CHEMICAL MODIFICATION OF SPIRAMYCINS

II. SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 4′-DEOXY DERIVATIVES OF NEOSPIRAMYCIN I AND THEIR 12-(*Z*)-ISOMERS

HIROSHI SANO, MICHI INOUE and SATOSHI ŌMURA*

School of Pharmaceutical Sciences, Kitasato University and The Kitasato Institute, Minato-ku, Tokyo 108, Japan

(Received for publication April 9, 1984)

4′-Deoxy derivatives of neospiramycin I and their 12-(*Z*)-isomers were synthesized by reductive dechlorination *via* 4′-*epi*-chloro derivatives. The 12-(*Z*)-derivatives were more active against bacteria *in vitro* than the corresponding 12-(*E*)-derivatives in spite of their low affinities to ribosomes.

Neospiramycin I[1,2] is a demycarosyl derivative of spiramycin I,[3] a 16-membered macrolide antibiotic, and consists of two aminosugars, mycaminose and forosamine, and 16-membered lactone. Recently, 16-membered macrolides in which desosamine (4′-deoxymycaminose) is substituted for mycaminose, *e.g.* rosamicin,[4,5] mycinamicins[6] and demycarosyl-4′-deoxytylosin[7] were found to be active against some strains of Gram-negative bacteria and macrolide-resistant Gram-positive bacteria. The 4′-deoxy derivative of neospiramycin I which is different from these antibiotics in its structure at 3, 14 and 15 positions, provides an interesting object for chemical modification.

In this report we wish to describe the synthesis and antibacterial activities of 4′-deoxyneospiramycin I, its 4-*O*-tetrahydrofuranyl (THF) derivatives and 3-deoxy-2-eno derivative and their 12,13-stereoisomers which were found to be produced during the deoxygenation.

Synthesis

For the purpose of 4′-deoxygenation of neospiramycin I, 2′-*O*-acetyl-3-*O*-tetrahydrofuranylneospiramycin I[8] (**2**), which is derived from 2′-*O*-acetylneospiramycin I (**1**), is suitable as a protected derivative. Treatment of **2** with benzylsulfonyl chloride in pyridine gave 4′-benzylsulfonate (**3**) which was converted to 4′-*epi*-chloro-4′-deoxy derivative (**4**) by the reflux with lithium chloride in acetone - chloroform. The structures of **3** and **4** were confirmed by comparison of ^{13}C-chemical shifts of C-3′, 4′ and 5′ with those of **2** (Table 1). 4′-Mesylate (**5**) was obtained by treatment of **2** with mesyl chloride in pyridine. Hydrolysis of **5** gave 4′-*epi* derivative (**6**), which was deacetylated to give 4′-*epi*-3-*O*-tetrahydrofuranylneospiramycin I (**7**) but **5** was too labile in chlorination.

Reductive dechlorination of **4** with tributyltin hydride and α,α'-azobisisobutyronitrile in toluene at 80°C, followed by silica gel column chromatography, gave 3-*O*-*a*- and *b**-tetrahydrofuranyl-4′-deoxy derivatives (**8** and **9**) and their unusual 12-(*E*)→12-(*Z*) isomerized products (**10** and **11**), respectively.

Removal of 2′-*O*-acetyl groups of **8** and **10** by methanolysis gave 4′-deoxy-3-*O*-*a*-tetrahydrofuranylneospiramycin I (**12**) and its geometrical isomer (**14**), respectively. The pure 3-*O*-*b*-tetrahydrofuranyl derivatives (**13** and **15**) could not be obtained by the methanolysis of **9** and **11** because of their instability.

* Absolute configuration of C-1 of THF group 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.

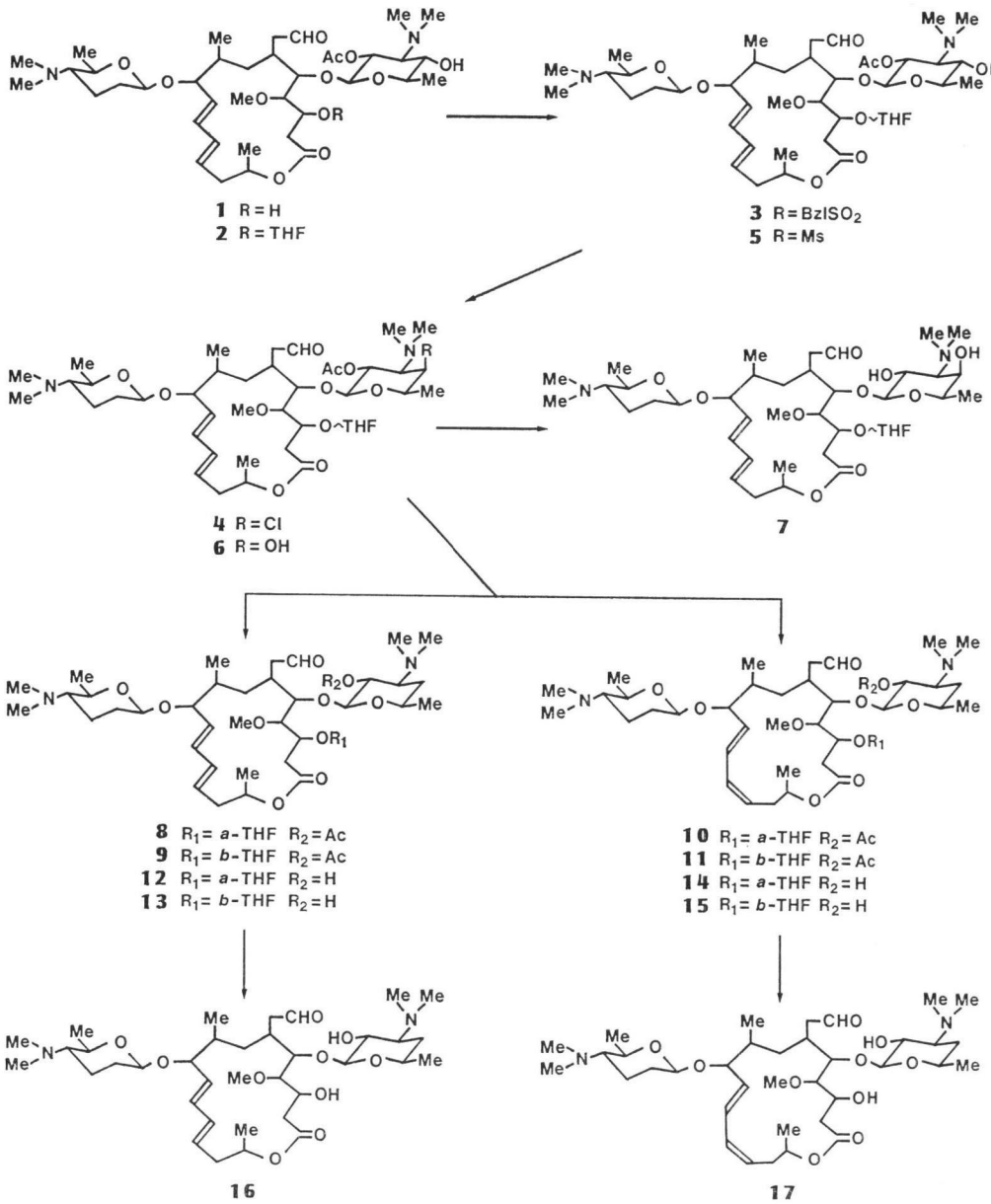
Carbon No.	**2**	**3**[*1]	**4**	**5**[*2]	**7**
1	170.5 171.9	170.4 171.8	170.4	170.4 171.8	171.2 171.8
2	39.5	39.5	40.4	39.2 39.5	39.4 40.0
3	75.3 75.4	75.4 75.5	75.4	75.4 75.6	75.1
4	85.6 85.7	85.7	85.7	85.6 85.7	83.8 84.0
5	76.5	77.1	75.6	72.2	79.8
6	29.3 29.7	30.1	29.8	29.3	31.7
7	30.2 30.3	30.1	29.8	30.1	32.1
8	31.1 31.6	31.6	31.5	31.1 31.6	32.9
9	78.7	78.6	78.5	78.8	79.5
10	127.3 127.9	127.3 127.9	127.2	127.5 127.8	128.5 128.8
11	134.4 134.8	134.3 134.8	134.4	134.3 134.8	133.3 134.1
12	132.0 132.9	132.0 132.8	132.1	132.1 132.8	132.3 132.6
13	130.9 131.8	130.8 131.8	131.8	131.1 131.8	131.1 131.2
14	42.8	42.8	40.7	41.3 42.8	41.1
15	69.4 69.8	69.4 69.8	69.9	69.4 69.9	69.5 69.7
16	20.3 20.7	20.3 20.6	20.7	20.3 20.6	20.4 20.6
17	44.4	44.6	42.6	43.4 44.6	44.8
18	202.1 204.1	201.8 203.4	201.8	201.8 203.5	202.9 203.5
19		15.2	15.1	15.2 15.3	15.7
20	61.7 62.0	61.7 62.0	61.9	61.7 62.0	61.1 61.4
1′	100.7 101.1	100.4 100.7	100.8	100.4 100.8	102.8 103.1
2′	70.8 70.9	70.8	71.1	70.8	70.8
3′	69.2	67.0	69.9	67.0	65.6
4′	70.5	80.6	60.9	80.7	66.3
5′	72.9	70.4	73.5	70.4	71.0 71.1
6′	17.9	17.6	18.3	17.6	16.9 17.0
3′-N(CH_3)$_2$	41.3	41.1	41.1	41.0	43.6
1″	99.6 99.8	99.6 99.8	99.5	99.6 99.8	100.2
2″	31.3	31.2	31.2	31.1	31.2
3″	18.5	18.5	18.5	18.6	18.6
4″	64.9	64.9	64.9	65.0	64.9
5″	73.7 73.8	73.6	73.8	73.4	73.3 73.8
6″	19.0	19.0	19.0	19.0	19.0
4″-N(CH_3)$_2$	40.7	40.6	40.6	40.6	40.7
O*C*OCH$_3$	168.9 169.1	168.6 168.7	169.3	168.6 168.7	
OCO*C*H$_3$	21.6	23.5 23.9	23.5	21.5	
3-1‴	104.5 105.8	104.5 105.8	105.8	104.6 105.8	103.5 105.9
3-2‴	32.6 33.0	32.6 33.0	33.0	32.6 33.0	32.6 32.7
3-3‴	23.5 23.9	23.5 23.9	23.5	23.5 23.9	23.5 23.8
3-4‴	67.4	67.4	67.4	67.4	67.2 67.4

*1 57.7 ($PhCH_2$), 128.8 (C-1 and C-2 of Ph), 130.6 (C-3 of Ph), 128.6 (C-4 of Ph).
*2 39.1 (SO_2Me).

The removal of tetrahydrofuranyl groups of **12**~**15** by hydrolysis gave 4′-deoxyneospiramycin I (**16**) and its 12-(*Z*)-isomer (**17**), respectively. The structures of the deoxy derivatives (**8**~**11**, **12**, **14**, **16** and **17**) were confirmed from the behavior of the chemical shifts of C-3′,4′ and 5′ in the ^{13}C NMR spectra (Table 2). The ^1H NMR spectra of 12-(*Z*)-isomers (**10**, **11**, **14**, **17**) (Table 4) showed the coupling constants, $J_{10,11}=15$, $J_{11,12}=11$ and $J_{12,13}=11$ in contrast to $J_{10,11}=15$, $J_{11,12}=10$ and $J_{12,13}=14$ Hz of the natural 12-(*E*)-isomers, **8**, **9**, **12** and **16**, and neospiramycin I.

4′-Deoxyneospiramycin I (**16**) was also obtained through an another protected derivative of neo-

Scheme 1.



spiramycin I. Treatment of neospiramycin (NSPM) I with *t*-butyldimethylsilyl (TBDMS) chloride and imidazole gave unique 3,18-*O*-TBDMS acetal (**18**), 2′-*O*-TBDMS-3,18-*O*-TBDMS acetal (**19**), 2′,4′-di-*O*-TBDMS-3,18-*O*-TBDMS acetal (**20**) and 18-*O*-TBDMS enol (**21**) of neospiramycin I, the structures of which were confirmed by ^{13}C NMR spectra (Table 3).

19, which was obtained as main product by controling the reaction conditions, was treated with mesyl chloride in pyridine to give 4′-*epi*-chloro derivative (**22**). 4′-*O*-Mesylation followed by SN_2 substitution by chloride anion formed from mesyl chloride seems to occur in the reaction.

Desilylation of **22** by tetrabutylammonium fluoride gave the free 4′-*epi*-chloride (**23**), which under-

Table 2. ^{13}C NMR chemical shifts for 4′-deoxy derivatives of neospiramycin I and their 12-(*Z*)-isomers.

Carbon No.	8	9	10	11	12	14	16	17
1	170.5	172.0	170.5	171.6	170.6	170.5	174.4	171.9
2	33.0	39.6		39.2	40.7		37.6	32.6
3	75.5	73.9	75.9	72.1	75.5	76.1	69.0	70.4
4	86.0	85.7	86.6	86.6	85.5	86.1	85.3	85.4
5	74.9	76.4	75.9	74.9	76.7	77.3	78.4	80.2
6	29.9	30.6	29.2	30.5	29.1	28.8	30.3	30.8
7	30.7	30.9	30.3	30.5	30.5	29.5	30.7	29.9
8	31.3	31.3	32.9	31.7	32.6	32.9	31.4	33.6
9	78.5	78.7	78.8	75.8	78.8	79.0	78.4	79.3
10	127.1	127.9	125.5	124.8	128.0	125.6	128.4	125.0
11	134.5	134.8	131.9	132.6	134.2	132.0	134.8	132.9
12	132.1	133.0	131.9	132.3	131.4	131.9	133.0	132.1
13	131.9	130.8	128.0	128.2	131.9	128.0	130.8	128.0
14	41.0	41.3	40.7	43.6	41.0	40.7	42.0	39.8
15	69.9	69.0	69.0	69.0	69.3	69.3	68.3	69.0
16	20.7	20.3	20.9	20.8	20.7	21.1	20.1	18.4
17	42.6	44.3	43.1	44.1	42.9	43.2	42.9	43.9
18	202.2	204.4	202.1	203.7	202.4	202.3	203.5	203.4
19	15.1	15.2	16.0	16.2	15.1	15.9	15.1	16.9
20	62.0	62.2	62.0	62.0	62.1	62.3	61.9	61.8
1′	101.2	100.8	101.1	101.0	103.9	103.8	103.9	104.1
2′	71.6	69.4	70.8	71.0	70.4	70.3	69.4	69.4
3′	63.3	63.4	63.4	63.3	65.6	65.6	65.6	65.6
4′	29.3	30.2	29.3	30.5	30.2	30.5	28.6	28.8
5′	69.0	71.7	71.6	71.6	69.8	70.8	70.2	70.2
6′	20.9	20.9	18.0	18.1	21.0	18.0	18.4	21.1
3′-N(CH_3)$_2$	40.7	40.5	40.72	40.6	40.5	40.3	40.2	40.3
1″	99.4	99.7	99.8	99.7	99.7	99.9	100.0	101.2
2″	31.3	31.3	31.3	31.3	31.3	31.3	31.3	31.3
3″	18.5	18.5	18.5	18.5	18.5	18.5	18.4	18.5
4″	64.9	65.0	65.0	64.9	64.9	64.9	64.9	65.0
5″	78.9	73.8	73.9	73.8	73.9	73.9	73.8	73.8
6″	19.0	18.9	18.9	18.9	19.0	18.9	18.9	19.1
4″-N(CH_3)$_2$	40.7	40.7	40.66	40.7	40.7	40.7	40.7	40.7
O*C*OCH$_3$	169.9	169.7	169.8	169.7				
OCO*C*H$_3$	21.4	21.4	21.4	21.4				
3-1‴	105.8	104.5	106.3	101.9	105.7	106.3		
3-2‴	33.0	32.6	32.8	32.7	33.0	32.9		
3-3‴	23.5	23.9	23.4	24.1	23.5	23.4		
3-4‴	67.4	67.3	67.4	67.4	67.4	67.4		

went reductive dechlorination to give **16** in a low yield. The reductive dechlorination of **22** gave the protected 4′-deoxy derivative (**24**), but removal of 2′-TBDMS group from **24** was not achieved in an usual manner.

3,4′-Dideoxy-2-enoneospiramycin I (**29**) and its 12-(*Z*)-isomer (**30**) were synthesized from 2′-*O*-acetylneospiramycin I (**1**) bearing a free 3-hydroxyl group. Mesylation of **1** followed by substitution by chloride anion, giving 4′-*epi*-chloro-3-*O*-mesyl derivative (**25**). Alkaline treatment of **25** gave 3-deoxy-2-eno derivative (**26**). The structures of **25** and **26** were confirmed by their ^{13}C NMR and mass spectra (Tables 3 and 5).

Table 3. ^{13}C NMR chemical shifts for TBDMS derivatives and 3,4′-dideoxy-2-eno derivatives of neospiramycin I.

Carbon No.	18*1	19*2	20*3	21*4	22*5	23	24*6	25*7	26	29	30
1	170.0	170.0	169.7	174.1	170.0	174.4	170.3	169.9	169.2	165.4	164.8
2	37.0	36.4	36.2	37.0	30.0	37.5	37.2	38.0	140.9	140.7	142.3
3	70.1	70.0	69.3	68.1	70.4	69.1	70.4	77.4	125.6	126.5	124.2
4	86.5	86.7	86.5	84.9	87.2	85.3	87.1	83.8	83.2	82.5	83.5
5	83.2	82.2	82.1	80.2	82.2	78.9	82.5	79.3	79.2	82.3	82.7
6	32.8	33.0	32.9	30.4	33.3	30.2	33.4	30.3	28.7	29.6	29.7
7	33.7	33.4	32.9	30.9	36.7	30.7	33.7	31.1	29.9	29.9	30.8
8	39.4	39.6	39.4	38.7	36.9	31.5	40.0	31.6	31.2	30.1	32.4
9	82.4	80.4	80.0	78.9	81.1	78.4	80.2	77.8	78.4	77.6	79.0
10	126.8	126.5	126.3	128.2	126.5	128.4	126.7	128.0	127.6	127.3	125.3
11	138.4	138.6	138.6	134.5	138.6	134.8	139.2	134.6	134.8	135.1	132.3
12	134.7	134.6	134.7	132.7	134.7	133.0	135.0	132.5	133.9	133.9	131.6
13	127.5	127.3	127.2	130.9	127.3	130.9	127.3	131.3	130.3	130.4	128.8
14	40.7	40.7	40.7	41.7	40.1	42.0	40.9	41.4	41.8	41.8	40.7
15	70.1	69.7	69.8	69.3	69.9	68.2	70.1	69.2	69.2	69.3	69.4
16	19.8	20.1	20.0	20.1	20.0	20.1	20.3	20.2	20.2	20.1	18.1
17	41.5	41.6	41.6	116.5	41.6	43.0	41.6	43.2	42.2	42.3	42.8
18	99.8	98.3	98.0	128.8	98.8	203.1	98.7	203.7	201.7	201.9	202.1
19	18.0	21.6	17.9	15.2	21.4	15.1	18.2	15.4	15.1	15.0	16.2
20	58.6	57.7	57.4	61.7	58.5	62.0	58.2	62.0	56.4	56.2	57.4
1′	103.9	103.4	103.4	105.5	103.3	103.1	103.3	100.7	101.8	105.8	105.6
2′	71.0	72.1	72.0	71.2	72.9	71.9	68.9	71.1	70.8	69.3	69.9
3′	70.5	71.2	72.7	70.3	72.6	70.4	66.5	69.2	69.6	65.3	65.3
4′	70.5	71.9	73.3	70.5	61.3	60.0	28.0	60.8	60.9	29.2	29.4
5′	73.5	72.7	73.9	73.7	73.1	73.8	71.9	73.5	73.3	70.5	70.5
6′	19.2	18.6	18.9	17.7	18.4	18.1	17.7	18.2	18.4	21.1	21.1
3′-N(CH_3)$_2$	41.9	41.6	41.6	41.7	41.6	41.2	41.2	41.1	41.1	40.7	40.7
1″	101.9	100.1	99.4	99.7	100.7	100.1	101.1	100.1	99.3	99.4	100.3
2″	31.2	31.2	31.3	31.4	31.2	31.3	31.3	31.3	31.2	31.2	31.3
3″	18.6	18.3	18.3	18.4	18.4	18.5	18.6	18.4	18.4	18.5	18.5
4″	65.0	65.1	65.0	64.9	65.1	64.9	65.3	65.0	64.8	64.9	64.9
5″	74.0	73.7	73.6	74.0	73.7	73.8	73.8	73.7	73.8	73.8	73.8
6″	19.2	19.2	19.1	19.2	19.2	18.9	19.3	18.9	18.9	19.0	19.0
4″-N(CH_3)$_2$	40.7	40.7	40.7	40.7	40.7	40.7	40.9	40.6	40.7	40.5	40.5
O*C*OCH$_3$								169.2	165.5		
OCO*C*H$_3$								21.2	21.2		

*1 Si(Me)$_2$: −3.8, −5.1 (C-18); SiC(*Me*)$_3$: 25.9 (C-18); Si*C*(Me)$_3$: 18.1 (C-18).

*2 Si(Me)$_2$: −1.9, −3.4 (C-2′), −3.8, −4.9 (C-18); SiC(*Me*)$_3$: 25.9 (C-18), 26.5 (C-2′); Si*C*(Me)$_3$: 18.0, 18.3 (C-2′, 18).

*3 Si(Me)$_2$: −1.6, −3.0 (*C*-2′), −3.1, −4.5 (C-4′), −3.8, −4.9 (C-18); SiC(*Me*)$_3$: 25.9 (C-18), 26.5 (*C*-2′), 26.1 (C-4′); Si*C*(Me)$_3$: 18.2, 18.3 (C-2′, 4′, 18).

*4 Si(Me)$_2$: −5.1, −5.3 (C-18); SiC(*Me*)$_3$: 25.5 (C-18); Si*C*(Me)$_3$: 18.4 (C-18).

*5 Si(Me)$_2$: −3.4, −4.6 (C-2′), −3.8, −5.0 (C-18); SiC(*Me*)$_3$: 25.9 (C-18), 26.1 (C-2′); Si*C*(Me)$_3$: 18.0, 18.2 (C-2′,18).

*6 Si(Me)$_2$: −3.3, −3.7 (C-2′), −4.2, −4.8 (C-18); SiC(*Me*)$_3$: 26.1 (C-18), 26.4 (C-2′); Si*C*(Me)$_3$: 18.2 (C-2′, 18).

*7 SO_2Me: 38.9.

Scheme 2.

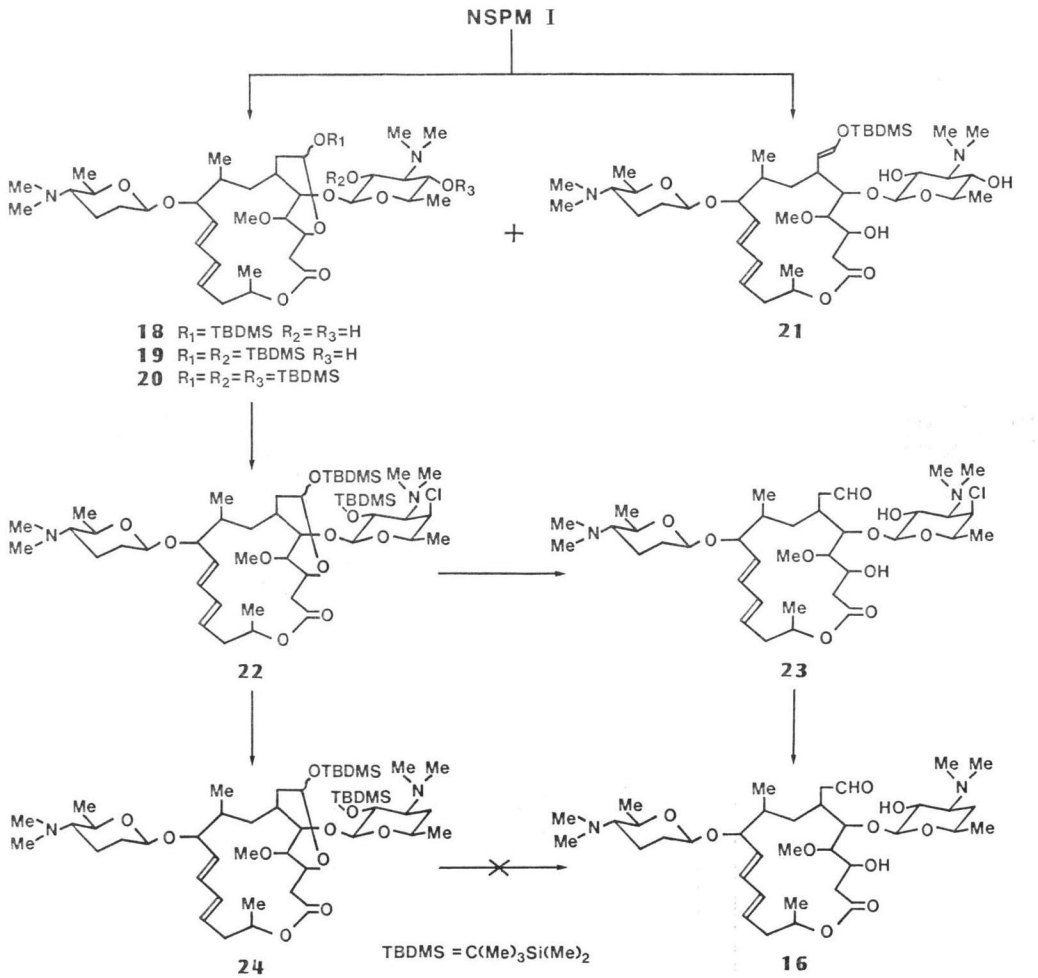


Table 4. ^{1}H NMR chemical shifts and coupling constants of H-10, 11, 12 and 13 of 4′-deoxy derivatives of neospiramycin I, their 12-(*Z*)-isomers, and neospiramycin I (NSPM I).

	NSPM I	8	9	10	11	12	14	16	17	29	30
Proton No.											
10	5.69	5.74	5.66	5.74	5.72	5.78	5.74	5.67	5.73	5.68	5.72
11	6.25	6.05	6.04	6.53	6.59	6.28	6.57	6.28	6.54	6.41	6.55
12	6.02	6.29	6.36	6.16	6.19	6.08	6.21	6.03	6.18	6.02	6.23
13	5.57	5.62	—	—	—	5.77	—	5.54	5.55	—	5.63
Coupling constant (Hz)											
$J_{9,10}$	10.0	9.0	10.0	9.0	8.0	11.5	10.0	9.0	8.5	9.5	8.5
$J_{10,11}$	15.5	14.5	14.5	15.0	14.0	15.5	15.0	14.0	15.5	14.0	14.5
$J_{11,12}$	11.0	9.5	11.0	11.0	11.0	10.0	11.0	10.0	11.5	11.0	11.0
$J_{12,13}$	15.5	14.0	15.0	11.0	11.0	14.0	11.0	14.5	11.5	14.5	11.0

Reductive dechlorination of **26** followed by silica gel column chromatographic separation, to give 4′-deoxy derivative (**27**) and its 12-(*Z*)-isomer (**28**). Both compounds were deprotected by methanolysis giving 3,4′-dideoxy-2-enoneospiramycin I (**29**) and its 12-(*Z*)-isomer (**30**), respectively. The ^{13}C NMR

Scheme 3.

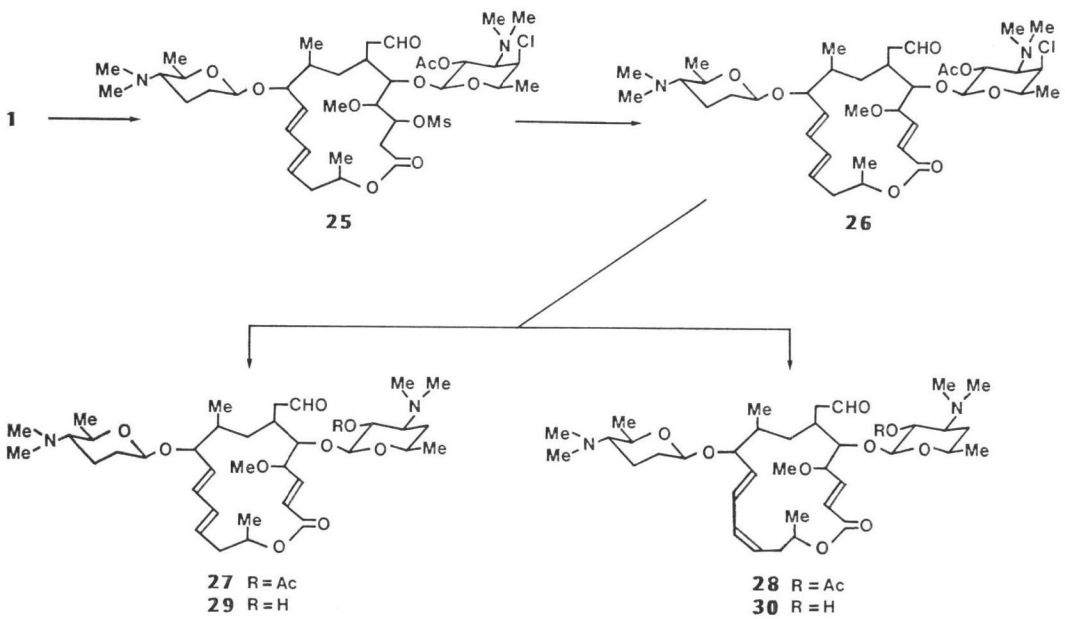
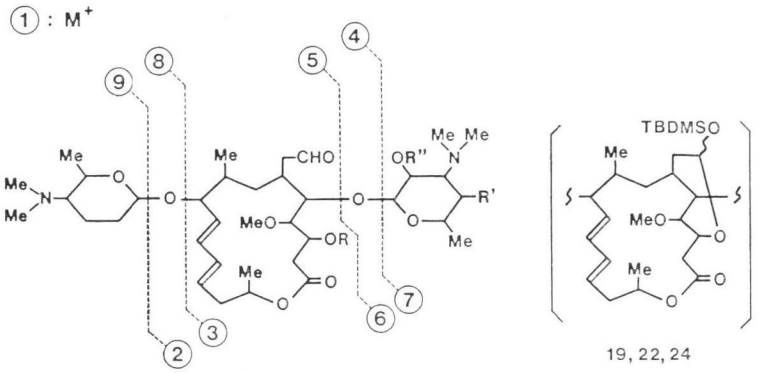


Table 5. Diagnostic mass fragmentation for neospiramycin I derivatives.



	2, 6	4	8~11	12~15	16, 17	19	22
1	810	826, 828	794	752	682	926	944, 946
2	—	—	—	610	—	784	802, 804
3	+H 653	670, 672	636	+H 595	540	—	786, 788
4	+H 595	—	—	+H 595	+H 525	638	638
5	+H 579	578	578	578	508	622	622
6	—	—	216	174	—	—	322, 324
7	216	234, 236	200	158	158	288	306, 308
8	158	—	158	158	158	—	158
9	142	142	142	142	142	142	142

	23	24	25	26	27, 28	29, 30
1	716, 718	910	—	740, 742	706	664
2	—	768	—	—	564	522
3	—	+H 753	−MsOH−H 581, 583	582, 584	548	+H 507
4	—	638	—	—	506	+H 507
5	508	622	−MsOH 490	490	490	490
6	208, 210	288	—	250, 252	216	174
7	192, 194	272	234, 236	234, 236	200	158
8	—	—	—	—	158	158
9	142	142	−H 141	142	142	142

spectra (Table 3) of **29** and **30** showed additional signals of olefinic carbons assigned to C-2 and 3 and of 4′-methylene and an up field shift of the signals of 3′ and 5′ carbons, respectively. The coupling constants, $J_{10,11}$, $J_{11,12}$ and $J_{12,13}$ were 14, 11 and 11 Hz in **30** in comparison of 14, 11 and 14 Hz in **29** in their ^{1}H NMR spectra (Table 4), thus confirming their structures.

Antibacterial Activity

The derivatives of neospiramycin I were evaluated by antibacterial activity (MIC), affinity to ribosomes (ID_{50}) and retention time (RT) in HPLC, as shown in Table 6. Since it is known that the binding of a macrolide to the 50S subunit of bacterial ribosome causes inhibition of protein synthesis, the ID_{50} value for ribosome binding is one of the parameters for the evaluation of derivatives at a level of the bacterial target. It would be possible to know the change of permeability to cell membrane from correlation between ID_{50} and MIC. RT in HPLC using a reverse phase system corresponds to lipophilicity[12] which is one of the indications of pharmacokinetics such as absorption and distribution. 4′-Deoxy derivatives (**12**, **13**, **16** and **29**) are equal in MIC to neospiramycin I and its derivatives which have a hydroxyl group at 4′-position, but they do not show any enhancement in activity against macrolide-resistant *Staphylococcus aureus* contrary to expectation. 12-(*Z*)-Isomers (**14**, **15**, **17** and **30**) of 4′-deoxy neospiramycin I derivatives are effective in antimicrobial activity in spite of their low affinities to ribosomes compared with that of each compound bearing natural geometry, indicating that the conformational change of the aglycone moiety based on the geometrical isomerism at the 12-position results in a higher permeability to bacterial cells. Among the derivatives, 12-(*Z*)-4′-deoxyneospiramycin I (**17**) is the most active. 3-Deoxy-2-eno compounds are less active than 3-OH compounds.

Recently, it has been reported that 10-(*Z*)- derivatives of carbomycin, deltamycin and 4″-phenylacetyldeltamycin show higher antimicrobial activity,[13] which is interesting in the similarity to 12-(*Z*)-derivatives of neospiramycin I.

Table 6. MIC, ID_{50} and RT of deoxy derivatives of neospiramycin I.

	Compounds	MIC (μg/ml)*							ID_{50} (μM)	RT (minutes)
		SA	SA^r	BS	BC	ML	EC	KP		
12	4′-Deoxy-3-*O*-*a*-THF	1.56	>100	3.12	6.25	0.78	100	50	—	—
14	12-(*Z*)-4′-Deoxy-3-*O*-*a*-THF	1.56	>100	3.12	3.12	0.78	>100	>100	—	—
13	4′-Deoxy-3-*O*-*b*-THF	6.25	>100	6.25	6.25	1.56	>100	>100	1.9	4.8
15	12-(*Z*)-4′-Deoxy-3-*O*-*b*-THF	3.12	>50	6.25	6.25	0.78	>50	50	7.0	4.3
16	4′-Deoxy	3.12	—	6.25	3.12	0.78	50	12.5	1.0	3.0
17	12-(*Z*)-4′-Deoxy	1.56	—	3.12	3.12	0.78	50	6.25	2.3	2.9
29	3,4′-Dideoxy-2-eno	12.5	>100	25	25	1.56	100	>100	4.8	4.1
30	12-(*Z*)-3,4′-Di-deoxy-2-eno	12.5	>50	25	25	6.25	100	>100	4.2	3.8
	Neospiramycin I	3.12	>100	3.12	3.12	0.2	50	12.5	1.2	3.2
	Spiramycin I	3.12	>100	1.56	3.12	0.1	100	>100	1.0	4.3

* SA: *Staphylococcus aureus* KB210 (ATCC 6538P), SA^r: *Staphylococcus 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).

Experimental

NMR spectra were measured on a Jeol FX-100 spectrometer in $CDCl_3$ solution (Tables 1~3). Mass spectra were obtained on a Jeol D-100 and DX-300 spectrometer at 20 eV (Table 5). 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 without cited. 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.[6]

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.[14] RT was recorded at 1 ml/minute of flow rate with a UV monitor (231 nm).

2′-*O*-Acetyl-4′-*O*-benzylsulfonyl-3-*O*-tetrahydrofuranylneospiramycin I (3)

To a solution of 2′-*O*-acetyl-3-*O*-tetrahydrofuranylneospiramycin I[8] (**2**) (1.00 g) in pyridine (20 ml), benzylsulfonyl chloride (517 mg) was added and stood for 1.5 hours at room temp. The reaction mixture was poured into H_2O (100 ml) and extracted with $CHCl_3$ (100 ml×3). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residual solid was dissolved in toluene - Me_2CO, 4: 1 (3 ml) and the insoluble solid of **3** (681 mg) was filtered. The filtrate was chromatographed on a silica gel column with toluene - Me_2CO, 4: 1, to give a colorless powder of **3** (137 mg) (total yield 818 mg, 68.7%). TLC Rf 0.50; $[\alpha]_D^{23}$ −45.4° (*c* 1.0, $CHCl_3$).

Anal Calcd for $C_{49}H_{45}N_2O_{10}S$: C 60.41, H 7.86, N 2.88, S 3.29.
Found: C 59.93, H 8.09, N 2.86, S 3.03.

2′-*O*-Acetyl-4′-*epi*-chloro-4′-deoxy-3-*O*-tetrahydrofuranylneospiramycin I (4)

To a solution of **3** (600 mg) in $CHCl_3$ - Me_2CO, 1: 3 (16 ml), lithium chloride (264 mg) and triethylamine (0.07 ml) were added and heated to reflux for 5 hours. The reaction mixture was diluted with $CHCl_3$ (60 ml) and washed with H_2O (100 ml). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to give a powder, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 4: 1, to give a colorless powder, 278 mg (53.9%). TLC Rf 0.50; $[\alpha]_D^{23}$ −34.2° (*c* 1.0, $CHCl_3$).

2′-*O*-Acetyl-4′-*O*-mesyl-3-*O*-tetrahydrofuranylneospiramycin I (5)

To a solution of **2** (760 mg) in pyridine (24 ml), mesyl chloride (0.41 ml) was added and stood for 45 minutes at room temp. The reaction mixture was poured into H_2O (100 ml) and extracted with $CHCl_3$ (100 ml×3). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to give a powder, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 2: 1, to give a colorless powder, 588 mg (70.3%). TLC Rf 0.71.

2′-*O*-Acetyl-4′-*epi*-3-*O*-tetrahydrofuranylneospiramycin I (6)

A solution of **5** (580 mg) in 50% Me_2CO (23 ml) was held for 8 days at room temp. The reaction mixture was poured into a saturated solution of sodium hydrogen carbonate (60 ml) and extracted with $CHCl_3$ (60 ml×3). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to give a powder, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 1: 1, to give a colorless powder, 320 mg (59.5%). TLC Rf 0.41; $[\alpha]_D^{23}$ −26.2° (*c* 1.0, $CHCl_3$).

4′-*epi*-3-*O*-Tetrahydrofuranylneospiramycin I (7)

A solution of **6** (206 mg) in MeOH (8 ml) was heated at 45°C for 5 days. The reaction mixture was evaporated to give a powder, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH, 15: 1, giving a colorless powder, 103 mg (84.3%). TLC Rf 0.37; $[\alpha]_D^{24}$ −44.1° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 232 (21,900); High MS 768.479 (Calcd for $C_{40}H_{68}N_2O_{12}$: 768.477).

2′-*O*-Acetyl-4′-deoxy-3-*O*-*a*-tetrahydrofuranylneospiramycin I (**8**), 2′-*O*-Acetyl-4′-deoxy-3-*O*-*b*-tetrahydrofuranylneospiramycin I (**9**), 12-(*Z*)-2′-*O*-Acetyl-4′-deoxy-3-*O*-*a*-tetrahydrofuranylneospiramycin I (**10**) and 12-(*Z*)-2′-*O*-Acetyl-4′-deoxy-3-*O*-*b*-tetrahydrofuranylneospiramycin I (**11**)

To a solution of **4** (169 mg) in toluene (3.4 ml), tributyltin hydride (1.0 ml) and α,α'-azobisisobutyronitrile (11 mg) were added and heated at 80°C for 1 hour under a stream of nitrogen gas. The reaction mixture was diluted with $CHCl_3$ (20 ml) and washed with H_2O. The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated, to give an oily residue. The oil was chromatographed on a column of silica gel with C_6H_6 - Me_2CO, 2: 1, to give **10**, **11**, **8** and **9** in the order of elution.

8, colorless powder, 24 mg (14.9%). TLC Rf 0.31; $[\alpha]_D^{23}$ −28.4° (*c* 0.5, $CHCl_3$).

9, colorless powder, 17 mg (10.5%). TLC Rf 0.27; $[\alpha]_D^{23}$ −60.8° (*c* 0.5, $CHCl_3$).

10, colorless powder, 29 mg (17.6%). TLC Rf 0.34; $[\alpha]_D^{23}$ −55.5° (*c* 0.8, $CHCl_3$).

11, colorless powder, 13 mg (8.2%). TLC Rf 0.32; $[\alpha]_D^{23}$ −75.3° (*c* 0.3, $CHCl_3$).

4′-Deoxy-3-*O*-*a*-tetrahydrofuranylneospiramycin I (**12**)

A solution of **8** (25 mg) in MeOH (1.0 ml) was held for 2 days at room temp. The reaction mixture was evaporated, to give a powder, which was purified by a preparative silica gel TLC with $CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.01, giving a colorless powder, 16 mg (66%). TLC Rf 0.21; $[\alpha]_D^{24}$ −8.3° (*c* 0.05, MeOH); UV λ_{max}^{MeOH} nm (ε) 232 (20,700).

4′-Deoxy-3-*O*-*b*-tetrahydrofuranylneospiramycin I (**13**)

A solution of **9** (40 mg) in MeOH (1.6 ml) was held for 2 days at room temp. The reaction mixture was evaporated, to give a crude powder, 26 mg, TLC Rf 0.19. Further purification failed because of its instability.

12-(*Z*)-4′-Deoxy-3-*O*-*a*-tetrahydrofuranylneospiramycin I (**14**)

A solution of **10** (30 mg) in MeOH (1.3 ml) was held for 2 days at room temp. The reaction mixture was evaporated and the residual powder was chromatographed on a preparative silica gel TLC plate with $CHCl_3$ - MeOH - conc NH_4OH, 10: 1: 0.01, to give a colorless powder, 19 mg (66%). TLC Rf 0.24; $[\alpha]_D^{24}$ −38.3° (*c* 0.05, MeOH); UV λ_{max}^{MeOH} nm (ε) 233 (11,000).

12-(*Z*)-4′-Deoxy-3-*O*-*b*-tetrahydrofuranylneospiramycin I (**15**)

A solution of **11** (40 mg) in MeOH (1.6 ml) was held for 2 days at room temp. The reaction mixture was evaporated to give a crude powder, 24 mg, TLC Rf 0.23, which could not be purified because of its instability.

4′-Deoxyneospiramycin I (**16**) and 12-(*Z*)-4′-Deoxyneospiramycin I (**17**)

To a solution of a mixture of **12**~**15** (123 mg, prepared from **4** in a similar way described above without separation of each epimers arising from tetrahydrofuranyl group) in dioxane - H_2O, 1: 1 (2.5 ml), pyridinium *p*-toluenesulfonate (41 mg) was added and heated at 45°C for 40 hours. The reaction mixture was diluted with H_2O (15 ml) and extracted with $CHCl_3$ (15 ml×3). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to give a powder, which was chromatographed on a silica gel preparative TLC plate with a lower layer of $CHCl_3$ - MeOH - 1.5 M NH_4OH, 2: 1: 1, giving **16** and **17**.

16, colorless powder, 21 mg (19%). TLC Rf 0.33; $[\alpha]_D^{24}$ −18.4° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 231 (12,000); High MS 682.441 (Calcd for $C_{36}H_{62}N_2O_{10}$: 682.440).

17, colorless powder, 17 mg (15%). TLC Rf 0.26; $[\alpha]_D^{24}$ −28.3° (*c* 0.4, MeOH); UV λ_{max}^{MeOH} nm (ε) 236 (21,000); High MS 682.439.

t-Butyldimethylsilylation of Neospiramycin I

To a solution of neospiramycin I (251 mg) in DMF (0.63 ml), *t*-butyldimethylsilyl chloride (216 mg) and imidazole (195 mg) were added and held for 25 hours at room temp. After addition of a few drops of MeOH, the reaction mixture was poured into H_2O (25 ml) and extracted with $CHCl_3$ (25 ml×3). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to give an oil, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH, 10: 1, giving 2′,4′-di-*O*-*t*-butyldimethylsilylneospiramycin I 3,18-(*O*-*t*-butyldimethylsilyl)acetal (**20**), 2′-*O*-*t*-butyldimethylsilylneospiramycin I 3,18-(*O*-*t*-butyldimethylsilyl)acetal (**19**), neospiramycin I (*O*-*t*-butyldimethylsilyl)enol (**21**) and neo-

spiramycin I 3,18-(*O-t*-butyldimethylsilyl)acetal (**18**), in the order of elution.

18, colorless powder, 70 mg (24.0%); TLC Rf 0.19; $[\alpha]_D^{23}$ −10.8° (*c* 1.0, $CHCl_3$).

19, colorless powder, 77 mg (23.3%); TLC Rf 0.39; $[\alpha]_D^{23}$ −8.0° (*c* 0.3, $CHCl_3$); High MS 926.608 (Calcd for $C_{48}H_{90}N_2O_{11}Si_2$: 926.608).

20, colorless powder, 7 mg (1.9%); TLC Rf 0.62; $[\alpha]_D^{23}$ +10.2° (*c* 1.0, $CHCl_3$).

21, colorless powder, 94 mg (32.3%); TLC Rf 0.25.

19 was obtained in 51.4% yield by the prolonged reaction time.

2′-*O-t*-Butyldimethylsilyl-4′-*epi*-chloro-4′-deoxyneospiramycin I 3,18-(*O-t*-Butyldimethylsilyl)acetal (**22**)

To a solution of **18** (1.00 g) in pyridine (20 ml), mesyl chloride (0.97 ml) was added and stood for 6 days at room temp. The reaction mixture was poured into H_2O (100 ml) and extracted with $CHCl_3$ (100 ml×3). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to give a solid, which was chromatographed on a silica gel with C_6H_6 - Me_2CO, 15: 1, to give a colorless powder, 0.38 g (36.9%). TLC Rf 0.32; $[\alpha]_D^{24}$ −1.0° (*c* 1.0, MeOH).

4′-*epi*-Chloro-4′-deoxyneospiramycin I (**23**)

22 (100 mg) was dissolved in 1 M solution of tetrabutylammonium fluoride in THF (0.8 ml) and held for 24 hours at room temp. The reaction mixture was diluted with $CHCl_3$ (10 ml) and washed with H_2O. The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to give an oily residue, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH, 30: 1, to give a colorless powder, 41 mg (46.9%). TLC Rf 0.43 ($CHCl_3$ - MeOH - conc NH_4OH, 47: 1: 0.01); $[\alpha]_D^{24}$ −47.4° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 232 (19,300); High MS 716.402, 718.394 (Calcd for $C_{36}H_{61}N_2O_{10}Cl$: 716.401, 718.398).

4′-Deoxy-2′-*O-t*-butyldimethylsilylneospiramycin I 3,18-(*O-t*-Butyldimethylsilyl)acetal (**24**)

To a solution of **22** (200 mg) in toluene (4 ml), tributyltin hydride (0.74 ml) and α,α′-azobisisobutyronitrile (7.3 mg) were added and heated at 80°C for 1.5 hours under a nitrogen atmosphere. The reaction mixture was diluted with $CHCl_3$ (20 ml) and washed with H_2O. The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated, to give an oily residue, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 9: 1, giving 113 mg (58.9%). TLC Rf 0.29 (lower layer of $CHCl_3$ - MeOH - 1.5 N NH_4OH, 2: 1: 1); $[\alpha]_D^{24}$ −1.1° (*c* 1.0, MeOH).

2′-*O*-Acetyl-4′-*epi*-chloro-4′-deoxy-3-*O*-mesylneospiramycin I (**25**)

To a solution of **1** (17.0 g) in pyridine (340 ml), mesyl chloride (3.2 ml) was added and held for 1 hour at room temp. The reaction mixture was poured into H_2O (1.5 liters) and extracted with $CHCl_3$ (1.5 liters×3). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated, to give a powder. To a solution of the powder in $CHCl_3$ - Me_2CO, 1: 1 (360 ml), lithium chloride (9.80 g) and triethylamine (2.3 ml) were added and heated to reflux for 2.5 hours. The reaction mixture was diluted with $CHCl_3$ (1.0 liter) and washed with H_2O (1.5 liters). The $CHCl_3$ layer was dried over anhydrous sodium sulfate and evaporated, and the residual powder was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 2: 1, giving a colorless powder, 9.00 g (46.3%). TLC Rf 0.44; $[\alpha]_D^{24}$ −42.5° (*c* 1.0, MeOH).

Anal Calcd for $C_{36}H_{55}N_2O_{13}SCl$: C 55.93, H 7.82, N 3.35, S 3.83, Cl 4.23.
Found: C 55.45, H 7.98, N 3.20, S 3.74, Cl 4.75.

2′-*O*-Acetyl-4′-*epi*-chloro-2-eno-3,4′-dideoxyneospiramycin I (**26**)

A solution of **25** (3.00 g) in a saturated solution of sodium carbonate in MeOH (60 ml) was stirred for 3 hours at room temp. The reaction mixture was evaporated and the residue was dissolved in $CHCl_3$ (300 ml) and washed with H_2O (300 ml). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated, to give a powder, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH, 45: 1, to give a colorless powder, 2.39 g (90.0%). TLC Rf 0.51; $[\alpha]_D^{24}$ −28.9° (*c* 1.0, MeOH); High MS 740.402, 742.398 (Calcd for $C_{38}H_{61}N_2O_{10}Cl$: 740.401, 742.398).

2′-*O*-Acetyl-3,4′-dideoxy-2-enoneospiramycin I (**27**) and 12-(*Z*)-2′-*O*-Acetyl-3,4′-dideoxy-2-enoneospiramycin I (**28**)

To a solution of **26** (2.50 g) in toluene (50 ml), tributyltin hydride (17.7 ml) and α,α'-azobisisobutyronitrile (177 mg) were added and heated at 80°C for 1 hour under a stream of nitrogen gas. The reaction mixture was diluted with $CHCl_3$ (250 ml) and washed with H_2O (250 ml). The $CHCl_3$ solution was dried over anhydrous sodium sulfate, evaporated, and oily residue was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 2: 1, giving **28** and **27** in the order of elution.

27, a colorless powder, 264 mg (11.1%). TLC Rf 0.42, $[\alpha]_D^{24}$ −21.0° (*c* 1.0, MeOH); High MS 706.440 (Calcd for $C_{38}H_{62}N_2O_{10}$: 706.440).

28, a colorless powder, 332 mg (13.9%). TLC Rf 0.46; $[\alpha]_D^{24}$ −59.5° (*c* 1.0, MeOH); High MS 706.439.

3,4'-Dideoxy-2-enoneospiramycin I (**29**)

A solution of **27** (40 mg) in MeOH (1.6 ml) was held for 2 days at room temp. The reaction mixture was evaporated, and the residue was chromatographed on a silica gel column with $CHCl_3$ - MeOH, 6: 1, to give a colorless powder of **29**, 55 mg (47%). TLC Rf 0.24; $[\alpha]_D^{24}$ −14.3° (*c* 0.04, MeOH); UV λ_{max}^{MeOH} nm (ε) 226 (12,400); High MS 664.427 (Calcd for $C_{36}H_{60}N_2O_9$: 664.430).

12-(*Z*)-3,4'-Dideoxy-2-enoneospiramycin I (**30**)

28 (24 mg) was treated in a similar manner with the preparation of **29**, to give a colorless powder of **30**, 14 mg (61%). TLC Rf 0.26; $[\alpha]_D^{24}$ −45.2° (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm (ε) 229 (20,400); High MS 664.432 (Calcd for $C_{36}H_{60}N_2O_9$: 664.430).

Acknowledgment

The authors wish to thank Dr. H. TANAKA, Kitasato University, for ribosome-binding assays, Mr. R. MASUMA, 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.

References

1) TAKAHIRA, H.: The studies on spiramycin derivatives. I. Jpn. J. Antibiotics 23: 424~428, 1970
2) PAUL, R. & S. TCHELITCHEFF: Structure de la spiramycin. I. Etude des produits de degradation: Caracterisation du mycarose. Bull. Soc. Chim. France 1957: 443~447, 1957
3) PINNERT-SINDICO, S.; L. NINET, J. PREUD'HOMME & C. COSAR: A new antibiotic — spiramycin. Antibiot. Annual 1954/1955: 724~727, 1955
4) WAGMAN, G. H.; J. A. WAITZ, J. MARQUEZ, A. MURAWSKHI, E. M. ODEN, R. T. TESTA & M. J. WEINSTEIN: A new *Micromonospora*-produced macrolide antibiotic, rosamicin. J. Antibiotics 25: 641~646, 1972
5) REIMANN, H. & R. S. JARET: Structure of rosamicin, a new macrolide from *Micromonospora rosaria*. J. Chem. Soc., Chem. Commun. 1972: 1270, 1972
6) HAYASHI, M.; M. OHNO & S. SATOI: Structures of mycinamicins. J. Chem. Soc., Chem. Commun. 1980: 119~121, 1980
7) TANAKA, A.; A. WATANABE, T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Syntheses of 4'-deoxy-demycarosyl tylosin and its analogues. J. Antibiotics 34: 1381~1384, 1981
8) SANO, H.; M. INOUE, K. YAMASHITA, R. OKACHI & S. ŌMURA: Chemical modification of spiramycins. I. Synthesis of the acetal derivatives of neospiramycin I. J. Antibiotics 36: 1336~1344, 1983
9) 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
10) CORCORAN, J. W.: Erythromycin and the bacterial ribosome. *In* Drug Action and Drug Resistance in Bacteria. *Ed.* S. MITSUHASHI, pp. 177~200, University of Tokyo Press, Tokyo, 1971
11) OLEINICK, N. L.: The Erythromycins. *In* Antibiotics. III. *Ed.* J. W. CORCORAN & F. E. HAHN, pp. 396~419, Springer-Verlag, New York, 1975
12) BRENT, D. A.; J. J. SABATKA, D. J. MINICK & D. W. HENRY: A simplified high-pressure liquid chromatography method for determining lipophilicity for structure-activity relationships. J. Med. Chem. 26: 1014~1020, 1983
13) SAKAMOTO, M.; Y. MUTOH, Y. FUKAGAWA, T. ISHIKURA & J. LEIN: Deepoxidation of 16-membered epoxyenone macrolide antibiotics. III. *In vitro* and *in vivo* evaluation of deepoxidation products of carbomycin A, deltamycin A_1, 4''-phenylacetyldeltamycin, angolamycin and rosamicin. J. Antibiotics 37: 130~135, 1984
14) MOUROT, D.; B. DELÉPINE, J. BOISSEANU & G. GAYOT: Reversed-phase high-pressure liquid chromatography of spiramycin. J. Chromatogr. 161: 386~388, 1978