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Stereoselective *Pseudomonas cepacia* lipase mediated synthesis of α-hydroxyamides

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Abstract: A new method for the synthesis of α-hydroxyamides via the *Pseudomonas cepacia* lipase catalyzed amidation of α-hydroxyesters in non-aqueous media is described. Reactivities of α-hydroxy benzyl esters are excellent, resulting in rapid conversions to good yields of α-hydroxyamides, while ethyl and methyl esters react more slowly, and some hindered esters show no reactivity under the conditions studied. Some benzyl esters react stereoselectively, producing excellent yields of asymmetric α-hydroxyamides. © 1997 Elsevier Science Ltd

The α-hydroxyamide functionality is frequently found in biologically active molecules and is valuable as a synthetic intermediate. Mycalamides, for example, are a class of α-hydroxyamides which inhibit both protein and DNA synthesis at subnanomolar levels.[1] More recently, several second generation α-hydroxyamide HIV protease inhibitors have been prepared which show improved antiviral activity.[2] Wipf and Kim demonstrated the utility of an α-hydroxyamide as a protected α-ketoamide in their synthesis of Cyclotheonamide A.[3] The use of α-hydroxyamides as intermediates in the synthesis of oxazolidinone α-adrenergic anatagonists[4] and morpholine derivatives[5,6] has also been reported.

Several methods for the synthesis of chiral α-hydroxyamides are known. Intermolecular transformation of α-hydroxyacids to α-hydroxyamides in reasonable yield has been achieved at high temperature,[7] high pressure,[8] by Lewis acid catalysis,[9,10] and in simple cases by protection/deprotection (frequently a multi-step process) of the α-hydroxyl with an acetyl group,[11] trimethylsilyl group,[12] or as a cyclic derivative.[13,14] Such conditions are not always suitable for chiral molecules. Generally, reactions of α-hydroxyacid derivatives such as α-hydroxyesters require lengthy reaction periods and forcing conditions. Even though the syntheses of chiral α-hydroxyamides under milder conditions have been achieved via antibody-catalyzed α-ketoamide reduction[15] and by oxidation of amide enolates with oxaziridines,[16] there is a need for a mild, regioselective method for the direct conversion of α-hydroxyesters to α-hydroxyamides.

Lipases have become increasingly useful as catalysts for industrial chemical reactions. These biocatalysts work under extremely mild conditions, frequently demonstrate good regioselectivity and stereoselectivity, are active in the same organic solvents needed for substrate solubility, produce few by-products, and are generally environmentally benign.[17–19] Originally observed to catalyze the hydrolysis of fatty acid esters, lipases have now been shown to be capable of catalyzing the amidation of a variety of ester types.[20,21] α-Hydroxyesters, however, have not been studied in lipase catalyzed amidations. This is not unexpected, in light of the preference that lipase demonstrates for non-polar, lipophilic substrates, which might appear to make α-hydroxyesters less amenable to lipase catalyzed amidation. We believed, however, that use of a highly lipophilic benzyl ester would provide a preferred substrate for the lipase-catalyzed reaction of ester substrates with amines. The previously mentioned utility of lipase in stereoselective transformations presented the intriguing possibility that simultaneous resolution of racemic α-hydroxyesters to form asymmetric α-hydroxyamides might also be achieved

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Table 1. *Pseudomonas cepacia* lipase catalyzed synthesis of α-hydroxyamides from α-hydroxyesters

Entry	α-Hydroxyester	Amine	% Conversion 24 hr	72 hr	144 hr
1.	benzyl R-lactate	benzylamine	66	89	98
2.	benzyl S-lactate	benzylamine	79	95	>99
3.	benzyl R-lactate	2-phenethylamine	56	84	89
4.	benzyl S-lactate	2-phenethylamine	65	91	93
5.	methyl R-phenyllactate	2-phenethylamine	12	41	58
6.	methyl S-phenyllactate	2-phenethylamine	55	60	75
7.	benzyl R-phenyllactate	2-phenethylamine	36	54	67
8.	benzyl S-phenyllactate	2-phenethylamine	86	96	>99
9.	ethyl R-mandelate	BOCethylenediamine	5	18	56
10.	ethyl S-mandelate	BOCethylenediamine	5	19	58
11.	benzyl R-mandelate	BOCethylenediamine	29	70	97
12.	benzyl S-mandelate	BOCethylenediamine	38	82	>99

during the amidation reaction. This report first details the successful lipase-catalyzed amidation of α-hydroxyesters, allowing the synthesis of α-hydroxyamides in good yield without racemization, and second demonstrates the asymmetric induction observed during the amidation reaction.

A variety of different α-hydroxyesters and amines were utilized in our study of the efficacy of Amano *Pseudomonas cepacia* lipase to catalyze amidations in isopropyl ether. Lactate, phenyllactate, and mandelate esters were chosen as substrates, since both enantiomeric acids and esters were available commercially in enantiomerically pure form. Use of an aromatic ester or aromatic amine partner provided the UV active chromophore essential for ease of monitoring of both the ester and amide reaction components by TLC and reversed-phase HPLC. Finally, since variations between the reactivity of different enantiomers and ester types were expected, the same amine was utilized for all phenyllactates and mandelates so that conversions could be accurately compared.

As Table 1 above indicates, good to excellent conversions of esters to amides were rapidly achieved in most cases.[22] While unsubstituted amines such as benzylamine and 2-phenethylamine reacted smoothly (entries 1–8), diamines containing a BOC protective group (entries 9–12) were also well tolerated in the reaction. In all cases tested, benzyl esters showed greater reactivity than the corresponding methyl or ethyl esters (entries 5 vs. 7, 6 vs. 8, 9 vs. 11, and 10 vs. 12), in line with the greater lipophilicity that benzyl esters demonstrate vs. methyl or ethyl esters. Observable for each pair of enantiomeric benzyl or methyl ester substrates was a preference of *Pseudomonas cepacia* lipase for the (S)-enantiomer (entries 1 vs. 2, 3 vs. 4, 5 vs. 6, 7 vs. 8, 11 vs. 12), resulting in higher reactivities for the (S)-α-hydroxyesters in the amidation reaction. Interestingly, the optical isomers of ethyl mandelate (entries 9 and 10) showed no distinguishable difference in reactivity.

The enantiomeric benzyl perhydromandelates and the non-optically active benzyl 2-hydroxyisobutyrate were also tested in lipase catalyzed amidations, but showed no observable reaction under these conditions. Presumably, the secondary center adjacent to the hydroxyl and the tertiary hydroxyl center both provide too hindered an ester substrate for lipase.

The amidation products from the reactions described above were further examined for enantiomeric purity by normal phase chiral HPLC.[23] Analogous to the starting benzyl esters, the (S)-amide eluted more rapidly than the (R)-amide. We were gratified to find that no racemization at the hydroxyl center had occurred during the lipase-catalyzed amidation.

We then studied the stereoselectivity of the amidation reaction with racemic mixtures of the three previously tested α-hydroxy benzyl esters and 2-phenethylamine. At the appropriate level of conversion

Table 2. *Pseudomonas cepacia* amidations of racemic α-hydroxyesters with 2-phenethylamine

Ester	% Conversion	Product (%) S	Product (%) R	Recovered Ester (%) S	Recovered Ester (%) R
benzyl phenyllactate	49	93	7	9	91
benzyl lactate	42	91	9	10	90
benzyl mandelate	44	73	27	29	61

(≈50%, monitoring by reversed phase HPLC as described above), the lipase was filtered and washed with acetonitrile. The filtrate was purified by reversed-phase preparative HPLC, producing unreacted benzyl ester starting material and asymmetric α-hydroxy amides which were analyzed by normal phase chiral HPLC. The identity of the ester and amide peaks were confirmed by coelution with samples of the optically pure compounds synthesized above. As shown in Table 2, after 49% of the starting material had been consumed, the S-(−)-phenethyl phenyllactamide product demonstrated 86% enantiomeric excess. While racemic benzyl lactate showed analogous results, yielding predominantly one amide product at slightly less than 50% conversion of starting ester, racemic benzyl mandelate showed lower selectivity. Thus, in a truly competitive reaction where both enantiomers are present at the same concentration, the lipase demonstrates a strong preference for the S enantiomer. Therefore, preliminary work indicates that the simultaneous amidation/resolution of racemic α-hydroxyesters into chiral α-hydroxyamides is possible in some cases with *Pseudomonas cepacia* lipase.

In conclusion, we have demonstrated that *Pseudomonas cepacia* lipase (Amano PS-30) is a synthetically useful enzyme for the previously unreported lipase mediated transformation of α-hydroxyesters to α-hydroxyamides. This amidation method accomodates a variety of different amines, including those with the BOC protective group, and produces good yields of α-hydroxyamides under mild conditions without racemization. The enzyme is also capable of stereoselective amidation, producing good yields of asymmetric α-hydroxyamides.

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22. In a typical example, 66 μmol of each enantiomeric benzyl phenyllactate was separately reacted with 300 μmol phenethylamine and 15 mg lipase in isopropyl ether (Table 1, entries 7 and 8). The reactions were monitored by reversed-phase HPLC (8×100 mm radial compression μBondapak column, 50% CH_3CN: 50% 0.05% CF_3COOH/H_2O, 2 mL/min, UV detection at 220 nm) at the times indicated. The reactions were terminated by filtering the lipase and washing it with acetonitrile. The combined filtrates were purified directly by column chromatography (silica gel, EtOAc/hexane mixtures) or by preparative reversed phase HPLC. The phenethyl phenyllactamides showed the following data: ^1H NMR ($CDCl_3$) δ 7.35–7.19 (m, 5H), 6.52 (br s, 1H), 4.26 (dd, 1H, J=8.2 Hz, J=4.1 Hz), 3.49 (m, 2H), 3.19 (dd, 1H, J=13.9 Hz, J=4.1 Hz), 2.86 (dd, 1H, J=13.9 Hz, J=8.3 Hz), 2.76 (m, 2H), 2.54 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 172.6, 138.8, 136.9, 128.9, 128.8, 128.7, 127.7, 126.6, 72.8, 40.8, 40.1, 35.6; ESMS $(M+H)^+$ at 270; chiral HPLC (see reference 23 for conditions) retention times 17.6 mins (S), 39.8 mins (R).
23. A Regis 250×4.6 mm Chiralcel OD column, eluted with 1%–2% iPrOH/hexanes for esters and 5% iPrOH/hexanes for amides, was employed.

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