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Peptidic Aldehydes Based on α- and β-Amino Acids: Synthesis, Inhibition of m-Calpain, and Anti-Cataract Properties

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We present a new synthesis of SJA6017 (a potent m-calpain inhibitor) and its adaptation in order to prepare analogues in which the constituent Leu and Val residues are systematically replaced with their corresponding β-amino acids and/or the *N*-terminal fluorophenylsulfonyl group is replaced by a water solubilizing *N*-pyridin-3-ylmethoxycarbonyl group. All compounds have been assayed against m-calpain, and the best inhibitor, SJA6017, has been shown to inhibit the development of opacity in a lens culture system design to mimic cataract.

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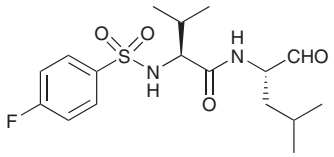
Introduction

Calpains (EC 3.4.22.17) are cytosolic, non-lysosomal, cysteine proteases that are activated by elevated levels of intracellular calcium ions, a feature that distinguishes them from other proteases in the papain superfamily.[1–5] Various isoforms of calpain have been identified and in mammals these are classified into two main categories; ubiquitous and tissue-specific. Of the ubiquitous enzymes, μ-calpain (calpain I) and m-calpain (calpain II) are the predominant isoforms and have been implicated in a variety of important diseases. For example, μ-calpain has been identified as the major isoform present during pathological conditions of the nervous tissue such as Alzheimer's, motor neuron damage, muscular dystrophy, and stroke.[6–8] On the other hand, m-calpain has recently been associated with the development of cataract, a condition whereby the lens of an eye becomes increasingly clouded and eventually results in complete blindness.[9–11] Here, a sustained activation of the Ca^{2+}-activated calpain results in precipitation of degraded lens proteins, predominantly α- and β-crystallins.[9] Cataract is one of the leading causes of world blindness, and to date the only treatment available is surgical replacement of the lens with a prosthesis.[12] Therefore, application of a potent calpain inhibitor in an eye-drop formulation is a desirable objective for the prevention or delay of cataract progression.

Several classes of calpain inhibitor are known, but none so far have proven useful as therapeutic agents.[9,11,13–15] While many of these peptidic inhibitors possess neuroprotective properties, few if any, have anticataract properties.[15–25] However, Senju Pharmaceuticals recently patented a dipeptidyl aldehyde, SJA6017 (Scheme 1), a potent m-calpain inhibition that is effective against selenite-induced cataract in rats.[9] Unfortunately, compounds of this type have been shown to possess limited therapeutic potential because of their poor stability, solubility, and selectivity.[9] We have recently embarked on a program that attempts to address some of these shortcomings.



Scheme 1. SJA6017.

In this paper we present results of a systematic study in which β-amino acids are introduced into SJA6017, where residues of this type are known to increase the biostability of peptide-based drugs.[26] We also present work on the incorporation of an *N*-pyridin-3-ylmethoxycarbonyl protecting group (see compounds **12** in Scheme 4) in place of the fluorophenylsulfonyl group in SJA6017 (Scheme 1) as a group of this type is known to enhance the water solubility, and hence potential bioavailability, of peptide-based protease inhibitors.

Results and Discussion

Synthesis

The key β-amino acid esters β-valine **2** and β-leucine **4** were prepared from their respective α-amino acids by an Arndt–Eistert homologation sequence (Scheme 2).[27] In particular, reaction of L-valine with triethylamine and ethylchloroformate gave the mixed anhydride, which was subsequently treated with an ethereal diazomethane solution to afford

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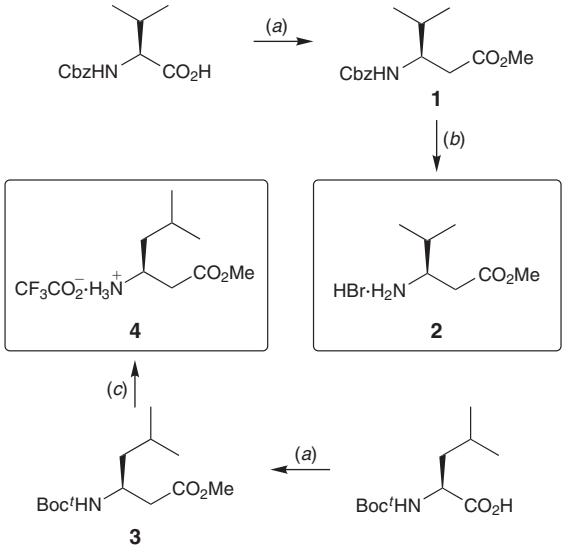
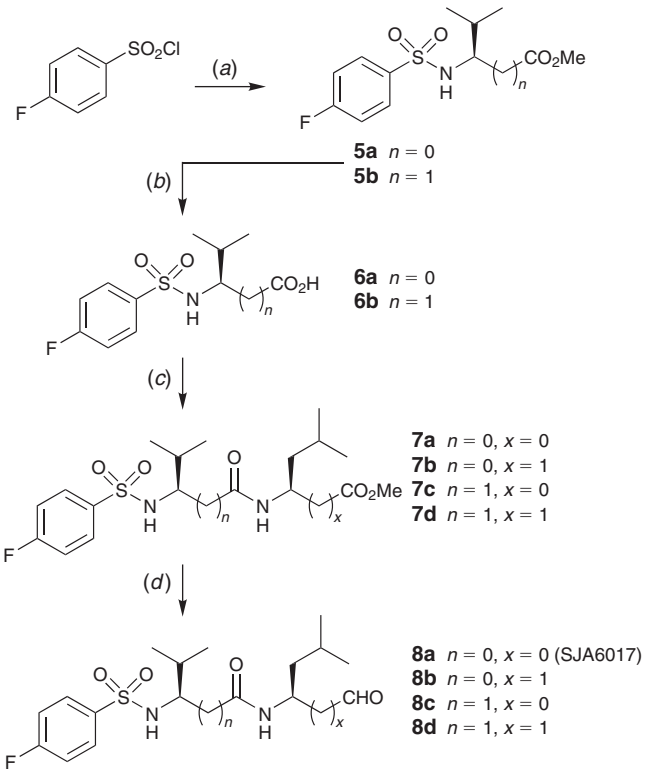
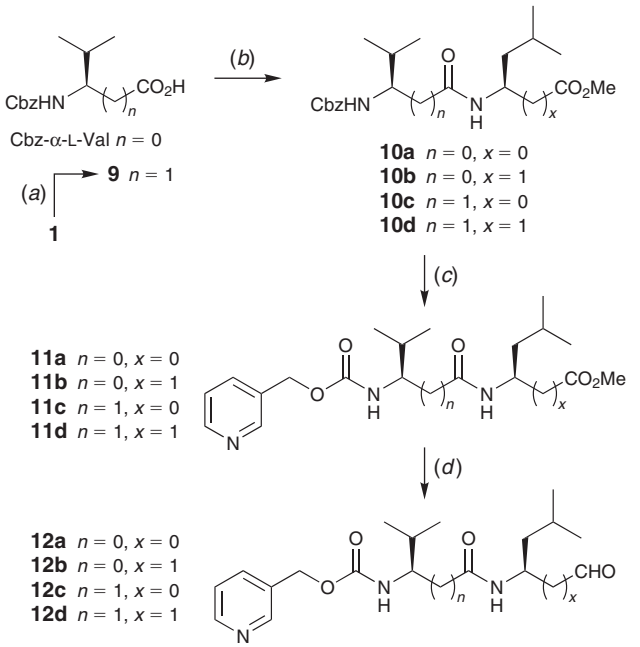


Scheme 2. Reagents and conditions: (*a*) Et_3N, EtOCOCl, CH_2N_2, then MeOH, Et_3N, AgOCOPh; (*b*) HBr, AcOH; (*c*) 95% TFA.



Scheme 3. Reagents and conditions: (*a*) α-L-Val-OMe or **2**, DIPEA, CH_2Cl_2; (*b*) KOH, THF/H_2O; (*c*) α-L-Leu-OMe or **4**, BOP, DIPEA, CH_2Cl_2; (*d*) DIBAL-H, CH_2Cl_2.

the corresponding diazoketone. Reaction with silver benzoate and triethylamine in methanol then gave Cbz-β-L-valine methyl ester **1** (59% yield), which was N-deprotected upon treatment with HBr/acetic acid to give the amine salt **2**. The Boc^t-β-L-leucine methyl ester **3** was similarly prepared from Boc^t-α-L-leucine in 63% yield, and the Boc^t protecting group was removed with 95% trifluoroacetic acid (TFA) to give **4** as the TFA salt.

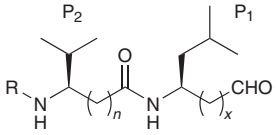


Scheme 4. Reagents and conditions: (*a*) KOH, THF/H_2O; (*b*) α-L-Leu-OMe or **4**, BOP, DIPEA, CH_2Cl_2; (*c*) HBr/AcOH, then sat. HCO_3^-, triphosgene, toluene, CH_2Cl_2, 3-pyridylcarbinol; (*d*) DIBAL-H, CH_2Cl_2. It should be noted that **12a** is less potent than **8a** by a factor of four. Therefore, the effective concentration of **12a** is less than that of **8a** in these experiments.

We then set about systematically preparing SJA6017 analogues **8b**–**8d** (Scheme 3) in which the P1 (Leu) and P2 (Val) residues[28] were replaced with the corresponding β-amino acids **2** and **4**. This was achieved by coupling 4-fluorosulfonyl chloride with either α-L-valine methyl ester or β-L-valine methyl ester **2** to give methyl esters **5a** and **5b**, respectively. Hydrolysis of the methyl esters proceeded smoothly with KOH to afford the corresponding acids **6a** and **6b**. The N-protected amino acid **6a** was then coupled with either α-L-leucine methyl ester or β-L-leucine methyl ester **4** in the presence of BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate) to give dipeptides **7a** and **7b**, respectively. Similarly, separate samples of **6b** were allowed to react with either α-L-leucine methyl ester or β-L-leucine methyl ester **4** to afford **7c** and **7d**, respectively. Finally, methyl esters **7a**–**7d** were reduced with diisobutyl aluminium hydride (DIBAL-H) to give the desired peptidic aldehydes **8a**–**8d** in satisfactory yield. SJA6017 (**8a**, see Scheme 1) has been prepared previously by coupling lucinol to *N*-phenylsulfonyl-L-valine and subsequently oxidizing the amino alcohol to the aldehyde.[9] The new method presented in this paper is more versatile in that it allows ready access to derivatives that contain non-natural amino acids, particularly β-amino acids.

The analogous *N*-pyridin-3-ylmethoxycarbonyl-protected inhibitors (**12a**–**12d**, Scheme 4) were prepared by BOP-mediated peptide coupling of Cbz-α-L-valine with either the hydrochoride salt of α-L-leucine or β-amino acid **4** to give dipeptides **10a** and **10b**, respectively. β-L-Valine **9**, which was prepared by KOH hydrolysis of **1**, was similarly coupled to either the hydrochoride salt of α-L-leucine

Table 1. Inhibition of m-calpain



No.	R	P2 (*n*)	P1 (*x*)	IC_{50} [μM]
8a	$FPhSO_2$	α (0)	α (0)	0.05
8b	$FPhSO_2$	α (0)	β (1)	2.3
8c	$FPhSO_2$	β (1)	α (0)	6.5
8d	$FPhSO_2$	β (1)	β (1)	>500
12a	$PyrCH_2OCO$	α (0)	α (0)	0.2
12b	$PyrCH_2OCO$	α (0)	β (1)	37
12c	$PyrCH_2OCO$	β (1)	α (0)	200
12d	$PyrCH_2OCO$	β (1)	β (1)	105

or **4** to give the dipeptides **10c** and **10d**, respectively. The Cbz protecting groups of **10a**–**10d** were then removed with HBr/acetic acid and the free amines were treated with triphosgene/$NaHCO_3$ to give the corresponding isocyanates. These were coupled with 3-pyridylcarbinol and the resultant *N*-pyridin-3-ylmethoxycarbonyl-protected methyl esters **11a**–**11d** were reduced with DIBAL-H to give the desired peptidic aldehydes **12a**–**12d**.

Enzyme Inhibition and Lens Culture

The peptidic aldehydes **8a**–**8d** and **12a**–**12d** were assayed against m-calpain using a fluorescence-based assay[29] and the results of these studies are presented in Table 1. It is apparent from these data that the replacement of an α-amino acid with a β-amino acid at P1 and/or P2[28] results in a systematic reduction in potency. For example, the introduction of a single β-amino acid, be it β-Leu (P1) or β-Val (P2), results in a reduction in potency for both the *N*-phenylsulfonyl protected (compare **8a**/**8b** 46-fold decrease, and **8a**/**8c** 130-fold decrease) and *N*-pyridin-3-ylmethoxycarbonyl-protected (compare **12a**/**12b** 185-fold decrease, and **12a**/**12c** 1000-fold decrease) series. Replacement of the second α-amino acid of the *N*-phenylsulfonyl protected derivatives **8b** and **8c** to give the all β-derivative **8d** results in a further and dramatic reduction in potency. It is interesting to note that the effect of this second replacement is not as pronounced for the *N*-pyridin-3-ylmethoxycarbonyl-protected series in which the all β-derivative **12d** is less potent than **12b** by a factor of approximately three, but is in fact slightly more potent than **12c** (twofold). This observation has relevance for the future design of β-amino acid-based inhibitors of calpain (see below for further discussion).

The data presented in Table 1 also reveals that replacement of an *N*-phenylsulfonyl group with an *N*-pyridin-3-ylmethoxycarbonyl group results in a decrease in inhibitor potency. A comparison of the activity of the α–α, α–β, and β–α derivatives upon the introduction of an *N*-pyridin-3-ylmethoxycarbonyl protecting group across both series (**8a**/**12a**, **8b**/**12b**, and **8c**/**12c**, respectively) indicates a four-, 16-, and 31-fold decrease in activity. However, once again the all β-derivatives provide an outlier whereby **12d** is more potent than **8d**. This, together with the earlier observation that the introduction of a second β-amino acid is better tolerated in the *N*-pyridin-3-ylmethoxycarbonyl series suggests that a β-amino acid-based peptidic inhibitor may in fact be accommodated in the active site of calpain if an appropriate choice of N-protecting group is made. Further work is required to optimize the best combination of amino acid and N-protecting group. The work presented here provides a lead for the optimization of the potency of inhibition and other properties necessary for the development of a successful cataract therapeutic. These properties include: (*a*) biostability, which is for example associated with the incorporation of a β-amino acid; and (*b*) bioavailability, which is influenced by the nature of the N-protecting group. For example, the *N*-pyridin-3-ylmethoxycarbonyl protecting group in the current study is known to enhance water solubility.

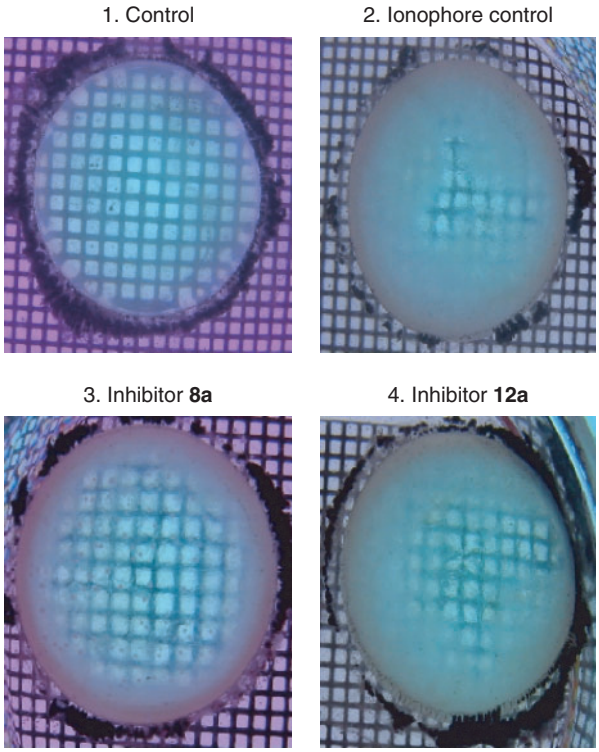


Fig. 1. Representative photomicroscopy of calcium ionophore-induced cataract after 48 h: (1) without added ionophore (no cataract); (2) with ionophore added (cataract); (3) with addition of ionophore followed by **8a**; and (4) with addition of ionophore followed by **12a**.

Finally, we studied the effect of administering two of the best inhibitors, **8a** and **12a**, to cataract-induced sheep lenses that were cultured in Eagle's minimal essential medium. The calcium ionophore ionomycin was added to lenses 2–4 to induce cataract formation[30] and 100 μM of inhibitors **8a** and **12a** were added to lenses three and four, respectively.* After 48 hours all the lenses were photographed under a dissecting microscope (Fig. 1). It is apparent from these preliminary experiments that **8a** reduces the development of cataract. Lens 3 is noticeably more

* It should be noted that **12a** is less potent than **8a** by a factor of four. Therefore, the effective concentration of **12a** is less than **8a** in these experiments.

transparent than the cataract-induced control (lens 2), but is still somewhat less transparent than the cataract-free lens 1. Inhibitor **12a** was less effective in inhibiting the development of opacity. Full details of these experiments will be published elsewhere in due course.

In conclusion, we report a new synthesis of SJA6017, a potent inhibitor of m-calpain which is associated with the development of cataract. This method has been used to construct a series of analogues in which: (*a*) the Leu and Val residues are systematically replaced with their corresponding β-amino acids; and/or (*b*) the N-terminal fluorophenylsulfonyl group is replaced by a water solubilizing *N*-pyridin-3-ylmethoxycarbonyl group. In vitro testing of these compounds against m-calpain has shown that the incorporation of a β-amino acid at the P1 and/or P2 positions results in an additive effect to significantly reduce inhibitor potency. The incorporation of an *N*-pyridin-3-ylmethoxycarbonyl group also results in a loss of potency, although this group is somewhat better tolerated in combination with a β-amino acid(s). Finally, SJA6017 has been shown to effectively suppress the development of Ca^{2+} ionophore-induced cataract in a lens culture system.

Experimental

Proton NMR spectra were acquired on an Inova 500 spectrometer operating at 500 MHz. Carbon NMR spectra were obtained on a Varian Unity XL 300 MHz Fourier transform spectrometer operating at 75 MHz. Spectra were obtained at a temperature of 23°C and chemical shifts are reported in parts per million (ppm, δ) referenced relative to tetramethylsilane (Me_4Si). Molecular masses were detected by electron-impact (EI) mass spectrometry at 4 kV and 70 eV ionization energy using a Kratos MS80 RFA spectrometer with a 250°C source. IR spectra were obtained using a Shimadzu 8201PC series FTIR and compounds were pressed into a KBr disk for analysis. Melting points were obtained on an electrothermal melting point apparatus and are uncalibrated. Thin-layer chromatography (TLC) was performed on aluminium-backed Merck Kieselgel KG60F silica plates that were visualized by short-wavelength ultraviolet light. Flash column chromatography was carried out under positive pressure using Merck silica gel 60 (230 to 400 mesh). The key starting materials **1**,[31] **2**,[32] and **3**[33] were prepared as described in the literature.

*Methyl (*S*)-3-Amino-5-methylhexanoate **4***[34]

A solution of **3** (0.597 g, 2.3 mmol) in 95% TFA (60 mL) was stirred slowly at room temperature for 30 min. The solvent was removed under vacuum and the residue was washed with light petroleum (3 × 50 mL). The residue was dried under high vacuum for 24 h to afford **4** as a pale brown solid (0.617 g, 95%) which was not purified further (data as in ref. [34]) (Found: 160.1331. $C_8H_{18}NO_2$ requires $[M+H]^{+\bullet}$ 160.1338). δ_H (D_2O) 0.77 (3H, d, *J* 7.8, CH_3), 0.79 (3H, d, *J* 7.8, CH_3), 1.41 (2H, m, CH_2), 1.54 (1H, m, CH), 2.58 (1H, dd, *J* 7.8 and 17.0, C*H*H), 2.73 (1H, dd, *J* 4.4 and 17.6, CH*H*), 3.57 (1H, m, CH), 3.61 (3H, s, CO_2Me).

*Methyl (*S*)-2-(4-Fluorobenzenesulfonylamino)-3-methylbutanoate **5a***

4-Fluorobenzenesulfonyl chloride (4.48 g, 0.023 mol) and L-valine methyl ester (4.25 g, 25.0 mmol) were suspended in freshly distilled dichloromethane (30 mL) under an atmosphere of argon. *N*,*N*-Diisopropylethylamine (8.81 mL, 0.057 mol) was added dropwise, the mixture was heated at reflux for 4 h, and was then cooled to room temperature. Ethyl acetate (50 mL) was added and the solution was washed with 10% aqueous HCl (3 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), saturated NaCl (2 × 50 mL), and dried over $MgSO_4$. The solvent was then removed under reduced pressure to give **5a** (6.35 g, 95%) as a white solid (Found: C 49.6, H 5.7, N 4.8%, 289.0784. $C_{12}H_{16}FNO_4S$ requires C 49.9, H 5.6, N 4.9%, $M^{+\bullet}$ 289.0784). δ_H ($CDCl_3$) 0.86 (3H, d, *J* 6.8, CH_3), 0.95 (3H, d, *J* 6.8, CH_3), 2.03 (1H, m, CH), 3.48 (3H, s, CO_2Me), 3.73 (1H, dd, *J* 4.8 and 9.8, CH), 5.09 (1H, d, *J* 9.8, NH), 7.15 (2H, m, 2 ArH), 7.83 (2H, m, 2 ArH). δ_C ($CDCl_3$) 17.3, 18.8, 31.3, 52.1, 61.0, 116.0 (d, *J* 22.8), 129.9 (d, *J* 9.3), 135.7 (d, *J* 3.1), 163.4 (d, *J* 254.7), 171.5.

*Methyl (*S*)-3-(4-Fluorobenzenesulfonylamino)-4-methylpentanoate **5b***

Reaction of **2** (0.22 g, 1.0 mmol) with 4-fluorobenzenesulfonyl chloride (0.172 g, 0.9 mmol) as described for **5a** above gave **5b** (0.264 g, 98%) as a yellow-orange oil. δ_H ($CDCl_3$) 0.79 (3H, d, *J* 6.3, CH_3), 0.81 (3H, d, *J* 6.8, CH_3), 1.79 (1H, m, CH), 2.33 (1H, m, C*H*H), 2.41 (1H, m, CH*H*), 3.35 (1H, m), 3.57 (3H, s, CO_2Me), 5.29 (1H, d, *J* 8.8, NH), 7.16 (2H, m, 2 ArH), 7.88 (2H, m, 2 ArH). δ_C ($CDCl_3$) 18.4, 18.7, 31.7, 36.3, 51.8, 56.3, 116.1 (d, *J* 22.3), 129.7 (d, *J* 8.8), 137.1, 164.9 (d, *J* 254.7), 171.8.

*(*S*)-2-(4-Fluorobenzenesulfonylamino)-3-methylbutanoic Acid **6a***

To a solution of methyl ester **5a** (2.5 g, 8.6 mmol) in water/THF (1 : 1, 150 mL) was added KOH (1.94 g, 0.035 mol). The resultant mixture was heated at 40°C for 3 h, cooled to room temperature, and washed with ethyl acetate (150 mL). The aqueous layer was acidified to pH 1 by the dropwise addition of concentrated HCl, the product was extracted with ethyl acetate (3 × 100 mL), dried over $MgSO_4$, and the solvent was removed under vacuum to give **6a** (0.24 g, 97%) as a pale pink solid (Found: C 47.9, H 5.5, N 5.1%, 276.0706. $C_{11}H_{14}FNO_4S$ requires C 47.8, H 5.2, N 4.8%, $[M+H]^{+\bullet}$ 276.0706). δ_H ([D_6]acetone) 0.87 (3H, d, *J* 6.8, CH_3), 0.96 (3H, d, *J* 6.8, CH_3), 2.10 (1H, m, CH), 3.78 (1H, dd, *J* 4.8 and 10.3, CH), 5.19 (1H, d, *J* 10.3, NH), 7.15 (2H, m, 2 ArH), 7.84 (2H, m, 2 ArH). δ_C ([D_6]acetone) 17.2, 18.8, 31.2, 61.3, 116.0 (d, *J* 22.8), 130.2 (d, *J* 9.3), 137.7 (d, *J* 3.1), 165.0 (d, *J* 251.1), 171.8.

*(*S*)-3-(4-Fluorobenzenesulfonylamino)-4-methylpentanoic Acid **6b***

A solution of **5b** (0.967 g, 0.330 mol) in water/THF was hydrolyzed with KOH (0.074 g, 1.3 mmol) as described for **6a** to give **6b** (91 mg, 94%) as an off-white solid (Found: $[M+H]^{+\bullet}$ 290.0867. $C_{12}H_{16}FNO_4S$ requires $[M+H]^{+\bullet}$ 290.0862). δ_H ([D_6]acetone) 0.97 (3H, d, *J* 4.4, CH_3), 0.98 (3H, d, *J* 4.4, CH_3), 1.98 (1H, m, CH), 2.42 (1H, dd, *J* 5.9 and 15.6, C*H*H), 2.57 (1H, dd, *J* 6.8 and 15.6, CH*H*), 3.67 (1H, m, CH), 6.60 (1H, d, *J* 8.8, NH), 7.45 (2H, m, 2 ArH), 8.05 (2H, m, 2 ArH). δ_C ([D_6]acetone) 17.2, 18.4, 31.8, 36.7, 56.3, 116.1 (d, *J* 22.8), 130.0 (d, *J* 9.3), 138.7 (d, *J* 3.6), 164.8 (d, *J* 251.1), 172.0.

*Methyl (2′*S*,2*S*)-2-[2-(4-Fluorobenzenesulfonylamino)-3-methylbutanoylamino]-4-methylpentanoate **7a***

The acid **6a** (100 mg, 0.4 mmol), L-leucine methyl ester (73 mg, 0.4 mmol), and BOP (180 mg, 0.4 mmol) were dissolved in freshly distilled dichloromethane (5 mL). *N*,*N*-Diisopropylethylamine (0.14 mL, 0.8 mmol) was added dropwise and the solution was stirred under an atmosphere of argon with the exclusion of light for 24 h. The mixture was diluted with ethyl acetate (20 mL), washed successively with 10% aqueous HCl (20 mL), saturated $NaHCO_3$ (20 mL), and saturated NaCl (20 mL), and was dried over $MgSO_4$. The organic solvent was removed under reduced pressure and the resultant white solid was purified by column chromatography (light petroleum/ethyl acetate 1 : 1) to give **7a** (0.13 g, 89%) as a white solid (Found: C 53.4, H 7.1, N 6.9. $C_{18}H_{27}FN_2O_5S$ requires C 53.7, H 6.8, N 7.0%). δ_H ($CDCl_3$) 0.80 (3H, d, *J* 6.3, CH_3), 0.84 (6H, d, *J* 6.3, 2 CH_3), 0.96 (3H, d, *J* 6.8, CH_3), 1.30 (2H, m, CH_2), 1.47 (1H, m, CH), 2.02 (1H, m, CH), 3.52 (1H, dd, *J* 4.8 and 8.8, CH), 3.70 (3H, s, CO_2Me), 4.40 (1H, m, CH), 5.40 (1H, d, *J* 8.8, NH), 5.84 (1H, d, *J* 8.3, NH) 7.15 (2H, m, 2 ArH), 7.85 (2H, m, 2 ArH). δ_C ($CDCl_3$) 17.0, 19.1, 21.6, 22.6, 24.6, 32.0, 41.5, 50.7, 52.4, 61.5, 116.2 (d, *J* 22.8), 130.1 (d, *J* 9.3), 135.7, 167.5 (d, *J* 255.3), 169.7, 172.8. *m/z* (EI) 343, 231, 159 (100%).

*Methyl (2′*S*,3*S*)-3-[2-(4-Fluorobenzenesulfonylamino)-3-methylbutanoylamino]-5-methylhexanoate **7b***

The acid **6a** (100 mg, 0.4 mmol) was allowed to react as described above with **4** (113 mg, 0.4 mmol). Workup and purification of the resultant

residue by chromatography (as described above) gave **7b** (0.14 g, 94%) as a white solid (Found: C 54.6, H 7.4, N 6.7. $C_{19}H_{29}FN_2O_5S$ requires C 54.8, H 7.0, N 6.7%). δ_H ($CDCl_3$) 0.78 (3H, d, *J* 5.9, CH_3), 0.80 (3H, d, *J* 5.9, CH_3), 0.81 (3H, d, *J* 6.3, CH_3), 0.92 (3H, d, *J* 6.3, CH_3), 1.11 (2H, m, CH_2), 1.22 (1H, m, CH), 1.98 (1H, m, CH), 2.44 (2H, m, CH_2), 3.43 (1H, dd, *J* 3.9 and 8.3, Val α-CH), 3.66 (3H, s, CO_2Me), 4.12 (1H, m, β-Leu-CH), 5.44 (1H, d, *J* 8.3, Val-NH), 6.15 (1H, d, *J* 9.3, β-Leu-NH), 7.13 (2H, m, 2 ArH), 7.83 (2H, m, 2 ArH). δ_C ($CDCl_3$) 16.9, 19.2, 21.9, 22.7, 24.7, 31.9, 38.2, 42.9, 44.3, 51.8, 61.6, 116.2 (d, *J* 22.8), 130.0 (d, *J* 9.3), 135.5, 165.0 (d, *J* 253), 169.1, 172.2. *m/z* (EI) 230, 159 (100%).

*Methyl (2*S*,3′*S*)-2-[3-(4-Fluorobenzenesulfonylamino)-4-methylpentanoylamino]-4-methylpentanoate **7c***

The acid **6b** (51 mg, 0.2 mmol) was allowed to react as described above with L-leucine methyl ester (35 mg, 0.2 mmol). Workup and purification of the resultant residue by chromatography (as described above) gave **7c** (59 mg, 81%) as a white solid (Found: C 55.1, H 7.5, N 6.5. $C_{19}H_{29}FN_2O_5S$ requires C 54.8, H 7.0, N 6.7%). δ_H ($CDCl_3$) 0.82 (6H, d, *J* 6.8, 2 CH_3), 0.91 (3H, d, *J* 6.3, CH_3), 0.92 (3H, d, *J* 6.4, CH_3), 1.24–1.62 (3H, m, CH_2 and CH), 1.84 (1H, m, CH), 2.27 (2H, m, CH_2), 3.23 (1H, m, β-Val-CH), 3.73 (3H, s, CO_2Me), 4.51 (1H, m, Leu α-CH), 5.84 (1H, d, *J* 8.3, NH), 5.84 (1H, d, *J* 5.8, NH), 7.14 (2H, m, 2 ArH), 7.88 (2H, m, 2 ArH). δ_C ($CDCl_3$) 19.00, 19.04, 21.9, 22.6, 24.8, 31.5, 36.8, 41.4, 50.6, 52.3, 57.1, 116.1 (d, *J* 22.8), 129.7 (d, *J* 9.3), 137.1 (d, *J* 3.2), 164.8 (d, *J* 254.7), 170.6, 173.2. *m/z* (EI) 373, 202, 159, 146 (100%).

*Methyl (3*S*,3′*S*)-3-[3-(4-Fluorobenzenesulfonylamino)-4-methylpentanoylamino]-5-methylhexanoate **7d***

The acid **6b** (60 mg, 0.2 mmol) was allowed to react as described above with **4** (52 mg, 0.2 mmol). Workup and purification of the resultant residue by chromatography (as described above) gave **7d** (64.5 mg, 78%) as a white solid. δ_H ($CDCl_3$) 0.78 (3H, d, *J* 6.8, CH_3), 0.82 (3H, d, *J* 6.8, CH_3), 0.87 (3H, d, *J* 7.8, CH_3), 0.87 (3H, d, *J* 6.4, CH_3), 1.23 (1H, m, C*H*H), 1.42 (1H, m, CH*H*), 1.48 (1H, m, CH), 1.79 (1H, m, CH), 2.15 (2H, m, CH_2), 2.46 (2H, m, CH_2), 3.22 (1H, m, β-Val-CH), 3.65 (3H, s, CO_2Me), 4.23 (1H, m, β-Leu-CH), 6.07 (1H, d, *J* 8.8, NH), 6.11 (1H, d, *J* 6.8, NH), 7.14 (2H, m, 2 ArH), 7.88 (2H, m, 2 ArH). δ_C ($CDCl_3$) 19.0 (2C), 22.1, 22.7, 25.0, 31.5, 36.8, 38.3, 42.9, 44.1, 51.7, 57.2, 116.1 (d, *J* 22.8), 129.7 (d, *J* 9.3), 137.2, 164.8 (d, *J* 254.2), 170.2, 172.3. *m/z* (EI) 387 (100%), 355, 202, 159.

*(1′*S*,2*S*)-2-(4-Fluorobenzenesulfonylamino)*-N-*(1-formyl-3-methylbutyl)-3-methylbutyramide **8a** (SJA6017)*

A solution of ester **7a** (40 mg, 0.1 mmol) in freshly distilled dichloromethane (10 mL) under an atmosphere of argon was cooled to −78°C. Diisobutyl aluminium hydride (0.09 mL, 0.5 mmol) was added dropwise and the mixture was stirred at −78°C for 3 h. Superdry methanol (10 mL), which had been precooled to −78°C, was added dropwise and the mixture was stirred for a further 25 min at −78°C. The cooling bath was removed and 10% aqueous HCl (10 mL) was added. The organic layer was separated from the resultant white precipitate, diluted with ethyl acetate (20 mL), washed with 10% aqueous HCl (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and saturated aqueous NaCl (10 mL), dried over $MgSO_4$, and concentrated under vacuum to give a cream oil which was purified by column chromatography (ethyl acetate/light petroleum, 2 : 3) to give **8a** (27 mg, 73%) as a white solid[28] (Found: C 54.3, H 7.0, N 7.3%, 373.1603. $C_{17}H_{25}FN_2O_4S$ requires C 54.7, H 7.0, N 7.5%, $[M+H]^{+\bullet}$ 373.1597). υ_{max}/cm^{-1} (KBr) 1725, 1638. δ_H ($CDCl_3$) 0.84 (3H, d, *J* 7.3, CH_3), 0.86 (3H, d, *J* 6.8, CH_3), 0.89 (3H, d, *J* 6.8, CH_3), 0.94 (3H, d, *J* 6.8, CH_3), 1.22–1.27 (2H, m, CH_2), 1.55 (1H, m, CH), 2.06 (1H, m, $CHMe_2$), 3.56 (1H, dd, *J* 4.8 and 8.3, Val α-CH), 4.42 (1H, m, Leu α-CH), 5.31 (1H, d, *J* 8.8, Val NH), 5.97 (1H, d, *J* 7.3 Hz, Leu-NH), 7.15 (2H, m, 2 ArH), 7.86 (2H, m, 2 ArH), 9.49 (1H, s, CHO). δ_C ($CDCl_3$) 16.9, 19.2, 21.7, 22.9, 24.6, 31.9, 37.9, 57.4, 61.6, 116.2 (d, *J* 22.3), 130.1 (d, *J* 9.3), 170.2, 198.5.

*(1′*S*,2*S*)-2-(4-Fluorobenzenesulfonylamino)-3-methyl-*N-*[3-methyl-(1-oxoethyl)butyl]butyramide **8b***

The ester **7b** (49 mg, 0.1 mmol) was reduced in the manner described above with DIBAL-H (0.11 mL, 0.6 mmol). Workup and purification of the resultant residue by chromatography (as described above) gave **8b** (26 mg, 58%) as a white solid (Found: C 54.8, H 7.4, N 6.9%, 387.1759. $C_{18}H_{27}FN_2O_4S{\cdot}0.5H_2O$ requires C 54.5, H 7.4, N 7.1%, $[M+H]^{+\bullet}$ 387.1754). υ_{max}/cm^{-1} (KBr) 1715, 1668. δ_H ($CDCl_3$) 0.78 (3H, d, *J* 6.3, CH_3), 0.79 (3H, d, *J* 5.4, CH_3), 0.83 (3H, d, *J* 5.9, CH_3), 0.86 (3H, d, *J* 6.8, CH_3), 1.18–1.36 (3H, m, CH_2 and CH), 1.96 (1H, m, $CHMe_2$), 2.58 (2H, m, CH_2), 3.41 (1H, dd, *J* 4.4 and 7.8, Val α-CH), 4.19 (1H, m, Leu α-CH), 5.30 (1H, d, *J* 7.8, Val-NH), 5.97 (1H, d, *J* 8.8, Leu-NH), 7.15 (2H, m, 2 ArH), 7.84 (2H, m, 2 ArH), 9.69 (1H, s, CHO). δ_C ($CDCl_3$) 16.8, 19.2, 21.7, 22.8, 24.8, 31.7, 43.1, 43.7, 48.3, 61.7, 116.2 (d, *J* 22.8), 130.1 (d, *J* 9.3), 169.4, 200.9.

*(1′*S*,3*S*)-3-(4-Fluorobenzenesulfonylamino)-*N-*(1-formyl-3-methylbutyl)-4-methylpentamide **8c***

The ester **7c** (45 mg, 0.1 mmol) was reduced in the manner described above with DIBAL-H (0.096 mL, 0.5 mmol). Workup and purification of the resultant residue by chromatography (as described above) gave **8c** (28 mg, 67%) as a white solid (Found: C 54.6, H 7.3, N 6.9%, 387.1751. $C_{18}H_{27}FN_2O_4S{\cdot}0.5H_2O$ requires C 54.5, H 7.4, N 7.1%, $[M+H]^{+\bullet}$ 387.1754). υ_{max}/cm^{-1} (KBr) 1734, 1659. δ_H ($CDCl_3$) 0.82 (3H, d, *J* 6.8, CH_3), 0.83 (3H, d, *J* 6.3, CH_3), 0.96 (6H, d, *J* 6.3, 2 CH_3), 1.40 (1H, m, CH), 1.68 (2H, m, CH_2), 1.83 (1H, m, $CHMe_2$), 2.34 (2H, m, CH_2), 3.23 (1H, m, Val α-CH), 4.52 (1H, m, Leu α-CH), 5.73 (1H, d, *J* 7.8, NH), 5.93 (1H, m, NH), 7.16 (2H, m, 2 ArH), 7.88 (2H, m, 2 ArH), 9.53 (1H, s, CHO). δ_C ($CDCl_3$) 18.6, 18.7, 21.8, 22.9, 24.7, 31.6, 37.2, 37.4, 56.9, 57.2, 116.1 (d, *J* 22.3), 129.7 (d, *J* 9.3), 136.9 (d, *J* 3.7), 164.8 (d, *J* 254.7), 171.3, 199.8.

*(1′*S*,3*S*)-3-(4-Fluorobenzenesulfonylamino)-4-methyl-*N-*[3-methyl-1-(2-oxoethyl)butyl]pentamide **8d***

The ester **7d** (59 mg, 0.1 mmol) was reduced in the manner described above with DIBAL-H (0.122 mL, 0.7 mmol). Workup and purification of the resultant residue by chromatography (as described above) gave **8d** (35 mg, 64%) as a white solid (Found: C 56.1, H 7.9, N 6.6%, 401.1909. $C_{19}H_{29}FN_2O_4S{\cdot}0.5H_2O$ requires C 55.9, H 7.6, N 6.8%, $[M+H]^{+\bullet}$ 401.1910). υ_{max}/cm^{-1} (KBr) 1715, 1649. δ_H ($CDCl_3$) 0.77 (3H, d, *J* 6.8, CH_3), 0.81 (3H, d, *J* 6.8, CH_3), 0.90 (6H, d, *J* 6.3, 2 CH_3), 1.30 (1H, m, C*H*H), 1.53 (2H, m, CH and CH*H*), 1.76 (1H, m, CH), 2.20 (2H, m, CH_2), 2.63 (2H, m, CH_2), 3.20 (1H, m, β-Val-CH), 4.32 (1H, m, β-Leu-CH), 5.82 (1H, d, *J* 8.3, NH), 5.94 (1H, d, *J* 8.8, NH), 7.15 (2H, m, 2 ArH), 7.91 (2H, m, 2 ArH), 9.73 (1H, s, CHO).

*Methyl (2′*S*,3*S*)-3-(2-Benzyloxycarbonylamino-3-methylbutanoylamino)-5-methylhexanoate **10b***

To a mixture of Cbz-L-valine (200 mg, 0.8 mmol) and **4** (248 mg, 0.9 mmol) was added BOP (387 mg, 0.9 mmol) in the manner described above for **7a**. Workup and purification of the resultant residue through a small plug of silica (ethyl acetate) gave **10b** (276 mg, 88%) as a white solid (Found: 415.2208. $C_{21}H_{32}N_2O_5$ requires $[M+Na]^{+\bullet}$ 415.2209). δ_H ($CDCl_3$) 0.88 (6H, d, *J* 6.8, 2 CH_3), 0.93 (6H, d, *J* 6.8, 2 CH_3), 1.26 (1H, m, CH), 1.51 (2H, m, CH_2), 2.10 (1H, m, CH), 2.49 (2H, m, CH_2), 3.65 (3H, s, CO_2Me), 3.93 (1H, m, Val α-CH), 4.32 (1H, m, β-Leu-CH), 5.09 (2H, s, CH_2), 5.36 (1H, d, *J* 8.3, NH), 6.36 (1H, d, *J* 8.3, NH), 7.32 (5H, m, ArH). δ_C ($CDCl_3$) 17.7, 19.2, 22.0, 22.8, 24.9, 30.9, 38.6, 43.0, 44.4, 51.7, 60.5, 67.0, 128.0, 128.1, 128.5, 136.3, 156.3, 170.6, 172.1.

*Methyl (2*S*,3′*S*)-(3-Benzyloxycarbonylamino-4-methylpentanoylamino)-4-methylpentanoate **10c***

To a solution of ester **1** (600 mg, 2.2 mmol) in THF/water (1 : 1 v/v, 50 mL) was added KOH (482 mg, 8.6 mmol), and the resultant mixture was heated at 40°C for 3 h. The reaction mixture was allowed to cool to room temperature, ethyl acetate (30 mL) was added, and the aqueous phase was acidified to pH 1 by the dropwise addition of

concentrated HCl. The aqueous phase was further extracted with ethyl acetate (3 × 30 mL), the combined organic fractions were dried over $MgSO_4$, and the solvent was removed under vacuum to give **9** as a white solid that was used without further purification. This material (250 mg) was allowed to react with L-leucine methyl ester (188 mg, 1.0 mmol) and BOP (460 mg, 1.0 mmol) in the manner described for **7a**. Workup and purification of the resultant residue through a small plug of silica (ethyl acetate) gave **10c** (382 mg, 100%) as a white solid (Found: 393.2387. $C_{21}H_{32}N_2O_5$ requires $[M+H]^{+\bullet}$ 393.2387). δ_H ($CDCl_3$) 0.91 (6H, d, *J* 4.4, 2 CH_3), 0.92 (6H, d, *J* 6.8, 2 CH_3), 1.50 (1H, m, CH), 1.60 (2H, m, CH_2), 1.85 (1H, m, CH), 2.47 (2H, m, CH_2), 3.70 (3H, s, CO_2Me), 3.72 (1H, m, β-Val-CH), 4.57 (1H, m, Leu α-CH), 5.06 (2H, m, CH_2), 5.40 (1H, d, *J* 9.3, NH), 6.23 (1H, d, *J* 7.3, NH), 7.32 (5H, m, ArH). δ_C ($CDCl_3$) 18.6, 19.4, 21.8, 22.8, 24.8, 31.8, 38.7, 41.3, 50.7, 52.3, 54.2, 66.7, 127.9, 128.0, 128.5, 136.5, 156.4, 170.4, 171.9.

*Methyl (3*S*,3′*S*)-3-(3-Benzyloxycarbonylamino-4-methylpentanoylamino)-5-methylhexanoate* ***10d***

To a mixture of acid **9** (150 mg, 0.6 mmol) (prepared as detailed above) and **4** (176 mg, 0.6 mmol) was added BOP (270 mg, 0.6 mmol) in the manner described for **7a**. Workup and purification of the resultant residue through a small plug of silica (ethyl acetate) gave **10d** (174 mg, 76%) as a white solid (Found: 407.2546. $C_{22}H_{34}N_2O_5$ requires $[M+H]^{+\bullet}$ 407.2546). δ_H ($CDCl_3$) 0.87 (6H, d, *J* 6.8, 2 CH_3), 0.90 (6H, d, *J* 6.8, 2 CH_3), 1.25 (1H, m, CH), 1.42 (1H, m, C*H*H), 1.44 (1H, m, CH*H*), 1.83 (1H, m, CH), 2.46 (4H, m, 2 CH_2), 3.64 (3H, s, CO_2Me), 3.72 (1H, m, β-Val-CH), 4.31 (1H, m, β-Leu-CH), 5.06 (2H, m, CH_2), 5.48 (1H, d, *J* 9.3, NH), 6.23 (1H, d, *J* 8.8, NH), 7.32 (5H, m, ArH). δ_C ($CDCl_3$) 18.6, 19.4, 22.0, 22.8, 24.9, 31.9, 38.8, 43.0, 44.3, 51.6, 54.2, 66.6, 127.9, 128.0, 128.4, 136.5, 156.4, 170.7, 172.2.

*(1*S*,1′*S*)-[1-(1-Formyl-3-methylbutylcarbamoyl)-2-methylpropyl]carbamic Acid Pyridin-3-ylmethyl Ester* ***12a***

Cbz-L-valine (1.25 g, 5.0 mmol) and L-leucine methyl ester (1.00 g, 6.0 mmol) were coupled with BOP (2.435 g, 6.0 mmol) in the manner described above for **7a**. Workup and purification of the resultant residue through a small plug of silica (ethyl acetate) gave **10a** (1.866 g, 99%) as a white solid that was not purified further. δ_H ($CDCl_3$) 0.92 (12H, m, 4 CH_3), 1.52 (1H, m, CH), 1.60 (2H, m, CH_2), 2.10 (1H, m, CH), 3.71 (3H, s, CO_2Me), 4.04 (1H, m, Val α-CH), 4.60 (1H, m, β-Leu-CH), 5.09 (2H, s, CH_2), 5.42 (1H, d, *J* 8.8, NH), 6.37 (1H, d, *J* 7.3, NH), 7.33 (5H, m, ArH).

A solution of **10a** (1.0 g, 2.6 mmol) in 33% (v/v) HBr in acetic acid (4 mL) was stirred vigorously at room temperature for 30 min. Diethyl ether (precooled to 0°C) was then added to afford a white precipitate. The mixture was stirred for a further 5 min and was then placed in an ice bath for 30 min. The precipitate was subsequently filtered off and rinsed with diethyl ether (100 mL). The remaining solid was added to dichloromethane (3 mL) and saturated aqueous $NaHCO_3$ (3 mL). The suspension was stirred at 0°C for 10 min, a solution of triphosgene (56 mg, 0.2 mmol) in toluene (0.5 mL) was added dropwise, and the mixture was stirred for an additional 2.5 h. The aqueous phase was separated and extracted with dichloromethane (3 × 15 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated under vacuum to give the crude isocyanate as a milky oil.

To a solution of this residue in freshly distilled dichloromethane (3 mL) was added dropwise 3-pyridylcarbinol (0.03 mL, 0.31 mmol), and the resultant mixture was stirred for 18 h. The solvent was then removed under vacuum and the crude product was purified by column chromatography (ethyl acetate/light petroleum, 4 : 1) to afford **11a** (59 mg, 52%) as a colourless oil. Compound **11a** (59 mg) was dissolved in freshly distilled dichloromethane (15 mL) under an atmosphere of argon and the resultant solution was cooled to −78°C. Diisobutyl aluminium hydride (0.135 mL, 0.8 mmol) was then added dropwise and the solution was stirred at −78°C for 3 h. Super-dry methanol (10 mL), precooled to −78°C, was then added dropwise and the reaction mixture was stirred for a further 25 min at −78°C. The cooling bath was removed and 10% aqueous HCl was added followed by ethyl acetate (20 mL). The organic phase was extracted with 10% HCl (3 × 20 mL) and the combined aqueous fractions were basified to pH 14 with 1 M aqueous NaOH and then extracted with ethyl acetate (3 × 20 mL). The combined organic fractions were washed with saturated aqueous $NaHCO_3$ (10 mL), saturated aqueous NaCl (10 mL), dried over $MgSO_4$, and concentrated under vacuum to give a cream oil. The crude product was purified by column chromatography (100% ethyl acetate) to give **12a** (23 mg, 41%) as a white solid (Found: 350.2080. $C_{18}H_{27}N_3O_4$ requires $[M+H]^{+\bullet}$ 350.2080). υ_{max}/cm^{-1} (KBr) 1717, 1661. δ_H ([D_6]acetone) 1.02 (3H, d, *J* 6.3, CH_3), 1.04 (3H, d, *J* 6.3, CH_3), 1.06 (3H, d, *J* 6.8, CH_3), 1.10 (3H, d, *J* 6.8, CH_3), 1.60–1.87 (3H, m, CH_2 and CH), 2.27 (1H, m, CH), 4.21 (1H, dd, *J* 6.3 and 8.8, Val α-CH), 4.45 (1H, m, Leu α-CH), 5.24 (2H, s, CH_2), 6.55 (1H, m, Val-NH), 7.48 (1H, dd, *J* 4.4 and 7.8, pyridyl-H), 7.74 (1H, m, Leu-NH), 7.89 (1H, d, *J* 7.8, pyridyl-H), 8.64 (1H, d, *J* 4.4, pyridyl-H), 8.73 (1H, s, pyridyl-H), 9.64 (1H, s, CHO). δ_C ([D_6]acetone) 17.5, 19.1, 21.0, 22.8, 24.5, 31.1, 37.0, 57.4, 60.5, 63.8, 123.6, 133.0, 135.6, 149.4, 149.5, 156.4, 171.9, 200.4.

*(1*S*,1′*S*)-{2-Methyl-1-[3-methyl-1-(2-oxoethyl)butylcarbamoyl]propyl}carbamic Acid Pyridin-3-ylmethyl Ester* ***12b***

A solution of **10b** (0.270 g, 0.7 mmol) in 33% (v/v) HBr in acetic acid (3 mL) was stirred vigorously at room temperature for 30 min before the acetic acid was removed under reduced pressure. The residue was dissolved in water (20 mL) and was washed with diethyl ether (20 mL). The aqueous phase was concentrated under vacuum to give an orange oily solid to which were added dichloromethane (5 mL) and saturated aqueous $NaHCO_3$ (5 mL). The suspension was allowed to react with a solution of triphosgene (0.113 g, 0.4 mmol) in toluene (1 mL) in the manner described above for **12a** to give the crude isocyanate as a milky oil. The residue was then dissolved in freshly distilled dichloromethane (6 mL), 3-pyridylcarbinol (0.061 mL, 0.6 mmol) was added dropwise, and the reaction mixture was stirred for 24 h. The solvent was removed and the crude product was purified by column chromatography (ethyl acetate/light petroleum, 4.5 : 1) to give **11b** (0.103 g, 43%) as a white solid. δ_H ([D_6]acetone) 1.00 (12H, m, 4 CH_3), 1.40 (1H, m, C*H*H), 1.60 (1H, m, CH*H*), 1.79 (1H, m, CH), 2.20 (1H, m, CH), 2.60 (2H, m, CH_2), 3.73 (3H, s, CO_2Me), 4.06 (1H, m, Val α-CH), 4.45 (1H, m, β-Leu-CH), 5.23 (2H, s, CH_2), 6.45 (1H, br s, NH), 7.23 (1H, br s, NH), 7.47 (1H, m, pyridyl-H), 7.89 (1H, d, *J* 7.3, pyridyl-H), 8.64 (1H, m, pyridyl-H), 8.73 (1H, s, pyridyl-H). δ_C ([D_6]acetone) 17.4, 19.0, 21.4, 22.9, 24.8, 31.1, 39.8, 43.6, 44.7, 51.0, 60.7, 63.8, 123.6, 135.6, 149.4, 149.6, 170.6, 171.5.

The above solid (100 mg) was reduced with DIBAL-H (0.211 mL, 1.2 mmol) in the manner described above for **12a**. The resultant crude product was purified by column chromatography (100% ethyl acetate) to give **12b** (56 mg, 60%) as a colourless oil (Found: 364.2239. $C_{19}H_{29}N_3O_4$ requires $[M+H]^{+\bullet}$ 364.2236). υ_{max}/cm^{-1} (KBr) 1720, 1649. δ_H ([D_6]acetone) 0.99 (3H, d, *J* 5.4, CH_3), 1.00 (3H, d, *J* 6.3, CH_3), 1.01 (3H, d, *J* 6.3, CH_3), 1.03 (3H, d, *J* 5.4, CH_3), 1.40 (1H, m, CH*H*), 1.65 (1H, m, CH*H*), 1.81 (1H, m, CH), 2.20 (1H, m, CH), 2.65 (2H, m, CH_2), 4.05 (1H, dd, *J* 6.3 and 8.8, Val α-CH), 4.59 (1H, m, β-Leu-CH), 5.24 (2H, s, CH_2), 6.52 (1H, m, Val-NH), 7.38 (1H, m, Leu-NH), 7.51 (1H, m, pyridyl-H), 7.91 (1H, d, *J* 7.3, pyridyl-H), 8.68 (1H, m, pyridyl-H), 8.76 (1H, s, pyridyl-H), 9.82 (1H, s, CHO). δ_C ([D_6]acetone) 17.5, 19.1, 21.3, 22.8, 24.7, 31.0, 43.1, 43.9, 49.8, 60.8, 63.8, 135.8, 149.2, 149.4, 156.3, 170.9, 201.0.

*(1′*S*,2*S*)-[1-(1-Formyl-3-methylbutylcarbamoyl)-3-methylbutyl]-carbamic Acid Pyridin-3-ylmethyl Ester* ***12c***

A solution of **10c** (0.366 g, 0.9 mmol) in 33% (v/v) HBr in acetic acid (4 mL) was allowed to react in the manner described above for **12b**. The resultant orange solid was dissolved in dichloromethane (5 mL) and saturated $NaHCO_3$ (5 mL), and was then allowed to react with a solution of triphosgene (0.160 g, 0.5 mmol) in toluene (1 mL) in the manner described above for **12a** to afford the crude isocyanate as a milky oil. The resultant oil was dissolved in freshly distilled dichloromethane (7 mL), 3-pyridylcarbinol (0.086 mL, 0.9 mmol) was added dropwise, and the reaction mixture was stirred for 24 h. The solvent was then removed under vacuum and the crude product was purified by column chromatography (ethyl acetate/light petroleum 4.5 : 1) to give **11c** (0.15 g,

41%) as a white solid. δ_H ([D$_6$]acetone) 1.00 (12H, m, 4 CH$_3$), 1.66 (2H, m, CH$_2$), 1.80 (1H, m, CH), 1.96 (1H, m, CH), 2.60 (2H, m, CH$_2$), 3.76 (3H, s, CO$_2$Me), 3.95 (1H, m, CH), 4.60 (1H, m, CH), 5.26 (2H, s, CH$_2$), 6.50 (1H, br s, NH), 7.57 (1H, br s, NH), 7.62 (1H, m, pyridyl-H), 8.05 (1H, d, *J* 7.8, pyridyl-H), 8.71 (1H, m, pyridyl-H), 8.80 (1H, s, pyridyl-H).

The above solid (30 mg) was dissolved in freshly distilled dichloromethane (7.5 mL) and was reduced with DIBAL-H (0.063 mL, 0.4 mmol) in the manner described for **12a**. The crude product was then purified by column chromatography (100% ethyl acetate) to give **12c** (12 mg, 43%) as a white solid (Found: 364.2229. $C_{19}H_{29}O_4N_3$ requires $[M+H]^{+\bullet}$ 364.2236). υ_{max}/cm^{-1} (KBr) 1720, 1655. δ_H ([D$_6$]acetone) 0.99 (12H, m, 4 CH$_3$), 1.42 (1H, m, C*H*H), 1.60 (1H, m, C*H*H), 1.78 (1H, m, CH), 1.85 (1H, m, CH), 2.50 (2H, m, CH$_2$), 3.85 (1H, m, CH), 4.25 (1H, m, CH), 5.18 (2H, s, CH$_2$), 6.45 (1H, m, NH), 7.58 (2H, m, pyridyl-H and NH), 7.98 (1H, d, *J* 7.3, pyridyl-H), 8.60 (1H, m, pyridyl-H), 8.65 (1H, s, pyridyl-H), 9.45 (1H, s, CHO).

(1′S,2S)-{(2-Methyl-1-[3-methyl-1-(2-oxoethyl)butylcarbamoyl]-3-methylbutyl}carbamic Acid Pyridin-3-ylmethyl Ester ***12d***

A solution of **10d** (0.161 g, 0.4 mmol) in 33% (v/v) HBr in acetic acid (1.2 mL) was allowed to react in the manner described above for **12b** to afford an oil. 2,2-Dimethoxypropane (7.3 mL), concentrated HCl (0.6 mL), and then methanol (2 mL) were added to the residue, the reaction mixture was heated at reflux for 2 h, then stirred at room temperature for 18 h. The solvent was removed under vacuum and the residue was washed several times with pentane. The resultant oil was dissolved in dichloromethane (4 mL) and saturated aqueous NaHCO$_3$ (4 mL), and was allowed to react with a solution of triphosgene (0.111 g, 0.4 mmol) in toluene (1 mL) in the manner described above for **12a** to give the crude isocyanate as a milky oil. The resultant oil was dissolved in freshly distilled dichloromethane (6 mL), 3-pyridylcarbinol (0.06 mL, 0.6 mmol) was added dropwise, and the reaction mixture was stirred for 24 h. The solvent was removed under vacuum and the crude product was purified by column chromatography (ethyl acetate/light petroleum, 4.5 : 1) to give **11d** (0.034 g, 14% over three steps) as a white solid. δ_H ([D$_6$]acetone) 1.00 (12H, m, 4 CH$_3$), 1.40 (1H, m, C*H*H), 1.60 (1H, m, C*H*H), 1.80 (1H, m, CH), 1.96 (1H, m, CH), 2.51 (4H, m, 2 CH$_2$), 3.71 (3H, s, CO$_2$Me), 3.94 (1H, m, CH), 4.43 (1H, m, CH), 5.21 (2H, m, CH$_2$), 6.56 (1H, d, *J* 9.3, NH), 7.23 (1H, d, *J* 8.3, NH), 7.46 (1H, m, pyridyl-H), 7.88 (1H, d, *J* 7.8, pyridyl-H), 8.64 (1H, m, pyridyl-H), 8.72 (1H, s, pyridyl-H).

The above solid (27 mg) was dissolved in freshly distilled dichloromethane (7 mL) and was reduced with DIBAL-H (0.055 mL, 0.31 mmol) in the manner described for **12a**. The crude product was purified by column chromatography (100% ethyl acetate) to give **12d** (8 mg, 31%) as a white solid (Found: 378.2393. $C_{20}H_{31}O_4N_3$ requires $[M+H]^{+\bullet}$ 378.2393). υ_{max}/cm^{-1} (KBr) 1717, 1649. δ_H ([D$_6$]acetone) 1.00 (12H, m, 4 CH$_3$), 1.40 (1H, m, CH*H*), 1.60 (1H, m, CH*H*), 1.80 (1H, m, CH), 1.93 (1H, m, CH), 2.50 (2H, m, CH$_2$), 2.60 (2H, m, CH$_2$), 3.94 (1H, m, CH), 4.57 (1H, m, CH), 5.22 (2H, m, CH$_2$), 6.50 (1H, d, *J* 8.8, Val-NH), 7.26 (1H, d, *J* 7.8, Leu-NH), 7.49 (1H, br s, pyridyl-H), 7.90 (1H, d, *J* 7.3, pyridyl-H), 8.66 (1H, m, pyridyl-H), 8.75 (1H, s, pyridyl-H), 9.78 (1H, s, CHO). δ_C ([D$_6$]acetone) 17.9, 19.0, 21.4, 22.9, 24.8, 32.1, 38.5, 43.9, 44.0, 49.9, 54.4, 63.5, 124.0, 135.6, 149.3, 149.4, 156.0, 170.5, 201.1.

Assay for m-Calpain Inhibition[29]

Inhibitor effectiveness was determined by the ability of the compound to reduce the activity of m-calpain using BODIPY-FL casein (Molecular Probes E-6638) as a substrate. The m-calpain was partially purified from sheep lung by ion-exchange chromatography and was diluted to give a linear response over the course of the assay. The substrate solution (0.0005% BODIPY-FL casein in 10 mM tris-HCl, pH 7.5 containing 10 mM $CaCl_2$, 0.1 mM NaN_3, and 0.1% mercaptoethanol) was prepared fresh each day. The calpain assays were performed in 96-well black ELISA plates. The calpain control assays contained 50 μL m-calpain in sample buffer (20 mM tris-HCl, pH 7.5 containing 1 mM EDTA, 1 mM EGTA, and 2 mM dithiothreitol) and 50 μL of sample buffer, while 100 μL of substrate solution was used to start the reaction. The progress of the reaction at 25°C was followed for 10 min in a BMG Fluorostar with excitation of 485 nm and emission of 530 nm. For the inhibitor assays the sample buffer was replaced by 50 μL of inhibitor diluted in the sample buffer. The percentage inhibition was determined as 100 times the activity with inhibitor present divided by the activity of the control assay.

Lens Culture

Lenses (24) were dissected from the eyes of sheep collected at the local abattoir. After an initial incubation to sterilize the lenses, each lens was maintained at 35°C in 10 mL of Eagle's minimum essential medium for up to 10 days. After 48 h, 100 μM of inhibitors **8a** and **12a** were added to selected lenses (six for each inhibitor) and 24 h later 5 μM ionomycin was added to the lenses with inhibitor and to six other lenses. All lenses were photographed every 48 h.

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