

⁶⁴Cu-labeled LyP-1 dendrimer for PET-CT imaging of atherosclerotic plaque targeting p32 proteins on macrophages

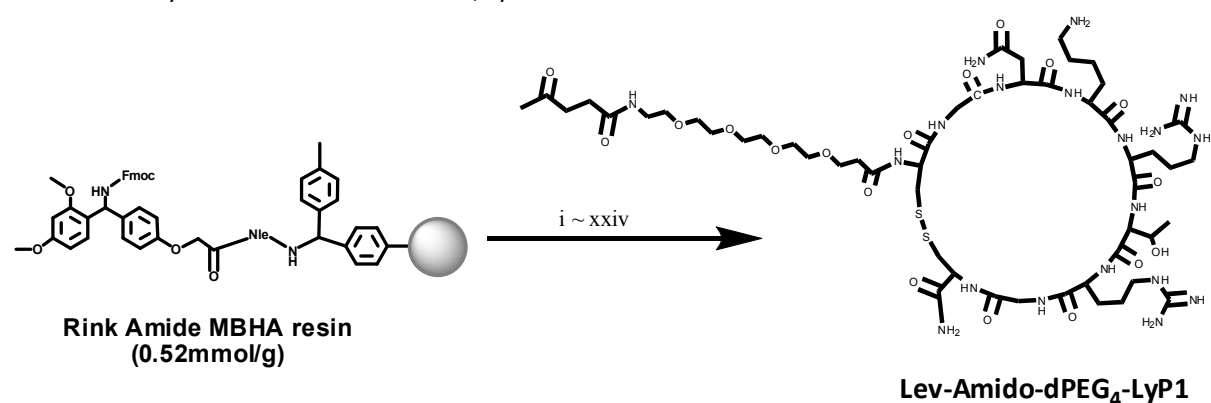
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Supporting Information

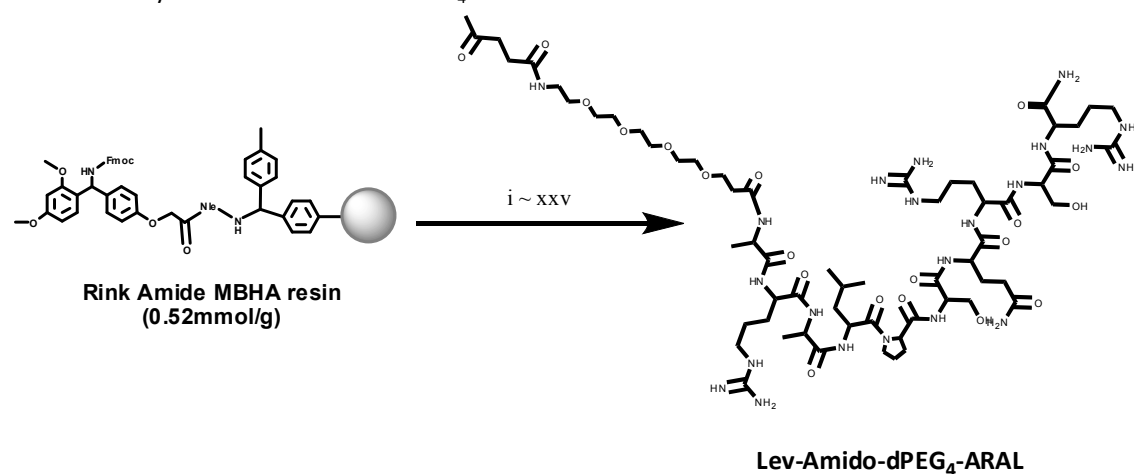
SI-1. Synthesis of each building block.

Scheme S1. Synthesis of Lev-amido-dPEG₄-LyP1



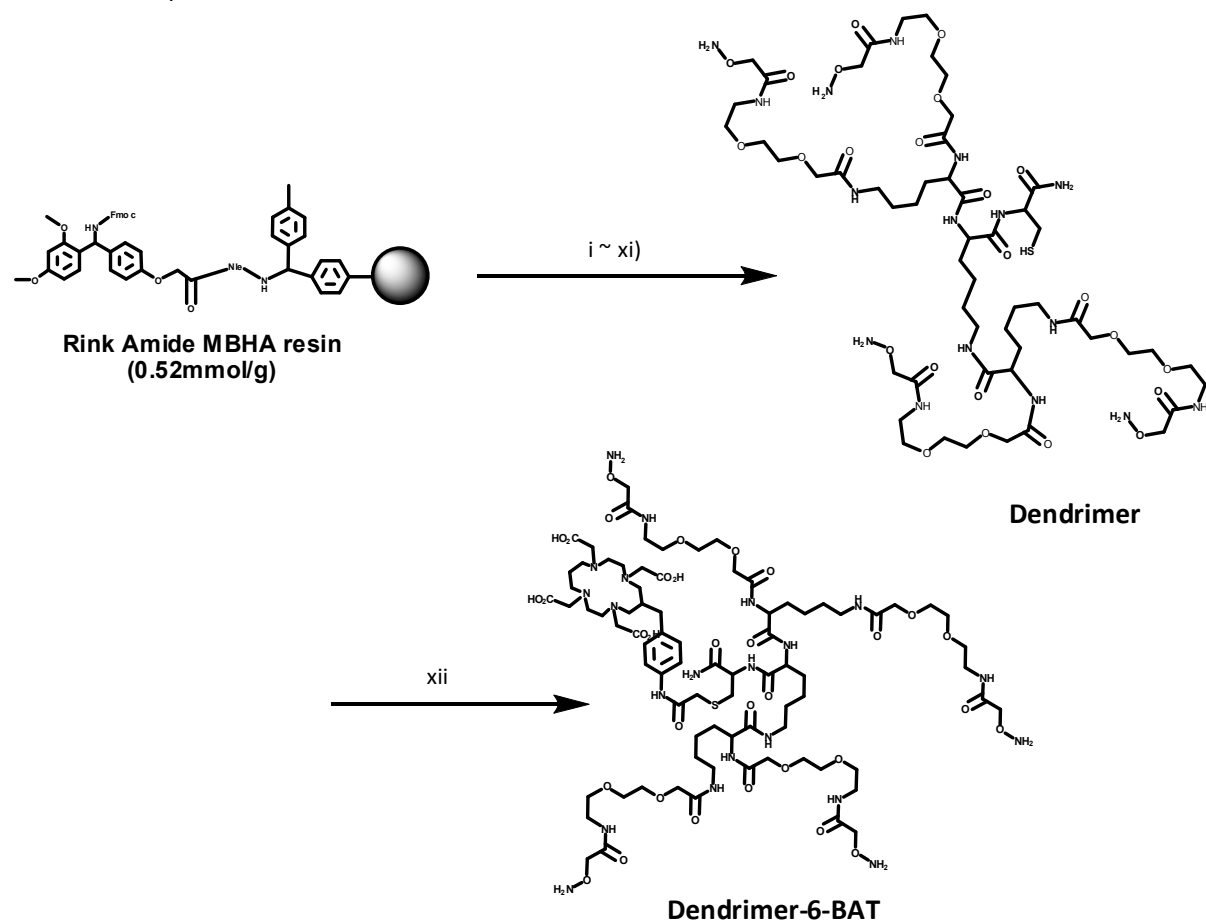
Reagents: i) 20% piperidine, 10min, ii) Fmoc-Cys(Trt)-OH, HATU, DIPEA, DMF, iii) 20% piperidine, 10min, ix) Fmoc-Gly-OH, HATU, DIPEA, DMF, v) 20% piperidine, 10min, vi) Fmoc-Arg(Pbf)-OH, HATU, DIPEA, DMF, vii) 20% piperidine, 10min, viii) Fmoc-Thr(tBu)-OH, HATU, DIPEA, DMF, ix) 20% piperidine, 10min, x) Fmoc-Arg(Pbf)-OH, HATU, DIPEA, DMF, xi) 20% piperidine, 10min, xii) Fmoc-Lys(Boc)-OH, HATU, DIPEA, DMF, xiii) 20% piperidine, 10min, xiv) Fmoc-Asn(Trt)-OH, HATU, DIPEA, DMF, xv) 20% piperidine, 10min, xvi) Fmoc-Gly-OH, HATU, DIPEA, DMF, xvii) 20% piperidine, 10min, xviii) Fmoc-Cys(Trt)-OH, HATU, DIPEA, DMF, xix) I₂, DMF, xx) 20% piperidine, 10min, xxi) Fmoc-Amido-dPEG₄-OH, HATU, DIPEA, DMF, xxii) 20% piperidine, 10min, xxiii) Levulinic acid, HATU, DIPEA, DMF, xxiv) cleavage, TFA:TIS:H₂O(9.5:0.25:0.25, v/v %).

Scheme S2. Synthesis of Lev-amido-dPEG₄-ARAL



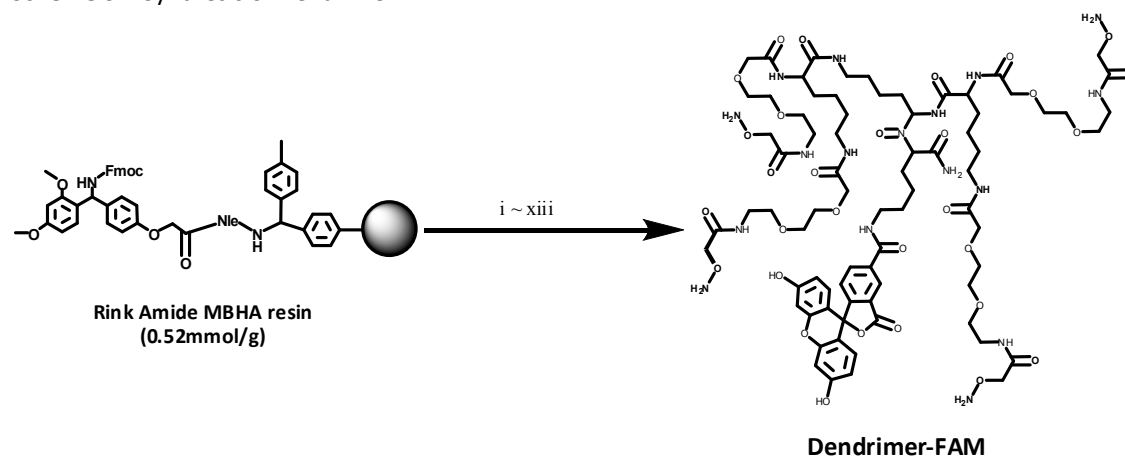
Reagents: i) 20% piperidine, 10min, ii) Fmoc-Arg(Pbf)-OH, HATU, DIPEA, DMF, iii) 20% piperidine, 10min, iv) Fmoc-Ser(tBU)-OH, HATU, DIPEA, DMF, v) 20% piperidine, 10min, vi) Fmoc-Arg(Pbf)-OH, HATU, DIPEA, DMF, vii) 20% piperidine, 10min, viii) Fmoc-Gln(Trt)-OH, HATU, DIPEA, DMF, ix) 20% piperidine, 10min, x) Fmoc-Ser(tBU)-OH, HATU, DIPEA, DMF, xi) 20% piperidine, 10min, xii) Fmoc-Pro-OH, HATU, DIPEA, DMF, xiii) 20% piperidine, 10min, xiv) Fmoc-Leu-OH, HATU, DIPEA, DMF, xv) 20% piperidine, 10min, xvi) Fmoc-Ala-OH, HATU, DIPEA, DMF, xvii) 20% piperidine, 10min, xviii) Fmoc-Arg(Pbf)-OH, HATU, DIPEA, DMF, xix) 20% piperidine, 10min, xx) Fmoc-Ala-OH, HATU, DIPEA, DMF, xxi) 20% piperidine, 10min, xxii) Fmoc-Amido-dPEG₄-OH, HATU, DIPEA, DMF, xxiii) 20% piperidine, 10min, xxiv) Levulinic acid, HATU, DIPEA, DMF, xxv) cleavage, TFA:TIS:H₂O(9.5:0.25:0.25).

Scheme S3. Synthesis of Dendrimer-6-BAT.



Reagents: i) 20% piperidine, 10min, ii) Fmoc-Cys(Trt)-OH, HATU, DIPEA, DMF, iii) 20% piperidine, 10min, iv) Fmoc-Lys(Fmoc)-OH, HATU, DIPEA, DMF, v) 20% piperidine, 10min, vi) Fmoc-Lys(Fmoc)-OH, HATU, DIPEA, DMF, vii) 20% piperidine, 10min, viii) Fmoc-mini-PEG-OH, HATU, DIPEA, DMF, ix) 20% piperidine, 10min, x) Boc-Aoa-OH, HATU, collidine, DMF, xi) Cleavage, TFA:TIS:H₂O(9.5:0.25:0.25), xii) 6-BAT, 0.1M Ammonium acetate, r.t., 6hrs.

Scheme S4. Synthesis of Dendrimer-FAM



Reagent: i) 20% piperidine, 10min, ii) Fmoc-Lys(Mmt)-OH, HATU, DIPEA, DMF, iii) 20% piperidine, 10min, iv) Fmoc-Lys(Fmoc)-OH, HATU, DIPEA, DMF, v) 20% piperidine, 10min, vi) Fmoc-Lys(Fmoc)-OH, HATU, DIPEA, DMF, vii) 20% piperidine, 10min, viii) Fmoc-minPEG-OH, HATU, DIPEA, DMF, ix) 20% piperidine, 10min, x) Boc-Aoa-OH, HATU, collidine, DMF, xi) deprotect Mmt with AcOH/TFE/DCM (1:2:7), xii) 5(6)-carboxy fluorescein, HOBt, DIC, DMF, xiii) Cleavage, TFA:TIS:H₂O(9.5:0.25:0.25, v/v %).

SI-2. MALDI spectrum of synthesized compounds

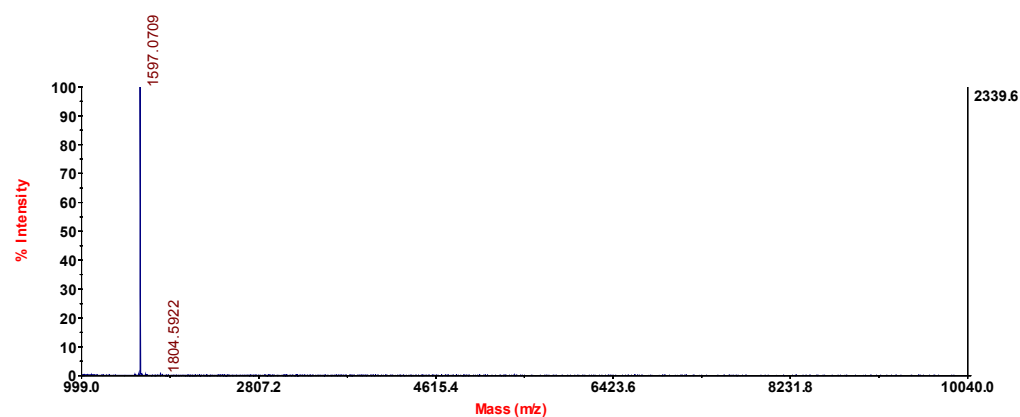


Figure SI-2.1. Lyp-1-FAM

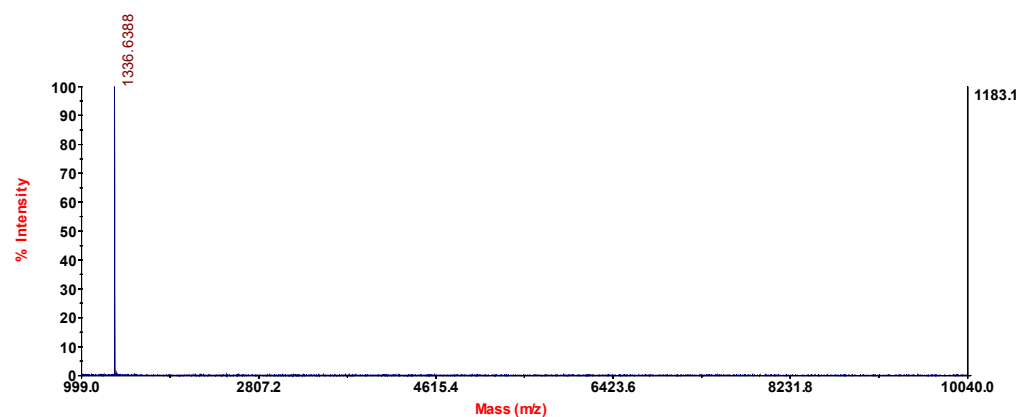


Figure SI-2.2. Lev-Amido-dPEG4-cLyP-1 (1)

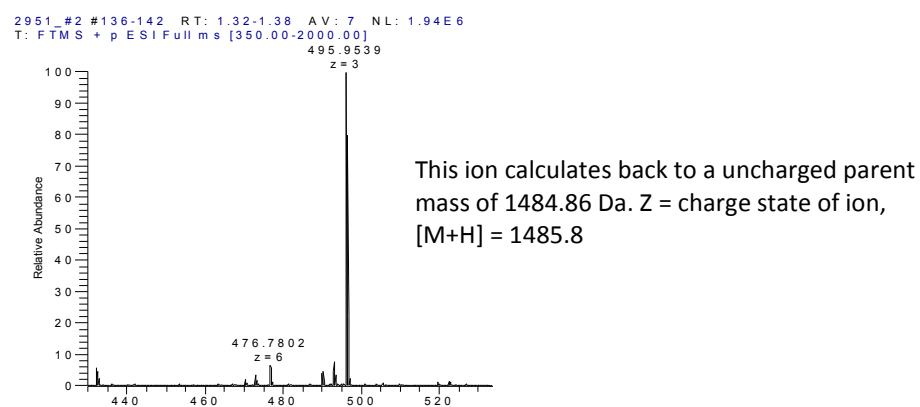


Figure SI-2.3. Lev-Amido-dPEG4-ARAL (2)

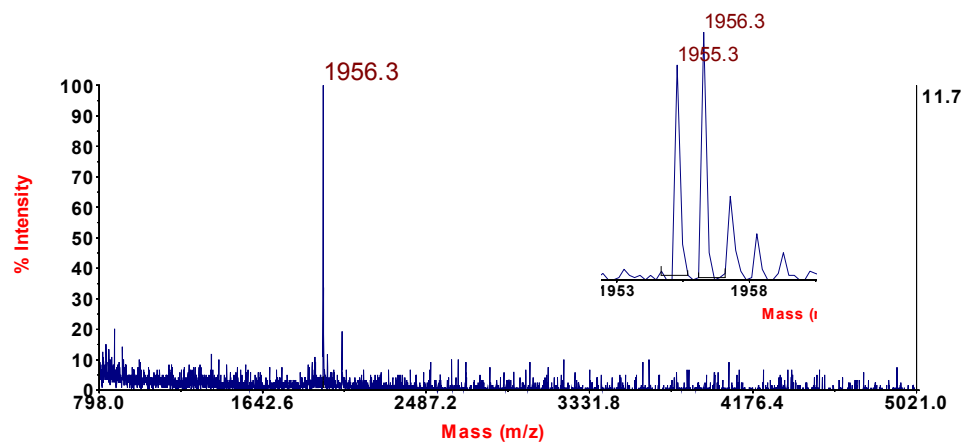


Figure SI-2.4. 6-BAT dendrimer (3)

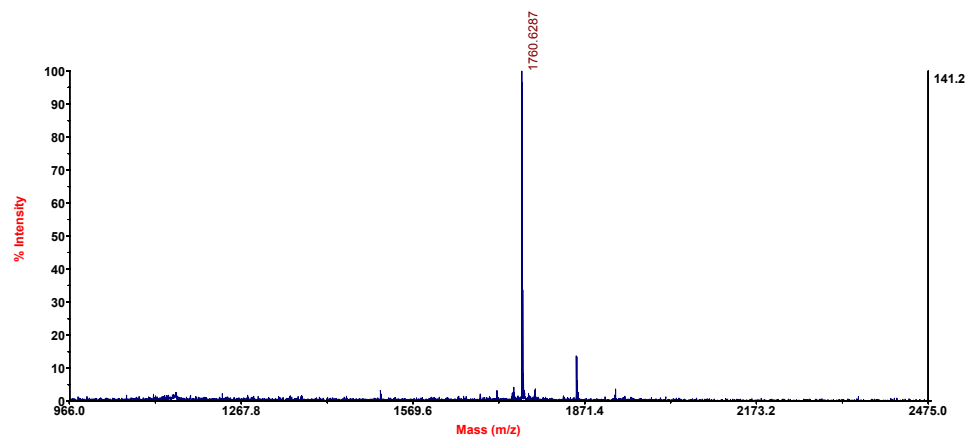


Figure SI-2.5. FAM-dendrimer (4)

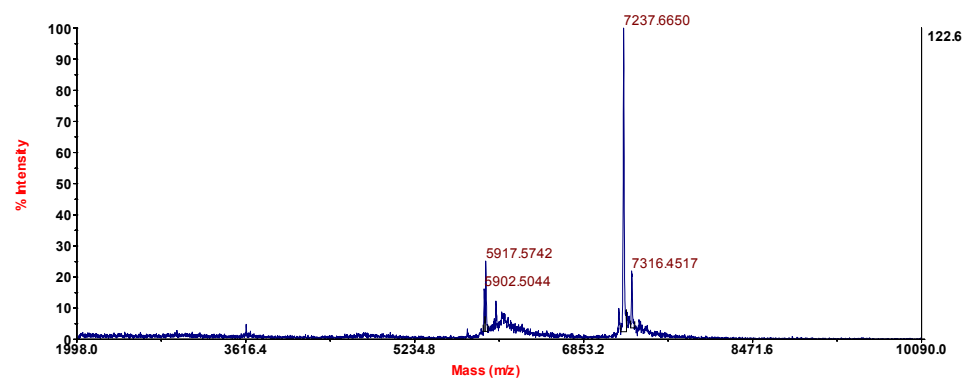


Figure SI-2.6. (LyP-1)₄-dendrimer-6-BAT (5)

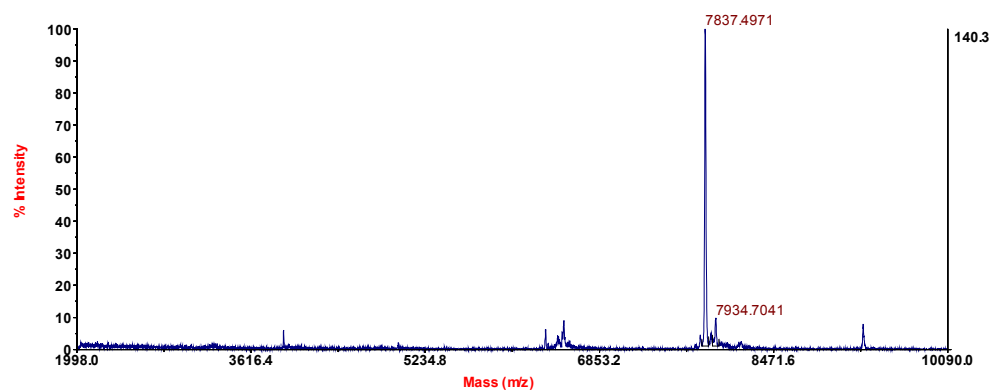


Figure SI-2.7. (ARAL)₄-dendrimer-6-BAT (6)

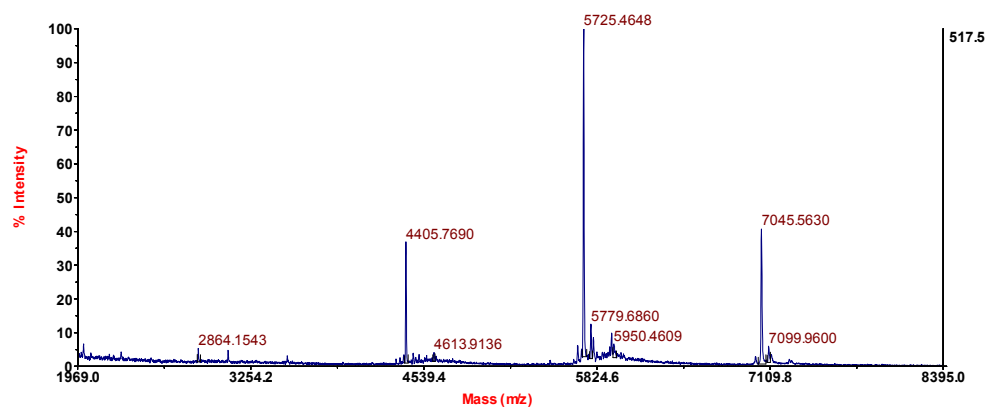


Figure SI-2.8. (LyP-1)₄-Dendrimer-FAM (7)

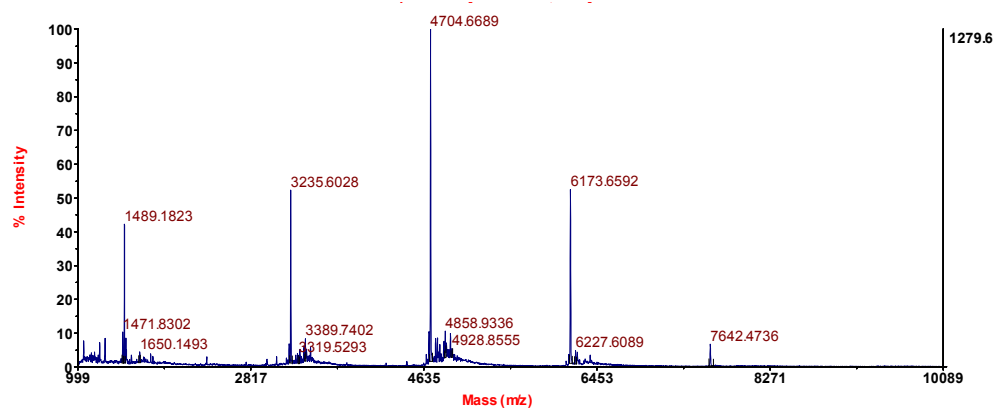


Figure SI-2.9. (ARAL)₄-Dendrimer-FAM (8)

SI-3. Determination of radiochemical purity

Table SI-1. Radio-TLC condition

Eluent	TLC phase	Rf	
		Cu-64	⁶⁴ Cu-dendrimers
10% NH ₄ OAc:MeOH (1:1)	Normal	0.8 (EDTA)	0
0.1%TFA ACN:water (1:9) x3	C18	0.4	0

abbreviation: TFA (trifluoroacetic acid), ACN (acetonitrile)

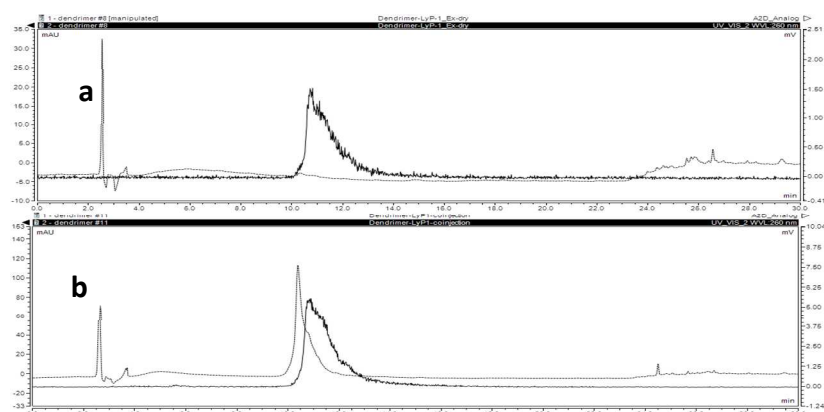


Figure SI-3.1. HPLC chromatogram of (LyP-1)₄-dendrimer-⁶⁴Cu (a) UV Dotted and black lines in chromatogram represent UV absorbance at 280 and radioactivity, respectively. (b) The co-injection chromatogram of (LyP-1)₄-dendrimer-⁶⁴Cu with cold (LyP-1)₄-dendrimer-6-BAT. (Gradient: 10-60% acetonitrile (0.1%TFA) from 0 to 30 min with 0.1% TFA water, flow rate 1 ml/min, analytical column: C12 4.6 x 250 mm).

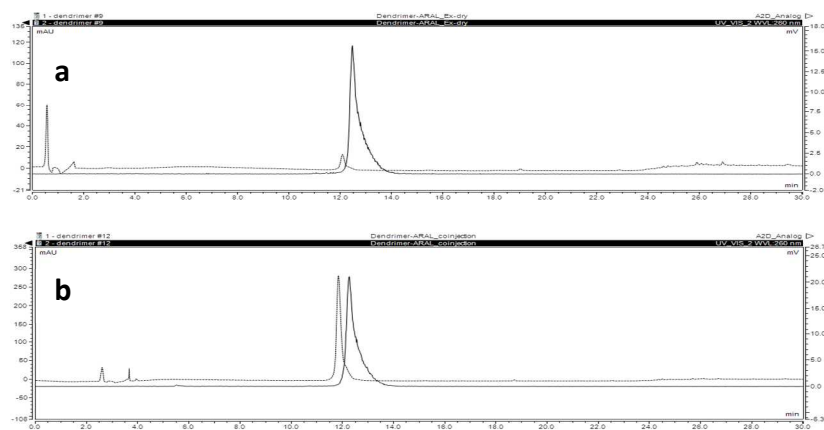


Figure SI-3.2. HPLC chromatogram of (ARAL)₄-dendrimer-⁶⁴Cu (a) UV Dotted and black lines in chromatogram represent UV absorbance at 280 and radioactivity, respectively. (b) The co-injection chromatogram of (ARAL)₄-dendrimer-⁶⁴Cu with cold (ARAL)₄-dendrimer-6-BAT. (Gradient: 10-60% acetonitrile (0.1%TFA) from 0 to 30 min with 0.1% TFA water, flow rate 1 ml/min, analytical column: C12 4.6 x 250 mm).

SI-4. Biodistribution and optical study of dendrimers

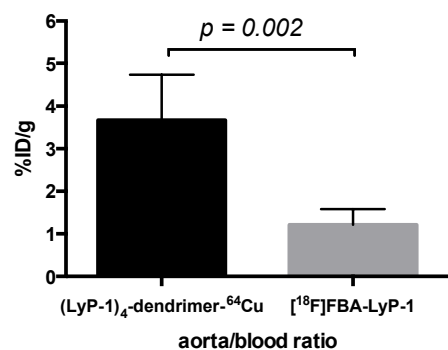


Figure SI-4.1. Comparison of aorta/blood ratio after three hours circulation of (LyP-1)₄-dendrimer-⁶⁴Cu and [¹⁸F]FBA-LyP-1

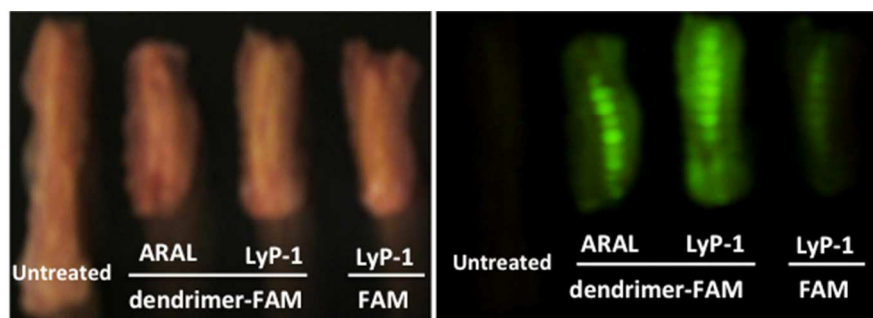


Figure SI-4.2. Ex vivo confocal microscope images of spinal cord after 1 hour circulation of (ARAL)₄-dendrimer-FAM, (LyP-1)₄-dendrimer-FAM, and LyP-1-FAM. Green channel: FAM labeled dendrimer or peptide

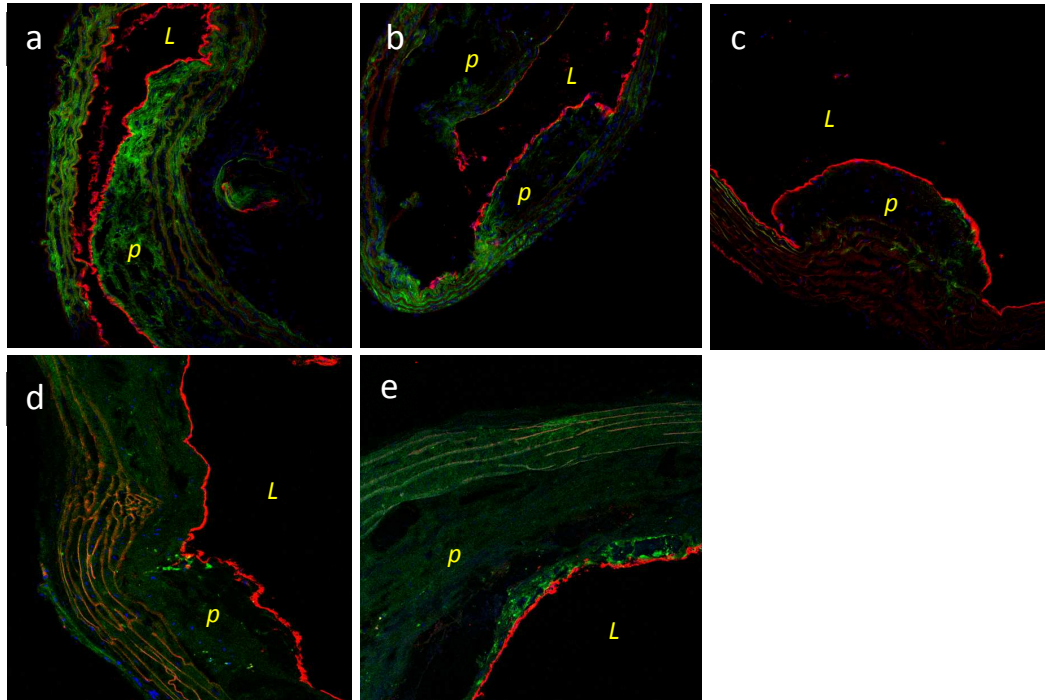


Figure SI-4.3. Sliced aorta images after circulation of (LyP-1)₄- ((a) and (b)), (ARAL)₄-dendrimer-FAM (c), and LyP-1-FAM ((d) and (e)). Green channel: FAM labeled dendrimer or peptide, Red channel: luminal endothelium (anti-CD31), Blue channel: nuclide (DAPI). *P* and *L* represents a plaque and lumen, respectively.

SI-5. Saturation binding assay (K_d)

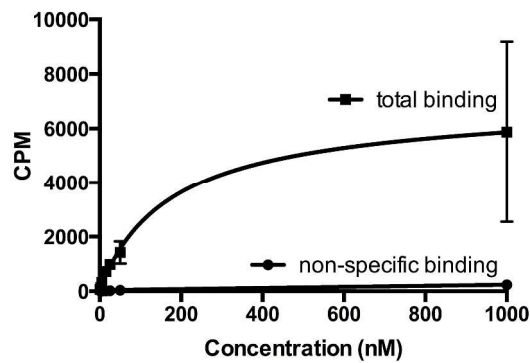


Figure SI-5.1. Binding affinity (K_d) of LyP-1 peptide with p32 protein. Binding of increasing amounts of (LyP-1)₄-dendrimer-⁶⁴Cu to immobilized p32 protein was detected on gamma counter. Nonspecific binding was subtracted from total binding.