Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information

^{99m}Tc-DTPA-bis-c(RGDfK) a potential alpha(v)beta3 integrin based homobivalent radioligand for imaging neoangiogenesis in malignant glioma and melanoma

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Experimental procedure for cell binding assays

Binding assays on U87MG cells were performed using increasing concentrations of labeled DTPA-bis-c(RGDfK). Cells were plated in 48-well plate at a density of 12,000 cells per well in triplicates. On the day of experiment, media were aspirated from all wells and 1 mL Hank's balanced salt solution (HBSS) was added to each well. Increasing concentration (0.001-10 nM) ^{99m}Tc-DTPA-bis-c(RGDfK) was added to each well sample, which was tested in triplicate unless otherwise noted. Cells were incubated in the presence and absence of 100 fold excess c(RGDfK) for 40 min at 37 °C. Following the incubation, cells were washed 4 times with washing buffer (50 mM Tris–HCl, 0.2% BSA, 30 mM NaCl). Scatchard plot was generated using [Bound/Free] versus Bound ligand concentration.

Experimental procedure for cytotoxicity study

Methylthiazole tetrazolium (MTT) assay was performed measuring the cell metabolic activity of U87MG and HEK cells. Monolayer cultures of normal embryonic kidney cells, HEK, in DMEM (Sigma, USA) supplemented with 10% fetal bovine serum (GIBCO), 50 U/mL penicillin, 2 µg/mL nystatin and 50 µg/mL streptomycin sulfate were maintained at 37 °C in a humidified CO_2 incubator (5% CO_2, 95% air). Exponentially growing cells were seeded in a 96 well microtiter plate at a cell density of 5000 cells per well and were allowed to adhere for 24 h at 37°C. Then, cells were exposed to various drug concentrations (32 pM-1 mM) for different time intervals 2, 4, 6, 12 h. At the end of treatment, both the treated cells and negative control were incubated with MTT at a final concentration of 0.1 mg/ml for 2 h at 37˚C. The MTT assay based on the conversion of yellow tetrazolium salt to purple-formazan crystals by metabolically active cells provides a quantitative determination of viable cells. Triplicate wells from each treatment were lysed and the formazan crystals were dissolved using 150 µL of DMSO for 30 min. Absorbance was read at 570 nm with 630 nm as reference filter. Surviving fraction was calculated by normalizing control and was plotted against concentration at 2 h and 4 h as a function of surviving fraction and IC_{50} value was calculated.

Figure S1. Cells from HEK cell line were treated with various concentrations of DTPA-bis-c(RGDfK) for different time intervals 2, 4, 6, 12 h. At the end of the treatment period, cell viability was measured using an MTT assay. Values are the means of three different experiments. IC_{50} value was 1.02 ± 0.03 mM at 2h and 4h; No significant difference was observed compared to control at 1 mM concentration at 6 h and 12 h.

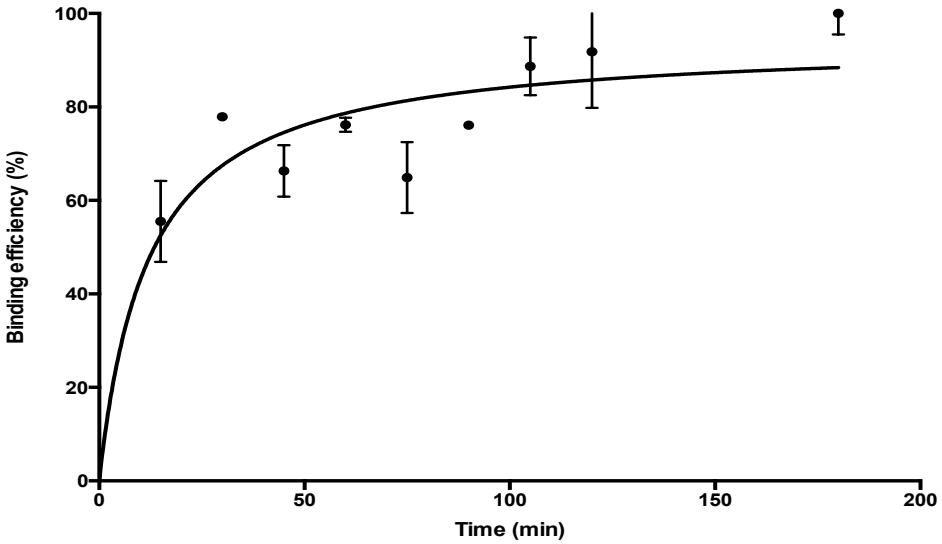
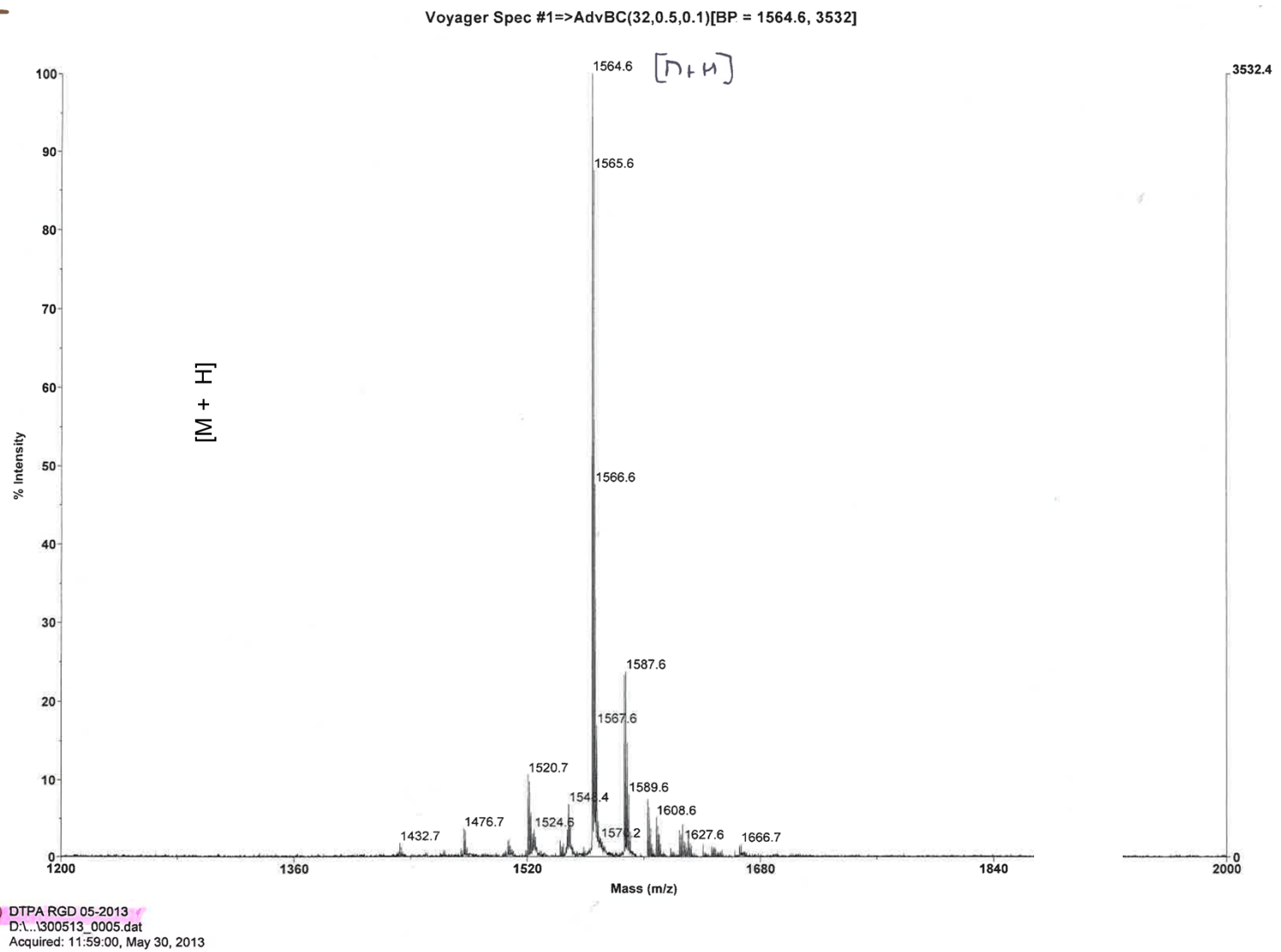


Figure S2. Time course and kinetics of ^{99m}Tc-DTPA-bis-c(RGDfK) in B16F10 cells. Uptake was monitored for 180 min and equilibrium was obtained at 90 min.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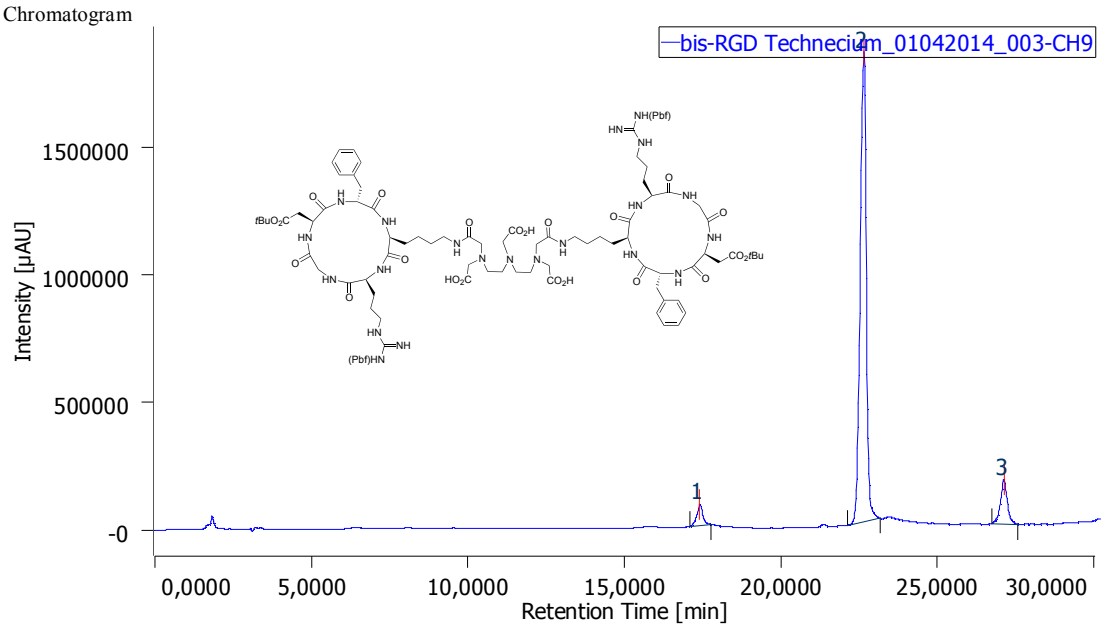
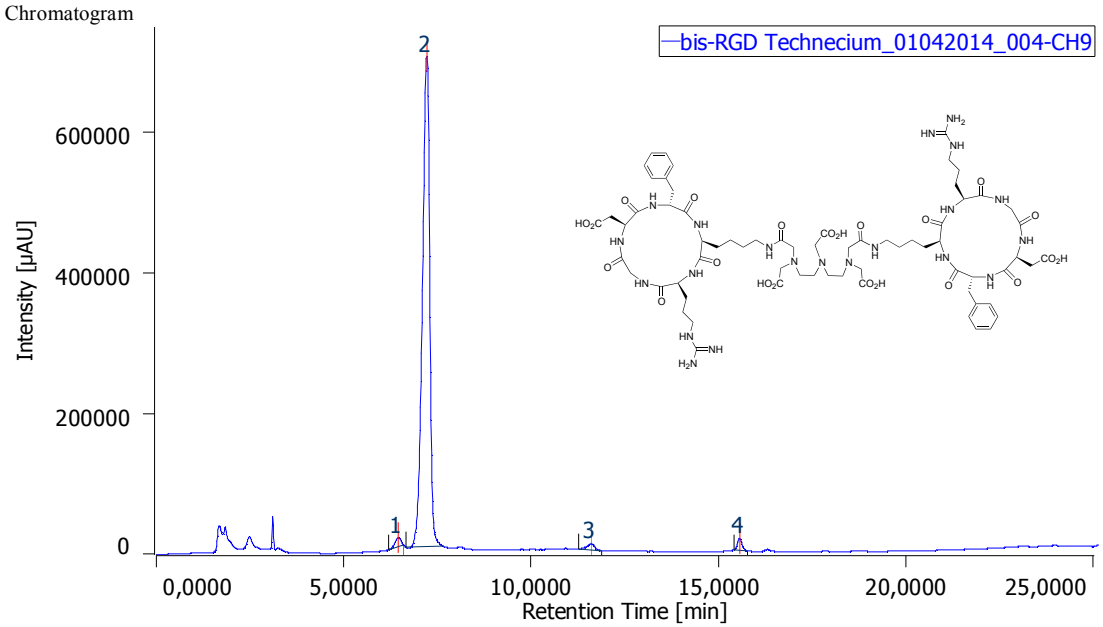


Channel & Peak Info.Table

Chromatogram Name: bis-RGD Technecium_01042014_003-CH9
Sample Name: bis cRGDfK prot 2013
Channel Name: 220,0nm

#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Symmetry Factor
1	Unknown	9	17,387	1063966	80920	3,710	3,865	0,992
2	Unknown	9	22,613	25001322	1840539	87,170	87,912	0,884
3	Unknown	9	27,080	2615766	172168	9,120	8,223	0,975

Figure S4. HPLC profile of DTPA-bis-c(R(Pbf)GD(tBu)fK)



Channel & Peak Info.Table

Chromatogram Name: bis-RGD Technecium_01042014_004-CH9
Sample Name: bis cRGDfK 2013
Channel Name: 220,0nm

#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Symmetry Factor
1	Unknown	9	6,467	138915	12559	1,440	1,710	0,812
2	Unknown	9	7,227	9227653	695540	95,658	94,720	0,808
3	Unknown	9	11,600	131970	8920	1,368	1,215	0,839
4	Unknown	9	15,547	147974	17294	1,534	2,355	1,024

Figure S5. HPLC profile of DTPA-bis-c(RGDfK)