CHEMICAL MODIFICATION OF SPIRAMYCINS

VI. SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF 3,3″-DI-*O*-ACYL-4″-*O*-SULFONYL AND 3,3″-DI-*O*-ACYL-4″-*O*-ALKYL DERIVATIVES OF SPIRAMYCIN I

HIROSHI SANO, TOSHIAKI SUNAZUKA, HARUO TANAKA, KINYA YAMASHITA†, RYO OKACHI† and SATOSHI ŌMURA*

School of Pharmaceutical Sciences, Kitasato University and The Kitasato Institute, Minato-ku, Tokyo 108, Japan
†Pharmaceutical Research Laboratory, Kyowa Hakko Kogyo Co., Ltd., Nagaizumi-cho, Sunto-gun, Shizuoka-ken 411, Japan

(Received for publication May 9, 1985)

3,3″-Di-*O*-acyl-4″-*O*-sulfonyl and 3,3″-di-*O*-acyl-4″-*O*-alkyl derivatives of spiramycin I were synthesized and evaluated by four parameters, antibacterial activity, affinity to ribosomes, lypophilicity and therapeutic effects. Among them, 3,3″-di-*O*-acetyl-4″-*O*-mesyl and 3,3″-di-*O*-acetyl-4″-*O*-methylspiramycin I having relatively small substituents at 4″-position were the most effective in mouse protection tests, and the results were comparable to acetylspiramycin.

In a previous paper[1], we reported that 4″-sulfonates and 4″-alkylethers of spiramycin I, which were estimated to delay metabolism of spiramycin in the body, showed superior *in vitro* activities and were almost comparable to spiramycin I *in vivo*. In another paper[2], we also described that some of the 3,3″,4″-triacyl derivatives of spiramycin I were more effective *in vivo* than acetylspiramycin. From these findings, we have been very interested in the synthesis and activities of the 3,3″-diacylates of 4″-*O*-sulfonyl and 4″-*O*-alkylspiramycin I. Details of the synthesis and activities of the derivatives are described in this paper.

Synthesis

4″-*O*-Sulfonyl- and 4″-*O*-alkyl-2′-*O*-acetylspiramycin I 3,18-*O*-(*tert*-butyldimethylsilyl)acetal[1] were used as starting materials bearing a free hydroxyl group at 3″-position. However, acylation of 3″-hydroxyl group of 2′-*O*-acetyl-4″-*O*-mesylspiramycin I 3,18-*O*-(*tert*-butyldimethylsilyl)acetal (**1**) did not occur even under severe conditions because of hindrance from the *tert*-butyldimethylsilyl (TBDMS) protective group[2]. The TBDMS group of **1** was removed by treatment with tetrabutylammonium fluoride to give 2′-*O*-acetyl-4″-*O*-mesylspiramycin I (**2**). On the treatment of **2** with acetic anhydride and dimethylaminopyridine in $CHCl_3$ at refluxing temperature, similar to the conditions used for synthesis of 3,3″,4″-tri-*O*-acylspiramycin I[2], the desired 3″-acylate was not obtained. We therefore applied more drastic conditions which have been utilized for the preparation of 3″-acylleucomycin A_5[3].

Treatment of **2** with acetyl chloride in the presence of tribenzylamine in 1,2-dichloroethane at refluxing temperature gave predominantly 3,18,2′,3″-tetra-*O*-acetyl-17,18-enol-4″-*O*-mesylspiramycin I (**3**) and a small amount of 3,2′,3″-tri-*O*-acetyl-4″-*O*-mesylspiramycin I (**4**). Treatment of the mixture of compounds **3** and **4** with 70% MeOH resulted in removal of the enolacetyl group at the 18-

Scheme 1.

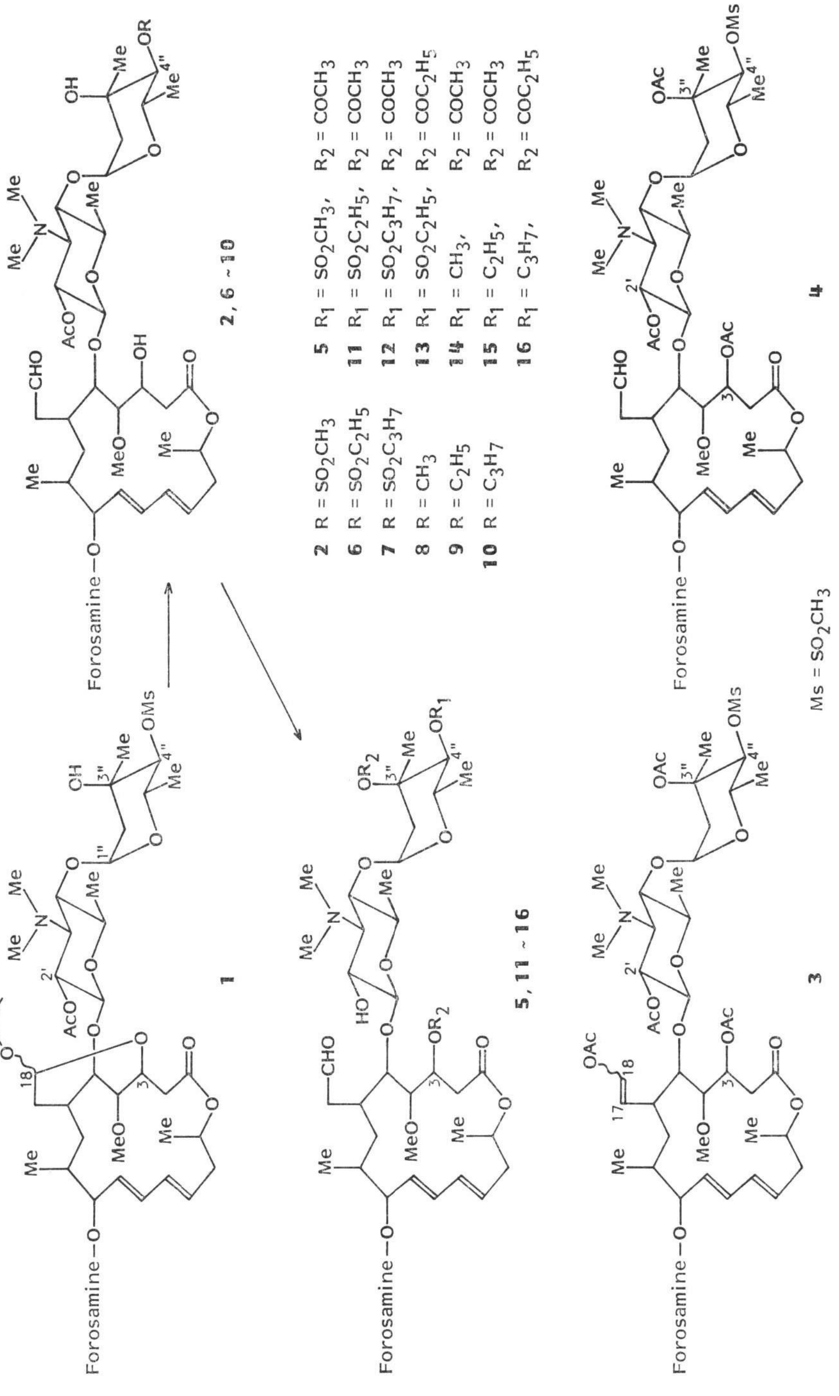

1
2, 6 ~ 10
2 R = SO2CH3
6 R = SO2C2H5
7 R = SO2C3H7
8 R = CH3
9 R = C2H5
10 R = C3H7
5 R1 = SO2CH3, R2 = COCH3
11 R1 = SO2C2H5, R2 = COCH3
12 R1 = SO2C3H7, R2 = COCH3
13 R1 = SO2C2H5, R2 = COC2H5
14 R1 = CH3, R2 = COCH3
15 R1 = C2H5, R2 = COCH3
16 R1 = C3H7, R2 = COC2H5
5, 11 ~ 16
3
4
Ms = SO2CH3

Table 1. ^{13}C NMR chemical shifts for spiramycin I derivatives.

Carbon No.	2[a]	3[b]	4[c]	5[d]	11[e]	12[f]	13[g]	14[h]	15[i]	16[j]
1	174.2	170.0	170.0	170.0	170.1	170.1	170.0	170.1	170.1	170.1
2	37.6	35.9	36.8	37.0	37.0	37.1	37.0	37.1	36.9	37.3
3	68.2	68.0	68.1	69.1	69.1	69.2	69.1	69.3	69.3	69.1
4	85.2	86.1	86.1	84.7	84.7	84.8	84.7	84.7	84.7	84.7
5	78.7	79.5	79.6	80.2	80.2	80.2	80.2	80.2	80.4	80.4
6	30.6	29.9	31.2	28.8	28.7	28.7	28.8	28.6	28.7	28.7
7	30.6	30.5	31.2	29.6	29.6	29.6	30.1	29.9	29.6	29.9
8	31.8	30.9	31.9	31.8	31.9	31.9	31.8	31.8	31.7	31.7
9	79.3	82.2	79.7	79.6	79.7	79.7	79.7	79.6	79.6	79.6
10	128.6	127.0	126.5	126.8	126.5	126.8	126.6	126.5	126.5	126.5
11	134.7	135.0	135.5	135.5	135.5	135.5	135.4	135.5	135.5	135.4
12	132.9	132.8	132.3	132.3	132.3	132.3	132.3	132.3	132.3	132.3
13	130.9	131.2	131.8	131.8	131.9	131.9	131.9	131.8	131.8	131.9
14	42.0	41.2	41.0	41.0	41.0	41.0	41.0	41.0	41.0	41.0
15	69.5	69.2	69.2	69.1	69.1	69.2	69.1	69.1	69.2	69.1
16	20.1	20.3	20.3	20.3	20.3	20.3	20.3	20.3	20.3	20.3
17	42.8	99.3	42.2	42.3	42.3	42.3	42.4	42.3	42.3	42.3
18	202.9	135.0	201.2	201.2	201.2	201.2	201.2	201.2	201.2	201.2
19	15.1	14.8	15.3	15.3	15.3	15.3	15.3	15.2	15.2	15.2
20	61.5	62.2	62.1	62.5	62.6	62.6	62.6	62.6	62.6	62.6
1′	100.8	100.6	100.9	103.7	103.6	103.6	103.6	103.4	103.4	103.4
2′	71.0	70.9	70.8	70.6	70.5	70.5	70.6	70.2	70.1	70.1
3′	67.9	68.8	69.2	69.1	69.1	69.2	69.1	69.1	69.2	69.1
4′	75.8	77.3	75.7	77.6	77.4	77.5	77.6	77.3	77.4	77.1
5′	72.7	73.0	72.7	73.0	73.0	73.0	73.1	73.2	73.1	73.2
6′	18.9	18.6	18.6	18.2	18.2	18.2	18.2	18.3	18.3	18.3
3′-NCH_3	41.6	41.5	41.5	41.6	41.6	41.5	41.5	41.4	41.4	41.4
1″	96.5	97.7	97.7	98.1	98.1	98.1	98.2	98.6	98.8	98.7
2″	40.7	36.9	37.1	37.0	37.0	37.1	37.3	36.7	36.9	37.0
3″	69.5	77.9	77.9	77.9	77.9	77.9	77.8	79.1	79.3	79.1
4″	85.0	84.6	85.2	85.7	84.7	84.8	86.1	88.5	86.9	86.8
5″	63.3	63.0	63.0	63.2	63.3	63.4	63.6	65.0	65.1	65.2
6″	18.4	18.2	17.9	18.0	18.0	18.0	17.9	17.9	18.0	18.0
7″	23.4	23.4	23.5	23.4	23.4	23.4	23.3	22.7	22.8	22.6
1‴	100.2	99.3	100.0	100.2	100.2	100.2	100.1	100.1	100.1	100.0
2‴	31.3	31.2	31.5	31.2	31.2	31.2	31.3	31.2	31.2	31.3
3‴	18.4	18.2	18.2	18.5	18.6	18.6	18.6	18.5	18.5	18.5
4‴	64.8	64.9	64.8	64.8	64.8	64.9	64.9	64.8	64.8	64.9
5‴	73.9	74.0	73.7	73.7	73.7	73.6	73.7	73.7	73.7	73.7
6‴	19.0	18.9	19.0	19.0	19.0	19.1	19.0	19.0	19.0	19.0
4‴-NCH_3	40.7	40.7	40.6	40.7	40.6	40.7	40.7	40.7	40.7	40.7

[a] 38.9 (SCH_3); 21.7, 168.7 (2′-$OCOCH_3$).

[b] 39.0 (SCH_3); 21.2, 170.6 (3-$COCH_3$); 20.9, 169.4 (18-$COCH_3$); 21.6, 168.7 (2′-$COCH_3$); 22.5, 170.1 (3″-$COCH_3$).

[c] 39.0 (SCH_3); 21.2, 170.7 (3-$COCH_3$); 21.7, 168.7 (2′-$COCH_3$); 22.4, 170.0 (3″-$COCH_3$).

[d] 38.9 (SCH_3); 21.2, 170.8 (3-$COCH_3$); 22.4, 170.1 (3″-$COCH_3$).

[e] 8.3, 46.4 (SCH_2CH_3); 21.2, 170.8 (3-$COCH_3$); 22.4, 170.1 (3″-$COCH_3$).

[f] 12.9, 17.3, 53.5 ($SCH_2CH_2CH_3$); 21.3, 170.8 (3-$COCH_3$); 22.4, 170.1 (3″-$COCH_3$).

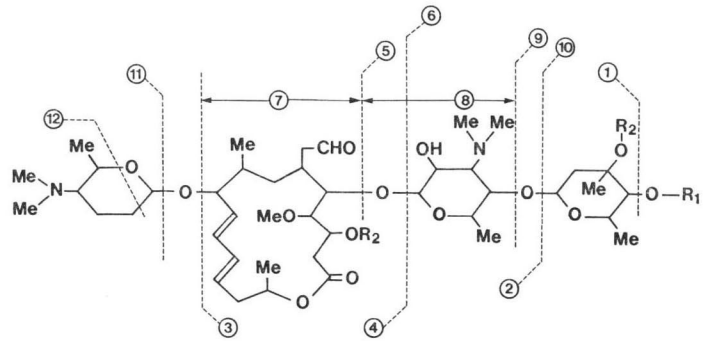
[g] 8.2, 46.4 (SCH_2CH_3); 9.0, 27.7, 173.9 (3-$COCH_2CH_3$); 9.2, 28.8, 173.5 (3″-$COCH_2CH_3$).

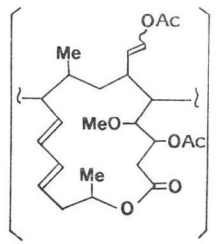
[h] 62.2 (CH_3); 21.3, 170.8 (3-$COCH_3$); 22.3, 170.1 (3″-$COCH_3$).

[i] 15.7, 69.8 (CH_2CH_3); 21.3, 170.8 (3-$COCH_3$); 22.4, 170.1 (3″-$COCH_3$).

[j] 10.5, 23.5, 76.1 ($CH_2CH_2CH_3$); 9.0, 27.7, 174.2 (3-$COCH_2CH_3$); 9.2, 28.7, 173.9 (3″-$COCH_2CH_3$).

Table 2. Diagnostic mass fragmentation for spiramycin I derivatives.





	2	3	4	5	11	12	13	14	15	16
M^+	962	1,088	1,046	1,004	−H 1,017	1,032	1,046	940	954	996
1	—	—	—	—	—	909	937	909	—	—
2+H	740	824	782	740	740	740	754	+H 741	740	754
3	−H 803	—	—	−H 845	—	—	—	782	—	+H 839
4	—	−H 607	566	−H 565	—	—	580	—	+2H 568	+H 581
5	454	496	—	454	—	—	+2H 484	—	404	—
6	438	480	480	438	452	466	466	374	388	416
7−H	349	—	391	391	+H 392	+H 392	405	391	391	405
8+H	216	216	216	174	174	−H 173	174	−H 173	174	174
9	239	281	281	281	—	—	—	−H 216	+2H 233	−H 258
10	223	265	265	265	279	—	—	201	215	243
10−R_1OH	—	205	—	205	219	—	219	141	155	169
11	142	142	142	−H 141	−H 141	142	142	142	142	142
12+H	114	114	114	114	114	114	114	114	114	114

Table 3. MIC, ID_{50}, survival % and retention time in HPLC (RT) of 3,3″-diacylates of 4″-*O*-sulfonyl and 4″-*O*-alkylspiramycin I.

Compound	3	3″	4″[a]	MIC (μg/ml)[b]							ID_{50} (μM)	Survival %			RT (minutes)
				SA	SA^r	BS	BC	ML	EC	KP		100[c]	60	40	
5	Ac	Ac	Ms	6.25	>100	6.25	3.12	1.56	>100	>100	2.0	100	60	20	11.5
11	Ac	Ac	Es	3.12	>100	3.12	6.25	0.78	>100	>100	2.5	60	30	0	13.4
12	Ac	Ac	Ps	3.12	>100	3.12	3.12	0.4	>100	>100	3.3	30	10	0	18.3
13	Pn	Pn	Es	1.56	>100	3.12	6.25	0.2	>100	>100	NT	70	10	0	21.4
14	Ac	Ac	Me	25	>100	3.12	12.5	0.4	>100	>100	1.8	100	40	20	10.8
15	Ac	Ac	Et	50	>100	12.5	12.5	1.56	>100	>100	2.1	40	0	0	11.9
16	Pn	Pn	Pr	6.25	>100	3.12	12.5	0.2	>100	>100	2.0	0	0	0	19.5
SPM I	H	H	H	3.12	>100	1.56	3.12	0.2	>100	>100	1.0	10	0	0	4.3
AcSPM				12.5	>100	3.12	6.25	0.4	>100	>100	1.9	80	60	0	

[a] Ac: $COCH_3$, Pn: COC_2H_5, Ms: SO_2CH_3, Es: $SO_2C_2H_5$, Ps: $SO_2C_3H_7$, Pr: C_3H_7.

[b] Test organism: SA; *Staphylococcus aureus* KB210 (ATCC 6538P), SA^r; *S. aureus* KB224 (MC^r, TC^r), BS; *Bacillus subtilis* KB211 (ATCC 6633), BC; *B. cereus* KB143 (IFO 3001), ML; *Micrococcus luteus* KB212 (ATCC 9341), EC; *Escherichia coli* KB213 (NIHJ), KP; *Klebsiella pneumoniae* KB214 (ATCC 10031).

[c] Test organism: *Streptococcus pneumoniae* III, dose (mg/kg).

position and the acetyl group at the 2′-position to give 3,3″-di-*O*-acetyl-4″-*O*-mesylspiramycin I (**5**). 3,3″-Diacetates of 4″-*O*-ethanesulfonyl (**11**), 4″-*O*-propanesulfonyl (**12**), 4″-*O*-methyl (**14**) and 4″-*O*-ethylspiramycin I (**15**), and 3,3″-dipropionates of 4″-*O*-ethanesulfonyl (**13**) and 4″-*O*-propylspiramycin I (**16**) were also synthesized in a similar manner.

The structure of **5** was confirmed by ^{13}C NMR (Table 1) and mass spectral evidence (Table 2). The ^{13}C NMR spectrum of **5** showed signals of two acetyl and one mesyl group, downfield shifts of 3- and 3″-carbons and upfield shifts of 1-, 18-, 2″-, 5″- and 7″-carbons compared with that of 4″-*O*-mesylspiramycin I, indicating that 3- and 3″-hydroxyl groups of 4″-*O*-mesylspiramycin I were acetylated. The mass spectral fragmentation (Table 2) also assisted this structure. The structures of **11**～**16** were also confirmed in the similar manner.

The chemical shifts of acyl carbons of all the derivatives agreed with the experimental rule between the bonding positions and the ^{13}C NMR chemical shifts of acyl groups in spiramycins[2].

Evaluation by Antibacterial Activity, Affinity to Ribosomes, Lipophilicity and Therapeutic Effect

Spiramycin I derivatives prepared as described above were evaluated by four parameters; *in vitro* antibacterial activity (MIC), affinity to ribosomes (ID_{50})[4], lipophilicity (retention time in HPLC[5]) and therapeutic effect in mice (survival %), as shown in Table 3. In *in vitro* antibacterial activity, 3,3″-di-*O*-acyl derivatives of 4″-*O*-sulfonylspiramycin I were comparable to spiramycin I but 4″-alkylethers showed considerably lower activity. Almost all the derivatives showed affinity to ribosomes comparable to or somewhat weaker than acetylspiramycin. A relationship between ribosome affinity and antibacterial activity was not found.

3,3″-Diacylates of 4″-*O*-sulfonyl and 4″-*O*-alkylspiramycin I except 3,3″-di-*O*-propionyl-4″-*O*-propylspiramycin I were more effective than spiramycin I in therapeutic effect. The size of 4″-substituent correlates with therapeutic effect; derivatives with a relatively small sulfonyl or alkyl group were more effective.

3,3″-Di-*O*-acetyl-4″-*O*-methyl (**14**) and 3,3″-di-*O*-acetyl-4″-*O*-ethylspiramycin I (**15**) showed good therapeutic effects in spite of their low antibacterial activity, which implies that pharmacokinetics of these derivatives was improved.

3,3″-Di-*O*-propionyl-4″-*O*-propylspiramycin I (**16**) was ineffective *in vivo* contrary to expectation, although its substituent bulkiness is similar to that of 3,3″,4″-tri-*O*-propionylspiramycin I[2] which is the most effective derivative among the 3,3″,4″-triacylates.

Among the derivative prepared, 3,3″-di-*O*-acetyl-4″-*O*-mesyl (**5**) and 3,3″-di-*O*-acetyl-4″-*O*-methylspiramycin I (**14**) were the best in therapeutic effect and comparable to acetylspiramycin.

Experimental

NMR spectra were measured on a Jeol FX-100 spectrometer in $CDCl_3$ solution. Mass spectra were obtained on a Jeol D-100 and DX-300 spectrometer at 20 eV. Optical rotations were measured with a Jasco DIP-181 polarimeter. UV spectra were taken with a Shimadzu UV-210A spectrometer. Thin-layer chromatography (TLC) was performed on pre-coated plates, Merck Kieselgel 60 F_{254} with $CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.01. Silica gel column chromatography was performed with Merck Kieselgel 60.

MIC Determination

The MIC values of each derivative against various bacteria were determined by the agar dilution

method using heart infusion agar (pH 7.0).

ID_{50} for the Binding to Ribosomes

The 50% inhibition doses (ID_{50}) of the derivatives for [10,11,12,13-^{3}H]tetrahydroleucomycin A_3 binding to *Escherichia coli* ribosomes were determined as described previously[4].

Retention Time (RT) in HPLC

HPLC was performed on a reverse phase silica gel column (Merck Lichrosorb RP-8, 4×250 mm) with CH_3CN - 0.2 M NaH_2PO_4, 1: 2, as a solvent system[5]. RT was recorded at 1 ml/minute of flow rate with a UV monitor (231 nm).

Therapeutic Effect in Experimental Mice Protection Test

The therapeutic effect was represented by survival percent. Mice (*dd*Y; ♂: 19±1 g, 10 mice per a group) were infected intraperitoneally with *Streptococcus pneumoniae* Type III. Compounds suspended in 0.3% sodium carboxymethyl cellulose were administered po immediately after infection. The survival percent values were recorded as percentages of the survival mice on the doses 100, 60 and 40 mg/kg at 7 day after infection.

2′-*O*-Acetyl-4″-*O*-mesylspiramycin I (**2**)

2′-*O*-Acetyl-4″-*O*-mesylspiramycin I 3,18-*O*-(*tert*-butyldimethylsilyl)acetal (**1**)[1] (1.42 g) was dissolved in 1 M solution of tetrabutylammonium fluoride in THF (1.58 ml) and stood for 1.3 hours at room temp. The reaction mixture was diluted with $CHCl_3$ (140 ml), washed with H_2O (140 ml), dried over Na_2SO_4, and evaporated to give an oily residue, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 3: 1, to give **2**, 1.04 g (81.9%). TLC Rf 0.33; $[\alpha]_D^{20}$ −70.2° (*c* 1.0, $CHCl_3$).

3,18,2′,3″-Tetra-*O*-acetyl-17,18-enol-4″-*O*-mesylspiramycin I (**3**) and 3,2′,3″-Tri-*O*-acetyl-4″-*O*-mesylspiramycin I (**4**)

To an ice-cooled solution of **2** (200 mg) in 1,2-dichloroethane (0.8 ml), tribenzylamine (632 mg) and acetyl chloride (0.16 ml) were mixed and heated at 75°C for 2 days. After addition of MeOH, the reaction mixture was diluted with $CHCl_3$ (20 ml) and washed with a $NaHCO_3$-saturated solution (20 ml) and then H_2O (20 ml). The $CHCl_3$ layer was dried over Na_2SO_4 and evaporated to give a residue, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 5: 1, to give **3** and **4** in the order of elution, as colorless powders.

3: 99 mg (41.4%); TLC Rf 0.46; $[\alpha]_D^{27}$ −11.4° (*c* 0.3, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 234 (34,000). High MS 1,046.522 (Calcd for $C_{50}H_{82}N_2O_{19}S$: 1,046.522).

4: 42 mg (18.3%); TLC Rf 0.42; $[\alpha]_D^{27}$ −78.6° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 232 (28,500). High MS 1,088.533 (Calcd for $C_{52}H_{84}N_2O_{20}S$: 1,088.533).

3,3″-Di-*O*-acetyl-4″-*O*-mesylspiramycin I (**5**)

To an ice-cooled solution of **2** (850 mg) in 1,2-dichloroethane (3.4 ml), tribenzylamine (2.69 g) and acetyl chloride (0.68 ml) were mixed and heated at 80°C for 2 days. After addition of MeOH, the reaction mixture was diluted with $CHCl_3$ (85 ml) and washed with a $NaHCO_3$-saturated solution (85 ml) and then H_2O (85 ml). The $CHCl_3$ layer was dried over Na_2SO_4 and evaporated to give a residue. The residue was dissolved in 70% MeOH (48 ml) and heated at 50°C for 22 hours. The reaction mixture was diluted with $CHCl_3$ (85 ml) and washed with a $NaHCO_3$-saturated solution (85 ml). The $CHCl_3$ layer was dried over Na_2SO_4 and evaporated to give a colorless solid, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 3: 1, to give **5**, 633 mg (71.4%). TLC Rf 0.36; $[\alpha]_D^{24}$ −60.2° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 232 (32,000). High MS 1,004.512 (Calcd for $C_{48}H_{80}N_2O_{18}S$: 1,004.512).

2′-*O*-Acetyl-4″-*O*-ethanesulfonylspiramycin I (**6**)

2′-*O*-Acetyl-4″-*O*-ethanesulfonylspiramycin I 3,18-(*O*-*tert*-butyldimethylsilyl)acetal[1] (964 mg) was treated with 1 M solution of tetrabutylammonium fluoride in THF (1.06 ml) as described in preparation of **2** to afford **8**, 408 mg (47.3%). TLC Rf 0.34; $[\alpha]_D^{20}$ −62.4° (*c* 1.0, $CHCl_3$).

3,3''-Di-*O*-acetyl-4''-*O*-ethanesulfonylspiramycin I (**11**)

6 (330 mg) was treated with tribenzylamine (1.07 g) and acetyl chloride (0.27 ml), and then 70% MeOH (16.5 ml) as described in preparation of **5** to afford **11**, 161 mg (46.6%). TLC Rf 0.37; $[\alpha]_D^{23}$ −54.8° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 235 (34,300). High MS 1,018.528 (Calcd for $C_{49}H_{82}N_2O_{18}S$: 1,018.528).

2'-*O*-Acetyl-4''-*O*-propanesulfonylspiramycin I (**7**)

2'-*O*-Acetyl-4''-*O*-propanesulfonylspiramycin I 3,18-(*O*-*tert*-butyldimethylsilyl)acetal[1] (570 mg) was treated with 1 M solution of tetrabutylammonium fluoride in THF (0.65 ml) as described in preparation of **2** to afford **7**, 244 mg (47.7%). TLC Rf 0.36; $[\alpha]_D^{20}$ −46.7° (*c* 1.0, $CHCl_3$).

3,3''-Di-*O*-acetyl-4''-*O*-propanesulfonylspiramycin I (**12**)

7 (573 mg) was treated with tribenzylamine (1.47 g) and acetyl chloride (0.46 ml), and then 70% MeOH (23.0 ml) as described in preparation of **5** to afford **12**, 113 mg (18.9%). TLC Rf 0.38; $[\alpha]_D^{23}$ −50.8° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 233 (34,300).

Anal Calcd for $C_{50}H_{84}N_2O_{18}S \cdot H_2O$: C 57.09, H 8.24, N 2.66.
Found: C 56.96, H 8.16, N 2.68.

3,3''-Di-*O*-propionyl-4''-*O*-ethanesulfonylspiramycin I (**13**)

6 (408 mg) was treated with tribenzylamine (1.25 g) and propionyl chloride (0.38 ml), and then 70% MeOH (16 ml) as described in preparation of **5** to afford **13**, 86 mg (19.7%). TLC Rf 0.43; $[\alpha]_D^{23}$ −55.8° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 234 (33,900). High MS 1,046.559 (Calcd for $C_{51}H_{86}N_2O_{18}S$: 1,046.559).

2'-*O*-Acetyl-4''-*O*-methylspiramycin I (**8**)

2'-*O*-Acetyl-4''-*O*-methylspiramycin I 3,18-(*O*-*tert*-butyldimethylsilyl)acetal[1] (373 mg) was treated with 1 M solution of tetrabutylammonium fluoride in THF (0.44 ml) as described in preparation of **2** to afford **8**, 237 mg (71.6%). TLC Rf 0.32; $[\alpha]_D^{20}$ −34.8° (*c* 1.0, $CHCl_3$).

3,3''-Di-*O*-acetyl-4''-*O*-methylspiramycin I (**14**)

8 (237 mg) was treated with tribenzylamine (846 mg) and acetyl chloride (0.22 ml), and then 70% MeOH (7.5 ml) as described in preparation of **5** to afford **14**, 118 mg (47.6%). TLC Rf 0.38; $[\alpha]_D^{23}$ −54.0° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 238 (32,700). High MS 940.550 (Calcd for $C_{48}H_{80}N_2O_{16}$: 940.550).

2'-*O*-Acetyl-4''-*O*-ethylspiramycin I (**9**)

2'-*O*-Acetyl-4''-*O*-ethylspiramycin I 3,18-(*O*-*tert*-butyldimethylsilyl)acetal[1] (1.83 g) was treated with 1 M solution of tetrabutylammonium fluoride in THF (2.14 ml) as described in preparation of **2** to afford **9**, 1.13 g (69.8%). TLC Rf 0.34; $[\alpha]_D^{20}$ −36.7° (*c* 1.0, $CHCl_3$).

3,3''-Di-*O*-acetyl-4''-*O*-ethylspiramycin I (**15**)

9 (800 mg) was treated with tribenzylamine (2.80 g) and acetyl chloride (0.71 ml), and then 70% MeOH (47 ml) as described in preparation of **5** to afford **15**, 661 mg (75.3%). TLC Rf 0.40; $[\alpha]_D^{23}$ −58.0° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 235 (32,800). High MS 954.566 (Calcd for $C_{49}H_{82}N_2O_{16}$: 954.566).

2'-*O*-Acetyl-4''-*O*-propylspiramycin I (**10**)

2'-*O*-Acetyl-4''-*O*-propylspiramycin I 3,18-(*O*-*tert*-butyldimethylsilyl)acetal[1] (914 mg) was treated with 1 M solution of tetrabutylammonium fluoride in THF (1.10 ml) as described in preparation of **2** to give **10**, 545 mg (52.4%). TLC Rf 0.35; $[\alpha]_D^{20}$ −39.4° (*c* 1.0, $CHCl_3$).

3,3''-Di-*O*-propionyl-4''-*O*-propylspiramycin I (**16**)

10 (348 mg) was treated with tribenzylamine (1.12 g) and propionyl chloride (0.39 ml), and then 70% MeOH (17.4 ml) as described in preparation of **5** to afford **16**, 212 mg (56.6%). TLC Rf 0.42; $[\alpha]_D^{23}$ −53.6° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 238 (33,700). High MS 996.613 (Calcd for $C_{52}H_{88}N_2O_{16}$: 996.613).

Acknowledgments

The authors wish to thank to Mr. R. MASUMA, The Kitasato Institute, for the MIC assay and Dr. K. SHIRAHATA and Mrs. M. YOSHIDA, Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd., for the NMR spectroscopy. The authors also thank to Messrs. K. KIKUTA and K. MAEBASHI for their technical assistance.

References

1) SANO, H.; T. SUNAZUKA, H. TANAKA, K. YAMASHITA, R. OKACHI & S. ŌMURA: Chemical modification of spiramycins. III. Synthesis and antibacterial activities of 4″-sulfonates and 4″-alkylethers of spiramycin I. J. Antibiotics 37: 750~759, 1984
2) SANO, H.; T. SUNAZUKA, H. TANAKA, K. YAMASHITA, R. OKACHI & S. ŌMURA: Chemical modification of spiramycins. IV. Synthesis and *in vitro* and *in vivo* activities of 3″,4″-diacylates and 3,3″,4″-triacylates of spiramycin I. J. Antibiotics 37: 760~772, 1984
3) SAKAKIBARA, H.; O. OKEKAWA, T. FUJIWARA, M. OTANI & S. ŌMURA: Acyl derivatives of 16-membered macrolides. I. Synthesis and biological properties of 3″-*O*-propionylleucomycin A_5 (TMS-19-Q). J. Antibiotics 34: 1001~1010, 1981
4) ŌMURA, S.; H. TANAKA, J. INOKOSHI, H. SAKAKIBARA & T. FUJIWARA: Binding of [^{3}H]tetrahydroleucomycin A_3 to *Escherichia coli* ribosomes and the effect of 3″-*O*-acyl derivatives of leucomycins on the binding. J. Antibiotics 35: 491~496, 1982
5) MOUROT, D.; B. DELÉPINE, J. BOISSEAU & G. GAYOT: Reverse-phase high-pressure liquid chromatography of spiramycin. J. Chromatogr. 161: 386~388, 1978