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Efficient production of S-(+)-2-chlorophenylglycine by immobilized penicillin G acylase in a recirculating packed bed reactor

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ABSTRACT

(S)-(+)-2-Chlorophenylglycine **1** is an important intermediate in the synthesis of Clopidogrel. A recirculating packed bed reactor (RPBR) was constructed for efficient production of (S)-**1** by kinetic resolution of racemic N-phenylacetyl-2- chlorophenylglycine **2** using immobilized penicillin G acylase (PGA). The immobilized PGA exhibited maximum activity at 50 °C and pH 8.0 with (R,S)-**2** as substrate. The kinetic constants (K_m and v_{max}) of immobilized PGA were calculated to be 20.61 mM and 83.2 mM/min/g, respectively. The substrate displayed inhibitory effect on immobilized PGA with inhibition constant of 221.23 mM. The immobilized PGA showed a strict enantiospecificity for substrate at different temperature, pH and substrate concentration examined. The performance and productivity of RPBR were evaluated by several critical parameters, including immobilized PGA load, substrate feeding rate, height to diameter ratio and so on. The kinetic resolution process shows higher initial reaction rate and conversion by recycling 100 mL of substrate solution (80 mM) through RPBRs packed with 6.0 g immobilized PGA with a feeding rate of 1.5 mL/min while the H/D ratio was 4.0. The immobilized PGA-catalyzed kinetic resolution of (R,S)-**2** was successfully operated in the RPBR for 60 batches, with an average productivity of 1.2 g/L/h for (S)-**1** in high optical purity (>97% enantiomeric excess) in semi-continuous operation. The residual (R)-**2** can be easily racemized and then used as substrate.

1. Introduction

(S)-(+)-2-Chlorophenylglycine [(S)-2-amino-(2-chlorophenyl) ethanoic acid] **1** is one of the key intermediates in the synthesis of Clopidogrel, an antiaggregatory and antithrombotic drug administered for the reduction of atherosclerotic events including myocardial infarction, ischemic stroke, and peripheral vascular disease, widely used in combination with aspirin after placement of intravascular stents [1–5]. The prevalence of hypertension and hyperlipemia is markedly increasing throughout the world. Facing such an increasingly worsening trend, the research and production of this drug are becoming more and more critical to reduce the health-care spending. Many chemical methods have been reported for the synthesis of clopidogrel, most routes utilize either the racemic 2-chloromandalate or 2-chlorophenylglycine derivatives as starting materials [6]. The final product is usually made in racemic form and resolved via fractional crystallization with a resolving agent such as camphor sulfonic acid. Economically, the use of an enantiomerically pure reagent such as (S)-**1** at the start of a synthetic sequence is more cost effective and less polluting (Scheme 1).

Several syntheses of (S)-**1** start from the resolution of (R,S)-2-chlorophenylglycine, or its esters, via the formation of diastereomeric salts with tartaric [7] or camphor sulfonic acid [8] followed by fractional crystallization. However, the chemical resolution methods need expensive resolving agents and result in low yield, which are unfavorable for commercial production. Biocatalysts play an important role in many industrial syntheses aiming for chiral molecules. Enzymes are highly valuable catalysts, and allow the manufacture of chiral chemicals on an industrial scale with high enantioselectivity, yield, volumetric productivity and little waste. Several enzymatic approaches have been developed for the production of (S)-**1**, including alcalase-catalyzed resolution of 2-chlorophenylglycine alkyl ester [9], resolution of (R,S)-N-Boc-2-chlorophenylglycine methyl ester by subtilisin [1], and resolution of (R,S)-2-chlorophenylglycine by penicillin G acylase (PGA) [10]. PGA-mediated pathway offers significant advantages over other routes because of its excellent enantioselectivity and cheap biocatalyst. More importantly, this method can provide theoretically 100% yield of the product since the unreacted substrate can be racemized easily and then used as the substrate, thereby being consumed and converted to (S)-**1** (Scheme 2).

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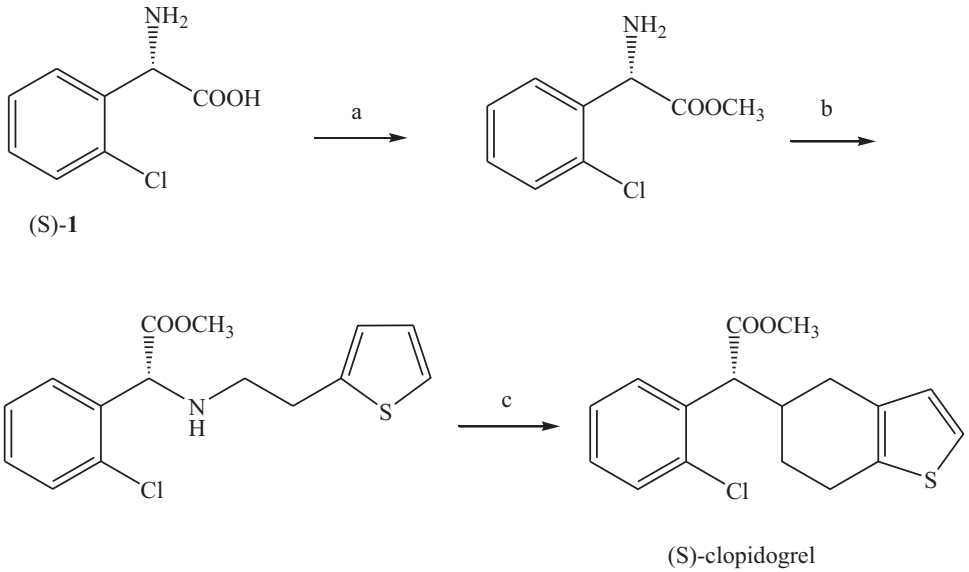


Scheme 1. Asymmetric synthesis of (S)-clopidogrel from (S)-**1**. Reagents and conditions: (a) CH_3OH, $SOCl_2$, 50 °C; (b) 2-(2-thienyl)ethyl toluene-*p*-sulphonate, CH_3CN, K_2HPO_4, 60–100 °C; (c) HCHO, HCl, 60 °C.

Recirculating packed-bed reactor (RPBR) is a kind of practical and efficient reactor, showing high conversion efficiency and long reaction time [11–13]. A major advantage of a RPBR in immobilized PGA-catalyzed reaction is that the enzyme loss can be reduced by the absence of collisions between enzyme particles and an impeller, and the reduction of liquid shearing when compared to a stirred tank reactor [14]. To date, no researches has been conducted for the production of (S)-**1** by kinetic resolution of (R,S)-**2** in bioreactors including RPBR in spite of their importance for large scale preparations. In this work, an immobilized PGA hydrolysis process for the semi-continuous production of (S)-**1** in RPBR was investigated for the first time. The properties of immobilized PGA for substrate (R,S)-**2** was firstly investigated. The performance and productivity of the immobilized PGA in RPBR were then evaluated as a function of a few critical parameters, such as immobilized PGA amount, feeding rate of substrate solution, H/D (height to diameter) of enzyme column, and operation stability.

2. Materials and methods

2.1. Materials

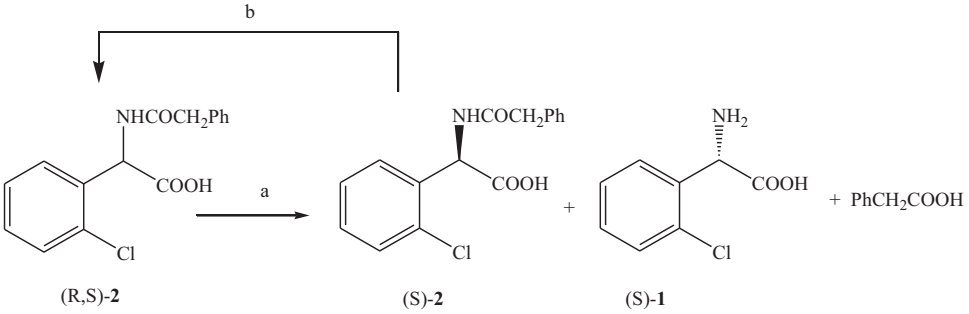
(R,S)-**1**, (R)-**1**, and (S)-**1** were purchased from Shanghai Haiqu Chemical Co. Ltd. (Shanghai, China). Phenylacetyl chloride was purchased from Shanghai Mayao Chemicals Co. Ltd. (Shanghai, China). Immobilized PGA was obtained from Zhejiang Shunfeng haider Co. Ltd. (Zhejiang, China). All other chemicals used were of analytical reagent grade and commercially available.

2.2. Synthesis of (R,S)-2 from (R,S)-1

(R,S)-**1** (18.56 g, 0.1 mol) was dissolved in NaOH solution (4.0 M, 100 mL), and the mixture was stirred in an ice bath. Phenylacetyl chloride (17.0 g, 0.11 mol) was added to the mixture dropwise. The reactants were stirred overnight after complete addition and extracted with dichloromethane. The aqueous layer was then cooled in an ice bath and acidified with 6.0 M HCl. The precipitate was filtered and washed with water to obtain (R,S)-**2** (28.35 g, 93.4% yield). ^{1}H NMR (500 MHz, DMSO) δ 13.10 (s, 1H), 8.95 (s, 1H), 7.48 (s, 1H), 7.37 (s, 2H), 7.28 (s, 5H), 7.22 (s, 1H), 5.79 (s, 1H), 3.56 (s, 2H).

2.3. Analytical methods

Direct reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed for the separation of enantiomers of 2-chlorophenylglycine and N-phenylacetyl-2-chlorophenylglycine. (S)-**1**, (R)-**1,** (S)-**2**, and (R)-**2** were analyzed by RP-HPLC on a Dionex HPLC system equipped with a P680 pump, an ASI-100 automated sample injector, an Ulti-Mate 3000 thermostatted column compartment and a UV–vis detector (Dionex, Sunnyvale, CA, USA). Enantiomers were separated without further



Scheme 2. Kinetic resolution of (R,S)-**2** to produce (S)-**1** by immobilized PGA. The residual (R)-**2** which is unreactive to the immobilized PGA can be racemized and then used as the substrate. Reagents and conditions: (a) Immobilized PGA, 30 °C, pH 8.0; (b) 150 °C, 30 min.