Enzyme-initiated domino (cascade) reactions

Sandra F. Mayer, Wolfgang Kroutil and Kurt Faber*

Department of Chemistry, Organic & Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, A-8010 Graz, Austria. E-mail: Kurt.Faber@kfunigraz.ac.at; Fax: +43-316-380-9840; Tel: +43-316-380-5332

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Domino or cascade reactions involve the transformation of materials through several inseparable steps, which often proceed *via* highly reactive intermediates. In the case where the reaction sequence is triggered by a biocatalyst, such as an enzyme, the cascade may proceed in a highly chemo- or stereoselective way. In this review, emphasis is laid on biocatalyzed domino reactions of non-natural compounds (rather than natural substrates) which have been aptly denoted as 'enzyme-initiated' (or -'triggered') domino (or cascade) reactions. Biosynthetic pathways involving biological cascade reactions are out of the scope of this review (see, for example, D. E. Cane, *Chem. Rev.*, 1990, 90, 1089).

1 Introduction

Synthetic organic chemistry has undergone deep changes during the past decade, during which molecules of increasing complexity have been synthesized on the basis of methods with improved regio-, chemo-, diastereo- and enantioselectivity. Despite this 'technical' success and the increasing importance of chemistry to our society, the public image of chemistry has deteriorated in the wake of public environmental concerns. Today the question of *what* we can synthesize is of lower importance than *how* we do it. Due to economic reasons, major concerns in chemical production are the handling of waste, the search for environmentally tolerable procedures, the preservation of resources and increased efficiency.[1]

The traditional procedure for the synthesis of an organic compound is the stepwise formation of individual bonds towards the construction of the target molecule. However, it would be much more efficient if several bonds could be formed in a single sequence without isolation of intermediates. It is obvious that this type of reaction would be more economic by requiring fewer reagents, solvents and adsorbents and less energy and labour together with a reduction of waste.

Reactions proceeding through more than a single step in a concurrent fashion have been described in various contexts and different terms have been used to describe them, which has caused some confusion. For the sake of clarity, the following definitions are used throughout this paper.

Sandra F. Mayer (born 1976) started her studies of chemistry at Graz University of Technology in 1994, where she received her MSc in chemistry in 1999. Then she joined the Department of Organic and Bioorganic Chemistry at the Karl-Franzens-University Graz, where she is currently working on her PhD under the supervision of K. Faber. Her project is focussed on enantio-convergent biotransformations using bacterial epoxide hydrolases and their application to the asymmetric synthesis of natural compounds.

Wolfgang Kroutil (born 1972) received his undergraduate training in chemistry at the University of Technology in Graz (Austria) and completed graduate studies in Exeter (UK) and Graz. Then he undertook two years of industrial experience in the biocatalysis research group at Syngenta (formerly Novartis CP) in Basel (Switzerland) and in the research and development department of Krems Chemie Chemical Services (Austria). In 2000 he became Assistant Professor in the research group of Professor Faber at the Department of Chemistry at the Karl-Franzens-University Graz.

Kurt Faber (born in 1953) received his PhD in chemistry at Graz University and switched (after a post-doc in Canada) to Graz University of Technology, where he became Associate Professor in 1997 after several long-term visits to foreign universities (Exeter, UK; Tokyo; Trondheim, Norway; Stockholm, Sweden). In 1998 he returned to Graz University as full professor. His main research interest is the development of biocatalysts for the chemo- and stereoselective transformation of non-natural organic compounds.

Sandra F. Mayer

Wolfgang Kroutil

Kurt Faber

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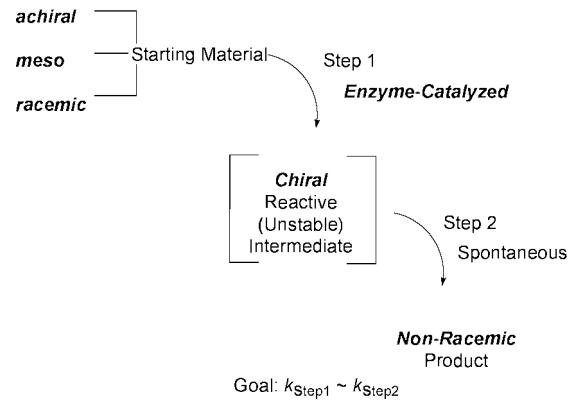
Type I processes are reactions in which the starting material undergoes a transformation *via* two (or more) reactions one after another in an inseparable fashion and are denoted 'domino' or 'cascade' reactions. The choice of words—*domino* or *cascade*—indicates that both individual reactions belong tightly together and are rather difficult to perform in a stepwise (independent) fashion. As a consequence, the intermediate between both steps is likely to be unstable and (often) eludes isolation and characterization.

Type II processes, in contrast, constitute 'sequential' or 'tandem' reactions, which are considered to be two-step reactions that proceed in a consecutive fashion where each of the steps can be performed separately. Thus, it can be anticipated that the intermediate species will be a rather stable compound.

Type I processes show a remarkable advantage: Despite the fact that the cascade reaction is likely to proceed *via* a highly reactive (unstable) intermediate, which is prone to elude isolation and characterization, the final product can often be isolated in good yields, because decomposition of the reactive intermediate is largely avoided since it is transformed in the same instant as it appears. As a consequence, in an ideal case (when the velocity of the domino reaction is much faster than the biotransformation) it does not occur in measurable concentrations. Unstable intermediates may constitute ionic or radical species and, by convention, they are usually drawn in square brackets.

An impressive number of chemo-catalyzed cascade reactions (especially those involving cyclizations) have been accomplished by using palladium.[2] Other types of typical cascade reactions (although they have been often denoted as 'tandem' reactions) have been extensively reviewed and classified according to their type of mechanism.[3] All of these reaction sequences were initiated by an organic or inorganic catalyst, or by thermal reactions. In contrast, only a few examples of cascade reactions have been reported in which the initiation of the reaction cascade consisted of a biotransformation.[4]

By making use of the advantages of biocatalysts, such as cheap resources, biocompatible reaction conditions and (most prominently) the unparalleled stereospecificity of enzymes, a biocatalyzed reaction cascade may be turned into an asymmetric process to provide a non-racemic product (Scheme 1).



Scheme 1 Principle of enzyme-initiated domino (or cascade) reactions.

2 Background: enzyme catalysis

During the past 3×10^9 years, the forces of natural selection have refined and improved the properties of biological macromolecules in order to contribute to the survival of their host organism. These adaptive forces have engendered enzymes with the ability to catalyze reactions at rate accelerations of up to 10^{17}-fold.[5]

It is commonly assumed that natural selection favors enzymes that have evolved active-site arrangements in order to conform to underlying chemical principles, thus energetic factors which govern structure and reactivity in solution also govern the basic features of enzyme-catalyzed reactions. Some features of contemporary enzymes might reflect the haphazard accidents of evolution, however, rather than adaptation for optimal catalytic power. Distinguishing between these possibilities is essential to our understanding of how enzymes achieve their enormous (and unparalleled) rate enhancements, and to the design and manipulation, according to our ability, of the structure and catalytic activity of biological macromolecules. Explanations for the extraordinary power of enzymes to accelerate chemical reactions have been sought ever since this behavior was observed. An important principle of enzyme catalysis is based on the strong binding interactions that are required to reduce the energy barriers along the chemical reaction pathway. Modern explanations of the (bio)catalytic process date from Haldane's classic treatise on enzymatic activity through comments made by Pauling in the 1940's that biocatalysis rests on the enzyme's ability to stabilize the transition-state structure of the substrate relative to that of the ground state. The transition-state theory rests on two basic assumptions: (1) that an 'activated complex' in a chemical reaction is formed from the reactant(s) as if in equilibrium with them, and (2) that the rate of the chemical reaction is governed by the decomposition of this activated complex to products.[6]

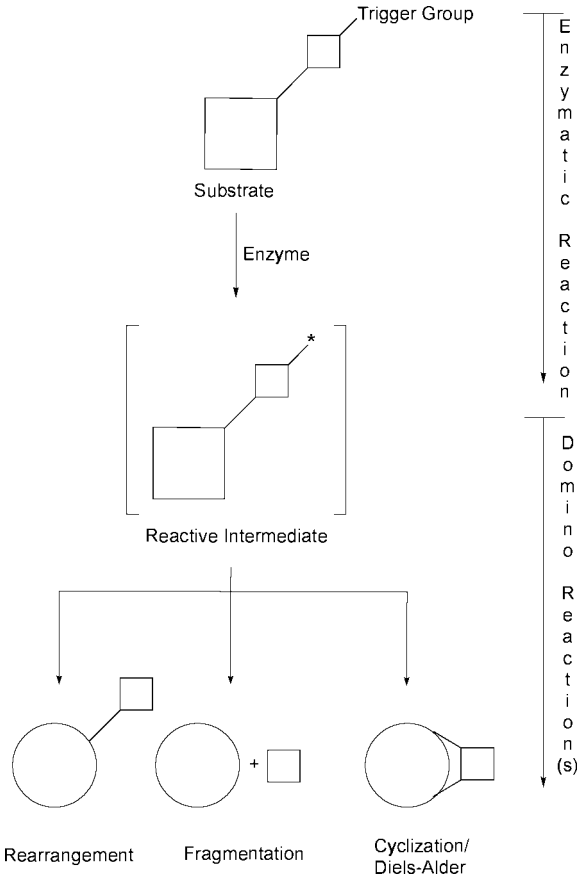
This theory functioned as the intellectual starting point for the development of catalytic antibodies. Although the binding forces exerted by enzymes and antibodies are fundamentally the same, Nature has nevertheless dictated that an enzyme, not an antibody, can use this binding energy to stabilize a transition state, thereby accelerating a chemical reaction. However, if one had an antibody that specifically bound the transition state of a given chemical reaction, it would catalyze that reaction to a certain extent. By mimicking the transition state of a reaction using a stable chemical (transition-state) analogue,† antibodies showing tailor-made catalytic activities can now be generated making use of the immune system's capacity to produce high-affinity binding sites for virtually any ligand.[7] In principle, catalytic antibodies would be ideal catalysts for asymmetric synthesis. In practice, however, the application of catalytic antibodies has met unsurmountable problems mainly for two reasons: (1) their large-scale production is cumbersome and exceedingly expensive and (2) their catalytic power is poor. Even the best catalytic antibodies are much less active than their natural enzymic counterparts by orders of magnitude.[8]

In contrast, the use of enzymes as catalysts for a variety of transformations has dramatically increased over the last two decades,[9] hand in hand with progress in biochemistry and protein chemistry, as well as in gene and fermentation technology.

3 Types of biocatalyzed cascade reactions

A survey of enzyme-triggered domino reactions published to date reveals a common picture (Scheme 2). In the first step, the enzyme modifies a group ('trigger' group) in the starting material (*e.g. via* oxidation, transesterification of an alcohol, hydrolysis of an ester or epoxide, respectively), giving access to a reactive intermediate that can undergo a subsequent domino reaction. These intermediates may be a diene or may bear a liberated negative charge, which can either push electrons into

† These compounds are denoted as haptens. Although the latter are not immunogenic by themselves, they are able to stimulate the formation of antibodies highly specific and complementary to their structure when these molecules are conjugated to a carrier protein.



Scheme 2 Types of enzyme-initiated domino reactions.

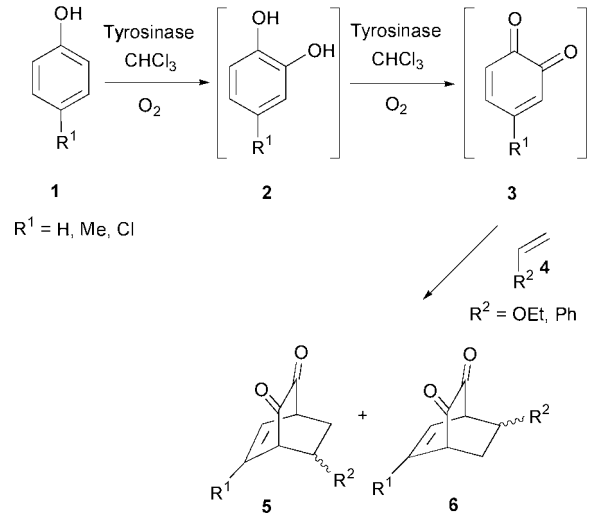
a π-electron system or act as a nucleophile. Consequently, the intermediate thus formed immediately undergoes a subsequent 'domino' reaction, which may consist of (i) a fragmentation, (ii) a rearrangement or (iii) a cyclization. The latter may involve a Diels–Alder reaction or an intramolecular S_N2 reaction by the nucleophile liberated by the enzyme. Different classes of enzymatic domino reactions and their types of follow-up reaction are summarized in Table 1.

The first report on a deliberate combination of a biotransformation with a chemical reaction to furnish a domino sequence appeared as early as 1981.[10,11] For the sake of clarity, enzyme-initiated domino reactions are grouped into the following subclasses: (i) Diels–Alder reactions as a domino reaction, (ii) enzyme-triggered skeleton rearrangements (excluding Diels–Alder), (iii) reactions initiated by an enzymatically liberated charge, and (iv) cyclizations involving enzymatically generated nucleophiles.

3.1 Enzyme-triggered Diels–Alder reaction

The Diels–Alder reaction is one of the most useful carbon–carbon bond forming transformations in organic chemistry, which involves the concerted [4+2] cycloaddition of a diene to a dienophile *via* a highly ordered cyclic transition state. Diels–Alder reactions are frequently found in chemical cascade or tandem reactions: they are employed either as the first step (*e.g.* combined with an aldol reaction), or as the second step (where the first step constitutes a Heck reaction). Another challenging strategy is the use of two consecutive Diels–Alder reactions during both steps. This was accomplished either by using a specially designed (double) dienophile or by employing a sequence consisting of a retro-hetero-Diels–Alder followed by a second (intramolecular) Diels–Alder reaction. In enzyme-triggered cascade reactions involving a Diels–Alder reaction, the enzymatic step is always first.[4]

3.1.1 Generation of a diene by enzymatic oxidation. *o*-Quinones are reactive compounds which can be generated from phenols by oxidation. Nature employs *o*-quinones during the synthesis of pigments and structural materials (*e.g.* the insect cuticle), and uses *inter alia* the enzyme tyrosinase [EC 1.14.18.1] for their generation. *o*-Quinones react readily with nucleophiles, electrophiles and dienophiles and therefore they serve as advantageous intermediates in domino sequences. A cascade reaction involving *o*-quinones obtained by an enzyme-initiated hydroxylation–oxidation sequence combined with a Diels–Alder reaction was recently published[12] (Scheme 3).



Scheme 3 Enzymatic oxidative generation of a diene followed by Diels–Alder reaction.

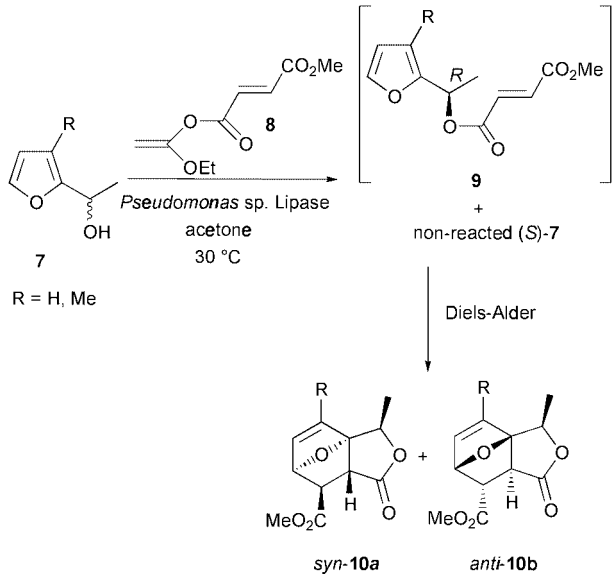
Phenols **1** were treated with tyrosinase in the presence of oxygen and a dienophile **4**. In the ensuing reactions the biocatalyst hydroxylated the *o*-position to the aromatic OH group to yield catechols **2**. These intermediates subsequently were further (bio)oxidized to the corresponding highly reactive *o*-quinones **3**. The latter underwent a Diels–Alder reaction with the dienophiles **4** to give the desired bicyclic cycloaddition products in high yields (up to 85%). Since *o*-quinones are prone to undergo polymerization, they eluded isolation but they could be trapped by reaction with a dienophile to form bicyclic

Table 1 Types of biocatalyzed domino reactions

Enzymatic trigger reaction	Effect of trigger reaction	Domino reaction(s)
Phenol oxidation	Diene formed	Diels–Alder
Transesterification of alcohol	Dienophile formed in kinetic resolution	Intramolecular Diels–Alder
Ester hydrolysis	Electron-donating group liberated	Retro-[2+2]cycloaddition/fragmentation
Ester hydrolysis	Electron-donating group liberated	Fragmentation
Ester hydrolysis	Electron-donating group liberated	Rearrangement
Ester hydrolysis	Nucleophile liberated ($-CO_2^-$)	Cyclization
Ester hydrolysis	Nucleophile liberated (–OH)	Cyclization
Epoxide hydrolysis	Nucleophile liberated (–OH)	Cyclization

products **5** and **6**. However, the overall processes were rather slow and required from several hours up to three days for completion. Although the products formed were intrinsically chiral, no asymmetric induction was noticed, since the cycloaddition reaction was of spontaneous nature and thus proceeded without the influence of the enzyme.

3.1.2 Generation of a dienophile *via* enzymatic kinetic resolution. An elegant case where a Diels–Alder reaction proceeded in an asymmetric fashion leading to non-racemic product(s) is depicted in Scheme 4. It is known that the use of



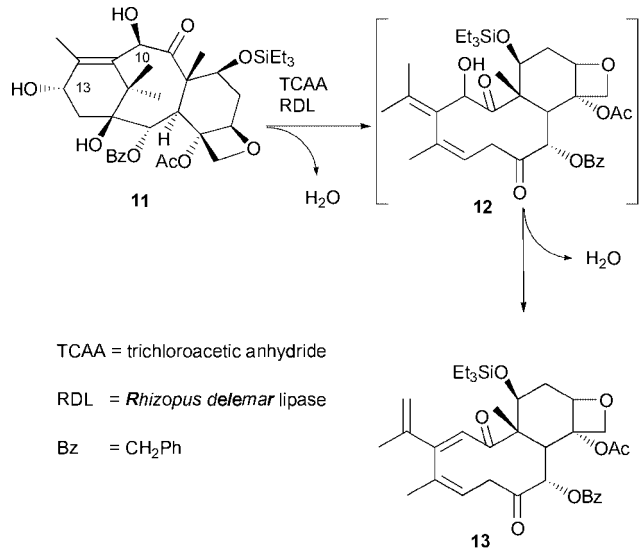
Scheme 4 Enzymatic linkage of diene and dienophile *via* kinetic resolution, followed by Diels–Alder reaction.

ethoxyvinyl esters **8** as acyl donors for enzymatic kinetic resolution of alcohols is advantageous in comparison to the commonly employed vinyl esters. Following this idea, kinetic resolution of *rac*-furfuryl alcohol derivatives **7** was accomplished *via* acyl transfer catalyzed by a *Pseudomonas* sp. lipase preparation, employing an enol ester **8** (ethoxyvinyl methyl fumarate) as acyl donor in the first step. By this method two goals were achieved in a single step, *i.e.* diene and dienophile were linked on to each other and asymmetry was introduced into the system by means of kinetic resolution. The second step constitutes an intramolecular Diels–Alder reaction of compound **9**, which provided 7-oxabicyclo[2.2.1]heptene derivatives *syn*-**10a** and *anti*-**10b** as the final products in low to moderate yields (18–43%) but in good ee's (79–93%). As may be expected from the spontaneous (non-biocatalyzed) nature of the cycloaddition, the diastereoselectivity was shown to depend solely on the substituent R, ranging from low (R = H, de ~25%) to excellent (R = Me, de >99%).[13]

3.2 Enzyme-triggered rearrangements (except Diels–Alder)

Skeleton rearrangements are of special interest for organic transformations because they often yield products of unrelated structure possessing several stereocenters. In the following section, enzyme-initiated rearrangement reactions are summarized.

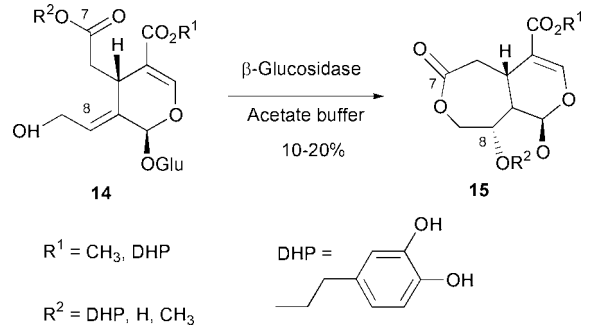
3.2.1 Enzymatic dehydration-initiated rearrangement. This enzymatically selective dehydration and rearrangement was observed during the development of a new strategy for the synthesis of Paclitaxel (Scheme 5).[14] Compound **11**, the



Scheme 5 Enzymatic selective dehydration and skeleton rearrangement of Paclitaxel precursors.

7-triethylsilyl derivative of 10-deacetylbaccatine III served as the starting material for this cascade reaction. The 13-hydroxy group of **11** was regioselectively acylated by *Rhizopus delemar* lipase (RDL) in the presence of trichloroacetic anhydride (TCAA) as acyl donor. At the onset of the reaction, the rearranged intermediate **12** could be detected but after a prolonged reaction time only **13** was isolated. It was assumed that after the first dehydration–rearrangement had formed **12**, the latter underwent a (slower) second dehydration (*i.e.* a 1,4-elimination of water).

3.2.2 β-Glucosidase-initiated rearrangement. The biohydrolysis of the glucose-moiety of multifloroside **14** and several analogues afforded the rearrangement product **15** (Scheme 6). It seems obvious that two concomitant steps are



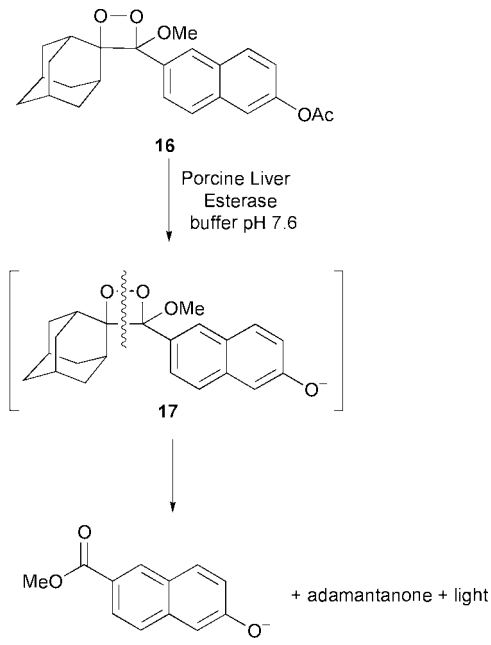
Scheme 6 Enzyme-triggered rearrangement of multifloroside **14**.

involved in this cascade reaction, *i.e.* lactonization involving the primary allylic alcohol and stereospecific alkoxy transfer from C-7 to C-8. These reactions could not be achieved by using dilute acid (*e.g.* AcOH or HCl). As a consequence, it was assumed that the rearrangement was catalyzed by an enzyme. A possible mechanism still awaiting proof was proposed.[15]

3.3 Domino reactions initiated by an enzymatically liberated (negative) charge

All of these reaction sequences have an enzyme-catalyzed hydrolytic starting step in common, during which a carboxy ester moiety is cleaved. The latter leads to the liberation of an anion (*e.g.* a phenolate or carboxylate), which does not participate itself in the subsequent reaction, but donates electrons into the molecule, thus initiating a domino reaction involving fragmentation or rearrangement.

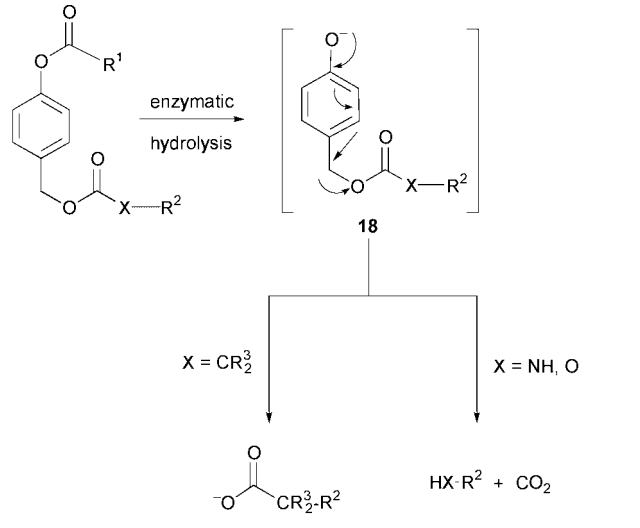
In the reaction in Scheme 7, the acetate ester of a naphthol derivative containing a highly reactive 1,2-dioxetane moiety



Scheme 7 Dioxetane fragmentation initiated by ester hydrolysis.

(**16**) was cleaved *via* hydrolysis by employing porcine liver esterase, thus liberating the free intermediate naphtholate anion **17**. The latter underwent an immediate fragmentation reaction which resulted in the formation of the naphthol methyl ester and adamantone and also in chemiluminescence.[16]

A related reaction sequence involving an enzyme-initiated fragmentation of a phenolate was developed in order to construct highly sophisticated protective groups for amino, hydroxy and carboxy moieties within sensitive target molecules, such as bioactive glyco- or lipopeptides (Scheme 8). The



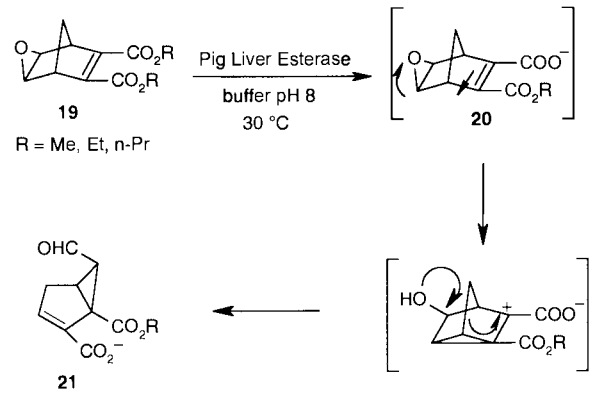
R^1(Trigger group)	Enzyme	X	R^2 (Target molecule)
CH_2Ph	Penicillin G Acylase	NH	Amine
CH_3, *n*-C_3H_7, *n*-C_7H_{15}	Lipase	O	Alcohol
		CR^3_2	Carboxylic acid

Scheme 8 Enzyme-triggered fragmentation of protective groups for amines, alcohols and carboxylic acids.

protective groups could be selectively removed *via* enzyme catalysis under mild conditions thus avoiding damage to the delicate bioactive compound. The protective group setup consisted of a phenol ester (R^1–CO–) and a central aromatic moiety, bearing the target molecule (–R^2) *via* an ester, carbonate or urethane linkage. Depending on the reactive group within the target molecule, liberation occurs with (amine, alcohol) or without (carboxylic acid) decarboxylation. The group triggered by the enzyme (R^1–CO–) may consist of a phenylacetate ester (which can be cleaved with absolute chemoselectivity using Penicillin G acylase) or of an aliphatic carboxylic ester (*e.g.* acetate, butanoate, octanoate), which may be selectively hydrolyzed by a lipase. The enzyme-triggered reaction liberated a phenolate anion **18**, which (at an appropriate pH) led to spontaneous fragmentation to form a *p*-quinomethane species along with the expulsion of the target molecule or a reactive intermediate, which consisted of a (hemi)carbonate or a carbamic acid, respectively. In the latter case, the intermediate underwent further spontaneous decarboxylation to furnish the non-protected target alcohol or amine derivative.[17]

This strategy is also applicable to solid-phase synthesis, if the aromatic moiety that is to build the scaffold is linked on to a macroscopic polymeric carrier *via* a spacer-arm, which acts as an enzymatically labile anchoring group.[18] The latter method is particularly useful for combinatorial chemistry and parallel synthesis for the creation of compound libraries attached to polymeric supports. It provides an efficient tool for the rapid generation of new substances with a predetermined profile of properties.

An unusual enzyme-triggered asymmetric rearrangement was observed by serendipity: when attempting to hydrolyze the symmetric tricyclic diester **19** in an asymmetric fashion using porcine liver esterase, the expected (chiral) monoester **20** was not obtained, but rather the product turned out to be a bicyclo[3.1.0]hexane framework (**21**, Scheme 9). Detailed



Scheme 9 Asymmetric ester hydrolysis followed by Meinwald rearrangement.

analysis of the reaction sequence revealed that the hemiester **20** was indeed formed, but immediately underwent Meinwald rearrangement to furnish **21** in near-quantitative yield. Since the enzyme-triggered reaction was expected to proceed in a stereoselective fashion, the product was analyzed for its enantiomeric composition, which turned out to be a moderate 48% ee for R being CH_3.[19]

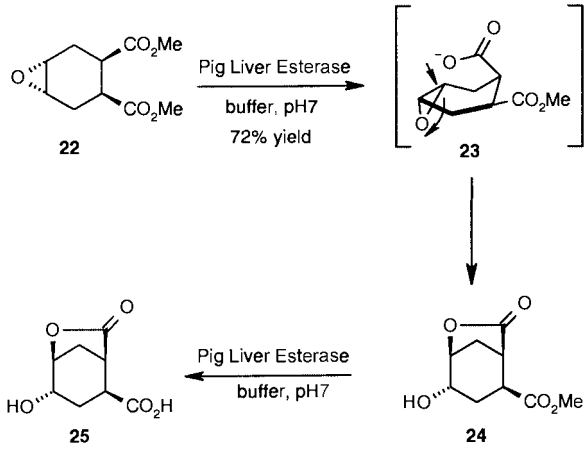
3.4 Domino reactions initiated by an enzymatically liberated nucleophile

Instead of undergoing a fragmentation or rearrangement reaction, the carboxylate or hydroxy group formed during (enzymatic) ester hydrolysis or epoxide ring-opening can also act as a nucleophile by attacking an electrophile during the cascade reaction. Most interestingly, other strong nucleophiles (such as amines or thiols) are seldom reported in this context. To

date, the electrophile usually consisted of an epoxide or a related species, such as halide.[20] Obvious analogues, such as toluene-*p*-sulfonate, or Michael acceptors (*e.g.* enones), *etc.* are waiting to be explored in this context.

Domino reactions of this type can start with the enzymatic hydrolysis of an ester or epoxide to liberate a nucleophile ($-CO_2^-$ or –OH), which opens an epoxide in an intramolecular S_N2 reaction in the second step. Thus, the final product formed is a hydroxy lactone ($-CO_2^-$ acting as Nu) or a (hydroxy)tetrahydrofuran (–OH acting as Nu) (see Schemes 12 and 13).

Such a cascade reaction was observed upon asymmetric hydrolysis of *meso*-epoxy diester **22** using porcine liver esterase (PLE) (Scheme 10).[21] It was found that the more accessible



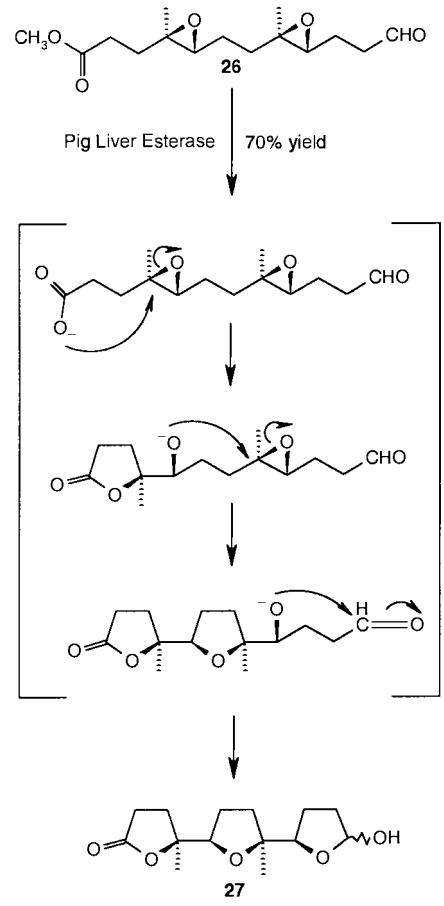
Scheme 10 γ-Lactone formation initiated by an enzymatically liberated Nu ($-CO_2^-$).

(equatorial) carboxy ester moiety was selectively hydrolyzed, thus liberating carboxylate anion **23**, which in turn acted as a nucleophile for opening the epoxide moiety to furnish the corresponding hydroxy γ-lactone **24**. In order to undergo lactone formation, the intermediate epoxy carboxylate has to undergo a conformational change, which turned the second (remaining) axial ester moiety into the more accessible equatorial position. As a consequence, it could now be hydrolyzed as well by PLE and (1*R*,2*S*,4*S*,5*S*)-4-hydroxy-7-oxo-6-oxabicyclo[3.2.1]octane-2-carboxylic acid **25** was obtained as the final product in 96% ee.

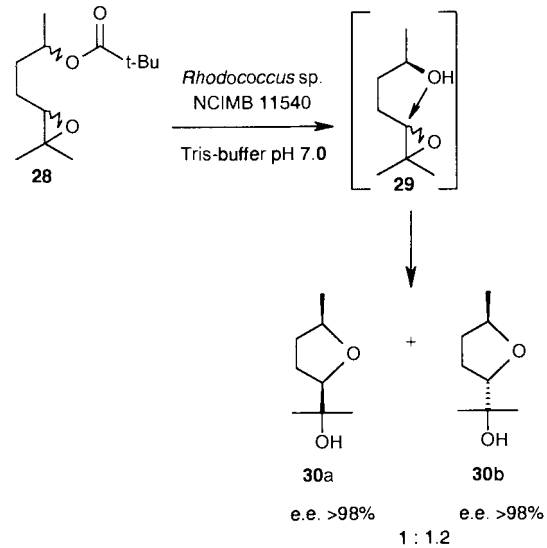
A related, but even more complex, domino reaction is depicted in Scheme 11. Again, the cascade was started by enzymatic hydrolysis of an ester **26** liberating a nucleophile ($-CO_2^-$), which opened an epoxide to furnish the corresponding lactone together with a free alkoxy moiety in the δ-position. The latter alkoxide underwent another (intramolecular) nucleophilic attack on the second epoxide to furnish a tetrahydrofuran derivative. At the end of this cascade, the resulting alkoxide was trapped by forming a hemiacetal **27** with an aldehyde bringing the cascade to a halt.[22]

Instead of an enzymatically generated carboxylate anion, an alcohol group (derived from a biocatalyzed ester or epoxide hydrolysis) may also serve as the nucleophile to open an epoxy moiety in a cascade reaction (Scheme 12). For instance, treatment of a diastereomeric mixture of (±)-epoxy ester **28** with a crude immobilized enzyme preparation (Novo SP 409)‡ or whole lyophilized cells of *Rhodococcus erythropolis* NCIMB 11540 gave compound **29** *via* kinetic resolution of the secondary alcohol moiety (ee >98%). The latter spontaneously opened the epoxide in an S_N2 fashion to furnish diastereomeric tetrahydrofuran derivatives **30a** and **30b**, which could be

‡ This crude immobilized enzyme preparation was initially designed for the biocatalytic hydrolysis of nitriles, but it was shown to contain several other enzymatic activities, such as carboxy ester and epoxide hydrolases.



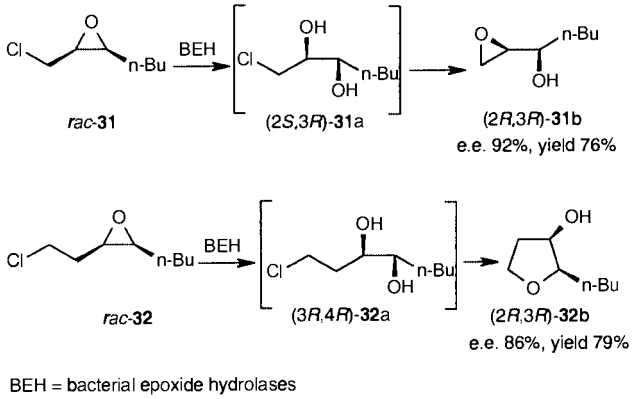
Scheme 11 Enzymatic liberation of Nu ($-CO_2^-$) followed by a three-step S_N2 cascade involving two epoxy groups.



Scheme 12 Cyclization initiated by enzymatically generated Nu (–OH) attacking an epoxide.

separated by conventional column chromatography.[23] Both compounds are bioactive constituents of bark beetle pheromones.

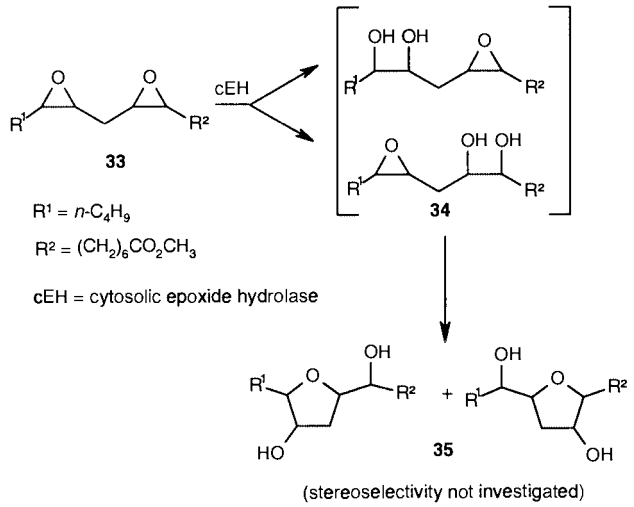
In all of the cases described above, the nucleophile acting during the cascade was liberated by hydrolysis of an ester. In the following examples, the nucleophile is generated by enzymatic hydrolysis of an epoxide to form the corresponding *vic*-diol. A recently developed biohydrolysis of (±)-2,3-disubstituted *cis*-chloroalkyl epoxides **31** and **32** turned out to initiate a cascade reaction (Scheme 13).[20] First, both enantiomers of the *rac*-



Scheme 13 Enzyme-initiated cascade reaction of (±)-2,3-disubstituted *cis*-chloroalkyl epoxides.

epoxide were hydrolyzed at pH 7.5 by various bacteria exhibiting epoxide hydrolase activity (*Mycobacterium paraffinicum* NCIMB 10420 for **31** and *Rhodococcus* sp. DSM 44541 for **32**) in an enantioconvergent fashion to furnish the (expected) *vic*-diols **31a** and **32a**, respectively. However, the latter underwent spontaneous ring closure to yield the cyclic products **31b** (ee 92%, yield 76%) and **32b** (ee 86%, yield 79%). The cyclization reaction showed some resemblance to a Payne-type rearrangement.§ Depending on the length of the haloalkyl substituent, the relative reaction rate of hydrolysis *versus* cyclization varied to a significant extent: Whereas the rate of epoxide formation to yield **31b** is in the same order of magnitude as the corresponding biohydrolysis, in case of the haloethyl derivative, **32a** was formed only in minute amounts, since cyclization to give **32b** was considerably faster than biohydrolysis of **32**. In both cases, the ring closure follows an *exo-tet* pattern. The huge difference in the relative rate of cyclization could be explained by energetic considerations, taking into account the large difference in ring strain between **31b** and **32b**.

A related picture shows the biotransformation of bis(epoxide) **33** using cytosolic epoxide hydrolase from rat liver (Scheme 14). In the first step, hydrolysis occurred to furnish the expected



Scheme 14 Liberation of Nu (–OH) *via* enzymatic hydrolysis of an epoxide, followed by intramolecular opening of another epoxy moiety.

epoxy-*vic*-diol **34**, which acted as a nucleophile in an intramolecular S_N2 fashion with the remaining epoxy moiety to finally yield tetrahydrofuran derivatives **35** in a manner related to that described above.[24] Although some asymmetric induction for the THF derivatives **35** might be expected in this cascade, the regio- and enantio-selectivity was not investigated in this study. As a consequence, these results could have also been obtained *via* chemical catalysis. In this context it should be mentioned that a related base-catalyzed cascade reaction of optically active tris(epoxide)s has been published.[25]

A careful survey of the literature reveals several transformations which cannot be accurately classified as they remain rather unclear in terms of the catalyst(s) involved and/or the intermediates that occur along the pathway. However, they illustrate the wide scope of the synthetic potential of biocatalyzed cascade reactions. In this context, the cyclization of non-conjugated cyclic dienes by root extracts of chicory (*Cichorium intybus*) to yield bicyclic products constitutes an example of an enzyme-triggered cyclization reaction. In this case, the biocatalyst involved as well as the mechanism are still unknown.[26]

The same holds true for the anomalous course of the enzymatic hydrolysis of highly strained bicyclic norbornene oxides, which did not lead to the formation of the expected *vic*-diols, but gave products with a rearranged carbon framework.[27]

4 Conclusion and outlook

The application of enzyme-triggered cascade reactions to the transformation of non-natural compounds offers two distinct advantages. Firstly, the final product can often be obtained in good yield, despite the fact that the reaction sequence involves several steps through highly reactive and thus ostensibly unstable intermediate species. Secondly, based on the exquisite diastereo- and enantio-selectivities of biocatalysts, pathways may often be followed in an asymmetric fashion, thus non-racemic products are obtained.

Until now, in all cascade reactions involving a biotransformation, the biocatalyst was employed in the first step. This is likely to be the most economic strategy, since asymmetry is introduced at the very beginning of the cascade. Since the design of enzyme-triggered cascade reactions is not trivial and (out of necessity) will always involve rather complex molecules, these processes are unlikely to become a general tool but they definitely offer an intelligent option for asymmetric syntheses. Given the knowledge obtained to date, this field as a whole is almost unexploited and, to our belief, its potential is underestimated. Detailed studies on this topic are being carried out in our laboratories.

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