ORGANIC LETTERS

2003
Vol. 5, No. 8
1209−1212

Lipase-Catalyzed Enantioselective Ring Opening of Unactivated Alicyclic-Fused β-Lactams in an Organic Solvent

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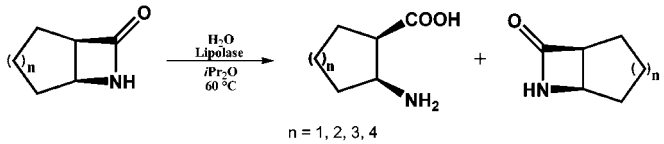
Received January 20, 2003

ABSTRACT



A highly efficient and very simple method was developed for the synthesis of enantiopure β-amino acids (e.g. *cispentacin*) and β-lactams through the enzyme-catalyzed enantioselective ring opening of unactivated alicyclic β-lactams in organic media. High enantioselectivity (*E* > 200) was observed when the Lipolase (lipase B from *Candida antarctica*)-catalyzed reactions were performed with H_2O (1 equiv) in diisopropyl ether at 60 °C. The resolved products, obtained in good chemical yield (36–47%), could be easily separated.

The β-amino acids and β-lactams are of biological and chemical importance. Some β-amino acids themselves exhibit antibacterial activity (e.g. *cispentacin*).[1] They can serve as precursors of β-lactams,[2] and can be used as building blocks for the synthesis of modified peptides with increased activity and stability,[3] and in drug research.[4] Well-defined three-dimensional structures (e.g. β-peptides with possible antibiotic activity) similar to those of natural peptides can also be formed from β-amino acids.[5] Alicyclic β-amino acids can be used in heterocyclic[6] and combinatorial[7] chemistry. Besides the utility of monocyclic β-lactams as antibiotic agents (e.g. monobactams), they are used as intermediates in β-amino acid synthesis,[8] and as building blocks for short peptide segments,[9] taxoid antitumor agents,[10] alkaloids,[11] heterocycles[12] and other types of compounds of biological and medicinal interest. Consequently, in the past few years

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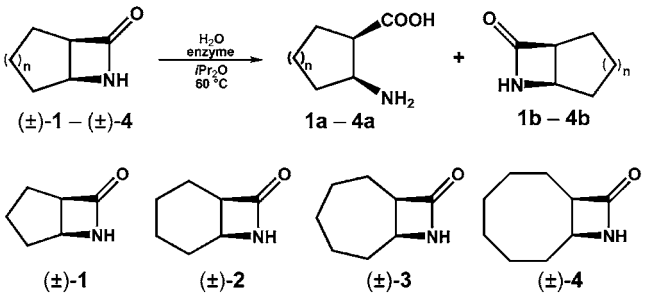
10.1021/ol034096o CCC: $25.00
Published on Web 03/19/2003

a large number of syntheses (enantioselective synthesis and enzymatic resolution) have been developed for enantiopure alicyclic β-amino acid[13] and β-lactam[14−16] derivatives. One good possibility for the preparation of enantiopure β-lactams is an enantioselective enzyme-catalyzed hydrolysis of activated β-lactams.[14] As an example, Adam et al. described the simultaneous preparation of enantiopure amino acids and β-lactams from α-methylene β-lactams by lipase-catalyzed kinetic resolution through hydrolysis.[14a] Achilles et al. described the enzyme-catalyzed hydrolysis of ethyl (±)-2-(2-oxo-4-phenylazetidin-1-yl)acetate with α-chymotrypsin.[14b] However, these ring-opening methods have been limited to activated β-lactams (activated with *N*-acyl-protecting groups such as *tert*-butoxycarbonyl or acetyl). Evans et al. investigated the enzymatic ring-opening reactions of unactivated (±)-6-azabicyclo[3.2.0]-hept-3-en-7-one, and found that lactamases in a special whole-cell preparation, ENZA-1 (*Rhodococcus equi NCIMB 40213*), catalyzed the enantioselective ring opening of this compound in water.[15] Interestingly, the saturated β-lactam remains intact under the same conditions. An important indirect enzymatic route for enantiopure alicyclic β-amino acid derivatives and β-lactams is through lipase-catalyzed asymmetric acylation of the *N*-hydroxymethylated β-lactams or lipase-catalyzed hydrolysis of the corresponding ester derivatives in an organic solvent.[16,17]

In this paper, we report a new direct method for the enantioselective ring opening of unactivated cyclic β-lactams (±)-**1**−(±)-**4**, yielding the ring-opened β-amino acids **1a**−**4a** and unreacted β-lactam enantiomers **1b**−**4b**, which could be easily separated (Scheme 1).

Scheme 1. Enantioselective Ring Opening of Unactivated Cyclic β-Lactams (±)-**1**−(±)-**4**



The alicyclic β-lactams (±)-**1**−(±)-**3** were prepared by 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate (CSI) to the corresponding cycloalkene,[18] while (±)-**4** was obtained by means of CSI cycloaddition to cyclooctadiene[19] and subsequent catalytic reduction in the presence of cyclohexene as a hydrogen donor.[17d]

Adam et al. reported on the highly enantioselective hydrolysis of the *N*-substituted β-lactam derivatives (*E* usually >200) with the lipase Chirazyme L-2, in water at 70 °C.[14a] Since they mentioned that the enzyme rapidly lost activity at higher temperatures, we started our experiments with Chirazyme L-2 (a carrier-fixed lipase B from *Candida antarctica*) at 60 °C, but instead of water the ring-opening reactions of (±)-**1**−(±)-**4** were performed in diisopropyl ether, using water (1 equiv) as nucleophile (Table 1, entries 1, 3, 5, and 16). High enantioselectivities but rather long reaction times were observed. An additional enzyme screening including lipase PS (*Pseudomonas cepacia*), lipase AK (*Pseudomonas fluorescens*), lipase AY (*Candida rugosa*),

Table 1. Conversion and Enantioselectivity of Ring Opening of (±)-**1**−(±)-**4**

entry	compd[a]	enzyme (50 mg mL^{-1})	H_2O (equiv)	conv (%)	ee_s[b] (%)	ee_p[c] (%)	*E*
1[d]	(±)-**1**	Chirazyme L-2	1	14	16	>95	>45
2[d]	(±)-**1**	Lipolase	1	19	22	>95	>48
3[d]	(±)-**2**	Chirazyme L-2	1	25	31	>95	>52
4[d]	(±)-**2**	Lipolase	1	29	39	>95	>57
5[d]	(±)-**3**	Chirazyme L-2	1	41	67	>95	>78
6[e]	(±)-**3**	Chirazyme L-2	2	32	45	>95	>60
7[e]	(±)-**3**	Chirazyme L-2	3	30	40	>95	>57
8[d]	(±)-**3**	Lipolase	1	48	89	>95	>117
9[e]	(±)-**3**	Lipolase	-	49	90	>95	>120
10[e]	(±)-**3**	Lipolase	2	46	82	>95	>99
11[e]	(±)-**3**	Lipolase	3	43	73	>95	>85
12[e]	(±)-**3**	Lipolase	4	30	40	>95	>57
13[e]	(±)-**3**	Lipolase	10	13	14	>95	>44
14[d]	(±)-**3**	Novozyme 435	1	40	63	>95	>74
15[d]	(±)-**3**	CAL-A	1	18	21	>95	>47
16[d]	(±)-**4**	Chirazyme L-2	1	19	22	>95	>48
17[d]	(±)-**4**	Lipolase	1	20	24	>95	>49

[a] 0.05 M substrate in diisopropyl ether, 60 °C. [b] According to GC. [c] Calculated by using an internal standard (hexadecane). [d] After 20 h. [e] After 23 h.

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Table 2. Lipolase-Catalyzed Ring Opening of (±)-**1**−(±)-**4**[a]

				β-lactam (**1b**−**4b**)				β-amino acid (**1a**−**4a**)			
	time (h)	conv (%)	E	yield[b] (%)	isomer	ee[c] (%)	$[\alpha]^{25}_D$	yield[b] (%)	isomer	ee[d] (%)	$[\alpha]^{25}_D$
(±)-**1**	249	48	>200	42	1*S*,5*R*	93	+35.9[e]	44	1*R*,2*S*	99	−9.1[f]
(±)-**2**	141	50	>200	45	1*S*,6*R*	98	−3.6[e]	45	1*R*,2*S*	98	−19.4[f,g]
(±)-**3**	31	50	>200	41	1*S*,7*R*	99	−5.1[e]	47	1*R*,2*S*	98	−7.2[f]
(±)-**4**	170	50	>200	36	1*S*,8*R*	99	−18[e]	43	1*R*,2*S*	95	+17.8[h]

[a] 50 mg mL^{-1} of enzyme in diisopropyl ether, 1 equiv of H_2O, 60 °C. [b] Yield 100% at 50% conversion. [c] According to GC. [d] Calculated by using an internal standard (hexadecane). [e] *c* = 0.5; $CHCl_3$. [f] *c* = 0.5; H_2O. [g] $[\alpha]^{25}_D$ −19.6 (*c* 0.25, H_2O).[17b] [h] *c* = 0.4; H_2O

PPL (porcine pancreatic lipase), CAL-A (lipase A from *Candida antarctica*), Novozym 435 (lipase B from *Candida antarctica* immobilized on a macroporous polyacrylic resin), and Lipolase (lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) in diisopropyl ether was performed for the model compound **3**. Lipase PS, AK, AY, and PPL did not exhibit any activity at 30 °C (no conversion after 46 h), while CAL-A even catalyzed the hydrolysis of **3** at 60 °C, but the reaction rate was low (Table 1, entry 15). Besides Chirazyme L-2 (Table 1, entry 5), Lipolase (Table 1, entry 8) and Novozym 435 (Table 1, entry 14) were all promising catalysts, directing the hydrolysis with the same high enantioselectivity. With regard to the reaction rates (Table 1, entries 5, 8, 14, and 15), Lipolase was chosen as the enzyme for further studies and for the gram-scale resolution.

Several solvents were tested to study the solvent effect in the Lipolase-catalyzed hydrolysis of (±)-**3** at 60 °C. Lipolase was practically inactive in acetone and tetrahydrofuran (conversion < 5% after 42 h). The reaction proceeded somewhat more slowly in *n*-hexane (conversion 47% after 22 h) and much more slowly in toluene (conversion 37% after 41 h) than that in diisopropyl ether (conversion 48% after 20 h), which all afforded high enantioselectivities (*E* > 68).

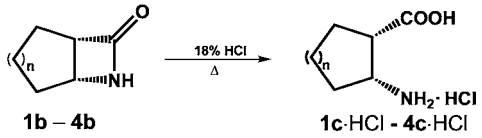
The presence of water in the reaction medium affects the enzymatic activity;[20] accordingly, the enzyme-catalyzed ring opening of (±)-**3** was performed with an increased amount of water (Table 1, entries 5−8 and 10−13). The catalytic activities of the tested Chirazyme L-2 and Lipolase were progressively lowered on increase of the amount of water, though the enantioselectivity was apparently not affected. Furthermore, the degree of hydrolysis of **3** in the presence of Lipolase was complete even without the addition of any water (Table 1, entry 9), but the water in the reaction medium (<0.1%) or present in the enzyme preparation (<5%) was sufficient for the lactam ring opening.

Even though the *E* value in the Lipolase-catalyzed ring opening is excellent, the reaction rate for the ring opening in the case of **3** clearly increases with increasing amount of enzyme (after 23 h, 1 equiv of H_2O, 20 mg mL^{-1} of Lipolase: conv = 42%, ee_s = 69%, ee_p > 95%; after 23 h, 1 equiv of H_2O, 75 mg mL^{-1} of Lipolase: conv. = 50%, ee_s = 94%, ee_p > 95%).

On the basis of the preliminary results, the gram-scale resolutions of (±)-**1**−(±)-**4** were performed with 1 equiv of water in the presence of Lipolase in diisopropyl ether at 60 °C. Despite the long reaction times (very different reaction rates, probably due to the different conformations of the cycloalkane rings), the products were characterized by an excellent enantiomeric excess at 50% conversion. The results are reported in Table 2 and the Supporting Information.

The transformations involving the ring opening of β-lactams **1b**−**4b** with 18% aqueous HCl resulted in the enantiomers of the β-amino acid hydrochlorides **1c**·HCl−**4c**·HCl (Scheme 2). Treatment of amino acids **1a**−**4a** with 22% HCl/EtOH resulted in enantiopure hydrochloride salts **1a**·HCl−**4a**·HCl.

Scheme 2. Ring Opening of β-Lactam Enantiomers **1b**−**4b** with Aqueous HCl



The absolute configurations in the cases of **1** and **2** were proved by comparing the [α] values with the literature data. The value of $[\alpha]^{25}_D$ −5.1 (*c* 0.5, H_2O) for **1a**·HCl and the literature value[21] for (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid hydrochloride, $[\alpha]^{25}_D$ −4.5 (*c* 0.5, H_2O), and the value of $[\alpha]^{25}_D$ −19.4 (*c* 0.5, H_2O) for **2a** and the literature value[17b] for (1*R*,2*S*)-2-aminocyclohexanecarboxylic acid, $[\alpha]^{25}_D$ −19.6 (*c* 0.25, H_2O), indicate (1*R*,5*S*) selectivity for the enzymatic ring opening of (±)-**1** and (1*R*,6*S*) selectivity for the enzymatic ring opening of (±)-**2**. When (1*S*,8*R*)-9-azabicyclo-[6.2.0]dec-4-en-10-one, recently prepared by our group,[17d] was reduced catalytically in the presence of cyclohexene as hydrogen donor, (1*S*,8*R*)-9-azabicyclo[6.2.0]decan-10-one was formed with $[\alpha]^{25}_D$ −16.3 (*c* 0.3, $CHCl_3$). This value is close to the $[\alpha]^{25}_D$ −19 (*c* 0.5, $CHCl_3$) reported for **4b**. Therefore, the absolute configuration for **4b** is (1*S*,8*R*) and that for **4a** is (1*R*,2*S*).

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The gas chromatograms indicate that the corresponding enantiomer of compound **3** reacts similarly as in the cases of **1**, **2**, and **4**. Additionally, it was proved that the ring opening follows the same selectivity for the five-, six-, and eight-membered systems **1**, **2**, and **4**, and it is therefore doubtless that the seven-membered **3** should similarly fit the active pocket of the enzyme. The (1*S*,7*R*) configuration is therefore accepted for the unreactive β-lactam **3b**, and (1*R*,2*S*) for β-amino acid **4a**.

In conclusion, a simple and efficient direct method was developed for the enantioselective ring opening of unactivated β-lactams **1**−**4** in an organic medium. The Lipolase-catalyzed highly enantioselective reactions ($E > 200$), when H_2O (1 equiv) is used in diisopropyl ether at 60 °C, produce β-amino acids (1*R*,2*S*)-**1a**−**4a** and β-lactam enantiomers (1*S*,5*R*)-**1b**, (1*S*,6*R*)-**2b**, (1*S*,7*R*)-**3b**, and (1*S*,8*R*)-**4b**, respectively, in good chemical yield (36−47%). The products could be easily separated. Transformations by ring opening of β-lactams **1b**−**4b** with 18% aqueous HCl resulted in enantiomers of β-amino acid hydrochlorides **1c**·HCl−**4c**·HCl (ee ≥ 99%). The present method of formation of (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid (*cispentacin*) proved to be a very simple, inexpensive route, and could be easily scaled up.

Acknowledgment. The authors acknowledge receipt of OTKA grants T 034901 and TS 040888, FKFP grant 0115/2001, and a Bolyai Fellowship for E.F. (grant No.181/2002).

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

OL034096O