Studies on the Biosynthesis of Clavulanic Acid. Part 4.[1] Synthetic Routes to the Monocyclic β-Lactam Precursor, Proclavaminic Acid

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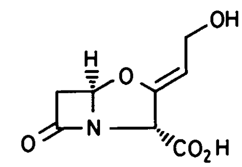
Aldol condensation of 3-substituted propionaldehydes with the lithium enolate of ethyl or benzyl (2-oxoazetidin-1-yl)acetate yielded derivatives of proclavaminic acid. The proportion of the *threo* diastereoisomer in the aldol product could be increased by thermodynamically controlled equilibration with 1,5-diazabicyclo[4.3.0]non-5-ene. In the case of benzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate the diastereoisomers were separated and the *threo* diastereoisomer was resolved by enzymatic hydrolysis of the ester by subtilisin Carlsberg [EC 3.4.21.14]. Catalytic reduction of the unhydrolysed *threo* enantiomer yielded (2*S*,3*R*)-5-amino-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate which had spectroscopic properties identical with those of natural proclavaminic acid and which was a substrate for clavaminic acid synthase. Two crystalline derivatives of (2*S*,3*R*)-proclavaminic acid were prepared for X-ray analysis.

Clavulanic acid (1) is a fused bicyclic β-lactam produced by *Streptomyces clavuligerus*.[2] It is a potent inhibitor of many bacterial β-lactamases[3] and is used clinically in formulation with amoxycillin or ticarcillin to treat infections caused by β-lactamase-producing bacteria. Recent preliminary communications from these laboratories have described the isolation[4] and characterisation of the monocyclic β-lactam proclavaminic acid (2),[4] and the bicyclic clavam clavaminic acid (3),[5] and have also reported their role as biosynthetic precursors of clavulanic acid.[6] The enzyme clavaminic acid synthase, a 2-oxoglutarate-linked oxygenase which converts proclavaminic acid (2) into clavaminic acid (3), has also been characterised.[4]



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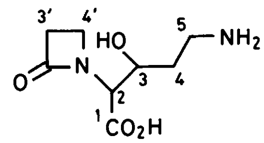

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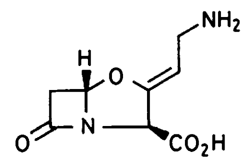

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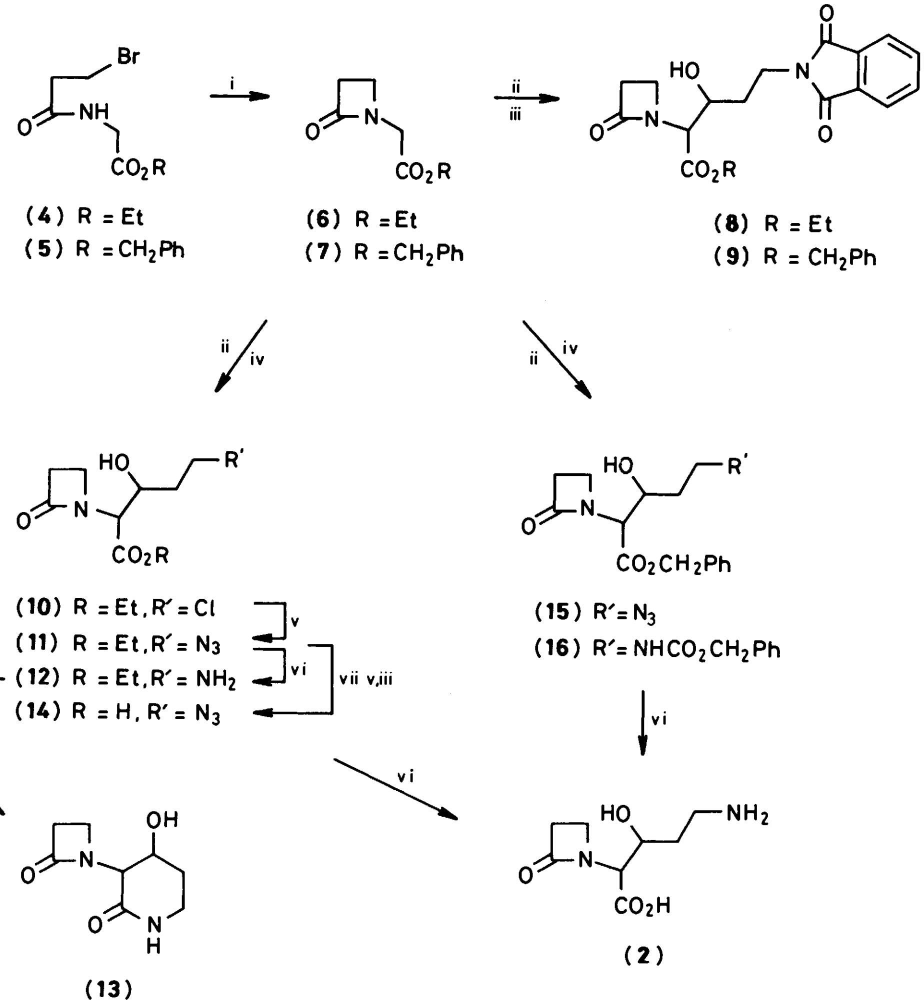
This paper describes studies directed to the synthesis of proclavaminic acid in order to confirm the structure assigned to the material isolated from *S. clavuligerus* and to establish a supply of material for further biochemical studies. Since these studies were completed we have achieved a synthesis of proclavaminic acid[7] described in full in the following paper, which indicates the absolute stereochemistry to be (2*S*,3*R*) and allows the stereochemistry to be assigned to compounds described herein.

The strategy adopted involved the aldol reaction of a suitably 3-protected propionaldehyde with an ester of (2-oxoazetidin-1-yl)acetate. It was anticipated that this approach would yield mixtures of the four possible stereoisomers which, after modification of protecting groups, separation of diastereoisomers, and a resolution would provide the enantiomer corresponding to natural proclavaminic acid. It was also hoped to prepare a crystalline derivative of this enantiomer and hence elucidate the absolute stereochemistry by *X*-ray crystallography.

Three routes to proclavaminic acid based on aldol condensation were attempted. The 3-bromopropionamidoacetates (4) and (5) were ring closed[8] to provide ethyl and benzyl (2-oxoazetidin-1-yl)acetates (6) and (7) from which the enolate anions were generated with lithium bis(trimethylsilyl)amide at −70 °C in dry tetrahydrofuran (THF).

In the first route, reaction of the anions of (6) or (7) with 3-phthalimidopropanal[9] provided the adducts (8) or (9) (Scheme 1), which on examination by ^{1}H or ^{13}C NMR or by HPLC were observed to be mixtures of diastereoisomers. In the case of the ethyl ester (8), which was obtained as an oil, the diastereoisomer ratio was 2:3. (Diastereoisomer ratios are quoted in order of elution from a silica HPLC system.) Trituration of this mixture with diethyl ether resulted in a differential solubilisation of diastereoisomers, leaving a crystalline solid (with a diastereoisomer ratio 5:95). Attempts to deprotect the phthalimides using hydrazine,[10] acid,[11] or base-plus-acid[12] conditions failed to produce the required amines cleanly.

In the second route a strategy was adopted which would provide a versatile intermediate to a range of terminal functionalities, including the required amino group. This involved the 5-chloro aldol adduct (10) (Scheme 1) which could be subjected to nucleophilic substitution with, for example, azide ion to give the 5-azido derivative (11); this in turn could be reduced to the required 5-amino functionality. Thus, compound (11) was successfully prepared; subsequent catalytic hydrogenation produced the target amino ester (12), but spontaneous intramolecular cyclisation resulted in a significant production of the bis-lactam (13). To circumvent this side reaction, the azide (11) was de-esterified by treatment with one molar equivalent of potassium carbonate, then converted into the free acid (14) by treatment with an ion-exchange resin. This latter process also removed the by-product formed by base hydrolysis of the β-lactam ring. Hydrogenation of compound (14) over palladium–carbon catalyst afforded the required compound (2) as a mixture of diastereoisomers (^{1}H and ^{13}C NMR). Incubation of this mixture with a cell-free preparation of clavaminic acid



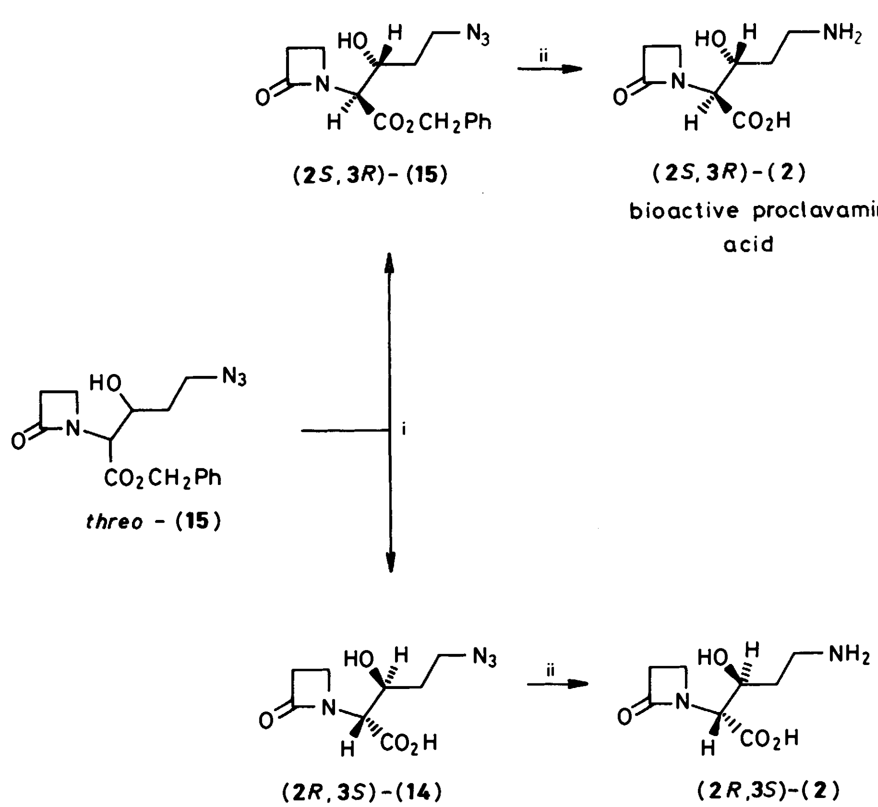
Scheme 1. *Reagents and conditions:* i, Bu_4NBr, KOH, DCM–MeCN; ii, $[(CH_3)_3Si]_2NLi$, THF, −70 °C; iii, 3-phthalimidopropanal; iv, $R'CH_2CH_2CHO$; v, NaN_3, DMSO; vi, H_2, 5% Pd–C, EtOH–water (2:1); vii, K_2CO_3, aq. THF; viii, Amberlite 1R-120 (H^+).

synthase[13] resulted in the production of clavaminic acid, thereby demonstrating that the structural assignment of natural proclavaminic acid had been correct. The quantity of clavaminic acid produced suggested that only one of the four stereoisomers was acting as a substrate for the enzyme, as would be expected.

The feasibility of using an azido group as a precursor to the primary amino functionality having been demonstrated, the third route adopted a more direct approach using 3-azidopropanal[14] in the aldol reaction. The resulting adduct (**15**) was successfully reduced and deprotected in a one-step catalytic hydrogenation to give the required compound (**2**) as a solid in quantitative yield. The aldol reaction consistently produced the product (**15**) as a mixture of diastereoisomers in the approximate ratio 2:3. Trituration of the mixture of diastereoisomers (**2**) with methanol dissolved all the minor diastereoisomer and some of the major diastereoisomer, leaving the bulk of the major component as a diastereoisomerically pure solid. The major component was not cyclised by clavaminic acid synthase to clavaminic acid but the minor, methanol-soluble component was cyclised. Since the absolute stereochemistry of the natural proclavaminic acid is now known to be (2*S*,3*R*), *i.e.* L-*threo*,[7] the product of the aldol reaction is therefore *threo*:*erythro* 2:3.

The minor *threo* diastereoisomer (**15**) resulting from the aldol reaction could be obtained in pure form by silica gel column chromatography. This diastereoisomer in turn was converted into *threo*-(**2**). The ^1H NMR spectra of the two diastereoisomers of compound (**2**) differed, especially with respect to the resonance of the proton alpha to the carboxylate. In the *threo* diastereoisomer spectrum the signal for this proton appeared as a doublet at δ 4.08 whereas the corresponding signal for the *erythro* diastereoisomer appeared at δ 4.18, coincident with the β-proton multiplet. The ^1H NMR spectrum of *threo*-(**2**) was identical with that of natural proclavaminic acid. In comparative cell-free experiments with clavaminic acid synthase, natural proclavaminic acid produced double the amount of clavaminic acid (**3**) as did the synthetic mixture of enantiomers of *threo*-(**2**). This was consistent with only one *threo* enantiomer being converted by the enzyme.

In order to increase the proportion of the required *threo* diastereoisomer, the mixture (**15**) resulting from the aldol reaction was equilibrated in the presence of 1,5-diazabicyclo-[4.3.0]non-5-ene (DBN), when it was observed that the *threo* diastereoisomer was thermodynamically favoured. Thus the ratio of diastereoisomers was changed from 2:3 (*threo*:*erythro*) to 3:1 (*threo*:*erythro*). When the aldol reaction was run at higher temperatures in the hope of achieving thermodynamically as opposed to kinetically controlled product, poor yields resulted. Use of the less accessible 3-benzyloxycarbonylaminopropionaldehyde[15] in the aldol reaction with benzyl ester (**7**) gave the adduct (**16**) (1:2 *threo*:*erythro*) in a similar ratio to that achieved with 3-azidopropionaldehyde. Treatment of adduct (**16**) with DBN also reversed the diastereoisomer ratio



Scheme 2. *Reagents and conditions:* i, subtilisin Carlsberg [EC 3.4.21.14], pH 6.5, water; ii, H_2, 5% Pd–C, EtOH–water (2:1).

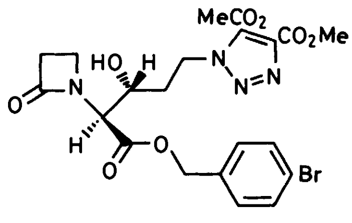
(to 4:1 *threo*:*erythro*). Catalytic reduction of each diastereoisomer of compound **(16)** yielded the corresponding diastereoisomers of proclavaminic acid **(2)**.

Separation of enantiomers of *threo*-**(15)** was accomplished by a stereospecific enzymic hydrolysis of the benzyl ester function (Scheme 2). Thus, an aqueous suspension of *threo*-**(15)** was hydrolysed with the protease subtilisin Carlsberg [EC 3.4.21.14], with the pH maintained at 6.5 with addition of dilute base from an automatic titrator. After the addition of a half-molar equivalent of base there was a sharp decrease in the rate of reaction. The resulting acid was separated from the remaining dextrorotatory ester which was shown to have high enantiomeric purity by ^{1}H NMR spectroscopy in the presence of the chiral solvating reagent (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol.[16] A mixture of enantiomers of *threo*-**(15)** in the presence of the chiral solvating reagent in the ^{1}H NMR mixture showed non-equivalence of the 2-H proton.

Each of the enzymic reaction products was hydrogenated to give compound **(2)**, when only the enantiomer resulting from the unhydrolysed ester was shown to be a substrate for clavaminic acid synthase. From the knowledge that the absolute stereochemistry of natural proclavaminic acid is (2*S*,3*R*),[7] it is clear that subtilisin Carlsberg has shown enantioselectivity for hydrolysis of the 2*R*-ester, a stereopreference which we have also seen for the action of α-chymotrypsin with *threo*-**(15)**. If the ester substrate is considered as a modified amino acid then this stereoselectivity is unusual since these enzymes normally show a marked stereopreference for *S*(L)-amino acids with the exception of a few cyclic analogues.[17] The above stereoselectivity of subtilisin Carlsberg was observed to decrease rapidly at substrate concentrations above 0.2% w/v.

The route through intermediates **(7)** and **(15)** described in Scheme 1 provides a convenient synthesis of proclavaminic acid with natural stereochemistry but does not lead to assignment of absolute stereochemistry. In attempts to elucidate the absolute stereochemistry of this compound by *X*-ray crystallographic techniques, several derivatives bearing bromine were prepared. The *p*-bromobenzyl ester analogue of compound **(15)** was synthesized from *p*-bromobenzyl (2-oxoazetidin-1-yl)acetate as Scheme 1 and the diastereoisomers were separated. The *threo* diastereoisomer crystallised easily and the enantiomer resulting from enzymic resolution exhibited comparable circular dichroic spectra to the corresponding (2*S*,3*R*)-**(15)**, indicating both compounds possessed the same absolute stereochemistry. However, the (2*S*,3*R*)-*p*-bromobenzyl ester analogue of compound **(15)** could not be induced to form crystals. Reaction of this material with dimethyl acetylenedicarboxylate (DMAD) gave a crystalline adduct resulting from 1,3-dipolar cycloaddition of the azide moiety to form a triazole **(17)**. This material, however, could not be induced to form single crystals suitable for *X*-ray analysis. Similarly, (2*S*,3*R*)-proclavaminic acid when treated successively with *p*-bromobenzenesulphonyl chloride and *p*-nitrobenzyl bromide, provided the crystalline derivative **(18)** which also proved unsuitable for *X*-ray structure determination due to crystal twinning.

In conclusion, the preferred chemical route through intermediates **(7)** and **(15)** described in Scheme 1 has produced a material which is stereochemically pure, possesses the same spectral properties as natural proclavaminic acid, and is a substrate for clavaminic acid synthase. Thus, the original[4] structural assignment of natural proclavaminic acid has been shown to be correct. This route has proved to be a viable method for producing quantities of proclavaminic acid of natural (*i.e.*, 2*S*,3*R*) stereochemistry for biochemical studies on clavulanic acid biosynthesis. Also, since the synthetic proclavaminic acid is built up from one C_2 plus two C_3-skeletons, the route was readily adapted for the preparation of ^{13}C-isotopically labelled samples for feeding experiments.[6] However, the route does have the disadvantage that separation of diastereoisomers and enantiomers is necessary, though recent



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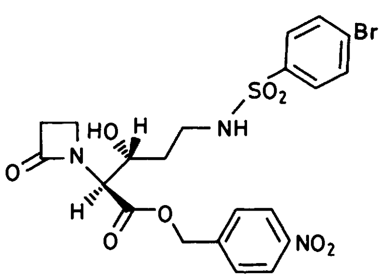

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developments in the field of enantioselective aldol reactions[18] may provide a direct enantioselective synthesis of natural proclavaminic acid.

Experimental

M.p.s were determined on a Reichert Micro Melting Point or Gallenkamp MF 370/11 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 983 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded either on a Bruker AM 250 or AM 400 spectrometer; except where otherwise stated $CDCl_3$ was used as solvent with tetramethylsilane as internal standard. *J*-Values are given in Hz. For non-equivalence measurements the ^1H NMR spectra were recorded using solutions of (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol and the compound under study in the ratio 10:1 by weight in $CDCl_3$ (0.5 ml). Racemates were always checked to confirm the separation of signals due to the proton at the 2-position. Mass spectra were recorded on a VG 7070F spectrometer using electron impact (EI) or chemical ionisation (CI); for fast-atom bombardment (FAB) spectra and high-resolution spectra a VG ZAB IF double-focusing instrument was used. Optical rotations were measured on a Perkin Elmer 141 polarimeter. CD spectra were recorded on a JASCO J600 spectropolarimeter. HPLC was performed using a Spherisorb 5μ silica column with ethyl acetate–acetic acid–hexane (45:0.1:64.9), dichloromethane (DCM)–acetonitrile (MeCN) (6:94), or tetrahydrofuran–DCM (4:96) mixtures as eluant, with detection at 254 nm. Analytical TLC was carried out on Merck pre-coated silica gel 60 F_{254} glass plates which were visualised with UV light and/or iodine vapour; TLC was carried out routinely on all reaction mixtures and final products. Column chromatography was carried out on Merck or Reidel-de-Haahn Kieselgel 60 (0.04–0.063 mm). Anhydrous magnesium sulphate was used for drying organic solutions.

Benzyl 3-Bromopropionamidoacetate (**5**).—A solution of benzyl glycine toluene-*p*-sulphonate (1.23 g, 3.6 mmol) in water (7 ml)–THF (7 ml) was stirred vigorously at 4 °C while a solution of 3-bromopropionyl chloride (0.37 ml, 3.6 mmol) in THF (2.5 ml) was added during 10 min and the pH was maintained between 5.5 and 6.5 with aqueous sodium hydroxide. The reaction mixture was stirred for 0.75 h and then extracted with ethyl acetate (75 ml). The organic layer was washed with saturated aqueous sodium chloride, dried, and evaporated to give a white solid which, after trituration with two aliquots (15 ml) of hexane, gave *benzyl 3-bromopropionamidoacetate* (**5**) (1.02 g, 93%), m.p. 71.5–72.5 °C (from EtOAc–hexane) (Found: C, 48.1; H, 4.7; N, 4.7; Br, 26.7. $C_{12}H_{14}BrNO_3$ requires C, 48.02; H, 4.70; N, 4.67; Br, 26.62%); ν_{max}(KBr) 3 310 (amide), 1 739 (CO_2), 1 652 (amide), and 1 551 cm^{-1} (amide); δ_H(90 MHz) 2.80 (2 H, t, *J* 7, C*H*$_2$CONH), 3.59 (2 H, t, *J* 7, CH_2Br), 4.07 (2 H, d, *J* 6, 2-H_2), 5.17 (2 H, s, C*H*$_2$Ph), 6.47 (1 H, br, s, NH), and 7.30 (5 H, s, Ph).

Benzyl (2-Oxoazetidin-1-yl)acetate (**7**).—Pulverised potassium hydroxide (5.05 g, 90 mmol) and tetrabutylammonium bromide (4.84 g, 15 mmol) were suspended in DCM–MeCN (19:1, 1.5 l). The suspension was stirred vigorously while a solution of benzyl 3-bromopropionamidoacetate (**5**) (21.5 g, 72 mmol) in DCM–MeCN (19:1, 1.5 l) was added during 6 h. The reaction mixture was stirred for a further 30 min, then the insoluble material was filtered off and the filtrate was evaporated to dryness. The resulting oil was chromatographed rapidly to remove the catalyst (eluant ether). Further chromatography with ethyl acetate–hexane (1:1) as eluant gave *benzyl (2-oxoazetidin-1-yl)acetate* (**7**) as an oil (6.0 g, 38%), ν_{max}(KBr) 1 750br cm^{-1} (CO and CO_2); δ_H(90 MHz) 2.98 (2 H, t, *J* 4, 3′-H_2), 3.36 (2 H, t, *J* 4, 4′-H_2), 3.99 (2 H, s, 2-H_2), 5.12 (2 H, s, C*H*$_2$Ph), and 7.30 (5 H, s, Ph). Distillation of this material at 185 °C and 1.5 mmHg gave an oil (Found: C, 65.45; H, 6.15; N, 6.3. $C_{12}H_{13}NO_3$ requires C, 65.74; H, 5.98; N, 6.39%).

Aldol Reactions.—The preparation of compound (**15**) is illustrative of the general method using the appropriate aldehyde.

Benzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (**15**). Under an atmosphere of dry nitrogen, a solution of benzyl (2-oxoazetidin-1-yl)acetate (**7**) (0.88 g, 3.98 mmol) in dry, distilled THF (30 ml) was stirred and cooled to −70 °C. To this was added a solution of lithium bis(trimethylsilyl)amide in THF (5 ml; 5 mmol) at a rate such that the temperature did not rise above −60 °C. After the mixture had been stirred for a further 15 min at −70 °C a solution of 3-azidopropanal[14] (0.57 g, 5.75 mmol) in THF (10 ml) was added, again with the temperature kept below −60 °C, and the reaction mixture was stirred at −70 °C for 2 h. A solution of acetic acid (0.57 ml, 10 mmol) in THF (5 ml) and then water (5 ml) were added to the cold reaction mixture, which was then diluted with ethyl acetate (200 ml) and allowed to reach ambient temperature. The organic phase was washed sequentially with saturated aqueous sodium chloride (20 ml) and saturated aqueous sodium hydrogen carbonate (2 × 15 ml), dried, and evaporated to give an oil. Column chromatography with ethyl acetate–hexane–ethanol (20:30:1) as eluant provided *benzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate* (**15**) as an oil (0.95 g, 74%), ν_{max}(KBr) 3 407 (OH), 2 100 (N_3), and 1 733 cm^{-1} (CO); δ_H(250 MHz) 1.66–1.84 (1 H, m) and 1.88–2.04 (1 H, m) (4-H), 2.99 (2 H, t, *J* 4.2, superimposed upon m, 3′-H_2), 3.26–3.38 (2 H, m, 4′-H_2), 3.39–3.53 (2 H, m, 5-H_2), 4.03 (1 H, d, *J* 3.3, 2-H), 4.22–4.30 (1 H, m, 3-H), 4.51 (1 H, d, *J* 4.5, OH, exch. D_2O), 5.23 (2 H, s, C*H*$_2$Ph), and 7.34 (5 H, s, Ph); *m*/*z* (FAB, thioglycerol) (Found: *M*H$^+$, 319.1397. $C_{15}H_{19}N_4O_4$ requires *m*/*z*, 319.1406). HPLC showed ratio of diastereoisomers 25:75 (*threo*:*erythro*).

Ethyl 3-Hydroxy-2-(2-oxoazetidin-1-yl)-5-phthalimidovalerate (**8**).—Obtained in 43% yield as an oil, ν_{max}(KBr) 3 459 (OH), 1 709 (CO), and 1 397 cm^{-1}; δ_H(250 MHz) 1.30 (3 H, 2 × t, *J* 7.1, Me), 1.77–2.18 (2 H, m, 4-H_2), 2.99 (t, *J* 4.2) and 3.04 (t, *J* 4.3)

(2 H, 3'-H_2), 3.37–3.52 (2 H, m, 4'-H_2), 3.75–3.95 (2 H, m, 5-H_2), 4.05–4.38 (4 H, m, CH_2Me, 2-H, and 3-H), 7.75 (2 H, m, ArH), and 7.84 (2 H, m, ArH). HPLC showed a ratio of diastereoisomers (36:64). Trituration of this material with ether gave a *white crystalline solid*, m.p. 115.5–117 °C (Found: C, 59.8; H, 5.6; N, 7.9. $C_{18}H_{20}N_2O_6$ requires C, 59.99; H, 5.59; N, 7.78%); δ_H(250 MHz) 1.29 (3 H, t, *J* 7.1, Me), 1.90–2.13 (2 H, m, 4-H_2), 2.99 (2 H, t, *J* 4.2, 3'-H_2), 3.36–3.45 (2 H, m, 4'-H_2), 3.85–3.95 (2 H, m, 5-H_2), 4.04–4.33 (4 H, m, 2-H, 3-H, and CH_2Me), 7.75 (2 H, m, ArH), and 7.84 (2 H, m, ArH); δ_C(63 MHz) 14.21, 32.64, 34.90, 36.53, 39.84, 61.12, and 61.47 (ratio 7:93), 61.76, 68.85, and 69.03 (ratio 7:93), 123.38, 132.09, 134.09, 168.23, 168.46, and 168.62. HPLC showed a ratio of diastereoisomers 5:95.

Benzyl 3-*Hydroxy*-2-(2-*oxoazetidin*-1-*yl*)-5-*phthalimidovalerate* (**9**).—Obtained in 13% yield as an *oil*, ν_{max}(KBr) 3 455 (OH), 1 707br (CO), and 1 397 cm^{-1}; δ_H(250 MHz) 1.88–2.16 (2 H, m, 4-H_2), 2.95 (t, *J* 4.2) and 3.03 (t, *J* 4.2) (2 H, 3'-H_2), 3.36 (2 H, m, 4'-H_2), 3.78–3.91 (2 H, m, 5-H_2), 4.05–4.14 (1 H, m, 3-H), 4.22 (1 H, d, *J* 3.9, 2-H), 4.33 (1 H, d, *J* 4.8, OH, D_2O exch.), 5.21 (2 H, s, CH_2Ph), 7.34 (5 H, s, Ph), 7.73 (2 H, m, ArH), and 7.84 (2 H, m, ArH) (Found: MH^+, 423.1556. $C_{23}H_{23}N_2O_6$ requires *m*/*z*, 423.1556).

Ethyl 5-*Chloro*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valerate* (**10**).—Obtained as an *oil* in 32% yield and distilled at 0.3 mmHg between 170–180 °C (Found: C, 48.5; H, 6.8; N, 5.7. $C_{10}H_{16}ClNO_4$ requires C, 48.10; H, 6.46; N, 5.61%); ν_{max}(KBr) 3 383 (OH) and 1 734 cm^{-1} (CO); δ_H(250 MHz) 1.31 (3 H, t, *J* 7.1, Me), 1.89–2.28 (2 H, m, 4-H_2), 3.03 (2 H, t, *J* 4.2, 3'-H_2), 3.32–3.47 (2 H, m, 4'-H_2), 3.62–3.78 (2 H, m, 5-H_2), and 3.99–4.50 (4 H + 1 H, D_2O exch., m, 2- and 3-H, CH_2Me, OH).

Benzyl 5-*Benzyloxycarbonylamino*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valerate* (**16**).—Obtained in 50% yield as an oil after a reaction time of 17 min. HPLC showed a diastereoisomer ratio 1:2 (*threo*:*erythro*). Column chromatography of the mixture of diastereoisomers (700 mg), with ethyl acetate–hexane (3:1) as eluant, yielded threo-*benzyl 5-benzyloxycarbonylamino-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate* (**16**) (138 mg) as an oil, ν_{max}(KBr) 3 400br (OH), 1 725 (CO), 1 527, 752, and 698 cm^{-1}; δ_H(250 MHz) 1.60–1.80 (2 H, m, 4-H_2), 3.01 (2 H, t, *J* 4, 3'-H_2), 3.20–3.60 (4 H, m, 4'- and 5-H_2), 4.14 (1 H, d, *J* 2.5, 2-H), 4.20–4.35 (1 H, m, 3-H), 4.49 (1 H, d, *J* 8.4, OH, D_2O exch.), 5.07 (2 H, s, CH_2Ph), 5.11 (1 H, br s, NH), 5.2 and 5.24 (2 H, ABq, *J* 11, CH_2Ph), and 7.36 (10 H, m, Ph) (Found: M^+, 426.1793. $C_{23}H_{26}N_2O_6$ requires *M*, 426.1791).

Further elution gave a mixture of diastereoisomers (40:60) *threo*:*erythro* (325 mg) followed by erythro-*benzyl 5-benzyloxycarbonylamino-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate* (**16**) (225 mg) as an oil which crystallised on being kept, m.p. 66–68 °C (from EtOAc–hexane) (Found: C, 64.8; H, 6.1; N, 6.55. $C_{23}H_{26}N_2O_6$ requires C, 64.77; H, 6.15; N, 6.57%); ν_{max}(KBr) 3 400br (OH), 1 725br (CO), 742 and 698 cm^{-1}; δ_H(250 MHz) 1.65–2.00 (2 H, m, 4-H_2), 2.98 (2 H, t, *J* 4, 3'-H_2), 3.18–3.6 (4 H, m, 4'- and 5-H_2), 4.09 (1 H, d, *J* 3.3, 2-H), 4.11–4.24 (1 H, m, 3-H), 4.69 (1 H, d, *J* 4, OH, D_2O exch.), 5.09 (2 H, s, CH_2Ph), 5.21 and 5.22 (2 H, ABq, *J* 12, CH_2Ph) superimposed on 5.10–5.25, 1 H, m, NH), and 7.35 (10 H, s, Ph); *m*/*z* (FAB, thioglycerol) (Found: MH^+, 427. $C_{23}H_{27}N_2O_6$ requires *m*/*z*, 427).

Ethyl 5-*Azido*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valerate* (**11**).—A solution of ethyl 5-chloro-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (**10**) (3.45 g, 13.8 mmol) and sodium azide (1.10 g, 17 mmol) in dry dimethyl sulphoxide (DMSO) (20 ml) was stirred at 55–65 °C for 5 h. The reaction mixture was diluted with DCM and washed three times with water before being dried and evaporated. The residue was chromatographed with ethyl acetate–hexane (2:1) as eluant to give *ethyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate* (**11**) as a pale yellow oil (2.59 g, 73%). Bulb-to-bulb distillation at 0.2 mmHg in the range 185–195 °C further purified the product to an oil (Found: C, 46.9; H, 6.5; N, 21.8. $C_{10}H_{16}N_4O_4$ requires C, 46.87; H, 6.29; N, 21.86%); ν_{max}(KBr) 3 405 (OH), 2 101 (N_3), and 1 733 cm^{-1} (CO); δ_H(250 MHz) 1.32 (3 H, t, *J* 7.1, Me), 1.75–1.87 (m) and 1.93–2.05 (m) (2 H, 4-H_2), 3.03 (2 H, t, *J* 4.2, 3'-H_2), 3.32–3.55 (4 H, m, 5- and 4'-H_2), 4.00 (1 H, d, *J* 3.3, 2-H), 4.17–4.33 (3 H, m, CH_2Me and 3-H), and 4.53 (1 H, d, *J* 4.8, OH, D_2O exch.).

4-*Hydroxy*-3-(2-*oxoazetidin*-1-*yl*)*piperidin*-2-*one* (**13**).—Ethyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (**11**) (260 mg, 1 mmol) was hydrogenated over 5% Pd–carbon catalyst (27 mg) in ethanol (10 ml) at ambient temperature for 1.5 h. After filtration and evaporation, the resultant yellow oil (100 mg) was boiled in ethanol (10 ml) for 0.5 h. Evaporation to a solid and recrystallisation gave *4-hydroxy-3-(2-oxoazetidin-1-yl)piperidin-2-one* (**13**) as a white solid (27.7 mg, 16%), m.p. 137.5–140.5 °C (from methanol–ether) (Found: C, 52.55; H, 6.55; N, 15.1. $C_8H_{12}N_2O_3$ requires C, 52.16; H, 6.57; N, 15.21%); ν_{max}(KBr) 3 087, 1 733 (CO), 1 655 (CO), and 1 493 cm^{-1}; δ_H(250 MHz; [$^2H_6$]DMSO) 1.70–1.80 (m) and 1.80–2.00 (m) (2 H, 5-H_2), 2.76–2.90 (2 H, m, 3'-H_2), 2.98–3.10 (2 H, m) and 2.26–3.40 (2 H, m) (6- and 4'-H_2), 3.78–3.82 (m), 4.12–4.14 (m), and 4.19 (d, *J* 3.0) (together 2 H, 3- and 4-H), 5.30 (1 H, d, *J* 3.5, OH, D_2O exch.), and 7.57 (br s, D_2O exch.) and 7.74 (br, s, D_2O exch.) (1 H, NH, ratio of integrals 28:72); δ_C(100 MHz; [$^2H_6$]DMSO) 28.36 and 30.20 (ratio 29:71), 36.31, 36.65, 36.79, 37.30, 37.40, 55.34, and 59.92 (ratio 29:71), 65.58 and 67.07 (ratio 29:71), 167.87, and 168.96.

5-*Azido*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valeric Acid* (**14**).—A solution of ethyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (**11**) (0.77 g, 3 mmol) in THF (13 ml)–water (13 ml) was treated with a solution of potassium carbonate (0.445 g, 3.2 mmol) in water (2.5 ml) in such a way that the pH of the reaction mixture did not rise above 11.5. After evaporation of the solvent, the product was extracted into ethanol, and the solution was filtered and chromatographed with ethanol as eluant, to give a white solid (396 mg). This solid (200 mg) was dissolved in water and passed through a column of Amberlite IR-120 (H^+) ion-exchange resin to give 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valeric acid (**14**) as an oil which slowly crystallised to a white solid (92 mg, 13%). This material was used without further purification; δ_H(250 MHz; [$^2H_6$]DMSO–D_2O) 1.56–1.79 (2 H, m, 4-H_2), 2.89 (2 H, t, *J* 2.9, 3'-H_2), 3.28–3.53 (4 H, m, 5- and 4'-H_2), 3.89 (1 H, m, 3-H), and 4.10 (1 H, d, *J* 6.6, 2-H).

Equilibration and Separation of the Diastereoisomers of Benzyl 5-*Azido*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valerate* (**15**).—A mixture of diastereoisomers of benzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (**15**) (950 mg, 2.98 mmol), prepared by aldol coupling, was dissolved in DCM (100 ml) and the solution was stirred with DBN (0.37 g, 2.98 mmol) for 1.5 h at room temperature. The reaction mixture was evaporated under reduced pressure and chromatographed with DCM–ethyl acetate–ethanol (60:10:1) as eluant to give the faster running threo-*benzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate* (**15**) (370 mg, 39%) as an oil which solidified after a time, m.p. 63–64 °C (from hexane) (Found: C, 56.6; H, 5.6; N, 17.5. $C_{15}H_{18}N_4O_4$ requires C, 56.59; H, 5.70; N, 17.60%); ν_{max}(KBr) 2 101 (N_3), 1 732br (CO and CO_2), 752, and 699 cm^{-1}; δ_H(400 MHz) 1.65–1.75 (1 H, m) and 1.78–1.90 (1 H, m) (4-H_2), 2.98–3.10 (2 H, m, 3'-H_2), 3.32–3.38 (1 H, m) and 3.41–3.46 (1 H, m) (4'-H_2), 3.46–3.55 (2 H, m, 5-H_2), 4.04 (1 H, d, *J* 2.9, OH), 4.28–4.37 (2 H, br s superimposed upon m, 2- and 3-H), 5.22 and 5.26

(2 H, ABq, *J* 12.2, CH_2Ph), and 7.37 (5 H, s, Ph); δ_C(100 MHz) 33.42, 36.14, 40.23, 48.08, 62.80, 67.59, 68.50, 128.23, 128.53, 128.64, 128.71, 135.06, 168.27, and 169.37. HPLC showed a single diastereoisomer.

Further elution gave a mixture of diastereoisomers (470 mg, 49%) in the ratio 55:45 (HPLC), which on further chromatography under the same conditions provided an oil (197 mg, 42%), predominantly the slower running *erythro* diastereoisomer, ν_{max}(KBr) 2 100 (N_3), 1 735br (CO and CO_2), 752, and 699 cm^{-1}; δ_H(250 MHz) 1.89–1.70 (1 H, m) and 1.90–2.07 (1 H, m) (4-H_2), 3.00 (2 H, t, *J* 4.2, 3'-H_2), 3.25–3.31 (1 H, m) and 3.32–3.40 (1 H, m) (4'-H_2), 3.50 (2 H, dd, *J* 8.0 and 5.6, 5-H_2), 4.03 (1 H, d, *J* 3.4, 2-H), 4.22–4.35 (1 H, m, 3-H), 4.52 (1 H, dd, *J* 4.9 and 1.1, D_2O exch., OH), 5.23 and 5.24 (2 H, ABq, *J* 12.3, CH_2Ph), and 7.37 (5 H, s, Ph); δ_C(100 MHz) 32.79, 36.35, 39.84, 48.12, 62.80, 67.52, 68.37, 126.30, 128.60, 128.67, 134.99, 167.93, and 168.81 [Found: MH^+, 319 (100%) and 291, MH^+ − 28 (16%). $C_{15}H_{19}N_4O_4$ requires *m/z* 319]. HPLC showed a ratio of diastereoisomers 10:90 (*threo*:*erythro*).

(2S,3R)-*Benzyl* 5-*Azido*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valerate* (**15**).—Finely powdered *threo*-benzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (**15**) (2 g, 6.3 mmol) was suspended in water (1 l) and the mixture was stirred at 36 °C with subtilisin Carlsberg (EC 3.4.21.14) (400 units) while the pH was maintained at 6.5 by the addition of 1M-NaOH. After 5.5 h, after alkali (3.7 ml) had been added, the reaction mixture was extracted three times with DCM. The extracts were bulked, dried, and evaporated under reduced pressure. The residual oil (1.25 g) was chromatographed with ethyl acetate–hexane–ethanol (20:30:1) as eluant to give (2S,3R)-*benzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate* (**15**) (0.78 g, 78%) as an oil, $[\alpha]_D^{20}$ +33.15° (*c* 2.0, $CHCl_3$); ν_{max} 2 100 (N_3), 1 724br (CO and CO_2), 753, and 699 cm^{-1}; δ_H(250 MHz) 1.60–1.95 (2 H, m, 4-H_2), 2.95–3.15 (2 H, m, 3'-H_2), 3.31–3.19 (1 H, m) and 3.40–3.48 (1 H, m) (4'-H_2), 3.51 (2 H, dd, *J* 7.4 and 5.6, 5-H_2), 4.03 (1 H, d, *J* 2.9, OH), 4.25–4.40 (2 H, m, 3- and 2-H), 5.22 and 5.26 (2 H, ABq, *J* 12, CH_2Ph), and 7.31 (5 H, s, Ph); addition of (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol to the NMR solution demonstrated the presence of a single enantiomer; *m/z* (FAB, thioglycerol) (Found: MH^+, 319. $C_{15}H_{19}N_4O_4$ requires *m/z*, 319); CD in MeCN $\Delta\varepsilon_{215}$ +2.7, $\Delta\varepsilon_{242.9}$ −0.088, and $\Delta\varepsilon_{284}$ −0.005.

5-*Amino*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valeric Acid* (**2**) *by Catalytic Reduction of Azide* (**14**).—5-Azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valeric acid (**14**) (58 mg, 0.25 mmol) was dissolved in a mixture of ethanol (4 ml) and water (2 ml), and hydrogenated with 5% Pd–carbon catalyst at ambient temperature for 1 h. After filtration and evaporation, the resulting gum was triturated with ether to give *5-amino-3-hydroxy-2-(2-oxoazetidin-1-yl)valeric acid* (**2**) as a white solid (47.6 mg, 93%) (Found: C, 46.7; H, 7.6; N, 11.0. $C_8H_{14}N_2O_4 \cdot 0.75\ C_2H_5OH$ requires C, 46.15; H, 7.54; N, 11.33%); ν_{max}(KBr) 3 425 (OH), 1 714 (CO), 1 639, and 1 575 cm^{-1}; δ_H(250 MHz; D_2O) 1.71–2.00 (2 H, m, 4-H_2), 2.99 (2 H, t, *J* 3.9, 3'-H_2), 3.08–3.21 (2 H, m, 5-H_2), 3.41–3.61 (2 H, m, 4'-H_2), and 4.05 (2 H, m, 2- and 3-H); signals for 0.75 mol equiv. of ethanol were observed in the spectrum; *m/z* (FAB, thioglycerol) (Found: MH^+, 203. $C_8H_{15}N_2O_4$ requires *m/z* 203).

The following compounds were prepared in a similar manner.

erythro-5-*Amino*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valeric Acid* (**2**).—Benzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)-valerate (**15**) (450 mg, 1.4 mmol) as a mixture of diastereoisomers was hydrogenated and the resulting solid was triturated with methanol. Filtration gave the *title compound* as a white solid (61 mg, 22%), m.p. 160–161 °C (Found: C, 45.9; H, 6.9; N, 13.0. $C_8H_{14}N_2O_4 \cdot 0.5\ H_2O$ requires C, 45.49; H, 7.16; N, 13.26%); ν_{max}(Nujol) 3 200 (OH), 1 730 (CO), and 1 600 cm^{-1} (NH_3 and CO_2); δ_H(250 MHz; D_2O) 1.81–1.98 (2 H, m, 4-H_2), 2.99 (2 H, t, *J* 3.9, 3'-H_2), 3.09–3.24 (2 H, m, 5-H_2), 3.43–3.59 (2 H, m, 4'-H_2), and 4.06–4.21 (2 H, m, 3- and 2-H).

threo-5-*Amino*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valeric Acid* (**2**).—*threo*-Benzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)-valerate (**15**) (536 mg, 1.7 mmol) was hydrogenated. The resulting solid was dissolved in water and the solution was re-evaporated to remove the ethanol of crystallisation, to yield threo-*5-amino-3-hydroxy-2-(2-oxoazetidin-1-yl)valeric acid* (**2**) as a solid (359 mg, 99%) (Found: C, 44.55; H, 7.2; N, 13.0. $C_8H_{14}N_2O_4 \cdot 0.75\ H_2O$ requires C, 44.54; H, 7.24; N, 12.99%); ν_{max}(KBr) 3 499br (OH), 1 722 (CO), 1 655, 1 610, and 1 577 cm^{-1}; δ_H(400 MHz; D_2O) 1.75–1.93 (2 H, m, 4-H_2), 3.00 (2 H, t, *J* 3.9, 3'-H_2), 3.06–3.20 (2 H, m, 5-H_2), 3.46–3.53 (1 H, m) and 3.54–3.60 (1 H, m) (4'-H_2), 4.05 (1 H, d, *J* 5.5, 2-H), and 4.18 (1 H, ddd, *J* 9.3, 5.3, and 4.0, 3-H); δ_C(100 MHz; D_2O) 31.63, 36.13, 37.81, 41.01, 63.1, 69.55, 172.81, and 174.96.

(2S,3R)-5-*Amino*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valeric Acid* (**2**), *Proclavaminic Acid*.—(2*S*,3*R*)-Benzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (**15**), which was recovered from the enzymic resolution, was hydrogenated to give a pale cream solid, m.p. 130–135 °C (from aq. MeOH), $[\alpha]_D^{20}$ +7.8° (*c* 1.0, water) (Found: C, 43.9; H, 7.4; N, 12.5. $C_8H_{14}N_2O_4$ requires C, 43.63, H, 7.32; N, 12.72%); ν_{max}(KBr) 3 411br (OH), 1 717 (CO), and 1 600br cm^{-1} (NH_3^+ and CO_2^-); δ_H(400 MHz; D_2O) 1.77–1.94 (2 H, m, 4-H_2), 3.01 (2 H, t, *J* 4.0, 3'-H_2), 3.10–3.23 (2 H, m, 5-H_2), 3.48–3.53 (1 H, m) and 3.55–3.62 (1 H, m) (4'-H_2), 4.08 (1 H, d, *J* 5.5, 2-H), and 4.20 (1 H, ddd, *J* 9.4, 5.4, and 3.9, 3-H); δ_C(100 MHz; D_2O) 31.6, 36.1, 37.8, 41.0, 63.2, 69.5, 172.8, and 174.9; *m/z* (FAB, glycerol) (Found: MH^+, 203. $C_8H_{15}N_2O_4$ requires *m/z* 203).

(2R,3S)-5-*Amino*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valeric Acid* (**2**).—The aqueous solution remaining after the extraction of (2*S*,3*R*)-benzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (2*S*,3*R*)-(**15**) from a similar subtilisin enzymation of racemic *threo*-(**15**) (300 mg) to that described above, was acidified to pH 3.0 with dil. HCl and extracted with ethyl acetate. The dried organic solution yielded (2*S*,3*R*)-(**14**) (83 mg) as an oil which was a single spot on TLC (R_f 0.6; 15% water–EtOH). Without further purification, this material was hydrogenated to give the title compound as a pale yellow solid [85 mg, 45% from *threo*-(**15**)] with NMR properties identical with those of the (2*S*,3*R*) enantiomer, $[\alpha]_D^{20}$ −4.6° (*c* 0.5, water).

Sodium (2-*Oxoazetidin*-1-*yl*)*acetate*.—Benzyl (2-oxoazetidin-1-yl)acetate (**7**) (412 mg, 1.88 mmol) and sodium hydrogen carbonate (158 mg, 1.88 mmol) in water (5 ml)–ethanol (10 ml) were hydrogenated with 10% Pd–carbon catalyst at ambient temperature for 0.75 h. The catalyst was removed by filtration and the filtrate was evaporated to dryness to give, on trituration with ether, a white solid, sodium (2-oxoazetidin-1-yl)acetate (271 mg, 95%), m.p. 203–205 °C (Found: C, 39.8; H, 3.9; N, 9.3. $C_5H_6NNaO_3$ requires C, 39.76; H, 4.00; N, 9.27%); ν_{max}(KBr) 1 743, 1 721, (CO), 1 617, and 1 596 cm^{-1}; δ_H(90 MHz; D_2O) 3.00 (2 H, t, *J* 4, 3'-H_2), 3.41 (2 H, t, *J* 4, 4'-H_2), and 3.80 (2 H, s, 2-H_2).

4-*Bromobenzyl* (2-*Oxoazetidin*-1-*yl*)*acetate*.—Sodium (2-oxoazetidin-1-yl)acetate (730 mg, 4.8 mmol) was stirred at room temperature for 18 h in dimethylformamide (DMF) (15 ml) with 4-bromobenzyl bromide (1.32 g, 5.28 mmol). The reaction mixture was treated with ethyl acetate (200 ml) and the resulting precipitate was filtered off. The filtrate was evaporated to dryness and the residue was chromatographed with ethyl acetate–hexane (1:1) as eluant to give *4-bromobenzyl (2-oxoazetidin-1-yl)acetate* as a clear oil which solidified after a time (1.1 g, 76%), m.p. 39.5–40.5 °C (Found: C, 48.5; H, 4.15; N, 4.7; Br, 27.0. $C_{12}H_{12}BrNO_3$ requires C, 48.34; H, 4.06; N, 4.70; Br, 26.80%); ν_{max}(KBr) 1 750br cm^{-1} (CO and CO_2); δ_H(90 MHz) 3.00 (2 H, t, *J* 4.5, 3'-H_2), 3.38 (2 H, t, *J* 4.5, 4'-H_2), 3.99 (2 H, s, 2-H_2), 5.08 (2 H, s, C*H*$_2$Ar), and 7.16 and 7.45 (4 H, ABq, *J* 8, ArH).

threo-*4-Bromobenzyl 5-Azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate*.—4-Bromobenzyl (2-oxoazetidin-1-yl)acetate (900 mg, 3.0 mmol) was coupled with 3-azidopropanal[14] by the general method, and the product mixture of diastereoisomers was equilibrated and separated in the same manner as the benzyl ester (**15**) to give, after repeated chromatography, threo-*4-bromobenzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate* as an oil which crystallised after a time (221 mg, 19%), m.p. 53–54.5 °C (from di-isopropyl ether) (Found: C, 45.59; H, 4.35; N, 14.11. $C_{15}H_{17}BrN_4O_4$ requires C, 45.35; H, 4.31; N, 14.11%); ν_{max}(KBr) 3 400br (OH), 2 101 (N_3), and 1 732br cm^{-1} (CO and CO_2); δ_H(250 MHz) 1.60–1.90 (2 H, m, 4-H_2), 2.95–3.15 (2 H, m, 3'-H_2), 3.30–3.39 (1 H, m) and 3.39–3.48 (1 H, m) (4'-H_2), 3.52 (2 H, dd, *J* 7.4 and 5.5, 5-H_2), 4.10 (1 H, d, *J* 2.5, OH), 4.25–4.40 (2 H, m, 2- and 3-H), 5.18 and 5.19 (2 H, ABq, *J* 12.7, C*H*$_2$Ar), and 7.25 and 7.51 (4 H, ABq, *J* 8.5, ArH). HPLC showed a single diastereoisomer. A mixture of diastereoisomers of 4-bromobenzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (150 mg, 12%) was also recovered from the column.

(2S,3R)-*4-Bromobenzyl 5-Azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate*.—*threo*-4-Bromobenzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (690 mg, 1.77 mmol) was resolved with subtilisin Carlsberg (EC 3.4.21.14) in a manner similar to the resolution of the *threo*-benzyl ester (**15**) to give (2S,3R)-*4-bromobenzyl-5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate* as an oil (320 mg, 92%), $[\alpha]_D^{20}$ +25.1° (*c* 2.0, $CHCl_3$) (Found: C, 45.8; H, 4.6; N, 13.7. $C_{15}H_{17}BrN_4O_4$ requires C, 45.35; H, 4.31; N, 14.11%); ν_{max}(KBr) 3 412br (OH), 2 100 (N_3), and 1 730br cm^{-1} (CO and CO_2); δ_H(250 MHz) 1.60–1.92 (2 H, m, 4-H_2), 2.95–3.13 (2 H, m, 3'-H_2), 3.31–3.39 (1 H, m) and 3.39–3.48 (1 H, m) (4'-H_2), 3.53 (2 H, dd, *J* 7.5 and 5.5, 5-H_2), 4.03 (1 H, d, *J* 2.7, OH), 4.29–4.38 (2 H, m, 2- and 3-H), 5.18 and 5.19 (2 H, ABq, *J* 13.2, C*H*$_2$Ar), and 7.25 and 7.51 (4 H, ABq, ArH). Addition of (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol to the NMR solution demonstrated the presence of a single enantiomer; CD in MeCN $\Delta\varepsilon_{217}$ +3.1, $\Delta\varepsilon_{243.3}$ −0.142, and $\Delta\varepsilon_{283}$ −0.001.

(2S,3R)-*4-Bromobenzyl 5-(4,5-Bismethoxycarbonyl-1,2,3-triazol-1-yl)-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate* (**17**).—A solution of (2*S*,3*R*)-4-bromobenzyl 5-azido-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (217 mg, 0.55 mmol) and DMAD (144 mg, 1 mmol) in toluene (8 ml) was heated under reflux for 1.25 h, cooled, and evaporated under reduced pressure. Column chromatography of the residual oil (ethyl acetate–hexane–EtOH, 35:15:1) followed by further chromatography with methyl acetate–cyclohexane (3:2) as eluant and recrystallisation from propan-2-ol gave the *title compound* (**17**) (134.4 mg, 46%) as prisms, m.p. 100 °C; $[\alpha]_D^{25}$ +20.3° (*c* 2, $CHCl_3$) (Found: C, 47.1; H, 4.3; N, 10.2; Br, 14.8%; M^+, 538.0698. $C_{21}H_{23}BrN_4O_8$ requires C, 46.76; H, 4.29; N, 10.38; Br, 14.82%; *M*, 538.0699); ν_{max}(KBr) 3 303br (OH), 1 718br (CO), and 1 228 cm^{-1}; δ_H(250 MHz) 2.09–2.27 (2 H, m, 4-H_2), 2.95–3.14 (2 H, m, 3'-H_2), 3.28–3.50 (2 H, m, 4'-H_2), 3.95–4.05 (7 H, m, 2 × Me and 2-H), 4.10–4.24 (1 H, m, 3-H), 4.44 (1 H, d, *J* 10, OH), 4.76 (2 H, t, *J* 7, 5-H_2), 5.15 (2 H, s, C*H*$_2$Ar), and 7.21 and 7.50 (4 H, ABq, *J* 8, ArH).

(2S,3R)-*4-Nitrobenzyl 5-(4-Bromophenylsulphonylamino)-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate* (**18**).—A stirred solution of (2*S*,3*R*)-5-amino-3-hydroxy-2-(2-oxoazetidin-1-yl)valeric acid (**2**) (proclavaminic acid) (134.8 mg, 0.66 mmol) in THF–water (1:1) (2 ml) was treated portionwise with a solution of 4-bromobenzenesulphonyl chloride (171 mg, 0.67 mmol) in THF (2 ml), with the pH maintained at 8 with 0.5M-caesium carbonate. A further aliquot of THF (2 ml) was added to maintain a homogeneous solution. The mixture was brought to pH 7 with 0.1M-hydrochloric acid, the solution was evaporated to dryness under reduced pressure, and DMF (2 ml) added and evaporated off. The evaporation with DMF (2 ml) was repeated. To the residue was added a solution of 4-nitrobenzyl bromide (144 mg, 0.67 mmol) in dry DMF (2 ml) and the mixture was stirred overnight under nitrogen. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The organic layer was washed with water, dried, and evaporated. The crude product was purified by chromatography with an ethyl acetate–hexane gradient eluant (3:2 to 4:1) then rechromatographed with acetone–chloroform as eluant (1:9) to yield (2S,3R)-*4-nitrobenzyl 5-(4-bromophenylsulphonylamino)-3-hydroxy-2-(2-oxoacetidin-1-yl)valerate* (**18**) as a solid (107.9 mg, 30%), m.p. 122–124 °C (from MeOH); $[\alpha]_D^{20}$ +1.28° (*c* 0.39, $CHCl_3$) (Found: C, 45.3; H, 3.8; N, 7.5. $C_{21}H_{22}BrN_3O_8S$ requires C, 45.33; H, 3.99; N, 7.55%); ν_{max}(KBr) 3 334br (OH and NH), 1 748, 1 713, 1 516, 1 347 (SO_2), 1 330, 739, and 607 cm^{-1}; δ_H(250 MHz) 1.65–1.80 (2 H, m, 4-H_2), 3.00–3.30 (4 H, m, 3'- and 5-H_2), 3.30–3.50 (2 H, m, 4'-H_2), 4.05 (1 H, d, *J* 3, 2-H), 4.45 (1 H, m, 3-H), 4.55 (1 H, d, *J* 10, OH), 4.96 (1 H, t, *J* 4, NH) 5.32 (2 H, s, C*H*$_2$Ar), 7.54 and 8.26 (2 H, ABq, *J* 9, ArH), and 7.73 and 7.76 (4 H, ABq, *J* 9, ArH).

threo-*4-Nitrobenzyl 5-(4-Bromophenylsulphonylamino)-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate*.—In a similar manner to the (3*R*,2*S*) derivative *this material* was prepared in 20% yield, m.p. 141 °C (from MeOH) (Found: C, 44.85; H, 3.8; N, 7.5; S, 5.7. $C_{12}H_{22}BrN_3O_8S$ requires C, 45.33; H, 3.99; N, 7.55; S, 5.76%). The IR and ^{1}H NMR data were identical with those for the (2*S*,3*R*)-enantiomer; *m/z* (FAB, thioglycerol) (Found: *M*H$^+$, 556. $C_{21}H_{23}BrN_3O_8S$ requires *m/z*, 556).

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