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Synthesis and Application of Enantioenriched Functionalized α-Tetrasubstituted α-Amino Acids from Biocatalytic Desymmetrization of Prochiral α-Aminomalonamides

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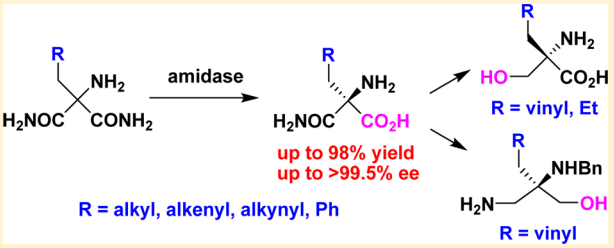
Supporting Information

ABSTRACT: Catalyzed by *Rhodococcus erythropolis* AJ270, an amidase-containing microbial whole cell catalyst in neutral phosphate buffer at 30 °C, a number of prochiral α-substituted α-aminomalonamides underwent highly efficient and enantioselective hydrolytic desymmetrization to afford functionalized α-tetrasubstituted α-amino acids in 74−98% chemical yields and 94.0 to >99.5% ee. The presence of a free α-amino (NH_2) substituent in the substrates was deemed important to ensure high biocatalytic efficiency and enantioselectivity. The synthetic application of biocatalytic desymmetrization was demonstrated by practical chemical transformations of (*R*)-2-amino-2-carbamoylpent-4-enoic acid to α-substituted serine analogues and a bioactive diamino alcohol derivative.



INTRODUCTION

Biotransformations using either enzymes or whole cell catalysts have become important and routinely applied preparative methods in asymmetric synthesis owing to their high efficiency and enantioselectivity under eco-environmentally benign conditions.[1] Biocatalytic desymmetrization of prochiral substrates, in particular, constitutes an elegant and powerful strategy for the generation of enantioenriched chiral compounds.[2] Unlike the kinetic resolution processes, desymmetrization provides the enabling technology to achieve a maximum yield of 100% with excellent enantiocontrol. Furthermore, the method, starting from readily available materials, generally furnishes highly enantiopure chiral compounds that are hardly accessible by other synthetic means. Esterase-, lipase-, and protease-catalyzed hydrolysis of prochiral malonates and 3-substituted glutarates, for example, leads to the formation of the corresponding monoesters with varied enantiomeric excess (ee) values, while enzymatic acylation of prochiral 2-substituted 1,3-alkanediols affords chiral alcohols in good yields with moderate to good enantiocontrol.[3] In addition to extensively used biocatalytic hydrolysis and formation of C−O bonds, desymmetric hydrolysis of prochiral dinitriles has also been reported in the past two decades.[4−8] In the presence of a nitrile-hydrolyzing biocatalyst such as *Rhodococcus rhodochrous* IFO 15564[4] and *Rhodococcus* sp. CGMCC 0497,[5] prochiral malononitriles undergo hydrolysis to produce a mixture of cyanoacetamides, cyanoacetic acids and carbamoylacetic acids with different ee values, while prochiral glutaronitriles bearing a 3-substituent have been biotransformed by *Rhodococcus rhodochrous* IFO 15564,[6] *Rhodococcus* sp. 361,[7] *Rhodococcus erythropolis* AJ270,[8] and nitrilase III[9] into 4-cyanobutyric acid products with moderate to excellent enantioselectivity depending on the nature of 3-substituent, biocatalysts, and additives used.

Amidases [3.5.1.4] catalyze the conversion of primary amides into carboxylic acids with the release of ammonia.[10] A large number of amidases and microorganisms that contain amidases have been reported to date, and some of them have been studied extensively in the kinetic resolution of racemic amides.[11] *Rhodococcus erythropolis* AJ270, a soil-derived nitrile hydratase and amidase-containing microbial strain,[12] for instance, has been shown to catalyze enantioselective biotransformation of a large number of structurally diverse racemic amides including amino-,[13] hydroxy- and alkoxy-,[14] alkenyl-,[15] alkynyl-,[16] allenyl-,[16] and azido-bearing[17] amides, cyclopropanecarboxamide,[18] and various heterocyclic amides[19−22] to produce highly enantiopure carboxylic acid and amide products. Surprisingly, biocatalytic enantioselective desymmetrization of prochiral dicarboxamides remains largely unexplored. To the best of our knowledge, there are only two literature reports.[5a,23] In the study of desymmetrization of malononitriles,[5b,c] Wu and Li[5a] found in 2003 that α-methyl-α-arylmethylmalonamides undergo biocatalytic hydrolysis to yield chiral malonamic acids with high enantioselectivity. Since the substrate structures are very simple, synthetic applications are

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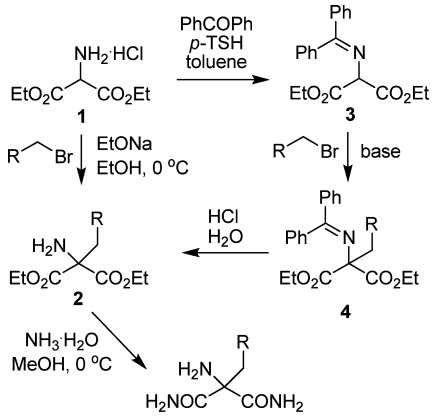
therefore limited.[5a] We have expanded very recently the scope of the substrates, demonstrating a practical synthesis of functionalized carbamoylacetic acids from *Rhodococcus erythropolis* AJ270-catalyzed desymmetrization of malonamides that contain functional groups.[23] On the basis of easy availability of prochiral dicarboxamide compounds, high catalytic efficiency and enantioselectivity of amidases, we envisioned that the amidase-catalyzed desymmetrization of prochiral dicarboxamides would provide a novel method for the synthesis of enantioenriched amido-substituted carboxylic acid compounds, invaluable nitrogen-containing chiral entities in organic synthesis.

Nonproteinogenic amino acids are important entities in organic and medicinal chemistry. α-Tetrasubstituted α-amino acids or α,α-disubstituted α-amino acids, for instance, are often used as building units in the construction of peptidomimetics of pharmaceutical importance[24] while incorporation of α-amino acids functionalized with a terminal alkene moiety into peptides enables preparation of stapled α-helical peptides.[25] α-Tetrasubstituted functional α-amino acids have also been used extensively in the synthesis of natural and bioactive products.[26] Although a plethora of chemical and enzymatic methods have been established for the synthesis of chiral quaternary, carbon-centered carboxylic acids,[26−28] surprisingly, desymmetrization of prochiral α-amino-substituted malonate diesters and their malonamide derivatives has never been reported. Enzymatic preparation of functionalized and α-tetrasubstituted α-amino acids documented in the literature involves mainly kinetic resolution of racemic substrates using various hydrolytic enzymes.[27] Our continuous interest in synthetic biocatalysis[11a−c] and in nonproteinogenic amino acids[13,19,20] led us to undertake the current study. We report herein the first example of highly efficient, enantioselective, and scalable amidase-catalyzed desymmetrization of α-substituted-α-amino malonamides. The reaction produces various functionalized α-tetrasubstituted α-amino acids in high yields with excellent enantiomeric excesses. The synthetic versatility of densely functional products resulting from biotransformation has been demonstrated by practical and expedient synthesis of enantiopure α-substituted serine analogues and diamino alcohol products of pharmaceutical interest.

RESULTS AND DISCUSSION

Starting prochiral α-substituted-α-amino malonamides **5** were easily prepared from diethyl α-aminomalonate **1**, a commercially available compound (Scheme 1). According to a reported method,[29] diethyl α-aminomalonate **1** reacted with allyl bromide, (*E*)-1-bromobut-2-ene, 3-bromo-2-methylprop-1-ene, 1-bromobut-2-yne, and methyl iodide in the presence of sodium ethoxide at 0 °C to produce the corresponding α-substituted-α-aminomalonates **2a−c**, **2g**, and **2j**. Other α-substituted-α-aminomalonates such as **2d**, **2e**, **2f**, and **2h** were obtained from the alkylation of imine of diethyl α-aminomalonate **1**[30] with (*E*)-(3-bromoprop-1-enyl)benzene, 4-bromobut-1-ene, 3-bromoprop-1-yne, and benzyl bromide, respectively, followed by hydrolysis of imines **4** under acidic conditions.[30] Diethyl α-amino-α-propylmalonate **2i** was synthesized from catalytic hydrogenation of diethyl α-allyl-α-aminomalonate **2a**. All malonates **2** were converted effectively into prochiral α-substituted-α-aminomalonamides **5a−j** by means of ammonolysis with aqueous ammonia in methanol at 0 °C.[31]

Scheme 1. Synthesis of Prochiral Malonamides 5



5a, R = CH_2=CH; **5b**, R = (*E*)-MeCH=CH;
5c, R = CH_2=CMe; **5d**, R = (*E*)-PhCH=CH;
5e, R = CH_2=$CHCH_2$; **5f**, R = HC≡C;
5g, R = MeC≡C; **5h**, R = Ph;
5i, R = Et; **5j**, R = Me

We initiated our biotransformation study with the examination of the reaction of prochiral α-allyl-α-aminomalonamide **5a**. Catalyzed by *Rhodococcus erythropolis* AJ270 whole cells in neutral phosphate buffer at 30 °C, malonamide **5a** underwent very rapid and highly enantioselective hydrolysis to afford, within 40 min, (*R*)-2-amino-2-carbamoylpent-4-enoic acid (*R*)-**6a** in 87% yield with 97.0% ee (entry 1, Table 1). Encouraged by the high efficiency and excellent enantioselectivity of the amidase observed in desymmetrization reaction of **5a**, a number of prochiral α-aminomalonamides that contained various substituents were subjected to biocatalysis. Gratifyingly, as compiled in Table 1, under identical mild biocatalytic conditions, almost all substrates tested were desymmetrically hydrolyzed, producing highly enantioenriched (*R*)-α-amino acids in good to excellent yields. For example, α-aminomalonamides that have an α-(*E*)-but-2-enyl (**5b**) and an α-2-methylallyl substituent (**5c**) were transformed almost quantitatively within 1 h into the corresponding α-amino acids **6b** and **6c** with ee's of 97.4% and 98.6%, respectively (entries 2 and 3, Table 1). Introduction of an (*E*)-cinnamyl group at the α-position led to equally enantioselective hydrolysis of **5d**, which yielded **6d** in 74% yield and 94.0% ee, albeit at a slower reaction rate due to most likely the steric effect of the phenyl group (entry 4, Table 1). Fast and almost complete conversion and enantioselectivity was observed in the desymmetrization of α-but-3-enyl-substituted α-aminomalonamide **5e**, a substrate contains a terminal olefin moiety γ-positioned to stereogenic center. Enantiopure *R*-**6e** was produced in 96% yield from incubation of **5e** with microbial cells in less than 30 min (entry 5, Table 1). It is noteworthy that α-aminomalonamides bearing substituents other than alkene were also excellent substrates that were recognized nicely by the amidase. The biotransformation of alkyne-bearing substrates **5f** and **5g**, which contained, respectively, a prop-2-ynyl and a but-2-ynyl group, thus proceeded efficiently around 30 min to furnish an excellent enantiomeric excess value (96.8%) of (*R*)-2-amino-2-carbamoylpent-4-ynoic acid **6f** in 80% yield and (*R*)-2-amino-2-carbamoylhex-4-ynoic acid **6g** in 94% yield (entries 6 and 7, Table 1). Interestingly, the amidase within *Rhodococcus erythropolis* AJ270 exhibited similarly excellent enantioselectivity against α-aminomalonamides that contain a simple alkyl group. This has been exemplified by the hydrolytic desymmet-

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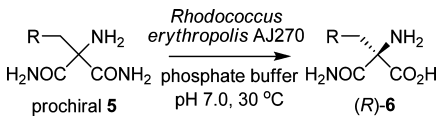
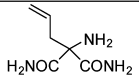
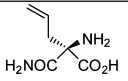
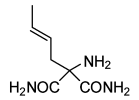
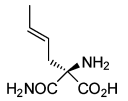
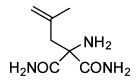
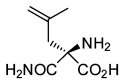
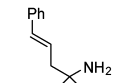
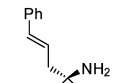
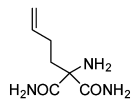
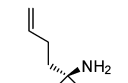
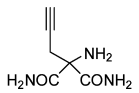
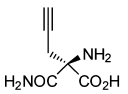
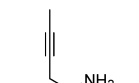
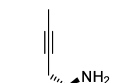
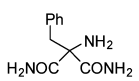
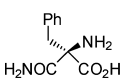
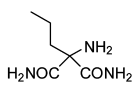
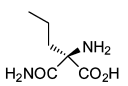
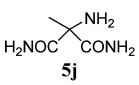
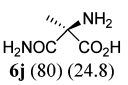
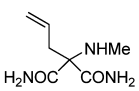
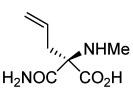
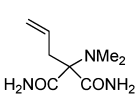
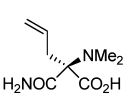
Table 1. Biocatalytic Hydrolysis of Malonamides 5[a]



Entry	Substrate	t (min)	6 (yield %)[b] (ee %)[c]
1	 **5a**	40	 **6a** (87) (97.0)
2	 **5b**	50	 **6b** (93) (97.4)
3	 **5c**	40	 **6c** (98) (98.6)
4	 **5d**	330	 **6d** (74) (94.0)
5	 **5e**	25	 **6e** (96) (>99.5)
6	 **5f**	25	 **6f** (80) (96.8)
7	 **5g**	34	 **6g** (94) (96.8)
8	 **5h**	60	 **6h** (83) (>99.5)
9	 **5i**	35	 **6i** (84) (97.8)
10	 **5j**	18	 **6j** (80) (24.8)
11	 **5k**	7 d	 **6k** (53)[d] (64.6%)
12	 **5l**	7 d	 **6l** (0)[e]

[a]Substrate **5** (2 mmol) was incubated with *Rhodococcus erythropolis* AJ270 cells (2 g wet weight) in phosphate buffer (*p*H 7.0) at 30 °C. [b]Isolated yield. [c]Determined by chiral HPLC analysis of their benzyl ester derivatives **7** (see Scheme 2). [d]Starting material **5k** (42%) was recovered. [e]Starting material **5l** (98%) was recovered.

rization of α-benzylated and α-propylated α-aminomalonamides **5h** and **5i**, which led to the high-yielding formation of (*R*)-2-amino-2-carbamoyl-3-phenylpropanoic acid **6h** and (*R*)-2-amino-2-carbamoylpentanoic acid **6i** in highly enantiomerically pure form (entries 8 and 9, Table 1). Only in the case of biotransformation of α-amino-α-methylmalonamide **5j** was low enantioselectivity obtained (entry 10, Table 1).

It is very interesting to note that *Rhodococcus erythropolis* AJ270-catalyzed desymmetrization reaction of prochiral α-substituted α-aminomalonamides **5** proceeds much more efficiently and enantioselectively than that of prochiral α-substituted α-methylmalonamides and α,α-unsymmetrically disubstituted malonamides.[23] In other words, all prochiral α-substituted α-aminomalonamides **5** tested in the current study are excellent substrates for the amidase involved in microbial whole cells. To shed light on the effect of amino group on the biocatalytic desymmetrization, α-methylamino- and α-dimethylamino-substituted malonamide substrates **5k** and **5l** were incubated with biocatalyst under identical conditions. Intriguingly, biocatalytic hydrolysis of **5k** proceeded very slowly, with only about half of the substrate being converted into product **6k** with 64.6% ee in 7 days (entry 11, Table 1). α-Dimethylamino-α-allylmalonamide **5l** was not accepted at all by the amidase, and nearly quantitative reactant was recovered after 7 days' incubation with biocatalyst (entry 12, Table 1). Although detailed mechanism of the amidase awaits further investigation, hypothetically, there is most probably an enantioselective pocket which recognizes the amino (NH_2) moiety of the substrates **5**.

As a microbial whole catalyst, *Rhodococcus erythropolis* AJ270 has been found to be robust and tolerant to high concentrations of many amide substrates and acid products, allowing the scaled-up synthesis of desired chiral products.[32] To demonstrate the practical preparative biotransformation, immobilized whole cell catalyst was employed and its recyclability was examined. Using *Rhodococcus erythropolis* AJ270 whole cells immobilized in alginate,[33] reiterative incubation of biocatalyst with **5a** (10 mmol ×8) afforded (*R*)-**6a** (9.3 g, 74%) with 95.8% ee.

Densely functionalized α-amino acids **6** from biocatalytic desymmetrization are conceivably versatile organic entities in synthesis based on functional group transformations. To demonstrate their potential in organic synthesis and also to obtain the enantiomeric excess values of products, all compounds **6** were first of all derivatized into their benzyl esters **7** (Scheme 2), which were successfully resolved on a column coated with chiral stationary phase (see Supporting Information). To further show synthetic application and to determine its absolute configuration as well, (*R*)-**6a** was converted conveniently into α-substituted serine analogues, compounds that are useful in the study of peptide or protein mimics.[24,25] As illustrated in Scheme 2, treatment of (*R*)-**6a** with diazomethane gives ester (*R*)-**8** in 78% yield. Selective reduction of the ester group of (*R*)-**8** using a combination of $NaBH_4$ and $LiCl{\cdot}H_2O$[34] followed by amide hydrolysis under basic conditions results in the quantitative formation of (*S*)-(−)-α-allylserine **9**. Catalytic hydrogenation of (*S*)-(−)-**9** affords (*S*)-(+)-α-propylserine **10** almost quantitatively. Comparison of the optical rotation of **10** with that reported in the literature[35] allowed us to assign the absolute configuration of (*S*)-**10** and therefore of (*R*)-**6a**. The synthetic versatility of (*R*)-**6a** was further demonstrated by the synthesis of diamino alcohol (*R*)-(+)-**12**, a key precursor to chiral cyclic

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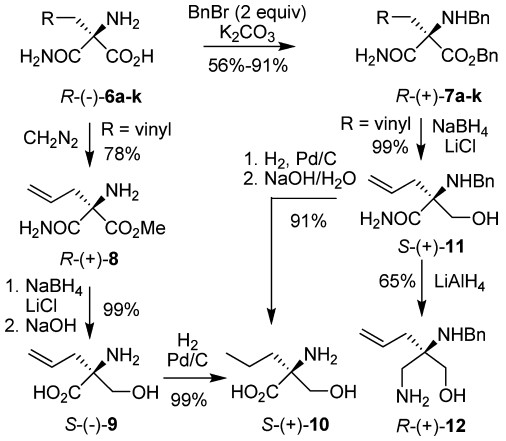
Scheme 2. Synthesis of α-Substituted Serine and Diamino Alcohol Derivatives



urea derivatives that are very potent and selective NK_1 receptor antagonists.[36] Thus, interaction of (*R*)-**6a** with 2 equiv of benzyl bromide gave rise to α-(benzylamino) ester intermediate (*R*)-(+)-**7a** in a high yield. Serine amide analogue (*S*)-(+)-**11** was obtained in a quantitative yield from selective reduction of ester of (*R*)-(+)-**7a** by $NaBH_4$ in the presence of $LiCl{\cdot}H_2O$.[34] Reduction of amide by $LiAlH_4$ furnished diamino alcohol product (*R*)-(+)-**12**. Catalytic hydrogenation of (*S*)-(+)-**11** led simultaneously to the reduction of the carbon−carbon double bond and the cleavage of the *N*-benzyl group to produce, after hydrolysis, (*S*)-(+)-α-propylserine **10** in 91% yield (Scheme 2).

CONCLUSION

In conclusion, we have shown a highly efficient and enantioselective biocatalytic desymmetrization reaction using *Rhodococcus erythropolis* AJ270 whole cell catalyst under mild conditions. The amidase-catalyzed hydrolysis of prochiral α-substituted α-aminomalonamides provides a convenient and straightforward synthetic route to densely functionalized α-tetrasubstituted α-amino acids in high yields with excellent enantiomeric excesses. The resulting enantioenriched products, which are not readily available by other chemical and biochemical means, are invaluable chiral building blocks in organic synthesis, and their applications have been demonstrated by the practical and expedient preparation of α-substituted or functionalized serine analogues and diamino alcohol.

EXPERIMENTAL SECTION

Preparation of α-Substituted α-Aminomalonates 2a−c, 2g, and 2j (Method A).[29] To a solution of sodium ethoxide (680 mg, 10 mmol) in EtOH (30 mL) was added the hydrochloric acid salt of diethyl α-aminomalonate **1** (1.05 g, 5 mmol). After the solution was stirred at 0 °C for 2 h, a solution of RBr or iodomethane (6 mmol) in EtOH (3 mL) was added through a syringe. The resulting mixture was stirred at 0 °C for another 3 h. The reaction was quenched by addition of water (100 mL) at room temperature and then extracted with ethyl acetate (3 × 50 mL). The organic layer was dried with anhydrous $MgSO_4$, and the solvent was removed under vacuum. The residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate (8:1−4:1) as the mobile phase to give product **2a** [0.54 g, 50%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.77−5.63 (m, 1H), 5.53−5.44 (m, 2H), 4.23 (q, *J* = 7.2 Hz, 4H), 3.19 (d, *J* = 7.2 Hz, 2H), 2.13 (s, 1H), 1.67 (d, *J* = 7.2 Hz, 6H), 1.67 (t, *J* = 7.2 Hz, 6H)], **2b** [0.33 g, 29%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.66−5.57 (m, 1H), 5.23−5.17 (m, 1H), 4.23 (q, *J* = 7.2 Hz, 4H), 3.19 (d, *J* = 7.2 Hz, 2H), 2.13 (s, 2H), 1.68 (t, *d* = 6.0 Hz, 3H), 1.29 (t, *J* = 7.2 Hz, 6H)], **2c** [0.38 g, 33%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.94 (s, 1H), 4.84 (s, 1H), 4.22 (q, *J* = 7.2 Hz, 4H), 2.78 (s, 2H), 2.08 (s, 2H), 1.69 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 6H)], **2f** [0.51 g, 48%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.30−4.20 (m, 4H), 2.92 (d, *J* = 2.4 Hz, 2H), 2.28 (s, 2H), 2.08 (t, *J* = 2.4 Hz, 1H), 1.28 (t, *J* = 7.2 Hz, 6H)], or **2j** [0.30 g, 32%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.22 (q, *J* = 7.2 Hz, 4H), 2.08 (s, 2H), 1.54 (s, 3H), 1.27 (t, *J* = 7.2 Hz, 6H)]. The products were used directly for the next step without further characterization.

Preparation of α-Substituted α-Aminomalonates 2d, 2e, 2g, and 2h (Method B).[30] *Synthesis of Imine* ***3***. A mixture of the hydrochloric acid salt of diethyl α-aminomalonate **1** (1.05 g, 5 mmol), benzophenone (910 mg, 5 mmol), *p*-TSA (95 mg, 0.5 mmol), and toluene (50 mL) was refluxed overnight using a Dean−Stark apparatus to remove water. The solvent was then removed under vacuum. The residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate (15:1−10:1) as the mobile phase to give imine intermediate **3** (900 mg, 53%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69−7.18 (m, 10H), 4.85 (s, 1H), 4.30−4.20 (m, 4H), 1.28 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 167.2, 139.0, 135.5, 130.9, 129.3, 129.1, 128.7, 127.8, 69.7, 61.9, 14.1.

Alkylation of Imine ***3***. To a solution of sodium hydride (0.65 g, 26 mmol) in dry THF (125 mL) and DMF (15 mL) or potassium carbonate (3.59 g, 26 mmol) in acetone (125 mL) or acetonitrile (125 mL) was added imine intermediate **3** (8.5 g, 25 mmol). After the mixture was stirred at room temperature for 1 h, RBr (30 mmol) was added through a syringe. After the resulting mixture was stirred at room temperature or at refluxing temperature for another 12 h, the reaction was quenched by addition of water (300 mL) and then extracted with ethyl acetate (3 × 150 mL). The organic layer was dried with anhydrous $MgSO_4$, and the solvent was removed under vacuum. The residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate (30:1−20:1) as the mobile phase to give **4d** (8.19 g, 72%), **4e** (6.29 g, 64%), **4g** (3.71 g, 38%), or **4 h** (8.47 g, 79%). All intermediates were used directly for the next step without characterization.

Hydrolysis of Imine ***4***. To a mixture of ether (5 mL) and hydrochloric acid (1 N, 1 mL) was added **4** (1 mmol). After the mixture was stirred at room temperature overnight, a solution of sodium hydroxide (2 N) was added to basify the mixture to pH = 8. Water (10 mL) was added, and the mixture was then extracted with ethyl acetate (3 × 20 mL). The organic layer was dried with anhydrous $MgSO_4$, and the solvent was removed under vacuum. The residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate (3:1−1:1) as the mobile phase to give product **2d** (215 mg, 74%), **2e** [199 mg, 87%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.86−5.76 (m, 1H), 5.08−4.97 (m, 2H), 4.22 (q, *J* = 7.2 Hz, 4H), 2.07−2.05 (m, 6H), 1.27 (t, *J* = 7.2 Hz, 6H)], **2g** [127 mg, 56%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.27−4.20 (m, 4H), 2.86 (q, *J* = 2.4 Hz, 2H), 2.26 (s, 2H), 1.77 (t, *J* = 2.4 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 6H)], or **2h** [209 mg, 79%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29−7.16 (m, 5H), 4.24 (q, *J* = 7.2 Hz, 4H), 3.32 (s, 2H), 1.94 (s, 2H), 1.28 (t, *J* = 7.2 Hz, 6H)]. Compounds were used directly without further characterization.

Preparation of α-Substituted α-Aminomalonates 2i (Method C). In the presence of Pd/C (10%, 200 mg), **2a** (2.15 g, 10 mmol) in methanol (50 mL) was hydrogenated using a hydrogen balloon. When the starting material was consumed, the catalyst was filtered and the filtrate was concentrated to give diethyl α-amino-α-propylmalonate **2i** (2.15 g, 99%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.22 (q, *J* = 7.2 Hz, 4H), 2.02 (s, 2H), 1.96−1.90 (m, 2H), 1.36−1.25 (m, 8H), 0.95 (t, *J* = 7.2 Hz, 3H). It was used directly for the next step without further characterization.

Preparation of α-Substituted α-Aminomalonamides 5.[31] A mixture of α-substituted α-aminomalonates **2a−j** (2 mmol), ammonia solution ($NH_3{\cdot}H_2O$, 25% w/w, 4 mL), and MeOH (4 mL) was stirred at 0 °C until the reactant was consumed. The reaction mixture was then neutralized with hydrochloric acid (1 N). After removal of solvent under vacuum, the residue was chromatographed on a silica gel column using ethyl acetate as the mobile phase to give product **5a−j**.

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Except for **5h**, all products **5** are new compounds. Spectroscopic data and microanalyses of **5a–j** were listed as follows.

*2-Allyl-2-aminomalonamide (**5a**).* 160 mg, 51%; mp 106–107 °C; IR (KBr) ν 3430, 3339, 1667 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.82 (s, 2H), 5.96 (s, 2H), 5.75–5.61 (m, 1H), 5.25–5.17 (m, 2H), 70 (d, J = 7.2 Hz, 2H), 2.16 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 174.8, 131.4, 121.0, 63.8, 45.4; MS (ESI) m/z 180 $[M + Na]^+$ (67), 158 $[M + H]^+$ (100). Anal. Calcd for $C_6H_{11}N_3O_2$: C, 45.85; H, 7.05; N, 26.74. Found: C, 45.68; H, 7.12; N, 26.66.

*(E)-2-Amino-2-(but-2-en-1-yl)malonamide (**5b**).* 161 mg, 47%; mp 135–136 °C; IR (KBr) ν 3395, 3187, 1687, 1352 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 7.46 (s, 2H), 7.32 (s, 2H) 5.56–5.48 (m, 1H), 5.31–5.23 (m, 1H), 2.47 (d, J = 7.2 Hz, 2H), 2.12 (s, 2 H), 1.62 (d, J = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 174.0, 129.3, 125.3, 64.1, 42.1, 17.9; MS (ESI) m/z 194 $[M + Na]^+$ (20), 172 $[M + H]^+$ (100). Anal. Calcd for $C_7H_{13}N_3O_2$: C, 49.11; H, 7.65; N, 24.54. Found: C, 49.29; H, 7.63; N, 24.94.

*2-Amino-2-(2-methylallyl)malonamide (**5c**).* 154 mg, 45%; mp 133–134 °C; IR (KBr) ν 3409, 3269, 1670, 1573 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 7.52 (s, 2H), 7.38 (s, 2H) 4.85 (s, 1H), 4.77 (s, 1H), 2.57 (s, 2H), 2.14 (s, 2H), 1.66 (s, 3H); ^{13}C NMR (75 MHz, DMSODMSO-d_6) 174.2, 141.0, 115.7, 63.3, 46.4, 22.7; MS (ESI) m/z 194 $[M + Na]^+$ (20), 172 $[M + H]^+$ (100). Anal. Calcd for $C_7H_{13}N_3O_2$: C, 49.11; H, 7.65; N, 24.54. Found: C, 49.16 7.63; N, 24.69.

*2-Amino-2-cinnamylmalonamide (**5d**).* 224 mg, 48%; mp 120–121 °C; IR (KBr) ν 3430, 3252, 1683 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 7.36 (s, 2H), 7.34–7.28 (m, 6H), 7.2–7.19 (m, 1), 6.49 (d, J = 15.0 Hz, 1H), 6.17–6.07 (m, 1H), 2.71 (d, J = 7.3, 2H), 2.27 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) 173.9, 136.9, 133.3, 128.5, 127.2, 126.0, 124.8, 64.5, 42.3; MS (ESI) m/z 256 $[M + Na]^+$ (19), 234 $[M + H]^+$ (100). Anal. Calcd for $C_{12}H_{15}N_3O_2$: C, 61.79; H, 6.48 N, 18.01. Found: C, 62.10; H, 6.54; N, 17.85.

*2-Amino-2-(but-3-en-1-yl)malonamide (**5e**):* 109 mg, 32%; mp 133–134 °C; IR (KBr) ν 3416, 1657 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 7.82 (s, 2H), 5.91 (s, 2H), 5.85–5.72 (m, 1H), 5.08–4.97 (m, 2H), 2.14–2.01 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) 172.4, 134.2, 113.1, 62.2, 37.2, 25.5; MS (ESI) m/z 194 $[M + Na]^+$ (38), 172 $[M + H]^+$ (100). Anal. Calcd for $C_7H_{13}N_3O_2$: C, 49.11; H, 7.65; N, 24.54. Found: C, 48.91; H, 7.36; N, 24.41.

*2-Amino-2-(prop-2-yn-1-yl)malonamide (**5f**):* 167 mg, 54%; mp 122–123 °C; IR (KBr) ν 3400, 1689 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 7.42 (s, 2H), 7.38 (s, 2H), 2.87(s, 2H), 2.73 (d, J = 2.2, 2H), 2.33 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) 172.5, 80.5, 73.7, 63.9, 28.6; MS (ESI) m/z 156 $[M + H]^+$ (100). Anal. Calcd for $C_6H_9N_3O_2$: C, 46.45; H, 5.85; N, 27.08. Found: C, 46.43; H, 5.87; N, 26.78.

*2-Amino-2-(but-2-yn-1-yl)malonamide (**5g**):* 176 mg, 52%; mp 168–169 °C; IR (KBr) ν 3394, 3181, 1680 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 7.40 (s, 2H), 7.34 (s, 2H), 2.69 (d, J = 2.4 Hz, 2H), 2.29 (s, 2H), 1.73 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 172.8, 78.5, 75.0, 64.0, 29.2, 3.2; MS (ESI) m/z 192 $[M + Na]^+$ (68), 170 $[M + H]^+$ (100). Anal. Calcd for $C_7H_{11}N_3O_2$: C, 49.70; H, 6.55; N, 24.84. Found: C, 49.75; H, 6.43; N, 24.70.

*2-Amino-2-benzylmalonamide (**5h**):* 207 mg, 50%; mp 175–176 °C; IR (KBr) ν 3421, 3206, 1687 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 7.49 (s, 2H), 7.41 (s, 2H), 7.29–7.21 (m, 5H), 3.11 (s, 2H), 2.06 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) 173.7, 136.3, 130.2, 127.9, 126.6, 65.3, 43.8; MS (ESI) m/z 230 $[M + Na]^+$ (100), 208 $[M + H]^+$ (90). Anal. Calcd for $C_{10}H_{13}N_3O_2$: C, 57.96; H, 6.32; N, 20.28. Found: C, 57.79; H, 6.40; N, 20.49.

*2-Amino-2-propylmalonamide (**5i**):* 108 mg, 34%; mp 168–169 °C; IR (KBr) ν 3374, 1678 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 7.49 (s, 2H), 7.33 (s, 2H), 2.24 (s, 2H), 1.76–1.70 (m, 2H), 1.28–1.16 (m, 2H), 0.84 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 177.5, 66.3, 43.2, 18.2, 14.4; MS (ESI) m/z 160 $[M + H]^+$ (100). Anal. Calcd for $C_6H_{13}N_3O_2$: C, 45.27; H, 8.23; N, 26.40. Found: C, 45.30; H, 8.18; N, 26.18.

*2-Amino-2-methylmalonamide (**5j**):* 145 mg, 57%; mp 218–219 °C; IR (KBr) ν, 3396, 1673 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.41 (s, 2H), 7.24 (s, 2H), 2.30 (s, 2H), 1.37 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.3, 61.3, 26.1; MS (ESI) m/z 154 $[M + Na]^+$ (25), 132 $[M + H]^+$ (100). Anal. Calcd for $C_4H_9N_3O_2$: C, 36.64; H, 6.92; N, 32.04. Found: C, 36.69; H, 6.95; N, 31.95.

*Preparation of **5k** and **5l**.* A mixture of **1a** (157 mg, 1 mmol), K_2CO_3 (35 mg, 0.25 mmol), CH_3I (1 mL, 16 mmol), and DMF (1 mL) was stirred at room temperature for 2 h. After removal of solvent, the residue was chromatographed on a silica gel column eluted with a mixture of petroleum ether, ethyl acetate and methanol (1:2:0.05–0:2:0.05) to give pure **5k** and **5l** along with the recovery of **1a** (80 mg, 51%).

*2-Allyl-2-(methylamino)malonamide (**5k**):* 53 mg, 31%; 179 °C sublimation; IR (KBr) ν, 3389, 1658 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 2H), 5.80–5.64 (m, 3H), 5.19–5.14 (m, 2H), 2.67 (d, J = 7.2 Hz, 2H), 2.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 131.2, 119.7, 69.6, 42.3, 30.6; MS (ESI) m/z 194 $[M + Na]^+$ (17), 172 $[M + H]^+$ (100). Anal. Calcd for $C_7H_{13}N_3O_2$: C, 49.11; H, 7.65; N, 24.54. Found: C, 49.02; H, 7.65; N, 24.36.

*2-Allyl-2-(dimethylamino)malonamide (**5l**):* 32 mg, 17%; mp 101–102 °C; IR (KBr) ν, 3432, 3279, 1658 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.66 (s, 2H), 7.33 (s, 2H), 5.77–5.67 (m, 1H), 5.09–4.97 (m, 2H), 2.61 (d, J = 7.2 Hz, 2H), 2.32 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.4, 134.0, 117.4, 72.7, 40.3, 38.7; MS (ESI) m/z 208 $[M + Na]^+$ (43), 286 $[M + H]^+$ (100). Anal. Calcd for $C_8H_{15}N_3O_2$: C, 51.88; H, 8.16; N, 22.69. Found: C, 51.63; H, 7.92; N, 22.62.

General Procedure for the Biocatalytic Desymmetrization of Prochiral Dicarboxamides 5. To an Erlenmeyer flask (150 mL) with a screw cap were added *Rhodococcus erythropolis* AJ270 cells[12a,14] (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. A prochiral diamide substrate **5a–l** (2 mmol) 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 and GC, was quenched after a specified period of time (Table 1) by filtration of the mixture through a Celite pad. The aqueous filtrate was freeze-dried, and the residue was chromatographed on a reversed-phase silica gel column (SP-120-50-ODS-A) using water as mobile phase or on an ion-exchange resin column (Dowex50wx8) using aqueous ammonium solution (10%) as mobile phase to give product **6a–k**. Except for **6j**, products **6** are new compounds. All products were fully characterized by means of spectroscopic data and microanalyses.

*(R)-2-Amino-2-carbamoylpent-4-enoic acid (**6a**):* 275 mg, 87%; mp 145–146 °C; $[\alpha]^{25}_D$ −22.4 (c 1.70, H_2O); IR (KBr) ν 3397, 1668, 1516 cm^{-1}; ^1H NMR (300 MHz, D_2O) 5.67–5.53 (m, 1H), 5.25–5.19 (m, 2H), 2.84–2.69 (m, 2H); ^{13}C NMR (75 MHz, D_2O) 170.9, 170.5, 129.3, 122.2, 67.0, 40.3; MS (ESI) m/z 157 $[M - H]^-$ (100), calcd for $C_6H_{10}N_2O_3$ 159.0764 $[M + H]^+$, found (P-SIMS) 159.0765 $[M + H]^+$.

*(R,E)-2-Amino-2-carbamoylhex-4-enoic acid (**6b**):* 320 mg, 93%; mp 148–149 °C; $[\alpha]^{25}_D$ −18.7 (c 1.50, H_2O); IR (KBr) ν 3326, 1685, 1508 cm^{-1}; ^1H NMR (300 MHz, D_2O) 5.78–5.66 (m, 1H), 5.32–5.22 (m, 1H), 2.87–2.66 (m, 2H), 1.61 (d, J = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, D_2O) 170.9, 170.6, 134.1, 121.2, 67.3, 39.4, 17.3; MS (ESI) m/z 195 $[M + Na]^+$ (33), 173 $[M + H]^+$ (100); calcd for $C_7H_{12}N_2O_3$ 173.0921 $[M + H]^+$, found (P-SIMS) 173.0922 $[M + H]^+$.

*(R)-2-Amino-2-carbamoyl-4-methylpent-4-enoic acid (**6c**):* 337 mg, 98%; mp 139–140 °C; $[\alpha]^{25}_D$ −20.6 (c 1.55, H_2O); IR (KBr) ν 3128, 1685, 1501 cm^{-1}; ^1H NMR (300 MHz, D_2O) 5.01 (s, 1H), 4.87 (s, 1H), 4.87 (s, 1H), 2.91 (d, J = 14.4 Hz, 1H), 2.78 (d, J = 14.4 Hz, 1H), 1.69 (s, 3H); ^{13}C NMR (75 MHz, D_2O) 171.4, 170.9, 138.7, 117.9, 66.3, 43.7, 21.8; MS (ESI) m/z 171 $[M - H]^-$ (100), calcd for $C_7H_{12}N_2O_3$ 173.0921 $[M + H]^+$, found (P-SIMS) 173.0923 $[M + H]^+$.

*(R,E)-2-Amino-2-carbamoyl-5-phenylpent-4-enoic acid (**6d**):* 346 mg, 74%; mp 162–163 °C; $[\alpha]^{25}_D$ +11.8 (c 0.85, DMF); IR (KBr) ν 3140, 1682, 1507 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 7.76 (s, 2H), 7.40–7.20 (m, 5H), 6.47 (d, J = 12.8 Hz, 1H), 6.21–6.11 (m, 1H), 2.85–2.81 (m, 2H); ^{13}C NMR (75 MHz, MeOD/DMSO-d_6) 170.4, 166.9, 136.9, 133.4, 128.4, 127.3, 126.2, 123.5, 66.4, 40.2; MS (ESI)

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m/z 233 $[M - H]^-$ (100); cCalcd for $C_{12}H_{14}N_2O_3$ 235.1077 $[M + H]^+$, found (P-SIMS) 235.1079 $[M + H]^+$.

*(R)-2-Amino-2-carbamoylhex-5-enoic acid (**6e**):* 330 mg, 96%; mp 158−159 °C; $[\alpha]^{25}_D$ −28.1 (c 1.85, H_2O); IR (KBr) ν 3332, 1682, 1508 cm^{-1}; ^1H NMR (300 MHz, D_2O) 5.86−5.72 (m, 1H), 5.09−4.98 (m, 2H), 2.18−2.14 (m, 2H), 2.08−2.05 (m, 2H); ^{13}C NMR (75 MHz, D_2O) 171.2, 170.7, 136.3, 116.1, 67.6, 35.2, 27.6; MS (ESI) m/z 171 $[M - H]^-$ (100); calcd for $C_7H_{12}N_2O_3$ 173.0921 $[M + H]^+$, found (P-SIMS) 173.0922 $[M + H]^+$.

*(R)-2-Amino-2-carbamoylpent-4-ynoic acid (**6f**):* 251 mg, 80%; mp 125−126 °C; $[\alpha]^{25}_D$ −5.7 (c 1.75, H_2O); IR (KBr) ν 3395, 1671, 1513 cm^{-1}; ^1H NMR (300 MHz, D_2O) 2.98 (s, 2H), 2.49 (s, 1H); ^{13}C NMR (75 MHz, D_2O) 171.4, 170.9, 77.1, 74.0, 66.0, 26.5; MS (ESI) m/z 155 $[M - H]^-$ (100); calcd for $C_6H_8N_2O_3$ 157.0608 $[M + H]^+$, found (P-SIMS) 157.0609 $[M + H]^+$.

*(R)-2-Amino-2-carbamoylhex-4-ynoic acid (**6g**):* 320 mg, 94%; mp 129−130 °C; $[\alpha]^{25}_D$ −27.7 (c 1.56, H_2O); IR (KBr) ν 3382, 1697, 1335 cm^{-1}; ^1H NMR (300 MHz, D_2O) 2.91 (s, 2H), 1.69 (s, 3H); ^{13}C NMR (75 MHz, D_2O) 171.0, 170.6, 82.4, 71.1, 66.5, 26.9, 2.5; MS (ESI) m/z 169 $[M - H]^-$ (100); calcd for $C_7H_{10}N_2O_3$ 171.0764 $[M + H]^+$, found (P-SIMS) 171.0762 $[M + H]^+$.

*(R)-2-Amino-2-carbamoyl-3-phenylpropanoic acid (**6h**):* 347 mg, 83%; mp 148−149 °C; $[\alpha]^{25}_D$ −23.3 (c 0.60, DMF); IR (KBr) ν 3377, 1682, 1503 cm^{-1}; ^1H NMR (300 MHz, D_2O) 7.34−7.22 (m, 5H), 3.44 (d, J = 14.4 Hz, 1H), 3.33 (d, J = 14.4 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) 171.0, 170.6, 132.9, 129.9, 129.1, 128.3, 68.3, 41.6; MS (ESI) m/z 207 $[M - H]^-$ (100); calcd for $C_{10}H_{12}N_2O_3$ 209.0921 $[M + H]^+$, found (P-SIMS) 209.0921 $[M + H]^+$.

*(R)-2-Amino-2-carbamoylpentanoic acid (**6i**):* 269 mg, 84%; mp 163−164 °C; $[\alpha]^{25}_D$ −22.1 (c 1.90, H_2O); IR (KBr) ν 3441, 1681, 1508 cm^{-1}; ^1H NMR (300 MHz, D_2O) 2.05−1.88 (m, 2H), 1.32−1.08 (m, 2H), 0.81 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, D_2O) 171.5, 171.1, 67.8, 38.1, 16.9, 12.9; MS (ESI) m/z 159 $[M - H]^-$ (100); calcd for $C_6H_{12}N_2O_3$ 161.0921 $[M + H]^+$, found (P-SIMS) 161.0921 $[M + H]^+$.

*(R)-2-Amino-2-carbamoylpropanoic acid (**6j**):* 211 mg, 80%; mp 216−217 °C; $[\alpha]^{25}_D$ +6.1 (c 1.65, H_2O); IR (KBr) ν 3370, 1634, 1097 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.62 (s, 3H); ^{13}C NMR (75 MHz, D_2O) δ 172.0, 171.7, 63.8, 21.8; MS (ESI) m/z 133 $[M + 1]^+$ (100); calcd for $C_4H_8N_2O_3$ 133.0608 $[M + 1]^+$, found (P-SIMS) 133.0608 $[M + 1]^+$.

*(R)-2-Carbamoyl-2-(methylamino)pent-4-enoic acid (**6k**):* 182 mg, 53%; mp 177−178 °C; $[\alpha]^{25}_D$ −10.0 (c 0.60, H_2O); IR (KBr) ν 3352, 1676, 1399 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 5.69−5.55 (m, 1H), 5.32−5.23 (m, 2H), 2.93−2.80 (m, 2H), 2.59 (s, 3H); ^{13}C NMR (75 MHz, D_2O) δ 170.2, 169.5, 129.0, 122.0, 72.6, 37.4, 29.0; MS (ESI) m/z 171 $[M + H]^+$ (100); calcd for $C_7H_{10}N_2O_3$ 173.0921 $[M + 1]^+$, found (P-SIMS) 173.0921 $[M + 1]^+$.

General Procedure for the Conversion of Acid Products 6 into Esters 7. To a solution of **6** (100 mg) in DMF (3 mL) were added K_2CO_3 (200 mg) and benzyl bromide (0.2 mL). The resulting mixture was allowed to stir at room temperature overnight. Water (25 mL) was added, and the mixture was extracted with ethyl acetate (3 × 25 mL). The organic phase was dried with anhydrous $MgSO_4$. After removal of the solvent under vacuum, the residue was chromatographed on a silica gel column with a mixture of petroleum ether and ethyl acetate (3:1) as the mobile phase to give products **7**.

*Benzyl (R)-2-(benzylamino)-2-carbamoylpent-4-enoate (**7a**):* 196 mg, 91%; mp 145−146 °C; $[\alpha]^{25}_D$ +17.9 (c 1.90, $CHCl_3$); ee = 97.0% (chiral HPLC analysis); IR (KBr) ν 3437, 1742, 1689, 1202 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.32−7.19 (m, 10H), 7.08 (s, 1H), 5.80−5.66 (m, 2H), 5.29−5.13 (m, 4H), 3.64 (d, J = 12.0 Hz, 1H), 3.56 (d, J = 12.0 Hz, 1H), 2.97−2.84 (m, 2H), 2.31 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 172.2, 170.7, 135.4, 131.5, 128.63, 128.58, 128.51, 128.47, 128.2, 128.1, 127.5, 119.7, 70.5, 67.5, 47.6, 36.7; MS (ESI) m/z 339 $[M + H]^+$ (100). Anal. Calcd.for $C_{20}H_{22}N_2O_3$: C, 70.99; H, 6.55; N, 8.28. Found: C, 70.81; H, 6.57; N, 8.25.

*Benzyl (R,E)-2-(benzylamino)-2-carbamoylhex-4-enoate (**7b**):* 127 mg, 62%; mp 88−89 °C; $[\alpha]^{25}_D$ +17.5° (c 1.60 $CHCl_3$); ee = 97.4% (chiral HPLC analysis); IR (KBr) ν 3433, 1745, 1683, 1202 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.36−7.20 (m, 10H), 7.11 (s, 1H), 5.75 (s, 1H), 5.62−5.50 (m, 1H), 5.36−5.15 (m, 3H), 3.63 (d, J = 12.3 Hz, 1H), 3.56 (d, J = 12.0 Hz, 1H), 2.91−2.76 (m, 2H), 2.31 (s, 1H), 1.63 (d, J = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 172.3, 170.8, 139.0, 135.4, 130.5, 128.6, 128.55, 128.47, 128.2, 127.4, 123.7, 70.6, 67.4, 47.6, 35.6, 18.1; MS (ESI) m/z 375 $[M + Na]^+$ (17), 353 $[M + H]^+$ (100). Anal. Calcd for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95. Found: C, 71.50; H, 6.87; N, 8.02.

*Benzyl (R)-2-(benzylamino)-2-carbamoyl-4-methylpent-4-enoate (**7c**):* 137 mg, 67%; mp 139−140 °C; $[\alpha]^{25}_D$ +7.62 (c 1.45, $CHCl_3$); ee = 98.6% (chiral HPLC analysis); IR (KBr) ν 3382, 1740, 1680, 1213 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.35−7.21 (m, 11H), 5.67 (s, 1H), 5.26 (d, J = 12.3 Hz, 1H), 5.19 (d, J = 12.3 Hz, 1H), 4.87 (d, J = 17.4 Hz, 2H), 3.67 (d, J = 12.0 Hz, 1H), 3.61 (d, J = 12.0 Hz, 1H), 2.97 (d, J = 12.0 Hz, 1H), 2.91 (d, J = 12.0 Hz, 1H), 2.34 (s, 1H), 1.76 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 172.3, 170.9, 140.3, 139.0, 135.2, 128.60, 128.55, 128.47, 128.4, 128.2, 127.4, 115.6, 70.6, 67.5, 47.9, 39.7, 23.7; MS (ESI) m/z 375 $[M + Na]^+$ (17), 353 $[M + H]^+$ (100). Anal. Calcd for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95. Found: C, 71.59; H, 6.78; N, 7.81.

*Benzyl (R,E)-2-(benzylamino)-2-carbamoyl-5-phenylpent-4-enoate (**7d**):* 131 mg, 74%; mp 125−126 °C; $[\alpha]^{25}_D$ +16.9 (c 1.30, $CHCl_3$); ee = 94.0% (chiral HPLC analysis); IR (KBr) ν 3423, 1727, 1696, 1190 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.37−7.22 (m, 15H), 7.10 (s, 1H), 6.48 (d, J = 15.9 Hz, 1H), 6.11−6.00 (m, 1H), 5.76 (s, 1H), 5.26 (d, J = 12.0 Hz, 1H), 5.19 (d, J = 12.0 Hz, 1H), 3.70 (d, J = 12.3 Hz, 2H), 3.62 (d, J = 12.3 Hz, 1H), 3.13−2.97 (m, 2H), 2.36 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 172.1, 170.7, 138.9, 137.0, 135.4, 134.5, 128.7, 128.60, 128.58, 128.52, 128.2, 127.52, 127.49, 126.3, 122.9, 70.9, 67.6, 47.8, 36.0; MS (ESI) m/z 415 $[M + H]^+$ (100). Anal. Calcd for $C_{26}H_{26}N_2O_3$: C, 75.34; H, 6.32; N, 6.76. Found: C, 75.14; H, 6.29; N, 6.83.

*Benzyl (R)-2-(benzylamino)-2-carbamoylhex-5-enoate (**7e**):* 126 mg, 61%; mp 78−79 °C; $[\alpha]^{25}_D$ +12.4 (c 1.45, $CHCl_3$); ee >99.5% (chiral HPLC analysis); IR (KBr) ν 3439, 1729, 1644, 1221 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.64−7.19 (m, 10H), 7.09 (s, 1H), 5.83−5.72 (m, 2H), 5.27 (d, J = 12.0 Hz, 1H), 5.21 (d, J = 12.0 Hz, 1H), 5.15−4.95 (m, 2H), 3.59 (d, J = 12.0 Hz, 1H), 3.48 (d, J = 12.0 Hz, 1H), 2.23−2.02 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) 172.4, 171.1, 138.8, 137.2, 135.4, 128.63, 128.59, 128.50, 128.46, 128.2, 127.5, 115.3, 70.5, 67.4, 47.8, 31.2, 27.7; MS (ESI) m/z 375 $[M + Na]^+$ (12), 353 $[M + H]^+$ (100). Anal. Calcd for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95. Found: C, 71.56; H, 6.77; N, 8.02.

*Benzyl (R)-2-(benzylamino)-2-carbamoylpent-4-ynoate (**7f**):* 127 mg, 59%; mp 113−114 °C; $[\alpha]^{25}_D$ +24.6 (c 1.30, $CHCl_3$); ee = 96.8% (chiral HPLC analysis); IR (KBr) ν 3430, 1729, 1701, 1026 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.35−7.26 (m, 10H), 7.21 (s, 1H), 5.76 (s, 1H), 5.25 (d, J = 12.3 Hz, 1H), 5.20 (d, J = 12.3 Hz, 1H), 3.67 (d, J = 12.0 Hz, 1H), 3.61 (d, J = 12.0 Hz, 1H), 3.22 (dd, J = 17.7, 2.4 Hz, 1H), 3.06 (dd, J = 17.7, 2.4 Hz, 1H), 2.65 (s, 1H), 2.07 (t, J = 2.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 170.9, 169.3, 138.7, 135.1, 128.6, 128.5, 128.3, 128.2, 127.5, 78.3, 72.4, 69.6, 67.9, 47.3, 22.5; MS (ESI) m/z 359 $[M + Na]^+$ (40), 337 $[M + H]^+$ (100). Anal. Calcd for $C_{20}H_{20}N_2O_3$: C, 71.41; H,5.99; N, 8.33. Found: C, 71.62; H, 6.07; N, 8.25.

*Benzyl (R)-2-(benzylamino)-2-carbamoylhex-4-ynoate (**7g**):* 121 mg, 59%; mp 95−96 °C; $[\alpha]^{25}_D$: +31.1° (c 1.80, $CHCl_3$); ee = 96.8% (chiral HPLC analysis); IR (KBr) ν 3430, 3252, 1702, 1185 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.33−7.21 (m, 11H), 6.11 (s, 1H), 5.21 (s, 2H), 3.67 (d, J = 12.0 Hz, 1H), 3.59 (d, J = 12.0 Hz, 1H), 3.16 (dd, J = 17.3, 1.8 Hz, 1H), 2.89 (dd, J = 17.3, 1.8 Hz, 1H), 2.59 (s, 1H), 1.73 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 171.5, 169.7, 139.1, 135.3, 128.5, 128.3, 128.2, 128.1, 127.4, 79.8, 72.8, 69.9, 67.6, 47.2, 23.0, 3.6; MS (ESI) m/z 373 $[M + Na]^+$ (22), 351 $[M + H]^+$ (100). Anal. Calcd for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 7.99. Found: C, 71.98; H, 6.18; N, 8.15.

*Benzyl (R)-2-(benzylamino)-2-carbamoyl-3-phenylpropanoate (**7h**):* 104 mg, 56%; mp 81−82 °C; $[\alpha]^{25}_D$ +16.0 (c 2.00, $CHCl_3$); ee >99.5% (chiral HPLC analysis); IR (KBr) ν 3440, 1714, 1680, 1186 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.35−7.14 (m, 15H), 6.89 (s,

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1H), 5.45 (s, 1H), 5.25 (d, J = 12.3 Hz, 1H), 5.16 (d, J = 12.3 Hz, 1H), 3.79 (d, J = 12.2 Hz, 1H), 3.69 (d, J = 12.2 Hz, 1H), 3.47 (s, 2H), 2.19 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 171.9, 170.6, 135.2, 130.1, 128.64, 128.63, 128.56, 128.4, 128.1, 127.5, 127.1, 72.0, 67.6, 48.1, 38.0; MS (ESI) m/z 389 $[M + H]^+$ (100). Anal. Calcd. for $C_{24}H_{24}N_2O_3$: C, 74.21; H, 6.23; N, 7.21. Found: C, 74.17; H, 6.26; N, 7.10.

*Benzyl (R)-2-(benzylamino)-2-carbamoylpentanoate (**7i**):* 123 mg, 56%; mp 163−164 °C; $[\alpha]^{25}_D$ +16.5 (c 1.70, $CHCl_3$); ee = 97.8% (chiral HPLC analysis); IR (KBr) ν 3440, 1728, 1642, 1218 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) 7.33−7.16 (m, 10H), 7.12 (s, 1H), 5.93 (s, 1H), 5.27 (d, J = 12.3 Hz, 1H), 5.16 (d, J = 12.3 Hz, 1H), 3.57 (d, J = 12.0 Hz, 1H), 3.47 (d, J = 12.0 Hz, 1H), 2.28 (s, 1H), 2.10−2.05 (m, 2H), 1.34−1.25 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 172.8, 171.4, 135.5, 128.61, 128.56, 128.5, 128.4, 128.2, 127.5, 70.8, 67.3, 47.8, 34.2, 16.8, 14.1; MS (ESI) m/z 341 $[M + H]^+$ (100); calcd for $C_{20}H_{24}N_2O_3$ 341.1860 $[M + 1]^+$, found (P-SIMS) 341.1857 $[M + 1]^+$.

*Benzyl (R)-2-(benzylamino)-2-carbamoylpropanoate (**7j**):* 121 mg, 51%; mp 207−208 °C; $[\alpha]^{25}_D$ +10.0 (c 0.60, $CHCl_3$); ee = 24.8% (chiral HPLC analysis); IR (KBr) ν 3325, 1690, 1261 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27−7.11 (m, 10H), 7.02 (s, 1H), 5.55 (s, 1H), 5.16 (dd, J = 36.3, 12.3 Hz, 2H), 3.70−3.33 (m, 2H), 2.30 (s, 1H), 1.59 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 171.7, 139.1, 135.5, 128.62, 128.57, 128.4, 128.3, 128.2, 127.4, 67.9, 67.4, 48.3, 20.5; MS (ESI) m/z 335 $[M + Na]^+$ (55), 313 $[M + H]^+$ (100); calcd for $C_{18}H_{20}N_2O_3$ 313.1547 $[M + 1]^+$, found (P-SIMS) 313.1547 $[M + 1]^+$.

*Benzyl (R)-2-(benzyl(methyl)amino)-2-carbamoylpent-4-enoate (**7k**):* 133 mg, 65%; oil; $[\alpha]^{25}_D$ +23.3 (c 2.15, $CHCl_3$); ee = 64.6% (chiral HPLC analysis); IR (KBr) ν 3460, 2924, 1690, 1215 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.23 (m, 10H), 6.84 (s, 1H), 5.98−5.86 (m, 2H), 5.32−5.13 (m, 4H), 3.85 (d, J = 14.2, Hz, 1H), 3.63 (d, J = 14.2, Hz, 1H), 3.00−2.85 (m, 2H) 2.24 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 169.0, 138.5, 135.3, 132.1, 128.3, 128.1, 127.4, 126.9, 76.0, 66.8, 57.2, 38.5, 36.3; MS (ESI) m/z (%) 357 $[M + Na]^+$ (45), 353 $[M + H]^+$ (100); calcd for $C_{21}H_{24}N_2O_3$ 353.1860 $[M + 1]^+$, found (P-SIMS) 353.1852 $[M + 1]^+$.

Procedure for Biotransformation of 5a Using an Immobilized Whole Cell Catalyst. The immobilized *Rhodococcus erythropolis* AJ270 cells (4 g wet weight) in alginate capsules[33] were activated in sodium bicarbonate buffer solution (pH 7.25, 100 mL) at 30 °C for 30 min. A solution of **5a** (1.57 g, 10 mmol) in bicarbonate buffer solution (pH 7.25, 50 mL) was added. The resulting mixture was incubated at 30 °C with orbital shaking for 30 min. The immobilized catalyst was filtered and reused for another seven times. The combined aqueous solution was worked up, and (*R*)-**6a** (9.3 g, 74% yield, 95.8% ee) was obtained.

*Synthesis of (R)-Methyl 2-Amino-2-carbamoylpent-4-enoate (**8**).* To a solution of *R*-(−)-**6a** (316 mg, 2 mmol) in methanol (20 mL) was added an ether solution of CH_2N_2 (2 M, 10 mL) at 0 °C for 0.5 h. The mixture was stirred at room temperature overnight. The solvent was removed under vacuum. The residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate (1:1−1:2) as the mobile phase to give (*R*)-(+)-**8** (269 mg, 78%): mp 66−67 °C; $[\alpha]^{25}_D$ = +5.0 (c 2.00, $CHCl_3$); IR (KBr) ν 3407, 1730, 1633 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) 7.42 (s, 1H), 6.27 (s, 1H), 5.79−5.71 (m, 1H), 5.23−5.18 (m, 2H), 3.81 (s, 3H), 2.85−2.78 (m, 1H), 2.69−2.62 (m, 1H), 2.10 (s, 2H); 13C NMR (75 MHz, $CDCl_3$) 173.7, 172.4, 131.9, 120.8, 65.0, 53.2, 41.7; MS (ESI) m/z 173 $[M + H]^+$ (100); calcd for $C_7H_{12}N_2O_3$ 173.0921 $[M + 1]^+$, found (P-SIMS) 173.0915 $[M + 1]^+$.

*Synthesis of (S)-2-Amino-2-(hydroxymethyl)pent-4-enoic Acid (**9**).* To a solution of (*R*)-(+)-**8** (50 mg, 0.3 mmol) in THF (4 mL) and EtOH (4 mL) were added LiCl·H_2O (30 mg, 0.5 mmol) and $NaBH_4$ (38 mg, 1 mmol), and the resulting mixture was stirred at room temperature. When the starting material was consumed, the solvent was removed under vacuum. The residue was mixed with MeOH (1 mL) and aqueous NaOH (2 N, 1 mL). After being heated at 60 °C for 5 h, the mixture was then neutralized with hydrochloric acid (1 N) at room temperature. The solvent was removed under vacuum. The residue was chromatographed on an ion-exchange resin (Dowex 50WX8, 200−400 mesh) column with aqueous ammonia (10%) as mobile phase to give products (*S*)-(−)-**9** (46 mg, 99%): mp 178−179 °C (lit.[37] mp 215−220 °C); $[\alpha]^{25}_D$ = −34.7° (c 0.75, H_2O); IR (KBr) ν 3080, 1620 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) 5.72−5.58 (m, 1H), 5.23−5.18 (m, 2H), 3.87 (d, J = 12.3 Hz, 1H), 3.64 (d, J = 12.3 Hz, 1H), 2.53 (dd, J = 14.4, 6.3 Hz, 1H), 2.37 (dd, J = 14.4, 8.4 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) 173.8, 129.9, 1214, 65.7, 63.9, 36.7; MS (ESI) m/z 168 $[M + Na]^+$ (23), 146 $[M + H]^+$ (100).

*Synthesis of (S)-2-Amino-2-(hydroxymethyl)pentanoic Acid (**10**).* In the presence of Pd/C catalyst (10%, 10 mg), compound (*S*)-(−)-**9** (30 mg, 0.2 mmol) in methanol (2 mL) was hydrogenated using a hydrogen balloon. After the starting material was consumed, the catalyst was filtered, and the filtrate was concentrated to give (*S*)-(+)-**10** (30 mg, 99%). Alternatively, compound (*S*)-(+)-**10** was obtained from (*S*)-(+)-**11**. Thus, in the presence of Pd/C catalyst (10%, 20 mg), compound (*S*)-(+)-**11** (234 mg, 1 mmol) in methanol (5 mL) was hydrogenated using a hydrogen balloon. After the starting material was consumed, the catalyst was removed by filtration, and the filtrate was concentrated. The residue was mixed with aqueous NaOH (2 N, 1 mL) and MeOH (3 mL). After being heated at 60 °C for 5 h, the reaction mixture was then neutralized with hydrochloric acid (1 N) at room temperature. The solvent was removed under vacuum. The residue was chromatographed on an ion-exchange resin (Dowex50wx8, 200−400 mesh) column using aqueous ammonia (10%) as the mobile phase to give products *S*-(+)-**10** (133 mg, 91%): mp 188−189 °C (lit.[38] mp 279 °C); $[\alpha]^{25}_D$ = +9.5 (c 1.05, 1 N HCl) (lit.[35] $[\alpha]^{25}_D$ = +10 (c 0.160, 1 N HCl)); IR (KBr) ν 3140, 3044, 1405 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) 3.78 (d, J = 12.0 Hz, 1H), 3.53 (d, J = 12.0 Hz, 1H), 1.67−1.46 (m, 2H), 1.30−1.04 (m, 2H), 0.77 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, D_2O) 174.5, 66.4, 64.3, 34.3, 16.5, 13.4; MS (ESI) m/z 148 $[M + H]^+$ (100).

*Synthesis of (S)-2-(Benzylamino)-2-(hydroxymethyl)pent-4-enamide (**11**).* To a mixture of compound (*R*)-(+)-**7a** (170 mg, 0.5 mmol) in THF (5 mL) and EtOH (5 mL) were added LiCl·H_2O (30 mg, 0.5 mmol) and $NaBH_4$ (38 mg, 1 mmol), and the resulting mixture was kept stirring at room temperature. When the starting material was consumed, water (20 mL) was added, and the mixture was extracted with ethyl acetate (3 × 20 mL). The organic layer was dried with anhydrous $MgSO_4$. After removal of the solvent under vacuum, the residue was chromatographed on a silica gel column with a mixture of petroleum ether and ethyl acetate (1:1−1:2) as the mobile phase to give product (*S*)-(+)-**11** (116 mg, 99%): mp 95−96 °C; $[\alpha]^{25}_D$ = +2.05 (c 1.95, $CHCl_3$); IR (KBr) ν 3443, 3285, 1659 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) 77.36−7.24 (m, 6H), 6.00 (s, 1H), 5.92−5.78 (m, 1H), 5.20−5.15 (m, 2H), 3.84 (s, 2H), 3.79 (d, J = 12.3 Hz, 1H), 3.72 (d, J = 12.3 Hz, 1H), 2.58−2.41 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 178.1, 139.9, 132.4, 128.6, 128.0, 127.3, 119.4, 65.1, 64.1, 46.9, 38.2; MS (ESI) m/z 257 $[M + Na]^+$ (28), 235 $[M + H]^+$ (100). Anal. Calcd for $C_{13}H_{18}N_2O_2$: C, 66.64; H, 7.74; N, 11.96. Found: C, 66.72; H, 7.67; N, 11.92.

*Synthesis of (R)-2-(Aminomethyl)-2-(benzylamino)pent-4-en-1-ol (**12**).* To a solution of (*S*)-(+)-**11** (117 mg, 0.5 mmol) in dry THF (10 mL) under argon protection was added through a syringe a solution of $LiAlH_4$ in THF (1 N, 5 mL). The resulting mixture was refluxed for 48 h. The reaction was quenched by addition of EtOH (2 mL) and NaOH (2 N, 3 mL) at room temperature, and then the mixture was filtered and washed with EtOH (3 × 10 mL). The filtrate was concentrated under vacuum, and the residue was chromatographed on a silica gel column with a mixture of ethyl acetate, ethanol, and ammonia (25% w/w) (100:5:1) as the mobile phase to give products (*R*)-(+)-**12** (72 mg, 65%): mp 49−50 °C; $[\alpha]^{25}_D$ = +10.0 (c 1.60, MeOH); IR (KBr) ν 3359, 2864, 1639 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, J = 5.7 Hz, 2H), 7.31 (t, J = 5.7 Hz, 2H), 7.24 (q, J = 5.7 Hz, 1H), 5.97−5.86 (m, 1H), 5.21−5.13 (m, 2H), 3.72 (d, J = 9.0 Hz, 1H), 3.68 (d, J = 9.0 Hz, 1H), 3.55 (d, J = 8.4 Hz, 1H), 3.51 (d, J = 8.4 Hz, 1H), 2.72 (d, J = 9.9 Hz, 1H), 2.68 (d, J = 9.9 Hz, 1H), 2.28 (d, J = 5.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.4, 133.3, 128.11, 128.06, 126.7, 117.5, 63.0, 58.5, 45.1, 43.6, 36.1; MS (ESI) $m/$

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z 221 $[M + H]^+$ (100); calcd for $C_{13}H_{20}N_2O$: 221.1648 $[M + 1]^+$, found (P-SIMS) 221.1656 $[M + 1]^+$.

■ ASSOCIATED CONTENT

Supporting Information

Chiral HPLC analysis and ^1H and ^{13}C spectra of all products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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