A New Enantioselective Synthesis of (2*R*,3*S*)-3-(4-Methoxyphenyl)glycidic Ester *via* the Enzymatic Hydrolysis of *erythro*-*N*-Acetyl-β-(4-methoxyphenyl)serine

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Received January 13, 1993

Enantioselective synthesis of (2*R*,3*S*)-3-(4-methoxyphenyl)glycidic ester ((2*R*,3*S*)-1) *via* the enzymatic hydrolysis of *erythro*-*N*-acetyl-β-(4-methoxyphenyl)serine (9) was investigated. Treatment of the obtained α-amino acid (−)-10 with $NaNO_2$–KBr–dilute H_2SO_4, esterification, and subsequent oxiran-ring closure of the halohydrin gave the target compound (2*R*,3*S*)-1 with high enantiomeric excess (94% ee).

Keywords enantioselective synthesis, (2*R*,3*S*)-3-(4-methoxyphenyl)glycidic ester; aminoacylase; *erythro*-*N*-acetyl-β-(4-methoxyphenyl) serine; enzymatic hydrolysis

Glycidic esters are good intermediates for the synthesis of many useful compounds. In particular, optically active *trans*-3-(4-methoxyphenyl)glycidic ester ((2*R*,3*S*)-**1**) is an efficient and practical key intermediate for the enantioselective syntheses of diltiazem (**4**), which is a typical calcium channel blocker used as an antianginal and antihypertensive agent,[1,2] and of clentiazem (**5**), the 8-chloro isomer of **4**, which is a cerebral vasodilator and antihypertensive agent,[3] as shown in Chart 1.

In the preceding paper,[4] we reported a new, efficient synthesis of (2*R*,3*R*)-**1** *via* the asymmetric reduction of 3-(4-methoxyphenyl)-3-oxopropionic esters, as a part of our studies on the asymmetric reduction of various aromatic ketones. Some other approaches involving conventional optical resolution of 3-(4-methoxyphenyl)glycidic acid,[5] enzymatic kinetic resolution of (±)-**1**,[6] and Darzens reaction in the presence of lithium salt of chiral organic base[7] have been reported by several research groups. On the other hand, in view of the excellent enantiodifferentiating ability of many aminoacylases not only in the laboratory but also industrially, the enzymatic hydrolysis of the corresponding *N*-acyl-α-amino acids seemed to be an obvious and facile approach to the above optically active molecule **1**.

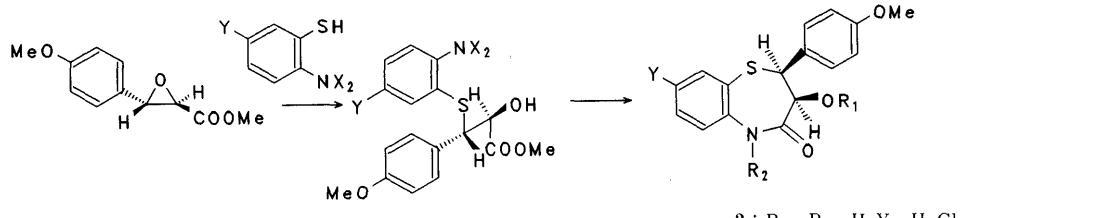
In this paper, we wish to describe a new, efficient synthesis of (2*R*, 3*S*)-**1** *via* the enzymatic hydrolysis of *erythro*-*N*-acetyl-β-(4-methoxyphenyl)serine (**9**) by using aminoacylase, as shown in Chart 2.

erythro-β-(4-Methoxyphenyl)serine ethyl ester (**8**) was prepared by nitrosating anisoylacetic ester (**6**)[8] and then hydrogenating the resulting 3-hydroxyimino compound (**7**) in an acidic medium in the presence of Pd–C[9] in high yield. According to Chang and Hartung[9a] the hydrogenation of the α-hydroxyimino-ketones in an acidic medium gave the *erythro*-aminoalcohol as a sole product *via* a rigid ring-like substrate–catalyst complex, which was probably formed by the coordination of the nitrogen and oxygen atoms of the substrate molecule to the catalyst (Pd).

The hydroxyamino ester (**8**) was converted to the *N*-acetamino acid (**9**) either by hydrolysis of the ester group and then *N*-acetylation or conversely by *N*,*O*-diacetylation and then hydrolysis in overall yields of 78.3 and 41.1%, respectively.

Enantioselective enzymatic hydrolysis[10] of the *N*-acetyl group of **9** using aminoacylase (Amano Pharmaceutical Co.) in the presence of $CoCl_2$[11] at 37±4 °C in aqueous solution (pH 7.0) gave the (−)-α-amino acid ((−)-**10**, 100% ee) and the (+)-α-acetamino acid ((+)-**9**) in 31 and 35% yields, respectively. Generally, aminoacylase hydrolyzes exclusively the *N*-acyl group of an α-amino acid of L-configuration, but not the corresponding D-enantiomer. Consequently, the absolute configuration of (−)-**10** obtained above was presumed to be 2*S*, 3*S*, and this was confirmed by derivation to the desired (2*R*, 3*S*)-glycidic ester ((2*R*, 3*S*)-**1**).

Deamination of (−)-**10** by treatment with sodium nitrite in 1.25 N H_2SO_4 in the presence of KBr as a halogen source under ice-cooling, followed by esterification with diazomethane, gave the bromohydrin ((+)-**11**) as an oil in 40% yield. Use of KCl in concentrated HCl instead of KBr in H_2SO_4 afforded the chlorohydrin derivative in almost the same yield. Deamination reaction of α-amino acid is known to proceed with nearly complete retention of the



1 2

3 : $R_1=R_2=H$, Y=H, Cl
diltiazem (**4**) : R_1=Ac ; $R_2=(CH_2)_2N(CH_3)_2$, Y=H
clentiazem (**5**) : R_1=Ac ; $R_2=(CH_2)_2N(CH_3)_2$, Y=Cl

Chart 1

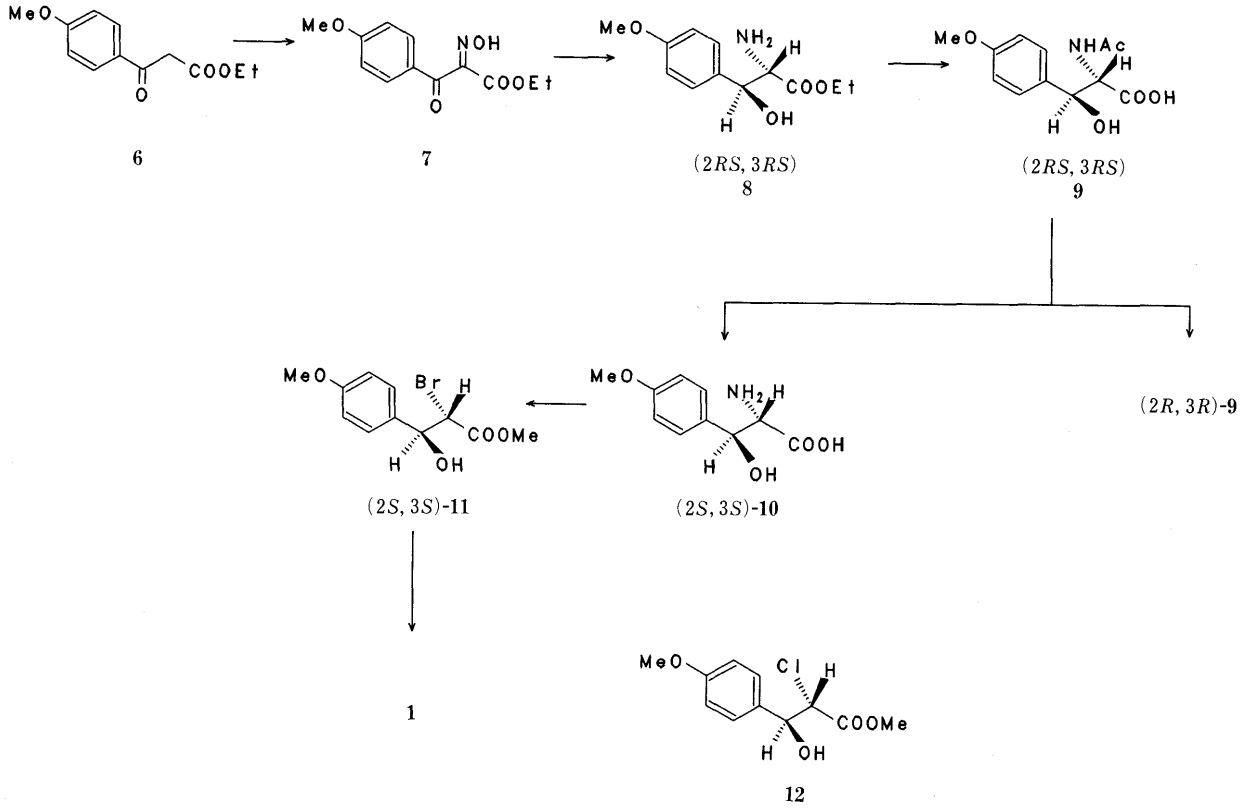


Chart 2

Table I. Oxiran Ring-Closure of **11** and **12**

Starting material	Base	Solvent	Conditions	Yield of **1** (%)
(±)-**11**	K_2CO_3	Et_2O–MeOH[a]	0—5°C, 30 min	94
(±)-**11**	$KHCO_3$	Et_2O–MeOH[a]	r.t., 6 h	96
(±)-**11**	Na_2CO_3	Et_2O–MeOH[a]	r.t., 6 h	—[b]
(+)-**11**	K_2CO_3	Et_2O–MeOH[a]	0—5°C, 30 min	74
(±)-**12**	K_2CO_3	Et_2O–MeOH[a]	0—5°C, 1 h	80

a) Without MeOH the reaction proceeded very slowly. *b*) No reaction was observed. r.t. = room temperature.

configuration at C_2 through anchimeric assistance of the adjacent carboxylic acid function.[12] The *erythro*-stereochemistry of the bromohydrin ((+)-**11**) was confirmed by comparison of its NMR spectra with that of a known compound, methyl *erythro*-2-chloro-3-hydroxy-3-(4-methoxyphenyl)propionate.[4]

Finally, epoxy ring-closure of (+)-**11** with 2 eq of K_2CO_3 in MeOH under ice-cooling gave the desired (2*R*, 3*S*)-**1** of 94% ee in 74% yield. The results of oxiran ring-closure of the halohydrins **11** and **12** by treatment with a base such as K_2CO_3, $KHCO_3$, or Na_2CO_3 are summarized in Table I.

Experimental

All melting points are uncorrected. IR spectra were taken with a Hitachi IR-215 instrument. ^{1}H-NMR spectra were recorded on a JEOL PMX60 or JEOL JNM-FX100 spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were measured using a JEOL JMS-HX100 mass spectrometer. Microanalysis was performed on a Perkin Elmer 240B C, H, N analyzer and a Yokogawa IC-100 ion chromatographic analyzer.

***erythro*-β-(4-Methoxyphenyl)serine Ethyl Ester (8)** i) A solution of $NaNO_2$ (22.4 g, 0.32 mol) in H_2O (47 ml) was added to a solution of **6**[13] (67.3 g, 0.30 mol) in AcOH (40 ml) at 10—20 °C during a period of 1 h. After being stirred for 2 h, the reaction mixture was extracted with Et_2O. The extracts were combined, washed with water, $NaHCO_3$, and water successively, dried, and concentrated, and the residue was recrystallized from iso-Pr_2O–*n*-hexane to give ethyl α-hydroxyimino-β-oxo-4-methoxybenzenepropionate (**7**) (61.4 g, 88%), mp 118—120 °C (lit.,[8b] mp 113—114 °C).

ii) A solution of **7** (15.0 g, 64.9 mmol) in EtOH (90 ml) and concentrated HCl (15 ml, 180 mmol) was hydrogenated in the presence of 10% Pd–C (2.23 g) under ordinary pressure at ambient temperature overnight. The reaction mixture was diluted with water to dissolve the precipitated HCl salt of **8** and Pd–C was filtered off. The filtrate was concentrated and the residue was recrystallized from 95% EtOH to give **8**·HCl (15.1 g, 85%), mp 167—170 °C (dec.). IR (Nujol): 3220, 3190, 3120, 2800—2500, 1735 cm^{-1}. MS *m/z*: 239 (M^+), 222, 166, 148, 137, 103, 74. ^{1}H-NMR (DMSO-d_6–D_2O) δ: 1.04 (3H, t, *J* = 7 Hz, CH_3), 3.75 (3H, s, OCH_3), 4.10 (1H, d, *J* = 3.3 Hz, C<u>H</u>NH_2), 4.04 (2H, q, *J* = 7 Hz, CH_2), 5.18 (1H, d, *J* = 3.3 Hz, C<u>H</u>OH), 6.90 (2H, d, *J* = 9 Hz, aromatic H), 7.29 (2H, d, *J* = 9 Hz, aromatic H). *Anal.* Calcd for $C_{12}H_{18}ClNO_4$: C, 52.27; H, 6.58; Cl, 12.86; N, 5.08. Found: C, 52.35; H, 6.40; Cl, 12.87; N, 5.00.

(±)-*erythro*-*N*-Acetyl-β-(4-methoxyphenyl)serine ((±)-9) Method A: A suspension of **8**·hydrochloride (14.0 g, 50.8 mmol) in aqueous NaOH (NaOH (7.31 g, 0.183 mol) and water (39 ml)) was stirred at room temperature for 30 min.[14] To the obtained clear solution was added Ac_2O (6.74 g, 66.0 mmol) at 30—35 °C over a period of 30 min. The reaction mixture was stirred for 1 h, then acidified with concentrated HCl, saturated with NaCl, and extracted with $CHCl_3$. The extracts were combined, dried over $MgSO_4$, and concentrated. The residual solid was recrystallized from Et_2O to give (±)-**9** (9.42 g), mp 82—85 °C. The aqueous layer was charged on a Mitsubishi Diaion HP-20 column, eluted with water to remove inorganic compounds, and then eluted with MeOH–H_2O (1 : 1) to give additionl (±)-**9** (650 mg after recrystallization, total yield 78%). IR ($CHCl_3$): 3300, 3000—2200, 1720, 1640, 1600 cm^{-1}. MS *m/z*: 253 (M^+), 137 (base peak), 135. ^{1}H-NMR ($CDCl_3$) δ: 1.98 (3H, s, $COCH_3$), 3.77 (3H, s, OCH_3), 4.88 (1H, dd, *J* = 6.3, 7.0 Hz), 5.19 (1H, d, *J* = 6.3 Hz), 6.60 (1H, d, *J* = 7.0 Hz), 6.83 (2H, d, *J* = 8.6 Hz, aromatic H), 7.25 (2H, d, *J* = 8.6 Hz, aromatic H). *Anal.* Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.87; H, 5.86; N, 5.60.

Method B: Acetyl chloride (7.10 g, 90.4 mmol) was added to a mixture of **8**·hydrochloride (8.00 g, 29.0 mmol), triethylamine (13.1 g, 0.129 mol), and CH_2Cl_2 (50 ml) under ice-cooling. After being stirred at room

temperature overnight, the reaction mixture was diluted with water, acidified with dilute HCl, and extracted with AcOEt. The extracts were combined, washed with water, dried over $MgSO_4$, and concentrated to give an oil, which was purified by chromatography (silica gel, eluted with $CHCl_3$–AcOEt (8:2)) to give (±)-*erythro*-*N*,*O*-diacetyl-β-(4-methoxyphenyl)serine ethyl ester (8.27 g, 88%) as an oil. IR (liquid): 3340, 3270, 1740, 1660, 1600 cm^{-1}. MS *m/z*: 323 (M^+), 264, 222, 218, 179, 137. ^{1}H-NMR ($CDCl_3$): 1.26 (3H, t, *J*=7 Hz), 2.00 (3H, s), 2.10 (3H, s), 3.80 (3H, s), 4.18 (2H, q, *J*=7 Hz), 5.18 (1H, dd, *J*=4.4, 8.8 Hz), 5.94 (1H, d, *J*=8.8 Hz, NH), 6.13 (1H, d, *J*=4.4 Hz), 6.87 (2H, d, *J*=8.8 Hz), 7.22 (2H, d, *J*=8.8 Hz).

The obtained diacetyl ester (8.27 g, 25.9 mmol) was hydrolyzed by stirring in a solution of EtOH (5 ml), H_2O (20 ml), and NaOH (2.04 g, 51.2 mmol) at room temperature for 30 min. The reaction mixture was worked up in a usual manner to give (±)-**9** (3.02 g, 47%).

(2*S*, 3*S*)-(−)-β-(4-Methoxyphenyl)serine ((−)-10) A suspension of (±)-**9** (10.0 g, 39.5 mmol) in water (200 ml) was converted to a solution by adjusting its pH to 7.0 with 2 N NaOH. To this solution, $CoCl_2 \cdot 6H_2O$ (20 mg) and acylase (Amano Pharmaceutical Co., Ltd.) (150 mg) were added, and the mixture was stirred at 37±4 °C. Stirring was continued for 6 h, additional acylase (150 mg) was added, and the pH was adjusted to 7.0. Stirring was continued for 24 h, then the reaction mixture was concentrated under reduced pressure until the volume of the mixture became *ca.* 20 ml. The residual oil was acidified with concentrated HCl and extracted with $CHCl_3$. The extracts were combined, dried over $MgSO_4$, and concentrated to give (2*R*, 3*R*)-**9** (3.54 g, 35%)[15] as an oil, which was identical with (±)-**9** on TLC (silica gel, $CHCl_3$–EtOH–HOAc–H_2O (65:30:3:2)).

The aqueous layer was made basic with concentrated NH_4OH and concentrated until the volume of the solution became 5—10 ml. The precipitated crystals were collected on a filter, washed with a small amount of water, and recrystallized from aqueous MeOH to give (−)-**10** (2.82 g, 31%), mp 163—166 °C (dec.). $[\alpha]_D^{20}$ −2.73 (*c*=0.586, MeOH–H_2O (1:1)), 100% ee as determined by HPLC [column, MCI gel CRS 10W (Mitsubishi Kasei); eluent, 2 mM $CuSO_4$/MeOH–H_2O (1:9); flow rate, 1 ml/min; retention times, 15.1 and 19.6 min for (+)-**10** and (−)-**10**, respectively; detector, 254 nm]. IR (Nujol): 3470, 3220, 3000—2500, 1670, 1620 cm^{-1} (HCl salt). *Anal.* Calcd for $C_{10}H_{13}NO_4 \cdot H_2O$: C, 52.39; H, 6.60; N, 6.11. Found: C, 52.51; H, 6.54; N, 6.18.

Methyl (2*S*, 3*S*)-(+)-2-Bromo-3-hydroxy-3-(4-methoxyphenyl)propionate ((+)-11) A mixture of (−)-**10** (1.91 g, 8.32 mmol), KBr (3.48 g, 29.1 mmol), 1.25 N H_2SO_4 (17.3 ml, 21.6 mmol), and Et_2O (4 ml) was treated with $NaNO_2$ (920 mg, 13.3 mmol) over a period of 1 h under ice-cooling. After being stirred at room temperature for 2 h, the reaction mixture was extracted with Et_2O. The extracts were combined, washed with saturated aqueous NaCl, and treated with a solution of diazomethane in Et_2O [prepared from nitrosomethylurea (1.0 g, 10 mmol), 50% aqueous KOH (6.43 g, 57.4 mmol), and Et_2O]. The reaction mixture was stirred at room temperature for 10 min, then concentrated under reduced pressure and the residual oil (1.77 g) was purified by column chromatography (silica gel, eluted with $CHCl_3$–EtOH (99:1)) to give (+)-**11** (970 mg, 40%) as an oil. IR (liquid): 3470, 1740 cm^{-1}. MS *m/z*: 290, 288 (M^+), 137. ^{1}H-NMR ($CDCl_3$) δ: 3.04 (1H, d, *J*=5 Hz, OH), 3.81 (6H, s, OCH_3), 4.35 (1H, d, *J*=8.3 Hz, CHBr), 5.04 (1H, dd, *J*=8.3, 5.0 Hz, CHOH), 6.90 (2H, d, *J*=8.8 Hz, aromatic H), 7.31 (2H, d, *J*=8.8 Hz, aromatic H). $[\alpha]_D^{20}$ +9.62° (*c*=0.239, MeOH), 98% ee as determined by HPLC [column, Chiralpak AD (Daicel Chemical Industry); eluent, hexane–iso-PrOH (90:10); flow rate, 1 ml/min; retention times, 17.0 and 20.5 min for (−)-**11** and (+)-**11**, respectively; detector, 220 nm]. Compound (±)-**10** was also converted to (±)-**11** in 42% yield as an oil in the same manner as described above.

When (±)-**10** (300 mg, 1.42 mmol) was treated with KCl (240 mg, 3.22 mmol), concentrated HCl (1.0 ml, 12 mmol), Et_2O (5 ml), and $NaNO_2$ (170 mg, 2.46 mmol) and then diazomethane at room temperature for 2 h in the same manner as described above, the (±)-chlorohydrin (**12**) was obtained in 32% yield.

Methyl (2*R*,3*S*)-3-(4-Methoxyphenyl)glycidate((−)-1) Under ice-cooling, a mixture of (+)-**11** (169 mg, 0.585 mmol), Et_2O (1 ml), MeOH (2 ml), and K_2CO_3 (powder, 170 mg, 1.23 mmol) was stirred for 30 min, diluted with ice-water, and extracted with $CHCl_3$. The extracts were combined, washed with water, dried over $MgSO_4$, and distilled *in vacuo* after removal of the $CHCl_3$ to give (−)-**1** (90 mg, 74%) as an oil, bp 130—140 °C (0.3 mmHg). $[\alpha]_D^{20}$ −168.6° (*c*=0.690, EtOH), 94% ee as determined by HPLC [column, Chiralcel OJ (Daicel Chemical Industry); eluant, *n*-hexane–iso-PrOH (70:30); flow rate, 1 ml/min; retention times, 13.9 and 17.0 min for (+)-**1** and (−)-**1**, respectively; detector, 254 nm]. IR (liquid): 1730 cm^{-1}. mp 88—89 °C.

Acknowledgement We are grateful to Dr. S. Yamada, Professor Emeritus of Tokyo University, and Dr. S. Saito, Senior Managing Director of Tanabe Seiyaku Co., Ltd., for their interest and fruitful suggestions. Thanks are also due to the staff of the analytical section of this laboratory for spectral and elemental analysis and to Dr. H. Abe for HPLC analysis.

References and Notes

1) *a*) H. Kugita, H. Inoue, M. Ikezaki, M. Konda, S. Takeo, *Chem. Pharm. Bull.*, **19**, 595 (1971); *b*) H. Inoue, S. Takeo, M. Kawazu, H. Kugita, *Yakugaku Zasshi,* **93**, 729 (1973); *c*) M. Sato, T. Nagao, I. Yamaguchi, H. Nakajima, A. Kiyomoto, *Arzneim. Forsch.*, **21**, 1338 (1971); *d*) H. Nakajima, M. Hoshiyama, K. Yamashita, A. Kiyomoto, *Jpn. J. Pharmacol.*, **26**, 571 (1976); *e*) K. Abe, H. Inoue, T. Nagao, *Yakugaku Zasshi*, **108**, 716 (1988).

2) *a*) H. Kugita, H. Inoue, M. Ikezaki, M. Konda, S. Takeo, *Chem. Pharm. Bull.*, **18**, 2028 (1970); *b*) *Idem*, *ibid.*, **18**, 2284 (1970); *c*) T. Hashiyama, H. Inoue, M. Konda, M. Takeda, *J. Chem. Soc., Perkin Trans. 1*, **1984**, 1725; *d*) T. Hashiyama, H. Inoue, M. Takeda, *ibid.*, **1985**, 421.

3) *a*) H. Inoue, M. Konda, T. Hashiyama, H. Otsuka, K. Takahashi, M. Gaino, T. Date, M. Aoe, M. Takeda, S. Murata, H. Narita, T. Nagao, *J. Med. Chem.*, **34**, 675 (1991); *b*) H. Narita, S. Murata, H. Yabana, K. Kikkawa, Y. Akimoto, T. Nagao, *Arzneim. Forsch./Drug Res.*, **38**, 515 (1988); *c*) H. Narita, K. Kikkawa, H. Yabana, T. Nagao, *ibid.*, **38**, 521 (1988); *d*) K. Kikkawa, S. Murata, T. Nagao, *ibid.*, **38**, 526 (1988).

4) K. Matsuki, M. Sobukawa, A. Kawai, H. Inoue, M. Takeda, *Chem. Pharm. Bull.*, **41**, 643 (1993).

5) *a*) H. Sawai, J. Yoshinaga, Japan Kokai 60-13775 and 60-13776 (1985) [*Chem. Abstr.*, **103**, 71179*w* and 71180*q* (1985)]; *b*) M. Hayashi, M. Yamamoto, Japan Kokai 61-145159 and 61-145160 (1986) [*Chem. Abstr.*, **106**, 4672*a* and 32600*u* (1987)]; *c*) M. Hayashi, M. Yamamoto, Japan Kokai 61-145173 and 61-145174 (1986) [*Chem. Abstr.*, **106**, 32811*p* and 32810*m* (1987)]; *d*) M. Yamamoto, M. Hayashi, M. Masaki, H. Nohira, *Tetrahedron Asym.*, **2**, 403 (1991).

6) *a*) T. Shibatani, K. Nakamichi, H. Matsumae, European Patent Appl., EP 362556 (1990) [*Chem. Abstr.*, **113**, 96133*w* (1990)]; *b*) L. A. Hulshof, J. H. Roskam, Eur. Patent Appl. EP 343714 (1989) [*Chem. Abstr.*, **113**, 76603 *w* (1990)]; *c*) D. R. Dodds, J. L. Lopez, C. M. Zepp, S. Brandt, R. F. Rossi, PCT. Int. Appl. WO 9004643 (1990) [*Chem. Abstr.*, **113**, 76620*z* (1990)].

7) *a*) K. Koga, H. Kawasaki, Japan Kokai 1-226881 (1989) [*Chem. Abstr.*, **112**, 158034*e* (1990)]; *b*) M. Okamura, H. Kawasaki, K. Koga, Abstracts of Papers, The 108 th Annual Meeting of Pharmaceutical Society of Japan, Hiroshima, April 1988, p. 68.

8) *a*) H. Adkins, E. W. Reeve, *J. Am. Chem. Soc.*, **60**, 1328 (1938); *b*) A. Wahl, C. Silberzweig, *Bull. Soc. Chim. Fr.*, **11**, 28 [*Beilstein*, **10** I, 490 (1932)].

9) *a*) Y. Chang, W. H. Hartung, *J. Am. Chem. Soc.*, **75**, 89 (1953); *b*) C. Tanaka, N. Saito, *Yakugaku Zasshi*, **83**, 136 (1962).

10) Y. Masaoka, M. Sakakibara, K. Mori, *Agric. Biol. Chem.*, **46**, 2319 (1982).

11) Co^{2+} specifically activates aminoacylase: T. Tosa, T. Mori, N. Fuse, I. Chibata, *Enzymologia*, **32**, 153 (1967).

12) *a*) Y. Shimohigashi, M. Waki, N. Izumiya, *Bull. Chem. Soc. Jpn.*, **52**, 949 (1979); *b*) R. Amouroux, B. Gerin, M. Chastrette, *Tetrahedron*, **41**, 5231 (1985); *c*) K. Hintzer, B. Koppenhoefer, V. Schurig, *J. Org. Chem.*, **47**, 3850 (1982).

13) H. Gilman, H. S. Broadbent, *J. Am. Chem. Soc.*, **70**, 2755 (1948).

14) When the reaction mixture was worked up in a usual manner, (±)-**10** (mp 155—157 °C (dec.)) was obtained in 94% yield. IR (Nujol): 3230, 3050—2500, 1640, 1610, 1570, 1500 cm^{-1}. *Anal.* Calcd for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.20; N, 6.63. Found: C, 56.77; H, 6.15; N, 6.74.

15) Estimation of the optical purity of (2*R*,3*R*)-**9** was unsuccessful.