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Efficient synthesis and biological activity of enantiomeric pairs of thiolactomycin and its 3-demethyl derivative☆

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ABSTRACT

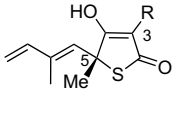
The title total synthesis was achieved by employing deconjugative asymmetric α-sulfenylation of the chiral 3-(α,β,γ,δ-unsaturated acyl)oxazolidin-2-one with a 3,3-dimethoxypropyl methanethiosulfonate as a key step. From the biological activity assay carried out using the title compounds, it appeared evident that in vitro antibacterial and mammalian type I FAS inhibitory activity can be cleanly separated by changing not only the substituent at the C_3-position but also the absolute configuration at the C_5-position, and that unnatural (*S*)-(−)-3-demethylthiolactomycin and its congeners might be usable as selective mammalian type I FAS inhibitors.

1. Introduction

(*R*)-(+)-Thiolactomycin (**1**), a thiolactone antibiotic isolated from a soil bacterium, *Nocardia* sp.,[2] shows moderate in vitro activity against a number of pathogens, including Gram-positive and Gram-negative bacteria,[3,4] *Mycobacterium tuberculosis*[5,6] and malaria parasites[7,8] (Fig. 1). According to the published reports, **1** exhibits inhibitory activity against bacterial and plant type II fatty acid synthase (FAS) but not mammalian type I FAS.[9–12] From the more detailed experiments, it appeared evident that **1** shows its activity via the inhibition of β-ketoacyl-acyl carrier protein synthases.[5,10,13] These inhibitory activities are considered to explain the antibacterial and antiparasitical properties observed for **1**. Interestingly, Townsend et al. recently disclosed that **1** and its derivatives also show inhibitory activity against mammalian type I FAS.[14] Therefore, synthesis of **1** and its congeners has attracted much attention, because these compounds seem to constitute promising drug candidates with a hitherto unexplored mode of action for cancer and obesity treatments as well as for infective diseases.

Reflecting the inherent interest in these compounds, one racemic synthesis of **1**, one synthesis of unnatural *ent*-**1**, and three syntheses of natural **1** have hitherto been reported.[15–19] Thus, Wang and Salvino reported the first total synthesis of racemic **1** in which alkylation of the thiotetronic acid dianion is employed as a key step.[15] Starting with (*S*)-ethyl lactate, the first asymmetric synthesis of unnatural *ent*-**1** was accomplished in 19 steps by Thomas and Chambers.[16] In this total synthesis, stereoselective [3,3]-rearrangement of the allyl xanthate to dithiocarbonate was characterized as a key step, leading to determination of the absolute stereochemistry of **1**. In 2002, Townsend et al. developed a nine-step asymmetric synthesis of naturally occurring **1** by employing (*R*)-alanine as the origin of chirality.[17] Takabe et al. also synthesized **1** via lipase-catalyzed kinetic resolution of the thiotetronic acid derivative derived from methyl propionate in 12 steps.[18] Most recently, Brückner and Dormann completed a seven-step synthesis of **1** featuring Sharpless asymmetric epoxidation and stereoselective thiolysis.[19]

Taking into account the potential of **1** and its derivatives as promising drug candidates, we also embarked on exploration of a novel synthetic route to **1**, which might be more efficient and flexible than reported.[15–19] This report details our efficient total synthesis of the enantiomeric pairs of thiolactomycin (**1** and *ent*-**1**) and its 3-demethyl derivative (**2** and *ent*-**2**) accomplished by



(*R*)-(+)-thiolactomycin (**1**): R = Me
(*R*)-(+)-3-demethylthiolactomycin (**2**): R = H

Figure 1. Structures of (*R*)-(+)-thiolactomycin (**1**) and (*R*)-(+)-3-demethylthiolactomycin (**2**).

☆ See Ref. 1.

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employing novel deconjugative asymmetric α-sulfenylation as a key step.[1a] This report also investigates novel aspects of the structure–activity relationship explored by subjecting **1**, *ent*-**1**, **2**, and *ent*-**2** to in vitro antibacterial and type I FAS inhibitory activity assays.[1b]

2. Results and discussion

2.1. Synthetic strategy

Our novel synthetic strategy to **1** is outlined in Scheme 1, in which deconjugative asymmetric α-sulfenylation of the chiral 3-(α,β,γ,δ-unsaturated acyl)oxazolidin-2-one **7**[20] with an *S*-3,3-dimethoxypropyl thiosulfonate **6**[21] (vide infra) is employed as a key step. It has been well established that thiosulfonates are prominent electrophilic sulfenylating agents, which efficiently react with carbanion species.[22] Thus, deprotonation of **7** will afford trienolate **A**, which on treatment with **6** undergoes deconjugative asymmetric α-sulfenylation under influence of the chiral auxiliary, producing the α-sulfenylated product **5**.[23] It is expected that, in addition to the absolute stereochemistry, the geometry at the C-3′ position may be controlled to have an (*E*)-configuration due to steric and/or electronic effects. To our knowledge, this sort of electrophilic deconjugative asymmetric α-sulfenylation of carboxylic acid derivatives that can directly construct a quaternary asymmetric center bearing a sulfur atom has not hitherto been reported.[24] Subsequent imide-ester exchange,[25,26] deacetalization, retro-Michael reaction, and acylation readily afford α-propionylthio ester **3**. Dieckmann condensation of **3** following the reported protocol[17] gives rise to **1**. The chiral oxazolidin-2-one **7** is readily obtainable from tiglic aldehyde **8**. Preparation of **6** can be accomplished starting with 3-bromopropionaldehyde dimethylacetal based on the previously reported procedures with some modification.[15,27] According to this designed synthetic scheme, the 3-demethyl derivative **2** of **1** will be obtained similar to **1** when acylation is performed using acetyl chloride instead of propionyl chloride. It is also obvious that use of the optically active oxazolidin-2-one derivative enantiomeric to that utilized for the preparation of **7** can furnish *ent*-**1** and *ent*-**2** in the same manner as for the preparation of **1** and **2**.

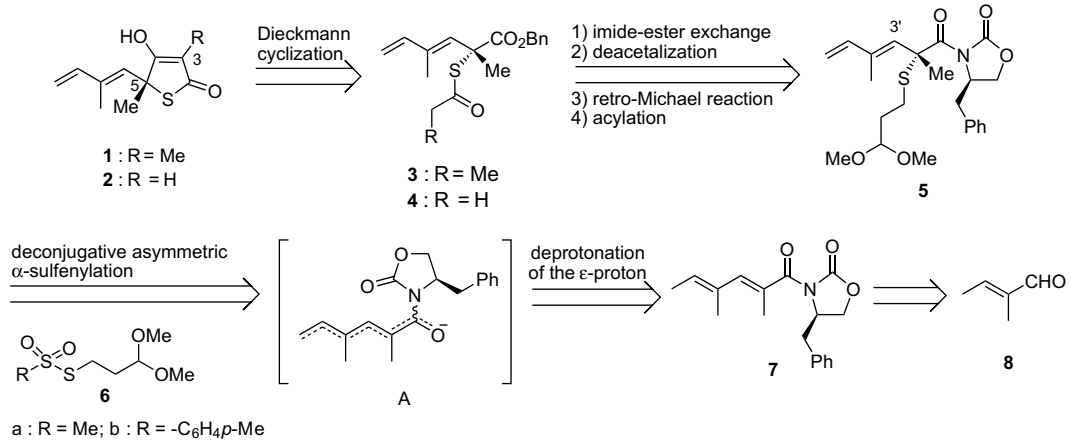
2.2. Synthesis of natural (*R*)-(+)-thiolactomycin 1

Following the novel synthetic scheme designed above, we embarked on the synthesis of **1** and **2**. As shown in Scheme 2, Horner–Wadsworth–Emmons reaction of **8**[28] followed by alkaline hydrolysis afforded hexadienoic acid **9** in 87% yield. This was converted to **7** in 92% yield by sequential reaction with pivaloyl chloride and (*R*)-4-benzyloxazolidin-2-one.[20,29] With **7** in hand, the electrophilic deconjugative asymmetric α-sulfenylation, which constitutes the key synthetic step, was next examined.

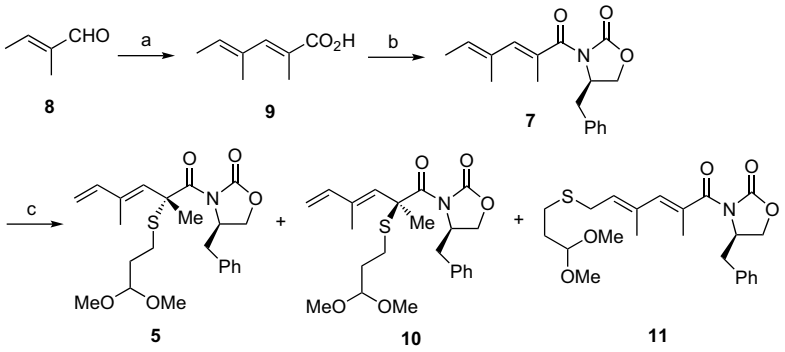
As sulfenylating agents that can act as excellent electrophilic agents with functional group readily transformable into a thiol, *S*-3,3-dimethoxypropyl thiosulfonates **6** were employed for this sulfenylation reaction[21,22] (vide supra). Preparation of **6a** was accomplished starting with 3-bromopropionaldehyde dimethylacetal **12** using the previously reported procedures[15,27] with some modification (Scheme 3). Thus, commercially available **12** was converted to thioacetylated compound **13** using thiolacetic acid in a good yield.[15] Deacetylation of **13** followed by dimerization of the resulting thiol with iodine quantitatively produced disulfide **14**. Following the reported procedure,[27] reaction of **14** with sodium *p*-toluenesulfinate and iodine gave rise to **6a**. Synthesis of **6b** was achieved using **14** and sodium methanesulfinate and a manner similar to that described for the preparation of **6a**.

After several experiments were carried out by changing the sulfenylating agent and chiral auxiliary,[20,21] sulfenylation of **7** was attempted using tosylate **6a** and sodium bis(trimethylsilyl)amide (NaHMDS) in the presence of hexamethylphosphoramide (HMPA) in tetrahydrofuran (THF), furnishing α-sulfenylated products **5** and **10** in 64% yield along with ε-sulfenylated product **11** in 25% yield (Table 1, run 1). The formation ratio of α- to ε-sulfenylated product [(**5**+**10**)/**11**] was estimated as ca. 2.6:1. ^{1}H NMR analysis of α-sulfenylated products clearly disclosed that the formation ratio of **5** to **10** was 6:1 and **5** carrying (3′*E*)-configuration contained 5–10% of the undesired (3′*Z*)-isomer. In order to explore the steric effect of the sulfenylating agent, sulfenylation of **7** was further examined using sterically less hindered mesylate **6b** in place of **6a**. To our delight, sulfenylation of **7** with **6b** and NaHMDS in the presence of HMPA in THF took place with a more improved regioselectivity to afford a mixture of **5** and **10** and **11** in 57% and 7% yields [(**5**+**10**)/**11**=ca. 8:1], respectively. In this case too, the formation ratio of **5** to **10** was 5:1 and **5** was contaminated by ca. 5–10% of the (3′*Z*)-isomer.

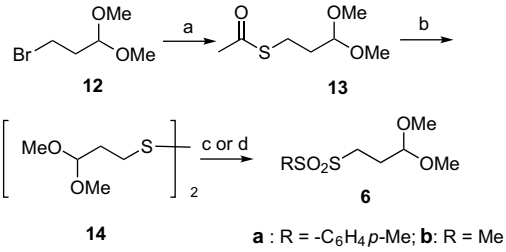
While the α- to ε-regioselectivity of sulfenylation reaction was successfully improved by using a sterically less hindered sulfenylating agent **6b**, the yield and diastereoselectivity of the desired α-sulfenylated product (ratio of **5** to **10**) were unsatisfactory. With an aim to increase the chemical yield as well as the stereo- and regioselectivity, the sulfenylation reaction of **7** was further attempted by changing bases, additives, and solvents. Taking into account both the chemical yield and the stereo- and regioselectivity, NaHMDS and lithium bis(trimethylsilyl)amide (LiHMDS) were found to be the metal bases of choice (Table 1, runs 2–6). Examination of the effect of HMPA as an additive clearly disclosed



Scheme 1. Synthetic design of natural (*R*)-(+)-thiolactomycin (**1**) and its 3-demethyl derivative (**2**) in which the deconjugative asymmetric α-sulfenylation is employed as a key step.



Scheme 2. Deconjugative asymmetric α-sulfenylation of the chiral 3-(α,β,γ,δ-unsaturated acyl)oxazolidin-2-one **7**; (a) (i) *t*-BuOLi, $(EtO)_2P(O)CHMeCO_2Et$, hexane, rt; (ii) 10% NaOH aq, EtOH, 50 °C, 92%; (b) *t*-BuCOCl, Et_3N, THF, −15 °C, then LiCl, (*R*)-4-benzyl-2-oxazolidinone, rt, 91%; (c) see Table 1.



Scheme 3. Syntheses of *S*-3,3-dimethoxypropyl thiosulfonates; (a) CH_3COSH, TEA, rt, 100%; (b) (i) NaOMe, MeOH, rt; (ii) I_2, 98%; (c) *p*-$TolSO_2Na$, I_2, CH_2Cl_2, rt, 87% for **6a**; (d) $MeSO_2Na$, I_2, CH_2Cl_2, rt, 86% for **6b**.

that HMPA is indispensable for this deconjugative asymmetric α-sulfenylation reaction (Table 1, runs 2 and 7). Increasing the amount of HMPA turned out to give better reactivity and stereoselectivity (Table 1, runs 2 and 8); however, use of a 14 equiv of HMPA, which corresponded to the same volume of THF used as a solvent, resulted in lower chemical yield and diastereoselectivity (Table 1, runs 2, 8, and 9). Use of *N*,*N*′-dimethylpropyleneurea (DMPU) and 18-crown-6 as additive in place of HMPA resulted in no improvement for the chemical yield and regio- and stereoselectivity (Table 1, runs 8, 10, and 11). Finally, the effect of the reaction solvent was studied. Although the sulfenylation using diethyl ether (Et_2O) as a solvent afforded a mixture of **5** and **10** and **11** in a chemical yield and regio- and stereoselectivity similar to those observed for the reaction in THF (Table 1, runs 9 and 12), the sulfenylation in toluene or dichloromethane (CH_2Cl_2) gave a lower regio- and stereoselectivity or no reactivity (Table 1, runs 9, 13, and 14). For runs 3–13 in Table 1, **5** was contaminated by 5–10% of the undesired (3′*Z*)-isomer similar to the cases for runs 1 and 2. Based on the experiments delineated above, the best reaction condition described below was established (Table 1, run 8).

Thus, on treatment with NaHMDS (1.2 equiv) in the presence of HMPA (4.0 equiv) in THF at −78 °C, **7** smoothly underwent deprotonation, producing trienolate **A**. Subsequent addition of a THF solution of **6b** (1.5 equiv) followed by gradual warming up to room temperature over 2 h afforded a mixture of **5** and **10** (ca. 8:1 by ^1H NMR and HPLC analysis) in 90% yield along with **11** in 8% yield after aqueous workup and separation by column chromatography (SiO_2). The C4′- and/or the *O*-sulfenylated compounds, which may constitute other by-products were not detected at all. Separation of diastereomeric **5** and **10** could not be accomplished by the usual column chromatography (SiO_2). The ^1H NMR spectrum of the mixture of **5** and **10** clearly showed that **5** consists of the desired (3′*E*)- and the undesired (3′*Z*)-isomer in a ratio of ca.12:1. Further separation of the mixture of **5**, the (3′*Z*)-isomer of **5** and **10** by preparative HPLC gave pure samples of **5**, the (3′*Z*)-isomer of **5** and **10** in 74%, 6%, and 9% yields, respectively. While the (3′*Z*)-isomer of **5** could be separated by preparative HPLC, the (3′*Z*)-isomer of **10** was not detected, probably because it was present in such an extremely minute quantity. The structures of **5**, (3′*Z*)-isomer of **5**, and **10** were rigorously determined by their ^1H NMR spectra. Thus, the NOESY spectra of **5** and **10** were observed between C-3′H and C-5′H, and not between C-3′H and the protons

Table 1
Deconjugative asymmetric α-sulfenylation of the chiral 3-(α,β,γ,δ-unsaturated acyl)oxazolidin-2-one **7** with *S*-3,3-dimethoxypropyl thiosulfonates **6**

Run	Base	Additive (equiv)	R	Solvent	Yield of the α-sulfenylated products[a] (**5**+**10**, %)	dr[b] (**5**/**10**)	Yield of the ε-sulfenylated product **11** (%)
1	NaHMDS	HMPA (3)	*p*-Tol (**6a**)	THF	64	6:1	25
2	NaHMDS	HMPA (3)	Me (**6b**)	THF	57	5:1	7
3	LiHMDS	HMPA (3)	Me (**6b**)	THF	62	4:1	9
4	KHMDS	HMPA (3)	Me (**6b**)	THF	31	1:1	6
5	LDA	HMPA (4)	Me (**6b**)	THF	20	4:1	Trace
6	LiTMP	HMPA (4)	Me (**6b**)	THF	28	6:1	Trace
7	NaHMDS	HMPA (0)	Me (**6b**)	THF	3	1:2	0
8	NaHMDS	HMPA (4)	Me (**6b**)	THF	90	8:1	8
9	NaHMDS	HMPA (14)	Me (**6b**)	THF	54	3:1	0
10	NaHMDS	DMPU (4)	Me (**6b**)	THF	32	3:1	4
11	NaHMDS	18-Crown-6 (4)	Me (**6b**)	THF	58	4:1	17
12	NaHMDS	HMPA (4)	Me (**6b**)	Et_2O	83	8:1	7
13	NaHMDS	HMPA (4)	Me (**6b**)	Toluene	60	3:1	22
14	NaHMDS	HMPA (4)	Me (**6b**)	CH_2Cl_2	0	—	—

[a] This sample contained the (3′*Z*)-isomer of **5**, which corresponds to 5–10% of the (3′*E*)-isomer **5**. Presence of the (3′*Z*)-isomer of **10** was not detected probably due to its extremely minute amount.
[b] Diastereomeric ratio of 5–10 was determined by ^1H NMR spectrum of the mixture.

of the C-4′ methyl group. The structure of the (3′*Z*)-isomer of **5** was similarly determined by the NOESY spectrum observed between C-3′H and the protons of the C-4′ methyl group, and not between C-3′H and C-5′H.

In consideration of the fact that the structure of **7** is elaborated to **1** (vide infra) and HMPA is used as an additive, the deconjugative asymmetric α-sulfenylation might proceed through the non-chelated (*E*)-trienolate **I** with the sterically less hindered α face approach of **6b** (Fig. 2).

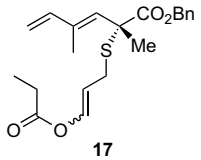
Since the trienolate produced by deprotonation of the ε-proton in the presence of HMPA cannot chelate with an alkali metal, formation of the chelated (*E*)-trienolate **II** and (*Z*)-trienolate **IV** are considered to be disadvantageous. It is also speculated that the formation of **I** occurred more predominantly than that of **III** due to the steric and electrostatic stability of enolate.[30] Based on these considerations, it is most plausible that the α-sulfenylated compound **5** was produced via enolate **I**.

Since the designed asymmetric reaction could be optimized, transformation of **5** into the target molecule **1** was next attempted.[31,32] Treatment of **5** with titanium isopropoxide [Ti(O*i*-Pr)$_4$] in benzyl alcohol effected an imide–ester exchange,[25,26] affording benzyl ester **15** in 83% yield. Acidic hydrolysis of the acetal moiety in **15** gave rise to α-(2-formylethylthio) ester **16** in 97% yield.

With **16** in hand, retro-Michael reaction of **16** followed by acylation of the produced thiol functionality was next studied as shown in Table 2. Treatment of **16** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave rise to the *S*-propionylated product **3** in 35% yield along with the *O*-propionylated compound **17** as a by-product in 38% yield (Table 2, run 1). Compound **17** was found to be a mixture of the geometric isomers, and the ratio of the (*E*)- to the (*Z*)-isomer was determined as 3:4 by the ^{1}H NMR spectrum.[33] Lowering the reaction temperature could not improve the yield of **3** (Table 2, run 2). On the other hand, the formation of **17** was clearly suppressed when the reaction was performed at higher temperature (Table 2, runs 2 and 3). After treating **16** with various alkali metal bases,[34] it was finally found that when **16** was allowed to react with cesium carbonate (Cs_2CO_3, 10 equiv) and the formed cesium thiolate was treated with propionyl chloride in the presence of triethylamine (Et_3N) without isolation, the desired α-propionylthio ester **3** was produced in 75% yield. The formation of **17** might be explained by an insufficient retro-Michael reaction. The successful result obtained with Cs_2CO_3 might be due to the completion of the retro-Michael reaction induced by an increase in the thiophilicity of the cesium cation.

Table 2
Sequential retro-Michael reaction of **16** and acylation of the formed thiol with propionyl chloride in the presence of a base

Run	Base	Solvent	Temp	Yield (%)	
				3	**17**
1	DBU	THF	rt	35	38
2	DBU	DMF	−60 °C	Trace	43
3	DBU	Toluene	100 °C	41	0
4	NaH	Et_2O	0 °C	11	0
5	Cs_2CO_3	EtOH	0 °C	75	0



17

With **3** in hand, Dieckmann condensation of **3** was finally attempted. Thus, according to the reported protocol,[17] treatment of **3** with LiHMDS smoothly gave optically pure natural (*R*)-(+)-thiolactomycin **1** in 63% yield. The spectral properties of **1** were found to be identical to those reported previously.[17,35] As delineated above, efficient synthesis of **1** was completed by employing novel deconjugative asymmetric α-sulfenylation as a key step.

2.3. Synthesis of unnatural (*S*)-(−)-thiolactomycin *ent*-1, (*R*)-(+)-3-demethylthiolactomycin 2, and (*S*)-(−)-3-demethylthiolactomycin *ent*-2

In an attempt to clarify the relationship between the absolute configuration and the biological activities—including antibacterial and FAS inhibitory activity for **1**—as well as to demonstrate the flexibility and efficiency of our explored synthetic route to **1**, we next planned to synthesize the enantiomer of **1**, *ent*-**1**, by utilizing *ent*-**7** instead of **7** as a chiral substrate for deconjugative asymmetric α-sulfenylation. Using the same synthetic scheme described above, unnatural (*S*)-(−)-thiolactomycin (*ent*-**1**) was readily prepared. The physical and spectral properties of *ent*-**1** were identical to those previously reported.[16] Subsequently, the synthesis of the enantiomeric pair of 3-demethyl derivative **2** and *ent*-**2** was examined to further demonstrate the flexibility and efficiency of our synthetic scheme (Scheme 4). Thus, when acetyl chloride was

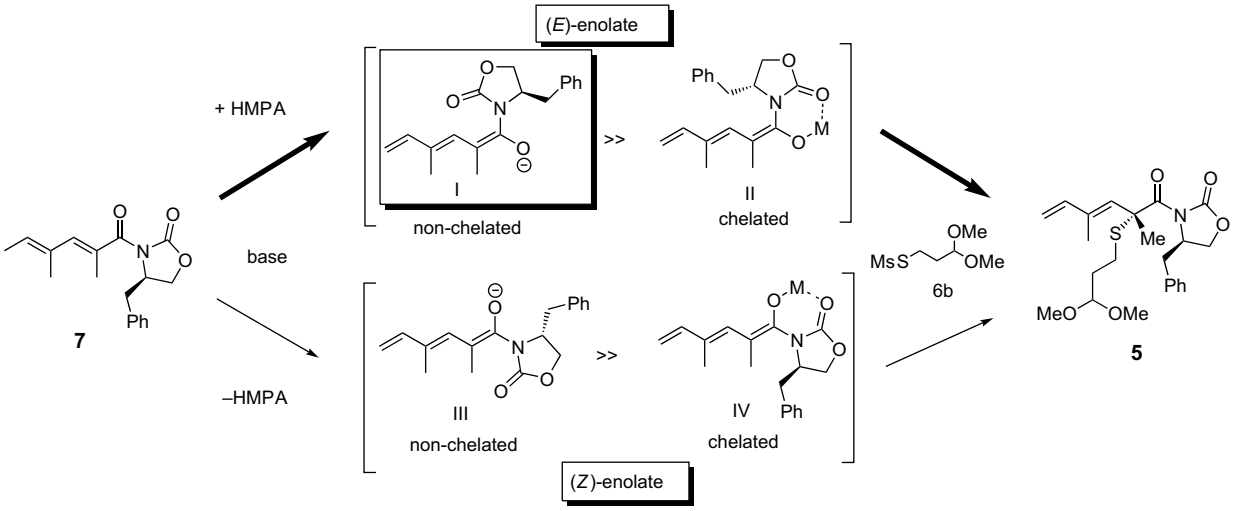
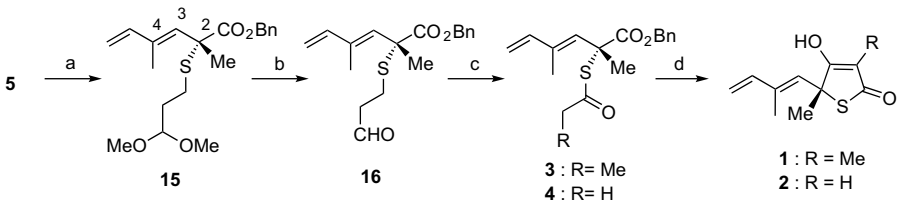


Figure 2. Plausible mechanism of deconjugative asymmetric α-sulfenylation of **7** in the presence or the absence of HMPA.



Scheme 4. Synthesis of (*R*)-(+)-thiolactomycin **1** and its 3-demethyl derivative **2**; (a) Ti(O*i*-Pr)$_4$, BnOH, 70 °C, 82%; (b) 6% HCl aq, THF, rt, 95%; (c) see Table 2, (i) Cs_2CO_3, EtOH, 4 °C; (ii) EtCOCl or AcCl, TEA, CH_2Cl_2, 4 °C, 75% for **3**, 68% for **4**; (d) LiHMDS, THF, −78 °C to rt, 63% for **1**, 59% for **2**.

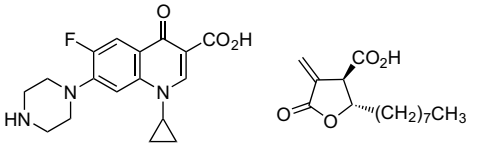
allowed to react with the crude thiol produced by the retro-Michael reaction of **16** in place of propionyl chloride, α-acetylthio ester **4** was similarly obtained. In a manner similar to that for the synthesis of **1**, Dieckmann condensation of **4** gave rise to (*R*)-(+)-3-demethylthiolactomycin **2** in 59% yield. (*S*)-(−)-3-demethylthiolactomycin *ent*-**2** was similarly obtained from *ent*-**7** by way of *ent*-**16** and *ent*-**4**.

2.4. In vitro antibacterial and type I FAS inhibitory activity of enantiomeric pairs of thiolactomycin 1 and *ent*-1 and 3-demethylthiolactomycin 2 and *ent*-2

With completion of the synthesis of **1**, *ent*-**1**, **2**, and *ent*-**2**, their in vitro antibacterial activity against various strains of bacteria[36] and inhibitory activity against mammalian type I FAS[37] were evaluated. For the in vitro antibacterial activity assay, ciprofloxacin, one of the most potent quinolone antibacterial agents,[38] was employed as a reference compound. As a reference compound for type I FAS inhibitory activity, C75, one of the potent inhibitors so far reported,[39] was also used. While in vitro antibacterial activity and inhibitory activity against type I and type II FAS were reported for **1**,[3,11,14,40,41] there has been no report describing the biological activity of *ent*-**1**. When **1**, *ent*-**1**, **2**, and *ent*-**2** were subjected to the biological activity assay mentioned above, some novel features were disclosed as shown in Table 3. Thus, although it is well known that **1** shows moderate in vitro antibacterial activity,[3] it appeared that the other three congeners, *ent*-**1**, **2**, and *ent*-**2**, exhibit very weak (for **2**) or no antibacterial activity (for *ent*-**1** and *ent*-**2**). Quite interestingly, while **1** and **2** bearing a natural (*R*)-configuration were found to exhibit no inhibitory activity against the type I FAS, their enantiomers *ent*-**1** and *ent*-**2** clearly responded to the type I FAS inhibitory assay. Since *ent*-**2** lacking the C_3-methyl group exhibited no in vitro antibacterial activity and showed inhibitory activity against type I FAS that was more than one-third of that of C75, it became evident that *ent*-**2** and its congeners might be usable as selective mammalian type I FAS inhibitors.

Table 3
In vitro antibacterial and mammalian type I FAS inhibitory activity of enantiomeric pairs of thiolactomycin and its 3-demethyl derivative (**1**, *ent*-**1**, **2**, and *ent*-**2**)

Compound	In vitro antibacterial activity, MIC (μg/mL)				Mammalian type I FAS inhibitory activity, IC_{50} (μg/mL) HepG2 ^{14}C
	Staphylococcus aureus Smith	*Moraxella catarrhalis* ATCC 25238	*Heamophilus influenzae* IID 983	*Bacteroides fragilis* GAI 5560	
1	128	0.25	2	1	>80
ent-**1**	>128	>128	N.T.[a]	N.T.[a]	43.7
2	>128	16	32	128	>80
ent-**2**	>128	>128	N.T.[a]	N.T.[a]	19.0
Ciprofloxacin	0.063	0.031	0.008	4	NT[a]
C75	NT[a]	NT[a]	NT[a]	NT[a]	7.4



ciprofloxacin C75

[a] NT; not tested.

3. Conclusion

As described above, we have succeeded in exploring a novel synthetic route to enantiomeric pairs of optically pure thiolactomycin (**1** and *ent*-**1**) and its 3-demethyl derivative (**2** and *ent*-**2**) by employing electrophilic deconjugative asymmetric α-sulfenylation of the chiral 3-(α,β,γ,δ-unsaturated acyl)oxazolidin-2-one (**7** and *ent*-**7**) as a key step. The successful synthesis of enantiomeric pairs of optically pure **1** and **2** obviously showed the efficiency and flexibility of the developed synthetic scheme. From the biological activity assay carried out using the enantiomeric pairs of **1** and **2**, it appeared evident that in vitro antibacterial and mammalian type I FAS inhibitory activity can be cleanly separated by changing not only the substituent at the C_3-position but also the absolute configuration of the C_5-position. Based on these biological studies, it was disclosed that unnatural *ent*-**2** and its congeners might be usable as selective mammalian type I FAS inhibitors. Studies to further explore the characteristic features of the biological activity of thiolactomycin congeners are in progress.

4. Experimental

4.1. General

All melting points were determined with a Yanaco MP-500 melting point apparatus and are uncorrected. Measurements of optical rotations were carried out using a JASCO P-1020 automatic digital polarimeter. Infrared spectra were recorded with a JASCO FT/IR-5300 spectrometer or a Perkin–Elmer spectrum 100 spectrometer. ^1H NMR spectra were measured with a JEOL JNM-ECA-400 (400 MHz) spectrometer. Measurements of ^{13}C NMR spectra were carried out using a JEOL JNM-ECA-400 (100 MHz) spectrometer. The chemical shifts are expressed in parts per million (δ value) downfield from tetramethylsilane, using tetramethylsilane (δ=0) and/or residual solvents such as chloroform (δ=7.26) as an internal standard. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak. Measurements of mass spectra were performed with a JEOL JMS-SX102X mass spectrometer. Data for elemental analyses are within ±0.3% of the theoretical values, and were determined by a Yanaco CHN-corder MT-6. Unless otherwise noted, all the experiments were carried out using anhydrous solvents under an atmosphere of argon. Throughout this study, Merck precoated TLC plates (Silica gel 60 F_{254}, 0.25 mm) were used for thin layer chromatographic (TLC) analysis, and all the spots were visualized using UV light followed by coloring with phosphomolybdic acid or anisaldehyde. Silica gel

60N (40–50 μm, neutral; Kanto Chemical Co., Inc., Tokyo, Japan) or Chromatorex® NH DM2035 (200–350 mesh; Fuji Silysia Chemical, Ltd., Aichi, Japan) was used for the flash column chromatography. Analytical and preparative HPLC was carried out using an apparatus equipped with a Hitachi L-7405 UV-detector, a Hitachi L-7100 HPLC pump, a Hitachi D-7500 chromato integrator, a GL Sciences UV702 UV–vis detector, a GL Sciences PU716 HPLC pump, and a CO705 column oven. The following abbreviations are used for solvents and reagents: ethanol (EtOH), methanol (MeOH), sodium sulfate (Na_2SO_4), ethyl acetate (AcOEt), chloroform ($CHCl_3$), and trifluoroacetic acid (TFA).

4.2. (2*E*,4*E*)-2,4-Dimethylhexa-2,4-dienoic acid (9)

To a solution of triethyl 2-phosphono-propionate (21.3 g, 89.4 mmol) in hexane (180 mL), lithium *tert*-butoxide (1.0 mol/L solution in hexane, 94 mL, 94 mmol) was added dropwise at room temperature, and the resulting mixture was stirred at the same temperature for 30 min. *trans*-2-Methyl-2-butenal **8** (8.4 mL, 82.6 mmol) was added dropwise to the mixture at 0 °C, and stirring was continued for 30 min at room temperature. After quenching the reaction by adding water (100 mL), the mixture was extracted with hexane (100 mL×3). The organic extracts were combined, dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. The residue was dissolved in EtOH (90 mL), and 10% sodium hydroxide (70 mL) was added to the ethanolic solution. The mixture was heated at 50 °C for 16 h with stirring. After cooling, the reaction mixture was washed with hexane (100 mL×3). The aqueous layer was made acidic (pH 1) by adding 2 mol/L HCl to precipitate **9**. Filtration followed by drying in vacuo gave **9** (10.1 g, 87%) as a colorless solid. Mp 75–76 °C (from H_2O/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.77 (d, J=6.7 Hz, 3H), 1.87 (s, 3H), 2.02 (s, 3H), 5.80 (q, J=6.7 Hz, 1H), 7.26 (s, 1H), 11.9 (br s, 1H). IR (KBr): 1664, 1273 cm^{-1}. MS (EI$^+$) m/z: 140 (M^+). Anal. Calcd for $C_8H_{12}O_2$: C, 68.54; H, 8.63. Found: C, 68.60; H, 8.60.

4.3. (*R*)-4-Benzyl-3-[(2*E*,4*E*)-2,4-dimethylhexa-2,4-dienoyl]oxazolidin-2-one (7) and its enantiomer (*ent*-7)

(a) *Preparation of* **7**. To a solution of **9** (2.00 g, 14.3 mmol) and Et_3N (4.8 mL, 34.4 mmol) in THF (72 mL), pivaloyl chloride (1.9 mL, 15.4 mmol) was added dropwise at −15 °C, and the resulting mixture was stirred at the same temperature for 15 min. Lithium chloride (730 mg, 17.2 mmol) and (*R*)-4-benzyloxazolidin-2-one (3.20 g, 17.2 mmol) were added to the mixture at the same temperature, and stirring was continued for 16 h at room temperature. After quenching the reaction by adding saturated aqueous ammonium chloride solution (100 mL), the mixture was extracted with AcOEt (50 mL×2). The organic extracts were combined, washed with saturated aqueous sodium hydrogen carbonate solution (50 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash column chromatography (hexane/AcOEt=4:1) of the residue gave **7** (3.93 g, 92%) as a colorless solid. Mp 81–81.5 °C (from Et_2O). [α] −87.6 (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.75 (d, J=7.3 Hz, 3H), 1.87 (s, 3H), 2.08 (s, 3H), 2.83 (dd, J=13, 9.2 Hz, 1H), 3.35 (dd, J=13, 3.1 Hz, 1H), 4.73 (m, 1H), 5.75 (q, J=7.3 Hz, 1H), 6.44 (s, 1H), 7.19–7.35 (m, 5H). IR (KBr): 1786, 1674 cm^{-1}. MS (EI$^+$) m/z: 299 (M^+). Anal. Calcd for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68. Found: C, 72.07; H, 7.00; N, 4.70.

(b) *Preparation of ent*-**7**. Compound *ent*-**7** (3.83 g, 89%) was prepared as a colorless solid from (*S*)-4-benzyloxazolidin-2-one (2.00 g, 14.3 mmol) in the same manner as described in (a). Mp 88.9–89.1 °C (from Et_2O). [α] +88.8 (*c* 1.0, MeOH). ^{1}H NMR, IR and MS spectra of this sample were identical to those described in (a). Anal. Calcd for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68. Found: C, 72.12; H, 7.07; N, 4.59.

4.4. *S*-3,3-Dimethoxypropyl ethanethioate (13)

To a solution of 3-bromopropionaldehyde dimethylacetal **12** (11.5 mL, 81.7 mmol) and Et_3N (13.7 mL, 98.3 mmol) in THF (500 mL), thiolacetic acid (6.3 mL, 86.4 mmol) was added dropwise at −10 °C, and the resulting mixture was stirred at room temperature for 15 h. The insoluble materials were separated by filtration and washed with Et_2O. The filtrates were combined and concentrated in vacuo. Flash column chromatography (hexane/AcOEt=10:1) of the residue gave **13** (14.8 g, 100%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.85–1.91 (m, 2H), 2.33 (s, 3H), 2.90 (t, J=7.3 Hz, 2H), 3.34 (s, 6H), 4.42 (t, J=5.5 Hz, 1H). IR (NaCl): 1694, 1125 cm^{-1}. MS (EI$^+$) m/z: 147 $(M-OCH_3)^+$. HRMS (EI$^+$) m/z: calcd for $C_6H_{11}O_2S$ $[(M-OCH_3)^+]$: 147.0480. Found: 147.0516.

4.5. 1,2-Bis(3,3-dimethoxypropyl)disulfane (14)

To a solution of **13** (22.3 g, 125 mmol) in MeOH (310 mL), sodium methoxide (9.24 g, 162 mmol) was added dropwise at 4 °C, and the resulting mixture was stirred at room temperature for 50 min. Iodine (15.9 g, 62.6 mmol) was added and stirring was continued for 30 min at the same temperature. The reaction mixture was concentrated in vacuo, and water (200 mL) was added to the residue. The mixture was extracted with AcOEt (100 mL×3). The organic extracts were combined, washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash column chromatography (hexane/AcOEt=4:1) of the residue gave **14** (16.6 g, 98%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96–2.05 (m, 2H), 2.72 (t, J=7.3 Hz, 2H), 3.34 (s, 6H), 4.48 (t, J=5.5 Hz, 1H). IR (NaCl): 1122 cm^{-1}. MS (EI$^+$) m/z: 270 (M^+). HRMS (EI$^+$) m/z: calcd for $C_{10}H_{22}O_4S_2$ (M^+): 270.0960. Found: 270.0978.

4.6. *S*-3,3-Dimethoxypropyl *p*-toluenethiosulfonate (6a)

To a solution of **14** (583 mg, 2.53 mmol) in CH_2Cl_2 (17.0 mL), sodium *p*-toluenesulfinate (1.47 g, 8.08 mmol) and iodine (1.29 g, 5.08 mmol) were added at room temperature, and the resulting mixture was stirred at room temperature for 3 h. After quenching by adding 1 mol/L aqueous sodium thiosulfate until the reaction mixture became colorless, the mixture was extracted with CH_2Cl_2 (20 mL×2). The organic extracts were combined, washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash column chromatography (hexane/AcOEt=4:1) of the residue gave **6a** (1.28 g, 87%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.89–1.99 (m, 2H), 2.45 (s, 3H), 3.02 (t, J=7.3 Hz, 2H), 3.29 (s, 6H), 4.36 (t, J=5.5 Hz, 1H), 7.35 (d, J=7.9 Hz, 2H), 7.82 (d, J=7.9 Hz, 2H). IR (ATR): 1322, 1138, 1120 cm^{-1}. MS (CI$^+$) m/z: 291 $[(M+H)^+]$. HRMS (CI$^+$) m/z: calcd for $C_{12}H_{19}O_4S_2$ $[(M+H)^+]$: 291.0725. Found: 291.0708.

4.7. *S*-3,3-Dimethoxypropyl methanethiosulfonate (6b)

To a solution of **14** (305 mg, 1.13 mmol) in CH_2Cl_2 (7.0 mL), sodium methanesulfinate (400 mg, 3.64 mmol) and iodine (580 mg, 2.29 mmol) were added at room temperature, and the resulting mixture was stirred at room temperature for 2 h. After quenching by adding 1 mol/L aqueous sodium thiosulfate until the reaction mixture became colorless, the mixture was extracted with CH_2Cl_2 (50 mL). The organic extracts were combined, washed with water (50 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash column chromatography (hexane/AcOEt=2:1) of the residue gave **6b** (209 mg, 86%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.09 (m, 2H), 3.22 (t, J=6.7 Hz, 2H), 3.33 (s, 3H), 3.36 (s, 6H), 4.47 (t, J=5.5 Hz, 1H). IR (NaCl): 1319,

1132 cm^{-1}. MS (EI^+) *m*/*z*: 183 [$(M-OCH_3)^+$]. HRMS (EI^+) *m*/*z*: calcd for $C_5H_{11}O_3S_2$ [$(M-OCH_3)^+$]: 183.0150. Found: 183.0163.

4.8. Deconjugative asymmetric α-sulfenylation of (*R*)- and (*S*)-4-benzyl-3-[(2*E*,4*E*)-2,4-dimethylhexa-2,4-dienoyl]oxazolidin-2-one (7 and *ent*-7). (*R*)-4-Benzyl-3-[(*R*,*E*)-2-(3,3-dimethoxypropylthio)-2,4-dimethylhexa-3,5-dienoyl]-oxazolidin-2-one (5), its (*R*,*Z*)-isomer [(3′*Z*)-5], its (*S*,*E*)-diastereoisomer (10), (*R*)-4-benzyl-3-[(2*E*,4*E*)-6-(3,3-dimethoxypropylthio)-2,4-dimethylhexa-2,4-dienoyl]oxazolidin-2-one (11) and their enantiomers (*ent*-5, *ent*-[(3′*Z*)-5], *ent*-10, and *ent*-11)

(a) *Preparation of* ***5****, (3′Z)-****5****,* ***10****, and* ***11*** (Table 1, run 8). To a solution of **7** (598 mg, 2.00 mmol) and HMPA (1.4 mL, 8.05 mmol) in THF (10 mL), NaHMDS (1.0 mol/L solution in THF, 2.4 mL, 2.40 mmol) was added dropwise at −78 °C, and the resulting mixture was stirred at the same temperature for 30 min. A solution of **6b** (645 mg, 3.01 mmol) in THF (2.0 mL) was added to the reaction mixture at the same temperature, and the resulting mixture was allowed to slowly warm to room temperature. After quenching the reaction by adding saturated aqueous ammonium chloride solution (15 mL), the mixture was extracted with AcOEt (15 mL×3). The organic extracts were combined, washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash column chromatography (hexane/AcOEt=4:1) of the residue gave a mixture of **5**, (3′*Z*)-**5**, and **10** (780 mg, 90%) and a pure sample of **11** (70 mg, 8%), both as an oil. The mixture of **5**, (3′*Z*)-**5**, and **10** was further separated by HPLC [Daicel Chiralpak AD-H, ϕ 2.0 cm×25 cm: hexane/2-propanol/EtOH=93:4:3, flow rate 10 mL/min, HPLC analysis; Daicel Chiralpak AD-H, ϕ 0.46 cm×25 cm, hexane/2-propanol=95:5, flow rate 1.0 mL/min; t_R 10.4 min ((3′*Z*)-**5**), 14.7 min (**5**), 25.1 min (**10**)] to give pure samples of **5**, (3′*Z*)-**5**, and **10**, all as an oil.

Compound **5** (641 mg, 74%). [α] −253 (*c* 0.5, MeOH). ^1H NMR (400 MHz, $CDCl_3$): δ 1.72 (d, *J*=1.2 Hz, 3H), 1.82–1.89 (m, 2H), 1.95 (s, 3H), 2.62 (t, *J*=7.3 Hz, 2H), 2.72 (dd, *J*=13, 10 Hz, 1H), 3.318 (s, 3H), 3.324 (s, 3H), 3.27–3.38 (m, 1H), 4.09–4.14 (m, 2H), 4.48 (t, *J*=5.5 Hz, 1H), 4.65–4.71 (m, 1H), 5.01 (d, *J*=11 Hz, 1H), 5.14 (d, *J*=17 Hz, 1H), 5.72 (s, 1H), 6.36 (dd, *J*=17, 11 Hz, 1H), 7.22–7.36 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.6, 24.4, 25.7, 31.9, 38.0, 52.9, 53.3, 55.0, 57.4, 66.2, 103.3, 112.7, 127.4, 129.0, 129.4, 133.7, 133.9, 135.4, 140.9, 150.9, 173.1. IR (NaCl): 1790, 1680 cm^{-1}. MS (EI^+) *m*/*z*: 433 (M^+). HRMS (EI^+) *m*/*z*: calcd for $C_{23}H_{31}NO_5S$ (M^+): 433.1923. Found: 433.1941.

Compound (3′*Z*)-**5** (51 mg, 6%). ^1H NMR (400 MHz, $CDCl_3$): δ 1.83 (d, *J*=1.2 Hz, 3H), 1.83–1.90 (m, 2H), 1.96 (s, 3H), 2.65–2.74 (m, 3H), 3.318 (s, 3H), 3.324 (s, 3H), 3.29–3.37 (m, 1H), 4.04 (q, *J*=7.9 Hz, 1H), 4.09 (dd, *J*=8.6, 2.4 Hz, 1H), 4.48 (t, *J*=6.1 Hz, 1H), 4.59–4.63 (m, 1H), 5.11 (d, *J*=11 Hz, 1H), 5.22 (d, *J*=17 Hz, 1H), 5.60 (s, 1H), 6.62 (dd, *J*=17, 11 Hz, 1H), 7.22–7.36 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.7, 24.8, 26.2, 32.0, 38.0, 53.0, 53.3, 54.8, 57.6, 66.4, 103.4, 115.7, 127.3, 128.9, 129.5, 131.4, 132.4, 133.2, 135.5, 151.1, 173.9. IR (NaCl): 1790, 1680 cm^{-1}. MS (EI^+) *m*/*z*: 433 (M^+). HRMS (EI^+) *m*/*z*: calcd for $C_{23}H_{31}NO_5S$ (M^+): 433.1923. Found: 433.1901.

Compound **10** (77 mg, 9%). ^1H NMR (400 MHz, $CDCl_3$): δ 1.71 (d, *J*=1.2 Hz, 3H), 1.84 (s, 3H), 1.84–1.91 (m, 2H), 2.59–2.70 (m, 3H), 3.313 (s, 3H), 3.318 (s, 3H), 3.34 (dd, *J*=13, 3.1 Hz, 1H), 4.06–4.14 (m, 2H), 4.47 (t, *J*=5.5 Hz, 1H), 4.65–4.70 (m, 1H), 5.01 (d, *J*=11 Hz, 1H), 5.13 (d, *J*=18 Hz, 1H), 5.77 (s, 1H), 6.36 (dd, *J*=18, 11 Hz, 1H), 7.24–7.37 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.6, 24.3, 25.2, 32.0, 37.6, 53.1, 53.2, 53.7, 57.4, 66.0, 103.3, 112.5, 127.3, 129.0, 129.4, 133.2, 134.3, 135.6, 141.0, 150.8, 172.3. IR (NaCl): 1790, 1676 cm^{-1}. MS (EI^+) *m*/*z*: 433 (M^+). HRMS (EI^+) *m*/*z*: calcd for $C_{23}H_{31}NO_5S$ (M^+): 433.1923. Found: 433.1902.

Compound **11**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.86–1.96 (m, 5H), 2.05 (s, 3H), 2.55 (t, *J*=7.3 Hz, 2H), 2.84 (dd, *J*=13, 9.2 Hz, 1H), 3.27 (d, *J*=7.3 Hz, 2H), 3.29–3.38 (m, 7H), 4.14–4.19 (m, 1H), 4.26 (t, *J*=9.5 Hz, 1H), 4.49 (t, *J*=5.5 Hz, 1H), 4.68–4.76 (m, 1H), 5.70 (t, *J*=7.3 Hz, 1H), 6.38 (s, 1H), 7.20–7.37 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.4, 16.2, 26.5, 29.5, 32.7, 37.5, 53.2, 55.4, 66.5, 103.3, 127.4, 128.9, 129.5, 129.9, 130.9, 134.2, 135.1, 139.3, 150.0, 172.5. IR (NaCl): 1788, 1678 cm^{-1}. MS (FAB^-) *m*/*z*: 432 [$(M-H)^-$]. HRMS (FAB^-) *m*/*z*: calcd for $C_{23}H_{30}NO_5S$ [$(M-H)^-$]: 432.1845. Found: 432.1868.

(b) *Preparation of ent-****5****.* Compound *ent*-**5** (870 mg, 67%) was prepared as a colorless oil from *ent*-**7** (900 mg, 3.01 mmol) in the same manner as described in (a). [α] +257 (*c* 0.5, MeOH). Three types of by-products enantiomeric to (3′*Z*)-**5** [*ent*-(3′*Z*)-**5**], **10** (*ent*-**10**), and **11** (*ent*-**11**) were obtained similar to the case described in (a). Compounds *ent*-(3′*Z*)-**5** (47 mg, 5%), *ent*-**10** (101 mg, 12%), and *ent*-**11** (101 mg, 12%). ^1H NMR, IR, and MS spectra of these samples were identical to those described in (a). HRMS (EI^+) *m*/*z*: calcd for $C_{23}H_{31}NO_5S$ (M^+) (for *ent*-**5**, *ent*-(3′*Z*)-**5**, and *ent*-**10**): 433.1923. Found: 433.1881 (for *ent*-**5**), 433.1931 [for *ent*-(3′*Z*)-**5**], 433.1902 (for *ent*-**10**). HRMS (FAB^-) *m*/*z*: calcd for $C_{23}H_{30}NO_5S$ [$(M-H)^-$] (*ent*-**11**): 432.1845. Found: 432.1869 (for *ent*-**11**).

4.9. (*R*,*E*)-Benzyl 2-(3,3-dimethoxypropylthio)-2,4-dimethylhexa-3,5-dienoate (15) and its enantiomer (*ent*-15)

(a) *Preparation of* ***15****.* To benzyl alcohol (8.8 mL, 85.0 mmol), $Ti(OiPr)_4$ (1.3 mL, 4.40 mmol) was added and the resulting mixture was stirred at room temperature for 5 h under a reduced pressure (0.5–1.0 mmHg). The mixture was added to **5** (893 mg, 2.06 mmol), and the whole mixture was stirred at 70 °C for 16 h. After cooling, the mixture was diluted with CH_2Cl_2 (30 mL) and the reaction was quenched by adding 1 mol/L HCl (10 mL). The insoluble materials, which appeared were removed by filtration through a pad of Celite and washed with CH_2Cl_2. The filtrates were combined, diluted with H_2O (10 mL), and extracted with CH_2Cl_2 (25 mL×3). The organic extracts were combined, washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash column chromatography (hexane/AcOEt=10:1) of the residue gave **15** (619 mg, 82%) as a colorless oil. [α] +4.4 (*c* 0.5, MeOH). ^1H NMR (400 MHz, $CDCl_3$): δ 1.66 (s, 3H), 1.70 (d, *J*=1.2 Hz, 3H), 1.73–1.79 (m, 2H), 2.50–2.65 (m, 2H), 3.28 (s, 6H), 4.36 (t, *J*=5.5 Hz, 1H), 5.03 (d, *J*=11 Hz, 1H), 5.15–5.23 (m, 3H), 6.34 (dd, *J*=17, 11 Hz, 1H), 7.30–7.38 (m, 5H). IR (NaCl): 1726 cm^{-1}. MS (EI^+) *m*/*z*: 364 (M^+). HRMS (EI^+) *m*/*z*: calcd for $C_{20}H_{28}O_4S$ (M^+): 364.1708. Found: 364.1710.

(b) *Preparation of ent-****15****.* Compound *ent*-**15** (590 mg, 81%) was prepared as a colorless oil from *ent*-**5** (870 mg, 2.01 mmol) in the same manner as described in (a). [α] −4.3 (*c* 0.5, MeOH). ^1H NMR, IR, and MS spectra of this sample were identical to those described in (a). HRMS (EI^+) *m*/*z*: calcd for $C_{20}H_{28}O_4S$ (M^+): 364.1708. Found: 364.1737.

4.10. (*R*,*E*)-Benzyl 2,4-dimethyl-2-(3-oxopropylthio)-hexa-3,5-dienoate (16) and its enantiomer (*ent*-16)

(a) *Preparation of* ***16****.* To a solution of **15** (805 mg, 2.21 mmol) in THF (9.0 mL), 6% HCl (7.0 mL) was added at room temperature, and the resulting mixture was stirred at the same temperature for 6 h. After quenching the reaction by adding saturated aqueous sodium hydrogen carbonate solution (20 mL) under cooling in an ice bath, the mixture was extracted with AcOEt (15 mL×3). The organic extracts were combined, washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash column chromatography (hexane/AcOEt=6:1) of the residue gave **16** (667 mg, 95%) as a colorless oil. [α] +27.5 (*c* 0.4, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.66 (s, 3H), 1.72 (d, *J*=1.2 Hz, 3H), 2.56 (t, *J*=7.3 Hz, 2H), 2.76–2.86 (m, 2H), 5.05 (d, *J*=11 Hz, 1H), 5.20 (d, *J*=17 Hz, 1H), 5.20 (q, *J*=12 Hz, 2H), 5.74 (s, 1H), 6.34 (dd, *J*=17,

11 Hz, 1H), 7.30–7.39 (m, 5H), 9.61 (s, 1H). IR (NaCl): 1726 cm^{-1}. MS (EI^+) *m*/*z*: 318 (M^+). HRMS (EI^+) *m*/*z*: calcd for $C_{18}H_{22}O_3S$ (M^+): 318.1290. Found: 318.1308.

(b) *Preparation of ent-**16***. Compound *ent*-**16** (501 mg, 99%) was prepared as a colorless oil from *ent*-**15** (580 mg, 1.59 mmol) in the same manner as described in (a). [α] −27.2 (*c* 0.4, $CHCl_3$). ^1H NMR, IR, and MS spectra of this sample were identical to those described in (a). HRMS (EI^+) *m*/*z*: calcd for $C_{18}H_{22}O_3S$ (M^+): 318.1290. Found: 318.1277.

4.11. (*R*,*E*)-Benzyl 2,4-dimethyl-2-(propionylthio)hexa-3,5-dienoate (3) and its enantiomer (*ent*-3)

(a) *Preparation of **3*** (Table 2, run 5). To a suspension of Cs_2CO_3 (6.81 g, 20.9 mmol) in EtOH (105 mL), a solution of **16** (666 mg, 2.09 mmol) in EtOH (7.0 mL) was added dropwise at 4 °C, and the resulting mixture was stirred at the same temperature for 20 min. The reaction mixture was poured into a mixture of saturated aqueous ammonium chloride solution and 1 mol/L HCl (3:1, 120 mL), and the aqueous mixture was extracted with Et_2O (20 mL×3). The organic extracts were combined, dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. The residue was diluted with CH_2Cl_2 (21 mL). Et_3N (0.45 mL, 3.23 mmol) and propionyl chloride (0.24 mL, 2.69 mmol) were added dropwise to the resulting CH_2Cl_2 solution at 4 °C, and the mixture was stirred at the same temperature for 30 min. After quenching the reaction by adding saturated aqueous ammonium chloride solution (50 mL), the mixture was extracted with CH_2Cl_2 (30 mL×3). The organic extracts were combined, washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash column chromatography (hexane/AcOEt=20:1) of the residue gave **3** (501 mg, 75%) as a colorless oil. [α] +22.0 (*c* 0.2, MeOH). ^1H NMR (400 MHz, $CDCl_3$): δ 1.10 (t, *J*=7.3 Hz, 3H), 1.77 (s, 3H), 1.90 (s, 3H), 2.49 (d, *J*=7.3 Hz, 2H), 5.04 (d, *J*=10 Hz, 1H), 5.18 (s, 2H), 5.18 (d, *J*=17 Hz, 1H), 5.75 (s, 1H), 6.30 (dd, *J*=17, 10 Hz, 1H). IR (NaCl): 1738, 1694 cm^{-1}. MS (EI^+) *m*/*z*: 318 (M^+). HRMS (EI^+) *m*/*z*: calcd for $C_{18}H_{22}O_3S$ (M^+): 318.1290. Found: 318.1310.

(b) *Preparation of ent-**3***. Compound *ent*-**3** (290 mg, 77%) was prepared as a colorless oil from *ent*-**16** (380 mg, 1.19 mmol) in the same manner as described in (a). [α] −24.7 (*c* 0.2, MeOH). ^1H NMR, IR, and MS spectra of this sample were identical to those described in (a). HRMS (EI^+) *m*/*z*: calcd for $C_{18}H_{22}O_3S$ (M^+): 318.1290. Found: 318.1273.

4.12. (*R*,*E*)-Benzyl 2,4-demethyl-2-[(*E*,*Z*)-3-(propionyloxy)prop-2-enylthio]hex-3,5-dienolate (17) (Table 2, run 1)

To a solution of **16** (36 mg, 0.113 mmol) in THF (1.0 mL), propionyl chloride (20 μL, 0.224 mmol) was added at 4 °C, and the resulting mixture was stirred at room temperature for 1 h. DBU (20 μL, 0.134 mmol) was added to the mixture, and stirring was continued at 40 °C until the TLC analysis showed complete disappearance of **16**. After quenching the reaction by adding water (5 mL) under cooling in an ice bath, the mixture was extracted with AcOEt (10 mL×3). The organic extracts were combined, washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Separation of the residue by preparative TLC (hexane/AcOEt=4:1) gave **3** (12.6 mg, 35%) as a colorless oil and oily **17** (16.0 mg, 38%) as a 3:4 mixture of the (*E*)- and the (*Z*)-isomer.

Compound **17** (a 3:4 mixture of the (*E*)- and the (*Z*)-isomer). ^1H NMR (400 MHz, $CDCl_3$): δ 1.16 (t, *J*=7.3 Hz, 4/7×3H), 1.16 (t, *J*=7.3 Hz, 3/7×3H), 1.66 (s, 3/7×3H), 1.68 (s, 4/7×3H), 1.71–1.73 (m, 3H), 2.35–2.42 (m, 2H), 3.17 (td, *J*=7.9, 1.2 Hz, 3/7×2H), 3.34 (td, *J*=7.9, 1.2 Hz, 4/7×2H), 4.83–4.90 (m, 4/7×1H), 5.04 (d, *J*=11 Hz, 1H), 5.15–5.27 (m, 3H), 5.30–5.36 (m, 3/7×1H), 5.74 (s, 3/7×1H), 5.76 (s, 4/7×1H), 6.30–6.38 (m, 1H), 7.07 (td, *J*=5.7, 1.2 Hz, 4/7×1H), 7.15 (td, *J*=13, 1.2 Hz, 3/7×1H), 7.31–7.39 (m, 5H). MS (EI^+) *m*/*z*: 374 (M^+).

4.13. (*R*,*E*)-4-Hydroxy-3,5-dimethyl-5-(2-methylbuta-1,3-dienyl)thiophen-2(5*H*)-one [(*R*)-(+)-thiolactomycin] (1) and its enantiomer [(*S*)-(−)-thiolactomycin] (*ent*-1)

(a) *Preparation of **1***. To a solution of **3** (498 mg, 1.56 mmol) in THF (78 mL), LiHMDS (1.0 mol/L solution in THF, 4.0 mL, 4.00 mmol) was added dropwise at −78 °C, and the resulting mixture was allowed to slowly warm to room temperature over 3.5 h. The mixture was poured into a solution of 1 mol/L HCl (40 mL), and the aqueous mixture was extracted with Et_2O (35 mL×3). The organic extracts were combined, dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. The residue was added to a saturated aqueous sodium hydrogen carbonate solution (20 mL). The aqueous mixture was washed with Et_2O (20 mL×2), then made acidic (pH1) by adding 1 mol/L HCl. The resulting aqueous mixture was extracted with Et_2O (25 mL×2) and AcOEt (25 mL×2). The organic extracts were combined, dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo to give **1** (300 mg, 63%) as a colorless solid. Mp 119–120 °C (diisopropyl ether) [lit.,[17] mp 119.5–121 °C; lit.,[34] mp 120 °C]. [α] +198 (*c* 1.0, MeOH) [lit.,[17] [α] +174 (*c* 0.6, MeOH); lit.,[34] [α] +176 (*c* 1.0, MeOH)]. The optical purity of **1** obtained here was determined to be >99% ee by HPLC analysis with a chiral column [Daicel Chiralcel OJ φ 0.46 cm×25 cm, hexane/2-propanol/TFA=95:5:0.2, flow rate 1.0 mL/min, t_R 11.0 min (**1**), 14.9 min (*ent*-**1**), detection at 238 nm]. ^1H NMR (400 MHz, CD_3OD): δ 1.70 (s, 3H), 1.71 (d, *J*=1.2 Hz, 3H), 1.80 (s, 3H), 5.02 (d, *J*=10 Hz, 1H), 5.26 (d, *J*=17 Hz, 1H), 5.63 (s, 1H), 6.37 (dd, *J*=16.5, 9.8 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 7.7, 12.0, 30.2, 56.4, 110.1, 113.7, 131.5, 140.4, 142.2, 183.2, 197.5. IR (KBr): 1605 cm^{-1}. MS (EI^+) *m*/*z*: 210 (M^+). Anal. Calcd for $C_{11}H_{14}O_2S$: C, 62.83; H, 6.71. Found: C, 62.68; H, 6.54.

(b) *Preparation of ent-**1***. Compound *ent*-**1** (89 mg, 42%) was prepared as a colorless solid from *ent*-**3** (320 mg, 1.00 mmol) in the same manner as described in (a). Mp 119–120 °C (diisopropyl ether). [α] −187 (*c* 1.0, MeOH) [lit.,[16] [α] −172 (*c* 0.2, MeOH)]. The optical purity of *ent*-**1** prepared here was estimated to be >99% ee using a method similar to that described in (a). ^1H NMR, ^{13}C NMR, IR, and MS spectra of this sample were identical to those described in (a). Anal. Calcd for $C_{11}H_{14}O_2S$: C, 62.83; H, 6.71. Found: C, 62.51; H, 6.68.

4.14. (*R*,*E*)-Benzyl 2,4-dimethyl-2-(acetylthio)hexa-3,5-dienoate (4) and its enantiomer (*ent*-4)

(a) *Preparation of **4***. Treatment of **16** (510 mg, 1.60 mmol) in a manner similar to those described for the preparation of **3** from **16** using acetyl chloride (0.12 mL, 1.66 mmol) in place of propionyl chloride afforded **4** (332 mg, 68%) as a colorless oil. [α] +13.8 (*c* 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.76 (d, *J*=1.2 Hz, 3H), 1.91 (s, 3H), 2.24 (s, 3H), 5.04 (d, *J*=11 Hz, 1H), 5.18 (s, 2H), 5.20 (d, *J*=18 Hz, 1H), 6.30 (dd, *J*=17, 10 Hz, 1H), 7.30–7.36 (m, 5H). IR (NaCl): 1738, 1694 cm^{-1}. MS (EI^+) *m*/*z*: 304 (M^+). HRMS (EI^+) *m*/*z*: calcd for $C_{17}H_{20}O_3S$ (M^+): 304.1133. Found: 304.1159.

(b) *Preparation of ent-**4***. Compound *ent*-**4** (391 mg, 82%) was prepared as a colorless oil from *ent*-**16** (500 mg, 1.57 mmol) in the same manner as described in (a). [α] −13.6 (*c* 0.5, $CHCl_3$). ^1H NMR, IR, and MS spectra of this sample were identical to those described in (a). HRMS (EI^+) *m*/*z*: calcd for $C_{17}H_{20}O_3S$ (M^+): 304.1133. Found: 304.1114.

4.15. (*R*,*E*)-4-Hydroxy-5-methyl-5-(2-methylbuta-1,3-dienyl)thiophen-2(5*H*)-one [(*R*)-(+)-3-demethylthiolactomycin] (**2**) and its enantiomer [(*S*)-(−)-3-demethylthiolactomycin] (*ent*-**2**)

(a) *Preparation of* ***2***. Treatment of **4** (318 mg, 1.04 mmol) in the same manner as described for the preparation of **1** gave **2** (121 mg, 59%) as a colorless solid. Mp 120–121 °C (diisopropyl ether). [α] +186 (*c* 0.5, MeOH). The optical purity of **2** prepared here was determined to be >99% ee by HPLC analysis with a chiral column [Daicel Chiralcel OJ φ 0.46 cm×25 cm, hexane/EtOH/TFA=99:1:0.1, flow rate 1.0 mL/min, t_R 17.9 min (**2**) and 22.5 min (*ent*-**2**) detection at 238 nm]. ^{1}H NMR (400 MHz, CD_3OD): δ 1.79 (d, *J*=1.2 Hz, 3H), 1.83 (s, 3H), 5.06 (d, *J*=10.4 Hz, 1H), 5.27 (d, *J*=17.7 Hz, 1H), 5.66 (s, 1H), 6.36 (dd, *J*=17.1, 11.0 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 12.4, 30.3, 58.8, 113.7, 131.4, 140.5, 142.1, 190.1, 197.2. IR (KBr): 1607 cm^{-1}. MS (EI$^+$) *m*/*z*: 318 (M^+). Anal. Calcd for $C_{10}H_{12}O_2S$: C, 61.20; H, 6.16. Found: C, 60.90; H, 6.07.

(b) *Preparation of ent-****2***. Compound *ent*-**2** (210 mg, 55%) was prepared as a colorless solid from *ent*-**4** (590 mg, 1.94 mmol) in the same manner as described in (a). Mp 119–121 °C (diisopropyl ether). [α] −185 (*c* 0.5, MeOH). The optical purity of *ent*-**2** prepared here was estimated to be >99% ee using a method similar to that described in (a). ^{1}H NMR, ^{13}C NMR, IR, and MS spectra of this sample were identical to those described in (a). Anal. Calcd for $C_{10}H_{12}O_2S$: C, 61.20; H, 6.16. Found: C, 60.98; H, 6.08.

4.16. Biological assay

In vitro antibacterial activity assay. The MIC (mg/mL) was determined by the ager dilution method[36] with Muller-Hinton agar (Difco Laboratories, Detroit, MI). The MIC was defined as the lowest concentration of an antibacterial agent that inhibited visible growth after incubation at 35 °C for 18 h.

In vitro inhibitory activity assay for mammalian type I fatty acid synthase. Fatty acid synthesis was evaluated by measuring the incorporation of [1-^{14}C] acetate into cellular fatty acid as previously described[37] with some modification. Human HepG2 cells were seeded in a 12-well plate and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2 atmosphere at 37 °C for 24 h. The medium was removed and replaced with serum-free DMEM. After 24 h incubation, the cells were treated with various concentrations of the test compound for 4 h in Krebs–Ringer phosphate HEPES buffer (pH7.4). [1-^{14}C] acetate (0.4 uCi/mL; 56.5 mCi/mmol; Perkin–Elmer Inc., Norwalk, CT) was added to the medium, which was then incubated at 37 °C for 2 h. The metabolic reaction was stopped by the addition of ethanolic KOH, and the samples were left at 80 °C for 2 h. After extracting nonsaponifiable lipids with petroleum ether, the water-soluble residual layer was acidified to pH<1 by the addition of HCl. Total fatty acids were extracted with petroleum ether, and the combined organic extracts were dried and concentrated in vacuo. The residue was dissolved with methanol and was transferred into a scintillation vial. Radioactivity of total fatty acids was counted by a liquid scintillation counter (Perkin–Elmer Inc.). IC_{50} values were calculated by the auto-analysis program GraphPad Prism, version 4.00 (GraphPad Software Inc., La Jolla, CA).

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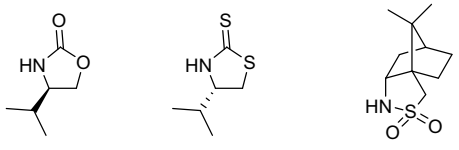
20. In the preliminary experiments, (*R*)-4-isopropyloxazolidin-2-one (**i**), (*S*)-4-isopropyl-thiazolidin-2-thione (**ii**), and (1*R*)-2,10-camphur-sultam (**iii**) were examined as chiral auxiliaries in addition to (*R*)-4-benzyloxazolidin-2-one. However, when **i** was employed as a chiral source, the deconjugative asymmetric α-sulfenylation usually took place with a decreased diastereoselectivity and in a lower chemical yield than those obtained using (*R*)-4-benzyloxazolidin-2-one. In the case where **ii** was used as a chiral source, the attempted asymmetric reaction under various conditions always gave complex mixtures of the products. The asymmetric reaction employing **iii** as a chiral source afforded the ε-sulfenylated product corresponding to **11** (*see text*) as a sole product. Based on these numerous experiments, (*R*)-4-benzyloxazolidin-2-one was selected as the most promising chiral auxiliary. Ohata, K.; Terashima, S., unpublished results.



(**i**) (**ii**) (**iii**)

21. Prior to selecting **6** as a sulfenylating agent, various *S*-electrophiles were examined in the preliminary experiments. Thus, acetylsulfenyl chloride, which was considered to be one of the most attractive *S*-electrophiles at the outset, turned out not to react with **7** at all. While methyl methanethiosulfonate ($MeSSO_2Me$) and (2-trimethylsilyl)ethyl *p*-toluenethio-sulfonate ($TMSCH_2CH_2SSO_2C_6H_4$*p*-Me) underwent deconjugative asymmetric α-sulfenylation to give α-sulfenylated products in 84% and 53% yield, respectively, the methylsulfenyl and the (2-trimethylsilyl)ethylsulfenyl group introduced into the reaction products could not be elaborated to a thiol or an acylsulfenyl group. Ohata, K.; Terashima, S., unpublished results.
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24. Deconjugative asymmetric allylation of a dienolate with formation of a quaternary asymmetric center was recently reported by Kobayashi, et al. Abe, T.;

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31. In our preliminary experiments, Dieckmann condensation of the imide compound derived from **5** by sequential deacetalization, retro-Michael reaction, and acylation without transesterification were attempted to produce **1**. However, since the leaving ability of the imide moiety toward nucleophilic attack of the α-carbanion of thioester was insufficient for Dieckmann condensation under various conditions, we decided to subject the corresponding ester **3**, which had a leaving ability superior to that of the imide compound, to Dieckmann condensation.
32. In addition to benzyl ester **15**, some other esters (methyl, ethyl, and isopropyl ester) were synthesized in a similar manner. Since the thiols derived from these esters by sequential deacetalization and retro-Michael reaction were fairly volatile and unstable, the chemical yield of the acylated compound carrying a methyl, ethyl or isopropyl ester was found to be lower than that for **3** bearing a benzyl ester.
33. The structures of (*E*)- and (*Z*)-isomer were determined by the coupling constant (*J* value) of olefinic protons in the vinyl ester moiety in the ^{1}H NMR spectrum (*see* Experimental).
34. In addition to utilizing NaH and Cs_2CO_3, further attempts were made using potassium bis(trimethylsilyl)amide (KHMDS), $BaCO_3$, $CaCO_3$, and K_2CO_3 as an alkali metal base. However, all these bases were found to be not effective for this retro-Michael reaction.
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