Introduction of Chiral Centers to $\alpha$- and/or $\beta$-Positions of Carbonyl Groups by Biocatalytic Asymmetric Reduction of $\alpha,\beta$-Unsaturated Carbonyl Compounds

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Abstract
Biocatalytic asymmetric reductions of acyclic and cyclic $\alpha,\beta$-unsaturated carbonyl compounds are favorable protocols for introduction of chiral centers to $\alpha$- and/or $\beta$-positions of the carbonyl groups. Representative biocatalytic reductions of electron deficient olefins are compiled from a synthetic point of view according to compound types from the papers in 2012 to early 2022. Applications to syntheses of some enantiomerically enriched perfumery ingredients are presented to show the feasibility of the biocatalytic reductions.

Keywords
asymmetric conjugate reduction, $\alpha,\beta$-unsaturated carbonyl compound, biocatalytic reduction, ene reductase, perfumery ingredient

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Introduction of chiral centers to organic molecules is an important task, especially for total syntheses of physiologically active compounds. Carbonyl groups have been utilized to introduce chiral centers at the $\alpha$- or $\beta$-position by asymmetric nucleophilic reactions of enolates,\(^1\) asymmetric protonation\(^2\),\(^3\) or asymmetric conjugate additions to $\alpha,\beta$-unsaturated carbonyl compounds (Figure 1).\(^4\),\(^5\) Catalytic asymmetric hydrogenation of a double bond employing chiral organometallic catalysts has an alternative advantage, especially in the reduction of $\alpha,\beta$-unsaturated carbonyl compounds, which play a major role not only on a laboratory scale but also on a process scale to introduce up to two stereogenic centers in a single step.\(^6\) Compared to other asymmetric inductions, the catalytic reductions do not require any extra reagents other than catalysts, which is superior in atom economy, benign and sustainable, with less waste. A simple synthetic operation is an extra advantage.

In addition to the catalytic asymmetric hydrogenation to introduce chiral centers at the $\alpha$- and/or $\beta$-positions of carbonyl groups, biocatalytic asymmetric reductions of $\alpha,\beta$-unsaturated carbonyl compounds have attracted more and more attention employing enzymes or whole cells, which proceed in aqueous solvents under neutral conditions at room temperature under atmospheric pressure. The reaction condition is benign and environmentally friendly. An additional advantage is that there is no contamination of heavy metals in the products and wastes, which is different from catalytic hydrogenations. Chemoselectivity of the reduction is high, because the reaction proceeds only with electron-deficient olefins. Isolated double bonds, carbonyl, formyl, nitro, and cyano groups, and benzyl ethers are left intact under the reaction conditions, which could be reduced by catalytic hydrogenations. Although selective reduction of a double bond of an $\alpha,\beta$-unsaturated carbonyl compound in the presence of an isolated double bond could be carried out chemoselectively by electron transfer reduction with alkaline metals, the reaction is not suitable for chiral induction.

These characteristic features of biocatalytic reductions are favorable for syntheses of physiologically active compounds including not only flavor and fragrance substances,\(^7\) but also pharmaceutical ingredients.\(^8\) The quality of perfumery ingredients highly depends on the absolute stereochemistries, their enantiomeric purities, and on the character of very small amounts of contaminants. The

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This paper is dedicated to Professor Dr DHC Yoshinori Asakawa on the occasion on his 80th birthday.

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benign nature of biocatalytic reduction might be suitable to apply the flavor substances to human bodies. Some representative flavor and fragrance substances having chiral centers at α- or β-positions of carbonyl groups are highlighted in Figure 2.

A great deal of research data has been accumulated on the biocatalytic asymmetric reduction of electron-deficient olefins,9–14 and during preparation of this manuscript, a comprehensive review on the topic by Hollmann, Opperman and Paul has appeared.13 Synthetic organic chemists are familiar with reagent-based reductions, in spite of the big advantage of biocatalytic reduction. In this regard, the present review deals with developments in enantioselective biocatalytic reduction of electron-deficient olefins to emphasize the synthetic utility of biocatalytic reduction, in which more specific examples of bioreductions and applications to the syntheses of some perfumery ingredients and physiologically active small molecules are listed to exemplify the further feasibility of bioreduction in organic synthesis.

Among recent progress in biocatalytic asymmetric reductions, old yellow enzymes (OYE: NADPH dehydrogenase, EC 1.6.99.1) have been studied to reduce electron-deficient olefins, such as α,β-unsaturated carbonyl compounds. A variety of OYEs are found in a variety of plants such as tomatoes and microorganisms, which are relatively stable and available on a large scale. Their structures are well established by X-ray crystallography.15,16 With the development of genetic engineering,17 variants by genetic modification and homologues by cloning have been created to elucidate the reaction mechanism, to expand the scope of applications, and to improve the reaction conditions.

The mechanism of the reduction by OYE is considered as shown in Figure 3.9,18–20 The substrate taken up in the enzyme is reduced in trans-fashion by 1,4-addition of the hydride by the flavin residue present in the protein, which is in contrast to the cis-type addition in the catalytic reduction in the presence of metal catalyst under a hydrogen atmosphere. The oxidized flavin residue is reduced by a cofactor NADPH, which starts the reduction cycle again. The resulting NADP⁺ is reduced back to NADPH by a combination of glucose and glucose dehydrogenase to turn the catalytic cycle. A combination of glucose-6-phosphate and glucose-6-phosphate dehydrogenase, formate and formate dehydrogenase, 2-propanol and alcohol dehydrogenase,

Figure 1. Introduction of chiral centers at α- and/or β-positions of carbonyl groups.
or phosphite and phosphite dehydrogenase can also be used in this cofactor recycling process.

**Biocatalytic Reductions of Cyclic α, β-Unsaturated Ketones**

Cyclic ketones having chiral centers at α- and/or β-positions of ketones are important chiral building blocks for total syntheses of natural products. Some representative results to introduce chiral centers by bioreductions at β-positions of β-substituted cyclic enones are shown in entries 1~8, Table 1. In entry 1, whole cells of *E. coli* BL21(DE3)(pOYE-pET3b) were employed to reduce 3-methylcyclohexenone in 100% conversion with 96% enantioselectivity (ee) without adding NADP⁺ and GDH. Cyclohexenones having larger substituents also resulted in high enantioselectivity, but in lower yield. β-Cyclodextrin was added to promote solubility of the substrate.

![Figure 2](image)

**Figure 2.** Some representative flavor ingredients having chiral centers at α- and/or β-positions of carbonyl groups and their related derivatives.
in aqueous media. In entry 2, glucose was added to maintain the strain. In entry 3, OYE homologue, YqjM, enabled the reduction of cyclohexenone having a longer substituent. In entries 4 and 5, both enantiomers were obtained by changing ene reductases, and also in 6 and 7 by changing YqjM mutants. (R)-(-)-Muscone (2) is a precious perfumery component, as well as a medicinal ingredient to activate the cardiovascular system. Entry 8 shows that the ene reductase from *S. salmonicolor* TPU 2001 furnished unnatural (S)-(+) -muscone in 95% yield and 100% ee without adding glucose and GDH.25 The catalyst was specific for the reduction of (E)-3-methyl-2-cyclopentadecenone, but not active for the (Z)-enone, along with enones of smaller ring size. Development of a new catalytic system to provide the precious natural (R)-(−)-muscone (2) is expected. The synthetic pathway leading to (E)-enone is shown in Figure 4, which involves intramolecular cyclization in vapor phase in the process scale.42 The enzyme was compatible for reduction of either (E)-2-cyclohexeneone or (E)-2-cyclopentadecenone.

Some examples of asymmetric bioreductions of α-substituted cyclic enones to introduce chiral centers at α-positions are presented in entries 9–19. The same reaction condition in entry 1 was applicable to the reduction of 2-methylcyclohexeneone in entry 9, with the same efficiency. The same enone was reduced by 90 kDa reductase from *Nicotiana tabacum* plant culture in entry 10, while the opposite enantiomer was obtained in entry 12 by 44 kDa reductase from the same plant. In entry 11, the bioreduction was accomplished without addition of cofactor, NADH, in which photo-excited rose bengal reduced the oxidized OYE to turn the catalytic cycle. The oxidized rose bengal was driven back to rose bengal by triethanolamine (TEOA). The process is clean, cost effective and favorable for large scale preparation. In entry 13, the same reaction conditions to reduce 3-methylcyclohexeneone in entry 1 was applied to the reduction of 2-methylcyclohexeneone. The same (R)-2-methylcyclohexanone was obtained from 2-methylencyclohexanone in entry 14. Similarly, 2-methylcyclopentenone was reduced by CER and OPR1 in entries 14 and 15. The Baylis–Hillman substrate was reduced by OYE 2.6 in entry 16. Among various mutants investigated, the wild type mutant exhibited the best performance. In entry 18 and 19, the bioreductions were carried out without employing the GDH recycling system, in which the reduction of entry 18 was scaled up to 400 mg of the substrate.

Bioreduction of ketoisophorone (oxophorone) (17) has been the most widely investigated, as shown in entries 20–26 as a model reaction to test new reduction conditions. The product, levodione (18), has been employed for the synthesis of carotenoids as a key starting material.43–45 In entries 20 and 21, OPR1 and CER were applicable not only to the bioreduction of cyclic enones (entries 5, 15 and 16), but also ketoisophorone. Entry 22 shows cofactor free bioreduction using whole cells of recombinant *S. cerevisiae* BY4741ΔOye2. The cofactor free process is cost effective and might be useful for large-scale reduction. In entry 23, an alternative

![Figure 3. Representative OYE catalyzed reduction of electron deficient olefins by NADPH recycling with glucose. EWG = electron-withdrawing group. GDH = Glucose dehydrogenase.](Image)
| Entry | Substrate | Product | Enzyme | Reaction conditions | Yields | Ref. |
|-------|-----------|---------|--------|---------------------|--------|------|
| 1     |           |         | Whole cells of *E. coli* BL21(DE3)(pOYE-pET3b) | Ampicillin, IPTG, β-cyclodextrin | 100% conv., 96% ee | 21   |
| 2     |           |         | OYE from *Candida sake* DBVPG6162 | pH 7.0, glucose, EtOH | 75%, 98% ee | 22   |
| 3     |           |         | YqiM mutant C26D/I69T | NADP⁺, GDH, glucose | 92%, 99% ee | 23   |
| 4     |           |         | YqiM C26G | | 100% conv., 96% ee | 23   |
| 5     |           |         | OPR1 | DMSO or EtOH, GDH, NADH, pH 7.5 | 99% conv., 99% ee | 24   |
| 6     |           |         | YqiM wild type | NADP⁺, GDH, glucose | 100% conv., 93% ee | 23   |
| 7     |           |         | YqiM C26G | | 100% conv., 96% ee | 23   |
| 8     |           |         | Reductase from *Sporidiobolus salmonicolor* TPU 2001 (S)-Muscone (16) | pH 7.0, DMSO, NADPH | 95%, 100% ee | 25   |
| 9     |           |         | Whole cells of *E. coli* BL21(DE3)(pOYE-pET3b) | Ampicillin, IPTG, β-cyclodextrin | 100%, 94% ee | 21   |
| 10    |           |         | 90 kDa reductase from *Nocentiana tabacum* | NADPH, pH 8.0, 2-mercaptoethanol, dithiothreitol | 95% conv., 99% ee | 26   |
| 11    |           |         | YqiM | Rose bengal, TEOA, CaCl₂, white LED, pH 7.5 | 84%, 99% ee | 27   |
| 12    |           |         | 44 kDa reductase from *N. tabacum*, | NADPH, pH 8.0, 2-mercaptoethanol, dithiothreitol | 80% conv., >99% ee | 26   |
| 13    |           |         | Whole cells of *E. coli* BL21(DE3)(pOYE-pET3b) | Ampicillin, IPTG, β-cyclodextrin | 100% conv., 96% ee | 21   |
| 14    |           |         | 74 kDa reductase from *N. tabacum* | NADPH, pH 8.0, 2-mercaptoethanol, dithiothreitol | 99% conv., 97% ee | 26   |
| 15    |           |         | Purified CER | GDH, glucose, NADP⁺, pH 6.0 | >99% conv., 93% ee | 28   |
Table 1. Continued

| Entry | Substrate | Product | Enzyme | Reaction conditions | Yields | Ref. |
|-------|-----------|---------|--------|---------------------|--------|------|
| 16    |           |         | OPR1   | NAD⁺, GDH, glucose  | 69%, 72% ee | 29   |
| 17    |           | Purified OYE 2.6 wild type | pH 7.0, NADP⁺, glucose, GDH | >99% >98% ee | 30   |
| 18    | OYE1      |        | NADH, tris-HCl buffer | 69% conv., 94% ee | 31   |
| 19    | XeNA      |        | DMSO or EtOH, NADH, pH 7.5 | 99%, 99% ee | 24   |
| 20    | OPR1      |        | NADP⁺, G6PDH, glucose-6-phosphate | 95%, 91% ee | 29   |
| 21    | Crude CER | GDH, glucose, NADP⁺, pH 6.0 | >99% conv., 96% ee | 28   |
| 22    | Recombinant whole cells from \textit{Saccharomyces cerevisiae} BY4741ΔOye2 | Glucose, pH 7.0, EtOH | 89%, 80% ee | 22   |
| 23    | Progesterone 5β-reductase (At5β-24StR) from \textit{Arabidopsis thaliana} | GDH, glucose, NADPH, pH 7.5 | 99%, 88% ee | 32   |
| 24    | NCR       | Degased tris-HCl buffer pH 7.0, under argon | 98%, 88% ee | 33   |
| 25    | YqjM      | FMN, methylviologen, CdSe quantum dots, TEOA, Xe lamp (410-520 nm) | No yield, 64% ee | 34   |
| 26    | TiOYE     | Au-TiO₂, FMN, Xe-lamp (200 W), 50 °C, pH 7.0 | 66% conv., 86% ee | 35   |
| 27    | Purified CER | GDH, glucose, NADP⁺, pH 6.0 | 99% conv., 93% de | 28   |
| 28    | LaeER from \textit{Lactobacillus casei} | GDH, glucose, NADP⁺, pH 7.0, DMSO | 99%, 98% de | 36   |
nicotinamide-independent bioreductase, progesterone 5β-reductase, was employed. In entry 24, an equivalent amount of N-Boc-pyrrolidinone was used as a hydrogen donor under anaerobic conditions to prevent generation of H₂O₂, which may cause epoxidation of the double bond of the substrate. N-Boc-pyrrolidinone was favorable, because the co-product, 3-hydroxypyrole, may not inhibit the activity of the catalyst.

In entry 25, CdSe quantum dots, methylviologen and triethanolamine (TEOA) were used to reduce cofactor flavin mononucleotide (FMN) under visible light irradiation, which was followed by reduction of ketoisophorone with YqjM. Although a variety of attempts have been reported on the bioreduction of ketoisophorone, only (R)-levodione was obtained by ene reductase reduction. In entry 26, the bioreduction was carried out without adding NADP⁺, in which ene reductase was regenerated via a photocatalytic oxidation cycle of water employing titanium dioxide based photocatalyst. FMN plays a dual role as an enzyme prosthetic group and a redox mediator delivering electrons from the photocatalyst to the enzyme. Medium enantioselectivity might be the result of asymmetric hydrogenation by H₂ generated by the oxidation of water.

Dihydrocarvone (1) is a very versatile chiral building block for natural product syntheses, because it has a six-membered ring, two chiral centers and two functional groups for further transformations. Chemo- and diastereoselective bioreductions of the enone moiety of carvone are listed in entries 27∼32, in which both enantiomers were obtained. Although selective reduction of an enone moiety in the presence of either a non-conjugated double bond or ketone is usually carried out by an electron transfer process to avoid over-reduction of such substituents, stereoselectivity of the process is not always satisfactory. Compared to such a process, purified ClER was active in reducing both (R)- and (S)-carvones to corresponding dihydrocarvones in entries 27 and 31, with high efficiency. LacER from Lactobacillus casei and NostocER1 from the cyanobacterium, Nostoc sp. PCC 7120 in entry 29 provided (2R,5R)-dihydrocarvone (1) efficiently. Biphasic bioreduction in the presence of hydrophobic ionic liquid, [BMIM][PF₆] 10% (v/v) or resin XAD4 (AR), pH 6.3 suppressed a host cell-mediated epimerization of (2R,5R)-dihydrocarvone (1) in entry 30 and enabled large scale bioreduction on a liter scale. No further reduction of the carbonyl group occurred. Heterogeneous reduction on resin XAD4 (AR) was also effective, resulting in a shorter time scale. Progesterone was reduced stereoselectively in entry 32.

Biocatalytic Reduction of Acyclic α, β-Unsaturated Ketones

In entry 1, Table 2, bioreduction by microorganism, S. cerevisiae, was carried out by adsorbing the substrate in filter paper. On the other hand, the opposite enantiomer was obtained by the cascade reaction in entry 1, Table 2, which was applied to the total synthesis of (S)-tropinal® (6) in Figure 5. Acyclic Baylis-Hillman product was reduced by YqjM variant in entry 2. Bioreduction of doubly activated double bonds in entries 3 and 4 proceeded enantioselectively
to afford both enantiomers according to the geometry of the double bonds of the substrates. Phosphonate group was also effective as a double activating group in entry 5. Enantiomerically pure α-haloketone is difficult to prepare owing to higher acidity of the α-proton. In entry 6, α-bromoketone was obtained, in which enantioselectivity could not be determined due to isomerization. However, enantioselectivity was maintained in the case that the present process was combined to cascade process, in which subsequent reduction of the carbonyl group in one pot operation led to bromohydrin in entry 4, Table 2.

**Biocatalytic Reductions of α,β-Unsaturated Aldehydes**

Some aldehydes of low molecular weight are versatile fragrance components due to their volatility. Introduction of chiral centers
Table 3. Biocatalytic Reductions of α,β-Unsaturated Aldehydes.

| Entry | Substrate | Product | Enzyme | Reaction conditions | Yields | Ref. |
|-------|-----------|---------|--------|---------------------|--------|-----|
| 1     | Citral(23) | (R)-Citronellal(4) | FMN-binding protein from *Pyrococcus horikoshii* (PhENR) | Tris-HCl buffer, NADH or NADPH | 19% conv., 96% ee | 16 |
| 2     | Progesterone | 5β-reductase (At5β-StR) from *A. thaliana* | pH 7.5, NADPH | 99% conv., 99% ee | 32 |
| 3     | OPR1 | NADH or NADPH | Degassed Tris-HCl buffer, pH 7.0 | 99% conv., 99% ee | 29 |
| 4     | NCR | PETNR342C | (1 eq) [Ru(bpy)2(Clcbpy)]Cl2, [MV2+]Cl2, pH 8.0, NADP+, G6PDH, O2 free, glucose-6-phosphate, pH 8.0, n-octanol, isoctane, hr 150 W | 65%, 85% ee | 51 |
| 5     | OYE3 | XAD 1180 resin, Et2O Evaporation GDH, NADP+, pH 7.0 | 100%, 99% ee | 52 |
| 6     | OYE3 | pH 7.5, t-BuOMe | 32% conv., >95% ee | 53 |
| 7     | (S)-Lilial® | (S)-Tropional®, (Helional) (6) | pH 7.5, t-BuOMe | 99%, >95% ee | 53 |

Figure 5. Synthesis of (R)-tropional (6), a flavor of lily of the valley.47.
at α- or β-position of a formyl group is not well exploited because of inherent sensitive nature of a formyl group toward various chemical transformations. An asymmetric catalytic hydrogenation is not feasible due to the possibility of reduction of the formyl group into primary alcohol. Citronellal (4) in Figure 2 is a very important perfumery ingredient and a useful synthon to install a chiral secondary methyl group distal to a functional group in natural product syntheses. Reduction of citral with PhENR
provided natural citronellal (4) in high enantioselectivity albeit in low yield in entry 1, Table 3. On the other hand, its antipode was obtained in high yield with high enantioselectivity in entry 2 by progesterone reductase. In entry 3, isoenzyme OPR obtained from tomato was also effective for the reduction. The α,β-unsaturated aldehydes having a substituent at α-position were successfully reduced in entries 5~8. In entry 5, by employing [Ru(bpy)3]2+(Clbpy)Cl2 as a photosensitizer and methyl viologen dichloride ([MV2]Cl2) as an electron transfer mediator, light-driven biocatalytic reduction was carried out to realize regeneration of costly redox coenzymes in 100 turnover frequency with 65% yield and 85% ee, which might be useful for process scale reduction. Electron rich unsaturated aldehyde was reduced by OYE3 in gram scale in entry 6 by impregnation of the aldehyde on XAD 1180 resin with Et3O. Subsequent oxidation of the formyl group led to a key precursor of anti-diabetic drugs of the PPAR-α/g agonists such as tesaglitazar. Two olfactory principles of the lily-of-the-valley, lilial® (5) and tropional® (6), were obtained in entries 7 and 8. Slow reaction rate in buffer increased by carrying out the bioreduction in a biphasic system.

Biocatalytic Reductions of α,β-Unsaturated Acids, Esters and Lactones

Unsaturated acids and esters, especially those having extra electron- withdrawing groups (EWGs), are suitable for bioreduction, in which the EWG groups make the reduction easier by both lowering the barrier and stabilizing the product so shifting the equilibrium compared to substrates with electron donating groups (Table 4).

Regio- and asymmetric reduction of (E)-β-cyanoacrylic acid is depicted in entry 1, in which the presence of γ,δ-unsaturation was vital for substrate acceptance. The (Z)-double bond isomer was not reduced. Catalytic hydrogenation of the product led to the γ,γ′-aminoester (5)-pregabalin (25), an analogue of the neurotransmitter GABA (Figure 6).

In entry 2, by employing an oxidized OYE, XenA, the unsaturated acid, led to (R)-naproxen, an antipode of the anti-inflammatory drug, (S)-naproxen. Development to afford the pharmacologically important (S)-isomer is expected.

Electron deficient (E)-β-cyanoacrylate was reduced by OYE3 in entry 3. Bioreduction of the corresponding (Z)-isomer was less efficient. The present protocol was applied to the synthesis of a GABA analogue (26), after hydride reduction and protection, in one pot (Figure 7).

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When Trp in position 116 in OYE1 was replaced by Leu, the reduction of β-cyanoacrylate provided (R)-3-cyano-3-phenylpropanoate (27) Installed for the synthesis of a GABA analogue (26). When Trp in position 116 in OYE1 was replaced by Leu, the reduction of β-cyanoacrylate provided (R)-3-cyano-3-phenylpropanoate in entry 4; replacement with Ala led to the (S)-enantiomer in entry 5. Similarly, both enantiomers were obtained from Baylis-Hillman product by two alternative YqM variants in entries 6 and 7. In entries 8 and 9, (Z)- and (E)-2-methylsuccinate furnished by the same enzyme (R)- and (S)-enantiomers, respectively. The isolated double bond was stable under the same reaction conditions in entry 10. It is worthy of note that both (Z)- and (E)-α,bromocrotonate were reduced by OYE3 quite efficiently in entry 11 to provide (Z)-2-bromobutanoate, which is difficult to prepare by other means, such as catalytic hydrogenation, owing to the labile nature of the C–Br bond toward hydrogenation, along with easy racemization. α-Chlorocrotonate was also reduced by the same enzyme to afford the (S)-enantiomer highly efficiently. In spite of the absence of an electron-withdrawing group, β-substituted butenolides were reduced in entry 12. Both enantiomers of β-methyl-β-valerolactone were prepared in entries 13 and 14, which is superior than transition metal catalyzed asymmetric reduction. Scale-up synthesis of the (R)-enantiomer by OYE2 in entry 13 was accomplished in a 1.3 L batch reactor to result in a final concentration of 1.0 g L−1.

Cascade Reductive Biotransformations

Synthesis by multiple biotransformations in a one pot operation is very favorable in terms of pot economy. Several efforts have been reported by either combining two different enzymes, or a chemical reaction and enzyme.

Entry 1 in Table 5 shows that reduction of an acetoxy-enone provided (R)-α-methylketone in 63% and 96% ee in a 5 mmol scale. The reduction was carried out by adsorption of the substrate on filter paper. The product was applied to the synthesis of (R)-tropional® (6), a flavor of lily of the valley, which is an important fragrance ingredient (Figure 5). Haloform reaction of the product, followed by treatment with chloroformate, led to a mixed anhydride, which was reduced to primary alcohol, and subsequent TEMPO oxidation furnished (R)-tropional® (6) in 49% overall yield and 94% ee. The authors proposed that the initial reaction proceeded via S_{2}′ elimination of the acetoxy group by the attack of the hydride from flavin mononucleotide to provide α-methylene ketone, which was reduced to give (R)-methylketone. It is interesting to note that the opposite (S)-enantiomer was obtained under the same enzymatic reaction conditions in entry 1, Table 2, when the substrate without the acetoxy group was employed.

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![Figure 6](image6.png)

![Figure 7](image7.png)

**Figure 6.** Synthesis of (5)-pregabalin (25).**

**Figure 7.** Synthesis of γ,γ′-aminoester (26).**
| Entry | Substrate | Product | Enzyme | Reaction conditions | Yields | Ref. |
|-------|-----------|---------|--------|---------------------|--------|------|
| 1     | S. cerevisiae type II | H₂O | The substrate was adsorbed on filter paper. | 63%, 96% ee | 47 |
| 2     | 1. YqM, GDH 2. Prelog-ADH | glucose, NADP⁺, pH 7.0 | 75%, >99% ee | 66 |
| 3     | 1. YqM, glucose GDH, 2. ADH₁K | NADP⁺, pH 7.0 | 85%, >99% ee | 66 |
| 4     | OYE3 from Saccharomyces cerevisiae, ADH EVO030 (6:1) | DMSO, GDH, glucose, NAD⁺, NADP⁺, pH 7.0 | 99% conv., 99% syn | 50 |
| 5     | Mutant of Candida macedoniensis old yellow enzyme (CmOYE) | DMSO, GDH, glucose, NAD⁺, NADP⁺, pH 7.0 | 90% ee, unknown | 67 |
| 6     | NiDBR, MMR | GDH, glucose, NADP⁺, pH 7.0, 79%, de | 79%, de unknown | 68 |
| 7     | NiDBR, MNMR | GDH, glucose, NADP⁺, pH 7.0, 90%, de | 90%, de unknown | 68 |
| 8     | Recombinant S. cerevisiae BY4741ΔOye2 | pH 7.0, EtOH | 100%, 98% ee | 22 |
| 9     | Baker’s yeast | D-glucose, EtOH | 49%, 97% ee | 69 |
| 10    | 1. OYE3 2. (S)-ω-transaminase ATA-113 | 1. DMSO, GDH, glucose, pH 7.0, NADP⁺ 2. i-Pr₂N, PLP | >99% conv., 96% ee | 70 |
| 11    | 1. OYE3 2. (R)-ω-transaminase ATA-251 | 1. DMSO, GDH, glucose, pH 7.0, NADP⁺ 2. i-Pr₂N, PLP | >99% conv., 96% ee | 70 |
| 12    | 1. OYE1 2. (S)-ω-transaminase ATA-237 | 1. DMSO, GDH, glucose, pH 7.0, NADP⁺ 2. i-Pr₂N, PLP | 78%, >99% ee | 70 |
| 13    | CgeAlcOx, OYE2, horseradish peroxidase, catalase | glucose, NADP⁺, pH 8.0, 1% acetone | 95% conv., 99% ee | 71 |

(Continued)
In entries 2 and 3, after confirming the reduction of enone by the ene reductase, alcohol dehydrogenase was added sequentially in one pot to reduce the carbonyl group diastereoselectively, in which alcohols having two chiral centers were obtained with high enantioselectivity. OYE3 and alcohol dehydrogenase were added sequentially to reduce the carbonyl group diastereoselectively, in which endogeneous carbonyl reductase subsequently reduced the aldehyde. The resulting syn-bromohydrin was transformed to the tetrahydrofuran (28), the most pleasant roasted meat flavor, via enzymatic hydrolysis of the benzoate by lipase in a one pot operation, followed by cyclization and S_{2}2 substitution (Figure 8).

(4R,6R)-Actinol (29) is an important doubly chiral building block for the synthesis of carotenoids such as xanthoxin, zeaxanthin, and related compounds.\textsuperscript{12} Reduction of ketiospororone (17) by the mutant of \textit{Candida macedoniensis} old yellow enzyme (CmOYE) in entry 5 afforded (4R,6R)-actinol (29) in 90% yield, in which the chemical yield was improved compared with the two-enzyme system employing CmOYE and \textit{Corynebacterium aquaticum} (6R)-levodione reductase (LVR). Menthols are known to be important precursors in the production of several pharmaceutical and commodity chemicals, such as fragrances, perfumes and flavors. Sequential diastereoselective reduction of pulegone (31) was carried out by combining ene reductase from \textit{Nicotiana tabacum} (NtDBR) and (−) menthone: (+)-neomenthol reductase from \textit{Mentha piperita}; MMR, (−)-menthone: (−)-menthol reductase from \textit{Mentha piperita}; NtDBR, ene reductase from \textit{Nicotiana tabacum}; PLP, pyridoxal-5′-phosphate.

Transamination was combined with enone reduction in entries 10~12. Two diastereomers were obtained independently by employing (5)-α- or (R)-α-transaminase in entries 10 and 11. Sequential addition of two enzymes was favorable for good conversion in entries 10 and 11, whereas mixing the two enzymes at one time was also effective in entry 12. In entry 13, a one-pot biocatalytic cascade from inexpensive geraniol (33) was carried out by combining a copper radical oxidase (GgrAlcOx) and OYE2 to afford (R)-citronellal (4) in 95% conversion with 96% ee. Alternatively, in entry 14, combination with GluER provided (S)-citronellal (24) in 95% conversion with 99% ee. In entry 15,
the resulting intermediary (S)-citronellal (24), after the ene reduction, was oxidized to (S)-citronelllic acid (32) with the co-existing aldehyde dehydrogenase in a one pot operation, in which NADPH was regenerated with aldehyde dehydrogenase to recycle the ene reductase. The process is called a hydrogen-borrowing cascade since it does not require a hydride source such as glucose in combination with glucose dehydrogenase, which makes the process cost effective. The oxidation process was effective also by separate oxidation of citronellal (24) to citronelllic acid (32) with comparably high efficiency. Similar one pot two-enzyme reduction-oxidation systems have been reported without adding a hydride source (entry 16). The process was also applicable to other α-substituted aldehydes. Combination of allylic C-H oxidation, followed by ene reduction was realized in entry 17. Cyclohexenecarboxylate was treated with BOU730 cells containing P450-BM3 mutant to promote allylic C–H oxidation, and subsequent allyl alcohol oxidation led to cyclohex-2-enone. Addition of BOU730 cells containing YgjM (R)-selective mutant in one pot enabled enone reduction in high yield and high enantioselectivity to provide β-methoxycarbonylcyclohexanone. A combination of chemical reaction and enzymatic reaction was reported in entry 18. A Rh-catalyzed diazocoupling reaction led to the (E)-succinate derivative, which was subjected to ene reduction without isolation after evaporation of dichloromethane to realize turnover number (TON) 440. The side product in diazocoupling, (Z)-alkene, was not reduced.
Conclusions

The introduction of chiral centers into the α- and/or β-positions of carbonyl compounds is an important task in organic synthesis, and a variety of methods are known, in which asymmetric alkylation or protonation at the α-position of the carbonyl group, catalytic hydrogenation, and conjugate addition to the β-position of an α,β-unsaturated carbonyl compound has been well investigated. Among them, the reduction of α,β-unsaturated carbonyl compounds using asymmetric organometallic catalysts has been widely studied and achieved in recent years, because the reaction proceeds cleanly and atom economically, does not require extra reagents, and produces less waste, and the operation is simple compared with other methods, which is a promising protocol for process scale asymmetric synthesis. In addition to the introduction of chiral centers by catalytic asymmetric hydrogenation, the biocatalytic asymmetric reduction of α,β-unsaturated carbonyl compounds is an alternative method that should be focused on.

In this review, the asymmetric biocatalytic reduction of α,β-unsaturated carbonyl compounds is summarized according to compound type from the viewpoint of synthetic organic chemistry. The progress in biocatalytic asymmetric reduction has been remarkable in recent years, and a great deal of research data have been accumulated. In particular, much effort has been devoted to the asymmetric reduction of electron-deficient olefins employing OYE s, and many remarkable studies have been reported, including the development of reaction conditions and the preparation of OYE variants by genetic engineering. Compared to the asymmetric catalytic hydrogenation with a chiral organometallic catalyst, only the electron-deficient olefins are reduced chemoselectively, in which isolated double bonds, formyl groups, ketones, benzyl ethers, etc remained intact under the reaction conditions. The reaction proceeds at ambient temperature under atmospheric pressure in aqueous solvent. There is no contamination of heavy metal in either the product or waste. These characteristic benign features are suitable for preparation of chiral building blocks as a starting material for natural products, syntheses of chiral fragrance compounds of low molecular weight, and for transformations of compounds with multifunctional groups, especially in the final stage of syntheses of pharmaceuticals. At the same time, various variants of OYE s have been prepared by genetic engineering and used either as purified enzymes or in whole cells, which led to the expansion of substrate scope and improvement of the reaction conditions. Development in the reduction using OYE s has made improvements in co-factor regeneration in various ways.

In spite of the abundance of research work on the biocatalytic reduction of α,β-unsaturated carbonyl compounds, there still remains some issues to be considered. Owing to the higher substrate specificity of the enzymes, diversity of substrates is limited. Only one enantiomer can be obtained by a specific enzyme, and only one enantiomer can be accepted by an enzyme. Substrates of higher molecular weight having more complex structures are difficult to be reduced. Asymmetric biocatalytic reduction of a tetra-substituted olefin is rare introducing two chiral centers at α- and β-positions of a carbonyl group at the same time. In a process scale reaction, recycling of the catalyst system and improvement of the turnover number are desired. In order to stabilize unstable enzymes and make them reusable, immobilization on solid or liquid support is suggested, which might be useful also for flow synthesis. Even though the reduction by enzymes is useful in organic synthesis, synthetic organic chemists are not used to isolate or bioengineer enzymes on a preparative scale. Utility of these enzymes like stock reagents is expected. How to introduce efficiently chiral centers into the α- and/or β-positions of carbonyl compounds is a long-lasting topic in synthetic organic chemistry. It is a desired challenge to improve these issues. Since biocatalytic reactions are basically sustainable and atom economical, their potential is high. Further progress in this area is expected.

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