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Two-step desymmetrization of dipyrazolidyl 3-phenylglutarate via lipase-catalyzed hydrolysis in organic solvents

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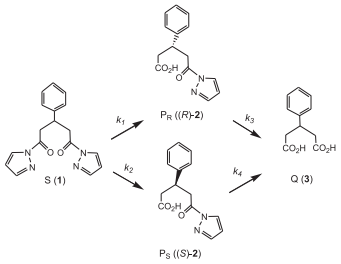
HIGHLIGHTS

- A sufficient condition for obtaining enantiomerically enriched product was proposed.
- Dipyrazolidyl 3-phenylglutarate but not the diester was employed as the substrate.
- Results agreed with the experimental data from the kinetic and thermodynamic analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

A theoretical analysis on comparing the enzyme performance in a single-step desymmetrization, single-step kinetic resolution, and two-step desymmetrization (i.e. a single-step desymmetrization followed by a sequent kinetic resolution) is reported. On the basis of $ee^* \geqq 0.95$, a sufficient condition of $E_3E_2^{-1} \geqq 10$ and $E_1 \geqq 2$ is proposed for obtaining an acceptable yield of $X_{2R}^* > 0.412$ for the desired enantiomer in the two-step desymmetrization process, in comparison with $E_1 \geqq 39$ for the single-step desymmetrization and $E_3E_2^{-1} \geqq 20$ for the single-step kinetic resolution. With the CALB-catalyzed hydrolytic desymmetrization of dipyrazolidyl 3-phenylglutarate (**1**) in MTBE as the model system, enantiomerically pure (*R*)-monopyrazolidyl 3-phenylglutarate ((*R*)-**2**) can then bfdee prepared. Moreover from the kinetic analysis, the best reaction condition of using 20% water-saturated MTBE as the medium at 45 °C is selected for improving the enzyme activity and stereoselectivity. The thermodynamic analysis also indicates that the enzyme stereo-discrimination in the desymmetrization and sequent kinetic resolution is mainly entropic-driven.

1. Introduction

Lipases (E.C. 3.1.1.3) have been employed as the biocatalysts for synthesizing a variety of lipids, food and flavors, pharmaceuticals, fine chemicals, cosmetics, biodiesels, and polymers (Hasan et al., 2006; Kobayashi, 2009; Tan et al., 2010; Soumanou et al., 2013). A very useful feature of these enzymes is the enantiodiscrimination with which the preparation of single enantiomer of alcohol, acid, and amine is fulfilled (Bornscheuer and Kazlauskas, 2006; Ghanem, 2007; Kamal et al., 2008; Faber, 2011; Paravidino et al., 2012). Many important intermediates and building blocks for various synthetic applications belong to diol, diamine, dicarboxylic acid, or anhydride that contains one or more prochiral or chiral centers. When using a symmetrical prochiral or *meso* compound as the substrate, the first-step desymmetrization followed by a subsequent kinetic resolution can generally increase the optical purity, yet with the price of decreasing the yield of the required enantiomer (Bornscheuer and Kazlauskas, 2006; Faber, 2011). Quantitative analysis in enzyme-catalyzed two-step desymmetrization of prochiral or *meso* compounds is more complicated than that in a single-step desymmetrization or kinetic resolution, as at least two

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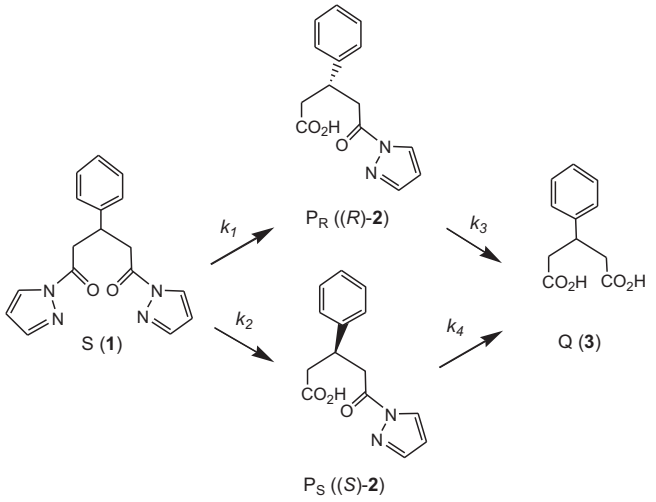
more kinetic parameters should be considered. This formulation remains still unclear and the relation of the theoretical to the experimental analysis has not been clearly discussed.

Enantiomerically pure 3-substituted glutarates are key structural elements of many drug intermediates and building blocks in organic synthesis (Fryszkowska et al., 2005; Gopinath et al., 2012; Jung et al., 2013). Enantiomerically enriched 3-arylglutarates have been prepared from alcoholic ring-opening of cyclic anhydrides via organocatalysts or enzymes (Chaubey et al., 2008; Park et al., 2010; Roy et al., 2014; Fryszkowska et al., 2006; García-Urdiales et al., 2011; Palomo and Cabrera, 2012; Liu et al., 2014) and enzyme-catalyzed hydrolysis, alcoholysis, aminolysis, or ammonolysis of dialkyl 3-arylglutarates (Yu et al., 2000; Homann et al., 2001; Lopez-Garcia et al., 2003; Cabrera et al., 2008; B. Wang et al., 2010; P.Y. Wang et al., 2010; Cabrera and Palomo, 2011; Liu et al., 2012; Nojiri et al., 2013). In general it is difficult to develop an efficient desymmetrization process leading to high enantiomeric purity and yields for the desired enantiomer at the mild reaction condition after inspecting the experimental data reported in the references of this paragraph. It is not only about the reaction conditions but also about the biocatalysts.

As a part of our ongoing efforts toward using azolides as the substrate for preparing optically active compounds (Wang et al., 2009, B. Wang et al., 2010; P.Y. Wang et al., 2010; Cheng et al., 2012; Tsai, in press), we aimed to employ dipyrazolidyl 3-phenylglutarate (**1**) as the model substrate for preparing an enantiomer of high enantiomeric purity and yield via CALB-catalyzed hydrolysis in MTBE (Scheme 1). A thorough kinetic analysis is firstly performed for proposing a sufficient condition leading to the efficient desymmetrization process giving $ee^* \geqq 0.95$ and $X_{2R}{}^* > 0.412$. From the kinetic analysis, the kinetic constants shown in Scheme 1 are estimated from experimental data for selecting the best reaction condition. The thermodynamic analysis is moreover addressed and elucidated.



Scheme 1. Two-step desymmetrization of dipyrazolidyl 3-phenylglutarate in MTBE containing different water contents via lipase-catalyzed hydrolysis.

2. Model development

By using an excess of water for the hydrolysis and assuming the substrate concentrations to be much lower than the Michaelis-Menten constants, an irreversible first-order kinetics for 1, (*R*)-2, and (*S*)-2 can be derived and solved for the time-course molar fractions as follows (Faber, 2011):

$$X_1^* = \exp(-t*) \tag{1}$$

$$X_{2\,R}{}^* = \frac{E_1[\exp(-E_2 t*) - \exp(-t*)]}{(1+E_1)(1-E_2)} \tag{2}$$

$$X_{2\,S}{}^* = \frac{\exp(-E_3 t*) - \exp(-t*)}{(1+E_1)(1-E_3)} \tag{3}$$

$$X_3^* = 1 - X_1^* - X_{2\,R}{}^* - X_{2\,S}{}^* \tag{4}$$

The dimensionless parameters are defined as: $t^* = (k_1+k_2)t$ as dimensionless time, $E_1 = k_1 k_2{}^{-1}$ as stereoselectivity for the single-step desymmetrization, $E_2 = k_3(k_1+k_2)^{-1}$, $E_3 = k_4(k_1+k_2)^{-1}$, $ee^* = (X_{2R}{}^* - X_{2S}{}^*)(X_{2R}{}^* + X_{2S}{}^*)^{-1}$ as enantiomeric excess for (*R*)-**2**, and hence $E_3 E_2{}^{-1} = k_4 k_3{}^{-1}$ as enantiomeric ratio for the second-step kinetic resolution. The kinetic parameter combination (k_1+k_2) may be firstly estimated from the regression of time-course data of $X_1{}^*$ to Eq. (1), and then k_2 and k_4 from the time-course $X_{2S}{}^*$ and Eq. (3). With the known k_1 value, k_3 is then regressed from the time-course $X_{2R}{}^*$ and Eq. (2).

Apparently for $E_2 = E_3 = 0$, Eqs. (2) and (3) reduce to Eqs. (5) and (6) for a single-step desymmetrization, and lead to a constant enantiomeric excess of $ee^* = (E_1\text{-}1)(E_1+1)^{-1}$ throughout the reaction. Similarly when performing a single-step kinetic resolution by using (*R*,*S*)-**2** as the substrate, one obtains Eqs. (7) and (8), with which the enantiomeric excess for (*R*)-**2** defined as $ee = (X_{2R}\text{-}X_{2S})(X_{2R}+X_{2S})^{-1}$ may vary from zero to one.

$$X_{2\,R}{}^* = \frac{E_1[1-\exp(-t*)]}{1+E_1} \tag{5}$$

$$X_{2\,S}{}^* = \frac{1-\exp(-t*)}{1+E_1} \tag{6}$$

$$X_{2\,R} = 0.5\exp(-E_2 t*) \tag{7}$$

$$X_{2\,S} = 0.5\exp(-E_3 t*) \tag{8}$$

3. Materials and methods

3.1. Materials

Novozym 435 (*Candida antarctica* lipase B (CALB) immobilized on acrylic resins, containing 1–2% (w/w) water and has 7000 PLU/g by using lauric acid and 1-propanol as substrates at 60 °C) was purchased from Novozymes (Bagsvaerd, Denmark). 1 PLU is the amount of enzyme activity which generates 1 μmol of propyl laurate per minute under the defined conditions. Other chemicals of analytical grade were commercially available: pyrazole and 1 H-benzotriazole from Acros (Geel, Belgium); thionyl chloride from Seedchem (Camberwell, Australia); chloroform-D from Cambridge Isotope Laboratories (Andover, MA); calcium hydride from Sigma-Aldrich (St. Louis, MO); acetic acid glacial (AA), benzene, isopropanol (IPA), methyl *tert*-butyl ether (MTBE), *n*-hexane (HEX) and triethylamine from Tedia (Fairfield, OH); 3-phenylglutaric acid from Alfa (Ward Hill, MA). 100% Water-saturated MTBE was prepared from a biphasic aqueous-MTBE solution kept in a water bath of specified temperature and with stirring for more than 24 h. Anhydrous MTBE was made by adding calcium hydride to the solvent for 24 h. Both solvents were then employed for preparing MTBE containing different water contents. For example, 20% water-saturated MTBE containing 94.5 mM of water at 45 °C was prepared by mixing 1:4 (v/v) of 100% water-saturated MTBE containing 472.7 mM of water and anhydrous MTBE (Alkandary et al., 2001).