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An Efficient and Practical Chemoenzymatic Preparation of Optically Active Secondary Amines

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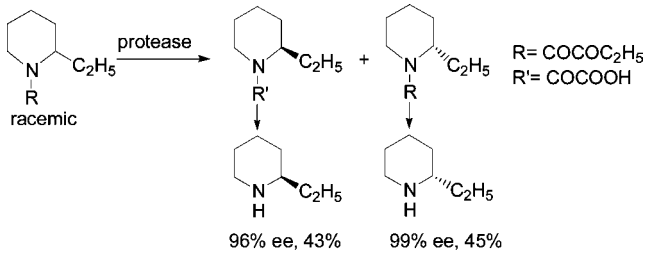
ABSTRACT



An efficient and practical chemoenzymatic method was developed for the preparation of a variety of chiral secondary amines. Here, oxalamic esters were identified as unique derivatives amenable to the enzyme-catalyzed kinetic resolution of racemic secondary amines. Both enantiomers of the amines were produced in high optical purity and yields after the cleavage of the oxalamic groups.

Optically pure secondary amines are important building blocks in the synthesis of biologically active compounds. For example, many current drugs contain optically pure secondary amine moieties. The development of general, efficient, and practical methods for the production of enantiomerically pure secondary amines is still a challenging task.

Although a few methods have been reported for the synthesis of optically pure secondary amines using chiral auxiliaries,[1] asymmetric hydrogenation,[2a,b] or hydrosilylation[2c] of imines, classical diastereomeric resolution is still the preferred choice due to its practicality and economic efficiency.[3] On the other hand, enzyme-catalyzed hydrolysis of secondary amides is difficult probably due to the steric hindrance in the enzyme active site. Despite many enzymatic methods available for the preparation of chiral primary amines,[4a,b] only a few examples have been reported for the enzymatic preparation of chiral secondary amines, and most of them rely on the kinetic acylation of racemic amines with narrow substrate spectrum and low reactivity.[4c,d]

We disclose here an efficient and highly enantioselective enzymatic preparation of chiral aryl−alkyl secondary amines in addition to a variety of *endo*- and *exo*cyclic alkyl-alkyl secondary amines. The oxalamic esters were identified as the unique derivatives amenable to enzyme-catalyzed stereoselective hydrolysis of racemic secondary amines.[5a] Enzymatic resolution of oxalamic esters of a tertiary alco-

(1) (a) Guerrier, L.; Royer, J.; Grierson, D.; Husson, H.-P. *J. Am. Chem. Soc*. **1983**, *105*, 7754. (b). Munchhof, M. J.; Meyers, A. I. *J. Am. Chem. Soc*. **1995**, *117*, 5399.

(2) (a) For a review, see: Ohkuma, T.; Noyori, R. *Comprehensive Asymmetric Catalysis*, Supplement; Springer: New York, 2004; p 43. (b). Willoughby, C. A,; Buchwald, S. L. *J. Am. Chem. Soc*. **1992**, *114*, 7562. (c). Verdaguer, X.; Lange, U. E. W.; Reding, M. T.; Buchwald, S. L. *J. Am. Chem. Soc*. **1996**, *118*, 6784.

(3) (a) Sheldon, R. A. *J. Chem. Technol. Biotechnol*. **1996**, *67*, 1. (b). Vries, T.; Wynberg, H.; van Echten, E.; Koek, J.; ten Hoeve, W.; Kellogg, R. M.; Broxtermann, Q. B.; Minnaard, A.; Kaptein, B.; van der Sluis, S.; Hulshof, L.; Kooistra, J. *Angew. Chem., Int. Ed*. **1998**, *37*, 2349.

(4) (a) For a review, see: van Rantwijk, F.; Sheldon, R. A. *Tetrahedron* **2004**, *60*, 501. (b). Carr, R.; Alexeeva, M.; Enright, Eve, T. S. C.; Dawson, M. J.; Turner, N. J. *Angew. Chem., Int. Ed*. **2003**, *42*, 4807. (c). Orsat, B.; Alper, P. B.; Moree, W.; Mak, C.-P.; Wong, C.-H. *J. Am. Chem. Soc*. **1996**, *118*, 712. (d). Morgan, W. B.; Zaks, A.; Dodds, D. R.; Liu, J.; Jain, R.; Megati, S.; Njoroge, F. G.; Girijavallabhan, V. M. *J. Org. Chem*. **2000**, *65*, 5451 and references therein.

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hol or a primary amine was described in the literature previously.[5b,c]

The general strategy is shown in Scheme 1, using 2-ethylpiperidine (**5a**) as an example.[6] First, the racemic compound **5a** was acylated with ethyl chlorooxoacetate to give **5b**. The formation of oxalamic esters in general proceeds with high yields and chemical purities, and no chromatographic purification is required (see Supporting Information). Subsequent enzymatic hydrolysis showed excellent enantioselectivity ($E > 200$) for both the acid (*R*)-**5c** and ester (*S*)-**5b**, producing optically pure amines (*R*)-**5a** and (*S*)-**5a** after deprotection. The best enzyme *Aspergillus* protease highly selectively hydrolyzes the ester bond rather than the amide function in the oxalamic derivative, which thus produced only the corresponding oxalamic acid, and no free amine was detected from the reaction mixture. The pH of the reaction was kept at 7.0−7.5, and background chemical hydrolysis was suppressed under such conditions. The cleavage of the oxalamic ester from the leftover starting materials or the oxalamic acid from products by refluxing with 4 N HCl proceeds with high yields (90−99% yields, see Supporting Information).

The exploration of the scope of this methodology was assisted by automated enzyme screening in 96-well plate format developed in our lab.[7] The reactions are then analyzed by automated HPLC station to identify the ideal enzyme(s) with the desired reactivity and enantioselectivity.

Interestingly, in almost all cases only proteases were identified with high enantioselectivity (Table 1). Although the esters hydrolyzed are three bonds away from the chiral center, high enantiomeric excesses (ee's) were generally obtained for both the oxalamic esters and acids when the reactions were stopped at a conversion of about 50%.

Scheme 1. Chemoenzymatic Preparation of Enantiomerically Pure (*R*)- and (*S*)-2-ethylpiperidines

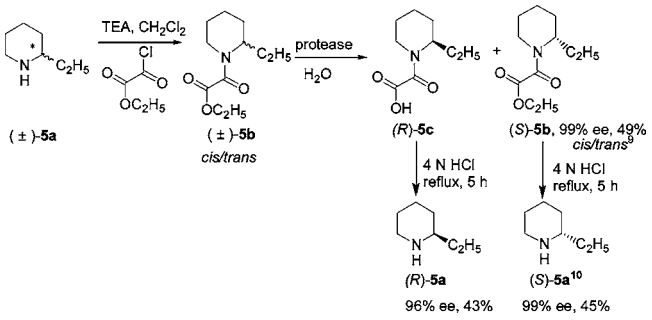
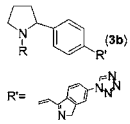


Table 1. Preparation of Optically Pure Secondary Amines via Enantioselective Hydrolysis of Oxalamic Esters

No.	oxalamic ester R=$COCO_2C_2H_5$	protease	conv[a]	ee[b]	E[c]	config[g]
1	 (1b)	*Aspergillus species*	50 (20)[d]	99	>200	R
2	 (2b)	*Aspergillus species*	51 (48)	92	53	R
3	 (3b) R'=	*Bacillus lentus*	48 (50)	91	145[e]	R
4	 (4b)	*Aspergillus species*	55 (42)	99	48	R
5	 (5b)	*Aspergillus species*	51 (49)	99	>200	R
6	 (6b)	*Streptomyces griseus*	50 (37)	99	>200	R
7	 (7b)	*Streptomyces griseus*	53 (47)	94	38	n.d.[h]
8	 (8b)	*Bacillus licheniformis*	43 (57)	77	~145	n.d.
9	 (9b)	*Streptomyces griseus*	53 (47)	95	43	S
10	 (10b)	β-chymotrypsin	42 (58)	58[f]	12	n.d.
11	 (11b)	β-chymotrypsin	50 (49)	95	145	R
12	 (12b)	*Streptomyces sp.*	51 (50)	97	>200	R

[a] Conversions to the corresponding oxalamic acids were calculated from HPLC. Isolated yields for the leftover oxalamic esters are given in parentheses. [b] In most cases, chiral chromatographic methods can be developed more straightforwardly for the oxalamic esters than the corresponding oxalamic acids. The accurate ee was thus only determined for the remaining esters by chiral HPLC and GC methods. For details, please see Supporting Information. [c] The *E* value was calculated from the ee of the oxalamic ester and the conversion.[10] [d] Lower yields were due to material loss in workup. [e] The *E* value was calculated from the ee of oxalamic acid (96% ee). [f] The ee was determined by chemical derivatization of free secondary amine upon deprotection. [g] The absolute configurations of the oxalamic acid were determined by comparing with a chiral standard or a reported optical rotation in the literature.[2] [h] Not determined.

(5) (a) Enzymatic hydrolysis of amides, carbonates, or other linkages has little success for the preparation of chiral secondary amines. (b). Brackenridge, I.; McCague, R.; Roberts, S. M.; Turner, N. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1093. (c). Chapman, D. T.; Crout, D. H. G.; Mahmoudian, M.; Scopes, D. I. C.; Smith, P. W. *Chem. Commun*. **1996**, 2415.

(6) **Typical Procedure.** To a potassium phosphate buffer (43 mL, pH 7.0, 0.1 M) was added *Aspergillus species* protease (2.0 g). Then, **5b** (1.06 g, 5.0 mmol) in acetonitrile (5.0 mL) was added to the solution, and the reaction mixture was stirred vigorously at 23 °C and pH 7.0 controlled by a continuous addition of 1 N NaOH. After about 50% conversion as shown in HPLC (within 24 h), the reaction was quenched and extracted with CH_2Cl_2. After removal of the solvent, the remaining ester **5b** was recovered (0.52 g, 49%). The aqueous layer was adjusted to pH 4.0 and extracted with CH_2Cl_2 to afford the acid **5c** (0.41 g). Then, **5b** and **5c** were refluxed separately with 4 N HCl for 5 h. The reaction mixture was washed with CH_2Cl_2, and the aqueous layer was then brought to pH 10 and extracted with CH_2Cl_2. After evaporation of the solvent, the amines (*R*)-**5a** and (*S*)-**5a** were produced in good yields and optical purity (*R*-**5a**: 43%, 99% ee; *S*-**5a**: 45%, 96% ee).

(7) Yazbeck, D. R.; Tao, J.; Martinez, C. A.; Kline, B. J.; Hu, S. *Adv. Synth. Catal*. **2003**, *345*, 524.

Protease-catalyzed resolution of remote stereocenters from the reacting carbonyl group was reported previously.[8] As shown in Table 1, proteases prefer to hydrolyze the oxalamic esters with (*R*)-stereochemistry.

A variety of (*R*) and (*S*)-2-substituted pyrrolidines can be prepared by this protocol (entries 1−3). Like the pyrrolidines, 2-alkyl and aryl piperidines can also be obtained in high ee's (entries 4−8). It seems that the enzymes are less sensitive to the size of α-substituent. To further examine the scope of this method, an alkyl-aryl amine **9a** was studied, and it was found that the hydrolysis proceeded with good enantioselectivity ($E = 43$). Furthermore, this protocol can also be used to prepare chiral amines where substituents are remote from the amino group (entry 10, 3-substituted piperidine). It is difficult to obtain these two classes of compounds by existing strategies.[1,2]

In addition to endocyclic alkyl-alkyl and aryl-alkyl amines, this methodology can also be successfully applied to the preparation of exocyclic chiral secondary amines (entries 11, 12), where both enantiomers of the amines were produced in high optical purity and yields.

In summary, we have developed an efficient chemoenzymatic route for the preparation of optically pure secondary amines. The proteases disclosed here display a broad acceptance to a variety of amines compatible with a variety of substitutes and functional groups, including *endo*- and *exo*cylic alkyl-alkyl, aryl-alkyl 2-substituted and 3-substituted secondary amines. The intrinsic enantioselectivity of these proteases for most amines tested is high (*E* value ranging from 40 to >200).

One limitation of the current method is that kinetic resolution comes with a maximum of 50% yield. However, as all of the proteases identified here and many racemic amines are inexpensive, this method can be quite economic and practical for large-scale production of optically pure secondary amines and offers advantages over classic resolution where the yields are generally lower. Moreover, the method provides a general and fast way to access both enantiomers from one racemic amine, ideal for drug discovery to identify leads, where both enantiomers are highly desirable for activity and toxicity studies.

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

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(8) Lee, T.; Jones, J. B. *J. Am. Chem. Soc.* **1996**, *118*, 502.

(9) The ratio of *cis* and *trans* for the oxalamic ester (**5b**) is not changed during the enzymatic hydrolysis by ^{1}H NMR, presumably because of the rapid equilibrium between the two conformations under the reaction conditions.

(10) The absolute configurations of the amines were determined by comparing with commercially available standards.

(11) Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.