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Ene-Reductases for Organic Synthesis Applications: Chemoselective Hydrogenation of Alkenes in the Presence of Alkynes for the Homologation of 2-Alkynals into 4-Alkynals/Alkynols

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Abstract. No chemical methods are available for the chemoselective hydrogenation of alkenes in the presence of alkynes. The possibility to accomplish this reaction would enrich the tool-kit available to organic chemists for the development of effective synthetic routes, and the creation of novel structural motifs. The reduction of activated alkene bonds by ene-reductases (ERs) is completely chemoselective, because of the mechanism of the reaction. Thus, we investigated the use of ERs belonging to the Old Yellow Enzyme family for the reduction of α,β-unsaturated aldehydes with a conjugated C≡C triple bond at the γ position. This reaction was exploited as the key step for the development of an effective homologation route to convert aryl and alkyl substituted propynals into 4-alkynals and 4-alkynols, showing several advantages with respect to known methods. Furthermore, the experimental data on the bioreduction of C≡C triple bonds conjugated to a carbonyl group were enriched by studying the OYE-mediated reduction of phenylpropynal, one of the starting aldehydes of the homologation route.

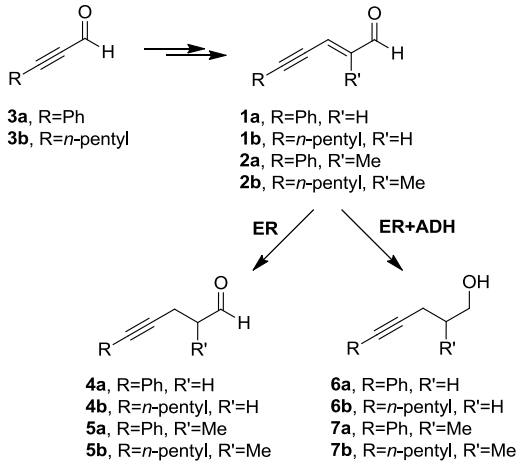
Keywords: alkynes; biocatalysis; green chemistry; oxidoreductases; bioreduction

Introduction

Organic chemists are ever since fascinated by the capability of enzymes to catalyse chemical reactions with excellent chemo-, regio- and stereoselectivity.[1] Among the enzymatic variants of classical organic transformations, the enantioselective reduction of activated alkenes mediated by ene-reductases (ERs) is of great interest for synthetic applications.[2] The reaction has been extensively investigated in the last years to establish mechanism and substrate-scope.[3] Typical substrates are functionalised alkenes bearing on the C=C bond at least one electron-withdrawing group (EWG) capable of establishing hydrogen bonds with specific amino acid residues in the active site of the enzyme. The biocatalyst portfolio is continuously expanding, thanks to both the isolation of new ERs,[4] and the creation of tailor-made variants by protein engineering.[5] Commercial kits of ERs are now also available.
On the side of synthetic applications, this reaction has been successfully exploited for the preparation of enantiopure chiral intermediates to be used in the synthesis of active pharmaceutical ingredients and fragrances.[6] A number of research programs are on-going to employ ER-mediated steps in multi-enzymatic and chemo-enzymatic procedures for the optimisation of standard protocols that can be included in more complex synthetic schemes.[7] Great efforts are being devoted to showing how the use of ERs (as well as many other biocatalysts) can provide optimal and sustainable solutions to specific synthetic necessities that are hardly satisfied by classical chemical methods.[8]

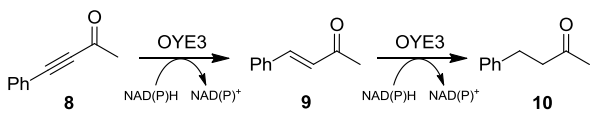
As an example, the chemoselective hydrogenation of alkenes in the presence of alkynes cannot be accomplished by traditional reducing agents. The possibility to perform this transformation could offer novel interesting synthetic approaches and enrich the variety of molecular skeletons that can be created. With this aim, we investigated the use of ERs belonging to the Old Yellow Enzyme (OYE) family for the reduction of α,β-unsaturated aldehydes of type **1** and **2** (Scheme 1), bearing a conjugated carbon-carbon triple bond at the γ position. This reaction

represents the key step for the development of an effective homologation route to convert aryl and alkyl substituted propynals **3** into 4-alkynals and alkynols **4-7**.



Scheme 1. Homologation of 2-alkynals to afford 4-alkynals and 4-alkynols.

The screening against our panel of OYEs was also extended to derivatives **3**, in order to expand upon the experimental data on the bioreduction of C≡C triple bonds conjugated to carbonyl groups. The first report on this topic, published by Rosche *et al.* [9] in 2007, described the capability of OYE1-3 to convert 4-phenyl-3-butyn-2-one **8** into (*E*)-benzalacetone **9** (Scheme 2).



Scheme 2. OYE3-mediated reduction of **8** and **9**

OYE3 showed a much higher rate for the reduction of **8** than for the reduction of **9**, which accumulated in the first 5 hours of reaction, and was then completely reduced to 3-phenylbutan-2-one **10** in 11.5 h overall reaction time. The authors described the reaction as an environmental friendly alternative for *trans*-selective conversion of alkynes into alkenes. To the best of our knowledge, in spite of the extensive research on synthetic applications of ERs, no further development of the topic appeared since then in the literature.

Thus, we report herein on the results of our studies on the synthesis and ER-mediated bioreduction of alkyne-substituted substrates of type **1-3**, achieving in particular a novel chemo-enzymatic procedure for the homologation of aryl and alkyl propynals **3** to 4-alkynals/alkynols.

Results and Discussion

Chemo-enzymatic homologation of alkynals 3 into derivatives 4-7

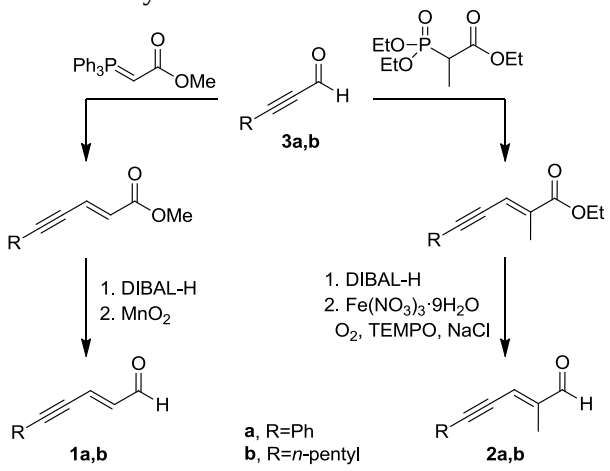
The chemoselective reduction of a C=C double bond in the presence of a C≡C triple bond cannot be accomplished by chemical methods. The reactivity of alkenes towards reducing agents is higher than that of alkynes, both in hydrogenation with molecular hydrogen and hydrogen transfer reactions. By contrast, the reaction catalysed by ERs occurs with complete chemoselectivity: the mechanism of the reaction consists in the *anti* hydrogen addition to the multiple bond linked to the activating EWG, which is involved in the formation of hydrogen bonds within the active site of the enzyme. Firstly, a hydride from the reduced flavin mononucleotide (FMN) cofactor adds to the substrate carbon atom in β position with respect to the EWG and positioned above the cofactor. Then, protonation of the other carbon atom of the multiple bond occurs from the opposite face of the alkene, mediated by a tyrosine side-chain.[10]
We envisaged the possibility to exploit this chemoselection for optimising a two-carbon homologation of alkynals **3** to 2-substituted-4-alkynals **4-5** and alkynols **6-7**, based on the ER-mediated reduction of 2-alken-4-ynals **1** and **2** (Scheme 1).

Compounds of general formula **4-7,** with R = alkyl or aryl and R' = H or Me, occur in the literature as key intermediates for the synthesis of natural products,[11] pheromones,[12] cosmetics[13] and drugs.[14] Their known syntheses typically involve the chemistry of terminal alkynes: (i) coupling of terminal alkynes with acrolein in the presence of $Pd(OAc)_2$ and PMe_3;[13] (ii) coupling of terminal alkynols with aryl bromides using CuI and $Pd(PPh_3)_2Cl_2$;[11e,11f] (iii) reaction of the lithium salt of terminal alkynes with allyl bromide (followed by hydroboration of the C=C double bond)[11b] or with *O*-protected bromoalkanols.[12a,12b,11g]

We developed an alternative synthetic procedure to derivatives of type **4-7**, selecting as target compounds those having R = Ph or *n*-pentyl and R' = H or Me. These compounds are representative of aromatic and aliphatic propynals that are transformed into achiral (R' = H) or chiral (R' = Me) derivatives. For this purpose propynals **3a,b** were prepared by oxidation of the corresponding commercially available alcohols, following literature procedures based on the use of $Fe(NO_3)_3{\cdot}9H_2O$ in the presence of O_2, TEMPO and NaCl, or activated MnO_2, respectively (see the Supporting Information for detailed methods and references).

The conversion of starting alkynals **3a,b** into alkenynals **1a,b** and **2a,b** was accomplished according to the synthetic route reported in Scheme 3 (see the Supporting Information for details and references). A standard protocol was employed for the generation of unsaturated aldehydes: Wittig or Horner-Wadsworth-Emmons

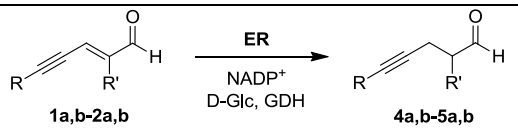
(HWE) reaction, followed by DIBAL-H reduction and final allylic oxidation.



Scheme 3. Synthesis of 2-alken-4-ynals **1a,b-2a,b**.

Compounds **1a,b-2a,b** were submitted to bioreduction with OYE1-3 from *Saccharomyces* sp., using glucose and glucose dehydrogenase (GDH) from *B. megaterium* for NADPH regeneration, to afford the corresponding 4-alkynals **4a,b-5a,b** according to the conversion values reported in Table 1. OYE1-3 were found to be very effective catalysts for the C=C reduction of all the derivatives tested. The presence of the conjugated triple bond does not inhibit the nucleophilic attack of the hydride to the olefinic carbon atom. The loss of conformational mobility of the molecular skeleton, due to the introduction of the linear triple bond, does not prevent the binding of the unsaturated aldehyde to the enzyme active site.

Table 1. OYE-mediated reactions of substrates **1a,b-2a,b**.



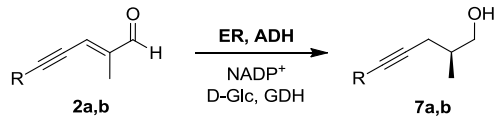
Substrate	Product	OYE1 c (%)[a)]	OYE2 c (%)[a)]	OYE3 c (%)[a)]
1a	**4a**	99	99	99
R=Ph, R'=H				
2a	**5a**	99	99	99
R=Ph, R'=Me				
1b	**4b**	99	99	99
R=*n*-pentyl, R'=H				
2b	**5b**	99	13	39
R=*n*-pentyl, R'=Me				

Expt. cond.: 5 mM substrate, 20 mM D-Glc, 0.1 mM $NADP^+$, 0.1 mg mL^{-1} ER, 4 U mL^{-1} GDH, 50 mM KP_i, pH 7.0, 30°C, 24 h.
[a)] Conversion calculated on the basis of GC analysis.

The biocatalysed reduction of intermediates **1a,b** and **2a,b** was then performed on a preparative scale, affording **4a,b-5a,b** with overall isolation yields in the range 33-47% from aldehydes **3a,b**.

In the case of substrates **2a,b**, the bioreduction occurs with the formation of a stereogenic centre. The enantiomeric excess (ee) of aldehyde **5a** could be determined by HPLC analysis (chiral stationary phase, see Experimental Section) of the corresponding alcohol **7a**, obtained upon addition of a commercial alcohol dehydrogenase (ADH, EVO30 from EVOXX) to the reaction mixture at the end of the OYE-mediated reduction. In this way, artefacts due to the alkaline medium of $NaBH_4$ reduction could be avoided. The following ee values were obtained: 37% (OYE1), 48% (OYE2), and 44% (OYE3). The poor enantiomeric purity of compound **5a** is due to the lability of the stereogenic centre in α position to the aldehydic group in the biotransformation conditions. The same phenomenon has been observed previously during the OYE-mediated reduction of other α-substituted α,β-unsaturated aldehydes.[15] Very likely aldehyde **5b**, for which the ee values could not be determined, undergoes epimerisation as well. In order to avoid loss of enantiomeric purity due to configurational instability, the reduction of the alkene mediated by OYEs was coupled with the concomitant conversion of the aldehyde into a primary alcohol catalysed by an alcohol dehydrogenase (ADH). A screening was performed (see Experimental Section) to select an ADH capable of reducing selectively the saturated aldehyde with respect to the unsaturated one, because the formation of the allylic alcohol would prevent ER-mediated reduction. A commercially available screening kit of 18 ADHs (from EVOXX) was employed, obtaining best results with EVO200. Cascade reactions performed on **2a,b** with OYEs and ADH EVO200 added together to the reaction medium from the beginning afforded the results reported in Table 2.

Table 2. Two-enzyme cascade synthesis of alcohols **7a,b**.

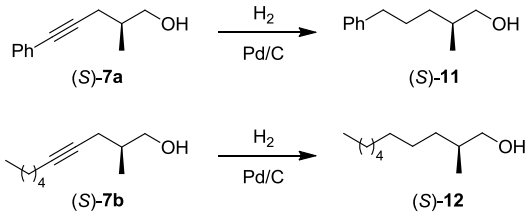


ER	7a, R=Ph c (%)[a)], ee (%)[b)]	7b, R=*n*-pentyl c (%)[a)], ee (%)[c)]
OYE1	99, 87 (*S*)	99, 90 (*S*)
OYE2	99, 84 (*S*)	55, n.d.[d)]
OYE3	99, 95 (*S*)	75, n.d

Expt. cond.: 5 mM substrate, 20 mM D-Glc, 0.1 mM NAD^+, 0.1 mM $NADP^+$, 0.1 mg mL^{-1} ER, 0.2 mg mL^{-1} ADH, 4 U mL^{-1} GDH, 50 mM KP_i, pH 7.0, 30°C, 24 h.
[a)] Conversion calculated on the basis of GC analysis.

[b] Enantiomeric excess calculated on the basis of HPLC analysis on a chiral stationary phase.
[c] Optical purity calculated on the basis of the optical rotation value of **7b**.[17]
[d] Not determined.

Employing this strategy, 4-alkynols **7a,b** could be obtained in good enantiomeric purity. Their absolute configuration was determined by chemical correlation, by converting them into the corresponding saturated derivatives (*S*)-**11**[16] and (*S*)-**12**[17] of known absolute configuration, by hydrogenation with molecular hydrogen and Pd/C as a catalyst (Scheme 4). The enantioselectivity of the reaction was in agreement with that observed for the OYE-catalysed reduction of α-alkyl-α,β-unsaturated aldehydes,[15] with the substrate binding to the enzyme active site in a "flipped" mode.[18]



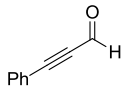
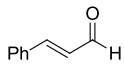
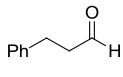
Scheme 4. Conversion of alcohols (*S*)-**7a** and (*S*)-**7b** into derivatives of known absolute configuration; i) H_2, Pd/C.

The same cascade procedure was also successfully applied to substrates **1a,b**, to afford the corresponding alcohols **6a,b** in quantitative conversion.
The ER/ADH-mediated reduction was run at a preparative scale on intermediates **1a,b-2a,b**, to give **6a,b-7a,b** with overall isolation yields in the range 35 - 44% from aldehydes **3a** and **3b**.

ER-mediated reduction of alkynals 3a,b

Having aldehydes **3a,b** available, we decided to investigate their behaviour in the biotransformations catalysed by ERs. Compound **3b** was left unreacted by OYE1-3, whereas phenylpropiolic aldehyde **3a** was reduced to (*E*)-cinnamaldehyde **13** as shown in Table 3. The possibility to reduce C≡C triple bonds using OYE1-3 seemed to be limited to aryl-substituted propynals and butynones, such as **3a** and **8**.
The best results were obtained with OYE3. The reaction was investigated after 5 and 24 h, and (*E*)-cinnamaldehyde **13** was observed to accumulate in the reaction medium (achieving a maximum conversion of nearly 50%) even when the amount of the biocatalyst is doubled.

Table 3. OYE-mediated reactions of aldehyde **3a**.

ER	Time (h)	Product distribution (%)[a]		
		 **3a**	 **13**	 **14**
OYE1	24	94	6	-
OYE2	24	83	17	-
OYE3	5	64	33	3
OYE3	24	53	44	3
OYE3[b]	5	63	35	2
OYE3[b]	24	54	44	2

Expt. cond.: 5 mM substrate, 20 mM D-Glc, 0.1 mM $NADP^+$, 0.1 mg mL^{-1} ER, 4 U mL^{-1} GDH, 50 mM KP_i, pH 7.0, 30°C, 5-24 h.
[a] Conversion calculated on the basis of GC analysis.
[b] Enzyme loading doubled (0.2 mg mL^{-1}).

This is a very surprising observation, considering that **13** is a standard model substrate for ER activity assays, reduced quickly and completely, even with low enzyme concentrations. This unexpected result was also confirmed by using a 1:1 mixture of **3a** and **13** as substrate for OYE3: only partial reduction of **3a** to **13** was observed, and a negligible amount of saturated product **14** could be detected by GC.
Further investigations are on-going to understand the lack of reactivity of the unsaturated aldehyde, although, in principle, this procedure could constitute an environmentally friendly alternative to metal-catalysed reductions of alkynes to *trans*-alkenes.

Conclusion

The excellent selectivity of enzymes gives organic chemists the opportunity to explore new synthetic paths, that have been until now precluded by classical organic synthesis. This work illustrates how a novel chemo-enzymatic sequence involving an easy C=C bond formation reaction followed by an enzyme-mediated chemoselective hydrogenation can be exploited for the addition of a CH_2CHO/CH_2CH_2OH moiety to an alkynal. This approach avoids the disadvantages of the known routes to derivatives **4-7**: the use of metal-based reagents and catalysts, the troublesome formation and use of alkynyllithium salts, and the need for protection/deprotection steps.
Starting aldehydes of type **3** can be prepared by oxidation of the corresponding substituted propargyl alcohols, many of which are already available as commercial products. For the sake of simplicity we chose the most well-established approach for the stereoselective synthesis of

alkenes, based on Wittig or HWE reactions. The sustainability of these processes has been recently improved, by optimising catalytic variants of the Wittig reaction, to reduce the amount of the required phosphine,[19] and solvent-free protocols of the HWE olefination.[20]

The OYE-mediated reduction of compounds **1** and **2** proceeds smoothly in quantitative yields. The peculiar linear geometry and rigidity of the C≡C triple bond and the π-conjugation between the multiple bonds do not seem to hinder the efficiency of the binding of such substrates to the enzyme active site and of hydride transfer.

Furthermore, our homologation procedure could be successfully applied to the synthesis of chiral molecules and extended to the preparation of alcohol derivatives, courtesy of the concomitant use of an ER and a suitable ADH.

Work is in progress to improve this synthetic route by studying the enzymatic oxidation of the alcohols employed as precursors of aldehydes **1-3**. As for the synthesis of derivative **4a,** the first attempts to run the Wittig condensation with PPh_3=CHCHO and the OYE-mediated reduction in the same reaction medium, using water as a solvent, unfortunately failed. The resulting unsaturated aldehyde **1a** underwent a second Wittig condensation with no reduction of the alkene bond.

In this study, we also confirmed the capability of OYEs to promote hydrogen *anti* addition to C≡C bonds activated by a carbonyl group (either an aldehyde or a ketone) and substituted with an aromatic ring. Under non-optimised reaction conditions, phenylpropiolic aldehyde afforded cinnamaldehyde and, surprisingly, further reduction to 3-phenylpropanal was strongly inhibited.

Experimental Section

General methods

GC-MS analyses were performed using a HP-5MS column (30 m × 0.25 mm × 0.25 µm, Agilent). The following temperature program was employed: 60°C (1 min) / 6°C min^{-1} / 150°C (1 min) / 12°C min^{-1} / 280°C (5 min). The enantiomeric excess values of compounds **7a** and **11** were determined by HPLC analysis on a Chiralcel OD column (4.6 mm × 250 mm, Daicel), using the following conditions: hexane : *i*-PrOH 95:5, λ = 215 nm, 0.6 mL min^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz spectrometer, and the chemical shift scale was based on internal tetramethylsilane. TLC analyses were performed on Merck Kieselgel 60 F254 plates. All the chromatographic separations were carried out on a PuriFlash XS-420+ (Interchim) using Purezza-Daily Standard Flash cartridges (Sepachrom, Italy).

Sources of enzymes

OYEs (OYE1 from *Saccharomyces pastorianus*, OYE2 and OYE3 from *Saccharomyces cerevisiae*) fused with a His_6-tag and GDH (from *Bacillus megaterium*) fused with a His_6-tag were overproduced in *Escherichia coli* BL21(DE3) strains, harboring the following plasmids: pET-30a-OYE1 containing the *oye1* gene provided by Neil C. Bruce[21] pET-30a-OYE2 and pET-30a-OYE3 containing the *oye2* and *oye3* genes cloned from *S. cerevisiae* BY474[22] pKTS-GDH containing the *gdh* gene cloned from *B. megaterium* DSM509.[22] The screening panel of ADHs (containing 18 enzymes) was obtained from EVOXX.

Production of the enzymes in *E. coli* BL21(DE3)

LB medium (15 mL) supplemented with the appropriate antibiotic (50 µg mL^{-1} kanamycin for pET-30a, 100 µg mL^{-1} ampicillin for pKTS) was inoculated with a single colony from a fresh plate and grown overnight at 37°C and 220 rpm. This starter culture was used to inoculate 1.5 L of LB medium, which was incubated at 37°C and 220 rpm until OD_{600} reached 0.4-0.5, then enzyme expression was induced by the addition of IPTG (0.1 mM final concentration). For the pKTS-GDH plasmid anhydrotetracycline was also added (50 ng mL^{-1} final concentration). After 5 h, the cells were harvested by centrifugation (5000 *g*, 20 min, 4°C), resuspended in 50 mL of lysis buffer (20 mM potassium phosphate buffer pH 7.0, 300 mM NaCl, 10 mM imidazole), disrupted by sonication (Omni Ruptor 250 ultrasonic homogeniser, five sonication cycles, 15 s each, 50% duty) and centrifuged (20000 *g*, 20 min, 4°C). The cell-free extract of His-tagged OYE1-3 and GDH, were purified by affinity chromatography on IMAC stationary phase (Ni-Sepharose Fast Flow, GE Healthcare) with a mobile phase composed of 20 mM potassium phosphate buffer pH 7.0, 300 mM NaCl and a 10-300 mM imidazole gradient. Protein elution was monitored at 280 nm, the fractions were collected according to the chromatogram and dialysed twice against 1.0 L of 50 mM potassium phosphate buffer pH 7.0 (12 h each, 4°C) to remove imidazole and salts. Purified protein aliquots were stored frozen at –80°C.

General procedure for OYE-mediated reductions of substrates 1-3 (screening)

A solution of the substrate in DMSO (10 µL, 500 mM) was added to a KP_i buffer solution (1.0 mL, 50 mM, pH 7.0) containing glucose (20 µmol), $NADP^+$ (0.1 µmol), GDH (4 U) and the required purified OYE (80-120 µg). The mixture was incubated for 24 h in an orbital shaker (150 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. Two replicates were performed for each biotransformation: no significant differences (<5%) were observed for conversion and enantiomeric excess values.

General procedure for OYE-mediated reductions of substrates 1-2 (preparative scale)

A solution of the suitable substrate (50 mg) in *i*-PrOH (150-200 µL) was added to a KP_i buffer solution (5 mL, 50 mM, pH 7.0) containing the required OYE (4-5 mg), GDH (100 U), glucose (4 eq.) and $NADP^+$ (10 µmol, 7.4 mg). The reaction was monitored by GC until complete conversion (generally 24 h). The mixture was then extracted with EtOAc (3 × 3 mL), dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (*n*-hexane with increasing amounts of EtOAc).

General procedure for the one-pot conversion of substrates 1-2 into alcohols 6-7 (screening)

The same procedure for the screening of OYE-mediated reduction was followed, adding ADH (200 µg) and NAD^+ (0.1 µmol) to the reaction mixture. Of the 18 commercially available ADHs (from EVOXX),

EVO200 was selected on the basis of the complete chemoselectivity in the quantitative conversion of intermediate aldehydes **4-5** into alcohols **6-7**.

General procedure for the one-pot conversion of substrates 1-2 into alcohols 6-7 (preparative scale)

The same procedure for the OYE-mediated reduction at the preparative scale was followed, adding ADH EVO200 (3-4 mg) and NAD^+ (10 μmol, 6.5 mg) to the reaction mixture.

5-Phenylpent-4-ynal (4a)

From aldehyde (*E*)-**1a** (50 mg, 0.32 mmol), by reaction with OYE3, aldehyde **4a** (38.9 mg, 77%) was obtained: ^{1}H NMR (400 MHz, $CDCl_3$)[23] δ 9.85 (m, 1H, *CHO*), 7.40 – 7.34 (m, 2H, aromatic hydrogens), 7.30 – 7.24(m, 3H, aromatic hydrogen), 2.80 -2.70 (m, 4H, CH_2CH_2); ^{13}C NMR (101 MHz, $CDCl_3$)[23] δ 200.5, 131.7, 128.4, 128.0, 123.5, 87.8, 81.7, 42.8, 12.9; GC-MS (EI) t_r = 14.8 min, *m/z* 158 (M^+, 27), 129 (100), 115 (100), 102 (90).

5-Phenylpent-4-yn-1-ol (6a)

From aldehyde (*E*)-**1a** (50 mg, 0.32 mmol), by reaction with OYE3 and EVO200, alcohol **6a** was obtained (36.8 mg, 72%): ^{1}H NMR (400 MHz, $CDCl_3$)[24] δ 7.42 – 7.34 (m, 2H, aromatic hydrogens), 7.30 – 7.23 (m, 3H, aromatic hydrogen), 3.82 (q, *J* = 5.9 Hz, 2H, CH_2OH), 2.54 (t, *J* = 6.9 Hz, 2H, CH_2C≡C), 1.87 (quintuplet, $J_{\_}$ = 6.6 Hz, 2H, CH_2), 1.51 (t. $J_{\_}$ = 5.9 Hz, 1H, OH); ^{13}C NMR (101 MHz, $CDCl_3$)[24] δ 131.7, 128.4, 127.8, 123.9, 89.4, 81.3, 62.0, 31.6, 16.2; GC-MS (EI) t_r = 16.3 min, *m/z* 160 (M^+, 32), 141 (73), 128 (55), 115 (100).

(*S*)-2-Methyl-5-phenylpent-4-ynal ((*S*)-5a)

From aldehyde **2a** (50 mg, 0.29 mmol), by reaction with OYE3, aldehyde **5a** (36.9 mg, 74%) was obtained: ^{1}H NMR (400 MHz, $CDCl_3$)[25] δ 9.77 (m, 1H, *CHO*), 7.42 – 7.34 (m, 2H, aromatic hydrogens), 7.30 – 7.22 (m, 3H, aromatic hydrogens), 2.80 – 2.54 (m, 3H, CH_2CH), 1.29 (d, *J* = 7.1 Hz, 3H, $CHCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$)[25] δ 203.4, 131.7, 128.4, 128.0, 123.5, 86.5, 82.7, 45.5, 21.0, 13.4; GC-MS (EI) t_r = 18.3 min, *m/z* 172 (M^+, 13), 157 (25), 129 (100), 115 (79), 102 (67).

(*S*)-2-Methyl-5-phenylpent-4-yn-1-ol ((*S*)-7a)

From aldehyde **2a** (50 mg, 0.29 mmol), by reaction with OYE3 and EVO200, alcohol (*S*)-**7a** (34.8 mg, 69%) was obtained: ee = 96% (HPLC), $[\alpha]_D$ = – 11.7 (*c* 2.5, $CHCl_3$) (lit. ref. [25] $[\alpha]_D$ = -12.5 (*c* 7.2, CH_2Cl_2) for (*S*)-**7a**); ^{1}H NMR (400 MHz, $CDCl_3$)[25] δ 7.44 – 7.34 (m, 2H, aromatic hydrogens), 7.32 – 7.22 (m, 3H, aromatic hydrogen), 3.70 -3.58 (m, 2H, CH_2OH), 2.54 – 2.38 (m, 2H, CH_2C≡C), 2.09 – 1.92 (m, 1H, $CHCH_3$), 1.07 (d, *J* = 6.8 Hz, 3H, $CHCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$)[25] δ 131.6, 128.2, 127.7, 123.9, 88.3, 82.0, 67.0, 35.4, 23.3, 16.3; GC-MS (EI) t_r = 19.6 min, *m/z* 174 (M^+, 20), 159 (25), 128 (35), 115 (100). HPLC: t_r (*R*)-**7a** = 16.9 min, t_r (*S*)- **7a** = 20.7 min. The same reaction was repeated with OYE1 and OYE2 to establish the enantiomeric excess of the final reduced alcohol **7a.**

(*S*)-2-Methyl-5-phenylpentan-1-ol ((*S*)-11)

Alcohol (–)-**7a** (20.0 mg,0.11 mmol) was reduced with H_2 in the presence of Pd/C to afford (*S*)-**11** (17.0 mg, 87%): ee = 90 % (HPLC), $[\alpha]_D$ = – 9.8 (*c* 1.4, EtOH) [lit. ref.[16] $[\alpha]_D$ = + 10.9, *c* 3.0, EtOH) for (*R*)-**11** ee = 95%)]; ^{1}H NMR (400 MHz, $CDCl_3$)[16] δ 7.30 – 7.22 (m, 2H, aromatic hydrogens), 7.20 – 7.12 (m, 3H, aromatic hydrogens), 3.48 (dd, *J* = 10.5 and 5.8 Hz, *CH*HOH), 3.40 (dd, *J* = 10.5 and 6.5 Hz, *CH*HOH), 2.68 – 2.52 (m, 2H, PhCH_2), 1.80 -1.52 (m, 3H, CH_2 and *CH*), 1.50 -1.40 (m, 1H, *CH*H-CH), 1.50 -1.40 (m, 1H, *CH*H-CH), 0.92 (d, *J* = 6.7 Hz, 3H, $CHCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$)[16] δ 142.7, 128.5, 128.4, 125.8, 68.3, 36.3, 35.8, 32.9, 29.0, 16.6; GC-MS (EI) t_r = 16.2 min, *m/z* 178 (M^+, 3), 160 (27), 104 (100). HPLC:[16] t_R (*S*)-**11** = 18.1 min, t_R (*R*)-**11**= 20.8 min.

Dec-4-ynal (4b)

From aldehyde **1b** (50 mg, 0.33 mmol), by reaction with OYE1, aldehyde **4b** (38.5 mg, 76%) was obtained: ^{1}H NMR (400 MHz, $CDCl_3$)[26] δ 9.79 (t, *J* = 1.2 Hz, 1H, *CHO*, 2.65 – 2.56 (m, 2H, CH_2CHO), 2.52 – 2.44 (m, 2H, CH_2C≡C), 2.12 (tt, *J* = 7.1 and 2.4 Hz, 2H, CH_2C≡C), 1.55 – 1.40 (m, 2H, CH_2), 1.40 – 1.28 (m, 4H, 2CH_2), 0.90 (t, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$)[26] δ 201.2, 81.8, 77.8, 43.1, 31.2, 28.7, 22.3, 18.8, 14.1, 12.3; GC-MS (EI) t_r = 12.3 min, *m/z* 151 (M^+ - 1, 1), 123 (7), 109 (28), 95 (100).

Dec-4-yn-1-ol (6b)

From aldehyde **1b** (50 mg, 0.33 mmol), by reaction with OYE1 and EVO200, alcohol **6b** (39.1 mg, 77%) was obtained: ^{1}H NMR (400 MHz, $CDCl_3$)[27] δ 3.76 (t, *J* = 6.1 Hz, 2H, CH_2OH), 2.28 (tt, *J* = 6.9 and 2.4 Hz, CH_2C≡C), 2.13 (tt, *J* = 7.1 and 2.4 Hz, CH_2C≡C), 1.74 (quintuplet, *J* = 7.0 Hz, 2H, $CH_2CH_2CH_2$), 1.52 – 1.43 (2H, m, CH_2), 1.40 – 1.28 (4H, m, 2CH_2), 0.90 (t, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$)[27] δ 81.3, 79.4, 62.3, 31.8, 31.2, 28.9, 22.4, 18.9, 15.6, 14.1; GC-MS (EI) t_r = 13.9 min, *m/z* 153 (M^+ - 1, 1), 111 (12), 97 (70), 79 (100).

2-Methyldec-4-ynal (5b)

From aldehyde **2b** (50 mg, 0.30 mmol), by reaction with OYE1, aldehyde **5b** (32.4 mg, 65%) was obtained: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.70 (d, *J* = 0.9 Hz, 1H, *CHO*), 2.54 – 2.42 (m, 2H, *CH*CHO + *CH*HC≡C), 2.41 – 2.31 (m, 1H, *CH*HC≡C), 2.12 (tt, *J* = 7.1 and 2.4 Hz, 2H, CH_2C≡C), 1.51 – 1.41 (m, 2H, CH_2), 1.37 – 1.27 (m, 4H, 2CH_2), 1.19 (d, *J* = 6.8 Hz, 3H, $CHCH_3$), 0.89 (t, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.9, 82.8, 76.4, 45.7, 31.2, 28.7, 22.3, 20.5, 18.8, 14.1, 13.2; GC-MS (EI) t_r = 13.4 min, *m/z* 165 (M^+ - 1, 5), 151 (20), 123 (25), 109 (100), 95 (100).

(*S*)-2-Methyldec-4-yn-1-ol (*S*)-7b

From aldehyde **2b** (50 mg, 0.30 mmol), by reaction with OYE1 and EVO200, alcohol **7b** (37.3 mg, 74%) was obtained: $[\alpha]_D$ = – 3.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.55 -3.45 (m, 2H, CH_2OH), 2.15 – 2.11 (m, 2H, CH_2C≡C), 2.11 – 2.03 (m, 2H, CH_2C≡C), 1.84 – 1.72 (m, 1H, $CHCH_3$), 1.49 – 1.36 (m, 2H, CH_2), 1.34 – 1.20 (m, 4H, 2CH_2), 0.91 (d, *J* = 6.8 Hz, 3H, $CHCH_3$) 0.83 (t, *J* = 7.0 Hz, 3H, CH_3CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 82.1, 78.1, 67.5, 35.5, 31.2, 28.9, 22.9, 22.3, 18.8, 16.4, 14.1; GC-MS (EI) t_r = 14.7 min, *m/z* 168 (M^+, 1), 111 (89), 97 (92), 79 (100). Compound (–)-**7b** (25.0 mg, 0.15 mmol) was hydrogenated with molecular H_2 in the presence of Pd/C to afford (*S*)-(–)-**12** (22.7 mg, 88%): $[\alpha]_D$ = – 7.9 (*c* 0.8, $CHCl_3$), [lit. ref. [17] $[\alpha]_D$ = + 8.7, *c* 2.5, $CHCl_3$) for (*R*)-**12**]; ^{1}H NMR (400 MHz, $CDCl_3$)[28] δ 3.51 (dd, *J* = 10.5, 5.8 Hz, 1H, *CH*HOH), 3.41 (dd, *J* = 10.5, 6.5 Hz, 1H, *CH*HOH), 1.65 – 1.55 (1H, m, $CHCH_3$), 1.45 – 1.20 (m, 14H, 7CH_2), 0.95 – 0.80 (d+t, *J* = 6.7 and 7.0 Hz, 6H, 2CH_3); ^{13}C NMR (101 MHz, $CDCl_3$)[28] δ 68.6, 35.9, 33.3, 32.0, 30.1, 29.8, 29.5, 27.1, 22.8, 16.7, 14.2; GC-MS (EI) t_r = 14.8 min, *m/z* 154 (M^+ - 18, 2), 111 (20), 85 (45), 57 (100).

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Ene-Reductases for Organic Synthesis Applications: Chemoselective Hydrogenation of Alkenes in the Presence of Alkynes for the Homologation of 2-Alkynals into 4-Alkynals/Alkynols

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