Synthesis of Optically Active β - Alkyl - α - methylene - γ - butyrolactones from Enantioselective Biotransformation of Nitriles, an Unusual Inversion of Enantioselectivity†

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A new approach to optically active β-alkyl-α-methylene-γ-butyrolactone derivatives was reported from the *Rhodococcus* sp. AJ270-catalyzed hydrolysis of appropriate nitriles. The inversion of enantioselectivity of the amidase has been observed when a methyl protection was introduced into the hydroxy group of the parent substrate.

Keywords biotransformation, nitrile hydratase, amidase, optically active β-alkyl-α-methylene-γ-butyrolactone, inversion of enantioselectivity

Introduction

Biotransformations of nitriles, either through a nitrilase-catalyzed direct conversion to the carboxylic acids or via a nitrile hydratase-catalyzed hydration to the amides followed by the hydrolysis to the acids mediated by an amidase, have provided a useful and environmentally benign approach to the optically active carboxylic acids and their derivatives.[1] *Rhodococcus* sp. AJ270, a novel strain recently isolated from a soil sample,[2] is a powerful and robust nitrile hydratase/amidase-containing microorganism. It has been demonstrated, for example, that *Rhodococcus* sp. AJ270 shows broad enzymatic activity against almost all types of nitriles including aromatic, heterocyclic and aliphatic ones, and both amides and acids can be obtained in high yields from appropriate nitriles.[3] It also displays excellent regioselectivity in hydrolyzing aromatic dinitriles and a variety of aliphatic dinitriles bearing a suitably placed second chelating moiety.[4] Very recently, we have found that *Rhodococcus* sp. AJ270 acted as an efficient enantioselective biocatalytic system able to transform some racemic nitriles such as α-substituted arylacetonitriles,[5,6] 2-arylcyclopropanecarbonitriles[7] and α-amino nitriles[8] into the corresponding amides and acids in enantiomerically enriched form. Our interest in understanding the mechanism of catalysis of the nitrile hydratase and the amidase involved in *Rhodococcus* sp. AJ270, particularly the enantioselectivity of these two enzymes against nitrile and amide substrates, and in exploring the application of *Rhodococcus* sp. AJ270 microbial whole-cell system in asymmetric synthesis has led us to study the biotransformation of nitriles bearing a chiral center remote to the cyano function.

Although a number of enantioselective biotransformations of nitriles have been investigated since the past decade, the substrate types, however, are mainly confined to nitriles having an adjacent chiral center. Except for the prochiral dinitriles containing a chiralgenic center at β-[9] or γ-position,[10-12] the enantioselective biotransformation of racemic nitriles bearing a remote chiral center remains decisively unclear. For example, *Rhodococcus* sp. 361, an immobilized whole-cell catalyst from Novo Industri A/S, efficiently catalyzed the hydrolysis of a variety of nitriles bearing a remote chiral center but no stereoselectivity was reported.[13] The nitrilase involved in *Rhodochrous rhodochrous* NCIMB 11216 did not show

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enantioselection against either enantiomers of β-phenylbutyronitrile.[14] Using *Rhodococcus rhodochrous* IFO 15564, Ohta and his coworkers[15] recently achieved efficient kinetic resolution of racemic 3-benzoyloxypentanenitrile, a nitrile substrate containing a β-chiral center. The same microbial strain has been shown to induce low to moderate enantioselectivity when it catalyzed the hydrolysis of γ-hydroxylated alkylnitriles.[16]

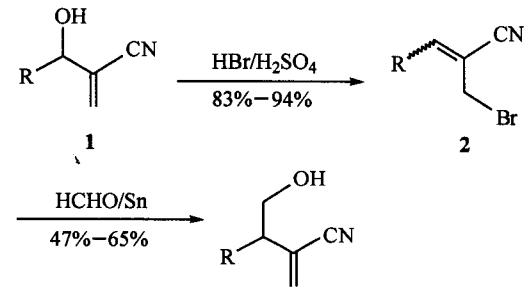
During the study of biotransformations of nitriles a few years ago, we[3b] discovered an interesting *ortho*-substituent effect. Catalyzed by *Rhodococcus* sp. AJ270, acrylonitrile was rapidly hydrolyzed to give acrylic acid without accumulation of acrylamide intermediate while methacrylonitrile was efficiently transformed into methacrylamide and methacrylic acid in a short incubation period. Considering the slower reaction of amide hydrolysis and enantio-discriminative nature of the amidase we envisaged that the β-substituted α-methylenepropionitriles, the nitrile substrates bearing a β-chiralgenic center, would be suitable for enantioselective biotransformation. Furthermore, if such a substrate containing an additional functional group such as hydroxy, the biotransformation would provide a novel approach to optically active α-methylene-γ-butyrolactone that is the key intermediate in organic synthesis.[17] Moreover, these racemic substrates, different from more extensively studied α-substituted chiral nitriles, would also serve as unique tools to probe the active sites of the enzymes.

Results and discussion

The required substrates, β-alkyl-γ-hydroxy-α-methylenebutyronitriles (**3**), were prepared from the Baylis-Hillman nitriles[18] (**1**) (Scheme 1). Treatment of **1** with hydrobromic acid in the presence of sulfuric acid afforded allylic bromide derivatives (**2**) in excellent yields.[19] The reaction[20] of **2** with formaldehyde, promoted by tin, led to nitriles **3**.

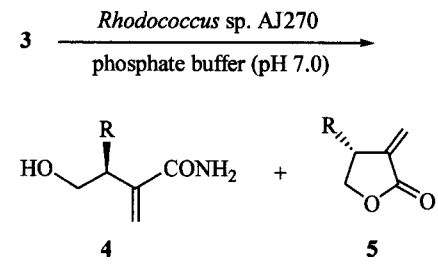
Catalyzed by the *Rhodococcus* sp. AJ270 cells, nitriles **3** underwent hydrolysis to give optically active *S*-(+)-β-substituted-γ-hydroxy-β-methylenebutyramides (**4**) and *R*-(+)-β-substituted-α-methylene-γ-butyrolactones (**5**) (Scheme 2). No free acid product was isolated or detected. Although it was difficult to rule out the possibility of a very rapid chemical lactonization reaction of the acid precursor, the formation of γ-butyrolactone (**5**) most likely resulted from intramolecular cyclization of the acyl-enzyme intermediate.[16] Chemical hydrolysis of **4** in hydrochloric acid (2 mol/L) at ambient temperature afforded *S*-(−)-β-substituted-α-methylene-γ-butyrolactones (**6**) (Scheme 3). The configurations of products **4**—**6** were determined by the comparison of optical rotation of lactones **5** and **6** with that of (*R*)-(+)-β-methyl-α-methylene-γ-butyrolactone,[21] assuming the change of β-alkyl group does not change the direction of the optical rotation.

Scheme 1 Preparation of nitriles **3**

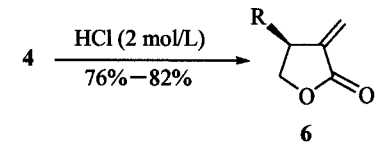


a, R=C_2H_5; **b**, R=$CHMe_2$; **c**, R=C_5H_{11}

Scheme 2 Biotransformation of nitriles **3**



Scheme 3 Chemical hydrolysis of amides **4**



The biocatalytic hydration of the nitriles **3** was found very efficient, and in almost all cases they were transformed into the amides **4** in a few hours. The conversion of the amides **4** into the lactones **5**, however, appeared slower. As tabulated in Table 1, the nature of the substituent R played an important part in determining the

biotransformation efficiency. For the substrate **3a** containing a small alkyl group such as ethyl, a rapid and complete hydrolysis was observed. Quenching the reaction in 3 h led to the kinetic resolution to give **4a** and **5a** in good yields, albeit with low enantioselectivity. With the increase of the bulkiness of the substituent R, the biotransformation became less effective; only after the interaction with microbial cells for several days, did the isopropyl-substituted nitrile **3b** give an appreciable amount of lactone product **4b**. It is interesting to note that the bulkier the substituent R, the slower the reaction, and the higher the enantioselectivity. This noticeable substituent effect indicated that the amidase involved in *Rhodococcus* sp. AJ270 is more sensitive than the nitrile hydratase towards the steric effect of the substrates, even with the substituent being remote from the reactive functionality. It should also be noted that the amidase displays its *R*-enantioselectivity against amide **4** containing a chiralgenic center at the β-position, in contrast to the *S*-enantioselectivity that has always been observed for the amide substrate bearing an α-chiralgenic center.[1,5-8]

To understand the role of the free hydroxy played in the biotransformation, the nitriles **3** were reacted with methyl iodide with the aid of sodium hydride, and the subsequent β-substituted γ-methoxy-α-methylenebutyronitriles (**7**) were then fed to the *Rhodococcus* sp. AJ270 cells. With a methyl protection group on the hydroxy, the biohydrolysis of **7** proceeded in a longer period to afford the corresponding amide **8** and the acid **9** (Table 2). To determine the absolute configurations and the enantiomeric excesses of the products, both **8** and **9** were treated with hydroiodic acid to give demethylative lactonization products **5** and **6**, respectively (Scheme 4). Except for **7a**, introduction of methyl protection group into the substrates **7b** and **7c** did not improve the enantioselectivity of the biotransformation. It was striking to us,

Scheme 4 Biotransformation of nitriles **7**

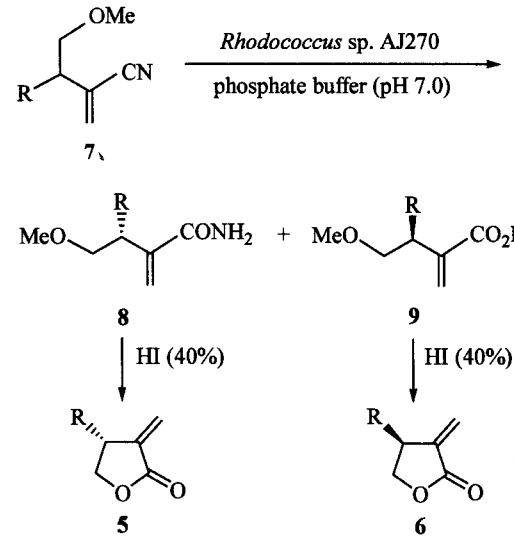


Table 1 Biotransformation of nitriles **3**

Entry	Nitrile	R	Conditions[a]	Yield (%)[b] **4**	*ee* (%)[c] **4**	Yield (%)[b] **5**	*ee* (%)[c] **5**
1	**3a**	C_2H_5	1 mmol, 3 h	47	20	51	27
2	**3a**	C_2H_5	1 mmol, 4.5 h	27	31	64	22
3	**3b**	$CHMe_2$	1 mmol, 7 d	51	82	33	77
4	**3c**	n-C_5H_{11}	1 mmol, 7 d	58	52	30	70

[a] *Rhodococcus* sp. AJ270 cells (2 g wet weight) in phosphate buffer (0.1 mol/L, 50 mL) were used. The reaction conditions were not optimized. [b] Isolated yield. [c] Determined by chiral HPLC analysis of the corresponding lactones.

Table 2 Biotransformation of nitriles **7**

Entry	Nitrile	R	Conditions[a]	Yield (%)[b] **8**	*ee* (%)[c] **8**	Yield (%)[b] **9**	*ee* (%)[c] **9**
1	**7a**	C_2H_5	1 mmol, 22 h	50	56	45	56
2	**7b**	$CHMe_2$	1 mmol, 7 d	59	49	40	69
3	**7c**	n-C_5H_{11}	1 mmol, 7 d	73	2	24	23

[a] *Rhodococcus* sp. AJ270 cells (2 g wet weight) in phosphate buffer (0.1 mol/L, 50 mL) were used. The reaction conditions were not optimized. [b] Isolated yield. [c] Determined by chiral HPLC analysis of the corresponding lactones.

however, that the biotransformation of **7a**—**7c** led to the inversion of enantioselectivity. In contrast to the biotransformation of free hydroxylated nitriles **3a**—**3c**, the reaction of methyl protected analogs **7a**—**7c** gave optically active *R*-amide and *S*-acid, as proved by the isolation of enantiomerically enriched *R*- and *S*-configurated γ-butyrolactones from the demethylative lactonization reaction of *R*-amides **8** and *S*-acids **9**, respectively. To our knowledge, the switch of enantioselectivity of the amidase from *R* to *S* or *vice versus* resulting from protection or deprotection of the substrates has not been reported. It is also very rare in other enzyme-catalyzed reactions. The change of the enantioselectivity from *R* to *S* is most probably due to the alteration of chiral recognition of the amidase towards the change of the structure of **4** to structure of **8**, and the detailed mechanism still awaits further investigation. Nevertheless, it opens a new avenue allowing us to control the stereochemistry of the biotransformation of nitriles and amides simply utilizing the protection strategy.[22]

Conclusion

Catalyzed by the *Rhodococcus* sp. AJ270 whole cells under very mild conditions, β-alkyl-γ-hydroxy-α-methylenebutyronitriles (**3**) underwent enantioselective hydrolysis to form the corresponding optically active *S*-amides (**4**) and *R*-γ-butyrolactones (**5**), while the hydrolysis of β-alkyl-γ-methoxy-α-methylenebutyronitriles (**7**) afforded enantiomerically enriched *R*-amides (**8**) and *S*-acids (**9**). The amidase involved in *Rhodococcus* sp. AJ270 has been shown to display opposite enantioselectivity depending only on the methylation of the hydroxy group of the parent substrate. This study has also provided a new method for the preparation of optically active β-alkyl-α-methylene-γ-butyrolactone derivatives.

Experimental

Both melting points, which were determined using a Reichert Kofler hot-stage apparatus, and boiling points were uncorrected. IR spectra were obtained on a Perkin-Elmer 782 instrument as liquid films or KBr discs. NMR spectra were recorded on a Bruker AM 300 spectrometer. Mass spectra were measured on an AEI MS-50 mass spectrometer and microanalyses were carried out by the Analytical Laboratory of the Institute.

Racemic lactones (+/−)-**5** were obtained from chemical hydrolysis of nitriles **3** in refluxing hydrochloric acid (6 mol/L). The configurations of amides **4** and **8** and acids **9** were determined by converting them into the corresponding lactones **5** followed by the comparison of the direction of optical rotation with that of authentic samples.[21]

Polarimetry was carried out using an optical activity AA-10R polarimeter and the measurements were made at the sodium D-line with a 5-cm pathlengh cell. Concentrations (*c*) are given in g/100 mL. The enantiomeric excesses of products were determined from that of the corresponding lactones **5** and **6** with a Shimadzu LC-10AVP HPLC system using a Chiralcel OD column at a flow rate 0.8 mL/min, with hexane:2-propanol (180:1, *V*:*V*) as the mobile phase. The retention time values of both enantiomers of lactones **5** are as follows: **5a**: t_S = 22.92 min, t_R = 24.37 min; **5b**: t_S = 20.89 min, t_R = 22.16 min; **5c**: t_S = 19.45 min, t_R = 21.07 min.

Preparation of β-substituted γ-hydroxy-α-methylenebutyronitriles (**3a**—**3c**)

Bromination of the Baylis-Hillman nitriles (**1**)

To an ice-water bath-cooled solution of the Baylis-Hillman nitrile **1** (0.1 mol) was added consecutively hydrobromic acid (40%, 45 mL) and sulfuric acid (98%, 28 mL). The mixture was stirred overnight at room temperature. Dichloromethane and water were then added slowly to the mixture at 0 ℃. The organic layer was separated and the aqueous phase was extracted twice with dichloromethane. The combined organic phase was washed twice with water and dried over anhydrous Na_2SO_4. After removal of the solvent the residue was distilled under vacuum to give allylic bromide derivative (**2**) for the next step without further purification.

2-Bromomethylpent-2-enenitrile (**2a**)[19] Oil, yield 94%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.51 (t, *J* = 7.3 Hz, 1H), 4.14 (s, 2H), 2.49—2.29 (m, 2H), 1.18—1.01 (m, 3H); IR (KBr) ν: 2247, 1685 cm^{-1}; MS (EI) *m*/*z*: 176 (M^+ + 2, 21), 174 (M^+, 22), 94 (100), 67 (45), 54 (18), 41 (30).

2-Bromomethyl-4-methylpent-2-enenitrile (**2b**)[19] Oil, yield 83%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.34—6.30 (m, 1H), 4.02—3.98 (m, 2H), 2.96—2.70 (m, 1H), 1.27—1.08 (m, 6H); IR (KBr) ν:

2224, 1633 cm^{-1}; MS (EI) *m/z* (%): 190 (M^+ +2, 13), 188 (M^+, 14), 108 (100), 93 (12), 91 (10), 81 (35).

2-Bromomethyloct-2-enenitrile (**2c**)[19] Oil, yield 94%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 6.58—6.51 (m, 1H), 4.03 (s, 2H), 2.47—2.40 (m, 2H), 1.38—1.32 (m, 6H), 0.95—0.91 (m, 3H); IR (KBr) ν: 2224, 1634 cm^{-1}; MS (EI) *m/z* (%): 218 (M^+ +2, 25), 216 (M^+, 27), 136 (100), 119 (19), 109 (26), 94 (30), 80 (52), 55 (41), 41 (77).

Reaction of **2a—2c** *with formaldehyde*

To a suspension of tin powder (60.5 mmol) and aluminum powder (60.5 mmol) in diethyl ether (100 mL) was added aqueous formaldehyde solution (37%, 25 mL), allylic bromide **2** (55 mmol) and a catalytic amount of acetic acid (0.05 mmol). The mixture was kept refluxing gently for 3 d. The organic layer was separated and the aqueous layer was extracted twice with diethyl ether (100 mL). After drying with anhydrous Na_2SO_4 and the removal of solvent under vacuum, the pure nitrile **3** was obtained from the column chromatography using a silica gel column with a mixture of ethyl acetate and petroleum ether (1:5, *V*:*V*) as the mobile phase.

3-Hydroxymethyl-2-methylenevaleronitrile (**3a**) Oil, yield 71%; ^1H NMR ($CDCl_3$) δ: 6.02 (s, 1H, C=C**H**H), 5.84 (s, 1H, C=CH**H**), 3.62—3.73 (m, 2H, CH_2O), 2.34—2.39 (m, 1H, CH), 2.04 (brs, 1H, OH), 1.41—1.63 (m, 2H, CH_2), 0.96 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 130.9, 122.6, 115.6, 62.0, 47.7, 20.4, 9.6; IR (KBr) ν: 3441 (OH), 2224 (CN), 1622 cm^{-1} (C=C); MS (EI) *m/z* (%): 126 (M+1, 32), 125 (M^+, 28), 113 (100), 98 (62), 97 (50), 96 (70), 95 (40), 94 (75); HRMS calcd for $C_7H_{12}NO$ 126.0913, found 126.0913 (M+H).

3-Hydroxymethyl-4-methyl-2-methylenevaleronitrile (**3b**) Oil, yield 49%; ^1H NMR ($CDCl_3$) δ: 6.03 (s, 1H, C=C**H**H), 5.81 (s, 1H, C=CH**H**), 3.68—3.88 (m, 2H, CH_2O), 2.13—2.21 (m, 1H, CH), 1.74—1.89 (m, 1H, CH), 1.69 (brs, 1H, OH), 0.94—0.98 (m, 6H, $2CH_3$); ^{13}C NMR ($CDCl_3$) δ: 133.3, 124.1, 117.9, 62.3, 54.6, 27.9, 20.6, 20.4; IR (KBr) ν: 3445 (OH), 2223 (CN), 1620 (C=C) cm^{-1}; MS (EI) *m/z* (%): 139 (M^+, 28), 109 (54), 108 (31), 94 (70), 79 (100). HRMS calcd for $C_8H_{14}NO$ 140.1070, found 140.1070 (M+H).

3-Hydroxymethyl-2-methyleneoctanenitrile (**3c**) Oil, yield 47%; ^1H NMR ($CDCl_3$) δ: 5.99 (s, 1H, C=C**H**H), 5.83 (s, 1H, C=CH**H**), 3.60—3.71 (m, 2H, CH_2O), 2.39—2.49 (m, 1H, CH) 2.00 (brs, 1H, OH), 1.38—1.49 (m, 2H, CH_2), 1.30 (brs, 6H $(CH_2)_3$), 0.88 (t, J = 5.5 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 130.6, 122.7, 115.5, 61.9, 45.8, 29.6, 27.1, 24.5, 20.5, 12.0; IR (KBr) ν: 3440 (OH), 2223 (CN), 1623 (C=C) cm^{-1}; MS (EI) *m/z* (%): 167 (M^+, 1), 137 (40), 122 (23), 108 (41), 95 (85), 94 (75), 81 (100), 80 (78). HRMS calcd for $C_{10}H_{18}NO$ 168.1383, found 168.1383 (M+H).

Preparation of β-substituted γ-methoxy-α-methylenebutyronitriles (**7a—7c**)

To a solution of nitrile **3** (3 mmol) in dry tetrahydrofuran was added, under cooling, two equiv. of NaH and five equiv. of methyl iodide in sequence. After the starting nitrile was converted, water was added and the mixture was extracted with diethyl ether. After the organic layer was dried (anhydrous Na_2SO_4) and the solvent was removed under vacuum, the nitrile **7** was obtained and purified from silica gel column chromatography.

3-Methoxymethyl-2-methylenevaleronitrile (**7a**) Oil, yield 50%; ^1H NMR ($CDCl_3$) δ: 5.94 (s, 1H, C=C**H**H), 5.76 (s, 1H, C=CH**H**), 3.41 (d, J = 6.8 Hz, 2H, OCH_2), 3.34 (s, 3H, OCH_3), 2.39—2.46 (m, 1H, CH), 1.39—1.62 (m, 2H, CH_3CH_2), 0.91 (t, J = 7.2 Hz, 3H, CH_3CH_2); ^{13}C NMR ($CDCl_3$) δ: 132.1, 125.1, 117.7, 74.2, 59.2, 47.4, 22.9, 11.6; IR (KBr) ν: 2222 (CN), 1622 (C=C) cm^{-1}; MS (EI) *m/z* (%): 139 (M^+), 107 (12), 94 (5), 79 (4), 67 (4), 53 (6), 45 (100).

3-Methoxymethyl-4-methyl-2-methylenevaleronitrile (**7b**) Oil, yield 58%; ^1H NMR δ: 5.97 (s, 1H, C=C**H**H), 5.74 (s, 1H, C=CH**H**), 3.44—3.59 (m, 2H, OCH_2), 3.36 (s, 3H, OCH_3), 2.21—2.28 (m, 1H, CH), 1.79—1.86 (m, 1H, $(CH_3)_2CH$), 0.98 (d, J = 6.7 Hz, 3H, CH_3), 0.94 (d, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 132.2, 124.4, 117.9, 72.4, 58.9, 52.0, 28.3, 20.4, 20.3; IR (KBr) ν: 2221 (CN), 1620 (C=C) cm^{-1}; MS (EI) *m/z* (%): 153 (M^+), 121 (16), 108 (4), 96 (4),

79 (14), 45 (100). Anal calcd for $C_9H_{15}NO$: C 70.55, H 9.87, N 9.14; found C 70.19, H 10.01, N 9.12.

3-Methoxymethyl-2-methyleneoctanenitrile (**7c**) Oil, yield 66%; ^{1}H NMR ($CDCl_3$) δ: 5.94 (s, 1H, C=CHH), 5.78 (s, 1H, C=CHH), 3.42 (d, J=6.9 Hz, 2H, OCH_2), 3.36 (s, 3H, OCH_3), 2.56—2.47 (m, 1H, CH), 1.39—1.50 (m, 2H, $CH_3(CH_2)_3CH_2$), 1.30 (brs, 6H, $(CH_2)_3$), 0.90 (t, J=5.9 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 131.4, 124.9, 117.2, 73.9, 58.8, 45.3, 31.4, 29.3, 26.3, 22.3, 13.8; IR (KBr) ν: 2222 (CN) cm^{-1}; MS(EI) m/z (%): 181 (M^+, 1), 45 (100). Anal calcd for $C_{11}H_{19}NO$: C 72.88, H 10.56, N 7.73; found C 72.45, H 10.77, N 7.98.

General procedure for biotransformation of nitriles (**3a**—**3c**) and (**7a**—**7c**)

To an Erlenmeyer flask (100 mL) with a screw cap were added *Rhodococcus* sp. AJ270 cells (2 g wet weight) and the potassium phosphate buffer (0.1 mol/L, pH 7.0, 50 mL) and the resting cells were activated at 30 ℃ for 0.5 h with orbital shaking. Nitrile **3** or **7** (Tables 1 and 2) was added in one portion to the flask and the mixture was incubated at 30 ℃ using an orbital shaker (200 rpm). The reaction, monitored by TLC, was quenched after a period of time (Tables 1 and 2) by removing the biomass through a Celite pad filtration. The resulting aqueous solution was extracted with ethyl acetate. After drying and concentraction ($MgSO_4$), the residue was chromatographied on a silica gel column. For compounds **4** and **5**, ethyl acetate/petroleum ether (1:12, V:V) and later methanol/ethyl acetate (1:50, V:V) were used as the mobile phase, while for **8** and **9** a mixture of acetic acid, ethyl acetate and petroleum ether (1:50:300, V:V:V) was applied as an eluent. All products were characterized by their spectral data and comparison of the melting points and optical rotary power with the known compounds, which are listed below, or by full characterization.

(*S*)-(+)-*3-Hydroxymethyl-2-methylenevaleramide* (**4a**) Solid, yield 27%; m.p. 67—69 ℃; $[\alpha]_D^{25}$ +6.7 (c 1.50, CH_3OH); *ee* 31% (HPLC); ^{1}H NMR ($CDCl_3$) δ: 6.43 (brs, 1H, CONHH), 6.27 (brs, 1H, CONHH), 5.74 (s, 1H, C=CHH), 5.37 (s, 1H, C=CHH), 3.56—3.72 (m, 2H, CH_2O), 3.35 (brs, 1H, OH), 2.48—2.53 (m, 1H, CH), 1.46—1.59 (m, 2H, CH_2), 0.90 (t, J=7.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 173.9, 147.7, 120.5, 67.2, 47.7, 23.6, 12.9; IR (KBr) ν: 3323 (NH_2, OH), 3155 (NH_2), 1675 (C=O), 1600 (C=C), 1590 cm^{-1}; MS (EI) m/z (%): 143 (M^+, 5), 126 (10), 123 (12), 113 (28), 108 (21), 106 (15), 98 (32), 97 (35), 96 (42), 95 (42), 94 (89), 80 (49), 67 (100). Anal calcd for $C_7H_{13}NO_2$: C 58.72, 9.15, N 9.78; found C 58.72, H 9.20, N 9.79.

(*R*)-(+)-*β-Ethyl-α-methylene-γ-butyrolactone* (**5a**) Oil,[21,23] yield 64%, $[\alpha]_D^{25}$ +16 (c 1.00, $CHCl_3$), *ee* 22% (HPLC); ^{1}H NMR ($CDCl_3$) δ: 6.28 (d, J=2.7 Hz, 1H, C=CHH), 5.62 (d, J=2.3 Hz, 1H, C=CHH), 4.70 (t, J=8.6 Hz, 1H, OCHH), 4.01 (dd, J=5.5, 9.0 Hz, 1H, OCHH), 2.97—3.04 (m, 1H, CH) 1.68—1.79 (m, 1H, CH_3CHH), 1.52—1.64 (m, H, CH_3CHH), 0.98 (t, J=7.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 171.0, 138.2, 122.0, 70.9, 40.1, 26.7, 10.6; IR (KBr) ν: 1765 (C=O), 1665 (C=C) cm^{-1}; MS (EI) m/z (%): 126 (M^+, 49), 98 (56), 97 (82), 96 (100).

(*S*)-(+)-*3-Hydroxymethyl-4-methyl-2-methylenevaleramide* (**4b**) Solid, yield 51%; m.p. 138—139 ℃; $[\alpha]_D^{25}$ +43.1 (c 1.30, CH_3OH); *ee* 82% (HPLC); ^{1}H NMR ($CDCl_3$) δ: 6.10 (brs, 1H, CONHH), 5.80 (brs, 1H, CONHH), 5.71 (s, 1H, C=CHH), 5.40 (s, 1H, C=CHH), 3.73—3.86 (m, 2H, CH_2O), 2.19—2.25 (m, 1H, CH), 1.93—2.00 (m, 1H, CH), 1.00 (d, J=6.6 Hz, 3H, CH_3), 0.88 (d, J=6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 172.6, 147.3, 120.4, 64.6, 53.9, 26.9, 21.7, 20.5; IR (KBr) ν: 3314 (NH_2, OH), 3130 (NH_2), 1677 (C=O), 1590 cm^{-1}; MS (EI) m/z (%): 139 (M^+, 2), 127 (15), 98 (100). Anal calcd for $C_8H_{15}NO_2$: C 61.12, H 9.62, N 8.91; found C 61.01, H 9.66, N 8.87.

(*R*)-(+)-*α-Isopropyl-β-methylene-γ-butyrolactone* (**5b**) Oil,[21] yield 33%; $[\alpha]_D^{25}$ +56 (c 1.05, $CHCl_3$); *ee* 77% (HPLC); ^{1}H NMR δ:6.32 (d, J=2.4 Hz, 1H, C=CHH), 5.61 (d, J=2.1 Hz, 1H, C=CHH), 4.34 (t, J=8.7 Hz, 1H, OCHH), 4.15 (dd, J=4.0, 9.3 Hz, 1H, OCHH), 2.92—2.99 (m, 1H, CH), 1.87—1.95 (m, 1H, CH), 0.94 (d, J=7.0 Hz, 3H, CH_3), 0.91 (d, J=6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 171.1, 137.0, 123.0, 68.3, 44.6, 31.4, 19.1, 17.7; IR (KBr) ν: 1765

(C=O), 1661 (C=C) cm^{-1}; MS (EI) *m/z* (%): 98 (M^+ - 42, 100), 69 (15), 43 (25), 41 (18).

(*S*)-(+)-3-*Hydroxymethyl*-2-*methyleneoctamide* (**4c**) Solid, yield 58%; m.p. 69—70.5 ℃; $[\alpha]_D^{25}$ +11.2 (*c* 5.35, CH_3OH); *ee* 53% (HPLC); ^1H NMR ($CDCl_3$) δ: 6.27 (brs, 1H, CONHH), 6.15 (brs, 1H, CONHH), 5.73 (s, 1H, C=CHH), 5.39 (s, 1H, C=CHH), 3.72 (dd, *J* = 3.9, 10.5 Hz, 1H, OCHH), 3.60 (dd, *J* = 7.2, 10.5 Hz, 1H, OCHH), 2.57—2.64 (m, 1H, CH), 1.49 (brs, 2H, CH_2), 1.27 (brs, 6H, $(CH_2)_3$), 0.87 (t, *J* = 5.9 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 172.8, 147.1, 119.4, 66.7, 45.2, 31.7, 29.5, 27.0, 22.4, 13.9; IR (KBr) ν: 3322 (NH_2), 3144 (NH_2, OH), 1676 (C=O), 1593 (C=C) cm^{-1}; MS (EI) *m/z* (%): 122 (6), 112 (21), 108 (17), 99 (86), 98 (52), 95 (41), 81 (38), 67 (37), 55 (59), 43 (100); HRMS calcd for $C_{10}H_{20}NO_2$ 186.1488, found 186.1489 (M+H).

(*R*)-(+)-*α*-*Methylene*-*β*-*pentyl*-*γ*-*butyrolactone* (**5c**) Oil,[21,23] yield 30%; $[\alpha]_D^{25}$ +93 (*c* 2.5, $CHCl_3$); *ee* 70% (HPLC); ^1H NMR ($CDCl_3$) δ: 6.19 (d, *J* = 2.8 Hz, 1H, C=CHH), 5.53 (d, *J* = 2.5 Hz, 1H, C=CHH), 4.39 (t, *J* = 8.5 Hz, 1H, OCHH), 3.91 (q, *J* = 5.6, 8.9 Hz, 1H, OCHH), 2.94—3.01 (m, 1H, CH) 1.59—1.65 (m, 1H, CHH), 1.40—1.47 (m, 1H, CHH), 1.17—1.29 (m, 6H, $(CH_2)_3$), 0.82 (t, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 171.0, 138.5, 121.8, 71.3, 38.8, 33.7, 31.6, 26.0, 22.5, 14.0; IR (KBr) ν: 1766 (C=O), 1663 (C=C) cm^{-1}; MS (EI) *m/z* (%): 139 (M - 29, 9), 112 (7), 99 (100), 98 (46).

(*R*)-3-*Methoxymethyl*-2-*methylenevaleramide* (**8a**) Oil, yield 50%; $[\alpha]_D^{25}$ -2.15 (*c* 3.25, $CHCl_3$); *ee* 56% (HPLC analysis of the corresponding lactone); ^1H NMR ($CDCl_3$) δ: 6.47 (brs, 1H, CONHH), 5.90 (brs, 1H, CONHH), 5.83 (s, 1H, C=CHH), 5.35 (s, 1H, C=CHH), 3.41—3.52 (m, 2H, OCH_2), 3.34 (s, 3H, OCH_3), 2.61—2.71 (m, 1H, CH), 1.51—1.63 (m, 2H, CH_3CH_2), 0.92 (t, *J* = 7.4 Hz, 3H, CH_3CH_2); ^{13}C NMR δ: 171.9, 146.2, 119.0, 75.9, 58.5, 43.4, 23.3, 11.5; IR (KBr) ν: 3416 (NH_2, OH), 3200 (NH_2), 1664 (C=O), 1628, 1600 cm^{-1}; MS (EI) *m/z* (%): 157 (M^+), 140 (M - 17, 5), 125 (13), 110 (9), 96 (3), 81 (4), 67 (6), 53 (5), 45 (100). HRMS calcd for $C_8H_{16}NO_2$ 158.1175, found 158.1177 (M+H).

(*S*)-3-*Methoxymethyl*-2-*methylenevaleric acid* (**9a**) Oil, yield 45%; $[\alpha]_D^{25}$ +17.8 (*c* 1.80, $CHCl_3$); *ee* 56% (HPLC analysis of the corresponding lactone); ^1H NMR ($CDCl_3$) δ: 9.90 (brs, 1H, COOH), 6.41 (s, 1H, C=CHH), 5.67 (s, 1H, C=CHH), 3.37—3.51 (m, 2H, OCH_2), 3.31 (s, 3H, OCH_3), 2.75—2.80 (m, 1H, CH), 1.49—1.63 (m, 2H, CH_3CH_2), 0.86 (t, *J* = 7.4 Hz, 3H, CH_3CH_2); ^{13}C NMR ($CDCl_3$) δ: 172.0, 140.0, 127.4, 74.9, 58.4, 42.1, 23.4, 11.3; IR (KBr) ν: 2590—3440 (brs, COOH), 1694 (C=O), 1624 (C=C) cm^{-1}; MS (EI) *m/z* (%): 140 (M - 18, 12), 126 (6), 111 (4), 96 (11), 81 (4), 67 (10), 53 (6), 45 (100). HRMS calcd for $C_8H_{13}O_3$ 157.0870, found 157.0873 (M-H).

(*R*)-3-*Methoxymethyl*-4-*methyl*-2-*methylenevaleramide* (**8b**) Oil, yield 59%; $[\alpha]_D^{25}$ -4.91 (*c* 2.85, $CHCl_3$); *ee* 49% (HPLC analysis of the corresponding lactone); ^1H NMR ($CDCl_3$) δ: 6.60 (brs, 1H, CONHH), 5.90 (brs, 1H, CONHH), 5.81 (s, 1H, C=CHH), 5.32 (s, 1H, C=CHH), 3.53 (d, *J* = 6.1 Hz, 2H, OCH_2), 3.32 (s, 3H, OCH_3), 2.40—2.43 (m, 1H, CH), 1.91—1.94 (m, 1H, $(CH_3)_2CH$), 0.94 (t, *J* = 6.6 Hz, 3H, CH_3), 0.88 (t, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 171.9, 146.1, 119.8, 74.2, 58.5, 49.1, 28.2, 20.9, 20.3; IR (KBr) ν: 3352 (NH_2, OH), 3202 (NH_2), 1665 (C=O), 1627, 1600 (C=C) cm^{-1}; MS (EI) *m/z* (%): 171 (M^+), 154 (2), 139 (4), 121 (10), 108 (4), 106 (4), 96 (5), 79 (13), 67 (6), 45 (100). HRMS calcd for $C_9H_{18}NO_2$ 172.1332, found 172.1331 (M+H).

(*S*)-3-*Methoxymethyl*-4-*methyl*-2-*methylenevaleric acid* (**9b**) Oil, yield 40%; $[\alpha]_D^{25}$ +13.3 (*c* 3.00, $CHCl_3$); *ee* 69% (HPLC analysis of the corresponding lactone); ^1H NMR ($CDCl_3$) δ: 9.80 (brs, 1H, COOH), 6.41 (s, 1H, C=CHH), 5.65 (s, 1H, C=CHH), 3.45—3.56 (m, 2H, OCH_2), 3.28 (s, 3H, OCH_3), 2.56—2.63 (m, 1H, CH), 1.83—1.90 (m, 1H, $(CH_3)_2CH$), 0.87 (d, *J* = 6.7 Hz, 3H, CH_3), 0.84 (d, *J* = 6.7 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 172.4, 140.4, 127.9, 73.3, 58.5, 47.1, 28.7, 20.6, 20.2; IR (KBr) ν: 2950—3437 (brs, COOH), 1693 (C=O), 1623 (C=C) cm^{-1}; MS (EI) *m/z* (%): 154 (M - 18, 13), 140 (6), 127 (9), 125

(7), 110 (6), 98 (34), 81 (8), 69 (12), 53 (7), 45 (100). HRMS calcd for $C_9H_{15}O_3$ 171.1027, found 171.1026 (M - H).

(*R*)-3-*Methoxymethyl*-2-*methyleneoctamide* (**8c**) Oil, yield 73%; $[\alpha]_D^{25}$ - 0.52 (*c* 6.50, $CHCl_3$); *ee* 2% (HPLC analysis of the corresponding lactone); ^1H NMR ($CDCl_3$) δ: 6.52 (brs, 2H, $CONH_2$), 5.74 (s, 1H, C = C**H**H), 5.27 (s, 1H, C = CH**H**), 3.32—3.41 (m, 2H, OCH_2), 3.25 (s, 3H, OCH_3), 2.64—2.68 (m, 1H, CH), 1.43 (brs, 2H, $CH_3(CH_2)_3C\mathbf{H}_2$), 1.20 (brs, 6H, $(CH_2)_3$), 0.80 (t, *J* = 6.1 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 172.0, 146.5, 118.9, 76.3, 58.5, 41.6, 31.6, 30.4, 26.7, 22.2, 13.8; IR (KBr) ν: 3351 (NH_2, OH), 3200 (NH_2), 1662 (C = O), 1627, 1600 (C = C) cm^{-1}; MS (EI) *m/z* (%): 182 (M - 18, 1), 167 (2), 154 (2), 149 (2), 134 (2), 124 (3), 110 (3), 94 (4), 81 (3), 79 (3), 67 (3), 55 (5), 45 (100). HRMS calcd for $C_{11}H_{22}NO_2$ 200.1645, found 200.1646 (M + H).

(*S*)-3-*Methoxymethyl*-2-*methyleneoctanoic acid* (**9c**) Oil, yield 24%; $[\alpha]_D^{25}$ + 2.46 (*c* 1.30, $CHCl_3$); *ee* 23% (HPLC analysis of the corresponding lactone); ^1H NMR ($CDCl_3$) δ: 6.46 (s, 1H, C = C**H**H), 5.74 (s, 1H, C = CH**H**), 3.52 (dd, *J* = 7.3, 9.0 Hz, 1H OC**H**H), 3.44 (dd, *J* = 6.2, 9.3 Hz, 1H, OCH**H**), 3.68 (s, 3H, OCH_3), 2.87—2.92 (m, 1H, CH), 1.50—1.63 (m, 2H, $CH_3(CH_2)_3C\mathbf{H}_2$), 1.30 (brs, 6H, $(CH_2)_3$), 0.90 (t, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 172.3, 141.0, 127.7, 75.6, 58.7, 40.8, 31.8, 30.7, 26.8, 22.5, 14.0; IR (KBr) ν: 3120—3438 (brs, COOH), 1694 (C = O), 1622 (C = C) cm^{-1}; MS (EI) *m/z* (%): 182 (M - 18, 22), 168 (5), 139 (12), 125 (13), 109 (8), 99 (14), 95 (10), 82 (11), 81 (11), 67 (14), 55 (15), 45 (100). HRMS calcd for $C_{11}H_{19}O_3$ 199.1340, found 199.1339 (M - H).

Chemical hydrolysis of **4a—4c**

Amide (0.2 mmol) was stirred in hydrochloric acid (2 mol/L) till it was consumed. Extraction with chloromethane, drying with Na_2SO_4 and flash chromatography gave lactone **6**.

(*S*)-(-)-*β-Ethyl-α-methylene-γ-butyrolactone* (**6a**) Oil, yield 76%; $[\alpha]_D^{25}$ - 40 (*c* 1.0, $CHCl_3$); *ee* 31% (HPLC). The same spectra as those of **5c** were obtained.

(*S*)-(-)-*β-Isopropyl-α-methylene-γ-butyrolactone* (**6b**) Oil, yield 76%; $[\alpha]_D^{25}$ - 87.5 (*c* 1.6, $CHCl_3$); *ee* 82% (HPLC). The same spectra as those of **5b** were obtained.

(*S*)-(-)-*α-Methylene-β-pentyl-γ-butyrolactone* (**6c**) Oil, yield 79%; $[\alpha]_D^{25}$ - 49.0 (*c* 1.55, $CHCl_3$); *ee* 52% (HPLC). The same spectra as those of **5c** were obtained.

Demethylative lactonization of **8** *and* **9**

To a solution of amide **8** or acid **9** (0.12—0.33 mmol) in chloroform (1 mL) was added hydroiodic acid (40%, 1 mL), and the resulting mixture was refluxed overnight. The organic layer was separated and the aqueous layer was extracted with chloroform. The combined organic layer was then washed with saturated $Na_2S_2O_3$ solution, and dried over anhydrous Na_2SO_4. After concentration and column chromatography, lactone **5** or **6** was obtained.

Demethylative lactonization of **8a** gave (*R*)-*β*-ethyl-*α*-methylene-*γ*-butyrolactone (**5a**): oil, yield 44%; *ee* 56% (HPLC).

Demethylative lactonization of **8b** gave (*R*)-*β*-isopropyl-*α*-methylene-*γ*-butyrolactone (**5b**): oil, yield 15%; *ee* 49% (HPLC).

Demethylative lactonization of **8c** gave (*R*)-*α*-methylene-*β*-pentyl-*γ*-butyrolactone (**5c**): oil, yield 68%; *ee* 2% (HPLC).

Demethylative lactonization of **9a** gave (*S*)-*β*-ethyl-*α*-methylene-*γ*-butyrolactone (**6a**): oil, yield 73%; *ee* 56% (HPLC). The same spectra as those of **5a** were obtained.

Demethylative lactonization of **9b** gave (*S*)-*β*-isopropyl-*α*-methylene-*γ*-butyrolactone (**6b**): oil, yield 66%; *ee* 69% (HPLC). The same spectra as those of **5b** were obtained.

Demethylative lactonization of **9c** gave (*S*)-*α*-methylene-*β*-pentyl-*γ*-butyrolactone (**6c**): oil, yield 74%; *ee* 23% (HPLC). The same spectra as those of **5c** were obtained.

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