4'''-*N*-Demethylspiramycin Derivatives: Synthesis and Evaluation of Effectiveness against Drug-resistant Bacteria

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Abstract 18-Amino-4''-*O*-benzoyl-4'''-*N*-demethyl-18-deoxospiramycins were designed and synthesized. Synthetic strategy involved selective demethylation of the dimethylamino group in forosamine, benzylation of the hydroxyl group at the C4'' position and reductive *N*-amination of the formyl group. Antibacterial characteristics of spiramycin derivatives were tested. The derivatives exhibited promising activity against drug-resistant bacterial strains.

Keywords spiramycin, MRSA, demethylation, reductive *N*-amination

Introduction

Spiramycin I (**1**) is a 16-membered-ring macrolide with two aminosugars, mycaminose and forosamine together with one neutral sugar mycarose (Fig. 1). It shows antibacterial activities against Gram-positive bacteria and mycoplasmas [1]. Our group has continuously studied the synthesis and biological activity of spiramycin derivatives to elucidate their structure-activity relationships [2–7]. Recently, the spiramycin derivatives in our chemical library have been re-investigated in the course of our screening for antibacterial agents. The spiramycin derivative **2a** [4], one of by-products in the silylacetal formation, was found to show

moderate activity for drug-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), whereas the parent compound **1** is inactive. Structurally, **2a** has 3,18-(*O*-silyl)acetal, 4''-*O*-TBDMS and 4'''-methylamino groups. However, the 3,18-(*O*-silyl)acetal and 4''-*O*-TBDMS groups are not appropriate for medicinal uses because of their lability under acid conditions. Nevertheless, we found that the TBDMS ether **2a** can be converted to esters **2b** and **2c**, which maintain activity against MRSA strains [8]. Moreover, acetylene compound **2c** is useful for producing 1,2,3-triazole analogues *via* click chemistry [9]. If the remaining silylacetal moiety in **2** is converted to other functional groups without reducing activity, it could be a promising lead compound to combat drug-resistant bacteria. Herein, we report the design, synthesis and antibacterial activity of 4'''-*N*-demethylspiramycin derivatives **3** bearing various amines instead of the 3,18-(*O*-silyl)acetal moiety found in **2**.

Chemistry

Synthesis of spiramycin derivatives **3** is summarized in Scheme 1. Selective 2'-acetylation of **1** with acetic anhydride yielded 2'-*O*-acetylspiramycin **4**, which was converted to 4''-benzoyl compound **5** in 72% yield through treatment with benzoic acid and DCC in the presence of catalytic DMAP. In this acylation step, the use of benzoic anhydride or benzoyl chloride as acylating agents gave lower yields of **5**, accompanied by 3,4''-dibenzoyl derivative. Next, we attempted selective *N*-demethylation of the 4'''-dimethylamino group in **5** using our method previously reported [6]. However, *N*-demethylation with NBS and NaN₃ caused decomposition of **5**. After several attempts, the reaction of **5** with FmocCl and NaHCO₃ in EtOAc under reflux smoothly proceeded to provide 4'''-

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Fig. 1 Structures of spiramycin I (**1**), and compounds **2a**, **2b**, **2c** and **3**.

Fmoc protected spiramycin **6** in quantitative yield via *N*-demethylation of the 4''-dimethylamino group without formation of 3'-Fmoc protected spiramycin. The selectivity of 4''-*N*-demethylation is due to less steric hindrance around the dimethylamino group of forosamine compared to that of mycaminoside. It is noted that benzylation of the 4''-hydroxyl group did not work when **4** was protected with Fmoc group in advance. This result indicates that introduction of 4''-*N*-Fmoc group causes a conformational change that forosamine is close to mycarose, and then the Fmoc group shields around 4''-hydroxyl group in mycarose from attack of acylating agents. The formyl group of **6** was treated with a variety of amines in the presence of NaBH(OAc)₃ and AcOH to afford amine compounds **7** in high yields [10]. Finally, deprotection of the Fmoc group with piperidine, followed by methanolysis of the acetyl group provided spiramycin derivatives **3** in good yields. DBU can be used for deprotection of the Fmoc group without decomposition of the spiramycin skeleton.

Results and Discussion

The *in vitro* antibacterial activities of spiramycin derivatives **3** were tested by the standard agar dilution method against *S. aureus* FDA209P (susceptible strain), *S. aureus* Smith (susceptible strain), MRSA HH-1 (methicillin-resistant *S. aureus*), MRSA HH-1 HR-1 (methicillin-resistant *S. aureus*), MRSA 70 (methicillin-resistant *S. aureus*), MRSA 92-1191 (methicillin-resistant

S. aureus), *S. aureus* ISP447 (inducibly resistant strain), *S. aureus* ISP217 (constitutively resistant strain), *S. aureus* 8325 (pEP2104) (encoded by *erm* gene), *S. aureus* 8325 (pMS97) (encoded by *erm* and *mef* genes) shown in Table 1.

Spiramycin I (**1**) displayed antibacterial activity against *S. aureus* FDA209P and *S. aureus* Smith with an MIC value of 2.0 and 4.0 µg/ml respectively, but no activity for MRSA strains. Dibenzylamino spiramycin **3a** was less active against EM susceptible strains compared to **1**, while **3a** showed antibacterial activity against MRSA strains with MIC values of 8.0~16 µg/ml. However, **3a** showed weak activity compared to vancomycin. Compound **3b** was slightly less active than **3a**. These results indicate that the replacement of the 3,18-(*O*-silyl)acetal moiety in **2a** and **2b** with a suitable amino group at C18 does not reduce activity significantly. Exceptionally, monobenzylamino spiramycin **3c** lost activity against all tested strains. The phenyl group seems to have a crucial effect on antibacterial activity because pyridine derivatives **3d** and **3e** were inactive against all susceptible and resistant strains. The significance of the position and orientation of the phenyl group is emphasized because **3c** and **3f** having the phenyl group showed no activity. Furthermore, indoline **3g** and tetrahydroquinoline **3i** displayed moderate activity against MRSA strains, whereas isoindoline **3h** and tetrahydroisoquinoline **3j** diminished activity against all tested strains. To examine the effect of the three functional groups in **3g** on anti-MRSA activity, spiramycin derivatives **8**, **9** and **10** were prepared (Fig. 2). Surprisingly, **8**, **9** and **10**

a) Ac_2O , acetone, rt, 5 hours, quant.; b) Benzoic acid, DCC, DMAP, CH_2Cl_2 , $0^\circ C \sim rt$, 20 hours, 72%; c) FmocCl, $NaHCO_3$, EtOAc, reflux, 3.5 hours, quant.; d) amine, AcOH, $NaBH(OAc)_3$, $CHCl_3$, rt, 81%~quant.; e) piperidine, EtOAc, rt or DBU, EtOAc, rt; f) MeOH, reflux, 60%~quant. in 2 steps; DCC = *N,N'*-dicyclohexylcarbodiimide, DMAP = 4-(dimethylamino)pyridine, Fmoc = 9-Fluorenyl-methyloxycarbonyl.

Scheme 1 Synthesis of spiramycin derivatives **3**.

showed no antibacterial activity, suggesting that combination of the 18-*N*-indolino, 4''-*O*-benzoyl and 4''-*N*-methylamino groups bestows effectiveness against drug-resistant strains.

Conclusion

In conclusion, we have demonstrated the synthesis and antibacterial activity of 18-amino-4''-*O*-benzoyl-4''-*N*-demethyl-18-deoxospiramycins. The structural features of the potent compound, **3g** include the 18-*N*-indolino, 4''-*O*-

benzoyl and 4''-*N*-methylamino groups. Although **3a**, **3b**, **3g** and **3i** are less potent than vancomycin (MIC: MRSA strains; $0.5 \sim 1.0 \mu g/ml$), this result may offer useful information to help design 16-membered-ring macrolide derivatives with effectiveness against drug-resistant strains. Based on these results, further spiramycin derivatives are being investigated. The *in vivo* biological activity will be reported elsewhere.

Table 1. *In vitro* antibacterial activities of spiramycin derivatives and vancomycin

Strain/Compound	MIC ($\mu\text{g/ml}$)														
	SPM	VCM	2a	2b	2c	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j
<i>S. aureus</i> FDA209P ¹⁾	2	1	4	8	16	8	16	128	128	>64	64	8	>64	8	128
<i>S. aureus</i> Smith ²⁾	4	1	4	16	16	16	—	>128	>128	>64	128	8	>64	16	>128
MRSA HH-1 ³⁾	>256	≤ 0.5	4	8	16	8	8	>128	>128	>64	64	4	>64	8	128
MRSA HH-1 HR-1 ⁴⁾	>256	≤ 0.5	4	8	16	16	16	>128	>128	>64	64	4	>64	8	128
MRSA 70 ⁵⁾	>256	≤ 0.5	4	8	16	8	16	>128	>128	>64	64	64	>64	8	128
MRSA 92-1191 ⁶⁾	—	1	4	16	32	16	32	>128	>128	>64	128	4	>64	8	>128
<i>S. aureus</i> ISP447 ⁷⁾	8	1	4	16	32	16	32	>128	>128	>64	128	8	>64	16	>128
<i>S. aureus</i> ISP217 ⁸⁾	>256	≤ 0.5	4	8	16	8	32	>128	>128	>64	64	8	>64	8	64
<i>S. aureus</i> 8325 (pEP2104) ⁹⁾	4	1	>64	8	8	>128	>64	>128	32	≤ 0.5	64	2	>128	8	64
<i>S. aureus</i> 8325 (pMS97) ¹⁰⁾	4	1	>64	8	8	>128	>64	>128	8	1	64	2	32	8	128

¹⁾ *Staphylococcus aureus* FDA209P (susceptible strain), ²⁾ *S. aureus* Smith (susceptible strain), ³⁾ MRSA HH-1 (methicillin-resistant *S. aureus*), ⁴⁾ MRSA HH-1 HR-1 (methicillin-resistant *S. aureus*), ⁵⁾ MRSA 70 (methicillin-resistant *S. aureus*), ⁶⁾ MRSA 92-1191 (methicillin-resistant *S. aureus*), ⁷⁾ *S. aureus* ISP447 (inducibly resistant strain), ⁸⁾ *S. aureus* ISP217 (constitutively resistant strain), ⁹⁾ *S. aureus* 8325 (pEP2104) (encoded by *erm* gene), ¹⁰⁾ *S. aureus* 8325 (pMS97) (encoded by *erm* and *mef* genes).

SPM: spiramycin; VCM: vancomycin.

Fig. 2 Structures of spiramycin derivatives **8**, **9** and **10**.

Experimental

Reagents were purchased at highest commercial quality and used without further purification, unless specified otherwise. Reactions were monitored by TLC using Merck F60₂₅₄ silica gel plates. The plates were visualized with UV light and stained with phosphomolybdic acid. Silica gel chromatography was performed on Merck Kieselgel 60 (Art. 1.09385).

FT-IR spectra were recorded on a Horiba FT-210 spectrometer. Mass spectra were recorded on a JEOL JMS-AX505 HA Mass Spectrometer. ¹H-NMR spectra were recorded on a JEOL JNM-EX270 spectrometer in CDCl₃, CD₃OD or pyridine-*d*₅. ¹H-NMR spectral data are reported as follows: chemical shifts relative to chloroform (7.26 ppm), multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad), coupling and

integration.

2'-O-Acetylsiramycin I (**4**)

To a solution of spiramycin I (**1**) (6.70 g, 7.95 mmol) in Me₂CO (9.9 ml) was added acetic anhydride (2.25 ml, 23.8 mmol), and the mixture was stirred at room temperature. After stirring for 5 hours, the solvent was concentrated under reduced pressure. The residue was diluted with CHCl₃ and washed with saturated aq NaHCO₃ and H₂O. The organic layer was dried over anhydrous Na₂SO₄, filtered and evaporated to give **4** (7.03 g, quant.) as a colorless powder.

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₄₅H₇₇N₂O₁₅: 885.5324 [M+H], found: *m/z* 885.5328 [M+H]⁺.

IR ν_{max} (KBr) cm⁻¹: 3466, 2935, 1726.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 9.73 (s, 1H, H-18), 6.17 (dd, *J*=14.9, 10.5 Hz, 1H, H-11), 5.91

(dd, $J=14.9$, 9.7 Hz, 1H, H-12), 5.61 (dd, $J=15.1$, 9.5 Hz, 1H, H-10), 5.48 (ddd, $J=15.7$, 13.5 , 2.7 Hz, 1H, H-13), 5.21 (m, 1H, H-15), 4.98 (d, $J=3.0$ Hz, 1H, H-1''), 4.90 (dd, $J=10.3$, 7.6 Hz, 1H, H-2'), 4.60 (d, $J=7.8$ Hz, 1H, H-1'), 4.30 (d, $J=8.4$ Hz, 1H, H-1'''), 4.05~3.94 (m, 3H, H-5, H-9, H-5''), 3.71 (d, $J=11.3$ Hz, 1H, H-3), 3.38 (s, 3H, 4-OCH₃), 3.34 (m, 1H, H-5'''), 3.23~3.21 (m, 2H, H-4', H-5'), 2.92 (d, $J=8.9$ Hz, 1H, H-4), 2.86 (d, $J=10.0$ Hz, 1H, H-4''), 2.32 (s, 3H, 3'-N(CH₃)₂), 2.30 (s, 3H, 3'-N(CH₃)₂), 2.18 (s, 6H, 4'''-N(CH₃)₂), 1.96 (s, 3H, 2'-OCOCH₃), 1.23 (d, $J=6.5$ Hz, 3H), 1.21 (d, $J=6.5$ Hz, 3H), 1.16 (m, 15H, 15-CH₃, 3''-CH₃, 5'-CH₃, 5''-CH₃, 5'''-CH₃), 0.89 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.81 (m, 1H, H-7).

2'-O-Acetyl-4''-O-benzoylspiramycin I (5)

To a solution of **4** (3.03 g, 3.42 mmol) in CH₂Cl₂ (34 ml) was added benzoic acid (2.09 g, 17.0 mmol, DMAP (207 mg, 1.70 mmol and DCC (3.51 g, 17.0 mmol at 0°C under N₂. After stirring for 1 hour, the mixture was warmed to room temperature and further stirred for 19 hours. The mixture was cooled to 0°C and then saturated aq NH₄Cl was added. The resulting mixture was extracted with EtOAc, washed with saturated aq NaHCO₃ and brine, dried over anhydrous Na₂SO₄, filtered and evaporated. The residue was purified by column chromatography on silica gel with an eluent (CH₂Cl₂: MeOH: NH₄OH=80: 1: 0.1 to 60: 1: 0.1) to give **5** (2.43 g, 72%) as a colorless powder.

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₅₂H₈₁N₂O₁₆: 989.5586 [M+H], found: *m/z* 989.5598 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm⁻¹: 3505, 2975, 2935, 2875, 2842, 1749, 1724.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 9.80 (s, 1H, H-18), 8.11~8.04 (m, 2H, 4''-OCOPh), 7.55 (m, 1H, 4''-OCOPh), 7.49~7.39 (m, 2H, 4''-OCOPh), 6.23 (dd, $J=16.2$, 10.5 Hz, 1H, H-11), 5.96 (dd, $J=14.4$, 10.5 Hz, 1H, H-12), 5.65 (dd, $J=15.1$, 9.6 Hz, 1H, H-10), 5.53 (ddd, $J=15.0$, 11.0 , 3.9 Hz, 1H, H-13), 5.27 (m, 1H, H-15), 5.11 (d, $J=3.2$ Hz, 1H, H-1''), 4.98 (dd, $J=10.4$, 7.6 Hz, 1H, H-2'), 4.81 (d, $J=10.0$ Hz, 1H, H-4''), 4.70 (d, $J=7.6$ Hz, 1H, H-1'), 4.51 (dq, $J=10.1$, 6.5 Hz, 1H, H-5''), 4.34 (d, $J=7.8$ Hz, 1H, H-1'''), 4.13~4.01 (m, 2H, H-5, H-9), 3.78 (d, $J=11.3$ Hz, 1H, H-3), 3.45 (s, 3H, 4-OCH₃), 3.40 (dq, $J=9.5$, 6.2 Hz, 1H, H-5'''), 3.34~3.31 (m, 2H, H-4', H-5'), 2.98 (d, $J=9.0$ Hz, 1H, H-4), 2.44 (s, 6H, 3'-N(CH₃)₂), 2.25 (s, 6H, 4'''-N(CH₃)₂), 2.04 (s, 3H, 2'-OCOCH₃), 1.29 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.23 (d, $J=6.5$ Hz, 3H, 5'-CH₃), 1.18 (d, $J=5.1$ Hz, 3H, 5''-CH₃), 1.14 (d, $J=6.5$ Hz, 3H, 5'''-CH₃), 1.18 (s, 3H, 3''-CH₃), 0.95 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.87 (m, 1H, H-7).

2'-O-Acetyl-4''-O-benzoyl-4'''-N-demethyl-4'''-N-(9-fluorenylmethoxycarbonyl)spiramycin I (6)

To a solution of **5** (3.13 g, 3.17 mmol) in EtOAc (32 ml) were added NaHCO₃ (1.34 g, 17.0 mmol) and 9-fluorenylmethylchloroformate (2.85 g, 11.0 mmol), and the mixture was stirred under reflux. After stirring for 3.5 hours, the mixture was cooled to room temperature. The mixture was diluted with EtOAc and washed with water and brine, dried over anhydrous Na₂SO₄, filtered and evaporated. The residue was purified by column chromatography on silica gel with an eluent (hexane: EtOAc=3: 1 to 1: 1) to give **6** (3.80 g, quant.) as a colorless powder.

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₆₆H₈₉N₂O₁₈: 1197.6110 [M+H], found: *m/z* 1197.6152 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm⁻¹: 3500, 2975, 2935, 2842, 1749, 1722, 1704.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 9.80 (s, 1H, H-18), 8.08 (d, $J=7.3$ Hz, 2H, 4''-OCOPh), 7.76 (d, $J=7.3$ Hz, 2H, Fmoc), 7.56~7.52 (m, 3H, 4''-OCOPh), 7.45~7.28 (m, 6H, Fmoc), 6.23 (dd, $J=15.1$, 10.5 Hz, 1H, H-11), 5.97 (dd, $J=14.9$, 10.5 Hz, 1H, H-12), 5.65 (dd, $J=15.1$, 10.0 Hz, 1H, H-10), 5.54 (m, 1H, H-13), 5.27 (m, 1H, H-15), 5.11 (d, $J=3.0$ Hz, 1H, H-1''), 4.97 (m, 1H, H-2'), 4.83 (d, $J=10.3$ Hz, 1H, H-4''), 4.69 (m, 1H, H-1'), 4.53 (m, 1H, H-5''), 4.42~4.25 (m, 3H, H-1''', Fmoc), 4.23~4.00 (m, 3H, H-5, H-9, Fmoc), 3.79 (d, $J=11.0$ Hz, 1H, H-3), 3.59 (m, 1H, H-5'''), 3.47 (m, 3H, 4-OCH₃), 3.31~3.30 (m, 2H, H-4', H-5'), 2.98 (d, $J=8.9$ Hz, 1H, H-4), 2.72 (br.s, 3H, 4'''-NCH₃), 2.43 (s, 6H, 3'-N(CH₃)₂), 2.03 (s, 3H, 2'-OCOCH₃), 1.29 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.18~1.14 (m, 9H, 5'-CH₃, 3''-CH₃, 5''-CH₃), 1.08 (d, $J=6.5$ Hz, 3H, 5'''-CH₃), 0.96 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.86 (m, 1H, H-7).

2'-O-Acetyl-4''-O-benzoyl-4'''-N-demethyl-18-(*N,N*-dibenzyl)amino-4'''-N-(9-fluorenylmethoxycarbonyl)-18-deoxospiramycin I (7a)

To a solution of **6** (78.5 mg, 65.6 μ mol) in CHCl₃ (656 μ l) were added *N,N*-dibenzylamine (19.0 μ l, 98.4 μ mol) and AcOH (9.40 μ l, 164 μ mol), and the mixture was stirred at room temperature for 15 minutes. Then NaHB(OAc)₃ (21.0 mg, 98.4 μ mol) was added, and stirring continued for 30 minutes. The mixture was diluted with CHCl₃ and washed with saturated aq NaHCO₃ and brine, dried over anhydrous Na₂SO₄, filtered and evaporated. The residue was purified by column chromatography on silica gel with an eluent (hexane: EtOAc=3: 1 to 1: 1) to give **7a** (79.3 mg, 88%) as a colorless powder.

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₈₀H₁₀₄N₃O₁₇: 1378.7366 [M+H], found: *m/z* 1378.7379

[M+H]⁺.

IR $\nu_{\max}$ (KBr) cm^{-1} : 3668, 2975, 2935, 2842, 1749, 1722, 1704.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 8.10 (d, $J=7.3$ Hz, 2H, 4''-OCOPh), 7.75 (d, $J=7.3$ Hz, 2H, Fmoc), 7.59~7.54 (m, 3H, 4''-OCOPh), 7.47~7.23 (m, 16H, 18-N-(CH₂Ph)₂, Fmoc), 6.01~5.96 (m, 2H, H-11, H-12), 5.65 (dd, $J=15.1, 10.0$ Hz, 1H, H-10), 5.57 (m, 1H, H-13), 5.33 (m, 1H, H-15), 5.09 (d, $J=3.2$ Hz, 1H, H-1''), 4.99 (m, 1H, H-2'), 4.83 (d, $J=10.3$ Hz, 1H, H-4''), 4.74 (d, $J=7.3$ Hz, 1H, H-1'), 4.54 (m, 1H, H-5''), 4.43~4.38 (m, 3H, H-1''', Fmoc), 4.23~4.07 (m, 3H, H-5, H-9, Fmoc), 3.47 (s, 3H, 4-OCH₃), 3.29~3.26 (m, 2H, H-4', H-5'), 3.01 (d, $J=8.6$ Hz, 1H, H-4), 2.63 (br s, 3H, 4'''-NCH₃), 2.44 (s, 6H, 3'-N(CH₃)₂), 2.03 (s, 3H, 2'-OCOCH₃), 1.30 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.20~1.17 (m, 9H, 5'-CH₃, 5''-CH₃, 3''-CH₃), 1.08 (d, $J=6.5$ Hz, 3H, 5'''-CH₃), 0.96 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.81 (m, 1H, H-7).

2'-O-Acetyl-4''-O-benzoyl-4'''-N-demethyl-18-(N,N-dibenzyl)amino-18-deoxospiramycin I

To a solution of **7a** (70.8 mg, 51.4 μmol) in EtOAc (514 μl) was added piperidine (5.60 μl , 56.5 μmol) and the mixture was stirred at room temperature. After stirring for 6 hours, the mixture was diluted with a 1 : 1 mixture of EtOAc and hexane, washed with saturated aq NaHCO₃ and brine, dried over anhydrous Na₂SO₄, filtered and evaporated. The residue was purified by column chromatography on silica gel with an eluent (CHCl₃ : MeOH : NH₄OH = 50 : 1 : 0.1) to give the title compound (62.0 mg, quant.) as a colorless powder.

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₆₅H₉₄N₃O₁₅: 1156.6685 [M+H], found: m/z 1156.6741 [M+H]⁺.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 8.09 (d, $J=7.0$ Hz, 2H, 4''-OCOPh), 7.57 (m, 1H, 4''-OCOPh), 7.47~7.22 (m, 12H, 4''-OCOPh, 18-N-(CH₂Ph)₂), 6.02~5.94 (m, 2H, H-11, H-12), 5.63 (dd, $J=14.6, 9.7$ Hz, 1H, H-10), 5.52 (ddd, $J=14.3, 10.5, 3.8$ Hz, 1H, H-13), 5.31 (m, 1H, H-15), 5.08 (d, $J=3.5$ Hz, 1H, H-1''), 4.97 (dd, $J=10.4, 7.8$ Hz, 1H, H-2'), 4.82 (d, $J=10.3$ Hz, 1H, H-4''), 4.72 (d, $J=7.6$ Hz, 1H, H-1'), 4.53 (m, 1H, H-5''), 4.25 (dd, $J=9.5, 1.9$ Hz, 1H, H-1'''), 4.06 (d, $J=8.9$ Hz, 1H, H-5), 3.92 (m, 1H, H-9), 3.74 (d, $J=11.6$ Hz, 1H, H-3), 3.66 (br s, 2H, 18-N-(CH₂Ph)₂), 3.46 (s, 3H, 4-OCH₃), 3.40 (br s, 2H, 18-N-(CH₂Ph)₂), 3.29~3.25 (m, 2H, H-4', H-5'), 3.01 (m, 1H, H-5'''), 2.97 (d, $J=9.7$ Hz, 1H, H-4), 2.42 (s, 6H, 3'-N(CH₃)₂), 2.39 (s, 3H, 4'''-NHCH₃), 2.07 (s, 3H, 2'-OCOCH₃), 1.30 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.17 (m, 9H, 5'-CH₃, 3''-CH₃, 5''-CH₃), 1.05 (d, $J=6.5$ Hz, 3H, 5'''-CH₃), 0.95 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.77 (m, 1H, H-7).

4''-O-Benzoyl-4'''-N-demethyl-18-(N,N-dibenzyl)amino-18-deoxospiramycin I (3a)

A solution of 2'-O-acetyl compound (55.2 mg, 48.0 μmol) in MeOH (480 μl) was refluxed. After stirring for 12 hours, the solvent was removed. The residue was purified by column chromatography on silica gel with an eluent (CHCl₃ : MeOH : NH₄OH = 60 : 1 : 0.1 ~ 20 : 1 : 0.1) to give **3a** (46.9 mg, 88%) as a colorless powder.

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₆₃H₉₂N₃O₁₄: 1114.6579 [M+H], found: m/z 1114.6595 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm^{-1} : 3496, 2973, 2933, 1722.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 8.11 (d, $J=7.0$ Hz, 2H, 4''-OCOPh), 7.57 (m, 1H, 4''-OCOPh), 7.48~7.26 (m, 12H, 18-N-(CH₂Ph)₂, 4''-OCOPh), 5.99~5.94 (m, 2H, H-11, H-12), 5.61 (m, 1H, H-10), 5.52 (m, 1H, H-13), 5.33 (m, 1H, H-15), 5.10 (d, $J=3.0$ Hz, 1H, H-1''), 4.83 (d, $J=10.3$ Hz, 1H, H-4''), 4.62 (m, 1H, H-5''), 4.31 (d, $J=8.1$ Hz, 1H, H-1'), 4.04~4.01 (m, 2H, H-5, H-1'''), 3.94 (m, 1H, H-9), 3.76~3.71 (m, 3H, H-3, 18-N-(CH₂Ph)₂), 3.52 (m, 1H, H-2'), 3.50 (s, 3H, 4-OCH₃), 3.36 (d, $J=14.0$ Hz, 2H, 18-N-(CH₂Ph)₂), 2.51 (s, 6H, 3'-N(CH₃)₂), 2.42 (s, 3H, 4'''-NHCH₃), 1.31 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.17 (m, 12H, 5'-CH₃, 3''-CH₃, 5''-CH₃, 5'''-CH₃), 0.94 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.87 (m, 1H, H-7).

4''-O-Benzoyl-18-(N-benzyl-N-phenyl)amino-4'''-N-demethyl-18-deoxospiramycin I (3b)

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₆₂H₉₀N₃O₁₄: 1100.6423 [M+H], found: m/z 1100.6420 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm^{-1} : 3488, 2973, 2933, 2877, 1720.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 8.11 (d, $J=7.0$ Hz, 2H, 4''-OCOPh), 7.57 (m, 1H, 4''-OCOPh), 7.47~7.42 (m, 2H, 4''-OCOPh), 7.34~7.14 (m, 7H, 18-N-Ph(CH₂Ph)), 6.74~6.71 (m, 2H, 18-N-Ph(CH₂Ph)), 6.65 (m, 1H, 18-N-Ph(CH₂Ph)), 6.01~5.93 (m, 2H, H-11, H-12), 5.65 (m, 1H, H-10), 5.52 (m, 1H, H-13), 5.31 (m, 1H, H-15), 5.09 (d, $J=3.0$ Hz, 1H, H-1''), 4.83 (d, $J=10.3$ Hz, 1H, H-4''), 4.65 (m, 2H, 18-N-(CH₂Ph)), 4.63 (m, 1H, H-5''), 4.25~4.19 (m, 2H, H-1', H-1'''), 4.06~4.02 (m, 2H, H-5, H-9), 3.74 (m, 1H, H-3), 3.56 (m, 1H, H-2'), 3.51 (s, 3H, 4-OCH₃), 3.32~3.21 (m, 2H, H-4', H-5'), 3.09 (d, $J=8.6$ Hz, 1H, H-4), 2.53 (s, 6H, 3'-N(CH₃)₂), 2.39 (s, 3H, 4'''-NHCH₃), 1.30 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.19~1.16 (m, 12H, 5'-CH₃, 3''-CH₃, 5''-CH₃, 5'''-CH₃), 0.96 (d, $J=6.5$ Hz, 8-CH₃), 0.89 (m, 1H, H-7).

4''-O-Benzoyl-18-N-benzylamino-4'''-N-demethyl-18-deoxospiramycin I (3c)

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₅₆H₈₆N₃O₁₄: 1024.6110 [M+H], found: m/z 1024.6108 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm^{-1} : 3502, 2975, 2935, 2875, 1749, 1722, 1704.

$^1\text{H-NMR}$ (270 MHz, CDCl_3 , partial data) δ (ppm): 8.10 (d, $J=7.0$ Hz, 2H, 4''-OCOPh), 7.57 (m, 1H, 4''-OCOPh), 7.48–7.42 (m, 2H, 4''-OCOPh), 7.35–7.33 (m, 4H, 18-*N-CH}_2\text{Ph}*), 7.27 (m, 1H, 18-*N-CH}_2\text{Ph}*), 6.17 (dd, $J=14.9$, 10.5 Hz, 1H, H-11), 5.97 (dd, $J=14.7$, 10.8 Hz, 1H, H-12), 5.68 (dd, $J=14.9$, 9.5 Hz, 1H, H-10), 5.45 (ddd, $J=15.0$, 11.1, 3.5 Hz, 1H, H-13), 5.24 (m, 1H, H-15), 5.11 (d, $J=3.0$ Hz, 1H, H-1''), 4.83 (d, $J=10.3$ Hz, 1H, H-4''), 4.62 (m, 1H, H-5''), 4.48–4.46 (m, 2H, H-1', H-1'''), 4.15 (dd, $J=10.3$, 4.3 Hz, 1H, H-9), 4.07 (d, $J=8.9$ Hz, 1H, H-5), 3.83 (d, $J=13.2$ Hz, 1H, 18-*N-CH}_2\text{Ph}*), 3.76 (m, 1H, H-3), 3.75 (d, $J=13.2$ Hz, 1H, 18-*N-CH}_2\text{Ph}*), 3.56 (dd, $J=10.3$, 7.0 Hz, 1H, H-2'), 3.50 (s, 3H, 4-OCH₃), 3.32 (m, 2H, H-4', H-5'), 3.18 (m, 1H, H-5'''), 3.08 (d, $J=8.4$ Hz, 1H, H-4), 2.53 (s, 6H, 3'-N(CH₃)₂), 2.40 (s, 3H, 4'''-NHCH₃), 1.29 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.25–1.17 (m, 12H, 5'-CH₃, 3''-CH₃, 5''-CH₃, 5'''-CH₃), 0.97 (d, $J=6.5$ Hz, 8-CH₃), 0.91 (m, 1H, H-7).

4''-O-Benzoyl-4'''-N-demethyl-18-(*N,N*-di-2-picolyl)amino-18-deoxospiramycin I (3d)

HR-MS (FAB) (matrix; *m*-NBA): calcd. for $\text{C}_{61}\text{H}_{90}\text{N}_5\text{O}_{14}$: 1116.6484 [M+H], found: m/z 1024.6448 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm^{-1} : 3457, 2935, 1722.

$^1\text{H-NMR}$ (270 MHz, CDCl_3 , partial data) δ (ppm): 8.62–8.54 (m, 2H, 18-*N-CH}_2(2\text{-pyridine})*), 8.12–8.08 (m, 2H, 4''-OCOPh), 7.74–7.65 (m, 2H, 18-*N-CH}_2(2\text{-pyridine})*), 7.61–7.55 (m, 3H, 18-*N-CH}_2(2\text{-pyridine})*, 4''-OCOPh), 7.48–7.42 (m, 2H, 4''-OCOPh), 7.23–7.12 (m, 2H, 18-*N-CH}_2(2\text{-pyridine})*), 5.94 (m, 1H, H-11), 5.81 (m, 1H, H-12), 5.62 (dd, $J=14.0$, 9.7 Hz, 1H, H-10), 5.42 (m, 1H, H-13), 5.25 (m, 1H, H-15), 5.05 (d, $J=3.0$ Hz, 1H, H-1''), 4.81 (d, $J=9.7$ Hz, 1H, H-4''), 4.60 (m, 1H, H-5''), 4.30 (dd, $J=7.8$ Hz, 1H, H-1'), 4.16 (m, 1H, H-1'''), 4.00–3.88 (m, 5H, H-3, H-5, H-9, 18-*N-CH}_2(2\text{-pyridine})*), 3.59 (m, 1H, H-2'), 3.52 (s, 3H, 4-OCH₃), 3.43 (d, $J=14.0$ Hz, 2H, 18-*N-CH}_2(2\text{-pyridine})*), 3.27–3.11 (m, 2H, H-4', H-5'), 2.51 (s, 6H, 3'-N(CH₃)₂), 2.41 (s, 3H, 4'''-NHCH₃), 1.31 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.24–1.15 (m, 9H, 5'-CH₃, 3''-CH₃, 5''-CH₃), 1.09 (d, $J=6.2$ Hz, 3H, 5'''-CH₃), 0.93 (d, $J=6.8$ Hz, 3H, 8-CH₃), 0.84 (m, 1H, H-7).

4''-O-Benzoyl-4'''-N-demethyl-18-(*N,N*-di-3-picolyl)amino-18-deoxospiramycin I (3e)

HR-MS (FAB) (matrix; *m*-NBA): calcd. for $\text{C}_{61}\text{H}_{90}\text{N}_5\text{O}_{14}$: 1116.6484 [M+H], found: m/z 1024.6454 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm^{-1} : 3490, 2973, 2935, 2875, 1722.

$^1\text{H-NMR}$ (270 MHz, CDCl_3 , partial data) δ (ppm): 8.57–8.56 (m, 2H, 18-*N-CH}_2(3\text{-pyridine})*), 8.55–8.49 (m,

2H, 18-*N-CH}_2(3\text{-pyridine})*), 8.12–8.08 (m, 2H, 4''-OCOPh), 7.79–7.76 (m, 2H, 18-*N-CH}_2(3\text{-pyridine})*), 7.57 (m, 1H, 4''-OCOPh), 7.45 (m, 2H, 4''-OCOPh), 7.32–7.29 (m, 2H, 18-*N-CH}_2(3\text{-pyridine})*), 6.03–5.96 (m, 2H, H-11, H-12), 5.64 (m, 1H, H-10), 5.55 (m, 1H, H-13), 5.31 (m, 1H, H-15), 5.07 (d, $J=3.0$ Hz, 1H, H-1''), 4.82 (d, $J=10.3$ Hz, 1H, H-4''), 4.61 (m, 1H, H-5''), 4.34–4.27 (m, 2H, H-1', H-1'''), 4.03–3.92 (m, 2H, H-5, H-9), 3.73 (m, 1H, H-3), 3.71 (d, $J=13.0$ Hz, 2H, 18-*N-CH}_2(3\text{-pyridine})*), 3.55 (m, 1H, H-2'), 3.49 (m, 3H, 4-OCH₃), 3.73 (d, $J=13.0$ Hz, 2H, 18-*N-CH}_2(3\text{-pyridine})*), 3.15–3.05 (m, 2H, H-4', H-5'), 2.52 (s, 6H, 3'-N(CH₃)₂), 2.40 (s, 3H, 4'''-NHCH₃), 1.31 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.18–1.14 (m, 12H, 5'-CH₃, 3''-CH₃, 5''-CH₃, 5'''-CH₃), 0.95 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.84 (m, 1H, H-7).

4''-O-Benzoyl-4'''-N-demethyl-18-(4-phenylpiperazin-1-yl)-18-deoxospiramycin I (3f)

HR-MS (FAB) (matrix; *m*-NBA): calcd. for $\text{C}_{59}\text{H}_{91}\text{N}_4\text{O}_{14}$: 1079.6532 [M+H], found: m/z 1079.6543 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm^{-1} : 3494, 2973, 2935, 2875, 1722.

$^1\text{H-NMR}$ (270 MHz, CDCl_3 , partial data) δ (ppm): 8.10 (d, $J=7.0$ Hz, 2H, 4''-OCOPh), 7.57 (m, 1H, 4''-OCOPh), 7.48–7.42 (m, 2H, 4''-OCOPh), 7.29–7.23 (m, 2H, 18-*N-Ph*), 6.94–6.92 (m, 2H, 18-*N-Ph*), 6.85 (m, 1H, 18-*N-Ph*), 6.20 (dd, $J=14.8$, 10.5 Hz, 1H, H-11), 6.01 (dd, $J=14.8$, 10.5 Hz, 1H, H-12), 5.70 (dd, $J=14.9$, 9.5 Hz, 1H, H-10), 5.51 (ddd, $J=15.0$, 11.1, 3.5 Hz, 1H, H-13), 5.18 (m, 1H, H-15), 5.14 (d, $J=3.0$ Hz, 1H, H-1''), 4.83 (d, $J=10.3$ Hz, 1H, H-4''), 4.63 (m, 1H, H-5''), 4.45–4.48 (m, 2H, H-1', H-1'''), 4.17–4.08 (m, 2H, H-5), 3.78 (d, $J=10.5$ Hz, 1H, H-3), 3.59 (dd, $J=10.3$, 7.0 Hz, 1H, H-2'), 3.51 (s, 3H, 4-OCH₃), 2.54 (s, 6H, 3'-N(CH₃)₂), 2.41 (s, 3H, 4'''-NH(CH₃)), 1.31–1.25 (m, 12H, 15-CH₃, 5'-CH₃, 3''-CH₃, 5''-CH₃), 1.18 (d, $J=6.5$ Hz, 3H, 5'''-CH₃), 0.98 (d, $J=6.5$ Hz, 8-CH₃), 0.93 (m, 1H, H-7).

4''-O-Benzoyl-4'''-N-demethyl-18-(indolin-1-yl)-18-deoxospiramycin I (3g)

HR-MS (FAB) (matrix; *m*-NBA): calcd. for $\text{C}_{57}\text{H}_{86}\text{N}_3\text{O}_{14}$: 1036.6110 [M+H], found: m/z 1036.6145 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm^{-1} : 3490, 2935, 1733.

$^1\text{H-NMR}$ (270 MHz, CDCl_3 , partial data) δ (ppm): 8.07 (d, $J=7.2$ Hz, 2H, 4''-OCOPh), 7.53 (m, 1H, 4''-OCOPh), 7.43–7.38 (m, 2H, 4''-OCOPh), 7.03 (d, $J=7.0$ Hz, 1H, 18-*N-indoline*), 6.99 (d, $J=8.0$ Hz, 1H, 18-*N-indoline*), 6.58 (t, $J=7.3$ Hz, 1H, 18-*N-indoline*), 6.49 (d, $J=7.8$ Hz, 1H, 18-*N-indoline*), 6.00–5.93 (m, 2H, H-11, H-12), 5.63 (dd, $J=13.8$, 9.5 Hz, 1H, H-10), 5.47 (ddd, $J=13.9$, 11.1, 4.9 Hz, 1H, H-13), 5.24 (m, 1H, H-15), 5.09 (d, $J=4.1$ Hz, 1H, H-1''), 4.80 (d, $J=10.3$ Hz, 1H, H-4''), 4.63 (m, 1H, H-5''),

4.55 (d, $J=7.6$ Hz, 1H, H-1'), 4.19 (m, 1H, H-5), 3.96 (d, $J=8.6$ Hz, 1H, H-1''), 3.90 (dd, $J=9.5, 3.8$ Hz, 1H, H-9), 3.75 (d, $J=10.5$ Hz, 1H, H-3), 3.57 (dd, $J=10.3, 7.6$ Hz, 1H, H-2'), 3.50 (s, 3H, 4-OCH₃), 2.52 (s, 6H, 3'-N(CH₃)₂), 2.35 (s, 3H, 4'''-NHCH₃), 1.25 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.16~1.12 (m, 9H, 5'-CH₃, 3''-CH₃, 5'''-CH₃), 1.09 (d, $J=6.2$ Hz, 3H, 5''-CH₃), 0.94 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.86 (m, 1H, H-7).

4''-O-Benzoyl-4'''-N-demethyl-18-(isoindolin-2-yl)-18-deoxospiramycin I (3h)

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₅₇H₈₅N₃O₁₄Na: 1058.5929, found: m/z 1058.5973 [M+Na]⁺.

IR $\nu_{\max}$ (KBr) cm⁻¹: 3469, 2967, 2935, 1720.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 8.09 (d, $J=7.0$ Hz, 2H, 4''-OCOPh), 7.57 (m, 1H, 4''-OCOPh), 7.54~7.48 (m, 2H, 4''-OCOPh), 7.20 (s, 4H, 18-*N*-isoindoline), 6.18 (dd, $J=15.0, 10.3$ Hz, 1H, H-11), 6.00 (dd, $J=14.6, 10.5$ Hz, 1H, H-12), 5.70 (dd, $J=14.9, 9.7$ Hz, 1H, H-10), 5.56 (ddd, $J=14.7, 10.7, 4.1$ Hz, 1H, H-13), 5.22 (m, 1H, H-15), 5.12 (d, $J=3.2$ Hz, 1H, H-1''), 4.83 (d, $J=10.3$ Hz, 1H, H-4''), 4.69 (m, 2H, H-5''), 4.63 (d, $J=7.6$ Hz, 1H, H-1'), 4.52 (m, 1H, H-1''), 4.21~4.18 (m, 2H, H-9, H-5), 3.98 (s, 4H, 18-*N*-isoindoline), 3.77 (d, $J=10.5$ Hz, 1H, H-3), 3.60 (dd, $J=10.5, 7.6$ Hz, 1H, H-2'), 3.63 (s, 3H, 4-OCH₃), 3.43~3.35 (m, 2H, H-4', H-5'), 2.60 (s, 6H, 3'-N(CH₃)₂), 2.46 (s, 3H, 4'''-NHCH₃), 1.28 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.28~1.22 (m, 12H, 5'-CH₃, 3''-CH₃, 5'''-CH₃, 5'''-CH₃), 1.00 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.96 (m, 1H, H-7).

4''-O-Benzoyl-4'''-N-demethyl-18-(1,2,3,4-tetrahydroquinolin-1-yl)-18-deoxospiramycin I (3i)

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₅₈H₈₈N₃O₁₄: 1050.6266 [M+H], found: m/z 1050.6211 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm⁻¹: 3491, 2967, 2935, 2865, 1720.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 8.10 (d, $J=7.0$ Hz, 2H, 4''-OCOPh), 7.58 (m, 1H, 4''-OCOPh), 7.48~7.42 (m, 2H, 4''-OCOPh), 7.02 (t, $J=7.3$ Hz, 1H, 18-*N*-1,2,3,4-tetrahydroquinoline), 6.96 (d, $J=7.3$ Hz, 1H, 18-*N*-1,2,3,4-tetrahydroquinoline), 6.62 (d, $J=8.1$ Hz, 1H, 18-*N*-1,2,3,4-tetrahydroquinoline), 6.55 (t, $J=7.3$ Hz, 1H, 18-*N*-1,2,3,4-tetrahydroquinoline), 6.11~6.09 (m, 2H, H-11, H-12), 5.68 (dd, $J=14.2, 9.7$ Hz, 1H, H-10), 5.50 (ddd, $J=14.4, 11.0, 3.2$ Hz, 1H, H-13), 5.30 (m, 1H, H-15), 5.14 (d, $J=3.0$ Hz, 1H, H-1''), 4.84 (d, $J=10.0$ Hz, 1H, H-4''), 4.64 (m, 1H, H-5''), 4.57 (d, $J=7.6$ Hz, 1H, H-1'), 4.25 (d, $J=8.9$ Hz, 1H, H-5), 3.90 (d, $J=7.8$ Hz, 1H, H-1''), 3.80~3.73 (m, 2H, H-3, H-9), 3.58 (dd, $J=10.3, 7.6$ Hz, 1H, H-2'), 3.53 (s, 3H, 4-OCH₃), 3.48~3.28 (m, 4H, 18-*N*-1,2,3,4-tetrahydroquinoline, H-4', H-5'), 3.09 (d, $J=8.4$ Hz,

1H, H-4), 2.55 (s, 6H, 3'-N(CH₃)₂), 2.41 (s, 3H, 4'''-NHCH₃), 1.30 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.20~1.14 (m, 12H, 5'-CH₃, 3''-CH₃, 5'''-CH₃, 5'''-CH₃), 0.94 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.84 (m, 1H, H-7).

4''-O-Benzoyl-4'''-N-demethyl-18-(1,2,3,4-tetrahydroisoquinolin-2-yl)-18-deoxospiramycin I (3j)

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₅₈H₈₈N₃O₁₄: 1050.6266 [M+H], found: m/z 1050.6257 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm⁻¹: 3488, 2937, 1720.

¹H-NMR (270 MHz, CDCl₃) δ (ppm): 8.09 (d, $J=7.0$ Hz, 2H, 4''-OCOPh), 7.56 (m, 1H, 4''-OCOPh), 7.44 (m, 2H, 4''-OCOPh), 7.07 (m, 4H, 18-*N*-1,2,3,4-tetrahydroisoquinoline), 6.07~5.95 (m, 2H, H-11, H-12), 5.92 (dd, $J=14.2, 10.5$ Hz, 1H, H-10), 5.65 (dd, $J=14.4, 9.7$ Hz, 1H, H-13), 5.13 (m, 1H, H-15), 5.12 (d, $J=3.0$ Hz, 1H, H-1''), 4.82 (d, $J=10.3$ Hz, 1H, H-4''), 4.53 (m, 1H, H-5''), 4.56 (d, $J=7.6$ Hz, 1H, H-1'), 4.39 (dd, $J=9.3, 2.2$ Hz, 1H, H-1''), 4.16~4.13 (m, 2H, H-5, H-9), 4.07 (d, $J=10.8$ Hz, 1H, H-3), 3.50 (dd, $J=10.5, 7.6$ Hz, 1H, H-2'), 3.45 (s, 3H, 4-OCH₃), 3.34~3.11 (m, 2H, H-4', H-5'), 2.53 (s, 6H, 3'-N(CH₃)₂), 2.40 (s, 3H, 4'''-NHCH₃), 1.29 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.22~1.16 (m, 12H, 5'-CH₃, 3''-CH₃, 5'''-CH₃, 5'''-CH₃), 0.95 (d, $J=6.2$ Hz, 3H, 8-CH₃), 0.87 (m, 1H, H-7).

4'''-N-Demethyl-18-(indolin-1-yl)-18-deoxospiramycin I (8)

Title compound **8** was prepared from compound **4** by Fmoc protection, *N*-amination, Fmoc deprotection, and methanolysis (37%, 4 steps).

HR-MS (FAB) (matrix; *m*-NBA): calcd. for C₅₈H₈₈N₃O₁₄: 932.5848 [M+H], found: m/z 932.5814 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm⁻¹: 3478, 2933, 1727.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 7.06 (t, $J=7.5$ Hz, 1H, 18-*N*-indoline), 7.03 (d, $J=7.5$ Hz, 1H, 18-*N*-indoline), 6.62 (t, $J=7.5$ Hz, 1H, 18-*N*-indoline), 6.50 (d, $J=7.5$ Hz, 1H, 18-*N*-indoline), 6.02~5.97 (m, 2H, H-11, H-12), 5.65 (dd, $J=14.0, 10.0$ Hz, 1H, H-10), 5.47 (m, 1H, H-13), 5.28 (m, 1H, H-15), 5.06 (d, $J=3.0$ Hz, 1H, H-1''), 4.54 (d, $J=7.3$ Hz, 1H, H-1'), 4.22 (d, $J=8.9$ Hz, 1H, H-5''), 3.23 (m, 1H, H-1''), 3.90 (dd, $J=9.5, 3.8$ Hz, 1H, H-9), 3.79 (d, $J=10.5$ Hz, 1H, H-3), 3.57 (m, 1H, H-2'), 3.51 (s, 3H, 4-OCH₃), 2.47 (s, 6H, 3'-N(CH₃)₂), 2.40 (s, 3H, 4'''-NHCH₃), 1.30~1.22 (m, 12H, 15-CH₃, 5'-CH₃, 5'''-CH₃), 1.14 (d, $J=6.5$ Hz, 3H, 5''-CH₃), 0.96 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.84 (m, 1H, H-7).

4''-O-Benzoyl-18-(indolin-1-yl)-18-deoxospiramycin I (9)

Title compound **9** was prepared from compound **5** by *N*-amination followed by methanolysis (84%, 2 steps).

HR-MS (FAB) (matrix; *m*-NBA): calcd. for $C_{58}H_{88}N_3O_{14}$: 1050.6266 [M+H], found: m/z 1050.6223 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm^{-1} : 3450, 2935, 1724.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 8.09 (d, $J=7.2$ Hz, 2H, 4''-OCOPh), 7.57 (m, 1H, 4''-OCOPh), 7.47~7.42 (m, 2H, 4''-OCOPh), 7.05 (t, $J=7.5$ Hz, 1H, 18-*N*-indoline), 7.03 (d, $J=8.0$ Hz, 1H, 18-*N*-indoline), 6.61 (t, $J=7.3$ Hz, 1H, 18-*N*-indoline), 6.52 (d, $J=7.8$ Hz, 1H, 18-*N*-indoline), 6.03~5.96 (m, 2H, H-11, H-12), 5.67 (dd, $J=14.0, 10.0$ Hz, 1H, H-10), 5.47 (ddd, $J=13.9, 11.1, 4.9$ Hz, 1H, H-13), 5.28 (m, 1H, H-15), 5.13 (d, $J=3.5$ Hz, 1H, H-1''), 4.83 (d, $J=10.3$ Hz, 1H, H-4''), 4.63 (m, 1H, H-5''), 4.57 (d, $J=7.3$ Hz, 1H, H-1'), 4.46 (br s, 2H, 18-*N*-indoline), 3.23 (d, $J=8.6$ Hz, 1H, H-1'''), 4.03 (br s, 2H, 18-*N*-indoline), 3.86 (m, 1H, H-9), 4.19 (d, $J=8.1$ Hz, 1H, H-5), 3.79 (d, $J=10.5$ Hz, 1H, H-3), 3.59 (m, 1H, H-2'), 3.53 (s, 3H, 4-OCH₃), 2.55 (s, 6H, 3'-N(CH₃)₂), 2.25 (s, 6H, 4'''-N(CH₃)₂), 1.30 (d, $J=6.5$ Hz, 3H, 15-CH₃), 1.27 (d, $J=6.5$ Hz, 3H, 5'''-CH₃), 1.18 (d, $J=6.5$ Hz, 3H, 5'-CH₃), 1.16 (s, 3H, 3''-CH₃), 1.13 (d, $J=6.5$ Hz, 3H, 5''-CH₃), 0.96 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.88 (m, 1H, H-7).

4''-O-Benzoyl-4'''-N-demethylspiramycin I (10)

Title compound **10** was prepared from compound **6** by Fmoc deprotection followed by methanolysis (58%, 2 steps).

HR-MS (FAB) (matrix; *m*-NBA): calcd. for $C_{98}H_{149}N_4O_{28}$: 1830.0358 [2M+H], found: m/z 1830.0358 [M+H]⁺.

IR $\nu_{\max}$ (KBr) cm^{-1} : 3490, 2935, 1733.

¹H-NMR (270 MHz, CDCl₃, partial data) δ (ppm): 9.80 (s, 1H, H-18), 8.11~8.08 (m, 2H, 4''-OCOPh), 7.55 (m, 1H, 4''-OCOPh), 7.47~7.42 (m, 2H, 4''-OCOPh), 6.23 (dd, $J=16.2, 10.5$ Hz, 1H, H-11), 5.96 (dd, $J=14.4, 10.5$ Hz, 1H, H-12), 5.65 (dd, $J=15.1, 9.6$ Hz, 1H, H-10), 5.53 (ddd, $J=15.0, 11.0, 3.9$ Hz, 1H, H-13), 5.27 (m, 1H, H-15), 5.11 (d, $J=3.2$ Hz, 1H, H-1''), 4.98 (dd, $J=10.4, 7.6$ Hz, 1H, H-2'), 4.81 (d, $J=10.3$ Hz, 1H, H-4''), 4.72 (d, $J=7.6$ Hz, 1H, H-1'), 4.51 (dq, $J=10.1, 6.5$ Hz, 1H, H-5''), 4.34 (m, 1H, H-1'''), 4.10 (m, 1H, H-5), 4.02 (dd, $J=9.6, 3.8$ Hz, 1H, H-9), 3.78 (d, $J=11.1$ Hz, 1H, H-3), 3.45 (s, 3H, 4-OCH₃), 3.40 (dq, $J=9.5, 6.2$ Hz, 1H, H-5'''), 3.30 (m, 2H, H-4', H-5'), 2.98 (d, $J=9.0$ Hz, 1H, H-4), 2.43 (s, 6H, 3'-N(CH₃)₂), 2.20 (s, 3H, 4'''-NHCH₃), 1.28 (d, $J=6.2$ Hz, 3H, 15-CH₃), 1.22 (d, $J=6.5$ Hz, 3H, 5'-CH₃), 1.21 (s, 3H, 3''-CH₃), 1.18 (d, $J=6.5$ Hz, 3H, 5'''-CH₃), 1.14 (d, $J=6.5$ Hz, 3H, 5''-CH₃), 0.95 (d, $J=6.5$ Hz, 3H, 8-CH₃), 0.87 (m, 1H, H-7).

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