Preparation of Optically Active (2*RS*,3*SR*)-2-Amino-3-hydroxy-3-phenylpropanoic Acid (*threo*-β-Phenylserine) *via* Optical Resolutions by Replacing and Preferential Crystallization

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To obtain optically active *threo*-2-amino-3-hydroxy-3-phenylpropanoic acid (1) *via* optical resolutions by replacing and preferential crystallization, the racemic structure of (2*RS*,3*SR*)-1 hydrochloride [(2*RS*,3*SR*)-1·HCl] was examined based on the melting point, solubility, and infrared spectrum. (2*RS*,3*SR*)-1·HCl was indicated to exist as a conglomerate at room temperature, although it forms a racemic compound at the melting point. When, in optical resolution by replacing crystallization, L-phenylalanine methyl ester hydrochloride (L-2) was used as the optically active co-solute, (2*R*,3*S*)-1·HCl was preferentially crystallized from the supersaturated racemic solution; the use of D-2 as the co-solute afforded (2*S*,3*R*)-1·HCl with an optical purity of 95%. In addition, optical resolution by preferential crystallization was successfully achieved to give successively (2*R*,3*S*)- and (2*S*,3*R*)-1·HCl with optical purities of 90—92%. The (2*R*,3*S*)- and (2*S*,3*R*)-1·HCl purified by recrystallization from 1-propanol were treated with triethylamine in methanol to give optically pure (2*R*,3*S*)- and (2*S*,3*R*)-1.

Key words   *threo*-β-phenylserine; optical resolution; conglomerate; replacing crystallization; preferential crystallization

2-Amino-3-hydroxy-3-phenylpropanoic acid (**1**; β-phenylserine) that exists as four stereoisomers is a physiologically important α-amino acid.[1—3] (2*RS*,3*SR*)-**1**, DL-*threo*-β-phenylserine, is predominantly given by condensation of glycine with benzaldehyde under aqueous alkaline conditions.[4—6] However, (2*R*,3*S*)- and (2*S*,3*R*)-**1** enantiomers, non-proteinogenic α-amino acids, are difficult to produce commercially in large quantities. Therefore, *N*-benzoyl (2*RS*,3*SR*)-**1** was optically resolved by separation of diastereoisomeric salts with (1*S*,2*S*)- and (1*R*,2*R*)-2-amino-1-(4-nitrophenyl)-1,3-propanediol.[6] In addition, benzylammonium salt of *N*-benzoyl (2*RS*,3*SR*)-**1**, which was found to exist as a conglomerate, was subjected to optical resolution by preferential crystallization, with the aim of acquisition of (2*R*,3*S*)- and (2*S*,3*R*)-**1**.[6] However, hydrolysis of the *N*-benzoyl (2*R*,3*S*)- and (2*S*,3*R*)-**1** obtained gave (2*R*,3*S*)- and (2*S*,3*R*)-**1** in not a very high yields.[6] Therefore, we attempted to obtain (2*R*,3*S*)- and (2*S*,3*R*)-**1** by more simple and efficient optical resolution.

Optical resolutions by preferential and replacing crystallization are simple and useful procedures for separation of enantiomers from racemates. The optical resolution by preferential crystallization is achieved by providing a small amount of one enantiomer as seed crystals in a supersaturated racemic solution. On the other hand, the optical resolution by replacing crystallization is achieved by allowing an optically active co-solute to coexist in a supersaturated racemic solution without formation of diastereoisomers with the co-solute. The optical resolution by replacing crystallization does not require the enantiomer as seed crystals. However, although racemates exist in the form of racemic compounds, racemic solid solutions, and conglomerates, only conglomerates, which are defined as mechanical mixtures of crystals of both enantiomers and are much less common than racemic compounds, can be optically resolved by preferential and replacing crystallization. (2*RS*,3*SR*)-**1** was concluded to form a racemic compound which does not be optically resolved by preferential and replacing crystallization.[6] There-fore, we have been screening for a simple derivative, such as (2*RS*,3*SR*)-**1** hydrochloride [(2*RS*,3*SR*)-**1**·HCl], that exist as conglomerate and attempted to optically resolve the found conglomerate by preferential and replacing crystallization (Chart 1).

Results and Discussion

We examined the racemic structure of (2*RS*,3*SR*)-**1**·HCl by comparing the melting point, solubility, and IR spectrum of the (2*RS*,3*SR*)-**1**·HCl with those of (2*R*,3*S*)-**1**·HCl.[6,7] (2*RS*,3*SR*)-**1**·HCl had a lower melting point (144—145 °C) than (2*R*,3*S*)-**1**·HCl (150—151 °C). Although conglomerates are known to have such melting point characteristics,[6,7] the melting-point binary-phase diagram suggested that (2*RS*,3*SR*)-**1**·HCl forms a racemic compound, as shown in Fig. 1. On the other hand, the IR spectrum of (2*RS*,3*SR*)-**1**·HCl was identical to that of (2*R*,3*S*)-**1**·HCl. In addition, (2*RS*,3*SR*)-**1**·HCl was more soluble than the (2*R*,3*S*)-**1**·HCl, as summarized in Table 1. The solubility ternary-phase diagram also showed that (2*RS*,3*SR*)-**1**·HCl is expected to be a conglomerate, as shown in Fig. 2. The above results suggested that (2*RS*,3*SR*)-**1**·HCl exists as a conglomerate at room temperature and forms a racemic compound at the
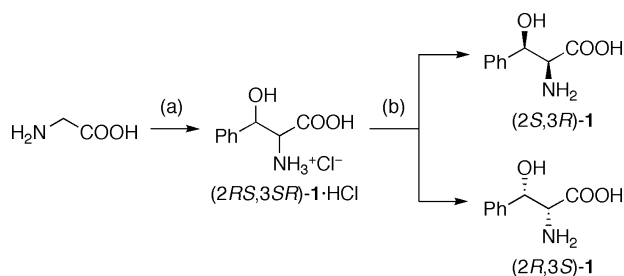
(a) i) benzaldehyde (2 eq), OH⁻, ii) recrystallization from water, iii) hydrochloric acid; (b) i) optical resolution by preferential and replacing crystallization, ii) recrystallization from 1-propanol, iii) triethylamine (pH 6), methanol.

Chart 1.   Preparation of Optically Active *threo*-2-Amino-3-hydroxy-3-phenylpropanoic Acid (**1**)

Table 1. Solubilities of (2RS,3SR)- and (2R,3S)-2-Amino-3-hydroxy-3-phenylpropanoic Acid Hydrochlorides[a]

1·HCl[b]	L- or D-2[c] as an optically active co-solute (mmol)	Solubility [g (100 cm³ of 1-propanol)⁻¹]		
(2RS,3SR)-1·HCl	—[d]	12.12	{ (2R,3S)-1·HCl 6.06	(2S,3R)-1·HCl 6.06 }
(2R,3S)-1·HCl	—[d]	6.13		
(2RS,3SR)-1·HCl	L-2 45.0	15.64	{ (2R,3S)-1·HCl 7.50	(2S,3R)-1·HCl 8.14 }
(2R,3S)-1·HCl	L-2 45.0	7.39		
(2R,3S)-1·HCl	D-2 45.0	7.97		

a) Temperature, 10 °C. b) 1·HCl: *threo*-2-Amino-3-hydroxy-3-phenylpropanoic acid hydrochloride. c) 2: Phenylalanine methyl ester hydrochloride. d) None.
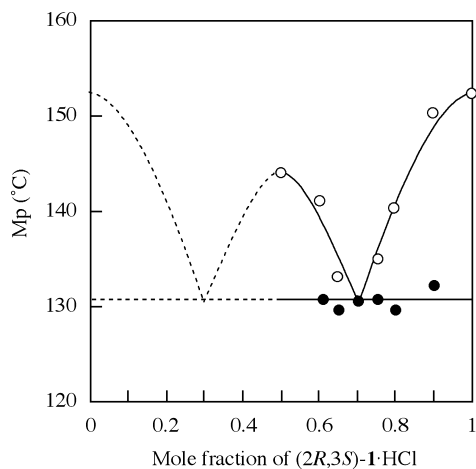

Fig. 1. Melting-Point Binary-Phase Diagram of *threo*-2-Amino-3-hydroxy-3-phenylpropanoic Acid Hydrochloride (1·HCl)
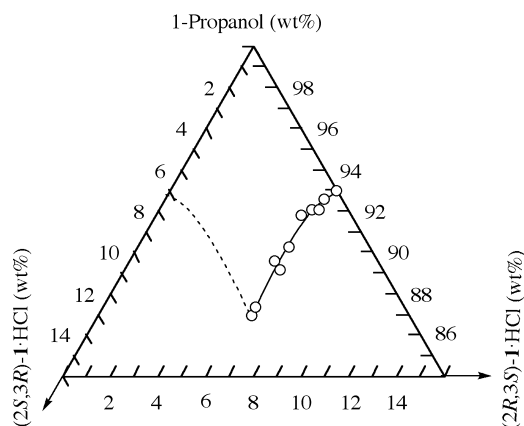
●: beginning of melting. ○: end of melting.

Fig. 2. Solubility Ternary-Phase Diagram of *threo*-2-Amino-3-hydroxy-3-phenylpropanoic Acid Hydrochloride (1·HCl)
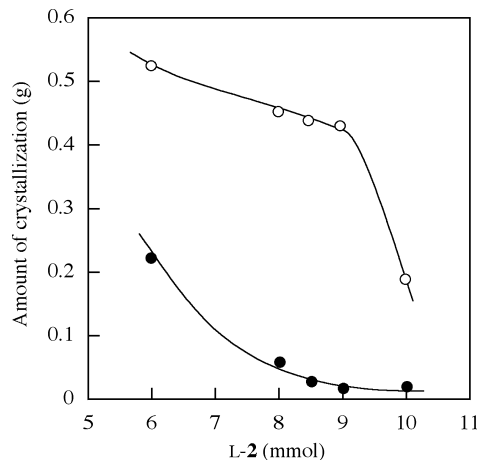
Conditions: temperature, 10 °C; solvent, 1-propanol.

Fig. 3. Relationship between the Amount of Crystallization and Amount of L-Phenylalanine Methyl Ester Hydrochloride (L-2) as Optically Active Co-Solute in Optical Resolution of (2RS,3SR)-2-Amino-3-hydroxy-3-phenylpropanoic Acid Hydrochloride [(2RS,3SR)-1·HCl]

Conditions: (2RS,3SR)-1·HCl, 4.527 g (20.8 mmol); solvent, 20 ml of 1-propanol; stirring time, 60 min; temperature, 10 °C. Amount of crystallization: ○ (2R,3S)-1·HCl; ● (2S,3R)-1·HCl.

melting point.

Based on the above results, the optical resolution by replacing crystallization of (2RS,3SR)-1·HCl was first attempted using various L-amino acid hydrochlorides and their methyl esters as optically active co-solute. Of these co-solutes, only L-phenylalanine methyl ester hydrochloride (L-2) gave a good result. When L-2 was present in the supersaturated solution of (2RS,3SR)-1·HCl, the solubilities summarized in Table 1 suggest that (2R,3S)-1·HCl is preferentially crystallized from the racemic solution. The optical resolution was attempted by stirring a mixture containing 20.8 mmol (4.527 g) of (2RS,3SR)-1·HCl and 6.00—10.0 mmol of L-2 in 20 ml of 1-propanol for 60 min at 10 °C. The yield of enantiomer [YE (g)] and amounts of crystallization [$AC_{(2R,3S)}$ (g) and $AC_{(2S,3R)}$ (g)] were calculated from

$$YE\,(g) = yield\,(g) \times OP\,(\%)/100$$

$$AC_{(2S,3R)}\,(g) = (1/2)[yield\,(g) - YE\,(g)]$$

$$AC_{(2R,3S)}\,(g) = YE\,(g) + AC_{(2S,3R)}\,(g)$$

where OP (%) is the optical purity of the obtained (2R,3S)-1·HCl and was calculated based on the specific rotation of optically pure (2R,3S)-1·HCl: $[\alpha]_D^{20}$ +34.4° (c=1.00, methanol). The results are shown in Fig. 3.

The ¹H-NMR spectra revealed that a trace of L-2 (below 2.5 mol%) was present in the 1·HCl crystallized; the L-2 content was determined by the intensity ratio of the methine proton signal (4.31 ppm) at the C-2 position of 1·HCl and methyl proton signal (3.84 ppm) of L-2 in the ¹H-NMR spectrum. However, the specific rotations of the 1·HCl obtained are little affected by the presence of L-2 because of such low content and the specific rotation of L-2 ($[\alpha]_D^{20}$ +15.9° (c=1.00, methanol)) which is half the specific rotation of (2R,3S)-1·HCl.

In the presence of 6.00—9.00 mmol of L-**2**, the amount of crystallization of (2R,3S)-**1**·HCl slightly decreased with increasing amount of L-**2**, whereas that of (2S,3R)-**1**·HCl rapidly decreased. When a larger amount (10.0 mmol) of L-**2** was present in the racemic solution, the amount of crystallization of (2R,3S)-**1**·HCl was rapidly decreased, and (2S,3R)-**1**·HCl was hardly crystallized. In addition, (2R,3S)-**1**·HCl and (2S,3R)-**1**·HCl were not crystallized in the presence of 11.0 mmol of L-**2** by stirring for 60 min. Therefore, (2R,3S)-**1**·HCl with an optical purity of 92% was obtained in a degree of resolution [DR (%)] of 54% in the presence of 9.00 mmol of L-**2**: DR (%) was calculated from

$$DR\,(\%) = YE\,(g)/[(4.527\,(g)/2) - 1.501\,(g)]$$

where the solubility of (2R,3S)-**1**·HCl is 1.501 g in the presence of 9.00 mmol of L-**2** in 20 ml of 1-propanol at 10 °C. The degree of resolution [DR (%)] is defined as the yield (%) of (2R,3S)-**1**·HCl enantiomer, based on the supersaturating portion of (2R,3S)-**1**·HCl in a racemic solution, and indicates the efficiency of optical resolution; the supersaturating portion of (2R,3S)-**1**·HCl means the theoretical yield (g) of (2R,3S)-**1**·HCl.

When the supersaturated racemic solution was stirred for 40—170 min in the presence of 9.00 mmol of L-**2**, as shown in Fig. 4, (2R,3S)-**1**·HCl was rapidly crystallized during the first 40—80 min, and then the amount of crystallization of (2R,3S)-**1**·HCl for 80—170 min was approximately constant. On the other hand, (2S,3R)-**1**·HCl was hardly crystallized under these conditions, although the amount of crystallization of (2S,3R)-**1**·HCl tended to slightly increase with prolonged stirring. Therefore, (2R,3S)-**1**·HCl of an optical purity with 97% was obtained in the highest degree of resolution (65.5%) by stirring for 80 min: $[\alpha]_D^{20}$ +33.5° (c=1.00, methanol). After collecting (2R,3S)-**1**·HCl by filtration, (2S,3R)-**1**·HCl was not crystallized from the filtrate despite vigorous stirring in an ice bath. Therefore, D-**2** (9.00 mmol) was employed as the optically active co-solute to obtain (2S,3R)-**1**·HCl with an optical purity of 95% in a degree of resolution of 64% by stirring the solution of (2RS,3SR)-**1**·HCl (4.527 g) in 20 ml of 1-propanol for 80 min at 10 °C: $[\alpha]_D^{20}$ −32.7° (c=1.00, methanol).

The (2R,3S)- and (2S,3R)-**1**·HCl partially resolved were recrystallized from 1-propanol to give optically pure (2R,3S)- and (2S,3R)-**1**·HCl, as described in the Experimental section. For example, optically pure (2R,3S)-**1**·HCl (2.45 g) was obtained from (2R,3S)-**1**·HCl (5.00 g) with an optical purity of 52%, and optically pure (2S,3R)-**1**·HCl (2.00 g) from (2S,3R)-**1**·HCl (4.00 g) with an optical purity of 56%. The ^{1}H-NMR spectra indicated that the recrystallized (2R,3S)-

and (2S,3R)-**1**·HCl were free from L- and D-**2**, respectively.

Next, we attempted the successive optical resolution by preferential crystallization of (2RS,3SR)-**1**·HCl by employing (2R,3S)- and (2S,3R)-**1**·HCl as the seed crystals: (2R,3S)- and (2S,3R)-**1**·HCl were obtained by the optical resolution described above. The optical resolution was carried out by stirring a racemic solution with a degree of supersaturation of 140%, as the initial solution, for 30 min (Table 2). The degree of resolution [DR (%)] of the obtained (2R,3S)- and (2S,3R)-**1**·HCl were calculated from

$$DR\,(\%) = [(\text{yield}\,(g) \times OP\,(\%)/100) - 0.050] \times 100/ \\ [\text{operation amount of }(2R,3S)\text{- or }(2S,3R)\text{-}\mathbf{1}\cdot\text{HCl}\,(g) - 1.212]$$

where the operation amount is the amount of (2R,3S)- and (2S,3R)-**1**·HCl in the solution used in the optical resolution and those in runs 2—4 in Table 2 were calculated based on the yields and optical purities of the (2R,3S)- and (2S,3R)-**1**·HCl obtained in runs 1—3, respectively. Half of the solubility of (2RS,3SR)-**1**·HCl is 1.212 g in 20 ml of 1-propanol at 10 °C.

The optical resolution afforded (2R,3S)- and (2S,3R)-**1**·HCl with optical purities of over 90% in degree of resolutions of 71—79%. The (2R,3S)- and (2S,3R)-**1**·HCl recrystallized from 1-propanol were treated with triethylamine in methanol to give optically pure (2R,3S)- and (2S,3R)-**1**.

Experimental

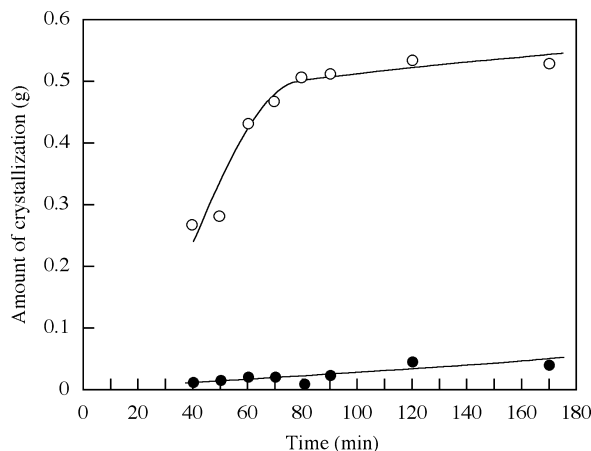**General** Specific rotations were measured at 589 nm and 20 °C with a

Fig. 4. Relationship between the Amount of Crystallization and Resolution Time in Optical Resolution (2RS,3SR)-2-Amino-3-hydroxy-3-phenylpropanoic Acid Hydrochloride [(2RS,3SR)-**1**·HCl]

Conditions: (2RS,3SR)-**1**·HCl, 4.527 g (20.8 mmol); L-phenylalanine methyl ester hydrochloride (L-**2**), 1.941 g (9.00 mmol); solvent, 20 ml of 1-propanol; stirring time, 40—170 min; temperature, 10 °C. Amount of crystallization: ○ (2R,3S)-**1**·HCl; ● (2S,3R)-**1**·HCl.

Table 2. Successive Optical Resolution by Preferential Crystallization of (2RS,3SR)-2-Amino-3-hydroxy-3-phenylpropanoic Acid Hydrochloride[a]

Run	Amount of (2RS,3SR)-**1**·HCl added (g)	Operation amount (g)		Resolution time (min)	Yield[b] (g)	Specific rotation[c]	DR[d] (%)
		(2R,3S)-**1**·HCl	(2S,3R)-**1**·HCl				
1	3.392	1.696	1.696	30	(2R,3S) 0.479	+31.0	78.9
2	0.428	1.504	1.886	40	(2S,3R) 0.619	−31.4	76.4
3	0.616	1.785	1.652	20	(2R,3S) 0.507	+31.6	72.6
4	0.500	1.598	1.881	20	(2S,3R) 0.580	−31.1	70.9

a) Conditions: Seed crystals of (2R,3S)- or (2S,3R)-**1**·HCl, 0.050 g; solvent, 20 ml of 1-propanol; temperature, 10 °C. b) The yield is the sum of the amounts of the crystallized **1**·HCl and seed crystals. c) $[\alpha]_D^{20}$ (c=1.00, methanol). d) DR, degree of resolution.

Horiba Seisakusho SEPA-300 auto polarimeter equipped with a quartz cell with a 5.00 cm path length. IR spectra were obtained in the range of 4000—400 cm^{-1} with a Perkin–Elmer Model 1600 FT-IR spectrometer by the KBr disk method. ^{1}H- and ^{13}C-NMR spectra were recorded on a JNM-FX270 FT NMR system in deuterium oxide with sodium 3-(trimethyl-silyl)propane-1-sulfonate (DSS) as an internal standard. Chemical shifts were reported in δ units downfield from DSS. Melting points were measured with a Yanaco MP-500 D micro melting point apparatus.

Glycine and L-**2** ([α]$_D^{20}$ $-3.75°$ (c=2.00, water), [α]$_D^{20}$ $+15.9°$ (c=1.00, methanol)) were purchased from Wako Pure Chemical Ind. Compound D-**2** was prepared by a general procedure,[8] namely, treatment of D-phenylalanine (19.8 g, 120 mmol), purchased from Wako Pure Chemical Ind., with thionyl chloride (13 ml) in methanol (100 ml); yield, 22.6 g (87.3%); [α]$_D^{20}$ $+3.95°$ (c=2.00, water), [α]$_D^{20}$ $-16.7°$ (c=1.00, methanol). ^{1}H-NMR (270 MHz, D$_2$O, DSS) δ: 7.44—7.28 (5H, m, arom. H), 4.45 (1H, dd, J=4.1, 5.1 Hz, >C$\underline{H}$(NH$_3^+$)), 3.84 (3H, s, –CH$_3$), 3.36 (1H, dd, J=4.1, 10.0 Hz, >C$\underline{H}$H), 3.24 (1H, dd, J=5.1, 10.0 Hz, >CH$\underline{H}$).

(2RS,3SR)-2-Amino-3-hydroxy-3-phenylpropanoic Acid Hydrochloride [(2RS,3SR)-1·HCl] (2RS,3SR)-**1** was prepared by the reaction of glycine with benzaldehyde in aqueous alkaline media.[6] After dissolving 90.6 g (500 mmol) of (2RS,3SR)-**1** in 2.5 mol/l hydrochloric acid (200 ml), the solution was evaporated to dryness $in\ vacuo$ at 60 °C and then the residue was dissolved in 300 ml of ethanol at 60 °C. The solution was allowed to stand overnight at 5 °C. The precipitated (2RS,3SR)-**1**·HCl was collected by filtration and dried.

(2RS,3SR)-**1**·HCl: Yield, 98.7 g (90.6%); mp 144—145 °C. IR (KBr) cm^{-1}: 2907, 1738, 1564, 1504, 1454, 1207, 1028, 737, 700, 502. ^{1}H-NMR (270 MHz, D$_2$O, DSS) δ: 7.49—7.41 (5H, m, arom. H), 5.45 (1H, d, J=4.1 Hz, >C$\underline{H}$(OH)), 4.31 (1H, d, J=4.1 Hz, >C$\underline{H}$(NH$_3^+$)). ^{13}C-NMR (67.5 MHz, D$_2$O, DSS) δ=170.5 (–COOH), 138.6 (arom. C), 129.7 (arom. C), 129.6 (arom. C), 129.5 (arom. C), 126.5 (arom. C), 126.4 (arom. C), 71.3 (>CH(OH)), 59.9 (>CH(NH$_3^+$)). $Anal$. Calcd for C$_9$H$_{12}$ClNO$_3$: C, 49.67; H, 5.56; N, 6.44. Found: C, 49.40; H, 5.40; N, 6.46.

Optical Resolution by Replacing Crystallization (2RS,3SR)-**1**·HCl (4.527 g, 20.8 mmol) and L-**2** (1.294—2.157 g, 6.00—10.0 mmol) were dissolved in 20 ml of 1-propanol at 60 °C. After cooling the solution to 10 °C over a period of 40 min, followed by stirring by a magnetic stirrer for 60 min at 100 rpm and 10 °C, the precipitated (2R,3S)-**1**·HCl was collected by filtration and dried.

(2R,3S)-**1**·HCl obtained using 6.00 mmol of L-**2**: yield, 0.747 g; [α]$_D^{20}$ $+13.7°$ (c=1.00, methanol). (2R,3S)-**1**·HCl obtained using 8.00 mmol of L-**2**: yield, 0.506 g; [α]$_D^{20}$ $+26.9°$ (c=1.00, methanol). (2R,3S)-**1**·HCl obtained using 8.50 mmol of L-**2**: yield, 0.465 g; [α]$_D^{20}$ $+30.0°$ (c=1.00, methanol). (2R,3S)-**1**·HCl obtained using 9.00 mmol of L-**2**: yield, 0.446 g; [α]$_D^{20}$ $+31.8°$ (c=1.00, methanol). (2R,3S)-**1**·HCl obtained using 10.0 mmol of L-**2**: yield, 0.204 g; [α]$_D^{20}$ $+29.3°$ (c=1.00, methanol).

Optical resolution was carried out for a solution of (2RS,3SR)-**1**·HCl (4.527 g, 20.8 mmol) in 20 ml of 1-propanol in the presence of L-**2** (1.941 g, 9.00 mmol) by stirring for 40—170 min at 10 °C in a manner similar to that described above.

(2R,3S)-**1**·HCl obtained at 40 min: yield, 0.275 g; [α]$_D^{20}$ $+32.6°$ (c=1.00, methanol). (2R,3S)-**1**·HCl obtained at 50 min: yield, 0.289 g; [α]$_D^{20}$ $+31.8°$ (c=1.00, methanol). (2R,3S)-**1**·HCl obtained at 70 min: yield, 0.481 g; [α]$_D^{20}$ $+32.0°$ (c=1.00, methanol). (2R,3S)-**1**·HCl obtained at 80 min: yield, 0.513 g; [α]$_D^{20}$ $+33.5°$ (c=1.00, methanol). (2R,3S)-**1**·HCl obtained at 90 min: yield, 0.531 g; [α]$_D^{20}$ $+31.4°$ (c=1.00, methanol). (2R,3S)-**1**·HCl obtained at 120 min: yield, 0.575 g; [α]$_D^{20}$ $+29.0°$ (c=1.00, methanol). (2R,3S)-**1**·HCl obtained at 170 min: yield, 0.566 g; [α]$_D^{20}$ $+29.7°$ (c=1.00, methanol).

Optical resolution was carried out using D-**2** (1.941 g, 9.00 mmol) as the optically active co-solute for a solution of (2RS,3SR)-**1**·HCl (4.527 g, 20.8 mmol) in 20 ml of 1-propanol by stirring for 80 min at 10 °C, in a manner similar to that described above, to give (2S,3R)-**1**·HCl: yield, 0.512 g; [α]$_D^{20}$ $-32.7°$ (c=1.00, methanol).

Successive Optical Resolution by Preferential Crystallization (2RS,3SR)-**1**·HCl (3.392 g) was dissolved in 20 ml of 1-propanol at 60 °C to prepare a 140% supersaturated solution at 10 °C. The solution was cooled to 10 °C over a period of 40 min and then seeded with 0.050 g of the (2R,3S)-**1**·HCl. After stirring the mixture for 30 min at 10 °C, (2R,3S)-**1**·HCl (0.479 g) was collected by filtration and dried (run 1 in Table 2). (2RS,3SR)-**1**·HCl (0.428 g) was dissolved in the filtrate at 60 °C and the resulting solution was cooled to 10 °C. After adding (2S,3R)-**1**·HCl (0.050 g) as seed crystals to the solution, followed by stirring the mixture for 40 min at 10 °C, (2S,3R)-**1**·HCl (0.619 g) was collected by filtration and dried (run 2 in Table

2). Optical resolution was carried out at 10 °C by adding further (2RS,3SR)-**1**·HCl to the filtrates in a way similar to that described above; the detailed conditions are shown in runs 3 and 4 in Table 2.

(2R,3S)- and (2S,3R)-2-Amino-3-hydroxy-3-phenylpropanoic Acid [(2R,3S)- and (2S,3R)-1] The partially resolved (2R,3S)- and (2S,3R)-**1**·HCl were recrystallized from 1-propanol in the following manner: (2R,3S)-**1**·HCl (5.00 g) ([α]$_D^{20}$ $+17.9°$ (c=1.00, methanol)) was added to 1-propanol (20 ml). After vigorously stirring the mixture for 1 h at 50 °C, followed by for 3 h at 10 °C, purified (2R,3S)-**1**·HCl was collected by filtration and dried.

(2R,3S)-**1**·HCl: Yield, 2.45 g; mp 149—151 °C; [α]$_D^{20}$ $+34.4°$ (c=1.00, methanol). $Anal$. Calcd for C$_9$H$_{12}$ClNO$_3$: C, 49.67; H, 5.56; N, 6.44. Found: C, 49.59; H, 5.38; N, 6.52. IR and ^{1}H- and ^{13}C-NMR spectra were virtually identical to those of (2RS,3SR)-**1**·HCl.

The partially resolved (2S,3R)-**1**·HCl (4.00 g) ([α]$_D^{20}$ $-19.4°$ (c=1.00, methanol)) was purified by recrystallization from 15 ml of 1-propanol in a manner similar to (2R,3S)-**1**·HCl described above.

(2S,3R)-**1**·HCl: Yield, 2.10 g; mp 150—151.5 °C; [α]$_D^{20}$ $-34.4°$ (c=1.00, methanol). IR and ^{1}H- and ^{13}C-NMR spectra were virtually identical to those of (2RS,3SR)-**1**·HCl.

A solution of (2R,3S)- or (2S,3R)-**1**·HCl (1.50 g) in 15 ml of methanol was adjusted with triethylamine to pH 6. After allowing the mixture to stand overnight at 5 °C, the precipitated (2S,3R)- or (2R,3S)-**1** was collected by filtration, washed with a small amount of chloroform, and dried.

(2R,3S)-**1**: Yield, 1.14 g; [α]$_D^{20}$ $+50.4°$ (c=0.500, 5 mol/l HCl). ^{1}H-NMR (270 MHz, D$_2$O, DSS) δ=7.50—7.38 (5H, m, arom. H), 5.29 (1H, d, J=4.3 Hz, >C$\underline{H}$(OH)), 3.91 (1H, d, J=4.3 Hz, >C$\underline{H}$(NH$_2$)). ^{13}C-NMR (67.5 MHz, D$_2$O, DSS) δ=174.3 (–COOH), 141.5 (arom. C), 131.4 (arom. C), 131.3 (arom. C), 131.0 (arom. C), 128.3 (arom. C), 128.2 (arom. C), 73.8 (>CH(OH)), 63.4 (>CH(NH$_2$)).

(2S,3R)-**1**: Yield, 1.15 g; [α]$_D^{20}$ $-50.5°$(c=0.500, 5 mol/l HCl) ([α]$_D$ $-50.3°$ (5 mol/l HCl)).[9] ^{1}H- and ^{13}C-NMR spectra were virtually identical to those of (2R,3S)-**1**.

Solubility and Phase Diagrams (2RS,3SR)-**1**·HCl (4.535 g) or (2R,3S)-**1**·HCl (3.00 g) was dissolved in 20 ml of 1-propanol at 60 °C. After vigorously stirring the solution for 10 h at 10 °C, the precipitated (2RS,3SR)- or (2R,3S)-**1**·HCl was rapidly collected by filtration and thoroughly dried. The solubility at 10 °C was calculated on the basis of the weight of the precipitated (2RS,3SR)- or (2R,3S)-**1**·HCl. Solubility of (2RS,3SR)-**1**·HCl at 10 °C: 12.115 g (100 ml of 1-propanol)$^{-1}$. Solubility of (2R,3S)-**1**·HCl at 10 °C: 6.125 g (100 ml of 1-propanol)$^{-1}$.

(2RS,3SR)-**1**·HCl (4.527 g) or (2R,3S)-**1**·HCl (3.00 g) was dissolved in a solution containing 1.941 g of L- or D-**2** in 20 ml of 1-propanol at 60 °C. After vigorously stirring the solution for 10 h at 10 °C, the precipitated **1**·HCl was rapidly collected by filtration and thoroughly dried. The solubility at 10 °C was calculated on the basis of the weight of **1**·HCl. For the dissolution of (2RS,3SR)-**1**·HCl, the solubilities of (2R,3S)- and (2S,3R)-**1**·HCl were estimated based on the optical purity of the **1**·HCl obtained by filtration and its weight. The solubilities were summarized in Table 1.

Preparing the solubility ternary-phase diagram, the solubilities of mixtures of (2RS,3SR)- and (2R,3S)-**1**·HCl were measured at 10 °C similar to the method described above. The solid **1**·HCl was filtered off and thoroughly dried and its specific rotation was measured. The amounts of (2R,3S)- and (2S,3R)-**1**·HCl in the solution were calculated based on the solubility of **1**·HCl and the specific rotation of the solid **1**·HCl.

In preparation of the melting-point binary-phase diagram, the melting points of the mixtures composed of (2RS,3SR)- and (2R,3S)-**1**·HCl were measured; after dissolving (2RS,3SR)- and (2R,3S)-**1**·HCl in an appropriate ratio in methanol, the mixtures were obtained by evaporating the solutions to dryness $in\ vacuo$. The melting-point binary-phase diagram was prepared from the temperatures at the beginning and end of melting.

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