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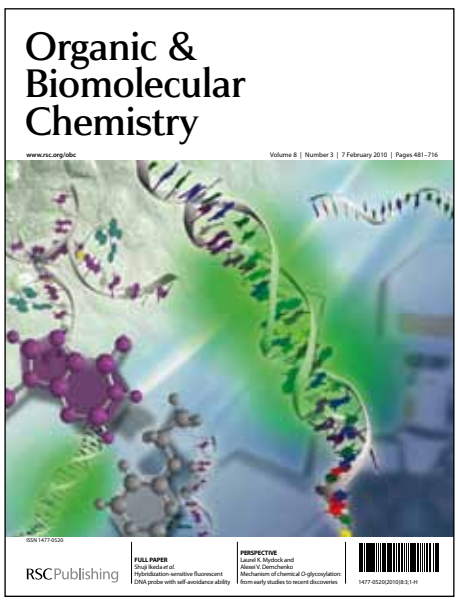

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PAPER

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Solid phase synthesis of peptides containing backbone-fluorinated amino acids†

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Backbone-fluorinated amino acids exhibit unique conformational behaviour, and have potential utility as components of bioactive shape-controlled peptides. However, methods for the elaboration of backbone-fluorinated amino acids have thus far been limited to solution phase peptide coupling reactions. In this paper, protocols are developed that allow the successful manipulation of backbone-fluorinated amino acids using Fmoc-strategy solid phase peptide synthesis. To exemplify this strategy, several fluorinated RGD peptide analogues were synthesised in moderate to good overall yields.

Introduction

Fluorinated amino acids have found a variety of applications in peptide and protein science.[1] One such application is in the creation of proteins with increased stability. For example, highly fluorinated analogues of canonical amino acids (eg. **1**) have been incorporated into "fluorous-core" proteins and "fluorous-face" alpha-helical dimers in order to investigate the importance of hydrophobic effects on protein structure and stability.[1] In another example, Raines and co-workers have demonstrated that the stability of the collagen triple helix is increased when the constituent 4-hydroxyproline residues are replaced with 4-fluoroproline (**2**);[2] the enhanced collagen stability was attributed (in part) to inter-strand dipolar interactions associated with the C–F bond.[3] Another application of fluorinated amino acids is in the area of protein NMR: for example, fluorinated tyrosine analogues (eg. **3**) have been employed as NMR indicators,[4] taking advantage of the fact that fluorine is absent from natural proteins. Another application of fluorinated amino acids is in the conformational control of peptides.[5] The highly polarised C–F bond participates in a variety of stereoelectronic interactions with neighbouring functional groups, and these can favour certain molecular conformations over others.[6] Taking advantage of this concept, Seebach and co-workers have incorporated α-fluoro-β-amino acids (eg. **4**) into β-heptapeptides, and have demonstrated that the configuration of the fluorinated stereocentre has a dramatic influence on the secondary structure of the β-peptide.[7] Hunter and co-workers have explored this concept with α,β-difluoro-γ-amino acids (eg. **5**);[8] the different stereoisomers of **5** are found to have contrasting conformations that offer potentially valuable biological applications.[9]

There are several synthetic methods for incorporating amino acids such as **1**–**5** into peptides and proteins. Fluorinated

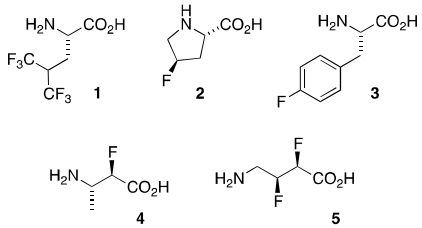
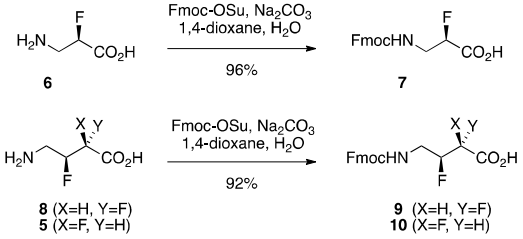


Fig. 1 Examples of fluorinated amino acids that have been incorporated into peptides and proteins.

analogues of the canonical amino acids (eg. **1**–**3**) can sometimes be incorporated into analogues of natural proteins by a microorganism's biosynthetic machinery, using feeding experiments with bacterial strains that are auxotrophic for the parent amino acid.[10] In practice, chemical methods are also commonly used for the elaboration of amino acids such as **1**–**3**, since these amino acids are readily amenable to solid phase peptide synthesis.[4] In contrast with the sidechain-fluorinated amino acids **1**–**3**, the backbone-fluorinated amino acids **4** and **5** do not resemble canonical amino acids, and hence chemical synthesis is the only option for manipulating these building blocks. Seebach[7] and Hunter[8] have previously elaborated amino acids such as **4** and **5** into peptides containing up to twenty amino acid residues,[11] using solution phase peptide coupling reactions. However it would be desirable to be able to employ these building blocks within the manifold of solid phase peptide synthesis, given the operational simplicity and the rapidity of this technique.[12] Challenges associated with solid phase peptide synthesis include strongly basic peptide coupling conditions, and the standard technique of employing a large reagent excess in each peptide coupling step; both of these issues could prove problematic with the non-commercial amino acids **4** and **5**. Accordingly, the aim of this study was to investigate whether it is



Scheme 1 Synthesis of Fmoc-protected building blocks.

possible to efficiently manipulate backbone-fluorinated amino acids such as **4** and **5** using Fmoc-strategy solid phase peptide synthesis.[12]

Results and discussion

The first task was to assemble the required collection of Fmoc-protected fluorinated amino acids. This was accomplished by treatment of the appropriate unprotected amino acids **5**, **6** and **8** with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-OSu) under standard conditions[13] (Scheme 1). The protected amino acids **7**, **9** and **10** were obtained in good yields, and were fully characterised according to the standard suite of analytical techniques. The NMR spectra[14] of **7**, **9** and **10** revealed the presence of rotamers at 300 K, the signals of which were observed to coalesce at elevated temperature (~330 K).

With building blocks **7**, **9** and **10** (and their enantiomers) in hand, attention was next turned toward their use in solid phase peptide synthesis. The peptides targeted in this study were analogues of the tetrapeptide sequence RGDF (arginine-glycine-aspartate-phenylalanine), where the glycine residue was to be replaced with various fluorinated amino acids. These peptide targets would allow the efficiency of peptide coupling at both the C- and N-termini of the fluorinated amino acids to be investigated, and would also allow the compatibility with some sidechain protecting groups to be determined.

Initial experiments were performed with the fluorinated amino acid **9** (Table 1, entry 1). 2-Chlorotrityl chloride resin loaded with aspartate-phenylalanine dipeptide (**11**) was treated for 2 h with a solution of **9** (1 equivalent relative to resin loading) and HBTU (1 equivalent) in a minimal volume of dimethylformamide containing 0.4 M diisopropylethylamine, and this coupling step was then repeated with 0.5 equivalents of **9**/HBTU for 2h. A standard sequence of deprotection, peptide coupling and cleavage steps followed; a double coupling of arginine was employed to compensate for any reduced nucleophilicity of the amino group of the fluorinated amino acid. This reaction sequence (Table 1, entry 1) eventually delivered the undesired product **13** which had suffered HF elimination (alkene geometry of **13** confirmed by $^3J_{HF}$ = 34.5 Hz).[15] The undesired elimination reaction likely occurred during HBTU/diisopropylethylamine activation of **9** prior to its addition to the resin; a dramatic colour change of the solution from colourless to dark orange was observed at this step. The presence of excess base was thought to be responsible for an efficient E2 elimination, therefore alternative conditions were investigated in which only one equivalent of base was employed (Table 1, entry 2). For this second experiment it was also decided to switch to Wang resin as the solid support (**12**), to offer a more acid-stable linkage in case small amounts of HF continued to be formed. When PyBOP and *N*-methylmorpholine were employed as the peptide coupling reagents (both equimolar relative to **9**), a moderate yield of the desired peptide **14** was eventually obtained, along with a smaller quantity of the undesired elimination product **13** (Table 1, entry 2).

Due to a temporary exhaustion of the available stock of Fmoc-protected amino acid **9**, further SPPS investigations were performed using the building block **10**. In the next experiment (Table 1, entry 3), a single coupling of **10** was employed, with 1.2 equivalents of **10** and an equimolar quantity of HOBt and DIC (ie. no base). Gratifyingly, a moderate yield of the desired peptide **15** was obtained (Table 1, entry 3). Finally, the yield of **15** was able to be increased to 83% by employing 1.5 equivalents of **10** (Table 1, entry 4). The absence of base in the key coupling step resulted in complete suppression of the undesired HF elimination pathway, and standard peptide coupling conditions (HBTU, diisopropylethylamine, DMF) were able to be used for all other coupling steps in the sequence. This suggests that the fluorinated amino acid is sensitive to HF elimination only when converted into the corresponding active ester; in constrast, once incorporated into a peptide the difluoro motif is stable even under basic conditions.

The optimised SPPS conditions (Table 1, entry 4) were then employed to generate a small library of fluorinated peptides (Figure 2). Overall yields ranged from moderate to good, although in most cases it was necessary purify the target peptide by preparative reverse-phase HPLC in order to remove minor impurities such as the Arg-Asp-Phe tripeptide (ie. lacking the fluorinated amino acid).

Peptides **14**–**22** are analogues of the RGD tripeptide, a sequence that is commonly found in extracellular matrix proteins and which is recognised by cell-surface receptors known as integrins.[16] There is considerable interest in creating RGD analogues that are selective for individual integrin receptor subtypes, because such molecules have a variety of potential therapeutic applications;[17] $\alpha_V\beta_3$ integrin is a target of particular interest because this receptor has a pro-angiogenic function and is upregulated in solid tumours.[18] It has previously been shown that $\alpha_V\beta_3$ integrin selectivity is exhibited by synthetic peptides in

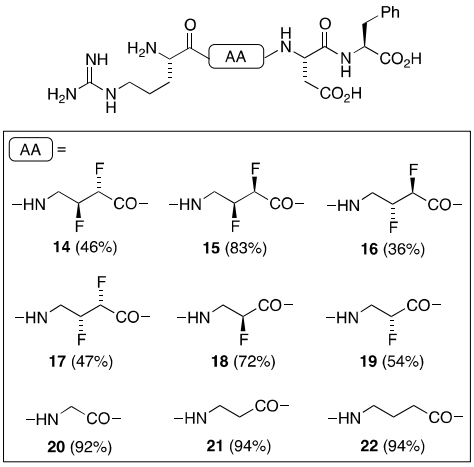
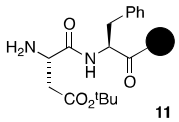
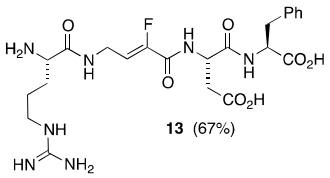
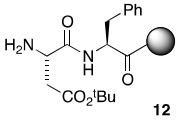
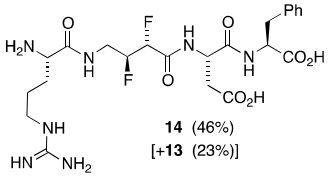
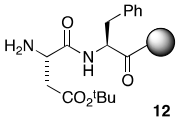
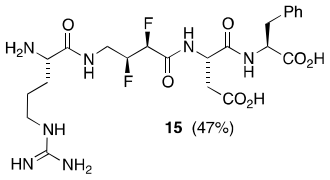
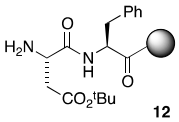
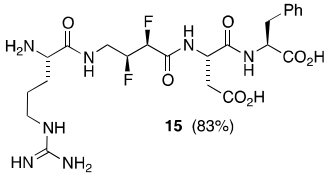


Fig. 2 Peptides prepared by SPPS in this study. Yields refer to purified materials and are based on the initial resin loading of Fmoc-phenylalanine.

Table 1 Investigation of different SPPS protocols.[a]

Entry	Starting material	Reaction conditions	Product (yield)
1	 **11**	(a) **9** (1+0.5 eq), HBTU (1+0.5 eq), DIPEA (0.4 M), DMF (b) Piperidine (10%), DMF (c) Fmoc-Arg(Pbf) (3+3 eq), HBTU (2.9+2.9 eq), DIPEA (0.4 M), DMF (d) Piperidine (10%), DMF (e) TFA (95%), TIS (2.5%), H_2O	 **13** (67%)
2	 **12**	(a) **9** (1+0.5 eq), PyBOP (1+0.5 eq), NMM (1+0.5 eq), DMF (b) Piperidine (10%), DMF (c) Fmoc-Arg(Pbf) (3+3 eq), PyBOP (3+3 eq), NMM (3+3 eq), DMF (d) Piperidine (10%), DMF (e) TFA (95%), TIS (2.5%), H_2O	 **14** (46%) [**+13** (23%)]
3	 **12**	(a) **10** (1.2 eq), HOBt (1.2 eq), DIC (1.2 eq), DMF (b) Piperidine (10%), DMF (c) Fmoc-Arg(Pbf) (3+3 eq), HBTU (2.9+2.9 eq), DIPEA (6+6 eq), DMF (d) Piperidine (10%), DMF (e) TFA (95%), TIS (2.5%), H_2O	 **15** (47%)
4	 **12**	(a) **10** (1.5 eq), HOBt (1.5 eq), DIC (1.5 eq), DMF (b) Piperidine (10%), DMF (c) Fmoc-Arg(Pbf) (3+3 eq), HBTU (2.9+2.9 eq), DIPEA (6+6 eq), DMF (d) Piperidine (10%), DMF (e) TFA (95%), TIS (2.5%), H_2O	 **15** (83%)

[a] Abbreviations: Solid black circle = 2-chlorotrityl chloride resin; shaded grey circle = Wang resin; HBTU = *O*-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate; DIPEA = diisopropylethylamine; DMF = dimethylformamide; Fmoc-Arg(Pbf) = *N*α-Fmoc-*N*ω-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginine; TFA = trifluoroacetic acid; TIS = triisopropylsilane; PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; NMM = *N*-methylmorpholine; HOBt = hydroxybenzotriazole; DIC = diisopropylcarbodiimide.

which the RGD sequence is constrained into a "bent" conformation.[19] Since the fluorinated amino acids employed in this study are known to adopt uniqe and well-defined conformations,[7,8] it became of interest to investigate whether any of the peptides **14**–**22** exhibited integrin binding, and/or $\alpha_V\beta_3$ integrin selectivity. Accordingly, cell adhesion assays were performed with $\alpha_V\beta_3$, $\alpha_V\beta_6$, $\alpha_V\beta_5$ and $\alpha_5\beta_1$ integrins.[20] Disappointingly however, only the control peptide **20** was found to exhibit any detectable integrin activity (at $\alpha_V\beta_3$ and $\alpha_V\beta_5$).[14]

The lack of activity of the fluorinated peptides **14**–**19** could be attributed to the altered length of the backbone-homologated amino acid residues;[21] in the future, it may be of interest to investigate peptide analogues in which one or more methylene groups are removed from the arginine sidechain in order to restore the natural distance between the reactive groups of the arginine and aspartate sidechains which provide key binding interactions at integrin receptors.[17] Indeed, work is currently underway in our laboratories towards the incorporation of backbone-fluorinated amino acids such as **9** and **10** into a variety of peptide systems, including a second generation of fluorinated RGD peptide analogues, using the solid phase synthesis methods developed here.

Conclusions

Protocols have been developed that allow backbone-fluorinated amino acids to be efficiently incorporated into peptides using Fmoc-strategy SPPS. Fluorinated peptides are obtained in moderate to good overall yields (suffering only minimal HF elimination), using only a slight excess of the non-commercial fluorinated amino acid. The peptides created in this study did not possess significant biological activity in the context of integrin receptor ligands; nevertheless, the synthetic methodology described herein should expedite the future creation of a variety of fluorinated peptides for other applications in biotechnology and medicine.

Experimental section

Solvents, reagents and instrumentation

Water was obtained from a Millipore filtration system. *N*,*N*-Dimethylformamide was purchased in peptide synthesis grade and stored over 4Å molecular sieves. All other reagents and solvents were purchased in the highest available quality and used as received. Eluting solvents for chromatography are reported as volume/volume mixtures. Where indicated, NMR signals were

assigned using information from COSY experiments. SAFETY PRECAUTION: due to the possibility that small amounts of HF could be formed as a side-product in these experiments, appropriate personal protective equipment was employed (including a tube of calcium gluconate gel kept close at hand).

General procedure A: synthesis of Fmoc-protected fluorinated amino acids

A solution of Fmoc-*O*-succinimide (1 mmol) in 1,4-dioxane (7.5 mL) was added to a solution of the appropriate fluorinated amino acid (1 mmol) and sodium carbonate (3 mmol) in water (7.5 mL) at 0 °C. The mixture was stirred at room temperature overnight, then acidified with dilute hydrochloric acid. The mixture was concentrated onto silica, and the crude product was purified by flash chromatography eluting with 90 : 8 : 2 chloroform/ methanol/ acetic acid.

General procedure B: SPPS: preparation of resin

Solid phase peptide synthesis was conducted manually in a sinter-fitted polypropylene syringe. Wang resin (100–200 mesh) pre-loaded with Fmoc-phenylalanine (0.65 mmol/g resin loading) was agitated in DCM for 1h, then drained and washed with DMF (3 × 1 min).

General procedure C: SPPS: Fmoc deprotection

The resin was washed with DMF (3 × 1 min). The resin was agitated with a solution of 10% piperidine in DMF (2 × 3 min), then washed with DMF (3 × 1 min), DCM (3 × 1 min) and DMF (3 × 1 min). The deprotection solutions were combined and diluted 100-fold with 10% piperidine in DMF, and the absorbance of the diluted solution was measured at 301 nm against 10% piperidine in DMF as reference. The resin loading was determined by calculating the concentration of the piperidine-fulvene adduct (ε = 7800 $M^{-1}cm^{-1}$) in the deprotection solution.

General procedure D: SPPS: peptide coupling (commercial amino acids)

The resin was washed with DMF (3 × 1 min). A solution was prepared of the appropriate Fmoc-protected amino acid (3 equiv. relative to resin loading) and HBTU (2.9 equiv. relative to resin loading) in minimal DMF. DIPEA (6 equiv. relative to resin loading) was added to this solution, and the mixture was immediately added to the resin and agitated for 1.5 h. The resin was drained and washed with DMF (3 × 1 min), DCM (3 × 1 min) and DMF (3 × 1 min).

General procedure E: SPPS: peptide coupling (fluorinated amino acids)

The resin was washed with DMF (3 × 1 min). A solution was prepared of the appropriate Fmoc-protected amino acid (1.5 equiv. relative to resin loading), HOBt (1.5 equiv. relative to resin loading) and DIC (1.5 equiv. relative to resin loading) in minimal DMF. This solution was stirred for 20 min, then added to the resin and agitated overnight. The resin was drained and washed with DMF (3 × 1 min), DCM (3 × 1 min) and DMF (3 × 1 min).

General procedure F: SPPS: cleavage of peptide from resin

After the last Fmoc deprotection, the resin was washed with DMF (3 × 1 min) and DCM (3 × 1 min). The resin was agitated with a solution of 95 : 2.5 : 2.5 TFA/TIS/H_2O for 2h. The resin was drained and washed with TFA (2 × 1 min). The combined cleavage solutions were concentrated *in vacuo*. The residue was dissolved in water (~20 mL per mmol of peptide) and this solution was washed four times with an equal volume of diethyl ether. The aqueous layer was freeze-dried to afford the crude peptide, which was purified if necessary by reverse-phase HPLC eluting with 0→30% acetonitrile/water (containing 0.1% v/v TFA) over 40 min and monitoring at 254 nm.

(*R*)-3-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-2-fluoropropanoic acid (7)

The title compound was prepared from (*R*)-3-amino-2-fluoropropanoic acid hydrochloride (**6**)[22] according to General Procedure A on 1.40 mmol scale. The product was obtained as a white solid (0.445 g, 96% yield); m.p. 138–139 °C; $[\alpha]_D$ –3.3 (*c* 0.94, MeOH); IR (neat) ν_{max} (cm^{-1}) 3305, 3062, 1718, 1535, 1450, 1259, 1156, 1104; ^{1}H NMR (300 MHz, MeOD) δ 7.80–7.59 (m, 4H, ArH), 7.42–7.26 (m, 4H, ArH), 5.00 (ddd, *J* = 49.1, 6.6, 3.5 Hz, 1H, α-CHF), 4.31 (d, *J* = 6.8 Hz, 2H, Fmoc CH_2), 4.16 (t, *J* = 6.8 Hz, 1H, Fmoc CH), 3.71 (ddd, *J* = 24.5, 14.7, 3.3 Hz, 1H, β-CHH), 3.58 (ddd, *J* = 22.2, 14.7, 6.6 Hz, β-CHH); ^{13}C {^{1}H} NMR (75 MHz, MeOD) δ 171.5 (d, *J* = 23.4 Hz, CO_2H), 158.8, 145.2, 142.5, 128.7, 128.1, 126.2, 120.9, 88.9 (d, *J* = 184.4 Hz, α-CHF), 68.0, 48.3, 43.7 (d, *J* = 21.5 Hz, β-CH_2); ^{19}F NMR (282 MHz, MeOD) δ –197.1 (ddd, *J* = 47.7, 23.9, 23.9 Hz, 1F); ^{19}F {^{1}H} NMR (282 MHz, MeOD) δ –197.1 (s, 1F); MS (ESI, +ve) *m*/*z* 352 (MNa^+, 52%); HRMS (ESI, +ve) $C_{18}H_{16}NO_4FNa^+$ requires *m*/*z* 352.0956, found 352.0954.

(*S*)-3-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-2-fluoropropanoic acid (*ent*-7)

The title compound was prepared from (*S*)-3-amino-2-fluoropropanoic acid hydrochloride (*ent*-**6**)[22] according to General Procedure A on 1.34 mmol scale. The product was obtained as a white solid (0.200 g, 45% yield); m.p. 127–132 °C; $[\alpha]_D$ +2.3 (*c* 1.54, MeOH); IR data, ^{1}H NMR data, ^{13}C {^{1}H} NMR data, ^{19}F NMR data, ^{19}F {^{1}H} NMR data and MS data identical to that of **7** above; HRMS (ESI, +ve) $C_{18}H_{16}NO_4FNa^+$ requires *m*/*z* 352.0956, found 352.0953.

(2*R*,3*S*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-2,3-difluorobutanoic acid (9)

The title compound was prepared from (2*R*,3*S*)-4-amino-2,3-difluorobutanoic acid hydrochloride (**8**)[9] according to General Procedure A on 0.18 mmol scale. The product was obtained as a white solid (0.059 g, 92% yield); m.p. 171–174 °C; $[\alpha]_D$ –4.5 (*c* 1.21, MeOH); IR (neat) ν_{max} (cm^{-1}) 3439, 1714, 1540, 1455, 1275; ^{1}H NMR (300 MHz, MeOD) δ 7.79–7.75 (m, 2H, ArH), 7.65–7.61 (m, 2H, ArH), 7.40–7.26 (m, 4H, ArH), 5.31–4.81 (m, 2H, α-CHF + β-CHF), 4.34 (d, *J* = 6.7 Hz, 2H, Fmoc CH_2), 4.18 (t, *J* = 6.7 Hz, 1H, Fmoc CH); ^{13}C {^{1}H} NMR (100 MHz, MeOD, 330 K) δ 168.4 (m, CO_2H), 158.0, 144.5, 141.8, 127.9, 127.3, 125.3, 120.1, 91.4 (dd, *J* = 180.3, 21.3 Hz, CHF), 88.7 (dd, *J* = 195.4, 23.8 Hz, CHF), 67.2, 47.8, 40.8 (dd, *J* = 26.6, 5.7 Hz, γ-CH_2); ^{19}F NMR (282 MHz, MeOD) δ –199.7 (m, 1F, β-CHF), –204.3 (m, 1F, α-CHF); ^{19}F {^{1}H} NMR (282 MHz, MeOD) δ

–199.7 (d, J = 13.3 Hz, 1F, β-CHF), –204.3 (d, J = 13.3 Hz, 1F, α-CHF); MS (ESI, +ve) m/z 384 (MNa^+, 100%); HRMS (ESI, +ve) $C_{19}H_{17}F_2NO_4Na^+$ requires m/z 384.1018, found 384.1013.

(2*S*,3*R*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-2,3-difluorobutanoic acid (*ent*-9)

The title compound was prepared from (2*S*,3*R*)-4-amino-2,3-difluorobutanoic acid hydrochloride (*ent*-**8**)[9] according to General Procedure A on 0.36 mmol scale. The product was obtained as a white solid (0.061 g, 46% yield); m.p. 172–176 °C; $[\alpha]_D$ +3.7 (*c* 1.08, MeOH); IR data, ^{1}H NMR data, ^{13}C {^{1}H} NMR data, ^{19}F NMR data, ^{19}F {^{1}H} NMR data and MS data identical to that of **9** above; HRMS $C_{19}H_{17}F_2NO_4Na^+$ requires m/z 384.1018, found 384.1022.

(2*S*,3*S*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-2,3-difluorobutanoic acid (10)

The title compound was prepared from (2*S*,3*S*)-4-amino-2,3-difluorobutanoic acid hydrochloride (**5**)[9] according to General Procedure A on 0.32 mmol scale. The product was obtained as a white solid (0.107 g, 92% yield); m.p. 142–148 °C; $[\alpha]_D$ –16.0 (*c* 0.094, MeOH); IR (neat) ν_{max} (cm^{-1}) 3320, 3065, 2949, 1705, 1538, 1429, 1265, 1126; ^{1}H NMR (300 MHz, MeOD) δ 7.78–7.74 (m, 2H, ArH), 7.64–7.60 (m, 2H, ArH), 7.39–7.25 (m, 4H, ArH), 5.22–4.82 (m, 2H, α-CHF + β-CHF); 4.37 (d, J = 6.7 Hz, 2H, Fmoc CH_2), 4.17 (t, J = 6.7 Hz, 1H, Fmoc CH), 3.59–3.44 (m, 2H, γ-C<u>H</u>H + γ-CH<u>H</u>); ^{13}C {^{1}H} NMR (75 MHz, MeOD) δ 170.5 (dd, J = 26.0, 6.1 Hz, CO_2H), 159.3, 145.6, 143.0, 129.2, 128.6, 126.6, 121.4, 91.9 (dd, J = 179.3, 18.5 Hz, CHF), 88.9 (dd, J = 182.1, 15.7 Hz, CHF), 68.3, 48.9, 42.1 (dd, J = 25.0, 4.6 Hz, γ-CH_2); ^{19}F NMR (282 MHz, MeOD) δ –204.1 (m, 1F, β-CHF), –211.8 (m, 1F, α-CHF); ^{19}F {^{1}H} NMR (282 MHz, MeOD) δ –204.1 (d, J = 8.7 Hz, 1F, β-CHF), –211.8 (d, J = 8.7 Hz, 1F, α-CHF); MS (ESI, +ve) m/z 384 (MNa^+, 100%); HRMS $C_{19}H_{17}F_2NO_4Na^+$ requires m/z 384.1018, found 384.1018.

(2*R*,3*R*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-2,3-difluorobutanoic acid (*ent*-10)

The title compound was prepared from (2*R*,3*R*)-4-amino-2,3-difluorobutanoic acid hydrochloride (*ent*-**5**)[9] according to General Procedure A on 0.10 mmol scale. The product was obtained as a white solid (0.032 g, 86% yield); m.p. 137–140 °C; $[\alpha]_D$ +16.9 (*c* 0.172, MeOH); IR data, ^{1}H NMR data, ^{13}C {^{1}H} NMR data, ^{19}F NMR data, ^{19}F {^{1}H} NMR data and MS data identical to that of **10** above; HRMS $C_{19}H_{17}F_2NO_4Na^+$ requires m/z 384.1018, found 384.1023.

L-Arginyl-[(*Z*)-4-amino-2-fluorobut-2-enoyl]-L-aspartyl-L-phenylalanine, TFA salt (13)

The title compound was prepared on 25 μmol scale according to the procedure described in Table 1, entry 1. The product was obtained as a fluffy white solid (11.2 mg, 67% overall yield based on initial resin loading); analytical HPLC (0→25% acetonitrile/water (containing 0.1% v/v formic acid) over 30 min) retention time = 7.3 min, >90% purity; ^{1}H NMR (300 MHz, D_2O) δ 7.31–7.15 (m, 5H, Ph), 5.97 (dt, J = 34.5, 7.0 Hz, 1H, GABA β-H), 4.70–4.58 (m, 2H, Asp α-H + Phe α-H), 4.14 (ddd, J = 16.1, 7.0, 1.6 Hz, 1H, GABA γ-C<u>H</u>H), 4.00–3.92 (m, 2H, GABA γ-CH<u>H</u> + Arg α-H), 3.19 (dd, J = 14.0, 5.4 Hz, 1H, Phe β-C<u>H</u>H), 3.12 (m, 1H, Arg δ-CH_2), 2.95 (dd, J = 14.0, 8.6 Hz, 1H, Phe β-CH<u>H</u>), 2.85 (dd, J = 17.1, 6.1 Hz, 1H, Asp β-C<u>H</u>H), 2.69 (dd, J = 17.1, 8.0 Hz, 1H, Asp β-CH<u>H</u>), 1.86 (m, 2H, Arg β-CH_2), 1.56 (m, 2H, Arg γ-CH_2); ^{19}F NMR (282 MHz, D_2O) δ –76.0 (s, 3F, TFA), –129.3 (d, J = 34.5 Hz, 1F, α-F); ^{19}F {^{1}H} NMR (282 MHz, D2O) δ –76.0 (s, 3F, TFA), –129.3 (s, 1F, α-F); MS (ESI, +ve) m/z 538 (MH^+, 100%).

L-Arginyl-[(2*R*,3*S*)-4-amino-2,3-difluorobutanoyl]-L-aspartyl-L-phenylalanine, TFA salt (14)

The title compound was prepared on 25 μmol scale according to General Procedures B–F, employing a double coupling of Fmoc-arginine. The product was obtained as a sticky white solid (7.3 mg, 46% overall yield based on initial resin loading); analytical HPLC (0→25% acetonitrile/water (containing 0.1% v/v formic acid) over 30 min) retention time = 8.8 min, >95% purity; $[\alpha]_D$ –16.4 (*c* 0.21, H_2O); IR (neat) ν_{max} (cm^{-1}) 3398, 2927, 1675, 1543, 1398, 1204, 1137; ^{1}H NMR (300 MHz, D_2O, COSY) δ 7.41–7.26 (m, 5H, Ph), 5.30 (ddd, J = 47.4, 18.1, 2.3 Hz, 1H, α-CHF), 5.02 (ddddd, J = 46.8, 22.3, 8.0, 3.1, 2.3 Hz, 1H, β-CHF), 4.82 (m, 1H, Asp α-H), 4.71 (m, 1H, Phe α-H), 4.05 (t, J = 6.4 Hz, 1H, Arg α-H), 3.68 (ddd, J = 29.3, 15.0, 3.1 Hz, 1H, CHFC<u>H</u>H), 3.59 (m, 1H, CHFCH<u>H</u>), 3.29 (dd, J = 14.2, 5.4 Hz, 1H, Phe β-C<u>H</u>H), 3.24 (t, J = 6.6 Hz, 2H, Arg δ-CH_2), 3.06 (dd, J = 14.0, 8.9 Hz, 1H, Phe β-CH<u>H</u>), 2.91 (dd, J = 17.1, 5.2 Hz, 1H, Asp β-C<u>H</u>H), 2.77 (dd, J = 17.1, 8.7 Hz, 1H, Asp β-CH<u>H</u>), 1.95 (m, 2H, Arg β-CH_2), 1.66 (m, 2H, Arg γ-CH_2); ^{13}C {^{1}H} NMR (75 MHz, D_2O) δ 172.5, 171.6, 168.8, 167.4, 165.1 (dd, J = 21.4, 8.4 Hz, CHF<u>C</u>O), 154.5, 134.3, 126.9, 126.3, 124.8, 87.7 (dd, J = 201.7, 21.8 Hz, CHF), 87.1 (dd, J = 192.7, 22.7 Hz, CHF), 51.9, 50.5, 47.3, 37.9, 36.1 (dd, J = 22.7, 10.0 Hz, CHF<u>C</u>H_2), 34.3, 32.9, 25.6, 21.1; ^{19}F NMR (282 MHz, D_2O) δ –76.0 (s, 3F, TFA), –198.7 (m, 1F, β-CHF), –202.1 (ddd, J = 47.6, 22.1, 13.3 Hz, 1F, α-CHF); ^{19}F NMR (282 MHz, D_2O) δ –76.0 (s, 3F, TFA), –198.7 (d, J = 13.3 Hz, 1F, β-CHF), –202.1 (d, J = 13.3 Hz, 1F, α-CHF); MS (ESI, +ve) m/z 558 (MH^+, 100%); HRMS (ESI, +ve) $C_{23}H_{34}F_2N_7O_7^+$ requires m/z 558.2482, found 558.2486.

L-Arginyl-[(2*S*,3*S*)-4-amino-2,3-difluorobutanoyl]-L-aspartyl-L-phenylalanine, TFA salt (15)

The title compound was prepared on 25 μmol scale according to General Procedures B–F, employing a double coupling of Fmoc-arginine. The product was obtained as a sticky white solid (13.8 mg, 83% overall yield based on initial resin loading); analytical HPLC (0→25% acetonitrile/water (containing 0.1% v/v formic acid) over 30 min) retention time = 8.7 min, >90% purity; $[\alpha]_D$ –11.6 (*c* 0.28, H_2O); IR (neat) ν_{max} (cm^{-1}) 3195, 2925, 2856, 1667, 1538, 1397, 1201, 1134; ^{1}H NMR (300 MHz, D_2O, COSY) δ 7.41–7.24 (m, 5H, Ph), 5.22 (ddd, J = 46.3, 29.6, 1.3 Hz, 1H, α-CHF), 5.04 (ddddd, J = 46.2, 27.2, 8.1, 3.6, 1.3 Hz, 1H, β-CHF), 4.81 (m, 1H, Asp α-H), 4.68 (m, 1H, Phe α-H), 4.07 (t, J = 6.5 Hz, 1H, Arg α-H), 3.86 (ddd, J = 28.9, 14.7, 3.6 Hz, 1H, CHFC<u>H</u>H), 3.63 (ddd, J = 14.7, 14.7, 8.2 Hz, 1H, CHFCH<u>H</u>), 3.23 (dd, J = 14.1, 5.2 Hz, 1H, Phe β-C<u>H</u>H), 3.19 (t, J = 6.8 Hz, 2H, Arg δ-CH_2), 3.06 (dd, J = 14.1, 8.2 Hz, 1H, Phe β-CH<u>H</u>), 2.92 (dd, J = 17.0, 5.7 Hz, 1H, Asp β-C<u>H</u>H), 2.80 (dd, J = 17.0, 8.2 Hz, 1H, Asp β-CH<u>H</u>), 1.95 (m, 2H, Arg β-CH_2), 1.66 (m, 2H, Arg γ-CH_2); ^{13}C {^{1}H} NMR (75 MHz, D_2O) δ 174.9, 174.3,

171.6, 170.3, 168.5 (dd, J = 21.3, 2.9 Hz, CHFCO), 157.1, 136.8, 129.7, 129.1, 127.5, 89.8 (dd, J = 181.3, 20.7 Hz, CHF), 89.7 (dd, J = 193.8, 22.8 Hz, CHF), 54.6, 53.3, 50.2, 40.7, 37.0, 35.4, 28.3, 23.8; ^{19}F NMR (282 MHz, D_2O) δ –76.0 (s, 3F, TFA), –203.3 (m, 1F, β-CHF), –209.6 (ddd, J = 46.2, 26.8, 10.4 Hz, 1F, α-CHF); ^{19}F {^{1}H} NMR (282 MHz, D_2O) δ –76.0 (s, 3F, TFA), –203.3 (d, J = 10.4 Hz, 1F, β-CHF), –209.6 (d, J = 10.4 Hz, 1F, α-CHF); MS (ESI, +ve) m/z 558 (MH^+, 100%); HRMS (ESI, +ve) $C_{23}H_{34}F_2N_7O_7^+$ requires m/z 558.2482, found 558.2481.

L-Arginyl-[(2*S*,3*R*)-4-amino-2,3-difluorobutanoyl]-L-aspartyl-L-phenylalanine, TFA salt (16)

The title compound was prepared on 25 μmol scale according to General Procedures B–F, employing a double coupling of Fmoc-arginine. The product was obtained as a sticky white solid (6.0 mg, 36% overall yield based on initial resin loading); analytical HPLC (0→100% acetonitrile/water (containing 0.1% v/v formic acid) over 10 min) retention time = 7.3 min, >90% purity; $[\alpha]_D$ +8.5 (*c* 0.20, H_2O); IR (neat) ν_{max} (cm^{-1}) 3358, 2959, 2926, 2874, 1684, 1527, 1464, 1407, 1205, 1139; ^{1}H NMR (300 MHz, D_2O, COSY) δ 7.41–7.26 (m, 5H, Ph), 5.29 (ddd, J = 47.2, 18.8, 2.3 Hz, 1H, α-CHF), 5.02 (ddddd, J = 47.2, 22.1, 8.5, 3.0, 2.3 Hz, 1H, β-CHF), 4.82 (m, 1H, Asp α-H), 4.65 (m, 1H, Phe α-H), 4.04 (t, J = 6.1 Hz, 1H, Arg α-H), 3.73 (ddd, J = 17.3, 14.9, 8.5 Hz, 1H, CHFCHH), 3.52 (ddd, J = 30.5, 14.9, 3.0 Hz, 1H, CHFCHH), 3.25 (dd, J = 14.0, 5.2 Hz, 1H, Phe β-CHH), 3.20 (t, J = 6.7 Hz, 2H, Arg δ-CH_2), 3.06 (dd, J = 14.0, 8.5 Hz, 1H, Phe β-CHH), 2.92 (dd, J = 17.1, 5.6 Hz, 1H, Asp β-CHH), 2.80 (dd, J = 17.1, 8.4 Hz, 1H, Asp β-CHH), 1.92 (m, 2H, Arg β-CH_2), 1.64 (m, 2H, Arg γ-CH_2); ^{13}C {^{1}H} NMR (75 MHz, D_2O) δ 175.5, 174.5, 171.6, 170.1, 167.8 (m, CHFCO), 157.1, 137.1, 129.7, 129.1, 127.5, 55.1, 53.3, 50.3, 40.7, 40.5 (dd, J = 9.1, 1.9 Hz, CHFCH$_2$), 37.1, 35.7, 28.3, 23.8 [2 × CHF signals overlapping or obscured]; ^{19}F NMR (282 MHz, D_2O) δ –76.0 (s, 3F, TFA), –198.2 (m, 1F, β-CHF), –201.7 (m, 1F, α-CHF); ^{19}F NMR (282 MHz, D_2O) δ –76.0 (s, 3F, TFA), –198.2 (d, J = 12.5 Hz, 1F, β-CHF), –201.7 (d, J = 12.5 Hz, 1F, α-CHF); MS (ESI, +ve) m/z 558 (MH^+, 100%); HRMS (ESI, +ve) $C_{23}H_{34}F_2N_7O_7^+$ requires m/z 558.2482, found 558.2474.

L-Arginyl-[(2*R*,3*R*)-4-amino-2,3-difluorobutanoyl]-L-aspartyl-L-phenylalanine, TFA salt (17)

The title compound was prepared on 25 μmol scale according to General Procedures B–F, employing a double coupling of Fmoc-arginine. The product was obtained as a sticky white solid (7.9 mg, 47% overall yield based on initial resin loading); analytical HPLC (0→100% acetonitrile/water (containing 0.1% v/v formic acid) over 10 min) retention time = 7.2 min, >95% purity; $[\alpha]_D$ –13.3 (*c* 0.26, H_2O); IR (neat) ν_{max} (cm^{-1}) 3206, 1651, 1556, 1531, 1415, 1178, 1129; ^{1}H NMR (300 MHz, D_2O, COSY) δ 7.41–7.25 (m, 5H, Ph), 5.19 (ddd, J = 46.2, 29.3, 1.3 Hz, 1H, α-CHF), 5.03 (ddddd, J = 46.5, 26.9, 8.5, 3.4, 1.3 Hz, 1H, β-CHF), 4.81 (m, 1H, Asp α-H), 4.68 (m, 1H, Phe α-H), 4.06 (t, J = 6.4 Hz, 1H, Arg α-H), 3.87 (ddd, J = 15.2, 14.7, 8.5 Hz, 1H, CHFCHH), 3.65 (ddd, J = 29.8, 14.7, 3.4 Hz, 1H, CHFCHH), 3.27 (dd, J = 14.2, 5.4 Hz, 1H, Phe β-CHH), 3.22 (t, J = 6.8 Hz, 2H, Arg δ-CH_2), 3.05 (dd, J = 14.0, 8.6 Hz, 1H, Phe β-CHH), 2.91 (dd, J = 16.9, 5.4 Hz, 1H, Asp β-CHH), 2.76 (dd, J = 16.9, 8.5 Hz, 1H, Asp β-CHH), 1.95 (m, 2H, Arg β-CH_2), 1.66 (m, 2H, Arg γ-CH_2); ^{13}C {^{1}H} NMR (75 MHz, D_2O) δ 175.5, 174.4, 171.5, 170.3, 168.4 (dd, J = 21.5, 3.6 Hz, CHFCO), 157.1, 137.1, 129.7, 129.0, 127.4, 90.1 (dd, J = 181.2, 18.0 Hz, CHF), 89.6 (dd, J = 194.5, 19.0 Hz, CHF), 54.9, 53.3, 50.2, 40.7, 39.5 (dd, J = 22.6, 6.6 Hz, CHFCH$_2$), 37.1, 35.8, 28.3, 23.8; ^{19}F NMR (282 MHz, D_2O) δ –73.5 (s, 3F, TFA), –201.1 (m, 1F, β-CHF), –207.0 (ddd, J = 46.1, 26.9, 10.7 Hz, 1F, α-CHF); ^{19}F NMR (282 MHz, D_2O) δ –73.5 (s, 3F, TFA), –201.1 (d, J = 10.7 Hz, 1F, β-CHF), –207.0 (d, J = 10.7 Hz, 1F, α-CHF); MS (ESI, +ve) m/z 558 (MH^+, 100%); HRMS (ESI, +ve) $C_{23}H_{34}F_2N_7O_7^+$ requires m/z 558.2482, found 558.2491.

L-Argininyl-[(*S*)-3-amino-2-fluoropropanoyl]-L-aspartyl-L-phenylalanine, TFA salt (18)

The title compound was prepared on 50 μmol scale according to General Procedures B–F, employing a double coupling of Fmoc-arginine. The product was obtained as a sticky white solid (22.9 mg, 72% overall yield based on initial resin loading); analytical HPLC (0→100% acetonitrile/water (containing 0.1% v/v formic acid) over 10 min) retention time = 7.3 min, >95% purity; $[\alpha]_D$ +4.0 (*c* 0.33, H_2O); IR (neat) ν_{max} (cm^{-1}) 3274, 2926, 2855, 1655, 1534, 1431, 1400, 1186, 1133; ^{1}H NMR (300 MHz, D_2O, COSY) δ 7.42–7.27 (m, 5H, Ph), 5.09 (dt, J = 47.5, 4.6 Hz, 1H, CHF), 4.76–4.64 (m, 2H, Asp α-H + Phe α-H), 4.00 (t, J = 6.4 Hz, 1H, Arg α-H), 3.71 (dd, J = 24.8, 4.3 Hz, 2H, CHFCH$_2$), 3.27 (dd, J = 14.0, 5.1 Hz, 1H, Phe β-CHH), 3.21 (m, 2H, Arg δ-CH_2), 3.06 (dd, J = 14.0, 8.9 Hz, 1H, Phe β-CHH), 2.90 (dd, J = 17.0, 5.8 Hz, 1H, Asp β-CHH), 2.78 (dd, J = 17.0, 7.9 Hz, 1H, Asp β-CHH), 1.89 (m, 2H, Arg β-CH_2), 1.64 (m, 2H, Arg γ-CH_2); ^{13}C {^{1}H} NMR (75 MHz, D_2O) δ 175.2, 174.3, 171.9, 170.2, 169.8 (d, J = 21.8 Hz, CHFCO), 157.2, 137.0, 129.7, 129.1, 127.5, 89.3 (d, J = 186.9 Hz, CHF), 54.7, 53.4, 50.1, 41.3 (d, J = 21.1 Hz, CHFCH$_2$), 40.7, 36.9, 35.6, 28.3, 23.9; ^{19}F NMR (282 MHz, D_2O) δ –76.0 (s, 3F, TFA), –195.9 (dt, J = 48.7, 25.0 Hz, 1F, CHF); ^{19}F {^{1}H} NMR (282 MHz, D_2O) δ –76.0 (s, 3F, TFA), –195.9 (s, 1F, CHF); MS (ESI, +ve) m/z 526 (MH^+, 100%); HRMS (ESI, +ve) $C_{22}H_{33}FN_7O_7^+$ requires m/z 526.2420, found 526.2424.

L-Argininyl-[(*R*)-3-amino-2-fluoropropanoyl]-L-aspartyl-L-phenylalanine, TFA salt (19)

The title compound was prepared on 50 μmol scale according to General Procedures B–F, employing a double coupling of Fmoc-arginine. The product was obtained as a sticky white solid (17.3 mg, 54% overall yield based on initial resin loading); analytical HPLC (0→100% acetonitrile/water (containing 0.1% v/v formic acid) over 10 min) retention time = 7.3 min, >95% purity; $[\alpha]_D$ –16.2 (*c* 0.26, H_2O); IR (neat) ν_{max} (cm^{-1}) 3215, 2926, 2855, 1655, 1533, 1429, 1186, 1134; ^{1}H NMR (300 MHz, D_2O, COSY) δ 7.42–7.27 (m, 5H, Ph), 5.10 (dt, J = 47.1, 4.1 Hz, 1H, CHF), 4.76–4.70 (m, 2H, Asp α-H + Phe α-H), 4.02 (t, J = 6.4 Hz, 1H, Arg α-H), 3.83–3.72 (m, 2H, CHFCH$_2$), 3.30 (dd, J = 14.1, 5.2 Hz, 1H, Phe β-CHH), 3.23 (m, 2H, Arg δ-CH_2), 3.06 (dd, J = 14.1, 9.0 Hz, 1H, Phe β-CHH), 2.88 (dd, J = 17.2, 5.1 Hz, 1H, Asp β-CHH), 2.75 (dd, J = 17.2, 8.8 Hz, 1H, Asp β-CHH), 1.92 (m, 2H, Arg β-CH_2), 1.65 (m, 2H, Arg γ-CH_2); ^{13}C {^{1}H} NMR (50 MHz, D_2O) δ 175.0, 174.3, 171.8, 170.2, 170.0 (d, J = 22.0 Hz, CHFCO), 157.2, 137.0, 129.7, 129.1, 127.5, 89.4 (d, J = 188.7 Hz, CHF), 54.4, 53.3, 50.0, 40.7, 40.6

(d, J = 31.0 Hz, CHFCH$_2$), 36.8, 35.5, 28.3, 23.9; ^{19}F NMR (282 MHz, D$_2$O) δ –76.0 (s, 3F, TFA), –196.4 (ddd, J = 47.5, 26.4, 23.7 Hz, 1F, CHF); ^{19}F {^{1}H} NMR (282 MHz, D$_2$O) δ –76.0 (s, 3F, TFA), –196.4 (s, 1F, CHF); MS (ESI, +ve) m/z 526 (MH$^+$, 100%); HRMS (ESI, +ve) $C_{22}H_{33}FN_7O_7^+$ requires m/z 526.2420, found 526.2423.

L-Argininyl-glycinyl-L-aspartyl-L-phenylalanine, TFA salt (20)

The title compound was prepared on 0.1 mmol scale according to General Procedures B–D & F. The product was obtained as a sticky white solid (0.053 g, 92% overall yield based on initial resin loading); analytical HPLC retention time = 7.0 min (0→25% acetonitrile/water (containing 0.1% v/v formic acid) over 30 min), >95% purity; [α]$_D$ –4.7 (c 0.53, H$_2$O); IR (neat) ν_{max} (cm^{-1}) 3203, 1645, 1531, 1416, 1026; ^{1}H NMR (300 MHz, D$_2$O, COSY) δ 7.40–7.26 (m, 5H, Ph), 4.71–4.60 (m, 2H, Asp α-H + Phe α-H), 4.11 (t, J = 6.4 Hz, 1H, Arg α-H), 4.04 (d, J = 16.7 Hz, 1H, Gly α-CHH), 3.92 (d, J = 16.7 Hz, 1H, Gly α-CHH), 3.28–3.20 (m, 3H, Arg δ-CH$_2$ + Phe β-CHH), 3.04 (dd, J = 13.8, 8.3 Hz, 1H, Phe β-CHH), 2.82 (dd, J = 16.9, 5.5 Hz, 1H, Asp β-CHH), 2.69 (dd, J = 16.9, 8.1 Hz, 1H, Asp β-CHH), 1.97 (m, 2H, Arg β-CH$_2$), 1.72 (m, 2H, Arg γ-CH$_2$); ^{13}C {^{1}H} NMR (75 MHz, D$_2$O) δ 175.7, 174.7, 172.0, 170.9, 170.5, 157.2, 137.2, 129.7, 129.1, 127.5, 55.1, 53.2, 50.6, 42.7, 40.8, 37.2, 36.0, 28.3, 23.8; MS (ESI, +ve) m/z 494 (MH$^+$, 100%); HRMS (ESI, +ve) $C_{21}H_{32}N_7O_7^+$ requires m/z 494.2358, found 494.2360.

L-Arginyl-β-alanyl-L-aspartyl-L-phenylalanine, TFA salt (21)

The title compound was prepared on 0.1 mmol scale according to General Procedures B–D & F. The product was obtained as a sticky white solid (0.053 g, 94% overall yield based on initial resin loading); analytical HPLC (0→25% acetonitrile/water (containing 0.1% v/v formic acid) over 30 min) retention time = 11.2 min, >95% purity; [α]$_D$ –5.6 (c 0.53, H$_2$O); IR (neat) ν_{max} (cm^{-1}) 3207, 1651, 1531, 1416, 1183, 1044; ^{1}H NMR (400 MHz, D$_2$O, COSY) δ 7.29–7.14 (m, 5H, Ph), 4.57–4.52 (m, 2H, Asp α-H + Phe α-H), 3.85 (t, J = 6.2 Hz, 1H, Arg α-H), 3.38 (m, 2H, β-Ala β-CH$_2$), 3.15 (dd, J = 13.9, 4.8 Hz, 1H, Phe β-CHH), 3.09 (t, J = 6.2 Hz, 2H, Arg δ-CH$_2$), 2.94 (dd, J = 13.9, 8.7 Hz, 1H, Phe β-CHH), 2.71 (dd, J = 17.0, 5.0 Hz, 1H, Asp β-CHH), 2.59 (dd, J = 17.0, 8.5 Hz, Asp β-CHH), 2.38 (t, J = 5.8 Hz, 2H, Gly α-CH$_2$), 1.78 (m, 2H, Arg β-CH$_2$), 1.51 (m, 2H, Arg γ-CH$_2$); ^{13}C {^{1}H} NMR (100 MHz, D$_2$O) δ 175.1, 174.1, 173.4, 171.9, 169.3, 156.6, 136.5, 129.2, 128.6, 127.0, 54.3, 52.7, 50.0, 40.2, 36.6, 35.8, 35.3, 34.5, 27.8, 23.4; MS (ESI, +ve) m/z 508 (MH$^+$, 100%); HRMS (ESI, +ve) $C_{22}H_{34}N_7O_7^+$ requires m/z 508.2514, found 508.2513.

L-Arginyl-GABA-L-aspartyl-L-phenylalanine, TFA salt (22)

The title compound was prepared on 0.1 mmol scale according to General Procedures B–D & F. The product was obtained as a sticky white solid (0.059 g, 94% overall yield based on initial resin loading); analytical HPLC (0→25% acetonitrile/water (containing 0.1% v/v formic acid) over 30 min) retention time = 11.0 min, >95% purity; [α]$_D$ –7.9 (c 0.59, H$_2$O); IR (neat) ν_{max} (cm^{-1}) 3212, 1651, 1556, 1416, 1180, 1128; ^{1}H NMR (300 MHz, D$_2$O, COSY) δ 7.38–7.23 (m, 5H, Ph), 4.70–4.63 (m, 2H, Asp α-H + Phe α-H), 3.98 (t, J = 6.4 Hz, 1H, Arg α-H), 3.27–3.14 (m, 5H, Arg δ-CH$_2$ + GABA γ-CH$_2$ + Phe β-CHH), 3.03 (dd, J = 14.7, 9.4 Hz, 1H, Phe β-CHH), 2.81 (dd, J = 16.8, 5.4 Hz, 1H, Asp β-CHH), 2.68 (dd, J = 16.8, 8.3 Hz, Asp β-CHH), 2.24 (t, J = 7.3 Hz, 2H, GABA α-CH$_2$), 1.91 (m, 2H, Arg β-CH$_2$), 1.74 (m, 2H, GABA β-CH$_2$), 1.63 (m, 2H, Arg γ-CH$_2$); ^{13}C {^{1}H} NMR (75 MHz, D$_2$O) δ 178.4, 177.4, 176.9, 174.9, 172.2, 159.7, 139.3, 132.2, 131.6, 130.1, 56.8, 55.9, 52.8, 43.2, 41.7, 39.4, 38.2, 35.6, 30.9, 27.6, 26.5; MS (ESI, +ve) m/z 522 (MH$^+$, 100%); HRMS (ESI, +ve) $C_{23}H_{35}N_7O_7^+$ requires m/z 22.2671, found 522.2675.

Cell adhesion assay

Reagents and methods were utilised mostly as described.[20] The following cell lines were used, with ligands in brackets: K562-$\alpha_5\beta_1$ (Fibronectin), K562-$\alpha_v\beta_3$ (LAP-β_1), K562-$\alpha_v\beta_5$ (Vitronectin), K562-$\alpha_v\beta_6$ (LAP-β_1). The divalent cation used to facilitate adhesion was 2 mM MgCl$_2$. Adhesion was quantified by cell labelling with the fluorescent dye BCECF-AM (Life Technologies), where cell suspensions at 6×10^6 cells/mL were incubated with 0.66 µL/mL of 30 mM BCECF-AM at 37 °C for 10 minutes, before dispensing into the assay plate. At the assay conclusion cells that adhered were lysed using 50 µl/well of 0.5% Triton X-100 in H$_2$O to release fluorescence. Fluorescence intensity was detected using an Envision® plate reader (Perkin Elmer). For active antagonists in the assay, data were fitted to a 4 parameter logistic equation for IC$_{50}$ determinations.

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Notes and references

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