Synthesis and Reaction of a New Chiral Pyridoxamine Analogue; Some Doubt about the Stereochemical Process Tentatively Proposed for a Nonenzymatic Transamination Reaction

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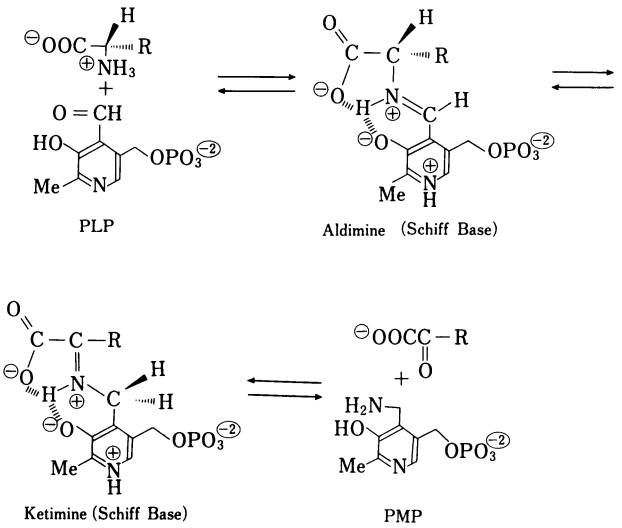
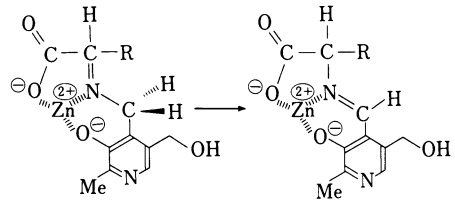
(Received August 8, 1988)

A pyridoxamine analogue-like chiral pyridinophane with two sulfonyl groups in the bridging chain, (*S*)-15-aminomethyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane *S,S,S',S'*-tetraoxide ((*S*)-**7**), was prepared by oxidation of the sulfide precursor, (*S*)-**2**. The amino group was successfully transferred from (*S*)-**7** to several 2-oxo carboxylic acids in methanol at room temperature in the presence of one-half equimolecular zinc(II) ion, giving (*R*)-amino acids in excess. The reaction rates of this nonenzymatic transamination using chiral (*S*)-**7** were much smaller than those of the corresponding reaction using chiral (*S*)-**2**. The enantiomeric excess of the amino acids obtained through the reactions of (*S*)-7 was compared with those of (*S*)-**2**, showing that (*S*)-**7** was more efficient than (*S*)-**2** for the preparation of (*R*)-alanine, but was less than that of (*S*)-**2** for the preparations of (*R*)-valine, (*R*)-leucine, and (*R*)-phenylalanine. These results aroused some doubt about the previous explanation of the stereochemical features of such nonenzymatic transamination reactions.

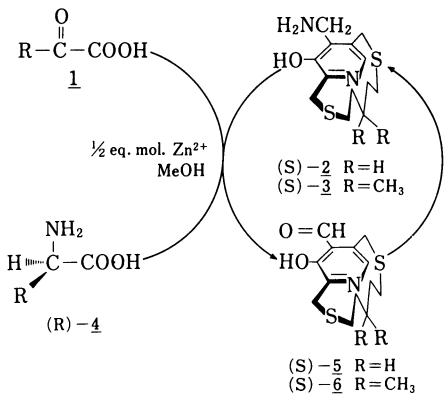
Transaminases, as well as other vitamin B_6-dependent enzymes, play a crucial role in amino acid metabolism, catalyzing the transfer of an amino group between amino acids and 2-oxo carboxylic acids. Two coenzymes, pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP), mediate the transfer of amino groups in such enzymatic reactions by forming Schiff bases with substrates, that undergo subsequent equilibration between their aldimine and ketimine forms (Scheme 1). The pioneering work of Snell[1] demonstrated that, in the simple model systems (in the absence of apoprotein), pyridoxal and pyridoxamine (with or without phosphate groups) duplicate the reaction of transaminases, and that the reaction rate is considerably increased when polyvalent metal cations are present. Martell[2] presented the first example of zinc(II) catalysis of a model transamination reaction in nonaqueous solvents, in which a simple isomerization from the ketimine to the aldimine reached near completion in the absence of large excesses of reagents (Scheme 2). Utilizing these fundamental discoveries, several groups[3] have pursued further development of new transaminase models capable of enantioface differentiation. We have succeeded in the preparation of pyridoxamine analogues with planar chirality, the (*R*) and (*S*) enantiomers of 15-aminomethyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane (**2**)[4] and 15-aminomethyl-14-hydroxy-5,5-dimethyl-2,8-dithia[9]-(2,5)pyridinophane (**3**).[5] Stereoselective transamination from chiral **3** (and **2** in a few cases) to various 2-oxo carboxylic acids (**1**) in methanol in the presence of



Scheme 1.



Scheme 2.



Scheme 3.

zinc(II), successfully produced α-amino acids (**4**) with enantiomeric excesses (e.e.) ranging from 60 to 96% depending on the substrates employed.[6] Interesting stereochemical features were observed in these transamination reactions. First, the reduction of the molar ratio of zinc(II) vs. chiral **3** from 1/1 to 0.5/1 resulted in a remarkable increase of the e.e. values of **4** produced, without affecting their chemical yields (about 70% in most cases). Second, the employment of the (*S*) enantiomer of **3** gave the (*R*) enantiomer of **4** in excess, and vice versa. In order to rationalize these phenomena, we tentatively proposed an octahedral zinc(II) chelate complex coordinated with two equimolecular Schiff base ligands as the key intermediate.[7] As is seen in Fig. 1,[8] a kinetically controlled stereoselective protonation would take place on one of the enantiofaces of the carbanion. The other enantioface is sterically hindered by one of the bulky sulfur atoms (circled in Fig. 1). If this hypothetical view is applicable to other cases, the replacement of the sulfur atoms of **3** (or **2**) with bulkier groups would result in the increase of the e.e. values of **4** produced by the model transamination reaction.

This paper describes syntheses of the racemate and the (*S*)-enantiomer of 15-aminomethyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane *S,S,S',S'*-tetraoxide (**7**) from **2**, and the zinc(II)-catalyzed transamination reactions of chiral **7** and chiral **2** with various kinds of **1**. The sulfonyl group in **7** is much bulkier than the thio group in **2** and would be expected to have little tendency to chelate metal ions, which is a necessary condition for the model transamination reactions to proceed smoothly. Although it has been shown that **3** shows higher efficiency in asymmetric induction than **2**,[6] the derivatives of **3** were generally much less soluble than those of **2**. Consequently, the asymmetric transaminations from **7** were compared with those from **2**, but not the sulfonyl analogue of **3** with **3**.

Synthesis of 7. Initially, racemic **7** was prepared for model studies. Acetylation of (*RS*)-**2** with acetic anhydride and pyridine at 100 °C gave crystalline 14-acetoxy-15'-diacetylamino derivative, (*RS*)-**9**, which revealed three carbonyl absorptions at 1690, 1710, and 1780 cm^{-1} but no NH absorption in the IR spectrum. Its ^1H NMR spectrum also revealed methyl protons of three acetyl groups around δ 2.30. In the spectrum, all gem-protons of the three pyridylmethylene groups appeared with the same characteristic pattern; i.e., one proton as a sharp doublet but the other as a broad doublet, suggesting that all acetyl groups undergo restricted rotation, and each rotamer influences one of the gem-protons in a different way. Oxidation of (*RS*)-**9** with *m*-chloroperbenzoic acid in chloroform gave almost equal amounts of two products in 82% total yield, which were separable by silica-gel chromatography. Both compounds revealed two absorption

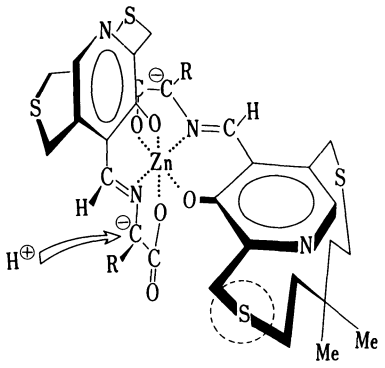
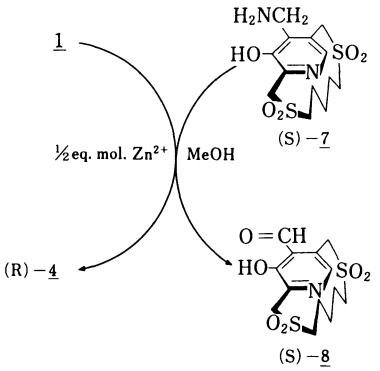
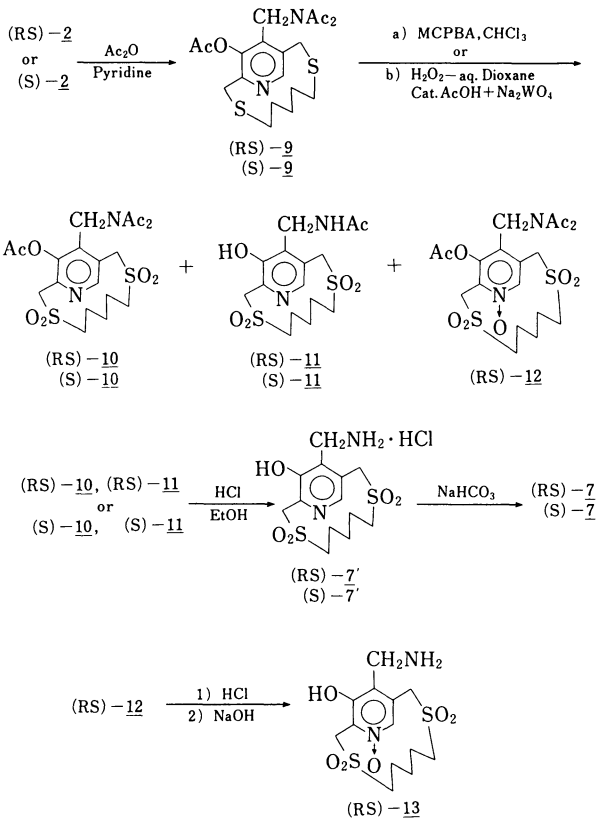


Fig. 1. *A*-Isomer of the octahedral zinc(II) chelate complex containing (*S*)-**3** Schiff base.



Scheme 4.



Scheme 5.

peaks of sulfonyl groups, at 1120—1130 and 1310—1320 cm^{-1} in their IR spectra. Elemental analyses of these products suggested that the less polar one was the expected (*RS*)-14-acetoxy-15-[(diacetylamino)methyl]-2,8-dithia[9](2,5)pyridinophane *S,S,S',S'*-tetraoxide ((*RS*)-**10**), and the other was *N,S,S,S',S'*-pentaoxide, (*RS*)-**12**. This identification was in good agreement with the results of our previous model oxidation experiment.[9] When the ^1H NMR spectrum of (*RS*)-**10** was compared with that of (*RS*)-**9**, four protons of 1- and 9-pyridylmethylene groups showed downfield shifts, appearing in the region of δ 4.36—5.24. One of the gem-protons of the (diacetylamino)methyl group, that appeared at 5.08 ppm as a broad doublet in the spectrum of **9**, was split into three sharp doublets at δ 5.07, 5.15, and 5.22 in the spectrum of **10**, with peak ratio 1:3:1. This presumably reflects the geometric proportion of the acetyl groups undergoing the restricted rotation. The ^1H NMR spectrum of (*RS*)-**12** revealed that one of the protons of the methylene group existing between the pyridine ring and the sulfonyl groups was missing, suggesting its easy replacement with the deuterium of the solvent (DMSO-d_6). Selective oxidation of the sulfur atoms in **9** to the corresponding sulfone was achieved by employing hydrogen peroxide as the oxidant. Although this oxidation also gave two compounds, the by-product was not **12** but **11**, a partially deacetylated derivative of **10**. Thus, when (*RS*)-**9** was treated in aqueous dioxane with hydrogen peroxide in the presence of catalytic amounts of acetic acid and sodium tungstate, (*RS*)-**10** and (*RS*)-15-[(acetylamino)-methyl]-14-hydroxy-2,8-dithia[9](2,5)pyridinophane *S,S,S',S'*-tetraoxide ((*RS*)-**11**) were obtained in a total yield of 62% with a ratio dependent on the reaction conditions employed. Both racemic **10** and **11** were deacetylated by treatment with hydrochloric acid, giving the same product, the racemic hydrochloride of **7** ((*RS*)-**7'**), which was quantitatively converted to the free base, (*RS*)-**7**, by neutralization with aqueous sodium hydrogencarbonate. In the ^1H NMR spectrum of (*RS*)-**7** using DMSO-d_6 as the solvent, all gem-protons of the pyridylmethylene groups appeared as three sets of sharp AB quartets, one of which was characteristic in showing a large difference of the chemical shifts between the A and B doublets (δ 3.97 and 4.98). (*RS*)-**12** could also be deprotected in a similar way, giving (*RS*)-**13**.

Chiral **7** was prepared from the corresponding **2** in the same way as the racemate model preparation. Thus, (*S*)-**2** was changed to (*S*)-**9**, which was oxidized by using the hydrogen peroxide–acetic acid–sodium tungstate system. Acidic hydrolysis of (*S*)-**10** and (*S*)-**11** gave (*S*)-**7'**, which was neutralized to give free (*S*)-**7** with the specific rotation of −352° at 589 nm.

Transamination Reactions. Transaminations from (*S*)-**7**, and from (*S*)-**2** were conducted employing four kinds of **1** as the reactants. The reaction conditions employed were the same, as those of the previous experiments mainly using chiral **3**,[6] except for the reaction time. A methanol solution containing the sodium salt of **1** (sodium pyruvate), (*S*)-**7** (or (*S*)-**2**), and one-half equivalent of zinc(II) was allowed to stand at room temperature, and the progress of the reaction was monitored by the change in its electronic absorption spectra with time (Fig. 2). The decrease in the initial absorption at 329 nm, due to the ketimine Schiff base zinc chelate complex, occurred simultaneously with an increase in the corresponding aldimine absorption at 390 nm. The time required for completion of the reaction reflected a large difference in the rate of the reactions of **2** and **7**. In the case of **2**, the isomerization from the ketimine to the aldimine was complete within 1 day, whereas it took more than 10 days in the reaction of **7**.

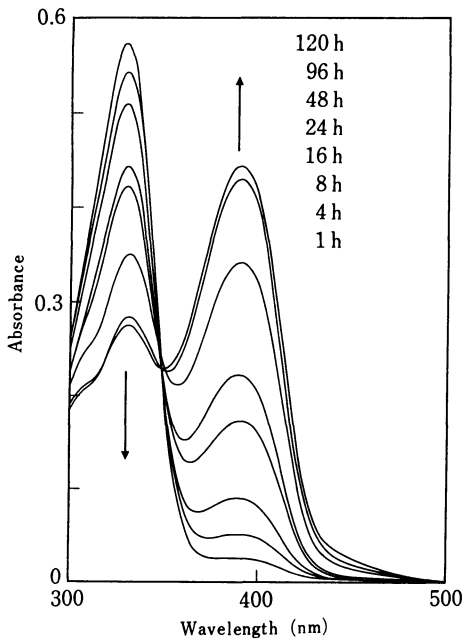


Fig. 2. Changes of the electronic absorption spectra with time for a methanol solution of (*S*)-**7** (0.1 mM), zinc perchlorate (0.05 mM), and sodium pyruvate (0.2 mM).

Various modifications of the procedures previously employed[6] were also required for isolation of amino acids from the zinc(II) chelate complexes of aldimine Schiff bases between **4** and **8** (Scheme 4). We have previously reported that the chelated aldimine intermediate derived from (*S*)-**2** was readily decomposed at room temperature by addition of dilute hydrochloric acid to the methanol solution of the reaction mixture. After extraction of (*S*)-**5** and excess of **1** with organic solvent, ion-exchange chromatography of the remaining aqueous solution gave (*R*)-**4**. Unexpectedly, this method failed in the present case, and the chelated aldimine intermediate derived from (*S*)-**7** resisted

Table 1. Transamination Reactions between (*S*)-**7** or (*S*)-**2** and RCOCOOH (**1**)

R	Reaction time/d		Recovered (*S*)-**7** or (*S*)-**2**/%		Products (amino acids) Major enantiomer		e.e./%		Chemical yield (isolation)/%		Name
	(*S*)-**7**	(*S*)-**2**	(*S*)-**7**	(*S*)-**2**	(*S*)-**7**	(*S*)-**2**	(*S*)-**7**	(*S*)-**2**	(*S*)-**7**	(*S*)-**2**	
Methyl	5	1	13	0	R	R	71	51	67 (77)[a]	80	Ala
Isopropyl	10	1	31	0	R	R	45	57	44 (64)	61	Val
Isobutyl	10	1	10	0	R	R	69	78	51 (58)	87	Leu
Benzyl	5	1	18	0	R	R	45	47	60 (73)	79	Phe

a) The values in the parentheses are the calculated ones on the basis of the amount of (*S*)-**7** consumed.

hydrolysis with dilute hydrochloric acid at room temperature, giving no amino acids. As the initial electronic absorption at 390 nm disappeared by addition of dilute hydrochloric acid it was replaced by a new stable absorption which appeared at 300 nm. It is possible that only zinc(II) ion was removed, leaving the acid-stable aldimine Schiff base in the solution. Therefore, the mixture in methanol–hydrochloric acid was evaporated and the residue redissolved in 1 M (1 M=1 mol dm^{-3}) hydrochloric acid. After heating at 100 °C for 5 h, extraction with ethyl acetate removed (*S*)-**8** and the excess of **1**. The aqueous solution was placed on a Dowex 50 W (H^+) column and successively eluted with water and aqueous ammonia to remove inorganic salts. In contrast to the case of transamination using (*S*)-**2**, concentration of the aqueous ammonia solution precipitated (*S*)-**7** unchanged. After filtration, the filtrate containing the amino acid **4** was passed through an Amberlite CG 50 (H^+) column, for complete removal of (*S*)-**7**. The combined yield of (*S*)-**7** recovered was in the range of 10—30%, depending upon the bulkiness of **1** employed. A small amount of brown-colored unidentified impurity was finally removed from the aqueous amino acid solution, using a LOBAR column.

Chemical yields and e.e. values of the amino acids isolated were determined on the basis of their weights and specific rotations measured. All results of the transamination reaction are tabulated in Table 1. The behavior of the *N,S,S,S',S'*-pentaoxide **13** in the transamination reaction is interesting, but no transamination was observed with (*RS*)-**13**.

Discussion

As mentioned above, the transamination reactions of (*S*)-**7** were different from those of (*S*)-**2** in several respects. For example, in the preparation of valine, the transamination using (*S*)-**2** was completed within 24 h and no (*S*)-**2** was left; whereas 31% of (*S*)-**7** was recovered even after standing for 10 days. Nevertheless, as the change of electronic absorption spectra showed, the reaction pattern producing chiral amino acids was essentially the same for both cases. The chemical yields of the amino acids produced by transamination using (*S*)-**2** or (*S*)-**7** did not differ much, when they

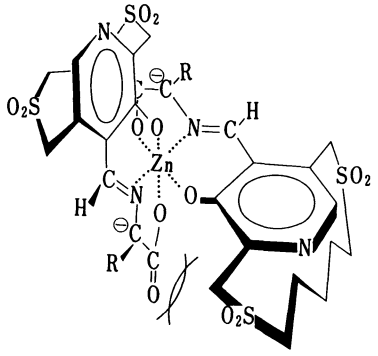


Fig. 3.

were calculated on the basis of the chiral pyridoxamine analogues consumed (the values in the parentheses for (*S*)-**7** in Table 1). The major enantiomer of amino acids obtained was *R* in all cases, suggesting that the transamination reaction using chiral **7** also obeys the rule established by the previous experiments using chiral **3** (and **2** in part); i.e., use of the (*S*)-enantiomer of the pyridoxamine analogues gives the (*R*)-amino acids in excess, and vice versa.[6] Contrary to the previous expectation of generally higher e.e. value by using (*S*)-**7** instead of (*S*)-**2**, it was actually so only in the single case of alanine. In the preparation of other amino acids such as valine, leucine, and phenylalanine, the efficiency of asymmetric induction by (*S*)-**7** was at most nearly equal to or lower than (*S*)-**2**. These unexpected results might be still rationalized with our view of the hypothetical reaction intermediate like Fig. 1, as described in the following. When the alkyl group in **1** is small like pyruvic acid (R=CH_3), the ketimine Schiff base resulted from its condensation with (*S*)-**7** might behave similarly to the Schiff base of (*S*)-**2**, forming a six-coordinated octahedral zinc(II) chelate complex. Isomerization from the ketimine to the aldimine involving the kinetically controlled stereoselective protonation would also take place within such chelate complex (see Fig. 1). These situations should have been considerably disturbed in the syntheses of other amino acids having bulkier alkyl groups (R in **1**). Namely, steric interaction should exist between the two bulky groups, R and sulfonyl group, as shown in

Fig. 3. This would labilize such zinc(II) chelate structure coordinated with two equimolecular Schiff base ligands. As a result, the geometry most favorably fixed for the ketimine-aldimine isomerization would be partially destroyed, lowering the steric effect of the sulfonyl group for enantioselectivity. This situation would lead not only to the increase of the amount of unchanged (*S*)-**7** but also to the decrease of the e.e. value of the amino acids.

The above speculative rationalization has been presented without any evidence. Therefore, an alternative process involving four- or five-coordinated zinc(II) chelate complex might better explain the whole stereochemical features of the nonenzymatic transamination reactions. Consideration of these possibilities and accumulation of the evidences for them will be made hereafter.

Experimental

General Procedures. Melting points were determined in a capillary tube using a Büchi melting point apparatus and are uncorrected. IR and UV-VIS absorption spectra were obtained with a Shimadzu IR 27G and a Varian-Cary 2290 spectrometer, respectively. Specific rotations were measured by using a Perkin-Elmer 241 polarimeter with a 1 dm 5 mL or a 1 dm 1 mL cell at room temperature. ^{1}H NMR spectra were obtained with a JEOL JNM GSX 500 s (500 MHz) using tetramethylsilane or sodium 3-(trimethylsilyl)propionate-d_4 as the internal references. Thin-layer chromatography (TLC) used precoated aluminium sheets from E. Merck (silica gel 60 F_{254}, 0.2 mm-thick for pyridinophanes, and cellulose F_{254}, 0.1 mm-thick for amino acids). The solvent systems used were as follows: chloroform–methanol for silica gel and 1-butanol–acetic acid–water for cellulose. The pyridinophanes on the silica gel layer were observed under a UV lamp or visualized by iodine vapor. The amino acids on the cellulose layer were visualized by ninhydrin–1-butanol and heating. Silica-gel column chromatography was performed using Merck silica gel 60 (0.063—0.200 mm in particle size) with solvent systems specified. Dowex 50 W×8 (100—200 mesh) and Amberlite CG 50 (Type 1) were purified in the usual way prior to use. Reversed-phase silica-gel column chromatography was carried out with a prepacked Merck LOBAR Column (Li Chroprep RP-8, 40—60 μm in particle size and 10×240 mm in column size) using water as the eluent. Ultrafiltration was performed using a Millex GS (0.22 μm in pore size and 4 cm^2 in effective area of filtration) purchased from Millipore, Japan Ltd.

(*RS*)-14-Acetoxy-15-[(diacetylamino)methyl]-2,8-dithia[9]-(2,5)pyridinophane ((*RS*)-9). A solution of (*RS*)-**2**[4] (10.00 g) in pyridine (100 mL) and acetic anhydride (100 mL) was stirred at 100 °C for 16 h, concentrated in vacuo at 70 °C, and poured into ice-water. The mixture was extracted with ethyl acetate and the extract was washed successively with aqueous sodium hydrogencarbonate and water, dried ($MgSO_4$), and concentrated in vacuo. The residual matter was chromatographed on silica gel with 100:1 (v/v) $CHCl_3$–MeOH as the eluent, giving (*RS*)-**9** (14.37 g, quantitative), mp 127—128 °C; UV ($CHCl_3$) 289 nm (ε 4.0×10^3); IR (KBr) 1780 (O–C=O) and 1710 and 1690 cm^{-1} (N–C=O); ^{1}H NMR (DMSO-d_6) δ=2.28 (s, 3H, $OCOCH_3$), 2.32 (s, 6H, $N(COCH_3)_2$), 3.52 (d, 1H, J=13.0 Hz, $=CCH_2S$), 3.75 (broad, d, 1H, J=ca. 13 Hz, $=CCH_2S$), 3.87 (d, 1H, J=13.7 Hz, $=CCH_2S$), 4.01 (broad, d, 1H, J=ca. 14 Hz, $=CCH_2S$), 4.87 (d, 1H, J=16.2 Hz, $=CCH_2N$), 5.08 (broad, d, 1H, J=ca. 16 Hz, $=CCH_2N$), and 8.42 (s, 1H, N=CH). Found: C, 55.43; H, 6.38; N, 6.73; S, 15.62%. Calcd for $C_{19}H_{26}N_2O_4S_2$: C, 55.59; H, 6.38; N, 6.82; S, 15.62%.

(*S*)-14-Acetoxy-15-[(diacetylamino)methyl]-2,8-dithia[9]-(2,5)pyridinophane ((*S*)-9). The same treatments of (*S*)-**2** as those of the racemate described above resulted in (*S*)-**9** in 87% yield: mp 147—148 °C; $[\alpha]_D^{27}$ −42.6° (*c* 0.384, $CHCl_3$). Found: C, 55.57; H, 6.36; N, 6.85; S, 15.48%. Calcd for $C_{19}H_{26}N_2O_4S_2$: C, 55.59; H, 6.38; N, 6.82; S, 15.62%.

Oxidation of (*RS*)-9. (a) With *m*-Chloroperbenzoic Acid: A solution of *m*-chroroperbenzoic acid (85% purity, 7.50 g, 37 mmol) in chloroform (75 mL) was added dropwise at 5—10 °C for 1 h with stirring to a solution of (*RS*)-**9** (2.00 g, 4.9 mmol) in chloroform (100 mL), giving a precipitate. The mixture was stirred at 5—10 °C for another 1 h and at room temperature overnight. Meanwhile the precipitate completely dissolved. After addition of 20—30 mL of 5% aqueous sodium hydrogensulfite, the mixture was extracted with aqueous sodium hydrogencarbonate for removal of *m*-chlorobenzoic acid. The chloroform layer was washed with water, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on silica gel, using 100:1 (v/v) $CHCl_3$–MeOH as the eluent, giving (*RS*)-**10** (0.95 g, 41%) and (*RS*)-**12** (0.97 g, 41%) in this elution order. (*RS*)-**10**: mp 202—204 °C; UV ($CHCl_3$) 282 nm (ε 4.2×10^3); IR (KBr) 1790 (O–C=O), 1710 and 1690 (N–C=O), and 1320 and 1120 cm^{-1} (SO_2); ^{1}H NMR (DMSO-d_6) δ=2.31 (s, 3H, $COOCH_3$), 2.32 (s, 6H, $N(COCH_3)_2$), 4.36—4.92 (m, 5H, $=CCH_2S$ and $=CCH_2N$), 5.07 (d, 0.2H, J=17 Hz, $=CCH_2N$), 5.14 (d, 0.6H, J=16 Hz, $=CCH_2N$), 5.22 (d, 0.2H, J=17 Hz, $=CCH_2N$), and 8.81 (s, 1H, N=CH). Found: C, 47.74; H, 5.51; N, 5.68; S, 13.64%. Calcd for $C_{19}H_{26}N_2O_8S_2$: C, 48.09; H, 5.52; N, 5.90; S, 13.51%.

(*RS*)-**12:** Mp 201—202 °C (decomp); UV ($CHCl_3$) 287 nm (ε 11.9×10^3); IR (KBr) 1780 (O–C=O), 1710 and 1690 (N–C=O), 1310 (SO_2), 1290 (N→O), and 1130 cm^{-1} (SO_2); ^{1}H NMR (DMSO-d_6) δ=2.31 (s, 3H, $OCOCH_3$), 2.32 (s, 6H, $N(COCH_3)_2$), 4.51 (d, 1H, J=13.5 Hz, $=CCH_2S$), 4.84 (d, 1H, J=14.2 Hz, $=CCH_2S$), 4.85 (d, 1H, J=15.9 Hz, $=CCH_2N$), 4.90 (d, 1H, J=14.2 Hz, $=CCH_2S$), 5.14 (d, 1H, J=15.9 Hz, $=CCH_2N$), and 8.81 (s, 1H, N=CH). Found: C, 46.33; H, 5.32; N, 5.67; S, 12.97%. Calcd for $C_{19}H_{26}N_2O_9S_2$: C, 46.52; H, 5.34; N, 5.71; S, 13.07%.

(b) With Hydrogen Peroxide: Aqueous hydrogen peroxide (30%, 2.43 mL, 24.5 mmol) was added dropwise with stirring at 60—65 °C for 30 min to a solution of (*RS*)-**9** (2.00 g, 4.9 mmol), acetic acid (ca. 15 mg), and sodium tungstate dihydrate (ca. 5 mg) in dioxane–water (2:1 v/v, 30 mL). The mixture was heated at 80—85 °C for 1 h, cooled, diluted with aqueous sodium hydrogensulfite (5%, 5—10 mL), and extracted with chloroform. The extract was dried ($MgSO_4$), concentrated, and chromatographed on silica gel with 100:1 (v/v) $CHCl_3$–MeOH as the eluent, giving (*RS*)-**10** (1.15 g, 50%). Further elution with 25:1 (v/v) $CHCl_3$–MeOH gave (*RS*)-**11** (0.22 g, 12%); mp>280 °C; UV ($CHCl_3$) 282 nm (ε 4.2×10^3); IR (KBr) 3350 (broad, NH, OH), 1650 (NHCO), and 1310 and 1140 cm^{-1} (SO_2); ^{1}H NMR

(DMSO-d_6) δ=1.87 (s, 3H, $NCOCH_3$), 4.34 (d, 1H, J=13.3 Hz, $=CCH_2S$), 4.35 (dd, 1H, J=14.8 and 6.1 Hz, $=CCH_2N$), 4.57 (dd, 1H, J=14.8 and 6.1 Hz, $=CCH_2N$), 4.67 (d, 1H, J=14.1 Hz, $=CCH_2S$), 4.95 (d, 1H, J=13.7 Hz, $=CCH_2S$), 5.00 (d, 1H, J=13.3 Hz, $=CCH_2S$), 8.37 (s, 1H, N=CH), 8.82 (t, 1H, J=5.8 Hz, NH), and 10.82 (s, 1H, OH). Found: C, 45.36; H,5.57; N, 6.89; S, 16.41%. Calcd for $C_{15}H_{22}N_2O_6S_2 \cdot 1/2H_2O$: C, 45.10; H, 5.80; N, 7.01; S, 16.05%.

Oxidation of (*S*)-9 with Hydrogen Peroxide. The same treatment of (*S*)-**9** with hydrogen peroxide-acetic acid-sodium tungstate gave a mixture of (*S*)-**10** and (*S*)-**11** in 33% and 30% yields, respectively. (*S*)-**10**: mp 204—205 °C; $[\alpha]_D^{31}$ −78° (*c* 0.363, $CHCl_3$). Found: C, 47.12; H, 5.40; N, 5.70; S, 13.45%. Calcd for $C_{19}H_{26}N_2O_8S_2 \cdot 1/2H_2O$: C, 47.19; H, 5.63; N, 5.79; S, 13.26%.

(*S*)-**11**: Mp 266—267 °C (decomp); $[\alpha]_D^{29}$ −168 ° (*c* 0.321, pyridine). Found: C, 43.97; H, 5.38; N, 6.68; S, 15.96%. Calcd for $C_{15}H_{22}N_2O_6S_2 \cdot H_2O$: C, 44.11; H, 5.92; N, 6.86; S, 15.70%.

(*RS*)-15-Aminomethyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane *S,S,S',S'*-Tetraoxide ((*RS*)-7) via Monohydrochloride of 7 ((*RS*)-7'). **(a) From (*RS*)-10:** A solution of (*RS*)-**10** (3.00 g, 7.3 mmol) in ethanol (15 mL) and 6 M hydrochloric acid (15 mL) was heated under reflux for 3 h and concentrated in vacuo. The residue was dissolved in a small amount of hot water, treated with charcoal, and diluted with ethanol, giving (*RS*)-**7'** (1.94 g, 80%): mp>280 °C; UV (H_2O) 333 (ε 3.7×10³), 301 (ε 4.1×10³), and 255 nm (ε 3.7×10³); IR (KBr) 3000, 1580, and 1500 (OH, $NH_2 \cdot HCl$) and 1320 and 1110 cm^{-1} (SO_2). Found: C, 40.56; H, 5.58; Cl, 8.85; N, 7.13; S, 16.54%. Calcd for $C_{13}H_{20}N_2O_5S_2 \cdot HCl$: C, 40.57; H, 5.50; Cl, 9.21; N, 7.28; S, 16.66%.

To a solution of (*RS*)-**7'** (1.414 g, 3.7 mmol) in water was added aqueous sodium hydrogencarbonate (310 mg, 3.7 mmol) and the mixture was stirred at room temperature for 30 min, giving (*RS*)-**7** (1.223 g, 91%) as a precipitate: mp 175—202 °C (decomp); UV (dioxane) 300 nm (ε 2.9×10³); IR (KBr) 3500 and 3350 (NH_2, OH) and 1300 and 1130 cm^{-1} (SO_2); ^1H NMR (DMSO-d_6)[10] δ=3.97 (d, 1H, J=12.4 Hz, $=CCH_2S$), 4.07 (d, 1H, J=15.6 Hz, $=CCH_2N$), 4.13 (d, 1H, J=15.3 Hz, $=CCH_2N$), 4.42 (d, 1H, J=14.0 Hz, $=CCH_2S$), 4.65 (d, 1H, J=13.7 Hz, $=CCH_2S$), 4.98 (d, 1H, J=12.4 Hz, $=CCH_2S$), and 7.78 (s, 1H, N=CH). Found: C, 42.58; H, 5.50; N, 7.57; S, 17.47%. Calcd for $C_{13}H_{20}N_2O_5S_2 \cdot H_2O$: C, 42.61; H, 6.05; N, 7.64; S, 17.50%.

(b) From (*RS*)-11: A solution of (*RS*)-**11** (0.85 g, 2.1 mmol) in ethanol (10 mL) and 6 M hydrochloric acid (10 mL) was heated under reflux for 3 h and worked up in the same way as described in (a), giving (*RS*)-**7'** (0.71 g, 86%), which was neutralized with sodium hydrogencarbonate to give (*RS*)-**7** (0.61 g, 90%).

(*S*)-15-Aminomethyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane *S,S,S',S'*-Tetraoxide ((*S*)-7) via Monohydrochloride of 7 ((*S*)-7'). Either (*S*)-**10** or (*S*)-**11** was treated with hydrochloric acid in the same way as the hydrolysis of the corresponding racemate, giving (*S*)-**7'** in 74% and 85% yields, respectively: mp>280 °C; $[\alpha]_D^{29}$ −317 ° (*c* 0.293, H_2O). Found: C, 40.40; H, 5.56; Cl, 9.10; N, 7.20; S, 16.59%. Calcd for $C_{13}H_{20}N_2O_5S_2 \cdot HCl$: C, 40.57; H, 5.50; Cl, 9.21; N, 7.28; S, 16.66%.

Neutralizing (*S*)-**7'** with equimolecular amount of sodium hydrogencarbonate similarly to the case of the racemate gave (*S*)-**7** in quantitative yield: mp 196—199 °C (decomp); $[\alpha]_D^{19}$ −352 ° (*c* 0.241, pyridine). Found: C, 42.69; H, 5.51; N, 7.52; S, 17.55%. Calcd for $C_{13}H_{20}N_2O_5S_2 \cdot H_2O$: C, 42.61; H, 6.05; N, 7.64; S, 17.50%.

(*RS*)-15-Aminomethyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane *N,S,S,S',S'*-Pentaoxide ((*RS*)-13). A solution of (*RS*)-**12** (1.92 g, 5.3 mmol) in ethanol (20 mL) and 6 M hydrochloric acid (20 mL) was heated under reflux for 3 h, treated with charcoal, and concentrated in vacuo. The residue was dissolved in a minimum amount of water and adjusted to pH 3 by addition of aqueous sodium hydroxide, giving (*RS*)-**13** (1.38 g, 97%) as a precipitate: mp>280 °C; UV (1 M HCl) 327 (ε 4.8×10³), 276 (ε 9.0×10³), and 240 nm (ε 23.7×10³); IR (KBr) 3400 (broad, NH_2, OH), 1360 (SO_2), 1280 (N→O), and 1130 cm^{-1} (SO_2); ^1H NMR (DCl-D_2O)[11] δ=3.86 (s, 2H, $=CCH_2N$), 4.20 (d, 1H, J=15.0 Hz, $=CCH_2S$), 4.46, (d, 1H, J=14.6 Hz, $=CCH_2S$), 4.50 (d, 1H, J=14.6 Hz, $=CCH_2S$), 5.02 (d, 1H, J=14.6 Hz, $=CCH_2S$), and 7.92 (s, 1H, N=CH). Found: C, 42.57; H, 5.58; N, 7.44; S, 17.43%. Calcd for $C_{13}H_{20}N_2O_6S_2$: C, 42.85; H, 5.53; N, 7.69; S, 17.65%.

Transamination Reactions. (a) Using (*S*)-2: A solution of (*S*)-**2** (85 mg, 0.3 mmol), sodium salts of **1** (0.6 mmol), and zinc perchlorate hexahydrate (56 mg, 0.15 mmol) in methanol (150 mL) was stirred at room temperature for 24 h. After addition of 1 M hydrochloric acid (6 mL), the resulting mixture was concentrated to dryness in vacuo. The residue was extracted with a mixture of water and ethyl acetate. The aqueous extract was concentrated to ca. 10 mL and put on a column packed with Dowex 50 W×8 (100—200 mesh,H^+ form, 50 mL). The column was successively eluted with water (1 L) and 0.1 M aqueous ammonia. The latter eluate was concentrated to give a residue, which was put on a column packed with Amberlite CG 50 (100—200 mesh, H^+ form, 20 mL). The column was eluted with water to collect the amino acid-containing fractions, and evaporated. Optical purities of **4** obtained were calculated on the basis of its measured optical rotations.

(b) Using (*S*)-7: The same reaction conditions were employed except for the longer reaction time. A solution of (*S*)-**7** (110 mg, 0.3 mmol), sodium salt of **1** (0.6 mmol), and zinc perchlorate hexahydrate (56 mg, 0.15 mmol) in methanol (150 mL) was stirred at room temperature, monitoring the reaction by the change of the electronic absorption spectrum. The reaction time employed was 5 days for preparations of alanine (from pyruvate) and phenylalanine (from phenylpyruvate), whereas 10 days were necessary for the preparation of valine (from 3-methyl-2-oxobutyrate) and leucine (from 4-methyl-2-oxovalerate). After the reaction was over, 1 M hydrochloric acid (6 mL) was added and the mixture was concentrated to dryness; the residue was dissolved again in 1 M hydrochloric acid (20 mL) and heated at 100 °C for 5 h. Concentration in vacuo followed by dilution with water was repeated several times to remove hydrochloric acid as much as possible. The final residue was extracted with a mixture of water and ethyl acetate. The aqueous extract was concentrated to a volume of 10 mL and put on a column packed with Dowex 50 W×8 (100—200 mesh, H^+ form, 50 mL). The column was successively eluted with water (1 L) and 0.1 M aqueous ammonia. The latter eluate was concentrated to a volume of 5 mL to give (*S*)-**7** unreacted as a precipitate, which was filtered off. The filtrate was put on a column packed with Amberlite CG 50

(100—200 mesh, H^+ form, 20 mL) and eluted with water. The amino acid-containing fractions were collected, concentrated, and put on a LOBAR column RP-8 (10×240 mm), which was eluted with water. Concentration of the eluate gave **4**. Before measurement of the optical rotations, the aqueous solutions of **4** obtained were passed through a membrane filter.

The authors wish to express their thanks to Miss Mutsuko Yoshida and her coworkers of this Institute for elemental analyses and to Dr. Jun Uzawa and Mrs. Tamiko Chijimatsu of this Institute for the measurements of ^1H NMR spectra.

This study was performed through Special Coordination Funds of the Science and Technology Agency of the Japanese Government.

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11) When ^1H NMR spectrum of **13** was obtained after **13** had been dissolved in DCl–D_2O as soon as possible, no replacement of the protons of the pyridylmethylene groups with deuterium of the solvents was observed.