CHEMICAL MODIFICATION OF SPIRAMYCINS

I. SYNTHESIS OF THE ACETAL DERIVATIVES OF NEOSPIRAMYCIN I

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Tetrahydrofuranyl and tetrahydropyranyl derivatives of neospiramycin I at 3 and/or 4′ position were synthesized. *In vitro* and *in vivo* activities of these derivatives were correlated with the position and configuration of acetal groups. The most effective derivative, 3-*a*, 4′-*a*-di-*O*-tetrahydrofuranylneospiramycin I was comparable to spiramycin I.

Spiramycins are sixteen membered macrolide antibiotics,[1,2] which are active against Gram-positive bacteria and mycoplasmas and the acetyl derivative[3] is used clinically. Neospiramycin, the demycarosyl derivative of spiramycin, is equal to or greater in activity than spiramycin *in vitro*[3,4], but practically inactive *in vivo*. It seems that the pharmacokinetics of neospiramycins differs from spiramycins. Spiramycins and neospiramycins consist mainly of three components, I (3-OH), II (3-*O*-acetyl), III (3-*O*-propionyl). Many reports of the chemical modification of macrolide antibiotics[5], mainly leucomycin[6,7] and tylosin type[8,9] antibiotics, have been published. However, few reports of the chemical modifications of spiramycins,[3] except acylation and hydrogenation have been reported. There have been no presentations of chemical modifications of neospiramycins. In this paper, we wish to report the synthesis of acetal derivatives of 3 and/or 4′ hydroxyl groups of neospiramycin I. These chemical modifications result in enhanced *in vivo* activity.

Synthesis

Neospiramycin I (**1**) has three hydroxyl groups at the 3, 2′ and 4′ positions, which possess little difference in reactivity. In order to modify the 3- and/or 4′-hydroxyl groups, it is necessary to protect the 2′-hydroxyl group. This was achieved by careful hydrolysis of 2′-*O*-acetylspiramycin I (**2**). **2**, obtained by selective acylation of spiramycin I, was hydrolyzed by 0.07 M hydrochloric acid (pH 2.0) at 42°C, to give 2′-*O*-acetylneospiramycin I (**3**) in a crystalline form. The position of the acetyl group was confirmed by the behavior of the chemical shifts of C-1′, 2′ and 3′ in the ^{13}C NMR spectrum. This procedure is also convenient for a facile preparation of neospiramycin I.

Pyridinium *p*-toluenesulfonate (PPTS)[10,11] is a good catalyst for introduction of tetrahydrofuranyl (THF) or tetrahydropyranyl (THP) groups to neospiramycin I which is labile to acid and base. Treatment of **3** with 2,3-dihydrofuran and PPTS afforded 2′-*O*-acetyl-3-*O*-tetrahydrofuranylneospiramycin I (**4**) and 3,4′-di-*O*-tetrahydrofuranyl derivative (**5**), as an epimeric mixture. The proportion of the

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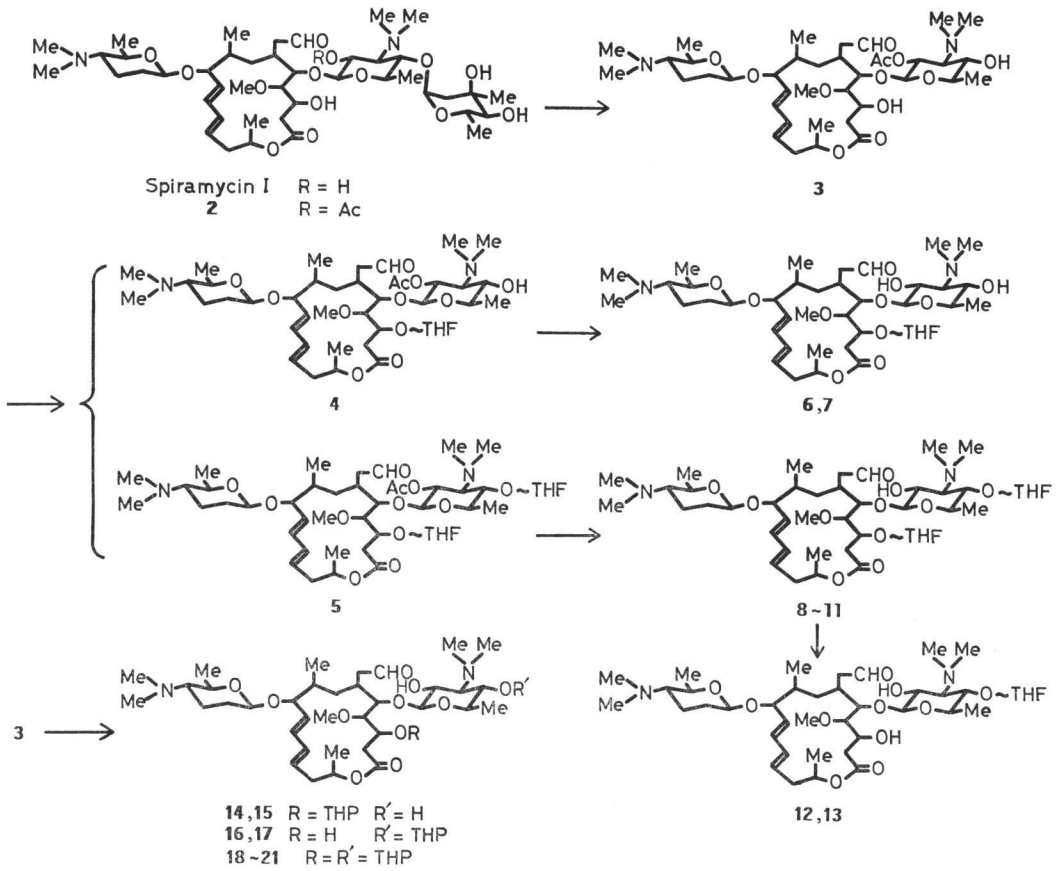
Scheme 1.



products **4** and **5** could be controlled by altering the molar ratio of reagents (see experimental section). **4** is also useful as a protected derivative for 4′-hydroxyl modification of neospiramycin I.

Methanolysis of **4** and **5** was followed by silica gel column chromatography to give two epimers of 3-*O*-tetrahydrofuranylneospiramycin I (**6** and **7**) and four epimers of 3,4′-di-*O*-tetrahydrofuranylneospiramycin I (**8**~**11**), respectively. 4′-*O*-Tetrahydrofuranylneospiramycin I (**12** and **13**) were produced by selective removal of the 3-*O*-THF group by treatment of the mixture of 3,4′-di-*O*-THF derivatives with PPTS in dioxane - water.

Each structure was confirmed by ^{13}C NMR and mass spectra (Tables 1 and 2). The position of the THF group and the relative configuration of C-1 of the THF group were correlated to the chemical shifts of C-1, 2, 3, 18, 2′, 3′, 4′, 5′ and 3′-*N*-methyl carbons and C-1~4 carbons of THF group in their ^{13}C NMR spectra. Their resonances occurred in the ranges shown in Tables 3 and 4. That is, in comparison with neospiramycin I, a 3-*a**-THF group caused an up field shift of C-1 and a down field shift of C-2 and 3 to a greater extent than 3-*b**. A 4′-*a*-THF group resulted in an up field shift of C-2′, 3′, 3′-*N*-methyl carbon and a down field shift of C-4′ to a greater extent than 4′-*b*, and an up field shift of C-5′ was larger in 4′-*b* than 4′-*a*. Mass spectral data also supported these structures.

* Absolute configuration of C-1 of THF and THP groups could not be determined. So we call one configuration *a* and the other *b*, in this paper.

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

Carbon No.	3	6	7	8	9	10	11	12	13
1	174.3	170.9	171.7	170.5	171.9	170.6	171.9	174.5	174.4
2	37.6	40.1	39.2	40.7	39.5	40.4	39.4	37.5	37.6
3	68.2	75.0	73.7	75.5	74.3	75.4	75.7	68.3	68.3
4	85.9	84.1	84.3	85.5	85.3	85.0	85.0	85.3	85.4
5	78.9	79.9	79.2	78.6	78.5	79.1	78.7	77.3	78.7
6	30.1	29.3	29.3	29.9	30.3	30.5	30.7	30.2	30.3
7	30.3	31.5	31.5	30.3	30.6	31.2	31.0	30.6	30.8
8	31.4	33.2	31.9	31.6	31.0	32.2	31.3	31.3	31.6
9	77.1	78.9	77.4	77.0	77.2	75.9	78.7	78.6	78.8
10	128.5	128.8	128.3	127.2	127.9	127.8	128.0	128.4	128.4
11	134.8	133.3	134.4	134.3	134.8	134.0	134.7	134.8	134.8
12	132.9	132.0	132.5	132.1	133.0	131.9	132.8	133.0	132.9
13	130.9	131.4	131.1	131.8	130.9	131.9	130.9	130.9	130.9
14	42.0	41.1	41.3	40.7	41.9	41.0	40.7	42.0	42.0
15	69.1	69.6	69.4	69.2	69.3	69.7	69.3	69.1	69.1
16	20.1	20.6	20.3	20.7	20.3	20.7	20.3	20.1	20.1
17	42.9	44.2	44.7	42.6	44.3	43.2	44.5	43.0	43.2
18	202.9	202.4	203.5	202.1	204.4	202.3	204.1	203.4	203.2
19	15.1	15.7	15.6	15.6	15.1	15.3	15.3	15.0	15.2
20	61.5	61.3	61.8	62.2	62.5	61.8	62.2	62.0	61.9
1′	100.8	104.4	103.9	104.9	104.6	104.3	104.3	105.1	104.2
2′	71.0	71.2	70.9	70.0	69.3	70.9	70.8	70.1	70.9
3′	67.9	70.2	70.3	69.8	69.7	70.2	70.3	69.1	70.1
4′	70.5	70.4	70.7	76.5		75.6	73.9		76.0
5′	72.9	73.2	73.3	73.0	73.2	72.6	72.7	73.2	72.7
6′	17.8	18.0	18.0	18.6	18.4	18.0	18.9	18.4	17.9
3′-NCH_3	41.3	41.9	41.8	40.8	40.7	41.5	41.4	40.7	41.4
1″	100.2	100.4	100.2	99.4	99.5	99.8	99.8	100.0	100.2
2″	31.3	31.2	31.2	31.1	31.2	31.2	31.3	31.3	31.2
3″	18.5	18.5	18.5	18.4	18.4	18.5	18.4	18.6	18.7
4″	64.9	64.9	64.9	64.9	64.9	64.9	64.9	64.9	64.9
5″	73.8	73.8	73.7	73.5	73.6	73.8	73.7	73.7	73.6
6″	19.0	19.0	19.0	19.1	19.0	19.0	18.9	19.0	19.1
4″-NCH_3	40.8	40.7	40.7	40.7	40.7	40.7	40.7	40.7	40.7
$COCH_3$	21.6								
$COCH_3$	168.9								
3-*O*-THF 1‴		105.7	104.5	105.6	104.8	105.7	104.3		
2‴		32.8	32.6	32.9	32.6	32.8	32.6		
3‴		23.5	23.9	23.5	23.9	23.5	23.9		
4‴		67.2	67.3	67.3	67.3	67.3	67.3		
4′-*O*-THF 1⁗				103.0	102.9	103.6	103.4	103.1	103.4
2⁗				32.9	32.9	32.8	32.8	33.0	32.9
3⁗				23.9	23.9	23.7	23.7	23.9	23.7
4⁗				67.7	67.6	67.3	67.3	67.7	67.4

THP derivatives were synthesized in a manner similar to that described in the preparation of THF derivatives. Treatment of **3** with 2,3-dihydro-4*H*-pyran and PPTS gave 3-, 4′- and 3,4′-di-*O*-THP derivatives in a single reaction. After removal of the acetyl group by methanolysis, all isomers, 3-*O*-tetrahydropyranyl (**14** and **15**), 4′-*O*-tetrahydropyranyl (**16** and **17**) and 3,4′-di-*O*-tetrahydropyranylneospiramycin I (**18**~**21**) were obtained by separation with silica gel column chromatography.

Table 1. (continued)

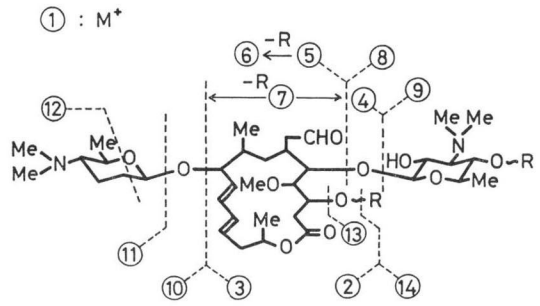
Carbon No.	14	15	16	17	18	19	20	21
1	171.6	171.6	174.3	174.4	171.1	171.8	171.2	171.8
2	39.9	39.1	37.5	37.6	40.3	39.4	40.2	39.4
3	75.3	73.8	68.2	63.8	75.8	73.9	75.5	73.8
4	83.1	84.3	85.3	85.2	84.2	85.3	83.5	84.8
5	80.9	79.4	77.6	77.4	77.7	77.6	80.3	78.4
6	29.7	31.4	30.2	30.2	31.1	29.9	31.0	30.8
7	31.0	31.6	30.5	30.6	31.6	31.7	31.4	31.3
8	32.3	31.8	31.7	31.6	31.6	31.7	32.0	31.6
9	80.4	79.2	78.3	78.6	79.6	78.1	79.1	78.8
10	130.2	128.5	128.3	128.7	128.8	128.0	129.6	128.3
11	132.6	134.1	134.7	134.8	133.4	134.6	132.9	134.4
12	132.4	132.6	132.9	133.0	132.2	133.3	132.3	133.0
13	130.7	130.9	130.8	130.9	131.2	130.5	130.9	130.7
14	41.1	41.1	42.0	42.0	41.1	40.9	41.2	41.1
15	69.5	69.4	69.0	69.1	69.6	69.3	69.4	69.4
16	20.5	21.5	20.1	20.1	20.6	20.3	20.5	20.4
17	45.4	44.0	42.9	43.0	44.3	42.9	45.0	43.6
18	202.7	203.9	203.3	203.4	202.9	205.3	203.1	204.7
19	16.2	15.5	15.0	15.0	15.7	15.0	15.9	15.3
20	60.9	61.7	61.9	62.1	61.6	62.4	61.1	62.0
1′	104.6	104.4	103.1	102.8	103.9	102.9	104.5	103.4
2′	72.1	70.8	69.1	70.9	69.5	69.3	71.0	70.8
3′	70.2	70.5	69.9	68.6	69.8	69.9	70.6	70.5
4′	69.7	70.7	76.4	75.9	76.5	76.5	76.5	75.8
5′	73.4	73.3	73.1	72.4	73.2	73.1	72.6	72.7
6′	18.2	18.0	18.3	18.0	18.6	18.3	18.6	18.4
3′-NCH_3	42.0	41.8	40.6	41.2	40.8	40.7	41.7	41.5
1″	101.0	100.0	99.9	99.8	100.1	99.5	100.6	99.9
2″	31.2	31.3		31.1	31.3	31.2	31.3	31.3
3″	18.6	18.4	18.4	18.7	18.6	18.4	18.5	18.5
4″	65.0	64.9	64.9	65.0	64.9	64.9	64.9	64.9
5″	73.7	73.8	73.7	73.3	73.8	73.0	73.9	73.9
6″	19.0	18.9	18.9	19.0	19.0	18.9	19.0	18.9
4″-NCH_3	40.7	40.7	40.7	40.7	40.7	40.7	40.7	40.7
3-*O*-THP 1‴	101.3	100.2			101.0	100.7	101.5	100.2
2‴	34.2	31.3			32.9	31.3	33.6	31.3
3‴	20.0	21.5			20.3	21.8	20.2	21.5
4‴	25.3	25.3			25.3	25.2	25.4	25.3
5‴	63.4	65.1			63.7	64.9	63.6	64.9
4′-*O*-THP 1⁗			101.6	102.1	101.5	101.5	100.9	100.8
2⁗			31.3	31.1	31.1	30.8	31.0	31.3
3⁗			21.8	22.0	21.3	21.8	20.7	20.8
4⁗			25.2	24.9	25.3	25.2	25.3	25.3
5⁗			65.2	66.4	64.7	65.3	64.0	64.1

In the ^{13}C NMR spectra of THP derivatives, a similar relationship in THF derivatives was observed between a bonding position and C-1 configuration of THP group and chemical shifts (Tables 3 and 4).

Antibacterial Activity

Minimum inhibitory concentrations (MIC) and therapeutic effects in mice were shown in Tables

Table 2. Diagnostic mass fragmentation for neospiramycin I derivatives.



	3	4	5	6, 7	8~11	12, 13	14, 15	16, 17	18~21
1	740	810	880	768	838	768	782	782	866
2	—	740	810	698	768	—	698	—	782
3+H	582	653	723	611	680	−2H 609	625	—	709
4	524	+H 595	+H 595	—	+2H 596	524	608	+2H 526	+2H 610
5	508	+H 579	+H 579	578	+H 579	−H 507	+H 593	+H 509	592
6	—	—	—	508	+H 509	—	+H 509	—	508
7	—	—	—	−H 349	−H 349	−H 349	−H 349	350	−H 349
8	—	—	—	190	260	+H 261	190	274	274
9	216	216	286	174	244	244	174	258	258
10	158	158	—	158	158	158	158	158	158
11	142	142	142	142	142	142	142	142	142
12	114	114	—	114	114	114	114	114	114
13	—	—	—	+H 87	+H 87	—	100	—	100
14	—	+H 71	+H 71	70	+H 71	—	84	—	84

Table 3. Characteristic ^{13}C NMR chemical shifts of acetal derivatives of neospiramycin I (NSPM I) (ppm from TMS).

Carbon	NSPM I	3-*a*	3-*b*	4′-*a*	4′-*b*
1	174.2	170.5~171.6	171.6~171.9	174.3~174.5	
2	37.8	39.9~ 40.7	39.1~ 39.5	37.2~ 37.6	
3	68.3	75.0~ 75.8	73.7~ 73.9	68.2~ 68.3	
18	202.9	202.1~203.1	204.1~205.3	203.2~203.4	
2′	71.0	70.9~71.2		69.1~70.1	70.8~71.0
3′	70.2	70.2~70.5		69.1~69.9	70.1~70.6
4′	70.8	70.4~70.8		76.4~76.5	75.6~76.0
5′	73.4	73.2~73.4		73.0~73.2	72.4~72.7
3′-N(Me)$_2$	41.7	41.8~42.0		40.6~40.8	41.2~41.7

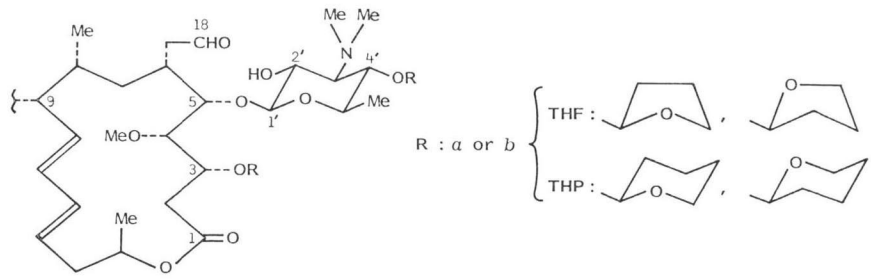


Table 4. ^{13}C NMR chemical shifts of acetal groups on neospiramycin I (ppm from TMS).

	3-*a*	3-*b*	4′-*a*	4′-*b*
THF 1	105.6~105.8	104.3~104.8	102.9~103.0	103.4~103.6
2	32.8	32.6	32.9~ 33.0	32.8~ 32.9
3	23.5	23.9	23.9	23.7
4	67.2~ 67.3	67.3	67.6~ 67.7	67.3
THP 1	101.0~101.5	100.2~100.7	101.5	100.8~100.9
2	32.9~ 33.6	31.3	30.8~ 31.1	31.1~ 31.3
3	20.0~ 20.3	21.5~ 21.8	21.3~ 21.8	20.7~ 20.8
4	25.3~ 25.4	25.2~ 25.3	25.2~ 25.3	24.9~ 25.3
5	63.4~ 63.7	64.9~ 65.1	64.7~ 65.3	64.0~ 64.1

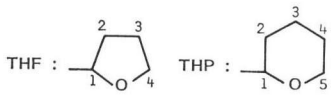


Table 5. Minimum inhibitory concentrations (MIC, μg/ml) of acetal derivatives of neospiramycin I.

Compounds		SA	BS	BC	ML	EC	KP
SPM I		0.78	0.4	1.56	<0.1	50	12.5
NSPM I		0.78	0.78	0.78	<0.1	25	6.25
Ac SPM		3.12	1.56	3.12	<0.1	>100	100
3-*b*-THF	**(7)**	3.12	1.56	3.12	0.78	100	50
3-*a*-THF	**(6)**	1.56	0.4	0.78	<0.1	25	6.25
3-*b*, 4′-*b*-THF	**(11)**	12.5	3.12	6.25	0.78	>100	>100
3-*a*, 4′-*b*-THF	**(10)**	6.25	1.56	3.12	0.78	100	100
3-*b*, 4′-*a*-THF	**(9)**	1.56	0.4	0.78	<0.1	100	50
3-*a*, 4′-*a*-THF	**(8)**	1.56	0.4	0.78	<0.1	50	50
4′-*b*-THF	**(13)**	6.25	3.12	12.5	3.12	100	100
4′-*a*-THF	**(12)**	3.12	1.56	3.12	0.4	6.25	6.25
3-*b*-THP	**(15)**	3.12	3.12	6.25	0.4	>100	50
3-*a*-THP	**(14)**	6.25	6.25	6.25	0.78	>100	50
4′-*b*-THP	**(17)**	25	6.25	12.5	1.56	>100	100
4′-*a*-THP	**(16)**	12.5	6.25	12.5	3.12	>100	>100
3-*b*, 4′-*b*-THP	**(21)**	25	3.12	6.25	0.78	>100	>100
3-*a*, 4′-*b*-THP	**(20)**	50	6.25	12.5	1.56	>100	>100
3-*b*, 4′-*a*-THP	**(19)**	12.5	6.25	6.25	1.56	>100	>100
3-*a*, 4′-*a*-THP	**(18)**	50	12.5	25	3.12	>100	>100

SA : *Staphylococcus aureus* KB 210, ML : *Micrococcus luteus* KB 212,
BS : *Bacillus subtilis* KB 211, EC : *Escherichia coli* KB 213,
BC : *Bacillus cereus* KB 143, KP : *Klebsiella pneumoniae* KB 214.

5 and 6, respectively. There is a relationship between the bonding position and configuration of THF and THP groups and activities. In the THF derivatives, the *a* configuration is more effective than the *b* configuration, and the 4′-*O*-THF group appears to contribute to an increase of activity more than the 3-*O*-THF group. It is worth noting that 3-*a*, 4′-*a* derivative (**8**) shows a similar therapeutic effect with spiramycin I, which seems to suggest one approach to improve the activity of neospiramycin *in vivo*. In THP derivatives, the *a* configuration is more active than *b* at the 4′ position, but at the 3 position the *b* configuration is more effective. The 4′-*O*-THP group appears to have a effect negative in this case.

Table 6. Therapeutic effects of neospiramycin I and its derivatives in experimental mice protection tests.

Compounds	*Staphylococcus aureus* Smith		*Streptococcus pneumoniae* Type III	
	ED_{50} (mg/kg)	MIC (μg/ml)	ED_{50} (mg/kg)	MIC (μg/ml)
Acetylspiramycin	110.7 (71.9~170.4)[1]	12.5	71.9 (55.0~ 94.3)	0.2
Spiramycin I	116.6 (71.7~189.6)	6.25	167.9 (131.5~214.3)	0.1
Neospiramycin I	>400	3.13	399.8 (297.6~537.1)	0.2
3-*b*-THF (**7**)	NT[2]		>400	0.39
3-*a*-THF (**6**)	NT		304.9 (242.6~382.4)	0.2
3-*b*, 4′-*b*-THF (**11**)	325.1 (211.5~499.6)	25	236.4 (173.2~322.8)[3]	0.2
3-*a*, 4′-*b*-THF (**10**)	269.3 (167.7~432.5)	25		
3-*b*, 4′-*a*-THF (**9**)	229.6 (166.5~316.6)	12.5	175.8 (111.0~278.5)	0.2
3-*a*, 4′-*a*-THF (**8**)	169.4 (122.9~233.3)	6.25	143.2 (98.5~208.1)	0.1

1) 95% Confidence limits.
2) Not tested.
3) Mixture of **10** and **11**.
Challenge dose: *S. aureus* Smith (4.8×10^4 cfu/ml, i.p.).
S. pneumoniae Type III (3.2×10^2 cfu/ml, i.p.).
Compounds were administered p.o. at 0 hour.

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 chloroform - methanol - conc. ammonia water, 10: 1: 0.01 (solvent I) and lower layer of chloroform - methanol - 1.5 M aqueous ammonia (solvent II). Silica gel column chromatography was performed with Merck Kiesel gel 60.

Minimum Inhibitory Concentrations

MIC values against various bacteria were determined by the agar dilution method using heart infusion agar (pH 7.0).

Therapeutic Effects in Experimental Mice Protection Tests

Mice (*dd*Y; ♂: 19±1 g) were infected intraperitoneally with 4.8×10^4 cfu/ml or 3.2×10^2 cfu/ml of *Staphylococcus aureus* Smith or *Streptococcus pneumoniae* Type III, respectively. Compounds suspended in 0.3%-sodium carboxymethyl cellulose were administered p.o. immediately post infection. ED_{50} values were determined by LITDEFIELD-WILCOXON method according to the mortality of mice at 7 days after infection.

2′-*O*-Acetylspiramycin I (**2**)

To an ice-cooled solution of spiramycin I (18.0 g) in chloroform (300 ml) and pyridine (20 ml), acetic anhydride (17.0 ml) was added and stirred for 3.5 hours. After addition of methanol, the reaction mixture was diluted with chloroform (1.0 liter) and washed with water (1.5 liters). The chloroform layer was dried over anhydrous sodium sulfate and evaporated to give a colorless solid, 18.3 g (97%). This material was pure enough for the next step of reaction, but further purification was accomplished by a column chromatography with benzene - acetone, 2: 1. TLC Rf: 0.33 (solvent I), $[\alpha]_D^{24}$ −86.1° (*c* 1.0, MeOH), high mass; 884.524 (Calcd. for $C_{45}H_{76}N_2O_{15}$: 884.524).

2′-*O*-Acetylneospiramycin I (**3**)

A solution of **2** (18.9 g) in 0.07 M hydrochloric acid (800 ml) (pH 1.9) was stirred for 5.5 hours at 42°C. The reaction mixture was neutralized with sodium hydrogen carbonate to pH 7.0 and diluted with water (1.0 liter), extracted with chloroform (2.0 liters×3). A chloroform solution was dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a residue which was crystallized from chloroform - ether afforded a colorless prisms, 11.0 g (69.5%), mp 156~158°C, TLC Rf:

0.28 (solvent I), $[\alpha]_D^{24}$ −38.8° (*c* 1.0, MeOH), high mass: 740.449 (Calcd. for $C_{38}H_{64}N_2O_{12}$: 740.446).

2′-*O*-Acetyl-3-*O*-tetrahydrofuranylneospiramycin I (**4**) and 2′-*O*-Acetyl-3,4′-di-*O*-tetrahydrofuranylneospiramycin I (**5**)

1) To a solution of **3** (10.0 g) and pyridinium *p*-toluenesulfonate (PPTS) (13.6 g) in dichloromethane (136 ml), 2,3-dihydrofuran (114 ml) was added, and stirred for 21 hours at room temperature. The reaction mixture was diluted with chloroform (1.0 liter) and washed with water (1 liter). The chloroform solution was dried over anhydrous sodium sulfate and evaporated to give an oily residue, which was chromatographed on a silica gel column with benzene - acetone, 2: 1, gave **5** and **4** in the order of elution. **4**: 6.0 g (54.8%), TLC Rf: 0.47 (solvent I), $[\alpha]_D^{24}$ −39.3° (*c* 1.0, MeOH). **5**: 1.8 g (15.1%), TLC Rf: 0.59 (solvent I), $[\alpha]_D^{24}$ −32.1° (*c* 1.0, MeOH).

2) To a solution of **3** (5.0 g) and PPTS (10.2 g) in dichloromethane (65 ml) was added, 2,3-dihydrofuran (97.1 ml), and the solution was stirred for 67 hours at room temperature. The reaction mixture was worked up in a manner similar to that described above to give **4**, 4.3 g (78.9%).

3-*O*-*a*-Tetrahydrofuranylneospiramycin I (**6**) and 3-*O*-*b*-Tetrahydrofuranylneospiramycin I (**7**)

A solution of **4** (1.40 g) in methanol was stirred for 24 hours at room temperature. The reaction mixture was evaporated and chromatographed on a silica gel column with chloroform - methanol, 6: 1, giving **7** and **6** in the order of elution. **6**: 0.36 g (27.2%), TLC Rf: 0.21 (solvent II), $[\alpha]_D^{24}$ +11.1° (*c* 1.0, MeOH), high mass: 768.974 (Calcd. for $C_{40}H_{68}N_2O_{12}$: 768.972). **7**: 0.39 g (29.0%), TLC Rf: 0·26 (solvent II), $[\alpha]_D^{24}$ −33.8° (*c* 1.0, MeOH), high mass: 768.972.

3-*a*,4′-*a*-, 3-*b*,4′-*a*-, 3-*a*,4′-*b*- and 3-*b*,4′-*b*-Di-*O*-tetrahydrofuranylneospiramycin I (**8**, **9**, **10**, **11**)

A solution of **5** (1.50 g) in methanol (60 ml) was heated at 45°C for 3 days. The reaction mixture was evaporated and the residue was chromatographed on a silica gel column with chloroform - methanol, 18: 1 ~ 9: 1 to give **11**, **10**, **9** and **8** in the order of elution. **8**: 0.32 g (22.5%), TLC Rf: 0.24 (solvent II), $[\alpha]_D^{24}$ −30.1° (*c* 1.0, MeOH), high mass: 838.519 (Calcd. for $C_{44}H_{74}N_2O_{13}$: 838.519). **9**: 0.31 g (21.8%), TLC Rf: 0.30 (solvent II), $[\alpha]_D^{24}$ −63.0° (*c* 1.0, MeOH), high mass: 838.518. **10**: 0.42 g (29.5%), TLC Rf: 0.50 (solvent II), $[\alpha]_D^{24}$ +8.3° (*c* 1.0, MeOH), high mass: 838.519. **11**: 0.36 g (25.2%), TLC Rf: 0.56 (solvent II), $[\alpha]_D^{24}$ −3.3° (*c* 1.0, MeOH), high mass: 838.521.

4′-*O*-*a*- and 4′-*O*-*b*-Tetrahydrofuranylneospiramycin I (**12** and **13**)

To a solution of a mixture of **8** ~ **11** (500 mg) in dioxane - water (1: 1, 10 ml), PPTS (143 mg) was added and stood for 39 hours at 45°C. To the reaction mixture, chloroform (50 ml) was added and washed with water (50 ml), dried over anhydrous sodium sulfate. The chloroform solution was evaporated to give a residue, which was chromatographed on a preparative silica gel TLC plate with solvent II to give **12** and **13**.

12, colorless powder, 21 mg (4.6%) TLC Rf: 0.41 (solvent II), $[\alpha]_D^{24}$ −38.5° (*c* 0.05, MeOH), high mass: 768.970 (Calcd. for $C_{40}H_{68}N_2O_{12}$: 768.972).

13, colorless powder, 20 mg (4.4%), TLC Rf: 0.45 (solvent II), $[\alpha]_D^{24}$ +3.3° (*c* 0.06, MeOH), high mass: 768.973.

3-*O*-*a*-, 3-*O*-*b*-, 4′-*O*-*a*-, 4′-*O*-*b*-, 3-*a*,4′-*a*-Di-*O*-, 3-*b*,4′-*a*-Di-*O*-, 3-*a*,4′-*b*-Di-*O*- and 3-*b*,4′-*b*-Di-*O*-tetrahydropyranylneospiramycin I (**14**, **15**, **16**, **17**, **18**, **19**, **20** and **21**)

To a solution of **3** (5.0 g) and PPTS (6.8 g) in dichloromethane (65 ml), 2,3-dihydro-4*H*-pyran (65 ml) was added, and the solution was heated to 45°C for 66 hours. To the reaction mixture, chloroform (500 ml) was added and washed with water (1.0 liter). The chloroform solution was dried over anhydrous sodium sulfate and evaporated to give an oily residue, which was chromatographed on a short column of silica gel with chloroform - methanol, 40: 1 to afford a crude powder.

The crude powder was dissolved in methanol (200 ml), and set for 3 days at room temperature. The solution was evaporated to give a solid, which was chromatographed on a silica gel column with chloroform - methanol, 44: 1 ~ 5: 1 to give **21**, **20**, **19**, **16**, **17**, **18**, **15** and **14** in the order of elution.

14: 20 mg (0.4%), TLC Rf: 0.21 (solvent II), $[\alpha]_D^{24}$ +12.0° (*c* 0.05, MeOH), high mass: 782.493 (Calcd. for $C_{41}H_{70}N_2O_{13}$: 782.492). **15**: 110 mg (2.1%), TLC Rf: 0.27 (solvent II), $[\alpha]_D^{24}$ −33.3° (*c* 0.05, MeOH), high mass: 782.489. **16**: 204 mg (3.9%), TLC Rf: 0.44 (solvent II), $[\alpha]_D^{24}$ −37.3° (*c* 1.0,

MeOH), high mass: 782.491. **17**: 63 mg (1.2%), TLC Rf: 0.41 (solvent II), $[\alpha]_D^{24}$ −24.0° (*c* 0.05, MeOH), high mass: 782.494. **18**: 30 mg (0.5%), TLC Rf: 0.34 (solvent II), $[\alpha]_D^{24}$ −41.6° (*c* 0.05, MeOH), high mass: 866.550 (Calcd. for $C_{46}H_{78}N_2O_{13}$: 866.550). **19**: 219 mg (3.7%), TLC Rf: 0.46 (solvent II), $[\alpha]_D^{24}$ −37.5° (*c* 1.0, MeOH), high mass: 866.553. **20**: 54 mg (0.9%), TLC Rf: 0.63 (solvent II), $[\alpha]_D^{24}$ +14.8° (*c* 1.0, MeOH), high mass: 866.552. **21**: 195 mg (3.3%), TLC Rf: 0.67 (solvent II), $[\alpha]_D^{24}$ −10.5° (*c* 1.0, MeOH), high mass: 866.548.

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