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Cascade coupling of ene-reductases and ω-transaminases for the stereoselective synthesis of diastereoisomerically enriched amines
--Manuscript Draft--

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Abstract:	One-pot sequential and cascade processes employing ene-reductases (ERs) together with ω-transaminases (ω-TAs) for the obtainment of diastereoisomerically enriched (R)- and (S)-amine derivatives containing an additional stereocenter were investigated. Using as substrates either α- or β-substituted unsaturated ketones and coupling purified ERs belonging to the Old Yellow Enzymes (OYEs) family with a panel of commercially available ω-TAs, the desired products were obtained in up to >99% conversion and >99% de. The sequential reactions were carried out in a one-pot fashion with no need of adapting the reaction conditions to the reductive amination step or purifying the reaction intermediate. Moreover, a high chemoselectivity of the tested ω-TAs for the saturated ketones was shown in the cascade reactions.
Response to Reviewers:	Respond to Reviewers: Reviewer 1: The manuscript titled "Cascade coupling of ene-reductases and ω-transaminases for the stereoselective synthesis of diastereoisomerically enriched amines" submitted by Daniela Monti, Maria Chiara Forchin, Michele Crotti, Fabio Parmeggiani, Francesco G. Gatti, Elisabetta Brenna, and Sergio Riva describes the enantioselective synthesis of chiral amines using sequential and cascade enzymatic processes. The method developed represents convenient, one-pot processes for conversion of enones to important chiral amines using a combination of ene-reductases and ω-transaminases. I would highly recommend publication of the manuscript as a communication in ChemCatChem. At the same time, I would also advice a couple of minor changes to the manuscript. 1. The ene-reductases (ERs) belonging to the Old Yellow Enzymes (OYEs) family catalyzes the first step of the sequential or cascade processes. The choice of ene-reductases would dictate the absolute stereochemistry of the reduction intermediates (e.g. (S)-2a, (S)-2b, and (S)-2c). It would be ideal if both enantiomers of the reduction intermediates, subsequently, all four diastereomers of the chiral amines, can be accessed. Information shall be provided to the reader. A: The following comment, together with suitable references, has been added to the manuscript (page 3, after Table 2): " Although in the present work only two of the four possible stereoisomers of the chiral amines 3a-c were prepared, it might be foreseen that the opposite enantiomer of the intermediate saturated ketone 2a-c could be obtained by using ER variants with opposite stereoselectivity,[9c,17] or employing suitable regioisomers of the starting

material in a substrate-engineering approach, as it was for example already demonstrated for the ER/ADH cascades.[18]"

2. The newly developed process was successfully applied on three substrates. I would suggest the authors try on more sophisticated substrates bearing various functional groups. For example, ethyl 2-methyl-4-oxo-pent-2-enoate would be an attractive substrate to test on. The proposed amine products would be precursors to gamma-butyrolactams (2-pyrrolidones).

A: We thank the Reviewer for the useful suggestion. The cited compound (ethyl 2-methyl-4-oxo-pent-2-enoate) has been used as a substrate with ER-ADH cascade systems by the group of Pietruszka (see for example Korpak and Pietruszka, Adv. Synth. Catal. 2011, 353, 1420–142; Classen et al., ACS Catal., 2014, 4, 1321–1331) to obtain chiral γ-butyrolactones. We have also recently studied the stereoselective synthesis of Nicotiana tabacum lactones by applying OYE-ADH cascades on the key intermediate ethyl 4-hydroxy-3-methylpentanoate (Brenna et al., J.Mol.Cat.B-Enzym.,2015, 114, 77–85). Further investigation are currently underway in our lab to expand the substrate scope of these cascade systems and to prove the generality of this approach for the obtainment of different chiral amines. The suggested molecule will be added to the group of substrates we are currently investigating and the results will be reported in a subsequent paper.

Reviewer 2:

The manuscript is well written and deals with the subject using an experimental design complete and well-thought-out. The topic is also addressed in a recent publication cited in the text (ref12) but the paper is still innovative enough for publication on ChemCatChem as communication with minor revisions.

Specific comments:

1. The diasteromeric excess of all final compounds was calculated using GC. I think that, when possible, it is better to use H-NMR (ref.12, or, more simply, Fracchiolla et al. ChemMedChem, 2007, 2, 641-654). The spectra should be attached to the supporting information, as pure diastereomer and as diastereomeric mixture. In particular the signal of CHNH2 of the compound 3b is suitable to be used for this purpose (for 3a and 3c is hard to do considerations without seeing spectra).

A: The use of GC/MS analysis allowed us to determine the conversions of the reactions and the diastereoisomeric excess values of the final products easily and quickly. It was also particularly useful either to follow the course of the sequential and cascade reactions, by monitoring the reduction of the C=C bond, and to verify that no side reactions were occurring (i.e. the formation of the unsaturated amines). Furthermore, in the case of 3a and 3c the distinction between the two diastereoisomers was not possible by 1H NMR spectra.

2. Check H-NMR in supporting information: multiplets should be indicated as interval and there is some inaccuracy (such as the signal at 6.82 in compound 3c).

A: All the 1H-NMR in the Supporting Information have been carefully checked and corrected as suggested by the Reviewer. In particular, multiplets have been indicated as intervals. In the spectrum of (1S,3'S)-3c, the signal at "6.82 (m, 2H, hydrogens)" has been replaced with "6.90 - 6.75 (m, 2H, aromatic hydrogens)". Also the reference to Skalden et al., 2015, has been up-dated with the final page numbers now available.

3. TOC should be revised: the caption "up to >99% conversion, up to >99% de" should be eliminated because it could be misleading. Not all diasereomers were obtained with

high percentage of conversion or de.

A: The TOC has been corrected as suggested by the Reviewer, i.e., the caption has been deleted.

Reviewer 3:

The manuscript demonstrate an impressive methodology for the synthesis of chiral amines.

There are a few issues that the authors may wish to address:

1. The authors use "conversion" in their tables. Keeping in mind the difference between yield and conversion, is there a reason for not showing the actual yield?

A: As indicated in the legends to the Tables, the results reported in the Tables are conversion values as determined by GC analysis of the crude reaction mixtures (1-mL scale standard reactions, details are available in the Experimental Section, see also below the answer to the last question).

2. In cases of low conversion, e.g. in Table 2 where conversions are about 60%, what is the nature of the reaction mixture? Is it product + remaining starting material?

A: As mentioned in the manuscript, e.g., page 3, right column, lines 16-19, in case of incomplete conversion, the reaction mixture consisted of the residual starting substrate and the desired saturated amine. The formation of the unsaturated amine derivative, as well as of other major by-products, was never observed. A note ("No by-products formation was observed.") has been added to the Tables legends.

3. The scale for the reactions is very small. The authors should give at least one example of a case on a preparatory scale, and illustrate the practicality of the reaction through actual isolation, chromatography, and obtaining a product sample that can be weighed.

A: Indeed the reactions were already scaled up (50 mg scale) in order to isolate and characterize the products. The detailed information (general procedure for preparative reactions, as well as the recovery yields of each characterized product after flash chromatography) was and is reported in the Supporting Information. In order to make this point clearer to the readers, a sentence has been added to the text (page 3, before Table 2): " Selected ER/ω-TA coupled reactions were scaled-up (50 mg) to isolate and characterize the amine products 3a-c (see Supporting Information for details)". If the editor and/or the reviewer judge that this information should be reported in the main text (discussion & experimental part), we will move the experimental procedures there.

Section/Category:	
Additional Information:	
Question	Response
Submitted solely to this journal?	Yes
Has there been a previous version?	No
Dedication	
Animal/tissue experiments?	No

COMMUNICATION   **WILEY-VCH**

Cascade coupling of ene-reductases and ω-transaminases for the stereoselective synthesis of diastereoisomerically enriched amines

Daniela Monti,[a] Maria Chiara Forchin,[a] Michele Crotti,[b] Fabio Parmeggiani,[b] Francesco G. Gatti,[b] Elisabetta Brenna,[b] and Sergio Riva*[a]

Abstract: One-pot sequential and cascade processes employing ene-reductases (ERs) together with ω-transaminases (ω-TAs) for the obtainment of diastereoisomerically enriched (*R*)- and (*S*)-amine derivatives containing an additional stereocenter were investigated. Using as substrates either α- or β-substituted unsaturated ketones and coupling purified ERs belonging to the Old Yellow Enzymes (OYEs) family with a panel of commercially available ω-TAs, the desired products were obtained in up to >99% conversion and >99% de. The sequential reactions were carried out in a one-pot fashion with no need to adapt the reaction conditions to the reductive amination step or to purify the reaction intermediate. Moreover, a high chemoselectivity of the tested ω-TAs for the saturated ketones was shown in the cascade reactions.

Chiral amines are valuable building blocks for the preparation of pharmaceutical agents that span a range of therapeutic areas including antihypertensives, antibiotics, antidepressants, antihistamines, and antidiabetics.[1] In the last two decades, biocatalysis has emerged as an interesting alternative to conventional chemical methods for generating chiral amine compounds. In particular, great efforts have been recently devoted to the exploitation of the so-called ω-transaminases (ω-TAs) for the preparation of optically pure primary amines.[2] These biocatalysts are pyridoxal-5'-phosphate (PLP)-dependent enzymes capable of performing reductive amination reactions using either an α-amino acid or simple aliphatic amines as amine donor. Therefore, they find several applications in the asymmetric synthesis of α-chiral primary amines, and enzymes with different substrate specificity are already available from commercial sources.

To make the enzymatic processes more attractive for their exploitation at an industrial level, multistep cascades, which involve the use of two or more enzymes in a defined reaction pathway, have been intensively investigated as well.[3] In fact, the possibility to perform the enzymatic reactions of a multi-step process in a "one-pot" fashion, *i.e.*, without the isolation of the intermediates, avoids time-consuming or yield-reducing isolation and purification steps and minimize the amounts of chemicals/solvents required. Therefore, it might result effective in reducing operation time, costs and environmental impact.

As far as ω-TAs concern, cascade reactions have been widely applied to shift the equilibrium toward product formation.[4] Moreover, to achieve the *in situ* formation of ketone substrates, ω-TAs have been coupled to other enzymatic activities such as alcohol dehydrogenases (ADHs),[5] oxidases,[6] transferases,[7] and lyases.[8] In few cases, the cascade process allowed also the obtainment of products containing multiple stereocenters, in particular chiral vicinal amino alcohols.[7,8]

During the last years, other enzymes that have been thoroughly investigated for their application in asymmetric synthesis are the ene-reductases (ERs) belonging to the Old Yellow Enzymes (OYEs) family.[9] They catalyze the stereospecific *anti* hydrogenation of different activated olefins, including α,β-unsaturated open-chain and cyclic ketones.

Recently, we have investigated the ERs-catalyzed enantioselective reduction of different open-chain α-alkyl-β-arylenones such as **1a**,[10] as well as its coupling with the ADHs-catalyzed synthesis of the corresponding alcohols to give the most odorous stereoisomers of the chiral commercial fragrance Muguesia® (Scheme 1A).[11]
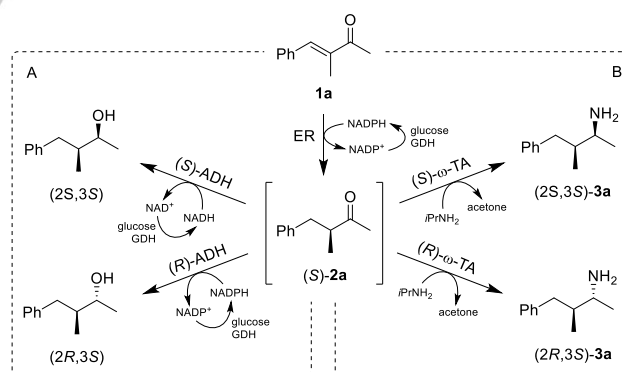
Scheme 1. ER/ADH (A) and ER/ω-TA (B) cascades starting from **1a**.

Specifically, in the presence of the *S. cerevisiae* ER OYE3 and a suitable D-glucose/glucose dehydrogenase (GDH) cofactor regeneration system, **1a** was stereoselectively reduced to give the (*S*)-**2a** product with an enantiomeric excess (ee) value of 98% in quantitative yields.[10] Subsequently, the cascade coupling with stereoselective ketone reductions was made possible by screening a set of commercially available (*S*)- and (*R*)-selective ADHs. By optimization of the biotransformation

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        Supporting information for this article is given via a link at the end of
        the document.((Please delete this text if not appropriate))

conditions, the corresponding *syn* and *anti* alcohols shown in Scheme 1A could be obtained in quantitative yields and 99% diastereomeric excesses (de) by adding all the enzymes from the beginning to the reaction mixture and without isolation of the intermediate saturated ketone (*S*)-**2a**.[11]

In the present work, we envisaged that, due to the structural similarity of **2a** with other common ω-TAs substrates,[2] the corresponding *syn* and *anti* amine derivatives **3a** could be obtained in a similar way by coupling the OYE3-mediated alkene reduction of **1a** with reductive amination reactions catalyzed by ω-TAs (Scheme 1B).

To test this hypothesis, we decided to screen a commercially available library of ω-TAs (Codex® ATA Screening Kit, obtained from Codexis) including 24 enzymatic preparations showing diverse substrate specificity and stereoselectivity. It is worth

mentioning that the coupling of ERs and ω-TAs in cascade reactions has not been deeply studied to date, and no reports were available when we started the present investigation. Only very recently, the first example of a ER/ω-TA cascade was reported by Bornscheuer *et al.*,[12] finalized to the stereoselective synthesis of 1-amino-3-methylcyclohexane diastereoisomers.

Racemic **2a** (prepared by chemical reduction of **1a**) was tested as substrate in reductive amination reactions catalyzed by the respective ω-TAs in the presence of an excess of *iso*-propylamine as amine donor (Table 1). As expected, most ω-TAs showed a good to excellent activity towards *rac*-**2a**, with conversion values up to 95% after 18 h. Only four of the enzymes tested (ATA-007, ATA-200, ATA-254, and ATA-301) were unable to catalyze the amine synthesis on either enantiomer of **2a** under these conditions.

Table 1. Screening of a panel of commercial ω-TAs for the transamination of *rac*-**2a** and their combined application with OYE3 on substrate **1a** (one-pot sequential or cascade).

ω-TA[a]	Typical selectivity[b]	ω-TAs screening (substrate *rac*-**2a**)	One-pot sequential OYE3/ω-TA (substrate **1a**)		Cascade OYE3/ω-TA (substrate **1a**)	
		Conv. (%)[c]	Conv. (%)[c]	de (%)[c]	Conv. (%)[c]	de (%)[c]
ATA-007	(*R*)	0	–	–	–	–
ATA-013	(*R*)	84	55	96 (2*R*,3*S*)-**3a**	20	96 (2*R*,3*S*)-**3a**
ATA-025	(*R*)	93	>99	96 (2*R*,3*S*)-**3a**	80	96 (2*R*,3*S*)-**3a**
ATA-113	(*S*)	93	>99	>99 (2*S*,3*S*)-**3a**	52	>99 (2*S*,3*S*)-**3a**
ATA-117	(*R*)	84	18	>99 (2*R*,3*S*)-**3a**	16	>99 (2*R*,3*S*)-**3a**
ATA-200	(*S*)	0	–	–	–	–
ATA-217	(*S*)	76	35	>99 (2*S*,3*S*)-**3a**	27	>99 (2*S*,3*S*)-**3a**
ATA-234	(*S*)	89	42	>99 (2*S*,3*S*)-**3a**	19	>99 (2*S*,3*S*)-**3a**
ATA-237	(*S*)	92	>99	95 (2*S*,3*S*)-**3a**	70	95 (2*S*,3*S*)-**3a**
ATA-238	(*S*)	88	55	>99 (2*S*,3*S*)-**3a**	13	>99 (2*S*,3*S*)-**3a**
ATA-251	(*S*)	92	>99	96 (2*S*,3*S*)-**3a**	83	96 (2*S*,3*S*)-**3a**
ATA-254	(*S*)	0	–	–	–	–
ATA-256	(*S*)	92	>99	98 (2*S*,3*S*)-**3a**	90	98 (2*S*,3*S*)-**3a**
ATA-260	(*S*)	95	34	94 (2*S*,3*S*)-**3a**	52	94 (2*S*,3*S*)-**3a**
ATA-301	(*R*)	0	–	–	–	–
ATA-303	(*R*)	49	9	>99 (2*R*,3*S*)-**3a**	5	>99 (2*R*,3*S*)-**3a**
ATA-412	(*R*)	83	67	>99 (2*R*,3*S*)-**3a**	28	>99 (2*R*,3*S*)-**3a**
ATA-415	(*R*)	89	57	95 (2*R*,3*S*)-**3a**	30	95 (2*R*,3*S*)-**3a**
ATA-P1-B04	(*S*)	93	87	97 (2*S*,3*S*)-**3a**	82	97 (2*S*,3*S*)-**3a**
ATA-P1-F03	(*S*)	76	35	>99 (2*S*,3*S*)-**3a**	15	>99 (2*S*,3*S*)-**3a**
ATA-P1-G05	(*S*)	89	52	>99 (2*S*,3*S*)-**3a**	26	>99 (2*S*,3*S*)-**3a**
ATA-P2-A01	(*R*)	92	27	93 (2*R*,3*S*)-**3a**	28	93 (2*R*,3*S*)-**3a**
ATA-P2-A07	(*R*)	90	80	96 (2*R*,3*S*)-**3a**	48	96 (2*R*,3*S*)-**3a**
ATA-P2-B01	(*R*)	94	90	93 (2*R*,3*S*)-**3a**	66	93 (2*R*,3*S*)-**3a**

[a] Numbering according to the Codex® ATA Screening Kit. [b] According to the manufacturer. [c] Determined by GC. No by-products formation was observed.

The synthesis of both diastereoisomers of **3a** by coupling the ER-catalyzed alkene reduction and the ω-TA-catalyzed reductive amination was then investigated in one-pot sequential processes. After performing the enantioselective reduction of **1a** to (*S*)-**2a** by OYE3-catalyzed bioreduction (>99% conv., 98% ee), the ω-TAs selected from the previous screening were simply added to the reaction mixture together with the PLP cofactor and the amine donor *iso*-propylamine.

As shown in Table 1, all the tested ω-TAs were capable to catalyze the formation of the amine derivative in these one-pot reactions without the need to change the reaction conditions or to purify the saturated reaction intermediate. As expected, in many cases the overall conversion values were somehow lower

than those observed during the previous ω-TAs screening on *rac*-**2a**, this fact being consistent with the lower amount of transaminase preparations employed in the one-pot reactions (2 mg mL⁻¹). However, in a few cases (ATA-025, ATA-113, ATA-237, ATA-251, and ATA-256), even quantitative conversions (>99%) were observed, suggesting an appreciable enantiopreference of these enzymes towards (*S*)-**2a** with respect to the other enantiomer.

Concerning the stereoselectivity, excellent results were obtained with all the tested ω-TAs, with de values between 93 and >99%. The previously described stereopreference with prochiral aromatic ketones [2] was perfectly conserved in all

cases, with (S)-selective ω-TAs forming (2S,3S)-**3a** while (2R,3S)-**3a** was obtained when applying (R)-selective ω-TAs.

Although the saturated α-chiral ketone (S)-**2a** is a very stable compound not posing any of the racemization issues observed with other ERs products, e.g., α-substituted aldehydes,[9f,13] the performances of OYE3/ω-TAs cascade reactions, i.e., with all the enzymes and reagents being present from the beginning, were investigated to further evaluate the compatibility between these enzymes and the respective reactions.

Although the conversion values obtained in the cascade reactions were in general slightly lower than those obtained in the one-pot sequential processes, GC analyses showed a good compatibility of the two reactions. Remarkably, the formation of the unsaturated amine derivative was never observed, thus showing a strict chemoselectivity of the tested ω-TAs for the saturated ketone (S)-**2a**. Moreover, the stereoselectivity was perfectly conserved, both in terms of de and of absolute configuration of the formed diastereoisomers.

To demonstrate the broad substrate scope of the ERs/ω-TAs coupled systems on structurally different substrates, two additional ketones were considered (Scheme 2). The β-substituted cyclic enone **1b** is a very well known substrate for OYEs,[9,14] and it has been recently used also to investigate the coupling of ERs with other enzymatic activities such as ADHs and Baeyer-Villiger monooxygenases,[15] and even ω-TAs, as mentioned above.[12] The chromanyl derivative **1c** was previously used by our group as a building block to prepare the (1R,3'S)-**3c** amine, a precursor of analogues of NK-1 tachykinin receptor antagonists,[16] via an ER/ADH cascade and subsequent chemical modification.[13c] The choice of these two additional substrates also required us to use different ERs in the alkene reduction step, OYE1 from S. pastorianus and OYE2 from S. cerevisiae being reported as the most stereoselective enzymes for the preparation of (S)-**2b** (>99% ee) [15] and (S)-**2c** (98% ee),[13c] respectively.
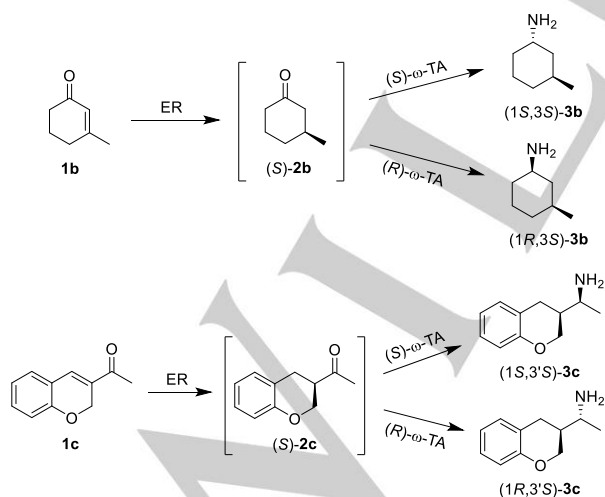
both compounds are very good substrates for the library of transaminases: rac-**2b** was accepted by all but three ω-TAs (ATA-007, ATA-117, ATA-217), while rac-**2c** was transformed into the corresponding amines by all the tested enzymes and in most cases with quantitative conversions (see Supporting Information for further details).

Subsequently, selected (R)- and (S)-selective ω-TAs were evaluated in one-pot sequential and cascade reactions aimed to the synthesis of diastereomerically enriched **3b** and **3c** derivatives starting from **1b** and **1c**, respectively.

As shown in Table 2, the desired products, specifically, the (1S,3S)-**3b** and (1R,3S)-**3b** amines and the (1S,3'S)-**3c** and (1R,3'S)-**3c** amines, were obtained in both types of coupled processes with similar conversions and up to 99% de values, thus demonstrating the general applicability of ERs/ω-TAs one-pot sequential and cascade reactions. As in the case of **1a**, the typical stereoselectivity shown by ω-TAs was again well conserved, and no formation of the unsaturated amines was observed in the cascade reactions with these substrates as well, thus confirming a high chemoselectivity of the ω-TAs toward the saturated ketones.

Selected ER/ω-TA coupled reactions were scaled-up (50 mg) to isolate and characterize the amine products **3a-c** (see Supporting Information for details).

Table 2. One-pot synthesis of optically pure diastereoisomers of amines **3b-c**.

Subs.	Enzymes	One-pot sequential OYE/ω-TA		Cascade OYE/ω-TA	
		Conv. (%)[a]	de (%)[a]	Conv. (%)[a]	de (%)[a]
1b	OYE1 / ATA-113	63	>99 (1S,3S)-**3b**	78	98 (1S,3S)-**3b**
1b	OYE1 / ATA-237	78	>99 (1S,3S)-**3b**	62	>99 (1S,3S)-**3b**
1b	OYE1 / ATA-025	70	90 (1R,3S)-**3b**	99	90 (1R,3S)-**3b**
1b	OYE1 / ATA-415	64	58 (1R,3S)-**3b**	62	72 (1R,3S)-**3b**
1c	OYE2 / ATA-256	63	79 (1S,3'S)-**3c**	95	88 (1S,3'S)-**3c**
1c	OYE2 / ATA-113	87	80 (1S,3'S)-**3c**	90	90 (1S,3'S)-**3c**
1c	OYE2 / ATA-013	65	80 (1R,3'S)-**3c**	68	84 (1R,3'S)-**3c**
1c	OYE2 / ATA-025	96	75 (1R,3'S)-**3c**	93	86 (1R,3'S)-**3c**

[a] Determined by GC. No by-products formation was observed.

Although in the present work only two of the four possible stereoisomers of the chiral amines **3a-c** were prepared, it might be foreseen that the opposite enantiomer of the intermediate saturated ketone **2a-c** could be obtained by using ER variants with opposite stereoselectivity,[9c,17] or employing suitable regioisomers of the starting material in a substrate-engineering approach, as it was for example already demonstrated for the ER/ADH cascades.[18]

In summary, our investigation showed that indeed the coupled use of ERs and ω-TAs can be exploited for the obtainment of diastereoisomerically enriched (R)- and (S)-amines with different structural features. The desired products could be obtained in one-pot sequential processes and even cascade processes, in up to >99% conversion and >99% de.

Scheme 2. ER/ω-TA cascades starting from **1b-c**.

A preliminary screening of the ω-TAs library on racemic mixtures of the saturated intermediates **2b** and **2c** showed that

Experimental Section

ERs (OYE1 from *Saccharomyces pastorianus* and OYE2 and OYE3 from *S. cerevisiae*) and *Bacillus megaterium* GDH were overexpressed in *Escherichia coli* BL21(DE3). Detailed methods are reported in the Supporting Information. ω-TA preparations were obtained from Codexis (Codex® ATA Screening Kit).

The screening of ω-TAs on racemic **2a-c** was performed by adding each substrate (10 μmol) dissolved in DMSO (20 μL) to a KP_i buffer solution (1.0 mL, 50 mM, pH 7.0) containing PLP (1 mM), *i*-PrNH$_2$ (1 M), and the respective ATA preparation (10 mg mL^{-1}). The mixture was incubated for 18 h in an orbital shaker (160 rpm, 30°C). The solution was extracted with EtOAc (2 × 250 μL), centrifuging after each extraction (15000 g, 1.5 min), and the combined organic solutions were dried over anhydrous Na_2SO_4 and analyzed by GC.

One-pot sequential ER/ω-TA reactions were carried out by adding substrates **1a-c** (10 μmol) dissolved in DMSO (20 μL) to a KP_i buffer solution (1.0 mL, 50 mM, pH 7.0) containing glucose (20 mM), NADP$^+$ (0.1 mM), GDH (4 U mL^{-1}), and the required OYE (60 μg mL^{-1}). After 18 h of incubation in an orbital shaker (160 rpm, 30°C), PLP (1 mM), *i*-PrNH$_2$ (1 M), and the respective ATA preparation (2 mg mL^{-1}) were added and the reaction mixture was incubated for additional 18 h under the same conditions. Samples preparation and analysis were carried out as described for the screening of ω-TAs on racemic substrates.

Cascade ER/ω-TA reactions on substrates **1a-c** were carried out by mixing from the beginning all the reagents and enzymes described in the one-pot sequential reaction. Samples were recovered after 36 h and analyzed as described for the screening of ω-TAs on racemic substrates.

Acknowledgements

Keywords: stereoselectivity • ene-reductases • transaminases • chiral amines • cascade biocatalysis

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Entry for the Table of Contents (Please choose one layout)

COMMUNICATION

The biocatalytic synthesis of diastereomerically enriched (*R*)- and (*S*)-amines was achieved by one-pot coupling of ene-reductases and ω-transaminases, in sequential and cascade processes. Using α- or β-substituted unsaturated ketones as substrates, up to >99% conversion and >99% de were obtained.
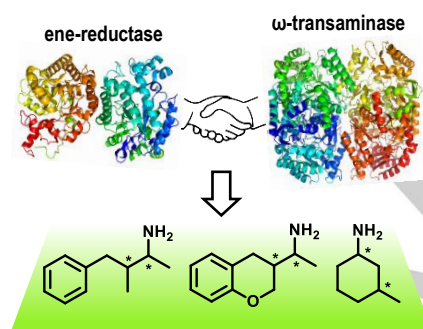
ene-reductase   ω-transaminase

Daniela Monti, Maria Chiara Forchin, Michele Crotti, Fabio Parmeggiani, Francesco G. Gatti, Elisabetta Brenna, Sergio Riva*

Page No. – Page No.

Cascade coupling of ene-reductases and ω-transaminases for the stereoselective synthesis of diastereoisomerically enriched amines