Tetrahedron: *Asymmetry* 26 (2015) 1095–1101

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

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

Synthesis of enantiopure L-(5-phenylfuran-2-yl)alanines by a sequential multienzyme process

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ARTICLE INFO

Article history:
Received 23 July 2015
Accepted 12 August 2015
Available online 28 August 2015

ABSTRACT

The enantioselective synthesis of unnatural amino acids is an attractive goal. Increasing attention has been given in recent years to the development of dynamic kinetic resolution processes, providing the desired enantiomer in almost quantitative yields and with high enantiopurity. Herein we describe an efficient sequential multi-enzyme process for the preparation of enantiopure 5-phenylfuran-2-ylalanines L-**4a–d**, starting from racemic 2-acetamido-3-(5-phenylfuran-2-yl)propanoic acids *rac*-**1a–d**. The first step, the CaL-B-mediated dynamic kinetic resolution of the racemic oxazolones provided the *N*- and *C*-protected L-amino acids L-**2a–d** (81–92% ee) with 100% theoretical yield. The protecting groups were removed in excellent yields by a second (mild non-stereoselective PLE mediated hydrolysis of the ester) and a third (Acylase I catalyzed stereoselective hydrolysis of the amide) enzymatic step, thus increasing the enantiomeric excess of the target compounds over 99%.

1. Introduction

Unnatural amino acids, non-genetically-coded amino acids that either occur naturally or are chemically synthesized, are important tools for modern drug discovery research. Due to their structural diversity and functional versatility, they are widely used as chiral building blocks and molecular scaffolds in constructing combinatorial libraries, especially in the synthesis of peptides with enhanced properties (e.g., proteolytic stability, biological activity, etc).[1] They are also commonly used as moieties in the rational design of chiral drugs such as anti-cancer compounds[2] and viral inhibitors[3] or as key intermediates of pharmaceuticals from a broad range of therapeutic fields such as hypertension, diabetes, HIV, migraine, etc.[4] Several non-proteinogenic amino acids, such as L-Dopa, L-homophenylalanine or D-2-naphthylalanine, are important pharmaceuticals,[5] while others such as D-phenylglycine, D-*para*-hydroxyphenylglycine, 2-thienylalanine, etc., are useful for the synthesis of certain drugs.[6] Accordingly, the enantioselective synthesis of amino acids is an attractive goal.

While natural L-amino acids can be obtained by fermentation or by applying natural enzymatic systems, for the synthesis of enantiopure unnatural amino acids, chiral chemical catalysis or biocatalysis is employed. Among the most common enantioselective synthetic procedures, such as reductive amination, transamination, asymmetric hydrogenation, kinetic resolution,[7] the biocatalytic approaches based on the use of aminoacylases, hydantoinases, aminotransferases, ammonia-lyases, and amino acid dehydrogenases offer valuable synthetic alternatives.[8] Despite the success of enzyme catalyzed kinetic resolutions for the synthesis of a wide range of chiral building blocks, there is an increasing demand to develop transformations which are not limited by a maximum yield of only 50% of the desired enantiomer of the product. Increasing attention has been given in recent years to the development of dynamic kinetic resolution processes[9] in which the unreactive enantiomer equilibrates in situ under the reaction conditions, with the more reactive antipode. Thus, dynamic kinetic resolution can result in almost quantitative yields, with enantiomeric excesses approaching 100%.

The first enzymatic dynamic kinetic resolution of oxazol-5(4*H*)-one was reported in 1990.[10] It was shown that due to the in situ racemization of the substrate during the Lypozyme TL IM-catalyzed butanolysis of 4-methyl-2-phenyloxazol-5(4*H*)-one, the *N*- and *C*-protected (*S*)-alanine was formed with moderate enantiomeric excess but with 100% conversion. It was demonstrated that oxazolones were excellent substrates for stereoselective dynamic kinetic resolution due to the low pK_a of the C-4 proton and their related reactivity toward lipase-catalyzed

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http://dx.doi.org/10.1016/j.tetasy.2015.08.004
0957-4166/

alcoholysis.[11] Furthermore, the same procedure was found to be adequate for the enzymatic dynamic kinetic resolution of other 4-substituted 2-phenyloxazol-5(4*H*)-ones and it was also shown that the nature of the solvent influenced the stereoselectivity of the reaction.[12] The efficiency of the enzymatic dynamic kinetic resolution of oxazolones was further improved by using catalytic amounts of organic bases in the reaction media, which enhanced the racemization rate of the substrates and the enantiomeric excesses of the isolated products.[13,14]

A reliable chemoenzymatic procedure for the preparation of enantiopure benzofuranyl- and benzothiophenylalanines, starting from racemic 2-acetamido-3-(heteroaryl)propanoic acids was previously described by us,[14] involving the Novozyme 435-mediated dynamic kinetic resolution of the oxazolones, which provided the *N*- and *C*-protected L-amino acids with 100% conversion. It was shown that the spontaneous racemization of these oxazolones was faster than the enzymatic alcoholysis, thus avoiding the need of an organic base in the reaction media. This unexpected feature of the mentioned 4-heteroaryl-2-methyl-oxazol-5(4*H*)-ones in relation to all of the other previously reported examples, prompted us to investigate the behavior of 4-heteroaryloxazolones with 5-phenylfuran-2-yl-substituents in their lipase catalyzed dynamic kinetic resolution in order to set up an optimized chemoenzymatic procedure for the preparative-scale synthesis of the corresponding enantiomerically pure L-(5-phenylfuran-2-yl)alanines.[14] This methodology would be more accessible for synthetic chemists, compared to the previously described[15] phenylalanine ammonia lyase (PAL) mediated stereoselective ammonia addition to (5-phenylfuran-2-yl)acrylates.

2. Results and discussion

2.1. Chemical synthesis

5-Arylfuran-2-carbaldehydes were used as starting materials. Their transformation via *rac*-2-acetamido-3-(5-phenylfuran-2-yl) propanoic acids *rac*-**1a–d** into racemic amino acids *rac*-**4a–d** was performed in accordance with the malonic synthesis protocol. Racemic oxazolones *rac*-**3a–d** and racemic 2-acetamido-3-(5-phenylfuran-2-yl)propanoic acid esters *rac*-**2a–d** were obtained from *rac*-**1a–d** using the methodology previously reported by us[14] (Scheme 1).

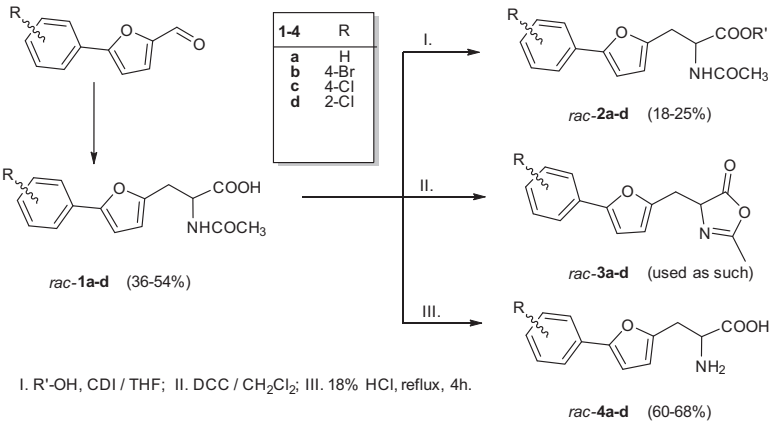
2.2. Enzymatic synthesis

In order to investigate the stereoselectivity and the optimal conditions of dynamic kinetic resolution based on lipase mediated alcoholysis of 2-methyl-4-[(5-phenylfuran-2-yl)methyl]oxazol-5(4*H*)-ones *rac*-**3a–d**, followed by the transformation of the 2-acetamido-3-(5-phenylfuran-2-yl)propanoic acid esters *rac*-**2a–d** into enantiopure amino acids L-**4a–d**, the chromatographic separation of the enantiomers of *rac*-**1–4a–d** was first established as reported in Section 4.

A large number of commercially available lipases exhibit high substrate tolerance, leading to products with various degrees of enantioselectivity. Accordingly, in order to obtain L-2-acetamido-3-(5-phenylfuran-2-yl)propanoic acid esters L-**2a–d** in good yields and with high selectivities, several enzymes were tested as potential biocatalysts for the dynamic kinetic resolution of *rac*-**3a–d** (Scheme 2a). The reactions were first performed in neat anhydrous alcohols at room temperature. Four different alcohols (methanol, ethanol, propan-1-ol, and butan-1-ol) were used as solvents and nucleophiles. While CaL-A and CrL were catalytically inactive, Lipozyme from *Mucor miehei* (LMM), PPL and lipase AK showed moderate selectivity (enantiomeric excesses of 20–49%) and reactivity (5–10% conversion, after 24 h) in all of the tested alcohols. All catalytically active lipases showed the same enantiopreference in the alcoholysis. Since CaL-B adsorbed on macroporous acrylic resin (Novozyme 435) showed the most promising properties in all tested alcohols (Table 1), this enzyme was applied to further investigations.

The decreasing enantiopurities of the produced L-**2a–d** with increasing reaction conversions indicated that the optimal conditions for an efficient dynamic kinetic resolution were not satisfactory. The spontaneous racemization of oxazolone enantiomers L- and D-**3a–d** was not efficient enough to provide the complete transformation of *rac*-**3a–d** into L-**2a–d** with high enantiopurity, that is, the enzymatic reactions of the less reactive enantiomers were faster than the racemization of the substrates.

For an efficient enzymatic dynamic kinetic resolution, three general requirements must be met simultaneously, namely: the enzyme must stay active throughout the reaction; the less reactive enantiomer must undergo rapid racemization under the reaction conditions where the product of the enzyme-catalyzed reaction is stable; and finally, the racemizing agent should not catalyze non-enzymatic secondary reactions, which could decrease the enantiopurity of the desired product. In this way, the substrate should always be racemic, thus allowing the maximum enantiopurity at the theoretical zero conversion of the more reactive enantiomer while the ee of the product should remain constant during the reaction. The second criterion is mandatory for an enzymatic reaction with low or medium stereoselectivity since the ee of the product will decrease with increasing conversion in an inefficient dynamic kinetic resolution.



Scheme 1. Synthesis of racemic heteroarylalanines and their derivatives.