Inhibitors of Human Leucocyte Elastase. Peptides Incorporating an α-Azanorvaline Residue or a Thiomethylene Linkage in Place of a Peptide Bond

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Peptides containing an α-azanorvaline residue at the *C*-terminus and *N*-[(1-methoxycarbonylalkyl)-carbamoyl] group at the *N*-terminus have been made as inhibitors of human leucocyte elastase. A number of analogues with an amide bond replaced by a thiomethylene group have also been prepared. The analogues were tested against leucocyte elastase using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as a substrate and the results were obtained as IC_{50} values. Both types of analogues inhibited the leucocyte elastase; the most potent of these was *N*-[(1-methoxycarbonylbutyl)-carbamoyl]-L-valyl-L-prolyl-α-azanorvaline phenyl ester (**2**) (IC_{50} 0.28 μM, K_i 0.02 μM).

Human leucocyte elastase has been implicated in the pathogenesis of pulmonary emphysema[1-3] and this hypothesis has led to a search for potent inhibitors of this enzyme. In addition to the naturally-occurring inhibitor, elasnin,[4] and some other heterocyclic inhibitors,[5] a number of peptide inhibitors of leucocyte elastase have been reported. These include trifluoroacetyl peptides,[6,7] peptide sulphonyl fluorides,[8] and peptide chloromethyl ketones.[9-11]

In an earlier publication,[12] we have reported inhibitors of porcine pancreatic elastase which had α-aza-amino acid residues at the *C*-terminus. These were based on peptide sequences, *e.g.* Gly-Val-Gly-Val, Gly-Val-Gly-Leu, present in bovine elastin, porcine tropoelastin and bovine ligamentum nuchae elastin.[13-15] The most potent inhibitors to originate from this work were of the following type: $HO_2CCH(Me)$-NHCO-Val-Gly-Azala-OCH_2Ph (IC_{50} 0.1 μM), MeOCOCH-(CH_2CHMe_2)NHCO-Val-Gly-Azala-OPh (IC_{50} 0.4 μM), and MeOCOCH(CH_2CHMe_2)NHCO-Val-Pro-Azala-OCH_2-Ph (IC_{50} 1.2 μM).

Although these analogues were much more potent than the known inhibitors of pancreatic elastase, *e.g.* Ac-Ala-Ala-Azala-ONp[16] (IC_{50} 5.7 μM), Ac-Ala-Ala-Pro-Ala-CH_2Cl[17] (IC_{50} 3.5 μM), none of these had any significant effect against human leucocyte elastase (only 30—60% inhibition at 400 μM).

We now describe new analogues of the above type which contain an α-azanorvaline residue in place of the α-aza-alanine residue at the *C*-terminus and an *N*-[(1-methoxycarbonylalkyl)-carbamoyl] group at the *N*-terminus [Table, compounds (**1**)—(**19**)]. A number of analogues in which the amide bonds have been replaced by a CH_2S group have also been made to study the effects of such a replacement on the enzyme inhibitory potency [Table, compounds (**20**)—(**30**)]. Two analogues [Table, compounds (**31**), (**32**)] which contain both an α-azanorvaline residue and a CH_2S linkage have also been prepared.

Synthesis.—*N*-[(1-Methoxycarbonylalkyl)carbamoyl]-valyl-X-α-azanorvaline-Y analogues (**1**)—(**11**) and (**16**); X = Gly, Ala, Val, or Pro; Y = OPh, OCH_2Ph, or Phe-OCH_2Ph) were prepared by the route shown in Scheme 1. *N*-t-Butoxycarbonyl-L-valyl-X-OH was coupled to benzyl 2-propylcarbazate by the DCCI—HOBt method.[18] The tripeptide derivatives, Boc-Val-X-Aznva-OCH_2Ph, were then deprotected with HCl in ethyl acetate and treated with the required *N*-carbonyl amino acid methyl esters to give *N*-[(1-methoxycarbonylalkyl)carbamoyl]-valyl-X-azanorvaline benzyl ester analogues (**1**), (**4**)—(**11**), and (**16**). The catalytic hydrogenolysis of *N*-[(1-methoxycarbonyl-

Table. Structures of synthetic peptides and their potency as inhibitors of leucocyte elastase.

No.	Comp.	IC_{50}* (μM) (Except where otherwise stated)
Modifications at position P_1'		
	MeOCOCH(CH_2CHMe_2)NHCO-Val-Pro-Aznva-X	
(1)	X = OCH_2Ph	0.88
(2)	OPh	0.28
(3)	Phe-OCH_2Ph	1.3
Modifications at position P_2		
	MeOCOCH(CH_2CHMe_2)NHCO-Val-X-Aznva-OCH_2Ph	
(4)	X = Val	23
(1)	Pro	0.88
(5)	Ala	4.8
	MeOCOCH$(CHMe_2)$NHCO-Val-X-Aznva-OCH_2Ph	
(6)	X = Pro	72% 100μM
(7)	Ala	34% 100μM
(8)	Gly	73% 100μM
	MeOCOCH(Me)NHCO-Val-X-Aznva-OCH_2Ph	
(9)	X = Pro	10
(10)	Ala	31
(11)	Gly	>67
Modifications at position P_3		
	X-Pro-Aznva-OCH_2Ph	
(12)	X = MeOCOCH(CH_2CHMe_2)NHCO	100% at 2mM
(13)	MeOCOCH$(CHMe_2)$NHCO	100% at 2mM
(14)	MeOCOCH(CH_3)NHCO	31% at 2mM
(15)	MeOCOCH(CH_2Ph)NHCO	77% at 2mM
Modifications at position P_4		
	X-Val-Pro-Aznva-OCH_2Ph	
(1)	X = MeOCOCH(CH_2CHMe_2)NHCO	0.88
(6)	MeOCOCH$(CHMe_2)$NHCO	72% at 100μM
(16)	MeOCOCH$(CHMe_2)$NHCO D	8.0
(9)	MeOCOCH(Me)NHCO	10

Table (*continued*)

No.	Comp.	IC_{50}* (μM) (Except where otherwise stated)
Modifications at position P_5		
	X-Gly-Val-Gly-Aznva-OCH_2Ph	
(17)	X = Boc	99
(18)	$MeOCOCH(CH_2CHMe_2)NHCO$	13
(19)	MeOCOCH(Me)NHCO	>130 (41% at 67μM)
Peptide bond modified analogues ($-CH_2S-$). Modifications at positions P_2 to P_1		
	Boc-Leu-X-OCH_2Ph	
(20)	X = $NHCH_2CH_2SCH(CH_2CHMe_2)CO$	26
(21)	$NHCH_2CH_2SCH(CH_2CHMe_2)CO$ D	22
	$MeOCOCH(CHMe_2)NHCO$-Leu-X-OCH_2Ph	
(22)	X = $NHCH_2CH_2SCH(CH_2CHMe_2)CO$	67
(23)	$NHCH_2CH_2SCH(CH_2CHMe_2)CO$ D	32
	$MeOCOCH(CH_2CHMe_2)NHCO$-Leu-X-OCH_2Ph	
(24)	X = $NHCH_2CH_2SCH(CH_2CHMe_2)CO$	19
(25)	$NHCH_2CH_2SCH(CH_2CHMe_2)CO$ D	13
Modifications at position P_3		
	X-Gly-ψ(CH_2S)-D-Leu-OCH_2Ph	
(21)	X = Boc-Leu	22
(26)	Boc-Val	33
(27)	$MeOCOCH(CH_2CHMe_2)NHCO$	23
(28)	$MeOCOCH(CHMe_2)NHCO$	37
	$MeOCOCH(CH_2CHMe_2)NHCO$-X-Gly-ψ(CH_2S)-D-Leu-OCH_2Ph	
(25)	X = Leu	13
(29)	Val	30
	$MeOCOCH(CHMe_2)NHCO$-X-Gly-ψ(CH_2S)-D-Leu-OCH_2Ph	
(23)	X = Leu	32
(30)	Val	60
Modifications at position P_4		
	X-Leu-Gly-ψ(CH_2S)-Leu-OCH_2Ph	
(24)	X = $MeOCOCH(CH_2CHMe_2)NHCO$	19
(22)	$MeOCOCH(CHMe_2)NHCO$	67
	X-Leu-Gly-ψ(CH_2S)-D-Leu-OCH_2Ph	
(25)	X = $MeOCOCH(CH_2CHMe_2)NHCO$	13
(23)	$MeOCOCH(CHMe_2)NHCO$	32
	X-Val-Gly-ψ(CH_2S)-D-Leu-OCH_2Ph	
(29)	X = $MeOCOCH(CH_2CHMe_2)NHCO$	30
(30)	$MeOCOCH(CHMe_2)NHCO$	60
	X-Leu-ψ(CH_2S)-Ala-Aznva-OCH_2Ph	
(31)	X = $MeOCOCH(CH_2CHMe_2)NHCO$	7.7
(32)	$MeOCOCH(CHMe_2)NHCO$	23

* Concentration at which 50% inhibition of leucocyte elastase is observed.

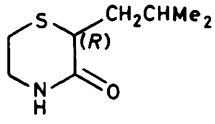
3-methylbutyl)carbamoyl]-valyl-prolyl-α-azanorvaline benzyl ester over 5% Pd–C followed by reaction with either phenyl chloroformate or *N*-carbonylphenylalanine benzyl ester gave compounds (**2**) and (**3**) respectively.

The MeOCOCH(R)NHCO-L-prolyl-α-azanorvaline benzyl ester analogues, (**12**—(**15**) (R = Me, $CHMe_2$, or CH_2CHMe_2), were prepared by treating prolyl-α-azanorvaline benzyl ester with the required *N*-carbonyl amino-acid methyl ester.

The tetrapeptide analogue (**17**) was prepared by coupling Boc-Gly-Val-Gly-OH with benzyl 2-propylcarbazate by the DCCI–HOBt method. The *N*-t-butoxycarbonyl group of (**17**) was cleaved by treatment with HCl in ethyl acetate and the resulting product was reacted with either *N*-carbonylleucine methyl ester or *N*-carbonylalanine methyl ester to give the analogues (**18**) and (**19**) respectively.

The analogues containing a CH_2S linkage in place of an amide bond between positions P_1 and P_2 were synthesized according to the route shown in Scheme 2. The procedures used for the synthesis of 2-(2-aminoethylthio)-4-methylpentanoic acid derivatives were based on the routes reported earlier for these compounds.[19,20] (*R*)-Or (*S*)-2-bromo-4-methylpentanoic acid derivatives were prepared from D-leucine or L-leucine respectively by treatment with sodium nitrite and potassium bromide in aqueous sulphuric acid. This reaction has been shown to proceed with retention of configuration. The reaction of these bromo acids with 2-aminoethanethiol in aqueous sodium hydrogen carbonate gave (*R*)- or (*S*)-2-(2-aminoethylthio)-4-methylpentanoic acids [Gly-ψ(CH_2S)-Leu]. Since the reaction of thiol derivatives with bromo acids is known to proceed with inversion of configuration, Gly-ψ(CH_2S)-L-Leu and Gly-ψ(CH_2S)-D-Leu derivatives were obtained starting from D-leucine and L-leucine respectively. The pseudo dipeptide analogues were converted to benzyl ester derivatives by refluxing in toluene with benzyl alcohol and toluene-*p*-sulphonic acid.

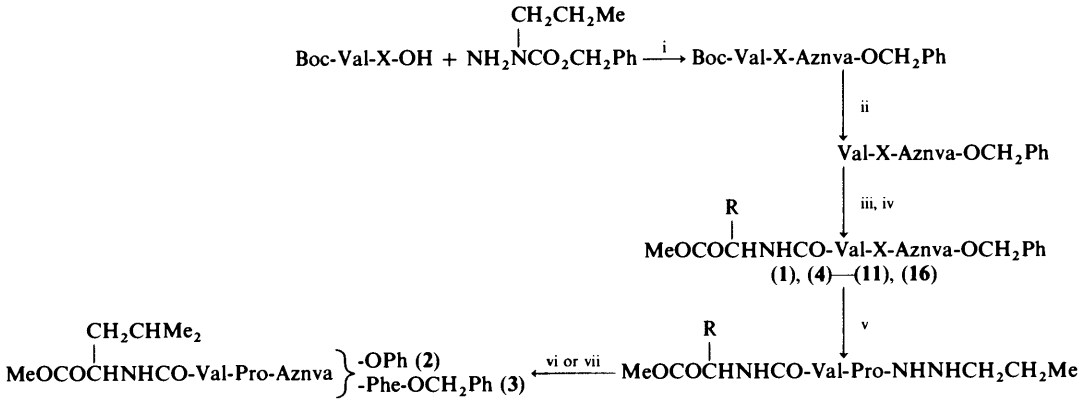
Although the benzyl ester toluene-*p*-sulphonate salt was stable, an attempt to prepare the corresponding hydrochloride salt by conversion of Gly-ψ(CH_2S)-D-Leu toluene-*p*-sulphonate to the free amine with 10% sodium carbonate followed by the addition of HCl–ethyl acetate gave the following cyclic derivative (2-isobutylthiomorpholin-3-one, m.p. 66—67 °C) as the major product (structure confirmed by n.m.r. spectroscopy).



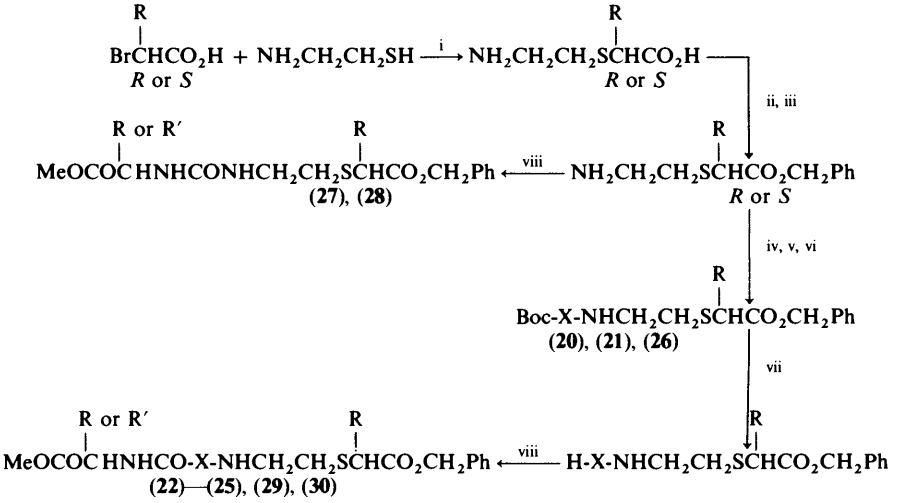
The sulphonate salts were therefore used either directly in coupling with Boc-Val-OH or Boc-Leu-OH by the DCCI–HOBt method with triethylamine to give compounds (**20**), (**21**), and (**26**), or were treated with *N*-carbonyl-L-leucine methyl ester or *N*-carbonyl-L-valine methyl ester and triethylamine to give compounds (**27**) and (**28**). The *N*-t-butoxycarbonyl group of compounds (**20**), (**21**), and (**26**) was removed by treatment with HCl in ethyl acetate and the resulting peptide hydrochlorides were then treated with *N*-carbonyl-L-leucine methyl ester or *N*-carbonyl-L-valine methyl ester and triethylamine to give compounds (**22**)—(**25**), (**29**), and (**30**).

The analogues containing a CH_2S linkage between positions P_2 and P_3 (**31**) and (**32**) were prepared by the route shown in Scheme 3.

4-Methyl-2-t-butoxycarbonylaminopentan-1-ol (Boc-L-leucinol) was tosylated by reaction with toluene-*p*-sulphonyl chloride in pyridine. The sulphonate was then treated successively with potassium *o*-ethyl dithiocarbonate[21] and ethylenediamine to give 4-methyl-2-t-butoxycarbonylaminopentanethiol. This thiol derivative was treated with (*R*)-2-bromopropanoic acid and the resulting compound, 2-(4-methyl-2-t-butoxycarbonylaminopentylthio)propionic acid [Boc-Leu-ψ(CH_2S)-Ala-OH], was coupled with benzyl 2-propylcarbazate to give Boc-Leu-ψ(CH_2S)-Ala-Aznva-OCH_2Ph. Removal of



Scheme 1. Synthesis of *N*-[(1-methoxycarbonylalkyl)carbamoyl]-Val-X-Aznva-Y analogues (X = Gly, Ala, Val or Pro; Y = OCH_2Ph, OPh, or Phe-OCH_2Ph). *Reagents:* i, DCCI–HOBt; ii, HCl–Ethyl acetate; iii, MeOCOCH(R)N=C=O (R = Me, $CHMe_2$, or CH_2CHMe_2); iv, triethylamine; v, H_2–5% Pd–C; vi, PhOCOCl; vii, $PhCH_2OCOCH(CH_2Ph)N{=}C{=}O$.



Scheme 2. Synthesis of *N*-[(1-methoxycarbonylalkyl)carbamoyl] analogues with the amide bond between P_1 and P_2 positions replaced by a CH_2S group. R = CH_2CHMe_2, R′ = $CHMe_2$, X = Leu or Val. *Reagents:* i, $NaHCO_3$; ii, $MeC_6H_4SO_3H{\cdot}H_2O$; iii, $PhCH_2OH$; iv, Boc-Val-OH or Boc-Leu-OH; v, DCCI; vi, HOBt; vii, HCl–ethyl acetate; viii, MeOCOCH(R or R′)–N=C=O.

the *N*-t-butoxycarbonyl group with HCl in ethyl acetate followed by reaction with either *N*-carbonyl-L-leucine methyl ester or *N*-carbonyl-L-valine methyl ester gave compounds (**31**) and (**32**).

Inhibition of the Leucocyte Elastase.—All of the analogues listed in the Table were tested as inhibitors of human leucocyte elastase using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the substrate and the results are expressed as IC_{50} (inhibitory concentration in μM to give 50% inhibition) values.

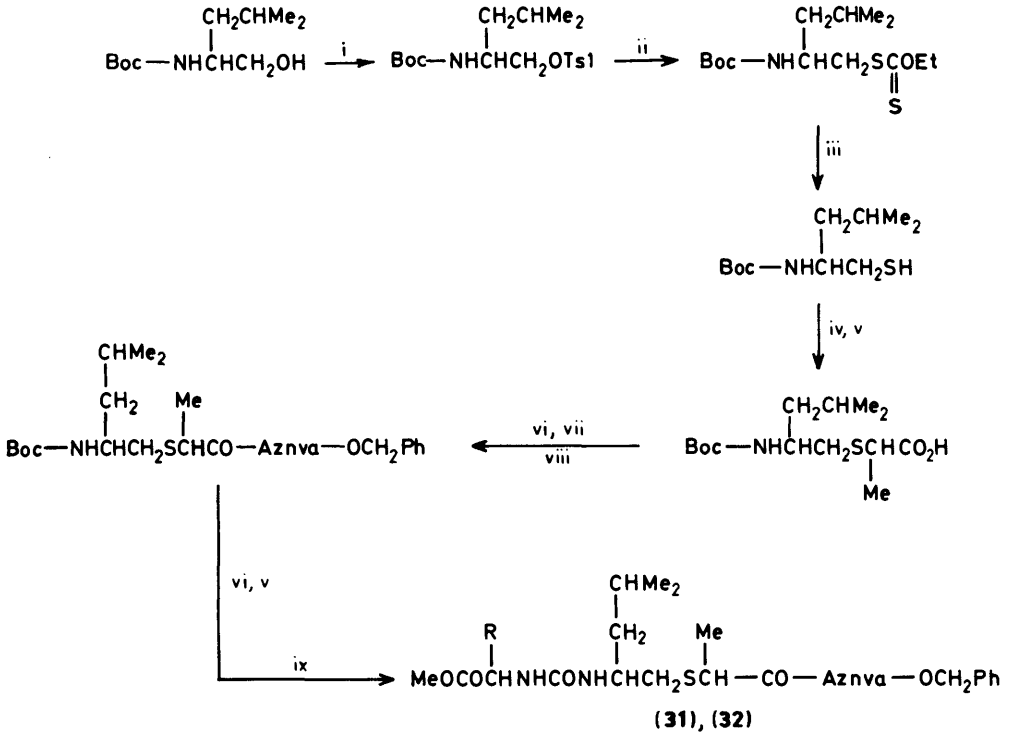
As mentioned in the earlier publication [12] it has been assumed that the analogues are binding to the enzyme in such a way that the *C*-terminal azanorvaline residue in compounds (**1**)—(**19**), (**31**), and (**32**) and the *C*-terminal leucine residue in compounds (**20**)—(**30**) are interacting at the P_1 binding site. In both series of compounds (Table) the analogues have been listed in such a way that the effects of a change in positions P_1' to P_5 can be studied individually. In order to make the structure-activity relationships more meaningful, the analogues modified in each position (P_1' to P_5) have been further divided so that the effects of single residue changes may be compared.

Effects of changes at position P_1'. Compounds (**1**)—(**3**) (Table) have a benzyl ester, a phenyl ester or a phenylalanine benzyl ester at the *C*-terminus. The phenyl ester analogue (**2**) (IC_{50} 0.28 μM), was about 3 to 4-fold more potent than the corresponding benzyl ester (**1**) or the Phe-OCH_2Ph analogue (**3**).

Effects of changes at position P_1. When the three sets of compounds which contain either Gly-ψ(CH_2S)-Leu or Gly-ψ(CH_2S)-D-Leu at the *C*-terminus [(**20**) and (**21**), (**22**) and (**23**), and (**24**) and (**25**)] are compared, the analogues do not appear to differ greatly from each other. The difference in the elastase inhibitory potency was less than 2-fold, but in each case the analogues with a Gly-ψ(CH_2S)-D-Leu residue were marginally more potent than the corresponding Gly-ψ(CH_2S)-Leu analogues. It appears that due to the presence of a CH_2S linkage, which is more flexible than an amide bond, the leucine side-chains in both the L and D analogues are able to interact with the enzyme in a similar manner.

When the potencies of each of the above six analogues (**20**)—(**25**), IC_{50} 20—30 μM) and MeOCOCH(CH_2CHMe_2)-NHCO-Val-Pro-Aznva-OCH_2Ph (**1**), (IC_{50} 0.88 μM) were compared with MeOCOCH(CH_2CHMe_2)NHCO-Val-Pro-Azala-OCH_2Ph (66% inhibition at 400 μM), the P_1 position appeared to prefer a bulky α-azanorvaline or a leucine residue rather than the smaller aza-alanine residue. The azanorvaline analogue (**1**) was about 400-fold more potent than the corresponding aza-alanine analogue.

Effects of changes at position P_2. In the three sets of



Scheme 3. Synthesis of *N*-[(1-methoxycarbonylalkyl)carbamoyl] analogues with the amide bond between P_2 and P_3 positions replaced by a CH_2S group. R = $CHMe_2$ or CH_2CHMe_2. *Reagents:* i, $MeC_6H_4SO_2Cl$ in pyridine; ii, EtOCSK; iii, $NH_2CH_2CH_2NH_2$; iv, (*R*)-BrCH(Me)CO_2H; v, triethylamine; vi, HCl-ethyl acetate; vii, DCCI–HOBt; viii, $NH_2N(CH_2CH_2Me)CO_2CH_2Ph$; ix, MeOCOCH(R)N=C=O (R = $CHMe_2$ or CH_2CHMe_2).

compounds containing a MeOCOCH(CH_2CHMe_2)NHCO (**1**), (**4**), and (**5**), an MeOCOCH($CHMe_2$)NHCO (**6**) and (**8**), or a MeOCOCH(Me)NHCO (**9**)—(**11**) group at the *N*-terminus, the analogues with a proline residue in position P_2 were the most potent. In the first set of compounds the proline analogue (**1**) was about five fold more potent than the alanine analogue (**5**) and about 25-fold more potent than the valine analogue (**4**). The analogues in the second set (**6**)—(**8**) were much less potent, making the comparison difficult but the proline (**6**) and the glycine (**8**) analogues were marginally more potent than the alanine analogue (**7**). The proline analogue (**9**) was again more potent than the corresponding alanine (**10**) and glycine (**11**) analogues in the third set of compounds.

Effects of Changes at position P_3. In the α-azanorvaline series of analogues (**12**)—(**15**), the compounds with a leucine or valine residue in position P_3 were more potent than those with a phenylalanine or an alanine residue in position P_3. In the CH_2S series (**21**), (**23**), (**25**)—(**30**) only leucine and valine residues were incorporated in the P_3 position. Although the analogues with an isobutyl side-chain were more potent than those with an isopropyl side-chain, the difference in potency was less than 2-fold.

Effects of changes at position P_4. In the α-azanorvaline series of analogues (**1**), (**6**), (**9**), and (**16**), compound (**1**) with an isobutyl side-chain in position P_4, was the most potent. The other three analogues with either a methyl or an isopropyl group in the side-chain were about 10-fold less potent. Similarly, in the CH_2S series of analogues (**22**)—(**25**), (**29**), and (**30**), the compounds with an isobutyl group in the side chain were more potent (2 to 3-fold) than the corresponding isopropyl analogues. The same pattern was observed in analogues (**31**) and (**32**) which had an azanorvaline residue in position P_1 and a CH_2S group between positions P_2 and P_3.

Effects of changes at position P_5. The analogue with a bulky leucine residue (**18**) in position P_5 was more potent (5 to 10-fold) than the corresponding alanine analogue (**19**) or the *N*-t-butoxycarbonyl analogue (**17**).

Effects of amide bond replacement. The effects of the amide bond replacement between positions P_1 and P_2, and P_2 and P_3, by a CH_2S group can be seen by comparing compound (**8**) with (**30**), (**4**), and (**5**) with (**29**), (**5**) with (**31**), and (**7**) with (**32**). Although some tentative conclusions can be drawn, the results should be interpreted with caution because these sets of compounds have at least one other change in addition to the amide bond replacement, *e.g.* compound (**8**) has an azanorvaline residue at position P_1 whereas compound (**30**) has a D-leucine residue in this position. The analogues (**8**) and (**30**), and (**4**) and (**29**), are of similar potency indicating that the amide bond between positions P_1 and P_2 is not necessary for binding to the enzyme, and the side-chains of the amino-acid residues in positions P_1 and P_2 could still be aligned properly to interact with the enzyme binding sites. Similar conclusions about the amide bond between the P_2 and P_3 positions can also be drawn by comparing compounds (**5**) and (**31**) which are of similar potency. A comparison of MeOCOCH($CHMe_2$)NHCO-Leu-ψ(CH_2S)-Ala-Aznva-OCH_2Ph (**32**), (IC_{50} 23 μM) with MeOCOCH($CHMe_2$)NHCO-Val-Ala-Aznva-OCH_2Ph (**7**) (34% inhibition at 0.1 mM) would indicate that in some cases the replacement of the amide bond may even improve the binding interactions with the enzyme, although in the case of compound (**32**) some of this improvement may be due to the leucine residue in position P_3.

Conclusions

On the basis of the above results, which show that inhibitors of human leucocyte elastase can be prepared by replacing the α-aza-alanine residue in $MeOCOCH(CH_2CHMe_2)NHCO$-Val-Pro-Azala-OCH_2Ph type compounds by an α-azanorvaline residue, the following conclusions can be drawn about the specificity of the various binding subsites of leucocyte elastase.

(*a*) The P_1 binding site in leucocyte elastase requires groups larger than a methyl, and both linear (α-azanorvaline) and branched side-chain (leucine) amino-acids are acceptable in this position.

(*b*) A proline residue in the P_2 position appears to be better than a glycine, alanine, or valine residue in the α-azanorvaline series of compounds.

(*c*) The P_3 to P_5 positions seem to require larger hydrophobic groups for better interaction with the enzyme. Analogues with a leucine side-chain in these positions are more potent than those with a valine, phenylalanine, or alanine residue.

(*d*) The amide bonds between positions P_1 and P_2 and P_2 and P_3 may also be replaced by a CH_2S group but these changes do not greatly affect the potency of these analogues.

Experimental

Details of solvent systems and spray reagents used for t.l.c. are described in an earlier paper.[22] Three new systems are L, ethyl acetate–toluene (9:1 v/v); M, ethyl acetate–toluene (3:1 v/v); N, ethyl acetate–toluene (1:1 v/v). All the evaporations were carried out under reduced pressure below 40 °C. Optical rotations were performed on a Perkin Elmer 241 polarimeter. Symbols and abbreviations used follow the IUPAC–IUB recommendations;[23] other abbreviations are as follows: DCCI, dicyclohexylcarbodi-imide; HOBt, 1-hydroxybenzotriazole; DMF, dimethylformamide; Aznva, $-NHN(CH_2CH_2Me)CO-$; Aznva-OCH_2Ph, $-NHN(CH_2CH_2Me)CO_2CH_2Ph$.

t-Butyl 3-Propylcarbazate.—Propanal (37.4 g, 653 mmol) was added slowly to a solution of Boc-$NHNH_2$ (85 g, 643 mmol) in ether (200 ml) keeping the temperature below 20 °C. The solution was left overnight at room temperature over anhydrous Na_2SO_4. The solvent was then evaporated and the residue, on trituration with light petroleum, gave the solid hydrazone which was washed with light petroleum and dried (89.8 g, 81.2%).

A solution of the above hydrazone in tetrahydrofuran was hydrogenated over 5% Pd–C at 25 °C and 25—30 lb in^{-2}. The catalyst was removed by filtration and the filtrate was evaporated to dryness to leave an oil which on fractional distillation (74—75 °C, 0.2 mmHg) gave the product as a colourless oil (55.9 g, 61.5%) (Found: C, 55.2; H, 10.4; N, 16.3. $C_8H_{18}N_2O_2$ requires C, 55.1; H, 10.4; N, 16.0%).

1-Benzyloxycarbonyl-1-propyl-2-t-butoxycarbonylhydrazine.—Benzyl chloroformate (45 ml, 320 mmol) in chloroform (50 ml) was added to a stirred and cooled (0 °C) solution of t-butyl 3-propylcarbazate (55.9 g, 320 mmol) and triethylamine (45 ml, 320 mmol), in chloroform (300 ml). After the reaction had been stirred overnight at room temperature, the chloroform solution was washed with water, 20% aqueous citric acid, saturated aqueous $NaHCO_3$, and water, and was then dried (Na_2SO_4) and evaporated to dryness. Any unchanged starting materials were removed by high vacuum distillation (*ca.* 80 °C, 0.2 mmHg) to leave the product as a yellow oil, (65.0 g, 65.9%) which was used directly in the next step.

Benzyl 2-Propylcarbazate·HCl.—A solution of 1-benzyloxycarbonyl-1-propyl-2-t-butoxycarbonylhydrazine (65 g, 210 mmol) in ethyl acetate (100 ml) was treated with 5M-HCl–ethyl acetate (100 ml, 500 mmol) at room temperature for 90 min. Most of the product precipitated out during this time. some of the ethyl acetate was removed, anhydrous ether (250 ml) was added and the hydrochloride was collected, washed with ether, and dried (46.6 g, 90.9%), m.p. 137 °C (decomp.), R_{FA} 0.82, R_{FB} 0.82, R_{FC} 0.75 R_{FD} 0.65, and R_{FF} 0.69 (Found: C, 53.7; H, 7.3; N, 11.6. $C_{11}H_{17}ClN_2O_2$ requires C, 53.9; H, 7.0; N, 11.4%).

N-t-Butoxycarbonyl-L-valyl-L-prolyl-α-azanorvaline Benzyl Ester.—DCCI (3.49 g, 16.9 mmol) was added to a cooled (0 °C) and stirred solution of Boc-Val-Pro-OH (5.07 g, 16.1 mmol), HOBt (4.32 g, 32 mmol), azanorvaline benzyl ester hydrochloride (3.67 g, 15 mmol) and triethylamine (2.1 ml, 15 mmol) in DMF (50 ml). The reaction mixture was stirred overnight at 4 °C. Dicyclohexylurea was then removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate, washed with water, 20% aqueous citric acid, saturated aqueous $NaHCO_3$ and water, and then dried (Na_2SO_4) and evaporated to dryness. The crude product was purified by silica gel column chromatography using chloroform as the eluant to give an oil (4.1 g, 54.2%), R_{FA} 0.69, R_{FC} 0.69, R_{FF} 0.70, R_{FH} 0.62, R_{FP} 0.66, and R_{FQ} 0.69 (Found: C, 61.9; H, 7.8; N, 10.9. $C_{26}H_{40}N_4O_6$ requires C, 61.9; H, 8.0; N, 11.1%).

N-t-Butoxycarbonyl-L-valylglycyl-α-azanorvaline Benzyl Ester.—This was prepared by coupling Boc-Val-Gly-OH and azanorvaline benzyl ester by the DCCI–HOBt method and purified by silica gel column chromatography as above to yield the product as a foam (77.3%), R_{FA} 0.71, R_{FB} 0.79, R_{FC} 0.72, R_{FD} 0.82, R_{FE} 0.58, R_{FF} 0.78, R_{FH} 0.64, R_{FP} 0.47, and R_{FQ} 0.62. (Found: C, 59.2; H, 8.0; N, 12.0. $C_{23}H_{36}N_4O_6$ requires C, 59.5; H, 7.8; N, 12.1%).

N-t-Butoxycarbonyl-L-valyl-L-alanyl-α-azanorvaline Benzyl Ester.—This compound was prepared from Boc-Val-Ala-OH and azanorvaline benzyl ester and purified as above. The product was obtained as a white solid (58.2%), m.p. 186—187 °C $[\alpha]_D^{25}$ −69.2° (*c* 2.5, in MeOH), R_{FC} 0.69, R_{FD} 0.82, R_{FE} 0.57, R_{FF} 0.72, R_{FH} 0.51, R_{FP} 0.58, and R_{FQ} 0.64 (Found: C, 60.4; H, 8.1; N, 11.6. $C_{24}H_{38}N_4O_6$ requires C, 60.2; H, 8.0; N, 11.7%).

N-t-Butoxycarbonyl-L-valyl-L-valyl-α-azanorvaline Benzyl Ester.—Preparation (from Boc-Val-Val-OH and Aznva-OCH_2Ph) and purification as above. The product was obtained as a white powder (55.4%), m.p. 170—171 °C, $[\alpha]_D^{25}$ −37.0° (*c* 3.0, in MeOH), R_{FA} 0.77, R_{FB} 0.80, R_{FC} 0.76, R_{FE} 0.67, R_{FH} 0.68, R_{FP} 0.66, and R_{FQ} 0.75 (Found: C, 61.9; H, 8.6; N, 11.1. $C_{26}H_{42}N_4O_6$ requires C, 61.6; H, 8.4; N, 11.1%).

N-[(1S)-(1-Methoxycarbonyl-3-methylbutyl)carbamoyl]-L-valyl-L-prolyl-α-azanorvaline Benzyl Ester (**1**).—*N*-Carbonyl-L-leucine methyl ester (1.71 g, 10 mmol) in chloroform (20 ml) was added to a suspension of Val-Pro-Aznva-OCH_2Ph·HCl (3.96 g, 9 mmol) and triethylamine (1.3 ml, 9 mmol) in chloroform (25 ml) at room temperature. A clear solution resulted in *ca.* 10 min. The solution was left standing overnight and then evaporated to dryness. The residue in ethyl acetate was washed with water, 20% aqueous citric acid, saturated aqueous $NaHCO_3$, and water, and then dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from ethyl acetate–light petroleum (60—80 °C) to give the *ester* (**1**) as a foam (3.9 g, 75.4%), $[\alpha]_D^{25}$ −105.0° (*c* 2, in MeOH), R_{FD} 0.8, R_{FE} 0.57, R_{FF} 0.60, R_{FH} 0.54, R_{FP} 0.48, and R_{FQ} 0.61 (Found: C, 60.4; H, 8.0; N, 11.9. $C_{29}H_{45}N_5O_7$ requires C, 60.5; H, 7.8; N, 12.1%).

N-[(1S)-(1-Methoxycarbonyl-3-methylbutyl)carbamoyl]-L-

valyl-L-prolyl-α-azanorvaline Phenyl Ester (**2**).—To a stirred solution of $MeOCOCH(CH_2CHMe_2)NHCO$-Val-Pro-$NHNHCH_2CH_2Me$ (0.55 g, 1.26 mmol) [obtained by the catalytic hydrogenolysis of (**1**)] in chloroform (5 ml) phenyl chloroformate (0.23 g, 1.5 mmol) was added and the solution was left at room temperature overnight. The chloroform was evaporated and the residue was purified by silica gel column chromatography using chloroform, and 1, 2, and 5% methanol in chloroform as the eluants. The product was obtained as a freeze-dried powder from 2-methylpropan-2-ol (89.3%), $[\alpha]_D^{25}$ −99.7° (*c* 1, in MeOH), R_{FA} 0.79, R_{FB} 0.68, R_{FC} 0.65, R_{FD} 0.77, R_{FE} 0.57, R_{FH} 0.65, R_{FP} 0.57, and R_{FQ} 0.65 (Found: C, 60.2; H, 7.9; N, 12.4. $C_{28}H_{43}N_5O_7$ requires C, 59.9; H, 7.7; N, 12.5%).

N-[(1S)-(1-*Methoxycarbonyl-3-methylbutyl*)*carbamoyl*]-L-*valyl-L-prolyl-α-azanorvalyl-L-phenylalanine Benzyl Ester* (**3**).—Prepared and purified as above from $MeOCOCH(CH_2CHMe_2)NHCO$-Val-Pro-$NHNHCH_2CH_2Me$ (0.55 g, 1.89 mmol) and *N*-carbonyl-L-phenylalanine benzyl ester (0.53 g, 1.89 mmol). The product was obtained as a freeze-dried powder from 2-methylpropan-2-ol (0.67 g, 73.6%), $[\alpha]_D^{25}$ −59.9° (*c* 2, in MeOH), R_{FA} 0.81, R_{FB} 0.73, R_{FC} 0.66, R_{FE} 0.57, R_{FH} 0.66, R_{FP} 0.57, and R_{FQ} 0.65 (Found: C, 63.0; H, 7.7; N, 11.4. $C_{38}H_{54}N_6O_8$ requires C, 63.1; H, 7.5; N, 11.6%).

N-[(1S)-(1-*Methoxycarbonyl-3-methylbutyl*)*carbamoyl*]-L-*valyl-L-valyl-α-azanorvaline Benzyl Ester* (**4**).—This was prepared by treating *N*-carbonyl-L-leucine methyl ester with Val-Val-Aznva-OCH_2Ph (obtained by treatment of the Boc derivative with HCl in EtOAc) by a method similar to that used for compound (**1**), (59.2%), m.p. 243—244 °C, $[\alpha]_D^{25}$ −47.1° (*c* 2, in MeOH), R_{FD} 0.88, R_{FE} 0.68, R_{FH} 0.80, R_{FP} 0.22, and R_{FQ} 0.48 (Found: C, 60.1; H, 8.2; N, 12.1. $C_{29}H_{47}N_5O_7$ requires C, 60.3; H, 8.2; N, 12.1%).

N-[(1S)-(1-*Methoxycarbonyl-3-methylbutyl*)*carbamoyl*]-L-*valyl-L-alanyl-α-azanorvaline Benzyl Ester* (**5**).—Prepared as above by treating *N*-carbonyl-L-leucine methyl ester with Val-Ala-Aznva-OCH_2Ph (obtained by HCl–ethyl acetate treatment of the Boc derivative), (43.3%), m.p. 228—229 °C, $[\alpha]_D^{25}$ −62.7° (*c* 1.5, in MeOH), R_{FE} 0.67, R_{FH} 0.45, R_{FQ} 0.52 (Found: C, 58.7; H, 7.9; N, 12.9. $C_{27}H_{43}N_5O_7$ requires C, 59.0; H, 7.8; N, 12.7%).

N-[(1S)-(1-*Methoxycarbonyl-2-methylpropyl*)*carbamoyl*]-L-*valyl-L-prolyl-α-azanorvaline Benzyl Ester* (**6**).—This was prepared as above from *N*-carbonyl-L-valine methyl ester and Val-Pro-Aznva-OCH_2Ph and purified by silica gel column chromatography using chloroform and 1 and 2% methanol in chloroform as the eluants to give the *ester* (**6**) as a foam (89.0%), R_{FA} 0.77, R_{FB} 0.72, R_{FC} 0.70, R_{FD} 0.77, R_{FE} 0.61, R_{FH} 0.65, R_{FP} 0.61, and R_{FQ} 0.75 (Found: C, 59.7; H, 7.6; N, 12.2. $C_{28}H_{43}N_5O_7$ requires C, 59.9; H, 7.7; N, 12.5%).

N-[(1S)-(1-*Methoxycarbonyl-2-methylpropyl*)*carbamoyl*]-L-*valyl-L-alanyl-α-azanorvaline Benzyl Ester* (**7**).—Prepared as above from *N*-carbonyl-L-valine methyl ester and Val-Ala-Aznva-OCH_2Ph and purified by silica gel column chromatography using chloroform, and 1, 2, and 5% methanol in chloroform as the eluants, 76.2%, m.p. 231—233 °C, $[\alpha]_D^{25}$ −65.8° (*c* 2, in MeOH), R_{FA} 0.74, R_{FB} 0.70, R_{FC} 0.70, R_{FF} 0.68, R_{FH} 0.62, and R_{FQ} 0.71 (Found: C, 57.9; H, 7.6; N, 12.8. $C_{26}H_{41}N_5O_7$ requires C, 58.3; H, 7.7; N, 13.1%).

N-[(1S)-(1-*Methoxycarbonyl-2-methylpropyl*)*carbamoyl*]-L-*valylglycyl-α-azanorvaline Benzyl Ester* (**8**).—Prepared as above from *N*-carbonyl-L-valine methyl ester and Val-Gly-Aznva-OCH_2Ph and purified by silica gel column chromatography using chloroform and 2% methanol in chloroform as the eluants, (78.5%), m.p. 154—155 °C, $[\alpha]_D^{25}$ −12.4° (*c* 2, in MeOH), R_{FD} 0.81, R_{FE} 0.49, R_{FF} 0.77, R_{FH} 0.60, R_{FP} 0.19, and R_{FQ} 0.52 (Found: C, 57.4; H, 7.9; N, 13.6. $C_{25}H_{39}N_5O_7$ requires C, 57.6; H, 7.5; N, 13.4%).

N-[(1S)-(1-*Methoxycarbonylethyl*)*carbamoyl*]-L-*valyl-L-prolyl-α-azanorvaline Benzyl Ester* (**9**).—This was prepared as above from *N*-carbonyl-L-alanine methyl ester and Val-Pro-Aznva-OCH_2Ph, (88.9%), foam, R_{FD} 0.68, R_{FE} 0.47, R_{FF} 0.57, R_{FH} 0.47, R_{FP} 0.37, and R_{FQ} 0.50 (Found: C, 58.8; H, 7.0; N, 13.3. $C_{26}H_{39}N_5O_7$ requires C, 58.5; H, 7.3; N, 13.1%).

N-[(1S)-(1-*Methoxycarbonylethyl*)*carbamoyl*]-L-*valyl-L-alanyl-α-azanorvaline Benzyl Ester* (**10**).—Prepared as above from *N*-carbonyl-L-alanine methyl ester and Val-Ala-Aznva-OCH_2Ph, (76.1%), m.p. 240—241 °C, R_{FA} 0.86, R_{FB} 0.77, R_{FC} 0.70 R_{FD} 0.77, R_{FE} 0.39, R_{FF} 0.80, R_{FH} 0.69, R_{FP} 0.25, and R_{FQ} 0.48 (Found: C, 56.8; H, 7.5; N, 14.0. $C_{24}H_{37}N_5O_7$ requires C, 56.8; H, 7.4; N, 13.8%).

N-[(1S)-(1-*Methoxycarbonylethyl*)*carbamoyl*]-L-*valylglycyl-α-azanorvaline Benzyl Ester* (**11**).—Prepared as above from *N*-carbonyl-L-alanine methyl ester and Val-Gly-Aznva-OCH_2Ph. The product was purified by silica gel column chromatography using chloroform and 2% methanol in chloroform as the eluants, (71.3%), m.p. 207—209 °C, $[\alpha]_D^{25}$ −15.9° (*c* 2, in MeOH), R_{FD} 0.74, R_{FE} 0.40, R_{FF} 0.77, R_{FH} 0.54, R_{FP} 0.17, and R_{FQ} 0.42 (Found: C, 55.8; H, 7.5; N, 14.4. $C_{23}H_{35}N_5O_7$ requires C, 56.0; H, 7.2; N, 14.2%).

N-*t-Butoxycarbonyl-L-prolyl-α-azanorvaline Benzyl Ester*.—To a cooled (0 °C) and stirred solution of Boc-Pro (19.3 g, 89.8 mmol), HOBt (24.3 g, 180 mmol), Aznva-OCH_2Ph·HCl (20.0 g, 81.7 mmol), and triethylamine (11.4 ml, 81.7 mmol) in DMF (200 ml) was added DCCI (18.5 g, 90 mmol) and the stirring was continued overnight at 4 °C. Dicyclohexylurea was removed by filtration and the filtrate was evaporated to dryness. The residue was taken up in ethyl acetate, washed with 20% aqueous citric acid, saturated aqueous $NaHCO_3$, and water and was then dried (Na_2SO_4). The ethyl acetate was then evaporated and the crude product was purified by silica gel column chromatography using ethyl acetate–toluene (1:1) as the eluting solvent. The pure product was obtained as a white solid (26.8 g, 80.9%), m.p. 98—99 °C, R_{FE} 0.61, R_{FH} 0.72, R_{FQ} 0.68 (Found: C, 62.1; H, 7.9; N, 10.4. $C_{21}H_{31}N_3O_5$ requires C, 62.2; H, 7.7; N, 10.3%).

N-[(1S)-(1-*Methoxycarbonyl-3-methylbutyl*)*carbamoyl*]-L-*prolyl-α-azanorvaline Benzyl Ester* (**12**).—*N*-Carbonyl-L-leucine methyl ester (0.71 g, 4.1 mmol) was added to a stirred solution of Pro-Aznva-OCH_2Ph·HCl (obtained from the above Boc compound by treatment with HCl in ethyl acetate) (1.28 g, 3.7 mmol) and triethylamine (0.52 ml, 3.7 mmol) in chloroform (20 ml). After 5 h at room temperature the reaction mixture was diluted with ethyl acetate, washed with water, 20% aqueous citric acid, and water, and was then dried (Na_2SO_4) and evaporated to dryness. The crude product was purified by silica gel column chromatography using chloroform and 2% methanol in chloroform as the eluting solvents, (72.8%), m.p. 125—126 °C, $[\alpha]_D^{25}$ −76.8° (*c* 2, in MeOH), R_{FD} 0.68, R_{FE} 0.46, and R_{FQ} 0.63 (Found: C, 60.4; H, 7.6; N, 11.4. $C_{24}H_{36}N_4O_6$ requires C, 60.4; H, 7.6; N, 11.7%).

N-[(1S)-(1-*Methoxycarbonyl-2-methylpropyl*)*carbamoyl*]-L-*prolyl-α-azanorvaline Benzyl Ester* (**13**).—Prepared from *N*-carbonyl-L-valine methyl ester, Pro-Aznva-OCH_2Ph·HCl, and triethylamine by a procedure similar to that described above for compound (**12**). The crude product was purified by silica gel

column chromatography using ethyl acetate–light petroleum (b.p. 40—60 °C) (3:2) as the eluant to give the product as an oil (1.51 g, 87.3%), R_{FD} 0.75, R_{FE} 0.49, R_{FF} 0.68, R_{FH} 0.50, R_{FP} 0.56, R_{FQ} 0.70 (Found: C, 59.5; H, 7.1; N, 12.2. $C_{23}H_{34}N_4O_6$ requires C, 59.7; H, 7.4; N, 12.1%).

N-[(1S)-(1-*Methoxycarbonylethyl*)*carbamoyl*]-L-*prolyl*-α-*azanorvaline Benzyl Ester* (**14**).—Prepared as above from *N*-carbonyl-L-alanine methyl ester and Pro-Aznva-OCH_2Ph and purified by silica gel column chromatography using chloroform and 1% methanol in chloroform as the eluants, (69.6%), foam, $[\alpha]_D^{25}$ −91.6° (*c* 2.5, in MeOH), R_{FD} 0.68, R_{FE} 0.43, R_{FF} 0.58, R_{FH} 0.40, R_{FP} 0.47, R_{FQ} 0.67 (Found: C, 57.9; H, 6.8; N, 12.8. $C_{12}H_{30}N_4O_6$ requires C, 58.0; H, 7.0; N, 12.9%).

N-[(1S)-(1-*Methoxycarbonyl*-2-*phenylethyl*)*carbamoyl*]-L-*prolyl*-α-*azanorvaline Benzyl Ester* (**15**).—Prepared as above from *N*-carbonyl-L-phenylalanine methyl ester and Pro-Aznva-OCH_2Ph and purified by silica gel column chromatography using chloroform and 2% methanol in chloroform as the eluants, (68.4%), foam, $[\alpha]_D^{25}$ −71.6° (*c* 2, in MeOH), R_{FD} 0.73, R_{FE} 0.49, R_{FF} 0.63, R_{FH} 0.46, R_{FP} 0.54, and R_{FQ} 0.68 (Found: C, 63.4; H, 6.6; N, 10.7. $C_{27}H_{34}N_4O_6$ requires C, 63.5; H, 6.7; N, 11.0%).

N-[(1R)-(1-*Methoxycarbonyl*-2-*methylpropyl*)*carbamoyl*]-L-*valyl*-L-*prolyl*-α-*azanorvaline Benzyl Ester* (**16**).—Prepared from *N*-carbonyl-D-valine methyl ester and Val-Pro-Aznva-OCH_2Ph by a procedure similar to that described for compound (**1**). The product after silica gel column chromatography using chloroform and 1 and 2% methanol in chloroform as the eluants was obtained as a foam (76%), $[\alpha]_D^{25}$ −90.8° (*c* 1, in MeOH), R_{FE} 0.59, R_{FH} 0.68, and R_{FQ} 0.56 (Found: C, 59.6; H, 7.6; N, 12.4. $C_{28}H_{43}N_5O_7$ requires C, 59.8; H, 7.7; N, 12.4%).

N-*t*-*Butoxycarbonylglycyl*-L-*valylglycyl*-α-*azanorvaline Benzyl Ester* (**17**).—Prepared by coupling Boc-Gly-Val-Gly-OH (5.4 g, 16.5 mmol) and Aznva-OCH_2Ph (3.5 g, 15 mmol) by the DCCI–HOBt method and purified by h.p.l.c. on Waters LC-500 using chloroform as the eluant (7.6 g, 97%), foam, $[\alpha]_D^{25}$ −13.3° (*c* 3, in MeOH), R_{FD} 0.71, R_{FE} 0.30, R_{FH} 0.50, R_{FP} 0.17, and R_{FQ} 0.41 (Found: C, 57.6; H, 7.3; N, 13.1. $C_{25}H_{39}N_5O_7$ requires C, 57.6; H, 7.5; N, 13.4%).

N-[(1S)-(1-*Methoxycarbonyl*-3-*methylbutyl*)*carbamoyl*]*glycyl*-L-*valylglycyl*-α-*azanorvaline Benzyl Ester* (**18**).—Boc-Gly-Val-Gly-Aznva-OCH_2Ph was treated with HCl in ethyl acetate for 45 min at room temperature to give Gly-Val-Gly-Aznva-OCH_2Ph·HCl. The hydrochloride (0.68 g, 1.5 mmol) was treated with *N*-carbonyl-L-leucine methyl ester (0.25 g, 1.5 mmol) and triethylamine (0.21 ml, 1.5 mmol) in chloroform (5 ml) for 4 h. Ethyl acetate (150 ml) was added and the solution was washed with water, 20% aqueous citric acid and water, and then dried (Na_2SO_4) and evaporated to dryness. The pure product was obtained by precipitation from methanol–ether (87.1%), m.p. 190—192 °C, $[\alpha]_D^{25}$ −20.7° (*c* 2.5, in MeOH), R_{FA} 0.56, R_{FC} 0.67, R_{FD} 0.62, R_{FH} 0.43, R_{FP} 0.19, and R_{FQ} 0.48 (Found: C, 56.5; H, 7.4; N, 13.9. $C_{28}H_{44}N_6O_8$ requires C, 56.7; H, 7.5; N, 14.2%).

N-[(1S)-(1-*Methoxycarbonylethyl*)*carbamoyl*]*glycyl*-L-*valylglycyl*-α-*azanorvaline Benzyl Ester* (**19**).—Prepared as above from *N*-carbonyl-L-alanine methyl ester and Gly-Val-Gly-Aznva-OCH_2Ph. The pure product was obtained by precipitation from ethyl acetate–light petroleum (b.p. 40—60 °C), (82.1%), m.p. 200—202 °C, $[\alpha]_D^{25}$ −20.7° (*c* 2, in MeOH), R_{FB} 0.63, R_{FC} 0.36, R_{FP} 0.20, and R_{FQ} 0.44 (Found: C, 54.3; H, 7.1; N, 14.9. $C_{25}H_{38}N_6O_8$ requires C, 54.5; H, 7.0; N, 15.3%).

(R)-2-*Bromo*-4-*methylpentanoic Acid*.—Sodium nitrite (24 g, 0.28 mol) in water (50 ml) was added dropwise over 4 h to a stirred solution of D-leucine (33 g, 0.25 mol) and potassium bromide (97.5 g, 0.82 mol) in aqueous sulphuric acid (28.25 ml of conc. H_2SO_4 in 300 ml water) at 0 °C. Stirring was continued for a further 2 h at 0 °C and overnight at room temperature. The product was extracted into ether (4 × 150 ml), back-washed with saturated aqueous sodium chloride (2 × 40 ml), and then dried ($MgSO_4$). Evaporation of the ether left an oil, which on distillation (b.p. 88—90 °C, 0.5 mmHg) gave the product as a colourless oil (38 g, 77.6%), $[\alpha]_D^{22}$ +39.6° (*c* 1.4, in MeOH) {lit.,[19] $[\alpha]_D^{22}$ +38.2° (*c* 2, in MeOH)}.

(S)-2-*Bromo*-4-*methylpentanoic Acid*.—This was prepared from L-leucine (131.2 g, 1 mol) in an analogous way. The product was obtained by distillation (87—88 °C, 0.5 mmHg) as an oil (150 6 g, 77%), $[\alpha]_D^{22}$ −38.9° (*c* 1, in MeOH {lit.,[24] $[\alpha]_D^{27}$ −34° (MeOH)}.

(S)-2-*Bromopropionic Acid*.—This was prepared from L-alanine (89.1 g, 1 mol) as above. The product was obtained by distillation (68—72 °C, 0.9 mmHg) as a colourless oil (94 g, 61.4%), $[\alpha]_D^{21}$ −29.8° (*c* 1, in MeOH) {lit.,[20,24] $[\alpha]_D^{27}$ −27.6° (MeOH)}.

(R)-2-(2-*Aminoethylthio*)-4-*methylpentanoic Acid* [Gly-ψ(CH_2S)-D-Leu].—(*S*)-2-Bromo-4-methylpentanoic acid (58.5 g, 300 mmol) was dissolved in 0.5M-aqueous $NaHCO_3$ (1 800 ml, 900 mmol) and the solution purged with nitrogen for 30 min. Still under nitrogen purge, 2-aminoethanethiol hydrochloride (102.2 g, 900 mmol) was added and the reaction solution was stirred for 1 h, stoppered and left for 2 days. The solution was acidified with 6M-HCl and then concentrated to *ca.* 750 ml. Butan-1-ol (750 ml) was added and after partitioning, the aqueous phase was transferred in a countercurrent fashion through eight separating funnels each containing butan-1-ol (500 ml) saturated with water. The organic phases were washed eight times with water saturated with butan-1-ol (200 ml). The combined organic phases were evaporated to an oil which was dissolved in water (750 ml) neutralised with 2M-NaOH and passed down a column of Bio-Rad AGI-X2 resin (hydroxide form; 360 ml) in two batches. The columns were washed with water and the product was eluted using a gradient of 10% $MeCO_2H$ (1 l) *vs.* water (1 l). The product containing fractions were collected, evaporated to dryness and the product crystallised from aqueous ethanol (25.8 g, 45%), m.p. 207—211 °C, $[\alpha]_D^{21}$ +22.6° (*c* 1.5, in water), R_{FA} 0.45, R_{FB} 0.5, R_{FK} 0.44 (Found: C, 50.4; H, 8.9; N, 7.4. Calc. for $C_8H_{17}NO_2S$: C, 50.2; H, 8.9; N, 7.3%) {lit.,[19] $[\alpha]_D$ +24.1° (*c* 2, in water)}.

(S)-2-(2-*Aminoethylthio*)-4-*methylpentanoic Acid* [Gly-ψ(CH_2S)-Leu].—2-Aminoethanethiol hydrochloride (43.69 g, 385 mmol) in water (50 ml) was purged with nitrogen for 30 min and then added to an ice-cold solution of (*R*)-2-bromo-4-methylpentanoic acid (25 g, 128 mmol) in DMF (200 ml) also under nitrogen purge. Triethylamine (53.9 ml, 385 mmol) in DMF (40 ml) was added and the reaction was stirred at 0 °C for 30 min. A solid started to separate from the reaction solution which after about 1.5 h became a solid mass. This solid was collected by filtration, washed with cold ethanol and then with ether. The solid was crystallised from methanol–propan-2-ol (10.6 g, 43%), m.p. 207—209 °C $[\alpha]_D^{26}$ −23.9° (*c* 1, in water), R_{FA} 0.46, R_{FB} 0.5, and R_{FK} 0.44 (Found: C, 50.3; H, 8.9; N, 7.3. Calc. for $C_8H_{17}NO_2S$: C, 50.2; H, 8.9; N, 7.3%) {lit.,[19] m.p. 205—210 °C, $[\alpha]_D^{21}$ −23.2° (*c* 2, in water)}.

(R)-2-(2-*Aminoethylthio*)-4-*methylpentanoic Acid Benzyl Ester* [Gly-ψ(CH_2S)-D-Leu-OCH_2Ph].—The pseudo dipeptide

Gly-ψ(CH_2S)-D-Leu (9.55 g, 50 mmol) in toluene (75 ml) was refluxed in a Dean and Stark trap with benzyl alcohol (15 ml) and toluene-*p*-sulphonic acid monohydrate (10.4 g, 54.7 mmol) for 2 h after which time no more water was collected. The solution was cooled, evaporated to an oil and triturated several times with light petroleum (b.p. 60—80 °C). The mobile oil was dissolved in ether (50 ml) and on addition of light petroleum (b.p. 60—80 °C) and scratching, the product was obtained as a waxy solid (24.4 g) which was dissolved in 50% aqueous methanol (500 ml) and passed through a column of Bio-Rad AGI-X2 resin (acetate form) (20 × 5 cm). The product-containing eluates were then passed through a column of Bio-Rex 70 (hydrogen form) resin (20 × 5 cm). The product was retained, unchanged benzyl alcohol was removed by washing through with 50% aqueous methanol, and the column was then eluted with a 10% solution of HCl in 50% aqueous methanol. The product containing fractions were evaporated and the residue was freeze-dried from aqueous 2-methylpropan-2-ol (12.6 g, 79.2%), R_{FQ} 0.2 and R_{FK} 0.83 (Found: C, 55.8; H, 7.4; N, 4.3; S, 10.2; Cl, 10.9. $C_{15}H_{23}NO_2S{\cdot}HCl{\cdot}\frac{1}{4}H_2O$ requires C, 55.87; H, 7.34; N, 4.34; S, 9.95; Cl, 11.0%).

(S)-2-(2-*Aminoethylthio*)-4-*methylpentanoic Acid Benzyl Ester* [Gly-ψ(CH_2S)-Leu-OCH_2Ph].—This was prepared as above from the corresponding pseudo dipeptide, Gly-ψ(CH_2S)-Leu (7 g, 36.6 mmol) but was purified as the toluene-*p*-sulphonate salt by preparative chromatography on a Waters LC-500 using 5 and 10% methanol in chloroform as the eluants, (12.5 g, 75%), R_{FQ} 0.17 and R_{FK} 0.7 (Found: C, 58.2; H, 6.6; N, 2.9; S, 13.9. $C_{22}H_{31}NO_5S_2$ requires C, 58.2; H, 6.9; N, 3.0; S, 14.1%).

N-t-*Butoxycarbonyl*-L-*leucylglycyl*-ψ(CH_2S)-*leucine Benzyl Ester* (**20**).—This was prepared by coupling Boc-Leu (3.2 g, 13.8 mmol) with Gly-ψ(CH_2S)-Leu-OCH_2Ph toluene-*p*-sulphonate (6.3 g, 13.8 mmol) in the presence of one equivalent of triethylamine in DMF (50 ml) by the DCCI–HOBt method. The procedure was similar to that described above for Boc-Pro-Aznva-OCH_2Ph. The product was obtained as an oil after silica gel column chromatography on Waters LC-500 using 2% methanol in chloroform as the eluant (6.5 g, 95%), $[\alpha]_D^{25}$ − 56.8° (*c* 2, in MeOH), R_{FP} 0.63, R_{FQ} 0.66, and R_{FK} 0.83 (Found: C, 63.1; H, 8.6; N, 5.8; S, 6.3. $C_{26}H_{42}N_2O_5S$ requires C, 63.1; H, 8.5; N, 5.6; S, 6.4%).

N-t-*Butoxycarbonyl*-L-*leucylglycyl*-ψ(CH_2S)-D-*leucine Benzyl Ester* (**21**).—Prepared from Boc-Leu (2.32 g, 10 mmol) and Gly-ψ(CH_2S)-D-Leu-OCH_2Ph (3.17 g, 10 mmol) by the DCCI–HOBt method. The crude product was purified on Waters LC-500 using 5% ethyl acetate in toluene as the eluant, (2.8 g, 57%), oil, $[\alpha]_D^{25}$ +23.4° (*c* 1, in MeOH) (Found: C, 62.9; H, 8.5; N, 5.4; S, 6.3. $C_{26}H_{42}N_2O_5S$ requires C, 63.1; H, 8.5; N, 5.6; S, 6.4%).

N-[(1S)-(1-*Methoxycarbonyl*-2-*methylpropyl*)*carbamoyl*]-L-*leucylglycyl*-ψ(CH_2S)-*leucine Benzyl Ester* (**22**).—Prepared by the reaction of *N*-carbonyl-L-valine methyl ester (1.0 g, 6.4 mmol), Leu-Gly-ψ(CH_2S)-Leu-$OCH_2Ph{\cdot}HCl$ (2.1 g, 4.87 mmol) and triethylamine (0.68 ml, 4.87 mmol) in chloroform (5 ml). The product was obtained as an oil after gel filtration on a column of Bio-Beads SX2 in tetrahydrofuran, (2.41 g, 90%), R_{FP} 0.36, R_{FQ} 0.46, and R_{FK} 0.95 (Found: C, 60.8; H, 8.0; N, 7.7; S, 5.6. $C_{28}H_{45}N_3O_6S$ requires C, 60.9; H, 8.2; N, 7.6; S, 5.8%).

N-[(1S)-(1-*Methoxycarbonyl*-3-*methylbutyl*)*carbamoyl*]-L-*leucylglycyl*-ψ(CH_2S)-D-*leucine Benzyl Ester* (**23**).—Prepared as above from *N*-carbonyl-L-valine methyl ester (232 mg, 1.5 mmol), Leu-Gly-ψ(CH_2S)-D-Leu-$OCH_2Ph{\cdot}HCl$ (470 mg, 1.09 mmol), and triethylamine (0.15 ml, 1.1 mmol) in chloroform (5 ml). The product was obtained as an oil after silica gel column chromatography using 5% methanol in chloroform as eluant, (524 mg, 87%), $[\alpha]_D^{25}$ +14.6° (*c* 2, in MeOH), R_{FH} 0.73, R_{FP} 0.63, R_{FQ} 0.73, and R_{FK} 0.93 (Found: C, 60.7; H, 8.1; N, 7.6; S, 5.6. $C_{28}H_{45}N_3O_6S$ requires C, 60.9; H, 8.2; N, 7.6; S, 5.8%).

N-[(1S)-(1-*Methoxycarbonyl*-2-*methylpropyl*)*carbamoyl*]-L-*leucylglycyl*-ψ(CH_2S)-*leucine Benzyl Ester* (**24**).—Preparation as above from *N*-carbonyl-L-leucine methyl ester (1 g, 5.8 mmol), Leu-Gly-ψ(CH_2S)-Leu-$OCH_2Ph{\cdot}HCl$ (2.1 g, 4.81 mmol) and triethylamine (0.68 ml, 4.87 mmol) in chloroform (25 ml). The product after silica gel column chromatography using 1.5% methanol in chloroform as the eluant was obtained as an oil (2.26 g, 82%), $[\alpha]_D^{25}$ −35.2° (*c* 2, in MeOH), R_{FP} 0.37, R_{FQ} 0.61, and R_{FK} 0.94 (Found: C, 61.3; H, 8.1; N, 7.4; S, 5.5. $C_{29}H_{47}N_3O_6S$ requires C, 61.5; H, 8.3; N, 7.4; S, 5.6%).

N-[(1S)-(1-*Methoxycarbonyl*-3-*methylbutyl*)*carbamoyl*]-L-*leucylglycyl*-ψ(CH_2S)-D-*leucine Benzyl Ester* (**25**).—Prepared as above from *N*-carbonyl-L-leucine methyl ester (256 mg, 1.5 mmol), Leu-Gly-ψ(CH_2S)-D-Leu-$OCH_2Ph{\cdot}HCl$ (479 mg, 1.1 mmol) and triethylamine (0.15 ml, 1.1 mmol) in chloroform (5 ml). The product was purified by silica gel column chromatography using 2% methanol in chloroform as the eluant, (599 mg, 96%), oil, $[\alpha]_D^{25}$ +14.6° (*c* 1, MeOH), R_{FH} 0.73, R_{FP} 0.63, R_{FQ} 0.73, and R_{FK} 0.93 (Found: C, 61.2; H, 8.4; N, 7.2; S, 5.5. $C_{29}H_{47}N_3O_6S$ requires C, 61.5; H, 8.3; N, 7.4; S, 5.6%).

N-*t*-*Butoxycarbonyl*-L-*valylglycyl*-ψ(CH_2S)-D-*leucine Benzyl Ester* (**26**).—A solution of DCCI (2.2 g, 10.6 mmol) in DMF (5 ml) was added to an ice-cold solution of Boc-Val (2.17 g, 10 mmol), HOBt (2.7 g, 20 mmol) and Gly-ψ(CH_2S)-D-Leu-$OCH_2Ph{\cdot}HCl$ (3.17 g, 10 mmol) in DMF (50 ml). Triethylamine (1.4 ml, 10 mmol) was added and the reaction was stirred at 0 °C for 1 h and then at room temperature overnight. The dicyclohexylurea was removed by filtration and the filtrate was evaporated to dryness to leave an oil which was partitioned between ethyl acetate and water. The organic phase was washed successively with 20% aqueous citric acid, saturated aqueous NaCl, saturated aqueous $NaHCO_3$, and saturated NaCl, and then dried over $MgSO_4$ and evaporated to an oil (4.38 g, 92%). Purification was by preparative chromatography on Waters LC-500 using ethyl acetate–toluene (1:9) as the eluant. The product was isolated as an oil, $[\alpha]_D^{25}$ +23.5° (*c* 1, in MeOH), R_{FL} 0.2, R_{FM} 0.49, R_{FN} 0.67 (Found: C, 62.6; H, 8.7; N, 5.6; S, 6.4. $C_{25}H_{40}N_2O_5S$ requires C, 62.4; H, 8.4; N, 5.8; S, 6.6%).

N-[(1S)-(1-*Methoxycarbonyl*-3-*methylbutyl*)*carbamoyl*]*glycyl*-ψ(CH_2S)-D-*leucine Benzyl Ester* (**27**).—Prepared by the reaction of *N*-carbonyl-L-leucine methyl ester (516 mg, 3 mmol) and Gly-ψ(CH_2S)-D-Leu-$OCH_2Ph{\cdot}HCl$ (644 mg, 2 mmol) in the presence of triethylamine (0.28 ml, 2 mmol) in chloroform (5 ml). The product was purified by chromatography on silica gel using 2.5% methanol in chloroform as the eluant followed by a gel filtration on a column of Bio-Beads SX2 (Bio-Rad) in tetrahydrofuran. The product was obtained as an oil (500 mg, 62%), $[\alpha]_D^{25}$ +28.1° (*c* 1, in MeOH), R_{FP} 0.41, R_{FQ} 0.53, and R_{FK} 0.90 (Found: C, 61.2; H, 8.2; N, 6.4; S, 7.1. $C_{23}H_{36}N_2O_5S$ requires C, 61.0; H, 8.0; N, 6.2; S, 7.0%).

N-[(1S)-(1-*Methoxycarbonyl*-2-*methylpropyl*) *carbamoyl*]-*glycyl*-ψ(CH_2S)-D-*leucine Benzyl Ester* (**28**).—Preparation from *N*-carbonyl-L-valine methyl ester (470 mg, 3 mmol), Gly-ψ(CH_2S)-D-Leu-$OCH_2Ph{\cdot}HCl$ (644 mg, 2 mmol) and triethylamine (0.28 ml, 2 mmol) and purification as above gave the product as an oil, (531 mg, 68%), $[\alpha]_D^{25}$ +40.3° (*c* 1, in MeOH),

R_{FP} 0.50, R_{FQ} 0.58, and R_{FK} 0.95 (Found: C, 59.9; H, 7.7; N, 6.4; S, 7.1. $C_{22}H_{34}N_2O_5S$ requires C, 60.2; H, 7.8; N, 6.4; S, 7.3%).

N-[(1S)-(1-*Methoxycarbonyl*-3-*methylbutyl*)*carbamoyl*]-L-*valylglycyl*-ψ(CH_2S)-D-*leucine Benzyl Ester* (**29**).—*N*-Carbonyl-leucine methyl ester (255 mg, 1.5 mmol) was added to an ice-cold, stirred solution of Val-Gly-ψ(CH_2S)-D-Leu-OCH_2Ph (422 mg, 1.01 mmol) (prepared by treating the above *N*-t-butoxycarbonyl derivative with HCl in ethyl acetate for 1 h at room temperature) and triethylamine (0.14 ml, 1 mmol) in chloroform (5 ml) and the reaction mixture was stirred in an ice-bath for 1 h and overnight at room temperature. The reaction mixture was diluted with chloroform (70 ml) and the organic phase washed with 20% aqueous citric acid and saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and evaporated to leave an oil. Silica gel column chromatography using 2% methanol in chloroform as the eluant gave the pure product as an oil (470 mg, 85%) R_{FP} 0.29, R_{FQ} 0.53, and R_{FK} 0.83 (Found: C, 60.6; H, 8.2; N, 7.4; S, 5.9. $C_{28}H_{45}N_3O_6S$ requires C, 60.9; H, 8.2; N, 7.6; S, 5.8%).

N-[(1S)-(1-*Methoxycarbonyl*-2-*methylpropyl*)*carbamoyl*]-L-*valylglycyl*-ψ(CH_2S)-D-*leucine Benzyl Ester* (**30**).—Prepared and purified as above from *N*-carbonyl-L-valine methyl ester (235 mg, 1.5 mmol), Val-Gly-ψ(CH_2S)-D-Leu-OCH_2Ph·HCl (406 mg, 0.97 mmol) and triethylamine (0.14 ml, 1 mmol), yield 493 mg (92%), oil, $[\alpha]_D^{25}$ +16.3° (*c* 2, in MeOH), R_{FP} 0.59, R_{FQ} 0.74 R_{FH} 0.75, and R_{FK} 0.95 (Found: C, 60.2; H, 8.0; N, 7.6; S, 5.7. $C_{27}H_{43}N_3O_6S$ requires C, 60.3; H, 8.0; N, 7.8; S, 5.9%).

2-(2-*t*-*Butoxycarbonylamino*-4-*methylpentylthio*)*propionic Acid* [Boc-Leu-ψ(CH_2S)-Ala].—2-t-Butoxycarbonylamino-4-methylpentanol (Boc-Leucinol) (118.5 g, 0.5 mol) was converted into the *O*-tosyl derivative by reaction with toluene-*p*-sulphonyl chloride (95.2 g, 0.5 mol) in pyridine (650 ml) at 4 °C overnight. The solvent was removed and the residue dissolved in ethyl acetate was washed with saturated aqueous $NaHCO_3$, water, 20% aqueous citric acid, and water. The ethyl acetate layer was dried (Na_2SO_4) and evaporated to dryness to leave an oil (164.6 g, 89%).

Potassium *O*-ethyl thiocarbonate (52 g, 0.33 mol) was added to the above tosyl derivative (110 g, 0.3 mol) in DMF (225 ml) and the reaction mixture was stirred at 45—50 °C for 4 h by which time the reaction mixture had solidified. After standing overnight at room temperature the solid was removed by filtration and the clear filtrate was cooled in an ice-bath, purged with nitrogen, and ethylenediamine (200 ml) was added dropwise under nitrogen with stirring. After 3 h the reaction mixture was stoppered and left overnight. The solvent was removed and the resulting oil, dissolved in nitrogen flushed ethyl acetate, was washed with nitrogen flushed $KHSO_4$ solution until acid, then with saturated aqueous NaCl, dried (Na_2SO_4) and evaporated to an oil (100 g). The 2-t-butoxycarbonylamino-4-methylpentanethiol was not purified any further as it is readily oxidised to the disulphide. The above thiol derivative (10 g, 42 mmol) in DMF (100 ml) was stirred and flushed with nitrogen and cooled in an ice-bath. Under constant nitrogen purge a solution of D-2-bromopropionic acid (6 g, 40 mmol) in water (5 ml) was added, followed by triethylamine (11.1 ml, 2 equivalents). After 3 h the pH of the solution was adjusted to 8 by the addition of triethylamine. After 4 days one more equivalent of the bromo acid (6 g) was added and after a further 24 h the reaction was terminated. The solvent was removed by evaporation and the residue was partitioned between ethyl acetate and water, the organic phase washed with 20% aqueous citric acid, with saturated aqueous NaCl, dried ($MgSO_4$) and evaporated to an oil (9 g). This was dissolved in butan-ol–methanol–water (BMW; 1:1:1 v/v) (400 ml) and the solution was passed down a column of Bio-Rad AG1-X2 resin (acetate form, 26 × 3 cm) pre-equilibrated with the same solvent. The column was eluted with BMW to remove the acidic products, then with a gradient of 10% acetic acid in BMW (400 ml) *vs.* BMW (400 ml) and fractions of about 12 ml were collected. The product containing fractions were combined, evaporated, redissolved in BMW and evaporated to leave a solid (4.34 g, 34%), R_{FH} 0.61, R_{FP} 0.27, R_{FQ} 0.45, and R_{FK} 0.82 (Found: C, 54.9; H, 8.8; N, 4.6; S, 10.5. $C_{14}H_{27}NO_4S$ requires C, 55.0; H, 8.9; N, 4.5; S, 10.5%).

N-*t*-*Butoxycarbonyl*-L-*leucyl*-ψ(CH_2S)-L-*alanyl*-α-*azanorvaline Benzyl Ester*.—This was prepared from Boc-Leu-ψ(CH_2S)-Ala-OH (711 mg, 2.33 mmol), azanorvaline benzyl ester hydrochloride (485 mg, 2.33 mmol) with DCCI–HOBt and one equivalent of triethylamine in DMF (10 ml). The crude product was then purified by silica gel column chromatography using 2% methanol in chloroform as the eluant. The product was obtained as an oil (703 mg, 66%), R_{FP} 0.58, R_{FQ} 0.64, and R_{FK} 0.92.

N-[(1S)-(1-*Methoxycarbonyl*-3-*methylbutyl*)*carbamoyl*]-L-*leucyl*-ψ(CH_2S)-L-*alanyl*-α-*azanorvaline Benzyl Ester* (**31**).—Boc-Leu-ψ(CH_2S)-Ala-Aznva-OCH_2Ph (703 mg, 1.5 mmol) was deblocked with 3M-HCl in ethyl acetate (20 ml) and the product obtained as an oil after triturating with ether and drying (KOH–P_2O_5) (627 mg, 94%). The hydrochloride (312 mg, 0.72 mmol) was treated with *N*-carbonyl-L-leucine methyl ester (0.17 g, 1 mmol) in chloroform (2 ml). The pure product was obtained after silica gel column chromatography using 2% methanol in chloroform as the eluant (82%), $[\alpha]_D^{25}$ −45.2° (*c* 2, in MeOH), R_{FH} 0.75, R_{FP} 0.42, R_{FQ} 0.61, and R_{FK} 0.96 (Found: C, 59.0; H, 8.3; N, 9.6; S, 5.6. $C_{28}H_{46}N_4O_6S$ requires C, 59.3; H, 8.2; N, 9.8; S, 5.6%).

N-[(1S)-(*Methoxycarbonyl*-2-*methylpropyl*)*carbamoyl*]-L-*leucyl*-ψ(CH_2S)-L-*alanyl*-α-*azanorvaline Benzyl Ester* (**32**).—Prepared and purified as above from *N*-carbonyl-L-valine methyl ester (0.15 g, 0.96 mmol) and Leu-ψ(CH_2S)-Ala-Aznva-OCH_2Ph (0.31 g, 0.72 mmol), (243 mg, 61%), $[\alpha]_D^{25}$ −51.7° (*c* 2, in MeOH), R_{FH} 0.75, R_{FP} 0.42, R_{FQ} 0.62, and R_{FK} 0.96 (Found: C, 58.4; H, 8.1; N, 9.9; S, 6.0. $C_{27}H_{44}N_4O_6S$ requires C, 58.6; H, 8.0; N, 10.1; S, 5.8%).

2-*Isobutylthiomorpholin*-3-*one*.—(2*R*)-2-(2-Aminoethylthio)-4-methylpentanoic acid benzyl ester hydrotoluene-*p*-sulphonate (5 g, 11 mmol) in EtOAc (100 ml) was treated with 10% Na_2CO_3 (3 × 25 ml) in a separating funnel, then washed with saturated NaCl solution, dried ($MgSO_4$) and evaporated to an oil. The expected product was the free base, which was then treated with 4M-HCl–EtOAc (3.5 ml, 1.1 equivalents) to form the hydrochloride salt. The title compound was obtained as an oil with excess benzyl alcohol on evaporation of this acid solution. The thiomorpholinone was purified on silica gel using 2% MeOH–$CHCl_3$ as the eluant, and obtained as a solid, m.p. 66—67 °C, R_{FP} 0.37 and R_{FQ} 0.56, δ_H (90 MHz; $CDCl_3$; $SiMe_4$), 0.98 (3 H, d, Me), 0.95 (3 H, d, Me), 1.5—2.0 (3 H, m, CH_2CHMe_2), 2.85 (3 H, m, CH_2SCH), 3.55 (2 H, m, CH_2NH), and 7.0 (1 H, br s, NH).

Enzyme Inhibition Assay.—The procedure used by Costillo *et al.*[25] was modified and used for the analogues reported here. MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide was synthesized by Mr. R. A. Wildonger, I.C.I. U.S. The human leucocyte elastase was prepared from sputum of cystic fibrosis patients[26] and was supplied by Dr. D. Holselaw, Hahnemann Medical College, Philadelphia, U.S.A.

The inhibitors, dissolved in dimethyl sulphoxide, were in-

cubated with human leucocyte elastase (enzyme concentration 40 nM) in buffer (0.05M-Tris–0.5M-NaCl; pH 8.0) for 10 min. The reaction was initiated by the addition of substrate (0.10 mM). Four minutes later the reaction was terminated by the addition of glacial acetic acid (100 μl) and the OD_{410} was determined. Percent inhibition was determined in the usual manner and the IC_{50} values (concentration of the inhibitor producing 50% inhibition) were determined by plotting % inhibition *vs.* inhibitor concentration.

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Received 10*th January* 1986; *Paper* 6/079