Journal of Molecular Catalysis B: Enzymatic 76 (2012) 68–74

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Enantioselective biocatalytic hydrolysis of β-aminonitriles to β-amino-amides using *Rhodococcus rhodochrous* ATCC BAA-870

Varsha Chhiba[a], Moira L. Bode[a,b], Kgama Mathiba[a], Wendy Kwezi[a], Dean Brady[a,*]

[a] *Enzyme Technologies, CSIR Biosciences, Pinelands, Ardeer Road, Modderfontein 1645, South Africa*
[b] *Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, Private Bag 3, PO WITS, 2050, South Africa*

ARTICLE INFO

Article history:
Received 30 October 2011
Received in revised form 9 December 2011
Accepted 9 December 2011
Available online 17 December 2011

Keywords:
Nitrile hydratase
Amidase
β-Aminonitrile
Biocatalysis
Biotransformation
Rhodococcus
Kinetic resolution

ABSTRACT

A range of β-aminonitriles (3-amino-3-phenylpropanenitrile and derivatives) were synthesised by reaction of various benzonitriles with acetonitrile and subsequent reduction of the resulting acrylonitrile products. These compounds were hydrolysed to the corresponding amides using the nitrile biocatalytic activity of *Rhodococccus rhodochrous* ATCC BAA-870. Results showed that the nitrile hydratase enzyme was enantioselective for these compounds, in particular 3-amino-3-*p*-tolylpropanenitrile and 3-amino-3-(4-methoxyphenyl)propanenitrile and the corresponding amides (up to 85% in one case). The reactions were performed at pH 9.0 after initial attempts at pH 7.0 were unsuccessful, most likely as a result of protonation of the 3-amino group at the lower pH.

1. Introduction

β-Amino-amides and acids can be used as building blocks for the synthesis of pharmaceutical intermediates. This includes biologically active peptides and small molecule pharmaceuticals [1]. β-Amino acids are constituents of compounds such as the antitumour drug Taxol [2], the antifungal antibiotic Cispentacin [3], and the antidiabetic drug Sitagliptin [4]. Another pharmaceutical application is for inclusion in peptidomimetics that may be of use as protease inhibitors against retroviruses such as HIV [5–7].

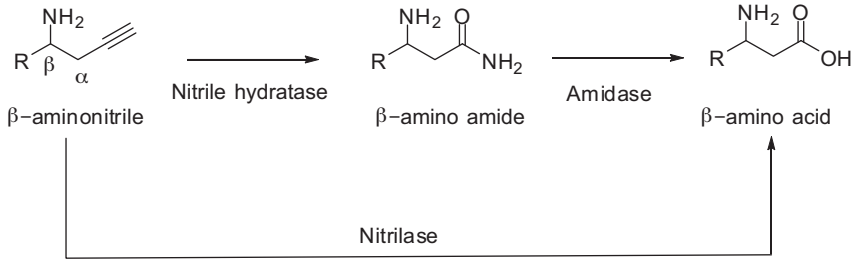
New potential applications for these compounds are being discovered continually. Wolin et al. [8] found that β-amino acid derivatives act as glycine transport inhibitors, while Zhu et al. [9] have discovered that others are proteasome inhibitors. Armour et al. [10] incorporated them into oxytocin inhibitors, Angelaud et al. [11] synthesised peptidase inhibitors and Imbriglio et al. included them in niacin receptor agonists for treatment of atherosclerosis and dyslipidemias [12].

There are a number of chemical synthesis methods for the preparation of racemic β^3-amino acids [13,14]. However, many applications require the β-amino substituted compounds as single enantiomers [1], and racemates thus need to be resolved. The classical method of resolving amino acids is through transformation of the racemate into a mixture of diastereomeric salts *via* complexation of the carboxylic acids with a chiral base, usually followed by multistep fractional recrystallisation.

Resolution may be achieved more simply through the application of biocatalysts, such as enzymatic resolution of β-amino esters and *N*-acyl derivatives [13] using lipases like CAL-B and the protease α-chymotrypsin; or by transesterification using lipase or acylase I [13]. Pousset et al. [15] demonstrated the application of *Burkholderia cepacia* lipase for hydrolytic resolution of heterocyclic β-amino acids, achieving greater than 99% ee. Tasnádi et al. [16–18] achieved ee values of 98–99% for both enantiomers of β-aryl-β-amino, β-arylalkyl-β-amino and β-heteroaryl-β-amino esters by enantioselective hydrolysis of the carboxylic acid ester using *B. cepacia* lipase (Amano PS) to hydrolyse the *S*-enantiomer. Li and Kanerva [19] used the lipase CAL-A to selectively acylate 3-amino-4-indolin-3-yl-butanenitrile, providing a 99% ee. A second option is to resolve the enantiomers through modification of the β-amine. *N*-acylation or deacylation can be performed by Acylase I and other enzymes [13]. Alternatively Forró et al. [20] and Tasnádi et al. [21] demonstrated enantioselective ring opening of β-lactams to provide β-amino acids with ee values of 99% using the lipase CAL-B. Aspartase can form β-amino acids through Michael addition. Hydantoinase hydrolysis of hydantoins, reductive amination by aminotransferase, and isomerisation by 2,3-aminomutase add

* Corresponding author at: CSIR Biosciences, Private bag X2, Modderfontein 1645, South Africa. Tel.: +27 82 467 6209.
E-mail address: dbrady@csir.co.za (D. Brady).

1381-1177/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2011.12.005



Scheme 1. Biocatalytic conversion of β-aminonitriles to β-amino amides and acids.

yet more options [13]. Recently phenylalanine aminomutase (PAM) has been applied in the enzymatic synthesis of (*R*)-β-arylalanines with high ee (>99%) [22].

Single enantiomer β-amino-amides and acids can also be obtained through the enzymatic hydrolysis of the related nitrile (Scheme 1). Nitrile hydrolysing enzymes (nitrilase or a combination of nitrile hydratase and amidase) provide a mild approach for the synthesis of carboxylic acids from nitriles [23]. β-Alanine, the only naturally occurring beta amino acid, can be derived from β-aminopropanenitrile using whole cell biocatalysts *Alcaligenes* sp. OMT-MY14, *Aminobacter* sp ATCC 23314 [24] and *Rhodococcus* sp [25]. β-Alanine is an achiral substrate, however nitrile and amide biocatalysts may act enantioselectively on chiral substrates, and this has been successfully applied to the resolution of *N*-protected cyclic β-amino acids [26–28]. Veitía et al. [29] demonstrated synthesis of *N*-protected β^3-amino acids using the Codexis range of nitrilases to hydrolyse the single enantiomer nitrile precursors. Biotransformation of alicyclic *N*-*p*-toluenesulfonyl- and *N*-butyloxycarbonyl protected β-aminonitriles to the *trans* amides and carboxylic acids using two strains of *Rhodococcus* (*Rhodococcus* sp. R312 and *R. erythropolis* NCIMB 11540) has been demonstrated [27]. With *Rhodococcus erythropolis* AJ270 cells 96.4% ee was achieved where the *N*-protected β-amino acid β-substituent was a c-Pr group [30].

β-Aminonitriles themselves can be prepared by a number of methods. Preiml et al. [26] used cyanide mediated ring opening of aziridines. González et al. [31] used a Mannich-type reaction to synthesise enantio-enriched (*R*)-unsubstituted β-aminonitriles through the organocatalytic addition of β-phenylsulfonylacetonitrile to either *N*-Boc-protected (*R*)-amido sulfones or imines.

Herein we synthesise various aryl substituted unprotected 3-amino-3-phenylpropanenitriles and demonstrate the enantioselective hydrolysis of these β-aminonitriles to the corresponding amides using *Rhodococcus rhodochrous* ATCC BAA-870, a nitrile hydrolysing organism isolated from soil [32,33], that expresses a benzamide induced cobalt type nitrile hydratase.

2. Experimental

2.1. General

Reactions were monitored by thin layer chromatography (TLC) on aluminium-backed Merck silica gel 60 F_{254} plates using an ascending technique. The plates were visualized both by UV at λ = 254 nm and then visually by colour development after treatment with ninhydrin spray. Gravity column chromatography was carried out on Merck silica gel 60 (70–230 mesh) and a mixture of ethyl acetate and hexane (or DCM and methanol) was used as eluent unless otherwise specified. Organic layers were dried over anhydrous $MgSO_4$ or anhydrous Na_2SO_4 before evaporation on a Büchi rotary evaporator RE 111 with a bath temperature of 40 °C or below, as required.

Nitrile and amide analysis was performed using a liquid chomatography (HPLC) system composed of a Waters 2690 separation module coupled with Waters diode array detector 996 (210–400 nm), a Waters X-Terra MS18 3.5 μm, 3.0 mm × 50 mm (ID × L) column (25 °C), with the isocratic eluent of 0.1% (v/v) trifluoroacetic acid in ultrapure water combined with acetonitrile (composition was varied according to the compound analysed) at a flow rate of 0.3–0.5 ml/min. The run time was 15–20.0 min, and all data handling was by Empower 2 Software.

Chiral nitrile analysis was performed using a liquid chromatography system composed of a Waters 600-MS Separation Module equipped with Waters 717 Autosampler, Waters 2486 UV/Visible Detector (210 nm) and Empower 2 software. Columns used were a Chiralpack AD-H, 250 mm × 4.6 mm, 5 μm and a Chiracel OD-H, 250 mm × 4.6 mm, 5 μm (Daicel Chemical Industries Ltd.). The eluent was n-hexane: isopropanol (both HPLC Grade) 90:10, % v/v and an isocratic flow rate of 1.00 ml/min at 25 °C with a run time of 35 min. The chromatographic system was conditioned for 1 h before the injection of samples. 3-Amino-3-phenylpropanenitrile (**1c**) enantiomers were eluted under these conditions at 12 and 13.6 min; 3-amino-3-*p*-tolylpropanenitrile (**2c**) at 11.0 and 12.7 min; 3-amino-3-(4-methoxyphenyl)propanenitrile (**3c**) at 19.0 and 23.0 min; 3-amino-3-(4-chlorophenyl)propanenitrile (**4c**) at 9.0 and 12.5 min; 3-amino-3-(4-bromophenyl)propanenitrile (**5c**) at 19.0 and 29.0 min; and 3-amino-3-(3-bromophenyl)propanenitrile (**6c**) at 19.0 and 29.8 min.

Chiral amide analysis was performed using a liquid chromatography system composed of a Waters 2690 Separation Module equipped with Photodiode Array Detector 996, and Empower 2 software. The column was a Crownpak CR (+), 150 mm × 4 mm (10 °C). The eluent used was 16.3 g/l perchloric acid, pH 2.00 in nano-pure water (MilliQ) at a flow rate of 0.25 ml/min over a run time of 20.0–60.0 min. The chromatographic system was conditioned for at least 1 h in advance with a column flow rate of 0.25 ml/min before the injection of samples. 3-Amino-3-phenylpropanamide (**1d**) enantiomers eluted at 10.9 and 12.9 min (210 nm); 3-amino-3-*p*-tolylpropanamide (**2d**) at 24.5 and 30.6 min (210 nm); 3-amino-3-(4-methoxy)phenylpropanamide (**3d**) at 20.0 and 23.4 min (225 nm), 3-amino-3-(4-bromophenyl)propanamide (**5d**) at 22.9 and 28.5 min (230 nm); and 3-amino-3-(3-bromophenyl)propanamide (**6d**) at 13.8 and 28.1 min (230 nm).

Uncorrected melting points were determined using a Reichert-Jung Thermovar hot-stage microscope or a Mettler FP62 capillary melting point apparatus.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded as either $CDCl_3$ or CD_3OD solutions with tetramethylsilane as an internal standard. Both a 400 MHz Varian Unity spectrometer as well as a 200 MHz spectrometer were used for all substrate and product analysis. The ^{13}C NMR spectra were recorded on the same instruments using tetramethylsilane as an internal standard. All chemical shifts were reported in ppm.