Superiority of the carbamoylmethyl ester as an acyl donor for the kinetically controlled amide-bond formation mediated by α-chymotrypsin †

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Received (in Cambridge, UK) 25th September 2001, Accepted 27th November 2001
First published as an Advance Article on the web 8th January 2002

The superiority of the carbamoylmethyl ester as an acyl donor for the α-chymotrypsin-catalysed kinetically controlled peptide-bond formation is demonstrated in the couplings of an inherently poor amino acid substrate, Ala, with various amino acid residues as amino components and in the couplings of non-protein amino acids such as halogenophenylalanines as carboxylic components. Furthermore, this approach is applied to the amide-bond formation between an amino acid residue and a chiral amine, which is highly diastereoselective.

Introduction

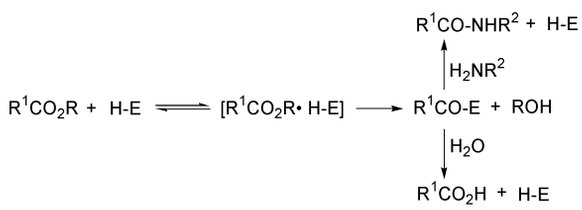
Protease-catalysed peptide synthesis has been intensively investigated during the last few decades,[1] because it has a potential advantage in circumventing the major problems associated with the chemical synthesis of peptides such as the risk of racemisation during the activation step and other undesirable side reactions. However, proteases' narrow substrate specificity is often considered as a main drawback from a synthetic standpoint which emphasises the general applicability of the reaction. In connection with this unsolved problem, we have recently reported on the broadening of substrate tolerance of α-chymotrypsin (EC 3.4.21.1) by taking advantage of such activated esters as the 2,2,2-trifluoroethyl (Tfe) or carbamoylmethyl (Cam) ester as acyl donors in kinetically controlled peptide synthesis.[2,3] Thus, as a typical example, the extremely low coupling efficiency obtained by employing the Me ester of an inherently poor amino acid substrate, Ala, was significantly improved by the use of the Cam ester.[3] The ameliorating effect of this ester was observed also in the couplings of other amino acid residues such as Gly and Ser as carboxylic components.

In the present work, first the effectiveness of the Cam ester was examined in the α-chymotrypsin-catalysed couplings of Ala with different amino acid amides as amino components. Next this approach was applied to the incorporation of non-protein amino acids such as halogenophenylalanines into peptides which had previously been attempted with only partial success by employing the Tfe ester.[2] Furthermore, the α-chymotrypsin-catalysed amide-bond formation between an amino acid residue and a chiral amine, not an amino acid derivative, was attempted by means of the Cam ester method.[4]

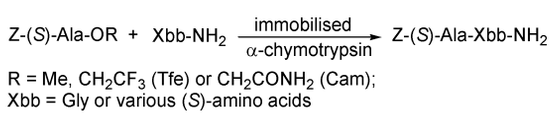
Results and discussion

Effectiveness of the carbamoylmethyl ester in the couplings of Ala with different amino acid nucleophiles

In the kinetically controlled peptide-bond formation mediated by α-chymotrypsin (Scheme 1), the ester substrate used is converted to the acyl-enzyme intermediate, which then reacts with an amino acid nucleophile in competition with water to afford the peptide product and the hydrolysis product of the donor ester, respectively.[1b] Even in a water-miscible organic solvent with low water content where the synthetic reaction is favoured due to the reduced water activity, the coupling efficiency must be affected by the amine nucleophile used. As the amino acid residue employed as the amino component had been restricted solely to (*S*)-Leu in the previous works,[2,3] we first intended to investigate the influence of the amino component in the α-chymotrypsin-catalysed couplings of Ala. Thus, the reactions of Z-(*S*)-Ala esters with several amino acid amides (Xbb-NH_2) were conducted in acetonitrile containing 4% (v/v) Tris buffer (pH 7.8) in the presence of the immobilised enzyme on Celite (Scheme 2).[3] The yields of the targeted peptide [Z-(*S*)-Ala-Xbb-NH_2] and hydrolysis product of the donor ester [Z-(*S*)-Ala] were quantified by HPLC analysis, and the results obtained after 48 h of incubation are compiled in Table 1. The yield of the desired peptide, as well as that of Z-Ala was extremely poor even after 48 h when the Me ester was used as the acyl donor, irrespective of the amine nucleophile. With the Tfe ester, the coupling efficiencies were generally improved, but the extents differed depending on the amine nucleophile



Scheme 1 Kinetically controlled peptide synthesis: H–E, α-chymotrypsin; R^1CO_2R, carboxylic component in the form of an ester; R^2NH_2, amino component.



Scheme 2

† Electronic supplementary information (ESI) available: elemental analyses and HPLC separation data. See http://www.rsc.org/suppdata/p1/b1/b108735p/

 DOI: 10.1039/b108735p

Table 1 Effect of donor esters on the α-chymotrypsin-catalysed couplings of Z-(*S*)-Ala-OR with various amino acid amides (Xbb-NH_2)[a]

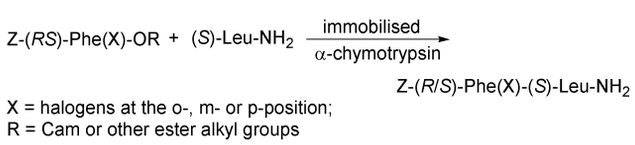
Xbb	R[b]	Yield (%)[c] Peptide	Z-(*S*)-Ala
Gly	Me	3	0
	Tfe	44	3
	Cam	95.6	4.4
(*S*)-Ala	Me	3.2	0
	Tfe	20	4
	Cam	96.8	2.5
(*S*)-Val	Me	2.9	0.9
	Tfe	17	7
	Cam	73.6	22.2
(*S*)-Leu[d]	Me	6.7	1.0
	Tfe	82.4	8.6[e]
	Cam	88.4	10.9[e]
(*S*)-Met	Cam	89.6	10.4
(*S*)-Phe	Me	4.3	0.6
	Tfe	55	8
	Cam	87.8	12.2

[a] Reactions were conducted using 0.05 mmol of Z-(*S*)-Ala-OR, 0.2 mmol of Xbb-NH_2·HCl, 0.2 mmol of TEA, and 150 mg of the immobilised enzyme in a solvent composed of 2 ml of acetonitrile and 83 μl of 0.05 M Tris buffer (pH 7.8) at 30 °C. [b] Tfe, 2,2,2-trifluoroethyl; Cam, carbamoylmethyl. [c] After 48 h of incubation. [d] Ref. 3. [e] Corrected for non-enzymatic hydrolysis.

employed: the peptide yield was only 20% at best when the amino component was (*S*)-Ala or (*S*)-Val. In this respect, (*S*)-Leu proved to be rather exceptional (82% peptide yield) among the amino acid residues examined as amino components. When the Cam ester was employed, the peptide yields were profoundly improved, reaching almost 90% in most cases. With (*S*)-Val as the amino component, the peptide yield was just over 70% and the production of Z-Ala was exceptionally large, suggesting the deteriorating effect of the bulky side chain of this amino acid residue on its nucleophilic attack toward the acyl-enzyme intermediate. Thus, the superiority of the Cam ester over other esters was once again ascertained in the couplings using several amino acids as amino components.

Incorporation of halogenophenylalanines into peptides using the carbamoylmethyl ester as an acyl donor

We reported in previous papers[2] that in the α-chymotrypsin-catalysed couplings of *N*-Z-(*RS*)-halogenophenylalanines [Z-(*RS*)-Phe(X)] with (*S*)-Leu-NH_2 the coupling yields were generally improved by the use of the Tfe esters as acyl donors instead of the conventional Me esters. However, with the amino acid residues carrying bulky substituents on the *ortho*-position of the benzene ring as the carboxylic components, the coupling efficiencies were still low [Phe(2Cl), 19% yield; Phe(2Br), 14% yield, after 1 h of incubation], though they themselves were prominent compared with the almost negligible yields obtained using the Me esters. As the deleterious effect of the *o*-Br substituent was especially noteworthy, we examined in the present study the couplings of Z-(*RS*)-Phe(2Br) by employing a series of its esters (Scheme 3). Table 2 summarises the yields of the



Scheme 3

desired peptide and the hydrolysis product of the donor ester after 1 h of incubation. When halogenated alkyl esters other than the Tfe ester were employed, the improvement in the coupling efficiency was not very significant. Once again it

Table 2 α-Chymotrypsin-catalysed couplings of Z-(*RS*)-Phe(X)-OR with (*S*)-Leu-NH_2[a]

X	R	Yield (%)[b] Peptide[c]	Z-Phe(X)
2Br	Me[d]	0.8	0.5
	CH_2CH_2Cl	2.3	0.9
	CH_2CCl_3	7.7	1.7
	Tfe[d]	13.9	3.4
	$CH_2CF_2CF_3$	7.0	1.3
	$N{=}CMe_2$	5.4[e]	2.1
	Cam	33.0	8.8
3Br	Cam	45.6	4.8
4Br	Cam	46.2	2.4
2F	Cam	47.5	2.9
3F	Cam	47.6	3.1
4F	Cam	49.2	1.9
2Cl	Cam	39.4	6.7
3Cl	Cam	47.1	4.7
4Cl	Cam	48.8	3.0

[a] Reactions were conducted using 0.1 mmol of Z-(*RS*)-Phe(X)-OR and 0.2 mmol of (*S*)-Leu-NH_2·HCl together with 0.2 mmol of TEA in the same manner as described in Table 1. [b] After 1 h of incubation. [c] *S-S* Isomer. [d] Ref. 2. [e] *S-S* Isomer, 5.2%; *R-S* isomer, 0.2%.

was gratifying to find that the peptide yield obtained using the Cam ester was 2.4-times greater than that with the Tfe ester. As the reaction profile in Fig. 1 shows, the amount of the donor

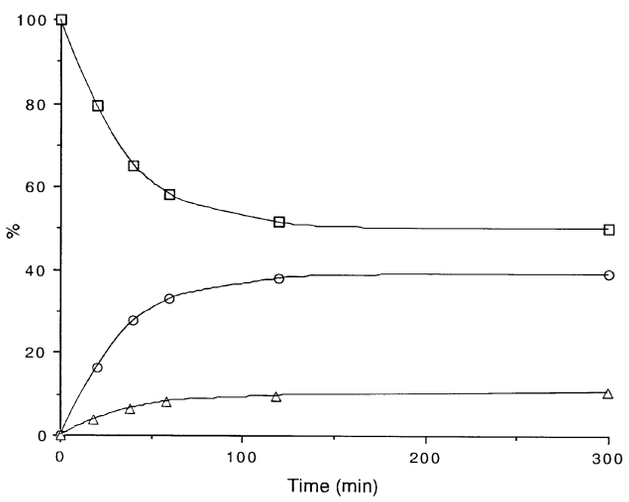


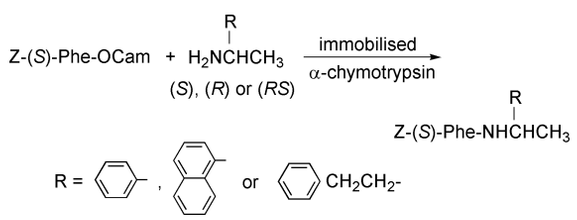
Fig. 1 Reaction profile in the α-chymotrypsin-catalysed coupling of Z-(*RS*)-Phe(2Br)-OCam with (*S*)-Leu-NH_2. Symbols: □, Z-(*RS*)-Phe(2Br)-OCam; ○, Z-(*S*)-Phe(2Br)-(*S*)-Leu-NH_2; △, Z-Phe(2Br).

ester decreased gradually, reaching nearly 50% of starting quantity after 2 h, and then remained constant afterwards. The yield of the *S-S* peptide reached a maximum (39.2%) after 5 h, while production of the *R-S* peptide was not detected. At this point in time the hydrolysis product of the donor ester [Z-Phe(2Br)] was formed in 10.8% yield and was found to be enantiomerically pure (*S*), which was confirmed by converting it to the Me ester followed by HPLC analysis on a chiral column.[5] These results indicate that the deacylation of the acyl-enzyme intermediate by the nucleophilic amino component or by water proceeded enantiospecifically within at least 5 h. Taking into consideration our previous report that in the coupling of Z-(*R*)-Ala-OCam with (*S*)-Leu-NH_2 the *R-S* peptide was produced in moderate yield (41%) after 48 h of incubation,[3] it follows that the (*S*)-Phe(2Br) residue is a better amino acid substrate than is the (*S*)-Ala residue and that the difference in reactivity of the enantiomers of Phe(2Br) is much greater than that of the enantiomers of Ala.

The couplings of other *N*-Z-halogenophenylalanines were next examined using their Cam esters as acyl donors. The results obtained after 1 h of incubation are compiled in Table 2. The behaviour of Phe(2Cl) resembled that of Phe(2Br): the peptide yield was moderate (39%), but still doubled that obtained using the Tfe ester. In all other cases, the yields of the *S*-*S* peptides were above 45% after 1 h, which were better than those obtained using the Tfe esters. Moreover, in some cases the reaction proceeded almost to completion within 1 h. Thus, incorporation of the non-protein amino acid residues was found to be best achieved by employing the Cam esters as acyl donors.

Enantioselective acylation of chiral amines with an *N*-protected amino acid ester as an acyl donor

If an amine is used as a nucleophile instead of an amino acid derivative in the kinetically controlled approach of protease-catalysed peptide synthesis as depicted in Scheme 1, the formation of an amide can be expected. To our best knowledge, however, there have been few reports concerning the protease-catalysed formation of an amide bond between an amino acid and an amine. Accordingly, the nucleophilicity or steric demand of amines during the reaction with the acyl-enzyme intermediate is not fully understood. Chinsky *et al.* reported on the chemoselective acylation of 6-aminohexan-1-ol with the 2-chloroethyl ester of *N*-Ac-(*S*)-Phe mediated by subtilisin Carlsberg in 2-methylbutan-2-ol.[6] On the other hand, in the case of α-chymotrypsin there had been no relevant reports until we reported preliminarily on the results of the first attempt to achieve the enantioselective acylation of chiral amines using an amino acid Cam ester as an acyl donor (Scheme 4).[4] Here we describe the relevant details.



Scheme 4

As shown below, the amines examined were found to be poor acyl acceptors compared with the amino acid amides employed previously[2,3] Consequently, the water content of the solvent must affect the amide-bond formation more severely and the minimal amount of water necessary for guaranteeing reasonable enzyme activity should be determined. Thus, the α-chymotrypsin-catalysed acylation of (*S*)-1-phenylethylamine with Z-(*S*)-Phe-OTfe was chosen as a model system and the influence of buffer content in acetonitrile on the yields of the desired amide and hydrolysis product of the donor ester was investigated. As shown in Table 3, even in the absence of water the enzyme showed some activity, but it was too low to give a practical yield of the amide within a reasonable time. Water content of 1.5% by volume was found to be the best, and further experiments were conducted using the solvent of this composition. When the amount of water was increased beyond this value, the yield of the product amide decreased due to competing hydrolysis of the donor ester.

Fig. 2 shows the comparison of reaction profiles of the α-chymotrypsin-catalysed acylations of (*S*)-1-phenylethylamine when different esters of Z-(*S*)-Phe were employed as acyl donors. The yield of the desired amide was extremely low when the Me ester was used. In contrast, the coupling efficiency was profoundly improved with the Tfe ester, while the competing hydrolysis of the donor ester was little accelerated [yield of Z-(*S*)-Phe after 1 h: Me ester, 0.5%; Tfe ester, 4.6%; Cam ester, 6.3%]. (*S*)-1-Phenylethylamine was found to be a poor acyl acceptor compared with the amino acid amides used previously [peptide yields (%) after 1 h during the couplings of Z-(*S*)-Phe-OTfe: Gly-NH_2, 99; (*S*)-Leu-NH_2, 98; (*S*)-Val-NH_2, 97].[2] Even with this amine, however, the Cam ester was a much better acyl donor than the halogenated alkyl ester, resulting in a marked increase of the amide yield (78.6% even after 15 min).

Table 3 Effect of water content of the solvent on the α-chymotrypsin-catalysed acylation of (*S*)-1-phenylethylamine with Z-(*S*)-Phe-OTfe[a]

Tris buffer (%)[b]	Yield (%)[c] Amide	Z-(*S*)-Phe
0	11.0	0.7
1	70.3	4.4
1.5	92.6	7.1
2	89.0	9.5
4	71.5	27.0

[a] Reactions were conducted using 0.05 mmol of Z-(*S*)-Phe-OTfe, 0.2 mmol of (*S*)-1-phenylethylamine and 150 mg of the immobilised α-chymotrypsin in a solvent composed of 2 ml of acetonitrile and 0 μl, 20 μl (water content, 1%), 31 μl (1.5%), 41 μl (2%), or 83 μl (4%) of 0.05 M Tris buffer (pH 7.8) at 30 °C. [b] By volume. [c] After 5 h of incubation.

Table 4 α-Chymotrypsin-catalysed acylation of homochiral amines with Z-(*S*)-Phe-OCam[a]

Amine	Yield (%)[b] Amide	Z-(*S*)-Phe
(*S*)-1-Phenylethylamine	92.5	6.8
(*R*)-1-Phenylethylamine	58.1	16.5
(*S*)-1-(1-Naphthyl)ethylamine	89.8	8.7
(*R*)-1-(1-Naphthyl)ethylamine	8.2	48.8

[a] Reactions were conducted in a solvent containing 1.5% water in the same manner as described in Table 3. [b] After 2 h of incubation.

Accordingly, employing Z-(*S*)-Phe-OCam as the acyl donor the acylation of each enantiomer of 1-phenylethylamine and 1-(1-naphthyl)ethylamine was examined. The results obtained after 2 h of incubation are compiled in Table 4. The (*S*)-enantiomer of 1-phenylethylamine was a more reactive nucleophile than the (*R*)-counterpart and the acyl donor was consumed almost completely after 2 h together with the concomitant reaction with water (6.8%). The proportion of the hydrolysis product of the donor ester increased when the less reactive (*R*)-enantiomer was employed as the nucleophile. In the case of 1-(1-naphthyl)ethylamine the difference in reactivity of the two enantiomers was much larger: the (*S*)-enantiomer was as reactive as (*S*)-1-phenylethylamine, while the (*R*)-counterpart was far less reactive as a nucleophile, resulting in the formation of a large quantity of the hydrolysis product of the donor ester.

Based on the above results, the enantioselective acylation of some racemic amines was examined using Z-(*S*)-Phe-OCam as the acyl donor (Table 5). The amount of the immobilised enzyme was reduced to one-fifth of that used in the above-mentioned investigations. The diastereomers of the resulting amides were separated well by normal-phase HPLC on a chiral column, which allowed the accurate determination of diastereomeric excess (d.e.)-values. The d.e.-value obtained was used together with the yield of the amide product to estimate the enantiomeric ratio *E*,[7] the discrimination between the two competing enantiomers of the amine nucleophile. The acylation of 1-phenylethylamine or 3-amino-1-phenylbutane was somewhat faster than that of 1-(1-naphthyl)ethylamine; the competing hydrolysis of the donor ester was at a low level (<6% yield). The *E*-values were good to excellent in all the cases examined and,

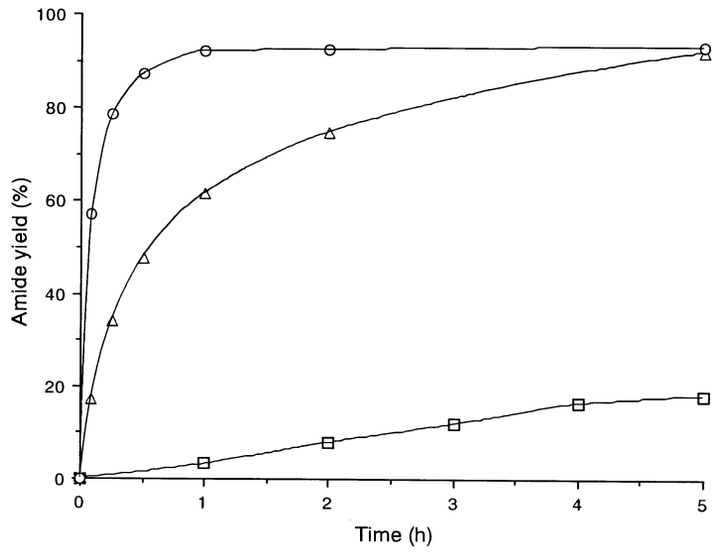


Fig. 2 Reaction profiles in the α-chymotrypsin-catalysed acylation of (*S*)-1-phenylethylamine with different *N*-Z-(*S*)-phenylalanine esters [Z-(*S*)-Phe-OR]. Symbols: ○, R = Cam; △, R = Tfe; □, R = Me.

Table 5 α-Chymotrypsin-catalysed acylation of racemic amines with Z-(*S*)-Phe-OCam[a]

Amine	Time/min	Amide			Z-(*S*)-Phe
		Yield (%)[b]	% d.e.[c]	*E*[d]	Yield (%)
1-Phenylethylamine	30	45.8	89.6	42	4.5
1-(1-Naphthyl)ethylamine	60	40.0	99.4	660	5.7
3-Amino-1-phenylbutane	30	44.5	85.1	25	5.0

[a] Reactions were conducted in a solvent containing 1.5% water in the same manner as described in Table 3. [b] Total yield of the *S*-*S* and *S*-*R* amides. [c] Diastereomeric excess of the *S*-*S* amide. [d] Enantiomeric ratio (Ref. 7). In this case $E = \ln [1 - c(1 + \text{d.e.})]/\ln [1 - c(1 - \text{d.e.})]$, where c is the total yield of the *S*-*S* and *S*-*R* amides.

among others, the enantiodiscrimination of 1-(1-naphthyl)-ethylamine proved to be extremely efficient. It is anticipated from the *E*-values obtained that if a racemic amine is used in an amount equivalent to the acyl donor, the enantiomeric excess of the untouched (*R*)-isomer will be >99% when the formation of amide is allowed to reach 50% yield with 1-(1-naphthyl)ethylamine, and 60% with 1-phenylethylamine or 3-amino-1-phenylbutane, respectively.

In summary, the results reported here together with those obtained previously[2,3] demonstrate the superiority of the Cam ester as an acyl donor in the α-chymotrypsin-catalysed formation of amide bonds *via* the kinetically controlled approach.

Experimental

General

^{1}H NMR spectra (300 MHz) were obtained on a Varian Unity 300 spectrometer using $CDCl_3$ or DMSO-d_6 as a solvent with TMS as an internal standard. Mps were determined on a Yamato MP-21 apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-4 digital polarimeter. $[\alpha]_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}. All solvents were distilled prior to use following standard protocols and dried over molecular sieves. Petroleum spirit refers to the fraction with distillation range 30–70 °C. Amino acid amide hydrochlorides were purchased from Kokusan Chemical Works. α-Chymotrypsin (type II, *ex* bovine pancreas) was purchased from Sigma and had a specific activity of 48 units per mg solid with Bz-Tyr-OEt. It was immobilised by dissolution in pH 7.8 Tris buffer, mixing with Celite No. 535 (Johns-Mansville Co.), and lyophilising as described before.[2] Elemental analysis data for all new compounds are available as Supplementary material. †

Preparation of *N*-Z-amino acid carbamoylmethyl esters

The following Cam esters of *N*-Z-amino acids were prepared *via* the reaction of their Cs salts with 2-chloroacetamide in DMF at 60 °C overnight as described previously for the preparation of Z-(*S*)-Ala-OCam.[3b] The crude products were purified by recrystallisation from EtOAc–petroleum spirit (60–86% yield).

Z-(*RS*)-Phe(2F)-OCam. Mp 121.5–122.5 °C; δ_H (DMSO-d_6) 2.84–3.27 (2H, d of AB q, *J* 14.1, 10.8 and 4.2), 4.38–4.44 (1H, m), 4.45 (2H, s), 4.95 (2H, s), 7.07–7.32 (10H, m), 7.39 (1H, br), 7.90 (1H, d, *J* 8.7).

Z-(*RS*)-Phe(3F)-OCam. Mp 107.5–108.5 °C; δ_H (DMSO-d_6) 2.83–3.22 (2H, d of AB q, *J* 14.1, 10.8 and 4.2), 4.39–4.45 (1H, m), 4.46 (2H, s), 4.96 (2H, s), 7.02–7.37 (10H, m), 7.43 (1H, br), 7.90 (1H, d, *J* 8.7).

Z-(*RS*)-Phe(4F)-OCam. Mp 119–120.5 °C; δ_H (DMSO-d_6) 2.80–3.18 (2H, d of AB q, *J* 13.8, 10.8 and 4.2), 4.34–4.44 (1H, m), 4.46 (2H, s), 4.96 (2H, s), 7.05–7.36 (10H, m), 7.42 (1H, br), 7.88 (1H, d, *J* 8.4).

Z-(*RS*)-Phe(2Cl)-OCam. Mp 148.5–149 °C; δ_H (DMSO-d_6) 2.90–3.37 (2H, d of AB q, *J* 13.8, 10.8 and 4.2), 4.43–4.51 (1H, m), 4.47 (2H, s), 4.94 (2H, s), 7.20–7.43 (11H, m), 7.94 (1H, d, *J* 8.7).

Z-(*RS*)-Phe(3Cl)-OCam. Mp 83.5–84.5 °C; δ_H (DMSO-d_6)

2.79–3.21 (2H, d of AB q, *J* 13.8, 11.1 and 4.2), 4.37–4.44 (1H, m), 4.46 (2H, s), 4.95 (2H, s), 7.20–7.36 (10H, m), 7.43 (1H, br), 7.89 (1H, d, *J* 8.7).

Z-(*RS*)-Phe(4Cl)-OCam. Mp 118.5–119.5 °C; δ_H (DMSO-d_6) 2.80–3.19 (2H, d of AB q, *J* 13.8, 11.1 and 4.2), 4.38–4.44 (1H, m), 4.47 (2H, s), 4.96 (2H, s), 7.22–7.34 (10H, m), 7.42 (1H, br), 7.87 (1H, d, *J* 8.4).

Z-(*RS*)-Phe(2Br)-OCam. Mp 164–165 °C; δ_H (DMSO-d_6) 2.92–3.33 (2H, d of AB q, *J* 14.1, 11.1 and 4.2), 4.44–4.53 (1H, m), 4.47 (2H, s), 4.95 (2H, s), 7.15–7.36 (10H, m), 7.58 (1H, d, *J* 8.1), 7.94 (1H, d, *J* 8.7).

Z-(*RS*)-Phe(3Br)-OCam. Mp 92.5–93 °C; δ_H (DMSO-d_6) 2.78–3.19 (2H, d of AB q, *J* 13.8, 11.1 and 4.2), 4.37–4.43 (1H, m), 4.46 (2H, s), 4.96 (2H, s), 7.20–7.43 (10H, m), 7.50 (1H, s), 7.89 (1H, d, *J* 8.4).

Z-(*RS*)-Phe(4Br)-OCam. Mp 122–123 °C; δ_H (DMSO-d_6) 2.76–3.17 (2H, d of AB q, *J* 13.8, 10.8 and 4.2), 4.34–4.42 (1H, m), 4.45 (2H, s), 4.95 (2H, s), 7.19–7.48 (11H, m), 7.87 (1H, d, *J* 8.4).

Z-(*S*)-Phe-OCam. Mp 88–89 °C; $[\alpha]_D^{25}$ −40.0 (*c* 1.0, DMF) {lit.,[8] mp 88.5–89.5 °C; $[\alpha]_D^{21.5}$ −38.5 (*c* 1.0, DMF)}; δ_H (DMSO-d_6) 2.80–3.18 (2H, d of AB q, *J* 13.8, 11.1 and 4.5), 4.34–4.42 (1H, m), 4.45 (2H, s), 4.95 (2H, s), 7.18–7.35 (11H, m), 7.39 (1H, br), 7.87 (1H, d, *J* 8.4).

Preparation of *N*-Z-(*RS*)-2-bromophenylalanine esters

The following esters were prepared through the reaction of Z-(*RS*)-Phe(2Br) (830 mg, 2.2 mmol) with the corresponding alcohol (2.1 mmol) in the presence of DMAP (135 mg, 1.1 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (460 mg, 2.4 mmol) in dichloromethane (DCM) (8 ml) according to the procedure of Dhaon *et al.*[9] The crude products were purified by recrystallisation from EtOAc–petroleum spirit (60–72% yield). The physical properties of Z-(*RS*)-Phe(2Br)-OTfe were described previously.[2b]

Z-(*RS*)-Phe(2Br)-$OCH_2CF_2CF_3$. Mp 82.5–83.5 °C; δ_H ($CDCl_3$) 3.11–3.40 (2H, d of AB q, *J* 14.1, 9.0 and 5.7), 4.58 (2H, t, *J* 13.2), 4.75–4.83 (1H, m), 5.06 (2H, s), 5.24 (1H, br d, *J* 8.4), 7.10–7.57 (9H, m).

Z-(*RS*)-Phe(2Br)-OCH_2CH_2Cl. Mp 54.5–56 °C; δ_H ($CDCl_3$) 3.14–3.39 (2H, d of AB q, *J* 13.8, 8.1 and 5.7), 3.61 (2H, t, *J* 5.7), 4.36 (2H, t, *J* 5.7), 4.71–4.78 (1H, q-like), 5.06 (2H, s), 5.30 (1H, br d, *J* ≈8), 7.08–7.56 (9H, m).

Z-(*RS*)-Phe(2Br)-OCH_2CCl_3. Mp 53–55 °C; δ_H ($CDCl_3$) 3.11–3.49 (2H, d of AB q, *J* 13.8, 8.7 and 5.1), 4.72–4.84 (2H, AB q, *J* 11.7), 4.78–4.89 (1H, m), 5.05 (2H, s), 5.28 (1H, br d, *J* ≈7), 7.09–7.57 (9H, m).

Z-(*RS*)-Phe(2Br)-$ON{=}C(CH_3)_2$. Mp 71.5–73 °C; δ_H ($CDCl_3$) 1.81 (3H, s), 2.02 (3H, s), 3.15–3.37 (2H, d of AB q, *J* 13.8, 8.1 and 6.6), 4.83–4.90 (1H, q-like), 5.06 (2H, s), 5.42 (1H, br d, *J* 8.4), 7.07–7.56 (9H, m).

Preparation of authentic dipeptides

The authentic samples of *N*-protected dipeptide amides, Z-(*S*)-Ala-Xbb-NH_2 [Xbb = Gly, (*S*)-Ala, (*S*)-Val, (*S*)-Met or (*S*)-Phe], were prepared through the coupling of Z-(*S*)-Ala and Xbb-NH_2·HCl [in the presence of an equimolar amount of triethylamine (TEA)] by the EDC–HOBT method in DMF as described previously for the preparation of Z-(*S*)-Ala-(*S*)-Leu-NH_2.[3b] As Z-(*S*)-Ala-Gly-NH_2 and Z-(*S*)-Ala-(*S*)-Ala-NH_2 were highly water-soluble, the washings were reduced to 1/5 of the usual quantity during the work-up. Z-(*S*)-Ala-Gly-NH_2: mp 118–119 °C (from aq. MeOH); $[\alpha]_D^{25}$ +7.5 (*c* 1.0, DMF) {lit.,[10] mp 119–121 °C; $[\alpha]_D^{23}$ +4.8 (*c* 2, DMF)}. Z-(*S*)-Ala-(*S*)-Val-NH_2: mp 243–246 °C (from MeOH); $[\alpha]_D^{25}$ +12.8 (*c* 1.0, DMF) (lit.,[11] mp 239–241 °C). Z-(*S*)-Ala-(*S*)-Phe-NH_2: mp 209–210 °C (from aq. MeOH); $[\alpha]_D^{25}$ −19.9 (*c* 1.0, DMF) (lit.,[11] mp 199–201 °C).

Z-(*S*)-Ala-(*S*)-Ala-NH_2. 50% yield; mp 224–225 °C (from EtOAc); $[\alpha]_D^{25}$ −5.4 (*c* 1.0, DMF); δ_H (DMSO-d_6) 1.18 (6H, d, *J* 7.2), 4.02 (1H, apparent quintet, *J* ≈7), 4.17 (1H, apparent quintet, *J* ≈7), 4.96–5.05 (2H, AB q, *J* 12.6), 7.00 (1H, s), 7.28 (1H, s), 7.29–7.38 (5H, m), 7.48 (1H, d, *J* 7.2), 7.84 (1H, d, *J* 7.2).

Z-(*S*)-Ala-(*S*)-Met-NH_2. 53% yield; mp 195.5–196 °C (from MeOH); $[\alpha]_D^{25}$ −7.9 (*c* 1.0, DMF); δ_H (DMSO-d_6) 1.19 (3H, d, *J* 7.2), 1.70–2.01 (2H, m), 2.01 (3H, s), 2.37–2.44 (2H, m), 3.98–4.08 (1H, quintet-like), 4.22–4.29 (1H, m), 5.00 (2H, s), 7.07 (1H, s), 7.30–7.34 (6H, m), 7.50 (1H, d, *J* 7.5), 7.88 (1H, d, *J* 7.8).

Peptide synthesis mediated by immobilised α-chymotrypsin

A mixture of an *N*-Z-amino acid ester [0.05 mmol for the (*S*)-amino acid derivatives or 0.1 mmol for the (*RS*)-amino acid derivatives], an amino acid amide hydrochloride (0.2 mmol), TEA (28 μl, 0.2 mmol) and the immobilized enzyme (150 mg, corresponding to 4.7 mg of α-chymotrypsin) was incubated with shaking (180 strokes min^{-1}) in a solvent composed of 2 ml of acetonitrile and 83 μl of 0.05 M Tris buffer (pH 7.8) at 30 °C. The amounts of the donor ester, desired peptide and hydrolysis product of the donor ester were determined by HPLC analysis on a GL Sciences PU-610 liquid chromatograph under the following conditions: column, Inertsil ODS 3 (5 μm; 1.5 mm id × 150 mm, GL Sciences); mobile phase, 30 or 35% aq. acetonitrile containing H_3PO_4 (0.01 M) or 55–65% aq. MeOH containing H_3PO_4 (0.01 M); flow rate, 0.1 ml min^{-1}; column temperature, 30 or 40 °C; detection, at 254 nm on a GL Sciences UV-620 variable-wavelength UV monitor; data acquisition and processing, Shimadzu C-R6A data processor. The diastereomers (*S*-*S* and *R*-*S*) of the resulting peptides were also separated on the same column by regulating the fraction of MeOH in the mobile phase (aq. MeOH). The HPLC separations of compounds relevant to some α-chymotrypsin-catalysed couplings are available as Supplementary material. †

An aliquot of the reaction mixture from Z-(*RS*)-Phe(2Br)-OCam + (*S*)-Leu-NH_2 was treated with an ethereal solution of diazomethane and the resulting ester Z-Phe(2Br)-OMe was analysed on a Chiralcel OD column (4.6 mm id × 250 mm, Daicel Chemical Industries; eluent, hexane–propan-2-ol) to determine its enantiomeric purity as described before.[5]

Preparation of authentic *N*-[*N*-Z-(*S*)-phenylalanyl]amines

The preparation of *N*-[Z-(*S*)-Phe]-(*S*)-1-(1-naphthyl)ethylamine is described as a typical example. To a stirred solution of Z-(*S*)-Phe (600 mg, 2.0 mmol), (*S*)-1-(1-naphthyl)ethylamine (340 mg, 2.0 mmol) and HOBT (270 mg, 2.0 mmol) in DCM (3 ml) was added EDC·HCl (380 mg, 2.0 mmol) under ice-cooling. After stirring of the mixture at this temperature for 3 h and then at room temperature for 3 days, the solvent was removed *in vacuo*. The residue was partitioned between EtOAc and water, and the organic layer was washed successively with 1 M HCl, water, 1 M aq. $NaHCO_3$, and brine, and dried over Na_2SO_4. Evaporation of the solvent *in vacuo* afforded crystals,

which were recrystallised from EtOAc–hexane; 77% yield; mp 183–185 °C; $[\alpha]_D^{25}$ +6.7 (*c* 1.0, DMF); δ_H ($CDCl_3$) 1.55 (3H, d, *J* 6.9), 2.89–3.09 (2H, d of AB q, *J* 13.8, 8.4 and 6.0), 4.31–4.38 (1H, q-like), 5.04 (2H, apparent s), 5.47 (1H, br d), 5.81–5.91 (1H, quintet-like, *J* 7.5), 6.03 (1H, br d), 6.97–8.05 (17H, m).

***N*-[Z-(*S*)-Phe]-(*S*)-1-phenylethylamine.** 79% yield; mp 186–188 °C; $[\alpha]_D^{25}$ −18.7 (*c* 1.0, DMF); δ_H ($CDCl_3$) 1.38 (3H, d, *J* 6.9), 2.93–3.11 (2H, d of AB q, *J* 13.6, 8.0 and 6.0), 4.31–4.40 (1H, q-like), 4.97–5.07 (1H, quintet-like), 5.08 (2H, apparent s), 5.40 (1H, br d), 5.88 (1H, br d), 7.07–7.33 (15H, m).

***N*-[Z-(*S*)-Phe]-(*R*)-1-methyl-3-phenylpropylamine.** 83% yield; mp 162–163 °C; $[\alpha]_D^{25}$ −14.8 (*c* 1.0, DMF); δ_H ($CDCl_3$) 0.95 (3H, d, *J* 6.9), 1.57–1.69 (2H, m), 2.53 (2H, t, *J* 7.8), 2.93–3.15 (2H, d of AB q, *J* 13.5, 8.1 and 6.0), 3.94 (1H, septet-like, *J* ≈7), 4.26 (1H, q-like, *J* ≈7), 5.09 (2H, s), 5.24 (1H, d, *J* 8.7), 5.29 (1H, br), 7.09–7.37 (15H, m).

The mixtures of diastereomers (*S*-*S* + *S*-*R*) of *N*-[Z-(*S*)-Phe]-amines were also prepared through the coupling of Z-(*S*)-Phe with(*RS*)-1-phenylethylamine, (*RS*)-1-(1-naphthyl)ethylamine or (*RS*)-1-methyl-3-phenylpropylamine by the EDC–HOBT method in DCM in the same manner as above and purified by recrystallisation from EtOAc–hexane. Each sample thus prepared showed only two peaks corresponding to both the diastereomers on normal-phase HPLC using the chiral column mentioned below. The slower eluting diastereomer proved to be *S*-*S* by comparison with the authentic sample.

Reactions of *N*-Z-(*S*)-phenylalanine esters with homochiral amines mediated by α-chymotrypsin

The reaction of Z-(*S*)-Phe-OTfe with (*S*)-1-phenylethylamine is described as a typical example. To a solution of Z-(*S*)-Phe-OTfe (0.05 mmol) and (*S*)-1-phenylethylamine (0.2 mmol) in acetonitrile (2 ml) was added the immobilised α-chymotrypsin on Celite (150 mg), followed by 31 μl of pH 7.8 Tris buffer (water content, 1.5% by volume). The mixture was incubated at 30 °C with shaking (180 strokes min^{-1}). The amounts of the donor ester, desired amide and Z-(*S*)-Phe in the reaction mixture were determined by HPLC analysis on a Shimadzu LC-10AS liquid chromatograph under the following conditions: column, Cosmosil $5C_{18}$ (4.6 mm id × 150 mm, Nacalai Tesque); mobile phase, 60% aq. MeOH containing H_3PO_4 (0.01 M); flow rate, 1.0 ml min^{-1}; column temperature, 40 °C; detection, at 254 nm on a Shimadzu SPD-10A UV monitor. Aliquots (10 μl each) of the reaction mixture were withdrawn at frequent intervals, diluted with AcOH (100 μl), and then injected onto the column. The retention times (min) of the relevant compounds are as follows: Z-(*S*)-Phe-OCam, 4.9; Z-(*S*)-Phe, 6.3; Z-(*S*)-Phe-OTfe, 17.1; *N*-[Z-(*S*)-Phe]-(*R*)-1-(1-naphthyl)ethylamine, 34.5; *N*-[Z-(*S*)-Phe]-(*S*)-1-(1-naphthyl)ethylamine, 35.4.

Reactions of *N*-Z-(*S*)-phenylalanine carbamoylmethyl ester with racemic amines mediated by α-chymotrypsin

A mixture of Z-(*S*)-Phe-OCam (0.05 mmol), a racemic amine (0.2 mmol) and the immobilised α-chymotrypsin (30 mg) was incubated, with shaking, in the same solvent as above at 30 °C. The progress of the reaction was monitored by reversed-phase HPLC analysis, and the diastereomers of the resulting amide were quantified by normal-phase HPLC on a Chiralpak AD column (4.6 mm id × 250 mm, Daicel Chemical Industries) using a Shimadzu LC-5A liquid chromatograph equipped with an SPD-2A UV monitor. The retention times (min) are as follows [mobile phase, hexane–propan-2-ol (9 : 1, v/v); flow rate, 1.0 ml min^{-1}; column temperature, 40 °C]: *N*-[Z-(*S*)-Phe]-(*R*)-1-phenylethylamine, 10.8; *N*-[Z-(*S*)-Phe]-(*S*)-1-phenylethylamine, 12.3; *N*-[Z-(*S*)-Phe]-(*R*)-1-(1-naphthyl)ethylamine, 13.0; *N*-[Z-(*S*)-Phe]-(*S*)-1-(1-naphthyl)ethylamine, 14.4; *N*-[Z-(*S*)-Phe]-(*R*)-3-amino-1-phenylbutane, 7.9; *N*-[Z-(*S*)-Phe]-(*S*)-3-amino-1-phenylbutane, 9.4.

Acknowledgements

This work was supported in part by a grant from the Hirao Taro Foundation of the Konan University Association for Academic Research.

References

1 For reviews, see: (*a*) K. Faber, *Biotransformations in Organic Chemistry*, Springer-Verlag, Berlin, 4th edn., 2000, p. 370; (*b*) H.-D. Jakubke, in *Enzyme Catalysis in Organic Synthesis*, ed. K. Drauz and H. Waldmann, VCH, Weinheim, 1995, B.2.5, p. 431; (*c*) C.-H. Wong and G. M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Pergamon, Oxford, 1994, p. 46.
2 (*a*) T. Miyazawa, S. Nakajo, M. Nishikawa, K. Imagawa, R. Yanagihara and T. Yamada, *J. Chem. Soc., Perkin Trans. 1*, 1996, 2867; (*b*) T. Miyazawa, S. Nakajo, M. Nishikawa, K. Hamahara, K. Imagawa, E. Ensatsu, R. Yanagihara and T. Yamada, *J. Chem. Soc., Perkin Trans. 1*, 2001, 82.
3 (*a*) T. Miyazawa, K. Tanaka, E. Ensatsu, R. Yanagihara and T. Yamada, *Tetrahedron Lett.*, 1998, **39**, 997; (*b*) T. Miyazawa, K. Tanaka, E. Ensatsu, R. Yanagihara and T. Yamada, *J. Chem. Soc., Perkin Trans. 1*, 2001, 87.
4 Preliminary communication: T. Miyazawa, N. Yabuuchi, R. Yanagihara and T. Yamada, *Biotechnol. Lett.*, 1999, **21**, 725.
5 T. Miyazawa, Y. Shindo, T. Yamada and S. Kuwata, *Anal. Lett.*, 1993, **26**, 457.
6 N. Chinsky, A. L. Margolin and A. L. Klibanov, *J. Am. Chem. Soc.*, 1989, **111**, 386.
7 C.-S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.*, 1982, **104**, 7294.
8 F. H. C. Stewart, *Aust. J. Chem.*, 1965, **18**, 1089.
9 M. K. Dhaon, R. K. Olsen and K. Ramasamy, *J. Org. Chem.*, 1982, **47**, 1962.
10 P. A. Jaquenoud, *Helv. Chim. Acta*, 1965, **48**, 1899.
11 M. Lobell and M. P. Schneider, *J. Chem. Soc., Perkin Trans. 1*, 1998, 319.