CHEMICAL MODIFICATION OF SPIRAMYCINS

III. SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF 4″-SULFONATES AND 4″-ALKYLETHERS OF SPIRAMYCIN I

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Among the derivatives protected with *t*-butyldimethylsilylether of spiramycin I, 2′-*O*-acetylspiramycin I 3,18-(*O*-*t*-butyldimethylsilyl)acetal was found to be a suitable intermediate for 4″-modification of spiramycin I. Seven 4″-sulfonates and four 4″-alkylethers were synthesized, which were more active against bacteria *in vitro* than spiramycin I. 4″-Substituted derivatives with relatively small sulfonyl and alkyl groups were comparable in therapeutic effect to spiramycin I.

Spiramycin is a complex of 16-membered macrolide antibiotics active against Gram-positive bacteria, mycoplasmas and toxoplasmas.[1] Spiramycin consists of four structural fragments, a 16-membered ring lactone and three sugars: mycaminose, mycarose and forosamine.[2] During some chemical modifications of spiramycins, certain 4″-*O*-acetyl derivative has been found to be more active *in vivo* than spiramycin.[3] It is estimated that the 4″-*O*-acetyl group delays a metabolism[4] of spiramycin in the body. Introduction of sulfonyl and/or alkyl groups, which are poorly hydrolyzed to the corresponding 4″-hydroxyl group would be an interesting approach to further chemical modifications of spiramycin.

Synthesis

Protection of Spiramycin I

Spiramycin I has four hydroxyl groups: 3, 2′, 3″ and 4″, and an aldehyde group in its molecule. In order to modify the 4″-hydroxyl group, it is necessary to protect the functional groups other than 3″-hydroxyl group, which is less reactive because it is a tertiary hydroxyl group and has a 1,3-diaxial relationship with the 1″ glycosidic linkage.

Since spiramycin I is labile in acid and base, the attachment and removal of protective groups must proceed around neutrality. A *t*-butyldimethylsilyl (TBDMS) group was found to be suitable for simultaneously protecting the 3-hydroxyl and 18-aldehyde functions by formation of the 3,18-*O*-TBDMS acetal.

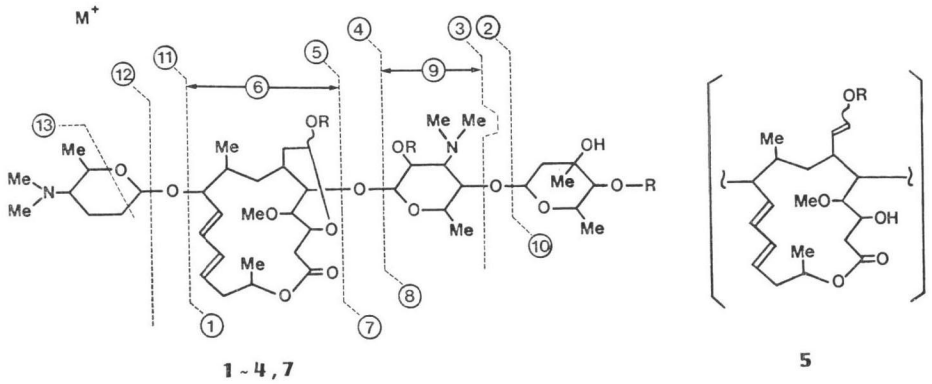
Treatment of spiramycin I with TBDMS chloride and imidazole gave 3,18-*O*-TBDMS acetal (**1**), di-TBDMS (**2**), tri-TBDMS (**3**) and a small amount of another tri-TBDMS (**4**) and *O*-TBDMS enol (**5**) derivatives in 18, 27, 14, 3 and 4% yield, respectively. Reactivity of hydroxyl groups on spiramycin I under these reaction conditions is in the order of 3,18-acetal, 2′ and 4″.

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

Carbon No.	1	2	3	4	5	7	8	16	19	27
1	170.0	169.9	170.0	170.0	171.5	169.9	169.9	169.9	174.1	174.3
2	33.7	33.4	33.4	33.4	36.6	32.8	32.7	32.7	37.7	37.7
3	70.1	70.1	70.1	69.8	68.5	68.1	68.1	68.0	68.2	68.3
4	83.2	82.1	82.1	82.0	82.8	86.3	86.2	86.2	79.3	85.3
5	86.6	86.6	86.7	87.0	79.3	82.5	82.6	82.4	85.2	79.1
6	39.4	39.5	39.6	39.5	31.4**	38.0	38.0	38.1	30.6	30.5
7	32.7	33.2	33.4	33.0	34.4**	34.5	34.7	34.7	30.7	30.7
8	37.1	36.3	36.6	36.6	34.5**	38.9	38.8	38.7	31.8	31.7
9	82.1	80.4	80.3	80.7	78.8	80.9	80.8	80.7	78.7	78.7
10	126.7	126.5	126.5	126.3	129.1	126.7	126.7	126.7	128.6	128.5
11	127.1	127.3	127.3	127.2	136.3	127.4	127.5	127.4	134.6	134.6
12	134.7	134.7	134.7	134.7	132.8	135.1	135.1	135.1	132.8	132.8
13	138.4	138.6	138.7	138.7	130.3	139.4	139.4	139.4	131.0	131.0
14	41.7	41.1	41.9	42.1	41.7	41.0	42.1	42.0	42.0	42.0
15	71.2	69.7	69.8	69.5	69.9	69.8	69.8	69.8	69.2	69.2
16	19.8	20.0	20.0	20.0	20.3	20.5	20.5	20.5	20.1	20.1
17	40.4	41.5	39.6	40.1	41.9	42.5	42.6	42.6	43.2	43.2
18	101.6	99.9	100.0	100.5	131.8	99.1	99.1	99.0	202.7	203.0
19	21.2	21.7	21.6	21.5	15.8	20.7	20.7	20.7	15.3	15.3
20	58.7	57.7	57.9	58.3	60.8	58.3	58.3	58.2	61.8	61.8
1′	103.8	103 5	103.4	103.5	104.4	102.9	102.9	102.8	103.9	103.7
2′	71.6	72.2	72.1	71.9	72.1	70.4	70.3	70.3	71.7	71.2
3′	68.8	70.8	70.4	70.5	69.1	70.4	70.5	70.5	68.7	68.8
4′	74.7	76.5	77.8	77.7	76.3	75.1	75.8	76.2	75.8	76.6
5′	73.3	72.3	72.2	72.2	73.3	73.0	72.9	72.9	72.9	73.2
6′	18.5	18.6	18.5	18.5	19.2*	19.2	18.9	18.9	19.0	18.8
3′-NCH_3	42.1	41.5	41.5	41.4	41.9	41.7	41.7	41.6	42.0	41.8
1″	96.2	96.7	96.9	97.5	97.0	96.5	96.6	97.3	96.5	97.3
2″	40.9	39.7	40.7	40.1	40.7	40.2	40.2	40.2	40.7	40.7
3″	69.4	69.5	69.8	69.5	69.8	69.5	69.6	70.3	69.5	70.2
4″	76.5	76.6	78.7	78.3	81.2	76.4	85.0	85.9	85.0	85.8
5″	66.0	66.2	65.8	66.0	65.5	66.0	63.3	64.9	63.8	65.2
6″	19.0	19.2	19.1	18.6	18.5*	18.4	18.5	18.5	18.4	18.4
7″	25.4	25.5	27.4	26.3	27.1	25.4	26.3	25.8	26.4	26.0
1‴	99.8	98.4	98.4	98.8	101.6	99.4	99.5	99.4	100.2	100.2
2‴	31.2	31.2	31.2	31.3	31.4	30.9	30.9	30.8	31.3	31.3
3‴	18.3	18.3	18.3	18.2	18.6*	18.4	18.5	18.5	18.9	18.5
4‴	64.9	65.1	65.1	65.1	65.0	64.9	64.9	64.9	64.8	64.9
5‴	74.0	73.7	73.6	73.6	73.3	73.8	73.9	73.7	73.9	73.8
6‴	19.3	19.3	19.2	19.2	19.3*	19.2	19.2	19.2	19.0	19.0
4‴-NCH_3	40.7	40.7	40.7	40.7	40.7	40.7	40.7	40.7	40.7	40.7
18-$Si(CH_3)_2$	−5.2	−4.9	−4.9	−5.0	−5.5	−5.2	−5.2	−5.2		
	−3.9	−3.7	−3.8	−4.0	−5.3	−4.0	−4.0	−4.0		
2′-$Si(CH_3)_2$		−3.2	−3.4	−3.4	−4.6	2′-$OCOCH_3$	$OCOCH_3$	$OCOCH_3$		
		−1.6	−2.1	−2.1	−3.2	21.5	21.5	21.5		
4″-$Si(CH_3)_2$			−3.6	−3.8	−5.0	168.4	168.5	168.5		
			−3.3	−2.7	−3.4					
18-Si*C*$(CH_3)_3$	18.0	18.0	18.1	18.0	18.0	18.1	18.1	18.1		
2′-Si*C*$(CH_3)_3$		18.3	18.4	18.4	18.5					
4″-Si*C*$(CH_3)_3$			18.4	18.4	18.6					
18-Si$C(CH_3)_3$	25.9	25.9	26.0	25.8	25.5	26.0	26.0	26.0		
2′-Si$C(CH_3)_3$		26.5	26.4	26.1	26.2		SO_2CH_3	OCH_2CH_3	SO_2CH_3	OCH_2CH_3
4″-Si$C(CH_3)_3$			26.1	25.9	25.9		38.9	15.8	38.9	15.8
								69.3		69.3

*,** Assignments of carbons may be interchangeable.

Table 2. Diagnostic mass fragmentation for TBDMS derivatives of spiramycin I.

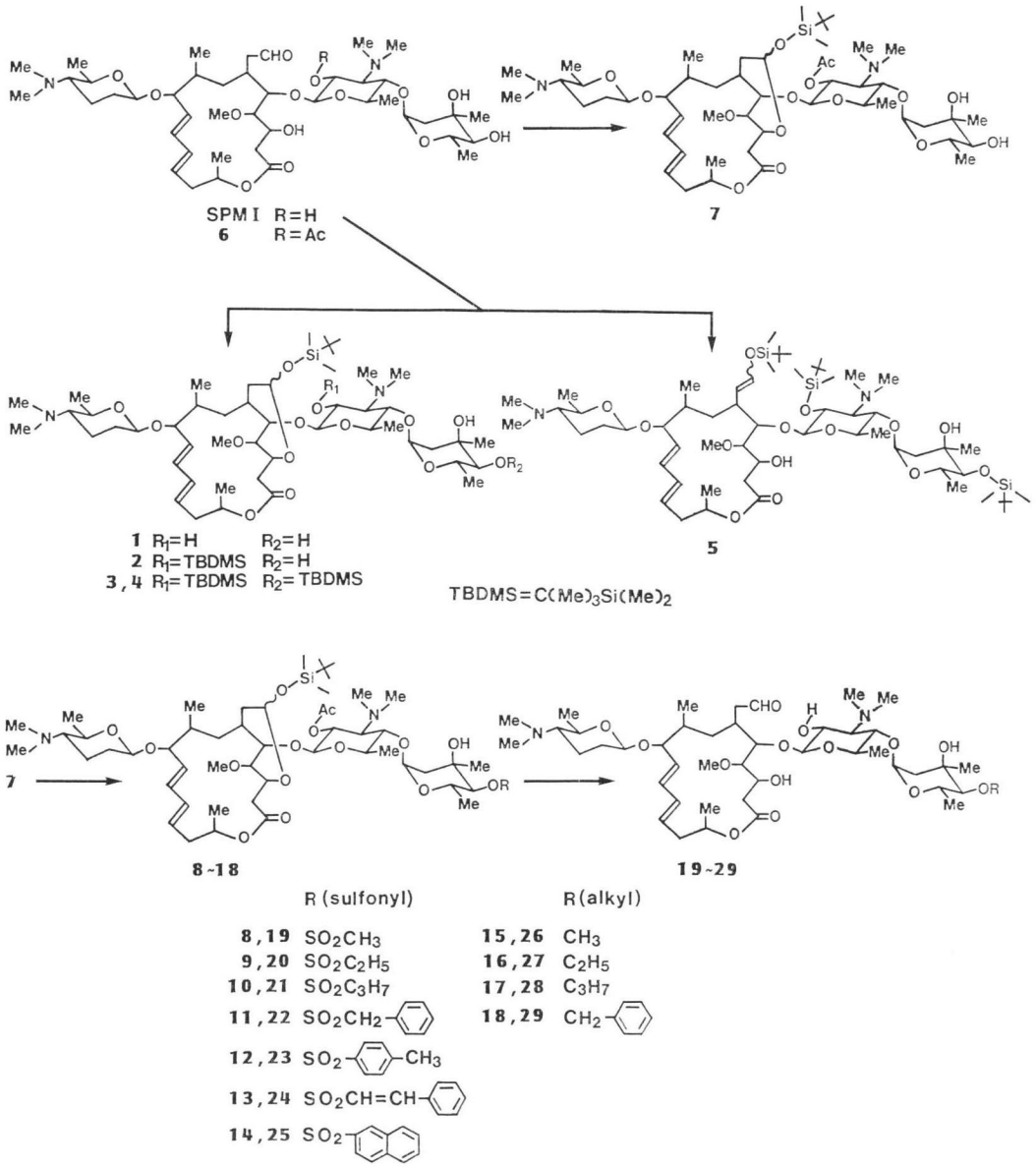


	1	2	3	4	5	7
M^+	956	1,070	1,184	1,184	1,184	998
1	−H 797	912	1,026	1,026	1,026	−H 854
2+H	+H 813	926	926	926	926	854
3+H	796	910	910	910	910	+H 839
4	638	638	638	638	638	638
5	622	−H 621	−H 621	−H 621	622	622
6−H	463	−H 462	−H 462	−H 462	463	−H 462
7	—	448	562	562	—	−H 375
8	318	432	546	546	546	360
9+H	174	288	288	288	288	216
10	145	145	259	259	259	—
11	158	158	158	158	158	158
12	142	142	142	142	142	142
13	114	114	114	114	114	114

The ^{13}C NMR spectrum of **1** (Table 1) showed $SiCH_3$, $SiC(CH_3)_3$ and $SiC(CH_3)_3$ signals of one TBDMS group, an additional acetal carbon and loss of aldehyde carbon observed in spiramycin I. Downfield shift of 3-carbon and upfield shift of 1- and 2-carbons implied the 3-substitution, thus confirming its structure. Conformation of the aglycone moiety of **1** seemed to be changed, considering the alteration of their chemical shifts, especially of carbons assigned to diene. Di-TBDMS (**2**) or two tri-TBDMS derivatives (**3** and **4**) showed additional carbons of TBDMS groups and changes of chemical shifts of 2′ or 2′, 4″ and 7″ carbon, respectively, in their ^{13}C NMR spectra. As both **3** and **4** were consistent with the structure of tri-TBDMS, they seemed to be diastereomers at 18-acetal carbon, each other. In the ^{13}C NMR spectra of **5**, two additional olefinic carbons assignable to 17,18-enol carbons and a loss of aldehyde carbon were observed. These observations in the ^{13}C NMR spectra accompanying the mass spectral results (Table 2) were well assisted their structures.

Although **2** was a derivative protected at 3-hydroxyl, aldehyde and 2′-hydroxyl groups, 2′-*O*-TBDMS group was scarcely removed under usual conditions. 2′-*O*-Acetyl group of 2′-*O*-acetylspiramycin I (**6**)[5] is easily removed by methanolysis participated neighboring dimethylamino group. Treatment of **6** with TBDMS chloride and imidazole afforded 2′-*O*-acetylspiramycin I 3,18-*O*-TBDMS acetal (**7**) as a sole product, which was a suitable protected derivative for 4″-modification. The structure of **7** was confirmed by the similarity of the ^{13}C NMR spectrum to that of **2**.

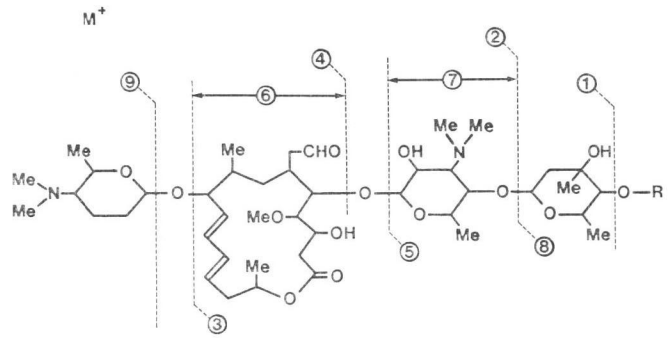
Scheme 1.



Sulfonylation and Alkylation at 4″-Position

Sulfonylation and alkylation of **7** were achieved on treatment of **7** with the corresponding sulfonyl chloride in pyridine, and alkyl iodide and sodium hydride in *N*,*N*-dimethylformamide, respectively. Mesyl (**8**), ethanesulfonyl (**9**), propanesulfonyl (**10**), benzylsulfonyl (**11**), tosyl (**12**), β-styrenesulfonyl (**13**) and β-naphthalenesulfonyl (**14**) derivatives and methyl (**15**), ethyl (**16**), propyl (**17**) and benzyl (**18**) derivatives were synthesized. Methanolysis of **8**～**18**, followed by treatment with tetrabutylammonium fluoride, gave free 4″-sulfonates (**19**～**25**) and 4″-alkylethers (**26**～**29**). The structures of **19**～**29** were confirmed by the signals of the corresponding sulfonyl and alkyl groups and downfield shift of 4″- and 7″-carbons and upfield shift of 5″-carbon observed in their ^{13}C NMR spectra (Table 1) and fragmenta-

Table 3. Diagnostic mass fragmentation for spiramycin I derivatives.



	19	20	21	22	23	24	25	26	27	28	29
M^+	—	—	—	—	—	—	—	856	870	−H 883	932
M^+-H_2O	—	—	—	—	—	—	—	838	852	−H 865	+H 915
1	−H 825	826	−H 825	−H 825	−2H 824	—	−H 825	—	—	−2H 824	−H 825
2+H	698	698	698	698	698	698	698	698	698	698	698
3	665	—	667	—	—	—	—	+H 699	—	+H 727	—
4	—	−H 507	−H 507	−H 507	+H 509	−H 507	—	−H 507	508	−H 507	−H 507
5	—	—	—	—	—	—	—	332	346	360	—
6−H	349	—	349	349	349	349	349	349	349	349	349
7+H	174	174	174	174	174	174	174	174	174	174	174
8	—	—	—	—	—	—	—	159	173	187	235
9	142	142	142	142	142	142	142	142	142	142	142

tion pattern in mass spectra (Table 3).

Biological Activities

Spiramycin I derivatives were evaluated by four parameters: MIC, affinity to ribosomes (ID_{50}),[6] retention time(RT) in HPLC[7] and therapeutic effects in mice (ED_{50}), as shown in Table 4. In the MIC values, 4″-sulfonates and 4″-alkylethers of spiramycin I were superior to spiramycin I regardless of the bulkiness of the substituents. Among them 4″-*O*-tosylspiramycin I (**23**) showed a considerable activity against macrolide-resistant *Staphylococcus aureus*.

In the ID_{50} values, ethanesulfonyl (**20**), propanesulfonyl (**21**), tosyl (**23**), β-naphthalenesulfonyl (**25**) derivatives were comparable to spiramycin I but the other derivatives showed somewhat higher ID_{50} than spiramycin I. Any relation with the structure was not found among them.

The ED_{50} values of the 4″-derivatives bearing relatively small sulfonyl groups, that are mesyl (**19**) and ethanesulfonyl (**20**), were similar to that of spiramycin I, whereas those of more bulky benzylsulfonyl (**22**) and tosyl (**23**) derivatives were larger. The ED_{50} values almost correlate the RT values.

4″-*O*-Ethylspiramycin I (**27**) is equally effective against *Streptococcus pneumoniae* III infection but less active against *Staphylococcus aureus* Smith infection.

4″-Substituted derivatives with relatively small sulfonyl and alkyl groups are interesting as an objective of further modification.

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

Table 4. MIC, ID_{50}, ED_{50} and RT of 4''-sulfonates and 4''-alkylethers of spiramycin I (SPM I).

Compound		MIC (μg/ml)*1							ID_{50} (μM)	ED_{50} (mg/kg)*2		RT (minutes)
No.	R	SA	SA^r	BS	BC	ML	EC	KP		SA	SP	
19	$-SO_2CH_3$	1.56	>100	1.56	1.56	<0.1	100	50	1.2	89.7	—	6.7
20	$-SO_2C_2H_5$	1.56	100	3.12	1.56	<0.1	>100	50	0.7	71.4	143	8.7
21	$-SO_2C_3H_7$	1.56	100	1.56	1.56	<0.1	>100	50	0.9	—	—	13.1
22	$-SO_2CH_2C_6H_5$	1.56	>100	1.56	1.56	<0.1	>100	12.5	1.2	216.8	—	26.3
23	$-SO_2C_6H_4CH_3$	1.56	12.5	1.56	1.56	0.2	100	50	0.8	>300	—	42.1
24	$-SO_2CH=CHC_6H_5$	1.56	>100	1.56	1.56	<0.1	>100	25	1.7	—	—	45.2
25	$-SO_2C_{10}H_8$	0.78	>100	1.56	1.56	<0.1	100	50	0.8	—	—	74.0
26	$-CH_3$	3.12	>100	3.12	6.25	0.4	>100	>100	1.8	—	—	6.7
27	$-C_2H_5$	1.56	>100	1.56	3.12	0.2	100	25	1.5	225.2	115	9.0
28	$-C_3H_7$	1.56	>100	1.56	1.56	<0.1	100	50	1.4	—	—	9.8
29	$-CH_2C_6H_5$	1.56	>100	1.56	1.56	<0.1	100	50	1.5	—	—	21.1
SPM I	–H	3.12	>100	1.56	3.12	<0.1	>100	>100	1.0	116.6	117	4.3
AcSPM		6.25	>100	3.12	3.12	<0.1	>100	>100	1.9	110.7	65	

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

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 Kiesel gel 60 F_{254} with $CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.01. Silica gel column chromatography was performed with Merck Kiesel gel 60.

Minimum Inhibitory Concentration

MIC values 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 dose (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.[7]

Therapeutic Effect in Experimental Mice Protection Test

Mice (*dd*Y; ♂: 19±1 g) were infected intraperitoneally with *Staphylococcus aureus* Smith or *Streptococcus pneumoniae* Type III, respectively. Compounds suspended in 0.3% sodium carboxymethyl cellulose were administered po immediately post infection. ED_{50} values were determined by Litchfield-Wilcoxon method according to the mortality of mice at 7 day after infection.

Retention Time (RT) in HPLC

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

Spiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**1**), 2′-*O*-*t*-Butyldimethylsilylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**2**), 2′,4″-Di-*O*-*t*-butyldimethylsilylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**3** and **4**) and 18,2′,4″-Tri-*O*-*t*-Butyldimethysilylspiramycin I Enol (**5**)

To a solution of spiramycin I (4.21 g) in DMF (20 ml), *t*-butyldimethylsilyl chloride (6.02 g) and imidazole (5.44 g) were added and stirred for 2 days at room temp. After addition of MeOH, the reaction mixture was diluted with $CHCl_3$ (200 ml) and washed with H_2O (200 ml). The $CHCl_3$ layer was dried over anhydrous sodium sulfate and evaporated to give a residue, which was chromatographed on a silica gel column with C_6H_6 - EtOAc, 1: 1, to give **3**, **4**, **2**, **5** and **1** in the order of elution, as a colorless powder, respectively.

1, 0.88 g (18.4%). TLC Rf 0.30; $[\alpha]_D^{17}$ −40.8° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 234 (24,700).
Anal Calcd for $C_{49}H_{88}N_2O_{14}Si$: C 61.48, H 9.26.
Found: C 61.00, H 9.34.

2, 1.46 g (27.3%). TLC Rf 0.49; $[\alpha]_D^{17}$ −19.2° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 234 (22,500).
Anal Calcd for $C_{55}H_{102}N_2O_{14}Si_2$: C 61.65, H 9.59, N 2.61.
Found: C 61.95, H 9.46, N 2.70.

3, 0.80 g (13.6%). TLC Rf 0.73; $[\alpha]_D^{17}$ −16.8° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 236 (21,800).
Anal Calcd for $C_{61}H_{116}N_2O_{14}Si_3$: C 61.80, H 9.86, N 2.36.
Found: C 61.47, H 9.95, N 2.34.

4, 0.18 g (3.1%). TLC Rf 0.67; $[\alpha]_D^{17}$ −14.4° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 235 (12,000).
Anal Calcd for $C_{61}H_{116}N_2O_{14}Si_3 \cdot H_2O$: C 60.88, H 9.88, N 2.33.
Found: C 61.00, H 9.78, N 2.33.

5, 0.23 g (4.3%). TLC Rf 0.39; $[\alpha]_D^{17}$ −46.8° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 232 (16,800).
Anal Calcd for $C_{61}H_{116}N_2O_{14}Si_3 \cdot H_2O$: C 60.87, H 9.88.
Found: C 60.73, H 9.55.

2′-*O*-Acetylspiramycin I (**6**)

2′-*O*-Acetylspiramycin I (**6**) was prepared in a similar way described previously.[5]

2′-*O*-Acetylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**7**)

To a solution of **6** (21.00 g) in DMF (105 ml), *t*-butyldimethylsilyl chloride (7.23 g) and imidazole (6.52 g) were added and stirred for 1 day. After addition of MeOH, the reaction mixture was diluted with $CHCl_3$ (1 liter) and washed with water (1 liter). The $CHCl_3$ layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was chromatographed on a silica gel

column with C_6H_6 - Me_2CO, 4: 1, to give **7**, 17.65 g (74.5%). TLC Rf 0.37; $[\alpha]_D^{20}$ −35.5° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 235 (34,600).

Anal Calcd for $C_{51}H_{90}N_2O_{15}Si$: C 61.30, H 9.07, N 2.80.
Found: C 61.40, H 8.99, N 3.12.

2′-*O*-Acetyl-4″-*O*-mesylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**8**)

To an ice-cooled solution of **7** (2.32 g) in $CHCl_3$ (58 ml) and pyridine (7.4 ml), mesyl chloride (3.1 ml) was added and stood for 24 hours at room temp. After addition of MeOH, the reaction mixture was diluted with $CHCl_3$ (500 ml) and washed with a saturated aqueous sodium hydrogen carbonate solution (500 ml). The $CHCl_3$ layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure, to give a colorless solid, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 3: 1, to give **8**, 2.12 g (84.8%). TLC Rf 0.39; $[\alpha]_D^{19}$ −64.6° (*c* 1.0, $CHCl_3$).

4″-*O*-Mesylspiramycin I (**19**)

A solution of **8** (65 mg) in MeOH (3 ml) was heated at 50°C for 3 days. The reaction mixture was evaporated to give a colorless solid. The dried solid was dissolved in 1 M solution of tetrabutylammonium fluoride in THF (0.14 ml) and stood for 1.5 hours at room temp. To the reaction mixture, $CHCl_3$ (6 ml) was added and washed with H_2O (6 ml), dried over anhydrous sodium sulfate. The $CHCl_3$ solution was evaporated to give an oily residue, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 1: 1, to give **19**, 39 mg (70.8%). TLC Rf 0.24; $[\alpha]_D^{24}$ −56.8° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 239 (30,900).

Anal Calcd for $C_{44}H_{76}N_2O_{16}S \cdot \frac{1}{2}H_2O$: C 56.81, H 8.34, N 3.01, S 3.44.
Found: C 56.64, H 8.34, N 2.89, S 3.05.

2′-*O*-Acetyl-4″-*O*-ethanesulfonylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**9**)

To a solution of **7** (300 mg) in pyridine (9 ml), ethanesulfonyl chloride (0.09 ml) was added and stood at room temp for 19 hours. The reaction mixture was worked up in a similar manner described in the preparation of **8**, to give **9**, 149 mg (45.0%). TLC Rf 0.40; $[\alpha]_D^{19}$ −53.4° (*c* 1.0, $CHCl_3$).

4″-*O*-Ethanesulfonylspiramycin I (**20**)

Treatment of **9** (107 mg) with MeOH (4.3 ml) and then 1 M solution of tetrabutylammonium fluoride in THF (0.10 ml) afforded **20**, 34 mg (37.0%). TLC Rf 0.26; $[\alpha]_D^{24}$ −47.7° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 236 (29,800).

Anal Calcd for $C_{45}H_{78}N_2O_{16}S \cdot \frac{1}{2}H_2O$: C 57.24, H 8.43, N 2.97, S 3.39.
Found: C 57.00, H 8.25, N 2.77, S 2.64.

2′-*O*-Acetyl-4″-*O*-propanesulfonylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**10**)

Treatment of **7** (1.30 g) with propanesulfonyl chloride (0.45 ml) in pyridine (19.5 ml) in a similar way described in a preparation of **8**, to afford **10**, 856 mg (59.5%). TLC Rf 0.40; $[\alpha]_D^{19}$ −33.2° (*c* 1.0, $CHCl_3$).

4″-*O*-Propanesulfonylspiramycin I (**21**)

A solution of **10** (800 mg) in MeOH (32 ml) was heated at 50°C for 4 days. The reaction mixture was evaporated to give a residue, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 3: 1, to give a colorless solid. The colorless solid was dissolved in 1 M solution of tetrabutylammonium fluoride in THF (0.65 ml) and stood for 1.5 hours at room temp. The reaction mixture was treated in a similar way described in a preparation of **19**, to afford **21**, 244 mg (35.1%). TLC Rf 0.26; $[\alpha]_D^{24}$ −65.8° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 233 (32,500).

Anal Calcd for $C_{46}H_{80}N_2O_{16}S \cdot H_2O$: C 57.12, H 8.54, N 2.89, S 3.31.
Found: C 57.06, H 8.20, N 2.72, S 2.55.

2′-*O*-Acetyl-4″-*O*-benzylsulfonylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**11**)

7 (200 mg) was treated with benzylsulfonyl chloride (84 mg) in a similar way described in a preparation of **8**, to afford **11**, 218 mg (96.0%). TLC Rf 0.45; $[\alpha]_D^{19}$ −18.0° (*c* 1.0, $CHCl_3$).

4″-*O*-Benzylsulfonylspiramycin I (**22**)

11 (184 mg) was treated with MeOH (7.4 ml) and then 1 M solution of tetrabutylammonium fluoride in THF (0.19 ml) in a similar way described in a preparation of **19**, to afford **22**, 100 mg (62.0%). TLC

Rf 0.27; $[\alpha]_D^{24}$ −47.6° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 242 (34,300).

Anal Calcd for $C_{50}H_{82}N_2O_{16}S \cdot \frac{1}{2}H_2O$: C 59.68, H 8.11, N 2.78, S 3.19.
Found: C 59.42, H 8.07, N 2.69, S 2.87.

2′-*O*-Acetyl-4″-*O*-*p*-toluenesulfonylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**12**)

7 (2.00 g) was treated with *p*-toluenesulfonyl chloride (915 mg) in a similar way described in a preparation of **8**, to afford **12**, 1.67 g (72.3%). TLC Rf 0.40; $[\alpha]_D^{19}$ −25.4° (*c* 1.0, $CHCl_3$).

4″-*O*-*p*-Toluenesulfonylspiramycin I (**23**)

12 (32 mg) was treated with MeOH (1.3 ml) and then 1 M solution of tetrabutylammonium fluoride in THF (0.04 ml) in a similar way described in a preparation of **19**, to afford **23**, 25 mg (88.0%). TLC Rf 0.27; $[\alpha]_D^{24}$ −57.8° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 238 (32,000).

Anal Calcd for $C_{50}H_{80}N_2O_{16}S \cdot \frac{1}{2}H_2O$: C 60.22, H 8.09, N 2.81, S 3.22.
Found: C 59.68, H 8.11, N 2.78, S 3.19.

2′-*O*-Acetyl-4″-*O*-β-styrenesulfonylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**13**)

7 (300 mg) was treated with β-styrenesulfonyl chloride (146 mg) in a similar way described in a preparation of **8**, to afford **13**, 193 mg (52.0%). TLC Rf 0.45; $[\alpha]_D^{19}$ −24.0° (*c* 1.0, $CHCl_3$).

4″-*O*-β-Styrenesulfonylspiramycin I (**24**)

13 (151 mg) was treated with MeOH (6 ml) and then 1 M solution of tetrabutylammonium fluoride in THF (0.14 ml) in a preparation of **19**, to afford **24**, 98 mg (80.0%). TLC Rf 0.29; $[\alpha]_D^{24}$ −50.4° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 206 (24,200), 234 (75,600).

Anal Calcd for $C_{51}H_{80}N_2O_{16}S \cdot H_2O$: C 59.62, H 8.04, N 2.72, S 3.12.
Found: C 59.46, H 7.96, N 2.61, S 2.84.

2′-*O*-Acetyl-4″-*O*-β-naphthalenesulfonylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**14**)

7 (200 mg) was treated with 2-naphthalenesulfonyl chloride (218 mg) in a similar way described in a preparation of **8**, to afford **14**, 164 mg (69.0%). TLC Rf 0.46; $[\alpha]_D^{19}$ −29.6° (*c* 1.0, $CHCl_3$).

4″-*O*-β-Naphthalenesulfonylspiramycin I (**25**)

14 (104 mg) was treated with MeOH (4.2 ml) and then 1 M solution of tetrabutylammonium fluoride in THF (0.12 ml) in a similar way described in a preparation of **19**, to afford **25**, 64 mg (75.0%). TLC Rf 0.30; $[\alpha]_D^{24}$ −66.5° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 239 (44,400), 276 (12,600), 313 (2,600), 324 (3,400).

Anal Calcd for $C_{53}H_{80}N_2O_{16}S \cdot H_2O$: C 60.55, H 7.86, N 2.66, S 3.05.
Found: C 60.43, H 7.55, N 2.31, S 2.43.

2′-*O*-Acetyl-4″-*O*-methylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**15**)

7 (200 mg) and 60% oily sodium hydride (12 mg) were dissolved in DMF (4 ml). After 10 minutes, methyl iodide (32 ml) was added to the solution and stood at room temp for 1.5 hours. After addition of MeOH, the reaction mixture was diluted with $CHCl_3$ (20 ml) and washed with H_2O (20 ml). The $CHCl_3$ layer was dried over anhydrous sodium sulfate and evaporated, giving a colorless solid, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 4: 1, gave **15**, 92 mg (48.8%). TLC Rf 0.39; $[\alpha]_D^{19}$ −20.2° (*c* 1.0, $CHCl_3$).

4″-*O*-Methylspiramycin I (**26**)

A solution of **15** (72 mg) in MeOH (2.9 ml) was heated at 50°C for 63 hours. The reaction mixture was evaporated to give a colorless solid. The dried solid was dissolved in 1 M solution of tetrabutylammonium fluoride in THF (0.09 ml) and stood for 2 hours at room temp. The reaction mixture was diluted with $CHCl_3$ (7 ml) and washed with H_2O (7 ml). The $CHCl_3$ layer was dried over anhydrous sodium sulfate and evaporated to give a residue, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 1: 1, to give **26**, 44 mg (72.0%). TLC Rf 0.24; $[\alpha]_D^{24}$ −47.2° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 233 (24,400); High MS 856.530 (Calcd for $C_{46}H_{78}N_2O_{13}$: 856.529).

2′-*O*-Acetyl-4″-*O*-ethylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**16**)

7 (3.00 g) was treated with ethyl iodide (0.64 ml) and 60% oily sodium hydride (180 mg) in a similar way described in a preparation of **15**, to afford **16**, 1.80 g (58.4%). TLC Rf 0.39; $[\alpha]_D^{19}$ −21.0° (*c* 1.0, $CHCl_3$).

4''-*O*-Ethylspiramycin I (**27**)

16 (1.40 mg) was treated with MeOH (56 ml) and then 1 M solution of tetrabutylammonium fluoride in THF (1.68 ml) in a similar way described in a preparation of **26**, to afford, **27**, 729 mg (61.0%). TLC Rf 0.24; $[\alpha]_D^{24}$ −57.6° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 231 (17,000); High MS 870.545 (Calcd for $C_{45}H_{75}N_2O_{14}$: 870.545).

2'-*O*-Acetyl-4''-*O*-propylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**17**)

7 (1.00 g) was treated with propyl iodide (0.96 ml) and 60% oily sodium hydride (240 mg) in a similar way described in a preparation of **15**, to afford **17**, 507 mg (48.7%). TLC Rf 0.40; $[\alpha]_D^{19}$ −22.8° (*c* 1.0, $CHCl_3$).

4''-*O*-Propylspiramycin I (**28**)

A solution of **17** (500 mg) was treated with MeOH (20 ml) and then 1 M solution of tetrabutylammonium fluoride in THF (0.6 ml) in a similar way described in a preparation of **26**, to afford **28**, 328 mg (77.2%). TLC Rf 0.25; $[\alpha]_D^{24}$ −52.6° (*c* 1.0, $CHCl_3$), UV λ_{max}^{MeOH} nm (ε) 237 (25,900); High MS 884.560 (Calcd for $C_{46}H_{80}N_2O_{14}$: 884.560).

2'-*O*-Acetyl-4''-*O*-benzylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**18**)

7 (200 mg) was treated with benzyl bromide (0.09 ml) and 60% oily sodium hydride (72 mg) in a similar way described in a preparation of **15**, to give **18**, 116 mg (53.3%). TLC Rf 0.41; $[\alpha]_D^{19}$ −19.0° (*c* 1.0, $CHCl_3$).

4''-*O*-Benzylspiramycin I (**29**)

18 (113 mg) was treated with MeOH (4.5 ml) and then 1 M solution of tetrabutylammonium fluoride in THF (0.12 ml) in a similar way described in a preparation of **26**, to afford **29**, 63 mg (69.0%). TLC Rf 0.26; $[\alpha]_D^{24}$ −43.0° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 238 (29,600); High MS 932.561 (Calcd for $C_{50}H_{80}N_2O_{14}$: 932.560).

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