Dynamic Chemistry

Efficient Asymmetric Synthesis of 1-Cyano-tetrahydroisoquinolines from Lipase Dual Activity and Opposite Enantioselectivities in α-Aminonitrile Resolution

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Abstract: Dual promiscuous racemization/amidation activities of lipases leading to efficient dynamic kinetic resolution protocols of racemic α-aminonitrile compounds are described. α-Aminonitrile products of high enantiomeric purity could be formed in high yields. Several lipases from different sources were shown to exhibit the dual catalytic activities, where opposite enantioselectivities could be recorded for certain substrates.

Enzyme-catalyzed chemical reactions have witnessed concurrent growth on both the laboratory and industrial scales in recent years, generally due to high reaction efficiencies and low negative environmental impact.[1–6] Enzymes are recognized as efficient catalysts for synthetic transformations, typically associated with high chemo-, regio-, and enantioselectivities, and have been used in preparations of key intermediates of high enantiomeric purities in, for example, the synthesis of pharmaceutically active species.[4,7] Although enzymes are highly specific to their catalytic transformations and substrate acceptances, it is highly challenging for enzymologists and organic chemists to extend the enzyme catalytic scope in organic synthesis. Owing to directed evolution methodologies, enzymes can, for example, often be modified to better suit certain transformations, reaction conditions, or classes of compounds.[8] Furthermore, discovery of new activities for a specific enzyme, known as enzyme catalytic promiscuity, has become increasingly important, generally based on detailed understanding of the catalytic mechanisms of the enzymes.[3,6,9–13]

Lipases, belonging to the hydrolase class of enzymes, possess several advantageous features and are widely applied in organic synthesis. These enzymes are commercially available from many sources, show high tolerance to many reaction conditions, and display broad substrate specificities.[13,14] Lipases are also known to be promiscuous enzymes, and have been used in a variety of transformations.[15–28]

A particularly useful methodology to enantipure compounds relies on dynamic kinetic resolution (DKR), a process based on the combination of in situ racemization and kinetic resolution.[29–33] The main challenge in the accomplishment of efficient DKR processes is finding racemization and kinetic-resolution processes that are mutually compatible under suitable reaction conditions.

Recently, we have reported a promiscuous dual activity of the lipase from Burkholderia cepacia, where the enzyme displayed both amidation and racemization activities towards N-methyl α-aminonitriles.[24] This resulted in the dynamic kinetic resolution of the corresponding N-methyl α-acetamidonitriles of high enantiomeric purities in high yields, where both steps were catalyzed by the same enzyme. Asymmetric synthesis of α-aminonitriles and their derivatives is of high interest in organic synthesis, because these compounds can be transformed into optically active α-amino acids and pharmaceutically interesting compounds.[34,35]

Herein, the scope of the promiscuous dual function of lipases in dynamic kinetic asymmetric resolution protocols is presented. Unexpectedly, it could be shown that lipases from different sources exhibited significantly different activities toward α-aminonitriles, providing the desired amide products with opposite configurations (Scheme 1). In addition, cyclic substrates based on the 1,2,3,4-tetrahydroisoquinoline motif proved to be

Scheme 1. Promiscuous dual activity of lipases resulting in dynamic kinetic asymmetric resolution of α-aminonitriles in a one-pot process, in which stereospecific amidation operates in sequel to racemization.
especially applicable to the concept, resulting in products of high enantiopurity in high yields.

The lipase-catalyzed racemization and asymmetric amidation was first applied to the transformation of α-aminonitriles 1a–d, 1-Aminocyclpentane-2,2,2-trifluoroethyl)acetone (TFEA), and ethyl acetate, were also applied to the proved conversions in the latter cases (11 and 4 %).

Different acyl donors: phenyl acetate, 2,2,2-trifluoroethyl acetate (TFEA), and ethyl acetate, were also applied to the enzyme-catalyzed reactions. The enantiomeric excess (ee) of amide product 2c from the reactions using ethyl acetate was slightly higher than using the other acyl donors. Moreover, 100 mg of lipase PS-C I and 50 mg of Novozyme 435 were sufficient to catalyze the transformation of amiononitriles 1a–d to the corresponding amide products 2a–d in similar reaction times.

After initial optimization of the dual lipase-catalyzed racemization and asymmetric amidation, the enzymatic reactions of α-aminonitriles 1a–d were performed in tert-butyl methyl ether (TBME) at room temperature by using ethyl acetate as acyl donor. The results of the reactions were followed by 1H NMR spectroscopy and chiral HPLC. Unexpectedly, amide products 2a–c formed under the same reaction conditions using the two different lipase preparations, Novozyme 435 and lipase PS-C I, provided the opposite absolute configuration, respectively, in all cases (entries 1–6, Table 1).

Table 1. Catalytic activities and stereoselectivities of lipase-catalyzed racemization and asymmetric amidation of compounds 1a–d.

| Entry | Product | Lipase Loading [mg] | Time [days] | Conversion [%] | ee [%] |
|-------|---------|---------------------|-------------|----------------|--------|
| 1     | 2a      | Novozyme 435        | 50          | 98 (94)        | (--) 83|
| 2     | PS-C I  | 100                 | 10          | 97 (92)        | (+) 15 |
| 3[4]  | 2b      | Novozyme 435        | 50          | 32 (30)        | (--) 97|
| 4[4]  |         | PS-C I              | 100         | 32 (33)        | (+) 52 |
| 5     | 2c      | Novozyme 435        | 50          | 10             | 95 (89) |
| 6     | PS-C I  | 100                 | 10          | 95 (90)        | (+) 37 |
| 7[4]  | 2d      | PS                  | 100         | 4              | 11 (+) 56|
| 8[4]  | PFL     | 100                 | 4           | 4              | (+) 62 |
| 9     | 2d      | PS                  | 100         | 10             | 95 (89) |
| 10    | PS-C I  | 100                 | 10          | quant. (93)    | (--) 37|

[a] Reactions carried out with compound 1 (0.05 mmol), ethyl acetate (3 equiv), TMSCN (0.01 equiv), and lipase in TBME at RT. (b) Followed by chiral HPLC analysis and 1H NMR spectroscopy. [c] Determined by chiral HPLC analysis on an OJ column; see the Supporting Information. [d] 63 % 2,3-bis(benzyldieneamino)-4-phenylacetamide was formed. [e] 59 % 2,3-bis(benzyldieneamino)-4-phenylacetamide was formed. [f] Reactions performed at 40 °C.

Using lipase preparation PS-C I, very similar yields/conversions as for Novozyme 435 were obtained, and amide products (+)-2a, (+)-2c, and (+)-2d were produced in 89–92 % yield, whereas product (+)-2b was formed at a lower rate (35 % yield). In the latter reaction from compound rac-1b, by-product 3 was again formed. However, the enantiomeric excesses of the products, of opposite configuration compared to the products formed using Novozyme 435, were lower than for the CAL-B-catalyzed reactions (0–37 % ee). This effect is likely due to lower rates of the racemization step using PS-C I, compared with higher rates of the asymmetric amidation step. Attempts to improve the results by using silica gel as additive in the enzymatic reaction using lipase preparation PS-C I did not result in any enhancement in these cases.

To further evaluate the lipase catalytic activities in racemization and asymmetric amidation, cyclic amiononitrile structures were subsequently probed. Thus, 1-cyano-1,2,3,4-tetrahydroisoquinolines 4a and 4b, representing important intermediates for isoquinoline alkaloid syntheses,[39] were applied as amiononitrile substrate candidates in the DKR process. Stereoselective acylation of this class of compounds, for example, accomplished by using metal-based catalysts,[40] organocatalysts,[41, 42] and chiral auxiliaries,[43] results in optically active isoquinoline Reissert-type products. In the present case, Novozyme 435, PS-C I, PS, and PFL were evaluated as racemizing and resolving agents, and the enzymatic reactions were performed in TBME at 40 °C by using phenyl acetate as acyl donor. The reactions were followed by 1H NMR spectroscopy and chiral HPLC (Table 2). To compare the catalytic activities, the different enzyme preparations were first applied in equal
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In conclusion, it has been demonstrated that combined, dual-function, lipase-catalyzed racemization and asymmetric amidation can be efficiently used to produce different α-aminonitrile products in good yield and enantiopurity. The dual function appears to be a general feature for several lipases, where preparation using lipases from _Candida_ and _Burkholderia_ gave the best results with excellent yield and enantiopurity. The product configurations did not vary between the enzymes in this case. These results showed that dual-function lipase promiscuity resulted in simultaneous racemization and asymmetric amidation of α-aminonitriles, thus providing a useful synthetic method to optically active α-aminonitrile amide derivatives.

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1. B. M. Nestl, S. C. Hammer, B. A. Nebel, B. Hauer, _Angew. Chem. Int. Ed._ 2014, 53, 3070–3095.
2. M. Rachwalski, N. Vermue, F. P. J. T. Rutjes, _Chem. Soc. Rev._ 2013, 42, 9268–9282.
