Inhibitors of Porcine Pancreatic Elastase. Peptides Incorporating α-Aza-amino Acid Residues in the P_1 Position

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Inhibitors of porcine pancreatic elastase based on one of the repeating peptide sequences (Gly-Val-Gly-Val-Ala) present in elastin have been prepared. Most of these contain an α-aza-amino acid benzyl ester group at the C-terminus and an *N*-[(1-methoxycarbonylalkyl)carbamoyl]- or an *N*-[(1-carboxyalkyl)-carbamoyl]-group at the N-terminus. The most potent analogue of the series, *N*-[(1-carboxyethyl)-carbamoyl]-valylglycyl-α-aza-alanine benzyl ester (**53**) was *ca.* 60-fold more potent than one of the azapeptide inhibitors of elastase (Ac-Ala-Ala-Azala-ONp) reported earlier.

Various forms of elastase (*e.g.* pancreatic,[1] leucocytic,[2] macrophagic[3]) have been implicated in the pathogenesis of pulmonary emphysema, atherosclerosis, arthritis, and pancreatitis, *etc.*[4,5] In order to investigate the role of this enzyme in these disorders we have prepared inhibitors of pancreatic and leucocytic elastase, and our results on porcine pancreatic elastase inhibitors are reported here.

The active site of elastase which cleaves the peptide bonds formed by the carboxyl groups of small aliphatic side-chain containing amino acids extends over several subsites.[6,7] The nomenclature used by Schechter and Berger[8] to identify these subsites (P and P′) has been used throughout this paper.

Known substrates and inhibitors of elastase are based on L-alanine containing oligopeptides. Acetyl-$(Ala)_3$-OMe and $(Ala)_5$-Lys-Phe have been shown to be good substrates for elastase,[6,7] whereas peptides containing an α-aza-amino acid,[9,10] a C-terminal chloromethyl ketone,[11,12] or an amino-aldehyde[13] residue at the C-terminus were reported to be inhibitors of elastase. Some other di- and tri-peptide derivatives have also been shown to be potent inhibitors of elastase.[14,15]

Since elastin is the physiological substrate for elastase, it occurred to us that inhibitors of elastase based on the amino acid sequences present in elastin itself might be more potent and selective, if the sequences which are recognised by the enzyme could be identified. An examination of the amino acid sequences of bovine elastin, porcine tropoelastin, and bovine ligamentum nuchae elastin revealed the presence of two types of sequences which were repeated several times within the primary structures of these elastin fragments.[5,16,17] In some of these repeating sequences, *e.g.* Gly-Val-Gly-Val, Gly-Val-Gly-Leu, and Gly-Ile-Gly-Val, the glycine residues alternate with hydrophobic amino acids, whereas in some other, *e.g.* Val-Gly-Gly-Val, Val-Gly-Gly-Leu, Val-Gly-Gly-Ile, the hydrophobic amino acids were separated by two glycine residues. We hypothesised that these repeating sequences could be significant with respect to the overall conformation of the elastin molecule and may also be responsible for the binding of the natural substrate (elastin) with enzyme (elastase).

All of the work reported here is based on the Gly-Val-Gly-Val sequence. Since elastase has an extended binding site and cleaves peptide bonds formed by the carboxyl groups of the hydrophobic aliphatic amino acid residues, the repeating sequences may bind with the enzyme in the following manner.

Site of cleavage ↓ (between P_1 and P_1')

	P_5	P_4	P_3	P_2	P_1	P_1'	P_2'
		Gly -	Val -	Gly -	Val -	Ala -	Pro
or	Gly -	Val -	Gly -	Val -	Ala -	Pro	

On the basis of this assumption, α-aza-amino acid residues have been incorporated into the peptides in place of the Val or Ala which we believe may occupy the P_1 position. The residues occupying various other positions (P_1'—P_4) have also been modified in the hope of obtaining inhibitors of elastase.

Before embarking on the synthesis of analogues, Gly-Val-Gly-Val-OBzl, Boc-Gly-Val-Gly-Val-OBzl, and Boc-Pro-Gly-Val-Gly-Val-OBzl were synthesised and compared with Boc-Ala-Ala-Ala-OBzl as inhibitors of porcine pancreatic elastase. All of these compounds resulted in 65—75% inhibition of elastase at 400 μM, thus indicating that inhibitors of elastase based on the Gly-Val-Gly-Val peptide sequence could be at least as potent as the inhibitors which are based on the sequence Ala-Ala-Ala.

The analogous reported here are listed in Table 1. The P_1' modifications include a formyl group, Lac-OEt, Lac-NH_2, Ala-OBzl, Phe-OMe, Phe-OBzl, phenyl ester, benzyl ester, and a number of other aromatic or aliphatic esters. The Lac-OEt and Lac-NH_2 residues were earlier incorporated into the P_1' position and the resulting compounds shown to be potent inhibitors of elastase.[10] The P_1 position changes include a number of α-aza-amino acid residues (Azgly, Azala, Azval, Aznva, Azile). The P_2 position has been modified by incorporating Gly, Ala, Val, and Pro residues, but the P_3 position is occupied by Val in all the analogues. The P_4 position has been substituted by *N*-[(1-methoxycarbonyl-X)carbamoyl]-, *N*-[(1-carboxyalkyl-X)carbamoyl]-, *N*-[(1-ethoxycarbonylethyl)-oxycarbonyl]-, or a number of other acyl groups. The *N*-[(1-carboxyalkyl-3-methylbutyl)carbamoyl]- group is located at the *N*-terminus of elastatinal, a naturally occurring inhibitor of elastase.[18] For comparison purposes some analogues based on the sequences $(Ala)_n$-Azala-, -Ala-Pro-Azala-, and -Ala-Pro-Azala-Lac-, already reported as inhibitors of elastase,[9,10] have also been synthesized.

Synthesis.—All of the peptides reported here are listed in Table 1. The compounds have been arranged in the table in such a way that the analogues modified at position P_1' are listed first, followed by those modified at positions P_1, P_2, and P_4.

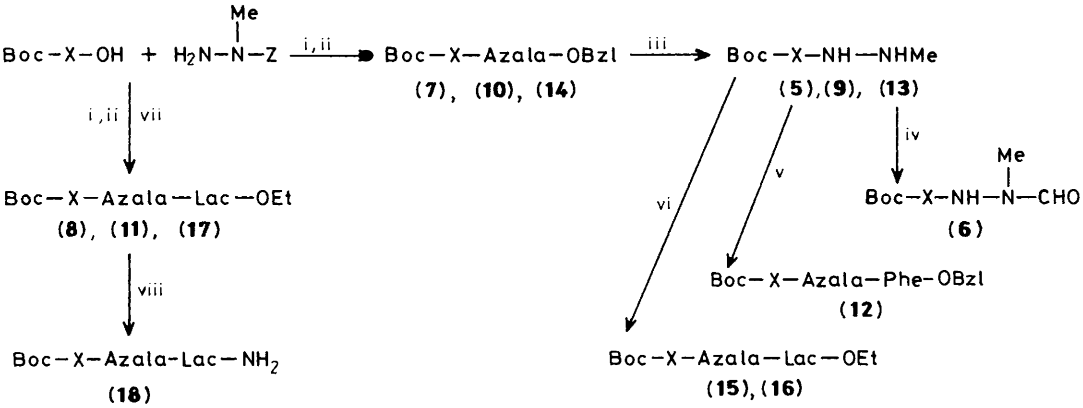
Compounds (**1**)—(**3**) were prepared *via* a stepwise coupling procedure starting from valine benzyl ester. *N*-t-Butoxycarbonyl protected amino acid derivatives were coupled using the DCCI–

Table 1. Structures of peptides and inhibition of porcine pancreatic elastase by these peptides

Compd.	Inhibition of pancreatic elastase IC$_{50}$/μM (%Inhibition)
(1) Gly-Val-Gly-Val-OBzl	400 (76.5)
(2) Boc-Gly-Val-Gly-Val-OBzl	400 (71.2)
(3) Boc-Pro-Gly-Val-Gly-Val-OBzl	400 (65.4)
(4) Boc-Ala-Ala-Ala-OBzl	400 (76.3)
Modifications at the P$_1$′ postion	
Boc-Val-Gly-X	
(5) X = -NH-NH-Me	>400
(6) -NH-N(Me)-CHO	>400
(7) -Azala-OBzl	66.0
(8) -Azala-Lac-OEt	>400
Boc-Gly-Val-Gly-X	
(9) X = -NH-NHMe	>400
(10) -Azala-OBzl	146
(11) -Azala-Lac-OEt	>400
(12) -Azala-Phe-OBzl	>400
Boc-Ala-Pro-X	
(13) X = -NH-NH-Me	>400
(14) -Azala-OBzl	14.0
(15) -Azala-Lac-OEt	23.0
(16) -Azala-Phe-OBzl	>400
Boc-Pro-Ala-Pro-X	
(17) X = -Azala-Lac-OEt	23.0
(18) -Azala-Lac-NH$_2$	22.8
MeOCO-CH(CH$_2$CHMe$_2$)-NH-CO-Val-Gly-Azala-X	
(19) X = –OCH$_2$C$_6$H$_4$NO$_2$-*p*	3.4
(20) –OC$_6$H$_4$NO$_2$-*p*	>400
(21) –C$_6$H$_4$NO$_2$-*p*	>400
(22) –OCH$_2$C$_6$H$_5$	3.3
(23) –CH$_2$OC$_6$H$_5$	>400
(24) –OC$_6$H$_5$	0.4
(25) –C$_6$H$_5$	>400
(26) –NHC$_6$H$_5$	>400
(27) –CH$_2$C$_6$H$_5$	>400
(28) 2-Thienyl	>400
(29) OBu^i	2.4
(30) -Phe-OMe	10^{-3}M (67.9)
(31) -Ala-OBzl	10^{-3}M (100)
Modifications at the P$_1$ position	
Boc-Val-Gly-X-OBzl	
(32) X = -Azgly-	>400
(7) -Azala-	66.0
(33) -Aznva-	8.9
(34) -Azile-	25.8
Boc-Ala-Ala-X-OBzl	
(35) X = -Azala-	13.2
(36) -Aznva-	4.1
(37) -Azile-	27.2
Boc-Pro-Ala-Pro-X-OBzl	
(14) X = -Azala-	14.0
(38) -Azval-	122.1
(39) -Aznva-	155.5
Boc-Pro-Ala-Pro-X-OBzl	
(40) X = -Azala-	42
(41) -Azval-	224
HO$_2$C-CH(CH-Me$_2$)-NH-CO-Ala-Pro-X-OBzl	
(42) X = -Azala-	22.5
(43) -Azval-	57.8
Modifications at the P$_2$ position	
Boc-Val-X-Azala-OBzl	
(7) X = -Gly-	66.0
(44) -Ala-	27.0
(45) -Pro-	0.4
(46) -Val-	61.2
Boc-Ala-X-Azala-OBzl	
(35) X = -Ala-	13.2
(14) -Pro-	14.0
MeOCO-CH(CH$_2$-CHMe$_2$)-NH-CO-Val-X-Azala-OBzl	
(22) X = -Gly-	3.3
(47) -Ala-	0.12
(48) -Pro-	1.24
(49) -Val-	1.28
Modifications at the P$_4$ position	
X-Val-Gly-Azala-OBzl	
(7) X = Boc-	66.0
(50) Me$_2$CH-CH$_2$-CO-	13.1
(51) Me$_3$CH-CO-	6.7
(52) MeOCO-CH(Me)-NH-CO-	15.4
(53) HO$_2$C-CH(Me)-NH-CO-	0.1
(54) MeOCO-CH(CHMe$_2$)NH-CO-	13.4
(55) HO$_2$C-CH(CHMe$_2$)NH-CO-	1.2
(22) MeOCO-CH(CH$_2$CHMe$_2$)NH-CO-	3.3
(56) HO$_2$C-CH(CH$_2$CHMe$_2$)NH-CO-	4.2
(57) MeOCO-CH(CH$_2$CO$_2$Me)NH-CO-	98.4
(58) HO$_2$C-CH(CH$_2$CO$_2$H)NH-CO-	28.4
(59) MeOCO-CH(CH$_2$CH$_2$CO$_2$Me)NH-CO-	29.3
(60) HO$_2$C-CH(CH$_2$CH$_2$CO$_2$H)NH-CO-	2.7
(61) EtOCO-CH(Me)OCO	>400
(62) CF$_3$CO-	57.1
(63) CCl$_3$CO-	7.0
(64) Bu^{i}CO-	13.1
(65) Bu^{t}CO-	6.7
(66) CH$_2$CH$_2$CH$_2$CH$_2$CO- (ring: first and fourth C linked)	2.4
(67) C$_6$F$_5$SO$_2$-	>400
(68) C$_6$H$_5$OCO-	>400
X-Ala-Pro-Azala-OBzl	
(14) X = Boc-	14
(69) MeOCO-CH(Me)NH-CO-	14
(70) MeOCO-CH(CHMe$_2$)NH-CO-	12
(42) HO$_2$C-CH(CHMe$_2$)NHCO-	22.5
(71) MeOCO-CH(CH$_2$CHMe$_2$)NHCO-	1.1
(72) MeOCO-CH(CH$_2$CH$_2$CO$_2$Me)NHCO-	3.4
(73) HO$_2$C-CH$_2$CH$_2$CO-	42
(74) EtOCO-CH(Me)O-CO-	18
(75) HO$_2$C-CH(Me)O-CO-	22
(76) MeCO-	22

HOBt method[19] and the t-butoxycarbonyl group at each step was removed by treatment with HCl in ethyl acetate. Boc-Ala-Ala-Ala-OBzl[20] **(4)** was prepared by a similar route starting from alanine benzyl ester.

The compounds modified in position P_1' **(5)**—**(18)** were prepared as shown in the Scheme. Boc-Val-Gly-OH, Boc-Gly-Val-Gly-OH, Boc-Ala-Pro-OH,[21-23] or Boc-Pro-Ala-Pro-OH,[23] were coupled with benzyl 2-methylcarbazate[24] by the mixed carbonic anhydride method to give the analogues incorporating an α-aza-alanine benzyl ester residue at the C-terminus **(7)**, **(10)**, and **(14)**. The removal of the ester group by catalytic hydrogenolysis (5% Pd/C) gave the 2-methylhydrazide derivatives **(5)**, **(9)**, and **(13)** which on further reaction with *N*-formyl imidazole, *N*-carbonyl L-phenylalanine benzyl ester, or ClOCOCH(Me)-CO_2Et[10] gave compounds **(6)**, **(12)**, **(15)**, and **(16)**. Compounds with Azala-Lac-OEt at the C-terminus [**(8)**, **(11)**, **(17)**] were prepared by coupling Boc-Val-Gly-OH, Boc-Gly-Val-Gly-OH, or Boc-Pro-Ala-Pro-OH with α-Azala-Lac-OEt[10] by the mixed anhydride method.



Scheme. Synthesis of P_1 modified compounds **(5)**—**(18)**. *Reagents:* i, $ClCO_2Et$; ii, *N*-methylmorpholine; iii, H_2/5% Pd-C; iv, *N*-formylimidazole; v, O=C=NCH(CH_2Ph)CO_2Bzl; vi, ClOCOCH(Me)CO_2Et; vii, Azala-Lac-OEt; viii, NH_3; X = -Val-Gly- for **(5)**—**(8)**; -Gly-Val-Gly-for **(9)**—**(12)**; -Ala-Pro- for **(13)**—**(16)**; and Pro Ala-Pro- for **(17)** and **(18)**. Lac = -OCH(Me)CO-.

The remaining P_1' position modified analogues **(19)**—**(31)** were prepared by treating *N*-[(1-methoxycarbonyl-3-methylbutyl)carbamoyl]-Val-Gly-NHNHMe with the required acid chloride or the chloroformate. The 2-methylhydrazide derivative itself was prepared by treating Val-Gly-Azala-OBzl with *N*-carbonyl-L-leucine methyl ester and then removing the C-terminal benzyl ester group by catalytic hydrogenolysis.

The analogues modified in position P_1, **(32)**—**(41)**, were prepared by coupling Boc-Val-Gly-OH, Boc-Ala-Ala-OH, Boc-Ala-Pro-OH, or Boc-Pro-Ala-Pro-OH with benzyl carbazate, benzyl 2-methylcarbazate, benzyl 2-(1-methylethyl)carbazate, benzyl 2-propylcarbazate, or benzyl 2-(1-methylpropyl)-carbazate by the mixed anhydride method to give compounds with an azaglycine, aza-alanine, azavaline, azanorvaline, or azaisoleucine residue, respectively, in position P_1. Benzyl 2-propylcarbazate was prepared by the route described earlier for the other substituted carbazates.[24] *N*-[(1-Carboxy-2-methylpropyl)-carbamoyl]-Ala-Pro-Azala-OBzl **(42)** and the corresponding azavalyl analogue **(43)** were prepared by treating Ala-Pro-Azala-OBzl or Ala-Pro-Azval-OBzl with *N*-carbonyl valine methyl ester, followed by saponification.

The analogues modified in position P_2 **(14)**, **(35)**, and **(44—46)** were prepared by coupling Boc-Ala-Ala-OH, Boc-Ala-Pro-OH, Boc-Val-Ala-OH, Boc-Val-Val-OH, or Boc-Val-Pro-OH with benzyl 2-methylcarbazate by the mixed anhydride method. Analogues **(47)**—**(49)** were synthesized by treating Val-X-Azala-OBzl (X = Ala, Pro, or Val) with *N*-carbonyl leucine methyl ester.

The analogues modified in position P_4, **(50)**—**(76)** were prepared by treating either Val-Gly-Azala-OBzl [**(50)**—**(68)**] or Ala-Pro-Azala-OBzl [**(69)**—**(76)**] with the appropriate acid chloride, sulphonyl chloride, anhydride, chloroformate, or isocyanate to give *N*-acyl [**(50)**, **(51)**, **(62—66)**, **(68)**, **(76)**], pentafluorophenylsulphonyl [**(67)**], succinyl [**(73)**], oxycarbonyl [**(61)**, **(74)**, **(75)**], or carbamoyl [**(52—60)**, **(69—72)**] derivatives. The carboxyalkyl analogues were prepared from the corresponding methoxycarbonyl analogues by saponification.

Results and Discussion

All of the analogues listed in Table 1 were tested as inhibitors of porcine pancreatic elastase using a synthetic substrate, succinyl-(L-Ala)$_3$-*p*-nitroanilide (Calbiochem). Initially the compounds were tested at 400 μM concentration and the IC_{50} values (concentration of the inhibitor producing 50% inhibition under the conditions of the assay) were then determined for the more active compounds. The potency of the compounds was compared with Ac-Ala-Ala-Azala-ONp[9] (IC_{50} 5.7 μM) and Ac-Ala-Ala-Pro-Ala-CH_2Cl[12] (IC_{50} 3.5 μM).

For the purposes of this discussion we have assumed that the analogues reported here are interacting with the enzyme in a similar manner to other reported inhibitors of elastase and that the α-aza-amino acid residue is located at P_1 postion.

Modifications at Position P_1'.—In the Boc-Val-Gly-NH-N(Me)R **(5)**—**(8)**, or Boc-Gly-Val-Gly-NH-N(Me)R **(9)**—**(12)** series of analogues, a benzyl ester moiety in this position appears to be essential for inhibitory activity. The two benzyl ester analogues, Boc-Val-Gly-Azala-OBzl **(7)** and Boc-Gly-Val-Gly-Azala-OBzl **(10)**, had IC_{50} values of 66 and 146 μM, respectively. The compounds with no substituent at position P_1' [**(5)** and **(9)**] or a formyl **(6)**, Lac-OEt **(8)**, **(11)**, or Phe-OBzl **(12)** residue were inactive at 400 μM.

As above, in the Boc-Ala-Pro-NH-N(Me)R **(13)**—**(16)** and Boc-Pro-Ala-Pro-NH-N(Me)R **(17,18)** series of compounds, the analogues with no P_1' substituent **(13)** or with a Phe-OBzl **(16)** residue in this position were inactive and the benzyl ester analogue, Boc-Ala-Pro-Azala-OBzl **(14)** was a potent inhibitor of elastase (IC_{50} 14 μM). The results with the Lac-OEt residue in position P_1' were quite different in Val-Gly- and Ala-Pro series of compounds. Boc-Val-Gly-Azala-Lac-OEt **(8)** and Boc-

Table 2. Analytical data for the *N*-[(1-methoxycarbonyl-3-methylbutyl)carbamoyl]-L-valylglycyl-NH-N(Me)-CO-X-analogues (**20**), (**21**), (**23**—**31**)

			T.l.c. (R_F)						Found (%) (Required)		
Compd. (Formula)	Yield (%)	M.p. (°C)	A	B	C	E	H	Q	C	H	N
(**20**) ($C_{23}H_{34}N_6O_9$)	92.3	110—111	0.82	0.84	0.70	0.40	0.70	0.44	51.4 (51.2)	6.5 (6.3)	15.7 (15.6)
(**21**) ($C_{23}H_{34}N_6O_8$)	73.4	209—210	0.78	0.81	0.66	0.31	0.60	0.36	52.8 (52.8)	6.5 (6.5)	15.7 (16.0)
(**23**) ($C_{24}H_{37}N_5O_7$)	76	199—202	0.76	0.80	0.68	0.34	0.71	0.21	56.7 (56.8)	7.5 (7.4)	13.5 (13.8)
(**24**) ($C_{23}H_{35}N_5O_7$)	70.9	145—146	0.63	0.59	0.49	0.53	0.51	0.18	55.8 (56.0)	7.1 (7.2)	13.9 (14.2)
(**25**) ($C_{23}H_{35}N_5O_6$)	79.1	205—206	0.75	0.71	0.63	0.36	0.64	0.13	57.8 (57.8)	7.6 (7.4)	14.5 (14.7)
(**26**) ($C_{23}H_{36}N_6O_6$)	93.2	226—227	0.75	0.81	0.63	0.28	0.58	0.18	56.2 (56.1)	7.4 (7.4)	17.1 (17.1)
(**27**) ($C_{24}H_{37}N_5O_6$)	63.4	165—166	0.73	0.76	0.59	0.36	0.62	0.23	58.4 (58.6)	7.7 (7.6)	13.9 (14.2)
(**28**) ($C_{21}H_{33}N_5O_6S$)	84.2	201—203	0.68	0.67	0.56	0.35	0.58		51.9 (52.1)	6.7 (6.8)	14.6 (14.4)
(**29**) ($C_{21}H_{39}N_5O_7$)	71.6	186—188	0.74	0.74	0.68	0.33	0.67	0.32	53.6 (53.3)	8.6 (8.3)	14.6 (14.8)
(**30**) ($C_{27}H_{42}N_6O_8$)	67.5	*a*	0.72	0.69	0.64	0.30	0.58	0.40	55.8 (56.0)	7.2 (7.3)	14.2 (14.5)
(**31**) ($C_{27}H_{42}N_6O_8$)	76	163—164	0.72	0.69	0.66	0.30	0.54	0.41	55.7 (56.0)	7.4 (7.3)	14.2 (14.5)

[a] Obtained as a freeze-dried powder.

Table 3. Analytical data for Boc-Val-X-α-Azala-OBzl and MeOCOCH(CH_2CHMe_2)-NH-CO-Val-Y-α-Azala-OBzl

			T.l.c. (R_F)					Found (%) (Required)		
Compd. (Formula)	Yield (%)	M.p. (°C)	E	F	H	P	Q	C	H	N
(**44**) ($C_{22}H_{34}N_4O_6$)	68	179—181	0.53	0.60	0.61	0.27	0.45	58.4 (58.6)	7.5 (7.6)	12.4 (12.4)
(**45**) ($C_{24}H_{36}N_4O_6$)	59.9	Foam	0.59	0.67	0.66	0.29	0.56	60.2 (60.4)	7.6 (7.6)	11.5 (11.7)
(**46**) ($C_{24}H_{38}N_4O_6$)	76	Foam	0.60	0.61	0.65	0.31	0.47	60.1 (60.2)	8.2 (8.0)	11.8 (11.7)
(**47**) ($C_{25}H_{39}N_5O_7$)	89.6	227—229	0.48	0.73	0.53	0.0	0.47	57.4 (57.6)	7.8 (7.5)	13.2 (13.4)
(**48**) ($C_{27}H_{41}N_5O_7$)	93.1	Foam	0.52	0.64	0.52	0.23	0.58	59.0 (59.2)	7.8 (7.5)	12.8 (12.7)
(**49**) ($C_{27}H_{43}N_5O_7$)	39.5	229—230	0.59	0.78	0.76	0.23	0.55	58.6 (59.0)	7.8 (7.9)	12.5 (12.7)

Gly-Val-Gly-Azala-Lac-OEt (**11**) were inactive, but similar analogues (**15**), (**17**), (**18**) in the Boc-Ala-Pro or Boc-Pro-Ala-Pro series were almost as potent as the corresponding benzyl ester analogue (**14**).

The effects of other aliphatic or aromatic substituents in the P_1' position were studied in the *N*-[(1-methoxycarbonyl-3-methylbutyl)carbamoyl]-Val-Gly-Azala-X series of analogues, (**19**)—(**31**). The C-terminal phenyl ester analogue (**24**) was the most potent of the series (IC_{50} 0.4 μM). The corresponding benzyl, *p*-nitrobenzyl, and isobutyl ester analogues (**22**), (**19**), and (**29**) were 5- to 10-fold less potent. The Phe-OMe (**30**) and Ala-OMe (**31**) analogues were much less potent and all the other analogues were inactive at 400 μM.

Modifications at Postion P_1.—The effects of various α-aza-amino acid residues in the P_1 position have been studied in Boc-Val-Gly-X-OBzl (**7**), (**32**)—(**34**), Boc-Ala-Ala-X-OBzl (**35**)—(**37**), Boc-Ala-Pro-X-OBzl (**14**), (**38**), (**39**), Boc-Pro-Ala-Pro-X-OBzl (**40**), (**41**), and HO_2C-CH($CHMe_2$)-NH-CO-Ala-Pro-X-OBzl (**42**), (**43**) series of analogues. In the Boc-Val-Gly-X-OBzl series of analogues the α-azanorvaline substitution gave the most potent analogue (**33**; IC_{50} 8.9 μM), followed by α-Azile (**34**) and α-Azala (**7**) analogues. The α-azaglycine analogue (**32**) was inactive. In the other series of compounds (**35**)—(**43**), analogues with an α-azavaline residue in position P_1 were invariably less potent than the other aza analogues, but the difference between an aza-alanine and an α-azanorvaline residue in the P_1 position was not so clear. Boc-Ala-Ala-α-Aznva-OBzl (**36**) was *ca.* 3-fold more potent than the corresponding aza-alanine analogue (**35**), but Boc-Ala-Pro-α-Aznva-OBzl (**39**) was about 10-fold less potent than the corresponding α-aza-alanine analogue (**14**).

Modifications at Postion P_2.—In the Boc-Ala-X-Azala-OBzl series of compounds, the analogues with an alanine (**35**) or a proline (**14**) residue in position P_2 were equipotent, but in the Boc-Val-X-Azala-OBzl series, the proline analogue (**45**), which was the most potent of the series (IC_{50} 0.4 μM), was almost 70-fold more potent than the corresponding alanine analogue (**44**), and about 150-fold more potent than the valine (**46**) or the glycine (**7**) analogues. In contrast to the above results, in the MeOCO-CH(CH_2-CH-Me_2)-NH-CO-Val-X-Azala-OBzl series, the compound containing an alanine residue (**47**) was

Tabel 4. Analytical data for the X-Val-Gly-Azala-OBzl analogues

Compd. (Formula)	Yield (%)	M.p. (°C)	T.l.c. (R_F) A	B	C	F	H	Q	Found(%) (Required) C	H	N
(50) ($C_{21}H_{32}N_4O_5$)	68	159—160	0.71	0.71	0.63	0.73	0.60	0.50	59.9 (59.9)	7.8 (7.7)	13.4 (13.3)
(51) ($C_{21}H_{32}N_4O_5$)	41.4	149—151	0.72	0.72	0.62	0.64	0.59	0.50	59.8 (59.9)	7.7 (7.7)	13.4 (13.3)
(52) ($C_{21}H_{31}N_5O_7$)	83.9	160—162	0.69	0.76	0.66	0.72	0.62	0.32	53.9 (54.1)	6.7 (6.7)	14.9 (15.0)
(53) ($C_{20}H_{29}N_5O_7$)	37.1	189—190	0.70	0.42	0.28	0.30	0.21	0.0	53.3 (53.2)	6.4 (6.4)	15.3 (15.5)
(54) ($C_{23}H_{35}N_5O_7$)	82.5	*a*	0.70	0.68	0.65	0.60	0.56	0.33	55.6 (55.9)	7.2 (7.1)	14.1 (14.1)
(55) ($C_{22}H_{33}N_5O_7$)	82	*a*	0.65	0.63	0.30				55.0 (55.1)	6.7 (6.9)	14.5 (14.6)
(56) ($C_{23}H_{35}N_5O_7$)	76.9	*a*	0.66	0.61	0.30				55.7 (55.9)	7.1 (7.1)	14.0 (14.1)
(57) ($C_{23}H_{33}N_5O_9$)	72	179—180 (decomp.)	0.68	0.73	0.62	0.66	0.57	0.43	52.5 (52.7)	6.4 (6.3)	13.5 (13.3)
(58) ($C_{21}H_{29}N_5O_9$)	96	*a*	0.51	0.48	0.11	0.21	0.10		50.7 (50.9)	6.1 (5.9)	14.2 (14.1)
(59) ($C_{24}H_{35}N_5O_9$)	81	137—138	0.68	0.75	0.64	0.69	0.59	0.47	53.4 (53.6)	6.4 (6.5)	13.2 (13.0)
(60) ($C_{22}H_{31}N_5O_9 \cdot H_2O$)	75.9	197—199 (decomp.)	0.65	0.62	0.08	0.22	0.10		50.1 (50.1)	6.1 (6.3)	13.3 (13.3)
(61) $C_{22}H_{32}N_4O_8$)	56.3	*a*	0.74	0.70	0.60	0.61	0.59	0.51	55.2 (54.9)	6.5 (6.7)	11.8 (11.6)
(62) ($C_{18}H_{23}N_4O_5F_3$)	71.8	156—160	0.70	0.74	0.66	0.75	0.68	0.40	49.8 (50.0)	5.5 (5.4)	12.7 (13.0)
(63) ($C_{18}H_{23}N_4O_5Cl_3$)	76.9	*a*	0.64	0.60	0.58	0.63	0.57	0.52	44.6 (44.9	4.7 (4.8)	11.4 (11.6)
(64) ($C_{21}H_{32}N_4O_5$)	68	159—160	0.71	0.71	0.63	0.73	0.60	0.50	59.9 (59.9)	7.8 (7.7	13.4 (13.3)
(65) ($C_{21}H_{32}N_4O_5$)	41.4	149—151	0.71	0.72	0.62	0.64	0.59	0.50	59.8 (59.9)	7.7 (7.7)	13.4 (13.3)
(66) ($C_{21}H_{30}N_4O_5$)	35.6	186—187	0.78	0.65	0.68	0.72	0.58	0.16	60.0 (60.3)	7.4 (7.2)	13.1 (13.4)
(67) ($C_{22}H_{23}N_4O_6F_5S$)	52.8	174—175	0.83	0.79	0.67	0.72	0.68	0.26	46.3 (46.6)	4.2 (4.1)	9.7 (9.9)
(68) ($C_{23}H_{28}N_4O_6$)	43.8	*a*	0.82	0.78	0.68	0.72	0.72	0.29	60.6 (60.5)	6.3 (6.2)	12.1 (12.3)

[a] Obtained as freeze-dried powders from 2-methylpropan-2-ol.

Table 5. Analytical data for the X-Ala-Pro-Azala-OBzl analogues

Compd. (Formula)	Yield (%)	M.p. (°C)	T.l.c. (R_F) A	B	C	F	H	Q	Found (%) (Required) C	H	N
(42) ($C_{23}H_{33}N_5O_7$)	77	*a*	0.67	0.72	0.64	0.61	0.57	0.29	56.2 (56.2)	6.8 (6.8)	14.2 (14.2)
(69) ($C_{22}H_{31}N_5O_7$)	68.2	*a*	0.69	0.86	0.59	0.71			55.5 (55.3)	6.7 (6.5)	14.6 (14.6)
(70) ($C_{24}H_{35}N_5O_7$)	75.2	*a*				0.63	0.48	0.45	56.8 (57.0)	7.1 (7.0)	13.8 (13.8)
(71) ($C_{25}H_{37}N_5O_7$)	57.7	*a*	0.65	0.69	0.47	0.59	0.64		57.7 (57.7)	7.3 (7.1)	13.2 (13.4)
(72) ($C_{25}H_{35}N_5O_9$)	52.2	*a*	0.68	0.81	0.59	0.71	0.58		54.8 (54.6)	6.5 (6.4)	12.5 (12.7)
(73) ($C_{21}H_{28}N_4O_7$)	76.5	167—168	0.59	0.68	0.21	0.42	0.21		56.1 (56.2)	6.3 (6.3)	12.5 (12.5)
(74) ($C_{23}H_{32}N_4O_8$)	82.3	*a*	0.75	0.76	0.72	0.70	0.54	0.45	55.9 (56.1)	6.6 (6.5)	11.5 (11.4)
(75) ($C_{21}H_{28}N_4O_8$)	87.5	Foam	0.54	0.64	0.23				54.1 (54.3)	6.0 (6.1)	11.9 (12.1)
(76) ($C_{19}H_{26}N_4O_5$)	80.1	Foam	0.54	0.66	0.56	0.52	0.45	0.25	58.3 (58.4)	6.8 (6.7)	14.3 (14.3)

[a] Obtained as freeze-dried powders from 2-methylpropan-2-ol.

about 10-fold more potent than the compounds containing a proline (**48**) or a valine (**49**) residue. Compounds (**48**) and (**49**) were equipotent and only marginally better than the analogue (**22**) with a glycine residue in the P_2 position.

Modifications at Position P_4.—When the N-terminal t-butoxycarbonyl group in Boc-Val-Gly-Azala-OBzl (**7**, IC_{50} 66 μM) was replaced by the *N*-[(1-methoxycarbonyl-X)-carbamoyl]- residues, the elastase inhibitory potency was significantly altered. For compounds in which X was -CH(Me)- (**52**), -CH(CHMe$_2$)- (**54**), or -CH(CH$_2$CHMe$_2$)- (**22**), the potency was enhanced but when the X was -CH(CH$_2$CO$_2$Me)- (**57**), or -CH(CH$_2$-CH$_2$-CO$_2$Me)- (**59**), the compounds were somewhat less potent. In the carbamoyl series of analogues (**22**), (**52**)—(**59**), apart from (**22**) and (**56**), the analogues with an N-terminal carboxyalkyl group (**53**), (**55**), (**58**) and (**60**) were more potent than the corresponding methoxycarbonyl (**52**), (**54**), (**57**), and (**59**). The most potent analogue of the series, *N*-[(1-carboxyalkyl-1-methyl)-carbamoyl]-Val-Gly-Azala-OBzl (**53**) (IC_{50} 0.1 μM) was about 150-fold more potent than the corresponding methoxycarbonyl analogue (**52**; IC_{50} 15.4 μM). When the carbamoyl linkage in (**52**) was replaced by an oxycarbonyl linkage the resulting compound (**61**) did not inhibit elastase up to 400 μM concentration.

The N-terminal Boc-group in (**7**) was also replaced by a number of other acyl groups. The most potent of these, (**66**), containing the cyclobutyl-carbonyl group, was about 24-fold less potent than (**53**).

In the X-Ala-Pro-Azala-OBzl series of analogues (**14**), (**69**)—(**75**), the potency of the *N*-[(1-methoxycarbonyl-2-methylpropyl)-carbamoyl]- analogue (**70**) was slightly higher than the corresponding carboxyalkyl analogue (**42**). The most significant difference between the X-Val-Gly-Azala-OBzl and X-Ala-Pro-Azala-OBzl series of compounds was seen in the case of the *N*-terminal oxycarbonyl compounds (**61**) and (**74**). *N*-[(1-ethoxycarbonylethyl)oxycarbonyl]-Val-Gly-Azala-OBzl (**61**) was inactive at 400 μM, but *N*-[(1-ethoxycarbonylethyl)oxycarbonyl]-Ala-Pro-Azala-OBzl (**74**) was nearly as active as the corresponding carbamoyl analogue (**69**).

The differences mentioned above, in the Val-Gly and Ala-Ala or Ala-Pro series of analogues as a result of similar substitutions (mainly in the P_1' and P_4 positions), may be the result of the two series of compounds binding in different manners. It is also possible that the conformational changes induced by these substitutions in the two series of analogues are quite different.

A comparison of the elastase inhibitory potency of the analogues reported here with Ac-Ala-Ala-Azala-ONp (IC_{50} 5.7 μM) and Ac-Ala-Ala-Pro-Ala-CH$_2$Cl (IC_{50} 3.5 μM) shows that inhibitors of pancreatic elastase, more potent than the two above inhibitors, can be obtained by various modifications of a Gly-Val-Gly-Val sequence. A number of analogues (**19**), (**22**), (**24**), (**29**), (**45**), (**47**)—(**49**), (**53**), (**55**), (**60**), and (**66**) were either equipotent with, or more potent than, Ac-Ala-Ala-Pro-Ala-CH$_2$Cl. The most potent inhibitor from our series of analogues, HO$_2$C-CH(CH$_3$)-NH-CO-Val-Gly-Azala-OBzl (IC_{50} 0.1 μM) was about 35-fold more potent than the above chloromethyl ketone and about 57-fold more potent than Ac-Ala-Ala-Azala-ONp.

Experimental

The following solvent systems were used for ascending t.l.c. on precoated silica gel plates (Merck Kieselgel 60 F254): butanol–acetic acid–water (4:1:5 v/v) (R_{FA}); butanol–acetic acid–water–pyridine (15:3:12:10) (R_{FB}); butan-2-ol–3% ammonium hydroxide (3:1) (R_{FC}); acetonitile–water (3:1) (R_{FD}); acetone–chloroform (1:1) (R_{FE}); ethanol–chloroform (4:1) (R_{FF}); cyclohexane–ethyl acetate–methanol (1:1:1) (R_{FH}); chloroform–methanol–water (11:8:2) (R_{FK}); chloroform–methanol (19:1) (R_{FP}); and chloroform–methanol (9:1) (R_{FQ}). Spots were revealed by u.v. light, ninhydrin, and Cl$_2$/starch–KI. Symbols and abbreviations used follow the IUPAC-IUB recommendations;[25] other abbreviations used are as follows: DCCI, dicylohexylcarbodi-imide; HOBt, 1-hydroxybenzotriazole; Lac, -OCH(Me)CO-; α-Azgly, -NHNHCO-; α-Azala, -NHN(Me)CO-; α-Azval, -NHN(CHMe$_2$)CO-; α-Aznva, -NHN(Pr)CO-; α-Azile, -NHN(CHMeEt)CO-; DMF, dimethylformamide. All the evaporations were carried out under reduced pressure below 40 °C. Ac-Ala-Ala-Pro-Ala-CH$_2$Cl and Ac-Ala-Ala-Azala-ONp used as standards were a gift from Dr. J. Powers, Dept. of Chemistry, Georgia Institute of Technology, U.S.A.

N-*t-Butoxycarbonylglycyl-*L*-valine Benzyl Ester*.—A solution of Boc-Gly (26.28 g, 150 mmol), HOBt (20.25 g, 150 mmol), and valine benzyl ester *p*-toluenesulphonate (56.92 g, 150 mmol) in DMF was cooled to 0 °C. To the stirred solution was added triethylamine (21.3 ml, 159 mmol) followed by DCCI (34.04 g, 165 mmol). The reaction mixture was stirred overnight at 4 °C. Dicyclohexylurea was filtered off and the filtrate evaporated to dryness. The residue was dissolved in ethyl acetate (800 ml) and washed with water, 20% aqueous citric acid, water, saturated aqueous NaHCO$_3$, and water. The ethyl acetate solution was dried (Na$_2$SO$_4$) and evaporated to dryness. The product was obtained as an oil which still contained some dicyclohexylurea. It was dissolved in ether, the urea was filtered off, and the filtrate was evaporated to leave an oil (48.6 g, 89.0%) (lit.,[26] oil), R_{FD} 0.79, R_{FE} 0.66, R_{FH} 0.78, R_{FP} 0.69, and R_{FQ} 0.74.

*Glycyl-*L*-valine Benzyl Ester Hydrochloride*.—The above benzyl ester (48 g, 131.8 mmol) was dissolved in ethyl acetate (200 ml) and a 6M solution of hydrogen chloride in ethyl acetate (100 ml, 600 mmol) was added to it. After 1 h at room temperature the solvent was removed and the residue was triturated with anhydrous ether to leave the product as a solid which was collected, washed with ether, and dried (37.1 g, 93.7%), m.p. 180—182 °C, R_{FF} 0.60, and R_{FH} 0.48 (Found: C, 55.6; H, 7.0; N, 9.0. $C_{14}H_{21}ClN_2O_3$ requires C, 55.9; H, 7.0; N, 9.3%).

N-*t-Butoxycarbonyl-*L*-valylglycyl-*L*-valine Benzyl Ester*.—Prepared by coupling Boc-Val (30.42 g, 140 mmol) and glycyl-L-valine benzyl ester•HCl (35 g, 132 mmol) by the DCCI–HOBt method using the procedure already described. Yield 58.2 g, (89.8%), oil, R_{FD} 0.71, R_{FE} 0.60, R_{FH} 0.63, R_{FP} 0.53, and R_{FQ} 0.63 (Found: C, 62.3; H, 8.0; N, 9.3. $C_{24}H_{37}N_3O_6$ requires C, 62.2; H, 8.0; N, 9.0%).

N-*t-Butoxycarbonylglycyl-*L*-valylglycyl-*L*-valine Benzyl Ester* (**2**).—The above protected tripeptide (46.3 g, 100 mmol) was treated with trifluoroacetic acid (250 ml) at room temperature for 30 min. The trifluoroacetic acid was evaporated and the peptide trifluoroacetate was coupled with Boc-Gly (17.5 g, 100 mmol) using triethylamine (14.5 ml, 100 mmol), DCCI (20.6 g, 100 mmol), and HOBt (13.5 g, 100 mmol) by the procedure described above for Boc-Gly-Val-OBzl. The product was crystallised from propan-2-ol (41.5 g, 79.8%), m.p. 209—210 °C, R_{FD} 0.76, R_{FE} 0.50, R_{FH} 0.75, and R_{FQ} 0.58 (Found: C, 59.9; H, 7.5; N, 10.9 $C_{26}H_{40}N_4O_7$ requires C, 59.9; H, 7.7; N, 10.7%).

N-*t-Butoxycarbonyl-*L*-prolylglycyl-*L*-valylglycyl-*L*-valine Benzyl Ester* (**3**).—The above Boc-tetrapeptide benzyl ester (5 g, 9.6 mmol) was dissolved in trifluoroacetic acid (10 ml) and the solution was left at room temperature for 30 min. The trifluoroacetic acid was removed and the residue was triturated with ether, collected, washed with ether, and dried to give Gly-Val-

Gly-Val-OBzl (**1**) as a trifluoroacetate salt, R_{FD} 0.60, R_{FF} 0.36, and R_{FK} 0.70 (Found: C, 51.6; H, 6.3; N, 10.5. $C_{23}H_{33}F_3N_4O_7$ requires C, 51.7; H, 6.2; N, 10.5%).

The above trifluoroacetate salt was coupled to Boc-Pro (2.03 g, 9.6 mmol) with DCCI (2.06 g, 10 mmol) and HOBt (2.6 g, 19.2 mmol) after adding triethylamine (1.35 ml, 9.6 mmol). The work up procedure was similar to that described for Boc-Val-Gly-OBzl. The crude product was purified by silica gel (200 g) column chromatography using chloroform and 2.5% methanol in chloroform as eluants. Yield 3.6 g (61.9%), m.p. 185—188 °C, R_{FD} 0.60, R_{FE} 0.37, R_{FF} 0.66, R_{FH} 0.55, and R_{FQ} 0.44 (Found: C, 60.4; H, 7.6; N, 11.5. $C_{31}H_{47}N_5O_8$ requires C, 60.2; H, 7.6; N, 11.3%).

N-*t-Butoxycarbonyl*-L-*alanyl*-L-*alanine Benzyl Ester*.—Boc-Ala (28.3 g, 150 mmol) was coupled with alanine benzyl ester *p*-toluenesulphonate (47.7 g, 150 mmol) using triethylamine (21.3 ml, 150 mmol), HOBt (40.5 g, 300 mmol), and DCCI (34.0 g, 165 mmol) in DMF (250 ml). The procedure was similar to that employed for the synthesis of Boc-Gly-Val-OBzl. The product was obtained as an oil (43.7 g, 83.2%), R_{FD} 0.77, R_{FE} 0.62, R_{FH} 0.72, R_{FP} 0.67, and R_{FQ} 0.75.

N-*t-Butoxycarbonyl*-L-*alanyl*-L-*alanyl*-L-*alanine Benzyl Ester* (**4**).—The above dipeptide benzyl ester (17.5 g, 50 mmol) was dissolved in ethyl acetate (50 ml) and 6M HCl in ethyl acetate (50 ml) was added. After 30 min at room temperature, the ethyl acetate was evaporated and the remaining oil, which showed a single spot on t.l.c. (R_{FD} 0.67, R_{FH} 0.45, R_{FK} 0.91), was coupled to Boc-Ala (9.4 g, 50 mmol) in DMF (150 ml) by the DCCI (10.3 g, 50 mmol)–HOBt (6.8 g, 50 mmol) method using the procedure described for Boc-Gly-Val-OBzl. The product was crystallised from methanol–water (14.2 g, 66.4%), m.p. 137—138 °C (lit.,[20] 140—141 °C), R_{FD} 0.70, R_{FE} 0.55, R_{FH} 0.73, R_{FP} 0.46, and R_{FQ} 0.62 (Found: C, 59.7; H, 7.3; N, 9.7. Calc. for $C_{21}H_{31}N_3O_6$: C, 59.8; H, 7.4; N, 10.0%).

N-*t-Butoxycarbonyl*-L-*valylglycine Ethyl Ester*.—A solution of Boc-Val (65.1 g, 300 mmol) and *N*-methylmorpholine (33 ml, 300 mmol) in DMF (200 ml) was cooled to −20 °C. To the stirred reaction mixture was added ethyl chloroformate (28.5 ml, 300 mmol), the temperature being maintained below −20 °C. After 2 min, a precooled (−20 °C) mixture of Gly-OEt•HCl (41.8 g, 300 mmol) and *N*-methylmorpholine (33 ml, 300 mmol) in DMF (250 ml) was added and the reaction mixture was stirred below 0 °C for 2 h and then overnight at room temperature. The DMF was then evaporated and the residue in ethyl acetate washed with water, 20% aqueous citric acid, water, and saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and evaporated to dryness. The crude peptide was crystallised from hot cyclohexane, (68.1 g, 75%), m.p. 167—168 °C (lit.,[27,28] 169—170 and 167 °C), R_{FD} 0.70, R_{FE} 0.64, R_{FF} 0.70, R_{FH} 0.69, R_{FP} 0.59, and R_{FQ} 0.56 (Found: C, 55.5; H, 8.7; N, 9.4. Calc. for $C_{14}H_{26}N_2O_5$: C, 55.6; H, 8.6; N, 9.2%).

N-*t-Butoxycarbonyl*-L-*valylglycine*.—The above ethyl ester (35.3 g, 117 mmol) was dissolved in ethanol (250 ml) and a solution of NaOH (5.16 g, 129 mmol) in water (30 ml) was added and the reaction mixture was stirred at room temperature for 90 min. Most of the ethanol was then evaporated and water (150 ml) was added. The aqueous phase was extracted with ethyl acetate, acidified with citric acid (to pH 4), and extracted again with ethyl acetate. The organic phase was washed with water, dried (Na_2SO_4), and evaporated to give the product as a foam (31.2 g, 97.3%), R_{FA} 0.64, R_{FB} 0.61, R_{FC} 0.40, R_{FD} 0.52 and R_{FH} 0.40 (Found: C, 52.3; H, 8.1; N, 10.1. $C_{12}H_{22}N_2O_5$ requires C, 52.5; H, 8.1; N, 10.2%).

N-*t-Butoxycarbonyl*-L-*valylglycyl-α-aza-alanine Benzyl Ester* (**7**).—Boc-Val-Gly-OH (8.2 g, 30 mmol) was coupled with benzyl 2-methylcarbazate (5.4 g, 30 mmol) by the mixed anhydride method. The procedure was similar to that described above for Boc-Val-Gly-OEt. The crude peptide was purified by silica gel column chromatography using chloroform and 1% methanol in chloroform as eluants to give the pure tripeptide derivative as a foam (9.7 g, 74.3%), R_{FA} 0.73, R_{FD} 0.67, R_{FE} 0.47 R_{FH}0.65, R_{FP} 0.41, and R_{FQ} 0.53 (Found: C, 57.5; H, 7.4; N, 12.6. $C_{21}H_{32}N_4O_6$ requires C, 57.8; H, 7.4; N, 12.8%).

Ethyl N-*t-Butoxycarbonyl*-L-*valylglycyl-α-aza-alanyl-lactate* (**8**).—This was prepared from Boc-Val-Gly-OH (4.12 g, 15 mmol) and Azala-Lac-OEt (2.85 g, 15 mmol) by the mixed anhydride method. The procedure used was similar to that employed for Boc-Val-Gly-OEt. The crude peptide was purified by silica gel column chromatography using chloroform and 1% methanol in chloroform as eluants to yield compound (**8**) as a foam (5.08 g, 75.8%), R_{FA} 0.63, R_{FB} 0.72, R_{FC} 0.72, R_{FE} 0.45, R_{FH} 0.61, R_{FP} 0.44, and R_{FQ} 0.56 (Found: C, 51.3; H, 7.6; N, 12.4. $C_{19}H_{34}N_4O_8$ requires C, 51.1; H, 7.6; N, 12.5%).

N-*t-Butoxycarbonyl*-L-*valylglycine 2-Methylhydrazide* (**5**).—Boc-Val-Gly-Azala-OBzl (4 g, 9.16 mmol) was dissolved in methanol (50 ml) and 5% Pd/C (0.8 g) in water (10 ml) was added. Hydrogen gas was bubbled through for 6 h. The catalyst was then filtered off and the filtrate was evaporated to dryness. The residue was triturated with ether, collected, washed with ether, and dried to yield compound (**5**) (2.5 g, 93%), R_{FA} 0.51, R_{FB} 0.64, R_{FC} 0.59, R_{FF} 0.51, R_{FH} 0.50, and R_{FQ} 0.26 (Found: C, 51.6; H, 8.7; N, 18.3. $C_{13}H_{26}N_4O_4$ requires C, 51.6; H, 8.7; N, 18.5%).

N-*t-Butoxycarbonyl*-L-*valylglycine 2-Formyl-2-methylhydrazide* (**6**).—Carbonyl di-imidazole (1.1 equiv.) was added to formic acid in chloroform and the solution was stirred for 10 min. This solution of *N*-formyl imidazole (0.5 mmol/ml) was added to a solution of Boc-Val-Gly-NH-NHMe (250 mg, 0.83 mmol) in chloroform (10 ml). After stirring for 2 h at room temperature the solution was evaporated to dryness. The crude product was purified by gel filtration on Sephadex G-10 in water and then by silica gel column chromatography using chloroform and 1 and 2% methanol in chloroform as eluants to give compound (**6**) (200 mg, 73.2%) (Found: C, 50.7; H, 8.2; N, 16.0. $C_{14}H_{26}N_4O_5$ requires C, 51.0; H, 8.2; N, 15.9%).

N-*t-Butoxycarbonylglycyl*-L-*valylglycine Ethyl Ester*.—Val-Gly-OEt•HCl (34 g, 146 mmol) (prepared from Boc-Val-Gly-OEt by HCl in ethyl acetate treatment) was coupled to Boc-Gly (28 g, 160 mmol) using the DCCI–HOBt method as described for Boc-Gly-Val-OBzl. Yield 36.1 g, (68.8%), m.p. 145—147 °C, R_{FD} 0.65, R_{FE} 0.46, R_{FH} 0.56, R_{FP} 0.45, and R_{FQ} 0.42 (Found: C, 53.8; H, 8.4; N, 11.7. $C_{16}H_{29}N_3O_6$ requires C, 53.5; H, 8.1; N, 11.7%).

N-*t-Butoxycarbonylglycyl*-L-*valylglycine*.—Boc-Gly-Val-Gly-OEt (3.59 g, 10 mmol) in ethanol (50 ml) was treated with 1M NaOH (12.0 ml, 12 mmol) for 1 h. The solution was then passed through a Biorex 70 (H^+ form) ion exchange resin column, evaporated to dryness, and triturated with ether to give the product as a gelatinous solid (2.5 g, 75.7%), R_{FA} 0.66, R_{FB} 0.56, R_{FC} 0.25, R_{FD} 0.52, and R_{FK} 0.52 (Found: C, 50.6; H, 7.3; N, 12.4. $C_{14}H_{25}N_3O_6$ requires C, 50.7; H, 7.6; N, 12.7%).

N-*t-Butoxycarbonylglycyl*-L-*valylglycyl-α-aza-alanine Benzyl Ester* (**10**).—This was prepared by coupling Boc-Gly-Val-Gly-OH (4.97 g, 15 mmol) and benzyl 2-methylcarbazate (2.7 g, 15 mmol) by the mixed anhydride method as described above for

Boc-Val-Gly-OEt. The crude peptide was purified by silica gel column chromatography using chloroform and 1% methanol in chloroform as eluants. The product was obtained as a foam (4.8 g, 66%), R_{FA} 0.84, R_{FB} 0.84, R_{FC} 0.75, R_{FE} 0.33, R_{FH} 0.56, and R_{FQ} 0.27 (Found: C, 56.1; H, 7.3; N, 14.2. $C_{23}H_{35}N_5O_7$ requires C, 56.0; H, 7.2; N, 14.2%).

Ethyl N-*t-Butoxycarbonylglycyl*-L-*valylglycyl*-α-*aza-alanyl-lactate* (**11**).—This was prepared from Boc-Gly-Val-Gly-OH (2.65 g, 8 mmol) and Azala-Lac-OEt (1.53 g, 8 mmol) by the mixed anhydride method as described above for Boc-Val-Gly-OEt. The product was purified by silica gel column chromatography using chloroform and 2% methanol in chloroform as eluants to give (**11**) as a foam (1.46 g, 36.2%), R_{FA} 0.78, R_{FB} 0.79, R_{FC} 0.70, R_{FD} 0.65, R_{FE} 0.35, R_{FH} 0.55, and R_{FQ} 0.38 (Found: C, 49.9; H, 7.5; N, 13.6. $C_{21}H_{37}N_5O_9$ requires C, 50.1; H, 7.4; N, 13.9%).

N-*t-Butoxycarbonylglycyl*-L-*valylglycine* 2-*Methylhydrazide* (**9**).—This was prepared by a method similar to that used for the preparation of Boc-Val-Gly-NH-NH-Me, yield 90.7%, R_{FA} 0.46, R_{FB} 0.68, R_{FC} 0.52, R_{FD} 0.46, R_{FF} 0.48, and R_{FH} 0.36 (Found: C, 49.9; H, 8.1; N, 19.4. $C_{15}H_{29}N_5O_5$ requires C, 50.1; H, 8.1; N, 19.4%).

N-*t-Butoxycarbonylglycyl*-L-*valylglycyl*-α-*aza-alanyl*-L-*phenylalanine Benzyl Ester* (**12**).—To a stirred suspension of Boc-Gly-Val-Gly-NH-NH-Me (0.40 g, 1.1 mmol) in chloroform (5 ml), *N*-carbonylphenylalanine benzyl ester (0.31 g, 1.2 mmol) was added. A clear solution, obtained in a few min, was left at room temperature overnight. The solvent was removed and the residue was purified by silica gel column chromatography using chloroform and 1 and 2% methanol in chloroform as eluants to give (**12**) (0.54 g, 76.7%), R_{FA} 0.70, R_{FC} 0.62, R_{FE} 0.22, R_{FH} 0.65, and R_{FQ} 0.39 (Found: C, 59.8; H, 6.8; N, 13.2. $C_{32}H_{44}N_6O_8$ requires C, 59.9; H, 6.9; N, 13.1%). Amino acid analysis (16 h acid hydrolysate): Gly 1.99, Val 1.0, Phe 0.97.

N-*t-Butoxycarbonyl*-L-*alanyl*-L-*prolyl*-α-*aza-alanine Benzyl Ester* (**14**).—Prepared by coupling Boc-Ala-Pro-OH [22] (10.9 g, 40 mmol) with benzyl 2-methylcarbazate (8.3 g, 40 mmol) in DMF by the mixed anhydride method as described above for Boc-Val-Gly-OEt. The pure product was obtained as a foam after silica gel (500 g) column chromatography using chloroform and 2% methanol in chloroform as eluants (15.03 g, 83.7%), R_{FA} 0.89, R_{FB} 0.83, R_{FC} 0.77, R_{FE} 0.58, R_{FH} 0.61, and R_{FQ} 0.57 (Found: C, 59.0; H, 7.4; N, 12.4. $C_{22}H_{32}N_4O_6$ requires C, 58.9; H, 7.2; N, 12.5%).

N-*t-Butoxycarbonyl*-L-*alanyl*-L-*proline* 2-*Methylhydrazide* (**13**).—This was prepared by a method similar to that used for the preparation of Boc-Val-Gly-NH-NH-Me. The product was crystallised from ether–light petroleum (60—80 °C) to give (**13**) (3.19 g, 80%), m.p. 99—100 °C R_{FA} 0.46, R_{FB} 0.68, R_{FC} 0.52, R_{FD} 0.46, R_{FF} 0.49, and R_{FH} 0.36 (Found: C, 52.3; H, 8.3; N, 17.4. $C_{14}H_{26}N_4O_4$ requires C, 52.0; H, 8.4; N, 17.3%).

Ethyl N-*t-Butoxycarbonyl*-L-*alanyl*-L-*prolyl*-α-*aza-alanyl-lactate* (**15**).—A solution of Cl-CO-Lac-OEt [10] (0.72 g, 4 mmol) in chloroform (5 ml) was added to a solution of Boc-Ala-Pro-NH-NH-Me (1.2 g, 3.8 mmol) and triethylamine (0.53 ml, 3.8 mmol) and the reaction mixture was left overnight at room temperature. It was evaporated to dryness and the residue was purified by silica gel column chromatography using chloroform as eluant. The product was obtained as a foam (1.3 g, 74.6%), R_{FA} 0.65, R_{FB} 0.69, R_{FC} 0.62, R_{FE} 0.45, R_{FF} 0.64, and R_{FQ} 0.50 (Found: C, 52.3; H, 7.6; N, 12.2. $C_{20}H_{34}N_4O_8$ requires C, 52.4; H, 7.5; N, 12.2%).

N-*t-Butoxycarbonyl*-L-*alanyl*-L-*prolyl*-α-*aza-alanyl*-L-*phenylalanine Benzyl Ester* (**16**).—This was prepared by treating Boc-Ala-Pro-NH-NH-Me (1.2 g, 3.8 mmol) with *N*-carbonylphenylalanine benzyl ester (1.12 g, 4 mmol) in chloroform (10 ml). After 18 h at room temperature the solution was evaporated to dryness and the pure product was obtained by silica gel column chromatography using chloroform and 1 and 2% methanol in chloroform as eluants (1.95 g, 86%), m.p. 177—178 °C, R_{FA} 0.75, R_{FB} 0.76, R_{FC} 0.70, R_{FE} 0.43, R_{FH} 0.62, and R_{FQ} 0.40 (Found: C, 62.6; H, 6.9; N, 11.9. $C_{31}H_{41}N_5O_7$ requires C, 62.5; H, 6.9; N, 11.8%).

N-*t-Butoxycarbonyl*-L-*prolyl*-L-*alanyl*-L-*proline Methyl Ester*.—L-Ala-Pro-OMe·HCl (11.8 g, 50 mmol), prepared by the catalytic hydrogenolysis (5% Pd/C) of Z-Ala-Pro-OMe,[22] was coupled to Boc-Pro-OH (10.76 g, 50 mmol) by the mixed anhydride method. The procedure was similar to that used for the preparation of Boc-Val-Gly-OEt and the product was obtained as an oil (10.2 g, 51.4%), R_{FE} 0.44, R_{FH} 0.52, R_{FP} 0.49, and R_{FQ} 0.57 (Found: C, 57.2; H, 7.8; N, 10.4. $C_{19}H_{31}N_3O_6$ requires C, 57.4; H, 7.8; N, 10.5%).

N-*t-Butoxycarbonyl*-L-*prolyl*-L-*alanyl*-L-*proline*.—A solution of Boc-Pro-Ala-Pro-OMe (10.1 g, 25.5 mmol) in methanol (50 ml) was treated with 2M NaOH (14 ml, 28 mmol) for 2 h at room temperature. The work-up method was similar to that used for the preparation of Boc-Gly-Val-Gly-OH, described above. Yield 93.3%, foam (lit.,[23] glassy solid), R_{FA} 0.61, R_{FB} 0.61, R_{FC} 0.20, and R_{FK} 0.57. Amino acid analysis (16 h acid hydrolysate): Ala 1, Pro 1.96.

Ethyl N-*t-Butoxycarbonyl*-L-*prolyl*-L-*alanyl*-L-*prolyl*-α-*aza-alanyl-lactate* (**17**).—A procedure similar to that used for the preparation of Boc-Ala-Pro-Azala-Lac-OEt described above was employed except that the silica gel column was eluted with chloroform and 1% methanol in chloroform to give the pure product as a foam (58.7%), R_{FA} 0.70, R_{FB} 0.79, R_{FC} 0.70, R_{FD} 0.65, R_{FE} 0.42, and R_{FH} 0.50 (Found: 53.3; H, 7.6; N, 12.4. $C_{25}H_{41}N_5O_9·0.5H_2O$ requires C, 53.2; H, 7.5; N, 12.4%). Amino acid analysis: Ala 1, Pro 1.98.

N-*t-Butoxycarbonyl*-L-*prolyl*-L-*alanyl*-L-*prolyl*-α-*aza-alanyl-lactamide* (**18**).—Compound (**17**) (2.56 g, 4.61 mmol) dissolved in methanol saturated with ammonia (25 ml) was left at room temperature for 3 days. Methanol was then removed and the residue after silica gel column chromatography using chloroform and 1 and 2% methanol in chloroform as eluants gave the pure amide (**18**) as a foam (1.4 g, 57.8%), R_{FA} 0.51, R_{FB} 0.65, R_{FC} 0.52, R_{FF} 0.54, and R_{FH} 0.31 (Found: C, 50.7; H, 7.2; N, 15.2. $C_{23}H_{38}N_6O_8$ requires C, 50.7; H, 7.4; N, 15.4%). Amino acid analysis: Ala 1, Pro 1.97.

N-[(1-*Methoxycarbonyl*-3-*methylbutyl*)*carbamoyl*]-L-*valylglycylaza-alanine Benzyl Ester* (**22**).—A mixture of Val-Gly-Azala-OBzl·HCl (25 g, 67.1 mmol) (prepared by the HCl in acetic acid treatment of the Boc-derivative for 1 h at room temperature) and triethylamine (9.5 ml, 68 mmol) in chloroform (500 ml) was stirred and cooled in an ice bath. *N*-Carbonyl L-leucine methyl ester (11.98 g, 70 mmol) was added and the reaction mixture was stirred in an ice bath for 2 h and then left at room temperature overnight. The chloroform was then evaporated and the residue in ethyl acetate (1 l) was washed with water, 20% aqueous citric acid and water, dried (Na_2SO_4), and evaporated to dryness. The product was crystallised from hot ethyl acetate–light petroleum (60—80 °C) (28.8 g, 84.7%), m.p. 178—179 °C, R_{FA} 0.81, R_{FB} 0.78, R_{FC} 0.70, R_{FE} 0.40, R_{FF} 0.69, R_{FH} 0.62, and R_{FQ} 0.27 (Found: C, 56.6; H, 7.4; N, 13.7. $C_{24}H_{37}N_5O_7$ requires C, 56.8; H, 7.3; N, 13.8%).

N-[(1-*Methoxycarbonyl*-3-*methylbutyl*)*carbamoyl*]-L-*valylglycine* 2-*Methylhydrazide*.—Prepared from the above benzyl ester by the procedure used for Boc-Val-Gly-NH-NH-Me. The product was collected washed with ether and dried (92.4%) (Found: C, 51.5; H, 8.4; N, 18.9. $C_{16}H_{31}N_5O_5$ requires C, 51.4; H, 8.3; N, 18.7%).

N-[(1-*Methoxycarbonyl*-3-*methylbutyl*)*carbamoyl*]-L-*valylglycylaza-alanine* p-*Nitrobenzyl Ester* (**19**).—*p*-Nitrobenzyl chloroformate (430 mg, 2 mmol) was added to an ice-cold stirred solution of *N*-[(1-methoxycarbonyl-3-methylbutyl)-carbamoyl]-L-valylglycine 2-methylhydrazide (373 mg, 1 mmol) in pyridine (5 ml). The reaction mixture was left at room temperature overnight. Ethyl acetate (200 ml) and water (25 ml) were then added and the organic phase was washed with water, 20% aqueous citric acid and water, dried (Na_2SO_4), and evaporated to dryness. Crystallisation from hot ethyl acetate gave (**19**) (357 mg, 64.6%), m.p. 207—208 °C (decomp.), R_{FA} 0.81, R_{FB} 0.79, R_{FC} 0.70, R_{FD} 0.73, R_{FH} 0.64, and R_{FQ} 0.40 (Found: C, 52.3; H, 6.6; N, 15.0 $C_{24}H_{36}N_6O_9$ requires C, 52.1; H, 6.5; N, 15.2%).

Compounds (**20**), (**21**), and (**23**)—(**31**) were also prepared by the above procedure using the appropriate acid chloride, chloroformate or isocyanate; the data for these compounds are listed in Table 2.

N-*t*-*Butoxycarbonyl*-L-*valylglycyl*-α-*azaglycine Benzyl Ester* (**32**).—Prepared by coupling Boc-Val-Gly-OH and benzyl carbazate by the mixed anhydride method as described above for Boc-Val-Gly-OEt. Yield 81.7%, m.p. 91—92 °C, R_{FA} 0.40, R_{FB} 0.51, R_{FD} 0.64, R_{FE} 0.33 and R_{FQ} 0.33 (Found: C, 56.6; H, 7.2; N, 12.9. $C_{20}H_{30}N_4O_6$ requires C, 56.8; H, 7.1; N, 13.2%).

N-*t*-*Butoxycarbonyl*-L-*valylglycyl*-α-*azanorvaline Benzyl Ester* (**33**).—The preparation was similar to that described for Boc-Val-Gly-Azgly-OBzl. Yield 60.8%, m.p. 52—54 °C, R_{FA} 0.53, R_{FB} 0.65, R_{FC} 0.55, R_{FE} 0.47, R_{FF} 0.52, and R_{FQ} 0.54 (Found: C, 59.2 H, 8.0; N, 12.0. $C_{23}H_{36}N_4O_6$ requires C, 59.4; H, 7.8; N, 12.0%).

N-*t*-*Butoxycarbonyl*-L-*valylglycyl*-α-*azaisoleucine Benzyl Ester* (**34**).—This was prepared by a method similar to that used for the preparation of Boc-Val-Gly-Azgly-OBzl using Boc-Val-Gly-OH and benzyl 2-(s-butyl)-carbazate. Yield 82.2% m.p. 65—66 °C, R_{FA} 0.72, R_{FB} 0.74, R_{FC} 0.61, R_{FE} 0.19, R_{FF} 0.65, and R_{FQ} 0.49 (Found: C, 60.4; H, 8.3; N, 11.9. $C_{24}H_{38}N_4O_6$ requires C, 60.2; H, 8.0; N, 11.7%).

N-*t*-*Butoxycarbonyl*-L-*alanyl*-L-*alanyl*-α-*aza-alanine Benzyl Ester* (**35**).—This was prepared as above by coupling Boc-Ala-Ala-OH[21] with benzyl 2-methyl carbazate. Crystallisation from hot isopropanol–ether gave pure (**35**) (66%), m.p. 195—197 °C, R_{FA} 0.83, R_{FB} 0.75, R_{FC} 0.72, R_{FD} 0.69, R_{FE} 0.49, R_{FH} 0.63, and R_{FQ} 0.42 (Found: C, 56.8; H, 7.3; N, 13.4. $C_{20}H_{30}N_4O_6$ requires C, 56.9; H, 7.2; N, 13.3%).

N-*t*-*Butoxycarbonyl*-L-*alanyl*-L-*alanyl*-α-*azanorvaline Benzyl Ester* (**36**).—This was prepared by coupling Boc-Ala-Ala-OH to benzyl 2-propylcarbazate by the DCCI–HOBt method. The crude peptide was purified by silica gel column chromatography using chloroform and 1% methanol in chloroform as eluants. The products was obtained as a foam (85%), R_{FA} 0.87, R_{FB} 0.82, R_{FC} 0.75, R_{FE} 0.57, R_{FH} 0.67, and R_{FQ} 0.49 (Found: C, 58.6; H, 7.8; N, 12.2. $C_{22}H_{34}N_4O_6$ requires C, 58.6; H, 7.60; N, 12.43%).

N-*t*-*Butoxycarbonyl*-L-*alanyl*-L-*alanyl*-α-*azaisoleucine Benzyl Ester* (**37**).—Preparation and purification by silica gel column chromatography was similar to Boc-Ala-Ala-Aznva-OBzl, yield, 68.3%, foam, R_{FA} 0.73, R_{FB} 0.68, R_{FC} 0.53, R_{FE} 0.43, R_{FH} 0.54, and R_{FQ} 0.52 (Found: C, 59.6; H, 8.0; N, 11.8. $C_{23}H_{36}N_4O_6$ requires C, 59.4; H, 7.8; N, 12.0%).

N-*t*-*Butoxycarbonyl*-L-*alanyl*-L-*prolyl*-α-*azavaline Benzyl Ester* (**38**).—This was prepared by coupling Boc-Ala-Pro-OH to benzyl 2-isopropyl carbazate by the mixed anhydride method. The product was obtained as a foam after silica gel column chromatography using chloroform and 0.5% methanol in chloroform as eluants (59.4%), R_{FA} 0.83, R_{FB} 0.77, R_{FC} 0.67, R_{FE} 0.53, R_{FH} 0.55, and R_{FQ} 0.57 (Found: C, 60.3; H, 7.7; N, 11.6. $C_{24}H_{36}N_4O_6$ requires C, 60.5; H, 7.6; N, 11.8%).

N-*t*-*Butoxycarbonyl*-L-*alanyl*-L-*prolyl*-α-*azanorvaline Benzyl Ester* (**39**).—This was prepared by coupling Boc-Ala-Pro-OH to benzyl 2-propylcarbazate by the DCCI-HOBt method. The product after silica gel column chromatography using chloroform and 1% methanol in chloroform as eluants was obtained as a foam (54.8%), R_{FD} 0.74, R_{FE} 0.59, R_{FP} 0.54, and R_{FQ} 0.58 (Found: C, 60.3; H, 7.3; N, 12.0. $C_{24}H_{36}N_4O_6$ requires C, 60.5; H, 7.6; N, 11.8%).

N-*t*-*Butoxycarbonyl*-L-*prolyl*-L-*alanyl*-L-*prolyl*-α-*aza-alanine Benzyl Ester* (**40**).—This was prepared by coupling Boc-Pro-Ala-Pro-OH to benzyl 2-methylcarbazate by the mixed anhydride method. The crude product was purified by silica gel column chromatography using chloroform and 2% methanol in chloroform as eluants to yield (**40**) as a foam (67.3%), R_{FA} 0.78, R_{FD} 0.65, R_{FE} 0.42, R_{FH} 0.53, and R_{FQ} 0.47 (Found: C, 59.4; H, 7.3; N, 12.7. $C_{27}H_{39}N_5O_7$ requires C, 59.4; H, 7.2; N, 12.8%).

N-*t*-*Butoxycarbonyl*-L-*prolyl*-L-*alanyl*-L-*prolyl*-α-*azavaline Benzyl Ester* (**41**).—This was prepared by coupling Boc-Pro-OH to Ala-Pro-Azval-OBzl by the DCCI–HOBt method. The crude peptide was purified by silica gel column chromatography using chloroform and 1 and 3% methanol in chloroform as eluants. Yield 75.8%, foam, R_{FA} 0.74, R_{FB} 0.78, R_{FD} 0.63, R_{FE} 0.44, R_{FH} 0.59, R_{FP} 0.47, and R_{FQ} 0.54 (Found: C, 60.7; H, 7.5; N, 12.2. $C_{29}H_{43}N_5O_7$ requires C, 60.7; H, 7.6; N, 12.2%).

N-*t*-*Butoxycarbonyl*-L-*valyl*-*X*-α-*aza-alanine Benzyl Ester* (X = Ala, Pro, Val) (**44**)—(**46**).—All compounds were prepared by coupling Boc-Val-X-OH to benzyl 2-methylcarbazate by a procedure similar to that used for the preparation of Boc-Val-Gly-Azala-OBzl described above. The characterisation data for these compounds are summarised in Table 3.

N-[(1-*Methoxycarbonyl*-3-*methylbutyl*)*carbamoyl*]-L-*valyl*-*Y*-α-*aza-alanine Benzyl Ester* (Y = Ala, Pro, Val) (**47**)—(**49**).—The t-butoxycarbonyl protecting group from compunds (**44**)—(**46**) was cleaved by a treatment with HCl in ethyl acetate and the resulting peptides were reacted with *N*-carbonyl L-leucine methyl ester by a procedure similar to that used for the preparation of (**22**). Table 3 summarises the characterisation data.

X-L-*Valylglycyl*-α-*aza-alanine Benzyl Ester* (**50**)—(**68**).—All of these analogoues were prepared by treating Val-Gly-Azala-OBzl with the appropriate acid chloride, isocyanate [MeOCOCH(R)N=C=O], or chloroformate [EtOCOCH(Me)-OCOCl]. The data are summarised in Table 4.

The *N*-terminal carboxyalkyl derivatives were prepared by saponification of the corresponding methyl esters. The general procedure used was as follows. The methoxycarbonyl analogue was dissolved in methanol and 1M NaOH (2 equiv.) was added. The reaction mixture was stirred at room temperature for 2 h. Methanol was then removed and the residue was dissolved in water acidified with solid citric acid (pH 2—3). Citric acid and salts were then removed by countercurrent distribution using butanol–water (10 transfers). The product containing fractions were evaporated to dryness and the residue, dissolved in a mixture of butanol–methanol–water (1:1:1 v/v), was passed through an anion exchange column (AGI X-2, acetate form). The column was eluted with increasing concentrations of acetic acid (0.01—0.1M) in butanol–methanol–water (1:1:1 v/v) and the pure peptides were obtained as freeze-dried powders.

X-L-Alanyl-L-prolyl-α-aza-alanine Benzyl Ester (**42**), (**69**)—(**76**).—The preparation was similar to that used for the above X-Val-Gly-Azala-OBzl analogues. Data are summarised in Table 5.

Inhibition of Pancreatic Elastase.—A method similar to that described by Beith *et al.*[29] was used. Porcine pancreatic elastase (7.5 μg, Worthington Biochemicals) was combined with either vehicle or inhibitor contained in 0.2M Tris buffer (2.5 ml, pH 8.0). Following a 30 min incubation period, the reaction was initiated by the addition of succinyl-Ala-Ala-Ala-*p*-nitroanilide (Calbiochem) (20 μl, 125 mM). After 15 min, the reaction was quenched by the addition of glacial acetic acid (100 μl) and optical density (O.D.) was measured at 410 nm.

Percent inhibition was calculated as follows:

$$\%\ \text{inhibition} = \frac{a - b}{a} \times 100 \text{ where}$$

a = O.D. in absence of inhibitor
b = O.D. in presence of inhibitor

The IC_{50} values were then determined from a plot of % inhibition *vs.* log concentration of the inhibitor.

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