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ORIGINAL ARTICLE

Synthesis and antibacterial activity of novel lincomycin derivatives. IV. Optimization of an *N*-6 substituent

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The design and synthesis of lincomycin derivatives modified at the C-6 and C-7 positions are described. A substituent at the C-7 position is a 5-aryl-1,3,4-thiadiazol-2-yl-thio group that generates antibacterial activities against macrolide-resistant *Streptococcus pneumoniae* and *Streptococcus pyogenes* carrying an *erm* gene. An additional modification at the C-6 position was explored in application of information regarding pirlimycin and other related compounds. These dual modifications were accomplished by using methyl α-thiolincosaminide as a starting material. As a result of these dual modifications, the antibacterial activities were improved compared with those of compounds with a single modification at the C-7 position. The antibacterial activities of selected compounds in this report against macrolide-resistant *S. pneumoniae* and *S. pyogenes* with an *erm* gene were superior to those of telithromycin.
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INTRODUCTION

Macrolide antibiotics have had a significant role in treatment of respiratory infections. Clarithromycin[1] and azithromycin[2] synthesized from 14-membered erythromycin are clinically important macrolides and widely used over many years (Figure 1). However, prevalence of macrolide resistance in Gram-positive bacteria such as *S. pneumoniae* has been causing serious clinical problems.[3,4] Clarithromycin and azithromycin are inactive against resistant strains of *S. pneumoniae*, which have a mechanism of ribosome methylation by an *erm* gene and decreased antibacterial activities against resistant strains with a *mef* gene, which expresses the efflux pump. Although telithromycin (TEL)[5] derived from natural erythromycin has improved antibacterial activities against resistant *S. pneumoniae* with an *erm* gene and/or a *mef* gene, safety problems[6] of TEL made its clinical use difficult. Another promising example is a group of 16-membered azalides[7,8] that are effective against resistant *S. pneumoniae* with an *erm* gene. However, these compounds are still in the research phase and have not entered development. Novel therapeutic agents that are effective against the resistant *S. pneumoniae* without concerns of adverse events are required in clinical sites.

Lincomycin (LCM)[9] (Figure 2) is one of secondary metabolites of *Streptomyces lincolnensis* and has been used as antibacterial agent mainly against Gram-positive bacteria. Chemical modification of LCM led to clindamycin (CLDM),[10] which has an enhanced antibacterial activity against *S. pneumoniae* and an improved pharmacokinetic profile. Although LCM and CLDM are distinct from macrolide antibiotics in their chemical structures, they have cross-resistance against *S. pneumoniae* with an *erm* gene.[11] The cross-resistance is consistent with the result of X-ray crystallographic studies that revealed the overlap of binding site on 50S ribosome. Thus, CLDM shows almost no antibacterial activity against these resistant pathogens as shown in Table 1. CLDM, however, is attractive because of its safety profile and effectiveness against resistant pathogens expressing a drug efflux pump. In addition, CLDM has no gastrointestinal side effect caused by modulating motilin receptor.[12] Furthermore, CLDM has been reported to be effective for invasive group A streptococcal infections caused by *S. pyogenes*.[13] A simple chemical structure of CLDM compared with that of macrolide is attractive from the viewpoint of production cost. Based on these reasons, we have been expanding chemical modifications of lincosamide, in order to generate a novel antibacterial agent that is effective against resistant *S. pneumoniae* and *S. pyogenes* with *erm* and *mef* genes.

It has been reported that modifications at the C-7 position of LCM tend to provide comparable antibacterial activity to that of LCM.[14,15] against susceptible strains. We reported chemical modifications of LCM and clarified that (7*S*)-7-deoxy-7-thiolincomycin derivatives[16–22] exhibited moderate to strong antibacterial activities against *S. pneumoniae* and *S. pyogenes* with an *erm* gene. Compound **1** that has a phenyl-thiadiazol-2-yl-thio moiety to the C-7 position showed response (very weak antibacterial activities) against those resistant pathogens (Table 1). A nitro group at the 2-position and methoxy groups at the 4- and 5-positions in the benzene ring of **1** had a key role for enhancement of the antibacterial activity against *S. pneumoniae* with an *erm* gene.[21,22]

The ring-size modification of a proline moiety linked to the N-6 position of lincosamides was reported by several groups[23–27]. As for the piperidine derivatives, pirlimycin[23] has been used for animal drugs and VIC-105555[24–26] displays enhanced *in vitro* and *in vivo* activities

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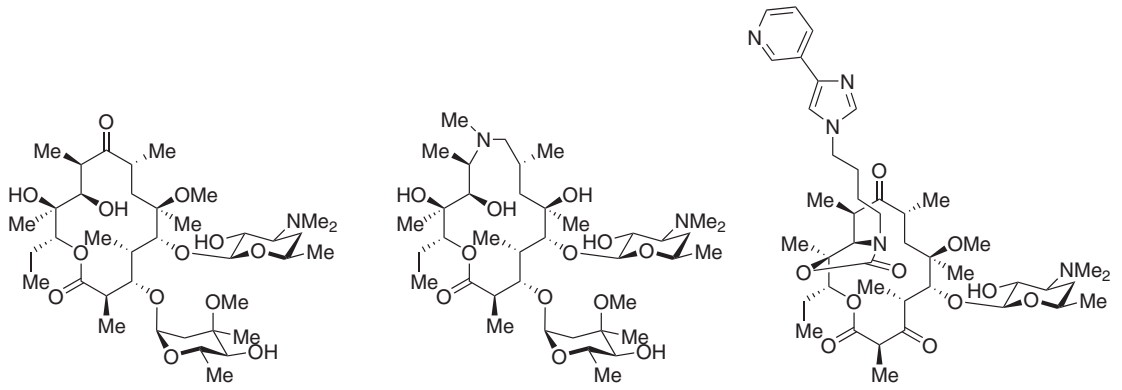
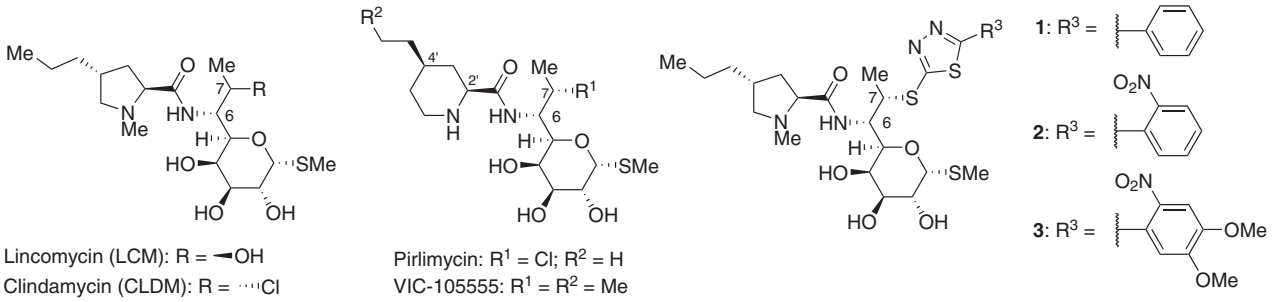


Clarithromycin (CAM) Azithromycin (AZM) Telithromycin (TEL)

Figure 1 Structures of widely used macrolides, clarithromycin and azithromycin, and telithromycin.



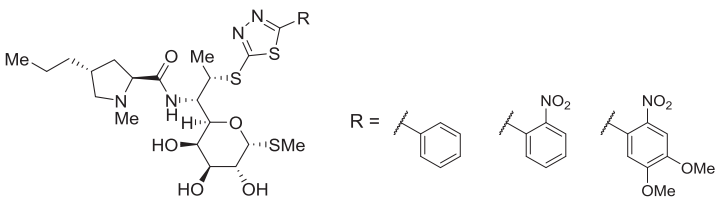
Lincomycin (LCM): R = –OH
Clindamycin (CLDM): R = ···Cl

Pirlimycin: R^1 = Cl; R^2 = H
VIC-105555: R^1 = R^2 = Me

1: R^3 =
2: R^3 =
3: R^3 =

Figure 2 Structures of lincomycin, clindamycin, pirlimycin, VIC-105555, compounds **1**, **2** and **3**.

Table 1 Antibacterial activities of lincomycin derivatives previously reported by our group and known antibiotics (MIC; μg ml^{-1})[a]

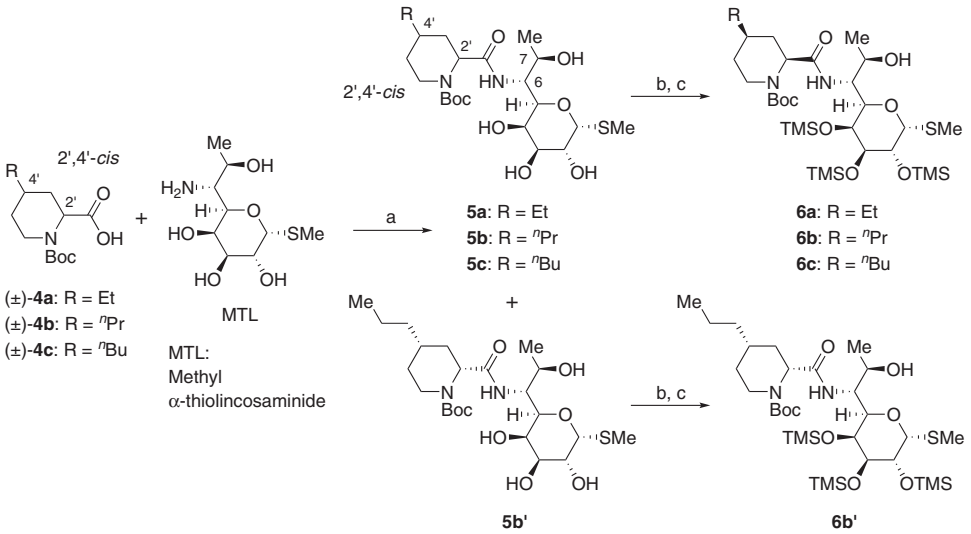


No.	*Test organism*[b]	*Characteristics*	**1**	**2**	**3**	*CLDM*	*CAM*	*TEL*
1	*S. pneumoniae* DP1 TypeI	susceptible	0.13	0.06	0.015	0.13	0.03	≤0.008
2	*S. pneumoniae* #2	susceptible	0.06	0.06	0.015	0.13	0.03	≤0.008
3	*S. pneumoniae* #3	susceptible	0.06	0.13	0.015	0.13	0.015	≤0.008
4	*S. pneumoniae* #4	*ermB methylase* (c)	64	4	0.5	>128	>128	0.5
5	*S. pneumoniae* #5	*ermB methylase* (c)	32	8	0.25	>128	>128	1
6	*S. pneumoniae* #6	*ermB methylase* (c) + *mefE*	128	64	1	>128	>128	1
7	*S. pneumoniae* #7	*ermB methylase* (i)	16	4	0.25	>128	>128	0.06
8	*S. pneumoniae* #8	*ermB methylase* (i)	16	4	0.25	>128	>128	0.06
9	*S. pneumoniae* #9	*mefE efflux*	0.06	0.06	≤0.008	0.13	0.5	0.06
10	*S. pneumoniae* #10	*mefE efflux*	0.06	0.06	0.015	0.13	0.5	0.06
11	*S. pyogenes* Cook	susceptible	0.06	0.06	0.03	0.13	0.015	0.015
12	*S. pyogenes* #2	*ermB methylase* (c)	4	4	0.25	>128	>128	16
13	*S. pyogenes* #3	*mefE efflux*	0.13	0.13	0.015	0.13	8	0.5
14	*H. influenzae* #1	susceptible	16	16	2	8	2	0.5
15	*H. influenzae* #2	susceptible	16	8	2	8	4	2
16	*H. influenzae* #3	susceptible	64	64	2	32	8	1

Abbreviations: c, constitutive; i, inducible; CAM, clarithromycin; CLDM, clindamycin; TEL, telithromycin.

[a]All antibacterial evaluations were performed as hydrochloride. Gray shading strains are target strains.

[b]All strains except standard organisms were clinically isolated.



Scheme 1 Synthesis of key intermediates having a 4-substituted piperidine 2-carbonyl group. Conditions: (**a**) DCC, HOBt, DMF, rt; (**b**) TMSCl, HMDS, pyridine, rt; (**c**) 6N AcOH, MeOH, rt.

with superior efficacy over clindamycin in several experimental models (Figure 2). Although these compounds did not show antibacterial activities against resistant Gram-positive bacteria with an *erm* gene, *in vitro* and/or *in vivo* properties of (7*S*)-7-deoxy-7-thiolincomycin derivatives are expected to be improved by the modification of the proline moiety. In this article, we report optimization of the proline moiety of (7*S*)-7-deoxy-7-(5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio)lincomycin derivatives, in order to generate novel LCM derivatives exhibiting stronger antibacterial activities than TEL.

Chemistry

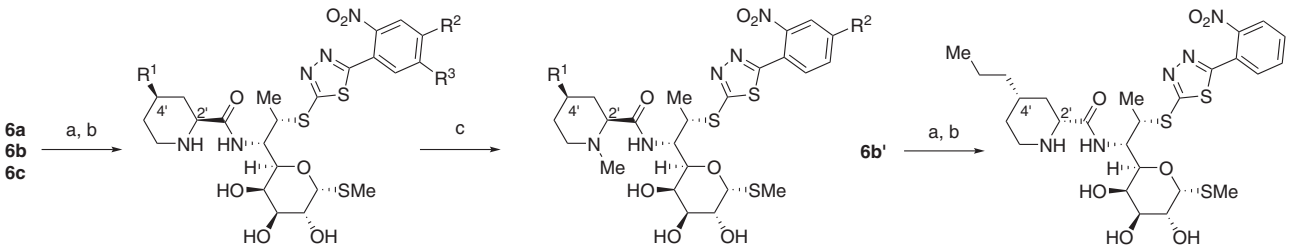
Synthesis of important intermediates that have 4′-substituted piperidine-2′-carbonyl group instead of a natural 4′-propyl proline part is shown in Scheme 1. A racemic mixture of 2,4-*cis*-piperidinecarboxylic acids was prepared by a reported procedure[28] from 4-alkylpyridine. The protection of an amine gave carboxylic acids (±)-**4a**, (±)-**4b** and (±)-**4c**. Condensation of these carboxylic acids with methyl α-thiolincosaminide[29] gave 1′-*N*-Boc protected compounds **5a**, **5b** and **5c** as a 1:1 diastereomeric mixture. These mixtures showed two spots on TLC, but complete separation by silica gel chromatography was difficult. After exploring a solvent for selective precipitation, we found that less polar isomer was precipitated from ethyl acetate and more polar isomer was enriched in mother liquor. Birkenmeyer *et al.*[23] reported that more active 2′-β-4′-β-*cis* isomers of pirlimycin derivatives showed more polar profile on TLC. Based on the report, the more polar isomer of **5b** was assigned as a 2′-β-4′-β-*cis* isomer. The 2′-β-4′-β-*cis* isomers of **5b** were converted to tetra-*O*-trimethylsilyl intermediate, which was followed by regioselective deprotection of the 7-*O*-trimethylsilyl group and silica gel column chromatography, afforded **6b**. The other 2′-α-4′-α-*cis* isomer **6b**′ was prepared from less polar isomer (**5b**′) as well. Regarding 4′-ethyl and 4′-*n*-butyl derivatives, 2′-β-4′-β-*cis* isomers **6a** and **6c** were obtained in the similar manner to **6b**.

Synthesis of (7*S*)-7-deoxy-7-(5-phenyl-1,3,4-thiadiazol-2-yl-thio)-LCM derivatives modified at the C-6 position is shown in Scheme 2. Introductions of the 5-substituted 1,3,4-thiadiazol-2-yl-thio group at the C-7 position were achieved by Mitsunobu reaction and subsequent deprotection led to target compounds **7a–7k**. In order to obtain information on the relationship between stereochemistry in the piperidine moiety and antibacterial activity, 2′-α-4′-α-*cis* isomer **7b**′ was also synthesized in the similar manner to **7a–7k** from **6b**′. Compound **7b** was more polar than **7b**′ on TLC as in the case of **6b** and **6b**′. A major reason for relatively low yield of these Mitsunobu reactions was explained by generation of an *N*-connected byproduct in the thiadiazole moiety instead of the desired *S*-connected derivative. 1′-Methylated compounds **8d**, **8e** and **8f** were prepared by reductive *N*-methylation of the corresponding secondary amine. Synthesis of VIC-105555 and **10** were conducted based on the reported procedure[24] as shown in Scheme 3. The reduction of **9** gave a diastereomeric mixture of VIC-105555 and **10**. We separated these isomers and a more polar isomer was assigned as VIC-105555.

RESULTS AND DISCUSSION

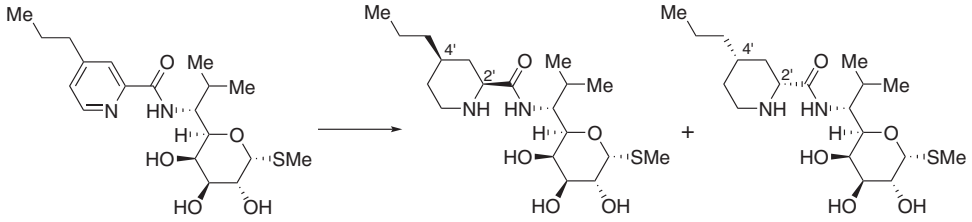
Antibacterial activities of LCM derivatives that have the 5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio group at the C-7 position and the 4′-alkyl piperidin-2′-carbonylamino group at the C-6 position are shown in Table 2. Regarding stereoisomers **7b** and **7b**′, which are 4′-propyl derivatives, antibacterial activities of **7b** were clearly more potent than those of **7b**′ as expected. According to the result, the assignment of stereochemistry in the piperidine moiety was also supported by antibacterial activity. Similarly, VIC-105555 showed reported antibacterial activities against *S. pneumoniae* and *S. pyogenes* without an *erm* gene and antibacterial activities of stereoisomer **10** was much weaker than those of VIC-105555. Compared with a natural proline analog **2**, transformation to 4′-*n*-propylpiperidine-2′-carbonyl analog remarkably improved the antibacterial activities against resistant *S. pneumoniae* and *S. pyogenes* with an *erm* gene. 4′-Ethyl analog (**7a**) showed improved antibacterial activities against susceptible strains of *S. pneumoniae* and *S. pyogenes* compared to **2**. However, its antibacterial activities against resistant *S. pneumoniae* with an *erm* gene were weaker than those of **7b**. In contrast, 4′-*n*-butyl analog (**7c**) exhibited more potent antibacterial activities than **7b** against all of the test organisms. Antibacterial activities of these derivatives were significantly superior to TEL against *S. pyogenes* with an *erm* gene.

Table 3 shows antibacterial activities of 4-fluoro-2-nitrophenyl analogs. The antibacterial activities of **7d**, **7e** and **7f** indicated that the effect of a fluorine atom on antibacterial activity against *S. pneumoniae* and *S. pyogenes* was unclear. However, antibacterial



7a: R^1 = Et, R^2 = R^3 = H
7b: R^1 = ^{n}Pr, R^2 = R^3 = H
7c: R^1 = ^{n}Bu, R^2 = R^3 = H
7d: R^1 = Et, R^2 = F, R^3 = H
7e: R^1 = ^{n}Pr, R^2 = F, R^3 = H
7f: R^1 = ^{n}Bu, R^2 = F, R^3 = H
7g: R^1 = ^{n}Pr, R^2 = H, R^3 = NHMe
7h: R^1 = ^{n}Pr, R^2 = H, R^3 = OMe
7i: R^1 = Et, R^2 = R^3 = OMe
7j: R^1 = ^{n}Pr, R^2 = R^3 = OMe
7k: R^1 = ^{n}Bu, R^2 = R^3 = OMe
8d: R^1 = Et, R^2 = F
8e: R^1 = ^{n}Pr, R^2 = F
8f: R^1 = ^{n}Bu, R^2 = F

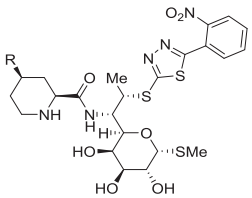
Scheme 2 Synthesis of (7*S*)-7-deoxy-7-(5-phenyl-1,3,4-thiadiazol-2-yl-thio)-LCM derivatives having a 4-substituted piperidine 2-carbonyl group. Conditions: (**a**) ArSH, DEAD, PPh_3, THF or toluene, 0 °C to rt; (**b**) TFA, DCM, −20 °C to rt; (**c**) 36% HCHO, $NaBH(OAc)_3$, AcOH, MeOH or EtOH, rt.



9 VIC-105555 **10**

Scheme 3 Synthesis of VIC-105555 and its 2′-α-4′-α-*cis* isomer **10**. Condition: H_2, PtO_2, conc. HCl, MeOH-H_2O, rt.

Table 2 Antibacterial activities of novel lincomycin derivatives (MIC; μg ml^{-1})[a]



R = Et ^{n}Pr ^{n}Bu

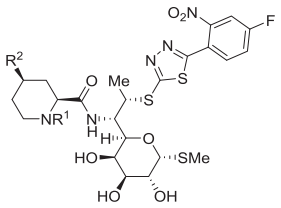
No.	Test organism[b]	Characteristics	2	7a	7b	7b'	7c	*VIC-105555*	10
1	*S. pneumoniae* DP1 TypeI	susceptible	0.06	≤0.008	0.03	8	0.015	≤0.008	2
2	*S. pneumoniae* #2	susceptible	0.06	≤0.008	0.03	8	0.015	0.015	4
3	*S. pneumoniae* #3	susceptible	0.13	≤0.008	0.015	4	0.015	0.03	4
4	*S. pneumoniae* #4	*ermB methylase* (c)	4	4	0.5	>128	0.25	128	>128
5	*S. pneumoniae* #5	*ermB methylase* (c)	8	4	0.5	>128	0.25	128	>128
6	*S. pneumoniae* #6	*ermB methylase* (c) + *mefE*	64	4	1	>128	0.25	128	>128
7	*S. pneumoniae* #7	*ermB methylase* (i)	4	0.5	0.25	128	ND	32	>128
8	*S. pneumoniae* #8	*ermB methylase* (i)	4	0.5	0.25	128	ND	32	>128
9	*S. pneumoniae* #9	*mefE efflux*	0.06	≤0.008	0.015	4	ND	0.015	4
10	*S. pneumoniae* #10	*mefE efflux*	0.06	≤0.008	0.03	8	0.015	0.015	4
11	*S. pyogenes* Cook	susceptible	0.06	≤0.008	0.015	4	0.03	0.015	4
12	*S. pyogenes* #2	*ermB methylase* (c)	4	0.06	0.5	128	0.13	64	>128
13	*S. pyogenes* #3	*mefE efflux*	0.13	0.015	0.03	8	0.03	0.015	4
14	*H. influenzae* #1	susceptible	16	32	8	>128	4	8	>128
15	*H. influenzae* #2	susceptible	8	32	8	>128	4	16	>128
16	*H. influenzae* #3	susceptible	64	128	16	>128	8	64	>128

Abbreviations: c, constitutive; i, inducible.

[a]All antibacterial evaluations were performed as hydrochloride. Gray shading strains are target strains.

[b]All strains except standard organisms were clinically isolated.

Table 3 Antibacterial activities of novel lincomycin derivatives (MIC; μg ml^{-1})[a]



	R^1 =	H	Me	H	Me	H	Me
	R^2 =	Et	Et	^{n}Pr	^{n}Pr	^{n}Bu	^{n}Bu

No.	*Test organism*[b]	*Characteristics*	**7d**	**8d**	**7e**	**8e**	**7f**	**8f**	*TEL*
1	*S. pneumoniae* DP1 TypeI	susceptible	≤0.008	0.015	0.03	0.06	0.015	0.03	≤0.008
2	*S. pneumoniae* #2	susceptible	≤0.008	≤0.008	0.03	0.06	0.015	0.03	≤0.008
3	*S. pneumoniae* #3	susceptible	≤0.008	≤0.008	0.03	0.06	0.03	0.06	≤0.008
4	*S. pneumoniae* #4	*ermB methylase* (c)	1	>128	0.5	4	0.13	1	0.5
5	*S. pneumoniae* #5	*ermB methylase* (c)	2	64	1	4	0.25	1	1
6	*S. pneumoniae* #6	*ermB methylase* (c) + *mefE*	4	>128	1	4	0.25	2	1
7	*S. pneumoniae* #7	*ermB methylase* (i)	1	8	0.25	ND	0.06	0.25	0.06
8	*S. pneumoniae* #8	*ermB methylase* (i)	1	4	0.25	ND	0.06	0.25	0.06
9	*S. pneumoniae* #9	*mefE efflux*	≤0.008	0.015	≤0.008	ND	≤0.008	0.03	0.06
10	*S. pneumoniae* #10	*mefE efflux*	≤0.008	0.015	0.015	0.06	0.015	0.03	0.06
11	*S. pyogenes* Cook	susceptible	≤0.008	0.015	0.03	0.13	0.015	0.03	0.015
12	*S. pyogenes* #2	*ermB methylase* (c)	0.25	1	0.5	2	0.13	0.5	16
13	*S. pyogenes* #3	*mefE efflux*	0.015	0.03	0.03	0.13	0.03	0.03	0.5
14	*H. influenzae* #1	susceptible	8	64	4	16	2	4	0.5
15	*H. influenzae* #2	susceptible	8	32	2	8	1	2	2
16	*H. influenzae* #3	susceptible	32	128	4	16	2	4	1

Abbreviations: c, constitutive; i, inducible; TEL, telithromycin.

[a]All antibacterial evaluations were performed as hydrochloride. Gray shading strains are target strains.

[b]All strains except standard organisms were clinically isolated.

activities of **7f** against *S. pneumoniae* with an *erm* gene were generally more potent than those of TEL as well as **7c**. On the other hand, **7d**, **7e** and **7f** exhibited enhanced antibacterial activities against *Haemophilus influenzae* compared with the corresponding compounds **7a**, **7b** and **7c** without a fluorine atom. Compounds **8d**, **8e** and **8f**, which have a methyl group at the 1′-position showed weaker antibacterial activities against resistant *S. pneumoniae* and *S. pyogenes* with an *erm* gene than corresponding 1′-demethyl compounds, respectively.

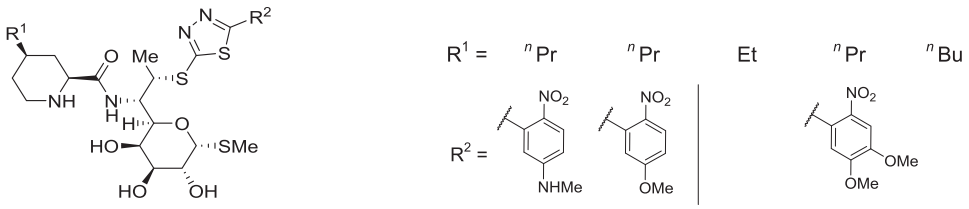
Antibacterial activities of 2-nitrophenyl derivatives that have substituent(s) in the benzene ring are shown in Table 4. We previously reported[22] that the introduction of a substituent such as a methylamino group and a methoxy group to the 5-position of the benzene ring of LCM analog possessing a natural 4′-propyproline moiety dramatically improved the antibacterial activities and their antibacterial activities were further enhanced by additional substitution at the 4-position of the benzene ring. According to the antibacterial activities of **7g** and **7h**, ring size expansion from a pyrrolidine ring found in the original LCM to the piperidine ring enhanced antibacterial activities against *S. pneumoniae* and *S. pyogenes* as in the case of the 2-nitrophenyl derivatives and 4-fluoro-2-nitrophenyl derivatives. In addition, the conversion of 4,5-dimethoxy-2-nitrophenyl derivative **3** (Figure 2) to a piperidine analog gave **7j** that exhibited apparently more potent antibacterial activities against resistant *S. pneumoniae* and *S. pyogenes* with an *erm* gene than TEL. Although antibacterial activities of 4′-ethyl derivative (**7i**) against resistant *S. pneumoniae* with an *erm* gene were remarkably weakened, 4′-*n*-butyl derivative (**7k**) showed more potent antibacterial activities against resistant *S. pneumoniae* and *S. pyogenes* with an *erm* gene than TEL. It should be noted that antibacterial activities of **7k** against resistant strains with an *erm* gene are almost same as those against susceptible strains. These results encouraged us to investigate the antibacterial activities of *Mycoplasma pneumoniae*. It is reported that macrolide resistant *M. pneumoniae* has been prevailing in China, Japan and other countries.[30] Compounds tested showed potent antibacterial activities against resistant *M. pneumonia*, except for **7h** (#18).

In summary, we previously reported that **1** possessing the 5-phenyl-1,3,4-thiadiazol-2-yl-thio group at the C-7 position showed weak antibacterial activities against the resistant pathogens. In the course of our continuous chemical derivatization, target antibacterial activities were significantly improved. Compound **3** that has two methoxy groups at the benzene ring exhibited potency comparable to that of TEL. In this article, an additional modification at the C-6 position led to **7k** possessing the 4-*n*-butylpiperidine-2-carbonyl group instead of the 4-*n*-propylpyrrolidin-2-carbonyl group at the C-6 position. Selected compound **7k** exhibited significantly potent antibacterial activities against the resistant pathogens than TEL. This series of compounds has a nitro group at the benzene ring. Although it is known that a nitro group has a risk for mutagens and carcinogens, there are drugs and drug candidates that have a nitro group.

CONCLUSIONS

A series of LCM derivatives that have the 5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio moiety at the C-7 position in *S*-configuration and the 4-alkylpipelidine-2-carbonylamino group at the C-6 position was synthesized. Introductions of a substituent at the C-7 position were accomplished by the Mitsunobu reaction, and stereochemistry of

Table 4 Antibacterial activities of novel lincomycin derivatives (MIC; μg ml^{-1})[a]



No.	Test organism[b]	Characteristics	7g	7h	7i	7j	7k	TEL
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3	*S. pneumoniae* #3	susceptible	0.06	0.015	0.015	0.06	0.03	≤0.008
4	*S. pneumoniae* #4	*ermB methylase* (c)	0.25	0.13	1	0.06	0.03	0.5
5	*S. pneumoniae* #5	*ermB methylase* (c)	0.5	0.25	1	0.06	0.03	1
6	*S. pneumoniae* #6	*ermB methylase* (c) + *mefE*	0.5	0.25	1	0.13	0.03	1
7	*S. pneumoniae* #7	*ermB methylase* (i)	0.5	0.06	0.25	0.03	0.015	0.06
8	*S. pneumoniae* #8	*ermB methylase* (i)	0.25	0.06	0.25	0.03	0.015	0.06
9	*S. pneumoniae* #9	*mefE efflux*	0.03	≤0.008	0.015	≤0.008	0.015	0.06
10	*S. pneumoniae* #10	*mefE efflux*	0.06	≤0.008	0.015	0.015	0.015	0.06
11	*S. pyogenes* Cook	susceptible	0.13	0.03	0.015	0.03	0.03	0.015
12	*S. pyogenes* #2	*ermB methylase* (c)	0.5	0.13	0.13	0.13	0.06	16
13	*S. pyogenes* #3	*mefE efflux*	0.13	0.03	0.015	0.03	0.06	0.5
14	*H. influenzae* #1	susceptible	4	4	4	2	2	0.5
15	*H. influenzae* #2	susceptible	4	2	4	2	2	2
16	*H. influenzae* #3	susceptible	4	4	16	2	2	1
17	*M. pneumoniae* #1	susceptible	0.03	0.03	0.015	≤0.008	0.015	0.001
18	*M. pneumoniae* #2	A2063G	0.13	1	0.13	≤0.06	≤0.06	32

Abbreviations: c, constitutive; i, inducible; TEL, telithromycin.

[a]All antibacterial evaluations were performed as hydrochloride. Gray shading strains are target strains.

[b]All strains except standard organisms were clinically isolated.

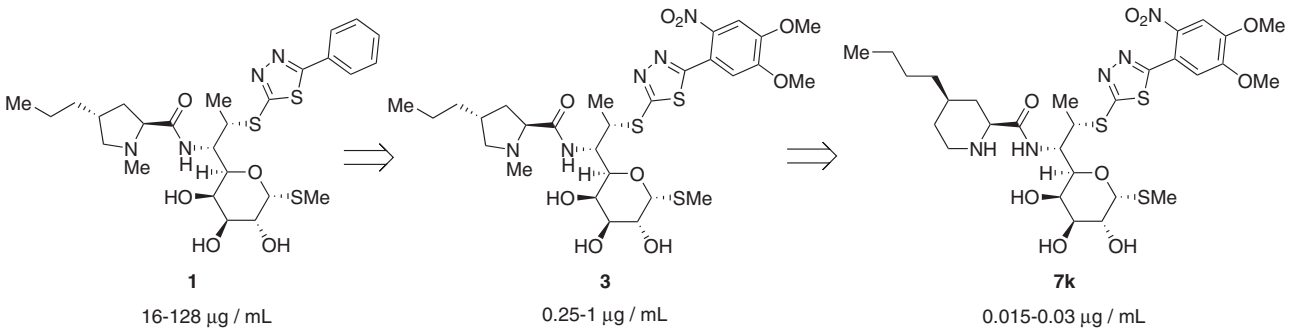

Figure 3 Summary of SAR between potency (MIC against resistant *S. pneumoniae* with an *erm* gene) and (7*S*)-5-aryl-1,3,4-thiadiazol-2-yl-thio-LCM derivatives.

4-alkylpiperidine-2-carbonyl moiety was assigned by the reported information on the relationship between stereochemistry and polarity. Additional modification at the C-6 position improved antibacterial activities of (7*S*)-1,3,4-thiadiazol-2-yl-thio LCM derivatives. In this study, we found the 4-*n*-butylpiperidine-2-carbonylamino group had the most potent functionality at the C-6 position against resistant *S. pneumoniae* and *S. pyogenes* with an *erm* gene. In particular, compound **7k** exhibited the most potent antibacterial activities among all our (7*S*)-1,3,4-thiadiazol-2-yl-thio-LCM derivatives and apparently more potent *in vitro* than TEL against the resistant strains. Summary of SAR between antibacterial activity against resistant *S. pneumoniae* with an *erm* gene and (7*S*)-7-(5-aryl-1,3,4-thiadiazol-2-yl-thio)-LCM derivatives is shown in Figure 3. This series of LCM derivatives is promising to overcome maclolide-resistant *S. pneumoniae* and *S. pyogenes* with an *erm* gene.

EXPERIMENTAL PROCEDURES

General

^{1}H NMR spectra were measured with Varian Gemini-300 (Varian, Inc., Palo Alto, CA, USA) for 300 MHz, JEOL JNM-GSX 400 (JEOL Ltd, Tokyo, Japan)

for 400 MHz or BRUKER Ascend 400 NMR spectrometer (BRUKER Corporation, Coventry, UK) for 400 MHz in $CDCl_3$ or CD_3OD with 0.03% tetramethylsilane as an internal standard. MS spectra were obtained on a JEOL JMS-FABmate spectrometer or JEOL JMS-700 mass spectrometer or Agilent Technologies 6530-Q-TOF LC/MS mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The optical rotations were recorded with Jasco P-2300 digital polarimeter (Jasco, Tokyo, Japan). Column chromatography was performed with silica gel 60 N (Kanto Chemical, Tokyo, Japan, spherical, neutral).

Mixture 5a of methyl 6-*N*-[(2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-ethylpiperidine-2′-carbonyl]-α-thiolincosaminide and methyl 6-*N*-[(2′*R*, 4′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-ethylpiperidine-2′-carbonyl]-α-thiolincosaminide

To a solution of (±)-**4a** (6.66 g, 25.9 mmol) in *N,N*′-dimethylformamide (45 ml) were added 1-hydroxybenzotriazole (3.50 g, 25.9 mmol), *N,N*′-dicyclohexylcarbodiimide (6.05 g, 29.3 mmol), methyl α-thiolincosaminide (7.37 g, 29.1 mmol) and triethylamine (10.0 ml, 71.7 mmol), and stirred at room temperature for overnight. To the mixture was added H_2O and filtrated. Ethyl acetate was added to the filtrate and washed with saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 50/50 to ethyl acetate, then ethyl acetate to ethyl acetate/MeOH = 90/10) to afford methyl 6-*N*-[1′-*N*-(*tert*-butoxycarbonyl)-4′-ethylpiperidine-2′-carbonyl]-α-thiolincosaminide (18.5 g, 84.9%, (2′*S*, 4′*R*) isomer: (2′*R*, 4′*S*) isomer = ca 50:50) as a colorless solid. To this colorless solid was added ethyl acetate, and insoluble matter was filtrated off and ethyl acetate solution was concentrated under reduced pressure to afford mixture **5a** (7.31 g, 60% de ((2′*S*, 4′*R*): (2′*R*, 4′*S*) = 80:20)) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.72 (br s, 0.8H), 6.55 (d, J = 8.6 Hz, 0.2H), 5.30–5.36 (m, 1H), 4.58–4.65 (m, 0.2 H), 4.46 (br s, 0.8H), 4.07–4.26 (m, 5H), 3.95–3.98 (m, 0.8H), 3.90 (br s, 0.2H), 3.47–3.62 (m, 2H), 3.31–3.41 (m, 1H), 2.80–3.03 (m, 1H), 2.55–2.70 (m, 2H), 2.17 (s, 3H), 1.96–2.04 (m, 1H), 1.78–1.88 (m, 1H), 1.61–1.74 (m, 1H), 1.44–1.49 (m, 9H), 1.28–1.38 (m, 4H), 1.20–1.24 (m, 3H), 0.91 (t, J = 7.3 Hz, 3H).

Methyl 6-*N*-[(2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-ethylpiperidine-2′-carbonyl]- 2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (6a)

To a solution of mixture **5a** (7.31 g, 14.8 mmol, 60% de ((2′*S*, 4′*R*):(2′*R*, 4′*S*) = 80:20)) in pyridine (38 ml) were added trimethylchlorosilane (8.0 ml, 6.3 mmol) and hexamethyldisilazane (6.0 ml, 2.8 mmol), and stirred at room temperature for overnight. The mixture was added to saturated aqueous $NaHCO_3$ and was extracted with ethyl acetate, washed with brine. The organic phase was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. To the resulting residue were added methanol (45 ml) and 6 N acetic acid (2.7 ml), and stirred at room temperature for 3 h. The mixture was added to saturated aqueous $NaHCO_3$ and concentrated under reduced pressure. The resulting mixture was extracted with ethyl acetate, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 19/1 to 3/1) to afford **6a** (5.56 g, 30% from (±)-**4a**) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.40 (d, J = 9.2 Hz, 1H), 5.17 (d, J = 5.5 Hz, 1H), 4.30–4.38 (m, 1H), 4.19 (dd, J = 9.0, 6.3 Hz, 1H), 4.12 (dd, J = 9.5, 5.5 Hz, 1H), 4.07 (d, J = 8.3 Hz, 1H), 3.93–4.02 (m, 1H), 3.90–3.93 (m, 1H), 3.62 (dd, J = 9.5, 2.6 Hz, 1H), 3.48–3.59 (m, 1H), 3.31–3.40 (m, 1H), 2.83–2.89 (m, 1H), 2.05 (s, 3H), 1.99–2.03 (m, 1H), 1.78–1.89 (m, 1H), 1.57–1.63 (m, 1H), 1.47 (s, 9H), 1.29–1.39 (m, 2H), 1.20–1.29 (m, 1H), 1.17 (d, J = 6.4 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H), 0.19 (s, 9H), 0.13–0.16 (m, 18H); MS (ESI) *m/z* 709 $(M+H)^+$.

Methyl 6-*N*-[(2′*R*, 4′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-propyl) piperidine-2′-carbonyl] -α-thiolincosaminide (5b′) and mixture 5b of methyl 6-*N*-[(2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-propyl) piperidine-2′-carbonyl]-α-thiolincosaminide and methyl 6-*N*-[(2′*R*, 4′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-propyl)piperidine-2′-carbonyl]-α-thiolincosaminide

Compound (±)-**4b** (11.7 g, 43.1 mmol), 1-hydroxybenzotriazole (7.55 g, 55.8 mmol), *N,N*′-dicyclohexylcarbodiimide (11.0 g, 53.3 mmol) and methyl α-thiolincosaminide (14.2 g, 56.1 mmol) in *N,N*′-dimethylformamide (100 ml) were treated for 12 h according to the similar procedure as described for the preparation of mixture **5a** to afford methyl 6-*N*-[1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-propyl)piperidine-2′-carbonyl]-α-thiolincosaminide (18.5 g, 84.9%, (2′*S*, 4′*R*) isomer: (2′*R*, 4′*S*) isomer = *ca.* 50:50) as a colorless solid. To this colorless solid was added ethyl acetate and insoluble matter was collected by filtration to afford **5b**′ (3.2 g, 15%) as a colorless solid and ethyl acetate solution was concentrated under reduced pressure to afford mixture **5b** (13.5 g, 62%, 20% de ((2′*S*, 4′*R*): (2′*R*, 4′*S*) = 60:40)) as a colorless solid. **5b**′: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.55 (d, J = 8.6 Hz, 1H), 5.34 (d, J = 5.5 Hz, 1H), 4.62 (br s, 1H), 4.11–4.27 (m, 4H), 3.99 (d, J = 9.4 Hz, 1H), 3.90 (br s, 1H), 3.48–3.57 (m, 2H), 3.32–3.42 (m, 1H), 2.85–2.94 (m, 2H), 2.64 (br s, 1H), 2.16 (s, 3H), 1.94–2.03 (m, 1H), 1.77–1.87 (m, 1H), 1.62–1.69 (m, 1H), 1.50–1.59 (m, 1H), 1.47 (s, 9H), 1.23–1.38 (m, 5H), 1.22 (d, J = 6.4 Hz, 3H), 0.89 (t, J = 6.9 Hz, 3H); MS (ESI) *m/z* 507 $(M+H)^+$. **5b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (br s, 0.6H), 6.54 (d, J = 8.7 Hz, 0.4H), 5.30–5.35 (m, 1H), 4.60 (br s, 0.4H), 4.45 (br s, 0.6H), 3.88–4.27 (m, 6H), 3.47–3.62 (m, 2H), 3.28–3.42 (m, 1H), 2.61–3.06 (m, 3H) 2.16 (m, 3H), 1.92–2.02 (m, 1H), 1.74–1.86 (m, 1H), 1.61–1.67 (m, 1H), 1.49–1.54 (m, 1 H), 1.42–1.49 (m, 9H), 1.25–1.37 (m, 5H), 1.19–1.25 (m, 3H), 0.86–0.91 (m, 3H).

Methyl 6-*N*-[(2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-propyl) piperidine-2′-carbonyl]-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (6b)

Mixture **5b** (13.5 g, 26.6 mmol, 20% de ((2′*S*, 4′*R*): (2′*R*, 4′*S*) = 60:40), trimethylchlorosilane (17.0 ml, 133 mmol) and hexamethyldisilazane (27.9 ml, 133 mmol) in pyridine (50 ml) were treated for 40 min according to the similar procedure as described for the preparation of **6a** and the crude tetrakis-*O*-trimethylsilyl intermediate and 6 N acetic acid (5.8 ml) in MeOH (138 ml) were treated for 2.5 h according to the similar procedure as described for the preparation of **6a** to afford **6b** (9.28 g, 48% in 2 steps from diastereomeric mixture **5b**) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.36 (d, J = 9.4 Hz, 1H), 5.17 (d, J = 5.5 Hz, 1H), 4.30–4.37 (m, 1H), 4.09–4.19 (m, 2H), 4.06 (d, J = 8.3 Hz, 1H), 3.95–4.03 (m, 1H), 3.98–3.92 (m, 1H), 3.61 (dd, J = 9.5, 2.6 Hz, 1H), 3.44–3.55 (m, 1H), 3.3–3.41 (m, 1H), 2.92 (d, J = 6.5 Hz, 1H), 2.06 (s, 3H), 2.00–2.05 (m, 1H), 1.78–1.90 (m, 1H), 1.49–1.64 (m, 2H), 1.42–1.49 (m, 9H), 1.20–1.37 (m, 5H), 1.17 (d, J = 6.4 Hz, 3H), 0.85–0.92 (m, 3H), 0.19–0.20 (m, 9H), 0.13–0.15 (m, 18H); MS (ESI) *m/z* 723 $(M+H)^+$.

Methyl 6-*N*-[(2′*R*, 4′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-propyl) piperidine-2′-carbonyl]-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (6b′)

Compound **5b**′ (1.00 g, 1.97 mmol), trimethylchlorosilane (1.26 ml, 9.87 mmol) and hexamethyldisilazane (2.07 ml, 9.87 mmol) in pyridine (4.0 ml) were treated for 30 min according to the similar procedure as described for the preparation of **6a** and the crude tetrakis-*O*-trimethylsilyl intermediate was treated with 6 N acetic acid (0.42 ml) in MeOH (10 ml) for 1 h according to the similar procedure as described for the preparation of **6a** to afford **6b**′ (926 mg, 65% in 2 steps) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.56 (d, J = 8.9 Hz, 1H), 5.21 (d, J = 5.5 Hz, 1H), 4.24–4.31 (m, 1H), 4.11–4.20 (m, 2H), 4.08 (d, J = 8.1 Hz, 1H), 4.02–4.04 (m, 1H), 3.63 (dd, J = 9.5, 2.6 Hz, 1H), 3.32–3.51 (m, 3H), 2.04 (s, 3H), 1.86–1.93 (m, 1H), 1.70–1.85 (m, 2H), 1.44–1.49 (m, 10H), 1.21–1.38 (m, 5H), 1.15 (d, J = 6.5 Hz, 3H), 0.89 (t, J = 6.9 Hz, 3H), 0.19 (s, 9H), 0.15 (s, 9H), 0.14 (s, 9H); MS (ESI) *m/z* 723 $(M+H)^+$.

Mixture 5c of methyl 6-*N*-[(2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-butyl)piperidine-2′-carbonyl]-α-thiolincosaminide and methyl 6-

N-[(2′*R*, 4′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-butyl)piperidine-2′-carbonyl]-α-thiolincosaminide

Compound (±)-**4c** (12.6 g, 44.2 mmol), 1-hydroxybenzotriazole (7.77 g, 57.5 mmol), *N*,*N*′-dicyclohexylcarbodiimide (11.0 g, 53.3 mmol) and methyl α-thiolincosaminide (14.6 g, 57.5 mmol) in *N*,*N*′-dimethylformamide (120 ml) were treated for 20 h according to the similar procedure as described for the preparation of mixture **5a** to afford methyl 6-*N*-[1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-butyl)piperidine-2′-carbonyl]-α-thiolincosaminide (20.0 g, 87%, (2′*S*, 4′*R*) isomer: (2′*R*, 4′*S*) isomer = ca 50:50) as a colorless solid. To this colorless solid (14.5 g) was added ethyl acetate and insoluble matter was filtrated off and ethyl acetate solution was concentrated under reduced pressure to afford mixture **5c** (8.15 g, 80% de ((2′*S*, 4′*R*): (2′*R*, 4′*S*) = 90:10)) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (br s, 0.9H), 6.58 (d, J = 8.7 Hz, 0.1H), 5.30–5.34 (m, 1H), 4.64 (br s, 0.1H), 4.46 (br s, 0.9H), 3.93–4.25 (m, 6H), 3.51–3.63 (m, 2H), 3.28–3.38 (m, 1H), 2.80–2.98 (m, 2H), 2.14–2.16 (m, 3H), 1.93–2.02 (m, 1H), 1.68–1.86 (m, 3H), 1.54 (br s, 1H), 1.44–1.47 (m, 9H), 1.19–1.35 (m, 7H), 1.19–1.25 (m, 3H), 0.86–0.91 (m, 3H).

Methyl 6-*N*-[(2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-butyl) piperidine-2′-carbonyl]-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (6c)

Mixture **5c** (8.15 g, 15.7 mmol, 80% de ((2′*S*, 4′*R*): (2′*R*, 4′*S*) = 90:10), trimethylchlorosilane (100 ml, 78.3 mmol) and hexamethyldisilazane (16.4 ml, 78.3 mmol) in pyridine (30 ml) were treated for 20 min according to the similar procedure as described for the preparation of **6a** and the crude tetrakis-*O*-trimethylsilyl intermediate and 6 N acetic acid (3.4 ml) in MeOH (88 ml) were treated for 40 min according to the similar procedure as described for the preparation of **6a** to afford **6c** (8.20 g, 71% in 2 steps from diastereomeric mixture **5c**) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.34 (d, J = 9.3 Hz, 1H), 5.17 (d, J = 5.5 Hz, 1H), 4.30–4.36 (m, 1H), 4.09–4.15 (m, 2H), 4.05 (d, J = 8.3 Hz, 1H), 3.95–4.03 (m, 1H), 3.88–3.90 (m, 1H), 3.61 (dd, J = 9.5, 2.6 Hz, 1H), 3.43–3.44 (m, 1H), 3.32–3.44 (m, 1H), 2.96 (d, J = 6.5 Hz, 1H), 2.05 (s, 3H), 2.00–2.04 (m, 1H), 1.79–1.89 (m, 1H), 1.49–1.87 (m, 1H), 1.46 (s, 9H), 1.19–1.33 (m, 7H), 1.16 (d, J = 6.4 Hz, 3H), 0.85–0.92 (m, 3H), 0.17–0.19 (m, 9H), 0.12–0.15 (m, 18H); MS (ESI) *m/z* 737 $(M+H)^+$.

Methyl (7*S*)-7-deoxy-6-*N*-[(2′*S*, 4′*R*)-4′-ethylpiperidine-2′-carbonyl]-7-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-α-thiolincosaminide (7a)

To a solution of **6a** (270 mg, 0.381 mmol) in tetrahydrofuran (5 ml) at 0 °C were added triphenylphosphine (200 mg, 0.762 mmol) and diethlazodicarboxylate (0.150 ml, 0.823 mmol), and stirred at 0 °C for 10 min, and 5-(2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (200 mg, 0.836 mmol) was added and stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure and added methanol (10 ml), 5 N hydrochloric acid (0.1 ml) and stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure. To the resulting residue was added trifluoroacetic acid (3.0 ml) and stirred at room temperature for 30 min. The mixture was concentrated under reduced pressure and the resulting residue was purified by preparative TLC ($CHCl_3$/CH_3OH/28% aq NH_4OH = 20/1/0.1) to afford **7a** (52.5 mg, 22%) as colorless solid. $[\alpha]_D^{25}$ +100° (*c* 1.3, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.10 (d, J = 7.6 Hz, 1H), 7.76–7.86 (m, 3H), 5.28 (d, J = 5.6 Hz, 1H), 4.67 (dd, J = 10.1, 2.5 Hz, 1H), 4.46 (qd, J = 6.8, 2.5 Hz, 1H), 4.38–4.43 (m, 1H), 4.07–4.12 (m, 1H), 3.87–3.89 (m, 1H), 3.56 (dd, J = 10.4, 3.3 Hz, 1H), 3.26–3.31 (m, 1H), 3.13–3.20 (m, 1H), 2.56–2.67 (m, 1H), 1.99 (s, 3H), 1.95–1.98 (m, 1H), 1.68–1.75 (m, 1H), 1.56 (d, J = 6.8 Hz, 3H), 1.36–1.50 (m, 1H), 1.25–1.34 (m, 2H), 0.98–1.11 (m, 2H), 0.89–0.95 (m, 3H); MS (FAB) *m/z* 614 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{25}H_{36}N_5O_7S_3$ 614.1771, found 614.1768 $(M+H)^+$.

Methyl (7*S*)-7-deoxy-7-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-6-*N*-[(2′*S*, 4′*R*)-4′-(*n*-propyl)piperidine-2′-carbonyl]-α-thiolincosaminide (7b)

Reaction of **6b** (200 mg, 0.277 mmol) with 5-(2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (114 mg, 0.476 mmol) afforded **7b** as a colorless solid in 24% yield by the similar procedure to **7a**. $[\alpha]_D^{26}$ +83° (*c* 0.17, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.09–8.13 (m, 1H), 7.77–7.86 (m, 3H), 5.29 (d, J = 5.6 Hz, 1H), 4.63–4.69 (m, 1H), 4.41–4.47 (m, 1H), 4.43 (d, J = 10.2 Hz, 1H), 4.06–4.12 (m, 1H), 3.87–3.91 (m, 1H), 3.56 (dd, J = 10.2, 3.4 Hz, 1H), 3.25–3.31 (m, 1H), 3.19–3.25 (m, 1H), 3.08–3.18 (m, 1H), 2.58–2.68 (m, 1H), 1.99 (s, 3H), 1.65–1.72 (m, 1H), 1.56 (d, J = 7.1 Hz, 3H), 1.24–1.34 (m, 5H), 0.98–1.11 (m, 2H), 0.85–0.95 (m, 3H); MS (FAB) *m/z* 628 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{38}N_5O_7S_3$ 628.1928, found 628.1926 $(M+H)^+$.

Methyl (7*S*)-7-deoxy-7-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]- 6-*N*-[(2′*R*, 4′*S*)-4′-(*n*-propyl)piperidine-2-carbonyl]-α-thiolincosaminide (7b′)

Reaction of **6b′** (200 mg, 0.277) mmol) with 5-(2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (104 mg, 0.435 mmol) afforded **7b′** as a light yellow solid in 25% yield by the similar procedure to **7a**. $[\alpha]_D^{26}$ +110° (*c* 0.21, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.08–8.12 (m, 1H), 7.75–7.85 (m, 3H), 5.27 (d, J = 5.9 Hz, 1 H), 4.66 (dd, J = 10.1, 2.5 Hz, 1H), 4.39–4.49 (m, 2H), 4.07–4.13 (m, 1H), 3.83–3.85 (m, 1H), 3.56 (dd, J = 10.2, 3.3 Hz, 1H), 3.24–3.29 (m, 1H), 3.09–3.15 (m, 1H), 2.58–2.66 (m, 1H), 2.01 (s, 3H), 1.94–1.99 (m, 1H), 1.65–1.73 (m, 1H), 1.58 (d, J = 6.9 Hz, 3H), 1.18–1.42 (m, 5H), 0.99–1.12 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H); MS (FAB) *m/z* 628 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{38}N_5O_7S_3$ 628.1928, found 628.1921 $(M+H)^+$.

Methyl (7*S*)-6-*N*-[(2′*S*, 4′*R*)-4′-(*n*-butyl)piperidine-2′-carbonyl]-7-deoxy-7-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-α-thiolincosaminide (7c)

Reaction of **6c** (500 mg, 0.678 mmol) with 5-(2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (211 mg, 0.882 mmol) afforded **7c** as a colorless solid in 21% yield by the similar procedure to **7a**. $[\alpha]_D^{25}$ +93° (*c* 0.48, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.08–8.12 (m, 1H), 7.76–7.87 (m, 3H), 5.29 (d, J = 5.6 Hz, 1H), 4.68 (dd, J = 10.1, 2.3 Hz, 1H), 4.46 (dd, J = 6.8, 2.4 Hz, 1H), 4.42 (d, J = 10.2 Hz, 1H), 4.11 (dd, J = 10.2, 5.6 Hz, 1H), 3.88–3.91 (m, 1H), 3.59–3.63 (m, 1H), 3.57 (dd, J = 10.2, 3.2 Hz, 1H), 3.38 (dd, J = 11.9, 2.9 Hz, 1H), 3.15–3.22 (m, 1H), 2.63–2.72 (m, 1H), 1.99 (s, 3H), 1.68–1.76 (m, 1H), 1.52–1.58 (m, 4H), 1.24–1.34 (m, 6H), 1.02–1.17 (m, 3H), 0.84–0.92 (m, 3H); MS (FAB) *m/z* 642 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{40}N_5O_7S_3$ 642.2084, found 642.2092 $(M+H)^+$.

Methyl (7*S*)-7-deoxy-6-*N*-[(2′*S*, 4′*R*)-4′-ethylpiperidine-2′-carbonyl]-7-[5-(4-fluoro-2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-α-thiolincosaminide (7d)

Reaction of **6a** (180 mg, 0.253 mmol) with 5-(4-fluoro-2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (80.0 mg, 311 mmol) afforded **7d** as a light yellow solid in 39% yield by the similar procedure to **7a**. $[\alpha]_D^{25}$ +89° (*c* 0.53, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (dd, J = 8.3, 2.5 Hz, 1H), 7.85 (dd, J = 8.7, 5.3 Hz, 1H), 7.64 (ddd, J = 8.7, 7.7, 2.5 Hz, 1H), 5.27 (d, J = 5.6 Hz, 1H), 4.67 (dd, J = 10.0, 2.4 Hz, 1H), 4.46 (qd, J = 6.8, 2.4 Hz, 1H), 4.41 (dd, J = 10.1, 0.9 Hz, 1H), 4.10 (dd, J = 10.3, 5.9 Hz, 1H), 3.88 (dd, J = 3.2, 0.9 Hz, 1H), 3.55 (dd, J = 10.3, 3.2 Hz, 1H), 3.26–3.30 (m, 1H), 3.14–3.20 (m, 1H), 2.64 (td, J = 12.8, 2.8 Hz, 1H), 1.99 (s, 3H), 1.95–1.98 (m, 1H), 1.68–1.75 (m, 1H), 1.56 (d, J = 6.8 Hz, 3H), 1.37–1.50 (m, 1H), 1.26–1.35 (m, 2H), 1.00–1.11 (m, 2H), 0.92 (t, J = 7.5 Hz, 3H); MS (FAB) *m/z* 632 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{25}H_{35}FN_5O_7S_3$ 632.1677, found 632.1674 $(M+H)^+$.

Methyl (7*S*)-7-deoxy-6-*N*-[(2′*S*, 4′*R*)-4-ethyl-1′-*N*-methylpiperidine-2′-carbonyl]-7-[5-(4-fluoro-2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-α-thiolincosaminide (8d)

To a solution of compound **7d** (52 mg, 0.082 mmol) in MeOH (3 ml) at 0 °C were added 36% aqueous formaldehyde (100 µl, 1.2 mmol), acetic acid (50 µl, 0.79 mmol) and $NaBH(OAc)_3$ (120 mg, 0.57 mmol) and stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure. Ethyl acetate was added to the residue and washed with saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The resulting residue was purified by preparative TLC ($CHCl_3$/MeOH/28% aq NH_4OH = 10/1/0.1) to afford **8d** (41 mg, 77%) as a

light yellow solid. $[\alpha]_D^{25}$ +80° (*c* 0.92, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 7.98 (dd, *J*=8.3, 2.6 Hz, 1H), 7.85 (dd, *J*=8.7, 5.3 Hz, 1H), 7.61–7.67 (m, 1H), 5.28 (d, *J*=5.6 Hz, 1H), 4.69 (dd, *J*=10.1, 2.6 Hz, 1H), 4.48 (qd, *J*=6.9, 2.6 Hz, 1H), 4.43 (dd, *J*=10.1, 0.8 Hz, 1H), 4.12 (dd, *J*=10.2, 5.6 Hz, 1H), 3.83–3.87 (m, 1H), 3.57 (dd, *J*=10.2, 2.9 Hz, 1H), 2.93–3.99 (m, 1H), 2.58–2.64 (m, 1H), 2.25 (s, 3H), 2.06–2.15 (m, 1H), 1.98–2.01 (m, 3H), 1.85–1.91 (m, 1H), 1.69–1.76 (m, 1H), 1.58 (d, *J*=6.9 Hz, 3H), 1.23–1.34 (m, 5H), 0.87–0.94 (m, 3H); MS (FAB) *m/z* 646 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{39}FN_5O_7S_3$ 646.1834, found 646.1833 $(M+H)^+$.

Methyl (7*S*)-7-deoxy-7-[5-(4-fluoro-2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-6-*N*-[(2′*S*, 4′*R*)-4′-(*n*-propyl)piperidine-2′-carbonyl]-α-thiolincosaminide (7e)

Reaction of **6b** (2.80 g, 3.87 mmol) with 5-(4-fluoro-2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (1.19 mg, 4.59 mmol) afforded **7e** as a light yellow solid in 39% yield by the similar procedure to **7a** except for using toluene as a solvent of Mitunobu reaction. $[\alpha]_D^{26}$ +93° (*c* 0.20, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 7.99 (dd, *J*=8.3, 2,7 Hz, 1H), 7.85 (dd, *J*=8.6, 5.2 Hz, 1H), 7.60–7.69 (m, 1H), 5.27 (d, *J*=5.6 Hz, 1H), 4.66 (dd, *J*=10.1, 2.6 Hz, 1H), 4.43–4.50 (m, 1H), 4.41 (d, *J*=10.1 Hz, 1H), 4.10 (dd, *J*=10.3, 5.6 Hz, 1H), 3.85–3.90 (m, 1H), 3.55 (dd, *J*=10.3, 3.3 Hz, 1H), 3.12–3.19 (m, 1H), 2.57–2.67 (m, 1H), 1.99 (s, 3H), 1.92–1.97 (m, 1H), 1.65–1.74 (m, 1H), 1.56 (d, *J*=6.8 Hz, 3H), 1.19–1.42 (m, 5H), 0.99–1.12 (m, 2H), 0.90 (t, *J*=7.2 Hz, 3H); MS (FAB) *m/z* 646 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{37}FN_5O_7S_3$ 646.1834, found 646.1836 $(M+H)^+$.

Methyl (7*S*)-7-deoxy-7-[5-(4-fluoro-2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-6-*N*-[(2′*S*, 4′*R*)-1′-*N*-methyl-4′-(*n*-propyl)piperidine-2′-carbonyl]-α-thiolincosaminide (8e)

The title compound was synthesized from **7e** (55 mg, 0.086 mmol) as a light yellow solid in 37% yield by the similar procedure to **8d**. $[\alpha]_D^{26}$ +88° (*c* 0.17, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 7.99 (dd, *J*=5.2 Hz, 1H), 7.85 (dd, *J*=8.9, 5.2 Hz, 1H), 7.61–7.67 (m, 1H), 5.28 (d, *J*=5.6 Hz, 1H), 4.69 (dd, *J*=10.2, 2.4 Hz, 1H), 4.45–4.52 (m, 1H), 4.43 (d, *J*=10.2 Hz, 1H), 4.12 (dd, *J*=10.2, 5.7 Hz, 1H), 3.84–3.86 (m, 1H), 3.57 (dd, *J*=10.2, 3.2 Hz, 1H), 2.92–3.00 (m, 1H), 2.57–2.65 (m, 1H), 2.25 (s, 3H), 2.10–2.18 (m, 1H), 2.00 (s, 3H), 1.83–1.90 (m, 1H), 1.68–1.74 (m, 1H), 1.57 (d, *J*=7.6 Hz, 3H), 1.20–1.40 (m, 7H), 0.86–0.92 (m, 3H); MS (FAB) *m/z* 660 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{39}N_5O_7S_3$ 660.1990, found 660.1995 $(M+H)^+$.

Methyl (7*S*)-6-*N*-[(2′*S*, 4′*R*)-4′-(*n*-butyl)piperidine-2′-carbonyl]-7-deoxy-7-[5-(4-fluoro-2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-α-thiolincosaminide (7f)

Reaction of **6c** (500 mg, 0.678 mmol) with 5-(4-fluoro-2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (224 mg, 0.882 mmol) afforded **7f** as a light yellow solid in 30% yield by the similar procedure to **7a**. $[\alpha]_D^{26}$ +100° (*c* 0.27, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 7.98 (dd, *J*=8.2, 2,6 Hz, 1H), 7.85 (dd, *J*=8.6, 5.2 Hz, 1H), 7.60–7.68 (m, 1H), 5.28 (d, *J*=5.6 Hz, 1H), 4.67 (dd, *J*=10.2, 2.4 Hz, 1H), 4.43–4.51 (m, 1H), 4.41 (d, *J*=10.2 Hz, 1H), 4.10 (dd, *J*=10.2, 5.6 Hz, 1H), 3.87–3.91 (m, 1H), 3.56 (dd, *J*=10.2, 3.2 Hz, 1H), 3.32–3.36 (m, 1H), 3.14–3.21 (m, 1H), 2.61–2.71 (m, 1H), 2.00–2.04 (m, 1H), 1.99 (s, 3H), 1.68–1.75 (m, 1H), 1.56 (d, *J*=7.1 Hz, 3H), 1.23–1.34 (m, 6H), 0.99–1.14 (m, 2H), 0.86–0.91 (m, 3H); MS (FAB) *m/z* 660 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{39}FN_5O_7S_3$ 660.1990, found 660.1994 $(M+H)^+$.

Methyl (7*S*)-6-*N*-[(2′*S*, 4′*R*)-4′-(*n*-butyl)-1′-*N*-methylpiperidine-2′-carbonyl]-7-deoxy-7-[5-(4-fluoro-2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-α-thiolincosaminide (8f)

The title compound was synthesized from **7f** (72 mg, 0.11 mmol) as a light yellow solid in 85% yield by the similar procedure to **8d**. $[\alpha]_D^{26}$ +94° (*c* 0.22, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 7.98 (dd, *J*=8.2, 2,6 Hz, 1H), 7.85 (dd, *J*=8.6, 5.2 Hz, 1H), 7.60–7.68 (m, 1H), 5.28 (d, *J*=5.6 Hz, 1H), 4.70 (dd, *J*=10.1, 2.7 Hz, 1H), 4.45–4.53 (m, 1H), 4.43 (d, *J*=10.1 Hz, 1H), 4.12 (dd, *J*=10.2, 5.6 Hz, 1H), 3.82–3.87 (m, 1H), 3.57 (dd, *J*=10.2, 3.3 Hz, 1H), 2.93–3.01 (m, 1H), 2.62–2.68 (m, 1H), 2.27 (s, 3H), 2.09–2.18 (m, 1H), 2.00 (s, 3H), 1.83–1.92 (m, 1H), 1.67–1.76 (m, 1H), 1.57 (d, *J*=7.1 Hz, 3H), 1.21–1.42 (m, 9H), 0.85–0.93 (m, 3H); MS (FAB) *m/z* 674 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{28}H_{41}FN_5O_7S_3$ 674.2147, found 674.2160 $(M+H)^+$.

Methyl (7*S*)-7-deoxy-7-[5-(5-methylamino-2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-6-*N*-[(2′*S*, 4′*R*)-4′-(*n*-propyl)piperidine-2′-carbonyl]-α-thiolincosaminide (7g)

Reaction of **6b** (200 mg, 0.277 mmol) with 5-(5-methylamino-2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (96.6 mg, 0.360 mmol) afforded **7 g** as a yellow solid in 26% yield by the similar procedure to **7a**. $[\alpha]_D^{24}$ +91° (*c* 0.41, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, *J*=8.7 Hz, 1H), 6.62–6.72 (m, 2H), 5.33 (d, *J*=5.7 Hz, 1H), 4.46–4.53 (m, 1H), 4.31 (d, *J*=9.9 Hz, 1H), 4.11–4.25 (m, 2H), 3.87–3.91 (m, 1H), 3.56–3.68 (m, 1H), 3.37–3.46 (m, 1H), 3.16–3.24 (m, 1H), 2.91 (s, 3H), 2.68–2.90 (m, 2H), 2.61–2.68 (m, 1H), 2.19 (s, 3H), 2.00–2.09 (m, 1H), 1.64–1.72 (m, 1H), 1.51 (d, *J*=6.9 Hz, 3H), 1.16–1.36 (m, 4H), 0.98–1.12 (m, 2H), 0.79–0.89 (m, 3H); MS (FAB) *m/z* 657 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{41}N_6O_7S_3$ 657.2193, found 657.2193 $(M+H)^+$.

Methyl (7*S*)-7-deoxy-7-[5-(5-methoxy-2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-6-*N*-[(2′*S*, 4′*R*)-4′-(*n*-propyl)piperidine-2′-carbonyl]-α-thiolincosaminide (7h)

Reaction of **6b** (200 mg, 0.277 mmol) with 5-(5-methoxy-2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (97.0 mg, 0.360 mmol) afforded **7h** as a light yellow solid in 24% yield by the similar procedure to **7a**. $[\alpha]_D^{24}$ +79° (*c* 0.12, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.11–8.20 (m, 1H), 7.07–7.16 (m, 2H), 6.62–6.72 (m, 2H), 5.36 (d, *J*=5.7 Hz, 1H), 4.47–4.53 (m, 1H), 4.09–4.35 (m, 3H), 3.94 (s, 3H), 3.77–3.89 (m, 1H), 3.55–3.70 (m, 1H), 3.36–3.51 (m, 1H), 3.16–3.29 (m, 1H), 2.65–2.79 (m, 2H), 2.15 (s, 3H), 1.98–2.09 (m, 1H), 1.64–1.73 (m, 1H), 1.52 (d, *J*=6.9 Hz, 3H), 1.17–1.36 (m, 4H), 0.98–1.13 (m, 2H), 0.77–0.89 (m, 3H); MS (FAB) *m/z* 658 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{40}N_5O_8S_3$ 658.2034, found 658.2038 $(M+H)^+$.

Methyl (7*S*)-7-deoxy-6-*N*-[(2′*S*, 4′*R*)-4′-ethylpiperidine-2′-carbonyl]-7-[5-(4,5-dimethoxy-2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-α-thiolincosaminide (7i)

Reaction of **6a** (158 mg, 0.223 mmol) with 5-(4,5-dimethoxy-2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (80.0 mg, 0.267 mmol) afforded **7i** as a light yellow solid in 11% yield by the similar procedure to **7a**. $[\alpha]_D^{25}$ +89° (*c* 0.46, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 7.75 (s, 1H), 7.21 (s, 1H), 5.28 (d, *J*=5.6 Hz, 1H), 4.67 (dd, *J*=10.0, 2.4 Hz, 1H), 4.40–4.48 (m, 2H), 4.10 (dd, *J*=10.3, 5.6 Hz, 1H), 3.98 (s, 3H), 3.96 (s, 3H), 3.88–3.91 (m, 1H), 3.56 (dd, *J*=10.3, 3.4 Hz, 1H), 3.14–3.20 (m, 1H), 2.64 (td, *J*=12.9, 2.7 Hz, 1H), 2.01 (s, 3H), 1.98 (br s, 1H), 1.69–1.75 (m, 1H), 1.55 (d, *J*=6.8 Hz, 3H), 1.39–1.49 (m, 1H), 1.24–1.34 (m, 2H), 1.00–1.12 (m, 2H), 0.92 (t, *J*=7.3 Hz, 3H); MS (FAB) *m/z* 674 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{40}N_5O_9S_3$ 674.1983, found 674.1988 $(M+H)^+$.

Methyl (7*S*)-7-deoxy-7-[5-(4,5-dimethoxy-2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-6-*N*-[(2′*S*, 4′*R*)-4′-(*n*-propyl)piperidine-2′-carbonyl]-α-thiolincosaminide (7j)

Reaction of **6b** (200 mg, 0.277 mmol) with 5-(4,5-dimethoxy-2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (99.4 mg, 0.332 mmol) afforded **7j** as a light yellow solid in 10% yield by the similar procedure to **7a**. $[\alpha]_D^{26}$ +97° (*c* 0.30, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 7.77 (s, 1H), 7.22 (s, 1H), 5.28 (d, *J*=5.6 Hz, 1H), 4.67 (dd, *J*=10.2, 2.4 Hz, 1H), 4.39–4.48 (m, 2H), 4.10 (dd, *J*=10.2, 5.6 Hz, 1H), 3.99 (s, 3H), 3.96 (s, 3H), 3.87–3.90 (m, 1H), 3.56 (dd, *J*=10.2, 3.0 Hz, 1H), 3.32–3.38 (m, 1H), 3.15–3.20 (m, 1H), 2.63–2.71 (m, 1H), 2.02 (s, 3H), 1.97–2.01 (m, 1H), 1.68–1.75 (m, 1H), 1.55 (d, *J*=7.1 Hz, 3H), 1.31–1.40 (m, 2H), 1.20–1.28 (m, 2H), 1.02–1.15 (m, 3H), 0.90 (t, *J*=7.2 Hz, 3H); MS (FAB) *m/z* 688 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{28}H_{42}N_5O_9S_3$ 688.2139, found 688.2153 $(M+H)^+$.

Methyl (7*S*)-6-*N*-[(2′*S*, 4′*R*)-4′-(*n*-butyl)piperidine-2′-carbonyl]-7-deoxy-7-[5-(4,5-dimethoxy-2-nitrophenyl)-1,3,4-thiadiazol-2-yl-thio]-α-thiolincosaminide (7k)

Reaction of **6c** (200 mg, 0.271 mmol) with 5-(4,5-dimethoxy-2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (106 mg, 0.353 mmol) afforded **7k** as a light yellow solid in 19% yield by the similar procedure to **7a**. $[\alpha]_D^{26}$ +96° (*c* 0.49, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 7.75 (s, 1H), 7.21 (s, 1H), 5.28 (d, *J* = 5.7 Hz, 1H), 4.63–4.69 (m, 1H), 4.38–4.47 (m, 2H), 4.10 (dd, *J* = 10.1, 5.7 Hz, 1H), 3.98 (s, 3H), 3.96 (s, 3H), 3.87–3.90 (m, 1H), 3.54–3.58 (m, 1H), 3.27–3.29 (m, 1H), 3.12–3.18 (m, 1H), 2.58–2.67 (m, 1H), 2.01 (s, 3H), 1.92–2.00 (m, 1H), 1.65–1.74 (m, 1H), 1.55 (d, *J* = 6.8 Hz, 3H), 1.46–1.52 (m, 1H), 1.20–1.34 (m, 6H), 0.99–1.11 (m, 2H), 0.86–0.93 (m, 3H); MS (FAB) *m/z* 702 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{29}H_{44}N_5O_9S_3$ 702.2296, found 702.2294 $(M+H)^+$.

Methyl 7-deoxy-7-methyl-6-*N*-[(2′*S*, 4′*R*)-4′-(*n*-propyl)piperidine-2′-carbonyl]-α-thiolincosaminide (VIC-105555) and methyl 7-deoxy-7-methyl-6-*N*-[(2′*R*, 4′*S*)-4′-(*n*-propyl)piperidine-2′-carbonyl]-α-thiolincosaminide (10)

To a solution of compound **9** (1.36 g, 3.41 mmol) in MeOH-H_2O (5:2, 100 ml) were added conc. hydrochloric acid (410 µl, 4.90 mmol), platinum (IV) oxide (1.16 g, 5.12 mmom) and stirred under a hydrogen pressure of 3.9 MPa at room temperature for 24 h. The mixture was filtered and concentrated under reduced pressure. The resulting residue was added saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/28% aq NH_4OH = 95/5/0.5 to 85/15/1.5), to afford the title compounds as colorless solids (VIC-105555; more polar, 630 mg, 46%: **10**; less polar, 653 mg, 47%). VIC-105555: $[\alpha]_D^{26}$ +170° (*c* 0.91, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 5.24 (d, *J* = 5.6 Hz, 1H), 4.16 (d, *J* = 10.0, 3.1 Hz, 1H), 4.08 (dd, *J* = 10.3, 5.6 Hz, 1H), 4.04 (d, *J* = 10.0 Hz, 1H), 3.79–3.81 (m, 1H), 3.51 (dd, *J* = 10.3, 3.2 Hz, 1H), 3.24–3.29 (m, 1H), 3.10–3.16 (m, 1H), 2.62 (td, *J* = 12.8, 2.8 Hz, 1H), 2.12–2.20 (m, 1H), 2.11 (s, 3H), 1.89–1.96 (m, 1H), 1.66–1.73 (m, 1H), 1.46–1.57 (m, 1H), 1.32–1.41 (m, 2H), 1.21–1.28 (m, 2H), 0.98–1.10 (m, 2H), 0.89–0.96 (m, 9H); MS (FAB) *m/z* 405 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{19}H_{37}N_2O_5S$ 405.2418, found 405.2422 $(M+H)^+$. **10**: $[\alpha]_D^{26}$ +200° (*c* 1.2, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 5.25 (d, *J* = 5.7 Hz, 1H), 4.14 (dd, *J* = 9.9, 3.6 Hz, 1H), 4.09 (dd, *J* = 10.2, 5.7 Hz, 1H), 4.06 (d, *J* = 9.9 Hz, 1H), 3.76–3.79 (m, 1H), 3.52 (dd, *J* = 10.2, 3.5 Hz, 1H), 3.24 (dd, *J* = 11.6, 2.8 Hz, 1H), 3.08–3.14 (m, 1H), 2.61 (td, *J* = 12.7, 2.7 Hz, 1H), 2.12–2.19 (m, 1H), 2.11 (s, 3H), 1.93–1.99 (m, 1H), 1.65–1.72 (m, 1H), 1.42–1.55 (m, 1H), 1.31–1.42 (m, 2H), 1.20–1.29 (m, 2H), 0.96–1.11 (m, 2H), 0.88–0.96 (m, 9H); MS (FAB) *m/z* 405 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{19}H_{37}N_2O_5S$ 405.2418, found 404.2423 $(M+H)^+$.

In vitro antibacterial activity

MIC was determined by the agar dilution method. Test strains were subjected to seed culture using sensitivity test broth (Nissui Pharmaceutical, Tokyo, Japan) cultured on blood agar plate for *S. pneumoniae*, *S. pyogenes* and *H. influenzae*. A 5 µl portion of cell suspension of the test strains having about 10^6 CFU ml^{-1} was inoculated into sensitivity disk agar (Nissui Pharmaceutical) supplemented with 5% horse blood and incubated at 37 °C for 20 h. Then, MIC was defined as the lowest drug concentration that prevented visible growth.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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