Optical Resolution of (*RS*)-Mercaptosuccinic Acid and Syntheses of Four Stereoisomers of 2-Amino-3-[(1,2-dicarboxyethyl)sulfanyl]propanoic Acid

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To synthesize four stereoisomers of 2-amino-3-[(1,2-dicarboxyethyl)sulfanyl]propanoic acid (ADC), (*RS*)-mercaptosuccinic acid [(*RS*)-MSA] was optically resolved using (1*S*,2*S*)-2-amino-1-phenyl-1,3-propanediol and (*R*)- and (*S*)-1-phenylethylamine as resolving agents to yield (*R*)- and (*S*)-MSA with optical purities of 100%. In addition, the racemic structures of 1-propylammonium and 1-butylammonium salts of (*RS*)-MSA were examined based on melting point, solubility, infrared spectra, and binary and ternary phase diagrams, with the aim of optical resolution by preferential crystallization of (*RS*)-MSA. Results indicated that the 1-butylammonium salt of (*RS*)-MSA [(*RS*)-BA salt] exists as a conglomerate and that the 1-propylammonium salt forms a racemic compound. Optical resolution by preferential crystallization of (*RS*)-BA salt yielded (*R*)- and (*S*)-BA salts with optical purities of over 90%. The (*R*)- and (*S*)-MSA obtained by optical resolution were condensed with (*R*)- and (*S*)-2-amino-3-chloropropanoic acid hydrochlorides to give (2*R*,1′*R*)-, (2*S*,1′*S*)-, (2*R*,1′*S*)-, and (2*S*,1′*R*)-ADC. In addition, these stereoisomers were also synthesized from D- and L-cysteine and optically active bromosuccinic acid.

Key words 2-amino-3-[(1,2-dicarboxyethyl)sulfanyl]propanoic acid; mercaptosuccinic acid; optical resolution; conglomerate; preferential crystallization; separation of diastereoisomeric mixture

2-Amino-3-[(1,2-dicarboxyethyl)sulfanyl]propanoic acid (ADC) has been isolated from human urine, guinea pig kidney, calf lens, and *Asparagus officinalisare*.[1—3] ADC from the fungus *Amanita pantherina* has been reported to show antagonistic action against *N*-methyl-D-aspartic acid-sensitive glutamate receptors in rat brain and spinal motoneurons.[4] ADC has been synthesized by electrophilic addition of L-cysteine (L-Cys) to (*E*)-ethenedioic acid (fumaric acid).[1,2,4] By this method, ADC is obtained as a diastereoisomeric mixture of (2*R*,1′*R*)- and (2*R*,1′*S*)-ADC, and it is difficult to completely separate the mixture into both diastereoisomers. Therefore, we attempted to prepare the four stereoisomers of ADC (Fig. 1) by nucleophilic substitution reaction.

We designed a method to synthesize ADC by reaction of (*R*)- and (*S*)-mercaptosuccinic acids [(*R*)- and (*S*)-MSA] with (*R*)- and (*S*)-2-amino-3-chloropropanoic acid hydrochlorides [(*R*)- and (*S*)-ACP·HCl] and by reaction of D- and L-Cys with (*R*)- and (*S*)-bromosuccinic acids [(*R*)- and (*S*)-BSA] (Chart 1). L-Cys is commercially available, D-Cys can be obtained from L-Cys by the literature method,[5] and (*R*)- and (*S*)-BSA by optical resolution of (*RS*)-BSA.[6] (*R*)- and (*S*)-ACP·HCl were synthesized starting from L- and D-serine (L- and D-Ser), respectively.[7,8] Although (*RS*)-MSA is commercially available and has been employed as a metallochromic indicator for volumetric estimation of copper ion,[9] it is difficult to obtain optically active MSA. Therefore, we attempted to obtain both MSA enantiomers by optical resolution. Preferential crystallization and separation of a diastereoisomeric mixture has been employed for optical resolution.[10—12] We first attempted to optically resolve (*RS*)-MSA using (*R*)- and (*S*)-1-phenylethylamine [(*R*)- and (*S*)-PEA] and (1*S*,2*S*)-2-

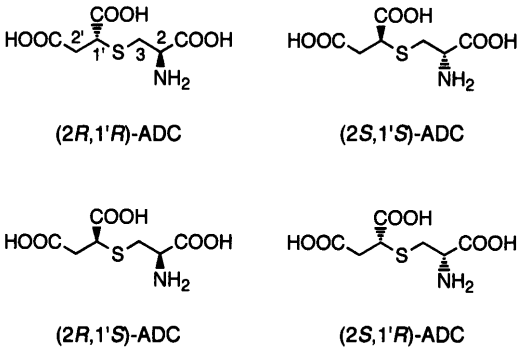

Fig. 1. Four Stereoisomers of 2-Amino-3-[(1,2-dicarboxyethyl)sulfanyl]propanoic Acid (ADC)

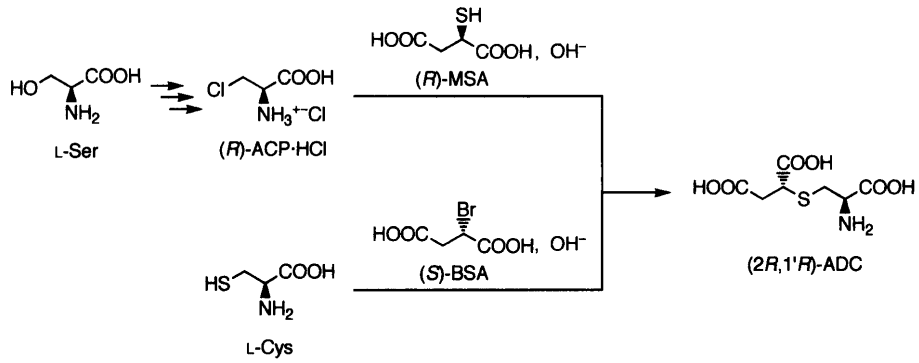

Chart 1. Synthetic Routes to Optically Active 2-Amino-3-[(1,2-dicarboxyethyl)sulfanyl]propanic Acid (ADC)

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amino-1-phenyl-1,3-propanediol [(*S*)-APP] as resolving agents. We also attempted to optically resolve (*RS*)-MSA by preferential crystallization. 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, can be optically resolved by preferential crystallization. Since (*RS*)-MSA seemed to form a racemic compound which cannot be optically resolved by preferential crystallization, we examined the racemic structures of some organic ammonium salts of (*RS*)-MSA to screen for a salt that exists as a conglomerate. The 1-butylammonium salt of (*RS*)-MSA was thus concluded to exist as a conglomerate. Therefore, we attempted to optically resolve the 1-butylammonium salt by preferential crystallization to obtain both MSA enantiomers. (*R*)- and (*S*)-MSA obtained by optical resolution were then subjected to reaction with (*R*)- and (*S*)-ACP·HCl to synthesize the four stereoisomers of ADC.

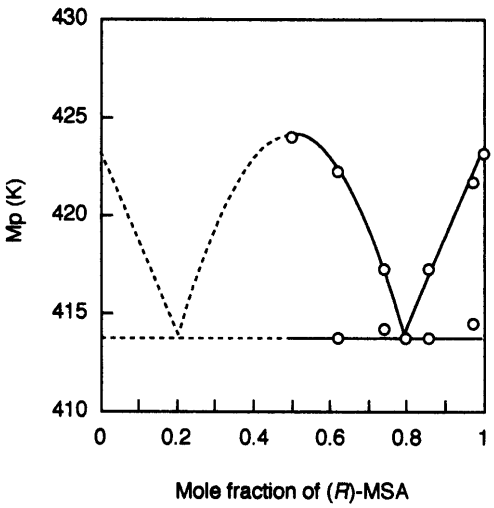

Fig. 2. Binary Melting Point Phase Diagram of Mercaptosuccinic Acid

Results and Discussion

Optical Resolution of (*RS*)-Mercaptosuccinic Acid by Separation of Diastereoisomeric Salt When (*RS*)-MSA was optically resolved using an equimolar amount of (*S*)-PEA as the resolving agent in 1-propanol, the (*R*)-MSA·(*S*)-PEA salt was preferentially crystallized as the less soluble diastereoisomeric salt. IR and ^{1}H-NMR spectra showed that the (*R*)-MSA·(*S*)-PEA salt was composed of equimolar amounts of (*R*)-MSA and (*S*)-PEA and showed IR absorption bands at 1717 cm^{-1}, due to a free carboxy group, and at 1540 cm^{-1} due to a COO^- group. (*S*)-MSA·(*S*)-PEA salt was obtained from the filtrate as a syrupy residue. (*R*)-MSA·(*S*)-PEA salt was recrystallized from 1-propanol and an aqueous solution of the salt was then treated with cation-exchange resin to give (*R*)-MSA with 100% optical purity in 66% yield, based on half the starting amount of (*RS*)-MSA, $[\alpha]_D^{20}$ +64.8° (c=1.00, ethanol). On the other hand, the (*S*)-MSA·(*S*)-PEA salt was treated with cation-exchange resin without purification to give (*S*)-MSA with an optical purity of 62%, $[\alpha]_D^{20}$ −40.1° (c=1.00, ethanol). The (*S*)-MSA obtained was again treated with an equimolar amount of (*R*)-PEA to obtain the (*S*)-MSA·(*R*)-PEA salt as the less soluble diastereoisomeric salt. After recrystallization from 1-propanol, followed by treatment with cation-exchange resin, optically pure (*S*)-MSA was obtained in 75% yield.

When (*S*)-APP was used as the resolving agent, better optical resolution was obtained than with optically active PEA. In our previous study (*RS*)-BSA, which is the same dibasic acid as (*RS*)-MSA, was demonstrated to form a diastereoisomeric salt composed of 1-molar amount of BSA and 2-molar amounts of (*S*)-APP.[6] Therefore, (*S*)-APP was used at double the molar amount of (*RS*)-MSA in the optical resolution. (*R*)-MSA·(*S*)-APP salt crystallized as the less soluble diastereoisomeric salt from methanol solution. After collecting this salt by filtration, the (*S*)-MSA·(S)-APP salt was obtained from the filtrate as a syrupy residue. (*RS*)- and (*R*)-MSA showed IR absorption bands at 1698 cm^{-1} due to free carboxy groups. On the other hand, (*R*)-MSA·(*S*)-APP salt showed a band at 1558 cm^{-1} due to COO^- groups, but no band at around 1700 cm^{-1}. Therefore, the IR spectra and elemental analyses demonstrated that (*R*)-MSA·(*S*)-APP salt was composed of 1-molar amount of MSA and 2-molar amounts of (*S*)-APP. (*R*)-MSA·(*S*)-APP and (*S*)-MSA·(*S*)-APP salts were then treated with cation-exchange resin in aqueous solution. The (*R*)- and (*S*)-MSA thereby obtained were recrystallized from water to give optically pure (*R*)- and (*S*)-MSA in 81 and 77% yields, respectively. However, it should be noted that (*S*)-APP is not commercially available at present.

Racemic Structure of Organic Ammonium Salt with (*RS*)-Mercaptosuccinic Acid (*RS*)-MSA has a slightly higher melting point than (*R*)-MSA and shows a different IR spectrum from (*R*)-MSA. Since racemic compounds are known to have the above characteristics,[10,11] (*RS*)-MSA is determined to form a racemic compound. This result was also supported by the binary melting point phase diagram of MSA, as shown in Fig. 2.[10,11]

Salts of (*RS*)- and (*R*)-MSA were formed with several organic amines to screen for racemic salts existing as conglomerates. Amongst the organic ammonium salts of (*RS*)-MSA that were prepared, the 1-propylammonium salt [(*RS*)-PA salt] and 1-butylammonium salt [(*RS*)-BA salt] were obtained as crystalline salts. The racemic salts were demonstrated by ^{1}H-NMR spectra to be composed of equimolar amounts of MSA and amines; (*RS*)-PA and (*RS*)-BA salts also showed IR absorption bands at 1688 and 1700 cm^{-1} due to free carboxy groups and at 1576 and 1635 cm^{-1} due to COO^- groups, respectively. These (*RS*)-salts had lower melting points than the corresponding (*R*)-salts, and conglomerates are known to have such melting point characteristics.[10,11] However, in the binary melting point phase diagrams, as shown in Figs. 3 and 4, the mole ratio of (*R*)-PA salt at the eutectic point was 0.65 (eutectic temperature, 349 K), whereas the BA salt had a eutectic point of 0.5 (racemic composition). The IR spectrum of the (*RS*)-BA salt was identical to that of the (*R*)-BA salt, whereas the (*RS*)-PA salt showed an IR spectrum different from the (*R*)-PA salt. In addition, the (*RS*)-BA salt was more soluble than the (*R*)-BA salt, as described in the experimental section. The ternary solubility phase diagram also showed the expected features for a conglomerate,[10,11] as shown in Fig. 5. The above results indicated that (*RS*)-BA salt exists as a conglomerate and that (*RS*)-PA salt forms a racemic compound.

Optical Resolution by Preferential Crystallization of

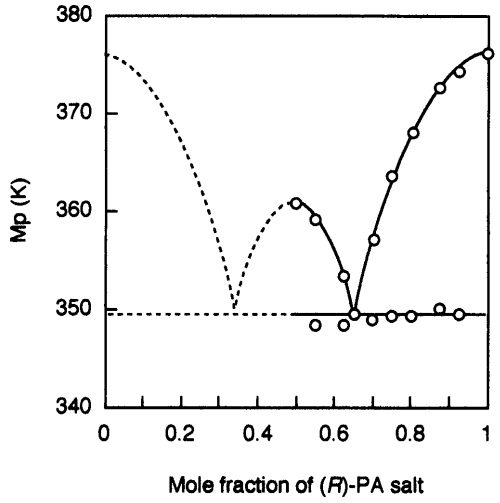

Fig. 3. Binary Melting Point Phase Diagram of 1-Propylammonium Salt of Mercaptosuccinic Acid

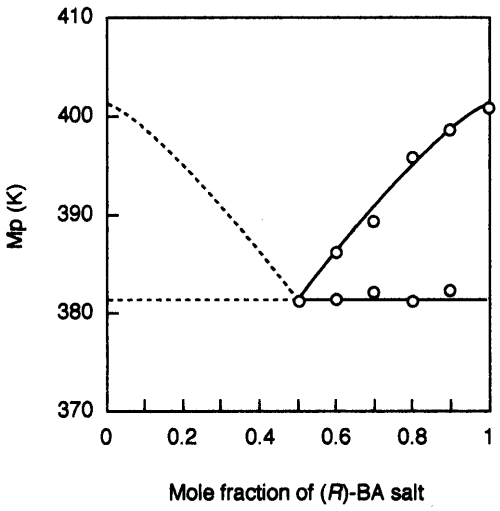

Fig. 4. Binary Melting Point Phase Diagram of 1-Butylammonium Salt of Mercaptosuccinic Acid

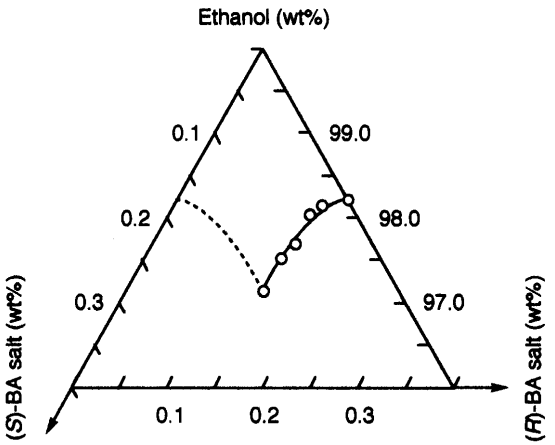

Fig. 5. Ternary Solubility Phase Diagram of 1-Butylammonium Salt of Mercaptosuccinic Acid

Conditions: solvent, ethanol; temperature, 10 °C.

the 1-Butylammonium Salt of (*RS*)-Mercaptosuccinic Acid (*RS*)-BA salt was optically resolved by preferential crystallization in ethanol at 10 °C. To optimize conditions,

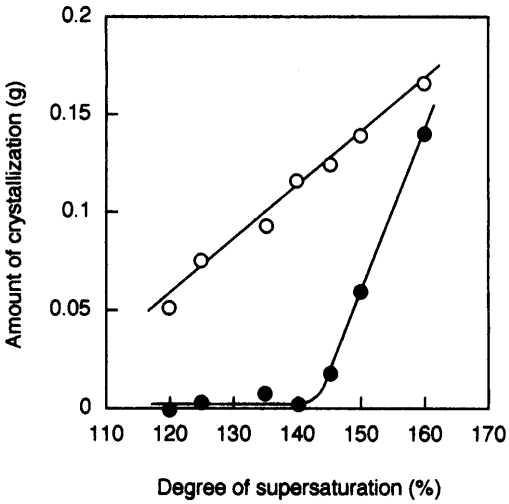

Fig. 6. Relationship between Amount of Crystallization and Degree of Supersaturation in Optical Resolution of (*RS*)-1-Butylammonium Salt of Mercaptosuccinic Acid

BA salt: 1-butylammonium salt. Conditions: (*RS*)-BA salt, 1.402 (120% supersaturation), 1.460 (125%), 1.577 (135%), 1.636 (140%), 1.694 (145%), 1.752 (150%). and 1.869 g (160%); seed crystals, 0.030 g of (*R*)-BA salt; solvent, 50 cm^3 of ethanol; stirring time, 30 min; temperature, 10 °C. Amount of crystallization: ○, (*R*)-BA salt; ●, (*S*)-BA salt.

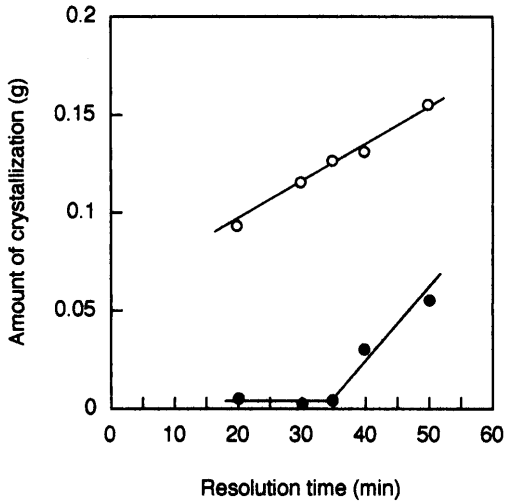

Fig. 7. Relationship between Amount of Crystallization and Resolution Time in Optical Resolution of (*RS*)-1-Butylammonium Salt of Mercaptosuccinic Acid

BA salt: 1-butylammonium salt. Conditions: (*RS*)-BA salt, 1.636 g (140% supersaturation); seed crystals, 0.030 g of (*R*)-BA salt; solvent, 50 cm^3 of ethanol; stirring time, 20—50 min; temperature, 10 °C. Amount of crystallization: ○, (*R*)-BA salt; ●, (*S*)-BA salt.

the optical resolution was conducted by stirring 120—160% supersaturated solutions for 20—50 min; (*R*)-BA salt (0.030 g) was employed as seed crystals. The results are shown in Figs. 6 and 7.

When 120—140% supersaturated solutions were employed, (*R*)-BA salts with optical purities of 90—100% were obtained after a resolution time of 30 min (Fig. 6). When 140 and 150% supersaturated solutions were employed, optical resolution gave (*R*)-BA salts with low optical purities. From these results, optical resolution of the 140% supersaturated solution was carried out for resolution times of 20—50 min (Fig. 7). The unseeded (*S*)-BA salt began to crystallize rapidly at 40 min, but not during the first 35 min. Therefore, optical resolution at 35 min gave the (*R*)-BA salt with an op-

Table 1. Successive Optical Resolution by Preferential Crystallization of the 1-Butylammonium Salt of (*RS*)-Mercaptosuccinic Acid[a]

Run	Added amount of (*RS*)-BA salt (g)	Operation amounts of (*R*)- and (*S*)-BA salt[b] (g)		Resolution time (min)	BA salt obtained		
		(*R*)-BA salt	(*S*)-BA salt		*Yield*[c] (g)	*OP*[d] (%)	*DR*[e] (%)
1	1.636	0.818	0.818	35	(*R*) 0.160	94.5	51.7
2	0.130	0.758	0.879	40	(*S*) 0.184	96.6	50.2
3	0.154	0.832	0.805	30	(*R*) 0.173	92.9	52.8
4	0.143	0.767	0.871	25	(*S*) 0.201	91.4	53.7

a) Conditions: seed crystals 0.030 g of (*R*)- or (*S*)-BA salt; solvent, 50 cm^3 of ethanol; temperature, 10 °C. *b*) The operation amounts in runs 2, 3, and 4 were calculated from the results in runs 1, 2, and 3, respectively. *c*) The *Yield* is the sum of the amounts of the crystallized BA salt and seed crystals. *d*) *OP*, optical purity. *e*) *DR*, degree of resolution.

tical purity of 94.5% at the highest degree of resolution (52%). Based on the above results, successive optical resolution was attempted by stirring the 140% supersaturated solution, as the initial solution, for 35 min. These results are summarized in Table 1.

Optical resolution afforded (*R*)- and (*S*)-BA salts with optical purities of 91—97% at over 50% degree of resolution. The obtained (*R*)- and (*S*)-BA salts were recrystallized from ethanol and then treated with cation-exchange resin to give optically pure MSA enantiomers.

Syntheses of Four Stereoisomers of 2-Amino-3-[(1,2-dicarboxyethyl)sulfanyl]propanoic Acid The four stereoisomers of ADC have already been obtained by reaction of D- and L-Cys with fumaric acid1,[2,4] followed by chromatographic separation of the diastereoisomeric mixtures.[4] However, the obtained ADC did not appear to be completely separated into each individual diastereoisomer. Therefore, (*R*)- and (*S*)-BSA were first condensed with D- and L-Cys under alkaline conditions and a nitrogen atmosphere to give (2*R*,1′*R*)-, (2*S*,1′*S*)-, (2*R*,1′*S*)-, and (2*S*,1′*R*)-ADC in 50—61% yields. Although all stereoisomers of ADC formed good crystalline adducts with ethanol, residual solvent could be removed by repeated evaporation of aqueous solutions of the adducts *in vacuo*, followed by drying of the residues over diphosphorus pentoxide. The ^{1}H-NMR and IR spectra of the obtained ADC isomers were identical to those reported previously.[4] The ADC samples obtained in this work showed specific rotations whose absolute values were larger than those of ADC from Cys and fumaric acid.[4] In addition, the absolute values of the specific rotations of the obtained ADC isomers agreed with those of the corresponding enantiomers. BSA reacted with Cys *via* a S$_N$2 mechanism; for example, the reaction of (*S*)-BSA with L-Cys afforded (2*R*,1′*R*)-ADC. However, the reaction of BSA with Cys may have proceeded with partial epimerization at the C-1′ position, if the S$_N$2 reaction is in competition with a S$_N$1 mechanism. Therefore, the four stereoisomers of ADC were synthesized from the (*R*)- and (*S*)-MSA obtained by optical resolution and (*R*)- and (*S*)-ACP·HCl in 42—55% yields, since these reactions do not have the possibility of partial epimerization at the C-1′ position. The obtained ADC showed specific rotations with absolute values slightly larger than those of the corresponding ADC from BSA and Cys. Based on their specific rotations, the ADC samples obtained from BSA and Cys were estimated to have optical purities of 96.5%. Therefore, the reaction of BSA and Cys was estimated to proceed with inversion plus slight epimerization at the C-1′ position.

Two diastereoisomers of ADC that were separated from *Amanita pantherina* were deduced to have (2*R*,1′*R*)- and (2*R*,1′*S*)-configurations by comparing the specific rotation of (*R*)-3-(methylsulfanyl)succinamide with that of L-Cys.[4] The absolute configurations were clarified by the specific rotations of the ADC samples obtained here. The four stereoisomers of ADC synthesized from MSA and ACP·HCl will be useful as authentic samples for determination of unknown configurations of natural ADC.

Experimental

General Specific rotations were measured at 589 nm with a Horiba Seisakusho SEPA-200 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-NMR spectra were recorded on a JNM-FX270 FT NMR SYSTEM with sodium 3-(trimethylsilyl)propanesulfonate (DSS) as an internal standard. Chemical shifts are reported in δ units downfield from DSS. The refractive indexes were measured with a Shimadzu refractometer Abbe 3L. Melting points were measured with a Yanaco MP-500 D micro melting point apparatus.

D-Cys was prepared from L-Cys by asymmetric transformation[5]; $[\alpha]_D^{20}$ −6.5° (c=2.00, 5 mol dm^{-3} HCl). L-Cys was purchased from Wako Pure Chemicals Ind. (*R*)- and (*S*)-ACP·HCl were synthesized starting from L- and D-Ser.[7,8] (*R*)-ACP·HCl: mp 193—194 °C (dec.), $[\alpha]_D^{20}$ +10.4° (c=2.00, methanol). (*S*)-ACP·HCl: mp 192—194 °C (dec.), $[\alpha]_D^{20}$ −10.4° (c=2.00, methanol). L- and D-Ser were purchased from Wako Pure Chemicals Ind. (*S*)-APP was purchased from Tokyo Chemical Ind., Co., Ltd. and (*R*)- and (*S*)-PEA from Wako Pure Chemicals Ind. (*RS*)-BSA was purchased from Tokyo Chemical Ind. Co., Ltd. and was recrystallized from water, mp 163—164 °C. (*R*)- and (*S*)-BSA were obtained by optical resolution of (*RS*)-BSA.[6] (*R*)-BSA: mp 178—179 °C, $[\alpha]_D^{20}$ +73.5° (c=1.00, ethyl acetate). (*S*)-BSA: mp 178—179 °C, $[\alpha]_D^{20}$ −73.5° (c=1.00, ethyl acetate). The (*RS*)-MSA purchased from Tokyo Chemical Ind., Co., Ltd. was recrystallized from water, mp 150—151 °C. IR (KBr) cm^{-1}: 2910, 1698, 1425, 1313, 1176, 939, 677, 612, 549, 447.

Optical Resolution with (1*S*,2*S*)-2-Amino-1-phenyl-1,3-propanediol (*RS*)-MSA (13.5 g, 90.0 mmol) and (*S*)-APP (30.1 g, 180 mmol) were dissolved in 125 cm^3 of methanol. After stirring the solution for 2.5 h at room temperature, the precipitated (*R*)-MSA·(*S*)-APP salt (21.4 g) was collected by filtration and dried. The filtrate was evaporated *in vacuo* at 30 °C to give (*S*)-MSA·(*S*)-APP salt as a syrupy residue. (*R*)-MSA·(*S*)-APP salt was recrystallized from 90 cm^3 of methanol to give the purified salt (20.7 g). To solutions of (*R*)-MSA·(*S*)-APP and (*S*)-MSA·(*S*)-APP salts in 45 cm^3 of water was added Amberlite IR-120B ion exchange resin in the H^+ form (2.5 g per 1 g of the salt), and the mixtures allowed to stand for 1 d at room temperature with occasional stirring. After removing the ion exchange resin by filtration, the filtrates were evaporated in vacuo to give (*R*)- and (*S*)-MSA as a crude solid residues, in 6.31 and 6.35 g yields, respectively. The (*R*)- and (*S*)-MSA thus obtained were recrystallized from 15 cm^3 of water.

(*R*)-MSA·(*S*)-APP Salt: Yield 20.7 g, mp 129—131 °C, $[\alpha]_D^{20}$ +34.7° (c=1.00, water). IR (KBr) cm^{-1}: 3182, 2924, 1558, 1408, 1377, 1056, 760, 700, 546. *Anal.* Calcd for $C_{22}H_{32}N_2O_8S \cdot H_2O$: C, 52.58; H, 6.82; N, 5.57. Found: C, 52.37; H, 6.87; N, 5.39.

(*R*)-MSA: Yield 5.47 g (81.0%, based on half of the starting amount of (*RS*)-MSA), mp 149—150 °C, $[\alpha]_D^{20}$ +64.8° (c=1.00, ethanol) (lit.,[13] $[\alpha]_D^{17}$ +64.4° (ethanol)). IR (KBr) cm^{-1}: 2920, 1698, 1420, 1311, 1178, 940, 679,

613, 549, 438. (*S*)-MSA: yield 5.22 g (77.3%), mp 149—150 °C, $[\alpha]_D^{20}$ −64.8° (*c*=1.00, ethanol) (lit.,[13] $[\alpha]_D^{17}$ −64.8° (ethanol)). The IR spectrum of (*S*)-MSA was virtually identical to that of (*R*)-MSA.

Optical Resolution with Optically Active 1-Phenylethylamine (*RS*)-MSA (15.0 g, 100 mmol) and (*S*)-PEA (12.1 g, 100 mmol) were dissolved in 80 cm^3 of 1-propanol. After standing overnight at −10 °C, the precipitated (*R*)-MSA·(*S*)-PEA salt (14.5 g) was collected by filtration, washed with a small amount of cold 1-propanol, and dried. This salt was then recrystallized three times from 1-propanol (40 cm^3) to obtain the purified (*R*)-MSA·(*S*)-PEA salt (9.25 g). After removal of the (*R*)-MSA·(*S*)-PEA salt, the filtrates were combined and evaporated *in vacuo* at 30 °C to give (*S*)-MSA·(*S*)-PEA salt as a syrupy residue. Solutions of the purified (*R*)-MSA·(S)-PEA salt and crude (*S*)-MSA·(*S*)-PEA salt in 100 cm^3 of water were treated with Amberlite IR-120B ion exchange resin in the H^+ form in a similar manner to that described for MSA·(*S*)-APP salt to give (*R*)- and (*S*)-MSA. The obtained (*S*)-MSA (9.36 g, 62.3 mol) and (*R*)-PEA (7.55 g, 62.3 mol) were dissolved in 40 cm^3 of 1-propanol and the resulting solution allowed to stand overnight at −10 °C. The precipitates (12.2 g) was recrystallized from 40 cm^3 of 1-propanol to give purified (*S*)-MSA·(*R*)-PEA salt (10.9 g). Treatment of this salt with Amberlite IR-120B gave (*S*)-MSA.

(*R*)-MSA·(*S*)-PEA Salt: Yield 9.25 g, $[\alpha]_D^{20}$ +21.6° (*c*=1.00, water). IR (KBr) cm^{-1}: 2875, 1717, 1619, 1540, 1404, 1389, 1266, 1220, 1185, 770, 704. ^{1}H-NMR (270 MHz, 1 mol dm^{-3} DCl, DSS) δ: 7.49 (5H, s, C_6H_5), 4.57 (1H, q, *J*=6.9 Hz, –C<u>H</u>(NH_3^+)–), 3.82 (1H, dd, *J*=5.9, 8.6 Hz, –C<u>H</u>(SH)–), 3.03 (1H, dd, *J*=8.6, 17.3 Hz, –C<u>H</u>H–), 2.92 (1H, dd, *J*=5.9, 17.2 Hz, –CH<u>H</u>–), 1.66 (3H, d, *J*=6.9 Hz, $-CH_3$).

(*R*)-MSA: Yield 4.98 g (66.4%, based on half of the starting amount of (*RS*)-MSA), $[\alpha]_D^{20}$ +64.8° (*c*=1.00, ethanol). (*S*)-MSA: yield 5.65 g (75.3%), $[\alpha]_D^{20}$ −64.8° (*c*=1.00, ethanol).

Preparation of Organic Ammonium Salts of Mercaptosuccinic Acid (*RS*)-MSA (1.50 g, 10.0 mmol) and 1-propylamine (0.591 g, 10.0 mmol) or 1-butylamine (0.731 g, 10.0 mmol) were dissolved in 5 cm^3 of methanol. After adding diethyl ether (50 cm^3) to the solution, followed by stirring the mixture for 2 h at room temperature, the precipitated salt was collected by filtration. (*R*)-PA salt and (*R*)- and (*S*)-BA salts were also prepared by a method similar to that described above.

(*RS*)-PA Salt: Yield 1.68 g, mp 87—88 °C. IR (KBr) cm^{-1}: 3032, 2973, 1688, 1576, 1374, 1341, 1303, 1239, 1192, 994. ^{1}H-NMR (270 MHz, D_2O, DSS) δ: 3.66 (1H, dd, *J*=6.9, 7.8 Hz, –C<u>H</u>(SH)–), 2.95 (2H, t, *J*=7.6 Hz, –C<u>H</u>$_2$$NH_3^+$), 2.90 (1H, dd, *J*=7.8, 16.5 Hz, –C<u>H</u>HCOOH), 2.74 (1H, dd, *J*=7.6, 16.5 Hz, –CH<u>H</u>COOH), 1.66 (2H, sextet, *J*=7.6 Hz, –C<u>H</u>$_2$$CH_3$), 0.96 (3H, t, *J*=7.6 Hz, $-CH_3$). (*R*)-PA salt: yield 1.85 g, mp 102—103 °C, $[\alpha]_D^{20}$ +31.4° (*c*=1.00, water). IR (KBr) cm^{-1}: 3100, 2960, 1718, 1630, 1521, 1325, 1274, 1192, 1155, 856, 624, 484. The 1H-NMR spectrum of the (*R*)-PA salt was virtually identical with that of the (*RS*)-PA salt.

(*RS*)-BA Salt: Yield 1.93 g, mp 107—108 °C. IR (KBr) cm^{-1}: 3095, 1700, 1635, 1521, 1342, 1269, 1195, 868, 623. ^{1}H-NMR (270 MHz, D_2O, DSS) δ: 3.66 (1H, dd, *J*=6.9, 7.8 Hz, –C<u>H</u>(SH)–), 2.99 (2H, t, *J*=7.5 Hz, –C<u>H</u>$_2$$NH_3^+$), 2.90 (1H, dd, *J*=7.8, 16.6 Hz, –C<u>H</u>HCOOH), 2.74 (1H, dd, *J*=6.9, 16.5 Hz, –CH<u>H</u>COOH), 1.63 (2H, quintet, *J*=7.5 Hz, –CH_2<u>C</u>$H_2$$CH_2$–), 1.38 (2H, sextet, *J*=7.4 Hz, –CH_2<u>C</u>H_3), 0.92 (3H, t, *J*=7.4 Hz, $-CH_3$). *Anal.* Calcd for $C_8H_{17}NO_4S$: C, 43.03; H, 7.67; N, 6.27. Found: C, 43.00; H, 7.70; N, 6.23. (*R*)-BA salt: yield 2.02 g, mp 122—124 °C, $[\alpha]_D^{20}$ +29.3° (*c*=1.00, water). Found: C, 42.92; H, 7.57; N, 6.21. The IR and ^{1}H-NMR spectra of the (*R*)-BA salt were virtually identical with those of the (*RS*)-BA salt. (*S*)-BA salt: yield 2.00 g, mp 122—124 °C, $[\alpha]_D^{20}$ −29.3° (*c*=1.00, water). Found: C, 42.85; H, 7.50; N, 6.18. The IR and ^{1}H-NMR spectra of (*S*)-BA salt were virtually identical with those of (*RS*)-BA salt.

Optical Resolution by Preferential Crystallization (*RS*)-BA salt (1.402, 1.460, 1.577, 1.636, 1.694, 1.752, or 1.869 g) was dissolved in 50 cm^3 of ethanol at 40 °C to prepare 120, 125, 135, 140, 145, 150 and 160% supersaturated solutions at 10 °C. The solutions were cooled to 10 °C over a period of 30 min and then seeded with 0.030 g of (*R*)-BA salt. After stirring the mixture for 20, 30, 35, 40 or 50 min at 10 °C, (*R*)-BA salt was collected by filtration and dried. The yield of enantiomer [*YE* (g)], degree of resolution [*DR* (%)] of the obtained (*R*)-BSA salt, and the amount of crystallization [$AC_{(S)}$ and $AC_{(R)}$ (g)] were calculated from

$$YE\ (g) = [Yield\ (g) \times OP\ (\%)/100] - 0.030,$$

$$DR\ (\%) = YE\ (g) \times 100/(1/2)[\text{amount of } (RS)\text{-BA salt } (g) - 1.168],$$

$$AC_{(S)}\ (g) = (1/2)[Yield\ (g) - YE\ (g) - 0.030],$$

$$AC_{(R)}\ (g) = Yield\ (g) - AC_{(S)}\ (g) - 0.030,$$

where *OP* is the optical purity of the obtained (*R*)-BA salt and the solubility of (*RS*)-BA salt is 1.168 g in 50 cm^3 of ethanol at 10 °C. *Yield* is the sum of the amounts of the crystallized BA salt and seed crystals.

Successive Optical Resolution by Preferential Crystallization (*RS*)-BA salt (1.636 g) was dissolved in 50 cm^3 of water at 40 °C to prepare a 140% supersaturated solution at 10 °C. The solution was cooled to 10 °C over a period of 30 min and then seeded with 0.030 g of (*R*)-BA salt. After stirring the mixture for 35 min at 10 °C, (*R*)-BA salt (0.160 g) was collected by filtration and dried (run 1 in Table 1). (*RS*)-BA salt (0.130 g) was dissolved in the filtrate at 40 °C and the resulting solution was cooled to 10 °C. After adding (*S*)-BA salt (0.030 g) as seed crystals to the solution, followed by stirring the mixture for 40 min at 10 °C, (*S*)-BA salt (0.184 g) was collected by filtration and dried (run 2 in Table 1). Optical resolution was carried out at 10 °C by adding further (*RS*)-BA salt to the filtrates in a way similar to that described above; the detailed conditions are shown in runs 3 and 4 in Table 1. The degrees of resolution [*DR* (%)] of the (*R*)- and (*S*)-BA salt obtained were calculated from

$$DR\ (\%) = YE\ (g) \times 100/[\text{operation amount of } (R)\text{- or } (S)\text{-BSA } (g) - 0.584],$$

where the operation amount is the amount of (*R*)- or (*S*)-BA salt in the solution used in the optical resolution and those in runs 2—4 in Table 1 were calculated based on the yields and optical purities of the (*R*)- or (*S*)-BA salt obtained in runs 1—3, respectively. The amount for half solubility of (*RS*)-BA salt is 0.584 g in 50 cm^3 of ethanol at 10 °C.

Recrystallization and Isolation The obtained (*R*)- and (*S*)-BA salts were recrystallized from ethanol. For example, (*R*)- and (*S*)-BA salts (3.00 g) of 77% optical purity were dissolved in 29.5 cm^3 of ethanol at 40 °C. After vigorously stirring the solutions for 3 h at 10 °C, the crystallized (*R*)- and (*S*)-BA salts were collected by filtration. (*R*)-BA salt: yield 2.15 g, $[\alpha]_D^{20}$ +29.3° (*c*=1.00, water). (*S*)-BA salt: yield 2.21 g, $[\alpha]_D^{20}$ −29.3° (*c*=1.00, water).

Solutions of the purified (*R*)- and (*S*)-BA salts (2.00 g) in 15 cm^3 of water were treated with Amberlite IR 120B, in a way similar to MSA·(*S*)-APP salt, to give (*R*)- and (*S*)-MSA. (*R*)-MSA: yield 1.21 g, $[\alpha]_D^{20}$ +64.8° (*c*=1.00, ethanol). (*S*)-MSA: yield 1.25 g, $[\alpha]_D^{20}$ −64.8° (*c*=1.00, ethanol).

General Procedure for Synthesis of 2-Amino-3-[(1,2-dicarboxyethyl)sulfanyl]propanoic Acids from Optically Active Mercaptosuccinic Acid and 2-Amino-3-chloropropanoic Acid Hydrochloride. (2*R*,1′*R*)-2-Amino-3-[(1,2-dicarboxyethyl)sulfanyl]propanoic Acid To a solution of (*R*)-ACP·HCl (3.20 g, 20.0 mmol) and (*R*)-MSA (3.00 g, 20.0 mmol) in 16 cm^3 of water was added dropwise 5 mol dm^{-3} aqueous sodium hydroxide (20 cm^3), maintaining pH 12—13, over a period of 20 h at room temperature under a nitrogen atmosphere. After stirring the solution for a further 2 d, 5 mol dm^{-3} hydrochloric acid (24 cm^3) was added to the solution. After evaporation *in vacuo*, followed by addition of 70 cm^3 of ethanol to the residue, sodium chloride was removed by filtration. The filtrate was adjusted with triethylamine to pH 3 and crude (2*R*,1′*R*)-ADC collected by filtration, washed with ethanol, and dried. (2*R*,1′*R*)-ADC was washed twice by stirring in 60 cm^3 of ethanol for 2 h at 10 °C to give (2*R*,1′*R*)-ADC as an adduct with ethanol. After dissolving (2*R*,1′*R*)-ADC in 30 cm^3 of water, followed by evaporation *in vacuo* at 50 °C, the residue was dried over diphosphorus pentoxide for 24 h; this procedure was repeated three times to thoroughly remove ethanol.

(2*R*,1′*R*)-ADC: Yield 2.29 g (48.3%), mp 103—105 °C (lit.,[4] 125—126 °C), $[\alpha]_D^{20}$ +55.0° (*c*=0.500, water) (lit.,[4] $[\alpha]_D$ +30.0° (*c*=0.447, water)). IR (KBr) cm^{-1}: 3150—2800, 1716, 1620, 1503, 1398, 1226. ^{1}H-NMR (270 MHz, D_2O, DSS) δ: 4.09 (1H, dd, *J*=4.5, 7.4 Hz, H-2), 3.81 (1H, dd, *J*=6.4, 8.4 Hz, H-1′), 3.34 (1H, dd, *J*=4.3, 14.9 Hz, H-3), 3.22 (1H, dd, *J*=7.4, 14.7 Hz, H-3), 2.99 (1H, dd, *J*=8.6, 17.2 Hz, H-2′), 2.88 (1H, dd, *J*=6.2, 17.3 Hz, H-2′). *Anal.* Calcd for $C_7H_{11}NO_6S$: C, 35.44; H, 4.67; N, 5.90. Found: C, 35.44; H, 4.77; N, 5.91.

The following analogs, (2*S*,1′*S*)-, (2*R*,1′*S*)-, and (2*S*,1′*R*)-ADC were prepared from (*S*)-ACP·HCl and (*S*)-MSA, (*R*)-ACP·HCl and (*S*)-MSA, and (*S*)-ACP·HCl and (*R*)-MSA, respectively, in a similar manner to (2*R*,1′*R*)-ADC.

(2*S*,1′*S*)-ADC: Yield 2.22 g (46.8%), mp 104—105 °C (lit.,[4] 115—117 °C), $[\alpha]_D^{20}$ −55.0° (*c*=0.500, water) (lit.,[4] $[\alpha]_D$ −34.1° (*c*=0.270, water)). The ^{1}H-NMR and IR spectra were virtually identical to those of (2*R*,1′*R*)-ADC. Found: C, 35.44; H, 4.76; N, 6.02.

(2*R*,1′*S*)-ADC: Yield 1.99 g (42.0%), mp 98—101 °C (lit.,[4] 125—127 °C), $[\alpha]_D^{20}$ −110° (*c*=0.500, water) (lit.,[4] $[\alpha]_D$ −96.4° (*c*=0.671, water)). IR (KBr) cm^{-1}: 3180—2800, 1716, 1622, 1505, 1400, 1225. ^{1}H-

NMR (270 MHz, D_2O, DSS) δ: 4.09 (1H, dd, J=4.0, 8.4 Hz, H-2), 3.80 (1H, dd, J=6.3, 8.6 Hz, H-1'), 3.38 (1H, dd, J=4.1, 14.7 Hz, H-3), 3.13 (1H, dd, J=8.4, 14.7 Hz, H-3), 3.00 (1H, dd, J=8.7, 17.3 Hz, H-2'), 2.84 (1H, dd, J=6.3, 17.5 Hz, H-2'). Found: C, 35.44; H, 4.81; N, 5.84.

(2*S*,1'*R*)-ADC: Yield 2.61 g (55.1%), mp 97—100 °C (lit.,[4] 115—116 °C), $[\alpha]_D^{20}$ +110° (c=0.500, water) (lit.,[4] $[\alpha]_D$ +84.0° (c=0.617, water)). The ^{1}H-NMR and IR spectra were virtually identical to those of (2*R*,1'*S*)-ADC. Found: C, 35.44; H, 4.78; N, 5.89.

Synthesis of 2-Amino-3-[(1,2-dicarboxyethyl)sulfanyl]propanoic Acids from Optically Active Bromosuccinic Acid and Cysteine Four stereoisomers of ADC were synthesized by reaction of (*R*)- and (*S*)-BSA (3.94 g, 20.0 mmol) with D- and L-Cys (2.42 g, 20.0 mmol), in a similar manner to that used to obtain ADC from ACP·HCl and MSA. (2*R*,1'*R*)-, (2*S*,1'*S*)-, (2*R*,1'*S*)-, and (2*S*,1'*R*)-ADC were obtained from (*S*)-BSA and L-Cys, (*R*)-BSA and D-Cys, (*R*)-BSA and L-Cys, and (*S*)-BSA and D-Cys, respectively.

(2*R*,1'*R*)-ADC: Yield 2.88 g (60.8%), mp 96—99 °C, $[\alpha]_D^{20}$ +52.1° (c= 0.500, water). The ^{1}H-NMR and IR spectra were virtually identical to those of (2*R*,1'*R*)-ADC from (*R*)-ACP·HCl and (*R*)-MSA. Found: C, 35.44; H, 4.74; N, 5.92.

(2*S*,1'*S*)-ADC: Yield 2.73 g (57.6%), mp 94—97 °C, $[\alpha]_D^{20}$ −52.1° (c= 0.500, water). The ^{1}H-NMR and IR spectra were virtually identical to those of (2*R*,1'*R*)-ADC from (*R*)-ACP·HCl and (*R*)-MSA. Found: C, 35.44; H, 4.85; N, 5.85.

(2*R*,1'*S*)-ADC: Yield 2.35 g (49.5%), mp 93—95 °C, $[\alpha]_D^{20}$ −107° (c= 0.500, water). The ^{1}H-NMR and IR spectra were virtually identical to those of (2*R*,1'*S*)-ADC from (*R*)-ACP·HCl and (*S*)-MSA. Found: C, 35.41; H, 4.81; N, 5.87.

(2*S*,1'*R*)-ADC: Yield 2.79 g (58.9%), mp 94—96 °C, $[\alpha]_D^{20}$ +107° (c= 0.500, water). The ^{1}H-NMR and IR spectra were virtually identical to those of (2*R*,1'*S*)-ADC from (*R*)-ACP·HCl and (*S*)-MSA. Found: C, 35.29; H, 4.83; N, 5.82.

Solubility and Ternary and Binary Phase Diagrams (*RS*)- or (*R*)-BA salt (4.00 g) was dissolved in 50 cm^3 of ethanol at 40 °C. After vigorously stirring the solution at 10 °C, samples of the solution were removed from the mixture, avoiding contamination with the solid BA salt, and the refractive index was measured at 25 °C. The mixture was stirred at 10 °C until the refractive index showed a constant value. The solubility was determined based on the calibration curves previously prepared. Solubility of (*RS*)-BA salt at 10 °C: 2.336 g (100 cm^3 ethanol)$^{-1}$. Solubility of (*R*)-BA salt at 10 °C: 1.460 g (100 cm^3 ethanol)$^{-1}$.

For preparing a ternary solubility phase diagram of the BA salt, the solubilities of mixtures of (*RS*)- and (*S*)-BA salts were measured at 10 °C, in a similar way to the method described above. After the refractive index reached a constant value, the solid BA salt was filtered off, thoroughly dried, and the specific rotation was measured. The amounts of (*R*)- and (*S*)-BA salts in the solution were calculated based on the solubility and the specific rotation of the solid BA salt.

For prepaing binary melting point phase diagrams, the melting points of mixtures composed of (*RS*)- and (*R*)-PA salts, (*RS*)- and (*R*)-BA salts, and (*RS*)- and (*R*)-MSA were measured accurately. The binary phase diagrams were prepared from their temperatures at the beginning and end of melting.

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