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

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3″,4″-Diacylates and 3,3″,4″-triacylates of spiramycin I were synthesized and evaluated by the four parameters, MIC against bacteria, affinity to ribosomes, retention time in HPLC and therapeutic effect. Among them, 3,3″,4″-tri-*O*-propionyl and 3,4″-di-*O*-acetyl-3″-*O*-butyryl-spiramycin I were the most active *in vivo*, which were superior to acetylspiramycin.

Spiramycin,[1] a 16-membered macrolide antibiotics complex, consists of three major components differentiated in the substituent at 3-position, namely I (3-OH), II (3-*O*-acetyl) and III (3-*O*-propionyl) and contains no components acylated at 4″ position. 4″-*O*-Acetylspiramycin II[2] which was synthesized from spiramycin I is known to show a stronger therapeutic effect than that of spiramycin I. Several kinds of 3″-acylates of 16-membered macrolides, such as leucomycin A_3[3] and midecamycin,[4] have also been reported to be more effective *in vivo* than their parent antibiotics. Spiramycin I was reported to be inactivated through a lactone ring opening by metabolism which is inhibited by a 3-*O*-acetyl group.[5] From these findings, we have been very interested in the synthesis and activities of the 3,4″-di- and 3,3″, 4″-triacylates of spiramycin I, and wish to report the details in this paper.

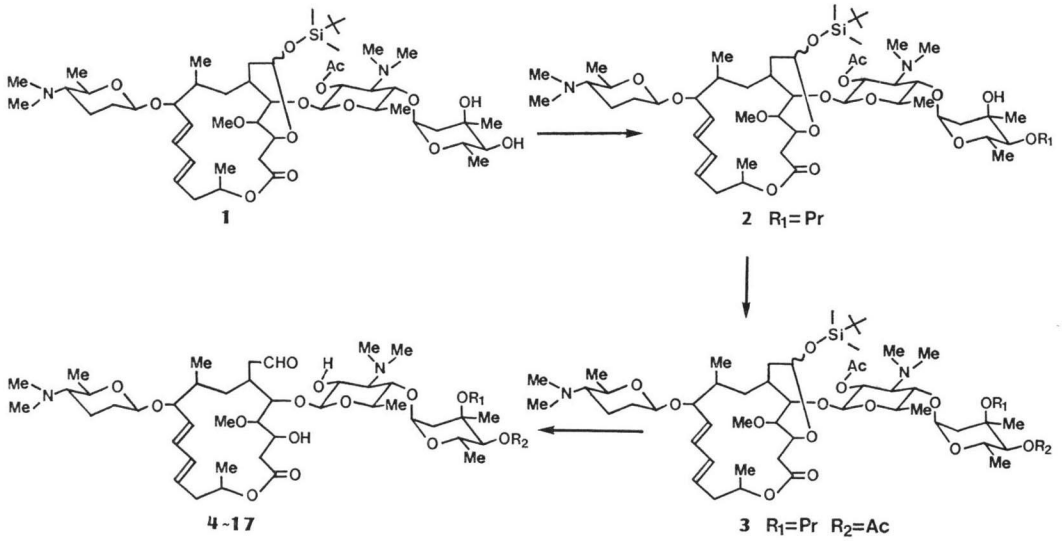
Synthesis

Spiramycin I has four hydroxyl groups, 3, 2′, 3″ and 4″ in its molecule, in which the order of reactivity to acylation under basic conditions is 2′>4″>3>3″. The 3″-hydroxyl group of spiramycin I is less reactive, because it is a tertiary alcohol and there lies a 1,3-diaxial interaction between 1″- and 3″-positions. Two alternative devices for acylation of 3″-hydroxyl group of mycarose were reported; one is a direct acylation using acyl chloride and tribenzylamine,[3] and the other is a acyl migration from 4″→3″-position.[4]

In order to synthesize a derivative bearing a free hydroxyl group at 3-position, protection of 3-hydroxyl group is necessary. 2′-*O*-Acetylspiramycin I 3,18-(*O*-*t*-butyldimethylsilyl)acetal (**1**)[6] was suitable for this purpose. Treatment of **1** with propionyl chloride in pyridine gave 4″-propionate (**2**). Direct acetylation of 3″-hydroxyl group of **2** could not be achieved under various reaction conditions. The 3″-hydroxyl group of 3,18-(*O*-*t*-butyldimethylsilyl)acetal protected derivative seems to be highly hindered sterically in addition to its low reactivity.

The 3″-acylate could be synthesized applying the acyl migration reaction conditions. 3″-*O*-Propionyl-2′,4″-di-*O*-acetylspiramycin I 3,18-(*O*-*t*-butyldimethylsilyl)acetal (**3**) was afforded in a low

Scheme 1.



yield on treatment of **2** with acetic anhydride in pyridine at refluxing temperature, which is the conditions for the synthesis of 3″-acylate of midecamycin.[4] **3** was afforded almost quantatively under rather mild conditions, heating **2** with acetic anhydride in the presence of 4-dimethylaminopyridine and triethylamine in chloroform. It is certain that acylation proceeds through an 4″→3″ acyl migration reaction, because spiramycin I derivative bearing the same protective groups as those of **3** and the 4″-*O*-substituent which is known not to migrate, *e.g.* 2′-*O*-acetyl-4″-*O*-mesylspiramycin I 3,18-*O*-(*t*-butyldimethylsilyl)acetal,[6] could not be acylated under the reaction conditions described above,[7] and from the structural evidence confirmed by ^{13}C NMR spectrum, as described below.

2′-Acetyl and *t*-butyldimethylsilyl protective groups were removed by methanolysis and treatment with tetrabutylammonium fluoride, respectively, to give 4″-*O*-acetyl-3″-*O*-propionylspiramycin I (**8**). **8** was also synthesized without any purification of the intermediates. The other 3″,4″-diacylates were prepared by a similar manner and are summarized in Table 4.

The ^{13}C NMR spectrum of **8** showed signals of one acetyl and one propionyl group, downfield shifts of 3″- and 4″-carbons, and upfield shifts of 2″-, 5″- and 7″-carbons comparing with that of spiramycin I, indicating that 3″- and 4″-hydroxyl groups of spiramycin I were acylated by acetyl and propionyl groups. The mass spectral fragmentation also assisted the structure elucidation. From the comparison of the ^{13}C NMR spectra of 2′-, 2′,4″-di-, 3,2′,4″-tri-, 3″,4″-di- and 3,3″,4″-triacylates of spiramycin I, it was found that carbons of acyl groups resonanced at the corresponding region correlated with their bonding positions (Table 2). 4″-*O*-Acetyl-3″-*O*-propionyl structure of **8** was decided from this relationship between the bonding position of acyl groups in spiramycin I derivatives and ^{13}C NMR chemical shifts of acyl carbons. The structure of the other 3″,4″-diacylates of spiramycin I were also confirmed in the same manner.

3,3″,4″-Triacylates of spiramycin I were synthesized through selective acylation and selective removal of acyl groups. Spiramycin I was acylated with propionic anhydride in pyridine at room tem-

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

Carbon No.*1	SPM I	2*2	3*3	8	19	22	24
1	174.1	169.9	170.0	174.2	174.2	169.9	170.1
2	37.8	32.7	32.7	37.7	37.5	37.2	37.2
3	68.3	68.1	68.2	68.2	68.2	68.8	69.2
4	85.3	86.2	86.0	85.2	86.0	85.2	84.8
5	79.3	82.6	82.5	79.3	76.8	75.1	77.9
6	30.6	38.7	38.6	30.4	30.1	28.6	28.8
7	30.7	34.7	34.9	30.6	30.2	29.5	30.0
8	31.8	38.1	38.3	31.6	31.2	31.7	31.9
9	78.7	80.7	80.4	78.6	78.5	79.3	79.8
10	128.6	126.7	126.7	128.5	128.4	126.5	126.6
11	134.6	127.1	127.3	134.6	134.7	135.4	135.5
12	132.8	135.1	135.2	132.8	132.9	132.2	132.3
13	131.0	139.4	139.7	130.9	130.9	131.8	131.9
14	42.0	41.7	40.3	42.0	42.0	41.6	41.1
15	69.2	69.8	69.8	69.1	69.1	69.2	69.2
16	20.1	20.5	20.5	20.1	20.1	20.3	20.4
17	43.3	42.6	42.7	43.2	42.9	42.3	42.3
18	202.7	99.1	99.1	202.9	202.9	201.2	201.2
19	15.3	20.7	20.8	15.2	15.1	15.2	15.3
20	61.8	58.2	58.1	61.9	61.4	61.9	62.6
1′	103.9	102.9	102.9	103.7	100.7	100.6	103.5
2′	71.7	70.3	70.4	70.6	71.0	70.9	70.5
3′	68.8	70.5	70.6	69.1	67.8	67.8	69.2
4′	75.0	75.8	78.3	77.9	75.9	75.9	77.5
5′	73.1	73.0	73.3	73.2	72.7	72.7	73.1
6′	19.0	18.9	18.3	18.2	18.7	18.7	18.3
3′-$N(CH_3)_2$	42.0	41.7	41.5	41.5	41.6	41.6	41.5
1″	96.4	96.9	98.0	98.4	96.9	96.9	98.6
2″	40.9	40.2	36.5	36.6	41.6	40.9	36.7
3″	69.4	69.4	77.9	77.6	69.4	69.4	77.7
4″	76.4	77.1	79.3	80.2	77.2	77.1	80.3
5″	66.0	63.4	63.1	63.3	63.4	63.4	63.4
6″	18.3	17.8	17.5	17.3	17.9	17.8	17.4
7″	25.4	25.3	22.4	22.4	25.2	25.2	22.4
1‴	100.2	99.5	99.5	100.2	100.1	99.8	100.2
2‴	31.3	30.8	30.8	31.3	31.3	31.2	31.2
3‴	18.5	18.5	18.5	18.5	18.5	18.5	18.7
4‴	64.8	64.9	65.0	64.8	64.9	64.9	64.9
5‴	73.8	73.8	73.8	73.7	73.8	73.7	73.7
6‴	19.0	19.2	19.2	18.9	18.9	19.0	19.1
4‴-$N(CH_3)_2$	40.7	40.7	40.7	40.7	40.7	40.7	40.6

*1 Signals of acyl carbons are listed in Table 2.

*2 18-Si$(CH_3)_2$, −5.2, −4.0; 18-Si$C(CH_3)_3$, 18.1; 18-Si$C(CH_3)_3$, 26.0.

*3 18-Si$(CH_3)_2$, −5.2, −4.0; 18-Si$C(CH_3)_3$, 18.2; 18-Si$C(CH_3)_3$, 26.0.

perature to give 2′,4″-dipropionate (**19**). 3,2′,4″-Tripropionate (**22**) was produced on treatment of spiramycin I with the same reagents at 50°C for 5 days. 2′,4″-Diacetate (**18**), 2′,4″-dibutylate (**20**) and 3,2′,4″-triacetate (**21**) were also synthesized in a similar manner. In the ^{13}C NMR spectrum of 2′,4″-dipropionate (**19**), signals assigned two propionate carbons and downfield shifts of 2′- and 4″-carbon signals and upfield shifts of 1′-, 3′- and 5″-carbon signals were observed. The ^{13}C NMR spectrum of

Fig. 1. Diagnostic mass fragmentation of 4″-*O*-acetyl-3″-*O*-propionylspiramycin I (**8**).

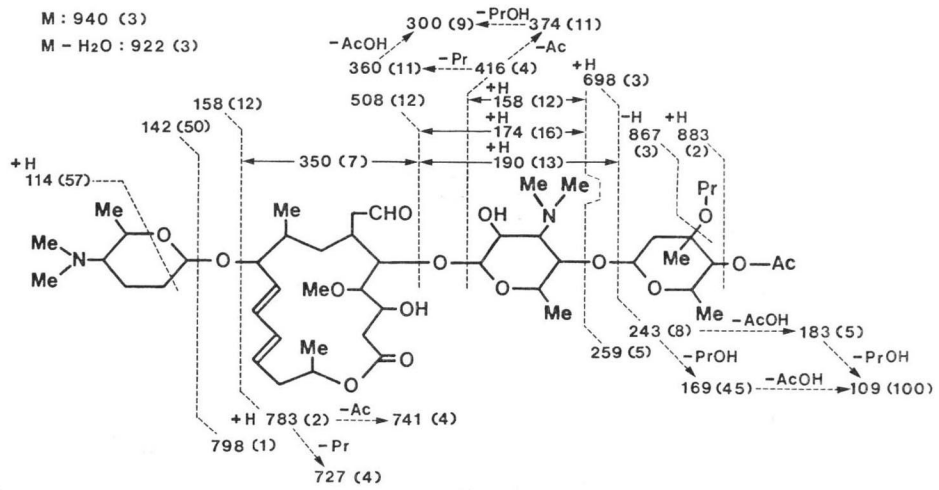


Table 2. ^{13}C NMR chemical shifts of acyl groups on spiramycin I derivatives.

Acyl carbon No.		Bonding position			
		3	2′	3″	4″
Acetyl	1	170.8	168.5~168.7	170.4~170.5	170.5~170.8
	2	21.2~21.3	21.5~21.7	22.4~22.7	20.7~20.9
Propionyl	1	173.7~173.9	172.3	173.6~173.8	173.9~174.3
	2	27.5~27.7	28.0~28.1	28.6~28.8	27.5~27.6
	3	8.9~9.0	8.9~9.0	9.0~9.2	9.2~9.3
Butyryl	1	—	171.5	172.8	173.1~173.4
	2	—	36.5	37.3~37.4	36.1~36.2
	3	—	18.0	18.2	18.5
	4	—	13.8	13.8	13.7~13.8
Isovaleryl	1	—	—	172.2~172.3	172.6
	2	—	—	44.5~44.6	43.3
	3	—	—	25.3	25.5
	4	—	—	22.7	22.4

3,2′,4″-tripropionate (**22**) showed additional propionate carbons, downfield shift of 3-carbon signal and upfield shifts of 1-, 2- and 18-carbon signals in comparison with spiramycin I, thus confirming their structure. The structures of **18**, **20** and **21** were also ascertained by their ^{13}C NMR spectra.

Treatment of **19** and **22** with acetic anhydride in the presence of 4-dimethylaminopyridine and triethylamine followed by methanolysis gave 3,4″-di-*O*-acetyl-3″-*O*-propionyl (**24**) and 4″-*O*-acetyl-3,3″-di-*O*-propionyl (**29**) derivatives of spiramycin I, respectively. 3″-*O*-Acylation occurred through acyl migration reaction as well as in the synthesis of 3″,4″-diacylates. The structures of **24** and **29** were confirmed by the relation between the bonding position of acyl groups in spiramycin I derivatives and ^{13}C NMR chemical shifts of acyl carbons and the other ^{13}C NMR spectral behavior. The other 3,3″,4″-triacylates were synthesized by a similar manner and are summarized in Table 6.

Evaluation of the Derivatives Obtained

The acyl derivatives of spiramycin I were evaluated by four parameters:[5] MIC, affinity to ribo-

Scheme 2.

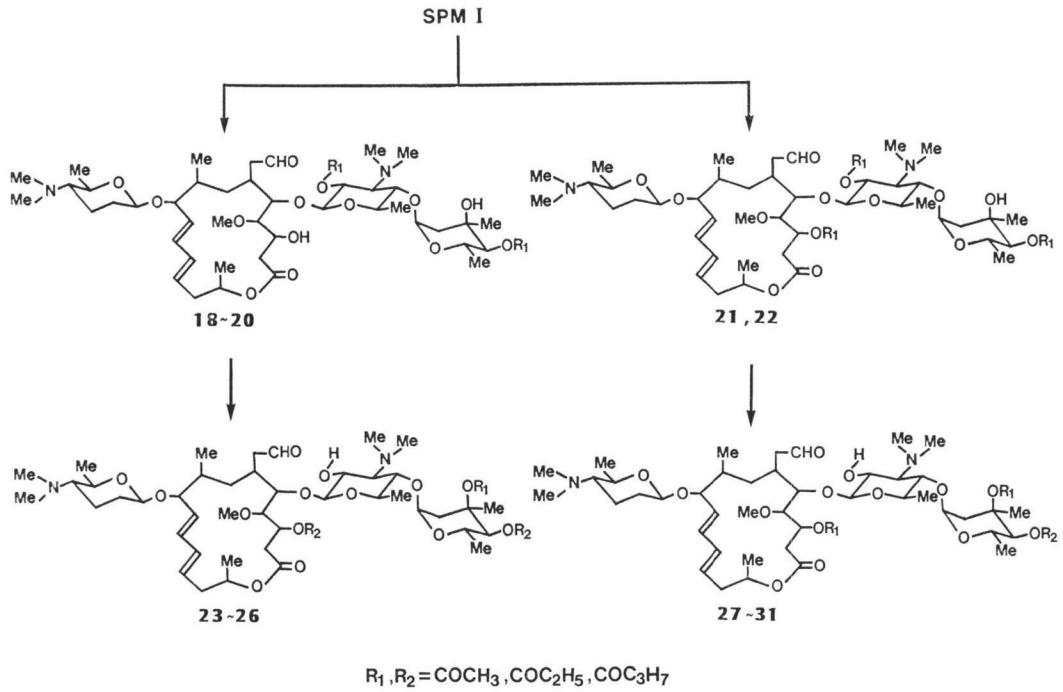
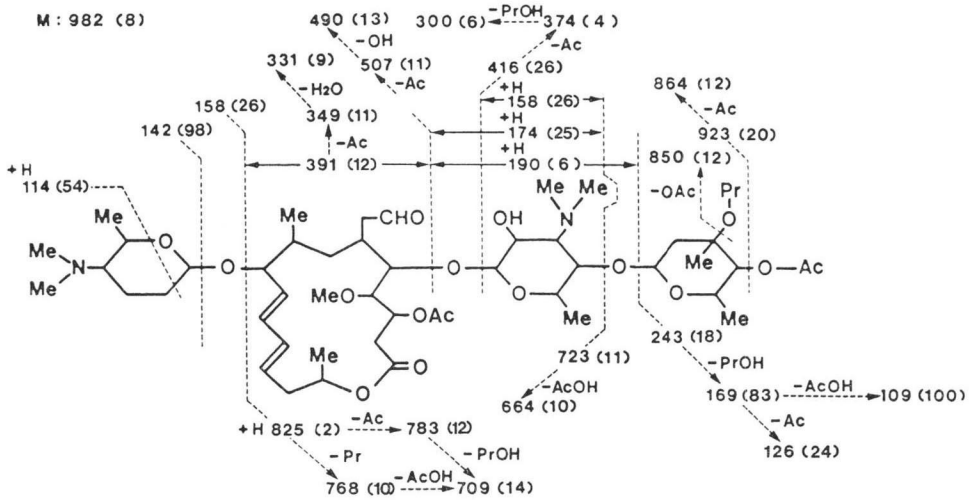


Fig. 2. Diagnostic mass fragmentation of 3,4″-di-*O*-acetyl-3″-propionylspiramycin I (**24**).



somes (ID_{50}),[9] retention time in HPLC[10] and therapeutic effects in mice (survival %), as shown in Table 3.

Among the derivatives, 3″-*O*-acetyl-4″-*O*-butyryl (**6**), 3″-*O*-acetyl-4″-*O*-isovaleryl (**7**), 3″,4″-di-*O*-propionyl (**9**), 3″-*O*-propionyl-4″-*O*-butyryl (**10**), 3-*O*-butyryl-4″-*O*-propionyl (**13**), 3″-*O*-isovaleryl-4″-*O*-butyryl (**17**), 3,3″-di-*O*-acetyl-4″-*O*-butyryl (**28**) and 3,3″,4″-tri-*O*-propionylspiramycin I (**30**) showed relatively high activity. The ID_{50} values of 3,3″,4″-tri-*O*-propionyl (**30**) and 3,4″-di-*O*-acetyl-3″-*O*-butyrylspiramycin I (**25**) were comparable to that of spiramycin I but the others showed the weaker ones.

Table 3. The MIC, ID_{50}, survival % and RT of 3″,4″-diacylates and 3,3″,4″-triacylates of spiramycin I.

Compound No.	Acyl group*1			MIC (μg/ml)*2							ID_{50} (μM)	Survival %*3			RT (minutes)
	3	3″	4″	SA	SA^r	BS	BC	ML	EC	KP		100	60	40	
4	H	Ac	Ac	3.12	100	1.56	1.56	<0.1	>100	100	1.8	NT	NT	NT	13.3
5	H	Ac	Pr	6.25	>100	3.12	3.12	0.2	>100	100	1.9	100	20	0	24.6
6	H	Ac	Bu	1.56	100	1.56	1.56	<0.1	>100	100	2.0	90	0	NT	38.7
7	H	Ac	*i*Va	1.56	50	1.56	1.56	<0.1	>100	100	2.6	NT	NT	NT	70.1
8	H	Pr	Ac	3.12	50	3.12	1.56	<0.1	>100	100	2.0	100	40	NT	19.5
9	H	Pr	Pr	1.56	>100	1.56	1.56	<0.1	>100	100	1.9	90	40	NT	39.1
10	H	Pr	Bu	1.56	>100	1.56	1.56	<0.1	>100	100	2.9	90	40	NT	61.4
11	H	Pr	*i*Va	6.25	100	3.12	3.12	0.2	>100	100	2.6	90	10	0	119
12	H	Bu	Ac	6.25	>100	1.56	1.56	<0.1	>100	>100	2.1	90	30	10	34.7
13	H	Bu	Pr	1.56	>100	1.56	1.56	<0.1	>100	>100	1.9	90	20	0	61.4
14	H	Bu	Bu	3.12	>100	3.12	3.12	0.2	>100	>100	2.3	60	10	NT	119
15	H	*i*Va	Ac	12.5	>100	6.25	3.12	0.2	>100	>100	2.7	90	0	0	61.2
16	H	*i*Va	Pr	3.12	>100	3.12	3.12	0.2	>100	>100	2.6	60	20	NT	112
17	H	*i*Va	Bu	1.56	>100	3.12	3.12	<0.1	>100	>100	2.4	50	0	NT	190
27	Ac	Ac	Pr	3.12	>100	1.56	3.12	<0.1	>100	>100	1.8	100	50	NT	27.0
23	Pr	Ac	Pr	3.12	>100	3.12	3.12	0.2	>100	>100	2.5	NT	NT	NT	27.3
28	Ac	Ac	Bu	1.56	>100	1.56	1.56	<0.1	>100	>100	2.0	100	50	NT	39.7
24	Ac	Pr	Ac	3.12	>100	3.12	1.56	<0.1	>100	>100	2.0	100	30	0	19.5
29	Pr	Pr	Ac	3.12	>100	3.12	3.12	<0.1	>100	>100	1.9	100	50	NT	35.9
30	Pr	Pr	Pr	1.56	>100	1.56	1.56	<0.1	>100	>100	1.2	100	100	20	58.7
31	Pr	Pr	Bu	3.12	>100	3.12	3.12	0.2	>100	>100	2.5	NT	NT	NT	85.3
25	Ac	Bu	Ac	6.25	>100	3.12	1.56	0.2	>100	>100	1.4	100	100	10	36.1
26	Pr	Bu	Pr	3.12	>100	1.56	1.56	<0.1	>100	>100	2.1	100	50	0	95.5
SPM I	H	H	H	1.56	>100	0.78	1.56	<0.1	100	100	1.0	10	0	0	4.3
AcSPM				6.25	>100	3.12	3.12	0.2	>100	>100	1.9	80	60	0	NT

*1 Ac: $COCH_3$, Pr: $COCH_2CH_3$, Bu: $COCH_2CH_2CH_3$, *i*Va: $COCH_2CH(CH_3)_2$

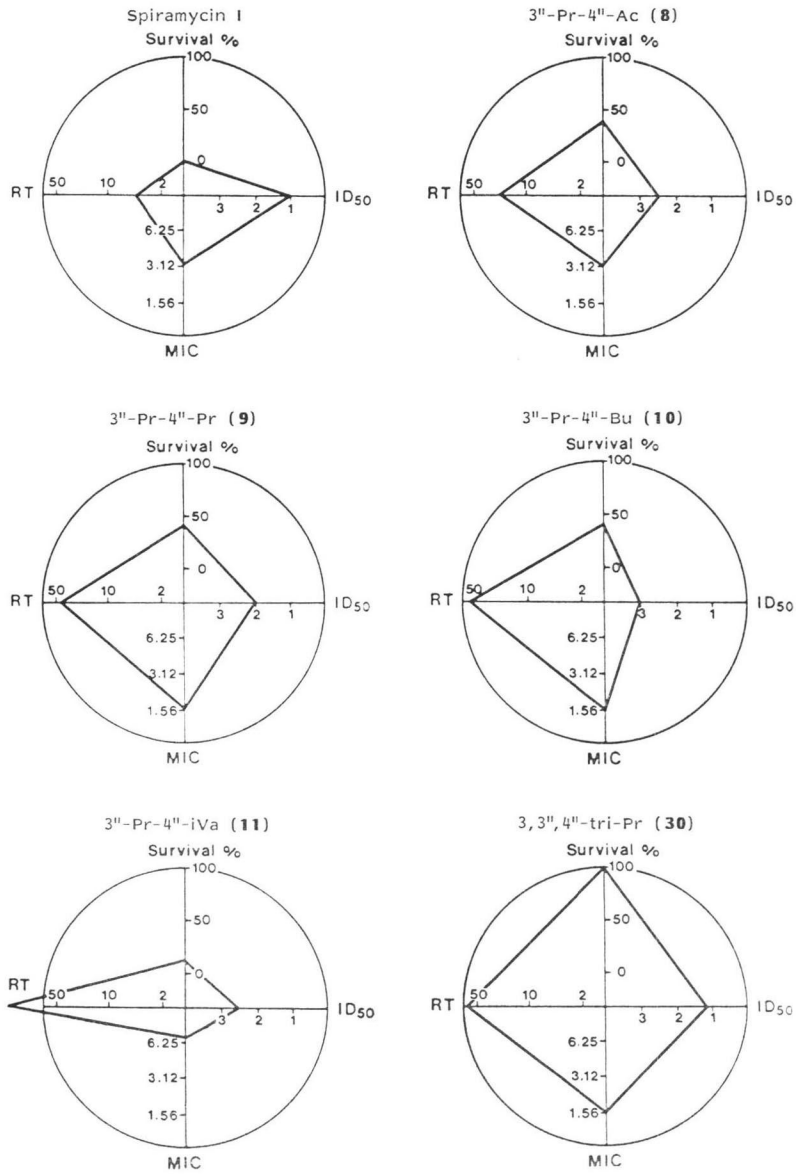
*2 SA : *Staphylococcus aureus* KB210 (ATCC 6538P)
SA^r : *S. aureus* KB224 (MC^r, TC^r)
BS : *Bacillus subtilis* KB211 (ATCC 6633)
BC : *Bacillus cereus* KB143 (IFO 3001)
ML : *Micrococcus luteus* KB212 (ATCC 9341)
EC : *Escherichia coli* KB213 (NIHJ)
KP : *Klebsiella pneumoniae* KB214 (ATCC 10031)

*3 Test organism: *Streptococcus pneumoniae* III, dose (mg/kg)
NT: Not tested

The retention time (RT) values increased with an increase in the length of alkyl chain of the 3″- and 4″-acyl groups but 3-acyl groups scarcely affected the RT. Several derivatives (**7**, **10**, **13** and **15**) with 40~70 minutes of RT were found to possess a strong antibacterial activity, although the affinities to ribosomes were low, which implies that the permeability of these derivatives to bacterial cells is superior.

In survival percent, the diacylates were more effective than spiramycin I. 3″-*O*-Propionyl-4″-*O*-acetyl (**8**), 3″,4″-di-*O*-propionyl (**9**), 3″-*O*-propionyl-4″-*O*-butyrylspiramycin I (**10**) and all the triacylates were almost comparable to acetylspiramycin. Especially, 3,3″,4″-tri-*O*-propionyl (**30**) and 3,4″-di-*O*-acetyl-3″-*O*-butyrylspiramycin I (**25**) showed higher therapeutic effects than that of acetylspiramycin.

Fig. 3. Graphic representation of the properties indicated by four parameters of spiramycin I and its acyl derivatives.



Drawing the four parameters of a derivative in a graph is useful to understand the characteristics of the compound. Fig. 3 shows the graphs for spiramycin I and its acyl derivatives in which propionyl group is introduced to 3″-position. The relationships between RT or MIC, and size of acyl group can be understood from these graphs. In therapeutic effect indicated by survival percent, the first three derivatives (**8**, **9** and **10**) are equivalent each other, whereas the isovaleryl derivative (**11**) is weaker. When the third propionyl group is introduced to the 3-position of 3″,4″-dipropionate (**9**), the survival percent is significantly increased though the MIC is not changed as shown in the last graph. These findings would indicate that pharmacological characteristics such as absorption, distribution and metabolism of these derivatives much affect the therapeutic activities and that the introduction of acyl groups of a suitable size to 3- and 3″-positions is highly effective to the therapeutic effects. The property of the whole molecule arising from the combination of 3″- and 4″-, or 3-, 3″- and 4″-substituents is important for the therapeutic effect, and it is difficult to discuss about each substituent separately.

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. 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.

MIC Determination

The MIC for 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 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.[9]

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.[10] 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*-propionylspiramycin I 3,18-*O*-(*t*-Butyldimethylsilyl)acetal (**2**)

To an ice-cooled solution of **1**[6] (5.00 g) in pyridine (75 ml), propionyl chloride (2.1 ml) was added and set for 3 hours at room temp. After addition of a few drops of H_2O, the reaction mixture was diluted with $CHCl_3$ (500 ml) and washed with H_2O (500 ml). The $CHCl_3$ layer was dried over anhydrous sodium sulfate and evaporated to give a brown solid, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 2: 1, to give a colorless solid, 4.17 g (79.0%). TLC Rf 0.40; $[\alpha]_D^{24}$ −49.8° (*c* 1.0, $CHCl_3$); MS *m/z* 1,054 (M^+); UV λ_{max}^{MeOH} nm (ε) 236 (40,900).

2′,4″-Di-*O*-acetyl-3″-*O*-propionylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**3**)

To a solution of **2** (757 mg) and 4-dimethylaminopyridine (63 mg) in $CHCl_3$ (20.5 ml), triethylamine (2.17 ml) and acetic anhydride (1.61 ml) were added and heated to reflux for 68 hours. After addition of MeOH, the reaction mixture was diluted with $CHCl_3$ (75 ml) and washed with saturated sodium

Table 4. Reaction conditions of the preparation of 3″,4″-diacylates of spiramycin I.

Product			4″-Acylation		Acyl migration				Methanolysis	De-TBDMS	
					Reagent (mole equivalent)			Reaction time (days)	Reaction time (days)	Reaction time (hours)	Yield (%)
No.	3″(R_1)	4″(R_2)	Reagent	Reaction time	$(R_2)_2O$	DMAP*	Et_3N				
5	Ac	Pr	Ac_2O	2 days	22	0.71	30	3	2	0.5	44.4
6	Ac	Bu			26	0.84	36	3	2	0.5	59.1
7	Ac	*i*Va			26	0.84	36	7	3	1	32.3
9	Pr	Pr	Pr_2O	6 days	26	0.84	37	3	3	0.5	67.9
10	Pr	Bu			33	1.07	47	3	2	0.5	66.4
11	Pr	*i*Va			44	1.42	61	3	3	0.5	26.7
12	Bu	Ac	Bu_2O	5 days	26	0.84	38	6	2	0.5	56.7
13	Bu	Pr			26	0.84	37	4	2	0.7	68.9
14	Bu	Bu			39	1.26	57	3	2	0.5	41.3
15	*i*Va	Ac	*i*VaCl	1.5 hours	26	0.84	38	9	3	2	22.2
16	*i*Va	Pr			26	0.84	38	9	5	1.5	48.5
17	*i*Va	Bu			39	1.26	57	10	4	7	34.8

* DMAP, 4-dimethylaminopyridine.

hydrogen carbonate solution and then H_2O. The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to give a yellow powder, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 5: 1, to give a colorless powder of **3**, 590 mg (75.0%). TLC Rf 0.39; $[\alpha]_D^{24}$ −12.6° (*c* 0.2, $CHCl_3$); MS *m/z* 1,096 (M^+); UV λ_{max}^{MeOH} nm (ε) 226 (32,100).

4″-*O*-Acetyl-3″-*O*-propionylspiramycin I (**8**)

A solution of **3** (3.23 g) in MeOH (130 ml) was heated at 50°C for 4 days and evaporated to give a colorless solid.

Well dried solid was dissolved in 1 M solution of tetrabutylammonium fluoride in THF (3.77 ml) and held for 1.5 hours at room temp. The reaction mixture was diluted with $CHCl_3$ (300 ml) and washed with H_2O. The $CHCl_3$ solution 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, 3: 2, to give a colorless powder of **8**, 1.80 g (65.0%).

The other 3″,4″-diacylates (**5**~**17**) (Table 5) were prepared in a similar manner applying the reaction conditions listed in Table 4 without a purification of the intermediates.

2′,4″-Di-*O*-acetylspiramycin I (**18**)

To an ice-cooled solution of spiramycin I (2.00 g) in pyridine (30 ml), acetic anhydride (2.4 ml) was added and held for 20 hours at room temp. After addition of MeOH, the reaction mixture was diluted with $CHCl_3$ (100 ml) and washed with saturated solution of sodium hydrogen carbonate and H_2O, respectively. The $CHCl_3$ layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure, to give a brown solid, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 3: 1~2: 1 (gradually changed), to afford colorless powder, 1.02 g (46.3%). TLC Rf 0.51; $[\alpha]_D^{21}$ −88.1° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 232 (25,900).

Anal Calcd for $C_{47}H_{78}N_2O_{16}\cdot 2H_2O$: C 58.61, H 8.58, N 2.91.
Found: C 58.82, H 8.14, N 2.90.

2′,4″-Di-*O*-propionylspiramycin I (**19**)

19 was prepared from spiramycin I (5.05 g) and propionic anhydride (7.8 ml) as described in the preparation of **18**. Colorless powder, 3.13 g (54.7%). TLC Rf 0.49; $[\alpha]_D^{19}$ −88° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 230 (19,500).

Anal Calcd for $C_{49}H_{82}N_2O_{16}\cdot \frac{1}{2}H_2O$: C 61.04, H 8.68, N 2.91.
Found: C 60.86, H 8.68, N 3.32.

2′,4″-Di-*O*-Butyrylspiramycin I (**20**)

20 was prepared from spiramycin I (5.05 g) and butyric anhydride (14.3 ml) as described in the preparation of **18**. Colorless powder, 3.26 g (55.3%). TLC Rf 0.46; $[\alpha]_D^{19}$ −91° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 233 (35,900).

Table 5. Physico-chemical characteristics of 3″,4″-di-*O*-acylspiramycin I.

Compound No.	TLC Rf	$[\alpha]_D^{25}$ °(*c* 1.0, $CHCl_3$)	UV λ_{max}^{MeOH} nm (ε)	High MS	
				Found	Calcd (Molecular formula)
5	0.35	−72.8	232 (17,600)	940.550	940.550 ($C_{48}H_{80}N_2O_{16}$)
6	0.36	−61.6	231 (15,900)	954.566	954.566 ($C_{49}H_{82}N_2O_{16}$)
7	0.37	−57.6	231 (12,100)	968.582	968.582 ($C_{50}H_{84}N_2O_{16}$)
8	0.36	−34.0	232 (22,900)	940.551	940.551 ($C_{48}H_{80}N_2O_{16}$)
9	0.37	−66.8	233 (32,900)	954.566	954.566 ($C_{49}H_{82}N_2O_{16}$)
10	0.37	−66.0	232 (25,300)	968.582	968.582 ($C_{50}H_{84}N_2O_{16}$)
11	0.38	−63.0	231 (31,200)	982.597	982.597 ($C_{51}H_{86}N_2O_{16}$)
12	0.37	−65.8	232 (21,700)	954.566	954.566 ($C_{49}H_{82}N_2O_{16}$)
13	0.38	−75.6	232 (28,900)	968.582	968.582 ($C_{50}H_{84}N_2O_{16}$)
14	0.38	−61.0	232 (19,800)	982.597	982.597 ($C_{51}H_{86}N_2O_{16}$)
15	0.38	−59.4	232 (34,700)	968.582	968.582 ($C_{50}H_{84}N_2O_{16}$)
16	0.38	−58.8	231 (38,100)	982.597	982.597 ($C_{51}H_{86}N_2O_{16}$)
17	0.39	−60.0	234 (40,200)	996.613	996.613 ($C_{52}H_{88}N_2O_{16}$)

Table 6. Reaction time, yield and physico-chemical characteristics of 3,3″,4″-tri-*O*-acylspiramycin I.

Compound				Reaction time		Yield (%)	TLC Rf	$[\alpha]_D^{19}$ ° (*c* 1.0, $CHCl_3$)	UV λ_{max}^{MeOH} nm (ε)	*Anal* Found / Calcd			Formula
No.	3	3″	4″	Acyl migration (hours)	Methanolysis (hours)					C	H	N	
23	Pr	Ac	Pr		*		0.57	−62.6	239 (38,600)	60.08 60.34	8.12 8.54	2.77 2.76	$C_{51}H_{84}N_2O_{17}\cdot H_2O$
24	Ac	Pr	Ac	60	98	35.8	0.42	−62.6	234 (35,300)	60.03 59.98	8.60 8.46	2.85 2.80	$C_{50}H_{82}N_2O_{17}\cdot H_2O$
25	Ac	Bu	Ac	75	67	44.3	0.44	−58.2	231 (24,500)	59.55 59.28	8.45 8.58	2.83 2.71	$C_{51}H_{84}N_2O_{17}\cdot 2H_2O$
26	Pr	Bu	Pr	97	44	29.5	0.50	−51.2	235 (22,500)	60.29 59.98	8.81 8.73	2.93 2.64	$C_{53}H_{88}N_2O_{17}\cdot 2H_2O$
27	Ac	Ac	Pr	98	62	41.4	0.45	−54.0	231 (24,400)	59.08 58.92	8.42 8.50	2.46 2.75	$C_{50}H_{82}N_2O_{17}\cdot 2H_2O$
28	Ac	Ac	Bu	89	86	31.8	0.46	−59.0	231 (22,000)	60.05 59.81	8.54 8.56	2.70 2.74	$C_{51}H_{84}N_2O_{17}\cdot 1.5\,H_2O$
29	Pr	Pr	Ac	164	56	15.1	0.46	−66.0	232 (29,500)	60.40 60.34	8.45 8.54	2.81 2.76	$C_{51}H_{84}N_2O_{17}\cdot H_2O$
30	Pr	Pr	Pr	84	120	28.3	0.51	−85.3	231 (22,500)	60.22 60.68	8.58 8.61	2.56 2.72	$C_{52}H_{86}N_2O_{17}\cdot H_2O$
31	Pr	Pr	Bu	96	50	31.5	0.57	−61.0	233 (37,700)	61.04 61.02	8.52 8.69	2.68 2.69	$C_{53}H_{88}N_2O_{17}\cdot H_2O$

* See Experimental

Anal Calcd for $C_{51}H_{86}N_2O_{16}\cdot\frac{1}{2}H_2O$: C 61.80, H 8.85, N 2.83.
Found: C 61.85, H 8.90, N 3.09.

3,2′,4″-Tri-*O*-acetylspiramycin I (**21**)

To an ice-cooled solution of spiramycin I (5.05 g) in pyridine (60 ml), acetic anhydride (6.0 ml) was added and heated to 50°C for 5 days. After addition of MeOH, the reaction mixture was diluted with $CHCl_3$ (500 ml) and washed with H_2O. The $CHCl_3$ solution was evaporated to give a brown solid, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 5: 2, giving a colorless powder of **21**, 636 mg (11.0%). TLC Rf 0.88; $[\alpha]_D^{19}$ −93.6° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 239 (18,000).

Anal Calcd for $C_{49}H_{80}N_2O_{17}$: C 60.73, H 8.32, N 2.89.
Found: C 60.56, H 8.51, N 3.14.

3,2′,4″-Tri-*O*-propionylspiramycin I (**22**)

22 was synthesized from spiramycin I (5.05 g) and propionic anhydride (12.0 ml) as described in the synthesis of **21**. Colorless powder, 1.40 g (23.1%). TLC Rf 0.89; $[\alpha]_D^{19}$ −85.8° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 231 (24,300).

Anal Calcd for $C_{52}H_{86}N_2O_{17}\cdot 2.5\ H_2O$: C 59.13, H 8.68, N 2.65.
Found: C 59.32, H 8.25, N 3.20.

3″-*O*-Acetyl-3,4″-di-*O*-propionylspiramycin I (**23**)

To a solution of **18** (1.02 g) and 4-dimethylaminopyridine (113 mg) in $CHCl_3$ (27 ml), triethylamine (3.5 ml) and propionic anhydride (3.7 ml) were added and heated to reflux for 74 hours. After addition of MeOH, the reaction mixture was diluted with EtOAc (100 ml) and washed with saturated solution of sodium hydrogen carbonate and H_2O, respectively. The EtOAc solution was dried over anhydrous sodium sulfate and evaporated, to give a residue.

The residual solid was dissolved in 70% MeOH (49 ml) and heated at 50°C for 96 hours. The reaction mixture was diluted with $CHCl_3$ (70 ml) and washed with H_2O. The organic layer was dried over anhydrous sodium sulfate and evaporated, to give a residue, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH - conc NH_4OH, 50: 1: 0.01, giving a colorless powder of **23**, 419 mg (38.9%).

The other 3,3″,4″-triacylates of spiramycin I (**24**～**31**) were synthesized in a similar manner using 0.84, 33 and 26 molar equivalent of 4-dimethylaminopyridine, triethylamine and corresponding acid anhydride, respectively. Reaction time, yield and physico-chemical characteristics of the 3,3″,4″-triacylates are listed in Table 6.

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