

**Multivalent Bifunctional Chelator Scaffolds for Gallium-68 Based Positron Emission
Tomography Imaging Probe Design: Signal Amplification *via* Multivalency**

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Supporting Information

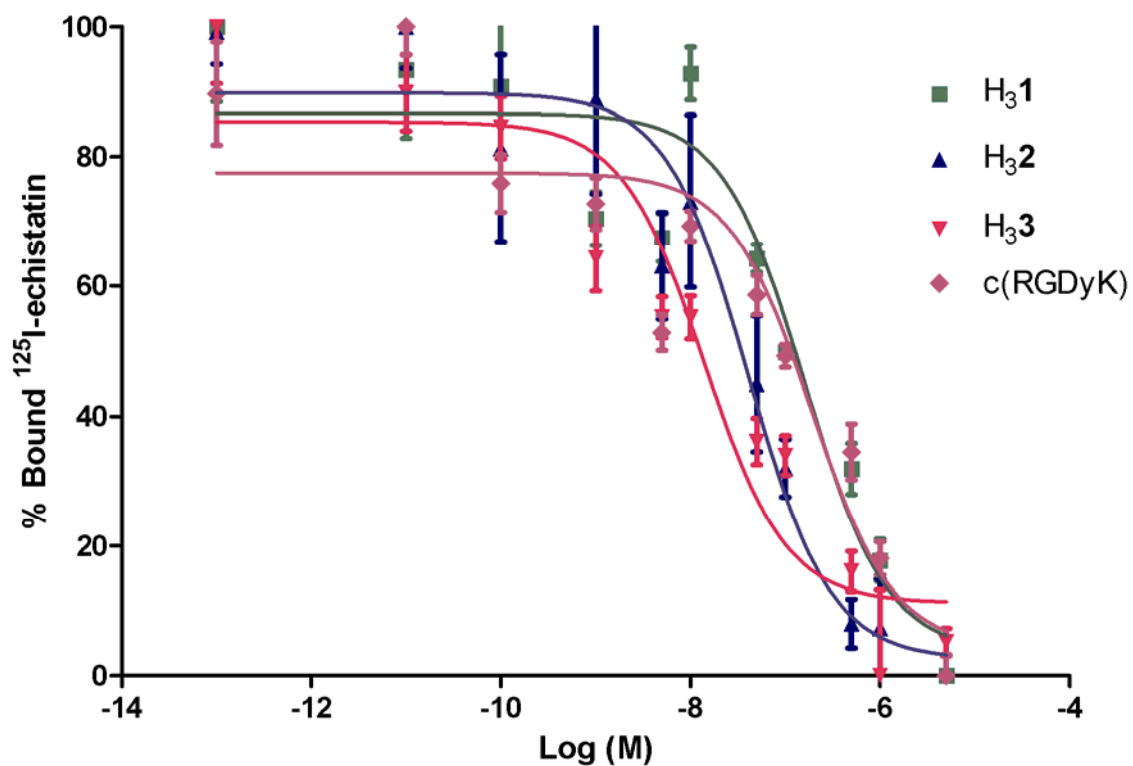


Figure S1. The integrin $\alpha_v\beta_3$ binding affinities of c(RGDyK), H₃₁, H₃₂, and H₃₃ measured by a competitive cell-binding assay using ¹²⁵I-echistatin as the integrin-specific radioligand. The IC₅₀ values were calculated to be 204 ± 76 nM, 171 ± 60 nM, 43.9 ± 16.1 nM, and 14.7 ± 5.0 nM for c(RGDyK), H₃₁, H₃₂, and H₃₃, respectively (two-tailed t-test: $p < 0.01$). Goodness of fit (R^2): 0.80 – 0.82.

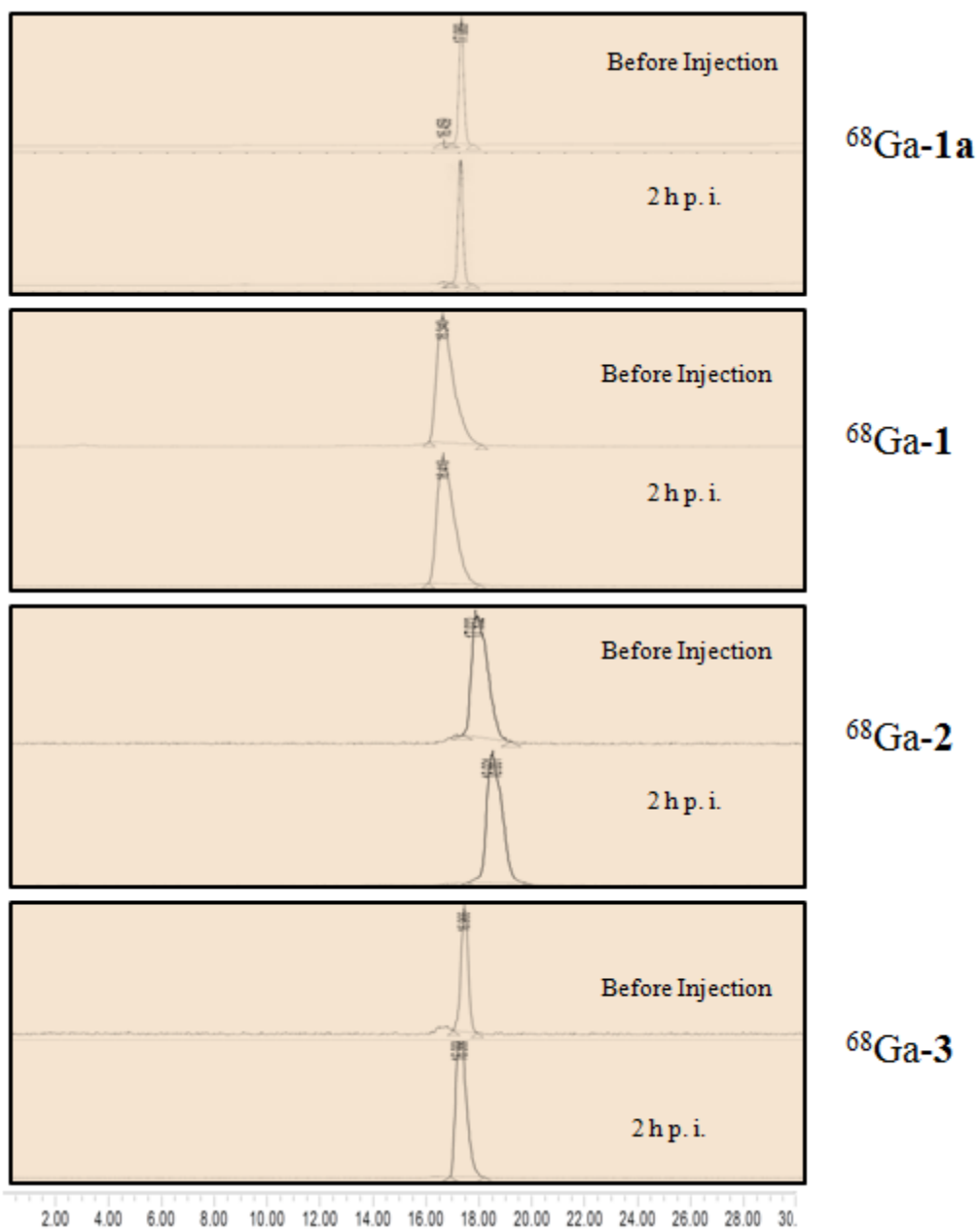


Figure S2. Radio-HPLC analysis of the metabolites of $^{68}\text{Ga-1a}$, $^{68}\text{Ga-1}$, $^{68}\text{Ga-2}$ and $^{68}\text{Ga-3}$ in mouse urine at 2 h p.i. (n = 4)

Table S1. Uptake of $^{68}\text{Ga-1}$, $^{68}\text{Ga-1a}$, $^{68}\text{Ga-2}$ and $^{68}\text{Ga-3}$ at 30 min and 2 h p.i. in major organs and tumor determined by quantitative PET imaging analysis. Data are presented as %ID/g \pm s.d. (n = 3).

Organ	$^{68}\text{Ga-1a}$		$^{68}\text{Ga-1}$		$^{68}\text{Ga-2}$		$^{68}\text{Ga-3}$	
	(30 min)	(2 h)	(30 min)	(2 h)	(30 min)	(2 h)	(30 min)	(2 h)
Tumor	3.24 \pm 0.41	1.40 \pm 0.36	3.26 \pm 0.70	1.66 \pm 0.15	3.06 \pm 0.21	1.90 \pm 0.10	3.55 \pm 0.85	2.55 \pm 0.50
Heart	4.48 \pm 0.74	0.86 \pm 0.25	3.16 \pm 0.50	0.89 \pm 0.17	3.44 \pm 0.51	0.95 \pm 0.18	3.26 \pm 0.81	1.14 \pm 0.21
Lung	2.76 \pm 0.35	0.73 \pm 0.15	2.13 \pm 0.30	0.73 \pm 0.03	2.22 \pm 0.10	0.85 \pm 0.19	2.6 \pm 0.72	1.07 \pm 0.31
Liver	3.92 \pm 0.70	1.92 \pm 0.71	3.16 \pm 0.32	2.03 \pm 0.25	3.5 \pm 0.55	2.04 \pm 0.35	3.88 \pm 0.92	2.70 \pm 0.39
Kidney	12.52 \pm 4.0	2.34 \pm 0.60	9.73 \pm 5.0	1.77 \pm 1.01	11.22 \pm 3.8	2.22 \pm 0.73	9.23 \pm 3.78	3.90 \pm 1.60
Muscle	1.03 \pm 0.32	0.37 \pm 0.17	0.77 \pm 0.63	0.51 \pm 0.07	0.85 \pm 0.33	0.27 \pm 0.13	1.34 \pm 0.55	0.50 \pm 0.34

Table S2. Uptake of $^{68}\text{Ga-1}$, $^{68}\text{Ga-1a}$, $^{68}\text{Ga-2}$ and $^{68}\text{Ga-3}$ co-injected with c(RGDyK) (10mg/kg) at 30 min and 2 h p.i. in major organs and tumor determined by quantitative PET imaging analysis. Data are presented as %ID/g \pm s.d. (n = 3).

Organ	$^{68}\text{Ga-1a}$		$^{68}\text{Ga-1}$		$^{68}\text{Ga-2}$		$^{68}\text{Ga-3}$	
	(30 min)	(2 h)	(30 min)	(2 h)	(30 min)	(2 h)	(30 min)	(2 h)
Tumor	2.40 \pm 0.12	0.64 \pm 0.21	2.55 \pm 0.32	0.3 1 \pm 0.02	2.98 \pm 1.5	0.44 \pm 0.19	1.69 \pm 0.42	0.51 \pm 0.15
Heart	3.81 \pm 1.00	0.55 \pm 0.21	4.09 \pm 1.00	0.45 \pm 0.02	4.33 \pm 3.2	0.60 \pm 0.08	3.18 \pm 0.04	0.77 \pm 0.46
Lung	2.14 \pm 0.46	0.37 \pm 0.04	1.93 \pm 0.37	0.30 \pm 0.05	2.32 \pm 1.7	0.45 \pm 0.43	1.70 \pm 0.02	0.48 \pm 0.21
Liver	2.81 \pm 0.66	0.81 \pm 0.10	2.89 \pm 0.47	0.92 \pm 0.10	2.81 \pm 1.9	0.93 \pm 0.75	2.23 \pm 0.00	0.91 \pm 0.26
Kidney	6.92 \pm 3.17	1.95 \pm 0.33	9.97 \pm 5.61	1.55 \pm 0.77	8.26 \pm 7.2	1.54 \pm 0.40	7.09 \pm 2.53	3.40 \pm 1.52
Muscle	1.49 \pm 0.11	0.22 \pm 0.05	1.51 \pm 0.20	0.15 \pm 0.04	1.77 \pm 0.97	0.17 \pm 0.04	1.31 \pm 0.02	0.35 \pm 0.18

Table S3. Biodistribution data of $^{68}\text{Ga-1}$, $^{68}\text{Ga-1a}$, $^{68}\text{Ga-2}$ and $^{68}\text{Ga-3}$ at 30 min and 2 h p.i. in major organs and tumor. Data are presented as %ID/g \pm s.d. (n = 3) (S. Int.: Small intestines; L. Int = Large intestines).

Organ	$^{68}\text{Ga-1a}$		$^{68}\text{Ga-1}$		$^{68}\text{Ga-2}$		$^{68}\text{Ga-3}$	
	(30min)	(2 h)	(30min)	(2 h)	(30min)	(2 h)	(30min)	(2 h)
Tumor	2.84 \pm 0.24	1.63 \pm 0.19	3.04 \pm 0.24	1.67 \pm 0.16	3.16 \pm 0.22	1.94 \pm 0.32	5.58 \pm 0.45	4.41 \pm 1.00
Blood	1.31 \pm 0.11	0.16 \pm 0.06	1.43 \pm 0.25	0.23 \pm 0.03	1.24 \pm 0.09	0.51 \pm 0.60	1.21 \pm 0.28	0.18 \pm 0.00
Heart	1.00 \pm 0.07	0.47 \pm 0.21	0.96 \pm 0.25	0.50 \pm 0.05	1.08 \pm 0.02	0.55 \pm 0.08	1.78 \pm 0.23	1.00 \pm 0.14
Lung	2.74 \pm 0.11	1.24 \pm 0.42	2.62 \pm 0.24	1.25 \pm 0.13	2.93 \pm 0.10	1.31 \pm 0.15	5.29 \pm 1.25	2.94 \pm 0.12
Liver	1.98 \pm 0.29	1.43 \pm 0.53	1.91 \pm 0.30	1.72 \pm 0.09	2.07 \pm 0.05	1.80 \pm 0.14	3.10 \pm 0.77	2.81 \pm 0.26
Spleen	2.66 \pm 0.94	2.10 \pm 1.08	3.17 \pm 0.70	2.55 \pm 0.84	2.44 \pm 0.54	2.78 \pm 0.19	3.42 \pm 0.91	3.14 \pm 0.20
Kidney	6.25 \pm 0.61	2.95 \pm 0.76	5.40 \pm 0.61	3.14 \pm 0.13	3.18 \pm 2.76	3.86 \pm 1.80	8.41 \pm 0.99	7.50 \pm 0.95
Fat	0.40 \pm 0.21	0.42 \pm 0.09	1.34 \pm 0.74	0.45 \pm 0.04	0.66 \pm 0.28	0.39 \pm 0.08	1.08 \pm 0.51	0.61 \pm 0.42
Bone	0.97 \pm 0.17	0.44 \pm 0.09	0.94 \pm 0.05	0.52 \pm 0.06	0.91 \pm 0.81	0.37 \pm 0.21	1.23 \pm 0.10	1.00 \pm 0.24
Muscle	0.70 \pm 0.10	0.42 \pm 0.14	0.62 \pm 0.02	0.30 \pm 0.03	0.91 \pm 0.73	0.41 \pm 0.27	1.57 \pm 0.24	1.17 \pm 0.31
Brain	0.08 \pm 0.01	0.05 \pm 0.00	0.07 \pm 0.01	0.06 \pm 0.01	0.06 \pm 0.02	0.06 \pm 0.02	0.13 \pm 0.01	0.10 \pm 0.03
S.Int	2.01 \pm 0.18	2.39 \pm 0.88	2.01 \pm 0.22	2.20 \pm 0.28	2.01 \pm 0.19	1.59 \pm 0.15	3.97 \pm 0.30	3.14 \pm 0.69
L.Int	0.84 \pm 0.03	0.92 \pm 0.08	1.04 \pm 0.10	0.98 \pm 0.24	1.07 \pm 0.68	1.40 \pm 0.19	1.96 \pm 0.17	1.60 \pm 0.65
Stomach	0.82 \pm 0.33	1.83 \pm 0.18	0.75 \pm 0.07	0.99 \pm 0.11	0.59 \pm 0.02	1.20 \pm 0.34	3.36 \pm 0.43	1.86 \pm 0.61