Dynamic Chemistry

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Racemase Activity of *B. cepacia* Lipase Leads to Dual-Function Asymmetric Dynamic Kinetic Resolution of α-Aminonitriles**

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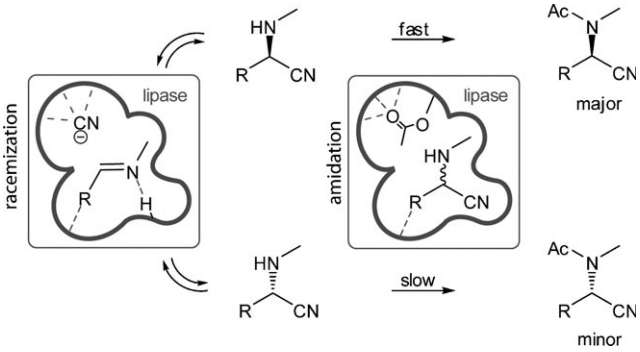
Chirality is certainly one of the most intriguing phenomena in nature. It generally leads to specific activities and properties that differentiate stereoisomers. As a result, there is a high demand for chiral building blocks, and the search for new and efficient methods for the synthesis of enantiomerically pure compounds has been a major area of research in chemistry.[1] Of the many methods developed, the use of biocatalysts remains especially attractive, and enzyme-enabled resolution has become one of the most common ways to prepare enantiomerically pure compounds on an industrial scale, owing to high economic efficiency as well as low environmental impact.[2] In this context, the lipase family of enzymes occupies a privileged position and possesses many advantages for synthetic applications. These enzymes have high commercial availability, are generally robust and amenable to recycling, and can accommodate a broad range of substrates.[2–4] Most examples involve secondary alcohols,[5] but the lipase-mediated asymmetric acylation of primary amines is becoming increasingly common.[6–10] Surprisingly, however, only rare examples concerning secondary amines have been reported.[11–17] We recently addressed this deficiency and demonstrated a method for the generation and screening of dynamic N-substituted α-aminonitrile systems through kinetically controlled lipase-mediated amidation.[17] The core α-aminonitrile (Strecker) structures are synthetically very versatile. For example, the hydrolysis, reduction, or alkylation of the nitrile functionality leads to a multitude of building blocks.[18] Nevertheless, the enzymatic resolution of this class of compounds has not been much explored.[17,19,20] Accordingly, the information that we gained on substrate selectivity and stereoselectivity from the screening process in our study on lipase-mediated amidation prompted us to develop a practical method for the preparation of optically active *N*-methyl α-aminonitrile derivatives.

During these studies, we discovered a novel racemase-type activity of the lipase.[21] Such catalytic promiscuity of enzymes has become an important subject for both enzymologists and organic chemists,[22,23] and could potentially lead to new synthetic organic methodologies.[24] We also found that the racemase activity could be used together with the acylation activity in a coupled process (Scheme 1). Herein, we describe the use of this dual enzyme functionality for the asymmetric synthesis of α-aminonitrile amides in good yields with high enantiomeric purity. A mechanism supported by experimental and computational studies is also proposed for the racemization process.



Scheme 1. General concept of the dual-function lipase-mediated dynamic asymmetric resolution of *N*-methyl α-aminonitriles. Both the acylase activity and the racemase activity of the lipase are operating in the process, in which stereoselective acyl transfer to the secondary amine is accompanied by racemization of the remaining enantiomer.

Dynamic kinetic resolution (DKR) has proven to be a powerful method for the preparation of enantiomerically pure compounds.[25] It circumvents the drawback of traditional kinetic resolution (KR) to give the desired product in greater than 50% yield with reduced need for the separation of unreacted starting materials. The combination of a kinetic resolving agent, for example, an enzyme or a chemocatalyst, with in situ racemization, has thus been applied in a variety of DKR processes.[26] However, the requirement for compatibility between the two processes often presents a major obstacle in the development of such reactions.[27]

In the present study, we initially used compound **1a** (Table 1) to optimize a KR protocol. Reactions were performed at 40 °C in *tert*-butyl methyl ether (TBME) with phenyl acetate as an acyl donor and the lipase from *Burkholderia cepacia* (previously *Pseudomonas cepacia*) as the resolving agent. The reaction was monitored by ^{1}H NMR

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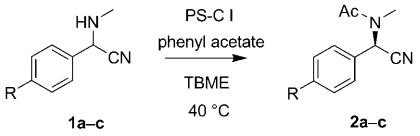
Table 1: Dynamic kinetic resolution of *N*-methyl α-aminonitriles **1a–c** through the lipase-catalyzed amidation/racemization one-pot process.[a]



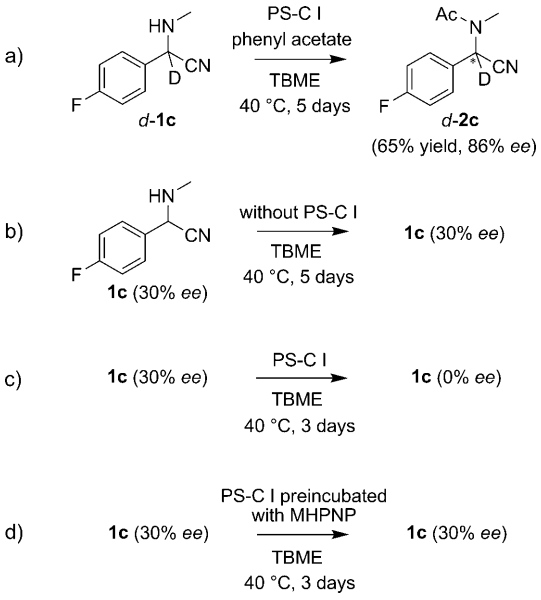
1a–c **2a–c**

Entry	R	Product	*t* [days] ($t_{1/2}$ [days])	Yield[b] [%] (Conv.[c] [%])	*ee*[d] [%]
1	H	**2a**	8 (1.34)	90 (94)	85
2	OCH_3	**2b**	7 (1.13)	89 (92)	86
3	F	**2c**	13 (2.36)	87 (92)	88

[a] Reactions were carried out with **1** (0.05 mmol), lipase PS-C I (400 mg), and phenyl acetate (0.15 mmol) in TBME (1.5 mL) at 40°C. [b] Yield of the isolated product. [c] The conversion was determined by ^{1}H NMR spectroscopy. [d] The *ee* value was determined by HPLC analysis on an OD-H or OJ column.

spectroscopy and HPLC. Surprisingly, conversion into the final product **2a** exceeded the maximum KR yield but still proceeded with high enantioselectivity. Thus, after two days, a conversion of 60% and an enantiomeric purity of 88% *ee* were recorded, whereas the remaining starting material **1a** remained racemic. The process reached completion (94% conversion into **2a** with 85% *ee*) after a reaction time of eight days (Table 1, entry 1). This result clearly indicates that the transformation of compound **1a** into **2a** occurred through a DKR process, rather than the expected KR process, under the conditions used. Other *N*-methyl α-aminonitrile derivatives, **1b** and **1c**, were subsequently subjected to the same reaction conditions. The results for these structures were similar to those for compound **1a**, although the reaction rates were different. Similar rates of DKR were observed for compounds **1a** ($t_{1/2} = 1.34$ days) and **1b** ($t_{1/2} = 1.13$ days), which contain unsubstituted or electron-rich aromatic moieties (Table 1, entries 1 and 2). In contrast, compound **1c**, with an electron-withdrawing aromatic substituent, showed the slowest rate ($t_{1/2} = 2.36$ days); the reaction required 13 days to reach completion (Table 1, entry 3).[28] A similar longer reaction time was observed for the corresponding 3-methoxy-substituted aromatic *N*-methyl α-aminonitrile derivative, the reaction of which also resulted in lower enantiomeric purity (see the Supporting Information). The reaction of primary amines resulted in good conversions, but no enantioselectivity was observed under these conditions.

In principle, racemization of the starting compounds **1a–c** must be a key step in the DKR process. Two possible mechanisms were hypothesized for this racemization: 1) abstraction of the α hydrogen atom of the starting *N*-methyl α-aminonitrile by a base, or 2) in situ bond cleavage through a retro-Strecker reaction. We performed various control experiments to challenge these hypotheses. First, the stability of the α hydrogen atom of the starting compounds was examined. Our previous studies demonstrated that the α hydrogen atom of the final products is sensitive to the presence of a base and relatively easily abstracted, which results in racemization.[17] Moreover, previous KR studies with *Candida antarctica* lipase B showed generally low enantioselectivity for the formation of the acylated product. This low enantioselectivity was explained in terms of unexpected turnover-related racemization through abstraction of the α hydrogen atom.[20] We thus tested compound **1c** with a variety of organic acids and bases together with D_2O to probe the potential deuterium exchange at the α position. However, no exchange was observed under these conditions, even with strong acids or bases at elevated temperatures.[28] Next, α-deuterated **1c** (*d*-**1c**) was prepared and subjected to the DKR conditions (Scheme 2a). Residual water and/or protein acidic



Scheme 2. Control experiments supporting the proposed lipase-mediated retro-Strecker racemization mechanism.

hydrogen atoms in the system would then serve as proton donors. The same yield and enantioselectivity were observed as for **1c**, and no deuterium/proton exchange was observed for compound *d*-**1c** or the corresponding product *d*-**2c**.[24] It has also been proposed that aldehydes may catalyze this process. Therefore, deuterium-exchange experiments were carried out in the presence of 4-fluorobenzaldehyde (up to 30%). The presence of the aldehyde did not, however, lead to any exchange (and hence to racemization) under these conditions. These results reject the first hypothesis that the racemization mechanism will pass through abstraction of the α-proton of the starting Strecker compounds, where neither enzymatic nor non-enzymatic processes are productive.

To test the second hypothesis, we prepared solutions of the nonracemic compound **1c** (30% *ee*) in situ and subjected them to three different potential sets of racemization conditions (see the Supporting Information for details). The first experiment was performed under non-enzymatic conditions. The solution of compound **1c** (30% *ee*) was thus allowed to stand at 40°C for up to five days. No racemization was observed under these conditions during this period (Scheme 2b). In contrast, when the solution of compound **1c** (30% *ee*) was treated with the *B. cepacia* lipase at the same

temperature, racemization occurred. As expected, the *ee* value dropped from 30 to 10% in two days; 0% *ee* was reached within three days (Scheme 2c). These results indicate that the racemization process is exclusively mediated by the lipase.

To elucidate the importance of the active site of the enzyme, we performed a third racemization test. The lipase was thus first inactivated by methyl 4-nitrophenyl hexylphosphonate (MNPHP), an established irreversible inhibitor of lipases.[5c,29] The resulting enzyme preparation exhibited no catalytic activity under the acylation conditions used. The inhibited enzyme was then treated with the solution of compound **1c** (30% *ee*) at 40°C for three days. No racemization was observed (Scheme 2d), which implies that the racemization and the transacylation processes take place at the same site of the enzyme. In addition to these studies, where a commercial preparation of *B. cepacia* was used, a preparation involving a pure lipase sample immobilized on the same carrier was analyzed.[28] As expected, the same results were observed with this preparation, and it could be concluded that the racemase activity emanated from the lipase. From these control experiments, the racemase activity could be confirmed. The results also indicate that the transformation occurs specifically with the starting Strecker compounds, a conclusion further supported by experiments in which cyanohydrins were used instead of Strecker compounds under the DKR conditions; in this case, no racemization was observed. Other structures based on the N-substituted α-aminonitrile core structure are, however, suitable substrates, which extends the scope of the reaction. In addition, the racemization can also be observed using other lipases, both immobilized and non-immobilized, not affecting the acylation selectivity, in contrast to the results for *Candida antarctica* lipase B.[20] All these results support the hypothetical lipase-mediated retro-Strecker racemization mechanism (see Figure S12 in the Supporting Information for more details).

Additional support for the racemase-type activity was acquired from molecular dynamics (MD) simulations and density functional theory (DFT) calculations. MD simulations were performed for both aminonitrile enantiomers complexed to the *B. cepacia* lipase.[28] The results showed that both enantiomers are tightly bound to the oxyanion hole through hydrogen bonds to the cyano group (Figure 1a). Strong hydrogen bonding is also observed between the N^{ε} atom of His286 and the substrate amino group (S3). This interaction locks the substrates in a rather rigid position in the active site. Moreover, a fluctuating hydrogen bond observed between Ser87 O^{γ} and the α nitrogen atom (S4) may play a role in the transition state. The hydrogen-bond network of each enantiomer is consistent with a general-base-catalyzed elimination mechanism in which His286 abstracts the proton of the amino group, and the transition state and the intermediate cyanide ion are stabilized by the oxyanion hole.

We subsequently performed hybrid DFT calculations to validate the reaction mechanism.[28] The model system, taken from the simulation for the *R* aminonitrile enantiomer, is displayed in Figure 1b and shows the optimized geometry of the transition state at the B3LYP/6-31+G(d) level. The calculations indicate a concerted mechanism in which proton abstraction from the amino group precedes the leaving of the cyanide ion. The whole catalytic machinery of the lipase is active; however, Ser87 evidently acts more like an auxiliary hydrogen-bond donor than a nucleophile, as predicted by the MD simulations. The activation energy was calculated to be 21 kcal mol^{-1} for the rate-limiting transition state. This value is in reasonable agreement with the experimentally observed rate.

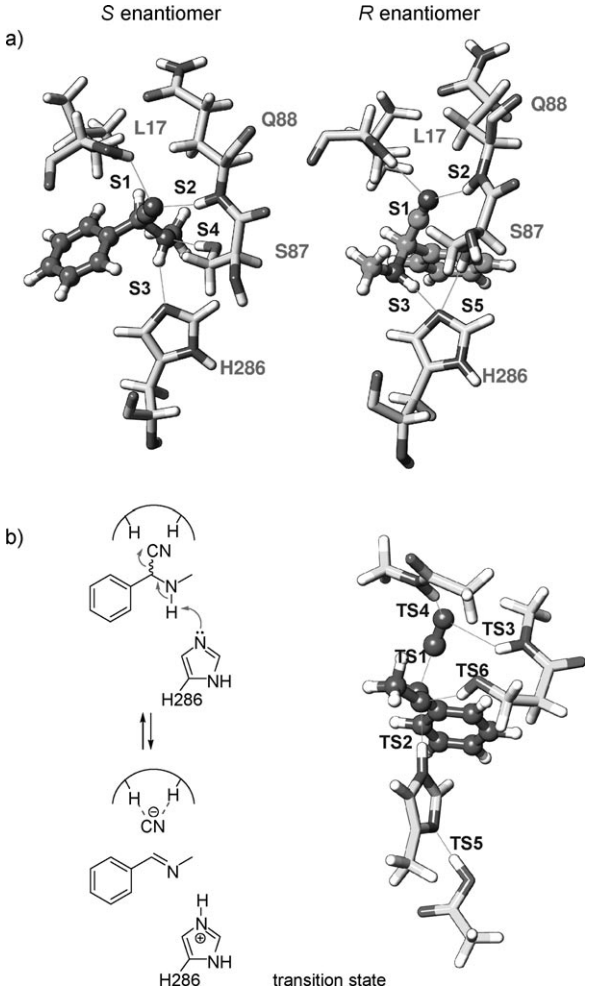


Figure 1. a) Snapshot of MD simulations of the *S* (left) and *R* aminonitriles (right) with selected active-site residues. Hydrogen bonds are depicted as thin lines. b) Model transition state (*R* aminonitrile). Annotated distances [Å]: TS1 = 2.02, TS2 = 1.79, TS3 = 2.89, TS4 = 2.05, TS5 = 1.62, TS6 = 1.91 (see the Supporting Information for details).

In conclusion, we have discovered that a wild-type lipase enzyme shows racemase-type activity, specifically for the N-substituted α-aminonitrile (Strecker) core structure. The racemization mechanism is proposed to proceed through a C–C bond-breaking/forming retro-Strecker/Strecker reaction catalyzed at the hydrolase active site. Moreover, we have demonstrated a convenient approach to the asymmetric synthesis of *N*-methyl α-aminonitrile derivatives by a lipase-catalyzed direct one-pot reaction that relies on the combined racemase activity and normal lipase transesterification activity of the enzyme. The spontaneous communication between

these two processes through a single catalyst led to the desired products in good yields with high enantiomeric purity.

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