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Efficient Route to (*S*)-Azetidine-2-carboxylic Acid

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A new and efficient route to (*S*)-azetidine-2-carboxylic acid (>99.9% ee) in five steps and total yield of 48% *via* malonic ester intermediates was established. As the key step, efficient four-membered ring formation (99%) was achieved from dimethyl (*S*)-(1′-methyl)benzylaminomalonate by treating with 1,2-dibromoethane (1.5 eq) and cesium carbonate (2 eq) in DMF. Krapcho dealkoxycarbonylation of dimethyl (1′*S*)-1-(1′-methyl)benzylazetidine-2,2-dicarboxylate, the product of this cyclization procedure, proceeded with preferential formation (2.7:1, 78% total yield) of the desired (2*S*,1′*S*)-monoester, with the help of a chiral auxiliary which was introduced on the nitrogen atom. The undesired (2*R*,1′*S*)-isomer could be converted to that with proper stereochemistry, by a deprotonation and subsequent reprotonation step. Finally, lipase-catalyzed preferential hydrolysis of the (2*S*,1′*S*)-monoester and subsequent deprotection provided enantiomerically pure (*S*)-azetidine-2-carboxylic acid in a 91% yield from the mixture of (2*S*,1′*S*)- and (2*R*,1′*S*)-isomers.

Key words: cyclic amino acid; azetidine-2-carboxylate; azetidine-ring formation; Krapcho dealkoxycarbonylation; diastereofacially selective protonation

(*S*)-Azetidine-2-carboxylic acid (**1a**), a non-proteogenic cyclic amino acid, is the key component of deoxymugineic acid and nicotianamine, which are potent plant-origin promoters for the uptake of iron from soil.[1–3] Acid **1a** also works as the starting material for a nicotinic receptor tracer.[4] Preparation of the pure (*S*)-enantiomer has been achieved by synthesis from chiral pools,[5–7] optical resolution,[8,9] and enzyme-catalyzed kinetic resolution.[10,11] Among these, Sumitomo's group has reported the separation of diastereomers (2*S*,1′*S*)- and (2*R*,1′*S*)-**2a**,[9] which had been prepared in an equimolar ratio from racemic methyl 2,4-dibromobutanoate. Our synthesis, inspired by the foregoing intermediates, is shown in Scheme 1. The key feature is the use of the (1′*S*)-1-(1′-methyl)benzylazetidine-2,2-dicarboxylate ester (**3a**), a cyclic malonic acid ester intermediate, which facilitated 1) the formation of a four-membered ring, taking advantage of efficient alkylation at the α-position of the acyclic malonic ester precursor (**4a**), and 2) the asymmetric Krapcho dealkoxycarbonylation[12] assisted by an α-methylbenzyl chiral auxiliary.[13]

Toward this end, DBU-catalyzed transesterification of known aminomalonic ester **4b**[14] provided **4a** (86%).[15] It was found from systematic studies that the proper combination of the amine and haloester, in regard to steric hindrance, nucleophilicity, electrophilicity, and stability, was very important. For example, an attempt to directly prepare **4a** from (*S*)-α-methylbenzylamine and dimethyl bromomalonate only resulted in very low yield. An α-methyl substituent in the benzylamine moiety increased the nucleophilicity, in spite of the steric hindrance. Indeed, benzylamine[16] itself showed much lower reactivity toward alkylation.

The first keystep, azetidine ring formation, was successful (99%) by applying 1,2-dibromoethane (1.5 eq) and cesium carbonate (2 eq) in DMF. The only detected by-product was an eight-membered ring compound (**5**). Steric hindrance of the diester moiety affected the rate of cyclization. When the substrate was changed to diethyl ester **4b**, *C*-alkylated intermediate **6** was isolated in a substantial amount, and the addition of tetra-*n*-butylammonium iodide (TBAI) was necessary to ensure cyclization to azetidine **3b** (95%). Efficient deprotonation of the malonic ester was also essential to promote the first step of cyclization. When a weaker base, potassium carbonate, was applied to **4b**, the only isolable product was imine **7**, which originated from the initial attack of the amine lone-pair electron on the bromine atom of 1,2-dibromoethane, accompanied with ethylene formation and subsequent dehydrobromination of the resulting *N*-bromo intermediate.

Conventional Krapcho dealkoxycarbonylation on **3a** by applying sodium chloride in aqueous DMSO[12] at as high a temperature as 160 °C only resulted in a moderate yield of **2a** (65%), probably due to undesired hydrolysis of either the starting material or the product. The attempted dealkoxycarbonylation of more stable diethyl ester **3b** under similar conditions was very slow. This situation was improved (78%) by the combined use of lithium chloride in DMSO[17] under strictly anhydrous conditions in the presence of molecular sieves 3A. Instead of water, 2,6-di-*tert*-butyl-4-methylphenol (BHT) was added as a proton source. The yields of

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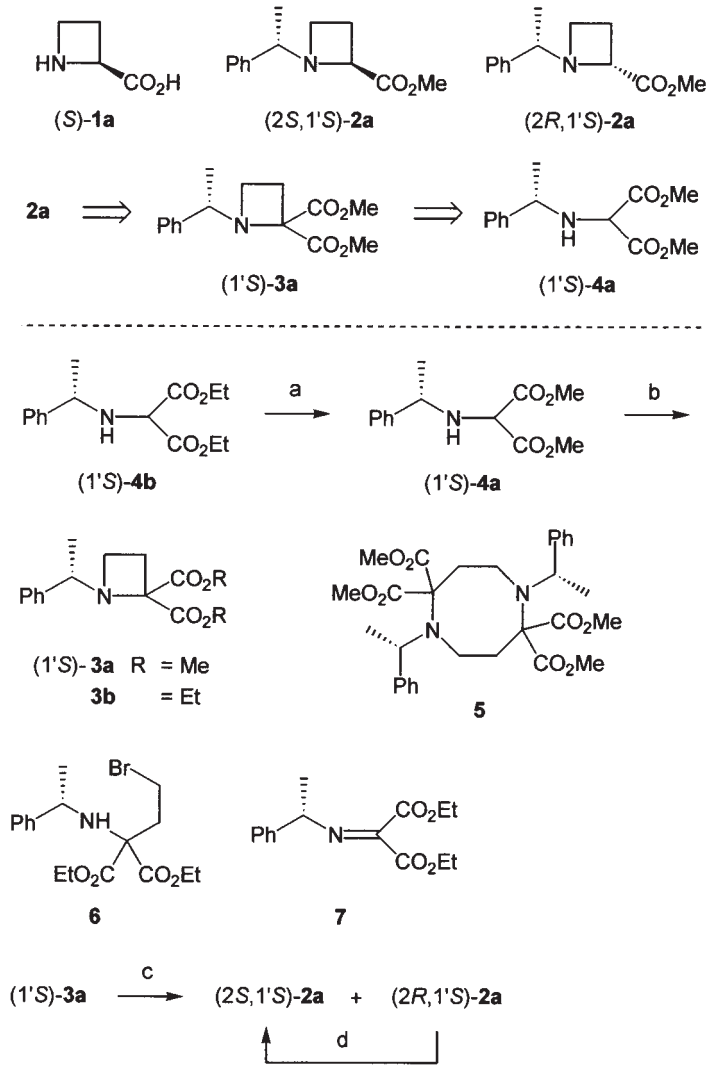


Scheme 1. Reagents: a) DBU, MeOH, 86%; b) Cs_2CO_3 (2.0 eq), $BrCH_2CH_2Br$ (1.5 eq), 99%; c) LiCl (3.2 eq), BHT (3.2 eq), 140 °C, 78%; d) 1) LDA, THF, −78 °C; 2) aq. NH_4Cl, −78 °C, 82%.

(2*S*,1′*S*)-**2a** and (2*R*,1′*S*)-**2a** were 57% and 21%, respectively, and both could be easily separated by silica gel column chromatography. On the other hand, attempts at selective hydrolysis[18,19] of one of the two esters prior to decarboxylation of the α-alkoxycarbonyl acid only resulted in a complex mixture of highly polar, water-soluble materials.

We have some comments on the preferential formation (2.7:1) of desired (2*S*,1′*S*)-**2a**. The intermediate from dealkoxycarbonylation was an enolate, and two possible stable conformers, **A** and **B**, which are postulated by means of the Newman projection, are shown in Fig. 1. Protonation from the less sterically hindered sides, the *re*-face on **A** and the *si*-face on **B**, would provide (2*S*,1′*S*)-**2a** and (2*R*,1′*S*)-**2a**, respectively. Gauche repulsion between the phenyl group in the α-methylbenzyl chiral auxiliary and the α-oriented hydrogen atom at the azetidine C-4 position would reduce the stability of conformer **B**, and protonation would then preferentially proceed on conformer **A**. As a high

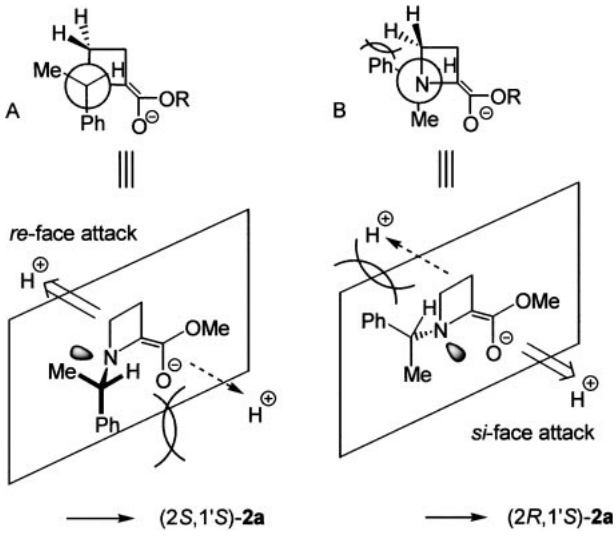
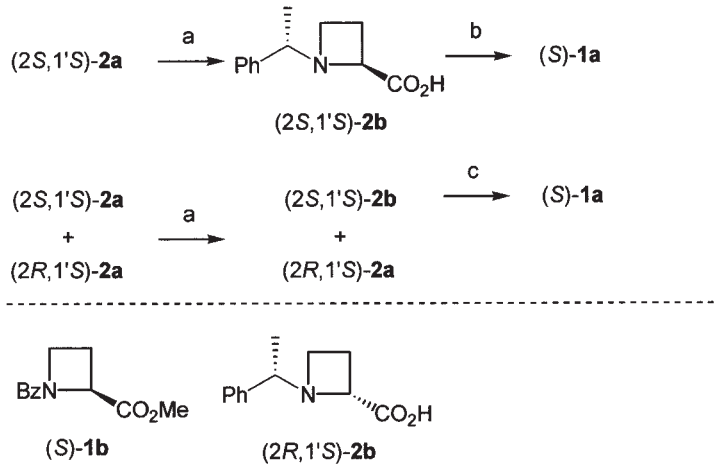


Fig. 1.



Scheme 2. Reagents: a) Chirazyme L-2, H_2O, quant.; b) H_2, Pd-C, EtOH–H_2O, 75%.; c) same as b, 91% from the mixture of (2*S*,1′*S*)- and (2*R*,1′*S*)-**2a**.

temperature was required for the dealkoxycarbonylation (160 °C), the difference between the foregoing two pathways is rather small (2.7:1). However, when the same enolate was independently generated[20] by the action of LDA in THF on product **2a**, protonation with an aqueous ammonium chloride solution proceeded in a 6:1 ratio at 0 °C. This ratio was further improved to 6.7:1 under a lower (−78 °C) temperature. In turn, by treating with sodium methoxide or DBU in refluxing methanol, no interconversion between the two dealkoxycarbonylation products, (2*S*,1′*S*)-**2a** and (2*R*,1′*S*)-**2a**, occurred. This means that re-abstraction of the α-proton from the resulting products in the presence of a proton source was not feasible, even under basic conditions at high temperature. The combined results suggest that the ratio of the two products was determined under kinetic control, and not under thermodynamic control, as Krapcho dealkoxycarbonylation proceeded under nearly neutral conditions.

Based on the foregoing comments, the property of the proton source would be important under non-aqueous conditions. Among such bulky and hydrophobic candidates as BHT, 2,4,6-trichlorophenol and Amberlyst-15, only BHT worked well as was shown before. The similar phenol, (*S*)-binaphthol, also promoted the reaction (23%), but the existing chirality of the proton source[21] did not affect the product ratio (2.8:1).

Product (2*S*,1′*S*)-**2a** isolated by chromatography was hydrolyzed with *Candida antarctica* lipase B (Chirazyme L-2)[8] under neutral conditions to provide acid (2*S*,1′*S*)-**2b** in a 75% yield. Finally, catalytic hydrogenolysis of the chiral auxiliary enabled (*S*)-azetidine-2-carboxylic acid (**1a**) to be obtained in quantitative yield (Scheme 2). Its high ee (>99.9%) was confirmed by an HPLC analysis of corresponding ester (*S*)-**1b**, to which a benzoyl group had been introduced on the free amine. Lipase-catalyzed hydrolysis also worked in a diastereoselective manner on the 2.7:1 mixture of (2*S*,1′*S*)- and (2*R*,1′*S*)-**2a** to give (2*S*,1′*S*)-**2b** with some contamination by (2*R*,1′*S*)-**2b** (*ca.* 3%). This was eventually converted to (*S*)-**1** (91% from the mixture of **2a**) with >99.9% ee, after recrystallization at the final stage.[10] As already stated, undesired (2*R*,1′*S*)-**2a** was epimerized into a 6.7:1 mixture of (2*S*,1′*S*)- and (2*R*,1′*S*)-**2a** in an 82% yield.

In conclusion, a new and efficient route to (*S*)-azetidine-2-carboxylic acid (**1a**) *via* the malonic ester intermediates, **3a** and **4a**, was established.

Experimental

All boiling point (bp) and melting point (mp) data are uncorrected. IR spectra were measured as films for oil and as KBr disks for solids with a Jasco FT/IR-410 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with a Jeol JNM GX-270 or GX-400 spectrometer. High-resolution mass spectra were recorded by a Jeol JMS-700 spectrometer. HPLC data were recorded by an SSC-5410 liquid chromatograph (Senshu Scientific Co., Ltd.). Optical rotation data were recorded on a Jasco DIP 360 polarimeter. Silica gel 60 (spherical, 100–210 μm) from Kanto Chemical Co. was used for column chromatography. Preparative TLC was performed with E. Merck silica gel 60 F_{256} plates (0.5 mm thickness, No. 5744).

*Dimethyl (1′S)-(1′-methyl)benzylaminomalonate (**4a**).* Known crude diester **4b**[14] (1.30 g, 4.68 mmol) was dissolved in MeOH (65 ml), and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU, 70 μl, 0.47 mmol) was added dropwise at room temperature, before the mixture was stirred under reflux for 3 h. The reaction mixture was concentrated *in vacuo*. The residue was poured into a phosphate buffer (0.1 M, pH 8.0, 100 ml), and the mixture was extracted three times with ethyl acetate. The extracts were combined, washed with brine, dried with anhydrous sodium sulfate, and concentrated *in vacuo* to give **4a** (1.01 g, 86%, from diethyl bromomal-

onate) as a yellow oil. This was employed for the next step without further purification.

A small portion was purified by preparative TLC [developed with hexane–EtOAc (4:1) with a trace of triethylamine]. $[\alpha]^{22}_{D}$ −64.5° (*c* 1.1, EtOH); IR ν_{max} cm^{-1}: 3338, 1757, 1739, 1225, 1153; NMR δ_H (270 MHz, $CDCl_3$): 1.33 (3H, d, $J = 6.6$ Hz), 2.18 (1H, br), 3.62 (3H, s), 3.69 (3H, s), 3.71 (1H, q, $J = 6.6$ Hz), 3.87 (1H, s), 7.15–7.29 (5H, m); NMR δ_C (67.5 MHz, $CDCl_3$): 24.46, 52.67 (×2), 56.41, 62.54, 126.70 (×2), 127.24, 128.36 (×2), 143.47, 168.42, 169.33. The IR and NMR spectra were in good accordance with those of the racemate reported previously.[14)]

*Dimethyl (1′S)-1-(1′-methyl)benzylazetidine-2,2-dicarboxylate (**3a**).* Cesium carbonate (134 mg, 0.41 mmol) was added to a solution of **4a** (45.7 mg, 0.18 mmol) in DMF (0.7 ml), and the mixture was stirred for 10 min at room temperature. 1,2-Dibromoethane (23 µl, 0.267 mmol) was added, and the reaction mixture was stirred for 14 h at room temperature and then for 22 h at 40 °C. The reaction mixture was next diluted with a phosphate buffer (0.1 M, pH 7.5) and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give a crude product. Purification by preparative TLC [developed with hexane–EtOAc (4:1) with a trace of triethylamine] afforded **3a** (49.9 mg, 99%) as a yellow oil. Bulb-to-bulb distillation gave an analytical sample. Bp 160–180 °C at 0.15 mmHg (bath temp.); $[\alpha]^{21}_{D}$ −112.5° (*c* 1.0, EtOH); IR ν_{max} cm^{-1}: 1763, 1734, 1255, 1103; NMR δ_H (270 MHz, $CDCl_3$): 1.26 (3H, d, $J = 6.3$ Hz), 2.41–2.56 (2H, m), 2.98 (1H, ddd, $J = 3.0$, 6.5, 7.9 Hz), 3.12 (1H, ddd, $J = 7.4$, 7.4, 7.9 Hz), 3.70 (1H, q, $J = 6.3$ Hz), 3.73 (3H, s), 3.76 (3H, s), 7.20–7.33 (5H, m); NMR δ_C (67.5 MHz, $CDCl_3$): 21.83, 25.87, 48.40, 52.10, 52.17, 61.86, 72.99, 126.98, 127.47 (×2), 127.92 (×2), 142.67, 169.40, 170.80. *Anal.* Found: C, 64.81; H, 6.94; N, 4.97%. Calcd. for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05%.

Eight-membered by-product resulting from the double *C*- and *N*-alkylation (**5**): NMR δ_H (400 MHz, $CDCl_3$): 1.37 (6H, d, $J = 6.8$ Hz), 2.27 (2H, ddd, $J = 5.8$, 9.8, 15.1 Hz), 2.56 (2H, ddd, $J = 5.9$, 9.6, 15.1 Hz), 3.12 (2H, ddd, $J = 5.8$, 9.6, 9.9 Hz), 3.23 (2H, ddd, $J = 5.9$, 9.8, 9.9 Hz), 3.46 (6H, s), 3.63 (1H, q, $J = 6.8$ Hz), 3.74 (6H, s), 7.20–7.35 (10H, m); FAB-HRMS ($M^+ + Na^+$, *m/z*): 577.2502; calcd. for $C_{30}H_{38}N_2O_8Na$, 577.2526.

*Diethyl (1′S)-1-(1′-methyl)benzylazetidine-2,2-dicarboxylate (**3b**).* Cesium carbonate (2.11 g, 6.47 mmol) was added to a solution of **4b** (408 mg, 1.42 mmol) in DMF (5.0 ml), and the mixture was stirred for 10 min at room temperature. 1,2-Dibromoethane (0.25 ml, 2.90 mmol) and tetra-*n*-butylammonium iodide (TBAI, 55 mg, 0.15 mmol) were then added, and the reaction mixture was stirred for 15.5 h at room temperature. Another portion of TBAI (479 mg, 1.30 mmol) was added, and the mixture was heated to 40 °C for 7 h. A similar workup and purification to that described for **3a** gave **3b** as a pale yellow oil (417 mg, 95%). IR ν_{max} cm^{-1}: 1761, 1732, 1252, 1101; NMR δ_H (270 MHz, $CDCl_3$): 1.26 (6H, t, $J = 7.1$ Hz), 1.28 (3H, d, $J = 6.4$ Hz), 2.39–2.55 (2H, m), 3.00 (1H, ddd, $J = 3.0$, 6.5, 7.9 Hz), 3.14 (1H, ddd, $J = 7.4$, 7.4, 7.9 Hz), 3.73 (1H, q, $J = 6.4$ Hz), 4.06–4.39 (4H, m), 7.14–7.34 (5H, m).

When the reaction was quenched prior to cyclization under a low dose of TBAI, the *C*-alkylated form (**6**) was isolated as an intermediate: NMR δ_H (270 MHz, $CDCl_3$): 1.10 (3H, t, $J = 7.1$ Hz), 1.22 (3H, t, $J = 7.1$ Hz), 1.34 (3H, d, $J = 6.8$ Hz), 2.14–2.26 (1H, m), 2.43–2.54 (1H, m), 3.05–3.17 (2H, m), 3.73 (1H, q, $J = 6.8$ Hz), 7.17–7.26 (5H, m).

Another by-product with an imine structure (**7**): NMR δ_H (270 MHz, $CDCl_3$): 1.47 (3H, d, $J = 6.4$ Hz), 1.23 (3H, t, $J = 7.3$ Hz), 1.24 (3H, t, $J = 7.3$ Hz), 4.24 (2H, q, $J = 7.3$ Hz), 4.27 (2H, q, $J = 7.3$ Hz), 4.58 (1H, q, $J = 6.6$ Hz), 7.10–7.28 (5H, m).

*Methyl (2S,1′S)-1-(1′-methyl)benzylazetidine-2-carboxylate (**2a**) and methyl (2R,1′S)-1-(1′-methyl)benzylazetidine-2-carboxylate (**2a**).* Lithium chloride (23 mg, 0.54 mmol), BHT (120 mg, 0.55 mmol) and molecular sieves 3A (150 mg) were added to a solution of **3a** (47 mg, 0.17 mmol) in DMSO (0.25 ml), and the mixture was stirred for 1 h at room temperature. The reaction mixture was then heated at 140 °C for a further 2 h and, after cooling, was diluted with a phosphate buffer (pH 6.0, 0.1 M) and extracted three times with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by preparative thin-layer column chromatography [developed with hexane–EtOAc (2:1) with a trace of triethylamine] to afford a mixture of (2*S*,1′*S*)-**2a** and (2*R*,1′*S*)-**2a** (total 27.2 mg, 78%) as a pale yellow oil. The diastereomeric ratio (2.7:1) was estimated from the ^{1}H-NMR spectra as shown next. These two components were further purified by the same preparative TLC and subsequent bulb-to-bulb distillation.

(2*S*,1′*S*)-**2a** (Rf 0.40): Bp 150–170 °C at 0.18 mmHg (bath temp.); $[\alpha]^{22}_{D}$ −118.7° (*c* 1.1, EtOH); IR ν_{max} cm^{-1}: 1753, 1730, 1196, 1174; NMR δ_H (270 MHz, $CDCl_3$): 1.21 (3H, d, $J = 6.6$ Hz), 2.16 (1H, dddd, $J = 2.8$, 8.0, 8.4, 8.6 Hz), 2.25 (1H, dddd, $J = 8.0$, 8.0, 8.4, 8.6 Hz), 2.78 (1H, ddd, $J = 8.0$, 8.0, 8.0 Hz), 3.09 (1H, ddd, $J = 2.8$, 8.0, 8.0 Hz), 3.43 (1H, q, $J = 6.6$ Hz), 3.74 (3H, s), 3.74 (1H, dd, $J = 8.4$, 8.4 Hz), 7.18–7.33 (5H, m); NMR δ_C (67.5 MHz, $CDCl_3$): 20.85, 20.99, 49.62, 51.87, 63.89, 67.21, 127.04, 127.30 (×2), 128.14 (×2), 142.27, 173.44. *Anal.* Found: C, 71.26; H, 7.74; N, 6.35%. Calcd. for $C_{13}H_{17}NO_2$: C, 71.21; H, 7.81; N, 6.39%. The ^{1}H-NMR spectrum was identical with that reported previously.[9)]

(2*R*,1′*S*)-**2a** (Rf 0.15): Bp 150–170 °C at 0.18 mmHg (bath temp.); $[\alpha]^{19}_{D}$ +48.9° (*c* 1.1, EtOH); IR ν_{max}

cm^{-1}: 1745, 1196, 1173; NMR δ_H (270 MHz, $CDCl_3$): 1.27 (3H, d, $J = 6.6$ Hz), 2.12 (1H, dddd, $J = 3.0$, 8.1, 8.8, 9.2 Hz), 2.28 (1H, dddd, $J = 9.2$, 9.2, 9.2, 9.2 Hz), 2.99 (1H, ddd, $J = 8.1$, 8.1, 9.2 Hz), 3.31 (3H, s), 3.34 (1H, q, $J = 6.6$ Hz), 3.56 (1H, ddd, $J = 3.0$, 8.1, 9.2 Hz), 3.56 (1H, dd, $J = 8.8$, 9.2 Hz), 7.17–7.27 (5H, m); NMR δ_C (67.5 MHz, $CDCl_3$): 19.74, 20.85, 50.74, 51.45, 64.40, 68.09, 127.31, 127.79 (×2), 127.86 (×2), 141.42, 172.38. *Anal.* Found: C, 71.13; H, 7.78; N, 6.30%. Calcd. for $C_{13}H_{17}NO_2$: C, 71.21; H, 7.81; N, 6.39%. The NMR spectrum was identical with that reported previously.[9)]

*Isomerization of (2R,1′S)-**2a**.* An LDA solution was prepared by adding *n*-butyllithium (2.7 M in hexane, 150 µl, 0.41 mmol) to a solution of diisopropylamine (117 µl, 0.83 mmol) in THF (0.5 ml) at 0 °C. After cooling to −78 °C, a solution of (2*R*,1′*S*)-**2a** (45.4 mg, 0.21 mmol) in THF (0.5 ml) was added, and the mixture was stirred for 2 h. The reaction was quenched by adding MeOH at −78 °C, and the conventional workup and purification already described to give a mixture of (2*S*,1′*S*)- and (2*R*,1′*S*)-**2a** (37.1 mg, 82%, 6.7:1).

*(2S,1′S)-1-(1′-Methyl)benzylazetidine-2-carboxylic acid (**2b**) from pure (2S,1′S)-**2a**.* Chirazyme L-2 (c–f, 40 mg) was added to (2*S*,1′*S*)-**2a** (36.4 mg, 0.12 mmol) in water (1.5 ml), which had been ultrasonically emulsified in advance. The mixture was stirred for 2 h at 40 °C. The reaction mixture was then filtered, and the filtrate was extracted with diethyl ether. The aqueous layer was concentrated *in vacuo* to give (2*S*,1′*S*)-**2b** (38.7 mg, quant) as a pale yellow amorphous solid. $[\alpha]^{20}_D$ −48.9° (*c* 1.0, EtOH); IR ν_{max} cm^{-1}: 3411, 1626, 1404; NMR δ_H (270 MHz, $CDCl_3$): 1.69 (3H, d, $J = 6.9$ Hz), 2.40 (1H, dddd, $J = 3.6$, 9.3, 9.6, 9.9 Hz), 2.66 (1H, dddd, $J = 9.3$, 9.3, 9.6, 9.9 Hz), 3.34 (1H, ddd, $J = 9.3$, 9.3, 9.3 Hz), 3.89 (1H, ddd, $J = 3.6$, 9.3, 9.3 Hz), 4.25 (1H, q, $J = 6.9$ Hz), 4.42 (1H, dd, $J = 9.6$, 9.6 Hz), 7.34–7.52 (5H, m); NMR δ_C (67.5 MHz, $CDCl_3$): 18.57, 21.04, 47.43, 65.12, 66.86, 121.94, 129.08 (×2), 129.13 (×2), 135.38, 170.84; FAB-HRMS ($M^+ + Na^+$, *m/z*): 228.0974; calcd. for $C_{12}H_{15}NO_2Na$, 228.1001. The ^{1}H-NMR spectrum was identical with that reported previously.[9)]

*(S)-Azetidine-2-carboxylic acid (**1a**).* *N*-Protected amino acid **2b** (112 mg, 0.55 mmol) was dissolved in a mixture of water and ethanol (1:1, 2.5 ml). Palladium on carbon (10%, 120 mg) was added, and the mixture was vigorously stirred under hydrogen for 46 h. The resulting suspension was filtered, and the solid residue was washed with water. The solvent was evaporated *in vacuo*, and the solid residue (41.8 mg, 75%) was ultrasonically suspended in methanol (0.5 ml), before the mixture was left to stand overnight at −20 °C. The solid was recovered and washed with methanol to afford **1a** (30.0 mg, 54%) as colorless fine needles. Mp above 200 °C (dec.) [lit.[7)] 210 °C]; $[\alpha]^{21}_D$ −121.7° (*c* 0.5, H_2O) [lit.[7)] $[\alpha]^{21}_D$ −120° (*c* 1, H_2O); IR ν_{max} cm^{-1}: 2976, 2688, 2517, 1637, 1593, 1410, 1306, 1288, 1254; NMR δ_H (270 MHz, D_2O): 2.28–2.44 (1H, m), 2.54–2.69 (1H, m), 3.75 (1H, ddd, $J = 6.0$, 10.0, 10.0 Hz), 3.91 (1H, ddd, $J = 8.8$, 10.0, 10.0 Hz), 4.62 (1H, dd, $J = 8.3$, 10.2 Hz). All the physical and spectral properties were coincident with those of a commercially available authentic sample (Sigma, A0760).

A small portion of (*S*)-**1a** was converted to corresponding *N*-benzoyl methyl ester **1b** by successively treating with benzoyl chloride in an aqueous sodium hydroxide solution and then with diazomethane in diethyl ether in the conventional manner to determine its enantiomeric excess (ee). HPLC: column, Daicel Chiralcel OD, 0.46 cm × 25 cm; eluent, hexane-isopropyl alcohol = 7/1; flow rate, 0.5 ml/min; Rt 63.4 min [for (*S*)-**1b** as a single peak]. Rt 56.7 min for (*R*)-**1b**.

*(S)-Azetidine-2-carboxylic acid (**1a**) from a mixture of (2S,1′S)- and (2R,1′S)-**2a**.* Chirazyme L-2 (c–f, 117 mg) was added to a mixture of **2a** [115 mg, total 0.52 mmol, (2*S*,1′*S*)/(2*R*,1′*S*) = 2.7:1 by NMR] in water (2.5 ml), which had been ultrasonically emulsified in advance. The mixture was stirred for 10 h at 17 °C. The reaction mixture was filtered, and the resulting filtrate was extracted several times with toluene to remove the unreacted materials. Concentration of the combined organic layer and purification by preparative thin-layer column chromatography yielded **2a** (23.4 mg, 20% recovery). Its NMR spectrum showed that the unreacted material was the pure (2*R*,1′*S*)-isomer.

The aqueous layer was concentrated *in vacuo* to give (2*S*,1′*S*)-**2b** (108 mg, quant.) as a pale yellow amorphous solid. This contained (2*R*,1′*S*)-**2b** (*ca.* 3%), judging from its NMR spectrum with the signal at δ_H 3.54 (1H, ddd, $J = 8.9$, 8.9, 8.9 Hz). This was hydrogenated in a similar manner to that just described to give crude **1a** (51.1 mg, quant.). Preferential crystallization from methanol (0.5 ml) afforded (*S*)-**1a** (35.2 mg, 91% from the mixture of **2a**), whose ee was >99.9% after conversion to **1b** as already described. The acid (*S*)-**1a** recovered from the mother liquor of the final crystallization showed 87.5% ee.

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References

1) Ripperger, H., and Schreiber, K., The "normalizing factor" for the tomato mutant chloronerva. XXV (1). Synthesis of 2-epinicotianamine and nicotianamine. *J. Prakt. Chem.*, **329**, 231–234 (1987).
2) Shioiri, T., Irako, N., Sakakibara, S., and Hamada, Y., A new efficient synthesis of nicotianamine and 2′-deoxymugineic acid. *Heterocycles*, **44**, 519–530 (1997).
3) Miyakoshi, K., Oshita, J., and Kitahara, T., Expeditious synthesis of nicotianamine and 2′-deoxymugineic acid. *Tetrahedron*, **57**, 3355–3360 (2001).
4) Brenner, E., Baldwin, R. M., and Tamagnan, G., Synthesis of a new precursor to the nicotinic receptor tracer 5-IA-85380 precursor using trimethylsilyl iodide as deblocking agent. *Tetrahedron Lett.*, **45**, 3607–3610 (2004).
5) Kondo, T., and Ueyama, N., process for producing optically active azetidine-2-carboxylic acid. PCT Int. Appl. WO 2003014081 (2003); CA 138: 170068.
6) Takano, N., Ushio, H., and Seko, S., Preparation of optically active *N*-substituted azetidine-2-carboxylic acids. Jpn. Kokai Tokkyo Koho JP 10120648 (1998); CA 129: 41069.
7) Miyoshi, M., Sugano, H., Fujii, T., Ishihara, T., and Yoneda, N., A novel synthesis of optically active azetidine-2-carboxylic acids. *Chem. Lett.*, 5–6 (1973).
8) Rodebaugh, R. M., and Cromwell, N. H., Resolution of DL-azetidine-2-carboxylic acid. *J. Heterocycl. Chem.*, **6**, 993–994 (1969).
9) Ushio, H., Takano, N., Honda, Y., Seko, S., and Hazama, M., Preparation of optically active *N*-alkylbenzylazetidine-2-carboxylic acids. Eur. Pat. Appl. EP 827954 (1998); CA 128: 230235.
10) Kudo, J., Hazama, M., and Hirata, N., Process for producing optically active azetidine-2-carboxylic acid derivative. Eur. Pat. Appl. EP 855446 (1998); CA 129: 160706.
11) Starmans, W. A. J., Doppen, R. G., Thijs, L., and Zwanenburg, B., Enzymatic resolution of methyl *N*-alkyl-azetidine-2-carboxylates by *Candida antarctica* lipase-mediated ammoniolysis. *Tetrahedron: Asymmetry*, **9**, 429–435 (1998).
12) Krapcho, P. A., Synthetic applications of dealkoxycarbonylations of malonate esters, β-keto esters, α-cyano esters and related compounds in dipolar aprotic media—part 1. *Synthesis*, 805–822 (1982).
13) Juaristi, E., León-Romo, J. L., Reyes, A., and Escalante, J., Recent applications of α-phenylethylamine (α-PEA) in the preparation of enantiopure compounds. Part 3: α-PEA as chiral auxiliary. Part 4: α-PEA as chiral reagent in the stereodifferentiation of prochiral substrates. *Tetrahedron: Asymmetry*, **10**, 2441–2495 (1999).
14) Sugiyama, S., Watanabe, S., Inoue, T., Kurihra, R., Itou, T., and Ishii, K., A novel dynamic kinetic resolution accompanied by intramolecular transesterification: asymmetric synthesis of 4-hydroxymethyl-2-oxazolidinone from serinol derivatives. *Tetrahedron*, **59**, 3417–3425 (2003).
15) Husinec, S., Juranic, I., Liobera, A., and Porter, A. E. A., Bis(methoxycarbonyl)carbene insertion into N–H bonds: a facile route to *N*-substituted aminomalonic esters. *Synthesis*, 721–723 (1988).
16) Simig, G., Doleschall, G., Hornyák, G., Fetter, J., Lempert, K., Nyitrai, J., Huszthy, P., and Gizur, T., Simple and condensed β-lactams-I. The application of diketene in β-lactam synthesis. The synthesis and functional group manipulations of diethyl 3-acetyl-4-oxoacetidine-2,2-dicarboxylate. *Tetrahedron*, **40**, 479–484 (1984).
17) Qian, X., Zheng, B., Burke, B., Saindane, M. T., and Kronenthal, D. R., A Stereoselective synthesis of BMS-262084, an azetidinone-based tryptase inhibitor. *J. Org. Chem.*, **67**, 3595–3600 (2002).
18) Toshima, H., and Ichihara, A., Practical stereoselective syntheses of all four stereoisomers of coronamic acid (2-ethyl-1-aminocyclopropane-1-carboxylic acid). *Biosci. Biotechnol. Biochem.*, **59**, 497–500 (1995).
19) Niwayama, S., Highly efficient selective monohydrolysis of symmetric diesters. *J. Org. Chem.*, **65**, 5834–5836 (2000).
20) Wasserman, H. H., Lipshutz, B. H., Tremper, A. W., and Wu, J. S., Preparation of β-lactams from azetidine-2-carboxylic acids and esters. *J. Org. Chem.*, **46**, 2991–2999 (1981).
21) Duhamel, L., Duhamel, P., and Plaquevent, J.-C., Enantioselective protonations: fundamental insights and new concepts. *Tetrahedron: Asymmetry*, **15**, 3653–3691 (2004).