



Expanding the utility of proteases in synthesis: broadening the substrate acceptance in non-coded amide bond formation using chemically modified mutants of subtilisin

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Abstract—The strategy of combined site directed mutagenesis and chemical modification creates chemically modified mutants (CMMs) with greatly broadened substrate specificities. We have previously reported that the CMMs of subtilisin *Bacillus lentus* (SBL) are efficient catalysts for the coupling of both L- and D-amino acids. We now report that these powerful catalysts also allow amide bond formation between a variety of non-coded carboxylic acids, including β -alanine and β -amino homologues of phenylalanine, with both L- and D-amino acid nucleophiles. As a guide to enzyme efficiency, a hydrolysis assay indicating pH change has been employed. CMMs selected by this screen furnished higher yields of coupling products compared to the wild-type enzyme (WT). Furthermore, both WT and CMM enzymes allow highly stereoselective aminolysis of a *meso* diester with an amino acid amine. These results highlight the utility of CMMs in the efficient formation of non-coded amides as potential peptide isosteres. © 2001 Published by Elsevier Science Ltd.

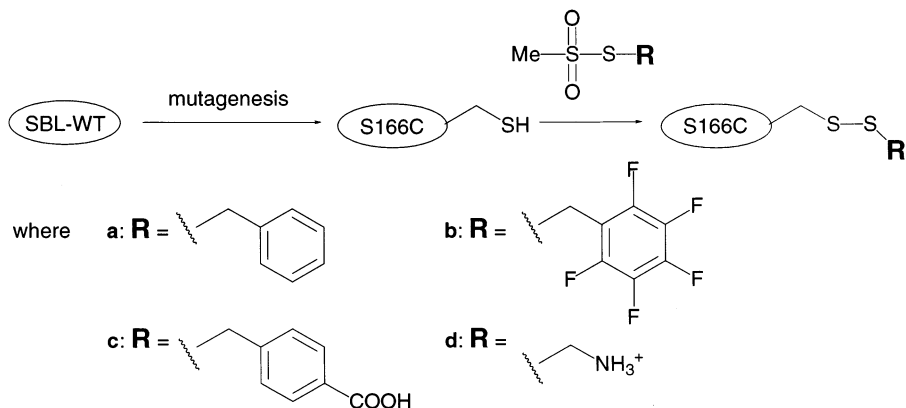
1. Introduction

The use of enzymes as biocatalysts for transformations in both forward and reverse hydrolyses is now an attractive and widely accepted synthetic method.^{1,2} Hydrolytic enzymes operate with high efficiency under mild conditions that minimise undesirable side reactions and the need for extensive protection regimes. In particular, serine proteases allow the ready construction of a variety of acyl linkages including peptide ligation, esterification and amide bond formation. For example, their use allows racemisation-free peptide ligation without the need for side chain protection. However, despite these demonstrated advantages their natural chemo-, regio- and stereospecificities can sometimes confine their use in synthesis to a limited range of substrates. Several strategies have been employed in an attempt to overcome these restrictions. As alternative catalysts with typically broader specificities, lipases have been employed but their lower activities in aqueous solvents negate this broader substrate specificity with a disad-

vantageous solvent specificity.³ Alternatively, substrate engineering or mimicry^{4–6} has been employed, but by definition requires the synthesis of specifically designed substrates that typically are not readily available. Impressive protein engineering methods, such as site directed mutagenesis^{7,8} or forced evolution^{9–11} have also successfully altered the specificities of proteases for use in synthesis. However, these methods do not routinely allow the creation of large numbers of catalysts in sufficient quantities for synthesis.

To overcome these problems, biocatalysts with broad specificities for amide synthesis that are readily created, that may be used in simple solvent systems and that employ standard readily available substrates are required. Using the serine protease subtilisin *Bacillus lentus* (SBL), we have successfully exploited a combined site directed mutagenesis and chemical modification approach for enhancing enzyme activity and tailoring enzyme specificity (Scheme 1).^{12–23} This strategy involves the introduction of a cysteine residue through site directed mutagenesis and the reaction of its thiol side chain with chemoselective methanethiosulfonate reagents^{24,25} to produce chemically modified mutant

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Scheme 1. The creation of CMM biocatalysts for use in amide bond formation.

(CMM) enzymes (Scheme 1). Since wild-type (WT) SBL contains no natural cysteines the site of modification corresponds only to the site of mutagenesis. The ease of this method is such that large arrays of different CMM biocatalysts can be created and screened in a short space of time.²⁶ In this way, groups intended to influence the specificity of SBL were introduced to position 166 at the base of the primary specificity determining S_1 ²⁷ pocket through the creation of a wide range of S166C-CMMs.

Such modification of SBL provided CMMs that display broadened structural as well as stereospecificities when used as synthetic catalysts. Recently, we reported the improved synthetic utility of both CMM-catalysed transesterifications²⁸ and peptide ligations.²⁹ These reactions also allowed successful incorporation of D-amino acid esters as acyl donors and α -branched amino acid amides as acyl acceptors. This expanded specificity prompted us to investigate further the application of CMMs in other challenging coupling reactions with non-coded acids. We now report the high synthetic utility of CMMs of SBL in the coupling of representative esters derived from β -amino acids and chiral carboxylic acids as unnatural acyl donors with both L- and D-amino acid acyl acceptors.

2. Results and discussion

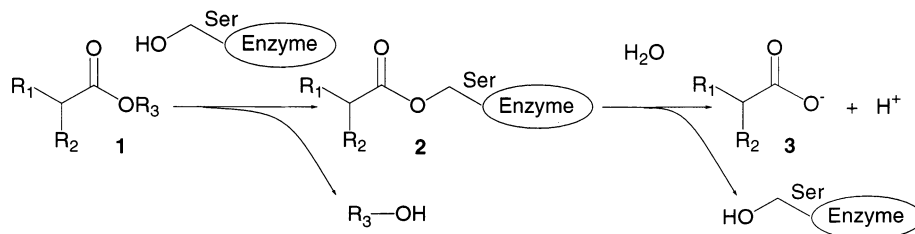
2.1. pH Indicator-based screening of CMMs

A fast screening method was developed to identify enzymes suitable for amide formation reactions from our CMM library. Monitoring the progress of enzyme-

catalysed reactions by assaying the pH change during the reaction course has been used by several research groups³⁰ and was chosen as a suitable method for our system. The assay developed is based on the mechanism of serine protease-catalysed ester hydrolysis as shown in Scheme 2.

Productive binding of the acyl donor **1** leads to the formation of the acyl-enzyme intermediate **2**. Deacylation of **2** in aqueous media leads to hydrolysis and generates **3** and a hydronium ion, which may be detected using a pH indicator. It is the aminolysis of **2** that, in the presence of an amine acyl acceptor, leads to amide bond formation. Therefore, an assay that measures formation of **3** gives a valuable indication of which CMMs successfully form the acyl-enzyme intermediate **2** with a given acyl donor **1**. The reaction conditions for the assay were chosen in such a way that the degree of colour change of the indicator system was a qualitative indication of the concentration of **3** that was formed. Therefore, the pH of the buffering system was chosen to match the pK_a of the indicator. From a variety of indicators tested, bromothymol blue [$pK_a = 7.1$, pH range 6.0 (yellow)–7.6 (blue)] in phosphate buffer (pH 7.1) mixed with DMF was selected.

Using this screening system with representative acyl donors **1a–m** (Scheme 3), CMMs S166C-S-a–d (Scheme 1) were identified. Their selection corresponded well with the results of previous detailed analyses of amidase and esterase activities, using standard substrates suc-AAPF-*p*-NA and suc-AAPF-SBn, respectively, that by virtue of their high esterase activity to amidase activity ratios (E/A) had identified them as excellent candidates for efficient amide bond formation.^{20,26}

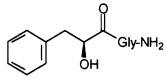
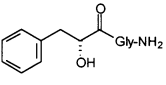
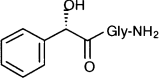
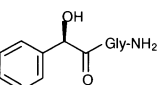
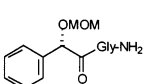
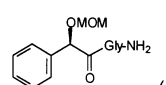
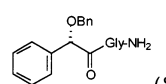
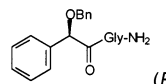
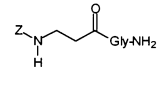
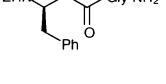
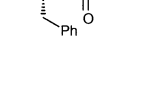
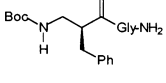
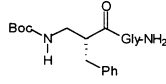


Scheme 2. The mechanism of serine protease-catalysed ester hydrolysis.

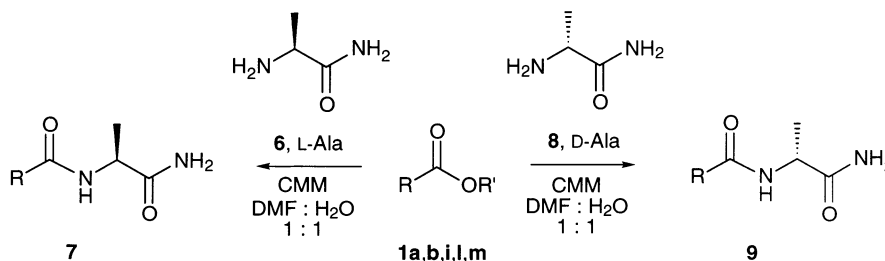
such are not only potentially useful in vivo peptidomimetics, but are also testing substrates for protease-catalysed peptide ligation.^{39,40} SBL-catalysed coupling

of acyl donor *Z*- β -Ala-OBn **1i** successfully formed amide **5i**, containing naturally occurring but non-coded amino acid β -alanine, in moderate yields. CMMs

Table 1. Coupling of non-coded chiral carboxylic acid or β -amino acid esters **1** with glycylamide **4** catalysed by SBL-WT and SBL-CMMs S166C-S-a-d

Acyl Donor	Product	Yield [%] with				
		WT	S166C-S-a	S166C-S-b	S166C-S-c	S166C-S-d
1a	 (<i>S</i>)- 5a ^a	30	59	69	50	50
1b	 (<i>R</i>)- 5b ^b	20	48	60	64	51
1c	 (<i>S</i>)- 5c ^e	nr	nr	nr	nr	nr
1d	 (<i>R</i>)- 5d ^e	nr	nr	nr	nr	nr
1e	 (<i>S</i>)- 5e ^d	15	22	21	52	63
1f	 (<i>R</i>)- 5f ^e	12	21	27	28	33
1g	 (<i>S</i>)- 5g ^c	7	12	15	17	14
1h	 (<i>R</i>)- 5h ^d	trace	15	15	13	18
1i	 5i ^a	39	49	33	23	45
1j	 (<i>R</i>)- 5j ^e	nr	nr	nr	nr	nr
1k	 (<i>S</i>)- 5k ^e	nr	nr	nr	nr	nr
1l	 (<i>S</i>)- 5l ^a	51	71	79	61	60
1m	 (<i>R</i>)- 5m ^b	9	32	36	27	40

nr = no reaction of acyl donor, ^a reaction for 24 h., ^b reaction for 48 h., ^c reaction for 96 h., ^d reaction for 120 h., ^e reaction for 168 h.



Scheme 4. CMM-catalysed coupling of D- and L-alaninamide with a variety of non-coded carboxylic acid esters **1**.

S166C-SBn (**-a**) and S166C-SEtNH₃⁺ (**-d**) gave improved yields of 49 and 45%, respectively, when used as catalysts compared with SBL-WT (39%). Despite these positive indications for the coupling of β -amino acids, the use of acyl donors with an additional β -benzyl substituent as phenylalanine homologues proved dramatically detrimental and no reaction of (*R*)- or (*S*)-*Z*- β -benzyl- β -Ala-OEt **1j** and **1k**⁴¹ was observed even after 5 days. The substituent at the β -position of compounds **1j** and **1k** seems to be responsible for the failure of this reaction. Corresponding α -benzyl substituted esters **1l**, and **1m**, which were synthesised by slight modification of a literature procedure,⁴² proved to be good substrates. SBL-WT shows a strong stereospecificity for (*S*)-Boc- α -benzyl- β -Ala-OBn **1l** (51% yield of **5l**) over (*R*)-Boc- α -benzyl- β -Ala-OBn **1m** (9% yield of **5m**). The use of CMMs greatly enhances the synthetic utilities of these reactions and S166C-S-**a-d** catalyse the formation of **5l** in good yields of 60–79%. Similarly, the syntheses of **5m** were significantly improved with CMMs as catalysts and up to 40% yield of product was obtained using S166C-SEtNH₃⁺ (**-d**). This four-fold broadening of stereospecificity to accept both (*S*)- and (*R*)-isomers as acyl donors in fair yield closely mirrors that found for L- and D-phenylalanine esters.²⁹

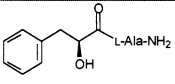
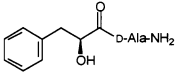
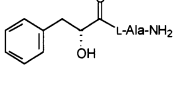
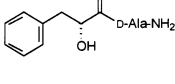
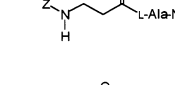
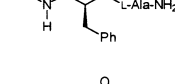
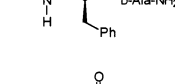
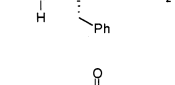
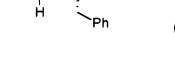
2.3. Coupling of **1** with L- and D-alaninamide: probing the specificity of the S₁' pocket

Having determined that the CMM strategy successfully creates catalysts S166C-S-**a-d** with broadened P₁ specificities in the coupling of non-coded acids **1a-m**, we probed the altered specificities of the S₁' pocket in these same catalysts using α -branched amino acid alaninamide as an acyl acceptor. In addition to probing the ability of these catalysts to accept α -branched P₁ substrates, parallel reactions using both L-**6** and D-**8** alaninamide also tested the P₁ stereochemical preference (Scheme 4 and Table 2). As representative acyl donors **1a**, **1b**, **1i**, **1l** and **1m** were chosen on the basis of superior yields in couplings with glycine.

The results of these coupling reactions are shown in Table 2. In agreement with our previous studies,²⁹ these reactions using Ala-NH₂ **6,8** as the acyl acceptors typically required longer reaction times than for those using Gly-NH₂ **4**. As for the glycine couplings, the WT enzyme accepts both (*S*)-**1a** and (*R*)-**1b**

phenyllactates as acyl donors in coupling reactions with L-Ala-NH₂ **6** with an approximate two-fold stereospecificity for (*S*)- (27% yield of **7a**) over (*R*)- (12% yield of **7b**) that reflects the natural P₁ stereospecificity for L-phenylalanine over D-phenylalanine. However, the stringent P₁ stereospecificity of SBL-WT was demonstrated by the complete absence of coupling product in these reactions using D-Ala-NH₂ as the acyl acceptor with either donor **1a,b**. In contrast, corresponding CMM-catalysed amide formation was dramatically enhanced. In all cases the yields of amides **7a,b** and **9a,b** were greater than those for SBL-WT. Indeed, the specificities of S166C-S-**b,c** were sufficiently broadened that they allowed the synthesis of **7a** from **1a** in excellent yields of 88% that were, in fact, superior to those obtained in glycine couplings of **1a**. Excitingly, S166C-S-**a-d** were also capable of catalysing coupling reactions (which SBL-WT will not) of both (*S*)-**1a** and (*R*)-**1b** acyl donors with D-Ala-NH₂ to give **9a,b**, respectively, in low yields of 14–25%. Reaction of β -amino ester *Z*- β -Ala-OBn **1i** as the acyl donor allowed coupling to L-Ala-NH₂ **6** in good yields that were similar or even, in the case of S166C-S-**b-d**, superior to those found for coupling to glycine **4**. Addition of a benzyl group at the α -carbon of this β -amino acid scaffold gives the more challenging acyl donors (*S*)-**1l** and (*R*)-**1m** Boc- α -benzyl- β -Ala-OBn. Little or no coupling was observed in the reaction of these β -alaninyl esters **1l**, and **1m** with D-Ala-NH₂ **8** in the presence of SBL-WT, which is in line with the strict P₁ stereospecificity observed for the SBL-WT catalysed coupling of phenyllactates, although low yields of **7l** and **7m** (20 and 7%, respectively) were obtained with L-Ala-NH₂ **6**. Gratifyingly, in all cases S166C-S-**a-d** were superior catalysts for these reactions. Reaction of **1l,m** with L-Ala-NH₂ **6** gave yields only slightly lower than those obtained for glycine **4** as an acyl acceptor (64% yield of **7l**, using S166C-S-**b** as a catalyst and 20% of **7m**, using S166C-S-**d**). Moreover, the broadening of the P₁ stereospecificity of these S166C CMMs was further demonstrated by the successful coupling of D-Ala-NH₂ **8** as an acyl acceptor with **1l,m** to give **9l** in 41% yield (using S166C-S-**b** as a catalyst) and **9m** in 13% yield (using S166C-S-**c** as a catalyst). This demonstrates that these catalysts tolerate not only α -branched acyl acceptors in their P₁ subsites but also those with either (*R*)- or (*S*)-configurations at α -stereocentres.

Table 2. Coupling of chiral carboxylic acids or β -amino acids **1** with alaninamide catalysed by SBL-WT and SBL-CMMs S166C-S-a-d

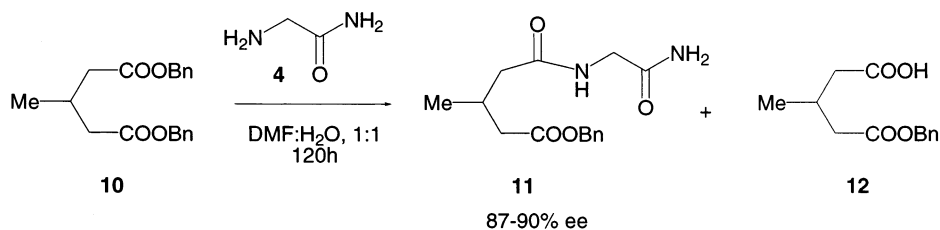
Acyl Donor	Acyl Acceptor	Product	Yield [%] with				
			WT	S166C-S-a	S166C-S-b	S166C-S-c	S166C-S-d
1a	6, L-Ala-NH ₂	 (S,S)- 7a ^b	27	66	88	88	72
1a	8, D-Ala-NH ₂	 (S,R)- 9a ^b	0	16	25	21	22
1b	6, L-Ala-NH ₂	 (R,S)- 7b ^b	12	36	34	40	33
1b	8, D-Ala-NH ₂	 (R,R)- 9b ^d	0	14	22	16	16
1i	6, L-Ala-NH ₂	 7i ^a	38	44	47	52	62
1l	6, L-Ala-NH ₂	 (S,S)- 7l ^b	20	35	64	52	50
1l	8, D-Ala-NH ₂	 (S,R)- 9l ^c	trace ^e	20	41	35	37
1m	6, L-Ala-NH ₂	 (R,S)- 7m ^b	7	13	13	15	20
1m	8, D-Ala-NH ₂	 (R,R)- 9m ^d	0	10	8	13	12

^a reaction for 24 h., ^b reaction for 48 h., ^c reaction for 72 h., ^d reaction for 96 h., ^e detected by TLC.

2.4. Probing the selectivity of CMMs in the aminolysis of a prochiral non-coded diester

Following the successful coupling of amino acid acyl acceptors **4,6,8** with a variety of non-coded acid esters described in Sections 2.2 and 2.3, we investigated the potential of CMMs of SBL as catalysts for the selective amidation of a prochiral *meso*-diester substrate **10**

(Scheme 5). Such reactions offer the potential for very efficient generation of optically active materials and mono amino acid-glutaramides such as **11** have shown potent activity as important endopeptidase inhibitors.⁴³ As Table 3 shows, in all cases SBL-catalysed coupling of the dibenzyl ester of glutaric acid **10** as the acyl donor and glycineamide **4** as the acyl acceptor proceeded with good stereoselectivity to yield monoamide **11** in



Scheme 5. CMM-catalysed enantiotopic aminolysis of prochiral *meso*-diester **10**.

Table 3. CMM-catalysed coupling reactions of **10** with glycinamide **4**

Enzymes	% Yield of 11	% E.e. of 11 ^a	% Yield of 12
WT	6	90	Not determined
S166C-S-b	11	91	22
S166C-S-c	10	87	16

^a Column: Chiralcel OJ, hexane/*i*-PrOH=75/25, flow rate: 0.5 mL/min.

high enantiomeric excess (87–91%) as determined by chiral HPLC. Although rather slow and low in yield, these first examples of selective amidation of prochiral *meso*-diesters by proteases clearly demonstrate the potential of such enzyme catalysed reactions. Further studies to improve the yield of **11** by enhancing conversion levels⁴⁴ and reducing competing hydrolysis to mono-ester **12** are in progress.

3. Conclusions

CMMs S166C-S-a–d of the serine protease subtilisin *Bacillus lentus* (SBL) are powerful catalysts in the amidation of important non-coded chiral carboxylic acids and amino acids. The modification of position 166 at the base of the primary specificity determining S₁ pocket in these enzymes broadens not only P₁ structural and stereospecificities, but also P₁' structural and stereospecificities. They allowed the coupling of phenyl-lactates, hydroxyl-protected mandelates and β-amino acids. Highest yields were obtained for α-benzylcarboxylate acyl donors **1a,b,l,m**, consistent with the natural P₁ preference of SBL for phenylalanine. Although much broadened over SBL-WT, the stereochemical preference of these CMMs typically favours the configuration of the α-benzyl stereocentre in these donors that is homochiral with that of L-phenylalanine. The dramatically broadened P₁' specificity has allowed the use of single CMMs to construct all four possible stereoisomers of both phenyllactylalaninamide (**7a,b** and **9a,b**) and α-benzyl-β-alaninyl-alaninamide (**7l,m** and **9l,m**) as examples of potentially important non-coded peptide isosteres. Furthermore, although low in yield, SBL-WT and SBL-CMMs catalysed the first examples of a stereoselective amidation of a prochiral *meso*-diester with an amino acid.

4. Experimental

4.1. General methods

WT-subtilisin *Bacillus lentus* and mutant enzyme S166C were purified and prepared as previously reported.^{12,13,20} Protected amino acids were purchased from Sigma or Bachem and were used as received. (*R*)-**1j** and (*S*)-3-(benzyloxycarbonylamino)-4-phenylbutanoate, **1k**, were prepared according to literature methods.⁴¹ All solvents were reagent grade and distilled prior to use. Thin-layer chromatography analy-

sis and purification were performed on pre-coated Merck silica gel plates (60 F-254, 250 μm) visualised with UV light or iodine. ¹H and ¹³C NMR spectra were recorded on a Varian Gemini 200 (200 MHz for ¹H and 50.3 MHz for ¹³C) or Unity 400 (400 MHz for ¹H and 100 MHz for ¹³C) spectrometer and chemical shifts are given in ppm (δ) using CDCl₃, acetone-*d*₆ or DMSO-*d*₆ as an internal standard. High resolution mass spectra (HRMS) were recorded using Micromass ZAB-SE (FAB⁺). Optical rotations were measured with a Perkin–Elmer 243B polarimeter.

4.2. CMM screen using pH indicator

The screen was carried out on a 96 well plate with an overall volume of 200 μL per well. The assay solutions were prepared by mixing phosphate buffer (45 μL of a 0.02 M solution, pH 7.1), DMF (125–140 μL), substrate (5 μL of a 1 M solution in DMF), and bromothymol blue [45 μL of a 1.9 M solution in phosphate buffer (0.1 M, pH 7.1)] in the well. Enzyme [5–20 μL of a solution in MES-buffer (1–3 mg/mL enzyme, 10 mmol MES, 1 mM CaCl₂, pH 5.8)] was added and all components were carefully mixed. The enzymes were used in the same concentrations and were evaluated qualitatively as good, poor and not working hydrolysis catalysts by comparing the time needed for colour change. The enzyme concentrations necessary for a reasonably fast assay were substrate dependent and had to be tested individually. The concentrations given can be used as a guide. Controls without enzyme were employed for better detection.

4.3. General procedure for the coupling reaction of acyl donors and amino acids as the acyl acceptors catalysed by CMMs

To a solution of acyl donor (0.1 mmol) in DMF (0.4 mL) and water (0.4 mL), glycinamide hydrochloride **4** (0.3 mmol) or L-(**6**) or D-(**8**) alaninamide hydrochloride (0.2 mmol) and Et₃N (0.083–0.125 mL, 0.3–0.4 mmol) were added, followed by addition of a solution of 1 mg of active enzyme (0.0037 mmol, 0.037 equiv.), as determined by titration with PMSF.⁴⁵ The resulting volume was 1.0–1.2 mL. The reaction was left stirring at rt for the period indicated in Tables 1 and 2. In the case of reaction times longer than 48 h, a solution containing 1 mg more of active enzyme as well as an equal volume of DMF were added at 48 h. Although activity decreases over time due to autolysis, variations in rates of autolysis between CMMs are minimal.²⁹ After the reaction had finished, the mixture was extracted with EtOAc and brine and then the organic layer was dried (MgSO₄) and concentrated in vacuo. The residue was purified by preparative TLC (2–5% MeOH in CH₂Cl₂).

4.4. (*S*)-Phenyllactic-Gly-NH₂ **5a**

A mixture of (*S*)-phenyllactic acid (0.4 g, 2.5 mmol), benzyl alcohol (0.27 g, 2.5 mmol) and *p*-TsOH (cat.) in benzene (50 mL) was refluxed for 3 h. The water was removed from the reaction using a Dean–Stark trap.

The reaction was worked up by removing solvent, diluting with CH_2Cl_2 and washing with water and brine. The organic layer was dried (MgSO_4) and the solvent was removed in vacuo. The crude product was purified by column chromatography (gradient elution with EtOAc in hexane) to yield 0.55 g (86%) of (*S*)-benzyl phenyllactate **1a**. ^1H NMR (CDCl_3) δ 2.40 (brs, 1H, OH), 2.99 (dd, $J=4.5$, 14 Hz, 1H, $\text{CHH}'\text{CHOH}$), 3.15 (dd, $J=7$, 14 Hz, 1H, $\text{CHH}'\text{CHOH}$), 4.50 (dd, $J=4.5$, 7 Hz, 1H, CHOH), 5.21 (s, 2H, OCH_2Ph), 7.11–7.52 (m, 10H, 2 \times Ph); ^{13}C (CDCl_3) δ 41.0, 67.9, 71.8, 127.3, 128.9, 129.1, 129.2, 130.5, 135.6, 136.7, 174.5. $[\alpha]_{\text{D}}^{27} = -15.8$ (c 0.89, MeOH) {lit. enantiomer,⁴⁶ $[\alpha]_{\text{D}}^{25} = +13.6$ (c 1, MeOH)}.

The coupling reaction of (*S*)-benzyl phenyllactate **1a** and glycinamide hydrochloride was carried out using the general procedure and **5a** was obtained in the yield as indicated in Table 1. ^1H NMR ($\text{DMSO}-d_6$) δ 2.68 (dd, $J=8$, 13 Hz, 1H, $\text{CHH}'\text{CHOH}$), 2.99 (dd, $J=4$, 13 Hz, 1H, $\text{CHH}'\text{CHOH}$), 3.62 (dd, $J=5$, 14 Hz, 1H, $\text{CONHCHH}'\text{CONH}_2$), 3.70 (dd, $J=7$, 14 Hz, 1H, $\text{CONHCHH}'\text{CONH}_2$), 4.10 (m, 1H, CHOH), 5.74, 7.11 (2 \times brs, 3H, NH_2 , OH), 7.16–7.28 (m, 5H, Ph), 7.95 (m, 1H, NH); ^{13}C (acetone- d_6) δ 41.6, 42.7, 73.8, 127.0, 128.9, 130.5, 139.5, 171.6, 174.3. HRMS (MH^+) calcd for $\text{C}_{11}\text{H}_{14}\text{N}_2\text{O}_3$, 223.1083; found 223.1100. $[\alpha]_{\text{D}}^{26} = +72.1$ (c 0.19, MeOH).

4.5. (*R*)-Phenyllactic-Gly-NH₂ **5b**

(*R*)-Benzyl phenyllactate **1b** was prepared in the same manner as (*S*)-benzyl phenyllactate from (*R*)-phenyllactic acid (96% yield). ^1H and ^{13}C data were identical to (*S*)-benzyl phenyllactate. $[\alpha]_{\text{D}}^{26} = +16.2$ (c 1.0, MeOH) {lit.,⁴⁶ $[\alpha]_{\text{D}}^{25} = +13.6$ (c 1, MeOH)}.

Using the general procedure, **5b** was obtained in the yield as indicated in Table 1. ^1H NMR and ^{13}C data were identical to **5a**. HRMS (MH^+) calcd for $\text{C}_{11}\text{H}_{14}\text{N}_2\text{O}_3$, 223.1083; found 223.1097. $[\alpha]_{\text{D}}^{26} = -72.9$ (c 0.23, MeOH).

4.6. (*S*)-*O*-MOM-Mandelic-Gly-NH₂ **5e**

(*S*)-Benzyl mandelate **1c** was prepared in the same manner as **1a** from (*S*)-mandelic acid (1.52 g, 10 mmol), benzyl alcohol (1.08 g, 10 mmol) and *p*-TsOH (cat.) in benzene (50 mL). The crude product was purified by column chromatography (10% EtOAc in hexane) to obtain pure (*S*)-benzyl mandelate **1c**⁴⁷ (1 g, 41%). ^1H NMR (CDCl_3) δ 3.58 (d, $J=7$ Hz, 1H, OH), 5.14 (dd, $J=12$ Hz, 1H, $\text{OCHH}'\text{Ph}$), 5.24 (d, $J=7$ Hz, 1H, CHOH), 5.25 (d, $J=12$ Hz, 1H, $\text{OCHH}'\text{Ph}$), 7.20–7.45 (m, 10H, 2 \times Ph); ^{13}C (CDCl_3) δ 67.8, 73.1, 126.7, 128.1, 128.5, 128.6, 128.7, 128.8, 135.1, 138.3, 173.6. $[\alpha]_{\text{D}}^{26} = +49.2$ (c 0.455, MeOH) {lit.,⁴⁸ $[\alpha]_{\text{D}}^{23} = +55.7$ (c 1, CHCl_3)}.

To the mixture of **1c** (0.367 g, 1.5 mmol) and *i*-Pr₂NET (0.52 mL, 3 mmol) in CH_2Cl_2 (10 mL) at 0°C, ClCH_2OMe (0.23 mL, 3 mmol) was added. The reaction was then warmed up to rt and was stirred for 24 h.

The mixture was diluted with CH_2Cl_2 and washed with water and brine. Purification by column chromatography (gradient elution with EtOAc in hexane) provides (*S*)-*O*-MOM-benzyl mandelate **1e** (0.32 g, 75%). ^1H NMR (CDCl_3) δ 3.34 (s, 3H, OCH_3), 4.67 (d, $J=12$ Hz, 1H, $\text{OCHH}'\text{O}$), 4.75 (d, $J=12$ Hz, 1H, $\text{OCHH}'\text{O}$), 5.10 (d, $J=12$ Hz, 1H, $\text{OCHH}'\text{Ph}$), 5.17 (d, $J=12$ Hz, 1H, $\text{OCHH}'\text{Ph}$), 5.20 (s, 1H, CHOMOM), 7.18–7.45 (m, 10H, 2 \times Ph); ^{13}C (CDCl_3) δ 56.2, 67.0, 76.9, 95.2, 127.5, 128.0, 128.4, 128.6, 128.8, 128.9, 135.6, 136.1, 170.8. $[\alpha]_{\text{D}}^{26} = +50.0$ (c 1.01, MeOH).

Using the general procedure, **5e** was obtained in the yield indicated in Table 1. ^1H NMR (CDCl_3) δ 3.35 (s, 3H, OCH_3), 3.92 (dd, $J=6$, 12 Hz, 1H, $\text{CONHCHH}'\text{CONH}_2$), 4.00 (dd, $J=4$, 12 Hz, 1H, $\text{CONHCHH}'\text{CONH}_2$), 4.62 (d, $J=12$ Hz, 1H, $\text{OCHH}'\text{O}$), 4.70 (d, $J=12$ Hz, 1H, $\text{OCHH}'\text{O}$), 5.07 (s, 1H, OCHPh), 5.63, 6.41, 7.57 (3 \times brs, NH, NH₂), 7.26–7.40 (m, 5H, Ph); ^{13}C (CDCl_3) δ 42.5, 56.3, 78.0, 94.7, 127.5, 128.8, 128.9, 136.7, 170.9, 171.5. HRMS (MH^+) calcd for $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_4$, 253.1188; found 253.1184. $[\alpha]_{\text{D}}^{24} = +56.7$ (c 1.78, MeOH).

4.7. (*R*)-*O*-MOM-Mandelic-Gly-NH₂ **5f**

(*R*)-Benzyl mandelate **1d** was prepared in the same manner as (*S*)-benzyl mandelate **1c** from (*R*)-mandelic acid (41% yield). ^1H and ^{13}C data were identical to **1c**. $[\alpha]_{\text{D}}^{24} = -49.2$ (c 1.51, MeOH).

(*R*)-*O*-MOM-benzyl mandelate **1f** (75% yield) was prepared in the same manner as (*S*)-*O*-MOM-benzyl mandelate **1e**. ^1H and ^{13}C data were identical to **1e**. $[\alpha]_{\text{D}}^{24} = -49.2$ (c 1.4, MeOH).

Using the general procedure, **5f** was obtained in the yield as indicated in Table 1. ^1H NMR and ^{13}C data were identical to **5e**. HRMS (MH^+) calcd for $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_4$, 253.1188; found 253.1191. $[\alpha]_{\text{D}}^{26} = -57.6$ (c 1.54, MeOH).

4.8. (*S*)-*O*-Bn-Mandelic-Gly-NH₂ **5g**

To a solution of (*S*)-benzyl mandelate **1c** (0.367 g, 1.5 mmol) in THF (20 mL) at 0°C, NaH (80% in oil, 0.023 g, 0.75 mmol) was added. The reaction mixture was stirred for 15 min, then a solution of benzyl bromide (0.18 mL, 1.5 mmol) in THF (5 mL) was added. After stirring at rt for 5 h the reaction was worked up by adding water and extracting with CH_2Cl_2 . The organic layer was washed with brine, dried over MgSO_4 and the solvent was removed in vacuo. The crude product was purified by column chromatography (0.4% EtOAc in hexane) to yield (*S*)-*O*-Bn-benzyl mandelate **1g**⁴⁹ (0.12 g, 49%). ^1H NMR (CDCl_3) δ 4.59 (br s, 2H, OCH_2Ph), 4.97 (s, 1H, OCHPh), 5.12 (d, $J=12$ Hz, 1H, $\text{OCHH}'\text{Ph}$), 5.19 (d, $J=12$ Hz, 1H, $\text{OCHH}'\text{Ph}$), 7.20–7.47 (m, 15H, 3 \times Ph); ^{13}C (CDCl_3) δ 66.9, 71.3, 79.7, 127.6, 128.1, 128.2, 128.4, 128.6, 128.7, 128.8, 128.9, 135.6, 136.3, 137.2, 170.8. $[\alpha]_{\text{D}}^{26} = +19.0$ (c 0.83, MeOH).

Using the general procedure, (*S*)-*O*-Bn-mandelic-Gly-NH₂ **5g** was obtained in the yield as indicated in Table 1. ¹H NMR (CDCl₃) δ 3.92 (br d, 2H, *J*=7 Hz, CH₂CONH₂), 4.43 (d, *J*=11 Hz, 1H, OCHHPh), 4.58 (d, *J*=11 Hz, 1H, OCHHPh), 4.85 (s, 1H, OCHPh), 5.55, 5.99 (brs×2, 1H×2, NH₂), 7.23–7.47 (m, 11H, 2×Ph, NH); ¹³C NMR (CDCl₃) δ 42.7, 71.4, 81.0, 127.3, 128.3, 128.7, 128.8, 128.9, 129.0, 136.7, 136.8, 171.1, 171.6. HRMS (MH⁺) calcd for C₁₇H₁₈N₂O₃, 299.1391; found 299.1391. [α]_D²⁴=+6.8 (*c* 0.78, MeOH).

4.9. (*R*)-*O*-Bn-Mandelic-Gly-NH₂ **5h**

(*R*)-*O*-Bn-Benzyl mandelate **1h** (60% yield) was prepared according to the procedure described for (*S*)-*O*-Bn-benzyl mandelate **1g**. The ¹H and ¹³C NMR were identical to **1g**. [α]_D²⁴=−18.3 (*c* 1.53, MeOH).

Using the general procedure, **5h** was obtained in the yield as indicated in Table 1. The ¹H and ¹³C NMR were identical to the **5g**. HRMS (MH⁺) calcd for C₁₇H₁₈N₂O₃, 299.1391; found 299.1386. [α]_D²⁴=−6.1 (*c* 1.29, MeOH).

4.10. *N*-*Z*-β-Alaninyl-Gly-NH₂ **5i**⁵⁰

To a solution of β-alanine (1.8 g, 20 mmol) in 2N NaOH (10 mL, 20 mmol) at 0°C, benzyl chloroformate (3.16 mL, 22 mmol) and 2N NaOH (11 mL) were added alternatively. After the addition was finished, the reaction was stirred at rt for a further 0.5 h. The mixture was then worked up by washing with ether (3×50 mL). The aqueous layer was acidified with 2.5N HCl and extracted with CH₂Cl₂ (3×50 mL). The combined organic layer was dried (MgSO₄) and the solvent was removed in vacuo. The crude product was used without further purification.

The esterification of *N*-*Z*-β-Ala was carried out according to the procedure used for the synthesis of (*S*)-benzyl phenyllactate **1a**, using *N*-*Z*-β-Ala (2.2 g, 10 mmol), benzyl alcohol (1.18 g, 10 mmol) and *p*-TsOH (cat.) in benzene (100 mL). Purification by column chromatography furnished 2.1 g (67%) of *N*-*Z*-β-Ala-OBn **1i**; mp 22°C [lit.⁵¹ mp 22°C]. ¹H NMR (CDCl₃) δ 2.59 (t, *J*=6 Hz, 2H, CH₂CH₂CO), 3.49 (q, *J*=6 Hz, 2H, NHCH₂CH₂), 5.08, 5.12 (2×s, 2H×2, 2×OCH₂Ph), 5.30 (br m, 1H, NH) 7.24–7.37 (m, 10H, 2×Ph); ¹³C (CDCl₃) δ 34.6, 36.7, 66.7, 66.9, 127.7, 128.2, 128.3, 128.4, 128.5, 128.6, 135.8, 136.3, 156.4, 172.2.

Using the general procedure, **5i** was obtained in the yield as indicated in Table 1. ¹H NMR (DMSO-*d*₆+CDCl₃) δ 2.35 (t, *J*=7 Hz, 2H, CH₂CH₂CO), 3.33 (q, *J*=7 Hz, 2H, NHCH₂CH₂), 3.72 (d, *J*=6 Hz, 2H, CH₂CONH₂), 5.01 (s, 2H, CH₂OPh), 7.05, 7.23, 8.08 (3×m, 3×1H, NH, NH₂), 7.30–7.40 (m, 5H, Ph); ¹³C (DMSO-*d*₆+CDCl₃) δ 35.6, 37.1, 41.9, 65.3, 127.7, 128.4, 137.2, 156.1, 170.7, 171.1. HRMS (MH⁺) calcd for C₁₃H₁₇N₃O₄, 280.1297; found 280.1294.

4.11. (2*S*)-3-[(*t*-Butyloxycarbonyl)amino]-2-benzyl propanoic-Gly-NH₂ **5l**⁴²

Dimethyl malonate (11.9 g, 90 mmol) in THF (120 mL) was added to a stirred suspension of NaH (80% in oil, 2.7 g, 90 mmol) in THF (60 mL) at 0°C. After warming to rt and stirring for 0.5 h, a solution of benzyl bromide (9.5 mL, 80 mmol) in THF (120 mL) was added. The reaction was stirred for 2 h and then quenched with satd NH₄Cl. The organic layer was separated and the aqueous phase was extracted with EtOAc (2×50 mL). The combined organic layers were dried (MgSO₄) and the solvent was removed in vacuo. Purification by column chromatography (gradient elution with EtOAc in hexane) furnished 15.4 g (82%) of 2-benzyl dimethylmalonate. 2-Benzyl dimethylmalonate (5.4 g, 24.3 mmol) in THF (20 mL) was added to a stirred suspension of LiAlH₄ (0.95 g, 25 mmol) in THF (100 mL) at 0°C. The mixture was stirred at rt for 3 h and quenched by adding satd NaSO₄ until all the suspension solid became white. The solid was filtered out and the organic layer was dried (MgSO₄). The solvent was removed in vacuo to yield 3.81 g (95%) of 2-benzyl-1,3-propanediol, which was pure by NMR and was used in the next step without further purification. ¹H NMR (CDCl₃) δ 2.05 (m, 1H, CHCH₂Ph), 2.61 (d, *J*=7 Hz, 2H, CH₂Ph), 3.67 (dd, *J*=7, 11 Hz, 2H, CH₂OH), 3.80 (dd, *J*=4, 11 Hz, 2H, CH₂OH), 7.17–7.29 (m, 5H, Ph); ¹³C (CDCl₃) δ 34.4, 44.0, 65.9, 126.3, 128.6, 129.1, 140.0.

2-Benzyl-1,3-propanediol (1.84 g, 11 mmol) was treated with Ac₂O (4 mL) and Et₃N (4 mL) and DMAP (0.244 g, 2 mmol) at rt for 2 h. The mixture was diluted with 1N HCl and extracted with CH₂Cl₂. The solution was washed with satd NaHCO₃, dried (MgSO₄) and the solvent removed in vacuo. The crude product was purified by column chromatography (gradient elution with EtOAc in hexane) to give 2.4 g (100%) of the corresponding diacetate. ¹H NMR (CDCl₃) δ 2.04 (s, 6H, 2×CH₃), 2.32 (m, 1H, CHCH₂Ph), 2.68 (d, *J*=7 Hz, 2H, CH₂Ph), 4.00 (dd, *J*=6, 11 Hz, 2H, CH₂OAc), 4.04 (dd, *J*=4, 11 Hz, 2H, CH₂OAc), 7.13–7.30 (m, 5H, Ph); ¹³C (CDCl₃) δ 21.0, 34.8, 39.2, 63.9, 126.6, 128.7, 129.1, 138.8, 171.1.

The diacetate (2.4 g, 10 mmol) was suspended in a 70:30 mixture (100 mL) of 0.1 M phosphate buffer (pH 7.0) and *i*-Pr₂O. Lipase PS-30 (Amano) (600 mg) was added at rt and the mixture was stirred overnight. The mixture was filtered and extracted with CH₂Cl₂. The extract was washed with brine, dried (MgSO₄) and the solvent was removed in vacuo. The crude product was separated from unreacted starting material by column chromatography (gradient elution with EtOAc in hexane) to yield 1.1 g (58%) of (2*S*)-benzyl-1-acetoxypropane-3-ol. ¹H NMR (CDCl₃) δ 2.08 (s, 3H, CH₃), 2.11 (m, 1H, CHCH₂Ph), 2.62 (m, 2H, CH₂Ph), 3.49 (dd, *J*=7, 12 Hz, 1H, CHHOH), 3.59 (dd, *J*=4, 11 Hz, 1H, CHHOH), 4.06 (dd, *J*=7, 12 Hz, 1H, CHHOAc), 4.17 (dd, *J*=7, 11 Hz, 1H, CHHOAc), 7.16–7.30 (m, 5H, Ph); ¹³C (CDCl₃) δ 21.1, 34.4, 42.6, 62.1, 64.1, 126.4, 128.7, 129.2, 139.5, 171.9. [α]_D²⁴=−26.2 (*c* 0.98, MeOH).

HN₃ [generated by NaN₃ (1.5 g, 23 mmol) and 50% H₂SO₄ (10 mL) in benzene (15 mL)] and diethyl azodicarboxylate (DEAD) (1.2 mL, 6 mmol) was added to a solution of (2*S*)-benzyl-1-acetoxypropane-3-ol (1 g, 5.15 mmol) and PPh₃ (1.6 g, 6 mmol) in THF (50 mL) at –35°C. The mixture was stirred at this temperature for 0.5 h and then at rt for 2 h. After evaporation the residue was dissolved in EtOAc (50 mL) and di-*t*-butyl dicarbonate (1.13 g, 6 mmol) and Pd-C (250 mg) were added. The reaction mixture was hydrogenated at rt and at atmospheric pressure overnight. The catalyst was removed by filtering through Celite. The solvent was removed and the crude product was purified using column chromatography (gradient elution with EtOAc in hexane) to yield 1.0 g (70%) of (2*S*)-2-benzyl-3-[(*t*-butyloxycarbonyl)amino]propyl acetate. ¹H NMR (CDCl₃) δ 1.41 (s, 9H, *t*-Bu), 2.04 (s, 3H, CH₃), 2.08 (m, 1H, CHCH₂Ph), 2.65 (m, 2H, CH₂Ph), 3.08 (m, 1H, CHHN), 3.22 (m, 1H, CHHN), 3.90 (dd, *J*=4, 11 Hz, 1H, CHHOAc), 4.10 (dd, *J*=6, 11 Hz, 1H, CHHOAc), 7.16–7.32 (m, 5H, Ph); ¹³C (CDCl₃) δ 21.1, 28.5, 35.7, 40.5, 41.7, 64.2, 79.5, 126.5, 128.7, 129.2, 139.3, 156.2, 171.4. [α]_D²⁵ = –6.2 (*c* 0.79, MeOH).

K₂CO₃ (0.94 g, 7.2 mmol) was added to a solution of (2*S*)-2-benzyl-3-[(*t*-butyloxycarbonyl)amino]propyl acetate (1 g, 3.6 mmol) in 5% aq. MeOH (50 mL). The reaction mixture was stirred at rt for 2 h and then worked up by extraction with water and CH₂Cl₂ to obtain the crude 0.56 g (62%) of (2*S*)-2-benzyl-3-[(*t*-butyloxycarbonyl)amino]propanol. The product was used in the next step without further purification. ¹H NMR (CDCl₃) δ 1.46 (s, 9H, *t*-Bu), 1.91 (m, 1H, CHCH₂Ph), 2.49 (dd, *J*=8, 14 Hz, 1H, CHHPh), 2.62 (dd, *J*=7, 13 Hz, 1H, CHHPh), 3.08 (dd, *J*=7, 14 Hz, 1H, CHHN), 3.22 (dd, *J*=4, 14 Hz, 1H, CHHN), 3.41 (dd, *J*=4, 11 Hz, 1H, CHHOH), 3.58 (dd, *J*=7, 11 Hz, 1H, CHHOH), 7.19–7.41 (m, 5H, Ph); ¹³C (CDCl₃) δ 28.5, 35.5, 40.4, 43.7, 62.0, 80.1, 126.3, 128.6, 129.1, 140.1, 157.8. [α]_D²⁶ = –29.8 (*c* 0.43, MeOH).

Jones reagent (5 mL) was added to a solution of (2*S*)-2-benzyl-3-[(*t*-butyloxycarbonyl)amino]propanol (0.56 g, 2.25 mmol) in acetone (45 mL) at 0°C. The reaction mixture was stirred at rt for 1 h, then quenched with *i*-PrOH until the colour of the reaction mixture turned to green. After dilution with water and extraction with CH₂Cl₂ (3×50 mL), the combined organic layers were dried (MgSO₄) and the solvent was removed in vacuo to obtain crude (2*S*)-2-benzyl-3-[(*t*-butyloxycarbonyl)amino]propanoic acid (0.54 g, 90%). ¹H NMR (CDCl₃) δ 1.42 (s, 9H, *t*-Bu), 2.41–3.60 (m, 5H, CH₂Ph, CH₂N, CHCH₂Ph), 7.18–7.38 (m, 5H, Ph), 9.21 (brs, 1H, COOH).

(2*S*)-3-[(*t*-Butyloxycarbonyl)amino]propanoic acid (0.29 g, 1.1 mmol) was dissolved in DMF (20 mL) and then CsCO₃ (0.39 g, 1.21 mmol) was added. The mixture was evaporated in vacuo to dryness. DMF (20 mL) was added to the remaining solid and the solvent was removed again in vacuo. The residue was dissolved in DMF (20 mL) and treated with benzyl bromide (0.15 mL, 1.21 mmol). The mixture was stirred at rt

overnight. The reaction was worked up by extraction with water and CH₂Cl₂. The organic layer was dried (MgSO₄) and the solvent was removed in vacuo. The crude product was purified by column chromatography (gradient elution with EtOAc in hexane) to obtain benzyl (2*S*)-3-[(*t*-butyloxycarbonyl)amino]-2-benzylpropanoate **11** (0.29 g, 70%). ¹H NMR (CDCl₃) δ 1.42 (s, 9H, *t*-Bu), 2.82–3.01 (m, 3H, CH₂Ph, CHCO), 3.28 (m, 1H, CHHN), 3.38 (m, 1H, CHHN), 4.83 (m, 1H, NH), 5.06 (s, 2H, OCH₂Ph), 7.11–7.35 (m, 10H, 2×Ph); ¹³C (CDCl₃) δ 28.5, 36.1, 41.9, 47.6, 66.6, 79.6, 126.7, 128.3, 128.4, 128.7, 129.0, 135.8, 138.3, 155.9, 174.3. [α]_D²⁴ = –7.2 (*c* 0.43, MeOH).

Using the general procedure for CMM catalysed coupling reactions, reaction of benzyl (2*S*)-3-[(*t*-butyloxycarbonyl)amino]-2-benzylpropanoate **11** with glycineamide hydrochloride gave **51** in the yield as indicated in Table 1. ¹H NMR (DMSO-*d*₆) δ 1.35 (s, 9H, *t*-Bu), 2.60–2.80 (m, 3H, CH₂Ph, CHCO), 3.06 (m, 2H, CH₂N), 3.62 (m, 2H, CH₂CONH₂), 6.68, 7.00, 8.01 (m×3, 1H×3, NH, NH₂), 7.05–7.27 (m, 5H, Ph); ¹³C (DMSO-*d*₆) δ 28.3, 35.5, 40.1, 41.9, 47.5, 77.8, 126.1, 128.2, 128.9, 139.6, 155.6, 171.1, 173.3. HRMS (MH⁺) calcd for C₁₇H₂₅N₃O₄, 336.1917; found 336.1915. [α]_D²⁵ = –3.8 (*c* 0.16, MeOH).

4.12. (2*R*)-3-[(*t*-Butyloxycarbonyl)amino]-2-benzylpropanoic-Gly-NH₂ **5m**

A mixture of 2-benzyl-1,3-propanediol (0.92 g, 5.5 mmol), vinyl acetate (0.52 mL, 11 mmol) and lipase PS-30 (Amano) (0.92 g, 1 g per 1 g of the substrate) in THF (25 mL) was stirred at rt for 2 h. The reaction was then filtered and the solvent was removed to yield 1 g (94%) of 2(*R*)-benzyl-1-acetoxypropane-3-ol. [α]_D²⁶ = +25.4 (*c* 0.54, MeOH); {lit.,⁵² [α]_D²⁰ = +27.7 (*c* 1.3, CHCl₃); lit.,⁵³ [α]_D²³ = +20.6 (*c* 1.0, CHCl₃)}.

Benzyl-2(*R*)-3-[(*t*-butyloxycarbonyl)amino]-2-benzylpropanoate **1m** was prepared from 2(*R*)-benzyl-1-acetoxypropane-3-ol in the same manner as for **11** from 2(*S*)-benzyl-1-acetoxypropane-3-ol. ¹H NMR and ¹³C data were identical to **11**. [α]_D²⁴ = +7.6 (*c* 2.11, MeOH).

Using the general procedure for CMM catalysed coupling reactions, reaction of **1m** with glycineamide hydrochloride gave **5m** in the yield as indicated in Table 1. ¹H NMR and ¹³C data were identical to **51**. HRMS (MH⁺) calcd for C₁₇H₂₅N₃O₄, 336.1917; found 336.1921. [α]_D²⁶ = +2.9 (*c* 0.70, MeOH).

4.13. (*S*)-Phenyllactic-L-Ala-NH₂ **7a**

Using the general procedure for CMM-catalysed coupling reactions, reaction of (*S*)-benzyl phenylacetate **1a** with L-alanineamide hydrochloride **6** gave **7a** in the yield as indicated in Table 2. ¹H NMR (acetone-*d*₆) δ 1.38 (d, *J*=7 Hz, 3H, CH₃), 2.81 (dd, *J*=8, 12 Hz, 1H, CHH-CHOH), 3.20 (dd, *J*=4, 12 Hz, 1H, CHH'CHOH), 4.25 (br dd, *J*=4, 8 Hz, 1H, CHOH), 4.42 (quin., *J*=7 Hz, 1H, CHCONH₂), 4.83, 6.48, 6.87, 7.61 (4×brs, NH, NH₂, OH), 7.19–7.35 (m, 5H, Ph); ¹³C (acetone-*d*₆) δ

19.1, 41.6, 48.8, 73.6, 127.1, 128.9, 130.5, 139.5, 173.4, 174.7. HRMS (MH⁺) calcd for C₁₂H₁₆N₂O₃, 237.1239; found 237.1231. [α]_D²⁷ = -55.1 (*c* 1.65, MeOH).

4.14. (S)-Phenyllactic-D-Ala-NH₂ 9a

Using the general procedure, **9a** was obtained in the yield as indicated in Table 2. ¹H NMR (DMSO-*d*₆) δ 1.18 (d, *J* = 7 Hz, 3H, CH₃), 2.73 (dd, *J* = 8, 14 Hz, 1H, CHHCHOH), 2.98 (dd, *J* = 4, 14 Hz, 1H, CHHCHOH), 4.14 (ddd, *J* = 4, 6, 8 Hz, 1H, CHOH), 4.22 (quin, *J* = 7 Hz, 1H, CHCONH₂), 5.79 (d, OH, *J* = 6 Hz), 7.15, 7.42, 7.61 (3×brs, NH, NH₂, OH), 7.20–7.38 (m, 5H, Ph); ¹³C (acetone-*d*₆) δ 19.6, 40.9, 48.9, 73.8, 127.4, 129.3, 130.2, 139.9, 173.0, 175.8. HRMS (MH⁺) calcd for C₁₂H₁₆N₂O₃, 237.1239; found 237.1241. [α]_D²⁷ = -52.5 (*c* 1.19, MeOH).

4.15. (R)-Phenyllactic-L-Ala-NH₂ 7b

Using the general procedure, **7b** was obtained in the yield as indicated in Table 2. ¹H NMR (DMSO-*d*₆) and ¹³C (acetone-*d*₆) data were identical to **9a**. HRMS (MH⁺) calcd for C₁₂H₁₆N₂O₃, 237.1239; found 237.1229. [α]_D²⁷ = +52.6 (*c* 1.25, MeOH).

4.16. (R)-Phenyllactic-D-Ala-NH₂ 9b

Using the general procedure, **9b** was obtained in the yield as indicated in Table 2. ¹H NMR (acetone-*d*₆) and ¹³C (acetone-*d*₆) data were identical to **7a**. HRMS (MH⁺) calcd for C₁₂H₁₆N₂O₃, 237.1239; found 237.1227. [α]_D²⁷ = +54.0 (*c* 0.71, MeOH).

4.17. N-Z- β -Alaninyl-L-Ala-NH₂ 7i

Using the general procedure, **7i** was obtained in the yield as indicated in Table 2. ¹H NMR (DMSO-*d*₆) δ 1.16 (d, *J* = 7 Hz, 3H, CH₃), 2.29 (m, 2H, CH₂CH₂CO), 3.19 (q, *J* = 7 Hz, 2H, NHCH₂CH₂), 4.18 (quin, *J* = 7 Hz, 1H, CH₃CHCONH₂), 5.00 (s, 2H, CH₂OPh), 6.96, (brs, 2H, NH₂), 7.21 (t, *J* = 7 Hz, 1H, NHZ), 7.29–7.36 (m, 5H, Ph), 8.01 (d, *J* = 7 Hz, 1H, NH); ¹³C (DMSO-*d*₆) δ 18.3, 35.5, 37.1, 48.0, 65.2, 127.8, 127.9, 128.4, 137.3, 156.0, 170.0, 174.4. HRMS (MH⁺) calcd for C₁₄H₁₉N₃O₄, 294.1454; found 294.1445.

4.18. (2S)-3-[(*t*-Butyloxycarbonyl)amino]-2-benzyl propanoic-L-Ala-NH₂ 7l

Using the general procedure, **7l** was obtained in the yield as indicated in Table 2. ¹H NMR (DMSO-*d*₆) δ 1.16 (d, *J* = 7 Hz, 3H, CH₃CH), 1.37 (s, 9H, *t*-Bu), 2.57–3.16 (m, 5H, CH₂Ph, CHCO, CH₂N), 4.18 (quin, *J* = 7 Hz, 1H, CHCONH₂), 6.70 (t, *J* = 7 Hz, 1H, NHBoc), 6.91, 6.95 (2×brs, 2H, NH₂), 7.18–7.26 (m, 5H, Ph), 7.82 (d, *J* = 7 Hz, 1H, NH); ¹³C (DMSO-*d*₆) δ 18.4, 28.3, 35.4, 42.1, 47.2, 48.0, 77.7, 126.1, 128.2, 128.9, 139.6, 155.5, 172.4, 174.2. HRMS (MH⁺) calcd for C₁₈H₂₇N₃O₄, 350.2073; found 350.2082. [α]_D²⁴ = -11.3 (*c* 0.46, MeOH).

4.19. (2S)-3-[(*t*-Butyloxycarbonyl)amino]-2-benzyl propanoic-D-Ala-NH₂ 9l

Using the general procedure, **9l** was obtained in the yield as indicated in Table 1. ¹H NMR (DMSO-*d*₆) δ 1.10 (d, *J* = 7 Hz, 3H, CH₃CH), 1.38 (s, 9H, *t*-Bu), 2.55–3.13 (m, 5H, CH₂Ph, CHCO, CH₂N), 4.01 (quin, *J* = 7 Hz, 1H, CHCONH₂), 6.95, 6.98, 7.04, 7.80 (4×brs, 4H, NH, NHBoc, NH₂), 7.15–7.27 (m, 5H, Ph); ¹³C (DMSO-*d*₆) δ 18.3, 28.0, 35.0, 41.0, 47.0, 48.0, 77.9, 126.0, 128.2, 128.8, 139.5, 155.4, 172.4, 174.1. HRMS (MH⁺) calcd for C₁₈H₂₇N₃O₄, 350.2073; found 350.2075. [α]_D²⁴ = -9.4 (*c* 0.47, MeOH).

4.20. (2R)-3-[(*t*-Butyloxycarbonyl)amino]-2-benzyl propanoic-L-Ala-NH₂ 7m

Using the general procedure, **9m** was obtained in the yield as indicated in Table 1. ¹H NMR and ¹³C data were identical to **9l**. HRMS (MH⁺) calcd for C₁₈H₂₇N₃O₄, 350.2073; found 350.2066. [α]_D²⁴ = +9.7 (*c* 0.64, MeOH).

4.21. (2R)-3-[(*t*-Butyloxycarbonyl)amino]-2-benzyl propanoic-D-Ala-NH₂ 9m

Using the general procedure, **9m** was obtained in the yield as indicated in Table 1. ¹H NMR and ¹³C data were identical to **7l**. HRMS (MH⁺) calcd for C₁₈H₂₇N₃O₄, 350.2073; found 350.2069. [α]_D²⁴ = +11.7 (*c* 1.33, MeOH).

4.22. Monobenzyl 3-methylglutaryl-Gly-NH₂ 11

To a suspension of NaH (80% in oil, 843 mg, 28.1 mmol) in THF (10 mL), BnOH (2.91 mL, 28.1 mmol) was added at rt under nitrogen atmosphere and the resulting mixture was stirred for 15 min. After addition of a solution of 3-methylglutaric anhydride (3.00 g, 23.4 mmol) in THF (40 mL) at rt the mixture was stirred overnight. The reaction was quenched with water, and the pH was adjusted to ca. 8 with 0.1 M NaOH. After extraction with EtOAc, the pH of the water layer was adjusted with 1 M HCl, the solution was saturated with NaCl and extracted with EtOAc. After evaporation, crude monobenzyl 3-methylglutarate was obtained as a colourless oil. This compound was used in the next step without further purification. ¹H NMR (CDCl₃) δ 1.05 (d, *J* = 5.5 Hz, 3H, CH₃), 2.22–2.40 (m, 2H, CH₂CO₂), 2.40–2.58 (m, 2H, CH₂CO), 2.46–2.52 (m, 1H, CHCH₃), 5.12 (s, 2H, CH₂Ph), 7.30–7.48 (m, 5H, Ph), 10.5 (brs, 1H, CO₂H).

To a solution of monobenzyl 3-methylglutarate and BnOH (2.23 g, 20.6 mmol) in benzene (50 mL) a catalytic amount of *p*-TsOH was added, and the mixture was refluxed using a Dean–Stark trap and stirred overnight. The reaction was quenched with satd NaHCO₃, extracted with EtOAc, washed with brine and dried over MgSO₄. After evaporation the residue was purified by column chromatography on silica gel (hexane:EtOAc, 9:1) to afford dibenzyl 3-methylglutarate **10** as a colourless oil (5.34 g, 70%). ¹H NMR

(CDCl₃) δ 1.02 (d, $J=6.5$ Hz, 3H, CH₃), 2.31 (dd, $J=7.5, 15.0$ Hz, 2H, CH₂CO₂), 2.44–2.60 (m, 1H, CHCH₃), 2.45 (dd, $J=6.0, 15.0$ Hz, 2H, CH₂CO₂), 5.12 (s, 4H, CH₂Ph), 7.32–7.41 (m, 10H, Ph); ¹³C NMR (CDCl₃) δ 20.4, 28.0, 41.3, 66.7, 128.7, 128.9, 129.1, 136.5, 172.6.

Using the general procedure, **11** was obtained in the yields and % e.e. as indicated in Table 3. ¹H NMR (DMSO-*d*₆) δ 0.87 (d, $J=6.5$ Hz, 3H, CH₃), 2.00–2.50 (m, 4H, CH₂CO), 2.20–2.40 (m, 1H, CHCH₃), 3.60 (d, $J=5.5$ Hz, 2H, NHCH₂CO), 5.08 (s, 2H, CH₂Ph), 6.99 (brs, 1H, NH₂), 7.28 (brs, 1H, NH₂), 7.30–7.45 (m, 5H, Ph), 8.01 (t, $J=5.5$ Hz, 1H, NH); ¹³C NMR (acetone-*d*₆) δ 20.4, 29.1, 30.5, 41.7, 43.4, 66.8, 129.2, 129.3, 129.7, 137.9, 172.7, 173.0, 173.3.

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