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Enzyme-catalyzed enantiomeric resolution of *N*-Boc-proline as the key-step in an expeditious route towards RAMP

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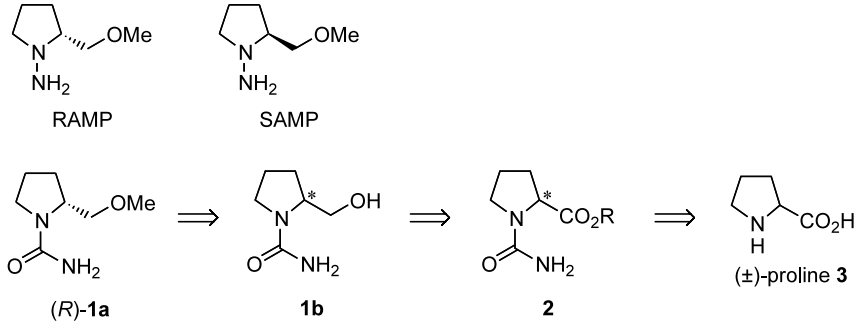
Abstract—For the preparation of both enantiomers of *N*-carbamoyl-2-methoxymethylpyrrolidine, the precursors of Enders' chiral auxiliaries, SAMP and RAMP, enzyme-catalyzed kinetic resolution of racemic *N*-carbamoyl, *N*-Boc, *N*-Cbz proline esters and prolinols were examined. *B. licheniformis* protease (subtilisin) preferentially hydrolyzed the (*R*)-carbamoylproline ester with an enantiomeric ratio (E) of 10. To a hydrophobic *N*-Cbz proline ester, subtilisin showed lower selectivity ($E=2.8$), and in contrast, a purified protease (isozyme A) from the earthworm showed the preference of (*S*)-enantiomer ($E=13.6$). In a practical sense, *C. antarctica* lipase B (Chirazyme L-2) was effective for the hydrolysis of both *N*-Boc and *N*-Cbz derivatives with E >100. The e.e. of (*R*)-*N*-carbamoyl-2-methoxymethylpyrrolidine was raised to be >99.9% by recrystallization at the *N*-Boc-prolinol stage, which was derived from the (*R*)-*N*-Boc-proline methyl ester (98.7% e.e.) through a preparative-scale enzyme-catalyzed resolution (49% yield) of the racemic substrate.

1. Introduction

α-Substituted pyrrolidines have significant importance in asymmetric synthesis in organic chemistry.[1] Among them, 1-amino-2-methoxymethylpyrrolidine in enantiomerically enriched forms, SAMP and RAMP, developed by Enders, play important roles for the derivatisation of prochiral carbonyl compounds to hydrazones, which allow a number of diastereotopic group-selective reactions as well as diastereofacially selective reactions.[2] SAMP and RAMP have so far been prepared from the pure enantiomers of proline, by way of carbamoyl derivatives **1a** as the key intermediates.[3] Our recent efforts on the study of the synthesis and reaction of carbamoylamino compounds[4] prompted an expeditious way toward (*R*)- and (*S*)-**1a**, based on the enzyme-catalyzed resolution of the appropriate intermediates **1b** or **2** from a racemic form of proline **3** (Scheme 1).



Scheme 1.

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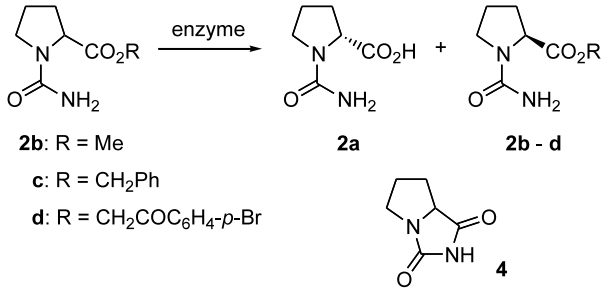
2. Attempted kinetic resolution of *N*-carbamoyl derivatives

Our first approach was an attempted kinetic resolution of derivatives with esters **2** and primary alcohols **1**, both of which bear an *N*-carbamoyl protecting group, seldom used so far. Among commercially available enzymes, protease from *Bacillus licheniformis* (subtilisin, Sigma)[5] performed the hydrolysis of esters **2b–d** (Scheme 2) with preference for the (*R*)-enantiomers as shown in Table 1 (entries 1–3). The enantioselectivity (*E* value[6]) was 8–10, and did not significantly depend upon the size or hydrophobicity of the ester moiety. The closely related substrate, hydantoin **4** was not hydrolyzed either by protease or hydantoinase.[7]

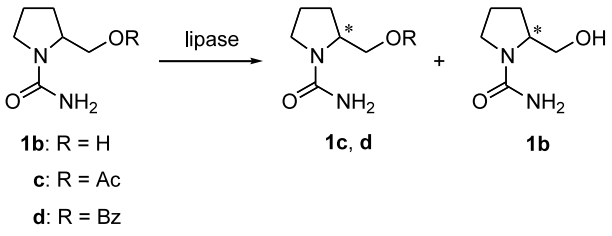


Scheme 2.

Next, the lipase-catalyzed kinetic resolution of hydroxymethyl derivatives (Scheme 3) was attempted, and the results are shown in Table 2. In the case of *Candida antarctica* lipase B-catalyzed reaction, the enantioselectivity observed in the hydrolysis of the acetate was higher ($E=4.8$, entry 2) than that in the acetylation of alcohol in organic solvent ($E=2.4$, entry 1).[8] The introduction of benzoate affected the reaction rate, and the hydrolysis became very slow. In contrast, *Candida rugosa* lipase-catalyzed hydrolysis of the same substrate **1d** proceeded; the enantioselectivity, however, was as low as 3.0 compared with those of examples reported so far.[9]

3. Effect of the protecting group and source of enzymes on selectivity

At this stage, we took the property of an *N*-protecting



Scheme 3.

carbamoyl group into account (Fig. 1). It has been suggested that the hydrogen atom on the acylamino group of the substrate has a considerable importance for the enantiomeric recognition through a hydrogen bond formation between peptide residue of the enzyme in the active site to fix the orientation of the side chain.[10] As the result, for example, Ghisalba reported an excellent enantioselectivity ($E>200$)[5g] on the racemic *N*-acetyl-*tert*-leucine chloroethyl ester, with the (*S*)-enantiomer being preferentially hydrolyzed.

In our case, however, there is no hydrogen atom. If the *N*-carbamoyl group acts as the bulky substituent, instead of the side chain of the normal amino acid, the inverted preference on the (*R*)-enantiomer is understandable. In this fitting model, it is possible that hydrogen bonding between carbamoyl NH and the peptide has another effect on the molecular recognition. This hypothesis prompted us to introduce a hydrophobic Boc or Cbz group instead of the carbamoyl group. Previously, an example demonstrating that the property of the *N*-protecting group affected the selectivity has been observed in pig liver esterase-catalyzed hydrolysis of substrates related to β-amino acid structure.[11]

The enantioselectivity was nearly lost ($E=1.3$, entry 2, Table 3) in the case of a bulky but hydrophobic group, Boc derivative **5b** (Scheme 4). Similarly, the Cbz group also weakened the preference for the (*R*)-enantiomer ($E=2.8$, entry 3) compared with the original carbamoyl substrate ($E=9.4$, entry 1). At this juncture, we became interested in another source of enzymes, from the earthworm,[12] far from the microbial origin, and applied some partially purified isozymes to the substrates above. Although an enzyme (isozyme C) did not work on the *N*-carbamoyl substrate **2b** (entry 4), one of the major isozymes[13] (isozyme A) showed a preference for

Table 1. Enzymatic hydrolysis of *N*-carbamoylproline esters

Entry	Substrate	Enzyme	Product		Recovery		E^a
			Yield (%)	% e.e.	Yield (%)	% e.e.	
1	**2b**	Protease[b]	47.0	64.0	24.0	86.6	9.4
2	**2c**	[c]	ND	ND	41.3	81.1	8.8
3	**2d**	[c]	ND	ND	40.5	87.0	9.8
4	**4**	Hydantoinase[d]	No reaction				

[a] E(P) for entry 1, E(S) for entries 2 and 3. For the definition of E(P) and E(S), see Ref. 6.
[b] From *B. licheniformis* (subtilisin, Sigma, P5459).
[c] From *B. licheniformis* (subtilisin, Sigma, P5380).
[d] From Azuki bean (Sigma, H4028).

Table 2. Enzymatic hydrolysis and acetylation of *N*-carbamoylprolinol and esters

Entry	Substrate	Lipase	Conversion[a] (%)	Product (% e.e.)	Recovery (% e.e.)	E[a]
1	**1b**	*C. antarctica*	86.4	12.2	77.2	2.4
2	**1c**	*C. antarctica*	20.3	60.9	15.6	4.8
3	**1d**	*C. antarctica*	No reaction			
4	**1d**	*C. rugosa*	53.6	34.8	40.2	3.0

[a] Conversion and E value were calculated from the e.e.s of the product and the recovered substrate.

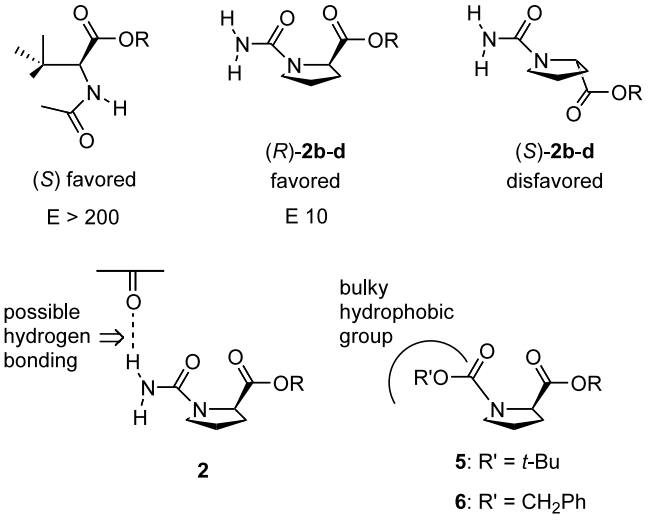
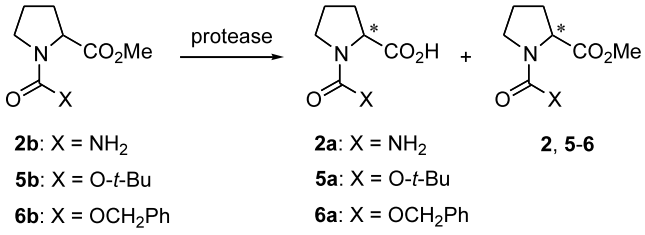


Figure 1.

the (*S*)-enantiomer with $E=13.6$ on substrate **6b** (entry 5). Enhancement of the activity at a slightly raised pH (8.5) was attempted, however, the background non-enzymatic hydrolysis brought about an apparently lowered enantioselectivity ($E=2.7$, entry 6). To our surprise, isozyme C (entry 7) showed the preference of the (*R*)-enantiomer with a similar inclination with *Bacillus* protease (entry 3). The structural similarities and catalytic functions of enzyme proteins indicated that isozyme A is classified as trypsin-type protease, while isozyme C is attributed to elastase-type.[14] This is the first example of examination of substrate specificity and enantioselectivity of the purified earthworm proteases toward artificial compounds other than naturally occurring peptide substrates. Although the specific activity was low, overexpression of enzymes[15] combined with further screening of substrates would be promising as a new tool for biotransformation, as the diversity is suggested through the entries shown in Table 3.



Scheme 4.

At the very outset of this study, many lipases were dropped as candidates, by the screening of hydrolytic enzymes on the *N*-carbamoyl substrate **2b** (Scheme 5). The diversity of stereoselectivity as well as the reactivity observed in proteases led us to reexamine *Candida antarctica* lipase B on the substrates with a hydrophobic protecting group. This was also suggested from the successful results reported on structurally related compounds.[16] This lipase-catalyzed hydrolysis worked very well with a preference for the (*S*)-enantiomer shown in Table 4. In both *N*-Boc (**5b**, entry 2) and *N*-Cbz (**6b**, entry 3) substrates, the E values exceeded 100, and the enzyme activity was kept throughout the reaction to achieve a nearly 50% conversion. Unnatural (*R*)-proline derivatives became available in 98.7% e.e. in the preparative scale (see Section 6).

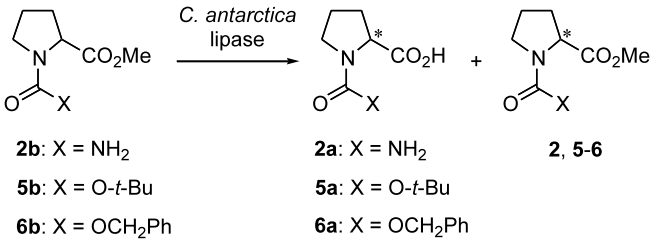
4. Transformation to *N*-carbamoyl-2-methoxymethylpyrrolidine

The final task was the transformation of enzymatically

Table 3. Protease-catalyzed hydrolysis of *N*-protected forms of proline

Entry	Substrate	Protease	Preferred enantiomer	Conversion (%)	Product (% e.e.)	Recovery (% e.e.)	E
1	**2b**	Subtilisin	*R*	53.2	64.0	86.6	9.4[a]
2	**5b**	Subtilisin	*S*	23.3	11.2	3.4	1.3
3	**6b**	Subtilisin	*R*	23.4	42.9	13.1	2.8
4	**2b**	Isozyme C[b]	No reaction				
5	**6b**	Isozyme A[b]	*S*	1.6	85.9	1.4	13.6
6	**6b**	Isozyme A[c]	*S*	9.0	44.5	4.4	2.7
7	**6b**	Isozyme C[b]	*R*	4.0	43.7	1.7	2.4
8	**6b**	Isozyme E[b]	No reaction				

[a] See Table 1, entry 1.
[b] Protease isozymes from *Lumbricus rubellus*.
[c] This reaction was under pH 8.5, while all other reactions were carried out at 7.0.



Scheme 5.

resolved highly pure enantiomers to *N*-carbamoyl-2-methoxymethylpyrrolidine **1a**. The reduction of carboxylic esters in both **5b** and **6b** smoothly proceeded with $NaBH_4$–LiCl[17] in 93 and 97% yield, respectively (Scheme 6). Recrystallization of one of the resulting alcohols **7a** provided the pure (*R*)-enantiomer. In the methylation step of the resulted hydroxymethyl group, the advantage of *N*-Boc compound **7a** was emphasized. In the case of *N*-carbamoyl derivative **1b**, which has the closest relationship to **1a**, however, *N*-methylated by-product **1e** also formed (15%) together with **1a** (68%) even under optimised conditions in terms of the equivalent of the methylating reagent (NaH–MeI) as well as the reaction temperature (–78°C to rt). In turn, an oxazolidinone **9** was a by-product (33%) in the case of *N*-Cbz precursor **8b**. Finally, no problem of significant by-product formation (**9**: <3%) was found for **7a** giving **7b** in 94% yield. The product was converted to **1a** via one-pot conversion[18] involving deprotection of the Boc group under acidic conditions and subsequent *N*-carbamoylation in 98% yield.

5. Conclusion

An expeditious route to both enantiomers of *N*-carbamoyl-2-methoxymethylpyrrolidine **1a** was established by means of the lipase-catalyzed enantioselective hydrolysis of racemic *N*-Boc proline esters. The properties of the *N*-protecting group greatly affected the enantioselectivity.

6. Experimental

6.1. General

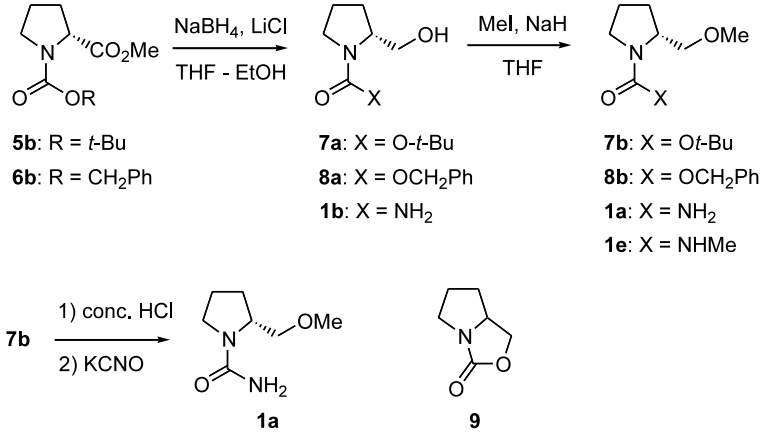
All melting points were uncorrected. IR spectra were measured as thin films for oils or KBr disks of solids on a JASCO FT/IR-410 spectrometer. ^{1}H NMR spectra were measured at 270 MHz on a JEOL JNM EX-270 spectrometer and at 400 MHz on a JEOL JNM GX-400 spectrometer. ^{13}C NMR spectra were measured at 100 MHz on a JEOL JNM GX-400 spectrometer. Mass spectra were recorded on Hitachi M-80B spectrometer at 70 eV. HPLC analyses were performed with a SSC-5410 (Senshu Scientific Co., Ltd.) liquid chromatographs and Chiralcel OJ (0.46×25 cm, Daicel Chemical Ind.). Optical rotations were recorded on a JASCO DIP 360 polarimeter. Silica gel 60 (spherical, 100–210 μm, 37558-79) of Kanto Chemical Co. was used for column chromatography.

6.2. (±)-*N*-Carbamoylproline methyl ester (±)-2b

A stirred solution of (±)-*N*-carbamoylproline[19] **2a**, (1.50 g, 9.48 mmol) in MeOH (150 mL) was treated with

Table 4. *C. antarctica* lipase-catalyzed hydrolysis of *N*-protected forms of proline

Entry	Substrate	Preferred enantiomer	Conversion (%)	Product (% e.e.)	Recovery (% e.e.)	*E*
1	**2b**	No reaction				
2	**5b**	*S*	49.7	>99.9	98.7	>100
3	**6b**	*S*	49.5	99.8	97.8	>100



Scheme 6.

Amberlyst-15 (1.50 g) and refluxed for 2 h. After cooling to room temperature, the reaction mixture was filtered. The filtrate was concentrated in vacuo and the residue was purified by column chromatography (silica gel, 38 g). Elution with $CHCl_3$–EtOH (10:1) afforded (±)-**2b** (98%) as a colorless fine powder. Mp 170.0–170.5°C; ^{1}H NMR (270 MHz, D_2O) δ 4.23 (dd, 1H, $J=3.7$, 6.6 Hz), 3.58 (s, 3H), 3.25 (m, 2H) 2.12 (m, 1H), 1.85 (m, 3H); ^{13}C NMR (100 MHz, D_2O; acetonitrile δ 1.7 as internal standard) δ 176.5, 159.9, 59.8, 53.6, 47.3, 30.3, 24.9; IR (KBr) 3400, 3179, 1739, 1651, 1610, 1600, 1470, 1456, 1213, 1200, 1179, 1104, 996, 778, 609 cm^{-1}. Anal. calcd for $C_7H_{12}N_2O_3$: C, 48.83; H, 7.03; N, 16.27. Found: C, 48.80; H, 6.99; N, 16.30.

6.3. (±)-*N*-Carbamoylproline benzyl ester (±)-2c

KCNO (0.757 g, 9.33 mmol) was added to a solution of (±)-proline (1.02 g, 8.88 mmol) in water (5.0 mL), and the mixture was stirred at 70°C for 1 h. After cooling to room temperature, THF (11.0 mL), BnBr (1.0 mL, 8.44 mmol), KI (0.153 g, 0.92 mmol) and DMF (15.0 mL) were added and the mixture was stirred at room temperature for 161 h. The remaining BnBr was quenched with aq. NH_3 soln (total 54.0 μL, 0.88 mmol) for 1 h at room temperature and the mixture was concentrated in vacuo. The residue was dissolved in a mixture of water and AcOEt, and then the organic layer was separated. The aqueous layer was extracted with AcOEt, and the combined organic layer was washed with 10% aq. $Na_2S_2O_3$ soln and brine, dried over Na_2SO_4, and concentrated in vacuo. The solid residue was further washed with Et_2O and the precipitate was filtered and dried in vacuo to give (±)-**2c** (two steps, 71%) as a white solid. Mp 147.5–148.3°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (m, 5H), 5.18 (s, 2H), 4.62 (br s, 2H), 4.40 (m, 1H), 3.46 (m, 2H), 2.28–1.90 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 157.1, 135.6, 128.5, 128.1, 127.9, 66.8, 59.1, 46.4, 29.9, 24.5; IR (KBr) 3404, 3176, 2963, 2879, 1739, 1650, 1609, 1598, 1494, 1473, 1455, 1378, 1212, 1170, 1102, 970, 754, 699, 597 cm^{-1}. Anal. calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.80; H, 6.43; N, 11.33.

6.4. (±)-*N*-Carbamoylproline *p*-bromophenacyl ester (±)-2d

(±)-*N*-Carbamoylproline[19] **2a** (0.159 g, 0.950 mmol) was dissolved in 5% aq. NaOH soln (0.8 mL). After the solution was stirred at room temperature for 30 min, EtOH (1.5 mL) and *p*-bromophenacyl bromide (0.279 g, 1.01 mmol) were added to the mixture and stirred at reflux for 5 h. The mixture was concentrated in vacuo. The residue was dissolved in a mixture of water and AcOEt, and then the organic layer was separated. The aqueous layer was extracted with AcOEt, and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 12 g). Elution with AcOEt–EtOH (1:0 to 8:1) afforded (±)-**2d** (78%) as a white solid. Mp 163.3–164.0°C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.76 (m, 2H), 7.64 (m, 2H), 5.47 (d, 1H, $J=16.3$ Hz), 5.26 (d, 1H, $J=16.3$ Hz), 4.72 (br s, 2H), 4.51 (m, 1H), 3.50 (m, 2H), 2.30–1.95 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.1, 172.2, 157.2, 132.6, 132.2, 129.2, 66.1, 58.9, 46.6, 30.3, 24.5; IR (KBr) 3398, 3185, 2949, 2882, 1746, 1700, 1649, 1609, 1596, 1472, 1455, 1399, 1366, 1236, 1179, 1103, 1071, 1010, 971, 809, 776, 612 cm^{-1}; MS (70 eV) *m*/*e* (rel. int.,%): 355 (M+1, 2), 183 (30), 171 (4), 113 (35), 90 (4), 70 (base peak), 41 (17); HR-MS (70 eV): M^+ 354.0238 (calcd. 354.0215). Anal. calcd for $C_{14}H_{15}BrN_2O_4$: C, 47.34; H, 4.26; N, 7.89. Found: C, 47.14; H, 4.05; N, 7.57.

6.5. Subtilisin-catalyzed hydrolysis of *N*-carbamoylproline esters. (*R*)-*N*-Carbamoylproline (*R*)-2a and (*S*)-*N*-carbamoylproline methyl ester (*S*)-2b

Subtilisin (Sigma, P5459, 300 μL, 199 units) was added to a solution of the methyl ester **2b** (1.00 g, 5.81 mmol) in 0.1 M phosphate buffer (pH 6.5, 60 mL), and the mixture was stirred at 24°C for 25 h, while keeping its pH to 6.5 by a pH controller. The reaction was monitored by TLC analysis (AcOEt–EtOH = 1:1, $R_f=0.50$ for **2b**; *n*-BuOH–AcOH–H_2O = 3:1:1, $R_f=0.45$ for **2a**). A small portion of the residue was dissolved in D_2O and its ^{1}H NMR spectrum of the mixture was measured. The ratio of the expecting product (*R*)-**2a** to the starting material (*S*)-**2b** was estimated by comparing the H-2 signal of the substrate (δ 4.23) with that of the product (δ 3.94). The reaction mixture was filtered through Amicon YM 10 ultrafiltration membrane (62 mm ϕ). Purification was performed by filtration through an anion-exchange resin (HCO_3^- form). The column was eluted with H_2O and a gradient from 0 to 1.0 M aq. NH_4HCO_3 soln. Fractions containing (*R*)-**2a** were combined and concentrated in vacuo to give a white solid. The acid (*R*)-**2a** was further derived to the benzyl ester (*R*)-**2c** (two steps, 47%) for the evaluation of enantiomeric excess. Unreacted (*S*)-**2b** was recorved in 24% yield.

(*R*)-**2c**: Solidified upon standing: mp 75.0–76.0°C; $[\alpha]_D^{18}$ +41.7 (*c* 1.02, $CHCl_3$). This was estimated to be 64.0% e.e. based on specific rotation which was compared with that of an authentic (*R*)-enantiomer, prepared from the commercial (*R*)-proline (Aldrich Co. 85,891-9) in the same manner as before [mp 78.5–79.3°C, $[\alpha]_D^{19}$ +65.7 (*c* 1.00, $CHCl_3$)]. Its NMR spectrum was identical with that of this authentic specimen.

(*S*)-**2b**: Solidified upon standing: mp 136.0°C; $[\alpha]_D^{18}$ −53.5 (*c* 1.00, MeOH). This was estimated to be 86.6% e.e. based on specific rotation which was compared with that of an authentic (*S*)-enantiomer, prepared from the commercial (*S*)-proline (Aldrich Co. 13,154-7) in the same manner as before [mp 135.0–136.0°C, $[\alpha]_D^{20}$ −61.8 (*c* 1.00, MeOH)]. Its NMR spectrum was identical with that of this authentic specimen.

6.6. (*S*)-*N*-Carbamoylproline benzyl ester (*S*)-2c

Subtilisin (Sigma, P5380, 25.0 mg, 240 units) was added to a suspension of the benzyl ester (**2c**, 400 mg, 1.61 mmol) in 0.1 M phosphate buffer (pH 7.0, 5.6 mL) and acetone (2.4 mL), and the mixture was stirred at 25°C

for 6 h, while keeping its pH to 6.5 by pH controller. The reaction mixture was extracted with AcOEt, and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 15 g). Elution with $CHCl_3$–MeOH (20:1) afforded (*S*)-**2c** (41%) as a white solid. In this case, another product (*R*)-**2a** stayed in the aqueous phase and was not recovered.

(*S*)-**2c**: $[\alpha]_D^{22}$ −53.3 (*c* 1.00, $CHCl_3$). This was estimated to be 81.1% e.e. based on specific rotation which was compared with that of an authentic (*R*)-**2c** as before. Its NMR spectrum was identical with this authentic specimen.

6.7. (*S*)-*N*-Carbamoylproline *p*-bromophenacyl ester (*S*)-2d

Subtilisin (Sigma, P5380, 74.7 mg, 710 units) was added to a suspension of the *p*-bromophenacyl ester (**2d**, 399 mg, 1.12 mmol) in 0.1 M phosphate buffer (pH 7.0, 10.6 mL) and DMSO (4.4 mL), and the mixture was stirred at 30°C for 44 h, while keeping its pH to 6.5 by pH controller. The reaction mixture was extracted with AcOEt, and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 22 g). Elution with AcOEt–EtOH (1:0 to 8:1) afforded (*S*)-**2d** (41%) as a white solid. In this case also, the product (*R*)-**2a** remained in the aqueous phase.

(*S*)-**2d**: $[\alpha]_D^{21}$ −57.9 (*c* 0.96, EtOH). This was estimated to be 85.8% e.e. based on the specific rotation which was compared with that of an authentic (*S*)-**2d**, prepared from (*S*)-proline in the same manner as described for racemic form [mp 71.6–72.5°C, $[\alpha]_D^{20}$ −67.5 (*c* 0.98, EtOH)]. Its NMR spectrum was identical with that of this authentic specimen.

6.8. Attempt for D-hydantoinase-catalyzed hydrolysis of (*R*)-proline-hydantoin (*R*)-4

D-Hydantoinase (Sigma, H4028, 30.0 mg, 9.07 units) was added to a solution of (*R*)-proline-hydantoin[19] **4** (224 mg, 1.60 mmol) in 0.16 M borate buffer (pH 9.0, 10 mL), and the mixture was stirred at 40°C for 6 days. The acid **2a** was estimated to be 1.28 mmol, which indicated that the progress of the reaction was 80% yield. In the control experiment without D-hydantoinase at pH 9.0, however, the spontaneous consumption (72%) of substrate was observed.

Details of the analysis for reaction progress were as follows: a 400 μL aliquot of the reaction mixture was taken and the reaction was terminated by the addition of 700 μL of 12% (w/v) trichloroacetic acid, followed by 100 μL of 10% (w/v) *p*-dimethylaminobenzaldehyde in 6 M HCl. After the cells and debris were removed by centrifugation, the absorbance at 450 nm was measured.

6.9. (±)-*N*-Carbamoylprolinol (±)-1b

KCNO (1.02 g, 12.5 mmol) and AcOH (1.16 mL) were added to a solution of (±)-prolinol (0.510 g, 5.00 mmol) in DMF (3.0 mL), and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo, and the residual solid was thoroughly extracted with $CHCl_3$. The extract was concentrated in vacuo. On the other hand, the residual solid at the stage of $CHCl_3$ extraction was dissolved in water, and the mixture was desalted by AC-110-10 on Asahi Chemical Micro Acylyzer S1. At the initial stage, the conductivity was 62 mS (4.3 V) and after the desaltation at the 3.4 V, it reached 619 μS (26 h). The recovered solution was frozen and lyophilized. This lyophilizate was combined with the $CHCl_3$ extract, and further purified by column chromatography (silica gel, 20 g). Elution with AcOEt–EtOH (3:1) afforded (±)-**1b** (84%) as a white solid. Mp 115.2–116.0°C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.64 (br s, 3H), 4.01 (m, 1H), 3.58 (m, 2H), 3.40 (m, 2H), 2.07–1.86 (m, 3H), 1.60 (m, 1H); IR (KBr) 3358, 3253, 3195, 2975, 2877, 1646, 1593, 1484, 1456, 1353, 1096, 1040, 903, 770, 612, 584 cm^{-1}. Its NMR spectrum was identical with that reported previously.[20]

6.10. (±)-*N*-Carbamoylprolinol *O*-acetate (±)-1c

Ac_2O (0.340 g, 3.33 mmol) was added to the solution of the alcohol (±)-**1b** (0.309 g, 2.14 mmol) in pyridine (3.5 mL), and the mixture was stirred overnight at room temperature. Water was added to the reaction mixture and was stirred for 30 min to decompose excess Ac_2O. After the mixture was concentrated in vacuo, then toluene and EtOH were added and further evaporated until pyridine and AcOH were removed. The residue was purified by column chromatography (silica gel, 20 g). Elution with AcOEt–EtOH (8:1) afforded (±)-**1c** (84%) as a white solid. Mp 139.5–140.2°C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.62 (s, 2H), 4.18 (m, 1H), 3.95 (m, 1H), 3.81 (m, 1H), 3.30 (m, 2H), 2.01 (s, 3H), 1.89 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 157.7, 65.4, 55.6, 46.4, 28.7, 23.4, 21.2; IR (KBr) 3395, 3194, 2957, 2880, 1740, 1650, 1604, 1454, 1384, 1366, 1252, 1240, 1097, 1039, 778, 580 cm^{-1}. Anal. calcd for $C_8H_{14}N_2O_3$: C, 51.61; H, 7.58; N, 15.04. Found: C, 51.21; H, 7.59; N, 15.07.

6.11. (±)-*N*-Carbamoylprolinol *O*-benzoate (±)-1d

Bz_2O (1.71 g, 7.54 mmol) and DMAP (0.049 g, 0.40 mmol) were added to a solution of the alcohol (±)-**1b** (0.543 g, 3.77 mmol) in pyridine (6.0 mL), and the mixture was stirred overnight at room temperature. 3-(Dimethylamino)propylamine (0.5 mL, 4.01 mmol) was added to destroy the excess Bz_2O, and the mixture was poured into ice-cooled 2 M HCl. The mixture was extracted with AcOEt, and the combined organic layer was washed with aq. $NaHCO_3$ soln and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 20 g). Elution with AcOEt–EtOH (8:1) afforded (±)-**1d** (90%) as a white solid. Mp 162.7–163.5°C; ^{1}H NMR

(270 MHz, $CDCl_3$) δ 7.96 (m, 2H), 7.51 (m, 1H), 7.38 (m, 2H), 4.72 (s, 2H), 4.44 (m, 1H), 4.09 (m, 2H), 3.37 (m, 2H), 1.92 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 157.6, 133.1, 129.6, 129.5, 128.3, 65.6, 55.7, 46.5, 28.8, 23.5; IR (KBr) 3396, 3195, 2970, 2886, 1718, 1650, 1605, 1449, 1385, 1316, 1279, 1177, 1117, 1025, 776, 709, 586 cm^{-1}. Anal. calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.66; H, 6.44; N, 11.15.

6.12. Lipase-catalyzed resolution of *N*-carbamoylprolinol and esters. (*S*)-*N*-Carbamoylprolinol *O*-acetate (*S*)-1c and (*R*)-*N*-carbamoylprolinol (*R*)-1b: lipase-catalyzed transesterification

Candida antarctica lipase (Chirazyme L-2, 25.0 mg) was added to a suspension of the alcohol **1b** (50.0 mg, 0.347 mmol), vinyl acetate (1.0 mL), *tert*-butylamine (0.10 mL), and small portion of BHT in THF (0.75 mL), and the mixture was stirred at 20°C for 14 h. The reaction mixture was filtered and the filtrate was evaporated. The residue was purified by column chromatography (silica gel, 10 g). Elution with AcOEt–EtOH (8:1 to 3:1) afforded (*S*)-**1c** (72%) as a white solid and (*R*)-**1b** (12%) as a white solid. The e.e.s of both (*S*)-**1c** and (*R*)-**1b** were estimated to be 12.2 and 77.2%, respectively, by the HPLC analysis of **1d**. The conditions were as follows: eluent, hexane/*i*-PrOH = 6/1; flow rate, 0.5 mL/min; retention time, 36.1 and 44.8 min. The absolute configurations of present **1b** and **1c** were confirmed by comparing its retention time of the resulted **1d** that of the authentic (*R*)-**1d** (44.6 min), prepared from the commercial (*R*)-prolinol (Tokyo Kasei Kogyo Co., Ltd. P1274) in the same manner as before.

6.13. (*S*)-*N*-Carbamoylprolinol (*S*)-1b and (*R*)-*N*-carbamoylprolinol *O*-acetate (*R*)-1c: lipase-catalyzed hydrolysis

Candida antarctica lipase (Chirazyme L-2, 201 mg) was added to a solution of the acetate (**1c**, 502 mg, 2.69 mmol) in 0.02 M phosphate buffer (pH 7.0, 20 mL), and the mixture was stirred at 24°C for 51 h, while keeping its pH to 7.0 by pH controller. The reaction mixture was filtered and the filtrate was lyophilized. The residue was purified by column chromatography (silica gel, 30 g). Elution with AcOEt–EtOH (8:1 to 3:1) afforded (*R*)-**1c** (74%) as a white solid and (*S*)-**1b** (24%) as a white solid.

(*S*)-**1b**: $[\alpha]_D^{19}$ −33.6 (*c* 0.50, EtOH), [an authentic specimen: mp 92.7–93.5°C, $[\alpha]_D^{22}$ +57.2 (*c* 0.54, EtOH) for (*R*)-**1b**, which was prepared from (*R*)-prolinol in the same manner as described for racemic form], 60.9% e.e. by the HPLC analysis of **1d**. Its NMR spectrum was identical with that reported previously.[20]

(*R*)-**1c**: $[\alpha]_D^{20}$ +8.2 (*c* 1.00, EtOH) [an authentic specimen: mp 58.5–59.3°C, $[\alpha]_D^{18}$ −50.0 (*c* 0.97, EtOH) for (*S*)-**1c**, which was prepared from the commercial (*S*)-prolinol (Tokyo Kasei Kogyo Co., Ltd. P1087) in the same manner as described for racemic form], 15.6% e.e. by the HPLC analysis of **1d**. Its NMR spectrum was identical with that of this authentic specimen.

6.14. (*R*)-*N*-Carbamoylprolinol [(*R*)-1b] and (*S*)-*N*-carbamoylprolinol *O*-benzoate (*S*)-1d: lipase-catalyzed hydrolysis

Candida rugosa lipase (Meito OF, 504 mg, 181,400 units) was added to a suspension of the benzoate **1d** (505 mg, 2.03 mmol) in 0.02 M phosphate buffer (pH 7.0, 100 mL), and the mixture was stirred at 35°C for 24 h, while keeping its pH to 7.0 by pH controller. The mixture was diluted with AcOEt and filtered through Celite pad. The filtrate was extracted with Et_2O and AcOEt, and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 10 g). Elution with AcOEt–EtOH (8:1) afforded (*S*)-**1d** (32%) as a white solid. After the extraction of (*S*)-**1d**, the aqueous layer was desalted and lyophilized. The residue was purified by column chromatography (silica gel, 20g). Elution with AcOEt–EtOH (3:1) afforded (*R*)-**1b** (52%) as a white solid.

(*R*)-**1b**: $[\alpha]_D^{20}$ +18.7 (*c* 0.54, EtOH), 34.8% e.e. by HPLC analysis as above. Its NMR spectrum was identical with that reported previously.[20]

(*S*)-**1d**: $[\alpha]_D^{24}$ −24.8 (*c* 1.01, EtOH) [an authentic specimen: mp 94.3–95.0°C, $[\alpha]_D^{25}$ −56.0 (*c* 1.00, EtOH) for (*S*)-**1d**, which was prepared from (*S*)-prolinol in the same manner as described for racemic form], 40.2% e.e. by HPLC analysis as above. Its NMR spectrum was identical with that of this authentic specimen.

6.15. (±)-*N*-*tert*-Butoxycarbonylproline methyl ester (±)-5b

According to the reported procedure,[21c] free NH group of (±)-proline (1.40 g, 12.2 mmol) was protected with Boc group. K_2CO_3 (5.03 g, 36.4 mmol) was added to a solution of the crude **5a** in DMF (35.0 mL), and the mixture was stirred at room temperature for 30 min. MeI (2.40 mL, 38.5 mmol) was then added and the reaction mixture was stirred for 10 h at room temperature. The solvent was evaporated and the residue was diluted with water and AcOEt, then the organic layer was separated. The aqueous layer was extracted with AcOEt, and the combined organic layer was washed with water, 10% aq. $Na_2S_2O_3$ soln and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 105 g). Elution with hexane–AcOEt (7:1 to 5:1) afforded (±)-**5b** (two steps, 91%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 4.25 (m, 1H), 3.70 (s, 3H), 3.50 (m, 2H), 2.20 (m, 1H), 2.00–1.80 (m, 3H), 1.40 (s, 9H); IR (neat) 2977, 2881, 1748, 1698, 1395, 1162, 1121, 1089, 1034, 999, 974, 888, 858, 772 cm^{-1}. Its NMR and IR spectra were identical with those reported previously.[21b]

6.16. (±)-*N*-Benzyloxycarbonylproline methyl ester (±)-6b

In a similar manner as above, racemic *N*-Cbz-proline[22] (±)-**6a** (1.17 g, 4.69 mmol) was treated with K_2CO_3 (1.96 g, 14.2 mmol) and MeI (1.46 mL, 23.5 mmol) in

DMF (22.0 mL) to give crude (±)-**6b**. This was purified by column chromatography (silica gel, 30 g). Elution with hexane–AcOEt (4:1) afforded (±)-**6b** (98%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.34 (m, 5H), 5.15 (m, 2H), 4.35 (m, 1H), 3.86–3.40 (m, 5H), 2.30–1.80 (m, 4H); IR (neat) 3033, 2953, 2882, 1747, 1713, 1416, 1353, 1281, 1201, 1179, 1119, 1089, 1002, 919, 769, 699, 612, 554 cm^{-1}. Its NMR and IR spectra were identical with those reported previously.[22]

6.17. Subtilisin-catalyzed hydrolysis of *N*-substituted proline esters. (*S*)-*N*-*tert*-Butoxycarbonylproline (*S*)-5a and (*R*)-*N*-*tert*-butoxycarbonylproline methyl ester (*R*)-5b

Subtilisin (Sigma, P5380, 15.1 mg, 140 units) was added to an emulsion of the methyl ester **5b** (150 mg, 0.653 mmol) in 0.1 M phosphate buffer (pH 7.0, 3.0 mL), and the mixture was stirred at 24°C for 23 h. The reaction mixture was acidified by 2 M HCl to pH 2.5 and was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 10 g). Elution with hexane–AcOEt (5:1 to 4:1) afforded (*R*)-**5b** (70%) as a colorless oil, and further elution with hexane–AcOEt (1:1) gave (*S*)-**5a** (25%) as a white solid. The acid (*S*)-**5a** was further derived to (*S*)-**5b** by the treatment with $TMSCHN_2$ for analysis.

(*S*)-**5b**: $[\alpha]_D^{23}$ −7.3 (*c* 1.40, MeOH). This was estimated to be 11.2% e.e. based on specific rotation which was compared with the reported value [lit.[21d] $[\alpha]_D^{23}$ −65.0 (*c* 2.00, MeOH)].

(*R*)-**5b**: $[\alpha]_D^{24}$ +2.2 (*c* 3.20, MeOH), 3.4% e.e. in a similar way as above.

6.18. (*R*)-*N*-Benzyloxycarbonylproline (*R*)-6a and (*S*)-*N*-benzyloxycarbonylproline methyl ester (*S*)-6b

Subtilisin (Sigma, P5380, 11.5 mg, 106 units) was added to an emulsion of the methyl ester **6b** (107 mg, 0.407 mmol) in 0.1 M phosphate buffer (pH 7.0, 2.1 mL), and the mixture was stirred at 30°C for 48 h. The reaction was monitored by TLC analysis (hexane–AcOEt = 1:1, R_f = 0.75 for **6b**; R_f = 0.10 for **6a**). The ester (*S*)-**6b** (63%) and the acid (*R*)-**6a** (22%) were obtained. The acid (*R*)-**6a** was further derived to (*R*)-**6b** by the treatment with $TMSCHN_2$ for analysis.

(*R*)-**6b**: This was estimated to be 42.9% e.e. based on HPLC analysis. The conditions were as follows: eluent, hexane/*i*-PrOH = 9/1; flow rate, 0.5 mL/min; retention time, 41.7 (*R*) and 50.0 (*S*) min.

(*S*)-**6b**: This was estimated to be 12.6% e.e. by HPLC analysis as above.

6.19. Earthworm protease isozyme-catalyzed hydrolysis of *N*-substituted proline esters. Attempt for hydrolysis of *N*-carbamoylproline methyl ester 2b

Earthworm protease (isozyme C, 10.0 mg) was added to a solution of (*R*)-**2b** (2.0 mg, 0.012 mmol) in 0.05 M Tris–HCl buffer (pH 7.0, 1.0 mL), and the mixture was stirred at 37°C for 72 h. To (*S*)-enantiomer, same reaction was performed. The reaction was monitored by TLC analysis, however, any trace of **2a** was not observed in either case.

6.20. Isozyme A-catalyzed hydrolysis of (±)-*N*-benzyloxycarbonylproline methyl ester (±)-6b

Earthworm protease (isozyme A, 30.0 mg) was added to an emulsion of the methyl ester **6b** (28.0 mg, 0.106 mmol) in 0.05 M Tris–HCl buffer (pH 7.0, 0.5 mL), and the mixture was stirred at 37°C for 72 h. The reaction mixture was acidified by 2 M HCl to pH 2.0 and was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 11 g). Elution with hexane–AcOEt (3:1) afforded (*R*)-**6b** (93%) as a colorless oil and further elution with pure AcOEt gave (*S*)-**6a** (5%) as a colorless oil. The e.e.s of both (*S*)-**6a** and (*R*)-**6b** were estimated to be 1.4 and 85.9%, respectively, by the HPLC analysis of **6b** as described before. The same reaction was performed at pH 8.5 at 37°C for 120 h. The ester (*R*)-**6b** (89%, 4.4% e.e.) and the acid (*R*)-**6a** (6%, 44.5% e.e.) were obtained.

6.21. Isozyme C-catalyzed hydrolysis of (±)-*N*-benzyloxycarbonylproline methyl ester (±)-6b

Isozyme C (10.0 mg) was added to an emulsion of the methyl ester **6b** (20.0 mg, 0.076 mmol) in 0.05 M Tris–HCl buffer (pH 7.0, 1.0 mL), and the mixture was stirred at 37°C for 72 h. The ester (*S*)-**6b** (83%, 1.7% e.e.) and (*R*)-**6a** (7%, 43.7% e.e.) were obtained.

6.22. Attempt for isozyme E-catalyzed hydrolsis of (±)-*N*-benzyloxycarbonylproline methyl ester (±)-6b

In a similar manner as above, isozyme E (28 mg) was incubated with **6b** (28 mg, 0.106 mmol) for 72 h, however, any trace of **6a** was not observed by TLC analysis.

6.23. Lipase-catalyzed hydrolysis of *N*-protected forms of proline. Attempt for lipase-catalyzed hydrolysis of *N*-carbamoylproline methyl ester 2b

Candida antarctica lipase (Chirazyme L-2) was incubated with both enantiomers of **2b** by applying enzyme (7.7 mg) to substrate (21.7 mg, 0.13 mmol) in 0.1 M phosphate buffer (pH 7.0, 1.0 mL), and the mixture was stirred at 30°C for 72 h. The reaction was monitored by TLC analysis, but any trace of **2a** was not observed.

6.24. (*S*)-*N*-*tert*-Butoxycarbonylproline (*S*)-5a and (*R*)-*N*-*tert*-butoxycarbonylproline methyl ester (*R*)-5b

Candida antarctica lipase (Chirazyme L-2, 0.882 g) was added to an emulsion of the methyl ester **5b** (2.19 g, 9.55 mmol) in 0.02 M phosphate buffer (pH 7.0, 110

mL), and the mixture was stirred at 30°C for 25 h, while keeping its pH to 6.8 by pH controller. The reaction mixture was acidified by 2 M HCl to pH 2.5. The mixture was diluted with AcOEt and filtered through Celite pad. The filtrate was extracted with AcOEt and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 105 g). Elution with hexane–AcOEt (5:1 to 4:1) afforded (*R*)-**5b** (49%) as a colorless oil, and further elution with hexane–AcOEt (1:1) gave (*S*)-**5a** (50%) as a white solid.

(*S*)-**5a**: Mp 135.0–135.7°C (lit.[21c] 133.5–135.0°C); $[\alpha]_D^{20}$ −60.3 (*c* 2.02, AcOH) [lit.[21a] $[\alpha]_D^{25}$ −60.2 (*c* 2.01, AcOH) for (*S*)-**5a**]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (br s, 1H), 4.30 (m, 1H), 3.50 (m, 2H), 2.35–1.90 (m, 4H), 1.43 (s, 9H); IR (KBr) 3463, 2977, 2897, 1739, 1639, 1480, 1432, 1367, 1218, 1188, 1164, 1131, 902, 775 cm^{-1}. Its NMR and IR spectra were identical with those reported previously.[21c] Anal. calcd for $C_{10}H_{17}NO_4$: C, 55.80; H, 7.96; N, 6.51. Found: C, 55.79; H, 7.97; N, 6.32. A small portion of **5a** was treated with $TMSCHN_2$ to afford **5b**. Then the methyl ester **5b** was reduced (see below) and further derived to a benzoate **7c** for the HPLC analysis, in a conventional manner. ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.00 (m, 2H), 7.55 (m, 1H), 7.43 (m, 2H), 4.40 (m, 1H), 4.20 (m, 2H), 3.40 (m, 2H), 2.10–1.90 (m, 4H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 154.3, 132.9, 132.8, 129.9, 129.5, 128.3, 79.7, 79.5, 65.2, 65.0, 55.6, 46.7, 46.5, 28.9, 28.5, 27.9, 23.9, 23.1; IR (neat) 2975, 2879, 1722, 1695, 1452, 1393, 1366, 1314, 1272, 1172, 1108, 1027, 908, 865, 773, 712 cm^{-1}; HR-MS (70 eV): M^++H 306.1659 (calcd. 306.1704). Anal. calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.97; H, 7.59; N, 4.56. The e.e. of (*S*)-**5a** was estimated to be 99.9% by the HPLC analysis of **7c**. The conditions were as follows: eluent, hexane/*i*-PrOH = 39/1; flow rate, 0.5 mL/min; retention time, 15.4 (*S*) and 17.0 (*R*) min.

(*R*)-**5b**: $[\alpha]_D^{20}$ +63.6 (*c* 1.55, MeOH) [lit.[21d] $[\alpha]_D^{23}$ −65.0 (*c* 2.00, MeOH) for (*S*)-**5b**], 98.7% e.e. by the HPLC analysis of **7c** as above. Anal. calcd for $C_{11}H_{19}NO_4$: C, 57.63; H, 8.35; N, 6.11. Found: C, 57.55; H, 8.39; N, 5.99.

6.25. (*S*)-*N*-Benzyloxycarbonylproline (*S*)-6a and (*R*)-*N*-benzyloxycarbonylproline methyl ester (*R*)-6b

Candida antarctica lipase (Chirazyme L-2, 96.5 mg) was added to an emulsion of the methyl ester **6b** (241 mg, 0.917 mmol) in 0.1 M phosphate buffer (pH 7.0, 12.0 mL), and the mixture was stirred at 30°C for 38 h. The ester (*R*)-**6b** (46%) and the acid (*S*)-**6a** (46%) were obtained. The acid (*S*)-**6a** was further derived to (*S*)-**6b** by the treatment with $TMSCHN_2$ for analysis.

(*R*)-**6b**: $[\alpha]_D^{20}$ +54.9 (*c* 1.92, MeOH) [lit.[22] $[\alpha]_D^{20}$ −58.1 (*c* 1.80, MeOH) for (*S*)-**6b**], 97.8% e.e. by HPLC analysis as above. Anal. calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 64.10; H, 6.54; N, 5.20.

(*S*)-**6b**: $[\alpha]_D^{20}$ −55.2 (*c* 1.23, MeOH), 99.8% e.e. by HPLC analysis as above. Anal. calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.74; H, 6.69; N, 5.00.

6.26. (*R*)-*N*-*tert*-Butoxycarbonylprolinol (*R*)-7a

According to the reported procedure,[17b] the methyl ester (*R*)-**5b** (0.941 g, 4.10 mmol) was treated with $NaBH_4$ (0.381 g, 10.3 mmol) and LiCl (0.386 g, 9.11 mmol) to give (*R*)-**7a** (quant.). The product was recrystallized from hexane–Et_2O to afford colorless prisms (93%, >99.9% e.e. by the HPLC analysis of **7c**). Mp 60.5–60.8°C (lit.[17b] 58.5–59.4°C); $[\alpha]_D^{20}$ +54.4 (*c* 1.02, MeOH) [lit.[17b] $[\alpha]_D^{25}$ −53.9 (*c* 1.04, MeOH) for (*S*)-**7a**]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99 (m, 1H), 3.70 (br s, 1H), 3.58 (m, 2H), 3.40 (m, 1H), 3.25 (m, 1H), 2.05–1.70 (m, 4H), 1.42 (s, 9H). Its NMR spectrum was identical with that reported previously.[17b] Anal. calcd for $C_{10}H_{19}NO_3$: C, 59.68; H, 9.52; N, 6.96. Found: C, 59.87; H, 9.54; N, 7.07.

6.27. (*R*)-*N*-Benzyloxycarbonylprolinol (*R*)-8a

In a similar manner as above,[17a] the methyl ester (*R*)-**6b** (1.23 g, 4.67 mmol) was treated with $NaBH_4$ (0.345 g, 9.12 mmol) and LiCl (0.398 g, 9.39 mmol) to give crude (*R*)-**8a**. This was purified by column chromatography (silica gel, 30 g). Elution with hexane–AcOEt (5:1 to 2:1) afforded (*R*)-**8a** (97%) as a colorless oil. $[\alpha]_D^{20}$ +45.4 (*c* 1.05, MeOH); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.36 (m, 5H), 5.16 (s, 2H), 4.03 (m, 1H), 3.65 (m, 2H), 3.55 (m, 1H), 3.40 (m, 1H), 2.24 (s, 1H), 2.10–1.79 (m, 4H); IR (neat) 3429, 2953, 2879, 1699, 1417, 1359, 1193, 1104, 913, 769, 698 cm^{-1}. Its NMR and IR spectra were identical with those reported previously.[23]

6.28. (±)-*N*-Carbamoyl-2-methoxymethylpyrrolidine (±)-1a

NaH (10.6 mg, 0.443 mmol) and MeI (30.0 μl, 0.482 mmol) were added to a suspension of the alcohol **1b** (49.5 mg, 0.343 mmol) in THF (1.0 mL) at −78°C, and the cooling bath was removed. The mixture was stirred at room temperature for 4 h and saturated aq. NH_4Cl soln was then added. The mixture was extracted with $CHCl_3$ to ensure the complete recovery, and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 10 g). Elution with AcOEt–EtOH (8:1) afforded (±)-**1a** (68%) as a white solid and further *N*-methylated form [(±)-**1e**, 15%] as a white solid.

(±)-**1a**: Mp 153.9–154.6°C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.10 (br s, 2H), 3.96 (m, 1H), 3.56–3.17 (m, 4H), 3.30 (s, 3H), 2.10–1.75 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.2, 76.8, 59.1, 57.4, 46.8, 29.1, 23.8; MS (70 eV) *m*/*e* (rel. int.,%): 159 (M+1, 4), 126 (13), 113 (base peak), 70 (61), 45 (9); IR (KBr) 3386, 3185, 2982, 2873, 1653, 1603, 1460, 1115, 974, 782, 678 cm^{-1}. Its NMR and IR spectra were identical with the reported data.[18a]

(±)-**1e**: Mp 138.8–139.6°C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.52 (br s, 1H), 3.90 (m, 1H), 3.60 (m, 1H), 3.42–3.35 (m, 5H), 2.90 (m, 1H), 2.75 (s, 3H), 2.10–1.79 (m, 4H); HR-MS (70 eV): M^+ 172.1192 (calcd. 172.1211).

6.29. (*R*)-*N*-*tert*-Butoxycarbonyl-2-methoxymethylpyrrolidine (*R*)-7b

MeI (0.35 mL, 5.62 mmol) and NaH (0.132 g, 5.50 mmol) were added to a solution of the alcohol (*R*)-**7a** (0.658 g, 3.27 mmol) in THF (13.0 mL) at −78°C. Then the temperature was slowly raised to room temperture, and the mixture was stirred overnight. The reaction mixture was cooled to 0°C and saturated aq. NH_4Cl soln was then added. The mixture was extracted with AcOEt and the combined organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 20 g). Elution with hexane–AcOEt (9:1 to 6:1) afforded (*R*)-**7b** (94%) as a colorless oil. $[\alpha]_D^{19}$ +68.1 (*c* 1.35, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (m, 1H), 3.50 (m, 1H), 3.40–3.20 (m, 6H), 2.00–1.76 (m, 4H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 79.7, 73.5, 72.9, 59.0, 56.2, 46.8, 46.4, 28.7, 28.5, 27.9, 23.7, 22.9; IR (neat) 2976, 2878, 1695, 1478, 1455, 1393, 1255, 1173, 1102, 975, 907, 867, 773 cm^{-1}. Anal. calcd for $C_{11}H_{21}NO_3$: C, 61.37; H, 9.83; N, 6.51. Found: C, 61.49; H, 9.66; N, 6.31.

6.30. (*R*)-*N*-Benzyloxycarbonyl-2-methoxymethylpyrrolidine (*R*)-8b

In a similar way as above, the alcohol (*R*)-**8a** (0.728 g, 3.09 mmol) was treated with MeI (1.17 mL, 18.8 mmol) and NaH (0.156 g, 6.5 mmol) in THF (7.3 mL) under −25°C for 15 h and for overnight at room temperature to give crude (*R*)-**8b**. This was purified by column chromatography (silica gel, 10 g). Elution with hexane–AcOEt (8:1 to 1:1) afforded (*R*)-**8b** (58%) as a colorless oil and an oxazolidinone **9** (33%) as a colorless oil.

(*R*)-**8b**: $[\alpha]_D^{22}$ +57.5 (*c* 1.44, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 5H), 5.15 (s, 2H), 4.00 (m, 1H), 3.55–3.20 (m, 7H), 2.00–1.75 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.8, 136.8, 128.3, 127.7, 127.6, 73.4, 72.7, 66.6, 66.5, 59.0, 56.9, 56.3, 46.9, 46.6, 28.7, 27.9, 23.8, 22.9; IR (neat) 2976, 2879, 1704, 1453, 1412, 1358, 1198, 1098, 974, 914, 769, 748, 698 cm^{-1}. Anal. calcd for $C_{14}H_{19}NO_3$: C, 67.45; H, 7.68; N, 5.62. Found: C, 67.30; H, 7.45; N, 5.48.

9: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.50 (dd, 1H, J=8.9, 7.9 Hz), 4.18 (dd, 1H, J=7.9, 3.5 Hz), 3.92 (m, 1H), 3.67 (m, 1H), 3.20 (m, 1H), 2.18–1.90 (m, 3H), 1.50 (m, 1H); IR (neat) 3487, 2975, 2914, 1748, 1481, 1394, 1327, 1231, 1049, 988, 775 cm^{-1}. Its NMR and IR spectra were identical with those reported previously.[24]

6.31. (*R*)-*N*-Carbamoyl-2-methoxymethylpyrrolidine (*R*)-1a

The ether (*R*)-**7b** (0.551 g, 2.56 mmol) was dissolved in conc. HCl (6.0 mL), and the mixture was stirred for 2 h at room temperature. After saturated aq. KOH soln was added to the reaction mixture under 15°C until the pH of the mixture reached 2.8, KCNO (0.435 g, 5.37 mmol) was added and the resulting mixture was stirred at room temperature for 48 h. As mentioned before, due to the hydrophilicity of the product, the mixture was extracted several times with $CHCl_3$. The crude material was purified by column chromatography (silica gel, 20 g). Elution with AcOEt–EtOH (9:1 to 6:1) afforded (*R*)-**1a** (98%) as a white solid. Mp 61.7–62.5°C (lit.[18a] 60.5–61.5°C); $[\alpha]_D^{22}$ +65.1 (*c* 2.00, EtOH) [lit.[18a] $[\alpha]_D^{20}$ −6.7 (*c* 2.00, EtOH) for (*S*)-**1a**]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.20 (br s, 2H), 3.96 (m, 1H), 3.58–3.20 (m, 4H), 3.30 (s, 3H), 2.10–1.79 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3, 76.7, 59.0, 57.4, 46.7, 29.1, 23.7; IR (KBr) 3389, 3200, 2968, 2881, 1663, 1614, 1451, 1361, 1197, 1110, 905, 778, 679 cm^{-1}. Anal. calcd for $C_7H_{14}N_2O_2$: C, 53.15; H, 8.92; N, 17.71. Found: C, 52.76; H, 8.93; N, 17.41. Its NMR and IR spectra were identical with those reported previously.[18a]

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