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ORIGINAL ARTICLE

Synthesis and antibacterial activity of novel lincomycin derivatives. I. Enhancement of antibacterial activities by introduction of substituted azetidines

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The synthesis and antibacterial activity of (7*S*)-7-sulfur-azetidin-3-yl lincomycin derivatives are described. Modification was achieved by a simple reaction of (7*R*)-7-*O*-methanesulfonyllincomycin and the corresponding substituted azetidine-2-thiol. Several compounds first showed moderate antibacterial activity against *Streptococcus pneumoniae* and *Streptococcus pyogenes* with *erm* gene as lincomycin derivatives.
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INTRODUCTION

Lincosamide antibiotics are prokaryotic translation inhibitors that act on the 50S ribosome in a similar manner to macrolide antibiotics. Clindamycin (CLDM) derived from lincomycin (LCM) is a useful semisynthetic antibiotic (Figure 1) that is effective against staphylococcal and streptococcal infections. However, those lincosamides show almost no antibacterial activity against resistant pathogens such as *Streptococcus pneumoniae* and *Streptococcus pyogenes* with *erm* gene. Erm methyltransferases methylate A2058Ec of rRNA and diminish the affinity of clinically important macrolides, lincosamides and streptogramin B.[1] Emergence of resistant bacteria is a serious concern in clinical sites.[2] The crystal structures of bacterial 23S ribosomal rRNA complexed with CLDM showed several hydrogen bonds between a sugar moiety of CLDM and the peptidyl transferase cavity composed of such as A2058Ec, G2520Ec and A2059Ec.[3] This finding explains the reason why chemical modifications of the sugar moiety of lincosamide result in drastic loss of antibacterial activity.[4] On the other hand, there is a hydrophobic space around the C-7 position of CLDM in the crystal structure and it is consistent with the fact that modifications at the C-7 position of LCM tend to give comparable antibacterial activity to that of LCM.[5,6] Sztaricskai *et al.*[5] reported compounds **1** and **2**, which have a heteroaryl group via sulfur atom with (7*R*) configuration. We supposed that (7*S*) configuration is more suitable for target interaction from reported structure-activity relationships (SAR)[6] and the three-dimensional structural information. So far, we synthesized many (7*S*)-7-sulfur-substituted LCM analogues,[7] and we describe the first-generation derivatives among them in this report. Thus, we intended to incorporate an azetidine ring instead of the aromatic heterocycle. The azetidine ring is sometimes used in antibiotics chemistry, for example, β-lactam antiinfective, new quinolone and aminoglycoside: tebipenem,[8] delafloxacin[9] and a tobramycin derivative[10] (Figure 2). Although some of these compounds have different mode of actions from lincosamide, we attempted to incorporate the azetidine ring to lincosamide at the C-7 position via sulfur atom with (7*R*) configuration to generate novel lincomycin antibiotics that are active against *S. pneumoniae* and *S. pyogenes* with *erm* gene.

Chemistry

Although synthesis of lincosamide derivatives that have a sulfide group at the C-7 position with (7*R*) configuration was reported by a simple S_N2 reaction using CLDM,[5,11] preparation of lincosamide derivatives that have a sulfide group with (7*S*) configuration is limited because a starting material is a natural compound with a single stereochemistry at the C-7. Bannister[12] reported introduction of an alkyl thiol to the C-7 position with (7*S*) configuration utilizing epimine prepared form LCM. This method is unique but requires many steps, and a source of sulfide is limited to dithioacetals or monothioacetals. To establish practical synthetic route, we utilized 2,3,4-tris-*O*-(trimethylsilyl)lincomycin (**5**),[13] which was prepared in two steps from LCM as shown in Scheme 1. After methanesulfonylation of the 7-OH of LCM, nucleophilic substitution of **6** with **4** afforded **7** in 48% yield. Removal of trimethylsilyl groups followed by the deprotection of the amine afforded **8** in 73% yield. For the synthesis of *N*-substituted derivatives, **8** was converted to **9** by reprotecting hydroxyl groups of **8**. *N*-Aryl analogues **10a**, **10b** and **10d** were prepared by an S_NAr reaction between **9** and aryl fluoride or chloride. *N*-Alkyl analogues (**11a–c**) were synthesized via addition of **9** to acrylic acid derivatives and reaction of **9** with corresponding acid chloride furnished urea derivatives and amides (**12a–e**).

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Compound **10e** that has the side chain of tebipenem was prepared from **6** with 3-(4,5-dihydrothiazol-2-yl)azetidine-1-thiol[8] in a similar manner as shown in Scheme 2.

RESULTS AND DISCUSSION

The antibacterial activities of novel lincomycin derivatives against *S. pneumoniae*, *S. pyogenes* and *Haemophilus influenzae* are summarized in Table 1. CLDM showed potent activity against susceptible pathogens, but MICs against the resistant strains were larger than 128 μg ml^{-1}. Compound **8** exhibited comparable antibacterial activities to LCM against susceptible Gram-positive strains. Although the antibacterial activities of **8** against resistant bacteria were not detected, the result prompted us to explore the SAR of (7*S*)-7-sulfur-azetidin-3-yl-LCM derivatives. As for the compounds **10a–d** that have an aryl group directly to azetidine, all the compounds except **10c** showed more potent antibacterial activities against susceptible Gram-positive strains and resistant strains with *mef* gene than CLDM. It is notable that **10b**, **10d** and **10e** slightly exhibit antibacterial activity against resistant strains with *erm* gene. As for *N*-alkyl derivatives (**11a–c**), antibacterial activities were weak and almost the same as those of *N*-unsubstituted compound **8**, and their weak activities were probably due to their high hydrophilicity. On the other hand, amides **12d** and **12e** derived from acid chlorides showed similar antibacterial profile to **10b**, **10d** and **10e**. Furthermore, urea derivatives **12b** and **12c** showed stronger activities against resistant *S. pneumoniae* and *S. pyogenes* with *erm* gene than **10b**, **10d** and **10e**. The antibacterial activities of **12b** against susceptible Gram-positive strains are the strongest in this series, and **12c** showed stronger antibacterial activities against *H. influenzae* than CLDM.

CONCLUSIONS

To generate novel lincomycin derivatives, which are effective against resistant *S. pneumoniae* and *S. pyogenes* with *erm* gene, we synthesized a series of (7*S*)-7-sulfur-azetidin-3-yl-LCM derivatives by utilizing the simple substitution reaction. *N*-Unsubstituted compound **8** showed comparable antibacterial activities to LCM against susceptible Gram-positive strains. *N*-Aryl, amide and urea derivatives showed weak antibacterial activities against *S. pneumoniae* and *S. pyogenes* with *erm* gene. Especially, urea derivatives **12b** and **12c** first exhibited moderate antibacterial activity against those resistant strains as lincomycin derivatives. Thus, we confirmed that the (7*S*)-7-sulfur-azetidin-3-yl-LCM analogue was an attractive framework against the target pathogens.

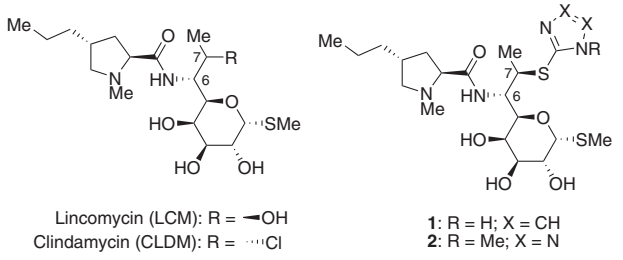


Figure 1 Structures of lincosamides.

EXPERIMENTAL PROCEDURE

General

^{1}H NMR spectra were measured with a JEOL JNM-GSX 400 (Tokyo, Japan, 400 MHz) spectrometer in $CDCl_3$ or CD_3OD with 0.03% tetramethylsilane as an internal standard. Mass spectra were obtained on a JEOL JMS-FABmate spectrometer or JEOL JMS-700 mass spectrometer or Agilent Technologies 6530-Q-TOF LC/MS mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The optical rotations were recorded with Jasco P-2300 digital polarimeter (Jasco, Tokyo, Japan). Column chromatography was performed with silica gel (Kanto Chemical, Tokyo, Japan: 60N; spherical, neutral).

tert-Butyl 3-mercaptoazetidine-1-carboxylate (4)

To a solution of **3** (2.48 g) in tetrahydrofuran (35 ml) were added triphenylphosphine (5.64 g), diethyl azodicarboxylate (3.92 ml) and the mixture was stirred at room temperature for 20 min. To the mixture was added thiobenzoic acid (2.53 ml) and the mixture was stirred at room temperature for 1 h. The mixture was concentrated in vacuo and the residue was purified by silica gel column chromatography (hexane–AcOEt) to give a colorless amorphous (6.0 g). To a stirred solution of the compound obtained above (6.0 g) in MeOH (50 ml) was added NaOMe (772 mg) and the reaction mixture was stirred for 1 h. Saturated aqueous $NaHCO_3$ was added to the mixture and extracted with AcOEt and the organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–AcOEt) to afford **4** (1.81 g, 67%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.32–4.37 (m, 2H), 3.77–3.82 (m, 2H), 3.61–3.71 (m, 1H), 2.00 (d, J=8.5 Hz, 1H), 1.39–1.50 (m, 9H).

7-*O*-Methanesulfonyl-2,3,4-tris-*O*-(trimethylsilyl)lincomycin (6)

To a cold (0 °C) solution of **5** (4.0 g) in $CHCl_3$ (20 ml) were added triethylamine (2.45 ml) and methanesulfonyl chloride (990 μl) and the mixture was stirred at room temperature for 3 h. The mixture was diluted with $CHCl_3$ and washed with 10% aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–AcOEt) to afford **6** (4.2 g, 93%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J=11.0 Hz, 1H), 5.16 (d, J=5.6 Hz, 1H), 5.10–5.15 (m, 1H), 4.75 (dt, J=3.3, 10.3 Hz, 1H), 4.15 (dd, J=5.6, 9.5 Hz, 1H), 3.90 (d, J=9.7 Hz, 1H), 3.76 (d, J=2.3 Hz, 1H), 3.52 (dd, J=2.3, 9.4 Hz, 1H), 3.15–3.20 (m, 1H), 3.09 (s, 3H), 2.99 (dd, J=3.7, 10.2 Hz, 1H), 2.40 (s, 3H), 2.11 (s, 3H), 1.92–2.09 (m, 3H), 1.79–1.89 (m, 1H), 1.40 (d, J=6.8 Hz, 3H), 1.23–1.35 (m, 4H), 0.89 (t, J=6.7 Hz, 3H), 0.17 (s, 9H), 0.14 (s, 9H), 0.13 (s, 9H); MS (ESI) *m/z* 701 (M+H)$^+$.

7(*S*)-7-Deoxy-7-[1-(tert-butoxycarbonyl)azetidin-3-ylthio]-2,3,4-tris-O-(trimethylsilyl)lincomycin (7)

To a solution of **6** (10.5 g) and K_2CO_3 (4.15 g) in *N*,*N*-dimethylformamide (50 ml) was added **4** (1.9 g) and the mixture was stirred at 80 °C for 15 h. After cooled to room temperature, the mixture was diluted with AcOEt and washed with brine. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography

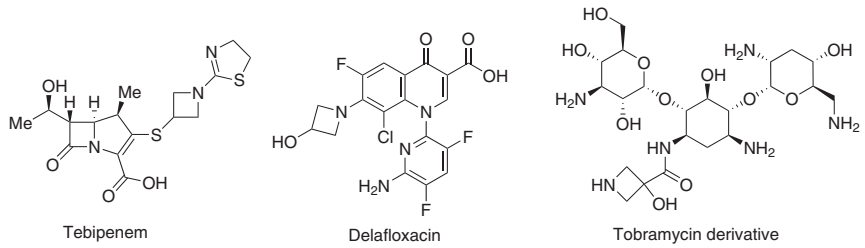
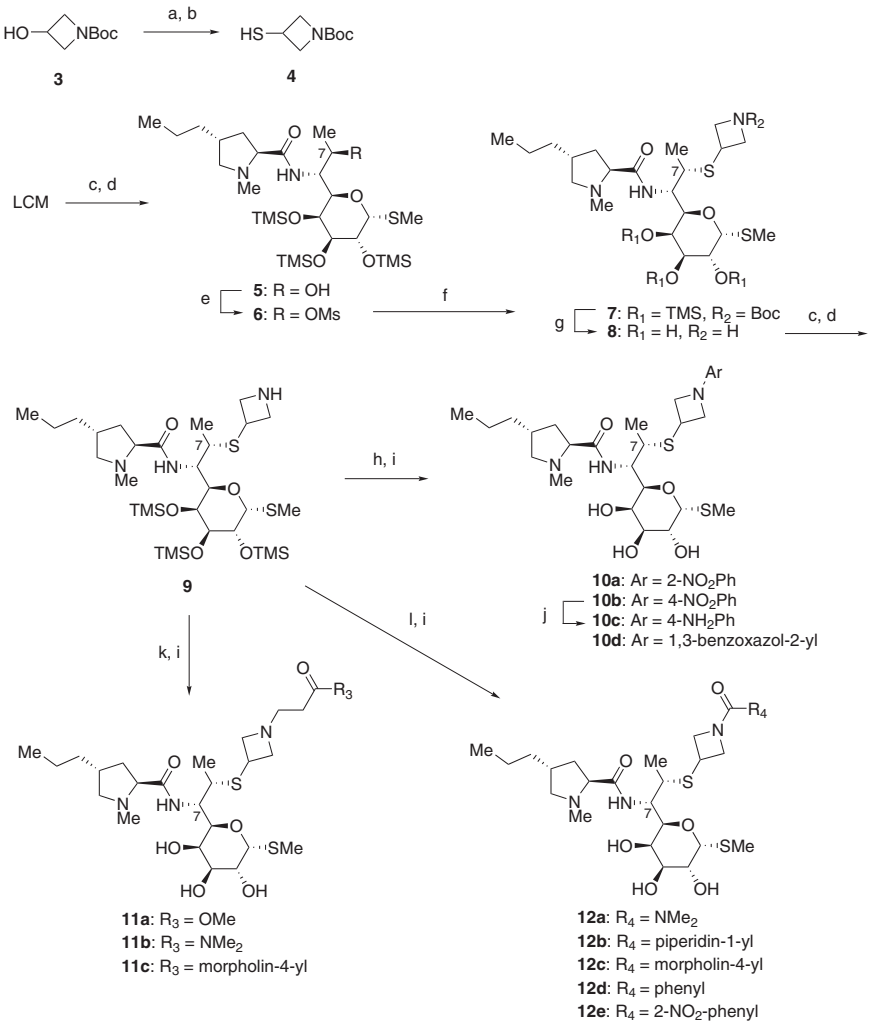
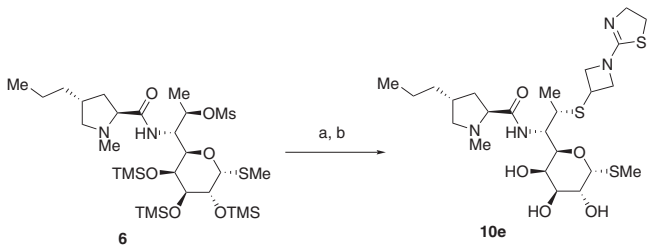


Figure 2 Structures of antibiotics that have an azetidine ring.



Scheme 1 Synthesis of 7(*S*)-sulfur-substituted lincomycin derivatives. Reagents: (a) thiobenzoic acid, DEAD, PPh_3, THF; (b) NaOMe MeOH; (c) TMSCl, HMDS, pyridine; (d) 6 N AcOH, MeOH; (e) MsCl, TEA, $CHCl_3$; (f) 4, K_2CO_3, DMF; (g) 1N HCl, MeOH then trifluoroacetic acid; (h) XAr, TEA, DMF; (i) 1 N HCl, MeOH; (j) H_2, Pd/C, MeOH; (k) CH_2CHCOR_3, EtOH; (l) R_4COCl, DMAP, TEA, $CHCl_3$.



Scheme 2 Synthesis of **10e**. Reagents: (a) 3-(4,5-dihydrothiazol-2-yl) azetidine-1-thiol hydrochloride, K_2CO_3, DMF; (b) 1 N HCl/AcOEt, MeOH.

(hexane–AcOEt) to afford **7** (5.7 g, 48%) as a colorless solid. $[\alpha]_D^{30}$ +76° (*c* 0.46, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J*=10.7 Hz, 1H), 5.21 (d, *J*=5.6 Hz, 1H), 4.50 (dt, *J*=1.7, 10.3 Hz, 1H), 4.29–4.16 (m, 2H), 4.15 (dd, *J*=5.6, 9.5 Hz, 1H), 3.98 (d, *J*=9.9 Hz, 1H), 3.75–3.80 (m, 2H), 3.64–3.71 (m, 2H), 3.59 (dd, *J*=2.4, 9.5 Hz, 1H), 3.35 (dq, *J*=1.7, 7.1 Hz, 1H), 3.14–3.19 (m, 1H), 2.97 (dd, *J*=3.7, 10.7 Hz, 1H), 2.40 (s, 3H), 2.17 (s, 3H), 1.89–2.05 (m, 2H), 1.77–1.87 (m, 1H), 1.41 (s, 9H), 1.24–1.30 (m, 4H), 1.22 (d, *J*=7.1 Hz, 3H), 0.84–0.89 (m, 3H), 0.16 (m, 9H), 0.12 (m, 9H), 0.11 (m, 9H); MS (ESI) *m/z* 794 $(M+H)^+$.

Synthesis of 7(*S*)-7-(azetidin-3-ylthio)-7-deoxylincomycin (8)

To a solution of **7** (280 mg) in MeOH (2 ml) was added 1 N hydrochloric acid (1 ml) and the mixture was stirred at room temperature for 20 min. The mixture was diluted with AcOEt and washed with 10% aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo to give white solid (205 mg). Trifluoroacetic acid (1 ml) was added to the solid and stirred at room temperature for 20 min and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (AcOEt–MeOH) to afford **8** (99 mg, 73%) as a colorless solid. $[\alpha]_D^{29}$ +118° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.91 (s, 1H), 5.26 (d, *J*=5.6 Hz, 1H), 4.36–4.50 (m, 3H), 4.21 (d, *J*=9.7 Hz, 1H), 4.12–4.17 (m, 1H), 4.09 (dd, *J*=5.6, 10.3 Hz, 1H), 3.98 (ddd, *J*=3.5, 7.4, 10.8 Hz, 2H), 3.78 (d, *J*=3.4 Hz, 1H), 3.59–3.66 (m, 1H), 3.56 (dd, *J*=3.4, 10.4 Hz, 1H), 3.36–3.51 (m, 3H), 2.61 (s, 3H), 2.34–2.45 (m, 1H), 2.34–2.44 (m, 1H), 2.19–2.30 (m, 1H) 2.18 (s, 3H), 1.96–2.14 (m, 2H), 1.33–1.44 (m, 4H), 1.31 (d, *J*=6.8 Hz, 3H), 0.93 (t, *J*=7.1 Hz, 3H); MS (FAB (fast atom bombardment)) *m/z* 477 $(M+H)^+$; high resolution mass spectrometry (HRMS) (FAB) *m/z* calcd for $C_{21}H_{40}N_3O_5S_2$ 478.2409, found 478.2408 $(M+H)^+$.

7(*S*)-7-(Azetidin-3-ylthio)-7-deoxy-2,3,4-tris-*O*-(trimethylsilyl) lincomycin (9)

To a cold (0 °C) solution of **8** (3.48 g) in pyridine were added trimethylsilyl chloride (4.59 ml) and hexamethyldisilazane (7.5 ml) and the mixture was

Table 1 Antibacterial activities of novel lincomycin derivatives against *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Haemophilus influenzae* (MIC (μg ml^{-1}))[a]

No.		*Characteristics*	*LCM*	*CLDM*	***8***	***10a***	***10b***	***10c***	***10d***	***10e***	***11a***	***11b***	***11c***	***12a***	***12b***	***12c***	***12d***	***12e***
1	*S. pneumoniae* DP1 type I	Susceptible	1	0.13	1	0.03	0.03	0.13	0.03	0.03	0.5	0.5	0.25	0.13	0.015	0.13	0.06	0.06
2	*S. pneumoniae* #2	Susceptible	1	0.13	1	0.06	0.03	0.13	0.06	0.06	1	1	0.5	0.25	0.03	0.13	0.13	0.06
3	*S. pneumoniae* #3	Susceptible	1	0.13	0.5	0.03	0.015	0.13	0.03	0.06	0.5	0.5	0.25	0.25	0.03	0.13	0.06	0.06
4	*S. pneumoniae* #4	*ermB methylase* (c)	> 128	> 128	> 128	128	128	> 128	64	64	> 128	> 128	> 128	128	32	16	32	128
5	*S. pneumoniae* #5	*ermB methylase* (c)	> 128	> 128	> 128	128	64	128	32	32	> 128	128	> 128	128	8	8	16	64
6	*S. pneumoniae* #6	*ermB methylase* (c)	> 128	> 128	> 128	> 128	128	> 128	64	128	> 128	> 128	> 128	> 128	32	16	128	> 128
7	*S. pneumoniae* #7	*ermB methylase* (i)	> 128	> 128	128	128	32	16	16	32	> 128	64	128	64	4	8	8	32
8	*S. pneumoniae* #8	*ermB methylase* (i)	> 128	> 128	> 128	64	32	16	16	64	> 128	> 128	128	64	8	ND	32	32
9	*S. pneumoniae* #9	*mefE efflux*	1	0.13	0.5	0.03	0.03	0.13	0.06	0.03	0.5	0.5	0.5	0.25	0.03	0.13	0.06	0.06
10	*S. pneumoniae* #10	*mefE efflux*	1	0.13	0.5	0.03	0.03	0.13	0.06	0.06	ND	ND	0.5	0.25	0.03	0.13	ND	0.13
11	*S. pyogenes* Cook	Susceptible	0.13	0.13	0.5	⩽0.008	0.015	0.13	0.03	0.06	0.5	0.25	0.25	0.25	0.015	0.13	0.06	0.06
12	*S. pyogenes* #2	*ermB methylase* (c)	> 128	> 128	> 128	32	16	64	16	32	> 128	64	128	32	8	4	16	64
13	*S. pyogenes* #3	*mefE efflux*	0.25	0.13	0.5	0.03	0.015	0.13	0.03	0.13	0.5	0.5	0.25	0.25	0.03	0.13	0.13	0.13
14	*H. influenzae* #1	Susceptible	8	8	> 128	64	64	64	32	16	64	16	32	32	8	4	32	64
15	*H. influenzae* #2	Susceptible	16	8	128	32	16	16	16	8	32	64	64	32	8	8	16	32
16	*H. influenzae* #3	Susceptible	16	32	> 128	128	64	64	64	16	128	128	128	64	32	16	64	128

Abbreviations: c, constitutive; i, inducible; CLDM, clindamycin; LCM, lincomycin; ND, not determined.
[a]All antibacterial evaluations were performed as hydrochloride. Grey shading strains are target strains.

stirred at room temperature for 1 h. The reaction mixture was poured into 10% aqueous $NaHCO_3$ and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The resulting residue was dissolved in MeOH (30 ml) and 6 N AcOH (3.59 ml) was added to the solution. After the mixture was stirred at room temperature for 3.5 h, the mixture was poured into 10% aqueous $NaHCO_3$ and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The resulting residue was purified by NH silica gel column chromatography (hexane–AcOEt) to afford **9** (3.0 g, 60%) as a colorless solid. $[\alpha]_D^{28}$ +78° (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J=11.0 Hz, 1H), 5.23 (d, J=5.6 Hz, 1H), 4.47 (dt, J=1.8, 10.5 Hz, 1H), 4.17 (dd, J=5.6, 9.5 Hz, 1H), 4.00 (d, J=10.2 Hz, 1H), 3.83–3.90 (m, 2H), 3.73–3.81 (m, 1H), 3.70 (d, J=2.2 Hz, 1H), 3.57–3.65 (m, 3H), 3.36 (dq, J=1.8, 7.1 Hz, 1H), 3.14–3.20 (m, 1H), 2.98 (dd, J=3.7, 11 Hz, 1H), 2.43 (s, 3H), 2.41 (d, J=6.1 Hz, 1H), 2.18 (s, 3H), 1.92–2.09 (m, 3H), 1.73–1.90 (m, 1H), 1.68 (br s, 2H), 1.25–1.32 (m, 4H), 1.23 (d, J=7.1 Hz, 3H), 0.89 (t, J=6.9 Hz, 3H), 0.17 (s, 9H), 0.14 (s, 9H), 0.12 (s, 9H); MS (ESI) *m/z* 694 $(M+H)^+$.

7(*S*)-7-[1-(Benzo[*d*]oxazol-2-yl)azetidin-3-ylthio]-7-deoxylincomycin (10d)

To a solution of **9** (60 mg) in *N,N*-dimethylformamide (500 μl) were added 2-chlorobenzoxazole (9.9 μl), triethylamine (12.1 μl) and the mixture was stirred at room temperature for 3 h The mixture was diluted with AcOEt and washed with 10% aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. To the resulting residue were added MeOH (1 ml) and 1 M hydrochloric acid (1 ml) and the reaction mixture was stirred at room temperature for 10 min. The mixture was diluted with AcOEt and washed with 10% aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (AcOEt–MeOH) to afford **10d** (51 mg, 99%) as a colorless solid. $[\alpha]_D^{28}$ +103° (*c* 0.99, $CHCl_3$); H NMR (400 MHz, CD_3OD) δ 7.29–7.35 (m, 2H), 7.19 (dt, J=1.1, 7.5 Hz, 1H), 7.09 (dt, J=1.1, 7.5 Hz, 1H), 5.27 (d, J=5.6 Hz, 1H), 4.62–4.72 (m, 2H), 4.33 (dd, J=2.9, 9.7 Hz, 1H), 4.07–4.21 (m, 5H), 3.71–3.75 (m, 1H), 3.57 (dd, J=3.4, 10.2 Hz, 1H), 3.52 (dq, J=2.9, 6.8 Hz, 1H), 3.23 (dd, J=5.6, 8.0 Hz, 1H), 2.99 (dd, J=4.4, 10.5 Hz, 1H), 2.43 (s, 3H), 2.17 (s, 3H), 1.95–2.15 (m, 3H), 1.80–1.90 (m, 1H), 1.27–1.38 (m, 7H), 0.88–0.94 (m, 3H); MS (FAB) *m/z* 595 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{28}H_{43}N_4O_6S_2$ 595.2624, found 595.2633 $(M+H)^+$.

7(*S*)-7-Deoxy-7-[1-(2-nitrophenyl)azetidin-3-ylthio]lincomycin (10a)

Reaction of **9** with 2-fluoronitrobenzene gave **10a** as a yellow solid in 57% yield by a similar procedure to **10d**. $[\alpha]_D^{29}$ +53° (*c* 0.75, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.78 (dd, J=1.6, 8.3 Hz, 1H), 7.45 (ddd, J=1.6, 7.1, 8.5 Hz, 1H), 6.80 (ddd, J=1.2, 7.1, 8.3 Hz, 1H), 6.72 (dd, J=1.2, 8.5 Hz, 1H), 5.26 (d, J=5.6 Hz, 1H), 4.39 (t, J=8.2 Hz, 1H), 4.27–4.36 (m, 2H), 4.16 (d, J=9.7 Hz, 1H), 4.09 (dd, J=5.6, 10.2 Hz, 1H), 3.91–4.01 (m, 1H), 3.72–3.78 (m, 2H), 3.70–3.72 (m, 1H), 3.56 (dd, J=3.2, 10.2 Hz, 1H), 3.51 (dq, J=2.7, 7.0 Hz, 1H), 3.23 (dd, J=5.6, 8.0 Hz, 1H), 2.99 (dd, J=4.6, 10.7 Hz, 1H), 2.41 (s, 3H), 2.15 (s, 3H), 2.03–2.14 (m, 2H), 1.93–2.03 (m, 1H), 1.84 (td, J=10.2, 12.8 Hz, 1H), 1.29–1.35 (m, 7H), 0.87–0.94 (m, 3H); MS (FAB) *m/z* 599 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{27}H_{43}N_4O_7S_2$ 599.2573, found 599.2572 $(M+H)^+$.

7(*S*)-7-Deoxy-7-[1-(4-nitrophenyl)azetidin-3-ylthio]lincomycin (10b)

Reaction of **9** with 4-fluoronitrobenzene gave **10b** as a yellow solid in 56% yield by a similar procedure to **10d**. $[\alpha]_D^{30}$ +94° (*c* 0.87, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 8.06–8.12 (m, 2H), 6.40–6.46 (m, 2H), 5.27 (d, J=5.4 Hz, 1H), 4.50 (t, J=8.0 Hz, 1H), 4.45 (t, J=8.0 Hz, 1H), 4.33 (dd, J=2.7, 9.7 Hz, 1H), 4.19 (d, J=9.7 Hz, 1H), 4.05–4.13 (m, 2H), 3.84–3.91 (m, 2H), 3.71–3.74 (m, 1H), 3.57 (dd, J=3.2, 10.2 Hz, 1H), 3.52 (dq, J=2.7, 7.3 Hz, 1H), 3.23 (dd, J=5.5, 7.9 Hz, 1H), 3.00 (dd, J=4.5, 10.6 Hz, 1H), 2.41 (s, 3H), 2.16 (s, 3H), 2.04–2.14 (m, 2H), 1.94–2.04 (m, 1H), 1.85 (td, J=10.2, 13.0 Hz, 1H), 1.27–1.37 (m, 7H), 0.87–0.94 (m, 3H); MS (FAB) *m/z* 599 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{27}H_{43}N_4O_7S_2$ 599.2573, found 599.2567 $(M+H)^+$.

7(*S*)-7-[1-(4-Aminophenyl)azetidin-3-ylthio]-7-deoxylincomycin (10c)
To a solution of **10b** (127 mg) in MeOH (4 ml) was added PtO_2 (77.2 mg) and the mixture was stirred in hydrogen atmosphere at room temperature for 1 h. The mixture was filtered through Celite and the filtrate was concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (AcOEt–MeOH) to afford a colorless solid (77 mg, 64%). $[\alpha]_D^{30}$ +104° (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 6.64–6.75 (m, 2H), 6.33–6.44 (m, 2H), 5.27 (d, *J*=5.6 Hz, 1H), 4.28 (dd, *J*=2.4, 9.7 Hz, 1H), 4.18–4.25 (m, 1H), 4.16 (d, *J*=9.7 Hz, 1H), 4.09 (dd, *J*=5.6, 10.2 Hz, 2H), 3.89–3.98 (m, 1H), 3.70 (d, *J*=3.2 Hz, 1H), 3.56 (dd, *J*=3.2, 10.2 Hz, 2H), 3.51–3.54 (m, 1H), 3.49 (dq, *J*=2.4, 7.1 Hz, 1H), 3.23 (dd, *J*=5.5, 7.7 Hz, 1H), 3.00 (dd, *J*=4.5, 10.5 Hz, 1H), 2.42 (s, 3H), 2.17 (s, 3H), 2.03–2.14 (m, 2H), 1.94–2.03 (m, 1H), 1.85 (td, *J*=10.2, 12.8 Hz, 1H), 1.29–1.36 (m, 7H), 0.87–0.95 (m, 3H); MS (FAB) *m/z* 569 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{27}H_{45}N_4O_5S_2$ 569.2831, found 569.2838 $(M+H)^+$.

7(*S*)-7-Deoxy-7-[1-(4,5-dihydrothiazol-2-yl)azetidin-3-ylthio] lincomycin (10e)
To a solution of **6** (150 mg) and K_2CO_3 (88.7 mg) in *N*,*N*-dimethylformamide (1.5 ml) was added 3-(4,5-dihydrothiazol-2-yl)azetidine-1-thiol hydrochloride (90.2 mg) and the mixture was stirred at 80 °C for 5 h. After cooled to room temperature, the mixture was diluted with AcOEt and washed with brine. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–AcOEt) to give a colorless solid (95 mg). To a solution of the compound obtained above (50 mg) in MeOH was added 1 M hydrochloric acid (1 ml) and the reaction mixture was stirred at room temperature for 5 min. The mixture was diluted with AcOEt and extracted with H_2O. The aqueous phase was neutralized with 10% aqueous $NaHCO_3$ and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo to afford **10e** (30 mg, 47%) as a colorless solid. $[\alpha]_D^{28}$ +103° (*c* 0.99, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.26 (d, *J*=5.6 Hz, 1H), 4.40 (t, *J*=8.0 Hz, 1H), 4.35 (t, *J*=8.2 Hz, 1H), 4.30 (dd, *J*=2.6, 9.6 Hz, 1H), 4.15 (d, *J*=9.6 Hz, 1H), 4.09 (dd, *J*=5.6, 10.2 Hz, 1H), 3.98 (tt, *J*=5.5, 7.8 Hz, 1H), 3.94 (t, *J*=7.5 Hz, 2H), 3.79–3.85 (m, 2H), 3.69–3.72 (m, 1H), 3.56 (dd, *J*=3.2, 10.2 Hz, 1H), 3.47 (dq, *J*=2.6, 7.1 Hz, 1H), 3.38 (t, *J*=7.5 Hz, 2H), 3.24 (dd, *J*=5.5, 7.9 Hz, 1H), 2.99 (dd, *J*=4.6, 10.7 Hz, 1H), 2.42 (s, 3H), 2.19 (s, 3H), 2.05–2.18 (m, 2H), 1.95–2.03 (m, 1H), 1.85 (td, *J*=10.2, 12.8 Hz, 1H), 1.31–1.37 (m, 4H), 1.29 (d, *J*=7.1 Hz, 3H), 0.88–0.95 (m, 3H); MS (FAB) *m/z* 563 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{24}H_{43}N_4O_5S_2$ 563.2396, found 563.2388 $(M+H)^+$.

7(*S*)-7-Deoxy-7-{1-[2-(dimethylaminocarbonyl)ethyl]azetidin-3-ylthio}lincomycin (11b)
To a solution of **9** (60 mg) in EtOH (1 ml) was added *N*,*N*-dimethylacrylamide (8.9 µl) and the mixture was stirred at room temperature for 15 min and at 50 °C for 3 h. The mixture was concentrated in vacuo and the residue was dissolved in MeOH (1 ml) and 1 M hydrochloric acid (1 ml) was added to the solution. The mixture was stirred at room temperature for 10 min and diluted with AcOEt and extracted with H_2O. The aqueous phase was neutralized with 10% aqueous $NaHCO_3$ and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo to afford **11b** (37 mg, 74%) as a colorless solid. $[\alpha]_D^{29}$ +90° (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.26 (d, *J*=5.6 Hz, 1H), 4.23 (dd, *J*=2.4, 9.7 Hz, 1H), 4.14 (d, *J*=9.7 Hz, H), 4.09 (dd, *J*=5.6, 10.2 Hz, 1H), 3.62–3.82 (m, 5H), 3.55 (dd, *J*=3.2, 10.2 Hz, 1H), 3.42 (dq, *J*=2.4, 6.9 Hz, 1H), 3.19–3.26 (m, 1H), 3.02–3.09 (m, 4H), 2.93–3.02 (m, 1H), 2.91 (s, 3H), 2.74 (t, *J*=7.3 Hz, 2H), 2.42 (s, 3H), 2.39 (t, *J*=7.3 Hz, 2H), 2.18 (s, 3H), 2.03–2.16 (m, 2H), 1.94–2.02 (m, 1H), 1.84 (td, *J*=10.0, 12.9 Hz, 1H), 1.30–1.37 (m, 4H), 1.28 (d, *J*=6.9 Hz, 3H), 0.87–0.95 (m, 3H); MS (FAB) *m/z* 577 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{26}H_{49}N_4O_6S_2$ 577.3094, found 577.3089 $(M+H)^+$.

7(*S*)-7-Deoxy-7-{1-[2-(methoxycarbonyl)ethyl]azetidin-3-ylthio} lincomycin (11a)
Reaction of **9** with methyl 3-butenoate gave **11a** as a colorless solid in 74% yield by a similar procedure to **11b**. $[\alpha]_D^{30}$ +86° (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.25 (d, *J*=5.6 Hz, 1H), 4.24 (dd, *J*=2.5, 9.7 Hz, 1H), 4.14 (d, *J*=9.7 Hz, 1H), 4.08 (dd, *J*=5.6, 10.4 Hz, 1H), 3.61–3.82 (m, 7H), 3.55 (dd, *J*=3.2, 10.4 Hz, 1H), 3.41 (dq, *J*=2.5, 7.0 Hz, 1H), 3.24 (dd, *J*=5.2, 8.0 Hz, 1H), 2.96–3.05 (m, 3H), 2.75 (t, *J*=7.2 Hz, 2H), 2.42 (s, 3H), 2.36 (t, *J*=7.2 Hz, 2H), 2.17 (s, 3H), 2.04–2.16 (m, 2H), 1.93–2.03 (m, 1H), 1.80–1.90 (m, 1H), 1.30–1.37 (m, 4H), 1.28 (d, *J*=7.0 Hz, 3H), 0.87–0.95 (m, 3H); MS (FAB) *m/z* 564 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{25}H_{46}N_3O_7S_2$ 564.2777, found 564.2770 $(M+H)^+$.

7(*S*)-7-Deoxy-7-{1-[2-(morpholin-4-yl)ethyl]azetidin-3-ylthio} lincomycin (11c)
Reaction of **9** with 4-acryloylmorpholine gave **11c** as a colorless solid in 70% yield by a similar procedure to **11b**. $[\alpha]_D^{29}$ +91° (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.26 (d, *J*=5.6 Hz, 1H), 4.24 (dd, *J*=2.4, 10.1 Hz, 1H), 4.14 (d, *J*=10.1 Hz, 1H), 4.08 (dd, *J*=5.6, 10.7 Hz, 1H), 3.71–3.81 (m, 2H), 3.61–3.71 (m, 6H), 3.49–3.58 (m, 5H), 3.42 (dq, *J*=2.4, 6.9 Hz, 1H), 3.20–3.26 (m, 1H), 3.01–3.09 (m, 2H), 2.99 (dd, *J*=4.6, 10.5 Hz, 1H), 2.75 (t, *J*=7.6 Hz, 2H), 2.42 (s, 3H), 2.41 (t, *J*=7.6 Hz, 2H), 2.18 (s, 3H), 2.05–2.16 (m, 2H), 1.94–1.99 (m, 1H), 1.80–1.89 (m, 1H), 1.30–1.37 (m, 4H), 1.28 (d, *J*=6.9 Hz, 3H), 0.88–0.95 (m, 3H); MS (FAB) *m/z* 619 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{28}H_{51}N_4O_7S_2$ 619.3199, found 619.3192 $(M+H)^+$.

7(*S*)-7-Deoxy-7-[1-(dimethylaminocarbonyl)azetidin-3-ylthio] lincomycin (12a)
To a solution of **9** (100 mg) in $CHCl_3$ (700 µl) were added triethylamine (20.2 µl), dimethylcarbamoyl chloride (13.2 µl) and *N*,*N*-dimethylaminopyridine (17.6 mg) and the mixture was stirred at room temperature for 3.5 h. The mixture was diluted with AcOEt and washed with 10% aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–AcOEt) to give a colorless solid (95 mg). To a stirred solution of the compound obtained above (95 mg) in MeOH (1 ml) was added 1 M hydrochloric acid (1 ml) and the reaction mixture was stirred at room temperature for 10 min. The mixture was diluted with AcOEt and extracted with H_2O. The aqueous phase was neutralized with 10% aqueous $NaHCO_3$ and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo to afford **12a** (55 mg, 70%) as a colorless solid. $[\alpha]_D^{29}$ +66° (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.26 (d, *J*=5.6 Hz, 1H), 4.32–4.42 (m, 2H), 4.29 (dd, *J*=2.6, 9.7 Hz, 1H), 4.15 (dd, *J*=0.73, 9.7 Hz, 1H), 4.09 (dd, *J*=5.6, 10.4 Hz, 1H), 3.80–3.88 (m, 3H), 3.69–3.71 (m, 1H), 3.56 (dd, *J*=3.2, 10.4 Hz, 1H), 3.46 (dq, *J*=2.6, 7.1 Hz, 1H), 3.24 (dd, *J*=5.5, 7.9 Hz, 1H), 2.99 (dd, *J*=4.6, 10.5 Hz, 1H), 2.84 (s, 6H), 2.42 (s, 3H), 2.19 (s, 3H), 2.04–2.16 (m, 2H), 1.94–2.02 (m, 1H), 1.84 (td, *J*=10.1, 12.9 Hz, 1H), 1.31–1.37 (m, 4H), 1.29 (d, *J*=7.1 Hz, 3H), 0.89–0.94 (m, 3H); MS (FAB) *m/z* 549 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{26}H_{44}N_5O_6S_3$ 549.2781, found 549.2782 $(M+H)^+$.

7(*S*)-7-Deoxy-7-{1-[(morpholin-4-yl)carbonyl]azetidin-3-ylthio} lincomycin (12c)
Reaction of **9** with 4-morpholinylcarbonyl chloride gave **12c** as a colorless solid in 70% yield by a similar procedure to **12a**. $[\alpha]_D^{29}$ +69° (*c* 0.86, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.26 (d, *J*=5.6 Hz, 1H), 4.33–4.45 (m, 2H), 4.29 (dd, *J*=2.7, 9.5 Hz, 1H), 4.15 (d, *J*=9.5 Hz, 1H), 4.09 (dd, *J*=5.6, 10.4 Hz, 1H), 3.83–3.91 (m, 3H), 3.70 (d, *J*=3.3 Hz, 1H), 3.59–3.64 (m, 4H), 3.55 (dd, *J*=3.3, 10.4 Hz, 1H), 3.46 (dq, *J*=2.7, 7.0 Hz, 1H), 3.27–3.33 (m, 4H), 3.23 (dd, *J*=5.2, 7.9 Hz, 1H), 2.99 (dd, *J*=4.6, 10.7 Hz, 1H), 2.42 (s, 3H), 2.18 (s, 3H), 2.04–2.17 (m, 2H), 1.94–2.02 (m, 1H), 1.79–1.89 (m, 1H), 1.31–1.37 (m, 4H), 1.29 (d, *J*=7.0 Hz, 3H), 0.88–0.95 (m, 3H); MS (FAB) *m/z* 591 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{26}H_{47}N_4O_7S_2$ 591.2886, found 591.2891 $(M+H)^+$.

7(*S*)-7-Deoxy-7-[1-(phenylcarbonyl)azetidin-3-ylthio]lincomycin (12d)
Reaction of **9** with benzoyl chloride gave **12d** as a colorless solid in 67% yield by a similar procedure to **12a**. $[\alpha]_D^{30}$ +90° (*c* 0.74, $CHCl_3$); ^{1}H NMR

(400 MHz, CD_3OD) δ 7.84–7.90 (m, 2H), 7.72–7.79 (m, 1H), 7.65–7.72 (m, 2H), 5.21 (d, $J=5.6$ Hz, 1H), 4.22 (dd, $J=2.7$, 9.3 Hz, 1H), 4.19–4.11 (m, 2H), 4.06 (dd, $J=5.6$, 10.3 Hz, 1H), 4.05–4.02 (m, 1H), 3.68–3.77 (m, 1H), 3.66 (d, $J=3.2$ Hz, 1H), 3.54–3.58 (m, 2H), 3.52 (dd, $J=3.2$, 10.3 Hz, 1H), 3.33 (dq, $J=2.7$, 6.8 Hz, 1H), 3.19 (dd, $J=5.2$, 7.9 Hz, 1H), 2.94 (dd, $J=4.6$, 10.7 Hz, 1H), 2.34 (s, 3H), 2.10–2.16 (m, 1H), 2.08 (s, 3H), 2.02–2.07 (m, 1H), 1.89–2.00 (m, 1H), 1.76–1.87 (m, 1H), 1.26–1.35 (m, 4H), 1.16–1.21 (m, 3H), 0.87–0.94 (m, 3H); MS (FAB) *m/z* 618 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{27}H_{44}N_3O_7S_3$ 618.2341, found 618.2340 $(M+H)^+$.

7(*S*)-7-Deoxy-7-[1-(2-nitrophenylcarbonyl)azetidin-3-ylthio] lincomycin (12e)

Reaction of **9** with 2-nitrobenzoyl chloride gave **12e** as a yellow solid in 72% yield by a similar procedure to **12a**. $[\alpha]_D^{30}$ +88° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD) δ 8.17–8.23 (m, 1H), 7.79–7.85 (m, 1H), 7.69–7.74 (m, 1H), 7.52–7.57 (m, 1H), 5.19–5.28 (m, 1H), 4.55–4.66 (m, 1H), 4.25–4.41 (m, 2H), 3.98–4.20 (m, 3H), 3.78–3.86 (m, 1H), 3.68–3.73 (m, 1H), 3.41–3.63 (m, 2H), 3.17–3.27 (m, 1H), 3.17–3.27 (m, 1H), 2.93–3.02 (m, 1H), 2.36–2.44 (m, 3H), 2.03–2.24 (m, 5H), 1.92–2.03 (m, 1H), 1.78–1.90 (m, 1H), 1.22–1.37 (m, 7H), 0.87–0.95 (m, 3H); MS (FAB) *m/z* 627 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{28}H_{43}N_4O_8S_2$ 627.2522, found 627.2516 $(M+H)^+$.

7(*S*)-7-Deoxy-7-[1-(piperidin-1-yl)azetidin-3-ylthio]lincomycin (12b)

To a cold (0 °C) solution of **9** (80 mg) in CH_2Cl_2 (0.5 ml) were added piperidine (12.5 µl), pyridine (20.5 µl) and triphosgene (12.6 mg), the mixture was stirred at room temperature for 1 h. The mixture was diluted with AcOEt and washed with 10% aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–AcOEt) to give a colorless amorphous (22 mg). To a stirred solution of the compound obtained above (22 mg) in MeOH (1 ml) was added 1 M hydrochloric acid (1 ml) and the reaction mixture was stirred at room temperature for 10 min. The mixture was diluted with AcOEt and washed with 10% aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo to afford **12b** (16 mg, 23%) as a colorless solid. $[\alpha]_D^{29}$ +100° (*c* 0.72, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD) δ 5.26 (d, $J=5.6$ Hz, 1H), 4.39 (t, $J=7.8$ Hz, 1H), 4.34 (t, $J=7.8$ Hz, 1H), 4.29 (dd, $J=2.6$, 9.6 Hz, 1H), 4.16 (d, $J=9.6$ Hz, 1H), 4.09 (dd, $J=5.6$, 10.4 Hz, 1H), 3.80–3.90 (m, 3H), 3.70 (d, $J=3.2$ Hz, 1H), 3.56 (dd, $J=3.2$, 10.4 Hz, 1H), 3.46 (dq, $J=2.6$, 7.1 Hz, 1H), 3.22–3.30 (m, 5H), 2.99 (dd, $J=4.6$, 10.7 Hz, 1H), 2.42 (s, 3H), 2.19 (s, 3H), 2.05–2.17 (m, 2H), 1.95–2.03 (m, 1H), 1.85 (td, $J=10.2$, 12.8 Hz, 1H), 1.59–1.66 (m, 2H), 1.49–1.57 (m, 4H), 1.31–1.38 (m, 4H), 1.29 (d, $J=7.1$ Hz, 3H), 0.90–0.94 (m, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 178.0, 164.0, 90.5, 72.0, 71.3, 70.4, 69.9, 69.5, 63.9, 61.3, 60.1, 54.3, 46.9, 42.9, 42.3, 39.2, 38.7, 37.0, 33.2, 27.0, 25.5, 22.6, 21.0, 14.6, 14.5; MS (EI) *m/z* 588 M^+; HRMS (FAB) *m/z* calcd for $C_{27}H_{49}N_4O_6S_2$ 589.3094, found 589.3096 $(M+H)^+$.

In vitro antibacterial activity

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

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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