Asymmetric Synthesis of α-Deuterated α-Amino Acids through Nonenzymatic Transamination Reaction and the Determination of Their Enantiomeric Excesses

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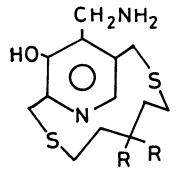
(Received April 13, 1983)

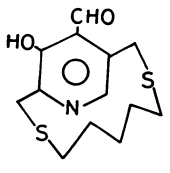
Optically active α-deuterated α-amino acids were prepared in methanol-*d* through Zn^{2+}-catalyzed transamination reaction between the chiral pyridoxamine analogs, (*R*)- or (*S*)-15-aminomethyl-14-hydroxy-5,5-dimethyl-2,8-dithia[9](2,5)pyridinophane and various α-keto acids with enantiomeric excesses ranging from 40 to 94%. Aliphatic α-keto acids gave α-deuterated α-amino acids, whereas aromatic ones possessing a methylene group between the carbonyl group (ketone) and the aromatic ring underwent concomitant deuterium substitution at the active methylene group to give the amino acids deuterated at both α- and β-positions of the carboxyl group. The use of the (*S*)-pyridoxamine analog gave the (*R*)-deuterated α-amino acids in excess and *vice versa*. The enatiomeric excesses of the amino acids were determined through the analyses of ^1H NMR spectra of the Schiff bases produced by the condensation of the amino acids with the chiral pyridoxal analog, (*R*)- or (*S*)-15-formyl-14-hydroxy-2,8-dithia[9]-(2,5)pyridinophane. The azomethine protons of the diastereomeric Schiff bases were clearly resolved in the spectrum and their intensities well reflected the amount of the diastereomers.

Chiral α-amino acids labelled at α- or β-position of the carboxyl group have been valuable for determinig the steric course of a variety of biosynthetic transformations and conformational analyses of peptides.[1-3] However, there are only a few reports concerning the preparation of these deuterated amino acids.[4-6]

In the course of our continuous study of vitamin B_6-dependent enzyme models, we have recently reported the asymmetric synthesis of α-amino acids through the enantioselective transamination reaction between the chiral pyridoxamine analogs with a pyridinophane structure, (*R*)- or (*S*)-15-aminomethyl-14-hydroxy-5,5-dimethyl-2,8-dithia[9](2,5)pyridinophane ((*R*)- or (*S*)-**1**) or (*S*)-15-aminomethyl-14-hydroxy-2,8-dithia[9](2,5)-pyridinophane ((*S*)-**2**), and various α-keto acids in the presence of Zn^{2+}.[7,8] We have also found that the α-proton of the amino acids comes directly from the methanol employed as solvent in the nonenzymatic transamination reaction.[9] In the present paper, we wish to describe the extended application of the nonenzymatic transamination system to the asymmetric synthesis of α-deuterated α-amino acids by employing methanol-*d* as the solvent and the determination of their enantiomeric excesses by analyzing the ^1H NMR spectra of the Schiff bases prepared from the deuterated amino acids and a chiral pyridoxal analog, (*R*)- or (*S*)-15-formyl-14 hydroxy-2,8-dithia[9](2,5)pyridinophane ((*R*)- or (*S*)-**3**).[10]



1: R=CH_3
2: R=H



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Results and Discussion

The nonenzymatic transamination reactions in methanol-*d* (MeOD) were slower than those in methanol and took several days to be completed because of the solvent isotope effect.[11] The isolation of the deuterated α-amino acid produced was carried out in a similar way to that described previously for the general preparation of amino acids.[7] The NMR spectra of the products showed that aliphatic α-keto acids such as pyruvic acid, 3-methyl-2-oxobutanoic acid, and 4-methyl-2-oxopentanoic acid gave the corresponding α-deuterated α-amino acid through reaction with the pyridoxamine analog ((*R*)- or (*S*)-**1**) in the presence of Zn^{2+}. On the other hand, the employment of aromatic α-keto acids such as phenylpyruvic acid and indole-3-pyruvic acid under the same reaction conditions resulted in the corresponding amino acids deuterated at both α- and β-positions of their carboxyl group (Fig. 1). The NMR spectra of deuterated leucine, valine, and phenylalanine are shown in Figs. 2,3, and 4. The results of the elemental analyses and mass spectra of these deuterated α-amino acids are shown in Table 1. Deuteration of the active methylene group between the keto and the aromatic groups of sodium phenylpyruvate or sodium indole-3-pyruvate took place spontaneously even if the pyridoxamine analog and the metal ion were absent. However, the chelated ketimine group formed in the presence of the pyridoxamine analog and the metal ion is more electron-withdrawing than the original keto group and more strongly prompts elimination of the protons from the neighboring methylene group, resulting in deuterio substitution at that place.

Determination of the enantiomeric excess of the deuterated amino acids was not easy because the specific rotations of the authentic specimens were unknown. The utilization of the diastereomeric Schiff base mixture produced by condensation of the deuterated amino acids with some chiral aldehydes might be a promising way for the enantiomeric excess determination. Our pyridoxal analog ((*R*)- or (*S*)-**3**) could

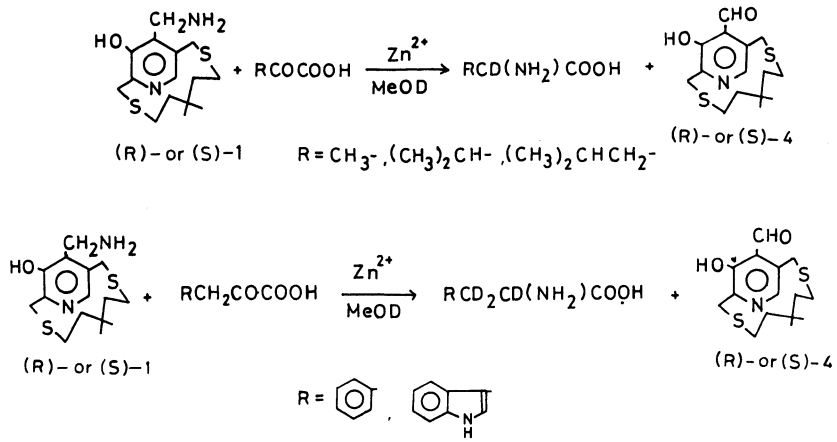

Fig. 1. Transamination reaction between pyridoxamine analogs ((*R*)- or (*S*)-**1**) and α-keto carboxylic acids in methanol-*d*.

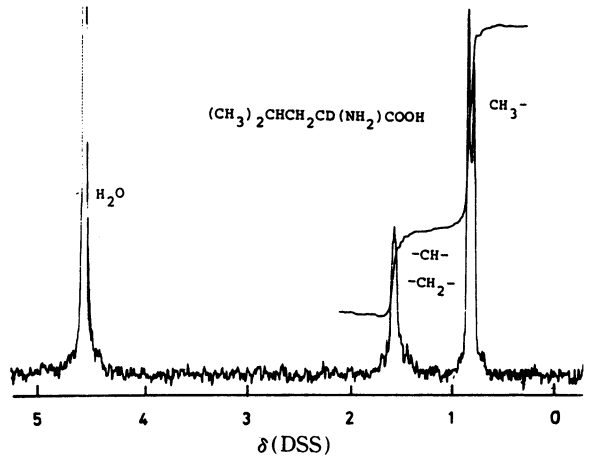

Fig. 2. ^{1}H 100-MHz NMR spectrum of $(CH_3)_2CHCH_2$-$CD(NH_2)COOH$ in D_2O (DSS internal standard).

be regarded as a candidate for the chiral aldehyde because **3** readily condensed with free amino acids in methanol at room temperature to give an aldimine type of Schiff base. We confirmed that no racemization of the general amino acid moieties in the Schiff bases took place under such mild conditions, since the amino acids recovered after acidic hydrolysis of the Schiff bases retained the full original chirality. Judging from the isotope effect, these results obtained by using general chiral amino acids would be also applicable to deuterated amino acids. Therefore, the deuterated amino acids obtained were converted into Schiff bases by condensation with (*R*)- or (*S*)-**3**. Figure 5 shows the 400 MHz ^{1}H NMR spectrum of the C_6D_6 solution of the diastereomeric mixture of the Schiff bases resulted from the reaction of the racemic α-deuterated leucine with (*R*)-**3** in methanol. The signals at δ 8.77 (b) and δ 9.00 (d) were assigned to the aldimine protons of the pyridoxal analog moiety.[12-16] The signals at δ 8.02 (a), δ 8.17 (c), and δ 8.26 (e) should be assigned to the 6-H protons on the pyridine ring.[17-18] The assignment of those signals (b—e) was confirmed by comparing them with the NMR spectra of the Schiff bases prepared from authentic sample of general (*S*)- or (*R*)-leucine and (*R*)-**3**, respectively.[19] The Schiff

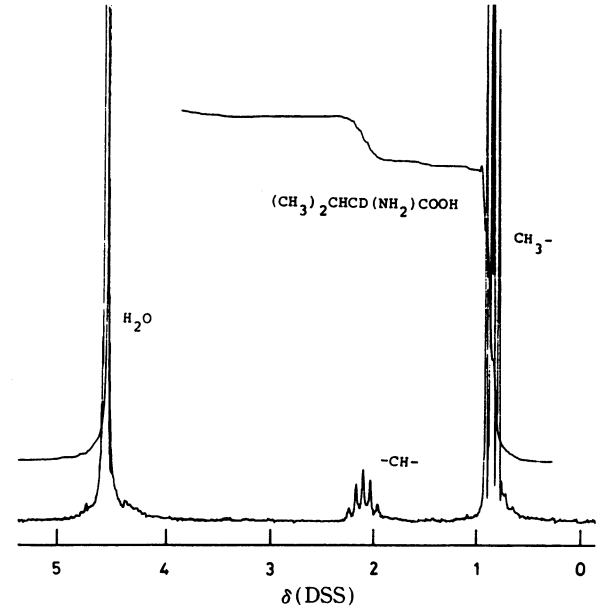

Fig. 3. ^{1}H 100-MHz NMR spectrum of $(CH_3)_2CHCD$-$(NH_2)COOH$ in D_2O (DSS internal standard).

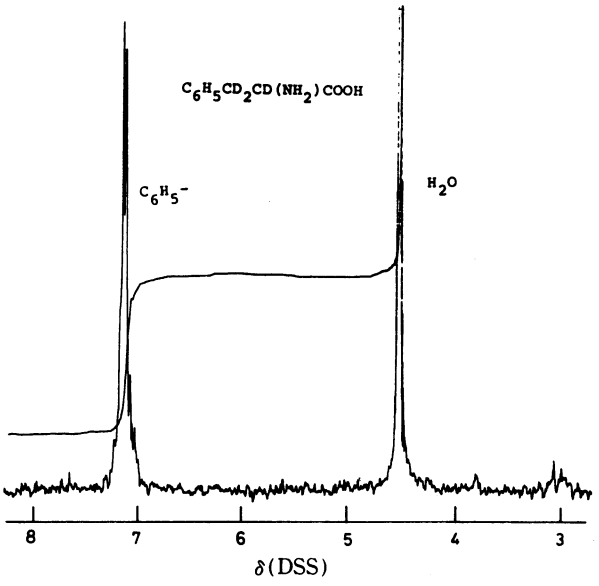

Fig. 4. ^{1}H 100-MHz NMR spectrum of $C_6H_5CD_2CD$-$(NH_2)COOH$ in D_2O (DSS internal standard).

TABLE 1. ELEMENTAL ANALYSES AND MASS SPECTRA OF DEUTERATED AMINO ACID, $RCD(NH_2)COOH$[a)]

R	Mass (*m/e*) Found	Mass (*m/e*) Calcd	Formula	Calcd (%) C	Calcd (%) H+D	Calcd (%) N	Found (%) C	Found (%) H+D[b)]	Found (%) N
$(CH_3)_2CHCH_2$	133 $(M+1)^+$	132 (M)	$C_6H_{12}DNO_2$	54.52	10.67	10.60	54.94	10.57	10.65
$(CH_3)_2CH$	119 $(M+1)^+$	118 (M)	$C_5H_{10}DNO_2$	50.82	10.23	11.86	50.62	10.43	11.72
$C_6H_5CD_2$	168 $(M)^+$	168 (M)	$C_9H_8D_3NO_2$	64.21	8.38	8.32	64.26	8.23	8.32

a) The deuterated amino acids were obtained through nonenzymatic transamination reaction between (*R*)-**1** and RCOCOOH. b) The determined values of H+D were calculated from the weight of H_2O+HOD produced on the assumption that the H : D atomic ratio was that of the compound analyzed.

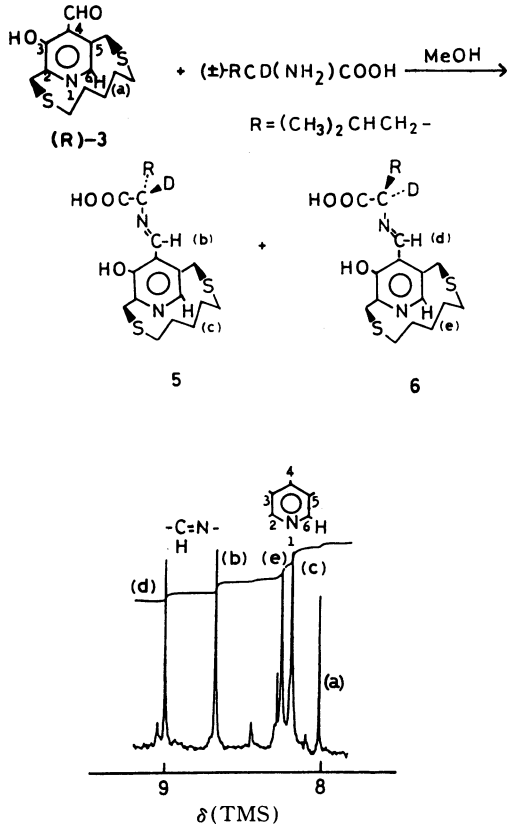

Fig. 5. ^1H 400-MHz NMR spectrum of C_6D_6 solution of the Schiff bases derived from (*R*)-**3** and racemic deuterated leucine in a molar ratio of 1.2/1 in MeOH (TMS internal standard). The signals (a—e) are; (a): 6-H proton of (*R*)-**3**. (b), (d); Azomethine protons of **5** and **6**. (c), (e); 6-H protons of **5** and **6**.

TABLE 2. CHEMICAL SHIFTS OF AZOMETHINE (–CH=N–) AND PYRIDINE RING PROTONS OF THE SCHIFF BASES PREPARED FROM $RCD(NH_2)COOH$ AND (*R*)-**3**

R (Configuration)		Chemical shift[a)] –CH=N–	Chemical shift[a)] Pyridine ring
$(CH_3)_2CHCH_2$	(*R*)	9.00	8.26
$(CH_3)_2CHCH_2$	(*S*)	8.77	8.17
$(CH_3)_2CH$	(*R*)	8.80	8.23
$(CH_3)_2CH$	(*S*)	8.54	8.17
$C_6H_5CD_2$	(*R*)	8.42[b)]	8.07[b)]
$C_6H_5CD_2$	(*S*)	8.30[b)]	8.04[b)]

a) δ from TMS in C_6D_6 (400 MHz). b) The high-field shift of the peaks is probably due to the anisotropic effect of the phenyl ring.

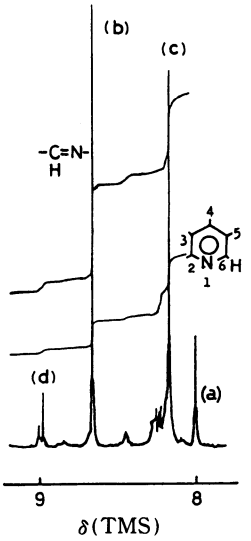

Fig. 6. ^1H 400-MHz NMR spectrum of C_6D_6 solution of the Schiff bases derived from (*R*)-**3** and deuterated leucine enriched in *S*-configuration in a molar ratio of 1.2/1 in MeOH (TMS internal standard). The signals (a—d) are; (a): 6-H proton of (*R*)-**3**, (b), (d): azomethine protons of **5** and **6**. (c): 6-H proton of **5**.

bases produced from (*S*)- or (*R*)-leucine and (*R*)-**3** can be isolated and characterized by elemental analyses, IR, and NMR spectra. When (*S*)-leucine was condensed with (*R*)-**3**, the resulting Schiff base revealed the azomethine and the pyridine ring protons at δ 8.73 and δ 8.13, respectively. When (*R*)-leucine was condensed with (*R*)-**3**, the signals for the azomethine and the pyridine ring protons appeared at δ 9.00 and 8.20. Therefore, the signals, (b) and (d), should be assigned to the azomethine protons of the Schiff bases containing (*S*)- and (*R*)-deutero leucine residues, respectively. In the same manner, the signals, (c) and (e), were apparently assignable to the protons of the pyridine ring of the Schiff bases constructed from (*S*)- and (*R*)-α-deuterated leucines, respectively. The difference of the chemical shifts between signals (b) and (d) was δ 0.23 which was enough resolution to make the accurate integration of each signal possible. Accordingly, the enantiomeric excess of the α-deuterated leucine obtained through the transamination reaction in MeOD was calculated from the ratio of the integrated intensities of the two diastereotopic azomethine protons, (b) and (d). Figure 6 shows the 400-MHz NMR spectrum of the Schiff bases prepared from the α-deuterated leucine, the product of transami-

Table 3. Enantiomeric excesses and yields of deuterated α-amino acids obtained through Zn^{2+}-catalyzed transamination reaction between (*R*)- or (*S*)-**1** and RCOCOOH in methanol-*d*

R	(*R*)- or (*S*)-**1**	Major enantiomer	$[\alpha]_D$/° (c, H_2O)	Temp/°C	e.e./%	Yield/%
$(CH_3)_2CHCH_2$	(*S*)-**1**	*R*	+9.2 (0.80)	22	94[a] 92[b]	66
$(CH_3)_2CHCH_2$	(*R*)-**1**	*S*	−8.4 (0.88)	23	90[a]	60
$(CH_3)_2CH$	(*R*)-**1**	*S*	+22.5 (0.66)[c]	19	80[a]	49
$C_6H_5CH_2$	(*R*)-**1**	*S*	−18.8 (0.56)	20	40[a]	56

a) (*R*)-**3** was used as the chiral pyridoxal analog. b) (*S*)-**3** was used as the chiral pyridoxal analog. c) 1 M HCl.

Table 4. Comparison of the enantiomeric excesses of amino acids determined by optical rotation and NMR analyses of diastereomeric Schiff bases

Amino acid (Configuration)[a]	Enantiomeric exescess (e.e./%)	
	Optical rotation	NMR method[b]
Leucine (*S*)	100	100[c]
Leucine (*R*)	100	100[c]
Leucine (*S*)[d]	96	90
Leucine (*S*)[d]	86	81
Leucine (*R*)[d]	73	78
Leucine (*R*)[d]	43	49
Valine (*S*)	100	100[c]
Valine (*R*)	100	100[c]
Valine (*S*)[d]	78	75
Valine (*R*)[d]	44	49

a) Major enantiomer. b) (*R*)-**3** was used as the chiral pyridoxal analog. c) Signals for only one diastereomer were observed. d) The amino acids were obtained through the nonezymatic transamination reaction.[8]

nation reaction using (*R*)-**1**, and (*R*)-**3**. Apparent predominance of the intensity of the signal at δ 8.77 over that at δ 9.00 indicates that the transamination reaction gave (*S*)-α-deuterated leucine in excess. The enantiomeric excess was calculated as described above. Similarly, the employment of (*S*)-enantiomer of **1** gave (*R*)-α-deuterated leucine in excess. The diastereotopic azomethine protons of Schiff bases prepared from the deuterated phenylalanine and valine, and (*R*)-**3** were also clearly resolved in the 400-MHz NMR spectra (Table 2). Consequently, the enantiomeric excess of the deuterated phenylalanine and valine could be determined as has been described in the case of the deuterated leucine. These results are shown in Table 3. The (*S*)-enantiomer of the pyridoxal analog (**3**) was also employed for quantitative measurement of enantiomeric excess of the deuterated amino acids. The enantiomeric excess of the deuterated alanine and tryptophane could not be determined by this procedure because of the overlapping of the diastereotopic azomethine protons.

This method can be applied to the determination of the enantiomeric excess of amino acids in general. Table 4 compares the enantiomeric excesses of several amino acids determined by the measurement of the optical rotations and by the NMR method described above. The results obtained by the two methods are consistent, confirming the reliability of the NMR method for the determination of enantiomeric excesses of amino acids.

Experimental

The melting points were determined with a Yamato capillary melting point apparatus and are uncorrected. The optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. The IR spectra were recorded on a Shimadzu IR-27 instrument. Mass spectra were recorded on a Hitachi M-80 mass spectrometer. The ^1H NMR spectra were recorded with a Varian HA-100D apparatus or a Japan Electron Optics Laboratory JEOL FX-400 with TMS or DSS as the internal standard. Chiral pyridoxamine analogs, (*R*)- or (*S*)-15-aminomethyl-14-hydroxy-5,5-dimethyl-2,8-dithia [9] (2,5)pyridinophane ((*R*)- or (*S*)-**1**),[20] and chiral pyridoxal analogs, (*R*)- or (*S*)-15-formyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane ((*R*)- or (*S*)- **3**),[10] were prepared as reported previously. Microanalyses of deuterated amino acids obtained by the conventional C and H method gave the total H+D percentages as determined by the weight of H_2O and HOD formed. The determined values of H+D were calculated from the weight of H_2O+HOD produced on the assumption that the H : D atomic ratio was that of the compound analyzed.

Preparation of Deuterated Amino Acid. A typical procedure for the preparation of the deuterated amino acid is described in the following. A mixture of sodium 4-methyl-2-oxopentanoate (106 mg, 0.70 mmol), $Zn(ClO_4)_2 \cdot 6H_2O$ (67 mg, 0.18 mmol), and pyridoxamine analog ((*R*)-**1**) (112 mg, 0.36 mmol) was stirred in methanol-*d* (100 ml) at room temperature for 4 d. The methanol-*d* solution was acidified to pH 1 with 1 M hydrochloric acid (1 M = 1 mol dm^{-3}) and evaporated *in vacuo*. Distilled water (50 ml) was added to the residue and the resulting mixture was extracted with ethyl acetate (200 ml) to remove the pyridoxal analog ((*R*)-**4**) produced. The aqueous layer was placed on Dowex-50 (H^+ form, 50 ml) column and eluted with distilled water (1000 ml) and 0.1 M aqueous ammonium hydroxide successively. The ammonium hydroxide effluent was evaporated *in vacuo* and the residue was redissolved in distilled water (10 ml). The solution was put on Amberlite IRC-50 (H^+ form, 15 ml) column and eluted with distilled water in order to remove trace of pyridoxamine analog ((*R*)-**1**). The effluent was concentrated to dryness *in vacuo* to give α-deuterated leucine (31.4 mg).

Determination of the Enantiomeric Excesses. A typical procedure for the determination of the enantiomeric excesses of the deuterated amino acids is exemplified by the case of leucine: a methanol solution (5 ml) of (*R*)-**3** (20.4 mg, 0.07 mmol) and the deuterated leucine (7.9 mg, 0.06 mmol) obtained was stirred at room temperature overnight and evaporated. The resulting residue was dissolved in C_6D_6 (1 ml) and the solution underwent recording the NMR spectrum at 400 MHz.

Spontaneous Deuteration at the β-Position of the Aromatic α-Keto Acids. Sodium phenylpyruvate (100 mg) was dissolved in methanol-d_4 (5 ml) and stirred at room temperature. The aliquots of the solution were taken for recording ^{1}H NMR spectrum (100 MHz). The ratio of the deuteration of the β-protons of the keto acid was determined by comparing the integral intensity of the β-protons with that of phenyl ring protons of the keto acid. The β-protons of sodium phenylpyruvate were gradually deuterated (44% at 24 h, 52% at 48 h, and 100% at 144 h, respectively). The β-protons of sodium indole-3-pyruvate were completely deuterated at 48 h.

Preparation of Schiff Base from General α-Amino Acid and Chiral Pyridoxal Analog. A mixture of (*S*)-leucine (66 mg, 0.5 mmol) and (*R*)-**3** (142 mg, 0.5 mmol) was stirred in methanol (150 ml) at room temperature overnight. After the methanol was recovered *in vacuo*, the resulting residue was extracted with benzene and the benzene solution was concentrated to about 10 ml. To the solution hexane was added gradually until a precipitate separated out. The precipitate was collected and recrystallized from a mixture of benzene and hexane to give the Schiff-base of (*R*)-pyridoxal analog-(*S*)-leucine in 65% yield: mp 100—102 °C. $[\alpha]_D^{23}$ +231° (*c* 0.13, $CHCl_3$). IR (KBr disk) 1715 (C=N) and 1625 cm^{-1} (C=O): ^{1}H NMR (400 MHz, C_6D_6) δ=8.73 (1H, s, C=N) and 8.13 (1H, s, 6-H). Found: C, 57.58; H, 7.28; N, 7.07; S, 16.41%. Calcd for $C_{19}H_{28}N_2O_3S_2$: C, 57.58; H, 7.06; N, 7.07; S, 16.18%.

The authors are grateful to Dr. Haruo Homma and his staff for performing elementary analyses, and to Dr. Jun Uzawa and Mrs. Tamiko Chijimatsu for ^{1}H NMR spectra measurement.

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