Highly enantioselective and efficient synthesis of methyl (*R*)-*o*-chloromandelate with recombinant *E. coli*: toward practical and green access to clopidogrel†

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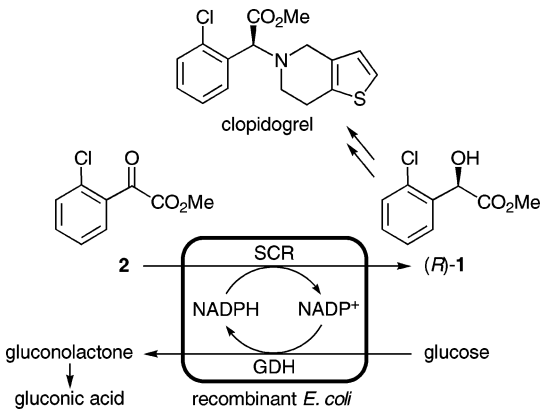
Methyl (*R*)-*o*-chloromandelate ((*R*)-1), which is an intermediate for a platelet aggregation inhibitor named clopidogrel, was obtained in >99% ee by the asymmetric reduction of methyl *o*-chlorobenzoylformate (2) (up to 1.0 M) with recombinant *E. coli* overproducing a versatile carbonyl reductase.

Clopidogrel is a platelet aggregation inhibitor widely administered to atherosclerotic patients with the risk of a heart attack or stroke caused by the formation of a clot in the blood. Worldwide sales of Plavix (clopidogrel bisulfate) amounted to $6.4 billion per year (data for the 12 months ending June 2006), which ranks second.[1] The key enantiomer, methyl (*R*)-*o*-chloromandelate ((*R*)-**1**), can be prepared from the corresponding (*R*)-carboxylic acid,[2] which can be obtained by the fractional crystallization of the racemic mixture. A more advanced method for obtaining (*R*)-**1** would be the direct asymmetric reduction of α-keto ester **2**. Surprisingly, only one report on the asymmetric reduction of **2** has been disclosed; Genet and co-workers have reported the Ru-catalyzed asymmetric hydrogenation of **2** to afford (*R*)-**1** with 50% ee at most.[3] Zhang and co-workers have reported the asymmetric hydrogenation of the corresponding ethyl ester with enantioselectivity of up to 76% ee.[4] No biotransformations of **2** to **1** have been reported. Although an alternative synthetic route to clopidogrel *via* the asymmetric hydrocyanation of *o*-chlorobenzaldehyde using *R*-selective hydroxynitrile lyases has been proposed,[5,6] the asymmetric reduction of **2** is much safer, more straightforward, and more advantageous because of the asymmetric induction in the step closer to the final product.

Our research has centered on versatile biocatalysts capable of showing high enantioselectivity and broad substrate specificity simultaneously. A carbonyl reductase called SCR showed catalytic activity for various ketones, such as α-chloro ketones, α-acetoxy ketones, α-keto esters, β-keto esters, γ-keto esters, and β-diketones, and 13 out of 20 alcohols obtained had enantiomeric purities of >98% ee.[7,8] The gene encoding SCR has recently been cloned and expressed in *E. coli*, and the asymmetric reduction of various ketones with the recombinant *E. coli* cells has afforded 20 synthetically useful alcohols, 11 of which had enantiomeric purities of >98% ee.[9,10] We considered the asymmetric reduction of **2** as a good test for the further evaluation of the power of the versatile biocatalyst. Here we report the highly enantioselective and efficient synthesis of (*R*)-**1** using the recombinant *E. coli* (Scheme 1).



Scheme 1

The *E. coli* strain coproducing SCR and GDH (glucose dehydrogenase) (BL21(DE3) harboring pESCR and pABGD), which has previously been reported,[10] was used in this study. α-Keto ester **2** (0.60–1.98 g, 3.0–10.0 mmol), which was prepared by the one-pot oxidation of methyl *o*-chlorophenylacetate according to the literature,[11] was added to a mixture of glucose (2 equiv.), $NADP^+$ (10 mg, 12 μmol), and *E. coli* wet cells (2.0 g) in 0.1 M phosphate buffer (pH 7.0, 10 mL). The mixture was stirred at a regulated temperature for 24 h, during which 2 N NaOH was added to neutralize the solution acidified upon formation of gluconic acid. The product was extracted with EtOAc and purified by column chromatography. The results are shown in Table 1.

We first employed the reaction conditions previously optimized for β-diketones.[10] To our delight, the desired alcohol (*R*)-**1** was

Table 1 Asymmetric reduction of **2** with recombinant *E. coli*[a]

Entry	[**2**]/M	[**2**]/g L^{-1}	*T*/°C	*C* (%)[b]	Yield (%)[c]	Ee (%)[d]
1	0.3	60	30	92	76	>99
2	0.3	60	25	>99	88	>99
3	0.6	120	25	94	88	>99
4	1.0	198	25	90	85	>99
5	1.0	198	20	99	89	>99
6	1.0	198	15	86	82	>99

[a] Conditions: **2** (0.60–1.98 g, 3.0–10.0 mmol), wet cells of *E. coli* BL21(DE3) harboring pESCR and pABGD (2.0 g), glucose (2 equiv.), $NADP^+$ (10 mg, 12 μmol), 0.1 M phosphate buffer (pH 7.0, 10 mL). [b] Conversion determined by ^1H NMR. [c] Isolated yield of (*R*)-**1**. [d] Determined by HPLC (Chiralpak AD-H, hexane/*i*-PrOH (9 : 1)).

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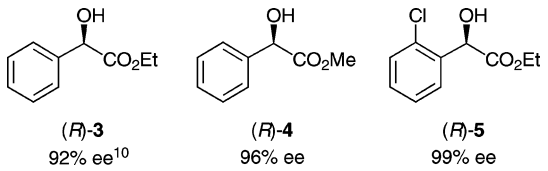
obtained in 76% yield with >99% ee (entry 1). This result was surprising because the sterically demanding chlorine atom at the *ortho* position might hinder the reaction and deteriorate the enantioselectivity and because α-hydroxy ester **1**, bearing the three electron-withdrawing groups at the stereocenter, might be subject to racemization. Indeed, the difficulty of asymmetric reduction of **2** can be inferred from the fact that Genet and co-workers gained (*R*)-**1** with 50% ee at most despite various attempts to increase the enantioselectivity in the Ru-catalyzed asymmetric hydrogenation of **2**.[3]

In view of the industrial utility of (*R*)-**1** as a synthetic intermediate for clopidogrel, we next turned our attention to the efficiency of this biotransformation. We tried to find the best reaction temperature, at which the enzyme denaturation caused by a large amount of substrate/product as well as temperature is suppressed well, and at which the asymmetric reduction of **2** proceeds smoothly, giving the highest productivity of (*R*)-**1**. Table 1 outlines how we optimized the productivity by changing the substrate concentration and the reaction temperature. When the reaction temperature was decreased by 5 °C, the conversion and isolated yield increased (entry 2), which prompted us to double the substrate concentration. Even at the substrate concentration of 0.6 M, the conversion reached 94% (entry 3). Therefore, we further increased the substrate concentration up to 1.0 M, which resulted in 90% conversion (entry 4). Finally, we further lowered the reaction temperature (entries 5 and 6) to find the best temperature giving the highest conversion at the same substrate concentration. Thus, the whole-cell reduction of 1.0 M of **2** at 20 °C gave 99% conversion and 1.78 g of isolated product (*R*)-**1** (entry 5), which corresponds to a productivity of 178 g L^{-1} (weight of isolated product per litre of initial reaction volume). Such a remarkable temperature effect on productivity was beyond our expectation although other researchers had gained the highest productivity at 20 °C in the whole-cell asymmetric reduction of ethyl 4-chloroacetoacetate.[12] Because only a few examples of microbial reduction systems capable of giving productivity higher than 100 g L^{-1} have been reported,[13–18] the present whole-cell reduction is quite promising. Moreover, the enantiomeric purities of (*R*)-**1** obtained under various conditions in Table 1 were >99% ee in all cases.

Previously, we have obtained ethyl (*R*)-mandelate ((*R*)-**3**) with 92% ee using recombinant *E. coli* overproducing SCR.[10] The fact that the (*R*)-enantiomer of **3** was obtained could not be explained well by the stereochemical trend observed for a series of products.[8] Before the attempt to reduce **2**, therefore, we could not predict how the enantioselectivity would change due to the structural modifications in the analogous compound. To investigate the factors responsible for the highly enantioselective production of (*R*)-**1**, we determined the enantiomeric purities of **4** and **5** obtained by the whole-cell reduction of the corresponding ketones at 30 °C. As a result, (*R*)-**4** and (*R*)-**5** were obtained in good yields with 96 and 99% ee, respectively (Scheme 2). Clearly, the presence of the chlorine atom and the replacement of the ethyl group by the methyl group each contributed to the enhancement of enantioselectivity, and the two modifications led to the production of (*R*)-**1** with >99% ee.



(*R*)-**3** 92% ee[10] (*R*)-**4** 96% ee (*R*)-**5** 99% ee

Scheme 2

In summary, the present biotransformation provides an efficient and green method for the synthesis of methyl (*R*)-*o*-chloromandelate ((*R*)-**1**). The hydride source is glucose, which is cheap biomass, and the catalyst is *E. coli*, which can be multiplied easily and inexpensively. The reaction is performed in an aqueous solution under air. This is the first example of the direct asymmetric synthesis of (*R*)-**1** with >99% ee. Excellent productivity as high as 178 g L^{-1} has been achieved. Because of the pharmaceutical value of the downstream product, clopidogrel, this bioprocess has good potential for an industrial application.

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