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An Efficient Synthesis of Chiral Amino Acid and Peptide Alkylamides via CLEC-Subtilisin Catalyzed Coupling and *in situ* Resolution

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Abstract: CLEC-Subtilisin efficiently catalyzed the synthesis of optically active alkylamides of amino acids and peptides. The high enantioselectivity of the catalyst toward L-amino acids and S-amines resulted in formation of the S,S-alkylamide regardless of the optical purity of the substrates. The catalyst accepts a broad range of substrates, including peptides, natural and unnatural amino acids. The acyl donor could be either as methyl, ethyl or benzyl ester. The N-protecting groups of acyl donor could be either acetyl, Boc or Cbz.

Chiral alkylamides of peptides and amino acids are important building blocks of many marketed and potential pharmaceuticals such as HIV protease inhibitors[1], enkephalins[2] and antibiotics.[3] Many methods for the preparation of optically pure alkylamides have been reported. In chemical procedures, the processes of coupling and resolution are usually separated: first optically pure components are prepared, then the coupling is performed. In general, chemical methods suffer from partial racemization, modest yield when coupled with bulky amines[4] and the difficulties of removing by-products produced during coupling reactions, of which dicyclohexyl urea, arising from the commonly-used dicyclohexylcarbodiimide (DCC) is a particularly troublesome example.[5] Several enantioselective peptide syntheses, where coupling and resolution are performed in one step, have been reported. In these cases, the resolution of amines occurs during the coupling of active esters of N-protected amino acids, such as N-hydroxytartarimide[6], or N-hydroxycamphorimide[7] with racemic amines. These methods, however, are rarely used in practice due to the unpredictable and often modest enantioselectivity, long reaction times and necessity of extra steps to prepare the active esters.[8]

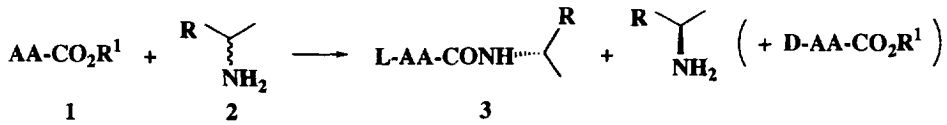
Enantioselective synthesis of alkylamides can also be achieved by enzyme-catalyzed coupling in organic solvents[9] accompanied by the *in situ* resolution of either ester, amines or both.[10] Surprisingly, the enantioselective synthesis of chiral amino acid alkylamides from racemic substrates, has not been reported.

We have recently found that cross-linked enzyme crystals (CLECs)[11] of thermolysin and *Candida rugosa* lipase are efficient, heterogeneous catalysts for many enantioselective transformations. Here we report the use of another catalyst, subtilisin-CLEC [12] in the synthesis of optically active alkylamides of amino acids and peptides. This catalyst exhibits excellent enantio- and diastereoselectivities in the coupling of both natural and unnatural amino acids with racemic amines.

In a representative procedure, a solution of S-**1a** (195 mg, 0.5 mmol) and amine R,S-**2b** (343 mg, 2 mmol) in 25 mL of acetonitrile was mixed with 165 mg of subtilisin CLECs and incubated for 8 hours at 40 ^{o}C. HPLC analysis[13] indicated the ester substrate was completely consumed and the products included amide **3a**, benzyl alcohol and the excess amine substrate. The catalyst was filtered off and washed with acetonitrile. Ethyl acetate was added and the excess amine was removed by extraction with 0.5 N aqueous HCl. After

drying over sodium sulfate, the organic solvents were evaporated off to give a residue which was separated by silica gel column chromatography to give 213 mg (94 %) of amide S,S-**3a**.[14]

Table 1 summarizes the coupling results. We found that the use of optically pure substrates was not necessary. The high enantioselectivity of the catalyst toward L-amino acids and S-amines resulted in formation of the S,S-alkylamide regardless of the optical purity of the substrates. Indeed, optically pure alkylamides S,S-**3** were obtained via *in situ* resolution when amines R,S-**2**, or even both amines and amino acids (R,S-**1a**; R,S-**1g**), were used in the racemic form. In the latter case the diastereomeric excess of product was greater than 98 %.



1 **2** **3**

1a N-Cbz Phe OBzl
1b N-Ac Phe OEt
1c N-Ac Ala OMe
1d N-Ac Leu OMe
1e N-Ac Val OMe

2a α-methyltryptamine
2b α-(1-naphtyl)ethylamine

1f N-Boc Tyr OMe
1g N-Boc-3-(2-Naphthyl)alanine OMe
1h N-Cbz-Ala-Ser OMe
1i N-Cbz-Val-Phe OMe
1k Trifluoroethyl butanoate

Table 1. Synthesis of Optically Active Alkylamides via Subtilisin-CLEC Catalyzed Coupling

Acyl donor (mmol)	Nucleophile (mmol)	Catalyst (mg/mL)	Solvent[a]	Time (h)	Product	Yield[b] (%)	ee or de[f] (%)
S-**1a** (0.1)	R,S-**2b** (0.2)	6.6	A	8	S,S-**3a**	>98[d] (94)[c]	>98
R,S-**1a** (0.2)	R,S-**2b** (0.4)	6.6	A	6	S,S-**3a**	95[d]	>98
S-**1b** (1.0)	R,S-**2a** (1.0)	5	3MP	24	S,S-**3b**	82[e]	89 (>98)[g]
S-**1b** (0.1)	R,S-**2a** (0.4)	5	A	32	S,S-**3b**	88[d]	89
S-**1c** (1.0)	R,S-**2a** (1.0)	5	3MP	24	S,S-**3c**	74[e]	90
S-**1d** (1.0)	R,S-**2a** (1.0)	5	3MP	24	S,S-**3d**	66[e]	91
S-**1e** (1.0)	R,S-**2a** (1.0)	5	3MP	24	S,S-**3e**	4.8[e]	---
S-**1f** (0.1)	R,S-**2b** (0.2)	6.6	A	96	S,S-**3f**	94[d]	98
S-**1g** (0.1)	R,S-**2b** (0.4)	6.6	A	96	S,S-**3g**	91[d]	>98
R,S-**1g** (0.2)	R,S-**2b** (0.4)	6.6	A	6	S,S-**3g**	87[d]	>98
S,S-**1h** (0.1)	R,S-**2b** (0.4)	6.6	A	6	S,S,S-**3h**	>98[d]	>98
S,S-**1i** (0.1)	R,S-**2b** (0.4)	6.6	A	6	S,S,S-**3i**	>98[d]	>98
1k (0.8)	R,S-**2a** (0.4)	16	3MP	22	S-**3k**	80[e]	90[h]

a. A, acetonitrile; 3MP, 3-methyl-3-pentanol; reaction volume was 5 mL. b. Yields were based on the HPLC conversions. c. Isolated yield. d. Based on the S-ester conversion. e. Based on the S-amine conversion. f. Determined by HPLC analysis (Ref. 13). g. After recrystallization. h. Determined by HPLC on Chiracel® OJ column.

We found that the catalyst accepts a broad range of substrates, including different amino acids (Ala, Phe, Leu, Tyr, Ser), different esters (methyl, ethyl, benzyl) and different N-protecting groups (acetyl, Boc and CBZ). In all cases, coupling proceeded with high stereoselectivity and good yield. The only exception was reaction of N-Ac L-valine methyl ester (S-**1e**) with R,S-**2a**. The yield was only 4.8% after 24 hour incubation.

The slow reaction rate was most probably due to the steric hindrance of the isopropyl group of valine combined with the bulky amine substrate. The coupling of peptides with racemic amines was also examined. Both N-Cbz-L-Val-L-Phe OMe, **1i** and N-Cbz-L-Ala-L-Ser OMe, **1h** reacted with R,S-**2b** to give the corresponding S,S,S-amides in high chemical yields. The diastereomeric excess of both **3h** and **3i** was greater than 98%. Significantly, subtilisin-CLEC also accepted an unnatural amino acid ester. S,S-amide **3g** was obtained via coupling of either optically pure or racemic N-Boc-(2-naphthyl)alanine methyl ester, **1g** with racemic amine **2b**. The optical purity of S,S-**3g** was >98 %.

To examine the solvent effect on the reaction, several different organic solvents including acetonitrile, *tert*-butanol, 3-methyl-3-pentanol, ethyl acetate, THF, pyridine, isopropanol, ethanol, acetone and DMF were examined in the reaction of N-Cbz phenylalanine benzyl ester, S-**1a** with **2b**. We found acetonitrile to give the best results, in terms of both reaction rates and product yields. Although *tert*-butanol and 3-methyl-3-pentanol also gave good reaction rates the yields in these solvents were lower due to the hydrolysis of ester substrate. The major product of the reactions in isopropanol and ethanol was the corresponding isopropyl and ethyl esters formed from the transesterification of S-**1a** with solvents. No reaction occurred when DMF was used as solvent.

The combination of high activity and stability of CLEC-catalyst in organic solvents leads to high productivity in the above mentioned reactions. Thus, seven 22h cycles in the syntheses of S-**3k** were conducted without a noticeable loss of enantioselectivity or yield.[15]

In conclusion, an efficient and convenient method for the preparation of optically pure alkylamides of amino acids and peptides from racemic starting materials has been developed. Since the catalyst accepts various natural and unnatural amino acids and amines and is applicable to peptide fragments without racemization, the application of this method may provide chemists with an efficient route to optically pure alkylamides of amino acids and peptidomimetics.

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12. Subtilisin- CLEC (PeptiCLEC™-BL) is a commercial product of Altus Biologics.
13. HPLC conditions: Microsorb C_{18} column (4.6 x 50 mm, 5 m, 300A), Mobile phase: gradient, ratio (v/v) of water (with 0.1 % trifluoroacetic acid) and acetonitrile (with 0.1 % trifluoroacetic acid) was 90:10 at 0 minute, 20:80 at 10 minute, 20:80 at 12 minute and 90:10 at 15 minute; Flow rate: 1 mL/ minute; UV detection at 254 nm; Retention times: amine, **2b**: 3.88 min., NCbz Phe OBzl: 9.43 min., alkylamide, S,S-**3a**: 9.18 min., S,R-**3a**: 9.21 min., benzylalcohol: 2.72 min.
14. All products have been characterized by ^1H NMR
15. After each reaction cycle, the catalysts were filtered off and washed with 3-methyl-3-pentanol. The washed catalysts were directly used in the next cycle.

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