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Synthesis and biological activity of enantiomeric pairs of 5-vinylthiolactomycin congeners

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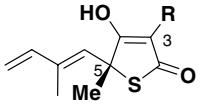
Abstract—Twelve enantiomeric pairs of 5-vinylthiolactomycin congeners were synthesized by employing our efficient synthetic route previously explored for the synthesis of enantiomeric pairs of thiolactomycin and its 3-demethyl derivative. From the biological activity assay carried out using the obtained congeners along with enantiomeric pairs of thiolactomycin and its 3-demethyl derivative previously prepared, it appeared evident that in vitro antibacterial and mammalian type I FAS inhibitory activity of thiolactomycin congeners can be cleanly separated by changing not only the structure but also the absolute configuration of the side chain at the C_5-position. These studies led us to explore (*S*)-3-demethyl-5-(pent-1-enyl)thiolactomycin derivative [(*S*)-4-hydroxy-5-methyl-5-(pent-1-enyl)-5H-thiophen-2-one] which exhibits type I FAS inhibitory activity equal to that of C75, the potent inhibitor so far reported, with complete loss of in vitro antibacterial activity.

(*R*)-(+)-Thiolactomycin (TLM, **1**) is a thiolactone antibiotic isolated from a soil bacterium, *Nocardia* sp.,[1] and shows moderate in vitro activity against a number of pathogens including Gram-positive and Gram-negative bacteria,[2] *Mycobacterium tuberculosis*,[3] and malaria parasites.[4] It was also disclosed that **1** exhibits inhibitory activity against fatty acid synthase (FAS).[5] Although it appeared that **1** inhibits bacterial and plant type II FAS but not mammalian type I FAS,[6] Townsend et al. recently reported that **1** and its derivatives also show inhibitory activity against type I FAS.[7] Since **1** and its congeners seem to constitute promising drug targets for cancer and obesity treatments as well as for infective diseases, syntheses and screenings of various structural types of TLM congeners have so far been reported.[3,4,7,8] However, probably due to lack of an efficient synthetic route,[9] studies on biological activity examined by using optically active compounds are quite limited.[8a]

We have recently reported an efficient total synthesis of **1** and its 3-demethyl derivative (3-demethyl TLM **2**) by featuring a novel deconjugative asymmetric α-sulfenylation of the chiral 3-(α,β/γ,δ-unsaturated acyl)oxazolidin-2-one with a methanethiosulfonate as a key step.[10] Flexibility of the explored synthetic route has been realized by the expeditious synthesis of (*S*)-TLM (*ent*-**1**) and (*S*)-3-demethyl TLM (*ent*-**2**) in addition to that of **1** and **2**. In the course of our studies on **1** and its congeners from the viewpoint of medicinal chemistry, we paid attention to the effects of the structure and the absolute configuration of the side chain at the C_5-position of **1** on in vitro antibacterial and mammalian type I FAS inhibitory activity. Therefore, enantiomeric pairs of 5-vinyl TLM and 3-demethyl-5-vinyl TLM congeners (**3**, *ent*-**3**, **4**, and *ent*-**4**) were designed in order to perform extensive studies on the structure–activity relationships of the C_5-position of **1** and to substantiate the efficiency of our previously developed synthetic route for synthesizing various structural types of TLM congeners (Figs. 1 and 2).



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

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

Keywords: Antibiotic; Inhibitor of mammalian type I fatty acid synthase; 5-Vinylthiolactomycin.
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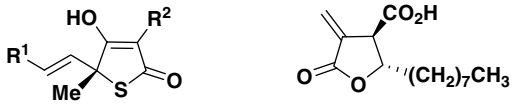


3a-f: R^2=Me; **4a-f**: R^2=H C75

a: R^1=H; **b**: R^1=Me; **c**: R^1=Et;
d: R^1=*n*Pr; **e**: R^1=*n*Bu; **f**: R^1=*n*Hex

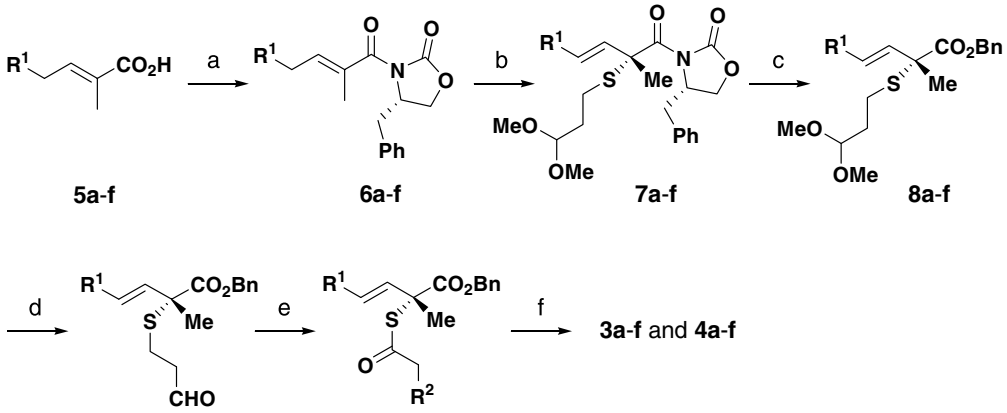
Figure 2. Structures of (*R*)-5-vinyl and (*R*)-3-demethyl-5-vinylthiolactomycin derivatives (**3a**–**f** and **4a**–**f**) and C75.

We wish to report here a concise synthesis of **3**, *ent*-**3**, **4**, and *ent*-**4** and their in vitro antibacterial and mammalian type I FAS inhibitory activity as well as that for **1**, *ent*-**1**, **2**, and *ent*-**2** previously reported by us.[10] These studies clearly disclosed novel aspects of the structure–activity relationships of TLM congeners and led us to explore (*S*)-3-demethyl-5-(pent-1-enyl)thiolactomycin derivative (*ent*-**4d**) which exhibits type I FAS inhibitory activity equal to that of C75, the potent inhibitor so far reported,[7,11] with complete loss of in vitro antibacterial activity.

Following the synthetic scheme previously established,[10] we commenced the synthesis of (*R*)-5-vinyl TLM and (*R*)-3-demethyl-5-vinyl TLM (**3** and **4**) (Scheme 1). α,β-Unsaturated carboxylic acids **5a**–**f** were allowed to react with pivaloyl chloride followed by treatment of the resulting mixed anhydrides with (*S*)-4-benzyloxazolidin-2-one, giving rise to (*S*)-*N*-acyloxazolidin-2-ones **6a**–**f**. Among **5a**–**f**, while **5a**–**c** were commercially available, **5d**–**f** were prepared from the corresponding aldehydes by sequential Horner–Wadsworth–Emmons reaction and alkaline hydrolysis in a similar manner to that reported.[10] Asymmetric deconjugative α-sulfenylation of **6a**–**f** with 3,3-dimethoxypropyl methanethiosulfonate took place highly diastereoselectively (>80%) using KHMDS as a base, affording α-sulfenylated products **7a**–**f**.[12,13] In the synthesis previously reported,[10] asymmetric deconjugative α-sulfenylation of the *N*-acyloxazolidin-2-one was effected using NaHMDS in the presence sof HMPA. However, when **6a**–**f** were treated under the same conditions as those employed before,[10] the reaction was found to occur in low yield along with low regio- and diastereo-selectivity. After some experimentation, it was found that the use of KHMDS in the absence of HMPA was fairly effective, cleanly affording **7a**–**f**.[12,13]

The α-sulfenylated products **7a**–**f** were transformed into benzyl esters **8a**–**f** by imide-ester exchange using titanium benzyloxide. Acidic hydrolysis of the dimethyl acetal moieties in **8a**–**f** gave rise to aldehydes **9a**–**f**. Retro-Michael reaction of **9a**–**f** using Cs_2CO_3 as a base followed by treatment of the resulting cesium thiolates with propionyl or acetyl chloride provided α-acylthio esters **10a**–**f** or **11a**–**f**. They were subjected to Dieckmann condensation using LiHMDS as a base, furnishing (*R*)-5-vinyl and (*R*)-3-demethyl-5-vinyl TLM derivatives **3a**–**f** and **4a**–**f**.[14,15] By the completely same synthetic scheme as delineated above, the enantiomers of **3a**–**f** and **4a**–**f** (*ent*-**3a**–**f**, and *ent*-**4a**–**f**) were synthesized from *ent*-**6a**–**f** prepared from **5a**–**f** and (*R*)-4-benzyloxazolidin-2-one.[13–15]

With completion of the synthesis of **3a**–**f**, *ent*-**3a**–**f**, **4a**–**f**, and *ent*-**4a**–**f**, their in vitro antibacterial activity against various strains of bacteria[16] and inhibitory activity against mammalian type I FAS[17] were evaluated along with those for **1**, *ent*-**1**, **2**, and *ent*-**2**, the optically pure samples of which had been obtained by our previous synthetic studies.[10] While in vitro antibacterial activity and inhibitory activity against type I and type II FAS



Scheme 1. Reagent and condition: (a) *t*-BuCOCl, Et_3N, THF, −15 °C, then LiCl, (*S*)4-benzyl-2-oxazolidinone, rt 83% for **6a**, 96% for **6b**, 90% for **6c**, 87% for **6d**, 81% for **6e**, 85% for **6f**; (b) KHMDS, −78 °C, then 3,3-dimethoxypropyl methanethiosulfonate, −78 to 0 °C, 18% for **7a**, 56% for **7b**, 60% for **7c**, 50% for **7d**, 80% for **7e**, 59% for **7f**; (c) Ti(O*i*-Pr)$_4$, BnOH, 70 °C, 82% for **8a**, 88% for **8b**, 95% for **8c**, 95% for **8d**, 92% for **8e**, 98% for **8f**; (d) 6% HCl aq THF, rt, 98% for **9a**, 98% for **9b**, 98% for **9c**, 93% for **9d**, 100% for **9e**, 88% for **9f**; (e) Cs_2CO_3, EtOH, 0 °C, then CH_3CH_2COCl or CH_3COCl, Et_3N, CH_2Cl_2, 0 °C, 81% for **10a**, 74% for **10b**, 50% for **10c**, 61% for **10d**, 76% for **10e**, 90% for **10f**, 69% for **11a**, 68% for **11b**, 52% for **11e**, 73% for **11d**, 37% for **11e**, 83% for **11f**; (f) LiHMDS, THF, −78 to 0 °C, 70% for **3a**, 74% for **3b**, 65% for **3c**, 64% for **3d**, 39% for **3e**, 26% for **3f**, 86% for **4a**, 89% for **4b**, 89% for **4c**, 77% for **4d**, 89% for **4e**, 89% for **4f**.

were reported for **1**,[2–8] there has been no report describing biological activity of *ent*-**1**. As shown in Table 1, very interesting features were disclosed when **1**, *ent*-**1**, **2**, and *ent*-**2** were subjected to the biological activity assay mentioned above. Thus, although it has been well known that **1** shows moderate in vitro antibacterial activity,[2] 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** carrying 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 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, it becomes evident that *ent*-**2** and its congeners may be usable as a selective mammalian type I FAS inhibitor.

Next, 5-vinyl and 3-demethyl-5-vinyl TLM congeners and their enantiomers (**3a**–**f**, *ent*-**3a**–**f**, **4a**–**f**, and *ent*-**4a**–**f**) were subjected to the same biological activity assays as those for **1**, **2** and their enantiomers *ent*-**1** and *ent*-**2**. Being different from the case delineated above, all the tested compounds were found to lack in vitro antibacterial activity against all the tested strains of bacteria even if **3a**–**f** and **4a**–**f** bear the same (*R*)-configuration as **1** and **2**. However, as for mammalian type I FAS inhibitory activity, seven compounds, that is, *ent*-**3d**,**e**, **4e**,**f**, and *ent*-**4c**–**e**, were found to show type I FAS inhibitory activity equal to or more potent than that recorded for *ent*-**2**. Especially, the inhibitory activity of *ent*-**4d** was found to be of almost the same level as that of C75, the potent type I FAS inhibitor so far reported.[7,11] Summing up the results shown in Table 1, it might be concluded that, in general, the (*S*)-enantiomers show more potent type I FAS inhibitory activity than the corresponding (*R*)-enantiomers (see the cases for R^1 = Et and *n*-Pr) and the activity of C_3-demethyl congeners is higher than that of the corresponding C_3-methyl compounds (see the cases for R^1 = Et, *n*-Pr, and *n*-Bu). As for the length of the C_5-vinyl moiety, type I FAS inhibitory activity seems to gradually increase

Table 1. In vitro antibacterial and mammalian type I FAS inhibitory activity of enantiomeric pairs of TLM and its congeners (**1**, *ent*-**1**,**2**, *ent*-**2**, **3a**–**f**, *ent*-**3a**–**f**, **4a**–**f**, and *ent*-**4a**–**f**)

Compound R^1 (for **3** and **4**)		In vitro antibacterial activity, MIC (μg/mL)				Mammalian type I FAS inhibitory activity IC_{50} (μg/mL)
		S. aureus Smith	*M. catarrhalis* ATCC 25238	*H. influenzae* IID983	*B. fragilis* GAI 5560	HepG2 ^{14}C
	TLM(**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
H	**3a**	>128	>128	>128	>128	>80
	ent-**3a**	>128	>128	>128	>128	>80
	4a	>128	>128	>128	>128	>80
	ent-**4a**	>128	>128	>128	>128	70.1
Me	**3b**	>128	>128	>128	>128	72.3
	ent-**3b**	>128	>128	>128	>128	37.1
	4b	>128	>128	>128	>128	72.1
	ent-**4b**	>128	>128	>128	>128	>80
Et	**3c**	>128	>128	>128	>128	57.5
	ent-**3c**	>128	>128	>128	>128	47.0
	4c	>128	>128	>128	>128	40.3
	ent-**4c**	>128	>128	>128	>128	18.9
n-Pr	**3d**	>128	>128	>128	>128	41.5
	ent-**3d**	>128	>128	>128	>128	20.0
	4d	>128	>128	>128	>128	41.6
	ent-**4d**	>128	>128	>128	>128	8.8
n-Bu	**3e**	>128	>128	>128	>128	33.7
	ent-**3e**	>128	>128	>128	>128	19.9
	4e	>128	>128	>128	>128	18.6
	ent-**4e**	>128	>128	>128	>128	21.0
n-Hex	**3f**	>128	>128	>128	>128	57.0
	ent-**3f**	>128	>128	>128	>128	34.8
	4f	>128	>128	>128	>128	20.1
	ent-**4f**	>128	>128	>128	>128	38.7
	CPFX[b]	0.063	0.031	0.008	4	N.T.[a]
	C75[c]	N.T.[a]	N.T.[a]	N.T.[a]	N.T.[a]	7.4

[a] N.T., not tested.
[b] Ciprofloxacin.
[c] See Figure 2.

until the number of carbon atoms reaches five and to slightly decrease when it exceeds five. This tendency was most apparent for *ent*-**4a**–**f**. In addition, it was found that, as the C_5-vinyl side chain becomes longer, the absolute configuration renders less effect on type I FAS inhibitory activity (see the cases for R^1 = *n*-Pr, *n*-Bu, and *n*-Hex).

In conclusion, we have succeeded in synthesizing twelve enantiomeric pairs of 5-vinyl TLM congeners (**3a**–**f**, *ent*-**3a**–**f**, **4a**–**f**, and *ent*-**4a**–**f**) using our efficient synthetic route previously explored for the total synthesis of TLM (**1**), 3-demethyl TLM (**2**), and their enantiomers (*ent*-**1** and *ent*-**2**).[10] Among them, (*S*)-3-demethyl-5-(pent-1-enyl) TLM [(*S*)-4-hydroxy-5-methyl-5-(pent-1-enyl)-5H-thiophen-2-one] (*ent*-**4d**) was found to exhibit inhibitory activity against mammalian type I FAS equal to that of C75, the potent inhibitor so far reported.[7,11] From the studies on biological activity carried out using optically active TLM and its congeners, it appeared evident that in vitro antibacterial and mammalian type I FAS inhibitory activity can be cleanly separated by changing not only the structure but also the absolute configuration of the side chain at the C_5-position of **1** and its congeners. Studies aiming at further exploring the characteristic features of biological activity for TLM are in progress.

Acknowledgments

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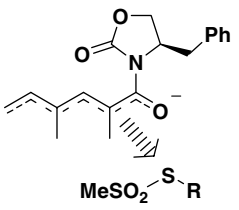
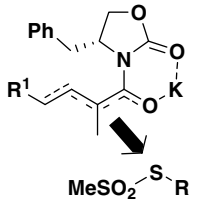
12. Being different from the previous case in which (*R*)-*N*-acyloxazolidin-2-one derived from (*R*)-4-benzyloxazolidin-2-one provided (*R*)-α-sulfenylated product by way of the non-chelated (*E*)-trienolate (**I**),[10] asymmetric deconjugative α-sulfenylation of (*S*)-*N*-acyloxazolidin-2-one **6a**–**f** prepared from (*S*)-4-benzyloxazolidin-2-one gave (*R*)-sulfenylated products **7a**–**f**. The latter reaction carried out in the absence of HMPA was anticipated to proceed through the chelated (*E*)-dienolate (**II**) due to the absence of HMPA.



I



II

13. The absolute stereochemistry of **7a**–**f** was rigorously assigned to have (*R*)-configuration by preliminary synthesis of known (*S*)-**3b**[9b] from *ent*-**7b** as well as the ^{1}H NMR spectra of **7a** and **7c**–**f** similar to that of **7b**. Based on these preliminary results, (*S*)-4-benzyloxazolidin-2-one was employed as a chiral auxiliary for preparing **3** and **4** instead of its (*R*)-enantiomer.

14. Physical data of **3a**–**f**, **4a**–**f**, and *ent*-**4d** are as follows. **3a**: colorless solid, mp 109–118 °C, $[\alpha]_D^{25}$ +42.1° (*c* 0.31, MeOH) (>90%ee[15]); **3b**: colorless solid, mp 128–131 °C, $[\alpha]_D^{25}$ +55.1° (*c* 0.31, MeOH) (>99%ee[15]); **3c**: colorless solid, mp 103.5–105 °C, $[\alpha]_D^{23}$ +52.1° (*c* 0.30, MeOH) (>90%ee[15]); **3d**: colorless solid, mp 54–55 °C, $[\alpha]_D^{25}$ +48.0° (*c* 0.30, MeOH) (>90%ee[15]); **3e**: colorless oil, $[\alpha]_D^{20}$ +69.1° (*c* 0.89, MeOH) (91%ee[15]); **3f**: colorless oil, $[\alpha]_D^{21}$ +57.2° (*c* 1.06, MeOH) (94%ee[15]); **4a**: colorless solid, mp 73–75 °C, $[\alpha]_D^{25}$ +23.3° (*c* 0.31, MeOH) (>90%ee[15]); **4b**: colorless solid, mp 97–98.5 °C, $[\alpha]_D^{25}$ +10.1° (*c* 0.30, MeOH) (>99%ee[15]); **4c**: colorless solid, mp 102–104 °C, $[\alpha]_D^{25}$ +23.6° (*c* 0.30, MeOH) (>99%ee[15]); **4d**: colorless solid, mp 90–92 °C, $[\alpha]_D^{23}$ +112° (*c* 0.30, $CHCl_3$) (>99%ee[15]); *ent*–**4d**: colorless solid, mp 90–92.5 °C, $[\alpha]_D^{24}$ −102° (*c* 0.30, $CHCl_3$) (>99%ee[15]); **4e**: colorless oil, $[\alpha]_D^{22}$ +85.7° (*c* 0.85, $CHCl_3$) (91%ee[15]); **4f**: colorless oil, $[\alpha]_D^{22}$ +78.7° (*c* 0.84, $CHCl_3$) (91%ee[15]).

15. Optical purities of **3b**,**e**,**f**, *ent*-**3b**,**e**,**f**, **4b**–**f**, and *ent*-**4b**–**f** were determined by HPLC analysis with a chiral column. Some representative data are as follows. Daicel Chiralpak AS ϕ 0.46 cm × 25 cm, hexane/*i*-PrOH/TFA = 90:10:0.1, flow rate 1.0 mL/min, t_R 10.5 min (**3b**), 14.4 min (*ent*-**3b**). Daicel Chiralpak AS–H ϕ 0.46 cm × 25 cm, hexane/*i*PrOH/TFA = 95:5:0.1, flow rate 0.45 mL/min, t_R 12.1 min (**3f**), 16.1 min (*ent*-**3f**). Daicel Chiralpak IA ϕ 0.46 cm × 25 cm, hexane/*i*-PrOH/TFA = 98:2:0.1, flow rate 0.5 mL/min, t_R 19.6 min (**4d**), 21.2 min (*ent*-**4d**). Daicel Chiralpak OD–H ϕ 0.46 cm × 25 cm, hexane/*i*-PrOH/TFA = 98:2:0.1, flow rate 0.35 mL/min, t_R 21.3 min (**4f**), 24.5 min (*ent*-**4f**). Optical purities of **3a**,**c**,**d**, *ent*-**3a**,**c**,**d**, **4a**, and *ent*-**4a** which could not be determined by HPLC analysis were calculated based on the diastereomeric excess of the corresponding **7a**,**c**,**d**, and *ent*-**7a**,**c**,**d** determined by their ^{1}H NMR spectra, **7a** >90%de; *ent*-**7a** >99%de; **7c** >90%de; *ent*-**7c** >99%de; **7d** >95%de; and *ent*-**7d** >95%de.

16. Determination of MICs by agar dilution methods was performed according to the guideline M7-A6 of the Clinical and Laboratory Standards Institute (2003).

17. Fatty acid synthesis was evaluated measuring incorporation of [1-^{14}C]acetate into cellular fatty acid as previously described with some modifications. See: Murakami, K.; Tobe, K.; Ide, T.; Mochizuki, T.; Ohashi, M.; Akanuma, Y.; Yazaki, Y.; Kadowaki, T. *Diabetes* **1998**, *47*, 1841.