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Microbial desymmetrization of 3-arylglutaronitriles, an unusual enhancement of enantioselectivity in the presence of additives

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Abstract

In the presence of an organic additive such as acetone or β-cyclodextrin or in a biphasic system of hexane and aqueous phosphate buffer, microbial desymmetrization of 3-arylglutaronitriles catalyzed by *Rhodococcus* sp. AJ270 cells proceeded regiospecifically and enantioselectively to produce *S*-(+)-3-aryl-4-cyanobutyric acids in high enantiomeric excess. Convenient chemoenzymatic syntheses of optically active *R*-(−)-4-amino-3-phenylbutyric acid and 4*R*-(−)-4-phenyltetrahydropyran-2-one are described.

Desymmetrization of prochiral compounds is one of the most important methods in asymmetric synthesis and much progress has been made during the past decade.[1] Enzymatic desymmetrization of prochiral diesters and diols has been extensively studied and used in the preparation of homochiral compounds in enantiopure form.[2] Desymmetrization of prochiral dinitriles, however, was not known until recently. Ohto[3] and Turner[4] and their co-workers independently investigated hydrolysis of *O*-substituted 3-hydroxyglutaronitriles using different *Rhodococcus* whole-cell catalysts and obtained the corresponding monocyanocarboxylic acids in good enantiomeric excess. When 3-benzylglutaronitrile was used as substrate, the stereoselectivity of the reaction was, however, extremely low.[3] It is also interesting to note that biotransformation of a disubstituted malononitrile catalyzed by *Rhodococcus rhodochrous* IFO15564 yielded an amido-acid with excellent enantioselectivity.[5]

Our previous studies have demonstrated that *Rhodococcus sp*. AJ270 is a powerful and robust nitrile hydratase/amidase-containing microorganism. It can efficiently hydrolyze a variety of nitriles[6] and dinitriles[7] with excellent chemo- and regioselectivity. It also showed high enantioselectivity against racemic nitriles such as α-substituted phenylacetonitriles[8] and *trans*-2-arylcyclo-

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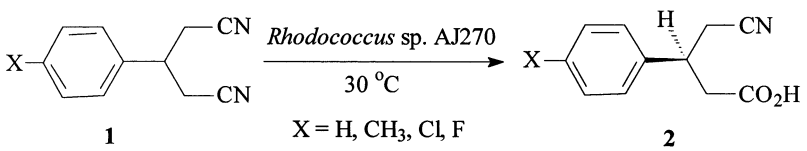
propanecarbonitriles.[9] To further explore its potential in asymmetric synthesis we undertook the current study of desymmetrization of 3-arylglutaronitriles, envisaging a simple and convenient synthesis of optically active 3-aryl-4-cyanobutyric acids which are important intermediates for the synthesis of chiral β-aryl-γ-aminobutyric acids (β-aryl-GABA) such as Baclofen.[10] We wish to report herein the *Rhodococcus* sp. AJ270-catalyzed desymmetrization of 3-arylglutaronitriles and an unusual enhancement of enantioselectivity caused by additives.

Interaction of 3-arylglutaronitriles **1** with *Rhodococcus* sp. AJ270 whole cells under various conditions yielded *S*-(+)-3-aryl-4-cyanobutyric acids **2** as the sole isolated product[11] (Scheme 1). In contrast to this observed regiospecificity, however, both the chemical conversion and enantioselectivity of the reaction were strongly dependent upon the nature of the substituent X at the *para* position of the aromatic ring and upon the reaction conditions employed (Table 1). The presence of either a bulkier electron-donating or electron-withdrawing group resulted in the decrease of the conversion rate, indicating a steric limitation of the nitrile hydratase although the substituent X is remote from the cyano function. Halogen substituents usually gave low enantioselectivity. Prolonged incubation times led to a slight increase in chemical conversion, but to a dramatic decrease of the enantiomeric excess of **2**. Surprisingly, addition of acetone (3 ml) as a co-solvent in the reaction gave rise to a significant improvement of enantioselectivity in all cases. Similar enhancement of enantioselectivity was also observed when β-cyclodextrin (β-CD) was used as an additive or when the incubation was performed in a biphasic system of hexane and aqueous phosphate buffer. The precise reason for the improvement of enantioselectivity in the presence of an organic additive is not clear at the moment, although these



Scheme 1.

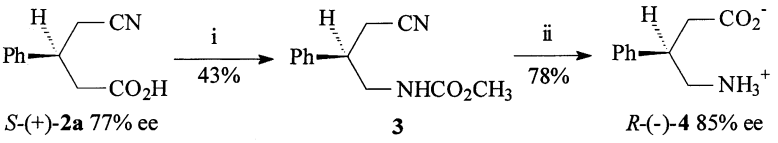
Table 1
Microbial desymmetrization of 3-arylglutaronitriles **1**[a]

Entry	X	Time (h)	Additive	**2** (%[b])	Ee (%[c])	**1** (%[b])
1	H	12	–	**2a** (56)	61	32
2	H	24	–	**2a** (88)	39	2
3	H	24	Acetone	**2a** (67)	88	21
4	H	12	β-CD	**2a** (61)	80	21
5	H	8	Hexane	**2a** (48)	84	39
6	CH_3	24	–	**2b** (42)	64	44
7	CH_3	24	Acetone	**2b** (25)	95	61
8	Cl	72	–	**2d** (37)	26	49
9	Cl	72	Acetone	**2d** (25)	63	63
10	F	24	–	**2e** (81)	25	6
11	F	24	Acetone	**2e** (16)	76	70

[a] Reaction was carried out in an aqueous phosphate buffer solution.
[b] Isolated yield.
[c] Determined by chiral HPLC analysis.[12]

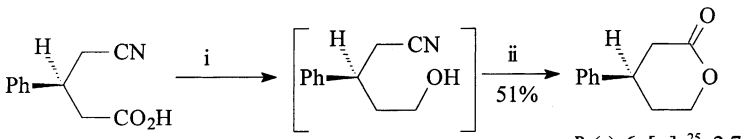
compounds may possibly act as inhibitors to deactivate a racemase. It should be pointed out that in some cases addition of acetone or hexane may also inhibit the nitrile hydratase, leading to lower catalytic efficiency.

S-(+)-3-Aryl-4-cyanobutyric acids **2** are useful chiral intermediates in organic synthesis. A straightforward Curtius rearrangement of **2a**, followed by acidic hydrolysis therefore afforded *R*-(−)-4-amino-3-phenylbutyric acid **4**, a clinically used mood elevator and tranquillizer,[10a] whilst the reduction of carboxylic acid ester of **2a**, followed by intramolecular hydrolytic cyclization yielded 4*R*-(−)-4-phenyltetrahydropyran-2-one **6**, a very useful intermediate in natural products synthesis[13] (Scheme 2).



S-(+)-**2a** 77% ee **3** *R*-(-)-**4** 85% ee

i. (a) $ClCO_2Et$, Et_3N, -5 °C; (b) NaN_3; (c) MeOH, reflux 3h. ii. HCl/H_2O reflux 48h



S-(+)-**2a** 72% ee **5** *R*-(-)-**6** $[\alpha]_D^{25}$-2.7

i. (a) CH_2N_2, Et_2O; (b) $LiBH_4$, THF reflux 8h; ii. (a) NaOH/H_2O; (b) *p*-$CH_3C_6H_4SO_3H$, THF

Scheme 2.

In summary, desymmetrization of 3-arylglutaronitriles catalyzed by *Rhodococcus* sp. AJ270 gave optically active *S*-(+)-3-aryl-4-cyanobutyric acids and the enantiomeric selectivity was improved dramatically by adding additives such as acetone and β-cyclodextrin or by using a biphasic system of hexane and an aqueous phosphate buffer. We have also provided novel and convenient chemoenzymatic methods for the preparation of optically active *R*-(−)-4-amino-3-phenylbutyric acid and 4*R*-(−)-4-phenyltetrahydropyran-2-one.

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References

1. For a useful review, see: Schoffers, E.; Golebiowski, A.; Johnson, C. R. *Tetrahedron* **1996**, *52*, 3769–3826.
2. For a comprehensive treatise, see: *Biotechnology*; Rehm, H.-J.; Reed, G.; Pühler, A.; Stadler, P., Eds.; Wiley-VCH: Weinheim, 1998; Vol. 8a.
3. Kakeya, H.; Sakai, N.; Yokoyama, M.; Sugai, T.; Ohta, H. *Chem. Lett.* **1991**, 1823–1284.
4. Beard, T.; Cohen, M. A.; Parratt, J. S.; Turner, N. J.; Crosby, J.; Moilliet, N. J. *Tetrahedron*: *Asymmetry* **1993**, *4*, 1085–1104.

5. Yokoyama, M.; Sugai, T.; Ohta, H. *Tetrahedron Lett*. **1993**, *4*, 1081–1084.
6. Meth-Cohn, O.; Wang, M.-X. *Tetrahedron Lett*. **1995**, *36*, 9561–9654; Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1099–1104.
7. Meth-Cohn, O.; Wang, M.-X. *Chem. Commun*. **1997**, 1041–1042; Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3197–3204.
8. Wang, M.-X.; Lu, G.; Ji, G.-J.; Huang, Z.-T.; Meth-Cohn, O.; Colby, J. *Tetrahedron*: *Asymmetry* **2000**, *11*, 1123–1135.
9. Wang, M.-X.; Feng, G.-Q. *Tetrahedron Lett*. **2000**, *41*, 6501–6505.
10. For stereoselective synthesis of β-aryl-γ-aminobutyric acids, see: (a) Allan, R. D.; Bates, M. C.; Drew, C. A.; Duke, R. K.; Hambley, T. W.; Johnston, G. A. R.; Mewett, K. N.; Spence, I. *Tetrahedron* **1990**, *46*, 2511–2524; (b) Herdeis, C.; Hubmann, H. P. *Tetrahedron: Asymmetry* **1992**, *3*, 1213–1221; (c) Schoenfelder, A.; Mann, A.; Le Coz, S. *Synlett* **1993**, 63–64; (d) Chenevert, R.; Desjardins, M. *Can. J. Chem*. **1994**, *72*, 2312–2317.
11. General procedure for the biotransformation: Dinitrile (2 mmol) was incubated with *Rhodococcus* sp. AJ270 (2 g wet weight) in an aqueous phosphate buffer (pH 7.0) at 30°C for a period of time. After removal of biomass through filtration, the resulting solution was acidified using HCl (2 M) to pH 2 and extracted with diethyl ether. Acid was obtained after removal of the solvent. All products were characterized by their spectral data and microanalysis.
12. Chiral HPLC analysis of monocyano-acid products was performed using a Chiracel OD column with hexane:2-propanol [180:1 or 90:10] as the solvent at a flow rate of 0.2 or 0.8 ml/min.
13. (+)-(*S*)-4-Phenyltetrahydropyran-2-one, 42% ee, $[\alpha]_D^{25}$ 1.73° (*c* 6); 98% ee, $[\alpha]_D^{25}$ 3.80°. See: (a) Lam, L. K. P.; Hui, R. A. H. F.; Jones, J. B. *J. Org. Chem*. **1986**, *51*, 2047–2050. (b) Meyers, A. I.; Smith, R. K.; Whitten, C. E. *J. Org. Chem*. **1979**, *44*, 2250.