Synthesis of *d*-Biotin Chiral Intermediates via a Biochemical Method

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The enzyme-catalyzed kinetic resolution of (±)-(3aα,4α,6aα)-4-acetoxy-1,3-dibenzyl-3a,4,6,6a-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**3a**)] was examined. Lipase B from *Pseudomonas fragi* and rabbit liver esterase gave (−)-[3a*R*-(3aα,4α,6aα)]-1,3-dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*R*)-(**2**)], while *Streptomyces rochei* var. *volubilis* gave the alcohol (*S*)-(**2**), which is a key intermediate in the synthesis of *d*-biotin, with high enantioselectivity.

Total synthesis of *d*-biotin, which is referred to as Vitamin H, has been a subject of interest for years, and many synthetic strategies have been reported. Most of the strategies are devised to prepare chiral intermediates effectively.[1] Recently, it has been realized that enzymatic transformations are promising ways to obtain chiral compounds. Two groups have independently reported the asymmetric synthesis of *d*-biotin intermediates, (**5**) and (**6**), utilizing enzymatic transformation (Fig. 1).[2] Although their enantiomeric excess (e.e.) was not sufficient, their findings encouraged us to investigate this promising methodology which uses enzymatic transformation.

We assumed that the easily obtainable *meso*-compound (**1**), which is prepared from fumaric acid,[3] would be suitable as a starting material, and the introduction of a hydroxyl group into the *meso*-compound (**1**) and the successive optical resolution to obtain the alcohol (*S*)-(**2**) would be crucial in *d*-biotin synthesis. The optical resolution of the alcohol (*RS*)-(**2**) seems in our view to be best carried out by enzymatic transformation. The reaction scheme is depicted in Fig. 2.

Results and Discussion

The ester (*RS*)-(**3a**) was easily prepared by the conventional acetylation of the alcohol (*RS*)-(**2**). The relative stereochemistry of the ester (*RS*)-(**3a**) was confirmed based on X-ray crystallographic analysis, as shown in Fig. 3.

Enantioselective hydrolysis of the ester (*RS*)-(**3a**) was performed using commercially available enzymes. The representative results are listed in Table 1. Lipase B from *Pseudomonas fragi* (Wako) and esterase from rabbit liver (Sigma E-9636) exhibited excellent enantioselectivity; however, they gave the undesired enantiomer, the alcohol (*R*)-(**2**).[5] The (*R*)-preferred hydrolysis is in good agreement with the prediction based on the sizes of the substituents at the chiral center.[6] The desired enantiomer, the alcohol (*S*)-(**2**), was obtained by the hydrolysis of the remaining the ester (*S*)-(**3a**); nevertheless, troublesome separation of the ester (*S*)-(**3a**) from the reaction mixture by column chromatography and base-catalyzed hydrolysis was inevitable. Accordingly, (*S*)-preferred hydrolysis is required for effective way of *d*-biotin intermediate synthesis.

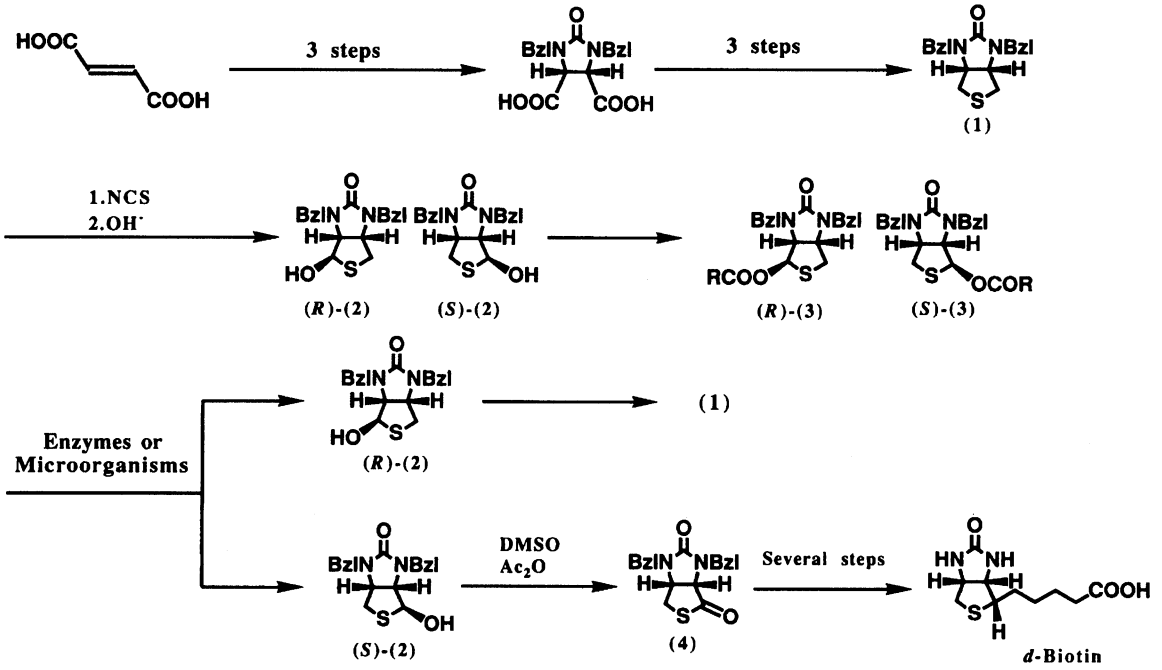
Fig. 1. The compound **5** and **6**.

Thus, we screened more than 100 strains of microorganisms, such as bacteria, fungi and yeasts to obtain the alcohol (*S*)-(**2**) predominantly. As a result, some microorganisms were found to hydrolyze the ester (*RS*)-(**3a**) enantioselectively. Among them, microorganisms giving *E*-values[4] of more than 15 are listed in Table 2. Most of the microorganisms gave the alcohol (*R*)-(**2**), while *Acetobacter rancens* IFO 3298 and *Streptomyces rochei* var. *volubilis*[7] gave the desired alcohol (*S*)-(**2**) with high enantioselectivity.

To determine the effect of acyl groups on the enantioselectivity, the ester (*RS*)-(**3b**—**f**) were synthesized and subjected to hydrolysis by *Pseudomonas aeruginosa* IFO3447 and *Streptomyces rochei* var. *volubilis*. The results are summarized in Table 3. The acyl groups affected conversion and enantioselectivity drastically. The elongation of acyl groups and the presence of a phenyl or carboxyl group had a detrinental effect on reactivity and enantioselectivity. Among the acyl groups tested, acetyl (R=CH_3) was found to be most suitable for our purpose.

Considering above mentioned preliminary results, we carried out the optical resolution of the ester (*RS*)-(**3a**) by use of *Streptomyces rochei* var. *volubilis* to isolate the alcohol (*S*)-(**2**). The hydrolysis proceeded with 27% conversion and 92% enantiomeric excess based on HPLC analysis. In general, separation of enantiomerically pure compounds from reaction mixtures is often troublesome as for optical resolution, especially in large scale production. However, the separation of the alcohol (*S*)-(**2**) was readily accomplished by crystallization from the reaction mixture to afford the alcohol (*S*)-(**2**) crystals (94% e.e.).

The alcohol (*S*)-(**2**) was oxidized to the known thiolactone (**4**), which is commercially utilized as a *d*-biotin intermediate,[8] by Swern oxidation[9] or more con-



3	R in acyl group of (3)
3a	CH_3
3b	C_2H_5
3c	C_3H_7
3d	C_4H_9
3e	CH_2COOH
3f	*p*-$NO_2C_6H_4$

Fig. 2. Reaction scheme.

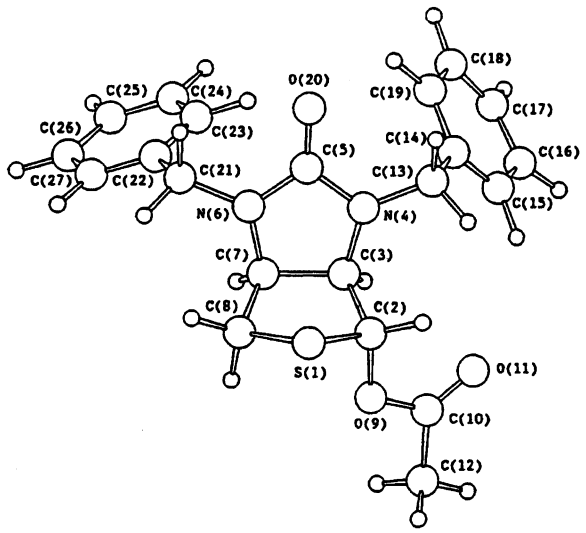


Fig. 3. X-Ray crystallographic analysis of the ester **3a**.

veniently by Albright–Goldman oxidation.[10] The undesired alcohol (*R*)-(**2**) and the ester (*R*)-(**3a**) can be reutilized as a starting material (**1**) by dehydroxylation.[11] Hence, the strategy described here provides an efficient synthetic method for *d*-biotin.

Experimental

Apparatus and Procedures. All melting points are uncorrected. Infrared (IR) spectra were obtained on a Shimadzu IR-260-10 infrared spectrometer. ^{1}H NMR spectra were recorded on a Hitachi R-90H (90 MHz) or on a JEOL JNM-GSX 270 (270 MHz) with tetramethylsilane as an internal reference. MS was recorded with a JEOL JMS-AX-505W.

(±)-(3aα,4α,6aα)-4-Acetoxy-1,3-dibenzyl-3a,4,6,6a-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(3a)]. (±)-(3aα,4α,6aα)-1,3-Dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**2**)] (47.8 g) was dissolved in pyridine (200 ml), and acetic anhydride (200 ml) was added dropwise over 1 h at 25 °C. The reaction mixture was stirred for an additional 1 h and concentrated under reduced pressure to give an oil. Crystallization from diethyl ether afforded (±)-(3aα,4α,6aα)-4-acetoxy-1,3-dibenzyl-3a,4,6,6a-tetrahydro-1*H*-thieno-[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**3a**)] (52.4 g, 97.7%). Mp 72—74 °C. MS *m/z* M^+=382. IR (KBr) 1745, 1700, 1455, 1240, 960, 700 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=1.96 (3H, s, Ac), 2.96 (1H, d, J=10.2 Hz, C(6)-*endo*-H), 3.03 (1H, dd, J=3.8, 10.2 Hz, C(6)-*exo*-H), 4.02 (1H, d, J=5.8 Hz, C(3a)–H), 4.20 (1H, m, C(6a)–H), 4.19, 4.24, 4.79, 4.84 (each 1H, d, J=15.4 Hz, CH_2Ph), 6.02 (1H, s, C(4)–H), 7.29 (10H, m, Ph). The crystal data are as follows: a=18.892(2),

Table 1. Enantioselective Hydrolysis of the Ester (**3a**) by Enzymes[a)]

Enzyme	React. time / h	Conf.[b)] / *R*/*S*	Conv.[b)] / %	e.e.[b)] / %	*E*[c)]
Lipase from porcine pancrease (Sigma L-3126)	20	*S*	33	24	2
Lipase from porcine pancrease (Wako)	20	*S*	26	55	4
Lipase from wheat germ (Sigma L-3001)	1	*R*	45	30	2
Lipase P from *Pseudomonas* sp. (Amano)	20	*R*	50	77	17
Lipase B from *Pseudomonas fragi* (Wako)	20	*R*	47	94	87
Lipase from *Rhizopus japonicus* (Saiken 100)	20	*R*	18	10	1
Lipase MY from *Candida cylindracea* (Meito)	20	*R*	50	57	6
Lipoprotein lipase from *Pseudomonas aeruginosa* (Toyobo)	20	*R*	53	83	37
Esterase from porcine liver (Amano PLE-A)	1	*R*	58	59	9
Esterase from porcine liver (Sigma E-3128)	1	*R*	56	30	3
Esterase from rabbit liver (Sigma E-9636)	1	*R*	35	95	65

a) The reaction mixtures containing the ester (**3a**) (5.0 mg), phosphate buffer (50 mM, pH 7.0, 900 ml), and enzyme (10—20 mg) were incubated at 30 °C on a rotary shaker. b) Determined by HPLC (Chiralcel OD; hexane (60), 2-propanol (40), 0.4 ml min^{-1}, 220 nm). c) Enantiomeric ratio.[4)]

Table 2. Enantioselective Hydrolysis of the Ester (**3a**) by Microorganisms[a)]

Microorganism	Conc.[b)] / mg ml^{-1}	React. time / h	Conf.[c)] / *R*/*S*	Conv.[c)] / %	e.e.[c)] / %	*E*[d)]
Bacillus cereus IFO3003	2	24	*R*	24	84	15
Bacillus megaterium IFO13498	2	24	*R*	35	96	82
Bacillus megaterium IFO12108	6	24	*R*	25	90	26
Pseudomonas aeruginosa IFO3445	4	24	*R*	30	86	19
Pseudomonas aeruginosa IFO3447	4	24	*R*	47	84	26
Pseudomonas aeruginosa IFO3447	8	24	*R*	37	88	26
Pseudomonas aeruginosa IFO3448	2	24	*R*	49	86	34
Acetobacter rancens IFO3298	2	24	*S*	16	80	10
Streptomyces rochei var. *volubilis*[7)]	2	1	*S*	41[e)]	82	18
Streptomyces rochei var. *volubilis*[7),f)]	2	1	*S*	27[e)]	92	33

a) Microorganisms were grown in a medium (pH 7.2, 2 ml) containing dextrin (1%), glucose (1%), peptone (0.5%), yeast extract (0.5%), meat extract (0.5%), sodium chloride (0.3%), and calcium carbonate (0.5%) at 28 °C for 2 d on a reciprocal shaker. The reaction mixtures containing the cultures (2 ml) and the ester (**3a**) (4.0—12 mg) were incubated at 28 °C on a reciprocal shaker. b) Initial concentration of the ester (**3a**) in the reaction mixtures. c) Determined by HPLC (Chiralcel OD; hexane (60), 2-propanol (40), 0.4 ml min^{-1}, 220 nm). d) Enantiomeric ratio.[4)] e) The reaction mixtures were incubated for an hour. f) The culture supernatant was used for the reaction.

b=10.781(1), c=9.980(2) Å, β=95.09(2)°, V=2024.7(6) $Å^3$, monoclinic, Space group Pc, Z=4, Density=1.255 g cm^{-3}, μ=1.84 cm^{-1}.

(±)-(3aα,4α,6aα)-1,3-Dibenzyl-3a,4,6,6a-tetrahydro-4-propionyloxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(3b)]. (±)-(3aα,4α,6aα)-1,3-Dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**2**)] (500 mg) was dissolved in pyridine (2.0 ml), and propionic anhydride (2.0 ml) was added dropwise over 1 h at 25 °C. The reaction mixture was stirred for an additional 1 h and concentrated under reduced pressure to give an oil. Crystallization from pentane afforded (±)-(3aα,4α,6aα)-1,3-dibenzyl-3a,4,6,6a-tetrahydro-4-propionyloxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**3b**)] (460 mg, 78%). IR (KBr) 1740, 1690, 1475, 1245, 960, 700 cm^{-1}. ^1H NMR ($CDCl_3$) δ=1.07 (3H, t, J=7.5 Hz, CH_3), 2.22 (2H, q, J=7.5 Hz, $COCH_2$), 2.95 (2H, m, CH_2S), 3.99 (1H, d, J=7.8 Hz, C(3a)–H), 4.2 (1H, m, C(6a)–H), 4.18, 4.24, 4.79, 4.85 (each 1H, d, J=15.4 Hz, CH_2Ph), 6.05 (1H, s, C(4)–H), 7.2—7.3 (10H, m, Ph).

Found: C, 66.72; H, 5.96; N, 7.13; S, 8.15%. Calcd for $C_{22}H_{24}N_2O_3S$: C, 66.64; H, 6.10; N, 7.06; S, 8.09%.

(±)-(3aα,4α,6aα)-1,3-Dibenzyl-4-butyryloxy-3a,4,6,6a-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(3c)]. (±)-(3aα,4α,6aα)-1,3-Dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**2**)] (340 mg) was dissolved in dichloromethane (5.0 ml), triethylamine (0.209 ml) and butyryl chloride (0.154 ml) were added. The reaction mixture was stirred for 1.5 h at room temperature and concentrated under reduced pressure to give an oil. Silica gel chromatography (hexane/ethyl acetate=2/1) gave (±)-(3aα,4α,6aα)-1,3-dibenzyl-4-butyryloxy-3a,4,6,6a-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**3c**)] (147 mg, 36%). IR (KBr) 1750, 1690, 1480, 1460, 1250 cm^{-1}. ^1H NMR ($CDCl_3$) δ=0.89 (3H, t, J=7.3 Hz, CH_3), 1.58 (2H, m, CH_2), 2.18 (2H, t, J=6.9 Hz, $COCH_2$), 2.99 (2H. m, CH_2S), 3.98 (1H, d, J=7.8 Hz, C(3a)–H), 4.2 (1H, m, C(6a)–H), 4.18, 4.23, 4.80, 4.87 (each 1H, d, J=15.3 Hz, CH_2Ph), 6.06 (1H, s, C(4)–H), 7.2—7.3 (10H, m, Ph).

(±)-(3aα,4α,6aα)-1,3-Dibenzyl-3a,4,6,6a-tetrahy-

Table 3. Effect of Acyl Groups on the Enantioselective Hydrolysis of the Ester (**3a**—**f**)[a]

Microorganism	R in acyl group of (**3**)	Conf.[b] *R*/*S*	Conv.[b] %	e.e.[b] %	*E*[c]
Pseudomonas aeruginosa	CH_3(**3a**)	*R*	50	80	22
	C_2H_5(**3b**)	*R*	57	60	9
	C_3H_7(**3c**)	*R*	37	14	1
	C_4H_9(**3d**)	*R*	42	66	8
	CH_2COOH(**3e**)	*R*/*S*	16	0	1
	PNP[d](**3f**)		0		
Streptomyces rochei var. *volubilis*	CH_3(**3a**)	*S*	28	90	27
	C_2H_5(**3b**)	*S*	8	90	21
	C_3H_7(**3c**)	*R*/*S*	1		
	C_4H_9(**3d**)	*R*/*S*	1		
	CH_2COOH(**3e**)	*R*/*S*	2		
	PNP[d](**3f**)		0		

a) Microorganisms were incubated in a medium (pH 7.2, 2 ml) containing dextrin (1%), glucose (1%), peptone (0.5%), yeast extract (0.5%), meat extract (0.5%), sodium chloride (0.3%), and calcium carbonate (0.5%) at 28 °C for 2 d. Reaction mixtures containing the culture or culture supernatant (2 ml) and the ester (**3a**) (4.0—8.0 mg) were incubated at 28 °C for 24 h (*Pseudomonas aeruginosa*) or 1 h (*Streptomyces rochei* var. *volubilis*) on a reciprocal shaker. b) Determined by HPLC (Chiralcel OD; hexane (60), 2-propanol (40), 0.4 ml min^{-1}, 220 nm). c) Enantiomeric ratio. d) *p*-nitrophenyl.

dro-4-valeryloxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(3d)]. (±)-(3aα,4α,6aα)-1,3-Dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**2**)] (340 mg) was dissolved in dietyl ether (5.0 ml), and triethylamine (0.209 ml) and valeryl chloride (0.198 ml) were added. The reaction mixture was stirred for 1.5 h at room temperature and concentrated under reduced pressure to give an oil. Silica-gel chromatography (hexane/ethyl acetate=2/1) gave (±)-(3aα,4α,6aα)-1,3-dibenzyl-3a,4,6,6a-tetrahydro-4-valeryloxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**3d**)] (111 mg, 26%). MS *m*/*z* 425 (MH^+). IR (KBr) 1735, 1700, 1450, 1240 cm^{-1}. ^1HNMR ($CDCl_3$) δ=0.87 (3H, t, *J*=6.4 Hz, CH_3), 1.16—1.62 (4H, m, CH_2CH_2), 2.21 (2H, t, *J*=7.4 Hz, $COCH_2$), 3.98 (1H, d, *J*=7.9 Hz, C(3a)–H), 4.2 (1H, m, C(6a)–H), 4.19, 4.22, 4.80, 4.87 (each 1H, d, *J*=15.4 Hz, CH_2Ph), 6.06 (1H, s, C(4)–H), 7.2—7.3 (10H, m, Ph).

(3aα,4α,6aα)-1,3-Dibenzyl-4-carboxyacetoxy-3a,4,6,6a-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(3e)]. (±)-(3aα,4α,6aα)-1,3-Dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**2**)] (340 mg) and Meldrum's acid (144 mg) were dissolved in toluene (1.0 ml), and the reaction mixture was stirred for 1.5 h at 100 °C. Then toluene (5.0 ml) and an aqueous solution (5.0 ml) of sodium hydrogencarbonate were added. The aqueous layer was acidified with dil. hydrochloric acid and extracted with dietyl ether. The organic layer was concentrated under reduced pressure to give an oil of (3aα,4α,6aα)-1,3-dibenzyl-4-carboxyacetoxy-3a,4,6,6a-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**3e**)] (279 mg, 65%). MS *m*/*z* 427 (MH^+).

IR (KBr) 3420, 2930, 1750, 1730, 1660, 1455, 1245 cm^{-1}. ^1HNMR ($CDCl_3$) δ=2.91 (2H, m, CH_2S), 3.29 (2H, s, CH_2COO), 4.05 (1H, d, *J*=8.0 Hz, C(3a)–H), 4.18, 4.26, 4.73, 4.76 (each 1H, d, *J*=15.4 Hz, CH_2Ph), 7.1—7.3 (10H, m, Ph).

(3aα,4α,6aα)-1,3-Dibenzyl-3a,4,6,6a-tetrahydro-4-(4-nitrobenzoyl)oxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(3f)]. Dietyl azodicarboxylate (1.40 ml), *p*-nitrobenzoic acid (2.23 g), and triphenylphosphine (2.32 g) were dissolved in benzene (43 ml) and stirred at 0 °C. To this solution, (±)-(3aα,4α,6aα)-1,3-dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**2**)] (2.00 g) dissolved in benzene (42 ml) and tetrahydrofuran (21 ml) were added dropwise and stirred at room temperature for 40 h. The resulting reaction mixture was concentrated under reduced pressure to give an oil. Silica-gel chromatography (100 g, benzene/chloroform=1/1) gave (3aα,4α,6aα)-1,3-dibenzyl-3a,4,6,6a-tetrahydro-4-(4-nitrobenzoyl)oxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**3f**)] (1.34 g, 47%). IR (KBr) 1715, 1695, 1530, 1270 cm^{-1}. ^1HNMR ($CDCl_3$) δ=3.07 (2H, m, CH_2S), 4.22, 4.35, 4.84, 4.88 (each 1H, d, *J*=15.3 Hz, CH_2Ph), 7.2—7.4 (10H, m, Ph), 8.05, 8.24 (each 2H, d, *J*=9.0 Hz, $PhNO_2$).

Found: C, 63.90; H, 4.68; N, 8.58; S, 6.58%. Calcd for $C_{26}H_{23}N_3O_5S$: C, 63.79; H, 4.74; N, 8.58; S, 6.55%.

Optical Resolution of (±)-(3aα,4α,6aα)-4-Acetoxy-1,3-dibenzyl-3a,4,6,6a-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(3a)] with *Streptomyces rochei* var. *volubilis*. Culture Conditions for *Streptomyces rochei* var. *volubilis*. *Streptomyces rochei* var. *volubilis*[7] was grown in a medium in Erlenmeyer flasks for 3 d at 28 °C on a reciprocal shaker. The medium (pH 7.2) contained 20 g of dextrin, 20 g of glucose, 20 g of glycerol, 10 g of peptone, 10 g of yeast extract, 10 g of meat extract, 6 g of sodium chloride, and 10 g of calcium carbonate in 2000 ml of deionized water. The culture was centrifuged to give supernatant solution.

Hydrolysis of (±)-(3aα,4α,6aα)-4-Acetoxy-1,3-dibenzyl-3a,4,6,6a-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(3a)] by *Streptomyces rochei* var. *volubilis*. (±)-(3aα,4α,6aα)-4-Acetoxy-1,3-dibenzyl-3a,4,6,6a-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-(**3a**)] (3.2 g) dissolved in dimethyl sulfoxide (40 ml)

was added to 1600 ml of the supernatant solution obtained above. The reaction mixture was incubated at 28 °C for 1h on a rotary shaker. The resulting mixture was extracted with ethyl acetate (1600 ml), and then the organic layer was washed with water and brine and concentrated under reduced pressure to afford a yellow oil. This oil was analyzed by high-performance liquid chromatography (HPLC) and found to contain (+)-[3a*S*-(3aα,4α,6aα)]-1,3-dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2-(3*H*)-one [(*S*)-(**2**)]. (25.5% yield) and the (*R*)-alcohol [(*R*)-(**2**)] (0.8% yield). The crystallization of the oil from acetone afforded the (*S*)-alcohol [(*S*)-(**2**)] (344 mg, 94%e.e.). Mp 166—168 °C. IR (KBr) 3300, 1680 cm^{-1}. ^1H NMR ($CDCl_3$) δ=1.7 (1H, br, s, OH), 2.86 (1H, d, J=12.7 Hz, C(6)–H), 3.01 (1H, dd, J=12.7, 4.7 Hz, C(6)–H), 4.02 (1H, d, J=7.9 Hz, C(3a)–H), 4.21 (1H, dd, J=7.9, 4.6 Hz, C(6)–H), 4.21, 4.32, 4.66, 4.76 (each 1H, d, J=15.5 Hz, CH_2Ph), 5.18 (1H, s, C(4)–H), 7.2—7.4 (10H, m, Ph). $[\alpha]_D^{30}$=+62.4 (c=0.78, chloroform).

(+)-[3a*S*-(3aα,6aα)]-1,3-Dibenzyl-6,6a-dihydro-1*H*-thieno[3,4-*d*]imidazole-2,4-dione [(*S*)-(4)]. A mixture of dimethyl sulfoxide (5.0 ml) and acetic anhydride (0.92 ml) was stirred at 50 °C for an hour under argon atmosphere. To this solution, (+)-[3a*S*-(3aα,4α,6aα)]-1,3-dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*S*)-(**2**)] (500 mg, 96%e.e.) was added followed by stirring at 50 °C for 1.5 h. The resulting reaction mixture was added to ice–water (100 ml) and stirred for an hour. The precipitated crystals were collected and washed with water to give crude (+)-[3a*S*-(3aα,6aα)]-1,3-dibenzyl-6,6a-dihydro-1*H*-thieno[3,4-*d*]imidazole-2,4-dione [(*S*)-(**4**)] (491 mg). This crude crystals were analyzed by high-performance liquid chromatography (HPLC) and found to contain the thiolactone (**4**) (87.0% yield). These crystals were recrystalized from ethyl acetate to give (+)-[3a*S*-(3aα,6aα)]-1,3-dibenzyl-6,6a-dihydro-1*H*-thieno[3,4-*d*]imidazole-2,4-dione [(*S*)-(**4**)] (238 mg, 48%). The mother liquor was subjected to silica-gel chromatography (hexane/ethyl acetate=2/1) to give (+)-[3a*S*-(3aα,6aα)]-1,3-Dibenzyl-6,6a-dihydro-1*H*-thieno[3,4-*d*]imidazole-2,4-dione [(*S*)-(**4**)] (119 mg, 24.0%). Mp 125—126 °C. IR (KBr) 1705, 1690, 1420, 1225 cm^{-1}. ^1H NMR ($CDCl_3$) δ=3.3 (2H, m,, C(6)–H), 3.79 (1H, d, J=7.8 Hz, C(3a)–H), 4.1 (1H, m, J=7.8 Hz, C(6)–H), 4.34, 4.35, 4.69, 5.03 (each 1H, d, J=15.2 Hz, CH_2Ph), 7.1—7.3 (10H, m, Ph). $[\alpha]_D^{22}$=+90.8 (c=1.00, chloroform).

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