Synthesis of Optically Active Homocysteine from Methionine and Its Use in Preparing Four Stereoisomers of Cystathionine

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In order to synthesize four stereoisomers of cystathionine (CYT), D- and L-homocysteines (D- and L-Hcy) were synthesized from methionine (Met) by a facile procedure. L-Met was reacted with dichloroacetic acid in concentrated hydrochloric acid under reflux to give (4*S*)-1,3-thiazane-2,4-dicarboxylic acid hydrochloride [(4*S*)-TDC·HCl]. L-Hcy was obtained by treatment of (4*S*)-TDC·HCl with hydroxylamine. D-Hcy was also synthesized from D-Met *via* (4*R*)-TDC·HCl intermediate. The obtained D- and L-Hcy were condensed with (*R*)- and (*S*)-2-amino-3-chloropropanoic acid hydrochlorides under alkaline conditions to give four stereoisomers of CYT.

Key words homocysteine; cystathionine; 1,3-thiazane-2,4-dicarboxylic acid; methionine

L-Cystathionine [(2*S*,2′*R*)-CYT; (2*S*,2′*R*)-2-amino-4-(2′-amino-2′-carboxyethylsulfanyl)butanoic acid] is a natural intermediate in the conversion of L-methionine (L-Met) to L-cysteine (L-Cys) *via* L-homocysteine [L-Hcy; (*S*)-2-amino-4-mercaptobutanoic acid] *in vivo*.[1] (2*S*,2′*R*)-CYT has been isolated from the leaves of *Astragalus pectinatus* and other plants, and found in the urine of cystathionuric patients.[2,3] The first synthesis of CYT, as a mixture of stereoisomers, was conducted by reacting methyl (*RS*)-2-amino-3-chloropropanoate with DL-Hcy.[4] The single stereoisomers of CYT were synthesized as follows: (2*S*,2′*R*)-, (2*R*,2′*S*)-, and (2*S*,2′*S*)-CYT were synthesized by reacting DL- or L-Hcy, which are generated from DL-Met[5] and *S*-benzyl-L-homocysteine[6] by treatment with sodium in liquid ammonia, with 2-acetamido-2-propenoic acid[5] or diethyl 2-acetamido-2-[(dimethylamino)methyl]malonate iodide.[6] L-Cystine is reacted with (*S*)- and (*R*)-3,6-bis(2-chloroethyl)piperazine-2,5-dione in liquid ammonia containing sodium to give (2*S*,2′*R*)- and (2*R*,2′*R*)-CYT.[7] Four stereoisomers of CYT have also been obtained by addition of *N*-acetyl-D- and -L-cysteines to 2-hydroxy-3-butenenitrile, followed by repetitions of recrystallization and enzymatic or chromatographic separation.[8] However, the obtained CYT stereoisomers did not be completely separated into each individual diastereoisomer. In addition, (2*S*,2′*R*)-CYT was synthesized by reacting L-Hcy with (*R*)-2-amino-3-chloropropanoic acid [(*R*)-ACP].[9] This reaction seemed to be the most simple procedure for obtaining CYT as a single stereoisomer. However, the other three stereoisomers of CYT have not yet been synthesized from optically active Hcy and ACP. Therefore, we attempted to synthesize four stereoisomers of CYT by reacting (*R*)- and (*S*)-ACP with D- and L-Hcy (Chart 1).

(*R*)- and (*S*)-ACP are easily prepared from L- and D-serine (L- and D-Ser),[10,11] whereas L- and D-Hcy, non-proteinogenic α-amino acids, are difficult to be available. L-Hcy is bio-synthesized as an intermediate in the conversion of L-Met to L-Cys.[1] L-Hcy is also useful as a key compound for the syntheses of pharmaceutical chemicals, such as benzo-fused azepinone and piperidinone compounds which are selective angiotensin-converting enzyme inhibitors.[12] Although homocystine which is reduced with sodium in liquid ammonia to Hcy has been synthesized by refluxing L-Met in sulfuric acid, the obtained homocystine undergoes partial racemization.[13] Furthermore, L-Met is refluxed in hydroiodic acid to give homocysteine thiolactone hydroiodide, which generates Hcy under alkaline conditions.[14] Although we attempted to synthesize the hydroiodide, the obtained product underwent complete racemization. Optically active Hcy is produced as an *N*-acyl derivative of homocysteine thiolactone by optical resolution of its racemate.[15] We also reported that optically active Hcy was obtained *via* asymmetric transformation of (*RS*)-1,3-thiazane-4-carboxylic acid [(*RS*)-THA] which is prepared from racemic homocysteine thiolactone hydrochloride.[16] However, (*RS*)-THA was not obtained in a high yield because of the production of 3,6-bis(2-mercaptoethyl)piperazine-2,5-dione as a by-product in a ring-opening reaction of (*RS*)-homocysteine thiolactone hydrochloride under alkaline conditions. We sought to avoid the use of the dangerous reagents such as sodium and liquid ammonia and to synthesize D- and L-Hcy in optically pure forms from Met by a more facile procedure. The obtained D- and L-Hcy were reacted with (*R*)- and (*S*)-ACP hydrochlorides [(*R*)- and (*S*)-ACP·HCl] to synthesize four stereoisomers of CYT.

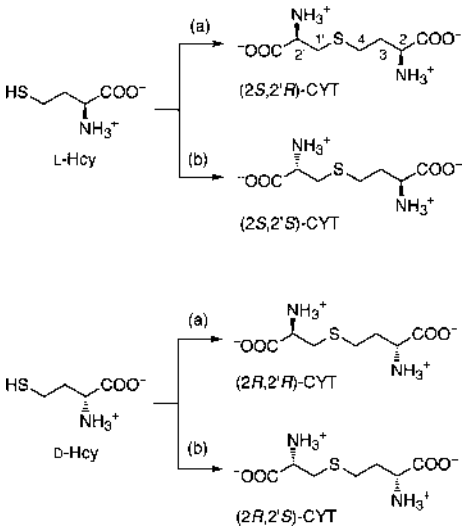

Chart 1. Syntheses of Four Stereoisomers of Cystathionine (CYT)

Reagents: (a) (*R*)-2-amino-3-chloropropanoic acid hydrochloride [(*R*)-ACP·HCl], OH^-; (b) (*S*)-ACP·HCl, OH^-.

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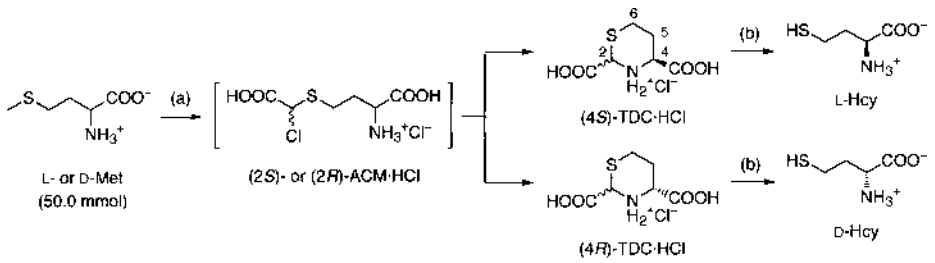

Chart 2. Synthetic Routes to Optically Active Homocysteine (Hcy)

Reagents: (a) dichloroacetic acid (DCA), conc. HCl, reflux; (b) hydroxylamine hydrochloride, triethylamine.

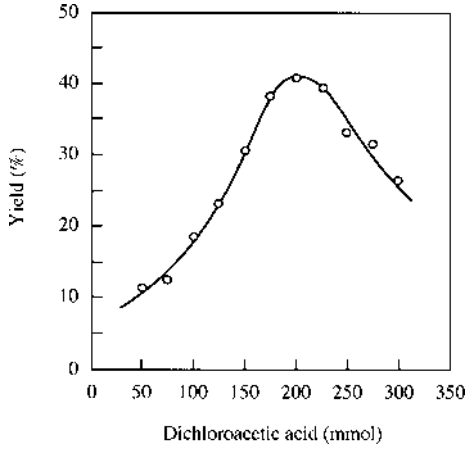

Fig. 1. Influence of Amount of Dichloroacetic Acid on the Synthesis of (4*S*)-1,3-Thiazane-2,4-dicarboxylic Acid Hydrochloride [(4*S*)-TDC · HCl]

Conditions: L-Met, 50.0 mmol; dichloroacetic acid (DCA), 50—300 mmol; conc. HCl, 100 cm^3; refluxing for 6 h.

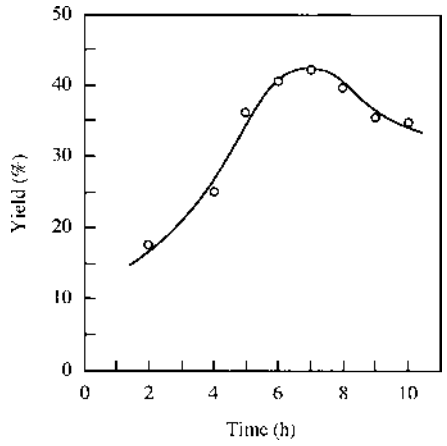

Fig. 2. Influence of Reaction Time on the Synthesis of (4*S*)-1,3-Thiazane-2,4-dicarboxylic Acid Hydrochloride [(4*S*)-TDC · HCl]

Conditions: L-Met, 50.0 mmol; dichloroacetic acid (DCA), 200 mmol; conc. HCl, 100 cm^3; refluxing for 2—10 h.

Results and Discussion

We attempted to construct a 1,3-thiazane ring from Met because the ring is easily opened by treatment with hydroxylamine to generate Hcy.[16] 2-Amino-4-(1-haloalkylsulfanyl)-butanoic acid seemed to be a precusor of THA derivative. L-Met has been reported to yield *S*-benzyl-L-homocysteine[17] and *S*-carboxymethyl-L-homocysteine[18] by refluxing with benzyl chloride and chloroacetic acid, respectively, in concentrated hydrochloric acid. Therefore, we first attempted to synthesize (2*S*)-2-amino-4-(carboxychloromethylsulfanyl)-butanoic acid hydrochloride [(2*S*)-ACM · HCl], which might yield 1,3-thiazane-2,4-dicarboxylic acid (TDC) by intramolecular condensation, by refluxing equimolar amounts (50.0 mmol) of L-Met and dichloroacetic acid (DCA) for 6 h in concentrated hydrochloric acid (Chart 2).[19]

The colorless product was crystallized from the reaction solution. However, its values from elemental analysis agreed with the values calculated for (4*S*)-TDC hydrochloride [(4*S*)-TDC · HCl], but not with those for (2*S*)-ACM · HCl. In addition, the ^{1}H- and ^{13}C-NMR spectra were identical to those of (4*S*)-TDC · HCl, which was separately synthesized by reacting L-Hcy[16] with glyoxylic acid monohydrate (GLA · H_2O). Based on the above results, the product was determined to be (4*S*)-TDC · HCl.

Next, L-Met (50.0 mmol) was reacted with 75—300 mmol of DCA for 2—10 h, as shown in Figs. 1 and 2, because the reaction using equimolar amounts of L-Met and DCA gave (4*S*)-TDC · HCl in a low yield (11%).[19]

When L-Met (50.0 mmol) was reacted with 200 mmol of DCA for 7 h, (4*S*)-TDC · HCl was obtained in the highest yield (42%), as shown in Fig. 2. However, the yield was not as high as expected. In this reaction, the reaction solution was tinged with brown-red during the reaction and the color deepened with the elapse of time and with increasing the starting amount of DCA. These facts seem to suggest the decomposition of the products because of the reaction under reflux in concentrated hydrochloric acid.

(4*S*)-TDC · HCl may be obtained as a mixture of two diastereoisomers, less-soluble and more-soluble ones, due to the generation of a new chiral center at the 2-position. The formation of the more-soluble diastereoisomer may be an another cause for the low yield because the more-soluble diastereoisomer has the potential of dissolving in the reaction solution. In the ^{1}H-NMR spectrum of the (4*S*)-TDC · HCl obtained by reaction of L-Hcy with GLA · H_2O, the singlet signals due to the 2-H protons of the both diastereoisomers appeared at 5.39 and 5.23 ppm at an intensity ratio of 1 : 66.5; the intensity ratio suggests that the reaction gives one diastereoisomer of (4*S*)-TDC · HCl in 97% *de*. On the other hand, in the reaction of L-Met with DCA, the (4*S*)-TDC · HCl crystallized from the reaction solution was judged to be a single diastereoisomer, because its ^{1}H-NMR spectrum showed the singlet signal due to the 2-H proton only at 5.23 ppm. Therefore, after the crystallized (4*S*)-TDC · HCl was filtered off, the filtrate was evaporated to dryness *in vacuo* to examine by its ^{1}H-NMR spectrum whether the

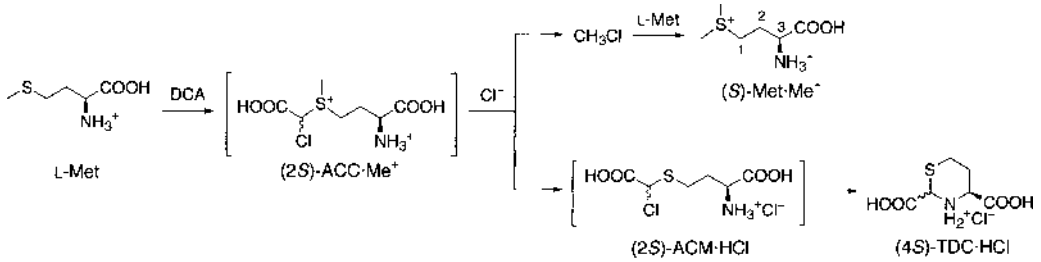

Chart 3. Formation of (4*S*)-1,3-Thiazane-2,4-dicarboxylic Acid Hydrochloride [(4*S*)-TDC·HCl] by Reaction of L-Methionine (L-Met) with Dichloroacetic Acid (DCA) in Concentrated Hydrochloric Acid

more-soluble diastereoisomer was present or not in the solution. The ^{1}H-NMR spectrum of the oily residue suggested that the reaction of L-Met and DCA gave selectively only one diastereoisomer of (4*S*)-TDC·HCl, because the proton signals due to the another diastereoisomer were not observed; the spectrum showed the proton signal due to the unreacted DCA at 6.27 ppm and those due to L-Met. In addition, the proton signals of (*S*)-(3-amino-3-carboxypropyl)dimethylsulfonium ion (L-Met·Me$^+$; L-methionine dimethyl sulfonium ion) were distinctly observed at 3.6—3.4 ppm (2H, m, 2-H) and 2.97 ppm (6H, s, $-S^+(CH_3)_2$), although the signals due to 1-H and 3-H protons could not be determined because of overlap with those due to the other compounds; the ^{1}H-NMR spectrum was compared with those of L-Met and L-Met·Me$^+$ iodide, which was separately synthesized by reacting L-Met with iodomethane.[20] When L-Met (50.0 mmol) was reacted with 200 mmol of DCA for 7 h, about 35% of the starting L-Met was estimated to be converted to L-Met·Me$^+$, based on the yield of (4*S*)-TDC·HCl and the intensity ratio of the methyl protons of L-Met·Me$^+$ and the unreacted L-Met. Therefore, (4*S*)-TDC·HCl is thought to be not obtained in a high yield owing to the formation of L-Met·Me$^+$, but the low yield is not owing to formation of the more-soluble diastereoisomer.

Based on the above results, the formation of (4*S*)-TDC·HCl can be estimated as follows (Chart 3): The reaction of L-Met with DCA seems to give (2*S*)-ACM·HCl and chloromethane, *via* formation of (2*S*)-(3-amino-3-carboxypropyl)(carboxychloromethyl)methylsulfonium ion [(2*S*)-ACC·Me$^+$].[17] (2*S*)-ACM·HCl immediately undergoes intramolecular condensation to afford (4*S*)-TDC·HCl. On the other hand, chloromethane is reacted with L-Met to give L-Met·Me$^+$.

The obtained (4*S*)-TDC·HCl was treated with hydroxylamine hydrochloride and triethylamine in ethanol without further purification to give L-Hcy in yields of over 80%. D-Hcy was also synthesized from D-Met *via* a (4*R*)-TDC·HCl intermediate, in a manner similar to that of L-Hcy.

As described above, D- and L-Hcy were easily synthesized starting from D- and L-Met. Although the yields of D- and L-Hcy were not very high (35% from Met), this synthesis seems to be a facile procedure for obtaining D- and L-Hcy in relatively large quantities from Met.

L-Hcy was condensed with (*S*)-ACP·HCl under alkaline conditions to give (2*S*,2′*S*)-CYT. The obtained (2*S*,2′*S*)-CYT showed a lower specific rotation than the reported value; $[\alpha]_D^{20}$ +21.2° (*c*=1.00, 1 mol dm^{-3} HCl) [lit, $[\alpha]_D^{26}$ +25.3° (*c*=1, 1 mol dm^{-3} HCl),[6] $[\alpha]_D^{21}$ +23.5° (*c*=1, 1 mol dm^{-3} HCl)[8]]. The lowering of the specific rotation did not seem to be due to epimerization, because the proton signals due to the diastereoisomer of (2*S*,2′*S*)-CYT were not observed in the ^{1}H-NMR spectrum. The crude (2*S*,2′*S*)-CYT was recrystallized from water to give (2*S*,2′*S*)-CYT, which showed a specific rotation of $[\alpha]_D^{20}$ +25.6° (*c*=1.00, 1 mol dm^{-3} HCl). Furthermore, (2*S*,2′*R*)-, (2*R*,2′*S*)-, and (2*R*,2′*R*)-CYT were prepared from (*R*)-ACP·HCl and L-Hcy, (*S*)-ACP·HCl and D-Hcy, and (*R*)-ACP·HCl and D-Hcy, respectively.

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 by the KBr disk method. ^{1}H- and ^{13}C-NMR spectra were recorded on a JNM-FX270 FT NMR system (270 MHz for ^{1}H and 67.5 MHz for ^{13}C) in deuterium oxide with sodium 3-(trimethylsilyl)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-500D micro melting point apparatus.

L- and D-Met and DCA were purchased from Wako Pure Chemicals Ind. (*R*)- and (*S*)-ACP·HCl were synthesized from L- and D-Ser;[11] (*R*)-ACP·HCl, $[\alpha]_D^{20}$ +10.4° (*c*=2.00, methanol); (*S*)-ACP·HCl, $[\alpha]_D^{20}$ −10.4° (*c*=2.00, methanol). L- and D-Ser were purchased from Wako Pure Chemicals Ind. L-Hcy ($[\alpha]_D^{20}$ +27.2° (*c*=1.00, water)) was obtained *via* asymmetric transformation of the (*RS*)-THA prepared from (*RS*)-homocysteine thiolactone hydrochloride,[16] which was purchased from Tokyo Kasei Kogyo Co.

Syntheses of (4*S*)- and (4*R*)-1,3-Thiazane-2,4-dicarboxylic Acid Hydrochlorides from Methionine DCA (6.45—38.7 g, 50.0—300 mmol) was added to a solution of L-Met (7.46 g, 50.0 mmol) in concentrated hydrochloric acid (100 cm^3). After refluxing for 6 h (104 °C), the solution was concentrated *in vacuo* at 60 °C to 30 cm^3 and then the mixture was allowed to stand overnight at 5 °C. The precipitated (4*S*)-TDC·HCl was collected by filtration, washed thoroughly with tetrahydrofuran (THF), and dried. After evaporation of the filtrate *in vacuo*, followed by adding ethanol (10 cm^3) to the oily residue, the mixture was allowed to stand overnight at 5 °C. The further (4*S*)-TDC·HCl was filtered off, washed thoroughly with THF, and dried.

(4*S*)-TDC·HCl obtained by reaction using 50.0 mmol of DCA: Yield 1.29 g (11.3%); mp 178—179 °C (decomp); $[\alpha]_D^{20}$ +6.5° (*c*=1.00, water). (4*S*)-TDC·HCl obtained by reaction using 75.0 mmol of DCA: Yield 1.44 g (12.6%); $[\alpha]_D^{20}$ +6.9° (*c*=1.00, water). (4*S*)-TDC·HCl obtained by reaction using 100 mmol of DCA: Yield 2.11 g (18.5%); $[\alpha]_D^{20}$ +6.9° (*c*=1.00, water). (4*S*)-TDC·HCl obtained by reaction using 125 mmol of DCA: Yield 2.63 g (23.1%); $[\alpha]_D^{20}$ +6.5° (*c*=1.00, water). (4*S*)-TDC·HCl obtained by reaction using 150 mmol of DCA: Yield 3.49 g (30.6%); $[\alpha]_D^{20}$ +6.3° (*c*=1.00, water). (4*S*)-TDC·HCl obtained by reaction using 175 mmol of DCA: Yield 4.34 g (38.1%); $[\alpha]_D^{20}$ +6.7° (*c*=1.00, water). (4*S*)-TDC·HCl obtained by reaction using 200 mmol of DCA: Yield 4.63 g (40.6%); mp 181—183 °C (decomp); $[\alpha]_D^{20}$ +6.6° (*c*=1.00, water). (4*S*)-TDC·HCl obtained by reaction using 225 mmol of DCA: Yield 4.48 g (39.3%); $[\alpha]_D^{20}$ +6.8° (*c*=1.00, water). (4*S*)-TDC·HCl obtained by reaction using 250 mmol of DCA: Yield 3.79 g (33.2%); $[\alpha]_D^{20}$ +6.8° (*c*=1.00, water). (4*S*)-TDC·HCl obtained by reaction using 275 mmol of DCA: Yield 3.61 g (31.6%); $[\alpha]_D^{20}$ +6.8° (*c*=1.00, water). (4*S*)-TDC·HCl obtained by reaction using 300 mmol of DCA: Yield 3.00 g (26.3%); $[\alpha]_D^{20}$ +6.5° (*c*=1.00, water).

(4*S*)-TDC·HCl was synthesized by reacting L-Met (7.46 g, 50.0 mmol)

with DCA (25.8 g, 200 mmol) for 2—10 h in concentrated hydrochloric acid, in a similar manner to the above.

(4*S*)-TDC·HCl obtained by reacting for 2 h: Yield 1.99 g (17.5%); $[\alpha]_D^{20}$ +6.7° (c=1.00, water). (4*S*)-TDC·HCl obtained by reacting for 4 h: Yield 2.86 g (25.1%); $[\alpha]_D^{20}$ +6.7° (c=1.00, water). (4*S*)-TDC·HCl obtained by reacting for 5 h: Yield 4.12 g (36.1%); $[\alpha]_D^{20}$ +6.7° (c=1.00, water). (4*S*)-TDC·HCl obtained by reacting for 7 h: Yield 4.80 g (42.1%); $[\alpha]_D^{20}$ +6.8° (c=1.00, water). (4*S*)-TDC·HCl obtained by reacting for 8 h: Yield 4.53 g (39.7%); $[\alpha]_D^{20}$ +6.9° (c=1.00, water). (4*S*)-TDC·HCl obtained by reacting for 9 h: Yield 4.03 g (35.4%); $[\alpha]_D^{20}$ +6.9° (c=1.00, water). (4*S*)-TDC·HCl obtained by reacting for 10 h: Yield 3.97 g (34.8%); $[\alpha]_D^{20}$ +6.8° (c=1.00, water).

The spectra and analytical data of (4*S*)-TDC·HCl obtained by reaction using 200 mmol of DCA for 6 h: IR (KBr) cm^{-1}: 2936, 2795, 2578, 2455, 1763, 1741, 1637, 1529, 1426, 1390, 1350, 1290, 1266, 1228, 1191, 1092, 1055, 1041, 1006, 995, 932, 909, 795, 762, 576. ^{1}H-NMR (D_2O) δ: 5.23 (1H, s, 2-H), 4.11 (1H, dd, J=2.8, 12.8 Hz, 4-H), 3.26—3.15 (1H, m, 5-H), 3.05—2.97 (1H, m, 5-H), 2.73—2.64 (1H, m, 6-H), 2.13—1.97 (1H, m, 6-H). ^{13}C-NMR (D_2O) δ: 172.9 (4-C-COOH), 170.3 (2-C-COOH), 61.3 (2-C), 60.3 (4-C), 29.7 (5-C), 29.4 (6-C). *Anal.* Calcd for $C_6H_{10}ClNO_4S$: C, 31.65; H, 4.43; N, 6.15%. Found: C, 31.43; H, 4.25; N, 6.14%. The ^{1}H- and ^{13}C-NMR spectra of the (4*S*)-TDC·HCl obtained under other conditions were virtually identical to the above spectra.

(4*R*)-TDC·HCl was synthesized by reacting D-Met (7.46 g, 50.0 mmol) with DCA (25.8 g, 200 mmol) for 6 h in concentrated hydrochloric acid, in a similar manner to that described for (4*S*)-TDC·HCl.

(4*R*)-TDC·HCl: Yield 4.73 g (41.5%); mp 176—179 °C (decomp.); $[\alpha]_D^{20}$ −6.5° (c=1.00, water). The IR, ^{1}H-, and ^{13}C-NMR spectra were virtually identical to those of (4*S*)-TDC·HCl. Found: C, 31.71; H,4.35; N, 6.14%.

Synthesis of (4*S*)-1,3-Thiazane-2,4-dicarboxylic Acid Hydrochloride from L-Homocysteine A mixture of L-Hcy (0.676 g, 5.00 mmol) and GLA·H_2O (0.460 g, 5.00 mmol) in 10 cm^3 of acetic acid was stirred for 2 h at 30 °C. After evaporation *in vacuo* at 50 °C, followed by dissolving the residue in 1 mol dm^{-3} hydrochloric acid (10 cm^3), the solution was further evaporated *in vacuo* to obtain (2*S*)-TDC·HCl as the residue. (2*S*)-TDC·HCl was washed by stirring in 50 cm^3 of THF for 30 min at room temperature, collected by filtration, and dried; yield 0.674 g (59.1%); $[\alpha]_D^{20}$ +5.8° (c=1.00, water). ^{1}H-NMR (D_2O) δ: 5.39 (s, 2-H), 5.23 (1H, s, 2-H), 4.7—4.6 (m, 4-H), 4.11 (1H, dd, J=2.8, 12.8 Hz, 4-H), 3.26—3.15 (1H, m, 5-H), 3.05—2.97 (1H, m, 5-H), 2.73—2.64 (1H, m, 6-H), 2.6—2.5 (m, 6-H), 2.3—2.4 (m, 6-H), 2.13—1.97 (1H, m, 6-H). The IR spectrum was identical to that of (4*S*)-TDC·HCl from L-Met.

Preparation of L- and D-Homocysteine (4*S*)- or (4*R*)-TDC·HCl (4.55 g, 20.0 mmol) was dissolved in 100 cm^3 of ethanol by adjusting with triethylamine to pH 7. To the solution was added 20 cm^3 of 0.5 mol dm^{-3} ethanolic hydroxylamine hydrochloride under reflux (78 °C) and then the mixture was immediately adjusted with triethylamine to pH 6—7. After refluxing the mixture for 25 min, 0.5 mol dm^{-3} ethanolic hydroxylamine hydrochloride (20 cm^3) was added to the mixture and then the mixture was adjusted with triethylamine to pH 6—7. After further refluxing the mixture for 1 h, followed by standing overnight at room temperature, the precipitated L- or D-Hcy was collected by filtration, washed with methanol, and dried.

L-Hcy: Yield 2.20 g (81.5%); mp 247—249 °C (decomp.); $[\alpha]_D^{20}$ +27.2° (c=1.00, 1 mol dm^{-3} HCl) (lit.,[16] $[\alpha]_D^{20}$ +26.8° (c=1, 1 mol dm^{-3} HCl)). IR (KBr) cm^{-1}: 3156, 2940, 1618, 1586, 1508, 1406, 1347, 1319, 1272, 1250, 1190, 1157, 966, 838, 754, 700, 654, 542. ^{1}H-NMR (D_2O) δ: 3.89 (1H, dd, J=5.9, 7.0 Hz, 2-H), 2.73—2.56 (2H, m, 4-H), 2.25—2.07 (2H, m, 3-H). ^{13}C-NMR (D_2O) δ: 176.7 (–COOH), 56.1 (2-C), 37.2 (3-C), 22.4 (4-C). *Anal.* Calcd for $C_4H_9NO_2S$: C, 35.54; H, 6.71; N, 10.36%. Found: C, 35.56; H, 6.41; N, 10.29%.

D-Hcy: Yield 2.27 g (84.1%); mp 248—250 °C (decomp.); $[\alpha]_D^{20}$ −27.2° (c=1.00, 1 mol dm^{-3} HCl) (lit.,[16] $[\alpha]_D^{20}$ −26.8° (c=1, 1 mol dm^{-3} HCl)). The IR, ^{1}H-, and ^{13}C-NMR spectra were virtually identical to those of L-Hcy. *Anal.* Found: C, 35.46; H, 6.46; N, 10.27%.

Preparation of (*S*)-(3-Amino-3-carboxypropyl)dimethylsulfonium Iodide After adding iodomethane (25.5 g, 180 mmol) and 300 cm^3 of methanol to a solution of L-Met (17.9 g, 120 mmol) in 600 cm^3 of water, the solution was stirred for 6 d at room temperature. The solution was evaporated *in vacuo* to give crude (*S*)-L-Met·Me^+ iodide as the residue. After refluxing a suspension of L-Met·Me^+ iodide (33.2 g) in 200 cm^3 of methanol for 30 min, L-Met·Me^+ iodide was collected by filtration and washed with methanol; yield 20.2 g; $[\alpha]_D^{20}$ +16.5° (c=1.00, water). After dissolving the obtained L-Met·Me^+ iodide in 120 cm^3 of water, followed by adding 400 cm^3 of methanol to the solution, the mixture was allowed stand overnight at 5 °C. The precipitated L-Met·Me^+ iodide was collected by filtration, washed with methanol, and dried; yield 12.9 g (37.0%); mp 155—159 °C (decomp.); $[\alpha]_D^{20}$ +17.0° (c=1.00, water). IR (KBr) cm^{-1}: 3021, 2996, 2907, 2826, 1616, 1567, 1542, 1438, 1410, 1372, 1342, 1328, 1274, 1050, 992, 871, 782, 762, 547, 441. ^{1}H-NMR (D_2O) δ: 3.90 (1H, dd, J=6.4, 6.9 Hz, 3-H), 3.60—3.39 (2H, m, 2-H), 2.98 (6H, s, $-S^+(CH_3)_2$), 2.38 (2H, dd, J=7.1, 15.0 Hz, 1-H). ^{13}C-NMR (D_2O) δ: 174.9 (–COOH), 56.9 (3-C), 55.3 (1-C), 42.0 (2-C), 27.6 ($-S^+(CH_3)_2$). *Anal.* Calcd for $C_6H_{14}INO_2S$: C, 24.75; H, 4.85; N, 4.81%. Found: C, 24.63; H, 4.89; N, 4.83%.

General Procedure for Syntheses of Four Stereoisomers of Cystathionine To a solution of L-Hcy (0.676 g, 5.00 mmol) in 5 mol dm^{-3} aqueous sodium hydroxide (4 cm^3) was added (*S*)-ACP·HCl (0.800 g, 5.00 mmol), stirring for 1 d at room temperature. After allowing to stand the solution overnight, 5 mol dm^{-3} hydrochloric acid (4 cm^3) was added to the solution. After evaporation *in vacuo*, followed by addition of 20 cm^3 of methanol to the residue, sodium chloride was removed by filtration. The filtrate was adjusted with triethylamine to pH 6 and crude (2*S*,2′*S*)-CYT was collected by filtration, washed with ethanol, and dried; yield 0.858 g; $[\alpha]_D^{20}$ +21.2° (c=1.00, 1 mol dm^{-3} HCl). After dissolving the (2*S*,2′*S*)-CYT in 150 cm^3 of water under refluxing, followed by standing the solution for 5 d at 5 °C, the precipitated (2*S*,2′*S*)-CYT was collected by filtration, and dried.

(2*S*,2′*S*)-CYT: Yield 0.493 g (44.4%); mp 285—288 °C (decomp.); $[\alpha]_D^{20}$ +25.6° (c=1.00, 1 mol dm^{-3} HCl) (lit., $[\alpha]_D^{26}$ +25.3° (c=1, 1 mol dm^{-3} HCl),[6] $[\alpha]_D^{21}$ +23.5° (c=1, 1 mol dm^{-3} HCl)[8]). IR (KBr) cm^{-1}: 3000, 2951, 2715, 1651, 1626, 1581, 1519, 1416, 1404, 1362, 1343, 1312, 1277, 1214, 1166, 1102, 1074, 920, 885, 846, 782, 757, 718, 699, 689, 554. ^{1}H-NMR (1 mol dm^{-3} DCl) δ: 4.36 (1H, dd, J=4.5, 7.1 Hz, 2′-H), 4.26 (1H, t, J=6.4 Hz, 2-H), 3.21 (1H, dd, J=4.6, 14.8 Hz, 1′-H), 3.10 (1H, dd, J=7.1, 14.9 Hz, 1′-H), 2.82 (2H, t, J=7.3 Hz, 4-H), 2.41—2.15 (2H, m, 3-H). ^{13}C-NMR (1 mol dm^{-3} DCl) δ: 174.1 (–COOH), 173.0 (–COOH), 54.8 (2′-C), 54.1 (2-C), 33.6 (1′-C), 32.0 (3-C), 29.7 (4-C). *Anal.* Calcd for $C_7H_{14}N_2O_4S$: C, 37.83; H, 6.35; N, 12.60%. Found: C, 37.68; H, 6.16; N, 12.54%.

The following analogs, (2*S*,2′*R*)-, (2*R*,2′*S*)-, and (2*R*,2′*R*)-CYT were prepared from (*R*)-ACP·HCl and L-Hcy, (*S*)-ACP·HCl and D-Hcy, and (*R*)-ACP·HCl and D-Hcy, respectively, in a similar manner to (2*S*,2′*S*)-CYT.

(2*S*,2′*R*)-CYT: Yield 0.403 g (36.3%); mp 282—283 °C (decomp.) (lit.,[8] over 300 °C); $[\alpha]_D^{20}$ +24.5° (c=1.00, 1 mol dm^{-3} HCl) (lit., $[\alpha]_D^{24}$ +23.5° (1 mol dm^{-3} HCl),[8] $[\alpha]_D^{24}$ +23.7° (1 mol dm^{-3} HCl)[9]). IR (KBr) cm^{-1}: 3037, 2657, 1632, 1545, 1423, 1394, 1344, 1314, 1217, 1155, 1072, 882, 842, 751, 586, 552, 528. ^{1}H-NMR (1 mol dm^{-3} DCl) δ: 4.34 (1H, dd, J=4.6, 7.0 Hz, 2′-H), 4.24 (1H, t, J=6.4 Hz, 2-H), 3.27 (1H, dd, J=4.6, 15.2 Hz, 1′-H), 3.16 (1H, dd, J=7.1, 15.0 Hz, 1′-H), 2.82 (2H, t, J=7.4 Hz, 4-H), 2.41—2.18 (2H, m, 3-H). ^{13}C-NMR (1 mol dm^{-3} DCl) δ: 173.9 (–COOH), 172.8 (–COOH), 54.8 (2′-C), 54.1 (2-C), 33.5 (1′-C), 31.9 (3-C), 29.7 (4-C). *Anal.* Found: C, 37.47; H, 6.25; N, 12.47%.

(2*R*,2′*S*)-CYT: Yield 0.391 g (35.2%); mp 281—284 °C (decomp.) (lit.,[8] over 300 °C); $[\alpha]_D^{20}$ −24.7° (c=1.00, 1 mol dm^{-3} HCl) (lit., $[\alpha]_D^{23}$ −21° (c=1, 1 mol dm^{-3} HCl),[5] $[\alpha]_D^{21}$ −23.0° (c=1, 1 mol dm^{-3} HCl)[8]). The IR, ^{1}H-, and ^{13}C-NMR spectra were virtually identical to those of (2*S*,2′*R*)-CYT. Found: C, 37.45; H, 6.18; N, 12.53%.

(2*R*,2′*R*)-CYT: Yield 0.360 g (32.4%); mp 284—288 °C (decomp.); $[\alpha]_D^{20}$ −25.5° (c=1.00, 1 mol dm^{-3} HCl) (lit., $[\alpha]_D^{21}$ −24.5° (c=1%, 1 mol dm^{-3} HCl),[7] $[\alpha]_D^{21}$ −22° (c=1, 1 mol dm^{-3} HCl)[8]). The IR, ^{1}H-, and ^{13}C-NMR spectra were virtually identical to those of (2*S*,2′*S*)-CYT. Found: C, 37.63; H, 6.16; N, 12.50%.

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