The Journal of Antibiotics (2018) 71, 298–317

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ORIGINAL ARTICLE

Synthesis and SARs of novel lincomycin derivatives Part 5: optimization of lincomycin analogs exhibiting potent antibacterial activities by chemical modification at the 6- and 7-positions

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In order to modify lincomycin at the C-6 and C-7 positions, we prepared target molecules, which have substituted pipecolinic acid at the 6-amino group and a *para*-substituted phenylthio group at the C-7 position, in application of palladium-catalyzed cross-coupling as a key reaction. As the result of structure-activity relationship (SAR) studies at the 6-position, analogs possessing 4′-*cis*-(cyclopropylmethyl)piperidine showed significantly strong antibacterial activities against *Streptococcus pneumoniae* and *Streptococcus pyogenes* with an *erm* gene. On the basis of SAR, we further synthesized novel analogs possessing 4′-*cis*-(cyclopropylmethyl)piperidine by transformation of a C-7 substituent. Consequently, novel derivatives possessing a *para*-heteroaromatic-phenylthio group at the C-7 position exhibited significantly strong activities against *S. pneumoniae* and *S. pyogenes* with an *erm* gene even when compared with those of telithromycin. Finally, *in vivo* efficacy of selected two derivatives was evaluated in a rat pulmonary infection model with resistant *S. pneumoniae* with *erm* + *mef* genes. One of them exhibited strong and constant *in vivo* efficacy in this model, and both compounds showed strong *in vivo* efficacy against resistant *S. pneumoniae* with a *mef* gene.
The Journal of Antibiotics (2018) **71,** 298–317; doi:10.1038/ja.2017.114; published online 1 November 2017

INTRODUCTION

Macrolide antibiotics have antibacterial activities against *S. pneumoniae*, *S. pyogenes*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae* and *Neisseria gonorrhoeae*, and have an acceptable safety profile as oral antibiotics. Consequently, macrolides have widely been used in clinical sites for bacterial respiratory infections. Recently, macrolide resistant bacteria with an *erm* gene have markedly increased.[1–3] Clarithromycin[4] and azithromycin[5,6] are not effective against *S. pneumoniae* and *S. pyogenes* with an *erm* gene and have low sensitivity against *S. pneumoniae* with a *mef* gene (Figure 1 and Table 1). Although telithromycin (TEL)[7] exhibits effective activities against *S. pneumoniae* with *erm* and/or *mef* genes, its activities are influenced by a *mef* gene. A serious liver damage[8,9] and loss of consciousness[10,11] were reported as side effects of TEL and medication with TEL was discontinued in Japan. Novel azalides reported by Miura *et al.*[12,13] are also effective against the above resistant pathogens, but these analogs are still under research process. Development of an oral antibiotic possessing potent antibacterial activities and an acceptable safety profile is strongly desired in clinical sites for respiratory infections.

Lincomycin (LCM)[14–17] and clindamycin (CLDM)[18] are effective against clinically isolated pathogens with a *mef* gene, but they are not effective against resistant bacteria with an *erm* gene (Figure 1, Table 1). As an overview, CLDM exhibits the following positive characters: (1) availability in p.o. and i.v. administrations (switch therapy is possible), (2) good distributions to the tissue and cells, (3) suppression[19] of toxin production by streptococcal strains and (4) expected reasonable production cost of its derivatives compared with that of ketolides with a complex chemical structure. Thus, LCM derivatives might be clinically more valuable than ketolide antibiotics, if they are effective against Gram-positive pathogens with an *erm* gene.

Chemical modifications at the C-7 position of LCM were reported by several research groups.[17,18,20-32] However, none of those compounds showed antibacterial activities against resistant Gram-positive pathogens with an *erm* gene. On the other hand, we reported that novel LCM derivatives modified at the C-7 position possessed antibacterial activities against resistant bacteria with an *erm* gene.[33–43] In particular, compound **1** (Wakiyama *et al.*[40]) (Figure 1) and its analogues (possessing a '1-methylpiperidin-3-yl' or '1-methyl-1,2,5,6-tetrahydropyridin-3-yl' moiety instead of a 'pyrimidin-5-yl' moiety in compound **1**) had significantly potent antibacterial activities against resistant bacteria with an *erm* gene.

Chemical modification at the C-6 position of LCM and/or CLDM was also performed by several research groups.[17,24,44–54] The C-6 side chain, originally 1′-*N*-methyl-4′-*trans*-*n*-propylproline, has the following characters: (1) diastereoisomers with 2′,4′-*trans* configuration were

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Received 20 May 2017; revised 15 August 2017; accepted 24 August 2017; published online 1 November 2017

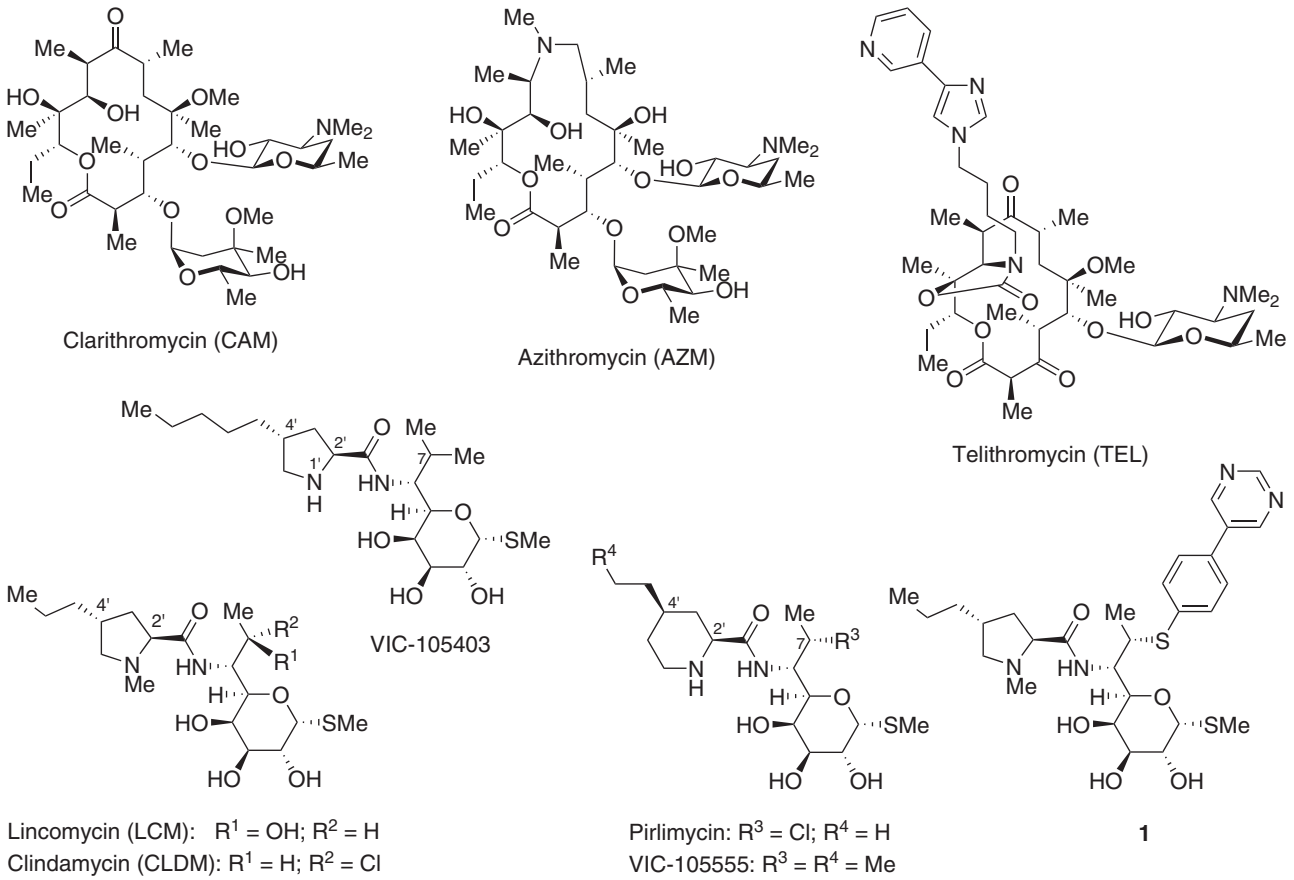


Figure 1 Chemical structures of macrolide derivatives and lincomycin/clindamycin derivatives.

Table 1 Antibacterial activities (MIC, μg ml^{-1}) of CAM, AZM, LCM, CLDM, VIC-105555, TEL and previously reported lincomycin derivative 1

Test organism[a]	*Characteristics*	*CAM*	*AZM*	*LCM*	*CLDM*	*VIC-105555*	*TEL*	***1***
S. pneumoniae DP1 TypeI	Susceptible	0.03	0.06	1	0.06	0.03	⩽0.008	⩽0.008
S. pneumoniae-2	Susceptible	0.03	0.03	1	0.12	0.06	⩽0.008	⩽0.008
S. pneumoniae-3	Susceptible	0.015	0.03	0.25	0.06	0.03	⩽0.008	⩽0.008
S. pneumoniae-4	*ermAM* methylase (c)	>128	>128	>128	>128	>128	0.5	0.5
S. pneumoniae-5	*ermAM* methylase (c)	>128	>128	>128	>128	>128	2	1
S. pneumoniae-6	*ermAM* methylase (c) + *mefE*	>128	>128	>128	>128	>128	1	2
S. pneumoniae-7	*ermAM* methylase (i)	>128	>128	128	128	128	0.03	0.25
S. pneumoniae-8	*ermAM* methylase (i)	>128	>128	128	128	128	0.03	0.25
S. pneumoniae-9	*mefE* efflux	0.5	0.5	1	0.12	0.015	0.06	⩽0.008
S. pyogenes Cook	susceptible	0.015	0.06	0.12	0.06	0.06	⩽0.008	⩽0.008
S. pyogenes-2	*ermAM* methylase (c)	>128	>128	>128	128	>128	16	0.5
S. pyogenes-3	*mefE* efflux	8	8	0.25	0.12	0.06	0.25	0.015
H. influenzae	Susceptible	2	0.25	8	16	32	0.5	4
H. influenzae-2	Susceptible	4	1	16	8	16	2	2
H. influenzae-3	Susceptible	8	2	16	16	32	1	8
H. influenzae-4	⊿acr	0.5	0.5	4	1	2	0.25	0.06

Abbreviations: AZM, azithromycin; c, constitutive; CAM, clarithromycin; CLDM, clindamycin; I, inducible Gray shading strains are target strains; LCM, lincomycin; TEL, telithromycin.
Gray shading strains are target strains.
[a]All strains except standard organisms were clinically isolated.

more potent than *cis* isomers; (2) 1′-*N*-demethylclindamycin was twice as active *in vitro* against *Sarcina lutea* as CLDM, but 1′-*N*-demethyl-lincomycin was about one twentieth as active as LCM;[21,44–45] (3) as for chain length (H, Me to octyl) at the 4′-position of LCM, the hexyl analog showed maximum *in vitro* antibacterial activity;[21,46] (4) introduction of hetero atoms to the 4′-side chain essentially lost activity;[17,21,47] and (5) VIC-105403 (Lewis *et al.*[24]) (Figure 1) had potent activities compared with CLDM.

As another background information on chemical modifications at the C-6, azetidine,[48–51] piperidine[48,49,52–54] and azepane analogs[48,49]

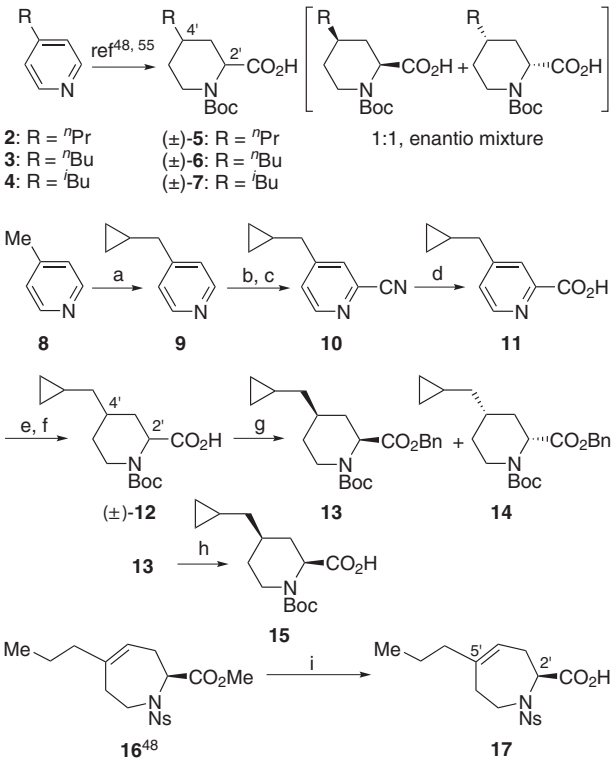
were synthesized accompanied with modifications at the C-7. Regarding azetidine derivatives, 3′-*trans*-cyclobutylethyl CLDM derivative showed significant antibacterial activities against sensitive *S. pneumoniae* compared with CLDM, but 3′-*trans*-cyclopropylmethyl, 3′-*trans*-*n*-propyl and 3′-*trans*-*n*-butyl analogs exhibited similar potency as CLDM. As a piperidine derivative, 4′-*cis*-ethyl CLDM analog, pirlimycin, is used in mastitis therapy for cattle in the European countries and United States. On the other hand, VIC-105555 (Figure 1) was selected as a candidate, which exhibited preferable pharmacokinetics and characteristic *in vitro* antibacterial activities against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis*. Furthermore, azepane-type CLDM analogs were also synthesized and 5′-(3-fluorobutyl) analog was 32 times as active (MIC: 0.25 μg ml^{-1}) against *H. influenzae* as CLDM (MIC: 8 μg ml^{-1}).[48]

None of the C-6-modified compounds were disclosed to possess activities against Gram-positive-resistant bacteria with an *erm* gene. We reported that novel (7*S*)-substituted analogs[42] modified at the N-1′ and C-4′ positions in a proline moiety had potent activities against Gram-positive-resistant bacteria with an *erm* gene. We further pursued modifications of LCM with a combination manner at the C-6 and C-7 positions in order to generate novel LCM derivatives exhibiting as strong antibacterial activities as TEL. Then, we synthesized novel (7*S*)-substituted analogs attached with piperidine or azepane instead of pyrrolidine (a part of proline) at the C-6 position. We have found three representative molecules so far and we chose a 'pyrimidin-5-yl'-phenyl derivative (**1**) (Figure 1) as a C-7 side chain for optimization of a C-6 moiety, because a '1-methylpiperidin-3-yl' moiety has a chiral center (anxiety for relatively complex production) and a '1-methyl-1,2,5,6-tetrahydropyridin-3-yl' moiety has an isolated double bond (anxiety for potential instability).

RESULTS AND DISCUSSION

Synthesis of the substituted piperidines and 2,3,6,7-tetrahydro-1*H*-azepine

Syntheses of substituted piperidines and 2,3,6,7-tetrahydro-1*H*-azepine are shown in Scheme 1. Substituted piperidines (±)-**5**-**7** and 2,3,6,7-tetrahydro-1*H*-azepine **16** were synthesized by methods reported by Shuman *et al.*[55] and Lewis *et al.*[48] Compound (±)-**12** was prepared from 4-methylpyridine (**8**) in improved reaction conditions based on reported methods[48,55] shown in Scheme 1. It was reported that hydrogenation of disubstituted pyridine in the presence of PtO_2 resulted in a racemate of *cis*-products as major products by Lewis *et al.*[48] Going back in time, Shuman *et al.*[55] proved that 2,4-disubstituted piperidine prepared from disubstituted pyridine by hydrogenation had *cis*-configuration by NOE experiments. At the beginning of this research, we used (±)-*cis*-carboxylic acids **5**-**7** and **12**, but later on we could separate (±)-**12** into each *cis*-enantiomer for efficient synthetic study. Carboxylic acid (±)-**12** was protected by a benzyl group for the purpose of optical resolution by HPLC and both enantiomers were purified by chiral column chromatography to obtain a desired compound **13**. Stereochemistry of compounds **13** and **14** was assigned as following. Pirlimycin and VIC-105555 are reported as representative LCM derivatives possessing a substituted piperidine moiety (Figure 1). Absolute stereochemistry of pirlimycin was clarified by X-ray crystallographic studies.[49] Absolute stereochemistry of VIC-105555 was reported by Vicuron at 44th Interscience Conference on Antimicrobial Agents and Chemotherapy.[54] Both compounds have 2′-β-4′-β-configuration and they showed remarkable polarity (lower Rf value) and stronger potency compared with the corresponding 2′-α-4′-α-diastereoisomer, respectively. When we coupled a substituted pipecolic acid with methyl α-thiolincosaminide (MTL), we assigned

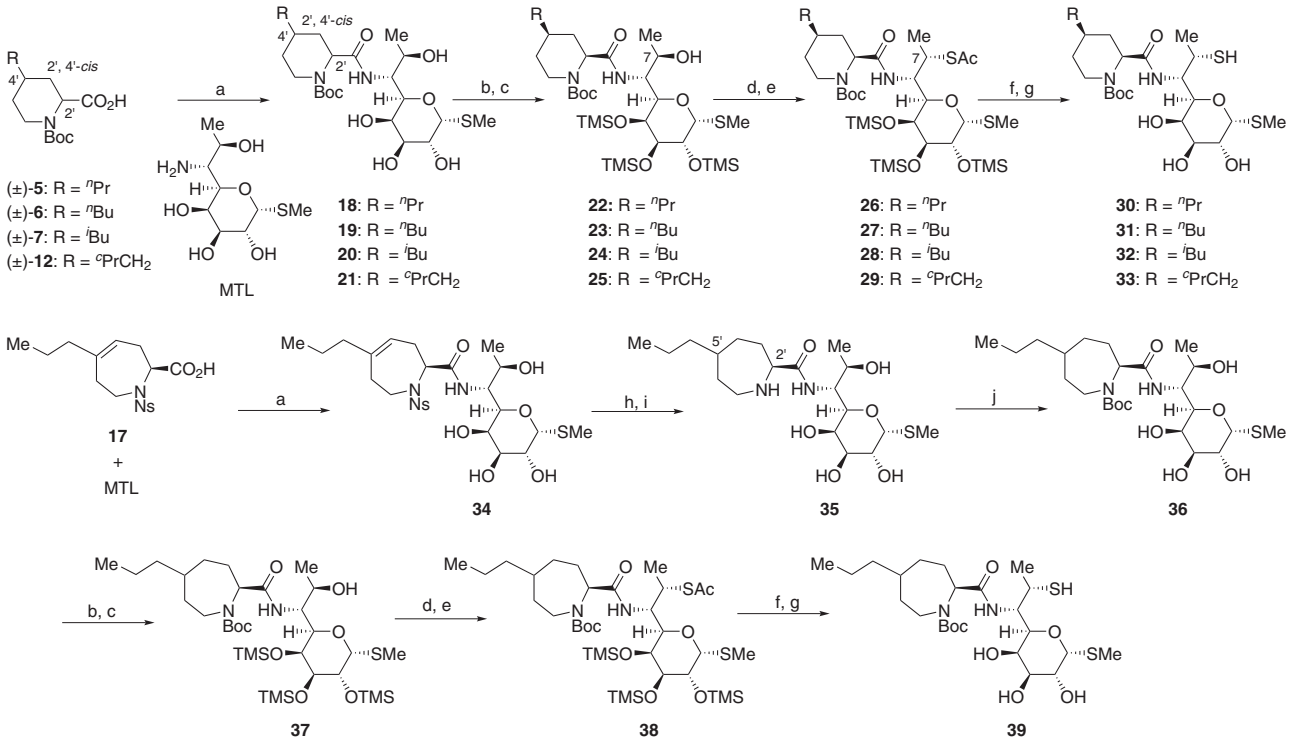


Scheme 1 Synthesis of substituted piperidines and 2,3,6,7-tetrahydro-1*H*-azepine. Conditions: (a) bromocyclopropane, lithium diisopropylamide, THF, −78 °C, 1 h; (b) *m*-chloroperoxybenzoic acid (*m*CPBA), CH_2Cl_2, 0 °C to r.t., 1 h; (c) TMSCN, Me_2NCOCl, CH_2Cl_2, 20 °C 40 min, then r.t.,17 h; (d) 5 N NaOH, MeOH, 50 °C, 8 h; (e) H_2, PtO_2, AcOH, r.t., 24 h; (f) Boc_2O, 2 N NaOH, dioxane, r.t., 15 h; (g) BnBr, $^iPr_2NEt$, CH_3CN, r.t., 48 h; (h) H_2, Pd/C, MeOH, r.t., 1 h; (i) LiOH·H_2O, dioxane:H_2O = 4:1, r.t. 5 h.

2′-β-4′-β-configuration for a polar product. The benzyl group in **13** was removed by hydrogenolysis to give a key intermediate **15**. A seven-membered intermediate (**17**) was prepared by basic hydrolysis of **16**.

Synthesis of key intermediates 30–33 and 39

Syntheses of key intermediates **30–33** and **39** are shown in Scheme 2. Diastereomeric mixtures **18–21** and compound **34** were synthesized by coupling of compounds (±)-**5**-**7**, (±)-**12** and **17** with MTL, respectively. MTL was prepared by a reported method.[56] Although each isomer was almost one to one mixture except **17** when the coupling reactions were completed, precipitation process gave 2′-β-4′-β-rich *cis*-isomers. As 'Experimental procedure' reported, ratio of diastereoisomeric mixture was difference for each compound. Tetra-*O*-trimethylsilylation of mixtures **18–21** and regioselective deprotection of the 7-*O*-TMS group followed by silica gel column chromatography finally gave single compounds **22–25**[48,57] as 2′-β-4′-β-pure *cis*-isomers. Methanesulfonylation of the 7-OH group and then SN2 reaction by potassium thioacetate gave compounds **26**-**29**. Key intermediates **30**-**33**[33–35,37–38,40,42] were prepared by deprotections of all TMS groups and an acetyl group. On the other hand, the Ns group of compound **34** was deprotected by 4-bromobenzenethiol under the basic condition, and then the olefin group was reduced by hydrogenation to give an azepane intermediate **35** (stereochemistry at the C-5′ position is not assigned). An amino group of **35**



Scheme 2 Synthesis of key intermediates **30** to **33** and **39**. Conditions: (a) *N,N′*-dicyclohexylcarbodiimide (DCC) or EDC·HCl, HOBt, DMF, r.t., 6–20 h; (b) TMSCl, HMDS, pyridine, r.t., 20 min–1 h; (c) 6 N AcOH or 2 N AcOH, MeOH, r.t., 40 min–6 h; (d) MsCl, NEt_3, CH_2Cl_2, 0 °C to r.t., 1 h; (e) AcSK, DMF, 80 °C, 1.5–3 h; (f) 1 N HCl, MeOH, r.t. or 0 °C, 5–100 min; (g) NaOMe, MeOH, r.t., 15 min–3 h; (h) 4-bromobenzenethiol, Cs_2CO_3, DMF, r.t., 2 h; (i) H_2 (0.95 MPa), Pd/C, MeOH, 40 °C, 70 h; (j) Boc_2O, LiOH·H_2O, dioxane:H_2O = 1:1, r.t. 3 h.

was protected by a Boc group to give **36**, and a key intermediate **39** was synthesized from **36** by the similar procedures as described for the preparation of **30**.

Synthesis of novel (7*S*)-4-(pyrimidin-5-yl)phenylthio LCM derivatives possessing piperidine or azepane as the C-6 side chain

Syntheses of novel (7*S*)-4-(pyrimidin-5-yl)phenylthio LCM derivatives possessing piperidine or azepane as the C-6 side chain are shown in Scheme 3. Compounds **40**-**43** and **48** were synthesized from key intermediates **30**-**33** and **39** by palladium-catalyzed cross-coupling reaction with 5-(4-bromophenyl)pyrimidine, respectively.[33-35,38,40,42,58,] The Boc group of **40**-**43** and **48** was finally removed with TFA to give desired compounds **44**-**47** and **49**.

Synthesis of divergent intermediate 54 and novel LCM derivatives possessing a 4-(2-(dimethylamino)ethyl)phenylthio group at the C-7 position

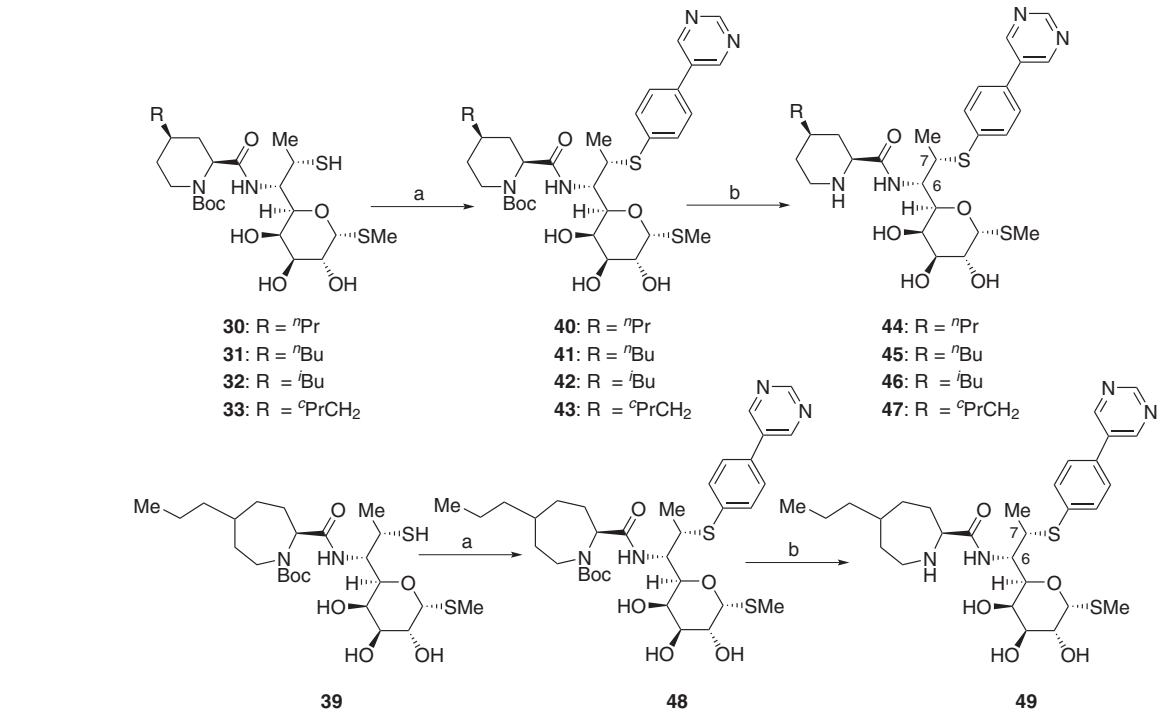
Because we had to develop a more divergent synthetic route than those exemplified in Schemes 2 and 3, we decided to apply the next key intermediate **54**. Syntheses of divergent intermediate **54** and novel LCM derivatives possessing a 4-(2-(dimethylamino)ethyl)phenylthio group at the C-7 position are shown in Scheme 4. Compound **50**[59] was synthesized by trifluoroacetylation of an amino group of MTL, and tetra-*O*-trimethylsilylation of all OH groups of **50** gave compound **51**. Divergent intermediate **54** was synthesized from **51** by the similar procedures as described for preparation of **30**. Palladium-catalyzed cross-coupling reaction of **54** with 2-(4-bromophenyl)-*N*,*N*-dimethylethanamine gave **55**, which was hydrolyzed in the presence of phase transfer catalyst under the basic condition to give diamine **56**. A coupling reaction of **56** with enantio-pure **15** provided desired **57** with all carbon's framework. Deprotection of the Boc group finally gave **58** and its reductive *N*-methylation provided compound **59**.

Synthesis of novel 4′-*cis*-(cyclopropylmethyl)piperidine LCM derivatives possessing a 4-substituted phenylthio group at the C-7 position

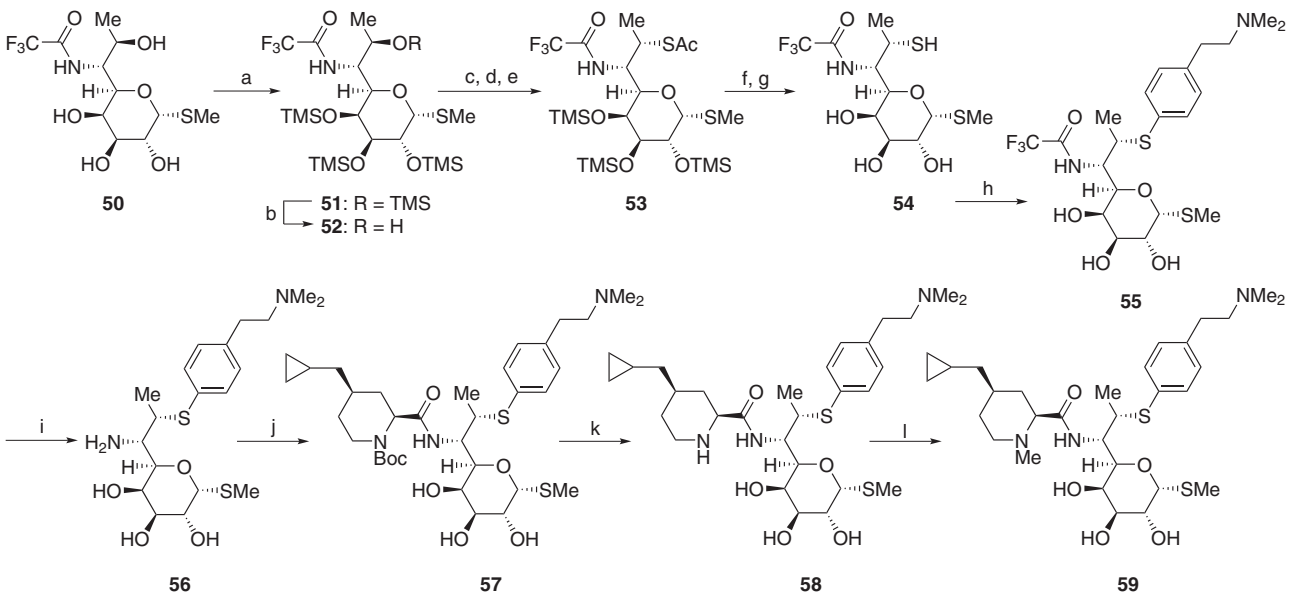
Syntheses of novel 4′-*cis*-(cyclopropylmethyl)piperidine LCM derivatives possessing a 4-substituted phenylthio group at the C-7 position are shown in Scheme 5. Compounds **60, 61** and **63**-**65** were synthesized from the key intermediate **33** by palladium-catalyzed cross-coupling reaction with the corresponding 4-substituted phenyl bromides. Reduction of **61** afforded saturated *N*-methylpiperidine **62** as a mixture of diastereoisomers at an *N*-methylpiperidine ring. The first half of desired compounds **66, 68**, **70**, **72**, **74** and **77** were prepared by deprotection of a Boc group and their free secondary amine was methylated by reductive alkylation to give the second half of desired compounds **67**, **69**, **71**, **73**, **75** and **78**, respectively. Compound **76** was also synthesized from **47** with the similar procedure. We confirmed that compound **76** derived from compound **33** had 4′-*cis*-stereochemistry by ROESY experiments. As the above, 4′-*cis*-stereochemistry of compound **12** was assigned.

SAR analysis of C-6 modified and (7*S*)-7-(4-(pyrimidin-5-yl)phenyl)thio-substituted LCM derivatives 44-47 and 49

We reported potent antibacterial activities of **1** possessing the (7*S*)-(4-(pyrimidin-5-yl)phenyl)thio group at the C-7 position. For the purpose of generating novel compounds possessing more potent antibacterial activities against resistant Gram-positive pathogens with

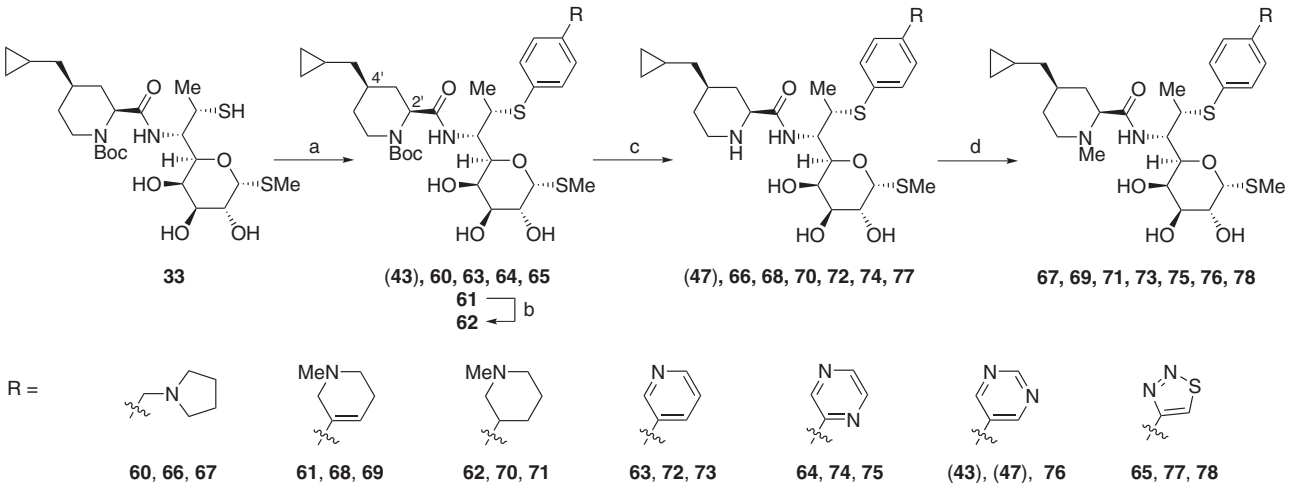


Scheme 3 Synthesis of novel (7*S*)-4-(pyrimidin-5-yl)phenylthio LCM derivatives possessing piperidine or azepane as the C-6 side chain. Conditions: (a) 5-(4-bromophenyl)pyrimidine, $Pd_2(dba)_3$, Xantphos, $^iPr_2NEt$, dioxane, reflux, 2-6 h; (b) TFA, CH_2Cl_2, −20 °C to r.t., 1.5–6 h.



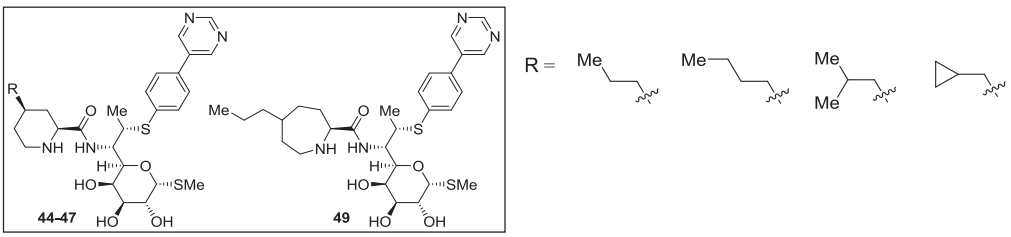
Scheme 4 Synthesis of divergent intermediate **54** and novel LCM derivatives possessing a 4-(2-(dimethylamino)ethyl)phenylthio group at the C-7 position. Conditions: (a) TMSCl, HMDS, pyridine, r.t., 1 h; (b) 6 N AcOH, MeOH, r.t., 15 min; (c) MsCl, NEt_3, $CHCl_3$, r.t., 1 h; (d) AcSK, DMF, 80 °C, 1.5 h; (e) TMSCl, HMDS, pyridine, r.t., 3 h; (f) 1 N HCl, MeOH, r.t., 10 min; (g) NaOMe, MeOH, r.t., 15 min; (h) 2-(4-bromophenyl)-*N*, *N*-dimethylethanamine, $Pd_2(dba)_3$, Xantphos, $^iPr_2NEt$, dioxane, reflux, 17 h; (i) 20% aq. KOH, *N*-benzyl-*N*, *N*, *N*-triethylammonium bromide, r.t., 4 h; (j) **15**, EDC·HCl, HOBt, DMF, r.t., 5.5 h; (k) TFA, CH_2Cl_2, 0 °C, 3.5 h; (l) 36% HCHO, $NaBH(OAc)_3$, AcOH, MeOH, r.t., 1 h.

an *erm* gene, we performed an SAR analysis of C-6-modified and (7*S*)-7-(4-(pyrimidin-5-yl)phenyl)thio-substituted LCM derivatives **44–47** and **49** (Table 2). According to our reported SAR studies, (7*S*) stereochemistry was selected among all novel derivatives.[40] Compound **44**, which possesses *n*-propyl-piperidine instead of *n*-propyl-pyrrolidine as the C-6 side chain, showed stronger activities against resistant *S. pneumoniae* with an *erm* gene than **1**. However, its antibacterial activity against resistant *S. pneumoniae* with both *erm* and *mef* genes (*S. pneumoniae*-6) was not sufficient (MIC: 1 μg ml^{-1}). Because there were a couple of reports[21,42,46] stating that elongation of a side chain in a piperidine ring enhanced antibacterial activity, we synthesized alternative derivatives with a longer carbon chain



Scheme 5 Synthesis of novel 4′-*cis*-(cyclopropylmethyl)piperidine LCM derivatives possessing an aliphatic- or aromatic-phenylthio group at the C-7 position. Conditions: (a) the corresponding 4-substituted phenylbromides $Pd_2(dba)_3$, Xantphos, $^iPr_2NEt$, dioxane, reflux, 4–5 h; (b) 4-methylbenzenesulfonohydrazide, toluene, reflux, 5.5 h; (c) TFA, CH_2Cl_2, −20 °C to r.t., 0.5–5 h; (d) 36% HCHO, $NaBH(OAc)_3$, AcOH, MeOH, r.t., 0.5–2 h.

Table 2 Antibacterial activities (MIC, μg ml^{-1}) of novel lincomycin derivatives modified at the C-6 position



Test organism[a]	*Characteristics*	44	45	46	47	49	*TEL*
Streptococcus pneumoniae DP1 TypeI	susceptible	0.015	0.015	0.015	≦0.008	≦0.008	≦0.008
S. pneumoniae -2	susceptible	0.015	0.015	0.03	≦0.008	≦0.008	≦0.008
S. pneumoniae -3	susceptible	0.015	0.03	0.015	0.015	≦0.008	≦0.008
S. pneumoniae -4	*ermAM* methylase (c)	0.5	1	2	0.03	0.12	0.5
S. pneumoniae -5	*ermAM* methylase (c)	0.25	1	2	0.03	0.12	2
S. pneumoniae -6	*ermAM* methylase (c) + *mefE*	1	2	2	0.06	0.25	1
S. pneumoniae -7	*ermAM* methylase (i)	0.06	0.25	NT	0.015	≦0.008	0.03
S. pneumoniae -8	*ermAM* methylase (i)	0.03	0.12	NT	0.015	NT	0.03
S. pneumoniae -9	*mefE* efflux	≦0.008	≦0.008	NT	≦0.008	≦0.008	0.06
Streptococcus pyogenes Cook	susceptible	0.015	≦0.008	0.015	0.015	≦0.008	≦0.008
S. pyogenes -2	*ermAM* methylase (c)	0.25	0.5	0.06	0.03	0.03	16
S. pyogenes -3	*mefE* efflux	0.015	0.03	0.015	0.015	≦0.008	0.25
Haemophilus influenzae	susceptible	2	4	16	1	2	0.5
H. influenzae -2	susceptible	2	4	16	1	2	2
H. influenzae -3	susceptible	8	16	>64	2	4	1
H. influenzae -4	⊿acr	0.06	0.12	0.5	0.03	0.03	0.25

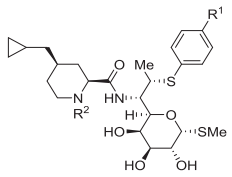
Abbreviations: c, constitutive; i, inducible; NT, not tested; TEL, telithromycin.
Gray shading strains are target strains.
[a]All strains except standard organisms were clinically isolated.

or a branched side chain. However, antibacterial activities of compounds **45** and **46** were not improved. On the other hand, both compounds **47** and **49** possessing a 4′-*cis*-(cyclopropylmethyl)piperidine-2-carbonyl and 5′-*n*-propylazepane-2-carbonyl group at the C-6 position exhibited potent antibacterial activities against resistant *S. pneumoniae* with *erm* gene. Because the cyclopropylmethyl analog (**47**) especially exhibited stronger activities against Gram-positive pathogens with an *erm* gene even compared with TEL, we chose a 4′-*cis*-cyclopropylmethyl moiety as the C-6 side chain for further medicinal chemistry.

Antibacterial activities of novel LCM derivatives 58, 59 and 66–71 possessing an aliphatic amine at the para-position of phenylthio group at the C-7 position

For the purpose of accumulating detail information of SAR on (7*S*)-7-(4-substituted-phenylthio) LCM derivatives with a 4′-*cis*-(cyclopropylmethyl)piperidine moiety, we synthesized novel derivatives possessing various substituents at the C-7 position with a set of R^2 = both '*N*-H' and '*N*-Me' analogs (Table 3). Consequently, compounds **58**, **66** and **68–71** showed potent antibacterial activities against target pathogens

Table 3 Antibacterial activities (MIC, μg ml^{-1}) of novel lincomycin derivatives modified at the C-7 position with an aliphatic moiety



	R^2 =	H	Me	H	Me	H	Me	H	Me	
Test organism[a]	*Characteristics*	**58**	**59**	**66**	**67**	**68**	**69**	**70**	**71**	*TEL*
Streptococcus pneumoniae DP1 TypeI	susceptible	0.03	≦0.008	0.03	0.015	0.015	0.06	0.06	0.015	≦0.008
S. pneumoniae -2	susceptible	0.03	≦0.008	0.03	0.03	0.015	0.06	0.06	0.03	≦0.008
S. pneumoniae -3	susceptible	0.06	0.03	0.06	0.06	0.015	0.06	0.06	0.03	≦0.008
S. pneumoniae -4	*ermAM* methylase (c)	0.5	1	0.25	0.5	0.06	0.25	0.25	0.25	0.5
S. pneumoniae -5	*ermAM* methylase (c)	0.5	0.5	0.25	0.5	0.06	0.25	0.25	0.25	2
S. pneumoniae -6	*ermAM* methylase (c) + *mefE*	0.5	2	0.25	1	0.06	0.5	0.12	0.5	1
S. pneumoniae -7	*ermAM* methylase (i)	0.03	0.015	0.03	0.06	0.03	0.06	0.06	0.06	0.03
S. pneumoniae -8	*ermAM* methylase (i)	NT	NT	NT	NT	0.03	0.06	0.06	0.06	0.03
S. pneumoniae -9	*mefE* efflux	0.015	≦0.008	0.015	≦0.008	0.015	0.03	0.03	0.015	0.06
Streptococcus pyogenes Cook	susceptible	0.015	0.015	0.06	0.06	0.015	0.03	0.03	0.03	≦0.008
S. pyogenes -2	*ermAM* methylase (c)	0.12	0.5	0.12	0.5	0.03	0.12	0.06	0.12	16
S. pyogenes -3	*mefE* efflux	0.03	0.015	0.06	0.12	0.015	0.03	0.03	0.03	0.25
Haemophilus influenzae	susceptible	2	2	1	4	0.5	2	1	2	0.5
H. influenzae -2	susceptible	4	1	4	2	1	2	4	4	2
H. influenzae -3	susceptible	4	4	4	8	2	4	4	8	1
H. influenzae -4	⊿acr	0.12	0.12	0.12	0.12	0.06	0.12	0.12	0.12	0.25

Abbreviations: c, constitutive; i, inducible; NT, not tested; TEL, telithromycin.
Gray shading strains are target strains.
[a]All strains except standard organisms were clinically isolated.

with an *erm* gene and their activities were relatively stronger even when compared with those of TEL. In addition, antibacterial activity of all compounds against *S. pyogenes* with an *erm* gene was significantly potent than that of TEL. We confirmed that combination of chemical modifications with the 4′-*cis*-(cyclopropylmethyl)piperidine group at the C-6 position and an aliphatic amine to the *para*-position of a phenylthio group at the C-7 position was important to enhance antibacterial activities against *S. pneumoniae* and *S. pyogenes* with an *erm* gene.

Antibacterial activities of novel LCM derivatives 47 and 72–78 possessing an aromatic amine at the *para*-position of a phenylthio group at the C-7 position

In order to expand possibilities of the combination modification at both the C-6 and C-7 positions, we synthesized novel derivatives possessing various aromatic amines as a substituent on the phenyl group with a set of both '*N*-H' and '*N*-Me' analogs (Table 4). All their antibacterial activities against target Gram-positive pathogens with an *erm* gene were also relatively stronger than those of TEL. To be more precise, compounds **47** and **72** showed potent activities in '*N*-H' analogs and compound **76** exhibited the strongest activities among all '*N*-Me' analogs in this article. As pharmacokinetic property must be different between '*N*-H' and '*N*-Me' analogs, it is important to select these two types of analogs for further development. Furthermore, antimicrobial activity of compounds **72**, **47** and **77** against *H. influenzae* was relatively strong among all LCM derivatives we reported, and their potency was stronger than that of clarithromycin and catching up with that of TEL. We also investigated antibacterial activity against *M. pneumoniae* (Table 4), because resistant *M. pneumoniae* is causing problems for respiratory infections in clinical sites. All evaluated compounds including **47** and **76** had significant antibacterial activity against resistant *M. pneumoniae*, which TEL was not effective against. We could generate several novel LCM derivatives exhibiting very strong antibacterial activities against resistant Gram-positive pathogens with *erm* and/or *mef* genes by combination modification at the C-6 position (the proline moiety) and the C-7 position. These derivatives were also effective against resistant *M. pneumoniae*.

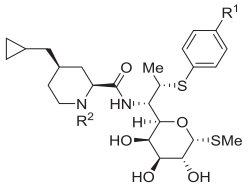
In vitro antibacterial activity (sensitivity distribution analysis) of selected compounds against sixty clinical isolates of *S. pneumoniae*

We evaluated the antibacterial activity of compounds **47**, **68**, **72**, **76**, **77** and TEL against 60 clinical isolates of *S. pneumoniae* including susceptible strains and resistant strains with *erm* and/or *mef* genes for sensitivity distribution analysis (Figure 2). MIC_{90} values of five novel LCM derivatives (0.06–0.125 μg ml^{-1}) were relatively smaller than that of TEL (0.25 μg ml^{-1}). Notably, **47** and **72** were significantly potent among tested compounds. These results reflect MIC values in Table 4 and it was suggested that these derivatives would also be effective against *S. pneumoniae* in clinical sites.

In vivo efficacy of 47 and 76 (subcutaneous administration) in rat pulmonary infection model with resistant *S. pneumoniae* with *erm* + *mef* genes and a *mef* gene

We finally investigated the *in vivo* efficacy of selected compounds in rat pulmonary infection model with resistant *S. pneumoniae* with *erm* + *mef* genes. Among derivatives reported in this study, *in vitro* activities of compounds **47** and **72** are rather strong (Figure 2). On the other hand, we had to clarify *in vivo* efficacy in the set of '*N*-H' and '*N*-Me' in the piperidine moiety (to evaluate '**72** and **73**' or '**47** and **76**'). As *in vitro* activities of **73** were slightly weaker than those of **76**, we decided to select the set of compounds **47** and **76** for *in vivo* evaluation. Compound **72** had weak hemolytic activity and thus compound **72** might not be appropriate for further evaluation. Compounds **47**, **76** and TEL were subcutaneously administered (10 mg kg^{-1}) to rats at 2 h after bacterial infection, and *in vivo* efficacies are shown in Figure 3a. Compound **47** exhibited strong *in vivo* efficacy (3 log reduction or more) against resistant *S. pneumoniae* with *erm* + *mef* genes and its efficacy was constant (small s.d. value) compared with that of TEL (<2 log reduction). For our

Table 4 Antibacterial activities (MIC, μg ml^{-1}) of optimized novel lincomycin derivatives modified at the C-7 position with an aromatic moiety



Test organism[a]	*Characheristics*	**72**	**73**	**74**	**75**	**47**	**76**	**77**	**78**	*TEL*
Streptococcus pneumoniae DP1 TypeI	susceptible	0.015	0.03	≦0.008	0.03	≦0.008	≦0.008	≦0.008	≦0.008	≦0.008
S. pneumoniae -2	susceptible	0.015	0.03	≦0.008	0.03	≦0.008	≦0.008	≦0.008	0.015	≦0.008
S. pneumoniae -3	susceptible	0.015	0.06	0.015	0.06	0.015	0.03	0.015	0.015	≦0.008
S. pneumoniae -4	*ermAM* methylase (c)	0.03	0.5	0.06	0.5	0.03	0.25	0.06	0.5	0.5
S. pneumoniae -5	*ermAM* methylase (c)	0.06	0.25	0.06	0.5	0.03	0.12	0.12	0.5	2
S. pneumoniae -6	*ermAM* methylase (c) + *mefE*	0.06	0.5	0.12	1	0.06	0.5	0.25	0.5	1
S. pneumoniae -7	*ermAM* methylase (i)	0.015	0.06	0.03	0.25	0.015	0.015	0.03	0.25	0.03
S. pneumoniae -8	*ermAM* methylase (i)	NT	NT	0.03	0.25	0.015	0.03	0.03	0.25	0.03
S. pneumoniae -9	*mefE* efflux	≦0.008	0.015	≦0.008	0.03	≦0.008	≦0.008	≦0.008	≦0.008	0.06
Streptococcus pyogenes Cook	susceptible	≦0.008	0.015	≦0.008	0.015	0.015	0.015	≦0.008	≦0.008	≦0.008
S. pyogenes -2	*ermAM* methylase (c)	0.06	0.25	0.03	0.25	0.03	0.12	0.03	0.12	16
S. pyogenes -3	*mefE* efflux	0.015	0.03	0.015	0.03	0.015	≦0.008	0.015	0.015	0.25
Haemophilus influenzae	susceptible	1	4	2	4	1	4	1	4	0.5
H. influenzae -2	susceptible	2	4	2	4	1	2	1	2	2
H. influenzae -3	susceptible	2	8	4	8	2	4	2	4	1
H. influenzae -4	⊿acr	0.03	0.12	0.03	0.12	0.03	0.06	0.03	0.03	0.25
Mycoplasma pneumoniae -1	susceptible	NT	NT	≦0.004	NT	≦0.004	≦0.004	≦0.004	NT	≦0.004
M. pneumoniae -2	A2063G	NT	NT	≦0.03	NT	≦0.03	≦0.03	≦0.03	NT	64

Abbreviations: c, constitutive; i, inducible; NT, not tested; TEL, telithromycin.
Gray shading strains are target strains.
[a]All strains except standard organisms were clinically isolated.

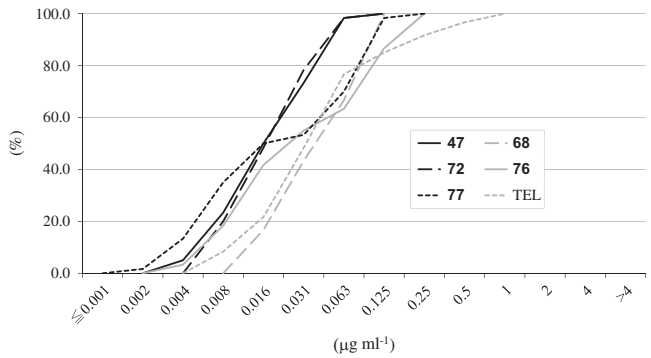


Figure 2 *In vitro* antibacterial activity (sensitivity distribution) of compounds **47**, **68**, **72**, **76**, **77** and TEL against 60 clinical isolates of *S. pneumoniae*.

references, we also evaluated *in vivo* efficacy of those by subcutaneous administration (3 mg kg^{-1}) to rats at 2 h after bacterial infection with *S. pneumoniae* with a *mef* gene, because resistant strains with a *mef* gene have increased in the US.[60] As a result shown in Figure 3b, **47** and **76** had significantly strong *in vivo* efficacy as expected on the basis of *in vitro* evaluation. *In vivo* efficacy of **76** (5 log reduction) was very constant (0 s.d. value) compared with that of TEL. Clinical efficacy of these novel LCM derivatives is expected from the above fundamental experimental data.

CONCLUSION

As the result of SAR studies at the 6-position of **44–47** and **49**, compound **47** possessing 4′-*cis*-(cyclopropylmethyl)piperidine showed significantly strong antibacterial activities against *S. pneumoniae* and *S. pyogenes* with an *erm* gene. On the basis of SAR, we synthesized novel analogs possessing 4′-*cis*-(cyclopropylmethyl)piperidine by transformation of a C-7 substituent. Consequently, compounds **47**, **68**, **72**, **76** and **77** (Figure 4) exhibited significantly strong activities against *S. pneumoniae* and *S. pyogenes* with an *erm* gene even when compared with those of TEL. Then, the *in vitro* antibacterial activities of compounds **47**, **68**, **72**, **76**, **77** and TEL were evaluated (sensitivity distribution analysis) against 60 clinical isolates of *S. pneumoniae* containing sensitive bacteria and resistant bacteria with *erm* and/or *mef* genes. As a result, compounds **47** and **72** showed relatively strong activities than that of TEL. Finally, the *in vivo* efficacy of compound **47** and its 1′-*N*-Me-derivative **76** was evaluated in the rat pulmonary infection model (subcutaneous administration) with resistant *S. pneumoniae* with *erm* + *mef* genes. Compound **47** exhibited strong and constant *in vivo* efficacy. Moreover, compounds **47** and **76** showed strong *in vivo* efficacy against resistant *S. pneumoniae* with a *mef* gene. These two compounds are under consideration toward next developing stage.

EXPERIMENTAL PROCEDURE

General methods

^{1}H NMR spectra were measured with a BRUKER Ascend 400 NMR spectrometer (BRUKER Corporation, Coventry, UK) for 400 MHz, JEOL JNM-GSX 400 NMR spectrometer (JEOL Ltd, Tokyo, Japan) for 400 MHz or a Varian Gemini 300 NMR spectrometer (Varian Inc., Palo Alto, CA, USA) for 300 MHz in $CDCl_3$ or CD_3OD. TMS (0 p.p.m.) in $CDCl_3$ or CD_3OD was used as an internal reference standard. Mass spectra were obtained on a JEOL JMS-700 mass spectrometer (JEOL Ltd) 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 Corporation, Tokyo, Japan). Column chromatography was performed with silica gel (Wakogel C200, Wako Pure Chemical Industries Ltd, Osaka, Japan). Preparative TLC was performed with silica gel (Merck, Darmstadt, Germany: TLC plates Silica gel 60 F254). All organic extracts were dried over anhydrous

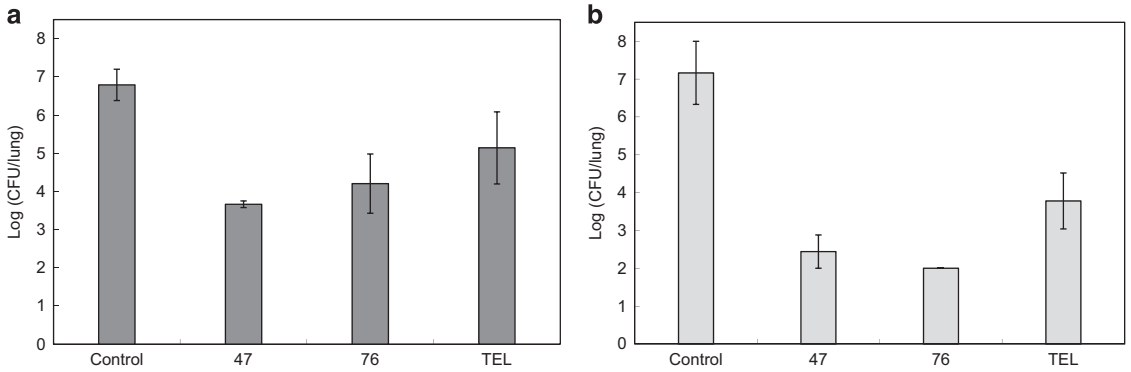


Figure 3 (**a**) *In vivo* efficacy of **47** and **76** in a rat pulmonary infection model with *S. pneumoniae* MSC06856 (*erm* + *mef*). (**b**) *In vivo* efficacy of **47** and **76** in a rat pulmonary infection model with *S. pneumoniae* MSC6729 (*mef*). Comparison of the efficacy of novel lincomycin derivatives **47** and **76** in a rat pulmonary neutropenic infection model with *S. pneumoniae* MSC06856 (*erm* + *mef*) and *S. pneumonia*e MSC06729 (*mef*). Three rats per group were rendered neutropenic and 10^6 CFU per rat of *S. pneumoniae* MSC06856 or *S. pneumonia*e MSC06729 was injected into the lung, followed by s.c. administration of the test compounds at 2 h after infection. The mean log10 CFU per lung recovered from the infected lung after 24 h is shown. Error bars represent the s.d.

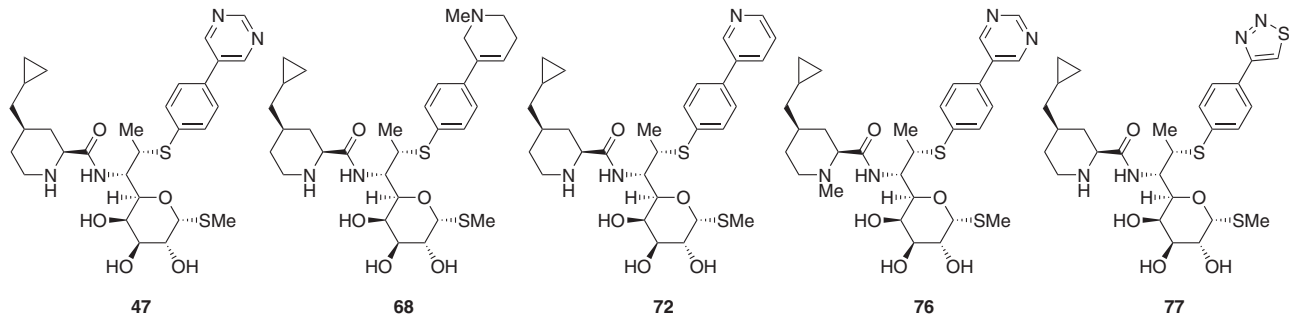


Figure 4 Structures of novel lincomycin derivatives possessing strong *in vitro* antibacterial activity.

$MgSO_4$ and the solvent was removed with a rotary evaporator under reduced pressure.

4-(Cyclopropylmethyl)pyridine (9)

To a solution of **8** (19.0 g, 204 mmol) in THF (136 ml) at −78 °C was added 2.0 M lithium diisopropylamide in tetrahydrofuran (THF) solution (204 ml, 408 mmol) and then was stirred in argon atmosphere at −40 °C for 20 min. The mixture was cooled to −78 °C. Then, bromocyclopropane (16.3 ml, 204 mmol) was added with dropwise to the solution. After stirring for 1 h, the solution was poured into saturated aqueous NH_4Cl. The desired compound was extracted with ethyl acetate, was washed with brine and then the organic phase was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The resulting residue was purified by distillation under reduced pressure (84 °C/8 mm Hg) to obtain the title compound (13.8 g, 51%) as colorless oil. Fast atom bombardment (FAB)–MS *m/z* 134 $(M+H)^+$ as $C_9H_{11}N$; ^1H NMR (400 MHz, $CDCl_3$) δ 0.18–0.26 (m, 2 H), 0.54–0.62 (m, 2 H), 0.93–1.05 (m, 1 H), 2.54 (d, *J*=7.1 Hz, 2 H), 7.17–7.23 (m, 2 H), 8.47–8.53 (m, 2 H).

4-(Cyclopropylmethyl)picolinonitrile (10)

To a solution of **9** (25.5 g, 191 mmol) in CH_2Cl_2 (300 ml) at 0 °C was added *m*-chloroperoxybenzoic acid (50.8 g, 191 mmol) and stirred at room temperature for 1 h. To the mixture was added $Na_2S_2O_3$ solution (75 g in 150 ml of H_2O). The solution was added to mixture of saturated aqueous $NaHCO_3$ (500 ml), saturated aqueous K_2CO_3 (40 ml) and $CHCl_3$ (500 ml). The organic phase was separated and then further extracted twice with $CHCl_3$ (500 ml)-isopropanol (100 ml), the organic phase was dried over Na_2SO_4, filtrated and concentrated under reduced pressure to obtain 4-(cyclopropylmethyl)pyridine *N*-oxide (30.7 g as crude). ^1H NMR (400 MHz, $CDCl_3$) δ 0.15–0.26 (m, 2 H), 0.54–0.66 (m, 2 H), 0.90–1.02 (m, 1 H), 2.54 (d, *J*=7.1 Hz, 2 H), 7.11–7.24 (m, 2 H), 8.08–8.18 (m, 2 H).

To a solution of 4-(cyclopropylmethyl)pyridine *N*-oxide (30.7 g) in CH_2Cl_2 (350 ml) were added trimethylsilanecarbonitrile (30.6 ml, 0.229 mmol) and dimethylcarbamic chloride (7.03 ml, 76.3 mmol) at room temperature. Then, dimethylcarbamic chloride (7.03 ml, 76.3 mmol) was added in two portions after 20 min interval to the mixture at 20 °C. The mixture was stirred at room temperature for 17 h. The solution was added to 10% aqueous K_2CO_3. The desired compound was extracted with CH_2Cl_2 and then 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 to hexane/ethyl acetate=17/3) to obtain the title compound (25.5 g, 84% in 2 steps) as a colorless oil. EI–MS *m/z* 158 $(M)^+$ as $C_{10}H_{10}N_2$; ^1H NMR (400 MHz, $CDCl_3$) δ 0.22–0.28 (m, 2 H), 0.60–0.70 (m, 2 H), 0.92–1.06 (m, 1 H), 2.61 (d, *J*=7.1 Hz, 2 H), 7.41–7.46 (m, 1 H), 7.64 (br dd, *J*=1.7, 0.7 Hz, 1 H), 8.55–8.64 (m, 1 H).

4-(Cyclopropylmethyl)picolinic acid (11)

To a solution of **10** (25.5 g, 161 mmol) in MeOH (300 ml) was added 5 M aqueous NaOH (250 ml) and stirred at 50 °C for 8 h. The mixture was cooled down to 0 °C, added to 5 M aqueous HCl (250 ml) at 0 °C and then concentrated under reduced pressure to remove MeOH. The solution was adjusted at pH 3 by 1 M aqueous HCl, extracted with $CHCl_3$ (500 ml)-isopropanol (150 ml) and then the organic phase was dried over Na_2SO_4, filtrated and concentrated under reduced pressure to obtain the title compound (27.6 g, 97%) as a colorless solid. FAB–MS *m/z* 178 $(M+H)^+$ as $C_{10}H_{11}NO_2$; ^1H NMR (400 MHz, $CDCl_3$) δ 0.22-0.30 (m, 2 H), 0.60–0.67 (m, 2 H), 0.95–1.11 (m, 1 H), 2.68 (d, *J*=7.1 Hz, 2 H), 7.48–7.54 (m, 1 H), 8.15-8.20 (m, 1 H), 8.58 (d, *J*=5.1 Hz, 1 H).

N-(*tert*-Butoxycarbonyl)-4-(cyclopropylmethyl)piperidine-2-carboxylic acid ((±)-12)

To a solution of **11** (6.90 g, 38.9 mmol) in AcOH (62 ml) was added PtO_2 (442 mg) and then vigorously stirred in hydrogen atmosphere at room temperature for 24 h. The mixture was filtrated with celite and concentrated under reduced pressure to obtain 4-(cyclopropylmethyl)piperidine-2-carboxylic acid (5.3 g as crude). For the qualified analytical purpose, the above crude compound was purified by reverse-phase column chromatography (0.1% aqueous TFA/CH_3CN = 90/10 to 10/90) to obtain the highly purified 4-(cyclopropylmethyl)piperidine-2-carboxylic acid TFA salt as a colorless solid. ESI–MS *m/z* 184 $(M+H)^+$ as $C_{10}H_{17}NO_2$; ^1H NMR (400 MHz, D_2O) δ −0.08 to 0.02 (m, 2 H), 0.31–0.41 (m, 2 H), 0.58–0.72 (m, 1 H), 1.10–1.43 (m, 4 H), 1.71–1.85 (m, 1 H), 1.92–2.03 (m, 1 H), 2.35–2.45 (m, 1 H), 2.91–3.03 (m, 1 H), 3.39–3.48 (m, 1 H), 3.85–3.94 (m, 1 H).

To a solution of the above disubstituted piperidine (5.3 g) in 1,4-dioxane (100 ml) were added 2 M aqueous NaOH (74.0 ml, 148 mmol) and di-*tert*-butyl dicarbonate (14.3 ml, 62.2 mmol) and then stirred at room temperature for 15 h. The mixture was concentrated under reduced pressure to remove 1,4-dioxane. The solution was adjusted at pH 8 by1 M aqueous NaOH. Then, to the aqueous phase was added H_2O, washed with Et_2O, adjusted at pH 4 by 1 M aqueous HCl, extracted with ethyl acetate and then the organic phase was dried over Na_2SO_4, filtrated and concentrated under reduced pressure to obtain the title compound (10.5 g, 96% in 2 steps) as a racemate of *cis*-isomers as a colorless solid. It was reported that hydrogenation of disubstituted pyridine in the presence of PtO_2 resulted in an approximately 1:1 mixture of two isomeric *cis*-products by Birkenmeyer *et al.*[49] ^1H NMR (400 MHz, $CDCl_3$) δ −0.04 to 0.08 (m, 2 H), 0.36–0.50 (m, 2 H), 0.60–0.73 (m, 1 H), 1.12–1.32 (m, 2 H), 1.35–1.55 (m, 1 H), 1.45 (s, 9 H), 1.66–1.89 (m, 3 H), 2.02–2.15 (m, 1 H), 3.55–3.60 (m, 2 H), 4.22–4.35 (m, 1 H).

2-Benzyl (2*S*, 4*R*)-*N*-(*tert*-butyl)-4-(cyclopropylmethyl)piperidine-1,2-dicarboxylate (13) 2-Benzyl (2*R*, 4*S*)-*N*-(*tert*-butyl)-4-(cyclopropylmethyl)piperidine-1,2-dicarboxylate (14)

To a solution of (±)-**12** (113 mg, 0.399 mmol) in CH_3CN (1 ml) were added diisopropylethylamine (0.104 ml, 0.599 mmol) and benzylbromide (0.652 ml, 0.439 mmol), and then stirred at room temperature for 48 h. The solution was added to saturated aqueous $NaHCO_3$. The desired compound was extracted with ethyl acetate and then the organic phase was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The resulting residue was purified by preparative TLC (hexane/ethyl acetate = 5/1) to obtain 2-benzyl *N*-(*tert*-butyl) 4-(cyclopropylmethyl)piperidine-1,2-dicarboxylate (133 mg, 89.3%) as a colorless oil. The above colorless oil (32.0 g) was further purified by column chromatography (Chiralpak AD-H, *n*-hexane/IPA = 98/2) to obtain **13** (11.1 g, 35%) and **14** (11.0 g, 34%) as a colorless solid both. These enantiomers could be independently analyzed by the following condition: Chiralpak AD-H, 0.46 cm I.D ×25 cm, *n*-hexane/IPA = 98/2, 1.0 ml min^{-1}, 40 °C, 208 nm. **13**: $[\alpha]_D^{27}$ −24.8° (*c* 0.65, $CHCl_3$); ESI–MS *m/z* 374 $(M+H)^+$ as $C_{22}H_{31}NO_4$; ^1H NMR (400 MHz, $CDCl_3$) δ −0.12 to −0.02 (m, 2 H), 0.32–0.44 (m, 2 H), 0.52–0.66 (m, 1 H), 0.99–1.18 (m, 2 H), 1.30–1.50 (m, 1 H), 1.41 (s, 9 H), 1.69–1.88 (m, 3 H), 2.04 (ddd, *J* = 13.5, 6.5, 4.6 Hz, 1 H), 3.08–3.70 (m, 2 H), 4.38 (t, *J* = 6.3 Hz, 1 H), 5.08–5.23 (m, 2 H), 7.27–7.42 (m, 5 H). **14**: $[\alpha]_D^{26}$ +24.2° (*c* 1.03, $CHCl_3$); ESI–MS *m/z* 374 $(M+H)^+$ as $C_{22}H_{31}NO_4$. Compound **14** showed the exactly same ^1H NMR spectrum with that of **13**.

(2*S*, 4*R*)- *N*-(*tert*-Butoxycarbonyl)-4-(cyclopropylmethyl)piperidine-2-carboxylic acid (15)

To a solution of **13** (1.51 g, 4.04 mmol) in MeOH (45 ml) was added Pd/C (0.166 g) and then vigorously stirred in hydrogen atmosphere at room temperature for 1 h. The mixture was filtrated with celite and concentrated under reduced pressure to obtain the title compound (1.15 g, quant) as a colorless solid. $[\alpha]_D^{27}$ −16.3° (*c* 0.40, MeOH); ESI–MS *m/z* 284 $(M+H)^+$ as $C_{15}H_{25}NO_4$; time-of-flight (TOF)–ESI–HR-MS $(M-H)^-$ calcd for $C_{15}H_{25}NO_4$: 282.1705, found: 282.1718 ; Compound **15** showed the exactly same ^1H NMR spectrum with that of (±)-**12**.

(2*S*, *Z*)-1-(2-Nitrophenylsulfonyl)-5-*n*-propyl-2, 3, 6, 7-tetrahydro-1*H*-azepine-2-carboxylic acid (17)

To a solution of **16** (72 mg, 0.19 mmol) in 1,4-dioxane (0.8 ml)-H_2O (0.2 ml) was added $LiOH{\cdot}H_2O$ (23.7 mg, 0.56 mmol) and then stirred at room temperature for 5 h. The mixture was diluted with H_2O, washed with Et_2O. The aqueous phase was adjusted at pH 3 by citric acid. The desired compound was extracted with ethyl acetate and then the organic phase was dried over Na_2SO_4, filtrated and concentrated under reduced pressure to obtain the title compound (70 mg as crude). This crude compound including a trace amount of citric acid could not be purified. ESI–MS *m/z* 369 $(M+H)^+$ as $C_{16}H_{20}N_2O_6S$; ^1H NMR (400 MHz, CD_3OD) δ 0.73 (t, *J* = 7.4 Hz, 3 H), 1.16–1.31 (m, 2 H), 1.73–1.82 (m, 2 H), 2.15–2.26 (m, 2 H), 2.41–2.51 (m, 1 H), 2.62–2.70 (m, 1 H), 3.42 (ddd, *J* = 14.5, 7.8, 5.2 Hz, 1 H), 3.72 (dt, *J* = 14.6, 4.7 Hz, 1 H), 4.71 (dd, *J* = 7.1, 3.7 Hz, 1 H), 5.37 (t, *J* = 6.5 Hz, 1 H), 7.56–7.71 (m, 3 H), 7.95-8.03 (m, 1 H).

Mixture 18 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

To a solution of (±)-**5** (11.7 g, 43.1 mmol) in DMF (100 ml) were added 1-hydroxybenzotriazole (7.55 g, 55.8 mmol), *N,N*′-dicyclohexylcarbodiimide (10.7 g, 51.9 mmol) and MTL (14.2 g, 56.1 mmol) and stirred at room temperature for 12 h. To the mixture was added H_2O and then the solution was filtrated, and ethyl acetate and saturated aqueous $NaHCO_3$ were added to the filtrate. The desired compound was extracted with ethyl acetate, extracted with $CHCl_3$ and then 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 obtain 6-*N*-(*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 filtrated off and ethyl acetate solution was concentrated under reduced pressure to obtain the mixture **18** (13.5 g, 20% de ((2′*S*, 4′*R*) : (2′*R*, 4′*S*) = 60:40)) as a colorless solid.

Mixture 19 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 (±)-**6** (12.6 g, 44.2 mmol), 1-hydroxybenzotriazole (7.77 g, 57.5 mmol), *N,N*′-dicyclohexylcarbodiimide (11.0 g, 53.3 mmol) and MTL (14.6 g, 57.5 mmol) in DMF (120 ml) were treated for 20 h according to the similar procedure as described for the preparation of mixture **18** to afford 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 (14.53 g) was added ethyl acetate, and insoluble matter was filtrated off and ethyl acetate solution was concentrated under reduced pressure to obtain the mixture **19** (8.15 g, 80% de ((2′*S*, 4′*R*) : (2′*R*, 4′*S*) = 90:10)) as a colorless solid.

Mixture 20 of methyl 6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*i*-butyl)piperidine-2′-carbonyl)-α-thiolincosaminide and methyl 6-*N*-((2′*R*, 4′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*i*-butyl)piperidine-2′-carbonyl)-α-thiolincosaminide

To a solution of (±)-**7** (6.00 g, 21.0 mmol) in DMF (57 ml) were added 1-hydroxybenzotriazole (2.84 g, 21.0 mmol), *N,N*′-dicyclohexylcarbodiimide (5.20 g, 25.2 mmol) and MTL (5.40 g, 21.3 mmol), and stirred at room temperature for 6 h. To the mixture were added H_2O and ethyl acetate, and then the mixture was filtrated. The desired compound was extracted with ethyl acetate, washed with saturated aqueous $KHCO_3$ and then the organic phase was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. To the resulting residue was added ethyl acetate, and insoluble matter was filtrated off and ethyl acetate solution was concentrated under reduced pressure. To the

resulting residue was added toluene, and insoluble matter was filtrated off and toluene solution was concentrated under reduced pressure to obtain the mixture **20** (5.7 g, 90% de ((2′*S*, 4′*R*) : (2′*R*, 4′*S*) = 95:5)) as a colorless solid.

Mixture 21 of methyl 6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-α-thiolincosaminide and methyl 6-*N*-((2′*R*, 4′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-α-thiolincosaminide

Compound (±)-**12** (44.2 g, 156 mmol), 1-hydroxybenzotriazole monohydrate (28.6 g, 187 mmol), *N,N′*-dicyclohexylcarbodiimide (35.8 g, 174 mmol) and MTL (47.4 g, 187 mmol) in DMF (300 ml) were treated for 13 h. To the mixture were added ethyl acetate and acetone, and then the solution was filtrated and concentrated under reduced pressure. To the resulting residue were added ethyl acetate, and the organic layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, and then filtrated and concentrated under reduced pressure. To the resulting residue was added ethyl acetate, and insoluble matter was filtrated off and ethyl acetate solution was concentrated under reduced pressure. To the resulting residue was added toluene, and insoluble matter was filtrated off and toluene solution was concentrated under reduced pressure to obtain the mixture **21** (36 g, 80% de ((2′*S*, 4′*R*) : (2′*R*, 4′*S*) = 90:10)) as a colorless solid. FAB–MS *m/z* 519 $(M+H)^+$ as $C_{24}H_{42}N_2O_8S$.

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 (22)

To a solution of mixture **18** (13.5 g, 26.6 mmol, 20% de ((2′*S*, 4′*R*) : (2′*R*, 4′*S*) = 60:40)) in pyridine (50 ml) were added trimethylchlorosilane (17.0 ml, 133 mmol) and hexamethyldisilazane (27.9 ml, 133 mmol), and stirred at room temperature for 40 min, then the solution was added to saturated aqueous $NaHCO_3$. The desired compound was extracted with ethyl acetate, washed with saturated aqueous NaCl and then the organic phase was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. To the resulting residue were added methanol (138 ml) and 6 N acetic acid (5.8 ml), and stirred at room temperature for 2.5 h. The mixture was added to saturated aqueous $NaHCO_3$ and concentrated under reduced pressure to remove MeOH. The desired compound was extracted with ethyl acetate, and then 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 = 19/1 to 3/1) to obtain the title compound (9.28 g, 48% (80% based on (2′*S*, 4′*R*) isomer) in 2 steps from mixture **18**) as a colorless solid. ESI–MS *m/z* 723 $(M+H)^+$ as $C_{32}H_{66}N_2O_8SSi_3$; ^{1}H NMR (400 MHz, CD_3OD) δ 0.14 (s, 9 H), 0.16 (s, 9 H), 0.20 (s, 9 H), 0.90 (t, *J* = 7.0 Hz, 3 H), 1.16 (d, *J* = 6.2 Hz, 3 H), 1.22-1.39 (m, 4 H), 1.40–1.49 (m, 1 H), 1.46 (s, 9 H), 1.52–1.74 (m, 2 H), 1.75–1.87 (m, 1 H), 1.90–2.01 (m, 1 H), 2.04 (s, 3 H), 3.41–3.61 (m, 2 H), 3.75 (dd, *J* = 9.6, 2.5 Hz, 1 H), 3.78–3.87 (m, 1 H), 4.07–4.20 (m, 3 H), 4.23–4.32 (m, 2 H), 5.17 (d, *J* = 5.4 Hz, 1 H).

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 (23)

Mixture **19** (8.15g, 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 **22**, and then the crude compound 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 **22** to afford **23** (8.20 g, 71% (79% based on (2′*S*, 4′*R*) isomer) in 2 steps from mixture **19**) as a colorless solid. ESI–MS *m/z* 737 $(M+H)^+$ as $C_{33}H_{68}N_2O_8SSi_3$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.14 (s, 18 H), 0.19 (s, 9 H), 0.82-0.93 (m, 3 H), 1.16 (d, *J* = 6.6 Hz, 3 H), 1.18–1.36 (m, 7 H), 1.38–1.55 (m, 2 H), 1.46 (s, 9 H), 1.77–1.89 (m, 1 H), 2.00–2.10 (m, 1 H), 2.06 (s, 3 H), 2.90–3.03 (m, 1 H), 3.30–3.54 (m, 2 H), 3.60 (dd, *J* = 9.6, 2.6 Hz, 1 H), 3.85–3.92 (m, 1 H), 3.94–4.07 (m, 2 H), 4.07–4.17 (m, 1 H), 4.26–4.40 (m, 1 H), 5.17 (d, *J* = 5.4 Hz, 1 H), 6.32 (br d, *J* = 9.0 Hz, 1 H).

Methyl 6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*i*-butyl) piperidine-2′-carbonyl)-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (24)

Mixture **20** (5.70 g, 10.9 mmol, 90% de ((2′*S*, 4′*R*) : (2′*R*, 4′*S*) = 95:5), trimethylchlorosilane (13.2 ml, 103.6 mmol) and hexamethyldisilazane (21.7 ml, 104 mmol) in pyridine (21 ml) were treated for 1 h according to the similar procedure as described for the preparation of **22**, and then the crude compound and 6 N acetic acid (2.4 ml) in MeOH (61 ml) were treated for 6 h according to the similar procedure as described for the preparation of **22** to afford **24** (7.25 g, 90% (95% based on (2′*S*, 4′*R*) isomer) in 2 steps from mixture **20**) as a colorless solid. ESI–MS *m/z* 737 $(M+H)^+$ as $C_{33}H_{68}N_2O_8SSi_3$; ^{1}H NMR (400 MHz, CD_3OD) δ 0.14 (s, 9 H), 0.16 (s, 9 H), 0.20 (s, 9 H), 0.88 (d, *J* = 6.5 Hz, 6 H), 1.08–1.33 (m, 3 H), 1.17 (d, *J* = 6.4 Hz, 3 H), 1.46 (s, 9 H), 1.53–1.73 (m, 3 H), 1.77–1.88 (m, 1 H), 1.92–2.01 (m, 1 H), 2.05 (s, 3 H), 3.44–3.58 (m, 2 H), 3.74 (dd, *J* = 9.6, 2.6 Hz, 1 H), 3.78–3.88 (m, 1 H), 4.05–4.17 (m, 3 H), 4.23–4.32 (m, 2 H), 5.17 (d, *J* = 5.4 Hz, 1 H).

Methyl 6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (25)

Mixture **21** (35.0 g, 67.5 mmol, 80% de ((2′*S*, 4′*R*) : (2′*R*, 4′*S*) = 90:10), trimethylchlorosilane (43.1 ml, 337 mmol) and hexamethyldisilazane (70.6 ml, 337 mmol) in pyridine (130 ml) were treated for 1 h according to the similar procedure as described for the preparation of **22**, and then the crude compound and 6 N acetic acid (16.9 ml) in MeOH (350 ml) were treated for 140 min according to the similar procedure as described for the preparation of **22** to afford **25** (30.6 g, 62% (69% based on (2′*S*, 4′*R*) isomer) in 2 steps from mixture **21**) as a colorless solid. FAB–MS *m/z* 735 $(M+H)^+$ as $C_{33}H_{66}N_2O_8SSi_3$; ^{1}H NMR (400 MHz, CD_3OD) δ −0.04 to 0.04 (m, 2 H), 0.13 (s, 9 H), 0.16 (s, 9 H), 0.20 (s, 9 H), 0.40–0.49 (m, 2 H), 0.65–0.78 (m, 1 H), 1.17 (d, *J* = 6.2 Hz, 3 H), 1.17–1.35 (m, 2 H), 1.34–1.47 (m, 1 H), 1.47 (s, 9 H), 1.64–1.91 (m, 3 H), 1.98–2.10 (m, 1 H), 2.05 (s, 3 H), 3.40–3.66 (m, 2 H), 3.75 (dd, *J* = 9.7, 2.6 Hz, 1 H), 3.76–3.85 (m, 1 H), 4.12 (dd, *J* = 9.7, 5.4 Hz, 1 H), 4.13–4.19 (m, 1 H), 4.20–4.35 (m, 3 H), 5.18 (d, *J* = 5.4 Hz, 1 H).

Methyl (7*S*)-7-acetylthio-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-propyl)piperidine-2′-carbonyl)-7-deoxy-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (26)

To a solution of **22** (500 mg, 0.69 mmol) in CH_2Cl_2 (2 ml) at 0 °C were added Et_3N (291 μl, 2.08 mmol) and methanesulfonyl chloride (107 μl, 1.38 mmol), and stirred at room temperature for 1 h. The mixture was added to saturated aqueous $NaHCO_3$, extracted with ethyl acetate, dried over Na_2SO_4 and concentrated under reduced pressure to obtain methyl 6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-propyl)piperidine-2′-carbonyl)-7-*O*-methanesulfonyl-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide as a crude compound (530 mg). To a solution of this crude compound (530 mg) in DMF (3.0 ml) was added AcSK (396 mg, 3.47 mmol) and stirred at 80 °C for 2 h. The mixture was added to saturated aqueous $NaHCO_3$, then extracted with ethyl acetate, washed with 10% aqueous NaCl, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane to hexane/ethyl acetate = 85/15) to obtain the title compound (357 mg, 66% in 2 steps from **22**) as a colorless solid. FAB–MS *m/z* 781 $(M+H)^+$ as $C_{34}H_{68}N_2O_8S_2Si_3$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.126 (s, 9 H), 0.13 (s, 9 H), 0.18 (s, 9 H), 0.88 (t, *J* = 6.9 Hz, 3 H), 1.10–1.21 (m, 1 H), 1.23–1.38 (m, 4 H), 1.35 (d, *J* = 6.8 Hz, 3 H), 1.42–1.60 (m, 2 H), 1.49 (s, 9 H), 1.80–1.94 (m, 1 H), 1.95–2.08 (m, 1 H), 1.99 (s, 3 H), 2.29 (s, 3 H), 3.00–3.18 (m, 1 H), 3.57 (dd, *J* = 9.5, 2.2 Hz, 1 H), 3.63-3.83 (m, 2 H), 3.87–4.04 (m, 2 H), 4.13 (dd, *J* = 9.5, 5.6 Hz, 1 H), 4.22–4.33 (m, 1 H), 4.50–4.62 (m, 1 H), 5.15 (d, *J* = 5.6 Hz, 1 H), 6.04–6.37 (m, 1 H).

Methyl (7*S*)-7-acetylthio-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-butyl)piperidine-2′-carbonyl)-7-deoxy-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (27)

Compound **23** (1.01 g, 1.37 mmol), Et_3N (490 μl, 3.48 mmol) and methanesulfonyl chloride (220 μl, 2.79 mmol) in CH_2Cl_2 (20 ml) at 0 °C were treated

for 1 h according to the similar procedure as described for the preparation of **26**, and then a crude mesylate (1.17 g) and AcSK (992 mg, 8.68 mmol) in DMF (13 ml) at 80 °C were treated for 3 h according to the similar procedure as described for the preparation of **26** to afford **27** (565 mg, 52% in 2 steps from **23**) as a colorless solid. FAB–MS *m/z* 795 $(M+H)^+$ as $C_{35}H_{70}N_2O_8S_2Si_3$; ^1H NMR (400 MHz, $CDCl_3$) δ 0.11 (s, 9 H), 0.12 (s, 9 H), 0.17 (s, 9 H), 0.78–0.95 (m, 3 H), 1.07–1.32 (m, 7 H), 1.34 (d, *J*=6.9 Hz, 3 H), 1.38–1.60 (m, 2 H), 1.48 (s, 9 H), 1.78–1.95 (m, 1 H), 1.95–2.11 (m, 1 H), 1.97 (s, 3 H), 2.27 (s, 3 H), 3.00–3.18 (m, 1 H), 3.54 (dd, *J*=9.6, 2.2 Hz, 1 H), 3.62–3.85 (m, 2 H), 3.85–4.05 (m, 2 H), 4.06–4.16 (m, 1 H), 4.21-4.31 (m, 1 H), 4.50–4.62 (m, 1 H), 5.14 (d, *J*=5.5 Hz, 1 H), 6.05–6.48 (m, 1 H).

Methyl (7*S*)-7-acetylthio-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*i*-butyl)piperidine-2′-carbonyl)-7-deoxy-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (28)

Compound **24** (1.01 g, 1.37 mmol), Et_3N (930 µl, 6.85 mmol) and methanesulfonyl chloride (210 µl, 2.74 mmol) in CH_2Cl_2 (20 ml) at 0 °C were treated for 1 h according to the similar procedure as described for the preparation of **26**, and then the crude mesylate (1.17 g) and AcSK (470 mg, 4.11 mmol) in DMF (6 ml) at 80 °C were treated for 3 h according to the similar procedure as described for the preparation of **26** to afford **28** (598 mg, 55% in 2 steps from **24**) as a colorless solid.

Methyl (7*S*)-7-acetylthio-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (29)

Compound **25** (7.40 g, 10.1 mmol), Et_3N (4.24 ml, 30.3 mmol) and methanesulfonyl chloride (1.56 ml, 20.2 mmol) in $CHCl_3$ (70 ml) at 0 °C were treated and then the solution was stirred at room temperature for 1 h according to the similar procedure as described for the preparation of **26** and then, the crude mesylate and AcSK (5.76 g, 50.5 mmol) in DMF (75 ml) at 80 °C were treated for 1.5 h according to the similar procedure as described for the preparation of **26** to afford **29** (4.30 g, 54% in 2 steps from **25**) as a colorless solid. ESI–MS *m/z* 793 $(M+H)^+$ as $C_{35}H_{68}N_2O_8S_2Si_3$; ^1H NMR (400 MHz, $CDCl_3$) δ −0.05 to 0.03 (m, 2 H), 0.125 (s, 9 H), 0.131 (s, 9 H), 0.18 (s, 9 H), 0.38–0.45 (m, 2 H), 0.60-0.73 (m, 1 H), 1.17–1.29 (m, 3 H), 1.35 (d, *J*=6.8 Hz, 3 H), 1.50 (s, 9 H), 1.59–1.72 (m, 2 H), 1.86–1.97 (m, 1 H), 2.00 (s, 3 H), 2.06–2.14 (m, 1 H), 2.29 (s, 3 H), 3.03–3.18 (m, 1 H), 3.58 (dd, *J*=9.6, 2.3 Hz, 1 H), 3.67–3.84 (m, 2 H), 3.88–4.02 (m, 2 H), 4.12 (dd, *J*=9.5, 5.4 Hz, 1 H), 4.30–4.38 (m, 1 H), 4.52–4.60 (m, 1 H), 5.16 (d, *J*=5.4 Hz, 1 H), 6.10–6.45 (m, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-propyl)piperidine-2′-carbonyl)-7-deoxy-7-mercapto-α-thiolincosaminide (30)

To a solution of **26** (341 mg, 0.436 mmol) in MeOH (4 ml) was added 1 N HCl (2.5 ml) and stirred at room temperature for 5 min. The mixture was added to saturated aqueous $NaHCO_3$, concentrated under reduced pressure to remove MeOH until half volume, extracted with ethyl acetate, dried over Na_2SO_4 and concentrated under reduced pressure to obtain methyl (7*S*)-7-acetylthio-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-propyl)piperidine-2′-carbonyl)-7-deoxy-α-thiolincosaminide (246.5 mg, quant) as a colorless solid. To a solution of this intermediate (244 mg, 0.432 mmol) in MeOH (2.5 ml) was added 28% NaOMe/MeOH solution (251 µl, 1.30 mmol), stirred at room temperature for 20 min. The mixture was added to a saturated aqueous NH_4Cl, extracted with ethyl acetate, washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane to hexane/ethyl acetate=96/4) to obtain the title compound (234 mg, 96%) as a colorless solid. ESI–MS *m/z* 523 $(M+H)^+$ as $C_{23}H_{42}N_2O_7S_2$; ^1H NMR (400 MHz, CD_3OD) δ 0.92 (t, *J*=7.0 Hz, 3 H), 1.24–1.41 (m, 5 H), 1.30 (d, *J*=7.0 Hz, 3 H), 1.46 (s, 9 H), 1.52–1.64 (m, 2 H), 1.77–1.90 (m, 1 H), 1.94–2.06 (m, 1 H), 2.15 (s, 3 H), 3.40–3.64 (m, 2 H), 3.46 (dq, *J*=7.0, 2.4 Hz, 1 H), 3.54 (dd, *J*=10.3, 3.4 Hz, 1 H), 3.94–4.01 (m, 1 H), 4.06 (dd, *J*=10.3, 5.6 Hz, 1 H), 4.18–4.26 (m, 2 H), 4.32 (dd, *J*=9.8, 2.4 Hz, 1 H), 5.25 (d, *J*=5.6 Hz, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-butyl)piperidine-2′-carbonyl)-7-deoxy-7-mercapto-α-thiolincosaminide (31)

To a solution of **27** (565 mg, 0.711 mmol) in MeOH (5.6 ml) was added 1 N HCl (5.6 ml) and stirred at room temperature for 100 min. The mixture was added to 8% aqueous $NaHCO_3$, extracted with ethyl acetate, washed with 25% aqueous NaCl, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=30/1) to obtain methyl (7*S*)-7-acetylthio-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-butyl)piperidine-2′-carbonyl)-7-deoxy-α-thiolincosaminide (378 mg, 91.8%) as a colorless solid. To a solution of this intermediate (378 mg, 0.652 mmol) in MeOH (4 ml) was added NaOMe (115 mg, 2.02 mmol) and stirred at room temperature for 3 h. The mixture was added to 8% aqueous $NaHCO_3$, extracted with ethyl acetate, washed with 25% aqueous NaCl, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=40/1) to obtain the title compound (373 mg, quant) as a colorless solid. ESI–MS *m/z* 537 $(M+H)^+$ as $C_{24}H_{44}N_2O_7S_2$; ^1H NMR (400 MHz, CD_3OD) δ 0.87–0.95 (m, 3 H), 1.23-1.40 (m, 7 H), 1.30 (br d, *J*=7.2 Hz, 3 H), 1.46 (s, 9 H), 1.50–1.65 (m, 2 H), 1.77–1.89 (m, 1 H), 1.96–2.06 (m, 1 H), 2.15 (s, 3 H), 3.42–3.60 (m, 2 H), 3.45 (dq, *J*=7.1, 2.3 Hz, 1 H), 3.54 (dd, *J*=10.3, 3.4 Hz, 1 H), 3.94–4.00 (m, 1 H), 4.06 (dd, *J*=10.1, 5.6 Hz, 1 H), 4.10–4.26 (m, 2 H), 4.32 (dd, *J*=9.8, 2.3 Hz, 1 H), 5.24 (d, *J*=5.6 Hz, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*i*-butyl)piperidine-2′-carbonyl)-7-deoxy-7-mercapto-α-thiolincosaminide (32)

To a solution of **28** (565 mg, 0.711 mmol) in MeOH (5.6 ml) was added 5 N HCl (0.3 ml) and stirred at room temperature for 30 min. The mixture was added to 8% aqueous $NaHCO_3$, extracted with ethyl acetate, washed with 25% aqueous NaCl, dried over Na_2SO_4 and concentrated under reduced pressure to obtain methyl (7*S*)-7-acetylthio-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*i*-butyl)piperidine-2′-carbonyl)-7-deoxy-α-thiolincosaminide (410 mg) as a crude compound. Then, the crude compound (410 mg) in MeOH (13 ml) was added and 5 N NaOMe (430 µl, 2.15 mmol) in MeOH at room temperature were treated for 1 h according to the similar procedure as described for the preparation of **31** to afford **32** (362 mg, 88% in 2 steps from **28**) as a colorless solid. ESI–MS *m/z* 537 $(M+H)^+$ as $C_{24}H_{44}N_2O_7S_2$; ^1H NMR (400 MHz, CD_3OD) δ 0.89 (d, *J*=2.8 Hz, 3 H), 0.90 (d, *J*=2.9 Hz, 3 H), 1.12–1.27 (m, 3 H), 1.30 (d, *J*=7.1 Hz, 3 H), 1.46 (s, 9 H), 1.49–1.60 (m, 1 H), 1.60–1.74 (m, 2 H), 1.75–1.88 (m, 1 H), 1.95–2.04 (m, 1 H), 2.15 (s, 3 H), 3.40–3.59 (m, 2 H), 3.45 (dq, *J*=7.1, 2.3 Hz, 1 H), 3.54 (dd, *J*=10.2, 3.4 Hz, 1 H), 3.93–4.02 (m, 1 H), 4.06 (dd, *J*=10.2, 5.6 Hz, 1 H), 4.11–4.26 (m, 2 H), 4.32 (dd, *J*=9.8, 2.3 Hz, 1 H), 5.25 (d, *J*=5.6 Hz, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-mercapto-α-thiolincosaminide (33)

To a solution of **29** (5.20 g, 6.55 mmol) in MeOH (70 ml) was added 1 N HCl (26.2 ml) and stirred at room temperature for 5 min. The mixture was added to 10% aqueous $NaHCO_3$ and then concentrated under reduced pressure until half volume to remove MeOH, extracted with ethyl acetate, washed with 25% aqueous NaCl, dried over Na_2SO_4 and concentrated under reduced pressure to obtain methyl (7*S*)-7-acetylthio-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-α-thiolincosaminide (4.70 g) as a crude compound. Then, the crude compound (4.70 g) and 28% NaOMe/MeOH solution (3.79 ml, 1.06 mmol) in MeOH (38 ml) at room temperature were treated for 15 min according to the similar procedure as described for the preparation of **30** to afford **33** (3.45 g, 99% in 2 steps from **29**) as a colorless solid. FAB–MS *m/z* 535 $(M+H)^+$ as $C_{24}H_{42}N_2O_7S_2$; ^1H NMR (400 MHz, CD_3OD) δ 0.01–0.06 (m, 2 H), 0.39–0.49 (m, 2 H), 0.63–0.78 (m, 1 H), 1.16–1.28 (m, 2 H), 1.30 (d, *J*=7.1 Hz, 3 H), 1.34–1.43 (m, 1 H), 1.47 (s, 9 H), 1.55–1.76 (m, 2 H), 1.82–1.93 (m, 1 H), 2.05–2.14 (m, 1 H), 2.15 (s, 3 H), 3.40–3.66 (m, 2 H), 3.45 (dq, *J*=7.1, 2.4 Hz, 1 H), 3.54 (dd, *J*=10.3, 3.4 Hz, 1

H), 3.94–4.01 (m, 1 H), 4.06 (dd, $J=10.3$, 5.6 Hz, 1 H), 4.13–4.25 (m, 2 H), 4.32 (dd, $J=9.7$, 2.4 Hz, 1 H), 5.25 (d, $J=5.6$ Hz, 1 H).

Methyl 6-*N*-((2′*S*, *Z*)-1′-*N*-(2′′-nitrophenylsulfonyl)-5′-*n*-propyl-2′,3′,6′,7′-tetrahydro-1*H*-azepine-2′-carbonyl)-α-thiolincosaminide (34)

To a solution of **17** (481.5 mg, 1.31 mmol) in DMF (5 ml) were added 1-hydroxybenzotriazole (265.0 mg, 1.96 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (375.8 mg, 1.96 mmol) and MTL (496.8 mg, 1.96 mmol), and stirred at room temperature for 14 h. To the mixture were added ethyl acetate and saturated aqueous $NaHCO_3$. The desired compound was extracted with ethyl acetate, washed with H_2O and then 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 ($CHCl_3$/MeOH = 50/1 to 30/1) to obtain the title compound (660 mg, 84%) as a colorless solid. FAB–MS *m/z* 604 $(M+H)^+$ as $C_{25}H_{37}N_3O_{10}S_2$; ^1H NMR (400 MHz, CD_3OD) δ 0.83 (t, $J=7.3$ Hz, 3 H), 1.15 (d, $J=6.3$ Hz, 3 H), 1.25–1.37 (m, 2 H), 1.08 (br t, $J=7.4$ Hz, 2 H), 2.05 (s, 3 H), 2.23–2.56 (m, 3 H), 2.68–2.80 (m, 1 H), 3.58 (dd, $J=10.2$, 3.4 Hz, 1 H), 3.73–3.88 (m, 3 H), 4.02–4.12 (m, 3 H), 4.36–4.41 (m, 1 H), 4.74 (dd, $J=8.0$, 3.7 Hz, 1 H), 5.22 (d, $J=5.6$ Hz, 1 H), 5.39 (br t, $J=6.3$ Hz, 1 H), 7.74–7.86 (m, 3 H), 8.09–8.17 (m, 1 H).

Methyl 6-*N*-((2′*S*)-5′-*n*-propylazepane-2′-carbonyl)-α-thiolincosaminide (35) (stereochemistry at the C-5′ position is not assigned)

To a solution of **34** (1.15 g, 1.90 mmol) in DMF (10 ml) at 0 °C were added 4-bromobenzenethiol (721 mg, 3.81 mmol) and cesium carbonate (1.25 g, 3.84 mmol), and then stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane to hexane/ethyl acetate = 50/50, then $CHCl_3$/CH_3OH/28% aq NH_4OH = 20/1/0.1) to obtain methyl 6-*N*-((2′*S*, *Z*)-5′-*n*-propyl-2′,3′,6′,7′-tetrahydro-1*H*-azepine-2′-carbonyl)-α-thiolincosaminide (649.1 mg, 81.4%) as a colorless solid. To this intermediate (649.1 mg, 1.55 mmol) in MeOH (30 ml) was added Pd/C (324 mg) and then vigorously stirred in hydrogen atmosphere of 0.95 MPa at 40 °C for 3.5 h. The mixture was filtrated off with celite and the mother liquor was concentrated under reduced pressure. To the resulting residue were added Pd/C (324 mg) and MeOH (30 ml), and then the mixture was vigorously stirred in hydrogen atmosphere of 0.95 MPa at 40 °C for 65 h. The mixture was filtrated with celite and concentrated under reduced pressure to obtain the title compound (560 mg, 86%) as a colorless solid. FAB–MS *m/z* 421 $(M+H)^+$ as $C_{19}H_{36}N_2O_6S$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{19}H_{36}N_2O_6S$: 421.2372, found: 421.2370; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (t, $J=7.2$ Hz, 3 H), 1.18 (d, $J=6.6$ Hz, 3 H), 1.20–1.48 (m, 7 H), 1.59–1.71 (m, 1 H), 1.77–1.88 (m, 1 H), 1.93–2.04 (m, 2 H), 2.08 (s, 3 H), 2.73–2.84 (m, 1 H), 3.03 (ddd, $J=13.8$, 5.5, 2.1 Hz, 1 H), 3.53–3.62 (m, 2 H), 3.94–4.05 (m, 2 H), 4.10 (dd, $J=10.2$, 5.6 Hz, 1 H), 4.15–4.20 (m, 1 H), 4.22–4.26 (m, 1 H), 5.24 (d, $J=5.4$ Hz, 1 H).

Methyl 6-*N*-((2′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-5′-*n*-propylazepane-2-carbonyl)-α-thiolincosaminide (36) (stereochemistry at the C-5′ position is not assigned)

To a solution of **35** (560 mg, 1.34 mmol) in 1,4-dioxane (10 ml)-H_2O (10 ml) were added LiOH·H_2O (84.0 mg, 2.00 mmol) and di-*tert*-butyl dicarbonate (0.37 ml, 1.6 mmol), and then stirred at room temperature for 3 h. The mixture was added to saturated aqueous $NaHCO_3$, extracted with ethyl acetate and then 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 ($CHCl_3$/CH_3OH/28% aq NH_4OH = 20/1/0.1) to obtain the title compound (483.3 mg, 70%) as a colorless solid. ESI–MS *m/z* 521 $(M+H)^+$ as $C_{24}H_{44}N_2O_8S$; ^1H NMR (400 MHz, CD_3OD) δ 0.85–0.96 (m, 3 H), 1.12–1.38 (m, 7 H), 1.47 (s, 9 H), 1.50–1.65 (m, 4 H), 1.66–1.80 (m, 1 H), 1.84–2.14 (m, 2 H), 2.07 (s, 3 H), 3.43–3.55 (m, 2 H), 3.59 (dd, $J=10.1$, 3.3 Hz, 1 H), 3.75–3.90 (m, 1 H), 3.98–4.18 (m, 3 H), 4.26–4.45 (m, 2 H), 5.24 (d, $J=5.6$ Hz, 1 H).

Methyl 6-*N*-((2′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-5′-*n*-propylazepane-2-carbonyl)-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (37) (stereochemistry at the C-5′ position is not assigned)

Compound **36** (894.0 mg, 1.72 mmol), trimethylchlorosilane (1.09 ml, 8.59 mmol) and hexamethyldisilazane (1.80 ml, 8.59 mmol) in pyridine (10 ml) were treated for 30 min according to the similar procedure as described for the preparation of **22**, and then the crude fully protected intermediate and 2 N acetic acid (2.23 ml) in MeOH (36 ml) were stirred at room temperature for 1 h. The mixture was added to saturated aqueous $NaHCO_3$, extracted with ethyl acetate and then the organic phase was dried over Na_2SO_4, filtrated and concentrated under reduced pressure to afford **37** as a crude compound.

Methyl (7*S*)-7-acetylthio-6-*N*-((2′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-5′-*n*-propylazepane-2-carbonyl)-7-deoxy-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (38) (stereochemistry at the C-5′ position is not assigned)

Crude **37**, Et_3N (1.20 ml, 8.58 mmol) and methanesulfonyl chloride (0.53 ml, 6.9 mmol) in $CHCl_3$ (20 ml) were treated at room temperature for 1 h according to the similar procedure as described for the preparation of **26**, and then the crude mesylate and AcSK (1.19 g, 10.4 mmol) in DMF (9.8 ml) at 80 °C were treated for 2 h according to the similar procedure as described for the preparation of **26** to afford **38** (768.6 mg, 56% in 4 steps from **36**) as a colorless solid. FAB–MS *m/z* 795 $(M+H)^+$ as $C_{35}H_{70}N_2O_8S_2Si_3$.

Methyl 6-*N*-((2′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-5′-*n*-propylazepane-2-carbonyl)-(7*S*)-7-mercapto-α-thiolincosaminide (39) (stereochemistry at the C-5′ position is not assigned)

To a solution of **38** (768.6 mg, 0.966 mmol) in MeOH (16 ml) at 0 °C was added 1 N HCl (1.6 ml) and then the mixture was stirred at 0 °C for 30 min. The mixture was concentrated under reduced pressure to obtain methyl (7*S*)-7-acetylthio-6-*N*-((2′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-5′-*n*-propylazepane-2-carbonyl)-7-deoxy-α-thiolincosaminide as a crude compound and then, the crude intermediate and 28% NaOMe/MeOH soultion (0.238 ml, 0.966 mmol) in MeOH (16 ml) at room temperature were treated for 30 min according to the similar procedure as described for the preparation of **30** to afford **39** (170 mg, 33% in 2 steps from **38**) as a colorless solid. ESI–MS *m/z* 537 $(M+H)^+$ as $C_{24}H_{44}N_2O_7S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{24}H_{44}N_2O_7S_2$: 537.2668, found: 537.2668; ^1H NMR (400 MHz, CD_3OD) δ 0.92 (br t, $J=6.6$ Hz, 3 H), 1.20–1.80 (m, 12 H), 1.48 (s, 9 H), 1.88–2.07 (m, 2 H), 2.15 (s, 3 H), 3.38–3.74 (m, 4 H), 3.76–3.94 (m, 1 H), 4.00–4.12 (m, 2 H), 4.23–4.34 (m, 1 H), 4.36–4.56 (m, 1 H), 5.24 (d, $J=5.6$ Hz, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-propyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl) phenylthio)-α-thiolincosaminide (40)

To a solution of 5-(4-bromophenyl)pyrimidine (103.4 mg, 0.440 mmol), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos) (18.8 mg, 0.0315 mmol) and tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$) (13.4 mg, 0.0146 mmol) in 1,4-dioxane (2.0 ml) were added compound **30** (152.1 mg, 0.291 mmol) and *N*,*N*-diisopropylethylamine (0.100 ml, 0.576 mmol), and refluxed for 6 h. The mixture was filtrated by either Chromatodisc (0.45 μm) (Kurabo Industries Ltd, Osaka, Japan) or celite, concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/28% aq NH_4OH = 10/1/0.1) to obtain the title compound (163.0 mg, 83%) as an off white solid. FAB–MS *m/z* 677 $(M+H)^+$ as $C_{33}H_{48}N_4O_7S_2$.

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*n*-butyl) piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl)phenylthio)-α-thiolincosaminide (41)

Compound **31** (150.1 mg, 0.280 mmol), 5-(4-bromophenyl)pyrimidine (99.3 mg, 0.422 mmol), Xantphos (17.3 mg, 0.029 mmol), $Pd_2(dba)_3$ (13.4 mg, 0.0146 mmol) and *N*,*N*-diisopropylethylamine (97.0 μl, 0.559 mmol) in 1,4-dioxane (2.0 ml) were treated for 6 h according to the similar procedure

as described for the preparation of **40** to afford **41** (150.8 mg, 78%) as a colorless solid.

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(*i*-butyl) piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl)phenylthio)-α-thiolincosaminide (42)

Compound **32** (100 mg, 0.186 mmol), 5-(4-bromophenyl)pyrimidine (50.0 mg, 0.213 mmol), Xantphos (10.0 mg, 0.0173 mmol), $Pd_2(dba)_3$ (10.0 mg, 0.0109 mmol) and *N,N*-diisopropylethylamine (60.0 μl, 0.353 mmol) in 1,4-dioxane (1.3 ml) were treated for 6 h according to the similar procedure as described for the preparation of **40** to afford **42** (105.0 mg, 82%) as a colorless solid.

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl)phenylthio)-α-thiolincosaminide (43)

Compound **33** (1.35 g, 2.52 mmol), 5-(4-bromophenyl)pyrimidine (771 mg, 3.28 mmol), Xantphos (151 mg, 0.253 mmol), $Pd_2(dba)_3$ (117 mg, 0.128 mmol) and *N,N*-diisopropylethylamine (0.875 ml, 5.04 mmol) in 1,4-dioxane (20 ml) were treated for 6 h according to the similar procedure as described for the preparation of **40** to afford **43** (1.63 g, 93%) as an off white solid. ESI–MS *m/z* 689 $(M+H)^+$ as $C_{34}H_{48}N_4O_7S_2$.

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(*n*-propyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl)phenylthio)-α-thiolincosaminide (44)

To a solution of **40** (163 mg, 0.241 mmol) in CH_2Cl_2 (3.3 ml) at −20 °C was added 2,2,2-trifluoroacetic acid (0.36 ml) and then the solution was stirred at room temperature for 4 h. The solution was concentrated under reduced pressure. The resulting residue was purified by preparative TLC ($CHCl_3$/MeOH/28% aq NH_4OH = 9/2/0.2) to obtain the title compound (122.8 mg, 88%) as a colorless solid. $[\alpha]_D^{22}$ +85.2° (*c* 1.02, MeOH); ESI–MS *m/z* 577 $(M+H)^+$ as $C_{28}H_{40}N_4O_5S_2$; TOF–ESI–HR-MS (M+H)+ calcd for $C_{28}H_{40}N_4O_5S_2$: 577.2518, found: 577.2516; ^{1}H NMR (400 MHz, CD_3OD) δ 0.90 (t, *J* = 7.3 Hz, 3 H), 1.02–1.15 (m, 2 H), 1.20–1.29 (m, 2 H), 1.31–1.42 (m, 2 H), 1.35 (d, *J* = 6.8 Hz, 3 H), 1.49–1.63 (m, 1 H), 1.68–1.77 (m, 1 H), 1.93 (s, 3 H), 1.96–2.05 (m, 1 H), 3.19 (dt, *J* = 10.9, 2.0 Hz, 1 H), 3.15-3.23 (m, 1 H), 3.40 (dd, *J* = 11.9, 2.8 Hz, 1 H), 3.58 (dd, *J* = 10.3, 3.3 Hz, 1 H), 3.88 (br dd, *J* = 3.3, 0.8 Hz, 1 H), 3.93 (dq, *J* = 6.8, 2.4 Hz, 1 H), 4.09 (dd, *J* = 10.3, 5.5 Hz, 1 H), 4.44 (br dd, *J* = 10.0, 0.8 Hz, 1 H), 4.61 (dd, *J* = 10.0, 2.4 Hz, 1 H), 5.27 (d, *J* = 5.5 Hz, 1 H), 7.52–7.58 (m, 2 H), 7.66–7.71 (m, 2 H), 9.06 (s, 2 H), 9.11 (s, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(*n*-butyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl)phenylthio)-α-thiolincosaminide (45)

Compound **41** (150.8 mg, 0.218 mmol) and 2,2,2-trifluoroacetic acid (0.33 ml) in CH_2Cl_2 (3.0 ml) were treated at −20 °C for 10 min and then treated room temperature for 2.5 h according to the similar procedure as described for the preparation of **44** to afford **45** (102.6 mg, 80%) as a colorless solid. $[\alpha]_D^{23}$ +81.2° (*c* 0.78, MeOH); ESI–MS *m/z* 591 $(M+H)^+$ as $C_{29}H_{42}N_4O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{29}H_{42}N_4O_5S_2$: 591.2675, found: 591.2669; ^{1}H NMR (400 MHz, CD_3OD) δ 0.85–0.96 (m, 3 H), 1.09–1.22 (m, 2 H), 1.23–1.41 (m, 6 H), 1.36 (d, *J* = 6.8 Hz, 3 H), 1.51–1.66 (m, 1 H), 1.74–1.84 (m, 1 H), 1.93 (s, 3 H), 2.04-2.12 (m, 1 H), 2.78 (dt, *J* = 12.9, 2.8 Hz, 1 H), 3.21–3.29 (m, 1 H), 3.52 (dd, *J* = 12.1, 2.9 Hz, 1 H), 3.58 (dd, *J* = 10.2, 3.3 Hz, 1 H), 3.89 (br dd, *J* = 3.3, 0.8 Hz, 1 H), 3.93 (dq, *J* = 6.8, 2.5 Hz, 1 H), 4.09 (dd, *J* = 10.2, 5.6 Hz, 1 H), 4.45 (br dd, *J* = 10.0, 0.8 Hz, 1 H), 4.63 (dd, *J* = 10.0, 2.5 Hz, 1 H), 5.27 (d, *J* = 5.6 Hz, 1 H), 7.51–7.58 (m, 2 H), 7.65–7.72 (m, 2 H), 9.06 (s, 2 H), 9.11 (s, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(*i*-butyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl)phenylthio)-α-thiolincosaminide (46)

Compound **42** (100 mg, 0.145 mmol) and 2,2,2-trifluoroacetic acid (0.50 ml) were treated at 0 °C for 1 h according to the similar procedure as described for the preparation of **44** to afford **46** (55.0 mg, 64%) as a colorless solid. $[\alpha]_D^{24}$ +93.9° (*c* 0.83, MeOH); ESI–MS *m/z* 591 $(M+H)^+$ as $C_{29}H_{42}N_4O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{29}H_{42}N_4O_5S_2$: 591.2675, found: 591.2674; ^{1}H NMR (400 MHz, CD_3OD) δ 0.91 (d, *J* = 5.7 Hz, 3 H), 0.90 (d, *J* = 5.7 Hz, 3 H), 1.08–1.20 (m, 3 H), 1.36 (d, *J* = 7.0 Hz, 3 H), 1.64–1.82 (m, 4 H), 1.93 (s, 3 H), 2.03–2.12 (m, 1 H), 2.76-2.86 (m, 1 H), 3.21–3.28 (m, 1 H), 3.51–3.61 (m, 2 H), 3.89 (br dd, *J* = 3.2, 0.8 Hz, 1 H), 3.93 (dq, *J* = 7.0, 2.4 Hz, 1 H), 4.09 (dd, *J* = 10.2, 5.6 Hz, 1 H), 4.45 (br dd, *J* = 10.0, 0.8 Hz, 1 H), 4.63 (dd, *J* = 10.0, 2.4 Hz, 1 H), 5.27 (d, *J* = 5.6 Hz, 1 H), 7.52–7.57 (m, 2 H), 7.66–7.71 (m, 2 H), 9.06 (s, 2 H), 9.11 (s, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl)phenylthio)-α-thiolincosaminide (47)

Compound **43** (1.63 g, 2.36 mmol) and 2,2,2-trifluoroacetic acid (3.5 ml) in CH_2Cl_2 (32 ml) were treated at −20 °C for 20 min, and then treated room temperature for 5.5 h according to the similar procedure as described for the preparation of **44** to afford **47** (1.12 g, 81%) as a colorless solid. $[\alpha]_D^{24}$ +86.1° (*c* 0.25, MeOH); ESI–MS *m/z* 589 $(M+H)^+$ as $C_{29}H_{40}N_4O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{29}H_{40}N_4O_5S_2$: 589.2518, found: 589.2517; IR (KBr) cm^{-1} 1046, 1078, 1415, 1508, 1602, 1653, 1671, 1698, 2338, 2360, 3001, 3347 and 3690 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ −0.01 to 0.08 (m, 2 H), 0.43–0.52 (m, 2 H), 0.67–0.78 (m, 1 H), 1.14–1.23 (m, 1 H), 1.24–1.40 (m, 3 H), 1.36 (d, *J* = 6.8 Hz, 3 H), 1.75-1.89 (m, 1 H), 1.90–2.00 (m, 1 H), 1.93 (s, 3 H), 2.28–2.37 (m, 1 H), 2.96 (dt, *J* = 13.1, 3.0 Hz, 1 H), 3.33–3.40 (m, 1 H), 3.59 (dd, *J* = 10.2, 3.1 Hz, 1 H), 3.75 (dd, *J* = 12.4, 3.0 Hz, 1 H), 3.90 (br dd, *J* = 3.1, 0.8 Hz, 1 H), 3.93 (dq, *J* = 6.8, 2.5 Hz, 1 H), 4.10 (dd, *J* = 10.2, 5.6 Hz, 1 H), 4.46 (br dd, *J* = 10.0, 0.8 Hz, 1 H), 4.65 (dd, *J* = 10.0, 2.5 Hz, 1 H), 5.28 (d, *J* = 5.6 Hz, 1 H), 7.51–7.57 (m, 2 H), 7.65–7.73 (m, 2 H), 9.06 (s, 2 H), 9.11 (s, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 5.1, 9.2, 13.8, 20.7, 33.8, 38.2, 38.8, 43.5, 44.8, 46.4, 53.8, 61.1, 69.6, 69.9, 71.0, 72.1, 90.2,128.6, 131.7, 133.0, 135.3, 138.6, 155.9, 157.9 and 176.5.

Methyl (7*S*)-6-*N*-((2′*S*)-1′-*N*-(*tert*-butoxycarbonyl)-5′-*n*-propylazepane-2-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl) phenylthio)-α-thiolincosaminide (48) (stereochemistry at the C-5′ position is not assigned)

Compound **39** (15.0 mg, 0.0279 mmol), 5-(4-bromophenyl)pyrimidine (13.1 mg, 0.0558 mmol), Xantphos (3.2 mg, 5.58 μmol), $Pd_2(dba)_3$ (5.1 mg, 5.6 μmol) and *N,N*-diisopropylethylamine (10.0 μl, 0.0558 mmol) in 1,4-dioxane (0.2 ml) were treated for 2 h according to the similar procedure as described for the preparation of **40** to afford **48** (17.0 mg, 88%) as a colorless solid. ESI–MS *m/z* 691 $(M+H)^+$ as $C_{34}H_{50}N_4O_7S_2$.

Methyl (7*S*)-6-*N*-((2′*S*)-5′-*n*-propylazepane-2-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl)phenylthio)-α-thiolincosaminide (49) (stereochemistry at the C-5′ position is not assigned)

Compound **48** (15.0 mg, 0.0217 mmol) and 2,2,2-trifluoroacetic acid (0.3 ml) were treated at 0 °C for 20 min and then treated room temperature for 1 h according to the similar procedure as described for the preparation of **44** to afford **49** (10.0 mg, 78%) as a colorless solid. $[\alpha]_D^{24}$ +82.9° (*c* 0.84, MeOH); ESI–MS *m/z* 591 $(M+H)^+$ as $C_{29}H_{42}N_4O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{29}H_{42}N_4O_5S_2$: 591.2675, found: 591.2667; ^{1}H NMR (400 MHz, CD_3OD) δ 0.91 (t, *J* = 7.1 Hz, 3 H), 1.21–1.45 (m, 5 H), 1.37 (d, *J* = 6.8 Hz, 3 H), 1.45–1.55 (m, 1 H), 1.55–1.68 (m, 1 H), 1.80–1.91 (m, 1 H), 1.92–2.01 (m, 1 H), 1.94 (s, 3 H), 2.07–2.22 (m, 2 H), 2.98–3.08 (m, 1 H), 3.32–3.39 (m, 1 H), 3.60 (dd, *J* = 10.2, 3.3 Hz, 1 H), 3.90 (dd, *J* = 3.3, 0.8 Hz, 1 H), 3.95 (dq, *J* = 6.8, 2.4 Hz, 1 H), 3.98 (dd, *J* = 6.4, 5.1 Hz, 1 H), 4.10 (dd, *J* = 10.2, 5.6 Hz, 1 H), 4.46 (dd, *J* = 10.0, 0.8 Hz, 1 H), 4.64 (dd, *J* = 10.0, 2.4 Hz, 1 H), 5.29 (d, *J* = 5.6 Hz, 1 H), 7.52–7.59 (m, 2 H), 7.65–7.74 (m, 2 H), 9.07 (s, 2 H), 9.12 (s, 1 H).

Methyl 6-*N*-(2,2,2-trifluoroacetyl)-2,3,4,7-tetrakis-*O*-(trimethylsilyl)-α-thiolincosaminide (51)

Compound **50** (6.88 g, 19.7 mmol), trimethylchlorosilane (12.6 ml, 98.5 mmol) and hexamethyldisilazane (20.6 ml, 98.5 mmol) in pyridine (40 ml) were treated at room temperature for 1 h according to the similar procedure as

described for the preparation of **22** to afford **51** (8.87 g, 71%) as a colorless solid. FAB–MS *m/z* 638 $(M+H)^+$ as $C_{23}H_{50}F_3NO_6SSi_4$.

Methyl 6-*N*-(2,2,2-trifluoroacetyl)-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (52)

To a solution of compound **51** (8.87 g, 13.9 mmol) in MeOH (65 ml) was added 6 N acetic acid (4.17 ml) and stirred at room temperature for 15 min. The mixture was added to saturated aqueous $NaHCO_3$ and concentrated under reduced pressure to remove MeOH. The desired compound was extracted with ethyl acetate, and then 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 = 95/5 to 80/20) to obtain the title compound (7.21 g, 91% in 2 steps) as a colorless solid. ESI–MS *m/z* 566 $(M+H)^+$ as $C_{20}H_{42}F_3NO_6SSi_3$; ^{1}H NMR (400 MHz, CD_3OD) δ 0.133 (s, 9 H), 0.134 (s, 9 H), 0.14 (s, 9 H), 1.13 (d, *J* = 6.5 Hz, 3 H), 2.06 (s, 3 H), 3.66 (dd, *J* = 9.6, 2.4 Hz, 1 H), 3.92 (d, *J* = 2.4 Hz, 1 H), 4.04 (dq, *J* = 6.5, 4.5 Hz, 1 H), 4.14 (dd, *J* = 9.6, 5.5 Hz, 1 H), 4.19 (d, *J* = 9.6 Hz, 1 H), 4.40 (dd, *J* = 9.6, 4.5 Hz, 1 H), 5.21 (d, *J* = 5.5 Hz, 1 H).

Methyl (7*S*)-7-acetylthio-7-deoxy-6-*N*-(2,2,2-trifluoroacetyl)-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (53)

Compound **52** (4.42 g, 7.82 mmol), Et_3N (21.8 ml, 15.6 mmol) and methanesulfonyl chloride (1.21 ml, 15.6 mmol) in $CHCl_3$ (20 ml) were treated at room temperature for 1 h according to the similar procedure as described for the preparation of **26** to afford methyl 7-*O*-methaneslufonyl-6-*N*-(2,2,2-trifluoroacetyl)-2,3,4-tris-*O*-(trimethylsilyl)-α-thiolincosaminide (5.46 g, quant) as a colorless solid. To a solution of this mesylate (5.46 g, 7.82 mmol) in DMF (40 ml) was added AcSK (2.68 g, 23.4 mmol) and stirred at 80 °C for 1.5 h. The mixture was concentrated under reduced pressure, diluted with ethyl acetate and saturated aqueous $NaHCO_3$, and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. To the resulting residue were added pyridine (16 ml), trimethylchlorosilane (6.35 ml, 50.0 mmol) and hexamethyldisilazane (10.5 ml, 50.0 mmol), and stirred at room temperature for 3 h. The mixture was added to saturated aqueous $NaHCO_3$, extracted with ethyl acetate and then 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 to hexane/ethyl acetate = 10/1) to obtain the title compound (2.88 g) as a crude compound. The crude compound (306.3 mg) was suspended in hexane, and then the solid was filtered, washed with hexane to obtain the title compound (129 mg, 25% in 3 steps) as a colorless solid. As compound **53** is partially soluble in hexane, a yield of the third step was low. ESI–MS *m/z* 624 $(M+H)^+$ as $C_{22}H_{44}F_3NO_6S_2Si_3$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.15 (s, 9 H), 0.16 (s, 9 H), 0.22 (s, 9 H), 1.41 (d, *J* = 7.1 Hz, 3 H), 2.01 (s, 3 H), 2.33 (s, 3 H), 3.65 (dd, *J* = 9.5, 2.7 Hz, 1 H), 3.76–3.86 (m, 1 H), 3.97–4.04 (m, 1 H), 4.10 (dd, *J* = 9.5, 5.4 Hz, 1 H), 4.14–4.21 (m, 1 H), 4.34-4.44 (m, 1 H), 5.18 (d, *J* = 5.4 Hz, 1 H), 7.20 (br dd, *J* = 9.5 Hz, 1 H).

Methyl (7*S*)-7-deoxy-7-mercapto-6-*N*-(2,2,2-trifluoroacetyl)-α-thiolincosaminide (54)

Compound **53** (2.83 g, 4.54 mmol) and 1 N HCl (18.1 ml) in MeOH (30 ml) were treated at room temperature for 10 min according to the similar procedure as described for the preparation of **30**, and then the crude intermediate and 28% NaOMe/MeOH solution (2.63 ml, 8.68 mmol) in MeOH (25 ml) at room temperature were treated for 15 min according to the similar procedure as described for the preparation of **30** to afford **54** (1.65 g, 99% in 2 steps from **53**) as a colorless solid. ESI–MS *m/z* 366 $(M+H)^+$ as $C_{11}H_{18}F_3NO_5S_2$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.29 (d, *J* = 7.0 Hz, 3 H), 2.16 (s, 3 H), 3.45 (dq, *J* = 7.0, 2.2 Hz, 1 H), 3.54 (dd, *J* = 10.2, 3.2 Hz, 1 H), 3.82 (dd, *J* = 3.2, 1.0 Hz, 1 H), 4.08 (dd, *J* = 10.2, 5.6 Hz, 1 H), 4.39 (dd, *J* = 9.9, 1.0 Hz, 1 H), 4.55 (dd, *J* = 9.9, 2.2 Hz, 1 H), 5.26 (d, *J* = 5.6 Hz, 1 H).

Methyl (7*S*)-7-deoxy-7-((4-(2-dimethylaminoethyl)phenyl)thio-6-*N*-(2,2,2-trifluoroacetyl)-α-thiolincosaminide (55)

Compound **54** (3.44 g, 9.42 mmol), 2-(4-bromophenyl)-*N*,*N*-dimethylethanamine (3.22 g, 14.1 mmol), Xantphos (544.8 mg, 0.942 mmol), $Pd_2(dba)_3$ (431.1 mg, 0.471 mmol) and *N*,*N*-diisopropylethylamine (3.28 ml, 18.8 mmol) in 1,4-dioxane (37 ml) were treated for 17 h according to the similar procedure as described for the preparation of **40** to afford **55** (3.85 g, 80%) as a colorless solid. FAB–MS *m/z* 513 $(M+H)^+$ as $C_{21}H_{31}F_3N_2O_5S_2$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.27 (d, *J* = 6.9 Hz, 3 H), 2.01 (s, 3 H), 2.36 (s, 6 H), 2.58-2.68 (m, 2 H), 2.74-2.84 (m, 2 H), 3.59 (dd, *J* = 10.3, 3.2 Hz, 1 H), 3.76 (dq, *J* = 6.9, 2.8 Hz, 1 H), 3.87-3.92 (m, 1 H), 4.09 (dd, *J* = 10.3, 5.6 Hz, 1 H), 4.59 (dd, *J* = 9.4, 0.7 Hz, 1 H), 4.64 (dd, *J* = 9.4, 2.8 Hz, 1 H), 5.28 (d, *J* = 5.6 Hz, 1 H), 7.16–7.22 (m, 2 H), 7.33-7.39 (m, 2 H).

Methyl (7*S*)-7-deoxy-7-((4-(2-dimethylaminoethyl)phenyl)thio-α-thiolincosaminide (56)

To a solution of **55** (3.85 g, 7.51 mmol) in CH_2Cl_2 (73 ml) were added *N*-benzyl-*N*, *N*, *N*-triethylammonium bromide (171.1 mg, 0.751 mmol) and 20% aqueous potassium hydroxide (5.1 ml), stirred at room temperature for 4 h. To a solution of mixture was added 1 N HCl to adjust at pH 7 and then the solution was concentrated under reduced pressure. The resulting residue was diluted with MeOH, filtrated, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/28% aq NH_4OH = 20/1/0.1 to 10/1/0.1) to obtain the title compound (2.94 g, 94%) as off white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 1.41 (d, *J* = 7.1 Hz, 3 H), 1.92 (s, 3 H), 2.30 (s, 6 H), 2.51–2.59 (m, 2 H), 2.73–2.81 (m, 2 H), 3.20 (dd, *J* = 8.8, 2.7 Hz, 1 H), 3.59 (dd, *J* = 10.3, 3.4 Hz, 1 H), 3.63 (dq, *J* = 7.1, 2.7 Hz, 1 H), 4.04–4.13 (m, 2 H), 4.23 (dd, *J* = 8.8, 1.2 Hz, 1 H), 5.22 (d, *J* = 5.8 Hz, 1 H), 7.14–7.20 (m, 2 H), 7.31–7.37 (m, 2 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-((4-(2-dimethylaminoethyl)phenyl)thio-α-thiolincosaminide (57)

Compound **15** (1.15 g, 4.04 mmol), **56** (2.02 g, 4.85 mmol), 1-hydroxybenzotriazole (0.819 g, 6.06 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.16 g, 6.06 mmol) in DMF (20 ml) were treated for 5.5 h according to the similar procedure as described for the preparation of **34** to afford the title compound (2.00 g, 73%) as a colorless solid. ESI–MS *m/z* 682 $(M+H)^+$ as $C_{34}H_{55}N_3O_7S_2$.

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-((4-(2-dimethylaminoethyl)phenyl)thio-α-thiolincosaminide (58)

Compound **57** (2.00 g, 2.93 mmol) and 2,2,2-trifluoroacetic acid (3.25 ml) in CH_2Cl_2 (5.0 ml) were treated at 0 °C for 3.5 h according to the similar procedure as described for the preparation of **44** to afford **58** (1.69 g, 99%) as a colorless solid. $[\alpha]_D^{23}$ +101.3° (*c* 0.65, MeOH); ESI–MS *m/z* 582 $(M+H)^+$ as $C_{29}H_{47}N_3O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{29}H_{47}N_3O_5S_2$: 582.3035, found: 582.3032; ^{1}H NMR (400 MHz, CD_3OD) δ −0.02 to 0.06 (m, 2 H), 0.40–0.50 (m, 2 H), 0.67–0.78 (m, 1 H), 1.02–1.25 (m, 4 H), 1.25 (d, *J* = 7.0 Hz, 3 H), 1.60–1.74 (m, 1 H), 1.75–1.84 (m, 1 H), 1.97 (s, 3 H), 2.04–2.12 (m, 1 H), 2.31 (s, 6 H), 2.52–2.61 (m, 2 H), 2.62–2.72 (m, 1 H), 2.73–2.81 (m, 2 H), 3.13–3.21 (m, 1 H), 3.34 (dd, *J* = 11.8, 2.9 Hz, 1 H), 3.56 (dd, *J* = 10.3, 3.3 Hz, 1 H), 3.76 (dq, *J* = 7.0, 2.4 Hz, 1 H), 3.85 (dd, *J* = 3.3, 0.8 Hz, 1 H), 4.08 (dd, *J* = 10.3, 5.6 Hz, 1 H), 4.40 (dd, *J* = 9.9, 0.8 Hz, 1 H), 4.52 (dd, *J* = 9.9, 2.4 Hz, 1 H), 5.25 (d, *J* = 5.6 Hz, 1 H), 7.15–7.19 (m, 2 H), 7.29–7.41 (m, 2 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)-1′-*N*-methylpiperidine-2′-carbonyl)-7-deoxy-7-((4-(2-dimethylaminoethyl)phenyl)thio-α-thiolincosaminide (59)

To a solution of **58** (1.19 g, 2.04 mmol) in MeOH (21 ml) were added 36% aqueous HCHO (0.51 ml, 6.12 mmol), AcOH (0.35 ml, 6.12 mmol) and $NaBH(OAc)_3$ (2.59 g, 12.2 mmol), and stirred at room temperature for 1 h. The mixture was diluted with ethyl acetate and saturated aqueous $NaHCO_3$,

extracted with ethyl acetate/MeOH = 5/1. 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 ($CHCl_3$/MeOH/28% aq NH_4OH = 20/1/0.1) to obtain the title compound (1.10 g, 91%) as a colorless solid. $[\alpha]_D^{22}$ +88.4° (*c* 1.58, MeOH); ESI–MS *m/z* 596 $(M+H)^+$ as $C_{30}H_{49}N_3O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{30}H_{49}N_3O_5S_2$: 596.3192, found: 596.3188; ^{1}H NMR (400 MHz, CD_3OD) δ −0.05 to 0.06 (m, 2 H), 0.38–0.49 (m, 2 H), 0.63–0.76 (m, 1 H), 1.13–1.38 (m, 4 H), 1.27 (d, *J* = 7.0 Hz, 3 H), 1.40–1.57 (m, 1 H), 1.75–1.84 (m, 1 H), 1.92–2.10 (m, 1 H), 1.99 (s, 3 H), 2.10–2.21 (m, 1 H), 2.26 (s, 3 H), 2.41 (s, 6 H), 2.60–2.73 (m, 3 H), 2.77–2.86 (m, 2 H), 2.92–3.02 (m, 1 H), 3.58 (dd, *J* = 10.1, 3.2 Hz, 1 H), 3.75–3.85 (m, 2 H), 4.10 (dd, *J* = 10.1, 5.6 Hz, 1 H), 4.41 (br dd, *J* = 9.8, 0.6 Hz, 1 H), 4.54 (dd, *J* = 9.8, 2.6 Hz, 1 H), 5.27 (d, *J* = 5.6 Hz, 1 H), 7.16–7.23 (m, 2 H), 7.33–7.39 (m, 2 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrrolidin-1-ylmethyl)phenylthio)-α-thiolincosaminide (60)

Compound **33** (40.2 mg, 0.0739 mmol), 1-(4-bromobenzyl)pyrrolidine (64.6 mg, 0.269 mmol), Xantphos (4.6 mg, 0.0077 mmol), $Pd_2(dba)_3$ (4.0 mg, 4.3 μmol) and *N,N*-diisopropylethylamine (38.5 μl, 0.22 mmol) in 1,4-dioxane (1 ml) were treated for 4 h according to the similar procedure as described for the preparation of **40** to afford **60** (41.4 mg, 81%) as a colorless solid. FAB–MS *m/z* 694 $(M+H)^+$ as $C_{35}H_{55}N_3O_7S_2$.

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)phenylthio)-α-thiolincosaminide (61)

Compound **33** (222.4 mg, 0.416 mmol), 3-(4-bromophenyl)-1-methyl-1,2,5,6-tetrahydropyridine (125.6 mg, 0.498 mmol), Xantphos (25.8 mg, 0.043 mmol), $Pd_2(dba)_3$ (19.4 mg, 0.021 mmol) and *N,N*-diisopropylethylamine (144 μl, 0.83 mmol) in 1,4-dioxane (3.5 ml) were treated for 5 h according to the similar procedure as described for the preparation of **40** to afford **61** (251.9 mg, 86%) as a colorless solid. ESI–MS *m/z* 706 $(M+H)^+$ as $C_{36}H_{55}N_3O_7S_2$.

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(1-methylpiperidin-3-yl)phenylthio)-α-thiolincosaminide (62) (a diastereo mixture at an *N*-methylpiperidine ring)

To a solution of **61** (201.7 mg, 0.286 mmol) in toluene (10 ml) was added 4-methylbenzenesulfonohydrazide (1.10 g, 5.72 mmol) at room temperature and then refluxed for 3 h. To the mixture was further added 4-methylbenzenesulfonohydrazide (1.09 g, 5.70 mmol) and then stirred for 2.5 h under the reflux condition. The solution was added to 1 N NaOH, extracted with ethyl acetate. The organic phase was dried over Na_2SO_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 obtain the title compound (27.7 mg, 14%) as a colorless solid. ESI–MS *m/z* 708 $(M+H)^+$ as $C_{36}H_{57}N_3O_7S_2$.

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyridin-3-yl)phenylthio)-α-thiolincosaminide (63)

Compound **33** (30.0 mg, 0.0561 mmol), 3-(4-iodophenyl)pyridine (18.9 mg, 0.0673 mmol), Xantphos (7.8 mg, 0.0135 mmol), $Pd_2(dba)_3$ (5.1 mg, 5.6 μmol) and *N,N*-diisopropylethylamine (19.5 μl, 0.112 mmol) in 1,4-dioxane (0.5 ml) were treated for 5 h according to the similar procedure as described for the preparation of **40** to afford **63** (39.3 mg as crude).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrazin-2-yl)phenylthio)-α-thiolincosaminide (64)

Compound **33** (80 mg, 0.147 mmol), 2-(4-bromophenyl)pyrazine (50.0 mg, 0.213 mmol), Xantphos (10 mg, 0.0173 mmol), $Pd_2(dba)_3$ (10 mg, 0.0109 mmol) and *N,N*-diisopropylethylamine (60 μl, 0.35 mmol) in 1,4-dioxane (1.5 ml) were treated for 4 h according to the similar procedure as described for the preparation of **40** to afford **64** as a crude compound.

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-1′-*N*-(*tert*-butoxycarbonyl)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(1,2,3-thiadiazol-4-yl)phenylthio)-α-thiolincosaminide (65)

Compound **33** (80.0 mg, 0.147 mmol), 4-(4-bromophenyl)-1,2,3-thiadiazole (50 mg, 0.207 mmol), Xantphos (10 mg, 0.0173 mmol), $Pd_2(dba)_3$ (10 mg, 0.011 mmol) and *N,N*-diisopropylethylamine (60 μl, 0.35 mmol) in 1,4-dioxane (1.5 ml) were treated for 4 h according to the similar procedure as described for the preparation of **40** to afford **65** as a crude compound. ESI–MS *m/z* 695 $(M+H)^+$ as $C_{32}H_{46}N_4O_7S_3$.

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrrolidin-1-ylmethyl)phenylthio)-α-thiolincosaminide (66)

Compound **60** (41.4 mg, 0.0597 mmol) and 2,2,2-trifluoroacetic acid (0.09 ml) in CH_2Cl_2 (0.9 ml) were treated at −20 °C for 5 min and then treated at room temperature for 5 h according to the similar procedure as described for the preparation of **44** to afford **66** (28.8 mg, 81%) as a colorless solid. $[\alpha]_D^{23}$ +68.9° (*c* 0.20, MeOH); ESI–MS *m/z* 594 $(M+H)^+$ as $C_{30}H_{47}N_3O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{30}H_{47}N_3O_5S_2$: 594.3035, found: 594.3031; ^{1}H NMR (400 MHz, CD_3OD) δ −0.01 to 0.06 (m, 2 H), 0.39–0.50 (m, 2 H), 0.66–0.79 (m, 1 H), 1.02-1.26 (m, 4 H), 1.28 (d, *J* = 6.8 Hz, 3 H), 1.60–1.74 (m, 1 H), 1.74–1.87 (m, 5 H), 1.93 (s, 3 H), 2.05–2.17 (m, 1 H), 2.49–2.60 (m, 4 H), 2.62–2.74 (m, 1 H), 3.12–3.24 (m, 1 H), 3.37 (dd, *J* = 11.8, 2.8 Hz, 1 H), 3.57 (dd, *J* = 10.2, 3.3Hz, 1 H), 3.63 (s, 2 H), 3.81 (dq, *J* = 6.8, 2.3 Hz, 1 H), 3.86 (br dd, *J* = 3.3, 0.7 Hz, 1 H), 4.08 (dd, *J* = 10.2, 5.6 Hz, 1 H), 4.38–4.46 (m, 1 H), 4.55 (dd, *J* = 10.1, 2.3 Hz, 1 H), 5.26 (d, *J* = 5.6 Hz, 1 H), 7.26–7.32 (m, 2 H), 7.34–7.39 (m, 2 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)-1′-*N*-methylpiperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrrolidin-1-ylmethyl)phenylthio)-α-thiolincosaminide (67)

Compound **66** (16.4 mg, 0.0276 mmol), 36% aqueous formaldehyde (21 μl, 0.28 mmol), AcOH (16 μl, 0.28 mmol) and $NaBH(OAc)_3$ (61.4 mg, 0.281 mmol) in MeOH (1.0 ml) were treated at room temperature for 2 h according to the similar procedure as described for the preparation of **59** to afford **67** (14.7 mg, 88%) as a colorless solid. $[\alpha]_D^{22}$ +65.9° (*c* 0.11, MeOH); ESI–MS *m/z* 608 $(M+H)^+$ as $C_{31}H_{49}N_3O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{31}H_{49}N_3O_5S_2$: 608.3192, found: 608.3187; ^{1}H NMR (400 MHz, CD_3OD) δ −0.05 to 0.07 (m, 2 H), 0.37–0.51 (m, 2 H), 0.64–0.78 (m, 1 H), 1.10–1.22 (m, 2 H), 1.23–1.39 (m, 2 H), 1.30 (d, *J* = 6.8 Hz, 3 H), 1.41–1.58 (m, 1 H), 1.73–1.88 (m, 5 H), 1.91–2.01 (m, 1 H), 1.95 (s, 3 H), 2.07–2.19 (m, 1 H), 2.56 (s, 3 H), 2.53–2.66 (m, 5 H), 2.92–3.01 (m, 1 H), 3.57 (dd, *J* = 10.2, 3.2 Hz, 1 H), 3.65 (s, 2 H), 3.79–3.89 (m, 2 H), 4.10 (dd, *J* = 10.2, 5.6 Hz, 1 H), 4.37–4.44 (m, 1 H), 4.56 (dd, *J* = 9.9, 2.6 Hz, 1 H), 5.26 (d, *J* = 5.6 Hz, 1 H), 7.26-7.33 (m, 2 H), 7.35–7.43 (m, 2 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)phenylthio)-α-thiolincosaminide (68)

Compound **61** (26.6 mg, 0.37 mmol) and 2,2,2-trifluoroacetic acid (60 μl) in CH_2Cl_2 (0.6 ml) were treated at −20 °C for 20 min and then treated room temperature for 4 h according to the similar procedure as described for the preparation of **44** to afford **68** (21.5 mg, 94%) as a colorless solid. $[\alpha]_D^{22}$ +91.4° (*c* 1.82, MeOH); ESI–MS *m/z* 606 $(M+H)^+$ as $C_{31}H_{47}N_3O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{31}H_{47}N_3O_5S_2$: 606.3035, found: 606.3012; ^{1}H NMR (400 MHz, CD_3OD) δ −0.07–0.03 (m, 2 H), 0.38–0.48 (m, 2 H), 0.62–0.75 (m,

1 H), 1.07–1.25 (m, 4 H), 1.25 (d, J=6.8 Hz, 3 H), 1.63–1.78 (m, 1 H), 1.78–1.87 (m, 1 H), 1.90 (s, 3 H), 2.10–2.19 (m, 1 H), 2.33–2.41 (m, 2 H), 2.44 (s, 3 H), 2.66 (t, J=5.9 Hz, 2 H), 2.80 (dt, J=12.9, 2.7 Hz, 1 H), 3.21–3.39 (m, 1 H), 3.32–3.38 (m, 2 H), 3.53–3.62 (m, 2 H), 3.80 (dq, J=6.8, 2.4 Hz, 1 H), 3.89 (dd, J=3.2, 0.7 Hz, 1 H), 4.09 (dd, J=10.3, 5.6 Hz, 1 H), 4.43 (dd, J=10.0, 0.7 Hz, 1 H), 4.57 (dd, J=10.0, 2.4 Hz, 1 H), 5.27 (d, J=5.6 Hz, 1 H), 6.17–6.23 (m, 1 H), 7.29–7.40 (m, 4 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)-1′-*N*-methylpiperidine-2′-carbonyl)-7-deoxy-7-(4-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)phenylthio)-α-thiolincosaminide (69)

Compound **68** (38.4 mg, 0.0634 mmol), 36% aqueous formaldehyde (48.0 µl, 0.645 mmol), AcOH (36.5 µl, 0.638 mmol) and $NaBH(OAc)_3$ (141.2 mg, 0.633 mmol) in MeOH (2.2 ml) were treated at room temperature for 1 h according to the similar procedure as described for the preparation of **59** to afford **69** (36.0 mg, 92%) as an off white solid. $[\alpha]_D^{23}$ +67.4° (*c* 2.48, MeOH); ESI–MS *m/z* 620 $(M+H)^+$ as $C_{32}H_{49}N_3O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{32}H_{49}N_3O_5S_2$: 620.3192, found: 620.3187; ^{1}H NMR (400 MHz, CD_3OD) δ −0.05 to 0.06 (m, 2 H), 0.38-0.49 (m, 2 H), 0.63–0.76 (m, 1 H), 1.10–1.23 (m, 2 H), 1.24–1.43 (m, 2 H), 1.30 (d, J=7.0 Hz, 3 H), 1.52–1.67 (m, 1 H), 1.81–1.90 (m, 1 H), 1.94 (s, 3 H), 2.00–2.09 (m, 1 H), 2.31–2.46 (m, 1 H), 2.41 (s, 3 H), 2.46–2.56 (m, 2 H), 2.69 (s, 3 H), 2.90–3.00 (m, 3 H), 3.06–3.15 (m, 1 H), 3.59 (dd, J=10.2, 3.2 Hz, 1 H), 3.62–3.69 (m, 2 H), 3.78–3.87 (m, 2 H), 4.10 (dd, J=10.2, 5.6 Hz, 1 H), 4.43 (dd, J=9.9, 0.6 Hz, 1 H), 4.59 (dd, J=9.9, 2.6 Hz, 1 H), 5.26 (d, J=5.6 Hz, 1 H), 6.23–6.29 (m, 1 H), 7.32–7.42 (m, 4 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(1-methylpiperidin-3-yl)phenylthio)-α-thiolincosaminide (70) (a diastero mixture at an *N*-methylpiperidine ring)

Compound **62** (6.9 mg, 9.75 µmol) and 2,2,2-trifluoroacetic acid (20 µl) in CH_2Cl_2 (0.2 ml) were treated at −20 °C for 20 min, and then treated room temperature for 4 h according to the similar procedure as described for the preparation of **44** to afford **70** (4.5 mg, 76%) as a colorless solid. $[\alpha]_D^{23}$ +81.6° (*c* 0.65, MeOH); ESI–MS *m/z* 608 $(M+H)^+$ as $C_{31}H_{49}N_3O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{31}H_{49}N_3O_5S_2$: 608.3192, found: 608.3175; ^{1}H NMR (400 MHz,CD_3OD) δ −0.06–0.04 (m, 2 H), 0.37–0.47 (m, 2 H), 0.60–0.75 (m, 1 H), 1.08–1.28 (m, 4 H), 1.22 (d, J=6.9 Hz, 3 H), 1.38–1.52 (m, 1 H), 1.63–1.78 (m, 2 H), 1.78–1.89 (m, 3 H), 1.90 (s, 3 H), 2.12–2.24 (m, 3 H), 2.37 (s, 3 H), 2.72–2.83 (m, 2 H), 2.94–3.04 (m, 2 H), 3.18–3.26 (m, 1 H), 3.46–3.56 (m, 2 H), 3.73 (dq, J=6.9, 2.3 Hz, 1 H), 3.79–3.85 (m, 1 H), 4.03 (dd, J=10.2, 5.6 Hz, 1 H), 4.37 (m, 1 H), 4.50 (dd, J=9.9, 2.3 Hz, 1 H), 5.21 (d, J=5.6 Hz, 1 H), 7.12-7.19 (m, 2 H), 7.28-7.34 (m, 2 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)-1'-*N*-methylpiperidine-2′-carbonyl)-7-deoxy-7-(4-(1-methylpiperidin-3-yl)phenylthio)-α-thiolincosaminide (71) (a diastero mixture at an *N*-methylpiperidine ring)

Compound **70** (9.4 mg, 0.016 mmol), 36% aqueous formaldehyde (12 µl, 0.16 mmol), AcOH (9.0 µl, 0.16 mmol) and $NaBH(OAc)_3$ (37.1 mg, 0.166 mmol) in MeOH (0.6 ml) were treated at room temperature for 1.5 h according to the similar procedure as described for the preparation of **59** to afford **71** (9.4 mg, 98%) as a colorless solid. $[\alpha]_D^{22}$ +70.1° (*c* 0.15, MeOH); ESI–MS *m/z* 622 $(M+H)^+$ as $C_{32}H_{51}N_3O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{32}H_{51}N_3O_5S_2$: 622.3348, found: 622.3342; ^{1}H NMR (400 MHz, CD_3OD) δ −0.28 to −0.16 (m, 2 H), 0.29–0.39 (m, 2 H), 0.55–0.67 (m, 1 H), 1.04–1.14 (m, 2 H), 1.15–1.31 (m, 2 H), 1.19 (d, J=6.8 Hz, 3 H), 1.38–1.57 (m, 2 H), 1.65–1.78 (m, 2 H), 1.79–1.89 (m, 2 H), 1.86 (s, 3 H), 1.89–1.97 (m, 1 H), 2.09–2.20 (m, 1 H), 2.23 (s, 3 H), 2.32–2.45 (m, 2 H), 2.47 (s, 3 H), 2.65 (br dd, J=11.5, 2.4 Hz, 1 H), 2.73–2.84 (m, 1 H), 2.90–2.97 (m, 1 H), 3.05–3.15 (m, 2 H), 3.48 (dd, J=10.2, 3.2 Hz, 1 H), 3.64–3.76 (m, 2 H), 4.00 (dd, J=10.2, 5.6 Hz, 1 H), 4.30 (d, J=9.8 Hz, 1 H), 4.46 (dd, J=9.8, 2.6 Hz, 1 H), 5.16 (d, J=5.6 Hz, 1 H), 7.09-7.16 (m, 2 H), 7.25-7.34 (m, 2 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyridin-3-yl)phenylthio)-α-thiolincosaminide (72)

Compound **63** (39.3 mg, 0.0571 mmol) and 2,2,2-trifluoroacetic acid (0.5 ml) in CH_2Cl_2 (0.1 ml) were treated at 0 °C for 1 h according to the similar procedure as described for the preparation of **44** to afford **72** (17.2 mg, 52% in 2 steps from **33**) as a colorless solid. $[\alpha]_D^{23}$ +92.4° (*c* 1.12, MeOH); ESI–MS *m/z* 588 $(M+H)^+$ as $C_{30}H_{41}N_3O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{30}H_{41}N_3O_5S_2$: 588.2566, found: 588.2560; ^{1}H NMR (400 MHz, CD_3OD) δ −0.04 to 0.05 (m, 2 H), 0.39–0.49 (m, 2 H), 0.65–0.76 (m, 1 H), 1.09–1.25 (m, 4 H), 1.35 (d, J=7.0 Hz, 3 H), 1.65–1.79 (m, 1 H), 1.79–1.88 (m, 1 H), 1.95 (s, 3 H), 2.10–2.20 (m, 1 H), 3.23 (dt, J=10.9, 1.9 Hz, 1 H), 3.18–3.27 (m, 1 H), 3.49 (dd, J=12.0, 2.9 Hz, 1 H), 3.59 (dd, J=10.2, 3.4 Hz, 1 H), 3.86–3.95 (m, 2 H), 4.10 (dd, J=10.2, 5.6 Hz, 1 H), 4.45 (br dd, J=9.9, 0.7 Hz, 1 H), 4.61 (dd, J=9.9, 2.4 Hz, 1 H), 5.28 (d, J=5.6 Hz, 1 H), 7.46-7.55 (m, 3 H), 7.58-7.66 (m, 2 H), 8.07 (ddd, J=7.9, 2.3, 1.5 Hz, 1 H), 8.50 (dd, J=4.9, 1.5 Hz, 1 H), 8.79 (dd, J=2.3, 0.8 Hz, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)-1′-*N*-methylpiperidine-2′-carbonyl)-7-deoxy-7-(4-(pyridin-3-yl) phenylthio)-α-thiolincosaminide (73)

Compound **72** (20.0 mg, 0.034 mmol), 36% aqueous formaldehyde (25 µl, 0.34 mmol), AcOH (19 µl, 0.30 mmol) and $NaBH(OAc)_3$ (18 mg, 0.08 mmol) in MeOH (2.2 ml) were treated at 0 °C for 1 h according to the similar procedure as described for the preparation of **59** to afford **73** (20.0 mg, 98%) as a colorless solid. $[\alpha]_D^{24}$ +57.4° (*c* 0.28, MeOH); ESI–MS *m/z* 602 $(M+H)^+$ as $C_{31}H_{43}N_3O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{31}H_{43}N_3O_5S_2$: 602.2722, found: 602.2714; ^{1}H NMR (400 MHz, CD_3OD) δ −0.05 to 0.05 (m, 2 H), 0.38–0.48 (m, 2 H), 0.62–0.74 (m, 1 H), 1.07–1.43 (m, 4 H), 1.37 (d, J=7.0 Hz, 3 H), 1.54–1.69 (m, 1 H), 1.83–1.92 (m, 1 H), 1.96 (s, 3 H), 2.03–2.11 (m, 1 H), 2.40–2.55 (m, 1 H), 2.47 (s, 3 H), 2.98–3.08 (m, 1 H), 3.10–3.20 (m, 1 H), 3.59 (dd, J=10.3, 3.3 Hz, 1 H), 3.85 (br dd, J=3.2, 0.9 Hz, 1 H), 3.91 (dq, J=6.9, 2.6 Hz, 1 H), 4.10 (dd, J=10.2, 5.6 Hz, 1 H), 4.46 (br dd, J=9.9, 0.7 Hz, 1 H), 4.64 (dd, J=9.9, 2.6 Hz, 1 H), 5.27 (d, J=5.5 Hz, 1 H), 7.50–7.56 (m, 2 H), 7.51 (ddd, J=8.0, 4.9, 0.8 Hz, 1 H), 7.60–7.67 (m, 2 H), 8.08 (ddd, J=8.0, 2.3, 1.5 Hz, 1 H), 8.51 (dd, J=4.9, 1.5 Hz, 1 H), 8.79 (br dd, J=2.3, 0.8 Hz, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrazin-2-yl)phenylthio)-α-thiolincosaminide (74)

Compound **64** (crude) and 2,2,2-trifluoroacetic acid (0.5 ml) were treated at 0 °C for 30 min according to the similar procedure as described for the preparation of **44** to afford **74** (41.1 mg, 48% in 2 steps from **33**) as a colorless solid. $[\alpha]_D^{22}$ +78.5° (*c* 1.20, MeOH); ESI–MS *m/z* 589 $(M+H)^+$ as $C_{29}H_{40}N_4O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{29}H_{40}N_4O_5S_2$: 589.2518, found: 589.2514; ^{1}H NMR (400 MHz, CD_3OD) δ −0.03 to 0.04 (m, 2 H), 0.40–0.48 (m, 2 H), 0.64–0.75 (m, 1 H), 1.09–1.27 (m, 4 H), 1.38 (d, J=6.9 Hz, 3 H), 1.65–1.78 (m, 1 H), 1.79–1.87 (m, 1 H), 1.91 (s, 3 H), 2.10–2.18 (m, 1 H), 2.70–2.81 (m, 1 H), 3.19–3.26 (m, 1 H), 3.49 (dd, J=12.0, 2.9 Hz, 1 H), 3.59 (dd, J=10.2, 3.2 Hz, 1 H), 3.89 (dd, J=3.2, 0.8 Hz, 1 H), 3.94 (dq, J=6.9, 2.4 Hz, 1 H), 4.10 (dd, J=10.2, 5.6 Hz, 1 H), 4.45 (dd, J=10.0, 0.8 Hz, 1 H), 4.63 (dd, J=10.0, 2.4 Hz, 1 H), 5.28 (d, J=5.6 Hz, 1 H), 7.48-7.55 (m, 2 H), 7.99-8.06 (m, 2 H), 8.50 (d, J=2.6 Hz, 1 H), 8.64 (dd, J=2.6, 1.5 Hz, 1 H), 9.08 (d, J=1.5 Hz, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)-1′-*N*-methylpiperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrazin-2-yl) phenylthio)-α-thiolincosaminide (75)

Compound **74** (31.0 mg, 0.050 mmol), 36% aqueous formaldehyde (12 µl, 0.15 mmol), AcOH (17 µl, 0.30 mmol) and $NaBH(OAc)_3$ (31.6 mg, 0.15 mmol) in MeOH (0.5 ml) were treated at room temperature for 30 min according to the similar procedure as described for the preparation of **59** to afford **75** (28.4 mg, 94%) as a colorless solid. $[\alpha]_D^{25}$ +72.4° (*c* 0.64, MeOH);

ESI–MS *m/z* 603 $(M+H)^+$ as $C_{30}H_{42}N_4O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{30}H_{42}N_4O_5S_2$: 603.2675, found: 603.2672; ^{1}H NMR (400 MHz, CD_3OD) δ −0.06 to 0.04 (m, 2 H), 0.37–0.47 (m, 2 H), 0.60–0.74 (m, 1 H), 1.08–1.22 (m, 2 H), 1.25–1.38 (m, 2 H), 1.39 (d, *J*=7.0 Hz, 3 H), 1.46–1.60 (m, 1 H), 1.78–1.85 (m, 1 H), 1.92 (s, 3 H), 1.95–2.02 (m, 1 H), 2.20–2.30 (m, 1 H), 2.34 (s, 3 H), 2.77 (br dd, *J*=11.4, 2.1 Hz, 1 H), 2.99–3.07 (m, 1 H), 3.59 (dd, *J*=10.2, 3.2 Hz, 1 H), 3.85 (dd, *J*=3.2, 0.6 Hz, 1 H), 3.96 (dq, *J*=6.9, 2.6 Hz, 1 H), 4.11 (dd, *J*=10.2, 5.6 Hz, 1 H), 4.45 (br dd, *J*=9.9, 0.6 Hz, 1 H), 4.64 (dd, *J*=9.9, 2.6 Hz, 1 H), 5.27 (d, *J*=5.6 Hz, 1 H), 7.50–7.56 (m, 2 H), 8.01–8.07 (m, 2 H), 8.50 (d, *J*=2.5 Hz, 1 H), 8.65 (dd, *J*=2.5, 1.5 Hz, 1 H), 9.09 (d, *J*=1.5 Hz, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)-1′-*N*-methylpiperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl) phenylthio)-α-thiolincosaminide (76)

Compound **47** (894.4 mg, 1.52 mmol), 36% aqueous formaldehyde (1.10 ml, 14.8 mmol), AcOH (0.85 ml, 14.8 mmol) and $NaBH(OAc)_3$ (3.31 g, 14.8 mmol) in MeOH (44 ml) were treated at room temperature for 1 h according to the similar procedure as described for the preparation of **59** to afford **76** (916.3 mg, quant) as a colorless solid. $[\alpha]_D^{22}$ +75.4° (*c* 0.96, MeOH); ESI–MS *m/z* 603 $(M+H)^+$ as $C_{30}H_{42}N_4O_5S_2$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{30}H_{42}N_4O_5S_2$: 603.2675, found: 603.2672; IR (KBr) cm^{-1} 1077, 1415, 1508, 1653, 2361, 3020 and 3690; ^{1}H NMR (400 MHz, CD_3OD) δ −0.06 to 0.04 (m, 2 H), 0.37–0.46 (m, 2 H), 0.63–0.73 (m, 1 H), 1.12-–1.19 (m, 2 H), 1.25–1.38 (m, 2 H), 1.37 (d, *J*=6.8 Hz, 3 H), 1.44–1.58 (m, 1 H), 1.75–1.84 (m, 1 H), 1.92–2.02 (m, 1 H), 1.94 (s, 3 H), 2.18 (dt, *J*=12.1, 2.3 Hz, 1 H), 2.30 (s, 3 H), 2.69 (dd, *J*=11.6, 2.7 Hz, 1 H), 2.95-3.03 (m, 1 H), 3.59 (dd, *J*=10.2, 3.1 Hz, 1 H), 3.85 (br dd, *J*=3.1, 0.7 Hz, 1 H), 3.95 (dq, *J*=6.8, 2.7 Hz, 1 H), 4.11 (dd, *J*=10.2, 5.6 Hz, 1 H), 4.43 (br dd, *J*=9.9, 0.7 Hz, 1 H), 4.64 (dd, *J*=9.9, 2.7 Hz, 1 H), 5.27 (d, *J*=5.6 Hz, 1 H), 7.52–7.58 (m, 2 H), 7.65–7.72 (m, 2 H), 9.06 (s, 2 H), 9.11 (s, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 5.1, 9.3, 13.9, 20.8, 30.8, 32.8, 37.3, 38.5, 42.6, 44.6, 44.8, 53.8, 56.8, 69.5, 70.2, 70.8, 70.9, 72.2, 90.3, 128.6, 131.9, 133.0, 135.2, 138.4, 155.9, 157.9 and 176.3.

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(1,2,3-thiadiazol-4-yl)phenylthio)-α-thiolincosaminide (77)

Compound **65** (crude) and 2,2,2-trifluoroacetic acid (0.50 ml) were treated at 0 °C for 30 min according to the similar procedure as described for the preparation of **44** to afford **77** (44.3 mg, 51% in 2 steps from **33**) as a colorless solid. $[\alpha]_D^{27}$ +80.4° (*c* 0.36, MeOH); ESI–MS *m/z* 595 $(M+H)^+$ as $C_{27}H_{38}N_4O_5S_3$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{27}H_{38}N_4O_5S_3$: 595.2083, found: 595.2073; ^{1}H NMR (400 MHz, CD_3OD) δ 0.01–0.09 (m, 2 H), 0.43–0.54 (m, 2 H), 0.66–0.78 (m, 1 H), 1.15–1.22 (m, 1 H), 1.25–1.45 (m, 3 H), 1.38 (d, *J*=6.9 Hz, 3 H), 1.77–1.91 (m, 1 H), 1.94 (s, 3 H), 1.96–2.04 (m, 1 H), 2.32–2.41 (m, 1 H), 3.02 (dt, *J*=13.1, 3.1 Hz, 1 H), 3.37-3.45 (m, 1 H), 3.59 (dd, *J*=10.2, 3.2 Hz, 1 H), 3.38 (dd, *J*=12.6, 3.1 Hz, 1 H), 3.89 (dd, *J*=3.2, 0.9 Hz, 1 H), 3.94 (dq, *J*=6.9, 2.5 Hz, 1 H), 4.10 (dd, *J*=10.2, 5.6 Hz, 1 H), 4.47 (dd, *J*=9.9, 0.9 Hz, 1 H), 4.66 (dd, *J*=9.9, 2.5 Hz, 1 H), 5.28 (d, *J*=5.6 Hz, 1 H), 7.49–7.56 (m, 2 H), 8.01–8.09 (m, 2 H), 9.22 (s, 1 H).

Methyl (7*S*)-6-*N*-((2′*S*, 4′*R*)-4′-(cyclopropylmethyl)-1′-*N*-methylpiperidine-2′-carbonyl)-7-deoxy-7-(4-(1,2,3-thiadiazol-4-yl) phenylthio)-α-thiolincosaminide (78)

Compound **77** (29.7 mg, 0.050 mmol), 36% aqueous formaldehyde (12 μl, 0.15 mmol), AcOH (17 μl, 0.30 mmol) and $NaBH(OAc)_3$ (31.6 mg, 0.15 mmol) in MeOH (0.5 ml) were treated at room temperature for 30 min according to the similar procedure as described for the preparation of **59** to afford **78** (27.3 mg, 90%) as a colorless solid. $[\alpha]_D^{23}$ +48.9° (*c* 0.28, MeOH); ESI–MS *m/z* 609 $(M+H)^+$ as $C_{28}H_{40}N_4O_5S_3$; TOF–ESI–HR-MS $(M+H)^+$ calcd for $C_{28}H_{40}N_4O_5S_3$: 609.2239, found: 609.2231; ^{1}H NMR (400 MHz, CD_3OD) δ −0.14 to 0.04 (m, 2 H), 0.30–0.40 (m, 2 H), 0.52–0.65 (m, 1 H), 1.00–1.18 (m, 2 H), 1.25–1.38 (m, 2 H), 1.30 (d, *J*=7.0 Hz, 3 H), 1.50–1.67 (m, 1 H), 1.78–1.88 (m, 1 H), 1.86 (s, 3 H), 1.97–2.06 (m, 1 H), 2.49 (s, 3 H), 2.50–2.64 (m, 1 H), 3.10–3.19 (m, 2 H), 3.51 (dd, *J*=10.2, 3.2 Hz, 1 H), 3.77 (br dd, *J*=3.2, 0.9 Hz, 1 H), 3.84 (dq, *J*=7.0, 2.6 Hz, 1 H), 4.01 (dd, *J*=10.2, 5.6 Hz, 1 H), 4.40 (br dd, *J*=9.9, 0.9 Hz, 1 H), 4.57 (dd, *J*=9.9, 2.6 Hz, 1 H), 5.18 (d, *J*=5.6 Hz, 1 H), 7.42–7.49 (m, 2 H), 7.93-7.99 (m, 2 H), 9.14 (s, 1 H).

In vitro antibacterial activity

MIC (μg/ml) was determined by the agar dilution method, which was described in Clinical and Laboratory Standards Institute (M07-07 in 2006). Test strains of *S. pneumoniae* and *S. pyogenes* were subjected to seed culture using brain heart infusion agar (Becton Dickinson and Company, Tokyo, Japan) and 5% defibrinated horse blood. Test strains of *H. influenzae* were subjected to seed culture using sensitivity disk agar-N 'Nissui' (Nissui, Tokyo, Japan), 5% defibrinated horse blood, 5 μg ml^{-1} Hemin and 15 μg ml^{-1} nicotinamide adenine dinucleotide. A 5 μl portion of cell suspension of the test strains having about 10^6 CFU ml^{-1} was inoculated into sensitivity disk agar supplemented with 5% defibrinated horse blood, 5 μg ml^{-1} Hemin and 15 μg ml^{-1} nicotinamide adenine dinucleotide, and incubated at 37 °C for 18–22 h. Then, the MIC was measured.

In vitro antibacterial activity (sensitivity distribution against *S. pneumoniae* of 60 strains)

The MIC for *S. pneumoniae* was determined by the twofold microdilution broth method using cation-adjusted Mueller–Hinton broth (Difco Laboratories, Detroit, MI, USA) supplemented with 2% lysed horse blood recommended by the Clinical and Laboratory Standards Institute.[61] The inoculum was prepared by making a direct colony Suspension, equivalent to a 0.5 McFarland standard, with isolated colonies selected from Mueller–Hinton agar (Difco Laboratories) supplemented with 5% defibrinated sheep blood. Fifty microliters of the adjusted inoculum was added to each well already containing 50 μl of antimicrobial agent in the dilution series. The final test concentration of bacteria was approximately 5×10^4 CFU per well. The MIC was determined as the lowest concentration that prevented visible growth of bacteria after incubation at 35 °C for 20 h.

Neutropenic rat lung infection model

The study and its protocol were complied with Guidelines on the Management of Animal Experiments established by the Pharmaceutical Research Center, Meiji Seika Pharma Co., Ltd and approved by the Animal Experiment Management Committee of it. Rats used in this study are 6-week-old, specific-pathogen-free, male Sprague-Dawley rats (SD-rats) (*n*=3) (Charles River Laboratories Japan, Inc., Kanagawa, Japan) weighing 160–180 g. These rats were bred under controlled conditions (temperature, 21–25 °C; humidity, 50–70%; lighting hours, 0700–1900 h), and feed (CRF-1; Oriental Yeast Co., Ltd, Tokyo, Japan) and water were available *ad libitum*. The rats were allowed to acclimatize for 1 week before the study. The rats were rendered neutropenic by intraperitoneal administration of cyclophosphamide (Sigma-Aldrich) 4 days and the day before infection (80 mg kg^{-1} of body weight). The rats were infected with *S. pneumoniae* by the injection of 10^6 CFU into lung through trachea under anesthesia with mixture of ketamine hydrochloride and xylazine hydrochloride (5:1) by injection intramuscularly. The rats were treated by subcutaneous administration of the test compound at 2 h after infection and were killed 24 h after infection by injection of excessive amounts of pentobarbital. The lung was removed and homogenized. Each homogenate was diluted 10-fold serially with physiological saline and an aliquot of each initial homogenate and dilution series was smeared onto a plate of brain heart infusion agar with 5% horse blood. After was incubation at 35 °C for 24 h, the number of colonies grown on the plate was counted. The detection limit was set at <2.0 log10 CFU per lung; if no colonies were detected in the initial homogenate, the value of 2.0 log10 CFU per lung was adopted. The data were expressed as the mean ± s.d. log10 CFU per lung.

DEDICATION

Dedicated to Professor K. C. Nicolaou and his outstanding contributions to complex natural product total synthesis and chemical biology.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Mr A. Tamura, Dr E. Shitara and Dr T. Yoshida for encouragement and valuable discussion. We are grateful to Professor Emeritus Dr M. Konno for supervision through our in-house drug discovery program in LCM field. We also thank Dr T. Murata for ROESY analysis; Ms M. Ishii for direction in intellectual properties; Mr T. Watanabe for computational chemistry; Ms T. Miyara, Ms S. Miki and Ms K. Kaneda for analytical and synthetic chemistry; Mr Y. Takayama and Ms K. Yamada for biological studies; and Ms M. Takagi and Ms Y. Saito for English manuscript.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)