The Synthesis of Peptide β-Lactams as Potential Protease Inhibitors

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β-Lactam analogues of a peptide which is a substrate/inhibitor of Angiotensin Converting Enzyme (ACE) have been designed as potential inhibitors of the enzyme. The synthesis of a series of these compounds is described and their absolute stereochemistry has been assigned by a combination of *X*-ray crystallography, chemical shift correlations, and nuclear Overhauser effect (n.O.e.) studies.

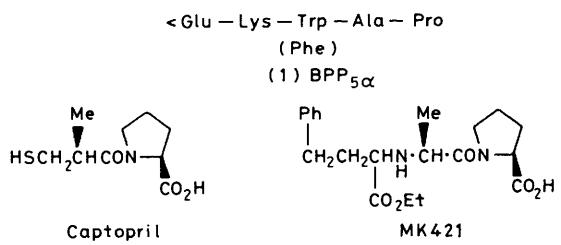
The function of all proteases is to cleave the peptidic amide bond in the substrate. The *specificity* of this process resides in the interactions of those amino-acid residues of substrate which are proximal to the cleavable bond, with the active-site of the particular enzyme. The *mechanism* whereby this process occurs varies from one enzyme to another and, indeed, has formed a basis for classification of the proteases; however the underlying process is the same and involves nucleophilic attack on the carbonyl carbon atom of the cleavable amide bond by a nucleophile which is either a part of the enzyme active site (*e.g.* OH of Ser, SH of Cys, CO_2H of Glu) or which is an enzyme-activated water molecule. Amide bond cleavage follows as a consequence; in the former case to give an acyl-enzyme intermediate (which is broken down in a subsequent step) or in the latter case to give the product acid and amine directly. Finally, separate diffusion of these fragments (which may in some instances be rate-limiting) from the active-site occurs.

Specific inhibitors of proteases have large therapeutic potential although problems relating to specificity have been recognised.[1] There is now significant effort aimed at many target enzymes using a multitude of design processes, but perhaps the most outstanding example of ' protease ' inhibition is shown by the penicillins. These bicyclic compounds inhibit the transpeptidases and carboxypeptidases which cross-link the bacterial cell wall.[2a] The resultant damage to the peptidoglycan ultimately results in cell-lysis and death and forms the basis of the potent bactericidal action of these antibiotics. The detailed biochemistry of this action is still not clear, but probably involves the acylation of an active-site Ser residue giving a relatively stable enzyme-inhibitor intermediate, which stops access of the normal substrate to the active-site. Comparison of molecular models of the penicillin structure with the D-Ala-D-Ala end group of the peptidoglycan substrate shows significant similarities between the two; moreover, this similarity is improved if the amide bond of the substrate is twisted out of its normal planar configuration. This implies that the antibiotic mimics the strained amide bond which is being broken, *i.e.* the penicillin may be a ' transition-state ' analogue. More recently monocyclic β-lactams such as the nocardicins [2a] and the monobactams [2b] have shown antibacterial activity so it is clear that the additional strain inherent in the bicyclic system is not an essential prerequisite for biological activity. The rigidity of the β-lactam molecule also maintains the other functionalities in a fixed conformation which may be important for recognition and binding. The use of lactams as conformational constraints in peptides has been described recently.[3]

Although so successful in this case, the general concept of inhibition of proteases by β-lactams has not been widely exploited. A β-lactam analogue of a peptide substrate for a particular protease enzyme could conceivably cause inhibition of that enzyme by several mechanisms if the molecule can indeed be recognised. These possibilities are as follows. (1) That the compound could act as a competitive reversible inhibitor by mimicking the natural substrate. ' Transition-state ' character would enhance the binding. (2) That the enzyme could be acylated, leading to (pseudo) [2a] irreversible inhibition, depending, of course, on the nature of the incoming nucleophile. (3) That normal substrate-like cleavage of the β-lactam could occur. This would give a product where the acid and amine ' fragments ' are still joined and separate diffusion from the active-site would then be impossible. This may effectively ' freeze ' the reaction co-ordinate, again resulting in tight binding.

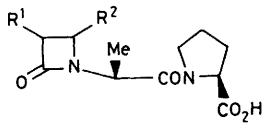
This paper describes our preliminary attempts to apply this idea to one protease of interest—Angiotensin Converting Enzyme (ACE). This enzyme is a carboxydipeptidase which cleaves angiotensin I (A_I) to the potent vasoconstrictor angiotensin II (A_{II}). The mode of action of the enzyme is generally assumed to be similar to that of the zinc metalloenzyme carboxypeptidase A, where the active-site nucleophile is either the carboxy group of Glu (270) or an enzyme activated water molecule. Inhibition of ACE reduces A_{II} levels and thereby lowers blood pressure and the rational development of Captopril as a novel antihypertensive agent has been well documented [4] as illustrating this.

Both Captopril and MK421,[5] another potent inhibitor of ACE, can be considered, not as analogues of the substrate, A_I, but rather as analogues of a tripeptide Phe-Ala-Pro, which in turn can be derived from the carboxy tripeptide portion of $BPP_{5\alpha}$, (1), a snake venom peptide [4] which is a powerful inhibitor and substrate (with low $V_{max.}$) of the enzyme. The importance of the binding of the phenyl group of MK421 to the putative enzyme binding site which recognises Phe in the above tripeptide or the analogous Trp in $BPP_{5\alpha}$ is reflected in the high potency of MK421.



Our starting point also has been to use this tripeptide as the basic binding template. The first target incorporating a β-lactam ring at the cleavable amide bond is the structure (2)

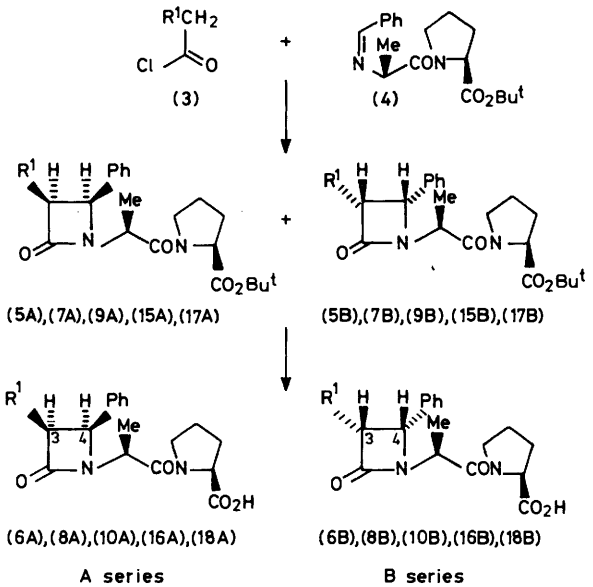
where R^1 and R^2 have been designed to serve a variety of functions.



(2)

From a preliminary inspection of molecular models it was clear that the phenyl group of the conformationally flexible tripeptide Phe-Ala-Pro could overlay either R^1 *or* R^2 although the former seemed more likely. Because it is synthetically convenient to have R^2 = Ph the first part of this investigation has been to make examples of (2) with a variety of substituents at R^1 with R^2 = Ph to see whether these compounds would bind to the enzyme. The details of the synthesis and stereochemistry of these compounds is described below.

After a survey of the methods available for constructing monocyclic β-lactams and after several unsuccessful attempts to construct (2) by more modern methods [6–9] we have used the early [10] acid chloride–imine condensation route shown in Scheme 1. Although the yields from these reactions have been uniformly low they have proved adequate for our needs and consequently no attempts have been made to improve them. One limitation on this route, however, is that the acid chloride (3) requires activation by an electron-withdrawing group at the α-position–hence the limited variation given for R^1 below. Benzylidene-L-Ala-L-ProOBu^t (4), the imine starting material for all of these reactions has been fully characterised, but is generally used without purification immediately after preparation.



(5),(6) R^1 = PhO
(7),(8) R^1 = $PhCH_2O$
(9),(10) R^1 = HO
(15),(16) R^1 = Cl
(17),(18) R^1 = Br

Scheme 1.

Condensation of phenoxyacetyl chloride (3; R^1 = PhO) with the imine (4) gave, after chromatography, two isomeric β-lactam esters (5A) and (5B) in poor yield. These were shown to be diastereoisomeric *cis* β-lactams from the n.m.r. coupling constants (J 4.2 Hz) of the methine protons of the β-lactam ring.[10]

Deprotection of each of the separated esters (5A) and (5B) was carried out using trifluoroacetic acid under standard conditions to give the respective target acids (6A) and (6B).

In order to relate these potential inhibitor structures more closely to the tripeptide template L-Phe-L-Ala-L-Pro it seemed important that the *absolute* stereochemistry of the β-lactam ring of these compounds was identified and to this end X-ray diffraction data were collected on the crystalline ester (5A).

Although no attempt was made to assign absolute stereochemistry from the X-ray data this follows from the relative configurational assignment because of the chiral nature of the dipeptide starting material (4). No racemisation has been observed in condensations of this type.[11] Unequivocal assignment of the stereochemistry of (5A) as shown in Figure 1 and Scheme 1 follows from this.

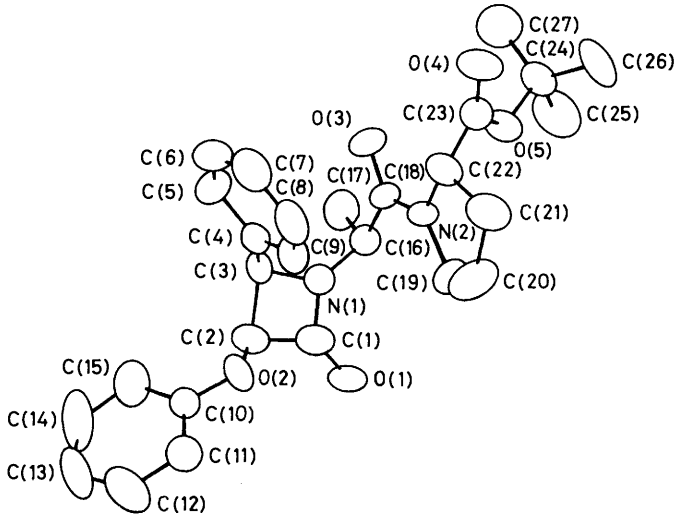


Figure 1. Structure of compound (5A) determined by X-ray diffraction

The absolute configuration of the other *cis*-isomer (5B) follows as a consequence of this.

Because of the poor isolated yields recorded in this reaction (and in others described below) we attach no mechanistic significance to the isolation of only the *cis* β-lactam isomers.

Detailed comparison of Dreiding models of (6A) and (6B) with L-Phe-L-Ala-L-Pro showed good overlap of both of these fairly rigid diastereoisomers with the tripeptide. However if the phenyl group of Phe in the tripeptide overlays R^1 of the β-lactam then it is clear that there is no counterpart of the synthetically expedient R^2 (Ph) group in the tripeptide and also that the R^1 (PhO) substituent seems to be too short to overlap optimally. If, however, the phenyl group of the tripeptide overlays R^2 (Ph) of the β-lactam then a small substituent is required at R^1. Whereas the problem of removal of 4-phenyl group (*i.e.* R^2) has not been addressed in this series of compounds it seemed clear that extension of the phenoxy (PhO) substituent (R^1) to benzyloxy ($PhCH_2O$) would improve the overlap. As the benzyloxy series would also serve as potential precursors to the target structures having R^1 = OH (*i.e.* small R^1), the synthetic effort was next directed to these compounds.

Benzyloxyacetyl chloride (3; R^1 = $PhCH_2O$) when con-

densed with the imine (4) gave the two *cis* β-lactam esters (7A) and (7B). Careful inspection of the ^{1}H n.m.r. spectra of these compounds has proved useful in making a tentative assignment of *absolute* configuration by comparisons with the stereochemically defined series (5A) and (5B). Knowing the absolute configurations of (5A) and (5B) and comparing the chemical shifts of the alanine methyl and methine protons of the two isomers in *both* series it is possible to assign the *absolute* stereochemistry of (7A) and (7B) with some confidence as shown from the correlation which is observed (Table 1). This correlation has been further used and is discussed below. Deprotection with trifluoroacetic acid gave the target acids (8A) and (8B).

Hydrogenolysis of the benzyl groups of (7A) and (7B) would provide the other target β-lactam structures (9A) and (9B); however it has been reported [11] that cleavage of this type of β-lactam ring occurs under mild hydrogenolysis conditions and indeed this reaction has been used to good effect by Ojima [11,12] for a stereospecific synthesis of dipeptides. Accordingly our hydrogenolysis reaction was carefully controlled; even so, hydrogenolysis of (7A) gave the desired β-lactam (9A) (*i.e.* ' x ' cleavage—see Scheme 2) after chromatography as the minor product only, with the ring opened product (11A) (' x ' and ' y ' cleavage, Scheme 2) being isolated as the major product. Both of the esters (9A) and (11A) were converted into the acids (10A) and (12A) respectively, by treatment with trifluoroacetic acid (TFA).

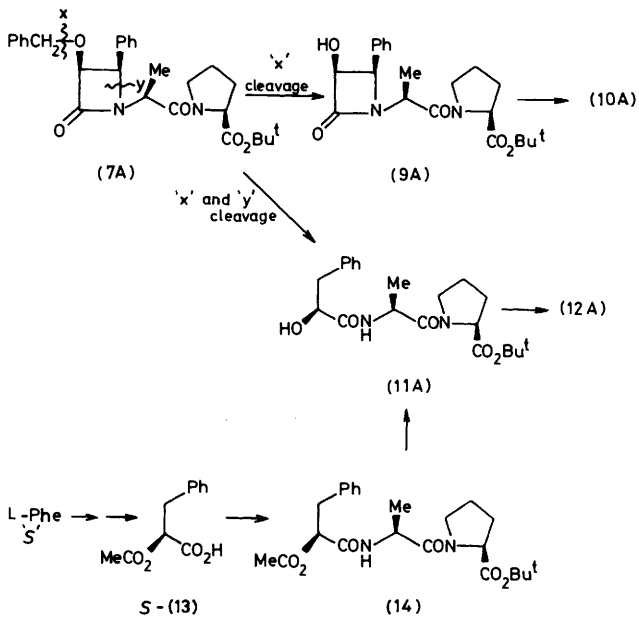
In an identical manner the ester (7B) was hydrogenolysed to the β-lactam (9B) and the ring-opened product (11B) but all attempts to cleave (9B) to the acid (10B) gave intractable mixtures. The ring-opened acid (12B) was, in contrast, satisfactorily produced by cleavage of (11B).

The alternative approach to the β-lactam acids (10A) and (10B) by direct hydrogenolytic cleavage of the acids (8A) and (8B) was unsuccessful in both cases giving only the ring-opened acids (12A) and (12B). Consequently it has not been possible to make the β-lactam acid (10B).

The formation of the ring-opened products has proved useful however in that it has enabled an unequivocal assignment of absolute stereochemistry to be made in the 3-benzyloxy substituted series [*i.e.* esters (7A) and (7B)] which has confirmed the chemical shift correlations based on the *X*-ray structures obtained in the 3-phenoxy series. This has been achieved by the synthesis of (11A) from stereochemically defined starting materials (see Scheme 2). The hydrogenolysis of (7A) to give (11A) removes the chirality at C-4 and (we assume) retains the configuration at C-3, so it only remains to define the absolute stereochemistry of this centre in order to define that of the β-lactam (7A) as a whole.

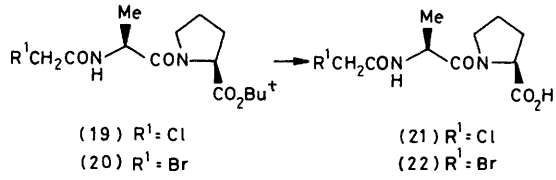
L-Phe (*S* configuration) was transformed by nitrous acid to 2-hydroxy-3-phenylpropionic acid with retention of configuration.[13] Protection of the hydroxy group by acetylation and condensation of the *S* product (13) with L-Ala-L-ProO-Bu^t gave (14) which on saponification with dilute alkali gave only one product which was shown to be identical with (11A) [and different from (11B)] by comparison of h.p.l.c. data, i.r., and n.m.r. spectra, and by m.p.s. Furthermore *in situ* acetylation of (11A) and (11B) and comparison of the products so formed with (14) showed that the latter was identical with the acetylation product of (11A) and different from that of (11B). This serves to define the absolute stereochemistry of (11A) and (11B) and, therefore, also that of the β-lactams (7A) and (7B) as shown in Scheme 2 and coincides with the chemical-shift assignment.

3-Chloro substituted β-lactams have been made by condensation of (4) with chloroacetyl chloride (3; R^1 = Cl) giving the *cis* β-lactam (15) after chromatography. The amide (19), presumably resulting from the reaction of acid chloride with



Scheme 2.

L-Ala-L-ProOBu^t formed from prior hydrolysis of the imine (4), was also isolated from the reaction mixture. Deprotection of the esters (15) and (19) to the corresponding acids (16) and (21) was carried out with TFA. A similar sequence of reactions was carried out using bromoacetyl bromide.



The α-halogenoamide acids (21) and (22) derived from the fortuitously formed esters (19) and (20) could be active-site directed irreversible inhibitors of the enzyme and are, therefore, interesting in their own right.

Using the chemical-shift correlation discussed above it is possible to assign these halogeno substituted β-lactams to the A series (see Table 1).

The good correlation observed for the use of this chemical shift data to assign absolute stereochemistry in these two diastereoisomeric series, even with data from different n.m.r. solvents, suggests that this observation may be generally predictive for structures of the type (2) above (see Table 1).

The last structural variation of the β-lactam (2) arose from our aim to search for an enzyme nucleophile *at* or close to the active site. Incorporation of a further reactive group into the existing structure seemed to be a reasonable approach and led to the potential Michael acceptor structures, the α-methylene-β-lactams. The synthesis of these compounds has followed literature precedent.[14–16]

Condensation of (4) with 2-phenylselenopropanoyl chloride and careful chromatography gave three isomeric β-lactams (23), (24), and (25) in poor yield. No further work was attempted on (25) because of the lack of material. Esters (23) and (24) were converted into the corresponding acids (26) and (27) using TFA in the usual way. Acid (26) was then con-

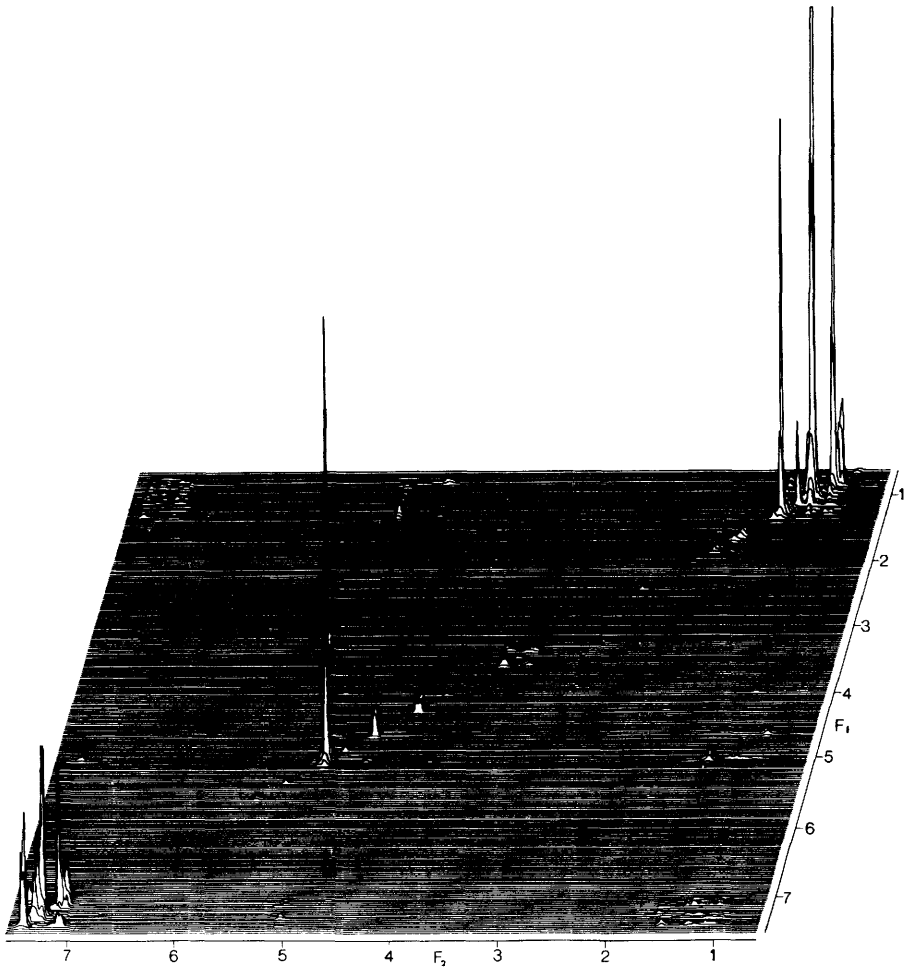


Figure 2. 3-D Stacked plot of N.O.E.S.Y. data on compound (25)

Table 1.

	A series			B series		
Compd.	Ala-CH_3 δ	Ala-CH δ	Solvent *	Ala-CH_3 δ	Ala-CH δ	Solvent
5	1.58	4.47	C	1.22	4.98	C
6	1.35	4.56	S	1.00	4.67	S
7	1.50	4.40	C	1.08	4.79	C
8	1.30	4.50	S	1.00 1.08	4.56 4.79	S C
9	1.45	4.40	C	0.95	4.60	S
10	1.31	4.45	S			
15	1.50	4.33	S	—	—	—
16	1.48	4.38	S	—	—	—
17	1.53	4.30	S	—	—	—
18	1.48	4.35	S	—	—	—

* C = ($CDCl_3$) and S = ([$^2H_6$]-DMSO).

verted into the target α-methylene-β-lactam (28). An analytical-scale transformation of the isomer (27) under identical conditions also gave (28) which serves to establish that (26) and (27) have the same relative stereochemistry at C-4 of the β-lactam ring.

Because of the different substitution pattern on the β-lactam rings of compounds (23), (24), and (25) the chemical-shift correlation illustrated in Table 1 is not applicable and, consequently, no assignment of absolute stereochemistry has been made in this series. However the relative stereochemistry of the substituents on the β-lactam rings of these compounds has been assigned from differential nuclear Overhauser effect (n.O.e.) enhancements.[17] The data are summarised in Table 2.

Table 2. Nuclear Overhauser enhancements for (23), (24), and (25) observed by difference spectroscopy

	Signal irradiated	Signal(s) enhanced †
(23)	Ala-Me	4-H, Ala H(q)
	3-Me	—
(24)	Ala-Me, 3-Me *	4-H, Ala H(q) 4-H
(25)	Ala-Me	—
	3-Me	4-H

* Overlap of signals precluded unequivocal separate irradiation of the methyl groups. † Small enhancements of the aromatic envelope were always observed.

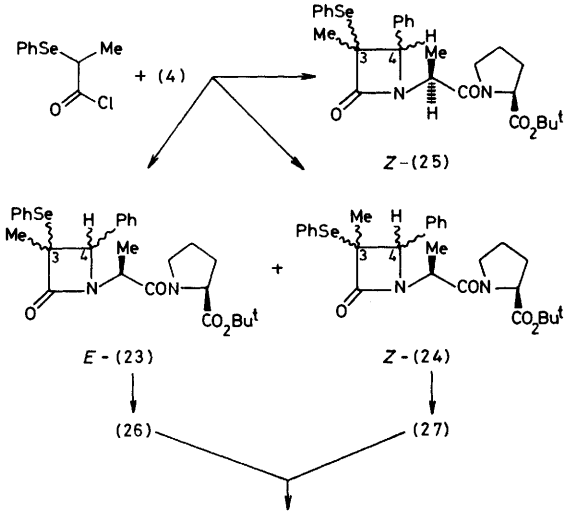
The n.O.e. experiments were carried out by irradiation at the ring 3-methyl group in each of (23), (24), and (25) which gave enhancements of different size on the adjacent 4-methine proton. No enhancement was observed in compound (23) indicating a *trans* disposition of the two substituents in this molecule (*i.e.* *E* configuration). Compounds (24) and (25) both gave enhancements on the 4-methine proton indicating *cis* disposition [*i.e.* both (24) and (25) have the *Z* configuration

on the ring]. In compound (24) the similarities of the chemical shifts of the 3-methyl group (δ 1.66) and the alanine methyl doublet (δ 1.62) made unequivocal irradiation of the former signal difficult in this n.O.e. experiment but the *Z* configurational assignment for (24) is confirmed from the fact that (23) and (24) have the same stereochemistry at C-4 (*vide supra*) and hence must be different at C-3.

Because the n.O.e. enhancement was small in compound (25) the effect was confirmed by a two dimensional N.O.E.S.Y. experiment. The 3-D stacked plot of the data (Figure 2) clearly shows the interaction between the 4-methine (δ 5.16) and the 3-methyl group as off-diagonal peaks which occur symmetrically at either side of the diagonal contour spectrum.

It is of interest that irradiation of the alanine methyl group in each of compounds (23), (24), and (25), gave n.O.e. enhancements on the ring 4-methine proton in (23) and (24) which have the same configuration at this centre, but very little enhancement on this proton in (25) where the configuration at C-4 must be inverted relative to (23) and (24). The implications of these results are that with the availability of one reference absolute configurational assignment (presumably from *X*-ray crystallography) it would be possible to predict the *absolute* configurations of the β-lactam ring substituents from the n.O.e. experiments using the alanine methyl group as a reference point of defined absolute configuration.

The relative configurational assignments of (23), (24), (25), and the derived (28) are shown in Scheme 3.



Scheme 3. *Note.* The substituents shown connected vertically to C-3 and C-4 in this Scheme are *cis* with respect to each other

Testing of these target β-lactam structures against ACE has shown no potent inhibition of the enzyme, although some of the acyclic derivatives described herein have significant inhibitory activity.

The next rational step is to remove the 4-Ph group from the β-lactam structures to investigate whether this precludes binding but it is important also to investigate the possibility that the presence of a tertiary nitrogen at the cleavable amide bond is not acceptable to the enzyme. This latter point is being addressed by movement of the ' Ala-Pro ' recognition function from nitrogen to the adjacent carbon in the ring.

Experimental

I.r. spectra were determined with a Perkin-Elmer 157G or 297 instrument for KBr discs, Nujol mulls, or liquid films. ^{1}H N.m.r. spectra were recorded using a Perkin-Elmer R12B (60 MHz) or a Jeol JNM-PMX60 (60 MHz) spectrometer operating in the continuous-wave mode and a Bruker WP-80 (80 MHz) or a Bruker HFX-90 (90 MHz) instrument operating in the Fourier-transform mode [tetramethylsilane or 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionic acid, sodium salt as internal standard]. Resonances are reported as p.p.m. downfield from the tetramethylsilane position on the δ scale.

All products were routinely checked for homogeneity by t.l.c. on silica-gel plates (E. Merck, 60F254, 0.25 mm) using the solvents indicated in the text. The spots were located either by filtered u.v. light ($\lambda_{max.}$ 254 and 365 nm), iodine, 1% t-butyl hypochlorite in cyclohexane followed by 1% starch/1% potassium iodide in water. Optical rotations were determined on a Bendix N.P.L. automatic polarimeter. Water of hydration reported in the microanalytical figures was confirmed either by n.m.r. or by loss on drying and re-analysis. Reaction times were not optimised.

L-Alanyl-L-proline t-butyl ester was prepared following the procedure of Hugeunin and Guttmann [18] and was characterised as the diacetate (Found: C, 53.45; H, 8.45; N, 7.85. Calc. for $C_{16}H_{30}N_2O_7$: C, 53.0; H, 8.35; N, 7.75%).

Preparation of N-*Benzylidene*-L-*alanyl*-L-*proline t-Butyl Ester* (4).—L-Alanyl-L-proline t-butyl ester (Ala-Pro-OBu^t) diacetate (28.0 g), triethylamine (43 ml), benzaldehyde (7.9 ml) and anhydrous magnesium sulphate (11.16 g) in dry dichloromethane (130 ml) were refluxed for 22 h, with exclusion of atmospheric moisture. The reaction mixture was filtered, the residual solid washed with dichloromethane and the combined filtrates washed with water (3 × 70 ml) and dried (Na_2SO_4). Filtration and evaporation gave the imine (4) (18.01 g, 71%); $\nu_{max.}$ (KBr disc) 2 975, 1 735, and 1 643 cm^{-1}; δ ($CDCl_3$) 1.43 (12 H, m, CMe_3 and CH_3CH), 1.76—2.40 (4 H, m, $NCH_2CH_2CH_2$), 3.40—4.00 (2 H, m, $NCH_2CH_2CH_2$), 4.28—5.00 (2 H, m, $CHCO_2CMe_3$ and CH_3CH), 7.20—8.00 (5 H, m, Ph), and 8.06 and 8.20 (1 H, two singlets in ratio 2 : 7, PhC*H*=).

Preparation of Phenoxyacetyl Chloride (3; R^1 = PhO).—Phenoxyacetic acid (50 g) and thionyl chloride (29 ml) in chloroform (150 ml) were refluxed for 3 h, with exclusion of atmospheric moisture. Volatile material was removed under reduced pressure at 40 °C and the residue was distilled at the water-pump; the product (21.4 g, 38%) had b.p. 115—118 °C; $\nu_{max.}$ (liq. film) 1 810 cm^{-1}; δ ($CDCl_3$) 4.74 (2 H, s, CH_2) and 6.40—7.20 (5 H, m, Ph).

Benzyloxyacetyl Chloride (3; R^1 = $PhCH_2O$).—Benzyloxyacetic acid [19] (11.0 g) and thionyl chloride (9.5 ml) were refluxed for 45 min with exclusion of atmospheric moisture. Excess of thionyl chloride was removed under reduced pressure at 40 °C and the residue was distilled to give three fractions; b.p. 49—59 °C/0.27 mmHg; 59—67 °C/0.27 mmHg (2.37 g); b.p. 67 °C/0.27 mmHg (5.685 g). These were identical [by i.r. $\nu_{max.}$ (liq. film) 1 800 cm^{-1}], and were combined (8.43 g, 69%); δ ($CDCl_3$) 4.32 (2 H, s), 4.56 (2 H, s), and 7.21 (5 H, s, Ph).

2-*Phenylselenopropanoyl Chloride*.—2-Phenylselenopropanoic acid [20] (15.0 g), thionyl chloride (5.7 ml), and dry DMF

(10 drops) in dry ether (200 ml) were stirred for 17 h at room temperature with the exclusion of atmospheric moisture. Removal of volatile material under reduced pressure at 40 °C and distillation of the residue gave the product (12.82 g, 79%) as a mobile yellow oil, b.p. 74—76 °C/0.08 mmHg, $\nu_{max.}$ (liq. film) 1 770 cm^{-1}; δ ($CDCl_3$) 1.52 (3 H, d, *J* 7.2 Hz, Me), 3.90 (1 H, q, *J* 7.2 Hz, CH), and 7.08—7.68 (5 H, m, Ph).

N-[2-(2-*Oxo*-3-*phenoxy*-4-*phenylazetidin*-1-*yl*)*propanoyl*]-L-*proline t-Butyl Ester* (5A) *and* (5B).—To a solution of *N*-benzylidene-L-alanyl-L-proline t-butyl ester (4) (1.0 g) and triethylamine (0.42 ml) in dry dichloromethane (25 ml) stirred at 0 °C was added phenoxyacetyl chloride (3; R^1 = PhO) (0.52 g) in dry dichloromethane (25 ml) during 35 min. The reaction mixture was then stirred for 1 h at 0 °C and for 48 h at room temperature. Washing of the reaction mixture with water (2 × 30 ml) and subsequent drying (Na_2SO_4) of the organic layer, filtration, and evaporation gave a crude white solid (1.107 g). Recrystallisation of this from chloroform–ether gave a first crop of (5A) (0.238 g, 17%), m.p. 185—187 °C; $[\alpha]_D^{15}$ = −91.9° ($CHCl_3$, *c* 0.356) (Found: C, 70.0; H, 6.7; N, 5.9. $C_{27}H_{32}N_2O_5$ requires C, 69.82; H, 6.90; N, 6.03%); $\nu_{max.}$ (KBr disc) 1 760, 1 720, and 1 660 cm^{-1}; δ ($CDCl_3$) 1.13 and 1.37 (9 H, 2 singlets in ratio 1 : 9 * CMe_3), 1.58 (3 H, d, *J* ≃ 6.5 Hz, CH_3), 1.67—2.0 (4 H, m, NCH_2CH_2-CH_2), 3.13—3.65 (2 H, m, $N^-CH_2CH_2CH_2$), 3.65—3.88 (1 H, m, $CHCO_2CMe_3$), 4.47 (1 H, q, *J* ≃ 6.3 Hz, CHMe), 5.00 (1 H, d, *J* 4.2 Hz) and 5.42 (1 H, d, *J* 4.2 Hz) (*cis*-coupled β-lactam methine protons), and 6.56—7.40 (10 H, m, 2 × Ph). The *X*-ray structure determination of (5A) is described later.

Evaporation of the mother-liquor from the recrystallisation gave a solid (0.685 g) shown by t.l.c. to be a mixture of (5A) and another product. These were separated by chromatography on silica gel (ART 9385, Kieselgel 60, Merck) using ethyl acetate–n-hexane (1 : 1) at 4 ml/min flow rate as eluant and monitoring absorbance at 280 nm.

The first eluted product was recrystallised from chloroform–ether to give (5B) (0.096 g, 7%), m.p. 164—165 °C, $[\alpha]_D^{15}$ = −44.6° ($CHCl_3$, *c* 0.358) (Found: C, 69.65; H, 7.0; N, 6.2. $C_{27}H_{32}N_2O_5$ requires C, 69.82; H, 6.90; N, 6.03%); $\nu_{max.}$ (KBr disc) 1 765, 1 723, and 1 646 cm^{-1}; δ ($CDCl_3$) 1.22 (3 H, d, *J* 7.3 Hz, CH_3), 1.56 (9 H, s, CMe_3), 1.95—2.44 (4 H, m, $NCH_2CH_2CH_2$), 3.50—3.95 (2 H, m, $NCH_2CH_2CH_2$), 4.47—4.70 (1 H, m, $CHCO_2CMe_3$), 4.98 (1 H, q, *J* 7.3 Hz, CH_3CH), 5.58 (1 H, d, *J* 4.2 Hz), and 5.67 (1 H, d, *J* 4.2 Hz) (*cis* coupled β-lactam methine protons), and 6.72—7.65 (10 H, m, 2 × Ph).

N-[2-(2-*Oxo*-3-*phenoxy*-4-*phenylazetidin*-1-*yl*)*propanoyl*]-L-*proline* (6A) *and* (6B).—Compound (5A) (0.428 g) was stirred in trifluoroacetic acid (3 ml) for 50 min with the exclusion of atmospheric moisture. Volatile material was removed under reduced pressure at 40 °C and the resulting gum was treated with water to give a solid which was recrystallised from acetone–n-hexane to give (6A) (0.194 g, 52%), m.p. 200.5—204 °C (Found: C, 67.7; H, 6.1; N, 6.8. $C_{23}H_{24}N_2O_5$ requires C, 67.65; H, 5.88; N, 6.86%); $\nu_{max.}$ (KBr disc) 1 800, 1 740, and 1 652 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.35 (3 H, d, *J* ≃ 6.5 Hz, Me), 1.65—2.20 (4 H, m, $NCH_2CH_2CH_2$), 3.00—3.79 (3 H + H_2O, broad hump + m, $NCH_2CH_2CH_2CH$ + H_2O), 4.56 (1 H, q, *J* ≃ 6.5 Hz, MeC*H*), 4.95 and 6.14 (1 H, 2d in ratio 1 : 14, *J* ≃ 4.4 Hz, for each, coalescence at 85 °C, PhC*H* *cis*/*trans* proline isomers), 5.50 and 5.63 (1 H, 2d in ratio 1 : 11, *J* ≃ 4.4 Hz for each, coalescence at 85 °C, PhOC*H* *cis*/*trans* proline isomers), and 6.60—7.43 (10 H, m, 2 × Ph).

In an identical manner (5B) was converted into the acid (6B) in 60% yield, m.p. 207—208 °C (Found: C, 67.4; H, 5.85; N, 6.95. $C_{23}H_{24}N_2O_5$ requires C, 67.65; H, 5.88; N, 6.86%); $\nu_{max.}$ (KBr disc) 1 770, 1 755sh, and 1 630 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.00 (3 H, d, *J* ≃ 7 Hz, *Me*), 1.63—2.23 (4 H, m, $NCH_2CH_2CH_2CH$), 3.00—3.77 (broad hump + m, $^-NCH_2$-CH_2CH_2 + H_2O), 4.12—4.33 (1 H, m, $CHCO_2H$), 4.67 (1 H, q, *J* ≃ 7 Hz, MeC*H*), 5.20 and 5.29 (1 H, 2 d in ratio 1 : 6, *J* ≃ 5 Hz for each, coalescence at 75 °C to 1 d at δ 5.33, PhOC*H* *cis*/*trans* proline isomers), 5.40 and 5.53 (1 H, 2 d in ratio 1 : 4, *J* ≃ 5 Hz for each, coalescence at 75 °C to 1 d at δ 5.59, PhC*H* *cis*/*trans* proline isomers), and 6.58—7.45 (10 H, m, 2 × Ph).

N-[2-(3-*Benzyloxy*-2-*oxo*-4-*phenylazetidin*-1-*yl*)*propanoyl*]-L-*proline t-Butyl Ester* (7A) *and* (7B).—To a stirred solution of *N*-benzylidene-L-alanyl-L-proline t-butyl ester (4) (14.7 g) and triethylamine (6.2 ml) in dry dichloromethane (1 000 ml) was added at 0 °C benzyloxyacetyl chloride (8.22 g) in dry dichloromethane (500 ml) during 2 h. The reaction mixture was stirred for a further 17 h at room temperature after which it was washed with water (2 × 250 ml), dried (Na_2SO_4), filtered, and evaporated to give a solid (19.768 g) which was recrystallised twice from chloroform–light petroleum (b.p. 60—80 °C) to give (7A; R^1 = $PhCH_2O$): first crop 1.441 g, m.p. 173—175 °C; second crop, 0.609 g, m.p. 172—173 °C (total yield 10%). For the first crop (Found: C, 70.1; H, 7.15; N, 5.55. $C_{28}H_{34}N_2O_5$ requires C, 70.29; H, 7.11; N, 5.86%), $\nu_{max.}$ (KBr disc) 1 748, 1 725, and 1 652 cm^{-1}; δ ($CDCl_3$) 1.18 and 1.40 (9 H, 2 s in ratio 1 : 16, CMe_3 in *cis*/*trans* proline isomers), 1.50 (3 H, d, *J* ≃ 6.5 Hz, *Me*CH), 1.65—2.60 (4 H, br m, $NCH_2CH_2CH_2$), 3.20—3.92 (3 H, br m, $CH_2CH_2CH_2CH$), 4.15 and 4.40 (2 H, 2 d, *J* 11 Hz, $PhCH_2O$), 4.40 (1 H, q, *J* 6.5 Hz, MeC*H*), 4.64 and 4.82 (2 H, 2 d, *J* 3.4 Hz, *cis* $PhCH_2OCH^-CH$Ph), and 6.60—7.60 (10 H, m, 2 × Ph).

Evaporation of the mother-liquor afforded a gum (13.37 g) which was chromatographed on silica gel (ART 9385) using ethyl acetate–cyclohexane (1 : 2) as eluant at a flow rate of 4 ml/min. The first band eluted gave a gum which crystallised when set aside at room temperature for 1 week to give a waxy solid (7B) (1.253 g, 6%), m.p. 80—84 °C (Found: C, 70.4; H, 7.15; N, 5.65. $C_{28}H_{34}N_2O_5$ requires C, 70.29; H, 7.11; N, 5.86%); $\nu_{max.}$ (KBr disc) 1 755, 1 725, and 1 655 cm^{-1}; δ ($CDCl_3$), 1.06 and 1.10 (3 H, 2 d, *J* ≃ 6 Hz, *Me*CH *cis*/*trans* proline isomers), 1.44 and 1.50 (9 H, 2 s, CMe_3 in *cis*/*trans* proline isomers), 1.76—2.30 (4 H, br m, $NCH_2CH_2CH_2$), 3.33—3.86 (2 H, br m, $NCH_2CH_2CH_2$), 4.07 and 4.28 (2 H, 2 d, *J* 11 Hz, $PhCH_2O$), 4.33—4.53 (1 H, br m, $CHCO_2CMe_3$), 4.79 (1 H, q, *J* ≃ 6 Hz, *H*CMe), 4.88 (1 H, d, *J* 4.2 Hz, *cis*-$PhCH_2$-OC*H*), 5.33 (1 H, d, *J* 4.2 Hz, *cis*-PhC*H*), and 6.79—7.58 (10 H, br m, 2 × Ph).

A second band eluted from the column gave a solid (0.334 g, 2%) shown by t.l.c. to be identical with (7A).

N-[2-(3-*Benzyloxy*-2-*oxo*-4-*phenylazetidin*-1-*yl*)*propanoyl*]-L-*proline* (8A) *and* (8B).—(7A) (0.5 g) was stirred with trifluoroacetic acid (3 ml) for 2 h with exclusion of atmospheric moisture. Evaporation left a gum which solidified on treatment with water. The solid was filtered off and recrystallised from acetone–n-hexane to give the product (8A) (R^1 = $PhCH_2O$) (0.2 g, 45%), m.p. 154—155.5 °C, $[\alpha]_D^{22}$ = −143.4° (MeOH, *c* 0.07) (Found: C, 68.25; H, 6.3; N, 6.4. $C_{24}H_{26}N_2O_5$ requires C, 68.25; H, 6.16; N, 6.64%); $\nu_{max.}$ (KBr disc) 1 754, 1 621, and 1 160 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.30 (3 H, d, *J* ≃ 7 Hz, *Me*CH), 1.63—2.00 (4 H, m, $NCH_2CH_2CH_2$), 3.00—3.80

* The *two* singlets shown for the t-butyl signal reflect the fact that at ambient temperature these dipeptides exist as *cis*- and *trans*-proline amides. This has been confirmed in other compounds (see below) where coalescence of these signals is observed when the temperature is raised.

(br m, NCH_2CH$_2$CH$_2$CH + H_2O), 3.88 and 4.25 (2 H, 2 d, J 11 Hz, PhCH_2O), 4.50 (1 H, q, $J \simeq 7$ Hz, CHMe), 4.88 and 4.98 (2 H, 2 d, J 5.4 Hz, for each, *cis*-PhCH$_2$OCH–CHPh), 6.78—7.00 (2 H, m), and 7.13—7.31 (3 H, m) (*Ph*CH$_2$O), and 7.38 (5 H, s, Ph).

The acid (8B) was prepared similarly in 54% yield from (7B), m.p. 48—56 °C, $[\alpha]_D^{25} = +23.6°$ (MeOH, *c* 0.06) (Found: C, 65.35; H, 6.2; N, 6.05. $C_{24}H_{26}N_2O_5 \cdot 1H_2O$ requires C, 65.45; H, 6.36; N, 6.36%); $\nu_{max.}$ (KBr disc) 1 750 and 1 645 cm^{-1}; δ (CDCl$_3$), 1.08 (3 H, d, $J \simeq 7$ Hz, *Me*CH), 1.79—2.40 (4 H, br m, NCH$_2$CH_2CH$_2$), 3.42—3.84 (2 H, br m, NCH_2CH$_2$CH$_2$), 4.05 and 4.26 (2 H, 2 d, J 11 Hz, PhCH_2O), 4.44—4.67 (1 H, m, CHCO$_2$H), 4.74 (1 H, q, J 7 Hz, CHMe), 4.91 and 5.24 (2 H, 2 d, J 4.4 Hz for each, *cis*-PhCH$_2$OCH–CHPh), 6.79—7.02 (2 H, m) and 7.09—7.28 (3 H, m) (*Ph*CH$_2$O), and 7.40 (5 H, s, Ph); δ ([$^2H_6$]-DMSO) 1.00 (3 H, d, $J \simeq 8$ Hz, *Me*CH), 1.58—2.44 (4 H, m, NCH$_2$CH_2CH_2), 3.10—3.73 (*ca.* 4 H, m, NCH_2CH$_2$CH$_2$ + H_2O), 3.84 and 4.18 (2 H, 2 d, J 11 Hz, non equivalent PhCH_2O protons), 4.22—4.60 (1 H, m, CHCO$_2$H), 4.56 (1 H, q, J 8 Hz, CHMe), 4.89 and 5.13 (2 H, 2 d, J 4 Hz, *cis*-PhCH$_2$OCH–CHPh), 6.64—6.91 (2 H, m) and 7.07—8.24 (3 H, m) (*Ph*CH$_2$O), and 7.36 (5 H, s, Ph).

Hydrogenolysis of N-[2-(3-*Benzyloxy*-2-*oxo*-4-*phenylazetidin*-1-*yl*)*propanoyl*]-L-*proline t-Butyl Esters* (7A) *and* (7B).—Compound (7A) (0.883 g) was hydrogenolysed under hydrogen (1 atm) in methanol (150 ml) containing 10% palladium on charcoal catalyst (0.1 g) for 96 h. One equivalent of hydrogen was consumed in this time. Filtration and evaporation of the filtrate gave a mixture of two products which were separated by fractional crystallisation from chloroform–ether. The first product *N*-[2-(3-hydroxy-2-oxo-4-phenylazetidin-1-yl)propanoyl]-L-proline t-butyl ester (9A) (0.07 g, 8%) had m.p. 215 °C (decomp.) (Found: C, 64.5; H, 7.2; N, 7.0. $C_{21}H_{28}N_2O_5$ requires C, 64.95; H, 7.22; N, 7.22%); $\nu_{max.}$ (KBr disc) 1 745, 1 660sh, and 1 657 cm^{-1}; δ (CDCl$_3$) 1.05 and 1.30 (9 H, 2 s in ratio 1 : 7.5, Me$_3$C), 1.45 (3 H, d, J 7 Hz, *Me*CH), 1.60—1.90 (4 H, br m, NCH$_2$CH_2CH_2), 2.10 (1 H, d, J 8 Hz, OH), 3.25—3.60 (2 H, br m, NCH_2CH$_2$CH$_2$), 3.70—4.03 (1 H, m, CHCO$_2$CMe$_3$), 4.40 (1 H, q, J 7 Hz, CHMe), 4.90 (1 H, d, J 5 Hz, CHPh), 5.03 (1 H, dd, J_{CH-CH} 5 Hz, J_{CHOH} 8 Hz, CHOH), and 7.35 (5 H, s, Ph).

The second product was *N*-(2-hydroxy-3-phenylpropanoyl)-L-alanine-L-proline t-butyl ester (11A), first crop (0.109 g), m.p. 132.5—134 °C; second crop (0.154 g), m.p. 129—131 °C (total yield 37%). For the first crop (Found: C, 64.9; H, 7.7; N, 7.05. $C_{21}H_{30}N_2O_5$ requires C, 64.62; H, 7.69; N, 7.18%), $\nu_{max.}$ (KBr disc) 1 721, 1 657, and 1 632 cm^{-1}; δ (CDCl$_3$) 1.32 (3 H, d, J 7 Hz, *Me*CH), 1.47 (9 H, s, Me$_3$C), 1.81—2.26 (4 H, br m, NCH$_2$CH_2CH_2), 2.95 (1 H, d, J 5 Hz, exchangeable with D$_2$O, OH), 2.93 (1 H, dd, J_{gem} 15 Hz, J_{CH_2CH} 7 Hz, CH_AH$_B$Ph), 3.26 (1 H, dd, J_{gem} 15 Hz, J_{CH_2CH} 4 Hz, CH$_A$H$_B$Ph), 3.42—3.81 (2 H, br m, NCH_2CH$_2$CH$_2$), 4.28 (1 H, m, dd on addition of D$_2$O, J_{CHCH_A} 7 Hz, J_{CHCH_B} 4 Hz, CHOH), 4.35 (1 H, t, J 5 Hz, CHCO$_2$CMe$_3$), 4.56—4.74 (1 H, m, MeCHNH), 7.28 (5 H, s, Ph), and 7.40 (1 H, d, J 8 Hz, NH).

Compound (7B) was hydrogenolysed in an identical manner to give (9B) in 13% yield, m.p. 156—157.5 °C (Found: C, 65.1; H, 7.3; N, 7.2. $C_{21}H_{28}N_2O_5$ requires C, 64.95; H, 7.22; N, 7.22%); $\nu_{max.}$ (KBr disc) 1 720 and 1 653 cm^{-1}; δ ([$^2H_6$]-DMSO) 0.95 (3 H, d, J 7 Hz, *Me*CH), 1.35 and 1.44 (9 H, 2 s in ratio 7.5 : 1, coalescence at 100 °C to δ 1.38, s, CMe$_3$), 1.63—2.16 (4 H, br m, NCH$_2$CH_2CH_2), 3.37—3.65 (2 H, br m, NCH_2CH$_2$CH$_2$), 4.05—4.23 (1 H, br m, CHCO$_2$CMe$_3$), 4.60 (1 H, br m which sharpens to a quartet at 100 °C, CHMe), 4.77 (2 H, m, CHOH), 5.05 (1 H, br s which sharpens to a doublet at 100 °C, J 5 Hz, CHPh), and 7.33 (5 H, s, Ph).

The second product obtained by fractional crystallisation was (11B) in 17% yield, m.p. 138.5—140 °C (Found: C, 64.55; H, 7.7; N, 7.15. $C_{21}H_{30}N_2O_5$ requires C, 64.62; H, 7.69; N, 7.18%); $\nu_{max.}$ (KBr disc) 1 720, 1 645sh, and 1 638 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.18 (3 H, d, J 7 Hz, *Me*CH), 1.38 (9 H, s, CMe$_3$), 1.60—2.20 (4 H, br m, NCH$_2$CH_2CH_2), 2.55—3.15 (2 H, br m, non equivalent PhCH_2), 3.38—3.80 (2 H, br m, –NCH$_2$–), 3.90—4.33 (2 H, br m, HOCH and CHCO$_2$CMe$_3$), 4.55 (1 H, quint., J_{CHNH} 8 Hz, J_{CHMe} 8 Hz, CHMe), 5.60 (1 H, d, J 5 Hz, exchangeable in D$_2$O, OH), 7.25 (5 H, s, Ph), and 7.83 (1 H, d, J 8 Hz, exchangeable in D$_2$O, NH).

N-[2-(3-*Hydroxy*-2-*oxo*-4-*phenylazetidin*-1-*yl*)*propanoyl*]-L-*proline* (10A).—Compound (9A) (0.15 g) was stirred with trifluoroacetic acid (2 ml) for 1 h with exclusion of atmospheric moisture. Evaporation of the acid afforded a gum. Treatment with water gave a white solid which was recrystallised from ethanol–light petroleum (b.p. 60—80 °C) to give (10A) (0.045 g, 36%), m.p. 195 °C (decomp.) (Found: C, 61.4; H, 6.2; N, 8.15. $C_{17}H_{20}N_2O_5$ requires C, 61.45; H, 6.02; N, 8.43%); $\nu_{max.}$ (KBr disc) 1 755, 1 724, and 1 620 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.31 (3 H, d, J 7 Hz, *Me*CH), 1.65—2.06 (4 H, br m, NCH$_2$–CH_2CH_2), 3.0—3.7 (m, NCH_2 and CHCO$_2$CMe$_3$ under HOD peak), 4.45 (1 H, q, J 7 Hz, CHMe), 4.70 (1 H, d, J 5 Hz, CHPh), 4.93 (1 H, d, J 5 Hz, CHOH), and 7.26 (5 H, s, Ph).

All attempts to convert (9B) into the acid (10B) with trifluoroacetic acid gave intractable mixtures.

Hydrogenolysis of the benzyloxy acids (8A) and (8B) gave only the ring-opened compounds (12A) and (12B) respectively.

N-(2-*Hydroxy*-3-*phenylpropanoyl*)-L-*alanine*-L-*prolines* (12A) *and* (12B).—The ester (11A) (0.2 g) was stirred with TFA (2 ml) at room temperature for 3 h with exclusion of atmospheric moisture. Evaporation of TFA afforded a gum which solidified on treatment with water and was recrystallised from ethanol and water (51 mg, 30%), m.p. 228—229 °C (Found: C, 61.1; H, 6.6; N, 8.3. $C_{17}H_{22}N_2O_5$ requires C, 61.08; H, 6.59; N, 8.38%); $\nu_{max.}$ (KBr disc) 3 375, 1 727, and 1 620 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.02 and 1.10 (total 3 H, 2 d, J 6.3 Hz, CH_3CH *cis*/*trans* proline isomers), 1.56—2.30 (4 H, m, NCH$_2$CH_2CH_2), 2.58—3.00 (2 H, m, nonequivalent PhCH_2), 3.00—3.67 (NCH_2 + CO$_2$$H$ + HOD), 4.00—4.72 (3 H, m, CHCH$_3$ + CHCO$_2$H + HOCH), 5.70 (1 H, d, J 5 Hz, disappears on D$_2$O shake, OH), 7.19 (5 H, s, Ph), 7.56 and 7.65 (total 1 H, 2 d, J 8 Hz, disappear on D$_2$O shake, NH *cis*/*trans* proline isomers).

In a similar manner ester (11B) (0.145 g) was converted into the acid (12B) (0.029 g, 23%), m.p. 146.5—147.5 °C (Found: C, 60.75; H, 6.6; N, 8.25. $C_{17}H_{22}N_2O_5$ requires C, 61.08; H, 6.59; N, 8.38%); $\nu_{max.}$ (KBr disc) 3 380, 1 725, 1 685, and 1 620 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.15 (3 H + solvent impurity, d, J 7 Hz, CH_3CH), 1.63—2.25 (4 H, m, NCH$_2$CH_2CH_2), 2.63—3.00 (2 H, m, nonequivalent PhCH$_2$), 3.00—3.80 (NCH_2 + HOD), 3.95—4.40 (2 H, m, CHCO$_2$H + HOCH), 4.58 (1 H, 2 overlapping quartets each with J 7 Hz, CH$_2$–CHNH, *cis*/*trans* proline isomers), 5.63 (1 H, d, J 6 Hz, OH), 7.30 (5 H, s, Ph), and 7.78 and 7.83 (total 1 H, 2 d, J 7 Hz, NH, *cis*/*trans* proline isomers).

Synthesis of (2S)-N-(2-*Hydroxy*-3-*phenylpropanoyl*)-L-*alanine*-L-*proline t-Butyl Ester*.—(2*S*)-2-Hydroxy-3-phenylpropanoic acid was a gift from Dr. S. Wilkinson of these Laboratories. This material was made from L-phenylalanine by the method described by Shildneck.[21]

(2*S*)-2-Acetoxy-3-phenylpropanoic acid (13) was made by treating the above acid with acetic anhydride.[22] The product was isolated as an oil which would not crystallise. The n.m.r. spectrum showed the presence of a small amount of acetic

acid. The purity of this material was adequate for the subsequent transformations.

(2S)-N-(2-*Acetoxy*-3-*phenylpropanoyl*)-L-*alanine*-L-*proline t-Butyl Ester* (14).—Compound (13) (2.28 g) and *N*-methylmorpholine (1.11 g) were dissolved in methylene chloride (20 ml) and cooled to −25 °C under positive nitrogen pressure. Isobutyl chloroformate (1.43 g) dissolved in methylene chloride (5 ml) was added and the reaction mixture stirred for 3 min at −25 °C. A solution of L-alanyl-L-proline t-butyl ester diacetate (3.62 g) and *N*-methylmorpholine (2.02 g) in methylene chloride (30 ml) was then added and the reaction mixture stirred for 4 h at 0—10 °C. The reaction mixture was washed with 10% citric acid solution (2 × 30 ml), saturated aqueous sodium hydrogen carbonate (2 × 30 ml), and brine (2 × 30 ml). The methylene chloride layer was then dried ($MgSO_4$) and evaporated to give (14) as a thick oil (5.02 g >100% yield—contains solvent impurity and hydrocarbon grease); δ ($CDCl_3$), 0.28 and 0.92 (impurity), 1.26 (3 H, d, *J* 7 Hz, *Me*CH), 1.44 (9 H, s, Me_3C), 1.80—2.32 (4 H, br m, $NCH_2CH_2CH_2$), 2.05 (3 H, s, CH_3CO_2), 3.14 (2 H, d, *J* 6 Hz, $PhCH_2$), 3.40—3.84 (2 H, br m, $NCH_2CH_2CH_2$), 4.16—4.52 (1 H, br m, $CHCO_2CMe_3$), 4.72 (1 H, q, *J* 7 Hz, *CH*Me), 5.36 (1 H, t, *J* 6 Hz, $CHCH_2Ph$), 6.96—7.38 (6 H, br s, Ph and NH). This material showed as a single peak by h.p.l.c. reverse-phase chromatography on a 25 cm × 4.6 mm Zorbax C8 column using 40% acetonitrile–water as the mobile phase monitoring u.v. absorbance of eluate at 254 nm.

Acetylation of 1 mg samples of (11A) and (11B) was carried out by heating with acetic anhydride for 3 h at 100 °C.

H.p.l.c. chromatography of these acetylated samples under the above conditions established that the synthetic *S*-product was identical with (11A) and different from (11B). This was confirmed by chromatography of mixtures of the samples.

(2S)-N-(2-*Hydroxy*-3-*phenylpropanoyl*)-L-*alanine*-L-*proline t-Butyl Ester*.—Compound (14) (0.864 g) was suspended in methanol (2 ml) and water (3 ml) and stirred at 0 °C. 1M-Sodium hydroxide (2.1 ml) was added dropwise during 10 min at 0 °C and then the reaction mixture was stirred for 4 h at room temperature. After this time t.l.c. (cyclohexane–ethyl acetate, 1 : 1) indicated complete reaction. Water (10 ml) was added and the mixture was extracted with ethyl acetate (3 × 20 ml). The latter was dried ($MgSO_4$) and evaporated to give a solid (0.46 g, 59% yield), which was recrystallised from chloroform–ether to give (2*S*)-*N*-(2-hydroxy-3-phenylpropanoyl)-L-alanine-L-proline t-butyl ester (0.18 g), m.p. 127—128 °C (Found: C, 64.65; H, 7.8; N, 7.1. $C_{21}H_{30}N_2O_5$ requires C, 64.6; H, 7.69; N, 7.18%).

The i.r. spectrum (KBr disc) was identical in all respects with that of (11A) and different from that of (11B). The n.m.r. spectrum ([$^2H_6$]-DMSO) was fully in accord with the above structure and was identical with the spectrum of (11A) and different from that of (11B) (both in [$^2H_6$]-DMSO) particularly regarding the chemical shifts of *Me*CH and in $PhCH_AH_B$. H.p.l.c. using the system and solvent described for the acetyl compound (14) above confirmed, using sample mixtures, that this synthetic 2*S*-product was identical with (11A) and different from (11B).

N-[2-(3-*Chloro*-2-*oxo*-4-*phenylazetidin*-1-*yl*)*propanoyl*]-L-*proline t-Butyl Ester* (15A).—To a solution of *N*-benzylidene-L-alanine-L-proline t-butyl ester (4) (12.69 g) and triethylamine (7.77 g, 10.7 ml) in dry benzene (1 000 ml) stirred and cooled at 5—10 °C was added chloroacetyl chloride (8.69 g, 6.12 ml) in dry benzene (600 ml) during 1.5 h under positive nitrogen pressure. After completion of the addition the mixture was allowed to warm to room temperature and stirred for 72 h. The solid was filtered off and identified as triethylamine hydrochloride (8.25 g, 78% based on triethylamine). The filtrate was washed with water (3 × 100 ml), dried (Na_2SO_4), filtered, and evaporated to leave a red gum (17.20 g) which was separated into two components by column chromatography (silica gel, ART 9385 Kieselgel 60, Merck) using cyclohexane–ethyl acetate (1 : 1) as eluant. The first product, recrystallised from chloroform–light petroleum (b.p. 60—80 °C), was (15A) [0.299 g, 2% based on imine (4)], m.p. 154—156 °C (Found: C, 62.15; H, 6.75; N, 6.75. $C_{21}H_{27}ClN_2O_4$ requires C, 61.99; H, 6.64; N, 6.89%); $\nu_{max.}$ (KBr disc) 1 782sh, 1 770, 1 745, and 1 655 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.08 and 1.25 (9 H, 2 s, coalescence at 100 °C to 1 s at δ 1.35, Me_3CH *cis*/*trans* proline isomers), 1.43 and 1.60 (3 H, 2 d, coalescence at 100 °C to 1 d at δ 1.50, *J* 7 Hz, *Me*CH), 1.68—2.30 (4 H, m, $NCH_2CH_2CH_2$), 3.38—3.63 (2 H, m, NCH_2), 3.73—4.05 (1 H, m, $CHCO_2CMe_3$), 4.43 (1 H, q, *J* 7 Hz, signal shifts to δ 4.33 at 100 °C, *CH*Me), 4.95 and 5.13 (1 H, 2 d, coalescence at 100 °C to 1 d at δ 5.10, *J* 5 Hz, ClCH), 5.45 and 5.55 (1 H, 2 d, coalescence at 100 °C to 1 d at δ 5.38, *J* 5 Hz, PhC*H*), and 7.43 (5 H, s, Ph).

The second product from the column crystallised from chloroform–light petroleum (b.p. 60—80 °C) was *N*-chloroacetyl-L-alanyl-L-proline t-butyl ester (19) (0.749 g, 6% based on imine), m.p. 84—86.5 °C; $\nu_{max.}$ (KBr disc) 1 740, 1 695, 1 635sh, and 1 615 cm^{-1}; δ ($CDCl_3$) 1.16—1.68 (12 H, m, *Me*CH and CO_2CMe_3), 1.68—2.40 (4 H, m, $NCH_2CH_2CH_2$), 3.24—3.88 (2 H, m, NCH_2), 3.98 (2 H, s, $ClCH_2$), 4.12—5.00 (2 H, m, $CHCO_2CMe_3$ and MeC*H*), 7.54 (1 H, br d, slowly exchanges in D_2O, NH).

N-[2-(3-*Chloro*-2-*oxo*-4-*phenylazetidin*-1-*yl*)*propanoyl*]-L-*proline* (16A).—The ester (15A) (100 mg) was stirred with trifluoroacetic acid (2 ml) for 50 min at room temperature with exclusion of atmospheric moisture. Evaporation of the excess of acid and treatment of the residual yellow gum with water afforded a white solid which was recrystallised from chloroform and light petroleum (b.p. 60—80 °C) to give (16A) (42 mg, 49%), m.p. 171—173 °C (Found: C, 58.0; H, 5.4; N, 7.7. $C_{17}H_{19}ClN_2O_4$ requires C, 58.21; H, 5.42; N, 7.99%); $\nu_{max.}$ (KBr disc), 1 740, 1 652, and 1 180 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.40 and 1.58 (3 H, 2 d, coalescence at 100 °C to 1 d at δ 1.48, *J* 7 Hz, *Me*CH), 1.68—2.20 (4 H, m, $NCH_2CH_2CH_2$), 2.83—3.68 (m, $NCH_2 + H_2O$), 3.68—4.08 (1 H, m, $CHCO_2CMe_3$), 4.45 (1 H, q, *J* 7 Hz, signal shifts to δ 4.38 at 100 °C, MeC*H*), 4.95 and 5.10 (1 H, 2 d, coalescence at 100 °C to 1 d at δ 5.10, *J* 5 Hz, ClCH), 5.28 and 5.48 (1 H, 2 d, coalescence at 100 °C to 1 d at δ 5.40, *J* 5 Hz, PhC*H*), and 7.38 (5 H, s, Ph).

N-[2-(3-*Bromo*-2-*oxo*-4-*phenylazetidin*-1-*yl*)*propanoyl*]-L-*proline t-Butyl Ester* (17A).—To a solution of (4) (25.0 g) and triethylamine (15.4 g, 21 ml) in dry benzene (2 000 ml) at 5—10 °C was added bromoacetyl bromide (30.6 g, 13.2 ml) in dry benzene (500 ml) during 7.5 h under positive nitrogen pressure. The reaction mixture was stirred at room temperature for 3 d. Triethylamine hydrobromide (26.91 g, 97% based on triethylamine) was removed by filtration and the filtrate was washed with water (3 × 300 ml), dried, and evaporated to a gum (33.32 g) which was fractionated by column chromatography using cyclohexane–ethyl acetate (2 : 1) as eluant. The first product, recrystallised from chloroform–light petroleum (b.p. 60—80 °C) was (17A) (0.184 g, 0.5% based on amine), m.p. 147.5—150 °C (Found: C, 55.75; H, 6.0; N, 6.05. $C_{21}H_{27}BrN_2O_4$ requires C, 55.88; H, 5.99; N, 6.21%); $M^{+\cdot}$ 450.1154 ($C_{21}H_{27}{}^{79}BrN_2O_4$ requires 450.4537); $\nu_{max.}$ (KBr disc) 1 783, 1 760, 1 740, and 1 654 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.08 and 1.30 (9 H, 2 s, coalescence at 110 °C to 1 s at δ 1.35, CO_2CMe_3, *cis*/*trans* proline isomers), 1.43 and 1.60 (3 H, 2 d,

coalescence at 110 °C to 1 d at δ 1.53, *J* 7 Hz, *Me*CH), 1.70—2.18 (4 H, m, $NCH_2CH_2CH_2$), 3.35—3.65 (2 H, m, NCH_2), 3.70—4.10 (1 H, m, $CHCO_2CMe_3$), 4.38 (1 H, q, shifts to δ 4.30 at 110 °C, *J* 7 Hz, MeC*H*), 4.88 and 5.05 (1 H, 2 d, coalescence at 110 °C to 1 d at δ 5.08, *J* 5 Hz, BrCH), 5.53 and 5.58 (1 H, 2 d, coalescence at 110 °C to 1 d at δ 5.50, *J* 5 Hz, PhC*H*), and 7.18—7.53 (5 H, m, coalescence at 110 °C to 1 s at δ 7.40, Ph).

The second product, recrystallised from chloroform–light petroleum (b.p. 60—80 °C) was (20) (0.615 g, 2.6% based on imine), m.p. 69.5—71 °C (Found: C, 46.55; H, 6.35; N, 7.6. $C_{14}H_{23}BrN_2O_4$ requires C, 46.28; H, 6.34; N, 7.71%); $\nu_{max.}$ (KBr disc) 1 736, 1 690, and 1 632 cm^{-1}; δ ($CDCl_3$) 1.44 (3 H, d, *J* 6 Hz, *Me*CH), 1.50 (9 H, s, CMe_3), 1.71—2.40 (4 H, m, $NCH_2CH_2CH_2$), 3.40—3.80 (2 H, m, NCH_2), 3.86 (2 H, s, $BrCH_2$), 4.20—4.96 (2 H, m, C*H*Me and $CHCO_2CMe_3$), and 7.48 (1 H, br d, *J* 6 Hz, NH).

N-[2-(3-*Bromo*-2-*oxo*-4-*phenylazetidin*-1-*yl*)*propanoyl*]-L-*proline* (18A).—Compound (17A) (80 mg) was stirred with trifluoroacetic acid (1 ml) for 1 h at room temperature, with exclusion of atmospheric moisture. The excess of acid was evaporated and the orange residue triturated with water to give an orange solid. This was recrystallised from ethanol–light petroleum (b.p. 60—80 °C) to give (18A) (23 mg, 32%), m.p. 159.5—162 °C (Found: C, 51.65; H, 4.9; N, 6.9. $C_{17}H_{19}BrN_2O_4$ requires C, 51.65; H, 4.81; N, 7.09%); $\nu_{max.}$ (KBr disc) 1 748, 1 737, and 1 652 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.40 and 1.55 (3 H, 2 d, coalescence at 110 °C to 1 d at δ 1.48, *J* 7 Hz, *Me*CH *cis*/*trans* proline isomers), 1.68—2.25 (4 H, m, $NCH_2CH_2CH_2$), 3.0—3.65 (m, $NCH_2 + H_2O$), 3.80—4.13 (1 H, m, $CHCO_2CMe_3$), 4.45 (1 H, q, *J* 7 Hz, signal shifts at 110 °C to δ 4.35, C*H*Me), 4.95 and 5.15 (1 H, 2 d, coalescence at 110 °C to 1 d at δ 5.09, *J* 5 Hz, BrCH), 5.55 and 5.59 (1 H, 2 d, coalescence at 110 °C to 1 d at δ 5.48, *J* 5 Hz, PhC*H*), and 7.18—7.58 (5 H, m, Ph).

N-[2-(3-*Methyl*-2-*oxo*-3-*phenylseleno*-4-*phenylazetidin*-1-*yl*)-*propanoyl*]-L-*proline t-Butyl Esters* (23), (24), *and* (25).—To a solution of (4) (34 g) and triethylamine (20.8 g, 29 ml) in dry benzene (1 500 ml) was added a solution of 2-phenylselenopropanoyl chloride (51.0 g) in dry benzene (800 ml) during 4.5 h at 5—10 °C under positive nitrogen pressure. The reaction mixture was stirred for 45 h at room temperature. Triethylamine hydrochloride (26.7 g, 94% based on triethylamine) was filtered off and the filtrate was washed with water (2 × 250 ml), dried (Na_2SO_4), and evaporated to give an orange gum (78.6 g). This complex mixture was separated into four fractions by chromatography (silica gel, ART 9385, Merck, ethyl acetate–cyclohexane, 1 : 1). Fraction 1 (2.175 g) was shown to be 2-phenylselenopropanoic acid by comparison with an authentic sample.

Fraction 2 (7.014 g) containing (23), (25), and 2-phenylselenopropanoic acid was recrystallised to give (23) (0.247 g). The mother-liquor was chromatographed (ethyl acetate–cyclohexane, 1 : 2) to give (25) contaminated with 2-phenylselenopropanoic acid. The latter was removed by filtration of the mixture dissolved in chloroform–ether (1 : 1) through type O alumina. A further purification step for the purposes of complete characterisation was achieved by h.p.l.c. on a Zorbax silica column using 33% ethyl acetate–cyclohexane as the mobile phase and monitoring the u.v. absorbance at 280 nm of the eluate.

Fraction 3 (38.64 g) containing the main bulk of (23) and (24) was recrystallised twice from chloroform–ether to give (24) (2.172). Sequential chromatography on two separate columns (ethyl acetate–cyclohexane 1 : 1 and 1 : 2) of the mother-liquors gave incomplete separation; thus, the fractions containing both compounds were rechromatographed to give (23) (3.47 g) and (24) (3.81 g).

Fraction 4 (1.99 g) containing mainly (24) was chromatographed (ethyl acetate–cyclohexane, 1 : 2) to purify this material (0.158 g obtained).

These isomers exhibited the following properties. The β-*lactam* (23) was obtained by recrystallisation from ether–light petroleum (b.p. 60—80 °C)–chloroform (3.715 g, 6.7%), m.p. 150.5—152 °C (Found: C, 61.9; H, 6.3; N, 5.1. $C_{28}H_{34}N_2O_4Se$ requires C, 62.11; H, 6.28; N, 5.18%); $\nu_{max.}$ (KBr disc) 2 980, 1 749, 1 654, 1 431, 1 372, and 1 160 cm^{-1}; δ ([$^2H_6$]-DMSO) 0.95 (3 H, d, *J* 6.7 Hz, *Me*CH), 1.03 (3 H, s, *Me*CSe), 1.37 (9 H, s, CMe_3), 1.58—2.23 (4 H, m, $NCH_2CH_2CH_2$), 3.42—3.70 (2 H, m, NCH_2), 3.70—3.93 (1 H, m, $CHCO_2CMe_3$), 4.40 (1 H, q, *J* 6.3 Hz, C*H*Me), 4.81 (1 H, s, PhC*H*), 7.10—7.56 and 7.56—7.88 (10 H, 2 m, 2 × Ph).

The β-*lactam* (24) was obtained similarly by recrystallisation (6.698 g, 12%), m.p. 154—156 °C (Found: C, 62.15; H, 6.3; N, 5.05. $C_{28}H_{34}N_2O_4Se$ requires C, 62.11; H, 6.28; N, 5.18%); $\nu_{max.}$ (KBr disc) 2 980, 1 741, 1 660, 1 653, and 1 150 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.04 and 1.36 (9 H, 2 s, CMe_3), 1.50 (3 H, d, *J* 7 Hz, *Me*CH), 1.61 (3 H, s, *Me*CSe), 1.68—2.15 (4 H, m, $NCH_2CH_2CH_2$), 3.38—3.78 (2 H, m, NCH_2), 3.85—4.13 (1 H, m, $CHCO_2CMe_3$), 4.20—4.55 (1 H, m, MeC*H*), 4.67 (1 H, s, PhC*H*), and 7.15—7.65 (10 H, m, 2 × Ph).

The β-*lactam* (25) (0.167 g, 0.3%), m.p. 157.5—159 °C (Found: C, 62.35; H, 6.3; N, 4.95. $C_{28}H_{34}N_2O_4Se$ requires C, 62.11; H, 6.28; N, 5.18%); $\nu_{max.}$ (KBr disc) 1 757, 1 735sh, 1 650sh, 1 640, 1 367, and 1 152 cm^{-1}; δ ([$^2H_6$]-DMSO), 1.08 (3 H, d, *J* 7 Hz, *Me*CH), 1.33 and 1.43 (9 H, 2 s, CMe_3), 1.58 (3 H, s, MeCSe), 1.68—2.25 (4 H, m, $NCH_2CH_2CH_2$), 3.43—3.78 (2 H, m, NCH_2), 4.05—4.35 (1 H, m, $CHCO_2CMe_3$), 4.78 (1 H, q, *J* 7 Hz, C*H*Me), 5.06 (1 H, s, PhC*H*), and 7.08—7.70 (10 H, m, 2 × Ph).

Nuclear Overhauser Effect Studies.—The 360 MHz ^1H n.m.r. spectra were obtained on a Bruker WM 360 instrument. All samples were run in $CDCl_3$ at 20 °C; for the n.O.e. measurements samples were degassed by bubbling nitrogen through them.

The one-dimensional spectra were run with a spectral width of 4 000 Hz and 32 K data points giving a digital resolution of 0.244 Hz/point. The pulse width was 2 μs (30°), the acquisition time was 4.096 s; no relaxation delay was applied. Quadrature detection and phase cycling were used in all cases and 64 transients recorded for each spectrum, no window function was applied before transformation.

For the n.O.e. difference spectra a 1.0 s relaxation delay was used and the decoupler gated on for 5.0 s prior to acquisition to generate the n.O.e.s. The decoupler power level was varied until sufficient selectivity together with adequate n.O.e.s were obtained. Control spectra were run with the decoupler frequency set at the edge of the spectral range. Spectra were subtracted rather than F.I.D.s.

The two dimensional N.O.E.S.Y. experiment was obtained using the pulse sequence [23] 90°-t_1-90°-t_m-90-acq(t_2). A standard Bruker pulse program and phase-cycling sequence was used for the Aspect 2000 pulse programmer. The sweep width in the t_2,F_2 dimension was 2 500 Hz with 1 024 data points and in the t_1,F_1 dimension ±1 250 Hz with 256 F.I.D.s (32 transients each +2 dummy scans) recorded, giving 256 data points; this was zero filled to 512 before transformation. The spectral digital resolution was 4.883 Hz/point in both dimensions. The 90° pulse width was 6.0 μs. A recycle time of 3.0 s was used and the mixing time t_m was 0.8 s which was randomly varied by 15% to suppress scalar coupling effects.[24] A sine bell squared window function was applied in both dimensions prior to transformation and symmetry correlation was applied

Table 3. Fractional positional parameters *

Atom	x	y	z	Atom	x	y	z
O(1)	−0.141 4(4)	0.791 0(0)	0.090 0(3)	C(11)	−0.542 6(6)	0.780(1)	0.143 8(4)
O(2)	−0.368 8(4)	0.618 2(9)	0.148 2(3)	C(12)	−0.662 3(6)	0.785(2)	0.118 7(5)
O(3)	0.047 1(5)	0.152 7(8)	0.256 9(3)	C(13)	−0.712 0(6)	0.641(2)	0.070 3(5)
O(4)	0.190 0(4)	0.189 4(9)	0.459 9(3)	C(14)	−0.648 6(7)	0.477(2)	0.045 3(6)
O(5)	0.251 8(4)	0.356 3(9)	0.353 8(3)	C(15)	−0.529 6(7)	0.457(2)	0.071 4(5)
N(1)	−0.119 2(4)	0.454 2(10)	0.142 5(3)	C(16)	0.001 9(5)	0.406(1)	0.152 9(4)
N(2)	0.041 5(4)	0.480 3(9)	0.299 4(3)	C(17)	0.031 9(6)	0.244(2)	0.092 3(4)
C(1)	−0.175 0(6)	0.623(1)	0.109 3(4)	C(18)	0.032 6(5)	0.337(1)	0.240 0(4)
C(2)	−0.289 3(6)	0.519(1)	0.104 9(4)	C(19)	0.031 5(7)	0.707(1)	0.290 1(4)
C(3)	−0.220 7(5)	0.326(1)	0.147 6(4)	C(20)	0.005 5(9)	0.783(1)	0.375 9(5)
C(4)	−0.245 2(5)	0.268(1)	0.232 1(4)	C(21)	0.065 3(7)	0.626(2)	0.429 7(4)
C(5)	−0.274 8(6)	0.066(1)	0.248 8(5)	C(22)	0.065 3(6)	0.416(1)	0.384 1(4)
C(6)	−0.296 3(6)	0.013(1)	0.327 7(5)	C(23)	0.172 3(5)	0.304(1)	0.403 2(4)
C(7)	−0.281 4(6)	0.148(2)	0.391 0(4)	C(24)	0.366 8(6)	0.265(2)	0.360 8(4)
C(8)	−0.252 5(6)	0.347(1)	0.374 0(4)	C(25)	0.418 5(7)	0.368(2)	0.290 6(6)
C(9)	−0.232 9(6)	0.411(1)	0.296 2(4)	C(26)	0.429 4(7)	0.342(2)	0.443 8(5)
C(10)	−0.480 2(5)	0.617(1)	0.119 9(4)	C(27)	0.360 0(8)	0.029(2)	0.354 4(6)

* Numbers in parentheses are estimated standard deviations in the least significant digits.

Table 4. Bond distances (Å) *

O(1)–C(1)	1.212(6)	C(1)–C(2)	1.506(7)	C(13)–C(14)	1.385(11)
O(2)–C(2)	1.394(6)	C(2)–C(3)	1.610(7)	C(14)–C(15)	1.436(8)
O(2)–C(10)	1.353(5)	C(3)–C(4)	1.502(6)	C(16)–C(17)	1.515(7)
O(3)–C(18)	1.232(6)	C(4)–C(5)	1.387(8)	C(16)–C(18)	1.518(7)
O(4)–C(23)	1.198(6)	C(4)–C(9)	1.402(7)	C(19)–C(20)	1.563(8)
O(5)–C(23)	1.349(5)	C(5)–C(6)	1.399(8)	C(20)–C(21)	1.484(9)
O(5)–C(24)	1.475(6)	C(6)–C(7)	1.364(9)	C(21)–C(22)	1.552(8)
N(1)–C(1)	1.361(6)	C(7)–C(8)	1.366(9)	C(22)–C(23)	1.466(7)
N(1)–C(3)	1.467(6)	C(8)–C(9)	1.393(7)	C(24)–C(25)	1.518(8)
N(1)–C(16)	1.458(5)	C(10)–C(11)	1.368(8)	C(24)–C(26)	1.577(8)
N(2)–C(18)	1.348(6)	C(10)–C(15)	1.400(8)	C(24)–C(27)	1.535(11)
N(2)–C(19)	1.475(7)	C(11)–C(12)	1.436(7)		
N(2)–C(22)	1.462(6)	C(12)–C(13)	1.327(10)		

* Numbers in parentheses are estimated standard deviations in the least significant digits.

Table 5. Bond angles (°) *

C(2)–O(2)–C(10)	120.1(4)	C(3)–C(4)–C(9)	121.8(5)	O(3)–C(18)–N(2)	119.9(5)
C(23)–O(5)–C(24)	122.9(4)	C(5)–C(4)–C(9)	118.4(5)	O(3)–C(18)–C(16)	121.1(5)
C(1)–N(1)–C(3)	96.2(4)	C(4)–C(5)–C(6)	119.5(6)	N(2)–C(18)–C(16)	119.0(4)
C(1)–N(1)–C(16)	130.6(4)	C(5)–C(6)–C(7)	122.4(6)	N(2)–C(19)–C(20)	103.8(5)
C(3)–N(1)–C(16)	132.2(4)	C(6)–C(7)–C(8)	117.6(5)	C(19)–C(20)–C(21)	101.8(5)
C(18)–N(2)–C(19)	127.3(4)	C(7)–C(8)–C(9)	122.3(6)	C(20)–C(21)–C(22)	109.2(4)
C(18)–N(2)–C(22)	119.9(4)	C(4)–C(9)–C(8)	119.5(5)	N(2)–C(22)–C(21)	102.1(4)
C(19)–N(2)–C(22)	112.8(5)	O(2)–C(10)–C(11)	115.3(5)	N(2)–C(22)–C(23)	115.4(4)
O(1)–C(1)–N(1)	131.8(5)	O(2)–C(10)–C(15)	122.7(5)	C(21)–C(22)–C(23)	111.7(4)
O(1)–C(1)–C(2)	134.8(5)	C(11)–C(10)–C(15)	121.9(5)	O(4)–C(23)–O(5)	123.5(5)
N(1)–C(1)–C(2)	93.4(4)	C(10)–C(11)–C(12)	118.5(6)	O(4)–C(23)–C(22)	123.8(4)
O(2)–C(2)–C(1)	114.6(5)	C(11)–C(12)–C(13)	121.6(7)	O(5)–C(23)–C(22)	112.5(4)
O(2)–C(2)–C(3)	117.9(4)	C(12)–C(13)–C(14)	119.8(6)	O(5)–C(24)–C(25)	101.7(5)
C(1)–C(2)–C(3)	85.0(4)	C(13)–C(14)–C(15)	121.4(7)	O(5)–C(24)–C(26)	107.0(5)
N(1)–C(3)–C(2)	85.3(4)	C(10)–C(15)–C(14)	116.6(7)	O(5)–C(24)–C(27)	110.5(5)
N(1)–C(3)–C(4)	115.4(4)	N(1)–C(16)–C(17)	111.1(4)	C(25)–C(24)–C(26)	109.8(6)
C(2)–C(3)–C(4)	117.7(4)	N(1)–C(16)–C(18)	108.4(4)	C(25)–C(24)–C(27)	113.9(7)
C(3)–C(4)–C(5)	119.7(5)	C(17)–C(16)–C(18)	112.1(4)	C(26)–C(24)–C(27)	113.1(6)

* Numbers in parentheses are estimated standard deviations in the least significant digits.

afterwards, this proved essential to reduce noise in the F_1 dimension.[25]

N-[2-(3-*Methyl*-2-*oxo*-3-*phenylseleno*-4-*phenylazetidin*-1-*yl*)-*propanoyl*]-L-*proline* (26).—Compound (23) (4.309 g) was stirred with TFA (10 ml) with exclusion of atmospheric moisture. Evaporation gave a gum which solidified on trituration with water. The product was recrystallised from chloroform–light petroleum (b.p. 60—80 °C) to give (26) (3.327 g, 86%), m.p. 184—185 °C (Found: C, 59.65; H, 5.35; N, 5.4. $C_{24}H_{26}N_2O_4Se$ requires C, 59.38; H, 5.36; N, 5.77%); $\nu_{max.}$ (KBr disc) 1 742, 1 720, and 1 655 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.48 (3 H, d, *J* 7 Hz, *Me*CH), 1.60 (3 H, s, MeCSe), 1.67—2.33 (4 H, m, $CH_2CH_2CH_2$), 2.93—3.70 (2 H + H_2O, m, NCH_2 +

H_2O), 3.74—4.12 (1 H, m, $CHCO_2CMe_3$), 4.37 (1 H, q, *J* 7 Hz, C*H*Me), 4.65 (1 H, s, PhC*H*), and 7.12—7.58 (10 H, m, 2 × Ph).

Similar conversion of the isomeric ester (24) gave (27) in 95% yield, m.p. 131—133 °C (Found: C, 58.55; H, 5.45; N, 5.6. $C_{24}H_{26}N_2O_4Se{\cdot}0.5H_2O$ requires C, 58.30; H, 5.47; N, 5.69%); $\nu_{max.}$ (KBr disc) 1 751, 1 741, 1 656, and 1 613 cm^{-1}; δ ([$^2H_6$]-DMSO) 0.83 (3 H, d, *J* 7 Hz, *Me*CH), 1.00 (3 H, s, MeCSe), 1.55—2.09 (4 H, m, $NCH_2CH_2CH_2$), 3.45—3.73 (2 H, m, NCH_2), 3.73—4.08 (1 H, m, $CHCO_2CMe_3$), 4.48 (1 H, q, *J* 7 Hz, *CH*Me), 4.78 (1 H, s, PhC*H*), 7.05—7.58 and 7.73—7.95 (10 H, 2 m, 2 × *Ph*).

N-[2-(3-*Methylene*-2-*oxo*-4-*phenylazetidin*-1-*yl*)*propanoyl*]-L-*proline* (28).—To a solution of (26) (3.16 g) in dichloromethane (200 ml) and pyridine (1.03 g, 1.05 ml) was added 30% aqueous hydrogen peroxide (2.2 ml) and the mixture was refluxed for 2.5 h; it was then washed with water (3 × 50 ml), dried (Na_2SO_4), filtered, and evaporated to give a brown gum which solidified after removal of residual pyridine by azeotroping with water. Recrystallisation from ethyl acetate–ether–light petroleum (b.p. 60—80 °C) gave (28) (0.512 g, 24%), m.p. 121—122 °C (Found: C, 65.45; H, 6.05; N, 8.3. $C_{18}H_{20}N_2O_4$ requires C, 65.85; H, 6.1; N, 8.5%); $\nu_{max.}$ (KBr disc) 1 745, 1 652, and 1 623 cm^{-1}; δ ([$^2H_6$]-DMSO) 1.33 and 1.50 (3 H, 2 d, coalescence at 100 °C to 1 d at δ 1.50, *J* 7 Hz, *Me*CH), 1.63—1.93 (4 H, m, $NCH_2CH_2CH_2$), 3.00—3.70 (3 H, m, NCH_2 and $CHCO_2CMe_3$), 4.54 (1 H, q, *J* 7 Hz, C*H*Me), 5.11 and 5.14 (2 H, 2 s, at 100 °C each signal is resolved into a doublet with *J* 2 Hz, $=CH_2$), 5.68 (1 H, dd, $J \simeq 2$ Hz, PhC*H*), and 7.36 (5 H, s, Ph).

Isomer (27) was cleanly converted in an identical manner on an analytical scale into a product which was identical with (28) by t.l.c.

X-*Ray Structure Determination of* (5A).—Crystals of (5A), $C_{27}H_{32}N_2O_5$, $M = 464.57$, are monoclinic *P*2, $a = 11.822(1)$, $b = 6.464(1)$, $c = 16.555(1)$ Å, $\beta = 95.62(1)°$ from diffractometer measurements [Cu-K_α radiation ($\lambda = 1.541\,84$ Å), graphite monochromated], $V = 1\,259.1$ Å^3, $Z = 2$, μ(Cu-K_α) = 6.9 cm^{-1}. Approximate crystal dimensions 0.05 × 0.15 × 0.40 mm, $D_c = 1.23$ g/cm^3. The colourless needle-like crystal was mounted parallel to the needle axis. Systematic absence $0k0$: $k = 2n + 1$ indicated space group *P*2.

Data collection was on an Enraf-Nonius CAD4 computer-controlled kappa axis diffractometer by the ω—θ scan method with θ scan width = 0.7 + 0.300 tan θ. The ω scan rate varied from 2 to 20° min^{-1} for the weakest to the strongest reflections respectively. Data were collected to a maximum 2θ of 150°. This gave 2 943 reflections of which 2 821 were unique. Lorentz and polarisation corrections, and a secondary extinction correction, but no absorption correction were applied. The structure was solved by direct methods with the Enraf-Nonius structure determination package [26] as well as a private suite of programs (Molecular Structure Corporation). Scattering factors were taken from Cromer and Waber.[27] Anomalous dispersion was included in F_o, the values for Δf′ and Δf″ are those of Cromer.[27] Weighted full-matrix least-squares converged at $R = 0.078$ for 1 822 reflections [$I > 3\sigma(I)$]; $(R = \Sigma[|F_o| - |F_c|]/\Sigma|F_o|)$; $R_\omega = 0.098$ $[R_\omega = \Sigma([|F_o| - |F_c|]\cdot\omega^{\frac{1}{2}}/\Sigma(|F_o|\cdot\omega^{\frac{1}{2}})]$. Hydrogen atoms were not included in the refinement. The largest feature (0.40 e Å^{-3}) of the final difference Fourier map was consistent with an expected hydrogen atom position. Table 3 lists the fractional atomic co-ordinates. Tables 4 and 5 give the bond lengths and bond angles respectively. Temperature and structure factors have been deposited as a Supplementary Publication [SUP No. 23747 (17 pp.)]. *

Acknowledgements

The *X*-ray structure determination was performed as a service by the crystallographic staff of Molecular Structure Corporation, 3304 Longmire Drive, College Station, Texas 77840, U.S.A. We thank Mrs. J. M. Williams for n.m.r. data; Mr. P. R. W. Baker and his staff for microanalytical data; Dr. D. W. Young of the University of Sussex, and our colleagues in the Department of Medicinal Chemistry, Beckenham for their help and advice during the course of this work and Mrs. S. Mitchell for help in the preparation of the manuscript.

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* For details of the supplementary publications scheme see Instruction for Authors (1984) in *J. Chem. Soc., Perkin Trans. 1*, 1984, Issue 1.

Received 16*th May* 1983; *Paper* 3/781