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Novel azalides derived from 16-membered macrolides. III. Azalides modified at the C-15 and 4″ positions: Improved antibacterial activities

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ABSTRACT

The design and synthesis of 16-membered azalides modified at the C-15 and 4″ positions are described. The compounds we report here are characterized by an arylpropenyl group attached to the C-15 position of macrolactone and a carbamoyl group at the C-4″ position in a neutral sugar. Introduction of alkylcarbamoyl groups to the C-4″ position was regioselectively achieved by unique and convenient methods via acyl migration. As a result of optimization at the C-3 and 15 positions, several compounds were found to have potent activity against *mef*- and *erm*-resistant bacterial strains. These results suggest that 16-membered azalides could be promising compounds as clinical candidates.

1. Introduction

Macrolide antibiotics have played an important role in treatment of respiratory tract infections. However, an emergence of resistance against macrolides in Gram-positive bacteria, especially *Streptococcus pneumoniae*, is a serious problem at clinical sites. Clarithromycin[1] (CAM) and azithromycin[2] (AZM) (Fig. 1), which are representative macrolides derived from 14-membered erythromycin, are inactive against resistant strains of *S. pneumoniae* which have a mechanism of ribosome methylation by an *erm* gene, and show low susceptibility against efflux-resistant *S. pneumoniae* with a *mef* gene. Although telithromycin[3] (TEL) (Fig. 1) synthesized from 14-membered erythromycin exhibits improved antibacterial activities against *erm*- and *mef*-resistant *S. pneumoniae*, it can be still a substrate of efflux pumps in *mef*-resistant *S. pneumoniae*. On the other hand, miokamycin[4] (MOM) and rokitamycin[5] (RKM) (Fig. 1) derived from 16-membered macrolides are not influenced by efflux pumps. There is a possibility that medicinal chemistry leads to a discovery of novel 16-membered macrolides via maximum utilization of this feature, overcoming *erm*-resistant *S. pneumoniae*.

We recently reported that novel azalides derived from 16-membered macrolides exhibited antibacterial activities against *erm*-resistant *S. pneumoniae*.[6] 16-membered azalides having an arylalkyl group at the C-15 position were attractive from the viewpoint of antibacterial activities. That is, some compounds exhibited activities against constitutive-resistant *S. pneumoniae*, even though they did not possess a substituent at the 3″-hydroxyl group in a neutral sugar (e.g., **1a** and **1b**, Fig. 2).[6b] These results encouraged us to explore further chemical modifications of C-15 substituted 16-membered azalides.

By the way, 16-membered macrolides such as MOM and RKM do not always exhibit satisfactory pharmacokinetics.[7] One explanation for such poor pharmacokinetics involves a sequence of in vivo deacylation that starts from metabolism of an acyl group at the C-4″ position in a neutral sugar.[8] For example, the propionyl moiety at the C-4″ position of compound **1a** could be easily metabolized in vivo. Therefore, a neutral sugar moiety had to be modified to improve metabolic stability and to maintain antibacterial activity in parallel with optimization of a heteroaryl group at the C-15 position. In this paper, we report the design and synthesis of novel 15-substituted 4″-*O*-carbamoyl azalides derived from 16-membered macrolides.

2. Results and discussion

2.1. Preparation of 4″-*O*-ethylcarbamoyl azalides in application of acyl migration

Kurihara et al. described that 3″,4″-di-*O*-alkyl analogues of 16-membered macrolides exhibited excellent pharmacokinetics,[9] however alkylation for synthesis of these compounds required complicated steps. By the way, an existence of two acyl groups at

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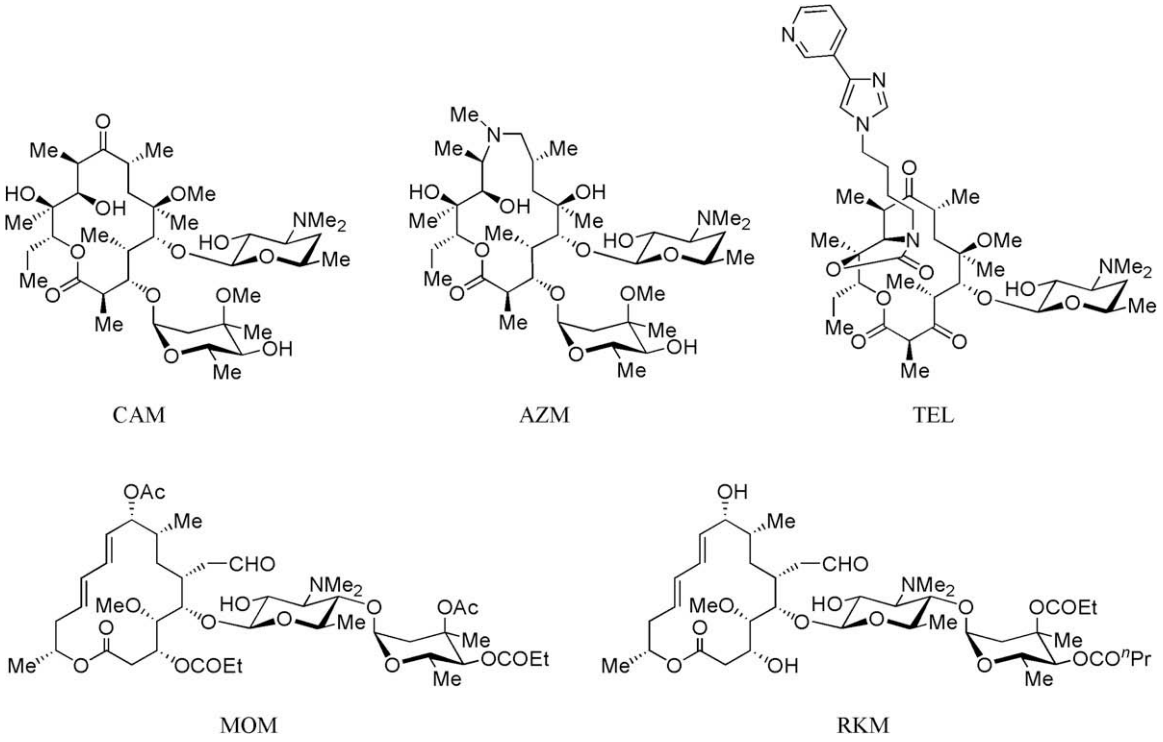
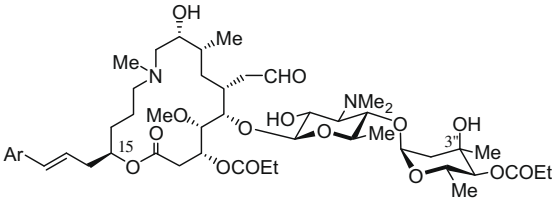


Figure 1. Structures of representative macrolides.



1a: Ar = quinolin-3-yl
1b: Ar = naphthalen-2-yl

Figure 2. Structures of azalides derived from a 16-membered macrolide.

the neutral sugar moiety such as MOM or RKM, unlike midecamycin A_1 (MDM), leads to an increase of antibacterial activities against *erm*-resistant *S. pneumoniae*, and 3″-*O*-acetyl 15-membered azalides derived from MOM actually exhibit superior activities against *erm*-resistant *S. pneumoniae* than the corresponding 3″-hydroxyl azalides.[6a] We therefore designed new azalides possessing an acyl group at the C-3″ position and a carbamoyl group at the C-4″ position. The carbamoyl group is expected to show resistance against the metabolism as an isostere of the 4″-*O*-acyl group from the aspect of antibacterial activities.

Omoto et al. reported an attractive reaction, that is, treatment of 9,2′-di-*O*-acetyl-MDM with acetic anhydride in pyridine at 100 °C afforded 4″-*O*-acetyl-3″-*O*-propionyl analogue via acetylation at the 4″-hydroxyl group after migration of the 4″-*O*-propionyl group to the 3″-hydroxyl group.[4] Then, we decided to use the acyl migration protocol to prepare 4″-*O*-carbamoyl-3″-*O*-propionyl azalides.

A synthetic method of 15-substituted 4″-*O*-ethylcarbamoyl azalides is shown in Scheme 1. Carbamoyl formation of the 15-allyl intermediate (**2**)[6b] with ethylisocyanate in pyridine at 100 °C gave the 4″-*O*-ethylcarbamoyl analogue (**3**) in 37% yield accompanied with an almost equal amount of undesired 3″-*O*-ethylcarbamoyl analogue, which made isolation of **3** difficult. As a result of investigating reaction conditions, we found that regioselective carbamoyl formation at the C-4″ position proceeded in 93% yield using 1,4-diazabicyclo[2.2.2]octane (DABCO), which activated the isocyanate,[10] at low temperature (method A). On the other hand, an alternative method was explored to prepare compound **3** without using isocyanate. Treatment of **2** with 1,1′-carbonyldiimidazole (CDI) in the presence of DABCO provided the 4″-*O*-acylimidazolyl-3″-*O*-propionyl intermediate (**4**) via acyl migration of the 4″-*O*-propionyl group (method B).

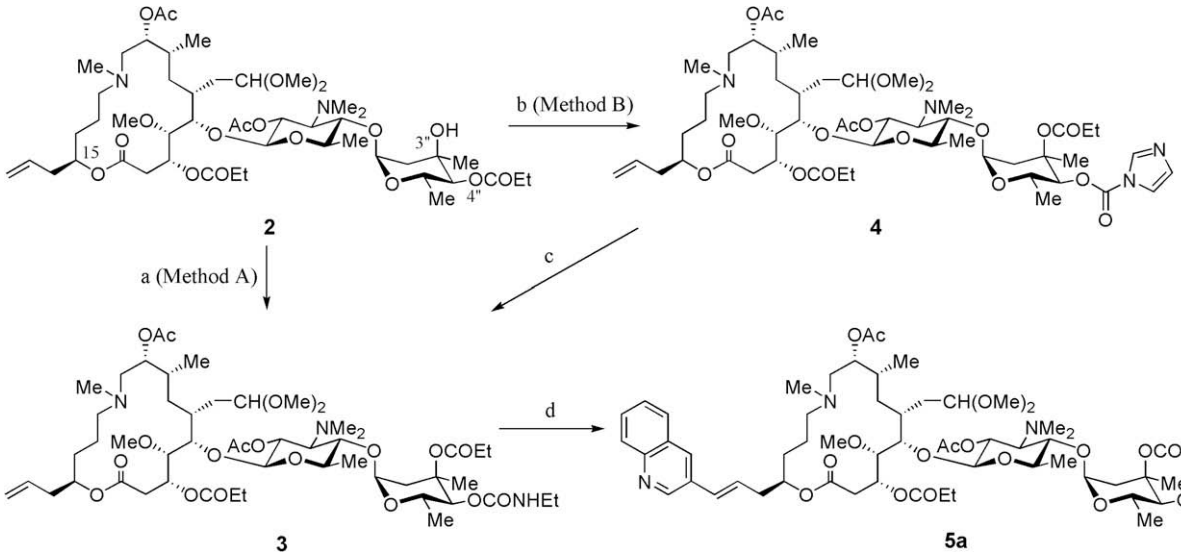
The structure of **3** was determined by HMBC, where correlations were observed between the proton at the C-4″ and the carbonyl carbon in the ethylcarbamoyl group, and also between the carbonyl carbon and the methylene proton in the ethylcarbamoyl group.

We introduced a 3-quinolinyl group as a characteristic heterocycle, which had been proved to be one of the most effective substituents for antibacterial activities,[6b] to side-chain terminus at the C-15 position. Heck reaction of **3** and 3-bromoquinoline with tri-*tert*-butyl phosphine[11] afforded the 3-(3-quinolinyl)-2-propenyl analogue (**5a**).

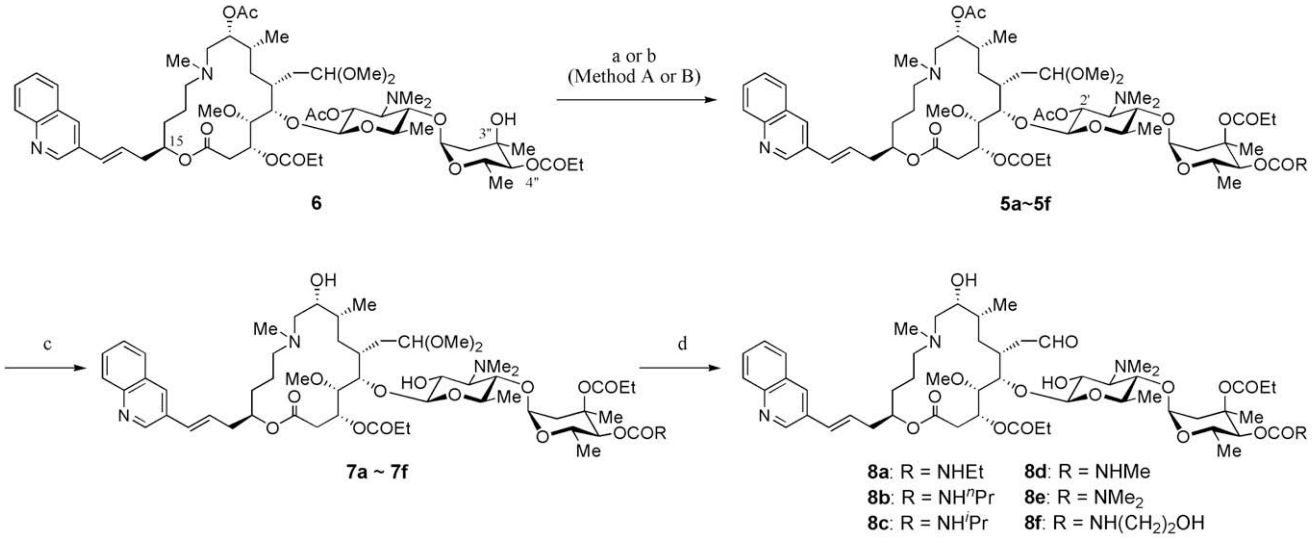
2.2. Preparation and evaluation of 4″-*O*-alkylcarbamoyl-3,3″-di-*O*-propionyl azalides

A variety of 4″-*O*-alkylcarbamoyl analogues with a 3-(3-quinolinyl)-2-propenyl group tethered to the C-15 position were synthesized from the protected 3-quinolinyl intermediate (**6**).[6b] As shown in Scheme 2, introduction of an alkylcarbamoyl group at the C-4″ position of **6** was accomplished by a similar method to as described for **3**. All reactions of **6** with isocyanates (method A) or CDI (method B) successfully proceeded to provide the corresponding alkylcarbamoyl analogues (**5b–f**) in good yield. Removal of 9- and 2′-*O*-acetyl groups of **5a–f** in MeOH gave deacetylated analogues (**7a–f**) that were converted into final products (**8a–f**) by treatment with difluoroacetic acid.

As shown in Table 1, antibacterial activities of these compounds against *mef*-resistant *S. pneumoniae* (#10 and 11) were generally



Scheme 1. Regioselective ethylcarbamoyl formation at the C-4″ position. Reagents and conditions: (a) method A: ethylisocyanate, DABCO, pyridine, sealed tube, 60 °C, 47 h, 93%; (b) method B: CDI, DABCO, pyridine, sealed tube, 60 °C, 69 h, (c) $EtNH_2$·HCl, Et_3N, DMF, 0 °C to rt, 56 h, 61% in two steps; (d) $Pd_2(dba)_3$, *t*-Bu_3P, Cy_2NMe, 3-bromoquinoline, 1,4-dioxane, 50 °C, 52 h, 68%.



Scheme 2. Synthesis of 15-substituted-4″-*O*-alkylcarbamoyl-3,3″-di-*O*-propionyl azalides. Reagents and conditions: (a) method A: the corresponding isocyanates, DABCO, pyridine, sealed tube, 60 °C, 61–120 h, 80–87% for **5b** and **5c**; (b) method B: (1) CDI, DABCO, pyridine, sealed tube, 60 °C, 17 h; (2) the corresponding amines, Et_3N, DMF, −15 °C to rt, 15–21 h, 79%–quant. in two steps for **5d**–**f**; (c) MeOH, rt∼50 °C, 17–96 h, 33–58%; (d) CHF_2COOH, MeCN–H_2O (1:1), rt, 17–23 h, 55–85%.

comparable with compound **1a** as expected. Antibacterial activities against *Haemophilus influenzae* in all compounds slightly decreased, however, their activities against *erm*-resistant *S. pneumoniae* (#5–9) were enhanced as we had expected. Although the methylcarbamoyl analogue (**8d**) was the most effective against *H. influenzae*, the ethylcarbamoyl analogue (**8a**) seemed to exhibit the best-balanced antibacterial spectrum.

2.3. Preparation and evaluation of 15-arylpropenyl-4″-*O*-ethylcarbamoyl-3,3″-di-*O*-propionyl azalides

We next examined possibilities of 4″-*O*-ethylcarbamoyl-3,3″-di-*O*-propionyl azalides to be enhanced in antibacterial activities. A series of 4″-*O*-ethylcarbamoyl azalides with a variety of arylpropenyl groups tethered to the C-15 position were prepared from **3**. As shown in Scheme 3, Heck reactions of **3** with various aryl bromides afforded 15-substituted analogues (**5g**–**k**), and subsequent deprotections of them gave desired **8g**–**k**. In the Heck reaction with 2-amino-5-bromopyridine, the desired product (**5k**) was isolated in a very low yield (17%, see experimental) due to its poor reactivity.

Antibacterial activities of **8g**–**k** are shown in Table 2. To our surprise, conversion of the propionyl to the ethylcarbamoyl group at the C-4″ position in the 2-naphthalenyl analogues (**1b** vs **8g**) did not improve antibacterial activities against constitutive-resistant *S. pneumoniae* (#5–7) unlike in the case of the 3-quinolinyl analogues (**1a** vs **8a**). In addition, 4-quinolinyl and 3-pyridinyl analogues (**8h** and **8j**) had less activities against *erm*-resistant *S. pneumoniae* (#5–9) than 3-quinolinyl analogue (**8a**).

On the other hand, 4-isoquinolinyl and 3-(6-aminopyridinyl) analogues (**8i** and **8k**) were potent, and their activities against constitutive-resistant *S. pneumoniae* were stronger than those of **8a**. One of drawbacks of these 3-*O*-propionyl azalides was a weak activity against *H. influenzae*.

Table 1
Antibacterial activities of 4″-*O*-alkylcarbamoyl azalides with a 3-(3-quinolinyl)-2-propenyl group at the C-15 position

Test organisms	Characteristics	(MIC, μg/ml)						
		8a	**8b**	**8c**	**8d**	**8e**	**8f**	**1a**
Staphylococcus aureus 209P JC-1	Susceptible	0.25	0.5	0.5	0.25	0.25	0.5	0.5
Streptococcus pneumoniae DP1 TypeI	Susceptible	0.015	0.03	0.03	<0.008	0.03	0.015	0.015
S. pneumoniae #5	*ermB* methylase (c)[a]	16	16	16	32	32	>32	128
S. pneumoniae #6	*ermB* methylase (c) + *mefA*[a]	16	32	16	32	>32	32	128
S. pneumoniae #7	*ermB* methylase (c)[a]	16	16	16	32	>32	32	128
S. pneumoniae #8	*ermB* methylase (i)[b]	0.13	0.25	0.25	0.25	0.25	0.25	8
S. pneumoniae #9	*ermB* methylase (i)[b]	0.25	0.25	0.25	0.5	0.5	0.5	8
S. pneumoniae #10	*mefA* efflux	0.015	0.06	0.06	0.015	0.06	0.015	0.06
S. pneumoniae #11	*mefA* efflux	0.015	0.06	0.13	0.03	0.06	0.015	0.03
Streptococcus pyogenes Cook	Susceptible	0.06	0.13	0.13	0.06	0.06	0.06	0.06
S. pyogenes #2	*ermB* methylase (c)[a]	32	64	32	>32	>32	>32	>128
S. pyogenes #3	*mefA* efflux	0.13	0.25	0.25	0.06	0.06	0.13	0.5
Haemophilus influenzae #1	Susceptible	8	8	8	4	8	4	2
H. influenzae #2	Susceptible	16	32	32	8	32	16	8
H. influenzae #3	Susceptible	16	32	32	8	32	16	8

[a] Constitutive.
[b] Inducible.

2.4. Preparation and evaluation of 3-hydroxyl azalides

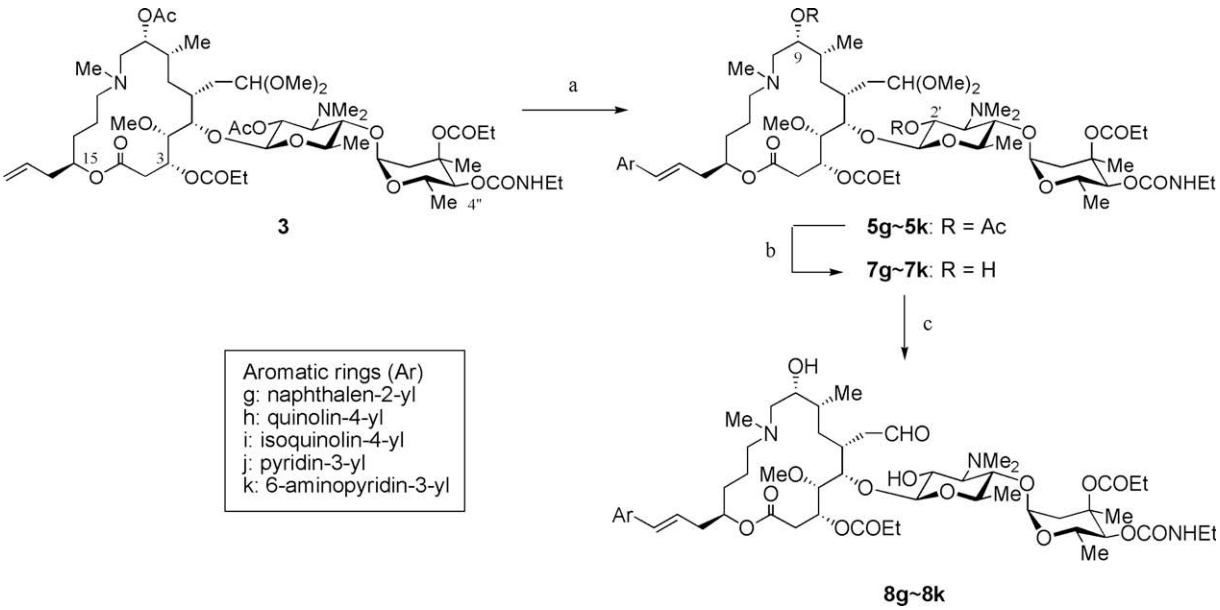
In general, 3-hydroxyl analogues tend to be more potent *in vitro* than the corresponding 3-*O*-acyl analogues in 16-membered macrolides. We therefore decided to prepare 3-hydroxyl azalides having an arylpropenyl and an ethylcarbamoyl group at the C-15 and C-4″ position, respectively, starting from leucomycin A_7[12] (LM-A_7). We chose the 3-quinolinyl, 4-isoquinolinyl and 6-amino-3-pyridinyl groups which were found to be potent side chains at the C-15 position in antibacterial activities against constitutive-resistant strains among the 3-*O*-propionyl azalide series (see Tables 1 and 2). Synthesis of the 3-hydroxyl azalides (**16a–c**) is shown in Scheme 4. The 15-allyl analogue (**12**) was synthesized from the previously reported intermediate (**9**)[13] by a basically similar method to as described for **2** in our previous report.[6b] A 3,18-silyl acetal protection in 16-membered macrolides has already been reported by Kitasato group.[14] After compound **9** was converted into the tetraol (**10**), oxidative cleavage of **10** with lead (IV) acetate followed by β-elimination of a generated dialdehyde with 1,8-diazabicyclo[5.4.0]undecene (DBU) afforded the 9-formylcarboxylic acid (**11**). Reductive amination of **11** with (*R*)-7-methylamino-1-hepten-4-ol[6b,c] gave a desired coupling product (seco acid of **12**), which was converted to the 15-allyl azalactone (**12**) by macrolactonization with 2-methyl-6-nitrobenzoic anhydride (MNBA).[15] Moreover, conversion of **12** to the 15-substituted ethylcarbamoyl azalides (**14a–c**) was achieved in two steps (Heck reaction and the next carbamoyl formation, or otherwise). Sequential deprotection of diacetyl and TBS groups provided the 3-hydroxyl azalides (**16a–c**). Three steps yield from **12** to **16c** was poor due to low yield of the Heck reaction between **15** and 2-amino-5-bromopyridine.

Furthermore, the biological results of **8i** and **8k** prompted us to introduce a 1-amino-4-isoquinolinyl group to the allyl group at the C-15 position, and we therefore planned to prepare **16d**. However, Heck reaction of **12** with 1-amino-4-bromoisoquinoline did not proceed. We thought that a free amino group in aryl bromides, such as 2-amino-5-bromopyridine or 1-amino-4-bromoisoquinoline, prevented the course of Heck reaction. We decided to prepare **16d** according to the modified method shown in Scheme 5. Treatment of **15** with **17** protected by the 2-(trimethylsilyl)ethyloxycarbonyl (Teoc) group under Heck condition provided the coupling products (**18**) as a mixture of inseparable regioisomers on the double bond. After deprotection of the acetyl groups, the 3,18-silyl acetal was cleaved by tetrabutylammonium fluoride (TBAF) to afford **20a** and **20b**. Deprotection of **20a** afforded the desired product (**16d**).

Antibacterial activities of the 3-hydroxyl azalides (**16a–c**) against constitutive-resistant *S. pneumoniae* (#5–7) and *H. influenzae* were stronger than those of corresponding 3-*O*-propionyl azalides (**8a**, **8i**, and **8k**) as shown in Table 3. The aminoisoquinolinyl azalide (**16d**) designed from **8i** and **8k** exhibited the strongest antibacterial activities against constitutive-resistant *S. pneumoniae* in this series, and its activities were comparable to those of TEL. Furthermore, **16d** was clearly superior to TEL against not only *mef*-resistant *S. pneumoniae* (#10 and 11) and *Streptococcus pyogenes* (#3) but also constitutive-resistant *S. pyogenes* (#2). The other azalides **16a** and **16b** were also more active than TEL against *mef*-resistant *S. pneumoniae* and *S. pyogenes*, and were comparable to TEL against constitutive-resistant *S. pyogenes*. On the other hand, **16a**, **16b**, and **16d** showed more potent antibacterial activities compared to CAM, MOM and RKM against *H. influenzae*, although they were a little less active than TEL.

3. Conclusion

A series of 4″-*O*-alkylcarbamoyl azalides having an arylpropenyl group at the C-15 position starting from 16-membered macrolides was designed and synthesized. Regioselective formation of an alkylcarbamoyl substituent at the C-4″position utilizing acyl migration protocol was achieved by two types of methods, using isocyanate or CDI with DABCO, to prepare the 4″-*O*-carbamoyl-3″-*O*-propionyl azalides from the 3″-hydroxy-4″-*O*-propionyl precursors. Although introduction of an alkylcarbamoyl group at the C-4″ position in the 3-*O*-propionyl series suggested improvement of activities against *erm*-resistant *S. pneumoniae*, this modification dramatically improved their metabolic stability.[16] On the other hand, structural modification at the C-15 position was also shown to be important for antibacterial activities against inducible resistant *S. pneumoniae*. As a result of optimization at the C-3 and C-15 positions, 3-hydroxyl azalides (**16a**, **16b**, and **16d**) exhibited significant improvement in antibacterial profiles, that is, the activities against *erm*-resistant *S. pneumoniae* and *H. influenzae*. Among them, **16d** was the most potent in antibacterial activities against constitutive-resistant *S. pneumoniae*. These compounds could be identified as promising candidates and next generation macrolides to overcome resistant pathogens.



Scheme 3. Synthesis of 15-arylpropenyl-4″-*O*-ethylcarbamoyl-3,3″-di-*O*-propionyl azalides. Reagents and conditions: (a) $Pd_2(dba)_3$, *t*-Bu_3P, Cy_2NMe, ArBr, 1,4-dioxane, 50–80 °C, 16–51 h, 17–67% for **5g**, **5h**, **5j**, and **5k** or microwave, 130–160 °C, 25 min, 42% for **5i**; (b) MeOH, rt to 40 °C, 36–89 h, 36–69%; (c) CHF_2COOH, MeCN–H_2O (1:1), rt, 8.5–28 h, 66–91%.

Table 2
Antibacterial activities of 15-arylpropenyl-4″-*O*-ethylcarbamoyl-3,3″-di-*O*-propionyl azalides

Test organisms	Characteristics	(MIC, μg/ml)					
		8g	**8h**	**8i**	**8j**	**8k**	**1b**
Staphylococcus aureus 209P JC-1	Susceptible	2	0.5	0.5	0.5	1	0.5
Streptococcus pneumoniae DP1 TypeI	Susceptible	0.06	0.015	0.015	0.015	0.03	0.06
S. pneumoniae #5	*ermB* methylase (c)[a]	16	64	8	32	8	32
S. pneumoniae #6	*ermB* methylase (c) + *mefA*[a]	32	64	8	32	8	32
S. pneumoniae #7	*ermB* methylase (c)[a]	32	64	8	32	8	32
S. pneumoniae #8	*ermB* methylase (i)[b]	0.5	0.5	0.13	0.5	0.25	8
S. pneumoniae #9	*ermB* methylase (i)[b]	0.5	0.5	0.25	0.5	0.5	8
S. pneumoniae #10	*mefA* efflux	0.13	0.03	0.03	0.06	0.06	0.13
S. pneumoniae #11	*mefA* efflux	0.13	0.03	0.03	0.06	0.06	0.13
Streptococcus pyogenes Cook	Susceptible	0.25	0.06	0.06	0.13	0.13	0.25
S. pyogenes #2	*ermB* methylase (c)[a]	16	>128	128	>64	>32	32
S. pyogenes #3	*mefA* efflux	0.5	0.25	0.25	0.5	0.5	1
Haemophilus influenzae #1	Susceptible	16	8	8	4	4	8
H. influenzae #2	Susceptible	16	32	16	16	16	16
H. influenzae #3	Susceptible	32	16	16	32	32	16

[a] Constitutive.
[b] Inducible.

4. Experimental

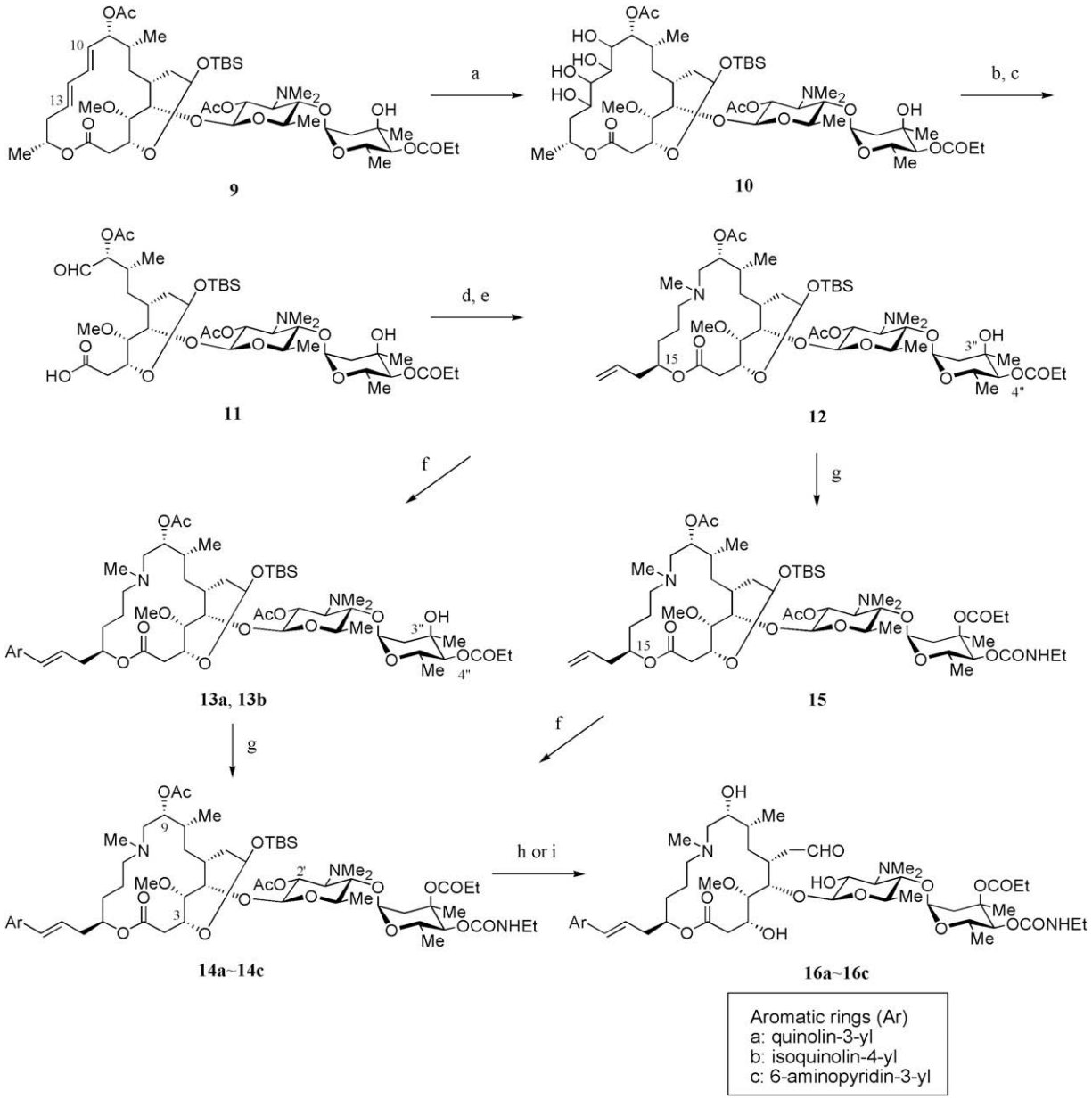
4.1. Chemistry

Optical rotations were measured on a Perkin–Elmer 241 polarimeter or JASCO DIP-370. Mass spectra were obtained on a JEOL JMS-700 for FAB-MS and HRMS or Agilent HP5989A for TSP-MS. ^1H NMR spectra were measured with a Varian Gemini-300 for 300 MHz in $CDCl_3$ using $CHCl_3$ as internal standard. ^{13}C NMR spectra were measured with a JEOL JNM-GSX 400 NMR spectrometer for 100 MHz. Silica gel chromatography and preparative TLC were performed on Wako C-300 and Merck TLC $60F_{254}$, respectively. A microwave-assisted reaction was carried out using a Biotage Initiator Sixty. Organic layer was dried with anhydrous Na_2SO_4, evaporation and concentrations were carried out under reduced pressure below 40 °C, unless otherwise noted.

4.1.1. Synthesis of 3

Method A: To a solution of **2**[6b] (150 mg, 152 μmol) in pyridine (450 μl), 1,4-diazabicyclo[2.2.2]octane (DABCO) (170 mg, 1.52 mmol) and ethylisocyanate (360 μl, 4.55 mmol) were added. After the reaction mixture was stirred in a sealed tube for 26 h at 60 °C, ethylisocyanate (240 μl, 3.03 mmol) was added, and the mixture was further stirred for 21 h at 60 °C. EtOAc and water were added to the reaction mixture, and the extract was washed with water and brine, dried and concentrated. The resulting residue was purified by silica gel chromatography [hexane/acetone/NH_4OH (40:10:0.1–30:10:0.1)] to afford **3** (149 mg, 93%) as a colorless solid.

Method B: To a solution of **2** (30.0 mg, 30.3 μmol) in pyridine (600 μl), DABCO (102 mg, 909 μmol) and 1,1′-carbonyldiimidazole (CDI) (25 mg, 154 μmol) were added and the reaction mixture was stirred in a sealed tube for 69 h at 60 °C. EtOAc and water were added to the reaction mixture, and the extract was washed with water and

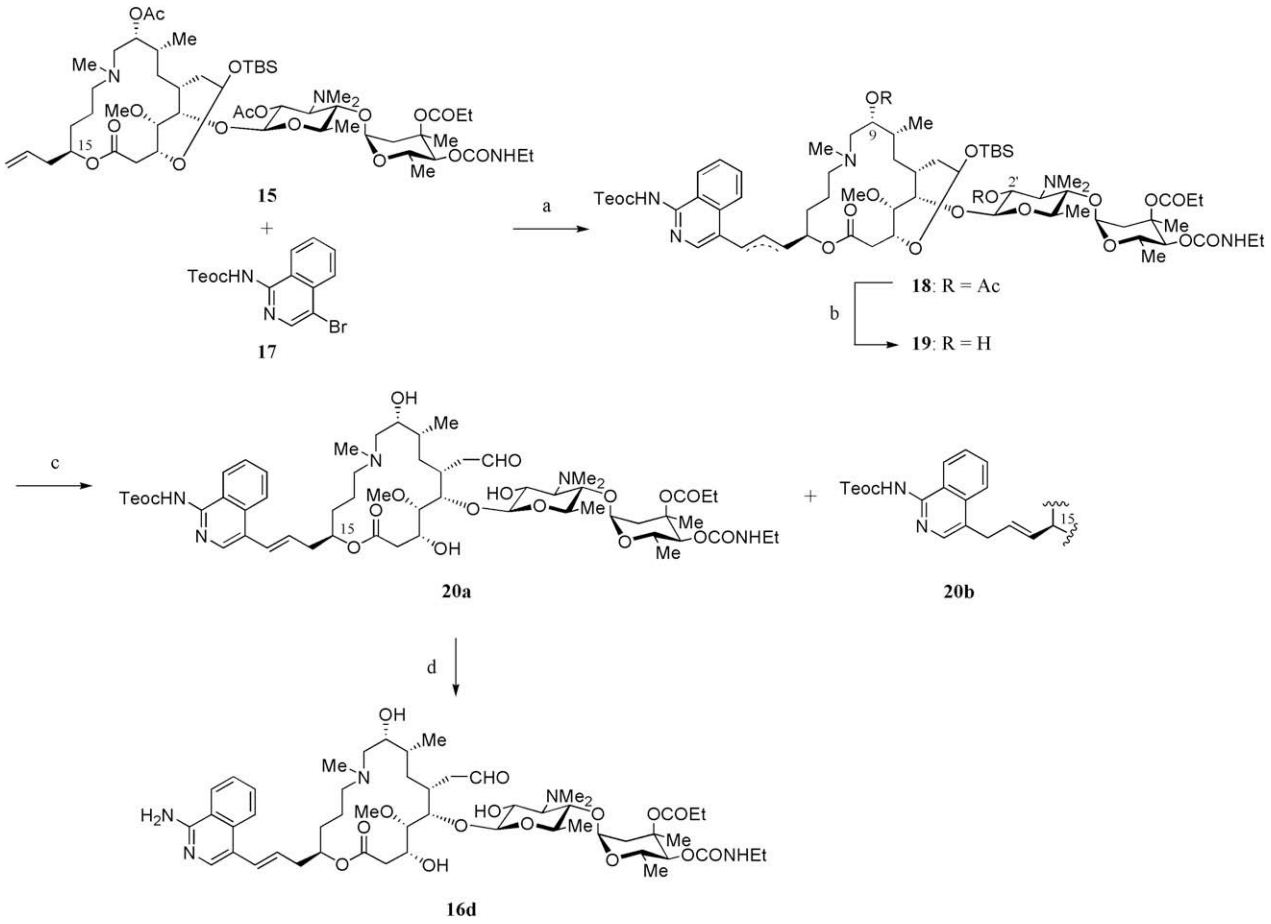


Scheme 4. Synthesis of 3-hydroxyl azalides. Reagents and conditions: (a) OsO_4, NMO, aq acetone, rt, 31 h; (b) $Pb(OAc)_4$, PhH, Na_2CO_3, rt, 15 min; (c) DBU, EtOAc, rt, 30 min, 44% in three steps from **9**; (d) (*R*)-7-methylamino-1-hepten-4-ol, molecular sieves 3A, DMF, rt, 5 h, and then $NaBH_4$, rt, 2 h, 36%; (e) MNBA, DMAP, THF, rt, 16 h, 68%; (f) ArBr, $Pd_2(dba)_3$, *t*-Bu_3P, Cy_2NMe, 1,4-dioxane, 70–90 °C, 17–69 h, 53–80%; (g) EtNCO, pyridine, DABCO, sealed tube, 60 °C, 27–46 h, 78–93%; (h) (1) MeOH, 50 °C, 24 h, 23% for **16a** and 63% for **16b**; (2) CHF_2COOH, MeCN–H_2O (1:1), rt, 15–40 h, 87% for **16a** and 72% for **16b**; (i) (1) MeOH, rt, 15 h; (2) HCl, MeCN–H_2O, rt, 5.5 h, 7.4% for **16c** in three steps.

brine, dried and concentrated to afford **4** (34 mg) as a colorless solid. To a solution of **4** (33 mg) in DMF (600 μl), Et_3N (30 μl, 215 μmol) and ethylamine hydrochloride (16.8 mg, 206 μmol) were added under ice cooling. After the reaction mixture was stirred for 29 h warming to room temperature, Et_3N (37.0 μl, 265 μmol) and ethylamine hydrochloride (21.0 mg, 258 μmol) were added under ice cooling, and the mixture was further stirred for 27 h at room temperature. EtOAc and water were added to the resulting mixture, and the extract was washed twice with water and once with brine. After the organic layer was dried and concentrated, the residue was purified by preparative TLC [hexane/acetone/NH_4OH (40:10:0.1)] to afford **3** (20 mg, 61%) as a colorless solid; $[\alpha]_D^{20}$ −58° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1060 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (3H, d, 8-CH_3), 1.11 (3H, d, 6″-H), 1.13 (3H, t, 3-$OCOCH_2CH_3$), 1.13 (3H, t, 3″-$OCOCH_2CH_3$), 1.15 (3H, t, $NHCH_2CH_3$), 1.19 (3H, d, 6′-H), 1.46 (3H, s, 3″-CH_3), 1.67 (1H, dd, 2″-Hax), 2.03 (3H, s, 9-$OCOCH_3$), 2.04 (3H, s, 2′-$OCOCH_3$), 2.22 (3H, s, 11-CH_3), 2.41 (6H, s, 3′-$N(CH_3)_2$), 2.85 (1H, dd, 2-H), 3.12 (1H, t, 4′-H), 3.14 (3H, s, $CH(OCH_3)_2$), 3.19 (1H, d, 2″-Heq), 3.23 (1H, m, 5′-H), 3.24 (3H, s, $CH(OCH_3)_2$), 3.61 (3H, s, 4-OCH_3), 3.63 (1H, br d, 4-H), 3.91 (1H, br d, 5-H), 4.41 (1H, m, 4″-H), 4.41 (1H, m, 5″-H), 4.54 (1H, dd, $CH(OCH_3)_2$), 4.67 (1H, d, 1′-H), 4.78 (1H, d, 1″-H), 4.89 (1H, m, 9-H), 4.96 (1H, dd, 2′-H), 5.03 (1H, br dd, 3-H), 5.03 (1H, m, 15-H), 5.07 (2H, m, $CH{=}CH_2$), 5.71 (1H, m, $CH{=}CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 173.4, 170.6, 170.1, 168.8, 156.1, 133.5, 117.9, 101.7, 100.6, 98.1, 79.4, 78.5, 78.3, 75.5, 75.3, 73.1, 73.0, 71.0, 70.2, 68.1, 63.4, 61.5, 57.3, 56.5, 53.8, 53.5, 49.4, 42.9, 41.5, 39.2, 36.6, 36.1, 36.0, 33.8, 33.3, 31.6, 31.3, 29.8, 28.7, 27.5, 22.3, 21.7, 21.3, 18.2, 17.5, 15.2, 9.19, 8.79.

4.1.2. Synthesis of 5a

To a solution of **3** (905 mg, 854 μmol) and 3-bromoquinoline (230 μl, 1.70 mmol) in 1,4-dioxane (2.8 ml), tris(dibenzylideneace-



Scheme 5. Synthesis of 15-[3-(aminoisoquinolin-4-yl)-2-propenyl]-3-hydroxyl azalide (**16d**). Reagents and conditions: (a) $Pd_2(dba)_3$, *t*-Bu_3P, Cy_2NMe, 1,4-dioxane, 50 °C, 40 h, 54%; (b) MeOH, rt to 40 °C, 72 h, 50%; (c) TBAF, THF, rt, 15 min, 46% for **20a** and 31% for **20b**; (d) KF, 18-crown-6, DMF, rt, 7 h, 81%.

tone)dipalladium (0) ($Pd_2(dba)_3$) (117 mg, 128 μmol) and dicyclohexylmethylamine (Cy_2NMe)(370 μl, 1.74 mmol) were added. The reaction vessel was purged with argon, and then a 0.5 M solution of tri-*t*-butylphosphine (*t*-Bu_3P) in 1,4-dioxane (510 μl, 255 μmol) was added to the reaction mixture. After the reaction mixture was stirred for 26 h at 50 °C, $Pd_2(dba)_3$ (195 mg, 213 μmol) and a 0.5 M solution of *t*-Bu_3P in 1,4-dioxane (850 μl, 425 μmol) were added, and the mixture was further stirred for 26 h at 50 °C. The catalyst was removed by filtration and the filtrate was concentrated to give a residue which was purified by silica gel chromatography [hexane/acetone/NH_4OH (20:10:0.1–30:20:0.1)] to afford **5a** (684 mg, 68%) as a colorless solid; $[\alpha]_D^{23}$ −58° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1187 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (3H, d, 8-CH_3), 1.12 (3H, d, 6″-H), 1.13 (3H, t, 3-$OCOCH_2CH_3$), 1.20 (3H, d, 6′-H), 1.47 (3H, s, 3″-CH_3), 1.62 (1H, dd, 2″-Hax), 2.04 (3H, s, 9-$OCOCH_3$), 2.07 (3H, s, 2′-$OCOCH_3$), 2.24 (3H, s, 11-CH_3), 2.42 (6H, s, 3′-$N(CH_3)_2$), 2.86 (1H, dd, 2-H), 3.15 (3H, s, $CH(OCH_3)_2$), 3.19 (1H, d, 2″-Heq), 3.25 (3H, s, $CH(OCH_3)_2$), 3.59 (3H, s, 4-OCH_3), 3.93 (1H, br d, 5-H), 4.41 (1H, m, 4″-H), 4.41 (1H, m, 5″-H), 4.55 (1H, dd, $CH(OCH_3)_2$), 4.68 (1H, d, 1′-H), 4.79 (1H, d, 1″-H), 4.92 (1H, m, 9-H), 4.97 (1H, dd, 2′-H), 5.07 (1H, br dd, 3-H), 5.15 (1H, m, 15-H), 6.37 (1H, dt, 15-CH_2CH=CH), 6.61 (1H, d, 15-CH_2CH=CH), 7.53 (1H, ddd, quinoline), 7.66 (1H, ddd, quinoline), 7.79 (1H, br d, quinoline), 8.02 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.95 (1H, d, quinoline).

4.1.3. Synthesis of 5b

Reaction of **6** with *n*-propylisocyanate gave **5b** as a colorless solid in 86% yield by a similar procedure to **3** (method A); $[\alpha]_D^{25}$ −58 (*c* 0.74, $CHCl_3$); FAB-MS *m*/*z* 1201 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (3H, d, 8-CH_3), 0.94 (3H, t, $NHCH_2CH_2CH_3$), 1.13 (3H, d, 6″-H), 1.20 (3H, d, 6′-H), 1.47 (3H, s, 3″-CH_3), 1.50–1.59 (2H, m, $NHCH_2CH_2CH_3$), 1.68 (1H, dd, 2″-Hax), 2.04 (3H, s, 9-$OCOCH_3$), 2.06 (3H, s, 2′-$OCOCH_3$), 2.24 (3H, s, 11-CH_3), 2.43 (6H, s, 3′-$N(CH_3)_2$), 2.86 (1H, dd, 2-H), 3.15 (3H, s, $CH(OCH_3)_2$), 3.19 (1H, d, 2″-Heq), 3.25 (3H, s, $CH(OCH_3)_2$), 3.59 (3H, s, 4-OCH_3), 3.93 (1H, br d, 5-H), 4.41 (1H, m, 4″-H), 4.41 (1H, m, 5″-H), 4.55 (1H, dd, $CH(OCH_3)_2$), 4.68 (1H, d, 1′-H), 4.79 (1H, d, 1″-H), 4.92 (1H, m, 9-H), 4.97 (1H, dd, 2′-H), 5.08 (1H, br dd, 3-H), 5.16 (1H, m, 15-H), 6.37 (1H, dt, 15-CH_2CH=CH), 6.61 (1H, d, 15-CH_2CH=CH), 7.53 (1H, br t, quinoline), 7.66 (1H, ddd, quinoline), 7.79 (1H, br d, quinoline), 8.02 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.95 (1H, d, quinoline).

4.1.4. Synthesis of 5c

Reaction of **6** with *i*-propylisocyanate gave **5c** as a colorless solid in 80% yield by a similar procedure to **3** (method A); $[\alpha]_D^{19}$ −58° (*c* 0.78, $CHCl_3$); FAB-MS *m*/*z* 1201 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (3H, d, 8-CH_3), 1.12 (3H, d, 6″-H), 1.18 (6H, d, $NHCH(CH_3)_2$), 1.47 (3H, s, 3″-CH_3), 1.68 (1H, dd, 2″-Hax), 2.04 (3H, s, 9-$OCOCH_3$), 2.06 (3H, s, 2′-$OCOCH_3$), 2.24 (3H, s, 11-CH_3), 2.42 (6H, s, 3′-$N(CH_3)_2$), 2.86 (1H, dd, 2-H), 3.14 (3H, s, $CH(OCH_3)_2$), 3.20 (1H, d, 2″-Heq), 3.25 (3H, s, $CH(OCH_3)_2$), 3.60 (3H, s, 4-OCH_3), 3.84 (1H, m, $NHCH(CH_3)_2$), 3.93 (1H, br d, 5-H), 4.41 (1H, m, 4″-H), 4.41 (1H, m, 5″-H), 4.56 (1H, dd, $CH(OCH_3)_2$), 4.68 (1H, d, 1′-H), 4.79 (1H, d, 1″-H), 4.92 (1H, m, 9-H), 4.97 (1H, dd, 2′-H), 5.07 (1H, br dd, 3-H), 5.16 (1H, m, 15-H), 6.37 (1H, dt, 15-CH_2CH=CH), 6.61 (1H, d, 15-CH_2CH=CH), 7.53 (1H, ddd, quinoline), 7.67 (1H,

Table 3
Antibacterial activities of 3-hydroxyl azalides

Test organisms	Characteristics	(MIC, μg/ml)							
		16a	**16b**	**16c**	**16d**	TEL	CAM	MOM	RKM
Staphylococcus aureus 209P JC-1	Susceptible	0.25	0.25	2	0.5	0.03	0.13	0.5	0.13
Streptococcus pneumoniae DP1 TypeI	Susceptible	<0.008	<0.008	0.03	0.015	<0.008	0.03	0.13	0.03
S. pneumoniae #5	*ermB* methylase (c)[a]	4	2	4	1	0.5	>128	>128	>128
S. pneumoniae #6	*ermB* methylase (c) + *mefA*[a]	4	4	4	1	1	>128	>128	>128
S. pneumoniae #7	*ermB* methylase (c)[a]	4	4	4	1	1	>128	>128	>128
S. pneumoniae #8	*ermB* methylase (i)[b]	0.06	0.06	0.25	0.06	0.06	>128	4	1
S. pneumoniae #9	*ermB* methylase (i)[b]	0.06	0.06	0.25	0.03	0.06	>128	8	1
S. pneumoniae #10	*mefA* efflux	0.015	0.015	0.06	0.015	0.06	1	0.25	0.06
S. pneumoniae #11	*mefA* efflux	0.015	0.015	0.13	0.015	0.06	1	0.25	0.06
Streptococcus pyogenes Cook	Susceptible	0.06	0.03	0.13	0.03	<0.008	0.03	0.13	0.06
S. pyogenes #2	*ermB* methylase (c)[a]	8	32	16	2	16	>128	>128	>128
S. pyogenes #3	*mefA* efflux	0.06	0.13	0.13	0.03	0.5	8	0.5	0.13
Haemophilus influenzae #1	Susceptible	2	2	4	2	0.5	2	4	2
H. influenzae #2	Susceptible	4	4	8	4	2	8	16	8
H. influenzae #3	Susceptible	4	4	16	8	1	8	16	8

[a] Constitutive.
[b] Inducible.

ddd, quinoline), 7.79 (1H, br d, quinoline), 8.02 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.95 (1H, d, quinoline).

4.1.5. Synthesis of 5d

Carbamoyl formation of **6** with methylamine hydrochloride gave **5d** as a colorless solid in 87% yield by a similar procedure to **3** (method B); $[\alpha]_D^{24}$ −63° (*c* 0.73, $CHCl_3$); FAB-MS *m*/*z* 1173 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (3H, d, 8-CH_3), 1.12 (3H, d, 6″-H), 1.13 (3H, t, 3-$OCOCH_2CH_3$), 1.20 (3H, d, 6′-H), 1.47 (3H, s, 3″-CH_3), 1.68 (1H, dd, 2″-Hax), 2.04 (3H, s, 9-$OCOCH_3$), 2.05 (3H, s, 2′-$OCOCH_3$), 2.24 (3H, s, 11-CH_3), 2.42 (6H, s, 3′-$N(CH_3)_2$), 2.84 (3H, d, $NHCH_3$), 2.86 (1H, dd, 2-H), 3.13 (1H, t, 4′-H), 3.15 (3H, s, $CH(OCH_3)_2$), 3.19 (1H, d, 2″-Heq), 3.25 (3H, s, $CH(OCH_3)_2$), 3.59 (3H, s, 4-OCH_3), 3.93 (1H, br d, 5-H), 4.41 (1H, m, 4″-H), 4.42 (1H, m, 5″-H), 4.55 (1H, dd, $CH(OCH_3)_2$), 4.68 (1H, d, 1′-H), 4.79 (1H, d, 1″-H), 4.90 (1H, m, 9-H), 4.97 (1H, dd, 2′-H), 5.07 (1H, br dd, 3-H), 5.16 (1H, m, 15-H), 6.37 (1H, dt, 15-CH_2CH═CH), 6.61 (1H, d, 15-CH_2CH═CH), 7.52 (1H, ddd, quinoline), 7.66 (1H, ddd, quinoline), 7.79 1H, (br d, quinoline), 8.01 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.95 (1H, d, quinoline).

4.1.6. Synthesis of 5e

Carbamoyl formation of **6** with dimethylamine hydrochloride gave **5e** as a colorless solid in quantitative yield by a similar procedure to **3** (method B); $[\alpha]_D^{24}$ −62° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1187 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (3H, d, 8-CH_3), 1.12 (3H, d, 6″-H), 1.14 (3H, t, 3-$OCOCH_2CH_3$), 1.15 (3H, t, 3″-$OCOCH_2CH_3$), 1.20 (3H, d, 6′-H), 1.46 (3H, s, 3″-CH_3), 1.69 (1H, dd, 2″-Hax), 2.04 (3H, s, 9-$OCOCH_3$), 2.05 (3H, s, 2′-$OCOCH_3$), 2.24 (3H, s, 11-CH_3), 2.43 (6H, s, 3′-$N(CH_3)_2$), 2.62 (1H, dd, 2-H), 2.86 (1H, dd, 2-H), 2.96 (6H, s, $CON(CH_3)_2$), 3.14 (1H, m, 4′-H), 3.15 (3H, s, $CH(OCH_3)_2$), 3.19 (1H, d, 2″-Heq), 3.25 (3H, s, $CH(OCH_3)_2$), 3.59 (3H, s, 4-OCH_3), 3.93 (1H, br d, 5-H), 4.42 (1H, m, 4″-H), 4.45 (1H, m, 5″-H), 4.55 (1H, m, $CH(OCH_3)_2$), 4.68 (1H, d, 1′-H), 4.80 (1H, d, 1″-H), 4.92 (1H, m, 9-H), 4.97 (1H, dd, 2′-H), 5.07 (1H, br dd, 3-H), 5.16 (1H, m, 15-H), 6.37 (1H, dt, 15-CH_2CH═CH), 6.61 (1H, d, 15-CH_2CH═CH), 7.53 (1H, ddd, quinoline), 7.66 (1H, ddd, quinoline), 7.79 (1H, br d, quinoline), 8.02 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.95 (1H, d, quinoline).

4.1.7. Synthesis of 5f

To a solution of **6** (330 mg, 296 μmol) in pyridine (3.3 ml), DABCO (500 mg, 4.46 mmol) and CDI (960 mg, 5.92 mmol) were added and the reaction mixture was stirred in a sealed tube for 16 h at 60 °C. EtOAc and water were added to the reaction mixture, and the extract was washed twice with water and once with brine, dried and concentrated to afford 4″-*O*-acylimidazolyl-3″-*O*-propionyl analogue of **6** (349 mg) as a colorless solid. To a solution of the imidazolide (55 mg) in DMF (1.1 ml), ethanolamine (28 μl, 468 μmol) was added under ice cooling and the reaction mixture was stirred for 5 h at the same temperature. EtOAc and water were added to the reaction mixture, and the extract was washed twice with water and once with brine. After the organic layer was dried and concentrated, the resulting residue was purified by preparative TLC [hexane/acetone/NH_4OH (15:15:0.1)] to afford **5f** (43 mg, 79%) as a colorless solid; $[\alpha]_D^{24}$ −58° (*c* 0.81, $CHCl_3$); FAB-MS *m*/*z* 1203 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (3H, d, 8-CH_3), 1.12 (3H, d, 6″-H), 1.14 (3H, t, 3-$OCOCH_2CH_3$), 1.20 (3H, d, 6′-H), 1.48 (3H, s, 3″-CH_3), 1.68 (1H, dd, 2″-Hax), 2.04 (3H, s, 9-$OCOCH_3$), 2.05 (3H, s, 2′-$OCOCH_3$), 2.24 (3H, s, 11-CH_3), 2.42 (6H, s, 3′-$N(CH_3)_2$), 2.86 (1H, dd, 2-H), 3.13 (1H, t, 4′-H), 3.15 (3H, s, $CH(OCH_3)_2$), 3.19 (1H, d, 2″-Heq), 3.25 (3H, s, $CH(OCH_3)_2$), 3.39 (2H, q, $NHCH_2$), 3.59 (3H, s, 4-OCH_3), 3.76 (2H, t, CH_2OH), 3.93 (1H, br d, 5-H), 4.40 (1H, d, 4″-H), 4.44 (1H, m, 5″-H), 4.55 (1H, dd, $CH(OCH_3)_2$), 4.68 (1H, d, 1′-H), 4.79 (1H, d, 1″-H), 4.91 (1H, m, 9-H), 4.97 (1H, dd, 2′-H), 5.07 (1H, br dd, 3-H), 5.15 (1H, m, 15-H), 6.37 (1H, dt, 15-CH_2CH═CH), 6.61 (1H, d, 15-CH_2CH═CH), 7.52 (1H, ddd, quinoline), 7.66 (1H, ddd, quinoline), 7.78 (1H, br d, quinoline), 8.02 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.95 (1H, d, quinoline).

4.1.8. Synthesis of 8a

Compound **5a** (129 mg, 109 μmol) was dissolved in methanol (5.1 ml), and the mixture was stirred for 42 h at 40 °C. After the reaction mixture was concentrated, and the residue was purified by silica gel chromatography [$CHCl_3$/MeOH/NH_4OH (100:1:0.1 to 60:1:0.1)] to obtain **7a** (60 mg, 50%). To a solution of **7a** (21 mg, 19.0 μmol) in acetonitrile and water (1:1) (0.5 ml), difluoroacetic acid (54 μl, 859 μmol) was added and the reaction mixture was stirred for 20 h at room temperature. Saturated aqueous $NaHCO_3$ was added to the reaction mixture and the aqueous layer was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried, and concentrated. The resulting

residue was purified by preparative TLC [$CHCl_3$/MeOH/NH_4OH (15:1:0.1)] to afford **8a** (11 mg, 55%) as a colorless solid; $[\alpha]_D^{18}$ −58° (*c* 0.30, $CHCl_3$); FAB-MS *m*/*z* 1057 $(M+H)^+$; FAB-HRMS: Calcd for $C_{55}H_{84}N_4O_{16}$: 1057.5961, Found: 1057.5947 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, d, 8-CH_3), 1.13 (3H, d, 6″-H), 1.16 (3H, t, $NHCH_2CH_3$), 1.34 (1H, m, 8-H), 1.47 (3H, s, 3″-CH_3), 1.69 (1H, dd, 2″-Hax), 2.31 (3H, s, 11-CH_3), 2.53 (6H, s, 3′-$N(CH_3)_2$), 2.84 (1H, dd, 2-H), 2.90 (1H, dd, 6-CH_2), 3.20 (1H, d, 2″-Heq), 3.25 (2H, q, $NHCH_2CH_3$), 3.39 (1H, dd, 2′-H), 3.66 (3H, s, 4-OCH_3), 3.87 (1H, br d, 5-H), 3.98 (1H, br d, 4-H), 4.38 (1H, d, 1′-H), 4.41 (1H, m, 4″-H), 4.42 (1H, m, 5″-H), 4.82 (1H, d, 1″-H), 5.13 (1H, m, 15-H), 5.58 (1H, m, 3-H), 6.36 (1H, dt, 15-CH_2CH═CH), 6.58 (1H, d, 15-CH_2CH═CH), 7.53 (1H, ddd, quinoline), 7.67 (1H, ddd, quinoline), 7.78 (1H, br d, quinoline), 8.00 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.94 (1H, d, quinoline), 9.65 (1H, s, CHO).

4.1.9. Synthesis of 8b

Reaction of **5b** with MeOH gave **7b** as a colorless solid in 58% yield by a similar procedure to **7a**. Subsequently, reaction of **7b** with difluoroacetic acid gave **8b** as a colorless solid in 61% yield by a similar procedure to **8a**; $[\alpha]_D^{19}$ −53° (*c* 0.70, $CHCl_3$); FAB-MS *m*/*z* 1071 $(M+H)^+$; FAB-HRMS: Calcd for $C_{56}H_{86}N_4O_{16}$: 1071.6117, Found: 1071.6129 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.93 (3H, t, $NHCH_2CH_2CH_3$), 1.13 (3H, t, 3-$OCOCH_2CH_3$), 1.14 (3H, t, 3″-$OCOCH_2CH_3$), 1.34 (1H, m, 8-H), 1.47 (3H, s, 3″-CH_3), 1.57 (2H, sext, $NHCH_2CH_2CH_3$), 1.69 (1H, dd, 2″-Hax), 2.32 (3H, s, 11-CH_3), 2.53 (6H, s, 3′-$N(CH_3)_2$), 2.84 (1H, dd, 2-H), 2.90 (1H, dd, 6-CH_2), 3.21 (1H, d, 2″-Heq), 3.37 (1H, m, 9-H), 3.25 (2H, q, $NHCH_2CH_3$), 3.39 (1H, dd, 2′-H), 3.66 (3H, s, 4-OCH_3), 3.87 (1H, br d, 5-H), 3.98 (1H, br d, 4-H), 4.38 (1H, d, 1′-H), 4.41 (1H, m, 4″-H), 4.44 (1H, m, 5″-H), 4.82 (1H, d, 1″-H), 4.84 (1H, t, NH), 5.13 (1H, m, 15-H), 5.57 (1H, br dd, 3-H), 6.35 (1H, dt, 15-CH_2CH═CH), 6.59 (1H, d, 15-CH_2CH═CH), 7.53 (1H, ddd, quinoline), 7.66 (1H, ddd, quinoline), 7.78 (1H, br d, quinoline), 8.00 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.94 (1H, d, quinoline), 9.65 (1H, s, CHO).

4.1.10. Synthesis of 8c

Reaction of **5c** with MeOH gave **7c** as a colorless solid in 58% yield by a similar procedure to **7a**. Subsequently, reaction of **7c** with difluoroacetic acid gave **8c** as a colorless solid in 81% yield by a similar procedure to **8a**; $[\alpha]_D^{23}$ −58° (*c* 0.70, $CHCl_3$); FAB-MS *m*/*z* 1071 $(M+H)^+$; FAB-HRMS: Calcd for $C_{56}H_{86}N_4O_{16}$: 1071.6117, Found: 1071.6110 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, d, 8-CH_3), 1.12 (3H, t, 3-$OCOCH_2CH_3$), 1.14 (3H, t, 3″-$OCOCH_2CH_3$), 1.18 (6H, d, $NHCH(CH_3)_2$), 1.34 (3H, m, 8-H), 1.47 (3H, s, 3″-CH_3), 1.69 (dd, 2″-Hax), 1.75 (1H, m, 14-H), 2.32 (3H, s, 11-CH_3), 2.53 (6H, s, 3′-$N(CH_3)_2$), 2.84 (1H, dd, 2-H), 2.90 (1H, dd, 6-CH_2), 3.15 (1H, m, 4′-H), 3.17 (1H, m, 5′-H), 3.21 (1H, d, 2″-Heq), 3.36 (1H, m, 9-H), 3.39 (1H, dd, 2′-H), 3.66 (3H, s, 4-OCH_3), 3.83 (1H, dq, $NHCH(CH_3)_2$), 3.87 (1H, br d, 5-H), 3.98 (1H, br d, 4-H), 4.38 (1H, d, 1′-H), 4.41 (1H, m, 4″-H), 4.44 (1H, m, 5″-H), 4.67 (1H, d, NH), 4.82 (1H, d, 1″-H), 5.13 (1H, m, 15-H), 5.57 (1H, br dd, 3-H), 6.35 (1H, dt, 15-CH_2CH═CH), 6.59 (1H, d, 15-CH_2CH═CH), 7.53 (1H, ddd, quinoline), 7.66 (1H, ddd, quinoline), 7.78 (1H, br d, quinoline), 8.00 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.94 (1H, d, quinoline), 9.65 (1H, s, CHO).

4.1.11. Synthesis of 8d

Reaction of **5d** with MeOH gave **7d** as a colorless solid in 40% yield by a similar procedure to **7a**. Subsequently, reaction of **7d** with difluoroacetic acid gave **8d** as a colorless solid in 84% yield by a similar procedure to **8a**; $[\alpha]_D^{25}$ −55° (*c* 0.54, $CHCl_3$); FAB-MS *m*/*z* 1043 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, d, 8-CH_3), 1.12 (3H, d, 6″-H), 1.13 (3H, t, 3-$OCOCH_2CH_3$), 1.14 (3H, d, 6′-H), 1.14 (3H, t, 3″-$OCOCH_2CH_3$), 1.34 (1H, m, 8-H), 1.47 (3H, s, 3″-CH_3), 1.69 (1H, dd, 2″-Hax), 2.32 (3H, s, 11-CH_3), 2.53 (6H, s, 3′-$N(CH_3)_2$), 2.84 (1H, dd, 2-H), 2.89 (1H, dd, 6-CH_2), 3.15 (1H, m, 4′-H), 3.18 (1H, m, 5′-H), 3.21 (1H, d, 2″-Heq), 3.36 (1H, m, 9-H), 3.39 (1H, dd, 2′-H), 3.66 (3H, s, 4-OCH_3), 3.87 (1H, br d, 5-H), 3.97 (1H, br d, 4-H), 4.38 (1H, d, 1′-H), 4.45 (1H, m, 4″-H), 4.47 (1H, m, 5″-H), 4.78 (1H, d, NH), 4.82 (1H, d, 1″-H), 5.13 (1H, m, 15-H), 5.57 (1H, br dd, 3-H), 6.35 (1H, dt, 15-CH_2CH═CH), 6.59 (1H, d, 15-CH_2CH═CH), 7.53 (1H, ddd, quinoline), 7.66 (1H, ddd, quinoline), 7.78 (1H, br d, quinoline), 8.00 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.94 (1H, d, quinoline), 9.65 (1H, s, CHO).

4.1.12. Synthesis of 8e

Reaction of **5e** with MeOH gave **7e** as a colorless solid in 33% yield by a similar procedure to **7a**. Subsequently, reaction of **7e** with difluoroacetic acid gave **8e** as a colorless solid in 85% yield by a similar procedure to **8a**; $[\alpha]_D^{25}$ −56° (*c* 0.58, $CHCl_3$); FAB-MS *m*/*z* 1057 $(M+H)^+$; FAB-HRMS: Calcd for $C_{55}H_{84}N_4O_{16}$: 1057.5961, Found: 1057.5958 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, d, 8-CH_3), 1.12 (3H, t, 3-$OCOCH_2CH_3$), 1.13 (3H, d, 6″-H), 1.16 (3H, t, 3″-$OCOCH_2CH_3$), 1.35 (1H, m, 8-H), 1.46 (3H, s, 3″-CH_3), 1.70 (1H, dd, 2″-Hax), 2.32 (3H, s, 11-CH_3), 2.54 (6H, s, 3′-$N(CH_3)_2$), 2.84 (1H, dd, 2-H), 2.90 (1H, dd, 6-CH_2), 2.96 (6H, s, $CON(CH_3)_2$), 3.18 (1H, m, 4′-H), 3.18 (1H, m, 5′-H), 3.22 (1H, d, 2″-Heq), 3.36 (1H, m, 9-H), 3.39 (1H, dd, 2′-H), 3.66 (3H, s, 4-OCH_3), 3.87 (1H, br d, 5-H), 3.97 (1H, br d, 4-H), 4.39 (1H, d, 1′-H), 4.43 (1H, d, 4″-H), 4.50 (1H, dq, 5″-H), 4.84 (1H, d, 1″-H), 5.13 (1H, m, 15-H), 5.57 (1H, br dd, 3-H), 6.35 (1H, dt, 15-CH_2CH═CH), 6.59 (1H, d, 15-CH_2CH═CH), 7.53 (1H, ddd, quinoline), 7.66 (1H, ddd, quinoline), 7.78 (1H, br d, quinoline), 8.00 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.94 (1H, d, quinoline), 9.65 (1H, s, CHO).

4.1.13. Synthesis of 8f

Reaction of **5f** with MeOH gave **7f** as a colorless solid in 40% yield by a similar procedure to **7a**. Subsequently, reaction of **7f** with difluoroacetic acid gave **8f** as a colorless solid in 83% yield by a similar procedure to **8a**; $[\alpha]_D^{25}$ −51° (*c* 0.51, $CHCl_3$); FAB-MS *m*/*z* 1073 $(M+H)^+$; FAB-HRMS: Calcd for $C_{55}H_{84}N_4O_{17}$: 1073.5910, Found: 1073.5913 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, d, 8-CH_3), 1.12 (3H, t, 3-$OCOCH_2CH_3$), 1.16 (3H, t, 3″-$OCOCH_2CH_3$), 1.48 (3H, s, 3″-CH_3), 1.69 (1H, dd, 2″-Hax), 2.32 (3H, s, 11-CH_3), 2.53 (6H, s, 3′-$N(CH_3)_2$), 2.84 (1H, dd, 2-H), 2.89 (1H, dd, 6-CH_2), 3.14 (1H, m, 4′-H), 3.17 (1H, m, 5′-H), 3.21 (1H, d, 2″-Heq), 3.39 (2H, m, $NHCH_2$), 3.66 (3H, s, 4-OCH_3), 3.75 (2H, t, CH_2OH), 3.87 (1H, br d, 5-H), 3.97 (1H, br d, 4-H), 4.38 (1H, d, 1′-H), 4.42 (1H, d, 4″-H), 4.48 (1H, dq, 5″-H), 4.82 (1H, d, 1″-H), 5.13 (1H, m, 15-H), 5.27 (1H, t, NH), 5.56 (1H, br dd, 3-H), 6.35 (1H, dt, 15-CH_2CH═CH), 6.59 (1H, d, 15-CH_2CH═CH), 7.53 (1H, ddd, quinoline), 7.66 (1H, ddd, quinoline), 7.78 (1H, br d, quinoline), 8.00 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.93 (1H, d, quinoline), 9.64 (1H, s, CHO).

4.1.14. Synthesis of 5g

Reaction of **3** with 2-bromonaphthalene gave **5g** as a colorless solid in 52% yield by a similar procedure to **5a**; $[\alpha]_D^{27}$ −59° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1186$(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (3H, d, 8-CH_3), 1.11 (3H, d, 6″-H), 1.13 (3H, t, 3-$OCOCH_2CH_3$), 1.13 (3H, t, 3″-$OCOCH_2CH_3$), 1.15 (3H, t, $NHCH_2CH_3$), 1.18 (3H, d, 6′-H), 1.46 (3H, s, 3″-CH_3), 1.67 (1H, dd, 2″-Hax), 2.02 (3H, s, 9-$OCOCH_3$), 2.04 (3H, s, 2′-$OCOCH_3$), 2.22 (3H, s, 11-CH_3), 2.41 (6H, s, 3′-$N(CH_3)_2$), 2.82 (1H, dd, 2-H), 3.14 (3H, s, $CH(OCH_3)_2$), 3.19 (1H, d, 2″-Heq), 3.22 (1H, m, 5′-H), 3.24 (3H, s, $CH(OCH_3)_2$), 3.57 (3H, s, 4-OCH_3), 3.62 (1H, br d, 4-H), 3.91 (1H, br d, 5-H), 4.41 (1H, m, 4″-H), 4.41 (1H, m, 5″-H), 4.54 (1H, dd, $CH(OCH_3)_2$), 4.66 (1H, d, 1′-H), 4.77 (1H, d, 1″-H), 4.90 (1H, m, 9-H), 4.95 (1H, dd, 2′-H), 5.06 (1H, br dd, 3-H), 5.12 (1H, m, 15-H), 6.23 (1H, dt, 15-CH_2CH═CH), 6.59 (1H, d, 15-CH_2CH═CH), 7.30–7.49 (4H, m,

naphthalene), 7.54 (1H, dd, naphthalene), 7.67 (1H, s, naphthalene), 7.75 (1H, dt, naphthalene).

4.1.15. Synthesis of 5h

Reaction of **3** with 4-bromoquinoline gave **5h** as a colorless solid in 46% yield by a similar procedure to **5a**; $[\alpha]_D^{24}$ −56° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1187 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, d, 8-CH_3), 1.09 (3H, d, 6″-H), 1.16 (3H, t, $NHCH_2CH_3$), 1.18 (3H, d, 6′-H), 1.45 (3H, s, 3″-CH_3), 1.66 (1H, dd, 2″-Hax), 2.01 (3H, s, 9-$OCOCH_3$), 2.04 (3H, s, 2′-$OCOCH_3$), 2.22 (3H, s, 11-CH_3), 2.40 (6H, s, 3′-$N(CH_3)_2$), 2.83 (1H, dd, 2-H), 3.13 (3H, s, $CH(OCH_3)_2$), 3.19 (1H, d, 2″-Heq), 3.23 (3H, s, $CH(OCH_3)_2$), 3.54 (3H, s, 4-OCH_3), 3.58 (1H, br d, 4-H), 3.91 (1H, br d, 5-H), 4.40 (1H, m, 4″-H), 4.40 (1H, m, 5″-H), 4.53 (1H, dd, $CH(OCH_3)_2$), 4.65 (1H, d, 1′-H), 4.77 (1H, d, 1″-H), 4.90 (1H, m, 9-H), 4.94 (1H, dd, 2′-H), 5.05 (1H, br dd, 3-H), 5.17 (1H, m, 15-H), 6.36 (1H, dt, 15-CH_2CH═CH), 7.13 (1H, d, 15-CH_2CH═CH), 7.39 (1H, d, quinoline), 7.54 (1H, ddd, quinoline), 7.68 (1H, ddd, quinoline), 8.06 (1H, br d, quinoline), 8.08 (1H, br d, quinoline), 8.81 (1H, d, quinoline).

4.1.16. Synthesis of 5i

To a 0.5–2 ml microwave reaction vial equipped with a magnetic stirring bar, compound **3** (45.2 mg, 42.6 μmol), 1,4-dioxane (2.8 ml), 4-bromoisoquinoline (17.8 mg, 85.5 μmol), ($Pd_2(dba)_3$) (3.9 mg, 4.26 μmol), Cy_2NMe (18.3 μl, 85.2 μmol) and a 0.5 M solution of *t*-Bu_3P in 1,4-dioxane (17.0 μl, 8.50 μmol) were added. After the reaction mixture was capped and irradiated with microwaves for 25 min at 130–160 °C, the catalyst was removed by filtration. The filtrate was concentrated to give a residue which was purified by preparative TLC [$CHCl_3$/EtOAc/MeOH (3:2:1)] to afford **5i** (21.2 mg, 42%) as a colorless solid; $[\alpha]_D^{20}$ −60° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1187 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (3H, d, 8-CH_3), 1.09 (3H, d, 6″-H), 1.17 (3H, t, $NHCH_2CH_3$), 1.18 (3H, d, 6′-H), 1.46 (3H, s, 3″-CH_3), 1.67 (1H, dd, 2″-Hax), 2.02 (3H, s, 9-$OCOCH_3$), 2.04 (3H, s, 2′-$OCOCH_3$), 2.23 (3H, s, 11-CH_3), 2.41 (s, 3′-$N(CH_3)_2$), 2.85 (1H, dd, 2-H), 3.13 (3H, s, $CH(OCH_3)_2$), 3.19 (1H, d, 2″-Heq), 3.23 (3H, s, $CH(OCH_3)_2$), 3.57 (3H, s, 4-OCH_3), 3.64 (1H, br d, 4-H), 3.92 (1H, br d, 5-H), 4.41 (1H, m, 4″-H), 4.41 (1H, m, 5″-H), 4.54 (1H, dd, $CH(OCH_3)_2$), 4.66 (1H, d, 1′-H), 4.77 (1H, d, 1″-H), 4.90 (1H, m, 9-H), 4.95 (1H, dd, 2′-H), 5.05 (1H, br dd, 3-H), 5.17 (1H, m, 15-H), 6.19 (1H, dt, 15-CH_2CH═CH), 7.01 (1H, d, 15-CH_2CH═CH), 7.60 (1H, ddd, isoquinoline), 7.71 (1H, ddd, isoquinoline), 7.95 (1H, br d, isoquinoline), 8.03 (1H, br d, isoquinoline), 8.51 (1H, s, isoquinoline), 9.12 (1H, s, isoquinoline).

4.1.17. Synthesis of 5j

Reaction of **3** with 3-bromopyridine gave **5j** as a colorless solid in 67% yield by a similar procedure to **5a**; $[\alpha]_D^{23}$ −62° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1137 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, d, 8-CH_3), 1.10 (3H, d, 6″-H), 1.17 (3H, t, $NHCH_2CH_3$), 1.18 (3H, d, 6′-H), 1.45 (3H, s, 3″-CH_3), 1.66 (1H, dd, 2″-Hax), 2.02 (3H, s, 9-$OCOCH_3$), 2.03 (3H, s, 2′-$OCOCH_3$), 2.21 (3H, s, 11-CH_3), 2.40 (6H, s, 3′-$N(CH_3)_2$), 2.54 (1H, dd, 2-H), 2.58 (1H, t, 3′-H), 2.83 (1H, dd, 2-H), 3.13 (3H, s, $CH(OCH_3)_2$), 3.19 (1H, d, 2″-Heq), 3.23 (3H, s, $CH(OCH_3)_2$), 3.57 (3H, s, 4-OCH_3), 3.90 (1H, br d, 5-H), 4.40 (1H, m, 4″-H), 4.40 (1H, m, 5″-H), 4.53 (1H, dd, $CH(OCH_3)_2$), 4.66 (1H, d, 1′-H), 4.77 (1H, d, 1″-H), 4.89 (1H, m, 9-H), 4.94 (1H, dd, 2′-H), 5.05 (1H, br dd, 3-H), 5.08 (1H, m, 15-H), 6.18 (1H, dt, 15-CH_2CH═CH), 6.41 (1H, d, 15-CH_2CH═CH), 7.19 (1H, dd, pyridine), 7.63 (1H, dt, pyridine), 8.42 (1H, d, pyridine), 8.52 (1H, d, pyridine).

4.1.18. Synthesis of 5k

Reaction of **3** with 2-amino-5-bromopyridine gave **5k** as a colorless solid in 17% yield by a similar procedure to **5a**; $[\alpha]_D^{20}$ −59° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1152 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (3H, d, 8-CH_3), 1.11 (3H, d, 6″-H), 1.17 (3H, t, $NHCH_2CH_3$), 1.19 (3H, d, 6′-H), 1.46 (3H, s, 3″-CH_3), 1.67 (1H, dd, 2″-Hax), 2.03 (3H, s, 9-$OCOCH_3$), 2.05 (3H, s, 2′-$OCOCH_3$), 2.25 (3H, s, 11-CH_3), 2.41 (6H, s, 3′-$N(CH_3)_2$), 2.84 (1H, dd, 2-H), 3.14 (3H, s, $CH(OCH_3)_2$), 3.19 (1H, d, 2″-Heq), 3.24 (3H, s, $CH(OCH_3)_2$), 3.59 (3H, s, 4-OCH_3), 3.61 (1H, br d, 4-H), 3.91 (1H, br d, 5-H), 4.41 (1H, m, 4″-H), 4.41 (1H, m, 5″-H), 4.54 (1H, dd, $CH(OCH_3)_2$), 4.67 (1H, d, 1′-H), 4.77 (1H, d, 1″-H), 4.91 (1H, m, 9-H), 4.95 (1H, dd, 2′-H), 5.05 (1H, br dd, 3-H), 5.06 (1H, m, 15-H), 5.91 (1H, dt, 15-CH_2CH═CH), 6.29 (1H, d, 15-CH_2CH═CH), 6.45 (1H, d, pyridine), 7.48 (1H, dd, pyridine), 7.96 (1H, d, pyridine).

4.1.19. Synthesis of 8g

Reaction of **5g** with MeOH gave **7g** as a colorless solid in 50% yield by a similar procedure to **7a**. Subsequently, reaction of **7g** with difluoroacetic acid gave **8g** as a colorless solid in 88% yield by a similar procedure to **8a**; $[\alpha]_D^{26}$ −55° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1056 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, d, 8-CH_3), 1.11 (3H, d, 6″-H), 1.13 (3H, t, 3-$OCOCH_2CH_3$), 1.13 (3H, t, 3″-$OCOCH_2CH_3$), 1.15 (3H, t, $NHCH_2CH_3$), 1.16 (3H, d, 6′-H), 1.46 (3H, s, 3″-CH_3), 1.68 (1H, dd, 2″-Hax), 2.30 (3H, s, 11-CH_3), 2.51 (6H, s, 3′-$N(CH_3)_2$), 2.63 (1H, dd, 2-H), 2.82 (1H, dd, 2-H), 2.90 (1H, dd, 6-CH_2), 3.21 (1H, d, 2″-Heq), 3.22 (1H, m, 5′-H), 3.39 (1H, dd, 2′-H), 3.63 (3H, s, 4-OCH_3), 3.85 (1H, br d, 5-H), 3.98 (1H, br d, 4-H), 4.36 (1H, d, 1′-H), 4.41 (1H, m, 4″-H), 4.44 (1H, m, 5″-H), 4.81 (1H, d, 1″-H), 5.12 (1H, m, 15-H), 5.55 (1H, m, 3-H), 6.22 (1H, dt, 15-CH_2CH═CH), 6.57 (1H, d, 15-CH_2CH═CH), 7.34–7.48 (4H, m, naphthalene), 7.53 (1H, dd, naphthalene), 7.65 (1H, s, naphthalene), 7.76 (1H, m, naphthalene), 9.63 (1H, s, CHO).

4.1.20. Synthesis of 8h

Reaction of **5h** with MeOH gave **7h** as a colorless solid in 69% yield by a similar procedure to **7a**. Subsequently, reaction of **7h** with difluoroacetic acid gave **8h** as a colorless solid in 78% yield by a similar procedure to **8a**; $[\alpha]_D^{26}$ −47° (*c* 0.82, $CHCl_3$); FAB-MS *m*/*z* 1057 $(M+H)^+$; FAB-HRMS: Calcd for $C_{55}H_{84}N_4O_{16}$: 1057.5961, Found: 1057.5958 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, d, 8-CH_3), 1.09 (3H, d, 6″-H), 1.17 (3H, d, 6′-H), 1.17 (3H, t, $NHCH_2CH_3$), 1.46 (3H, s, 3″-CH_3), 1.68 (1H, dd, 2″-Hax), 2.32 (3H, s, 11-CH_3), 2.52 (6H, s, 3′-$N(CH_3)_2$), 2.82 (1H, dd, 2-H), 2.90 (1H, dd, 6-CH_2), 3.20 (1H, dd, 2″-Heq), 3.37 (1H, dd, 2′-H), 3.60 (3H, s, 4-OCH_3), 3.85 (1H, br d, 5-H), 3.97 (1H, br d, 4-H), 4.37 (1H, d, 1′-H), 4.42 (1H, m, 4″-H), 4.43 (1H, m, 5″-H), 4.81 (1H, d, 1″-H), 5.17 (1H, m, 15-H), 5.55 (1H, m, 3-H), 6.35 (1H, dt, 15-CH_2CH═CH), 7.12 (1H, d, 15-CH_2CH═CH), 7.38 (1H, d, quinoline), 7.55 (1H, ddd, quinoline), 7.70 (1H, ddd, quinoline), 8.06 (1H, br d, quinoline), 8.08 (1H, br d, quinoline), 8.81 (1H, d, quinoline), 9.63 (1H, s, CHO).

4.1.21. Synthesis of 8i

Reaction of **5i** with MeOH gave **7i** as a colorless solid in 56% yield by a similar procedure to **7a**. Subsequently, reaction of **7i** with difluoroacetic acid gave **8i** as a colorless solid in 91% yield by a similar procedure to **8a**; $[\alpha]_D^{24}$ −44° (*c* 0.80, $CHCl_3$); FAB-MS *m*/*z* 1057 $(M+H)^+$; FAB-HRMS: Calcd for $C_{55}H_{84}N_4O_{16}$: 1057.5961, Found: 1057.5969 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, d, 8-CH_3), 1.09 (3H, d, 6″-H), 1.16 (3H, d, 6′-H), 1.17 (3H, t, $NHCH_2CH_3$), 1.46 (3H, s, 3″-CH_3), 1.68 (1H, dd, 2″-Hax), 2.33 (3H, s, 11-CH_3), 2.52 (6H, s, 3′-$N(CH_3)_2$), 2.83 (1H, dd, 2-H), 2.90 (1H, dd, 6-CH_2), 3.15 (1H, t, 4′-H), 3.21 (1H, d, 2″-Heq), 3.38 (1H, dd, 2′-H), 3.62 (3H, s, 4-OCH_3), 3.85 (1H, br d, 5-H), 3.97 (1H, br d, 4-H), 4.36 (1H, d, 1′-H), 4.39 (1H, d, 4″-H), 4.46 (1H, dq, 5″-H), 4.81 (1H, d, 1″-H), 5.16 (1H, m, 15-H), 5.55 (1H, m, 3-H), 6.19 (1H, dt, 15-CH_2CH═CH), 7.00 (1H, d, 15-CH_2CH═CH), 7.60 (1H, dt, isoquinoline), 7.72 (1H, dt, isoquinoline), 7.96 (1H, d, isoquinoline), 8.02 (1H, d, isoquinoline), 8.52 (1H, s, isoquinoline), 9.13 (1H, s, isoquinoline), 9.63 (1H, s, CHO).

4.1.22. Synthesis of 8j

Reaction of **5j** with MeOH gave **7j** as a colorless solid in 58% yield by a similar procedure to **7a**. Subsequently, reaction of **7j** with difluoroacetic acid gave **8j** as a colorless solid in 69% yield by a similar procedure to **8a**; $[\alpha]_D^{24}$ −47° (*c* 1.13, $CHCl_3$); FAB-MS *m*/*z* 1007 $(M+H)^+$; FAB-HRMS: Calcd for $C_{51}H_{82}N_4O_{16}$: 1007.5804, Found: 1007.5802 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, d, 8-CH_3), 1.10 (3H, d, 6″-H), 1.12 (3H, t, 3-OCOCH$_2$CH$_3$), 1.13 (3H, t, 3″-OCOCH$_2$CH$_3$), 1.16 (3H, t, NHCH$_2$CH$_3$), 1.17 (3H, d, 6′-H), 1.46 (3H, s, 3″-CH_3), 1.68 (1H, dd, 2″-Hax), 2.30 (3H, s, 11-CH_3), 2.52 (6H, s, 3′-N(CH_3)$_2$), 2.60 (1H, dd, 2-H), 2.81 (1H, dd, 2-H), 2.88 (1H, dd, 6-CH_2), 3.20 (1H, d, 2″-Heq), 3.38 (1H, dd, 2′-H), 3.64 (s, 4-OCH_3), 3.85 (1H, br d, 5-H), 3.95 (1H, br d, 4-H), 4.36 (1H, d, 1′-H), 4.43 (1H, m, 4″-H), 4.43 (1H, m, 5″-H), 4.81 (1H, d, 1″-H), 5.08 (1H, m, 15-H), 5.54 (1H, m, 3-H), 6.17 (1H, dt, 15-CH_2CH=CH), 6.40 (1H, d, 15-CH_2CH=CH), 7.20 (1H, dd, pyridine), 7.63 (1H, ddd, pyridine), 8.43 (1H, dd, pyridine), 8.52 (1H, br d, pyridine), 9.63 (1H, s, CHO).

4.1.23. Synthesis of 8k

Reaction of **5k** with MeOH gave **7k** as a colorless solid in 36% yield by a similar procedure to **7a**. Subsequently, reaction of **7k** with difluoroacetic acid gave **8k** as a colorless solid in 66% yield by a similar procedure to **8a**; $[\alpha]_D^{25}$ −37° (*c* 0.15, $CHCl_3$); FAB-MS *m*/*z* 1021 $(M+H)^+$; FAB-HRMS: Calcd for $C_{51}H_{83}N_5O_{16}$: 1022.5913, Found: 1022.5906 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.94 (3H, d, 8-CH_3), 1.11 (3H, d, 6″-H), 1.17 (3H, t, NHCH$_2$CH$_3$), 1.17 (3H, d, 6′-H), 1.46 (3H, s, 3″-CH_3), 2.32 (3H, s, 11-NCH_3), 2.53 (6H, s, 3′-N(CH_3)$_2$), 2.84 (1H, dd, 2-H), 2.89 (1H, dd, 6-CH_2), 3.20 (1H, d, 2″-Heq), 3.38 (1H, dd, 2′-H), 3.65 (3H, s, 4-OCH_3), 3.85 (1H, br d, 5-H), 3.93 (1H, br d, 4-H), 4.38 (1H, d, 1′-H), 4.43 (1H, m, 4″-H), 4.43 (1H, m, 5″-H), 4.82 (1H, d, 1″-H), 5.04 (1H, m, 15-H), 5.45 (1H, m, 3-H), 5.89 (1H, dt, 15-CH_2CH=CH), 6.28 (1H, d, 15-CH_2CH=CH), 6.45 (1H, d, pyridine), 7.47 (1H, dd, pyridine), 7.96 (1H, d, pyridine), 9.63 (1H, s, CHO).

4.1.24. Synthesis of 11

To a solution of **9**[13] (1.91 g, 2.00 mmol) in acetone (47 ml) and water (7.1 ml), *N*-methylmorpholine-*N*-oxide (940 μl, 4.01 mmol) and 4% aqueous osmium tetroxide (1.90 ml, 299 μmol) were added. After the reaction mixture was stirred for 23.5 h at room temperature, *N*-methylmorpholine-*N*-oxide (470 μl, 2.01 mmol) was added, and the mixture was further stirred for 7 h. After the reaction mixture was concentrated, EtOAc was added. The organic layer was successively washed with water, 5% aqueous $Na_2S_2O_3$ solution and brine. The extract was dried and concentrated to obtain crude **10** (2.10 g). To a solution of the crude **10** (200 mg) in benzene (3.0 ml) were added Na_2CO_3 (166 mg, 1.57 mmol) and lead tetraacetate (216 mg, 487 μmol). After the reaction mixture was stirred at room temperature for 15 min, the reaction mixture was filtered through Celite by using EtOAc (25 ml). To the filtrate was added 1,8-diazabicyclo[5.4.0]-7-undecene (45.0 μl, 302 μmol). After the solution was kept at room temperature for 30 min, the reaction mixture was concentrated to about 5 ml. The resulting solution containing **11** was purified by silica gel chromatography [$CHCl_3$/MeOH (80:1–20:1)] to afford **11** (75 mg, 44% in three steps from **9**) as a colorless solid; FAB-MS *m*/*z* 892 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.04 (3H, s, $SiCH_3$), 0.05 (3H, s, $SiCH_3$), 0.86 (9H, s, SiC(CH_3)$_3$), 1.02 (3H, d, 8-Me), 1.12 (3H, s, 3″-CH_3), 1.13 (3H, d, 6″-H), 1.18 (3H, t, 4″-OCOCH$_2$CH$_3$), 1.31 (3H, d, 6′-H), 1.44 (1H, br dd, 7-H), 1.68 (1H, m, 6-CH_2), 1.86 (1H, dd, 2″-Hax), 1.95 (1H, m, 6-H), 2.02 (1H, d, 2″-Heq), 2.05 (3H, s, 2′-$OCOCH_3$), 2.11 (1H, m, 6-CH_2), 2.17 (3H, s, 9-$OCOCH_3$), 2.36 (1H, m, 8-H), 2.41 (6H, s, 3′-N(CH_3)$_2$), 2.75 (1H, t, 3′-H), 2.82 (1H, dd, 2-H), 3.08 (1H, br d, 4-H), 3.37 (1H, t, 5′-H), 3.43 (3H, s, 4-OCH_3), 3.79 (1H, dd, 5-H), 4.01 (1H, br dd, 3-H), 4.40 (1H, dq, 5″-H), 4.42 (1H, d, 1′-H), 4.63 (1H, d, 4″-H), 4.93 (1H, d, 9-H), 5.00 (1H, m, CHOSi), 5.02 (1H, dd, 2′-H), 5.09 (1H, d, 1″-H), 9.58 (1H, s, CHO).

4.1.25. Synthesis of 12

To a solution of **11** (1.80 g, 2.02 mmol) and (*R*)-7-methylamino-1-hepten-4-ol[6c] (378 mg, 2.64 mmol) in DMF (36 ml), molecular sieves 3A (9.0 g) were added and the reaction mixture was stirred for 5 h at room temperature. Then, sodium borohydride (76 mg, 2.01 mmol) was added, and further stirred for 2 h at room temperature. The reaction mixture was filtered through Celite by using EtOAc. The filtrate was successively washed with water and brine. After the organic layer was dried and concentrated, the residue was purified by silica gel chromatography [$CHCl_3$/MeOH (50:1–15:1)] to afford seco acid of **12** (746 mg, 36%) as a pale yellow solid. Then, 2-methyl-6-nitrobenzoic anhydride (370 mg, 1.08 mmol) and 4-dimethylaminopyridine (262 mg, 2.15 mmol) were dissolved in THF (130 ml). A solution of the seco acid of **12** (730 mg, 716 μmol) in THF (90 ml) was dropwised over 2.5 h under ice cooling, and then stirred for 16 h at room temperature. Saturated aqueous ammonium chloride was added to the reaction mixture, and the mixture was concentrated. EtOAc was added to the resulting mixture, and the extract was washed with water and brine, dried and concentrated. The resulting residue was purified by silica gel column chromatography [hexane/EtOAc (2:1–1:1)] to afford **12** (487 mg, 68%) as a colorless solid; FAB-MS *m*/*z* 1001 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.11 (3H, s, $SiCH_3$), 0.12 (3H, s, $SiCH_3$), 0.63 (1H, br dd, 7-H), 0.89 (9H, s, SiC(CH_3)$_3$), 0.91 (3H, d, 8-CH_3), 1.12 (3H, s, 3″-CH_3), 1.13 (3H, d, 6″-H), 1.18 (3H, t, 4″-OCOCH$_2$CH$_3$), 1.28 (3H, d, 6′-H), 1.47 (1H, m, 13-H), 1.47 (1H, m, 6-CH_2), 1.62 (1H, m, 14-H), 1.85 (1H, dd, 2″-Hax), 2.02 (1H, d, 2″-Heq), 2.02 (3H, s, 9-$OCOCH_3$), 2.06 (3H, s, 2′-$OCOCH_3$), 2.15 (1H, br dd, 7-H), 2.26 (3H, s, 11-CH_3), 2.41 (6H, s, 3′-N(CH_3)$_2$), 2.59 (1H, dd, 2-H), 2.65 (1H, dd, 10-H), 2.76 (1H, t, 3′-H), 2.77 (1H, dd, 2-H), 3.02 (1H, dd, 4-H), 3.31 (1H, m, 4′-H), 3.32 (1H, m, 5′-H), 3.53 (3H, s, 4-OCH_3), 3.64 (1H, dd, 5-H), 4.12 (1H, br d, 3-H), 4.23 (1H, br s, 3″-OH), 4.41 (1H, dq, 5″-H), 4.54 (1H, d, 1′-H), 4.62 (1H, d, 4″-H), 4.86 (1H, m, 15-H), 4.90 (1H, dd, CHSi), 5.00 (1H, m, 9-H), 5.01 (1H, dd, 2′-H), 5.09 (1H, d, 1″-H), 5.10 (2H, m, CH=CH_2), 5.73 (1H, m, CH=CH_2).

4.1.26. Synthesis of 13a

Reaction of **12** with 3-bromoquinoline gave **13a** as a colorless solid in 53% yield by a similar procedure to **5a**; $[\alpha]_D^{23}$ −53° (*c* 0.50, $CHCl_3$); FAB-MS *m*/*z* 1128 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.11 (3H, s, $SiCH_3$), 0.12 (3H, s, $SiCH_3$), 0.66 (1H, br dd, 7-H), 0.89 (9H, s, SiC(CH_3)$_3$), 0.92 (3H, d, 8-CH_3), 1.12 (3H, s, 3″-CH_3), 1.13 (3H, d, 6″-H), 1.18 (3H, t, 4″-OCOCH$_2$CH$_3$), 1.29 (3H, d, 6′-H), 1.46 (1H, m, 6-CH_2), 1.85 (1H, dd, 2″-Hax), 2.02 (1H, d, 2″-Heq), 2.03 (3H, s, 9-$OCOCH_3$), 2.07 (3H, s, 2′-$OCOCH_3$), 2.15 (1H, br dd, 7-H), 2.29 (3H, s, 11-CH_3), 2.41 (6H, s, 3′-N(CH_3)$_2$), 2.55 (1H, dd, 2-H), 2.75 (1H, t, 3′-H), 2.79 (1H, dd, 2-H), 3.04 (1H, br d, 4-H), 3.31 (1H, m, 4′-H), 3.33 (1H, m, 5′-H), 3.54 (3H, s, 4-OCH_3), 3.64 (1H, dd, 5-H), 4.15 (1H, br dd, 3-H), 4.23 (1H, br s, 3″-OH), 4.41 (1H, dq, 5″-H), 4.54 (1H, d, 1′-H), 4.62 (1H, d, 4″-H), 4.93 (1H, dd, CHSi), 5.01 (1H, dd, 2′-H), 5.09 (1H, d, 1″-H), 6.36 (1H, dt, 15-CH_2CH=CH), 6.61 (1H, d, 15-CH_2CH=CH), 7.53 (1H, ddd, quinoline), 7.67 (1H, ddd, quinoline), 7.80 (1H, br d, quinoline), 8.02 (1H, d, quinoline), 8.07 (1H, br d, quinoline), 8.95 (1H, d, quinoline).

4.1.27. Synthesis of 13b

Reaction of **12** with 4-bromoisoquinoline gave **13b** as a colorless solid in 80% yield by a similar procedure to **5a**; $[\alpha]_D^{24}$ −70° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1128 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.11 (3H, s, $SiCH_3$), 0.12 (3H, s, $SiCH_3$), 0.67 (1H, br dd, 7-H), 0.89 (9H, s, SiC(CH_3)$_3$), 0.92 (3H, d, 8-CH_3), 1.12 (3H, s, 3″-CH_3), 1.13 (3H, d, 6″-H), 1.18 (3H, t, 4″-OCOCH$_2$CH$_3$), 1.29 (3H, d, 6′-H), 1.49 (1H, dd, 6-CH_2), 1.85 (1H, dd, 2″-Hax), 1.94 (1H, d, 2″-Heq), 2.03 (3H, s, 9-$OCOCH_3$), 2.08 (3H, s, 2′-$OCOCH_3$), 2.32 (3H, s, 11-CH_3), 2.41 (6H, s, 3′-N(CH_3)$_2$), 2.62 (1H, dd, 2-H), 2.78 (1H, dd, 2-H), 2.78 (1H, t, 3′-H), 3.05 (1H, br d, 4-H), 3.31 (1H, m, 4′-H), 3.33

(1H, m, 5′-H), 3.54 (3H, s, 4-OCH_3), 3.65 (1H, dd, 5-H), 4.16 (1H, br dd, 3-H), 4.22 (1H, br s, 3″-OH), 4.41 (1H, dq, 5″-H), 4.54 (1H, d, 1′-H), 4.62 (1H, d, 4″-H), 4.93 (1H, br d, CHOSi), 5.01 (1H, dd, 2′-H), 5.09 (1H, d, 1″-H), 6.22 (1H, dt, 15-CH_2CH=CH), 7.05 (1H, d, 15-CH_2CH=CH), 7.62 (1H, dd, isoquinoline), 7.73 (1H, dd, isoquinoline), 7.98 (1H, d, isoquinoline), 8.04 (1H, d, isoquinoline), 8.56 (1H, s, isoquinoline), 9.15 (1H, s, isoquinoline).

4.1.28. Synthesis of 14a

Reaction of **13a** with ethylisocyanate gave **14a** as a colorless solid in 85% yield by a similar procedure to **3** (method A); $[\alpha]_D^{24}$ −77° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1199 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.11 (3H, s, $SiCH_3$), 0.12 (3H, s, $SiCH_3$), 0.64 (1H, br dd, 7-H), 0.89 (9H, s, $SiC(CH_3)_3$), 0.91 (3H, d, 8-CH_3), 1.12 (3H, d, 6″-H), 1.14 (3H, t, 3″-$OCOCH_2CH_3$), 1.16 (3H, t, $NHCH_2CH_3$), 1.21 (3H, d, 6′-H), 1.47 (3H, s, 3″-CH_3), 1.69 (1H, dd, 2″-Hax), 2.02 (3H, s, 9-$OCOCH_3$), 2.06 (3H, s, 2′-$OCOCH_3$), 2.15 (1H, m, 7-H), 2.27 (3H, s, 11-CH_3), 2.42 (6H, s, 3′-$N(CH_3)_2$), 2.55 (1H, t, 3′-H), 2.59 (1H, dd, 2-H), 2.77 (1H, dd, 2-H), 3.06 (1H, br d, 4-H), 3.11 (1H, m, 4′-H), 3.14 (1H, m, 5′-H), 3.20 (1H, d, 2″-Heq), 3.24 (2H, dq, $NHCH_2CH_3$), 3.52 (3H, s, 4-OCH_3), 3.62 (1H, dd, 5-H), 4.14 (1H, br dd, 3-H), 4.41 (1H, d, 4″-H), 4.41 (1H, dq, 5″-H), 4.48 (1H, d, 1′-H), 4.81 (1H, d, 1″-H), 4.91 (1H, m, CHOSi), 4.94 (1H, dd, 2′-H), 5.02 (1H, m, 9-H), 6.36 (1H, dt, 15-CH_2CH=CH), 6.61 (1H, d, 15-CH_2CH=CH), 7.53 (1H, ddd, quinoline), 7.66 (1H, ddd, quinoline), 7.79 (1H, br d, quinoline), 8.02 (1H, d, quinoline), 8.07 (1H, br d, quinoline), 8.95 (1H, d, quinoline).

4.1.29. Synthesis of 14b

Reaction of **13b** with ethylisocyanate gave **14b** as a colorless solid in 78% yield by a similar procedure to **3** (method A); $[\alpha]_D^{22}$ −75° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1199 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.11 (3H, s, $SiCH_3$), 0.12 (3H, s, $SiCH_3$), 0.64 (1H, br dd, 7-H), 0.89 (9H, s, $SiC(CH_3)_3$), 0.92 (3H, d, 8-CH_3), 1.12 (3H, t, 3″-$OCOCH_2CH_3$), 1.13 (3H, d, 6″-H), 1.16 (3H, t, $NHCH_2CH_3$), 1.21 (3H, d, 6′-H), 1.48 (3H, s, 3″-CH_3), 1.69 (1H, dd, 2″-Hax), 2.28 (3H, s, 11-CH_3), 2.42 (6H, s, 3′-$N(CH_3)_2$), 2.59 (1H, t, 3′-H), 2.77 (1H, dd, 2-H), 3.07 (1H, br d, 4-H), 3.53 (3H, s, 4-OCH_3), 3.63 (1H, dd, 5-H), 4.16 (1H, br dd, 3-H), 4.41 (1H, m, 4″-H), 4.44 (1H, m, 5″-H), 4.49 (1H, d, 1′-H), 4.81 (1H, d, 1″-H), 4.88 (1H, t, NH), 4.93 (1H, m, CHOSi), 4.96 (1H, dd, 2′-H), 5.00 (1H, m, 15-H), 5.02 (1H, m, 9-H), 6.22 (1H, dt, 15-CH_2CH=CH), 7.05 (1H, d, 15-CH_2CH=CH), 7.62 (1H, ddd, isoquinoline), 7.73 (1H, ddd, isoquinoline), 7.98 (1H, br d, isoquinoline), 8.03 (1H, br d, isoquinoline), 8.56 (1H, s, isoquinoline), 9.15 (1H, s, isoquinoline).

4.1.30. Synthesis of 15

Reaction of **12** with ethylisocyanate gave **15** as a colorless solid in 93% yield by a similar procedure to **3** (method A); $[\alpha]_D^{21}$ −66° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1072 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.11 (3H, s, $SiCH_3$), 0.12 (3H, s, $SiCH_3$), 0.63 (1H, dd, 7-H), 0.89 (9H, s, $SiC(CH_3)_3$), 0.91 (3H, d, 8-CH_3), 1.12 (3H, d, 6″-H), 1.14 (3H, t, 3″-$OCOCH_2CH_3$), 1.16 (3H, t, $NHCH_2CH_3$), 1.20 (3H, d, 6′-H), 1.47 (3H, s, 3″-CH_3), 1.68 (1H, dd, 2″-Hax), 2.01 (3H, s, 9-$OCOCH_3$), 2.06 (3H, s, 2′-$OCOCH_3$), 2.29 (3H, s, 11-CH_3), 2.42 (6H, s, 3′-$N(CH_3)_2$), 2.74 (1H, dd, 2-H), 3.03 (1H, br d, 4-H), 3.20 (1H, d, 2″-Heq), 3.52 (3H, s, 4-OCH_3), 3.62 (1H, dd, 5-H), 4.11 (1H, m, 3-H), 4.39 (1H, d, 4″-H), 4.44 (1H, dq, 5″-H), 4.48 (1H, d, 1′-H), 4.81 (1H, d, 1″-H), 4.84 (1H, m, 15-H), 4.92 (1H, m, CHOSi), 4.95 (1H, dd, 2′-H), 5.02 (1H, m, 9-H), 5.08 (2H, m, $CH=CH_2$), 5.72 (1H, m, $CH=CH_2$).

4.1.31. Synthesis of 16a

Reaction of **14a** with MeOH gave the deacetylated analogue of **14a** as a colorless solid in 23% yield by a similar procedure to **7a**. Subsequently, reaction of this compound with difluoroacetic acid gave **16a** as a colorless solid in 87% yield by a similar procedure to **8a**; $[\alpha]_D^{23}$ −53° (*c* 0.50, $CHCl_3$); FAB-MS *m*/*z* 1001 $(M+H)^+$; FAB-HRMS: Calcd for $C_{52}H_{80}N_4O_{15}$: 1001.5698, Found: 1001.5696 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, d, 8-CH_3), 1.14 (3H, d, 6″-H), 1.14 (3H, t, 3″-$OCOCH_2CH_3$), 1.16 (3H, t, $NHCH_2CH_3$), 1.17 (3H, d, 6′-H), 1.47 (3H, s, 3″-CH_3), 1.51 (1H, m, 8-H), 1.70 (1H, dd, 2″-Hax), 2.31 (3H, s, 11-CH_3), 2.41 (1H, dd, 6-CH_2), 2.53 (6H, s, 3′-$N(CH_3)_2$), 2.94 (1H, dd, 6-CH_2), 3.21 (1H, d, 2″-Heq), 3.25 (1H, m, 5′-H), 3.38 (1H, m, 9-H), 3.40 (1H, dd, 2′-H), 3.60 (3H, s, 4-OCH_3), 3.64 (1H, br d, 4-H), 3.89 (1H, br d, 5-H), 4.35 (1H, d, 1′-H), 4.38 (1H, m, 3-H), 4.42 (1H, m, 4″-H), 4.45 (1H, m, 5″-H), 4.80 (1H, t, NH), 4.83 (1H, d, 1″-H), 5.12 (1H, m, 15-H), 6.38 (1H, dt, 15-CH_2CH=CH), 6.61 (1H, d, 15-CH_2CH=CH), 7.52 (1H, ddd, quinoline), 7.66 (1H, ddd, quinoline), 7.79 (1H, br d, quinoline), 8.02 (1H, d, quinoline), 8.06 (1H, br d, quinoline), 8.95 (1H, d, quinoline), 9.73 (1H, s, CHO).

4.1.32. Synthesis of 16b

Reaction of **14b** with MeOH gave the deacetylated analogue of **14b** as a colorless solid in 63% yield by a similar procedure to **7a**. Subsequently, reaction of this compound with difluoroacetic acid gave **16b** as a colorless solid in 72% yield by a similar procedure to **8a**; $[\alpha]_D^{25}$ −54° (*c* 1.0, $CHCl_3$); FAB-MS *m*/*z* 1001 $(M+H)^+$; FAB-HRMS: Calcd for $C_{52}H_{80}N_4O_{15}$: 1001.5698, Found: 1001.5696 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, d, 8-CH_3), 1.14 (3H, d, 6″-H), 1.14 (3H, t, 3″-$OCOCH_2CH_3$), 1.16 (3H, t, $NHCH_2CH_3$), 1.17 (3H, d, 6′-H), 1.24 (1H, br d, 7-H), 1.47 (3H, s, 3″-CH_3), 1.53 (1H, m, 8-H), 1.69 (1H, dd, 2″-Hax), 1.80 (1H, m, 14-H), 2.35 (3H, s, 11-CH_3), 2.42 (1H, dd, 6-CH_2), 2.52 (6H, s, 3′-$N(CH_3)_2$), 2.94 (1H, dd, 6-CH_2), 3.15 (1H, t, 4′-H), 3.21 (1H, d, 2″-Heq), 3.41 (1H, dd, 2′-H), 3.59 (3H, s, 4-OCH_3), 3.66 (1H, br d, 4-H), 3.88 (1H, br d, 5-H), 4.34 (1H, d, 1′-H), 4.41 (1H, d, 4″-H), 4.46 (1H, dq, 5″-H), 4.82 (1H, d, 1″-H), 5.16 (1H, m, 15-H), 6.23 (1H, dt, 15-CH_2CH=CH), 7.03 (1H, d, 15-CH_2CH=CH), 7.61 (1H, ddd, isoquinoline), 7.73 (1H, ddd, isoquinoline), 7.97 (1H, br d, isoquinoline), 8.04 (1H, br d, isoquinoline), 8.54 (1H, s, isoquinoline), 9.14 (1H, s, isoquinoline), 9.74 (1H, s, CHO).

4.1.33. Synthesis of 16c

Reaction of **15** with 2-amino-5-bromopyridine gave crude **14c** as a colorless solid by a similar procedure to **5a**. This compound (22 mg) was dissolved in methanol (2.0 ml). After the mixture was stirred for 15 h at room temperature, the reaction mixture was concentrated. The resulting residue was dissolved in acetonitrile (0.4 ml), and 1 M hydrochloric acid (0.4 ml, 400 μmol) was added. After the mixture was stirred for 5.5 h at room temperature, saturated aqueous $NaHCO_3$ was added to the reaction mixture. The aqueous layer was extracted with EtOAc and the extract was washed with brine. The organic layer was dried and concentrated to give a residue which was purified by preparative TLC [$CHCl_3$/MeOH/NH_4OH (5:1:0.1)] to afford **16c** (11 mg, 7.4% in three steps) as a colorless solid; $[\alpha]_D^{19}$ −69° (*c* 0.48, $CHCl_3$); FAB-MS *m*/*z* 966 $(M+H)^+$; FAB-HRMS: Calcd for $C_{48}H_{79}N_5O_{15}$: 966.5651, Found: 966.5643 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, d, 8-CH_3), 1.13 (3H, t, 3″-$OCOCH_2CH_3$), 1.16 (3H, t, $NHCH_2CH_3$), 1.17 (3H, d, 6′-H), 1.46 (3H, s, 3″-CH_3), 1.68 (1H, dd, 2″-Hax), 2.33 (3H, s, 11-CH_3), 2.51 (6H, s, 3′-$N(CH_3)_2$), 2.66 (1H, dd, 2-H), 2.93 (1H, dd, 6-CH_2), 3.16 (1H, t, 4′-H), 3.20 (1H, d, 2″-Heq), 3.39 (1H, dd, 2′-H), 3.59 (3H, s, 4-OCH_3), 3.63 (1H, br d, 4-H), 3.86 (1H, br d, 5-H), 4.34 (1H, d, 1′-H), 4.34 (1H, m, 3-H), 4.39 (1H, d, 4″-H), 4.44 (1H, dq, 5″-H), 4.81 (1H, d, 1″-H), 5.12 (1H, m, 15-H), 5.92 (1H, dt, 15-CH_2CH=CH), 6.29 (1H, d, 15-CH_2CH=CH), 6.45 (1H, d, pyridine), 7.48 (1H, dd, pyridine), 7.96 (1H, d, pyridine), 9.73 (1H, s, CHO).

4.1.34. Synthesis of 20a and 20b

Reaction of **15** with 4-bromo-1-[2-(trimethylsilyl)ethoxycarbonylamino]isoquinoline (**17**) gave **18** as a colorless solid in 54%

yield by a similar procedure to **5a**. Subsequently, reaction of **18** with MeOH gave **19** as a colorless solid in 50% yield by a similar procedure to **7a**. This compound (119 mg, 93.1 μmol) was dissolved in THF (2.4 ml), and 1 M tetrabutylammonium fluoride tetrahydrofuran solution (279 μl, 279 μmol) was added. After the reaction mixture was stirred for 15 min at room temperature, EtOAc and water were added to the mixture, and the extract was washed with brine. After the organic layer was dried and concentrated, the residue was purified by preparative TLC [$CHCl_3$/MeOH/NH_4OH (8:1:0.1)] to afford **20a** (50 mg, 46%) as a colorless solid and **20b** (34 mg, 31%) as a colorless solid; **20a**: FAB-MS *m*/*z* 1160 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.07 (9H, s, $Si(CH_3)_3$), 0.90 (3H, d, 8-CH_3), 1.14 (3H, d, 6″-H), 1.14 (3H, t, 3″-$OCOCH_2CH_3$), 1.16 (3H, t, $NHCH_2CH_3$), 1.47 (3H, s, 3″-CH_3), 1.51 (1H, m, 8-H), 1.69 (1H, dd, 2″-Hax), 2.38 (3H, s, 11-CH_3), 2.53 (6H, s, 3′-$N(CH_3)_2$), 2.97 (1H, dd, 6-CH_2), 3.22 (1H, m, 5′-H), 3.23 (1H, d, 2″-Heq), 3.25 (1H, m, 5′-H), 3.40 (1H, dd, 2′-H), 3.59 (3H, s, 4-OCH_3), 3.86 (1H, br d, 4-H), 4.35 (1H, d, 1′-H), 4.38 (1H, m, 3-H), 4.42 (1H, m, 4″-H), 4.45 (1H, m, 5″-H), 4.83 (1H, d, 1″-H), 5.12 (1H, m, 15-H), 6.71 (1H, dt, 15-CH_2CH═CH), 6.69 (1H, d, 15-CH_2CH═CH), 7.30 (1H, m, isoquinoline), 7.60 (1H, m, isoquinoline), 7.77 (1H, m, isoquinoline), 8.03 (1H, m, isoquinoline), 8.24 (1H, s, isoquinoline), 9.75 (1H, s, CHO). **20b**: FAB-MS *m*/*z* 1160 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.07 (9H, s, $Si(CH_3)_3$), 0.89 (3H, d, 8-CH_3), 1.10 (3H, d, 6″-H), 1.15 (3H, t, 3″-$OCOCH_2CH_3$), 1.16 (3H, t, $NHCH_2CH_3$), 1.47 (3H, s, 3″-CH_3), 1.51 (1H, m, 8-H), 1.70 (1H, dd, 2″-Hax), 2.37 (3H, s, 11-CH_3), 2.52 (6H, s, 3′-$N(CH_3)_2$), 2.95 (1H, dd, 6-CH_2), 3.20 (1H, m, 5′-H), 3.21 (1H, d, 2″-Heq), 3.24 (1H, m, 5′-H), 3.40 (1H, dd, 2′-H), 3.63 (3H, s, 4-OCH_3), 3.85 (1H, br d, 4-H), 4.35 (1H, d, 1′-H), 4.38 (1H, m, 3-H), 4.30 (1H, m, 5″-H), 4.41 (1H, m, 4″-H), 4.83 (1H, d, 1″-H), 5.30 (1H, m, 15-H), 5.59 (1H, dt, 15-CH═CH), 6.00 (1H, d, 15-CH═CH), 7.15 (1H, m, isoquinoline), 7.60 (1H, m, isoquinoline), 7.74 (1H, m, isoquinoline), 7.92 (1H, m, isoquinoline), 8.10 (1H, s, isoquinoline), 9.75 (1H, s, CHO).

4.1.35. Synthesis of 16d

To a solution of **20a** (50 mg, 43.1 μmol) in DMF (1.0 ml) were added potassium fluoride (25 mg, 431 μmol) and 18-crown-6 (114 mg, 431 μmol). After the reaction mixture was stirred for 7 h at room temperature, EtOAc and water were added, and the extract was washed with brine. The organic layer was dried and concentrated to give a residue which was purified by preparative TLC [$CHCl_3$/MeOH/NH_4OH (3:1:0.1)] to afford **16d** (35.5 mg, 81%) as a colorless solid; $[\alpha]_D^{24}$ −44° (*c* 0.30, $CHCl_3$); FAB-MS *m*/*z* 1016 $(M+H)^+$; FAB-HRMS: Calcd for $C_{52}H_{81}N_5O_{15}$: 1016.5807, Found: 1016.5802 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, d, 8-CH_3), 1.12 (3H, d, 6″-H), 1.13 (3H, t, 3″-$OCOCH_2CH_3$), 1.16 (3H, t, $NHCH_2CH_3$), 1.17 (3H, d, 6′-H), 1.46 (3H, s, 3″-CH_3), 1.69 (1H, dd, 2″-Hax), 2.32 (3H, s, 11-CH_3), 2.52 (6H, s, 3′-$N(CH_3)_2$), 2.67 (1H, dd, 2-H), 2.92 (1H, dd, 6-CH_2), 3.16 (1H, t, 4′-H), 3.21 (1H, d, 2″-Heq), 3.41 (1H, dd, 2′-H), 3.59 (3H, s, 4-OCH_3), 3.67 (1H, br d, 4-H), 3.88 (1H, br d, 5-H), 4.34 (1H, d, 1′-H), 4.40 (1H, d, 4″-H), 4.45 (1H, dq, 5″-H), 4.81 (1H, d, 1″-H), 5.11 (1H, m, 15-H), 6.01 (1H, dt, 15-CH_2CH═CH), 6.86 (1H, d, 15-CH_2CH═CH), 7.51 (1H, ddd, isoquinoline), 7.67 (1H, ddd, isoquinoline), 7.81 (1H, br d, isoquinoline), 7.93 (1H, br d, isoquinoline), 7.97 (1H, s, isoquinoline), 9.73 (1H, s, CHO).

4.2. Biology

4.2.1. In vitro antibacterial activity

Minimum inhibitory concentration (MIC) was determined by the agar dilution method. Test strains were subjected to seed culture using Sensitivity test broth (STB, Nissui Pharmaceutical) for *Staphylococcus aureus*, or cultured on blood agar plate for *S. pneumoniae, S. pyogenes* and *H. influenzae*. A 5 μl portion of cell suspension of the test strains having about 10^6 CFU/ml was inoculated into Sensitivity disk agar (SDA, 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.

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16. In vitro metabolic stability of the representative azalides and controls in human liver S9 were preliminary evaluated. The remaining percentage of parent compounds was measured after incubation for 1 h at 37 °C. The remaining percentages of **8a**, **8d**, **8g**, **1a**, MOM, and TEL were 79%, 78%, 82%, 28%, 5.1%, and 84%, respectively. The metabolic stability of the 4″-*O*-carbamoyl azalides (**8a**, **8d**, and **8g**) was definitely superior to that of 4″-*O*-acyl compounds (**1a** and MOM).