

Evaluating the Therapeutic Efficacy of Gastramide Theranostics Targeting Cholecystokinin-2 Receptors in a Preclinical Setting

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Supplemental Materials

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General Chemistry Methods:

Confirmation of peptide identity and purity as analyzed Analytical Radio-HPLC was performed using a Shimadzu HPLC system consisting of a SCL-10AVP system controller, SIL-0ADVP auto-injector, LC-10 ATVP solvent delivery unit, CV-10AL control valve, DGU-14A degasser, and SPD-10AVPV detector. This was coupled to a radiation detector consisting of an Ortec model 276 photomultiplier base with a 925-SCINTACE-mate preamplifier, amplifier, bias supply, SCA and a Bicron 1M11/2 photomultiplier tube. [^{177}Lu] LuCl_3 was calibrated using the CRC-15PET dose calibrator (Capintec). This was calibrated daily using Cs-137 and Co-57 sources (Isotope Products Laboratories).

Radiochemical purity was determined using a Kinetex XB-C18 column (5 μm , 100 $\text{\AA}$, 250 mm $\times$ 4.60 mm) with product eluted at 1 mL/min with a gradient of MeCN: 0.05% (v/v) TFA, commencing at 25% MeCN for 1 min, increased to 35% over 9 min, then immediately to 90%, maintained for 2 min, then returned to 25% MeCN. Specific activity (A_s) and molar activity (A_M) is determined at the end of synthesis (EOS).

Coupling of DOTA to peptides CP04, GA4 and GA13

DOTA (1.5 eq. relative to peptide) was pre-activated with *N*-hydroxysuccinimide (NHS) (2.25 eq.) using *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI) (2.25 eq.) and *N,N*-diisopropylethylamide (DIPEA) (3 eq.) in anhydrous dimethyl sulfoxide (DMSO) (500 μL). The reaction mixture was heated and gently sonicated at 50 $^{\circ}\text{C}$ for 30 min until starting material was dissolved. Peptides CP04, GA4 and GA13 (1 eq.) were dissolved in a minimum volume of anhydrous DMSO ($\sim$ 200–500 μL), then crude mixture was shaken for 30 min at room temperature. Reaction progress was monitored by LC-MS analysis, until completion was observed (usually

within 1 hour), observed by LC-MS analysis with the loss of the m/z peak of the starting peptide. Distilled water was added to form a 20:80 mixture of DMSO:water then crude peptide was purified using reverse phase HPLC.

Radiolabeling of peptides with ^{177}Lu

DOTA-peptides DOTA-CP04, DOTA-GA4, and DOTA-GA13 (30 μg , 14.6 nmol) were dissolved in a 0.5 M sodium acetate solution (100 μL , resulting in a peptide concentration of 0.3 $\mu\text{g}/\mu\text{L}$). This solution was then combined with 200 μL of 0.4 M ammonium acetate/0.24 M 2,5-dihydroxybenzoic acid (pH 4.5), along with 50 μL of ethanol and 50 μL of L-methionine (10 mg/mL solution in MilliQ water). Next, 500-1000 MBq of noncarrier-added [^{177}Lu]LuCl₃ in 0.04 M HCl (50-100 μL) was added to the crude mixture, which was then heated to 80 °C for 30 minutes. This process yielded the ^{177}Lu -labeled peptides, [^{177}Lu]Lu-CP04, [^{177}Lu]Lu-DOTA-GA4, and [^{177}Lu]Lu-DOTA-GA13 (Figure S1), with radiochemical purity of $\geq 94\%$ and quantitative yields (Table S1). All products showed over 90% purity by HPLC and TLC at 48 hours following the end of synthesis.

Table S1: Analytical data of [^{177}Lu]Lu-DOTA-CP04 , [^{177}Lu]Lu-DOTA-GA4 , [^{177}Lu]Lu-DOTA-GA13

Parameters	Acceptable specifications	[^{177}Lu]Lu-CP04	[^{177}Lu]Lu-DOTA-GA4	[^{177}Lu]Lu-DOTA-GA13
Appearance	Clear & colourless	pass	pass	pass
pH	4-8	5-6	5-6	5-6
Radiochemical purity (HPLC) ¹	$\geq 90\%$ labelled peptide	$\geq 94\%$	99%	99%
Radiochemical purity (TLC)	$\geq 98\%$ labelled peptide	$>98\%$	$>98\%$	$>98\%$
	$\leq 2\%$ free ^{177}Lu	$<2\%$	$<2\%$	$<2\%$
Specific Activity	≥ 3.0 MBq/ μg	16-35 MBq/ μg	16-35 MBq/ μg	16-35 MBq/ μg

¹HPLC conditions 1: Kinetex XB C18 column (5 μ m, 100 Å, 250 $\times$ 4.60 mm) eluted at 1 mL/min with a gradient of MeCN: 0.05% (v/v) TFA, starting at 25% MeCN for 1 min, increased to 35% over 9 min and then instantly increased to 90% and maintained at that for 2 min then back down to 25% MeCN.

Radio-HPLC trace of [¹⁷⁷Lu]Lu-CP04

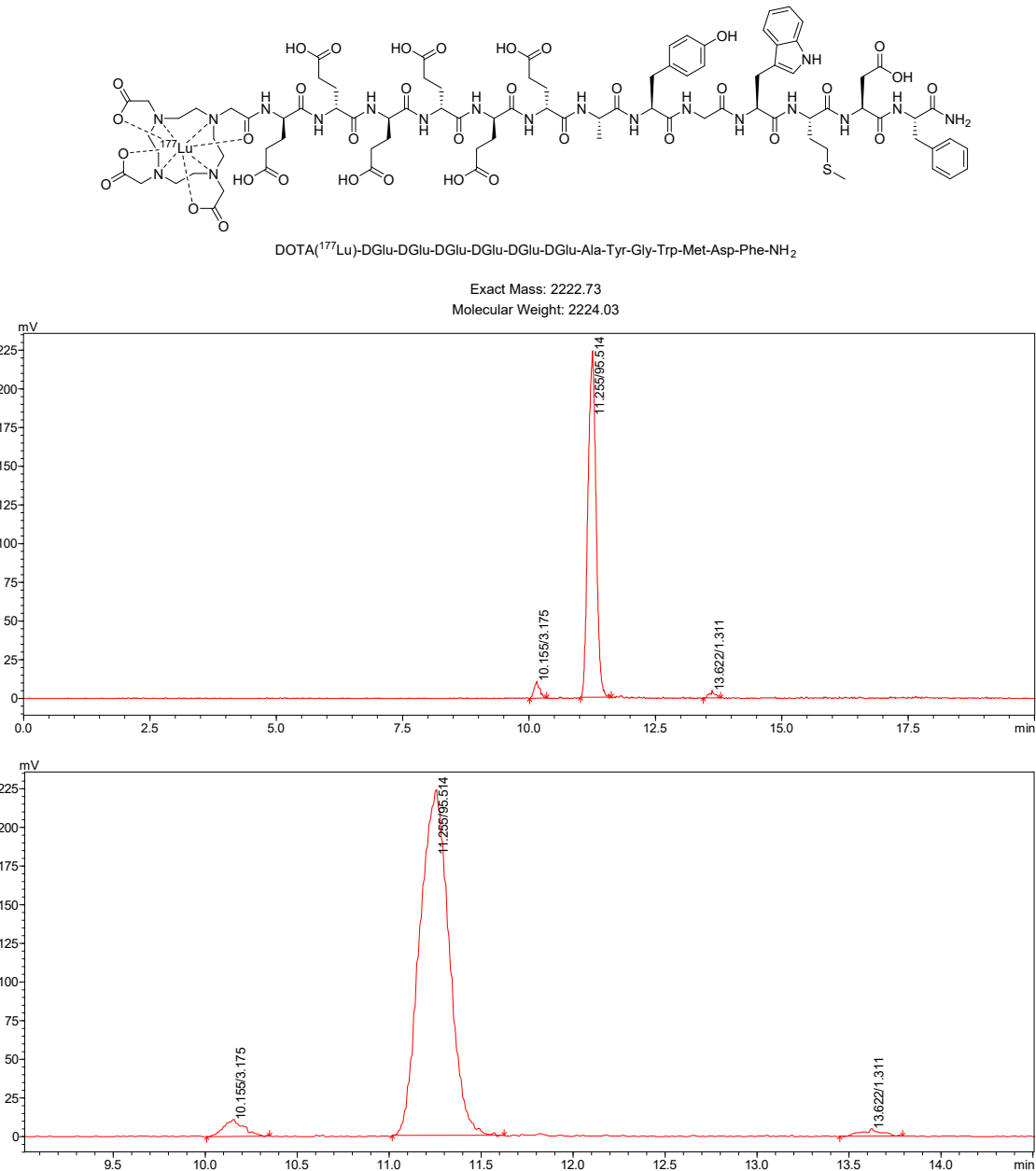


Figure S1: Radio-HPLC trace of [¹⁷⁷Lu]Lu-CP04.

Table S2: Peak retention time and AUC of [¹⁷⁷Lu]Lu-CP04 radio-HPLC.

Peak#	Ret. Time	Area	Area%
1	10.155	82125	3.175
2	11.255	2470725	95.514
3	13.622	33915	1.311
Total		2586764	100.000

Radio-HPLC trace of [¹⁷⁷Lu]Lu-DOTA-GA4

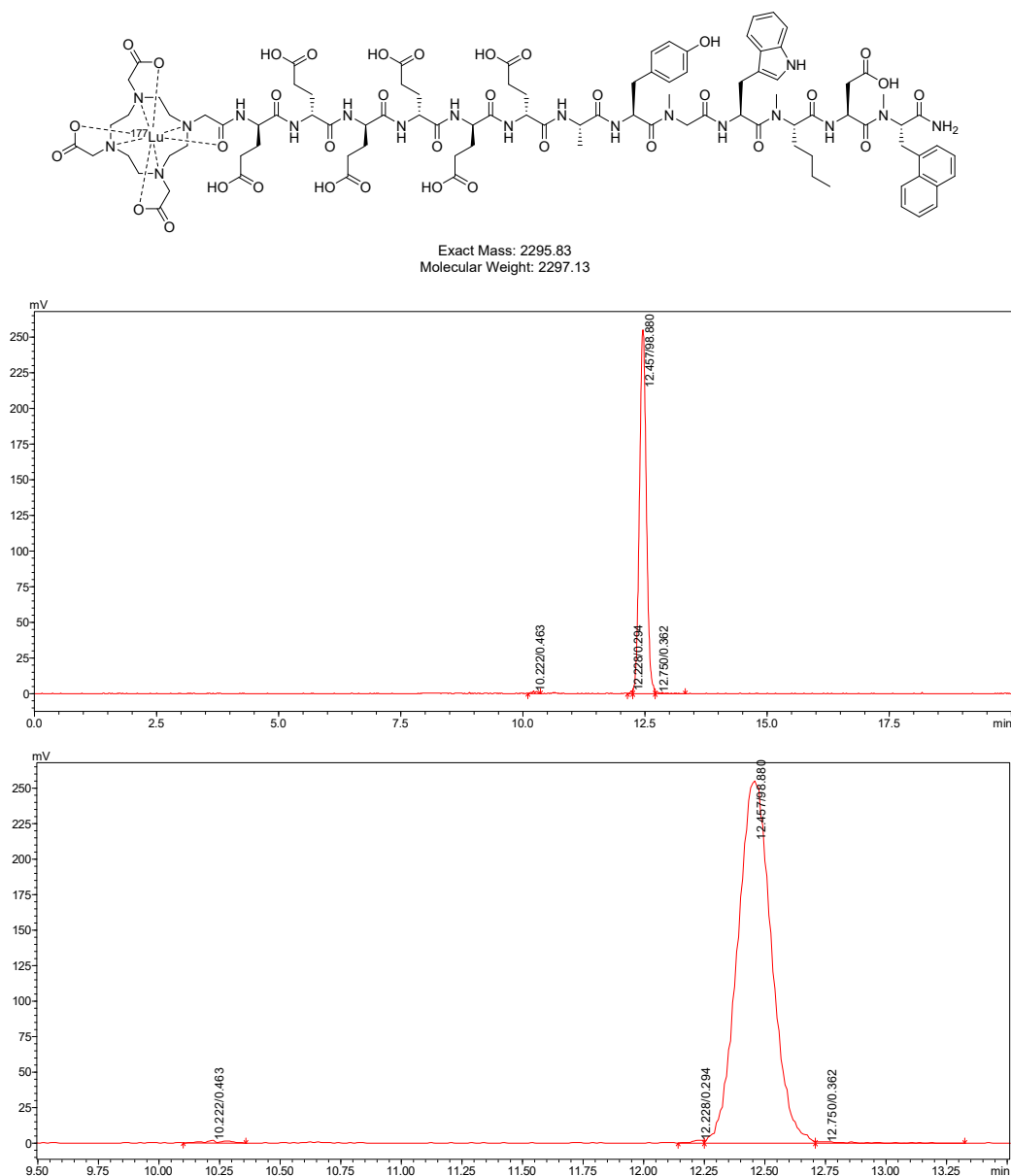


Figure S2: Radio-HPLC trace of [¹⁷⁷Lu]Lu-DOTA-GA4.

Table S3: Peak retention time and AUC of [¹⁷⁷Lu]Lu-DOTA-GA4 radio-HPLC.

Peak#	Ret. Time	Area	Area%
1	10.222	11659	0.463
2	12.228	7412	0.294
3	12.457	2488900	98.880
4	12.750	9112	0.362
Total		2517083	100.000

Radio-HPLC trace of [^{177}Lu]Lu-DOTA-GA13

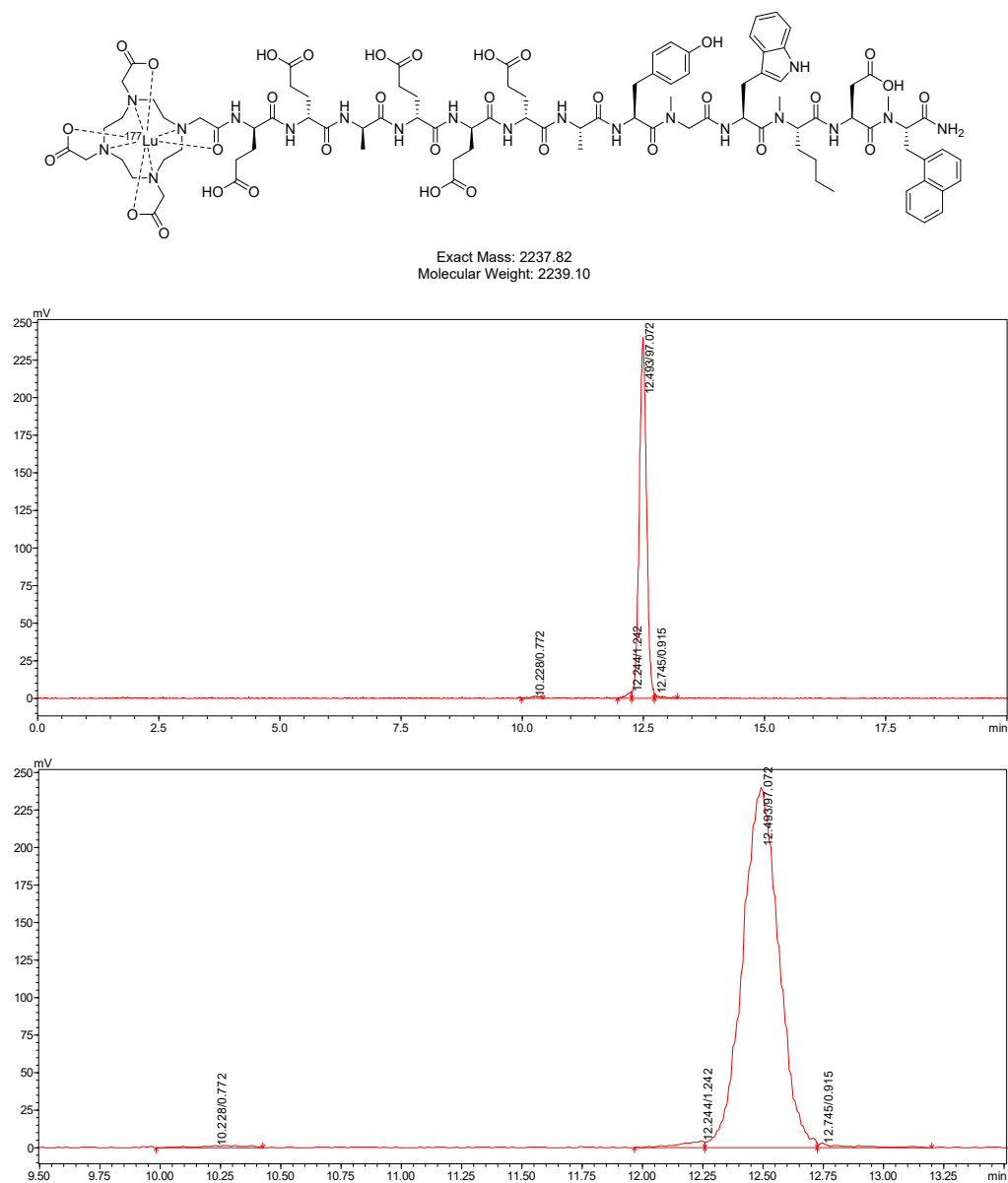


Figure S3: Radio-HPLC trace of [^{177}Lu]Lu-DOTA-GA13.

Table S4: Peak retention time and AUC of [^{177}Lu]Lu-DOTA-GA13 radio-HPLC.

Peak#	Ret. Time	Area	Area%
1	10.228	19791	0.772
2	12.244	31850	1.242
3	12.493	2489706	97.072
4	12.745	23468	0.915
Total		2564815	100.000

Radio-HPLC trace of [^{177}Lu]Lu-DTPA

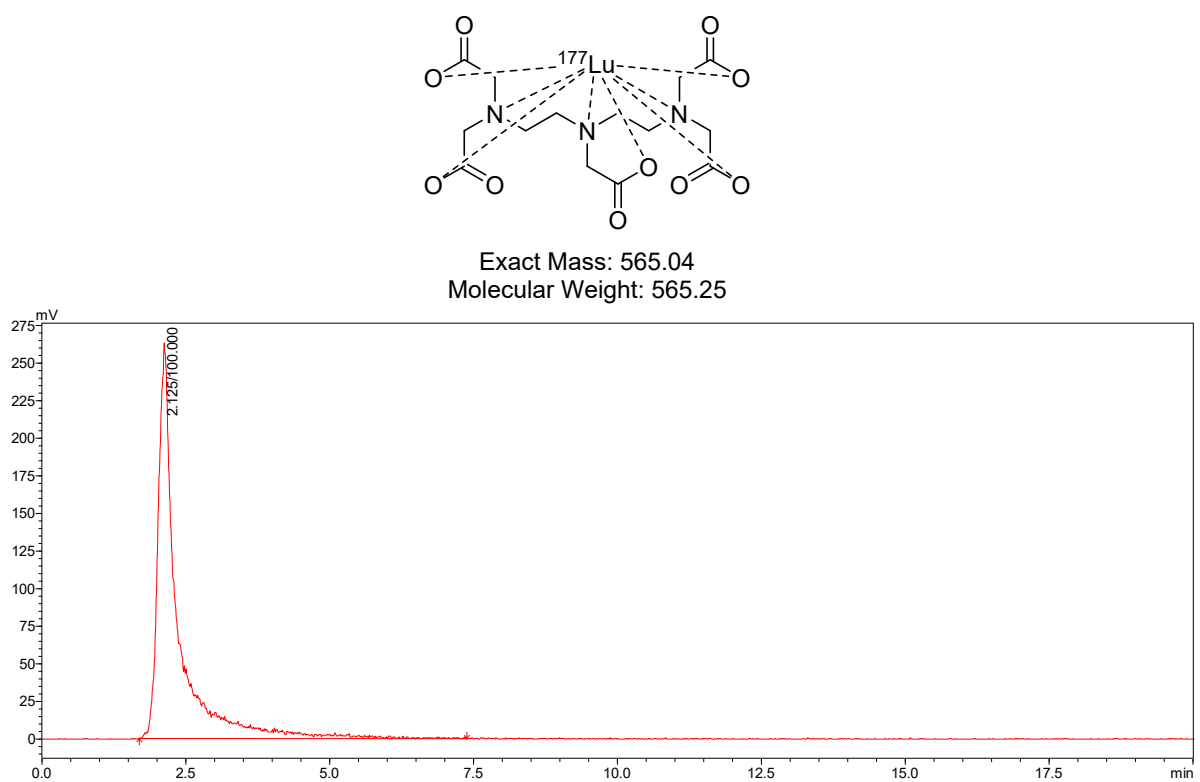


Figure S4: Radio-HPLC trace of [^{177}Lu]Lu-DTPA.

Mouse blood analysis: radio-HPLC traces of ligands at 15 minutes

Blood analysis: Metabolism of [^{177}Lu]Lu-CP04

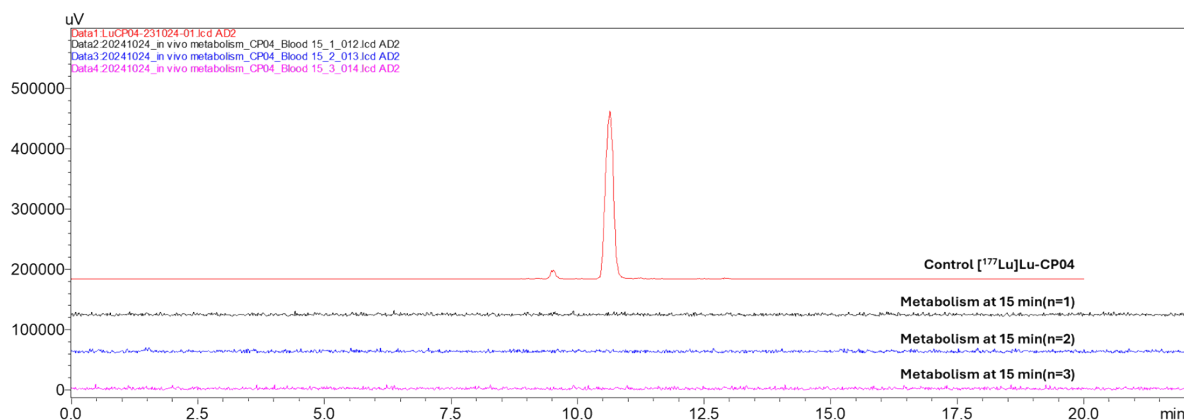


Figure S5: Overlaid radiochromatograms of control [^{177}Lu]Lu-CP04, and metabolized samples (n=3).

Blood analysis: Metabolism of [^{177}Lu]Lu-DOTA-GA4

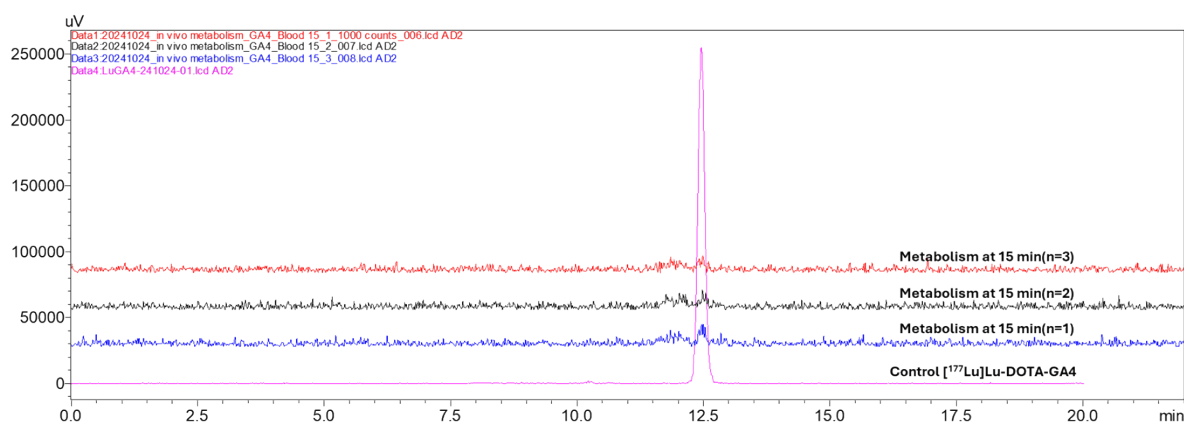


Figure S6: Overlaid radiochromatograms of control [^{177}Lu]Lu-DOTA-GA4, and metabolized samples (n=3).

Blood analysis: Metabolism of [^{177}Lu]Lu-DOTA-GA13

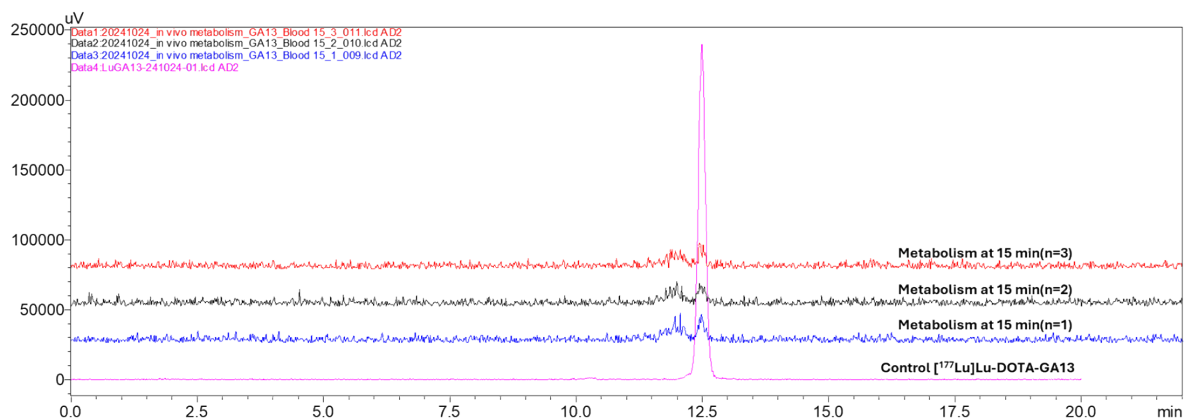


Figure S7: Overlaid radiochromatograms of control [^{177}Lu]Lu-DOTA-GA13, and metabolized samples (n=3).

Mouse urine analysis: radio-HPLC traces of ligands at 15 minutes

Urine analysis: Metabolism of [^{177}Lu]Lu-CP04

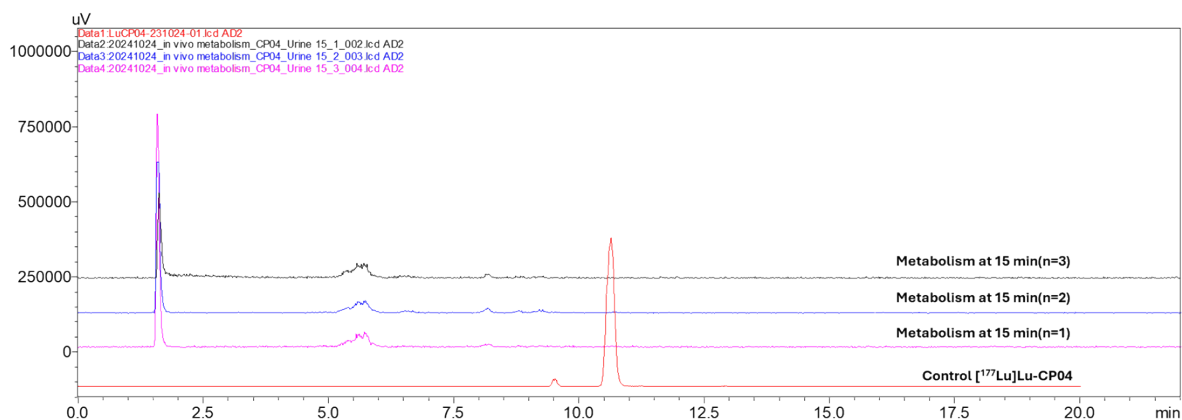


Figure S8: Overlaid radiochromatograms of control [^{177}Lu]Lu-CP04, and metabolized samples (n=3).

Urine analysis: Metabolism of [^{177}Lu]Lu-DOTA-GA4

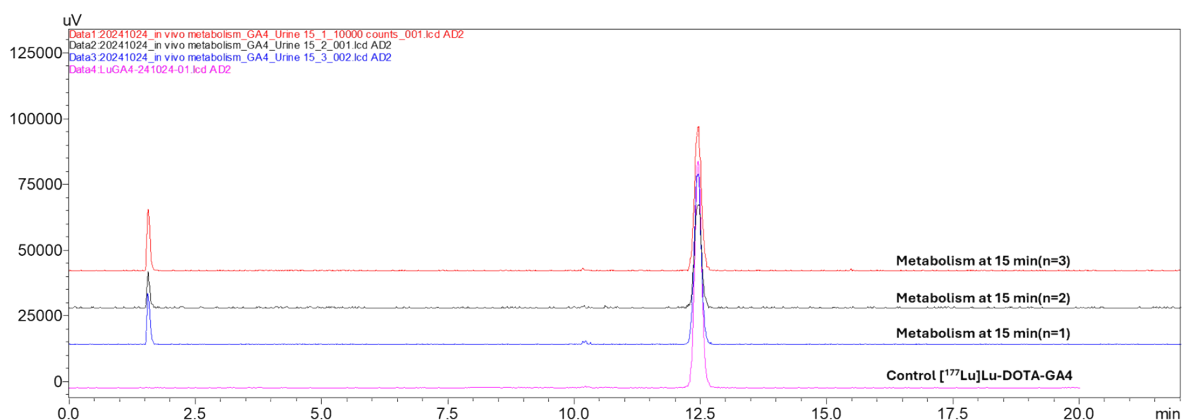


Figure S9: Overlaid radiochromatograms of control [^{177}Lu]Lu-DOTA-GA4, and metabolized samples (n=3).

Urine analysis: Metabolism of [^{177}Lu]Lu-DOTA-GA13

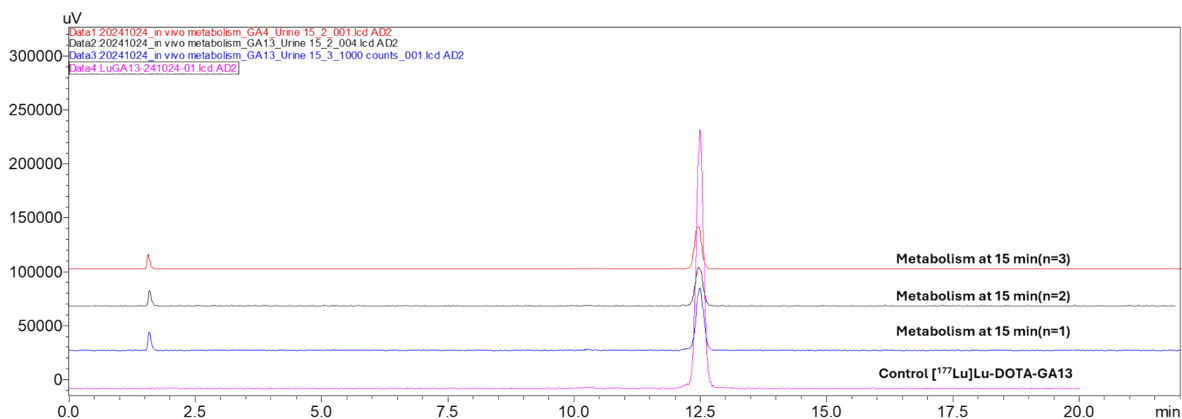


Figure S10: Overlaid radiochromatograms of control [^{177}Lu]Lu-DOTA-GA13, and metabolized samples (n=3).

Mouse weights measured for different treatments throughout the study

Table S5: Mouse weights during treatment with vehicle and 10 MBq [¹⁷⁷Lu]Lu-CP04, [¹⁷⁷Lu]Lu-DOTA-GA4 or [¹⁷⁷Lu]Lu-DOTA-GA13

Day	Vehicle						[¹⁷⁷ Lu]Lu-CP04						[¹⁷⁷ Lu]Lu-DOTA-GA4						[¹⁷⁷ Lu]Lu-DOTA-GA13					
Mouse #	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
0	18.8	19.5	19.3	18.4	18.8	18	18.5	16.9	17	19.3	17.3	18	19.4	15.1	16.9	17.3	17.3	18.6	18.8	16.5	14.2	17	17.2	19.5
3	19.2	19.8	19.4	18.5	19.1	17.7	18.5	16.4	16.7	19	17.3	18	19.2	14.9	16.1	17.1	17.4	18.2	16.2	14.4	13.2	15.1	14.5	17
4																			18.4	16.3	14	17.1	16.6	19.4
6	20.5	19.9	20.2	19.2	20.7	18.8	19.5	17	17.3	20.2	18.1	18.3	19.1	14.8	16.3	17.5	17.4	18.9	18.5	16.5	14.4	17.3	16.7	20.2
8	20.7	20.8	21	19.6	20.8	19.4	20.5	17.6	18	20.4	18.8	18.8	19.5	15.3	16.7	17.8	18.2	19.5	18.6	17.1	15	18.2	17.1	21
9	21.4	21.4	21.1	19.6	21.1	19.6																		
10			21.1	19.6		19.7		17.6	18.1	20.3	18.4	18.7	19.4	15.5	16.5	18	18.7	19.6	18.7	17.3	14.9	17.9	17	20.4
13			21.2	20.4				17.8	19	21.2	18.7	19	19.6	15.9	17	18.3	18.8	19.6	18.6	17.3	15.3	18.5	17.7	21.1
15				21.3				18		21.4	19.3	19.3	19.8	16.3	17.8	19.2	19.2	20.1	19	17.7	17	19.3	18.6	21.6
17								18.6		21.9	19.3	19.8	20.1	16	17.3		19.3	20.7	19.2	17.6	15.8	18.8	17.7	21.3
20											19.1	20.5		16.4	17.4		19.8	20.6		17.7	16.2	19	17.4	21.7
22											19.8			17	17.8		20.4	21		17.9	16.3	18.9	17.9	22.8
23																	20.8	20.9		17.8				23.1
24											19.6			17	17.4						16.1	18.9	17.7	
27											20.1			18.5	18.4						16.6	19.5	18.4	
28											19.9				18.3									
29											20.5											20.3		
30											20.6													
31																						19.6		
34																						19.7		

Table S6: Mouse weights during treatment with vehicle and 20 MBq [¹⁷⁷Lu]Lu-CP04, [¹⁷⁷Lu]Lu-DOTA-GA4 or [¹⁷⁷Lu]Lu-DOTA-GA13

Day	Vehicle						[¹⁷⁷ Lu]Lu-CP04						[¹⁷⁷ Lu]Lu-DOTA-GA4						[¹⁷⁷ Lu]Lu-DOTA-GA13					
Mouse #	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
0	18.8	19.5	19.3	18.4	18.8	18	16.9	20.4	19.3	17.6	17.4	19.9	19.7	18.8	19.0	18.0	16.0	18.4	18.4	18.2	18.8	17.7	17.9	18.0
3	19.2	19.8	19.4	18.5	19.1	17.7	17.2	20.3	18.8	17.6	17.8	19.5	17.9	18.9	19.0	18.0	15.9	18.1	18.7	18.1	18.2	17.2	18.5	18.4
6	20.5	19.9	20.2	19.2	20.7	18.8	17.3	20.6	19.1	18.0	17.9	19.6	18.1	19.2	18.9	18.2	16.1	18.3	18.8	18.4	18	17.3	18.2	18.6
8	20.7	20.8	21	19.6	20.8	19.4	17.9	21.1	19.3	18.4	18.2	20.1	19.0	19.7	19.1	18.8	16.5	18.8	19.6	18.9	18.6	17.7	19.2	19.5
9	21.4	21.4	21.1	19.6	21.1	19.6		21.3																
10			21.1	19.6		19.7	18.2		19.3	18.2	18.6	19.8	19.4	20.0	19.8	18.6	17.1	19	19.0	19.0	19.1	17.7	18.7	18.9
13			21.2	20.4			18.3		20.0	19.1	18.8	20.5	19.7	20.4	20.0	19.1	17.2	19.1	19.5	19.3	19.0	17.6	19.4	19.4
14							18.3																	
15				21.3			18.7		20.4	20.2	19.6	21.4	19.6	20.7	20.5	19.7	18	19.5	19.6	19.8	20.0	18.2	19.8	19.9
17									20.7		19.4	21.4	19.4	20.8	20.3	19.6	17.9	19.5	20.6	19.6	19.6	18.2	19.9	19.5
20									20.7			22.2	19.2	20.3	20	19.3	17.9	19.3	19.7	19.8	19.5	17.9	19.4	20.4
22									22.1			22.6	19.9	21.0	20.3	19.7	18.2	19.7	20.0	20.2	19.9	18.3	20.0	20.4
24													19.5	20.6	20.0	19.3	17.9	19.8	19.8	20	19.8	18.3	20.1	20.6
27													20.7	21.7	20.6	19.7	18.4	20.8	21.2	20.2	20	18.5	20.2	19.7
28																	19.1							
29													20.4	21.7	20.8	20.3			20.1	20.9	20.5	18.9	20.9	22.3
30																							20.5	
31													20.4	21.8	20.8	19.5			20.1	20.5	20.1	18.6		22.0
34													21.1		20.9	20.2			20.0	21.2	20.4	19.0		
35																					20.9			
36																					20.7			
38																					20.6			

Mean tumor volumes measured for each treatment throughout the study

Table S7: Mean tumor volumes (mm³) at Day 0 and after treatment with 10 MBq of [¹⁷⁷Lu]Lu-CP04, [¹⁷⁷Lu]Lu-DOTA-GA4 or [¹⁷⁷Lu]Lu-DOTA-GA13. Values in red indicate mice close to endpoint. Values in blue indicate measurements excluded from median tumor measurements.

Day	Vehicle						[¹⁷⁷ Lu]Lu-CP04						[¹⁷⁷ Lu]Lu-DOTA-GA4						[¹⁷⁷ Lu]Lu-DOTA-GA13					
Mouse #	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
0	205	164	119	67	127	75	190	214	127	70	95	51	108	139	60	225	69	137	303	155	127	70	61	98
3	380	391	190	144	454	172	484	181	205	148	114	38	270	260	61	583	112	266	438	243	208	78	89	145
6	780	694	332	302	672	377	724	334	433	181	179	58	450	207	90	602	112	252	524	388	345	89	134	139
8	1168	1014	434	333	956	412	1275	363	649	306	221	66	511	202	60	730	135	295	424	331	342	89	125	133
10			899	409		1078		427	758	397	215	112	447	217	88	654	170	346	456	382	246	71	112	182
13			1438	817				627	1398	595	207	207	617	269	66	943	197	334	706	348	168	129	90	205
15				1204				914		899	313	335	762	300	92	1284	316	427	725	340	268	116	107	245
17								1361		1171	383	773	1096	406	147		362	802	1099	513	273	147	163	374
20											491	1478		413	205		662	613		548	343	232	264	644
22											508			581	318		906	936		877	611	320	435	968
23																	1329	1464		1232				1264
24											591			810	597							421	657	
27											1091			1612	1009						1350	687	1200	
28											930				1364									
29											1160											850		
30											1284													
31																						830		
34																						1267		

Table S8: Mean tumor volumes (mm³) at Day 0 and after treatment with 20 MBq of [¹⁷⁷Lu]Lu-CP04, [¹⁷⁷Lu]Lu-DOTA-GA4 or [¹⁷⁷Lu]Lu-DOTA-GA13. Values in red indicate mice close to the endpoint. Values in blue indicate measurements excluded from median tumor measurements.

Day	Vehicle						[¹⁷⁷ Lu]Lu-CP04						[¹⁷⁷ Lu]Lu-DOTA-GA4						[¹⁷⁷ Lu]Lu-DOTA-GA13					
Mouse #	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
0	205	164	119	67	127	75	127	304	143	117	71	43	60	86	105	128	162	233	113	151	134	58	192	79
3	380	391	190	144	454	172	247	498	300	196	87	47	72	133	136	171	253	314	177	270	218	145	314	142
6	780	694	332	302	672	377	253	813	121	285	161	50	55	131	90	248	199	249	211	237	280	111	426	139
8	1168	1014	434	333	956	412	487	1093	146	435	221	69	32	136	124	258	256	238	131	282	168	141	302	114
10			899	409		1078	684		171	543	360	117	64	118	141	223	220	249	154	207	190	89	309	99
13			1438	817			1001		250	909	454	216	29	100	70	140	178	196	183	285	188	120	347	101
15				1203			1236		364	1357	689	215	49	162	124	259	145	268	188	207	173	127	309	139
17									527		1087	513	69	137	110	258	215	373	159	231	198	146	319	132
20									857			711	49	189	136	218	253	408	273	237	162	161	392	266
22									1430			1210	225	338	157	387	401	514	325	334	230	187	400	289
24													238	283	127	318	564	495	226	317	238	235	333	208
27													506	609	299	473	1048	1257	696	489	211	375	879	525
29													772	923	436	623			465	571	360	512	1011	921
31													853	1279	560	724			761	805	429	725		1329
34													1841		1239	1812			1417	1718	1145	1328		
35																					845			
36																					971			
38																					1241			