Electronic Supplementary Information

A Hydrazide-Anchored Dendron Scaffold for Chemoselective Ligation Strategies†

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1. General Experimental

All reagents and solvents were commercially available and used without further purification unless otherwise specified. NMR spectra were recorded on Bruker Avance III 400 MHz or 500 MHz NMR spectrometers. Data are expressed in parts per million downfield from SiMe$_4$ as an internal standard or relative to residual, non-deuterated solvent. J values are given in Hz. IR spectra were measured on a Perkin Elmer Spectrum RXI FT-IR spectrophotometer and absorptions reported as cm$^{-1}$. Mass spectra were obtained using Bruker micrOTOF spectrometers using electrospray ionization.

2. Chemical Synthesis

**Synthesis of side chain-protected RGD 1**

Synthesis was performed manually on 2-chlorotrityl polystyrene resin (1.00 g, 1.40 mmol). Loading of the resin was achieved by treating with a solution of Fmoc-Asp(OtBu)-OH (1.16 g, 2.80 mmol) and DIPEA (0.96 mL, 5.60 mmol) in CH$_2$Cl$_2$ for 90 min. Fmoc deprotection was achieved via treatment with piperdine/DMF (1:4 v/v) for 4 x 3 min. Peptide couplings were performed using Fmoc acid (2 equiv), PyBOP (2.0 equiv) and DIPEA (4 equiv) in DMF for 90 min, except for the coupling of Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH (2 equiv), PyBOP (2.0 equiv) and DIPEA (4 equiv) in DMF for 2 x 90 min were used. Solid phase reactions were monitored by use of the qualitative Kaiser test for the detection of primary amines. After coupling of Fmoc-Arg(Pbf)-OH, the Fmoc group was removed and cleavage from the resin was then effected by treatment with acetic acid/trifluoroethanol/CH$_2$Cl$_2$ (1:1:8 v/v/v) for 3 hr. The cleavage solution was concentrated *in vacuo* to yield the desired peptide 1 with side chain protecting groups intact (0.69 g, 1.05 mmol, 75% yield based on a 1.40 mmol starting resin). IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$ = 3521, 3029, 2927, 1783, 1697, 1452; $\delta_{\text{H}}$(CH$_3$OD) 1.48 (9 H, s, (CH$_3$)$_3$), 1.52 (6 H, s, 2 x CH$_3$), 1.63-1.77 (2 H, m, CH$_2$), 1.83-1.93 (1 H, m, CH$_2$), 1.99-2.08 (1 H, m, CH$_2$), 2.15 (3 H, s, CH$_3$), 2.52-2.58 (4 H, t and dd, CH$_3$ and CH), 2.65 (3 H, s, CH$_3$), 2.80-2.86 (1 H, dd, $J_2 = 4.8$ Hz, $J_3 = 16.3$ Hz, CH), 3.06 (2 H, s, CH$_2$), 3.14-3.21 (1 H, m, CH), 3.77-3.82 (2 H, m, $\alpha$-CH$_2$), 4.17-4.21 (1 H, m, $\alpha$-CH), 4.56-4.59 (1 H, t, $J = 4.6$ Hz, $\alpha$-CH); $\delta_{\text{C}}$(CH$_3$OD) 12.6 (CH$_3$), 18.5 (CH$_3$), 19.6 (CH$_3$), 21.4 (CH$_2$), 28.4 (C(CH$_3$)$_3$), 28.7 (2 x CH$_3$), 31.7 (CH$_2$), 39.4 (CH$_2$), 43.6
Synthesis of side chain-protected RGE 2

Synthesis was performed manually on 2-chlorotrityl polystyrene resin (1.00 g, 1.40 mmol). Loading of the resin was achieved by treating with a solution of Fmoc-Glu(OtBu)-OH (1.19 g, 2.80 mmol) and DIPEA (0.96 mL, 5.60 mmol) in CH₂Cl₂ for 90 min. Fmoc deprotection was achieved via treatment with piperidine/DMF (1:4 v/v) for 4 x 3 min. Peptide couplings were performed using Fmoc acid (2 equiv), PyBOP (2.0 equiv) and DIPEA (4 equiv) in DMF for 90 min, except for the coupling of Fmoc-Arg(Pbf)-OH, where Fmoc-Arg(Pbf)-OH (2 equiv), PyBOP (2.0 equiv) and DIPEA (4 equiv) in DMF for 2 x 90 min were used. Solid phase reactions were monitored by use of the qualitative Kaiser test for the detection of primary amines. After coupling of Fmoc-Arg(Pbf)-OH, the Fmoc group was removed and cleavage from the resin was then effected by treatment with acetic acid/trifluoroethanol/CH₂Cl₂ (1:1:8 v/v/v) for 3 hr. The cleavage solution was concentrated in vacuo to yield the desired peptide 2 with side chain protecting groups intact (0.58 g, 0.87 mmol, 62% yield based on a 1.40 mmol starting resin). IR (KBr) νmax/cm⁻¹ = 3521, 3029, 2927, 1783, 1697, 1452; δH(CH₃OD) 1.51 (9 H, s, (CH₃)₃), 1.53 (6 H, s, 2 x CH₃), 1.63-1.68 (4 H, m 2 x CH₂), 1.74-2.15 (2 H, m, CH₂), 2.15 (CH₃), 2.19-2.22 (2 H, m CH₂), 2.37-2.42 (2 H, m CH₂), 2.58 (CH₃), 2.64 (CH₃), 3.19 (2 H, s, CH₂), 3.20-3.27 (2 H, m CH₂), 3.96 (2 H, s, α-CH₂), 4.43-4.47 (1 H, t, J = 6.6 Hz, α-CH), 4.50-4.56 (1 H, s, α-CH); δC(CH₃OD) 12.5 (CH₃), 18.4 (CH₃), 19.6 (CH₃), 27.9 (CH₂), 28.4 (C(CH₃)₃), 28.7 (2 x CH₃), 30.0 (CH₂), 32.5 (CH₂), 43.3 (CH₂), 43.9 (α-CH₂), 52.9 (α-CH), 53.3 (α-CH), 79.5 (C(CH₃)₃), 81.8 (C), 163.8, 171.5, 173.8, 174.8 (all C=O); HRMS (ESI⁺): found 669.3283 ([M+H]+), C₃₀H₄₀N₆O₄S ([M+H]+) requires 669.3282.
Synthesis of Boc-protected hydrazide NHS ester 3

Protected hydrazide NHS ester 3 was synthesised as described in the literature.\textsuperscript{1} Spectroscopic data were consistent with literature values.

Synthesis of protected monomeric RGD hydrazide 4

Protected RGD peptide 1 (0.339 g, 0.518 mmol) in DMF (4 mL) was added to NHS ester 3 (0.100 g, 0.249 mmol) and DMAP (0.006 g, 0.050 mmol) and the solution stirred for 72 hr at room temperature. DMF was removed under reduced pressure and the crude product redissolved in CH$_2$Cl$_2$ (10 mL). Brine was added and the aqueous solution was extracted with CH$_2$Cl$_2$ (3 x 10 mL). The organic extracts were combined and dried over MgSO$_4$. The solution was evaporated to dryness and the residue purified by column chromatography using silica gel as absorbent with CH$_2$Cl$_2$/MeOH (8:2 v/v) with 0.1% TFA as eluent to yield 4 as a yellow oil (0.105 g, 45%). IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$ = 3512, 3367, 2247, 1681, 1459, 1204; $\delta_H$(CH$_3$OD) 1.51-1.52 (24 H, m, 2 x CH$_3$, and 2 x C(CH$_3$)$_3$), 1.66-1.76 (4 H, m, 2 x CH$_2$), 1.89-2.05 (2 H, m, CH$_2$), 2.15 (3 H, s, CH$_3$), 2.55-2.58 (5 H, m, CH$_3$ and CH$_2$), 2.62 (3 H, s, CH$_3$), 2.86-2.88 (2 H, m, CH$_2$), 3.04-3.08 (2 H, m, CH$_2$), 3.28-3.35 (2 H, m, CH$_2$), 3.37-3.41 (2 H, m, CH$_2$), 3.97-4.35 (3 H, m, 3 x $\alpha$-CH), 4.71-4.77 (1 H, m, $\alpha$-CH); $\delta_C$(CH$_3$OD) 12.5 (CH$_3$), 18.3 (CH$_3$), 19.6 (CH$_3$), 28.2 (CH$_3$), 28.6 (CH$_3$), 28.7 (CH$_3$), 30.0 (CH$_2$), 31.8 (CH$_2$), 37.0 (CH$_2$), 43.2 (CH$_2$), 43.4 (CH$_2$), 43.9 (CH$_3$), 51.0 (CH), 54.1 (CH), 82.1 (C(CH$_3$)$_3$), 83.6 (C(CH$_3$)$_3$), 88.1 (C), 115.9 (Ar-C), 118.8 (Ar-C), 157.7, 160.9, 161.3, 170.7, 170.9, 171.3, 171.5, 174.1, 174.4, 174.7 (all C=O); HRMS (ESI$^+$): found 962.4260 ([M+Na]$^+$), C$_{41}$H$_{63}$N$_9$O$_{14}$SNa ([M+Na]$^+$) requires 962.4269.

Synthesis of monomeric RGD hydrazide 5

Protected monomer 4 (0.050 g, 0.053 mmol) was dissolved in TFA (0.50 mL), triisopropylsilane (TIS) (0.025 mL) and water (0.025 mL) was added. The solution
was stirred for 2 hr at room temperature. Excess TFA was removed under reduced pressure and the crude product redissolved in 10% acetic acid (10 mL). The aqueous solution was extracted with chloroform (3 x 10 mL) and lyophilized to yield the deprotected RGD monomer 5 as white crystals (0.015 g, 53%). IR (KBr) ν\text{max}/cm\(^{-1}\) = 3230, 2200, 1640, 1380 cm\(^{-1}\); δ\text{H} (D\(_2\)O) 1.55-1.58 (2 H, m, CH\(_2\)), 1.61-1.65 (1 H, m CH), 1.68-1.82 (1 H, m, CH), 2.38-2.56 (6 H, m, 3 x CH\(_2\)), 2.85-2.87 (2 H, d, J = 5.9 Hz, CH\(_2\)), 3.10-3.14 (2 H, m, CH\(_2\)), 3.34-3.38 (2 H, m, CH\(_2\)), 3.87 (2 H, s, α-CH\(_2\)), 4.19-4.28 (1 H, m, α-CH\(_2\)), 4.65-4.68 (1 H, t, J = 5.7 Hz, α- CH\(_2\)); δ\text{C} (D\(_2\)O) 24.3 (CH\(_2\)), 27.9 (CH\(_2\)), 29.8 (CH\(_2\)), 34.8 (CH\(_2\)), 35.7 (CH\(_2\)), 40.4 (CH\(_2\)), 42.1 (CH\(_2\)), 49.2 (CH), 53.6 (CH), 156.7 (C), 162.8 (C), 170.8, 174.2, 174.3, 174.4 (C=O).

**Synthesis of protected monomeric RGE hydrazide 6**

Protected RGE peptide 2 (0.347 g, 0.518 mmol) in DMF (4 mL) was added to NHS ester 3 (0.100 g, 0.259 mmol) and DMAP (0.006 g, 0.050 mmol) and the solution stirred for 72 hr at room temperature. DMF was removed under reduced pressure and the crude product redissolved in CH\(_2\)Cl\(_2\) (10 mL). Brine was added and the aqueous solution was extracted with CH\(_2\)Cl\(_2\) (3 x 10 mL). The organic extracts were combined and dried over MgSO\(_4\). The solution was evaporated to dryness and the residue purified by column chromatography using silica gel as absorbent with CH\(_2\)Cl\(_2\)/MeOH (8:2 v/v) with 0.1% TFA as eluent to yield 6 as a yellow oil (0.100 g, 40%). IR (KBr) ν\text{max}/cm\(^{-1}\) = 3470, 2539, 2082, 1640, 1443, 969 cm\(^{-1}\); δ\text{H} (CH\(_3\)OD) 1.52 (18 H, s, 2 x C(CH\(_3\))\(_3\)) 1.53 (6 H, s, 2 x CH\(_3\)), 1.66-1.69 (2 H, m, CH\(_2\)), 1.74-1.77 (1 H, m CH), 1.81-1.89 (1 H, m CH), 1.97-2.04 (2 H, m, CH\(_2\)), 2.15 (3 H, s, CH\(_3\)), 2.19-2.24 (2 H, m CH\(_2\)), 2.38-2.41 (2 H, m, CH\(_2\)), 2.48-2.55 (4 H, m, 2 x CH\(_2\)), 2.55-2.57 (5 H, m, CH\(_3\) and CH\(_2\)), 2.64 (3 H, s, CH\(_3\)), 3.07 (2 H, s, CH\(_2\)), 3.27-3.29 (2 H, m, CH\(_2\)), 3.47-3.56 (2 H, m, CH\(_2\)), 3.91-4.03 (2 H, m, (α-CH\(_2\))), 4.26-4.29 (1 H, m, α-CH), 4.51-4.55 (1 H, m, α-CH); δ\text{C} (CH\(_3\)OD) 12.6 (CH\(_3\)), 18.3 (CH\(_3\)), 19.6 (CH\(_3\)), 27.9 (CH\(_3\)), 28.3 (CH\(_3\)), 28.5 (CH\(_3\)), 29.9 (CH\(_2\)), 31.7 (CH\(_2\)), 32.5 (CH\(_2\)), 37.0 (CH\(_2\)), 43.3 (CH\(_2\)), 43.9 (CH\(_2\)), 81.8 (C(CH\(_3\))\(_3\)), 135.4 (Ar-C), 173.8, 174.1 (all C=O); HRMS (ESI\(^+\)): found 993.4803 ([M+H+K\(^2+\)], C\(_{42}\)H\(_{68}\)N\(_8\)O\(_{14}\)SK ([M+H+K\(^2+\)]) requires 993.4244.
Synthesis of monomeric RGE hydrazide 7

Protected monomer 6 (0.050 g, 0.051 mmol) was dissolved in TFA (0.50 mL), TIS (0.025 mL) and water (0.025 mL) was added. The solution was stirred for 2 hr at room temperature. Excess TFA was removed under reduced pressure and the crude product redissolved in 10% acetic acid (10 mL). The aqueous solution was extracted with chloroform (3 x 10 mL). The aqueous solution was lyophilized to yield deprotected RGE monomer 7 as white crystals (0.012 g, 43%).

δ\text{H}(\text{D}_2\text{O}) 1.50-1.63 (2 H, m, CH\textsubscript{2}), 1.64-1.80 (2 H, m CH\textsubscript{2}), 1.85-1.96 (1 H, m, CH), 2.10-2.19 (2 H, m, CH\textsubscript{2}), 2.37-2.60 (8 H, m, 4 x CH\textsubscript{2}), 3.10-3.14 (2 H, t, J = 6.8 Hz, CH\textsubscript{2}), 3.33-3.37 (2 H, t, J = 6.8 Hz CH\textsubscript{2}), 4.18-4.22 (1 H, m, α-CH), 4.35-4.39 (1 H, m, α-CH); δ\text{C}(\text{D}_2\text{O}) 24.3 (CH\textsubscript{2}), 25.7 (CH\textsubscript{2}), 27.8 (CH\textsubscript{2}), 29.7 (CH\textsubscript{2}), 34.8 (CH\textsubscript{2}), 35.6 (CH\textsubscript{2}), 40.4 (α-CH), 42.2 (CH\textsubscript{2}), 43.4 (CH\textsubscript{2}), 51.8 (α-CH), 53.6 (α-CH), 156.6 (C), 162.7 (C), 163.0 (C), 171.1, 174.2, 174.3, 174.4, 176.6, 177.0 (all C=O); HRMS (ESI\textsuperscript{+}): found 546.2621 ([M+H]\textsuperscript{+}), C\textsubscript{20}H\textsubscript{36}N\textsubscript{9}O\textsubscript{9} ([M+H]\textsuperscript{+}) requires 546.2636.

Synthesis of Boc-protected diol 9

Diol 9 was synthesised as described previously in the literature.\textsuperscript{2,3} Spectroscopic data were consistent with literature values.

Synthesis of Boc-protected dinitrile 10

Acrylonitrile (0.65 mL, 9.84 mmol) was added to a stirred solution of tert-butyl (1,3-dihydroxy-2-methylpropan-2-yl)carbamate 9 (1.0 g, 4.87 mmol) in 1,4-dioxane (2 mL) and KOH (40%, 0.5 mL) over a period of 2.5 hr. The solution was allowed to stir at room temperature for a further 21 hr. 2 M HCl (1.2 mL) and CH\textsubscript{2}Cl\textsubscript{2} were added and the resultant brown precipitate filtered. The filtrate was dried over MgSO\textsubscript{4} and the solution was evaporated to dryness. The residue was purified by column chromatography using silica gel as absorbent with EtOAc/hexane (1:1 v/v) as eluent to afford dinitrile 10 as a yellow oil (0.72 g, 48%). R\textsubscript{f} (1:1 EtOAc/hexane) 0.50; δ\text{H}(\text{CDCl}_3) 1.27 (3H, s, CH\textsubscript{3}), 1.35 (9 H, s, (CH\textsubscript{3})\textsubscript{3}) 2.52-2.56 (4 H, t, J = 6.1 Hz, 3 x CH\textsubscript{2}), 3.44-3.46 (2 H, d, J = 8.9 Hz, CH\textsubscript{2}O), 3.57-3.59 (2 H, d, J = 8.9 Hz,
CH₂O), 3.60-3.63 (4 H, t, J = 6.1 Hz, 2 x CH₂); δC (CDCl₃) 18.7 (CH₂), 19.1 (CH₃), 28.2 ((CH₃)₃), 55.3 (C), 65.7 (CH₂), 72.7 (CH₂O), 79.1 (C), 117.8 (CN), 154.7 (C=O); HRMS (ESI⁺) found 334.1743 ([M+Na]⁺), C₁₅H₂₅N₃O₂Na ([M+Na]⁺) requires 334.1743.

Synthesis of Boc-protected diacid 12

Dinitrile 10 (0.50 g, 1.61 mmol) was dissolved in HCl (12 M, 0.5 mL) and the solution refluxed for 4 hr. Ethanol (1 mL) was added and the solution refluxed for a further 18 hr. The pH was adjusted to 8 using 5 M NaOH and the aqueous solution was extracted with CH₂Cl₂ (3 x 10 mL) and EtOAc (3 x 10 mL). The organic extracts were combined and dried over MgSO₄ before being evaporated to dryness to yield the crude diester 11, which was dissolved in THF (2 mL) and used without further purification. NaOH (5 M, 2 mL) and ethanol (1 mL) were then added the resulting solution stirred vigorously at 50 ºC for 18 hr. Most of the organic solvents were removed under reduced pressure and the pH of the remaining solution adjusted to 2 using 2 M HCl. The aqueous solution was extracted with CH₂Cl₂ (3 x 10 mL) and EtOAc (3 x 10 mL) and the organic extracts were then combined and dried over MgSO₄. The solution was evaporated to dryness to yield the diacid 12 as a colourless oil (0.28 g, 50% over two steps). Rf (EtOAc) 0.20; IR (KBr) νmax/cm⁻¹ = 3324, 2928, 2856, 1784, 1628, 1214 cm⁻¹; δH (CDCl₃) 1.24 (3 H, s, CH₃), 1.40 (9 H, s, (CH₃)₃), 2.57-2.62 (4 H, t, J = 10.3 Hz (CH₂)₂), 3.39-3.42 (2 H, d, J = 8.9 Hz, CH₂), 3.52-3.54 (2 H, d, J = 8.9 Hz, CH₂), 3.68-3.71 (4 H, t, J = 4.7 Hz, 2 x CH₂); δC (CDCl₃) 18.1 (CH₃), 28.3 (3 x CH₃), 34.7 (2 x CH₂), 55.3 (C), 58.3 (CH₂), 66.4 (CH₂), 73.0 (CH₂), 176.5 (C=O); HRMS (ESI⁺): found 372.1632 (M+Na)⁺, C₁₅H₂₇NO₈Na (M+Na)⁺ requires 372.1634 and 272.1081 ([M+Na-Boc]⁺), C₁₀H₁₉NNaO₆ ([M+Na-Boc]⁺) requires 272.1110.

Synthesis of Boc-protected di-NHS ester 13

To a solution of acid 12 (0.15 g, 0.43 mmol) and N-hydroxy succinimide (0.11 g 0.944 mmol) in anhydrous CH₂Cl₂ (2 mL) was added diisopropylcarbodiimide (0.119 g, 0.944 mmol) in anhydrous CH₂Cl₂ (2 mL). The solution was stirred for 72 hr at room temperature and quenched with brine. The aqueous layer was extracted with CH₂Cl₂ (2 x 10 mL), the organic layers were combined and
dried over MgSO₄. The solution was evaporated to dryness and the residue purified by column chromatography using silica gel as absorbent with EtOAc/hexane (1:1 v/v) to yield 13 as a colourless oil (0.100 g, 43%). \( R_f \) (1:1 EtOAc/hexane) 0.60; IR (KBr) \( \nu_{\text{max}}/\text{cm}^{-1} = 3674, 2988, 2902, 1734, 1402, 1185, 1078 \); \( \delta_H \) (CDCl₃) 1.31 (3 H, s, CH₃), 1.41 (9 H, s, (CH₃)₃), 2.81-2.87 (12 H, m, 4 x CH₂), 3.46-3.48 (2 H, d, \( J = 8.9 \) Hz, CH₂), 3.60-3.61 (2 H, d, \( J = 8.9 \) Hz, CH₂); \( \delta_C \) (CDCl₃) 18.9 (CH₃), 25.5 (C(CH₃)₃), 28.3 (4 x CH₂), 32.1 (2 x CH₂), 55.3 (C(CH₃)₃), 65.6 (2 x CH₂), 72.9 (2 x CH), 154.9 (C=O), 166.7 (C=O), 168.9 (C=O).

**Synthesis of sulphonate 14**

Sulphonate 14 was synthesised as described previously in the literature.²,⁵ Spectroscopic data were consistent with literature values.

**Synthesis of Boc-protected dibenzyl ester 15**

Di-NHS ester 13 (0.100 mg, 0.180 mmol) and β-alanine derivative (0.258 g, 0.735 mmol) were dissolved in CH₂Cl₂ (2 mL). Pyridine (0.5 mL) was added and the solution stirred for 48 hr at room temperature. Excess pyridine was removed under reduced pressure and 2 M HCl (10 mL) was added to the remaining organic solvent. The aqueous solution was extracted with CH₂Cl₂ (3 x 10 mL) and EtOAc (3 x 10 mL). The organic extracts were combined and dried (MgSO₄). The solution was evaporated to dryness and the residue purified by column chromatography using silica gel as absorbent with EtOAc as eluent. Evaporation of the fractions containing the second component yielded Boc-protected dibenzyl ester 15 as a yellow oil (0.09 g, 73%). \( R_f \) (EtOAc) 0.35; IR (KBr) \( \nu_{\text{max}}/\text{cm}^{-1} = 3358, 2992, 1681, 1532, 1255, 1149, 876 \); \( \delta_H \) (CDCl₃) 1.27 (3 H, s, CH₃), 1.39 (9 H, s, (CH₃)₃), 2.36-2.39 (4 H, t, \( J = 5.8 \) Hz, 2 x CH₂), 2.56-2.59 (4 H, t, \( J = 6.2 \) Hz, 2 x CH₂), 3.40-3.42 (2 H, m, CH₂), 3.49-3.52 (6 H, m, 3 x CH₂), 3.62-3.66 (4 H, t, 2 x CH₂), 5.10 (4 H, s, 3 x CH₂O), 6.72-6.75 (2 H, t, \( J = 5.7 \) Hz, 2 x NH), 7.25-7.32 (10 H, m, Ph-H); \( \delta_C \) (CDCl₃) 19.3 (CH₃), 28.3 ((CH₃)₃), 33.9 (CH₂), 34.9 (CH₂), 36.8 (CH₂), 55.4 (C), 66.5 (CH₂), 67.2 (CH₂), 154.9 (C=O), 166.7 (C=O), 168.9 (C=O).
73.5 (CH₂), 79.1 (C), 128.1, 128.3, 128.5 (all Ph-CH), 135.5 (C), 154.8 (C=O), 171.3 (C=O), 172.2 (C=O).

**Synthesis of deprotected dibenzyl ester 16**

Boc-protected dibenzyl ester 15 (0.200 g, 0.297 mmol) was dissolved in TFA/CH₂Cl₂ (4 ml, 1:1). The solution was stirred for 2 hr at room temperature, after which excess TFA was removed under reduced pressure to yield a crude residue which was basified to pH 8-9 using 5 M NaOH. The aqueous solution was extracted with CH₂Cl₂ (3 x 20 mL). The organic extracts were combined, dried over MgSO₄ and reduced to dryness to yield dibenzyl ester 15 as a yellow oil (0.155, 91%).

**Synthesis of Boc-protected hydrazide anchor 17**

Protected hydrazide 17 was synthesised as described previously in the literature.⁶ Spectroscopic data were consistent with literature values.

**Synthesis of dinitrile 19**

Acrylonitrile (12.40 mL, 189 mmol) was added to a stirred solution of 2-amino-2-methylpropane-1,3-diol (6.52 g, 62 mmol) in 1,4-dioxane (4 mL) and KOH (40%, 2 mL) over a period of 2.5 hr. The solution was allowed to stir at room temperature for a further 21 hr. 2 M HCl (1.2 mL) and CH₂Cl₂ were added and the resultant brown precipitate filtered. The filtrate was dried over MgSO₄ and the solution was evaporated to dryness. The residue was purified by column chromatography using silica gel as absorbent with MeOH/CH₂Cl₂ (1:9 v/v) as eluent to yield dinitrile 19 as a yellow oil (10.00 g, 76%).

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⁶ Spectroscopic data were consistent with literature values.

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bs, NH₂), 2.58-2.61 (4 H, t, J = 6.1 Hz, 2 x CH₂), 3.29-3.36 (4 H, q, J = 8.5 Hz, 2 x CH₂), 3.66-3.69 (4 H, t, J = 6.1 Hz, 2 x CH₂); δC (CDCl₃) 18.8 (CH₂), 22.4 (CH₃), 52.6 (C), 65.7 (CH₂), 76.4 (CH₂), 117.9 (CN); HRMS (ESI⁺): found 212.1398 ([M+H]+), C₁₀H₁₈N₃O₂ ([M+H]+) requires 212.1399.

**Synthesis of diester 20**

Dinitrile 19 (2.00 g, 9.47 mmol) was dissolved in HCl (3 mL, 12 M) and solution heated at reflux for 5 hr. The solution was allowed to cool and ethanol (20 mL) added. The solution was heated at reflux overnight. The solvent was removed under reduced pressure and HCl (0.2 M) was added to the crude residue to adjust the pH to 2. The aqueous solution was extracted with CH₂Cl₂ (3 x 20 mL) and EtOAc (3 x 20 mL). The organic extracts were combined and dried over MgSO₄ and the solution was evaporated to dryness. The residue was purified by column chromatography using silica gel as absorbent with EtOAc as eluent to yield diester 20 as a yellow oil (2.72 g, 94 %), which did not require further purification. Rf (EtOAc) 0.20; IR (KBr) νmax/cm⁻¹ = 3680, 2975, 2898, 1501, 1382, 1242, 1069 cm⁻¹; δH (CDCl₃) 1.19-1.23 (9 H, t and s, J = 7.1 Hz, CH₃CH₃ and CH₃), 2.53-2.56 (4 H, t, J = 6.4 Hz, 2 x CH₂), 3.42 (4 H, s, 2 x CH₂), 3.69-3.72 (4 H, t, J = 6.4 Hz, 2 x CH₂), 4.07-4.12 (4 H, q, J = 7.1 Hz, CH₃CH₂); δC (CDCl₃) 14.1 (CH₃), 18.3 (CH₃), 34.8 (CH₂), 55.6 (CH₂), 60.4 (CH₂), 66.9 (CH₂), 73.3 (CH₂), 171.4 (C=O), 171.5 (C=O); HRMS (ESI⁺): found 306.1964 ([M+H]+), C₁₄H₂₈NO₆ ([M+H]+) requires 306.1917.

**Synthesis of Boc-hydrazide diester 21**

To a solution of ester (0.100 g, 0.327 mmol), hydrazide (0.110 g, 0.473 mmol) and HOBt (0.066 g, 0.430 mmol) in DMF (2 mL), was added DIC (0.056 g, 0.443 mmol) in DMF (1 mL). The solution was stirred for 72 hr at room temperature. The solvent was removed under reduced pressure and the crude product redissolved in CH₂Cl₂ (10 mL). Brine was added and the aqueous solution was extracted with CH₂Cl₂ (3 x 10 mL) and EtOAc (3 x 10 mL). The organic extracts were combined and dried over MgSO₄ and the solution was evaporated to dryness. The residue was purified by column chromatography using silica gel as absorbent with EtOAc as eluent to yield 21 as a colourless oil (0.13 g, 77%). Rf (EtOAc) 0.35; IR (KBr) νmax/cm⁻¹ = 2982, 1734, 1601, 1528, 1402, 1229
cm\(^{-1}\); \(\delta_H(\text{CDCl}_3)\) 1.17-1.20 (6 H, t, \(J = 7.1\) Hz, \(\text{CH}_2\text{CH}_3\)), 1.23 (3 H, s, \(\text{CH}_3\)), 1.38 (9 H, s, \((\text{CH}_3)_3\)), 2.44-2.48 (8 H, m, 4 x \(\text{CH}_2\)), 3.37-3.39 (2 H, d, \(J = 9.1\) Hz, \(\text{CH}_2\)), 3.51-3.54 (2 H, d, \(J = 9.1\) Hz, \(\text{CH}_2\)), 3.61-3.64 (4 H, t, \(J = 6.2\) Hz, 2 x \(\text{CH}_2\)); \(\delta_C(\text{CDCl}_3)\) 14.1 (CH\(_2\text{CH}_3\)), 18.8 (CH\(_3\)), 28.0 ((CH\(_3\))\(_3\)), 31.7 (CH\(_2\)), 34.9 (CH\(_2\)), 56.7 (C), 60.4 (CH\(_2\)), 66.7 (CH\(_2\)), 72.6 (CH\(_2\)), 81.0 (C), 155.0, 171.5, 171.6, 171.9, 180.0 (all C=O); HRMS (ESI\(^+\)): found 520.2897 ([M+H]\(^+\)), \(\text{C}_{23}\text{H}_{42}\text{N}_3\text{O}_{10}\) ([M+H]\(^+\)) requires 520.2870.

**Synthesis of Boc-hydrazide diacid 22**

![Boc-Hydrazide Diacid](image)

Ester (0.25 g, 0.48 mmol) was dissolved in a solution of THF (2 mL) and NaOH (5 M, 2 mL), ethanol (1 mL) was added and the solution stirred vigorously at 50 °C for 18 hr. Most of the organic solvents were removed under reduced pressure and the pH of the remaining solution was adjusted to 2 using 2 M HCl. The aqueous solution was extracted with \(\text{CH}_2\text{Cl}_2\) (3 x 10 mL) and EtOAc (3 x 10 mL). The organic extracts were combined and dried over MgSO\(_4\). The solution was evaporated to dryness to yield compound 22 as a colourless oil (0.20 g, 90%). \(R_f\) (EtOAc) 0.35; IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\) = 3413, 3337, 3080, 1675, 1492, 1441, 1206 cm\(^{-1}\); \(\delta_H(\text{CDCl}_3)\) 1.28 (3 H, s, \(\text{CH}_3\)), 1.44 (9 H, s, \((\text{CH}_3)_3\)), 2.51-2.58 (8 H, m, 2 x \(\text{CH}_2\) and CO(\(\text{CH}_2\)_2\text{CO})), 3.51-3.57 (4 H, m, 2 x \(\text{CH}_2\)), 3.69-3.72 (4 H, t, \(J = 5.7\) Hz, \(\text{CH}_2\)); \(\delta_C(\text{CDCl}_3)\) 18.9 (CH\(_3\)), 27.1 ((CH\(_3\))\(_3\)), 29.7 (CH\(_2\)), 32.1 (CH\(_2\)), 34.7 (CH\(_2\)), 56.0 (C), 65.7 (CH\(_2\)), 72.1 (CH\(_2\)), 80.1 (C), 171.6 (C=O), 172.0 (C=O), 176.5 (C=O); HRMS (ESI\(^-\)): found 462.2073 ([M-H]\(^-\)), \(\text{C}_{19}\text{H}_{32}\text{N}_3\text{O}_{10}\) ([M-H]\(^-\)) requires 462.2088.

**Synthesis of Boc-hydrazide di-NHS ester 23**

![Boc-Hydrazide Di-NHS Ester](image)

To a solution of acid 22 (0.200 g, 0.430 mmol), and NHS (0.070 g, 0.650 mmol) in \(\text{CH}_2\text{Cl}_2\) (2.00 mL), was added diisopropylcarbodiimide (0.080 g, 0.650 mmol) in \(\text{CH}_2\text{Cl}_2\) (1.00 mL). The solution was stirred for 72 hr at room temperature and quenched with brine. The aqueous layer was extracted with \(\text{CH}_2\text{Cl}_2\) (2 x 10 mL) and the organic layers were combined and dried over MgSO\(_4\). The solution was evaporated to dryness and the residue purified by column chromatography using silica gel as absorbent with
MeOH/CH$_2$Cl$_2$ (1:9 v/v) as eluent to yield 23 as a colourless oil (0.16 g, 56%). $R_f$ (1:9 MeOH/CH$_2$Cl$_2$) 0.20; IR (KBr) $\nu_{\text{max}}$/cm$^{-1} = 3476, 2753, 2626, 1680, 1506, 1413, 1310, 1203 cm$^{-1}; \delta_{\text{H}}$(CDCl$_3$) 1.31 (3 H, s, CH$_3$), 1.42 (9 H, s, C(CH$_3$)$_3$), 2.44-2.46 (4 H, t, $J = 4.0$ Hz, (CH$_2$)$_2$), 2.82-2.83 (12 H, m, 6 x CH$_2$), 3.49-3.51 (2 H, d, $J = 9.1$ Hz, CH$_2$), 3.60-3.62 (2 H, d, $J = 9.1$ Hz, CH$_2$), 3.75-3.78 (4 H, m, (CH$_2$)$_2$); $\delta_{\text{C}}$(CDCl$_3$) 18.7 (CH$_3$), 25.6 (CH$_2$), 28.1 ((CH$_3$)$_3$), 29.6 (CH$_2$), 32.0 (CH$_2$), 32.1 (CH$_2$), 56.9 (C), 65.8 (CH$_2$), 72.6 (CH$_2$), 81.4 (C), 155.4, 167.0, 169.3, 172.2, 172.3, 172.5 (all C=O); HRMS (ESI$^+$): found 658.2587 ([M+H$^+$]), C$_{27}$H$_{46}$N$_3$O$_{14}$ ([M+H$^+$]) requires 658.2572.

**Synthesis of protected dimeric RGD hydrazide 24**

Side-chain protected RGD peptide 5 (0.298 g, 0.456 mmol) in DMF (4 mL) was added to NHS ester 23 (0.100 g, 0.149 mmol) and DMAP (0.006 g, 0.050 mmol) and the solution stirred for 72 hr at room temperature. DMF was removed under reduced pressure and the crude product redissolved in CH$_2$Cl$_2$ (10 mL). Brine was added and the aqueous solution was extracted with CH$_2$Cl$_2$ (3 x 10 mL). The organic extracts were combined and dried over MgSO$_4$ and the solution then evaporated to dryness. The residue was purified by column chromatography using silica gel as absorbent with CH$_2$Cl$_2$/MeOH (8:2 v/v) with 0.1% TFA as eluent to yield 24 as a yellow oil (0.030 g, 11%). $R_f$ (8:2 CH$_2$Cl$_2$/MeOH) 0.10; IR (KBr) $\nu_{\text{max}}$/cm$^{-1} = 3307, 2921, 2938, 2485, 1952, 1681 cm$^{-1}; \delta_{\text{H}}$(CH$_3$OD) 1.35 (3 H, s, CH$_3$), 1.50 (27 H, s, 3 x C(CH$_3$)$_3$), 1.54 (12 H, s, 4 x CH$_3$), 1.66-1.77 (m, 8 H, 4 x CH$_2$), 1.86-1.94 (2 H, m, CH$_2$), 2.16 (6 H, s, 2 x CH$_3$), 2.51-2.58 (12 H, m, 2 x CH$_3$ and 3 x CH$_2$), 2.64 (6 H, s, 2 x CH$_3$), 3.07 (4 H, s, 2 x CH$_2$CCH$_3$), 3.27-3.28 (4 H, m 2 x CH$_3$), 3.25-3.35 (4 H, m, 2 x CH$_2$), 3.55-3.59 (2 H, m, CH$_2$), 3.63-3.67 (2 H, m, CH$_2$), 3.76-3.77 (4 H, m, 2 x CH$_2$), 3.98 (4 H, s, 2 x $\alpha$-CH$_2$), 4.42-4.45 (2 H, t, $J = 6.6$ Hz, 2 x $\alpha$-CH), 4.81-4.84 (2 H, m, $J = 5.9$ Hz, 2 x $\alpha$-CH); $\delta_{\text{C}}$(CH$_3$OD) 12.6 (CH$_3$), 18.4 (CH$_3$), 19.5 (CH), 19.6 (CH$_3$), 28.3 ((CH$_3$)$_3$), 28.5 (CH$_3$), 28.7 (CH$_3$), 30.1 (CH$_2$), 30.7 (CH$_2$), 32.5 (CH$_2$), 37.3 (CH$_2$), 38.4 (CH$_2$), 43.3 ($\alpha$-CH$_2$), 44.0 (2 x CH$_2$), 50.3 ($\alpha$-CH), 58.1 ($\alpha$-CH), 68.5 (CH$_2$), 73.7 (2 x CH$_2$), 82.5 (C), 87.8 (C), 114.9 (Ph-C), 117.2 (Ph-C), 118.5 (Ph-C), 126.1 (Ph-C), 157.8 (Ph-C), 171.2, 171.3, 173.8, 174.3, 174.6 (all C=O); HRMS (ESI$^+$): found 1736.8120 ([M+H$^+$]), C$_{77}$H$_{122}$N$_{15}$O$_{26}$S$_2$ requires ([M+H$^+$]) 1736.8127.
Synthesis of dimeric RGD hydrazide 25

Protected dimer (0.050 g, 0.028 mmol) was dissolved in TFA (0.5 mL), water (0.025 mL and TIS (0.025 mL, 0.124 mmol) added. The solution was stirred for 3 hr at room temperature. Excess TFA was removed under reduced pressure and the resultant residue redissolved in 10% acetic acid (5 mL). The aqueous solution was extracted with chloroform (3 x 10 mL) and the aqueous fraction then lyophilized to yield the deprotected RGD dimer 25 as white crystals (0.023 mg, 79%). IR (KBr) \( \nu_{\text{max}}/\text{cm}^{-1} = 3290, 2856, 2200, 1670, 1081 \); \( \delta_{\text{H}}(\text{D}_{2}\text{O}) 1.14 (3 \text{H}, \text{s}, \text{CH}_3), 1.52-1.63 (4 \text{H}, \text{m}, 2 \times \text{CH}_2), 1.82-1.87 (4 \text{H}, \text{m}, 2 \times \text{CH}_2), 2.0 (5 \text{H}, \text{s}, \text{NH}), 2.43-2.47 (8 \text{H}, \text{m}, 4 \times \text{CH}_2), 2.86-2.87 (4 \text{H}, \text{m}, 2 \times \text{CH}_2), 3.09-3.12 (6 \text{H}, \text{m}, 2 \times \text{CH}_2), 3.84-3.86 (4 \text{H}, \text{s}, 2 \times \alpha-\text{CH}_2), 4.12-4.34 (2 \text{H}, \text{m}, 2 \times \alpha-\text{CH}), 4.68-4.69 (2 \text{H}, \text{m}, 2 \times \alpha-\text{CH}); \delta_{\text{C}} (\text{D}_{2}\text{O}) 18.6 (\text{CH}_3), 20.4 (\text{CH}), 23.5 (\text{CH}_2), 27.9 (\text{CH}_2), 35.6 (\text{CH}_2), 40.4 (\text{CH}_2), 40.6 (\text{CH}_2), 42.2 (\alpha-\text{CH}_2), 51.8 (\alpha-\text{CH}), 52.9 (\alpha-\text{CH}), 15.0 (\text{C}), 117.9 (\text{C}), 156.8 (\text{C}), 162.8 (\text{C}), 163.2 (\text{C}), 170.1, 170.5, 174.5, 176.7 (all \text{C}=\text{O}); \text{HRMS (ESI)}^+: \text{found } 1020.4594 ([\text{M+H}]^+), C_{38}H_{60}N_{15}O_{18} ([\text{M+H}]^+) \text{ requires } 1020.4710.

Synthesis of protected dimeric RGE hydrazide 26

Protected RGE peptide (0.178 g, 0.266 mmol) in DMF (4 mL) was added to NHS ester (0.070 g, 0.104 mmol) and DMAP (0.006 g, 0.050 mmol) and the solution stirred for 72 hr at room temperature. DMF was removed under reduced pressure and the crude product redissolved in CH$_2$Cl$_2$ (10 mL). Brine was added and the aqueous solution was extracted with CH$_2$Cl$_2$ (3 x 10 mL). The organic extracts were combined and dried over MgSO$_4$ and the solution then evaporated to dryness. The residue was purified by column chromatography using silica gel as absorbent and CH$_2$Cl$_2$/MeOH (8:2 v/v) with 0.1% TFA as eluent to yield 26 as a yellow oil (0.040 g, 21%). \( R_t \) (8:2 CH$_2$Cl$_2$/MeOH) 0.1; IR (KBr) \( \nu_{\text{max}}/\text{cm}^{-1} = 3307, 2921, 2938, 2485, 1952, 1681 \); \( \delta_{\text{H}}(\text{CH}_3\text{OD}) 1.34 (3 \text{H}, \text{s}, \text{CH}_3), 1.49 (27 \text{H}, \text{s}, 3 \times (\text{CH}_3)_3), 1.52 (12 \text{H}, \text{s}, 4 \times \text{CH}_3), 1.65-1.78 (6 \text{H}, \text{m}, 3 \times \text{CH}_2), 1.89-1.97 (2 \text{H}, \text{m}, \text{CH}_2), 1.99-2.11 (2 \text{H}, \text{m}, \text{CH}_2), 2.14 (6 \text{H}, \text{s}, 2 \times \text{CH}_3), 2.19-2.24 (2 \text{H}, \text{m}, \text{CH}_2), 2.37-2.41 (4 \text{H}, \text{t}, J = 7.5 \text{ Hz}, 2 \times \text{CH}_2), 2.57-2.59 (12 \text{H}, \text{m}, 2 \times \text{CH}_3).
and 3 x CH2), 2.63 (6 H, s, 2 x CH3), 3.07 (4 H, s, 2 x CH2), 3.21-3.33 (4 H, m, 2 x CH2), 3.57-3.58 (2 H, m, CH2), 3.65-3.66 (2 H, m, CH2), 3.71-3.76 (4 H, m, 2 x CH2), 3.93-4.04 (4 H, m, 2 x α-CH2), 4.38-4.39 (2 H, m, 2 x α-CH), 4.50-4.54 (2 H, m, 2 x α-CH); δc (DMSO) 12.6 (CH3), 18.5 (CH3), 19.6 (CH3), 27.9 (CH2), 28.2 (CH3), 28.4 ((CH3)3) 28.7 (CH3), 28.8 (CH3), 30.1 (CH2), 32.7 (CH2), 37.6 (CH2), 43.5 (α-CH2), 44.0 (CH2), 53.0 (α-CH), 54.9 (α-CH), 68.6 (CH2), 73.8 (2 x CH2), 81.9 (C), 88.0 (C), 118.7 (C), 126.4 (Ph-C), 157.7 (Ph-C), 171.6, 173.8, 174.4, 174.7 (all C=O); HRMS (ESI+): found 1786.8347 ([M+Na]+), requires 1786.8259.

**Synthesis of dimeric RGE hydrazide 27**

Protected dimer (0.050 g, 0.028 mmol) was dissolved in TFA (0.5 mL), water (0.025 mL) and TIS (0.025 mL, 0.124 mmol) added. The solution was stirred for 2 hr at room temperature. Excess TFA was removed under reduced pressure and the resultant residue redissolved in 10% acetic acid (5 mL). The aqueous solution was extracted with chloroform (3 x 10 mL) and the aqueous fraction then lyophilized to yield the deprotected RGE dimer 27 as white crystals (0.022 g, 75%). IR (KBr) νmax/cm⁻¹ = 3402, 3350, 2899, 2745, 1952, 1590 cm⁻¹; δH (D2O) 1.24 (3 H, s, CH3), 1.61-1.71 (4 H, m, 2 x CH2), 1.72-1.82 (2 H, m, CH2), 1.85-1.87 (2 H, m CH2), 1.97-2.09 (2 H, m CH2), 2.21-2.26 (2 H, m, CH2), 2.46-2.50 (4 H, m, (CH2)2), 2.53-2.58 (8 H, m, 4 x CH2), 3.20-3.23 (4 H, t, J = 6.4 Hz, 2 x CH2), 3.53-3.55 (2 H, m, CH2), 3.61-3.65 (2 H, m, CH2), 3.70-3.75 (4 H, m, 2 x CH2), 3.97 (4 H, s, 2 x α-CH2), 4.32-4.36 (2 H, t, J = 6.7 Hz, 2 x α-CH), 4.45-4.48 (2 H, m, 2 x α-CH); δC (D2O) 18.6 (CH3), 20.3 (CH), 24.4 (CH2), 25.1 (CH2), 25.2 (CH2), 25.7 (CH2), 28.0 (CH2), 29.8 (CH2), 35.6 (CH2), 40.4 (CH2), 42.2 (α-CH2), 51.8 (α-CH), 53.6 (α-CH), 56.9 (C), 67.3 (CH2), 72.2 (CH2), 114.8 (C), 117.7 (C), 156.6 (C), 171.1, 173.8, 174.3, 174.7, 176.6, 176.9 (C=O); HRMS (ESI+): found 1047.4877 ([M+]'), C40H69N15O18 ([M+]') requires 1046.4945.
Synthesis of Boc-hydrazide tetrameric ester 28

To a solution of diacid 22 (0.150 g, 0.324 mmol), \(N\)-(3-dimethylaminopropyl)-\(N'\)-ethylcarbodiimide (0.125 g, 0.81 mmol) and \(N\)-hydroxysuccinimide (0.093 g, 0.810 mmol) in DMF (2 mL) was added amine 20 (0.250 g, 0.820 mmol) in DMF (2 mL). The solution was stirred for 72 hr at room temperature. The solvent was removed under reduced pressure and the crude solid redissolved in CH\(_2\)Cl\(_2\) (10 mL). Brine was added and the aqueous solution was extracted with CH\(_2\)Cl\(_2\) (3 x 10 mL) and EtOAc (3 x 10 mL). The organic extracts were combined and dried over MgSO\(_4\) and the solution then evaporated to dryness. The residue was purified by column chromatography using silica gel as absorbent and EtOAc as eluent to yield the Boc-protected tetrameric ester hydrazide 28 as a colourless oil (0.150 g, 45%). \(R_f\) (EtOAc) 0.35; \(\delta_H\) (CDCl\(_3\)) 1.22-1.26 (12 H, t, \(J = 7.1 \text{ Hz}\), CH\(_2\)CH\(_3\)), 1.30 (9 H, s, (CH\(_3\))\(_3\)), 1.43 (9 H, s, 3 x CH\(_3\)), 2.34-2.37 (4 H, t, \(J = 5.7 \text{ Hz}\), (CH\(_2\))\(_2\)), 2.51-2.54 (12 H, t, \(J = 6.2 \text{ Hz}\), 6 x CH\(_2\)), 3.40-3.45 (6 H, m, 3 x CH\(_2\)), 3.57-3.67 (6 H, m, 3 x CH\(_2\)), 3.67-3.71 (12 H, t, \(J = 4.9 \text{ Hz}\) , 6 x CH\(_2\)), 4.09-4.14 (8 H, q, \(J = 7.1 \text{ Hz}\) , 4 x CH\(_2\)); \(\delta_C\) (CDCl\(_3\)) 14.1 (CH\(_2\)CH\(_3\)), 18.9 (CH\(_3\)), 28.1 (\((\text{CH}_3)_3\)), 30.0 (CH\(_2\)), 32.0 (CH\(_2\)), 34.9 (CH\(_2\)), 37.3 (CH\(_2\)), 56.6 (C), 60.4 (CH\(_2\)), 66.7 (CH\(_2\)), 67.4 (CH\(_2\)), 72.7 (CH\(_2\)), 81.1 (C), 155.3, 171.1, 171.5, 171.6, 172.8 (all C=O); HRMS (ESI\(^+\)): found 1038.5747 ([M+H]\(^+\)), C\(_{47}\)H\(_{84}\)N\(_5\)O\(_{20}\) ([M+H]\(^+\)) requires 1038.5704.

3. Cell-Based Experiments

Purification of Silk Fibroin Protein

Silk fibroin was isolated from silkworm cocoons using a method modified from that of Sofia, et al.\(^7\) Briefly, Bombyx mori silk cocoons (2 g) were heated to 100 °C in Na\(_2\)CO\(_3\) (0.02 M, 1 L) for 4 hr. The degummed silk fibres were washed with distilled water (5 x 1 L) and then allowed to air-dry overnight. The silk was dissolved to 10% w/v in LiBr solution (9 M) by heating at 60 °C for 4-5 hours with stirring. The resulting solution was filtered to remove any debris and dialysed against distilled water using SnakeSkin dialysis tubing (Pierce; MWCO
3,500) until no change in conductivity was detected. The solution was filtered and lyophilized to yield silk fibroin protein (1.5 g) as a white solid.

**Preparation and Functionalization of Silk Films**

Silk films were prepared by dissolving silk fibroin at 2% w/v in hexafluoroisopropanol and pipetting 50 µL of this solution per well into 96 well cell culture plates. The solvent was allowed to evaporate overnight and, following thorough washing with phosphate-buffered saline (PBS), films were incubated for 1 hr with 50 µL MeOH to convert the silk from its amorphous form to the insoluble silk II form. The films were then soaked in PBS for 2 hr to hydrate the surface before any further modification. To activate available carboxylic acids, films were incubated with 50 µL of a solution of EDC and NHS in PBS (36.5 and 17.4 mM, respectively) for 1 hour. After rinsing with PBS, activated surfaces were then decorated with amine functionality by incubation with 50 µL tetraethylene glycol diamine (NH₂(CH₂CH₂O)₃CH₂CH₂NH₂)(50 µM in 1:1 methanol/water) for 1 hour. Surfaces were then rinsed with PBS prior to introduction of aldehyde functionality via incubation with glutaraldehyde (0.01 M in PBS). Surfaces were then extensively rinsed (10 x) then incubated overnight with PBS to remove all traces of unreacted glutaraldehyde before further use. Aldehyde functionalization was confirmed by the chemoselective ligation of a fluorescent ligand, fluorescein-5-thiosemicarbazide (FTSC). Briefly, films modified with tetraethylene glycol diamine (negative control) or both tetraethylene glycol diamine and glutaraldehyde were incubated with FTSC solution (1 mg/mL in PBS; pH 5.5) at room temperature for 20 minutes. Following washing with ultrapure water, films were then observed using fluorescence microscopy (Leica DMI400B) to determine the relative extent of fluorescein ligation to the surfaces. Amine-functionalized films did not exhibit fluorescence, whereas aldehyde-functionalized surfaces did (Figure S1), confirming the successful generation of aldehyde-rich surfaces.

![Figure S1](image_url)

**Fig. S1** Typical fluorescence microscopy images of amine-functionalized silk films incubated with fluorescein-5-thiosemicarbazide following treatment with buffer (A) or 0.01M glutaraldehyde (B).
Modification of Silk Surfaces with Cell-Adhesive Species

Modified silk films were incubated with hydrazide-terminated monomers or dimers of both RGD and RGE (all 3 mg/mL in MES buffer; pH 5.5) for 3 hr at 37 °C. Following modification, surfaces were thoroughly washed prior to use in cell studies. Films were also incubated under the same conditions with commercially available RGD to determine the chemoselectivity of the reaction or these cell-adhesive species with aldehyde-rich surfaces.

Cell Growth on Modified Silk Films

Murine C2C12 myoblasts (ECACC, #91031101) were used as a model cell line and were cultured in Dulbecco's modified Eagle's medium supplemented with foetal bovine serum (10% v/v), penicillin (100 U/mL), streptomycin (100 µg/mL) and amphotericin B (250 ng/mL). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. Culture medium was replaced every two to three days and cells were sub-cultured when approximately 70 % confluent. For cell proliferation studies, each well was seeded with 1 x 10⁴ cells, and the relative cell number determined using the CellTiter 96® metabolic assay (Pierce), which measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) by metabolically active cells. At 24, 48 and 72 hours post seeding, MTS reagent (20 µL) was added to each well, without prior removal of medium, and the cells were incubated for a further 4 hr. The absorbance of the resultant soluble formazan crystals was then determined using a plate reader at 490 nm. Mean absorbance was determined from three replicate wells for each type of surface and the three independent experiments were performed. The resultant data were analysed by unpaired, two-tailed Students t-tests and are expressed as the mean ± standard error.

4. References

5. $^1$H And $^{13}$C NMR Spectra

$^1$H NMR, CH$_3$OD

$^{13}$C NMR, CH$_3$OD
$\text{H NMR, CH}_3\text{OD}$

$\text{13C NMR, CH}_3\text{OD}$
**H NMR, CH$_3$OD**

**1H NMR, CH$_3$OD**

**13C NMR, CH$_3$OD**
$^1$H NMR, D$_2$O

$^{13}$C NMR, D$_2$O
10

$^1$H NMR, CDCl$_3$

10

$^{13}$C NMR, CDCl$_3$
**H NMR, CDCl$_3$**

![NMR spectrum](image)

**13C NMR, CDCl$_3$**

![NMR spectrum](image)

13
C NMR, CDCl₃

1H NMR, CDCl₃

13C NMR, CDCl₃
**16**

$^{1}H$ NMR, CDCl$_3$

$^{13}C$ NMR, CDCl$_3$
$^1$H NMR, CDCl$_3$

$^1$C NMR, CDCl$_3$
$\text{H NMR, CDCl}_3$

$20$

$\text{C NMR, CDCl}_3$

$13\text{C NMR, CDCl}_3$
**1H NMR, CDCl₃**

![1H NMR spectrum](image)

**13C NMR, CDCl₃**

![13C NMR spectrum](image)
**H NMR, CDCl$_3$**

![H NMR spectrum](image)

**$^1$H NMR, CDCl$_3$**

**C NMR, CDCl$_3$**

![C NMR spectrum](image)

**$^{13}$C NMR, CDCl$_3$**
$^1$H NMR, CDCl$_3$

$^{13}$C NMR, CDCl$_3$
BocHN Arg(Pbf)-Gly-Asp(OtBu)-OH

24

$^1$H NMR, CH$_3$OD

BocHN Arg(Pbf)-Gly-Asp(OtBu)-OH

24

$^{13}$C NMR, CH$_3$OD
$^{1}H$ NMR, $D_2O$

$^{13}C$ NMR, $D_2O$
BocHN\text{Arg(Pbf)-Gly-Glu(OtBu)-OH} \quad 26

$^1$H NMR, CH$_3$OD

BocHN\text{Arg(Pbf)-Gly-Glu(OtBu)-OH} \quad 26

$^{13}$C NMR, CH$_3$OD
$^{1}$H NMR, D$_2$O

$^{13}$C NMR, D$_2$O
$^{1}H$ NMR, CDCl$_3$

$^{13}$C NMR, CDCl$_3$
### 6. IUPAC Names Of Novel Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>IUPAC name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-[2-(2-amino-5-[[3-[[2,2,4,6,7-pentamethyl-2,3-dihydro-1-benzofuran-5-yl]sulfonfonyl]carbamimidamido]pentanamido]acetamido]-4-(tert-butoxy)-4-oxobutanoic acid</td>
</tr>
<tr>
<td>2</td>
<td>2-[2-(2-amino-5-[[3-[[2,2,4,6,7-pentamethyl-2,3-dihydro-1-benzofuran-5-yl]sulfonfonyl]carbamimidamido]pentanamido]acetamido]-5-(tert-butoxy)-5-oxopentanoic acid</td>
</tr>
<tr>
<td>6</td>
<td>tert-butyl N-[1,3-bis-(2-cyanoethoxy)-2-methylpropan-2-yl]carbamate</td>
</tr>
<tr>
<td>7</td>
<td>ethyl 3-[[tert-butoxy]carbonyl]amino]-3-(3-ethoxy-3-oxopropoxy)-2-methylpropoxypropanoate</td>
</tr>
<tr>
<td>8</td>
<td>3-[[tert-butoxy]carbonyl]amino]-3-(2-carboxyethoxy)-2-methylpropoxypropanoate</td>
</tr>
<tr>
<td>9</td>
<td>2,5-dioxopyrrolidin-1-yl 3-2-[[tert-butoxy]carbonyl]amino]-3-[[3-[[2,5-dioxopyrrolidin-1-yl]oxy]-3-oxopropoxy]-2-methylpropoxypropanoate</td>
</tr>
<tr>
<td>12</td>
<td>ethyl 3-[2-amino-3-(2-cyanoethoxy)-2-methylpropoxypropanenitrile</td>
</tr>
<tr>
<td>13</td>
<td>ethyl 3-[2-amino-3-(3-ethoxy-3-oxopropoxy)-2-methylpropoxypropanoate</td>
</tr>
<tr>
<td>14</td>
<td>ethyl 3-[2-4-[[tert-butoxy]carbonyl]amino]-4-oxobutanamido]-3-(3-ethoxy-3-oxopropoxy)-2-methylpropoxypropanoate</td>
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<tr>
<td>15</td>
<td>3-[2-amino-3-(2-carboxyethoxy)-2-methylpropoxypropanoate</td>
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<tr>
<td>16</td>
<td>2,5-dioxopyrrolidin-1-yl 3-[2-[3-[[N'-(tert-butoxy)carbonyl]hydrazinecarbonyl]propanamido]-3-[3-[[2,5-dioxopyrrolidin-1-yl]oxy]-3-oxopropoxy]-2-methylpropoxypropanoate</td>
</tr>
<tr>
<td></td>
<td>Chemical Structure</td>
</tr>
<tr>
<td>---</td>
<td>-------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>26</td>
<td>5-(tert-butoxy)-2-[2-(3-{[4-(tert-butoxy)-1-carboxy-4-oxobutyl]carbamoyl}methyl)carbamoyl]-4-{1-[(2,2,4,6,7-pentamethyl-2,3-dihydro-1-benzofuran-5-yl)sulfonyl]carbamimidamido}butyl)carbamoyl)ethoxy]-2-(3-{N'-[(tert-butoxy)carbonyl]hydrazinecarbonyl)propanamido}-2-methylpropoxy)propanamido]-5-{3-{(2,2,4,6,7-pentamethyl-2,3-dihydro-1-benzofuran-5-yl)sulfonyl]carbamimidamido}pentanamido)acetamido]-5-oxopentanoic acid</td>
</tr>
<tr>
<td>27</td>
<td>2-{2-(5-carbamimidamido)-2-{3-2-(4-carbamimidamido)-1-([1,3-bis(3-ethoxy-3-oxopropoxy)-2-methylpropan-2-yl]carbamoyl)ethoxy}-2-[3-(hydrazinecarbonyl)propanamido]-2-methylpropoxy)propanamido]pentanamido)acetamido]pentanedioic acid</td>
</tr>
<tr>
<td>28</td>
<td>ethyl 3-(2-{3-2-{1,3-bis(3-ethoxy-3-oxopropoxy)-2-methylpropan-2-yl]carbamoyl}ethoxy)-2-[4-((tert-butoxy)carbonyl)amino]-4-oxobutanamido]-2-methylpropoxy)propanamido]-3-(3-ethoxy-3-oxopropoxy)-2-methylpropoxy)propanoate</td>
</tr>
</tbody>
</table>