Enantioselective Enzymatic Aminolysis and Ammonolysis of Dimethyl 3-Hydroxyglutarate. Synthesis of (*R*)-4-Amino-3-hydroxybutanoic Acid

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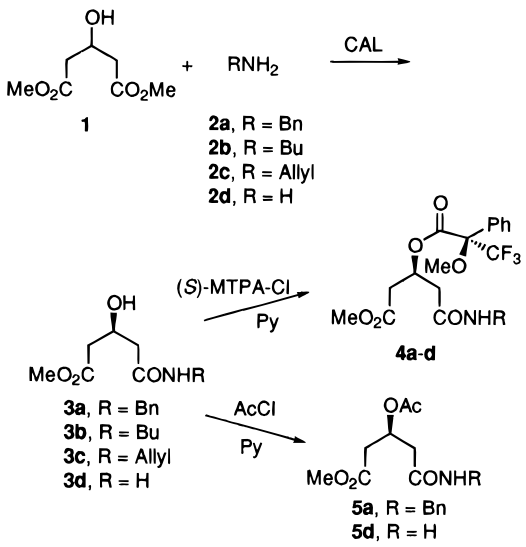
Enzymes are now widely recognized as practical catalysts for asymmetric synthesis,[1] their abilities to discriminate between enantiotopic groups or faces of a prochiral molecule being of particular importance. Hydrolysis, transesterification, or lactonization processes[2] have been applied to prochiral diesters and diols in order to prepare chiral synthons of high optical purity. However, the potential of enzymes, especially lipases, to catalyze the aminolysis of prochiral substrates has not hitherto been investigated.

With this in mind, the main purpose of this work is the study of the aminolysis and ammonolysis reactions of a prochiral diester, dimethyl 3-hydroxyglutarate (**1**). Optically active derivatives of **1** have proven utility in the synthesis of natural products; for example (*R*)- and (*S*)-3-hydroxyglutaric acid monoalkyl esters are starting materials in the synthesis of pimaricin,[3] the lactone portion of compactin, and other mevinic acids.[4] In addition, compounds derived of the aminolysis and ammonolysis of **1**, since they bear an amide function, could be suitable precursors of biologically active amino acids, amino alcohols, and other polyfunctionalized compounds.

We have chosen as biocatalyst the lipase from *Candida antarctica* (CAL), which has shown already a great efficiency in the resolution of both chiral esters[5] and chiral amines and diamines[6] through aminolysis and ammonolysis processes.

CAL-catalyzed aminolysis and ammonolysis reactions of **1** were carried out in 1,4-dioxane, with amines (**2a**−**c**) at 30 °C and ammonia (**2d**) at room temperature (Scheme 1), until disappearance of the starting diester (TLC monitoring). In these conditions, CAL proved to be an exceedingly effective catalyst either for the asymmetric aminolysis and ammonolysis of **1**, thus affording enantiopure monoamidation products (**3**) with very high yields. In all the cases, the reaction stopped at the amido ester stage and diamides never were detected. Other solvents such as hexane (for the aminolysis reactions) and diisopropyl ether and THF (for the ammonolysis) were also used but, for the same reaction time, the yields were much lower than in 1,4-dioxane; for instance, a 98% yield of **3a** was reached after 24 h in hexane, whereas only 9 h were required in 1,4-dioxane. However, the change of solvent did not influence on the enantioselectivity of the enzyme; in all the solvents CAL only transformed the *pro-R* ester grouping of **1**, affording compounds (*S*)-**3** independently of the amine used. Other lipases such as *Candida cylindracea* and *Pseudomonas cepacia* were checked in the reactions of **1** with **2a** and **2d** but, after 3 days of reaction, only starting materials were recovered.

Scheme 1



The enantiopurity of compounds **3** was determined by derivatization with (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride (MTPA-Cl),[7] to the corresponding esters **4**, whose ^{19}F NMR spectra showed only one fluorine signal. In contrast, MTPA-esters derived from *rac*-**3**[8] showed two well resolved fluorine resonances.[9]

To assign the absolute configuration of the enzymatically prepared amido esters **3**, product **3a** was conventionally acetylated to **5a** (Scheme 1), and this compound was compared with another sample obtained from a stereochemically well defined precursor, namely the acetylated monoacid **7** (Scheme 2). Thus, after chemical acetylation of **1**, the resulting acetylated diester **6** was subjected to CAL-catalyzed hydrolysis in phosphate

(1) (a) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Tetrahedron Organic Chemistry Series Vol. 12; Baldwin, J. E., Ed.; Pergamon: New York, 1994. (b) Faber, K. *Biotransformations in Organic Chemistry*, 2nd ed.; Springer-Verlag, New York, 1995.

(2) For typical examples see: (a) Toone, E. J.; Werth, M. J.; Jones, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 4946. (b) Shapira, M.; Gutman, A. L. *Tetrahedron: Asymmetry* **1994**, *5*, 1689. (c) Guanti, G.; Banfi, L.; Narisano, E. *J. Org. Chem.* **1992**, *57*, 1540. (d) Tsuji, K.; Terao, Y.; Achiwa, K. *Tetrahedron Lett.* **1989**, *30*, 6189. (e) Gutman, A. L.; Zuobi, K.; Bravdo, T. *J. Org. Chem.* **1990**, *55*, 3546.

(3) Brooks, D. W.; Palmer, J. T. *Tetrahedron Lett.* **1983**, *24*, 3059.

(4) (a) Rosen, T.; Heathcock, C. H. *Tetrahedron* **1986**, *42*, 4909. (b) Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Fehlhaber, H.-W.; Jendralla, H.; Kesseler, K.; Saric, R.; Schüssler, H.; Teetz, V.; Weber, M.; Wess, G. *Tetrahedron Lett.* **1988**, *29*, 2563.

(5) (a) García, M. J.; Rebolledo, F.; Gotor, V. *Tetrahedron: Asymmetry* **1993**, *4*, 2199. (b) Zoete, M. C.; Kock-van Dalen, A. C.; Rantwijk, F.; Sheldon, R. A. *J. Chem. Soc., Chem. Commun.* **1993**, 1831. (c) Zoete, M. C.; Ouwehand, A. A.; Rantwijk, F.; Sheldon, R. A. *Rec. Trev. Chim. Pays-Bas* **1995**, *114*, 171.

(6) (a) Astorga, C.; Rebolledo, F.; Gotor, V. *J. Chem. Soc. Perkin Trans.1* **1994**, 829. (b) García, M. J.; Rebolledo, F.; Gotor, V. *Tetrahedron* **1994**, *50*, 6935. (c) Reetz, M. T.; Dreisbach, C. *Chimia* **1994**, *48*, 570. (d) Chamorro, C.; González-Muñiz, R.; Conde, S. *Tetrahedron: Asymmetry* **1995**, *6*, 2343.

(7) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

(8) A small amount of *rac*-**3a**−**c** could be obtained when diester **1** and amines (**2a**−**c**) were allowed to react in hexane during 9 days in the absence of the enzyme. To obtain *rac*-**3d**, diester **1** was dissolved in a solution of ammonia in methanol and the mixture was kept at 5° C during 28 h.

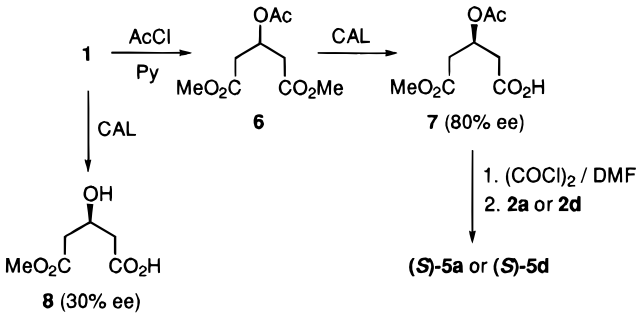
(9) As an example, ^{19}F NMR data for the diastereomeric MTPA-esters derived of *rac*-**3b**: δ −72.04 and −71.93 ppm ($CFCl_3$ as an external standard). For **4b** (obtained from optically active **3b**) only one signal to −72.04 ppm was observed.

(10) Configuration was assigned according to the sign of the previously reported optical rotation for (*R*)-(+)-monoacid **7**: Santaniello, E.; Chiari, M.; Ferraboschi, P.; Trave, S. *J. Org. Chem.* **1988**, *53*, 1567.

(11) The ee was determined by derivatization of (*S*)-**7** with (*R*)-α-methylbenzylamine using the strategy described below in the text, and ^{1}H NMR analysis of the obtained mixture of diastereomeric amides.

S0022-3263(96)00468-9 CCC: $12.00

Scheme 2



buffer-1,4-dioxane, which afforded only the acetylated monoacid (*S*)-(−)-**7**[10] with very good yield and 80% ee.[11] The one-pot successive treatments of (*S*)-**7** with oxalyl chloride and catalytic amounts of DMF, and with benzylamine (**2a**), led to the acetylated amido ester (*S*)-**5a**.

An alternative approach to **5a** from **1** by changing the order of the two first steps (first, CAL-catalyzed hydrolysis; second, acetylation) was discarded because the low optical purity (only 30% ee)[12] of the intermediate hydroxy monoacid **8**.

Comparison of the signs of the optical rotations of both compounds **5a** obtained according to Schemes 1 and 2 clearly establishes the *S*-configuration for our product **3a**. For **3b** and **3c** it is assumed the *S*-configuration by comparison of the ^{19}F NMR signals of the Mosher's esters **4a**−**c** with those of the derived from *rac*-**3a**−**c**; in all the cases the lower field signal disappears in the spectra of optically active amides. The *S*-configuration for **3d** was assigned following the same strategy as for **3a**.

In order to demonstrate the synthetic utility of optically active compounds **3**, the biologically active (*R*)-4-amino-3-hydroxybutanoic acid [(*R*)-GABOB] (**14**) was synthesized from **3d**. The method, outlined in Scheme 3, mainly involved a Hofmann rearrangement of the amide function and a hydrolysis step. Hofmann rearrangement of **3d** was effectively accomplished with $Hg(OAc)_2$ and NBS.[13] However, the hydrolysis of the resulting oxazolidinone **9** through the smooth procedure described by Kunieda et al.[14] (*N*-*tert*-butoxycarbonylation and subsequent treatment with Cs_2CO_3) did not lead to the desired cleavage of the ring, but to methyl *N*-Boc-4-aminocrotonate (**11**) as the sole product. Moreover, acid hydrolysis of **9** required drastic conditions (concd HCl, 24 h at 100 °C), which determined the formation of racemic GABOB together with its dehydration product.

These problems were resolved when the acetyl derivative **5d** was subjected to the Hofmann rearrangement instead of **3d**, thus avoiding the formation of the cyclic carbamate. To simplify the isolation and subsequent hydrolysis of the rearranged product, the Hofmann reaction of **5d** was carried out with $Hg(OAc)_2$, NBS, and benzyl alcohol. In these conditions, the benzyl carbamate **12** was achieved and later hydrolyzed with 2 N HCl, to give (*R*)-Cbz-GABOB (**13**). Finally, the hydrogenolysis of compound **13** gave enantiopure (*R*)-GABOB (**14**) in very good yield.

In summary, we have demonstrated that *C. antarctica* lipase is an excellent catalyst for the aminolysis of a prochiral diester. Our procedure allows the synthesis of enantiopure monoamides derived from dimethyl 3-hydroxyglutarate. These compounds (**3**) possess three differentiated functional groups and can be versatile chiral synthons. From **3d**, following a very simple procedure, both free (*R*)-GABOB and its Cbz-derivative can be obtained in enantiomerically pure forms. The availability of the starting material and the high yield and enantiopurity of the intermediate **3d** make this synthesis of (*R*)-GABOB an interesting alternative to other asymmetric syntheses of this compound[15] and, especially, to those syntheses of (*R*)-GABOB *via* optical resolution of racemates.[16]

Experimental Section

General. *C. antarctica* lipase, SP 435, was donated by Novo Nordisk Co. *C. cylindracea* and *P. cepacia* lipases were obtained from Sigma Chemical Co. and Amano Pharmaceutical Co., respectively. All reagents were purchased from Aldrich Chemie. Solvents were distilled over an adequate desiccant and stored under nitrogen. Flash chromatography was performed using Merck silica gel 60 (230−400 mesh).

General Procedure for the Aminolysis of 1. Dimethyl 3-hydroxyglutarate (0.30 mL, 2 mmol) and 2 mmol of the corresponding amine (**2a**−**c**) were added to a suspension of CA lipase (180 mg) in 1,4-dioxane (7 mL) under nitrogen atmosphere. The mixture was shaken at 30 °C and 250 rpm and monitored by TLC. At completion (reaction times are indicated in every case below), the enzyme was filtered and washed with dichloromethane, and the combined organic solvents were evaporated. The crude products **3a**−**c** were essentially pure by TLC and ^1H NMR analyses. For analytical purposes, compounds **3a**−**c** were purified by flash chromatography using hexane/ethyl acetate 1:2 as eluent.

Methyl (*S*)-4-(*N*-benzylcarbamoyl)-3-hydroxybutanoate (3a): reaction time, 9 h; yield, 98%; mp 61−63 °C; $[\alpha]^{20}_D$ −3.8 (*c* 1.1, $CHCl_3$); IR (Nujol) 1625, 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm) 2.37−2.63 (m, 4H, CH_2), 3.35 (bs, OH), 3.71 (s, 3H, CH_3), 4.34−4.55 (m, 3H, CH and CH_2-N), 6.55 (bs, 1H, NH), 7.20−7.45 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ (ppm) 40.58 (CH_2), 41.74 (CH_2), 43.10 (CH_2), 51.63 (OCH_3), 65.04 (CH), 127.20 (CH), 127.38 (CH), 128.43 (CH), 137.81 (C), 171.19 (C=O), 172.17 (C=O); MS (70 eV) *m*/*z* (relative intensity) 251 (M^+, 13), 106 (100), 91 (57). Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.31; H, 6.70; N, 5.45.

Methyl (*S*)-4-(*N*-butylcarbamoyl)-3-hydroxybutanoate (3b): reaction time, 9 h; yield, 96%; oil; $[\alpha]^{20}_D$ −6.9 (*c* 1.2, $CHCl_3$); IR (neat) 1643, 1738 cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm) 0.83−1.02 (t, 3H, CH_3), 1.21−1.58 (m, 4H, CH_2), 2.30−2.67 (m, 4H, CH_2), 3.18−3.32 (m, 2H, CH_2), 3.71 (s, 3H, CH_3), 4.25−4.55 (m, 2H, CH and OH), 6.42 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm) 13.36 (CH_3), 19.70 (CH_2), 31.07 (CH_2), 38.81 (CH_2), 40.70 (CH_2), 41.69 (CH_2), 51.44 (OCH_3), 65.04 (CH), 171.24 (C=O), 171.97 (C=O); HRMS *m*/*z* calcd for $C_{10}H_{19}NO_4$ 217.1314, found 217.1316. Anal. Calcd for $C_{10}H_{19}NO_4$: C, 55.28; H, 8.81; N, 6.45. Found: C, 55.12; H, 8.67; N, 6.58.

Methyl (*S*)-4-(*N*-allylcarbamoyl)-3-hydroxybutanoate (3c): reaction time, 8 h; yield, 95%; oil; $[\alpha]^{20}_D$ −3.8 (*c* 1.03, $CHCl_3$); IR (neat) 1642, 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm) 2.35−2.67 (m, 4H, CH_2), 3.71 (s, 3H, CH_3), 3.85−3.96 (m, 2H, CH_2), 4.36−4.49 (m, 1H, CH), 4.70 (bs, 1H, OH), 5.08−5.27 (m, 2H, =CH_2), 5.75−5.92 (m, 1H, =CH) 6.33 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm) 40.70 (CH_2), 41.38 (CH_2), 41.75 (CH_2), 51.48 (OCH_3), 64.96 (CH), 115.75 (CH_2), 133.61 (CH), 171.21 (C=O), 171.95 (C=O); HRMS *m*/*z* calcd for $C_9H_{15}NO_4$ 201.1001, found

(12) The ee for **8**, $[\alpha]^{20}_D$ +2.1 (*c* 1.0, $CHCl_3$), as well as the *S*-configuration were established by comparison of the optical rotation with reported data in the literature for (*R*)-(−)-**8**, $[\alpha]^{23}_D$ −6.1 (*c* 0.64, $CHCl_3$): Rosen, T.; Watanabe, M.; Heathcock, C. H. *J. Org. Chem.* **1984**, *49*, 3657.

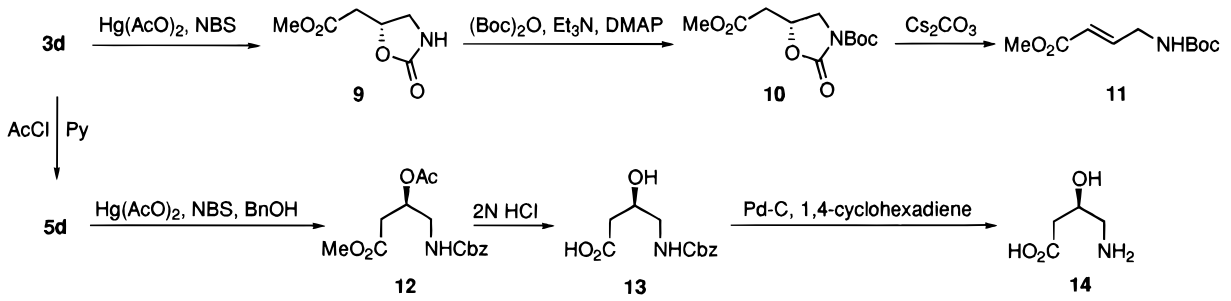
(13) Jew, S.-s.; Park, H. G.; Park, H.-J.; Park, M.-s.; Cho, Y.-s. *Tetrahedron Lett.* **1990**, *31*, 1559.

(14) Ishizuka, T.; Kunieda, T. *Tetrahedron Lett.* **1987**, *28*, 4185.

(15) (a) Kolb, H. C.; Bennani, Y. L.; Sharpless, K. B. *Tetrahedron: Asymmetry* **1993**, *4*, 133. (b) Gopalan, A. S.; Sih, C. J. *Tetrahedron Lett.* **1984**, *25*, 5235.

(16) Lu, Y.; Miet, C.; Kunesch, N.; Poisson, J. *Tetrahedron: Asymmetry* **1990**, *1*, 707.

Scheme 3



201.1002. Anal. Calcd for $C_9H_{15}NO_4$: C, 53.72; H, 7.51; N, 6.96. Found: C, 53.82; H, 7.43; N, 7.11.

Methyl (*S*)-4-Carbamoyl-3-hydroxybutanoate (3d). Ammonia was bubbled through 1,4-dioxane (7 mL) at 0 °C for 10 min, after which CAL (180 mg) and 0.30 mL (2 mmol) of **1** were added. The mixture was shaken at room temperature and 250 rpm for 5 h. Then, the enzyme was filtered and washed with dichloromethane, and the combined organic solvents were evaporated obtaining **3d** (0.316 g, 98%), essentially pure by TLC and ^{1}H NMR analyses. For analytical purposes, **3d** was purified by flash chromatography using hexane/ethyl acetate/propan-2-ol 2:8:1 as eluent. Mp 56−58 °C; $[\alpha]^{20}_D$ −4.2 (*c* 1.3, $CHCl_3$); IR (Nujol) 1665, 1726 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.33−2.69 (m, 4H, CH_2), 3.71 (s, 3H, CH_3), 4.09 (bs, 1H, OH), 4.32−4.52 (m, 1H, CH), 6.04 (bs, 1H, NH), 6.38 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm) 41.05 (CH_2), 41.93 (CH_2), 51.57 (OCH_3), 64.89 (CH), 172.25 (C=O), 174.76 (C=O); MS (70 eV) *m*/*z* (relative intensity) 161 (M^+, 1), 116 (38), 112 (41), 88 (62), 59 (100). Anal. Calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69. Found: C, 44.65; H, 6.80; N, 8.53.

Methyl (*S*)-3-acetoxy-4-(*N*-benzylcarbamoyl)butanoate (5a) was prepared from **3a** (0.125 g, 0.5 mmol) by reaction with acetyl chloride (71 μL, 1 mmol) in a mixture of pyridine (81 μL, 1 mmol) and THF (3 mL) and in the presence of catalytic amounts of DMAP (3 mg). At completion (TLC monitoring, 6 h at rt), the reaction mixture was poured into water and the product **5a** extracted with ethyl acetate. Evaporation of the organic solvent afforded 0.117 g (80%) of crude product, which was purified by flash chromatography (hexane/ethyl acetate 1:1); oil; $[\alpha]^{20}_D$ +3.3 (*c* 1.6, $CHCl_3$); IR (neat) 1651, 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.00 (s, 3H, CH_3), 2.62 (d, 2H, CH_2), 2.65−2.91 (m, 2H, CH_2), 3.68 (s, 3H, CH_3), 4.43 (d, 2H, CH_2), 5.40−5.56 (m, 1H, CH), 6.12 (bs, 1H, NH), 7.22−7.41 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ (ppm) 20.84 (CH_3), 37.97 (CH_2), 40.24 (CH_2), 43.46 (CH_2), 51.75 (OCH_3), 67.70 (CH), 127.42 (CH), 127.64 (CH), 128.57 (CH), 137.98 (C), 168.61 (C=O), 170.04 (C=O), 170.49 (C=O); HRMS *m*/*z* calcd for $C_{15}H_{19}NO_5$ 293.1263, found 293.1270.

Methyl (*S*)-3-acetoxy-4-carbamoylbutanoate (5d) was prepared as described for **5a**, with 2.5 h of reaction time. From 0.48 g (3 mmol) of **3d**, compound **5d** was obtained as an white solid in 86% yield. For analytical purposes **5d** was purified by flash chromatography (ethyl acetate/methanol 9:1).

(*S*)-3-Acetoxy-4-(methoxycarbonyl)butanoic Acid (7). To a mixture of phosphate buffer pH = 7 (4 mL) and 1,4-dioxane (1.5 mL) were added compound **6** (0.436 g, 2 mmol) and CAL (180 mg). When the reaction was completed (1.5 h, TLC monitoring), 3 N HCl and ethyl acetate were added. The organic layer was separated and the aqueous layer extracted again with ethyl acetate. The combined organic extracts were dried and evaporated to yield compound **7** (0.39 g, 96%), which was purified by flash chromatography (hexane/ethyl acetate 3:4). Oil, $[\alpha]^{20}_D$ −4.4 (*c* 1.2, $CHCl_3$), 80% ee. The spectral data for **7** were in accordance with literature values.[10]

(*R*)-4-[(Methoxycarbonyl)methyl]-2-oxooxazolidine (9). A solution of NBS (3 g, 16.8 mmol) in DMF (10 mL) was added under nitrogen to a solution of compound **3d** (2 g, 12.4 mmol) and $Hg(OAc)_2$ (4.8 g, 15 mmol) in DMF (15 mL) and MeOH (16 mL). The reaction was stirred for 20 h at 50 °C and immediately afterwards the white precipitate removed by filtration. The solution was evaporated and the solid residue extracted with dichloromethane. The organic solution was successively washed with 10% NH_4OH and water, dried, and concentrated. Flash chromatography of the crude product (hexane/ethyl acetate 1:3) gave 0.91 g (46%) of the pure product. Mp 51−52 °C; $[\alpha]^{20}_D$ −3.0 (*c* 1.2, $CHCl_3$); IR (neat) 1738 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.60−2.75 (dd, 1H, C*H*H), 2.78−2.93 (dd, 1H, CH*H*), 3.24−3.37 (dd, 1H, C*H*H), 3.67 (s, 3H, OCH_3), 3.71−3.83 (t, 1H, CH*H*), 4.88−5.03 (m, 1H, CH), 6.10 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm) 38.97 (CH_2), 45.67 (CH_2), 52.01 (OCH_3), 72.57 (CH), 159.50 (C=O), 169.67 (C=O). Anal. Calcd for $C_6H_9NO_4$: C, 45.28; H, 5.70; N, 8.80. Found: C, 45.38; H, 5.82; N, 8.65.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-4-[(methoxycarbonyl)-methyl]-2-oxooxazolidine (10). Et_3N (0.63 mL, 4.5 mmol) and DMAP (25 mg, 0.2 mmol) were added under nitrogen to a solution of **9** (0.6 g, 3.8 mmol) and $(Boc)_2O$ (1.1 g, 5.0 mmol) in THF (5 mL). After stirring at room temperature for 50 min, the mixture was dissolved in dichloromethane, successively washed with water, 2 N HCl, and water, and finally dried and concentrated. Flash chromatography of the crude product (hexane/ethyl acetate 3:1) gave 0.80 g (81%) of the pure product. Mp 84−86 °C; $[\alpha]^{20}_D$ +5.8 (*c* 0.97, $CHCl_3$); IR (Nujol) 1736, 1809 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.48 (s, 9H, CH_3), 2.58−2.73 (dd, 1H, C*H*H), 2.79−2.92 (dd, 1H, CH*H*), 3.54−3.65 (dd, 1H, C*H*H), 3.67 (s, 3H, OCH_3), 4.03−4.15 (dd, 1H, CH*H*), 4.73−4.88 (m, 1H, CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 27.81 (CH_3), 38.68 (CH_2), 48.39 (CH_2), 52.12 (OCH_3), 68.73 (CH), 83.93 (C), 149.14 (C=O), 151.24 (C=O), 169.24 (C=O); MS (70 eV) *m*/*z* 259 (M^+, <1), 244 (56), 203 (60), 159 (89), 57 (100). Anal. Calcd for $C_{11}H_{17}NO_6$: C, 50.96; H, 6.61; N, 5.40. Found: C, 50.88; H, 6.73; N, 5.32.

Methyl (*R*)-3-Acetoxy-4-[(benzyloxycarbonyl)amino]butanoate (12). BnOH (1.64 mL, 15.9 mmol) and a solution of NBS (1.9 g, 10.7 mmol) in DMF (10 mL) were added under nitrogen to a solution of **5d** (1.6 g, 7.9 mmol) and $Hg(OAc)_2$ (3.03 g, 9.5 mmol) in DMF (10 mL). The reaction was stirred for 22 h at 50 °C, and compound **12** was isolated using the same procedure described for **9**. The yield of **12** was 1.2 g (49%) after flash chromatography (hexane/ethyl acetate 2:1). Mp 39−41 °C; $[\alpha]^{20}_D$ +7.7 (*c* 0.94, $CHCl_3$); IR (Nujol) 1688, 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.03 (s, 3H, CH_3), 2.65 (d, 2H, CH_2), 3.40−3.56 (m, 2H, CH_2), 3.70 (s, 3H, OCH_3), 5.08 (bs, 1H, NH), 5.11 (s, 2H, CH_2), 5.20−5.37 (m, 1H, CH), 7.29−7.43 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ (ppm) 20.70 (CH_3), 36.06 (CH_2), 43.31 (CH_2), 51.71 (OCH_3), 66.67 (CH_2), 69.28 (CH), 127.93 (CH), 127.96 (CH), 128.31 (CH), 136.12 (C), 156.34 (C=O), 170.14 (C=O), 170.22 (C=O); MS (70 eV) *m*/*z* 309 (M^+, 26), 249 (59), 160 (78), 91 (100). Anal. Calcd for $C_{15}H_{19}NO_6$: C, 58.25; H, 6.19; N, 4.53. Found: C, 58.19; H, 6.26; N, 4.41.

(*R*)-4-Benzyloxycarbonylamino-3-hydroxybutanoic Acid (13). 2 N HCl (11 mL) was added to compound **12** (0.429 g, 1.39 mmol) and the mixture was heated at 50 °C. After 19 h, the acid solution was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were dried and concentrated to give **13** (0.303 g, 86%) as a white solid, which was washed with hexane and recristallyzed with ether−hexane. Mp 80−82 °C; $[\alpha]^{20}_D$ +4.7 (*c* 0.29, $CHCl_3$); IR (Nujol) 1672, 1696 cm^{-1}; ^{1}H NMR (D_2O) δ (ppm) 2.18−2.37 (dd, 1H, C*H*H), 2.37−2.53 (dd, 1H, CH*H*), 2.95−3.18 (m, 2H, CH_2), 3.88−4.07 (m, 1H, CH), 4.96 (s, 2H, CH_2), 7.15−7.40 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ (ppm) 38.39 (CH_2), 45.74 (CH_2), 67.02 (CH_2), 67.38 (CH), 128.03 (CH), 128.14 (CH), 128.46 (CH), 136.05 (C), 157.24 (C=O), 175.75 (C=O); MS (70 eV) *m*/*z* 253 (M^+, 2), 108 (29), 91 (100). Anal. Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.02; H, 5.91; N, 5.45.

(*R*)-4-Amino-3-hydroxybutanoic Acid (14). To a solution of 122 mg of **13** (0.48 mmol) in ethanol (4 mL) was added Pd−C (130 mg) and 1,4-cyclohexadiene (0.45 mL, 4.8 mmol), and the

mixture was heated under nitrogen at 40 °C for 4 h. Catalyst was filtered on Celite and washed with water. Removal of the solvents yielded compound **14** (55 mg, 96%) which was recrystallized in a mixture ethanol−water. Mp 215−217 °C, $[\alpha]^{20}_D$ −20.2 (*c* 1.0, H_2O), lit.[15a] mp 214 °C, $[\alpha]^{25}_D$ −20.7 (*c* 1.8, H_2O). The spectral data for **14** were in accordance with literature[15a] values: ^{1}H NMR (D_2O) δ (ppm) 2.37 (d, 2H, CH_2), 2.77 (dd, 1H, C*H*H), 3.00 (dd, 1H, CH*H*), 3.93−4.13 (m, 1H, CH); ^{13}C NMR (D_2O) δ (ppm) 43.01 (CH_2), 44.80 (CH_2), 66.22 (CH), 179.21 (C═O).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **5a** and **5d** (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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