

## Advances in biocatalytic synthesis. Enzyme-triggered asymmetric cascade reactions\*

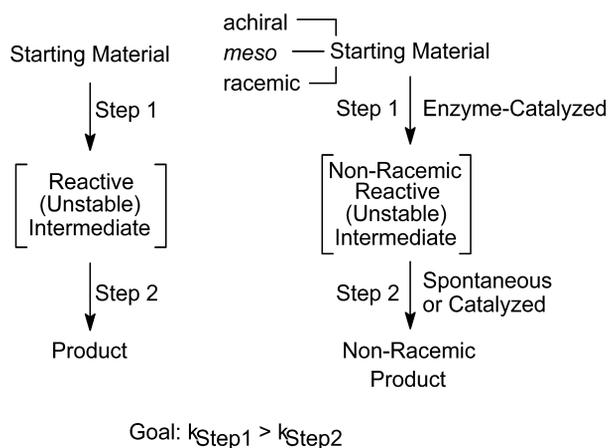
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**Abstract:** Organic compounds can be transformed through enzyme-triggered domino (or cascade) reactions via several (inseparable) consecutive steps in an asymmetric fashion to yield nonracemic products. Despite the fact that these sequences often involve the occurrence of highly reactive unstable intermediates, the overall efficiency of these processes can be high, provided that the reaction rates of the individual steps match each other in order to minimize side reactions.

### INTRODUCTION

Domino or cascade reactions involve the transformation of materials through several nonseparable steps in a concurrent fashion, which often proceed via highly reactive intermediates. The synthetic potential of this strategy has been well recognized for (traditional) organic synthesis [1]. However, the potential to conduct these processes in an asymmetric fashion is largely underexploited. The latter may conveniently be achieved by making use of the unparalleled chemo-, stereo-, and enantioselectivity of enzymes [2]. Thus, in case the sequence of events is triggered by a biocatalyst, the cascade may proceed in a highly asymmetric fashion to furnish products in nonracemic form (Fig. 1).



**Fig. 1** Principles of enzyme-initiated cascade reactions.

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A survey of enzyme-triggered domino-reactions published to date [3] reveal a common picture (Fig. 2). In a first step, the enzyme modifies an enzyme-labile trigger-group (Trig) within the starting material (e.g., via oxidation, hydrolysis/transesterification, etc.), giving access to a reactive intermediate. This, for instance, may bear a liberated negative charge, which can deliver electrons to a  $\pi$ -system or it may act as a nucleophile (Nu). Consequently, the intermediate thus formed immediately undergoes a subsequent domino-reaction, which may consist of a (i) fragmentation, (ii) rearrangement, (iii) Diels–Alder reaction, or (iv) an intramolecular nucleophilic substitution affecting cyclization.

These processes show a remarkable synthetic advantage despite the fact that the cascade is proceeding through one (or more) highly unstable intermediate(s), which are prone to decomposition reactions, the final product can often be isolated in good yields, because undesired side reactions of the reactive intermediate are largely avoided since the intermediate is transformed in the same instant as it appears, and, as a consequence, it does not occur in measurable concentrations. Best results are obtained when the reaction rates of the individual steps match each other, i.e., the initiation of the cascade must be rate-limiting to avoid an unfavorable accumulation of the reactive intermediate(s) ( $k_{\text{Step1}} < k_{\text{Step2}}$ , etc.). Thus, it is not surprising that cascade reactions show a general tendency to be “chemically clean” transformations. In addition, the separation and isolation of intermediates can be avoided, which minimizes waste and renders an improved economic balance.

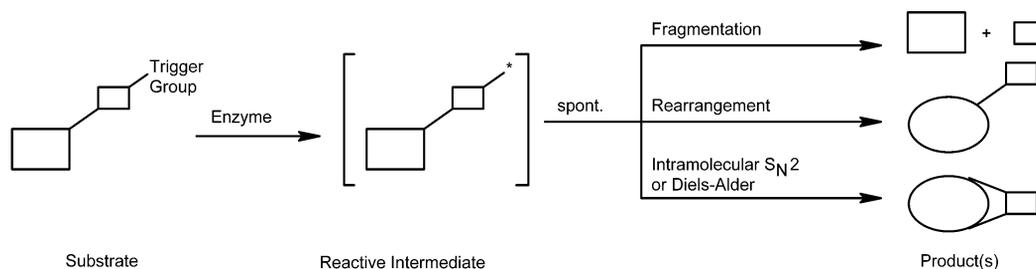


Fig. 2 Types of enzyme-initiated cascade reactions.

## ENZYME-TRIGGERED CYCLIZATION REACTIONS

By making use of the asymmetric catalytic potential of biocatalysts, enzyme-triggered cascade reactions may be turned into highly efficient protocols for the asymmetric synthesis of bioactive materials. Along these guidelines, the following synthetic concept was developed (Fig. 3).

From a precursor-substrate (containing an electrophile [E1] and a Nu masked with an enzyme-cleavable Trig), the latter is removed by the enzyme to liberate a reactive intermediate, which spontaneously undergoes cyclization. Based on the rules for ring closure [4], the formation of three-, five-, or six-membered ring structures are strongly favored.

The wide synthetic potential of this technique is illustrated by examples in the table to Fig. 3. Thus, a series of well-understood enzyme-catalyzed reactions may be used to liberate a (masked) Nu:

- i. For instance, asymmetric hydrolysis of epoxides leads to the formation of the corresponding *vic*-diol [5]. In several cases, this pathway was shown to occur in an enantio-convergent fashion, which allows for the transformation of a *rac*-substrate into a single stereoisomeric product in 100 % theoretical yield by completely avoiding the occurrence of an “undesired” stereoisomer [6].
- ii. The use of readily available carboxyl ester hydrolases, such as esterases and lipases [7] allows to liberate a (weakly nucleophilic) alcohol or carboxylate from the corresponding carboxylic ester. This technology is well developed and high asymmetric induction is usually accrued.

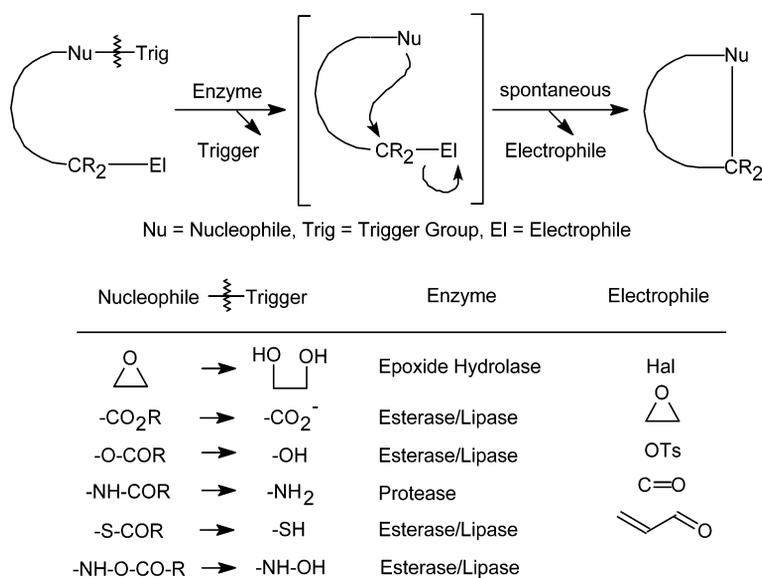


Fig. 3 Potential of enzyme-triggered cyclization reactions.

- iii. In a related fashion, highly nucleophilic amines may be formed by hydrolysis of carboxamides using proteases [7].
- iv. In certain cases, asymmetric hydrolysis of thioesters—liberating thiols—has been accomplished using esterases/lipases [8].

On the acceptor side, various well-known EIs may be used, such as halides, epoxides, activated ester groups, carbonyl moieties, and Michael acceptors.

Overall, O-, N- or S-heterocyclic structures—predominantly of epoxy-, tetrahydrofuran-, and pyran-type and the corresponding aza- and thia-analogs—are obtained as a result of the enzyme-triggered cascade reaction.

The preparative applicability of enzyme-triggered cascade reactions was recognized by chance during the asymmetric biohydrolysis of haloalkyl-substituted oxiranes by bacterial epoxide hydrolases [9] (Fig. 4). Thus, when 2,3-disubstituted haloalkyl oxiranes were subjected to biohydrolysis, the corresponding *vic*-diols were formed as expected. The latter, however were unstable at slightly alkaline pH and underwent spontaneous ring closure to form a three-membered ring product (from the halomethyl-derivative, [ $n = 1$ ]) and a tetrahydrofuran derivative was obtained from the haloethyl-analog [ $n = 2$ ]. During this process, two chiral centers could simultaneously be controlled in a highly stereoselective fashion. Based on the enantio-convergence of the biohydrolysis [10], both products were obtained as single stereoisomers in up to 92 % e.e. and 79 % yield [11].

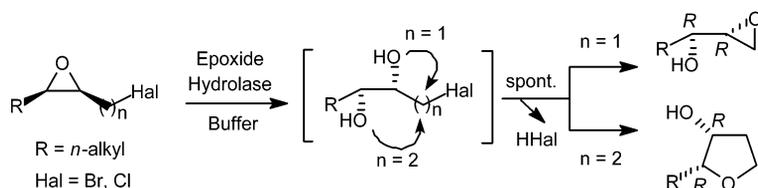
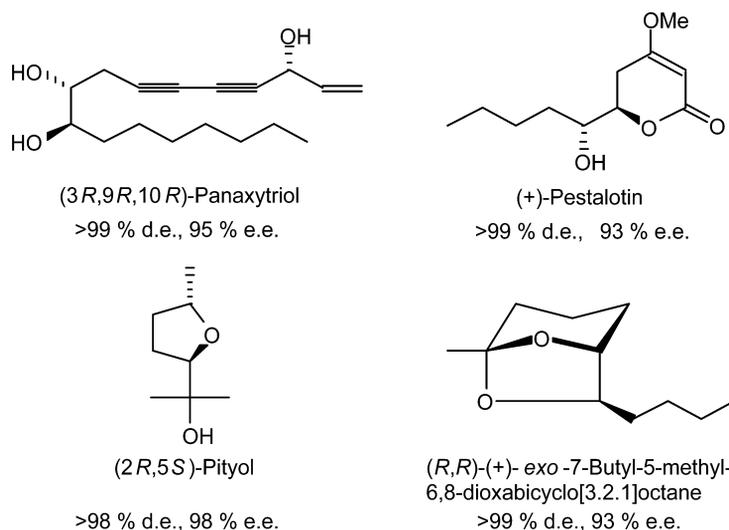


Fig. 4 Enzyme-triggered cyclization of haloalkyl oxiranes catalyzed by epoxide hydrolases.

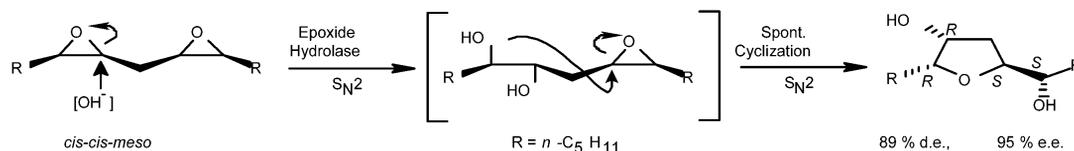
## APPLICATION TO BIOMIMETIC NATURAL PRODUCT SYNTHESIS

The synthetic potential of these building blocks was demonstrated by the asymmetric synthesis of four bioactive compounds, such as (3*R*,9*R*,10*R*)-panaxytriol [12] (an antileukemic constituent of ginseng roots), (+)-pestalotin (the antipode of a phytohormone) [13], (2*R*,5*S*)-pityol (a pheromone of the elm-bark beetle) [11], and a bicyclic acetal isolated from Jamaican rum aroma [13] (Fig. 5).

In order to test the limits of stereocontrol, the enzyme-triggered cyclization of *bis*-epoxides was investigated (Fig. 6). In this case, four enzymatic trigger-pathways are leading to four possible stereoisomeric epoxy-diols as intermediates based on the  $S_N2$ -type mechanism of epoxide hydrolysis. Each of the latter intermediates may undergo subsequent  $S_N2$ -type ring closure to furnish four possible stereoisomeric tetrahydrofuran products (i.e., enantiomeric pairs of two diastereomers) through two secondary pathways [14]. Careful elucidation of the products obtained showed that the *meso*-*cis*-*cis*-oxirane was converted through an enzyme-triggered cascade via a single dominant pathway [15] to furnish a dihydroxy-tetrahydrofuran derivative containing four stereogenic centers as the sole product in 89 % d.e. and 95 % e.e. Compounds of this type constitute the central core of *Annonaceous* acetogenins, which exhibit a range of biological effects, such as antitumor, antimalarial, pesticidal, and immunosuppressive activities. After all, it is very likely that the biosynthesis of these compounds proceeds via related enzyme-triggered cascade reactions. The analogous biotransformations of synthetic materials are thus aptly denoted as "biomimetic" [16].



**Fig. 5** Bioactive compounds synthesized via enzyme-triggered cascade reactions.



**Fig. 6** Enzyme-triggered rearrangement of *meso*-*bis*-epoxides.

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