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Construction of Chemoenzymatic Linear Cascades for the Synthesis of Chiral Compounds

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Juan Mangas Sanchez was nominated to be part of this collection by EurJOC Board Member Silvia Osuna Oliveras

Inspired by nature, synthetic chemists try to mimic the efficient metabolic networks in living organisms to build complex molecules by combining different types of catalysts in the same reaction vessel. These multistep cascade processes provide many advantages to synthetic procedures, resulting in higher productivities with lower waste generation and cost. However, combining different chemo- and biocatalysts can be challenging as reaction conditions might differ greatly. As a highly multidisciplinary field that benefits from advances in chemical catalysis, molecular biology and reaction engineering, this area of study is rapidly progressing. In this Review, we highlight recent trends and advances in the construction of multistep chemoenzymatic one-pot cascades to access chiral compounds as well as the different strategies to solve current challenges in the field.

1. Introduction

Cascade processes combine several consecutive synthetic steps, either in a multicomponent (concurrent) or sequential manner, where the product of a given reaction serves as the substrate for the next.[1] These systems allow for access to synthetically complex products with no intermediate purification or isolation required, reducing time, effort, and waste, as well as the environmental impact of the process. Highly reactive, labile, or toxic species can be readily transformed in the reaction mixture, allowing for higher productivities and yields. Additionally, enzymatic reactions often suffer from equilibrium and product inhibition issues. By coupling consecutive steps in the same reaction vessel, reactions can be driven to completion and inhibition issues minimised. The construction of multi-enzyme cascades has been a hot topic in the past two decades.[1,2] Enzymes are particularly convenient for these processes as they normally work under the same reaction conditions such as pH or temperature, and the outstanding chemoselectivity they display minimises the risk of cross-reactivities that can potentially lead to the formation of side products. In fact, auxiliary enzymatic cascades, i.e. cascades that convert byproducts to alter the overall thermodynamics of the process or for cofactor regeneration, are well-known and routinely applied.[3]

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However, the combination of different "catalytic worlds" can often be more challenging. Even though water is a desirable solvent for environmental, safety and cost reasons, transition metal (TM) and organocatalytic reactions are usually carried out in organic solvents, whereas enzyme chemistry is often conducted in aqueous media. Temperature, pressure, and other process requirements might also differ. Moreover, incompatibility and inhibition issues might also occur due to the complexity of these processes in which a large number of different components are often added to the reaction mixture. Nevertheless, recent advances in the design of new chemocatalysts for use in aqueous media, the rapid expansion of the range of reactions available using enzymes either by discovery or developed by evolution,[4,5] as well as advances in reaction engineering have led to a remarkable extension of these methodologies in the past decade. Several reviews have focused on different aspects of the combination of chemo and enzyme catalysis.[6–9] as well as on current strategies to overcome compatibility issues.[10] In this survey, we have selected recent examples of novel approaches to access synthetically interesting compounds by a one-pot combination of either TM or organocatalytic steps with biocatalysis.

2. Linear Cascades Combining Transition Metal Catalysis and Biocatalysis

By exploiting the metal d-orbitals, TM complexes have an outstanding ability to activate substrates and accelerate reactions leading to the formation/cleavage of single and multiple C–X bonds. Moreover, control of both reactivity and selectivity can be easily tuned by modification of their ligands. Some of these reactions are amongst the top 5 transformations performed by the pharmaceutical industry,[11] although the low solubility that organic molecules often present in aqueous media has always been a limitation for the implementation of

this chemistry in water. Advances in chemical engineering which include compartmentalisation strategies, immobilisation, or the use of non-conventional media, have opened the operational window to merge both worlds and prompted the integration of such systems to generate synthetic complexity more simply and efficiently. Examples developed in the past 5 years will be discussed in the following section.

2.1. Recent examples of the combination of enzyme catalysis and Suzuki-Miyaura coupling (SMC)

Cross-coupling and related reactions, generally promoted by Pd species, are amongst the most useful transformations for forming new C–C bonds.[12] The ability of such species to work under "enzyme-compatible" conditions has triggered the development of linear cascades to access a plethora of chiral compounds. Pioneering works by Gröger and others demonstrated the versatility of combining TM catalysts with ketoreductases (KREDs) to access chiral biaryl alcohols,[13–16] and these systems have also been applied to specific molecules of pharmaceutical relevance.[17,18] The discovery and development of new enzyme classes and the combination of these with other TM-mediated processes have led to a remarkable extension of these methodologies in recent years.

Halogenases have emerged as a powerful tool to incorporate halogen atoms into organic molecules employing halide salts as the halogen source, offering an alternative to traditional methodologies that often employ hazardous chemicals and harsh reaction conditions.[19,20] In 2016, Micklefield and coworkers exploited the exquisite regioselectivity of flavin-dependent halogenases (FDHs) to selectively brominate a series of aromatic compounds and then combined them with Pd-catalysed Suzuki Miyaura coupling (SMC) to access a diverse collection of biaryl compounds (Scheme 1a).[21] To mitigate catalyst incompatibility issues, a polydimethylsiloxane (PDMS) membrane was used to compartmentalise the reaction chamber. This strategy was firstly used by the Gröger lab and consists of a membrane which allows hydrophobic compounds to flux between both compartments and keeps other reaction components such as enzymes and metal ions separated, thus avoiding interaction.[22] Under this setup, the cascade was carried out sequentially with all components added initially in which the first step was performed at r.t. and the SMC step at 80 °C. The same group has recently expanded this cascade to develop a C–H bond cyanation process using potassium ferrocyanide as the CN source.[23] Frese *et al.* also exploited the exquisite regioselectivity displayed by different FDHs to selectively brominate tryptophan and generate biaryl derivatives via SMC with boronic acids using Na_2PdCl_4 as the Pd source and sSPhos as the ligand.[24]

Besides halogenases, other enzyme classes have recently been combined with cross-coupling reactions for the synthesis of chiral compounds. For instance, in 2018, Bornscheuer and coworkers developed a chemoenzymatic synthesis to access chiral biarylamines by combining the SMC with the asymmetric amino transfer catalysed by variants of the amine transaminase (TA) from *Aspergillus fumigatus* (4CHI-TA) (Scheme 1b).[25] In-silico studies were performed to determine relevant positions to be targeted in order to enlarge the active site to accommodate bulkier substrates. Two variants (F113A and I146A) were able to convert pyridine-containing biaryl substrates more efficiently, and one of them was immobilised and used to synthesise 1-(5-phenylpyridin-3-yl)ethan-1-amine using a continuous flow setup in 43% conversion and >99% *ee*. A year later, Gröger, González-Sabín and coworkers showed that

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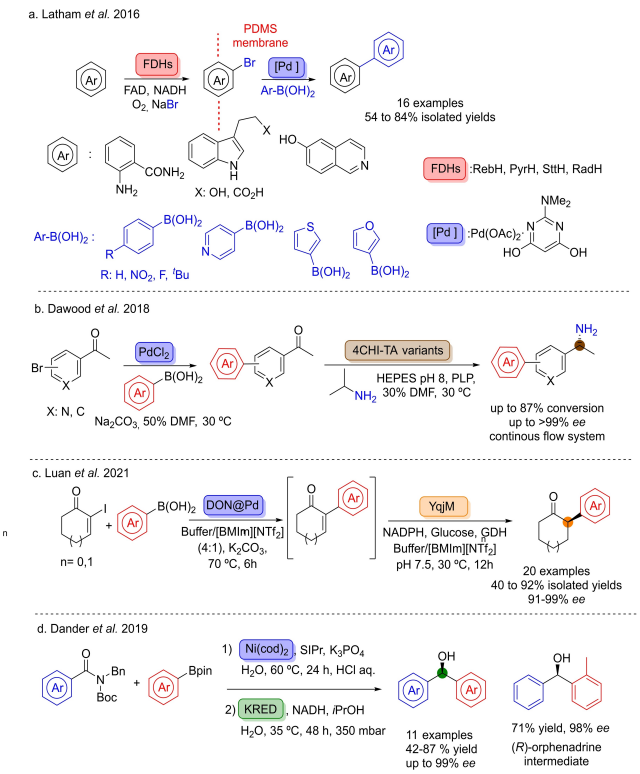
Juan Mangas-Sanchez obtained his Ph.D. at the University of Oviedo (Spain) under the supervision of Vicente Gotor-Fernandez working on new biocatalytic routes to optically active alcohols. Then he moved to Lund University (Sweden) to work in Professor Patrick Adlercreutz's group on the optimisation of chemoenzymatic processes to obtain biodiesel, tailored triglycerides and prebiotics using hydrolases. In 2015, he joined the group of Professor Nicholas Turner as a research associate at the Manchester Institute of Biotechnology to work on the discovery, engineering, characterisation, and applications of novel biocatalysts to produce chiral amines. He has recently been recruited by the Aragonese Foundation for Research & Development (ARAID) and now works as a senior scientist at the Institute of Chemical Synthesis and Homogeneous Catalysis (ISQCH-CSIC) in Zaragoza. He is interested in the development of sustainable synthetic routes to high value chemicals through chemoenzymatic cascades, enzyme evolution, and artificial metalloenzymes.



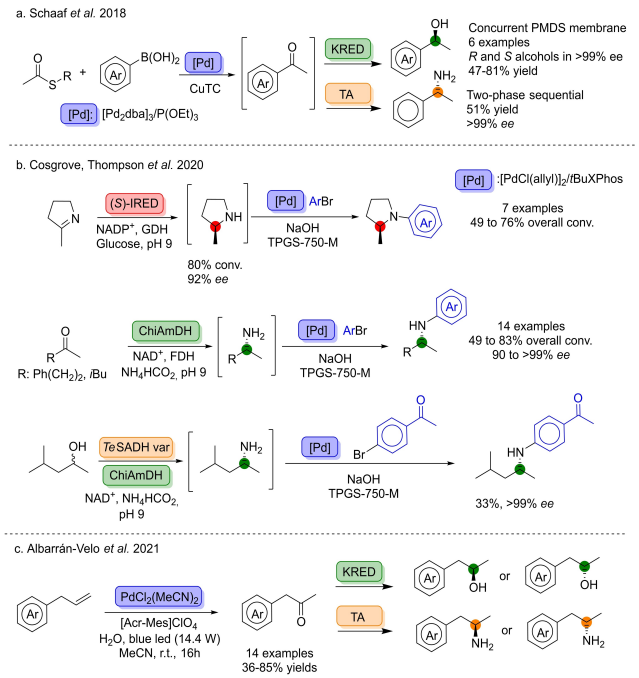
Scheme 1. Examples of cascade processes involving SMC coupling and biotransformations.

the use of non-conventional media such as deep eutectic solvents (DES) can increase the productivity of this cascade by improving substrate solubility.[26] Very recently, Luan *et al.* demonstrated that Pd-catalysed SMC can also be successfully coupled with ene-reductases (EREDs) to obtain a set of enantioenriched α-aryl-substituted cyclohexanones (Scheme 1c).[27] After observing a significant cosolvent effect in the ERED stereoselective step, a screening was performed in which the use of 20% v/v of the ionic liquid [BMIm][NTf_2] was found to be optimum. Initial enzyme inhibition due to unreacted boronic acid when Pd/C was employed was detected. The use of other homogeneous sources of Pd (II) such as $Pd(PPh_3)_4$ and $Pd(OAc)_2/PPh_3$ showed to inhibit the enzyme as well. By using dendritic organosilica nanoparticles as immobilisation support (DON@Pd), the coupling proceeded to completion and the cascade was successfully constructed in a sequential manner to afford the corresponding α-aryl-substituted cyclohexanones in good to excellent yields and optical purities. Besides the use of Pd species for cross-coupling, the combination of biocatalysis with Ni-catalysed SMC has also been recently explored. A new method to access chiral alcohols by Garg and coworkers was reported via $Ni(cod)_2$ catalysed SMC of aryl amides followed by ketoreductase (KRED) asymmetric reduction (Scheme 1d).[28] The process was run in a sequential manner, with the chemocatalytic step carried out in an aqueous medium at 60 °C followed by bioreduction at 35 °C and under reduced pressure to remove acetone and drive the reaction to completion.

2.2. Other Pd-mediated processes

The Liebeskind-Srogl reaction is a metal-catalysed coupling of thiol esters and boronic acids to access ketones.[29] In 2018, the Mihovilovic group investigated the combination of this process with ADH and TAs to access chiral alcohols and amines under either a concurrent or sequential approach (Scheme 2a).[30] For the synthesis of chiral alcohols, no compatibility issues were observed, and the cascade was proved to be efficient in both sequential and concurrent manners using a PDMS membrane to separate both reactions. The use of enantiocomplementary KREDs allowed access to both enantiomers in good to excellent yields. However, the biotransamination cascade proved to be more challenging, observing the formation of the desired chiral amine only when the process was run in a sequential manner in a biphasic system using heptane as the solvent and a super-absorber (sodium polyacrylate) to contain the components of the biocatalytic step.

Over the last two decades, extensive research has also been done on expanding the scope and conditions to access alkenes via the Heck reaction.[31] This has prompted the investigation of synthetic cascades to access chiral compounds by combining the Heck coupling with enzyme catalysis. After early examples developed by the Cacchi group,[32,33] in a recent report by Schwendenwein *et al.*, the combination of a Heck coupling reaction between aryltrifluoroborates and acrylic acids followed by consecutive double enzymatic reduction was reported.[34] After initial optimisation of the Pd-catalysed coupling, subsequent sequential combination with a whole-cell biocatalyst containing a carboxylic acid reductase from *Neurospora crassa* (*Nc*CAR) and an ene reductase from *Saccharomyces pastorianus*



Scheme 2. Examples of novel chemoenzymatic cascades involving other Pd-catalysed processes.

(OYE1), afforded the corresponding hydrocinnamaldehyde derivates. In this case, no apparent inhibition issues between catalysts were observed although the excess of acrylic and crotonic acid in the first step led to reduced yields due to competition with cinnamic acid substrates for *Nc*CAR and corresponding cofactors. Another interesting example on the combination of the Heck reaction with enzyme catalysis was recently reported by Grabner *et al.* in which they used the phenolic acid decarboxylase from *Bacillus subtilis* (*Bs*PAD) to generate 4-hydroxystyrene from the corresponding cinnamic acid followed by Pd-mediated Heck arylation with benzyl iodide to access (*E*)-4-hydroxy-stilbene.[35] In this case, both steps were completely segregated by running this process under a continuous flow system which allowed both reactions to be run at their optimal temperatures using a choline chloride-based DES to increase substrate solubility in aqueous media. This process was also explored by Cortes-Clerget *et al.* in a comprehensive report in which the use of surfactants in one-pot cascade processes involving both chemo- and biotransformations in aqueous media was extensively studied.[36] In this case, a diester consisting of racemic α-tocopherol and succinic acid (TPGS-750-M) was shown to be fully compatible with enzyme-catalysed reactions and to have a beneficial effect on productivities at high substrate concentrations in aqueous media.

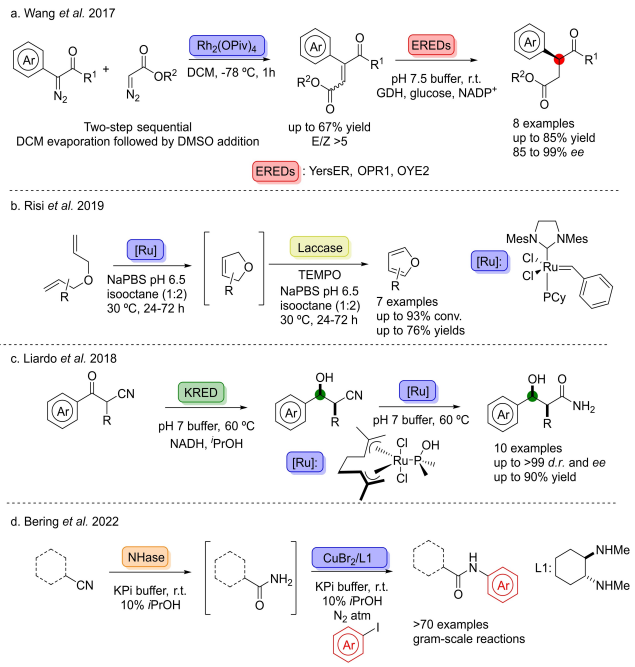
This surfactant was also proved to be advantageous in the combination of biocatalytic chiral amine synthesis with the Buchwald-Hartwig Amination (BHA) recently developed by Cosgrove, Thompson *et al.* (Scheme 2b).[37] In this study, amine dehydrogenases (AmDHs) and imine reductases (IREDs) were used to generate chiral amines for further Pd-mediated coupling with arylbromides following a sequential approach. This process was also implemented with a redox neutral H-borrowing cascade combining an AmDH and a KRED which allowed access to a chiral *N*-arylamine from a racemic alcohol. Very recently this methodology has also been extended by the Paradisi laboratory using ω-TAs.[38]

The combination of the Wacker-Tsuji oxidation with different enzymes to make chiral alcohols and amines has also been significantly studied using either Cu or Fe as the terminal oxidant.[22,39,40] An alternative approach has recently been reported by Gotor-Fernández and coworkers in which Pd(II) was regenerated by using the photosensitizer [Acr-Mes]ClO_4 and blue led light irradiation (Scheme 2c).[41] Sequential combination with enantiocomplementary KREDs and ω-TAs allowed access to a collection of chiral alcohols and primary amines, respectively, in moderate to high yields (42–83%) and excellent enantiomeric excesses (>99%) in all cases.

2.3. Recent examples of synthetic cascades combining other TM-mediated processes and enzyme catalysis.

The efficiency and ability of Grubbs-type Ru catalysts to work under enzyme-compatible conditions have also been used to construct chemoenzymatic cascades to make chiral and other industrially relevant compounds.[42] For instance, in a collaboration between the Hartwig and Zhao groups, the combination of NHC–Ru catalysts and cytochrome P450s to access chiral epoxides was studied.[43,44] The same groups have also reported the combination of TM-catalysed olefin synthesis via Rh-mediated diazocoupling with asymmetric alkene reduction using EREDs to synthesise chiral 2-aryl-substituted succinic acid derivatives (Scheme 3a).[45] The process was carried out in a two-step sequential manner in which DCM was evaporated after the coupling step and the reaction mixture redissolved in DMSO prior to the addition of biotransformation components. The optically active 2-aryl-substituted succinate derivatives were obtained in overall yields up to 62% and up to >99% *ee*. Also, Kourist and coworkers developed a chemoenzymatic cascade for the synthesis of stilbene-type antioxidants starting from cinnamic acids.[46] The phenolic acid decarboxylase from *B. subtilis* (*Bs*PAD) was used to generate the corresponding styrenes via decarboxylation. Subsequent Ru-catalysed metathesis yielded the corresponding stilbene compounds of interest. Finally, Castagnolo and coworkers have recently combined 2nd generation Grubbs catalyst-mediated ring-closing metathesis of diallyl ethers and laccase/TEMPO catalysed aromatisation to produce furans (Scheme 3b).[47] The cascade was run in a one-pot two-step manner, with isooctane as the cosolvent in a two-phase system to prevent enzyme deactivation caused by the metal catalyst.

Besides olefin metathesis, González-Sabín and coworkers have also studied Ru-enzyme cascades to design routes to access chiral alcohols. For instance, they combined a Ru (IV)-mediated isomerisation of α-vinylaryl alcohols with concurrent ADH reduction to obtain the corresponding saturated alcohols in high *ees*.[48] In 2018, they developed a new route to chiral β-hydroxyamides starting from β-ketonitriles via concomitant

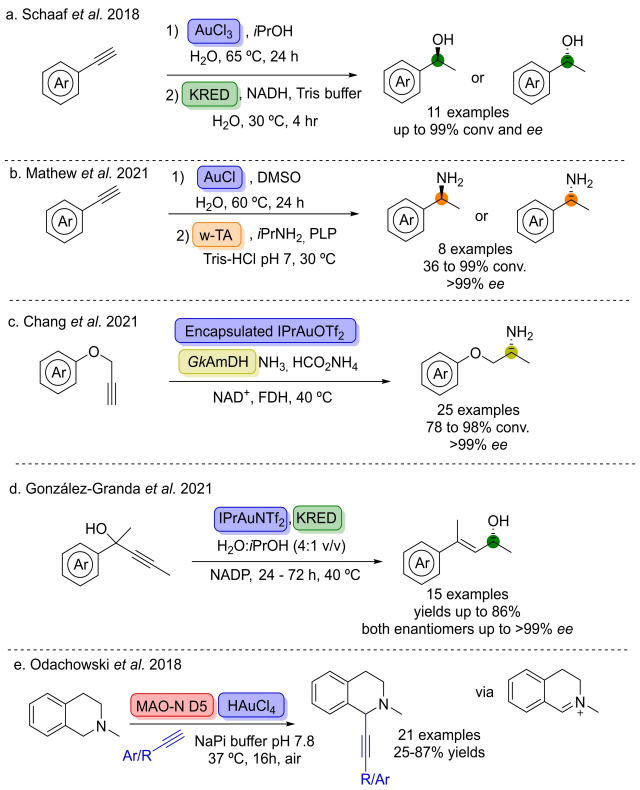


Scheme 3. Novel chemoenzymatic cascades involving other TM catalysts.

bioreduction/Ru nitrile hydration strategy (Scheme 3c).[49] The Micklefield lab has also recently developed a chemoenzymatic cascade for the synthesis of amides via nitrile hydration using nitrile hydratases (NHases) followed by Cu-catalysed amide arylation (Scheme 3d).[50] In this case, incompatibility issues were solved by running biotransformations using whole cell biocatalysts instead of purified enzymes, to create a physical barrier between both catalysts. By using the NHases from *Rhodopseudomanas palustris* CGA009 for aromatic nitriles and the one from *Rhodococcus erythropolis* AJ270 for aliphatic substrates, they could access a remarkably broad panel of arylamides with some reactions carried out at a gram scale. Like in previous reports herein described, the use of TPGS-750-M showed to be beneficial at high substrate loadings.

In the past two decades, gold species have proven to be useful catalysts for the electrophilic activation of alkynes, promoting a plethora of transformations for the construction of new C–C and C–X bonds.[51] The ability to work in aqueous media and under mild reaction conditions has also encouraged the development of one-pot cascade processes in combination with enzyme catalysis. In 2018, Schaaf *et al.* developed a one-pot sequential cascade process to access chiral alcohols from arylacetylenes via Au (III)-mediated hydration followed by subsequent KRED-catalysed asymmetric reduction (Scheme 4a).[52] By using enantiocomplementary enzymes, *i.e.* ADH-A from *Rhodococcus ruber* and *Lk*-ADH from *Lactobacillus kefir* they could access a set of chiral 1-phenylethanol derivatives in moderate to excellent conversions and in high *ee*. This process has now also been extended to the preparation of chiral amines using AuCl as the catalyst for the hydration step at very low loadings (0.2 mol%) in combination with different ω-transaminases to afford the corresponding enantioenriched α-methylbenzylamines (Scheme 4b).[53] Following a similar strategy, Chang *et al.* have recently reported the combination of gold-catalysed alkyne hydration of propargyl ethers followed by bioreductive amination employing the amine dehydrogenase from *Geobacillus kaustophilus* (*Gk*AmDH) to access a broad set of chiral aryloxy- and alkyloxy- propan-2-amines.[54] Due to compatibility issues, the Au/carbene catalyst (IPrAuOTf) was encapsulated in mesoporous silica, allowing the efficient implementation of both steps in the same vessel. Very recently, the Gotor-Lavandera group has described a sequential cascade combining a gold-mediated Meyer-Schuster rearrangement followed by subsequent asymmetric reduction to make chiral β,β-disubstituted allylic alcohols (Scheme 4c).[55]

Besides Au-mediated alkyne hydration, in a collaboration between the groups of Turner and Greaney, a variant of the monoamine oxidase from *Aspergillus niger* (MAO-N D5) was used to make different iminium ions starting from *N*-alkyl tetrahydroisoquinolines (THIQs) followed by gold-catalysed C–C bond formation to access a set of C-(1) functionalised N-alkyl THIQs in high yields, under mild conditions and with no compartmentalisation required (Scheme 4d).[56]



Scheme 4. Chemoenzymatic cascades involving Au-mediated processes.

3. Linear Cascades Combining Organocatalysis and Biocatalysis

The quest for green, environmentally friendly, synthetic procedures, is one of the most challenging tasks of contemporary organic chemistry. Organocatalysis, the use of small molecules to catalyse chemical transformations, has proven to be an excellent tool in asymmetric synthesis, providing access to a variety of functional groups under sustainable conditions.[57] In 2000, seminal works by MacMillan[58] and List[59] inspired the development of this field, for which they have been awarded the 2021 Nobel Prize in Chemistry . Despite the relatively late development when compared with other modalities of catalysis, the field has greatly expanded in the last 15 years and has proved to be a useful tool for asymmetric C–C bond formation under a variety of reaction conditions, showing high reactivity and selectivity. Despite the moderate similarities between small-molecule catalysis and biocatalysis, examples in the literature on the combination of both methodologies are still scarce compared to those of metal and enzyme catalysis.

3.1. Chemoenzymatic cascades involving asymmetric aldol reaction and biocatalytic steps.

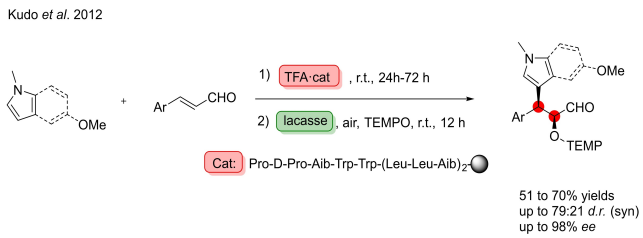
Organocatalytic aldol reactions are useful transformations for asymmetric C–C bond formation via enamine catalysis, and several of these catalysts can promote this transformation in the presence of water and under mild reaction conditions.[60]

Pioneering work by Gröger and coworkers demonstrated as a proof of concept that the reaction spectrum of asymmetric organo- and biocatalysis is complementary, thus enabling a range of unique synthetic cascades when combined. In a series of reports, they comprehensively studied the combination of the asymmetric aldol reaction using proline-type Singh's catalysts with the biocatalytic reduction of the aldol adduct employing the alcohol dehydrogenases from *Rhodococcus sp.* (ADH-A) and *Lactobacillus kefir* (*Lk*-KRED) to synthesise 1,3-diols with *d.r.* up to >25:1 and up to 99% *ee*. (Scheme 5a).[61,62] Subsequently, in 2017, they developed the first chemoenzymatic one-pot process in which both the organocatalytic aldol reaction and the enzymatic reduction proceeded in aqueous medium in a sequential manner.[63] A key feature of this one-pot synthesis is the high 500 mM loading of the aldehyde substrate required as a starting material due to kinetics of the reaction. Very recently, they performed the same chemoenzymatic cascade via both sequential-type and as a tandem-type flow cascade, which resulted in the synthesis of all four stereoisomers of the desired 1,3-diol product. Sequential flow approach provided the final products with conversions up to 76% over two steps and >99% *ee* in all cases. Tandem-type process performed both reactions simultaneously, leading to 51% conversion with >99% *ee* and 8:1 *d.r.* and representing a combination of the fields of asymmetric chemocatalysis, biocatalysis and flow chemistry.[64] In 2015 Suljic *et al.* developed an asymmetric bio- and organocatalytic cascade *one-pot* reaction for the α-arylation of aldehydes via aldol reaction.[65] Starting from 1,4-dihydroxybenzene, a laccase-catalysed aerobic oxidation provides the corresponding quinone, which is used as the electrophile in a Michael reaction with aldehydes catalysed by the Jørgensen catalyst to provide the desired products upon intramolecular cyclisation in moderate to good yields and moderate to excellent enantioselectivity (Scheme 5b).

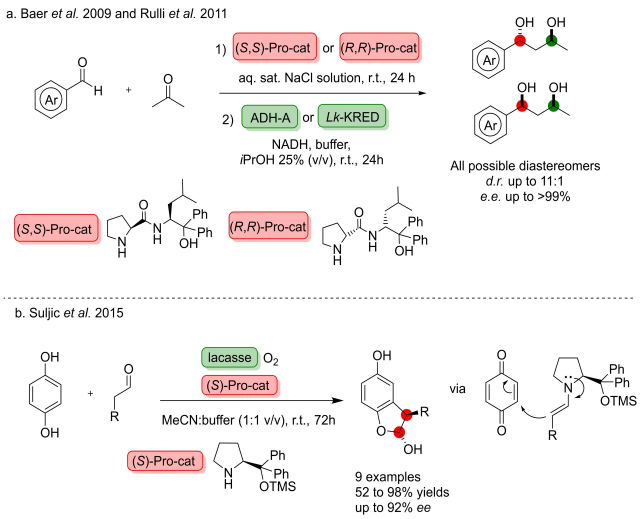
3.2. Other examples

Besides aldol reactions, one of the first examples of a cooperative process involving organo- and biocatalysis was reported by Kudo *et al.* in 2012. In this study, they combined an organocatalyic Friedel-Crafts-type alkylation of α,β-unsaturated aldehydes with indole and pyrrole derivatives using a resin-supported peptide-catalyst, followed by a laccase mediated α-oxyamination (Scheme 6).[66] The target products were obtained in moderate to good yields (51–70%) and high *ee*s, with the *syn* products formed preferentially.

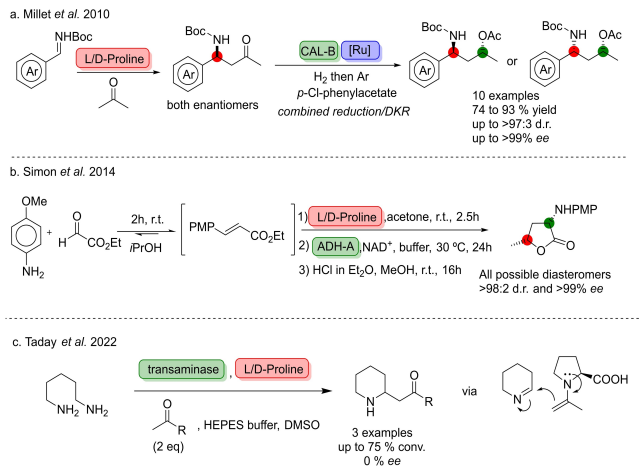
The Mannich reaction is a useful method for the synthesis of β-amino carbonyl compounds and their derivatives, and has been the target of many studies to develop asymmetric versions of this reaction using small organic molecules as catalysts.[67] Cascade processes combining organocatalytic Mannich-type reactions have also been recently combined with enzymatic steps to access chiral compounds. For instance, in 2010 Millet *et al.* took advantage of the ability of lipases to work in neat organic solvents to combine a proline mediated Mannich-type reaction with concomitant Ru-mediated hydrogenation/lipase dynamic kinetic resolution (DKR) process to access chiral 1,3-aminoalcohols (Scheme 7a).[68] In the first place, aldol reaction between acetone and different Boc-protected arylimines catalysed by either L- or D-proline afforded a series



Scheme 6. Chemoenzymatic cascade combining a peptide-catalyzed Friedel-Crafts-type alkylation of α,β-unsaturated aldehydes and enzymatic α-oxyamination.



Scheme 5. Novel chemoenzymatic cascades in aqueous medium involving proline-based organocatalysts.



Scheme 7. Chemoenzymatic cascades involving asymmetric organocatalytic Mannich-type reactions.

of chiral β-amino ketones. Upon Ru-mediated hydrogenation, the corresponding racemic β-amino alcohols were obtained which then via tandem lipase-Ru catalysed DKR were converted into the subsequent chiral 1,3-amino esters using *p*-chlorophenyl acetate as the acyl donor.

Another example of proline-catalysed Mannich reaction followed by a bioreduction step was reported by Simon *et al.* in 2014 to access the α-amino-γ-butyrolactone scaffold (Scheme 7b).[69] By combining D- and L-proline with enantiocomplementary ketoreductases they could synthesise all four diastereomers of *p*-methoxyphenyl-protected (PMP) α-amino-γ-butyrolactone in high yield and with excellent stereocontrol. Subsequent PMP deprotection step of one of the enantiomers yielded γ-hydroxynorvaline in 58% yield.

Very recently, Taday *et al.* reported a bio- organocatalytic cascade for the synthesis of a small panel of 2-substituted piperidines, employing a transaminase to generate an imine intermediate for the subsequent Mannich reaction using acetone with proline as the catalyst processes (Scheme 7c).[70] This approach enables access to the natural product pelletierine, via a *one-pot* setup, where acetone acts as both the amine acceptor in the biotransamination step and the nucleophile in the organocatalytic step. Although high proline equivalents (up to 10) were needed to reach high conversions and the products were obtained in racemic form, this study demonstrates the potential synthetic utility of organo- and biocatalytic cascades.

4. Summary and Outlook

In this review we surveyed recent examples for the synthesis of chiral compounds through chemocatalytic cascade processes involving TM or organocatalysts and enzyme catalysis. In the past decade, research on the development of new enzymes either by discovery or engineering as well and novel processes under mild reaction conditions have greatly expanded the operational window to combine the different catalytic worlds. We envisage that the growing need to provide the synthetic community with sustainable processes will continue to inspire the construction of new multistep catalytic systems. One-pot multistep approaches in a subsequent manner are the preferred setup in which the different components are added sequentially into the same reaction vessel, which allows changes in the reaction conditions such as temperature, pH or cosolvent use. Catalysts based on TM such as Ru, Pd, Fe or Au have so far shown great compatibility with enzymatic systems with several examples herein discussed proceeding in a concurrent manner. Most of this chemistry can be conducted under aqueous conditions, which facilitates the construction of multistep processes. However, Cu species have been demonstrated to cause inhibition issues with enzymatic systems, so compartmentalisation strategies are often required. Regarding the combination of asymmetric organo- and biocatalysis, compatibility issues resulting from catalysts interaction are less likely to affect the reaction outcome. Nevertheless, reaction conditions might differ substantially, especially concerning temperature and solvent. Hydrogen bonding and van der Waals interactions are often key aspects in the activation of substrates in small molecule catalysis, and those can be easily disrupted by water. The modification of these interactions might alter the catalytic activity and/or stereocontrol, therefore aqueous media are not generally considered as suitable solvents in these reactions.

Most of the examples to date are proof-of-concept processes designed to show the broad possibilities that this area can offer for the synthesis of chiral compounds. Researchers at Merck & Co. have recently demonstrated that multienzyme cascades can be designed and used at a large scale to manufacture specific compounds.[71] We believe multistep chemoenzymatic cascades will also be implemented in this manner. In cases where both steps cannot be carried out in the same vessel and compartmentalisation is required, the scalability of most strategies currently developed remains unclear. For instance, and even though PDMS membranes have been shown to be a feasible approach to solve incompatibility issues at small scale, we consider that the implementation of this strategy in large scale synthesis might be highly challenging. Other compartmentalisation strategies rely, for instance, on the use of whole cells to create a physical barrier between both reactions. Despite successful examples – some of them discussed in this survey – complicated downstream processes, mass transfer issues across cell membranes as well as incompatibility between certain TM and cell components are important limitations of this strategy. Catalyst encapsulation using novel materials, as described by Chang *et al.* for instance,[54] might be a feasible solution for large-scale use. However, these approaches do not solve other potential problems such as solvent compatibility or the use of inert atmosphere which is often required in some metal-catalysed processes. Alternatively, continuous flow processes have the potential to solve these issues. Under these systems, reactions are conducted in a continuous stream and the different reaction steps can be fully segregated. Despite being a relatively new area of study, it has become very popular in recent years.[72] Examples on the application of continuous flow setups in multistep enzymatic cascades have led to higher productivities and easier downstream processes, and have demonstrated that previously incompatible cascades can now be run efficiently.[73] We clearly believe that the combination of continuous flow systems and chemoenzymatic cascades will lead to a greater uptake and use of these multistep processes at scale. Besides TM and organocatalysts, the use of light or electrical energy to promote chemical transformations have recently emerged as new alternatives to these "classical" catalytic methodologies. Examples of such cascades have already been reported[74,75] and will also be the focus of extensive study in the coming years. The use of models to optimise multistep enzymatic cascades[76] can potentially be extended to multicatalytic processes to make them more efficient.

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Conflict of Interest

The authors declare no conflict of interest.

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