Tetrahedron 68 (2012) 9448–9455

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Scalable synthesis of an integrin-binding peptide mimetic for biomedical applications

Andrew G. Riches [a,b,*], Teresa Cablewski [a,b], Veronica Glattauer [a,b], Helmut Thissen [a,b], Laurence Meagher [a,b]

[a] CSIRO Materials Science and Engineering, Bayview Avenue, Clayton 3168 VIC, Australia
[b] CRC for Polymers, 8 Redwood Drive, Notting Hill 3168 VIC, Australia

ARTICLE INFO

Article history:
Received 11 May 2012
Received in revised form 16 August 2012
Accepted 3 September 2012
Available online 7 September 2012

Keywords:
RGD mimetic
Integrin
Biomaterials

ABSTRACT

A scalable, solution-phase synthesis of the selectively protected non-peptide RGD (arginine–glycine–aspartic acid) mimetic **6** is described. This synthesis serves as an alternative to the previously described solid-phase synthesis of this compound, thereby making this important integrin-binding mimetic readily accessible. The free carboxylic acid of **6** was conjugated to a protected diamine, followed by global deprotection to give a derivative **27**, suitable for immobilization onto amine-reactive surfaces. The RGD mimetic **28** demonstrated superior biological activity in comparison to a native linear RGD peptide and the semi-synthetic cyclic cRGDfK peptide in a cell attachment inhibition assay.

1. Introduction

The ability to control cell-material surface interactions effectively is essential to the development of new and improved biomaterials and biomedical devices for in vitro and in vivo applications such as diagnostics, drug delivery, implantable devices, and regenerative medicine.[1] The immobilization of bioactive molecules on material surfaces that enhance cell attachment is of particular interest. Traditional surface modification methods, which rely on the non-specific adsorption of proteins to mediate cell attachment are being replaced by improved methods, which display bioactive molecules, which are recognized by cell-surface receptors.[2] Here, the most important example is the recognition of extracellular matrix (ECM) proteins by the heterodimeric cell-surface receptors known as integrins.[3] These adhesive interactions are mediated by small integrin-binding motifs within ECM proteins, of which the arginine–glycine–aspartic acid (RGD) motif is the most widely studied.[4] The modification of biomaterial surfaces with small RGD peptides overcomes some of the disadvantages of immobilizing whole ECM proteins, which include control of orientation, immunogenicity, instability toward enzymatic degradation, sterilization conditions, and risk of pathogen transmission.[5,6] For example, the presentation of short linear RGD peptides on self-assembled monolayers of oligoethylene glycol has been shown to elicit biospecific attachment and survival of cells.[7] Small cyclic peptides are more stable in vivo than their linear counterparts and optimization of their selectivity for the αvβ3 integrin versus other integrins such as αIIbβ3 (platelet fibrinogen receptor) led to the development of potent and selective αvβ3 binding compounds such as cRGDfV[8] (f=D-phenylalanine) **1** and the antiangiogenic agent Cilengitide **2**[9] (Fig. 1). Immobilization of the cyclic pentapeptide cRGDfK **3**, through linkers attached to the lysine ε-amino group, onto poly(methyl methacrylate) substrates, resulted in materials which showed improved osteoblast adhesion in vitro and enhanced bone tissue ingrowth into a porous implant in an in vivo rabbit model.[10] Non-peptide mimetics are an attractive alternative to peptides due to their greater stability and lower cost of preparation on large scales. This is reflected by the effort that various groups have put into development of non-peptide RGD mimetics.[11–17] Kessler et al. designed a series of αvβ3-selective non-peptide RGD mimetics[18] in which the central glycine residue of the peptide was replaced by azaglycine (i.e., nitrogen replaces the α carbon of glycine).[19] The affinity for αvβ3 integrin of mimetic **4** (IC_{50}=0.1 nM) compares favorably with that of **1** (IC_{50}=2 nM),[18] **2** (IC_{50}=0.6 nM),[9a] and **3** (IC_{50}=4.2 nM).[8b] Linker-equipped mimetic **5** was immobilized onto titanium surfaces via the thiol group to give materials which demonstrated improved cell adhesion and proliferation.[20]

The excellent activity and selectivity profile shown by surface-bound compound **5**, together with its non-peptidic nature, prompted us to undertake studies toward the surface immobilization of this pharmacophore. Kessler prepared compound **5** by

* Corresponding author. Tel.: +61 3 9545 2540; fax: +61 3 9545 2446; e-mail address: andrew.riches@csiro.au (A.G. Riches).

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2012.09.002

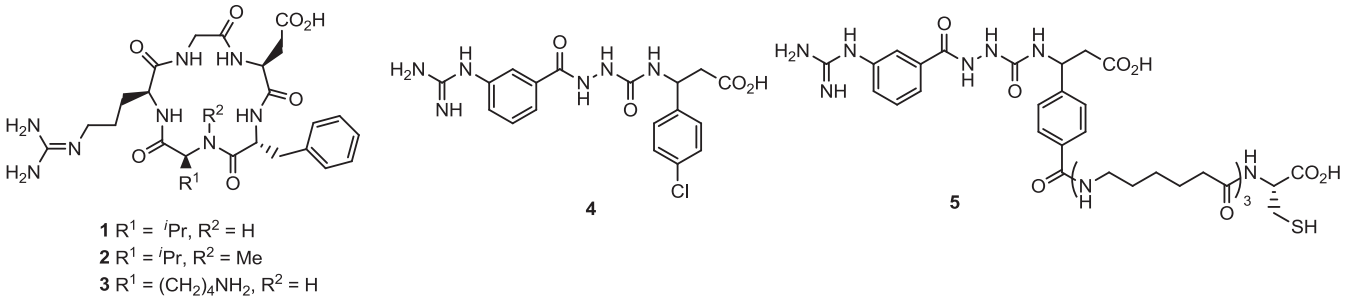
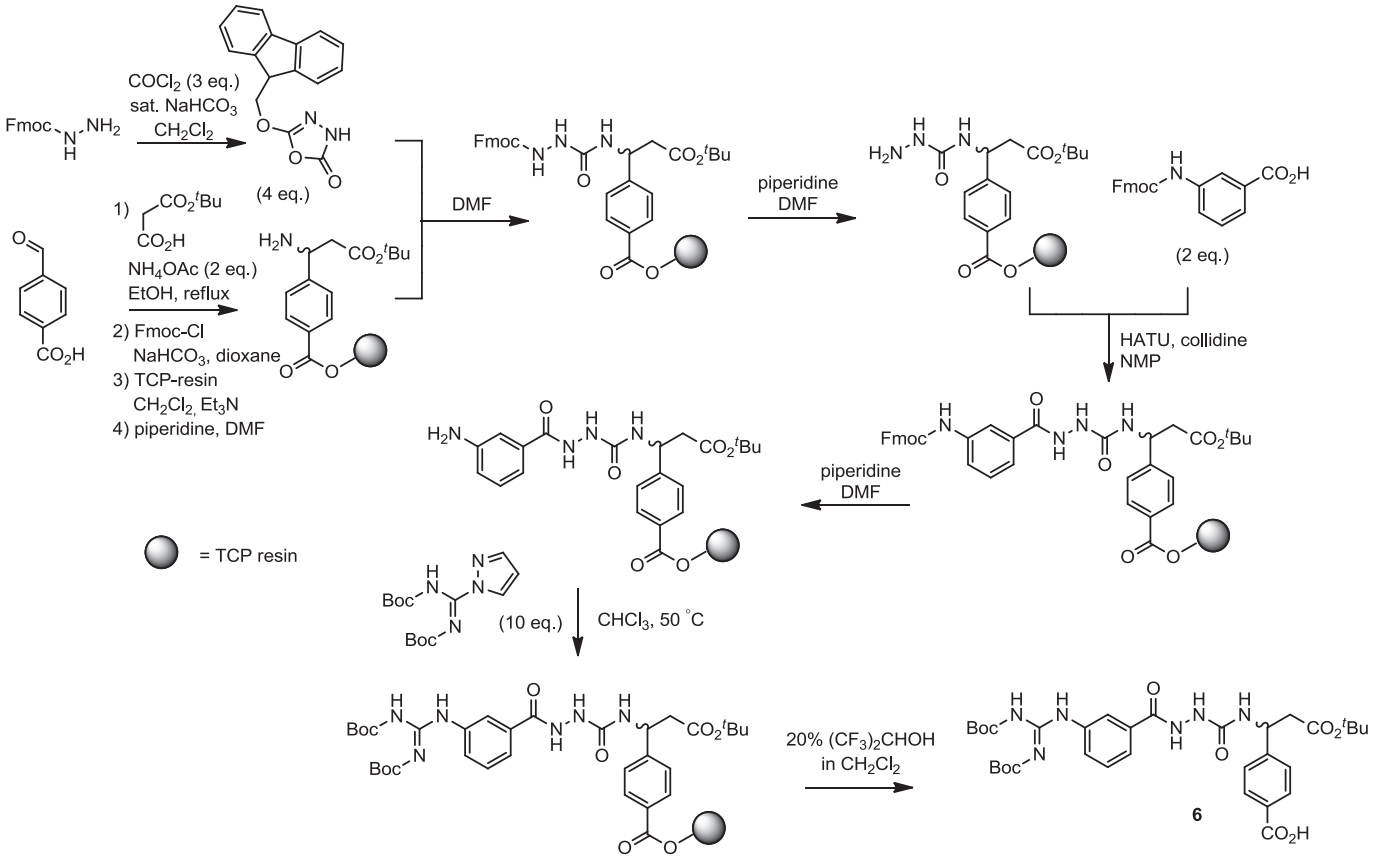


Fig. 1. Cyclic RGD peptides (**1**–**3**), non-peptide RGD mimetic (**4**), and mimetic equipped with a tether for surface immobilization (**5**).

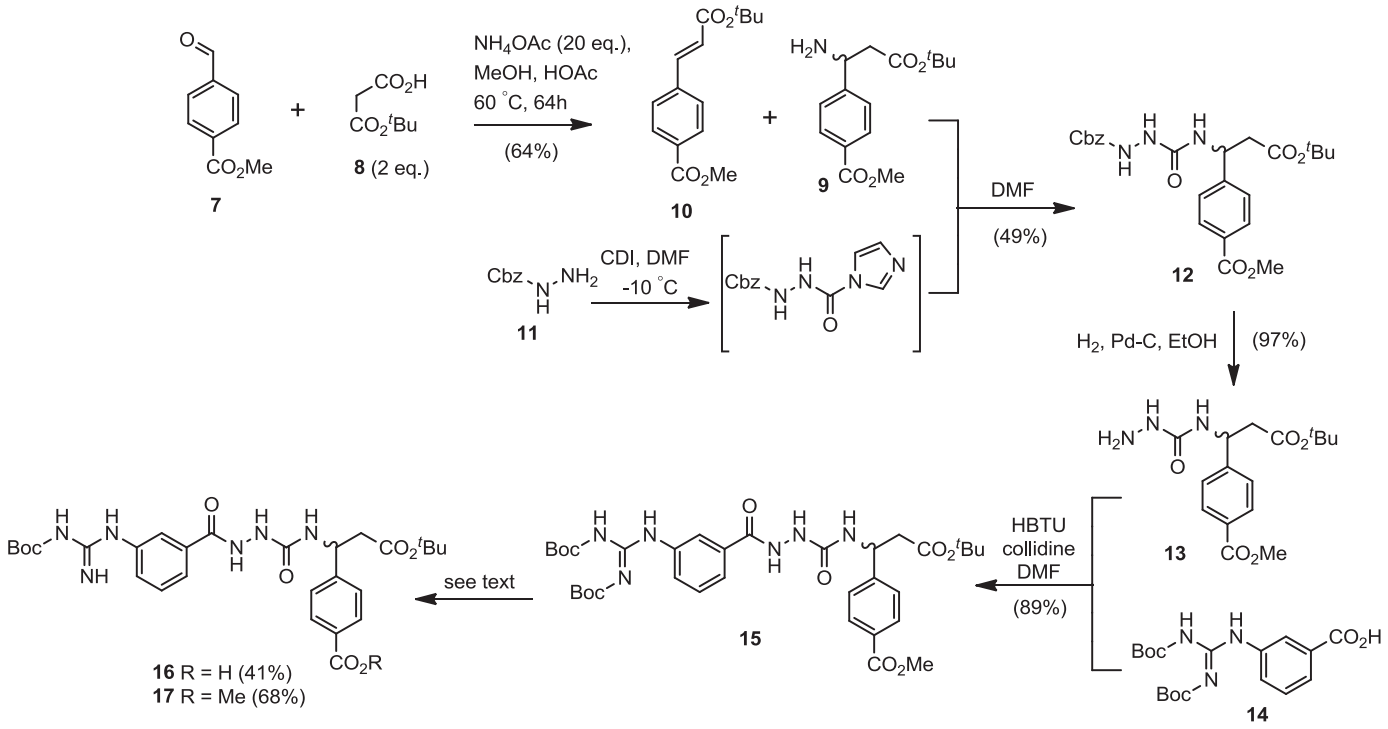
coupling amino-functionalized linkers to the carboxylic acid group of the triprotected mimetic **6** (Scheme 1).[20] Acid **6** was prepared by a solid-phase strategy in which 4-formylbenzoic acid was converted to the Fmoc-protected β-amino-*tert*-butyl ester and linked to the trityl chloride polystyrene (TCP) resin through the aryl carboxylic acid. Following Fmoc cleavage, Fmoc-protected azaglycine and amino benzoate fragments were successively added and deprotected before finally installing the protected guanidino group. Compound **6** was then released from the resin under mild acidic conditions (hexafluoroisopropanol in dichloromethane), which left the sensitive *tert*-butyl ester and *N*-Boc protecting groups intact. We required multi-gram quantities of carboxylic acid **6** for immobilization onto biomaterials. Due to the technical difficulty and expense involved in scaling up solid-phase chemistry, we sought to develop a scalable, solution-phase route to compound **6**.

2. Results and discussion

The resin used in the solid-phase synthesis was initially replaced by a methyl group, with the expectation that the methyl ester could be saponified following assembly of the protected mimetic without harm to the *tert*-butyl ester and the *N*-Boc groups (Scheme 2). Thus methyl 4-formylbenzoate **7** was reacted with ammonium acetate (2 equiv) and mono-*tert*-butyl malonate **8**[21] in refluxing ethanol to give a mixture of desired β-amino ester **9** (minor) and cinnamate ester **10** (major).[22] Pure racemic[23] amino ester **9** was readily isolated in 23% yield by acid–base extraction. The yield of **9** was improved to 64% by using 20 equiv of ammonium acetate and conducting the reaction in methanol containing 10% acetic acid at the lower temperature of 60 °C over a longer time (64 h). Lower reaction temperature (50 °C) resulted in incomplete decarboxylation of the intermediate Mannich adduct. The Fmoc



Scheme 1. Solid-phase synthesis of triprotected carboxylic acid **6** (adapted from Ref. 20).



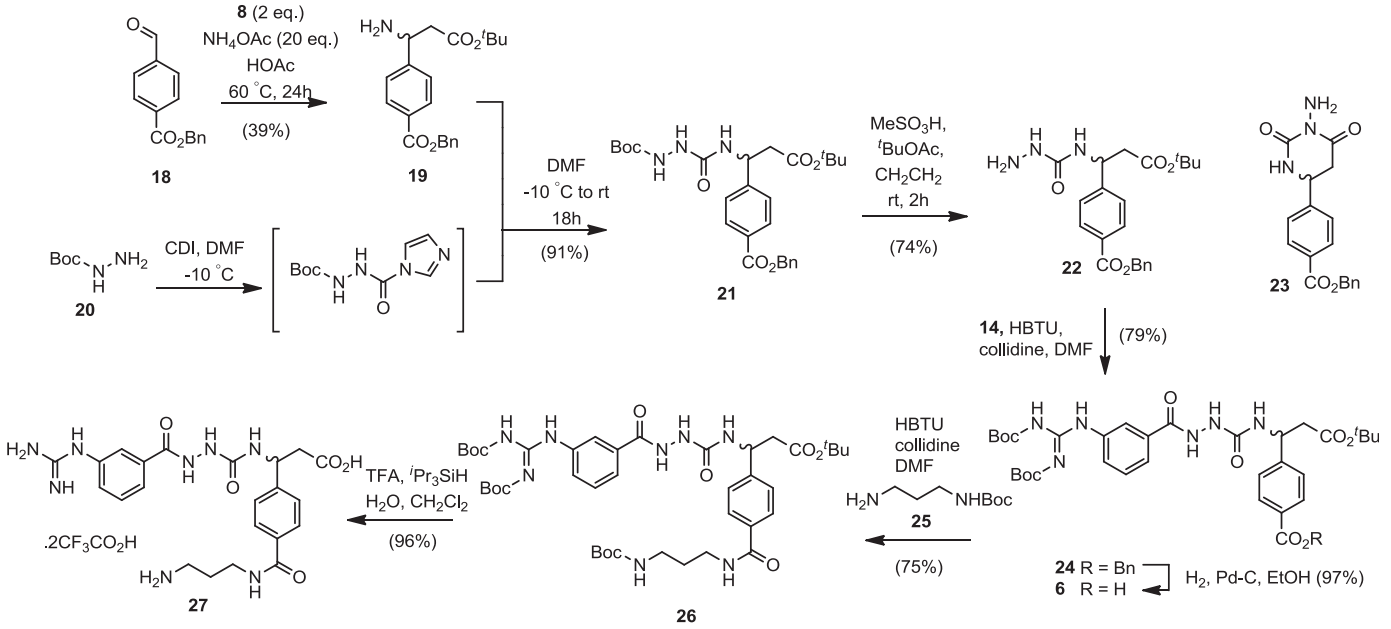
Scheme 2. Preparation of RGD mimetic, using a methyl ester to protect the aryl carboxylic acid.

protecting group used in the solid-phase synthesis was replaced by Cbz protection of the azaglycine in order to avoid non-volatile Fmoc-derived by-products. Thus reaction of benzyl carbazate **11** with an equimolar amount of *N*,*N*′-carbonyldiimidazole (CDI), followed by treatment of the resulting acyl imidazole in situ with the β-amino ester **9** gave compound **12**. Hydrogenolysis of the Cbz group of **12** under flow conditions using a ThalesNano H-Cube™ gave pure semicarbazide **13** in excellent yield. In contrast, conventional batch hydrogenolysis (1 atm H_2 over Pd—C) of **12**, gave additional side products within 2 h, the amount of which increased as reaction time increased.[24] Coupling of **13** with *N*,*N*′-di-Boc-3-guanidinobenzoic acid **14**[14] gave **15**. Selective cleavage of the methyl ester of **15** proved to be problematic. Treatment of **15** with lithium hydroxide hydrolyzed the ester, but also cleaved one of the Boc groups to give **16** in 41% yield.[25] Milder treatment of **15** with aq potassium carbonate in methanol at 45 °C for 6 h cleaved the Boc group in preference to the methyl ester to give **17** (68%) as the major product. Other hydrolytic methods such as barium hydroxide in methanol,[26,27] and nucleophilic methods including lithium iodide in refluxing pyridine,[28] sodium cyanide in DMSO,[29] and 4-aminothiophenol/cesium carbonate[30] all failed to yield **6**. While compound **16** is a potential substrate for amide coupling, the low isolated yield of **16** prompted the redesign of the protection strategy.

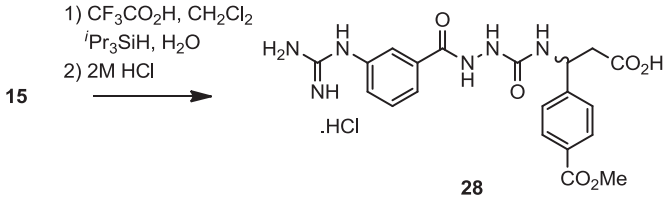
Benzyl ester **18** was identified as the starting material for a modified approach to **6** shown in Scheme 3, as benzyl esters undergo hydrogenolytic cleavage under conditions which should not affect the remaining protecting groups. Under the conditions previously developed for the preparation of methyl ester **9**, conversion of **18** to **19** was accompanied by significant transesterification of the benzoate ester with the methanol solvent to give an inseparable mixture of **9** and **19**. The use of acetic acid alone as the solvent for this reaction gave, after extractive workup, pure benzyl ester **19** in 39% yield. The remaining non-acid extractable fraction consisted of a complex mixture, which was not further characterized. The benzyl ester precluded hydrogenolytic removal of a Cbz group from the azaglycine portion of the system (cf. **12**→**13** in Scheme 2) and the azaglycine fragment was therefore incorporated in Boc-protected form by treatment of *tert*-butyl carbazate **20** with CDI, followed by **19** to give compound **21**. Attempts to selectively cleave the *N*-Boc group in the presence of the *tert*-butyl ester using either trifluoroacetic acid in dichloromethane[31,32] or anhydrous hydrochloric acid in various solvents[33] were unsuccessful. However, exposure of **21** to 3 equiv of methanesulfonic acid in *tert*-butyl acetate/dichloromethane for 2 h at room temperature gave the required semicarbazide **22** in 74% yield.[34] Careful monitoring of this reaction was necessary, as the product **22** slowly underwent further cyclization to **23**. The ^{1}H NMR spectrum of **23** showed the absence of the *tert*-butyl ester and the presence of two non-equivalent methylene protons. Coupling of semicarbazide **22** with carboxylic acid **14** gave **24** in 79% yield after chromatographic purification. Hydrogenolysis of **24** efficiently gave carboxylic acid **6**, spectroscopic data for which is reported here for the first time.

In order to prepare an amine-functionalized peptidomimetic, carboxylic acid **6** was coupled with mono-*N*-Boc-propane-1,3-diamine **25** to give tetraprotected conjugate **26** in 75% yield after purification by silica gel chromatography. Global deprotection of **26** using trifluoroacetic acid gave the highly polar, water-soluble bis-trifluoroacetate salt **27** in 96% yield after purification by reverse-phase chromatography. Studies in which **27** and related compounds are being used to functionalize surfaces are currently underway.

While compound **15** did not serve as a precursor to **6**, as originally planned, it afforded an opportunity to prepare an RGD mimetic suitable for testing in a cell attachment inhibition assay. Thus treatment of **15** with trifluoroacetic acid to cleave the *tert*-butyl ester and both *N*-Boc groups was followed by conversion of the resulting trifluoroacetate salt to the corresponding hydrochloride salt **28** (Scheme 4). The RGD mimetic **28** was assayed for inhibition of L929 fibroblast attachment to vitronectin-coated polystyrene plates. Compound **28** showed superior inhibition of cell attachment, i.e., effectively blocked the integrin receptor's ability to bind



Scheme 3. Preparation of RGD mimetic **27**, equipped with amine-bearing spacer.



Scheme 4. Preparation of RGD mimetic **28**.

to the epitope on vitronectin (EC_{50} 4.5 μM) when compared to both cRGDfK **3** (EC_{50} 92 μM) and linear GRGDS (EC_{50} 240 μM) (Fig. 2). As expected, the linear GRGDS peptide[8a] bound to and blocked the integrin receptor less effectively than the cRGDfK **3** peptide. The non-binding cyclic peptide cRADfK[35] served as a negative control

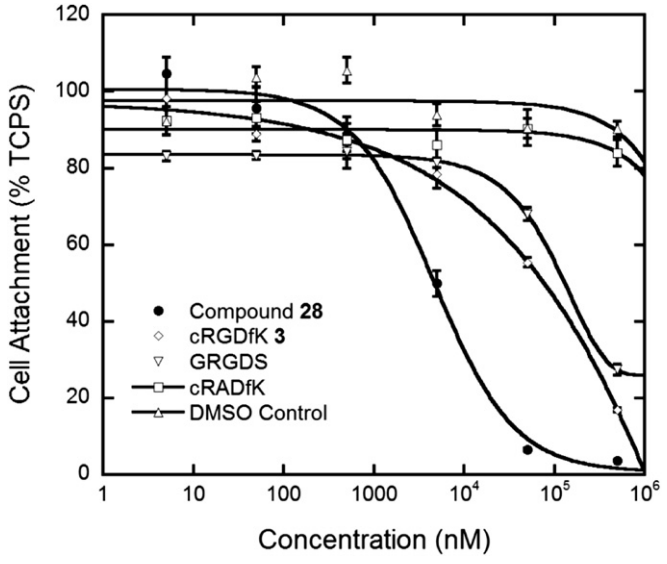


Fig. 2. Inhibition of L929 fibroblast attachment by mimetic **28** in comparison to **1** and linear RGD peptide.

and did not inhibit cell attachment. Also included in Fig. 2 are DMSO controls where DMSO only was present at the same concentration as in the solutions of compound **28**, cRGDfK **3**, linear GRGDS, and cRADfK. Thus inhibition of cell attachment could be attributed directly to the listed compounds and not to the presence of small DMSO concentrations, which could cause cell death.

3. Conclusion

This study successfully developed a scalable, solution-phase synthesis of Kessler's triprotected RGD peptide mimetic **6**. Two approaches using different protecting group strategies were evaluated, whereby the carboxylic acid was protected as either a methyl or a benzyl ester. The methyl ester route was compromised by the loss of an *N*-Boc group during the final ester cleavage step. In contrast, the benzyl ester was selectively removed at the final step to ultimately deliver a scalable synthesis of carboxylic acid **6** in six steps from 4-formylbenzoic acid. Conjugation of **6** with protected diamine linker **25** was followed by global deprotection to yield amine-functionalized mimetic **27**, which is particularly suitable for covalent attachment to surfaces equipped with amine-reactive groups. RGD mimetic ester **28** was prepared by trifluoroacetic acid deprotection of **15** and shown to have activity comparable to cyclic peptide **3** in a cell adhesion inhibition assay. It is anticipated that biomaterials modified with immobilizable RGD peptide mimetics such as **27** will exhibit desirable properties in regard to the modulation of cellular responses. By allowing cost-effective access to larger quantities of this type of surface-immobilizable RGD mimetic than have hitherto been available, the synthetic route described in this study will contribute to further expanding the applications of peptide mimetics in biomedical applications.

4. Experimental section

4.1. Synthesis

4.1.1. General. Melting points were determined on Büchi B-545 digital melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet 6700 FTIR instrument using

attenuated total reflectance (ATR) on a diamond crystal. The ^{1}H NMR spectra were recorded at 200 MHz (Bruker AC200SX), 400 MHz (Bruker Av400) or 500 MHz (Bruker DRX500). ^{13}C NMR spectra were recorded at 50, 100 or 125.7 MHz on the same instruments. The ^{19}F NMR spectra were recorded at 188.3 or 376 MHz on the same instruments. Chemical shifts (δ) are measured in parts per million using known solvent chemical shifts as an internal standard. $CDCl_3$ was used as solvent unless otherwise stated. Low-resolution electron impact (EI) positive ion mass spectra were run on a ThermoQuest MAT95XL mass spectrometer using an ionization energy of 70 eV. High-resolution EI mass spectra were obtained with a resolution of 5000–10,000 using PFK as the reference compound. Low-resolution positive and negative ion electrospray (ES) mass spectra were acquired either with a Shimadzu LCMS-2010EV mass spectrometer using a cone voltage of 50 V, the source was maintained at 80 °C using methanol containing 0.1% formic acid as solvent at a flow rate of 0.1 mL/min, or with a VG Platform mass spectrometer using a cone voltage of 50 or 30 V, the source was maintained at 80 °C using methanol solvent with a flow rate of 0.04 mL/min. High-resolution positive ion electrospray mass spectra were acquired either in house with a Micromass Q-TOF II mass spectrometer using a cone voltage of 35 V and a capillary voltage of 3.0 kV and the sample introduced by direct infusion at a rate of 2 μL/min using NaI as an internal calibrant, or by ChemicalAnalysis Pty Ltd. with an Agilent HPLC system, series 1100, Agilent LC/MSD TOF detector, using 0.1% formic acid in methanol as solvent at a flow rate of 0.3 mL/min and calibrating the spectrum using an Agilent TOF reference mass solution kit containing the reference ions of purine and HP-0921 (hexakis(1*H*,1*H*,3*H*-tetrafluoropropoxy)phosphazine). Thin layer chromatography (TLC) was performed on Merck pre-coated 0.25 mm silica F_{254} aluminum-backed plates (#5554) or RP-18 F_{254} plates (#5559). Column chromatography was performed using Merck (#9385, 230–400 mesh) silica gel 60, while radial chromatography was performed using a Harrison Research Chromatotron (#7924 T). Reverse-phase column chromatography was performed using Chromatarex C_{18} silica (Fuji Silysia Chemical Ltd). All anhydrous reactions were performed under a dry argon atmosphere. All extracts were dried over anhydrous magnesium sulfate unless otherwise stated. All inorganic solutions are aqueous unless otherwise specified. Anhydrous solvents were dried using a Pure Process Technology Glass Contour Solvent Purification System. Petroleum spirit (P.S.) refers to the fraction of bp 40–60 °C.

4.1.2. (±)-Benzyl-(1′-amino)-tert-butyl-4-propanoyl-benzoate ***9***. Acetic acid (25 mL) was added to a mixture of methyl 4-formylbenzoate **7** (9.57 g, 58.3 mmol), ammonium acetate (89.9 g, 1170 mmol), mono-*tert*-butyl malonate **8**[21] (18.7 g, 117 mmol), and methanol (120 mL) and the mixture heated to 60 °C for 64 h. The methanol was evaporated and the residue partitioned between EtOAc and satd $NaHCO_3$. The organic phase was extracted with 1 M aq citric acid (×3). The aqueous phase was made basic to approximately pH 9 with $NaHCO_3$ and extracted with EtOAc (×3). The combined extracts were washed with brine, dried ($MgSO_4$), and evaporated to give **9** (10.4 g, 64%) as a pale-yellow syrup, which crystallized on standing, mp 63–5 °C; ν_{max} 2978, 1716, 1611, 1436 cm^{-1}; δ_H 8.02 (d, *J*=8.3 Hz, 2H), 7.46 (d, *J*=8.3 Hz, 2H), 4.45 (t, *J*=6.8 Hz, 1H), 3.93 (s, 3H), 2.61 (d, *J*=6.8 Hz, 2H), 1.43 (s, 9H); δ_C 170.9, 166.8, 149.8, 129.8, 129.1, 126.3, 80.9, 52.5, 52.0, 45.0, 28.0; *m*/*z* (ES) 581 (8%, [2M]Na$^+$), 302 (100); HRMS (ES): [2M]Na$^+$, found 581.2834. $C_{30}H_{42}N_2NaO_8$ requires 581.2839.

4.1.3. (±)-Methyl-(1′-{[benzyloxyhydrazino]carbonyl}-amino)-tert-butyl-4-propanoyl-benzoate ***12***. A solution of 1,1′-carbonyldiimidazole (1.78 g, 11.0 mmol) in DMF (10 mL) was cooled to −10 °C and a solution of benzyl carbazate **11** (1.66 g, 10.0 mmol) in DMF (5 mL) was added over 5 min. Stirring was continued at −10 °C for 15 min, then a solution of amine **9** (2.80 g, 10.0 mmol) in DMF (15 mL) was added over for 10 min and the mixture was allowed to warm to room temperature overnight. The mixture was diluted with water (100 mL) and extracted twice with 1:1 EtOAc/P.S. (1 M aq citric acid (10 mL) was added to break initial emulsion). The combined organic phase was washed with water (×2), brine, dried (Na_2SO_4), and evaporated. The crude product was chromatographed through a short column of SiO_2, eluting with 40→60% EtOAc in P.S. The major fraction gave essentially pure **12** (2.29 g, 49%) as a colorless foam, mp 135–6 °C; R_f (60% EtOAc in P.S.) 0.28; ν_{max} 3317, 2979, 1714, 1664 (sh), 1612, 1532 cm^{-1}; δ_H 7.93 (*J*=8.3 Hz, d, 2H), 7.38–7.30 (m, 7H), 6.60 (br d, *J*=8.6 Hz, 1H), 6.57 (br s, 1H), 6.37 (br s, 1H), 5.29–5.24 (m, 1H), 5.16 (AB quartet, J_{AB}=12.1 Hz, 2H), 3.88 (s, 3H), 2.76–2.72 (m, 2H), 1.28 (s, 9H); δ_C 170.3, 166.8, 158.0, 157.1, 146.4, 135.5, 129.7, 129.0, 128.5, 128.3, 128.1, 126.2, 81.5, 67.8, 52.0, 50.3, 41.4, 27.8; *m*/*z* (ES$^+$) 494 (100%, MNa$^+$), 438 (41); HRMS (ES): MNa$^+$, found 494.1911. $C_{24}H_{29}N_3NaO_7$ requires 494.1903.

4.1.4. (±)-Methyl-(1′-{[hydrazino]carbonyl}-amino)-tert-butyl-4-propanoyl-benzoate ***13***. The Cbz protected semicarbazide **12** (201 mg, 0.426 mmol) was dissolved in ethanol (20 mL) and hydrogenated using a ThalesNano H-Cube™ (Pd–C CatCart™, 1 bar H_2, 50 °C, 1 mL/min). The effluent was evaporated to give **13** (139 mg, 97%) as a colorless solid, which was used without further purification, mp 149–52 °C; ν_{max} 3357, 2978, 1716, 1668, 1611, 1521, 1436 cm^{-1}; δ_H 8.01 (d, *J*=8.2 Hz, 2H), 7.43 (d, *J*=8.2 Hz, 2H), 7.14 (br d, *J*=8.3 Hz, 1H), 5.96 (br s, 1H), 5.37–5.32 (m, 1H), 3.93 (s, 3H), 3.78 (br s, 2H), 2.86–2.77 (m, 2H), 1.35 (s, 9H); δ_C 170.1, 166.7, 159.1, 146.9, 129.8, 129.1, 126.3, 81.4, 52.0, 49.9, 41.9, 27.9; *m*/*z* (ES) 360 (100%, MNa$^+$), 338 (8, MH$^+$), 304 (32); HRMS (ES): [2M]Na$^+$, found 697.3206. $C_{32}H_{46}N_6NaO_{10}$ requires 697.3173.

4.1.5. 3-{[Bis({[(tert-butoxy)carbonyl]amino})methylidene]amino}-benzoic acid ***14***. A mixture of 3-aminobenzoic acid (4.31 g, 31.4 mmol), *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (4.88 g, 15.7 mmol), triethylamine (4.77 g, 47.2 mmol), and methanol (60 mL) was heated at 40 °C for 20 h. The mixture was evaporated and the residue taken into ethyl acetate and washed with 1 M aq HCl, then brine, dried ($MgSO_4$), and evaporated. The crude product was chromatographed through a short column of SiO_2 (20→40% EtOAc in P.S.) to give **14**[25] (5.55 g, 93%) as a colorless solid, mp decomp. >240 °C; R_f (40% EtOAc in P.S.) 0.42; ν_{max} 2980, 1719, 1695 (sh), 1640, 1575 (sh), 1408 cm^{-1}; δ_H 11.58 (br s, 1H), 10.44 (br s, 1H), 8.12–8.06 (m, 2H), 7.83 (dt, *J*=7.8, 1.2 Hz, 1H), 7.44 (t, *J*=8.0 Hz, 1H), 1.51 (s, 18H); δ_C 171.4, 163.5 (br), 154.0, 153.5 (br), 137.3, 130.4, 129.4, 128.2, 126.7, 124.0, 84.2 (br), 80.2 (br), 28.3; *m*/*z* (ES) 402 (100%, MNa$^+$), 380 (23, MH$^+$), 346 (37), 268 (43).

4.1.6. (±)-Methyl-(1′-{[(N′-di-tert-butoxycarbonyl-guanidino-benzoyl)hydrazino]carbonyl}-amino)-tert-butyl-4-propanoyl-benzoate ***15***. The benzoic acid **14** (113 mg, 0.300 mmol) was dissolved in dry DMF (3 mL) and HBTU (136 mg, 0.360 mmol) was added, followed by collidine (364 mg, 3.00 mmol). After stirring at room temperature for 15 min, the semicarbazide **13** (100 mg, 0.300 mmol) was added and stirring continued with TLC monitoring. After 2 h the mixture was poured into 1 M aq citric acid and the resulting precipitate collected. Chromatography through a short silica column (40→80% ethyl acetate in P.S.) gave **15** (186 mg, 89%) as a colorless foam, mp decomp. >143 °C; R_f (60% EtOAc in P.S.) 0.41; ν_{max} 3256, 2979, 1719, 1628, 1568 cm^{-1}; δ_H [MeOH-d_4] 7.97–7.94 (m, 3H), 7.81 (d, *J*=7.9 Hz, 1H), 7.67 (d, *J*=7.9 Hz, 1H), 7.50–7.41 (m, 3H), 5.30 (t, *J*=6.7 Hz, 1H), 3.89 (s, 3H), 2.85–2.75 (m, 2H), 1.51 (br s, 18H), 1.34 (s, 9H); δ_C [MeOH-d_4] 171.6, 169.4, 168.3,

160.1, 155.3, 148.7, 138.3, 134.3, 130.8, 130.2, 127.9, 125.6, 123.5, 82.9 (br), 82.4, 52.7, 52.0, 42.8, 28.5, 28.4; *m/z* (ES) 721 (100%, MNa^+), 699 (25, MH^+); HRMS (ES): MNa^+, found 721.3185. $C_{34}H_{46}N_6NaO_{10}$ requires 721.3173.

4.1.7. (±)-(1′-{[(N′-tert-Butoxycarbonyl-guanidinobenzoyl)hydrazino]carbonyl}-amino)-tert-butyl-4-propanoyl-benzoic acid ***16****.* The methyl ester **15** (210 mg, 0.301 mmol) was dissolved in a mixture of methanol (2 mL), THF (2 mL) and water (1 mL) and lithium hydroxide monohydrate (50 mg, 1.19 mmol) was added. The mixture was stirred at room temperature for 18 h then acetic acid (1 mL) was added and the mixture evaporated (bath temperature ≤rt). The residue was subjected to reverse-phase chromatography through a column of C_{18} silica; 30→70% MeOH in H_2O (0.1% TFA). The major fraction gave **16** as a colorless oil (72 mg, 41%); R_f [RP-18 plate, 50% MeOH in H_2O (0.1% TFA)] 0.24; ν_{max} 3249 (br), 2983, 1665, 1585 (sh) cm^{-1}; δ_H [MeOH-d_4] 7.99 (d, *J*=8.2 Hz, 2H), 7.94 (d, *J*=7.8 Hz, 1H), 7.86 (br s, 1H), 7.64 (t, *J*=7.8 Hz, 1H), 7.58 (br d, *J*=8.2 Hz, 1H), 7.49 (d, *J*=8.2 Hz, 2H), 5.29 (t, *J*=6.8 Hz, 1H), 2.87–2.76 (m, 2H), 1.58 (s, 9H), 1.36 (s, 9H); δ_C [MeOH-d_4] 171.8, 169.7, 168.9, 160.1, 155.8, 153.6, 148.6, 136.0, 135.2, 131.8, 131.1, 129.0, 127.9, 127.0, 86.4, 82.6, 52.3, 43.0, 28.4, 28.3; δ_F [MeOH-d_4] 77.84 (s); *m/z* (ES) 607 (MNa^+), 585 (MH^+); HRMS (ES): MH^+, found 585.2672. $C_{28}H_{37}N_6O_8$ requires 585.2667.

4.1.8. (±)-Methyl-(1′-{[(N′-tert-butoxycarbonyl-guanidinobenzoyl)-hydrazino]carbonyl}-amino)-tert-butyl-4-propanoyl-benzoate ***17****.* The methyl ester **15** (38 mg, 0.0544 mmol) was dissolved in methanol (2 mL) and 1 M aq K_2CO_3 (60 μL) was added. The mixture was heated to 45 °C for 6 h, then evaporated. The residue was diluted with 1 M aq HCl and extracted with EtOAc (×3). The combined organic phase was washed with brine, dried ($MgSO_4$), and evaporated. Chromatography of the crude product through a short silica column [5→10% MeOH in CH_2Cl_2 (1% HOAc)] gave **17** (22 mg, 68%) as a colorless foam; R_f [5% MeOH in CH_2Cl_2 (1% HOAc)] 0.20; ν_{max} 3294, 2979, 1721, 1651, 1585 cm^{-1}; ^1H NMR [MeOH-d_4] δ 7.95 (d, *J*=8.3 Hz, 2H), 7.72 (s, 1H), 7.67 (d, *J*=7.4 Hz, 1H), 7.48 (d, *J*=8.3 Hz, 2H), 7.46–7.38 (m, 2H), 5.28 (t, *J*=6.7 Hz, 1H), 3.88 (s, 3H), 2.86–2.75 (m, 2H), 1.48 (s, 9H), 1.33 (s, 9H); δ_C [MeOH-d_4] 171.8, 169.6, 168.4, 160.2, 159.4, 157.7, 148.8, 139.8, 135.2, 131.1, 130.8, 130.4, 129.2, 128.0, 126.2, 124.7, 82.5, 82.4, 52.8, 52.2, 42.9, 28.6, 28.4; *m/z* (ES) 621 (100%, MNa^+), 599 (34, MH^+); HRMS (ES): MH^+, found 599.2822. $C_{29}H_{39}N_6O_8$ requires 599.2829.

4.1.9. Benzyl 4-formylbenzoate ***18****.* 4-Carboxybenzaldehyde (15.2 g, 101.2 mmol) was dissolved in dry DMF (100 mL) and Cs_2CO_3 (32.8 g, 101 mmol) was added, followed by benzyl bromide (15.7 g, 91.8 mmol). The mixture was stirred at room temperature for 3 h then filtered. The filtrate was diluted with 20% EtOAc in P.S. and washed successively with water (×2), satd $NaHCO_3$, and brine then dried ($MgSO_4$) and evaporated to give benzyl 4-formylbenzoate **18**[36] (21.3 g, 97%) as a colorless solid. This aldehyde oxidized on storage and was used immediately without further purification; δ_H 10.12 (s, 1H), 8.26 (d, *J*=8.3 Hz, 2H), 7.97 (d, *J*=8.3 Hz, 2H), 7.50–7.37 (m, 5H), 5.42 (s, 2H); δ_C 191.8, 165.6, 139.4, 135.7, 135.3, 130.5, 129.7, 128.9, 128.7, 128.5, 67.5.

4.1.10. (±)-Benzyl-(1′-amino)-tert-butyl-4-propanoyl-benzoate ***19****.* A mixture of 4-carboxybenzyl benzaldehyde **18** (20.3 g, 84.5 mmol), mono-*tert*-butyl malonate ammonium salt **8**[23] (29.9 g, 169 mmol), ammonium acetate (97.7 g, 1270 mmol), and acetic acid (150 mL) was heated to 60 °C for 24 h. The mixture was cooled and poured into ice-water. The pH was adjusted to ca. 9 by addition of concentrated aq ammonium hydroxide and extracted with EtOAc (×2). The organic extracts were diluted with an equal volume of P.S. and extracted with 1 M aq citric acid (×3). The combined citric acid extracts were made basic to pH 9 by addition of concentrated aq ammonium hydroxide then extracted with ethyl acetate (×3). The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated to give the β-amino ester **19** (11.8 g, 39%) as a pale-yellow oil; ν_{max} 2978, 1715, 1610 cm^{-1}; δ_H 8.06 (d, *J*=8.4 Hz, 2H), 7.49–7.45 (m, 4H), 7.39–7.29 (m, 3H), 5.38 (s, 2H), 4,45 (t, *J*=6.8 Hz, 1H), 2.60 (d, *J*=6.8 Hz, 2H), 1.44 (s, 9H); δ_C 171.1, 166.4, 150.3, 136.2, 130.2, 129.3, 128.8, 128.4, 128.3, 126.6, 81.2, 66.8, 52.8, 45.3, 28.3; *m/z* (ES) 378 (27%, MNa^+); HRMS (ES): MNa^+, found 378.1693; $C_{21}H_{25}NNaO_4$ requires 378.1681.

4.1.11. (±)-Benzyl-(1′-{tert-butoxycarbonyl[hydrazino]carbonyl}-amino)-tert-butyl-4-propanoyl-benzoate ***21****.* A solution of carbonyldiimidazole (6.40 g, 39.5 mmol) in dry DMF (40 mL) was cooled to −10 °C and a solution of *tert*-butyl carbazate **20** (4.74 g, 35.9 mmol) was added over 10 min. The mixture was stirred at −10 °C for 15 min and then a solution of the amino ester **19** (12.8 g, 35.9 mmol) in DMF (20 mL) was added over 10 min. The mixture was then allowed to stir at room temperature for 18 h. Aq citric acid (1 M) was added to give pH <5 and the mixture extracted twice with 1:1 EtOAc/P.S. The combined organic extracts were washed successively with water (×2), 1 M aq citric acid and brine, then dried (Na_2SO_4) and evaporated to give **21** (16.8 g, 91%) as a colorless foam, mp 61–3 °C; ν_{max} 3326, 2978, 1717, 1669 (sh), 1611, 1536 cm^{-1}; δ_H 7.98 (d, *J*=8.4 Hz, 2H), 7.43–7.30 (m, 7H), 6.71 (br d, *J*=8.6 Hz, 1H), 6.53 (br s, 1H), 6.45 (br s, 1H), 5.32 (s, 2H), 5.31–5.26 (m, 1H), 2.81–2.70 (m, 2H), 1.44 (s, 9H), 1.27 (s, 9H); δ_C 170.5, 166.4, 158.1, 156.0, 146.8, 136.3, 130.1, 129.3, 128.8, 128.4, 128.3, 126.5, 82.3, 81.8, 66.8, 50.2, 41.4, 28.3, 28.1; *m/z* (ES) 536 (100%, MNa^+); HRMS (ES): MNa^+, found 536.2375. $C_{27}H_{35}N_3NaO_7$ requires 536.2373.

4.1.12. (±)-Benzyl-(1′-{[hydrazino]carbonyl}-amino)-tert-butyl-4-propanoyl-benzoate ***22****.* The *N*-Boc-semicarbazide **21** (34.4 g, 67.0 mmol) was dissolved in dry dichloromethane (60 mL) and *tert*-butyl acetate (180 mL) was added. The mixture was cooled in an ice-water bath and methanesulfonic acid (19.5 g, 203 mmol) was added. After 5 min, the mixture was removed from the cold bath and allowed stir at room temperature for 2 h, after which time reaction was shown to be complete by TLC analysis. The mixture was diluted with an equal volume of P.S. and extracted with 1 M aq citric acid (×3). The combined citric acid extracts were washed with 1:1 EtOAc/PS and then cooled in ice as the pH was adjusted to ca. 10 by addition of concentrated aq ammonium hydroxide. The mixture was then extracted with dichloromethane (×3). The combined dichloromethane extracts were washed with brine, dried (Na_2SO_4), and evaporated to give semicarbazide **22** (20.60 g, 74%) as a white solid, mp 135–7 °C; ν_{max} 3358, 2978, 1717, 1675, 1611, 1523 cm^{-1}; δ_H 8.01 (d, *J*=8.4 Hz, 2H), 7.43–7.30 (m, 7H), 7.08 (br d, *J*=8.6 Hz, 1H), 6.05 (br s, 1H), 5.33 (s, 2H), 5.32–5.26 (m, 1H), 3.77 (br s, 2H), 2.82–2.72 (m, 2H), 1.31 (s, 9H); δ_C 170.3, 166.4, 159.7, 147.4, 136.3, 130.1, 129.3, 128.7, 128.4, 128.3, 126.5, 81.6, 66.8, 50.1, 42.1, 28.1; *m/z* (ES) 436 (100%, MNa^+), 380 (25); HRMS (ES): MNa^+, found 436.1845. $C_{22}H_{27}N_3NaO_5$ requires 436.1848.

4.1.13. (±)-Benzyl 4-(1-amino-2,6-dioxohexahydropyrimidin-4-yl)-benzoate ***23****.* Boc-protected semicarbazide **21** (1.03 g, 2.01 mmol) was treated with $MeSO_3H$ and ^tBuOAc as above except that the reaction mixture was allowed stir at room temperature for 11 days before workup. The crude product was purified by radial chromatography (5% MeOH in EtOAc) to give the cyclized compound **23** (289 mg, 42%) as a colorless solid, mp 58–59 °C; R_f (5% MeOH in EtOAc) 0.38; ν_{max} 3442, 2977, 2938, 2878, 1652, 1437 cm^{-1}; δ_H 8.00 (d, *J*=8.3 Hz, 2H), 7.41–7.24 (m, 7H), 6.86 (br s, 1H), 5.29 (s, 2H), 4.72–4.62 (m, 1H), 4.45 (br s, 2H), 2.91 (dd, *J*=16.6, 4.8 Hz, 1H), 2.69 (dd, *J*=16.6, 9.6 Hz, 1H); δ_C 166.0, 165.8, 153.7, 143.7, 135.9, 131.0, 130.9, 128.9, 128.6, 128.4, 126.3, 67.2, 50.9, 39.5; *m/z* (EI) 339 (47%,

$M^{+\cdot}$), 254 (100), 240 (61), 147 (52), 131 (47), 91 (90); HRMS (EI): $M^{+\cdot}$, found 339.1203. $C_{18}H_{17}N_3O_4$ requires 339.1214.

*4.1.14. (±)-1′-{[(N′-Di-tert-butoxycarbonyl-guanidinobenzoyl)hydrazino]carbonyl}-amino-tert-butyl-4-propanoyl-benzoic acid **24**.* The aryl carboxylic acid **14** (3.03 g, 7.99 mmol) was dissolved in dry DMF (25 mL), and HBTU (3.64 g, 9.60 mmol) and collidine (9.68 g, 79.9 mmol) were added, followed by the semicarbazide **22** (3.30 g, 7.99 mmol). The mixture was stirred at room temperature for 4 h, then partitioned between 1 M aq HCl and EtOAc. The combined organic phase was washed with water (×3), brine, dried ($MgSO_4$), and evaporated. The crude product was chromatographed through a short silica column [20→60% EtOAc in P.S. (1% HOAc)]. The major fraction gave the title compound **24** (4.89 g, 79%) as a colorless foam; R_f (60% EtOAc in P.S.) 0.31; ν_{max} 3255, 2980, 1717, 1627, 1569 cm^{-1}; δ_H 11.63 (br s, 1H); 10.34 (br s, 1H), 9.15 (br s, 1H), 7.95–7.81 (m, 5H), 7.52 (d, *J*=7.7 Hz, 1H), 7.44–7.23 (m, 8H), 6.79 (br d, *J*=8.1 Hz, 1H), 5.32 (s, 2H), 5.28–5.21 (m, 1H), 2.76–2.62 (m, 2H), 1.54 (br s, 9H), 1.47 (br s, 9H), 1.24 (s, 9H); δ_C 170.2, 166.6, 166.2, 163.3, 157.8, 154.0, 153.2, 146.8, 137.0, 136.2, 132.4, 130.0, 129.3, 129.0, 128.6, 128.2, 128.1, 126.6, 124.1, 121.9, 84.0, 81.4, 80.1, 66.6, 50.7, 41.6, 28.2, 27.9; *m/z* (ES) 797 (100%, MNa^+); HRMS (ES): MNa^+, found 797.3471. $C_{40}H_{50}N_6NaO_{10}$ requires 797.3486.

*4.1.15. (±)-1′-{[(N′-Di-tert-butoxycarbonyl-guanidinobenzoyl)hydrazino]carbonyl}-amino-tert-butyl-4-propanoyl-benzoic acid **6**.* A solution of the benzyl ester **24** (8.02 g, 10.4 mmol) in abs ethanol (200 mL) was hydrogenated over 10% Pd–C (448 mg), at 1 atm H_2 for 2 h. Evaporation gave **6** as a colorless syrup (6.86 g, 97%), which retained traces of ethanol even after prolonged drying in vacuo; R_f (60% EtOAc in P.S.) 0.19; ν_{max} 2978, 1715, 1640 cm^{-1}; δ_H (MeOH-d_4) 8.00–7.96 (m, 3H), 7.80 (br d, *J*=8.0 Hz, 1H), 7.67 (br d, *J*=7.8 Hz, 1H), 7.49–7.42 (m, 3H), 5.32–28 (m, 1H), 2.86–2.76 (m, 2H), 1.51 (s, 18H), 1.34 (s, 9H); δ_C (MeOH-d_4) 171.7, 169.7, 169.6, 160.2, 155.4, 148.5, 138.3, 134.5, 131.0, 130.3, 128.0, 127.8, 125.6, 123.6, 83.2(br), 82.4, 52.1, 42.9, 28.5, 28.4; *m/z* (ES) 707 (MNa^+); HRMS (ES): MNa^+, found 707.3016. $C_{33}H_{44}N_6NaO_{10}$ requires 707.3017.

*4.1.16. (±)-tert-Butyl-3-{[(N′-3-di-tert-butoxycarbonyl-guanidinobenzoyl)hydrazino]carbonyl}-amino-3-(4-(3-tert-butoxycarbonylaminopropyl)-carboxamidophenyl)propionoate **26**.* Carboxylic acid **25** (1.04 g, 1.52 mmol) was dissolved in dry DMF (10 mL) and HBTU (692 mg, 1.82 mmol) was added, followed by collidine (1.85 g, 15.3 mmol) and then 1-Boc-propane-1,3-diamine (392 mg, 2.25 mmol). The mixture was stirred at ambient temperature for 4 h (complete by TLC) and then partitioned between 1:1 EtOAc/P.S. and 1 M HCl. The combined organic phase was washed with water (×3), brine, dried ($MgSO_4$), and evaporated. The crude product was chromatographed through a short column of SiO_2, eluting with 60→100% EtOAc in P. S. (1% HOAc) to give the title product **26** as a colorless foam (964 mg, 75%); R_f (99:1 EtOAc/HOAc) 0.56; ν_{max} 3300, 2978, 1719, 1692, 1644, 1539 cm^{-1}; δ_H [MeOH-d_4] 7.95 (br s, 1H), 7.83–7.76 (3H, m), 7.67 (br d, *J*=7.9 Hz, 1H), 7.49–7.42 (m, 3H), 5.29 (t, *J*=6.8 Hz, 1H), 3.40 (t, *J*=6.8 Hz, 2H), 3.12 (t, *J*=6.8 Hz, 2H), 2.86–2.75 (m, 2H), 1.78–1.71 (m, 2H), 1.51 (s, 18H), 1.43 (s, 9H), 1.34 (s, 9H); δ_C [MeOH-d_4] 175.3, 171.8, 170.0, 169.7, 160.2, 158.7, 155.4, 147.1, 138.4, 134.7, 134.6, 130.3, 128.6, 128.1, 127.9, 125.7, 123.7, 83.3(br), 82.5, 80.2, 52.0, 42.9, 38.9, 38.4, 30.9, 28.9, 28.5, 28.4, 20.9; *m/z* (ES) 863 (100%, MNa^+); HRMS (ES): MNa^+, found 863.4285. $C_{41}H_{60}N_8NaO_{11}$ requires 863.4279.

*4.1.17. (±)-3-{[(N′-3-Guanidinobenzoyl)hydrazino]carbonyl}-amino-3-(4-(3-aminopropyl)-carboxamidophenyl)propionic acid bis-trifluoroacetate salt **27**.* Compound **26** (494 mg, 0.587 mmol) was treated with a mixture of TFA/CH_2Cl_2/$^iPr_3SiH$/H_2O (96:96:2:2) (10 mL) at room temperature for 3 h then the mixture was evaporated. The residue was partitioned between CH_2Cl_2 and H_2O. The aqueous phase was washed successively with CH_2Cl_2 and EtOAc then freeze-dried to give **27** as a colorless syrup (404 mg, 96%); R_f [RP-18 plate, 20% CH_3CN in H_2O (0.1% TFA)] 0.47; ν_{max} 3070, 2456, 1661, 1581 cm^{-1}; δ_H [MeOH-d_4] 7.85–7.78 (m, 4H), 7.58 (t, *J*=7.9 Hz, 1H), 7.52–7.46 (m, 3H), 5.30 (t, *J*=6.5 Hz, 1H), 3.49 (t, *J*=6.5 Hz, 2H), 2.99 (t, *J*=7.3 Hz, 2H), 2.93–2.82 (m, 2H), 1.99–1.92 (m, 2H); δ_C [MeOH-d_4] 173.1, 169.2, 167.9, 161.1 (q, *J*=37.1 Hz), 158.9, 156.7, 146.3, 135.5, 134.1, 132.8, 130.2, 128.6, 127.4, 126.5, 126.2, 124.2, 116.6 (q, *J*=202.0 Hz), 50.6, 40.3, 37.1, 36.3, 27.5; δ_F [MeOH-d_4] −77.4; *m/z* (ES) 485 (100%, MH^+); HRMS (ES): MH^+, found 485.2260. $C_{22}H_{28}N_8O_5$ requires 485.2261.

*4.1.18. (±)-3-{[(N′-3-Guanidinobenzoyl)hydrazino]carbonyl}-amino-3-(4-carboxamidophenyl)propionic acid hydrochloride salt **28**.* The methyl ester **15** (388 mg, 0.555 mmol) was treated with a mixture of TFA/CH_2Cl_2/$^iPr_3SiH$/H_2O (96:96:2:2) (5 mL) at room temperature for 3 h and then evaporated. The crude product was purified by reverse-phase chromatography through a short column of C_{18} silica (5→40% CH_3CN in H_2O, 0.1% CF_3CO_2H). The major fraction was collected and evaporated, and the residue dissolved in 2 M HCl and evaporated (×2) to give **28** (178 mg, 69%) as a colorless solid, mp decomp. >226 °C; R_f [RP-18 plate, 40% CH_3CN in H_2O (0.1% TFA)] 0.22; ν_{max} 3318, 3198, 1670, 1580 cm^{-1}; δ_H [DMSO-d_6] 10.25 (s, 1H), 10.14 (s, 1H), 8.19 (s, 1H), 7.91 (d, *J*=8.3 Hz, 2H), 7.77 (d, *J*=7.8 Hz, 1H), 7.71 (s, 1H), 7.66–7.48 (m, 7H), 7.41 (d, *J*=7.9 Hz, 1H), 7.19 (d, *J*=8.5 Hz, 1H), 5.18–5.11 (m, 1H), 3.84 (s, 3H), 2.83–2.71 (m, 2H); δ_C [DMSO-d_6] 171.8, 166.1, 165.6, 157.3, 155.9, 148.6, 135.5, 134.1, 129.8, 129.1, 128.2, 127.4, 126.8, 125.3, 123.2, 52.1, 50.0; *m/z* (ES) 443 (100%, MH^+), 194 (84); HRMS (ES): MH^+, found 443.1690. $C_{20}H_{23}N_6O_6$ requires 443.1674.

4.2. Cell culture

L929 mouse fibroblasts (cell line ATCC-CCL-1, Rockville, MD, USA) were used to investigate the ability of compound **28** to inhibit cell adhesion to vitronectin. Cells were cultured in MEM/Glutamax (Invitrogen) medium containing 10% fetal bovine serum with 1% non-essential amino acids at 37 °C, 5% CO_2 in air. Cells were collected by trypsinization (Mesencult®-ACF Dissociation kit, STEMCELL Technologies) and then extensively washed three times with serum free medium. Bovine vitronectin[37] was coated onto 96-well polystyrene plates (Nunc, Denmark) at 5 μg/mL in phosphate buffered saline (PBS) at 4 °C for 16 h. Prior to cell studies, plates were thoroughly washed three times with PBS and blocked in 1% bovine serum albumin (BSA) in PBS for 1 h. Stock solutions of cyclic RGDfK **3** (Peptides International Inc, USA), cyclic RADfK (Peptides International Inc, USA), GRGDS (GenScript, USA) peptides, and RGD mimetic **28** were made up at a concentration of 100 mM in DMSO then diluted 10 times in PBS and subsequently in cell culture media (without serum component). Peptides (140 μL, 100 mM–0 nM) were pre-incubated with cells (140 μL, 30×10^4 cells/mL) in a low binding plate (Corning® Ultra low attachment 96 well) at 37 °C for 1 h. Subsequently, 100 μL was transferred to a prepared vitronectin plate and cells further incubated at 37 °C for 90 min. Cell adhesion was quantitated by MTS colorimetric assay (Promega) and absorbance read in a Biotek reader.

Acknowledgements

We thank Drs. Roger Mulder, Jo Cosgriff, and Carl Braybrook for assistance with NMR and Mass spectrometry and Penny Bean (CSIRO) for a gift of bovine vitronectin.

Supplementary data

^1H and ^{13}C NMR spectra for compounds **9**, **12**–**19**, **21**–**24**, **6**, **26**–**28**. Supplementary data associated with this article can be

found in the online version, at http://dx.doi.org/10.1016/j.tet.2012.09.002.

References and notes

1. (a) Hook, A. L.; Voelcker, N. H.; Thissen, H. *Acta Biomater.* **2009**, *5*, 2350–2370; (b) Meyers, S. R.; Grinstaff, M. W. *Chem. Rev.* **2012**, *112*, 1615–1632.
2. Ameringer, T.; Fransen, P.; Bean, P.; Johnson, G.; Pereira, S.; Evans, R. A.; Thissen, H.; Meagher, L. *J. Biomed. Mater. Res., Part A* **2012**, *100*, 370–379.
3. Hynes, R. O. *Cell* **2002**, *110*, 673–687.
4. Ruoslahti, E.; Pierschbacher, M. D. *Cell* **1986**, *44*, 517–518.
5. Pierschbacher, M. D.; Ruoslahti, E. *Nature* **1984**, *309*, 30–33.
6. Hersel, U.; Dahmen, C.; Kessler, H. *Biomaterials* **2003**, *24*, 4385–4415.
7. Roberts, C.; Chen, C. S.; Mrksich, M.; Martinchonok, V.; Ingber, D. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1998**, *120*, 6548–6555.
8. (a) Aumailley, M.; Gurrath, M.; Muller, G.; Calvete, J.; Timpl, R.; Kessler, H. *FEBS Lett.* **1991**, *291*, 50–54; (b) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461–7472; (c) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem., Int. Ed.* **1997**, *36*, 1374–1389; (d) Pfaff, M.; Tangemann, K.; Müller, B.; Gurrath, M.; Müller, G.; Kessler, H.; Timpl, R.; Engel, J. *J. Biol. Chem.* **1994**, *269*, 20233–20238.
9. (a) Dechantsreiter, M. A.; Planker, E.; Mathä, B.; Lohof, E.; Hölzemann, G.; Jonczyk, A.; Goodman, S. L.; Kessler, H. *J. Med. Chem.* **1999**, *42*, 3033–3040; (b) Goodman, S. L.; Hölzemann, G.; Sulyok, G. A.; Kessler, H. *J. Med. Chem.* **2002**, *45*, 1045–1051; (c) Weide, T.; Modlinger, A.; Kessler, H. *Top. Curr. Chem.* **2007**, *272*, 1–50.
10. Kantlehner, M.; Finsinger, D.; Meyer, J.; Schaffner, P.; Jonczyk, A.; Diefenbach, B.; Nies, B.; Hölzemann, G.; Goodman, S. L.; Kessler, H. *ChemBioChem* **2000**, *1*, 107–114.
11. Gentilucci, L.; Cardillo, G.; Spampinato, S.; Tolomelli, A.; Squassabia, F.; De Marco, R.; Bedini, A.; Baiula, M.; Beloisi, L.; Civera, M. *J. Med. Chem.* **2010**, *53*, 106–118.
12. Rerat, V.; Dive, G.; Cordi, A. A.; Tucker, G. C.; Bareille, R.; Amédée, J.; Bordenave, L.; Marchand-Brynaert, J. *J. Med. Chem.* **2009**, *52*, 7029–7043.
13. Shannon, K. E.; Keene, J. L.; Settle, S. L.; Duffin, T. D.; Nickols, M. A.; Westlin, M. A.; Schroeter, S.; Ruminski, P. G.; Griggs, D. W. *Clin. Exp. Metastasis* **2004**, *21*, 129–138.
14. Vianello, P.; Cozzi, P.; Galvani, A.; Meroni, M.; Varasi, M.; Volpi, D.; Bandiera, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 657–661.
15. Yasuda, N.; Hsiao, Y.; Jensen, M. S.; Rivera, N. R.; Yang, C.; Wells, K. M.; Yau, J.; Palucki, M.; Tan, L.; Dormer, P. G.; Volante, R. P.; Hughes, D. L.; Reider, P. J. *J. Org. Chem.* **2004**, *69*, 1959–1966.
16. Corbett, J. W.; Graciani, N. R.; Mousa, S. A.; DeGrado, W. F. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1371–1376.
17. Nickols, M. A.; Settle, S. L.; Westlin, W. F.; Ruminski, P. G.; Nickols, G. A. A. *Cancer Res.* **1998**, *58*, 1930–1935.
18. Sulyok, G. A. G.; Gibson, S. L.; Goodman, G.; Hölzemann, G.; Wiesner, M.; Kessler, H. *J. Med. Chem.* **2001**, 1938–1950.
19. Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699–1720.
20. Dahmen, C.; Auernheimer, J.; Meyer, A.; Enderle, A.; Goodman, S. L.; Kessler, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 6649–6652.
21. Tararov, V. I.; Korostylev, A.; König, G.; Borner, A. *Synth. Commun.* **2006**, *36*, 187–191.
22. The ratio of β-amino carboxylate to cinnamate product is reported to be dependent on both the reaction solvent and substitution of the aryl ring: Tan, C. Y. K.; Weaver, D. F. *Tetrahedron* **2002**, *58*, 7449–7461.
23. For an example of enantioselective preparation of a related β-amino ester see Ref. 15. The aryl carboxylate esters employed in this work were not expected to be compatible with lithium amides.
24. Semicarbazide **12** could be separated from the mixture by acid–base extraction. The remaining by-products did not correspond to products of either cyclization (cf. **23**) or N-deamination
25. Analogous loss of a Boc group from a di-*N*-Boc protected guanidine has been reported to occur with aq NaOH in refluxing MeOH: Sircar, J. C.; Thomas, R. J.; Khatuya, H.; Nikoulin, I. WO 2005/123686.
26. Inoue, K.; Sakai, K. *Tetrahedron Lett.* **1977**, *46*, 4063–4066.
27. Paterson, I.; Yeung, K.-S.; Ward, R. A.; Smith, J. D.; Cumming, J. D.; Lamboley, S. *Tetrahedron* **1995**, *51*, 9467–9486.
28. Smith, A. B., III; Condon, S. M.; McCauley, J. A.; Leazer, J. L., Jr.; Leahy, J. W.; Maleczka, R. E., Jr. *J. Am. Chem. Soc.* **1997**, *119*, 962–973.
29. Zimmerman, S. C.; Zeng, Z.; Wu, W.; Reichert, D. E. *J. Am. Chem. Soc.* **1991**, *113*, 183–196.
30. Eren, D.; Keinan, E. *J. Am. Chem. Soc.* **1988**, *110*, 4356–4362.
31. Martin, C. L.; Overman, L. E.; Rohde, J. M. *J. Am. Chem. Soc.* **2010**, *132*, 4894–4906.
32. Martin, C. L.; Overman, L. E.; Rohde, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 7568–7569.
33. Gibson, F. S.; Bergmeier, S. C.; Rapoport, H. *J. Org. Chem.* **1994**, *59*, 3216–3218.
34. Lin, L. S.; Lanza, T.; de Laszlo, S. E.; Truong, Q.; Kamenecka, T.; Hagmann, W. K. *Tetrahedron Lett.* **2000**, *41*, 7013–7016.
35. Kok, R. J.; Schraa, A. J.; Bos, E. J.; Moorlag, H. E.; Ásgeirsdóttir, S. A.; Everts, M.; Meijer, D. K. F.; Molema, G. *Bioconjugate Chem.* **2002**, *13*, 128–135.
36. (a) Hu, H.; Mendoza, J. S.; Lowden, C. T.; Ballas, L. M.; Janzen, W. P. *Bioorg. Med. Chem.* **1997**, *5*, 1873–1882; (b) Gennari, C.; Ceccarelli, S.; Piarulli, U.; Aboutayab, K.; Donghi, M.; Paterson, I. *Tetrahedron* **1998**, *54*, 14999–15016.
37. Underwood, P. A.; Bean, P. A.; Mitchell, S. M.; Whitelock, J. M. *J. Immunol. Methods* **2001**, *247*, 217–224.