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

Morakot Sakulsombat,[a] Pornrapee Vongvilai,[a, b] and Olof Ramström*[a]

Abstract: Dual promiscuous racemization/amidation activities of lipases leading to efficient dynamic kinetic resolution protocols of racemic α-aminonitrile compounds are described. α-Amidonitrile 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.

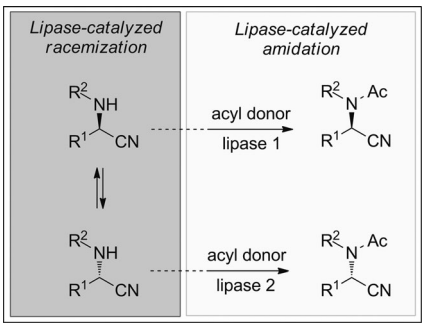
Enzyme-catalyzed chemical reactions have witnessed concurrent growth on both the laboratory and industrial scales in recent years, generally due to high reaction efficiencies and low negative environmental impact.[1–6] Enzymes are recognized as efficient catalysts for synthetic transformations, typically 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 enantiopure 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.

[a] *Dr. M. Sakulsombat, Dr. P. Vongvilai, Prof. Dr. O. Ramström*
KTH Royal Institute of Technology Department of Chemistry
Teknikringen 30, 10044 Stockholm (Sweden)
E-mail: ramstrom@kth.se

[b] *Dr. P. Vongvilai*
Present address: BioNet-Asia Co., Ltd. 19 Soi Udomsuk 37
Sukhumvit 103 road, Bangkok 10260 (Thailand)

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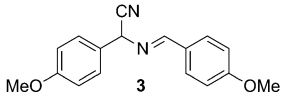
especially applicable to the concept, resulting in products of high enantiopurity in high yields.

The lipase-catalyzed racemization and asymmetric amidation was first applied to the transformation of α-aminonitriles **1a–d**. 1-Amino-2-(4-fluorophenyl) acetonitrile **1c** was selected as a candidate for the initial lipase screening (entries 5–8, Table 1). Four different lipase preparations were evaluated: Novozyme 435 (immobilized lipase CAL-B from *Candida antarctica*) lipase PS from *Burkholderia cepacia*, lipase PFL from *Pseudomonas fluorescens*, and immobilized PS-C I from *Burkholderia cepacia*. Of these, only reactions catalyzed by Novozyme 435 and lipase PS-C I provided good conversions (95%) to amide products **2c** at room temperature (entries 5–6), whereas no products were formed by using the PS and PFL preparations. Raising the temperature to 40 °C resulted in only marginally improved conversions in the latter cases (11 and 4%).

Different acyl donors: phenyl acetate, 2,2,2-trifluoroethyl acetate (TFEA),[36] 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 aminonitriles **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 ^{1}H 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). For the enzyme-catalyzed reactions using Novozyme 435, amide products (−)-**2a**, and (−)-**2c** were produced in very good to excellent yields (89–94%) and very good *ee* values (83–89%). Amide product (−)-**2b** was formed at lower yield (30%) under these conditions, but with an excellent enantiopurity (97% *ee*). Nitrile **3** was, in this case, formed as a by-product, resulting from the reaction between aminonitrile **1b** and (4-methoxyphenyl)methanimine, in turn formed from compound **1b** during the enzymatic reaction.[37] For the somewhat larger amide product (−)-**2d**, the contrary effect was instead recorded, quantitatively formed from *rac*-**1d** but with lower resulting enantiomeric excess (37%). This is likely due to an impaired racemization step in this case due to the larger substrate structure.



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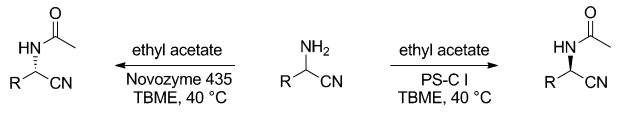
Using lipase preparation PS-C I, very similar yields/conversions as for Novozyme 435 were obtained, and amide products (+)-**2a**, (+)-**2c**, and (+)-**2d** were produced in 89–92% yield, whereas product (+)-**2b** was formed at a lower rate (35% yield). In the latter reaction from compound *rac*-**1b**, 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.[38]

To further evaluate the lipase catalytic activities in racemization and asymmetric amidation, cyclic aminonitrile structures were subsequently probed. Thus, 1-cyano-1,2,3,4-tetrahydroisoquinolines **4a** and **b**, representing important intermediates for isoquinoline alkaloid syntheses,[39] were applied as aminonitrile 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 ^{1}H NMR spectroscopy and chiral HPLC (Table 2).

To compare the catalytic activities, the different enzyme preparations were first applied in equal

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



(-)-**2a**; R = Ph
(-)-**2b**; R = 4-OMePh
(-)-**2c**; R = 4-FPh
(-)-**2d**; R = Ph(CH$_2$)$_2$

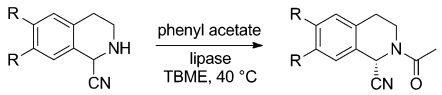
rac-**1a**; R = Ph
rac-**1b**; R = 4-OMePh
rac-**1c**; R = 4-FPh
rac-**1d**; R = Ph(CH$_2$)$_2$

(+)-**2a**; R = Ph
(+)-**2b**; R = 4-OMePh
(+)-**2c**; R = 4-FPh
(+)-**2d**; R = Ph(CH$_2$)$_2$

Entry	Product	Lipase	Loading [mg]	Time [days]	Conversion [%][b]	*ee* [%][c]
1	**2a**	Novozyme 435	50	9	98 (94)	(−) 83
2		PS-C I	100	10	97 (92)	(+) 15
3[d]	**2b**	Novozyme 435	50	12	33 (30)	(−) 97
4[e]		PS-C I	100	12	38 (35)	(+) 52
5	**2c**	Novozyme 435	50	10	95 (89)	(−) 89
6		PS-C I	100	10	95 (90)	(+) 37
7[f]		PS	100	4	11	(+) 56
8[f]		PFL	100	4	4	(+) 62
9	**2d**	Novozyme 435	50	10	quant. (93)	(−) 37
10		PS-C I	100	10	95 (89)	0

[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 ^{1}H NMR spectroscopy. [c] Determined by chiral HPLC analysis on an OJ column; see the Supporting information. [d] 63% α-Benylideneamino-α-phenylacetonitrile was formed. [e] 59% α-Benylideneamino-α-phenylacetonitrile was formed. [f] Reactions performed at 40 °C.

Table 2. Catalytic activities and stereoselectivities of lipase-catalyzed racemization and asymmetric amidation of cyclic compounds **4a** and **b** by using different lipase preparations.[a]



rac-**4a**; R = H
rac-**4b**; R = OMe

(-)-**5a**; R = H
(-)-**5b**; R = OMe

Entry	Product	Lipase	Loading [mg]	Time [days]	Conversion [%][b]	*ee* [%][c]
1	**5a**	Novozyme 435	100	3	quant. (95)	95
2			50	3	quant.	95
3			25	3	quant.	87
4		PS-C I	100	2	95	39
5			200	2	99	47
6			400	2	quant.	60
7			100	2	97	75[d]
8		PS	100	3	26	7
9		PFL	100	3	68	48
10	**5b**	Novozyme 435	100	2	quant. (92)	97
11			50	3	quant.	73
12			25	3	quant.	64
13		PS-C I	100	2	97	54
14			200	2	quant.	86
15		PS	100	3	30	0
16		PFL	100	3	65	60

[a] Reactions carried out with compound **1** (0.05 mmol), phenyl acetate (3 equiv, 0.15 mmol), TMSCN (0.01 equiv), and lipase in TBME at 40 °C [b] Followed by chiral HPLC analysis and ^{1}H NMR spectroscopy; isolated yields in parentheses [c] Determined by chiral HPLC analysis on an OD-H column; see the Supporting information [d] SiO_2 (10 mg) was added.

amounts (100 mg) under the same reaction conditions. In general, the enzyme-catalyzed reaction rates from compounds **4a** and **b** were considerably higher than those for compounds **1a**–**d**. Among the lipase preparations, Novozyme 435 and PS-C I proved again to be better than either PS or PFL, in which the reactions from compound **4a** reached completion within three days, providing Reissert-type product **5a** in 99 and 39% *ee*, respectively. In contrast to the results obtained for the noncyclic α-aminonitriles, the same absolute configuration were obtained with both enzyme preparations (entries 1 and 4, Table 2). Lipase preparations PFL and PS also exhibited dual catalytic activities in the resolution of compound **4a**, but their activities were less efficient. Attempts to improve the results by using silica gel were again performed for the enzymatic reaction using lipase preparation PS-C I.[38] In this case, this resulted in higher enantiomeric excess of amide product **5a**, leading to 97% conversion and 75% *ee* (entry 7, Table 2). The amount of enzyme loading was also screened in the enzymatic reactions using Novozyme 435 and PS-C I. With decreasing amounts of Novozyme 435, the catalytic rate remained unchanged, but the enantiomeric purities decreased. For lipase preparation PS-C I, the catalytic rate and the stereoselectivities decreased dramatically upon lower enzyme loading. However, the opposite effect was recorded with increased enzyme loading, from 100 to 400 mg, when the enantiomeric excess of product **5a** increased from 39 to 60% (entries 4–6, Table 2). These results are indicative of higher racemization rates with higher enzyme loading, thereby improving the product enantiopurity.

Lipase-catalyzed racemization and asymmetric amidation of 6,7-dimethoxy-1-cyano-1,2,3,4-tetrahydroisoquinoline **4b** using different enzyme preparations displayed the same trend as the results for compound **4a** (entries 10–16, Table 2). Novozyme 435 and lipase PS-C I (100 mg) provided complete transformations to compound **5b** within three days in 97 and 54% *ee*, respectively, with the same product configuration (entries 10 and 13). Similarly, lipase-preparation PFL exhibited fairly good asymmetric transformation of compound **4b** with 65% yield (60% *ee*) in three days. The result from lipase PS, on the other hand, showed poor activities with respect to both transformation and stereoselectivity, providing only 30% conversion without any enantiomeric preference of product **5b** (entry 15, Table 2). The amount of enzyme loading for Novozyme 435 was also varied, and the results indicate that 100 mg of Novozyme 435 was optimal for the asymmetric transformation of compound **4b** to **5b**. When increasing the amount of lipase preparation PS-C I to 200 mg, amide product **5b** was produced at quantitative conversion with 86% *ee*.

In conclusion, it has been demonstrated that combined, dual-function, lipase-catalyzed racemization and asymmetric amidation can be efficiently used to produce different α-amidonitrile products in good yield and enantiopurity. The dual function appears to be a general feature for several lipases, where preparation using lipases from *Candida*, *Burkholderia*, and *Pseudomonas sp.* all resulted in product formation. The lipase preparations Novozyme 435, and PS-C I were most efficient in the present cases. In addition, it could be shown that the enantiopreferences of the enzyme preparations varied, and noncyclic α-amidonitrile products of opposite configuration were formed by using Novozyme 435 and PSC I, respectively. Cyclic substrates were also evaluated, and 1-cyano-1,2,3,4-tetrahydroisoquinolines **4a**–**b** were efficiently transformed by using different lipase preparations. Novozyme 435 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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Keywords: amidation · asymmetric synthesis · dynamic chemistry · enzymes · lipases · racemization

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