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Spontaneous Enzymatically Mediated Dynamic Kinetic Resolution of 8-Amino-5,6,7,8-tetrahydroquinoline

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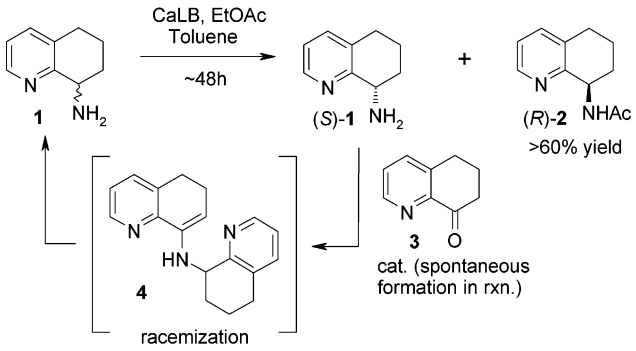
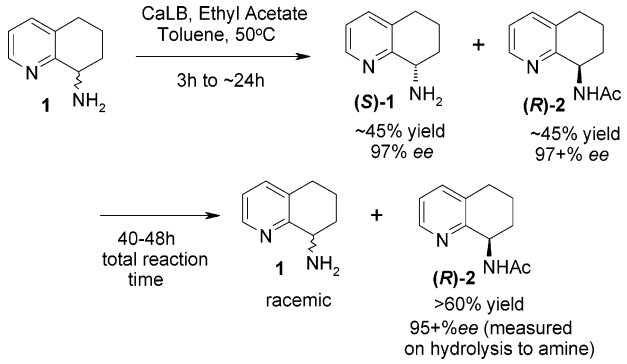


A spontaneous dynamic kinetic resolution of 8-amino-5,6,7,8-tetrahydroquinoline **1** was observed in the presence of *Candida antarctica* Lipase B, in which a >60% yield of (*R*)-acetamide [(*R*)-**2**] was isolated from the racemic amine. The spontaneous formation of ketone **3**, followed by a condensation/hydrolysis sequence with the remaining (*S*)-amine **1**, via enamine **4**, provides the necessary racemization pathway.

Although there are relatively few reports of the application of *Candida antarctica* Lipase B[1] (CaLB) in the resolution of primary amines,[2] the enzyme can provide a reliable and predictable route to access enantiopure amines from their racemic counterparts. Our evaluation of the kinetic resolution of racemic 8-amino-5,6,7,8-tetrahydroquinoline **1** with CaLB,[3] where the (*R*)-enantiomer is selectively acylated [to generate the acetamide (*R*)-**2**], has successfully provided the (*S*)-amine **1** and the (*R*)-acetamide **2** in 48% individual yields and in >98% ee.[4] The enantioselectivity (E)[5] of the reaction is >500.

SCHEME 1



Surprisingly, extended exposure (>36 h) of the amine **1** to CaLB resulted in an unexpected drop in enantiomeric excess of the unacylated amine (*S*)-**1**, such that the eventual reaction mixture was comprised of racemic amine **1** and (*R*)-acetamide **2**: HPLC analysis of an aliquot of the reaction at a 48 h timepoint typically shows 20−30% of amine **1** (<5% ee) and 60−70% of acetamide **2** (Scheme 1). The acetamide **2** can be isolated in a pure form from the reaction mixture in approximately 60% isolated yield (following chromatography) and converted to the corresponding amine (*R*)-**1** in 95+% ee (under hydrolysis conditions[3] which are known to not epimerize the chiral center). These results clearly demonstrate that a >50% yield of a single enantiomer was recovered from a racemic mixture, which requires a racemization mechanism for the (*S*)-amine **1**. In effect, the reaction is a spontaneous dynamic kinetic resolution (DKR).

A few comparisons to the literature can be made. In the case of the CaLB DKR of 1-phenylethylamine to generate (*R*)-*N*-(1-phenylethyl)acetamide, two metal-based hydrogen transfer catalysts have been used as amine racemization agents: Pd(C) (64% yield, 99% ee)[6] and a ruthenium-based hydrogen transfer catalyst (69% yield, >98% ee).[7] A complementary approach to amine racemization can occur via the formation of a Schiff base between a primary amine and a suitable aldehyde. As examples, the DKRs of amino acid esters have been achieved using pyridine carboxaldehydes as imine-forming racemization agents. The alkalase-catalyzed hydrolysis of phenylalanine esters (using 20 mol % of pyridoxal 5-phosphate)[8] and the CaLB-catalyzed ammonolysis of phenylglycine esters (using 0.1−2 mol % of pyridoxal or salicylaldehyde)[9] have been reported.

(1) Anderson, E. M.; Larsson, K. M.; Kirk, O. *Biocatal. Biotransform.* **1988**, *16*, 181−204. The enzyme is commercially available immobilized on polyacrylamide support as Novozym 435.

(2) (a) Van Rantwijk, F.; Hacking, M. A. P. J.; Sheldon, R. *Monatsh. Chem.* **2000**, *131*, 549−569. (b) Choi, Y. K.; Kim, M. J.; Ahn, Y.; Kim, M.-J. *Org. Lett.* **2001**, *3*, 4099−4101. (c) Gonzales-Sabin, J.; Gotor, V.; Rebolledo, F. *Tetrahedron: Asymmetry* **2002**, *13*, 1315−1320. (d) Iglesias, L. E.; Sanchez, V. M.; Rebolledo, F.; Gotor, V. *Tetrahedron: Asymmetry* **1997**, *8*, 2675−2677.

(3) (a) Skupinska, K. A.; McEachern, E. J.; Baird, I. R.; Skerlj, R. T.; Bridger, G. J. *J. Org. Chem.* **2003**, *68*, 3546−3551. (b) Bridger, G.; Skerlj, R.; Kaller, A.; Harwig, C.; Bogucki, D.; Wilson, T. R.; Crawford, J.; McEachern, E. J.; Atsma, B.; Nan, S.; Zhou, Y.; Schols, D.; Smith, C. D.; DiFluri, R. PCT Int. Appl. 2002, WO 0222600.

(4) For an alternative synthesis of 8-amino-5,6,7,8-tetrahydroquinoline via CaLB resolution of 5,6,7,8-tetrahydroquinolin-8-ol, see: Uenishi, J.; Hamada, M. *Synthesis* **2002**, 625−630.

(5) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299.

(6) Reetz, M. T.; Schimossek, K. *Chimia* **1996**, *50*, 668−669.

(7) Pàmies, O.; Éll, A. H.; Samec, J. S. M.; Hermanns, N.; Bäckvall, J. E. *Tetrahedron Lett.* **2002**, *43*, 4699.

(8) D,L-Phe-OBzl converted, for example, to L-Phe in 92% yield and 98% ee: Chen, S.-T.; Huang, W.-H.; Wang, K.-T. *J. Org. Chem.* **1994**, *59*, 7580−7581.

(9) D,L-Phenylglycine methyl ester converted to D-phenylglycine amide with 2% pyridoxal in 85% yield and 88% ee: Wegman, M. A.; Hacking, M. A. P. J.; Rops, J.; Pereira, P.; van Rantwijk, F.; Sheldon, R. A. *Tetrahedron: Asymmetry* **1999**, 1739−1750.

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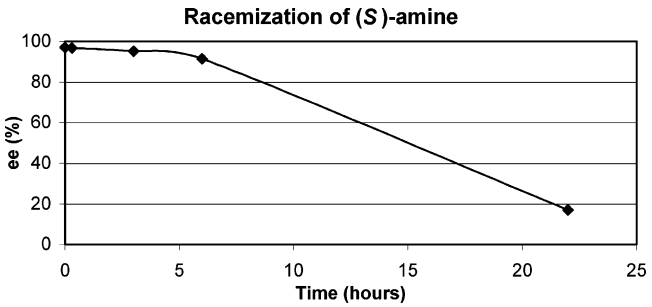
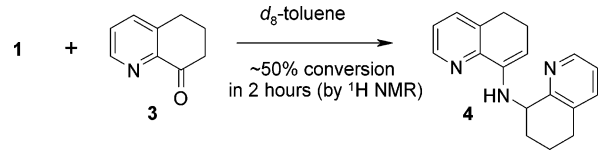


FIGURE 1. Racemization of (*S*)-8-amino-5,6,7,8-tetrahydroquinoline with CaLB.

SCHEME 2



In contrast to the above methods, there appeared to be no obvious chemical species in our resolution reaction mixture that could cause the catalytic racemization of the amine (*S*)-**1**: the reaction mixture is comprised of amine **1**, acetamide **2**, toluene, CaLB (Novozym 435), ethyl acetate, and molecular sieves. A systematic removal of each of the components showed that the Novozym 435 and the ethyl acetate are required for racemization to occur, which suggests that the enzyme is either involved in the racemization directly or is responsible for the generation of a side product which causes racemization. The course of racemization is clearly shown in Figure 1: enantiopure (*S*)-amine (97% ee) was subjected to the standard resolution conditions for an extended duration, and a subsequent drop in enantiomeric excess was measured by Chiral GC analysis of reaction aliquots.

Examination of the crude reaction mixtures by GC and LC-MS following racemization showed that extended exposure of the amine to CaLB in the presence of ethyl acetate resulted in the formation of 5,6,7,8-tetrahydroquinolin-8-one **3**[10] as a side product (in approximately 5% yield relative to the sum of the amine and acetamide). The ketone **3** is not present in measurable quantities (<0.1% by GC measurement) in the reagent amine **1** (in racemic or chiral form). The amine **1** is also stable to the reaction conditions toward oxidative degradation or racemization for >48 h in the absence of CaLB.[11] Hence, the conclusion can be made that the enzyme is responsible for the formation of the ketone **3** from amine **1**.[12] A point of note is that an analogous product of oxidative degradation of 1-phenylethylamine, acetophenone, was reported as a side product during the resolution with CaLB.[6] Presumably, the enzymatic oxidation of the primary amine to an imine, followed by hydrolysis, is responsible for the formation of the side products.

To confirm the condensation reactivity of the ketone **3** with amine **1**, a stoichiometrically equal amount of the two species

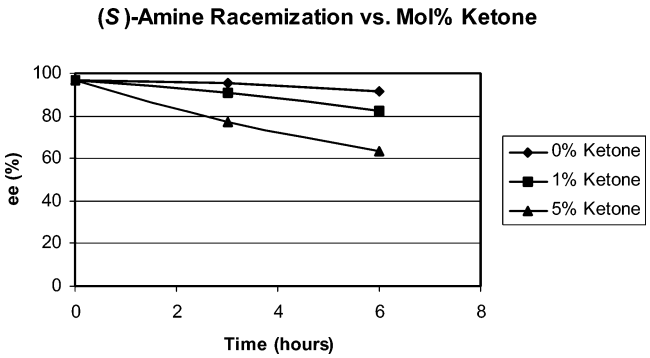


FIGURE 2. Racemization of (*S*)-amine **1** in the presence of ketone **3**.

was mixed in d_8-toluene and examined by ^{1}H NMR, where it was determined that, at room temperature, approximately a 50% conversion to the enamine **4** occurred over 2 h (Scheme 2). The enamine structure can be confirmed by examination of the ^{1}H NMR: the characteristic amine broad singlet appears at 5.82 ppm, along with a triplet at 5.12 ppm ($J = 4.6$ Hz) and a triplet at 4.47 ppm ($J = 4.8$ Hz).[13] Unfortunately, the enamine proved to be unstable toward extractive workups or silica gel chromatography and could not be isolated as a pure species for use as a reagent.

To evaluate the effect of the presence of ketone **3** on the rate of racemization of amine **1**, a series of resolution experiments were conducted in which the enantiopure (*S*)-amine **1** was spiked with varying levels of the ketone (in the presence of CaLB). The results are summarized in Figure 2, in which the rate of racemization of the (*S*)-amine is, in fact, dependent on the relative amount of the ketone: 5 mol % of ketone (relative to amine) gave 63% ee after 6 h, compared with 0 mol % of ketone giving 91% ee at the same timepoint. These results show that the presence of ketone is required in the rate-limiting step in the reaction leading to racemization. Because the condensation between **1** and **3** is spontaneous, it is likely that the enamine **4**, rather than the ketone itself, is actually involved.

Interestingly, the rate of racemization of the (*S*)-amine appears to be accelerated in the presence of an enzyme. In the absence of CaLB, a 24 h exposure of the amine (*S*)-**1** (97% ee) to 5 mol % of ketone in 50 °C toluene (with ethyl acetate) resulted in 89% ee for the amine **1**.

Because the racemization of **1** (via **4**) is accelerated in the presence of CaLB, a potential rationale can be developed in which the lipase catalyzes the hydrolysis of the enamine **4**, which, in turn, would provide a source of racemic amine **1**. From a practical standpoint, the racemization via the ketone-dependent process can be exploited by adding 5 mol % of ketone **3** to the racemic amine **1** prior to exposure to the enzyme. By doing so, the yield of (*R*)-acetamide **2** can be increased from ~60% in the absence of ketone (by HPLC analysis of the reaction mixture) to a 78% isolated yield of acetamide in >98% purity (Scheme 3). Similar to the results described above, the enantiopurity of the acetamide (*R*)-**2** was determined to be 95% ee via measurement of the corresponding amine (*R*)-**1** (following

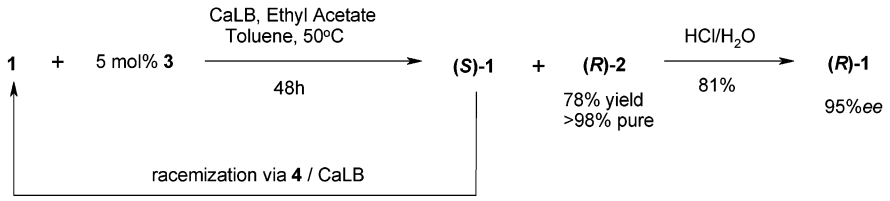
(10) Thummel, R. P.; Lefoulon, F.; Cantu, D.; Mahadevan, R. *J. Org. Chem.* **1984**, *49*, 2208−2212.

(11) (*S*)-**1** (99% pure, 97% ee) heated to 50 °C in 6:1 toluene/ethyl acetate for 48 h. Following concentration, the amine was measured (GC) as 96.5% ee and 98.5% pure.

(12) It should be noted that the (*S*)-amine **1** was stable towards exposure to Ovalbumin in toluene and ethyl acetate at 50 °C for 20 h, so it is not likely that a generic protein is responsible for the oxidative degradation of **1**.

(13) Spectral data from the ^{1}H NMR spectrum in $CDCl_3$: An analogous condensation between an acyl pyridine and a benzylamine to form an enamine has been reported with similar spectral characteristics: Zhong, H. M.; Cohen, J. H.; Abdel-Magid, A. F.; Kenney, B. D.; Maryanoff, C. A.; Shah, R. D.; Villani, F. J.; Zhang, F.; Zhang, X. *Tetrahedron Lett.* **1999**, *40*, 7721−7725.

SCHEME 3



acetamide hydrolysis). Hence, the enzyme maintains a high enantioselectivity for the acetylation of (*R*)-**1**.

Although the applicability of the methodology to other amine systems has not been examined, the results described herein offer a potentially complementary methodology to the existing metal- and enzyme-catalyzed dynamic kinetic resolution of amines.

Experimental Section

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded using $CDCl_3$ solvent with TMS as an internal standard. Column chromatography was performed using 230−400 mesh silica gel, and thin-layer chromatography (TLC) was performed on precoated silica plates. Commercial anhydrous solvents were used. The reagents **1**, (*S*)-**1**, (*R*)-**2**, and **3** were obtained from previously reported methods.[3]

GC data were obtained using a 5% phenyl methyl Si column, and chiral GC data were obtained using a Cylcosil-B column. In both cases, an FID detector was used, and identifications were made by comparison with retention times for pure samples of known origin.

Condensation of Ketone (3) with Amine (1) to give Enamine (4). The ketone (**3**) (500 mg, 3.4 mmol) and the amine (**1**) (500 mg, 3.4 mmol) were dissolved in toluene (10 mL) and heated to 50 °C for 16 h. The mixture was then concentrated. Owing to the hydrolytic instability of the enamine (**4**), chromatographic purification or HPLC analysis was not possible. ^{1}H NMR δ 1.8−2.2 (m, 4H), 2.40 (m, 2H), 2.78−2.85 (m, 5H), 4.82 (t, 1H, J = 4.8 Hz), 5.12 (t, 1H, J = 4.6 Hz), 5.82 (br s, 1H, N*H*), 7.00 (dd, 1H, J = 7.5, 4.8 Hz), 7.10 (dd, 1H, J = 7.8, 4.8 Hz), 7.27 (m, 1H), 7.38 (dd, 1H, J = 7.8, 4.5 Hz), 8.30 (d, 1H, J = 4.5 Hz), 8.48 (d, 1H, J = 4.8 Hz).

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Supporting Information Available: Representative experimental procedures for the resolution experiments, GC and chiral GC traces for isolated samples of **1**, and representative ^{1}H NMR spectra for **1**−**4** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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