Aminodeoxybestatin and *epi*-Aminodeoxybestatin: Stereospecific Synthesis and Aminopeptidase Inhibition

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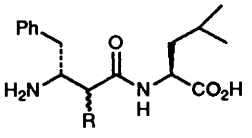
The synthesis of aminodeoxybestatin and *epi*-aminodeoxybestatin [(2*S*,3*R*)- and (2*R*,3*R*)-2,3-diamino-4-phenylbutanoyl-L-leucine; (2*S*,3*R*)- and (2*R*,3*R*)-DAPBA-L-Leu)], bestatin and *epi*-bestatin analogues, respectively, in which the hydroxy group has been replaced with an amino group, is described by two different methods. The first one involves the synthesis of bis-(*N*-Z)-DAPBA, by homologation of *N*-Z-phenylalanine, *via* a modified Strecker synthesis followed by subsequent coupling with the methyl ester of L-leucine and removal of the protecting groups. Following this procedure, 25% racemization at the C-3 centre of the DAPBA derivatives took place during the homologation reaction. The second method involves the stereospecific S_N2 nucleophilic substitution of the 2-hydroxy group of (2*R*,3*R*)- and (2*S*,3*R*)-3-(benzyloxycarbonyl)amino-2-hydroxy-4-phenylbutanoyl-L-leucine methyl esters [(2*R*,3*R*)- and (2*S*,3*R*)-*N*-Z-AHPBA-L-Leu-OMe], and subsequent saponification, azido reduction and removal of the *N*-Z-protecting group. Replacement of the hydroxy group of bestatin and *epi*-bestatin with an amino group results in a decrease in their aminopeptidase (AP-B, AP-M and Leu-AP)-inhibitory potencies.

The dipeptide bestatin, *N*-[(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine [**1a** (2*S*,3*R*)-APHPBA-L-Leu], isolated from *Streptomyces olivoreticuli*,[1] and in clinical use in Japan,[2] is a potent inhibitor of aminopeptidases (APs), namely cytosolic leucine aminopeptidase[3,4] (Leu-AP; EC 3.4.11.1), microsomal or membrane-bound leucine aminopeptidase[5] (AP-M; EC 3.4.11.2) and the cell surface-associated aminopeptidase B[1,3,6] (AP-B; EC 3.4.11.6). These inhibitory properties are related to the biological activities of bestatin[7] as immunomodifier,[8–10] antitumour agent[11–13] or analgesic.[14,15] Structure-modification studies on bestatin have established that the presence and stereochemistry of the (2*S*)-hydroxy group in AHPBA are critical for tight binding to APs.[3,16] The structural relationship between the sp^3 geometry of the C-2 hydroxy group of the AHPBA residue and the probable tetrahedral intermediate for amide bond hydrolysis led to the idea that bestatin may function as a transition-state analogue inhibitor.[6,17,18] Taking into account the structure-modification studies on bestatin, and that Leu-AP and AP-M are zinc metalloproteases[4,19,20] and that AP-B has also been proposed to be a zinc metalloenzyme,[3,6] two mechanisms of binding of this dipeptide to APs have been proposed. According to these mechanisms, the C-2 hydroxy group and other functionality (amino[16] or amide carbonyl[21]) are implicated in bidentate binding to the active-site zinc. In this sense, the replacement of the C-2 hydroxy group of bestatin by an amino group might help to give a better understanding about the importance of this group in mimicking the tetrahedral transition-state formed during substrate hydrolysis or in binding of the zinc in the active site. With this aim the stereospecific synthesis of aminodeoxybestatin **2a** and *epi*-aminodeoxybestatin **2b** and their inhibitory potencies against AP-B, AP-M and Leu-AP are described.



1a R = (2*S*)-OH
1b R = (2*R*)-OH
2a R = (2*S*)-NH_2
2b R = (2*R*)-NH_2

Results and Discussion

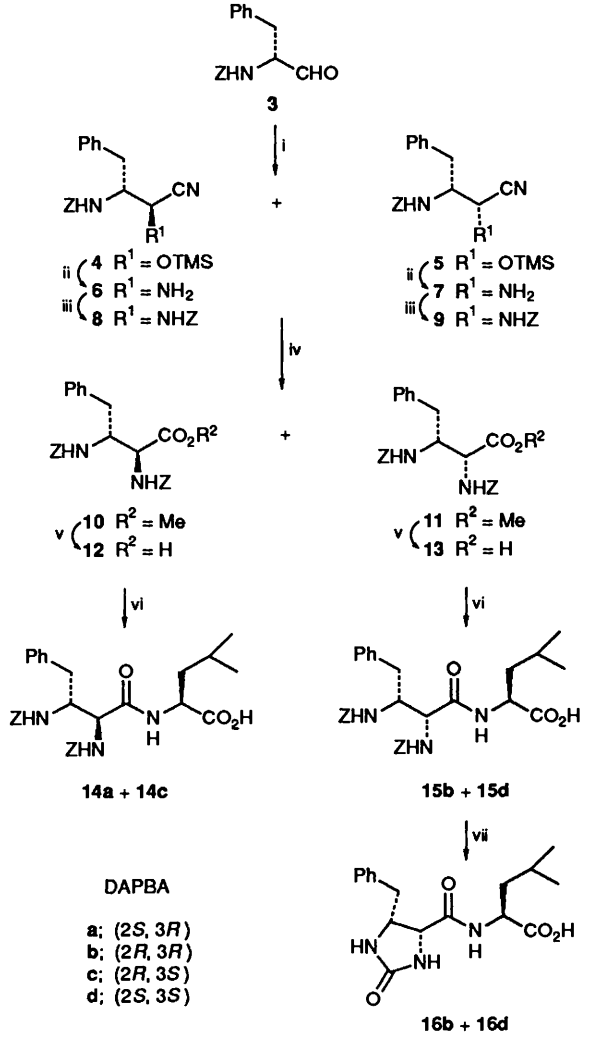
Synthesis.—Aminodeoxybestatin **2a** and *epi*-aminodeoxybestatin **2b** were firstly prepared following the synthetic route indicated in Scheme 1. This route essentially involves the homologation of *Z*-protected D-phenylalanine by a modified Strecker synthesis[22] and subsequent coupling of the resulting diastereoisomeric 2,3-diamino-4-phenylbutanoic acid (DAPBA) derivatives with the methyl ester of L-leucine. Thus, reaction of *N*-Z-D-phenylalaninal **3** with trimethylsilyl cyanide (TMSCN) gave a mixture of the *O*-(trimethylsilyl) cyanohydrins[23] **4** and **5** which was transformed *in situ* into the *threo*- and *erythro* 2,3-bis(benzyloxycarbonylamino) nitriles **8** and **9** by refluxing in methanolic ammonia for 10 h followed by Z-protection of the 2-amino group of the resulting 2-amino-3-(benzyloxycarbonylamino)butyronitrile derivatives **6** and **7**. Acid methanolysis (HCl–MeOH) of the mixture of diastereoisomeric amino nitriles **8** and **9** provided the corresponding methyl esters **10** and **11** in a 1:2 ratio, which, after chromatographic separation, were saponified to the carboxylic acids **12** and **13**, respectively. These acids were coupled with the methyl ester of L-leucine, *via* the dicyclohexylcarbodiimide (DCC) method in the presence of 1-hydroxybenzotriazole (HOBt),[24] to give the protected dipeptides **14** and **15**. The ^1H NMR spectra of these dipeptides revealed that each one was a 3:1 mixture of two diastereoisomers **14a** + **14c** and **15b** + **15d** with the same relative disposition at the C-2 and C-3 positions of the DAPBA residue. This disposition was established by conversion of the mixture **15b** + **15d** into the corresponding 2-oxoimidazolidines **16b** + **16c** by Z-deprotection and subsequent reaction with bis(trichloromethyl) carbonate. Thus, the coupling constants $J_{4,5}$ in the spectrum of the mixture of **16b** + **16d** were 9 and 8.9 Hz, respectively, consistent, in both cases, with a relative 4-H,5-H *cis* disposition. Racemization at C-3 of the DAPBA residue can be attributed to the easy racemization of the α-amino aldehyde **3**, in equilibrium with cyanohydrins **4** and **5**, during the slow transformation of cyanohydrins **4** and **5** into the 2-amino derivatives **6** and **7** in the basic ammonia medium.[25]

In view of the lack of stereoselectivity of the route

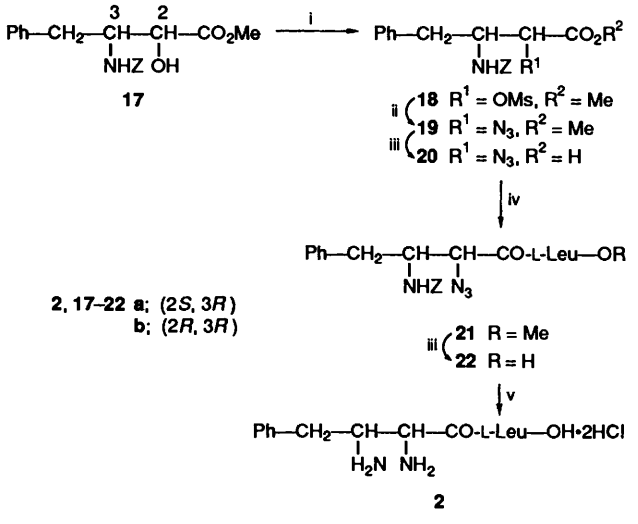
Table 1 Analytical and relevant spectroscopic data of the 3-amino-4-phenylbutanoic acid derivatives **18–20**

Compd.	Yield (%)	Formula	Found (%) (Required) C	H	N	Relevant ^{1}H NMR data (δ)[a] 2-H (d)	3-H (m)	$J_{2,3}$	4-H_2 (dd)	3-NH (d)	OMe (s)
18a	85	$C_{20}H_{23}NO_7S$	57.3 (57.0)	5.1 (5.5)	3.6 (3.3)	5.05	4.52	3	2.77, 2.91	5.01	3.56
18b	90	$C_{20}H_{23}NO_7S$	56.9 (57.0)	5.6 (5.5)	3.2 (3.3)	5.27	4.52	3	2.75, 2.82	5.03	3.58
19a	54	$C_{19}H_{20}N_4O_4$	61.8 (61.95)	5.5 (5.5)	15.4 (15.2)	4.02	4.41	3	2.75, 2.89	4.95	3.66
19b	60	$C_{19}H_{20}N_4O_4$	62.0 (61.95)	5.6 (5.5)	15.3 (15.2)	4.25	4.43	4	2.71, 2.75	5.02	3.63
20a	72	$C_{18}H_{18}N_4O_4$	60.8 (61.0)	5.4 (5.1)	15.7 (15.8)	4.01	4.50	2	2.75, 2.87	5.15	
20b	90	$C_{18}H_{18}N_4O_4$	60.9 (61.0)	5.4 (5.1)	15.7 (15.8)	4.31	4.43	4	2.84, 2.79	5.10	

[a] In $CDCl_3$.



Scheme 1 *Reagents:* i, TMSCN; ii, NH_3–MeOH; iii, Z-Cl; iv, HCl–MeOH; v, NaOH; vi, L-Leu-OMe·HCl, Et_3N, DCC, HOBt; vii, H_2, Pd/C; bis(trichlomethyl) carbonate



Scheme 2 *Reagents:* i, MsCl, DMAP; ii, LiN_3; iii, NaOH; iv, L-Leu-OMe·HCl, Et_3N, DDC, HOBt; v, H_2, Pd/C

shown in Scheme 1, an alternative route was devised which allowed us to synthesize stereospecifically the target compounds, **2a** and **2b** (Scheme 2). Thus, starting from the appropriate protected (2*R*,3*R*)- or (2*S*,3*R*)-AHPBA stereoisomer *N*,*C*-protected **17b** or **17a**, stereoselectively prepared from *N*-Z-D-Phe,[23,26] the (3*R*)-2-azido-3-(benzyloxycarbonyl-amino)-4-phenylbutanoic acid methyl esters **19a** and **19b** were stereospecifically obtained by an S_N2 nucleophilic substitution in the mesyl derivatives **18b** and **18a** with lithium azide. At this point, it is important to note that, when mesylation was carried out in the presence of 4-dimethylaminopyridine (DMAP) or pyridine, no racemization was observed, while when triethylamine was used, 25% racemization at C-2 took place as determined by measurement of the integrals of the two singlets corresponding to the OMe groups in the ^{1}H NMR spectra of the mesyl derivatives **18a** and **18b** (Table 1). Saponification of esters **19a** and **19b** and coupling of the resulting acids **20a** and **20b** with L-Leu-OMe by the DCC method afforded the fully protected dipeptide analogues **21a** and **21b**, which were partially deprotected to the corresponding acids **22a** and **22b**. Finally, removal of the Z-group and simultaneous azido reduction by hydrogenation in the presence of 10% Pd/C led to aminodeoxybestatin **2a** or *epi*-aminodeoxybestatin **2b**, respectively, in 30% overall yield from hydroxy ester **17b** or **17a**.

Aminopeptidase Inhibition.—The inhibitory potencies of aminodeoxybestatin **2a** and *epi*-aminodeoxybestatin **2b** against AP-B (associated with the surface of murine L cells), AP-M and Leu-AP are shown in Table 2. For comparative purposes bestatin **1a** was also tested and the reported AP-B and Leu-AP inhibition values for *epi*-bestatin **1b** are also included in Table 2. These results indicate that compound **2a** is essentially equi-

Table 2 Inhibition of AP-B, AP-M and Leu-AP by **1a, b** and **2a, b**

Compound	$IC_{50}(10^{-6}$ mol $dm^{-3})^a$		
	AP-B	AP-M	Leu-AP
1a	6.5	19.5	0.032
1b[b]	320		0.24
2a	698	39.8	53.2
2b	>1000	983	7.2

[a] Values are the mean of 4–5 experiments with 3–5 different concentrations of inhibitor. Standard errors were less than 10% of the mean. [b] Ref. 3.

potent to bestatin as an AP-M inhibitor, but this natural dipeptide is approximately 100- and 1000-fold better as an inhibitor of AP-B and Leu-AP, respectively. Except for Leu-AP, the (2*S*)-stereochemistry is preferred for inhibition of AP-B and AP-M by the 2-amino substituted derivatives **2a** and **2b** as happens with the hydroxy analogues **1a** and **1b**. It can be concluded that, in a similar way to statine-containing dipeptides [statine is (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid], also considered as transition-state analogue inhibitors, replacement of the hydroxy group of bestatin and *epi*-bestatin with an amino function results in a decrease or in a loss of effectivity in AP inhibition.[27,28]

Experimental

Chemistry.—M.p.s were taken on a Reichert-Jung Kofler micro hot stage apparatus, and are uncorrected. Elemental analyses were obtained using a Heraeus CHN-O-RAPID instrument. ^{1}H NMR spectra were recorded with a Varian XL-300 spectrometer (300 MHz), with Me_4Si as internal standard. *J*-Values are given in Hz. Optical rotations were measured on a Perkin–Elmer polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

Analytical TLC was performed on aluminium sheets coated with a 0.2 mm layer of silica gel 60 F_{254}, obtained from Merck. Silica gel 60 (230–400 mesh; Merck) was used for flash chromatography.

Synthesis of a Mixture of threo- *and* erythro-2,3-*Bis*(*benzyloxycarbonylamino*)-4-*phenylbutyronitrile* **8** *and* **9**.—TMSCN (2.5 cm^3, 20 mmol) was added to a solution of *N*-Z-D-phenylalaninal **3** (2.83 g, 10 mmol), freshly prepared by the method of Fehrentz and Castro,[29] in dry dichloromethane (60 cm^3), and the solution was stirred at room temperature for 5 h. After evaporation, the crude mixture of *threo*- and *erythro*-*O*-(trimethylsilyl) cyanohydrins **4** and **5** was dissolved in a saturated solution of ammonia in MeOH (100 cm^3), stirred at 65 °C for 5 h and evaporated to dryness. The residue of crude amino nitriles **6** and **7** was dissolved in dry dichloromethane (75 cm^3), the solution was cooled to 0 °C, and then propylene oxide (13 cm^3) and benzyl chloroformate (1.6 cm^3, 11 mmol) were added. After the mixture had been stirred at room temperature for 12 h and then evaporated, the residue was purified by flash chromatography with (3:1) hexane–ethyl acetate to afford a 1:2 mixture of the threo- and erythro-2,3-*diamino nitriles* **8** and **9**, respectively, as an oil (3.54 g, 80%) (Found: C, 70.6; H, 5.9; N, 9.2. $C_{26}H_{25}N_3O_4$ requires C, 70.4; H, 5.6; N, 9.5%); δ_H($CDCl_3$) 2.90–3.10 (2 H, m, 4-H_2), 4.30 (1 H, m, 3-H), 4.60 (1 H, m, 2-H), 5.08 [2 H, m, OCH_2 (Z)], 5.13 [2 H, s, OCH_2 (Z)], 6.20 (1 H, m, N*H*Z), 7.20 (1 H, m, N*H*Z) and 7.21–7.36 (15 H, m, Ph).

Synthesis of threo- *and* erythro-2,3-*Bis*(*benzyloxycarbonylamino*)-4-*phenylbutanoic Acid Methyl Esters* **10** *and* **11**.—The (1:2) mixture of *erythro*- and *threo*-2,3-diamino nitrile derivatives **8** and **9** (3.10 g, 7 mmol) was dissolved in a dry, cooled (0 °C), (3:1) dichloromethane–MeOH mixture (100 cm^3), previously saturated with HCl. This solution was stirred at below 5 °C for 24 h, and then was treated with ice–water (20 cm^3), while the temperature was kept below 10 °C, and the mixture was stirred at this temperature for 24 h. The reaction mixture was concentrated (≈20 cm^3), and extracted with dichloromethane (3 × 50 cm^3). The combined extracts were washed successively with water (30 cm^3) and brine (30 cm^3), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography with (7:1) hexane–ethyl acetate as eluent, to give the corresponding methyl esters of *N*,*N*′-di(Z)-DAPBA, **10** and **11**.

threo-2,3-*Bis*(*benzyloxycarbonylamino*)-4-*phenylbutanoic acid methyl ester* **10** (0.70 g, 21%), m.p. 99–101 °C (Found: C, 68.1; H, 6.0; N, 5.6. $C_{27}H_{28}N_2O_6$ requires C, 68.05; H, 5.9; N, 5.9%); δ_H($CDCl_3$) 2.72 (1 H, dd, *J* 9 and 14, 4-H), 2.91 (1 H, dd, *J* 5 and 14, 4-H′), 3.64 (3 H, s, OMe), 4.34 (1 H, m, 3-H), 4.40 (1 H, dd, *J* 3 and 8, 2-H), 4.83 (1 H, d, *J* 8, N*H*Z), 4.91 [2 H, s, OCH_2 (Z)], 5.06 [2 H, s, OCH_2 (Z)], 5.70 (1 H, d, *J* 8, N*H*Z) and 7.00–7.40 (15 H, m, Ph).

erythro-2,3-*Bis*(*benzyloxycarbonylamino*)-4-*phenylbutanoic acid methyl ester* **11** (1.36 g, 41%), m.p. 142–145 °C (Found: C, 68.3; H, 5.7; N, 6.0%); δ_H($CDCl_3$) 2.76 (2 H, m, 4-H_2), 3.55 (3 H, s, OMe), 4.34 (1 H, m, 3-H), 4.73 (1 H, dd, *J* 3 and 8, 2-H), 4.99 [2 H, s, OCH_2 (Z)], 5.04 [2 H, s, OCH_2 (Z)], 5.39 (1 H, d, *J* 8, N*H*Z), 5.85 (1 H, d, *J* 8, N*H*Z) and 7.20–7.50 (15 H, m, Ph).

Synthesis of threo *and* erythro-2,3-*Bis*(*benzyloxycarbonylamino*)-4-*phenylbutanoic Acid* **12** *and* **13**.—1 Mol dm^{-3} NaOH (2.2 cm^3, 2.2 mmol) was added to a solution of a methyl ester **10** or **11** (0.95 g, 2 mmol) in (1:1) 1,4-dioxane–water (60 cm^3). After being stirred for 12 h at room temperature, the reaction mixture was concentrated (≈25 cm^3), washed with dichloromethane (3 × 20 cm^3), and the aqueous phase was acidified to pH 3–4 with Dowex 50W-X4 resin. The resin was filtered off, and washed with ethyl acetate (20 cm^3). The aqueous phase was extracted with ethyl acetate (3 × 20 cm^3), and the combined organic phases were dried over Na_2SO_4 and evaporated to dryness, to give the corresponding *N*,*N*′-di(Z)-DAPBA **12** or **13**, respectively.

threo-2,3-*Bis*(*benzyloxycarbonylamino*)-4-*phenylbutanoic acid* **12** (0.64 g, 70%) (Found: C, 67.8; H, 5.6; N, 6.4. $C_{26}H_{26}N_2O_6$ requires C, 67.5; H, 5.7; N, 6.1%); δ_H[$(CD_3)_2SO$] 2.74 (2 H, m, 4-H_2), 4.08 (1 H, dd, *J* 3 and 9, 2-H), 4.32 (1 H, m, 3-H), 4.92 and 4.98 [2 H, 2 d, *J* 12.2, OCH_2 (Z)], 5.10 [2 H, s, OCH_2 (Z)] and 7.10–7.50 (15 H, m, Ph).

erythro-2,3-*Bis*(*benzyloxycarbonylamino*)-4-*phenylbutanoic acid* **13** (0.69 g, 75%) (Found: C, 67.7; H, 5.8; N, 6.2%); δ_H[$(CD_3)_2SO$] 2.71 (2 H, m, 4-H_2), 4.09 (1 H, m, 3-H), 4.30 (1 H, dd, *J* 6.5 and 9, 2-H), 4.92 [2 H, s, OCH_2 (Z)], 5.04 and 5.10 [2 H, 2 d, *J* 12.5, OCH_2 (Z)] and 7.10–7.50 (15 H, m, Ph).

Synthesis of the Dipeptides N-[threo- *and* erythro-2,3-*Bis*-(*benzyloxycarbonylamino*)-4-*phenylbutanoyl*]-L-*leucine Methyl Esters* **14** *and* **15**.—To a solution of the *N*,*N*′-di(Z)-DAPBA **12** or **13** (0.46 g, 1 mmol) and L-Leu methyl ester hydrochloride (0.22 g, 1.3 mmol) in dry tetrahydrofuran (THF) (10 cm^3) at 0 °C were added HOBt (0.18 g, 1.3 mmol) and triethylamine (0.13 cm^3, 1 mmol). The mixture was stirred at 0 °C for 30 min, then a solution of DCC (0.21 g 1 mmol) in dry dichloromethane (10 cm^3) was added, and the mixture was stirred at room temperature for 24 h. The dicyclohexylurea was then filtered off, and, after removal of the solvents under reduced pressure, the residue was purified by flash chromatography with (3:1) hexane–ethyl acetate to afford the corresponding dipeptide

methyl ester **14** or **15** (0.41 g, 70%). The ^{1}H NMR spectra of products **14** and **15** showed that both were a (3:1) mixture of diastereoisomers, **14a** + **14c** and **15b** + **15d**, respectively.

(3:1) *Mixture of* (2S,3R)- *and* (2R, 3S)-N,N′-*Di*(*Z*)-*DAPBA*-L-*Leu-OMe* **14a** + **14c** (Found: C, 67.0; H, 6.8; N, 7.1. $C_{33}H_{39}N_3O_7$ requires C, 67.2; H, 6.8; N, 7.1%); $\delta_H(CDCl_3)$ 0.90 [6 H, m, Me (Leu)], 1.00–1.80 [3 H, m, CH_2CH (Leu)], 2.81–2.98 [2 H, m, 4-H_2 (DAPBA)], 3.74 and 3.82 (3 H, 2 s, OMe), 4.27–4.38 [1 H, m, 3-H (DAPBA)], 4.46–4.55 [1 H, m, 2-H (DAPBA)], 4.63–4.70 [1 H, m, α-H (Leu)], 5.03–5.20 [4 H, m, OCH_2 (Z)], 5.39 and 5.50 (1 H, 2 d, *J* 7, NH), 6.40 and 6.50 (1 H, 2 d, *J* 7, NH), 6.65 and 6.80 [1 H, 2 d, *J* 8, NH (Leu)] and 7.10–7.50 (15 H, m, Ph).

(3:1) *Mixture of* (2R,3R)- *and* (2S,3S)-N,N′-*Di*(*Z*)-*DAPBA*-L-*Leu-OMe* **15b** + **15d** (Found: C, 67.3; H, 6.5; N, 7.3%); $\delta_H(CDCl_3)$ 0.90 [6 H, m, Me (Leu)], 1.00–1.70 [3 H, m, CH_2CH (Leu)], 2.80–3.00 [2 H, m, 4-H_2 (DAPBA)], 3.66 and 3.79 (3 H, 2 s, OMe), 4.19 [2 H, m, 2- and 3-H (DAPBA)], 4.51–4.55 [1 H, m, α-H (Leu)], 5.06–5.09 [4 H, m, OCH_2 (Z)], 5.80, 5.81 and 5.88 (2 H, 3 d, *J* 8, NHZ), 6.60 [1 H, d, *J* 9, NH (Leu)] and 7.20–7.70 (15 H, m, Ph).

Synthesis of a (3:1) *Mixture of* N-[(4R,5R)- *and* (4S,5S)-5-*Benzyl*-2-*oxoimidazolidin*-4-*yl*)*carbonyl*]-L-*leucine Methyl Esters* **16b** + **16d**.—A solution of the diastereoisomeric (3:1) mixture of dipeptide methyl esters **15b** + **15d** (0.23 g, 0.4 mmol) in a 0.1 mol dm^{-3} HCl solution in MeOH (10 cm^3) was hydrogenated in the presence of 10% Pd/C (20 mg), at 1 atm of H_2 pressure, and at room temperature for 3 h. The catalyst was filtered off, washed with MeOH (5 cm^3), and the solvent was removed under reduced pressure. The residue was taken up in dry THF (7 cm^3), and to this suspension, cooled at 0 °C, were added bis(trichloromethyl) carbonate (61 mg, 0.2 mmol) and triethylamine (0.28 cm^3, 2 mmol). The reaction mixture was stirred at 0 °C for 1 h and was then evaporated. The residue was dissolved in ethyl acetate (10 cm^3), and the solution was washed successively with water (5 cm^3) and brine (5 cm^3), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography with (3:1) hexane–ethyl acetate to afford a (3:1) *mixture* of the 2-oxoimidazolidines **16b** and **16d** (83 mg, 60%) (Found: C, 62.1; H, 7.3; N, 11.95. $C_{18}H_{25}N_3O_4$ requires C, 62.25; H, 7.2; N, 12.1%); $\delta_H[(CD_3)_2SO]$ 0.80–0.90 [6 H, m, Me (Leu)], 1.20–170 [3 H, m, CH_2CH (Leu)], 2.40–3.00 (2 H, m, 5-H_2), 3.66 and 3.78 (3 H, 2 s, OMe), 4.01–4.10 (1 H, m, 5-H), 4.28 and 4.29 (1 H, 2 d, *J* 8.9 and 9, 4-H), 4.52 [1 H, m, α-H (Leu)], 6.10 and 6.20 (1 H, 2 s, NH), 6.50 and 6.60 (1 H, 2 s, NH), 7.10–7.40 (5 H, m, Ph) and 8.22 and 8.29 [1 H, 2 d, *J* 8, NH (Leu)].

Synthesis of (2S,3R)- *and* (2R,3R)-3-(*Benzyloxycarbonylamino*)-2-*methylsulfonyloxy*-4-*phenylbutanoic Acid Methyl Esters* **18a** *and* **18b**.—Methanesulfonyl chloride (4 cm^3, 50 mmol) was added to a solution of the corresponding (2*S*,3*R*)-[23] or (2*R*,3*R*)-*N*-Z-3-amino-2-hydroxy-4-phenylbutanoic acid methyl ester[26] (**17a** or **17b**) (3.40 g, 10 mmol) and DMAP (6.04 g, 50 mmol) in dichloromethane (70 cm^3) at 0 °C. After being stirred at this temperature for 4 days, the solution was washed successively with water (20 cm^3) and brine (20 cm^3), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography with (5:1) hexane–ethyl acetate as eluent to yield the title methanesulfonate **18a** or **18b** as a foam, whose analytical and spectroscopic data are summarized in Table 1.

Synthesis of (2S,3R)- *and* (2R,3R)-2-*Azido*-3-(*benzyloxycarbonylamino*)-4-*phenylbutanoic Acid Methyl Esters* **19a** *and* **19b**.—Lithium azide (1.10 g, 23 mmol) was added to a solution of the (2*R*,3*R*)- or (2*S*,3*R*)-mesyl derivative **18b** or **18a** (3.30 g, 8 mmol) in *N*,*N*-dimethylformamide (40 cm^3). This solution was stirred at 50 °C for 24 h, and then the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (100 cm^3), and the solution was washed successively with water (20 cm^3) and brine (20 cm^3), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography with (5:1) hexane–ethyl acetate to afford the azide **19a** or **19b** whose analytical and spectroscopic data are listed in Table 1.

Synthesis of (2S,3R)- *and* (2R,3R)-2-*Azido*-3-(*benzyloxycarbonylamino*)-4-*phenylbutanoic Acids* **20a** *and* **20b**.—1 Mol dm^{-3} NaOH (5.4 cm^3, 5.4 mmol) was added to a solution of a methyl ester **19a** or **19b** (2.00 g, 5.4 mmol) in (1:1) 1,4-dioxane–water (100 cm^3). After being stirred for 12 h at room temperature, the reaction mixture was concentrated (≈30 cm^3), washed with dichloromethane (3 × 20 cm^3), and the aqueous phase was acidified to pH 3–4 with Dowex 50W-X4 resin. The resin was filtered off and washed with ethyl acetate (20 cm^3). The aqueous phase was extracted with ethyl acetate (3 × 20 cm^3), and the combined organic phases were dried over Na_2SO_4 and evaporated to dryness, to give the corresponding acid **20a** or **20b** as a foam whose analytical and spectroscopic data are shown in Table 1.

Synthesis of the Dipeptides N-[(2S,3R)- *and* (2R,3R)-2-*Azido*-3-(*benzyloxycarbonylamino*)-4-*phenylbutanoyl*]-L-*leucine Methyl Esters* **21a** *and* **21b**.—To a solution of a 2-azido-3-aminobutanoic acid derivative **20a** or **20b** (1.60 g, 4.5 mmol) and L-Leu methyl ester hydrochloride (0.90 g, 5.4 mmol) in dry THF (45 cm^3) at 0 °C were added HOBt (0.81 g, 5.4 mmol) and triethylamine (0.58 cm^3, 4.5 mmol). The mixture was stirred at this temperature for 30 min, then a solution of DCC (0.93 g, 4.5 mmol) in dry dichloromethane (45 cm^3) was added, and the reaction mixture was stirred at room temperature for 24 h. The dicyclohexylurea was filtered off, the solvents were removed, and the residue was purified by flash chromatography with (6:1) hexane–ethyl acetate to afford the corresponding dipeptide methyl ester **21a** or **21b** whose analytical and spectroscopic data are listed in Table 3.

Synthesis of N-[(2S,3R)- *and* (2R,3R)-2-*Azido*-3-(*benzyloxycarbonylamino*)-4-*phenylbutanoyl*]-L-*leucine* **22a** *and* **22b**.—1 Mol dm^{-3} NaOH (1.6 cm^3, 1.6 mmol) was added to a solution of the dipeptide methyl ester **21a** or **21b** (0.75 g, 1.6 mmol) in (1:1) 1,4-dioxane–water (60 cm^3). After being stirred for 12 h at room temperature, the reaction mixture was concentrated and worked up as in the case of the azides **20a** and **20b**. In this way, dipeptides **22a** and **22b** were obtained as foams, and their analytical and spectroscopic data are summarized in Table 3.

Synthesis of N-[(2S,3R)- *and* (2R,3R)-2,3-*Diamino*-4-*phenylbutanoyl*]-L-*leucine Dihydrochlorides* **2a** *and* **2b** (*Aminodeoxybestatin and* epi-*Aminodeoxybestatin*).—A solution of a protected dipeptide **22a** or **22b** (0.47 g, 1 mmol) in a 0.1 mol dm^{-3} HCl solution in MeOH (20 cm^3) was hydrogenated in the presence of 10% Pd/C (50 mg), at 1 atm of H_2 pressure, and at room temperature for 3 h. The catalyst was filtered off, and washed with MeOH (5 cm^3), and the solvent was removed under reduced pressure. The residue was dissolved in water (10 cm^3), and lyophilized to give aminodeoxybestatin **2a** or *epi*-aminodeoxybestatin **2b**.

Aminodeoxybestatin **2a** (0.33 g, 80%), m.p. 232–235 °C (Found: C, 46.1; H, 7.6; N, 9.9. $C_{16}H_{25}N_3O_3$·2HCl·2H_2O requires C, 46.15; H, 7.45; N, 10.1%); $[\alpha]_D^{20}$ +26 (*c* 1.05, water); $\delta_H(D_2O)$ 0.79 [3 H, d, *J* 6, Me (Leu)], 0.82 [3 H, d, *J* 6, Me (Leu)], 1.50–1.60 [3 H, m, CH_2CH (Leu)], 2.79 [1 H, dd, *J* 11

Table 3 Analytical and relevant spectroscopic data of the dipeptides **21** and **22**

Compd.	Yield (%)	M.p.[a] (°)	Formula	Found (%) (Required) C	H	N	Relevant ^{1}H NMR data (δ)[b] 3-Amino-2-azidobutanoic acid 2-H (d)	3-H (m)	$J_{2,3}$	4-H_2 (dd)	3-NH (d)	Leu-OR NH (d)	α-H (m)	OMe (s)
21a	70	140–142	$C_{25}H_{31}N_5O_5$	62.6 (62.35)	6.3 (6.5)	14.8 (14.5)	4.12	4.42	3	2.78, 2.85	5.33	6.74	4.57	3.69
21b	80	137–139	$C_{25}H_{31}N_5O_5$	62.7 (62.35)	6.4 (6.5)	14.3 (14.5)	4.28	4.40	3	2.76, 2.94	5.48	6.58	4.50	3.69
22a	73	*c*	$C_{24}H_{29}N_5O_5$	61.7 (61.7)	6.2 (6.25)	15.0 (15.0)	4.23	4.40	3	2.78, 2.85	5.40	7.01	4.58	
22b	80	*c*	$C_{24}H_{29}N_5O_5$	62.0 (61.7)	6.3 (6.25)	15.3 (15.0)	4.33	4.40	3	2.72, 2.93	5.41	6.90	4.58	

[a] From propan-2-ol. [b] In $CDCl_3$. [c] Foam.

and 15, 4-H (DAPBA)], 3.23 [1 H, dd, *J* 4 and 15, 4-H (DAPBA)], 4.03 [1 H, m, 3-H (DAPBA)], 4.32 [1 H, m, α-H (Leu)], 4.34 [1 H, d, *J* 5.5, 2-H (DAPBA)] and 7.20–7.40 (5 H, m, Ph).

epi-*Aminodeoxybestatin* **2b** (0.31 g, 75%), m.p. 136–140 °C (Found: C, 46.0; H, 7.6; N, 9.85%); $[\alpha]_D^{20}$ −15.6 (*c* 1.15, water); $\delta_H(D_2O)$ 0.85 [6 H, d, *J* 6, Me (Leu)], 1.29 [3 H, m, CH_2CH (Leu)], 2.88 [1 H, dd, *J* 9 and 15, 4-H (DAPBA)], 3.08 [1 H, dd, *J* 6 and 15, 4-H (DAPBA)], 4.01 [1 H, m, 3-H (DAPBA)], 4.29 [1 H, d, *J* 2.5, 2-H (DAPBA)], 4.30 [1 H, m, α-H (Leu)] and 7.10–7.40 (5 H, m, Ph).

Biological Methods

Materials.—The following commercial compounds were used: bestatin **1a**, Lys-NA (NA = 2-naphthylamide), Leu-NA and Fast Garnet GBC (Sigma UK), Microsomal (AP-M, EC 3.4.11.2) porcine kidney leucine aminopeptidase was purchased from Sigma (UK). Mouse L cells were grown in Dulbecco's modified Eagle's medium and 10% foetal calf serum. L Cells' media and serum were supplied by Flow Labs (UK).

AP-B Assays.—Cell Surface-associated AP-B activities were determined following the Aoyagi method.[30] The incubation mixture consisted of 2 mmol dm^{-3} L-Lys-NA (0.23 cm^3), Hank's balanced salt solution (0.65 cm^3), and distilled water (0.1 cm^3) with or without the inhibitor. After incubation (3 min; 37 °C) the mixture was added to monolayer cultures of mouse L cells (~5 × 10^5 cells), and the incubation was stopped after 30 min by addition of the stabilized diazonium salt Fast Garnet GBC (1 cm^3; 1 mg cm^{-3}) in acetic acid buffer at pH 4.2, containing 10% Tween 20. The mixture was left at room temperature for 15 min, and then was centrifuged, and its absorbance was measured at 525 nm.

AP-M and Leu-AP Assays.—This activity was determined by the described method.[1] A solution of 2 mmol dm^{-3} L-Leu-NA (0.25 cm^3) in 0.1 mol dm^{-3} Tris-HCl buffer (0.5 cm^3) at pH 7.0 was added to distilled water with or without an inhibitor in a series of test tubes in a 37 °C bath. After 3 min the enzyme solution (0.05 cm^3) was added and the solutions were mixed well. Exactly 30 min later the reaction was stopped by addition of a 1 mg cm^{-3} solution of the stabilized diazonium salt Fast Garnet GBC (1 cm^3) in acetic acid buffer at pH 4.2, containing 10% Tween 20. After storage for 15 min at room temperature, absorbance was read at 525 nm. The reaction was also carried out without enzyme solution, and the result was taken as the control blank.

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