Preparation of Optically Active Allothreonine *via* Optical Resolution by Replacing Crystallization

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An attempt was made to use a simple procedure to obtain D- and L-allothreonine (D- and L-aThr), which are non-proteinogenic α-amino acids and are useful as chiral reagents in asymmetric syntheses. DL-aThr that exists as a conglomerate was optically resolved by replacing crystallization with L-alanine (L-Ala) as an optically active co-solute. D-aThr was preferentially crystallized from an aqueous solution of DL-aThr in the presence of L-Ala, as was L-aThr in the presence of D-Ala. Furthermore, a diasteroisomeric mixture of D-aThr and L-threonine (L-Thr) and one of L-aThr and D-Thr were prepared, respectively, by epimerization of L- and D-Thr using salicylaldehyde as the catalyst in acetic acid. Based on the result of the replacing crystallization, D- and L-aThr were separated from aqueous solutions of the diastereoisomeric mixtures in the presence of L- and D-Ala. The partially resolved D- and L-aThr were recrystallized from water to yield the corresponding enantiomers in optically pure forms.

Key words allothreonine; optical resolution; alanine; threonine; epimerization

D- and L-Allothreonine [D- and L-aThr; (2*R*,3*R*)- and (2*S*,3*S*)-2-amino-3-hydroxybutanoic acid] are useful as chiral reagents in asymmetric syntheses.[1] However, D- and L-aThr, non-proteinogenic α-amino acids, are difficult to produce commercially in large quantities. Therefore, synthetic DL-aThr[2—4] has been subjected to optical resolution by separating the diastereoisomeric salts of DL-aThr derivatives to obtain the enantiomers.[5,6] In our previous paper,[7] DL-aThr was found to exist as a conglomerate and to be optically resolved by preferential crystallization and replacing crystallization of DL-aThr. The optical resolution by replacing crystallization is a procedure for obtaining an enantiomer from a conglomerate, and is achieved by allowing an optically active co-solute to coexist in a racemic supersaturated solution.[8] DL-aThr was optically resolved using 4-hydroxy-L-proline (L-Hyp) as the optically active cosolute, and D-aThr was allowed to preferentially crystallize from a supersaturated solution of DL-aThr.[7] Although L-aThr will be preferentially crystallized from the racemic solution in the presence of D-Hyp, L-Hyp is expensive and D-Hyp is not commercially available. Therefore, we selected D- and L-Ala, which are commercially available and are the most inexpensive optically active α-amino acids, as the optically active cosolutes in optical resolution by replacing crystallization of DL-aThr (Chart 1).

Optical resolution by replacing crystallization is based on different interactions between enantiomers and the optically active co-solute. Therefore, the solubilities (mole fractions) of D-, L-, and DL-aThr were first measured in the presence of L-Ala (16.0 mmol) in 100 cm^3 of water at 10 °C; these values are summarized in Table 1, together with the solubilities[7] in the absence of L-Ala.

When L-Ala was present in aqueous solutions of D-, L-, and DL-aThr, D-aThr was less soluble than L-aThr. In addition, D-, L-, and DL-aThr were each less soluble in the presence of L-

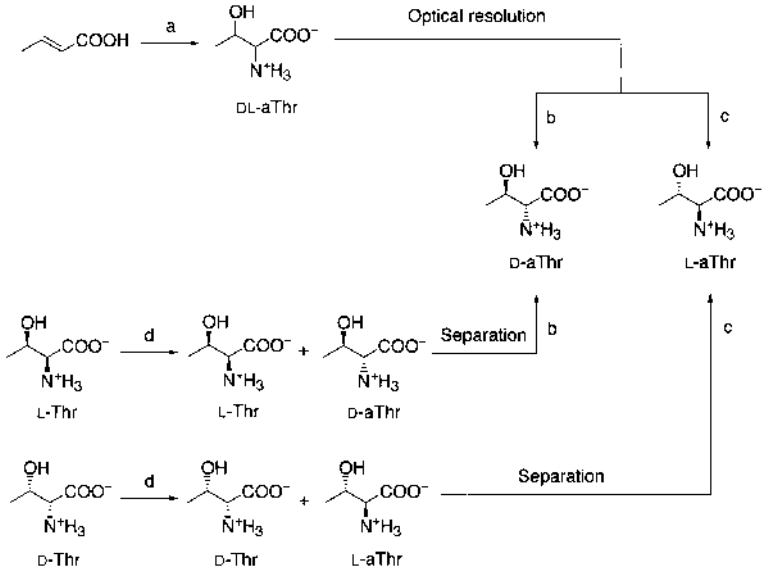

Chart 1. Preparation of Optically Active Allothreonine (aThr)

Reagents: (a) i) *N*-Bromosuccinimide, H_2O, ii) concentrated aqueous ammonia; (b) L-Ala as an optically active co-solute; (c) D-Ala as an optically active co-solute; (d) salicylaldehyde, acetic acid, 80 °C.

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Table 1. Solubilities of DL-, D-, and L-Allothreonine[a)]

Allothreonine	L-Ala as an optically active co-solute (mmol)	Solubility (g (100 cm^3 of water)$^{-1}$) [mole fraction]
DL-aThr[b)]	—[c)]	10.30 { D-aThr 5.15 [0.0077]; L-aThr 5.15 [0.0077] }
L-aThr[b)]	—[c)]	5.28 [0.0079]
DL-aThr	16.0	9.32 { D-aThr 4.51 [0.0067]; L-aThr 4.81 [0.0072] }
D-aThr	16.0	4.93 [0.0074]
L-aThr	16.0	5.06 [0.0076]

a) Conditions: Water, 100 cm^3; temperature, 10 °C. *b*) See ref. 7. *c*) None.

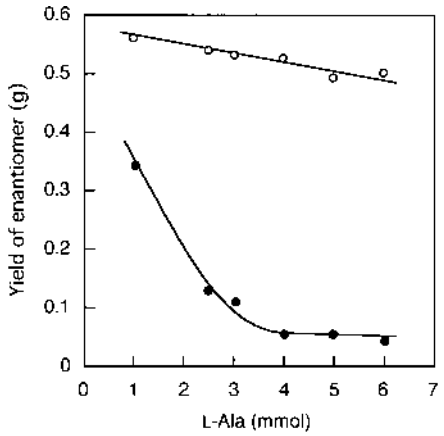

Fig. 1. Relationship between Yield of Enantiomer and L-Alanine in Optical Resolution of DL-Allothreonine

Conditions: DL-aThr, 4.384 g (36.8 mmol); solvent, 25 cm^3 of water; L-Ala, 0.0891—0.535 g (1.00—6.00 mmol); temperature, 10 °C; resolution time, 80 min. Yield of enantiomer: ○, D-aThr; ●, L-aThr.

Ala than in its absence. Therefore, a repulsive interaction is thought to occur between D-aThr and L-Ala, and between L-aThr and L-Ala, and the interaction between D-aThr and L-Ala may be stronger than that between L-aThr and L-Ala.[11)] These findings suggested that when L-Ala was present in the supersaturated aqueous solution of DL-aThr, D-aThr was preferentially crystallized from the solution.

Based on the above findings, the optical resolution of DL-aThr was attempted by stirring a mixture containing 36.8 mmol (4.384 g) of DL-aThr and 1.00—6.00 mmol of L-Ala, as the optically active co-solute, in 25 cm^3 of water for 80 min at 10 °C. The yields of enantiomers [YE_L(g) and YE_D(g)] of L- and D-aThr were calculated from

$$YE_L(g)=(1/2)[Yield(g)\times(100-OP(\%))/100] \quad (1)$$

and

$$YE_D(g)=Yield(g)-YE_L(g), \quad (2)$$

where *OP* (%) is the optical purity of obtained D-aThr, calculated based on the specific rotation of authentic D-aThr: $[\alpha]_D^{20}$ −32.8° (c=1.00, 1 mol dm^{-3} HCl).[7)] The results are shown in Fig. 1. The purity of the crystallized aThr was confirmed by its ^{1}H-NMR spectrum to be free of L-Ala.

When L-Ala was present as an optically active co-solute, D-aThr was preferentially crystallized from the racemic solution. The yield of D-aThr slightly decreased with increasing amounts of L-Ala. On the other hand, in the presence of 1.00—4.00 mmol of L-Ala, the yield of L-aThr rapidly decreased with increasing amounts of L-Ala. When a larger amount (4.00—6.00 mmol) of L-Ala was present in the racemic solution, the yield of L-aThr was approximately constant, and D-aThr of optical purity of 80—85% was obtained in yields of 0.54—0.58 g. Therefore, to optimize the conditions, the optical resolution was conducted by stirring mixtures containing 4.00 mmol of L-Ala with varying degrees of supersaturation from 174—214% for 80 min.

When 176—200% supersaturated solutions were used, L-aThr seemed to be hardly crystallized because the optical resolution afforded D-aThr of optical purities of 80—86% in yields of 0.37—0.59 g; the yield tended to increase with increasing supersaturation. When a 204% supersaturated solution was used, L-aThr began to rapidly crystallize, hence D-aThr of optical purity of 71% was obtained. In addition, the optical resolution for the 196% supersaturated solution was carried out by stirring for 70—180 min. Although almost no aThr was crystallized by stirring for 70 min, D-aThr of an optical purity of 81% was obtained in a yield of 0.580 g by stirring for 80 min. However, D-aThr, obtained by stirring for 90 min, showed a low optical purity (44%), because of the onset of rapid crystallization of L-aThr.

On the other hand, when D-Ala was used as the co-solute, L-aThr of an optical purity of 85% was obtained in a yield of 0.574 g from the solution containing DL-aThr (4.384 g) and D-Ala (4.00 mmol) in 25 cm^3 of water by stirring for 80 min.

The obtained D- and L-aThr were recrystallized from water to give optically pure D- and L-aThr. For example, optically pure D-aThr (3.90 g) was obtained from 5.00 g of D-aThr of an optical purity of 85%, and optically pure L-aThr (3.05 g) was obtained from 4.00 g of L-aThr of an optical purity of 81%, as described in the Experimental section.

Based on the above results, successive optical resolution was attempted by stirring the 196% supersaturated solution of DL-aThr with 4.00 mmol of L-Ala for 80 min. As described above for solubility, repulsive interactions are estimated to occur not only between D-aThr and L-Ala, but also between L-aThr and L-Ala. These interactions promote the crystallization of D- or L-aThr during the preferential crystallization of their enantiomers. Therefore, after optical resolution of the initial solution, a small amount of D- or L-aThr was added as seed crystals to the solution in subsequent optical resolutions. These results are summarized in Table 2. The degrees of resolution of D- and L-aThr [*DR*(%)] in Table 2 were calculated:

$$DR(\%)=[(Yield(g)\times OP(\%)/100-S_C)/\text{(operation amount of D- or L-aTh}-S_D \text{ or } S_L)]\times 100 \quad (3)$$

where S_L (1.232 g) is the solubility of L-aThr in DL-aThr in 25 cm^3 of water at 10 °C, and S_C (0.050 g) is the amount of seed crystals. The operation amounts (g) are the amounts of D- and L-aThr in the solution, and the values in runs 2—6 are calculated based on the results from runs 1—5, respectively.

A successive optical resolution was achieved to afford D-aThr of optical purities of 81—90%, and L-aThr with purities of 96 and 100% at 48—60% degrees of resolution. The partially resolved D-aThr was recrystallized from water to give optically pure D-aThr.

Next, we attempted to more simply obtain optically active aThr, based on the above result. In general, optically active

Table 2. Successive Optical Resolution by Replacing Crystallization of DL-Allothreonine[a)]

Run	Added amount of DL-aThr (g)	Operation amounts of D- and LaThr[b)] (g)		Resolution time (min)	aThr obtained		
		D-aThr	L-aThr		*Yield*[c)] (g)	Optical purity[d)] (%)	Degree of resolution (%)
1	4.384	2.192	2.192	80	D 0.580	81.4	47.7
2[e)]	0	1.666	2.138	90	L 0.552	100	55.4
3[f)]	0.500	1.916	1.886	60	D 0.507	89.6	56.6
4[e)]	0	1.485	1.860	80	L 0.428	100	60.2
5[f)]	0.380	1.675	1.672	70	D 0.315	88.2	48.2
6[e)]	0	1.429	1.653	90	L 0.296	96.1	55.7

a) Conditions: optically active co-solute, 0.356 g (4.00 mmol) of L-Ala; solvent, 25 cm^3 of water; temperature, 10 °C. *b*) The operation amounts in runs 2—6 were calculated from the results in 1—5, respectively. *c*) The *Yield* is the sum of the amounts of the crystallized aThr and seed crystals. *d*) The optical purities of D- and L-aThr obtained were calculated on the basis of the specific rotation of authentic D-aThr; lit.[7)] $[\alpha]_D^{26}$ −32.8° (c=1.00, 1 mol dm^{-3} HCl). *e*) Seed crystals, 0.050 g of L-aThr. *f*) Seed crystals, 0.050 g of D-aThr.

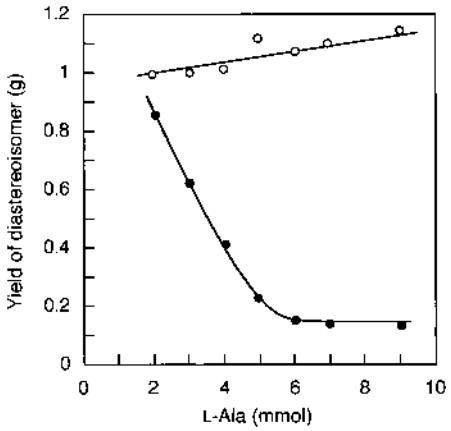

Fig. 2. Relationship between Yield of Diastereoisomer and L-Alanine in Separation of D-Allothreonine and L-Threonine

Conditions: Diastereoisomeric mixture (L-Thr in 18%*de*), 5.965 g (50.0 mmol); solvent, 25 cm^3 of water; L-Ala, 0.178—0.802 g (2.00—9.00 mmol); temperature, 10 °C; stirring time, 3 h. Yield of diastereoisomer: ○, D-aThr; ●, L-Thr.

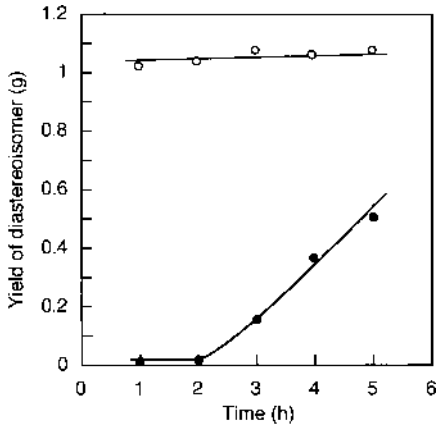

Fig. 3. Relationship between Yield of Diastereoisomer and Stirring Time in Separation of D-Allothreonine and L-Threonine

Conditions: Diastereoisomeric mixture (L-Thr in 18%*de*), 5.965 g (50.0 mmol); solvent, 25 cm^3 of water; L-Ala, 0.535 g (6.00 mmol); temperature, 10 °C; stirring time, 1—5 h. Yield of diastereoisomer: ○, D-aThr; ●, L-Thr.

α-amino acids undergo racemization with carbonyl compounds, such as salicylaldehyde, which acts as a catalyst in acetic acid.[9)] Therefore, epimerization at the C-2 position of threonine (Thr) will yield a diastereoisomeric mixture of Thr and aThr. However, Thr and aThr are difficult to separate from the mixture without transformation into their derivatives, such as *O*-methyl-*N*-acyl derivatives.[10)] In our previous paper,[11)] L-Thr was prevented from crystallizing from an aqueous solution of DL-Thr in the presence of L-Ala. Therefore, we attempted to separate D- and L-aThr from mixtures of Thr and aThr (Chart 1).

L-Thr was subjected to epimerization using salicylaldehyde as the catalyst, and was then stirred for 1—5 h in acetic acid at 80 °C. The epimerization gave a mixture of L-Thr and D-aThr in a molar ratio of 1 : 0.7, regardless of the reaction time after 1 h; the mixture was L-Thr with a diastereoisomeric excess (*de*) of about 17%. The molar ratio of L-Thr and D-aThr in the mixture was determined by the intensity ratios of the methine proton signals at the C-2 positions in the ^{1}H-NMR spectrum of the mixture. The yield of the diastereoisomeric mixture was approximately constant (1.1 g) during the first 1—3 h, then rapidly decreased with increasing reaction time, due to decomposition of L-Thr and D-aThr. Separation of D-aThr from the diastereoisomeric mixture was attempted by stirring 5.965 g (50.0 mmol) of the mixture (L-Thr of 17.5%*de*) in 25 cm^3 of water for 3 h at 10 °C in the presence of 2.00—9.00 mmol of L-Ala, as shown in Fig. 2.

The yield of D-aThr tended to gradually increase with increasing amounts of L-Ala. On the other hand, when L-Ala (2.00—6.00 mmol) was present, the yield of L-Thr rapidly decreased with increasing amounts of L-Ala. However, in the presence of 6.00—9.00 mmol of L-Ala, the yield of L-Thr was approximately constant. Therefore, D-aThr was crystallized in 74—78%*de* from a solution containing 6.00—9.00 mmol of L-Ala. When the solution of the mixture was stirred for 1—5 h in the presence of 6.00 mmol of L-Ala, the yield of D-aThr was approximately constant, as shown in Fig. 3. On the other hand, L-Thr was hardly crystallized during the first 1—2 h, and then rapidly crystallized with prolonged stirring. Therefore, D-aThr was obtained in 98%*de* and in a yield of more than 1 g by stirring for 1 and 2 h. In addition, the aqueous solution of the diastereoisomeric mixture prepared from D-Thr was stirred for 1 h in the presence of 6.00 mmol of D-Ala to give L-aThr in 97%*de*. D- and L-aThr obtained in low *de* were recrystallized from water to give D- and L-aThr as single diastereoisomers. For example, D-aThr (1.54 g) with 100%*de* and L-aThr (1.65 g) with 100%*de* were obtained from 2.00 g of D-aThr of 78%*de* and L-aThr of 84.5%*de*, respectively.

We reported optical resolution by preferential crystalliza-

tion of DL-aThr, which is another procedure for obtaining enantiomers from a conglomerate, and D- and L-aThr were obtained in yields of about 10% based on the amounts of D- and L-aThr in the supersaturated solution.[7] On the other hand, optical rersolution by replacing crystallization gave D- and L-aThr in yields of more than 20%. However, successive optical resolution required a small amount of L-aThr as seed crystals to promote the preferential crystallization of L-aThr. Separation of optically active aThr from the diastereoisomeric mixture was more simply achieved using D- and L-Ala as the co-solutes, and afforded D- amd L-aThr in high *de* and yields of more than 40%, based on the amounts of D- and L-aThr in the mixtues.

Experimental

General Specific rotation values were measured at 589 nm with a Horiba Seisakusho SEPA-300 auto-polarimeter equipped with a quartz cell with a 5.00 cm path length. ^{1}H-NMR spectra were recorded with a JNM-FX270 FT NMR system using sodium 3-(trimethylsilyl)propane-1-sulfonate (DSS) as an internal standard. Chemical shift values were reported in δ units downfield from DSS. Melting points were measured with a Yanaco MP-500 D micro melting point apparatus.

D- and L-Thr and D- and L-Ala were purchased from Wako Pure Chemical Ind. DL-aThr was synthesized starting from (*E*)-2-butenoic acid,[7] purchased from Wako Pure Chemical Ind.; mp 241—243 °C (decomp) (lit, mp 242—243 °C (decomp);[2] mp 260 °C (decomp);[3] mp 240—242 °C (decomp)).[7] ^{1}H-NMR (270 MHz, D_2O, DSS) δ: 4.36 (1H, qd, J=6.8, 4.1 Hz, 3-CH), 3.83 (1H, d, J=4.1 Hz, 2-CH), 1.20 (3H, d, J=6.8 Hz, 4-CH_3).

Optical Resolution by Replacing Crystallization DL-aThr (4.384 g, 36.8 mmol) and L-Ala (0.0891—0.535 g, 1.00—6.00 mmol) were dissolved in 25 cm^3 of water at 50 °C. After cooling the solution to 10 °C over 30 min, followed by stirring for 80 min with a blade (0.80 cm width; 2.5 cm length) at 100 rpm and 10 °C, the precipitated D-aThr was collected by filtration, washed with a small amount of methanol, and dried.

D-aThr obtained using 1.00 mmol of L-Ala: yield, 0.909 g; $[\alpha]_D^{20}$ −7.83° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained using 2.50 mmol of L-Ala: yield, 0.671 g; $[\alpha]_D^{20}$ −20.1° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained using 3.00 mmol of L-Ala: yield, 0.636 g; $[\alpha]_D^{20}$ −22.0° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained using 4.00 mmol of L-Ala: yield, 0.580 g; $[\alpha]_D^{20}$ −26.7° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained using 5.00 mmol of L-Ala: yield, 0.544 g; $[\alpha]_D^{20}$ −26.4° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained using 6.00 mmol of L-Ala: yield, 0.542 g; $[\alpha]_D^{20}$ −27.7° (c=1.00, 1 mol dm^{-3} HCl).

Optical resolution was carried out for the 176—214% supersaturated solutions of DL-aThr (3.931—4.765 g, 33.0—40.0 mmol) in the presence of L-Ala (0.356 g, 4.00 mmol) by stirring for 80 min at 10 °C in a manner similar to that described above.

D-aThr obtained from 176% supersaturated solution: yield, 0.371 g; $[\alpha]_D^{20}$ −26.4° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained from 182% supersaturated solution: yield, 0.449 g; $[\alpha]_D^{20}$ −26.7° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained from 187% supersaturated solution: yield, 0.516 g; $[\alpha]_D^{20}$ −28.3° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained from 192% supersaturated solution: yield, 0.552 g; $[\alpha]_D^{20}$ −26.9° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained from 200% supersaturated solution: yield, 0.588 g; $[\alpha]_D^{20}$ −26.1° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained from 204% supersaturated solution: yield, 0.631 g; $[\alpha]_D^{20}$ −23.3° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained from 209% supersaturated solution: yield, 0.654 g; $[\alpha]_D^{20}$ −22.5° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained from 214% supersaturated solution: yield, 0.728 g; $[\alpha]_D^{20}$ −18.6° (c=1.00, 1 mol dm^{-3} HCl).

Optical resolution was carried out for a solution of DL-aThr (4.384 g, 36.8 mmol) in the presence of L-Ala (0.356 g, 4.00 mmol) by stirring for 90—180 min at 10 °C in a manner similar to that described above.

D-aThr obtained at 90 min: yield, 0.830 g; $[\alpha]_D^{20}$ −14.5° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained at 120 min: yield, 1.079 g; $[\alpha]_D^{20}$ −6.41° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained at 150 min: yield, 1.215 g; $[\alpha]_D^{20}$ −3.32° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained at 180 min: yield, 1.279 g; $[\alpha]_D^{20}$ −3.10° (c=1.00, 1 mol dm^{-3} HCl).

Optical resolution was carried out for a solution of DL-aThr (4.384 g, 36.8 mmol) in the presence of D-Ala (0.356 g, 4.00 mmol) by stirring for 80 min at 10 °C in a manner similar to that described above.

L-aThr: yield, 0.574 g; $[\alpha]_D^{20}$ +27.9° (c=1.00, 1 mol dm^{-3} HCl).

The partially resolved D- and L-aThr were recrystallized from water in the following manner: D-aThr (5.00 g) ($[\alpha]_D^{20}$ −27.9° (c=1.00, 1 mol dm^{-3} HCl)) or L-aThr (4.00 g) ($[\alpha]_D^{20}$ +26.6° (c=1.00, 1 mol dm^{-3} HCl)) was added to water (7.5 cm^3). The mixture was vigorously stirred for 3 h at 10 °C before the purified D- or L-aThr was collected by filtration and dried.

D-aThr: yield, 3.90 g; $[\alpha]_D^{20}$ −32.8° (c=1.00, 1 mol dm^{-3} HCl). L-aThr: yield, 3.05 g; $[\alpha]_D^{20}$ +32.8° (c=1.00, 1 mol dm^{-3} HCl). The ^{1}H-NMR spectra of D- and L-aThr were virtually identical to that of DL-aThr.

Successive Optical Resolution DL-aThr (4.384 g, 36.8 mmol) and L-Ala (0.356 g, 4.00 mmol) were dissolved in 25 cm^3 of water at 50 °C. After cooling the solution to 10 °C, followed by stirring for 80 min at 10 °C, precipitated D-aThr (0.580 g) was collected by filtration and dried (run 1 in Table 2). After adding 0.050 g of L-aThr to the filtrate at 10 °C, followed by stirring for 90 min, precipitated L-aThr (0.552 g) was collected by filtration (run 2 in Table 2). DL-aThr (0.500 g) was dissolved in the filtrate at 50 °C. After adding 0.050 g of D-aThr to the filtrate at 10 °C, followed by stirring for 60 min, precipitated D-Thr (0.507 g) was collected by filtration (run 3 in Table 2). Optical resolution was carried out at 10 °C in a manner similar to that just described; the detailed conditions are given for runs 4—6 in Table 2.

Epimerization of Optically Active Threonine L-Thr (2.38 g, 20.0 mmol) was dissolved in 100 cm^3 of acetic acid at 80 °C. After adding salicylaldehyde (0.244 g, 2.00 mmol) to the solution, the mixture was stirred for 1—5 h at 80 °C. The mixture was concentrated *in vacuo* at 60 °C to give a mixture of L-Thr and D-aThr as the diastereoisomeric residue. After adding 50 cm^3 of methanol to the residue, followed by stirring for 0.5 h at 40 °C, the mixture was collected by filtration, washed thoroughly with methanol, and dried. The molar ratio of L-Thr and D-aThr in the mixture was determined by the intensity ratios of the methine proton signals at the C-2 positions in the ^{1}H-NMR spectrum of the mixture.

The mixture obtained at 1 h: yield, 1.13 g; $[\alpha]_D^{20}$ −21.9° (c=1.00, 1 mol dm^{-3} HCl); the mixture was composed of L-Thr and D-aThr in the molar ratio of 1 : 0.69. The mixture obtained at 1.5 h: yield, 1.12 g; $[\alpha]_D^{20}$ −22.0° (c=1.00, 1 mol dm^{-3} HCl); the mixture was composed of L-Thr and D-aThr at a molar ratio of 1 : 0.70. The mixture obtained at 2 h: yield, 1.11 g; $[\alpha]_D^{20}$ −22.1° (c=1.00, 1 mol dm^{-3} HCl); the mixture was composed of L-Thr and D-aThr in the molar ratio of 1 : 0.71. The mixture obtained at 2.5 h: yield, 1.11 g; $[\alpha]_D^{20}$ −22.2° (c=1.00, 1 mol dm^{-3} HCl); the mixture was composed of L-Thr and D-aThr in the molar ratio of 1 : 0.72. The mixture obtained at 3 h: yield, 1.10 g; $[\alpha]_D^{20}$ −22.2° (c=1.00, 1 mol dm^{-3} HCl); the mixture was composed of L-Thr and D-aThr in the molar ratio of 1 : 0.72. The mixture obtained at 4 h: yield, 0.884 g; $[\alpha]_D^{20}$ −22.0° (c=1.00, 1 mol dm^{-3} HCl); the mixture was composed of L-Thr and D-aThr in the molar ratio of 1 : 0.70. The mixture obtained at 5 h: yield, 0.515 g; $[\alpha]_D^{20}$ −21.9° (c=1.00, 1 mol dm^{-3} HCl); the mixture was composed of L-Thr and D-aThr in the molar ratio of 1 : 0.69. ^{1}H-NMR of the mixture obtained at 1.5 h (270 MHz, D_2O, DSS) δ: 4.36 (0.7H, qd, J=6.8, 4.1 Hz, 3-CH (D-aThr)), 4.25 (1H, qd, J=6.5, 4.9 Hz, 3-CH (L-Thr)), 3.83 (0.7H, d, J=4.1 Hz, 2-CH (D-aTr)), 3.58 (1H, d, J=4.9 Hz, 2-CH (L-Thr)), 1.32 (3H, d, J=6.6 Hz, 4-CH_3 (L-Thr)), 1.20 (2.1H, d, J=6.8 Hz, 4-CH_3 (D-aThr)). The ^{1}H-NMR spectra of the other mixtures were similar to that obtained at 1.5 h.

Epimerization of D-Thr (2.38 g, 20.0 mmol) was carried out at a reaction time of 1.5 h in a manner similar to L-Thr.

The mixture of D-The and L-aThr: yield, 1.14 g; $[\alpha]_D^{20}$ +20.1° (c=1.00, 1 mol dm^{-3} HCl); the mixture was composed of D-Thr and L-aThr at a molar ratio of 1 : 0.70. The ^{1}H-NMR spectrum was virtually identical to that of the mixture of L-Thr and D-aThr.

Separation of Optically Active Allothreonine from the Diastereoisomeric Mixture The diastereoisomeric mixture, composed of L-Thr and D-aThr at a molar ratio of 1 : 0.7, (5.955 g, 50.0 mmol) and L-Ala (0.178—0.802 g, 2.00—9.00 mmol) were dissolved in 25 cm^3 of water at 50 °C. After cooling the solution to 10 °C over 30 min, followed by stirring for 3 h with a blade (0.80 cm width; 2.5 cm length) at 100 rpm and 10 °C, the precipitated D-aThr was collected by filtration, washed with a small amount of methanol, and dried. The diastereoisomeric excess (%*de*) of the obtained D-aThr was determined based on the intensity ratios of the methine proton signals at the C-2 positions of L-Thr and D-aThr in the ^{1}H-NMR spectrum.

D-aThr obtained in 7.3%*de* using 2.00 mmol of L-Ala: yield, 1.86 g; $[\alpha]_D^{20}$ −24.3° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained in 23.3%*de* using 3.00 mmol of L-Ala: yield, 1.62 g; $[\alpha]_D^{20}$ −25.8° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained in 44.3%*de* using 4.00 mmol of L-Ala: yield, 1.45 g; $[\alpha]_D^{20}$ −27.7° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained in 66.2%*de* using 5.00 mmol of L-Ala: yield, 1.35 g; $[\alpha]_D^{20}$ −29.7° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained in 74.4%*de* using 6.00 mmol of L-Ala: yield, 1.23 g; $[\alpha]_D^{20}$ −30.5° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained in 77.3%*de* using

7.00 mmol of L-Ala: yield, 1.24 g; $[\alpha]_D^{20}$ −30.7° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained in 78.2%*de* using 9.00 mmol of L-Ala: yield, 1.29 g; $[\alpha]_D^{20}$ −30.8° (c=1.00, 1 mol dm^{-3} HCl).

Separation of D-aThr from the diastereoisomeric mixture was carried out in a solution of the mixture (4.384 g, 36.8 mmol) in the presence of L-Ala (0.535 g, 6.00 mmol) by stirring for 1—5 h in a manner similar to that described above.

D-aThr obtained in 98.2%*de* at 1 h: yield, 1.03 g; $[\alpha]_D^{20}$ −32.6° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained in 97.3%*de* at 2 h: yield, 1.06 g; $[\alpha]_D^{20}$ −32.6° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained in 48.7%*de* at 4 h: yield, 1.43 g; $[\alpha]_D^{20}$ −28.1° (c=1.00, 1 mol dm^{-3} HCl). D-aThr obtained in 36.2%*de* at 5 h: yield, 1.59 g; $[\alpha]_D^{20}$ −27.0° (c=1.00, 1 mol dm^{-3} HCl).

Separation of L-aThr from the mixture composed of D-Thr and L-aThr at a molar ratio of 1 : 0.7 was carried out in a solution of the mixture (4.384 g, 36.8 mmol) in the presence of D-Ala (0.535 g, 6.00 mmol) by stirring for 1 h in a manner similar to that described above.

L-aThr was obtained in 97.2%*de* at 1 h: yield, 1.07 g; $[\alpha]_D^{20}$ +32.5° (c=1.00, 1 mol dm^{-3} HCl).

The partially separated D- and L-aThr were recrystallized from water in the following manner: mixture of D-aThr (2.00 g) of 78%*de* or L-aThr (2.00 g) of 85%*de* in 10 cm^3 of water was vigorously stirred for 4 h at 10 °C, then the purified D- or L-aThr was collected by filtration and dried.

D-aThr: yield, 1.52 g; mp 270—272 °C (decomp); $[\alpha]_D^{20}$ −32.8° (c=1.00, 1 mol dm^{-3} HCl). L-aThr: yield, 1.65 g; mp 269—272 °C (decomp) (lit,[7] mp 269—270 °C (decomp)); $[\alpha]_D^{20}$ +32.8° (c=1.00, 1 mol dm^{-3} HCl). The ^{1}H-NMR spectra of D- and L-aThr were virtually identical to that of DL-aThr.

Solubility DL-, D-, or L-aThr (4.765 g, 40.0 mmol) was dissolved in a solution containing 0.356 g (4.00 mmol) of L-Ala in 25 cm^3 of water at 60 °C. After vigorously stirring the solution for 10 h at 10 °C, the precipitated aThr was rapidly collected by filtration and thoroughly dried. The solubility at 10 °C was calculated on the basis of the weight of aThr. For the dissolution of DL-aThr, the solubility of D- and L-aThr was estimated based on the optical purity of aThr obtained by filtration, and by its weight.

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