

# Introduction of Plural Asymmetric Centers by a $\beta$ -Keto Ester Reductase

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A  $\beta$ -keto ester reductase from bakers' yeast catalyzes asymmetric reduction accompanied by simultaneous kinetic resolution of dynamic and static optically active centers. The enzyme can recognize plural chiral centers to produce useful chiral building blocks containing plural asymmetric carbons.

**Keywords:** Asymmetric synthesis / Enzyme / Reductase / Chiral  $\beta$ -hydroxy ester

A number of purified alcohol dehydrogenases are now commercially available and used widely for the reduction of ketones to obtain chiral alcohols. The optically active alcohols are employed as useful chiral starting materials for the syntheses of biologically active compounds. In most cases so far reported, however, the results have been confined to the reduction of simple prochiral ketones, and enzymes introduce only one asymmetric carbon at the reaction center of the substrate [1]. Development of the method to introduce more than one chiral center in one stage of the reaction is of great value for the manufacture of natural compounds having a number of chiral centers. Recently, we reported the isolation and characterization of four  $\beta$ -keto ester reductases from bakers' yeast [2-4]. One of them, named L-enzyme-1 (YKER-I, Yeast Keto Ester Reductase-I), has an excellent stereoselectivity in  $\beta$ -keto ester reduction. This enzyme preferentially utilizes NADPH as the coenzyme and reduces  $\beta$ -keto esters to give L(S)-hydroxy

esters exclusively [2,5].  $\alpha$ -Substituted  $\beta$ -keto esters are also reduced by this enzyme [3,5,6]. Since these compounds enolyze and racemize easily in aqueous solutions, the substrate remains racemic throughout the reaction. The reduction of these compounds with L-enzyme-1 affords only one stereoisomer out of four possible stereoisomers in high yield [3,5,6].

This report will describe a new system of asymmetric synthesis; asymmetric reduction accompanied by simultaneous kinetic resolution of dynamic and static optically active centers [7]. Dynamic optically active center is defined as an optically active center, the configuration of which is mobile due to certain reason such as enolization, non-stereoselective exchange, pseudo-rotation, and so on. Static optically active center is configurationally rigid optically active center. The purpose of this method is introduction of three chiral centers in one stage of the reaction. We chose asymmetric reduction of  $\alpha$ -alkyl- $\beta$ -keto ester of secondary alcohol, **1**, as a typical

## BIOORGANIC CHEMISTRY — Bioorganic Reaction Theory —

### Scope of research

Biochemical reactions are studied from the viewpoint of physical organic chemistry. Namely, the reaction mechanism and stereochemistry of NAD-dependent oxidoreductases are explored. Stereospecific redox transformations mediated by certain biocatalysts such as microbes, enzymes, cultured tissues are also studied. The results will be applied to develop new organic reactions.

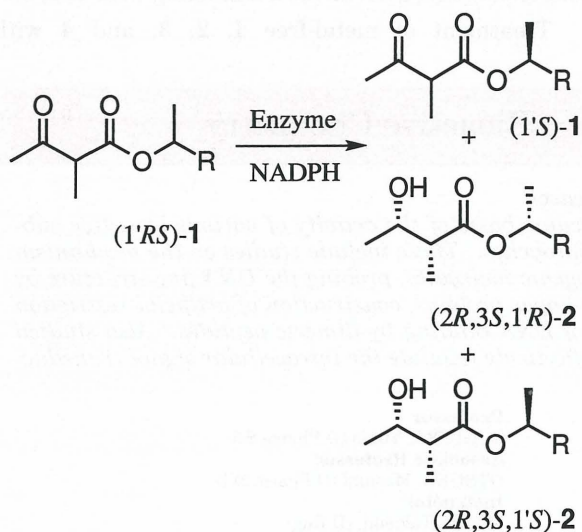


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example for this system. Microbial reduction of  $\beta$ -keto esters of secondary alcohols with unsatisfactory stereoselectivity has been reported [8].

Various keto esters, **1**, were subjected to the reduction by L-enzyme-1 and the results are summarized in Table 1. The reduction of keto ester **1** with L-enzyme-1 preferentially affords the (2*R*,3*S*,1'*R*)-hydroxy ester **2** in moderate to high diastereoselectivity. The reduced product was composed of two stereoisomers only, (2*R*,3*S*,1'*R*) and (2*R*,3*S*,1'*S*), in each case; no other six possible stereoisomers were detected. Thus, the enantiomeric excesses in the products are quantitative. The diastereoselectivity of this enzyme depends on the structure of alkoxy moiety. Steric bulk of the substituent plays an important part of the substrate recognition of this enzyme. Reduction of keto ester of acyclic aliphatic alcohol, **1a**, affords the corresponding hydroxy ester in low diastereoselectivity. Introduction of cyclic substituent such as phenyl or cyclohexyl, **1b** or **1k**, increases the diastereoselectivity. Further modification of the phenyl ring, **1c** - **1g**, however, does not alter the diastereoselectivity. A remarkable change in the diastereoselectivity can be seen in the esters having heteroatom(s) in the ring such as pyridyl or dithianyl, **1h** - **1j** and **1l**. Particularly, the reduction of dithianylethyl ester affords the (2*R*,3*S*,1'*R*)-hydroxy ester in more than 95 % purity out of possible eight stereoisomers. Starting from racemic compounds, this enzyme can produce hydroxy esters with three chiral centers in high stereoselectivity.



Scheme 1

In conclusion, introduction of three chiral centers in one stage of the reaction from racemic compounds was realized. The enzyme can recognize plural chiral centers to produce useful chiral building blocks containing plural asymmetric carbons.

Table 1. Stereoselectivity of Optical Resolution

	R	Yield %	d.e. <sup>a</sup> %	E <sup>b</sup>
a	Hex	33.9	65.6	6.5
b	Ph	44.6	73.6	13.3
c	2-Cl-Ph	34.6	69.7	8.2
d	2-Me-Ph	48.8	74.6	14.6
e	4-Cl-Ph	29.0	76.7	10.7
f	4-Me-Ph	39.6	74.6	12.2
g	4-NO <sub>2</sub> -Ph	36.1	80.4	15.9
h	2-Py	38.2	79.7	15.9
i	3-Py	43.0	86.3	29.0
j	4-Py	35.5	91.2	35.8
k	Cyclohexyl	34.5	77.6	12.2
l	1,3-Dithianyl	43.6	90.6	41.4

a: Ratio of (2*R*, 3*S*, 1'*R*) to (2*R*, 3*S*, 1'*S*).

b:  $E = [\text{Log}((1-ee(S))/(ee(S)+ee(P))) \times (1-ee(S))]/[\text{Log}((1-ee(S))/(ee(S)+ee(P))) \times (1+ee(S))]$ .

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