

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* advance online publication, 1 November 2017; doi:10.1038/ja.2017.114

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} Clarithromycin⁴ 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) suppression¹⁹ 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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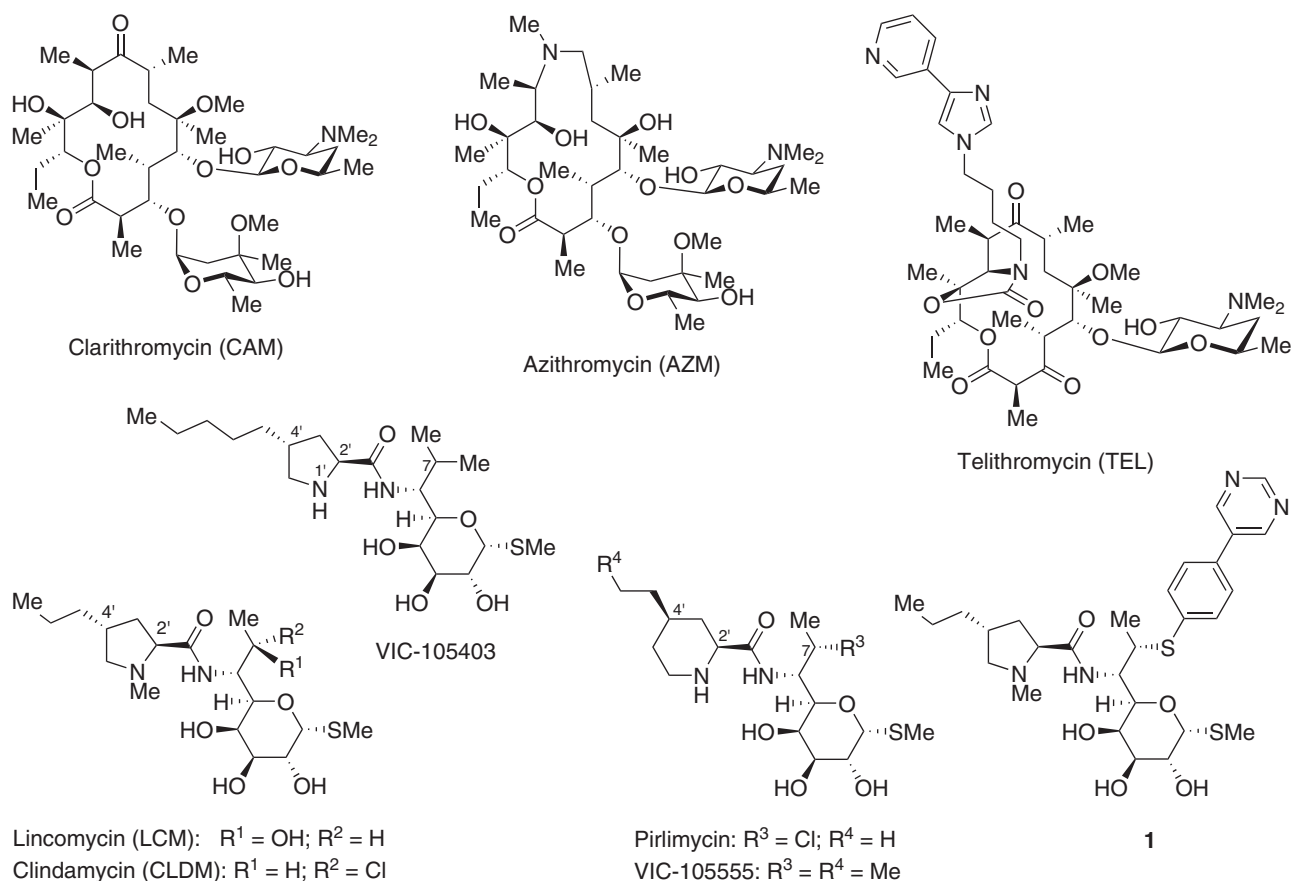


Figure 1 Chemical structures of macrolide derivatives and lincomycin/clindamycin derivatives.

Table 1 Antibacterial activities (MIC, $\mu\text{g ml}^{-1}$) 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 |
|--------------------------------|--|-------|-------|-------|-------|------------|--------------|--------------|
| <i>S. pneumoniae</i> DP1 Typel | Susceptible | 0.03 | 0.06 | 1 | 0.06 | 0.03 | ≤ 0.008 | ≤ 0.008 |
| <i>S. pneumoniae</i> -2 | Susceptible | 0.03 | 0.03 | 1 | 0.12 | 0.06 | ≤ 0.008 | ≤ 0.008 |
| <i>S. pneumoniae</i> -3 | Susceptible | 0.015 | 0.03 | 0.25 | 0.06 | 0.03 | ≤ 0.008 | ≤ 0.008 |
| <i>S. pneumoniae</i> -4 | <i>ermAM</i> methylase (c) | > 128 | > 128 | > 128 | > 128 | > 128 | 0.5 | 0.5 |
| <i>S. pneumoniae</i> -5 | <i>ermAM</i> methylase (c) | > 128 | > 128 | > 128 | > 128 | > 128 | 2 | 1 |
| <i>S. pneumoniae</i> -6 | <i>ermAM</i> methylase (c) + <i>mefE</i> | > 128 | > 128 | > 128 | > 128 | > 128 | 1 | 2 |
| <i>S. pneumoniae</i> -7 | <i>ermAM</i> methylase (i) | > 128 | > 128 | 128 | 128 | 128 | 0.03 | 0.25 |
| <i>S. pneumoniae</i> -8 | <i>ermAM</i> methylase (i) | > 128 | > 128 | 128 | 128 | 128 | 0.03 | 0.25 |
| <i>S. pneumoniae</i> -9 | <i>mefE</i> efflux | 0.5 | 0.5 | 1 | 0.12 | 0.015 | 0.06 | ≤ 0.008 |
| <i>S. pyogenes</i> Cook | susceptible | 0.015 | 0.06 | 0.12 | 0.06 | 0.06 | ≤ 0.008 | ≤ 0.008 |
| <i>S. pyogenes</i> -2 | <i>ermAM</i> methylase (c) | > 128 | > 128 | > 128 | 128 | > 128 | 16 | 0.5 |
| <i>S. pyogenes</i> -3 | <i>mefE</i> efflux | 8 | 8 | 0.25 | 0.12 | 0.06 | 0.25 | 0.015 |
| <i>H. influenzae</i> | Susceptible | 2 | 0.25 | 8 | 16 | 32 | 0.5 | 4 |
| <i>H. influenzae</i> -2 | Susceptible | 4 | 1 | 16 | 8 | 16 | 2 | 2 |
| <i>H. influenzae</i> -3 | Susceptible | 8 | 2 | 16 | 16 | 32 | 1 | 8 |
| <i>H. influenzae</i> -4 | Δacr | 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.

^aAll 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}

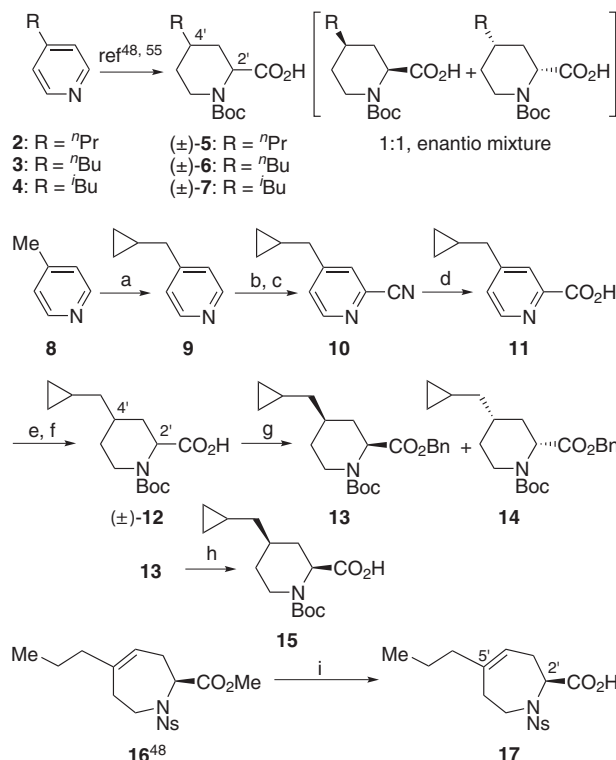
were synthesized accompanied with modifications at the C-7. Regarding azetidine derivatives, 3'-*trans*-cyclobutylethyl CLDM derivative showed significant antibacterial activities against sensitive *S. pneumoniae* compared with CLDM, but 3'-*trans*-cyclopropylmethyl, 3'-*trans*-*n*-propyl and 3'-*trans*-*n*-butyl 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 $\mu\text{g ml}^{-1}$) against *H. influenzae* as CLDM (MIC: 8 $\mu\text{g ml}^{-1}$).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 (7S)-substituted analogs⁴² 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 (7S)-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-1H-azepine

Syntheses of substituted piperidines and 2,3,6,7-tetrahydro-1H-azepine are shown in Scheme 1. Substituted piperidines (\pm)-**5**-**7** and 2,3,6,7-tetrahydro-1H-azepine **16** were synthesized by methods reported by Shuman *et al.*⁵⁵ and Lewis *et al.*⁴⁸ Compound (\pm)-**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 (\pm)-*cis*-carboxylic acids **5**-**7** and **12**, but later on we could separate (\pm)-**12** into each *cis*-enantiomer for efficient synthetic study. Carboxylic acid (\pm)-**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 pipercolic acid with methyl α -thiolincosaminide (MTL), we assigned

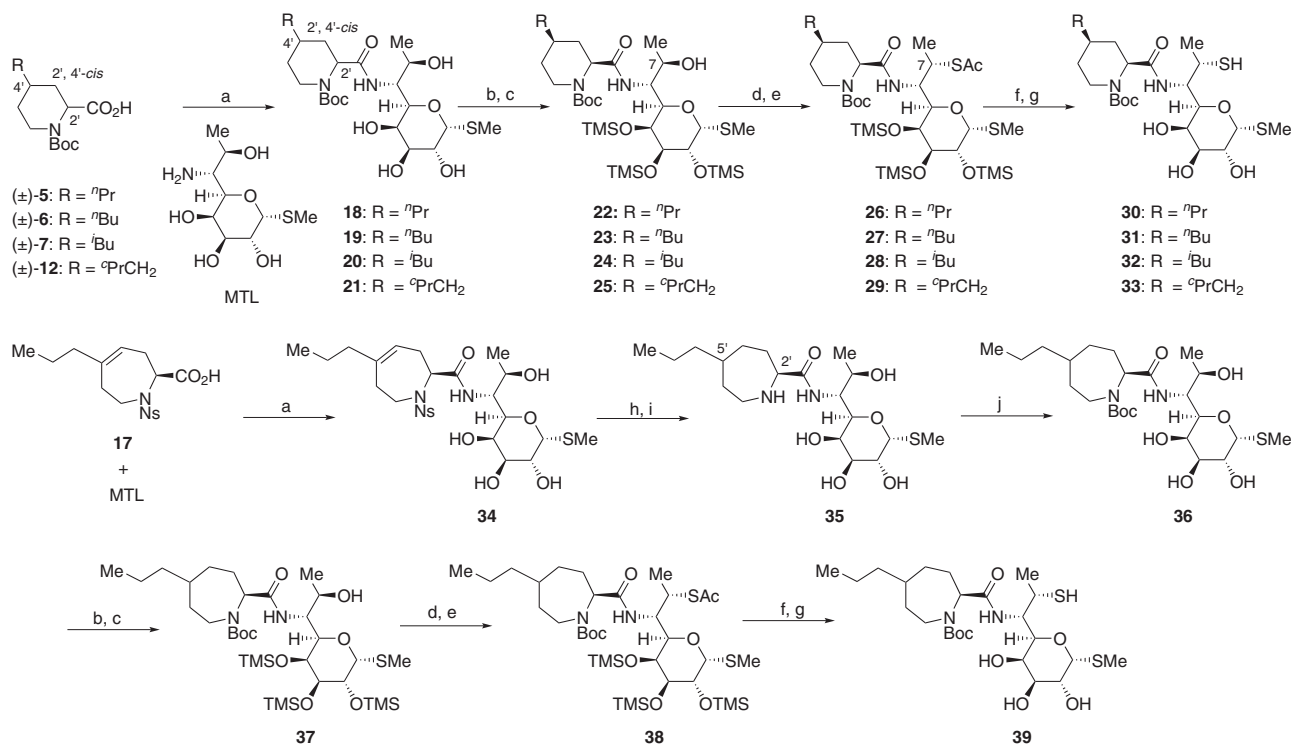


Scheme 1 Synthesis of substituted piperidines and 2,3,6,7-tetrahydro-1H-azepine. Conditions: (a) bromocyclopropane, lithium diisopropylamide, THF, -78°C , 1 h; (b) *m*-chloroperoxybenzoic acid (*m*CPBA), CH_2Cl_2 , 0°C to r.t., 1 h; (c) TMSCN , Me_2NCOCl , CH_2Cl_2 , 20°C 40 min, then r.t., 17 h; (d) 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 , $i\text{-Pr}_3\text{NEt}$, CH_3CN , r.t., 48 h; (h) H_2 , Pd/C, MeOH, r.t., 1 h; (i) $\text{LiOH}\cdot\text{H}_2\text{O}$, 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 (\pm)-**5**-**7**, (\pm)-**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 $\text{S}_\text{N}2$ 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, NEt₃, CH₂Cl₂, 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, Cs₂CO₃, DMF, r.t., 2 h; (i) H₂ (0.95 MPa), Pd/C, MeOH, 40 °C, 70 h; (j) Boc₂O, LiOH·H₂O, dioxane:H₂O = 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

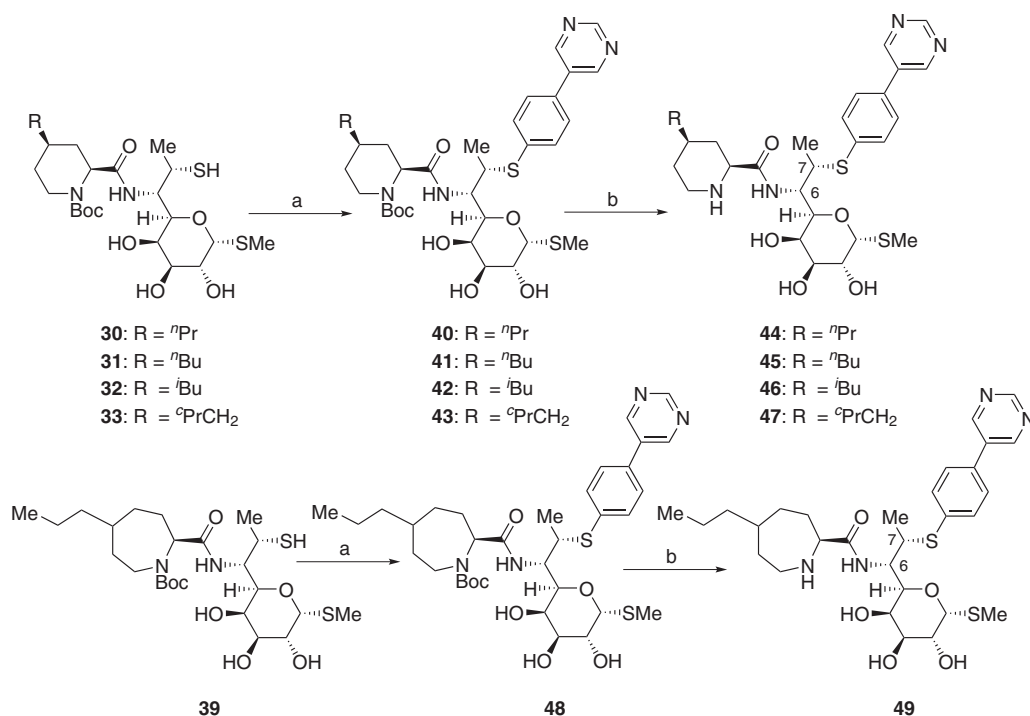
coupling reaction of **56** with enantio-pure **15** provided desired **57** with all carbon's framework. Deprotection of the Boc group finally gave **58** and its reductive *N*-methylation provided compound **59**.

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

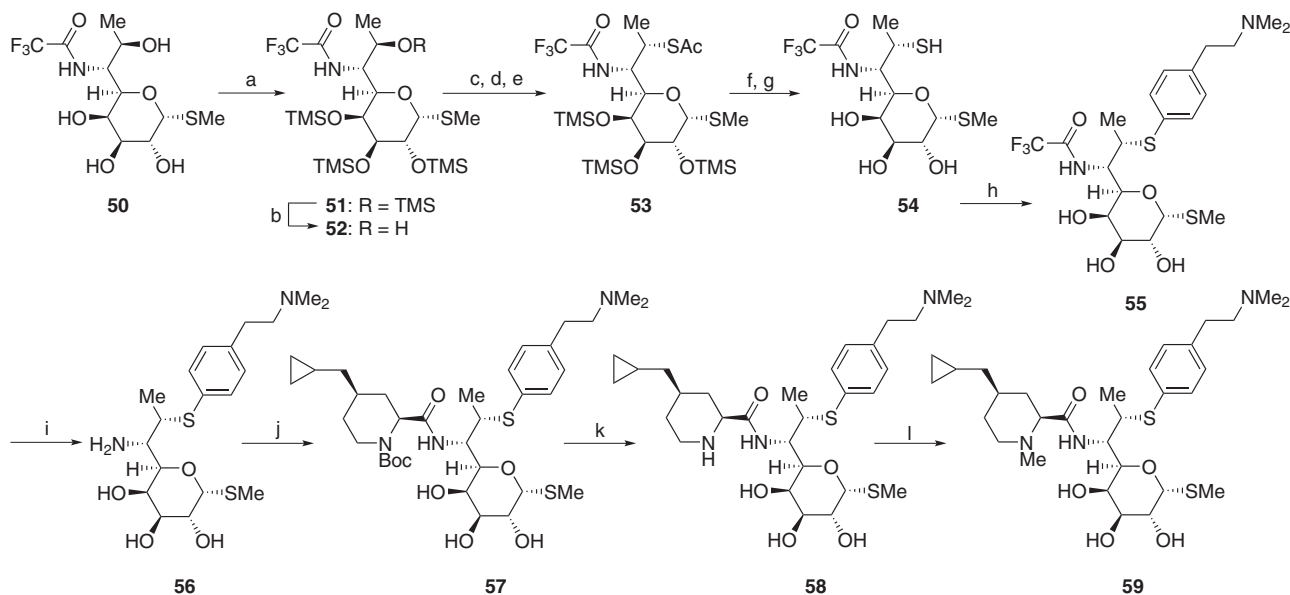
Syntheses of novel 4'-*cis*-(cyclopropylmethyl)piperidine LCM derivatives possessing a 4-substituted phenylthio group at the C-7 position are shown in Scheme 5. Compounds **60**, **61** and **63–65** were synthesized from the key intermediate **33** by palladium-catalyzed cross-coupling reaction with 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



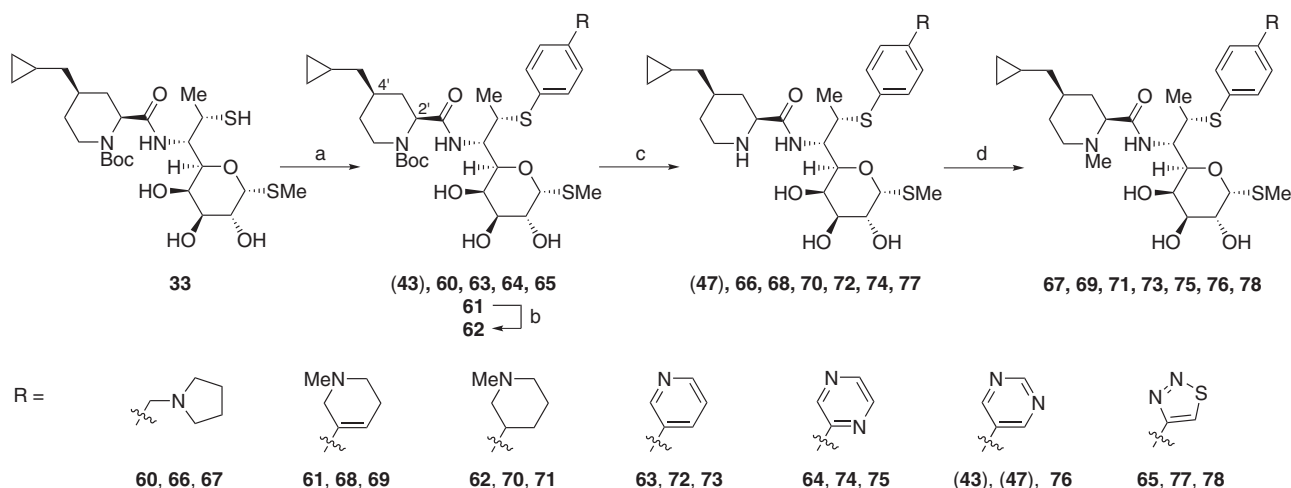
Scheme 3 Synthesis of novel (7S)-4-(pyrimidin-5-yl)phenylthio LCM derivatives possessing piperidine or azepane as the C-6 side chain. Conditions: (a) 5-(4-bromophenyl)pyrimidine, Pd₂(dba)₃, Xantphos, *i*Pr₂NEt, dioxane, reflux, 2–6 h; (b) TFA, CH₂Cl₂, –20 °C to r.t., 1.5–6 h.



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, NEt₃, CHCl₃, 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, Pd₂(dba)₃, Xantphos, *i*Pr₂NEt, 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, CH₂Cl₂, 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 (7S)-7-(4-(pyrimidin-5-yl)phenyl)thio-substituted LCM derivatives **44–47** and **49** (Table 2). According to our reported SAR studies, (7S) 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 $\text{Pd}_2(\text{dba})_3$, Xantphos, $i\text{Pr}_2\text{NEt}$, 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, $\text{NaBH}(\text{OAc})_3$, AcOH, MeOH, r.t., 0.5–2 h.

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

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

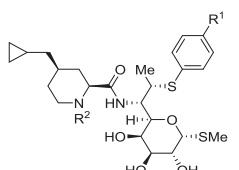

^aAll strains except standard organisms were clinically isolated.

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 (7S)-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, $\mu\text{g ml}^{-1}$) of novel lincomycin derivatives modified at the C-7 position with an aliphatic moiety

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

^aAll 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*.

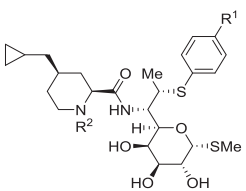




In vitro antibacterial activity (sensitivity distribution analysis) of selected compounds against sixty clinical isolates of *S. pneumoniae*

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). MIC₉₀ values of five novel LCM derivatives (0.06–0.125 $\mu\text{g ml}^{-1}$) were relatively smaller than that of TEL (0.25 $\mu\text{g ml}^{-1}$). 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

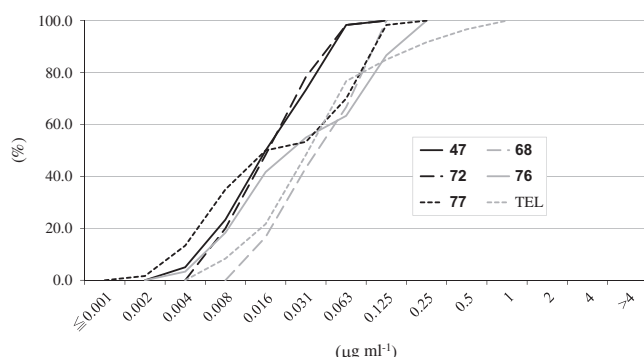
We finally investigated the *in vivo* efficacy of selected compounds in rat pulmonary infection model with resistant *S. pneumoniae* with *erm* + *mef* genes. Among derivatives reported in this study, *in vitro* activities of compounds 47 and 72 are rather strong (Figure 2). On the other hand, we had 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, $\mu\text{g ml}^{-1}$) of optimized novel lincomycin derivatives modified at the C-7 position with an aromatic moiety

|  | | $R^1 =$     | | | | | | | | |
|---|--|---|-------|--------|-------|--------|--------|--------|--------|--------|
| | | $R^2 =$ H Me H Me H Me H Me | | | | | | | | |
| Test organism ^a | Characheristics | 72 | 73 | 74 | 75 | 47 | 76 | 77 | 78 | TEL |
| <i>Streptococcus pneumoniae</i> DP1 TypeI | susceptible | 0.015 | 0.03 | ≤0.008 | 0.03 | ≤0.008 | ≤0.008 | ≤0.008 | ≤0.008 | ≤0.008 |
| <i>S. pneumoniae</i> -2 | susceptible | 0.015 | 0.03 | ≤0.008 | 0.03 | ≤0.008 | ≤0.008 | ≤0.008 | 0.015 | ≤0.008 |
| <i>S. pneumoniae</i> -3 | susceptible | 0.015 | 0.06 | 0.015 | 0.06 | 0.015 | 0.03 | 0.015 | 0.015 | ≤0.008 |
| <i>S. pneumoniae</i> -4 | <i>ermAM</i> methylase (c) | 0.03 | 0.5 | 0.06 | 0.5 | 0.03 | 0.25 | 0.06 | 0.5 | 0.5 |
| <i>S. pneumoniae</i> -5 | <i>ermAM</i> methylase (c) | 0.06 | 0.25 | 0.06 | 0.5 | 0.03 | 0.12 | 0.12 | 0.5 | 2 |
| <i>S. pneumoniae</i> -6 | <i>ermAM</i> methylase (c) + <i>mefE</i> | 0.06 | 0.5 | 0.12 | 1 | 0.06 | 0.5 | 0.25 | 0.5 | 1 |
| <i>S. pneumoniae</i> -7 | <i>ermAM</i> methylase (i) | 0.015 | 0.06 | 0.03 | 0.25 | 0.015 | 0.015 | 0.03 | 0.25 | 0.03 |
| <i>S. pneumoniae</i> -8 | <i>ermAM</i> methylase (i) | NT | NT | 0.03 | 0.25 | 0.015 | 0.03 | 0.03 | 0.25 | 0.03 |
| <i>S. pneumoniae</i> -9 | <i>mefE</i> efflux | ≤0.008 | 0.015 | ≤0.008 | 0.03 | ≤0.008 | ≤0.008 | ≤0.008 | ≤0.008 | 0.06 |
| <i>Streptococcus pyogenes</i> Cook | susceptible | ≤0.008 | 0.015 | ≤0.008 | 0.015 | 0.015 | 0.015 | ≤0.008 | ≤0.008 | ≤0.008 |
| <i>S. pyogenes</i> -2 | <i>ermAM</i> methylase (c) | 0.06 | 0.25 | 0.03 | 0.25 | 0.03 | 0.12 | 0.03 | 0.12 | 16 |
| <i>S. pyogenes</i> -3 | <i>mefE</i> efflux | 0.015 | 0.03 | 0.015 | 0.03 | 0.015 | ≤0.008 | 0.015 | 0.015 | 0.25 |
| <i>Haemophilus influenzae</i> | susceptible | 1 | 4 | 2 | 4 | 1 | 4 | 1 | 4 | 0.5 |
| <i>H. influenzae</i> -2 | susceptible | 2 | 4 | 2 | 4 | 1 | 2 | 1 | 2 | 2 |
| <i>H. influenzae</i> -3 | susceptible | 2 | 8 | 4 | 8 | 2 | 4 | 2 | 4 | 1 |
| <i>H. influenzae</i> -4 | Δ <i>acr</i> | 0.03 | 0.12 | 0.03 | 0.12 | 0.03 | 0.06 | 0.03 | 0.03 | 0.25 |
| <i>Mycoplasma pneumoniae</i> -1 | susceptible | NT | NT | ≤0.004 | NT | ≤0.004 | ≤0.004 | ≤0.004 | NT | ≤0.004 |
| <i>M. pneumoniae</i> -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.

^aAll 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

¹H 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 CDCl₃ or CD₃OD. TMS (0 p.p.m.) in CDCl₃ or CD₃OD 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

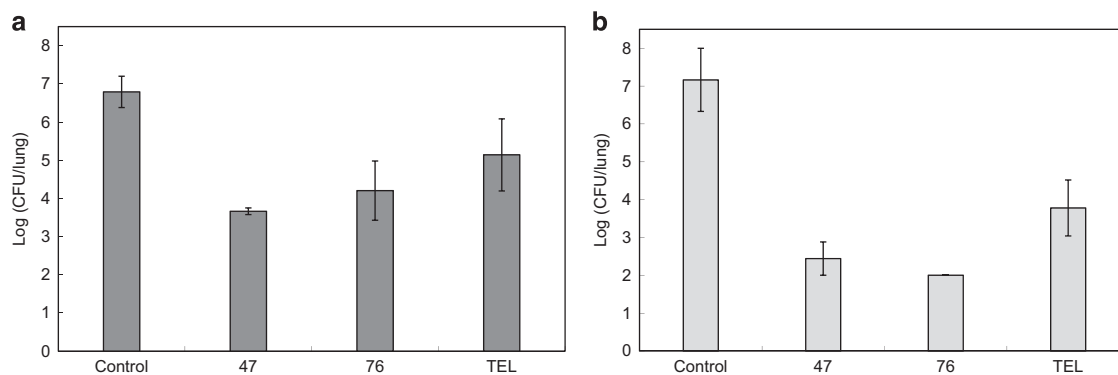


Figure 3 (a) *In vivo* efficacy of **47** and **76** in a rat pulmonary infection model with *S. pneumoniae* MSC06856 (*erm* + *mef*). (b) *In vivo* efficacy of **47** and **76** in a rat pulmonary infection model with *S. pneumoniae* MSC06729 (*mef*). Comparison of the efficacy of novel lincomycin derivatives **47** and **76** in a rat pulmonary neutropenic infection model with *S. pneumoniae* MSC06856 (*erm* + *mef*) and *S. pneumoniae* MSC06729 (*mef*). Three rats per group were rendered neutropenic and 10^6 CFU per rat of *S. pneumoniae* MSC06856 or *S. pneumoniae* 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.

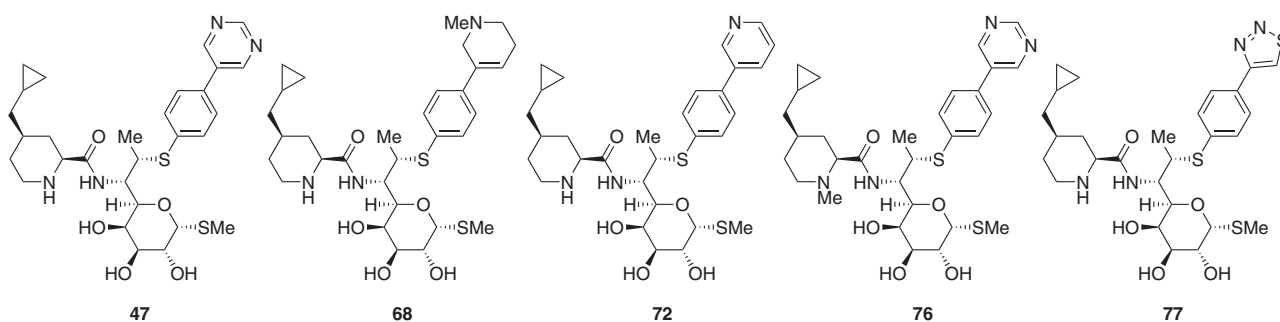


Figure 4 Structures of novel lincomycin derivatives possessing strong *in vitro* antibacterial activity.

MgSO₄ 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 NH₄Cl. The desired compound was extracted with ethyl acetate, was washed with brine and then the organic phase was dried over Na₂SO₄, filtrated and concentrated under reduced pressure. The resulting residue was purified by distillation under reduced pressure (84 $^{\circ}\text{C}/8$ 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 C₉H₁₁N; ¹H NMR (400 MHz, CDCl₃) 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 CH₂Cl₂ (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 Na₂S₂O₃ solution (75 g in 150 ml of H₂O). The solution was added to mixture of saturated aqueous NaHCO₃ (500 ml), saturated aqueous K₂CO₃ (40 ml) and CHCl₃ (500 ml). The organic phase was separated and then further extracted twice with CHCl₃ (500 ml)-isopropanol (100 ml), the organic phase was dried over Na₂SO₄, filtrated and concentrated under reduced pressure to obtain 4-(cyclopropylmethyl)pyridine *N*-oxide (30.7 g as crude). ¹H NMR (400 MHz, CDCl₃) 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 CH₂Cl₂ (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 K₂CO₃. The desired compound was extracted with CH₂Cl₂ and then the organic phase was dried over Na₂SO₄, 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 C₁₀H₁₀N₂; ¹H NMR (400 MHz, CDCl₃) 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 CHCl₃ (500 ml)-isopropanol (150 ml) and then the organic phase was dried over Na₂SO₄, 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 C₁₀H₁₁NO₂; ¹H NMR (400 MHz, CDCl₃) 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 PtO₂ (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/CH₃CN=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 C₁₀H₁₇NO₂; ¹H NMR (400 MHz, D₂O) 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 by 1 M aqueous NaOH. Then, to the aqueous phase was added H₂O, washed with Et₂O, adjusted at pH 4 by 1 M aqueous HCl, extracted with ethyl acetate and then the organic phase was dried over Na₂SO₄, 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 PtO₂ resulted in an approximately 1:1 mixture of two isomeric *cis*-products by Birkenmeyer *et al.*⁴⁹ ¹H NMR (400 MHz, CDCl₃) 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 CH₃CN (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 NaHCO₃. The desired compound was extracted with ethyl acetate and then the organic phase was dried over Na₂SO₄, 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**: [α]_D²⁷ -24.8° (c 0.65, CHCl₃); ESI-MS *m/z* 374 (M+H)⁺ as C₂₂H₃₁NO₄; ¹H NMR (400 MHz, CDCl₃) 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**: [α]_D²⁶ +24.2° (c 1.03, CHCl₃); ESI-MS *m/z* 374 (M+H)⁺ as C₂₂H₃₁NO₄. 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. [α]_D²⁷ -16.3° (c 0.40, MeOH); ESI-MS *m/z* 284 (M+H)⁺ as C₁₅H₂₅NO₄; time-of-flight (TOF)-ESI-HR-MS (M-H)⁻ calcd for C₁₅H₂₅NO₄: 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)-H₂O (0.2 ml) was added LiOH·H₂O (23.7 mg, 0.56 mmol) and then stirred at room temperature for 5 h. The mixture was diluted with H₂O, washed with Et₂O. 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 Na₂SO₄, 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 C₁₆H₂₀N₂O₆S; ¹H NMR (400 MHz, CD₃OD) 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 H₂O and then the solution was filtrated, and ethyl acetate and saturated aqueous NaHCO₃ were added to the filtrate. The desired compound was extracted with ethyl acetate, extracted with CHCl₃ and then the organic phase was dried over Na₂SO₄, 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 H₂O and ethyl acetate, and then the mixture was filtrated. The desired compound was extracted with ethyl acetate, washed with saturated aqueous KHCO₃ and then the organic phase was dried over MgSO₄, 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 (\pm)-**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 NaHCO₃ 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 C₂₄H₄₂N₂O₈S.

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 NaHCO₃. The desired compound was extracted with ethyl acetate, washed with saturated aqueous NaCl and then the organic phase was dried over Na₂SO₄, 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 NaHCO₃ and concentrated under reduced pressure to remove MeOH. The desired compound was extracted with ethyl acetate, and then the organic phase was dried over Na₂SO₄, 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 C₃₂H₆₆N₂O₈SSi₃; ¹H NMR (400 MHz, CD₃OD) 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 C₃₃H₆₈N₂O₈SSi₃; ¹H NMR (400 MHz, CDCl₃) 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 C₃₃H₆₈N₂O₈SSi₃; ¹H NMR (400 MHz, CD₃OD) 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 C₃₃H₆₆N₂O₈SSi₃; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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 CH₂Cl₂ (2 ml) at 0 °C were added Et₃N (291 μ l, 2.08 mmol) and methanesulfonyl chloride (107 μ l, 1.38 mmol), and stirred at room temperature for 1 h. The mixture was added to saturated aqueous NaHCO₃, extracted with ethyl acetate, dried over Na₂SO₄ 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 NaHCO₃, then extracted with ethyl acetate, washed with 10% aqueous NaCl, dried over Na₂SO₄, 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 C₃₄H₆₈N₂O₈S₂Si₃; ¹H NMR (400 MHz, CDCl₃) 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 (7S)-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), Et₃N (490 μ l, 3.48 mmol) and methane-sulfonyl chloride (220 μ l, 2.79 mmol) in CH₂Cl₂ (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 C₃₅H₇₀N₂O₈S₂Si₃; ¹H NMR (400 MHz, CDCl₃) 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 (7S)-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), Et₃N (930 μl, 6.85 mmol) and methane-sulfonyl chloride (210 μl, 2.74 mmol) in CH₂Cl₂ (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 (7S)-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), Et₃N (4.24 ml, 30.3 mmol) and methane-sulfonyl chloride (1.56 ml, 20.2 mmol) in CHCl₃ (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 C₃₅H₆₈N₂O₈S₂Si₃; ¹H NMR (400 MHz, CDCl₃) 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 (7S)-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 NaHCO₃, concentrated under reduced pressure to remove MeOH until half volume, extracted with ethyl acetate, dried over Na₂SO₄ and concentrated under reduced pressure to obtain methyl (7S)-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 NH₄Cl, extracted with ethyl acetate, washed with saturated aqueous NaHCO₃, dried over Na₂SO₄ 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 C₂₃H₄₂N₂O₇S₂; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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 NaHCO₃, extracted with ethyl acetate, washed with 25% aqueous NaCl, dried over Na₂SO₄ and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (CHCl₃/MeOH = 30/1) to obtain methyl (7S)-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 NaHCO₃, extracted with ethyl acetate, washed with 25% aqueous NaCl, dried over Na₂SO₄ and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (CHCl₃/MeOH = 40/1) to obtain the title compound (373 mg, quant) as a colorless solid. ESI–MS m/z 537 (M+H)⁺ as C₂₄H₄₄N₂O₇S₂; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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 NaHCO₃, extracted with ethyl acetate, washed with 25% aqueous NaCl, dried over Na₂SO₄ and concentrated under reduced pressure to obtain methyl (7S)-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 C₂₄H₄₄N₂O₇S₂; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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 NaHCO₃ and then concentrated under reduced pressure until half volume to remove MeOH, extracted with ethyl acetate, washed with 25% aqueous NaCl, dried over Na₂SO₄ and concentrated under reduced pressure to obtain methyl (7S)-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 C₂₄H₄₂N₂O₇S₂; ¹H NMR (400 MHz, CD₃OD) 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 NaHCO₃. The desired compound was extracted with ethyl acetate, washed with H₂O and then the organic phase was dried over Na₂SO₄, filtrated and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (CHCl₃/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 C₂₅H₃₇N₃O₁₀S₂; ¹H NMR (400 MHz, CD₃OD) 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 CHCl₃/CH₃OH/28% aq NH₄OH = 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 C₁₉H₃₆N₂O₆S; TOF–ESI–HR–MS (M+H)⁺ calcd for C₁₉H₃₆N₂O₆S: 421.2372, found: 421.2370; ¹H NMR (400 MHz, CD₃OD) 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)–H₂O (10 ml) were added LiOH·H₂O (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 NaHCO₃, extracted with ethyl acetate and then the organic phase was dried over Na₂SO₄, filtrated and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (CHCl₃/CH₃OH/28% aq NH₄OH = 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 C₂₄H₄₄N₂O₈S; ¹H NMR (400 MHz, CD₃OD) 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 NaHCO₃, extracted with ethyl acetate and then the organic phase was dried over Na₂SO₄, 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**, Et₃N (1.20 ml, 8.58 mmol) and methanesulfonyl chloride (0.53 ml, 6.9 mmol) in CHCl₃ (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 C₃₅H₇₀N₂O₈S₂Si₃.

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 solution (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 C₂₄H₄₄N₂O₇S₂; TOF–ESI–HR–MS (M+H)⁺ calcd for C₂₄H₄₄N₂O₇S₂: 537.2668, found: 537.2668; ¹H NMR (400 MHz, CD₃OD) 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) (Pd₂(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 μ m) (Kurabo Industries Ltd, Osaka, Japan) or celite, concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (CHCl₃/MeOH/28% aq NH₄OH = 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 C₃₃H₄₈N₄O₇S₂.

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), Pd₂(dba)₃ (13.4 mg, 0.0146 mmol) and *N,N*-diisopropylethylamine (97.0 μ l, 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 (7S)-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), Pd₂(dba)₃ (10.0 mg, 0.0109 mmol) and N,N-diisopropylethylamine (60.0 μ l, 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 (7S)-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), Pd₂(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 C₃₄H₄₈N₄O₇S₂.

Methyl (7S)-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 CH₂Cl₂ (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 (CHCl₃/MeOH/28% aq NH₄OH=9/2/0.2) to obtain the title compound (122.8 mg, 88%) as a colorless solid. [α]_D²² +85.2° (c 1.02, MeOH); ESI-MS *m/z* 577 (M+H)⁺ as C₂₈H₄₀N₄O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₂₈H₄₀N₄O₅S₂: 577.2518, found: 577.2516; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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 CH₂Cl₂ (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. [α]_D²³ +81.2° (c 0.78, MeOH); ESI-MS *m/z* 591 (M+H)⁺ as C₂₉H₄₂N₄O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₂₉H₄₂N₄O₅S₂: 591.2675, found: 591.2669; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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. [α]_D²⁴ +93.9° (c 0.83, MeOH); ESI-MS *m/z* 591 (M+H)⁺ as C₂₉H₄₂N₄O₅S₂; TOF-

ESI-HR-MS (M+H)⁺ calcd for C₂₉H₄₂N₄O₅S₂: 591.2675, found: 591.2674; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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 CH₂Cl₂ (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. [α]_D²⁴ +86.1° (c 0.25, MeOH); ESI-MS *m/z* 589 (M+H)⁺ as C₂₉H₄₀N₄O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₂₉H₄₀N₄O₅S₂: 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; ¹H NMR (400 MHz, CD₃OD) 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); ¹³C NMR (100 MHz, CD₃OD) 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 (7S)-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 μ mol), Pd₂(dba)₃ (5.1 mg, 5.6 μ mol) and N,N-diisopropylethylamine (10.0 μ l, 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 C₃₄H₅₀N₄O₇S₂.

Methyl (7S)-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. [α]_D²⁴ +82.9° (c 0.84, MeOH); ESI-MS *m/z* 591 (M+H)⁺ as C₂₉H₄₂N₄O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₂₉H₄₂N₄O₅S₂: 591.2675, found: 591.2667; ¹H NMR (400 MHz, CD₃OD) 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 C₂₃H₅₀F₃NO₆SSi₄.

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 NaHCO₃ and concentrated under reduced pressure to remove MeOH. The desired compound was extracted with ethyl acetate, and then the organic phase was dried over Na₂SO₄, 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 C₂₀H₄₂F₃NO₆SSi₃; ¹H NMR (400 MHz, CD₃OD) 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), Et₃N (21.8 ml, 15.6 mmol) and methane-sulfonyl chloride (1.21 ml, 15.6 mmol) in CHCl₃ (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*-methanesulfonyl-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 NaHCO₃, and extracted with ethyl acetate. The organic phase was dried over Na₂SO₄, 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 NaHCO₃, extracted with ethyl acetate and then the organic phase was dried over Na₂SO₄, 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 C₂₂H₄₄F₃NO₆S₂Si₃; ¹H NMR (400 MHz, CDCl₃) 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 C₁₁H₁₈F₃NO₅S₂; ¹H NMR (400 MHz, CD₃OD) 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*-dimethylethylamine (3.22 g, 14.1 mmol), Xantphos (544.8 mg, 0.942 mmol), Pd₂(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 C₂₁H₃₁F₃N₂O₅S₂; ¹H NMR (400 MHz, CD₃OD) 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 CH₂Cl₂ (73 ml) were added *N*-benzyl-*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 (CHCl₃/MeOH/28% aq NH₄OH=20/1/0.1 to 10/1/0.1) to obtain the title compound (2.94 g, 94%) as off white solid. ¹H NMR (400 MHz, CD₃OD) 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 C₃₄H₅₅N₃O₇S₂.

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 CH₂Cl₂ (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. [α]_D²³ +101.3° (*c* 0.65, MeOH); ESI–MS m/z 582 (M+H)⁺ as C₂₉H₄₇N₃O₅S₂; TOF–ESI–HR–MS (M+H)⁺ calcd for C₂₉H₄₇N₃O₅S₂: 582.3035, found: 582.3032; ¹H NMR (400 MHz, CD₃OD) 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 NaHCO₃,

extracted with ethyl acetate/MeOH = 5/1. The organic phase was dried over Na₂SO₄, filtrated and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (CHCl₃/MeOH/28% aq NH₄OH = 20/1/0.1) to obtain the title compound (1.10 g, 91%) as a colorless solid. $[\alpha]_D^{22} + 88.4^\circ$ (c 1.58, MeOH); ESI-MS m/z 596 (M+H)⁺ as C₃₀H₄₉N₃O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₃₀H₄₉N₃O₅S₂: 596.3192, found: 596.3188; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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), Pd₂(dba)₃ (4.0 mg, 4.3 μ mol) and *N,N*-diisopropylethylamine (38.5 μ l, 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 C₃₅H₅₅N₃O₇S₂.

Methyl (7S)-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), Pd₂(dba)₃ (19.4 mg, 0.021 mmol) and *N,N*-diisopropylethylamine (144 μ l, 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 C₃₆H₅₅N₃O₇S₂.

Methyl (7S)-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 Na₂SO₄, filtrated and concentrated under reduced pressure. The resulting residue was purified by preparative TLC (CHCl₃/MeOH/28% aq NH₄OH = 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 C₃₆H₅₇N₃O₇S₂.

Methyl (7S)-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), Pd₂(dba)₃ (5.1 mg, 5.6 μ mol) and *N,N*-diisopropylethylamine (19.5 μ l, 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 (7S)-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), Pd₂(dba)₃ (10 mg, 0.0109 mmol) and *N,N*-diisopropylethylamine (60 μ l, 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 (7S)-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), Pd₂(dba)₃ (10 mg, 0.011 mmol) and *N,N*-diisopropylethylamine (60 μ l, 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 C₃₂H₄₆N₄O₇S₃.

Methyl (7S)-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 CH₂Cl₂ (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. $[\alpha]_D^{23} + 68.9^\circ$ (c 0.20, MeOH); ESI-MS m/z 594 (M+H)⁺ as C₃₀H₄₇N₃O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₃₀H₄₇N₃O₅S₂: 594.3035, found: 594.3031; ¹H NMR (400 MHz, CD₃OD) 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, 3.3 Hz, 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 (7S)-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 μ l, 0.28 mmol), AcOH (16 μ l, 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. $[\alpha]_D^{22} + 65.9^\circ$ (c 0.11, MeOH); ESI-MS m/z 608 (M+H)⁺ as C₃₁H₄₉N₃O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₃₁H₄₉N₃O₅S₂: 608.3192, found: 608.3187; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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 μ l) in CH₂Cl₂ (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. $[\alpha]_D^{22} + 91.4^\circ$ (c 1.82, MeOH); ESI-MS m/z 606 (M+H)⁺ as C₃₁H₄₇N₃O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₃₁H₄₇N₃O₅S₂: 606.3035, found: 606.3012; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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. $[\alpha]_D^{23} + 67.4^\circ$ (c 2.48, MeOH); ESI-MS m/z 620 (M+H)⁺ as C₃₂H₄₉N₃O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₃₂H₄₉N₃O₅S₂: 620.3192, found: 620.3187; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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 CH₂Cl₂ (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. $[\alpha]_D^{23} + 81.6^\circ$ (c 0.65, MeOH); ESI-MS m/z 608 (M+H)⁺ as C₃₁H₄₉N₃O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₃₁H₄₉N₃O₅S₂: 608.3192, found: 608.3175; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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. $[\alpha]_D^{22} + 70.1^\circ$ (c 0.15, MeOH); ESI-MS m/z 622 (M+H)⁺ as C₃₂H₅₁N₃O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₃₂H₅₁N₃O₅S₂: 622.3348, found: 622.3342; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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 CH₂Cl₂ (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. $[\alpha]_D^{23} + 92.4^\circ$ (c 1.12, MeOH); ESI-MS m/z 588 (M+H)⁺ as C₃₀H₄₁N₃O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₃₀H₄₁N₃O₅S₂: 588.2566, found: 588.2560; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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. $[\alpha]_D^{24} + 57.4^\circ$ (c 0.28, MeOH); ESI-MS m/z 602 (M+H)⁺ as C₃₁H₄₃N₃O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₃₁H₄₃N₃O₅S₂: 602.2722, found: 602.2714; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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. $[\alpha]_D^{22} + 78.5^\circ$ (c 1.20, MeOH); ESI-MS m/z 589 (M+H)⁺ as C₂₉H₄₀N₄O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₂₉H₄₀N₄O₅S₂: 589.2518, found: 589.2514; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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. $[\alpha]_D^{25} + 72.4^\circ$ (c 0.64, MeOH);

ESI-MS m/z 603 (M+H)⁺ as C₃₀H₄₂N₄O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₃₀H₄₂N₄O₅S₂: 603.2675, found: 603.2672; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-6-*N*-((2′S, 4′R)-4′-(cyclopropylmethyl)-1′-*N*-methylpiperidine-2′-carbonyl)-7-deoxy-7-(4-(pyrimidin-5-yl)phenylthio)-α-thiolicosaminide (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. $[\alpha]_D^{22} +75.4^\circ$ (c 0.96, MeOH); ESI-MS m/z 603 (M+H)⁺ as C₃₀H₄₂N₄O₅S₂; TOF-ESI-HR-MS (M+H)⁺ calcd for C₃₀H₄₂N₄O₅S₂: 603.2675, found: 603.2672; IR (KBr) cm⁻¹ 1077, 1415, 1508, 1653, 2361, 3020 and 3690; ¹H NMR (400 MHz, CD₃OD) 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); ¹³C NMR (100 MHz, CD₃OD) 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 (7S)-6-*N*-((2′S, 4′R)-4′-(cyclopropylmethyl)piperidine-2′-carbonyl)-7-deoxy-7-(4-(1,2,3-thiadiazol-4-yl)phenylthio)-α-thiolicosaminide (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. $[\alpha]_D^{27} +80.4^\circ$ (c 0.36, MeOH); ESI-MS m/z 595 (M+H)⁺ as C₂₇H₃₈N₄O₅S₃; TOF-ESI-HR-MS (M+H)⁺ calcd for C₂₇H₃₈N₄O₅S₃: 595.2083, found: 595.2073; ¹H NMR (400 MHz, CD₃OD) 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 (7S)-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-thiolicosaminide (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. $[\alpha]_D^{23} +48.9^\circ$ (c 0.28, MeOH); ESI-MS m/z 609 (M+H)⁺ as C₂₈H₄₀N₄O₅S₃; TOF-ESI-HR-MS (M+H)⁺ calcd for C₂₈H₄₀N₄O₅S₃: 609.2239, found: 609.2231; ¹H NMR (400 MHz, CD₃OD) 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 10⁶ 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 × 10⁴ 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 10⁶ 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.

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- Reinert, R. R., van der Linden, M. & Al-Lahham, A. Molecular characterization of the first telithromycin-resistant *Streptococcus pneumoniae* isolate in Germany. *Antimicrob. Agents Chemother.* **49**, 3520–3522 (2005).
- Kim, S. H. *et al.* Changing trends in antimicrobial resistance and serotypes of *Streptococcus pneumoniae* isolates in Asian countries: an Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. *Antimicrob. Agents Chemother.* **56**, 1418–1426 (2012).
- Ajito, K., Miura, T., Furuuchi, T. & Tamura, A. Sixteen-membered macrolides: chemical modifications and future applications. *Heterocycles* **89**, 281–352 (2014).
- Morimoto, S., Takahashi, Y., Watanabe, Y. & Omura, S. Chemical modification of erythromycins I. Synthesis and antibacterial activity of 6-O-methylerythromycins A. *J. Antibiot.* **37**, 187–189 (1984).
- Slobodan, D. *et al.* Erythromycin series. Part 13. Synthesis and structure elucidation of 10-dihydro-10-deoxy-11-methyl-11-azaerythromycin A. *J. Chem. Res. Synop.* **40**, 152–153 (1988).
- Retsema, J. *et al.* Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against Gram-Negative Organisms. *Antimicrob. Agents Chemother.* **31**, 1939–1947 (1987).
- Denis, A. *et al.* Synthesis and antibacterial activity of HMR 3647 a new ketolide highly potent against erythromycin-resistant and susceptible pathogens. *Bioorg. Med. Chem. Lett.* **9**, 3075–3080 (1999).
- Clay, K. D. *et al.* Severe hepatotoxicity of telithromycin: three case reports and literature review. *Ann. Intern. Med.* **144**, 415–420 (2006).
- Ross, D. B. The FDA and the case of Ketek. *N. Engl. J. Med.* **356**, 1601–1604 (2007).
- Gleason, P. P., Walters, C., Heaton, A. H. & Schafer, J. A. Telithromycin: the perils of hasty adoption and persistence of off-label prescribing. *J. Manag. Care Pharm.* **13**, 20–25 (2007).
- Department of Health and Human Services. Telithromycin (marketed as Ketek) information available at <http://www.fda.gov/drugs/drugsafety/postmarketdrugsafetyinformationforpatientsandproviders/ucm107824.htm> Accessed 26 April 2007.
- Miura, T. *et al.* Novel azalides derived from sixteen-membered macrolides. I. Isolation of the mobile dialdehyde and its one-pot macrocyclization with an amine. *J. Antibiot.* **60**, 407–435 (2007).
- Miura, T. *et al.* Novel azalides derived from 16-membered macrolides. III. Azalides modified at the C-15 and 4'' positions: improved antibacterial activities. *Bioorg. Med. Chem.* **18**, 2735–2747 (2010).
- Mason, D. J., Dietz, A. & Deboer, C. Lincomycin, a new antibiotic I. Discovery and biological properties. *Antimicrob. Agents Chemother.* 554–559 (1962).
- Magerlein, B. J. & Lincomycin, X. The chemical synthesis of lincomycin. *Tetrahedron Lett.* **1**, 33–36 (1970).
- Howarth, G. B., Szarek, W. A. & Jones, J. K.N. The synthesis of lincomycin. *J. Chem. Soc. (c)* **16**, 2218–2224 (1970).
- Perlman, D. *Structure-Activity Relationships Among the Semisynthetic Antibiotics*. Academic Press: New York, San Francisco, London, A Subsidiary of Harcourt Brace Jovanovich Publishers, 1977, pp 600–651.
- Birkenmeyer, R. D. & Kagan, F. Lincomycin. XI. Synthesis and structure of clindamycin. A potent antibacterial agent. *J. Med. Chem.* **13**, 616–619 (1970).
- Shan, P. J., Vakil, N. & Kabakov, A. Role of intravenous immune globulin in streptococcal toxic shock syndrome and *Clostridium difficile* infection. *Am. J. Health Syst. Pharm.* **72**, 1013–1019 (2015).
- Hoeksema, H. Octoses from antibiotics. The Upjohn Company, Kalamazoo, Mich., Abstr. Pap. Division of Carbohydrate Chemistry, 149th Meet, American Chemical Society. *Am. Chem. Soc. Detroit, Mich* p 9C (1965).
- Magerlein, B. J., Birkenmeyer, R. D. & Kagan, F. Chemical modification of lincomycin. *Antimicrob. Agents Chemother.* 727–736 (1966).
- Sinkula, A. A., Morozowich, W., Lewis, C. & Mackellar, F. A. Synthesis and bioactivity of lincomycin-7-monoesters. *J. Pharm. Sci.* **58**, 1389–1392 (1969).
- Magerlein, B. J. & Kagan, F. Lincomycin. IX. 7-Thiol and thioamido analogs of lincomycin. *J. Med. Chem.* **12**, 974–977 (1969).
- Lewis, J. G. *et al.* Novel Antimicrobial 7-methyl Lincosamides: Prolamide Analogs. 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy Poster F-1388; Washington, DC, USA.
- Bannister, B. Modifications of lincomycin involving the carbohydrate portion. Part III. The 7-O-methyl and 6-de-(1-hydroxyethyl) analogues. *J. Chem. Soc. Perkin Trans. I* 1676–1682 (1973).
- Bannister, B. Modifications of lincomycin involving the carbohydrate portion. Part IV. (7S)-7-alkoxy-7-deoxy-analogues. *J. Chem. Soc. Perkin Trans. I* **1974**, 360–369 (1974).
- Bannister, B. & Mydlow, P. K. The S-alkylation of sulphides by an activated carbohydrate epimine under acidic catalysis: the formation of α -acetamido-sulphides. Part 5. The introduction of functionality into the sulphide substituent. *J. Chem. Res. (S)* **1989**, 90–91 (1989).
- Bannister, B. The S-alkylation of sulphides by an activated carbohydrate epimine under acidic catalysis: The formation of α -acetamido-sulphides. Part 4. Reaction with dithioacetals and monothioacetals. *J. Chem. Soc. Perkin Trans. I* **1980**, 540–552 (1980).
- Bannister, B. (7S)-7-deoxy-7-substituted-alkylthio-lincomycin. S-Alkylation of sulphides by an activated epimine under acidic catalysis: formation of α -acetamido-sulphides. *Tetrahedron* **40**, 1633–1660 (1984).
- Bannister, B. The Upjohn Company. Derivatives of lincomycin and its analogs and process. US Patent US3915954 A (1973).
- Bannister, B. The Upjohn Company. Derivatives of lincomycin and its analogs and process. Canadian Patent CA-971956 A1 (1972).
- Sztaricskai, F. *et al.* Semisynthetic modification of antibiotic lincomycin. *J. Antibiot.* **49**, 941–943 (1996).
- Umemura, E. *et al.* Lincomycin derivative and antibacterial agent containing the same as active ingredient. Japanese Patent WO/2007/066805 A1 (14 June 2007).
- Wakiyama, Y. *et al.* Lincomycin derivatives and antibacterial agents containing the same as the active ingredient. Japanese Patent WO/2008/146917 A1 (4 December 2008).
- Umemura, E. *et al.* Lincosamide derivative, and antibacterial agent comprising the same as active ingredient, WO/2008/146919 A1 (4 December 2008).
- Umemura, E. *et al.* Synthesis of novel lincomycin derivatives and their in vitro antibacterial activities. *J. Antibiot.* **66**, 195–198 (2013).
- Wakiyama, Y. *et al.* Synthesis and structure-activity relationships of novel lincomycin derivatives. Part 1. Newly generated antibacterial activities against Gram-positive bacteria with *erm* gene by C-7 modification. *J. Antibiot.* **69**, 368–380 (2016).
- Wakiyama, Y. *et al.* Synthesis and structure-activity relationships of novel lincomycin derivatives. Part 2. Synthesis of 7(S)-7-deoxy-7-(4-morpholinocarbonylphenylthio)lincomycin and its 3-dimensional analysis with rRNA. *J. Antibiot.* **69**, 428–439 (2016).
- Kumura, K. *et al.* Synthesis and antibacterial activity of novel lincomycin derivatives. I. Enhancement of antibacterial activities by introduction of substituted azetidines. *J. Antibiot.* **69**, 440–445 (2016).
- Wakiyama, Y. *et al.* Synthesis and structure-activity relationships of novel lincomycin derivatives Part 3: discovery of the 4-(pyrimidin-5-yl)phenyl group in synthesis of 7(S)-thiolincomycin analogs. *J. Antibiot.* **70**, 52–64 (2017).
- Kumura, K. *et al.* Synthesis and antibacterial activity of novel lincomycin derivatives. II. Exploring (7S)-7-(5-aryl-1,3,4-thiadiazol-2-yl-thio)-7-deoxylincomycin derivatives. *J. Antibiot.* **70**, 655–663 (2017).
- Wakiyama, Y. *et al.* Synthesis and structure-activity relationships of novel lincomycin derivatives Part 4. Synthesis of novel lincomycin analogs modified at the 6- and 7-positions and their potent antibacterial activities. *J. Antibiot.* **70**, 888–906 (2017).
- Kumura, K. *et al.* Synthesis and antibacterial activity of novel lincomycin derivatives. III. Optimization of a phenyl thiadiazole moiety. *J. Antibiot.* (e-pub ahead of print 5 July 2017; doi:10.1038/ja.2017.59).
- Argoudelis, A. D., Coats, J. H., Mason, D. J. & Sebek, O. K. Microbial transformation of antibiotics. III. Conversion of clindamycin to 1'-demethylclindamycin and clindamycin sulfoxide by *Streptomyces* species. *J. Antibiot.* **22**, 309–314 (1969).
- Magerlein, B. J., Birkenmeyer, R. D. & Kagan, F. Lincomycin. VI. 4'-alkyl analogs of lincomycin. Relationship between structure and antibacterial activity. *J. Med. Chem.* **10**, 355–359 (1967).
- Magerlein, B. J. Lincomycin. 14. An improved synthesis and resolution of the antimalarial agent, 1'-demethyl-4'-depropyl-4'-(R)- and -(S)-pentylclindamycin hydrochloride (U-24,729A). *J. Med. Chem.* **15**, 1255–1259 (1972).
- Magerlein, B. J. & Lincomycin. VII. 4'-depropyl-4'-ethoxylincomycins. *J. Med. Chem.* **10**, 1161–1163 (1967).
- Lewis, J. G. *et al.* Novel lincomycin derivatives possessing antimicrobial activity. WO/2006/055070 A2 (26 May 2006).
- Birkenmeyer, R. D., Kroll, S. J., Lewis, C., Stern, K. F. & Zurenko, G. E. Synthesis and antimicrobial activity of clindamycin analogues: pirlimycin, a potent antibacterial agent. *J. Med. Chem.* **27**, 216–223 (1984).
- O'Dowd, H. *et al.* Novel antibacterial azetidine lincosamides. *Bioorg. Med. Chem.* **18**, 2645–2648 (2008).
- O'Dowd, H. *et al.* Novel antibacterial azetidine lincosamides. 44th Interscience Conference on Antimicrobial Agents and Chemotherapy. Poster F-2037; Washington, DC, USA, 2004.
- Lewis, J. G. *et al.* Novel antimicrobial 7-methyl lincosamides: Pipecolamide analogs. 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy. Poster F-1389; Washington, DC, USA, 2004.
- Chen, T. *et al.* Novel 4'-cycloalkyl pipecolamide lincosamide analogs. 44th Interscience Conference on Antimicrobial Agents and Chemotherapy. Poster F-2036; Washington, DC, USA, 2004.
- Lopez, S. L. *et al.* Characterization of the spectrum of in vitro activity of VIC-105555, a new lincosamide. 44th Interscience Conference on Antimicrobial Agents and Chemotherapy. Poster F-2038; Washington, DC, USA, 2004.

- 55 Shuman, R. T., Ornstein, P. L., Paschal, J. W. & Gesellchen, P. D. An improved synthesis of homoproline and derivatives. *J. Org. Chem.* **55**, 738–741 (1990).
- 56 Schroeder, W., Bannister, B. & Hoeksema, H. Lincomycin. III. The structure and stereochemistry of the carbohydrate moiety. *J. Am. Chem. Soc.* **89**, 2448–2453 (1967).
- 57 Houtman, R. L. & Mich, P. The Upjohn Company. Trimethylsilyl ethers of lincomycin and its compounds. US Patent US3418414 (1966).
- 58 Itoh, T. & Mase, T. A general palladium-catalyzed coupling of aryl bromides/triflates and thiols. *Org. Lett.* **6**, 4587–4590 (2004).
- 59 Magerlein, B. J. & Kagan, F. Lincomycin. 8. 4'-Alkyl-1'-demethyl-4'-depropylclindamycins, potent antibacterial and antimalarial agents. *J. Med. Chem.* **12**, 780 (1969).
- 60 Farrell, D. J., Morrissey, I., Bakker, S. & Felmingham, D. Molecular characterization of macrolide resistance mechanisms among *Streptococcus pneumoniae* and *Streptococcus pyogenes* isolated from the PROTEKT 1999-2000 study. *J. Antimicrob. Chemother.* **50**(suppl_2), 39–47 (2002).
- 61 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI Document M100-S16*, Clinical and Laboratory Standards Institute, Wayne, PA, (2006).

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