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Highly efficient and enantioselective synthesis of L-arylglycines and D-arylglycine amides from biotransformations of nitriles

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Abstract—Under very mild conditions, the *Rhodococcus* sp. AJ270-catalysed biotransformation of arylglycine nitriles **1**, prepared easily from the reaction of substituted benzaldehydes, ammonium chloride and potassium cyanide, proceeded efficiently to produce optically active D-arylglycine amides **2** and L-arylglycines **3** in excellent yields with enantiomeric excesses higher than 99%.

Arylglycines, a type of nonproteinogenic amino acid, and their derivatives have attracted much attention because such compounds exhibit intriguing biological activity. A number of α-arylglycines, for example, have been used as antagonists of metabotropic glutamate receptors,[1] while D-phenylglycine amide and its 4-hydroxyphenylglycine amide analogue have been used successfully to produce penicillin and cephalosporin antibiotics.[2] Arylglycines and their amides are also powerful and versatile building blocks in organic synthesis, and they have been employed widely as chiral auxiliaries and ligands in asymmetric reactions.[3] Syntheses of optically active α-amino acids including arylglycines and their derivatives are well documented in the literature.[4] Although asymmetric chemical syntheses of amino acid derivatives have advanced tremendously in recent years, enzymatic reactions still play an important part in the preparation and production of amino acids. Lipase- and esterase-catalyzed kinetic resolution of racemic amino acid esters and of *N*-acylated amino acids, for instance, has been extensively studied.[2,5,6] D- or L-Hydantoinases catalyse an efficient ring opening reaction of hydantoin to afford D- or L-amino acids, respectively, a process that has been developed into commercial production of D-amino acids.[7] Amidase has also been reported to effect amino acid synthesis through kinetic resolution of a racemic amino amide.[8] All methods reported, however, are limited to certain types of substrate and often require the preparation of the precursors or derivatives of the amino acids, which are sometimes laborious. A general asymmetric synthesis of optically active arylglycines and their amides utilising readily available starting materials should be of great interest.

Nitriles are important organic compounds and they are easily prepared and chemically transformed.[9] For example, the formation of an α-amino nitrile from an aldehyde or ketone, ammonium chloride and sodium cyanide, followed by hydrolysis (Strecker synthesis), is a simple method for preparing racemic α-amino acids. Unfortunately, chemical hydrolysis of nitriles including amino nitriles needs harsh conditions such as using strong acid or base, which always results in a low chemical yield and by-products. Biotransformations of nitriles using both microbial cells and enzymes have been demonstrated as being unique and environmentally benign methods for the synthesis of chiral carboxylic acids and their derivatives due to the excellent selectivity and very mild reaction conditions.[10] Our earlier work[11] has demonstrated that *Rhodococcus* sp. AJ270, a robust nitrile hydratase/amidase-containing microorganism, was able to hydrolyse a wide range of structurally diverse mono- and di-nitriles with excellent chemo- and regioselectivities. Very recently, we have found that *Rhodococcus* sp. AJ270 could efficiently and enantioselectively catalyse the hydrolysis of a number of racemic α-substituted phenylacetonitriles[12] and 2-arylcyclopropanecarbonitriles[13] to produce the corresponding enantiopure carboxylic acids and amides in high yields. The nitrile hydratase and/or amidase involved in *Rhodococcus* sp. AJ270 could also recognise the enantiotopic cyano group during the course of desymmetrisation of 3-arylglutaronitriles.[14] Our interest in understanding the reaction scope and limitations of both nitrile hydratase and amidase involved in *Rhodo-*

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coccus sp. AJ270, and in preparing chiral arylglycines and their amides has led us to undertake the current study.

Few examples of enantioselective biotransformations of α-amino nitriles have been reported. The nitrilase of *Rhodococcus rhodochrous* PA-34[15] and a nitrilase-containing *Acinetobacter* sp. culture[16] have the ability to convert several aliphatic DL-α-amino nitriles into L-amino acids in moderate to excellent enantiomeric excesses, while the nitrilase-catalysed hydrolysis of DL-phenylglycine nitrile using *Aspergillus furmigatus* has been reported to form L-phenylglycine in 80% e.e.[17] Using a mutant of *Brevibacterium* sp. R312, Arnaud et al.[18] claimed to obtain a L-specific amidase which transformed aliphatic DL-amino amides into L-amino acids and D-amino amides.

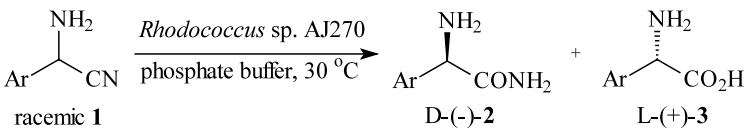
To begin our study, we first examined the biotransformation of DL-phenylglycine **1a**, a commercially available chemical, under various conditions (entries 1–4 in Table 1). Considering the optimal working pH for nitrile hydratase of *Rhodococcus* sp. AJ270 and to prevent the decomposition of phenylglycine nitrile in aqueous buffer, the biotransformation was performed in a phosphate buffer with pH 7.62. The hydration of nitrile was found to be very rapid with all the amino nitrile being consumed in 25 min. Quenching the reaction after 2.5 h gave D-phenylglycine amide **2a** and L-phenylglycine **3a** in ca. 50% yield each. The enantiomeric excesses obtained for amide **2a** and acid **3a** were not satisfactory, being 74 and 93%, respectively. The enantioselectivity of the biotransformation was then improved to >99% when the reaction was carried out in a phosphate buffer at pH 7.13. Although the transformation proceeded in 8 h at pH 7.13, which is slower than that at pH 7.62, no detrimental effect such as decomposition of the substrate **1a** was observed. The amidase involved in *Rhodococcus* sp. AJ270 appeared highly L-selective, as the complete conversion of both L- and D-amide was only effected after more than 1 day and with low substrate concentration. To test its usefulness in practical organic synthesis, we tried a gram-scale biotransformation of **1a** (6.75 mmol, 1.14 g), which produced almost identical chemical yields of **2a** and **3a** with e.e.s >99%.

In order to investigate the effect of the substituent on the phenyl ring on the reactivity and enantioselectivity of the biotransformation, we extended our range of substrates to other arylglycine nitriles that were prepared readily following the Strecker reaction from substituted benzaldehydes.[19] In the aqueous buffer with pH 7.13, however, the hydration of the substituted phenylglycine nitriles **1b–g** was slower than their parent analogue **1a**, and consequently they underwent decomposition to release aldehyde and cyanide that in turn caused inhibition of nitrile hydratase. The biotransformation of **1b–g** was then accelerated when the pH of the buffer was adjusted to 7.62 (Scheme 1). As illustrated in Table 1, the reaction rate and enantioselectivity were dependent on both the electronic and steric effects of the substituent. For example, the presence of a 2-methyl group on the phenyl ring retarded the reaction (entries 8–9), while 3-chlorophenylglycine nitrile **1c** led to amino acid **3c** in an enantiomeric excess of 87%. It is also worth noting that excellent enantioselectivities and high yields were obtained for most of the

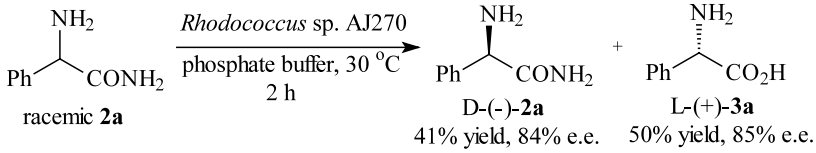
Table 1. Enantioselective biotransformation of arylglycine nitriles[20]

Entry	Sub. **1**	Ar	Conditions[a]	Arylglycine amide **2**		Arylglycine **3**	
				Yield[b] (%)	e.e.[c] (%)	Yield[b] (%)	e.e.[c] (%)
1	**1a**	C_6H_5	2 mmol, pH 7.62, 2.5 h	49	74	47	93
2	**1a**	C_6H_5	2 mmol, pH 7.13, 8 h	43	>99	52	>99
3	**1a**	C_6H_5	2 mmol, pH 7.13, 9.5 h	23	>99	58	81
4	**1a**	C_6H_5	1 mmol, pH 7.13, 25 h	–	–	93	5
5	**1b**	4-ClC_6H_4	2 mmol, pH 7.62, 4 h	42	>99	52	97
6	**1c**	3-ClC_6H_4	2 mmol, pH 7.62, 3 h	26	93	40	87
7	**1d**	4-MeC_6H_5	2 mmol, pH 7.62, 3 h	42	86	50	>99
8	**1e**	2-MeC_6H_5	2 mmol, pH 7.62, 5 h	62	17	30	>99
9	**1e**	2-MeC_6H_5	2 mmol, pH 7.62, 12 h	45	38	42	>99
10	**1f**	4-$MeOC_6H_5$	2 mmol, pH 7.62, 3.5 h	11	>99	47	>99
11	**1g**	3-$MeOC_6H_5$	2 mmol, pH 7.62, 1.5 h	47	71	46	>99
12	**1h**	3,4-$OCH_2OC_6H_3$	2 mmol, pH 7.62, 2 h	35	62	41	>99

[a] *Rhodococcus* sp. AJ270 cells (2 g wet weight) in phosphate buffer (0.1 M, 50 ml) were used. The reaction conditions were not optimised.
[b] Isolated yield.
[c] Determined by chiral HPLC analysis.[21]



Scheme 1.



Scheme 2.

arylglycines **3d–h**, while only with *para*-substituted phenylglycine nitriles such as **1b** and **1f** was good to excellent enantiocontrol observed for the corresponding amino amides.

To shed further light on the process, the racemic phenylglycine amide **2a**, obtained from the chemical hydration of phenylglycine nitrile **1a** using concentrated H_2SO_4 (98%), was fed to *Rhodococcus* sp. AJ270 under identical reaction conditions. The reaction was terminated at 50% conversion to give D-phenylglycine amide **2a** in 84% e.e. and L-phenylglycine **3a** in 85% e.e. (Scheme 2).

The outcome of the biotransformations of arylglycine nitriles **1** and of phenylglycine amide **2a** indicated that the amidase involved in *Rhodococcus* sp. AJ270 is highly L- or *S*-enantioselective, while the nitrile hydratase probably also exhibits L- or S-enantioselectivity but to a low degree.[22] Additionally, the enantioselectivity of nitrile hydratase is probably affected greatly by the structure of the amino nitriles. Therefore double *S*-enantioselections of nitrile hydratase and amidase resulted in the enantiopure arylglycines **3** and optically active arylglycine amides **2** with varied enantiomeric excesses depending on the substrates.

In conclusion, we have shown a very efficient, convenient and scale-upable method for the preparation of optically active L-arylglycines and D-arylglycine amides from the biotransformation of racemic arylglycine nitriles using *Rhodococcus* sp. AJ270 cells under very mild conditions. The overall enantioselectivity of the reaction was derived from the combined effects of a high L-enantioselective amidase and a low L-enantioselective nitrile hydratase involved in the cell.

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