Synthesis of hexapeptide and tetrapeptide analogues of the immunomodulating peptides

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Two hexapeptide and two tetrapeptide analogues of the bioactive hexapeptides [(Trp/Met/Phe)-Lys-Tyr-(Met/Val)-(Pro/Val)-Met] have been synthesized by incorporating novel peptide isosteres such as 2-isoxazoline, (*E*)-alkene, and reduced amide isosteres. These immunomodulating hexapeptides are known to stimulate the formation of inositol phosphates in lymphocyte cell lines.

Introduction

Peptides play important roles as hormones, enzyme inhibitors or substrates, growth promotors or inhibitors, neurotransmitters, and immunomodulators in living systems. Most peptides exhibit their biological activities through binding to corresponding acceptor molecules (receptors or enzymes). Each acceptor molecule shows a unique biological role, allowing the interaction of bioactive peptides with acceptor molecules to control specific physiological events. This characteristic can allow bioactive peptides to act as therapeutic agents.[1]

Recently, S. H. Ryu and co-workers[2] identified peptides which stimulated the formation of inositol phosphates (Ins*P*s) in lymphocyte cell lines by screening synthetic peptide libraries composed of random sequences of hexapeptides. These peptides also stimulated phosphoinositide hydrolysis and release of $[Ca^{2+}]_i$ in HL60 and U937 cell lines. For clinical applications of these peptides we planned to use peptidomimetics[3] which might be stable to enzymic degradation and have improved pharmacological and pharmacokinetic properties. We report the syntheses of hexapeptide and tetrapeptide analogues of the bioactive hexapeptides employing novel dipeptide isosteres.[4]

X-Lys-Tyr-(Met/Val)-(Pro/Val)-Met

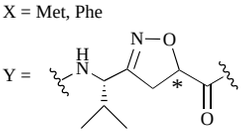
X = Trp, Met, Phe

Sequences of bioactive peptides identified by screening synthetic peptide libraries

Modification I X-Lys-Tyr-Y-Met

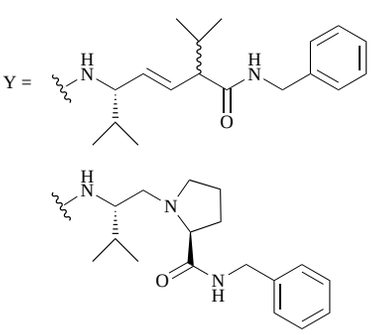
X = Met, Phe

Y =



Modification II Lys-Tyr-Y

Y =



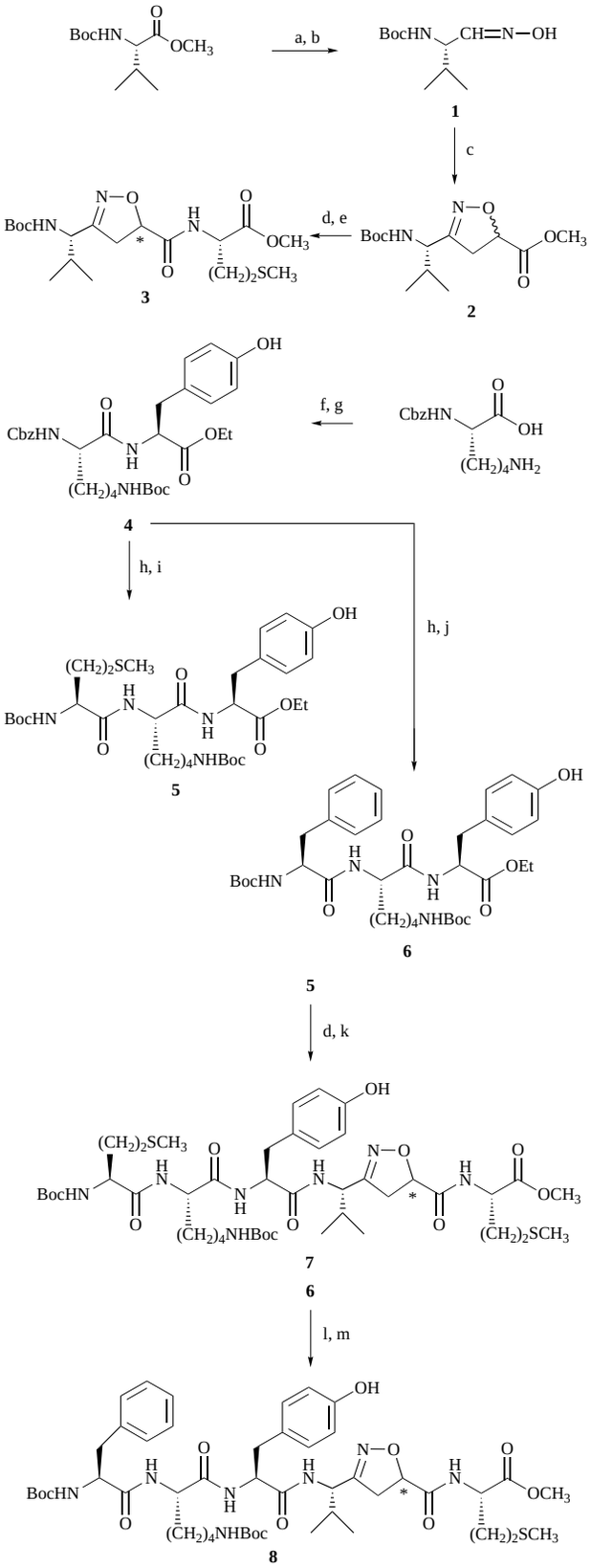
Modifications of bioactive peptides

Results and discussion

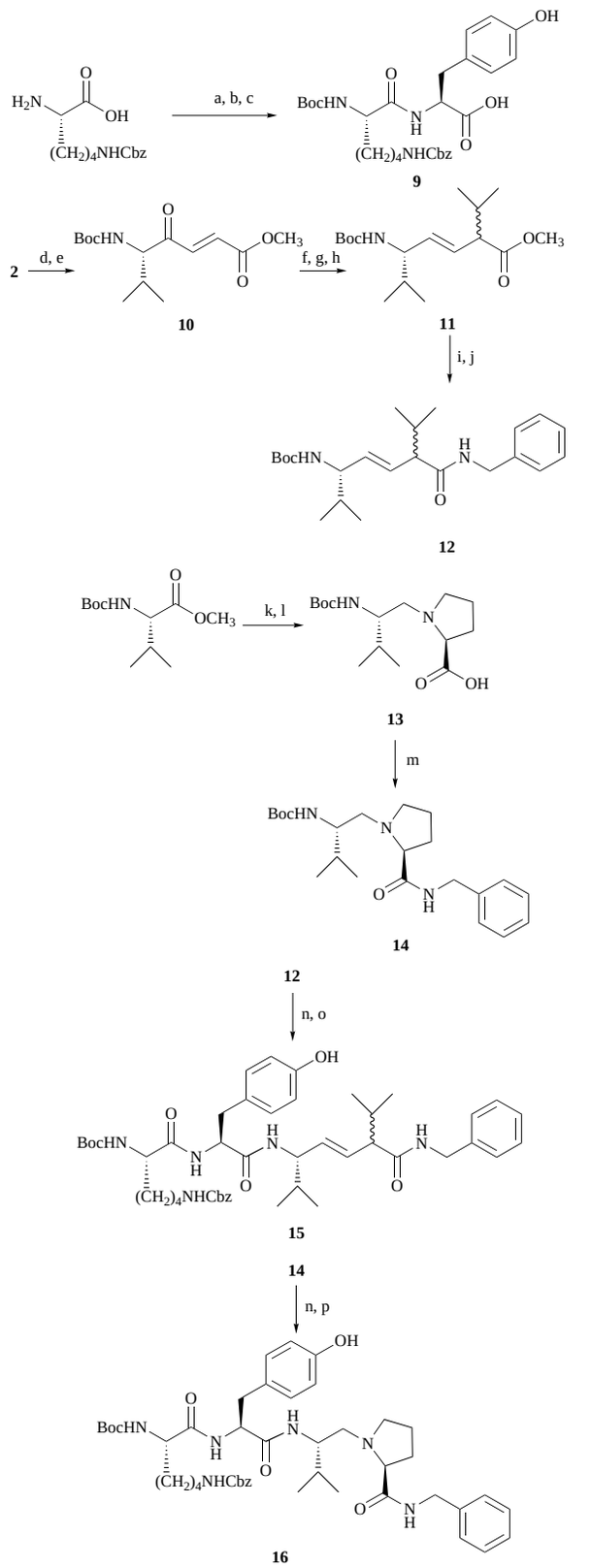
The reported sequences of hexapeptides were X-Lys-Tyr-(Met/Val)-(Pro/Val)-Met where X was Trp, Met or Phe.[2] To modify these peptides and reduce the synthetic targets we chose the (Met/Val)-(Pro/Val) moiety, the most variable part, for the transformation into dipeptide isosteres (Modifications). The terminal X and Met were conserved in Modification I but removed in Modification II.

Two modified hexapeptides in which a 2-isoxazoline ring was used as a dipeptide isostere[4] were synthesized as shown in Scheme 1. $BocValOCH_3$ was reduced with diisobutylaluminium hydride (DIBAL-H) to provide the corresponding aldehyde, which was treated with $NH_2OH{\cdot}HCl$ to give oxime **1**. Nitrile oxide cycloaddition of oxime **1** with methyl acrylate in the presence of NaOCl afforded a diastereomeric mixture (~1 : 1) of 2-isoxazoline dipeptide isostere **2**, which was inseparable using routine column chromatographic techniques. Hydrolysis of ester **2** followed by coupling with MetOMe·HCl using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC)[5] gave the corresponding tripeptide isosteres **3**. Fortunately, these two diastereomeric tripeptide isosteres could be separated by recrystallization. Other components, tripeptides **5** and **6**, were prepared in the usual manner. Boc protection of CbzLysOH followed by coupling with TyrOEt·HCl using EDC provided dipeptide **4**. The Cbz group of product **4** was removed using 10% Pd/C to give the corresponding amine, which was coupled with BocMetOH and BocPheOH to afford protected tripeptides **5** and **6**, respectively. Finally, the desired hexapeptide isosteres **7** and **8** were synthesized by hydrolysis of compounds **5** and **6** and coupling with the amine obtained from the deprotection of compound **3** with trifluoroacetic acid (TFA).

Additional modified peptides containing (*E*)-alkene isosteres[6] or reduced amide isosteres[7] were prepared as shown in Scheme 2. Boc protection of N^6-CbzLysOH followed by coupling with TyrOEt·HCl afforded the corresponding dipeptide, which was subsequently hydrolysed to give acid **9**. The 2-isoxazoline ring of dipeptide isostere **2** obtained previously was reduced using Curran's method[8] (Ra-Ni, H_2, H_3BO_3), and the resulting α-hydroxy ketomethylene dipeptide isostere[4b] was treated with MsCl in the presence of pyridine to provide ketovinyl dipeptide isostere **10**.[9] The olefinic geometry of compound **10** was assigned as *E* from the observed coupling

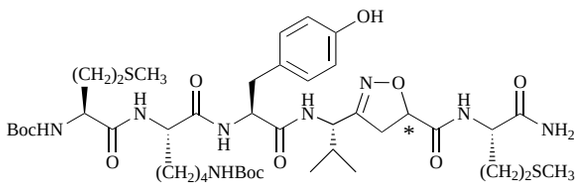


Scheme 1 *Reagents, conditions and yields:* (a) DIBAL-H, toluene, −78 °C; (b) $NH_2OH·HCl$, Na_2CO_3, MeOH–water (1:1), 84% (a and b); (c) methyl acrylate, NaOCl, EtOAc, 69%; (d) $LiOH·H_2O$, THF–water (5:1), 100%; (e) MetOMe·HCl, EDC, HOBt, NMM, CH_2Cl_2, 0 °C to room temp., 73%; (f) $(Boc)_2O$, 1 M NaOH, 1,4-dioxane, 0 °C; (g) TyrOEt·HCl, EDC, HOBt, NMM, CH_2Cl_2, 0 °C to room temp., 88% (f and g); (h) 10% Pd/C, H_2, MeOH, 98%; (i) BocMetOH, EDC, HOBt, CH_2Cl_2, 0 °C to room temp., 77%; (j) BocPheOH, EDC, HOBt, CH_2Cl_2, 0 °C to room temp., 53%; (k) (1) **3**, TFA, CH_2Cl_2, 99%, (2) EDC, HOBt, CH_2Cl_2, 0 °C to room temp., 58%; (l) $LiOH·H_2O$, THF–water (5:1), 85%; (m) (1) **3**, TFA, CH_2Cl_2, 99%, (2) EDC, HOBt, CH_2Cl_2, 0 °C to room temp., 56%



Scheme 2 *Reagents, conditions and yields:* (a) $(Boc)_2O$, Et_3N, MeOH; (b) TyrOEt·HCl, EDC, HOBt, NMM, CH_2Cl_2, 0 °C to room temp., 92% (a and b); (c) 1 M NaOH, MeOH, 100%; (d) Ra-Ni, H_2, H_3BO_3, THF–water (5:1); (e) MsCl, pyridine, CH_2Cl_2, 78% (d and e); (f) $NaBH_4$, $CeCl_3$, MeOH, −78 °C, 99%; (g) MsCl, pyridine, CH_2Cl_2, 0 °C, 96%; (h) Pr^iMgCl, CuCN, $BF_3·Et_2O$, THF, −78 °C, 83%; (i) 1 M NaOH, MeOH–THF (2.6:1); (j) benzylamine·HCl, EDC, HOBt, NMM CH_2Cl_2, 91% (i and j); (k) DIBAL-H, toluene, −78 °C; (l) L-Pro, $NaCNBH_4$, MeOH, 0 °C, 85% (k and l); (m) benzylamine·HCl, EDC, HOBt, NMM, CH_2Cl_2, 0 °C to room temp., 89%; (n) TFA, CH_2Cl_2; (o) **9**, EDC, HOBt, NMM, CH_2Cl_2, 0 °C to room temp., 87% (n and o); (p) **9**, EDC, HOBt, NMM, CH_2Cl_2, 0 °C to room temp., 56% (n and p)

Table 1 Effect of the compounds modified from WKYMVM-NH_2 on the PI hydrolysis in U937 cells



17

Compound	EC_{50} (μM)
WKYMVM-NH_2	0.030 ± 0.0068
7	>200
8	144 ± 58
15	Inactive
16	169 ± 74
17	23.16 ± 10.46

constant (*J* 15.5 Hz) between the two olefinic protons. Reduction of enone **10** with $NaBH_4$ in the presence of $CeCl_3$ gave the corresponding alcohol,[10] which was then mesylated. The *anti*-S_N2' displacement of the mesyl leaving group with $Pr^iCu{\cdot}BF_3$ according to Ibuka's method[11] provided (*E*)-alkene dipeptide isostere **11**, which was hydrolysed and coupled with benzylamine to afford amide **12**.

DIBAL-H reduction of $BocValOCH_3$ followed by reductive amination with L-Pro using $NaCNBH_4$ yielded reduced amide dipeptide isostere **13**. The final dipeptide isostere **14** was prepared by formation of the amide of acid **13** with benzylamine. Finally, compounds **12** and **14** were deprotected with TFA and coupled with dipeptide **9** to give the desired peptides **15** and **16**, respectively.

With two hexapeptides, two tetrapeptides, and one derivative of hexapeptide in hand, we carried out a bioassay for immunomodulating activity by measuring total inositol phosphates induced by peptides. The results are summarized in Table 1. It is noteworthy that functional-group conversion of the C-terminal ester (compound **7**) into a primary amide (compound **17**) gives a ~10-fold increase in activity. Further structural modification and bioassay results will be reported in due course.

Experimental

General details

Mps were determined with an 'electrothermal' capillary melting point apparatus and are uncorrected. IR spectra were measured with a Bruker Equinox 55 FTIR spectrometer. The ^1H (500 and 300 MHz) NMR and ^{13}C (125.8 and 75.4 MHz) NMR spectra were obtained on Bruker DRX 500 and Bruker DPX 300 spectrometers for samples in deuteriated solvents with trimethylsilane as the internal standard. *J*-Values are given in Hz. Mass spectra were obtained on a Kratos MS 25 RFA system and an HR Tandem MS spectrometer. Optical rotations were recorded on a Rudolph Autopol III automatic polarimeter. $[\alpha]_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}. Elemental analyses were performed by Galbraith Laboratories, Knoxville, USA. Column chromatography was performed on Merck silica gel 60. TLC was performed on Merck silica gel 60 F_{254}.

General coupling method using EDC

1-Hydroxybenzotriazole (HOBt) (1 mol equiv.), *N*-methylmorpholine (NMM) (1 mol equiv.), and EDC (1 mol equiv.) were added to a solution of the acid and the amine (0.1 M) in dry CH_2Cl_2 at 0 °C. The solution was stirred at room temp. until completion (TLC analysis). The mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography.

N-(*tert*-Butoxycarbonyl)-L-valinal oxime 1

A toluene solution of DIBAL-H (1.5 M; 40 cm^3) was added dropwise to a stirred solution of $BocValOCH_3$ (9.30 g, 40.23 mmol) in dry toluene (80 cm^3) at −78 °C. After 10 min MeOH (15 cm^3) was added carefully and the resulting mixture was stirred for 1 h. The mixture was poured into 10% aq. citric acid (20 cm^3) at 0 °C. After being stirred for 1 h, the mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the corresponding aldehyde, which was used immediately in the next step without further purification.

To a solution of the above aldehyde in MeOH–water (1:1, v/v; 40 cm^3) were added Na_2CO_3 (2.7 g, 25.3 mmol) and $NH_2OH{\cdot}HCl$ (3.1 g, 44.3 mmol) at 0 °C. After being stirred for 10 h, the mixture was concentrated *in vacuo* to half the original volume. The mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Recrystallization (CH_2Cl_2–EtOAc, 1:5) yielded *title oxime* **1** (8.28 g, 84%), mp 154–155 °C (Found: C, 55.73; H, 9.73; N, 13.28. $C_{10}H_{20}N_2O_3$ requires C, 55.53; H, 9.32; N, 12.95%); $[\alpha]_D^{25}$ +62.5 (*c* 1.07, CH_3OH); δ_H(300 MHz; CD_3OD) 0.91 (3 H, d, *J* 6.8), 0.93 (3 H, d, *J* 6.8), 1.43 (9 H, s), 1.91 (1 H, m), 4.66 (1 H, m) and 6.52 (1 H, d, *J* 6.8); δ_C(75.4 MHz; CD_3OD) 18.93, 19.48, 28.98, 32.44, 52.42, 80.39, 152.01 and 158.26; ν_{max}(KBr)/cm^{-1} 3344, 2969, 1682, 1526, 1315, 1250 and 1174.

(5*R*/*S*)-3-[(1*S*)-1-(*tert*-Butoxycarbonylamino)-2-methylpropyl]-4,5-dihydro-5-(methoxycarbonyl)isoxazole 2

Aq. (4%) NaOCl (113 cm^3) was added to a solution of oxime **1** (5.2 g, 21.12 mmol) in EtOAc (180 cm^3) over a period of 30 min. After being stirred for 2 h, the mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane–EtOAc, 2:1) to give *ester* **2** (4.81 g, 69%) as an inseparable mixture (~1:1) of diastereomers (Found: C, 56.17; H, 7.95; N, 9.30. $C_{14}H_{24}N_2O_5$ requires C, 55.99; H, 8.05; N, 9.33%); δ_H(300 MHz; $CDCl_3$) 0.98 (6 H, m), 1.44 (9 H, s), 2.06 (1 H, m), 3.25 (2 H, dd, *J* 8.9 and 3.7), 3.79 (3 H, s), 4.35 (1 H, m), 4.92 (1 H, m) and 5.01 (1 H, t, *J* 9.0); δ_C(75.4 MHz; $CDCl_3$) 18.14, 19.93, 28.92, 31.72, 41.05, 53.29, 54.58, 80.58, 156.09, 159.23 and 171.20; ν_{max}(film)/cm^{-1} 3347, 2965, 1707, 1516, 1367, 1170 and 1016; *m*/*z* (EI) 300 (M^+, 66%), 244 (88), 226 (53), 200 (100), 184 (87), 156 (90), 140 (89), 123 (71), 115 (80), 97 (88) and 69 (97).

(5ξ)-3-[(1*S*)-*tert*-Butoxycarbonylamino)-2-methylpropyl]-4,5-dihydro-5-(L-methioninocarbonyl)isoxazole methyl ester 3

$LiOH{\cdot}H_2O$ (380.9 mg, 9.80 mmol) was added to a solution of compound **2** (1.5 g, 4.54 mmol) in tetrahydrofuran (THF)–water (5:1, v/v; 60 cm^3). After being stirred for 2 h, the mixture was poured into water and acidified to pH 3 with saturated aq. $KHSO_4$. The aqueous layer was extracted with EtOAc, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford the corresponding acid.

The above acid and $MetOCH_3{\cdot}HCl$ (1.19 g, 5.94 mmol) were submitted to the conditions described in the general EDC method. After 12 h the mixture was worked up as outlined previously. Flash chromatography (CH_2Cl_2–EtOAc, 10:1 to 5:1) gave the product (1.32 g, 73%) as a diastereomeric mixture, which was separated by recrystallization (hexane–EtOAc, 2:1) to afford a more polar isomer **3** (603.5 mg, 33%) and a less polar isomer (451.1 mg, 25%). *Compound* **3** showed (HRMS: M^+ + H, 432.2150. $C_{19}H_{34}N_3O_6S$ requires *m*/*z*, 432.2168); $[\alpha]_D^{18}$ +76.5 (*c* 1.15, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 0.89 (3 H, d, *J* 6.8), 0.98 (3 H, d, *J* 6.8), 1.44 (9 H, s), 1.94–2.17 (6 H, m), 2.45 (2 H, t, *J* 7.5), 3.31 (2 H, m), 3.76 (3 H, s), 4.36 (1 H, br s), 4.68

(1 H, m), 4.96 (1 H, br s), 4.99 (1 H, dd, *J* 9.3 and 7.5), 7.29 (1 H, s); δ_C(75.4 MHz; $CDCl_3$) 15.81, 17.67, 19.71, 28.70, 30.28, 31.49, 31.89, 41.21, 51.63, 53.07, 54.18, 77.64, 78.57, 155.86, 160.58, 171.31 and 171.95; ν_{max}(film)/cm^{-1} 3402, 2973, 1743, 1711, 1502 and 1219; *m*/*z* (FAB) 432 (M^+ + 1, 46%), 376 (100), 301 (17), 268 (6), 213 (9), 154 (44) and 72 (98).

N^2-Benzyloxycarbonyl-N^6-(*tert*-butoxycarbonyl)-L-lysyl-L-tyrosine ethyl ester 4

A solution of $(Boc)_2O$ (1.87 g, 8.56 mmol) in 1,4-dioxane (15 cm^3) was added dropwise to a solution of N^2-CbzLysOH (2.0 g, 7.13 mmol) in 1 M NaOH (10 cm^3) at 0 °C. After being stirred for 4 h, the mixture was extracted with Et_2O. The aqueous layer was acidified to pH 4 with saturated aq. $KHSO_4$ at 0 °C. The solution was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give the corresponding N-protected acid. The above acid and TyrOEt·HCl (2.1 g, 8.56 mmol) were submitted to the conditions described in the general method. After 7 h the reaction mixture was worked up as outlined previously. Flash chromatography (hexane–EtOAc, 1:1) gave *title ester* **4** (3.60 g, 88%) (HRMS: M^+ + H, 572.2977. $C_{30}H_{42}N_3O_8$ requires *m*/*z*, 572.2972); $[\alpha]_D^{18}$ +14.0 (*c* 1.17, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 1.12–1.70 (6 H, m), 1.26 (3 H, t, *J* 7.2), 1.43 (9 H, s), 2.85–3.17 (4 H, m), 4.13 (1 H, br s), 4.17 (2 H, q, *J* 7.2), 4.70 (1 H, br s), 4.78 (1 H, dd, *J* 13.1 and 7.5), 5.08 (2 H, s), 5.42 (1 H, br s), 6.57 (1 H, br s), 6.73 (2 H, d, *J* 8.1), 6.92 (2 H, d, *J* 8.1), 7.05 (1 H, br s) and 7.33 (5 H, m); δ_C(75.4 MHz; $CDCl_3$) 14.52, 22.74, 28.83, 29.96, 32.48, 37.38, 40.46, 53.65, 55.35, 62.01, 67.58, 79.95, 116.06, 127.28, 128.49, 128.59, 128.93, 130.73, 136.50, 165.08, 156.64, 156.90, 171.92 and 171.99; ν_{max}(film)/cm^{-1} 3421, 3019, 1685, 1516 and 1215; *m*/*z* (FAB) 572 (M^+ + 1, 72%), 472 (100), 307 (20), 280 (18), 154 (59) and 91 (92).

***N*-(*tert*-Butoxycarbonyl)-L-methionyl-N^6-(*tert*-butoxycarbonyl)-L-lysyl-L-tyrosine ethyl ester 5**

10% Palladium on activated carbon (191.6 mg, 0.18 mmol) was added to a solution of compound **4** (1.03 g, 1.80 mmol) in MeOH (15 cm^3) and the reaction mixture was stirred under hydrogen (1 atm). After 5 h the mixture was filtered through Celite, and the filtrate was concentrated *in vacuo* to afford the corresponding amine (772.1 mg, 98%). The above amine and BocMetOH (538.3 mg, 2.16 mmol) were submitted to the conditions described in the general method. After 12 h the reaction mixture was worked up as outlined previously. Flash chromatography (hexane–EtOAc, 1:1) gave *title compound* **5** (924.1 mg, 77%) (HRMS: M^+ + H, 669.3535. $C_{32}H_{53}N_4O_9S$ requires *m*/*z*, 669.3533); $[\alpha]_D^{18}$ −3.6 (*c* 1.13, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 1.15–1.77 (6 H, m), 1.30 (3 H, t, *J* 7.1), 1.46 (18 H, s), 1.85–2.18 (2 H, m), 2.12 (3 H, s), 2.56 (2 H, t, *J* 7.1), 2.90–3.19 (4 H, m), 4.21 (2 H, q, *J* 7.1), 4.24 (1 H, br s), 4.34 (1 H, br s), 4.80 (2 H, m), 5.37 (1 H, br s), 6.65 (1 H, br s), 6.78 (2 H, d, *J* 8.4), 6.82 (1 H, br s), 6.97 (2 H, d, *J* 8.4), 7.19 (1 H, br s); δ_C(75.4 MHz; $CDCl_3$) 14.57, 15.68, 22.79, 28.73, 28.86, 29.81, 30.58, 31.66, 32.38, 37.25, 40.55, 53.67, 54.01, 62.01, 77.65, 79.81, 80.82, 116.16, 127.37, 130.74, 156.02, 156.12, 156.38, 171.41, 171.85 and 172.22; ν_{max}(film)/cm^{-1} 3325, 3019, 2981, 1691, 1516 and 1214; *m*/*z* (FAB) 669 (M^+ + 2, 76%) 569 (47), 513 (39), 469 (25), 321 (17), 210 (51) and 84 (100).

***N*-(*tert*-Butoxycarbonyl)-L-phenylalanyl-N^6-(*tert*-butoxycarbonyl)-L-lysyl-L-tyrosine ethyl ester 6**

10% Palladium on activated carbon (127.7 mg, 0.12 mmol) was added to a solution of compound **4** (684.1 mg, 1.2 mmol) in MeOH (10 cm^3) and the reaction mixture was stirred under hydrogen (1 atm). After 5 h the mixture was filtered through Celite, and the filtrate was concentrated *in vacuo* to afford the corresponding amine. The above amine and BocPheOH (318.2 mg, 1.2 mmol) were submitted to the conditions described in the general method. After 11 h the reaction mixture was worked up as outlined previously. Flash chromatography (hexane–EtOAc, 1:1) gave *title compound* **6** (433.0 mg, 53%) (HRMS: M^+ + H, 685.3814. $C_{36}H_{53}N_4O_9$ requires *m*/*z*, 685.3813); $[\alpha]_D^{18}$ −4.4 (*c* 1.08, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 1.05–1.74 (6 H, m), 1.27 (3 H, t, *J* 7.2), 1.40 (9 H, s), 1.44 (9 H, s), 2.86–3.15 (6 H, m), 4.19 (2 H, q, *J* 7.2), 4.29 (2 H, m), 4.64 (1 H, br s), 4.74 (1 H, dd, *J* 13.7 and 7.5), 4.96 (1 H, br s), 6.39 (2 H, br s), 6.52 (1 H, br s), 6.76 (2 H, d, *J* 8.1), 6.93 (2 H, d, *J* 8.1) and 7.18–7.30 (5 H, m); δ_C(75.4 MHz; $CDCl_3$) 14.57, 21.48, 22.66, 28.66, 28.86, 29.85, 32.39, 37.23, 40.56, 53.56, 53.66, 60.85, 61.99, 79.80, 80.94, 116.21, 127.39, 129.09, 129.71, 130.73, 136.91, 156.09, 156.75, 171.26, 171.82 and 171.91; ν_{max}(film)/cm^{-1} 3413, 3019, 2981, 1694, 1515 and 1216; *m*/*z* (FAB) 685 (M^+ + 2, 89%), 585 (59), 485 (37), 337 (23), 319 (23), 154 (73) and 84 (100).

(5ξ)-3-{(1*S*)-1-[*N*-(*tert*-Butoxycarbonyl)-L-methionyl-N^6-(*tert*-butoxycarbonyl)-L-lysyl-L-tyrosylamino]-2-methylpropyl}-4,5-dihydro-5-(L-methioninocarbonyl)isoxazole methyl ester 7

TFA (0.5 cm^3) was added to a solution of compound **3** (88.3 mg, 0.19 mmol) in CH_2Cl_2 (0.5 cm^3) and the solution was stirred for 30 min. The reaction mixture was evaporated to dryness *in vacuo* and the residue was dissolved in CH_2Cl_2. The solution was evaporated to dryness *in vacuo* and the residue was partitioned between CH_2Cl_2 and saturated aq. $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford the corresponding amine.

$LiOH·H_2O$ (50.3 mg, 1.20 mmol) was added to a stirred solution of compound **5** (265.5 mg, 0.40 mmol) in THF–water (5:1, v/v; 6 cm^3). After 3 h the reaction mixture was poured into water and the solution was extracted with Et_2O. The aqueous layer was acidified to pH 3 with saturated aq. $KHSO_4$ at 0 °C and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford the corresponding acid quantitatively.

The above amine and acid were submitted to the conditions described in the general method. After 6 h the mixture was worked up as outlined previously. Recrystallization (MeOH–Et_2O, 2:1) gave *title compound* **7** (105.3 mg, 58%) (HRMS: M^+ + H, 954.4675. $C_{44}H_{72}N_7O_{12}S_2$ requires *m*/*z*, 954.4680); δ_H(500 MHz; CD_3OD) *inter alia* 0.95 (3 H, d, *J* 6.7), 0.98 (3 H, d, *J* 6.7), 1.27 (2 H, m), 1.45 (9 H, m), 1.46 (2 H, m), 1.47 (9 H, m), 1.66 (2 H, m), 1.87 (1 H, m), 2.03 (2 H, m), 2.08 (3 H, s), 2.10 (3 H, s), 2.16 (2 H, m), 2.54 (4 H, m), 2.88 (1 H, dd, *J* 14.0 and 9.0), 3.00 (2 H, t, *J* 7.0), 3.08 (1 H, dd, *J* 9.0 and 5.4), 3.17 (2 H, d, *J* 8.5), 3.75 (3 H, s), 4.16 (1 H, dd, *J* 8.3 and 5.3), 4.23 (1 H, m), 4.52 (1 H, d, *J* 8.3), 4.58 (1 H, m), 4.64 (1 H, dd, *J* 9.0 and 4.8), 4.98 (1 H, dd, *J* 9.3 and 8.1), 6.72 (2 H, d, *J* 8.4) and 7.06 (2 H, d, *J* 8.4); δ_C(125.8 MHz; CD_3OD) 15.73, 15.83, 19.58, 20.52, 24.41, 29.29, 29.34, 31.05, 31.65, 31.70, 31.94, 32.13, 33.03, 33.12, 38.19, 41.26, 41.64, 53.06, 53.45, 54.49, 55.65, 55.76, 56.68, 79.88, 80.39, 81.38, 116.86, 129.51, 131.80, 157.80, 158.57, 159.00, 160.73, 173.85, 173.90, 174.29 and 175.45; ν_{max}(film)/cm^{-1} 3281, 2972, 1742, 1687, 1652, 1520 and 1253; *m*/*z* (FAB) 954 (M^+ + 1, 1.8%), 854 (26), 798 (9), 613 (4), 469 (20), 307 (100) and 289 (56).

(5ξ)-3-{(1*S*)-1-[*N*-(*tert*-Butoxycarbonyl)-L-phenylalanyl-N^6-(*tert*-butoxycarbonyl)-L-lysyl-L-tyrosylamino]-2-methylpropyl}-4,5-dihydro-5-(L-methioninocarbonyl)isoxazole methyl ester 8

Compounds **3** (97.3 mg, 0.21 mmol) and **6** (183.2 mg, 0.27 mmol) were submitted to the procedure described in the synthesis of analogue **7** to afford *title compound* **8** (117.5 mg, 56%) (HRMS: M^+ + H, 970.4974. $C_{48}H_{72}N_7O_{12}S$ requires *m*/*z*, 970.4960); δ_H(500 MHz; CD_3OD) *inter alia* 0.93 (3 H, d, *J* 6.7), 0.95 (3 H, *J* 6.7), 1.27 (2 H, m), 1.36 (9 H, s), 1.39 (2 H, m), 1.43 (9 H, s), 1.63 (2 H, m), 2.05 (2 H, m), 2.06 (3 H, s), 2.14 (1 H, m), 2.49 (2 H, m), 2.83 (2 H, m), 2.99 (2 H, t, *J* 7.0), 3.06 (2 H, m), 3.11 (2 H, d, *J* 8.8), 3.73 (3 H, s), 4.23 (1 H, m), 4.28 (1 H,

dd, *J* 9.3 and 4.9), 4.50 (1 H, d, *J* 8.0), 4.55 (1 H, dd, *J* 8.4 and 6.4), 4.62 (1 H, dd, *J* 8.9 and 4.8), 4.95 (1 H, t, *J* 8.7), 6.70 (2 H, d, *J* 8.4), 7.04 (2 H, d, *J* 8.2) and 7.24 (5 H, m); δ_C(125.8 MHz; CD_3OD) 15.85, 19.60, 20.66, 24.40, 29.36, 29.49, 31.09, 31.65, 31.97, 32.16, 33.45, 38.29, 39.37, 41.31, 41.66, 52.98, 53.50, 54.28, 55.24, 56.55, 57.85, 79.84, 80.12, 81.01, 116.89, 128.18, 129.55, 130.98, 131.91, 139.43, 157.83, 158.62, 160.70, 173.35, 173.58, 173.80, 173.94 and 174.60; ν_{max}(film)/cm^{-1} 3300, 2959, 1685, 1640, 1517 and 1266; *m*/*z* (FAB) 970 ($M^+ + 1$, 44%), 870 (100), 814 (30), 770 (13), 460 (10), 307 (79) and 289 (36).

N^6-Benzyloxycarbonyl-N^2-(*tert*-butoxycarbonyl)-L-lysyl-L-tyrosine 9

Et_3N (595 mm^3, 4.27 mmol) and $(Boc)_2O$ (900 mg, 4.27 mmol) were added to a solution of N^6-CbzLysOH (1 g, 3.56 mmol) in MeOH (8 cm^3). 1 M NaOH (5 cm^3) was then added and the solution was stirred for 2 h. The reaction mixture was evaporated *in vacuo* to afford a residue, which was subsequently dissolved in water. The solution was extracted with Et_2O. The aqueous layer was acidified to pH 2–3 with saturated aq. $KHSO_4$ and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*.

The above acid and TyrOEt·HCl (744 mg, 3.56 mmol) were submitted to the conditions described in the general method. After 24 h the mixture was worked up as outlined previously. Flash chromatography (hexane–EtOAc, 1:1) gave the corresponding dipeptide (1.86 g, 92%), which was subsequently dissolved in MeOH (10 cm^3) and 1 M NaOH (7.3 cm^3) was added at 0 °C. The solution was stirred for 3 h and evaporated *in vacuo*. The residue was dissolved in water and extracted with Et_2O. The aqueous layer was acidified to pH 2–3 with saturated aq. $KHSO_4$ and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give *title compound* **9** (1.93 g, 100%) (HRMS: $M^+ + H$, 544.2665. $C_{28}H_{38}N_3O_8$ requires *m*/*z*, 544.2659); $[a]_D^{18}$ +38.2 (*c* 1.12, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 1.17 (2 H, m), 1.42 (12 H, m), 1.59 (1 H, m), 3.06 (4 H, m), 4.00 (1 H, m), 4.79 (1 H, m), 5.07 (2 H, s), 5.16 (1 H, m), 5.52 (1 H, br d, *J* 7.4), 6.70 (2 H, m), 6.94 (3 H, m), 7.31 (5 H, m) and 7.61 (2 H, br s); δ_C(75.4 MHz; $CDCl_3$) 22.79, 28.72, 29.72, 32.53, 37.06, 41.06, 53.76, 54.75, 67.32, 81.07, 115.98, 127.40, 128.36, 128.53, 128.95, 130.96, 136.69, 155.92, 156.58, 157.47, 172.88 and 174.35; ν_{max}(film)/cm^{-1} 3331, 3008, 2938, 1716, 1516 and 1256; *m*/*z* (FAB) 544 ($M^+ + 1$, 21%), 444 (16), 391 (8), 307 (45), 220 (22), 154 (100) and 137 (59).

(5*S*)-5-(*tert*-Butoxycarbonylamino)-6-methyl-4-oxohept-2-enoic acid methyl ester 10

Boric acid (1.48 g, 23.9 mmol) and a catalytic amount of freshly activated Raney-Nickel were added to a solution of compound **2** (3.6 g, 11.9 mmol) in MeOH–water (5:1, v/v; 130 cm^3). The mixture was stirred under hydrogen (1 atm) until completion of reaction (TLC analysis). The reaction mixture was filtered through Celite and the filtrate was evaporated *in vacuo*. The residue was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the corresponding reduction product.

The above product was dissolved in pyridine (160 cm^3) and methanesulfonyl chloride (1.55 cm^3, 20 mmol) was added at 0 °C. After being stirred for 1 h, the mixture was diluted with Et_2O, washed successively with water and 1 M HCl, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash chromatography (hexane–EtOAc, 5:1) gave *title compound* **10** (2.2 g, 78%) (Found: C, 58.94; H, 8.12; N, 4.82. $C_{14}H_{23}NO_5$ requires C, 58.93; H, 8.12; N, 4.91%); $[a]_D^{24}$ +25.4 (*c* 2.07, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 0.80 (3 H, d, *J* 6.5), 1.02 (3 H, d, *J* 6.5), 1.44 (9 H, s), 2.17 (1 H, m), 3.82 (3 H, s), 4.52 (1 H, m), 5.13 (1 H, m), 6.80 (1 H, d, *J* 15.6) and 7.22 (1 H, d, *J* 15.5); δ_C(75.4 MHz; $CDCl_3$) 17.43, 20.43, 28.95, 30.79, 53.06, 64.15, 80.68, 132.25, 137.65, 156.49, 166.33 and 198.86; ν_{max}(film)/cm^{-1} 3327, 2966, 1732, 1684, 1532, 1366, 1319, 1250 and 1173.

(2*R*/*S*,5*S*)-5-(*tert*-Butoxycarbonylamino)-2-isopropyl-6-methylhept-3-enoic acid methyl ester 11

Ketone **10** (1.12 g, 3.85 mmol) and $CeCl_3·7H_2O$ (1.73 g, 4.62 mmol) were dissolved in MeOH (76 cm^3) and $NaBH_4$ (191 mg, 7.7 mmol) was added slowly at −78 °C. After stirring of the mixture for 5 min, water (150 cm^3) was added slowly and the solution was evaporated *in vacuo* to half of the original volume. The residue was extracted with EtOAc, washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash chromatography (hexane–EtOAc, 3:1) gave the corresponding alcohol (1.13 g, 99%).

To a solution of the above alcohol (460 mg, 1.6 mmol) in CH_2Cl_2 (2.6 cm^3) were added pyridine (1.6 cm^3) and methanesulfonyl chloride (500 mm^3, 6.4 mmol) at 0 °C. After being stirred for 11 h, the mixture was poured into 5% aq. $NaHCO_3$ and the aqueous layer was extracted with Et_2O–CH_2Cl_2 (4:1, v/v). The combined organic layers were washed successively with 5% aq. citric acid and 5% aq. $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash chromatography (hexane–EtOAc, 3:1) gave the corresponding methanesulfonyl derivative (577.8 mg, 96%).

Isopropylmagnesium chloride (2 M in Et_2O; 3.2 cm^3) was added to a stirred solution of copper(I) cyanide (565 mg, 6.3 mmol) in THF (1.6 cm^3) at −78 °C. The solution was warmed to 0 °C and stirred for 5 min before being cooled to −78 °C, and $BF_3·Et_2O$ (787 mm^3, 6.4 mmol) was added. After 5 min a solution of the above mesyl derivative (577 mg, 1.6 mmol) in THF (3 cm^3) was introduced. After 30 min, saturated aq. NH_4Cl–NH_4OH (2:1, v/v; 3 cm^3) was added. The aqueous layer was extracted with Et_2O. The combined organic layers were washed with water, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash chromatography (hexane–EtOAc, 4:1) gave title compound **11** (417 mg, 83%); δ_H(300 MHz; $CDCl_3$) 0.90 (12 H, m), 1.45 (9 H, s), 1.74 (1 H, m), 1.99 (1 H, m), 2.69 (1 H, t, *J* 8.9), 3.67 (3 H, s), 3.97 (1 H, m), 4.51 (1 H, m), 5.43 (1 H, dd, *J* 15.4 and 6.1) and 5.54 (1 H, dd, *J* 15.4 and 9.2); δ_C(75.4 MHz; $CDCl_3$) 18.63, 19.07, 20.18, 21.17, 28.76, 31.20, 32.76, 51.93, 57.28, 57.73, 79.60, 128.29, 133.29, 155.80 and 174.74; ν_{max}(film)/cm^{-1} 3375, 2966, 1715, 1502, 1466, 1390, 1296 and 1167; *m*/*z* (EI) 314 (M^+, 9%), 286 (73), 270 (62), 258 (61) 243 (93), 187 (66), 170 (89), 128 (51), 96 (85) and 81 (100).

(2*R*/*S*,5*S*)-5-(*tert*-Butoxycarbonylamino)-2-isopropyl-6-methylhept-3-enoic acid benzylamide 12

To a solution of ester **11** (410 mg, 1.3 mmol) in MeOH–THF (2.6:1, v/v; 3.6 cm^3) at 0 °C was added 1 M NaOH (2.8 cm^3). After being stirred for 20 min, the solution was evaporated *in vacuo* and the residue extracted with Et_2O. The aqueous layer was acidified to pH 2–3 with saturated aq. $KHSO_4$ and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the corresponding acid.

The above acid and benzylamine hydrochloride (206 mg, 1.32 mmol) were submitted to the conditions described in the general method. After 2 h the mixture was worked up as outlined previously. Flash chromatography (hexane–EtOAc, 3:1) gave *title amide* **12** (426 mg, 91%) (HRMS: $M^+ + H$, 389.2794. $C_{23}H_{37}N_2O_3$ requires *m*/*z*, 389.2804); δ_H(300 MHz; $CDCl_3$) 0.90 (12 H, m), 1.38 (4.5 H, s), 1.41 (4.5 H, s), 1.75 (1 H, m), 2.25 (0.5 H, m), 2.36 (0.5 H, m), 2.59 (0.5 H, m), 2.68 (0.5 H, m), 3.79 (0.5 H, q, *J* 7.0), 3.93 (0.5 H, m), 4.46 (3 H, m), 5.38–5.73 (2 H, m), 6.14 (0.5 H, br s), 6.70 (0.5 H, br s) and 7.22–7.35 (5 H, m); δ_C(75.4 MHz; $CDCl_3$) 18.70, 19.08, 19.62, 21.47, 21.56, 28.73, 29.67, 30.10, 32.16, 32.49, 43.77, 43.86, 57.56, 57.89, 58.40, 59.46, 79.73, 127.53, 127.72, 127.95, 128.16, 128.90, 129.01,

134.06, 134.78, 138.93, 139.27, 155.95, 156.09, 173.59 and 173.64; ν_{max}(film)/cm^{-1} 3309, 2957, 1685, 1654, 1545 and 1303; *m/z* (FAB) (M^+ + 1, 7%), 333 (7), 277 (5), 272 (10), 185 (57), 93 (100), 75 (23) and 57 (23).

1-[(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-methylbutyl]-L-proline 13

A toluene solution of DIBAL-H (1.5 M; 20 cm^3) was added dropwise to a stirred solution of BocValOCH$_3$ (3.1 g, 13 mmol) in dry toluene (30 cm^3) at −78 °C. After 10 min MeOH (6 cm^3) was added carefully and the resulting mixture was stirred for 1 h. The mixture was then poured into aq. (10%) citric acid (20 cm^3) at 0 °C. After being stirred for 1 h, the mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the corresponding aldehyde, which was used immediately in the next step without further purification.

$NaCNBH_4$ (660 mg, 10.5 mmol) was added to a solution of the above aldehyde (1.46 g, 7 mmol) and L-Pro (1 g, 8.4 mmol) in MeOH (80 cm^3) at 0 °C. After stirring of the mixture for 1 h, water was added, and evaporated *in vacuo* to give a residue, which was subsequently dissolved in water. The resulting solution was acidified to pH 2–3 with saturated aq. $KHSO_4$ and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Recrystallization (hexane–EtOAc) gave *title acid* **13** (1.78 g, 85%) (HRMS: M^+ + H, 301.2124. $C_{15}H_{29}N_2O_4$ requires *m/z*, 301.2127); $[\alpha]_D^{18}$ −9.1 (*c* 1.16, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 0.94 (6 H, m), 1.45 (9 H, s), 1.72–2.61 (5 H, m), 3.31 (3 H, m), 3.78 (2 H, m), 4.12 (1 H, m), 6.11 (1 H, br d, *J* 9.1) and 7.10 (1 H, br s); δ_C(75.4 MHz; $CDCl_3$) 17.98, 19.33, 23.21, 28.66, 31.51, 53.02, 54.82, 55.87, 68.17, 79.75, 156.23 and 171.27; ν_{max}(film)/cm^{-1} 3297, 2973, 1701, 1507 and 1215; *m/z* (FAB) 301 (M^+ + 1, 100%), 245 (59), 201 (12), 199 (10), 184 (3), 155 (2), 128 (13), 84 (14), 70 (12) and 57 (10).

1-[(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-methylbutyl]-L-proline benzylamide 14

Acid **13** (1 g, 3 mmol) and benzylamine hydrochloride (600 mg, 3.96 mmol) were submitted to the conditions described in the general method. After 2 h the mixture was worked up as outlined previously. Flash chromatography (hexane–EtOAc, 2:1) gave *title amide* **14** (1.14 g, 89%) (HRMS: M^+ + H, 390.2751. $C_{22}H_{36}N_3O_3$ requires M^+ + H, 390.2757); $[\alpha]_D^{24}$ −48.4 (*c* 1.58, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 0.69 (3 H, d, *J* 6.8), 0.84 (3 H, d, *J* 6.8), 1.39 (9 H, s), 1.63–1.91 (4 H, m), 2.16 (1 H, m), 2.42 (2 H, m), 2.71 (1 H, dd, *J* 12.9 and 6.4), 3.18 (2 H, m), 3.55 (1 H, m), 4.37 (2 H, m), 4.53 (1 H, dd, *J* 14.6 and 6.6), 7.27 (5 H, m) and 8.09 (1 H, br s); δ_C(75.4 MHz; $CDCl_3$) 16.89, 20.00, 25.28, 28.75, 29.88, 31.08, 43.21, 55.73, 56.02, 59.71, 69.78, 79.60, 127.51, 128.13, 128.89, 139.43, 156.57 and 175.30; ν_{max}(film)/cm^{-1} 3293, 2966, 1697, 1522 and 1172; *m/z* (FAB) 390 (M^+ + 1, 100%), 334 (9), 290 (12), 225 (29), 217 (16), 199 (27), 185 (22), 155 (9), 93 (37) and 70 (38).

(2*RlS*,5*S*)-5-[*N*^{6}-Benzyloxycarbonyl-*N*^{2}-(*tert*-butoxycarbonyl)-L-lysyl-L-tyrosylamino]-2-isopropyl-6-methylhept-3-enoic acid benzylamide 15

TFA (2.7 cm^3) was added to a solution of amide **12** (413 mg, 1.1 mmol) in CH_2Cl_2 (2.7 cm^3) and the solution was stirred for 2 h. The reaction mixture was evaporated to dryness *in vacuo* and the residue was dissolved in CH_2Cl_2. The solution was evaporated to dryness *in vacuo* and the residue was partitioned between CH_2Cl_2 and saturated aq. $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford the corresponding amine.

The above amine and acid **9** were submitted to the conditions described in the general method. After 36 h the mixture was worked up as outlined previously. Flash chromatography (EtOAc–CH_2Cl_2, 2:3) gave *title compound* **15** (0.79 g, 87%) (HRMS: M^+ + H, 814.4770. $C_{46}H_{64}N_5O_8$ requires *m/z*, 814.4755); δ_H(300 MHz; CD_3OD) *inter alia* 0.81–0.91 (12 H, m), 1.17–1.63 (6 H, m), 1.41 (9 H, s), 1.71 (1 H, m), 1.98 (1 H, m), 2.53 (1 H, q, *J* 8.7), 2.90 (2 H, m), 3.08 (2 H, t, *J* 6.8), 3.88 (1 H, m), 4.10 (1 H, m), 4.34 (2 H, m), 4.54 (1 H, m), 5.06 (2 H, s), 5.50 (2 H, m), 6.68 (2 H, two d, *J* 8.4), 7.01 (2 H, two d, *J* 8.4) and 7.19–7.34 (10 H, m); ν_{max}(film)/cm^{-1} 3300, 2960, 1685, 1640, 1517 and 1266; *m/z* (FAB) 814 (M^+ + 1, 28%), 714 (45), 680 (9), 452 (6), 398 (7), 272 (48), 136 (34) and 91 (100).

1-{(2*S*)-2-[*N*^{6}-Benzyloxycarbonyl-*N*^{2}-(*tert*-butoxycarbonyl)-L-lysyl-L-tyrosylamino]-3-methylbutyl}-L-proline benzylamide 16

Compound **14** (370 mg, 0.99 mmol) was submitted to the conditions described in the synthesis of compound **15**. Flash chromatography (CH_2Cl_2–EtOAc–Et_2O–MeOH, 10:10:5:1) gave *title compound* **16** (454 mg, 56%) (HRMS: M^+ + H, 815.4697. $C_{45}H_{63}N_6O_8$ requires *m/z*, 815.4707); $[\alpha]_D^{18}$ −26.0 (*c* 1.13, $CHCl_3$); δ_H(300 MHz; CD_3OD) *inter alia* 0.73 (3 H, d, *J* 6.8), 0.77 (3 H, d, *J* 6.8), 1.15–1.74 (10 H, m), 1.40 (9 H, s), 2.11 (1 H, m), 2.32 (2 H, m), 2.63 (1 H, dd, *J* 13.1 and 4.7), 2.86–3.09 (6 H, m), 3.66 (1 H, m), 3.87 (1 H, dd, *J* 8.0 and 5.5), 4.29–4.51 (3 H, m), 5.07 (2 H, s), 6.67 (2 H, d, *J* 8.4), 7.01 (2 H, d, *J* 8.4) and 7.22–7.34 (10 H, m); δ_C(75.4 MHz; CD_3OD) 17.18, 18.79, 22.87, 24.59, 27.76, 29.53, 30.62, 30.71, 31.56, 36.45, 40.33, 42.87, 55.21, 55.40, 55.77, 58.12, 66.36, 69.24, 79.85, 111.04, 115.42, 127.15, 127.56, 127.68, 127.79, 127.96, 128.47, 128.50, 130.43, 137.43, 139.34, 156.46, 157.16, 157.97, 172.28, 173.84 and 176.80; ν_{max}(film)/cm^{-1} 3302, 2965, 1696, 1648, 1517 and 1248; *m/z* (FAB) 815 (M^+ + 1, 26%), 725 (5), 681 (4), 546 (1), 437 (2), 282 (3) and 185 (22).

Measurement of total inositol phosphates induced by peptides

To measure the amount of total inositol phosphates induced by peptides, subconfluent U937 cells were labelled with *myo*-[^{3}H]inositol (1 μCi/10^6 cell, Amersham) for 24 h at 30 °C in inositol-free RPMI 1640 medium. Labelled cells were harvested, rinsed twice with inositol-free RPMI 1640, and incubated with LiCl medium (20 mM Hepes, pH 7.2, 20 mM LiCl, 0.1% BSA/PBS† in RPMI medium) for 20 min. After aliquoting of equal volumes into test tubes, a peptide was added for 30 min at room temp. Reactions were stopped by addition of 2% aq. $HClO_4$ and mixtures were then vigorously vortexed. After 30 min in an ice-bath, the samples were centrifuged and the supernatants were loaded onto Dowex AG 1-X8 anion-exchange columns (Bio-Rad). Subsequently, each column was washed with 2 cm^3 of distilled water and 10 cm^3 of 60 mM aq. ammonium formate containing 5 mM aq. disodium tetraborate. Total inositol phosphates were eluted with 2 cm^3 of 1 mM aq. ammonium formate and 0.1 M aq. formic acid. Eluted [^{3}H]-inositol phosphates were quantitated by counting in a liquid scintillation counter (Tri-Packard, Meriden, CT).

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† Bovine serum albumin/phosphate-buffered saline (9.6 g) in distilled water (1 l).

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