Preparation of Optically Active *threo*-2-Amino-3-hydroxy-3-phenylpropanoic Acid (*threo*-β-Phenylserine) *via* Optical Resolution

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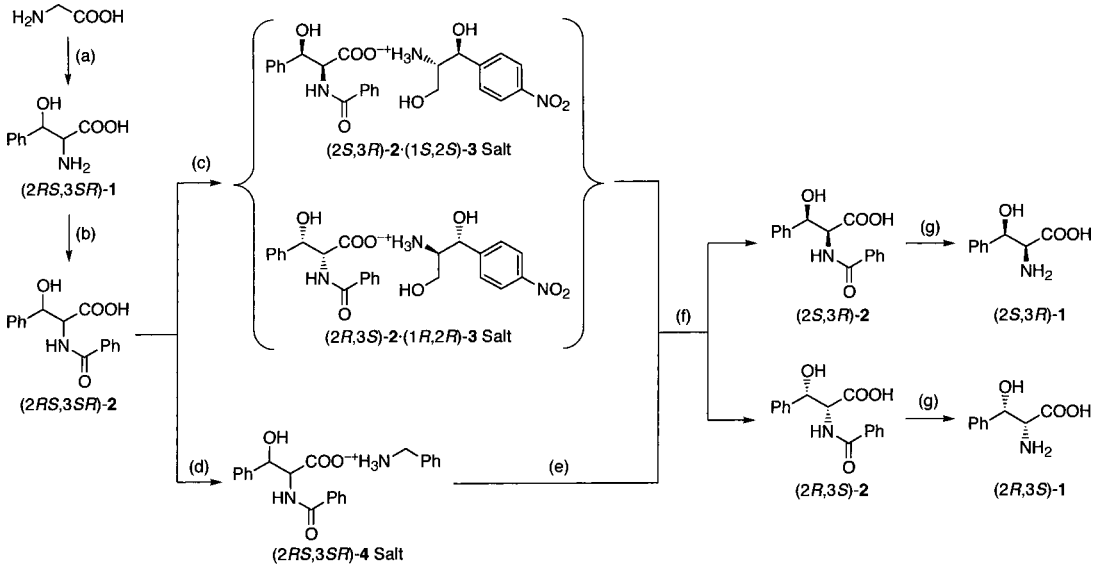
To obtain optically active *threo*-2-amino-3-hydroxy-3-phenylpropanoic acid (1), (2*RS*,3*SR*)-2-benzoylamino-3-hydroxy-3-phenylpropanoic acid [(2*RS*,3*SR*)-2] was first optically resolved using (1*S*,2*S*)- and (1*R*,2*R*)-2-amino-1-(4-nitrophenyl)-1,3-propanediol as the resolving agents to afford (2*R*,3*S*)- and (2*S*,3*R*)-2 in yields of 73% and 66%, based on half of the starting amount of (2*RS*,3*SR*)-2. Next, the racemic structures of ammonium and some organic ammonium salts of (2*RS*,3*SR*)-2 were examined based on melting point, solubility, and infrared spectrum, with the aim of optical resolution by preferential crystallization. The benzylammonium salt of (2*RS*,3*SR*)-2 was suggested to exist as a conglomerate at room temperature, although it forms a racemic compound at the melting point. The optical resolution by preferential crystallization of the racemic salt afforded the (2*R*,3*S*)- and (2*S*,3*R*)-salts with optical purities of 90—97%. The (2*R*,3*S*)- and (2*S*,3*R*)-2 obtained from the purified salts were hydrolyzed by reflux in hydrochloric acid to give (2*R*,3*S*)- and (2*S*,3*R*)-1.

Key words *threo*-β-phenylserine; optical resolution; conglomerate; diastereoisomeric separation

2-Amino-3-hydroxy-3-phenylpropanoic acid (**1**; β-phenylserine) that exists as four stereoisomers is a physiologically important α-amino acid.[1—3] L-Threonine aldolase has been reported to catalyze the condensation of glycine (Gly) and benzaldehyde to afford **1**, of which 60% is comprised of the (2*S*,3*R*)-form and 40% of the (2*S*,3*S*)-form.[3] Condensation of Gly and benzaldehyde also occurs nonenzymatically in aqueous alkaline media to give predominantly (2*RS*,3*SR*)-**1**, DL-*threo*-β-phenylserine.[4,5] Therefore we attempted to obtain (2*R*,3*S*)- and (2*S*,3*R*)-**1** by optical resolution (Chart 1).

Optical resolutions by preferential crystallization and separation of diastereoisomers have been successfully employed to obtain enantiomers from racemates.[6—9] Therefore the optical resolution of (2*RS*,3*SR*)-2-benzoylamino-3-hydroxy-3-phenylpropanoic acid [(2*RS*,3*SR*)-**2**] was first attempted by separation of its diastereoisomeric salts with optically active organic amines. In addition, we attempted to resolve (2*RS*,3*SR*)-**2** optically by preferential crystallization. Optical resolution by preferential crystallization is a simple and useful method for the large-scale separation of enantiomers from racemates and is achieved by providing a small amount of one enantiomer as seed crystals in a racemic supersaturated solution.[7,8,10] Racemates exist in the forms of racemic compounds, racemic solid solutions, and conglomerates. However, only conglomerates, which are defined as mechanical mixtures of crystals of both enantiomers, can be optically resolved by preferential crystallization. Therefore we examined the racemic structures of (2*RS*,3*SR*)-**1**, (2*RS*,3*SR*)-**2**, and ammonium and some organic ammonium salts of (2*RS*,3*SR*)-**2** to screen for a racemic salt that exists as a conglomerate. We



(a) i) benzaldehyde (2 eq), OH^-, ii) recrystallization from water; (b) benzoyl chloride, OH^-; (c) i) optical resolution by separation of diastereoisomeric salts of (2*RS*,3*SR*)-**2** with (1*S*,2*S*)-2-amino-1-(4-nitrophenyl)-1,3-propanediol [(1*S*,2*S*)-**3**], ii) salt formation of **2**, from filtrate, with (1*R*,2*R*)-**3**; (d) benzylamine; (e) optical resolution by preferential crystallization; (f) treatment with hydrochloric acid; (g) i) refluxing in 6 mol/l hydrochloric acid for 30 h, ii) triethylamine (pH 6—7), methanol.

Chart 1. Synthetic Route for Optically Active *threo*-2-Amino-3-hydroxy-3-phenylpropanoic Acid (**1**)

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attempted to resolve the racemic salt that exists as a conglomerate optically by preferential crystallization and then to obtain (2*R*,3*S*)- and (2*S*,3*R*)-**1** from the optically active **2**.

Results and Discussion

Reaction of Gly with benzaldehyde under alkaline conditions[4,5] afforded **1** of which 80% consisted of the (2*RS*,3*SR*)-form and 20% of the (2*RS*,3*RS*)-form. Compound **1** was recrystallized from water to give (2*RS*,3*SR*)-**1** in 100% *de* in a yield of 52%.

Compound (2*RS*,3*SR*)-**2**, which was obtained by *N*-benzoylation of (2*RS*,3*SR*)-**1**, was attempted to be resolved optically by formation of diastereoisomeric salts with basic resolving agents, such as optically active α-phenylethylamine, brucine, cinchonidine, and (1*S*,2*S*)- and (1*R*,2*R*)-2-amino-1-(4-nitrophenyl)-1,3-propanediol [(1*S*,2*S*)- and (1*R*,2*R*)-**3**]. Of these resolving agents, only optically active **3** gave a good result. A salt of (2*S*,3*R*)-**2** with (1*S*,2*S*)-**3** [(2*S*,3*R*)-**2**·(1*S*,2*S*)-**3** salt] was crystallized as a less-soluble diastereoisomeric salt. The purified (2*S*,3*R*)-**2**·(1*S*,2*S*)-**3** salt was treated with hydrochloric acid to give (2*S*,3*R*)-**2** in a yield of 66%, based on half of the starting amount of (2*RS*,3*SR*)-**2**: (2*S*,3*R*)-**2**, $[\alpha]_D^{20}$ −58.9° (c=1.00, ethanol). On the other hand, after collecting (2*S*,3*R*)-**2**·(1*S*,2*S*)-**3** salt by filtration, the filtrate was evaporated to dryness and the salt obtained as the residue was treated with hydrochloric acid to give the partially resolved (2*R*,3*S*)-**2**. The (2*R*,3*S*)-**2** obtained was reacted with (1*R*,2*R*)-**3** to yield the less-soluble diastereoisomeric (2*R*,3*S*)-**2**·(1*R*,2*R*)-**3** salt. The (2*R*,3*S*)-**2**·(1*R*,2*R*)-**3** salt was treated with hydrochloric acid to give (2*R*,3*S*)-**2** in a yield of 73%: $[\alpha]_D^{20}$ +58.6° (c=1.00, ethanol).

Next, we examined the racemic structures of (2*RS*,3*SR*)-**1** and -**2**, with the aim of optical resolution by preferential crystallization. Compounds (2*RS*,3*SR*)-**1** and -**2** have higher melting points than their enantiomers and show different IR spectra from their enantiomers. Since racemic compounds are known to have the above characteristics,[6—8] (2*RS*,3*SR*)-**1** and -**2** are determined to form racemic compounds. Therefore it was attempted to form ammonium, methylammonium, ethylammonium, 1-propylammonium, isopropylammonium, *tert*-butylammonium, and benzylammonium salts from (2*RS*,3*SR*)- and (2*S*,3*R*)-**2** to screen for racemic salts existing as conglomerates. The ammonium and organic ammonium salts of (2*RS*,3*SR*)-**2** were obtained as crystalline salts, but not crystalline salts of (2*S*,3*R*)-**2**, with the exception of the benzylammonium salt, [(2*S*,3*R*)-**4** salt], of (2*S*,3*R*)-**2**. Therefore the all salts except for the (2*RS*,3*SR*)-**4** salt are estimated to form racemic compounds because their racemic salts can be envisaged to have higher melting points than the optically active salts. On the other hand, the (2*RS*,3*SR*)-**4** salt had a lower melting point than the (2*S*,3*R*)-**4** salt. Although conglomerates are known to have such melting point characteristics,[6—8] the melting-point binary-phase diagram suggested that the (2*RS*,3*SR*)-**4** salt forms a racemic compound, as shown in Fig. 1. However, the IR spectrum of (2*RS*,3*SR*)-**4** salt was identical to that of the (2*S*,3*R*)-**4** salt. In addition, the (2*RS*,3*SR*)-**4** salt was more soluble than the (2*S*,3*R*)-**4** salt: solubility of the (2*RS*,3*SR*)-**4** salt at 10 °C was 1.486 g (100 ml of ethanol)$^{-1}$; and solubility of the (2*S*,3*R*)-**4** salt at 10 °C was 0.666 g (100 ml of ethanol)$^{-1}$. The ternary solubility diagram also showed that the (2*S*,3*R*)-**4** salt is expected to

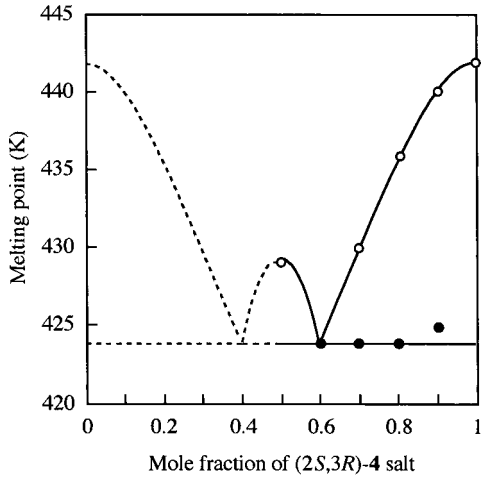

Fig. 1. Melting-Point Binary-Phase Diagram of Benzylammonium Salt of *threo*-2-Benzoylamino-3-hydroxy-3-phenylpropanoic Acid (**4** Salt)
●: Beginning of melting. ○: End of melting.

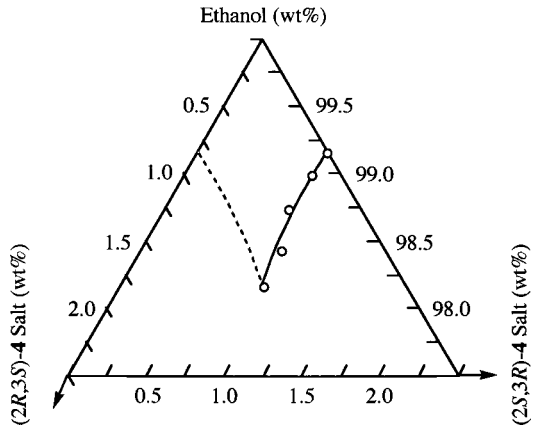

Fig. 2. Solubility Ternary-Phase Diagram of Benzylammonium Salt of *threo*-2-Benzoylamino-3-hydroxy-3-phenylpropanoic Acid (**4** Salt)
Conditions: Temperature, 10 °C; solvent, ethanol.

be a conglomerate (Fig. 2). The above results suggest that the (2*RS*,3*SR*)-**4** salt exists as a conglomerate at room temperature and forms a racemic compound at the melting point.

The (2*RS*,3*SR*)-**4** salt was optically resolved by preferential crystallization in ethanol at 10 °C. To optimize the conditions, the optical resolution was conducted by stirring solutions of the (2*RS*,3*SR*)-**4** salt in 50 ml of ethanol at 150—190% supersaturation for 30 min; the (2*S*,3*R*)-**4** salt (0.100 g) was employed as seed crystals. The results are shown in Fig. 3. The yield of enantiomer [*YE* (g)], degree of resolution [*DR* (%)], and the amount of crystallization [$AC_{(2S,3R)}$ and $AC_{(2R,3S)}$ (g)] were calculated from

$$YE\ (\mathrm{g}) = [\text{yield (g)} \times OP\ (\%)/100] - 0.100,$$

$$DR\ (\%) = YE\ (\mathrm{g}) \times 100/\{(1/2)[\text{amount of (2}RS,3SR\text{)-4 salt (g)} - 0.743]\},$$

$$AC_{(2R,3S)}\ (\mathrm{g}) = (1/2)[\text{yield (g)} - YE\ (\mathrm{g}) - 0.100],$$

$$AC_{(2S,3R)}\ (\mathrm{g}) = \text{yield (g)} - AC_{(2R,3S)}\ (\mathrm{g}) - 0.100,$$

where the optical purity [*OP* (%)] of the obtained (2*S*,3*R*)-**4** salt is calculated on the basis of the specific rotation ($[\alpha]_D^{20}$ −41.1° (c=0.500, ethanol)) of the (2*S*,3*R*)-**4** salt, the solubil-

ity of the (2*RS*,3*SR*)-**4** salt was 0.743 g in 50 ml of ethanol at 10 °C, and the yield is the sum of the amounts of the crystallized **4** salt and seed crystals. The degree of resolution [*DR* (%)] is defined as the yield (%) of the seeded enantiomer, based on half of the supersaturating portion of a racemate, and indicates the efficiency of optical resolution; half of the supersaturating portion of a racemate means the theoretical yield (g) of the seeded enantiomer.

When the 150—170% supersaturated solutions were employed, the (2*S*,3*R*)-**4** salts with optical purities of 97—99% were obtained with 58—83% degrees of resolution. Therefore the (2*RS*,3*SR*)-**4** salt was confirmed to exist as a conglomerate at room temperature. When the solutions with 180% and 190% supersaturation were employed, the optical resolutions gave poor results because of rapid crystallization of the unseeded (2*R*,3*S*)-**4** salt; the (2*S*,3*R*)-**4** salt obtained showed optical purities of 75% and 51%, respectively. From these results, the optical resolution for the 170% supersaturated solution was determined at resolution times of 15—60 min (Fig. 4).

Rapid crystallization of the unseeded (2*R*,3*S*)-**4** salt was not observed for the first 30 min, but the (2*R*,3*S*)-**4** salt began to crystallize rapidly at 45 min. Based on these results, successive optical resolution was attempted by stirring the 170% supersaturated solution, as the initial solution, for 30 min (Table 1). The degrees of resolution [*DR* (%)] of the (2*S*,3*R*)- and (2*R*,3*S*)-**4** salt obtained were calculated from

$$DR\,(\%) = YE\,(\mathrm{g}) \times 100 / [\text{operation amount of } (2S,3R)\text{- or } (2R,3S)\text{-}\mathbf{4} \text{ salt (g)} - 0.372]$$

where the operation amount is the amount of the (2*S*,3*R*)- and (2*R*,3*S*)-**4** salts in the solution used in the optical resolution and those in runs 2—7 in Table 1 were calculated based on the yields and optical purities of the (2*S*,3*R*)- and (2*R*,3*S*)-**4** salt obtained in runs 1—6, respectively. Half of the solubility of (2*RS*,3*SR*)-**4** salt is 0.372 g in 50 ml of ethanol at 10 °C.

The optical resolution afforded the (2*S*,3*R*)- and (2*R*,3*S*)-**4** salts with optical purities of 90—97% at 48—90% degrees of resolution. Although the (2*R*,3*S*)-**4** salt with 90% optical purity was obtained in run 2, the amount of crystallization was less than expected. Therefore the optical resolution in run 3 was carried out by again providing the (2*R*,3*S*)-**4** salt as seed crystals to afford the (2*R*,3*S*)-**4** salt of 90% optical purity with 58% degree of resolution. The (2*S*,3*R*)- and (2*R*,3*S*)-**4** salts obtained were recrystallized from ethanol, as described in the Experimental section, to obtain the optically pure (2*S*,3*R*)- and (2*R*,3*S*)-**4** salts. Treatment of the purified

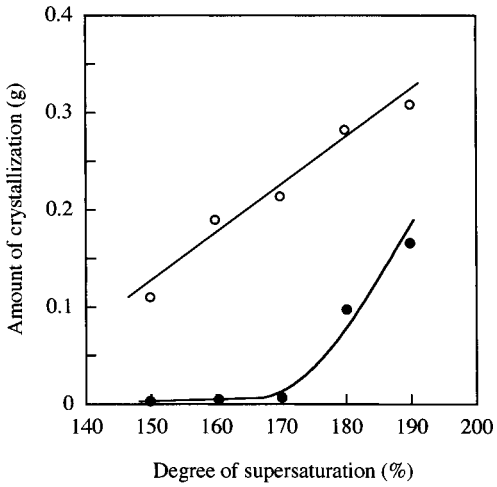

Fig. 3. Relationship between the Amount of Crystallization and Degree of Supersaturation in the Optical Resolution of Benzylammonium Salt of (2*RS*,3*SR*)-2-Benzoylamino-3-hydroxy-3-phenylpropanoic Acid [(2*RS*,3*SR*)-**4** Salt]

Conditions: (2*RS*,3*SR*)-**4** Salt, 1.115—1.412 g (150—190% supersaturation); seed crystals, 0.100 g of (2*S*,3*R*)-**4** salt; solvent, 50 ml of ethanol; stirring time, 30 min; temperature, 10 °C. Amount of crystallization: ○ (2*S*,3*R*)-**4** Salt; ● (2*R*,3*S*)-**4** salt.

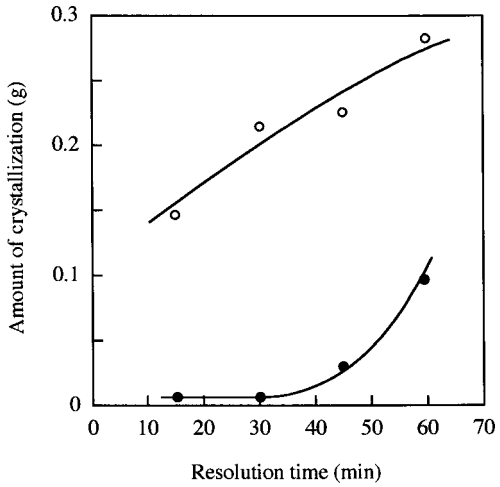

Fig. 4. Relationship between the Amount of Crystallization and Resolution Time in the Optical Resolution of Benzylammonium Salt of (2*RS*,3*SR*)-2-Benzoylamino-3-hydroxy-3-phenylpropanoic Acid [(2*RS*,3*SR*)-**4** Salt]

Conditions: (2*RS*,3*SR*)-**4** Salt, 1.263 g (170% supersaturation); seed crystals, 0.100 g of (2*S*,3*R*)-**4** salt; solvent, 50 ml of ethanol; stirring time, 15—60 min; temperature, 10 °C. Amount of crystallization: ○ (2*S*,3*R*)-**4** Salt; ● (2*R*,3*S*)-**4** salt.

Table 1. Successive Optical Resolution by Preferential Crystallization of Benzylammonium Salt of (2*RS*,3*SR*)-2-Benzoylamino-3-hydroxy-3-phenylpropanoic Acid[a)]

Run	Amount of (2*RS*,3*SR*)-**4** salt added (g)	Operation amount (g)		Yield[b)] (g)	Optical purity (%)	*YE*[c)] (g)	*DR*[d)] (%)
		(2*S*,3*R*)-**4** Salt	(2*R*,3*S*)-**4** Salt				
1	1.263	0.632	0.632	(2*S*,3*R*)-(−) 0.319	97	0.209	80
2	0.220	0.528	0.737	(2*R*,3*S*)-(+) 0.304	90	0.174	48
3	0.204	0.615	0.650	(2*R*,3*S*)-(+) 0.289	90	0.161	58
4	0.190	0.696	0.570	(2*S*,3*R*)-(−) 0.409	96	0.291	90
5	0.310	0.551	0.716	(2*R*,3*S*)-(+) 0.366	91	0.233	68
6	0.266	0.667	0.599	(2*S*,3*R*)-(−) 0.369	96	0.256	87
7	0.270	0.539	0.727	(2*R*,3*S*)-(+) 0.411	97	0.297	84

a) Conditions: Seed crystals of the (2*S*,3*R*)- or (2*R*,3*S*)-**4** salt, 0.100 g; solvent, 50 ml of ethanol; temperature, 10 °C; resolution time, 30 min. *b*) The yield is the sum of the amounts of crystallized **4** salt and seed crystals. *c*) *YE*, yield of enantiomer. *d*) *DR*, degree of resolution.

(2*S*,3*R*)- and (2*R*,3*S*)-**4** salts with hydrochloric acid quantitatively gave (2*S*,3*R*)- and (2*R*,3*S*)-**2**.

The obtained (2*S*,3*R*)- and (2*R*,3*S*)-**2** were hydrolyzed by refluxing for 30 h in 5 mol/l of hydrochloric acid, followed by treatment with triethylamine to give (2*S*,3*R*)- and (2*R*,3*S*)-**1**: (2*S*,3*R*)-**1**, $[\alpha]_D^{20}$ −50.5° (*c*=1.00, 5 mol/l HCl) ($[\alpha]_D$ −50.3° (5 mol/l HCl))[11]; (2*R*,3*S*)-**1**, $[\alpha]_D^{20}$ +50.5° (*c*=1.00, 5 mol/l HCl).

Experimental

General Specific rotations were measured at 589 nm and 20 °C with a 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 using the KBr disk method. ^{1}H- and ^{13}C-NMR spectra were recorded on a JNM-FX270 FT NMR system in deuterium oxide (D_2O) or dimethylsulfoxide-d_6 (DMSO-d_6) with sodium 3-(trimethylsilyl)propane-1-sulfonate (DSS) or tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in δ units downfield from DSS or TMS. Melting points were measured with a Yanaco MP-500 D micro melting point apparatus.

(2*RS*,3*SR*)-2-Amino-3-hydroxy-3-phenylpropanoic Acid [(2*RS*,3*SR*)-1] To the solution of Gly (56.3 g, 0.750 mol) in 5 mol/l aqueous sodium hydroxide (525 ml) 53.1 g (0.500 mol) each of benzaldehyde was added 3 times at 10-min intervals, keeping the temperature below 10 °C in an ice bath. The mixture was further stirred in an ice bath for 1 h and then at room temperature for about 30 min to give a solid condensation cake. After adding 5 mol/l of hydrochloric acid (750 ml) to the mixture in an ice bath, the resulting solution was evaporated to dryness *in vacuo* at 60 °C. Methanol (750 ml) was added to the residue and then the mixture was filtered to remove sodium chloride. The filtrate was adjusted with triethylamine to pH 6—7 to precipitate **1**. The crude **1** was collected by filtration, washed with methanol, and dried; the yield was 117 g (86.0%). ^{1}H-NMR (270 MHz, D_2O, DSS) δ: 7.51—7.38 (5H, m, C_6H_5–), 5.35 (0.19H, d, *J*=4.3 Hz, –C<u>H</u>(OH)–), 5.30 (0.81H, d, *J*=4.3 Hz, –C<u>H</u>(OH)–), 4.08 (0.19H, d, *J*=4.3 Hz, –C<u>H</u>(NH_2)–), 3.91 (0.81H, d, *J*=4.3 Hz, –C<u>H</u>(NH_2)–).

The obtained **1** was dissolved in water (3.75 ml g^{-1}) at 70 °C. After stirring the solution for 30 min in an ice bath, the precipitated (2*RS*,3*SR*)-**1** was collected by filtration and dried.

(2*RS*,3*SR*)-**1**: Yield, 70.0 g (51.5%); mp 189—190 °C. IR (KBr) cm^{-1}: 3615, 3137, 1644, 1488, 1402, 1352, 1017, 709, 526. ^{1}H-NMR (270 MHz, D_2O, DSS) δ: 7.51—7.38 (5H, m, arom. H), 5.30 (1H, d, *J*=4.3 Hz, >C<u>H</u>(OH)), 3.91 (1H, d, *J*=4.3 Hz, >C<u>H</u>(NH_2)). ^{13}C-NMR (67.5 MHz, D_2O, DSS) δ=174.3 (–COOH), 141.5 (arom. C), 131.4 (arom. C), 131.3 (arom. C), 131.8 (arom. C), 128.3 (arom. C), 128.2 (arom. C), 73.8 (>CH(OH)), 63.4 (>CH(NH_2)).

(2*RS*,3*SR*)-2-Benzoylamino-3-hydroxy-3-phenylpropanoic Acid [(2*RS*,3*SR*)-2] Benzoyl chloride (84.3 g, 0.600 mol) was added dropwise over a period of 1 h with stirring in an ice bath to the solution of (2*RS*,3*SR*)-**1** (90.6 g, 0.500 mol) in 5 mol/l of aqueous sodium hydroxide (300 ml). After stirring for 2 h in an ice bath and then for 1 h at room temperature, the reaction mixture was adjusted to pH 1 with 5 mol/l of hydrochloric acid. The precipitated (2*RS*,3*SR*)-**2** was collected by filtration, washed with diethyl ether (250 ml×4), and dried. The obtained (2*RS*,3*SR*)-**2** was dissolved in 100 ml of methanol and then water (1 l) was added to the solution. After stirring the mixture for 1 h at 20 °C, the purified (2*RS*,3*SR*)-**2** was collected by filtration and dried.

(2*RS*,3*SR*)-**2**: Yield, 101 g (70.6%); mp 146—148 °C. IR (KBr) cm^{-1}: 3244, 1732, 1602, 1548, 1397, 1257, 1215, 1066, 1024, 863, 697, 688, 546. ^{1}H-NMR (270 MHz, 0.5 mol/l NaOD, DSS) δ: 7.71—7.25 (10H, m, arom. H), 5.42 (1H, d, *J*=3.5 Hz, >C<u>H</u>(OH)), 4.73 (1H, dd, *J*=3.5, >C<u>H</u>–NH–). ^{13}C-NMR (67.5 MHz, 0.5 mol/l NaOD, DSS) δ=177.1 (–COOH), 170.3 (Ph-<u>C</u>O–), 142.5 (arom. C), 133.9 (arom. C), 132.7 (arom. C), 129.4 (arom. C), 129.2 (arom. C), 128.8 (arom. C), 128.3 (arom. C), 128.0 (arom. C), 127.6 (arom. C), 126.6 (arom. C), 72.6 (>CH(OH)), 58.9 (>CH–NH–). *Anal.* Calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.22; H, 5.34; N, 4.91.

Optical Resolution of (2*RS*,3*SR*)-2-Benzoylamino-3-hydroxy-3-phenylpropanoic Acid [(2*RS*,3*SR*)-2] by Separation of Diastereoisomeric Salts After adding (1*S*,2*S*)-**3** (10.6 g, 50.0 mmol) to the suspension of (2*RS*,3*SR*)-**2** (14.3 g, 50.0 mmol) in 150 ml of ethanol, the mixture was stirred for 2 h at room temperature. The precipitated (2*S*,3*R*)-**2**·(1*S*,2*S*)-**3** salt (9.41 g) was collected by filtration and dried; $[\alpha]_D^{20}$ +27.0° (*c*=0.500, ethanol). After dissolving the salt in ethanol (12 ml g^{-1}) at 60 °C, the solution was allowed to stand overnight at 5 °C. The precipitated salt was collected by filtration and dried. To the suspension of the purified (2*S*,3*R*)-**2**·(1*S*,2*S*)-**3** salt in water (4 ml g^{-1}) 5 mol/l of hydrochloric acid (0.8 ml g^{-1}) was added in an ice bath. After stirring the mixture for 2 h in an ice bath and then for 1 h at room temperature, the precipitated (2*S*,3*R*)-**2** was collected by filtration and then recrystallized from ethanol (1 ml g^{-1}).

(2*S*,3*R*)-**2**·(1*S*,2*S*)-**3** Salt: Yield, 8.24 g (66.5%); mp 169—171 °C; $[\alpha]_D^{20}$ +32.5° (*c*=0.500, ethanol). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS) δ: 8.22—7.12 (15H, m, arom. H, –NH–CO–), 5.20 (1H, d, *J*=3.8 Hz, >C<u>H</u>(OH) (**2**)), 4.85 (1H, d, *J*=7.3 Hz, >C<u>H</u>(OH) (**3**)), 4.51 (1H, dd, *J*=3.8, 7.6 Hz, >C<u>H</u>–NH– (**2**)), 3.51—3.45 (1H, m, –C<u>H</u>H–OH (**3**)), 3.28—3.22 (1H, m, –CH<u>H</u>–OH (**3**)), 3.16—3.10 (1H, m, >C<u>H</u>(NH_3^+) (**3**)). ^{13}C-NMR (67.5 MHz, DMSO-d_6, TMS) δ=165.3 (C_6H_5–<u>C</u>O–), 149.7 (arom. C), 146.8 (arom. C), 142.9 (arom. C), 134.5 (arom. C), 131.0 (arom. C), 128.3 (arom. C), 128.0 (arom. C), 127.9 (arom. C), 127.4 (arom. C), 127.3 (arom. C), 126.7 (arom. C), 126.6 (arom. C), 126.5 (arom. C), 126.2 (arom. C), 126.1 (arom. C), 125.4 (arom. C), 123.2 (arom. C), 123.1 (arom. C), 72.3 (>CH(OH) (**2**)), 69.9 (>CH(OH) (**3**)), 59.2 (>CH–NH– (**2**)), 58.7 (–CH_2–OH (**3**)), 58.0 (>CH(NH_3^+) (**3**)).

(2*S*,3*R*)-**2**: Yield, 4.70 g (65.9%); mp 125—126 °C; $[\alpha]_D^{20}$ −58.9° (*c*=1.00, ethanol). IR (KBr) cm^{-1}: 3213, 1736, 1641, 1340, 1265, 1219, 1059, 696. The ^{1}H- and ^{13}C-NMR spectra were virtually identical to those of (2*RS*,3*SR*)-**2**. *Anal.* Calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.32; H, 5.34; N, 5.00.

On the other hand, after collecting (2*S*,3*R*)-**2**·(1*S*,2*S*)-**3** salt by filtration, the filtrate was evaporated to dryness *in vacuo* at 50 °C to obtain the crude (2*R*,3*S*)-**2**·(1*S*,2*S*)-**3** salt (15.4 g) as the residue; $[\alpha]_D^{20}$ +0.1° (*c*=1.00, ethanol). (2*R*,3*S*)-**2**·(1*S*,2*S*)-**3** salt was treated with hydrochloric acid in a manner similar to (2*S*,3*R*)-**2**·(1*S*,2*S*)-**3** salt to give crude (2*R*,3*S*)-**2**; $[\alpha]_D^{20}$ +28.6° (*c*=1.00, ethanol). Compound (1*R*,2*R*)-**3** (6.51 g, 30.7 mmol) was added to the suspension of (2*R*,3*S*)-**2** (8.76 g, 30.7 mmol) in 90 ml of ethanol. After stirring the mixture for 2 h at room temperature, the precipitated (2*R*,3*S*)-**2**·(1*R*,2*R*)-**3** salt was collected by filtration and then was recrystallized from ethanol (12 ml g^{-1}). The purified (2*R*,3*S*)-**2**·(1*R*,2*R*)-**3** salt was treated with hydrochloric acid in a manner similar to (2*S*,3*R*)-**2**·(1*S*,2*S*)-**3** salt to give (2*R*,3*S*)-**2**.

(2*R*,3*S*)-**2**·(1*R*,2*R*)-**3** Salt: Yield, 9.27 g (74.8%); mp 169—170 °C; $[\alpha]_D^{20}$ −32.7° (*c*=1.00, ethanol). The ^{1}H- and ^{13}C-NMR spectra were virtually identical to those of the (2*S*,3*R*)-**2**·(1*S*,2*S*)-**3** salt.

(2*R*,3*S*)-**2**: Yield, 5.24 g (73.5%); mp 125—126 °C; $[\alpha]_D^{20}$ +58.6° (*c*=1.00, ethanol). The IR, ^{1}H- and ^{13}C-NMR spectra were virtually identical to those of (2*S*,3*R*)-**2**. *Anal.* Calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.25; H, 5.57; N, 4.75.

Benzylammonium Salt of 2-Benzoylamino-3-hydroxy-3-phenylpropanoic Acid (4 Salt) After adding benzylamine (0.536 g, 5.00 mmol) to a solution of (2*RS*,3*SR*)-, (2*S*,3*R*)-, or (2*R*,3*S*)-**2** (1.43 g, 5.00 mmol) in 15 ml of ethanol, the mixture was stirred for 1 h in an ice bath. The precipitated (2*RS*,3*SR*)-, (2*S*,3*R*)-, or (2*R*,3*S*)-**4** salt was collected by filtration and dried.

(2*RS*,3*SR*)-**4** Salt: Yield, 1.75 g (89.3%); mp 155—156 °C. IR (KBr) cm^{-1}: 3394, 3154, 1633, 1601, 1578, 1515, 1484, 1454, 1438, 1380, 1309, 1261, 1181, 1141, 1082, 1063, 926, 756, 735, 725, 708, 687, 593, 521. ^{1}H-NMR (270 MHz, DMSO-d_6, TMS) δ: 8.19—7.14 (16H, m, –C_6H_5, –NH–CO–), 5.13 (1H, d, *J*=3.7 Hz, >C<u>H</u>(OH)), 4.73 (1H, d, *J*=3.8 Hz, >C<u>H</u>–NH–), 3.88 (2H, s, –C<u>H</u>$_2$–C_6H_5). ^{13}C-NMR (67.5 MHz, DMSO-d_6, TMS) δ=172.8 (–COO^-), 165.4 (C_6H_5–<u>C</u>O–), 143.1 (C_6H_5–), 135.1 (C_6H_5–), 134.7 (C_6H_5–), 131.0 (C_6H_5–), 128.6 (C_6H_5–), 128.4 (C_6H_5–), 128.0 (C_6H_5–), 127.4 (C_6H_5–), 126.7 (C_6H_5–), 126.5 (C_6H_5–), 126.3 (C_6H_5–), 72.2 (>CH(OH)), 58.5 (>CH–NH–), 42.4 (–CH_2–NH_3^+). *Anal.* Calcd for $C_{23}H_{24}N_2O_4$: C, 70.39; H, 6.16; N, 7.14. Found: C, 70.33; H, 6.18; N, 7.05.

(2*S*,3*R*)-**4** Salt: Yield, 1.85 g (94.4%); mp 168—169 °C; $[\alpha]_D^{20}$ −41.1° (*c*=0.500, ethanol). IR and ^{1}H- and ^{13}C-NMR spectra were virtually identical to those of (2*RS*,3*SR*)-BZA salt. *Anal.* Calcd for $C_{23}H_{24}N_2O_4$: C, 70.39; H, 6.16; N, 7.14. Found: C, 70.21; H, 6.24; N, 7.11.

(2*R*,3*S*)-**4** Salt: Yield, 1.82 g (92.9%); mp 168—169 °C; $[\alpha]_D^{20}$ +41.1° (*c*=0.500, ethanol). IR and ^{1}H- and ^{13}C-NMR spectra were virtually identical to those of (2*RS*,3*SR*)-**4** salt. *Anal.* Calcd for $C_{23}H_{24}N_2O_4$: C, 70.39; H, 6.16; N, 7.14. Found: C, 70.31; H, 6.05; N, 7.14.

Optical Resolution by Preferential Crystallization The (2*RS*,3*SR*)-**4** salt (1.115—1.412 g) was dissolved in 50 ml of ethanol at 40 °C to prepare 150—190% supersaturated solutions at 10 °C. The solutions were cooled to 10 °C over a period of 30 min and then seeded with 0.100 g of the (2*S*,3*R*)-**4**

salt. After stirring the mixture for 15—60 min at 10 °C, the (2*S*,3*R*)-**4** salt was collected by filtration and dried.

(2*S*,3*R*)-**4** Salt obtained from 150% supersaturated solution at 30 min: Yield 0.210 g; $[\alpha]_D^{20}$ −40.8° (*c*=1.00, ethanol). (2*S*,3*R*)-**4** Salt obtained from 160% supersaturated solution at 30 min: Yield 0.293 g; $[\alpha]_D^{20}$ −39.9° (*c*=1.00, ethanol). (2*S*,3*R*)-**4** Salt obtained from 170% supersaturated solution at 15 min: Yield 0.252 g; $[\alpha]_D^{20}$ −39.3° (*c*=1.00, ethanol). (2*S*,3*R*)-**4** Salt obtained from 170% supersaturated solution at 30 min: Yield 0.319 g; $[\alpha]_D^{20}$ −39.8° (*c*=1.00, ethanol). (2*S*,3*R*)-**4** Salt obtained from 170% supersaturated solution at 45 min: Yield 0.355 g; $[\alpha]_D^{20}$ −34.1° (*c*=1.00, ethanol).

(2*S*,3*R*)-**4** Salt obtained from 170% supersaturated solution at 60 min: Yield 0.451 g; $[\alpha]_D^{20}$ −23.9° (*c*=1.00, ethanol). (2*S*,3*R*)-**4** Salt obtained from 180% supersaturated solution at 30 min: Yield 0.379 g; $[\alpha]_D^{20}$ −31.0° (*c*=1.00, ethanol). (2*S*,3*R*)-**4** Salt obtained from 190% supersaturated solution at 30 min: Yield 0.472 g; $[\alpha]_D^{20}$ −21.1° (*c*=1.00, ethanol).

Successive Optical Resolution by Preferential Crystallization The (2*RS*,3*SR*)-**4** salt (1.263 g) was dissolved in 50 ml of ethanol at 40 °C to prepare a 170% supersaturated solution at 10 °C. The solution was cooled to 10 °C over a period of 30 min and then seeded with 0.100 g of the (2*S*,3*R*)-**4** salt. After stirring the mixture for 30 min at 10 °C, the (2*S*,3*R*)-**4** salt (0.319 g) was collected by filtration and dried (run 1 in Table 1). The (2*RS*,3*SR*)-**4** salt (0.220 g) was dissolved in the filtrate at 40 °C and the resulting solution was cooled to 10 °C. After adding the (2*R*,3*S*)-**4** salt (0.100 g) as seed crystals to the solution, followed by stirring the mixture for 30 min at 10 °C, the (2*R*,3*S*)-**4** salt (0.304 g) was collected by filtration and dried (run 2 in Table 1). Optical resolution was carried out at 10 °C by adding further the (2*RS*,3*SR*)-**4** salt to the filtrates in a way similar to that described above; the detailed conditions are shown in runs 3—7 in Table 1.

(2*S*,3*R*)- and (2*R*,3*S*)-2-Amino-3-hydroxy-3-phenylpropanoic Acid [(2*S*,3*R*)- and (2*R*,3*S*)-1] The suspension of (2*S*,3*R*)- or (2*R*,3*S*)-**2** (2.85 g, 10.0 mmol) in 6 mol/l of hydrochloric acid (300 ml) was refluxed for 30 h. The reaction mixture was filtered to remove the precipitated benzoic acid. After evaporating the filtrate to dryness *in vacuo* at 60 °C, the crude (2*S*,3*R*)- or (2*R*,3*S*)-**1** hydrochloride obtained as the residue was washed with 50 ml of diethyl ether. A solution of (2*S*,3*R*)- or (2*R*,3*S*)-**1** hydrochloride in 100 ml of methanol was adjusted with triethylamine to pH 6—7. After evaporating the mixture to dryness *in vacuo* at 50 °C, chloroform (150 ml) was added to the residue. The precipitated (2*S*,3*R*)- or (2*R*,3*S*)-**1** was collected by filtration, washed with a small amount of chloroform, and dried.

(2*S*,3*R*)-**1**: Yield, 0.509 g (28.1%); mp 184—187 °C (decomp); $[\alpha]_D^{20}$ −34.4° (*c*=0.500, water); $[\alpha]_D^{20}$ −50.5° (*c*=0.500, 5 mol/l HCl) ($[\alpha]_D$ −50.3° (5 mol/l HCl)).[11] IR (KBr) cm^{-1}: 3047, 1666, 1609, 1520, 1398, 1350, 1016, 702, 536. The ^{1}H- and ^{13}C-NMR spectra were virtually identical to those of (2*RS*,3*SR*)-**1**. *Anal.* Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.45; H, 6.08; N, 7.71.

(2*R*,3*S*)-**1**: Yield, 0.547 g (30.2%); mp 185—187 °C (decomp); $[\alpha]_D^{20}$ +34.5° (*c*=0.500, water); $[\alpha]_D^{20}$ +50.5° (*c*=0.500, 5 mol/l HCl). The IR spectrum was virtually identical to that of (2*S*,3*R*)-**1** and ^{1}H- and ^{13}C-NMR spectra were identical to those of (2*RS*,3*SR*)-**1**. *Anal.* Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.44; H, 6.20; N, 7.74.

Solubility and Phase Diagrams The (2*RS*,3*SR*)-**4** salt (1.50 g) or (2*S*,3*R*)-**4** salt (1.20 g) was dissolved in 50 ml of ethanol at 60 °C. After vigorously stirring the solution for 12 h at 10 °C, the precipitated (2*RS*,3*SR*)- or (2*S*,3*R*)-**4** salt was rapidly collected by filtration and thoroughly dried. The solubility at 10 °C was calculated on the basis of the weight of the precipitated (2*RS*,3*SR*)- or (2*S*,3*R*)-**4** salt. Solubility of the (2*RS*,3*SR*)-**4** salt at 10 °C: 1.486 g (100 ml of ethanol)$^{-1}$. Solubility of the (2*S*,3*R*)-**4** salt at 10 °C: 0.666 g (100 ml of ethanol)$^{-1}$.

Preparing a ternary solubility diagram, the solubilities of mixtures of (2*RS*,3*SR*)- and (2*S*,3*R*)-**4** salts were measured at 10 °C similar to the method described above. The solid **4** salt was filtered off and thoroughly dried and the specific rotation was measured. The amounts of (2*R*,3*S*)- and (2*S*,3*R*)-**4** salts in the solution were calculated based on the solubility of **4** salt and the specific rotation of the solid **4** salt.

In preparation of the binary melting point diagram, the melting points of the mixtures composed of (2*RS*,3*SR*)- and (2*S*,3*R*)-**4** salts were measured; after dissolving (2*RS*,3*SR*)- and (2*S*,3*R*)-**4** salts 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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