Supporting Information

H_4octapa-Trastuzumab: Versatile Acyclic Chelate System for ^{111}In and ^{177}Lu Imaging and Therapy

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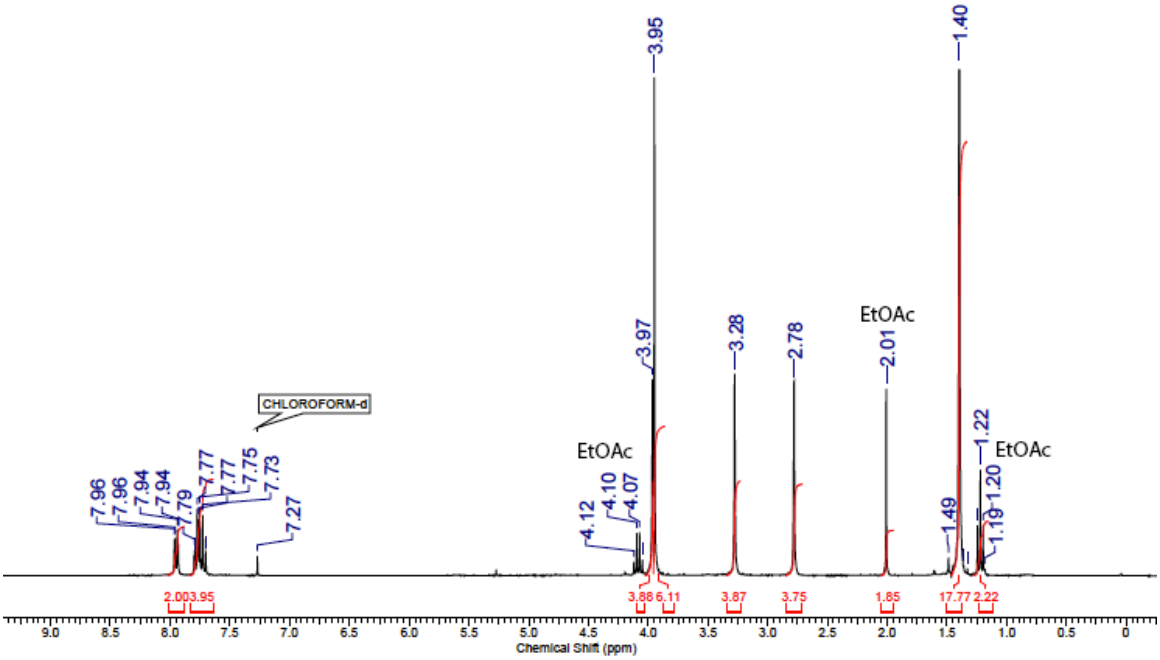
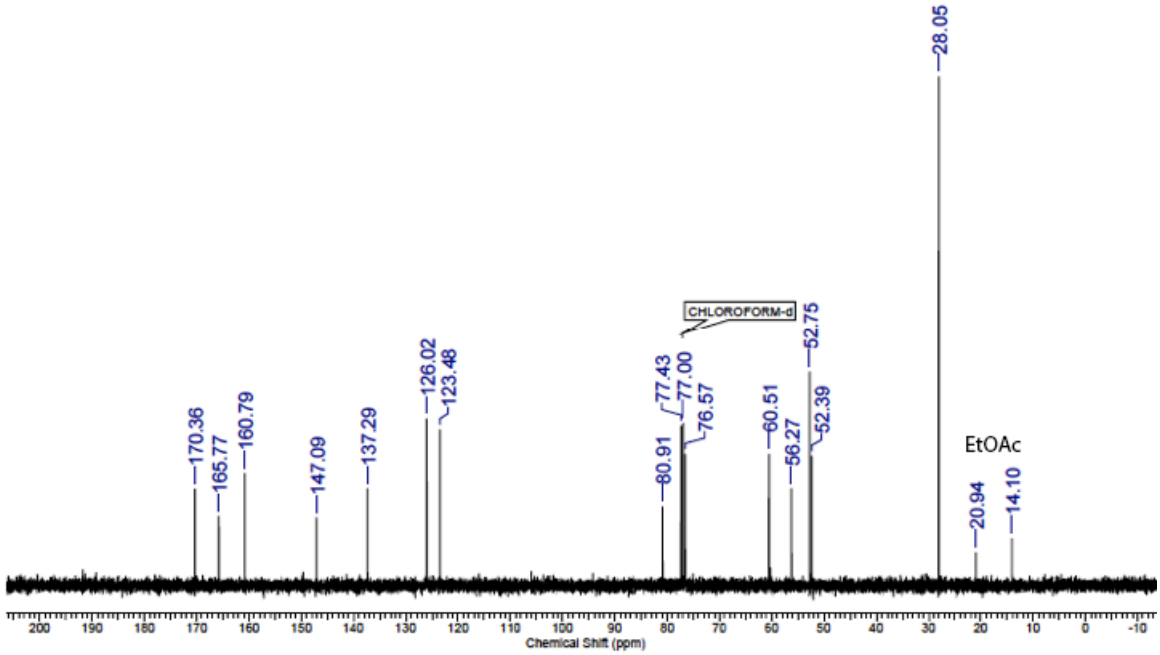
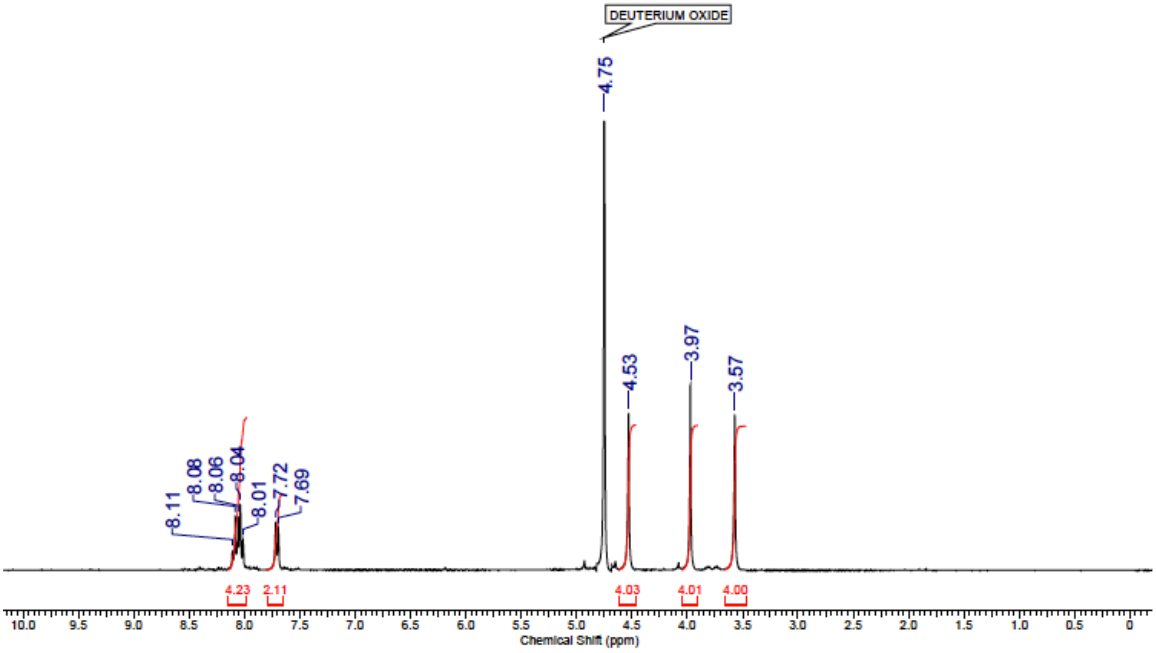


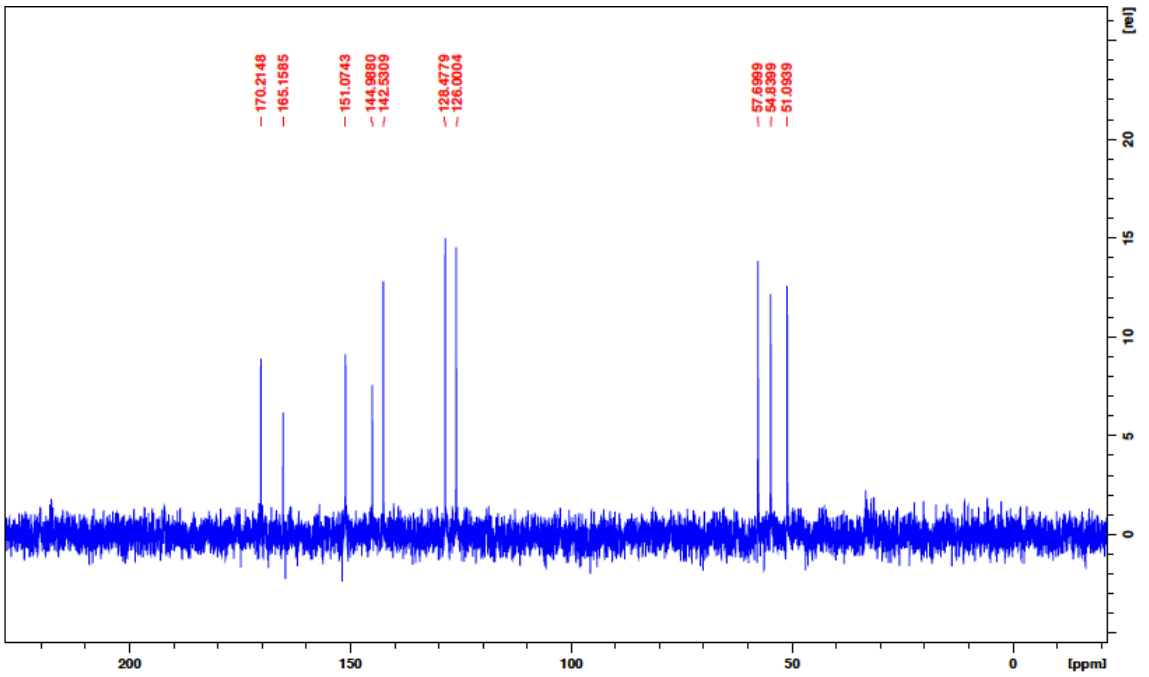
Supplementary Figure S1. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, RT) of **4**.



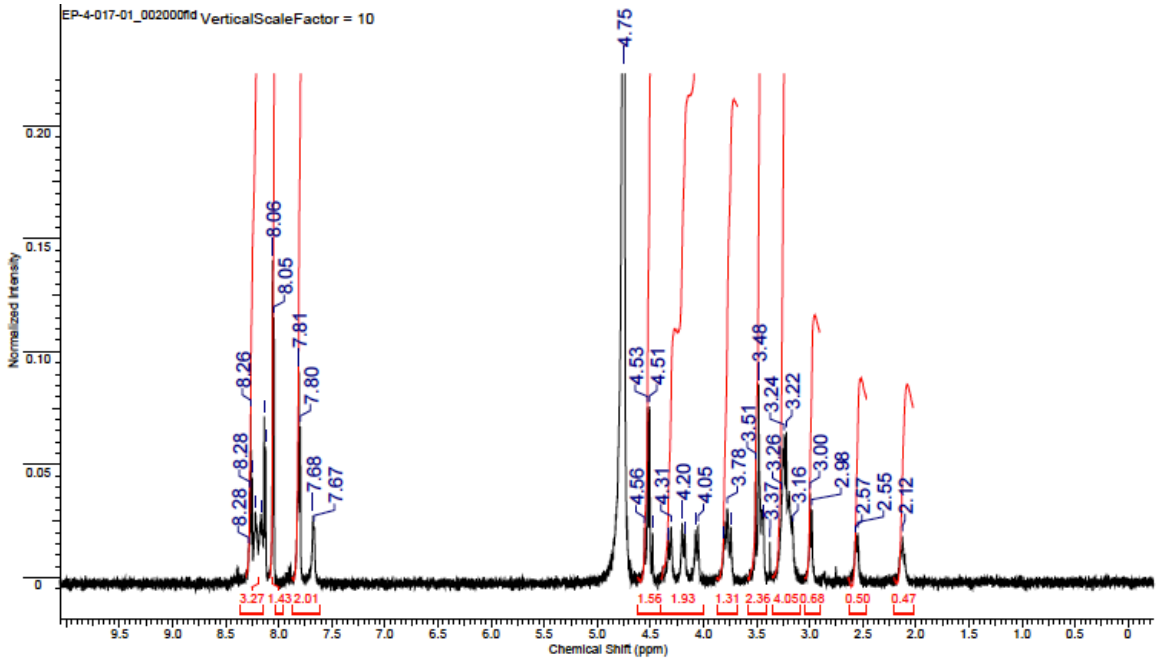
Supplementary Figure S2. ^{13}C NMR spectrum (75 MHz, $CDCl_3$, RT) of **4**.



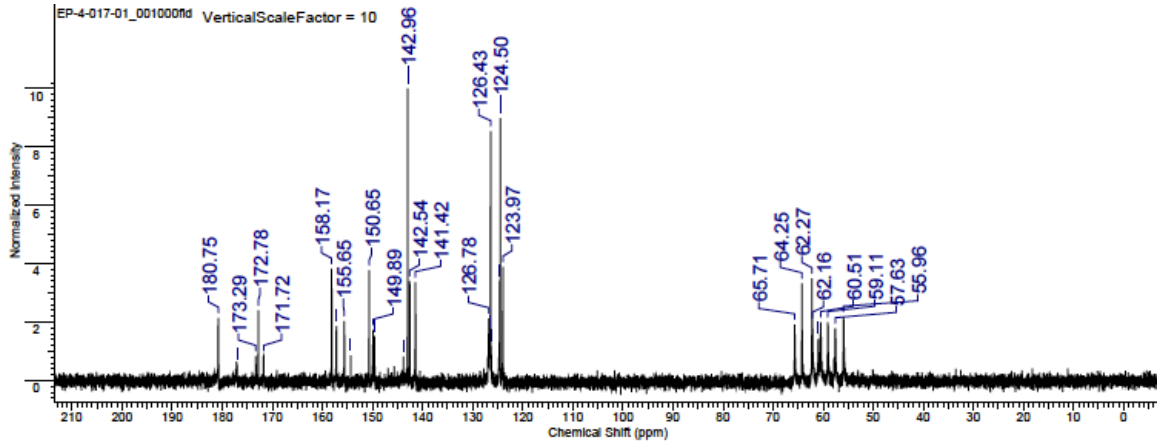
Supplementary Figure S3. ^{1}H NMR spectrum (300 MHz, D_2O, RT) of **H_4octapa (5)**.



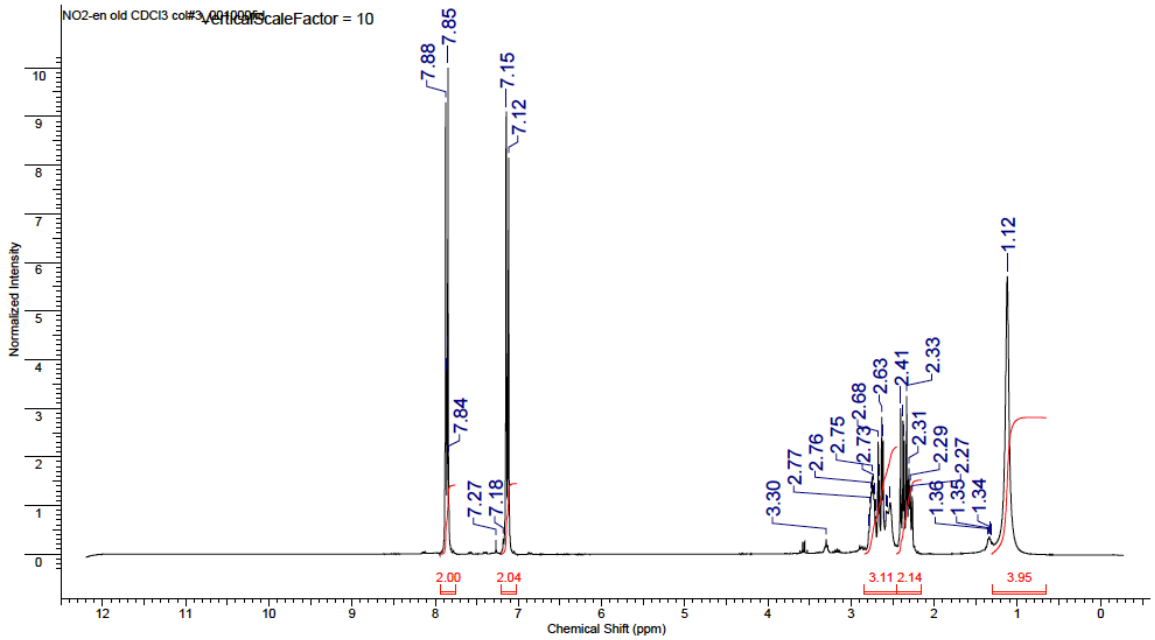
Supplementary Figure S4. ^{13}C NMR spectrum (75 MHz, D_2O, RT) of **H_4octapa (5)**, externally referenced to a solution of MeOH/D_2O (sr = -2.66).



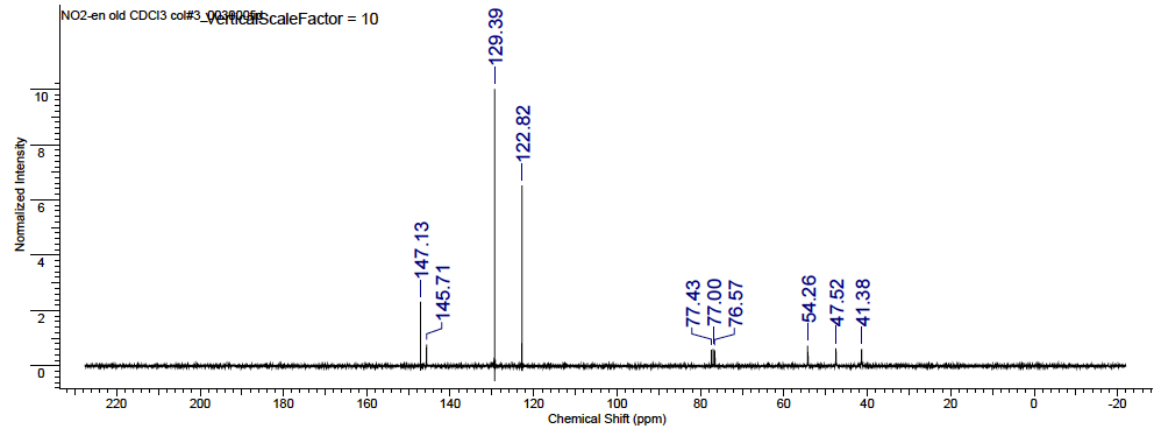
Supplementary Figure S5. ^{1}H NMR spectrum (600 MHz, D_2O, RT) of [**Lu(octapa)]$^-$ (6)**.



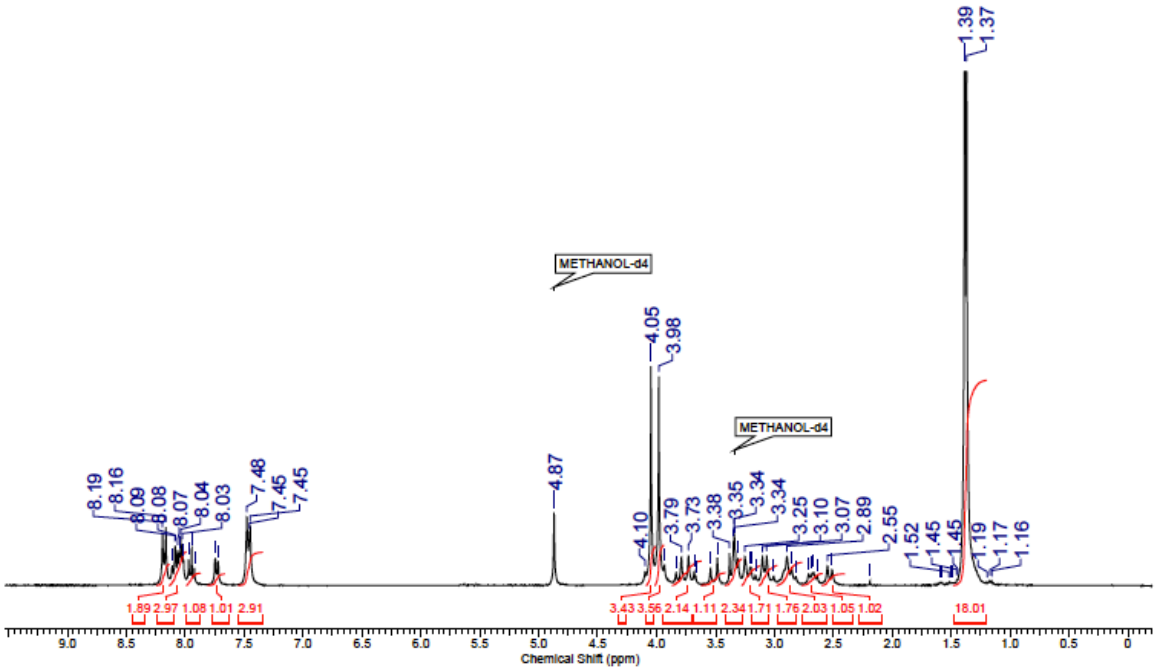
Supplementary Figure S6. ^{13}C NMR spectrum (150 MHz, D_2O, RT) of [**Lu(octapa)]$^-$ (6)**, externally referenced to a solution of MeOH/D_2O.



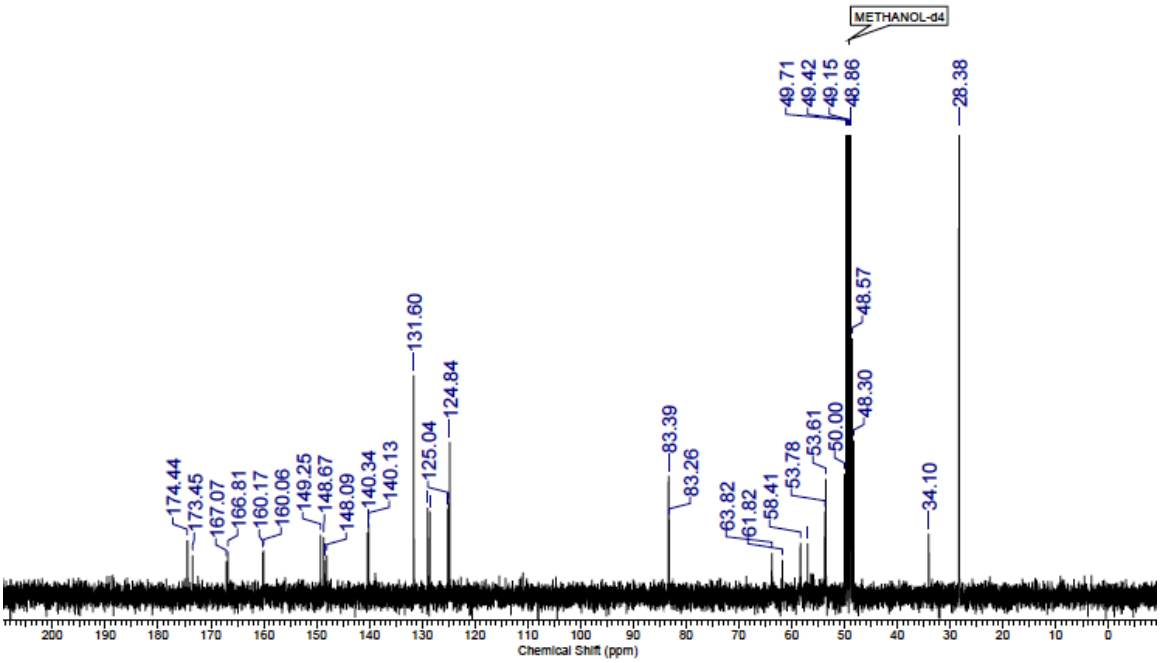
Supplementary Figure S7. ^{1}H NMR spectrum (300 MHz, $CDCl_3$, RT) of **7**.



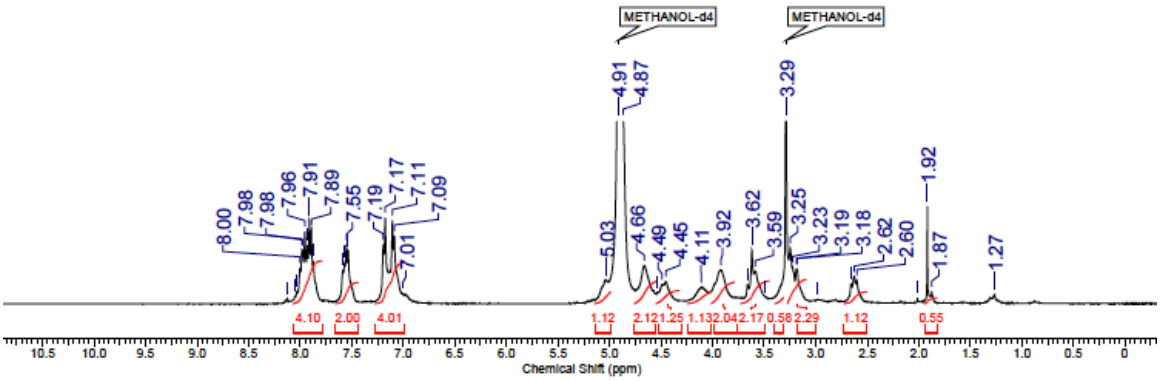
Supplementary Figure S8. ^{13}C NMR spectrum (75 MHz, $CDCl_3$, RT) of **7**.



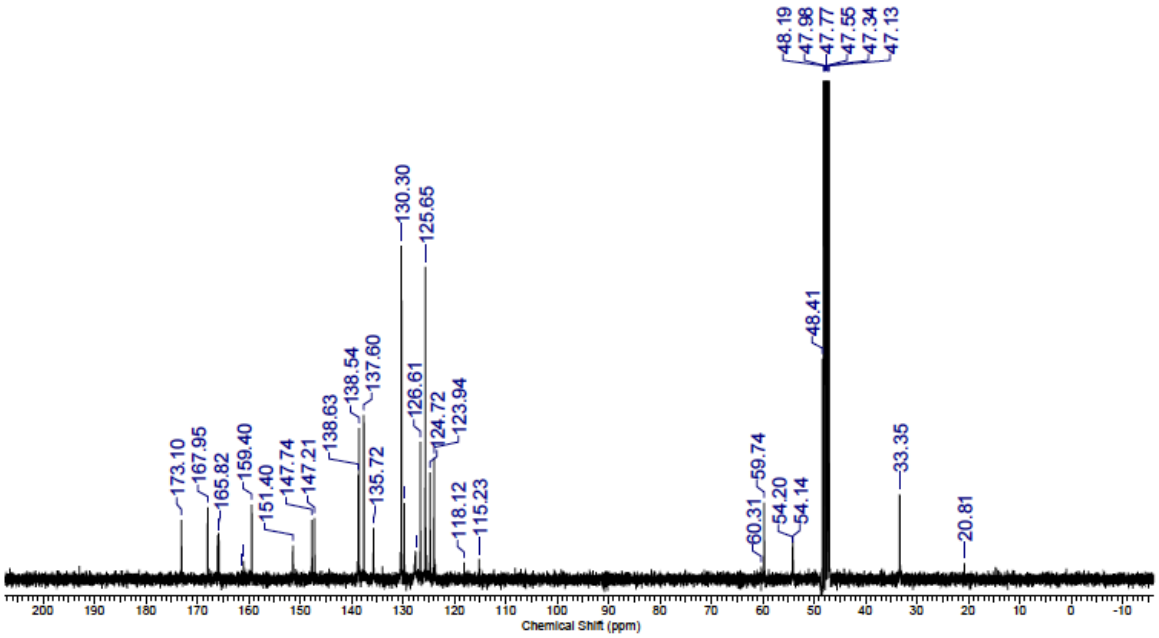
Supplementary Figure S9. ^{1}H NMR spectrum (300 MHz, MeOD, RT) of **11**.



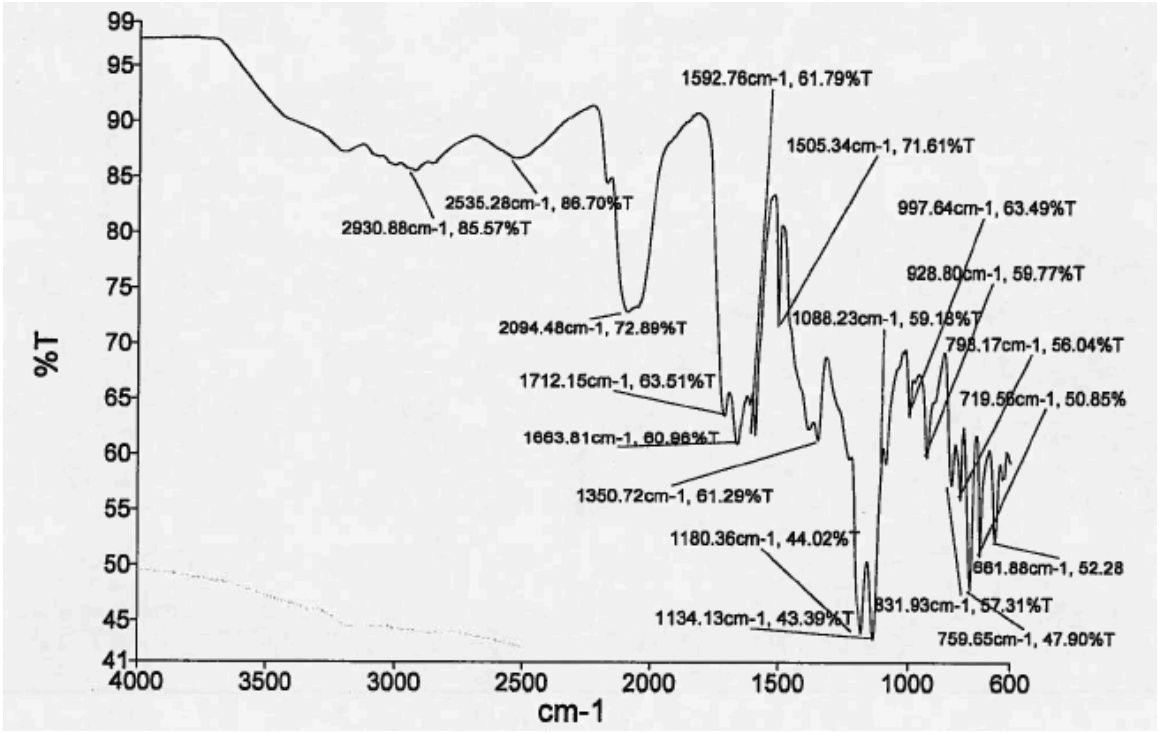
Supplementary Figure S10. ^{13}C NMR spectrum (75 MHz, MeOD, RT) of **11**.



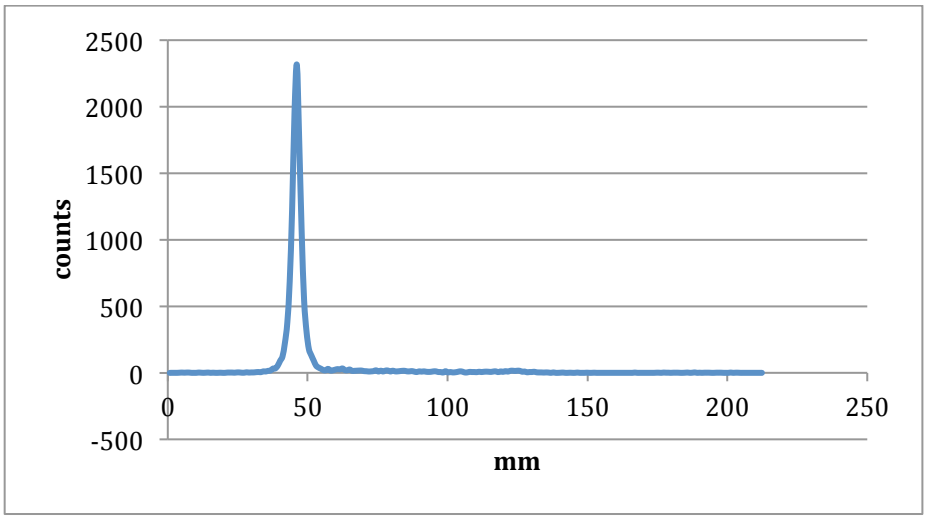
Supplementary Figure S11. ^{1}H NMR spectrum (400 MHz, MeOD, RT) of ***p*-SCN-Bn-H$_4$octapa (12)**.



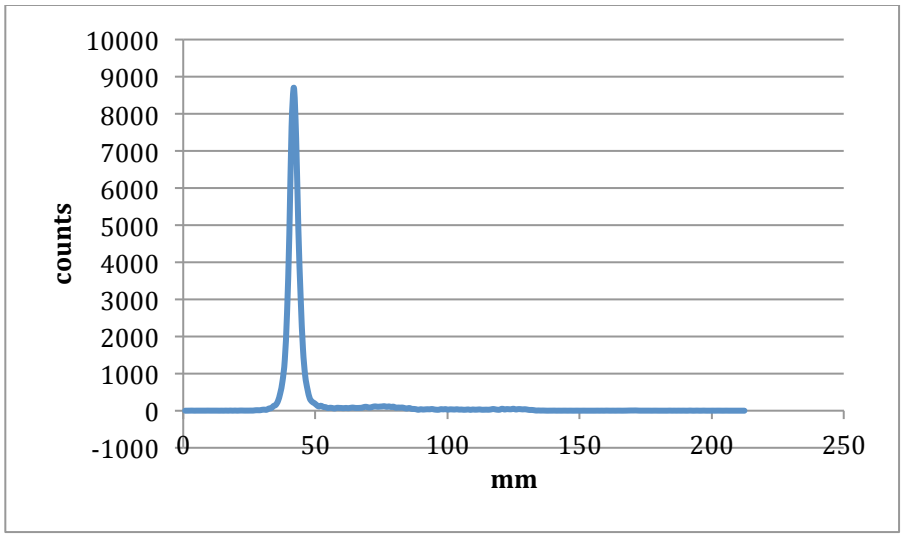
Supplementary Figure S12. ^{13}C NMR spectrum (100 MHz, MeOD, RT) of ***p*-SCN-Bn-H$_4$octapa (12)**.



Supplementary Figure S13. FT-ATR-IR spectrum (neat) of ***p*-SCN-Bn-H$_4$octapa (12)**.



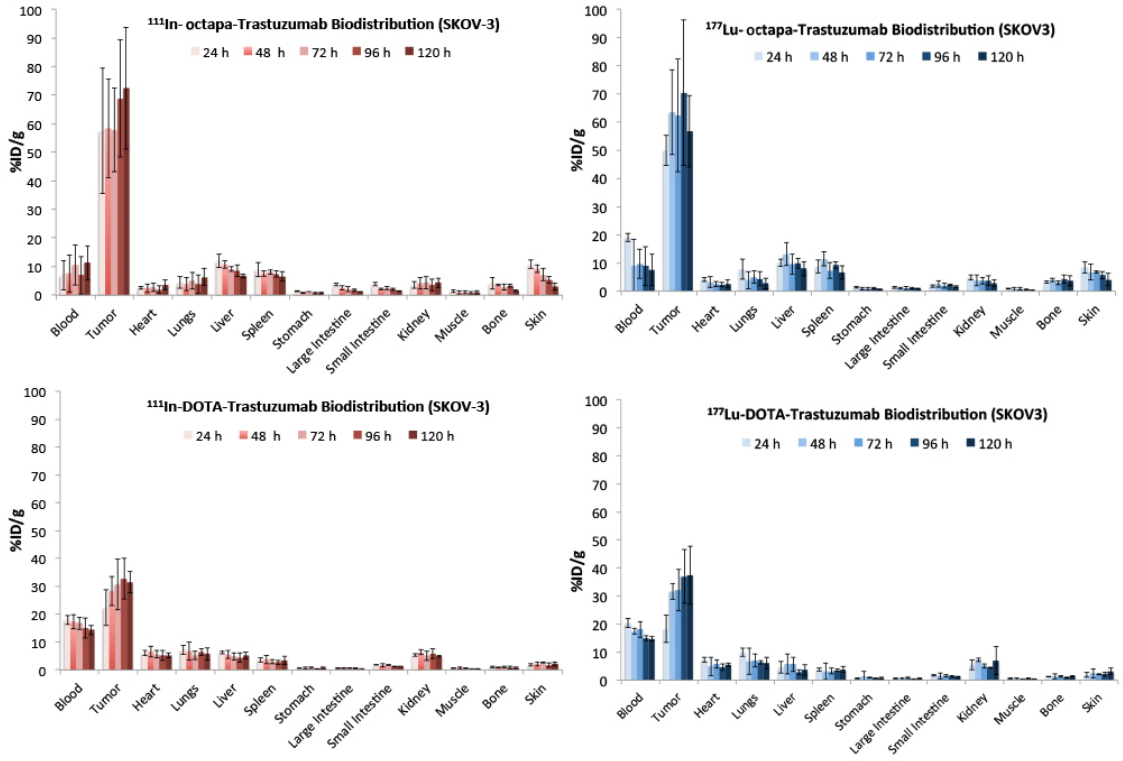
Supplementary Figure S14. Crude iTLC radiochromatograph of **^{111}In-octapa-trastuzumab** (radiolabeled in NH_4Ac buffer pH 5.5 100 mM, 15 min, RT and eluted with a mobile phase of 50 mM EDTA, pH 5) before purification, showing essentially quantitative radiolabeling yields. "Free" ^{111}In(EDTA) elutes near the solvent front ~150-200 mm.



Supplementary Figure S15. Crude iTLC radiochromatograph of **^{177}Lu-octapa-trastuzumab** (radiolabeled in NH_4Ac buffer pH 5.5 100 mM, 15 min, RT and eluted with a mobile phase of 50 mM EDTA, pH 5) before purification, showing essentially quantitative radiolabeling yields. "Free" ^{177}Lu(EDTA) elutes near the solvent front ~150-200 mm.

^{111}In-octapa-trastuzumab Biodistribution						^{111}In-DOTA-trastuzumab Biodistribution					
Organ	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**	**Organ**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
Blood	6.9 ± 5.1	7.6 ± 6.5	10.5 ± 7.1	6.9 ± 6.6	11.4 ± 6	Blood	18 ± 1.7	17.3 ± 2.6	16.8 ± 2.2	15 ± 3.6	14.3 ± 1.6
Tumor	57.4 ± 22	58.3 ± 17.2	57.8 ± 14.6	68.7 ± 20.5	72.4 ± 21.3	Tumor	22.3 ± 6.4	28.4 ± 5.2	30.6 ± 9.1	32.8 ± 7.3	31.5 ± 3.8
Heart	2.6 ± 0.5	2.5 ± 1.5	2.7 ± 1.4	1.9 ± 1.4	3.6 ± 1.6	Heart	6.1 ± 0.9	6.6 ± 2	5.6 ± 1.3	5.2 ± 1.8	5.2 ± 0.9
Lungs	4.4 ± 2.1	3.9 ± 2.3	5 ± 2.8	3.8 ± 3.3	6.3 ± 3	Lungs	7.2 ± 1.6	6.8 ± 3.3	5.2 ± 1.4	6.3 ± 1.2	5.7 ± 2.2
Liver	12 ± 2.4	10.6 ± 1.2	9.1 ± 0.8	8.5 ± 2	6.6 ± 0.6	Liver	6.2 ± 0.4	5.5 ± 1.4	4.8 ± 1.3	4.4 ± 1.7	5.2 ± 1.2
Spleen	8.9 ± 2.4	7.6 ± 1	8.1 ± 0.8	7.4 ± 1.1	6.5 ± 1.6	Spleen	3.6 ± 0.8	3.7 ± 1.6	3 ± 0.7	2.7 ± 0.7	3.4 ± 1.4
Stomach	1.4 ± 0.2	0.8 ± 0.2	1.2 ± 0.1	0.7 ± 0.3	0.7 ± 0.2	Stomach	0.7 ± 0.1	0.7 ± 0.4	0.8 ± 0.2	0.3 ± 0.2	0.6 ± 0.3
Large Intestine	3.7 ± 0.4	2.5 ± 0.7	2 ± 0.9	1.7 ± 0.4	1.2 ± 0.1	Large Intestine	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Small Intestine	3.8 ± 0.7	2.1 ± 0.4	2.5 ± 0.6	2.1 ± 0.4	1.5 ± 0.1	Small Intestine	1.9 ± 0.1	1.6 ± 0.5	1.7 ± 0.1	1.1 ± 0.2	1.3 ± 0.1
Kidney	3.6 ± 1.2	4.2 ± 2.1	4.3 ± 2.1	3.5 ± 2	4.3 ± 1.6	Kidney	5.3 ± 0.5	6.3 ± 0.9	5.1 ± 1.7	5.8 ± 1.7	4.9 ± 0.4
Muscle	1.3 ± 0.5	0.9 ± 0.5	1 ± 0.5	0.8 ± 0.5	0.9 ± 0.5	Muscle	0.7 ± 0.2	0.8 ± 0.3	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Bone	4.1 ± 2	3.5 ± 0.4	2.8 ± 1	3.3 ± 0.5	1.4 ± 0.3	Bone	1 ± 0.3	0.9 ± 0.1	1.1 ± 0.4	0.9 ± 0.4	0.6 ± 0.2
Skin	10.8 ± 1.4	9.3 ± 1.4	7.2 ± 2.1	5.4 ± 1.2	3 ± 1.1	Skin	1.8 ± 0.4	2.1 ± 0.7	2.6 ± 0.1	1.7 ± 0.8	2.2 ± 0.8
Tumor/Tissue	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**	**Tumor/Tissue**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
Tumor/Blood	8.3 ± 7	7.7 ± 6.9	5.5 ± 3.9	10 ± 10	6.4 ± 3.8	Tumor/Blood	1.2 ± 0.4	1.6 ± 0.4	1.8 ± 0.6	2.2 ± 0.7	2.2 ± 0.4
Tumor/Heart	22.3 ± 9.5	23.6 ± 16	21.4 ± 12.3	35.8 ± 27.5	20.1 ± 10.9	Tumor/Heart	3.6 ± 1.2	4.3 ± 1.5	5.5 ± 2.1	6.3 ± 2.6	6 ± 1.2
Tumor/Lung	13.1 ± 8	15.1 ± 10	11.6 ± 7.3	17.9 ± 16.4	11.5 ± 6.3	Tumor/Lung	3.1 ± 1.1	4.2 ± 2.2	5.9 ± 2.4	5.2 ± 1.5	5.5 ± 2.2
Tumor/Liver	4.8 ± 2.1	5.5 ± 1.7	6.4 ± 1.7	8.1 ± 3.1	10.9 ± 3.4	Tumor/Liver	3.6 ± 1.1	5.1 ± 1.6	6.4 ± 2.5	7.5 ± 3.3	6.1 ± 1.5
Tumor/Spleen	6.5 ± 3	7.7 ± 2.5	7.1 ± 1.9	9.3 ± 3.1	11.1 ± 4.2	Tumor/Spleen	6.2 ± 2.2	7.6 ± 3.6	10.3 ± 4	12 ± 4.2	9.2 ± 3.9
Tumor/Stomach	40.8 ± 16.9	76.1 ± 28.6	46.6 ± 12.9	92.6 ± 48.5	103.3 ± 43.3	Tumor/Stomach	32.7 ± 11.7	41.9 ± 26.2	40.3 ± 15.8	98.3 ± 52.2	49.9 ± 21.2
Tumor/LI	15.5 ± 6.2	23.2 ± 9.3	28.9 ± 14.5	39.8 ± 15.4	59.4 ± 18.6	Tumor/LI	43.6 ± 17.5	46.1 ± 12.3	46.2 ± 16.5	63.6 ± 19.9	63.5 ± 12.4
Tumor/SI	15 ± 6.3	27.5 ± 9.6	23 ± 7.9	33.4 ± 12.3	48 ± 14.8	Tumor/SI	11.8 ± 3.5	17.3 ± 6.4	17.9 ± 5.4	28.7 ± 8.8	23.7 ± 3
Tumor/Kidney	16.1 ± 8.3	14 ± 8.1	13.3 ± 7.3	19.6 ± 12.5	16.9 ± 8	Tumor/Kidney	4.2 ± 1.2	4.5 ± 1	6.1 ± 2.7	5.7 ± 2.1	6.4 ± 0.9
Tumor/Muscle	44.7 ± 23.7	64.7 ± 42.2	56.8 ± 33.2	89.3 ± 59.1	78.9 ± 52.2	Tumor/Muscle	33.2 ± 13.3	36.7 ± 14.9	63.4 ± 26.4	79.6 ± 26.5	90 ± 22.8
Tumor/Bone	14 ± 8.7	16.5 ± 5.2	20.8 ± 9.1	20.7 ± 6.9	50.4 ± 18.2	Tumor/Bone	22 ± 8.9	32 ± 7.6	29 ± 13.6	38.3 ± 21.6	48.6 ± 18.5
Tumor/Skin	5.3 ± 2.2	6.3 ± 2.1	8 ± 3.1	12.8 ± 4.8	24.2 ± 11.5	Tumor/Skin	12.3 ± 4.6	13.6 ± 5	11.7 ± 3.5	19.4 ± 9.8	14.6 ± 5.5
^{177}Lu-octapa-trastuzumab						^{177}Lu-DOTA-trastuzumab					
Organ	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**	**Organ**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
Blood	19.1 ± 1.5	9.1 ± 9.5	9.7 ± 5.2	8.9 ± 6.9	7.6 ± 5.7	Blood	20.4 ± 1.7	17.4 ± 1	18.1 ± 2.8	15 ± 0.8	14.7 ± 0.7
Tumor	50 ± 5.4	63.5 ± 15	62.4 ± 20	70.4 ± 25.8	56.6 ± 12.7	Tumor	18.3 ± 4.8	31.6 ± 2.9	32.1 ± 7.4	37 ± 9.5	37.4 ± 10.3
Heart	4.2 ± 0.7	3.2 ± 1.9	2.5 ± 0.9	2.3 ± 0.7	2.6 ± 1.4	Heart	7.2 ± 1	4.9 ± 3.3	5.8 ± 1.6	4.6 ± 1.1	5.4 ± 0.6
Lung	7.8 ± 3.6	3.9 ± 3.1	5 ± 2.3	4.4 ± 2.5	2.7 ± 1.8	Lungs	10 ± 1.5	6.7 ± 4.6	7.1 ± 2.3	6.3 ± 0.6	6 ± 2.1
Liver	10.1 ± 1.3	13.2 ± 4	9.5 ± 3.5	9.8 ± 1.7	8 ± 2.6	Liver	4.5 ± 2	5.7 ± 3.6	5.7 ± 2.5	2.8 ± 0.9	3.7 ± 1.9
Spleen	8.8 ± 2.3	11.5 ± 2.5	7.4 ± 2.8	9.2 ± 1.2	6.7 ± 2.4	Spleen	3.8 ± 0.5	3.1 ± 2.9	3.2 ± 1	3.5 ± 0.4	3.8 ± 1
Stomach	1.4 ± 0.3	1 ± 0.5	0.9 ± 0.4	1.1 ± 0.3	0.6 ± 0.2	Stomach	0.7 ± 0.2	1.3 ± 1.9	0.9 ± 0.2	0.8 ± 0.2	0.7 ± 0.2
Large Intestine	1.3 ± 0.3	1 ± 0.3	1.1 ± 0.5	1.1 ± 0.2	0.8 ± 0.2	Large Intestine	0.6 ± 0.1	0.5 ± 0.3	0.8 ± 0.2	0.5 ± 0.1	0.6 ± 0.2
Small Intestine	1.9 ± 0.5	2.5 ± 1.1	2 ± 0.9	2.3 ± 0.4	1.6 ± 0.4	Small Intestine	1.7 ± 0.2	1.5 ± 1	1.5 ± 0.4	1.4 ± 0.3	1.2 ± 0.1
Kidney	4.9 ± 0.9	3.8 ± 2	3.7 ± 1.2	3.8 ± 1.8	2.8 ± 1.2	Kidney	5.5 ± 1.7	7.3 ± 0.7	5.1 ± 0.8	4.4 ± 0.2	7.1 ± 5
Muscle	0.9 ± 0.2	0.7 ± 0.5	0.8 ± 0.5	0.5 ± 0.2	0.4 ± 0.2	Muscle	0.7 ± 0.2	0.5 ± 0.4	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
Bone	3.2 ± 0.5	4 ± 0.6	3.1 ± 0.7	4.2 ± 1.5	3.6 ± 1.7	Bone	1.2 ± 0	1.3 ± 0.8	1.3 ± 0.3	0.9 ± 0.2	1.4 ± 0.3
Skin	8.4 ± 2	6.8 ± 2.9	6.9 ± 0.5	5.6 ± 1.2	4.1 ± 2.2	Skin	1.9 ± 0.9	2.4 ± 1.7	2.1 ± 0.2	2.1 ± 0.7	3.1 ± 1.3
Tumor/Tissue	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**	**Tumor/Tissue**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
Tumor/Blood	2.6 ± 0.3	7 ± 7.5	6.4 ± 4	7.9 ± 6.8	7.5 ± 5.8	Tumor/Blood	0.9 ± 0.2	1.8 ± 0.2	1.8 ± 0.5	2.5 ± 0.7	2.5 ± 0.7
Tumor/Heart	11.8 ± 2.4	20 ± 13.1	25.1 ± 11.9	31.3 ± 15.4	21.7 ± 12.4	Tumor/Heart	2.5 ± 0.8	6.5 ± 4.5	5.5 ± 2	8 ± 2.9	7 ± 2.1
Tumor/Lung	6.4 ± 3	16.3 ± 13.5	12.5 ± 7	16 ± 10.7	21 ± 14.9	Tumor/Lung	1.8 ± 0.6	4.7 ± 3.3	4.5 ± 1.8	5.9 ± 1.6	6.2 ± 2.8
Tumor/Liver	4.9 ± 0.8	4.8 ± 1.9	6.5 ± 3.2	7.2 ± 2.9	7.1 ± 2.8	Tumor/Liver	4.1 ± 2.1	5.6 ± 3.5	5.7 ± 2.9	13.4 ± 5.7	10.2 ± 6
Tumor/Spleen	5.7 ± 1.6	5.5 ± 1.8	8.5 ± 4.2	7.6 ± 3	8.4 ± 3.6	Tumor/Spleen	4.8 ± 1.4	10 ± 9.4	9.9 ± 3.8	10.6 ± 3	9.8 ± 3.8
Tumor/Stomach	36.2 ± 8	66.8 ± 40.3	71.7 ± 39.7	64.6 ± 29.5	92.8 ± 38.7	Tumor/Stomach	27.4 ± 11.4	25.3 ± 37.9	34.6 ± 11.6	48.8 ± 16.1	52.1 ± 20.8
Tumor/LI	40 ± 12	64.8 ± 24.3	55.2 ± 31.4	65.2 ± 27.9	69.9 ± 21.9	Tumor/LI	31.9 ± 10	63.6 ± 39.3	42 ± 13	72.8 ± 20.8	61.7 ± 24.7
Tumor/SI	27 ± 7.2	25.1 ± 12.4	31.5 ± 17.3	31 ± 12.4	34.7 ± 11.5	Tumor/SI	10.7 ± 3.1	20.8 ± 14.2	20.8 ± 7.3	27.1 ± 9.2	30.5 ± 8.7
Tumor/Kidney	10.2 ± 2.2	16.7 ± 9.6	16.9 ± 7.6	18.8 ± 11.1	20.3 ± 9.9	Tumor/Kidney	3.4 ± 1.4	4.3 ± 0.6	6.3 ± 1.7	8.4 ± 2.2	5.3 ± 4
Tumor/Muscle	53.1 ± 10.7	86.9 ± 64.6	74.3 ± 52	138.1 ± 65.1	157.3 ± 81.5	Tumor/Muscle	28 ± 11.5	58.6 ± 39.5	71.5 ± 18.4	68.1 ± 27.6	74.3 ± 25.7
Tumor/Bone	15.8 ± 2.9	16.1 ± 4.5	20.3 ± 8.2	16.7 ± 8.4	15.8 ± 8.4	Tumor/Bone	15.1 ± 4	24.7 ± 16	24.2 ± 7.8	43.3 ± 14.5	26.4 ± 8.7
Tumor/Skin	5.9 ± 1.6	9.4 ± 4.6	9.1 ± 3	12.5 ± 5.3	13.9 ± 8.3	Tumor/Skin	9.4 ± 4.9	13.2 ± 9.5	15 ± 3.7	17.6 ± 7.1	11.9 ± 5.9

Supplementary Table S1. Biodistribution data from ^{111}In/^{177}Lu-octapa-trastuzumab and ^{111}In/^{177}Lu-DOTA-trastuzumab in female nude athymic mice (n = 4) with subcutaneous SKOV-3 xenografts (right shoulder, tumor volume ~ 100-150 mm^3) showing organ uptake as % ID/g with relevant tumor/tissue ratios, with the error expressed as standard deviation (SD).



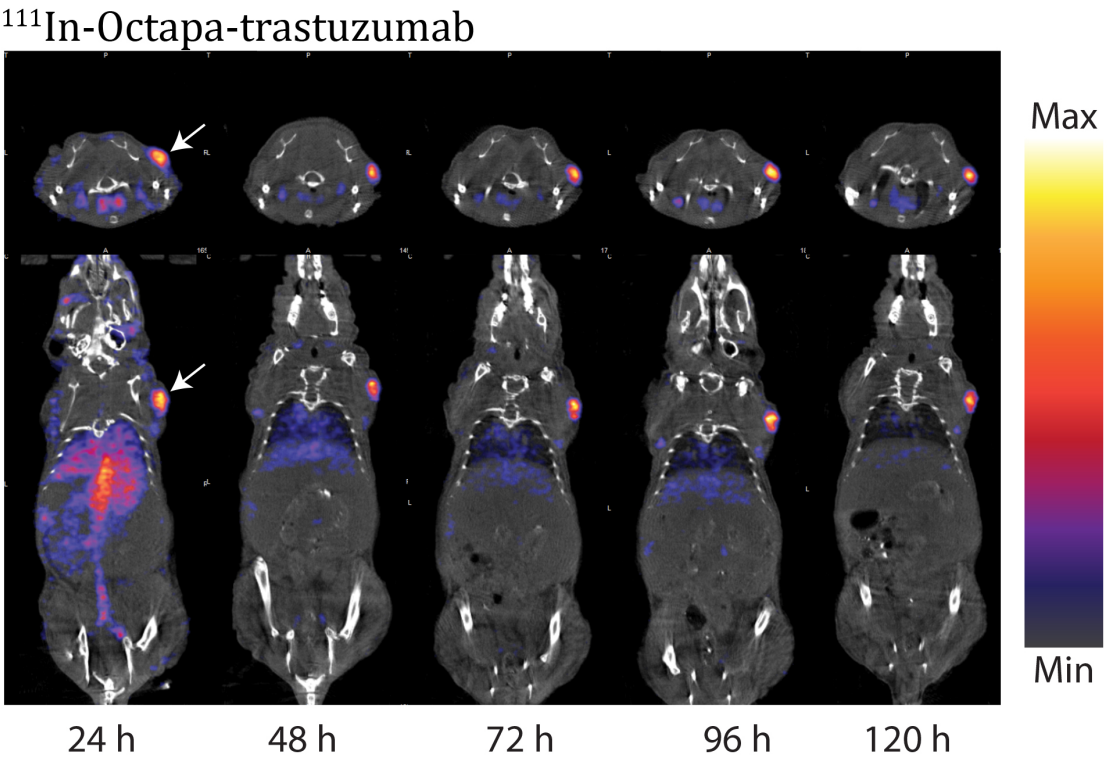
Supplementary Figure S16. Biodistribution data from ^{111}In/^{177}Lu-octapa-trastuzumab and ^{111}In/^{177}Lu-DOTA-trastuzumab in female nude athymic mice (n = 4) with subcutaneous SKOV-3 xenografts (right shoulder, 100-150 mm^3) showing organ uptake as % ID/g, with the error expressed as standard deviation (SD), see Table 1 and Supplementary Tables S18 and S19 for data.

Tissue	^{111}In-octapa DOTA-Trastuzumab 72 h	^{177}Lu-octapa DOTA-Trastuzumab 72 h	^{111}In-octapa DOTA-Trastuzumab 96 h	^{177}Lu-octapa DOTA-Trastuzumab 96 h	^{111}In-octapa DOTA-Trastuzumab 120 h	^{177}Lu-octapa DOTA-Trastuzumab 120 h
Blood	0.094	0.029	0.052	0.128	0.316	0.048
Tumor	0.027	0.030	0.038	0.051	0.033	0.057
Heart	0.026	0.011	0.023	0.014	0.091	0.010
Lungs	0.294	0.254	0.205	0.199	0.876	0.054
Liver	0.041	0.122	0.002	0.000	0.026	0.037
Spleen	0.000	0.033	0.001	0.000	0.027	0.072
Stomach	0.056	0.800	0.936	0.096	0.916	0.504
Large Intestine	0.031	0.234	0.001	0.004	0.001	0.147
Small Intestine	0.036	0.398	0.041	0.008	0.013	0.094
Kidney	0.531	0.095	0.403	0.495	0.331	0.147
Muscle	0.080	0.191	0.385	0.807	0.185	0.199
Bone	0.031	0.005	0.000	0.004	0.919	0.047
Skin	0.003	0.000	0.012	0.002	0.872	0.495

Supplementary Table S2. P-values from a students two-tailed T-Test comparing the data sets (n = 4) of biodistribution %ID/g values between ^{111}In and ^{177}Lu radiolabeled H_4octapa- and DOTA-based trastuzumab immunoconjugates.

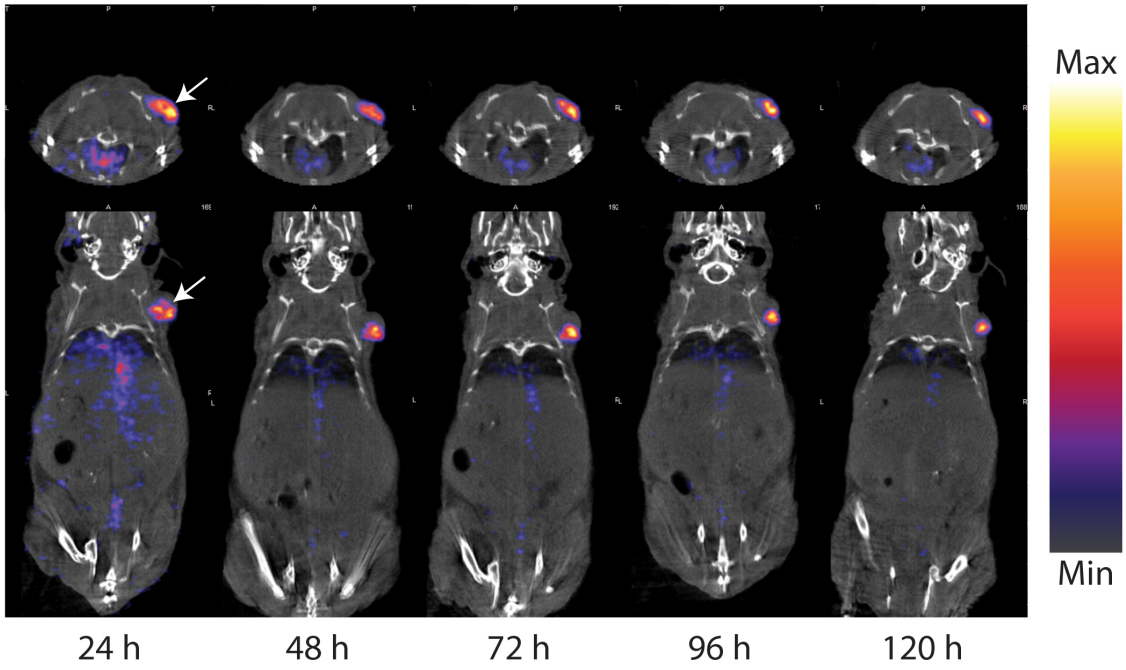
Complex	Serum stability 1.5 h (%)	Serum stability 24 h (%)
$[^{177}Lu(octapa)]^-$	88.1 ± 1.2	87.7 ± 0.7
$[^{177}Lu(DOTA)]^-$	87.7 ± 0.7	87.4 ± 2.1
$[^{177}Lu(DTPA)]^{2-}$	77.4 ± 1.2	81.6 ± 2.3

Supplementary Table S3. Human blood serum stability assays of ^{177}Lu(chelate) complexes (37.5 °C), analyzed via PD-10 size exclusion column elution, with % stability expressed as the % intact radiometal complex (n = 3).

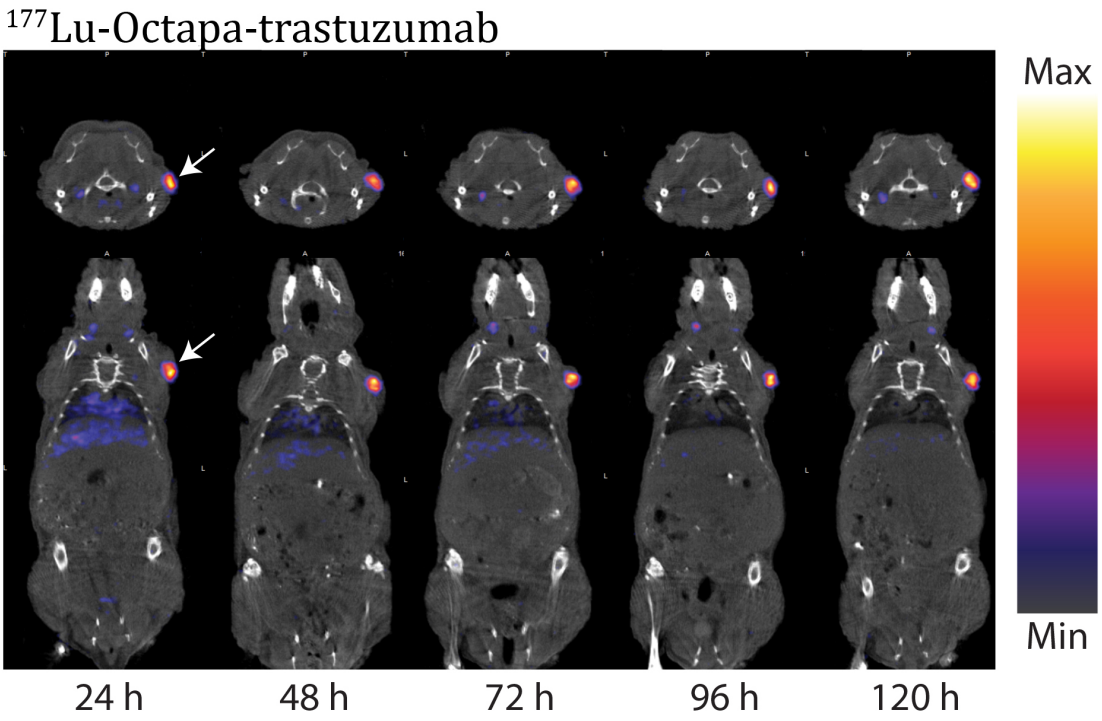


Supplementary Figure S17. SPECT/CT imaging of ^{111}In-octapa-trastuzumab in a female nude athymic mice (n = 2) bearing a subcutaneous SKOV-3 ovarian cancer xenograft (right shoulder, white arrow, tumor volume ~ 100-150 mm^3). The transverse (top) and coronal (bottom) planar images bisect the tumor and were collected at 24, 48, 72, 96, and 120 h post injection.

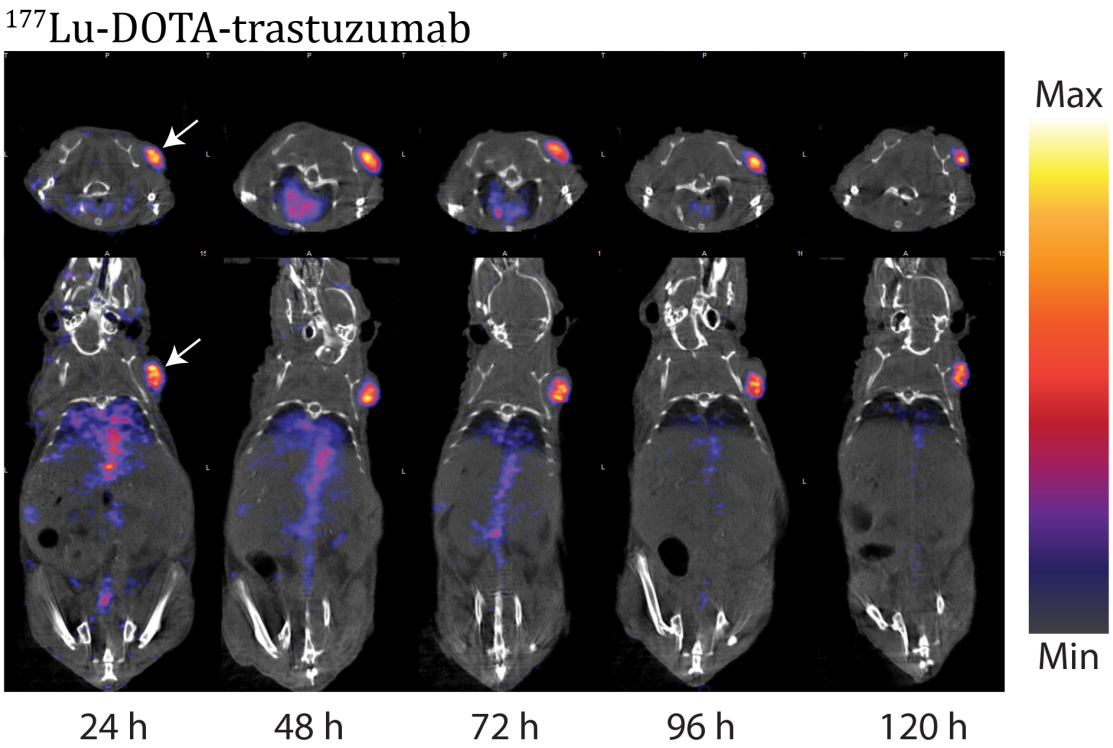
^{111}In-DOTA-trastuzumab



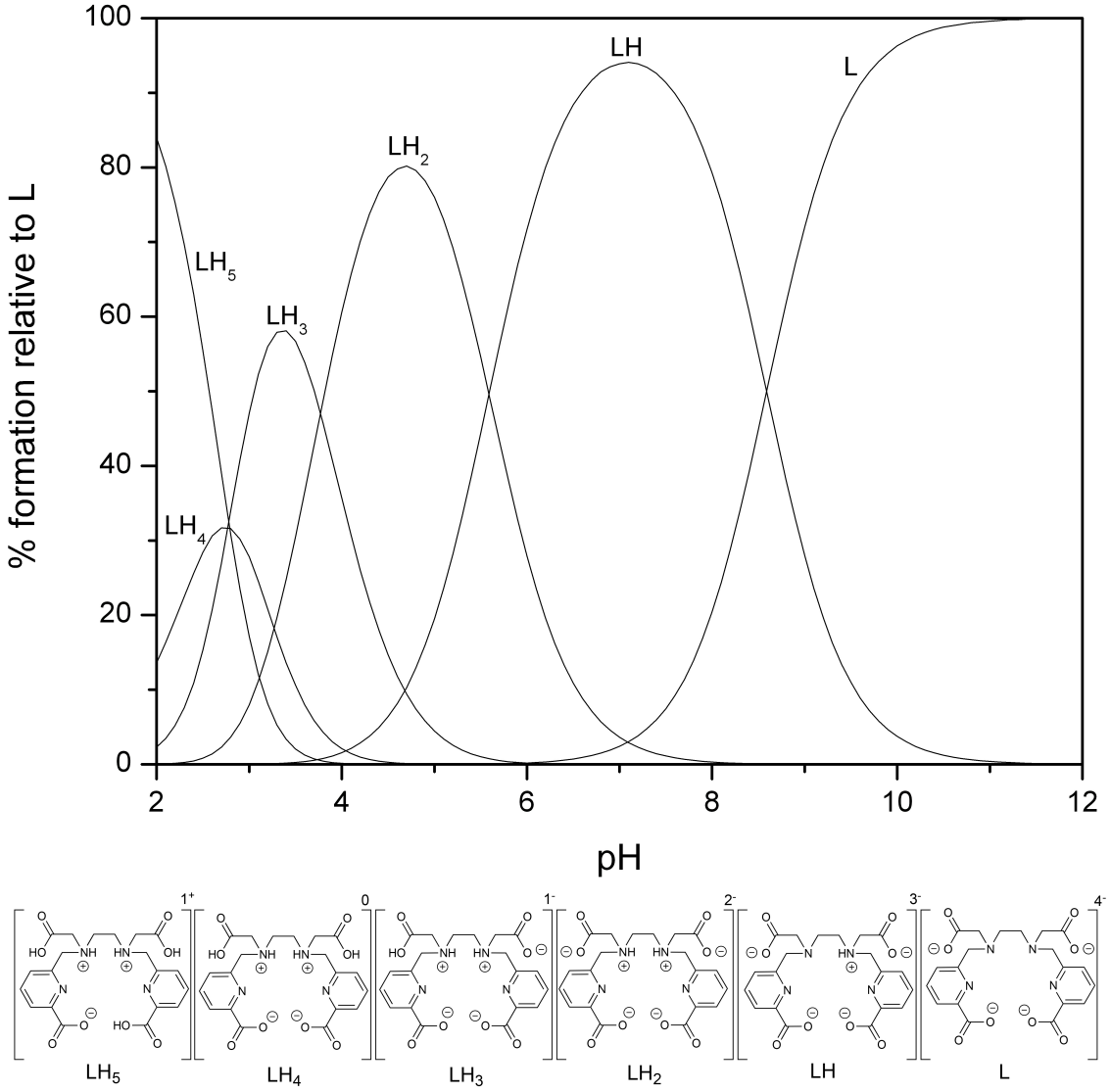
Supplementary Figure S18. SPECT/CT imaging of ^{111}In-DOTA-trastuzumab in a female nude athymic mice (n = 2) bearing a subcutaneous SKOV-3 ovarian cancer xenograft (right shoulder, white arrow, tumor volume ~ 100-150 mm^3). The transverse (top) and coronal (bottom) planar images bisect the tumor and were collected at 24, 48, 72, 96, and 120 h post injection.



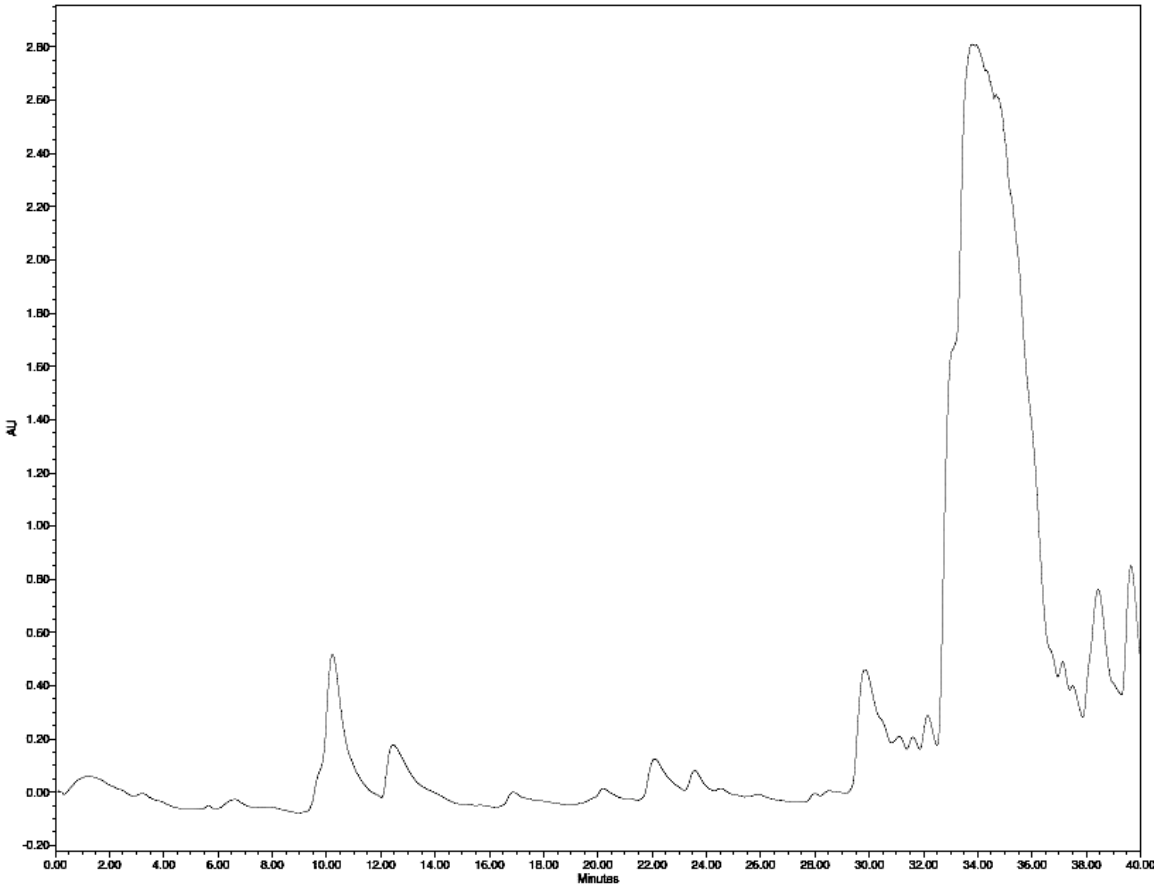
Supplementary Figure S19. SPECT/CT imaging of ^{177}Lu-octapa-trastuzumab in a female nude athymic mice (n = 2) bearing a subcutaneous SKOV-3 ovarian cancer xenograft (right shoulder, white arrow, tumor volume ~ 100-150 mm^3). The transverse (top) and coronal (bottom) planar images bisect the tumor and were collected at 24, 48, 72, 96, and 120 h post injection.



Supplementary Figure S20. SPECT/CT imaging of ^{177}Lu-DOTA-trastuzumab in a female nude athymic mice (n = 2) bearing a subcutaneous SKOV-3 ovarian cancer xenograft (right shoulder, white arrow, tumor volume ~ 100-150 mm^3). The transverse (top) and coronal (bottom) planar images bisect the tumor and were collected at 24, 48, 72, 96, and 120 h post injection.



Supplementary Figure S21. H_4octapa speciation curve, calculated from potentiometric titration experiments, showing the major species in solution at various pH, relative to 100% L.



Supplementary Figure S22. Crude HPLC chromatogram of *p*-SCN-Bn-octapa purified on a semi-preparatory column (see experimental methods). The product elutes as a broad peak at t_R of 34 minutes.