Pronase catalysed peptide syntheses

Mario Lobell [a] **and Manfred P. Schneider** [b]

[a] *Research School of Biosciences, University of Kent, Canterbury, UK CT2 7NJ*
[b] *Fb 9-Bergische Universität, 42097 Wuppertal, Germany*

1 PERKIN

A mixture of proteases from *Streptomyces griseus* (pronase), displaying a very broad substrate tolerance in the hydrolysis of peptides, has been studied for the first time systematically regarding their substrate specificity in peptide synthesis. It is demonstrated that pronase can be employed successfully for the formation of dipeptides with yields up to 95%. Pronase has also been employed successfully as catalyst for the enzyme assisted synthesis of a hexapeptide.

Introduction

Protease catalysed coupling reactions of suitably functionalised amino acids or peptide fragments offer many advantages over chemical methods. They can be carried out under mild conditions (room temperature, neutral pH), undesirable racemisations or formation of side products can usually be avoided. Routine application of protease catalysed peptide syntheses would require knowledge of a wide variety of protease subsite specifities and related synthetic procedures. Many efforts have been made in the past to obtain and publish these crucial data.[1–11] In 1989 V. Kasche[4] published a P_1–$P_{1'}$ correlation table linking 88 of the possible 400 P_1-$P_{1'}$-condensations to a collection of proteases. Those data were compiled from 35 publications on peptide hydrolysis and synthesis found in the literature. Unfortunately, Kasche's table does not allow each P_1–$P_{1'}$-condensation type to be linked to the corresponding reference. A more recent literature survey[12] crosslinks 178 different P_1–$P_{1'}$-condensation types with 20 proteases and 164 references. This database on protease catalysed peptide syntheses is fully searchable and can be accessed *via* the world wide web.[12] This survey reveals that in spite of much research a large number of unassigned P_1–$P_{1'}$-condensation types still exist. Some proteases are frequently used for the hydrolysis of peptides but have never been tested systematically as catalysts for peptide syntheses. Investigation of these proteases regarding their use as catalysts in enzymatic peptide syntheses might help to link more P_1–$P_{1'}$-condensation types to known proteases.

Pronase (EC 3.4.24.4, protein content of the preparation: 55%) is a commercially available (Boehringer Mannheim GmbH) mixture of several *Streptomyces griseus* proteolytic enzymes, endopeptidases and exopeptidases commonly used for the complete hydrolysis of peptides and proteins. It has also been used for the regio- and stereo-specific hydrolysis of amino acid esters[13,14] and amides[15] as well as for the hydrolysis of aspartame,[16] but, to the best of our knowledge, pronase has so far never been applied for enzymatic peptide syntheses. We therefore studied its suitability as catalyst for peptide synthesis and systematically investigated its substrate specificity. In the present paper we describe our studies aimed at the systematic investigation of this catalyst regarding its substrate specificity and suitability for the synthesis of peptides.

Enantioselective hydrolysis of *N*-acetylamino acid esters

In order to obtain reliable information regarding the substrate tolerance and enantioselectivities of this mixture of proteases, the capability of pronase for the hydrolytic resolution of a series of 12 different, racemic *N*-acetylamino acid esters was investigated (Scheme 1). The methyl esters of *N*-acetyl-DL-alloleucine and *N*-acetyl-DL-isoleucine were employed in this study as an equimolar mixture. The specific activities of the enzyme towards this series of substrates were determined using the titration method and are plotted in Fig. 1.

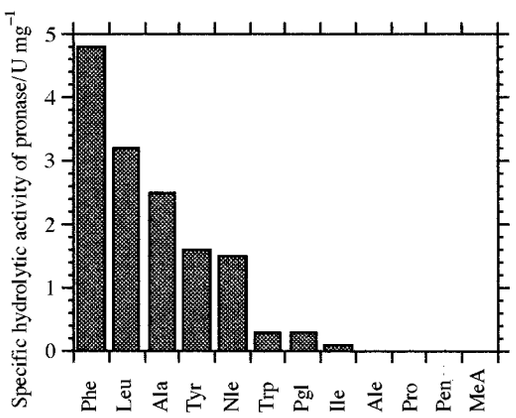
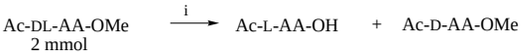


Fig. 1 Pronase catalysed kinetic resolution of *N*-acetyl-DL-amino acid esters leading to *N*-acetyl-L-amino acids and *N*-acetyl-D-amino acid esters. Conditions according to Scheme 1. Specific activity refers to activity per mg of protein preparation with a protein content of 55%. With the exception of Ac-DL-Ala-OMe all hydrolyses were enantioselective. Abbreviations of non-natural amino acids: Ale = alloleucine, Nle = norleucine, Pgl = phenylglycine, Pen = penicillamine, MeA = α-methylalanine.



Scheme 1 Enantioselective, pronase catalysed hydrolysis of racemic *N*-acetylamino acid esters (Ac = acetyl, AA = amino acid). *Reagents and conditions:* i, 20 mg pronase, 2 cm^3 THF, 20 cm^3 phosphate buffer (pH 7.0, 0.067 mol dm^{-3}), room temp.

Pronase displayed a very high selectivity when presented with an equimolar mixture of *N*-acetyl-DL-alloleucine methyl ester and *N*-acetyl-DL-isoleucine methyl ester. Only the *N*-acetyl-L-isoleucine methyl ester was hydrolysed yielding *N*-acetyl-L-isoleucine in 72% isolated yield. In 6 out of the remaining 10 experiments the attempted kinetic resolution was successful and the corresponding *N*-acetyl-L-amino acids and the unhydrolysed *N*-acetyl-D-amino acid methyl esters were isolated in yields ranging from 62 to 99%. The products were characterised by melting points (if isolated as solids) and optical rotations. The data obtained were in good agreement with data reported in the literature (Table 1). *N*-Acetyl-DL-alanine methyl ester was hydrolysed without selectivity leading to a racemic mixture of *N*-acetyl-DL-alanine. Based on this result one might have expected *N*-acetyl-α-methylalanine methyl ester to be hydrolysed by pronase, but no hydrolytic activity was observed with this substrate. The low or even absent hydrolytic activities of pronase towards the phenylglycine (0.3 U mg^{-1}), isoleucine

Table 1 Pronase catalysed kinetic resolution of *N*-acetyl-DL-amino acid esters leading to *N*-acetyl-L-amino acids and *N*-acetyl-D-amino acid esters. Conditions according to Scheme 1. Abbreviations: Nle = norleucine, Pgl = phenylglycine, Ref. = literature references

		Mp/°C		$[\alpha]_D^{20}/10^{-1}$ deg cm^2 g^{-1}	
Product	Yield (%)		Ref.		Ref.
Ac-D-Phe-OMe	93	80–82	89–91 [17]	−18.7 (*c* 2, MeOH)	−19 (*c* 2, MeOH) [17]
Ac-L-Phe	86	159–161	170–171 [18]	+47.3 (*c* 1, EtOH)	+47.6 (*c* 1, EtOH) [18]
Ac-D-Leu-OMe	91	oil		+44.3 (*c* 3.3, MeOH)	+42 (*c* 3.3, MeOH) [19]
Ac-L-Leu	79	177–179	185–186 [20]	−19.0 (*c* 1, EtOH)	−21.3 (*c* 2, EtOH) [21]
Ac-D-Tyr-OMe	62	137–139	135–139 [22]	−29.2 (*c* 0.4, MeOH)	−29.7 (*c* 0.4, MeOH) [23]
Ac-L-Tyr	85	oil		+7.1 (*c* 2, EtOH)	
Ac-D-Nle-OMe	96	47–48		+29.9 (*c* 1, MeOH)	
Ac-L-Nle	63	109–111	112 [24]	−24.4 (*c* 1, H_2O)	−20.0 (*c*1–2, H_2O) [24]
Ac-D-Trp-OMe	99	145–147	152.5 [25]	−12.4 (*c* 2, MeOH)	−12.0 (*c* 2, MeOH) [25]
Ac-L-Trp	99	oil		+29.3 (*c* 1, 1 equiv. NaOH)	+30 (*c* 1, 1 equiv. NaOH) [25]
Ac-D-Pgl-OMe	99	102–104		−151.6 (*c* 1, MeOH)	
Ac-L-Pgl	86	189–191	186–188 [26]	+215.6 (*c* 1, EtOH)	+217.9 (EtOH) [26]
Ac-L-Ile	72	134–136		+15.2 (*c* 1, EtOH)	+16.3 (*c* 2, EtOH) [27]

Table 2 Pronase catalysed peptide syntheses. Conditions according to Scheme 2

Product	*t*/h	Yield (%)	Mp/°C
Z-L-Leu-Gly-NH_2	24	41	164–166
Z-L-Met-Gly-NH_2	24	38	175–176
Z-L-Phe-Gly-NH_2	24	58	185–187
Z-L-Tyr-Gly-NH_2	24	82	154–156
Z-L-Ala-L-Ile-NH_2	24	57	226–227
Z-L-Leu-L-Ile-NH_2	24	19	228–229
Z-L-Met-L-Ile-NH_2	24	43	219–221
Z-L-Phe-L-Ile-NH_2	24	53	212–214
Z-L-Trp-L-Ile-NH_2	24	15	209–210
Z-L-Tyr-L-Ile-NH_2	24	68	219–221
Z-L-Phe-L-Met-NH_2	24	20	212–214
Z-L-Ala-L-Phe-NH_2	24	36	199–201
Z-L-His-L-Phe-NH_2	24	69	194–196
Z-L-Met-L-Phe-NH_2	24	39	185–186
Z-L-Phe-L-Phe-NH_2	24	27	221–222
Z-L-Thr-L-Phe-NH_2	24	53	178–180
Z-L-Trp-L-Phe-NH_2	24	31	209–212
Z-L-Tyr-L-Phe-NH_2	24	54	205–209
Z-L-Ala-L-Val-NH_2	24	19	239–241
Z-L-Phe-L-Val-NH_2	24	33	234–236
Z-L-Glu-(ε)-L-Lys-NH_2	24	25	186–190

Scheme 2 Equilibrium controlled, pronase catalysed peptide syntheses in a two-phase solvent system (Z = benzyloxycarbonyl). *Reagents and conditions:* i, 1 mg pronase, 0.3 cm^3 ethyl acetate, 2.7 cm^3 phosphate buffer (pH 7.0, 0.067 mol dm^{-3}), 100 μmol triethylamine, 30 °C, 24 h.

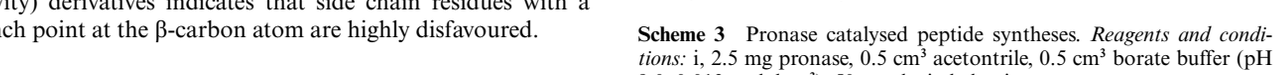
(0.1 U mg^{-1}), alloleucine (no activity) and penicillamine (no activity) derivatives indicates that side chain residues with a branch point at the β-carbon atom are highly disfavoured.

Equilibrium controlled dipeptide syntheses in an aqueous–organic two-phase solvent system

In order to determine the substrate tolerance of pronase as catalyst in the enzymatic peptide synthesis we studied the reactions of 22 different, Z-protected L-amino acids (Z-Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val; di-Z-Arg, Lys) with 18 different L-amino acid amides (Ala, Arg, Asn, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val, MeA-NH_2) (Scheme 2). The possible combinations thus resulted in 396 different experiments. In 21 of the 396 experiments the dipeptides formed precipitated spontaneously from the reaction mixture and could be isolated by simple filtration. The products were identified by ^{1}H NMR spectroscopy. The isolated products and their yields are summarised in Table 2. In the thus obtained dipeptides, Z-Phe, Z-Met and Z-Ala are frequently found in P_1-position while Phe-NH_2 and Ile-NH_2 often occupy the $P_{1'}$-position. These amino acid amides are obviously very good nucleophiles in the dipeptide synthesis employing the two phase solvent system described above.

Dipeptide syntheses using Z-protected amino acid methyl esters as donor substrates in an aqueous–organic single-phase solvent system

If acids are used as donor substrates the product yield of the peptide formed solely depends on the thermodynamic equilibrium constant of the reaction and these reactions are therefore called thermodynamically controlled or equilibrium controlled. However, if esters are used as donor substrates, it is often possible to obtain peptide yields above the level determined by the thermodynamic equilibrium, provided the reaction is terminated at maximum product yield. Usually esters are kinetically favoured as substrates over peptides or free acids so that the peptide formation becomes kinetically controlled. In order to determine the suitability of pronase as a catalyst for dipeptide synthesis under conditions favouring kinetic reaction control the reaction had to be optimised first regarding the optimal mixtures of water-miscible solvents (DMF, THF, ethanol, acetonitrile) and borate buffer of varying pH (9, 10). A mixture (1 cm^3, 1:1 v/v) of acetonitrile and borate buffer (pH 9.0, 0.013 mol dm^{-3}) in the presence of one equivalent of triethylamine proved to provide the optimal environment for the synthesis of a series of dipeptides (Scheme 3). The course of the reaction was followed by



Scheme 3 Pronase catalysed peptide syntheses. *Reagents and conditions:* i, 2.5 mg pronase, 0.5 cm^3 acetontrile, 0.5 cm^3 borate buffer (pH 9.0, 0.013 mol dm^{-3}), 50 μmol triethylamine, room temp.

reversed phase HPLC (Figs. 2–4). Using this method it was found that the consumption of the Z-protected amino acid esters was relatively slow. The yield of the formed dipeptide increased steadily reaching a maximum yet constant yield after about 5 h. Figs. 3 and 4 show the concentration of the initially formed Z-protected amino acid slowly declining after a reaction time of more than 2 h, revealing concominant peptide formation resulting from the Z-protected amino acid acting as donor substrate. The usually to be expected hydrolysis of the dipeptide product was not observed, most likely due to its precipitation from the reaction mixture. Therefore, the finally obtained product yield is equilibrium controlled rather than kinetically controlled. The precipitated

Table 3 Pronase catalysed peptide syntheses. Conditions according to Scheme 3

Product	*t*/h	Yield (%)	Mp/°C
Z-L-Tyr-L-Ile-NH_2	3.33	67	219–221
Z-L-Ala-L-Ile-NH_2	3.75	61	226–227
Z-L-Met-L-Ile-NH_2	3.75	70	219–221

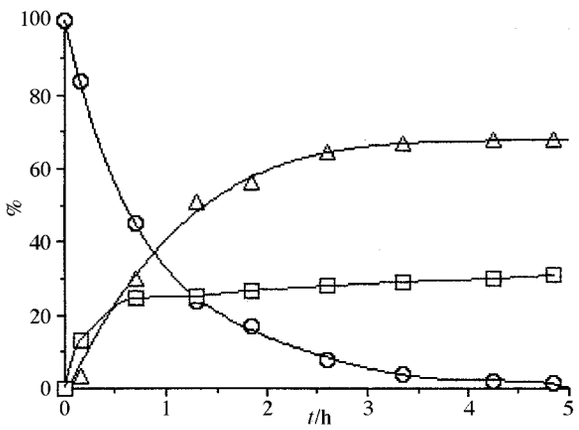


Fig. 2 Pronase catalysed synthesis of Z-L-Tyr-L-Ile-NH_2. ○, Z-L-Tyr-OMe; △, Z-L-Tyr-L-Ile-NH_2; □, Z-L-Tyr-OH. Conditions according to Scheme 2.

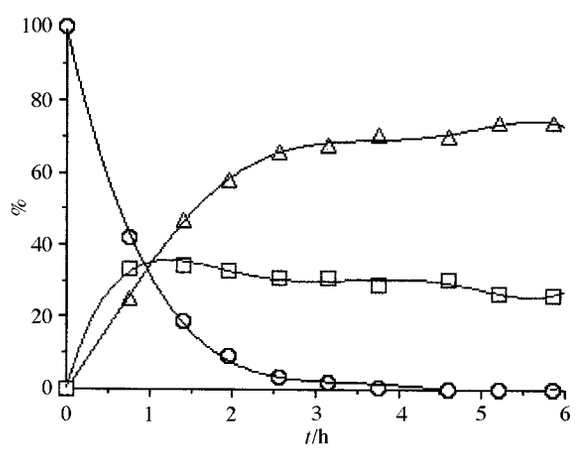


Fig. 3 Pronase catalysed synthesis of Z-L-Met-L-Ile-NH_2. ○, Z-L-Met-OMe; △, Z-L-Met-L-Ile-NH_2; □, Z-L-Met-OH. Conditions according to Scheme 2.

dipeptides were isolated by simple filtration and identified by ^{1}H NMR spectroscopy. The products obtained and their isolated yields are listed in Table 3.

Equilibrium controlled peptide syntheses in an aqueous–organic single-phase solvent system

From the above results—showing a favourable equilibrium controlled product yield—it can be expected that the single-phase solvent system (mixture of acetonitrile and borate buffer) described above could also be employed using amino-protected amino acids as donor substrates.

In order to investigate the full potential of pronase as a catalyst for equilibrium controlled dipeptide syntheses in this solvent system the reaction was first optimised using various mixtures of acetonitrile and borate buffer of varying pH (7–10). A mixture (1 cm^3, 1:9 v/v) of acetonitrile and borate buffer (pH 9.0, 0.013 mol dm^{-3}) in the presence of one equivalent of triethylamine proved to provide the optimal environment for the synthesis of a series of dipeptides (Scheme 4). Again the dipeptides formed precipitated from the reaction mixture and were isolated by simple filtration and identified by ^{1}H NMR

Table 4 Pronase catalysed peptide syntheses. Conditions according to Scheme 4

Product	*t*/h	Yield (%)	Mp/°C
Z-L-Ala-L-Ile-NH_2	6	84	226–227
Z-L-Asn-L-Ile-NH_2	12	63	226–228
Z-L-Gln-L-Ile-NH_2	12	64	234–236
Z-L-His-L-Ile-NH_2	12	42	176–178
Z-L-Leu-L-Ile-NH_2	6	87	228–229
Z-L-Met-L-Ile-NH_2	6	94	219–221
Ac-L-Nle-L-Ile-NH_2	6	70	>240
Z-L-Phe-L-Ile-NH_2	4	92	212–214
Boc-L-Phe-L-Ile-NH_2	4	95	197–198
Z-L-Thr-L-Ile-NH_2	24	16	215–218
Z-L-Trp-L-Ile-NH_2	24	95	209–210
Z-L-Tyr-L-Ile-NH_2	6	89	219–221
Z-L-Leu-L-Val-NH_2	25	77	214–216

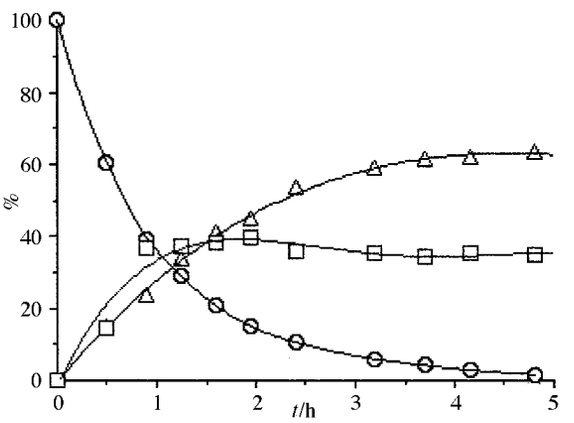


Fig. 4 Pronase catalysed synthesis of Z-L-Ala-L-Ile-NH_2. ○, Z-L-Ala-OMe; △, Z-L-Ala-L-Ile-NH_2; □, Z-L-Ala-OH. Conditions according to Scheme 2.

Scheme 4 Equilibrium controlled, pronase catalysed peptide syntheses in a single-phase solvent system (Pg = protecting group Ac, Boc or Z; Boc = *tert*-butoxycarbonyl). *Reagents and conditions:* i, 2.5 mg pronase, 0.1 cm^3 acetonitrile, 0.9 cm^3 borate buffer (pH 9.0, 0.013 mol dm^3), 100 μmol triethylamine, room temp.

spectroscopy. The thus obtained products and their isolated yields are listed in Table 4.

We were pleased to find that the product yields were exceptionally high (up to 95%) under these conditions. The substrate tolerance of pronase in the P_1-position is very broad. Ten different N-protected amino acids were used as acyl donors. Variations of the N-protecting group (Ac, Boc, Z) were also tolerated.

A crosscheck of the synthesised dipeptides (Tables 2–4) with our literature database[12] reveals that five of the P_1–$P_{1'}$-condensation types catalysed by pronase (Phe-Gln, Gly-Ile, Thr-Ile, Trp-Ile, Met-Trp) have not been previously reported in the literature. We have therefore contributed towards our goal to increase the number of P_1–$P_{1'}$-condensation types linked to known protease preparations.

Pronase catalysed fragment condensation

In order to demonstrate further the potential usefulness of pronase in enzyme catalysed fragment condensations we synthesised the fragment 3–8 of an octapeptide mimetic of calcium,[28] which can imitate the physiological effects of Ca^{2+}. The reaction conditions were chosen similar to the equilibrium controlled dipeptide syntheses in a mixture (1:9 v/v) of acetonitrile and borate buffer (pH 9.0, 0.013 mol dm^{-3}) (Scheme 5). The formed hexapeptide precipitated from the reaction mixture and

Table 5 ^{1}H NMR spectra of dipeptides synthesised

δ_H([$^2H_6$]DMSO, $SiMe_4$, *J*/Hz)

Z-L-Leu-Gly-NH_2

0.92 (3 H, d, *J* 3.3, CH-CH_3), 0.94 (3 H, d, *J* 3.4, CH-CH_3), 1.5–1.6 (2 H, m, CH-CH_2-CH), 1.6–1.8 [1 H, m, CH(CH_3)$_2$], 3.29 (2 H, s, NH-CH_2), 3.88–3.97 (1 H, m, CH-CH_2), 5.09 (2 H, s, C_6H_5-CH_2-O), 6.1–7.0 (1 H, br m, NH-CH_2), 7.17 (1 H, d, *J* 8.2, NH-CH), 7.22 (1 H, s, $CONH_2$), 7.3–7.5 (5 H, m, C_6H_5-CH_2-O), 7.57 (1 H, s, $CONH_2$)

Z-L-Met-Gly-NH_2

1.8–2.1 (2 H, m, CH_2-CH_2-S), 2.09 (3 H, s, S-CH_3), 2.52 (2 H, t, *J* 7.9, CH_2-S), 3.35 (2 H, s, NH-CH_2), 3.91–3.99 (1 H, m, CH-CH_2), 5.09 (2 H, s, C_6H_5-CH_2-O), 6.5–7.2 (1 H, br m, NH-CH_2), 7.08 (1 H, d, *J* 7.5, NH-CH), 7.28 (1 H, s, $CONH_2$), 7.3–7.5 (5 H, m, C_6H_5-CH_2-O), 7.62 (1 H, s, $CONH_2$)

Z-L-Phe-Gly-NH_2

2.87–2.96 (1 H, m, J_{AB} 13.5, C_6H_5-CH_2-CH), 3.13–3.20 (1 H, m, J_{AB} 13.5, C_6H_5-CH_2-CH), 3.38 (2 H, s, NH-CH_2), 4.04–4.12 (1 H, m, C_6H_5-CH_2-CH), 5.04 (2 H, s, C_6H_5-CH_2-O), 7.01 (1 H, d, *J* 7.8, NH-CH), 7.1–7.5 (12 H, m, C_6H_5-CH_2-O, C_6H_5-CH_2-CH, $CONH_2$, NH-CH_2), 7.67 (1 H, s, $CONH_2$)

Z-L-Tyr-Gly-NH_2

2.75–2.84 (2 H, m, J_{AB} 13.6, *H*O-C_6H_4-CH_2-CH), 3.00–3.07 (1 H, m, J_{AB} 13.6, HO-C_6H_4-CH_2-CH), 3.39 (2 H, s, NH-CH_2), 3.98–4.06 (1 H, m, NH-CH), 4.95–5.13 (2 H, m, C_6H_5-CH_2-O), 6.70 (2 H, d, J_{AB} 8.4, HO-C_6H_4-CH_2), 7.05 (3 H, m, J_{AB} 8.4, HO-C_6H_4-CH_2, NH-CH), 7.25–7.5 (6 H, m, C_6H_5-CH_2-O, $CONH_2$), 7.68 (1 H, s, $CONH_2$)

Z-L-Ala-L-Ile-NH_2

0.86–0.91 [6 H, m, CH(CH_3)CH_2CH_3], 1.0–1.4 (1 H, m, C$H_2$$CH_3$), 1.27 (3 H, d, *J* 7.1, NH-CH-CH_3), 1.4–1.6 (1 H, m, C$H_2$$CH_3$), 1.65–1.9 [1 H, m, CHC$H$($CH_3$)$CH_2$], 4.1–4.3 (2 H, m, 2 × NH-CH), 5.10 (2 H, s, C_6H_5-CH_2-O), 7.11 (1 H, s, $CONH_2$), 7.25–7.55 (5 H, m, C_6H_5-CH_2-O), 7.48 (1 H, s, $CONH_2$), 7.59 (1 H, d, *J* 7.7, NH-CH-CH_3), 7.67 (1 H, d, *J* 9.0, NH-CH-CH)

Z-L-Asn-L-Ile-NH_2

0.65–0.9 [6 H, m, CH(CH_3)$CH_2$$CH_3$], 0.95–1.15 (1 H, m, C$H_2$$CH_3$), 1.25–1.5 (1 H, m, C$H_2$$CH_3$), 1.6–1.85 [1 H, m, CHC$H$($CH_3$)$CH_2$], 2.3–2.6 (2 H, m, C$H_2$-$CONH_2$), 4.05–4.2 (1 H, m, NH-CH), 4.3–4.45 (1 H, m, NH-CH), 4.95–5.1 (2 H, m, C_6H_5-CH_2-O), 6.92 (1 H, s, $CONH_2$), 7.07 (1 H, s, $CONH_2$), 7.2–7.5 (7 H, m, C_6H_5-CH_2-O, $CONH_2$), 7.5–7.65 (2 H, m, 2 × NH-CH)

Z-L-Gln-L-Ile-NH_2

0.65–0.9 [6 H, m, CH(CH_3)CH_2CH_3], 0.9–1.15 (1 H, m, C$H_2$$CH_3$), 1.25–1.5 (1 H, m, C$H_2$$CH_3$), 1.55–1.8 [2 H, m, CHC$H$($CH_3$)$CH_2$, C$H_2$-$CH_2$-$CONH_2$], 1.8–1.95 (1 H, m, C$H_2$-$CH_2$-$CONH_2$), 1.95–2.25 (2 H, m, C$H_2$-$CONH_2$), 3.9–4.05 (1 H, m, NH-CH), 4.05–4.2 (1 H, m, NH-CH), 4.9–5.1 (2 H, m, C_6H_5-CH_2-O), 6.76 (1 H, s, $CONH_2$), 7.05 (1 H, s, $CONH_2$), 7.2–7.45 (m, 7 H, C_6H_5-CH_2-O, $CONH_2$), 7.50 (1 H, d, *J* 7.9, NH-CH), 7.62 (1 H, d, *J* 8.7, NH-CH)

Z-L-His-L-Ile-NH_2

0.65–0.9 [6 H, m, CH(CH_3)CH_2CH_3], 0.9–1.15 (1 H, m, C$H_2$$CH_3$), 1.2–1.5 (1 H, m, C$H_2$$CH_3$), 1.6–1.8 [1 H, m, CHC$H$($CH_3$)$CH_2$], 2.65–3.0 (2 H, m, CH-CH_2), 4.05–4.2 (1 H, m, NH-CH), 4.2–4.4 (1 H, m, NH-CH), 5.00 (2 H, m, C_6H_5-CH_2-O), 7.78 (1 H, s, $CONH_2$), 6.9–7.8 (m, 10 H, C_6H_5-CH_2-O, 2 × CH from imidazole ring, 2 × NH-CH, $CONH_2$)

Z-L-Leu-L-Ile-NH_2

0.86–0.96 [12 H, m, CH_2CH(CH_3)$_2$, CH(CH_3)CH_2CH_3], 1.0–1.3 (1 H, m, C$H_2$$CH_3$), 1.35–1.9 [5 H, m, C$H_2CH$($CH_3$)$_2$, CHC$H$($CH_3$)C$H_2$$CH_3$], 4.05–4.25 (2 H, m, 2 × NH-CH), 5.0–5.2 (2 H, m, C_6H_5-CH_2-O), 7.11 (1 H, s, $CONH_2$), 7.25–7.55 (5 H, m, C_6H_5-CH_2-O), 7.47 (1 H, s, $CONH_2$), 7.60 (1 H, d, *J* 8.4, NH-CH-CH_2), 7.66 (1 H, d, *J* 9.0, NH-CH-CH)

Z-L-Met-L-Ile-NH_2

0.86–0.92 [6 H, m, CH(CH_3)CH_2CH_3], 1.0–1.35 (1 H, m, C$H_2$$CH_3$), 1.35–1.6 (1 H, m, C$H_2$$CH_3$), 1.65–2.05 [3 H, m, CHCH(CH_3)CH_2, CH_2-CH_2-S], 2.10 (3 H, s, S-CH_3), 2.52 (2 H, t, *J* 8.2, CH_2-S), 4.1–4.3 (2 H, m, 2 × NH-CH), 5.11 (2 H, s, C_6H_5-CH_2-O), 7.12 (1 H, s, $CONH_2$), 7.3–7.55 (5 H, m, C_6H_5-CH_2-O), 7.50 (1 H, s, $CONH_2$), 7.64 (1 H, d, *J* 8.2, NH-CH-CH_2), 7.74 (1 H, d, *J* 8.9, NH-CH-CH)

Ac-L-Nle-L-Ile-NH_2

0.7–0.95 [9 H, m, CH_2CH_2CH_3, CH(CH_3)CH_2CH_3], 0.95–1.8 [9 H, m, (CH_2)$_3$-CH_3, CHCH(CH_3)CH_2CH_3], 1.83 (3 H, s, CH_3CO), 4.0–4.15 (1 H, m, NH-CH), 4.15–4.3 (1 H, m, NH-CH), 7.02 (1 H, s, $CONH_2$), 7.34 (1 H, s, $CONH_2$), 7.61 (1 H, d, *J* 8.9, NH-CH), 7.99 (1 H, d, *J* 7.8, NH-CH)

Z-L-Phe-L-Ile-NH_2

0.88–0.93 [6 H, m, CH(CH_3)CH_2CH_3], 1.0–1.3 (1 H, m, C$H_2$$CH_3$), 1.4–1.65 (1 H, m, C$H_2$$CH_3$), 1.65–1.9 [1 H, m, CHC$H$($CH_3$)$CH_2$], 2.77–2.86 (1 H, m, J_{AB} 13.7, C_6H_5-CH_2-CH), 3.04–3.11 (1 H, m, J_{AB} 13.7, C_6H_5-CH_2-CH), 4.22–4.28 (1 H, m, NH-CH-CH), 4.35–4.44 (1 H, m, NH-CH-CH_2), 4.97–5.08 (2 H, m, C_6H_5-CH_2-O), 7.13 (1 H, s, $CONH_2$), 7.2–7.5 (10 H, m, C_6H_5-CH_2-O, C_6H_5-CH_2-CH), 7.46 (1 H, s, $CONH_2$), 7.63 (1 H, d, *J* 8.7, NH-CH-CH_2), 7.88 (1 H, d, *J* 9.0, NH-CH-CH)

Boc-L-Phe-L-Ile-NH_2

0.80–0.85 [6 H, m, CH(CH_3)CH_2CH_3], 0.9–1.2 (1 H, m, C$H_2$$CH_3$), 1.30 [9 H, s, C($CH_3$)$_3$], 1.35–1.55 (1 H, m, C$H_2$$CH_3$), 1.6–1.8 [1 H, m, CHC$H$($CH_3$)$CH_2$], 2.6–2.8 (1 H, m, C_6H_5-CH_2-CH), 2.85–3.05 (1 H, m, C_6H_5-CH_2-CH), 4.22–4.28 (1 H, m, NH-CH-CH), 4.1–4.25 (2 H, m, 2 × NH-CH), 7.0–7.35 (7 H, m, C_6H_5-CH_2-CH, $CONH_2$, NH-CH-CH_2), 7.39 (1 H, s, $CONH_2$), 7.63 (1 H, d, *J* 9.0, NH-CH-CH)

Z-L-Thr-L-Ile-NH_2

0.75–0.9 [6 H, m, CH(CH_3)CH_2CH_3], 1.0–1.2 (1 H, m, C$H_2$$CH_3$), 1.04 [3 H, d, *J* 4.9, CH(OH)-CH_3], 1.3–1.5 (1 H, m, C$H_2$$CH_3$), 1.6–1.8 [1 H, m, CHC$H$($CH_3$)$CH_2$], 3.85–3.95 (1 H, m), 3.95–4.05 (1 H, m) and 4.05–4.2 (1 H, m) [2 × NH-CH, CH(OH)-CH_3], 4.83 [1 H, d, *J* 4.3, CH(O*H*)-CH_3], 5.03 (2 H, s, C_6H_5-CH_2-O), 7.0–7.15 [2 H, m, NH-CH-CH(OH), $CONH_2$], 7.25–7.5 (6 H, m, C_6H_5-CH_2-O, $CONH_2$), 7.66 [1 H, d, *J* 9.3, NH-CH-CH(CH_3)]

Table 5 (*Contd.*)

δ_H([$^2H_6$]DMSO, $SiMe_4$, *J*/Hz)

Z-L-Trp-L-Ile-NH_2

0.87–0.93 [6 H, m, CH(C*H*$_3$)CH$_2$C*H*$_3$], 1.0–1.35 (1 H, m, C*H*$_2$CH$_3$), 1.4–1.65 (1 H, m, C*H*$_2$CH$_3$), 1.7–1.9 [1 H, m, CHC*H*(CH$_3$)CH$_2$], 2.94–3.04 (1 H, m, J_{AB} 14.7, NH-CH-C*H*$_2$), 3.15–3.22 (1 H, m, J_{AB} 14.7, NH-CH-C*H*$_2$), 4.2–4.3 (1 H, m, NH-C*H*-CH), 4.35–4.5 (1 H, m, NH-C*H*-CH$_2$), 4.9–5.1 (2 H, m, C$_6$H$_5$-C*H*$_2$-O), 7.0–7.6 (12 H, m, C$_6$*H*$_5$-CH$_2$-O, $CONH_2$, =C*H*-NH, 4 aromatic protons from indole ring), 7.70 (1 H, d, *J* 7.7, N*H*-CH-CH$_2$), 7.84 (1 H, d, *J* 9.0, N*H*-CH-CH), 10.89 (1 H, s, NH from indole ring)

Z-L-Tyr-L-Ile-NH_2

0.87-0.93 [6 H, m, CH(C*H*$_3$)CH$_2$C*H*$_3$], 1.0–1.3 (1 H, m, C*H*$_2$CH$_3$), 1.4–1.6 (1 H, m, C*H*$_2$CH$_3$), 1.65–1.9 [1 H, m, CHC*H*(CH$_3$)CH$_2$], 2.64–2.74 (1 H, m, J_{AB} 13.9, HO-C$_6$H$_4$-C*H*$_2$-CH), 2.92–2.99 (1 H, m, J_{AB} 13.9, HO-C$_6$H$_4$-C*H*$_2$-CH), 3.1–3.8 (1 H, br m, *H*O-C$_6$H$_4$-CH$_2$), 4.21–4.33 (2 H, m, 2 × NH-C*H*), 5.03 (2 H, s, C$_6$H$_5$-C*H*$_2$-O), 6.72 (2 H, d, J_{AB} 8.3, HO-C$_6$*H*$_4$-CH$_2$), 7.14 (2 H, d, J_{AB} 8.3, HO-C$_6$*H*$_4$-CH$_2$), 7.2–7.5 (7 H, m, C$_6$*H*$_5$-CH$_2$-O, $CONH_2$), 7.55 (1 H, d, *J* 8.7, N*H*-CH-CH$_2$), 7.82 (1 H, d, *J* 9.0, N*H*-CH-CH)

Z-L-Phe-L-Met-NH_2

1.72–2.1 (2 H, m, C*H*$_2$-CH$_2$-S), 2.03 (3 H, s, S-CH$_3$), 2.44 (m, CH$_2$-S),* 2.71–2.81 (1 H, m, J_{AB} 14.0, C$_6$H$_5$-C*H*$_2$-CH), 2.99–3.07 (1 H, m, J_{AB} 14.0, C$_6$H$_5$-C*H*$_2$-CH), 4.2–4.35 (2 H, m, C$_6$H$_5$-CH$_2$-C*H*, C*H*-CH$_2$-CH$_2$-S), 4.95 (2 H, s, C$_6$H$_5$-C*H*$_2$-O), 7.05 (1 H, s, $CONH_2$), 7.1–7.45 (11 H, m, C$_6$*H*$_5$-CH$_2$-O, C$_6$*H*$_5$-CH$_2$-CH, $CONH_2$), 7.51 (1 H, d, *J* 8.2, N*H*-CH), 8.02 (1 H, d, *J* 8.5, N*H*-CH)

Z-L-Ala-L-Phe-NH_2

1.13 (3 H, d, *J* 7.1, CH-C*H*$_3$), 2.75–3.1 (2 H, m, C$_6$H$_5$-C*H*$_2$-CH), 3.95–4.1 (1 H, m, C*H*-CH$_3$), 4.35–4.4 (1 H, m, C$_6$H$_5$-CH$_2$-C*H*), 5.01 (2 H, s, C$_6$H$_5$-C*H*$_2$-O), 7.06 (1 H, s, $CONH_2$), 7.1–7.5 (12 H, m, C$_6$*H*$_5$-CH$_2$-O, C$_6$*H*$_5$-CH$_2$-CH, N*H*-CH, $CONH_2$), 7.80 (1 H, d, *J* 8.2, N*H*-CH)

Z-L-His-LPhe-NH_2

2.6–2.9 (3 H, m, =C-C*H*$_2$-CH, C$_6$H$_5$-C*H*$_2$-CH), 3.00–3.07 (1 H, m, J_{AB} 13.8, C$_6$H$_5$-C*H*$_2$-CH), 4.1–4.25 (1 H, m) and 4.3–4.5 (1 H, m) (2 × NH-C*H*), 4.99 (2 H, s, C$_6$H$_5$-C*H*$_2$-O), 6.75 (1 H, s, $CONH_2$), 7.05–7.75 (m, 14 H, C$_6$*H*$_5$-CH$_2$-O, C$_6$*H*$_5$-CH$_2$-CH, 2 × CH from imidazole ring, N*H*-CH, $CONH_2$), 7.97 (1 H, d, *J* 8.0, N*H*-CH), 11.4–12.1 (1 H, m, NH from imidazole ring)

Z-L-Met-L-Phe-NH_2

1.65–1.85 (2 H, m, C*H*$_2$-CH$_2$-S), 1.99 (3 H, s, S-CH$_3$), 2.36 (2 H, t, *J* 7.7, CH$_2$-S), 2.78–2.87 (1 H, m, J_{AB} 13.7, C$_6$H$_5$-C*H*$_2$-CH), 2.98–3.05 (1 H, m, J_{AB} 13.7 C$_6$H$_5$-C*H*$_2$-CH), 4.0–4.1 (1 H, m, C*H*-CH$_2$-CH$_2$-S), 4.4–4.55 (1 H, m, C$_6$H$_5$-CH$_2$-C*H*), 5.02 (2 H, s, C$_6$H$_5$-C*H*$_2$-O), 7.07 (1 H, s, $CONH_2$), 7.05–7.45 (11 H, m, C$_6$*H*$_5$-CH$_2$-O, C$_6$*H*$_5$-CH$_2$-CH, $CONH_2$), 7.46 (1 H, d, *J* 7.9, N*H*-CH), 7.85 (1 H, d, *J* 8.2, N*H*-CH)

Z-L-Phe-L-Phe-NH_2

2.55–3.1 (4 H, m, 2 × C$_6$H$_5$-C*H*$_2$-CH), 4.15–4.3 (1 H, m, NH-C*H*), 4.4–4.55 (1 H, m, NH-C*H*), 4.94 (2 H, s, C$_6$H$_5$-C*H*$_2$-O), 7.07 (1 H, s, $CONH_2$), 6.8–7.45 (16 H, m, C$_6$*H*$_5$-CH$_2$-O, $CONH_2$, 2 × C$_6$*H*$_5$-CH$_2$-CH), 7.44 (1 H, d, *J* 8.5, N*H*-CH), 7.99 (1 H, d, *J* 8.1, N*H*-CH)

Z-L-Thr-L-Phe-NH_2

0.95 (3 H, d, *J* 6.1, CH-C*H*$_3$), 2.78–2.87 (1 H, m, J_{AB} 13.7, C$_6$H$_5$-C*H*$_2$-CH), 3.00–3.08 (1 H, m, J_{AB} 13.7, C$_6$H$_5$-C*H*$_2$-CH), 3.8–4.0 (2 H, m, NH-C*H*-C*H*-OH), 4.4–4.55 (1 H, m, C$_6$H$_5$-CH$_2$-C*H*), 5.03 (2 H, s, C$_6$H$_5$-C*H*$_2$-O), 6.93 (1 H, d, *J* 7.8, N*H*-CH), 7.0–7.5 (12 H, m, C$_6$*H*$_5$-CH$_2$-O, C$_6$*H*$_5$-CH$_2$-CH, $CONH_2$), 7.94 (1 H, d, *J* 8.1, N*H*-CH)

Z-L-Trp-L-Phe-NH_2

2.7–3.1 (4 H, m, C$_6$H$_5$-C*H*$_2$-CH, NH-CH-C*H*$_2$-C=), 4.2–4.35 (1 H, m, C$_6$H$_5$-CH$_2$-C*H*), 4.45–4.6 (1 H, m, NH-C*H*-CH$_2$-C=), 4.95 (2 H, s, C$_6$H$_5$-C*H*$_2$-O), 6.95–7.45 (17 H, m, C$_6$*H*$_5$-CH$_2$-O, C$_6$*H*$_5$-CH$_2$-CH, $CONH_2$, =C*H*-NH, aromatic protons from indole ring), 7.58 (1 H, d, *J* 7.8, N*H*-CH), 7.95 (1 H, d, *J* 8.0, N*H*-CH), 10.76 (1 H, s, NH from indole ring)

Z-L-Tyr-L-Phe-NH_2

2.7–3.1 (4 H, m, C$_6$H$_5$-C*H*$_2$-CH, HO-C$_6$H$_4$-C*H*$_2$-CH), 4.05–4.2 (1 H, m, HO-C$_6$H$_4$-CH$_2$-C*H*), 4.4–4.55 (1 H, m, C$_6$H$_5$-CH$_2$-C*H*), 4.95 (2 H, s, C$_6$H$_5$-C*H*$_2$-O), 6.63 (2 H, d, J_{AB} 8.3, HO-C$_6$*H*$_4$-CH$_2$), 7.00 (2 H, d, J_{AB} 8.3, HO-C$_6$*H*$_4$-CH$_2$), 7.07 (1 H, s, $CONH_2$), 7.1–7.5 (12 H, m, C$_6$*H*$_5$-CH$_2$-O, C$_6$*H*$_5$-CH$_2$-CH, N*H*-CH, $CONH_2$), 7.95 (1 H, d, *J* 8.1, N*H*-CH)

Z-L-Ala-L-Val-NH_2

0.82 [3 H, d, *J* 6.7, CH(C*H*$_3$)$_2$], 0.84 [3 H, d, *J* 6.8, CH(C*H*$_3$)$_2$], 1.20 (3 H, d, *J* 7.2, NH-CH-C*H*$_3$), 1.85–2.05 [1 H, m, C*H*(CH$_3$)$_2$], 4.05–4.2 [2 H, m, C*H*CH(CH$_3$)$_2$, NH-C*H*-CH$_3$], 5.02 (2 H, s, C$_6$H$_5$-C*H*$_2$-O), 7.01 (1 H, s, $CONH_2$), 7.25–7.6 (8 H, m, C$_6$*H*$_5$-CH$_2$-O, N*H*-CH-CH$_3$, N*H*-CH-CH, $CONH_2$)

Z-L-Leu-L-Val-NH_2

0.7–0.95 [12 H, m, CH$_2$-CH(C*H*$_3$)$_2$, CH-CH(C*H*$_3$)$_2$], 1.3–1.55 (2 H, m, CH-C*H*$_2$-CH), 1.55–1.75 [1 H, m, CH$_2$-C*H*(CH$_3$)$_2$], 1.8–2.05 [1 H, m, CH-C*H*(CH$_3$)$_2$], 3.95–4.2 (2 H, m, 2 × NH-C*H*), 4.9–5.1 (2 H, m, C$_6$H$_5$-C*H*$_2$-O), 7.04 (1 H, s, $CONH_2$), 7.2–7.45 (6 H, m, C$_6$*H*$_5$-CH$_2$-O, $CONH_2$), 7.45–7.65 (2 H, m, 2 × N*H*-CH)

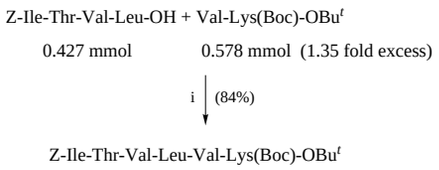
Z-L-Phe-L-Val-NH_2

0.84 [3 H, d, *J* 6.0, CH (C*H*$_3$)$_2$], 0.86 [3 H, d, *J* 6.0, CH(C*H*$_3$)$_2$], 1.85–2.05 [1 H, m, C*H*(CH$_3$)$_2$], 2.71–2.80 (1 H, m, J_{AB} 13.6, C$_6$H$_5$-C*H*$_2$-CH), 2.98–3.05 (1 H, m, J_{AB} 13.6, C$_6$H$_5$-C*H*$_2$-CH), 4.1–4.2 [1 H, m, C*H*CH(CH$_3$)$_2$], 4.25–4.4 (1 H, m, C$_6$H$_5$-CH$_2$-C*H*), 4.95 (2 H, s, C$_6$H$_5$-C*H*$_2$-O), 7.03 (1 H, s, $CONH_2$), 7.1–7.45 (11 H, m, C$_6$*H*$_5$-CH$_2$-O, C$_6$*H*$_5$-CH$_2$-CH, $CONH_2$), 7.53 (1 H, d, *J* 8.7, N*H*-CH), 7.73 (1 H, d, *J* 8.7, N*H*-CH)

Z-L-Glu-(ε)-L-Lys-NH_2

1.1–2.0 (8 H, m, C*H*$_2$-CH$_2$-COOH, NH-CH$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$), 2.15–2.35 (2 H, m, C*H*$_2$-COOH), 3.0–3.25 (2 H, m, NH-C*H*$_2$), 3.8–4.05 (2 H, m, NH-C*H*, H$_2$N-C*H*), 4.9–5.1 (2 H, m, C$_6$H$_5$-C*H*$_2$-O), 6.96 (1 H, d, *J* 7.3, N*H*-CH), 7.2–7.45 (5 H, m, C$_6$*H*$_5$-CH$_2$-O), 8.05 (1 H, t, *J* 5.7, N*H*-CH$_2$)

* No integral given because of overlying DMSO-signal.



Scheme 5 Equilibrium controlled, pronase catalysed synthesis of the 3–8-fragment of an octapeptide mimetic of calcium. *Reagents and conditions:* i, 5 mg pronase, 0.4 cm^3 acetonitrile, 3.6 cm^3 borate buffer (pH 9.0, 0.013 mol dm^{-3}), room temp., 64 h.

was isolated in high yield (84%) by simple filtration. The product was identified by ^{1}H NMR and mass spectroscopy.

Experimental

NMR spectra were obtained at 250 MHz using a Bruker HC-250 spectrometer. Mass spectra were obtained using a Finnigan MAT 90 spectrometer with fast atom bombardment (xenon, 7 keV, matrix: 3-nitrobenzyl alcohol) as ionisation method.

Enantioselective hydrolysis of *N*-acetylamino acid esters

2 mmol of the corresponding *N*-acetyl-DL-amino acid methyl ester was dissolved in THF (2 cm^3) and diluted with phosphate buffer (pH 7.0, 0.067 mol dm^{-3}; 20 cm^3). The hydrolysis was initiated by addition of pronase (20 mg). The pH of the reaction mixture was kept constant at pH 7 by continuous addition of aqueous NaOH (1 mol dm^{-3}) from an autoburette. The consumption of NaOH was continuously recorded and thus became a measure for the reaction progress. In most cases reactions came to a near stand still after consumption of 1 mmol of NaOH, indicating enantioselective hydrolysis. Products were isolated in the usual way by extraction with organic solvents as follows. First, the *N*-acetylamino acid methyl esters were extracted with three 50 cm^3 portions of *tert*-butyl methyl ether. The combined ether layers were washed with conc. aqueous NaCl (10 cm^3), dried (Na_2SO_4) and evaporated under reduced pressure. The aqueous phase was acidified by adding conc. aqueous HCl (3 cm^3). The *N*-acetylamino acids formed were then extracted with three 50 cm^3 portions of *tert*-butyl methyl ether. The combined ether layers were washed with aqueous HCl (2 mol dm^{-3}, 10 cm^3), dried (Na_2SO_4) and evaporated under reduced pressure.

Equilibrium controlled dipeptide syntheses in an aqueous–organic two-phase solvent system

Pronase (1 mg) was added to 100 μmol of the corresponding Z-protected amino acid, the respective amino acid amide hydrochloride and triethylamine, all dissolved in a mixture (3 cm^3, 9:1 v/v) of ethyl acetate and phosphate buffer (pH 7.0, 0.067 mol dm^{-3}) (Scheme 2). The reaction mixtures were shaken in stoppered vials at 30 °C at 250 rpm for 24 h. In 21 of the 396 experiments the dipeptides formed precipitated spontaneously from the reaction mixture and could be isolated by simple filtration. The products were identified by ^{1}H NMR spectroscopy (Table 5). The isolated products and their yields are summarised in Table 2.

Dipeptide syntheses using Z-protected amino acid methyl esters as donor substrates in an aqueous–organic single-phase solvent system

Pronase (2.5 mg) was added to 50 μmol of the corresponding Z-protected amino acid methyl ester, 100 μmol of the respective amino acid amide hydrochloride and 50 μmol triethylamine, all dissolved in a mixture (1 cm^3, 1:1 v/v) of acetonitrile and borate buffer (pH 9.0, 0.013 mol dm^{-3}) and stirred continuously at room temperature. The course of the reaction was followed by reversed phase HPLC (Figs. 2–4). The precipitated dipeptides were isolated by simple filtration and identified by ^{1}H NMR spectroscopy (Table 5). The products obtained and their isolated yields are listed in Table 3.

Equilibrium controlled peptide syntheses in an aqueous–organic single-phase solvent system

Pronase (2.5 mg) was added to 100 μmol of the corresponding amino-protected amino acid methyl ester, 200 μmol of the respective amino acid amide hydrochloride and 100 μmol triethylamine, all dissolved in a mixture (1 cm^3, 1:9 v/v) of acetonitrile and borate buffer (pH 9.0, 0.013 mol dm^{-3}) and stirred continuously at room temperature (Scheme 4). The dipeptides formed precipitated from the reaction mixture and were isolated by simple filtration and identified by ^{1}H NMR spectroscopy (Table 5). The products obtained and their isolated yields are listed in Table 4.

Z-L-Ile-L-Thr-L-Val-L-Leu-L-Val-L-Lys(Boc)-OBu^t

Pronase (5 mg) was added to Z-L-Ile-L-Thr-L-Val-L-Leu-OH (247 mg, 427 μmol) and L-Val-L-Lys(Boc)-OBu^t (232 mg, 578 μmol), all dissolved in a mixture (4 cm^3, 9:1 v/v) of acetonitrile and borate buffer (pH 9.0, 0.013 mol dm^{-3}) and stirred continuously at room temperature for 64 h (Scheme 5). The precipitated product was filtered off, washed with water (5 cm^3) and dried under reduced pressure overnight. The described procedure yielded Z-L-Ile-L-Thr-L-Val-L-Leu-L-Val-L-Lys(Boc)-OBu^t (346 mg, 84%); δ_H([$^2H_6$]DMSO, $SiMe_4$, *J*/Hz) 0.7–0.95 [24 H, m, CH(CH_3)-CH_2CH_3, 2 × CH-CH(CH_3)$_2$, CH_2-CH-(CH_3)$_2$], 1.01 [3 H, d, *J* 6.1, CH(OH)-CH_3], 1.05–2.1 [14 H, m, $CH(CH_3)$-CH_2CH_3, 2 × CH-$CH(CH_3)_2$, CH_2-$CH(CH_3)_2$, CH-(CH_2)$_3$], 1.37 [9 H, s, $C(CH_3)_3$], 1.39 [9 H, s, $C(CH_3)_3$], 3.8–4.4 (9 H, m, HO-CH, 6 × NH-CH, NH-CH_2), 4.85–4.95 (1 H, m, CH-OH), 5.03 (2 H, s, C_6H_5-CH_2-O), 7.2–7.5 (7 H, m, C_6H_5-CH_2-O, 2 × NH-CH), 7.5–7.75 (2 H, m, 2 × NH-CH), 7.81 (1 H, d, *J* 7.7, NH-CH), 7.95–8.15 (2 H, m, 2 × NH-CH); *m*/*z* (FAB, Xe, 7 keV) 660 ([Z-Ile-Thr-Val-Leu-Val-CO]$^+$, 5%), 561 ([Z-Ile-Thr-Val-Leu-CO]$^+$, 5), 448 ([Z-Ile-Thr-Val-CO]$^+$, 6), 430 ([Z-Ile-Thr-Val-CO − H_2O]$^+$, 5), 91 ([C_6H_5-CH_2]$^+$, 100).

Acknowledgements

We would like to thank the Boehringer Mannheim GmbH for supply of the enzyme and financial support of this work.

References

1 D. Konopinska and F. Muzalewski, *Mol. Cell. Biochem.*, 1983, **51**, 165.
2 H. D. Jakubke, P. Kuhl and A. Könnecke, *Angew. Chem., Int. Ed. Engl.*, 1985, **24**, 85.
3 W. Kullmann, *Enzymatic peptide synthesis*, CRC Press, Boca Raton, Florida, 1987.
4 V. Kasche, in *Proteases, a practical approach*, ed. R. Beynon, IRL-Press, Oxford, 1989, p. 125.
5 C.-H. Wong, *Science*, 1989, **244**, 1145.
6 A. M. Klibanov, *Trends Biochem. Sci.*, 1989, **14**, 141.
7 H. Gaertner and A. Puigserver, *Eur. J. Biochem.*, 1989, **181**, 207.
8 V. Schellenberger and H.-D. Jakubke, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1437.
9 C.-H. Wong and K. T. Wang, *Experimentia*, 1991, **47**, 1123.
10 C.-H. Wong, *Chimia*, 1993, **47**, 127.
11 D. Y. Jackson, J. Burnier, C. Quan, M. Stanley, J. Tom and J. A. Wells, *Science*, 1994, **266**, 243.
12 M. Lobell, PhD Thesis, University of Wuppertal, Germany, 1993 (http://biont.ukc.ac.uk/bio/database/proteases/Entry.html).
13 M. Pugnière, B. Castro, N. Domergue and A. Previero, *Tetrahedron: Asymmetry*, 1992, **3**, 1015.
14 J. E. Clark, D. P. Schumacher and D. Walker, *Int. Pat.*, WO 90/14434, 1990; Schering Corporation, (*Chem. Abstr.*, 1991, **115**, 69 924q).
15 I. A. Yamskov, T. V. Tichonova and V. A. Davankov, *Enzyme Microb. Technol.*, 1986, **8**, 241.
16 W. Hummel and U. Zervosen, *GIT Fachz. Lab.*, 1992, 199.
17 H. T. Huang, R. J. Foster and C. Niemann, *J. Am. Chem. Soc.*, 1952, **74**, 105.

18 R. S. Coffey, M. Green and M. W. Kenner, *J. Chem. Soc.*, 1959, 4100.
19 N. A. Smart, G. T. Young and M. W. Williams, *J. Chem. Soc.*, 1960, 3902.
20 H. D. De Witt and A. W. Ingersoll, *J. Am. Chem. Soc.*, 1951, **73**, 3359.
21 S. M. Birnbaum, L. Levintow, R. B. Kinsley and J. P. Greenstein, *J. Biol. Chem.*, 1952, **194**, 455.
22 T. H. Applewhite and C. Niemann, *J. Am. Chem. Soc.*, 1959, **81**, 2208.
23 E. L. Jackson, *J. Am. Chem. Soc.*, 1952, **74**, 837.
24 R. Marshall, S. M. Birnbaum and J. P. Greenstein, *J. Am. Chem. Soc.*, 1956, **78**, 4636.
25 H. T. Huang and C. Niemann, *J. Am. Chem. Soc.*, 1951, **73**, 1541.
26 C. T. Holdrege, *US Pat.* 3 464 985, 1969; Bristol-Meyers Co, *Chem. Abstr.*, 1969, **71**, 112 951h).
27 W. A. H. Huffman and A. W. Ingersoll, *J. Am. Chem. Soc.*, 1951, **73**, 3366.
28 J. Dillon, W. T. Woods, V. Guarcello, R. D. LeBoeuf and J. E. Blalock, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 9726.

Paper 7/04422D
Received 23*rd June* 1997
Accepted 20*th October* 1997