

Supplementary information for

**Preclinical evaluation of cationic DOTA-triarginine-lipid conjugates for
theranostic liquid brachytherapy**

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S1 – Preparation and TLC characterization of non-radioactive Cu-D3R-C16 and Cu-D3R-C18 reference compounds

Method

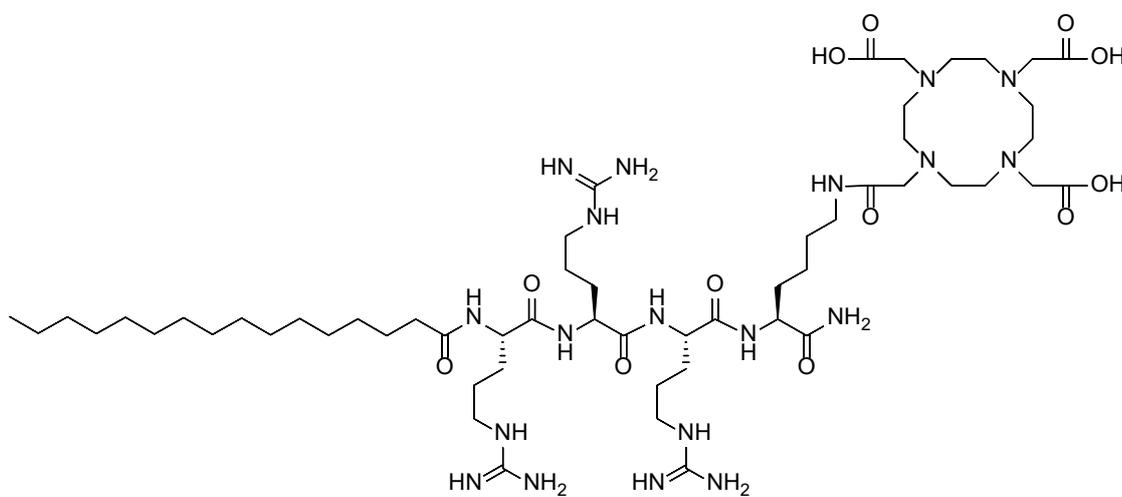
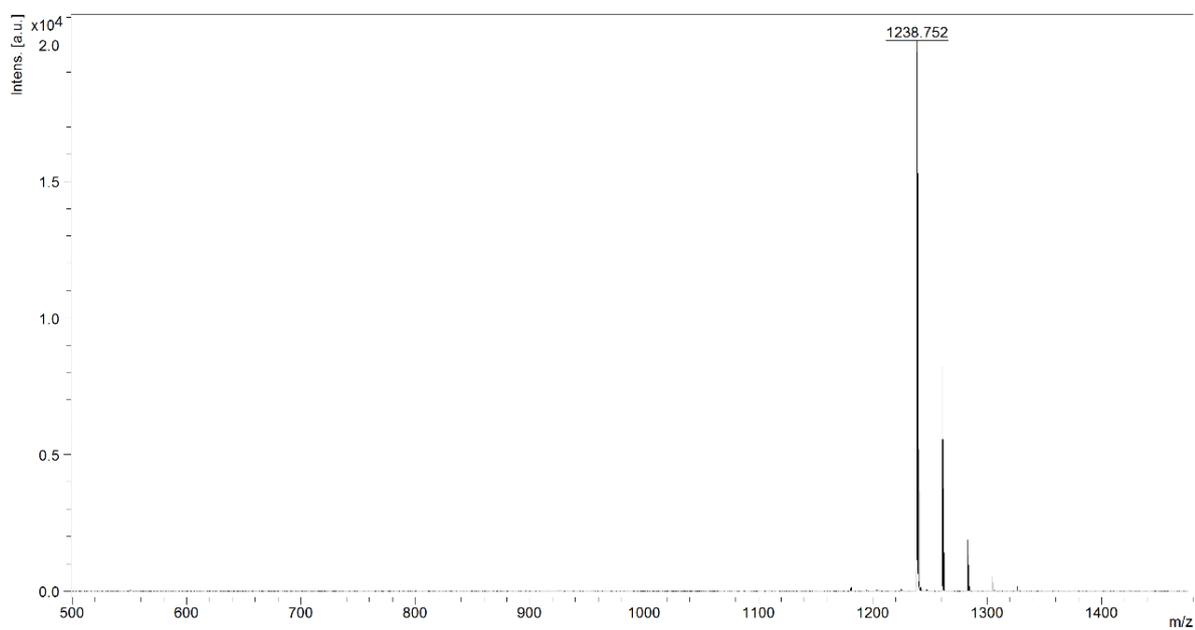
A micellar dispersion of D3R-C16 or D3R-C18 (200 μ M) in ISO-HEPES (NaCl, 500 μ L) was added to CuCl_2 (molar ratio Cu^{2+} :D3R-C16 or D3R-C18 10:1). The resulting mixtures were magnetically stirred at 55 $^{\circ}\text{C}$ for 30 minutes. 1 μ L of D3R-C16, D3R-C18, Cu-D3R-C16 or Cu-D3R-C18 was spotted on silica gel 60 F254 plates (Merck) and a solution of 5% (w/v) ammonium acetate (NH_4OAc) in water-methanol (1:3) was used as eluent. All the compounds were observed by using permanganate stain.

Results

Analysis by TLC gave R_f values 0.3-0.4 for Cu-D3R-C16 and 0.4-0.5 for Cu-D3R-C18. D3R-C16 and D3R-C18 have similar R_f of 0.6-0.7.

S2 – MALDI-TOF spectra for D3R-C16 and D3R-C18

D3R-C16



Exact Mass: 1237,83
Molecular Weight: 1238,59

S3 – Cytotoxicity of D3R-alkyl and non-radioactive Cu-D3R-alkyl

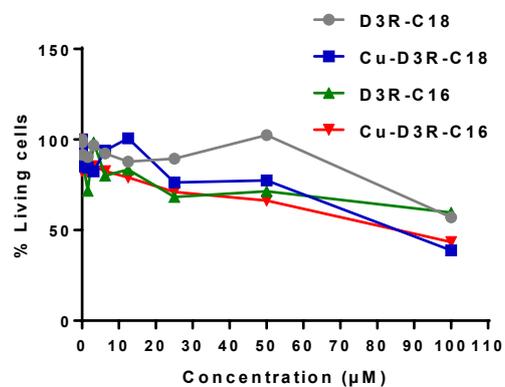


Figure S1. Cytotoxicity of C16/C18-3R-DOTA and their Cu^{2+} chelates with towards CT26 cells.

S4 – Partitioning kinetics of ^{64}Cu -D3R-C16 into POPC liposomes (0.36 mM)

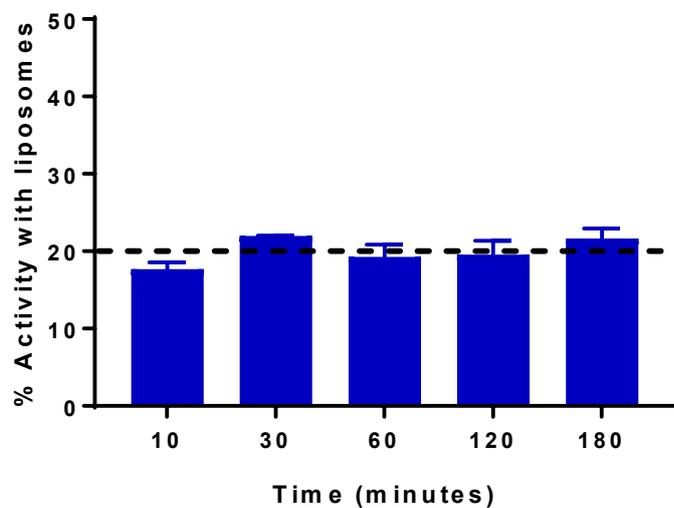


Figure S2. Partitioning kinetics of ^{64}Cu -D3R-C16 into liposomes as a function of time. The results are given as mean \pm SEM ($n = 3$).

S5 – Absolute retention of radioactivity in tumors

Absolute retention of radioactivity (%ID/g) in tumors as a function of time. Data are presented as mean \pm SEM.

Time	Free ^{64}Cu n = 3	^{64}Cu -DOTA n = 3	^{64}Cu -DOTA-3R-C18 n = 5	^{64}Cu -LIP n = 4
0.25 min	97 \pm 19	78 \pm 37	80 \pm 66	104 \pm 62
0.75 min	167 \pm 50	130 \pm 13	171 \pm 96	157 \pm 26
1.25 min	164 \pm 53	127 \pm 14	230 \pm 58	161 \pm 30
1.75 min	162 \pm 54	123 \pm 14	241 \pm 63	159 \pm 30
2.5 min	159 \pm 53	120 \pm 14	241 \pm 66	157 \pm 30
3.5 min	156 \pm 54	115 \pm 14	240 \pm 68	157 \pm 30
4.5 min	154 \pm 53	111 \pm 14	241 \pm 69	157 \pm 31
5.5 min	152 \pm 53	108 \pm 14	240 \pm 70	157 \pm 31
6.5 min	150 \pm 53	105 \pm 14	240 \pm 70	156 \pm 31
7.5 min	148 \pm 52	102 \pm 14	240 \pm 70	155 \pm 31
8.5 min	146 \pm 52	100 \pm 14	239 \pm 69	154 \pm 31
9.5 min	145 \pm 53	97 \pm 13	238 \pm 70	154 \pm 31
10.5 min	143 \pm 52	94 \pm 13	238 \pm 70	153 \pm 31
11.5 min	142 \pm 51	92 \pm 12	236 \pm 70	153 \pm 30
6 h	18 \pm 3	4 \pm 1	49 \pm 17	70 \pm 22
24 h	10 \pm 1	2 \pm 0.8	23 \pm 7	55 \pm 13

S6 - Well counting results: weights of tumor and organs, and accumulation of radioactivity (%ID/g) in relevant organs

Weights (g) of tumor and organs. Data are presented as mean \pm SEM.

	Tumor	Liver	Spleen
Free ^{64}Cu , n=3	0.14 \pm 0.079	0.39 \pm 0.091	0.15 \pm 0.11
^{64}Cu -DOTA, n = 3	0.42 \pm 0.069	0.43 \pm 0.10	0.085 \pm 0.0047
^{64}Cu -DOTA-3R-C18, n = 5	0.22 \pm 0.094	0.37 \pm 0.12	0.094 \pm 0.0064
LIP, n = 4	0.34 \pm 0.11	0.40 \pm 0.12	0.090 \pm 0.015

Accumulation of radioactivity (ID%/g) in relevant organs. Data are presented as mean \pm SEM

	Tumor	Liver	Spleen
Free ^{64}Cu , n=3	11 \pm 3	14 \pm 1	2 \pm 0.6
^{64}Cu -DOTA, n = 3	1 \pm 0.3	1 \pm 0.2	0.3 \pm 0.05
^{64}Cu -DOTA-3R-C18, n = 5	18 \pm 8	10 \pm 1	2 \pm 0.07
LIP, n = 4	43 \pm 31	9 \pm 2	9 \pm 5