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Microbial whole cell-catalyzed desymmetrization of prochiral malonamides: practical synthesis of enantioenriched functionalized carbamoylacetates and their application in the preparation of unusual α-amino acids

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ABSTRACT

Catalyzed by *Rhodococcus erythropolis* AJ270, an amidase-containing microbial whole cell catalyst, under very mild conditions, a number of functionalized prochiral malonamides underwent enantioselective desymmetrization reaction to afford high yield of carbamoylacetic acids with moderate to excellent enantioselectivity. The synthetic application has been demonstrated with a multi-gram scale biocatalytic preparation of *R*-(−)-α-allyl-α-methyl-carbamoylacetic acid and its conversions to varied α,α-di-substituted α-amino acids of the interest of medicinal chemistry.

1. Introduction

Desymmetrization of prochiral molecules constitutes an important strategy for the synthesis of chiral molecules. Biocatalytic desymmetrization of prochiral diols and diesters using lipases, esterases, and acylases, for example, has been widely used to prepare chiral products that are hardly accessible by other methods.[1] Amidase [3.5.1.4] is a type of hydrolytic enzyme catalyzing the hydrolysis of amides to produce carboxylic acids. A large number of studies have demonstrated that amidases derived from different microorganisms show a broad substrate spectrum and good enantioselectivity against racemic substrates. Amidases have been applied therefore to catalyze the kinetic resolution of a wide variety of racemic amides.[2] Surprisingly, the amidase-catalyzed selective desymmetrization of prochiral diamides has remained largely unexplored.[2,3] To the best of our knowledge, there is only one literature report in which Wu and Li[3] described in 2003 that α-methyl-α-arylmethylmalonamides undergo biocatalytic hydrolysis to yield chiral malonamic acids with high enantioselectivity. Since the substrate structures are simple and not functionalized, synthetic applications are limited.

Rhodococcus erythropolis AJ270 is a nitrile hydratase/amidase-containing microorganism.[4] It has been shown in the past decade that *Rh. erythropolis* AJ270 is a powerful microbial whole cell catalyst able to hydrolyze various racemic nitriles and amides. While the nitrile hydratase exhibits low or none enantioselectivity, the amidase involved in cell is highly enantioselective.[5] By means of *Rh. erythropolis* AJ270-catalyzed hydrolysis of nitriles and amides, a large number of enantioenriched carboxamides and carboxylic acids have been prepared.[5–11] To further explore the application of *Rh. erythropolis* AJ270 in organic synthesis, and to gain insight into the catalytic property of the amidase against prochiral diamides, we undertook the current study. We report herein the enantioselective bio-desymmetrization reaction of functionalized prochiral malonamides. Practical biocatalytic synthesis of highly enantiomerically pure α-allyl-α-methyl-carbamoylacetic acid in a multi-gram scale and its application in the preparation of unusual α,α-disubstituted α-amino acids are also demonstrated.

2. Results and discussion

We initiated our study with the examination of the biotransformation of prochiral α-allyl-α-methylmalonamide **1a** (Scheme 1). In the presence of *Rh. erythropolis* AJ270 whole cell catalyst in an aqueous phosphate buffer (pH=7.0), hydrolysis of **1a** was found to proceed efficiently at 30 °C. To facilitate the isolation of product, acid product was converted into methyl ester **2a** using diazomethane. As indicated in Table 1, enantioenriched carbamoylacetic ester (−)-**2a** was obtained in 85% with ee of 90.7% in 0.5 h (entry 1, Table 1). *Rh. erythropolis* AJ270 was able to catalyze the desymmetrization reaction of **1a** at a lower temperature. For example, when biotransformation was conducted at 20 °C and

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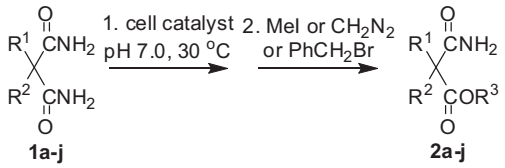
10 °C, the ee values of the product (−)-**2a** was improved to 93.8% and 95.0%, respectively, albeit the reaction rate was slightly decreased (entries 2 and 3, Table 1).



Scheme 1. Biotransformation of malonamides **1**.

Table 1
Biocatalytic desymmetrization of malonamides **1**[a]

Entry	1	R^1	R^2	R^3	*t* (h)	**2** (%)[b] (ee %)[c]
1	**1a**	Me	allyl	Bn	0.5	(−)-**2a** (85) (90.7)
2[d]	**1a**	Me	allyl	Bn	0.67	(−)-**2a** (76) (93.8)
3[e]	**1a**	Me	allyl	Bn	1.5	(−)-**2a** (59) (95.0)
4	**1b**	Me	Bn	Me	8	(+)-**2b** (99) (>99.5)
5	**1c**	Me	*n*-Pr	Bn	5	(−)-**2c** (80) (72.4)
6	**1d**	Me	2-Methylallyl	Bn	0.7	(−)-**2d** (85) (42.5)
7	**1e**	Me	*E*-But-2-enyl	Bn	1.3	(−)-**2e** (87) (97.8)
8	**1f**	Me	Prop-2-ynyl	Bn	25	(+)-**2f** (73) (43.8)
9	**1g**	Me	Cinnamyl	Bn	115	(−)-**2g** (84) (>99.5)
10	**1h**	allyl	Prop-2-ynyl	Bn	31.5	(−)-**2h** (88) (41.2)
11	**1i**	allyl	But-2-ynyl	Bn	104.5	(+)-**2i** (86) (25.2)
12[f]	**1j**	Bn	Prop-2-ynyl	Me	168	(−)-**2j** (<5) (85.0)

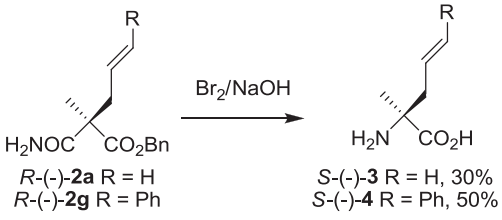
[a] Substrate **1** (1 mmol) was incubated with *Rh. erythropolis* AJ270 whole cell catalyst (2 g wet weight) in phosphate buffer (pH 7.0, 0.1 M, 50 mL) at 30 °C.
[b] Isolated yield.
[c] Determined using chiral HPLC.
[d] Incubation temperature was 20 °C.
[e] Incubation temperature was 10 °C.
[f] Starting material **1j** (83%) was recovered.

Encouraged by the enantioselective desymmetrization of **1a**, a number of functionalized prochiral malonamides **1b**–**j** were synthesized and their biocatalytic transformation was investigated (Scheme 1). Results tabulated in Table 1 show that most of the prochiral diamides underwent effective biocatalytic hydrolysis under mild conditions to produce carbamoylacetic acid products **2** in good to excellent yields. The reaction velocity and ee values of the products, however, were dependent dramatically upon the nature of substituents R^1 and R^2. The steric effect of the substituents R^1 and R^2 played an important role in determining reaction speed. For example, desymmetrization of malonamides containing one α-methyl group and one α-allyl (**1a**), benzyl (**1b**), *n*-propyl (**1c**), 2-methylallyl (**1d**), or *E*-but-2-enyl (**1e**) took place efficiently to afford the corresponding carbamoylacetic acid products in good to excellent yield yields within hours (entries 1–7, Table 1). Introduction of an α-prop-2-ynyl (**1f**), cinnamyl (**1g**) group in addition to an α-methyl led to a slower reaction rate, yielding products (+)-**2f** and (−)-**2g** in 73% and 84% after 25 and 115 h incubation, respectively (entries 8 and 9, Table 1). When both R^1 and R^2 were larger substituents, the reaction became very sluggish. This has been exemplified by the reaction of α-allyl-α-(but-2-ynyl)malonamide **1i**, which took more than 100 h to give product (+)-**2i** in 86% (entry 11). An extreme example was the reaction of α-benzyl-α-(prop-2-ynyl)malonamide **1j**, which yielded a very small amount of the product (−)-**2j** after a week's interaction with biocatalyst (entry 12, Table 1).

In contrast to reaction efficiency, the enantioselectivity of biocatalytic desymmetrization of **1** was dictated predominantly by the electronic effect of the substituents, and the combination of substituents R^1 and R^2. In the case of malonamides **1a**–**g** contain an α-methyl group, for instance, introduction of an alkenyl substituent, such as allyl (**1a**), *E*-but-2-enyl (**1e**), and cinnamyl (**1g**), or a benzyl group (**1b**) gave rise to highly enantioselective biotransformation (entries 1–4, 7, and 9, Table 1). The presence of a saturated α-propyl group (**1c**) or a prop-2-ynyl (**1f**), a carbon–carbon triple bond moiety, led to the alleviation of enantiocontrol of the reactions (entries 5 and 8). Surprisingly, reaction of α-(2-methylallyl)-substituted substrate **1d**, an isomer of **1e**, afforded (−)-**2d** with only 42.5% ee (entry 6, Table 1), reflecting the effect of isomerism on the enantioselectivity. Except α-benzyl-α-(prop-2-ynyl)-malonamide **1j**, which gave 85% ee of the product (entry 12, Table 1), diamides bearing both an allyl and a prop-2-ynyl (**1h**) or a but-2-ynyl (**1i**) underwent less enantioselective desymmetrization hydrolysis to form (−)-**2h** and (+)-**2i** with low ee values (entries 10 and 11, Table 1). The outcomes indicated clearly that the larger the steric difference between substitutes R^1 and R^2, the higher the enantioselectivity. Besides, the presence of a carbon–carbon triple bond leads a dramatic decrease of enantioselectivity. The beneficial effect of the carbon–carbon double bond of the substrates on the catalytic efficiency and enantioselectivity of biotransformations of amides has also been observed previously.[6d,6e]

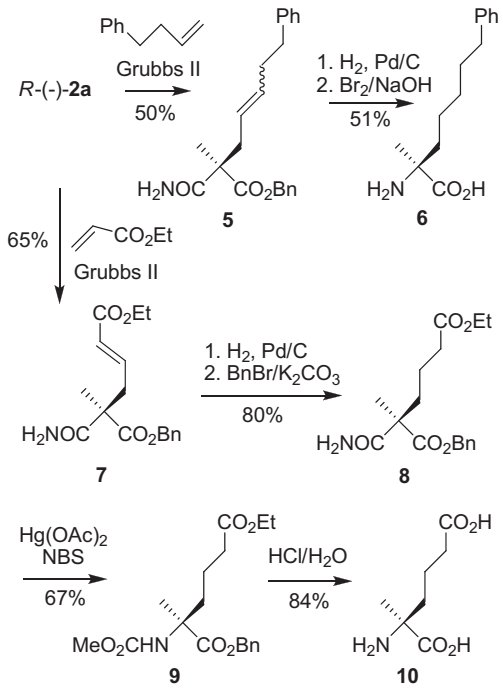
To examine the practical utility of biocatalytic desymmetrization, a large scale biotransformation of **1a** was tested using an immobilized *Rh. erythropolis* AJ270 whole cell catalyst.[12] To our delight, the immobilized biocatalyst was recycled for five times without noticeable decay of the activity and enantioselectivity, producing 8 g of (−)-**2a** with ee of 88.6%.

The highly enantiopure carbamoylacetates bearing carbon–carbon unsaturated functional groups are valuable chiral intermediates in organic synthesis. To demonstrate their synthetic applications, and also to determine their absolute configurations, both (−)-**2a** and (−)-**2g** were converted into α,α-disubstituted amino acids **3** and **4** through facile Hofmann rearrangement and hydrolysis (Scheme 2). The absolute configurations of *S*-**3** and *S*-**4**, that was determined by comparing their optical rotations with that of authentic samples,[13,14] revealed pro-*R*-enantioselectivity of the amidase involved in *Rh. erythropolis* AJ270.



Scheme 2. Synthesis of amino acids **3** and **4**.

Having had a large amount of highly enantioenriched α-allyl-α-methylcarbamoylacetate *R*-(−)-**2a** in hand, our interest in unusual amino acids led us to investigate its conversions to other α,α-disubstituted amino acids that are the useful building blocks in medicinal chemistry.[15–17] Illustrated in Scheme 3 are the synthesis of *S*-2-amino-2-methyl-7-phenylheptanoic acid **6** and *S*-2-amino-2-methylhexanedioic acid **10**. Cross metathesis between *R*-(−)-**2a** and 4-phenyl-but-1-ene in the presence of Grubbs II catalyst afforded a mixture of *E*- and *Z*-configured alkenes **5** in 50%. Hydrogenation reaction followed by typical Hofmann rearrangement produced compound **6**. Similar cross metathesis of *R*-(−)-**2a** with ethyl acrylate led to the formation of intermediate **7** in 65% yield. Catalytic hydrogenation, Hofmann rearrangement using $Hg(OAc)_2$ and NBS, and final hydrolysis under acidic conditions furnished the formation of product **10** (Scheme 3).



Scheme 3. Synthesis of amino acids **6** and **10**.

3. Conclusion

In summary, we have shown that *Rh. erythropolis* AJ270, an amidase-containing microbial cell catalyst, is able to catalyze the enantioselective hydrolysis of α,α-disubstituted malonamides under very mild conditions to afford high yield of carbamoylacetic acid products. Both the catalytic efficiency and enantioselectivity of desymmetrization of α,α-disubstituted malonamides were dependent upon the substituents on the substrate. The amidase-catalyzed enantioselective desymmetrization reaction provided an efficient and practical method for the preparation of highly enantiopure functionalized carbamoylacetic acids, and their synthetic applications have been demonstrated in the preparation of unusual α,α-disubstituted α-amino acids.

4. Experimental section

4.1. General information

^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer. Chemical shifts are reported in parts per million versus tetramethylsilane or the residual solvent resonance used as an internal standard. Melting points are uncorrected. Enantiomeric excess values of all compounds were obtained from chiral HPLC analyses (see Supplementary data). Prochiral diamides **1** were prepared from the chemical hydrolysis of dinitriles using H_2O_2 under basic conditions following the literature method.[6d,e]

4.2. Spectroscopic data of diamides 1

4.2.1. 2-Allyl-2-methylmalonamide ***1a***. Mp 163.0–164.0 °C; IR (KBr) ν 3382, 1643 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) 7.13 (s, 2H), 7.01 (s, 2H), 5.68–5.54 (m, 1H), 5.08–5.01 (m, 2H), 2,47 (d, J=7.2 Hz, 2H), 1.18 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 174.0, 134.5, 117.9, 52.5, 40.9, 19.7; MS (ESI) m/z (%) 179 [M+Na]$^+$ (100). Anal. Calcd for $C_7H_{12}N_2O_2$: C, 53.83; H, 7.74; N, 17.94. Found: C, 53.76; H, 7.67; N, 17.72.

4.2.2. 2-Benzyl-2-methylmalonamide ***1b***. Mp 211.0–212.0 °C (lit.[3] mp 195.4–196.4 °C); ^{1}H NMR (300 MHz, DMSO-d_6) 7.26–7.16 (m, 7H), 7.11 (s, 2H), 3.07 (s, 2H), 1.11 (s, 3H).

4.2.3. 2-Methyl-2-propylmalonamide ***1c***. Mp 179–180 °C (lit.[18] mp 182 °C); ^{1}H NMR (300 MHz, DMSO-d_6) 7.15 (s, 2H), 7.03 (s, 2H), 1.70–1.65 (m, 2H), 1.21–1.12 (m, 5H), 0.85 (t, J=7.2 Hz, 3H).

4.2.4. 2-Methyl-2-(2-methylallyl)malonamide ***1d***. Mp 158.0–159.0 °C; IR (KBr) ν 3382, 3217, 1641 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) 7.25 (s, 2H), 7.13 (s, 2H), 4.67 (s, 1H), 4.46 (s, 1H), 2.52 (s, 2H), 1.62 (s, 3H), 1.19 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 174.7, 142.0, 114.1, 51.9, 44.2, 23.0, 19.8; MS (ESI) m/z (%) 209 [M+K]$^+$ (13), 193 [M+Na]$^+$ (100), 171 [M+H]$^+$ (65). HRMS for $C_8H_{14}N_2O_2$: 170.1055 [M]$^+$. Found: 170.1057 [M]$^+$.

4.2.5. (E)-2-(But-2-en-1-yl)-2-methylmalonamide ***1e***. Mp 169.0–170.0 °C; IR (KBr) ν 3383, 3225, 1692, 1642 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) 7.10 (s, 2H), 7.05 (s, 2H) 5.48–5.41 (m, 1H), 5.26–5.21 (m, 1H), 2.40 (d, J=6.3 Hz, 2H), 1.60 (d, J=5.4 Hz, 3H), 1.16 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 174.2, 127.7, 126.8, 52.7, 19.7, 17.9, 12.9; MS (ESI) m/z (%) 209 [M+K]$^+$ (5), 193 [M+Na]$^+$ (100), 171 [M+H]$^+$ (9). Anal. Calcd for $C_8H_{14}N_2O_2$: C, 56.45; H, 8.29; N, 16.46. Found: C, 56.12; H, 8.16; N, 16.32.

4.2.6. 2-Methyl-2-(prop-2-yn-1-yl)malonamide ***1f***. Mp 169.0–170.0 °C; IR (KBr) ν 3385, 3275, 1693, 1639 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) 7.12 (s, 4H), 2.79 (t, J=2.4 Hz, 1H), 2.64 (d, J=2.4 Hz, 2H), 1.35 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 172.9, 81.4, 72.9, 52.4, 25.9, 19.9; MS (ESI) m/z (%) 193 [M+K]$^+$ (6), 177 [M+Na]$^+$ (100), 155 [M+H]$^+$ (13). Anal. Calcd for $C_7H_{10}N_2O_2$: C, 54.54; H, 6.54; N, 18.17. Found: C, 54.43; H, 6.53; N, 18.25.

4.2.7. 2-Cinnamyl-2-methylmalonamide ***1g***. Mp 174.0–175.0 °C; IR (KBr) ν 3409, 3197, 1658 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) 7.36–7.28 (m, 4H), 7.23–7.20 (m, 3H), 7.15 (s, 2H), 6.44 (d, J=15.6 Hz, 1H), 6.14–6.04 (m, 1H), 2,64 (d, J=7.2 Hz, 2H), 1.26 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 174.1, 137.0, 132.4, 128.5, 127.1, 126.1, 125.8, 53.0, 40.1 19.9; MS (ESI) m/z (%) 271 [M+K]$^+$ (66), 255 [M+Na]$^+$ (100), 233 [M+H]$^+$ (13). Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 67.27; H, 7.01; N, 12.14.

4.2.8. 2-Allyl-2-(prop-2-yn-1-yl)malonamide ***1h***. Mp 169.0–170.0 °C; IR (KBr) ν 3389, 3277, 1642 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) 7.23 (s, 2H), 7.19 (s, 2H) 5.64–5.50 (m, 1H), 5.13–5.04 (m, 2H), 2.79 (t, J=2.4 Hz, 1H), 2.66–2.62 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) 171.7, 133.5, 118.5, 80.9, 73.0, 55.7, 36.7, 22.1; MS (ESI) m/z (%) 219 [M+K]$^+$ (66), 203 [M+Na]$^+$ (100), 181 [M+H]$^+$ (13). Anal. Calcd for $C_9H_{12}N_2O_2$: C, 59.99; H, 6.71; N, 15.55. Found: C, 59.72; H, 6.70; N, 15.32.

4.2.9. 2-Allyl-2-(but-2-yn-1-yl)malonamide ***1i***. Mp 183.0–184.0 °C; IR (KBr) ν 3409, 3214, 1696, 1654 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) 7.19 (s, 2H), 7.12 (s, 2H) 5.61–5.52 (m, 1H), 5.12–5.01 (m, 2H), 2.63–2.57 (m, 4H), 1.71 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 172.2, 133.4, 118.4, 77.6, 75.4, 55.9, 36.9, 22.7, 3.1; MS (ESI) m/z (%) 217 [M+Na]$^+$ (100), 195 [M+H]$^+$ (13). Anal. Calcd for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.42. Found: C, 61.92; H, 7.37; N, 14.52.

4.2.10. 2-Benzyl-2-(prop-2-yn-1-yl)malonamide ***1j***. Mp 211.0–212.0 °C; IR (KBr) ν 3380, 3293, 3208, 1644 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) 7.34 (s, 2H), 7.18–7.23 (m, 7H), 3.21 (s, 2H), 2.94 (s, 1H), 2.50 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) 171.7, 137.0, 129.7, 127.9, 126.5, 81.3, 73.6, 57.2, 37.7, 22.1; MS (ESI) m/z (%) 269 [M+K]$^+$ (2), 253 [M+Na]$^+$ (100), 231

$[M+H]^+$ (48). Anal. Calcd for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.51; H, 6.14; N, 12.31.

4.3. General procedure for the biotransformation of α,α-disubstituted malonamides 1

To an Erlenmeyer flask (150 mL) with a screw cap were added *Rh. erythropolis* AJ270 cells[4,19] (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 solution of prochiral diamides **1** (1 mmol) or a solution of diamides **1c** and **1g** (1 mmol) in DMF (1 mL) 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 removing the biomass through a Celite pad filtration. The aqueous solution was freeze-dried, and the residue was dissolved in DMF (5 mL) and then treated with K_2CO_3 (270 mg, 2 mmol) and benzyl bromide (0.13 mL, 1.05 mmol) or methyl iodide (0.3 mL, 5 mmol). The mixture was kept stirring at room temperature overnight, and water (25 mL) was added and the mixture was extracted with ethyl acetate (3×25 mL). For the biotransformation of **1b**, the residue was dissolved in methanol (10 mL) followed by adding a solution of CH_2N_2 in ethyl ether at below 0 °C. After 4–6 h, water was added and the mixture was extracted with ethyl acetate (3×25 mL). Organic phase was dried over $MgSO_4$. 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 **2**.

*4.3.1. R-(−)-Benzyl 2-carbamyl-2-methylpent-4-enoate (**2a**).* Mp 25.0–25.5 °C; $[\alpha]_D^{25}$ −14.5 (*c* 0.8, $CHCl_3$); ee=90.7% (chiral HPLC analysis); IR (KBr) *ν* 3419, 3191, 1727, 1678 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.37–7.28 (m, 5H), 6.67 (s, 1H), 5.96 (s, 1H), 5.75–5.61 (m, 1H), 5.19 (s, 2H), 5.11–5.06 (m, 2H), 2.73 (dd, *J*=13.7, 7.2 Hz, 1H), 2.58 (dd, *J*=13.7, 7.2 Hz, 1H), 1.47 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 173.7, 173.4, 135.2, 132.6, 128.6, 128.5, 128.2, 119.3, 67.3, 53.7, 41.9, 20.6; MS (ESI) *m/z* (%) 270 $[M+Na]^+$ (100), 248 $[M+H]^+$ (6). Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 68.09; H, 6.96; N, 5.78.

*4.3.2. R-(+)-Methyl 3-amino-2-benzyl-2-methyl-3-oxopropanoate (**2b**).* Mp 111.0–112.0 °C (lit.[20] mp 104–106 °C); $[\alpha]_D^{25}$ +25 (*c* 2, $CHCl_3$); {lit.[20] $[\alpha]_D^{20}$ +9.8 (*c* 0.5, $CHCl_3$)}; ee >99.5% (chiral HPLC analysis); ^1H NMR (300 MHz, $CDCl_3$) 7.28–7.22 (m, 3H), 7.14–7.11 (m, 2H), 6.89 (s, 1H), 5.54 (s, 1H), 3.73 (s, 3H), 3.36 (d, *J*=13.2 Hz, 1H), 3.11 (d, *J*=13.2 Hz, 1H), 1.44 (s, 3H).

*4.3.3. (−)-Benzyl 2-carbamoyl-2-methylpentanoate (**2c**).* Mp 61.0–61.5 °C; $[\alpha]_D^{25}$ −28.0 (*c* 0.5, $CHCl_3$); ee=72.4% (chiral HPLC analysis); IR (KBr) *ν* 3418, 3187, 1716, 1686 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.39–7.37 (m, 5H), 6.87 (s, 1H), 5.58 (s, 1H), 5.20 (s, 2H), 1.97–1.79 (m, 2H), 1.48 (s, 3H), 1.26–1.24 (m, 2H), 0.90 (t, *J*=6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 174.6, 173.9, 135.4, 128.6, 128.4, 128.0, 67.1, 53.9, 40.3, 20.7, 18.3, 14.2; MS (ESI) *m/z* (%) 272 $[M+Na]^+$ (100), 250 $[M+H]^+$ (5). Anal. Calcd for $C_{14}H_{19}NO_3$: C, 67.45; H, 7.68; N, 5.62. Found: C, 67.33; H, 7.64; N, 5.61.

*4.3.4. (−)-Benzyl 2-carbamoyl-2,4-dimethylpent-4-enoate (**2d**).* Mp 49.0–49.5 °C; $[\alpha]_D^{25}$ −6.8 (*c* 0.5, $CHCl_3$); ee=42.5% (chiral HPLC analysis); IR (KBr) *ν* 3408, 3195, 1713, 1662 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.41–7.36 (m, 5H), 7.17 (s, 1H), 6.14 (s, 1H), 5.17 (s, 2H), 4.81(s, 1H), 4.67 (s, 1H), 2.85 (d, *J*=14.4 Hz, 1H), 2.56 (d, *J*=14.4 Hz, 1H), 1.66 (s, 3H), 1.48 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 174.6, 174.0, 141.2, 135.1, 128.6, 128.5, 128.2, 114.5, 67.4, 53.0, 45.1, 23.3, 21.8; MS (ESI) *m/z* (%) 284 $[M+Na]^+$ (100), 262 $[M+H]^+$ (18). Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 68.82; H, 7.41; N, 5.32.

*4.3.5. (−)-(E)-Benzyl 2-carbamoyl-2-methylhex-4-enoate (**2e**).* Mp 56.5–57.0 °C; $[\alpha]_D^{25}$ −21.9 (*c* 0.85, $CHCl_3$); ee=97.8% (chiral HPLC analysis); IR (KBr) *ν* 3424, 3183, 1622 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.35–7.33 (m, 5H), 6.64 (s, 1H), 5.62 (s, 1H), 5.61–5.42 (m, 1H), 5.31–5.24 (m, 1H), 5.23 (d, *J*=3 Hz, 2H), 2.67–2.61 (m, 1H), 2.52–2.45 (m, 1H), 1.61 (d, *J*=3 Hz, 3H), 1.43 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 174.0, 173.4, 135.4, 130.1, 128.6, 128.4, 128.2, 124.9, 67.2, 54.0, 41.0, 20.4, 18.0; MS (ESI) *m/z* (%) 284 $[M+Na]^+$ (100), 262 $[M+H]^+$ (9). Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 68.56; H, 7.23; N, 5.34.

*4.3.6. (+)-Benzyl 2-carbamoyl-2-methylpent-4-ynoate (**2f**).* Mp 48.0–49.0 °C; $[\alpha]_D^{25}$ +27.2 (*c* 0.5, $CHCl_3$); ee=43.8% (chiral HPLC analysis); IR (KBr) *ν* 3451, 3290, 1726, 1691 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.37 (s, 5H), 6.49 (s, 1H), 6.16 (s, 1H), 5.22 (s, 2H), 2.89 (dd, *J*=16.8, 2.4 Hz, 1H), 2.76 (dd, *J*=16.8, 2.4 Hz, 1H) 2.04 (t, *J*=2.4 Hz, 1H), 1.59 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 172.5, 172.4, 135.1, 128.6, 128.5, 128.1, 79.8, 71.4, 67.7, 53.4, 26.3, 21.1; MS (ESI) *m/z* (%) 284 $[M+K]^+$ (3), 268 $[M+Na]^+$ (100). Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.49; H, 6.15; N, 5.73.

*4.3.7. R-(−)-(E)-Benzyl 2-carbamoyl-2-methyl-5-phenylpent-4-enoate (**2g**).* Mp 60.5–61.0 °C; $[\alpha]_D^{25}$ −29.6 (*c* 0.5, $CHCl_3$); ee >99.5% (chiral HPLC analysis); IR (KBr) *ν* 3411, 3197, 1722, 1665 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.37 (s, 5H), 7.30–7.25 (m, 5H), 6.81 (s, 1H), 6.42 (d, *J*=15.7 Hz, 1H), 6.08–5.98 (m, 1H), 5.93 (s, 1H), 5.25 (d, *J*=12.3 Hz, 1H), 5.19 (d, *J*=12.3 Hz, 1H), 2.91 (dd, *J*=13.7, 7.2 Hz, 1H), 2.74 (dd, *J*=13.7, 7.2 Hz, 1H), 1.53 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 173.9, 173.4, 137.0, 135.3, 134.2, 128.7, 128.52, 128.49, 128.3, 127.5, 126.3, 124.1, 67.4, 54.2, 41.1, 21.0; MS (ESI) *m/z* (%) 362 $[M+K]^+$ (4), 346 $[M+Na]^+$, 324 $[M+H]^+$ (19). Anal. Calcd for $C_{20}H_{21}NO_3$: C, 74.28; H, 6.55; N, 4.33. Found: C, 74.28; H, 6.54; N, 4.48.

*4.3.8. (−)-Benzyl 2-carbamoyl-2-(prop-2-yn-1-yl)pent-4-enoate (**2h**).* Mp 35.5–36.0 °C; $[\alpha]_D^{25}$ −8.5 (*c* 0.4, $CHCl_3$); ee=41.2% (chiral HPLC analysis); IR (KBr) *ν* 3414, 3299, 1725, 1669 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.40–7.38 (m, 5H), 6.96 (s, 1H), 5.86 (s, 1H), 5.71–5.57 (m, 1H), 5.23 (s, 2H), 5.16–5.10 (m, 2H), 2.94–2,65 (m, 4H), 2.04 (t, *J*=2.4 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) 171.9, 171.1, 135.0, 131.5, 128.61, 128.56, 128.4, 120.0, 79.6, 71.4, 67.7, 57.3, 39.6, 24.1; MS (ESI) *m/z* (%) 310 $[M+K]^+$ (3), 294 $[M+Na]^+$ (100), 272 $[M+H]^+$ (3). Anal. Calcd for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16. Found: C, 70.93; H, 6.34; N, 5.00.

*4.3.9. (+)-Benzyl 2-allyl-2-carbamoylhex-4-ynoate (**2i**).* Mp 67.5–38.5 °C; $[\alpha]_D^{25}$ +5.6 (*c* 0.5, $CHCl_3$); ee=25.2% (chiral HPLC analysis); IR (KBr) *ν* 3415, 3206, 1728, 1672 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.36–7.34 (m, 5H), 6.95 (s, 1H), 5.91 (s, 1H), 5.71–5.57 (m, 1H), 5.2 (s, 2H), 5.20–5.05 (m, 2H), 2.85–2,61 (m, 4H), 1.70 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 172.2, 171.6, 135.3, 132.0, 128.5, 128.4, 128.2, 119.5, 79.0, 74.1, 67.4, 57.7, 39.4, 24.9, 3.5; MS (ESI) *m/z* (%) 324 $[M+K]^+$ (3), 308 $[M+Na]^+$ (100), 286 $[M+H]^+$ (6). Anal. Calcd for $C_{17}H_{19}NO_3$: C, 71.56; H, 6.71; N, 4.91. Found: C, 71.37; H, 6.73; N, 4.81.

*4.3.10. (−)-Methyl 2-benzyl-2-carbamoylpent-4-ynoate (**2j**).* Mp 108.5–109.5 °C; $[\alpha]_D^{25}$ −2.7 (*c* 0.6, $CHCl_3$); ee=85% (chiral HPLC analysis); IR (KBr) *ν* 3398, 3290, 1731, 1672 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.30–7.14 (m, 6H), 6.02 (s, 1H), 3.80 (s, 3H), 3.40 (d, *J*=13.4 Hz, 1H), 3.22 (d, *J*=13.4 Hz, 1H), 2.90 (dd, *J*=16.7, 2.4 Hz, 1H), 2.72 (dd, *J*=16.7, 2.4 Hz, 1H), 2.12 (t, *J*=2.4 Hz, 1H); ^{13}C NMR

(75 MHz, DMSO-d_6) 171.5, 170.3, 134.5, 128.7, 127.4, 126.3, 78.8, 70.6, 58.1, 51.7, 40.2, 23.6; MS (ESI) *m*/*z* (%) 246 $[M+H]^+$ (100). Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.45; H, 6.27; N, 5.80.

4.4. General procedure for a large scale biotransformation of 1a using an immobilized whole cell catalyst

The immobilized *Rh. erythropolis* AJ270 cells (4 g wet weight) in alginate capsules[12] were activated in sodium bicarbonate buffer solution (pH 7.25, 100 mL) at 30 °C for 30 min. A solution of **1a** (1.5 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 80 min. The immobilized catalyst was filtered and reused for another four times. The combined aqueous solution was worked up and (−)-**2a** (8 g, 65% yield, 88.6% ee) was obtained.

4.5. Synthesis of amino acids *S*-(−)-3 and *S*-(−)-4

A mixture of *R*-(−)-**2a** or *R*-(−)-**2g** (1 mmol) in an aqueous NaOH solution (2N, 10 mL) was stirred at room temperature until the completion of hydrolysis of ester. The mixture was then cooled down to 0 °C, and liquid bromine (0.13 mL, 2.5 mmol) was added. After 0.5 h, the mixture was warmed to 75 °C, and kept stirring for 4 h. The reaction mixture was then neutralized with hydrochloric acid. For the reaction of *R*-(−)-**2g**, white precipitate was formed and filtrated. Recrystallization in methanol gave pure *S*(−)-**4** (106 mg, 51%). For the reaction of *R*-(−)-**2a**, solvent was removed under vacuum and the residue was chromatographed on a column packed with reversed phase materials SP-120-50-ODS-A with water as mobile phase to give pure amino acid *S*-(−)-**3** (38 mg, 30%).

*4.5.1. S-(−)-2-Amino-2-methylpent-4-enoic acid (S-(−)-**3**).* 245 °C (sublimation) (lit.[14] mp 308 °C); $[\alpha]_D^{25}$ −24.6 (*c* 1.3, H_2O); {lit.[14] $[\alpha]_D^{25}$ −28.5 (*c* 1.3, H_2O)}; IR (KBr) *ν*, 3525, 3383, 1644, 1572 cm^{-1}; ^1H NMR (300 MHz, D_2O) 5.76–5.62 (m, 1H), 5.22–5.17 (m, 2H), 2.60 (dd, *J*=13.7, 7.2 Hz, 1H), 2.38 (dd, *J*=13.7, 7.2 Hz, 1H), 1.41 (d, *J*=1.5 Hz, 3H); ^{13}C NMR (75 MHz, D_2O) 176.4, 130.7, 121.3, 60.9, 41.4, 22.0. Anal. Calcd for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84. Found: C, 55.45; H, 8.70; N, 10.66.

*4.5.2. S-(−)-(E)-2-Amino-2-methyl-5-phenylpent-4-enoic acid (S-(−)-**4**).* Mp 249–250 °C (lit.[14] mp 249–250 °C); $[\alpha]_D^{25}$ −12.5 (*c* 1.0, MeOH); {lit.[14] $[\alpha]_D^{25}$ −12.9 (*c* 0.51, MeOH)}; IR (KBr) *ν* 3368, 1673, 1585 cm^{-1}; ^1H NMR (300 MHz, MeOD) 7.40 (d, *J*=7.5 Hz, 2H), 7.31 (t, *J*=7.5 Hz, 2H), 7.21 (t, *J*=7.5 Hz, 1H), 6.48 (d, *J*=15.9 Hz, 1H), 6.29–9.16 (m, 1H), 2.82–2.66 (m, 2H), 1.35 (s, 3H); ^{13}C NMR (75 MHz, acetone) 179.7, 138.6, 133.2, 129.4, 128.1, 127.8, 126.9, 56.2, 24.2, 21.6; MS (ESI) *m*/*z* (%) 204 $[M-1]^+$ (76).

4.6. Synthesis of 5

To a solution of *R*-(−)-**2a** (247 mg, 1 mmol) and 4-phenyl-but-1-ene (0.75 mL, 5 mmol) in dry toluene (10 mL) under argon protection was added through a syringe a solution of Grubbs II catalyst (40 mg, 5 mmol %) in dry toluene (5 mL). The resulting mixture was refluxed until *R*-(−)-**2a** was consumed. After reaction was quenched by adding water (0.5 mL), and removal of solvent under vacuum, the residue was chromatographed on a silica gel column using a mixture of petroleum ether, ethyl acetate, and 1,4-dioxane (15:5:1) as the mobile phase to give (*R*)-benzyl 2-carbamoyl-2-methyl-7-phenylhept-4-enoate **5** (176 mg, 50%): oil. IR (KBr) *ν* 3455, 3355, 1731, 1678 cm^{-1}; ^{13}C NMR (75 MHz, $CDCl_3$) 173.8, 173.6, 141.7, 140.3, 135.3, 134.6, 128.6, 128.4, 128.3, 128.2, 125.8, 124.6, 67.2, 53.9, 40.8, 35.6, 34.1, 20.5; MS (ESI) *m*/*z* (%) 374 $[M+Na]^+$ (100), 390 $[M+K]^+$ (8). HRMS for $C_{22}H_{25}NO_3$: 352.1907 $[M+1]^+$. Found: 352.1904 $[M+1]^+$.

4.7. Synthesis of 6

In the presence of Pd/C (10%, 12 mg), compound **5** (100 mg, 0.3 mmol) in methanol (10 mL) was hydrogenated using a hydrogen balloon. When the starting material was consumed, the catalyst was filtered and the filtrate was concentrated. The residue was mixed with aqueous NaOH (2 N, 4 mL), and liquid bromine (0.05 mL) was added at 0 °C. Following the procedure for the synthesis of **3** and **4**, (*S*)-2-amino-2-methyl-7-phenylheptanoic acid **6** (36 mg, 51%) was obtained from chromatography of a reversed phase column packed with SP-120-50-ODS-A using a mixture of water and methanol as mobile phase. Compound **6**: 235 °C (sublimation); $[\alpha]_D^{25}$ +3.6 (*c* 2.8, MeOH); IR (KBr) *ν* 3439, 1732, 1588 cm^{-1}; ^1H NMR (300 MHz, MeOD) 7.27–7.04 (m, 5H), 2.54 (t, *J*=7.5 Hz, 2H), 1.89–1.69 (m, 2H), 1.89–1.69 (m, 2H), 1.48 (s, 3H), 1.44–1.22 (m, 4H); ^{13}C NMR (75 MHz, MeOD) 173.8, 143.6, 129.4, 129.3, 126.8, 61.0, 38.4, 36.6, 32.3, 29.9, 24.4, 22.9; MS (ESI) *m*/*z* (%) 234 $[M-1]^+$ (100). HRMS for $C_{22}H_{25}NO_3$: 236.1645 $[M+1]^+$. Found: 236.1645 $[M+1]^+$.

4.8. Synthesis of 7

Following the same procedure for the preparation of **5**, (*R*,*E*)-6-benzyl 1-ethyl 5-carbamoyl-5-methylhex-2-enedioate **7** (206 mg, 65%) was obtained from the reaction between *R*-(−)-**2a** (247 mg, 1 mmol) and ethyl acrylate (0.54 mL, 5 mmol). Compound **7**: oil. $[\alpha]_D^{25}$ −3.0 (*c* 1.4, $CHCl_3$); IR (KBr) *ν* 3445, 3350, 1725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.31–7.27 (m, 5H), 6.76 (m, 1H), 6.54 (s, 1H), 5.91 (s, 1H), 5.76 (d, *J*=15.3 Hz, 1H), 5.11 (s, 2H), 4.08 (q, *J*=7.2 Hz, 2H), 2.82–2.60 (m, 2H), 1.40 (s, 3H), 1.19 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 173.1, 172.6, 165.9, 142.7, 135.0, 128.7, 128.6, 128.2, 125.2, 67.7, 60.4, 53.3, 39.3, 21.4, 14.2; MS (ESI) *m*/*z* (%) 342 $[M+Na]^+$ (100), 320 $[M+H]^+$ (63). HRMS for $C_{17}H_{21}NO_5$: 320.1492 $[M+1]^+$. Found: 320.1488 $[M+1]^+$.

4.9. Synthesis of 10

In the presence of Pd/C (10%, 24 mg), compound **7** (200 mg, 0.6 mmol) in methanol (10 mL) was hydrogenated using a hydrogen balloon. When the starting material was consumed, the catalyst was filtered and the filtrate was concentrated. The residue was mixed with K_2CO_3 (300 mg, 2.2 mmol) and benzyl bromide (0.2 mL, 1.5 mmol) in acetone (10 mL). After stirring at room temperature for 12 h, the solvent was removed under vacuum, The residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate (1:3) as the mobile phase to give (*R*)-1-benzyl 6-ethyl 2-carbamoyl-2-methylhexanedioate **8** (158 mg, 78%): oil. $[\alpha]_D^{25}$ −6.7 (*c* 0.9, $CHCl_3$); ee=85% (chiral HPLC analysis); IR (KBr) *ν* 3449, 3356, 1734, 1600 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.32–7.23 (m, 5H), 6.83 (s, 1H), 5.89 (s, 1H), 5.11 (s, 2H), 4.03 (q, *J*=7.2 Hz, 2H), 2.23–2.17 (m, 2H), 1.94–1.75 (m, 2H), 1.54–1.46 (m, 2H), 1.40 (s, 3H), 1.16 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 174.0, 173.8, 173.1, 135.3, 128.6, 128.4, 128.1, 67.3, 60.4, 53.7, 36.8, 34.0, 20.9, 20.3, 14.2; MS (ESI) *m*/*z* (%) 344 $[M+Na]^+$ (33), 322 $[M+H]^+$ (100). HRMS for $C_{17}H_{23}NO_5$: 322.1649 $[M+1]^+$. Found: 322.1640 $[M+1]^+$.

To a mixture of NBS (73 mg, 0.45 mmol) and $Hg(OAc)_2$ (134 mg, 0.42 mmol) in dry methanol (540 μL, 10 mmol) under argon protection was added a solution of **9** (100 mg, 0.35 mmol) in dry DMF (4 mL) through a syringe. The resulting mixture was stirred at room temperature overnight. Water (20 mL) was added and the mixture was extracted with ethyl acetate (3×25 mL). After drying with $MgSO_4$ and removal of the solvent, the residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl

acetate (5:1) as the mobile phase to give methyl (*S*)-2-((benzyloxy) carbonyl)-5-(ethoxycarbonyl)pentan-2-ylcarbamate **9** (82 mg, 67%): 190 °C (sublimation); $[\alpha]_D^{25}$ 20.0 (*c* 0.4, $CHCl_3$); ee=86.8% (chiral HPLC analysis); IR (KBr) *ν* 3358, 3299, 1726, 1519 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.30–7.25 (m, 5H), 5.57 (s, 1H), 5.13 (s, 2H), 4.03 (q, *J*=7.2 Hz, 2H), 3.55 (s, 3H), 2.20–2.05 (m, 2H), 2.02–2.00 (m, 1H), 1.83–1.73 (m, 1H), 1.83–1.44 (m, 4H), 1.40–1.20 (m, 1H), 1.30 (t, *J*=7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 173.9, 173.1, 155.3, 135.5, 128.6, 128.4, 128.2, 67.4, 60.3, 59.7, 51.8, 36.1, 33.8, 23.3, 19.4, 14.2; MS (ESI) *m*/*z* (%) 374 $[M+Na]^+$ (100), 352 $[M+H]^+$ (64). HRMS for $C_{18}H_{25}NO_6$: 352.1755 $[M+1]^+$. Found: 352.1748 $[M+1]^+$.

Compound **9** (56 mg, 0.15 mmol) was refluxed in hydrochloric acid (20%, 3 mL) for 4 h. After removal of the solvent, the residue was mixed with water (10 mL) and extracted with ethyl acetate (3×10 mL). The aqueous phase was concentrated under vacuum and then chromatographed on a reversed phase column packed with SP-120-50-ODS-A using water as mobile phase to give pure (*S*)-2-amino-2-methylhexanedioic acid **10** (22 mg, 84%) as white solid: mp 147.0–148.0 °C; $[\alpha]_D^{25}$ +16.0 (*c* 0.25, MeOH); IR (KBr) *ν* 3422, 3182, 1716, 1591 cm^{-1}; ^1H NMR (300 MHz, D_2O) 2.37 (t, *J*=7.2 Hz, 2H), 2.02–1.78 (m, 2H), 1.76–1.58 (m, 1H), 1.56–1.50 (m, 4H); ^{13}C NMR (75 MHz, D_2O) 177.5, 174.1, 60.0, 35.9, 33.0, 21.6, 18.5; MS (ESI) *m*/*z* (%) 174 $[M-1]^+$ (100). HRMS for $C_7H_{13}NO_4$: 176.0917 $[M+1]^+$. Found: 176.0920 $[M+1]^+$.

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Supplementary data

Chiral HPLC analysis of chiral products. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.05.103.

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