Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Self-assembling multivalency – Enhancing integrin binding through synthetically straightforward non-covalent ligand organisation

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SUPPLEMENTARY INFORMATION

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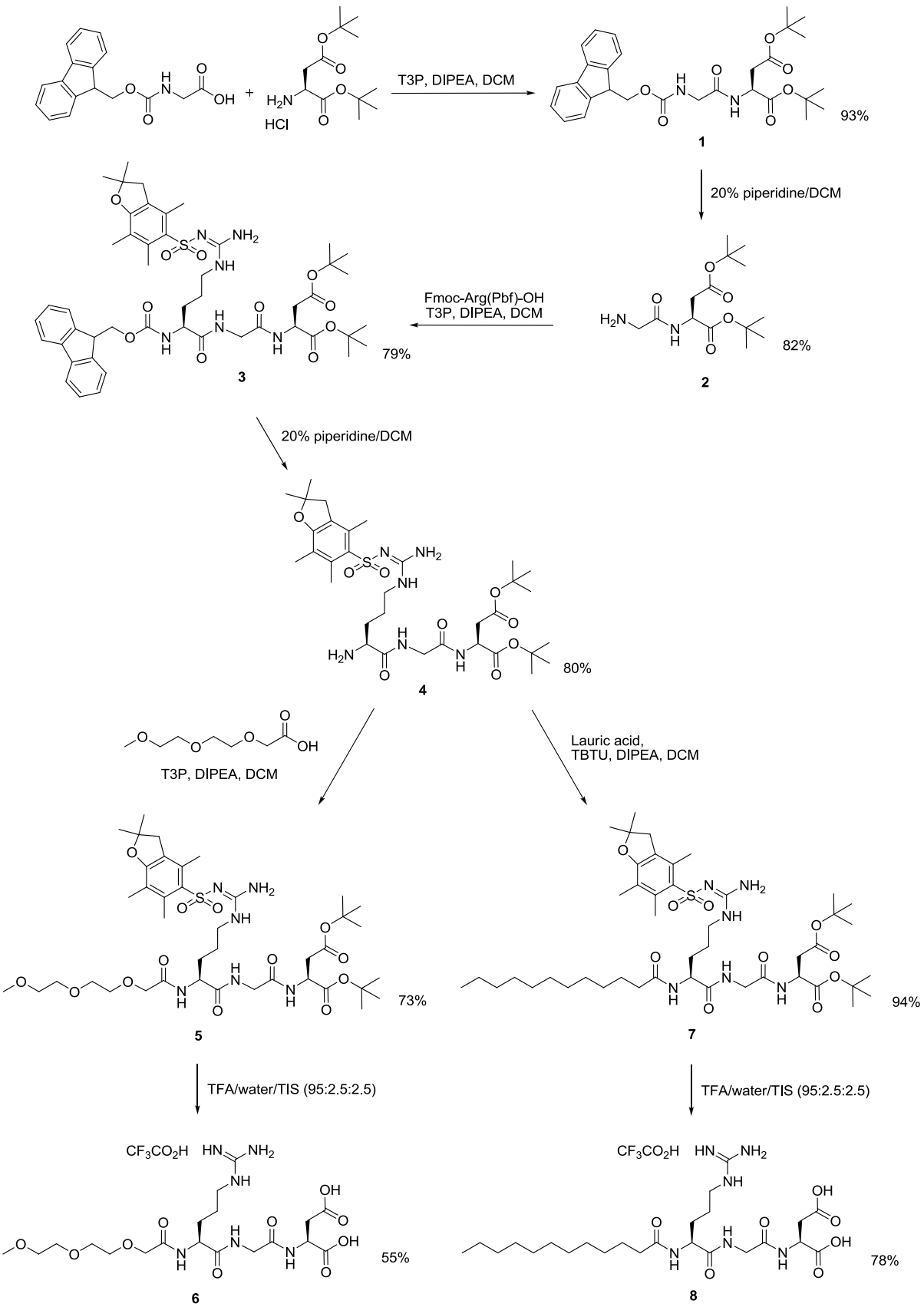
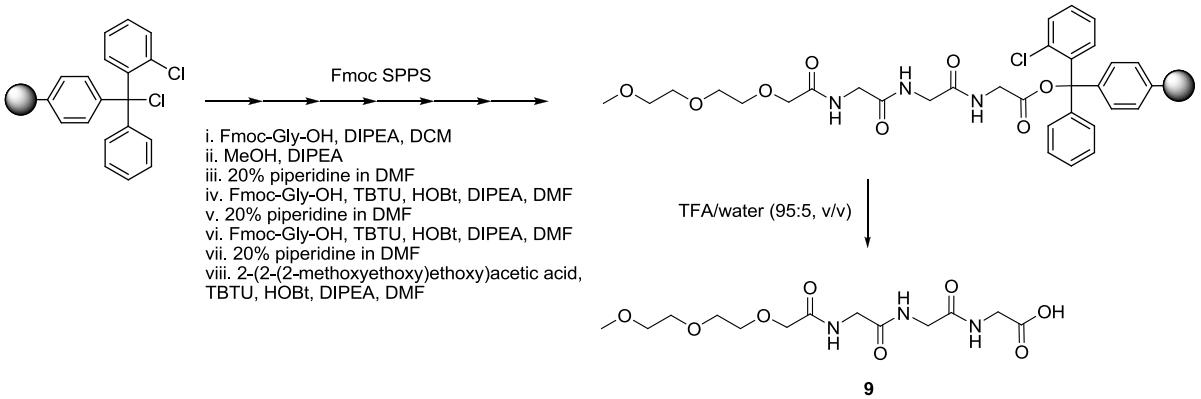
1. Synthetic Reaction Scheme





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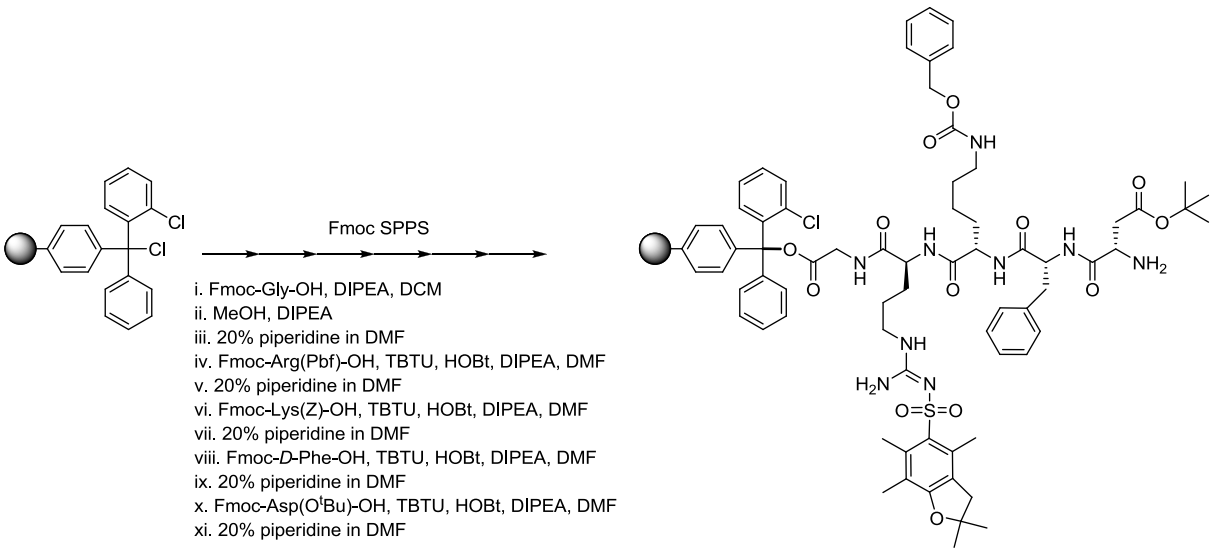

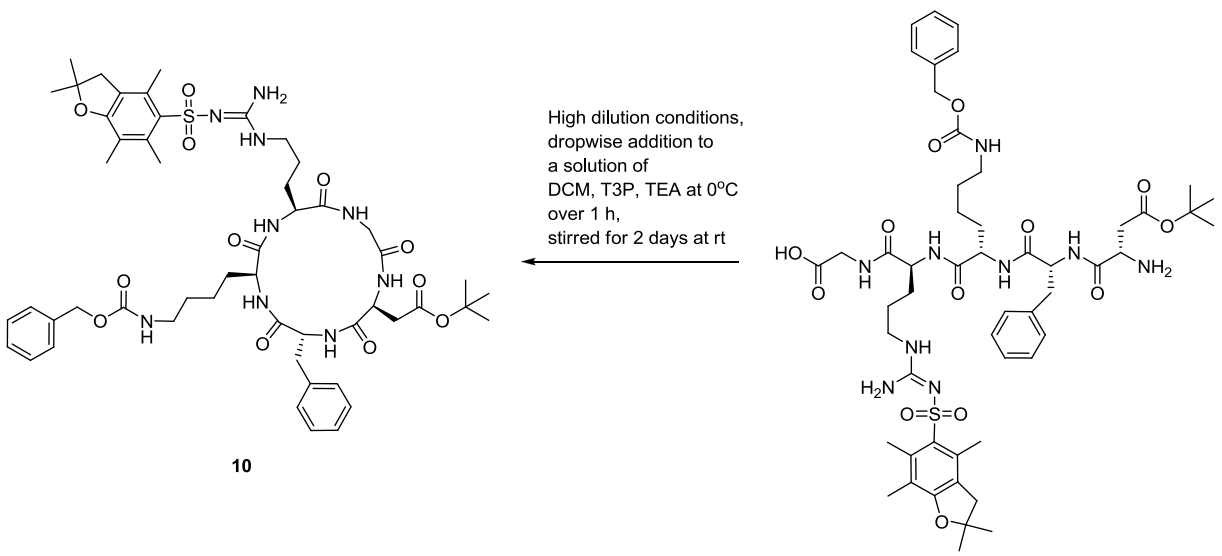

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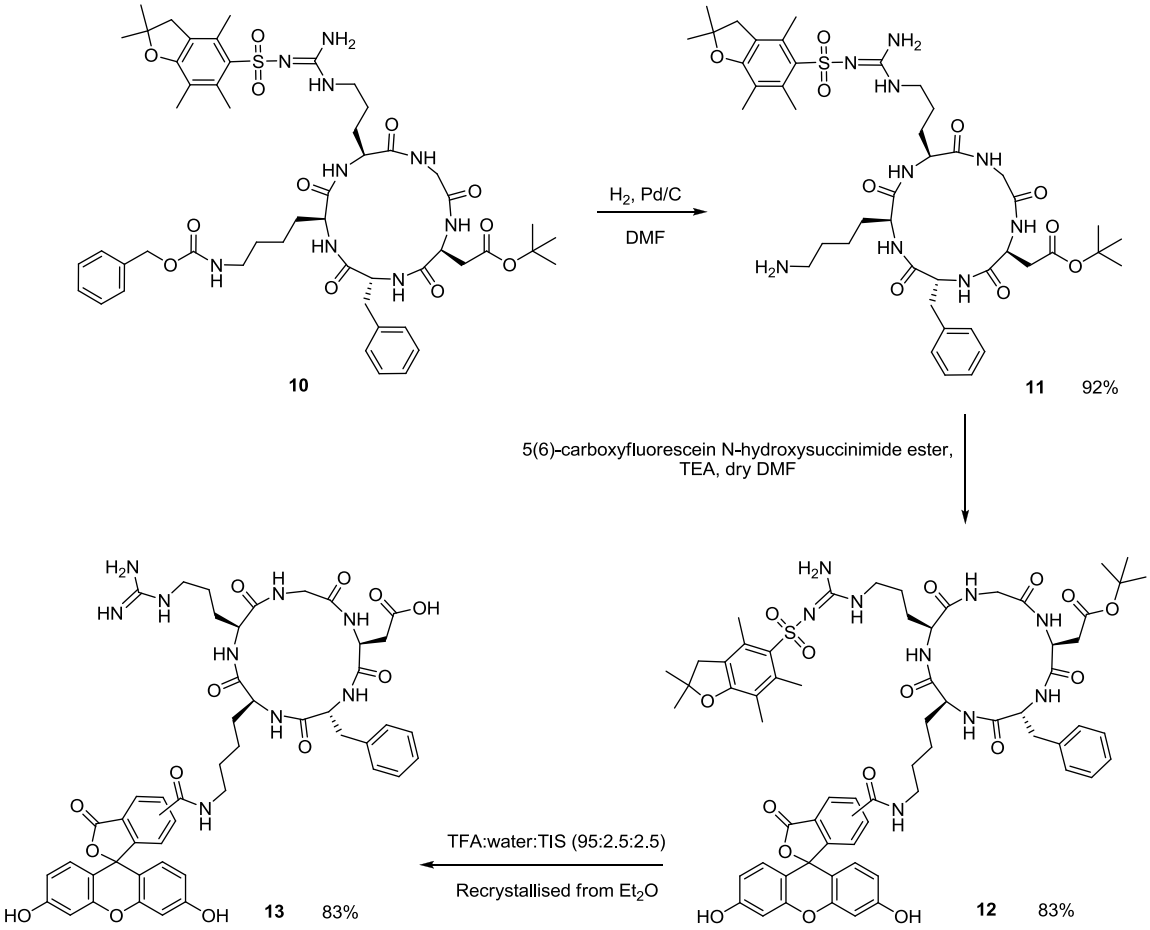


2. Synthetic Methods and Characterisation Data

Materials and Methods. All reagents were commercially available and used as supplied without further purification. Human integrin $\alpha_v\beta_3$ Triton X-100 formulation purified protein was purchased from Millipore and used without further purification. Column chromatography was performed on silica using silica gel 60 provided by Fluka Ltd. (35-70 μm) while TLC was performed on Merck aluminium-backed plates, coated with 0.25 mm silica gel 60. Spots were visualised either by UV, or by use of an appropriate stain (ninhydrin solution 0.2% (by mass) in ethanol, cerium molybdate stain: 180 ml H_2O, 20 ml conc. H_2SO_4, 24 g ammonium molybdate, 2 g cerium sulphate, or potassium permanganate stain: 1.5 g $KMnO_4$, 10 g K_2CO_3, 1.25 ml 10% NaOH in 200 ml water). Preparative gel filtration chromatography was carried out using Sephadex LH-20 purchased from Sigma Aldrich. Proton and carbon NMR chemical shifts (δ) are reported in ppm using residual solvent as internal reference, as noted, and peak assignments were deduced with DEPT-135 as well as 2D NMR experiments such as COSY and HSQC. All spectra were recorded on either a JEOL ECX400 or a JEOL ECS400 (^{1}H 400 MHz, ^{13}C 100 MHz) spectrometers or a JEOL EX270 (^{1}H 270 MHz, ^{13}C 68 MHz) spectrometer, as noted. ESI and HR ESI mass spectra were recorded on a Thermo-Finnigan LCQ, and a Bruker Daltonics Microtoff mass spectrometer respectively. Infrared spectra were recorded using a Shimadzu IRPrestige-21 FT-IR spectrometer. Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. Optical rotation was measured as $[\alpha]_D$ on a JASCO DIP-370 digital polarimeter. Nile Red fluorescence was measured on a Hitachi F-4500 spectrofluorimeter. Fluorescence polarisation data was collected on FluoroMax-3 and FluoroMax-4 spectrofluorimeters. The synthesis of compound **10** was adapted from liteature methods.[1] Compound **13** has been previously reported.[2]

Compound 1. H_2N-Asp(O^tBu)-O^tBu.HCl (2.85 g, 10.1 mmol, 1 eq) and Fmoc-Gly-OH (3.01 g, 10.1 mmol, 1 eq) were dissolved in DCM (100 ml) upon addition of DIPEA (3.52 ml, 20.2 mmol, 2 eq) with stirring. The solution was cooled in an ice-water bath to 0°C and then T3P (50 wt.% in EtOAc, 7.25 ml, 12.3 mmol, 1.2 eq) was added dropwise over 20 min. The ice-water bath was removed and the reaction mixture was stirred for 24 h. The reaction was quenched with water (100 ml), and then the organic layer was washed with saturated $NaHCO_3$ (100 ml), 1.33 M $NaHSO_4$ (100 ml), saturated $NaHCO_3$ (100 ml), neutralising to pH 7 and finally water (100 ml). The organic layer was dried over $MgSO_4$ and filtered before removing the solvent *in vacuo* to produce the product **1** as a white foam (4.90 g, 93%). No further purification was required. R_f 0.64 (9:1 DCM/MeOH, UV and cerium stain).

$[\alpha]_D$ = +22.9 (c = 1.0, $CHCl_3$). M.p: 54.4-60.2°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.76 (d, C*H* aromatic, *J* = 8.0 Hz, 2H); 7.60 (d, C*H* aromatic, *J* = 8.0 Hz, 2H); 7.40 (t, C*H* aromatic, *J* = 8.0 Hz, 2H); 7.31 (t, C*H* aromatic, *J* = 8.0 Hz, 2H); 6.86 (d, N*H* amide, *J* = 8.0 Hz, 1H); 5.45 (br s, N*H* carbamate, 1H); 4.70 (dt, Asp α-*H*, *J* = 8.0 Hz and 4.0 Hz, 1H); 4.39 (d, Fmoc CH_2, *J* = 7.0 Hz, 2H); 4.24 (t, Fmoc C*H*, *J* = 7.0 Hz, 1H); 3.98 (dd, Gly CH^A, *J* = 17.0 Hz and 5.0 Hz, 1H); 3.92 (dd, Gly CH^B, *J* = 17.0 Hz and 5.0 Hz, 1H); 2.91 (dd, Asp CH^A, *J* = 17.0 Hz and 4.5 Hz, 1H); 2.72 (dd, Asp CH^B, *J* = 17.0 Hz and 4.5 Hz, 1H); 1.45 (s, C(CH_3)$_3$, 9H); 1.42 (s, C(CH_3)$_3$, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.30, 169.60, 168.69 (*C*(O)O^{t}Bu × 2, *C*ONH amide); 156.53 (*C*ONH carbamate); 143.92, 141.35 (*C* aromatic); 127.80, 127.19, 125.24, 120.06 (*C*H aromatic); 82.66, 81.82 (*C*(CH$_3$)$_3$ × 2); 67.36 (Fmoc *C*H$_2$); 49.19 (Asp α-*C*H); 47.16 (Fmoc *C*H); 44.40 (Gly *C*H$_2$); 37.44 (Asp *C*H$_2$); 28.11, 27.97 (C(*C*H$_3$)$_3$ × 2). ν_{max} (cm^{-1}, solid): 3314*w* (N-H amide stretch); 3086*w* (C-H arene stretch); 2979*w* (C-H alkyl stretch); 1723*s* (C=O ester stretch); 1665*s* (C=O amide stretch); 1506br&*s* (N-H amide bend and C=C arene stretch); 1478*w*, 1450*m*, 1394*w*, 1367*m* (C-H alkyl bends); 1244*s*, 1224*s*, 1145*s*, 1045*m*, 1002*w* (C-O ester and C-N amide stretches, C-H arene bends); 948*w*, 845*m*, 759*s*, 739*s* (C-H arene bends). ESI-MS (m/z): Calc. for $C_{29}H_{36}N_2NaO_7$ 547.2415; found: 547.2411 (100%, $[M+Na]^+$); 491.1782 (15%, $[M+Na–C_4H_8]^+$); 435.1157 (79%, $[M+Na–2C_4H_8]^+$).

Compound 2. Compound **1** (4.58 g, 8.74 mmol) was stirred in a solution of 20% piperidine in DCM (30 ml). The solvent was removed *in vacuo* after stirring for 4 h to produce a pale yellow crude solid (6.62 g). The crude solid was purified by column chromatography (SiO_2, 9:1 DCM/MeOH) to yield **2** as a colourless oil (2.16 g, 82%). R_f 0.20 (9:1 DCM/MeOH, ninhydrin stain). $[\alpha]_D$ = +36.5 (c = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (d, N*H* amide, 8.0 Hz, 1H); 4.71 (dt, Asp α-*H*, *J* = 8.5 Hz and 4.5 Hz, 1H); 3.37 (d, Gly CH_2, *J* = 1.0 Hz, 2H); 2.89 (dd, Asp CH^A, *J* = 17.0 Hz and 4.5 Hz, 1H); 2.70 (dd, Asp CH^B, *J* = 17.0 Hz and 4.5 Hz, 1H); 1.53 (br s, NH_2, 2H); 1.45 (s, C(CH_3)$_3$, 9H); 1.44 (s, C(CH_3)$_3$, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.61, 169.95, 169.79 (*C*(O)O^{t}Bu × 2, *C*ONH amide); 82.10, 81.43 (*C*(CH$_3$)$_3$ × 2); 48.58 (Asp α-*C*H); 44.68 (Gly *C*H$_2$); 37.71 (Asp *C*H$_2$); 27.99, 27.86 (C(*C*H$_3$)$_3$ × 2). ν_{max} (cm^{-1}, oil): 3357*w* (N-H amine and amide stretches); 2978*w*, 2936*w* (C-H alkyl stretches); 1726*s* (C=O ester stretch); 1666*s* (C=O amide stretch); 1509*s* (N-H amide bend); 1480*w*, 1458*w*, 1394*w*, 1367*s*, 1350*m* (C-H alkyl bends); 1288*m*, 1249*m*, 1226*m*, 1145*s* (C-O ester and C-N stretches); 1079*w*; 1051*w*; 1032*w*; 845*m*; 752*w*; 732*w*. ESI-MS (m/z): Calc. for $C_{14}H_{26}N_2NaO_5$ 325.1734; found: 325.1739 (60%, $[M+Na]^+$); 303.1953 (100%, $[M+H]^+$); 247.129 (17%, $[M+H–C_4H_8]^+$); 191.0659 (10%, $[M+H–2C_4H_8]^+$).

Compound 3. Compound **2** (2.02 g, 6.68 mmol, 1 eq) and Fmoc-Arg(Pbf)-OH (4.33 g, 6.68 mmol, 1 eq) were dissolved in DCM (120 ml) upon addition of DIPEA (2.42 ml, 13.9 mmol, 2 eq) with stirring. The solution was cooled in an ice-water bath to 0°C and then T3P (50 wt. % in EtOAc, 5.00 ml, 8.49 mmol, 1.2 eq) was added dropwise over 20 min. The ice-water bath was removed and the reaction mixture was stirred for 24 h. The reaction was quenched with water (100 ml), and then the organic layer was washed with saturated $NaHCO_3$ (100 ml), 1.33 M $NaHSO_4$ (100 ml), saturated $NaHCO_3$ (100 ml), neutralising to pH 7 and finally water (100 ml) upon which the organic phase became milky white. The organic layer was dried over $MgSO_4$ and filtered before removing the solvent *in vacuo* to produce the product **3** as a white foam (4.90 g, 79%). No further purification was required. R_f 0.51 (9:1 DCM/MeOH, UV and cerium stain). $[\alpha]_D$ = +8.6 (c = 1.0, $CHCl_3$). M.p: 114.0-120.0°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (d, C*H* aromatic, *J* = 7.5 Hz, 2H); 7.70 (m, N*H* amide (Arg-Gly), 1H); 7.58 (m, C*H* aromatic, 2H); 7.36 (t, C*H* aromatic, *J* = 7.0 Hz, 2H); 7.26 (t, C*H* aromatic, *J* = 7.5 Hz, 2H); 7.11 (d, N*H* amide (Gly-Asp), *J* = 7.5 Hz, 1H); 6.33 (br s, NH_2 guanidine, 2H); 6.12 (br s, N*H* guanidine, 1H); 6.04 (d, N*H* carbamate, *J* = 7.5 Hz, 1H); 4.64 (dt, Asp α-*H*, *J* = 8.0 Hz and 5.0 Hz, 1H); 4.39 (m, Arg α-*H*, 1H); 4.34 (d, Fmoc CH_2, *J* = 7.0 Hz, 2H); 4.16 (t, Fmoc C*H*, *J* = 7.0 Hz, 1H); 4.06 (dd, Gly CH^A, *J* = 16.5 Hz and 5.5 Hz, 1H); 3.91 (dd, Gly CH^B, *J* = 17.0 Hz and 5.0 Hz, 1H); 3.42-3.28 (m, Arg CH^ANH, 1H); 3.24-3.14 (m, Arg CH^BNH, 1H); 2.92 (s, Pbf CH_2, 2H); 2.82 (dd, Asp CH^A, *J* = 17.0 Hz and 5.0 Hz, 1H); 2.66 (dd, Asp CH^B, *J* = 17.0 Hz and 4.5 Hz, 1H); 2.59 (s, Pbf CH_3Ar, 3H); 2.51 (s, Pbf CH_3Ar, 3H); 2.07 (s, Pbf CH_3Ar, 3H); 2.01-1.89 (m, Arg CHCH^A, 1H); 1.76-1.66 (m, Arg CHCH^B, 1H); 1.65-1.50 (m, Arg CH_2CH_2NH, 2H); 1.43 (s, Pbf CH_3 × 2, 6H); 1.40 (s, C(CH_3)$_3$ × 2, 18H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.07, 170.17, 169.82, 169.39 (*C*(O)O^{t}Bu × 2, *C*ONH amide × 2); 158.79, 156.68, 156.56 (Pbf Ar*C*O, *C*=N guanidine, *C*ONH carbamate); 143.96, 143.84, 141.30, 141.28 (Fmoc aromatic *C*); 138.46, 132.86, 132.37 (Pbf aromatic *C*); 127.73, 127.15, 125.31 (Fmoc aromatic *C*H); 124.67 (Pbf aromatic *C*); 119.97 (Fmoc aromatic *C*H); 117.56 (Pbf aromatic *C*); 86.42 (Pbf $CH_2C(CH_3)_2O$); 82.66, 81.73 (*C*(CH$_3$)$_3$ × 2); 67.14 (Fmoc *C*H$_2$); 54.33 (Arg α-*C*H); 49.45 (Asp α-*C*H); 47.14 (Fmoc *C*H); 43.28, 42.90, 40.20 (Pbf Ar*C*H$_2$, Arg *C*H$_2$NH, Gly *C*H$_2$); 37.37 (Asp *C*H$_2$); 29.88 (Arg CH*C*H$_2$ or Arg *C*H$_2$CH$_2$NH); 28.65 (Pbf $CH_2C(CH_3)_2O$); 28.08, 27.92 (C(*C*H$_3$)$_3$ × 2, multiple overlapping peaks); 25.28 (Arg CH*C*H$_2$ or Arg *C*H$_2$CH$_2$NH); 19.43, 18.08, 12.57 (Pbf Ar*C*H$_3$ × 3). ν_{max} (cm^{-1}, solid): 3422*w*, 3321*w* (N-H amide stretches); 2974*w*, 2932*w* (C-H alkyl and arene stretches); 1724*m* (C=O ester stretch); 1667*m*, 1620*m* (C=O amide stretches); 1543*br&s* (N-H amide bend and C=C arene stretch); 1450*m*, 1408*w*, 1393*w*, 1366*m* (C-H alkyl bend and S=O stretch); 1281*m*, 1246*s*, 1150*s*, 1103*s*, 1088*s* (C-O ester and C-N amide stretches, C-H arene

bends); 1034*m*; 991*m*; 849*m*, 818*w*, 756*w*, 737*m*, 660*m* (C-H arene bends); 640*m*; 617*m*; 598*m*. ESI-MS (m/z): Calc. for $C_{48}H_{65}N_6O_{11}S$ 933.4427; found: 933.4426 (100%, $[M+H]^+$).

Compound 4. Compound **3** (4.51 g, 4.84 mmol) was stirred in a solution of 20% piperidine in DCM (30 ml). The solvent was removed *in vacuo* after stirring for 3 h to produce a pale yellow crude solid (7.5 g). The crude solid was purified by column chromatography (SiO_2, 9:1 DCM/MeOH) to yield **4** as a fluffy white solid (2.75 g, 80%). R_f 0.15 (9:1 DCM/MeOH, UV and ninhydrin stain). $[\alpha]_D$ = +18.2 (c = 1.0, $CHCl_3$). M.p: 83.0-84.0°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (t, N*H* amide (Arg-Gly), *J* = 6.0 Hz, 1H); 7.10 (d, N*H* amide (Gly-Asp), *J* = 8.0 Hz, 1H); 6.32 (br s, NH_2 guanidine, 2H); 6.14 (br s, N*H* guanidine, 1H); 4.64 (dt, Asp α-*H*, *J* = 8.0 Hz and 4.5 Hz, 1H); 4.02 (dd, Gly CH^A, *J* = 17.0 Hz and 5.5 Hz, 1H); 3.90 (dd, Gly CH^B, *J* = 17.0 Hz and 6.0 Hz, 1H); 3.49-3.46 (m, Arg α-*H*, 1H); 3.27-3.19 (m, Arg CH_2NH, 2H); 2.95 (s, Pbf CH_2, 2H); 2.86 (dd, Asp CH^A, *J* = 17.0 Hz and 5.0 Hz, 1H); 2.70 (dd, Asp CH^B, *J* = 17.0 Hz and 4.5 Hz, 1H); 2.57 (s, Pbf CH_3Ar, 3H); 2.50 (s, Pbf CH_3Ar, 3H); 2.08 (s, Pbf CH_3Ar, 3H); 1.82-1.74 (m, Arg CHCH^A, 1H); 1.70-1.58 (m, Arg CHCH^B and Arg CH_2CH$_2$NH, 3H); 1.45 (s, Pbf CH_3 × 2, 6H); 1.42 (s, C(CH_3)$_3$ × 2, 18H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.41, 170.32, 169.79, 169.34 (*C*(O)O^{t}Bu × 2, *C*ONH amide × 2); 158.69, 156.49 (Pbf Ar*C*O, *C*=N guanidine); 138.30, 133.05, 132.23, 124.62, 117.48 (Pbf aromatic *C*); 86.42 (Pbf CH$_2$*C*(CH$_3$)$_2$O); 82.61, 81.76 (*C*(CH$_3$)$_3$ × 2); 54.54 (Arg α-*C*H); 49.27 (Asp α-*C*H); 43.28, 42.76, 40.61 (Pbf Ar*C*H$_2$, Arg *C*H$_2$NH, Gly *C*H$_2$); 37.38 (Asp *C*H$_2$); 32.08 (Arg CH*C*H$_2$ or Arg *C*H$_2$CH$_2$NH); 28.66 (Pbf CH$_2$C(*C*H$_3$)$_2$O); 28.09, 27.93 (C(*C*H$_3$)$_3$ × 2, multiple overlapping peaks); 25.42 (Arg CH*C*H$_2$ or Arg *C*H$_2$CH$_2$NH); 19.36, 18.02, 12.55 (Pbf Ar*C*H$_3$ × 3). ν_{max} (cm^{-1}, solid): 3320*w* (N-H amide stretch); 2972*w* (C-H alkyl stretch); 1735*w* (C=O ester stretch); 1647*br&w* (C=O amide stretch); 1540*br&w* (N-H amide bend, C-H alkyl bend, C=C arene stretch and S=O stretch); 1251*w*, 1145*w* (C-O ester and C-N amide stretches). ESI-MS (m/z): Calc. for $C_{33}H_{55}N_6O_9S$ 711.3746; found: 711.3761 (93%, $[M+H]^+$); 733.3608 (100%, $[M+Na]^+$); 367.1796 (73%, $[M+H+Na]^{2+}$).

Compound 5. 2-[2-(2-Methoxyethoxy)-ethoxy)acetic acid (93 mg, 0.52 mmol, 1 eq) and protected RGD peptide **4** (400 mg, 0.56 mmol, 1 eq) were suspended in DCM (10 ml) and DIPEA (200 µL, 1.12 mmol, 2 eq) was added with stirring. The solution was cooled in an ice-water bath to 0°C and then T3P (50 wt.% in EtOAc, 400 µL, 0.67 mmol, 1.2 eq) was added dropwise over 10 min. The ice-water bath was removed and the reaction mixture was stirred for 27 h. The solvent was removed *in vacuo* directly, without quenching with water or any prior acid/base workup, to leave a tacky, colourless crude solid (1

g) which was purified by column chromatography (SiO_2, 9:1 DCM/MeOH) to produce compound **5** as a fluffy white foam (330 mg, 73%). R_f 0.40 (9:1 DCM/MeOH, UV and cerium stain). $[\alpha]_D$ +36.0 (c = 1.0, $CHCl_3$). M.p: 64.0-68.0°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.69 (br s, N*H* amide (Arg-Gly), 1H); 7.48 (d, N*H* amide (PEG-Arg), *J* = 8.0 Hz, 1H); 7.04 (d, N*H* amide (Gly-Asp), *J* = 8.5 Hz, 1H), 6.28 (br s, NH_2 guanidine, 2H); 6.12 (br s, N*H* guanidine, 1H); 4.66-4.61 (m, Asp α-*H* and Arg α-*H*, 2H); 4.02 (dd, Gly CH^A, *J* = 17.0 Hz and 5.5 Hz, 1H); 4.01 (s, OCH_2C(O)NH, 2H); 3.86 (dd, Gly CH^B, *J* = 17.0 Hz and 5.5 Hz, 1H); 3.71-3.50 (m, $CH_3OCH_2CH_2OCH_2CH_2$, $CH_3OCH_2CH_2OCH_2CH_2$, $CH_3OCH_2CH_2OCH_2CH_2$, $CH_3OCH_2CH_2OCH_2CH_2$, 8H); 3.34 (s, CH_3O, 3H); 3.36-3.27 (m, Arg CH^ANH, 1H); 3.24-3.16 (m, Arg CH^BNH, 1H); 2.94 (s, Pbf CH_2, 2H); 2.83 (dd, Asp CH^A, *J* = 17.0 Hz and 5.0 Hz, 1H); 2.67 (dd, Asp CH^B, *J* = 17.0 Hz and 4.5 Hz, 1H); 2.58 (s, Pbf CH_3Ar, 3H); 2.51 (s, Pbf CH_3Ar, 3H); 2.07 (s, Pbf CH_3Ar, 3H); 2.01-1.91 (m, Arg CHCH^A, 1H); 1.75-1.66 (m, Arg CHCH^B, 1H); 1.61-1.51 (m, Arg CH_2CH$_2$NH, 2H); 1.44 (s, Pbf CH_3 × 2, 6H); 1.41, 1.40 (s × 2, C(CH_3)$_3$ × 2, 18H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.31, 170.54, 170.22, 169.78, 169.17 (*C*(O)O^{t}Bu × 2, *C*ONH amide × 3); 158.74, 156.62 (Pbf Ar*C*O, *C*=N guanidine); 138.48, 133.04, 132.41, 124.62, 117.51 (Pbf aromatic *C*); 86.41 (Pbf CH$_2$*C*(CH$_3$)$_2$O); 82.60, 81.74 (*C*(CH$_3$)$_3$ × 2); 71.84, 71.11, 70.59, 70.38, 70.32 (*C*H$_2$O's × 5); 59.00 (*C*H$_3$OCH$_2$); 51.99 (Arg α-*C*H); 49.37 (Asp α-*C*H); 43.34, 42.85 (Pbf Ar*C*H$_2$, Gly *C*H$_2$); 40.11 (Arg *C*H$_2$NH); 37.41 (Asp *C*H$_2$); 29.79 (Arg CH*C*H$_2$); 28.70 (Pbf CH$_2$C(*C*H$_3$)$_2$O); 28.12, 27.95 (C(*C*H$_3$)$_3$ × 2); 25.41 (Arg *C*H$_2$CH$_2$NH); 19.41, 18.07, 12.58 (Pbf Ar*C*H$_3$ × 3). ν_{max} (cm^{-1}, solid): 3322*br&w* (N-H amide stretch); 2976, 2930*br&w* (C-H alkyl stretches); 1730*m* (C=O ester stretch); 1655*br&m* (C=O amide stretch); 1543*br&s* (N-H amide bend and C=C arene stretch); 1452*w*, 1368*w* (C-H alkyl bend and S=O stretch); 1293*w*, 1278*w*, 1249*m*, 1202*w*, 1150*s*, 1094*s* (C-O ester and C-N amide stretches, C-H arene bends). ESI-MS (m/z): Calc. for $C_{40}H_{66}N_6NaO_{13}S$ 893.4301; found: 893.4318 (100%, $[M+Na]^+$).

Compound 6 (PEG-RGD). Compound **5** (56 mg, 0.064 mmol) was dissolved in a mixture of TFA, water and triisopropylsilane (TIPS) (500 μL, 95:2.5:2.5) and shaken for 2 h, after which time TLC indicated that the deprotection reaction was complete. The volatile organics were removed *in vacuo*, then the residue was dissolved in 10% aqueous acetic acid (3 ml) and washed three times with a twofold excess of chloroform to extract the non-polar by-products. The aqueous acetic acid layer was evaporated *in vacuo* and the residue was dissolved in water (5 ml), shell frozen and lyophilised to yield **PEG-RGD** as a hygroscopic, fluffy white powder which turned to a tacky solid upon standing (22 mg, 55% as TFA salt). R_f 0.00 (9:1 DCM/MeOH, cerium stain). $[\alpha]_D$ +10.1 (c = 1.0, CH_3OH). ^{1}H NMR

(D_2O, 400 MHz) δ 4.75 (t, Asp α-*H*, *J* = 6.0 Hz, 1H); 4.37 (dd, Arg α-*H*, *J* = 8.5 Hz and 5.5 Hz, 1H); 4.12 (d, OC*H*^{A}C(O)NH, *J* = 15.5 Hz, 1H); 4.08 (d, OC*H*^{B}C(O)NH, *J* = 15.5 Hz, 1H); 3.92 (s, Gly C*H*$_2$, 2H); 3.72-3.57 (m, $CH_3OCH_2CH_2OCH_2CH_2$, $CH_3OCH_2CH_2OCH_2CH_2$, $CH_3OCH_2CH_2OCH_2CH_2$, $CH_3OCH_2CH_2OCH_2CH_2$, 8H); 3.33 (s, CH_3O, 3H); 3.18 (t, Arg C*H*$_2$NH, *J* = 7.0 Hz, 2H); 2.92 (d, Asp C*H*$_2$, *J* = 5.5 Hz, 2H); 1.93-1.84 (m, Arg CHC*H*^{A}, 1H); 1.81-1.71 (m, Arg CHC*H*^{B}, 1H); 1.68-1.56 (m, Arg C*H*$_2$CH$_2$NH, 2H). ^{13}C NMR (D_2O, 100 MHz) δ 175.07, 174.81, 174.75, 173.64, 171.65, 157.43 (*C*(O)OH × 2, *C*ONH amide × 3, *C*=N guanidine); 71.16, 70.51, 69.70, 69.68, 69.58 (*C*H$_2$O's × 5, multiple overlapping peaks); 58.20 (*C*H_3OCH_2); 53.23 (Arg α-*C*H); 49.17 (Asp α-*C*H); 42.38 (Gly *C*H$_2$); 40.53 (Arg *C*H$_2$NH); 35.57 (Asp *C*H$_2$); 28.03 (Arg CH*C*H$_2$); 24.34 (Arg *C*H$_2$CH$_2$NH). ν_{max} (cm^{-1}, tacky solid): 3285*br&m*, 3198*br&m* (O-H acid and N-H amide/guanidino stretches); 2927*br&w* (C-H alkyl stretch); 1724*m* (C=O acid stretch); 1651*s* (C=O amide stretch); 1536*s* (N-H amide bend); 1407*w*, 1340*w* (C-H alkyl bends); 1202*br&m* (C-O acid stretch); 1083*s*, 1049*s* (C-O ether and C-N amide stretches). ESI-MS (m/z): Calc. for $C_{19}H_{35}N_6O_{10}$ 507.2409; found: 507.2386 (100%, $[M+H]^+$), 529.2197 (95%, $[M+Na]^+$), 551.2022 (16%, $[M+2Na-H]^+$).

Compound 7. Lauric acid (56 mg, 0.28 mmol, 1 eq) and protected RGD peptide **4** (200 mg, 0.28 mmol, 1 eq) were dissolved in DCM (10 ml), then DIPEA (100 µl, 0.56 mmol, 2 eq) was added and the reaction flask cooled over an ice-water bath. TBTU (90 mg, 0.28 mmol, 1 eq) was added as a solid and more DCM (2 ml) was used to wash out the vial and added to the reaction flask. The reaction was stirred at 0°C then rt for 3 days, then washed with hot 1M HCl (3 × 25 ml), hot 15% Na_2CO_3 (3 × 25 ml), and hot water (25 ml). The organic layer was dried over $MgSO_4$, filtered and the filtrate evaporated to yield compound **7** as a white solid (236 mg, 94%). R_f 0.42 (9:1 DCM/MeOH, UV and cerium stain). $[\alpha]_D$ = +5.2 (c = 1.0, $CHCl_3$). M.p: 70.5-78.6°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.87 (br t, N*H* amide (Arg-Gly), 1H); 7.41 (d, N*H* amide (Gly-Asp), *J* = 8.0 Hz, 1H); 7.06 (br s, N*H* amide (C12-Arg), 1H), 6.38 (br s, N*H*$_2$ guanidine, 2H); 6.26 (br s, N*H* guanidine, 1H); 4.63-4.58 (m, Asp α-*H*, 1H); 4.50-4.45 (m, Arg α-*H*, 1H); 4.00-3.80 (br m, Gly C*H*$_2$, 2H); 3.28-3.08 (m, Arg C*H*$_2$NH, 2H); 2.89 (s, Pbf C*H*$_2$, 2H); 2.73 (dd, Asp C*H*^{A}, *J* = 17.0 Hz and 5.0 Hz, 1H); 2.64 (dd, Asp C*H*^{B}, *J* = 17.0 Hz and 5.0 Hz, 1H); 2.51 (s, Pbf C*H*$_3$Ar, 3H); 2.44 (s, Pbf C*H*$_3$Ar, 3H); 2.15 (t, C*H*$_2$C(O)NH, *J* = 7.5 Hz, 2H); 2.02 (s, Pbf C*H*$_3$Ar, 3H); 1.89-1.77 (m, Arg CHC*H*^{A}, 1H); 1.70-1.59 (m, Arg CHC*H*^{B}, 1H); 1.57-1.45 (m, Arg C*H*$_2$CH$_2$NH and C12 C*H*$_2$ overlapping, 4H); 1.39 (s, Pbf C*H*$_3$ × 2, 6H); 1.35 (s, C(C*H*$_3$)$_3$ × 2, 18H); 1.25-1.15 (m, C12 C*H*$_2$'s, 16H); 0.81 (t, C12 C*H*$_3$, *J* = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.04, 172.92, 169.91, 169.74, 169.19 (*C*(O)O^{t}Bu × 2, *C*ONH amide × 3);

158.60, 156.54 (Pbf Ar*C*O, *C*=N guanidine); 138.23, 132.88, 132.15, 124.47, 117.34 (Pbf aromatic *C*); 86.25 (Pbf $CH_2C(CH_3)_2O$); 82.28, 81.36 ($C(CH_3)_3 \times 2$); 52.71 (Arg α-*C*H); 49.33 (Asp α-*C*H); 43.18 (Pbf ArCH_2); 42.76 (Gly CH_2); 40.15 (Arg CH_2NH); 37.30 (Asp CH_2); 36.22 (C12 $CH_2C(O)NH$); 31.84, 29.61, 29.57, 29.51, 29.36, 29.32, 29.29 (C12 CH_2's and either Arg CH*C*H_2 or Arg CH_2CH_2NH, multiple overlapping peaks); 28.54 (Pbf $CH_2C(CH_3)_2O$); 27.96, 27.80 ($C(CH_3)_3 \times 2$, multiple overlapping peaks); 25.61, 25.33, 22.61 (C12 CH_2's and either Arg CH*C*H_2 or Arg CH_2CH_2NH, multiple overlapping peaks); 19.27, 17.92 (Pbf Ar$CH_3 \times 2$); 14.07 (C12 CH_3); 12.42 (Pbf Ar$CH_3 \times 1$). ν_{max} (cm^{-1}, solid): 3317*br&m* (N-H amide stretch); 2973*w*, 2926*m*, 2853*w* (C-H alkyl stretches); 1733*m* (C=O ester stretch); 1648*br&s* (C=O amide stretch); 1544*br&s* (N-H amide bend and C=C arene stretch); 1455*w*, 1408*w*, 1393*w*, 1368*m* (C-H alkyl bends and S=O stretch); 1293*w*, 1276*w*, 1249*m*, 1152*s*, 1106*s*, 1091*s* (C-O ester and C-N amide stretches). ESI-MS (m/z): Calc. for $C_{45}H_{77}N_6O_{10}S$ 893.5416; found: 893.5424 (100%, $[M+H]^+$).

Compound 8 (C12-RGD). Compound **7** (206 mg, 0.23 mmol) was dissolved in a mixture of TFA, water and TIPS (2 ml, 95:2.5:2.5) and shaken for 3 h, after which time TLC indicated that the deprotection reaction was complete. The volatiles were removed *in vacuo*, then the residue was dissolved in the minimum amount of MeOH and recrystallised from Et_2O to yield **C12-RGD** as a white solid (116 mg, 78% as TFA salt). Sample then dissolved in water/^{t}BuOH, filtered over a PTFE membrane filter (0.2 μm), shell frozen and lyophilised to yield **C12-RGD** as a fluffy white powder. R_f 0.00 (9:1 DCM/MeOH, cerium stain). $[\alpha]_D$ = +4.8 (c = 1.0, CH_3OH). M.p: decomposed at 187.6°C. ^{1}H NMR (CD_3OD, 400 MHz) δ 4.74 (t, Asp α-*H*, *J* = 6.0 Hz, 1H); 4.29 (dd, Arg α-*H*, *J* = 8.0 and 5.5 Hz, 1H); 3.93 (d, Gly CH^A, *J* = 17.0 Hz, 1H); 3.86 (d, Gly CH^B, *J* = 17.0 Hz, 1H); 3.19 (t, Arg CH_2NH, *J* = 6.5 Hz, 2H); 2.90-2.79 (m, Asp CH_2, 2H); 2.27 (t, CH_2C(O)NH, *J* = 7.5 Hz, 2H); 1.91-1.83 (m, Arg CHCH^A, 1H); 1.79-1.57 (m, Arg CHCH^B, Arg CH_2CH$_2$NH and C12 CH_2 overlapping, 5H); 1.35-1.20 (m, C12 CH_2's, 16H); 0.88 (t, C12 CH_3, *J* = 7.0 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 176.86, 175.02, 174.06, 173.94, 171.46, 158.49 (*C*OOH × 2, *C*ONH amide × 3, *C*=N guanidine); 54.79 (Arg α-*C*H); 50.30 (Asp α-*C*H); 43.43 (Gly CH_2); 41.91 (Arg CH_2NH); 36.81, 36.73 (Asp CH_2, C12 $CH_2C(O)NH$); 33.06, 30.78, 30.75, 30.67, 30.53, 30.48, 30.43, 29.77, 26.82, 26.19, 23.73 (C12 CH_2's, Arg CH*C*H_2 and Arg CH_2CH_2NH, multiple overlapping peaks); 14.51 (C12 CH_3). ν_{max} (cm^{-1}, solid): 3287br&w (O-H acid and N-H amide/guanidino stretches); 2921w, 2853w (C-H alkyl stretches); 1728w (C=O acid stretch); 1648m (C=O amide stretch); 1539m (N-H amide bend); 1170br&m, 1045br&m (C-O acid and C-N amide stretches). ESI-MS (m/z) (positive ion mode): Calc. for

$C_{24}H_{45}N_6O_7$ 529.3344; found: 529.3331 (100%, $[M+H]^+$). ESI-MS (m/z) (negative ion mode): Calc. for $C_{24}H_{43}N_6O_7$ 527.3199; found: 527.3197 (100%, $[M-H]^-$).

Protected **G1-RGD$_3$** (see Scheme in main paper). First generation Z-protected 'Newkome-type' dendritic scaffold **(**Z/Newkome-G1/OH, 96 mg, 0.2 mmol, 1 eq) and protected RGD tripepetdie H_2N-Arg(Pbf)-Gly-Asp(O^{t}Bu)-O^{t}Bu (0.52 g, 0.73 mmol, 3.6 eq) were suspended in dry DCM (10 ml), then DIPEA (0.22 ml, 1.22 mmol, 6 eq) was added and the reaction flask cooled over an ice-water bath. T3P (50 wt. % in EtOAc, 0.44 ml, 0.73 mmol, 3.6 eq) was added dropwise over 20 min. The ice-water bath was removed and the reaction mixture was stirred for 22 h at rt. The reaction mixture was diluted with DCM (100 ml), quenched with water (50 ml), and then the organic layer was washed with saturated $NaHCO_3$ (100 ml), 1.33 M $NaHSO_4$ (100 ml), and finally water (100 ml). The organic layer was dried over $MgSO_4$ and filtered before removing the solvent *in vacuo* to produce *Protected* **G1-RGD$_3$** as a crude white solid/oil (~0.5 g, ~96% crude yield) which was purified by column chromatography (SiO_2, 9:1 DCM/MeOH) to produce the product as a white solid (0.4 g, 77%). R_f 0.36 (9:1 DCM/MeOH, UV and cerium stain). $[\alpha]_D$ -2.0 (c = 0.25, $CHCl_3$). M.p: 131.7-137.0°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70-7.68 (br m, N*H* amide × 3, 3H); 7.33-7.28 (m, N*H* amide × 6 and C*H* aromatic × 5, 11H); 6.35 (br s, N*H*$_2$ guanidine × 3, 6H); 6.22 (br s, N*H* guanidine × 3, 3H); 5.50 (br s, N*H* carbamate, 1H); 5.01 (s, C*H*$_2$ benzylic, 2H); 4.65 (dt, Asp α-*H* × 3, *J* = 8.0 Hz and 5.0 Hz, 3H); 4.54 (m, Arg α-*H* × 3, 3H); 4.00 (dd, Gly C*H*^{A} × 3, *J* = 17.0 Hz and 5.5 Hz, 3H); 3.85 (dd, Gly C*H*^{B} × 3, *J* = 17.0 Hz and 5.5 Hz, 3H); 3.68-3.57 (m, CC*H*$_2$O × 3, OC*H*$_2$CH$_2$ × 3, 12H); 3.30-3.15 (m, Arg C*H*$_2$NH × 3, 6H); 2.93 (s, Pbf C*H*$_2$ × 3, 6H); 2.78 (dd, Asp C*H*^{A} × 3, *J* = 17.0 Hz and 5.0 Hz, 3H); 2.68 (dd, Asp C*H*^{B} × 3, *J* = 17.0 Hz and 5.0 Hz, 3H); 2.56 (s, Pbf C*H*$_3$ × 3, 9H); 2.49 (s, Pbf C*H*$_3$ × 3, 9H); 2.47-2.40 (m, CH$_2$C*H*$_2$C(O) × 3, 6H); 2.07 (s, Pbf C*H*$_3$ × 3, 9H); 1.91-1.83 (m, Arg CHC*H*^{A} × 3, 3H); 1.76-1.67 (m, Arg CHC*H*^{B} × 3, 3H); 1.63-1.56 (m, Arg CH$_2$C*H*$_2$CH$_2$ × 3, 6H); 1.44 (s, [(Pbf C*H*$_3$ × 2)] × 3, 18H); 1.41, 1.40 (s, [(^{t}Bu C*H*$_3$ × 6)] × 3, 54H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.08, 172.47, 170.16, 169.99, 169.43 (*C*(O)O^{t}Bu × 2, *C*ONH amide × 3); 158.72, 156.59 (Pbf Ar*C*O, *C*=N guanidine); 138.35 (Pbf aromatic *C*); 136.67 (Benzyl aromatic *C*); 132.96, 132.27 (Pbf aromatic *C*); 128.55, 128.09 (Benzyl aromatic *C*H); 124.63, 117.50 (Pbf aromatic *C*); 86.42 (Pbf CH$_2$*C*(CH$_3$)$_2$O); 82.56, 81.64 (*C*(CH$_3$)$_3$ × 2); 69.38 (C*C*H$_2$O); 67.53 (O*C*H$_2$CH$_2$); 66.28 (*C*H$_2$ benzylic); 59.11 (*C*CH$_2$O); 53.15 (Arg α-*C*H); 49.44 (Asp α-*C*H); 43.28, 42.73, 40.33 (Pbf Ar*C*H$_2$, Arg *C*H$_2$NH, Gly *C*H$_2$); 37.44 (Asp *C*H$_2$); 36.63 (CH$_2$*C*H$_2$C(O)); 29.28 (Arg CH*C*H$_2$ or *C*H$_2$CH$_2$NH); 28.66 (Pbf CH$_2$C(*C*H$_3$)$_2$O); 28.08, 27.92 (C(*C*H$_3$)$_3$ × 2); 25.43 (Arg CH*C*H$_2$ or *C*H$_2$CH$_2$NH); 19.38, 18.03, 12.55

(Pbf Ar*C*H_3 × 3). ν_{max} (cm^{-1}) (solid): 3306*br&w* (N-H amide stretch); 2975*w* (C-H alkyl and arene stretches); 1727*m* (C=O ester stretch); 1648*br&m* (C=O amide stretch); 1543*br&s* (N-H amide bend and C=C arene stretch); 1453*w*, 1367*w* (C-H alkyl bend and S=O stretch); 1243*br&m*, 1151*s*, 1094*s* (C-O ether, C-O ester and C-N amide stretches, C-H arene bends). ESI-MS (m/z): Calc. for $C_{120}H_{185}N_{19}Na_2O_{35}S_3$ 1297.1114; found: 1297.1096 (100%, $[M+2Na]^{2+}$), 2572.2 (11%, $[M+Na]^+$).

G1-RGD$_3$ (see Scheme in main paper)**.** *Protected* **G1-RGD$_3$** (100 mg, 39 μmol) was dissolved in a mixture of TFA, water and triisopropylsilane (TIS) (500 μL, 95:2.5:2.5) and shaken for 2.5 h, after which time TLC indicated that the deprotection reaction was complete. The volatile organics were removed *in vacuo*, then the residue was dissolved in 10% aqueous acetic acid (2 ml) and washed three times with a twofold excess of chloroform to extract the non-polar by-products. The aqueous acetic acid layer was evaporated *in vacuo* and the residue was redissolved in water, shell frozen and lyophilised to yield **G1-RGD$_3$** as a fluffy white solid (72 mg, quantitative yield as TFA salt). R_f 0.00 (9:1 DCM/MeOH, cerium stain). $[\alpha]_D$ -10.8 (c = 0.5, D_2O). M.p: 80.9-88.9°C. ^{1}H NMR (D_2O, 400 MHz) δ 7.26-7.18 (m, C*H* aromatic, 5H); 4.89 (s, CH_2 benzylic, 2H); 4.61 (t, Asp α-*H* × 3, *J* = 6.0 Hz, 3H); 4.14 (dd, Arg α-*H* × 3, *J* = 8.5 Hz and 6.0 Hz, 3H); 3.77 (s, Gly CH_2 × 3, 6H); 3.59-3.39 (m, CCH_2O × 3, OCH_2CH_2 × 3, 12H); 2.99 (t, Arg CH_2NH × 3, *J* = 7.0 Hz, 6H); 2.78 (d, Asp CH_2 × 3, *J* = 5.5 Hz, 6H); 2.45-2.31 (m, CH_2CH_2C(O) × 3, 6H); 1.75-1.39 (m, Arg CHCH_2 × 3, CH_2CH_2CH_2 × 3, 12H). ^{13}C NMR (D_2O, 100 MHz) δ 174.70, 174.64, 174.54, 174.34, 171.16, 157.05 (*C*(O)OH × 2, *C*ONH amide × 3, O*C*(O)NH carbamate × 1); 137.50 (Benzyl aromatic *C*); 129.13, 128.72, 127.94 (Benzyl aromatic *C*H); 69.20, 67.76 (C*C*H_2O, O*C*H_2CH_2, *C*H_2 benzylic, multiple overlapping peaks); 59.44 (*C*CH_2O); 53.89 (Arg α-*C*H); 49.44 (Asp α-*C*H); 42.60, 40.87 (Arg *C*H_2NH, Gly *C*H_2); 36.17, 35.93 (Asp *C*H_2, CH_2*C*H_2C(O)); 28.59, 24.80 (Arg CH*C*H_2, *C*H_2CH_2NH). ν_{max} (cm^{-1}) (solid): 3690*br&w* (O-H acid and N-H amide/guanidino stretches); 3019*br&w* (C-H alkyl and arene stretches); 1735*w* (C=O acid stretch); 1656*m* (C=O amide stretch); 1543*m* (N-H amide bend and C=C arene stretch); 1475*w*, 1420*w* (C-H alkyl bends); 1181*m*, 1137*m*, 1110*m*, 1048*m* (C-O acid, C-O ether and C-N amide stretches, C-H arene bends). ESI-MS (m/z): Calc. for $C_{57}H_{91}N_{19}O_{26}$ 728.8186; found: 728.8169 (100%, $[M+2H]^{2+}$), 1456.4682 (2%, $[M+H]^+$).

Protected **G2-RGD$_9$** (see structure in main paper)**.** Z/Newkome-G2/OH (110 mg, 77 μmol, 1.0 eq) and protected RGD peptide **4** (0.98 g, 1.39 mmol, 18.0 eq) were suspended in dry DCM (10 ml), then DIPEA (0.24 ml, 1.39 mmol, 18.0 eq) was added and the reaction flask cooled over an ice-water bath.

T3P (50 wt. % in EtOAc, 0.82 ml, 1.39 mmol, 18.0 eq) was added dropwise over 10 min. The ice-water bath was removed and the reaction mixture was stirred for 2 days at rt. The reaction mixture was diluted with DCM (100 ml), quenched with water (100 ml), and then the organic layer was washed with saturated $NaHCO_3$ (100 ml), 1.33 M $NaHSO_4$ (100 ml), saturated $NaHCO_3$ (100 ml), 1.33 M $NaHSO_4$ (100 ml), and finally water (100 ml). The organic layer was dried over $MgSO_4$ and filtered before removing the solvent *in vacuo* to produce *Protected* **G2-RGD$_9$** as a crude white solid (~1.2 g) which was purified by column chromatography (SiO_2, 95:5 DCM/MeOH to 9:1 DCM/MeOH) to produce the product as a white solid (0.45 g, 76%). R_f 0.29 (9:1 DCM/MeOH, UV and cerium stain). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (br s, N*H* amide × 9, 9H); 7.54 (br s, N*H* amide × 9, 9H); 7.38 (d, N*H* amide × 9, 9H); 7.30-7.28 (m, C*H* aromatic × 5, 5H); 6.75 (br s, N*H* amide of branching × 3, 3H); 6.38 (br s, NH_2 guanidine × 9, 18H); 6.29 (br s, N*H* guanidine × 9, 9H); 5.81 (br s, N*H* carbamate, 1H); 5.02 (s, CH_2 benzylic, 2H); 4.65 (dt, Asp α-*H* × 9, *J* = 8.0 Hz and 5.0 Hz, 9H); 4.55 (m, Arg α-*H* × 9, 9H); 4.03 (dd, Gly CH^A × 9, *J* = 17.0 Hz and 5.0 Hz, 9H); 3.89 (dd, Gly CH^B × 9, *J* = 17.0 Hz and 5.0 Hz, 9H); 3.71-3.63 (m, CCH_2O × 3 gen. 1, CCH_2O × 9 gen. 2, OCH_2CH$_2$ × 3 gen. 1, OCH_2CH$_2$ × 9 gen. 2, 48H); 3.30-3.15 (m, Arg CH_2NH × 9, 18H); 2.93 (s, Pbf CH_2 × 9, 18H); 2.76 (dd, Asp CH^A × 9, *J* = 17.0 Hz and 5.5 Hz, 9H); 2.68 (dd, Asp CH^B × 9, *J* = 17.0 Hz and 5.5 Hz, 9H); 2.56 (s, Pbf CH_3 × 9, 27H); 2.49 (s, Pbf CH_3 × 9, 27H); 2.47-2.40 (m, CH$_2$CH_2C(O) × 3 gen.1, CH$_2$CH_2C(O) × 9 gen. 2, 24H); 2.07 (s, Pbf CH_3 × 9, 27H); 1.94-1.84 (m, Arg CHCH^A × 9, 9H); 1.79-1.69 (m, Arg CHCH^B × 9, 9H); 1.66-1.54 (m, Arg CH$_2$CH_2CH$_2$ × 9, 18H); 1.44 (s, [(Pbf CH_3 × 2)] × 9, 54H); 1.40 (s, [(^{t}Bu CH_3 × 6)] × 9, 162H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.15, 172.55, 172.43, 170.14, 170.00, 169.45 (*C*(O)O^{t}Bu × 2, *C*ONH amide × 4); 158.68, 156.60 (Pbf Ar*C*O, *C*=N guanidine); 138.31, 133.01, 132.25 (Pbf aromatic *C*); 128.55, 127.96 (Benzyl aromatic *C*H); 124.60, 117.45 (Pbf aromatic *C*); 86.39 (Pbf CH$_2$*C*(CH$_3$)$_2$O); 82.49, 81.57 (*C*(CH$_3$)$_3$ × 2); 69.38 (C*C*H$_2$O, gen. 1 and 2, multiple overlapping peaks); 67.56 (O*C*H$_2$CH$_2$, gen. 1 and 2, multiple overlapping peaks); 60.21 (*C*CH$_2$O gen. 1 and 2, multiple overlapping peaks); 53.23 (Arg α-*C*H, multiple overlapping peaks); 49.45 (Asp α-*C*H, multiple overlapping peaks); 43.28, 42.72, 40.45 (Pbf Ar*C*H$_2$, Arg *C*H$_2$NH, Gly *C*H$_2$, multiple overlapping peaks); 37.45 (Asp *C*H$_2$, multiple overlapping peaks); 36.46 (CH$_2$*C*H$_2$C(O), gen. 1 and 2, multiple overlapping peaks); 29.38 (Arg CH*C*H$_2$ or *C*H$_2$CH$_2$NH, multiple overlapping peaks); 28.66 (Pbf CH$_2$C(*C*H$_3$)$_2$O); 28.07, 27.91 (C(*C*H$_3$)$_3$ × 2); 25.56 (Arg CH*C*H$_2$ or *C*H$_2$CH$_2$NH, multiple overlapping peaks); 19.38, 18.04, 12.54 (Pbf Ar*C*H$_3$ × 3). ESI-MS (m/z): Calc. for $C_{357}H_{563}N_{58}O_{107}S_9$ 2555.9; found: 2555.9 (26%, $[M+3H]^{3+}$), 1916.9 (91%, $[M+4H]^{4+}$), 1534.0 (100%, $[M+5H]^{5+}$).

G2-RGD$_9$ (see structure in main paper). *Protected* **G2-RGD$_9$** (116 mg, 15 μmol) was dissolved in a mixture of TFA, water and triisopropylsilane (TIS) (500 μL, 95:2.5:2.5) and shaken for 4 h, after which time TLC indicated that the deprotection reaction was complete. The volatile organics were removed *in vacuo*, then the residue was dissolved in 10% aqueous acetic acid (2 ml) and washed three times with a twofold excess of chloroform to extract the non-polar by-products. The aqueous acetic acid layer was evaporated *in vacuo* and the residue was redissolved in water, shell frozen and lyophilised to yield **G2-RGD$_9$** as a fluffy white solid (73 mg, 89% as TFA salt). R_f 0.00 (9:1 DCM/MeOH, cerium stain). ^{1}H NMR (D_2O, 400 MHz) δ 7.17-7.09 (m, C*H* aromatic, 5H); 4.87 (s, CH_2 benzylic, 2H); 4.54 (t, Asp α-*H* × 9, *J* = 6.0 Hz, 9H); 4.08 (dd, Arg α-*H* × 9, *J* = 8.5 Hz and 6.0 Hz, 9H); 3.72 (s, Gly CH_2 × 9, 18H); 3.55-3.35 (m, CCH_2O × 12, OCH_2CH$_2$ × 12, 48H); 2.95 (t, Arg CH_2NH × 9, *J* = 7.0 Hz, 18H); 2.71 (d, Asp CH_2 × 9, *J* = 5.5 Hz, 18H); 2.40-2.20 (m, CH$_2$CH_2C(O) × 12, 24H); 1.66-1.57 (m, Arg CHCH^A × 9, 9H); 1.56-1.48 (m, Arg CHCH^B × 9, 9H); 1.47-1.32 (m, CH$_2$CH_2CH$_2$ × 9, 18H). ESI-MS (m/z): Calc. for $C_{168}H_{278}N_{58}O_{80}$ 731.3239; found: 627.3 (48%, $[M+7H]^{7+}$), 731.4926 (100%, $[M+6H]^{6+}$), 877.8 (48%, $[M+5H]^{5+}$), 1097.0 (36%, $[M+4H]^{4+}$), 1462.3 (7%, $[M+3H]^{3+}$).

Compound 9. Fmoc-Gly-OH (1.06 g, 3.57 mmol, 2 eq) and DIPEA (0.62 ml, 3.57 mmol, 2 eq) in dry DCM (25 ml) were added to 2-chlorotrityl chloride resin (1.19 g, 1.00-1.50 mmol/g, 1.19-1.79 mmol) and stirred for 2.25 h. DIPEA (0.4 ml) and MeOH (2 ml) were then added and shaken for 45 minutes to cap the unreacted sites on the resin. The resin was then filtered and washed with DMF (50 ml), DCM (50 ml), MeOH (50 ml), and finally Et_2O (50 ml) before drying in vacuo. A loading of 0.91 mmol of Fmoc-Gly was calculated from the resultant mass of the Resin-O-Gly-Fmoc (1.46 g). To remove the Fmoc-protecting group, a 20% solution of piperidine in DMF was twice added to the resin (50 ml in total) and shaken for 10 minutes each. Filtration of the resin, followed by washing with DMF (100 ml) then DCM (100 ml) until ninhydrin stain of the filtrate showed no visible spot for any residual piperidine or Fmoc-piperidine by-products, yielded Resin-O-Gly-NH_2 (1.22 g) after drying in vacuo. Fmoc-Gly-OH (0.68 g, 2.28 mmol, 2.5 eq with respect to the initial Fmoc-Gly loading), HOBt (0.31 g, 2.28 mmol, 2.5 eq), TBTU (0.73 g, 2.28 mmol, 2.5 eq), and DIPEA (1.1 ml, 6.3 mmol, 7 eq) were dissolved in dry DMF (25 ml) and added to a suspension of the resin in dry DMF (50 ml) and shaken overnight. Filtration of the resin, followed by washing with DMF (100 ml) then DCM (100 ml) before drying *in vacuo*, yielded Resin-O-Gly-Gly-Fmoc (1.59 g). The Fmoc-protecting group was removed as described above to yield Resin-O-Gly-Gly-NH_2. Fmoc-Gly-OH was coupled to the Fmoc deprotected resin as described above to yield Resin-O-Gly-Gly-Gly-Fmoc (1.58 g). The Fmoc-

protecting group was removed as described above to yield Resin-O-Gly-Gly-Gly-NH_2 (1.32 g). 2-[2-(2-methoxyethoxy)-ethoxy)acetic acid (0.41 g, 2.28 mmol, 2.5 eq with respect to the initial Fmoc-Gly loading), HOBt (0.31 g, 2.28 mmol, 2.5 eq), TBTU (0.73 g, 2.28 mmol, 2.5 eq), and DIPEA (1.1 ml, 6.3 mmol, 7 eq) were dissolved in dry DMF (25 ml) and added to a suspension of the resin in dry DMF (50 ml) and shaken overnight. A Kaiser Test performed on a small sample of beads taken from the reaction, to monitor the coupling, indicated that some free amines were still present on the beads. 2-[2-(2-methoxyethoxy)-ethoxy)acetic acid (0.41 g, 2.28 mmol, 2.5 eq with respect to the initial Fmoc-Gly loading), HOBt (0.31 g, 2.28 mmol, 2.5 eq), TBTU (0.73 g, 2.28 mmol, 2.5 eq), and DIPEA (1.1 ml, 6.3 mmol, 7 eq) were again dissolved in dry DMF (25 ml) and added to the reaction mixture and shaken for 6 days to try and drive the reaction to completion. Subsequently, a Kaiser Test was negative for free amines on the beads. Filtration of the resin, followed by washing with DMF (100 ml) then DCM (100 ml) before drying *in vacuo*, yielded Resin-O-Gly-Gly-Gly-PEG (1.50 g). The resin (1.50 g) was treated twice with TFA/water (95:5, v/v) (40 ml in total) for 10 minutes each to cleave the peptide from the resin. The resin was further shaken with DCM (3 × 20 ml) for 10 min each, then washed with DCM (100 ml) and all fractions were subsequently collected together and the solvent removed *in vacuo*. The crude peptide was obtained as a brown oil (~400 mg). Silica column purification (90:10, to 80:20, to 50:50 DCM/MeOH yielded a pale brown hygroscopic foam (320 mg, 0.9 mmol, near quantitative yield based on the initial Fmoc-Gly loading). The product was then dissolved in a ^{t}BuOH/water mixture, filtered over a 0.2 μm PTFE membrane filter, shell-frozen and lyophilised to yield a pale yellow foam. R_f = 0.17 (50:50 DCM/MeOH, $KMnO_4$ stain). ^{1}H NMR (CD_3OD, 400 MHz) 4.08, 3.98, 3.93, 3.86 (4 × s, 3 × Gly CH_2 and 1 × OCH_2C(O), 4 × 2H); 3.75-3.72 (m, PEG CH_2, 2H); 3.70-3.64 (m, 2 × PEG CH_2, 4H); 3.57-3.55 (m, PEG CH_2, 2H); 3.36 (s, CH_3O, 3H). ESI-MS (m/z) (positive ion mode): Calc. for $C_{13}H_{24}N_3O_8$ 350.1558; found: 350.1555 (97%, $[M+H]^+$); 372.1368 (100%, $[M+Na]^+$). ESI-MS (m/z) (negative ion mode): Calc. for 348.1412; found: 348.1418 (100%, $[M-H]^-$).

Compound 10. Fmoc-Gly-OH (1.08 g, 3.62 mmol, 2 eq) and DIPEA (0.63 ml, 3.62 mmol, 2 eq) in dry DCM (20 ml) were added to 2-chlorotrityl chloride resin (1.17 g, 1.55 mmol g^{-1}, 1.81 mmol) and stirred for 2.5 h. DIPEA (2 ml) and MeOH (10 ml) were then added and stirred for 30 minutes to cap the unreacted sites on the resin. The resin was then filtered and washed with DMF (50 ml), DCM (50 ml), MeOH (50 ml), and finally Et_2O (50 ml) before drying in vacuo. A loading of 1.75 mmol of Fmoc-Gly was calculated from the resultant mass of the Resin-O-Gly-Fmoc (1.69 g). To remove the Fmoc-protecting group, a 20 % solution of piperidine in DMF was twice added to the resin (25 ml in

total) and stirred for 20 minutes each. Filtration of the resin, followed by washing with DMF (200 ml) then DCM (200 ml) until ninhydrin stain of the filtrate showed no visible spot for any residual piperidine or Fmoc-piperidine by-products, yielded Resin-O-Gly-NH_2 (1.48 g). Fmoc-Arg(Pbf)-OH (2.27 g, 3.5 mmol, 2 eq wrt the initial Fmoc-Gly loading), HOBt (0.47 g, 3.5 mmol, 2 eq), TBTU (1.12 g, 3.5 mmol, 2 eq), and DIPEA (1.85 ml, 10.5 mmol, 6 eq) were added to the resin in dry DMF (25 ml) and stirred for 1.5 h. Filtration of the resin, followed by washing with DMF (200 ml) then DCM (200 ml) before drying in vacuo, yielded Resin-O-Gly-Arg(Pbf)-Fmoc (2.19 g). The Fmoc-protecting group was removed as described above to yield Resin-O-Gly-Arg(Pbf)-NH_2 (2.16 g). Fmoc-Lys(Z)-OH (1.76 g, 3.5 mmol, 2 eq wrt the initial Fmoc-Gly loading), HOBt (0.47 g, 3.5 mmol, 2 eq), TBTU (1.12 g, 3.5 mmol, 2 eq), and DIPEA (1.85 ml, 10.5 mmol, 6 eq) were added to the resin in dry DMF (25 ml) and stirred for 1.5 h. Filtration of the resin, followed by washing with DMF (200 ml) then DCM (200 ml) before drying in vacuo, yielded Resin-O-Gly-Arg(Pbf)-Lys(Z)-Fmoc (2.47 g). The Fmoc-protecting group was removed as described above to yield Resin-O-Gly-Arg(Pbf)-Lys(Z)-NH_2 (2.28 g). Fmoc-D-Phe-OH (1.36 g, 3.5 mmol, 2 eq wrt the initial Fmoc-Gly loading), HOBt (0.47 g, 3.5 mmol, 2 eq), TBTU (1.12 g, 3.5 mmol, 2 eq), and DIPEA (1.85 ml, 10.5 mmol, 6 eq) were added to the resin in dry DMF (25 ml) and stirred for 1.5 h. Filtration of the resin, followed by washing with DMF (200 ml) then DCM (200 ml) before drying in vacuo, yielded Resin-O-Gly-Arg(Pbf)-Lys(Z)-D-Phe-Fmoc (2.68 g). The Fmoc-protecting group was removed as described above to yield Resin-O-Gly-Arg(Pbf)-Lys(Z)-D-Phe-NH_2 (2.45 g). Fmoc-Asp(OtBu)-OH (1.44 g, 3.5 mmol, 2 eq wrt the initial Fmoc-Gly loading), HOBt (0.47 g, 3.5 mmol, 2 eq), TBTU (1.12 g, 3.5 mmol, 2 eq), and DIPEA (1.85 ml, 10.5 mmol, 6 eq) were added to the resin in dry DMF (25 ml) and stirred for 1.5 h. Filtration of the resin, followed by washing with DMF (200 ml) then DCM (200 ml) before drying in vacuo, yielded Resin-O-Gly-Arg(Pbf)-Lys(Z)-D-Phe-Asp(OtBu)-Fmoc (2.88 g). The Fmoc-protecting group was removed as described above to yield Resin-O-Gly-Arg(Pbf)-Lys(Z)-D-Phe-Asp(OtBu)-NH_2 (2.65 g). The resin (2.65 g) was treated twice with HFIP (1,1,1,3,3,3-hexafluoro-2-propanol)/DCM (1:4, v/v) (75 ml in total) for 30 minutes each to cleave the protected peptide from the resin. The resin was further washed with DCM (3 × 20 ml) for 10 min each and all fractions were subsequently collected together and the solvent removed in vacuo. The crude protected linear peptide was obtained as a brown solid (1.61 g, 86% crude yield as calculated from the initial Fmoc-Gly loading). Cyclisation of this compound was carried out without further purification of the protected linear peptide. The crude linear protected peptide (1.61 g, 1.51 mmol) dissolved in dry DCM (20 ml) was slowly added over 1 h to a solution of T_3P (50% w/w in EtOAc, 4.5 ml, 7.55 mmol, 5 eq), TEA (4.2 ml, 30.2 mmol, 20 eq) and DMAP (20 mg, 0.15 mmol, 0.1 eq) in dry DCM (500 ml), cooled to 0^oC in an ice-water bath. The

highly diluted solution subsequently turned from pale yellow to bright orange. The reaction was allowed to warm to room temperature and stirred for 2 days. Solvent was removed *in vacuo* to yield a crude, dark brown oil (6 g). Silica column chromatography (1:10, MeOH/EtOAc) was attempted on the crude material but the compound aggregated/crystallised out at the top of the column. The column was flushed with 100% MeOH and 3 g of the crude material was recovered. ESI-MS showed the molecular ion peaks at 1046.5 *m/z* $[M+H]^+$ and 1068.5 *m/z* $[M+Na]^+$ but also a peak at 660 *m/z* which was reasoned to be a short, linear oligopeptide impurity. Purification by gel filtration chromatography (Sephadex LH-20, DMF) was carried out to remove the impurity and this was evidenced by the reduced significance, or even disappearance on some attempts, of the peak at 660 *m/z* in the ESI MS. Compound **10** was recovered as a pale yellow solid (0.74 g, 40% yield based on the initial Fmoc-Gly loading). R_f 0.64 (1:10 MeOH/EtOAc, UV and cerium stain). $[\alpha]_D$ = -9.8 (c = 0.5, 1:1 $CHCl_3/CH_3OH$). M.p: decomposes at 198.7°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.33 (br s, N*H* amide, 1H); 8.04 (d, N*H* amide, *J* = 7.0 Hz, 1H); 7.95 (d, N*H* amide, *J* = 8.5 Hz, 1H); 7.91 (d, N*H* amide, *J* = 9.0 Hz, 1H); 7.50 (d, N*H* amide, *J* = 8.0 Hz, 1H); 7.38-7.28 (m, C*H* aromatic × 5, 5H); 7.26-7.15 (m, C*H* aromatic × 5, 5H); 7.08 (br s, N*H* carbamate, 1H); 6.70 (br s, N*H* guanidine, 1H); 6.46 (br s, NH_2 guanidine, 2H); 5.02 (s, CH_2 benzylic, 2H); 4.66-4.60 (m, α-*H*, 1H); 4.52-4.46 (m, α-*H*, 1H); 4.15-4.10 (m, α-*H*, 1H); 4.08-4.02 (m, Gly CH^A, 1H); 3.96-3.91 (m, α-*H*, 1H); 3.23-3.20 (Gly CH^B, 1H, and Arg CH_2NH, 2H, obscured by the water peak); 3.05-3.01 (m, Lys CH_2NH, 2H); 2.95 (s, Pbf CH_2, 2H); 2.93, 2.82 (dd × 2, Phe CH_2, *J* = 13.0 Hz and 6.5 Hz, 2H); 2.64, 2.36 (dd × 2, Asp CH_2, *J* = 15.5 Hz and 8.5 Hz, 2H); 2.48 (s, Pbf CH_3, 3H); 2.42 (s, Pbf CH_3, 3H); 2.01 (s, Pbf CH_3, 3H); 1.74-1.65, 1.60-1.52 (m × 2, Arg CHCH_2, 2H); 1.49-1.26 (m, Lys CHCH_2CH$_2$CH$_2$CH$_2$NH, Lys CHCH$_2$CH$_2$CH_2CH$_2$NH, 4H); 1.41 (s, Pbf CH_3 × 2, 6H); 1.37 (s, ^{t}Bu CH_3 × 3, 9H); 1.09-0.96 (m, Lys CHCH$_2$CH_2CH$_2$CH$_2$NH, 2H). ^{13}C NMR ($CDCl_3/CD_3OD$, 100 MHz) δ 174.03, 173.31, 172.99, 171.93, 171.40, 170.62, 159.35, 158.19, 157.23 (*C*(O)O^{t}Bu, *C*ONH amide × 5, *C*ONH carbamate, Pbf Ar*C*O, *C*=N guanidine); 138.93, 137.46, 137.03, 133.56, 132.91, 125.37, 118.09 (aromatic quaternary *C*'s for: Pbf × 5, Z group, phenylalanine); 129.75, 129.15, 129.02, 128.56, 128.35, 127.53 (aromatic *C*H's for: phenylalanine × 3, Z group × 3); 87.13 (Pbf CH$_2$*C*(CH$_3$)$_2$O); 82.12 (*C*(CH$_3$)$_3$); 67.07 (*C*H$_2$ benzyl Z group); 55.95, 55.68, 53.27, 50.33 (α-*C*H × 4); 44.41, 43.69, 40.84, 37.75, 37.09, (Gly *C*H$_2$, Pbf *C*H$_2$, benzyl *C*H$_2$ (phenylalanine), Arg *C*H$_2$NH, Lys *C*H$_2$NH, Asp *C*H$_2$, multiple overlapping peaks); 31.35, 29.60, 28.58, 26.38, 23.55 (Arg CH*C*H$_2$, Arg *C*H$_2$CH$_2$NH, Lys CH*C*H$_2$, Lys CH$_2$*C*H$_2$CH$_2$NH, Lys *C*H$_2$CH$_2$CH$_2$NH); 28.75 (Pbf CH$_2$C(*C*H$_3$)$_2$O); 28.20 (C(*C*H$_3$)$_3$ × 2); 19.55, 18.29, 12.62 (Pbf Ar*C*H$_3$ × 3). ν_{max} (cm^{-1}, solid): 3303*br&w* (N-H stretch); 2973*br&w*, 2932*br&w* (C-H alkyl and arene stretches); 1721*w*, 1678*m*,

1633*s* (C=O ester and C=O amide stretches); 1542*br&m* (N-H amide bends and C=C arene stretches); 1455*br&m*, 1368*w* (C-H alkyl bends and S=O stretches); 1244*br&m*, 1154*m*, 1091*br&m* (C-O ester and C-N amide stretches, C-H arene bends). ESI-MS (m/z): Calc. for $C_{52}H_{72}N_9O_{12}S$ 1046.5016; found: 1046.5018 (100%, $[M+H]^+$); 1068.5 (19%, $[M+Na]^+$).

Compound 11. Compound **10** (200 mg, 0.19 mmol) was dissolved in DMF followed by the addition of Pd/C (40 mg, 20%). The flask was evacuated from air, purged with H_2 and stirred for 24 h. The catalyst was filtered off over celite and carefully washed with DMF. The filtrate, still black in appearance, was passed through a syringe filter (0.45 μm, PTFE membrane) to try and remove the residual catalyst. The solvent was removed *in vacuo* to yield the product **11** as a black solid as some catalyst still remained due to the fine dispersion of Pd/C in DMF (160 mg, ~92%). R_f 0.00 (9:1 DCM/MeOH, UV and cerium stain). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.42-8.39 (m, N*H* amide, 1H); 8.11-8.03 (m, N*H* amide, 3H); 7.72 (d, N*H* amide, *J* = 7.5 Hz, 1H); 7.27-7.15 (m, C*H* aromatic × 5, 5H); 6.73 (br s, N*H* guanidine, 1H); 6.39 (br s, NH_2 guanidine, 2H); 4.65-4.59 (m, α-*H*, 1H); 4.50-4.44 (m, α-*H*, 1H); 4.16-4.10 (m, α-*H*, 1H); 4.07-4.01 (m, Gly CH^A, 1H); 3.98-3.93 (m, α-*H*, 1H); 3.45-3.21 (Gly CH^B, 1H, and Arg CH_2NH, 2H, obscured by the water peak); 3.05-3.29 (m, Lys CH_2NH, 2H); 2.96 (s, Pbf CH_2, 2H); 2.94, 2.79 (dd × 2, Phe CH_2, *J* = 13.0 Hz and 6.5 Hz, 2H); 2.63, 2.34 (dd × 2, Asp CH_2, *J* = 15.5 Hz and 8.5 Hz, 2H); 2.46 (s, Pbf CH_3, 3H); 2.41 (s, Pbf CH_3, 3H); 2.00 (s, Pbf CH_3, 3H); 1.74-1.63, 1.59-1.48 (m × 2, Arg CHCH_2, 2H); 1.47-1.19 (m, Lys CHCH_2CH$_2$CH$_2$CH$_2$NH, Lys CHCH$_2$CH$_2$CH_2CH$_2$NH, 4H); 1.40 (s, Pbf CH_3 × 2, 6H); 1.35 (s, ^{t}Bu CH_3 × 3, 9H); 1.09-0.95 (m, Lys CHCH$_2$CH_2CH$_2$CH$_2$NH, 2H). ESI-MS (m/z): Calc. for $C_{44}H_{65}N_9NaO_{10}S$ 934.4467; found: 934.4468 (100%, $[M+Na]^+$); 912.46 (68%, $[M+H]^+$).

Compound 12. Compound **11** (20 mg, 22 μmol, 1.2 eq) was dissolved in dry DMF (400 μl), then TEA (25 μl, 0.18 mmol, 10 eq) followed by 5(6)-carboxyfluorescein *N*-hydroxysuccinimide ester (9 mg, 18 μmol) were added. Additional dry DMF (600 μl) was used to wash the neck of the flask. The reaction was stirred at room temperature for 18 h. Solvent was removed *in vacuo* yielding a crude tacky black oil/solid with a yellow/green pigmentation. This residue was readily soluble in methanol and seemingly crashed out a black solid which was deemed to be the residual Pd/C catalyst from the previous hydrogenation step, with possible recrystallisation of the excess c[R(Pbf)GD(O^{t}Bu)fK] starting material also, which was known to be insoluble in methanol from previous reactions. The solution was filtered over cotton wool and the filtrate evaporated to yield a yellow/orange solid (~28 mg). Purification by gel filtration chromatography (Sephadex LH-20, DMF) yielded **12** as a yellow

solid (20 mg, 83%). One spot by TLC: R_f 0.67 (8:2 DCM/MeOH, UV and cerium stain). ESI-MS (m/z) (positive ion mode): Calc. for $C_{65}H_{77}N_9O_{16}S$ 635.7599; found: 635.7643 (100%, $[M+2H]^{2+}$); 1270.3665 (1%, $[M+H]^+$). ESI-MS (m/z) (negative ion mode): 1268.5 (82%, $[M-H]^-$), 1314.5 (100%, $[M-H+HCO_2H]^-$).

Compound 13. Compound **12** (16 mg, 12.6 μmol) was dissolved in a mixture of TFA, water and TIS (500 μl, 95:2.5:2.5) and shaken for 4 h, after which time TLC indicated that the deprotection reaction was complete. The volatiles were removed *in vacuo*, then the residue was dissolved in the minimum amount of methanol and recrystallised from cold Et_2O. The yellow solid was filtered over a cotton wool plug, washed with the minimum amount of cold Et_2O then flushed through the cotton wool plug using methanol to re-dissolve. The solvent was removed *in vacuo* to yield the product **13** as a yellow solid (10 mg, 83%). One spot by TLC: R_f 0.00 (9:1 DCM/MeOH, UV and cerium stain). ESI-MS (m/z) (positive ion mode): Calc. for $C_{48}H_{52}N_9O_{13}$ 962.3679; found: 962.3663 (100%, $[M+H]^+$). ESI-MS (m/z) (negative ion mode): 960.4 (100%, $[M-H]^-$); 982.3 (15%, $[M-2H+Na]^-$).

3. Fluorescence Polarisation (FP) Assay

The FP competition experiment was adapted from the method in reference [2]. To demonstrate that the probe **13** could bind integrin and produce a FP response, 10 nM of the probe was assayed as a function of increasing concentration of integrin. When no integrin was present the FP signal was around 35 mP (milli polarisation units). This is the background signal from intrinsic polarisation of the probe. This increased to over 100 mP when the concentration of integrin was more than 400 nM. This was envisaged as more integrin is available for binding to the probe and hence more [probe:protein] complex is formed. The calibration curve provides evidence that **13** binds to integrin $\alpha_v\beta_3$. The binding was found to be fast; incubating the mixed samples for longer than 5 min did not have any significant effect on the FP signal (data not shown).

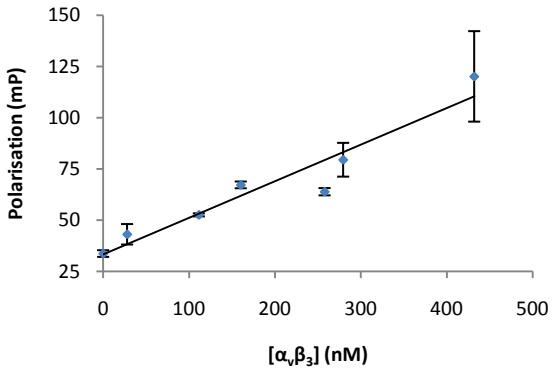


Fig. S1. Normalisation of FP Assay.

For the competition assay, a solution of 187 μM **5(6)-FL-c[RGDfK]** in PBS buffer (0.01 M phosphate, pH 7.4, 0.138 M NaCl, 0.0027 M KCl) was diluted with Tris buffer (50 mM TRIS, pH 7.4, 1 mM $CaCl_2$, 10 μM $MnCl_2$, 1 mM $MgCl_2$, 100 mM NaCl) to give a 100 nM stock. The assay mixture (200 μl in a 100 μl volume microcuvette) was composed of 280 nM integrin $\alpha_v\beta_3$ (13.25 μg) and 10 nM **13** in TRIS buffer. The cuvette was incubated at 29°C for 5 min and then the single-point fluorescence polarisation was obtained using λ_{ex} = 485 nm, λ_{em} = 510 nm. **5(6)-FL-c[RGDfK]** alone (10 nM) served as control and all subsequent data with the protein present was normalised to 100 mP units using this value. All data points (Fig. S1) are presented as mean values ± standard deviations from at least 5 independent scans.Competition experiments were performed with the synthetic ligands **G1-RGD$_3$**, **G2-RGD$_9$**, **PEG-RGD** and **C12-RGD**, with **PEG-GGG** and **SDS** serving as negative controls. Stocks of these compounds were made in PBS and diluted in the TRIS assay buffer to the desired concentration in the final stock titrant (100 μl) which also contained 280 nM integrin $\alpha_v\beta_3$ (6.625 μg)

and 10 nM **5(6)-FL-c[RGDfK]**, then incubated at 29°C in a water bath for at least 5 min before carrying out the titration experiment. The titrant was added to the assay mixture in microlitre aliquots, the cuvette shaken and incubated at 29°C for at least 5 min, and then the single-point FP value recorded, while incubation of the stock titrant was resumed at 29°C in between titrations. The mP values were plotted against ligand concentration in Excel and the effective concentration at which 50% displacement of the probe **5(6)-FL-c[RGDfK]**'s binding to integrin $\alpha_v\beta_3$ was achieved (EC_{50}) was extrapolated at 50 mP units from the normalised data. As stated in the Results and Discussion, this methodology is robust for the direct comparison of structurally related ligands, and avoids the use of radiolabel assays.

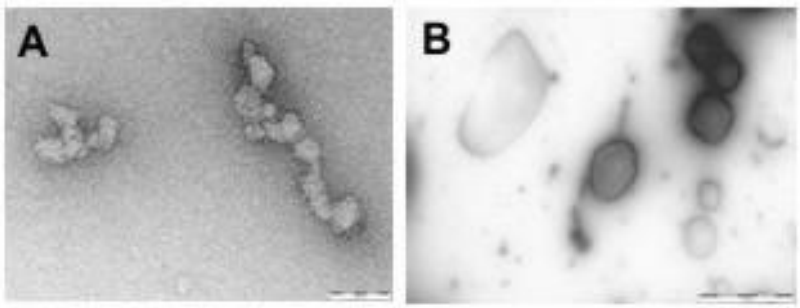
4. Nile Red Assay

The Nile Red encapsulation experiment was adapted from the methods in reference [3]. A 2.5 mM Nile Red (technical grade, Sigma) stock solution was made in EtOH and diluted 1000-fold in the surfactant system (i.e. 1 μL added to a 1 ml surfactant assay volume). A 1 mM **C12-RGD** stock solution was made in PBS buffer. Aliquots of the stock were taken and diluted to the desired concentration to make up a 1 ml assay volume in PBS buffer. Nile Red (1 μL) was added with swirling and the fluorescence emission measured immediately after mixing. Nile Red fluorescence was measured at room temperature using an excitation wavelength of 550 nm. Fluorescence emission was monitored from 550 to 700 nm at 1 nm intervals. Data are presented as mean values from 3 independent experiments (Fig. 2A). The critical aggregation concentration (CAC) was calculated from plotting the absorption of Nile Red at 635 nm against the log of the surfactant concentration (Fig. 2B), setting the equations from the two trendlines as equal to one another and solving for x at the turning point. As x is log concentration, 10^x yields the CAC in mol dm^{-3}. The same assay was applied to **G1-RGD$_3$**, **G2-RGD$_9$** and **PEG-RGD**, but these compounds did not exhibit any aggregation behavior at concentrations <1 mM.

5. TEM Images

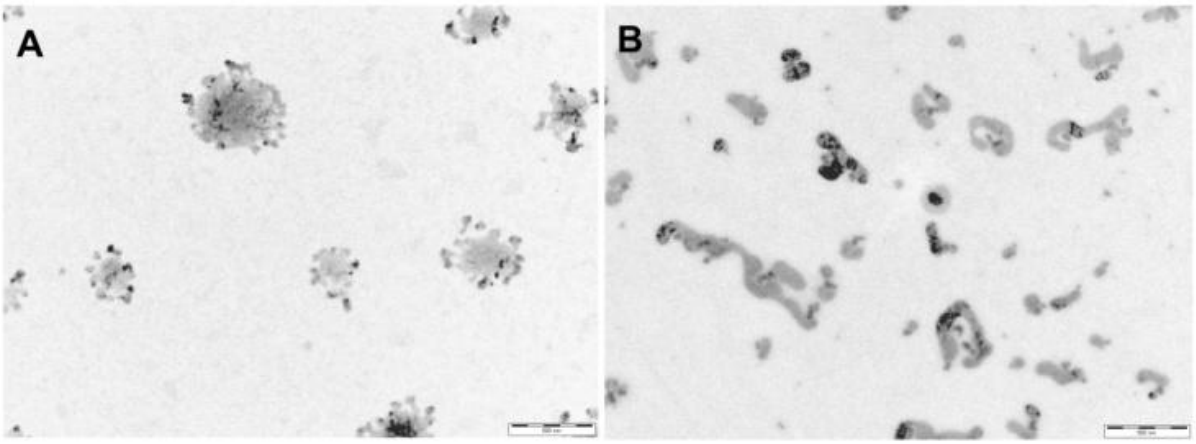
Samples were deposited onto a standard copper grid with Formvar and a carbon support film, allowed to equilibrate for several minutes before excess liquid was wicked off with filter paper. The grids were then washed with a few drops of deionized water to wash off residual salt crystals in the buffers, excess liquid was wicked off with filter paper, the grids were stained with uranyl acetate (1% in water, pH 4.5) before excess stain was wicked off with filter paper (N.B. no stain used for image in Fig. 6), then allowed to dry for 10 minutes. Imaging was performed immediately afterwards.

Images of Assemblies formed by C12-RGD



TEM images of assemblies formed by **C12-RGD** in TRIS buffer at: A) 1 mM, scale bar = 50 nm, and B) 400 μM, scale bar = 2 μm.

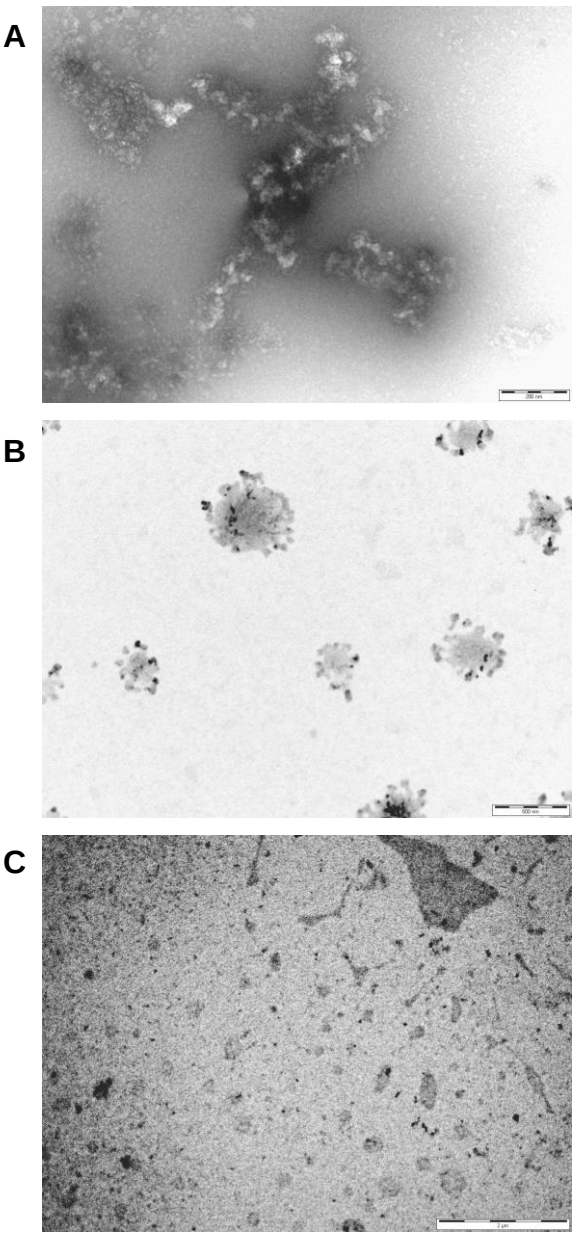
Imaging of C12-RGD with Triton/Integrin



TEM images of: A, integrin-triton aggregates, and B, integrin-triton assemblies in the presence of **C12-RGD**.Scale bar = 500 nm

Imaging of SDS Interactions with Triton/Integrin

SDS causes disruption of the assemblies of integrin, as is evident from image C – however, there is no binding between SDS and integrin itself, as shown in the FD assay data in the main paper.



TEM images of A) 1mM sodiumdodecylsulfate (SDS) in PBS buffer, scale bar = 200 nm; B) integrin-triton aggregates at assay concentration (66.25 ng/µl) in TRIS buffer, scale bar = 500 nm; C) integrin-triton in the presence of SDS at the end point of the FP assay, scale bar = 2 µm.

6. References

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