Bioorganic & Medicinal Chemistry 20 (2012) 5139–5149

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and biological evaluation of tyrosine modified analogues of the α4β7 integrin inhibitor biotin-R_8ERY

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ARTICLE INFO

Article history:
Received 9 May 2012
Revised 28 June 2012
Accepted 5 July 2012
Available online 21 July 2012

Keywords:
Peptidomimetics
Integrins

ABSTRACT

The α4β7 integrin is a well-known target for the development of drugs against various inflammatory disease states including inflammatory bowel disease, type 1 diabetes and multiple sclerosis. The synthesis of a small library of cell-permeable β7 integrin inhibitors based on the peptide biotin-R_8ERY is reported, in which the tyrosine residue has been modified by using the Suzuki-Miyaura cross-coupling reaction. The synthesised peptidomimetics were evaluated in a cell adhesion assay and shown to inhibit Mn^{2+}-activated adhesion of mouse TK-1 T cells to mouse MAdCAM-1. All of the synthesised peptidomimetics are more active than our previously reported lead compound biotin-R_8ERY with two of the analogues, **6** and **7**, exhibiting IC_{50} values of <15 μM.

1. Introduction

As described in our previous paper,[1] the α4 integrins α4β7 (LPAM-1) and α4β1 (VLA-4) are heterodimeric cell-surface receptors expressed on most leukocytes.[2,3] They consist of noncovalently-associated α and β transmembrane subunits with comparatively large extracellular domains and short cytoplasmic domains. They mediate fundamental cell-extracellular matrix and cell-cell adhesion events.[4] Ligands of the α4 integrin subfamily include fibronectin (Fn), vascular cell adhesion molecule-1 (VCAM-1), and mucosal addressin cell adhesion molecule-1 (MAdCAM-1).[5–7] α4β1 preferentially recognises VCAM-1, whereas α4β7 preferentially recognises MAdCAM-1. α4β7 binds to MAdCAM-1 expressed on high endothelial venules, which contributes to the homing of lymphocytes to gut-associated lymphoid tissues (GALT) such as Peyer's patches and the lamina propria.[8] The β7 subunit also associates with the integrin αE subunit, forming the receptor αEβ7 which mediates the binding of intestinal intraepithelial lymphocytes (iIEL) to E-cadherin expressed on gut enterocytes.[9] Mucosal T cell numbers are selectively reduced in αE gene knockout mice.

β7 integrin adhesion plays a crucial role in regulating gut immunity[10,11] and is therefore implicated in the pathogenesis and progression of inflammatory bowel diseases, such as Crohn's disease, ulcerative colitis[12–14] and intestinal graft-versus-host diseases (GVHD).[8,15] Furthermore, α4β7 integrins contribute to leukocyte infiltration into the islets of Langerhans in type 1 diabetes[16] and the central nervous system in demyelinating diseases such as multiple sclerosis.[17]

Currently, steroids and non-steroidal anti-inflammatory drugs (NSAIDs) are the mainstay treatments for the above diseases, but the long-term usage of such drugs has detrimental side-effects. The concept of developing selective drugs that would prevent the homing of lymphocytes to chronically inflamed tissues with minimal effects on normal immune surveillance has prompted a number of pharmaceutical companies to focus on the development of α4β1 and/or α4β7 antagonists, mainly by using small molecule inhibitors to block the binding of the integrins to their extracellular ligands.[7,18–20]

Krissansen and coworkers have previously shown that both L- and D-enantiomers of a cell-permeable peptide biotin-r_9YDRREY, residues 735–740 of the cytoplasmic tail of the β7 subunit, inhibited adhesion of Mn^{2+}-activated T cells to β7 integrin ligands. It was demonstrated that biotin-r_9YDRREY suppressed MAdCAM-1-induced clustering of α4β7 receptors on the cell surface, potentially by acting as a competitive substrate for src, FAK, and other tyrosine kinases and signalling molecules. It is postulated that peptide biotin-r_9YDRREY thereby interrupted the association of α4β7

Abbreviations: FAK, focal adhesion kinase; FBS, fetal bovine serum; GALT, gut-associated lymphoid tissues; GVHD, graft-versus-host disease; HBSS, Hank's buffered salt solution; HMP, linker 4-(hydroxymethyl)phenoxyacetic acid; iIEL, intestinal intraepithelial lymphocytes; LPAM-1, lymphocyte Peyer's patch adhesion molecule-1; MAdCAM-1, mucosal addressin cell adhesion molecule-1; NSAIDs, non-steroidal anti-inflammatory drugs; PBS, phosphate buffered saline; R_8, (L-arginine)$_8$; r_9, (D-arginine)$_9$; SPPS, solid phase peptide synthesis; src, sarcoma; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late activating antigen-4.

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http://dx.doi.org/10.1016/j.bmc.2012.07.010

with intracellular signalling molecules and cytoskeletal elements, which in turn prevented the ability of the receptors to aggregate, resulting in disruption of cell adhesion. The identity of the key β7 integrin ligand, the exact binding mechanism and the structure of the binding pockets of the β7 integrin ligand(s) are unknown to date. Nevertheless, the inhibitory activity of the biotin-r_9YDRREY peptide was shown to depend on the flanking tyrosine residues, as deletion of the tyrosines or their substitution with phenylalanine led to loss of activity.[21] We also found that the peptide biotin-R_8ERY, a short pseudo-analogue of the biotin-r_9YDRREY peptide, has similar activity, hence it was used as a lead motif. The tyrosine residue in the shortened tripeptide ERY was considered to be the key residue to modify, at least initially, in order to increase inhibitory activity. A number of commercially available tyrosine analogues were incorporated into the ERY* scaffold. The resulting trimers were fused to octa-arginine tags, which act as cationic carriers enabling crossing of the cell membrane and delivery of the inhibitors to the cytoplasm of activated T cells.[22–24] We previously demonstrated that substitution of tyrosine by 4-chlorophenylalanine, 4-nitrophenylalanine or, most interestingly, 4,4′-biphenylalanine afforded analogues with activity approaching that of the earlier-reported biotin-r_9YDRREY peptide (unpublished results). Furthermore it is known that oral bioavailability can be an issue with peptidic α4β7/α4β1 antagonists.[7] In an effort to enhance the activity of these cell-permeable peptides by decreasing their peptidic nature and using the 4,4′-biphenylalanine scaffold, we decided to use the versatile Suzuki-Miyaura cross-coupling reaction to synthesise a small library of biaryl tyrosine building blocks derived from tyrosine triflate **2**, which were then incorporated into the R_8ERY* peptide sequence by automated Fmoc SPPS.

The Suzuki-Miyaura reaction was first described in 1979,[25] providing the synthetic community with a facile method to effect the coupling of organoboron reagents with organo halides or pseudo halides. Today, Pd-catalysed cross-coupling reactions are amongst the most widely used reactions in the pharmaceutical industry.[26] The development of new ligands, for example the electron rich biarylmonophosphine SPhos by Buchwald[27] and of Pd(II) catalysts which exhibit greater air and moisture stability,[28] has broadened the scope of the Suzuki reaction. The coupling of less reactive substrates, such as aryl chlorides, electron deficient organoboron reagents,[29] electron rich aryl halides, or sterically encumbered systems[30] are now possible.

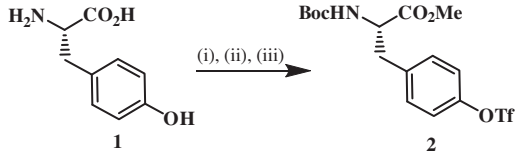
We have synthesised nine biaryl tyrosine analogues by using the Suzuki cross-coupling reaction and herein describe the synthesis and biological evaluation of cell permeable peptidomimetics based on this biotin-R_8ERY* motif, which have potential utility as anti-inflammatory agents for the treatment of chronic inflammatory diseases.

2. Results and discussion

2.1. Chemistry

The intermediate required for the Suzuki couplings was synthesised as shown in Scheme 1. Tyrosine **1** was esterified,[31] Boc-protected[32–34] then converted to the requisite triflate **2**,[35,36] which was then subjected to Pd-catalysed Suzuki reactions to produce the nine analogues shown in Table 1.

The reaction conditions were adjusted to meet the steric demands imposed by the boron reagents investigated. 4-Methylphenyl-, 4-ethylphenyl- and 4-biphenyl-boronic acids were cross-coupled with triflate **2** using $Pd(PPh_3)_4$ (Scheme 2, method **A**[7]), affording tyrosine mimetic building blocks **4a**,[37] **4b** and **4c**[38] for Fmoc SPPS after the appropriate deprotection/protection steps.[39,40] The more sterically demanding boron reagents, leading

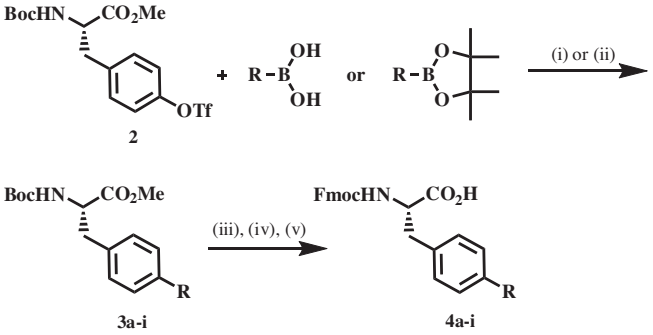


Scheme 1. Reagents and conditions: (i) $SOCl_2$, MeOH, reflux, 4 h; (ii) NEt_3, $(Boc)_2O$, MeOH, 0 °C, 12 h, N_2 (94%); (iii) pyridine, Tf_2O, DCM, −15 °C, 30 min, N_2 (90%).

Table 1
Synthetic building blocks **4** prepared by Suzuki coupling using tyrosine triflate **2**[7,30]

Compd	R=	Yield (%)	Method
4a	4-Methylphenyl[a]	90	A
4b	4-Ethylphenyl[a]	68	A
4c	Biphenyl[a]	68	A
4d	2-Methoxyphenyl[a]	73	B
4e	2,6-Dimethoxyphenyl[a]	73	B
4f	3,4-Dimethoxyphenyl[a]	29	B
4g	3-Nitrophenyl[a]	33	B
4h	4-Cyanophenyl[b]	74	B
4i	1′-Cyclohexenyl[b]	77	B

[a] Derived from requisite boronic acid.
[b] Derived from requisite boronic acid pinacol ester.



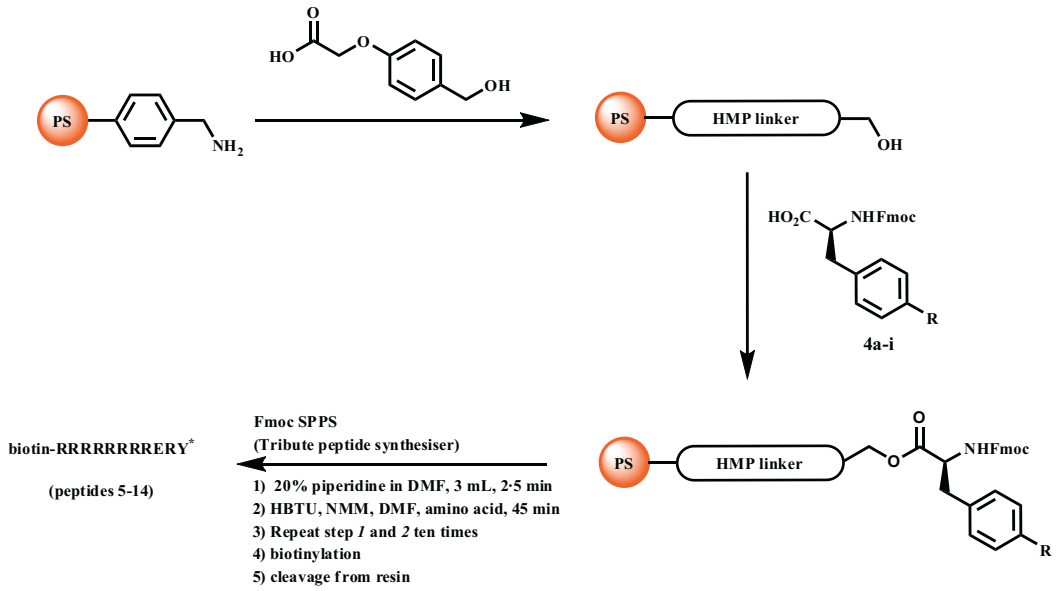
Scheme 2. Reagents and conditions: (i) $Pd(PPh_3)_4$, K_2CO_3, toluene/DMF, 80 °C, 12 h, N_2 (method **A**); (ii) $Pd(OAc)_2$, SPhos, K_3PO_4, toluene/DMF, 90 °C, N_2 (method **B**); (iii) NaOH, MeOH/THF (1/1), rt; (iv) TFA/DCM (1/1), 30 min; (v) Fmoc-OSu, $NaHCO_3$, THF/H_2O, rt, 12 h.

to building blocks **4d–f**, and the electron deficient boron reagents, giving **4g** and **4h**, were coupled to triflate **2** using $Pd(OAc)_2$ and SPhos (Scheme 2, method **B**[30]).

With the building blocks **4a–i** in hand, biotinylated R_8ERY* peptides **5–14** were then prepared on a 0.1 mmol scale following standard Fmoc SPPS procedures using a Tribute™ peptide synthesiser and aminomethylated polystyrene resin[41] derivatised with HMP linker (Scheme 3). Peptides **13** and **14** were obtained using the same building block **4i**, but applying different cleavage conditions. In order to obtain peptide **13** the cyclohexene moiety of building block **4i** was reduced to cyclohexane using 3,6-dioxa-1,8-octanedithiol (2.5% v/v) during cleavage from resin. The unsaturated analogue **14** was obtained, albeit in low yield, performing the cleavage with TFA/5% H_2O. All products were obtained in sufficient purity and yield for biological studies after purification by RP-HPLC (Table 2).

2.2. Biology

The $\alpha4\beta7^+$ TK-1 T cell line was chosen as a representative T cell as it is unique in that it does not express β1 integrins and hence can



Scheme 3. Fmoc SPPS of peptides **5**–**14** on aminomethylated PS resin.

Table 2
Biotin-R_8ERY* peptides and their percentage of inhibition in a cell adhesion assay

No.	Y*=	Yield[a] (%) (purity)	MS ($^{4+}$ion) Calcd/Obsd[b]	% Inhibition (50 μM)	% Inhibition (100 μM)
5	**4a**	13% (93%)	505.1/505.1	47.36	57.27
6	**4b**	35% (96%)	508.6/508.6	57.99	74.19
7	**4c**	15% (97%)	520.6/520.6	80.22	91.31
8	**4d**	47% (96%)	509.1/509.0	48.06	47.15
9	**4e**	53% (99%)	516.6/516.6	38.29	50.86
10	**4f**	43% (99%)	516.6/516.5	37.93	50.13
11	**4g**	34% (98%)	512.9/512.8	50.46	60.34
12	**4h**	23% (93%)	507.9/507.8	45.05	57.01
13 (Cyclohexyl)	**4i**	15% (91%)	503.1/503.1	71.72	64.85
14 (1′-Cyclohexenyl)	**4i**	3% (99%)	502.6/502.5	49.70	59.08
Biotin-R_8ERY	—	—	—	8.73	18.18
Biotin-r_9YDRREY	—	—	—	66.36	71.32

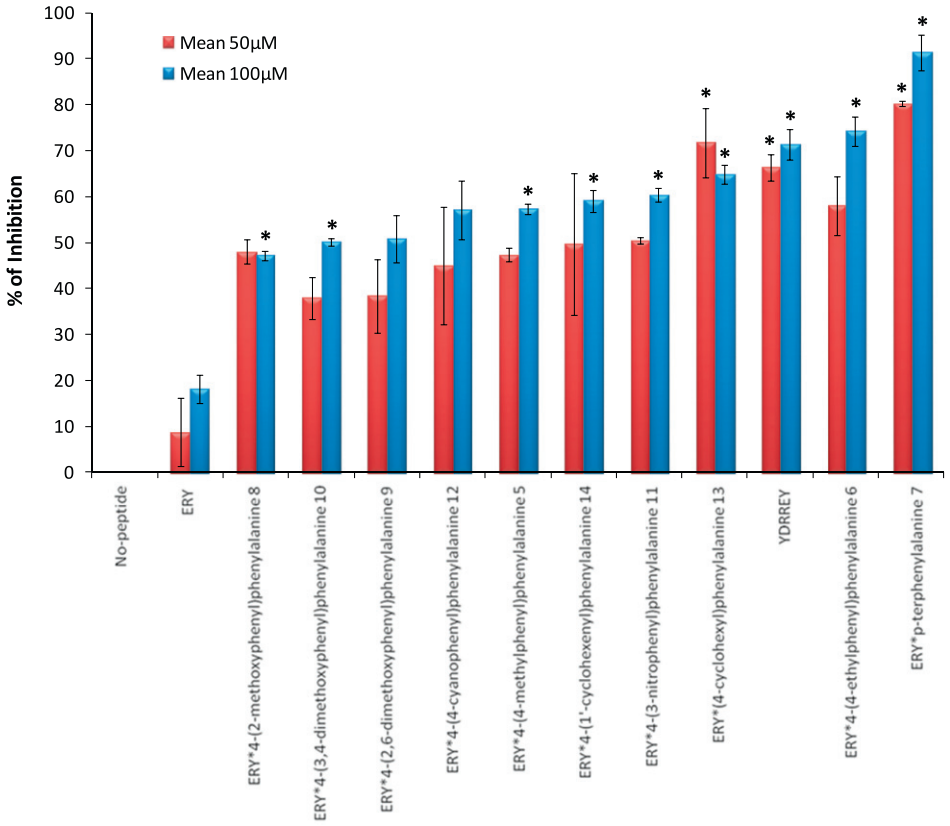
[a] Purified yield was based on calculated resin loading, see experimental section for further details.
[b] ESI-MS, see experimental section for further details.

be used to measure binding to MAdCAM-1 independently of α4β1, which shares α4β7 ligands. In the present work, the synthetic β7 tripeptides were tested for their ability to block the adhesion of Mn^{2+}-activated mouse TK-1 cells to mouse MAdCAM-1 coated onto chamber slides at a peptide concentration of 50 and 100 μM.

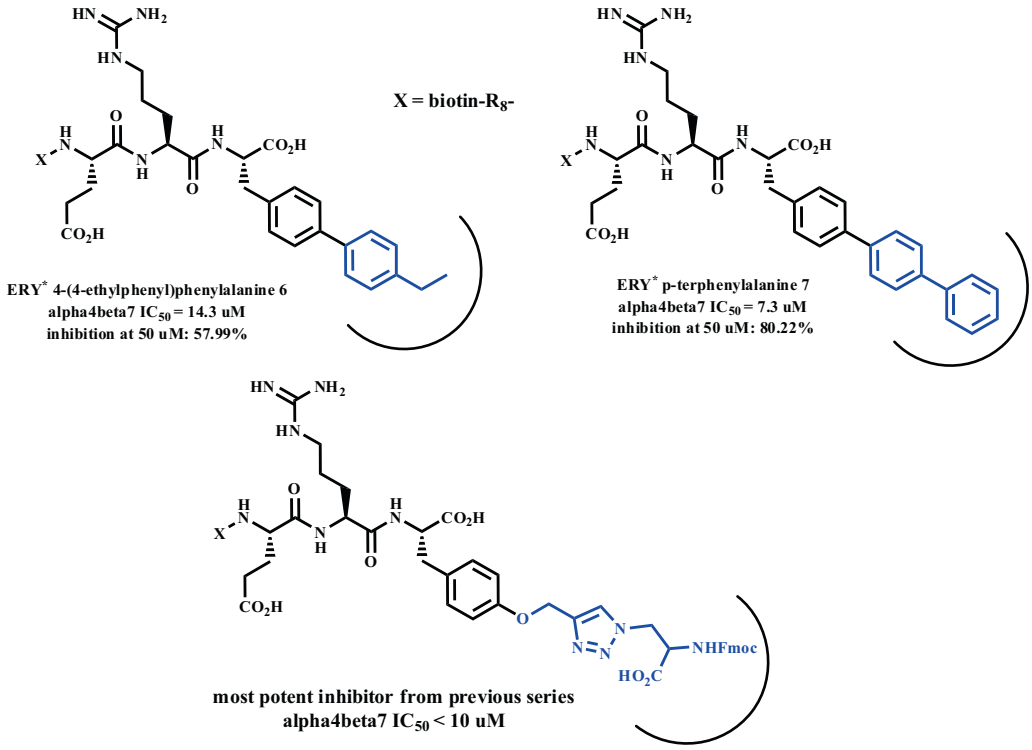
The inhibition assays were initially conducted at peptide concentrations of 50 and 100 μM (Fig. 1). Analogue **7**, carrying the *p*-terphenyl group, exhibited the strongest inhibition of TK-1 cell adhesion to MAdCAM-1 at both 50 and 100 μM, exceeding the activity of biotin-R_8ERY by 73% ($p = 0.01$) at 100 μM. Analogue **7** and compounds **13** and **6** exhibited the strongest inhibition in this library, which suggests that activity can be enhanced by incorporating large non-polar groups into the Y* side chain. Those modifications for compounds **7** and **6** rendered the trimeric ERY* peptide, which is considered to be the active unit of biotin-R_8ERY*, more active than the hexamer YDRREY, albeit the differences did not reach statistical significance [inhibition by analogue **7** at 50 μM approached significance ($p = 0.08$)]. This is also backed up by the results of the previous study where the biotin-R_8ERY* analogue carrying an Fmoc protecting group in the side chain of the altered tyrosine moiety was the most potent inhibitor (58% inhibition at 10 μM),[1] suggesting a 'deep' binding pocket and π–π stacking interactions between the peptide and the receptor (Scheme 4). When the size of the tyrosine substituent was decreased from biphenyl (**7**) to 4-ethylphenyl (**6**) the activity decreased by 22% and by changing to 4-methylphenyl (**5**) or 4-cyanophenyl (**12**) it decreased by 33% and 35%, respectively.

Though not statistically significant, the 4-cyclohexyl analogue **13** is slightly more active than the unsaturated 4-(1′-cyclohexenyl) analogue **14** at both concentrations and the methoxy substituted analogues **8–10** showed the poorest activity at 100 μM amongst the ten peptidomimetics. This is in accordance with the discovered steric demand for the 2-methoxyphenyl analogue **8** and 2,6-dimethoxy analogue **9**, as they lack a substituent at the 4-position of the second phenyl ring. Why the 3,4-dimethoxyphenyl analogue **10** does not display higher activity is not clear at this stage. The relatively high activity of the 3-nitrophenyl analogue **11** within this series at 100 μM could be an indication that the attachment of large aromatic substituents at the 3-position of the tyrosine ring could enhance activity. Whilst compound **13** appeared to be slightly more active at 50 μM than at 100 μM there is no statistically significant difference between the levels of inhibition at the two concentrations, indicating that inhibition is maximal at 50 μM.

In order to investigate the ability of the peptides to inhibit T cell adhesion at lower concentrations a dose response assay was conducted for the three most active peptides **6**, **7** and **13** at a wide



Figuer 1. Percent of inhibition of biotin-R_8ERY* peptides on Mn^{2+}-activated TK-1 cell adhesion to mouse MAdCAM-1-Fc (50 and 100 µM concentration). SEM of two experiments with duplicate wells. *$p \leqslant 0.05$, significantly more inhibitory than ERY.



Scheme 4. Comparison of the structures of the two most active peptides **6** and **7** of this study to the most potent inhibitor of the series published earlier.[1]

range of concentrations**.** The assay revealed that the two most active compounds **6** and **7** retained a good level of activity despite lowering the concentrations (Fig. 2). All three peptides were significantly more active at 50 µM (p <0.05–0.01) and 25 µM

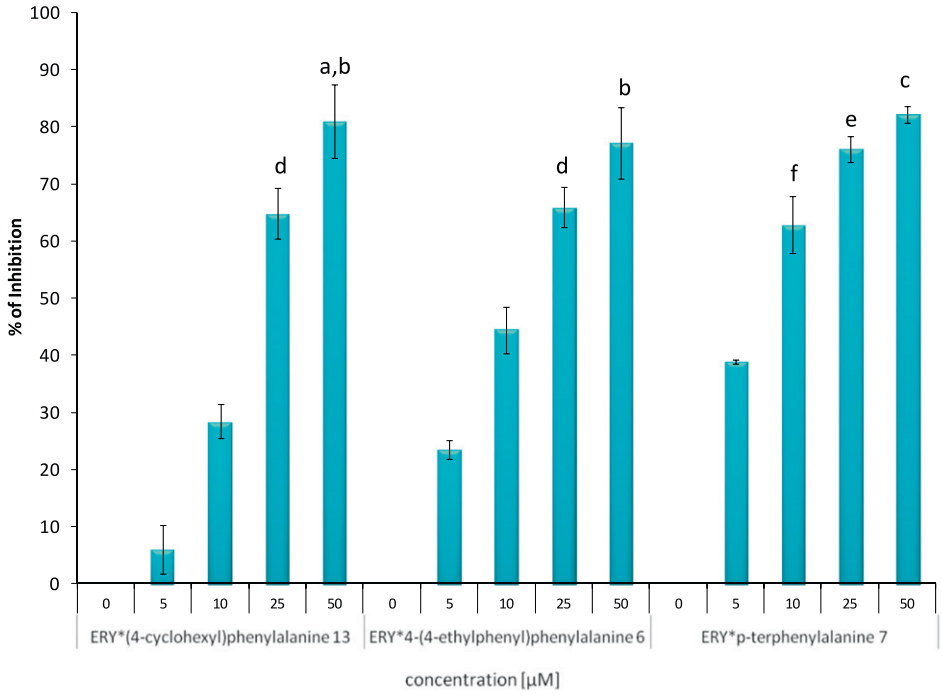


Figure 2. Dose response assay of the three most active peptides. Percent of inhibition of selected ERY* peptides on Mn^{2+} activated TK-1 cell adhesion to mouse MAdCAM-1-Fc. SEM of two experiments with duplicate samples. (a) 50 μM significantly different from 10 μM (p <0.05), (b) 50 μM significantly different from 5 μM (p <0.05), (c) 50 μM significantly different from 5 μM (p <0.01), (d) 25 μM significantly different from 5 μM (p <0.05), (e) 25 μM significantly different from 5 μM (p <0.01), (f) 10 μM significantly different from 5 μM ($p \leqslant 0.05$).

(p <0.05–0.01) than at 5 μM (p <0.05–0.01). Peptide **13** was significantly more active at 50 μM (p <0.05) and 25 μM (p <0.05) than at 10 μM. The dose-response analysis revealed that peptides **6**, **7**, and **13** had IC_{50s} of approximately 14.3, 7.3, and 19 μM. Peptide **13** lost inhibitory activity more significantly when its concentration was lowered.

3. Conclusion

Building on the previously reported library of α4β7 inhibitors[1] a series of ten novel, cell-permeable peptidomimetics incorporating the biotin-R_8ERY* motif, centered on aryl-substituted phenylalanine, was prepared. These tyrosine analogue building blocks were synthesised using Suzuki-Miyaura cross-coupling methodology then incorporated into the requisite peptide sequences, which were then tested for α4β7 antagonism in a lymphocyte cell-binding assay. Nine of the peptidomimetics exhibited statistically significant greater potency than the previously reported lead compound biotin-R_8ERY at 50 and 100 μM and a dose response assay showed an IC_{50} of <10 μM for the most active analogue **7**. Based on these promising results investigations to further improve the activity of the synthetic peptidomimetics are underway.

4. Experimental

4.1. Reagents and general methods

All reagents were purchased as reagent grade from commercial sources and used as supplied. Solvents were used as supplied unless otherwise stated or dried according to standard methods.[42] RP-HPLC solvents were purchased as HPLC grade and used without further purification. All amino acids were purchased as L-enantiomers.

Analytical thin layer chromatography (TLC) was performed on 0.2 mm aluminium plates of silica gel 60 F_{254} (Merck) and visualised by UV fluorescence. Flash chromatography was performed using Davisil® chromatographic silica (LC60Å 40–63 micron) (Grace GmbH & Co. KG) with indicated solvents. Infrared spectra were obtained using a Perkin Elmer spectrum One Fourier Transform infrared spectrometer with a universal ATR sampling accessory. Nuclear magnetic resonance (NMR) spectra were recorded as indicated on either a Bruker AVANCE DRX300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) or on a Bruker AVANCE DRX400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer at 298 K. Optical rotations were determined at 20 °C with a Perkin-Elmer 341 polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. Melting points were determined on a Electrothermal® melting point apparatus and are uncorrected. ESI-MS were recorded on a Thermo Finnigan Surveyor MSQ Plus spectrometer or a Bruker micrOTOF-Q II spectrometer. Semi-preparative RP-HPLC was performed on a Dionex Ultimate 3000 system using a Phenomenex Gemini C_{18} column (110 Å, 5 μM, 10 × 250 mm) eluting with a linear gradient of water/0.1% TFA and MeOH/0.1% TFA at a flow rate of 5 mL/min. Analytical RP-HPLC was performed on a Dionex P680 system using a Phenomenex Gemini C_{18} column (110 Å, 5 μM, 4.6 × 150 mm) with the same eluents at a flow rate of 1 mL/min. Gradient systems were adjusted according to the elution profiles and peak profiles obtained from the analytical RP-HPLC chromatograms.

5. Syntheses of building blocks using Suzuki-Miyaura reaction

5.1. General procedure A: Suzuki cross-coupling reaction

The requisite boron reagent (2.28 mmol) and K_2CO_3 (4.55 mmol, 580 mg) were combined and suspended in a degassed mixture of toluene/DMF (10:1, 15 mL). Boc–Tyr(OTf)–CO_2Me **2**

(1.75 mmol, 750 mg) was dissolved in the same solvent (4 mL) and added under N_2 followed by $Pd(PPh_3)_4$ (0.18 mmol, 202 mg) and the reaction was heated at 80 °C overnight. The black mixture was filtered through Celite and the filtrate concentrated. The residue was dissolved in EtOAc and the organic layer was washed with water, dried over Na_2SO_4 and concentrated to give the crude products **3a–c**, which were purified by flash column chromatography.

5.2. General procedure B: Suzuki cross-coupling reaction

The requisite boron reagent (2.63 mmol) and K_3PO_4 (5.25 mmol, 1.113 g) were combined and suspended in a degassed mixture of toluene/DMF (10:1, 15 mL). Boc–Tyr(OTf)–CO_2Me **2** (1.75 mmol, 750 mg) was added under N_2, followed by SPhos (12.5 mol %, 90 mg) and $Pd(OAc)_2$ (5 mol %, 20 mg) and the reaction was heated at 90 °C overnight. The black mixture was filtered through Celite and the filtrate concentrated. The residue was dissolved in EtOAc and the organic layer was washed with water, dried over Na_2SO_4 and concentrated to give the crude products **3d–i**, which were purified by flash column chromatography.

5.3. General procedure C: Fmoc protection

The Boc-protected amino acid methyl ester **3a–i** (1 equiv) was dissolved in MeOH/THF (1:1, 6 mL) and 1 N NaOH (5 equiv) was added. The reaction mixture was stirred at room temperature until complete hydrolysis was observed by TLC. The solution was acidified to pH 3 with 1 N HCl and then extracted with DCM. The organic layer was washed with water, dried over $MgSO_4$ and concentrated. The crude product was dissolved in DCM/TFA (1:1) and stirred at room temperature for 30 min whereupon the solvent was removed in vacuo.

The unprotected product was then dissolved in H_2O/THF, basified with $NaHCO_3$ to pH 10. Fmoc-OSu (1.05 equiv) was added slowly and the solution stirred at room temperature overnight. The solution was acidified to pH 3 with 5% citric acid, the precipitated product extracted into EtOAc and the organic layer was washed with water, dried over $MgSO_4$ and concentrated to give the crude product **4a–i**, which was purified by flash column chromatography.

5.4. NH_2–Tyr–CO_2Me

NH_2–Tyr–CO_2H **1** (16.6 mmol, 3 g) was dissolved in MeOH (12 mL) and neat $SOCl_2$ (18.3 mmol, 1.3 mL) added dropwise. The clear solution was heated under reflux for 4 h then cooled and the solvent removed in vacuo to give a white solid which was used without further purification in the next step. ^{1}H NMR (400 MHz, D_2O) δ 3.19 (1H, dd, J = 7.4, 14.7 Hz, βCH_2), 3.28 (1H, dd, J = 5.8, 14.7 Hz, βCH_2), 3.85 (3H, s, CO_2CH_3), 4.39 (1H, dd, J = 5.8, 7.4 Hz, αC*H*), 6.92 (2H, d, J = 8.5 Hz, 3,5-C*H*), 7.18 (2H, d, J = 8.6 Hz, 2,6-C*H*).

5.5. Boc–Tyr–CO_2Me

NH_2–Tyr–CO_2Me was dissolved in MeOH (40 mL), NEt_3 (24.9 mmol, 3.3 mL) added and the red solution cooled on ice under N_2. Boc_2O (18.3 mmol, 3.985 g) was added portionwise and the stirring continued overnight at room temperature under N_2. The solution was acidified with 1 N HCl (pH 3) and extracted with DCM. The organic layer was dried over $MgSO_4$, filtered and concentrated to give the crude product as an orange oil, which was purified by flash column chromatography (EtOAc/hexane 3/7, R_f 0.17) to give the desired product as a white foam (4.584 g, 94% over two steps): mp 108 °C (lit.[32] 100–102 °C); $[\alpha]_D^{20}$ +43.27 (*c* 0.1, $CHCl_3$) (lit.[32], +46.00 (*c* 1, $CHCl_3$)); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (9H, s, CH_3/Boc), 2.95 (1H, dd, J = 6.3, 14.1 Hz, βCH_2), 3.01 (1H, dd, J = 5.7, 13.9 Hz, βCH_2), 3.68 (3H, s, CO_2CH_3), 4.52 (1H, m, αC*H*), 5.09 (1H, d, J = 7.7 Hz, N*H*), 6.73 (2H, d, J = 8.1 Hz, 3-C*H*), 6.93 (2H, d, J = 8.4 Hz, 2-C*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.3 (3x CH_3/Boc), 37.6 (βCH_2), 52.3 (CO_2CH_3), 54.2 (αCH), 80.3 (*C*/Boc), 115.5 (3-CH), 127.4 (1-*C*), 130.3 (2-CH), 155.2, 155.4 (*C*=O/Boc, 4-C), 172.7 (CO_2CH_3); IR υ_{max} (cm^{-1}): 3305, 2982, 1760, 1686, 1508, 1448, 1369, 1209, 1147, 834; ESI-MS *m/z*: 296.1490 $(M+H)^+$ ($(M+H)^+$, $C_{15}H_{22}NO_5$ requires 296.1498), 318.1308 $(M+Na)^+$, 240.0870 $(M-C(CH_3)_3)^+$, 196.0970 $(M-Boc)^+$.

5.6. Boc–Tyr(OTf)–CO_2Me (2)

Boc–Tyr–CO_2Me (15.5 mmol, 4.584 g) was dissolved in DCM (50 mL), pyridine (38.8 mmol, 3.1 mL) added and the solution was stirred under N_2 at −15 °C. Triflic anhydride (18.6 mmol, 3.1 mL) was added dropwise and the red solution was stirred under N_2 at −15 °C for 30 min. To the reaction was added water and the layers were separated. The organic layer was washed with 0.5 N NaOH and 5% citric acid, dried over $MgSO_4$ and concentrated to give the crude product **2** as a red oil, which was purified by flash column chromatography (EtOAc/hexane 15/85, R_f 0.25) to give the desired product as a yellowish crystalline solid (5.972 g, 90%): mp 49 °C (lit.[35] 47–48 °C); $[\alpha]_D^{20}$ +34.80 (*c* 0.1, $CHCl_3$) (lit.[36] +33.60 (*c* 1, $CHCl_3$)); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (9H, s, CH_3/Boc), 3.04 (1H, dd, J = 6.5, 13.8 Hz, βCH_2), 3.17 (1H, dd, J = 5.7, 13.9 Hz, βCH_2), 3.71 (3H, s, CO_2CH_3), 4.60 (1H, m, αC*H*), 5.02 (1H, d, J = 7.7 Hz, N*H*), 7.21 (4H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.2 (3x CH_3/Boc), 37.9 (βCH_2), 52.4 (CO_2CH_3), 54.2 (αCH), 80.2 (C/Boc), 117.1 (C/OTf), 121.3 (3-CH), 131.1 (2-CH), 136.9 (1-C), 148.6 (4-C), 154.9 (*C*=O/Boc), 171.9 (CO_2CH_3); IR υ_{max} (cm^{-1}): 3382, 2984, 1733, 1690, 1502, 1517, 1424, 1248, 1129, 895; MS (EI) *m/z*: 450.0809 $(M+Na)^+$ ($(M+Na)^+$, $C_{16}H_{20}F_3NO_7SNa$ requires 450.0810), 372.0367 $(M-C(CH_3)_3)^+$, 328.0467 $(M-Boc)^+$.

5.7. Fmoc-4-(4-methylphenyl)phenylalanine (4a)

The intermediate **3a** was prepared following general procedure **A**, using 310 mg of 4-methylphenylboronic acid. The crude product (brown oil) was purified by flash column chromatography (EtOAc/hexane (1:9), R_f 0.14) to yield the desired product **3a** as a white solid (498 mg, 77%): mp 78–79 °C (lit.[36] 77–79 °C); $[\alpha]_D^{20}$ +50.00 (*c* 0.1, $CHCl_3$) (lit.[36] +52.24 (*c* 1, $CHCl_3$)); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (9H, s, CH_3/Boc), 2.39 (3H, s, CH_3), 3.09 (1H, dd, J = 6.3, 13.8 Hz, βCH_2), 3.19 (1H, dd, J = 5.6, 13.8 Hz, βCH_2), 3.73 (3H, s, CO_2CH_3), 4.65 (1H, m, αC*H*), 5.21 (1H, d, J = 8.2 Hz, N*H*), 7.21 (2H, d, J = 8.0 Hz, aromatic), 7.24 (2H, d, J = 8.1 Hz, aromatic), 7.48 (2H, d, J = 8.1 Hz, aromatic), 7.52 (2H, d, J = 8.1 Hz, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$): 21.1 (CH_3), 28.3 (3xCH_3/Boc), 37.9 (βCH$_2$), 52.2 (CO_2CH_3), 54.5 (αCH), 79.8 (C/Boc), 126.8, 127.0 (2, 3′-CH), 129.5, 129.7 (3, 2′-CH), 134.9, 137.0, 137.9, 139.8 (1, 4, 1′, 4′-C), 155.2 (*C*=O/Boc), 172.4 (CO_2CH_3); IR υ_{max} (cm^{-1}): 3313, 2976, 1734, 1696, 1498, 1209, 1158, 809; ESI-MS *m/z*: 370.2016 $(M+H)^+$ ($(M+H)^+$, $C_{22}H_{28}NO_4$ requires 370.2018), 392.1835 $(M+Na)^+$, 314.1395 $(M-C(CH_3)_3)^+$, 270.1498 $(M-Boc)^+$.

Intermediate **3a** (1.23 mmol, 476 mg) was converted into the corresponding Fmoc- protected building block using general procedure **C**. The crude product (orange oil) was purified by flash column chromatography (DCM then EtOAc/hexane (3:7 to neat EtOAc) + 1% acetic acid, R_f 0.35 in EtOAc/hexane (1:1) + 1% acetic acid) to yield the desired product **4a** as a white solid (557 mg, 90% over three steps): mp 130 °C; $[\alpha]_D^{20}$ +48.51 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.39 (3H, s, CH_3), 3.16 (1H, dd, J = 6.0, 13.8 Hz, βCH_2), 3.25 (1H, dd, J = 5.3, 14.0 Hz, βCH_2), 4.21 (1H, t, J = 7.1 Hz, CH/Fmoc), 4.38 (1H, dd, J = 6.8, 10.4 Hz, CH_2/Fmoc), 4.46 (1H, dd, J = 7.0, 10.7 Hz, CH_2/Fmoc), 4.75 (1H, m, αC*H*), 5.28 (1H, d,

J = 8.1 Hz, N*H*), 7.20 (2H, d, J = 8.0 Hz, aromatic), 7.23 (2H, d, J = 8.0 Hz, aromatic), 7.29 (2H, t, J = 7.4 Hz, 3-C*H*/Fmoc), 7.39 (2H, t, J = 7.4 Hz, 4-C*H*/Fmoc), 7.45 (2H, d, J = 7.9 Hz, aromatic), 7.50 (2H, d, J = 7.9 Hz, aromatic), 7.56 (2H, d, J = 5.4 Hz, 2-C*H*/Fmoc), 7.76 (2H, d, J = 7.5 Hz, 5-C*H*/Fmoc); ^{13}C NMR (100 MHz, $CDCl_3$): 21.1 (*C*H$_3$), 37.4 (βCH$_2$), 47.1 (*C*H/Fmoc), 54.7 (α*C*H), 66.9 (*C*H$_2$/Fmoc), 119.9, 125.1, 125.2, 126.8, 127.0, 127.1, 127.7, 129.5, 129.9 (16x *C*H/aromatic BiPhe + aromatic Fmoc), 134.9, 136.9, 137.8, 139.7, 141.3, 143.8, 143.9 (8x C/aromatic BiPhe + aromatic Fmoc), 155.8 (*C*=O/Fmoc), 174.1 (*C*O$_2$H); IR υ_{max} (cm^{-1}): 3347, 2924, 2497, 1723, 1689, 1529, 1254, 1216, 809, 738; ESI-MS *m/z*: 478.2003 (M+H)$^+$ ((M+H)$^+$, $C_{31}H_{28}NO_4$ requires 478.2018), 500.1819 (M+Na)$^+$.

5.8. Fmoc-4-(4-ethylphenyl)phenylalanine (4b)

The intermediate **3b** was prepared following general procedure **A**, using 342 mg 4-ethylphenylboronic acid. The crude product was purified by flash column chromatography (EtOAc/hexane (7:93), R_f 0.36) to yield the desired product **3b** as an off-white solid (339 mg, 51%): mp 88–89 °C; $[\alpha]_D^{20}$ +40.98 (*c* 0.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (3H, t, J = 7.6 Hz, C*H*$_3$), 1.47 (9H, s, C*H*$_3$/Boc), 2.72 (2H, q, J = 7.6 Hz, C*H*$_2$), 3.11 (1H, dd, J = 6.1, 13.7 Hz, βC*H*$_2$), 3.20 (1H, dd, J = 5.6, 13.8 Hz, βC*H*$_2$), 3.75 (3H, s, CO$_2$C*H*$_3$), 4.67 (1H, m, α*CH*), 5.17 (1H, bs, N*H*), 7.23 (2H, d, J = 7.9 Hz, aromatic), 7.29 (2H, d, J = 8.1 Hz, aromatic), 7.54 (4H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$): 15.6 (*C*H$_3$), 28.3 (3x *C*H$_3$/Boc), 28.5 (*C*H$_2$), 38.0 (β*C*H$_2$), 52.2 (CO$_2$*C*H$_3$), 54.4 (α*C*H), 79.9 (C/Boc), 126.9, 127.1, 128.3, 129.7 (2, 3, 2′, 3′-*C*H), 134.8, 138.1, 139.9, 143.4 (1, 4, 1′, 4′-C), 155.2 (*C*=O/Boc), 172.4 (*C*O$_2$CH$_3$); IR υ_{max} (cm^{-1}): 3362, 2965, 1735, 1682, 1513, 1498, 1299, 1158, 814; ESI-MS *m/z*: 384.2163 (M+H)$^+$ ((M+H)$^+$, $C_{23}H_{30}NO_4$ requires 384.2175), 406.1988 (M+Na)$^+$, 328.1531 (M−C(CH$_3$)$_3$)$^+$, 284.1628 (M−Boc)$^+$.

Intermediate **3b** (0.80 mmol, 215 mg) was converted into the corresponding Fmoc- protected building block using general procedure **C**. The crude product was purified by flash column chromatography (DCM to EtOAc/hexane (2:8) + 1% acetic acid, R_f 0.39 in EtOAc/hexane (1:1) + 1% acetic acid) to yield the desired product **4b** as a white solid (269 mg, 68% over three steps): mp 161 °C; $[\alpha]_D^{20}$ +45.19 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28 (3H, t, J = 7.6 Hz, C*H*$_3$) 2.69 (2H, q, J = 7.6 Hz, C*H*$_2$), 3.16 (1H, dd, J = 6.0, 13.8 Hz, βC*H*$_2$), 3.26 (1H, dd, J = 5.3, 14.2 Hz, βC*H*$_2$), 4.21 (1H, t, J = 6.8 Hz, C*H*/Fmoc), 4.39 (1H, dd, J = 6.5, 10.6 Hz, C*H*$_2$/Fmoc), 4.46 (1H, dd, J = 7.0, 10.6 Hz, C*H*$_2$/Fmoc), 4.75 (1H, m, α*CH*), 5.29 (1H, d, J = 8.0 Hz, N*H*), 7.20 (2H, d, J = 7.9 Hz, aromatic), 7.25–7.33 (4H, m, aromatic), 7.39 (2H, t, J = 7.4 Hz, 4-C*H*/Fmoc), 7.46–7.51 (4H, m, aromatic), 7.57 (2H, m, aromatic), 7.76 (2H, d, J = 7.5 Hz, 5-C*H*/Fmoc); ^{13}C NMR (100 MHz, $CDCl_3$): 15.6 (*C*H$_3$), 28.5 (*C*H$_2$), 37.3 (βCH$_2$), 47.1 (*C*H/Fmoc), 54.6 (α*C*H), 67.1 (*C*H$_2$/Fmoc), 120.0, 125.0, 126.9, 127.1, 127.2, 127.8, 128.3, 129.8 (16x *C*H/aromatic BiPhe + aromatic Fmoc), 134.2, 138.0, 140.1, 141.3, 143.5, 143.7, 143.8 (8x C/aromatic BiPhe + aromatic Fmoc), 155.8 (*C*=O/Fmoc), 175.9 (*C*O$_2$H); IR υ_{max} (cm^{-1}): 3323, 2967, 1717, 1692, 1530, 1263, 1228, 816, 731; ESI-MS *m/z*: 492.2177 (M+H)$^+$ ((M+H)$^+$, $C_{32}H_{30}NO_4$ requires 492.2175), 514.2001 (M+Na)$^+$.

5.9. Fmoc–(*p*-terphenyl)alanine–COOH (4c)

The intermediate **3c** was prepared following general procedure **A**, using 451 mg of 4-biphenylboronic acid. The crude product was purified by flash column chromatography (EtOAc/hexane (1:9–2:8), R_f 0.24 in EtOAc/hexane (15:85)) to yield the desired product **3c** as an off-white solid (450 mg, 60%): mp 185 °C (lit.[37], 189–190 °C); $[\alpha]_D^{20}$ +43.95 (*c* 0.1, $CHCl_3$) (lit.[37], + 54.81 (*c* 1, $CHCl_3$)); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (9H, s, C*H*$_3$/Boc), 3.12 (1H, dd, J = 5.9, 13.6 Hz, βC*H*$_2$), 3.20 (1H, dd, J = 5.6, 13.7 Hz, βC*H*$_2$), 3.76 (3H, s, CO$_2$C*H*$_3$), 4.66 (1H, m, α*CH*), 5.07 (1H, d, J = 7.0 Hz, N*H*), 7.23 (2H, d, J = 7.8 Hz, aromatic), 7.37 (1H, t, J = 7.1 Hz, 4″-C*H*), 7.47 (2H, t, J = 7.4 Hz, 3″-C*H*), 7.59 (2H, d, J = 8.1 Hz, aromatic), 7.65 (2H, d, J = 7.5 Hz, aromatic), 7.68 (4H, s, 2′,3′-C*H*); ^{13}C NMR (100 MHz, $CDCl_3$): 28.3 (3x *C*H$_3$/Boc), 38.0 (βCH$_2$), 52.3 (CO$_2$CH$_3$), 54.4 (α*C*H), 80.0 (C/Boc), 127.0, 127.2, 127.4, 127.5, 128.9, 129.8 (2, 3, 2′, 3′, 2″, 3″, 4″-CH), 135.2, 139.4, 139.7, 140.1, 140.7 (1, 4, 1′, 4′, 1″-C), 155.2 (*C*=O/Boc), 172.4 (*C*O$_2$CH$_3$); IR υ_{max} (cm^{-1}): 3382, 2981, 1742, 1697, 1516, 1485, 1298, 1167, 817, 762; ESI-MS *m/z*: 432.2182 (M+H)$^+$ ((M+H) $^+$, $C_{27}H_{30}NO_4$ requires 432.2175), 454.2002 (M+Na)$^+$, 376.1550 (M−C(CH$_3$)$_3$)$^+$, 332.1648 (M−Boc)$^+$.

Intermediate **3c** (1.04 mmol, 331 mg) was converted into the corresponding Fmoc- protected building block using general procedure **C**. The crude product was purified by flash column chromatography (DCM to EtOAc/hexane (1:1 to neat EtOAc) + 1% acetic acid, R_f 0.35 in EtOAc/hexane (1:1) + 1% acetic acid) to yield the desired product **4c** as a white solid (383 mg, 68% over three steps): mp 217 °C; $[\alpha]_D^{20}$ +50.77 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.93 (1H, dd, J = 10.8, 13.6 Hz, βC*H*$_2$), 3.15 (1H, dd, J = 4.4, 13.9 Hz, βC*H*$_2$), 4.15–4.29 (5H, m, C*H*/Fmoc + C*H*$_2$/Fmoc + α*CH* + N*H*), 7.26–7.42 (6H, m, aromatic), 7.48 (2H, t, J = 7.4 Hz, aromatic), 7.61–7.67 (4H, m, aromatic), 7.70–7.75 (6H, m, aromatic), 7.79 (1H, d, J = 8.5 Hz, 4″-C*H*), 7.87 (2H, d, J = 7.5 Hz, aromatic); ^{13}C NMR (100 MHz, DMSO-d_6): 46.5 (*C*H/Fmoc), 55.4 (α*C*H), 65.6 (*C*H$_2$/Fmoc), 120.0, 125.2, 126.3, 126.5, 126.9, 127.0, 127.1, 127.5, 127.6, 129.0, 129.8 (21x *C*H/aromatic TriPhe + aromatic Fmoc), 138.8, 138.9, 139.5, 140.6, 143.7 (9x C/aromatic TriPhe + aromatic Fmoc), 156.0 (*C*=O/Fmoc), 173.3 (*C*O$_2$H); IR υ_{max} (cm^{-1}): 3337, 2945, 1697, 1525, 1284, 1226, 814, 760, 738; ESI-MS *m/z*: 540.2160 (M+H)$^+$ ((M+H)$^+$, $C_{36}H_{30}NO_4$ requires 540.2175), 562.1976 (M+Na)$^+$.

5.10. Fmoc-4-(2-methoxyphenyl)phenylalanine (4d)

The intermediate **3d** was prepared following general procedure **B**, using 399 mg of 2-methoxyphenylboronic acid. The crude product was purified by flash column chromatography (EtOAc/hexane (2:8), R_f 0.13 in EtOAc/hexane (2:8)) to yield the desired product **3d** as a colourless foam (595 mg, 88%): $[\alpha]_D^{20}$ +39.10 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (9H, s, C*H*$_3$/Boc), 3.09 (1H, dd, J = 6.3, 13.9 Hz, βC*H*$_2$), 3.17 (1H, dd, J = 5.6, 13.8 Hz, βC*H*$_2$), 3.75 (3H, s, CO$_2$C*H*$_3$), 3.81 (3H, s, OC*H*$_3$), 4.64 (1H, m, α*CH*), 5.05 (1H, d, J = 8.0 Hz, N*H*), 6.98 (1H, d, J = 8.3 Hz, 3′-C*H*), 7.03 (1H, td, J = 0.9, 7.5 Hz, 5′-C*H*), 7.18 (2H, d, J = 8.0 Hz, aromatic), 7.31 (2H, m, aromatic), 7.48 (2H, d, J = 8.2 Hz, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$): 28.3 (3x *C*H$_3$/Boc), 38.0 (βCH$_2$), 52.2 (CO$_2$CH$_3$), 54.4 (α*C*H), 55.5 (OCH$_3$), 79.9 (C/Boc), 111.2 (3′-*C*H), 120.9 (5′-*C*H), 128.6, 129.0, 129.7 (2, 3, 4′-CH), 130.2 (1′-C), 130.8 (6′-*C*H), 134.6, 137.3 (1,4-C), 155.2 (*C*=O/Boc), 156.5 (2′-C), 172.5 (*C*O$_2$CH$_3$); IR υ_{max} (cm^{-1}): 3367, 2976, 1743, 1711, 1487, 1237, 1161, 1025, 752; ESI-MS *m/z*: 386.1957 (M+H)$^+$ ((M+H)$^+$, $C_{22}H_{28}NO_5$ requires 386.1967), 408.1778 (M+Na)$^+$, 330.1336 (M−C(CH$_3$)$_3$) $^+$, 286.1436 (M−Boc)$^+$.

Intermediate **3d** (1.50 mmol, 595 mg) was converted into the corresponding Fmoc- protected building block using general procedure **C**. The crude product was purified by flash column chromatography (DCM to EtOAc/hexane (3:7) + 1% acetic acid, R_f 0.31 in EtOAc/hexane (1:1) + 1% acetic acid) to yield the desired product **4d** as a white solid (542 mg, 73% over three steps): mp 184 °C; $[\alpha]_D^{20}$ +52.82 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.11 (1H, dd, J = 6.5, 13.9 Hz, βC*H*$_2$), 3.23 (1H, dd, J = 4.9, 14.0 Hz, βC*H*$_2$), 3.70 (3H, s, OC*H*$_3$), 4.16 (1H, t, J = 6.8 Hz, C*H*/Fmoc), 4.30–4.44 (2H, m, C*H*$_2$/Fmoc), 4.73 (1H, m, α*CH*), 5.56 (1H, d, J = 8.0 Hz, N*H*), 6.91 (1H, d, J = 8.3 Hz, 3′-C*H*), 6.96 (1H, t, J = 7.3 Hz, 5′-C*H*), 7.17 (2H, d, J = 7.8 Hz, aromatic), 7.25 (4H, m, aromatic), 7.33

(2H, t, J = 7.4 Hz, aromatic), 7.44 (2H, d, J = 7.7 Hz, aromatic), 7.53 (2H, m, aromatic), 7.44 (2H, d, J = 7.4 Hz, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$): 37.5 (βCH$_2$), 47.2 (*C*H/Fmoc), 54.8 (αCH), 55.5 (OCH$_3$), 67.2 (*C*H$_2$/Fmoc), 111.4 (3′-*C*H), 120.1, 121.0, 124.9, 125.0, 125.2, 125.3, 127.2, 127.8, 128.8, 129.2, 129.9, 130.2, 130.9 (15x *C*H/aromatic BiPhe + aromatic Fmoc), 134.4, 134.6, 137.5, 141.4, 143.8, 143.9 (7x C/aromatic BiPhe + aromatic Fmoc), 156.2, 156.5 (2′-C, *C*=O/Fmoc), 177.5 (*C*O$_2$H); IR υ_{max} (cm^{-1}): 3310, 2930, 1698, 1529, 1254, 738; ESI-MS *m/z*: 494.1956 (M+H)$^+$ ((M+H)$^+$, $C_{31}H_{28}NO_5$ requires 494.1967), 516.1787 (M+Na)$^+$.

5.11. Fmoc-4-(2,6-dimethoxyphenyl)phenylalanine (4e)

The intermediate **3e** was prepared following general procedure **B**, using 478 mg of 2,6-dimethoxyphenylboronic acid. The crude product was purified by flash column chromatography (EtOAc/hexane (2:8), R_f 0.18) to yield the desired product **3e** as a white solid (248 mg, 34%): mp 65 °C; $[\alpha]_D^{20}$ +24.60 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (9H, s, C*H*$_3$/Boc), 3.09–3.13 (2H, m, βC*H*$_2$), 3.72 (6H, s, OC*H*$_3$), 3.73 (3H, s, CO$_2$C*H*$_3$), 4.62 (1H, m, αC*H*), 5.05 (1H, d, J = 7.9 Hz, N*H*), 6.64 (2H, d, J = 8.4 Hz, 3′-C*H*), 7.16 (2H, d, J = 7.9 Hz, aromatic), 7.23 (1H, dd, J = 3.0, 13.4 Hz, 4′-C*H*), 7.28 (2H, d, J = 8.1 Hz, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$): 28.3 (3x *C*H$_3$/Boc), 38.1 (βCH$_2$), 52.2 (CO$_2$*C*H$_3$), 54.4 (α*C*H), 55.9 (2x OCH$_3$), 79.9 (C/Boc), 104.2 (3′-*C*H +1′-C), 128.6, 128.7 (2, 4′-*C*H), 131.2 (3-*C*H), 134.2 (1-C), 155.2 (*C*=O/Boc), 157.7 (2′-C), 172.6 (*C*O$_2$CH$_3$); IR υ_{max} (cm^{-1}): 3371, 2934, 1739, 1688, 1515, 1245, 1161, 1105, 1004, 781; ESI-MS *m/z*: 416.2053 (M+H)$^+$ ((M+H)$^+$, $C_{23}H_{30}NO_6$ requires 416.2073), 438.1879 (M+Na)$^+$, 316.1546 (M−Boc)$^+$.

Intermediate **3e** (0.60 mmol, 248 mg) was converted into the corresponding Fmoc- protected building block using general procedure **C**. The crude product was purified by flash column chromatography (DCM to EtOAc/hexane (3:7) + 1% acetic acid, R_f 0.34 in EtOAc/hexane (1:1) + 1% acetic acid) to yield the desired product **4e** as a white solid (227 mg, 73% over three steps): mp 116 °C; $[\alpha]_D^{20}$ +46.39 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.16 (1H, dd, J = 6.4, 14.1 Hz, βC*H*$_2$), 3.24 (1H, dd, J = 5.2, 14.1 Hz, βC*H*$_2$), 3.67 (6H, s, OC*H*$_3$), 4.21 (1H, t, J = 7.0 Hz, C*H*/Fmoc), 4.36 (1H, dd, J = 6.9, 10.2 Hz, C*H*$_2$/Fmoc), 4.43 (1H, dd, J = 7.5, 10.6 Hz, C*H*$_2$/Fmoc), 4.75 (1H, m, αC*H*), 5.45 (1H, d, J = 8.3 Hz, N*H*), 6.62 (2H, d, J = 8.4 Hz, 3′-C*H*), 7.19 (2H, d, J = 8.0 Hz, aromatic), 7.28 (5H, m, aromatic), 7.36 (2H, t, J = 7.4 Hz, 4-C*H*/Fmoc), 7.57 (2H, dd, J = 2.7, 7.1 Hz, 2-C*H*/Fmoc), 7.73 (2H, d, J = 7.4 Hz, 5-C*H*/Fmoc); ^{13}C NMR (100 MHz, $CDCl_3$): 37.5 (βCH$_2$), 47.2 (*C*H/Fmoc), 54.6 (αCH), 55.9 (2x OCH$_3$), 67.2 (*C*H$_2$/Fmoc), 104.3 (3′-CH), 119.0 (1′-C), 120.0, 125.2, 127.1, 127.8, 128.8, 131.3 (13x *C*H/aromatic BiPhe + aromatic Fmoc), 133.1, 133.9 (1, 4-C), 141.3, 143.8, 143.9 (1, 6-C/Fmoc), 156.0 (*C*=O/Fmoc), 157.6 (2′-C), 177.7 (*C*O$_2$H); IR υ_{max} (cm^{-1}): 3340, 2948, 2647, 1696, 1523, 1246, 724; ESI-MS *m/z*: 524.2058 (M+H)$^+$ ((M+H)$^+$, $C_{32}H_{30}NO_6$ requires 524.2073), 546.1877 (M+Na)$^+$.

5.12. Fmoc-4-(3,4-dimethoxyphenyl)phenylalanine (4f)

The intermediate **3f** was prepared following general procedure **B**, using 478 mg of 3,4-dimethoxyphenylboronic acid. The crude product was purified by flash column chromatography (MeOH/DCM 0 to 1% then EtOAc/hexane (2:8), R_f 0.14 in EtOAc/hexane (2:8)) to yield the desired product **3f** as a white solid (640 mg, 88%): mp 112 °C; $[\alpha]_D^{20}$ +48.10 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33 (9H, s, C*H*$_3$/Boc), 2.97 (1H, dd, J = 5.9, 13.6 Hz, βC*H*$_2$), 3.06 (1H, dd, J = 5.5, 13.7 Hz, βC*H*$_2$), 3.64 (3H, s, CO$_2$C*H*$_3$), 3.80 (3H, s, OC*H*$_3$), 3.84 (3H, s, OC*H*$_3$), 4.52 (1H, m, αC*H*), 5.03 (1H, d, J = 7.9 Hz, N*H*), 6.83 (1H, d, J = 8.2 Hz, 5′-C*H*), 7.00 (1H, s, 2′-C*H*), 7.02 (1H, d, J = 7.2 Hz, 6′-C*H*), 7.09 (2H, d, J = 7.8 Hz, aromatic), 7.39 (2H, d, J = 8.0 Hz, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$): 28.3 (3x *C*H$_3$/Boc), 37.8 (βCH$_2$), 52.2 (CO$_2$*C*H$_3$), 54.4 (αCH), 55.9 (OCH$_3$), 79.8 (C/Boc), 110.3, 111.5 (2′, 5′-*C*H), 119.2 (6′-CH), 126.9–129.7 (2, 3-*C*H), 133.7, 134.6, 139.7 (1, 4, 1′-C), 148.6, 149.1 (3′, 4′-C), 155.1 (*C*=O/Boc), 172.3 (*C*O$_2$CH$_3$); IR υ_{max} (cm^{-1}): 3383, 2993, 2969, 1742, 1688, 1504, 1254, 1170, 1145, 1018, 802; ESI-MS *m/z*: 416.2059 (M+H)$^+$ ((M+H)$^+$, $C_{23}H_{30}NO_6$ requires 416.2073), 438.1878 (M+Na)$^+$, 360.1432 (M−C(CH$_3$)$_3$)$^+$, 316.1530 (M−Boc)$^+$.

Intermediate **3f** (1.60 mmol, 640 mg) was converted into the corresponding Fmoc- protected building block using general procedure **C**. The crude product was purified by flash column chromatography (DCM to DCM + 1% MeOH, R_f 0.25 in EtOAc/hexane (1:1) + 1% acetic acid) to yield the desired product **4f** as a white solid (243 mg, 29% over three steps): mp 141 °C; $[\alpha]_D^{20}$ +44.58 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.16 (1H, dd, J = 6.3, 13.9 Hz, βC*H*$_2$), 3.26 (1H, dd, J = 5.3, 14.0 Hz, βC*H*$_2$), 3.91 (6H, s, OC*H*$_3$), 4.20 (1H, t, J = 6.9 Hz, C*H*/Fmoc), 4.36 (1H, dd, J = 7.0, 10.3 Hz, C*H*$_2$/Fmoc), 4.45 (1H, dd, J = 7.3, 10.7 Hz, C*H*$_2$/Fmoc), 4.74 (1H, m, αC*H*), 5.29 (1H, d, J = 8.6 Hz, N*H*), 6.91 (1H, d, J = 8.3 Hz, 5′-C*H*), 7.05 (1H, s, 2′-C*H*), 7.09 (1H, d, J = 8.4 Hz, 6′-C*H*), 7.20 (2H, d, J = 7.9 Hz, aromatic), 7.28 (2H, t, J = 7.9 Hz, 3-C*H*/Fmoc), 7.38 (2H, t, J = 7.4 Hz, 4-C*H*/Fmoc), 7.47 (2H, d, J = 7.9 Hz, aromatic), 7.55 (2H, dd, J = 3.3, 7.2 Hz, 2-C*H*/Fmoc), 7.75 (2H, d, J = 7.8 Hz, 5-C*H*/Fmoc); ^{13}C NMR (100 MHz, $CDCl_3$): 37.3 (βCH$_2$), 47.1 (*C*H/Fmoc), 54.6 (αCH), 55.9 (2x OCH$_3$), 67.1 (*C*H$_2$/Fmoc), 110.3, 111.5 (2′, 5′-*C*H), 119.3, 120.0, 125.0, 125.1, 127.1, 127.8, 129.8 (13x *C*H/aromatic BiPhe + aromatic Fmoc), 133.6, 134.1, 140.0, 141.3, 143.7 (7x C/aromatic BiPhe + aromatic Fmoc), 148.6, 149.1 (3′, 4′-C), 155.8 (*C*=O/Fmoc), 175.6 (*C*O$_2$H); IR υ_{max} (cm^{-1}): 3319, 2937, 2574, 1697, 1529, 1503, 1251, 1143, 737; ESI-MS *m/z*: 524.2078 (M+H)$^+$ ((M+H)$^+$, $C_{32}H_{30}NO_6$ requires 524.2073), 546.1901 (M+Na)$^+$.

5.13. Fmoc-4-(3-nitrophenyl)phenylalanine (4g)

The intermediate **3g** was prepared following general procedure **B**, using 438 mg of 3-nitrophenylboronic acid. The crude product was purified by flash column chromatography (EtOAc/hexane (1:9), R_f 0.16 in EtOAc/hexane (2/8)) to yield the desired product **3g** as an off-white solid (638 mg, 91%): mp 92 °C; $[\alpha]_D^{20}$ +34.60 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (9H, s, C*H*$_3$/Boc), 3.11 (1H, dd, J = 6.1, 13.7 Hz, βC*H*$_2$), 3.20 (1H, dd, J = 5.6, 13.7 Hz, βC*H*$_2$), 3.76 (3H, s, CO$_2$C*H*$_3$), 4.64 (1H, m, αC*H*), 5.08 (1H, d, J = 7.9 Hz, N*H*), 7.27 (2H, d, J = 8.2 Hz, aromatic), 7.56 (2H, d, J = 8.3 Hz, aromatic), 7.60 (1H, t, J = 8.0 Hz, 5′-C*H*), 7.89 (1H, d, J = 8.2 Hz, 6′-C*H*), 8.18 (1H, ddd, J = 0.8, 2.2, 8.2 Hz, 4′-C*H*), 8.43 (1H, dd, J = 2.0 Hz, 2′-C*H*); ^{13}C NMR (100 MHz, $CDCl_3$): 28.3 (3x *C*H$_3$/Boc), 38.0 (βCH$_2$), 52.3 (CO$_2$*C*H$_3$), 54.4 (αCH), 79.9 (C/Boc), 121.7, 121.9 (2′, 4′-*C*H), 127.2, 129.7, 130.1, 132.8 (2, 3, 5′, 6′-*C*H), 136.8, 137.2, 142.3 (1, 4, 1′- C), 148.7 (3′-C), 155.1 (*C*=O/Boc), 172.2 (*C*O$_2$CH$_3$); IR υ_{max} (cm^{-1}): 3350, 2987, 2946, 1750, 1689, 1518, 1347, 1164, 730; ESI-MS *m/z*: 401.1703 (M+H)$^+$ ((M+H)$^+$, $C_{21}H_{25}N_2O_6$ requires 401.1713), 423.1524 (M+Na)$^+$, 345.1079 (M−C(CH$_3$)$_3$)$^+$, 301.1182 (M−Boc)$^+$.

Intermediate **3g** (1.60 mmol, 638 mg) was converted into the corresponding Fmoc- protected building block using general procedure **C**. The crude product was purified by flash column chromatography (DCM to EtOAc/hexane (3:7) + 1% acetic acid, R_f 0.28 in EtOAc/hexane (1:1) + 1% acetic acid) to yield the desired product **4g** as a white solid (265 mg, 33% over three steps): mp 199 °C; $[\alpha]_D^{20}$ +38.96 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, acetone-d_6): δ 3.14 (1H, dd, J = 9.5, 13.7 Hz, βC*H*$_2$), 3.37 (1H, dd, J = 4.5, 13.9 Hz, βC*H*$_2$), 4.17 (1H, t, J = 7.1 Hz, C*H*/Fmoc), 4.26 (1H, dd, J = 7.1, 10.2 Hz, C*H*$_2$/Fmoc), 4.33 (1H, dd, J = 7.4, 10.3 Hz, C*H*$_2$/Fmoc), 4.44 (1H, m, αC*H*), 6.82 (1H, d, J = 8.6 Hz, N*H*), 7.30 (2H, m, aromatic),

7.39 (2H, t, J = 7.4 Hz, 4-C*H*/Fmoc), 7.49 (2H, d, J = 7.9 Hz, aromatic), 7.66–7.73 (5H, m, aromatic), 7.84 (2H, d, J = 7.5 Hz, 5-C*H*/Fmoc), 8.01 (1H, d, J = 7.7 Hz, 6′-C*H*), 8.19 (1H, dd, J = 1.5, 8.1 Hz, 4′-C*H*), 8.4 (1H, s, 2′-C*H*); ^{13}C NMR (100 MHz, acetone-d_6): 37.9 (βCH$_2$), 48.0 (*C*H/Fmoc), 56.0 (αCH), 67.2 (*C*H$_2$/Fmoc), 120.8, 122.0, 122.7, 126.1, 126.2, 127.9, 128.5, 131.0, 133.8 (16x CH/aromatic BiPhe + aromatic Fmoc), 137.7, 139.2, 142.0, 143.2, 145.0 (7x C/aromatic BiPhe + aromatic Fmoc), 149.8 (3′-C), 156.9 (*C*=O/Fmoc), 173.3 (*C*O$_2$H); IR υ_{max} (cm^{-1}): 3309, 3063, 1694, 1529, 1515, 1353, 1257, 741; ESI-MS m/z: 509.1710 (M+H)$^+$ ((M+H)$^+$, $C_{30}H_{25}N_2O_6$ requires 509.1713), 531.1531 (M+Na)$^+$.

5.14. Fmoc-4-(4-cyanophenyl)phenylalanine (4h)

The intermediate **3h** was prepared following general procedure **B**, using 601 mg of 4-cyanophenylboronic acid pinacol ester. The crude product was purified by flash column chromatography (EtOAc/hexane (1:9 to 2:8, R_f 0.17 in EtOAc/hexane (2:8)) to yield the desired product **3h** as a colourless foam (310 mg, 48%): $[\alpha]_D^{20}$ +37.90 (c 0.1, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ 1.42 (9H, s, C*H*$_3$/Boc), 3.08 (1H, dd, J = 6.0, 13.7 Hz, βC*H*$_2$), 3.19 (1H, dd, J = 5.6, 13.7 Hz, βC*H*$_2$), 3.75 (3H, s, CO$_2$C*H*$_3$), 4.63 (1H, m, αC*H*), 5.05 (1H, d, J = 7.9 Hz, N*H*), 7.25 (2H, d, J = 8.1 Hz, aromatic), 7.53 (2H, d, J = 8.3 Hz, aromatic), 7.66 (2H, d, J = 8.6 Hz, aromatic), 7.72 (2H, d, J = 8.4 Hz, aromatic); ^{13}C NMR (100 MHz, CDCl$_3$): 28.3 (3x *C*H$_3$/Boc), 38.1 (βCH$_2$), 52.3 (CO$_2$CH$_3$), 54.3 (αCH), 80.1 (C/Boc), 110.8 (4′-C), 118.9 (*C*N), 127.3, 127.6 (3, 2′-*C*H), 130.1 (2-*C*H), 132.6 (3′-*C*H), 136.9, 137.8 (1, 4-C), 145.2 (1′-C), 155.1 (*C*=O/Boc), 172.2 (*C*O$_2$CH$_3$); IR υ_{max} (cm^{-1}): 3368, 2977, 2227, 1743, 1709, 1497, 1249, 1161, 1055, 1006, 817; ESI-MS m/z: 381.1813 (M+H)$^+$ ((M+H)$^+$, $C_{22}H_{25}N_2O_4$ requires 381.1814), 403.1628 (M+Na)$^+$, 325.1197 (M−C(CH$_3$)$_3$)$^+$, 281.1300 (M−Boc)$^+$.

Intermediate **3h** (0.84 mmol, 310 mg) was converted into the corresponding Fmoc- protected building block using general procedure **C**. The crude product was purified by flash column chromatography (DCM to EtOAc/hexane (3:7) + 1% acetic acid, R$_f$ 0.28 in EtOAc/hexane (1:1) + 1% acetic acid) to yield the desired product **4h** as a white solid (303 mg, 74% over three steps): mp 173 °C; $[\alpha]_D^{20}$ +49.25 (c 0.09, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ 3.15 (1H, dd, J = 6.5, 13.9 Hz, βC*H*$_2$), 3.29 (1H, dd, J = 5.3, 14.0 Hz, βC*H*$_2$), 4.17 (1H, t, J = 6.8 Hz, C*H*/Fmoc), 4.36 (1H, dd, J = 6.9, 10.2 Hz, C*H*$_2$/Fmoc), 4.45 (1H, dd, J = 7.3, 10.2 Hz, C*H*$_2$/Fmoc), 4.76 (1H, m, αC*H*), 5.41 (1H, d, J = 8.1 Hz, N*H*), 7.24–7.30 (4H, m, aromatic), 7.39 (2H, t, J = 7.4 Hz, aromatic), 7.46 (2H, d, J = 7.9 Hz, aromatic), 7.53–7.58 (4H, m, aromatic), 7.67 (2H, d, J = 8.1 Hz, aromatic), 7.76 (2H, d, J = 7.5 Hz, aromatic); ^{13}C NMR (100 MHz, CDCl$_3$): 37.5 (βCH$_2$), 47.1 (*C*H/Fmoc), 54.6 (αCH), 67.1 (*C*H$_2$/Fmoc), 110.8 (4′-*C*H), 119.0 (*C*N), 120.1, 125.0, 125.1, 127.1, 127.4, 127.5, 127.8, 130.2, 132.6 (16x *C*H/aromatic BiPhe + aromatic Fmoc), 136.4, 138.0, 141.3, 143.7, 145.1 (7x C/aromatic BiPhe + aromatic Fmoc), 155.9 (*C*=O/Fmoc), 177.4(*C*O$_2$H); IR υ_{max} (cm^{-1}): 3307, 2948, 2227, 1691, 1539, 1257, 736; ESI-MS m/z: 489.1819 (M+H)$^+$ ((M+H)$^+$, $C_{31}H_{25}N_2O_4$ requires 489.1814), 511.1641 (M+Na)$^+$.

5.15. Fmoc-4-(1′-cyclohexenyl)phenylalanine (4i)

The intermediate **3i** was prepared following the general procedure **B**, using 0.6 mL of cyclohexene-1-boronic acid pinacol ester. The crude product was purified by flash column chromatography (EtOAc/hexane (1:9), R_f 0.19 in EtOAc/hexane (1:9)) to yield the desired product **3i** as a yellow oil (567 mg, 93%): $[\alpha]_D^{20}$ +41.00 (c 0.1, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ 1.41 (9H, s, C*H*$_3$/Boc), 1.61–1.67 (2H, m, C*H*$_2$/cyclohexene), 1.74–1.80 (2H, m, C*H*$_2$/cyclohexene), 2.19 (2H, m, C*H*$_2$/cyclohexene), 2.37 (2H, m, C*H*$_2$/cyclohexene), 3.02 (1H, dd, J = 6.1, 13.9 Hz, βC*H*$_2$), 3.08 (1H, dd, J = 5.8, 13.8 Hz, βC*H*$_2$), 3.71 (3H, s, CO$_2$C*H*$_3$), 4.56 (1H, m, αC*H*), 4.98 (1H, d, J = 8.1 Hz, N*H*), 6.10 (1H, bs, 2′-C*H*), 7.05 (2H, d, J = 8.1 Hz, aromatic), 7.30 (2H, d, J = 8.3 Hz, aromatic); ^{13}C NMR (100 MHz, CDCl$_3$): 23.0, 24.8, 25.9, 27.3 (3′, 4′, 5′, 6′-*C*H$_2$), 28.3 (3x *C*H$_3$/Boc), 37.8 (βCH$_2$), 52.2 (CO$_2$CH$_3$), 54.4 (αCH), 79.9 (C/Boc), 124.6, 125.0, 129.1 (2, 3, 2′-*C*H), 134.1, 136.1, 141.3 (1, 4, 1′-C), 155.1 (*C*=O/Boc), 172.4 (*C*O$_2$CH$_3$); IR υ_{max} (cm^{-1}): 3369, 2930, 1744, 1713, 1498, 1162, 732; ESI-MS m/z: 360.2166 (M+H)$^+$ ((M+H)$^+$, $C_{21}H_{30}NO_4$ requires 360.2175), 382.1979 (M+Na)$^+$, 304.1538 (M−C(CH$_3$)$_3$)$^+$, 260.1644 (M−Boc)$^+$.

Intermediate **3i** (1.63 mmol, 567 mg) was converted into the corresponding Fmoc- protected building block using general procedure **C**. The crude product was purified by flash column chromatography (DCM to EtOAc/hexane (3:7) + 1% acetic acid, R_f 0.44 in EtOAc/hexane (1:1) + 1% acetic acid) to yield the desired product **4i** as a white foam (588 mg, 77% over three steps): $[\alpha]_D^{20}$ +60.85 (c 0.1, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ 1.66 (2H, m, C*H*$_2$/cyclohexene), 1.76 (2H, m, C*H*$_2$/cyclohexene), 2.19 (2H, m, C*H*$_2$/cyclohexene), 2.37 (2H, m, C*H*$_2$/cyclohexene), 3.11 (1H, dd, J = 6.1, 13.9 Hz, βC*H*$_2$), 3.21 (1H, dd, J = 5.2, 13.8 Hz, βC*H*$_2$), 4.20 (1H, t, J = 6.9 Hz, C*H*/Fmoc), 4.36 (1H, dd, J = 7.2, 10.7 Hz, C*H*$_2$/Fmoc), 4.44 (1H, dd, J = 7.4, 10.5 Hz, C*H*$_2$/Fmoc), 4.72 (1H, m, αC*H*), 4.26 (1H, d, J = 8.2 Hz, N*H*), 6.11 (1H, bs, 2′-C*H*), 7.09 (2H, d, J = 7.9 Hz, aromatic), 7.31 (4H, m, aromatic), 7.40 (2H, t, J = 7.4 Hz, 3-C*H*/Fmoc), 7.56 (2H, t, J = 6.6 Hz, 4-C*H*/Fmoc), 7.77 (2H, d, J = 7.5 Hz, aromatic); ^{13}C NMR (100 MHz, CDCl$_3$): 22.1, 23.0, 25.9, 27.3 (3′, 4′, 5′, 6′-*C*H$_2$), 37.3 (βCH$_2$), 47.1 (*C*H/Fmoc), 54.6 (αCH), 67.1 (*C*H$_2$/Fmoc), 120.0, 124.9, 125.1, 127.1, 127.8, 129.2 (13x *C*H/aromatic Phe + aromatic Fmoc + 2′-*C*H), 133.6, 136.0, 141.3, 141.5, 143.7, 143.8 (7x C/aromatic Phe + aromatic Fmoc + 1′-C), 155.8 (*C*=O/Fmoc), 176.3 (*C*O$_2$H); IR υ_{max} (cm^{-1}): 2941, 1708, 1515, 1487, 1450, 1408, 1234, 738; ESI-MS m/z: 468.2163 (M+H)$^+$ ((M+H)$^+$, $C_{30}H_{30}NO_4$ requires 468.2175), 490.1979 (M+Na)$^+$.

6. Solid phase peptide synthesis

The peptides were assembled on a 0.1 mmol scale using standard Fmoc solid phase peptide synthesis (SPPS) on a Tribute™ peptide synthesiser (Protein Technologies, Inc.). The syntheses were performed on aminomethylated polystyrene resin (1.0 mmol/g) derivatised with HMP linker and all peptides were capped with D-biotin.[†]

6.1. Biotin-R$_8$ERY* (Y* = 4-(4-methylphenyl)phenylalanine) (5)

The peptide chain was assembled using building block **4a**. 16 mg of crude peptide were purified by semi-preparative HPLC using a linear gradient of 1% B to 80% B over 60 min, yielding 3 mg of purified peptide (13%) as a white solid in ca. 93% purity according to analytical HPLC. R_t 12.5 min (1–80% B over 30 min, 1 mL/min); m/z (ESI-MS): 337.0 [M+6H$^+$], 404.2 [M+5H$^+$], 505.1 [M+4H$^+$].

6.2. Biotin-R$_8$ERY* (Y* = 4-(4-ethylphenyl)phenylalanine) (6)

The peptide chain was assembled using building block **4b**. 17 mg of crude peptide were purified by semi-preparative HPLC using a linear gradient of 1% B to 80% B over 60 min, yielding 5 mg of purified peptide (35%) as a white solid in ca. 96% purity according to analytical HPLC. R_t 23.1 min (1–80% B over 30 min, 1 mL/min); m/z (ESI-MS): 339.4 [M+6H$^+$], 407.0 [M+5H$^+$], 508.6 [M+4H$^+$].

[†] See supporting information for details.

6.3. Biotin-R_8ERY* (Y* = (p-terphenyl)alanine) (7)

The peptide chain was assembled using building block **4c**. 12 mg of crude peptide were purified by semi-preparative HPLC using a linear gradient of 1% B to 80% B over 60 min, yielding 1 mg of purified peptide (15%) as a white solid in ca. 97% purity according to analytical HPLC. R_t 20.0 min (1–80% B over 30 min, 1 mL/min); *m*/*z* (ESI-MS): 347.4 [M+6H$^+$], 416.6 [M+5H$^+$], 520.6 [M+4H$^+$].

6.4. Biotin-R_8ERY* (Y* = 4-(2-methoxyphenyl)phenylalanine) (8)

The peptide chain was assembled using building block **4d**. 16.5 mg of crude peptide were purified by semi-preparative HPLC using a linear gradient of 1% B to 80% B over 60 min, yielding 9 mg of purified peptide (47%) as a white solid in ca. 96% purity according to analytical HPLC. R_t 14.9 min (1–80% B over 30 min, 1 mL/min); *m*/*z* (ESI-MS): 407.4 [M+5H$^+$], 509.0 [M+4H$^+$].

6.5. Biotin-R_8ERY* (Y* = 4-(2,6-dimethoxyphenyl)phenylalanine) (9)

The peptide chain was assembled using building block **4e**. 15 mg of crude peptide were purified by semi-preparative HPLC using a linear gradient of 1% B to 80% B over 60 min, yielding 6 mg of purified peptide (53%) as a white solid in ca. 99% purity according to analytical HPLC. R_t 15.3 min (1–80% B over 30 min, 1 mL/min); *m*/*z* (ESI-MS): 344.7 [M+6H$^+$], 413.4 [M+5H$^+$], 516.6 [M+4H$^+$].

6.6. Biotin-R_8ERY* (Y* = 4-(3,4-dimethoxyphenyl)phenylalanine) (10)

The peptide chain was assembled using building block **4f**. 14 mg of crude peptide were purified by semi-preparative HPLC using a linear gradient of 1% B to 80% B over 60 min, yielding 5 mg of purified peptide (43%) as a white solid in ca. 99% purity according to analytical HPLC. R_t 10.6 min (1–60% B over 20 min, 1 mL/min); *m*/*z* (ESI-MS): 344.7 [M+6H$^+$], 413.4 [M+5H$^+$], 516.5 [M+4H$^+$].

6.7. Biotin-R_8ERY* (Y* = 4-(3-nitrophenyl)phenylalanine) (11)

The peptide chain was assembled using building block **4g**. 18 mg of crude peptide were purified by semi-preparative HPLC using a linear gradient of 1% B to 80% B over 60 min, yielding 5 mg of purified peptide (34%) as a white solid in ca. 98% purity according to analytical HPLC. R_t 10.8 min (1–80% B over 30 min, 1 mL/min); *m*/*z* (ESI-MS): 342.2 [M+6H$^+$], 410.4 [M+5H$^+$], 512.8 [M+4H$^+$], 683.4 [M+3H$^+$].

6.8. Biotin-R_8ERY* (Y* = 4-(4-cyanophenyl)phenylalanine) (12)

The peptide chain was assembled using building block **4h**. 17 mg of crude peptide were purified by semi-preparative HPLC using a linear gradient of 1% B to 80% B over 60 min, yielding 4 mg of purified peptide (23%) as a white solid in ca. 93% purity according to analytical HPLC. R_t 16.3 min (1–80% B over 30 min, 1 mL/min); *m*/*z* (ESI-MS): 338.9 [M+6H$^+$], 406.4 [M+5H$^+$], 507.8 [M+4H$^+$], 714.7 [M+3H$^+$+TFA].

6.9. Biotin-R_8ERY* (Y* = (4-cyclohexyl)phenylalanine) (13)

The peptide chain was assembled using building block **4i**. 18 mg of crude peptide were purified by semi-preparative HPLC using a linear gradient of 1% B to 80% B over 60 min, yielding 3 mg of purified peptide (15%) as a white solid in ca. 91% purity according to analytical HPLC. R_t 22.5 min (1–80% B over 30 min, 1 mL/min); *m*/*z* (ESI-MS): 335.7 [M+6H$^+$], 402.6 [M+5H$^+$], 503.1 [M+4H$^+$].

6.10. Biotin-R_8ERY* (Y* = 4-(1′-cyclohexenyl)phenylalanine) (14)

The peptide chain was assembled using building block **4i**. 12.6 mg of crude peptide were purified by semi-preparative HPLC using a linear gradient of 1% B to 80% B over 60 min, yielding 0.6 mg of purified peptide (3%) as a white solid in ca. 99% purity according to analytical HPLC. R_t 13.9 min (1–90% B over 30 min, 1 mL/min); *m*/*z* (ESI-MS): 335.4 [M+6H$^+$], 402.2 [M+5H$^+$], 502.5 [M+4H$^+$].

7. Cell assays

7.1. Cell line

The mouse spontaneous AKR/Cum Y CD8 $\alpha4\beta7^+/\alpha4\beta1^-$ T lymphoma cell line TK-1 was purchased from the American Type Culture Collection, Rockville, MD. It was cultured at 37 °C in RPMI 1640 medium supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 200 μg/ml L-glutamine, 10% (v/v) FCS and 0.05 mM β-mercaptoethanol.

7.2. Recombinant MAdCAM-1

The production of soluble recombinant mouse MAdCAM-1-Fc from insect cells using a baculovirus expression system has been described previously.[43]

7.3. Cell adhesion assay

Lab-Tek 16-well glass slides (Nunc) were coated with purified mouse MAdCAM-1-Fc (10 μg/ml), and incubated at 4 °C overnight. Slides were washed once with PBS, and blocked with FBS for 2 h at room temperature. They were washed thrice with Hanks balanced salt solution (HBSS) containing 10 mM Hepes. TK-1 cells were washed thrice with HBSS containing 10 mM Hepes. They were resuspended in HBSS containing 10 mM Hepes and different concentrations of the β7 peptides or no peptide, and left standing for 1 h at room temperature. Integrins on the cells were activated by addition of $MnCl_2$ to 2 mM. The cells were added to the MAdCAM-1-coated slides at 10^6 cells/well and left to adhere for 30 min at room temperature. Non-adherent cells were removed by twice dipping slides gently into PBS. The adherent cells were fixed in PBS containing 2% (v/v) glutaraldehyde for at least 3 h, and stained with 0.1% methylene blue for 5 min, and destained with ethanol. The slides were air-dried and the absorbance at 495 nm was read and plotted, as a measure of cell adhesion. The adhesion assays were repeated at least thrice using duplicate wells.

7.4. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM), and a Student's t-test was used to evaluate statistical significance. *P* values $\leqslant 0.05$ were considered statistically significant.

Acknowledgments

We thank the Maurice Wilkins Centre for Molecular Biodiscovery for supporting the peptide synthesis laboratory and Auckland Uniservices Ltd. and the Foundation for Research, Science and Technology, New Zealand for financial support (AN).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.07.010.

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