A Practical Synthesis of *threo*-3-Amino-2-hydroxycarboxylic Acids[1)]

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(Received August 29, 1991)

An expeditious synthesis of (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutyric acid (**2**) and (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyric acid (**4**), the key components of the renin inhibitor (**1**) and bestatin (**3**), respectively, have been accomplished by featuring highly diastereoselective formation of cyanohydrin acetates from α-alkoxycarbonylamino aldehydes under phase-transfer conditions.

Optically active *threo*-3-amino-2-hydroxycarboxylic acids are often comprised in the medicinally important compounds as their key components. For example, one of the promising renin inhibitor (**1**)[2)] involves (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutyric acid (**2**) as its C-terminal moiety, and bestatin (**3**), the potent immunological response modifier,[3)] consists of (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyric acid (**4**) and (*S*)-leucine. Recently, we developed a practical method for the synthesis of these antipodal amino acids (**2** and **4**) from (*S*)- and (*R*)-phenylalanine (**5** and **16**), respectively, by employing diastereoselective formation of the cyanohydrin acetates from the α-alkoxycarbonylamino aldehydes (**10** and **20**), respectively, under phase-transfer conditions as a key step.[1)] We wish to report here full details of this synthesis.

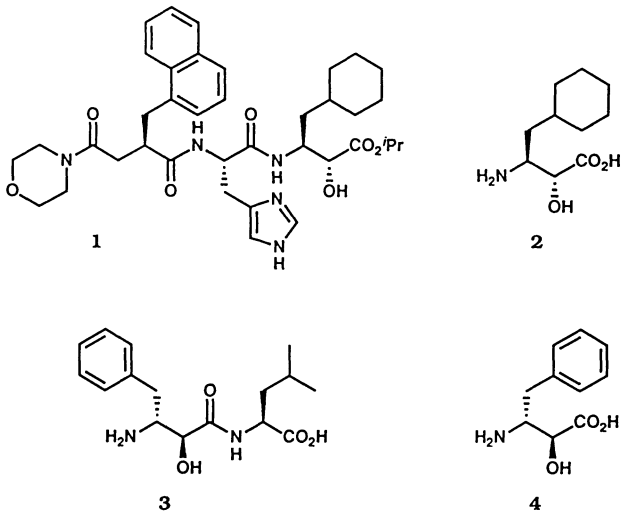
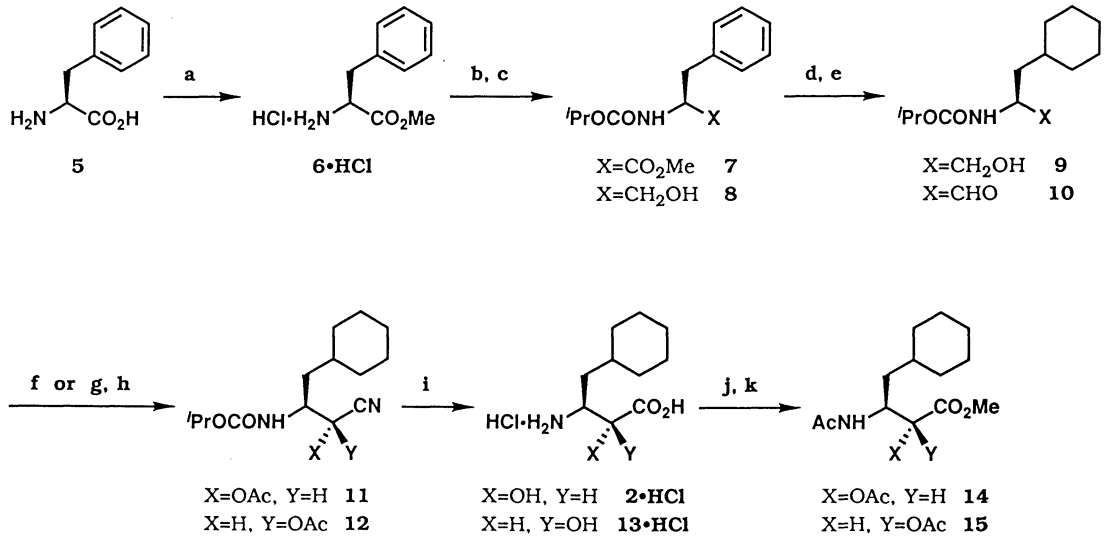


Fig. 1.



Scheme 1. a) $SOCl_2$, MeOH, 0 °C, 40 min→rt, 1 h→reflux, 2 h, 100%. b) ^{i}PrOCOCl, Et_3N, THF, 0 °C, 1 h, 91%. c) $NaBH_4$, LiCl, THF–EtOH, rt, 17 h, 98%. d) H_2 (4 atm), Rh–Al_2O_3, MeOH, rt, 5 h, 100%. e) SO_3·Py, Et_3N, DMSO, PhMe, rt, 20 min, 78%. f) NaCN, Ac_2O, $BnBu_3N$·Cl, CH_2Cl_2–H_2O, 0 °C, 3 h, 98% (a 6 : 1 mixture of **11** and **12**). g) NaCN, $BnBu_3N$·Cl, CH_2Cl_2–H_2O, 0 °C, 3 h. h) Ac_2O, DMAP, Py, rt, 3 h, 95% (a 1 : 1 mixture of **11** and **12**, 2 steps). i) AcOEt–6 mol dm^{-3} HCl, 100 °C, 2 h→12 mol dm^{-3} HCl, 80 °C, 12 h, 100% (6 : 1 and 1 : 1 mixtures of **2**·**HCl** and **13**·**HCl**), 52% (**2**·**HCl**). j) $SOCl_2$, MeOH, rt, 1 h→refux, 3 h. k) Ac_2O, DMAP, Py, rt, 16 h, 98% (a 6 : 1 mixture of **14** and **15**, 2 steps), 86% (a 1 : 1 mixture of **14** and **15**, 2 steps), 75% (**14**, 2 steps).

Results and Discussion

Synthesis of (2*R*,3*S*)-3-Amino-4-cyclohexyl-2-hydroxybutyric Acid. For the synthesis of (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutyric acid (**2**), the aldehyde (**10**), $[\alpha]_D^{20}$ +26.6° (*c* 0.939, $CHCl_3$), was synthesized from (*S*)-phenylalanine (**5**) in 5 steps according to the reported method (Scheme 1).[2b] Thus, after esterification of **5**, protection of the amino group of methyl (*S*)-phenylalaninate hydrochloride (**6**·**HCl**) followed by reduction of the methoxycarbonyl group of the ester (**7**) afforded the alcohol (**8**). Hydrogenation of the aromatic ring of **8** and subsequent Swern oxidation of the alcohol (**9**) gave **10**.

Direct formation of the cyanohydrin acetates from **10** with sodium cyanide and acetic anhydride under phase-transfer conditions[4] using benzyltributylammonium chloride as catalyst was found to take place in a highly diastereoselective manner, affording the desired *threo*-cyanohydrin acetate (**11**) as a major epimer along with a small amount of the *erythro*-isomer (**12**) (**11**:**12**=6:1 by ^{1}H NMR). In contrast, a 1:1 mixture (by ^{1}H NMR) of **11** and **12** was obtained when **10** was first allowed to react with sodium cyanide under the same conditions in the absence of acetic anhydride and the formed cyanohydrins were subsequently acetylated. These results obviously suggest that, under the phase-transfer conditions where acetic anhydride is present, the *threo*-cyanohydrin in situ produced as a kinetically more favored isomer can be immediately trapped with acetic anhydride prior to equilibrium with the *erythro*-isomer, resulting in the diastereoselective formation of **11**. Other phase-transfer catalysts such as benzyltriethylammonium chloride, tetrabutylammonium bromide, methyltrioctylammonium chloride, tetraoctylammonium bromide, (−)-*N*-benzylquininium chloride, and (+)-*N*-benzylquinidinium chloride, gave the same results. Thus, it appeared evident that utilization of a chiral catalyst, (−)-*N*-benzylquininium chloride or (+)-*N*-benzylquinidinium chloride, had no effect on the stereoselectivity.

Without separation, the 6:1 mixture of **11** and **12** prepared by direct formation of the cyanohydrin acetates from **10**, was hydrolyzed under acidic conditions, giving rise to a mixture of the *threo*-amino acid hydrochloride (**2**·**HCl**) and the *erythro*-isomer (**13**·**HCl**) in the same ratio as that for **11** and **12** (by ^{1}H NMR). The mixture was further converted into a mixture of the *N,O*-diacetyl methyl esters (**14** and **15**) via sequential esterification and acetylation without any change in the ratio of two diastereomers (by ^{1}H NMR). The ratio of *threo*- to *erythro*-isomers was rigorously determined to be 85:15 by measuring the ^{1}H NMR spectrum of the mixture. In the ^{1}H NMR spectrum, the signals due to C_2–H of **14** and **15** were observed as separate doublet peaks at δ=4.99 and 5.13, respectively. Furthermore, pure samples of **14**, $[\alpha]_D^{20}$ −60.3° (*c* 0.116, $CHCl_3$), and **15**, $[\alpha]_D^{20}$ −29.1° (*c* 0.103, $CHCl_3$), were obtained by separation of the mixture with preparative thin-layer chromatography on silica gel. Spectral (IR, ^{1}H NMR and MS) and chromatographic comparisons showed that **14** was identical with an authentic sample of **14** prepared as described below.

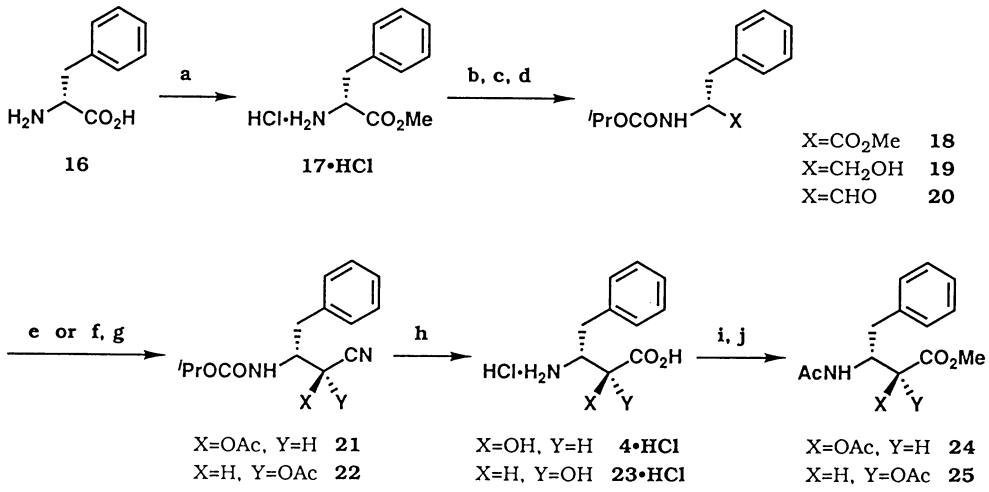
On the other hand, when the acidic reaction mixture produced by hydrolysis of the 6:1 mixture of **11** and **12** was concentrated to a small volume and set aside at 0 °C, white crystals separated. These were collected to afford a pure sample of **2**·**HCl**, mp 190 °C (decomp), $[\alpha]_D^{20}$ −12.4° (*c* 0.482, 1 mol dm^{-3} HCl), and $[\alpha]_D^{20}$ −12.2° (*c* 2.05, water). The IR, ^{1}H NMR, and MS spectra of **2**·**HCl** were identical with those of an authentic sample of **2**·**HCl**, mp 191 °C (decomp) and $[\alpha]_D^{20}$ −12.2° (*c* 0.490, 1 mol dm^{-3}HCl), synthesized according to the reported method.[2b] The melting point and optical rotation of **2**·**HCl** previously reported are mp 172—175 °C and $[\alpha]_D^{20}$ −11.2° (*c* 2.00, water), respectively.[2b] Moreover, the authentic sample of **14** was prepared from **2**·**HCl** by sequential esterification and acetylation. Thus, these results cleanly established stereochemistries of **11**, **12**, **2**·**HCl**, **13**·**HCl**, **14**, and **15**.

Similarly, the same conversion of the 1:1 mixture of **11** and **12** synthesized by the cyanohydrin formation of **10** followed by acetylation, afforded a mixture of **14** and **15**. ^{1}H NMR spectra of the mixture clearly disclosed that the ratio of *threo*- and *erythro*-isomers was 58:42 in this case.

Synthesis of (2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutyric Acid. Next, synthesis of (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyric acid (**4**) was examined by employing the explored method. Preparation of the aldehyde (**20**), mp 78—80 °C and $[\alpha]_D^{20}$ −61.1° (*c* 0.884, $CHCl_3$), was achieved starting from (*R*)-phenylalanine (**16**) in 4 steps following a similar procedure to that described for **10** without hydrogenation of the benzene ring (Scheme 2).

In the same manner, diastereoselctive formation of the cyanohydrin acetates from **20** occurred cleanly under the same phase-transfer conditions, affording a 4:1 mixture (by ^{1}H NMR) of the *threo*-cyanohydrin acetate (**21**) and the *erythro*-isomer (**22**). Furthermore, a 1:1 mixture (by ^{1}H NMR) of **21** and **22** was also obtained by stepwise formation of the cyanohydrin acetates.

Both of the 4:1 and 1:1 mixtures of **21** and **22** prepared by direct and stepwise formations of the cyanohydrin acetates from **20**, respectively, were converted into the mixtures of the *N,O*-diacetyl methyl esters (**24** and **25**) in the same ratios as those for **21** and **22** (by ^{1}H NMR) by similar sequential hydrolysis, esterification, and acetylation via mixtures of the *threo*-amino acid hydrochloride (**4**·**HCl**) and the *erythro*-isomer (**23**·**HCl**). Measurements of ^{1}H NMR spectra of the mixtures of **24** and **25** derived from the 4:1 and 1:1 mixtures of **21** and **22**, rigorously established that the ratios of *threo*- and *erythro*-isomers were 81:19 and 57:43, respectively. Separation of the 4:1 mixture of **24** and **25** by preparative thin-layer chromatography on



Scheme 2. a) $SOCl_2$, MeOH, 0 °C, 40 min→rt, 1 h→reflux, 2 h, 75%. b) ^{i}PrOCOCl, Et_3N, THF, 0 °C, 1 h, 89%. c) $NaBH_4$, LiCl, THF–EtOH, rt, 17 h, 98%. d) SO_3·Py, Et_3N, DMSO, PhMe, rt, 20 min, 81%. e) NaCN, Ac_2O, $BnBu_3N$·Cl, CH_2Cl_2–H_2O, 0 °C, 3 h, 100% (a 4 : 1 mixture of **21** and **22**). f) NaCN, $BnBu_3N$·Cl, CH_2Cl_2–H_2O, 0 °C, 3 h. g) Ac_2O, DMAP, Py, rt, 3 h, 93% (a 1 : 1 mixture of **21** and **22**, 2 steps). h) AcOEt–6 mol dm^{-3} HCl, 100 °C, 2 h→12 mol dm^{-3} HCl, 80 °C, 12 h, 100% (4 : 1 and 1 : 1 mixtures of **4**·**HCl** and **23**·**HCl**), 50% (**4**·**HCl**). i) $SOCl_2$, MeOH, rt, 1 h→reflux, 3 h. j) Ac_2O, DMAP, Py, rt, 16 h, 85% (a 4 : 1 mixture of **24** and **25**, 2 steps), 86% (a 1 : 1 mixture of **24** and **25**, 2 steps), 83% (**24**, 2 steps).

silica gel gave pure samples of **24**, $[\alpha]_D^{20}$ +61.4° (*c* 1.04, $CHCl_3$) and **25**, $[\alpha]_D^{20}$ 0.00° (*c* 1.00, $CHCl_3$). The spectral (IR, ^{1}H NMR, and MS) and chromatographic behaviors of **24** were identical with those of an authentic sample of **24** synthesized as described below.

Similarly to the case described for **2**·**HCl**, acidic hydrolysis of the 4 : 1 mrxture of **21** and **22** gave a pure sample of **4**·**HCl**, mp 191 °C (decomp) and $[\alpha]_D^{20}$ +25.8°(*c* 0.737, 1 mol dm^{-3} HCl), [lit,[3b] mp 219—221 °C and $[\alpha]_D^{20}$ +27.9° (0.717, 1 mol dm^{-3} HCl)], after direct crystallization from the concentrated reaction mixture. The ^{1}H NMR spectral data of **4**·**HCl** were identical with those previously reported.[3b] Esterification of **4**·**HCl** and subsequent acetylation were carried out in the same manner as described for **2**·**HCl**, affording the authentic sample of **24**. Therefore, stereochemistries of **21**, **22**, **4**·**HCl**, **23**·**HCl**, **24**, and **25** were confirmed unambiguously on the basis of these results. These assignments were further supported by comparisons of ^{1}H NMR spectra of these compounds with those of **11**, **12**, **2**·**HCl**, **13**·**HCl**, **14**, and **15**.

As described above, an expeditious synthesis of these antipodal amino acids (**2** and **4**) have been successfully achieved. The developed overall process may be applicable to industrial scale preparation of **2** and **4** due to its operational simplicity and uses of cheap reagents.

Experimental

General. All melting points were determined with a Yamato MP-21 melting point apparatus and are uncorrected. Measurements of optical rotations were carried out by using a Horiba SEPA-200 automatic digital polarimeter. IR spectral measurements were performed with a JASCO A-202 IR spectrometer. ^{1}H NMR spectra were measured with a Hitachi R-90H spectrometer (90 MHz) and a Bruker AM-400 spectrometer (400 MHz). All signals were expressed as ppm downfield from tetramethylsilane ($SiMe_4$) used as an internal standard (δ-value), unless otherwise noted. The following abbreviations are used: singlet (s), doublet (d), triplet (t), multiplet (m), broad (br). Assignments of peaks are indicated according to the numbering of IUPAC nomenclature. Mass spectra (MS) were taken with a Hitachi RMU-6MG mass spectrometer (EI-MS) and a Hitachi M-80A mass spectrometer (SI-MS). Wako Gel C-200 and Merck Silica Gel 60F_{254} were used for column chromatography and preparative thin-layer chromatography (TLC), respectively. The following abbreviations are used for solvents and reagents: acetic acid (AcOH), acetic anhydride (Ac_2O), benzene (PhH), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), 4-dimethylaminopyridine (DMAP), dimethyl sulfoxide (DMSO), ethanol (EtOH), ethyl acetate (AcOEt), methanol (MeOH), sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS), tetrahydrofuran (THF), toluene (PhMe).

Methyl (*S*)-Phenylalaninate Hydrochloride (6·HCl). (*S*)-Phenylalanine (**5**) (5.17 g, 31.3 mmol) was added to a stirred solution of $SOCl_2$ (7.50 g, 63.0 mmol) in MeOH (20 ml) cooled at −10 °C. The mixture was stirred at 0 °C for 40 min and at room temperature for 1 h, then heated at reflux for 2 h. Concentration in vacuo followed by azotropic removal of water using PhH gave pure **6**·**HCl** as a white solid (6.97 g, 100%), mp 160—161°C and $[\alpha]_D^{20}$ −3.80° (*c* 4.74, water) (recrystallized from hexane–MeOH) [lit,[5] mp 159—161°C and $[\alpha]_D^{20}$ −4.6° (*c* 5.0, water)].

Methyl (*S*)-*N*-(Isopropoxycarbonyl)phenylalaninate (7). Triethylamine (3.95 g, 39.0 mmol) and isopropyl chloroformate (2.45 g, 20.0 mmol) were successively added to a stirred suspension of **6**·**HCl** (3.90 g, 18.1 mmol) in THF (20 ml)

cooled at 0 °C. After stirring at the same temperature for 1 h, the mixture was concentrated in vacuo. The residue was dissolved in AcOEt, and the solution was washed successively with 1 mol dm^{-3} HCl, brine, sat. $NaHCO_3$, and brine, dried over Na_2SO_4, filtered, then concentrated in vacuo. The crude product was purified by column chromatography (SiO_2, hexane–AcOEt 10 : 1) to afford pure **7** as a white solid (4.35 g, 91%), mp 36—37°C and $[\alpha]_D^{20}$ +55.4° (*c* 1.32, $CHCl_3$). IR (KBr) 1740, 1685 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=1.21 (6H, d, *J*=6 Hz, CH*Me*$_2$) 3.11 (2H, d, *J*=6 Hz, PhC*H*$_2$), 3.72 (3H, s, CO_2Me), 4.50—5.20 (2H, m, C_2–H, NH), 4.92 (1H, quintet, *J*=6 Hz, C*H*Me$_2$), 7.04—7.48 (5H, m, Ph). EI-MS *m*/*z* 266 (M^++H), 206, 162, 131, 120.

(*S*)-*N*-(Isopropoxycarbonyl)phenylalaninol (8). LiCl (1.22 g, 28.8 mmol), $NaBH_4$ (1.09 g, 28.8 mmol), and EtOH (25 ml) were successively added to a solution of **7** (2.52 g, 9.47 mmol) in THF (12 ml) at room temperature. After stirring was continued at the same temperature for 17 h, the mixture was concentrated in vacuo, adjusted to pH 3 with 1 mol dm^{-3} HCl, then extracted with AcOEt. The combined extracts were successively washed with water and brine, dried over Na_2SO_4, filtered, then concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane–AcOEt 5 : 1→3 : 1), giving pure **8** as a white solid (2.21 g, 98%), mp 78—81 °C and $[\alpha]_D^{20}$ −25.2° (*c* 1.03, $CHCl_3$). IR (KBr) 1690 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=1.21 (6H, d, *J*=6 Hz, CH*Me*$_2$), 1.96—2.30 (1H, m, OH), 2.88 (2H, d, *J*=7 Hz, PhC*H*$_2$), 3.44—4.16 (3H, m, C_2–H, C*H*$_2$OH), 4.65—5.13 (1H, m, NH), 4.92 (1H, quintet, *J*=6 Hz, C*H*Me$_2$), 7.05—7.47 (5H, m, Ph). EI-MS *m*/*z* 237 (M^+) 206, 146, 120. Calcd for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90%. Found: C, 65.99; H, 8.03; N, 5.81%.

(*S*)-N-(Isopropoxycarbonyl)cyclohexylalaninol (9). A mixture of **8** (2.05 g, 8.65 mmol) and Rh–Al_2O_3 (400 mg) in a mixture of MeOH (6.0 ml) and AcOH (0.60 ml) was stirred at ambient temperature under a H_2 atmosphere (4 atm) for 5 h. The catalyst was flltered off and washed with MeOH. The combined filtrates were concentrated in vacuo, and purification of the residue by column chromatography (SiO_2, hexane–AcOEt 5 : 1) afforded pure **9** as a colorless oil (2.09 g, 100%), $[\alpha]_D^{20}$ −26.4° (*c* 1.78, $CHCl_3$) [lit,[2b] $[\alpha]_D^{20}$ −27.6° (*c* 1.06, $CHCl_3$)]. IR (neat) 1690 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=1.23 (6H, d, *J*=6 Hz, CH*Me*$_2$), 0.71—2.13 (13H, m, $C_6H_{11}CH_2$), 3.36—4.02 (3H, m, C_2–H, C*H*$_2$OH), 4.51—5.15 (1H, m, NH), 4.93 (1H, quintet, *J*=6 Hz, C*H*Me$_2$). EI-MS *m*/*z* 244 (M^++H), 212, 170, 126. Calcd for $C_{13}H_{25}NO_3 \cdot 0.1H_2O$: C, 63.69; H, 10.36; N, 5.71%. Found: C, 63.56; H, 10.39; N, 5.75%.

(*S*)-*N*-(Isopropoxycarbonyl)cyclohexylalaninal (10). Sulfur trioxide pyridine complex (491 mg, 3.08 mmol) was added to a solution of **9** (138 mg, 0.568 mmol) and triethylamine (312 mg, 3.08 mmol) in a mixture of PhMe (0.33 ml) and DMSO (0.67 ml) at room temperature with stirring. The mixture was stirred at the same temperature for 20 min, poured into ice-water, then extracted with AcOEt. The combined organic layers were washed successively with water and brine, dried over Na_2SO_4, filtered, then concentrated in vacuo. The crude product was purified by column chromatography (SiO_2, hexane–AcOEt 10 : 1→5 : 1), affording pure **10** as a colorless oil (107 mg, 78%), $[\alpha]_D^{20}$ +26.6° (*c* 0.939, $CHCl_3$). IR (neat) 1730, 1690 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=0.60—2.06 (13H, m, $C_6H_{11}CH_2$), 1.23 (6H, d, *J*=6 Hz, CH*Me*$_2$), 4.03—4.43 (1H, m, C_2–H), 4.93 (1H, quintet, *J*=6 Hz, C*H*Me$_2$), 5.20—5.52 (1H, m, NH), 9.56 (1H, s, CHO). EI-MS *m*/*z* 242 (M^++H), 212, 170, 126.

(2*R*,3*S*)-2-Acetoxy-4-cyclohexyl-3-(isopropoxycarbonylamino) butyronitrile (11) and Its (2*S*,3*S*)-Isomer (12). a) A 6 : 1 Mixture of 11 and 12. Ac_2O (86.6 mg, 0.848 mmol), water (0.37 ml), benzyltributylammonium chloride (4.4 mg, 0.014 mmol) and 3.00 mol dm^{-3} NaCN (0.280 ml, 0.840 mmol) were successively added to a stirred solution of **10** (61.5 mg, 0.255 mmol) in CH_2Cl_2 (0.65 ml) cooled at 0 °C. After stirring at the same temperature for 3 h, brine was added, then the mixture was extracted with CH_2Cl_2. The combined organic phases were washed successively with water and brine, dried over Na_2SO_4, filtered, then concentrated in vacuo. The residual oil purified by column chromatography (SiO_2, hexane–AcOEt 10 : 1) afforded a pure sample of the 6 : 1 mixture (by ^{1}H NMR) of **11** and **12** as a colorless oil (77.1 mg, 98%), IR (neat) 1755, 1690 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=0.68—1.96 (13H, m, $C_6H_{11}CH_2$), 1.28 (6H, d, *J*=6 Hz, CH*Me*$_2$), 2.20 (3H, s, $MeCO_2$), 3.80—4.32 (1H, m, C_3–H), 4.40—4.73 (1H, m, NH), 4.95 (1H, quintet, *J*=6 Hz, C*H*Me$_2$), 5.42 (1H, d, *J*=5 Hz, C_2–H). EI-MS *m*/*z* 311 (M^++H), 251, 212.

b) A 1 : 1 Mixture of 11 and 12. Water (0.29 ml), benzyltributylammonium chloride (3.5 mg, 0.011 mmol), and 3.00 mol dm^{-3} NaCN (0.210 ml, 0.630 mmol) were successively added to a stirred solution of **10** (49.0 mg, 0.203 mmol) in CH_2Cl_2 (0.50 ml) cooled at 0 °C, and stirring was continued at the same temperature for 3 h. After addition of brine, the mixture were extracted with CH_2Cl_2. The combined extracts were washed with water and brine, dried over Na_2SO_4, filtered, then concentrated in vacuo. DMAP (1.0 mg, 0.008 mmol) and Ac_2O (0.25 ml) were successively added to a solution of the residue in pyridine (0.50 ml) at ambient temperature. After stirring at the same temperature for 3 h, the mixture was poured into ice-water, extracted with ether. The ethereal layers were combined, washed successively with 1 mol dm^{-3} HCl, sat. $CuSO_4$, sat. $NaHCO_3$, water, and brine, dried over Na_2SO_4, filtered, then concentrated in vacuo. Purification of the crude product by column chromatography (SiO_2, hexane–AcOEt 10 : 1) gave a pure sample of the 1 : 1 mixture (by ^{1}H NMR) of **11** and **12** as a colorless oil (56.9 mg, 95%).

Methyl (2*R*,3*S*)-2-Acetoxy-3-acetylamino-4-cyclohexylbutyrate (14) and Its (2*S*,3*S*)-Isomer (15). a) From the 6 : 1 Mixture of 11 and 12. Six mol dm^{-3} hydrochloric acid (6.0 ml) was added to a solution of the 6 : 1 mixture of **11** and **12** (116 mg, 0.374 mmol) in AcOEt (3.0 ml) at room temperature. After heating at 100 °C for 2 h, 12 mol dm^{-3} HCl (1.5 ml) was further added, and the mixture was heated at 80 °C for 12 h. Concentration of the solution in vacuo afforded a pure sample of the 6 : 1 mixture (by ^{1}H NMR) of (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutyric acid hydrochloride (**2 · HCl**) and its (2*S*,3*S*)-isomer (**13 · HCl**) as a white solid (99.0 mg, 100%), which was dissolved in MeOH (5.0 ml). $SOCl_2$ (0.16 ml) was added to the solution cooled at 0 °C, and stirring was continued at room temperature for 1 h. The solution was heated at reflux for 3 h, then concentrated in vacuo. DMAP (2.0 mg, 0.016 mmol) and Ac_2O (0.50 ml) were added successively to a solution of the residue in pyridine (1.0 ml) at room temperature. After stirring at the same temperature for 16 h, the mixture was poured into ice-water, then extracted with AcOEt. The combined organic layers were washed successively with 1 mol dm^{-3} HCl, sat. $CuSO_4$, sat. $NaHCO_3$, water, and brine, dried over Na_2SO_4, filtered, then concentrated in vacuo, giving an almost pure sample of the mixture of **14** and

15 as a pale yellow oil. ^{1}H NMR spectra of this sample established rigorously that the ratio of *threo*- and *erythro*-isomers was 85 : 15. Purification of the residue by column chromatography (SiO_2, AcOEt) afforded a pure sample of the mixture of **14** and **15** as a colorless oil (77.1 mg, 98%). Further separation of the mixture by preparative TLC (SiO_2, AcOEt) gave pure samples of **14** and **15** as a colorless oil, respectively.

14: $[\alpha]_D^{20}$ −60.3° (*c* 0.116, $CHCl_3$). IR (Nujol) 1745, 1650,1540 cm^{-3}. ^{1}H NMR ($CDCl_3$) δ=0.81—1.86 (13H, m, $C_6H_{11}CH_2$), 1.97, 2.19 (each 3H, s, $MeCO_2$×2), 3.74 (3H, s, CO_2Me), 4.66 (1H, dddd, *J*=2.2, 6.4, 8.6, 9.8 Hz, C_3-H), 4.99 (1H, d, *J*=2.2 Hz, C_2-H), 5.53 (1H, brd, *J*=9.8 Hz, NH). EI-MS *m/z* 300 (M^++H), 257, 226, 198. High-resolution MS, Found: *m/z* 300.1783. Calcd for $C_{15}H_{26}NO_5$: M+H, 300.1808. The spectral (IR, ^{1}H NMR, and EI-MS) and chromatographic behaviors of this sample were identical with those of an authentic sample prepared as described below.

15: $[\alpha]_D^{20}$ −29.1° (*c* 0.103, $CHCl_3$). IR (Nujol) 1745, 1650, 1545 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=0.73—1.87 (13H, m, $C_6H_{11}CH_2$), 2.01, 2.17 (each 3H, s, $MeCO_2$×2), 3.78 (3H, s, CO_2Me), 4.61 (1H, ddt, *J*=9.3, 10.8, 3.7 Hz, C_3-H), 5.13 (1H, d, *J*=3.7 Hz, C_2-H), 5.45 (1H, brd, *J*=9.3 Hz, NH). EI-MS *m/z* 300 (M^++H), 299 (M^+), 257, 226, 198. High-resolution MS, Found: *m/z* 300.1798. Calcd for $C_{15}H_{26}NO_5$: M+H, 300.1808.

b) From the 1 : 1 Mixture of 11 and 12. The same hydrolysis of the 1 : 1 mixture of **11** and **12** (59.6 mg, 0.192 mmol) as mentioned in a) afforded a pure sample of the 1 : 1 mixture (by ^{1}H NMR) of **2**·**HCl** and **13**·**HCl**, as a white solid (53.2 mg, 100%) after concentration of the reaction mixture. Subsequent esterification of the mixture (53.2 mg, 0.192 mmol) and acetylation in the same manner as that described in a) gave rise to an almost pure sample of the mixture of **14** and **15** as a pale yellow oil after concentration of the combined AcOEt extracts in vacuo. The ratio of *threo*- and *erythro*-isomers was determined unambiguously to be 58 : 42 by measuring the ^{1}H NMR spectra of this sample. The mixture was further purified by column chromatography (SiO_2, AcOEt), affording a pure sample of the mixture of **14** and **15** as a colorless oil (47.9 mg, 86%).

(2*R*,3*S*)-3-Amino-4-cyclohexyl-2-hydroxybutyric Acid Hydrochloride (2·HCl). The 6 : 1 mixture of **11** and **12** (562 mg, 1.81 mmol) was hydrolyzed in the same manner as that mentioned above, and the solution was concentrated in vacuo to a volume of ca. 1 ml. After kept standing at 0 °C for 1 h, separated crystals were collected by decantation, triturated successively with PhMe and ether, then dried over KOH in vacuo, giving pure **2**·**HCl** as white crystals (224 mg, 52%), mp 190 °C (decomp), $[\alpha]_D^{20}$ −12.2° (*c* 2.05, water), and $[\alpha]_D^{20}$ −12.4° (*c* 0.482, 1 mol dm^{-3} HCl) [lit,[2b] mp 172—175 °C and $[\alpha]_D^{20}$ −11.2° (*c* 2.00, water)]. IR (KBr) 1725 cm^{-1}. ^{1}H NMR (D_2O, DSS as internal standard) δ=0.86—1.78 (13H, m, $C_6H_{11}CH_2$), 3.70 (1H, dt, *J*=7.2, 3.5 Hz, C_3-H), 4.36 (1H, d, *J*=3.5 Hz, C_2-H). SI-MS *m/z* 202 (M^++H−HCl), 156, 126,104. The IR, ^{1}H NMR, and SI-MS spectral data of this sample were identical with those of an authentic sample of **2**·**HCl**, mp 191 °C (decomp) and $[\alpha]_D^{20}$ −12.2° (*c* 0.490, 1 mol dm^{-3} HCl), prepared following the reported method.[2b]

Methyl (2*R*,3*S*)-2-Acetoxy-3-acetylamino-4-cyclohexylbutyrate (14). The same esterification of **2**·**HCl** (15.0 mg, 0.063 mmol) and subsequent acetylation as described for the mixture of **2**·**HCl** and **13**·**HCl** gave crude **14** after concentration of the combined AcOEt extracts in vacuo. This was purified by column chromatography (SiO_2, AcOEt), affording pure **14** as a colorless oil (14.2 mg, 75%).

Methyl (*R*)-Phenylalaninate Hydrochloride (17·HCl). The same esterification of (*R*)-phenylalanine (**16**) (5.03 g, 30.4 mmol) as described for **5** afforded pure **17**·**HCl** as a white solid (6.59 g, 100%), mp 159—160 °C and $[\alpha]_D^{20}$ +4.00° (*c* 2.25, water) (recrystallized from hexane–MeOH) [lit,[5] $[\alpha]_D^{20}$ +3.9° (*c* 2.7, water)], after concentration of the reaction mixture and azotropic removal of water employing PhH.

Methyl (*R*)-*N*-(Isopropoxycarbonyl)phenylalaninate(18). Acylation of **17**·**HCl** (2.01 g, 9.32 mmol) in the same manner as described for **6**·**HCl** gave rise to crude **18** after concentration of the combined AcOEt extracts. This was purified by column chromatography (SiO_2, hexane–AcOEt 10 : 1), affording pure **18** as a white solid (2.20 g, 89%), mp 36—37 °C and $[\alpha]_D^{20}$ −56.1° (*c* 1.03, $CHCl_3$). IR, ^{1}H NMR, and EI-MS spectral data of this sample were identical with those of **7**.

(*R*)-*N*-(Isopropoxycarbonyl)phenylalaninol (19). Reduction of **18** (2.06 g, 7.79 mmol) in the similar manner to that described for **7** afforded crude **19** after concentration of the combined AcOEt extracts in vacuo. Purification of the residue by column chromatography (SiO_2, hexane–AcOEt 5 : 1→3 : 1) gave pure **19** as a white solid (1.81 g, 98%), mp 75—78 °C and $[\alpha]_D^{20}$ +24.2° (*c* 1.08, $CHCl_3$). Calcd for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90%. Found: C, 65.52; H, 8.32; N, 5.96%. IR, ^{1}H NMR, and EI-MS spectra of this sample were identical with those of **8**.

(*R*)-*N*-(Isopropoxycarbonyl)phenylalaninal (20). The same oxidation of **19** (111 mg, 0.468 mmol) as described for **9** gave crude **20** after concentration of the combined AcOEt extracts in vacuo. This was chromatographed (SiO_2, hexane–AcOEt 5 : 1) to afford pure **20** as a white solid (88.9 mg, 81%). Recrystallization of this sample from hexane–ether gave an analytical sample of **20** as white crystals, mp 78—80 °C, $[\alpha]_D^{20}$ −61.1° (*c* 0.884, $CHCl_3$). IR (KBr) 1725, 1685 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=1.23 (6H, d, *J*=6 Hz, $CHMe_2$), 3.13 (2H, d, δ=7 Hz, $PhCH_2$), 4.30—4.64 (1H, m, C_2-H), 4.93 (1H, quintet, *J*=6 Hz, $CHMe_2$), 4.95—5.23 (1H, m, NH), 7.08—7.48 (5H, m, Ph), 9.62 (1H, s, CHO). EI-MS *m/z* 236 (M^++H), 206, 164, 120. Calcd. for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95%. Found: C, 66.54; H, 7.37; N, 5.91%.

(2*S*,3*R*)-2-Acetoxy-3-isopropoxycarbonylamino-4-phenylbutyronitrile (21) and Its (2*R*,3*R*)-Isomer (22). a) A 4 : 1 Mixture of 21 and 22. The same direct formation of the cyanohydrin acetates from **20** (51.6 mg, 0.220 mmol) as described for **10** gave a crude mixture of **21** and **22** after concentration of the combined CH_2Cl_2 extracts in vacuo. Purification of the mixture by preparative TLC (SiO_2, hexane–AcOEt 10 : 1) afforded a pure sample of the 4 : 1 mixture (by ^{1}H NMR) of **21** and **22** as a colorless oil (67.4 mg, 100%), IR (neat) 1760, 1700 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=1.05—1.38 (6H, m, $CHMe_2$), 2.19 (3H, s, $MeCO_2$), 2.80—3.15 (2H, m, $PhCH_2$), 4.15—4.57 (1H, m, C_3-H), 4.66—5.15 (2H, m, $CHMe_2$, NH), 5.45 (1H, d, *J*=5 Hz, C_2-H), 7.10—7.69 (5H, m, Ph). EI-MS *m/z* 304 (M^+) 245, 206.

b) A 1 : 1 Mixture of 21 and 22. Cyanohydrin formation of **20** (101 mg, 0.430 mmol) followed by acetylation in the same manner as described for **10** afforded a crude sample of the mixture of **21** and **22** after concentration of the combined ethereal layers in vacuo. This was purified by preparative TLC (SiO_2, hexane–AcOEt 10 : 1) giving rise to a pure sample of the 1 : 1 mixture (by ^{1}H NMR) of **21** and **22** as a colorless oil

(122 mg, 93%).

Methyl (2*S*,3*R*)-2-Acetoxy-3-acetylamino-4-phenylbutyrate (24) and Its (2*R*,3*R*)-Isomer (25). a) From the 4:1 Mixture of 21 and 22. The 4:1 mixture of **21** and **22** (60.6 mg, 0.199 mmol) was hydrolyzed in the same manner as that described for the mixture of **11** and **12**, affording a pure sample of the 4:1 mixture (by ^{1}H NMR) of (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyric acid hydrochloride (**4**·**HCl**) and its (2*R*,3*R*)-isomer (**23**·**HCl**) as a white solid (51.1 mg, 100%) after concentration of the reaction mixture. Subsequent esterification and acetylation of the mixture (51.1 mg, 0.199 mmol) in a similar manner to that described for the mixture of **2**·**HCl** and **13**·**HCl** gave rise to an almost pure sample of the mixture of **24** and **25** as a pale yellow oil after concentration of the combined AcOEt extracts in vacuo. The ^{1}H NMR spectra of this sample rigorously established that the ratio of *threo*- and *erythro*-isomers was 81:19. Purification of the residue by column chromatography (SiO_2, AcOEt) afforded a pure sample of the mixture of **24** and **25** as a colorless oil (49.8 mg, 85%). This was further separated by preparative TLC (SiO_2, AcOEt), giving pure samples of **24** and **25** as a colorless oil, respectively.

24: $[\alpha]_D^{20}$ +61.4° (*c* 1.04, $CHCl_3$). IR (neat) 1750, 1665, 1545 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=1.96, 2.23 (each 3H, s, $MeCO_2$×2), 2.79 (1H, dd, *J*=9.0, 13.6 Hz, PhC*H*), 2.94 (1H, dd, *J*=6.6, 13.6 Hz, PhC*H*), 3.69 (3H, s, CO_2Me), 4.70 (1H, ddt, *J*=6.6, 9.0, 2.2 Hz, C_3-H), 4.90 (1H, d, *J*=2.2 Hz, C_2-H), 5.73 (1H, brd, *J*=9.0 Hz, NH). EI-MS *m*/*z* 294 (M^++H), 293 (M^+) 234, 202, 192. High-resolution MS, Found: *m*/*z* 294.1347. Calcd for $C_{15}H_{20}NO_5$: M+H 294.1341. Spectral (IR, ^{1}H NMR and EI-MS) and chromatographic comparisons showed that this sample was identical with an authentic sample synthesized as mentioned below.

25: $[\alpha]_D^{20}$ 0.00° (*c* 1.00, $CHCl_3$). IR (neat) 1750, 1645, 1550 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=1.91, 2.18 (each 3H, s, $MeCO_2$ ×2), 2.87 (2H, d, *J*=7.2 Hz, PhCH_2), 3.70 (3H, s, CO_2Me), 5.11 (1H, d, *J*=4.3 Hz, C_2-H), 4.79 (1H, ddd, *J*=4.3, 7.2, 8.8 Hz, C_3-H), 5.56 (1H, brd, *J*=8.8 Hz, NH), 7.17—7.34 (5H, m, Ph). EI-MS *m*/*z* 294 (M^++H), 293 (M^+), 234, 202, 192. High-resolution MS, Found: *m*/*z* 294.1338. Calcd for $C_{15}H_{20}NO_5$: M+H, 294.1341.

b) From the 1:1 Mixture of 21 and 22. Hydrolysis of the 1:1 mixture of **21** and **22** (122 mg, 0.401 mmol) in a similar manner to that described for the mixture of **11** and **12** gave a pure sample of the 1:1 mixture (by ^{1}H NMR) of **4**·**HCl** and **23**·**HCl** as a white solid (102 mg, 100%) after concentration of the reaction mixture in vacuo. The same esterification and subsequent acetylation of the mixture (102 mg, 0.401 mmol) as those mentioned for the mixture of **2**·**HCl** and **13**·**HCl** afforded an almost pure sample of the mixture of **24** and **25** as a pale yellow oil. The ratio of *threo*- and *erythro*-isomers was determined unambiguously to be 57:43 by measuring the ^{1}H NMR spectra of this sample. This was further purified by column chromatography (SiO_2, AcOEt), affording a pure sample of the mixture of **24** and **25** as a colorless oil (101 mg, 86%).

(2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutyric Acid Hydrochloride (4·HCl). After hydrolysis of the 4:1 mixture of **21** and **22** (1.32 g, 4.34 mmol) in the same manner as described for the mixture of **11** and **12**, the reaction mixture was concentrated in vacuo to a volume of ca. 2 ml, then set aside at 0 °C for 1 h. Separated crystals were collected by decantation, triturated successively with PhMe and ether, and dried over KOH *in vacuo*, affording pure **4**·**HCl** as white crystals (500 mg, 50%), mp 191 °C (decomp) and $[\alpha]_D^{20}$ +25.8° (*c* 0.737, 1 mol dm^{-3} HCl) [lit,[3b] mp 219—221 °C and $[\alpha]_D^{20}$ +27.9° (*c* 0.717, 1 mol dm^{-3} HCl)]. IR (KBr) 1740 cm^{-1}. ^{1}H NMR (D_2O, DSS as internal standard) δ=3.01 (1H, dd, *J*=8.1, 14.1 Hz, PhC*H*), 3.16 (1H, dd, *J*=7.3, 14.1 Hz, PhC*H*), 3.94 (1H, dt, *J*=7.7, 3.3 Hz, C_3-H), 4.32 (1H, d, *J*=3.3 Hz, C_2-H), 7.33—7.47 (5H, m, Ph). SI-MS *m*/*z* 196 (M^++H−HCl), 150, 120, 104. The ^{1}H NMR spectral data of this sample were identical with those previously reporte.[3b]

Methyl (2*S*,3*R*)-2-Acetoxy-3-acetylamino-4-phenylbutyrate (24). Esterification of **4**·**HCl** (13.2 mg, 0.057 mmol) followed by acetylation in the same manner as described for the mixture of **2**·**HCl** and **13**·**HCl** gave crude **24** after concentration of the combined AcOEt extracts in vacuo. This was chromatographed (SiO_2, AcOEt), affording pure **24** as a colorless oil (13.8 mg, 83%).

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