Bioorganic Chemistry 80 (2018) 174–179

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Efficient chemoenzymatic synthesis of (*S*)-α-amino-4-fluorobenzeneacetic acid using immobilized penicillin amidase

Chao-Ping Lin, Xiao-Ling Tang, Ren-Chao Zheng*, Yu-Guo Zheng

Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, People's Republic of China

Engineering Research Center of Bioconversion and Biopurification of Ministry of Education, Zhejiang University of Technology, Hangzhou 310014, People's Republic of China

ARTICLE INFO

Keywords:
(*S*)-α-amino-4-fluorobenzeneacetic acid
Immobilized penicillin amidase
Kinetic resolution
Chemoenzymatic approach

ABSTRACT

An efficient chemoenzymatic route was developed for synthesis of (*S*)-α-amino-4-fluorobenzeneacetic acid, a valuable chiral intermediate of Aprepitant, using immobilized penicillin amidase catalyzed kinetic resolution of racemic *N*-phenylacetyl-4-fluorophenylglycine. The optimum temperature, pH and agitation rate of the reaction were determined to be 40 °C, 9.5 and 300 rpm, respectively. Kinetic resolution of 80 g L^{-1} *N*-phenylacetyl-4-fluorophenylglycine by immobilized amidase 20 g L^{-1} resulted in 49.9% conversion and > 99.9% *e.e.* within 3 h. The unreacted *N*-phenylacetyl-4-fluorophenylglycine can be easily racemized and then recycled as substrate. The production of (*S*)-α-amino-4-fluorobenzeneacetic acid was further amplified in 1 L reaction system, affording excellent conversion (49.9%) and enantioselectivity (99.9%). This chemoenzymatic approach was demonstrated to be promising for industrial production of (*S*)-α-amino-4-fluorobenzeneacetic acid.

1. Introduction

Aprepitant is a potent and orally active antagonist of human neurokinin-1 (NK-1) receptor in the treatment of chemotherapy-induced emesis, and also has good effect to release severe major depression, pain and migraine [1–3]. The compound **6** is a critical precursor of Aprepitant (Fig. 1), and its synthesis has aroused great interest. Several synthetic routes of **6** have been reported [4–8] (Fig. 2), and most of them introduced the chiral building block via direct addition reaction, or by asymmetric synthetic methodologies. One of the most economically attractive route for synthesis of **6** starts from (*R*)-α-methylbenzylamine [1] (Scheme 1). However, this method involved a reduction step where hazardous reagents like DIBALH and $NaBH_4$ are used. Besides, the poor optical purity of intermediate compound oxazinone and the redundant procedure restricted its industrial applications. Other potential synthetic routes using *N*-benzyl ethanolamine, *p*-fluorobenzaldehyde, 3, 5-bis (trifluoromethyl)-1-vinylben or 4-fluorophenylacetic acid as raw material also have been reported [7,9–15] (Schemes 2–5). However, the dangerous reagents and precious metal catalyst used in the synthesis of key chiral block resulted in security risks and cost pressures. Comparatively, an additional approach (Scheme 6) based on the improvement of Scheme 5 with (*S*)-α-amino-4-fluorobenzeneacetic acid **4** as raw material not only reduced the procedure, but also avoided using environmental unfriendly reagents.

Asymmetric synthesis and crystallisation-induced resolution are two main types of routes reported for synthesis of **4** [11,16,17]. However, those traditional chemical processes exhibited a lengthy procedure and required the used of toxic organic reagents. By comparison, the enzymatic process has become an appealing approach for producing **4**, because of its shorter process, greener solvent, and environmental friendliness [18–23]. The transaminase mediated synthesis of **4** using prochiral keto-acid as substrate has been reported [24] (Scheme 7a). Although with excellent enantioselectivity (98% *e.e.*), substrate loading of the enzymatic reaction was low (2.3 g L^{-1}) and reaction time was long (71 h). In addition, the reversible enzymatic process requires pyridoxal 5-phosphate as co-factor and aspartate as amino donor, resulting in increased cost and incomplete conversion. Biocatalytic deracemization in the preparation of **4** from **1** using whole cells (*Nocardia Corallina* CGMCC 4.1037) containing *R*-amino acid oxidase and *S*-aminotransferase has been reported [25] (Scheme 7b). The low substrate concentration (10 mM) and relatively inferior catalytic efficiency (63% yield) restrict its industrial applications. Therefore, developing a robust biocatalysts and an efficient synthesis strategy for producing **4** is urgent. A penicillin amidase which exhibited a high hydrolytic activity and excellent enantioselectivity toward *N*-phenylacetyl-4-fluorophenylglycine, was employed as biocatalyst for enzymatic synthesis of

* Corresponding author at: Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, People's Republic of China.
E-mail address: zhengrc@zjut.edu.cn (R.-C. Zheng).

https://doi.org/10.1016/j.bioorg.2018.06.020
Received 26 April 2018; Received in revised form 11 June 2018; Accepted 11 June 2018
Available online 15 June 2018

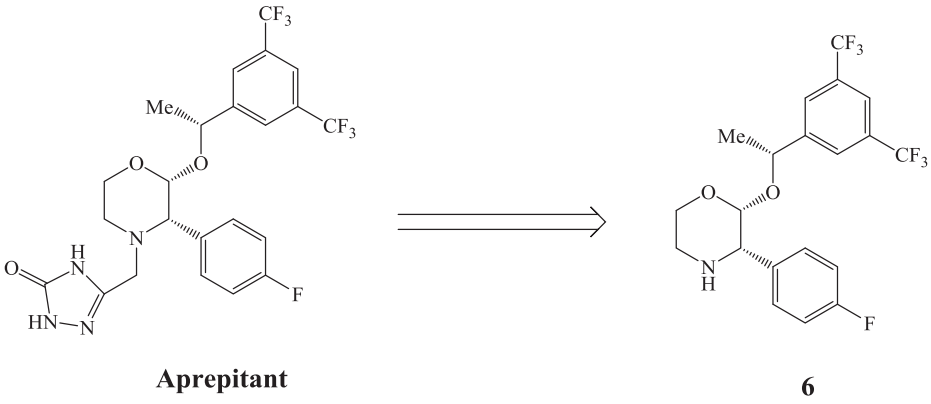


Fig. 1. Structures of Aprepitant and its chiral precursor **6**.

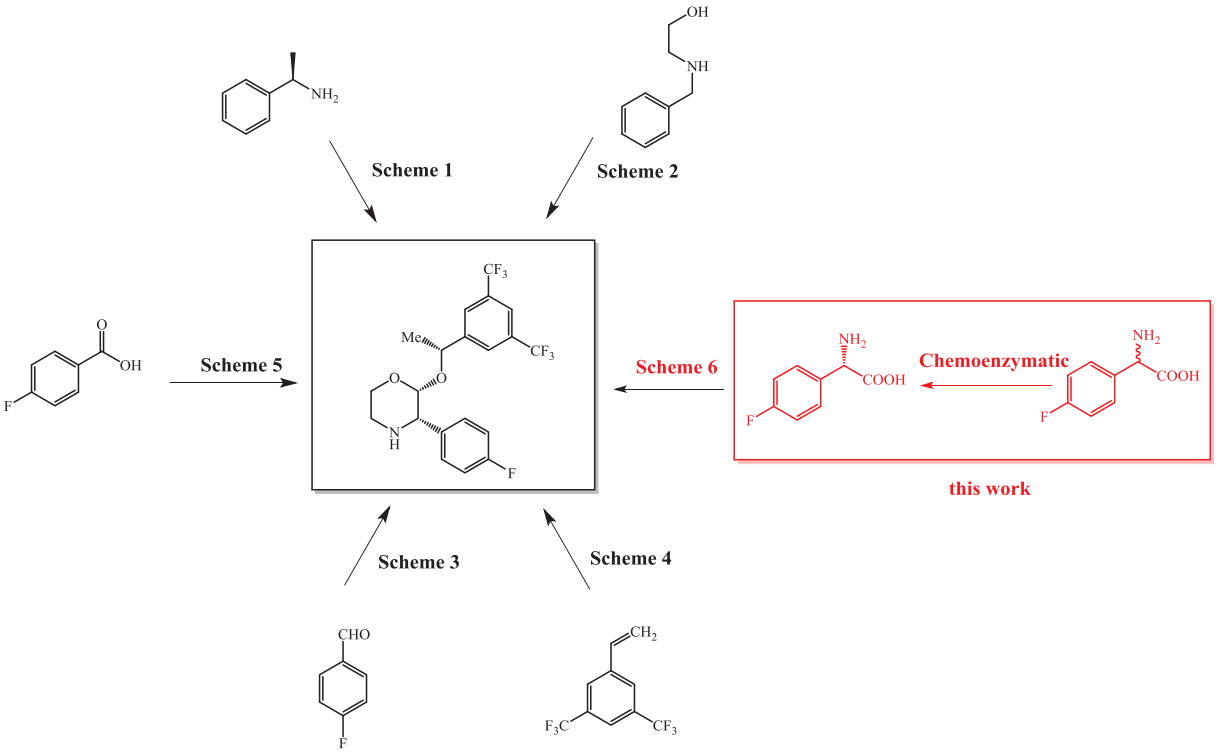
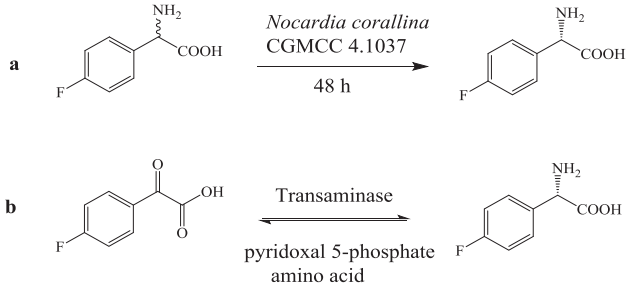


Fig. 2. Synthetic routes of **6**. The synthetic routes (Schemes 1–5) have been reported.



Scheme 7. Enzymatic synthesis of **4**.

4. However, free enzyme has some drawbacks such as poor stability, difficulty in separation and recycling. Fortunately, these problems could be overcome by immobilized enzyme.

In this work, we aim to develop a practical chemoenzymatic approach for efficient synthesis of **4** via kinetic resolution combined with flexible chemical racemization (Scheme 8). The biocatalyst (immobilized amidase) was prepared by covalent immobilization of the recombinant penicillin amidase from *Bacillus megaterium* onto epoxy resin. After optimization of the reaction parameters, the chemoenzymatic reaction was carried out with high substrate concentration, high conversion and excellent enantioselectivity. This highly efficient chemoenzymatic route appears to be industrial promising in the production of (*S*)-α-amino-4-fluorobenzeneacetic acid.

2. Materials and methods

2.1. General

Recombinant penicillin amidase was constructed by our laboratory [26]. 4-Fluorophenylglycine was purchased from Shanghai Rongli Chemical Co. Ltd. (Shanghai, China). Phenylacetyl chloride was purchased from Shanghai Mayao Chemicals Co. Ltd. (Shanghai, China). Epoxy resin (LX-1000EP) was purchased from Xian Lanxiao Technology Co. Ltd. (Xian, China). Analytical reagent grade solvents were purchased from TEDIA, USA. All other reagents were obtained from