Enantioconvergent Processes

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A Simple Enantioconvergent and Chemoenzymatic Synthesis of Optically Active α-Substituted Amides**

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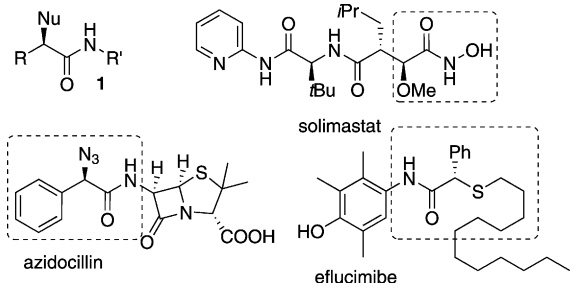
Enantiopure amides **1**, substituted in the α position with an azide or a simple N, O, or S substituent (Scheme 1), form an important class of compounds and are key intermediates in medicinal chemistry. The α-azidoamide motif (**1**, Nu = N_3) is found in bioactive molecules, such as the antibiotic azidocillin (Scheme 1). The azide group is considered to be an isostere of the amino group, however, it has increased bulkiness and is not positively charged at physiological pH values.[1] The introduction of an azide function into the α position of peptides is also aimed at the synthesis of enzyme inhibitors that, after binding to their targets and subsequent Staudinger-type ligation with biotin–phosphine, are subjected to streptavidin pull-down.[2] Furthermore, enantiopure α-azidoamides are important reagents for "click" chemistry[3] and valuable precursors in the synthesis of amino acids[4] and peptides.[5,6]



Scheme 1. General structure of **1** and examples of bioactive compounds derived from **1**.

The chiral scaffold of an amide with a simple O substituent (**1**, Nu = OR) in the α position can be found in numerous bioactive compounds, such as solimastat (Scheme 1), which is an inhibitor of matrix metalloproteinase,[7] and other anticancer[8] and anticonvulsant[9] agents. Bioactive molecules that bear a chiral amide motif with an S substituent (**1**, Nu = SR) include lipid-lowering agent eflucimibe,[10] TNFα convertase inhibitors,[11] and renin inhibitors.[12]

Considering the importance of the broad family of enantiopure α-heterosubstituted amides **1**, we decided to investigate the possibility of designing an enantioconvergent and chemoenzymatic process for their preparation. Herein, we present a time- and cost-efficient synthetic route that is characterized by high enantioselectivity and product versatility, and takes advantage of the intrinsically compatible product reactivity of haloalkane dehalogenase catalyzed reactions.

Enantioconvergent processes are transformations in which both enantiomers of a chiral substrate are converted into the same enantiomer of the product through different pathways.[13] Such processes allow the highly efficient transformation of cheap racemic starting materials into valuable enantiopure products by increasing the yield beyond the theoretical value of 50%, which is the yield that can be obtained from the common transformation of only one enantiomer, for example, by kinetic resolution. Chemoenzymatic enantioconvergent syntheses can be carried out in three general ways (Figure 1).

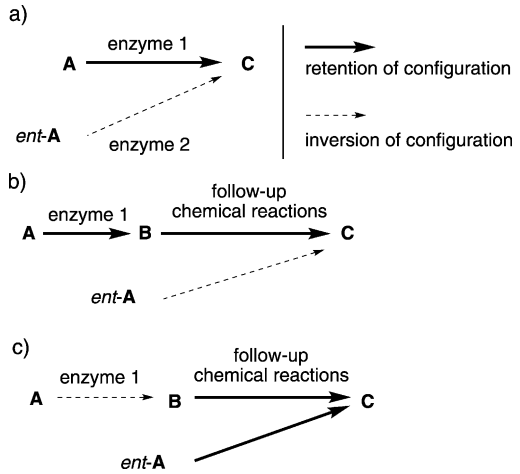


Figure 1. Strategies used in chemoenzymatic enantioconvergent processes.

The first approach (Figure 1 a) employs two enzymes that express opposite enantiopreferences. One enzyme catalyzes the transformation of substrate **A** to product **C** with retention of configuration, while the other one inverts the stereochemistry in the conversion of *ent*-**A** to **C**, usually by an S_N2-type process. This approach has been applied mainly for the hydrolysis of epoxides, by using two epoxide hydrolases[14,15]

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or one enzyme that catalyzes the convergent transformations of both enantiomers with distinct regioselectivity.[16–18]

The second approach (Figure 1b) uses one enzyme to perform a kinetic resolution of racemic substrate **A**. In a subsequent set of reactions, enantioenriched compounds *ent*-**A** and **B** are transformed into a single product **C**; one of these transformations proceeds with retention and the other with inversion of configuration. Such deracemizations can be performed, for example, by combining the lipase-catalyzed kinetic resolution of racemic esters with a subsequent stereoinversion of the obtained enantioenriched alcohols via sulfonyl esters[4,19,20] or nitrate esters,[19] or by following a Mitsunobu protocol.[21]

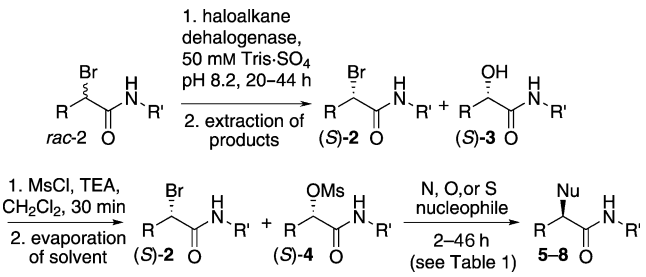
The third approach (Figure 1c) relies on the use of a single enantioinverting enzyme for the conversion of one of the enantiomers of substrate **A** into product **B**. In the follow-up reaction, this product and, optionally, the unreacted *ent*-**A**, are transformed into the final compound **C** with retention of configuration. Most recent examples of such syntheses include the use of inverting alkyl sulfatases for enantioselective sulfate ester hydrolysis, which produces an alcohol and an unreacted enantiomer of the sulfate ester, both with the same absolute configuration.[22–24] The follow-up chemical hydrolysis of the enantioenriched sulfate ester proceeds with retention of configuration and gives the same enantiomer of the alcohol as the one produced in the enzymatic inverting step.

Despite its apparent efficiency and elegance, the use of the first approach (Figure 1a) is severely limited by low availability of enzyme pairs that show complementary enantioselectivity. The efficiency of the latter approaches (Figure 1b,c) strongly depends on the high enantioselectivity of the enzymatic reaction and high atom economy of the chemical steps (which is especially low in case of the Mitsunobu protocol).[21] Furthermore, it would be advantageous if no separation of intermediates was needed and the purification steps were reduced to a minimum.[22]

Haloalkane dehalogenases are enzymes that catalyze the hydrolytic cleavage of carbon–halide bonds by employing a catalytic aspartate ion for the nucleophilic displacement of the halide in an S_N2-type reaction.[25] This process gives a covalent intermediate in which the product is bound to the enzyme by an ester bond. This bond is subsequently hydrolyzed to form the product and to recover the nucleophilic aspartate ion. Overall, the reaction proceeds with inversion of configuration, therefore the enzyme can be efficiently used in the process described in Figure 1c.

Additional possibilities stem from the fact that this reaction gives two enantiopure compounds, a haloalkane and an alcohol, both of which possess high intrinsic reactivity and outstanding potential for functionalization. In particular, both the halide (directly) and the hydroxide (upon simple activation) can be regarded as efficient leaving groups, which sets the stage for a number of nucleophilic displacements leading to diverse classes of products.

Taking advantage of this reactivity, we designed a chemoenzymatic enantioconvergent process (Scheme 2) for the synthesis of enantiopure amides **1** (Scheme 1) by using the general approach described in Figure 1c. This process uses

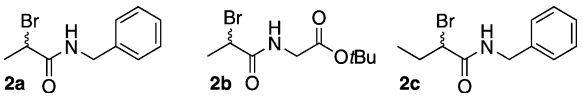


Scheme 2. Chemoenzymatic enantioconvergent preparation of (*R*)-**1** (compounds **5**–**8**) from rac-**2**. Ms = methanesulfonyl, TEA = triethylamine, Tris = tris(hydroxymethyl)aminomethane.

haloalkane dehalogenases as the inverting enzymes in the biocatalytic step, and sulfonyl esters for the follow-up chemical transformations.

We have recently established that easily prepared racemic α-bromoamides (*rac*-**2**) can be converted by haloalkane dehalogenases with high enantioselectivity (*E* values > 200).[26] Upon extraction of the aqueous enzymatic reaction mixture, both products (*S*)-**2** and (*S*)-**3** can be obtained with high overall yields (> 80%). We expected that no separation of intermediates would be needed prior to the conversion of alcohols (*S*)-**3** into methanesulfonyl esters (*S*)-**4**, and that this reaction mixture would also not have to be processed before the final step (Scheme 2). These simplifications to the procedure would fulfill the requirements of limiting the intermediate separation and purification steps in the processes described in Figure 1c. In the last reaction, in which the α-bromoamide (*S*)-**2** and the α-mesyloxyamide (*S*)-**4** are both converted in an S_N2-type process into final products (*R*)-**1**, we employed azide and benzylamine as N nucleophiles, ethanethiol as an S nucleophile, and phenol as an O nucleophile.

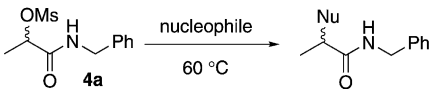
Racemic α-bromoamide **2a** (Scheme 3), which is a precursor of compound **4a**, undergoes kinetic resolution catalyzed by a haloalkane dehalogenase from *Bradyrhizobium japonicum* USDA110 (DbjA) with excellent enantioselectivity ($E > 200$).[26] To establish the best conditions for the reactions of model nucleophiles with mixtures of α-bromoamides (*S*)-**2** and α-methanesulfonyloxyamides (*S*)-**4**, we performed a study in which the racemic model compound **4a** was reacted to racemic products **5a**–**8a** under various conditions (Table 1).



Scheme 3. Racemic α-bromoamides.

The reaction of compound **4a** with sodium azide in DMF as the solvent resulted in the full conversion of the substrate in 2 h, and product **5a** was isolated in almost quantitative yield (Table 1, entry 1). When similar conditions were employed for the introduction of the N nucleophile benzylamine in the presence of potassium carbonate as the base, the reaction took 2 days to reach full conversion and the product was

Table 1: Model transformations of racemic compound **4a**.



5a: Nu = N_3
6a: Nu = NHBn
7a: Nu = OPh
8a: Nu = SEt

Entry	Nucleophile	Solvent	Amount of K_2CO_3	t	Product	Yield[a]
1	NaN_3 (5 equiv)	DMF	–	2 h	**5a**	96%
2	$BnNH_2$ (2 equiv)	DMF	2 equiv	46 h	**6a**	<50%
3	$BnNH_2$ (2 equiv)	MeCN	2 equiv	46 h	**6a**	71%
4	PhOH (2 equiv)	MeCN	2 equiv	20 h	**7a**	73%
5	EtSH (2 equiv)	MeCN	2 equiv	46 h	**8a**	71%
6	EtSH (2 equiv)	DMF	2 equiv	20 h	**8a**	70%

[a] Yield of isolated product. Bn = benzyl.

isolated with low yield because of the formation of side products (Table 1, entry 2). The use of acetonitrile instead of DMF as the solvent (Table 1, entry 3) resulted in a much cleaner reaction, and product **6a** was isolated in good yield after 46 h. When these conditions were used for the reaction of compound **4a** with phenol (Table 1, entry 4), full conversion of substrate was already achieved after 20 h. Under the same conditions, **4a** could be converted to the α-ethylthioamide **8a** in 71% yield within 46 h (Table 1, entry 5). Virtually the same yield was obtained with DMF as solvent, but the reaction time was shorter (Table 1, entry 6). On the basis of these results, the latter conditions were chosen for the planned enantioconvergent chemoenzymatic synthesis of compound **8a** and its analogues.

To study the feasibility of the transformations presented in Scheme 2, we focused on three model substrates (Scheme 3). Racemic compounds **2a–c** are readily accessible in more than 90% yield in one step from simple starting materials.[26] Furthermore, compound **2b**, which includes a moiety derived from glycine, the most simple amino acid, was chosen to show that our methodology has potential in the preparation of peptides. Moreover, compound **2c** features an ethyl substituent on the α position, which enabled us to assay the influence of steric hindrance on the efficiency of the final step of the transformation presented in Scheme 2.

Subsequently, we conducted a series of reactions aiming at the enantioconvergent chemoenzymatic preparation of compounds **5–8** (Scheme 2, Table 2). The racemic precursors *rac*-**2** were initially subjected to a haloalkane dehalogenase mediated kinetic resolution (Scheme 2), thus providing a selective transformation to α-hydroxyamides (*S*)-**3** by using DbjA and haloalkane dehalogenase from *Sphingomonas paucimobilis* UT26 (LinB). In the enzymatic reactions, conversions of 50%, which are crucial for the efficient enantioconvergent process,[27,28] were observed after 20–44 h. The enantiomeric excess of the products was found to be higher than 97% in all cases. Notably, only a small amount of enzyme, typically corresponding to 0.01 mol% of biocatalyst, was used for these conversions (Table 2). The reaction mixtures obtained from the biotransformations were extracted with ethyl acetate and no further product separation or purification was required. After evaporation of the solvent, the crude mixtures of enantiomerically enriched α-bromoamides (*S*)-**2** and α-hydroxyamides (*S*)-**3** were subjected to the reactions in

Table 2: Enantioconvergent chemoenzymatic preparation of compounds **5–8**.

Nucleophile	Substrate		
	rac-**2a**[a]	*rac*-**2b**[b]	*rac*-**2c**[c]
NaN_3	**5a**, 81%, 98% *ee*	**5b**, 73%, 97% *ee*	**5c**, 80%, 95% *ee*
$BnNH_2$	**6a**, 81%, 97% *ee*	**6b**, 96%, 91% *ee*	**6c**, 85%, 90% *ee*
PhOH	**7a**, 70%, 95% *ee*	**7b**, 57%, 93% *ee*	**7c**, 66%, 96% *ee*
EtSH	**8a**, 82%, 28% *ee*	**8b**, 76%, 92% *ee*	**8c**, 63%, 73% *ee*

[a] DbjA (1.7 wt%, 42 h). [b] DbjA (1.1 wt%, 20 h). [c] LinB (2.3 wt%, 44 h).

which compounds (*S*)-**3** were mesylated to give intermediates (*S*)-**4** (Scheme 2). We observed that these reactions proceeded cleanly and did not affect the α-bromoamides (*S*)-**2**.

Crude reaction mixtures obtained in the second step were concentrated in vacuo, and no purification or separation of intermediates (*S*)-**2** and (*S*)-**4** was required. This step was followed by the introduction of nucleophiles by using the optimized conditions for S_N2 reactions (Table 1, entries 1, 3, 4, and 6).

The reactions leading to α-azidoamides (*R*)-**5a–c** gave these products in 73–81% yield (based on *rac*-**2a–c**), thus providing the proof-of-principle for the proposed enantioconvergent synthetic route by showing that both products of the enzymatic reaction can be efficiently transformed into a single product (Table 2). The high enantiomeric excess (*ee* > 95%) of the obtained products renders our methodology suitable for the preparation of chiral α-azidoamides, which are important precursors for peptide synthesis.

Transformations in which benzylamine was used as a nucleophile led to α-(benzylamino)amides (*R*)-**6a–c** (Table 2) with even better yields than in the case of the azide nucleophile. The high enantiomeric excess of the products (up to 97% for (*R*)-**6a**) further confirms the potential of the presented methodology for the preparation of peptide analogues.

The yields obtained in the syntheses of α-phenoxyamides (*R*)-**7a–c** were generally lower (Table 2) than for the reactions with sodium azide and benzylamine, yet were still significantly superior to the maximum theoretical yield of 50% that could be obtained if only one product of the enzyme-catalyzed kinetic resolution was used for further transformations. Considering the high enantiomeric excess of the products (93–96% *ee*, Table 2), we conclude that the presented methodology can be efficiently used for the stereoselective preparation of chiral amides substituted with O nucleophiles.

In the transformations aimed at α-ethylthioamides (*R*)-**8a–c**, the products were obtained in good yields (Table 2), albeit with low *ee* values. In the least satisfying case, compound (*R*)-**8a** was obtained with only 28% *ee*. We suspect that this enhanced racemization may result from the ethylthio substituent, which stabilizes the intermediate carbanion and thus increases the acidity of the proton attached to the α position of compound **8**.[29,30] The deprotonation and subsequent reprotonation (enolization) of compound **8** under the basic reaction conditions (two equivalents of potassium carbonate) would lead to its racemization. We found that the

ee value of **8c** decreased from the initial value of 73% to 69% after two weeks of storage at 4°C, thus confirming our hypothesis. Subsequently, we incubated the compound for 5 h under the nucleophilic displacement reaction conditions (two equivalents of potassium carbonate, DMF) and found that the *ee* value further decreased to 65%. These data support our notion on the enhanced razemisation tendency of products **8**.

We have designed an efficient enantioconvergent process for the synthesis of various enantiopure α-substituted amides, which form an important class of chiral building blocks in medicinal chemistry. The process combines the high enantioselectivity of the haloalkane dehalogenase catalysed resolution with the efficient and convergent transformation of both the starting material and the biocatalysis product into a single enantiomer of the final compound **1**. The products are obtained in high yield (up to 96% after three steps) and with high enantiomeric excess (up to 98%) in the case of configurationally stable compounds **5**–**7**. In the whole process, the purification and separation steps are eliminated, thus rendering it a practical and time-efficient methodology. We are currently directing our efforts toward using directed evolution methodologies for the broadening of the substrate scope of haloalkane dehalogenases, and as a consequence fully enhancing the versatility of the new process.

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