Supporting information

Molecular Platform for Design and Synthesis of Targeted Dual-modality Imaging Probes

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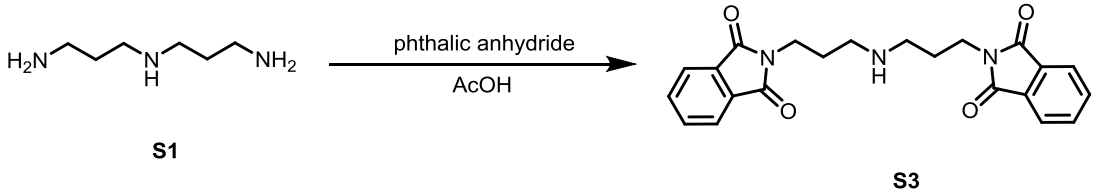
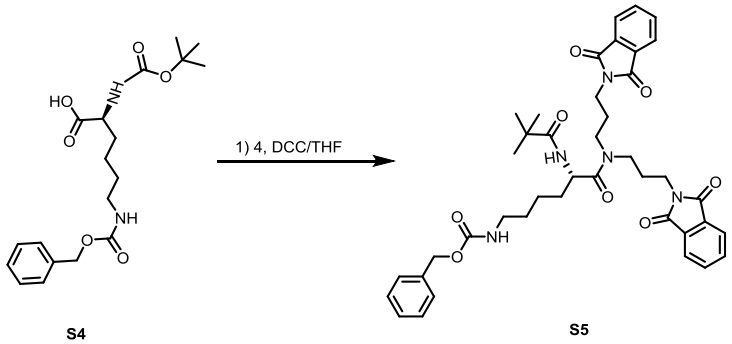
Synthesis Procedures



S1 S3

Compound S3. Bis(phthalimidylpropyl)amine (**S3**) was synthesized following a previously published report.[1] Briefly, phthalic anhydride (**S2**) (5.0 g, 33.8 mmol) was added to a solution of bis(3-aminopropyl)amine (**S1)** (2.03 g, 15.5 mmol) in toluene/DMF (50 mL/5 mL). The reaction mixture was stirred under reflux for 24 hours. The solvent was then evaporated and EtOH was (100 mL) added to the residue. The resultant mixture was stirred for 5 hours, the precipitate filtered, and dried to give compound **S3** (yield: 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83 (m, 4H), 7.70 (m, 4H), 3.75 (t, J = 6.8 Hz, 4H), 2.62 (t, J = 6.8 Hz, 4H), 1.84 (quintet, J = 6.8 Hz, 4H).



S4 S5

Compound S5. To a solution of protected lysine **S4** (2.0 g, 5.3 mmol) in THF (10 mL), were added the pthalimide protected secondary amine S**3** (2.1 g, 5.3 mmol), dicyclohexylcarbodiimide (1.1 g, 5.5 mmol) and triethylamine (0.6 g, 5.5 mmol). The resultant solution was stirred for 24 hours, filtered and the solvent evaporated. The crude product was purified by flash chromatography (ethyl acetate/hexane 1:1) to give lysine derivative **S5** (2.3 g, 3.0 mmol, 57%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65 (m, 3H), 7.54 (m, 3H), 7.18 (m, 4H), 5.47 (m, 1H), 5.27 (m, 1H), 4.94 (m, 1H), 4.36 (m, 1H), 3.72-3.34 (m, 6H), 3.23-2.89 (m, 4H), 2.03-1.63 (m, 5H), 1.62-1.35 (m, 4H), 1.33-1.11 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.5, 168.3, 168.1, 156.4, 155.4, 136.7, 133.8, 131.9, 128.3,127.9, 127.8, 123.2, 123.1, 79.3, 66.2, 49.6, 45.6, 43.9, 40.6, 35.6, 35.3, 33.1, 30.1, 30.5, 29.5, 29.1, 28.6, 28.2, 26.9, 22.4. MS (MALDI) m/z calcd for $C_{41}H_{47}N_5O_9$: 753.3; found: 776.6 ($[M + Na]^+$).

Compound 2. To a solution of a lysine derivative S5 (2.3 g, 3.0 mmol,) was added TFA (10 mL) and the solution was allowed to stir for 12 h. The solvent evaporated and the crude product was purified by flash chromatography (ethyl acetate) to give 2 as a white solid (1.8 g, 2.8 mmol, 93%). 1H NMR (400 MHz, CDCl3): δ = 7.73 (m, 3H), 7.61 (m, 3H), 7.23 (m, 4H), 5.87 (m, 1H), 5.58 (m, 1H), 4.97 (m, 1H), 4.40 (m, 1H), 3.72-3.37 (m, 6H), 3.35-2.82 (m, 4H), 2.10-1.68 (m,

5H), 1.59-1.29 (m, 4H). 13C NMR (100 MHz, CDCl3): δ 169.1, 168.3, 156.8, 136.7, 134.1, 133.9, 131.8, 131.7, 128.4,127.9, 123.3, 123.2, 66.4, 50.7, 45.5, 43.8, 40.1, 35.5, 35.1, 30.8, 29.7, 29.1, 27.7, 26.5, 21.4. MS (MALDI) m/z calcd for C36H39N5O7: 653.3; found: 654.6 ([M + H]+).

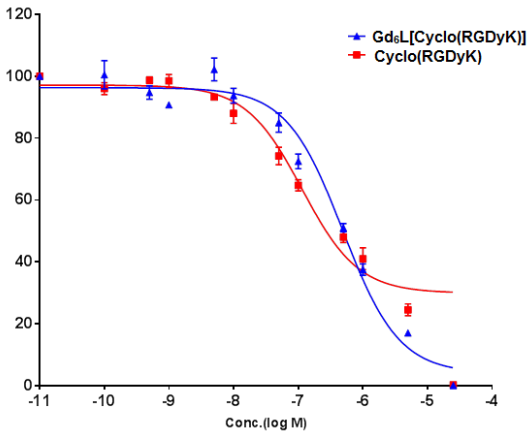
Determining the Gd to Ga ration in Gd_6LGa by ICP mass

Calibration curve was obtained by injecting various known concentrations of gallium and gadolinium salts in the ICP-mass. Various known concentrations of gallium and gadolinium salts were obtained by dissolving their chlorides in a 2.5 % HCl solution. All dilutions were performed in 2.5 % HCl solution. The solutions used for T_1 mapping (a1-c1) were then injected to the ICP-mass to get the concentration of the two salts. Replicates of each concentration were performed.

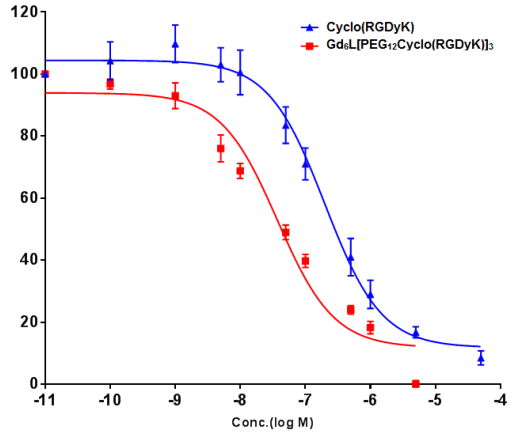
Samples	Gadolinium (mM)	Gallium (mM)	Ratio Gd:Ga
a-1	0.728471	0.121136	6.013634
a-2	0.734226	0.12062	6.087089
b-1	0.368362	0.062548	5.889323
b-2	0.37752	0.062892	6.002695
c-1	0.170531	0.028871	5.906574
c-2	0.188992	0.030865	6.123184

Integrin $\alpha_v\beta_3$ Receptor-Binding Assay for $Gd_6H_3L[c(RGDyK)]_3$ and $Gd_6H_3L[PEG_{12}c(RGDyK)]_3$

The $\alpha_v\beta_3$ integrin-binding affinities of c(RGDyK), $Gd_6\mathbf{H_3L}[c(RGDyK)]_3$ and $Gd_6\mathbf{H_3L}[PEG_{12}c(RGDyK)]_3$ were determined by a competitive cell-binding assay using ^{125}I-echistatin (PerkinElmer) as the $\alpha_v\beta_3$-specific radioligand. The experiments were performed on U87MG human glioblastoma cells following a previously reported method.[2] Briefly, U87MG cells were grown in RPMI 1640 medium supplemented with penicillin, streptomycin, and 10% (v/v) fetal bovine serum (FBS) at 37 °C under 5% CO_2. Suspended U87MG cells in binding buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 0.1% bovine serum albumin) were seeded on multiwell DV plates (Millipore) with 5×10^4 cells per well and then incubated with ^{125}I-echistatin (10 000 cpm/well) in the presence of increasing concentrations (0–5000 nM) of c(RGDyK) peptide conjugates for 2 h. The final volume in each well was maintained at 200 μL. At the end of incubation, unbound ^{125}I-echistatin was removed by filtration followed by three rinses with cold binding buffer. The retentate was collected and the radioactivity was measured using a γ-counter. The best-fit IC_{50} values (inhibitory concentration where 50% of the ^{125}I-echistatin bound on U87MG cells are displaced) of c(RGDyK), $Gd_6\mathbf{H_3L}[c(RGDyK)]_3$ and $Gd_6\mathbf{H_3L}[PEG_{12}c(RGDyK)]_3$ were calculated by fitting the data with nonlinear regression using GraphPad Prism (GraphPadSoftware, Inc.). Experiments were repeated with quintuplicate samples.



	Gd6L[Cyclo(RGDyK)]	Cyclo(RGDyK)
Best-fit values		
IC50	489 nM	115 nM
Goodness of Fit		
R square	0.9680	0.9608



	$Gd_6L[PEG_{12}Cyclo(RGDyK)]_3$	Cyclo(RGDyK)
Best-fit values		
IC50	37 nM	199 nM
Goodness of Fit		
R square	0.95	0.94

Figure S1. The calculated IC_{50} values of synthesized probes a) $Gd_6\mathbf{H_3L}[c(RGDyK)]_3$ and b) $Gd_6\mathbf{H_3L}[PEG_{12}c(RGDyK)]_3$

References

(1) Kang, S. O.; Day, V. W.; Bowman-James, K. *Organic Letters* 2009, *11*, 3654.

(2) Liu, W.; Hao, G.; Long, M. A.; Anthony, T.; Hsieh, J.-T.; Sun, X. *Angewandte Chemie International Edition* 2009, *48*, 7346.