.73
45.04
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1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
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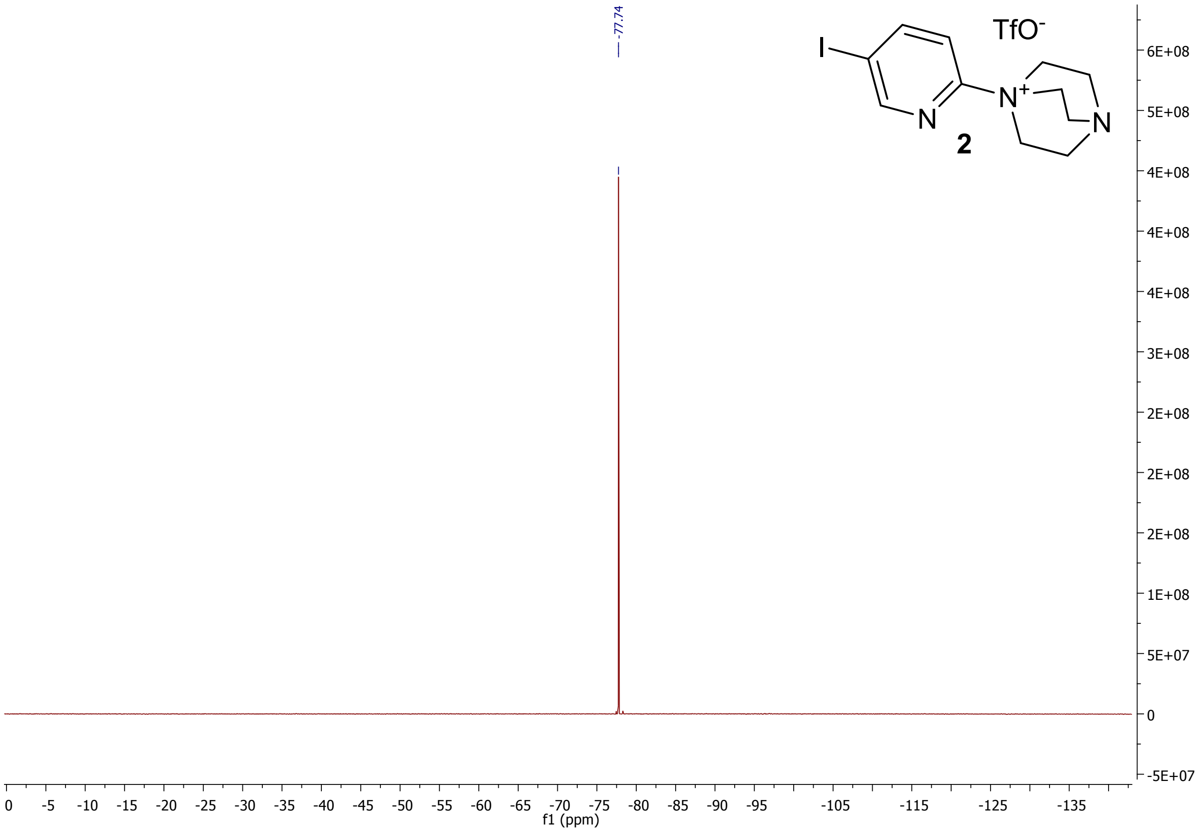
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)
I
N
N+
N
TfO-
2


2
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TfO-
I
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N+
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4E+08
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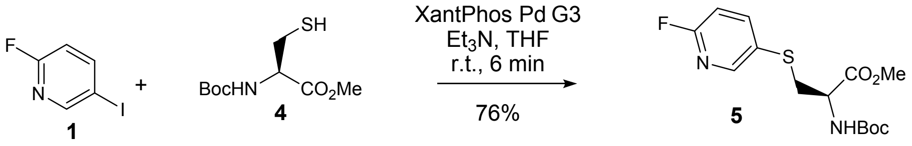
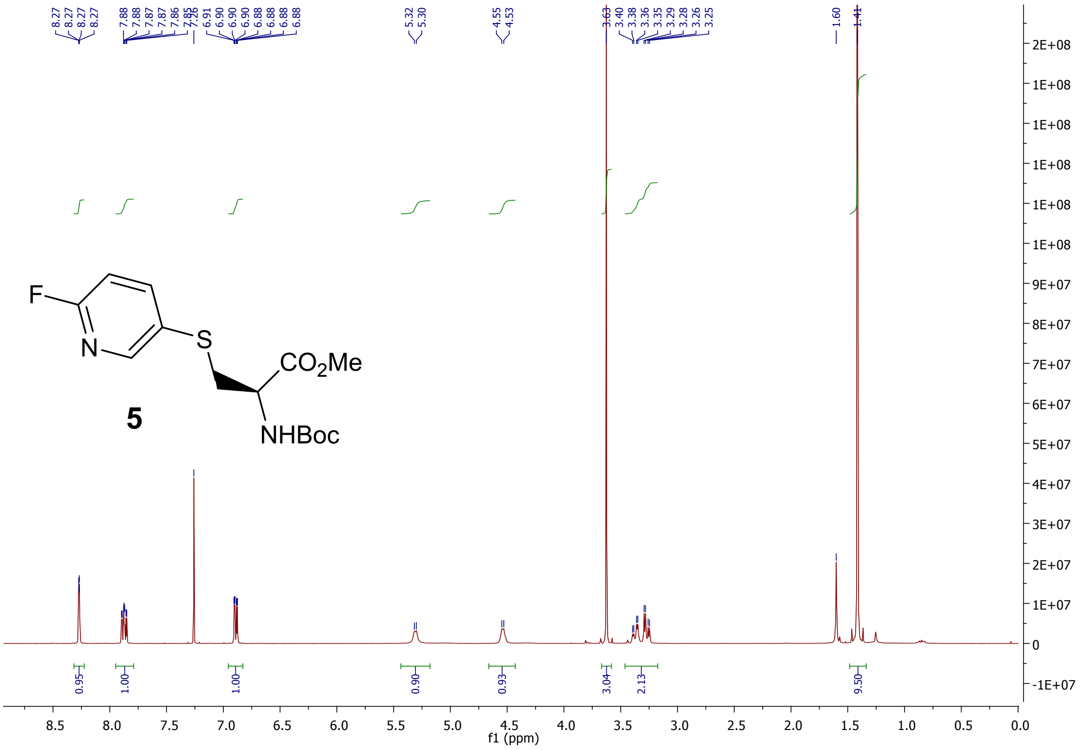
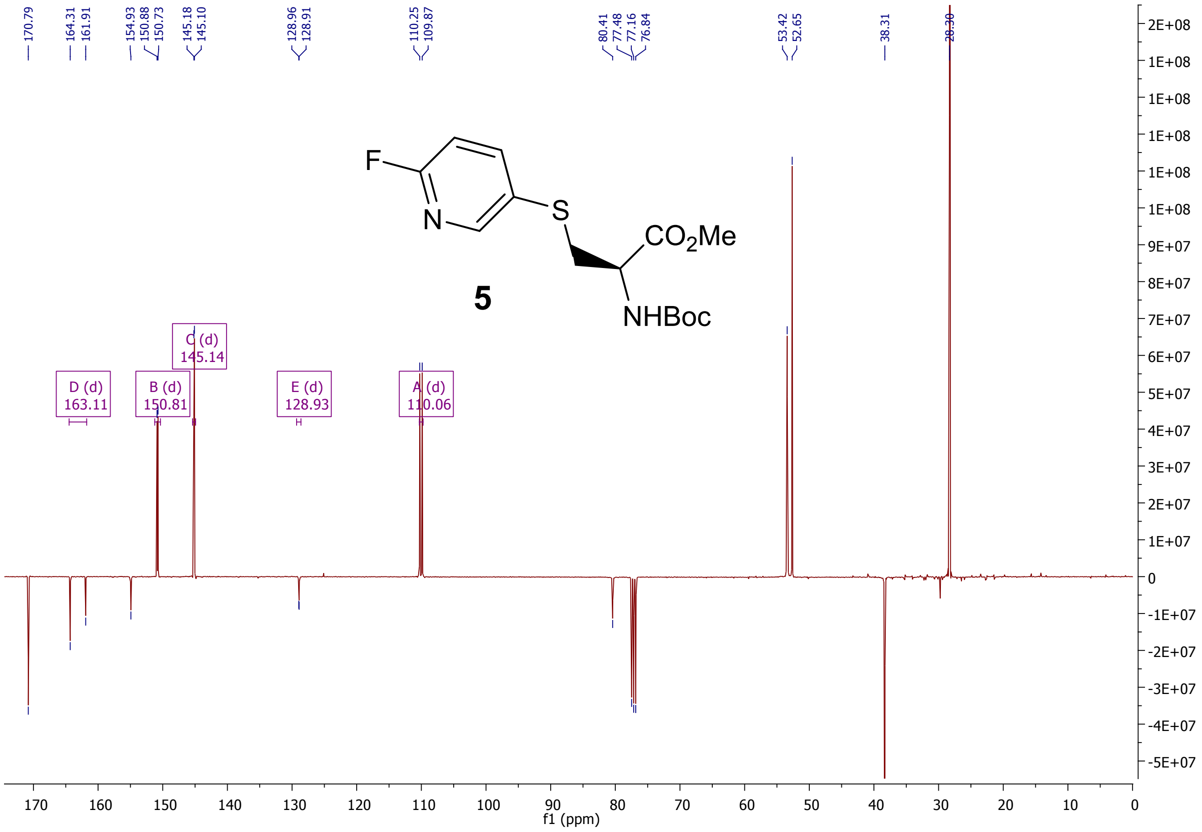
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
-5E+07
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
-60
-65
-70
-75
-80
-85
-90
-95
-105
-115
-125
-135
f1 (ppm)

2.3. *N*-(*tert*-Butoxycarbonyl)-*S*-(6-fluoropyridin-3-yl)-L-cysteine methyl ester (5)



The title compound was prepared using the protocol of Al-Shuaeeb *et al.*[3] as follows._5-Iodo-2-fluoropyridine (**1**) (45 mg, 0.20 mmol, 1.2 eq), Boc-Cys-OMe (**4**) (40 mg, 0.17 mmol, 1.0 eq), XantPhos Pd-G3 (3.2 mg, 2 mol%) were dissolved in anhydrous THF (240 µL) under Ar. The mixture was stirred at room temperature for 1 min, Et$_3$N (46.8 µL, 34 mg, 0.34 mmol, 2.00 eq) was added and the reaction mixture was stirred for further 5 min. The mixture was concentrated under reduced pressure and the residue purified by column chromatography (EtOAc/petroleum ether=1:4) to afford **5** as a colorless oil (45 mg, 0.13 mmol, 76%). ^{1}H-NMR (400 MHz, CDCl$_3$): 8.27 (dt, *J* = 2.5 Hz, *J* = 0.6 Hz, 1H), 7.90–7.81 (ddd, *J* = 8.5 Hz, *J* = 7.4 Hz, *J* = 2.8 Hz 1H), 6.89 (ddd, *J* = 8.5 Hz, *J* = 2.8 Hz, *J* = 0.6 Hz 1H), 5.31 (d, *J* = 6.6 Hz, 1H), 4.58–4.48 (m, 1H), 3.60 (s, 3H), 3.42–3.21 (m, 2H), 1.41 (s, 9H). ^{13}C-NMR (101 MHz, CDCl$_3$): 170.79, 163.11 (d, *J* = 241.0 Hz), 154.93, 150.81 (d, *J* = 15.0 Hz), 145.14 (d, *J* = 8.3 Hz), 128.87 (d, *J* = 4.4 Hz), 110.06 (d, *J* =38.0 Hz), 80.41, 53.42, 52.65, 38.31, 28.30. ^{19}F-NMR (376 MHz, CD$_3$OD): –69.05. HR-MS (ESI) *m/z:* [M + Na]$^+$ calcd for $C_{14}H_{19}FN_2NaO_4SNa^+$: 353.09418; found: 353.09420.

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150.73
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145.10
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128.91
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80.41
77.48
77.16
76.84
53.42
52.65
38.31
28.30
D (d) 163.11
B (d) 150.81
C (d) 145.14
E (d) 128.93
A (d) 110.06
F
N
S
CO2Me
NHBoc
5
2E+08
1E+08
1E+08
1E+08
1E+08
1E+08
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8E+07
7E+07
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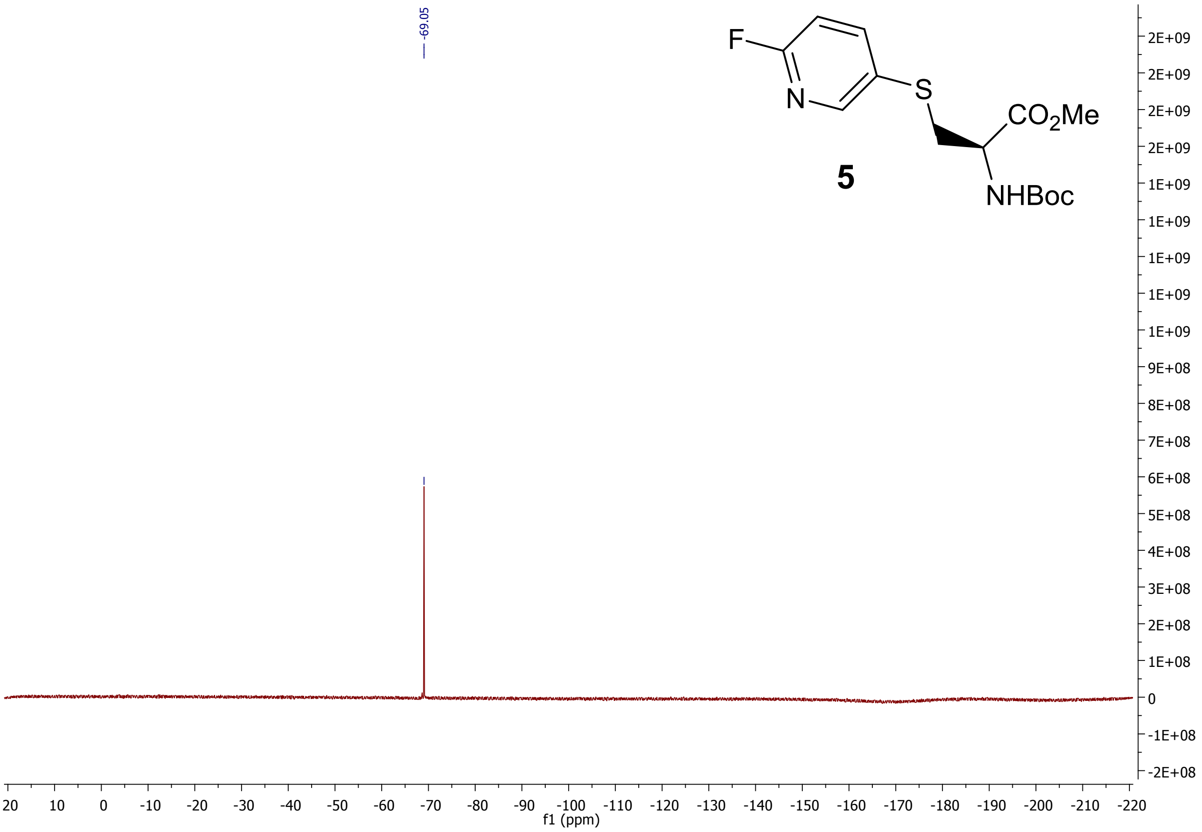
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-80
-90
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-110
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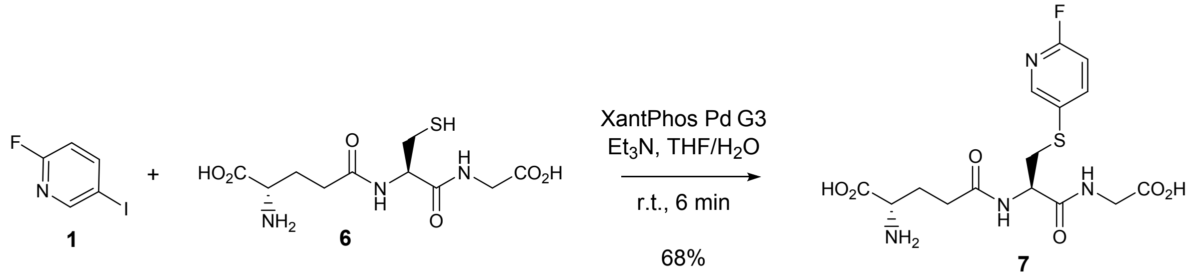
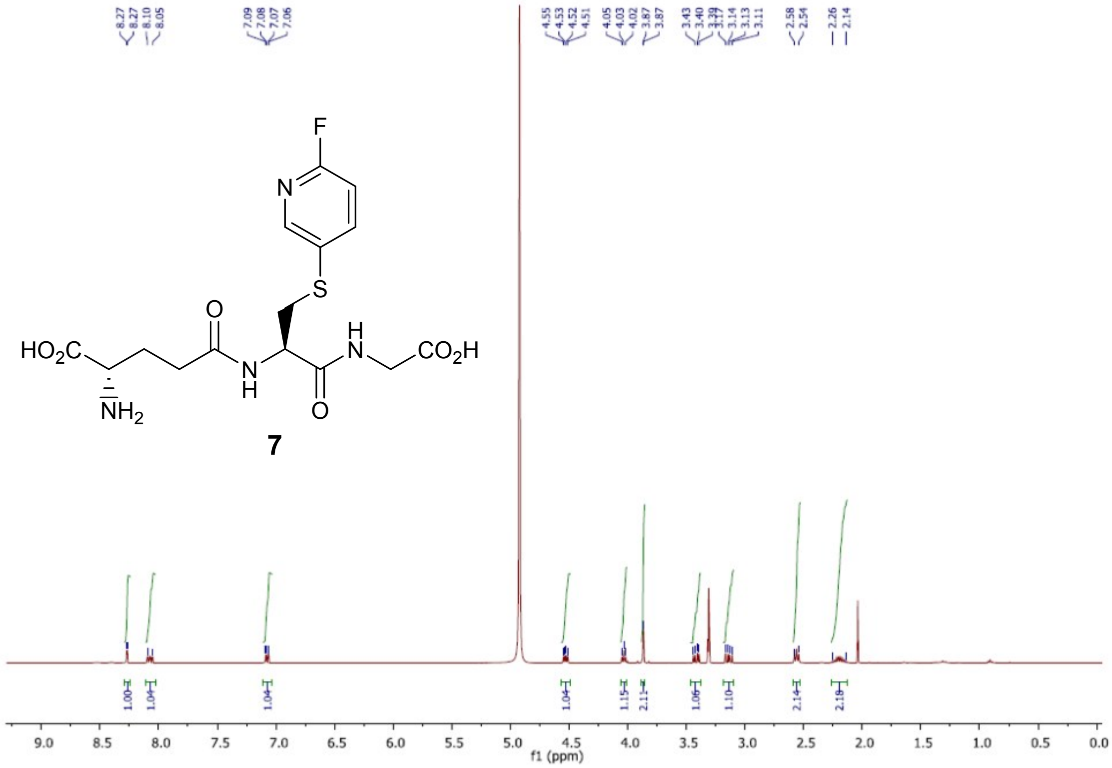
-140
-150
-160
-170
-180
-190
-200
-210
-220
f1 (ppm)

2.4. N_5-{(*R*)-1-[(Carboxymethyl)amino]-3-[(6-fluoropyridin-3-yl)thio]-1-oxopropan-2-yl}-(*S*)-glutamine (7)



The title compound was prepared using the protocol of Al-Shuaeeb *et al.*[3] as follows. 5-Iodo-2-fluoropyridine (5) (40 mg, 0.19 mmol, 3.2 eq), L-glutathion (6) (20 mg, 60 µmol, 1.00 eq), XantPhos Pd-G3 (1.7 mg, 2 mol%) were dissolved in 20% THF (480 µL) under Ar. The mixture was stirred at room temperature for 1 min, Et_3N (27.5 µL, 20 mg, 0.19 mmol, 3.00 eq) was added and the reaction mixture was stirred for 5 min. Thereafter the reaction mixture was concentrated under reduced pressure and the residue purified by semipreparative HPLC [column: Gemini C18(2), 250x10 mm, 5 µm/100 Å; eluent: 15% MeCN (0.1% TFA); flow rate: 6 mL/min] to afford 7 as a colorless solid (18 mg, 40 µmol, 67%). ^{1}H-NMR (400 MHz, CD_3OD): 8.27 (d, *J* = 2.6 Hz, 1H), 8.10–8.05 (m, 1H), 7.09–7.06 (dd, *J* = 8.7 Hz, *J* = 2.6 Hz, 1H), 4.55–4.51 (dd, *J* = 9.0 Hz, *J* = 5.1 Hz, 1H), 4.03 (t, *J* = 6.4 Hz, 1H), 3.87 (d, *J* = 1.7 Hz, 2H), 3.44–3.39 (dd, *J* = 14.1 Hz, *J* = 5.1 Hz, 1H), 3.17–3.11 (dd, *J* = 14.1 Hz, *J* = 9.0 Hz, 1H), 2.58–2.54 (m, 2H), 2.26–2.14 (m, 2H). ^{13}C-NMR (100 MHz, CD_3OD): 174.36, 172.65, 172.43, 171.51, 164.43 (d, *J* = 240.2 Hz), 151.20 (d, *J* = 14.3 Hz), 146.83 (d, *J* = 8.4 Hz), 130.61, 111.45 (d, *J* = 37.9 Hz), 53.84, 53.48, 41.74, 37.88, 32.36, 26.98. ^{19}F-NMR (376 MHz, CD_3OD): –77.22. HR-MS (ESI+) *m/z*: $[M + H]^+$ calcd for $C_{15}H_{20}FN_5O_6S^+$: 403.10821; found: 403.10827.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NH$_2$
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1.04
1.04
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1.06
1.10
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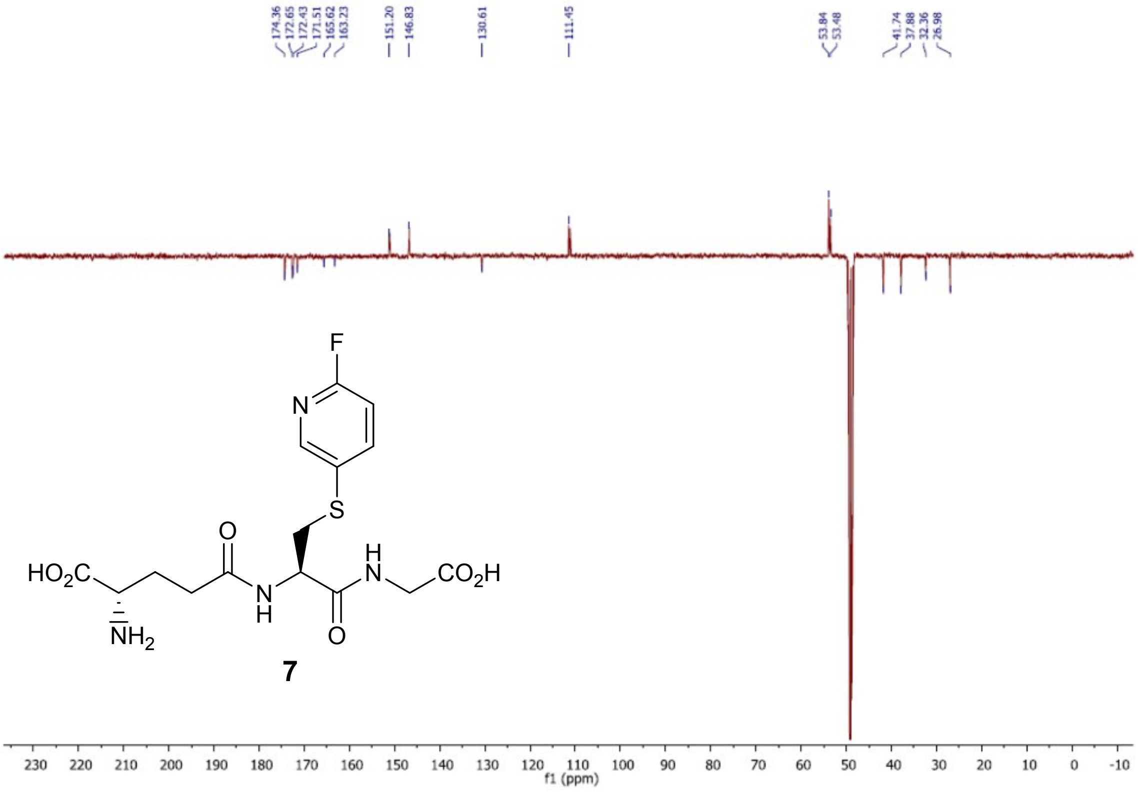
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26.98
F
N
S
O
H
HO$_2$C
N
CO$_2$H
N
H
O
NH$_2$
7
230
220
210
200
190
180
170
160
150
140
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120
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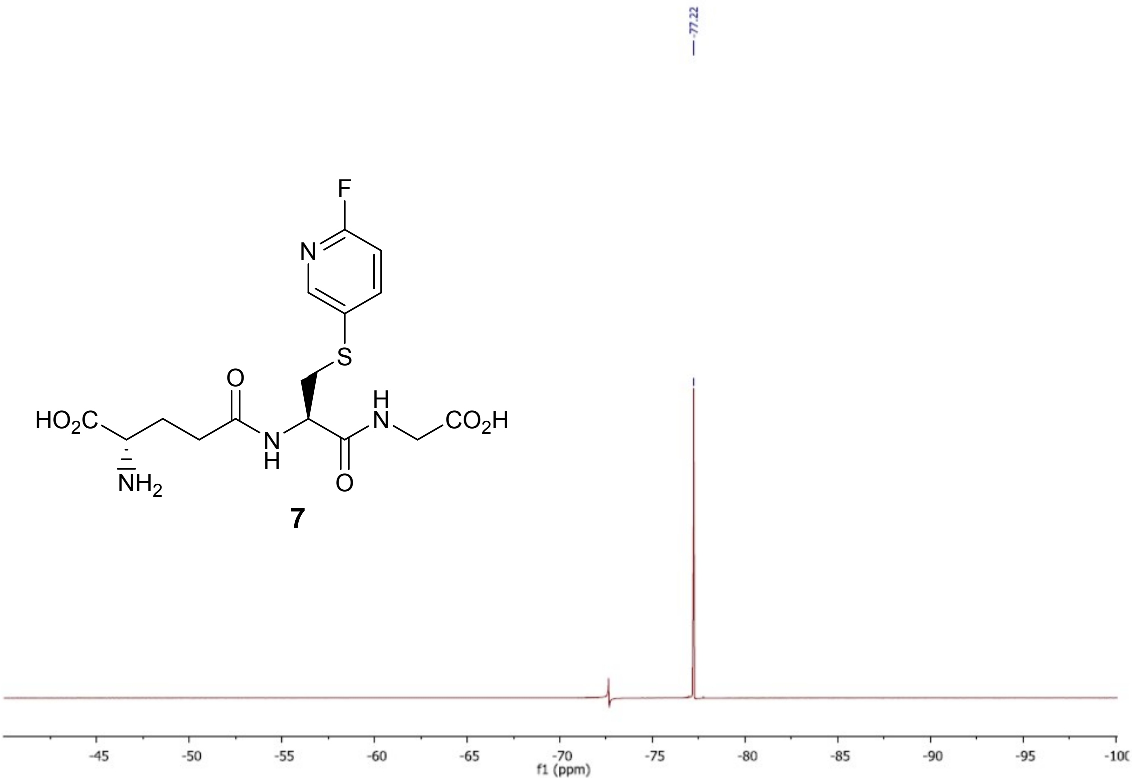
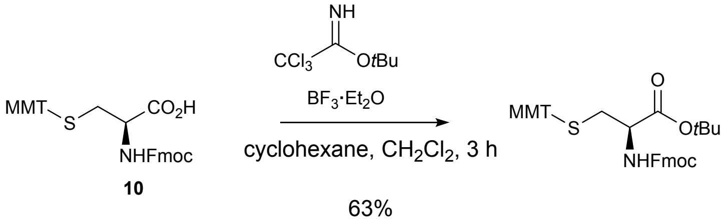
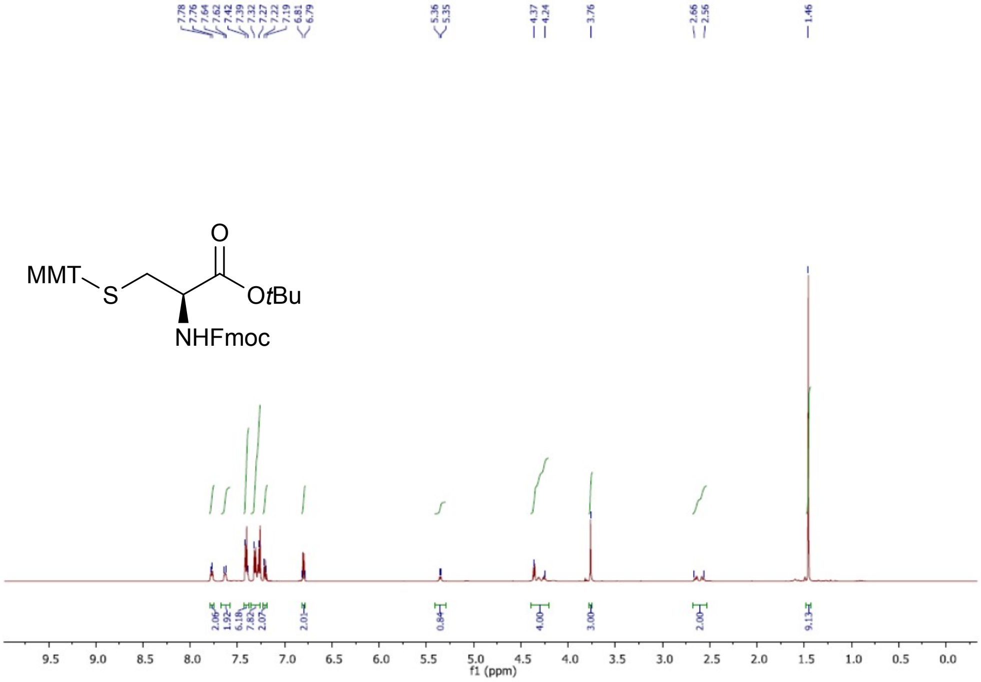
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

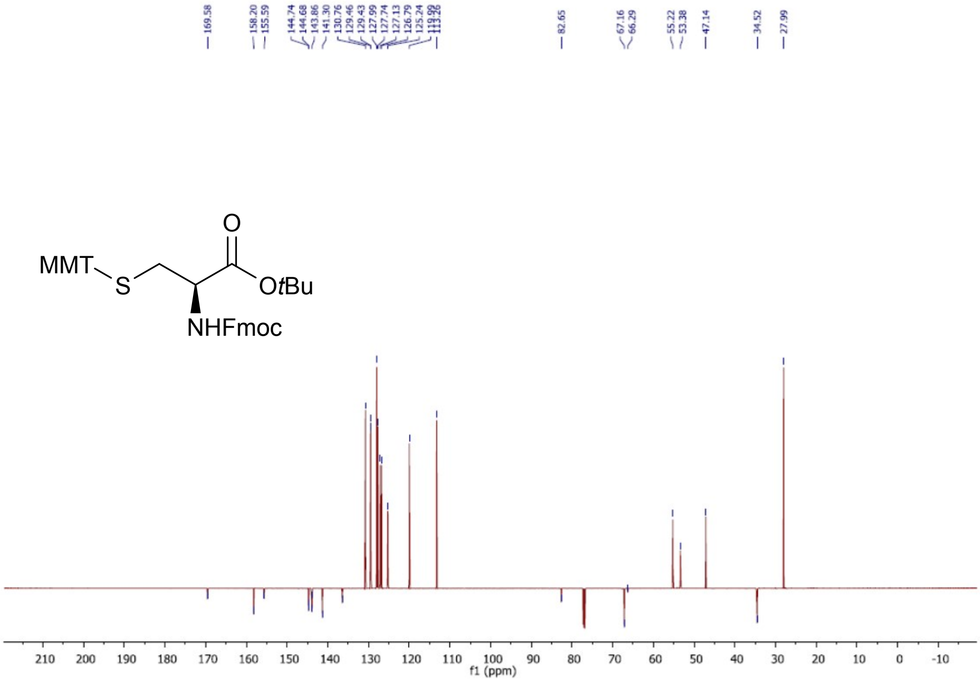


2.5. Fmoc-Cys(MMT)-O*t*Bu

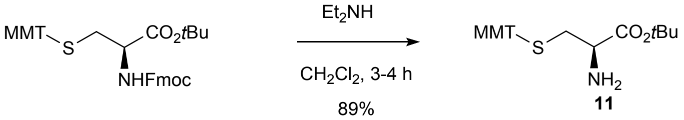


The title compound was prepared according to the protocol of Haag *et al.*[4] as follows. BF$_3$·Et$_2$O (80 µL) was added to a solution of *tert*-butyl-2,2,2-trichloroacetimidate (1.75 g, 8.00 mmol, 2.00 eq) and Fmoc-Cys(MMT)-OH (**10**) (2.46 g, 4 mmol, 1.00 eq) in anhydrous 33% CH$_2$Cl$_2$ in cyclohexane (18 mL) under Ar and the reaction mixture was stirred for 3 h at ambient temperature and neutralized by Et$_3$N. The mixture was concentrated under reduced pressure and the residue was purified by column chromatography (EtOAc/petroleum ether = 1:9), to afford the title compound as a colorless solid (1.71 g, 2.55 mmol, 63%). ^{1}H-NMR (400 MHz, CDCl$_3$): 7.78–7.76 (m, 2H), 7.64–762 (m, 2H), 7.42–7.39 (m, 6H), 7.32–7.27 (m, 8H), 7.22–7.19 (m, 2H), 6.81–6.79 (m, 2H), 5.36 (d, *J* = 8.2 Hz, 1H), 4.37–4.24 (m, 4H), 3.76 (s, 3H), 2.66–2.56 (m, 2H), 1.46 (s, 9H). ^{13}C-NMR (100 MHz, CDCl$_3$): 169.58, 158.20, 155.59, 144.74, 144.68, 143.96, 143.86, 141.30, 136.42, 130.76, 129.49, 129.46 (inequivalent C$_2$ and C$_6$ of the 4-anisyl group of the MMT residue); 127.99, 127.74, 127.13, 126.79, 125.24, 119.99, 113.26, 82.65, 67.16, 66.29, 55.22, 53.38, 47.14, 34.52, 27.99. HR-MS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{42}H_{41}NO_5SNa^+$: 694.26031; found: 694.25690.

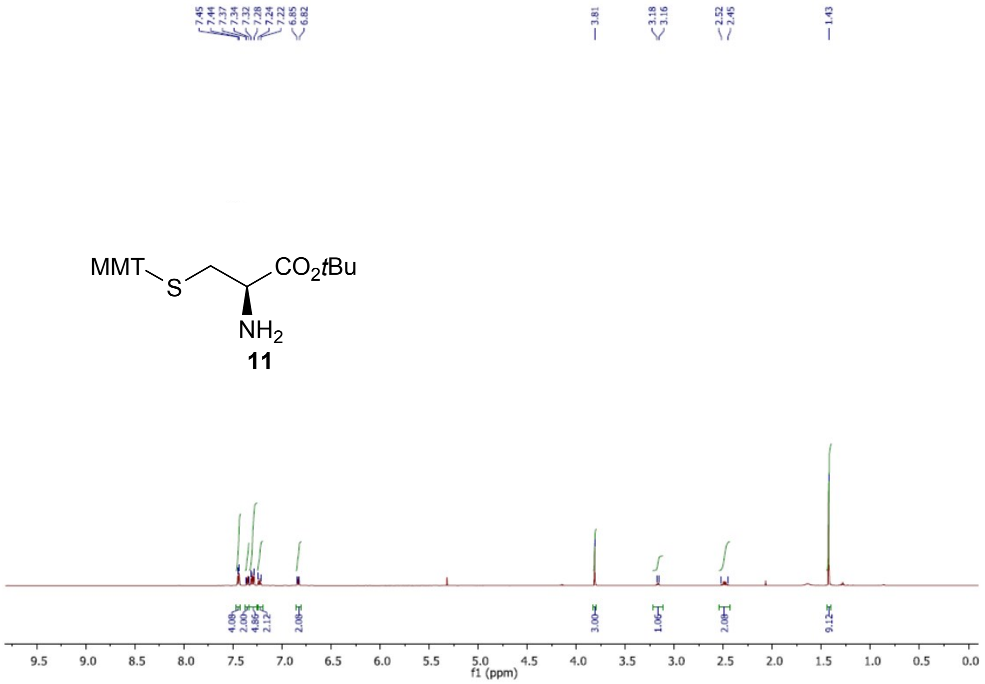




2.6. H-Cys(MMT)-O*t*Bu (11)



Et_2NH (1.23 mL, 0.87 g, 11.88 mmol, 5.00 eq) was added to a solution of Fmoc-Cys(MTT)-O*t*Bu (1.52 g, 2.37 mmol, 1.00 eq) in CH_2Cl_2 (10 mL) and the reaction mixture was stirred for 3-4 h at room temperature. The mixture was concentrated under reduced pressure and the residue was purified by column chromatography (petroleum ether/EtOAc = 3:1) to give **11** as a colorless oil (0.92 g, 2.12 mmol, 89%). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.45–7.44 (m, 4H), 7.37–7.34 (m, 2H), 7.32–7.28 (m, 5H), 7.24–7.22 (m, 2H), 6.85–6.82 (br, 2H), 3.81 (s, 3H), 3.18–3.16 (m, 1H), 2.52–2.45 (m, 2H), 1.43 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 173.05, 158.13, 144.98, 136.74, 130.80; 129.52, 129.51 (inequivalent C_2 and C_6 of the 4-anisyl group of the MMT residue); 127.90, 126.66, 113.18, 81.43, 66.27, 55.22, 54.51, 37.23, 27.97. HR-MS (ESI) *m/z*: $[2M + H]^+$ calcd for $C_{56}H_{63}N_2O_6S_2^+$: 899.41221; found: 899.41302.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7.44
7.37
7.34
7.32
7.28
7.24
7.22
6.85
6.82
3.81
3.18
3.16
2.52
2.45
1.43
MMT
S
CO2tBu
NH2
11
4.08
2.00
4.86
2.12
2.08
3.00
1.06
2.08
9.12
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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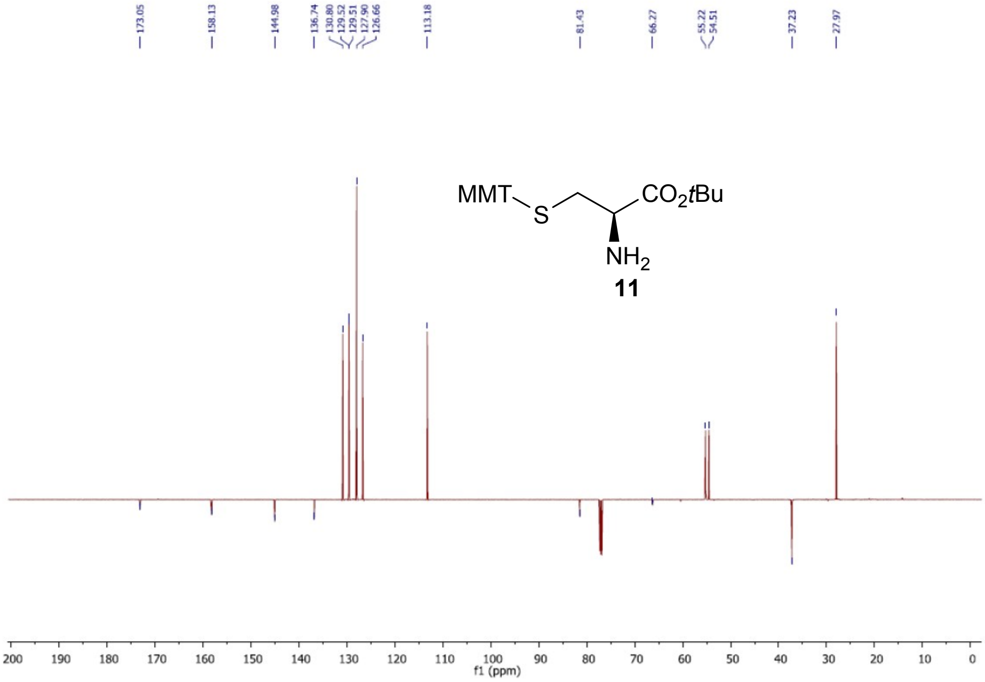
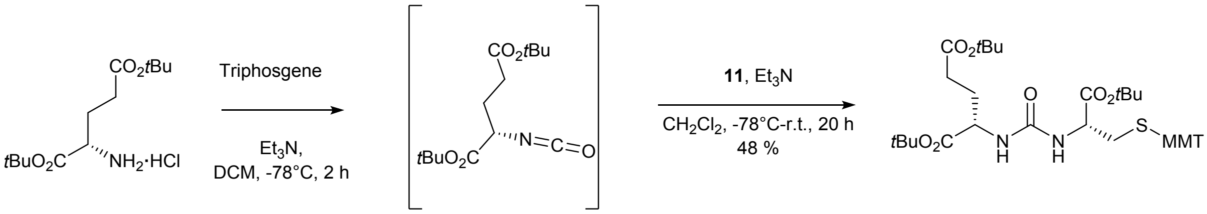
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

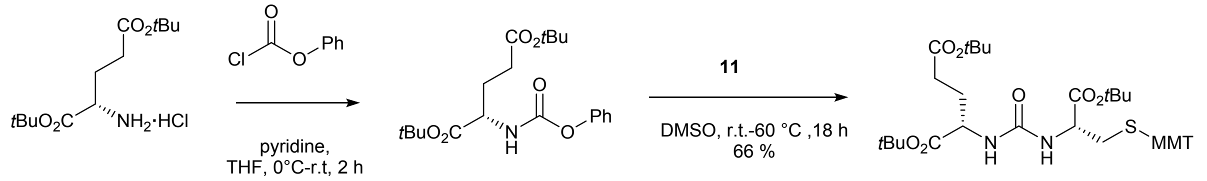

173.05
158.13
144.98
136.74
130.80
129.52
129.51
127.90
126.66
113.18
81.43
66.27
55.22
54.51
37.23
27.97
MMT
S
CO2tBu
NH2
11
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

2.7. Di-*tert*-butyl {[(*R*)-1-(*tert*-butoxy)-3-(((4-methoxyphenyl)diphenylmethyl)thio)-1-oxopropan-2-yl]carbamoyl-(*S*)-glutamic acid [Cys(MMT)-CO-Glu(O*t*Bu)$_2$] (Method A)

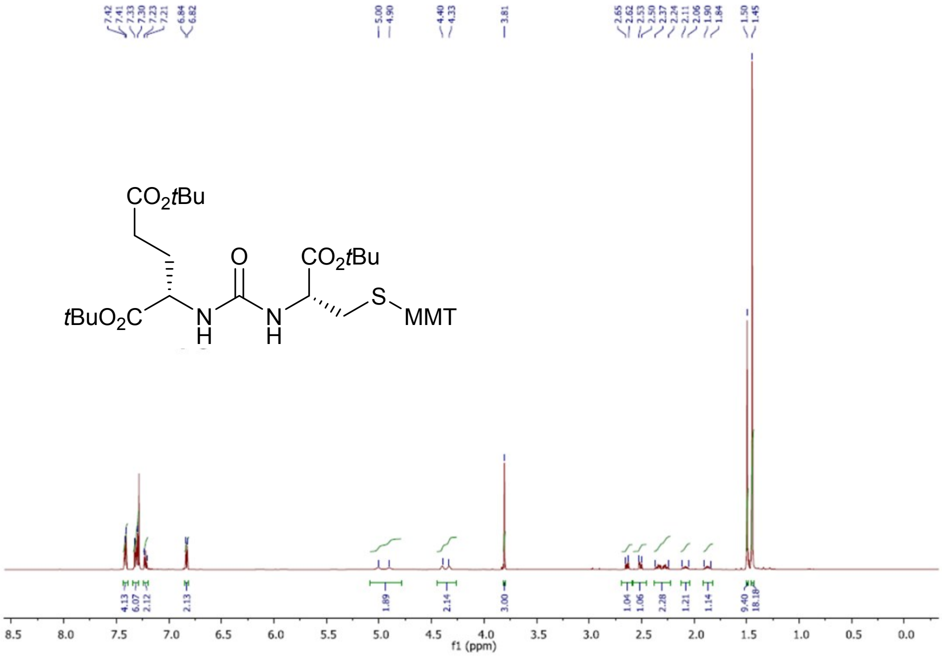


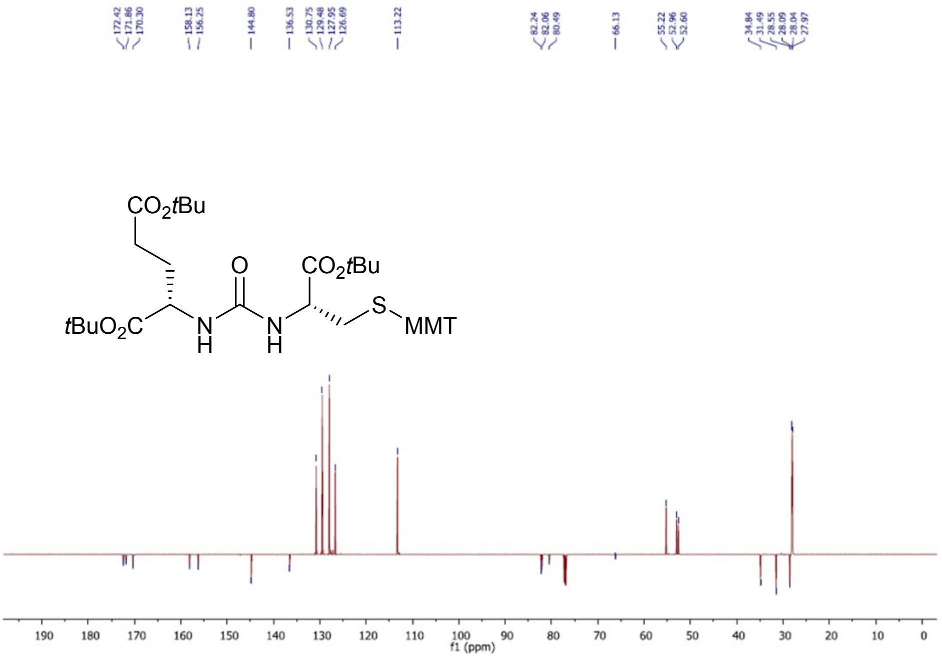
Et_3N (509 µL, 0.36 g, 3.54 mmol, 2.50 eq) was added to a solution of HCl·H-Glu(*t*Bu)$_2$ (0.42 g, 1.41 mmol, 1.00 eq) and triphosgene (0.14 g, 0.47 mmol, 0.33 eq) in anhydrous CH_2Cl_2 (10 mL) at –78 °C under Ar and the reaction mixture was stirred at the same temperature for 2 h. Thereafter, a solution of **11** (0.70 g, 1.55 mmol, 1.10 eq) and Et_3N (0.23 g, 2.33 mmol) in anhydrous CH_2Cl_2 (10 mL) was added and the mixture was allowed to warm to ambient temperature and stirred for further 16 h. The mixture was neutralized with 0.1 M HCl, the organic phase was separated and washed with H_2O (10 mL), brine (10 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography (on silica: 10 to 20% EtOAc in petroleum ether and afterwards on C_{18}-modified silica: 80% MeCN) furnishing the title compound a colorless waxy solid (0.50 g, 0.68 mmol, 48%).

2.8. Di-*tert*-butyl {[(*R*)-1-(*tert*-butoxy)-3-(((4-methoxyphenyl)diphenylmethyl)thio)-1-oxopropan-2-yl]carbamoyl-(*S*)-glutamic acid [Cys(MMT)-CO-Glu(O*t*Bu)$_2$] (Method B)

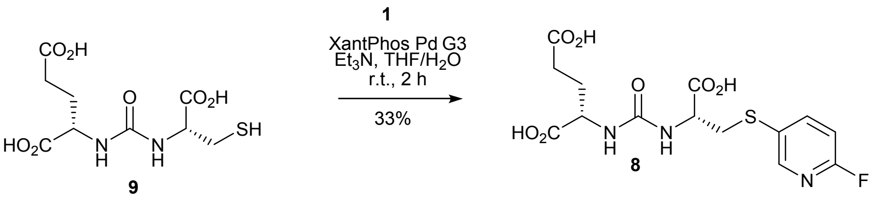


Phenyl chloroformiate (0.603 g, 3.85 mmol, 1.1 eq) was added dropwise over 5 minutes to a suspension of HCl·H-Glu(O*t*Bu)$_2$ (1.036 g, 3.50 mmol) in an ice-cold solution of pyridine (845 µL, 0.85 g, 3 eq.) in anhydrous THF (8 mL) under Ar. The reaction mixture was stirred for 5 minutes, allowed to warm up to room temperature and stirred for further 2 h (at this time point TLC analysis indicated complete conversion of the starting material). The mixture was diluted with EtOAc (60 mL), washed with 10 % citric acid (×2), saturated NaHCO3 (×2), brine (×2), dried and concentrated under reduced pressure affording the crude di-*tert*-butyl (phenoxycarbonyl)glutamate (1.215 g). A solution of **11** (1.42 g, 3.16 mmol, 1 eq.) in DMSO (5 mL) was added dropwise over 5 minutes to a solution of the latter in DMSO (5 mL) and the reaction mixture was stirred at 60 °C for 16 h and afterwards concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc/petroleum ether = 1:4) affording Cys(MMT)-CO-Glu(O*t*Bu)$_2$ (1.705 g, 66 %) as a colorless waxy oil. ^{1}H-NMR (400 MHz, CDCl$_3$): 7.42 (d, *J* = 7.7 Hz, 4H), 7.33–7.30 (m, 6H), 7.23–7.21 (m, 2H), 6.84–6.82 (m, 2H), 5.00–4.90 (br, 2H), 4.40–4.33 (m, 2H), 3.81 (s, 3H), 2.65–2.62 (m, 1H), 2.53–2.50 (m, 1H), 2.37–2.24 (m, 2H), 2.11–2.06 (m, 1H), 1.90–1.84 (m, 1H), 1.50 (s, 9H), 1.45 (2×s, 18H). ^{13}C-NMR (100 MHz, CDCl$_3$) δ (ppm) = 172.42, 171.86, 170.30, 158.13, 156.25, 144.80, 136.53, 130.75, 129.48, 127.95, 126.69, 133.22, 82.24, 82.06, 80.49, 66.13, 55.22, 52.96, 52.60, 34.84, 31.49, 28.55, 28.09, 28.04, 27.97. HR-MS (ESI) *m/z*: [M + Na]$^+$ calcd for $C_{41}H_{54}N_2O_8SNa^+$: 757.36009; found: 757.34853.

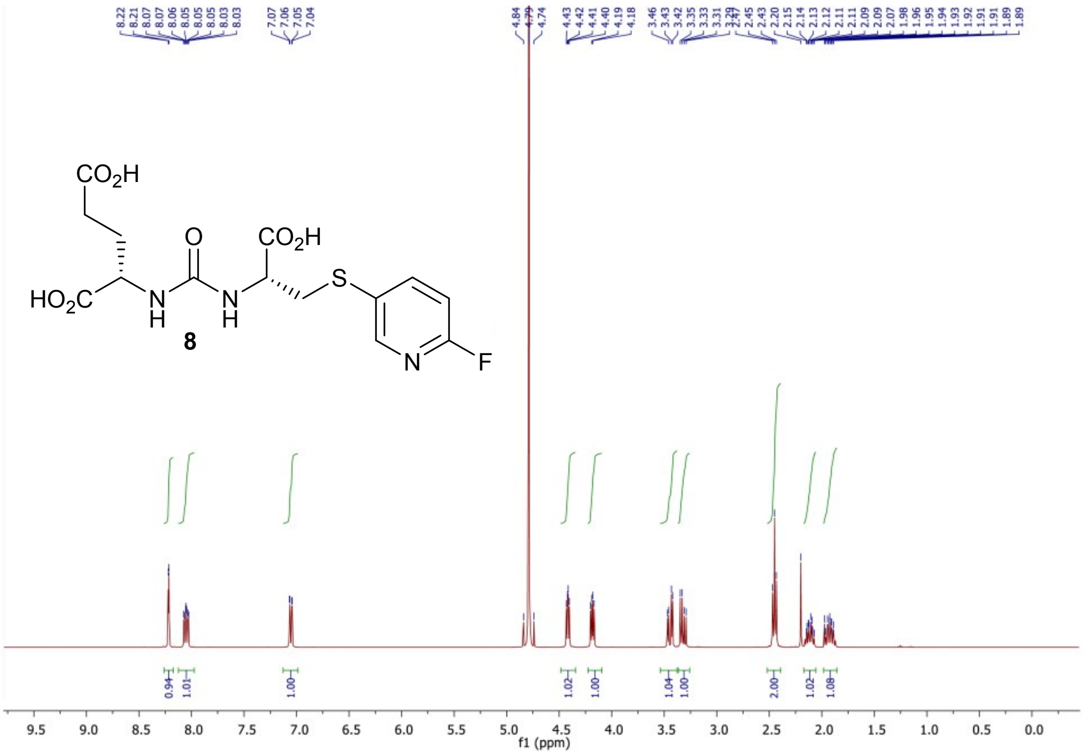




2.9 {[(*R*)-1-Carboxy-2-((6-fluoropyridin-3-yl)thio)ethyl]carbamoyl}-(*S*)-glutamic acid [Cys(FPy)-CO-Glu, 8]



Et_3N (~60 µL) was added portionwise to a solution of **9** (50 mg, 169 µmol) and XantPhos Pd G3 (1.6 mg, 0.169µmol, 1 mol%) in degassed 50% THF (1 mL) under Ar until slightly alkaline pH. 5-Iodo-2-fluoropyridine **5** (42 mg, 188 µmol, 1.1 eq) was added and the reaction mixture was stirred for 2 h. Volatiles were removed under reduced pressure and the residue was taken up in 15% MeCN and then acidified with TFA. The resulting suspension was sonicated and purified by semipreparative HPLC (column: Synergy Hydro-RP, 250 x 10 mm, 10 µm/100 Å; eluent: 15% MeCN (0.1% TFA); flow rate: 7.4 mL/min; t_R=10.3–12.0 min). The product containing fraction was concentrated under reduced pressure and the oily residue was diluted with H_2O (0.5 mL). Lyophilisation gave the title compound as a white, hygroscopic powder (22 mg, 33%). ^1H NMR (400 MHz, D_2O): 8.22 (d, *J* = 2.6 Hz, 1H), 8.05 (ddd, *J* = 8.6 Hz, *J* = 7.4 Hz, *J* = 2.6 Hz, 1H), 7.05 (dd, *J* = 8.6 Hz, *J* = 1.7 Hz, 1H), 4.42 (dd, *J* = 7.3 Hz, *J* = 4.5 Hz, 1H), 4.18 (dd, *J* = 8.8 Hz, *J* = 5.2 Hz, 1H), 3.44 (dd, *J* = 14.6 Hz, *J* = 4.5 Hz, 1H), 3.32 (dd, *J* = 14.6 Hz, *J* = 7.3 Hz, 1H), 2.45 (t, *J* = 7.3 Hz, 2H), 2.17 – 2.05 (m, 1H), 2.00 – 1.86 (m, 1H). ^{13}C NMR (101 MHz, D_2O): 177.15, 175.81, 174.17, 162.79 (d, *J* = 241.3 Hz), 158.44, 149.52 (d, *J* = 13.3 Hz), 146.31 (d, *J* = 8.9 Hz), 128.61 (d, *J* = 4.4 Hz), 110.63 (d, *J* = 36.5 Hz), 53.05, 52.35, 36.48, 29.86, 26.38. ^{19}F NMR (376 MHz, D_2O): – 72.12. HR-MS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{17}FN_3O_7S^+$: 390.07657; found: 390.07673.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4.19
4.18
3.46
3.43
3.42
3.35
3.33
3.31
3.29
2.47
2.45
2.43
2.20
2.15
2.14
2.13
2.12
2.11
2.11
2.09
2.09
2.07
1.98
1.96
1.95
1.94
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1.92
1.91
1.91
1.89
1.89
CO2H
O
CO2H
S
HO2C
N
H
N
H
N
F
8
0.94
1.01
1.00
1.02
1.00
1.04
1.00
2.00
1.02
1.08
9.5
9.0
8.5
8.0
7.5
7.0
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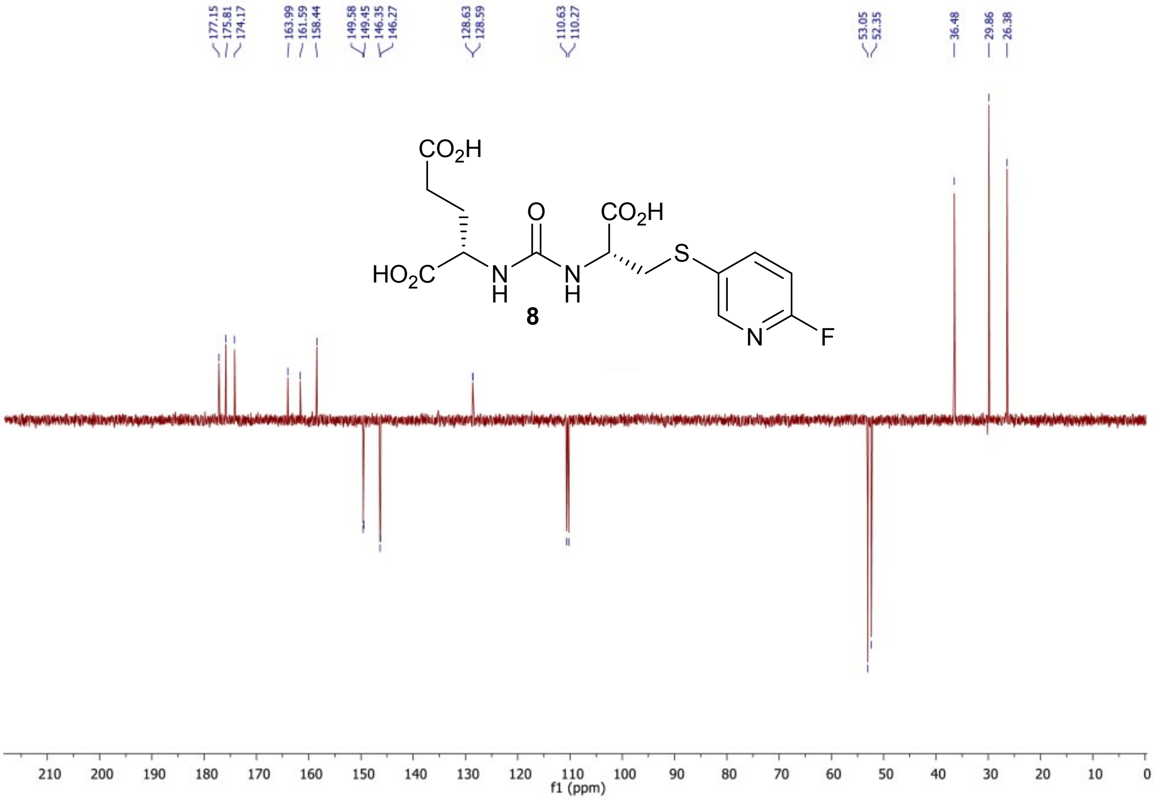
f1 (ppm)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149.45
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110.63
110.27
53.05
52.35
36.48
29.86
26.38
CO2H
O
CO2H
S
HO2C
N
H
N
H
N
F
8
210
200
190
180
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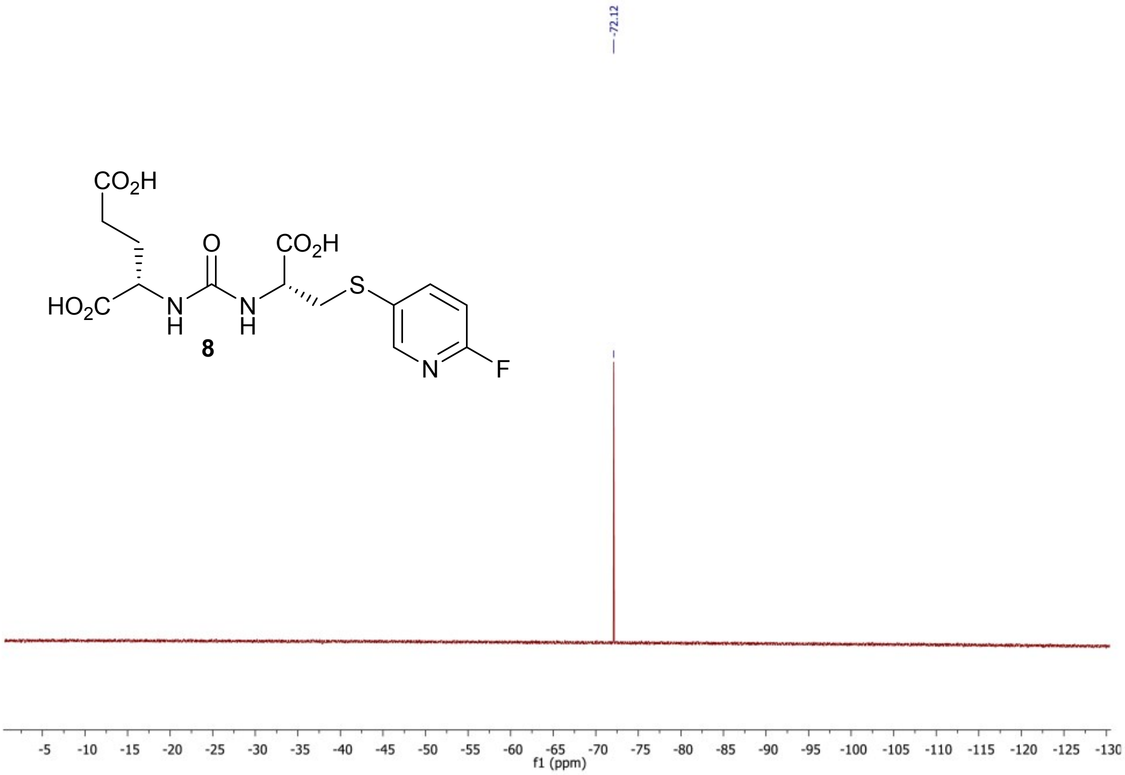
0
f1 (ppm)


-72.12
CO2H
O
CO2H
HO2C
N
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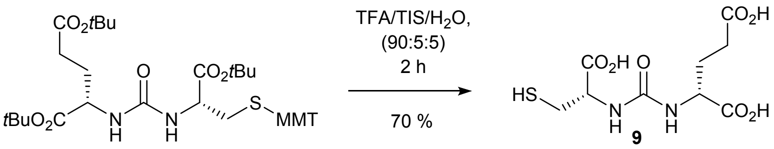
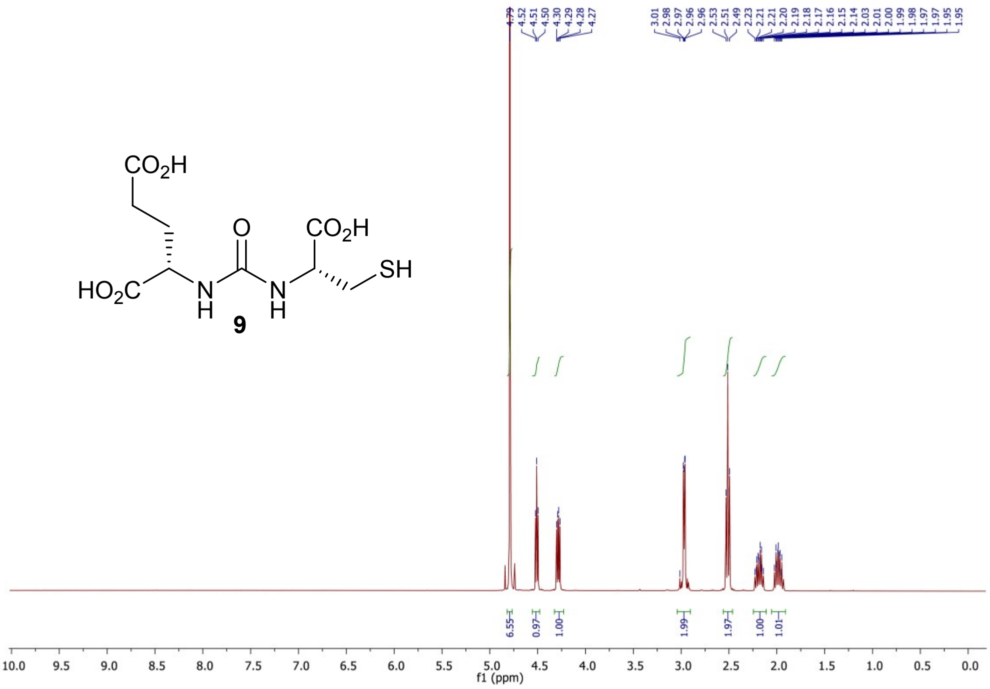
N
H
S
N
F
-5 -10 -15 -20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115 -120 -125 -130
f1 (ppm)

8

2.10. {[(*R*)-1-Carboxy-2-mercaptoethyl]carbamoyl}-(*S*)-glutamic acid (Cys-CO-Glu, 9)



Cys(MMT)-CO-Glu(O*t*Bu)$_2$ (0.805 g, 1.10 mmol) was added in portions over 5 min to a vigorously stirred TFA/TIS/H_2O mixture (90:5:5, 130 mL) and the resulting clear solution was stirred vigorously at ambient temperature for 2 h. The mixture was concentrated under reduced pressure to a few mL and the raw product precipitated by addition of *n*-hexane and sonication. The supernatant was removed by decantation and discarded. The solid residue was dissolved in H_2O (0.1% TFA) and purified by C18 flash chromatography [(5% MeCN, (0.1% TFA)]. The product fraction was then concentrated under reduced pressure and the resulting clear oil diluted with H_2O (2 mL) and lyophilized to afford the title compound as a white, hygroscopic powder (0.223 g, 70%). ^{1}H-NMR (400 MHz, D_2O): 4.51 (t, *J* = 5.3 Hz, 1H), 4.28 (dd, *J* = 9.1 Hz, *J* = 5.3 Hz, 1H), 2.98 (m, 2H), 2.51 (t, *J* = 7.3 Hz, 2H), 2.19 (m, 1H), 1.98 (m, 1H). ^{13}C-NMR (101 MHz, D_2O): 177.21, 176.15, 174.43, 159.04, 54.84, 52.50, 29.99, 26.18, 25.80. HR-MS (ESI) *m/z*: $[M + H]^+$ calcd for $C_9H_{15}N_2O_7S^+$: 295.05945; found: 295.05961.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2.49
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2.18
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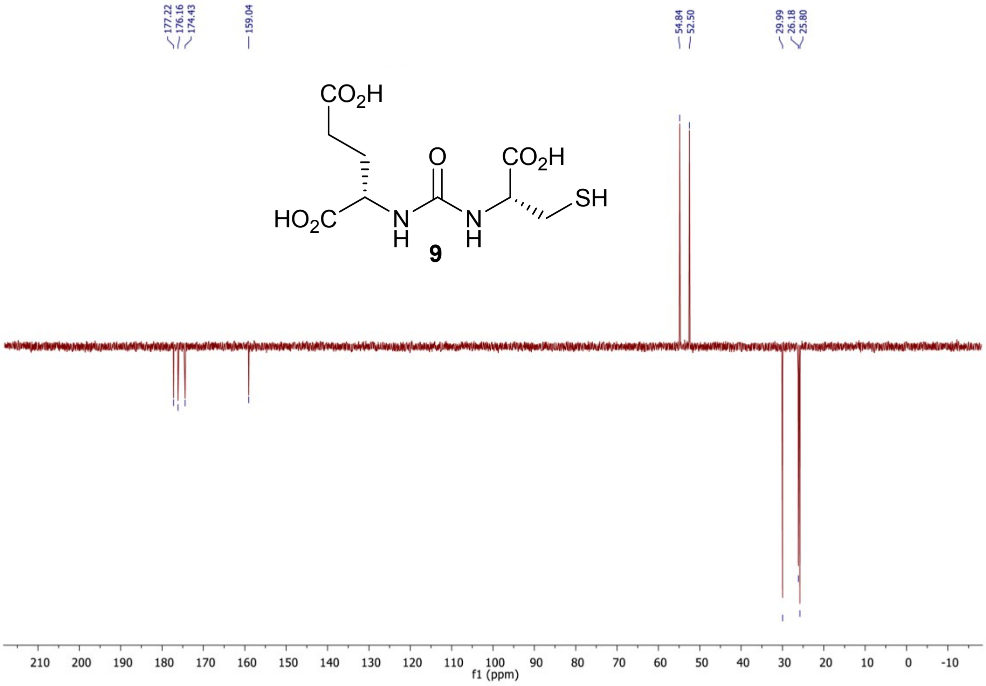
2.14
2.03
2.01
2.00
1.99
1.98
1.97
1.95
1.95
CO2H
O
CO2H
SH
HO2C
N
H
N
H
9
6.55
0.97
1.00
1.99
1.97
1.00
1.01
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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CO2H
O
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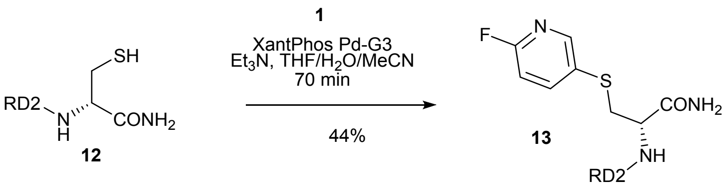
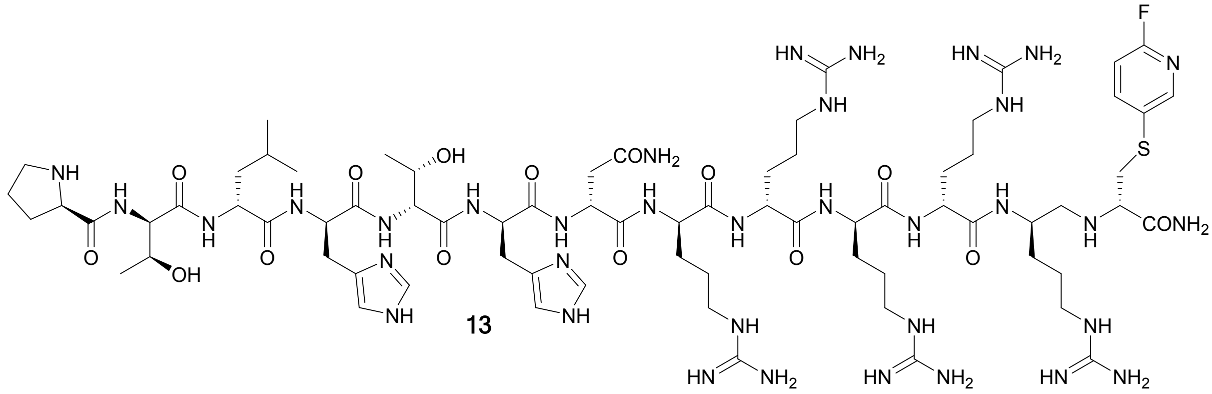
SH
HO2C
N
H
N
H
9
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

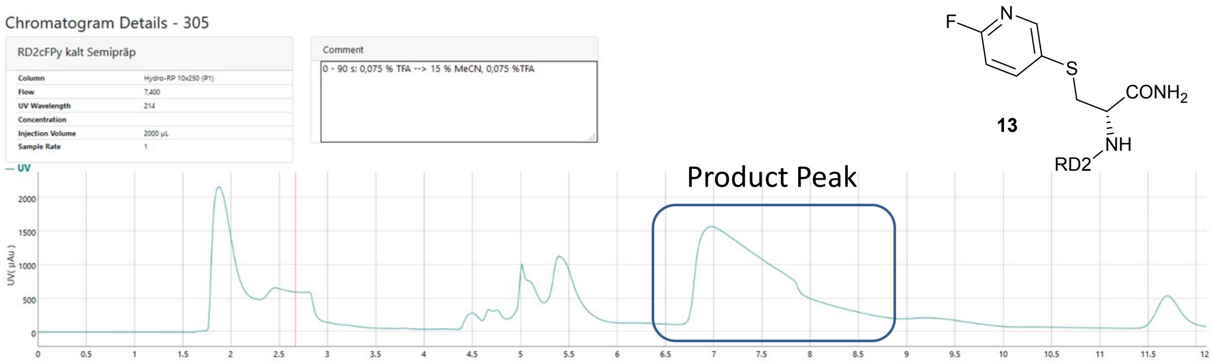
2.11. H-ptlhthnrrrrrc(2-fluoropyridin-5-yl)-NH$_2$ (13)





13

A solution of Et_3N (6 µL, 4.4 mg, 43.05 µmol) and H-RD2c-NH$_2$ (5 mg, 2.78 µmol) in degassed H_2O (100 µL) was added to a solution of Xantphos Pd-G3 (1 mg, 1.05 µmol) in degassed THF (100 µL). Degassed MeCN (100 µL) was added the biphasic reaction mixture and the resulting clear solution was stirred until HPLC indicated complete consumption of the H-RD2c-NH$_2$ (~70 min). The mixture was concentrated under reduced pressure, diluted with 0.5% TFA and purified by semipreparative HPLC (column: Synergy Hydro-RP, 250 x 10 mm, 10 µm/100 Å; gradient: 0–1.5 min: H_2O (0.075% TFA), 1.5–15 min: 15% MeCN (0.075% TFA); flow rate: 7.4 mL/min). The product fraction was concentrated under reduced pressure, dissolved in H_2O (600 µL) and lyophilized to afford the title compound as a colorless solid (2.3 mg, 44% based on RD2·5 TFA salt). MS (MALDI) *m/z*: $[M + H]^+$ calcd for $C_{73}H_{124}N_{35}O_{16}SF^+$: 1796.9586; found: 1796.9505.



Chromatogram Details - 305

RD2cFPy kalt Semipräp

Column	Hydro-RP 10x250 (P1)
Flow	7,400
UV Wavelength	214
Concentration	
Injection Volume	2000 µL
Sample Rate	1

Comment

0 - 90 s: 0,075 % TFA --> 15 % MeCN, 0,075 %TFA

Fig. S1. Semipreparative HPLC purification of **13**.

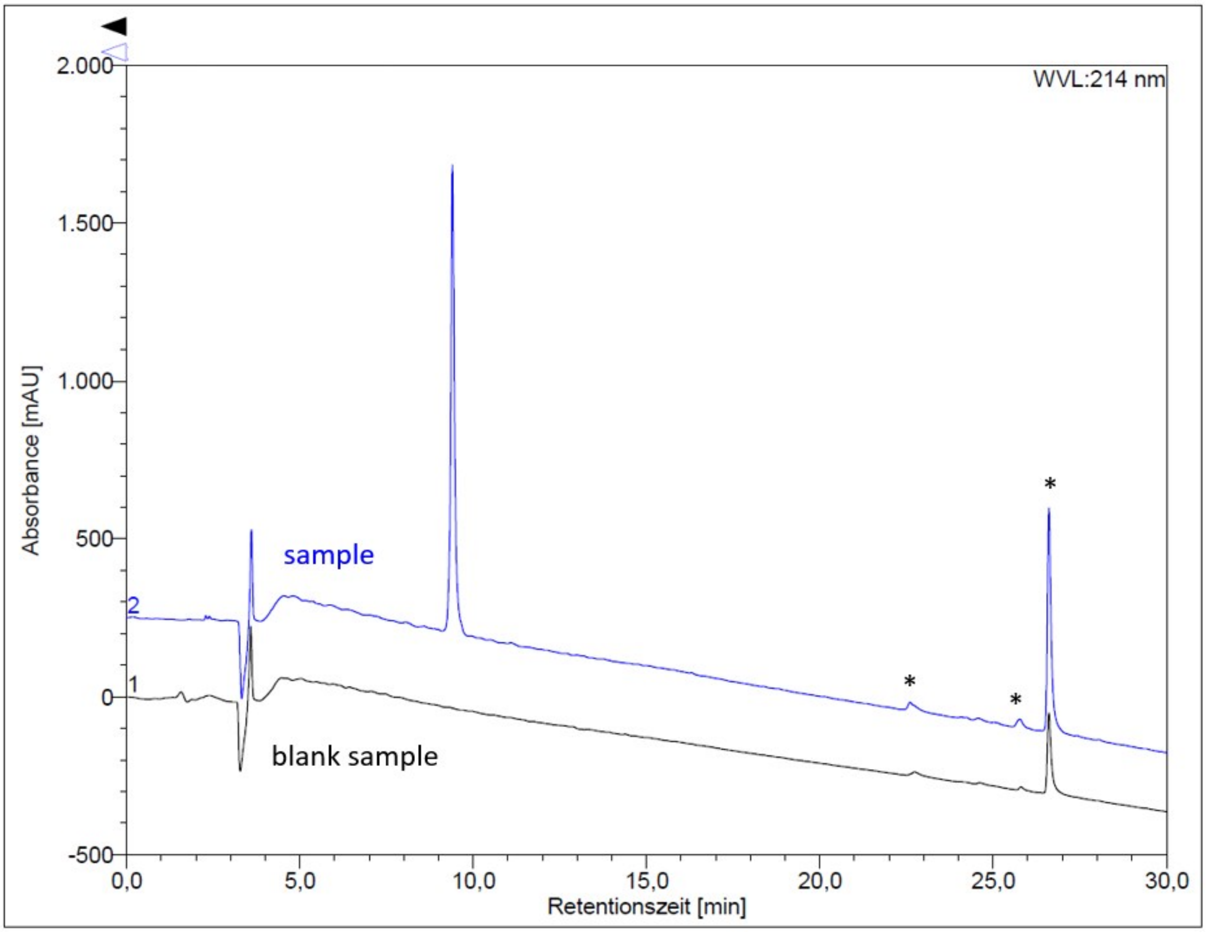


Fig. S2. HPLC trace of the purified **13**. Conditions: column: Synergy Hydro RP 4µ, 250 × 4.6 mm (Phenomenex); gradient: 0 min: 5% MeCN (0.1 % TFA), 30 min: 100 % MeCN (0.1 % TFA); flow rate: 1 mL/min; detection: UV: λ=214 nm. Blue trace: purified **13**, black trace: blank sample, *: eluent ghost peaks.

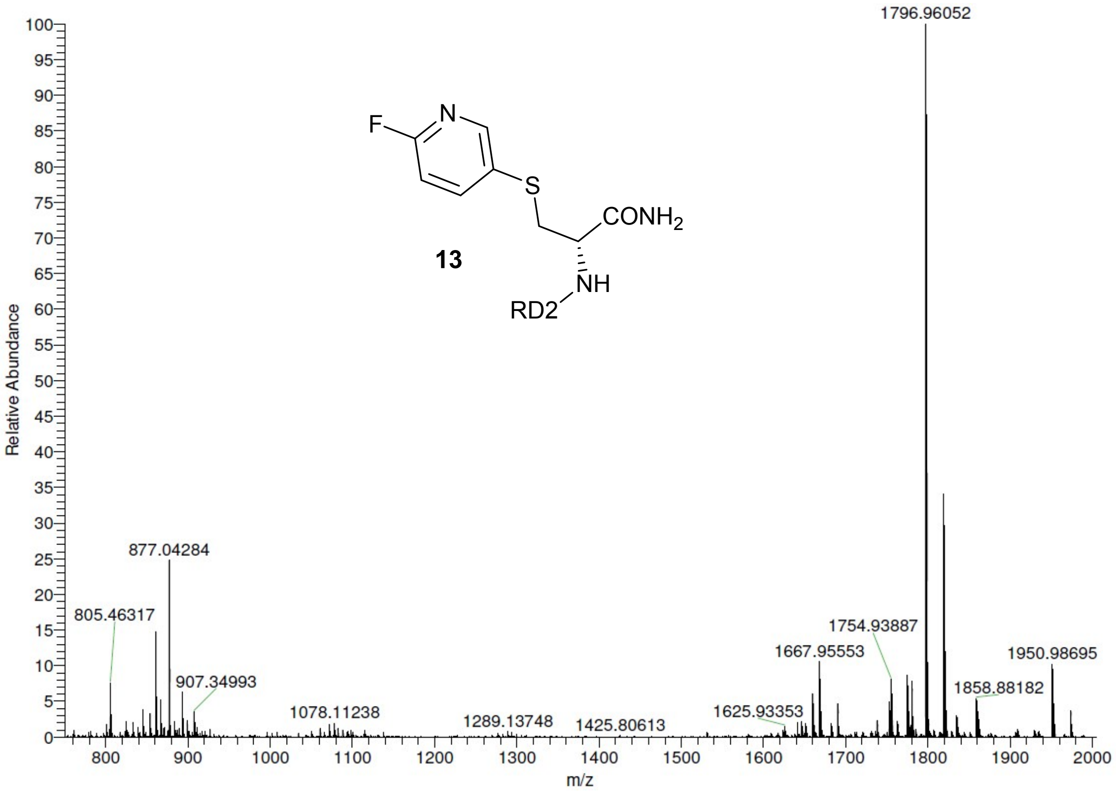
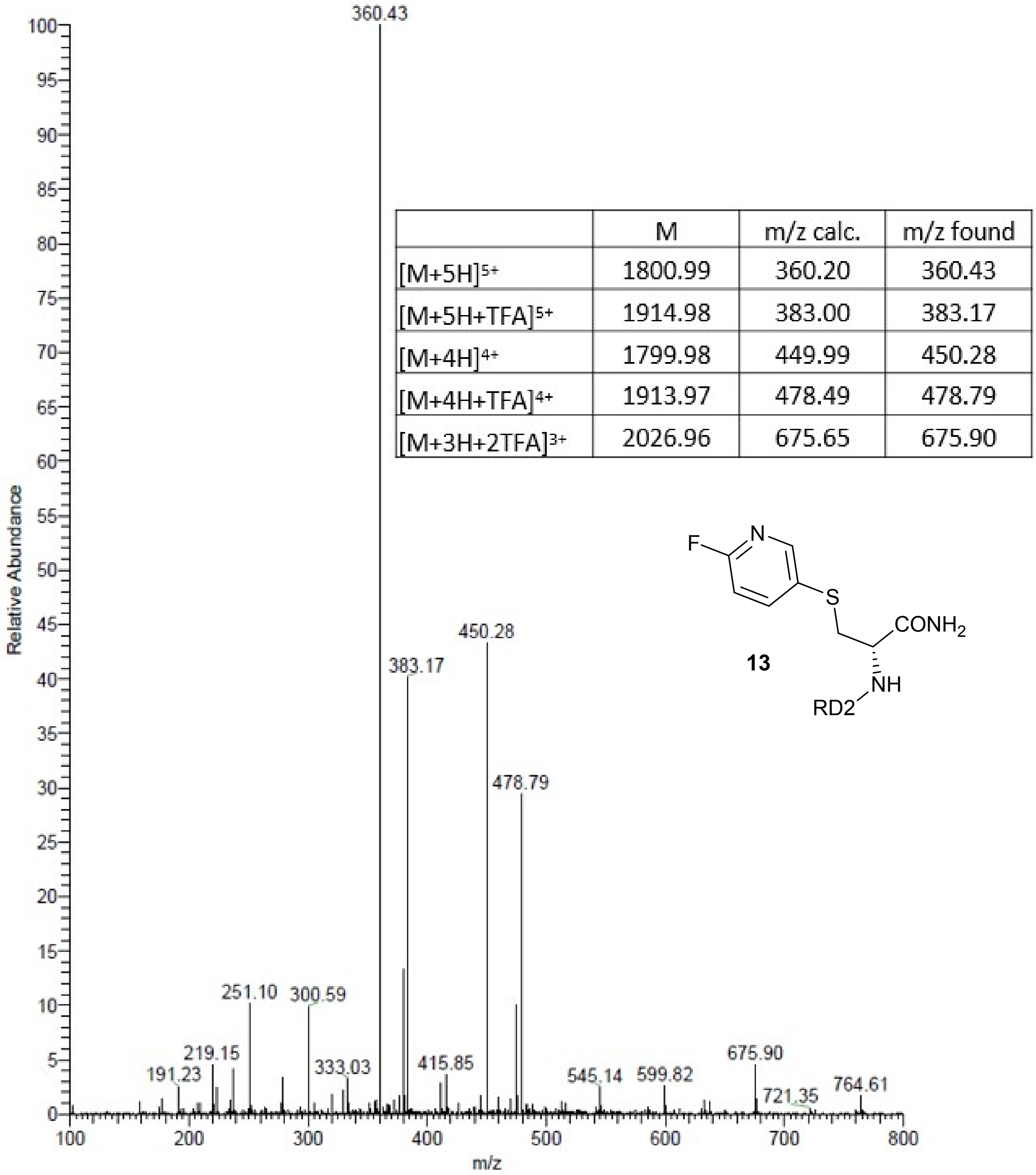


Fig. S3. MALDI-MS spectrum of **13**.

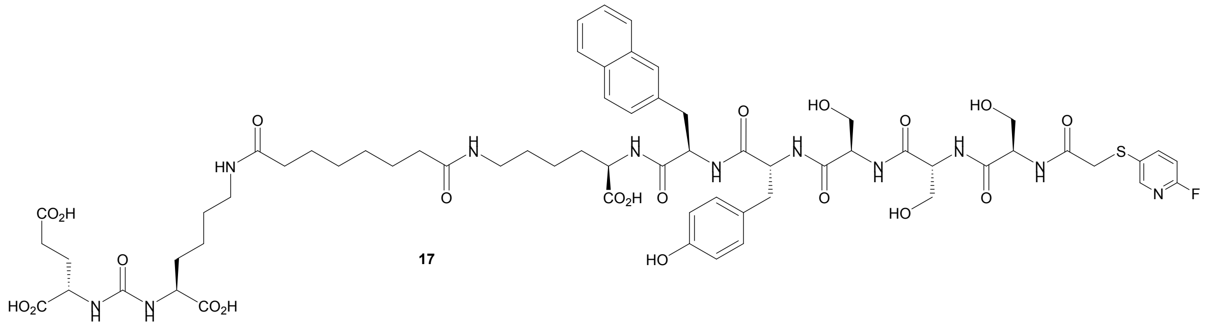


	M	m/z calc.	m/z found
$[M+5H]^{5+}$	1800.99	360.20	360.43
$[M+5H+TFA]^{5+}$	1914.98	383.00	383.17
$[M+4H]^{4+}$	1799.98	449.99	450.28
$[M+4H+TFA]^{4+}$	1913.97	478.49	478.79
$[M+3H+2TFA]^{3+}$	2026.96	675.65	675.90

13

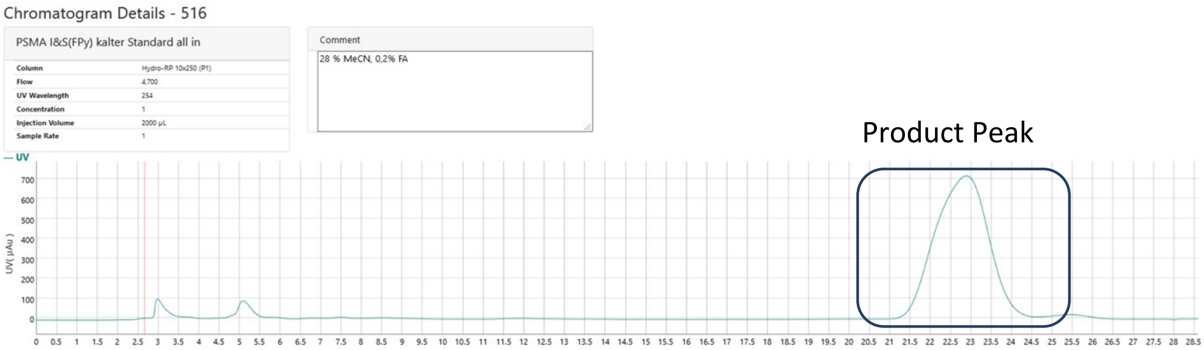
Fig. S4. ESI-MS spectrum of **13**.

2.12 PSMA I&S(FPy) (17)



17

2-Fluoro-5-iodopyridine (**1**) (38 µL of the 10 mg/mL stock solution in MeCN, 2 eq.), XantPhos Pd G3 (8 µL of the 20 mg/mL in MeCN, 0.1 eq.) and Et_3N (1.1 µL, 0.8 mg, 7.9 µmol, 5 eq.) were subsequently added to a solution of PSMA I&S (**16**) (2.2 mg, 1.7 µmol) in degassed 40% MeCN (150 µL) under Ar. The reaction mixture was stirred for 2 h and diluted with 2% HCO_2H (1 mL) and the desired product was isolated by semipreparative HPLC [Synergy Hydro RP, 10µ, 10 x 250 mm; solvent: 28% MeCN (0.2% HCO_2H); flow rate: 4.7 mL/min, detection: UV: λ=254 nm]. The product fraction (21.5–24.2 min) was collected and lyophilized furnishing **17** as hydroscopic colorless foam (0.5 mg, 22%). HRMS (TOF) m/z $[M + H]^+$ calcd for $C_{64}H_{85}FN_{11}O_{21}S^+$: 1394.5621; found: 1394.5637.


Chromatogram Details - 516

PSMA I&S(FPy) kalter Standard all in

Column	Hydro-RP 10x250 (P1)
Flow	4,700
UV Wavelength	254
Concentration	1
Injection Volume	2000 µL
Sample Rate	1

Comment

28 % MeCN, 0,2% FA

Fig. S5. Semipreparative HPLC purification of **17**.

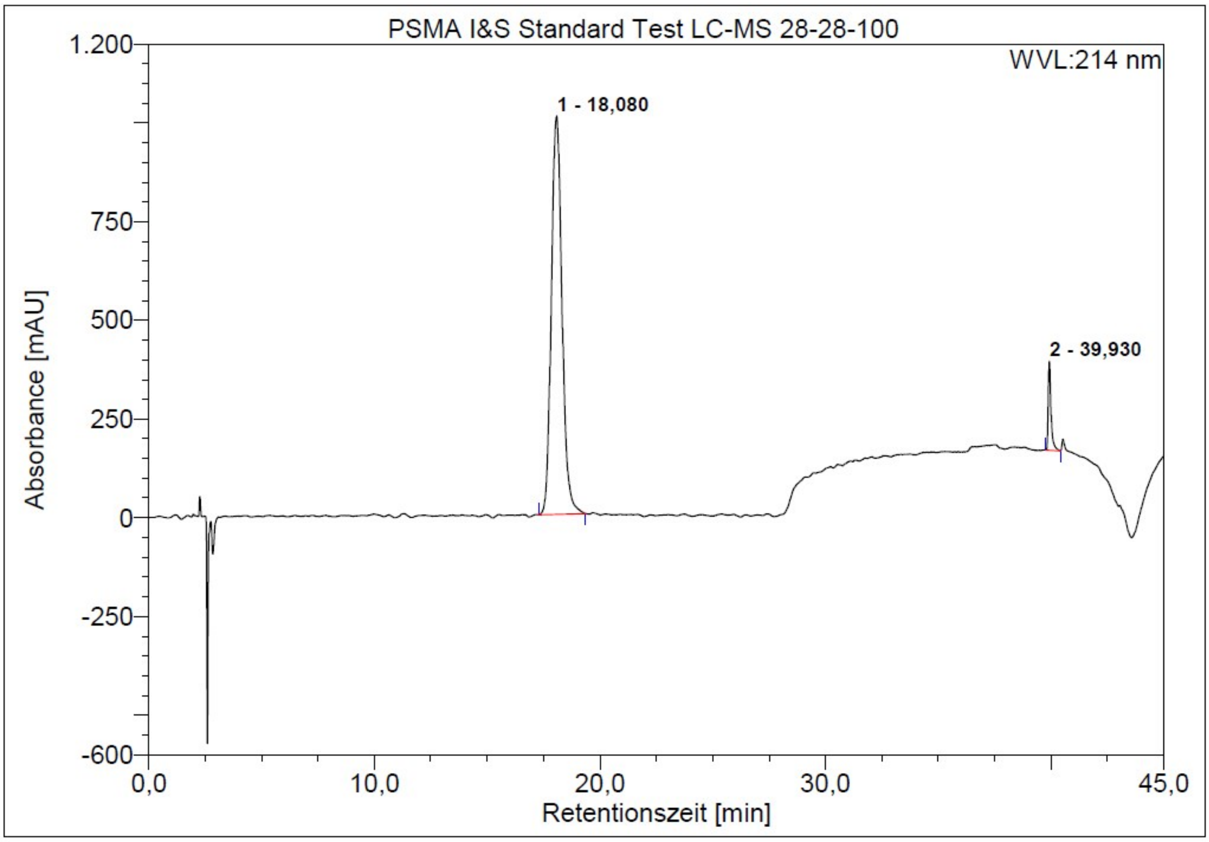
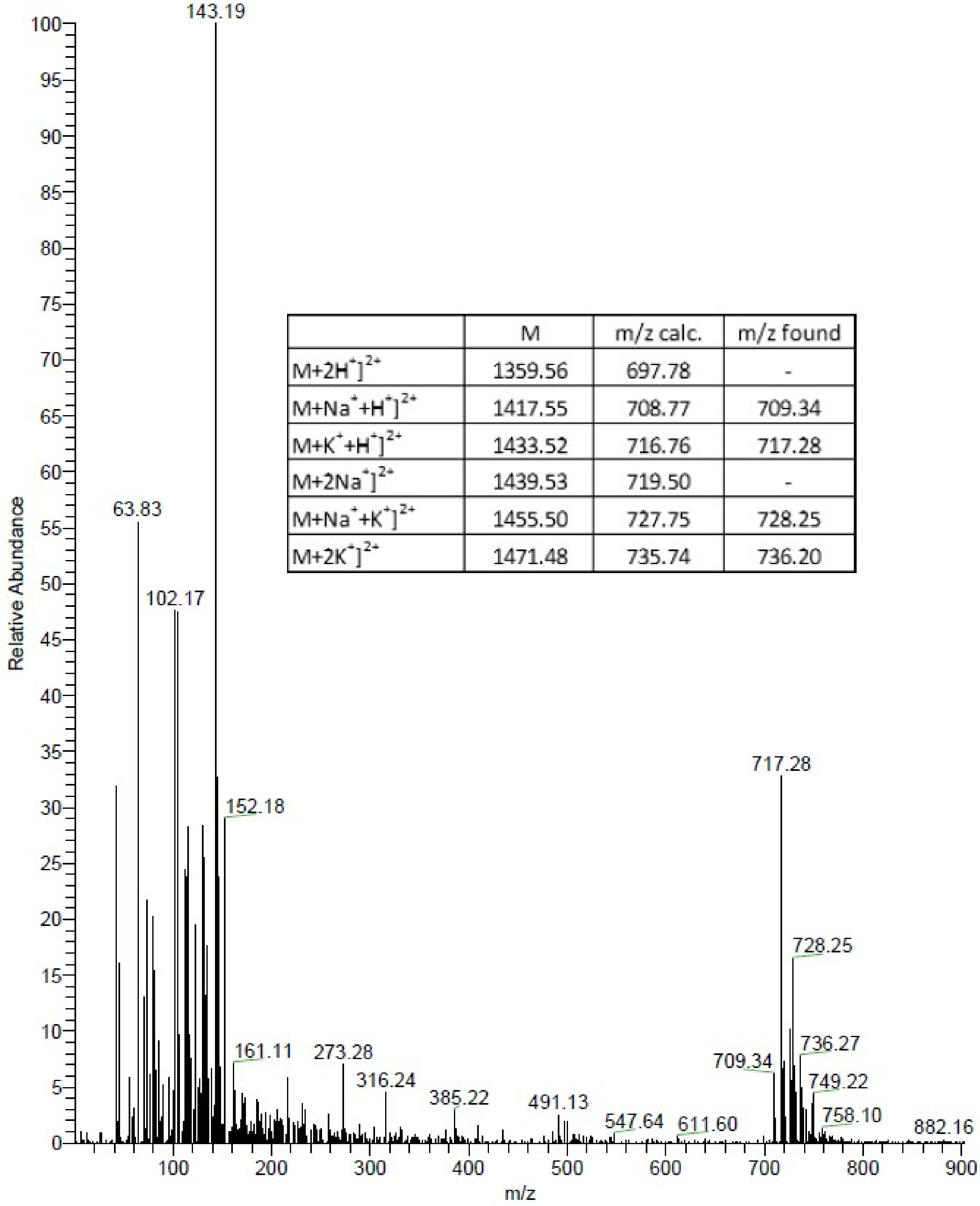


Fig. S6. HPLC trace of the purified **17**. Conditions: column: Synergy Hydro RP 4µ, 250 × 4.6 mm; gradient: 0–25 min: 28% MeCN (0.2% HCO_2H), 25–40 min: 28–100% MeCN (0.2% HCO_2H), 40–45 min: 100% MeCN (0.2% TFA); flow rate: 1 mL/min; detection: UV: λ=214 nm.



	M	m/z calc.	m/z found
$M+2H^{+}]^{2+}$	1359.56	697.78	-
$M+Na^{+}+H^{+}]^{2+}$	1417.55	708.77	709.34
$M+K^{+}+H^{+}]^{2+}$	1433.52	716.76	717.28
$M+2Na^{+}]^{2+}$	1439.53	719.50	-
$M+Na^{+}+K^{+}]^{2+}$	1455.50	727.75	728.25
$M+2K^{+}]^{2+}$	1471.48	735.74	736.20

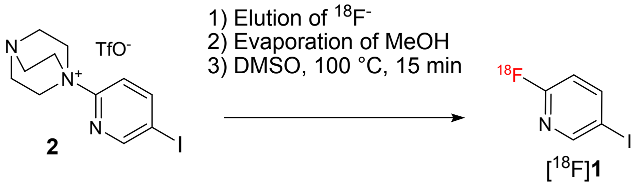
Fig. S7. ESI-MS spectrum of **17**.

3. Radiochemistry

[^{18}F]Fluoride was produced by the $^{18}O(p,n)^{18}F$ reaction by bombardment of enriched [^{18}O] water with 17 MeV protons at the BC1710 cyclotron (The Japan Steel Works, Tokyo, Japan) of the INM-5 (Forschungszentrum Jülich). Radioactivity was measured using a CRC-55tR Dose Calibrator from Capintec, Inc. (Florham Park, Netherlands). The following cartridges were used for radiosyntheses and solid phase extractions: Sep-Pak Plus C18 cartridges (130 mg sorbent, Part No WAT023501, preconditioned with 10 mL EtOH and 30 mL H2O), Sep-Pak Accell Plus QMA carbonate cartridges (130 mg sorbent, Part No 186004051; preconditioned with 1 mL H2O) and Oasis HLB Plus cartridges (225 mg sorbent, Part No 186000132; preconditioned with 5 mL EtOH and 20 mL H2O) from Waters GmbH (Eschborn, Germany) and Strata-X RP cartridges (30 mg sorbent, Part No 8B-S100-TAK; preconditioned with 1 mL EtOH and 20 mL H2O) from Phenomenex (Aschaffenburg, Germany). Automated radiosyntheses were performed in a home-made synthesis module using FKM valves (Christian Bürkert GmbH&Co. KG, Ingelfingen, Germany). All connections between the valves were made by means of PTFE tubes and PEEK fittings. Helium (Westfalen AG, Muenster, Germany) was used as operating gas.

The identity of radiolabeled products was confirmed by co-injection of the non-radioactive reference compounds. Radiochemical yields corrected for decay to the start of synthesis are provided for analytically pure radiolabeled compounds purified by HPLC ([^{18}F]**1**, [^{18}F]**5**, [^{18}F]**7**, [^{18}F]**8**, [^{18}F]**13**, [^{18}F]**17**), solid phase extraction (SPE) ([^{18}F]**1**) and gel permeation chromatography ([^{18}F]**14**). In an optimization study (described in Chapter 3.2.) RCYs of the *S*-arylation step were determined by HPLC. According to the “consensus nomenclature rules for radiopharmaceutical rules”[5] radiochemical yields determined by HPLC analysis after dilution of reaction mixtures with H_2O are referred as HRCY (HPLC RCY). In these cases, the radioactivity adsorbed on reactor walls was determined by the comparison of the total activity in the reaction vessel before and after removal of the complete reaction mixture (generally ≤ 5%). HRCY were determined by comparing of the decay corrected product peak area in chromatogram to that obtained by injection bypassing the column (corresponds to the total activity in the sample volume).

3.1. 5-Iodo-2-[^{18}F]fluoropyridine ([^{18}F]1)



Aqueous [^{18}F]fluoride (30–500 MBq) was loaded onto an anion-exchange resin (QMA cartridge) from the male side, the resin was washed in the same direction with MeOH (1.0–1.5 mL). Afterwards [^{18}F]F$^-$ was eluted into the reaction vial with a solution of the onium radiolabeling precursor salt **2** (3 mg, 6.4 µmol) in MeOH (0.5 mL) from the female side. MeOH was evaporated in the flow of Ar at 60 °C under reduced pressure (250 mbar) for 5 min, anhydrous DMSO (0.5 mL) was added and the reaction mixture was heated at 100 °C for 15 min. After cooling down, the mixture was diluted with H_2O and the crude [^{18}F]**1** was purified by SPE or HPLC as described below.

*3.1.1. SPE purification of [^{18}F]**1***

The reaction mixture was diluted with H_2O (2–3 mL), drawn up into a 20 mL single-use syringe containing H_2O (15 mL) and the resulting solution was loaded onto a pre-conditioned Strata-X RP cartridge. The cartridge was washed with 10 % MeCN (1 mL), dried in the flow of argon and [^{18}F]**1** was eluted with 200–500 µL of the organic solvent used for the subsequent conjugation reaction. The synthesis time including purification is ca. 25 min.

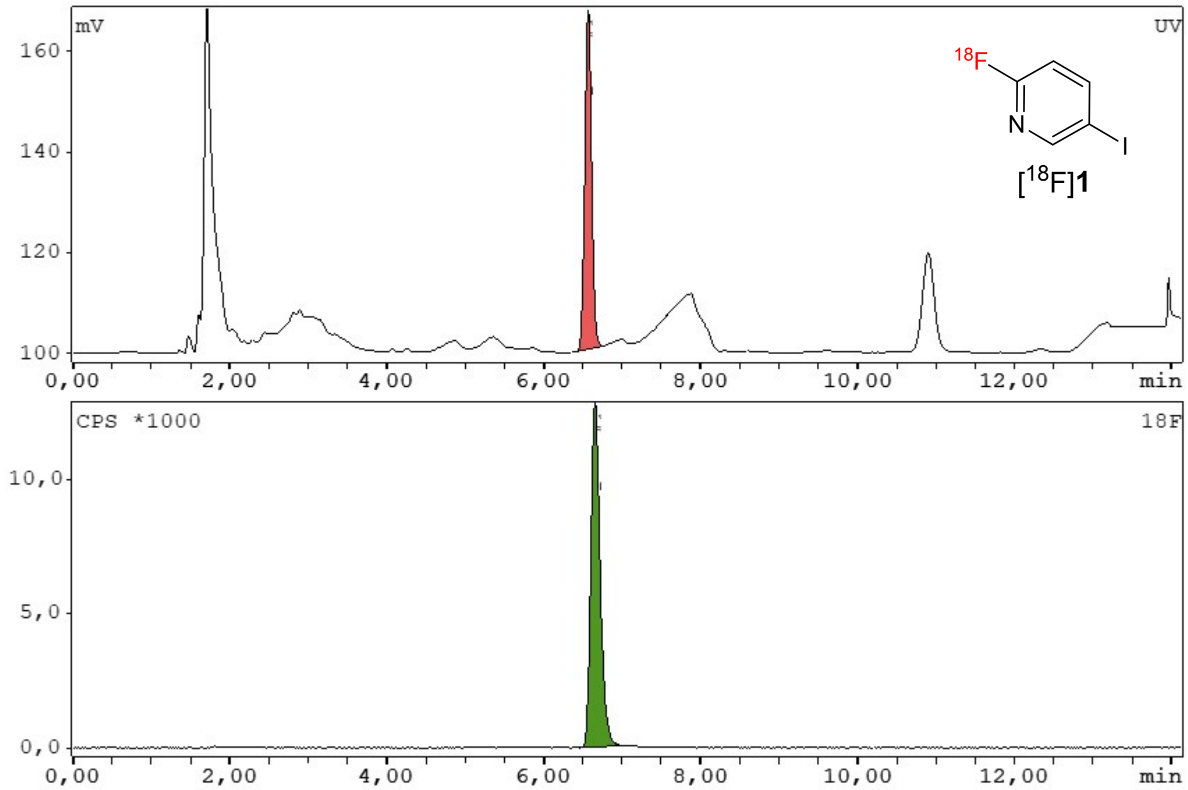


Fig. S8. HPLC trace of the SPE-purified [^{18}F]**1**, coinjected with the reference compound. HPLC conditions: column: Aeris Peptide XB C18 5µm, 4.6 × 250 mm; eluent: 40% MeCN; flow rate: 1.5 mL/min; detection: γ and UV: λ=254 nm.

*3.1.1.1. Determination of nonradioactive impurities in the SPE purified [^{18}F]**1***

For the identification of non-radioactive side products remaining in the solution of [^{18}F]**1** after SPE purification, the procedure described in 3.1. was performed without the addition of [^{18}F]fluoride. The reaction mixture was purified by SPE and analyzed by LR ESI-MS (Fig. S9). Additionally, two main impurities were isolated by HPLC and analyzed by LR ESI-MS (Fig. S10).

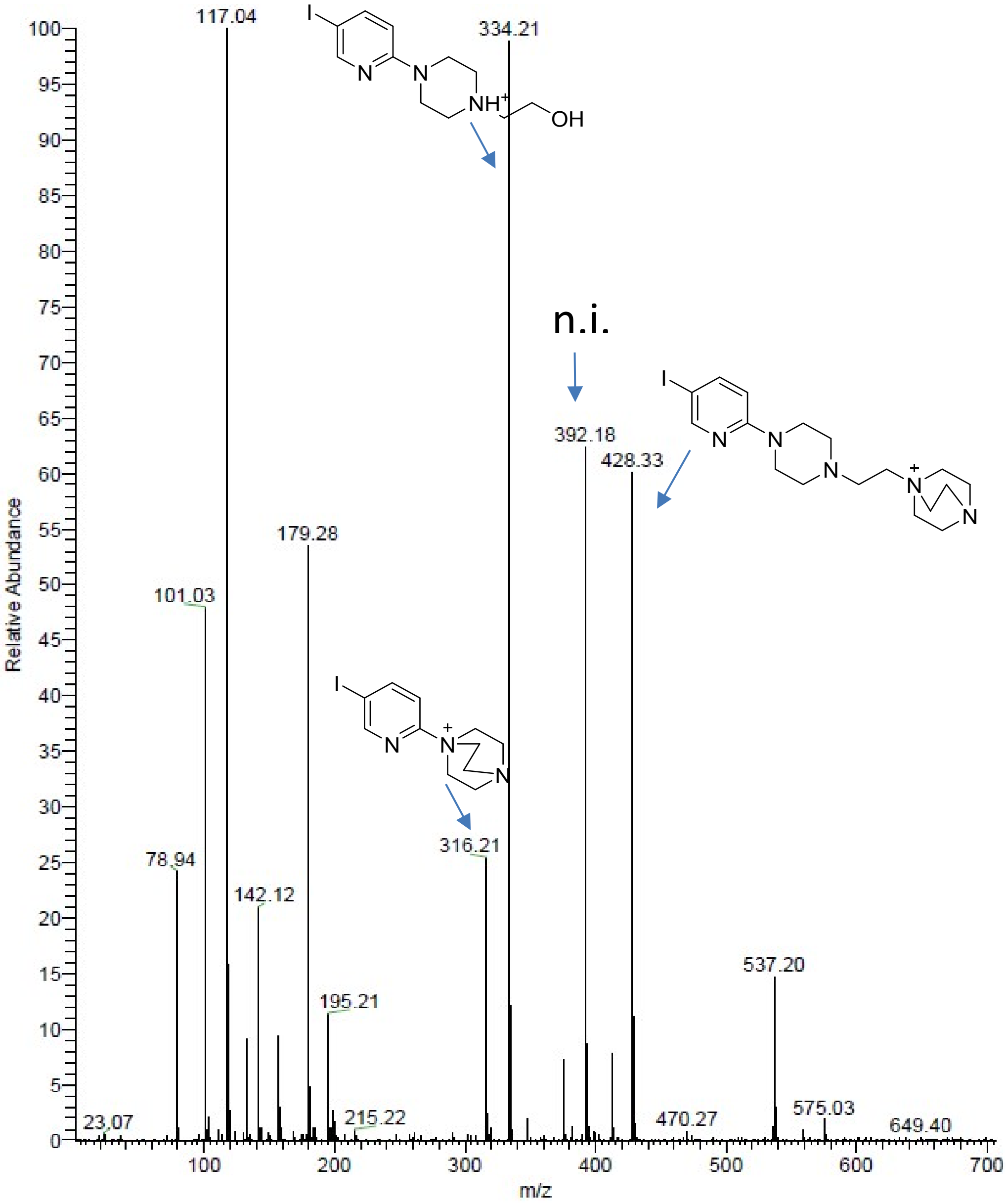


Fig. S9. LR ESI-MS spectrum of the SPE purified reaction mixture. Presumable contaminants are shown. Note that the detection sensitivity is much higher for ionic than for non-ionic compounds. n. i.: not identified.

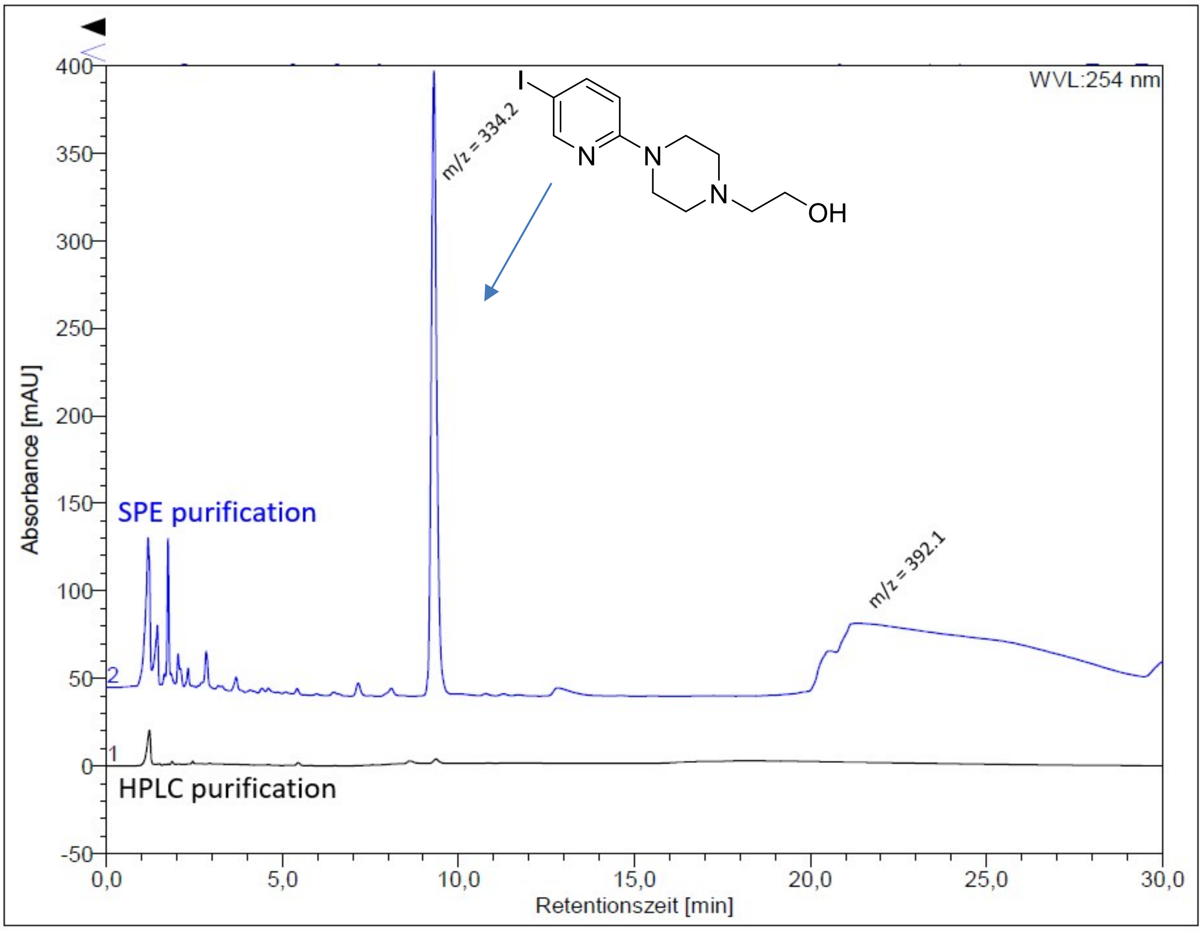


Fig. S10. UV-HPLC traces of the SPE-purified (blue trace) and HPLC-purified $[^{18}\text{F}]$**1** (black trace). For both radiosyntheses 4 mg of precursor **2** were used. HPLC conditions: column: Synergy Hydro RP 4µ, 250 × 4.6 mm; eluent: 50% MeCN; flow rate: 1.5 mL/min; detection: UV: λ=254 nm. Peaks for which m/z values are shown were isolated in a separate experiment and characterized after 24 h incubation (approx.. 10 half-lives of ^{18}F) in the fridge by LR ESI-MS.

3.1.2. HPLC purification of $[^{18}\text{F}]$***1***

The reaction mixture was diluted with H_2O (1.2 mL) and $[^{18}\text{F}]$**1** was isolated by semipreparative HPLC [column: Gemini C18, 250 × 10 mm, 5 µm/100 Å; eluent: 50% MeCN; flow rate: 7.4 mL/min]. The product fraction was diluted with 30 mL H_2O and $[^{18}\text{F}]$**1** was formulated using SPE as described in 3.1.1.. The synthesis time including purification was about 35 min.

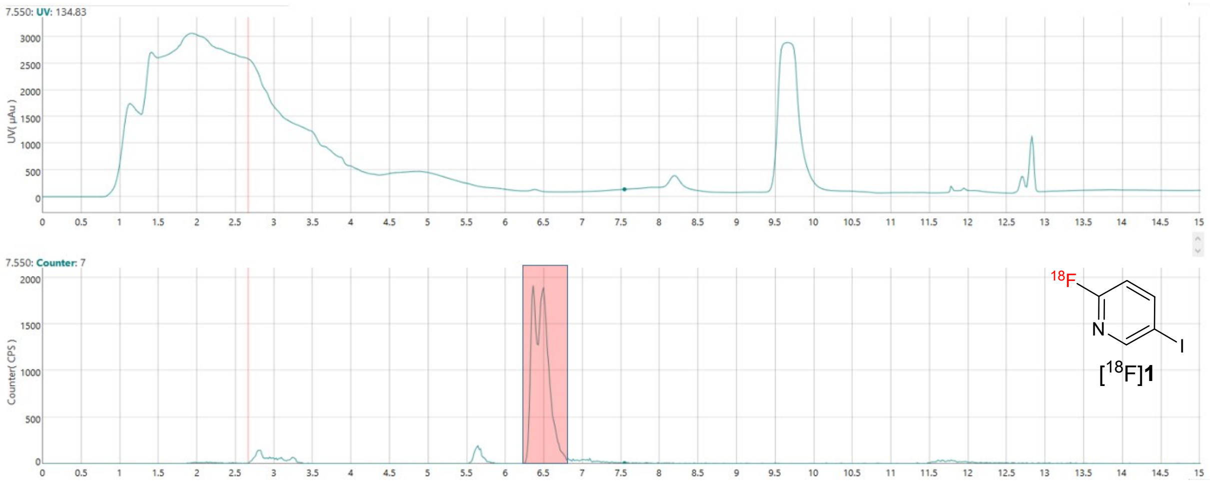


Fig. S11. HPLC purification of [^{18}F]**1**. The apparent double peak is caused by a detector overload.

3.2. Optimization study. Preparation of [^{18}F]5 and [^{18}F]7

*3.2.1. Preparation of [^{18}F]**5** and [^{18}F]**7** using small aliquots of [^{18}F]**1**. General Procedure 1 (GP1)*

A solution of XantPhos Pd-G3 (20 mg/mL in the corresponding organic solvent) followed by an aliquot of a solution of the SPE purified [^{18}F]**1** (typically 2–10 µL; 5–15 MBq) were added to a solution of Et_3N in the appropriate solvent or to a solution of the appropriate organic solvent in the appropriate buffer. Finally, a stock solution of the thiol-containing precursor in the corresponding solvent was added so that the final volume of the reaction mixture was 240 µL and the solvent composition corresponded to the appropriate entry in Table S1. The reaction mixture was gently shaken for 1–6 min at ambient temperature, diluted with MeCN (20% TFA, 100 µL) and HPLC eluent (1 mL), an aliquot (120 µL) was taken up and analyzed by HPLC (for [^{18}F]**5**: column: Gemini C18(2), 250 × 4.6 mm, 5 µm/100 Å; eluent: 55% MeCN; flow rate: 1.5 mL/min; k=2.6; for [^{18}F]**7**: column: Aeris PEPTIDE XB-C18, 250 × 4.6 mm, 5 µm/100 Å; eluent: 13% MeCN (0.1% TFA); flow rate: 1.5 mL/min; k=1.9). The residual radioactivity was measured, the reaction mixture was removed by syringe and the empty reaction vessel was again measured to determine radioactivity adsorbed on vessel walls (generally ≤ 5%).

Table S1. Optimization of reaction conditions for *S*-arylation of Boc-Cys-OMe (**4**) and GSH (**6**) with [^{18}F]**1**.[a,b]

Entry	Substrate	Amount (µmol)	Catalyst (µg)	Base (pH)	Solvent	Time (min)	Product	HRCY±SD (%)
1	Boc-Cys-OMe (**4**)	6l	120 (2 mol%)	Et_3N (>10)	THF	6	[^{18}F]**5**	93±2
2	⍰	⍰	500 (8 mol%)	⍰	⍰	⍰	⍰	99±1
3	⍰	3	500 (16 mol%)	⍰	⍰	⍰	⍰	97±1
4	⍰	2	500 (24 mol%)	⍰	⍰	⍰	⍰	96±1
5	L-glutathione (**6**)	3	60 (2 mol%)	⍰	THF/H_2O (1:1)	⍰	[^{18}F]**7**	93±10
6	⍰	⍰	120 (4 mol%)	⍰	⍰	⍰	⍰	93±2
7	⍰	1	40 (4 mol%)	⍰	⍰	⍰	⍰	70±2
8	⍰	⍰	80 (8 mol%)	⍰	⍰	⍰	⍰	95±1
9	⍰	0.5	40 (8 mol%)	⍰	⍰	⍰	⍰	71±31
10	⍰	⍰	500 (≥100 mol%)	⍰	⍰	⍰	⍰	40±29
11	⍰	⍰	⍰	(7.3)	THF/PB (1:1)	⍰	⍰	94±5
12	⍰	0.333	⍰	⍰	⍰	⍰	⍰	83±17
13	⍰	0.167	⍰	⍰	⍰	⍰	⍰	76±12
14	⍰	⍰	⍰	(6.7)	⍰	⍰	⍰	70±6
15	⍰	⍰	⍰	(7.8)	⍰	⍰	⍰	68±7
16	⍰	⍰	⍰	(7.3)	DMF/PB (1:1)	⍰	⍰	85±4
17	⍰	⍰	⍰	⍰	MeOH/PB (1:1)	⍰	⍰	86±2
18	⍰	⍰	⍰	⍰	MeCN/PB (1:1)	⍰	⍰	94±1
19	⍰	0.066	⍰	⍰	⍰	⍰	⍰	76±25
20	⍰	0.033	⍰	⍰	⍰	⍰	⍰	74±4
21	⍰	0.167	⍰	⍰	⍰	1	⍰	95±1

[a] For reaction conditions, see 3.2.1.. [b]All experiments were carried out at least in triplicate. PB – 0.1 M sodium phosphate buffer. HRCY – radiochemical yield determined by HPLC. SD – standard deviation.

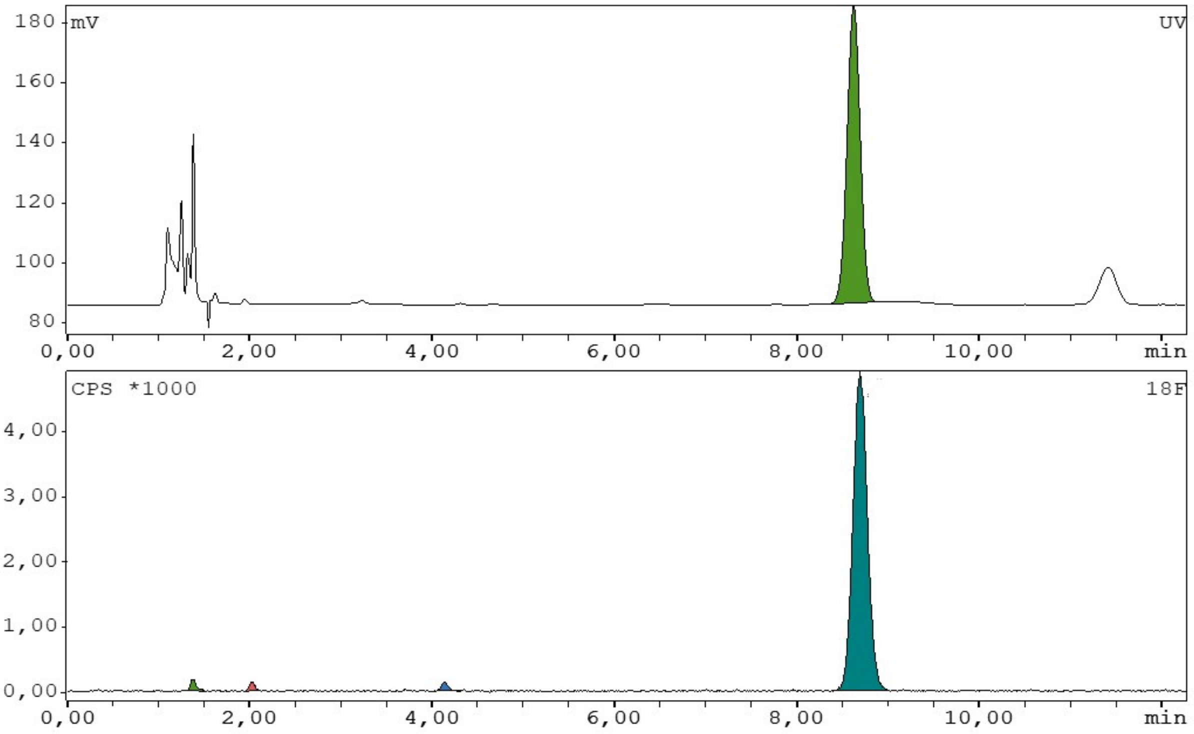


Fig. S12. Crude [^{18}F]**5** coinjected with the reference compound. HPLC conditions: column: Aeris Peptide XB C18 5 µ, 4.6 x 250 mm; eluent: 40% MeCN; flow rate: 2 mL/min;, detection: γ and UV: λ=270 nm. Reaction conditions: see 3.2.1. and Table S1, entry 2.

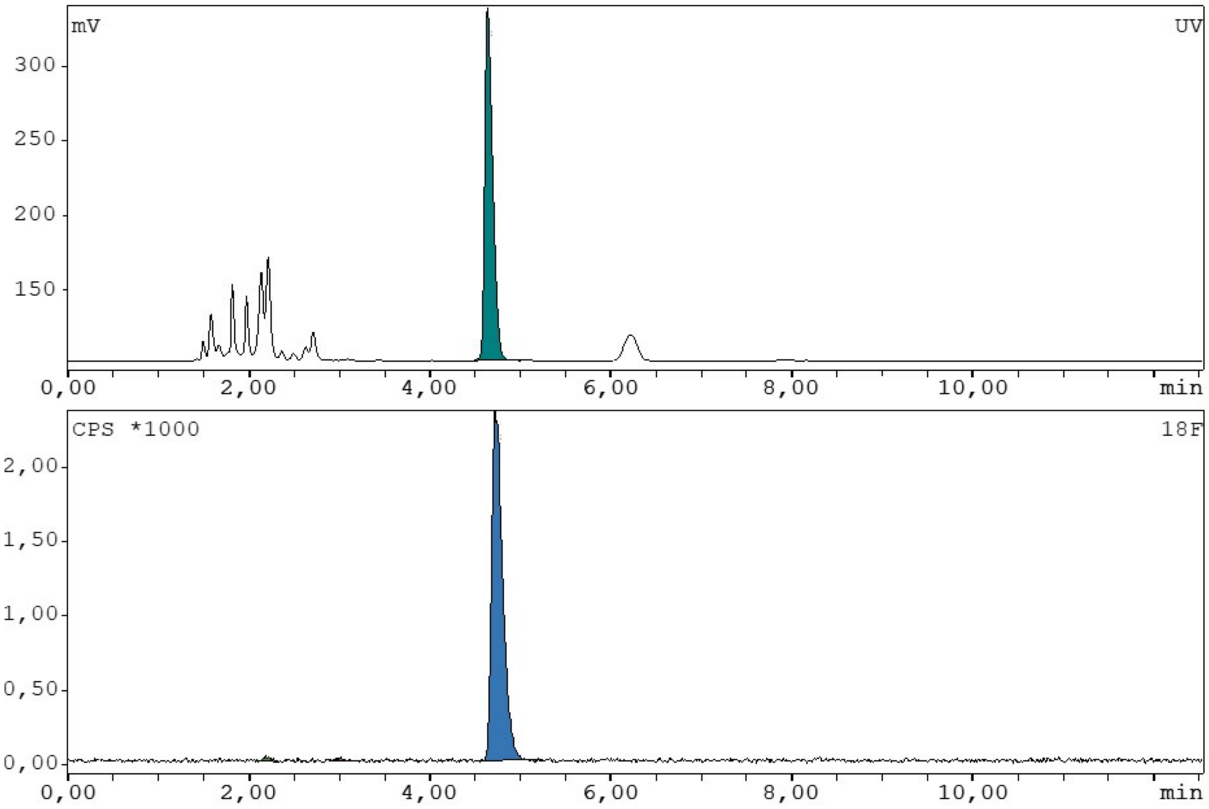


Fig. S13. Crude [^{18}F]**7**, coinjected with the reference compound. HPLC conditions: Column: Aeris Peptide XB C18 5µm, 4.6 x 250 mm; eluent: 13% MeCN (0,1 % TFA); flow rate: 1.5 mL/min; detection: γ and UV: λ= 254 nm. Reaction conditions: see 3.2.1. and Table S1, entry 11.

*3.2.2. Preparation of [^{18}F]**7** using full batches of [^{18}F]**1**. General Procedure 2 (GP1)*

[^{18}F]**1** (0.03–0.5 GBq) produced from **2** (0.5–4 mg) as described in 3.1. was purified by SPE as described in 3.1.1. and eluted from the Strata-X RP cartridge with MeCN (300 µL). An aqueous solution of GSH (270 µL, 0.1–1 µmol) followed by XantPhos Pd G3 (500 µg, 25 µL of the 20 mg/mL stock solution in MeCN) and 1 M sodium phosphate buffer (30 µL, pH 7.4, BBraun) were added and the mixture was shaken for 6 min. The reaction mixture was diluted with 2.5% TFA (1 mL), an aliquot (120 µL) was taken up and analyzed by HPLC as described in 3.2.1.. The residual radioactivity was measured, the reaction mixture was removed by syringe and the empty reaction vessel was again measured to determine radioactivity adsorbed on vessel walls (generally ≤ 5%).

Table S2: Optimization of RCY of [^{18}F]**7** over two steps in respect to amount of precursor **2** and GSH (**6**)[a,b,c]

Entry	**2** (mg)	EE±SD **(%)**	[^{18}F]**1**±SD (%)[d]	**6** (µmol)	(**6**+[^{18}F]**1**)±SD (%)[e]	RCY±SD (%)[f]
1	4	96±1	80±2	1	46±10	37±7
2	2	93±1	76±7	⍰	69±11	53±12
3	1	77±9	46±10	⍰	92±4	42±11
4	0.5	44±3	28±3	⍰	94±1	26±3
5	1	73±6	45±4	0.5	64±4	29±4
6	⍰	66±15	46±8	0.25	25±3	12±2
7	⍰	72±5	48±3	0.1	8±1	4±0

[a] [^{18}F]**1** was prepared as described in 3.1. and SPE purified as described in 3.1.1.. [b] *S*-Arylation was carried out as described in 3.2.2. [c] All experiments were carried out at least in triplicate. [d] Isolated yield of the SPE purified [^{18}F]**1**. [e] HRCY of the *S*-arylation step. [f] Calculated for each experiment by multiplication of RCY of [^{18}F]**1** and HRCY of [^{18}F]**7**. EE – [^{18}F]F^{-} elution efficacy. SD – standard deviation.

3.2.3. Statistical analysis of the results in Table S2

Statistical analysis of RCYs in Table S2 was performed using one-way ANOVA in GraphPad Prism 8 for MacOS. An effect of the amount of the DABCO salt precursor **2** used in the first reaction step (preparation of [^{18}F]**1**) on RCYs over two steps was found to be significant [F(3,8)=4.7; p=0.0362]. Tukey post-hoc comparison revealed a significant difference between the application of 0.5 mg and 2 mg **2** (p=0.0257).

3.3. Production of PSMA-specific ligand [^{18}F]8

*3.3.1. Manual radiosynthesis of [^{18}F]**8***

[^{18}F]**1** (0.05–5 GBq) was purified by SPE as described in 3.1.1. and eluted from the Strata-X RP cartridge with MeCN (300 µL) into a 2 mL Eppendorf vial. After addition of a solution of **9** (6 mg, 20 µmol) in 0.1 M sodium phosphate buffer (pH 7.3, 300 µL) and XantPhos Pd-G3 (25 µL of the 20 mg/mL stock solution in MeCN), the pH of the reaction mixture was adjusted to ~8 by addition of Et_3N (6 µL, 43 µmol) and the mixture was stirred for 6 min. The solution was then diluted with 0.5% H_3PO_4 (1.2 mL) and purified by semipreparative HPLC [column: Gemini C18, 250 x 10 mm, 5 µm/100 Å; eluent: 15% MeCN (0.17% H_3PO_4); flow rate: 6.0 mL/min]. The product fraction was diluted with H_2O (30 mL), loaded onto a pre-conditioned Oasis HLB Plus cartridge, washed with H_2O (2 mL) and dried in a stream of argon. The desired product was eluted with EtOH/0.9% NaCl/H_2O (10:7:3, 2 mL), of which the first 200–300 µL contained no product and were discarded. Eluent was removed under reduced pressure at 60 °C in a stream of Ar (~10 min at 40 mbar), the residue was taken up in H_2O (0.5 mL) containing Na phosphate buffer (B. Braun Melsungen, 1 M, pH=7.4, 2.5 µL) to obtain [^{18}F]**8** formulated for *in vivo* studies. The synthesis time starting from [^{18}F]fluoride including HPLC purification and SPE solvent exchange was 55 min. The reformulation step took 10–15 min. HPLC quality control: column: Kinetex F5, 250 × 4.6 mm, 5 µm/100 Å; eluent: 15% MeCN (0.1% TFA); flow rate: 1.5 mL/min; k=3.8.

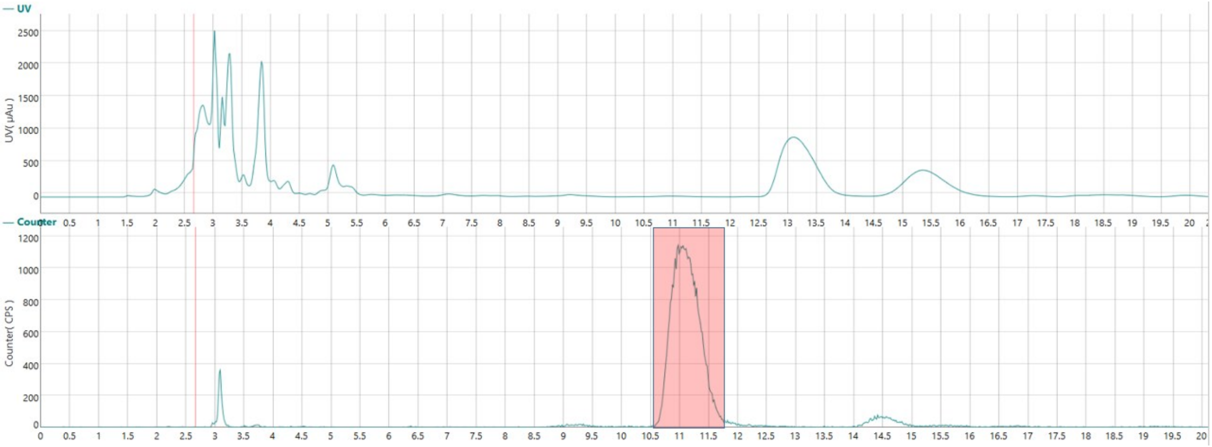


Fig. S14. Semipreparative purification of [^{18}F]**8**.

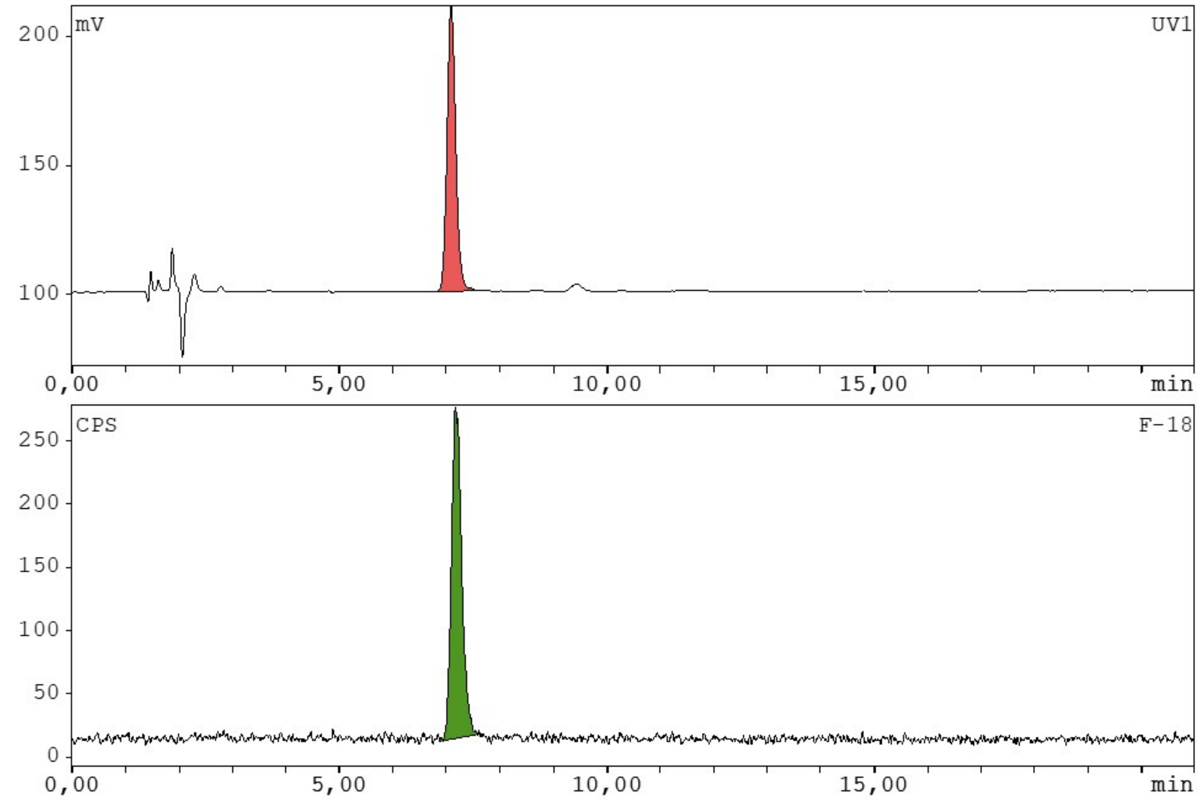


Fig. S15. HPLC trace of the purified [^{18}F]**8**, coinjected with the reference compound. HPLC conditions: column: Kinetex F5 5µm, 4.6 × 250 mm; eluent: 15% MeCN (0.1% TFA); flow rate: 1.5 mL/min; detection: γ and UV: λ= 254 nm.

*3.3.2. Automated radiosynthesis of [^{18}F]**8***

For production of larger activity amounts of [^{18}F]**8**, the radiosynthesis of was performed in an automated synthesis module using the flow scheme depicted below. Aqueous [^{18}F]fluoride (1–10 GBq) was loaded onto an anion-exchange resin (QMA cartridge) from the male side, the resin was washed in the same direction with MeOH (1.0–1.5 mL). Afterwards ^{18}F^{-} was eluted into the reaction vial with a solution of the onium radiolabeling precursor salt **2** (6 mg, 12.8 µmol) in MeOH (1 mL) from the female side of the cartridge into the reactor 1. MeOH was evaporated in the flow of He at 60 °C under reduced pressure (250 mbar) for 7 min, anhydrous DMSO (0.7 mL) was added and the reaction mixture was heated at 110 °C for 15 min. After addition of H_2O (13 mL) the reaction mixture was transferred onto a Strata-X SPE cartridge which was subsequently washed with H_2O (10 mL) and dried in a stream of He for 2 min. Purified [^{18}F]**1** was eluted with 10% Et_3N in THF (330 µL) into the reactor 2 charged with a solution of the precursor **9** (15 µmol) in H_2O (300 µL) and XantPhos Pd G3 (1.2 µmol) in 50% THF (100 µL). The reaction mixture was stirred at 37 °C for 8 min, diluted with 0.5% H_3PO_4 (3 mL) and [^{18}F]**8** was isolated by HPLC [column: Gemini C18, 250 x 10 mm, 5 µm/100 Å; eluent: 15% MeCN (0.17% H_3PO_4); flow rate: 6.0 mL/min]. The product was collected in a vessel charged with H_2O (30 mL). The resulting solution was loaded onto an Oasis HLB plus short cartridge, washed with H_2O and [^{18}F]**8** was eluted with EtOH/isotonic NaCl solution (1:1, 2 mL) to afford after dilution with phosphate buffered saline (20 mL) the desired PET-tracer [^{18}F]**8** in a ready-for-application form.

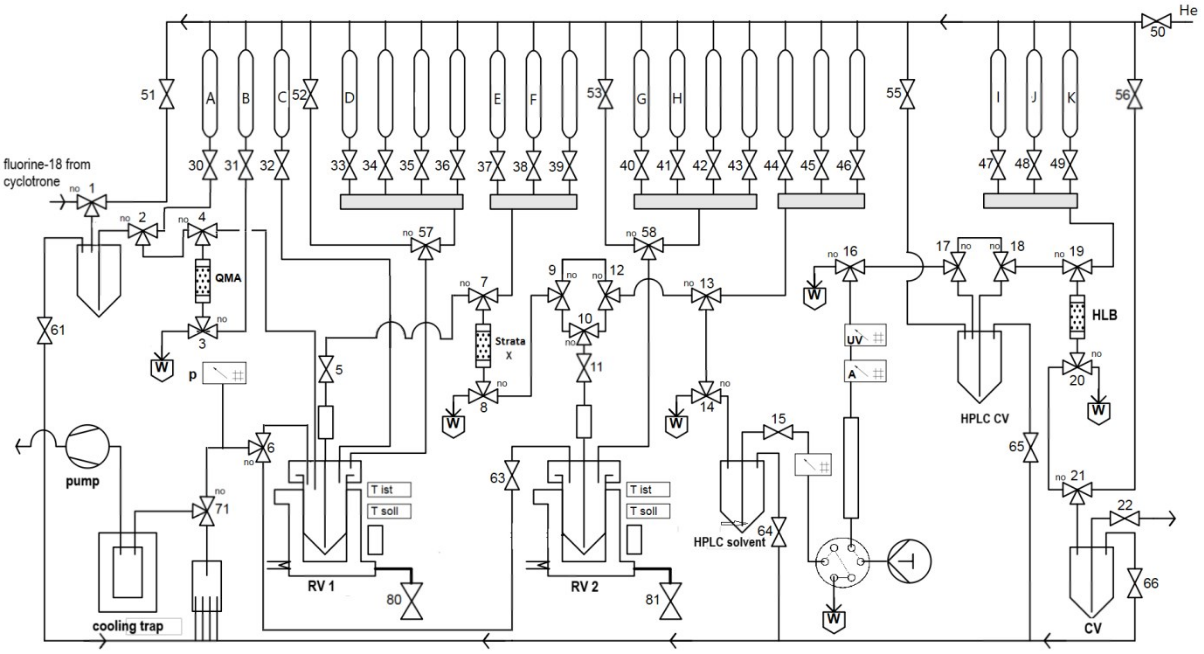


Fig. S16. A: MeOH (1.5 mL); B: radiolabeling precursor (12.8 µmol) in MeOH (1 mL); C: DMSO (0.7 mL); D: H_2O (13 mL); E: 10% Et_3N in THF (330 µL); F: H_2O (10 mL); G: Cys-CO-Glu (15 µmol) in H_2O (300 µL); H: 0.5% H_3PO_4; I: H_2O (20 mL); J: 50% EtOH in 0.9% NaCl (2 mL); K: phosphate buffered saline (20 mL); RV2: XantPhos Pd-G3 (1.2 µmol) in 50% THF (100 µL).

3.4. Preparation of H-ptlhthnrrrrrc(2-[^{18}F]fluoropyridin-5-yl)-NH$_2$ ([^{18}F]13)

*3.4.1. Radiosynthesis with HPLC-purified [^{18}F]**1***

0.1 M Na phosphate buffer (BBraun, pH 7.4, 200 µL), XantPhos Pd G3 (25 µL aliquot of the 20 mg/mL solution in MeCN) and a solution of RD2c peptide **12** (0.5 mg, 300 nmol) in H_2O (20 µL) were sequentially added to the HPLC-purified [^{18}F]**1** (see 3.1.2.) in MeCN (200 µL) and the reaction mixture was stirred for 12 min. Thereafter, it was diluted with 0.5% TFA (1.2 mL) and [^{18}F]**13** was isolated by semipreparative HPLC [column: Synergy Hydro-RP, 250 x 10 mm, 10 µm/100 Å; gradient: 0–1.5 min: H_2O (0.075% TFA), 1.5–15 min: 15% MeCN (0.075% TFA); flow rate: 7.4 mL/min]. The product fraction was diluted with H_2O (50 mL) and loaded onto a Sep-Pak C18 Plus cartridge. The latter was washed with H_2O (5 mL) and dried in a stream of Ar. [^{18}F]**13** was eluted with MeOH (0.5 mL). MeOH was removed in the stream of Ar at 40 °C under reduced pressure (250 mBar) and the residue was dissolved in PBS affording [^{18}F]**13** formulated for *in vivo* studies. The synthesis time starting from [^{18}F]fluoride including HPLC purification and SPE solvent exchange was about 65 min. The reformulation step took 10–15 min. HPLC quality control: column: Synergy Hydro RP, 250 × 4.6 mm, 5 µm/100 Å; eluent: 15% MeCN (0.1% TFA); flow rate: 1.0 mL/min; k=3.6.

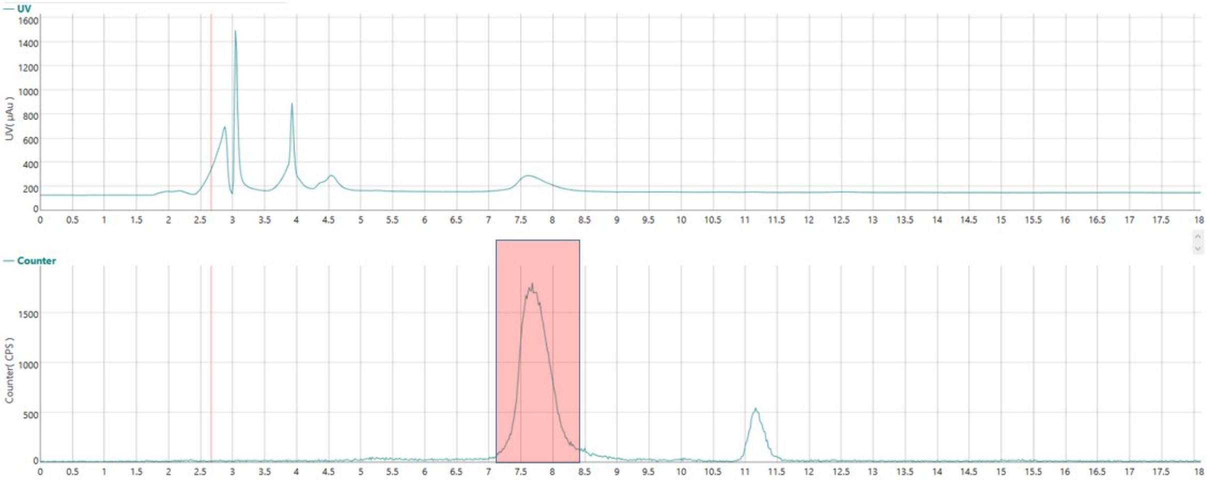


Fig. S17. Semipreparative purification of [^{18}F]**13**.

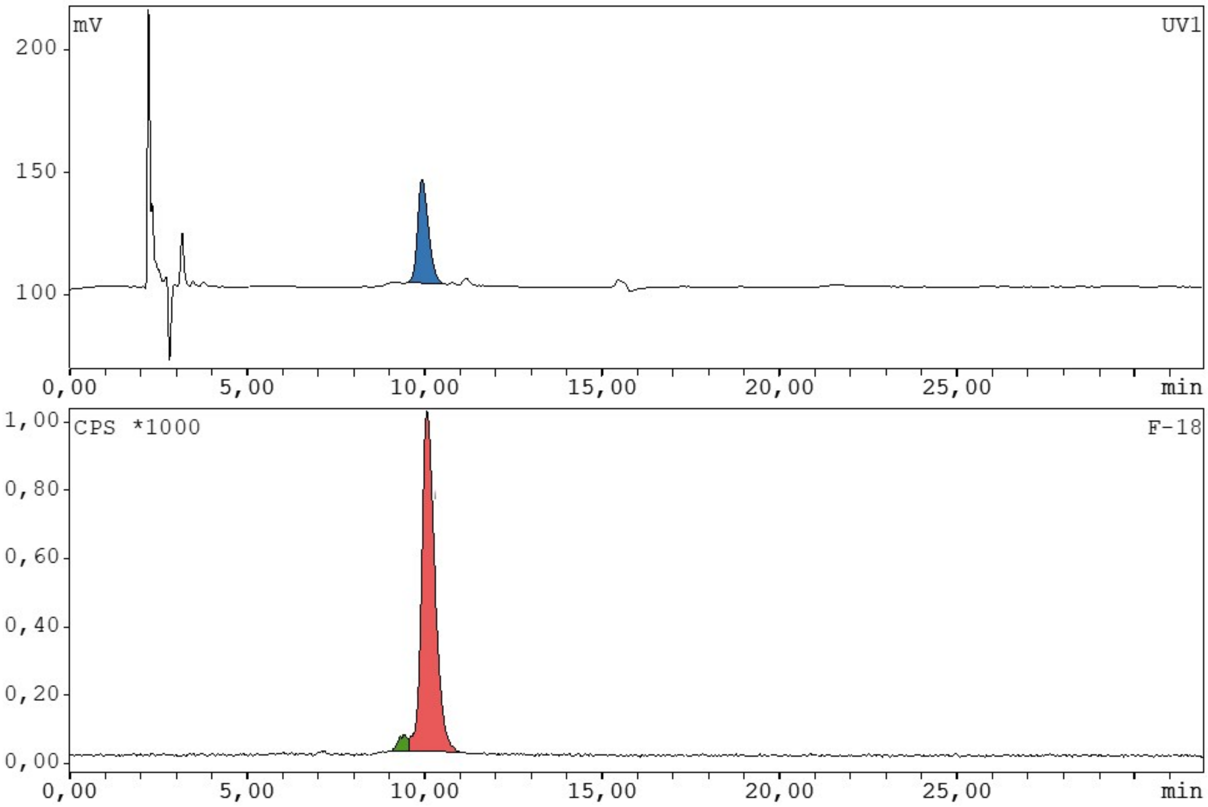


Fig. S18. HPLC trace of the purified [^{18}F]**13** coinjected with the reference compound. Conditions: Synergy Hydro-RP 5 µ, 4.6 × 250 mm; eluent: 15% MeCN (0.1% TFA); flow rate: 1.0 mL/min; detection: γ and UV: λ=214 nm.

*3.4.2. Simplified radiosynthesis of [^{18}F]**13** using SPE-purified [^{18}F]**1***

RD2c **12** (5 mg, 3 µmol) in 0.1 M Na phosphate buffer (300 µL, BBraun, pH 7.4) and XantPhos Pd-G3 (25 µL of the 20 mg/mL stock solution in MeCN) were sequentially added to a solution of the SPE-purified [^{18}F]**1** (see 3.1.1.) in MeCN (300 µL) and the mixture was gently shaken for 12 min and diluted with 0.5% TFA (1.2 mL). [^{18}F]**13** was isolated by semipreparative HPLC and formulated as described above (see 3.4.1.). The synthesis time starting from [^{18}F]fluoride including HPLC purification and SPE solvent exchange was about 55 min. The reformulation step took 10–15 min.

3.5. Radiolabeling of reduced BSA

BSA (40 µL of the 10 mg/mL stock solution in H_2O, 6 nmol) was added to 10 mM TCEP·HCl (6 µL, 10 eq.), the mixture was shaken at 37 °C for 1 h and the resulting solution of the reduced BSA was directly used for conjugation with [^{18}F]**1**. Alternatively, for the preparation of the GPC purified reduced BSA, BSA (460 µL of the 10 mg/mL stock solution in H_2O, 69 nmol) was added to 10 mM TCEP·HCl (69-690 µL, 10–100 eq.), the mixture was shaken at 37 °C for 1 h and the resulting solution of the reduced BSA was purified using Sephadex^{TM} PD-10 desalting columns (GE Healthcare) according to the gravity protocol provided by the manufacturer (*vide infra*) furnishing the purified protein fraction (3.5 mL) as a solution in 0.1 M sodium phosphate buffer (pH 7.0). Aliquots (40 µL) of this solution were applied for the subsequent conjugation. Afterwards, necessary amounts of 25 mM sodium borate buffer (pH 8.0), followed by MeCN, XantPhos Pd G3 (20 mg/mL stock solution in MeCN) and [^{18}F]**1** (2–20 µL of MeCN solution; ca. 5–10 MBq) were added to give 400 µL final volume of the reaction mixture at the given solvent composition (see Table S3) and the reaction mixture was incubated for 20 min at 37 °C. The protein fraction was isolated using Sephadex^{TM} PD-10 desalting columns (GE Healthcare) according to the gravity protocol provided by the manufacturer. Accordingly, 0.1 M sodium phosphate buffer (pH 7.0) was added to the mixture to a final volume of 2.5 mL and the resulting solution was passed through the column. To avoid unfavorable lipophilic interactions with the unreacted [^{18}F]**1**, the protein fraction was eluted using 20% MeCN in the same buffer (3.5 mL). Two control experiments were carried out: 1. without Pd-catalyst and 2. with native BSA (see Table S3, entries 10 and 11, respectively). The synthesis time including GPC purification was 30 min starting from the purified [^{18}F]**1**.

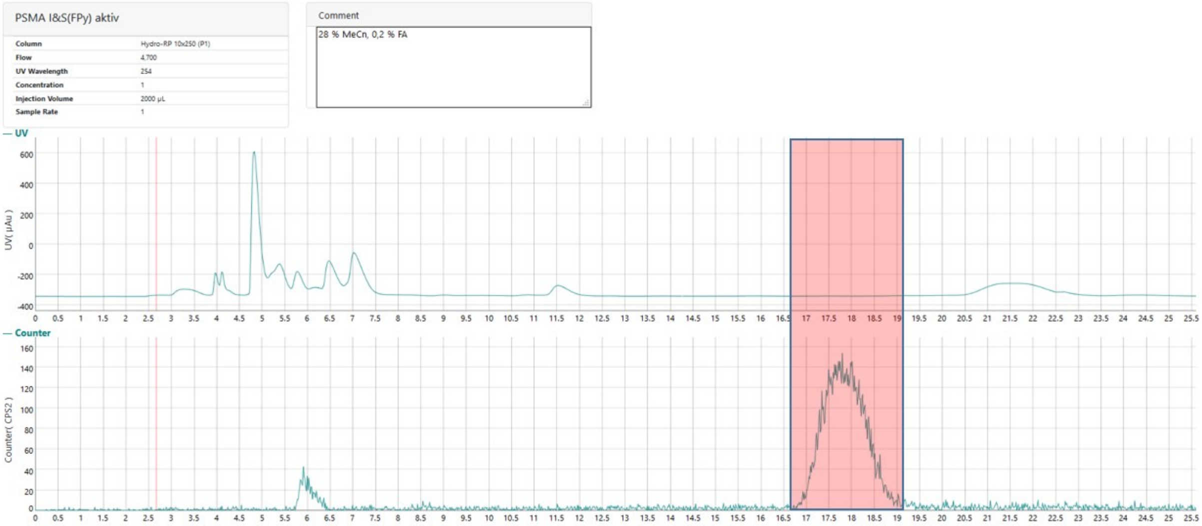
Table S3. Radiolabeling of BSA using [^{18}F]**1**.[a]

	Reduction			Radiolabeling			
Entry	TCEP (eq.)	Time (h)	Purif.[b]	MeCN (%)	[Pd] (µg)	RCY±SD (%)[c]	n[d]
1	10	1	no	10	50	17±3	3
2				25		34±11	9
3		2				31±1	3
4		1	yes			24±7	3
5	20					22±2	3
6	100					18±13	3
7	10		no		25	18±8	3
8					75	40±9	3
9					100	19±3	3
10					0	0	3
11	0	0			50	12±2	3

[a] Carried out as described in 3.5. [b] PD-10 SEC purification of the reduced protein before radiolabeling. [c] Determined as high molecular-weight fraction using PD-10 SEC. [d] Number of runs. SD – standard deviation.

3.6. Radiolabeling of PSMA I&S (16)

[^{18}F]**17** was produced according to the simplified protocol for the preparation of [^{18}F]**13** described in 3.4.2. using the SPE purified [^{18}F]**1** synthesized starting from 50–100 MBq [^{18}F]fluoride as described in 3.1. and 3.1.1.. After 12 min shaking the reaction mixture was diluted with 2.5% HCO_2H (1 mL) and [^{18}F]**17** was isolated by semipreparative HPLC [column: Hydro RP 10 µ, 10 x 250 mm; eluent: 28% MeCN (0.2% TFA); flow rate: 4,7 mL/min; detection: γ and UV: λ = 254 nm; fraction 16.5–19 min was collected]. The synthesis time starting from [^{18}F]fluoride including HPLC purification was 60 min. Analytical HPLC: column: Aeris Peptide XB C18 5 µ, 4.6 × 250 mm; eluent: 28% MeCN (0.2% TFA); flow rate: 1.5 mL/min; detection: γ and UV: λ = 220 nm.



PSMA I&S(FPy) aktiv

Column	Hydro-RP 10x250 (P1)
Flow	4,700
UV Wavelength	254
Concentration	1
Injection Volume	2000 µL
Sample Rate	1

Comment

28 % MeCn, 0,2 % FA

Fig. S19. Semipreparative purification of [^{18}F]**17**.

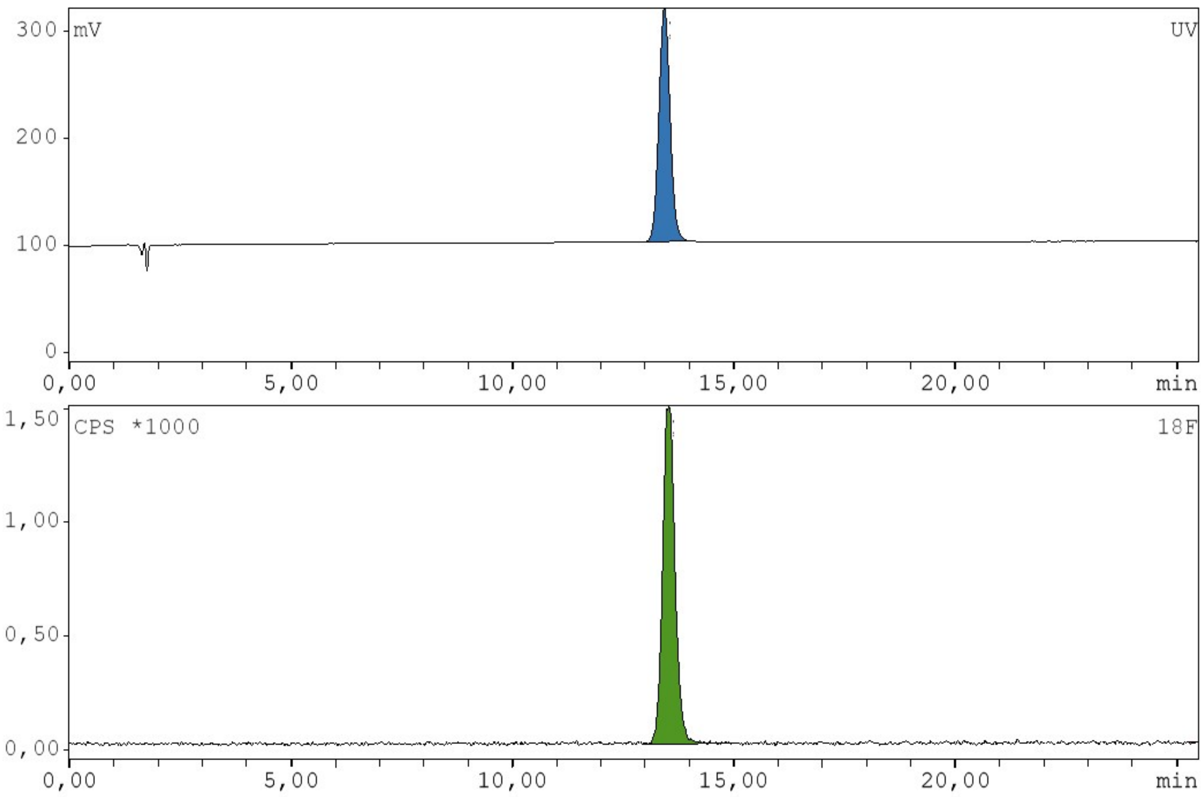


Fig. S20. HPLC trace of the purified [^{18}F]**17** coinjected with the reference compound. HPLC conditions: column: Aeris Peptide XB C18 5 µ, 4.6 × 250 mm; eluent: 28% MeCN (0.2% TFA); flow rate: 1.5 mL/min; detection: γ and UV: λ = 220 nm.

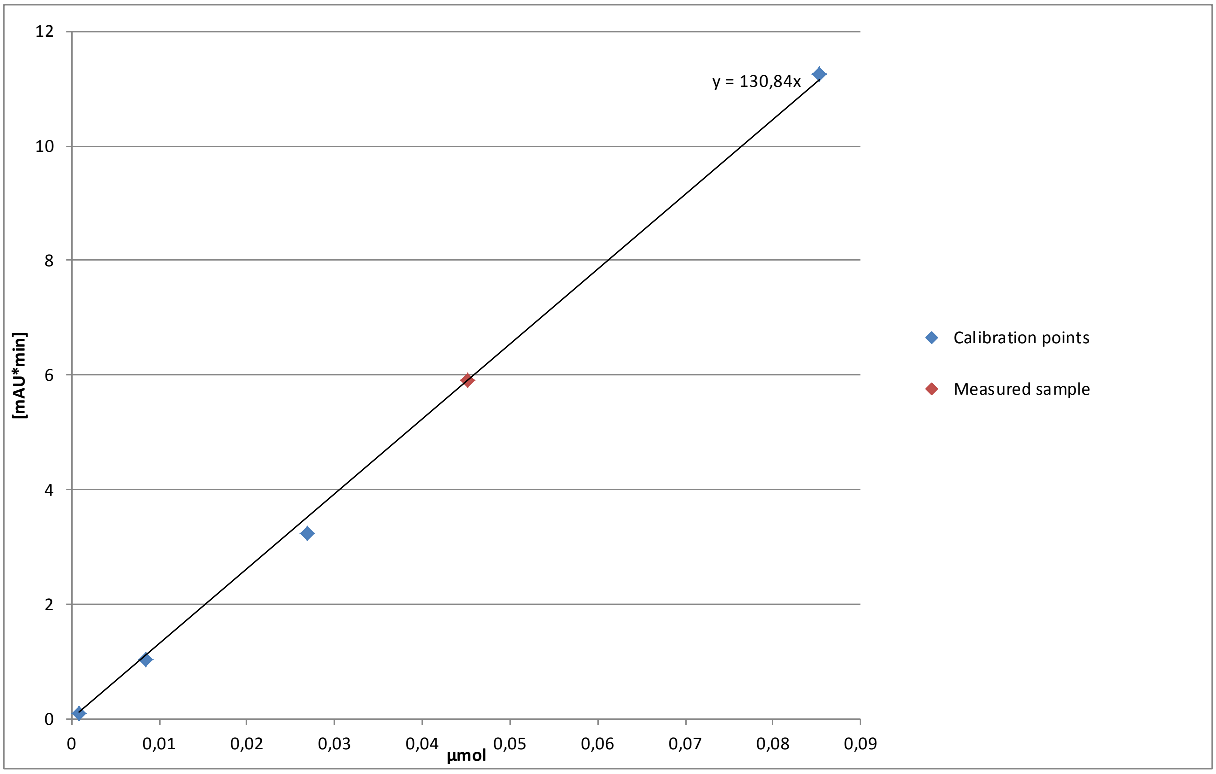
3.7. Determination of molar activity. General Procedure (GP 3)

An aliquot of the tracer obtained after HPLC purification (20 µL) or of the SPE purified building block [^{18}F]**1** was analyzed under HPLC conditions described in the respective chapter. Simultaneously, the tracer amount (in GBq) in the same size aliquot was measured. The peak area was determined and the molar activity was calculated according to calibration curves, which were obtained using at least four concentrations of the reference compounds.

$$A_M = \frac{A\,[\frac{GBq}{20\ \mu L}]}{c\,[\frac{\mu mol}{20\ \mu L}]}$$

*3.7.1. Determination of molar activity of [^{18}F]**1***

Calibration curve (measured at 220 nm):



Concentration µg/mL	µmol/mL	Peak area (mAU)
Calibration points		
190	0.8520	111.2603
19	0.08521	11.2408
6	0.02691	3.2504
1.9	0.008521	1.0484
0.19	0,0008521	0.0908
Measured sample		

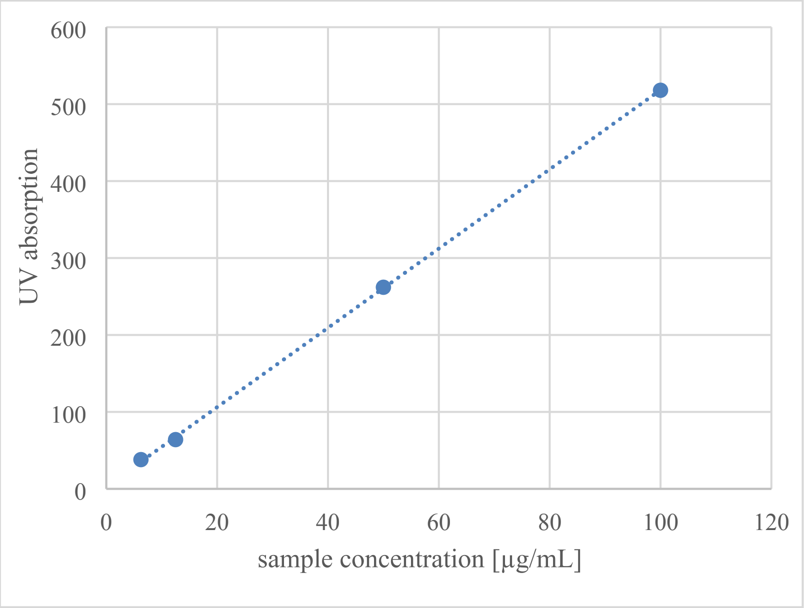
10.07	0.045254	5.9093

Probe:

Volume (mL)	1
Activity (GBq)	1376
Carrier amount (µmol)	0.045254
Molar Activity (GBq/µmol)	29.2

*3.7.2. Determination of molar activity of [^{18}F]**8***

Calibration curve (measured at 254 nm):



Concentration [µg/mL]	Peak area (mAU)
100	518
50	262
12.5	64
6.25	38

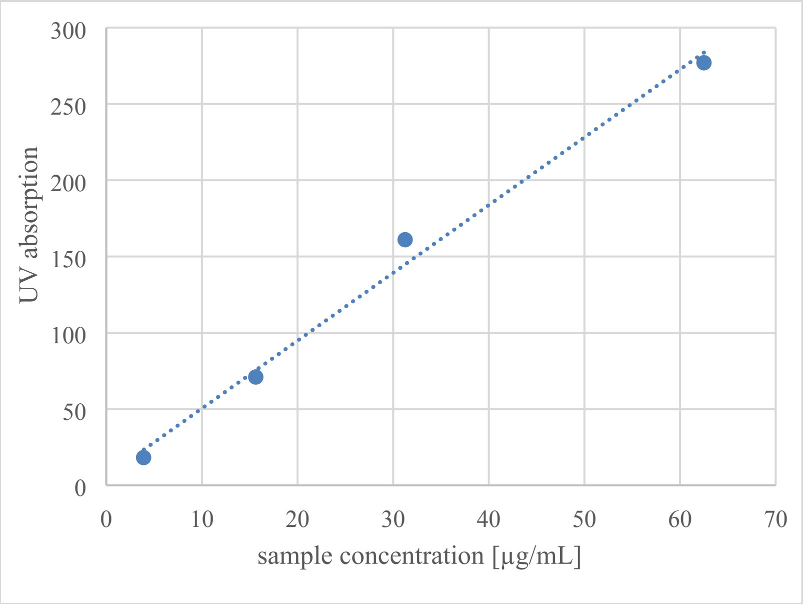
Molar activity of [^{18}F]**8**

Activity (GBq)	Molar activity (GBq/µmol)
0.37	70
0.015	11
1.84	78
1.81	57

3.77	75

*3.7.3. Determination of molar activity of [^{18}F]**13***

Calibration curve (measured at 254 nm):



Concentration [µg/mL]	Peak area (mAU)
62.5	277
31.25	161
15.63	71
3.9	18.2

Molar activity of [^{18}F]**13**

Activity (GBq)	Molar activity (GBq/µmol)
1.29	57
0.205	100
0.73	16
0.29	32
1.29	57

4. Small animal studies

4.1. Animals

Two healthy rats (283+555 g body weight; one female, one male) were used in the present study. Animals were housed in pair at a constant temperature (22 °C) with light on from 9.00 p.m. to 9.00 a.m. and ad libitum access to food and water. All animal experiments were performed in accordance with the EU directive 2010/63/EU for animal experiments and the German Animal Welfare Act (TierSchG, 2006) and were approved by the regional authorities (LANUV NRW), application number 84-02.04.2015.A240.

4.2. Positron emission tomography (PET)

For PET imaging, rats were anesthetized with isoflurane (5% for induction, 2% for maintenance) in O_2/air (3:7), and a catheter for tracer injection was inserted into the lateral tail vein. Rats were placed on an animal holder (Equipment Vétérinaire Minerve, Esternay, France) and fixed with a tooth bar in a respiratory mask. Body temperature was maintained at 37 °C by continuous warm air flow through the wall of the animal bed. Eyes were protected from drying with Bepanthen eye and nose ointment (Bayer). A dynamic PET scan in list mode was conducted using a Focus 220 micro PET scanner (CTI-Siemens, Erlangen, Germany) with a resolution at the center of field of view of 1.4 mm. Data acquisition started with intravenous injection of [^{18}F]**8** [57–66 MBq in PBS (500 µL)]. In one rat, the PSMA inhibitor 2-PMPA (23 mg/kg) was added to the tracer solution. Emission scan time was 120 min, followed by a 10 minute transmission scan using a ^{57}Co point source for attenuation correction. After the scan was finished, the catheter was removed and the rats were allowed to recover in their home cage. After Fourier rebinning, data were reconstructed in two frames of 60 min each, using the iterative OSEM3D/MAP algorithm with attenuation and decay correction. The resulting voxel sizes were 0.47 mm x 0.47 mm x 0.80 mm. Only the second frame was used for further analysis, using the software VINCI 4.72 (Max Planck Institute for Metabolism Research, Cologne, Germany). Images were Gauss filtered (1 mm FWHM) and standardized uptake values based on body weight (SUV_{bw}) were determined according to the following equation:

SUV_{bw}= radioactivity [Bq/g] × body weight [g] × 100 / injected dose [Bq]

Background activity for calculation of signal-to-noise ratios was measured in the back muscles.

5. References

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