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# Nitrile Biotransformations for the Synthesis of Highly Enantioenriched β-Hydroxy and β-Amino Acid and Amide Derivatives: A General and Simple but Powerful and Efficient Benzyl Protection Strategy To Increase Enantioselectivity of the Amidase

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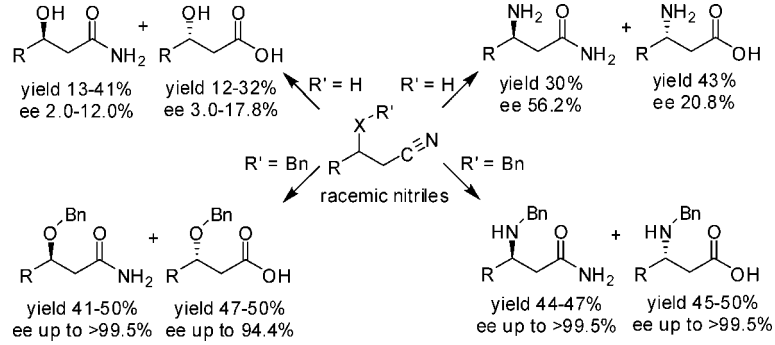


Biotransformations of a number of racemic β-hydroxy and β-amino nitrile derivatives were studied using *Rhodococcus erythropolis* AJ270, the nitrile hydratase and amidase-containing microbial whole cell catalyst, under very mild conditions. The overall enantioselectivity of nitrile biotransformations was governed predominantly by the amidase whose enantioselectivity was switched on remarkably by an *O*- and a *N*-benzyl protection group of the substrates. While biotransformations of β-hydroxy and β-amino alkanenitriles gave low yields of amide and acid products of very low enantiomeric purity, introduction of a simple benzyl protection group on the β-hydroxy and β-amino of nitrile substrates led to the formation of highly enantioenriched β-benzyloxy and β-benzylamino amides and acids in almost quantitative yield. The easy protection and deprotection operations, high chemical yield, and excellent enantioselectivity render the nitrile biotransformation a useful protocol in the synthesis of enantiopure β-hydroxy and β-amino acids.

## Introduction

Enantiomerically pure β-hydroxy and β-amino acids and their derivatives are important chemical entities because they are prevalent in natural products and synthetic pharmaceuticals.[1] They are also used as catalysts and the chiral ligands or essential building blocks for the construction of catalysts in asymmetric synthesis.[1,2] As a consequence, enormous efforts have been spent on the synthesis of optically active β-hydroxy and β-amino acids and their derivatives, and a large number of synthetic methods have been reported.[1−3] However, general approaches to the efficient and catalytic synthesis of enantiopure β-hydroxy and β-amino acids and their derivatives are still rare.

Biotransformations of nitriles,[4] either through a direct conversion from a nitrile to a carboxylic acid catalyzed by a nitrilase or through the nitrile hydratase-catalyzed hydration of a nitrile

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followed by the amide hydrolysis catalyzed by the amidase, have become effective and environmentally benign methods for the production of carboxylic acids and their amide derivatives. One of the well-known examples is the industrial production of acrylamide from biocatalytic hydration of acrylonitrile.[5] Recent studies have demonstrated that biotransformations of nitriles complement the existing asymmetric chemical and enzymatic methods for the synthesis of chiral carboxylic acids and their derivatives.[6] One of the distinct features of enzymatic transformations of nitriles is the straightforward generation of enantiopure amides, valuable organonitrogen compounds in synthetic chemistry, in addition to the formation of enantiopure carboxylic acids. For example, we[6c] have shown that *Rhodococcus erythropolis* AJ270,[7] a nitrile hydratase/amidase-containing whole cell catalyst, is able to efficiently and enantioselectively transform a variety of racemic nitriles into highly enantiopure carboxylic acids and amides. Using the nitrile biotransformation approach, many structurally diverse α-amino acids and amides of high enantiomeric purity have been synthesized.[8]

In contrast to the successful enantioselective nitrile biotransformations for the preparation of chiral carboxylic acids and amide derivatives that bear an α-stereocenter,[6c,9,10] biotransformations of substrates having a chiral center remote from the cyano or the amido functional group have been reported to proceed with, in most cases, disappointingly low enantioselectivity and chemical yield[11–13] except for some biocatalytic desymmtrization reactions of 3-substituted glutaronitrile derivatives.[14] Biotransformations of the Baylis−Hillman nitriles and their one-carbon homologated nitriles, for example, gave only moderate enantioselectivity,[12] whereas β-phenylbutyronitrile[11a] or β-, γ-, or δ-hydroxylated alkanenitriles yielded no or extremely low enantiocontrol.[11d]

It is generally believed that the movement of a stereocenter from the reactive site (α-position to functional group) to a remote place gives rise to the decrease of enantioselectivity in asymmetric reactions. However, this notion may not be true for enzymatic reactions since the chiral recognition site of an enzyme might be located in some distance to the catalytic center. In other words, the chiral recognition between the enzyme and a substrate might occur at a remote pocket from the reaction site. If this hypothesis works, it could lead to highly enantioselective biocatalytic reactions of the substrates that bear a remotely positioned chiral center. To test this hypothetic remote chiral recognition mechanism, and also to explore the application of nitrile biotransformations in the synthesis of enantiopure valuable β-hydroxy and β-amino acids and their amide derivatives, we undertook the current study.[15] We have revealed that the biotransformations of nitriles that contain a β-stereocenter can take place enantioselectively to afford the corresponding amide and acid products with excellent enantiomeric purity, provided the substrates are carefully engineered. Herein, we report a very simple, general, but powerful substrate engineering strategy to increase the enantioselectivity. Introduction of an *O*- and *N*-benzyl group into the β-hydroxy and β-amino nitrile and amide substrates, respectively, has been shown to enhance dramatically the enantioselectivity of the amidase and therefore to lead efficient biotransformations of nitriles to afford highly enantioenriched β-hydroxy and β-amino acid and amide derivatives.

## Results and Discussion

**Biotransformations of Racemic β-Hydroxy- and β-Benzyloxyalkanenitriles.** We initially investigated the biotransformations of racemic 3-hydroxy alkanenitriles **1**, expecting the

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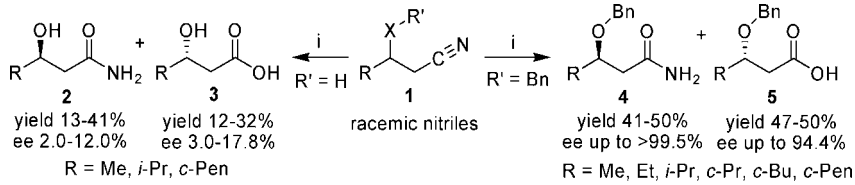
**SCHEME 1. Biotransformation of Racemic β-Hydroxy and β-Benzyloxy Nitriles**[a]



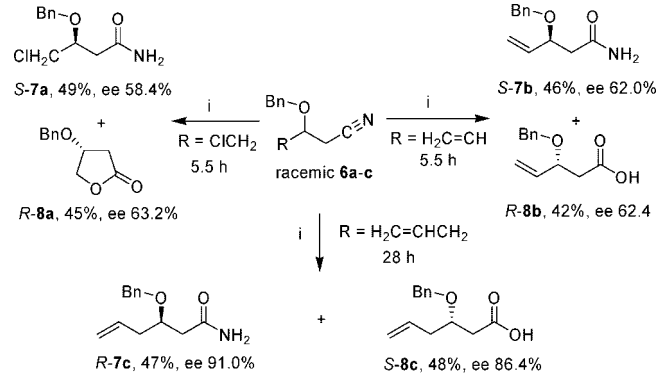

[a] Key: (i) *R. erythropolis* AJ270, phosphate buffer, pH 7.0, 30 °C.

formation of enantiomerically pure 3-hydroxy acids and amide derivatives. In the presence of *Rhodococcus erythropolis* AJ270 whole cell catalyst under very mild conditions, the biotransformation proceeded rapidly. Unfortunately, however, only disappointingly low enantiomeric excesses (ee) were obtained for amide **2** and acid **3** products along with the low chemical yields (Scheme 1). The low enantioselectivity of the biotransformations indicated that both the nitrile hydratase and the amidase involved in *R. erythropolis* AJ270 display low enantiodifferentiating power toward the 3-hydroxyalkanenitriles and 3-hydroxyalkaneamides, respectively, while the loss of mass balance was most probably attributable to the further metabolization of the resulting small β-hydroxy acid and amide derivatives, which was catalyzed by other enzymes within microbial whole cells, and to the difficulty of their extraction from aqueous reaction media because of their high polarity and hydrophilicity.

The observation of low enantioselectivity of the biotransformation of β-hydroxy nitriles was not totally unexpected. Except for highly enantioselective biotransformations of some prochiral dinitriles[14] and few racemic nitriles that bear a β-stereogenic center,[13] most of the hydrolyses of β-chiral center-containing racemic nitriles reported to date gave appallingly poor enantioselectivity.[11,12] The low enantiocontrol of biotransformation is mainly originated from less efficient chiral recognition of the substrate by the enzyme. It is most probably that neither the nitrile hydratase nor the amidase are able to discriminate a pair of alkanenitrile and alkaneamide enantiomers, respectively, that have a free hydroxyl group β-positioned to cyano and amido functionality.

Inspired by the docking strategy in drug discovery study and in biocatalysis,[17–19] we envisioned that the introduction of a removable protection group on the free hydroxyl moiety as a docking moiety in nitrile substrate might result in its enhanced chiral recognition by the enzyme. It might thus lead to the improved enantioselectivity in biotransformations. To choose a protection or a docking group, several criteria need to be considered. First, the *O*-protection group should be readily introduced and removed, and it should not cause contamination to the products. Second, no racemerization is allowed when the protection group is cleaved. This requires very mide conditions for deprotection reaction. Additionally, the protection group should be stable or resistant to other enzymes such as esterases, lipases, and oxidoreductases that are present in the whole

**SCHEME 2. Biotransformation of Functionalized β-Benzyloxy Nitriles 6a−c**[a]




[a] Key: (i) *R. erythropolis* AJ270, phosphate buffer, pH 7.0, 30 °C.

catalyst. Finally, for the biotransformations of UV-inactive 3-hydroxy alkanenitrile substrates, a UV-active protection group should be benificial for facile analysis and monitering of the reaction. To meet these criteria, a benzyl protection group appeared ideal.

To our delight, introduction of a benzyl group onto the hydroxy of nitrile substrates, which was readily achieved by the reaction with benzyl bromide in the presence of $Ag_2O$ (see the Supporting Information), led to a dramatic increase of enenatioselectivity of biotransformations albeit over a longer reaction period. In comparison to the biotransformations of hydroxylated nitriles **1** (R′ = H), for example, *R. erythropolis* AJ270-catalyzed hydrolysis of their corresponding *O*-benzylated 3-hydroxyalkanenitriles **1** (R′ = Bn) gave excellent enantioselectivity, affording highly enantioenriched amide **4** and acid **5** products (Scheme 1). In addition to the drastic enhancement of enantioselectivity, protection of the free hydroxy by a benzyl group also resulted in a remarkable increase of isolated yield of amide and acid products due to most probably the increase of the hydrophobicity of products (Scheme 1). The high enantioselectivity of the overall biotransformation of *O*-benzylated nitriles **1** is the result of combined actions of a highly enantioselective amidase and a lowly enantioselective nitrile hydratase, which was evidenced by the low enantioselectivity of the nitrile hydratase-catalyzed kinetic resolution of racemic nitriles.[15] Both amide and acid products underwent efficient debenzylation reaction to afford highly enantiopure β-hydroxy amides and acids. This also allowed us to assign the absolute configurations of amides **4** and acids **5** on the basis of the comparison of the optical rotation of β-hydroxy amides and acids with that of authentic samples.[15]

To further demonstrate the application of nitrile biotransformations in synthesis, functionalized β-benzyloxylated nitriles **6a−c** were subjected to *R. erythropolis* AJ270-catalyzed hydrolysis. As illustrated in Scheme 2, biotransformations of racemic 4-chloro-3-benzyloxypropanenitrile **6a** gave optically

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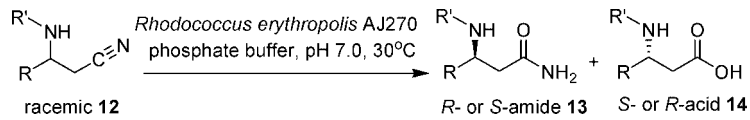
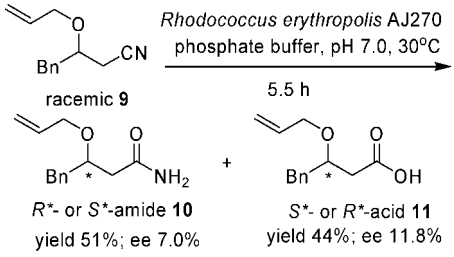
**TABLE 1. Biotransformations of Racemic β-Benzylamino Nitriles 12[a]**




| | | | | | 13 | | 14 | | |
|---|---|---|---|---|---|---|---|---|---|
| entry | 12 | R | R′ | time | yield[b] (%) | ee[c] (%) | yield[b] (%) | ee[c] (%) | $E^d$ |
| 1[e] | **12a** | Me | H | 100 min | 30 | 56.2 | 43 | 20.8[f] | |
| 2 | **12b** | Me | Bn | 80 min | 55 | 74.2 | 45 | 86.6 | 31 |
| 3 | **12b** | Me | Bn | 85 min | 46 | 93.2 | 53 | 61.4 | 13 |
| 4 | **12c** | Et | Bn | 2 h | 47 | >99.5 | 50 | >99.5 | >200 |
| 5[g] | **12d** | $^i$Pr | Bn | 2 h | 44 | 12.6 | | | |
| 6 | **12d** | $^i$Pr | Bn | 25 h | 45 | >99.5 | 48 | 90.2 | 99 |
| 7 | **12e** | *c*-Pr | Bn | 14 h | 46 | 92.4 | 45 | 96.4 | 161 |

[a] Biotransformation was carried out by incubating substrate (2 mmol) in a suspension of *Rhodococcus erythropolis* AJ270 cells (2 g wet weight) in phosphate buffer (50 mL, pH 7.0) at 30 °C. [b] Isolated yield. [c] Determined by chiral HPLC analysis. [d] Calculated following a literature method.[16] [e] Both amide and acid products were isolated as *N*-Cbz-protected derivatives. [f] Determined after esterification using $CH_2N_2$. [g] Nitrile **12d** (47% yield, ee 14.2%) was recovered.

**SCHEME 3. Biotransformation of Racemic Nitrile 9**

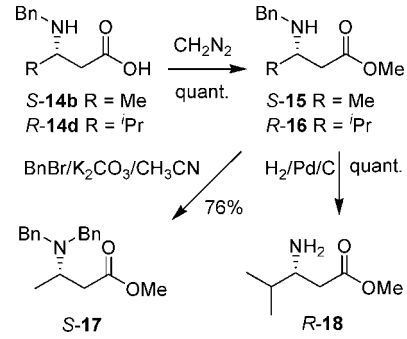



active amide *S*-**7a** and γ-lactone *R*-**8a** in excellent yields with ee of 58.4% and 63.2%, respectively. The formation of γ-lactone *R*-**8a** is most likely due to the spontaneous cyclization of acid formed from hydrolysis of nitrile **6a**. While the reaction of racemic 3-benzyloxypent-4-enenitrile **6b** afforded corresponding amide *S*-**7b** and acid *R*-**8b** with modest enantioselectivity, racemic 3-benzyloxyhex-5-enenitrile **6c** underwent effective biotransformations to furnish amide *R*-**7c** and acid *S*-**8c** in excellent chemical yields and high optical yields. Highly enantioenriched amide *R*-**7c** and acid *S*-**8c** were also prepared easily from a multigram scale biotransformation of racemic **6c** (see the Supporting Information).

It was interesting to note that, in sharp contrast to highly enantioselective biotransformations of nitrile **6c** (Scheme 2), the same biocatalytic reaction of racemic nitrile **9**, an isomer of **6c**, gave very low enantiopurity of amide **10** and acid **11** (Scheme 3). The results showed the sensitivity of the enzyme toward the structure of substrates in term of enantioselection. The interexchange of benzyl and allyl groups within nitrile structure led to a significant drop of enantioselectivity. It might imply a preferential binding domain of the enzyme for a benzyloxy moiety rather than for a benzyl group in chiral recognition.

**Biotransformations of Racemic β-Amino- and β-Benzylaminoalkanenitriles.** To examine the generality of the protection/docking strategy to improve the enantioselectivity of biocatalysis and also to prepare highly enantiopure β-amino acid and amide derivatives, *R. erythropolis* AJ270-catalyzed reaction of racemic β-amino- and β-benzylaminoalkanenitriles **12** was studied (Table 1). Analogous to the racemic β-hydroxy nitriles (Scheme 1), racemic 3-aminobutyronitrile **12a** underwent biocatalytic hydrolysis to give *R*-3-aminobutyramide **13a** and *S*-3-aminobutyric acid **14a** with ee of 56.2% and 20.8%, respectively (entry 1, Table 1). Introduction of a benzyl group into the free amino group of 3-aminobyturonitrile gave rise to a similar remarkable enhancement of enantioselectivity of the reaction of **12b**, affording a quantitative yield of amide *R*-**13b** and acid *S*-**14b** with high enantiomeric excess values (entry 2, Table 1). Under the identical biocatalytic conditions, all other racemic β-benzylaminoalkanenitriles examined were transformed efficiently into highly enantiopure β-benzylamino amides **13c**−**e** and β-benzylamino acids **14c**−**e** in excellent yield (entries 4, 6, and 7, Table 1). Similar to the biotransformations of β-benzyloxyalkanenitriles, the nitrile hydratase within *R. erythropolis* AJ270 did not show good enantioselectivity against β-benzylaminoalkanenitrile substrates. This has been exemplified by the observation of very poor enantioselectivity of the kinetic resolution of racemic 3-benzylamino-4-methylpentanenitrile **12d** (entry 5, Table 1). The excellent enantioselectivity of the overall nitrile biotransformations is therefore originated from the dominant effect of a highly enantioselective amidase within *R. erythropolis* AJ270.

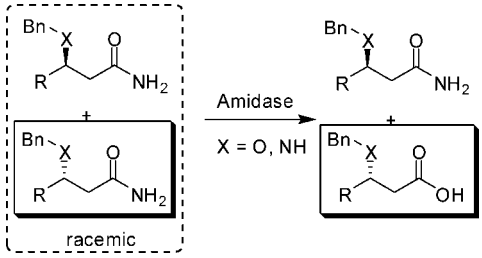
**SCHEME 4. Determination of Absolute Configurations of β-Amino Acids**




To determine the absolute configurations of β-benzylamino amide and acid products, the resulting enantioenriched acids **14b** and **14d** were converted into compounds *S*-**17**[20] and *R*-**18**[21] through esterification using $CH_2N_2$ followed by *N*-benzylation and *N*-debenzylation, respectively (Scheme 4). Comparison of their optical rotation directions with that of authentic samples[20,21] indicated the biotransformation products **14b** and **14d** have *S*-

(20) Etxebarria, J.; Vicario, J. L.; Badia, D.; Carrillo, L. *J. Org. Chem.* **2004**, *69*, 2588.
(21) Caputo, R.; Cassano, E.; Longobardo, L.; Palumbo, G. *Tetrahedron* **1995**, *51*, 12337.

**SCHEME 5. Enantiomeric Selection of the Amidase against *β*-Benzyloxy and *β*-Benzylamino Amides**




and *R*-configurations, respectively. The stereochemistry of nitrile biotransformations observed in current study suggests that the dominant amidase within *R. erythropolis* AJ270 whole cell catalyst exhibits the same sense of enantiomeric selection against both *β*-benzyloxy and *β*-benzylamino amides. In addition, the amidase shows excellent enantioselectivity toward both *β*-benzyloxy and *β*-benzylamino amides, irrespective of the nature of an ether (X = O) or imino (X = NH) linkage in substrates (Scheme 5). Apparently, the steric effect, viz. the fitting of the substrate into the chiral pocket of the enzyme, may play a crucial role in determining the enantioselectivity.

## Conclusion

In summary, we have shown a remarkable method of dramatically enhancing the enantioselectivity of biotransformations of nitriles using a general and simple but powerful protection/docking strategy. A simple and convenient protection of free hydroxyl and amino by a benzyl group gave rise to a significant increase of enantioselectivity of biocatalytic hydrolysis of *β*-hydroxy- and *β*-aminoalkanenitriles. The excellent enantioselectivity of overall nitrile biotransformations stemmed from the synergistic effect of a low enantioselective nitrile hydratase and a high enantioselective amidase within *R. erythropolis* AJ270. The current study provided an efficient approach to highly enantiopure *β*-hydroxy and *β*-amino acids and their amide derivatives that are valuable chiral intermediates in organic synthesis.

## Experimental Section

**General Procedure for the Biotransformations of Nitriles.** To an Erlenmeyer flask (150 mL) with a screw cap was added *Rhodococcus* sp. AJ270 cells[7,10a] (2 g wet weight), which were prepared in bulk from a 5 L fermentor using acetamide as both the carbon and nitrogen source and stored at −20 °C in a freezer, 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. Racemic nitriles were 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 or HPLC, was quenched after a specified period of time (see Table 1 and the Supporting Information) by removing the biomass through a Celite pad filtration. For biotransformations of *β*-hydroxy and *β*-benzyloxy nitriles, the resulting aqueous solution was adjusted to pH 12 with aqueous NaOH solution (2 N) and extracted with ethyl ether (3 × 50 mL). After drying ($MgSO_4$) and removal of the solvent under vacuum, the residue was subjected to a silica gel column using a mixture of petroleum ether and acetone as the mobile phase to give pure amide product and, in some cases, the recovered nitrile. The aqueous phase was then adjusted to pH 4 with hydrochloric acid (2 N) and extracted with ethyl ether (3 × 50 mL). The organic phase was combined. After drying ($MgSO_4$) and removal of the solvent under vacuum, the residue was chromatographed on a silica gel column using a mixture of petroleum ether and acetone as an eluant to give pure acid product. For biotransformations of *β*-benzylamino nitriles, the resulting aqueous solution was distilled under vacuum to remove water. Hydrochloric acid (1 N, 5 mL) was then added to the resulting residue. After being allowed to stand at room temperature for 30 min, the acidic solution was filtrated. The filtrate was loaded on a cation-exchange resin (10 mL) and eluted with water (200 mL) followed by aqueous ammonia solution (1%, 200 mL). The ammonia eluant was concentrated and then chromatographed on a C-18 silica gel column using a gradient eluant [from pure water to a mixture of methanol and water (7:3)] to give pure acid and amide products. The structures of all products were fully characterized by spectroscopic data and microanalyses. The ee values were determined by HPLC analyses using chiral stationary phase (see the Supporting Information).

For biotransformation of racemic **12c**, enantiopure *R*-**13c** and *S*-**14c** were obtained in 47% and 50% yield, respectively. **(*R*)-3-(Benzylamino)pentanoic acid amide (13c):** oil; [α] −21.4 (*c* 4.300, MeOH); ee >99.5% (chiral HPLC analysis of its *N*-methyl derivative using a Chiralcel OJH column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.5 mL/min as the mobile phase, oven temperature 15 °C, $t_1$ = 17.7 min, $t_2$ = 20.9 min); $^1$H NMR (300 MHz, DMSO-$d_6$) 7.40 (H, br), 7.34−7.20 (5H, m), 6.74 (1H, br), 3.69 (2H, s), 2.77 (1H, quin, $J$ = 6.0 Hz), 2.19 (1H, dd, $J$ = 14.3, 6.5 Hz), 2.13 (1H, dd, $J$ = 14.3, 6.1 Hz), 2.12 (1H, br), 1.46−1.37 (2H, m), 0.85 (3H, t, $J$ = 7.3 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 173.6, 141.2, 128.0, 127.8, 126.4, 55.1, 49.9, 39.3, 26.0, 9.6 ppm; IR (KBr) ν 3313, 3186 (NH, $CONH_2$), 1666 (C═O) cm$^{-1}$; MS (ESI$^+$) *m*/*z* 207.1 [M + 1]$^+$. Anal. Calcd for $C_{12}H_{18}N_2O$: C, 69.87; H, 8.80; N, 13.58. Found: C, 69.70; H, 8.87; N, 13.59. **(*S*)-3-(Benzylamino)pentanoic acid (14c):** mp152−153 °C; [α] +32.4 (*c* 2.655, $H_2O$); ee >99.5% (chiral HPLC analysis of its methyl ester using a Chiralcel OD column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.8 mL/min as the mobile phase, oven temperature 20 °C, $t_1$ = 7.0 min, $t_2$ = 8.8 min); $^1$H NMR (300 MHz, $D_2O$) 7.43 (5H, s), 4.21 (2H, s), 3.39−3.30 (1H, m), 2.60 (1H, dd, $J$ = 16.9, 4.9 Hz), 2.43 (1H, dd, $J$ = 16.9, 7.3 Hz), 1.86−1.77 (1H, m), 1.67−1.57 (1H, m), 0.91 (3H, t, $J$ = 7.5 Hz); $^{13}$C NMR (75 MHz, $D_2O$) δ178.2, 131.2, 129.5, 129.3, 56.8, 47.9, 35.4, 23.4, 8.9 ppm; IR (KBr) ν 3281−2375 (NH, COOH), 1697, 1594 cm$^{-1}$; MS (ESI$^+$) *m*/*z* 208.2 [M + 1]$^+$; HR-FAB *m*/*z* calcd for $C_{12}H_{17}NO_2$ 207.1259, found 208.1334.

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**Supporting Information Available:** Preparation of starting nitriles and their spectroscopic data; full characterization of products; $^1$H and $^{13}$C NMR spectra of **2**−**5**, **7**, **8**, **10**, **11**, **13**, and **14**; HPLC analysis of all chiral products. This material is available free of charge via the Internet at http://pubs.acs.org.

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