

# Bioorthogonal Chemistry Approach for Theranostics of GRPR-Expressing Cancers

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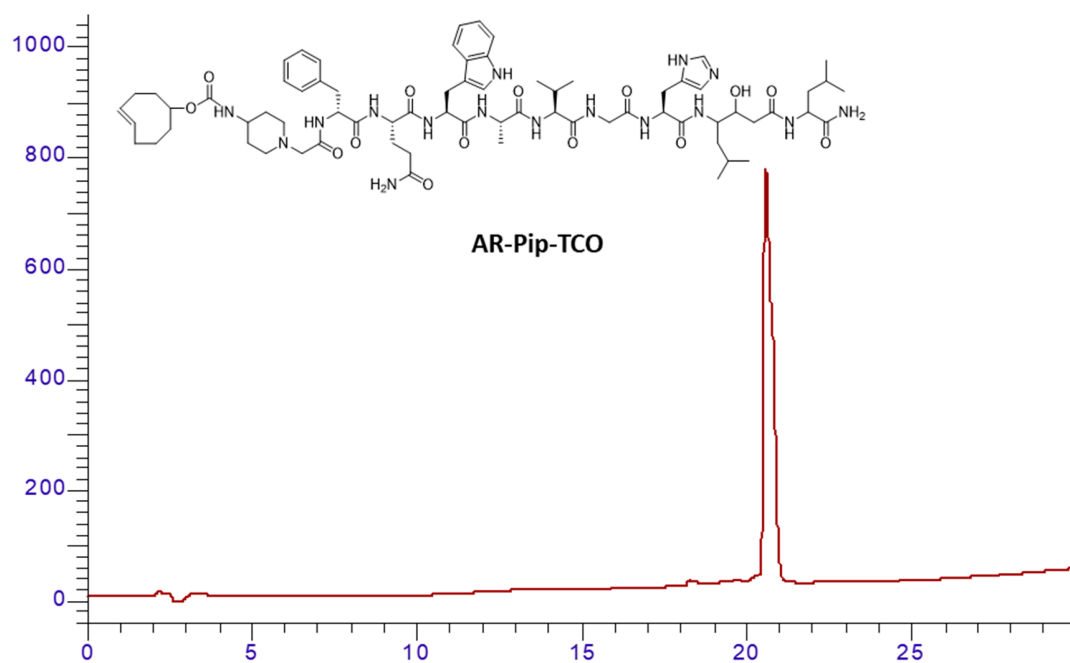
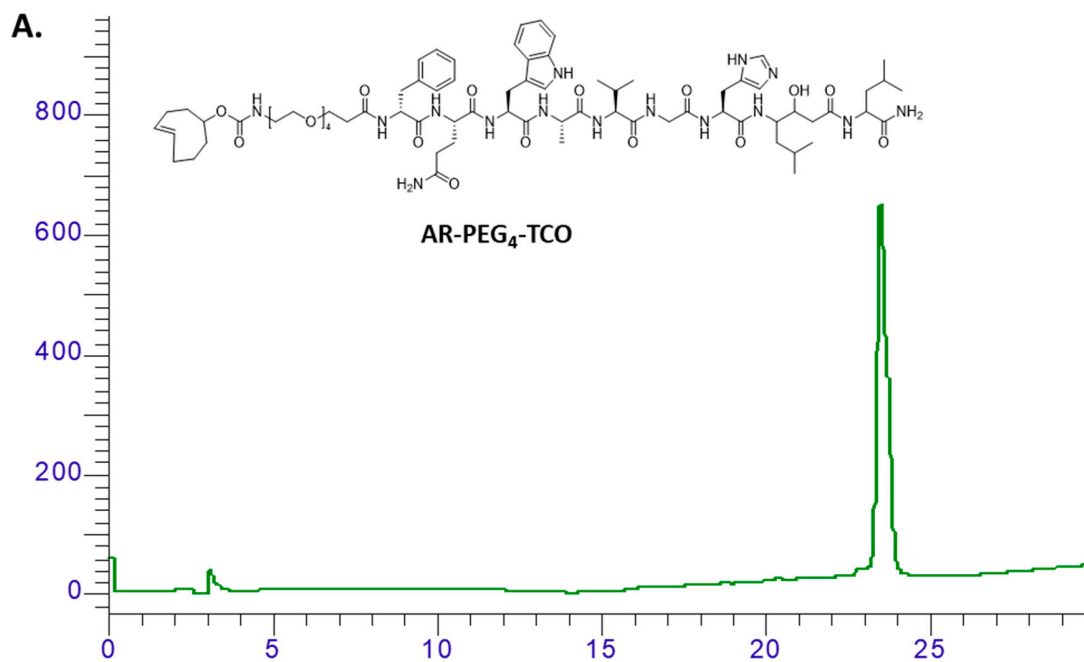
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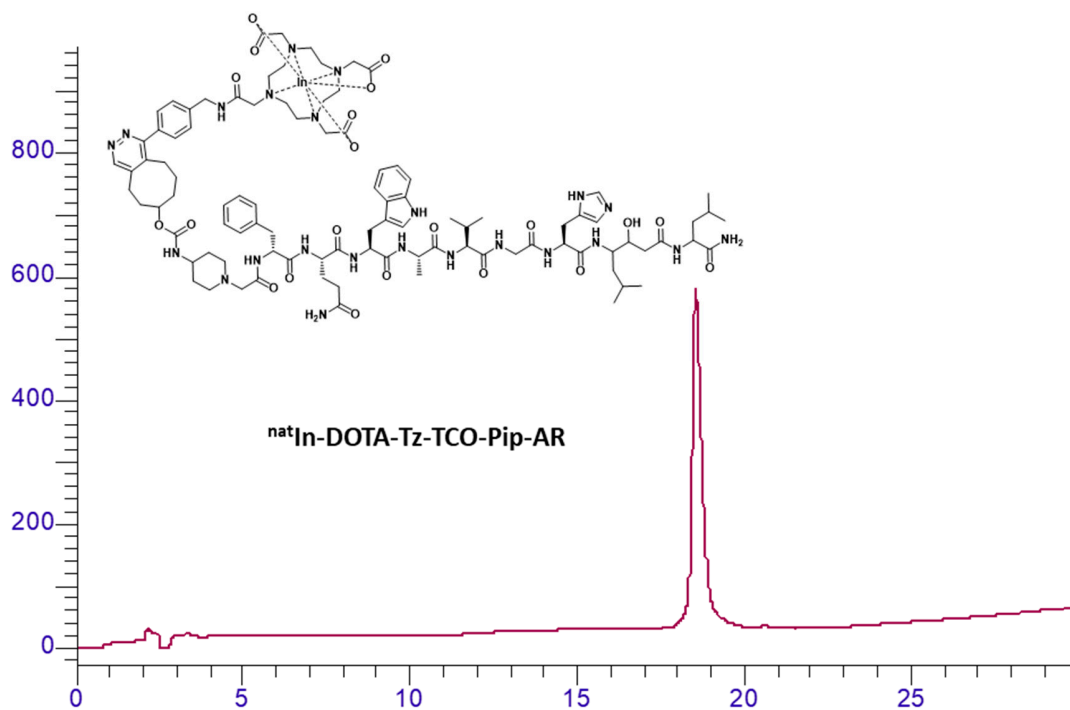
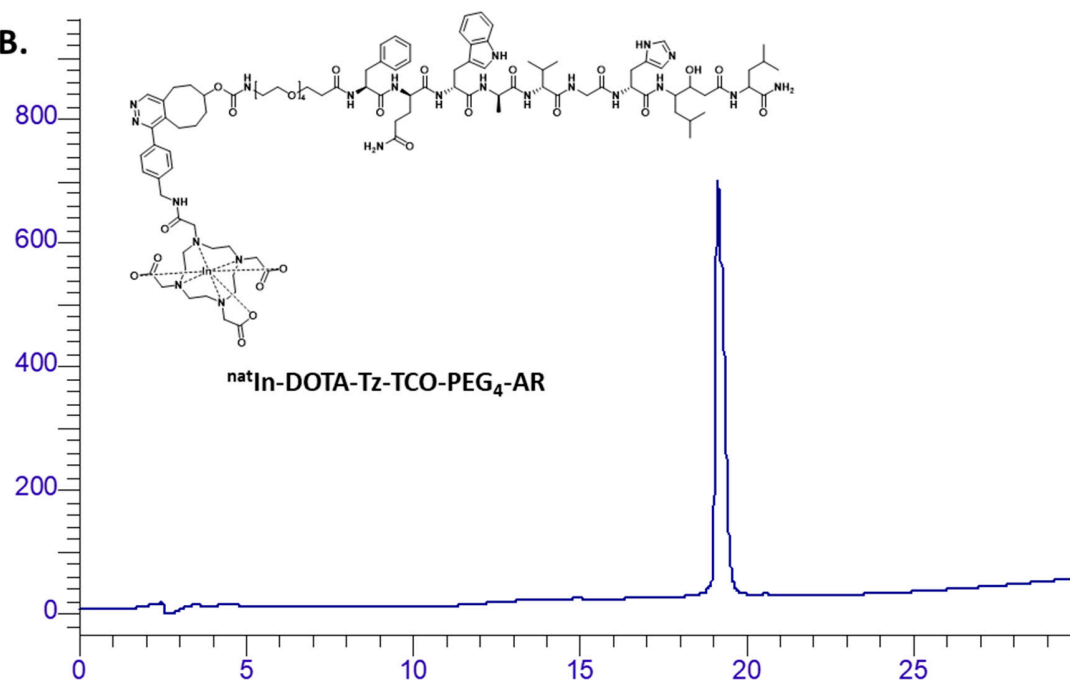
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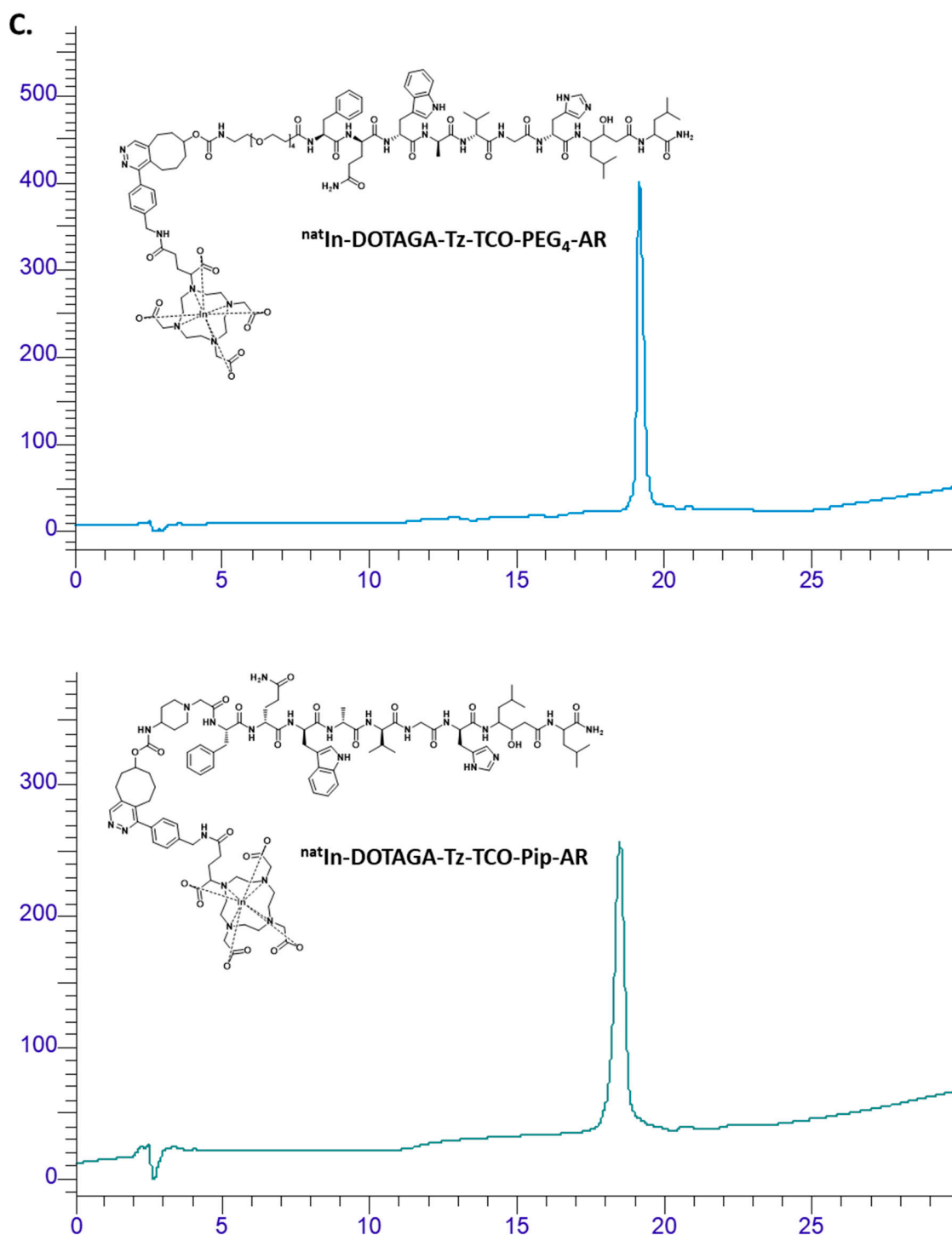
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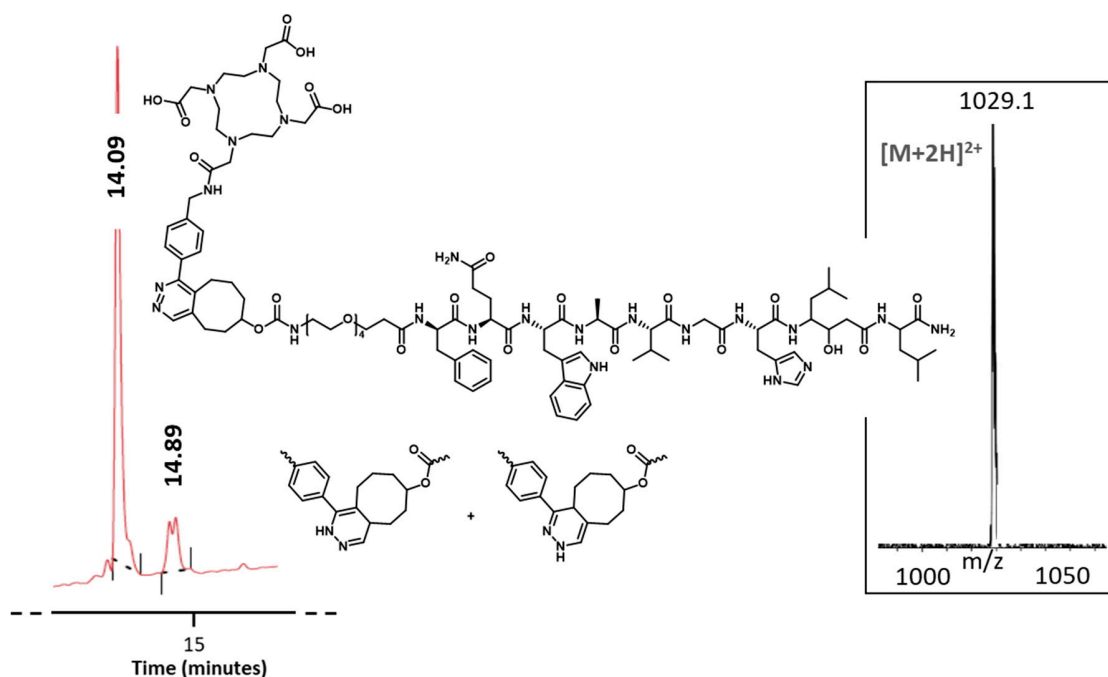


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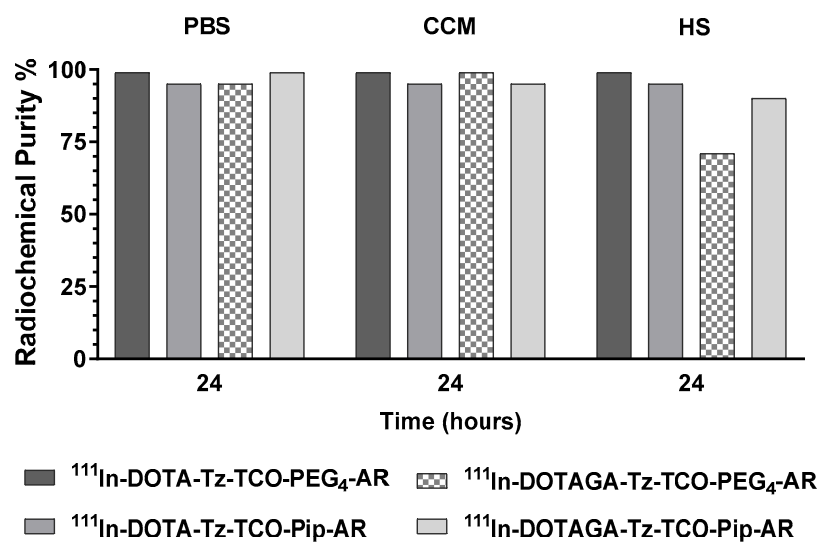




**Figure S1.** HPLC analysis (UV detection at 220 nm) of the **A.** TCO-bearing peptides with the PEG<sub>4</sub> and Pip linkers (Rt = 23.6 and 20.9 min, respectively) **B.** the corresponding cold DOTA-based <sup>nat</sup>In-conjugates (Rt = 19.1 and 18.5 min, respectively) and **C.** DOTAGA-based <sup>nat</sup>In-conjugates (Rt = 19.4 and 18.6 min, respectively).



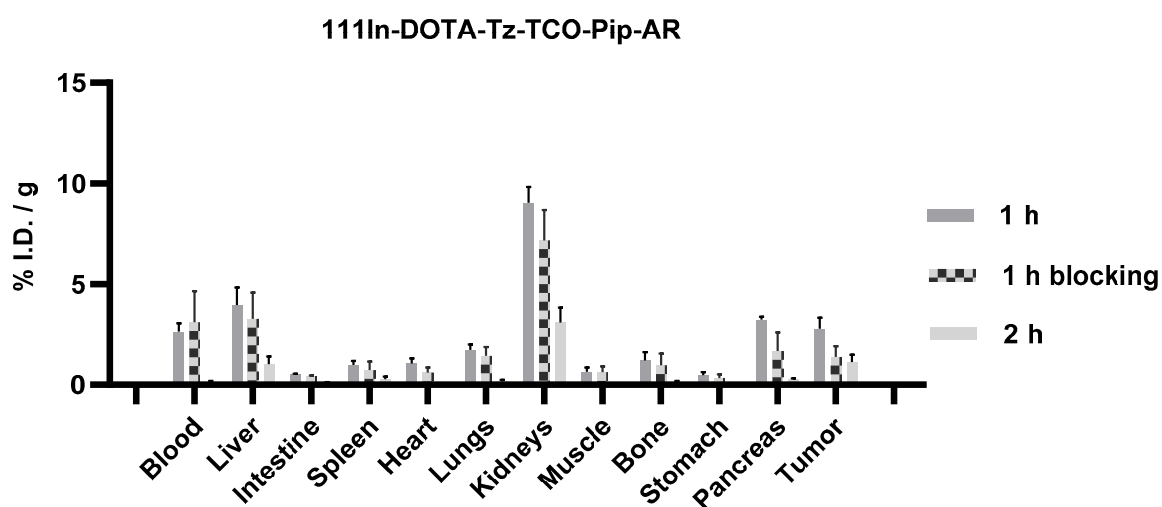
**Figure S2.** HPLC analysis of the formation of the dihydropyridazine isomers ( $t_R = 14.9$  min) upon click reaction leading to the formation of the compound DOTA-Tz-TCO-PEG4-AR ( $t_R = 14.1$  min).



**Figure S3.** Radiochemical purity (estimated by HPLC) of the radioconjugates after incubation in different media (PBS, RPMI 1640 medium supplemented with 10% FBS and 1% Penicillin/Streptomycin and Human Serum) at 37°C for 24 hours.

**Table S1.** Binding Affinity expressed as IC50 values obtained by non-linear regression analysis of competitive binding assay (two-sites).

	In-DOTA-Tz- TCO-PEG4-AR	In-DOTA-Tz- TCO-Pip-AR	In-DOTAGA-Tz- TCO-PEG4-AR	In-DOTAGA-Tz- TCO-Pip-AR	Tyr4-BBN
<b>IC50Hi</b>	6.20E-14	1.36E-12	5.89E-13	1.23E-13	/
<b>IC50Lo</b>	1.01E-10	6.19E-10	1.13E-10	9.00E-10	5.73E-10



**Figure S4.** Biodistribution data for [<sup>111</sup>In]In-DOTA-Tz-TCO-Pip-AR in PC3 xenografts bearing mice, expressed as % I.D./g of organ (mean ± S.D., n = 3).

**Table S2.** Biodistribution data of [<sup>111</sup>In]In-DOTA-Tz-TCO-PEG4-AR in PC3-xenografts bearing mice at 15 min, 1 h (with and without blocking), 2 h and 24 hours p.i. (data expressed as mean % I.D./g and SD, n = 3).

111In-DOTA-Tz-TCO-PEG4-AR		MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
		15 min		1 h		1 h blocking		2 h		24 h	
	Blood	6.7	1.4	2.62	0.74	3.08	0.46	1.7	0.7	0.16	0.01
	Liver	6	1.2	1.15	0.275	1.63	0.44	1.7	0.3	0.21	0.02
	Intestine	3.6	0.3	1.855	0.365	0.995	0.51	1.1	0.3	0.12	0.09
	Spleen	3.6	1.5	1.32	0.75	0.83	0.21	0.6	0.4	0.065	0.006
	Heart	5.4	2.1	0.815	0.19	1.11	0.18	0.5	0.2	0.065	0.005
	Lungs	9.6	0.1	1.615	0.43	2.135	0.51	1.1	0.4	0.12	0.02
	Kidneys	15.4	6.2	5.335	0.625	8.435	1.88	5.4	2.2	1.17	0.23
	Muscle	3.8	0.8	0.875	0.18	1.755	1.41	0.6	0.3	0.034	0.003
	Bone	7.2	3.3	0.85	0.23	1.395	0.75	0.7	0.4	0.038	0.0014
	Stomach	10.6	5.2	2.625	0.395	1.16	0.27	2.1	0.1	0.09	0.02
	Pancreas	25.2	3	13.875	1.595	1.685	0.59	11	2.5	0.14	0.09
	Tumor	8.4	0.5	9.575	1.705	4.445	1.97	13.1	1.7	0.76	0.09
	Excretion	17.4	9.8	74.85	7.05	51.85	14.35	84.7	8.7	96.8	0.2

**Table S3.** Biodistribution data of [<sup>111</sup>In]In-DOTA-Tz-TCO-Pip-AR in PC3-xenografts bearing mice at 1 h (with and without blocking) and 2h p.i. (data expressed as mean % I.D./g and SD, n = 3).

111In-DOTA-Tz-TCO-Pip-AR		MEAN	SD	MEAN	SD	MEAN	SD
		1 h		1 h blocking		2 h	
	Blood	2.63	0.42	3.11	1.55	0.16	0.03
	Liver	3.97	0.86	3.28	1.3	1.02	0.38
	Intestine	0.54	0.02	0.4	0.07	0.09	0.04
	Spleen	0.98	0.21	0.74	0.42	0.28	0.13
	Heart	1.06	0.26	0.6	0.26	0.08	0.01
	Lungs	1.74	0.26	1.44	0.44	0.18	0.06
	Kidneys	9.04	0.79	7.18	1.5	3.09	0.75
	Muscle	0.64	0.22	0.63	0.29	0.05	0.01
	Bone	1.2	0.43	0.97	0.58	0.13	0.06
	Stomach	0.48	0.14	0.34	0.17	0.05	0.02
	Pancreas	3.24	0.15	1.67	0.94	0.28	0.04
	Tumor	2.79	0.53	1.38	0.53	1.13	0.36
	Excretion	63.2	3.3	60.7	9	91.7	2.4

**Table S4.** Biodistribution data of [<sup>111</sup>In]In-DOTA-Tz in PC3-xenografts bearing mice at 15 min, 1 h and 24 hours p.i. (data expressed as mean % I.D./g and SD, n = 3).

111In-DOTA-Tz		MEAN	SD	MEAN	SD	MEAN	SD
		15 min		1 h		24 h	
	Blood	2.7	0.4	0.4	0.2	0.03	0.0002
	Liver	0.9	0.1	0.4	0.09	0.12	0.02
	Intestine	0.4	0.1	0.29	0.08	0.09	0.04
	Spleen	0.45	0.05	0.14	0.06	0.08	0.01
	Heart	0.9	0.1	0.17	0.06	0.02	0.005
	Lungs	1.4	0.4	0.38	0.1	0.07	0.02
	Kidneys	4.4	0.5	2.1	0.7	0.87	0.14
	Muscle	0.8	0.4	0.1	0.03	0.021	0.005
	Bone	0.8	0.2	0.2	0.1	0.04	0.01
	Stomach	0.4	0.2	0.17	0.06	0.03	0.01
	Pancreas	0.42	0.02	0.09	0.06	0.03	0.01
	Tumor	2.46	0.6	0.5	0.2	0.13	0.04
	Excretion	61.2	8	88	4.2	97.3	0.9

**Table S5.** Biodistribution data of the GRPR pre-targeting approach by injection of [<sup>111</sup>In]In-DOTA-Tz 4h p.i. of AR-PEG4-TCO (50 µg) in PC3-xenografts bearing mice at 15 min and 1 h (with and without blocking) p.i. (data expressed as mean % I.D./g and SD, n = 3).

111In-DOTA-Tz + AR-PEG4-TCO		MEAN	SD	MEAN	SD	MEAN	SD
		15 min		1 h		1 h blocking	
	Blood	5.3	0.75	0.96	0.2	1	0.5
	Liver	1.65	0.3	0.865	0.1	1.2	0.3
	Intestine	0.84	0.185	0.475	0.05	0.4	0.1
	Spleen	1.2	0.3	0.495	0.03	0.4	0.1
	Heart	2.05	0.55	0.33	0.008	0.51	0.03
	Lungs	3.75	0.95	0.63	0.07	1.1	0.2
	Kidneys	5.75	0.41	3.1	0.2	2.5	0.5
	Muscle	1.65	0.15	0.34	0.002	0.24	0.01
	Bone	1.6	0.5	1.07	0.001	0.3	0.03
	Stomach	1.35	0.55	0.25	0.008	0.16	0
	Pancreas	1.2	0.4	0.4	0.11	0.25	0.05
	Tumor	3.74	0.135	1.19	0.2	0.64	0.1
	Excretion	32.4	6.6	74.65	18.3	84.3	5.7