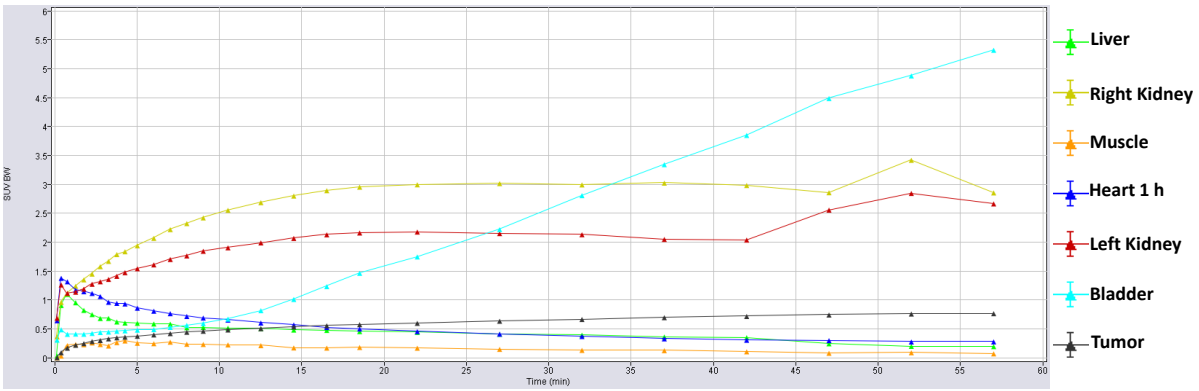


Supplemental Figure 1. Time-activity curves for tumor and relevant organs up to 1 h post injection of 0.2 nmol (100 µL -as injected volume of 0.9% saline) of ^{64}Cu-CA003 in a BALB/c *nu/nu* mouse bearing a C4-2 tumor xenograft. Data are mean standardized uptake value based on body weight-values (SUV_{BW}).

meanSUV	Heart	Liver	Kidneys	Bladder	Muscle	Tumor
T_1 = 1 h	0.25	0.19	4.0	5.3	0.07	0.76
T_2 = 2 h	0.04	0.19	1.3	23	0.01	1.2
T_3 = 4 h	0.04	0.12	0.70	2.8	0.01	1.0
T_4 = 20 h	0.02	0.08	0.15	0.44	0.01	0.92
T_5 = 45 h	0.01	0.06	0.08	0.11	0.00	0.66

Supplemental Table 1. Mean standardized uptake values (mSUV) derived from the time-activity curves from small-animal PET of ^{64}Cu-CA003 in a BALB/c *nu/nu* mouse bearing a C4-2 tumor xenograft.

meanSUV	Heart	Liver	Kidneys	Bladder	Muscle	Tumor
T_1 = 1 h	0.30	1.8	1.7	9.6	0.20	0.67
T_2 = 2 h	0.25	1.6	0.84	3.0	0.10	0.5
T_3 = 4 h	0.21	2.0	0.64	0.19	0.09	0.86
T_4 = 20 h	0.25	1.5	0.44	0.12	0.07	0.64
T_5 = 45 h	0.21	1.2	0.34	0.10	0.06	0.46

Supplemental Table 2. Mean standardized uptake values (mSUV) derived from the time-activity curves from small-animal PET of ^{64}Cu-PSMA-617 in a BALB/c *nu/nu* mouse bearing a C4-2 tumor xenograft.

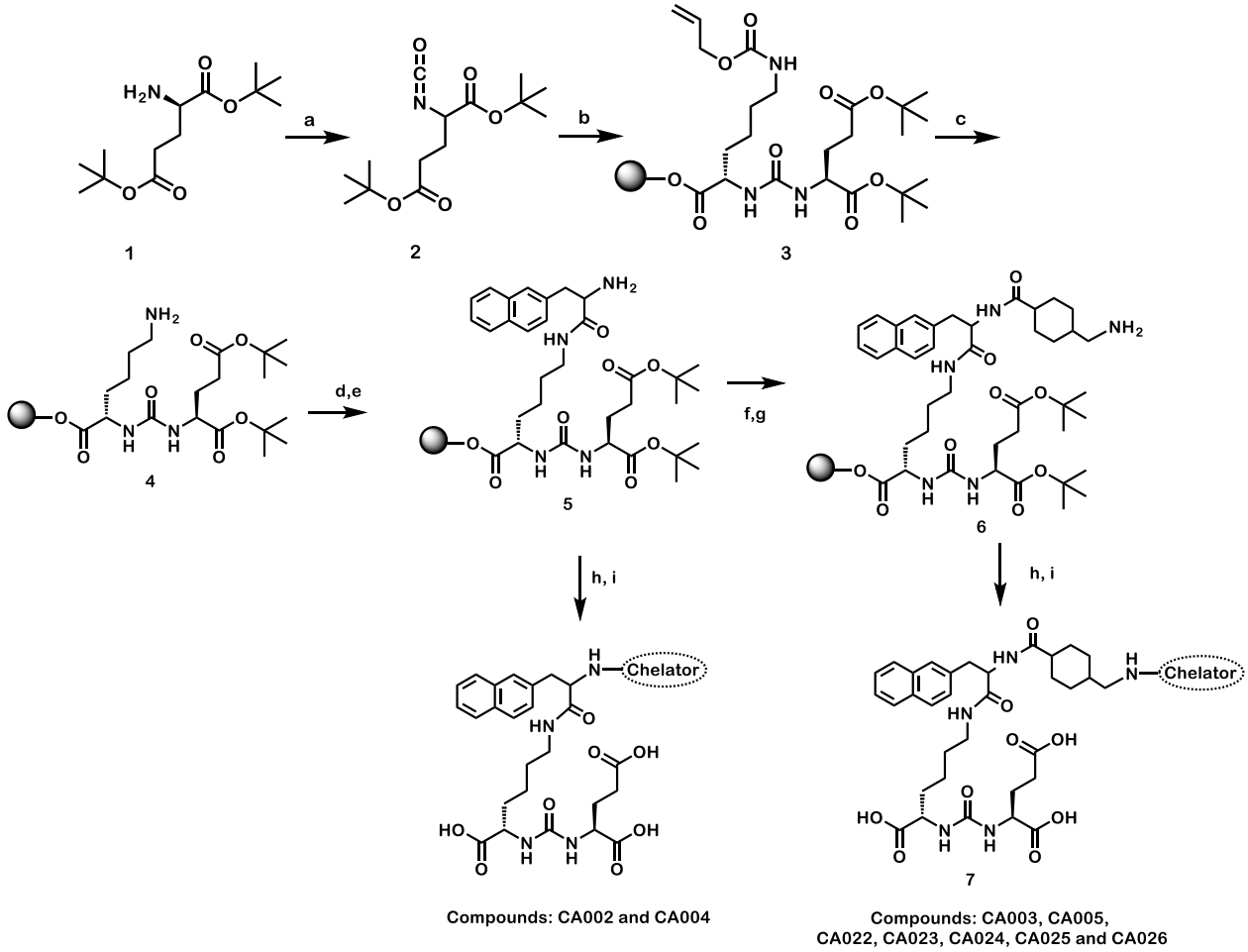
Organ Distribution for ^{64}Cu-CA003 in Tumor Bearing Mice

Tissue	Uptake (% ID/g) in:					
	10 min	1 h	1 h (block)	4 h	24 h	72 h
Blood	5 ± 2	3 ± 2	2 ± 1	1 ± 0	0 ± 0	0 ± 0
Heart	2 ± 1	1 ± 1	1 ± 0	1 ± 0	0 ± 0	0 ± 0
Lung	3 ± 1	3 ± 2	2 ± 1	2 ± 1	0 ± 0	0 ± 0
Spleen	4 ± 0	3 ± 2	1 ± 0	2 ± 1	0 ± 0	0 ± 0
Liver	2 ± 0	4 ± 2	2 ± 0	3 ± 1	1 ± 0	1 ± 0
Kidneys	44 ± 28	67 ± 21	4 ± 1	13 ± 3	8 ± 9	0 ± 0
Muscle	1 ± 0	1 ± 0	1 ± 0	1 ± 1	0 ± 0	0 ± 0
Small intestine	2 ± 1	2 ± 1	2 ± 2	1 ± 1	0 ± 0	0 ± 0
Brain	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Tumor	11 ± 4	31 ± 13	2 ± 0	32 ± 11	20 ± 6	4 ± 1

Organ distribution of 0.025 nmol of ^{64}Cu-CA003 at the time points: 10 min, 1 h, 4 h, 24 h and 72 h after injection. Values are expressed the range of % ID/g of tissue ± standard deviation; n = 3 for all tissues. In this blockade experiment, the radiotracer ^{64}Cu-CA003 (0.030 nmol) was injected at the same time as 2 mg of PSMA-617 per kilogram of body weight

Supplemental Table 3. Results of Organ Distribution experiment of ^{64}Cu-CA003.

Synthesis



Supplemental Figure 2. Reaction scheme for the synthesis of the PSMA-chelator conjugates. (a) triphosgene, DIPEA, CH_2Cl_2, 0 °C; (b) H-Lys(Alloc)-2CT-resin, CH_2Cl_2; (c) $Pd[P(C_6H_5)_3]_4$, morpholine, CH_2Cl_2; (d) Fmoc-2-Nal-OH, HBTU, DIPEA, DMF; (e) 50% piperidine, DMF; (f) trans-4-(Fmoc-aminomethyl)cyclohexanecarboxylic acid, Oxyma Pure, DIC, DMF; (g) 20% piperidine, DMF; (h) chelator, DIPEA, DMF; (i) 95% TFA, 2.5% H_2O and 2.5% TIPS.

The PSMA-binding motif was prepared by solid-phase synthesis on a 2-chlorotrityl resin (2 CT-resin). For this purpose Fmoc-Lys(Alloc)-OH was immobilized on an equimolar amount of 2-chlorotrityl resin. Afterwards, the isocyanate (2) of the glutamyl moiety by was generated using triphosgene. The ε-allyloxycarbonyl-protected lysine immobilized on 2-chloro-tritylresin was added and reacted for 16 h with careful agitation resulting in compound 3. The resin was filtered off and the allyloxycarbonyl-protecting group was cleaved to obtain (4). The coupling of Fmoc-2-naphthyl-L-alanine was proceeded to obtain (5).

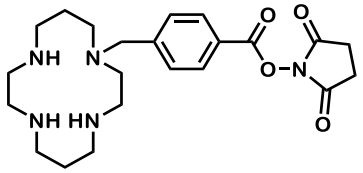
In order to obtain compounds CA002 and CA004 the respective chelator was coupled to this intermediate. Subsequently, the PSMA coupled to the chelator was cleaved from the resin. Alternatively, *trans*-4-(Fmoc-aminomethyl)-cyclohexanecarboxylic acid was coupled to obtain (6), the compound to which the respective chelator was coupled to obtain compounds CA003, CA005, CA022, CA023, CA024, CA025, CA026. Subsequently, the PSMA coupled to the chelator was cleaved from the resin. The compounds were evaluated by HPLC and MS-LC. The substances were isolated by preparative HPLC using water-acetonitrile gradients containing trifluoroacetic acid. For this, the compounds were purified using a gradient of 20–50% of acetonitrile in water over 15 min. The purified compounds were analyzed by analytical HPLC (0–100%) acetonitrile in water containing trifluoroacetic acid over 5 min, Monolith RP HPLC column 100 × 3 mm and LC/MS. The product fractions were pooled and lyophilized.

Specification for (CA002)

The product was obtained by incubating the resin (compound 5) with 1.5 equivalents of CTPA-NHS-ester (4-[(1,4,8,11-tetraazacyclotetradec-1-yl)-methyl] benzoic acid) and 10 equivalents of *N*,*N*-diisopropylamine (DIPEA) in 500 µL of *N*,*N*-dimethylformamide (DMF). The compound was purified and the final product was analyzed by HPLC as described above. HPLC-retention time: 2.38 min; ESI-MS (m/z): $[M+H]^+$ (calculated $C_{43}H_{61}N_8O_9$): 833.42 (833.45).

Specification for CA003

The product was obtained by incubating the resin (compound 6) with 1.5 equivalents of CTPA-NHS-ester (4-[(1,4,8,11-tetraazacyclotetradec-1-yl)-methyl] benzoic acid) and 10 equivalents of DIPEA in 500 µL of DMF. The compound was purified and the final product was analyzed by HPLC as described above. HPLC-retention time: 2.40 min; ESI-MS (m/z): $[M+H]^+$ (calculated $C_{51}H_{74}N_9O_{10}$): 972.52 (972.55)



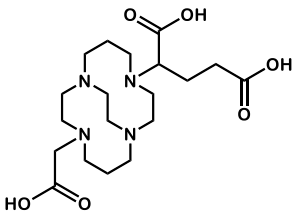
Supplemental Figure 3. Chemical structure of the chelator CTPA-NHS-ester, the compound used in the synthesis of CA002 and CA003.

Specification for CA004

The product was obtained by incubating the resin (compound 5) with 1.5 equivalents of cross bridged-TE2A chelator, 0.98 × n chelator 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 10 equivalents of DIPEA in 500 µL of DMF. The compound was purified and the final product was analyzed by HPLC as described above. HPLC-retention time: 2.12 min; ESI-MS (m/z): $[M+H]^+$ (calculated $C_{44}H_{64}N_8O_{13}$): 913.45 (913.47)

Specification for CA005

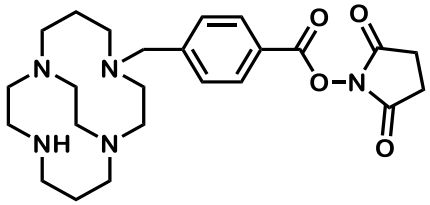
The product was obtained by incubating the resin (compound 6) with 1.5 equivalents of cross bridged-TE2A chelator, 0.98 × n chelator HBTU and 10 equivalents of DIPEA in 500 µL of DMF. The compound was purified and the final product was analyzed by HPLC as described above. HPLC-retention time: 2.33 min; ESI-MS (m/z): $[M+H]^+$ (calculated $C_{52}H_{78}N_9O_{14}$): 1052.62 (1052.56)



Supplemental Figure 4. Chemical structure of the chelator 8-carboxymethyl-cross bridged-TE2A, the compound used in the synthesis of CA005 and CA006.

Specification for CA022

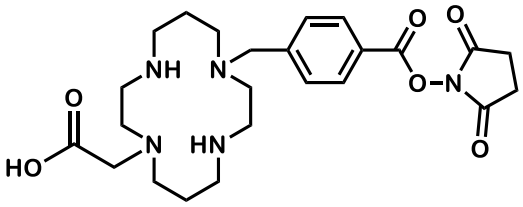
The product was obtained by incubating the resin (compound 6) with 1.5 equivalents of cross bridged-CTPA chelator and 10 equivalents of DIPEA in 500 µl of DMF. The compound was purified and the final product was analyzed by HPLC as described above. HPLC-retention time: 2.40 min; ESI-MS (m/z): $[M+H]^+$ (calculated $C_{53}H_{76}N_9O_{10}$): 998.56 (998.57)



Supplemental Figure 5. Chemical structure of the chelator cross-bridged-CTPA, the compound used in the synthesis of CA022

Specification CA023

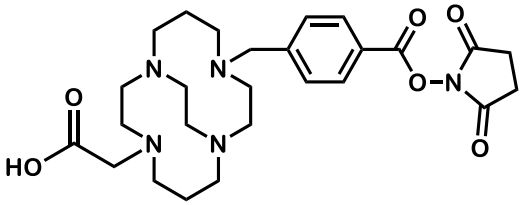
The product was obtained by incubating the resin (compound 6) with 1.5 equivalents of 8-carboxymethyl-CTPA chelator and 10 equivalents of DIPEA in 500 µL of DMF. The compound was purified and the final product was analyzed by HPLC as described above. HPLC-retention time: 2.39 min; ESI-MS (m/z): $[M+H]^+$ (calculated $C_{53}H_{76}N_9O_{12}$): 1030.55 (1030.56)



Supplemental Figure 6. Chemical structure of the chelator 8-carboxymethyl-CTPA, the compound used in the synthesis of CA023.

Specification for CA024

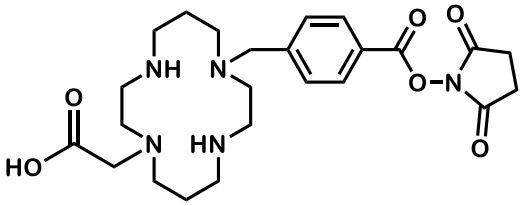
The product was obtained by incubating the resin (compound 6) with 1.5 equivalents of 8-carboxymethyl-cross bridged-CTPA chelator and 10 equivalents of DIPEA in 500 µL of DMF. The compound was purified and the final product was analyzed by HPLC as described above. HPLC-retention time: 2.46 min; ESI-MS (m/z): $[M+H]^+$ (calculated $C_{55}H_{78}N_9O_{12}$): 1056.56 (1056.57)



Supplemental Figure 7. Chemical structure of the chelator 8-carboxymethyl-cross bridged-CTPA, the compound used in the synthesis of CA024.

Specification for CA025

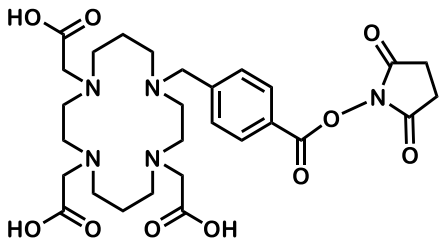
The product was obtained by incubating the resin (compound 6) with 1.5 equivalents of 8,11-bis(carboxymethyl)-CTPA chelator [CPTA = 4-[(1,4,8,11-tetraazacyclotetradec-1-yl)methyl]benzoic acid] and 10 equivalents of DIPEA in 500 µL of DMF. The compound was purified and the final product was analyzed by HPLC as described above. HPLC-retention time: 2.41 min; ESI-MS (m/z): $[M+H]^+$ (calculated $C_{55}H_{78}N_9O_{14}$): 1088.55 (1088.56)



Supplemental Figure 8. Chemical structure of the chelator 8,11- bis(carboxymethyl)-CTPA, the compound used in the synthesis of CA025.

Specification for CA026

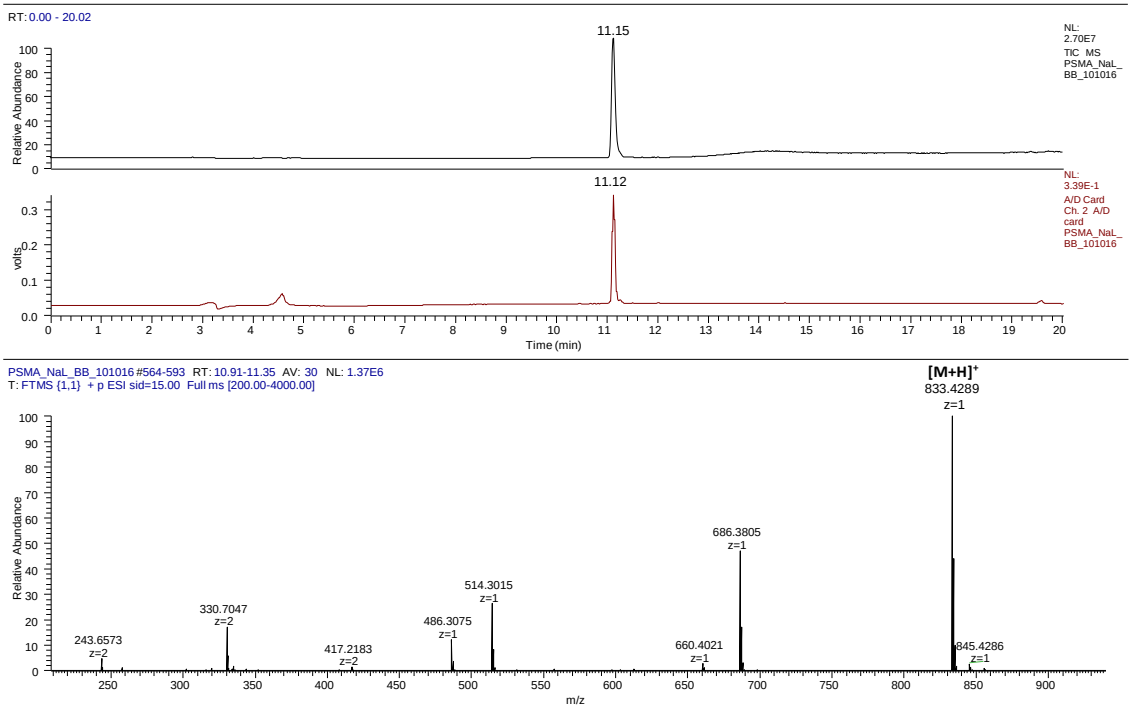
The product was obtained by incubating the resin (compound 6) with 1.5 equivalents of 8,11-bis(carboxymethyl)-CTPA chelator and 10 equivalents of DIPEA in 500 µL of DMF. The compound was purified and the final product was analyzed by HPLC as described above. HPLC-retention time: 2.41 min; ESI-MS (m/z): $[M+H]^+$ (calculated $C_{57}H_{80}N_9O_{16}$): 1146.56 (1146.57).



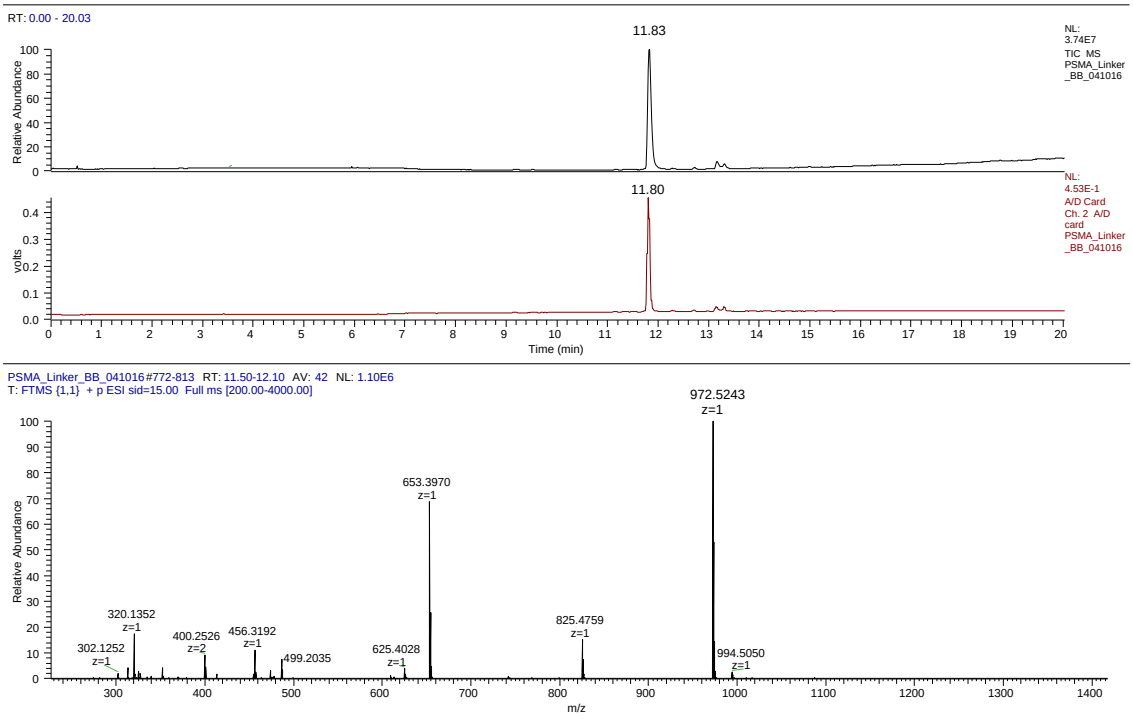
Supplemental Figure 9. Chemical structure of the chelator 4, 8,11-tris(carboxymethyl)-CTPA, the compound used in the synthesis of CA026.

Supplemental Figures 10-18. HPLC/MS analysis of the non-radioactive PSMA inhibitor. HPLC-MS (0-100% acetonitrile in water containing 0.05% trifluoroacetic acid) within 20 min on a Gold aq 200 × 2.1 mm column at a flow rate of 200 µL/min.

CA002



CA003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NL:
3.74E7
TIC MS
PSMA_Linker
_BB_041016
Relative Abundance
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NL:
4.53E-1
A/D Card
Ch. 2 A/D
card
PSMA_Linker
_BB_041016
volts
Time (min)
PSMA_Linker_BB_041016 #772-813 RT: 11.50-12.10 AV: 42 NL: 1.10E6
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653.3970
z=1
320.1352
z=1
400.2526
z=2
456.3192
z=1
302.1252
z=1
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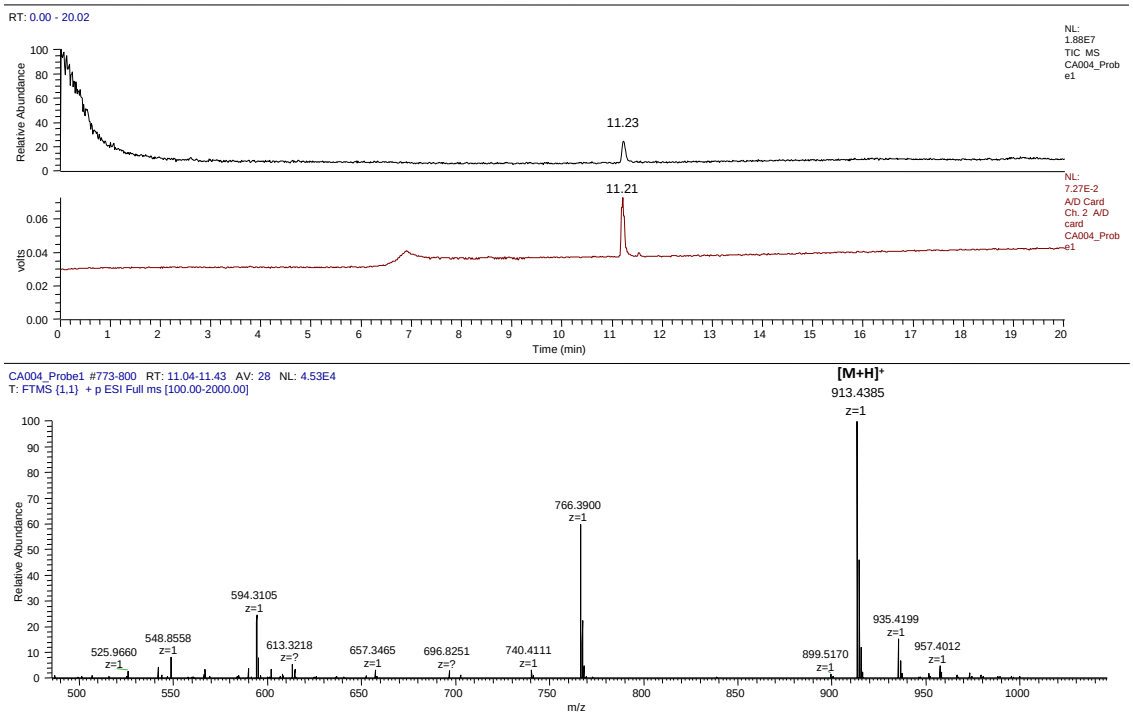
625.4028
z=1
825.4759
z=1
994.5050
z=1
Relative Abundance
m/z

CA004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NL:
1.88E7
TIC MS
CA004_Prob
e1
11.23
Relative Abundance
11.21
NL:
7.27E-2
A/D Card
Ch. 2 A/D
card
CA004_Prob
e1
volts
Time (min)
CA004_Probe1 #773-800 RT: 11.04-11.43 AV: 28 NL: 4.53E4
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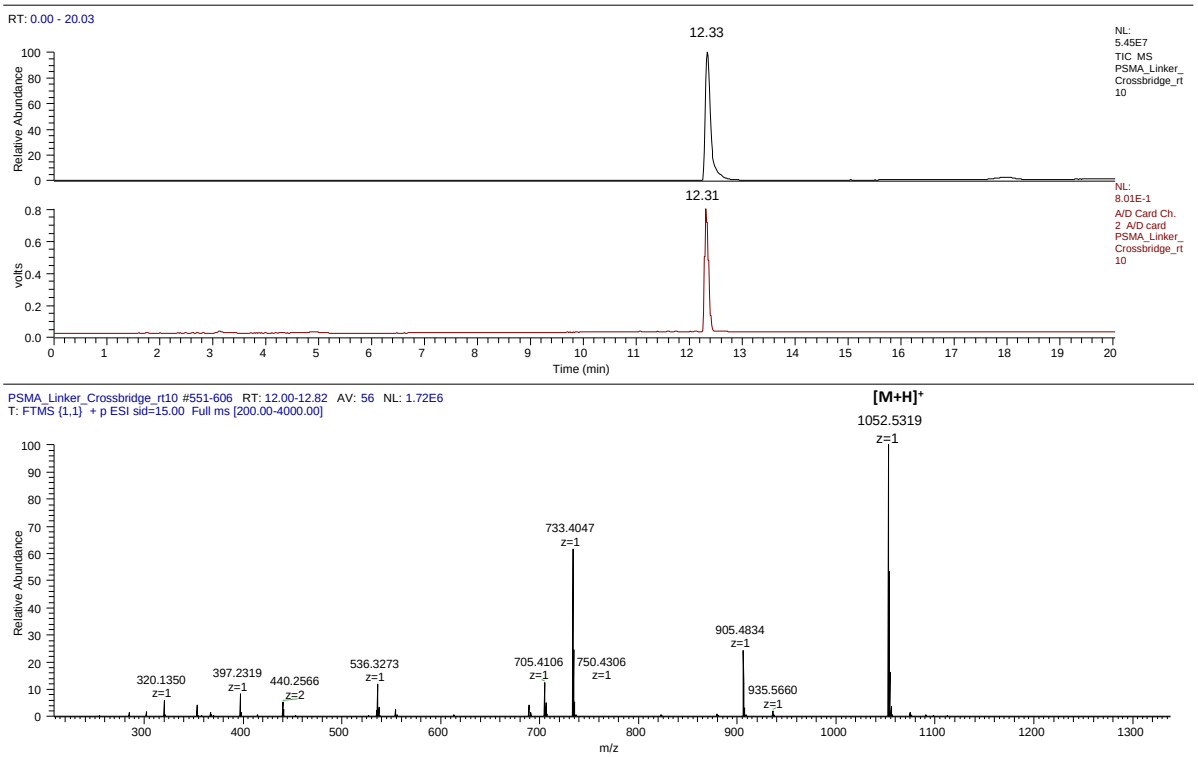
z=1
766.3900
z=1
594.3105
z=1
548.8558
z=1
525.9660
z=1
613.3218
z=?
657.3465
z=1
696.8251
z=?
740.4111
z=1
935.4199
z=1
899.5170
z=1
957.4012
z=1
Relative Abundance
m/z

CA005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12.33
NL:
5.45E7
TIC MS
PSMA_Linker_
Crossbridge_rt
10
Relative Abundance
12.31
NL:
8.01E-1
A/D Card Ch.
2 A/D card
PSMA_Linker_
Crossbridge_rt
10
volts
Time (min)
PSMA_Linker_Crossbridge_rt10 #551-606 RT: 12.00-12.82 AV: 56 NL: 1.72E6
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733.4047
z=1
905.4834
z=1
320.1350
z=1
397.2319
z=1
440.2566
z=2
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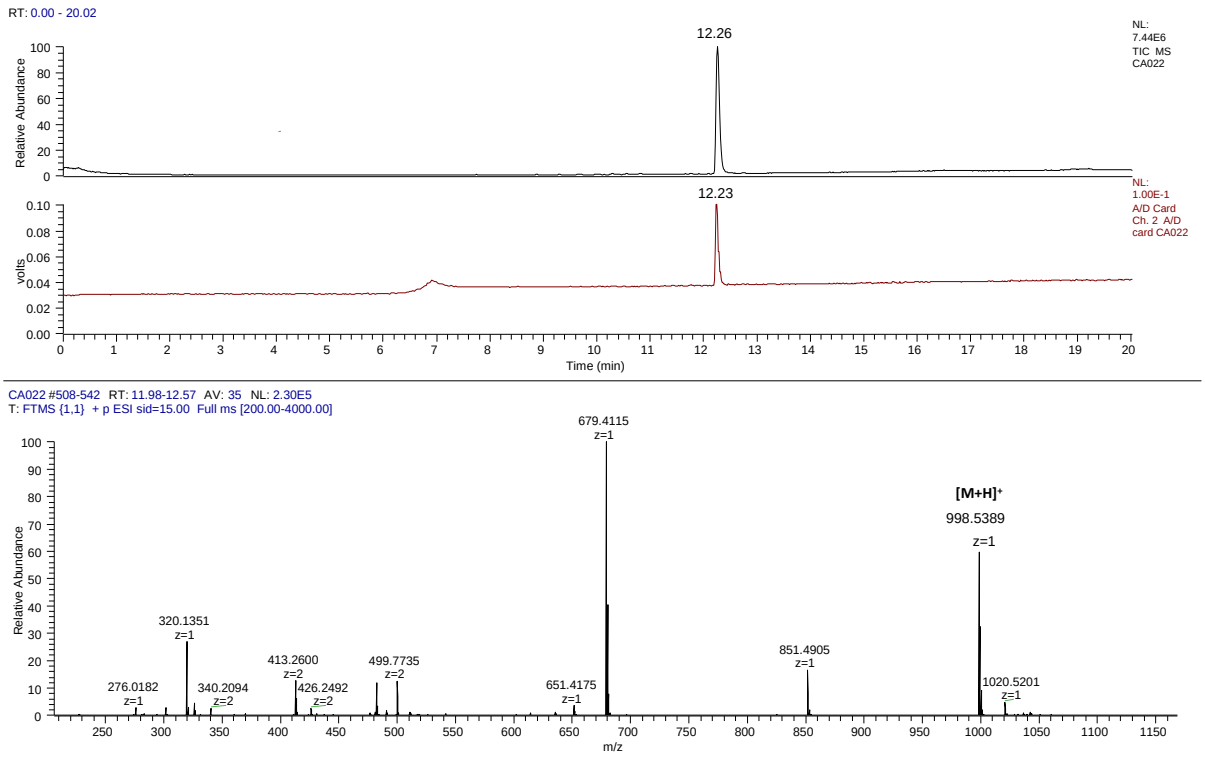
z=1
705.4106
z=1
750.4306
z=1
935.5660
z=1
m/z

CA022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12.26
NL:
7.44E6
TIC MS
CA022
Relative Abundance
12.23
NL:
1.00E-1
A/D Card
Ch. 2 A/D
card CA022
volts
Time (min)
CA022 #508-542 RT: 11.98-12.57 AV: 35 NL: 2.30E5
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320.1351
z=1
276.0182
z=1
340.2094
z=2
413.2600
z=2
426.2492
z=2
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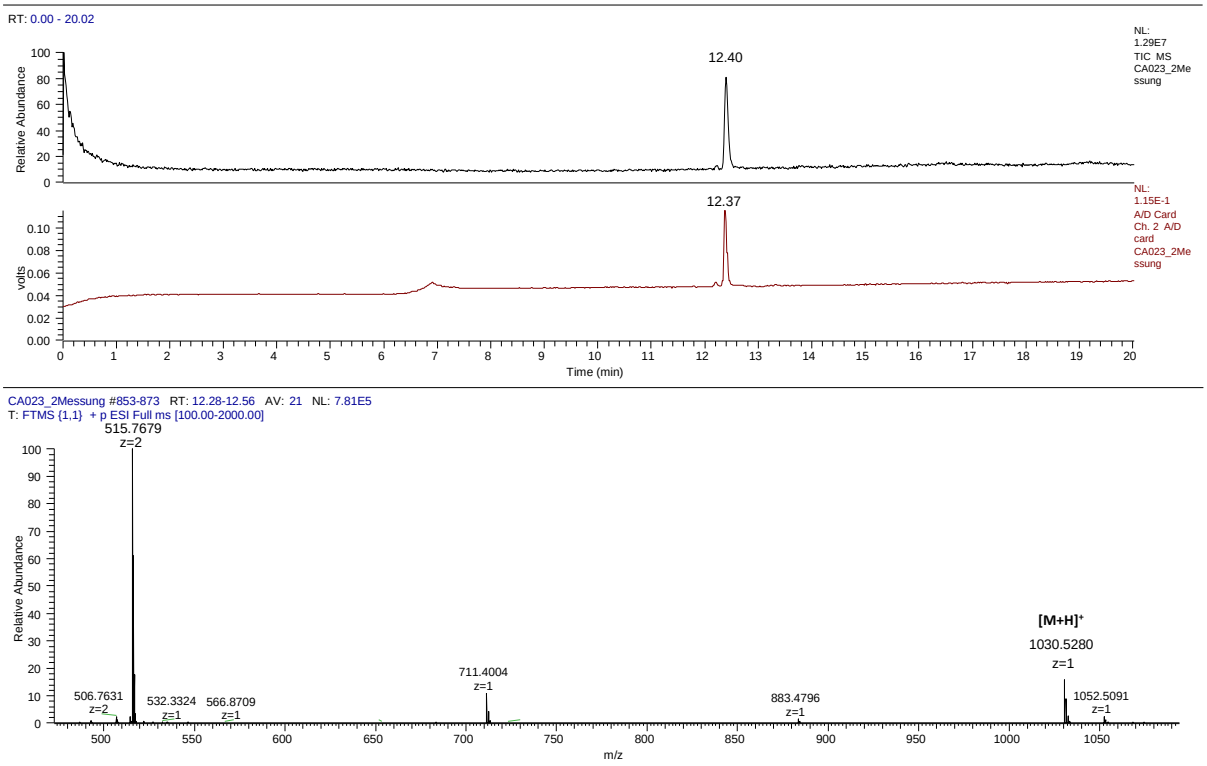
m/z

CA023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12.40
NL:
1.29E7
TIC MS
CA023_2Me
ssung
Relative Abundance
12.37
NL:
1.15E-1
A/D Card
Ch. 2 A/D
card
CA023_2Me
ssung
volts
Time (min)

CA023_2Messung #853-873 RT: 12.28-12.56 AV: 21 NL: 7.81E5
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[M+H]+
1030.5280
z=1
711.4004
z=1
506.7631
z=2
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z=1
566.8709
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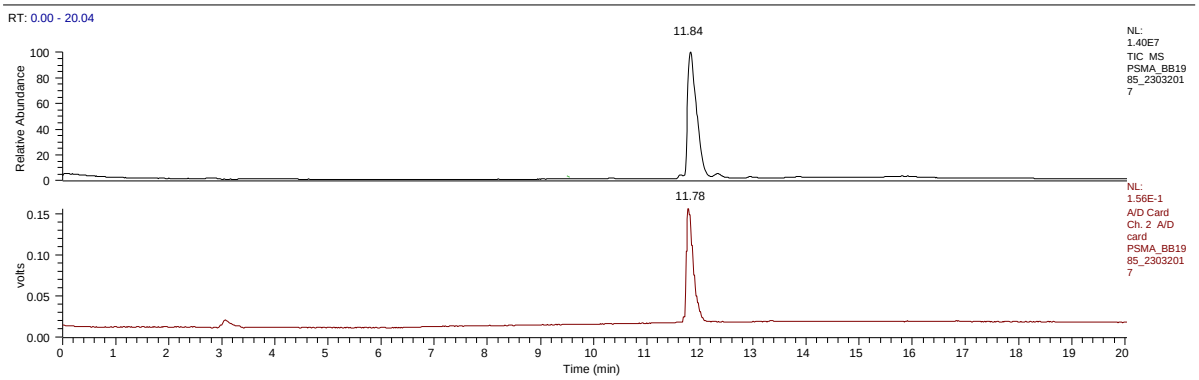
883.4796
z=1
1052.5091
z=1
Relative Abundance
m/z

CA024


RT: 0.00 - 20.04
11.84
NL:
1.40E7
TIC MS
PSMA_BB19
85_2303201
7
Relative Abundance
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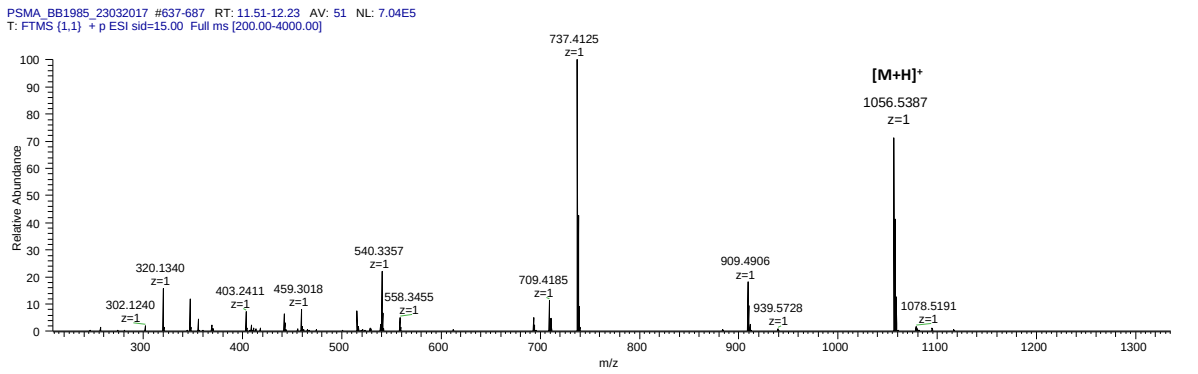
NL:
1.56E-1
A/D Card
Ch. 2 A/D
card
PSMA_BB19
85_2303201
7
volts
Time (min)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z=1
540.3357
z=1
320.1340
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558.3455
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709.4185
z=1
909.4906
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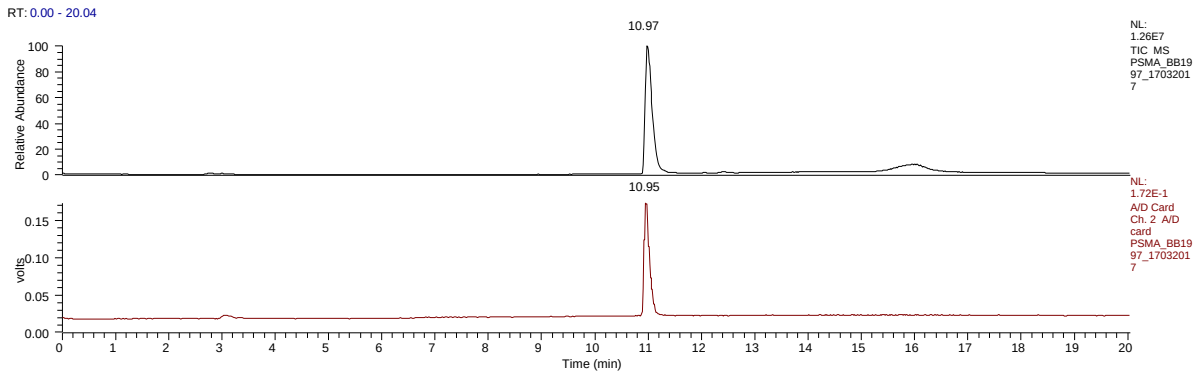
1078.5191
z=1
Relative Abundance
m/z

CA025


RT: 0.00 - 20.04
10.97
NL:
1.26E7
TIC MS
PSMA_BB19
97_1703201
7
Relative Abundance
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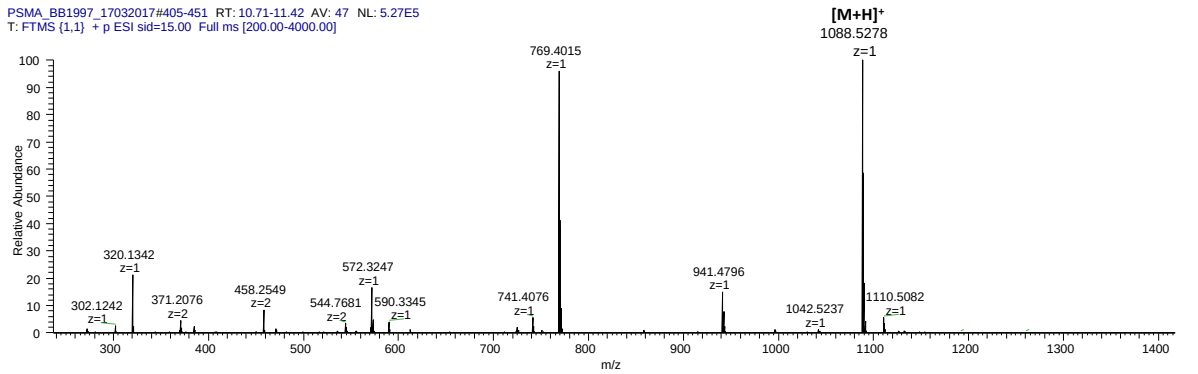
NL:
1.72E-1
A/D Card
Ch. 2 A/D
card
PSMA_BB19
97_1703201
7
volts
Time (min)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320.1342
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z=2
590.3345
z=1
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1110.5082
z=1
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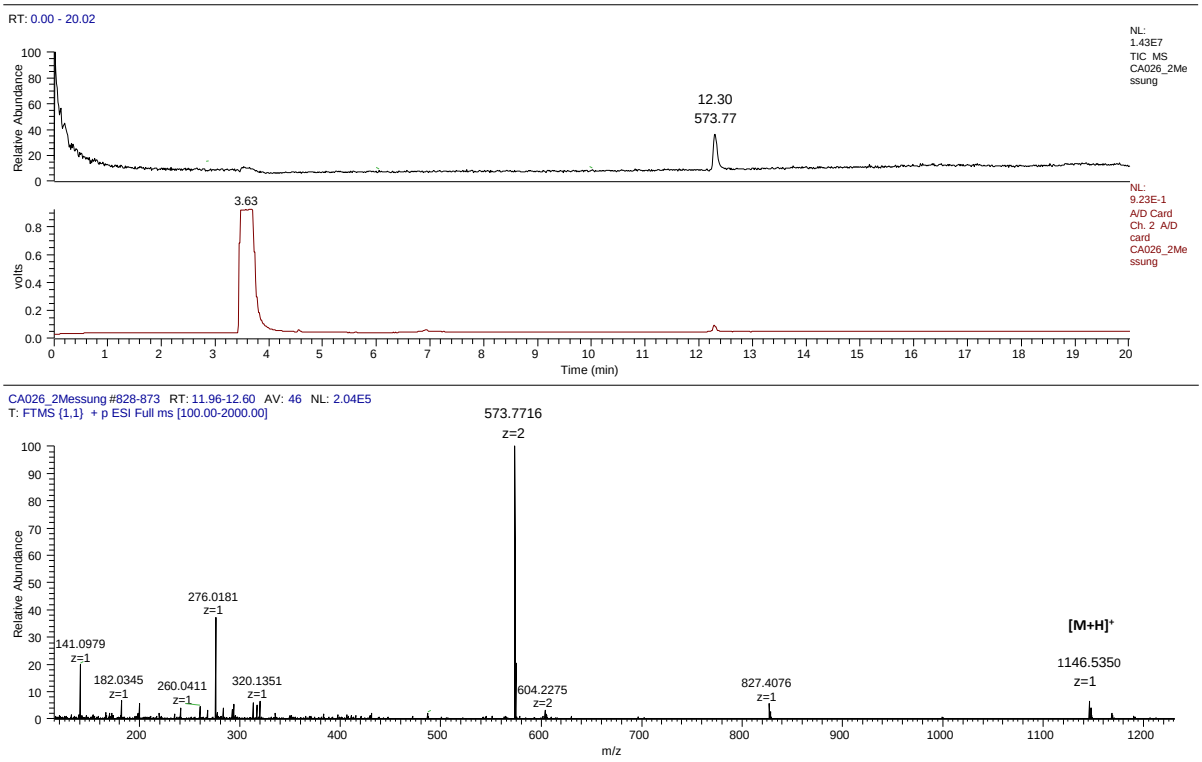
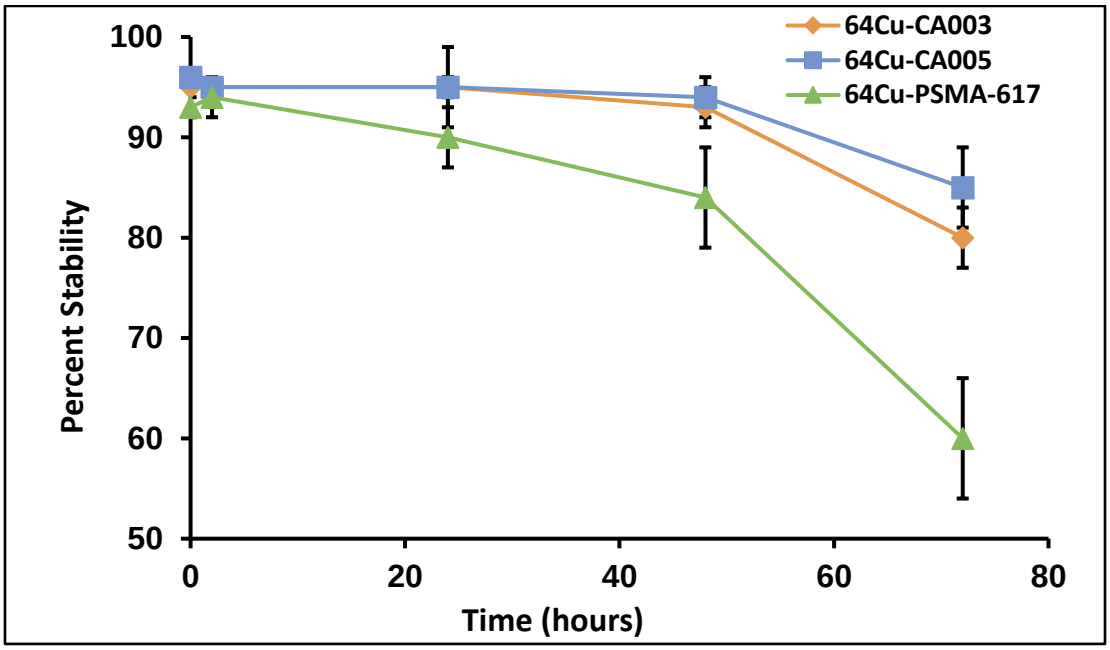
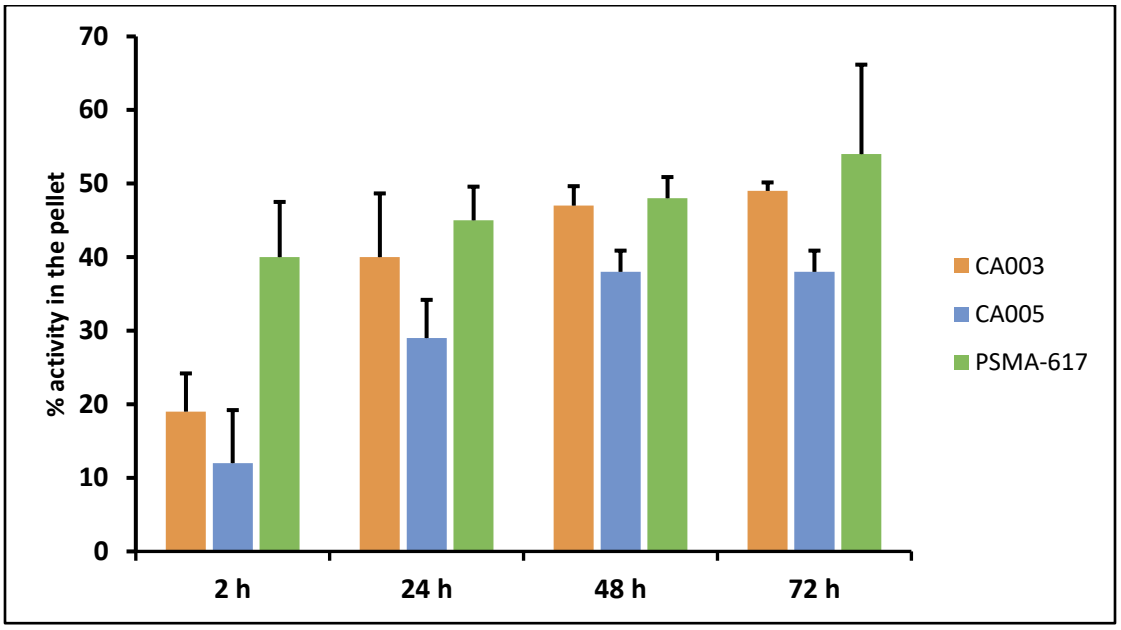
Relative Abundance
m/z

CA026

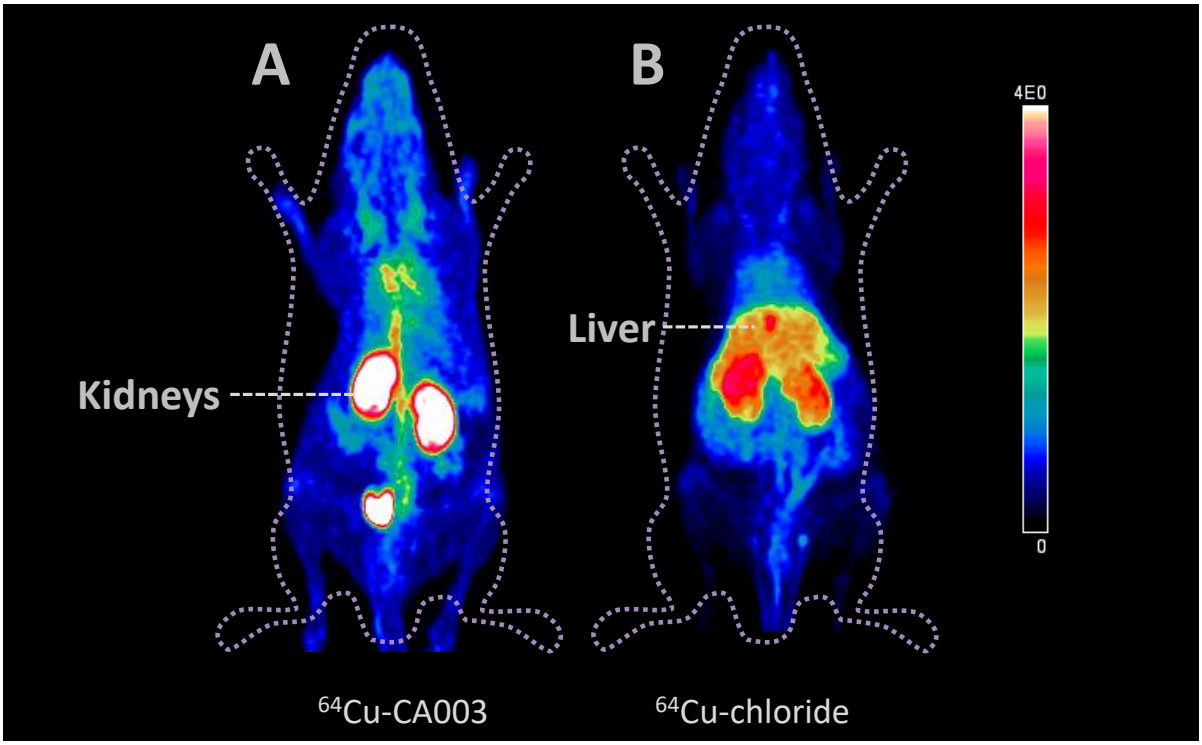

RT: 0.00 - 20.02
NL:
1.43E7
TIC MS
CA026_2Me
ssung
Relative Abundance
12.30
573.77
3.63
NL:
9.23E-1
A/D Card
Ch. 2 A/D
card
CA026_2Me
ssung
volts
Time (min)
CA026_2Messung #828-873 RT: 11.96-12.60 AV: 46 NL: 2.04E5
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]
573.7716
z=2
276.0181
z=1
141.0979
z=1
182.0345
z=1
260.0411
z=1
320.1351
z=1
604.2275
z=2
827.4076
z=1
[M+H]+
1146.5350
z=1
m/z



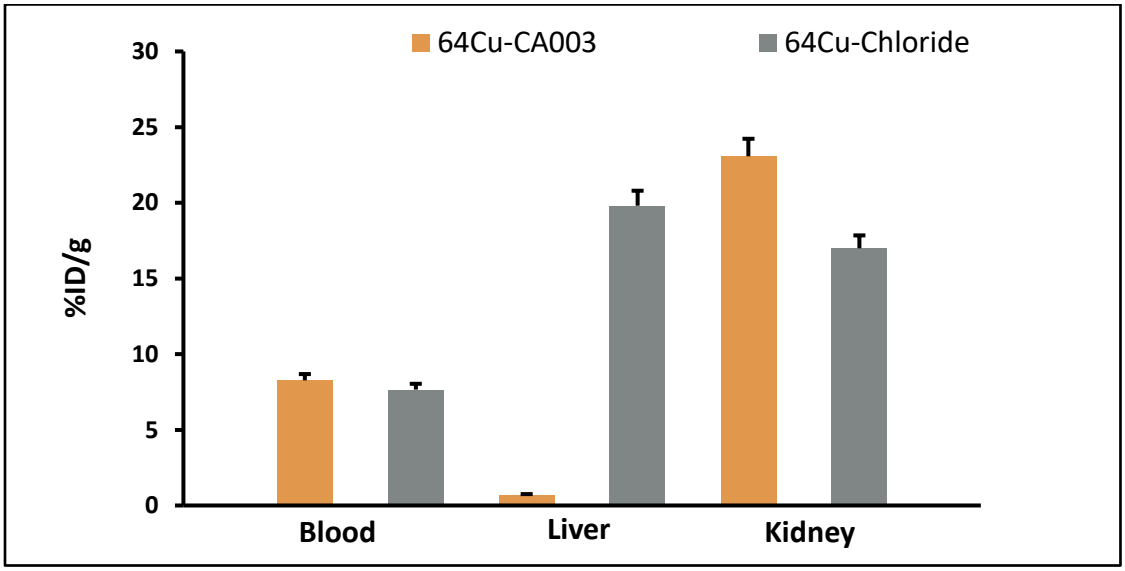
Supplemental Figure 19. Serum stability of ^{64}Cu-CA003, ^{64}Cu-CA005 and ^{64}Cu-PSMA-617 at 37 °C over 72 h (mean ± SD, n = 4) as determined by radio-ITLC.



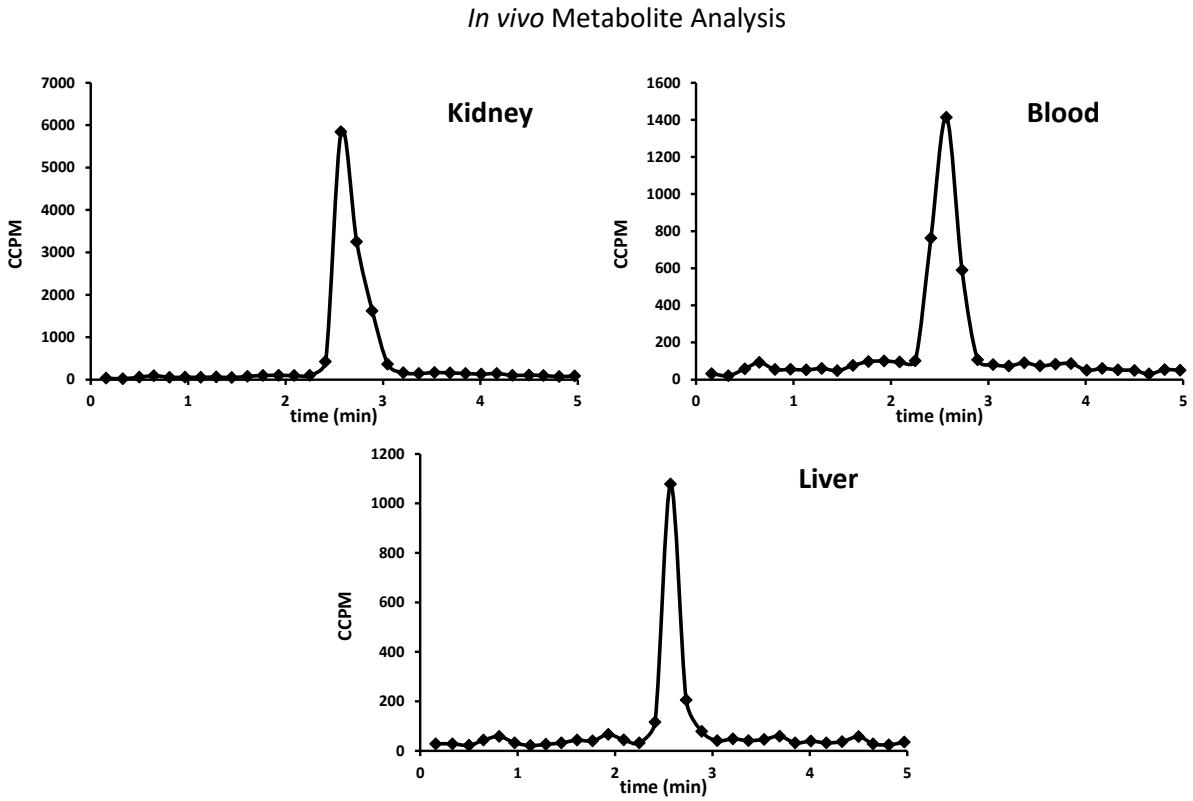
Supplemental Figure 20. Serum stability of ^{64}Cu-CA003, ^{64}Cu-CA005 and ^{64}Cu-PSMA-617 at 37 °C over 72 h (mean ± SD, n = 4) as determined by activity measurement.



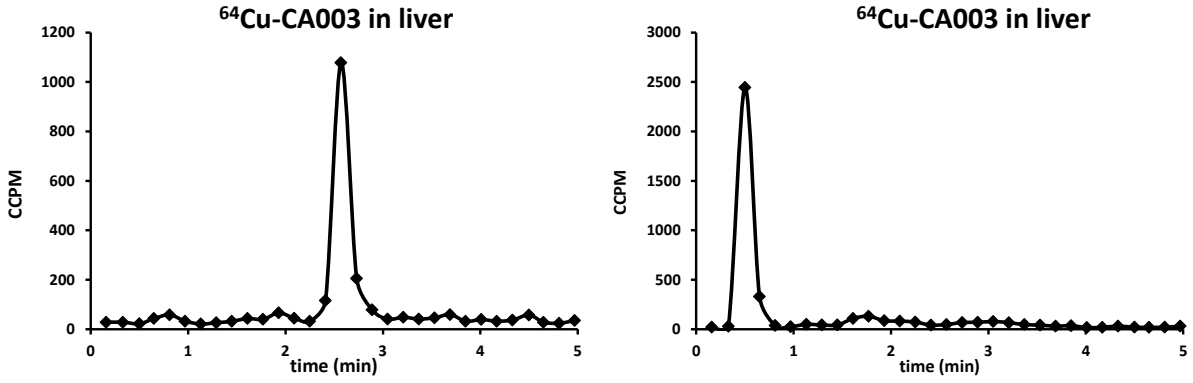
Supplemental Figure 21. (A) PET image of 9 MBq (0.30 nmol) ^{64}Cu-CA003 10 min post injection in a female Swiss mouse. The maximum intensity projection (MIP) illustrates circulation in the blood and renal uptake. (B) PET image of a female Swiss mouse at 10 min p.i. of 10 MBq ^{64}Cu 10 min post injection. The maximum intensity projection (MIP) illustrates a strong uptake in the liver and the kidneys.



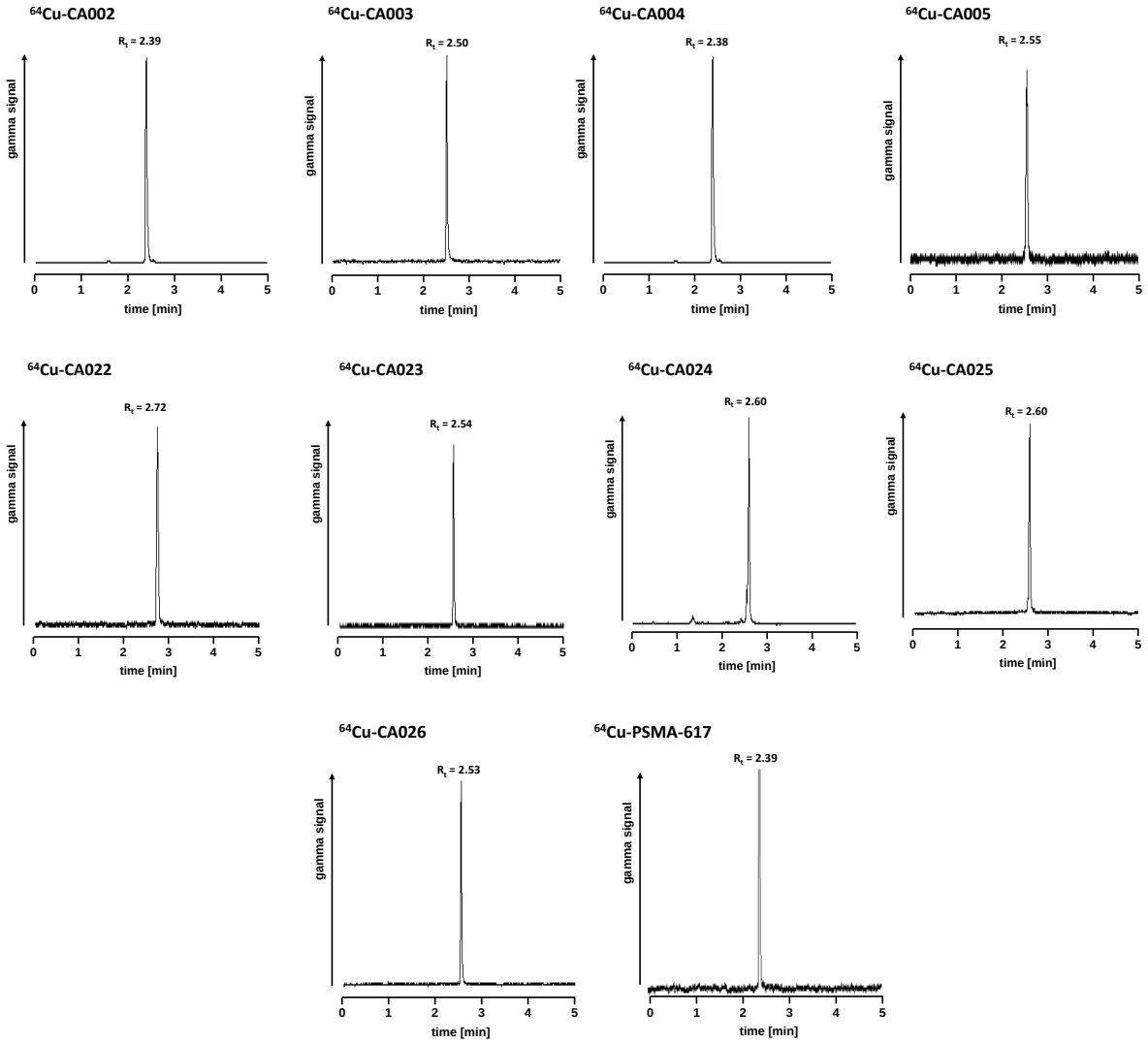
Supplemental Figure 22. Organ distribution of approximately 10 MBq (0.30 nmol) ^{64}Cu-CA003 and 10 MBq ^{64}Cu-chloride at 10 min post injection in non tumor bearing female Swiss mice (n = 3).



Supplemental Figure 23. *In vivo* metabolite analysis of ^{64}Cu-CA003 in a BALB/c nude mouse (no tumor) at 10 min *p.i.* Radio-HPLC chromatograms of extracts from the kidney, the blood and the liver show that the activity elutes at the retention time of the intact tracer. This proves the integrity of the copper complex within the main distribution period.



Supplemental Figure 24. Radio-HPLC chromatograms of extracts of ^{64}Cu-CA003 in liver in comparison with of ^{64}Cu-chloride in the liver in a BALB/c nude mouse (no tumor) at 10 min *p.i.*



Supplemental Figure 25. Radio-HPLC chromatograms of the novel compounds labeled with ^{64}Cu.