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Highly enantioselective biotransformations of 2-aryl-4-pentenenitriles, a novel chemoenzymatic approach to (*R*)-(−)-baclofen

Mei-Xiang Wang* and Sheng-Min Zhao

Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China

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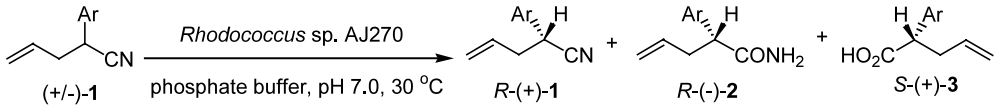
Abstract—Catalyzed by *Rhodococcus* sp. AJ270 microbial cells under mild conditions, a range of racemic 2-aryl-4-pentenenitriles **1** underwent effective hydrolysis to afford excellent yields of enantiomerically pure (*R*)-(−)-2-aryl-4-pentenamides **2** and (*S*)-(+)-2-aryl-4-pentenoic acids **3** in most cases. The application of this biotransformation has been shown by a two-step synthesis of (*R*)-(−)-baclofen.

Biotransformations of nitriles, either through a direct conversion from a nitrile to a carboxylic acid catalyzed by a nitrilase[1] or through the nitrile hydratase-catalyzed hydration of a nitrile followed by amide hydrolysis catalyzed by an amidase,[2] are effective and environmentally benign methods for the production of carboxylic acids and their amide derivatives. Microbial hydration of acrylonitrile to acrylamide, for instance, has become one of the largest industrial biotransformations in the world.[3] Recent endeavors by others[4] and us[5] have demonstrated that biotransformations of nitriles are also a unique complementary addition to the existing asymmetric chemical and enzymatic synthesis of carboxylic acids and their derivatives. One of the distinct features of enzymatic transformation of nitriles is not only the formation of enantiopure carboxylic acids, but also the straightforward generation of enantiopure amides, valuable organonitrogen compounds in organic synthesis.[6]

Rhodococcus sp. AJ270, a novel isolate from a soil sample,[7] appears to be a robust and useful nitrile hydratase/amidase-containing biocatalyst. Compared with other microbes reported, it displays broad enzymatic activity against almost all types of nitrile including aromatic, heterocyclic and aliphatic ones, and both amides and acids can be obtained in high yields from appropriate nitriles.[8] It shows excellent regioselectivity in hydrolyzing aromatic dinitriles and a variety of aliphatic dinitriles bearing a suitably placed second chelating moiety.[9] It has been demonstrated recently that *Rhodococcus* sp. AJ270 is an efficient enantioselective biocatalytic system able to transform some racemic nitriles such as α-alkyl[5a,b] and α-amino[5c] substituted arylacetonitriles, and 2-arylcyclopropanecarbonitriles[5d,e] into the corresponding amides and acids in enantiomerically pure form. Our interests in understanding the mechanism of enantioselective biotransformations of nitriles and in exploring this methodology to create unique and versatile chiral intermediates led us to undertake the current study. We wish to report herein a direct and convenient microbial production of 2-aryl-4-pentenoic acid derivatives and a new chemoenzymatic synthesis of (*R*)-(−)-baclofen.



Scheme 1. Biotransformations of racemic nitriles **1**.

Keywords: biotransformations; nitrile hydratase; amidase; chemoenzymatic synthesis; (*R*)-(−)-baclofen.
* Corresponding author. Tel.: +8610-62554628; fax: +8610-62569564; e-mail: mxwang@infoc3.icas.ac.cn

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To begin our study, we first examined the reaction of 2-phenyl-4-pentenenitrile **1a**. Catalyzed by *Rhodococcus* sp. AJ270 cells under very mild conditions,[10] **1a** underwent an efficient and a complete hydration reaction to form amide **2a** in a few hours. Highly optically pure (*R*)-(−)-2-phenyl-4-pentenamide **2a**[11] and (*S*)-2-phenyl-4-pentenoic acid **3a**[11] were obtained in almost quantitative yield after about 3 days incubation (Scheme 1). As illustrated in Table 1, an increase of and a decrease of the enantiomeric excesses of the amide **2a** and of the acid **3a**, respectively, during the progress of amide hydrolysis indicated a kinetic resolution of the amide effected by the *S*-enantioselective amidase involved in *Rhodococcus* sp. AJ270 cells.

To test the generality of this biotransformation and also to gain a better understanding of the enantioselectivity of nitrile hydratase and amidase enzymes, a number of 2-aryl-4-pentenenitrile analogs **1b**–**g** were synthesized[13] and subjected to biocatalytic hydrolysis.

The results summarized in Table 1 show a dramatic effect of the substituent attached to the benzene ring on both the reaction rate and enantioselectivity. When a *para*-fluorine group was introduced into the molecule, no detrimental influence was observed, as the hydrolysis of **1b** proceeded similarly and as efficiently to that of the parent substrate **1a** to give enantiopure (*R*)-amide **2b** and (*S*)-acid **3b** in excellent yield. Introduction of other substituents such as chlorine, methoxy and methyl at different positions on the benzene ring, however, resulted in slow conversions and, in some cases, low optical yield under identical reaction conditions. For example, after a week of exposure to the biocatalyst, the reaction of nitriles **1c**, **1d** and **1e** still gave a considerable amount of the starting materials (entries 5, 7 and 9). Only when the concentration of the substrate was halved and the incubation continued for a longer period, did the hydration reaction go to completion, with higher yields of the corresponding amide and acid being obtained (entries 6, 8 and 10). The slow hydration rate observed for these nitriles revealed that the nitrile hydratase is very sensitive to the steric effect of substituents, even remote from the cyano function. On the other hand, in most cases, the transformation of the amide into the acid was effective, except for **2g** which remained almost intact after 7 days reaction (entry 12). The sluggish hydrolysis of **2g** was most probably due to the crowding in the molecule caused by the *ortho*-methylphenyl group, which inhibited amidase action. It is interesting to note that substitution of the benzene ring with a fluorine or a methoxy group facilitated the conversion of amide into the acid while the presence of chlorine or methyl groups slowed down the amide hydrolysis.

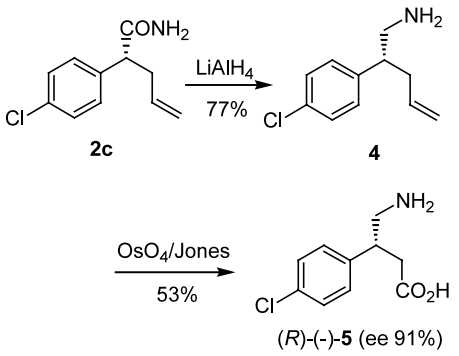
The low to moderate enantiomeric excess values obtained for the recovered nitriles (*R*)-(+)-**1c**–**e**[11] demonstrated a less effective enantioselectivity of the *S*-nitrile hydratase (entries 5, 7 and 9). In contrast, the amidase showed a very high *S*-enantioselection, which was exemplified by the excellent enantiomeric excess achieved for most of the (*S*)-acids. Only for (*S*)-(+)-acids **3d** and **3g**, did slightly lower optical yields result. It is worth noting that the overall enantioselectivity of the reaction is the result of the combined actions of *S*-nitrile hydratase and *S*-amidase. In other words, the double selections of two *S*-enantioselective enzymes account for the high enantiomeric excesses of the products.

The biotransformation of 2-aryl-4-pentenenitriles provides a simple and straightforward approach to enantiopure 2-aryl-4-pentenoic acids and their amide derivatives. The former compounds were only available from a somewhat tedious resolution[14] using a chiral base or from the less efficient multi-step asymmetric synthesis.[15] To demonstrate the synthetic potential of enantioselective biotransformations of nitriles which afford straightforwardly the enantiopure organonitrogen compounds, we carried out a synthesis of (*R*)-(−)-baclofen.

Table 1. Biotransformations of racemic 2-aryl-pentenenitriles **1**

Entry	Substrate	Ar	Conditions[a]	Nitrile **1**		Amide **2**		Acid **3**	
				Yield (%)[b]	Ee (%)[c]	Yield (%)[b]	Ee (%)[c]	Yield (%)[b]	Ee (%)[c]
1	**1a**	C_6H_5	1 mmol, 58 h	–	–	62	70.8	38	>99.5
2	**1a**	C_6H_5	1 mmol, 69 h	–	–	49	99.2	49	96.8
3	**1a**	C_6H_5	1 mmol, 83 h	–	–	39	>99.5	61	80.1
4	**1b**	4-F-C_6H_4	1 mmol, 60 h	–	–	50	>99.5	50	99.3
5	**1c**	4-Cl-C_6H_4	1 mmol, 7 d	36	59.1	20	99.2	39	99.2
6	**1c**	4-Cl-C_6H_4	0.5 mmol, 6 d	Trace	–	44	99.3	50	>99.5
7	**1d**	4-MeO-C_6H_4	1 mmol, 7 d	11	7.1	37	>99.5	49	89.7
8	**1d**	4-MeO-C_6H_4	0.5 mmol, 73 h	–	–	47	>99.5	51	87.4
9	**1e**	4-Me-C_6H_4	1 mmol, 8 d	41	47.0	28	3.7	26	>99.5
10	**1e**	4-Me-C_6H_4	0.5 mmol, 7 d	–	–	49	>99.5	49	94.3
11	**1f**	3-Me-C_6H_4	0.5 mmol, 6 d	–	–	49	>99.5	49	>99.5
12	**1g**	2-Me-C_6H_4	0.5 mmol, 7 d	Trace	–	93	3.2	4	78.5

[a] *Rhodococcus* sp. AJ270 cells (2 g wet weight) in phosphate buffer (0.1 M. pH 7.0, 50 ml) were used. The reaction conditions were not optimized.
[b] Isolated yield.
[c] Determined by chiral HPLC.[12]



Scheme 2. Synthesis of (*R*)-(−)-baclofen.

(*R*)-(−)-baclofen, as a specific $GABA_B$ (γ-aminobutyric acid) receptor agonist and a potent antispastic agent,[16] has attracted much attention in recent years. Syntheses of optically active (*R*)-(−)-baclofen have been reported either through the chymotrypsin mediated hydrolysis of dimethyl 3-(4-chlorophenyl)glutarate followed by several chemical transformations,[17] multi-step asymmetric reactions employing the Evans chiral enolate protocol,[18] or multi-step stereoselective transformations from (*S*)-glutamic acid[19] and (*S*)-3-(4-chlorophenyl)pyrrolidine.[20] With (*R*)-2-(4-chlorophenyl)-4-pentenamide **2c** in hand, we performed a direct and convenient two-step synthesis of (*R*)-(−)-baclofen utilizing routine reduction and oxidation reactions. Thus, in the presence of $LiAlH_4$, (*R*)-(−)-amide **2c** was reduced to (*R*)-2-(4-chlorophenyl)-4-pentenylamine **4**[21] in 77% yield. Direct oxidation of **4** using OsO_4 and Jones' agent led to the formation of (*R*)-(−)-baclofen **5**[22] in 53% yield (Scheme 2). To our knowledge, this is the shortest synthetic route to (*R*)-(−)-baclofen known so far.

In summary, we have shown a highly enantioselective synthesis of 2-aryl-4-pentenoic acids and their amides from the biotransformations of easily prepared[13] 2-aryl-4-pentenenitriles utilizing *Rhodococcus* sp. AJ270 cells under very mild conditions. The potential applications of this reaction have been demonstrated by the preparation of (*R*)-(−)-baclofen by a very short route. Further synthetic applications of enantiopure (*S*)-2-aryl-4-pentenoic acids and their (*R*)-amides are being actively investigated in this laboratory and will be published in due course.

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10. General procedure for the biotransformation reaction: To an Erlenmeyer flask (150 ml) with a screw cap was added *Rhodococcus* sp. AJ270 cells (2 g wet weight) and potassium phosphate buffer (0.1 M, pH 7.0, 50 ml), and the resting cells were activated at 30°C for 0.5 h with orbital shaking. Nitrile **1** was added in one portion to the flask and the mixture was incubated at 30°C using an orbital shaker (200 rpm). The reaction, monitored by TLC, was quenched after the specified period of time by removing the biomass by filtration through a Celite pad. The resulting aqueous solution was basified to pH 12 with aqueous NaOH (2 M). Extraction with ethyl acetate gave, after drying ($MgSO_4$), removal of solvent, and column chromatography, the unconverted nitrile **1** and amide **2**. The aqueous solution was then acidified using aqueous HCl (2 M) to pH 2 and extracted with ethyl acetate to give acid **3**.
11. The absolute configurations of (*S*)-2-aryl-4-pentenoic acids **3** were determined by measuring and comparing their specific rotations with that of authentic (*S*)-2-phenyl-4-pentenoic acid reported in the literature (see: Refs. 14 and 15). The absolute configurations of (*R*)-2-aryl-4-pentenamides **2** and of (*R*)-2-aryl-4-pentenenitriles amides **1** were obtained by converting them to the acids and then measuring the specific rotation. Selected data: Compound **2c**: solid (43%); Mp 99–100.3°C; $[\alpha]_D^{25}$ −71.0 (*c* 2.3, $CHCl_3$); ee 99.3%; IR (KBr) ν_{max} 3417, 3200 ($CONH_2$), 1653 (C=O), 1620 cm^{-1} (C=C); ^1H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, $J=8.3$ Hz, 2H, ArH), 7.24 (d, $J=8.3$ Hz, 2H, ArH), 5.99 (s, br., 1H, CON*H*H), 5.73–5.53 (m, 1H), 5.53 (s, br., 1H, CONH*H*), 5.05 (d, $J=12.3$ Hz, 1H), 5.00 (d, $J=10.9$ Hz, 1H), 3.44 (t, $J=7.5$ Hz, 1H), 2.90–2.80 (m, 1H), 2.53–2.46 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 175.1, 137.7, 135.2, 133.3, 129.4, 129.0, 117.3,

51.9, 37.3; MS (EI) m/z (%) 211 (M^++2, 5), 209 (M^+, 14), 168 (23), 167 (36), 166 (39), 165 (100), 153 (12), 150 (30), 142 (10), 140 (30), 131 (32), 130 (50), 129 (43), 128 (19), 127 (33), 125 (64). Found: C, 62.84; H, 5.71; N, 6.48. $C_{11}H_{12}NOCl$ requires: C, 63.01; H, 5.77; N, 6.68. Compound **3c**: oil (50%); $[\alpha]_D^{25}$ +49.8 (c 2.75, $CHCl_3$); ee>99.5%; IR (KBr) ν_{max} 3400–2800 (COOH), 1708 (C=O), 1644 cm^{-1} (C=C); ^1H NMR (300 MHz, $CDCl_3$) δ 11.4 (s, br., COOH), 7.37 (d, J=8.5 Hz, 2H, ArH), 7.32 (d, J=8.5 Hz, 2H, ArH), 5.80–5.69 (m, 1H), 5.14 (d, J=18.2 Hz, 1H), 5.09 (d, J=10.8 Hz, 1H), 3.70 (t, J=7.6 Hz, 1H), 2.92–2.82 (m, 1H), 2.73–2.53 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 179.3, 136.2, 134.4, 133.5, 129.4, 128.9, 117.6, 50.7, 37.0; MS (EI) m/z (%) 212 (M^++2, 4), 210 (M^+, 11), 171 (35), 169 (100), 167 (26), 165 (78), 143 (12), 141 (40), 129 (70), 128 (45), 77 (35). HRMS: 209.0372 (M–H). $C_{11}H_{10}O_2Cl$ requires 209.0374.

12. Enantiomeric excess values were obtained from HPLC analysis using a Chiracel OD, OJ or OB column with a mixture of n-hexane and 2-propanol (9:1) as the mobile phase.
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21. Selected data for compound **4**: oil (77.4%); $[\alpha]_D^{25}$ −12 (c 1, $CHCl_3$); IR (KBr) ν_{max} 1640 (C=C), 1595 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.31 (d, J=8.3 Hz, 2H, ArH), 7.13 (d, J=8.3 Hz, 2H, ArH), 5.74–5.60 (m, 1H), 5.00 (d, J=14.9 Hz, 1H), 4.96 (d, J=8.6 Hz, 1H), 2.95–2.99 (m, 1H), 2.81–2.88 (m, 1H), 2.67–2.70 (m, 1H), 2.30–2.44 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 141.4, 136.1, 132.2, 129.3, 128.7, 116.5, 48.6, 47.2, 38.2; MS (EI) m/z (%) 195 (M^+, 2), 30 (100). HRMS: 196.0889 (M+H). $C_{11}H_{15}NCl$ requires: 196.0887.
22. Selected data for compound **5**: solid (53.2%); Mp 161°C (dec.) (lit.[20] mp. 215°C for (*R*)-baclofen hydrochloride); $[\alpha]_D^{25}$ −2.0 (c 0.2, H_2O) [lit.[20] $[\alpha]_D^{25}$ −1.3 (c 0.2, H_2O)]; ee 91% (HPLC analysis using a CR(+) chiral column); IR (KBr) ν_{max} 3400–2800 (br.), 1627 (C=O), 1572, 1540 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 7.33 (d, J=7.7 Hz, 2H, ArH), 7.22 (d, J=7.5 Hz, 2H, ArH), 3.22 (d, J=11.3 Hz, 2H), 3.06–3.14 (m, 1H), 2.55–2.37 (m, 2H); ^{13}C NMR(D_2O) δ 179.4, 138.1, 132.9, 129.3, 129.1, 44.0, 42.1, 40.7.