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Organocatalysis and Biocatalysis Hand in Hand: Combining Catalysts in One-Pot Procedures

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Dedicated to Prof. Vicente Gotor on the occasion of his 70th birthday.

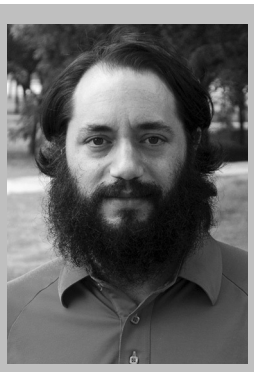
Abstract: Multi-step processes catalysed by several catalysts working concurrently have been developed in nature, thus improving reaction efficiency. The quest for novel and improved catalytic systems has led to the development of biocatalytic and later to organocatalytic procedures as very valuable tools in asymmetric synthesis while using mild reaction conditions in the absence of metal catalysts. As a timeless challenge, chemists are facing the need for process designs in which different sorts of catalysts can operate successfully in a one-pot concurrent fashion. Likewise, such designs bring about the best of each catalyst and, in certain cases, enable us to improve problematic issues, such as reactivity, selectivity, solubility, inhibition, *etc.* Specifically, to combine these two types of catalysts in one-pot, achieving high yields and selectivity, is a fascinating aspect of catalysis. This review covers representative advances in this field, in particular those in which biocatalysts and organocatalysts are employed either in sequential reactions or in simultaneous processes.

Keywords: concurrent processes; enantioselectivity; enzyme catalysis; multistep synthesis; one-pot procedures; organic catalysis

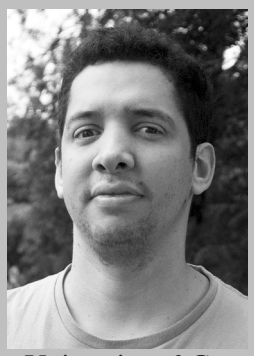
1 Introduction

In nature, the optimisation of a given process is driven by evolutionary pressures. Resource- and energy-saving maximisation became evolutionary pressures; hence, cellular machineries, *i.e.*, enzymatic networks dedicated to a specific or general cellular task, have evolved. In order to improve metabolic efficiency, living systems make use of several extremely selective catalysts working at the same time, thus forming complex biochemical networks. To achieve such a degree of success, a perfect regulation of the different catalysts working concomitantly, often in the same compartment, became of utmost importance. In this way, for a given biosynthetic pathway, the product of one reaction is the substrate of the following one, avoiding intermediate accumulation and, therefore, side reactions.[1]

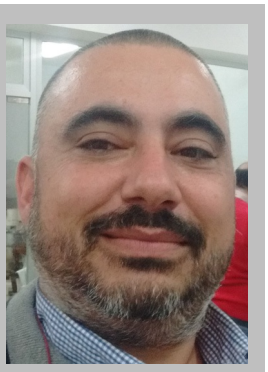
Often, chemists employ consecutive multistep chemical synthesis with catalytically efficient reactions thus improving the overall "atom economy" of the process.[2] These methodologies have been widely adopted in the industrial manufacture of fine chemicals and pharmaceutical intermediates. In this framework, strategies where multiple catalysts simultaneously work in "one-pot", avoiding the costly isolation and purification of chemical intermediates, are named concurrent.[1a,3] Therefore, reactions taking place in the cellular environment are in fact considered as concurrent. In organic chemistry, it has been largely



Fabricio R. Bisogno obtained his degree in biochemistry (2004) at San Luis National University (Argentina). In 2007, he spent half a year at the Weizmann Institute of Sciences (Israel, Prof. Meir Lahav) involved in research on the origin of homochirality. Then, he pursued Ph.D. studies at University of Oviedo (Spain, Prof. Iván Lavandera and Prof. Vicente Gotor), dealing with biocatalytic redox processes (2010). Besides, in 2012 he obtained another Ph.D. degree at San Luis National University (Argentina, Prof. Marcela Kurina-Sanz) working on fungal biotransformation of bioactive compounds. After postdoc periods in Oviedo and Córdoba (Argentina), in 2013 he joined Prof Alicia Peñéñory's group as researcher at Córdoba National University and INFIQC-CONICET (Argentina). His research interest comprises combination of biocatalysis with metal catalysis and organocatalysis for the construction of cooperative systems, along with exploration of novel reactivities for organo-sulfur- and organo-selenium-containing compounds in enzymatic or biomimetic processes.



Martín G. López-Vidal obtained his degree in biochemistry at Córdoba National University (2014, Argentina). In 2015, he joined Prof Alicia Peñéñory's group as a Ph.D. CONICET-fellow, under the guidance of Fabricio R. Bisogno at Córdoba National University. In 2016 he spent a three-months stay in the Laboratory of Biocatalysis at the University of Graz (Austria) exploring the reactivity of sulfur-containing compounds with ene-reductases under the supervision of Prof Mélanie Hall. His research interest is focused on the development of novel chemoenzymatic cascades for stereoselective preparation of organochalcogen-compounds.



Gonzalo de Gonzalo obtained his Ph.D. in 2003 (Prof. Vicente Gotor, University of Oviedo) working on the field of biocatalysis employing lipases and oxynitrilases. He spent his postdoctoral research at Consiglio Nazionale delle Ricerche (ICRM, Milano, Italy, Dr. Giacomo Carrea), moving back to University of Oviedo with a Juan de la Cierva Fellowship. After a one-year postdoctoral stage at University of Groningen (The Netherlands, Prof. Marco W. Fraaije) working in the research of novel oxidative biocatalysts, he spent two years at the R&D Department of the pharmaceutical company Antibióticos S.A.U. (León, Spain). He is currently a Ramón and Cajal Researcher (MINECO) at University of Sevilla. His research is focused on asymmetric synthesis by using different approaches, including biocatalytic and organocatalytic procedures, as well as the development of concurrent chemo- and biocatalytic reactions.

demonstrated that running multiple reactions in one-pot, either in sequential (also known as stepwise, when operations such as addition of catalysts/reagents, temperature/atmosphere modification, etc. are made during the course of the process to ensure the proper reactivity mode) or simultaneous (also known as cascade or domino reactions, in which conditions are not modified during the process and catalysts/reagents are added at the beginning, thus requiring only one operational step) mode, is challenging to a great extent given the diverging reaction conditions suitable for each single transformation.[4] Thus, tremendous efforts are made in order to find proper conditions to harmonically combine multiple catalytic reactions with no cross-spoiling effects.[5]

In a broad sense, organocatalysis can be defined as the acceleration of a chemical reaction using an organic compound in substoichiometric amounts in the absence of (transition) metals.[6] On the other hand, an acceptable definition of biocatalysis in organic synthesis can be the employment of a biomolecule (protein, antibody, ribozyme) or a living organism to carry out the transformation of a (non-)natural organic compound.[7]

Nowadays, both organocatalysis and biocatalysis are becoming mature and a mechanistic understanding of enzymatic reactions along with catalytically-productive organic associations have been deeply investigated. It is rather remarkable that the number of examples where both sorts of catalysis working concurrently were successfully applied is limited when compared with the far more explored combination of metal catalysed processes and biocatalysis.[8] Some reports have been published based on the combination of transition metals and organocatalysts;[9] but not so many examples are available dealing with the combination of organocatalysis and biocatalysis in one-pot procedures.[10]

It must be taken into account that organic substances are not always soluble in aqueous media, so a proper solvent selection is usually an issue in both organo- and biocatalytic systems. Notwithstanding, the use of cosolvents or additives (in the frame of a "medium engineering" concept), is a common practice in those catalytic methodologies, thus circumventing solubility and, in certain cases, reactivity problems.[11] For a combination of organo- and biocatalytic processes in one-pot procedures, the reaction medium must be carefully tailored in order to avoid cross-spoiling. Cosolvents and additives for the organocatalysed reaction need to be tested towards the biocatalysed reaction to prevent inhibitory effects.

From a synthetic point of view, chirality in the formed product can be installed or defined either through the enzymatic or the organocatalysed reaction or, even, both catalytic systems may define stereocentres in the same catalytic cycle, increasing the complexity of the final products.

With the settlement of technologies such as photochemistry, flow chemistry, mechanochemistry, among others, that can be incorporated into complex multicatalytic processes, the boundaries of combined catalysis are continuously being pushed forward.

In this review, the focus will be placed on selected representative examples that may give a general idea of the possibilities of combining organocatalysts and biocatalysts in one-pot including sequential and simultaneous methodologies. Special emphasis will be given to asymmetric processes.

2 Sequential Reactions Employing Organocatalysts and Biocatalysts

Initially, we shall deal with processes in which the organocatalyst and the biocatalyst are able to catalyse sequential reactions in order to have multistep one-pot procedures (Figure 1).[12] The selectivity of the final products when chiral compounds are synthesised, can be induced by only the organocatalyst, by only the biocatalyst or by both catalysts performing selective reactions in the sequential process.

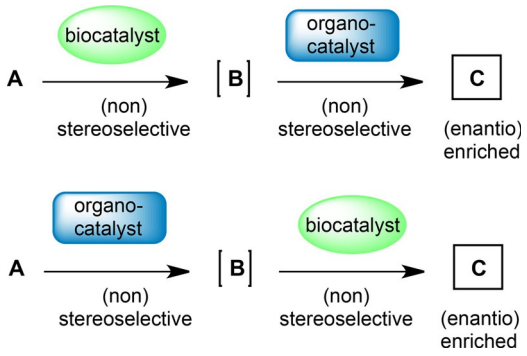
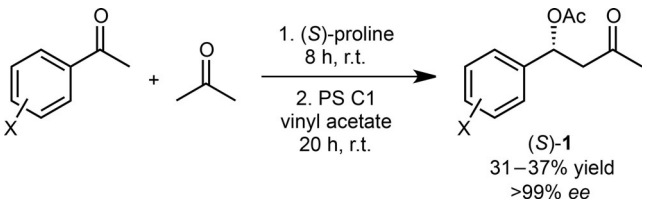


Figure 1. Schematic representation of a sequential one-pot process using organocatalysis and biocatalysis.

One of the first examples described in the literature of a sequential multistep one-pot process combining organocatalysts and enzymes, was published in 2004 for the synthesis of enantiomerically pure aldols, as shown in Scheme 1.[13]



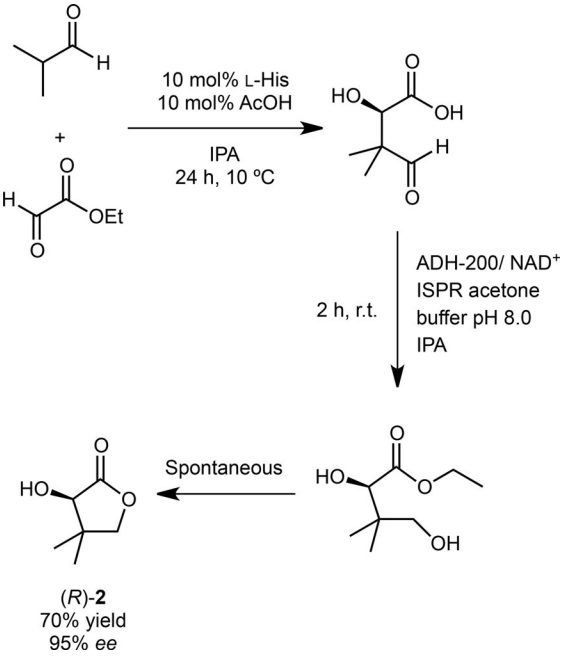
Scheme 1. Synthesis of optically active aldols by combining an organocatalytic aldol reaction with a biocatalysed acylation in a sequential one-pot process.[13]

In this process, the proline-catalysed aldol reaction between aromatic aldehydes and acetone was combined with the kinetic resolution of the resulting alcohols catalysed by lipases in the presence of vinyl acetate. Aldol reactions were performed in neat acetone, as the usual solvents for organocatalysed aldol reactions are toxic to lipases. Under these conditions, the aldol adducts were obtained with good yields and moderate selectivities. Such adducts were tested as substrates in biocatalysed acylations using vinyl ace-

tate as acyl donor. Lipase from *Pseudomonas cepacia* Amano I (PS C1) led to excellent selectivities in the kinetic resolutions, thus improving the *ee* obtained in the aldol reaction. In view of these results, the processes were carried out in one-pot. Acetone was removed from the reaction medium before the addition of vinyl acetate and the lipase. Likewise, the use of (*S*)-proline and lipase PS C1 led to the formation of the enantiopure (*S*)-acetates (*S*)-**1** with moderate yields (around 30–40%), These yields achieved in the one-pot procedures were slightly lower than those obtained in the stepwise reactions.

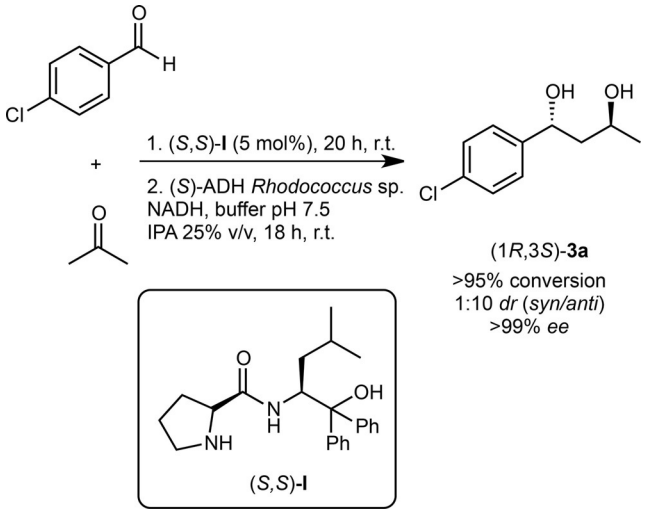
Other amino acids different from proline and its derivatives have been also studied as catalysts in aldol reactions. Thus, use of an L-histidine catalysed aldol reaction combined with an alcohol dehydrogenase (ADH) catalysed reduction,[14] has been reported towards the asymmetric synthesis of (*R*)-pantolactone (**2**).[15]

Firstly, the organocatalysed aldol reaction between isobutanal and ethyl glyoxylate in 2-propanol (IPA) was explored in order to obtain the enantioenriched aldol adduct. Several catalysts were studied, and among the tested amino acids and amino(thio)urea catalysts, L-histidine was chosen, displaying good activity with high enantioselectivity. An additional advantage of L-histidine towards more complex organocatalysts is its commercial availability in both enantiomeric forms. In the Brønsted acid cocatalyst screening, it turned out that acids with pK_a values between 4.0–5.0 showed the best results and the authors adopted acetic acid as cocatalyst. It has been described that those acids could increase the addition yield by facilitating the hydrolysis of the iminium ion formed between substrate and catalyst.[16] Reactions were performed using 2.5 M ethyl glyoxylate, 10 mol% of L-histidine, 10 mol% of acetic acid in a mixture water/alcohol 1:1 for 24 h at 10 °C leading to the formation of the (*R*)-α-hydroxy ester in up to 95% conversion, 85% yield and 79% *ee*. Further studies were focused on the enzymatic reduction of the aldol adduct (0.5 M) and further spontaneous lactonisation of the hydroxy ester, where ADH-200 from Evocatal (evo-1.1.200) showed higher conversions at pH 8.0 in buffer/2-propanol 20% v/v: up to 67% conversion and 95% *ee*. Removal of volatile compounds such as acetone (a by-product formed in the nicotinamide cofactor NAD^+ recycling) and non-converted aldehydes prior to the bioreduction step, increases significantly the conversion (64% *vs.* 33% without removal of volatile compounds under vacuum), probably due to competition of these compounds for the aldol adduct. In order to *in situ* remove the acetone (*In Situ Product Removal* – ISPR),[17] a continuous flow of air saturated with water/2-propanol (5% v/v) passing through the bioreduction has proved to be an effective strategy leading to 86% conversion of the aldol product and 95% *ee* after 2 h. The entire chemoenzymatic synthesis of (*R*)-pantolactone was performed in what the authors claimed as a "one-pot-like" process, as the volatile compounds of the aldol reaction are removed under vacuum prior to the bioreduction and ISPR during the enzymatic reaction. Starting from 1.28 mmol of ethyl glyoxate and 2.65 mmol of isobutanal in 0.41 mL of IPA, (*R*)-**2** was obtained with 55% conversion (referred to ethyl glyoxylate) and 95% *ee*, as shown in Scheme 2.

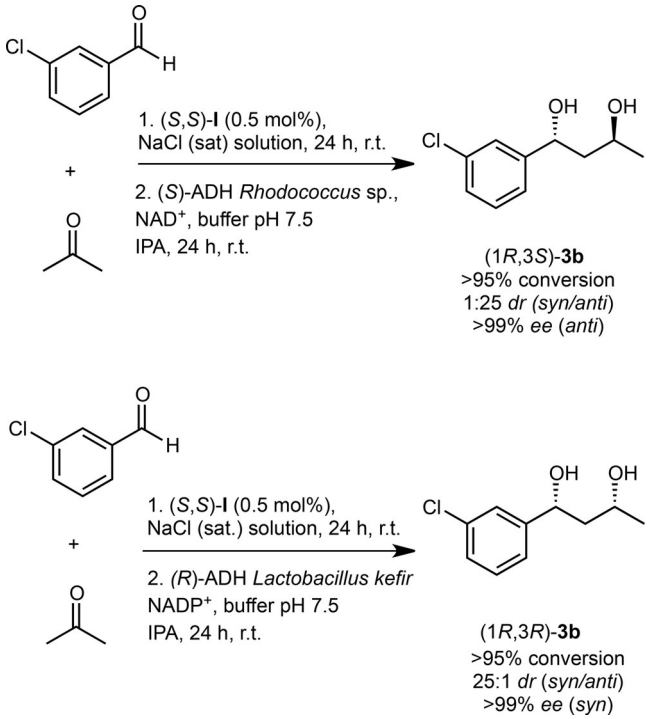


Scheme 2. One-pot preparation of (*R*)-pantolactone **2** by combining organocatalysed aldol reaction with enzymatic bioreduction in presence of ADH-200.[15]

In 2009, the sequential one-pot synthesis of chiral 1,3-diols combining an organocatalytic aldol reaction and an enzymatic keto reduction has been carried out in aqueous media (Scheme 3).[18] The proline-based catalyst **I**, developed by Singh in both homochiral diastereomeric forms,[19] has demonstrated to be an efficient catalyst for the enantioselective formation of β-hydroxy ketones with high yields and enantiomeric excesses. For this reason, preliminary studies were focused on the optimisation of the organocatalysed aldol reaction using both (*S*,*S*)-**I** and (*R*,*R*)-**I** as catalysts. Thus, employing *p*-chlorobenzaldehyde and acetone as model substrates, both enantiomeric forms of the corresponding β-hydroxy ketone were achieved with the same enantiomeric excess (83%). However, the obtained yields were lower in case of the (*R*)-isomer compared with the (*S*)-isomer (58 and 71%, respectively). Further studies were focused on the bioreduction of the aldol products. Either (*S*)-ADH from *Rhodococcus* sp. or (*R*)-ADH from *Lactobacil-*



Scheme 3. One-pot organocatalysed aldol reaction combined with biocatalysed reduction to yield the optically active diol (1*R*,3*S*)-**3a**.[18]



Scheme 4. Synthesis of optically active 1,3-diols by combining organocatalysed aldol reaction in the presence of (*S*,*S*)-**I** with bioreduction catalysed by (*S*)- or (*R*)-ADH in a sequential one-pot process to yield optically active diols (1*R*,3*S*)-**3b** and (1*R*,3*R*)-**3b**.[20]

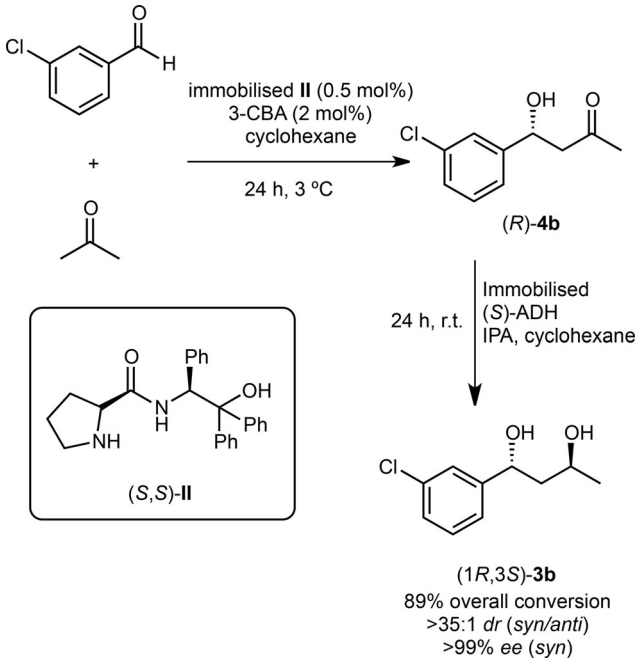
lus kefir using 2-propanol as cosubstrate in a substrate-coupled cofactor recycling system, allowed the authors to synthesise the corresponding four possible isomeric 1,3-diols **3** with excellent results (>95% conversion, >99% *ee* and 1:11 diastereomeric ratio).

In order to explore the substrate scope of this process, other substituents at the *para*-position of the aromatic ring were tested, where *p*-tolualdehyde showed similar results with only a slight decrease in the stereoselectivity of the aldol reaction (76% *ee*). Once the individual steps were optimised, for the proof-of-concept of the whole one-pot two-step system, *p*-chlorobenzaldehyde (0,5 mmol) and acetone were chosen as substrates. Thus, the corresponding β-hydroxy ketone was obtained using (*S*,*S*)-**I** in 20 hours at room temperature. After the aldol reaction, bioreduction employing the (*S*)-ADH was carried out in phosphate buffer pH 7.0 (67 mM of the substrate) containing 2-propanol 25% v/v as cosubstrate at room temperature, affording the desired (1*R*,3*S*)-**3a** with >99% *ee*, 1:10 *syn*/*anti* diastereomeric ratio after 18 hours, reaching >95% conversion as shown in Scheme 3.

Noteworthy, the generation of stereocentres is controlled only by the external catalyst, with marginal (if any) influence of the other chiral centre already present in the reactant. Thus, each stereocentre is possible to be defined enantioselectively by the sole modulation of the organo- and the biocatalysts.

Encouraged by these initial results, expansion of the substrate scope to *meta*-substituted aromatic aldehydes was attempted, as shown in Scheme 4.[20] Similarly, a sequential one-pot multistep system was set up using *m*-chlorobenzaldehyde as electrophile and 9 equivalents of acetone. Thus, with the chemoenzymatic one-pot synthesis as a final goal, the organocatalysed aldol reaction was optimised in saturated aqueous NaCl solution. As expected, the catalyst loading plays a critical role in the stereochemical outcome of the reaction and a significant decrease in enantioselectivity was observed using 5 mol% of (*S*,*S*)-**I** after 48 h. Hence, different catalyst loadings were tested, resulting in a high enantioselectivity at 0.5 mol%, while total reaction inhibition was seen at 10 mol% of **I**. The authors attributed this fact to a switch of the reaction control from a kinetic to a thermodynamic regime.

Thus, the combination of the organocatalysed aldol reaction with bioreduction using either (*S*)-ADH from *Rhodococcus* sp. or (*R*)-ADH from *Lactobacillus kefir* under optimised conditions, enabled the preparation of the corresponding optically active 1,3-diols **3b** starting from *m*-chlorobenzaldehyde and acetone in a one-pot, two-step fashion in 48 h. The products were obtained with >95% overall conversion, >25:1 diastereomeric ratio and >99% enantiomeric excess. As already mentioned, each catalyst or set of conditions must be optimised according to the required performance. In this case, different amounts of enzyme were used depending on the catalyst's activity towards the substrate, employing 10 units/mmol> or 480 units/mmol with of (*S*)- or (*R*)-ADH, respectively.



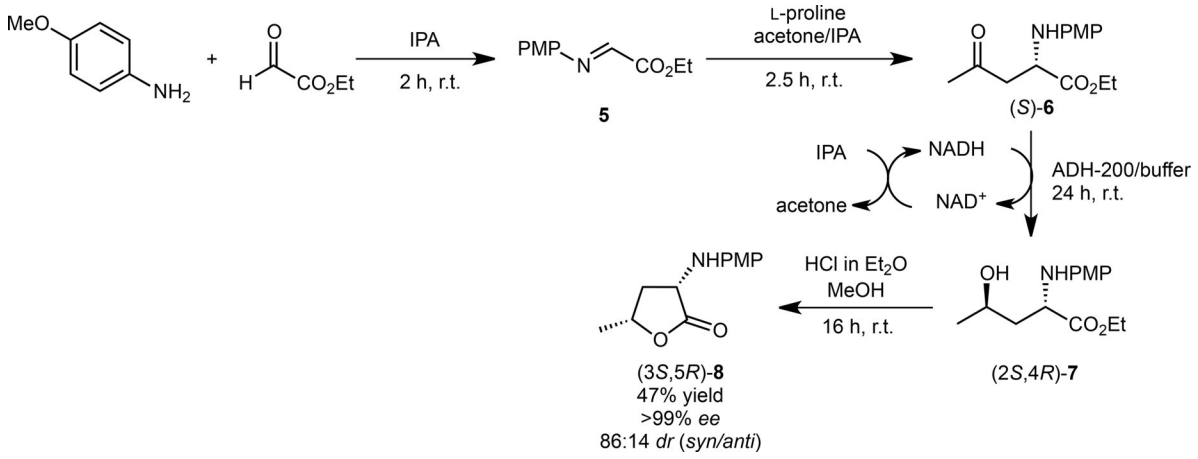
Scheme 5. Synthesis of (1*R*,3*S*)-**3b** in a one-pot procedure using both coimmobilised organocatalyst (*S*,*S*)-**II** and (*S*)-ADH from *Rhodococcus* sp. with its cofactor NAD^+ in organic solvent medium.[21]

Very recently, by taking advantage of this sequential process, the use of organic media was demonstrated in a similar one-pot process for an alternative preparation of chiral 1,3-diols, as shown in Scheme 5.[21] Immobilised organocatalyst **II** in acrylic polymeric beads catalysed the aldol reaction between aromatic aldehydes and acetone in organic media with similar results to those obtained with the free catalyst in aqueous media.[22] Likewise, employing immobilised **II**, it was possible to achieve the (*R*)-β-hydroxy ketone (*R*)-**4b** starting from *m*-chlorobenzaldehyde (1.5 M) as acceptor with 95% overall conversion and 95% *ee* after 24 h. However, 3-chlorobenzoic acid (3-CBA, 2 mol%) was needed as cocatalyst in order to overcome the low activity of (*S*,*S*)-**II** in non-aqueous conditions, and optimal performances were found at 3 °C in order to preserve a suitable stereorecognition in an organic solvent such cyclohexane.

The immobilised catalyst was recycled by decanting and evaporating the organic layer before the bioreduction step. Likewise, chiral (1*S*,3*R*)-**3b** was obtained using an (*S*)-ADH from *Rhodococcus* sp. coimmobilised with its cofactor (NAD^+) onto the superabsorbent polymer Favor SXM 9155 (Evonik Industries AG).[23] The desired diol was obtained with high conversion (89%) and excellent diastereo- and enantioselectivity (*dr* >35:1, >99% *ee*). Moreover, the free proline derivative catalyst **II** was also tested in this one-pot process and the same results were obtained in terms of activity and selectivity, making also suitable the combination in a one-pot process of a non-immobilised organocatalyst with an immobilised ADH in organic media.

α-Amino-γ-butyrolactones are valuable building blocks present in a set of natural compounds and pharmaceuticals. These compounds can be prepared by combining an L-proline-catalysed Mannich reaction to obtain an aminoketo ester, which can be further reduced to any diastereomer alcohol by choosing the suitable ADH.[24] The resulting amino alcohols cyclise, either spontaneously or under transesterification conditions (HCl-MeOH), to the desired aminolactones. The described synthesis comprises isolation of Mannich adduct intermediates, but the authors have also developed the one-pot reaction.

As shown in Scheme 6, the starting aldimine **5** is synthesised by mixing *p*-anisidine (0.53 mmol) with ethyl glyoxylate (0.55 mmol) in the presence of IPA (2.5 mL), which also serves as the solvent for the



Scheme 6. Synthesis of aminolactone (3*S*,5*R*)-**8** in a one-pot procedure by combing an L-proline-catalysed Mannich reaction with a biocatalysed reduction of the formed ketone (*S*)-**6**.[24]

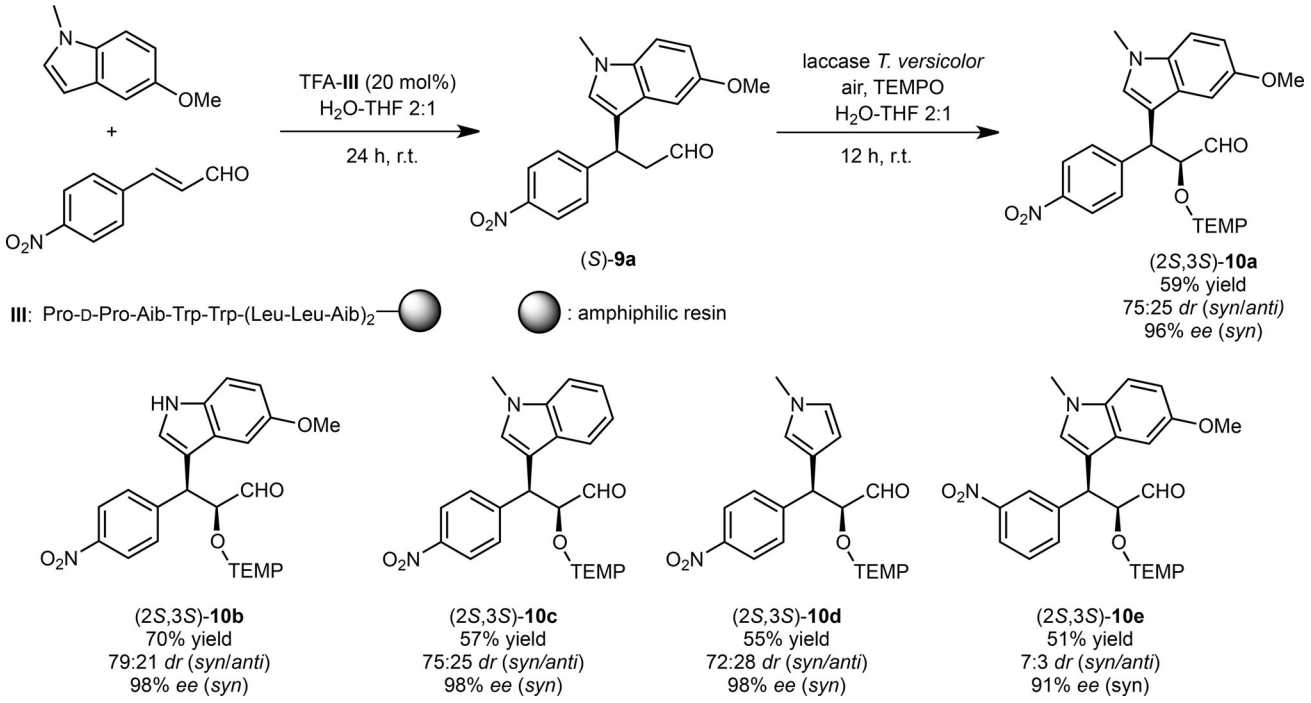
Mannich reaction catalysed by L-proline (0.13 mmol), and as hydrogen donor (cosubstrate) for the reduction of the formed ketone (*S*)-**6** to the corresponding amino alcohol (2*S*,4*R*)-**7** in the presence of ADH-200, after dilution of the reaction medium with aqueous buffer. The final transesterification in the presence of HCl in MeOH led to the enantiopure *syn*-(3*S*,5*R*)-lactone **8** with 47% yield and diastereomeric excess of 72%. This value, lower than that obtained in the step-by-step synthesis, can be due to the 2-propanol employed as the solvent for the organocatalysed reaction, which might impair the selectivity of this step.

In a similar one-pot approach, aldimine **5** (1.0 mmol) was employed in the Mannich reaction with acetone (5 mL) as solvent and L-proline (0.25 mmol) as organocatalyst. After 16 h reaction time, the solvent was evaporated and ketone (*S*)-**6** was selectively reduced in the presence of ADH-200 in buffer/IPA to amino alcohol (2*S*,4*R*)-**7**. Further hydrolysis and purification afforded aminolactone (3*S*,5*R*)-**8** with 51% yield.

Resin-supported peptides can be employed in organocatalysed reactions carried out in aqueous environment.[25] These catalysts have been also applied to the one-pot sequential synthesis of oxyfunctionalised indoles,[26] in combination with the laccase from *Trametes versicolor*.[27] The resin-supported peptide **III** catalysed the asymmetric Friedel–Crafts alkylation of the starting indole (0.1 mmol) with an α,β-unsaturated aldehyde (0.15 mmol), through an iminium intermediate, while the laccase was able to catalyse the selective α-oxyamination of the aldehyde (*S*)-**9a** formed in the first step, as shown in Scheme 7. The one-pot reaction was carried out in aqueous medium. After 24 h for the Friedel–Crafts alkylation at room temperature, laccase and TEMPO (2,2,6,6-tetramethylpiperidine 1-oxyl radical) as mediator were added to the reaction mixture which was stirred for additional 24 h. When employing 4-nitrocinnamaldehyde, the final product (2*S*,3*S*)-**10a** was recovered with a *syn*/*anti* ratio 75:25 and 96% *ee* for the major diastereomer, but unfortunately with a very low conversion. The use of THF as reaction cosolvent (1:2 ratio with water) allowed an increase of the substrate solubility, reaching a 59% yield for the final product with the same optical purity.

The reaction was then extended to other substituted indoles, to *N*-methylpyrrole and to 3-nitrocinnamaldehyde. For all the examples, the *syn*/*anti* ratios of the final compounds (2*S*,3*S*)-**10b–e** were around 75:25, reaching excellent optical purities for the *syn* diastereomer, while the yields were between 51 and 70%.

In 2012 the first one-pot sequential three-component reaction was described in which two C–C bonds were created.[28] In the first step, a diamine organocatalyst **IV** (10 mol%) was able to perform the aldol reaction between a glyoxylamide (0.1 mmol) and acetaldehyde (0.1 mmol). The second C–C bond formation was carried out by the E192N mutant of *N*-acetylneuraminic acid lyase (NAL), which catalysed the aldol condensation between the aldehyde formed in



Scheme 7. One-pot synthesis of indole derivatives **10a–e** by combining a resin-supported peptide-catalysed Friedel–Crafts alkylation with a laccase-catalysed α-oxyamination.[26]

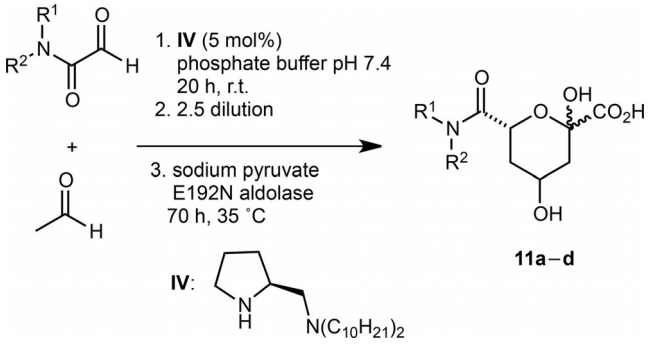
the first step and pyruvate (0.1 mmol). This linear product spontaneously cyclises to achieve the hemiketalic final products **11a–d**.

As the enzymatic reaction has to be carried out in aqueous buffer, the organocatalysed aldol condensation was studied in this reaction medium. It was observed that with 10 mol% **IV**, the reaction took place, but unfortunately with a modest diastereoselectivity and a drastic decrease in the enzymatic activity.

For this reason, the one-pot, three-component reaction was carried out in a reaction medium that was diluted with aqueous buffer after the first aldol reaction. Thus, a set of glyoxylamides reacted with 10 equivalents of acetaldehyde in the presence of 5 mol% organocatalyst in buffer pH 7.4 for 20 hours. After this time, a 2.5-fold dilution with buffer was performed and sodium pyruvate and E192N NAL were added. Reaction mixtures were shaken for other 70 h at 35 °C. The final products **11a–d** were recovered with yields around 40–51% and modest diastereoselectivities (Table 1). These lower values can be explained by two reasons: (i) the absence of selectivity in the organocatalysed step; and (ii) the low selectivity of the NAL-catalysed reaction.

A one-pot sequential organo- and biocatalysed process has been recently developed for the preparation of (3*R*,3*aS*,6*aR*)-hexahydrofuro[2,3-*b*]furan-3-ol (−)-**12** (bis-THF-alcohol, Scheme 8), a structural motif of different HIV-1 protease inhibitors such as Darunavir and Brecanavir. Firstly, the organocatalysed condensation of 1,2-dihydrofuran and glycoaldehyde dimer was studied in order to obtain a mixture of *syn* and *anti* diastereomers of bis-THF-alcohol.[29] Reactions were carried out in the presence of hexafluoroisopropyl alcohol (HFIP) as it has been described that this additive could increase the condensation yield by favouring the formation of glyceraldehyde monomer from its dimer. Among all the catalysts tested, Schreiner's thiourea (**V**) was the most active,[30] leading to a mixture of both *anti*-bis-THF-alcohol (the desired one to complete the synthesis) and the *syn* diastereomer with moderate yields.

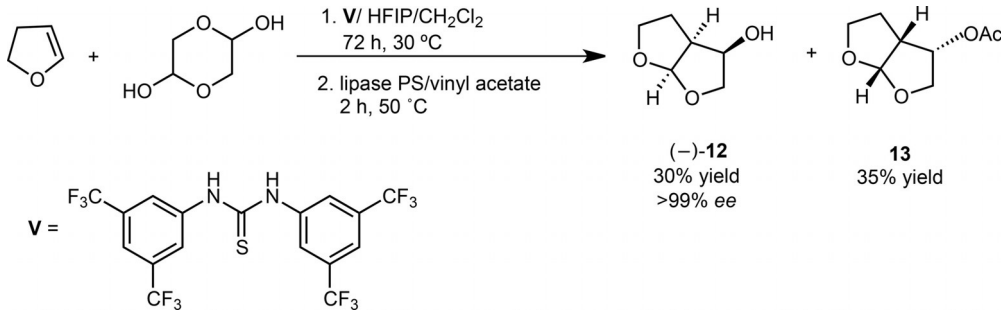
Table 1. One-pot three-component reaction catalysed by proline derivative **IV** and E192N NAL.[28]



Product	R^1	R^2	Yield [%][a]	*cis/trans*[b]
11a	Pr	Pr	40	78:22
11b	Me	Pr	48	57:43
11c	Me	Me	43	71:29
11d	Et	Et	51	61:39

[a] Based on glyoxylamide starting material.
[b] Determined by ^{1}H NMR.

Process optimization allowed the authors to obtain a 70% yield of *anti*-**12** by using 2 equivalents of 1,2-dihydrofuran and HFIP, 1 mol% of Schreiner's catalyst in dichloromethane at 30 °C. The catalysed acetylation of the *anti*-alcohol with vinyl acetate at 50 °C was tested in the presence of different lipases. Best results were achieved with lipase PS, recovering enantiopure *anti*-**12** with a 37% yield. Once the two catalytic processes had been optimised, the one-pot reaction was performed, first by carrying out the organocatalysed condensation between 1.0 mmol of dimer and 4.0 mmol of 1,2-dihydrofuran in 2 mL of CH_2Cl_2 in the presence of 4.0 mmol of HFIP and 2 mol% of thiourea **V** at 30 °C during 72 hours. After this time, vinyl acetate (2 mL) and lipase PS (260 mg) were added and the system was stirred at 50 °C for 24 h. After the crude purification, enantiopure *anti*-alcohol was obtained with 30% yield, while the *syn*-acyl ester **13** was obtained with 35% yield. Scaling up of the



Scheme 8. One-pot synthesis of the valuable synthon bis-THF-alcohol **12** by combining Schreiner's thiourea **V** with lipase PS.[29]

process up to the gram-scale (20 mmol of starting material) led to the same yield for the desired alcohol with 97% *ee*, demonstrating that this one-pot procedure can be practical for the preparation of pharmaceuticals.

3 Simultaneous One-Pot Processes Combining Organocatalysts and Biocatalysts

Apart from sequential one-pot reactions combining both organo- and biocatalysts, other designs have been performed in which all the reaction components are added at the beginning of the reaction (Figure 2).

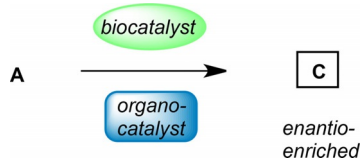
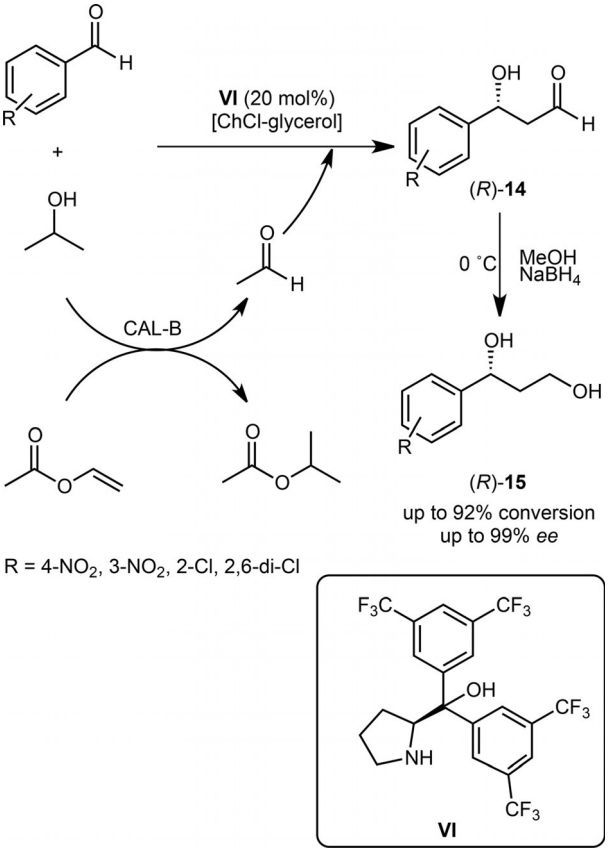


Figure 2. Typical simultaneous one-pot synthesis using organo- and biocatalysts.

The synthesis of diol (1*R*,3*S*)-**3b** (Scheme 4) has been recently performed by combining organocatalyst (*S*,*S*)-**I** and (*S*)-ADH from *Rhodococcus sp.* in a simultaneous way by adding both the organocatalyst and the biocatalyst at the beginning of the reaction.[31] Conditions for both the aldol reaction and the enzymatic reduction were optimised in order to develop an efficient process and to minimise possible side reactions such as the biocatalysed reduction of the aldehyde, the biooxidation of diol **3b** to ketone **4b**, or aldol condensations. By employing an aldehyde concentration of 500 mM, 5 equiv. of acetone and 2-propanol as cosubstrate for the ADH (28% v/v), enantiopure (1*R*,3*S*)-**3b** can be obtained in 60% conversion after 24 hours. Scaling up to 10 mmol of 3-chlorobenzaldehyde led to 50% conversion of the desired diol.

In 2014, an aldol reaction using an alternative source of aldehydes has been explored. In this context, the use of vinyl esters combined with lipases was proposed to generate acetaldehyde that will be employed in the organocatalysed synthesis of chiral 1,3-diols.[32] This strategy allows one to keep the acetaldehyde concentration low, therefore diminishing the deleterious effects of this compound towards the enzymatic catalyst and avoiding self-condensation of this aldehyde. The lipase-catalysed transesterification of vinyl acetate with 2-propanol leads to the formation of acetaldehyde, which will serve as substrate in an organocatalysed aldol reaction, leading to chiral β-hydroxyaldehydes (*R*)-**14**. All the reactions occurred in a simultaneous one-pot fashion. Moreover, this process can be coupled with a further sequential reduction of the aldehyde to the corresponding (*R*)-1,3-diol (**15**) in the presence of $NaBH_4$ (Scheme 9), minimising the retro-aldol reaction.



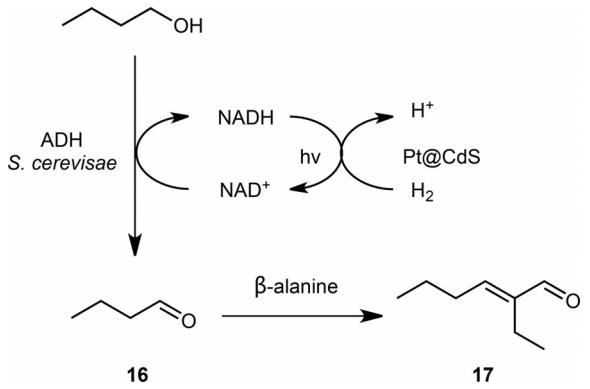
Scheme 9. Synthesis of (*R*)-1,3-diols in a one-pot process combining CAL-B and trifluoromethyl prolinol catalyst **VI** in DES.[32]

Thus, the one-pot reactions were performed using 1.0 mmol of aldehyde, 3.0 mmol of both vinyl acetate and propanol and 20 mol% of trifluoromethyl-substituted diphenylprolinol (**VI**) as organocatalyst. The deep eutectic solvent (DES) choline chloride-glycerol (1:2 molar ratio; 1.0 mL) was employed as reaction medium,[33] while immobilised lipase B from *Candida antarctica* (CAL-B) carried out the acetaldehyde generation. Conversions were very high after 48 h (up to 92%), with reasonably high yields (up to 70%), and excellent enantioselectivities (up to >99% *ee*). Substrates with electron-withdrawing substituents at any position of the aromatic ring were tested with similar good results.

The reaction was also extended to cinnamaldehyde derivatives such as α-bromocinnamaldehyde, with excellent *ee* (96%), although poor yield (14%). In addition, the use of aldehydes lacking electron-withdraw-

ing groups such as 4-pyridinecarboxaldehyde or cinnamaldehyde led to disappointingly low yields (up to 2%) and the enantioselectivity was fully suppressed. Furthermore, the mandatory use of acetaldehyde leads always to β-hydroxy aldehydes, therefore chiral 1,3-diols are obtained with only one stereocentre. In this case, the stereoselectivity is totally controlled by the organocatalyst. DES and CAL-B could be reused for up to six cycles without loss of enzymatic activity. Regarding the organocatalyst, yields were stable when fresh **VI** was added to the reaction medium while a slight loss of activity was observed in the absence of extra organocatalyst.

The combination of photo-, organo- and biocatalysis has recently allowed the conversion of *n*-butanol to 2-ethylhexenal (Scheme 10). In this simultaneous



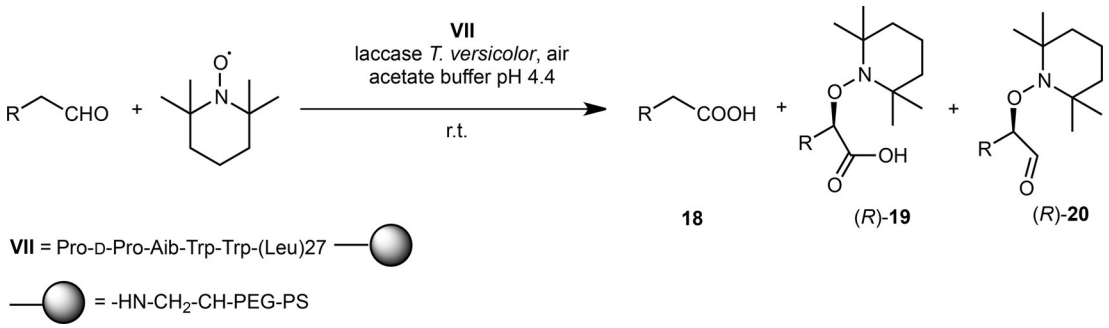
Scheme 10. Synthesis of 2-ethylhexenal **17** by combining three catalysts in a one-pot simultaneous procedure.[34]

one-pot process the ADH from *Saccharomyces cerevisae* catalysed the oxidation of *n*-butanol to *n*-butyraldehyde (**16**) using NAD^+ as cofactor. The photocatalyst platinium-seeded cadmium sulfide (Pt@CdS) Quantum Dot was employed for the cofactor regeneration. *n*-Butyraldehyde was converted to 2-ethylhexenal (**17**) in an aldol condensation catalysed by β-alanine.[34] The single reaction was performed by mixing 50 mM of *n*-butanol with 25 mM Pt@CdS, 3 mM NAD^+ and 550 mM β-alanine. After degassing this system for one hour, 100 units of the ADH were added to initiate the reaction. The system was photoirradiated for 3 hours and the crude material was extracted and analysed by ^{1}H NMR, with recovered of 1.5 mM of **16** and 1.8 mM of **17**.

The one-pot system was also carried out in a synthetic acetone-butanol-ethanol (ABE) solution, using 15 mM acetone, 30 mM butanol and 5 mM ethanol. The three catalysts and NAD^+ were added to this solution. After 3 h photoirradation, the solution was extracted and analysed by ^{1}H NMR. A mixture of 1.0 mM of acetaldehyde, 1.0 mM of butyraldehyde and 1.5 mM of 2-ethylhexenal was recovered.

Resin-supported peptides (see Scheme 7) have been also employed in the simultaneous asymmetric α-oxyamination of aldehydes in combination with the laccase from *Trametes versicolor* and TEMPO as mediator.[35] This reaction can be regarded as a formal enolate/enamine one-electron oxidation,[36] and further radical trapping by TEMPO. Initial studies were devoted to analyse the product outcome under different reaction conditions. Thus, the laccase-catalysed oxidation of 3-phenylpropanal in acetate buffer afforded the carboxylic acid (**18**) as sole product. When this biocatalysed reaction was carried out in the presence of pyrrolidine as base catalyst, the racemic α-oxyaminated carboxylic acid (**19**) was formed together with the α-unsubstituted carboxylic acid.

The pyrrolidine-catalysed reaction performed in a water/1,4-dioxane mixture led to the formation of α-unsubstituted carboxylic acid and two racemic oxyaminated products, the chiral aldehyde (*R*)-**20** and the carboxylic acid (*R*)-**19**. The use of the resin-supported peptide **VII** as catalyst (Scheme 11) in water allowed the formation of (*R*)-α-oxyaminated carboxylic acid (65% conversion) with 63% *ee* along with a smaller amount of **18** (35%). In view of these results, the one-pot reaction in the presence of **VII** and the enzymatic system was extended to 4-arylbutanals. Thus, oxidation of 4-phenylbutanal led only to the α-oxyaminated aldehyde with 71% yield and 82% *ee*. Similarly, complete conversion (53% yield) and 80% *ee* were ach-

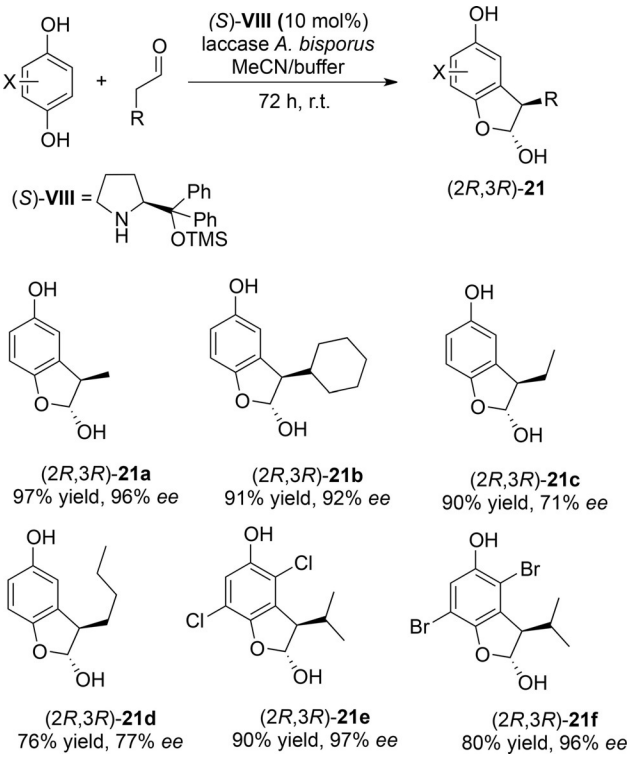


Scheme 11. Peptide-supported **VII**/laccase-catalysed simultaneous one-pot α-oxyamination of aldehydes to obtain the corresponding chiral (*R*)-α-oxyaminated carboxylic acids (**19**) and aldehydes (**20**).[35]

ieved in the reaction of 4-(4-methoxyphenyl)butanal. This yield could be improved by performing the reaction in acetate buffer (74%), while the addition of the surfactant Tween 80 to the reaction mixture after 2 hours resulted in the α-oxyaminated carboxylic acid as major product (81%) with 64% yield and 91% *ee*.

Taking into account these encouraging results, the asymmetric oxidation of different aldehydes with laccase and **VII** was performed in the absence and in the presence of Tween 80. Reactions were carried out with 0.05 mmol of aldehyde and TEMPO, while using 0.01 mmol of **VII** and 0.5 mg of laccase in 0.5 mL of acetate buffer. In the absence of surfactant, the oxyamination afforded the chiral aldehydes (*R*)-**20** in 1 h with good to moderate yields and optical purities close to 90%, while the presence of Tween 80 (1.0 μL) led to the carboxylic acids (*R*)-**19** with good yields and high enantioselectivities after 5–8 h. The system proved to be highly efficient since it was possible to reduce the amount of both catalysts to 5 mol% with negligible loss of activity and selectivity.

A set of 3-substituted-2,3-dihydrobenzofuran-2,5-diols (**21a–f**) was synthesised in a one-pot cascade procedure by combining an initial laccase-catalysed oxidation of 1,4-dihydroxybenzenes (hydroquinone derivatives), with the sequential aminocatalysed α-arylation of aldehydes, as shown in Scheme 12.[37] The

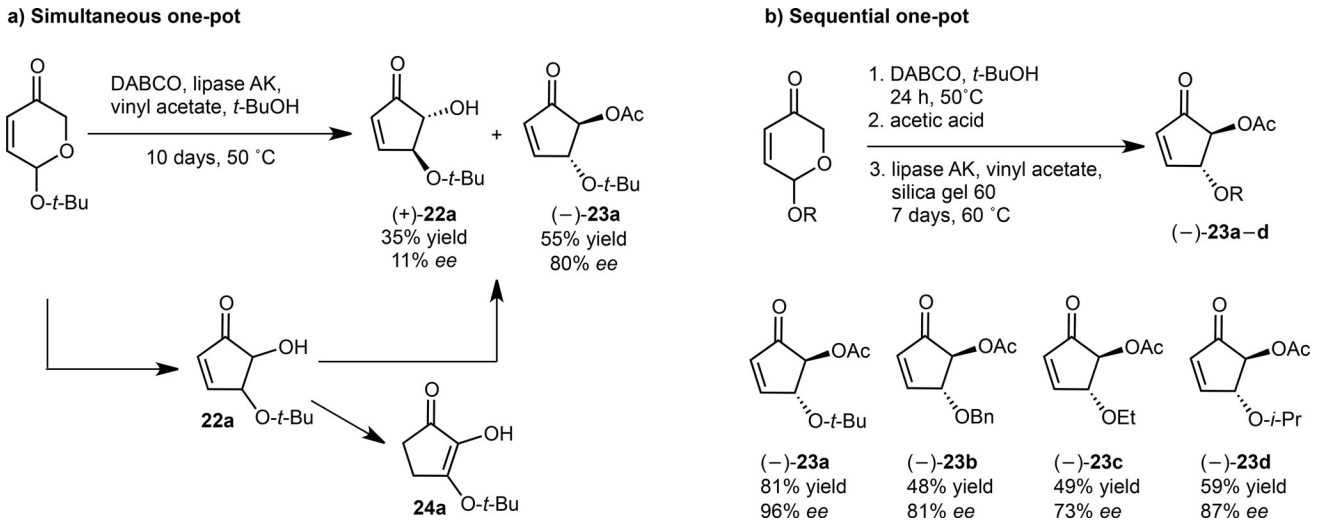


Scheme 12. One-pot synthesis of chiral 3-substituted 2,3-dihydrobenzafuran-2,5-diols (**21a–f**) employing the secondary amine (*S*)-**VIII** as organocatalyst and laccase from *Agaricus bisporus*.[37]

reaction features laccase-catalysed oxidation of the hydroquinone reagent giving rise to radical species that further add to the chiral enamine double bond. Reaction optimization was performed for the reaction between 3-methylbutanal and 1,4-dihydroxybenzene in the presence of (*S*)-2-[diphenyl(trimethylsilyloxy)-methyl]pyrrolidine (*S*)-**VIII** as organocatalyst and the laccase from *Agaricus bisporus*. The initial reaction was carried out with 0.25 mmol of 1,4-dihydroxybenzene in 0.5 mL buffer/acetonitrile pH 6.0 containing 10 mol% of (*S*)-**VIII**, 15 units of laccase and 5 equivalents of aldehyde per equivalent of 1,4-dihydroxybenzene.

After 72 hours, the final product **21a** was obtained with 76% yield and 87% *ee*. The use of 45 units of laccase had a really positive effect on the procedure, as the yield increased up to 97% yield in the same reaction time with 96% *ee*. The amount of organocatalyst had no effect on the cascade system. The reaction was scaled up to 2 mmol of 1,4-dihydroxybenzene, recovering the final hemiacetal with 84% yield and 92% *ee*. Another β-branched aldehyde such as 2-cyclohexylacetaldehyde has been successfully tested in this procedure (91% yield and 92% *ee* for **21b**). On the contrary, unbranched aldehydes (for instance **21c** and **d**) led to moderate to good yields (51% to 90%) and lower optical purities. It was observed that shorter reaction times afforded higher selectivities. Studies on this line suggested that the final product tautomerises, and thus the aldehyde forms an iminium ion with the aminocatalyst, leading to an enamine after deprotonation, which upon hydrolysis afforded again the hemiacetal. As branched aldehydes are more stable in the hemiacetalic form, this racemisation did not occur, while a significant amount of aldehyde is observed for the unbranched ones, which induces this decrease in the optical purity. The use of substituted 1,4-dihydroxybenzes such as the 2,6-dichloro (**21e**) or the 2,6-dibromo (**21f**) derivatives in the reaction with 3-methylbutanal allowed the authors to obtain the final products with good yields (around 90%) and excellent optical purities (around 95%).

Chiral functionalised cyclopentenones have been employed as precursors of natural products and biologically active compounds. Different methodologies have been proposed to synthesise them, but all suffered from a number of drawbacks. Therefore, a one-pot approach starting from a pyranone to achieve the optically active cyclopentenone has been recently developed. This methodology consisted in a sequential process in which an initial organocatalysed rearrangement converts the pyranone into a cyclopentenone presenting a hydroxy moiety, **22a**, which then underwent a lipase-catalysed kinetic resolution to yield the alcohol (+)-**22a** and the ester (-)-**23a** (Scheme 13a).[38] The rearrangement was optimised in order to have compatible conditions with the enzymatic reaction



Scheme 13. a) One-pot simultaneous synthesis of chiral cyclopentenones (+)-**22a** and (-)-**23a** by DABCO-catalysed rearrangement and lipase AK-catalysed kinetic resolution.[38] b) One-pot sequential synthesis and DKR of pyranones catalysed by DABCO and lipase AK for the preparation of acetylated cyclopentenones (−)-**23a–d**.[40]

and to prevent the decomposition of the obtained **22a** into the undesired enone (**24a**). Thus, after testing different reaction parameters, it was possible to obtain **23a** in 85% yield, with only 3% of enone side product, after a 24 h treatment of the starting material with 0.15 equivalents of the amine 4-diazabicyclo[2.2.2]octane (DABCO) at 50 °C in the presence of *tert*-butyl alcohol as solvent. When combining the rearrangement under the optimal conditions with the enzymatic resolution of the alcohol formed in the presence of vinyl acetate, several lipases were tested, the best results being achieved with lipase AK. Starting from 100 mg of pyranone, 5 equiv. of vinyl acetate, 30 mol% DABCO and 50 mg of lipase in 1.0 mL of *tert*-butyl alcohol led to chiral acetate (−)-**23a** which was isolated in 55% yield and 80% *ee* after 10 days, while the starting alcohol (+)-**22a** was recovered in 35% yield and only 11% *ee*. The low optical purity of the alcohol can be explained by its racemisation under the reaction conditions, which opens up the opportunity for developing a dynamic kinetic resolution (DKR) in order to obtain the acylated product **23a** with a high yield and optical purity (see Figure 3).[39]

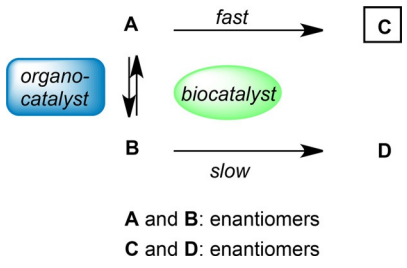
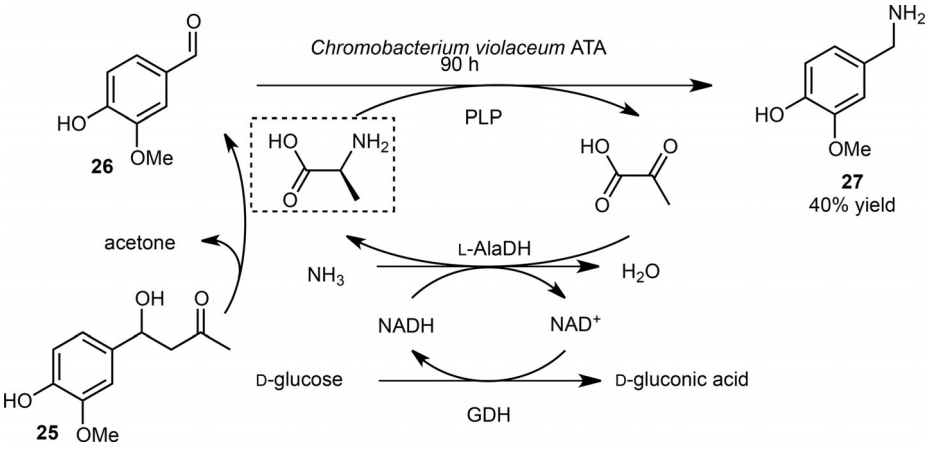


Figure 3. General representation for a dynamic kinetic resolution (DKR).

Thus, racemisation of alcohol (+)-**22a** was induced in the presence of a strong acidic medium (pH 1.0–2.0) in the reaction mixture, but these conditions have a negative effect on lipase activity. Racemisation is very effective under the acylation conditions, which can be likely due to the enzymatic activity or to the production of acetic acid by hydrolysis of vinyl acetate. It was also observed that the racemisation rate was improved by using silica gel 60 (5 mg/mg of lipase AK), which also presents an acidic character. In order to combine in a one-pot process the conditions for the racemisation and the biocatalysed acetylation, a sequential one-pot procedure was developed (Scheme 13b).[40] The pyranone (2.35 mmol) rearrangement was carried out during 24 h with DABCO (0.36 mmol) in *t*-BuOH (2.0 mL), after which the mixture was neutralised with acetic acid. Then, lipase AK (400 mg), silica gel 60 (2.0 g) and vinyl acetate (5.0 equiv.) were added and reacted for 7 days in order to achieve the acetylcyclopentenone (−)-**23a** with 81% yield and 95% *ee*, while the hydroxycyclopentenone was recovered with only 10% *ee* and a yield of 4%. This methodology was then extended to other (−)-cyclopentenones (**23b–d**) but in all cases, lower yields and optical purities were obtained.

A different approach to the organo- and biocatalysed one-pot systems has been recently described in the preparation of capsaicin analogues, compounds with high biological interest, starting from lignin-derived compounds using a multi-step procedure.[41] The last step of this synthesis is the biocatalysed acylation of vanillylamine (**27**). The authors have proposed several procedures for its preparation by combining different catalysts. One of the procedures consists in a simultaneous one-step process starting from 4-hydroxy-



Scheme 14. One-pot simultaneous synthesis of vanillylamine **27** employing L-alanine and aminotransferase from *Chromobacterium violaceum*.[41]

4-(4-hydroxy-3-methoxyphenyl)butan-2-one (**25**) in the presence of L-alanine as organocatalyst and the aminotransferase from *Chromobacterium violaceum* as biocatalyst using aqueous buffer. L-Alanine catalysed the retro-aldol reaction of **25** to vanillin (**26**) and is also employed as amino donor in the biotransformation of vanillin to **27** catalysed by the aminotransferase. An enzyme cascade system was employed in order to regenerate the L-alanine and to increase the reaction conversions, as shown in Scheme 14. During transamination of **26**, L-alanine is converted to L-pyruvate. In the presence of L-alanine dehydrogenase (L-AlaDH), L-alanine is regenerated using ammonia as nitrogen source. As L-AlaDH is an NADH-dependent enzyme, glucose dehydrogenase (GDH) is required to regenerate this cofactor by converting D-glucose into D-gluconic acid. The overall system to convert one equivalent of vanillin to vanillylamine requires one equivalent of ammonia and one equivalent of glucose. The combination of the organocatalyst (250 mM) and the enzymatic cascade system afforded vanillylamine from **25** (2.5 mM) substrate in HEPES buffer (1.0 mL) with complete conversion and 40% yield after 90 hours. The remaining 60% yield corresponds to the dehydrated aldol condensation product, obtained in a side reaction also catalysed by L-alanine, as suggested by the authors.

4 One-Pot Processes Combining Biocatalysts and Non-Traditional Organic Catalysts

4.1 Reactions Catalysed by Enzymes and Base Catalysts

In 1991 the one-pot synthesis of optically active cyanohydrin acetates was reported starting from aldehydes, by combining the anion exchange resin (OH^- form)-catalysed transcyanation of the aldehyde and cyanohydrin acetone and the lipase-catalysed kinetic resolution of the resulting cyanohydrin.[42] As the formation of the cyanohydrin is a reversible process, this compound will suffer a fast racemisation. Thus, the enzymatic acylation will afford a single enantiomer of the cyanohydrin acetate with high yield in a DKR.

After analysing different anion-exchange resins, the reactions were carried out with Amberlite IRA 904 and the lipase from *Pseudomonas* sp. M-12-33 from Amano, in the presence of isopropenyl acetate as acyl donor. After long reaction times (2–6 days), it was possible to obtain the chiral (*S*)-cyanohydrin acetates with high yields and optical purities, except for the 1-naphthyl derivative.

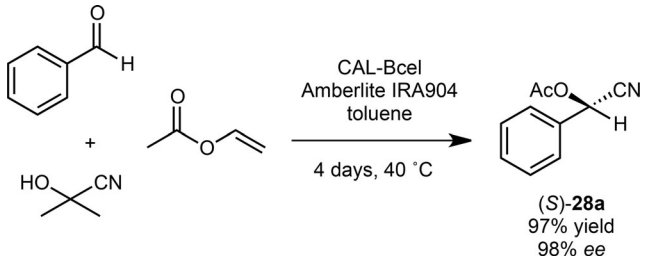
This kind of reaction has been exploited for the synthesis of other chiral cyanohydrin acetates. In 2003, it was performed for the preparation of optically active phenylfuran-based cyanohydrins esters, valuable building blocks of biologically active compounds, using Amberlite IRA 904 and lipase PS from *Pseudomonas cepacia*.[43] The basic exchange resin was able to perform the catalysed transcyanation between phenylfuranaldehydes and acetone cyanohydrin, while the cyanohydrins formed were selectively acylated in the presence of the biocatalyst and vinyl butanoate as acyl donor. The one-pot process afforded the final (*R*)-cyanohydrin esters with high yields and optical purities. The use of higher amounts of enzyme and higher temperatures led to much shorter reaction times, a result to be taken into account for the preparation of the desired products at the gram-scale. The authors have also performed the synthesis of chiral (*R*)-benzothiazol-based cyanohydrin acetates using the same procedure.[44] For these products, the best results were achieved again with Amberlite IRA 904 combined with the lipase from *Candida antarctica* A immobilised on Celite, which led to the best selectivi-

ties in the acetylation of the cyanohydrins with vinyl acetate. The one-pot processes were carried out with high selectivity and complete conversion after 2–3 days using 10 mg/mL of lipase preparation. Lower amounts led to longer times, while an excess of enzyme afforded a decrease in the optical purity of the (*R*)-cyanohydrin acetates as the racemisation step was not fast enough when compared with the enzymatic reaction.

In 2002, the enantioselective synthesis of (*S*)-mandelonitrile acetate (**28a**) through a DKR was developed starting from benzaldehyde (0.8 mmol), acetone cyanohydrin (2 equiv.) and isopropenyl acetate (3 equiv.) in toluene (8.0 mL). Amberlite IRA904 (0.25 equiv., OH^- form) and CAL-B (80 mg) were used in this simultaneous one-pot, three-step procedure.[45] Amberlite is able to perform the release of cyanide hydrogen from acetone cyanohydrin as well as the HCN addition to benzaldehyde. Both processes are reversible, something essential for the preparation and racemisation of mandelonitrile, which rapidly occurred at either 40 or 60 °C. The CAL-B-catalysed acetylation of mandelonitrile at these temperatures is a very selective process, in which enantiopure (*S*)-**28a** was recovered. Unfortunately, when the reaction was carried out in a one-pot approach, it was observed that the kinetic resolution of mandelonitrile is performed without racemisation of the starting material, indicating that under these conditions the Amberlite resin is deactivated. This is likely due to the presence of water in the reaction medium, responsible for the hydrolysis of isopropenyl acetate to acetic acid, which neutralises the alkaline resin. For this reason, an extra amount of basic resin was added to the reaction medium, but enantiopure (*S*)-**28a** was obtained with low yield (16%) after 45 h.

A further development in order to circumvent this low yield was made by immobilising CAL-B on Celite® (CAL-Bcel), a natural silicate able to adsorb water. The use of this biocatalyst preparation led to a 97% yield of almost enantiopure (*S*)-mandelonitrile acetate after 4 days reaction (Scheme 15).[46]

The combination in a simultaneous one-pot DKR of an organocatalysed nitroaldol (Henry) reaction



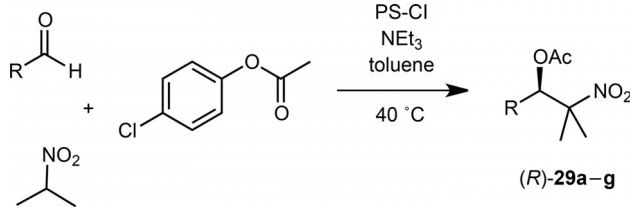
Scheme 15. One-pot, three-step procedure for the synthesis of (*S*)-**28a** using CAL-B supported on Celite® and the anion exchange resin Amberlite IRA904.[46]

with a lipase-catalysed acylation has been used for the preparation of a set of (*R*)-β-nitroalkyl acetates (**29a–g**) with high yields and optical purities.[47] As the Henry reaction requires the control of the equilibrium, this reaction has been used for two purposes: (i) synthesis of the β-nitro alcohol, the substrate of the enzymatic acylation; and (ii) racemisation of the non-reactive β-nitro alcohol enantiomer.

Several organic bases have been tested as catalysts for the nitroaldol reaction, the best results being achieved with triethylamine. Lipases from *Pseudomonas* led to better performance in the acylation step. This process was optimised by employing 30 mg of *Pseudomonas cepacia* lipase preparation (PS-CI) per 0.05 mmol of substrate in toluene (1.0 mL) at room temperature in the presence of 5 equivalents of vinyl acetate. When the one-pot DKR was conducted with an excess of nitroalkane, 2 equivalents of triethylamine, 1 equivalent of *p*-nitrobenzaldehyde, 5 equivalents of vinyl acetate and PS-CI, a 65% yield of the corresponding β-nitroalkyl acetate was recovered with 85% enantiomeric excess. A significant amount of two by-products was obtained. One of them is formed by the coupling of the acetaldehyde (generated as by-product in the acylation reaction) with the nitroalkane, while the other one was achieved by the acylation of this first by-product.

In order to improve the process yield, different acyl donors were tested in the PS-CI-catalysed acylation. Although *p*-chlorobenzyl acetate led to lower activities as compared with vinyl acetate, no side reactions were observed, so this compound was chosen for further development (Table 2). The simultaneous one-pot DKR starting from 2-nitropropane (0.5 mmol)

Table 2. Henry reaction and base-catalysed racemisation to obtain optically active (*R*)-β-nitroalkyl acetates in the presence of *Pseudomonas cepacia* lipase.[47]



Product	R	Time [days]	Yield [%][a]	*ee* [%][b]
29a	4-O_2N-C_6H_4	2	90	99
29b	4-F_3C-C_6H_4	3	89	97
29c	3-NC-C_6H_4	3	92	91
29d	4-F-C_6H_4	4	85	98
29e	4-Cl-C_6H_4	4	83	97
29f	Ph	4	79	91
29g	4-MeO-C_6H_4	4	28	99

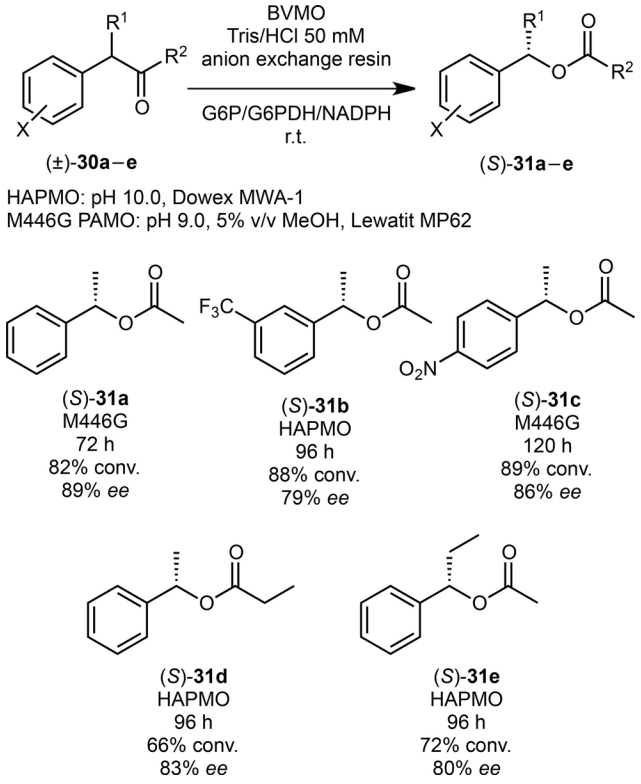
[a] Isolated yield.
[b] Determined by HPLC.

and *p*-nitrobenzaldehyde (0.125 mmol) in the presence of trimethylamine (0.25 mmol), PS-CI (90 mg) and *p*-chlorobenzyl acetate (106 mg) in toluene (0.25 mL) and molecular sieves (20 mg) at 40 °C afforded (*R*)-**29a** with 90% yield and complete selectivity. The biocatalyst can be recovered without loss of activity. This methodology was extended to other benzaldehydes by slightly modifying the reagent amounts, achieving excellent yields and selectivities for (*R*)-**29b–g**.

In the last few years, a number of examples have been reported regarding one-pot reactions in which an organic base catalyst promotes the racemisation of the biocatalytic reaction substrate, in order to achieve DKRs.

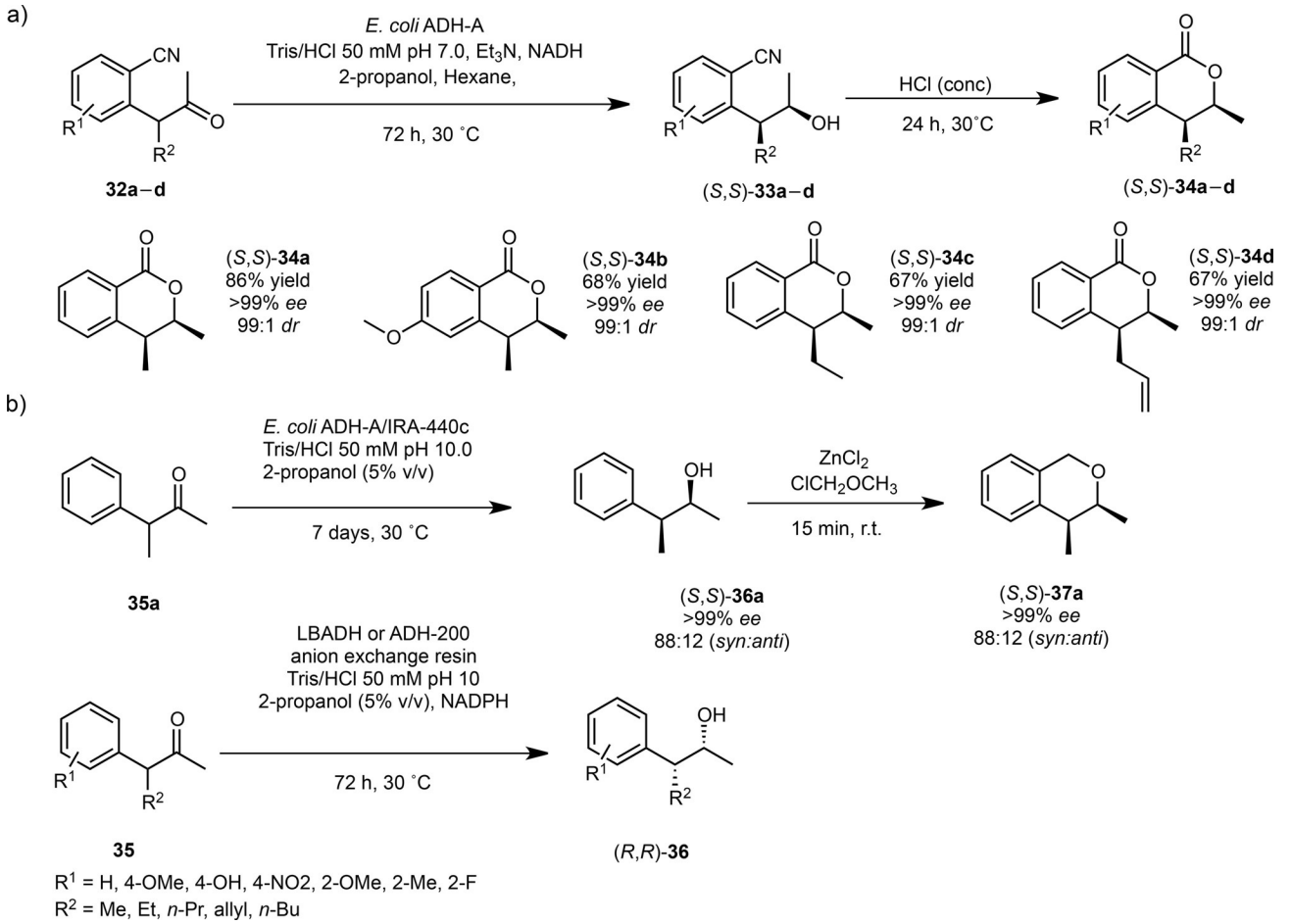
The selective Baeyer–Villiger oxidation of racemic benzyl ketones (±)-**30a–e** (50 mg) catalysed by the Baeyer–Villiger monooxygenase (BVMO)[48] 4-hydroxyacetophenone monooxygenase (HAPMO)[49] from *Pseudomonas fluorescens* ACB (2.0 μM), using glucose 6-phosphate (G6P, 20 mM) and glucose 6-phosphate dehydrogenase (G6PDH, 50 units) as NADPH (0.2 mM) cofactor regeneration system, was combined with the racemisation of the starting material in the presence of the weak anion exchange resin Dowex MWA-1 (100 mg). By using this simultaneous one-pot process benzyl esters (*S*)-**31a–e** were obtained with good yields and optical purities (Scheme 16).[50] The use of strong anion exchange resins allowed higher racemisation rates, whereas they negatively affected the enzymatic system. Reactions were performed in Tris/HCl buffer pH 10.0 in order to ensure a racemisation rate higher than the conversion of the slower ketone enantiomer. This procedure was then extended to the Baeyer–Villiger oxidations catalysed by the M446G mutant of phenylacetone monooxygenase (PAMO) from *Thermobifida fusca*.[51] For this biocatalyst, the best results were achieved in Tris/HCl buffer pH 9.0 containing 5% v/v of a cosolvent while using the weak anion exchange resin Lewatit MP62.[52] The space-time yield of the reaction, expressed as mmol of ketone consumed per hour and per litre of solution, increased up to 60. Higher substrate loading led to a decrease in this parameter, with no effect on the selectivity. The final (*S*)-benzyl esters were recovered with moderate to good yields and optical purities between 65 and 86%.

ADHs have been also tested in dynamic processes. These enzymes are valuable tools for the production of chiral alcohols with one or more stereocentres. The racemisation of the non-reactive stereocentre is possible in order to obtain multiple chiral centres in only one process. The epimerisable chiral centre is located in an adjacent position to the carbonyl moiety, containing an acidic proton which facilitates the racemisation, in the so-called dynamic reductive kinetic resolutions (DYRKRs).[53]



Scheme 16. Dynamic kinetic resolution of racemic benzyl ketones (±)-**30a–e** catalysed by Baeyer–Villiger monooxygenases and weak anion exchange resins.

A DYRKR has been described for the preparation of chiral 3,4-dialkyl-3,4-dihydroisocoumarins **34** starting from 2-(3-oxoalkyl)benzonitriles **32** through a simultaneous one-pot biocatalytic reduction combined with substrate racemisation (Scheme 17a).[54] 2-(3-Oxobutan-2-yl)benzonitrile (±)-**32a** was chosen as model substrate, being reduced in the presence of the Prelog alcohol dehydrogenase ADH-A from *Rhodococcus ruber* overexpressed in *E. coli*. The bioreduction carried out in Tris-HCl buffer pH 7.5 containing 5% v/v 2-propanol cosubstrate and 5% v/v hexane cosolvent and 30 °C afforded the chiral (*S*,*S*)-alcohol **33a** with an excellent enantio- and diastereoselectivity and a 56% conversion after 24 hours. As racemisation systems, two possible alternatives were studied: (i) triethylamine, or (ii) anion exchange resin Dowex MWA-1. The DYRKR process was studied with both systems, achieving a slightly higher conversion with the anion exchange resin to obtain enantiopure (*S*,*S*)-**33a** with 86% conversion after 92 hours. This procedure was performed with other ketones bearing different substituents in the aromatic ring or in the stereocentre, leading to the final enantiopure alcohols with good to excellent yields and complete diastereoselectivity. The process was further extended by carrying out the one-pot acid-catalysed cyclization of the (*S*,*S*)-alcohols obtained by DYRKR after 72 hours, leading



Scheme 17. a) DYRKR of 2-(3-oxoalkyl)benzonitriles to obtain dihydroisocoumarins (*S*,*S*)-**34a–d** using ADHs and trimethylamine as base catalyst.[54] b) DYRKR of 3-aryl-2-butanones to yield the corresponding chiral arylpropan-2-ols **36** and the isochroman (*S*,*S*)-**37a** by a one-pot process employing ADHs and an anion exchange resin.[55]

to the corresponding (*S*,*S*)-3-dialkyl-3,4-dihydroisocoumarins **34** with good yields in most of cases while excellent enantio- and diastereoselectivities were observed. Under the reaction conditions employed to obtain (*S*,*S*)**-34a–d**, starting from 0.3 mmol of **32a–d** in 13 mL Tris-HCl 50 mM pH 7.5 buffer, triethylamine (1% v/v) was chosen as racemisation reagent given its higher reliability against Dowex MWA-1.

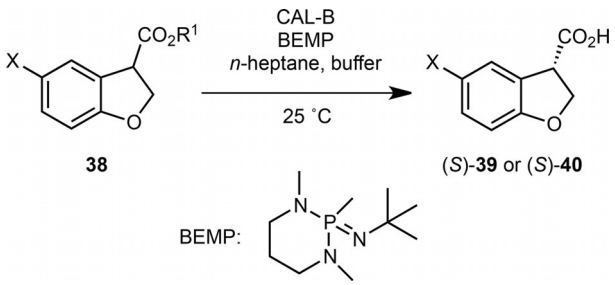
A similar methodology has been recently applied for the DYRKR of 3-arylalkan-2-ones **35** to obtain the corresponding chiral substituted propan-2-ols **36**,[55] which are converted to isochromans **37**, valuable building blocks in organic synthesis (Scheme 17b). The initial tests with racemic (±)-3-phenylbutan-2-one **35a** in the presence of the Prelog *E. coli*/ADH-A afforded (*S*,*S*)**-36a** with good selectivity values and conversions close to 50% at short reaction times (83:17 diastereomeric ratio for the *syn* diastereomer). This compound is transformed into the desired isochroman (*S*,*S*)**-37a** by treatment with zinc chloride in chloromethoxymethane at room temperature.

Substrate racemisation was studied in the presence of anion exchange resins such as DowexMWA-1 and Amberlite IRA-440C. A high pH was required to reach an effective racemisation, however, under these conditions the enzyme suffers from severe inactivation. In order to circumvent this drawback, a higher reaction temperature was used (30 °C), while ADH-A was added stepwise to the reaction medium. The optimal DYKRK conditions were applied for a set of racemic benzyl ketones (0.01 mmol). Bioreductions were carried out at 30 °C in Tris-HCl 50 mM pH 10.0 (0.35 mL) using one of the exchange resins, while ADH-A was added in portions over 3–4 days. Excellent enantio- and diastereoselectivities were observed for the C-2 substituted 3-arylbutan-2-ones. When Amberlite IRA-440C was employed, a significant decrease in the reactivity was observed for alkyl chains longer than methyl, while the use of Dowex MWA-1 led to better conversions, although with lower diastereomeric ratios.

Experiments were also performed with two anti-Prelog ADHs: alcohol dehydrogenase from *Lactobacillus brevis* (LBADH) and ADH-200, which led to the corresponding (*R*,*R*)-propan-2-ols **36**. It was observed that Amberlite IRA-440C caused inhibition in both biocatalysts, so the dynamic processes were carried out in the presence of Dowex MWA-1. For all the racemic benzyl ketones tested, moderate to good conversions were measured together with excellent selectivities and high diasteroselectivities for the (*R*,*R*)-alcohols obtained.

The chemoenzymatic synthesis of (*S*)-2,3-dihydrobenzo[*b*]furan-3-carboxylic acid (**39**) and (*S*)-5-chloro-2,3-dihydrobenzo[*b*]furan-3-carboxylic acid (**40**), valuable precursors in the synthesis of biologically active compounds, features as key step the sequential one-pot biocatalysed hydrolysis of the racemic methyl or ethyl esters (**38**) combined with the substrate racemisation in the presence of an organic base.[56] Initial screening on the enzymes for the kinetic resolution was performed by an HPLC-CD selectivity assay, leading to CAL-B and *Bacillus subtilis* esterase (BS3) as the best biocatalysts for this process. For both enzymes, enantioselectivities were excellent, recovering both ester and acid with 50% conversion and yields higher than 46%. While CAL-B hydrolysed the (*S*)-enantiomer of the ester (4.4 mmol) to yield (*S*)-**39** and (*S*)-**40**, BS3 hydrolysed the (*R*)-antipode in an enantiocomplementary fashion. Substrate racemisation was studied in the presence of different organic bases. The Schwesinger base 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,2,3-diazophosphorine (BEMP) was the more convenient for this process.[57] Depending on the substrate structure, different amounts of BEMP were required to achieve a satisfactory racemisation. As BEMP can be inactivated in aqueous medium, a reaction set-up for the one-pot reaction with separation of both processes was employed. Thus, a flask was connected with a peristaltic pump to a column containing immobilised BEMP. The reaction was carried out in a biphasic system buffer/*n*-heptane (60 mL, 2:1) in which the biocatalyst and the enantiopure acid product stand in the aqueous phase, while the ester (kept in the organic phase) was continuously pumped through the BEMP column. In this system, the ester is in a continuous racemisation while the acid accumulates in the aqueous phase. In order to avoid BEMP leaching, which can lead to side reactions and biocatalyst inactivation, the base was protected at the column with a second layer of ion-exchange resin. As shown in Table 3, the use of this system at a preparative scale (500 mg, 2.81 mmol of the corresponding ester) led to the chiral acids with high optical purities and good yields depending on the substrate structure.

Table 3. DKR of racemic esters in the presence of CAL-B and BEMP as racemisation reagent to obtain the (*S*)-2,3-dihydrobenzo[*b*]furans **39** or **40**.[56]



Product	X	R^1	Time [h]	BEMP [equiv.]	Yield [%]	*ee* [%][a]
39	H	Me	26	1.0	95	90
39	H	Me	24	1.5	92	>95
40	Cl	Me	24	2.0	71	>99
40	Cl	Et	24	1.0	82	>99

[a] Measured by HPLC.

4.2 Enzymatic Regeneration of a Redox Catalyst in a One-Pot Procedure

A significant advance in organocatalysed redox reactions has been experienced lately although it cannot be compared with the explosion of organocatalysed C–C bond forming reactions developed during the same period. Organocatalysed redox reactions are mostly focused on alkene epoxidations,[58] thioether sulfoxidations,[59] and Baeyer–Villiger oxidations[60] employing (a)chiral nitrosyl radical-, dioxirane- or oxaziridine-based catalysts, among others. More recently, successful organocatalytic asymmetric alkene reductions under hydrogen transfer conditions have been reported.[61]

With this scenario and considering the vast knowledge on biocatalysed oxidations,[62] it is reasonable to conceive fruitful combinations for the enzymatic regeneration of redox organocatalysts. Indeed, examples of such processes can be traced back to 1990.[63]

So far, two main strategies have been explored with a great deal of success (Figure 4): (i) the hydrolase-catalysed perhydrolysis of carboxylic acid/esters (the organocatalysts) giving rise to an organic peracid that performs as direct oxidising agent and, (ii) the versatile laccase/mediator system for several one- and two-electron oxidation processes.

In this hydrolase/carboxylic acid one-pot procedure, substoichiometric quantities of carboxylic derivative can be employed that, after peracid formation, should work as an oxo-transfer catalyst from hydrogen peroxide to the substrate (mostly ketones, olefins and sulfides).

The use of lipases in promiscuous reactions has been extensively reviewed in the last years.[64] One of

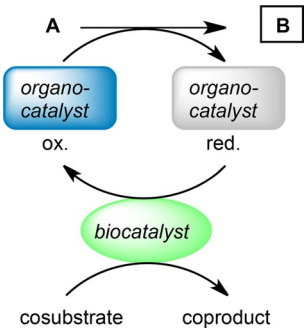


Figure 4. Biocatalysed regeneration of redox catalysts in a one-pot fashion.

these lipase-catalysed promiscuous processes is, as already mentioned, the reaction between an ester or carboxylic acid with H_2O_2 to render the corresponding peracid. When this carboxylic derivative is used in a substoichiometric quantity, a catalytic oxidation reaction can be coupled to the enzymatic perhydrolysis reaction.

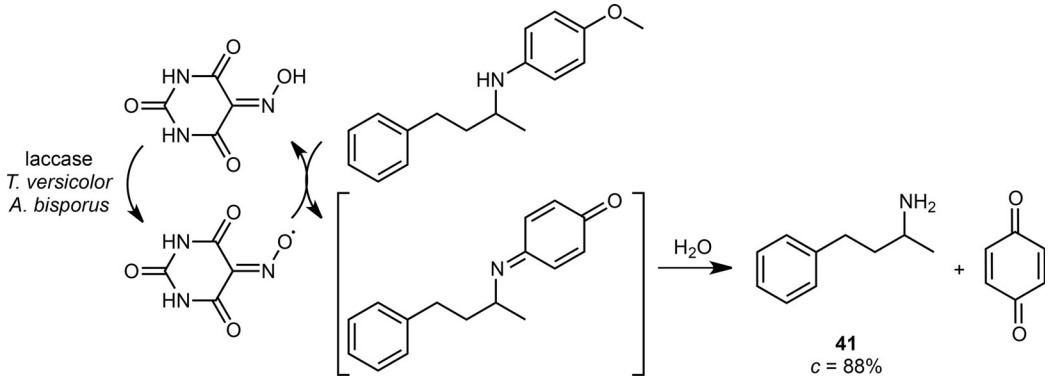
Remarkably, already in 1990 it was reported that immobilised CAL-B and octanoic acid (10% mol) in the presence of a slight molar excess of H_2O_2 (added in portions), results in the epoxidation of several alkenes under solvent-free conditions. The reactions were stopped at 15 h with almost full conversion in most cases.[63] Ten years later, a similar system was set up but taking place in ionic liquids, thus obtaining the cyclohexene oxide with high selectivity and 83% conversion.[65]

As expected, several reports have dealt with lipase-catalysed generation of percarboxylic acid to carry out reactions other than epoxidation.[66] However, in the vast majority of these cases, the carboxylic acid (here considered as the organocatalyst) is added in overstoichiometric quantities, thus, the sulfoxidation or the Baeyer–Villiger ketone oxidation are not strictly catalytic and therefore are beyond the scope of this review.

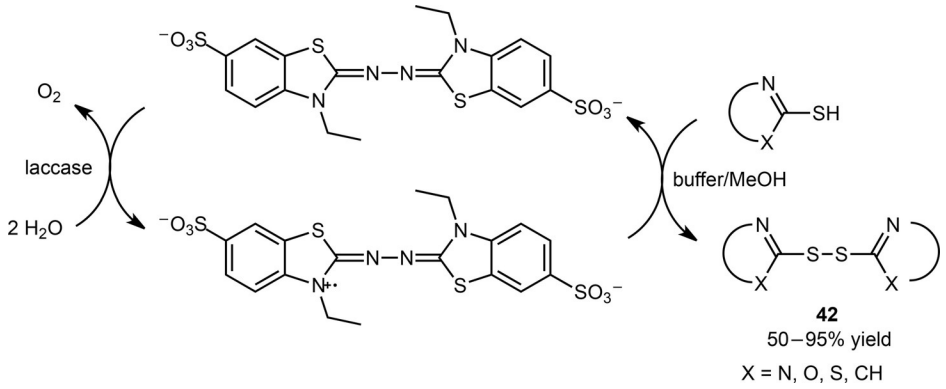
On the other hand, laccases are able to oxidise substrates using O_2 as terminal electron acceptor. When the enzyme redox potential is not enough for the oxidation of a given substrate, the chemist usually adopts a small organic molecule as an electron mediator, in the so-called "laccase/mediator system" (LMS).[27] This clean reaction is readily employed for the transient generation of highly reactive intermediates that can act as one- and two-electron shuttles between the substrate and O_2 to form H_2O. In a wide sense, this organic mediator employed in catalytic quantities or its reactive intermediate can be regarded as the actual organic catalyst.[67] Furthermore, these mediators can act by different mechanisms, such as electron-transfer (ET), radical hydrogen-atom-transfer (HAT) or polar mechanisms, depending on its electronic nature.

Several systems involving an oxidation step have been studied. The laccase-mediator couple has been shown as a reliable and mild system for the *N*-deprotection of *p*-methoxyphenyl (PMP)-protected amines.[68] Thus, oxidation of the protected amine by the laccases from *Trametes versicolor* or *Agaricus bisporus* leads to formation of the *p*-benzoquinone imine, which spontaneously hydrolyses in the aqueous reaction medium, furnishing the free amine and *p*-quinone, as shown in Scheme 18, using 0.92 mmol of the PMP-protected amine. Although high yields were obtained by employing the sole laccase, the use of catalytic amounts of mediators could expand the scope of this methodology, as demonstrated in the deprotection of *N*-PMP-protected 4-phenylbutan-2-amine (**41**), a non-benzylic amine, where no conversion was observed without a mediator. Best results were attained using violuric acid as a mediator after 48 h, achieving 88% conversion.

Another example of amine deprotection involves a laccase-mediator system in a similar fashion, but in this case employing TEMPO as organic electron mediator.[69] In this work, *N*-benzylamines can be deprotected in high chemo- and regioselectivity. Thus, oxidation of the amine transiently affords the corresponding imine, which is spontaneously hydrolysed, delivering the free amine. Hence, using laccase of *Trametes versicolor* and TEMPO, the reaction with *N*,*N*′-dibenzyl-4-aminopiperidine was highly regioselective



Scheme 18. Laccase-mediator (violuric acid) catalysed *N*-deprotection of *N*-PMP-protected amines.[68]



Scheme 19. Synthesis of a set of disulfides **42** employing laccase from *T. versicolor* in the presence of ABTS as mediator.[70]

to the secondary amine, obtaining as only product the corresponding free primary amine with complete conversion. Likewise, (±)-*trans*-*N*,*O*-dibenzyl-2-hydroxycyclohexylamine was successfully *N*-deprotected in a chemoselective fashion, yielding the *O*-benzyl derivative as sole product.

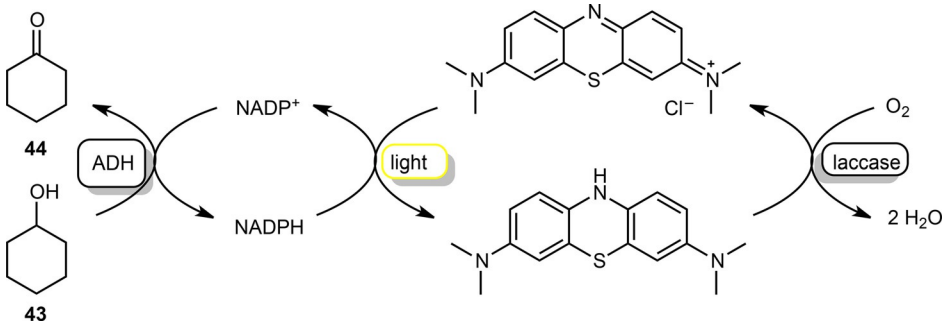
Taking advantage of laccases as mild and green oxidants, the synthesis of different disulfides **42** has been explored by homocoupling of heterocyclic thiols (Scheme 19).[70] Thus, employing catalytic ABTS as the organic mediator and the laccase from *Trametes versicolor*, one-electron oxidation leads to a thiyl radical, which collapses into the corresponding disulfide. Reactions were carried out in acetate buffer pH 4.4 containing MeOH (10% v/v). Disulfides were formed with yields ranging from 50 to 95% depending on the substrate structure, without formation of overoxidation side products.

This kind of laccase/mediator system has been also involved in alcohol oxidation. The oxidation of primary alcohols has been pursued in order to couple a secondary reaction employing *in-situ* the newly formed aldehyde.[71] A 1,4-diol or 1,5 diol can be oxidised by laccase/TEMPO, leading to a hydroxyaldehyde, that immediately cyclises affording a hemiacetal. Further oxidation by the same system allows the formation of stable butyro- and valerolactones in a one-pot, one-step fashion.

4.3 One-Pot Catalytic Combined Redox Processes Driven by Light

In certain cases, chromophoric organic redox mediators (here considered as organocatalysts) can be readily employed in biocatalysed processes under light irradiation. Photostimulation provides the chromophoric organocatalyst with a suitable redox potential to accept/donate electrons from/to molecules that otherwise would be difficult or even impossible. In this way, flavin and analogues have been successfully used as excitable organocatalysts with fair turnover when coupled to oxidative enzymatic transformations.

Likewise, it has been recently reported that pyrimidine cofactor regeneration to the oxidised form [NAD(P)$^+$] is feasible employing *Myceliophthora thermophila* laccase, (*Mtlac*), O_2 as final electron acceptor and dyes (methylene blue, methylene green and azure B) as mediators, as shown in Scheme 20.[72] In this report, the authors were able to demonstrate that, upon visible light irradiation, the NADH oxidation is three orders of magnitude faster than the obscure counterpart. The authors coupled this NAD(P)$^+$ regeneration system with two enzymes, namely *Thermus* sp. ATN1 ADH, and a commercially available glucose dehydrogenase (GDH) to successfully obtain more than 30% conversion in two hours of cyclohexanone (**44**) from cyclohexanol (**43**) and gluconic acid from



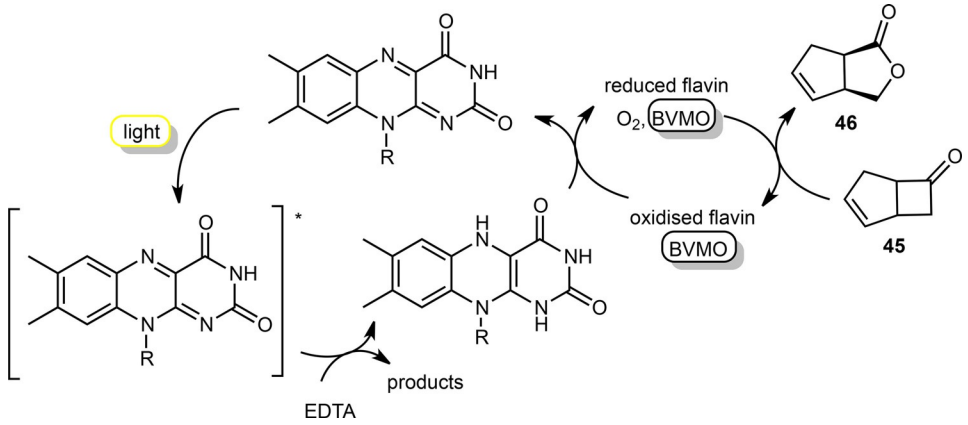
Scheme 20. Use of a bienzymatic system laccase-ADH coupled with dyes as mediators for the photooxidation of cyclohexanol to cyclohexanone.[72]

glucose, respectively. In the latter case, methylene blue was employed as organocatalyst showing a remarkable TTN of 2500.

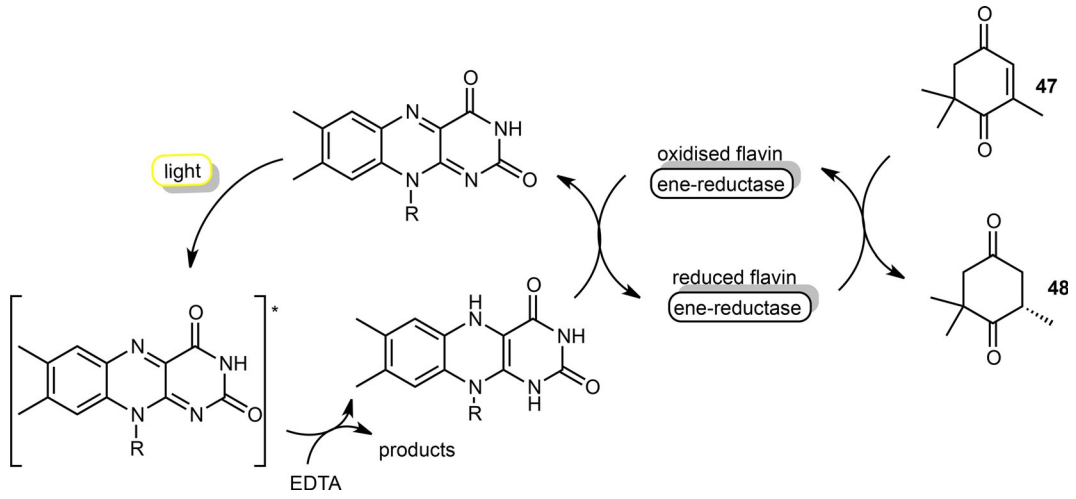
A similar approach has been shown in the *Caldariomyces fumago* chloroperoxidase (CPO)-catalysed sulfoxidation of thioanisole to enantiopure (*R*)-methyl phenyl sulfoxide employing FMN as chromophoric organocatalyst, EDTA as electron donor and molecular oxygen under photostimulation.[73] Hence, EDTA successively transfers electrons to the photoexcited FMN organocatalyst to be delivered to the heme prosthetic group of CPO. The authors improved biocatalyst stability and mass transfer issues by adopting a biphasic surfactant-stabilised system which permits an increase in substrate concentration and sulfoxide productivity.

Similarly, EDTA/flavin in the presence of light has been employed in the preparation of lactones starting from cyclic ketones catalysed by BVMOs (Scheme 21).[74] In this way, the NADPH cofactor is replaced by the system EDTA/flavin so, electrons coming from the sacrificial donor, pass to the diffusing excited flavin. Then, electrons are delivered to the BVMO-bound flavin to trigger formation of an enzyme-bound peroxyflavin, the actual oxo-transfer catalyst that finally introduces oxygen into the substrate. By using this methodology, a set of enantioenriched (>96% ee) γ- and ε-lactones was successfully prepared from the corresponding prochiral ketones.

On the contrary, reductive enzymatic reactions combined with light-driven organocatalysed electron supply are even less explored than the already described oxidative counterparts. A representative example is the use EDTA/flavin in the presence of light for the reduction of electron-deficient olefins into the corresponding saturated products by the action of an ene-reductase from *Bacillus subtilis* YqjM (Scheme 22).[75] In this case, under light irradiation, the excited flavin transfers two protons and two electrons from EDTA to the enzyme-bound flavin (pros-



Scheme 21. EDTA/flavin system employed in combination with BVMOs to catalyse Baeyer–Villiger oxidations. The scheme shows the oxidation of bicyclo[3.2.0]hept-2-en-6-one (**45**) to the corresponding lactone **46**.[74]



Scheme 22. Reduction of ketoisophorone **47** catalysed by the ene-reductase from *Bacillus subtilis* YqjM in the presence of EDTA and light.[75]

thetic group). Thus, YqjM with the prosthetic group in its reduced form is able to deliver a hydride to the beta position of the C=C bond in a Michael-type addition reaction. In this report, ketoisophorone (**47**) was chosen as model substrate and the reduction to diketone **48** took place with a similar stereoselectivity as for the standard reaction using NADPH and a normal cofactor regeneration system.

5 Outlook

Already organocatalysis and biocatalysis have reached a high degree of sophistication, as shown in the examples described in the present review. The interplay of both worlds, and therefore virtues from both sides, need further exploitation. The advancement in this area of catalysis shall necessarily come about by adopting interdisciplinary research, in which enzyme engineering will provide biocatalysts with enhanced robustness towards organic solvents and reaction temperature, while molecular design will bring novel organocatalysts that may exert high asymmetric induction in a temperature range that matches that of the involved enzymes. In the field of organocatalysis, aside from the system lipase/carboxylic acid/peroxide, in most of the reported examples concerning combination with biocatalysis, the organocatalyst employed behaves as a Lewis base. Therefore, it is tempting to explore novel cascade processes by targeting the use of Brønsted acid organocatalysts coupled with acidophilic enzymes. Moreover, metal-based catalysts can be efficiently incorporated into multicatalytic processes, thus allowing cross-coupling and cycloaddition reactions to take place concurrently with the organocatalytic and enzymatic transformations. The increasing number of artificial metalloenzymes[76] conceptually supports this idea and in the near future we may find broader partnerships in this area.[77] The employment of other technologies in combination with organo- and biocatalysis is worthy of further investigation. More complex photochemical approaches can be envisioned[78] that permit redox reactions to take place either on a catalyst or on a substrate/intermediate with a great deal of success. Finally, a general and reliable asymmetric version of the lipase/carboxylic acid/peroxide system is still elusive and a careful design of the carboxylic acid organocatalyst will be required.[79]

Acknowledgements

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