Electronic supplementary materials *Mendeleev Commun.*, 2022, **32**, 408–410

Al[^{18}F]F-HBED-CC-radiolabeling in a media of protic alkanolammonium ionic liquids

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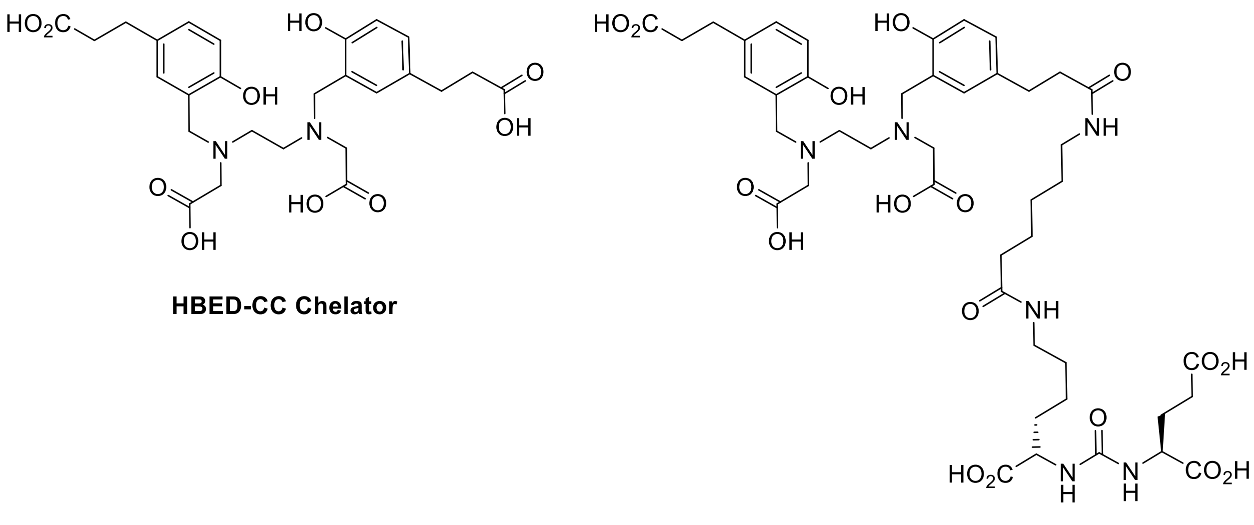
1. Materials

Protic alkanolammonium ionic liquids (PAAILs) containing triethanolammonium (TEA), tris(hydroxymethyl)methyl ammonium (TRIS), *N*-phenyldiethanolammonium (PDA), and triisopropanolammonium (TPA) cations were synthesized and characterized according to the literature procedure.[S1-S6]

Sodium Acetate trihydrate was purchased from Honeywell-Fluka. Acetic acid, 1-butanol, *N*,*N*-dimethylformamide(DMF), *N,N*-dimethylacetamide (DMA), 1,4-dioxane, 1-methylpyrrolidin-2-one (NMP), 1-propanol, 2-propanol, trifluoroacetic acid were purchased from Acros Organics. Acetone, acetonitrile (HPLC grade), dimethyl sulfoxide (DMSO), ethanol, methanol were purchased from Merck. Aluminium chloride hexahydrate, ammonium acetate, *tert*-butanol, pyridine, sulfolane were purchased from Sigma-Aldrich. Isotonic 0.9% saline solution was purchased from Solopharm (Saint-Petersburg, Russia). ^{18}O-Enriched water (97%) was purchased from CJSC ‘Global Scientific Technology’ (Saint-Petersburg, Russia). Chelator HBED-CC (Figure S1) was purchased from PharmSintez.Lab (Moscow, Russia). Peptide PSMA-HBED-CC (PSMA-11, Figure S1) was purchased from ABX (Radeberg, Germany). Thin-layer chromatography plates Sorbfil was purchased from Imid Ltd.(Krasnodar, Russia).

Only 18 MOhm deionized water (MilliQ) was used for preparation of aqueous solutions.

Radiolabeling reactions were performed in a sealed 2-mL PP microcentifuge tubes in Biosan TS-100 thermoshaker. All experiments were carried out at least in triplicates. All data are expressed as the mean of values ± standard deviation (mean ± SD). Prism 5 Software (GraphPad Software) was used to determine statistical significance at the 95% confidence level, with a P value of less than 0.05 being considered significant.


HBED-CC Chelator

Peptide PSMA-11

Figure S1. Structures of HBED-CC chelator and PSMA-11 peptide

2. Production of $[^{18}F]$Fluoride

$[^{18}F]$Fluoride was produced via the $^{18}O(p,n)^{18}F$ nuclear reaction by irradiation of $[^{18}O]H_2O$ (97% enrichment) in a water cyclotron target (niobium, 2.3 mL volume) using 16.4 MeV protons beam on PETTrace 800 cyclotron (GEHealthcare, Uppsala, Sweden). The radionuclide was transferred from the target by means of helium flow and collected in a screw cap closed vial. An aliquot of the aqueous $[^{18}F]$Fluoride (15-40 MBq) was used in small-scale radiolabeling.

3. Effect of buffer on the RCC of Al$[^{18}F]$F-HBED-CC complex formation (Figure 1).

3 µL of 2 mM $AlCl_3$ solution in 0.1 M sodium acetate (pH 4.0) buffer was loaded in 2 mL microcentrifuge tube, and then 100 µL buffer (0.9% NaCl, 0.1 M sodium acetate (pH 4.0) or 0.1 M aqueous [TEA]Benz) and 100 µL of EtOH were added. Afterward, 10 µL aqueous $[^{18}F]$fluoride and 2.5-30 µL of precursor HBED-CC (1 mg ml^{-1} solution in deionized water) were added to the reaction tube. The mixture was incubated in a sealed tube at 50 °C for 20 min (shaking 1000 RPM). After cooling, RCC was determined by radioTLC (Table S1).

Table S1. Buffer for radiolabeling screen

HBED-CC, µg	RCC (n=3), %		
	0.9% NaCl	0.2 M NaAc (pH 4.0)	0.1 M [TEA]Benz (pH 5.9)
2.5	0	12.2±5.0	13.9±1.8
5	4.6±1.8	13.4±9.5	50.1±7.6
7.5	4.7±1.2	12.6±2.8	46.8±3.5
10	4.2±0.8	30.8±5.3	64.4±1.9
20	5.8±1.2	41.0±4.7	63.1±4.3
30	6.4±0.7	57.7±0.8	62.8±2.4

4. Effect of [TEA]Benz concentration on the RCC of Al[^{18}F]F-HBED-CC complex formation (Figure 2).

3 µL of 2 mM $AlCl_3$ solution in 0.1 M sodium acetate (pH 4.0) buffer was loaded in 2 mL microcentrifuge tube, and then 100 µL of 0.025-1 M aqueous[TEA]Benz and 100 µL of EtOH were added. Afterward, 10 µL aqueous [^{18}F]fluoride and 10 µL of precursor HBED-CC (1 mg ml^{-1} solution in deionized water) were added to the reaction tube. The mixture was incubated in a sealed tube at 50 °C for 20 min (shaking 1000 RPM). After cooling, RCC was determined by radioTLC (Table S2).

Table S2.[TEA]Benz concentration screen

C, M	RCC (n=3), %
0.025	60.3±5.6
0.05	64.4±1.9
0.1	64.2±1.1
0.25	48.5±2.4
0.5	28.8±1.4
1.0	31.8±1.0

5. Effect of EtOH concentration on the RCC of Al[^{18}F]F-HBED-CC complex formation (Figure 2).

3 µL of 2 mM $AlCl_3$ solution in 0.1 M sodium acetate (pH 4.0) buffer was loaded in 2 mL microcentrifuge tube, and then 100 µL of 0.05 M aqueous [TEA]Benz and 0-400 µL of EtOH were added. Afterward, 10 µL aqueous [^{18}F]fluoride and 10 µL of precursor HBED-CC (1 mg ml^{-1} solution in deionized water) were added to the reaction tube. The mixture was incubated in a sealed tube at 50 °C for 20 min (shaking 1000 RPM). After cooling, RCC was determined by radioTLC (Table S3).

Table S3. EtOH concentration screen

EtOH, %	RCC (n=3), %
0 (0 µL)	27.1±4.6
18 (25 µL)	39.8±4.2
31 (50 µL)	50.1±6.5
47 (100 µL)	64.4±1.9
64 (200 µL)	49.0±5.1
78 (400 µL)	50.8±6.1

6. Effect of DMSO concentration on the RCC of Al[^{18}F]F-HBED-CC complex formation

3 µL of 2 mM $AlCl_3$ solution in 0.1 M sodium acetate (pH 4.0) buffer was loaded in 2 mL microcentrifuge tube, and then 100 µL of 0.05 M aqueous [TEA]Benz and 0-400 µL of DMSO were added. Afterward, 10 µL aqueous [^{18}F]fluoride and 10 µL of precursor HBED-CC (1 mg ml^{-1} solution in deionized water) were added to the reaction tube. The mixture was incubated in a sealed tube at 50 °C for 20 min (shaking 1000 RPM). After cooling, RCC was determined by radioTLC (Table S4, Figure S2).

Table S4. DMSO concentration screen

DMSO, %	RCC (n=3), %
0 (0 µL)	27.1±4.6
18 (25 µL)	76.3±0.8
31 (50 µL)	91.8±0.6
47 (100 µL)	99.5±0.3
64 (200 µL)	99.3±0.2
78 (400 µL)	99.7±0.1

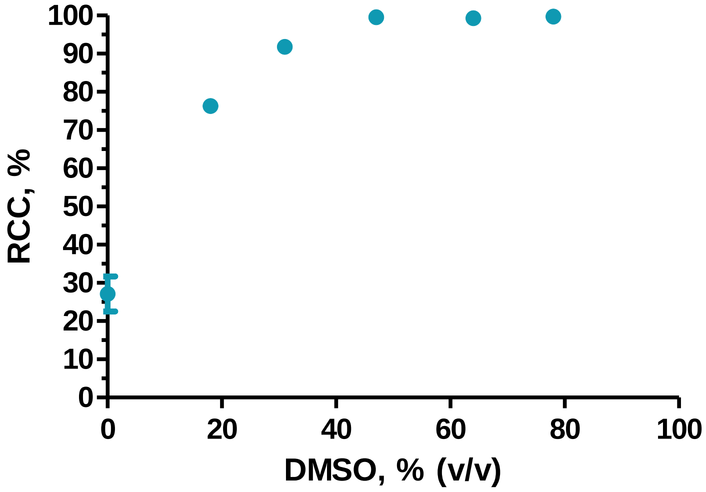


Figure S2. Dependence of RCC (%) from the amount of DMSO as co-solvent (% v/v; n=3 in all cases)

7. Effect of organic solvent nature on the RCC of Al[^{18}F]F-HBED-CC complex formation (Figure 3).

3 µL of 2 mM $AlCl_3$ solution in 0.1 M sodium acetate (pH 4.0) buffer was loaded in 2 mL microcentrifuge tube, and then 100 µL of 0.05 M aqueous [TEA]Benz and 100 µL of organic solvent (MeOH, EtOH, *n*-PrOH, *i*-PrOH, *n*-BuOH, *t*-BuOH, acetone, acetonitrile, DMSO, DMF, DMA, pyridine, NMP, sulfolane, 1,4-dioxane) were added. Afterward, 10 µL aqueous [^{18}F]fluoride and 10 µL of precursor HBED-CC (1 mg ml^{-1} solution in deionized water) were added to the reaction tube. The mixture was incubated in a sealed tube at 50 °C for 20 min (shaking 1000 RPM). After cooling, RCC was determined by radioTLC (Table S5).

Table S5. Organic solvent screen

Organic solvent	RCC (n=3), %
MeOH	73.5±0.8
EtOH	64.4±1.9
n-PrOH	69.3±3.5
i-PrOH	73.8±9.2
n-BuOH	32.8±17.0
t-BuOH	0
Acetone	75.8±4.6
Acetonitrile	78.9±4.2
DMSO	98.5±0.4
DMF	93.8±1.9
DMA	65.0±1.2
Pyridine	69.5±3.4
NMP	50.6±6.4
Sulfolane	73.7±5.9
1,4-Dioxane	81.4±3.0

8. Effect of PAAIL nature on the RCC of Al[^{18}F]F-HBED-CC complex formation (Figure 3).

3 µL of 2 mM $AlCl_3$ solution in 0.1 M sodium acetate (pH 4.0) buffer was loaded in 2 mL microcentrifuge tube, and then 100 µL of 0.05 M aqueous PAAIL solution and 100 µL of DMSO were added. Afterward, 10 µL aqueous [^{18}F]fluoride and 10 µL of precursor HBED-CC (1 mg ml^{-1} solution in deionized water) were added to the reaction tube. The mixture was incubated in a sealed tube at 50 °C for 20 min (shaking 1000 RPM). After cooling, RCC was determined by radioTLC (Table S6). The pH values of the reaction mixtures are presented in Table S7.

Table S6. PAAIL screen

Anion	RCC (n=3), % for various cations			
	TEA	TRIS	PDA	TPA
Benz	98.5±0.4	24.4±8.6	14.2±2.1	84.3±3.2
Cin	34.6±4.4	26.6±6.7	-	86.3±2.3
Sal	8.0±4.0	48.1±6.8	15.2±4.2	87.3±2.5
AcSal	73.9±5.1	42.2±2.4	0	86.5±2.1
Crez	6.8±4.4	0	19.3±16.1	96.8±0.6
ClCrez	0	48.5±9.5	0	95.3±1.6
HOx	0	0	0	0
Ox	0	0	0	0
HMal	0	0	0	0
Mal	0	0	0	0
HSuc	10.6±0.9	43.2±2.3	11.3±2.2	27.2±7.3
Suc	11.1±1.3	33.6±8.2	11.8±2.6	89.6±4.7
HMlt	8.4±1.7	0	16.8±2.0	11.9±1.9
Mlt	0	0	24.4±0.6	10.7±2.7

Table S7. pH values of reaction mixtures

Anion	pH of reaction mixture			
	TEA	TRIS	PDA	TPA
Benz	5.9	6.3	4.5	6.6
Cin	6.6	6.8	-	6.9
Sal	5.8	4.8	3.7	6.0
AcSal	5.4	4.3	3.7	6.3
Crez	6.2	6.4	3.9	6.2
ClCrez	6.3	5.2	4.1	6.2
HOx	4.2	5.7	3.0	5.2
Ox	6.7	8.1	3.5	6.6
HMal	5.7	6.4	4.4	5.8
Mal	7.4	8.0	4.6	6.7
HSuc	6.2	6.1	3.8	6.1
Suc	7.3	7.5	4.2	6.3
HMlt	5.5	5.4	4.0	5.1
Mlt	6.9	6.9	4.4	5.5

9. Effect of buffer's pH value on the RCC of Al[^{18}F]F-HBED-CC complex formation.

3 µL of 2 mM $AlCl_3$ solution in 0.1 M sodium acetate (pH 4.0) buffer was loaded in 2 mL microcentrifuge tube, and then 100 µL of 0.05 M aqueous [TEA]Benz (pH 2-11; adjusted with 1M HCl or 1M NaOH) and 100 µL of DMSO were added. Afterward, 10 µL aqueous [^{18}F]fluoride and 10 µL of precursor HBED-CC (1 mg ml^{-1} solution in deionized water) were added to the reaction tube. The mixture was incubated in a sealed tube at 50 °C for 20 min (shaking 1000 RPM). After cooling, RCC was determined by radioTLC (Table S8, Figure S3).

Table S8. Reaction mixture pH screen

pH	RCC (n=3), %
2.0	0
3.5	72.3±6.4
5.0	93.0±2.3
5.9	98.5±0.4
6.5	85.2±1.6
8.0	58.2±1.7
9.0	34.9±4.2
11.0	9.0±4.0

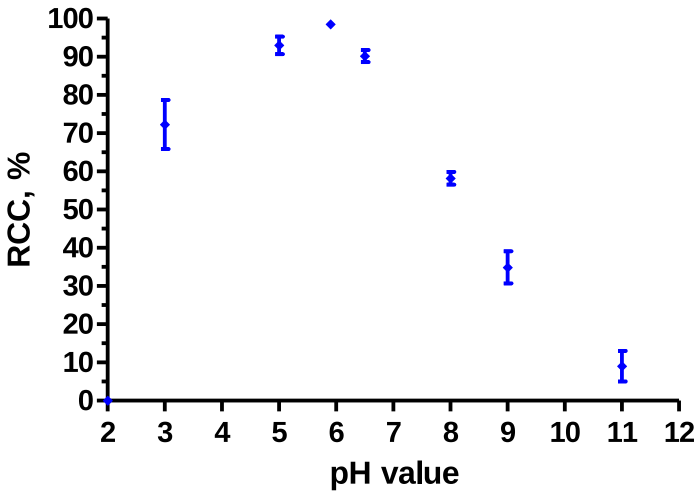


Figure S3. Dependence of RCC (%) from pH value of [TEA]Benz buffer (% v/v; n=3 in all cases)

10. Effect of QMA-Light precondition step on the ‘concentration-type Al[^{18}F]F-radiolabeling of HBED-CC chelator.

An aqueous [^{18}F]fluoride was loaded onto an QMA-Light (Cl-form) anion exchange cartridge from the male side. The cartridge was rinsed with 10 ml of deionized water in the same direction and dried by compressed air. [^{18}F]Fluoride was eluted from female side with 0.3 ml of 50 mM aqueous [TEA]Benz solution into the 2 ml PP tube containing 3 µL of 2 mM $AlCl_3$ solution in 0.1 M sodium acetate (pH 4.0) and 0.3 ml of 50 mM [TEA]Benz. Afterwards, 10 µL of HBED-CC (1 mg ml^{-1} solution in deionized water) in 150 µl of 50 mM [TEA]Benz and 0.75 ml of DMSO was added to the reaction mixture. The mixture was incubated in a sealed tube at 50 °C for 20 min (shaking 1000 RPM). After cooling, RCC was determined by radioTLC (Table S9).

Table S9. Preconditioning screen

Conditioning	RCC (n=3), %
1. 10 mL of deionized water	81.9±5.7
1. 5 mL of 0.2 M sodium acetate (pH 4.0) 2. 10 mL of deionized water	75.3±6.5
1. 5 mL of 0.5 M $NaHCO_3$ 2. 10 mL of deionized water	61.9±2.5

11. Al^{18}F-Radiolabeling of PSMA-11 in a media of PAAILs.

An aqueous [^{18}F]fluoride was loaded onto an QMA-Light (Cl-form) anion exchange cartridge from the male side. The cartridge was rinsed with 10 ml of deionized water in the same direction and dried by compressed air. [^{18}F]Fluoride was eluted from female side with 0.3 ml of 50 mM aqueous PAAIL ([TEA]Benz, [TPA]Crez; [TPA]ClCrez) solution into the 2 ml PP tube containing 3 µL of 2 mM $AlCl_3$ solution in 0.1 M sodium acetate (pH 4.0) and 0.3 ml of 50 mM [TEA]Benz. Afterwards, 20 µL of PSMA-11 peptide (1 mg ml^{-1} solution in deionized water) in 150 µl of 50 mM PAAIL ([TEA]Benz, [TPA]Crez; [TPA]ClCrez) solution and 0.75 ml of DMSO was added to the reaction mixture. The mixture was incubated in a sealed tube at 50 °C for 20 min (shaking 1000 RPM). After cooling, RCC was determined by radioTLC. For HPLC and TLC-analysis Al^{18}F-PSMA-11 was SPE-purified on Chromafix® HR-P (L) resin and reformulated with 1.7 ml of 70% EtOH and 10 ml of normal saline.

12. Radiochemical analysis

The RCCs of the reactions were determined by thin-layer chromatography using silica TLC plates (Sorbfil-HPTLC; 100 × 10 mm strips; origin – 10 mm, solvent front – 90 mm). The reaction solution (2 µL) was spotted on TLC plates and dried on dried on air before developing.

TLC condition: Sorbfil-HPTLC strip; eluent: 1 M ammonium acetate: methanol = 1:3; Rf's: unbounded $[^{18}F]F^-$ and $\{Al^{18}F\}^{2+}$ - 0.0 and $Al^{18}F$-HBED-CC– 0.8;$Al^{18}F$-PSMA-11 – 0.5 .

Analytical HPLC $Al^{18}F$-PSMA-11 was performed on a Dionex Ultimate-3000 series system equipped with a gradient pump, a variable wavelength UV detector and a Gabi-Nova radiodetector. Solvents were obtained as HPLC grade and degassed by ultra-sonication for 15–20 min before use. HPLC analyses were conducted on a Gemini® C18 110 Å, 250×4.6 mm reverse phase HPLC column (Phenomemex, USA) using the following conditions: 15 to 70% gradient (0.1% TFA + Acetonitrile (0.1% TFA)), flowrate: 2.0 mL/min, UV 220 nm: 0 – 4 min: 15 % CH_3CN isocratic; 4 - 11 min: 15 - 70% CH_3CN linear increase; 11 - 14 min: 70 - 15% CH_3CN linear decrease; 14 – 16 min: 15% CH_3CN isocratic. Retention time (t_R) for the radio-HPLC signal of PSMA-11 was 7.04 min.

RadioTLC/RadioHPLC data (Figures S4-S11)

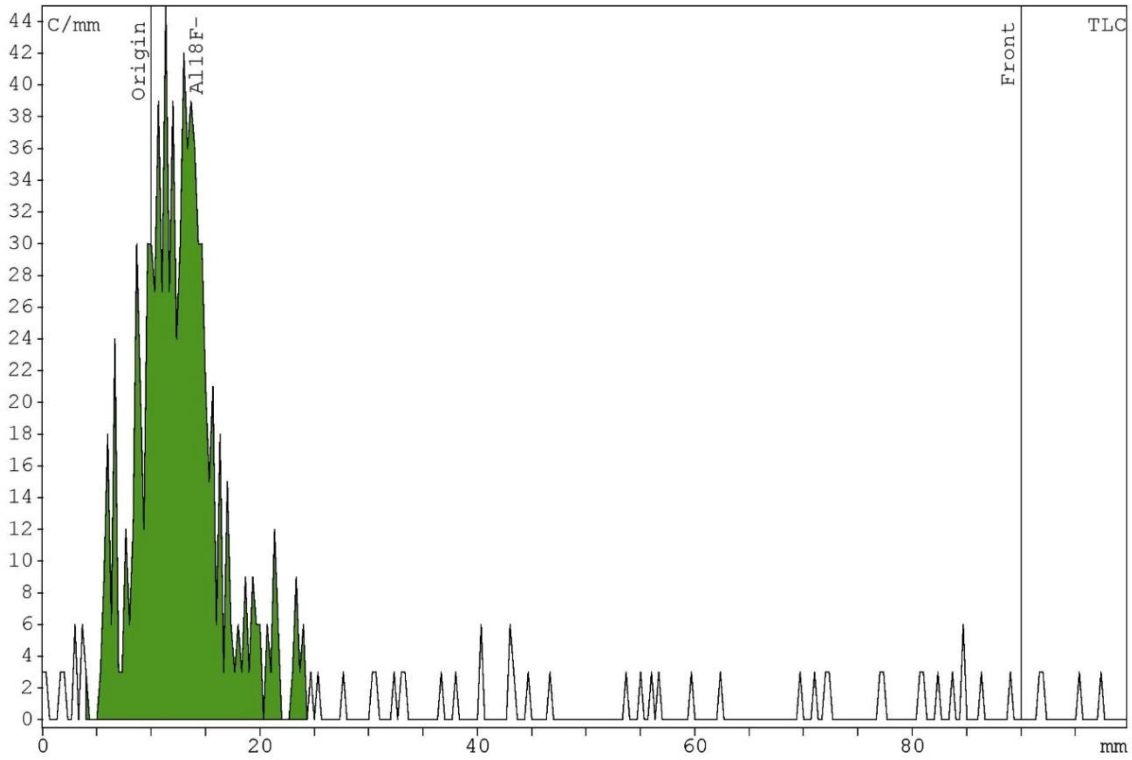


Figure S4. TLC chromatogram of the reaction mixture under the absence of HBED-CC or PSMA-11 in a media of [TEA]Benz

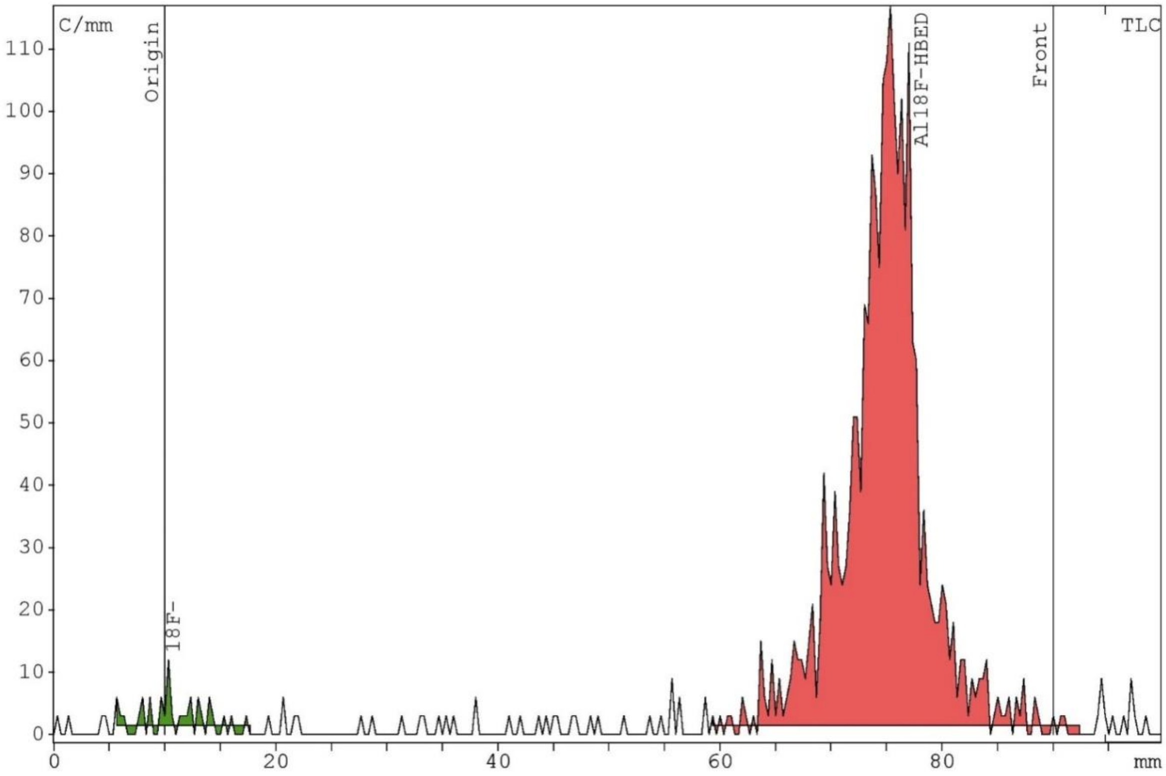


Figure S5. TLC chromatogram of Al^{18}F-HBED-CC reaction mixture

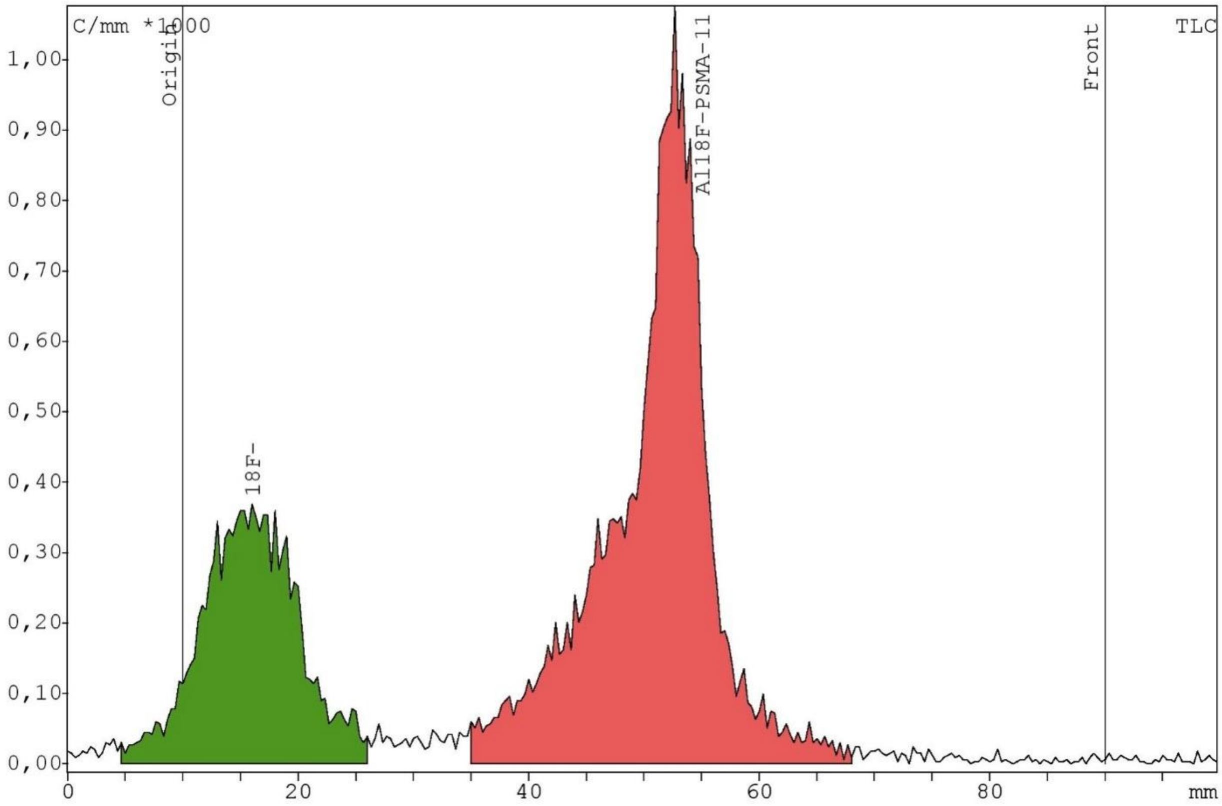


Figure S6. TLC chromatogram of $Al^{18}F$-PSMA-11 reaction mixture

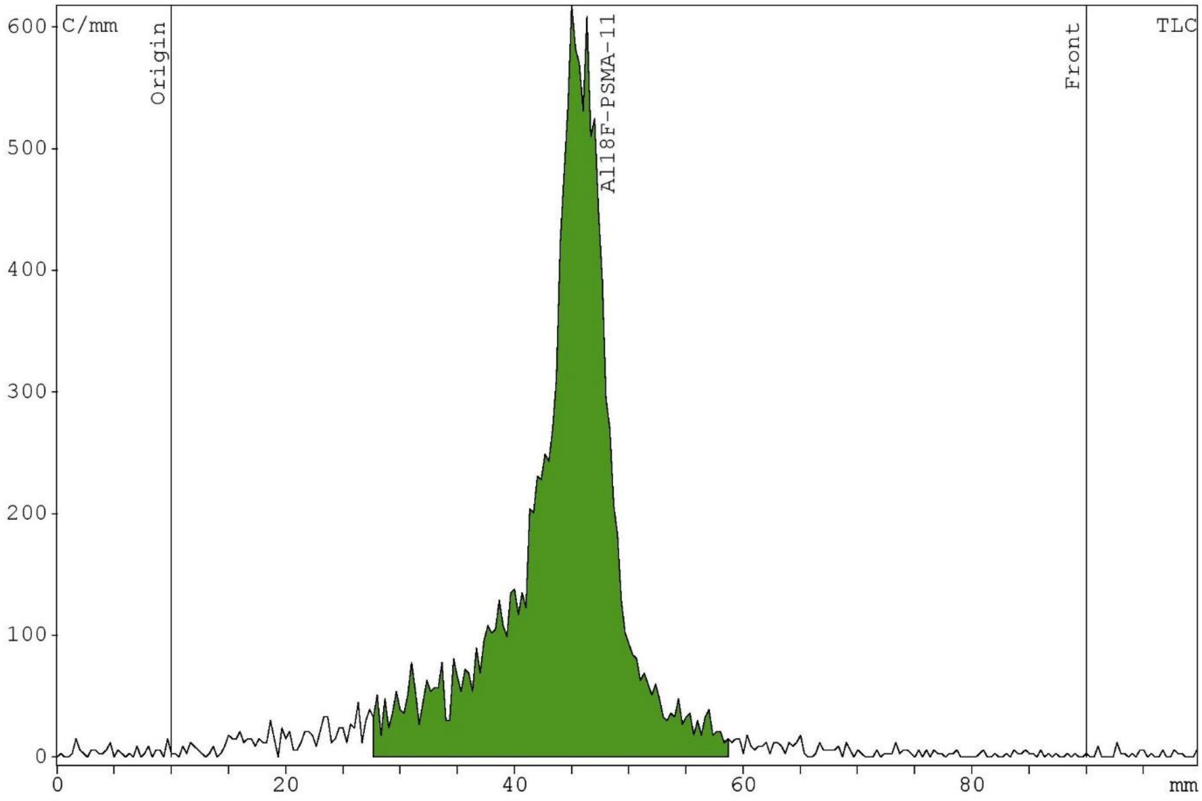


Figure S7. TLC chromatogram of $Al^{18}F$-PSMA-11 after SPE purification

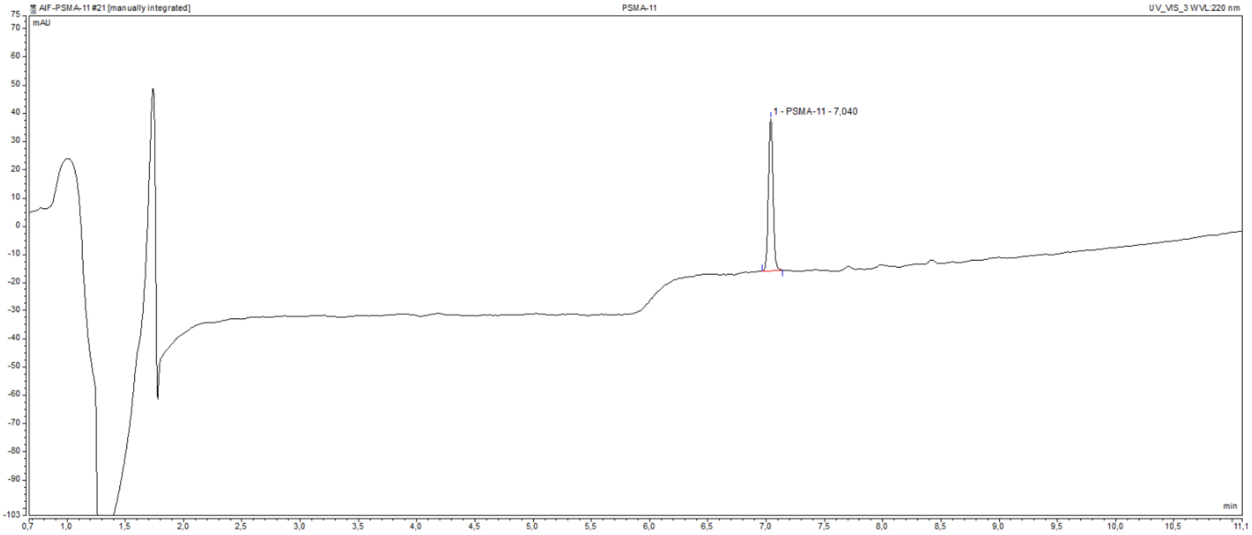


Figure S8. HPLC chromatogram of PSMA-11 (UV detector)

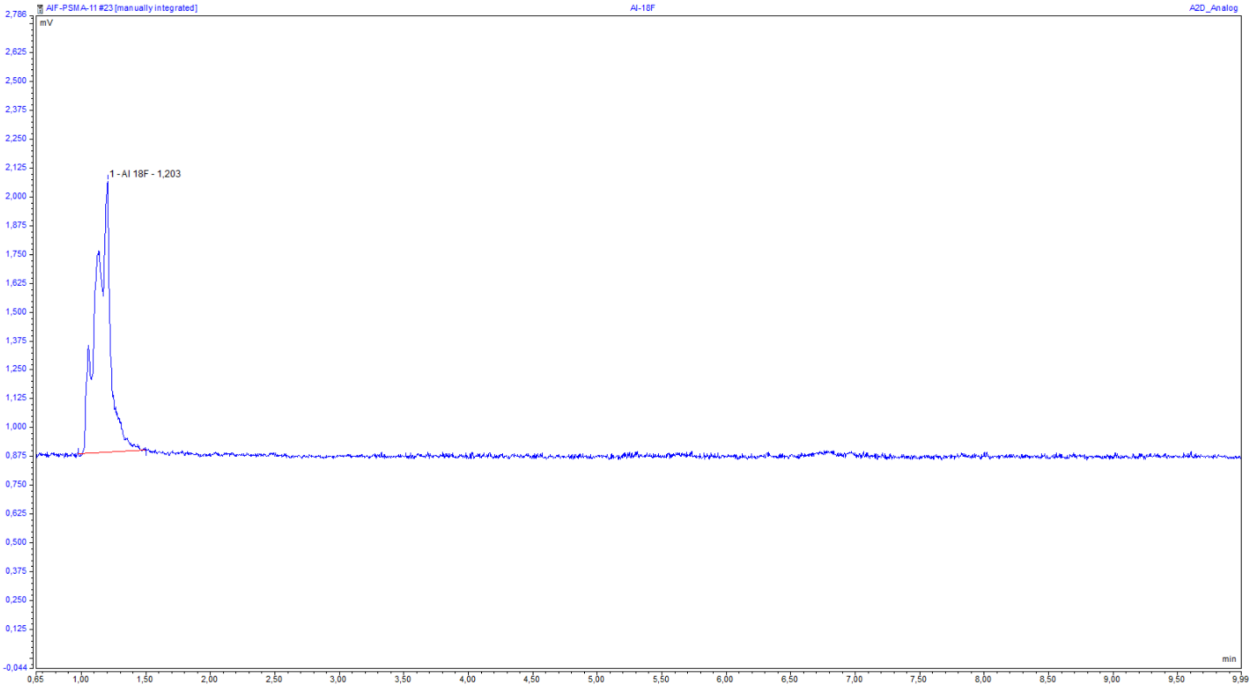


Figure S9. HPLC chromatogram of the reaction mixture under the absence of PSMA-11

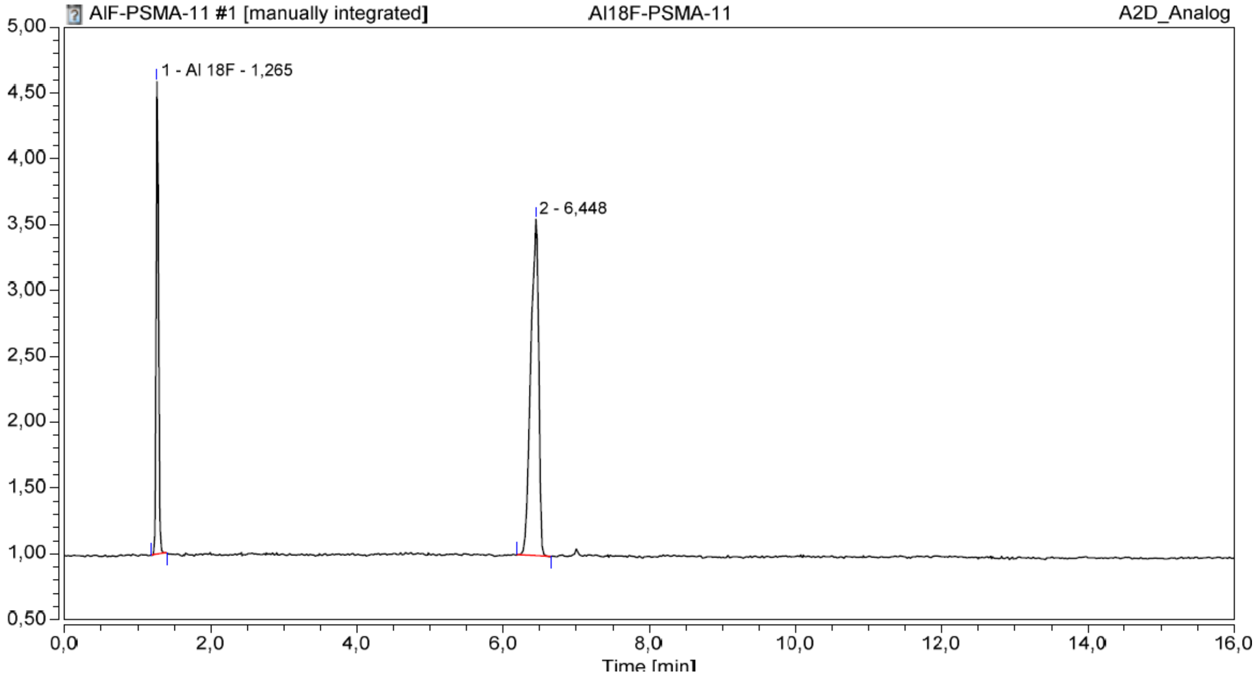


Figure S10. HPLC chromatogram of Al^{18}F-PSMA-11 reaction mixture

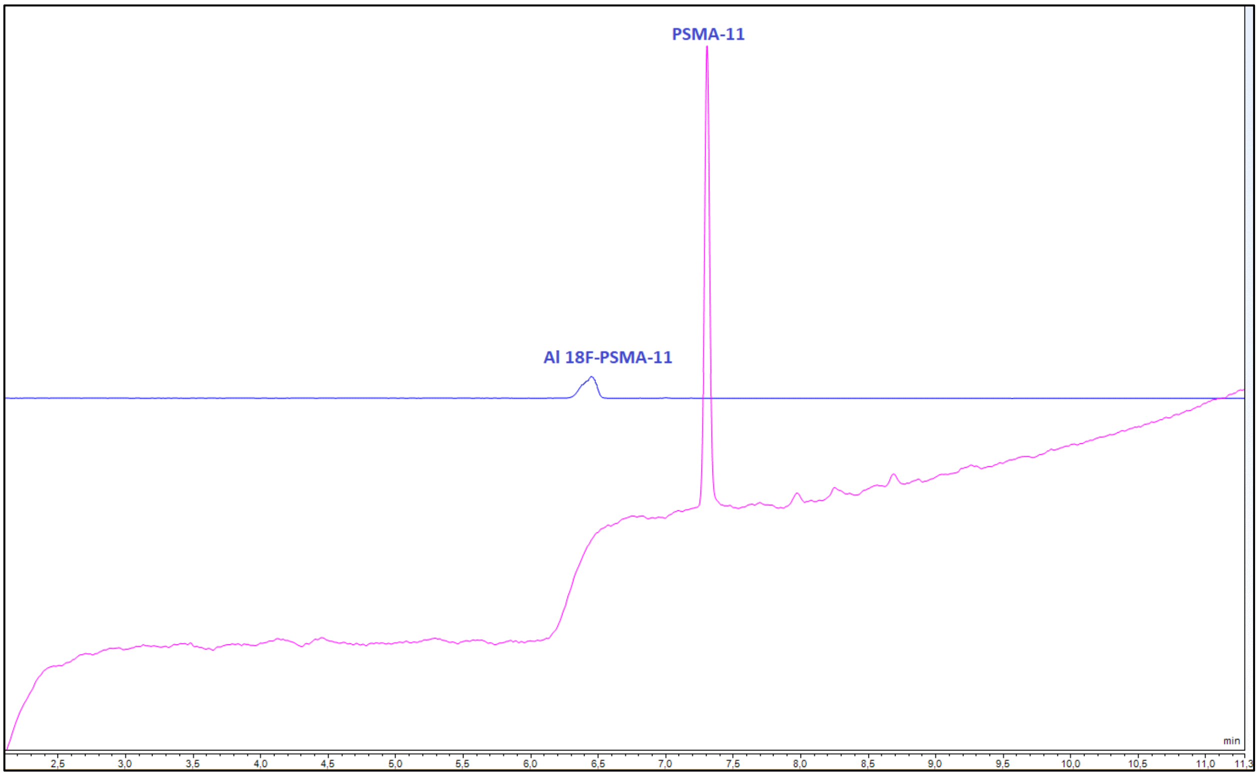


Figure S11. HPLC chromatogram of SPE-purified Al^{18}F-PSMA-11 reaction mixture (overlaid with UV signal of PSMA-11)

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