Kinetic Resolution of (±)-4-Acyloxy-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisoquinolines by Use of Immobilized Lipases in Organic Solvents[1)]

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Kinetic resolution of (±)-4-acyloxytetrahydroisoquinolines (5—7) by use of lipases immobilized on Celite in isooctane or cyclohexane saturated with water was carried out to give 4*S*-(+)-tetrahydroisoquinolin-4-ol (8) in a high optical purity. The absolute configuration of (+)-8 was determined by comparison of the spectral data and specific rotation with those of authentic 4*R*-(−)-tetrahydroisoquinolin-4-ol (8) derived from *R*-(−)-epinephrine (9).

Keywords (±)-4-acyloxytetrahydroisoquinoline; 4*S*-(+)-tetrahydroisoquinolin-4-ol; 4*R*-(−)-4-acyloxytetrahydroisoquinoline; 4*R*-(−)-tetrahydroisoquinolin-4-ol; *Candida cylindracea*; Celite-immobilized lipase; cyclohexane; isooctane; kinetic resolution; *R*-(+)-MTPA ester

It is well known that enzyme-catalyzed hydrolysis[2)] of esters and acetates constitutes one of the most useful methods for synthesis of optically active building blocks, which can be converted to naturally occurring compounds, such as antibiotics,[3)] terpenoids[4)] and so on. However, most of the reactions are performed with neutral or acidic esters and acetates and there are only a few reports[5)] on its application to basic substrates except for the synthesis of alkaloids[6)] by means of microbial conversion. In the course of our project aimed at the synthesis of optically active isoquinoline alkaloids, we examined the enzyme-catalyzed hydrolysis of (±)-4-acyloxytetrahydroisoquinolines, which might be applicable to a synthesis of optically active isoquinoline alkaloids having a hydroxyl group at their 4-position, such as (+)-steporphine (**1**),[7)] (+)-srilankine (**2**),[8)] (+)-cataline (**3**),[9)] and (+)-roemecarine (**4**).[10)] The present paper deals with successful kinetic resolution of (±)-4-acyloxytetrahydroisoquinolines (**5**—**7**) by the use of immobilized lipases in organic solvents.

(±)-4-Acetoxytetrahydroisoquinoline (**5**)[11)] was prepared by acetylation of (±)-tetrahydroisoquinolin-4-ol (**8**)[12)] derived from veratraldehyde and aminoacetaldehyde diethylacetal according to Bobbitt and Sih.[12)] The proton nuclear magnetic resonance (^{1}H-NMR) (400 MHz) spectrum of the *R*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (*R*-(+)-MTPA) esters of (±)-tetrahydroisoquinolin-4-ol (**8**), obtained by the reaction with *R*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA Cl),[13)] showed distinct N-methyl proton signals (δ 2.38 and 2.46) (see Experimental). Therefore, the optical purity (e.e.) of the unchanged (after chemical hydrolysis) and hydrolyzed compounds (**5** and **8**) could be readily determined by the ^{1}H-NMR spectral analysis from the ratio of the N-methyl proton signals.

Initially, enzymatic hydrolysis of (±)-5 with various commercially available lipases in a 0.1 M phosphate buffer solution (*p*H 7.25)[14)] was examined. The mixture was incubated with shaking at 33 °C while monitoring the reaction until about 50% hydrolysis of (±)-**5** was observed on thin layer chromatography (TLC). Usual work-up of the reaction mixture followed by silica gel column chromatography gave (−)-**5** and (+)-**8**, respectively. The former was hydrolyzed with 5% methanolic potassium hydroxide to give (−)-**8**. As mentioned above, each tetrahydroisoquinolin-4-ol was converted to the corresponding (+)-MTPA ester, and the ^{1}H-NMR spectral analysis of the ester allowed us to determine the optical purity of each alcohol (**8**). The results are shown in Table I.

As shown in Table I, although (+)- or (−)-**8** was formed by enzymatic hydrolysis, the optical purity in each case was extremely poor. With lipase OF-360 (*Candida cylindracea*), the optical purity of the recovered (−)-**5** was at best 32% e.e. Although with MY-30, Amano D-10, and Lusepase (+)-**5** and (+)-**8** were obtained, the optical purity was also poor.

In order to examine the effect of the acyl group, furthermore, (±)-5-phenylvalerate (**6**), prepared by valerylation[15)] of (±)-**8** with 5-phenylvaleric acid and diethyl chlorophosphate in the presence of triethylamine, was incubated under the same conditions as described for (±)-**5**. Although the reaction was slow (48 h), the optical purity of (+)- or (−)-**8** was also less than 20% e.e. The results are listed in Table II.

Such a nonselective hydrolysis may occur when the acyloxyl group is susceptible to the buffer solution. In the present reaction even without lipase, more than 50% of the

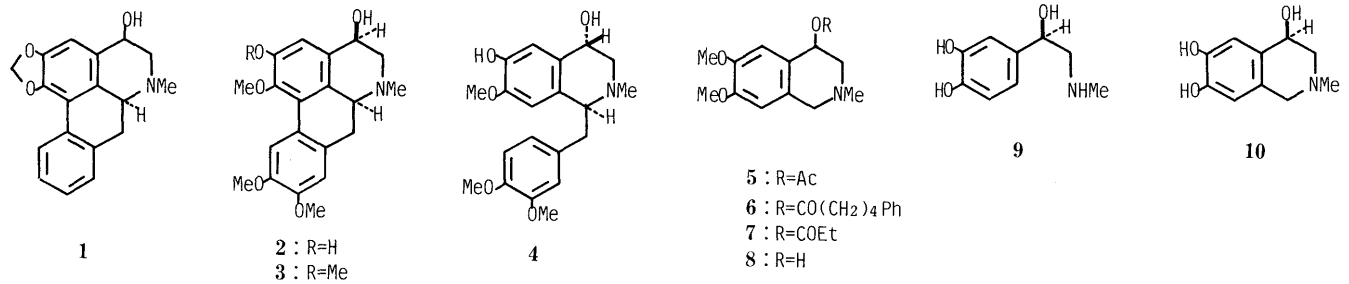

Chart 1

TABLE I. Reaction of (±)-4-Acetoxytetrahydroisoquinoline (**5**) with Lipase in Phosphate Buffer Solution

Lipase	Reaction time (h)	Chemical yield (%)		Optical purity (% e.e.)		Absolute config. of
		(−)-**5**	(+)-**8**	(−)-**5**	(+)-**8**	(+)-**8**
OF-360[a]	4.5	25	7	32	9	*S*
MY-30[b]	4.5	34[c]	16	6[c]	12	*S*
C.C. Sigma[d]	4.5	39	11[e]	6	4[e]	*R*[e]
Amano A-6[f]	4.0	37	60[e]	4	17[e]	*R*[e]
Amano P[g]	4.0	50	50	8	18	*S*
Amano M-10[h]	4.0	41	53[e]	7	11[e]	*R*[e]
Amano D-10[i]	4.0	41[c]	19	2[c]	11	*S*
R.D.[i]	4.0	38	18	6	7	*S*
Lusepase[j]	4.0	49[c]	21	14[c]	18	*S*
Amano GC[k]	4.5	21	51[e]	5	5[e]	*R*[e]
Pancreatin F[l]	4.5	52	20	9	4	*S*

a) *Candida cylindracea* OF-360 (Meito Sangyo Co., Ltd.). *b*) *C. cylindracea* MY-30 (Meito Sangyo Co., Ltd.). *c*) (+)-**5** was obtained. *d*) *C. cylindracea* (Sigma, L 1754). *e*) (−)-**8** was obtained. *f*) *Aspergillus niger* (Amano Pharmaceutical Co., Ltd.). *g*) *Pseudomonas* sp. (Amano Pharmaceutical Co., Ltd.). *h*) *Mucol japonicus* (Amano Pharmaceutical Co., Ltd.). *i*) *Rhizopus delemar* (Amano Pharmaceutical Co., Ltd.; D.R.: Biochemical Industry Co., Ltd.). *j*) *Rhizopus japonicus* (Osaka Saikin Kenkyusho). *k*) *Geotrichum candidum* (Amano Pharmaceutical Co., Ltd.). *l*) Porcine pancreas (Amano Pharmaceutical Co., Ltd.).

TABLE II. Reaction of (±)-5-Phenylvalerate (**6**) with Lipase in Phosphate Buffer Solution

Lipase	Reaction time (h)	Chemical yield (%)		Optical purity (% e.e.)		Absolute config. of
		(−)-**6**	(+)-**8**	(−)-**6**	(+)-**8**	(+)-**8**
OF-360	48	49	47	11	11	*S*
Amano A-6	48	76[a]	22	11[a]	20	*S*
Amano GC	48	45	44	5	2	*S*
Lusepase	48	50	46	7	1	*S*

a) (+)-**6** was obtained.

TABLE III. Reaction of (±)-4-Acetoxytetrahydroisoquinoline (**5**) with Immobilized Lipase in Organic Solvent

Lipase	Solvent	Reaction time (h)	Chemical yield (%)		Optical purity (% e.e.)		Absolute configuration of
			(−)-**5**	(+)-**8**	(−)-**5**	(+)-**8**	(+)-**8**
OF-360	I	24	44	40	93	94	*S*
	C	24	53	41	87	78	*S*
	B	24	70	3	17	26	*S*
MY-30	I	68	55	44	73	76	*S*
	C	68	25	47	61	80	*S*
C.C Sigma	I	68	51	48	80	87	*S*
	C	68	51	42	49	83	*S*
Amano A[a]	I	68	48	12[b]	15	1[b]	*R*[b]
	C	24	76	14[b]	21	60[b]	*R*[b]
Amano A-6	I	68	36	56[b]	62	30[b]	*R*[b]
	C	68	44	52[b]	55	22[b]	*R*[b]
Amano P	I	68	34	14	9	81	*S*
R.D.	I	68	79[c]	20	11[c]	26	*S*

I, isooctane; C, cyclohexane; B, benzene. *a*) *Aspergillus niger* (Amano Pharmaceutical Co., Ltd.). *b*) (−)-**8** was obtained. *c*) (+)-**5** was obtained.

acetate (**5**) was converted to the alcohol (**8**) under the above mentioned conditions after 4 h, although the reason is unclear.

This problem could presumably be solved by the use of immobilized enzymes,[16] because immobilization enables an enzyme to be used even in organic solvents,[17] requiring only small amounts of water. Thus, a mixture of (±)-**5** and each lipase immobilized on Celite[18] in an organic solvent (benzene, cyclohexane or isooctane) saturated with water was incubated at 33 °C with monitoring of the reaction by TLC. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* to give a residue, which was treated in the same manner as noted above. The results are listed in Table III.

The reaction rate was very slow (24 or 68 h), but as expected, the optical purity of the products was increased remarkably. In this case, lipases from *Aspergillus niger* gave (−)-**8**, while the other ones gave (+)-**8**. As for the solvent, benzene retarded the reaction, presumably because it deactivates the lipase. Among the lipases used, the reaction

TABLE IV. Reaction of (±)-5-Phenylvalerate (**6**) or (±)-Propionate (**7**) with Immobilized Lipase in Organic Solvent

Substrate	Lipase	Solvent	Reaction time (h)	Chemical yield (%)			Optical purity (% e.e.)			Absolute configuration of
				(−)-**6**	(−)-**7**	(+)-**8**	(−)-**6**	(−)-**7**	(+)-**8**	(+)-**8**
(±)-**6**	OF-360	I	24	50		48	89		95	*S*
		C	24	53		45	83		95	*S*
	C.C. Sigma	I	68	48		49	89[a]		92	*S*
		C	68	51		43	85		94	*S*
(±)-**7**	C.C. Sigma	I	72		70	16		40[b]	93	*S*
		C	72		77	15		27	89	*S*

I, isooctane; C, cyclohexane. *a*) $[\alpha]_D^{26}$ −116.7° (*c*=2.60, $CHCl_3$). *b*) $[\alpha]_D^{26}$ −17° (*c*=0.84, $CHCl_3$).

with immobilized lipase OF-360 in isooctane gave (+)-**8** (40%; 94% e.e.) and (−)-**8** (44%; 93% e.e.).

For the purpose of examining the utility of the method, similar hydrolysis of (±)-**6** and (±)-**7** with lipases OF-360 and C. C. Sigma was carried out. The results are shown in Table IV.

It is noteworthy that the kinetic resolution of (±)-**6** bearing a bulky acyl group proceeded in good chemical and optical yields.

The absolute configuration of (+)-**8** was determined as follows. Namely, *R*-(−)-epinephrine (**9**) was treated with 35% formalin according to Bates' method[19] to give the hydrochloride of 4*R*-(−)-1,2,3,4-tetrahydro-2-methylisoqionoline-4,6,7-triol (**10**), mp 165 °C (dec.), which was methylated with diazomethane in methanol to afford 4*R*-(−)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisoquinolin-4-ol (**8**), $[\alpha]_D^{25}$ −13.8°. The ^{1}H-NMR spectra of the (+)-alcohol (**8**) and 4*R*-(−)-**8** thus obtained were identical. As the specific rotation was reversed in sign, the absolute stereostructure of (+)-**8** was proved to be 4*S*-(+)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisoquinolin-4-ol.

In conclusion, kinetic resolution of (±)-4-acyloxytetrahydroisoquinolines (**5**—**7**) by the use of lipase (*Candida cylindracea*) immobilized on Celite in an organic solvent saturated with water was proved to proceed in good chemical and optical yields. Application of the present method to the synthesis of optically active isoquinoline alkaloids is in progress.

Experimental

All the melting points were measured on a Büchi 510 melting point measuring apparatus and are uncorrected. ^{1}H-NMR spectra were taken with a JEOL JNM-FX-100, GX-400 or GSX-500 instrument in $CDCl_3$ solution using tetramethylsilane as an internal standard, unless otherwise noted. Infrared (IR) spectra were taken with a Hitachi 260-10 instrument using KBr discs. Mass spectra (MS) were run on a Hitachi RMV-7M or M-80 instrument. Specific rotation was measured on a Perkin Elmer 241MC or JEOL DIP-360 polarimeter. Column chromatography was performed on Wakogel C-200 (Wako Pure Chemical Co., Ltd.). TLC and preparative TLC (PTLC) were carried out on Kieselgel 60F_{254} (Merck) and F_{254} (Merck).

Preparation of (±)-4-Acetoxy-, (±)-4-(5-Phenylvaleryloxy)-and (±)-4-Propionyloxy-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisoquinolines (5, 6 and 7) (±)-**5**: A mixture of (±)-**8**[12] (1.93 g, 7.7 mmol) and Ac_2O (1.20 g, 11.6 mmol) in pyridine (1 ml) was stirred overnight at room temperature. Usual work-up of the reaction mixture gave an oily residue, which was triturated in hexane to give (±)-**5** (1.53 g, 78%), 93—94 °C (lit.[11] 90—91.5 °C). IR *v*: 1725 cm^{-1}. ^{1}H-NMR (100 MHz) *δ*: 2.11 (3H, s, OAc), 2.48 (3H, s, NMe), 3.83, 3.84 (each 3H, s, 2×OMe), 5.89 (1H, t, *J*=3.2 Hz, 4-H), 6.51 6.59 (each 1H, s, 2×ArH).

(±)-**6**: Diethyl chlorophosphate[15] (2 ml, 14 mmol) was added to an ice-cooled, stirred solution of 5-phenylvaleric acid (2.14 g, 12 mmol) and Et_3N (2.43 g, 24 mmol) in benzene (15 ml) during 5 min and stirring was continued at room temperature for 12 h. A solution of (±)-**8** (2.23 g, 10 mmol) in benzene (60 ml) was added with stirring to the reaction mixture prepared above in an argon stream, and the whole was stirred at room temperature for 17 h. Water was added to the reaction mixture and the product was taken up in $CHCl_3$. Usual work-up of the $CHCl_3$ extract gave an oily residue (3.52 g), which was subjected to column chromatography with $CHCl_3$–MeOH (100:1) to give (±)-**6** (1.88 g, 49%), mp 52—54 °C (hexane). IR *v*: 1730 cm^{-1}. ^{1}H-NMR (100 MHz) *δ*: 1.68 (4H, m, CH_2CH_2), 2.44 (3H, s, NMe), 3.77, 3.84 (each 3H, s, 2×OMe), 5.91 (1H, t, *J*=3.4 Hz, 4-H) , 6.51, 6.75 (each 1H, s, 2×ArH), 7.00—7.20 (5H, m, C_6H_5). MS *m/z*: 383 (M^+). *Anal.* Calcd for $C_{23}H_{29}NO_4$: C, 72.04; H, 7.62; N, 3.65. Found: C, 71.77; H, 7.70; N, 3.84.

(±)-**7**: A mixture of (±)-**8** (1.79 g, 8 mmol), $(EtCO)_2O$ (1.25 g, 9.6 mmol) and 4-dimethylaminopyridine (0.49 g, 4 mmol) in pyridine (8 ml) was stirred at room temperature for 2 h. Usual work-up of the reaction mixture gave a solid (2.07 g, mp 77—79°C), which was subjected to column chromatography with $CHCl_3$–MeOH (100:1) to give (±)-**7** (1.91 g, 85.5%), mp 82—83 °C (hexane). IR *v*: 1730 cm^{-1}. ^{1}H-NMR (100 MHz) *δ*: 1.17 (3H, t, *J*=7.7 Hz, CH_2Me), 2.39 (2H, q, *J*=7.7 Hz, CH_2Me), 2.44 (3H, s, NMe), 3.81, 3.84 (6H, each s, 2×OMe), 5.91 (1H, t, *J*=3.2 Hz, 4-H), 6.51, 6.78 (each 1H, s, 2×ArH). MS *m/z*: 279 (M^+). *Anal.* Calcd for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.01. Found: C, 64.39; H, 7.50; N, 5.14.

Preparation of the *R*-(+)-MTPA Ester of (+)-8 A mixture of (±)-**8** (14.7 mg, 0.066 mmol) and *R*-(+)-MTPA Cl[13] (22.0 mg, 0.086 mmol) in pyridine (0.3 ml) was stirred at room temperature for 12 h. H_2O was added to the reaction mixture and the product was taken up in $CHCl_3$. Work-up of the $CHCl_3$ extract as usual gave an oily residue, which was purified by PTLC (developing solvent: $CHCl_3$: MeOH=15:1) to give a solid (19 mg, 65.6%), mp 88—89.5 °C. MS *m/z*: 439 (M^+). ^{1}H-NMR (400 MHz) *δ*: 2.38, 2.46 (1:1) (3H, each s, NMe), 6.10, 6.20 (1:1) (1H, each br t, 4-H), 6.48, 6.50, 6.54, 6.77 (1:1:1:1) (2H, each s, ArH). Assignment of the NMe signals [*δ* 2.38 for 4*S*-(+)-**8**-(+)-MTPA: *δ* 2.46 for 4*R*-(−)-**8**-(+)-MTPA] was performed by comparison of the ^{1}H-NMR spectrum with that of the (+)-MTPA ester of 4*S*-(+)-**8**, the absolute configuration of which was determined as described later.

General Procedure for Reaction in a 0.1 M Phosphate Buffer Solution A mixture of a (±)-4-acyloxytetrahydroisoquinoine (**5**—**7**) (100 mg) and lipase (50 mg) in a 0.1 M phosphate buffer solution (pH 7.25)[14] was incubated with shaking at 33 °C until about 50% hydrolysis of the substrate had occurred as determined by TLC monitoring. The product was taken up in $CHCl_3$. Work-up of the $CHCl_3$ extract as usual gave an oily residue, which was subjected to column chromatography. Elution with $CHCl_3$–MeOH (100:1) gave a recovered ester and elution with $CHCl_3$–MeOH (20:1) gave an alcohol. The former was hydrolyzed with 5% KOH–MeOH (2—5 ml) at room temperature for 0.5–1 h to give an alcohol. Each alcohol was treated with *R*-(+)-MTPA Cl (1.3—1.5 eq) in the same manner as noted above to afford the corresponding *R*-(+)-MTPA ester. The optical purity was determined on the basis of the area ratio of each NMe signal [*δ* 2.37—2.39 for 4*S*-(+)-**8**-(+)-MTPA; *δ* 2.46 for 4*R*-(−)-**8**-(+)-MTPA] in the ^{1}H-NMR (400 or 500 MHz) spectrum. The chemical and optical yields are listed in Tables I and II.

Preparation of Lipase Immobilized on Celite Celite 535 (Johns-Manville Co., Ltd.) (130 mg) was added to a mixture of lipase (50 mg) and H_2O (0.1 ml). The mixture was mixed well with a spatula at room temperature

for several minutes and then used for the following reaction.

General Procedure for Reaction in an Organic Solvent Saturated with Water A mixture of a (±)-substrate (**5**—**7**) (100 mg) and immobilized lipase prepared as described above in organic solvent (20 ml) saturated with water was incubated with shaking at 33 °C for 24 or 68 h. The Celite was filtered off and washed with MeOH (40—50 ml). The combined filtrate was dried over $MgSO_4$. Work-up of the filtrate in the manner as noted above gave the recovered ester (**5**—**7**) and (+)-alcohol (**8**). (−)-**5**: oil. $[\alpha]_D^{27}$ −127.7° (c=2.02, $CHCl_3$). MS m/z: 265 (M^+). The ^{1}H-NMR spectrum was identical with that of (±)-**5**. (−)-**6**: oil $[\alpha]_D^{26}$ −116.7° (c=2.60, $CHCl_3$), (−)-**7**: oil. $[\alpha]_D^{26}$ −17° (c=0.84, $CHCl_3$). ^{1}H-NMR spectra of (−)-**6** and (−)-**7** were identical with those of (±)-**6** and (±)-**7**. (+)-**8**: mp 111.5—112.0 °C (benzene–hexane). $[\alpha]_D^{27}$ +13.5° (c=0.48, $CHCl_3$). High resolution MS (m/z) Calcd for $C_{12}H_{17}NO_3$ (M^+): 223.1209. Found: 223.1204. ^{1}H-NMR (100 MHz) δ: 2.45 (3H, s, NMe), 3.85, 3.88 (6H, each s, 2 × OMe), 4.56 (1H, t, J=2.9 Hz, 4-H), 6.46, 6.94 (2H, each s, 2 × ArH). The optical purity of each product was estimated in the same manner as noted above. The chemical and optical yields are listed in Tables III and IV.

Absolute Configuration of (+)-1,2,3,4-Tetrahydro-6,7-methoxyisoquinolin-4-ol (8) A solution of commercially available *R*-(−)-epinephrine (**9**) (500 mg), $[\alpha]_D^{26}$ −52.4° (c=2.0, 1 N HCl), in 1 M HCl (2.4 ml) was adjusted to pH 2.0 with 1 M $NaHCO_3$ solution. The solution was treated with 35% formalin (1.2 ml) at room temperature for 18 h according to Bates' method.[19] Removal of the solvent *in vacuo* gave an oily residue, which dissolved in EtOH (10 ml). The solution was filtered through Celite and the solvent was removed *in vacuo*. The residue thus obtained was dissolved in EtOH (2 ml). Acetone (3 ml) was added to the solution to produce a precipitate, which was collected by filtration. The filtrate was concentrated *in vacuo* to give an oily residue, which was crystallized from acetone. The combined crystalline mass weighed 360 mg (57%). The hydrochloride of 4*R*-(−)-4,6,7-triol (**10**), mp 150—153 °C (dec.), $[\alpha]_D^{26}$ −17.5° (c=1.0, MeOH). MS m/z: 195 (M^+). ^{1}H-NMR (100 MHz) δ (D_2O): 3.08 (3H, s, NMe), 4.97 (1H, t, J=3 Hz, 4-H), 6.68, 6.93 (2H, each s, 2 × ArH). Analytical sample had mp 165 °C (dec.) (acetone–MeOH). *Anal.* Calcd for $C_{10}H_{13}NO_3 \cdot HCl \cdot 0.5H_2O$: C, 49.90; H, 6.24; N, 5.82. Found: C, 50.06; H, 6.39; N, 5.87. A solution of 4*R*-(−)-**10**·HCl (50 mg) was treated with CH_2N_2-ether (6 ml) in MeOH (6 ml) at room temperature for 19 h. Removal of the solvent *in vacuo* gave an oily product (17.8 mg), which was purified by PTLC (developing solvent: $CHCl_3$: MeOH = 20 : 1) to give 4*R*-(−)-**8** (9.5 mg, 20%). $[\alpha]_D^{25}$ −13.8° (c=0.63, $CHCl_3$). MS m/z: 223 (M^+). Its ^{1}H-NMR (100 MHz) spectrum was identical with that of (+)-**8**.

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