

Supplemental Information

**The importance of tyrosines in multimers of cyclic RGD
nonapeptides: towards $\alpha\beta6$ -integrin targeted
radiotherapeutics**

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Nguyen, Falco Reissig, Jakub Šimeček, Susanne Kossatz*, Johannes Notni*

1. HPLC and ESI-MS data

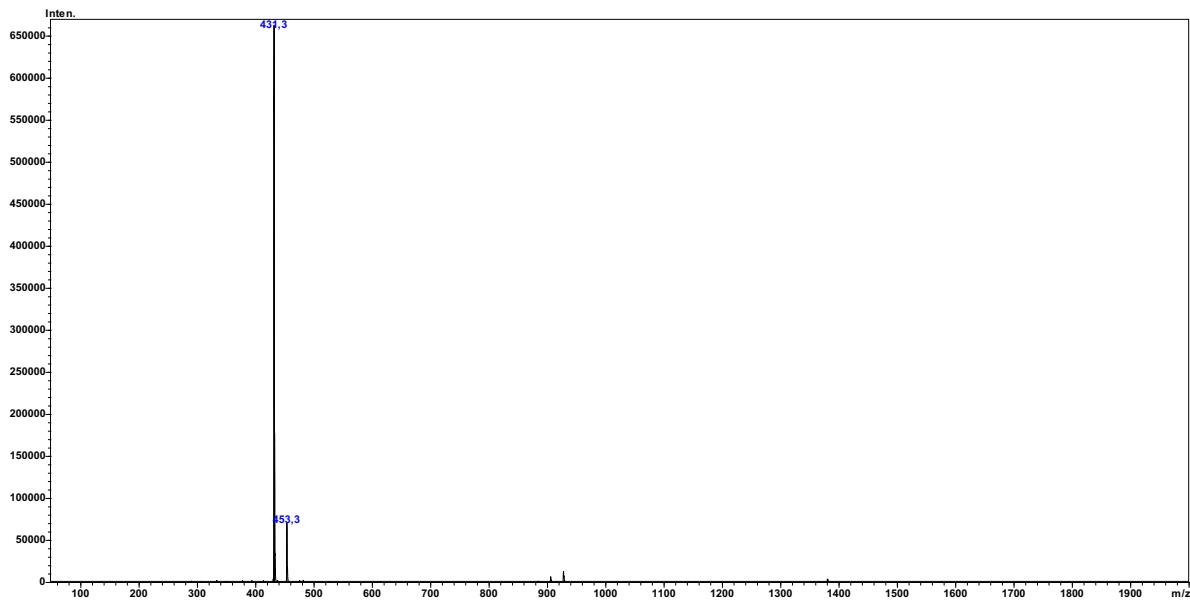


Figure S1. ESI-MS of GFK-alkyne; m/z corresponds to molecular composition as follows: 431.3 $[M+H^+]^+$, 453.3 $[M+Na^+]^+$.

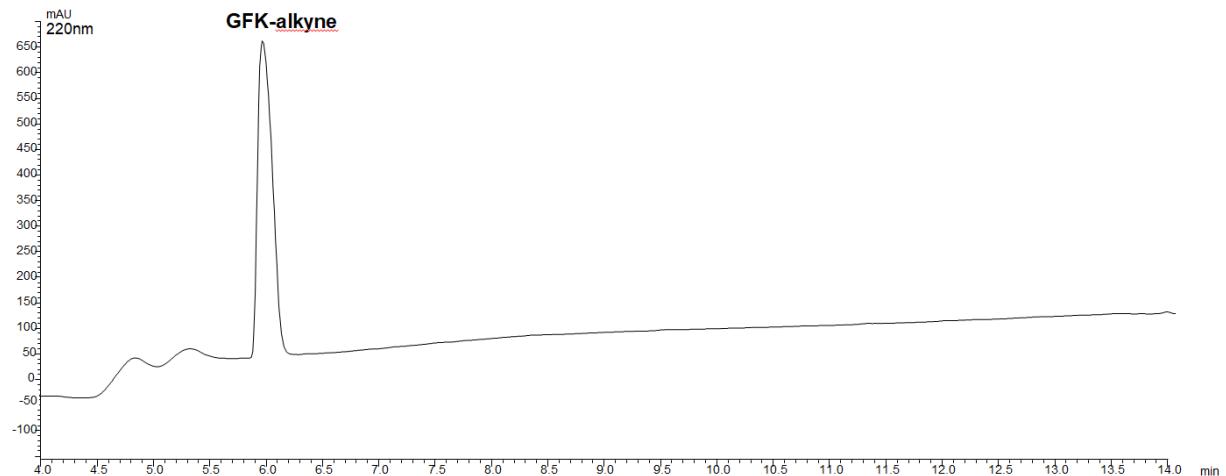


Figure S2. RP-HPLC of GFK-alkyne using analytical column AC2; 0.5 mL/min; gradient: 10–90% B in 15 min; $t_R = 6.0$ min.

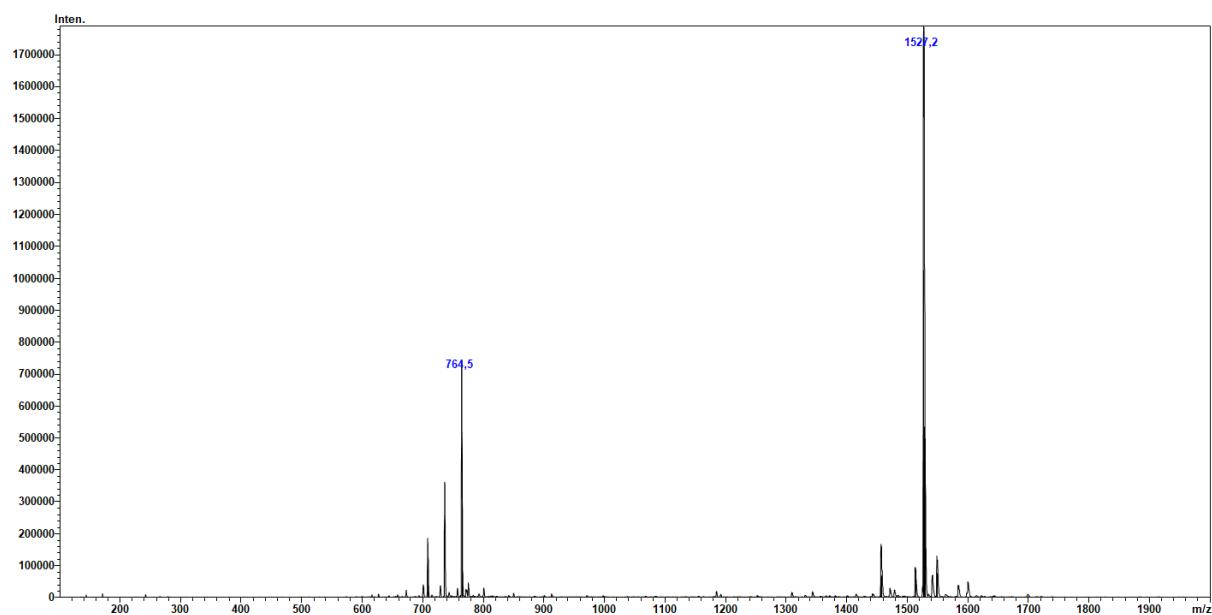


Figure S3. ESI-MS of FY-Suc; m/z corresponds to molecular composition as follows: 1527.2 $[M+H^+]^+$, 764.5 $[M+2H^+]^{2+}$.

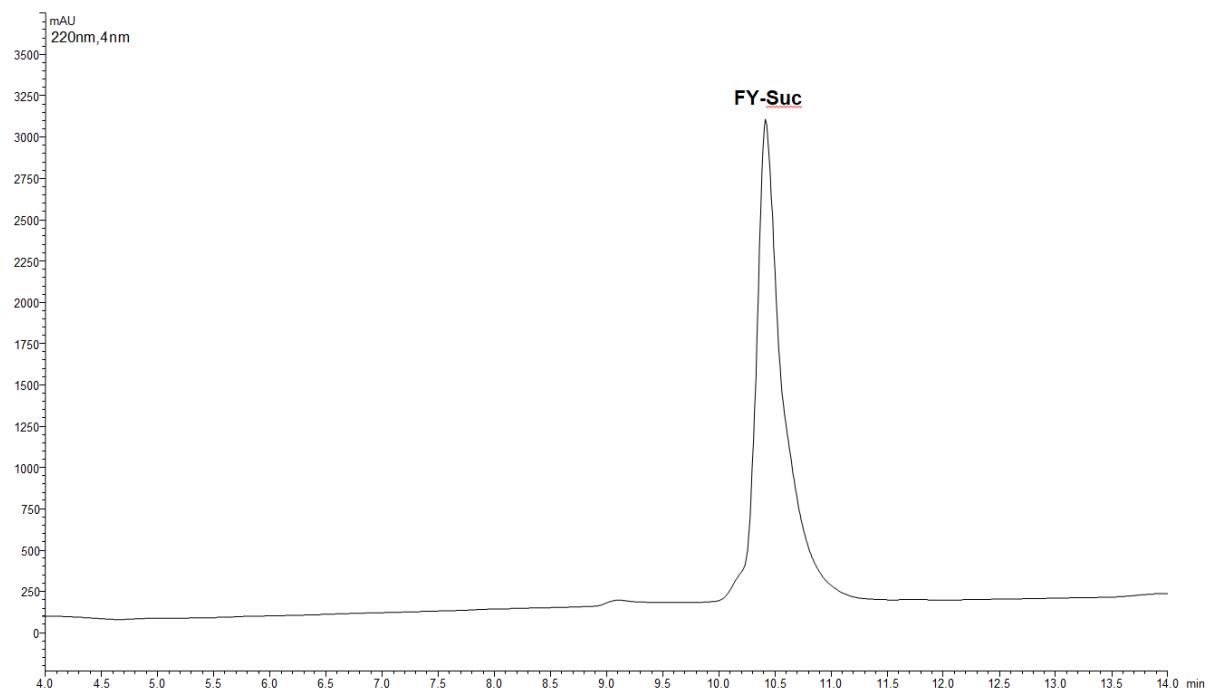


Figure S4. RP-HPLC of FY-Suc using analytical column AC2; 0.5 mL/min; gradient: 5–95 % B in 15 min; t_R = 10.5 min.

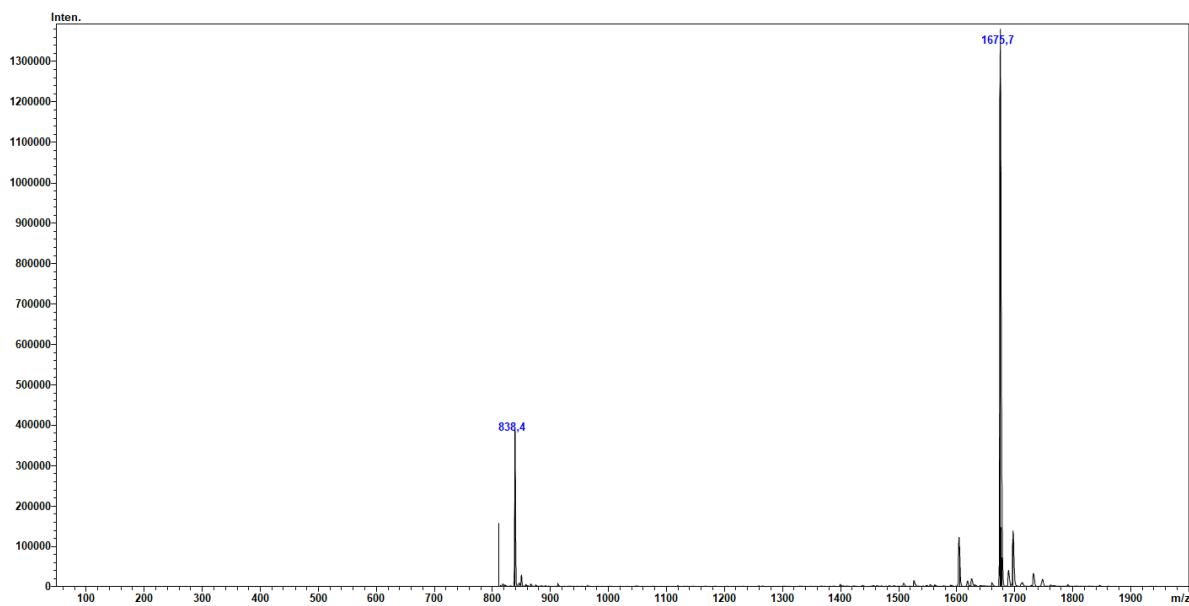


Figure S5. ESI-MS of FY-Suc-TFP. m/z corresponds to molecular composition as follows: 1675.7 [M+H]⁺, 838.4 [M+2H]²⁺.

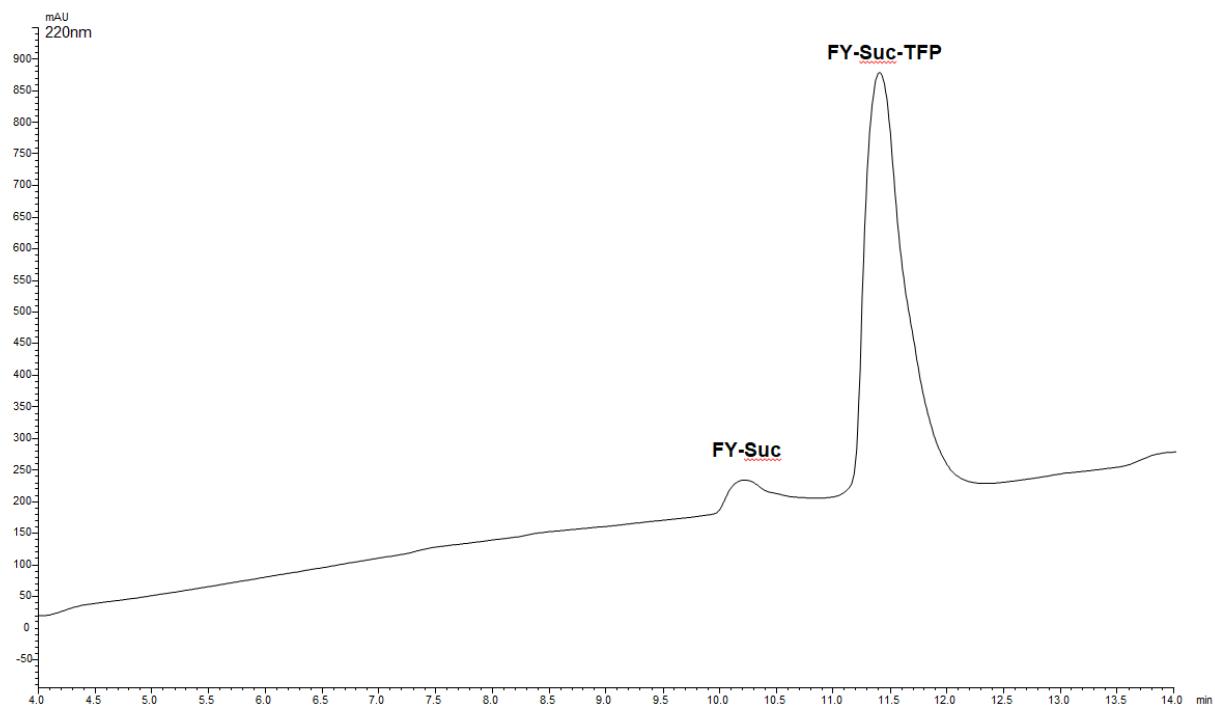


Figure S6. RP-HPLC of FY-Suc-TFP using analytical column AC2; 0.5 mL/min; gradient: 10–90 % B in 15 min; t_R = 11.5 min.

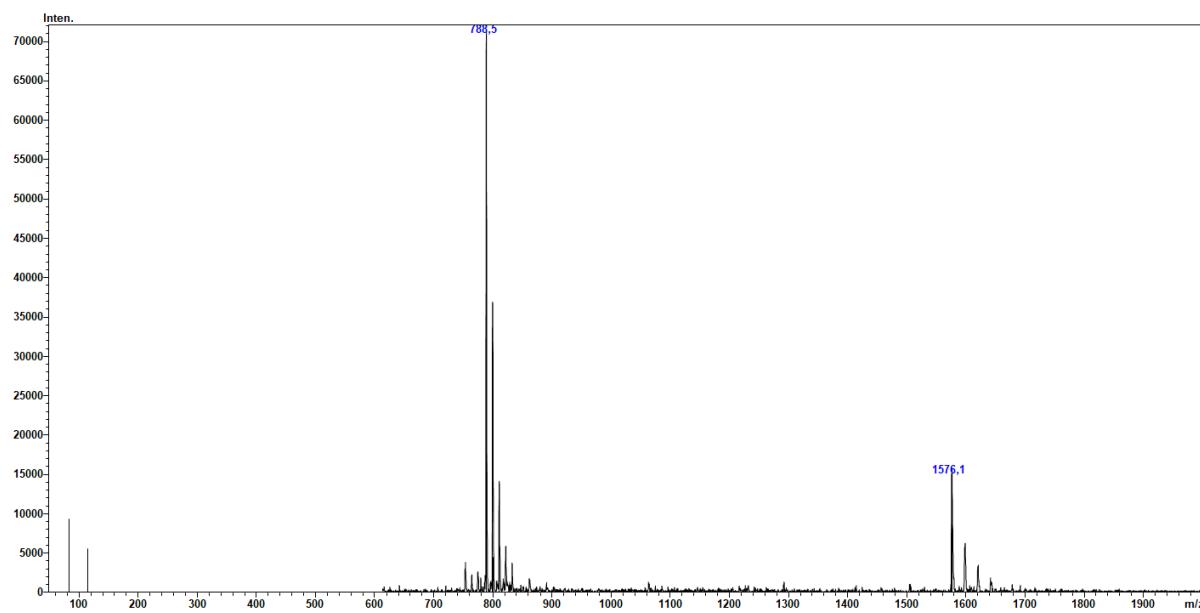


Figure S7. ESI-MS of GFK-FY. m/z corresponds to molecular composition as follows: 1576.1 $[M+H^+]^+$, 788.5 $[M+2H^+]^{2+}$.

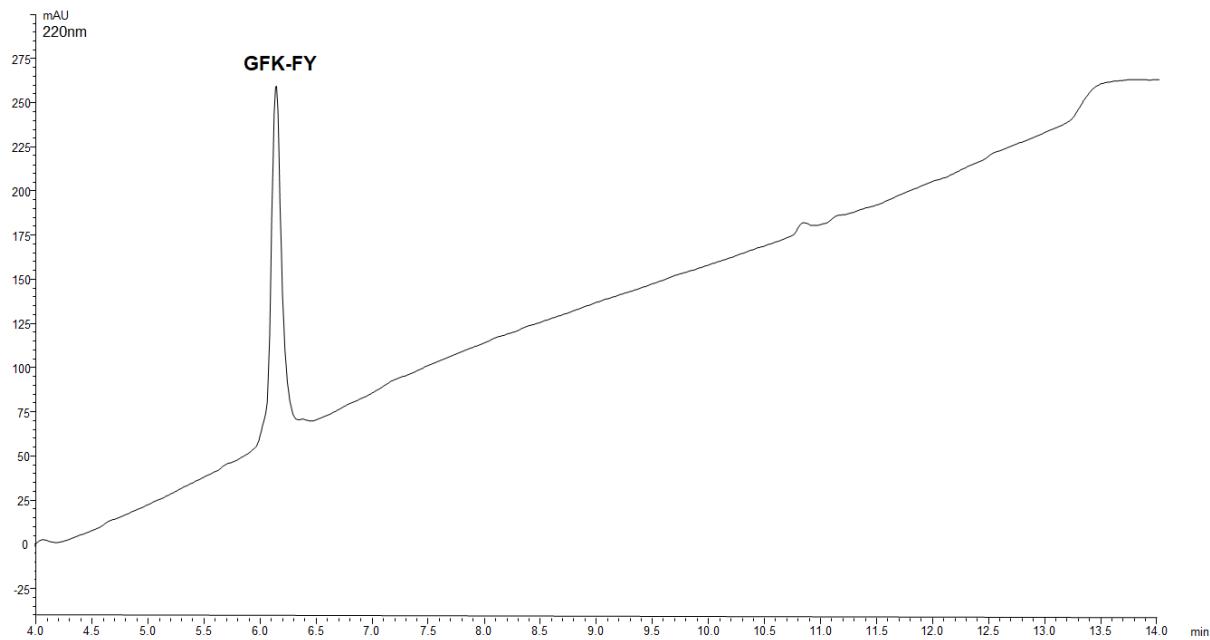


Figure S8. RP-HPLC of GFK-FY using analytical column AC2; 0.5 mL/min; gradient: 10–90 % B in 15 min; $t_R = 6.2$ min.

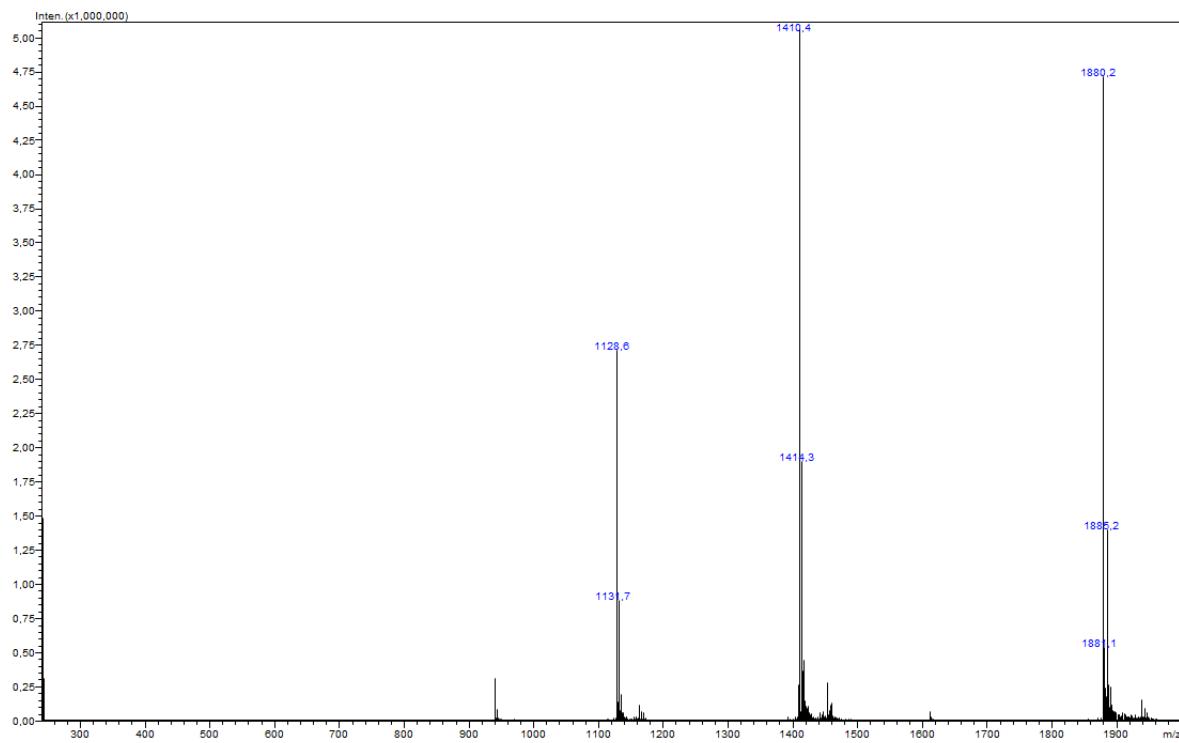


Figure S9. ESI-MS of Y0. m/z corresponds to molecular composition as follows: 1880.2 $[M+K^++2H^+]^{3+}$, 1410.4 $[M+K^++3H^+]^{4+}$, 1128.6 $[M+K^++4H^+]^{5+}$.

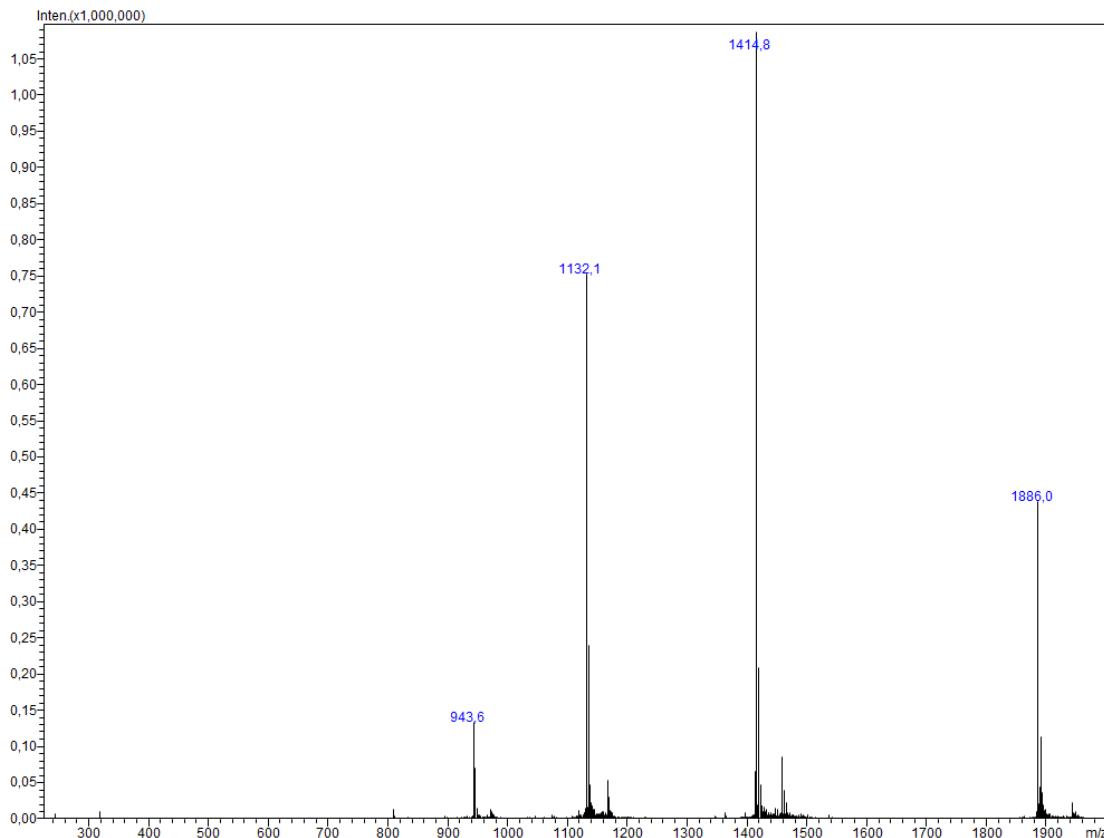


Figure S10. ESI-MS of Y3. m/z corresponds to molecular composition as follows: 1886.0 $[M+3H^+]^{3+}$, 1414.8 $[M+4H^+]^{4+}$, 1132.1 $[M+5H^+]^{5+}$, 943.6 $[M+6H^+]^{6+}$.

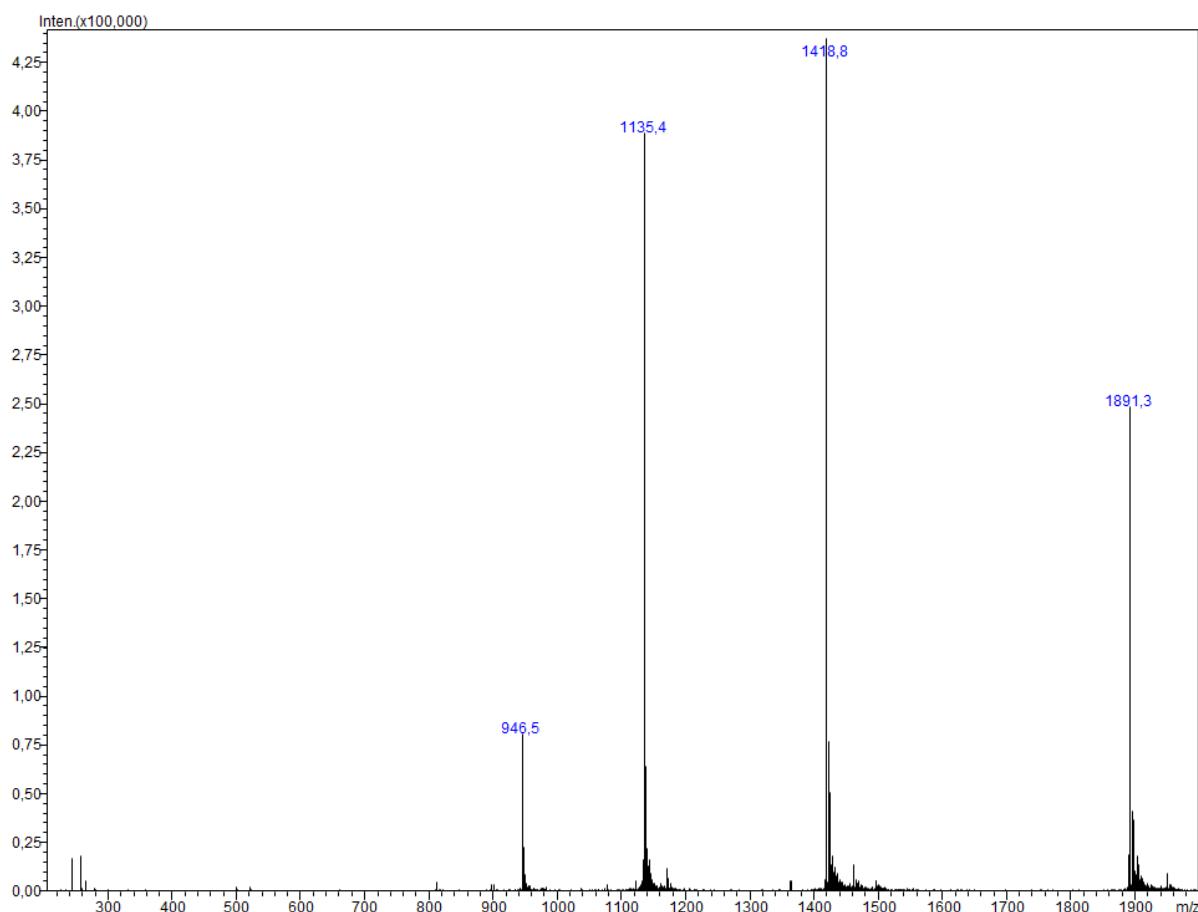


Figure S11. ESI-MS of **Y4**. m/z corresponds to molecular composition as follows: 1891.3 $[M+3H^+]^{3+}$, 1418.9 $[M+4H^+]^{4+}$, 1135.1 $[M+5H^+]^{5+}$, 946.5 $[M+6H^+]^{6+}$.

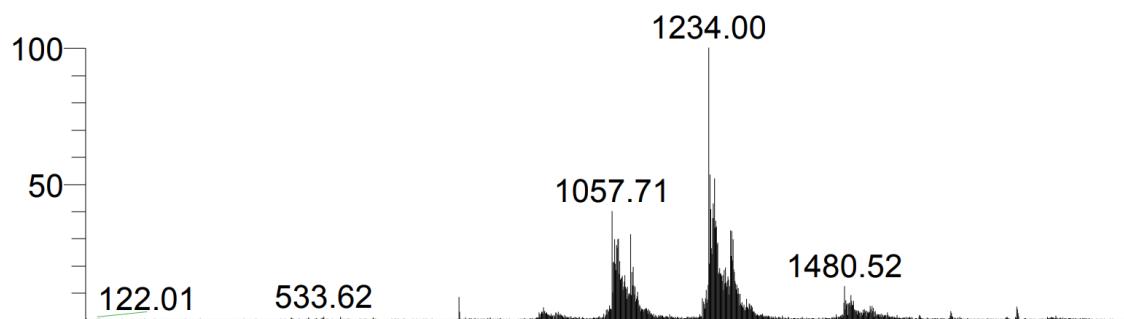


Figure S12. ESI-MS of **GFK-Y4**. m/z corresponds to molecular composition as follows: 1480.52 $[M+5H^+]^{5+}$, 1234.00 $[M+6H^+]^{6+}$, 1057.71 $[M+7H^+]^{7+}$.

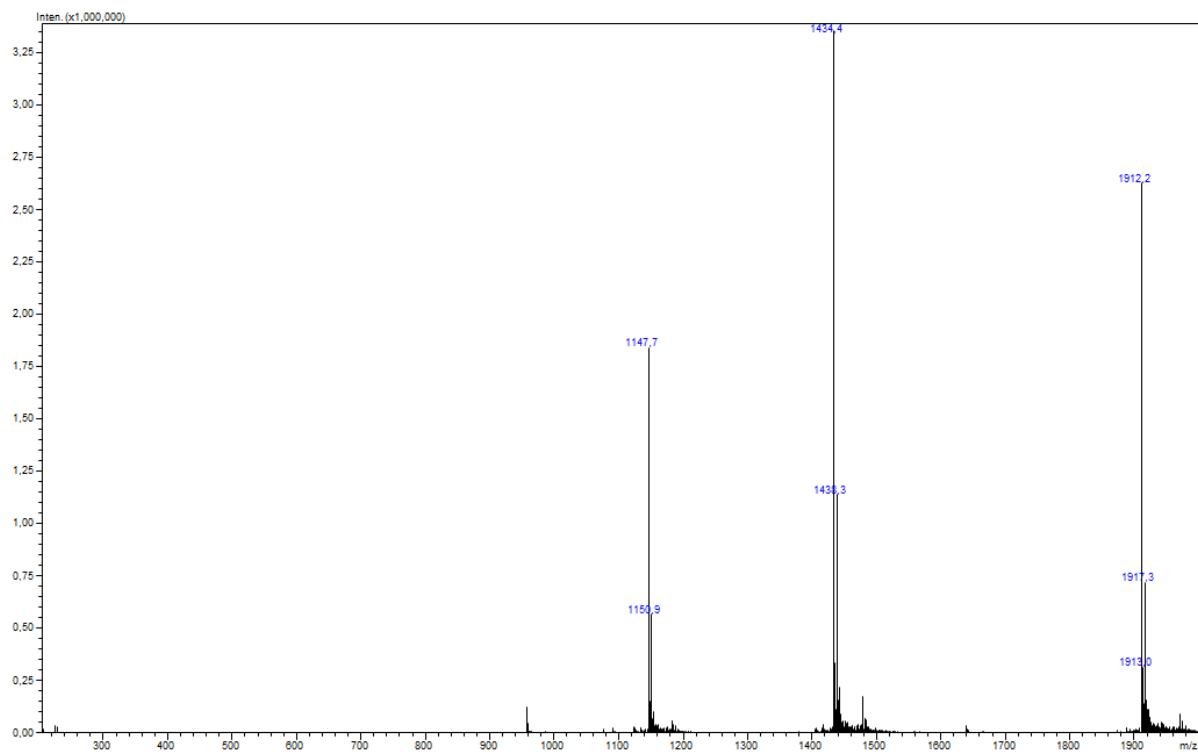


Figure S13. ESI-MS of Y8. m/z corresponds to molecular composition as follows: 1912.2 $[M+3H]^{3+}$, 1434.4 $[M+4H]^{4+}$, 1147.7 $[M+5H]^{5+}$.

2. Radio-HPLC

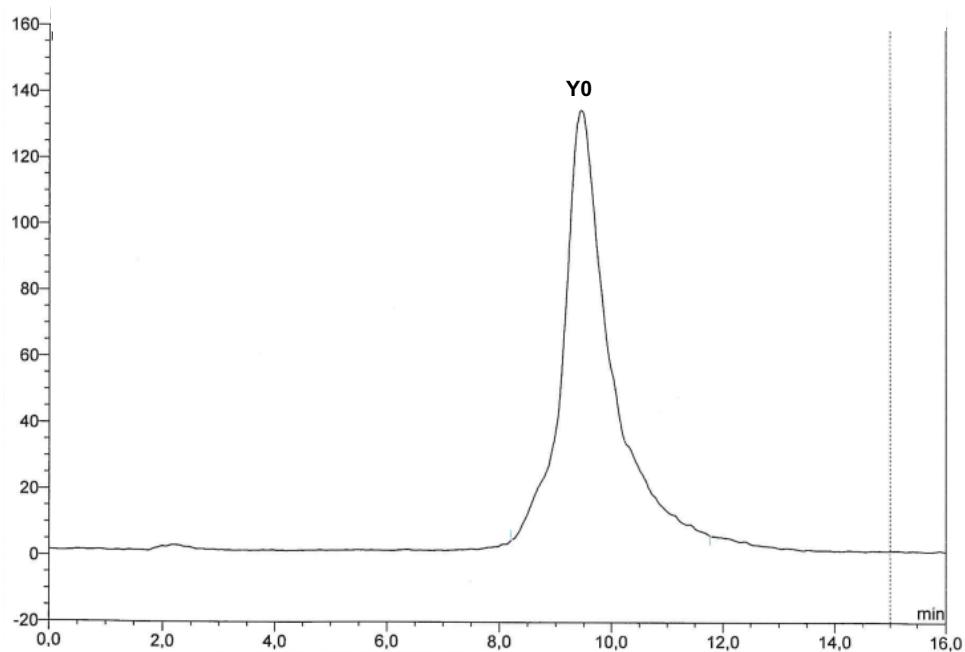


Figure S14. Radio-RP-HPLC of ^{177}Lu -Y0 performed using analytical column AC1; 1 mL/min; gradient: 0–99 % B in 15 min; $t_R = 9.5$ min. Radiochemical purity: 98%.

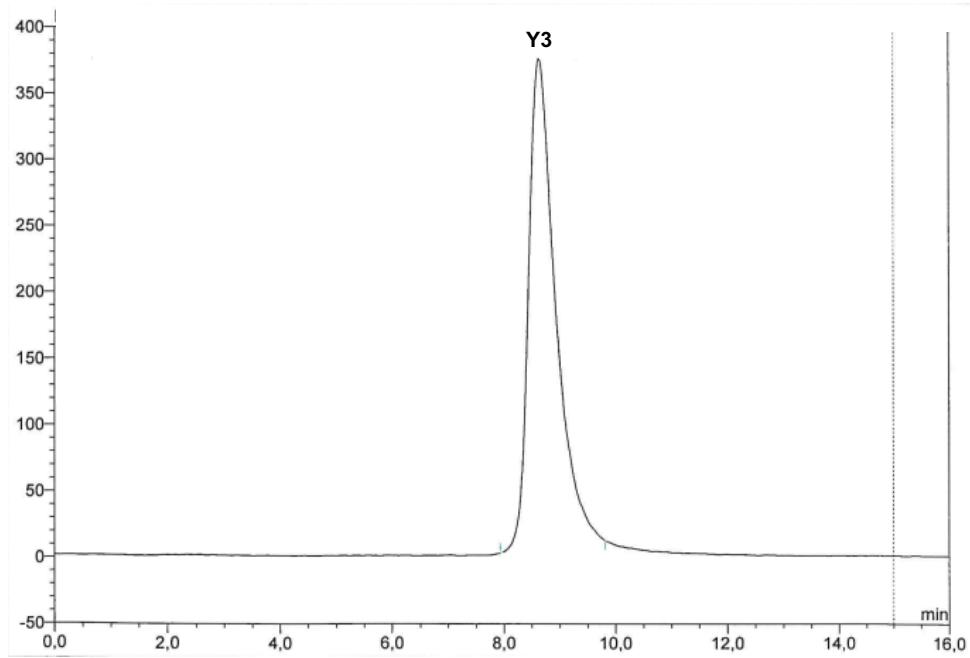


Figure S15. Radio-RP-HPLC of ^{177}Lu -Y3 performed using analytical column AC1; 1 mL/min; gradient: 0–99 % B in 15 min; $t_{\text{R}} = 8.6$ min. Radiochemical purity = 100%.

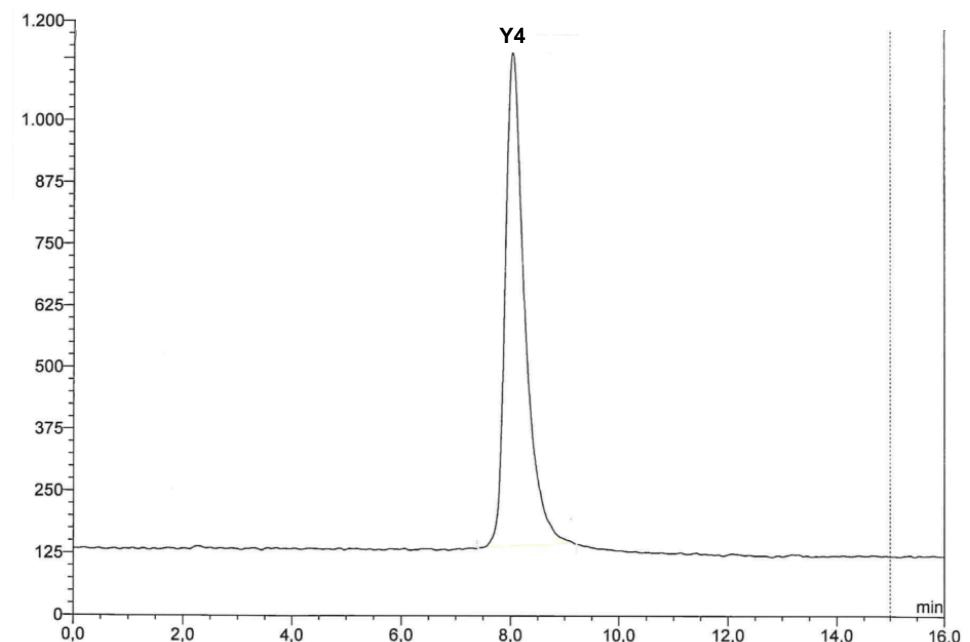


Figure S16. Radio-RP-HPLC of ^{177}Lu -Y4 performed using analytical column AC1; 1 mL/min; gradient: 0–99 % B in 15 mins; $t_{\text{R}} = 8$ min. Radiochemical purity = 100%.

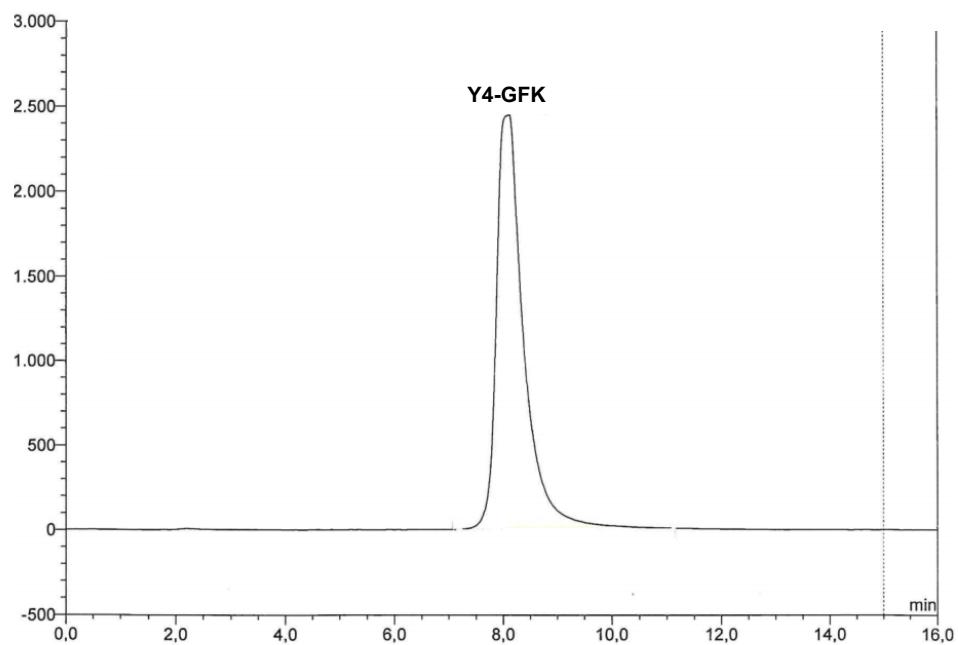


Figure S17. Radio-RP-HPLC of ^{177}Lu -GFK-Y4 performed using analytical column AC1; 1 mL/min; gradient: 0–99 % B 15 min.; t_R = 8.1 min. Radiochemical purity = 100%.

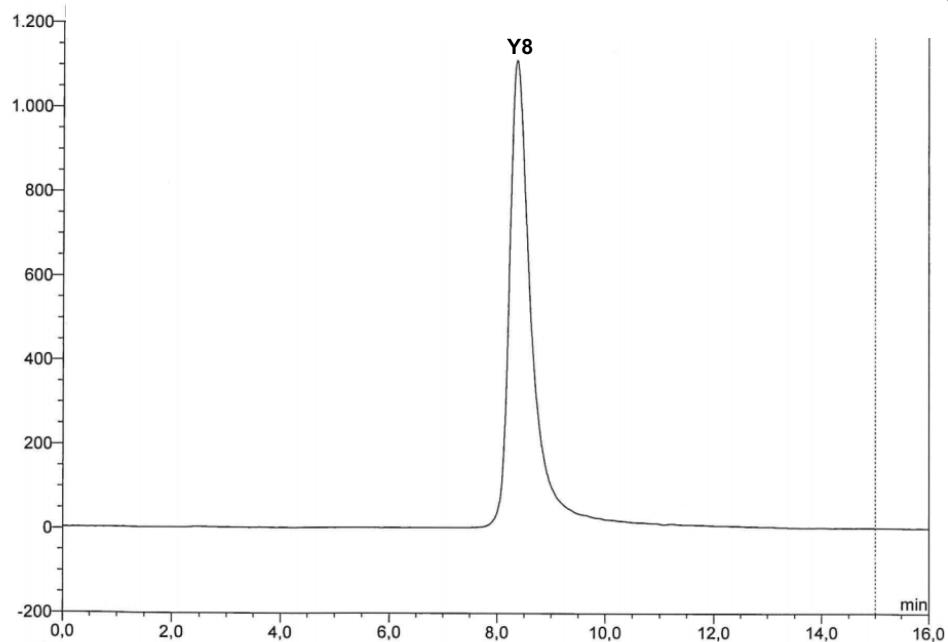
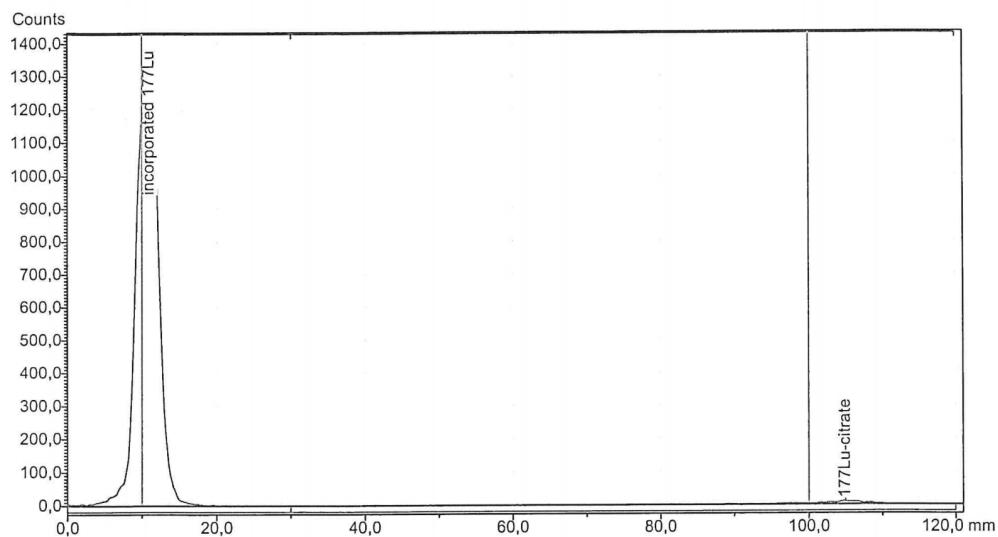


Figure S18. Radio-RP-HPLC of ^{177}Lu -Y8 performed using analytical column AC1; 1 mL/min; gradient: 0–99 % B in 15 min; t_R = 8.4 min. Radiochemical purity = 100%.

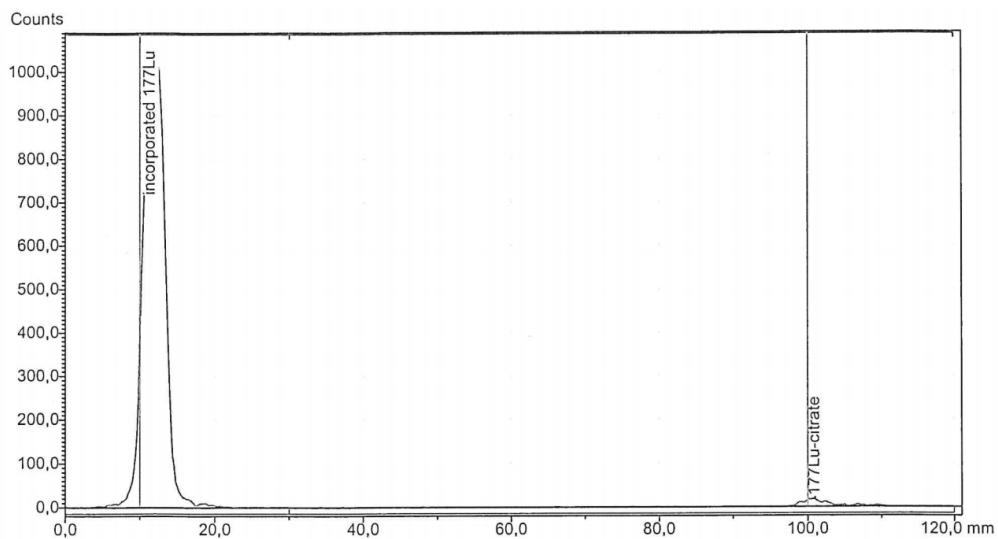
3. Radio-TLC



Regions: TLC

Name	Start (mm)	End (mm)	Retention (RF)	Height (Counts)	Area (Counts)	%ROI (%)	%Total (%)
incorporated 177Lu	0,0	30,0	0,0	1379,0	23718,0	98,60	98,60
177Lu-citrate	30,0	120,0	1,1	14,0	337,0	1,40	1,40
2 Peaks					24055,0	100,00	100,00

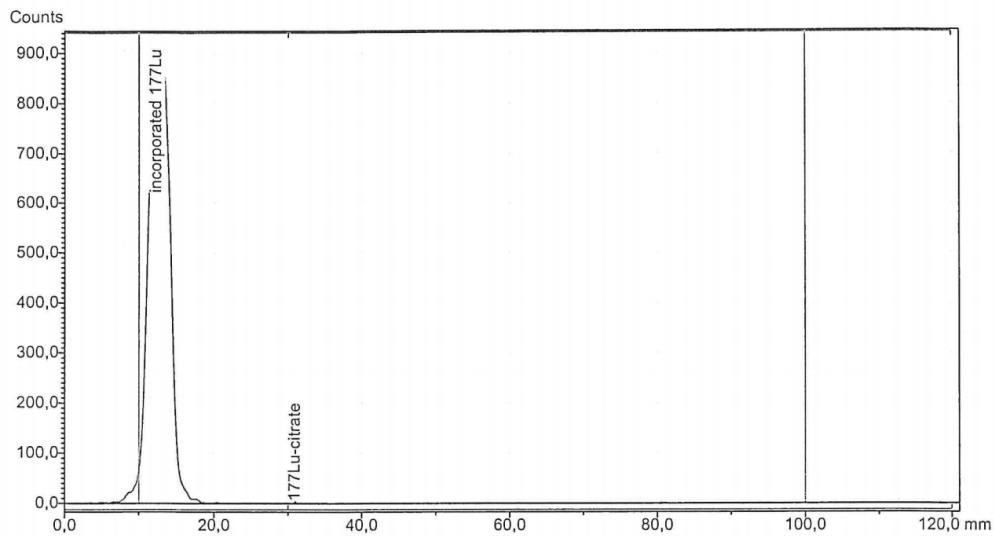
Figure S19. Radio-TLC for ^{177}Lu -Y0; mobile phase: 0.1 M trisodium citrate.



Regions: TLC

Name	Start (mm)	End (mm)	Retention (RF)	Height (Counts)	Area (Counts)	%ROI (%)	%Total (%)
incorporated 177Lu	0,0	30,0	0,0	1049,0	18362,0	97,72	97,72
177Lu-citrate	30,0	120,0	1,0	20,0	428,0	2,28	2,28
2 Peaks					18790,0	100,00	100,00

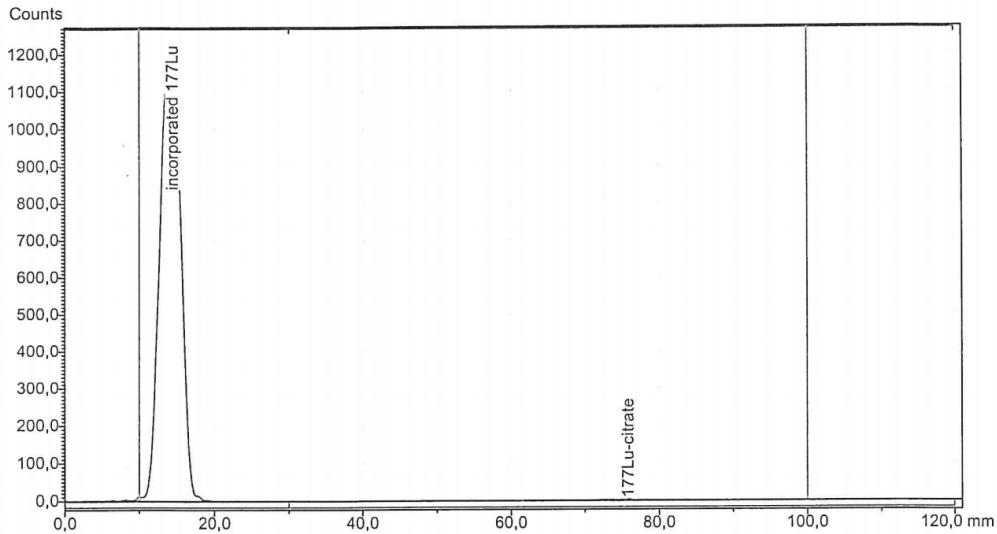
Figure S20. Radio-TLC for ^{177}Lu -Y3; mobile phase: 0.1 M trisodium citrate.



Regions: TLC

Name	Start (mm)	End (mm)	Retention (RF)	Height (Counts)	Area (Counts)	%ROI (%)	%Total (%)
incorporated 177Lu	0,0	30,0	0,0	908,0	15340,0	99,92	99,92
177Lu-citrate	30,0	120,0	0,2	3,0	12,0	0,08	0,08
2 Peaks						15352,0	100,00
%Total							

Figure S21. Radio-TLC for ^{177}Lu -Y4; mobile phase: 0.1 M trisodium citrate.

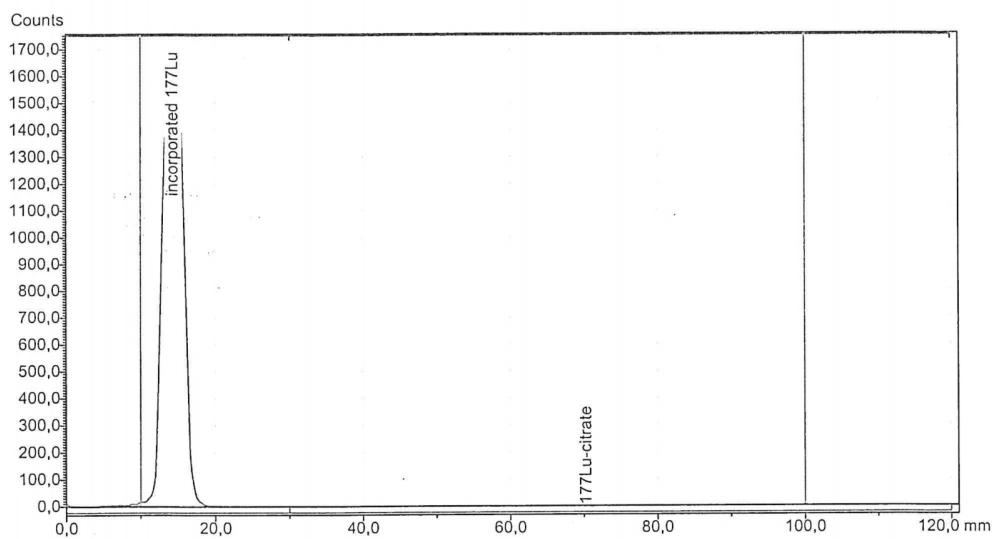


Regions: TLC

Name	Start (mm)	End (mm)	Retention (RF)	Height (Counts)	Area (Counts)	%ROI (%)	%Total (%)
incorporated 177Lu	0,0	30,0	0,1	1225,0	19340,0	99,78	99,78
177Lu-citrate	30,0	120,0	0,7	4,0	43,0	0,22	0,22
2 Peaks						19383,0	100,00
%Total							

Figure S22. Radio-TLC for ^{177}Lu -GFK-Y4; mobile phase: 0.1 M trisodium citrate.

Chromatogram: TLC



Regions: TLC

Name	Start (mm)	End (mm)	Retention (RF)	Height (Counts)	Area (Counts)	%ROI (%)	%Total (%)
incorporated 177Lu	0,0	30,0	0,0	1690,0	27045,0	99,95	99,95
177Lu-citrate	30,0	120,0	0,7	1,0	14,0	0,05	0,05
2 Peaks						100,00	100,00

Figure S23. Radio-TLC for ^{177}Lu -Y8; mobile phase: 0.1 M trisodium citrate.

4. Biodistribution data

Table S1: Biodistribution data for ^{177}Lu -Y0 ($n = 4$; 90 ± 24 pmol), in H2009 bearing SCID mice. Data are given as averages \pm standard deviation. %IA/g = percent injected activity per gram tissue.

^{177}Lu -Y0	90 min p.i.					
Organ/Tissue	%IA/g			tumor/organ ratio		
Blood	1.20	\pm	0.23	2.7	\pm	0.7
Heart (myocard)	1.13	\pm	0.62	3.0	\pm	0.5
Lung	3.59	\pm	1.01	0.9	\pm	0.1
Liver	5.03	\pm	1.98	0.7	\pm	0.2
Spleen	2.22	\pm	1.11	1.6	\pm	0.4
Pancreas	0.49	\pm	0.13	6.7	\pm	1.7
Stomach (empty)	4.15	\pm	1.03	0.8	\pm	0.2
Small intestine (empty)	1.67	\pm	0.41	2.0	\pm	0.6
Large intestine (empty)	3.00	\pm	0.32	1.1	\pm	0.4
Kidneys	208	\pm	65	0.0	\pm	0.0
Adrenals	1.36	\pm	0.79	2.9	\pm	1.3
Muscle	0.53	\pm	0.13	6.0	\pm	1.1
Bone	1.45	\pm	0.57	2.4	\pm	0.7
Tumor H2009	3.23	\pm	1.17			

Table S2: Biodistribution data for $^{177}\text{Lu-Y0}$ ($n = 5$; 92 ± 11 pmol), in H2009 bearing SCID mice. Data are given as averages \pm standard deviation. %IA/g = percent injected activity per gram tissue.

$^{177}\text{Lu-Y0}$			3 d p.i.			
Organ/Tissue	%IA/g		tumor/organ ratio			
Blood	0.02	\pm	0.00	97.5	\pm	10.4
Heart (myocard)	0.43	\pm	0.07	4.3	\pm	0.7
Lung	1.22	\pm	0.17	1.5	\pm	0.1
Liver	2.61	\pm	0.42	0.7	\pm	0.1
Spleen	1.58	\pm	0.26	1.1	\pm	0.2
Pancreas	0.31	\pm	0.09	6.3	\pm	1.9
Stomach (empty)	2.63	\pm	0.68	0.8	\pm	0.3
Small intestine (empty)	0.82	\pm	0.25	2.2	\pm	0.6
Large intestine (empty)	1.19	\pm	0.53	2.1	\pm	1.5
Kidneys	243	\pm	56	0.0	\pm	0.0
Adrenals	0.82	\pm	0.06	2.1	\pm	0.1
Muscle	0.29	\pm	0.11	8.2	\pm	6.4
Bone	0.42	\pm	0.14	5.0		2.4
Tumor H2009	1.72	\pm	0.07			

Table S3: Biodistribution data for $^{177}\text{Lu-Y3}$ ($n = 3$; 139 ± 59 pmol), in H2009 bearing SCID mice. Data are given as averages \pm standard deviation. %IA/g = percent injected activity per gram tissue.

$^{177}\text{Lu-Y3}$			90 min p.i.			
Organ/Tissue	%IA/g		tumor/organ ratio			
Blood	1.11	\pm	0.14	3.4	\pm	0.4
Heart (myocard)	0.65	\pm	0.12	5.8	\pm	0.7
Lung	4.91	\pm	0.51	0.8	\pm	0.1
Liver	7.59	\pm	3.21	0.6	\pm	0.3
Spleen	8.50	\pm	2.27	0.5	\pm	0.1
Pancreas	0.36	\pm	0.05	10.3	\pm	1.2
Stomach (empty)	7.42	\pm	0.32	0.5	\pm	0.0
Small intestine (empty)	2.49	\pm	0.46	1.5	\pm	0.2
Large intestine (empty)	3.43	\pm	0.68	1.1	\pm	0.2
Kidneys	141	\pm	30	0.0	\pm	0.0
Adrenals	1.04	\pm	0.44	3.9	\pm	1.2
Muscle	0.57	\pm	0.10	6.6	\pm	0.9
Bone	1.51	\pm	0.14	2.5		0.1
Tumor H2009	3.70	\pm	0.16			

Table S4: Biodistribution data for $^{177}\text{Lu-Y3}$ ($n = 5$; 42 ± 6 pmol), in H2009 bearing SCID mice. Data are given as averages \pm standard deviation. %IA/g = percent injected activity per gram tissue.

$^{177}\text{Lu-Y3}$			3 d p.i.			
Organ/Tissue	%IA/g		tumor/organ ratio			
Blood	0.02	\pm	0.00	128.6	\pm	22.2
Heart (myocard)	0.22	\pm	0.04	9.4	\pm	2.6
Lung	1.22	\pm	0.16	1.7	\pm	0.4
Liver	1.17	\pm	0.33	1.9	\pm	0.6
Spleen	1.36	\pm	0.54	1.7	\pm	0.6
Pancreas	0.25	\pm	0.17	10.6	\pm	4.8
Stomach (empty)	4.76	\pm	1.37	0.5	\pm	0.2
Small intestine (empty)	0.37	\pm	0.18	6.6	\pm	3.2
Large intestine (empty)	1.34	\pm	0.72	2.3	\pm	1.9
Kidneys	250	\pm	33	0.0	\pm	0.0
Adrenals	0.27	\pm	0.11	8.4	\pm	3.8
Muscle	0.47	\pm	0.23	5.3	\pm	2.6
Bone	0.35	\pm	0.17	6.6		2.5
Tumor H2009	2.03	\pm	0.29			

Table S5: Biodistribution data for $^{177}\text{Lu-Y4}$ ($n = 4$; 43 ± 7 pmol), in H2009 bearing SCID mice. Data are given as averages \pm standard deviation. %IA/g = percent injected activity per gram tissue.

$^{177}\text{Lu-Y4}$			90 min p.i.			
Organ/Tissue	%IA/g		tumor/organ ratio			
Blood	1.33	\pm	0.10	5.2	\pm	0.6
Heart (myocard)	0.74	\pm	0.19	9.6	\pm	1.6
Lung	6.74	\pm	0.71	1.0	\pm	0.1
Liver	1.06	\pm	0.13	6.6	\pm	1.3
Spleen	4.71	\pm	1.04	1.5	\pm	0.4
Pancreas	0.37	\pm	0.02	18.6	\pm	2.2
Stomach (empty)	10.65	\pm	3.09	0.7	\pm	0.2
Small intestine (empty)	3.05	\pm	1.08	2.6	\pm	1.5
Large intestine (empty)	6.99	\pm	0.20	1.0	\pm	0.1
Kidneys	219	\pm	17	0.0	\pm	0.0
Adrenals	0.52	\pm	0.20	13.9	\pm	3.4
Muscle	1.00	\pm	0.44	8.2	\pm	4.1
Bone	1.84	\pm	0.20	3.8		0.4
Tumor H2009	6.91	\pm	0.73			

Table S6: Biodistribution data for $^{177}\text{Lu-Y4}$ ($n = 5$; 64 ± 6 pmol), in H2009 bearing SCID mice. Data are given as averages \pm standard deviation. %IA/g = percent injected activity per gram tissue.

$^{177}\text{Lu-Y4}$			3 d p.i.			
Organ/Tissue	%IA/g		tumor/organ ratio			
Blood	0.02	\pm	0.01	108.2	\pm	40.0
Heart (myocard)	0.27	\pm	0.08	8.2	\pm	2.3
Lung	1.21	\pm	0.24	1.7	\pm	0.3
Liver	0.88	\pm	0.33	2.6	\pm	0.8
Spleen	1.31	\pm	0.33	1.7	\pm	0.4
Pancreas	0.19	\pm	0.04	11.3	\pm	1.3
Stomach (empty)	3.88	\pm	0.80	0.6	\pm	0.1
Small intestine (empty)	0.26	\pm	0.06	8.2	\pm	1.7
Large intestine (empty)	1.33	\pm	0.26	1.6	\pm	0.3
Kidneys	203	\pm	18	0.0	\pm	0.0
Adrenals	0.90	\pm	0.66	4.1	\pm	3.3
Muscle	0.28	\pm	0.12	9.1	\pm	5.4
Bone	0.55	\pm	0.07	3.8		0.8
Tumor H2009	2.07	\pm	0.16			

Table S7: Biodistribution data for $^{177}\text{Lu-GKF-Y4}$ ($n = 4$; 118 ± 40 pmol), in H2009 bearing SCID mice. Data are given as averages \pm standard deviation. %IA/g = percent injected activity per gram tissue.

$^{177}\text{Lu-GKF-Y4}$			90 min p.i.			
Organ/Tissue	%IA/g		tumor/organ ratio			
Blood	0.44	\pm	0.09	4.3	\pm	2.0
Heart (myocard)	0.32	\pm	0.01	5.5	\pm	1.6
Lung	2.88	\pm	0.37	0.6	\pm	0.2
Liver	1.16	\pm	0.56	1.7	\pm	0.6
Spleen	0.89	\pm	0.22	2.1	\pm	0.9
Pancreas	0.14	\pm	0.01	12.2	\pm	3.7
Stomach (empty)	3.29	\pm	0.53	0.6	\pm	0.2
Small intestine (empty)	0.73	\pm	0.14	2.5	\pm	1.0
Large intestine (empty)	1.76	\pm	0.55	1.1	\pm	0.7
Kidneys	274	\pm	75	0.0	\pm	0.0
Adrenals	0.95	\pm	0.66	2.2	\pm	0.9
Muscle	0.26	\pm	0.06	7.2	\pm	3.0
Bone	0.49	\pm	0.03	3.6		1.1
Tumor H2009	1.74	\pm	0.48			

Table S8: Biodistribution data for ^{177}Lu -GFK-Y4 ($n = 5$; 70 ± 39 pmol), in H2009 bearing SCID mice. Data are given as averages \pm standard deviation. %IA/g = percent injected activity per gram tissue.

^{177}Lu -GFK-Y4	3 d p.i.					
Organ/Tissue	%IA/g		tumor/organ ratio			
Blood	0.02	\pm	0.01	56.1	\pm	25.7
Heart (myocard)	0.12	\pm	0.03	6.9	\pm	2.1
Lung	0.50	\pm	0.26	1.8	\pm	0.7
Liver	0.48	\pm	0.22	2.0	\pm	1.1
Spleen	0.86	\pm	0.37	1.0	\pm	0.4
Pancreas	0.08	\pm	0.02	9.9	\pm	4.1
Stomach (empty)	2.42	\pm	0.83	0.4	\pm	0.1
Small intestine (empty)	0.15	\pm	0.04	5.6	\pm	1.9
Large intestine (empty)	0.78	\pm	0.42	1.4	\pm	1.0
Kidneys	169	\pm	37	0.0	\pm	0.0
Adrenals	0.25	\pm	0.10	3.7	\pm	0.9
Muscle	0.13	\pm	0.06	6.9	\pm	3.9
Bone	0.25	\pm	0.06	3.2		1.2
Tumor H2009	0.77	\pm	0.19			

Table S9: Biodistribution data (min p.i.) for ^{177}Lu -Y8 without ($n = 4$; 124 ± 24 pmol) and with ($n = 2$) addition of 50 nmol of non-labeled compound, in H2009 bearing SCID mice. Data are given as averages \pm standard deviation. %IA/g = percent injected activity per gram tissue.

^{177}Lu -Y8	90 min p.i.			+ 50 nmol cold		
	Organ/Tissue	%IA/g		tumor/organ ratio	10 min prior to activity	
Blood	2.54	\pm	0.56	2.4	\pm	0.5
Heart (myocard)	1.01	\pm	0.19	5.9	\pm	1.3
Lung	5.63	\pm	0.44	1.0	\pm	0.1
Liver	1.16	\pm	0.15	5.1	\pm	1.0
Spleen	8.41	\pm	1.93	0.7	\pm	0.3
Pancreas	0.42	\pm	0.08	14.3	\pm	3.6
Stomach (empty)	8.26	\pm	0.91	0.7	\pm	0.2
Small intestine (empty)	3.00	\pm	0.61	2.1	\pm	0.8
Large intestine (empty)	3.66	\pm	1.06	1.8	\pm	0.8
Kidneys	166	\pm	26	0.0	\pm	0.0
Adrenals	0.71	\pm	0.20	8.9	\pm	3.7
Muscle	0.87	\pm	0.35	7.4	\pm	2.7
Bone	3.24		0.20	1.8		0.4
Tumor H2009	5.89	\pm	1.09			1.71

Table S10: Biodistribution data for $^{177}\text{Lu-Y8}$ ($n = 5$; 154 ± 14 pmol), in H2009 bearing SCID mice. Data are given as averages \pm standard deviation. %IA/g = percent injected activity per gram tissue.

$^{177}\text{Lu-Y8}$		24 h p.i.		
Organ/Tissue	%IA/g	tumor/organ ratio		
Blood	0.04 \pm 0.01	59.6	\pm	9.9
Heart (myocard)	0.16 \pm 0.03	15.2	\pm	3.6
Lung	1.20 \pm 0.19	2.1	\pm	0.7
Liver	0.98 \pm 0.40	2.7	\pm	1.2
Spleen	1.89 \pm 0.77	1.4	\pm	0.6
Pancreas	0.14 \pm 0.02	18.4	\pm	6.0
Stomach (empty)	3.84 \pm 1.33	0.7	\pm	0.4
Small intestine (empty)	1.03 \pm 0.27	2.5	\pm	1.0
Large intestine (empty)	1.46 \pm 0.55	1.8	\pm	0.7
Kidneys	235 \pm 49	0.0	\pm	0.0
Adrenals	0.25 \pm 0.08	10.2	\pm	2.8
Muscle	0.36 \pm 0.34	9.5	\pm	4.5
Bone	0.68 \pm 0.08	3.6		1.0
Tumor H2009	2.42 \pm 0.58			

Table S11: Biodistribution data for $^{177}\text{Lu-Y8}$ ($n = 8$; 199 ± 84 pmol), in H2009 bearing SCID mice. Data are given as averages \pm standard deviation. %IA/g = percent injected activity per gram tissue.

$^{177}\text{Lu-Y8}$		3 d p.i.		
Organ/Tissue	%IA/g	tumor/organ ratio		
Blood	0.03 \pm 0.01	67.0	\pm	27.8
Heart (myocard)	0.13 \pm 0.02	12.2	\pm	4.0
Lung	0.73 \pm 0.13	2.2	\pm	0.8
Liver	0.70 \pm 0.14	2.4	\pm	1.0
Spleen	2.01 \pm 0.71	1.0	\pm	0.6
Pancreas	0.12 \pm 0.04	14.9	\pm	7.1
Stomach (empty)	2.65 \pm 1.11	0.8	\pm	0.5
Small intestine (empty)	0.16 \pm 0.05	11.4	\pm	5.7
Large intestine (empty)	0.48 \pm 0.17	3.6	\pm	1.4
Kidneys	237 \pm 42	0.0	\pm	0.0
Adrenals	0.22 \pm 0.04	4.5	\pm	2.6
Muscle	0.23 \pm 0.11	8.4	\pm	4.1
Bone	0.57 \pm 0.21	3.4		1.8
Tumor H2009	1.67 \pm 0.56			