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Kinetic resolution of (±)-1,2-*O*-isopropylidene-3,6-di-*O*-benzyl-*myo*-inositol by lipases: An experimental and theoretical study on the reaction of a key precursor of chiral inositols

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ABSTRACT

The study on kinetic resolution of two *myo*-inositol derivatives by lipases is reported. Treatment of the tri-ether derivative, (±)-1,2-*O*-isopropylidene-3,5,6-tri-*O*-benzyl-*myo*-inositol, with acylating agents in the presence of different lipases did not afford any detectable amount of acylated products. We speculate that the severe steric hindrance posed by this substrate precluded interaction with the enzymes' catalytic site. Conversely, diol (±)-1,2-*O*-isopropylidene-3,6-di-*O*-benzyl-*myo*-inositol, a key precursor of chiral *myo*-inositol derivatives, bearing one less benzyl protecting group, underwent a successful transesterification in EtOAc, catalyzed by CaL-B (Novozym 435). Thus, monoacetate L-(−)-1,2-*O*-isopropylidene-3,6-di-*O*-benzyl-5-*O*-acetyl-*myo*-inositol was regioselectively formed in >99% *ee*. Additionally, we developed theoretical models of the second tetrahedral intermediate (TI) complex of this reaction to explain the success of the CaL-B and the inactivity of RmL against the same substrate.

Abbreviations: Ac_2O, acetic anhydride; EtOAc, ethyl acetate; Et_2O, diethyl ether; CaL-B, *Candida antarctica* lipase-B; DMAP, 4-dimethylaminopyridine; *ee*, enantiomeric excesses; FT-IR, Fourier transform infra-red spectroscopy; HPLC, high performance liquid chromatography; MS-ESI, mass spectroscopy-electrospray ionization; NMR, nuclear magnetic resonance; PTFE, polytetrafluoroethylene; RmL, *Rhizomucor miehei* lipase; TI, tetrahedral intermediate; TLC, thin-layer chromatography; UV, ultraviolet.

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1. Introduction

A major challenge for studies on the synthesis of inositol derivatives, in the context of Medicinal Chemistry and Cell Biology, is the substantial number of steps involved [1–3]. Due to its availability, most of these studies employ *myo*-inositol itself, an achiral cyclitol, as starting material in the synthesis of chiral derivatives. Such strategy usually succeeds because of its practicality and the good repertoire of established protocols for differentiation of the hydroxyl groups in this molecule. Nevertheless, optical resolution of racemic intermediates is required in this case [1,2]. The usual procedure of generating mixtures of diastereomers, to be separated via chromatography or recrystallization, may be practical, but it adds steps and manipulations to the synthetic process, and that leads to decreased overall yields. Thus, the use of enzymes for kinetic resolution of racemic *myo*-inositol derivatives rises up as a valuable alternative.

Lipases, in particular, constitute a class of enzymes of great interest in the field of Biocatalysis applied to Organic Synthesis. These biocatalysts are reputed for their chemical selectivity and specificity [4]. Despite the potential of the use of lipases in chemoenzymatic synthesis of *myo*-inositol, few works have explored it. The number of different *myo*-inositol derivatives assayed as substrates in kinetic resolutions is even smaller. The diether derivative (±)-2,6-di-*O*-benzyl-*myo*-inositol was resolved by means of an immobilized *Candida antarctica* lipase-B (Novozym 435) in vinyl acetate. Thus, L-2,6-di-*O*-benzyl-5-*O*-acetyl-*myo*-inositol was regioselectively produced in 49% yield and 99% enantiomeric excess (*ee*) [5]. A more hindered derivative, 1,2:5,6-*O*-diisopropylidene-*myo*-inositol, was reacted with Ac_2O in Et_2O in the presence of *Candida rugosa* lipase. After a conversion of 48%, L-1,2:5,6-*O*-diisopropylidene-4-*O*-acetyl-*myo*-inositol was formed in moderate *ee* (84%) [6]. In a seminal investigation, the key synthetic precursor (±)-1,2-*O*-cyclohexylidene-*myo*-inositol was reacted with Ac_2O in 1,4-dioxane in the presence of *Pseudomonas sp*. lipase ("Amano" CES lipase). Monoacylated derivative L-2,3-*O*-cyclohexylidene-1-*O*-acetyl-*myo*-inositol was regioselectively produced in 49% yield and 98% *ee* [7,8; see also 9].

In general, all lipases (EC 3.1.1.3) share an evolutionarily conserved structure named α/β-hydrolase folding. In the active site, there is a catalytic triad composed of a nucleophilic residue (Ser) and two residues (Asp/Glu and His) playing the role of a charge relay system [10]. The active site is stabilized by hydrogen bond networks, for each particular enzyme, and the oxyanion hole is usually formed by two backbone nitrogen atoms (NH from the amide groups) close to the nucleophile [10]. Most of the lipases, such as RmL (but not CaL-B), have a structure in alpha-helix named "lid" or "flap" region that covers the active site, which has an important role in the enzyme activity, and a "tunnel" for the substrate recognition, which begins near the catalytic Ser and extends to the vicinity of the "lid" helix [10].

The Ping-Pong Bi–Bi type reaction mechanism, considering a transesterification reaction, is shown schematically in Fig. 1 (in CaL-B and RmL, the catalytic triad residues are numbered as Ser105–His224–Asp187 and Ser144–His257–Asp203, respectively), where EtOAc represents the first substrate and an alcohol (ROH), the second substrate. This reaction also involves two tetrahedral intermediate (TI) complexes, which are related to their correspondent transition states. The first TI complex results from the catalytic-Ser nucleophilic attack on the first substrate, and the second TI complex results from the second substrate nucleophilic attack on the acyl-enzyme complex (*i.e.*, first TI complex) [10]. The literature reports several molecular modeling studies on lipases (*e.g.*, CaL-B), based on classical force fields calculations, but using different approaches such as molecular docking [11], molecular dynamics [12], Monte Carlo [13], and three-dimensional quantitative structure-activity relationship (3D-QSAR) [14].

In the present work, we report the study of lipase-catalyzed optical resolution of two differentially protected racemic *myo*-inositol derivatives (Fig. 3), namely, (±)-3,5,6-*O*-tribenzyl-1,2-*O*-isopropylidene-*myo*-inositol (*rac*-**1**) and (±)-3,6-di-*O*-benzyl-1,2-*O*-isopropylidene-*myo*-inositol (*rac*-**2**). Such compounds (especially *rac*-**2**) are key synthetic intermediates in the synthesis of chiral inositol phosphates or other derivatives [15–21], and had not been previously assayed as substrates for lipases. The methodology used here enables the synthesis of such relevant materials in a cleaner and more efficient manner. In addition, we have docked *rac*-**2** on the CaL-B and RmL active sites, in order to model the second tetrahedral intermediate (TI) complex to explain the structural basis of the CaL-B mediated optical resolution and the inactivity of RmL.

2. Experimental

2.1. General

The synthetic acetylation reaction of product L-**3** was carried out under Ar atmosphere. Unless otherwise noted, commercially available reagents and solvents of analytical grade were used. *Myo*-inositol derivatives *rac*-**1** and *rac*-**2** were prepared according to the literature [22–24]. Ac_2O was treated with P_2O_5 and the mixture was heated at 50–60 °C for 1 h, cooled to room temperature and decanted. After being transferred by cannula to a dry distillation apparatus, the reagent was distilled under Ar. Pyridine was treated with CaH_2 and stirred at room temperature for several hours, and then, the same procedure applied to Ac_2O was carried out. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials.

Reactions were monitored by thin-layer chromatography (TLC), carried out on 0.25 mm E. Merck silica gel plates (60F-254), using UV light as visualizing agent, and/or an aqueous basic solution of $KMnO_4$, and heat as developing agents. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography.

NMR spectra were recorded on Bruker DRX-400 and Varian Gemini 200 instruments, and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. IR spectra were recorded on Nicolet Magna 760 FT-IR spectrometer. Elemental analyses were performed on a CHN 2400 unit. Mass spectra (ESI; aq. NH_4Cl sol.) were obtained in a simple quadrupole spectrometer. Specific rotations were recorded on a Jasco DIP-370 polarimeter.

Conversion analyses were carried out via HPLC on a Kromasil-C18 column (40 °C in a CTO-20A oven), eluted (0.5 mL/min) with acetonitrile/H_2O (60:40) by a Shimadzu LC-20AT pump. A Shimadzu SPD-M20A variable-wavelength UV/Vis detector was employed, with the detection set at 215 nm, and the Shimadzu LC solution software was used for chromatogram integration.

Chromatographic determinations of the enantiomeric excesses (*ee*) of L-(−)-**3** were done on the same equipment mentioned above, carrying a Chiralcel OD-H column, and eluted (0.8 mL/min) with hexane/2-propanol (9:1). The samples to be analysed were filtered through a 0.45 μm PTFE filter.

2.2. Enzymes

The commercial immobilized enzymes *C. antarctica* lipase-B (CaL-B, Novozym 435), *Rhizomucor miehei* lipase (RmL, Lipozyme RM IM) and *Thermomyces lanuginosus* lipase (Lipozyme TL IM)