CHEMICAL MODIFICATION OF SPIRAMYCINS

V. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 3′- OR 4‴-DE-*N*-METHYLSPIRAMYCIN I AND THEIR *N*-SUBSTITUTED DERIVATIVES

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

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

(Received for publication September 6, 1984)

The 3′- and 4‴-de-*N*-methylspiramycins were synthesized selectively, and then were converted to various *N*-substituted derivatives. 4‴-De-*N*-methyl derivatives were more active than 3′-de-*N*-methyl ones. Among the derivatives, 4‴-*N*-Fmoc-glycyl and 4‴-*N*-benzyl-4‴-de-*N*-methylspiramycin I were the most active *in vitro*, and were comparable to spiramycin I. 4‴-De-*N*-methylspiramycin I was about half as active as spiramycin I *in vivo*.

Spiramycin is a complex of 16-membered macrolide antibiotics,[1] which is active against Gram-positive bacteria, mycoplasmas and toxoplasmas. Spiramycin consists of four structural fragments, a 16-membered lactone, two aminosugars, mycaminose and forosamine, and one neutral sugar, mycarose.[2] Among 16-membered macrolide antibiotics spiramycin alone bears forosamine at the 9-position in its structure. During chemical modification of 16-membered macrolide antibiotics,[3] 3′-de-*N*-methyl derivative, its formyl and acetyl derivatives, and 3′-*N*-oxide of leucomycin A_3 have been prepared, and showed reduced antibacterial activity.[4] In this paper, we wish to describe the synthesis and antibacterial activity of de-*N*-methylspiramycins and their *N*-substituted derivatives.

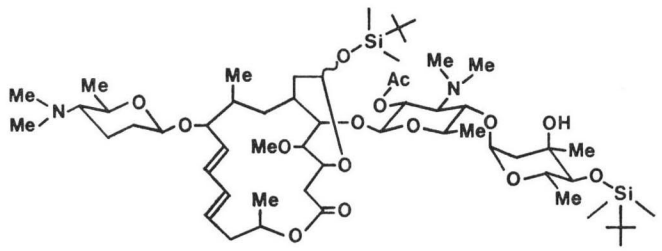
Synthesis

2′-*O*-Acetyl-4″-*O*-*t*-butyldimethylsilylspiramycin I 3,18-(*O*-*t*-butyldimethylsilyl)acetal (**1**), a protected derivative of spiramycin I in which aldehyde group and all hydroxyl function except the less reactive 3″-hydroxyl group are protected, was prepared from spiramycin I by selective acetylation[5] and *t*-butyldimethylsilylation. Treatment of **1** with *N*-bromosuccinimide (NBS) gave 3′-de-*N*-methyl derivative (**3**), selectively. The reaction may proceed through *N*-bromination, dehydrobromination and removal of a methylidene group. 4‴-De-*N*-methylation also occurred selectively by the treatment of **1** with NBS under basic conditions to afford **2**. Sodium azide was the best base among tested reagents.

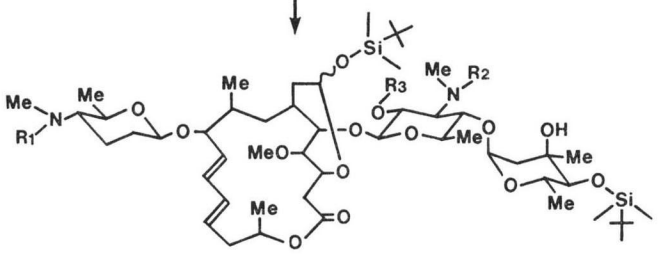
^{13}C NMR spectrum of **2** (Table 1) showed a disappearance of 4‴-*N*-methyl signal, up-field shift of 4‴-carbon and down-field shift of 5‴-carbon, thus confirming its structure. The structure of **3** was also confirmed in a similar manner.

Removal of the acetyl and *t*-butyldimethylsilyl (TBDMS) groups of **2** and **3** by methanolysis followed by treatment with tetrabutylammonium fluoride gave 3′- and 4‴-de-*N*-methylspiramycin I (**5** and **4**), respectively.

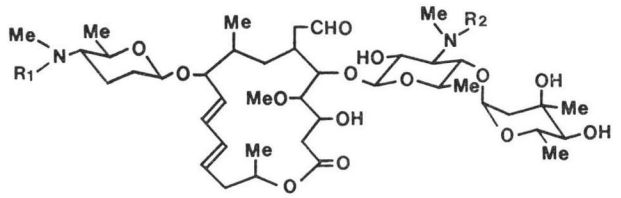
Scheme 1.



1



	R_1	R_2	R_3
2	H	Me	Ac
3	Me	H	Ac
12	$CH_2C_6H_5$	Me	Ac
14	$CO\text{-}OC_6H_4NO_2\text{-}p$	Me	H
15	$CON(Me)_2$	Me	H



	R_1	R_2
4	H	Me
5	Me	H
6	Fmoc	Me
7	Me	Fmoc
8	Ac	Me
9	BOC-Gly	Me
10	Fmoc-Gly	Me
11	Gly	Me
13	$CH_2C_6H_5$	Me
16	$CON(Me)_2$	Me

Compound **4** was also obtained from spiramycin I as described above for the preparation of **2**. However, selectivity between the two dimethylamino groups on spiramycin I is low and a fair amount of the 3′-de-*N*-methyl derivative (**5**) was produced, which was difficult to separate from the 4‴-de-*N*-methyl isomer by column chromatography. The mixture was subjected to silica-gel column chromatography after treatment with 9-fluorenylmethoxycarbonyl (Fmoc) chloride in pyridine to give 4‴- and 3′-*N*-Fmoc derivatives (**6** and **7**).

4‴-*N*-Acetyl (**8**), 4‴-*N*-*t*-butoxycarbonylglycyl (**9**) and 4‴-*N*-Fmoc-glycyl-4‴-de-*N*-methylspiramycin I (**10**) were prepared by coupling **4** with the corresponding carboxylic acid. Removal of Fmoc group of **10** gave 4‴-*N*-glycyl-4‴-de-*N*-methylspiramycin I (**11**). The treatment of the protected derivative of 4‴-de-*N*-methylspiramycin I (**2**) with benzyl bromide and silver oxide gave 4‴-*N*-benzyl derivative (**12**), followed by deprotection to afford **13**. 4‴-*N*-Dimethylcarbamoyl-4‴-de-*N*-methylspiramycin I (**16**) was synthesized *via* the 4‴-*N*-*p*-nitrophenoxycarbonyl derivative (**14**), which was prepared from 4″-*O*-TBDMS-spiramycin I 3,18-(*O*-TBDMS)-acetal. The structures of 4‴- and 3′-

Table 1. ^{13}C NMR chemical shifts for 3′- and 4‴-de-*N*-methylspiramycin I and their *N*-substituted derivatives.

	2*1	3*2	4	5	6*3	7*4	8*5	9*6
1	169.9	170.0	174.4	174.2	174.1	174.0	174.1	174.1
2	32.8	32.7	37.3	37.8	37.7	37.7	37.8	37.8
3	68.0	69.7	67.6	68.3	68.3	68.0	68.3	68.3
4	86.3	86.4	86.2	85.2	85.2	85.2	85.2	85.3
5	82.6	82.5	85.0	79.4	79.4		79.3	79.6
6	38.7	38.0	30.5	30.6	30.6	30.6	30.5	30.6
7	34.6	34.5	30.5	30.8	30.7	30.7	30.8	30.7
8	38.0	39.0	31.9	31.9	31.9	31.7	31.8	31.7
9	80.8	81.0	84.7	78.9	79.0	78.8	79.0	79.4
10	126.7	126.7	129.3	128.7	128.5	128.5	128.1, 128.4	128.3
11	127.4	127.3	134.2	134.6	134.7	134.6	134.6, 134.9	128.3
12	135.1	135.0	132.9	132.8	132.7	132.8	131.1, 131.4	131.3
13	139.4	139.4	130.6	131.1	131.2	131.1	131.1, 131.4	131.3
14	41.9	41.6	42.1	42.0	42.0	41.9	42.5	42.0
15	69.8	70.0	69.5	69.3	69.2	69.5	69.2	69.2
16	20.5	20.4	20.1	20.1	20.1	20.1	20.1	20.1
17	42.6	42.3	43.4	43.3	43.4	43.2	43.5	43.5
18	99.0	99.1	202.6	202.8	202.7	202.8	202.9	202.9
19	20.7	20.7	15.1	15.4	15.4	15.4	15.2	15.3
20	58.2	58.5	62.0	61.8	61.8	61.8	61.8	61.8
1′	102.8	103.0	102.8	104.0	103.9	103.4	103.8	103.9
2′	70.5	70.5	72.6	71.8	71.7	68.3	71.6	71.7
3′	70.5	63.1	68.4	68.8	68.8	70.0	68.8	68.8
4′	75.8	72.5	74.4	75.0	74.9	72.9	74.8	75.0
5′	73.0	72.4	73.1	73.2	73.1	72.6	73.0	73.1
6′	18.9	18.4	18.5	19.0	18.3	18.6	18.3	18.3
3′-NCH_3	41.6	29.3	42.1	29.3	42.0	47.2	42.0	42.0
1″	96.9	98.8	96.3	96.5	96.4	98.5	96.3	96.4
2″	40.2	40.1	41.0	40.9	40.9	41.0	40.9	41.0
3″	69.8	69.7	69.5	69.5	69.4	69.2	69.5	69.5
4″	78.7	78.4	76.5	76.5	76.4	76.3	76.4	76.5
5″	65.4	65.8	66.1	66.1	66.0	66.3	66.0	66.0
6″	18.9	18.9	18.3	18.3	18.3	18.3	18.2	18.2
7″	27.1	27.8	25.4	25.4	25.4	25.6	25.4	25.4
1‴	99.4	99.5	100.0	96.5	100.0	100.2	100.1	100.1
2‴	30.5	30.8	31.1	31.3	31.4	31.3	31.3	31.2
3‴		18.4	18.3	18.5	18.3	18.3	18.3	18.3
4‴	60.7	64.9	65.0	64.9	67.0	64.9	59.3	
5‴	75.8	73.8	73.7	73.9	72.1	73.8	72.3	72.0
6‴	19.3	19.2	19.2	19.1	19.1	19.0	19.0	19.1
4‴-NCH_3	33.9	40.7	34.1	29.3	47.4	40.7	31.8	

*1 −5.2, −4.0 (18-$SiCH_3$); −3.4, −3.2 (4″-$SiCH_3$); 18.1 (18-$SiC(CH_3)_3$); 18.4 (4″-$SiC(CH_3)_3$); 25.6 (4″-$SiC(CH_3)_3$); 26.2 (4″-$SiC(CH_3)_3$); 21.5 ($COCH_3$); 168.5 ($COCH_3$).

*2 −5.2, −4.0 (18-$SiCH_3$); −3.44, −3.38 (4″-$SiCH_3$); 18.1 ($SiC(CH_3)_3$); 18.4 (4″-$SiC(CH_3)_3$); 25.9 (18-$SiC(CH_3)_3$); 26.1 (4″-$SiC(CH_3)_3$); 21.1 ($COCH_3$); 169.2 ($COCH_3$).

*3 119.9 (×2), 124.9 (×2), 127.0 (×2), 127.7 (×2), 141.3 (×2), 144.0 (×2), 156.2 (Fmoc).

*4 119.9 (×2), 125.1 (×2), 127.0 (×2), 127.7 (×2), 141.3 (×2), 143.9 (×2), 156.9 (Fmoc).

*5 21.8, 22.4 ($COCH_3$); 170.8, 171.1 ($COCH_3$).

*6 42.9 (Gly–CH_2), 168.6 (Gly–CO), 27.7 (BOC–$C(CH_3)_3$), 155.8 (BOC–CO).

Table 1. (Continued)

	10*[7]	11*[8]	12*[9]	13*[10]	14*[11]	15*[12]	16*[13]
1	174.2	174.3	170.0	174.0	169.9	170.0	174.2
2	37.6	37.9	32.8	37.8	32.6	32.7	37.9
3	68.2	68.3	68.1	68.2	68.7	68.8	68.4
4	85.3	85.3	86.3	85.2	86.6, 86.7	86.7	85.3
5	79.5	79.5	82.5	79.5	82.2, 82.4	82.2	79.7
6	30.5	30.5	34.8	30.7	33.8	33.8	30.9
7	30.9	30.5	38.7	30.7	39.4	39.4	30.9
8	31.7	31.9	38.2	31.9	37.0	37.1	32.1
9	79.3	79.1	80.7	78.7	83.1, 83.4	83.2	79.2
10	128.4	128.4	126.8	128.7	127.0	126.9	128.7
11	134.8	134.8	127.5	134.5	127.3, 127.4	127.3	134.7
12	132.7	132.8	135.1	132.8	134.6	134.6	132.8
13	131.3	131.3	139.4	130.9	137.9	138.1	131.2
14	43.3	42.1	41.9	42.0	41.4	42.0	42.0
15	69.2	69.2	69.8	69.2	70.1	70.1	69.3
16	20.1	20.1	20.6	20.0			20.2
17	43.5	43.6	42.7	43.3	41.9	41.4	43.5
18	202.8	202.8		202.6		101.7	202.7
19	15.3	15.3	20.7	15.4	21.1	21.1	21.1
20	61.8	61.8	58.3	61.6	58.7, 58.8	58.8	61.8
1′	103.9	103.9	103.0	103.9	103.5	103.7	104.1
2′	71.7	71.7	70.6	71.7	71.4	71.3	71.7
3′	68.8	68.9	70.5	68.9	71.0	71.1	69.1
4′	74.9	75.1	75.8	74.8	75.8	75.8	74.8
5′	73.2	73.2	73.1	73.1	73.3	73.3	73.2
6′	18.3	18.3	18.9	18.2	18.3	18.3	18.3
3′-NCH_3	42.0	42.1	41.7	42.0	41.9	42.0	42.0
1″	96.4	96.3	96.9	96.3	96.8	96.7	96.5
2″	40.9	40.9	40.3	40.9	40.5	40.5	41.1
3″	69.5	69.5	69.9	69.5	69.8	69.8	69.6
4″	76.4	76.4	78.7	76.5	78.6	78.6	76.6
5″	66.1	66.1	65.5	66.1	65.6	65.6	66.3
6″	18.3	18.3	18.9	18.2	18.3	18.3	18.3
7″	25.4	25.4	27.2	25.4	27.1	27.1	25.5
1‴	100.1	100.1	99.5	100.2	99.8	99.7	100.4
2‴	31.3	31.3	31.0	31.4	31.2	31.5	31.7
3‴	18.3	18.3	19.7	19.6	19.8	19.8	18.3
4‴	67.1	66.1	64.1	63.5		59.5	59.5
5‴	71.9	72.0	74.0	73.9	71.9, 72.1	72.7	72.6
6‴	19.1	19.1	19.3	19.0	19.0	19.2	19.2
4‴-NCH_3	47.2	41.4	37.0	37.0		31.5	31.7

*[7] 42.9 (Gly–CH_2), 168.1, 168.4 (Gly–CO), 120.0 (×2), 125.1 (×2), 127.0 (×2), 127.7 (×2), 141.3 (×2), 143.9 (×2), 156.1 (Fmoc).

*[8] 169.2 (Gly–CO).

*[9] −5.2, −3.9 (18-$SiCH_3$); −3.4, −3.1 (4″-$SiCH_3$); 18.2 (18-Si$C(CH_3)_3$); 18.5 (4″-Si$C(CH_3)_3$), 26.0 (18-Si$C(CH_3)_3$), 26.2 (4″-Si$C(CH_3)_3$), 21.6 ($COCH_3$), 168.6 ($COCH_3$), 126.8, 128.2 (×2), 128.5 (×2), 140.0 (Bzl).

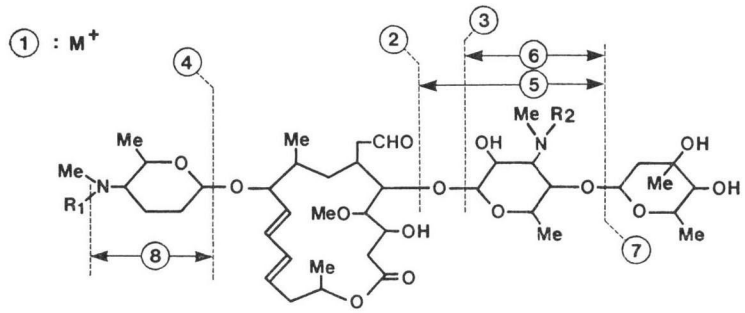
*[10] 58.7, 126.8, 128.1 (×2), 128.4 (×2), 139.8 (Bzl).

*[11] −5.2, −3.9 (18-$SiCH_3$); −3.4, −3.3 (4″-$SiCH_3$); 18.0 (18-Si$C(CH_3)_3$), 18.4 (4″-Si$C(CH_3)_3$), 25.9 (18-Si$C(CH_3)_3$), 26.2 (4″-Si$C(CH_3)_3$), 122.2, 125.0, 144.8, 144.9, 153.2, 153.5 (*p*-nitrophenyl), 156.2 (NCO–O).

*[12] −5.2, −3.9 (18-$SiCH_3$); −3.4, −3.2 (4″-$SiCH_3$), 18.0 (18-Si$C(CH_3)_3$), 18.4 (4″-Si$C(CH_3)_3$), 25.9 (18-Si$C(CH_3)_3$), 26.2 (4″-Si$C(CH_3)_3$), 165.7 (NCON=).

*[13] 38.7 ($CON(CH_3)_2$), 165.7 (NCON=).

Table 2. Diagnostic mass fragmentation of 3′- and 4‴-de-*N*-methylspiramycin I and their *N*-substituted derivatives.



	4	5	6	7	8	9	10	11	13	16
1	—	-mycarose668	—	—	—	—	—	—	919	—
2	494	508	-4+H369	—	-H535	—	—	—	584	+H565
3	-H317	—	—	—	318	—	—	—	-H317	-H317
4	126	142	+H349	-H141	+2H170	285	—	—	218	-H199
5+H	190	—	190	-Fmoc176	190	190	190	190	—	—
6	173	—	173	—	173	+H174	+H174	+H174	+H174	+H174
7	145	—	145	145	145	145	145	145	+H146	145
8+H	—	—	—	—	126	126	+H127	126	—	—

N-substituted derivatives were confirmed by the ^{13}C NMR behaviors (Table 1) and mass fragmentations (Table 2).

Evaluation by Antimicrobial Activity, Affinity to Ribosomes and Lipophilicity

3′- And 4‴-de-*N*-methylspiramycin I and their *N*-substituted derivatives were evaluated[6] by antibacterial activity (MIC), affinity to ribosomes (ID_{50})[7] and retention time (RT) in HPLC which is a parameter of lipophilicity.[8] Some of the derivatives were further evaluated by therapeutic effects in mice. Table 3 shows the MIC, ID_{50}, ED_{50} and RT values of the spiramycin I derivatives. Fig. 1 shows the correlation between average of −log MIC (M) against *Staphylococcus aureus* (SA) and *Bacillus subtilis* (BS) and pK_{50} (−log pID_{50}).

Most of the derivatives in which one methyl group of mycaminose or forosamine was substituted were less active in affinity to ribosomes and in antimicrobial activity than spiramycin I. The results and the finding (H. SANO and S. ŌMURA, unpublished data) that 4‴-*N*-oxide and 3′,4‴-di-*N*-oxide of spiramycin I also are less active in affinity to ribosomes and in antimicrobial activity than spiramycin I indicate that the dimethylamino groups of both mycaminose and forosamine are involved in the affinity of spiramycin I to bacterial ribosomes.

4‴-De-*N*-methylspiramycin I and its derivatives showed higher antimicrobial activity against Gram-positive bacteria than those of 3′-de-*N*-methylspiramycin I and its derivative. It seems that the bulk tolerance area exists around forosamine.

As shown in Fig. 1, almost all the derivatives tested exhibited relatively good correlation between affinity to ribosomes (pK_{50}) and antimicrobial activity [−log MIC (M) (SA, BS)]. However, some

Table 3. MIC, ID_{50}, ED_{50} and RT of 3′- and 4‴-de-*N*-methylspiramycin I and their *N*-substituted derivatives.

Compound	R	MIC (μg/ml)*1							ID_{50} (μM)	ED_{50} (mg/kg)*2		RT (minutes)	Average of −log MIC (M) (SA, BS)*3	pK_{50}*4
		SA	SA^r	BS	BC	ML	EC	KP		SA	SP			
	[4‴]													
4	H	12.5	>100	6.25	6.25	0.39	100	>100	2.3	204		3.2	4.97	5.6
6	Fmoc	25	>100	3.12	6.25	0.78	>100	>100	3.7		>300	9.0	5.08	5.4
8	Ac	12.5	>100	3.12	6.25	1.56	>100	>100	2.0	>300		7.9	5.14	5.7
9	BOC-Gly	25	>100	12.5	12.5	1.56	>100	>100	3.8			20.0	4.75	5.4
10	Fmoc-Gly	3.12	>100	1.56	3.12	0.39	100	100	2.5			8.8	5.68	5.6
11	Gly	12.5	>100	25	25	3.12	>100	>100	1.4		>250	3.7	4.70	5.9
13	Bzl	6.25	100	1.56	3.12	0.20	>100	>100	3.0			13.8	5.47	5.5
16	$CONMe_2$	12.5	>100	6.25	6.25	0.39	>100	>100	3.0			12.6	5.01	5.5
	[3′]													
5	H	25	>100	25	50	3.12	>100	>100	3.0			2.9	4.55	5.5
7	Fmoc	50	>100	25	50	6.25	>100	>100	7.4		>300	2.7	4.47	5.1
SPM I		3.12	>100	1.56	3.12	<0.10	>100	>100	1.0	117	168	4.3	5.58	6.0
AcSPM		6.25	>100	3.12	3.12	<0.10	>100	>100	1.9	111	70		5.30	5.7

Abbreviations: BOC, *t*-butoxycarbonyl; Fmoc, 9-fluolenylmethoxycarbonyl; SPM I, spiramycin I; AcSPM, acetylspiramycin.

Test organism

*1 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).

*2 SA: *Staphylococcus aureus* Smith, SP: *Streptococcus pneumoniae* III.

*3 Average of negative log MIC (M) against SA and BS.

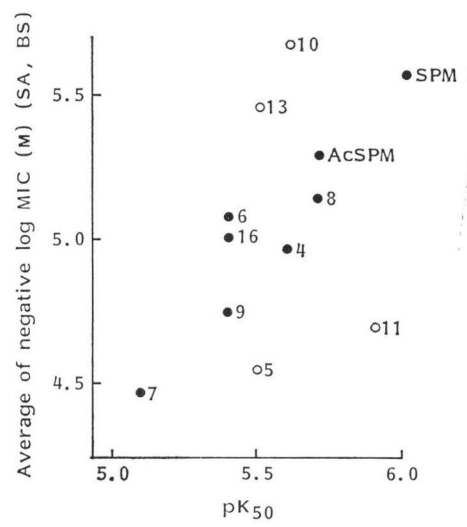
*4 Negative log of ID_{50}.

derivatives did not exhibit good correlation: 4‴-*N*-Fmoc-glycyl (**10**) and 4‴-*N*-benzyl derivative (**13**), substituted with rather lipophilic groups, had equivalent MIC values to those of spiramycin I although they had less affinity to ribosomes than that of spiramycin I suggesting that the derivatives are more permeable. On the other hand, 4‴-*N*-glycyl derivative (**11**) and 3′-de-*N*-methylspiramycin I (**5**) exhibited much lower antimicrobial activity than spiramycin I.

4‴-De-*N*-methylspiramycin I (**4**) showed about a half therapeutic effect on mice infected with *S. aureus* compared with that of spiramycin I, in spite of its relatively low *in vitro* activity.

Fig. 1. Average of negative log MIC (M) against *Staphylococcus aureus* and *Bacillus subtilis* as a function of pK_{50} of [^{3}H]tetrahydroleucomycin A_3 binding to ribosomes for 3′- and 4‴-de-*N*-methylspiramycin I and their *N*-substituted derivatives.

The numbers in the figure refer to the compounds as numbered in Table 3.



Experimental

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

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 *S. aureus* Smith or *Streptococcus pneumoniae* Type III, respectively. Compounds suspended in 0.3%-sodium carboxymethylcellulose 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 reversed 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.[9] RT was recorded at 1 ml/minute of flow rate with a UV monitor (231 nm).

2′-*O*-Acetyl-4‴-*O*-*t*-butyldimethylsilylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**1**)

To a solution of 2′-*O*-acetylspiramycin I (20.0 g)[5] in DMF (220 ml), *t*-butyldimethylsilyl chloride (22.5 g) and imidazole (20.3 g) were added and held for 12 days at room temp. *t*-Butyldimethylsilyl chloride (11.0 g) and imidazole (10.0 g) were added to the mixture and held for further 3 days at room temp. After an addition of MeOH (10 ml), the reaction mixture was evaporated to give an oily residue. The residue was dissolved in $CHCl_3$ (1.0 liter) and washed with saturated solution of $NaHCO_3$

(1.0 liter) and H_2O (1.0 liter), respectively. The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to afford a powder which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 7: 1, giving a colorless powder, 10.0 g (38.6%). TLC Rf 0.36; $[\alpha]_D^{25}$ −19.4° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 234 (19,000).

2′-*O*-Acetyl-4″-*O*-*t*-butyldimethylsilyl-4‴-de-*N*-methylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)-acetal (**2**)

To a mixture of a solution of **1** (3.40 g) in ethylene glycol dimethyl ether (37 ml) and a solution of sodium azide (998 mg) in H_2O (6 ml), *N*-bromosuccinimide (765 mg) was added within 15 minutes under cooling at −20~ −5°C and stirred for 1 hour at 0°C. The reaction mixture was diluted with $CHCl_3$ (300 ml) and washed with H_2O (300 ml). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated, to give a residual powder, which was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 6: 1, to afford a colorless powder, 1.91 g (56.6%). TLC Rf 0.34; $[\alpha]_D^{23}$ −24.8° (*c* 1.0, $CHCl_3$).

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

To a solution of **1** (556 mg) in ethylene glycol dimethyl ether (6 ml) and H_2O (1 ml), *N*-bromosuccinimide (155 mg) was added within 30 minutes under cooling at −10°C and stirred for 2.5 hours at 0~20°C. The reaction mixture was diluted with $CHCl_3$ (40 ml) and washed with saturated solution of sodium hydrogen carbonate (40 ml). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated, to give a residual powder. The powder was chromatographed on a silica gel column with C_6H_6 - Me_2CO, 4: 1~2: 1 (gradually changed), giving a colorless powder, 137 mg (25.0%). TLC Rf 0.39; $[\alpha]_D^{23}$ −10.6° (*c* 1.0, $CHCl_3$).

4‴-De-*N*-methylspiramycin I (**4**)

a) A solution of **2** (428 mg) in MeOH (17 ml) was heated at 42°C for 41 hours and evaporated, to give a powder.

The well dried powder was dissolved in 1 M solution of tetrabutylammonium fluoride in THF (0.46 ml) and held for 30 minutes at room temp. The reaction mixture was diluted with $CHCl_3$ (50 ml) and washed with H_2O (50 ml). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated, to afford a thick syrup, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH, 12: 1, giving a colorless powder, 80 mg (25.0%). TLC Rf 0.23 ($CHCl_3$ - MeOH - conc NH_4OH, 2: 1: 0.01); $[\alpha]_D^{16}$ −35.0° (*c* 0.2, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 235 (29,900).

Anal Calcd for $C_{42}H_{72}N_2O_{14}\cdot 5H_2O$: C 54.89, H 8.99, N 3.05.
Found: C 54.79, H 8.00, N 2.90.

b) To a stirred mixture of a solution of spiramycin I (25.3 g) in ethylene glycol dimethyl ether (270 ml) and sodium carbonate (8.0 g) in H_2O (50 ml), *N*-bromosuccinimide (8.0 g) was added within 20 minutes under cooling at −10~ −5°C, and stirred for 1 hour at 0~10°C. The reaction mixture was diluted with $CHCl_3$ (1.5 liters) and washed with H_2O (1.5 liters). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to give a solid, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH, 12: 1, giving a colorless powder of **4**, 4.40 g (17.8%).

3′-De-*N*-methylspiramycin I (**5**)

A solution of **3** (68 mg) in MeOH (2.7 ml) was heated at 50°C for 24 hours and evaporated, to give a powder, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH - conc NH_4OH, 20: 1: 0.01, giving a colorless powder.

A powder was dissolved in 1 M solution of tetrabutylammonium fluoride in THF (0.07 ml) and held for 45 minutes 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 $CHCl_3$ - MeOH - conc NH_4OH, 15: 1: 0.01, giving a colorless powder, 10 mg (19.7%). TLC Rf 0.26 ($CHCl_3$ - MeOH - conc NH_4OH, 2: 1: 0.01); $[\alpha]_D^{25}$ −53.2° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 232 (20,500).

4‴-De-*N*-methyl-4‴-*N*-fluorenylmethoxycarbonylspiramycin I (**6**) and 3′-De-*N*-methyl-3′-*N*-fluorenylmethoxycarbonylspiramycin I (**7**)

To a stirred mixture of a solution of spiramycin I (8.42 g) in ethylene glycol dimethyl ether (84 ml) and sodium azide (3.25 g) in H_2O (14 ml), *N*-bromosuccinimide (2.49 g) was added within 25 minutes under cooling at −15°C and stirred at 0~20°C for 3 hours. The reaction mixture was diluted with $CHCl_3$ (500 ml) and washed with H_2O (500 ml). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated, to give a yellow powder, which was chromatographed on a short column of silica gel with $CHCl_3$ - MeOH, 12: 1, to afford a colorless powder of a mixture of **4** and **5**, 2.28 g (27.7%).

To a stirred solution of a powder (747 mg) in dioxane (10 ml), 10% solution of sodium carbonate (1.6 ml) and 9-fluorenylmethoxycarbonyl chloride (259 mg) were added. After 2 hours, 10% sodium carbonate solution (0.8 ml) and fluorenylmethoxycarbonyl chloride (130 mg) were added and stirred overnight at room temp. The reaction mixture was diluted with $CHCl_3$ (70 ml) and washed with H_2O. The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated, to afford **6** and **7** in the order of elution.

6, a colorless powder, 92 mg (9.8%). TLC Rf 0.37; $[\alpha]_D^{16}$ −57.2° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 234 (39,800), 256 (29,700), 264 (33,500), 289 (9,500) and 300 (11,300).

Anal Calcd for $C_{57}H_{82}N_2O_{16}\cdot 2H_2O$: C 62.97, H 7.97, N 2.58.
Found: C 62.86, H 8.00, N 2.49.

7, a colorless powder, 130 mg (13.8%). TLC Rf 0.27; $[\alpha]_D^{17}$ −43.0° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 300 (19,300), 288 (16,500), 265 (57,200), 256 (54,100), 234 (76,000).

Anal Calcd for $C_{57}H_{82}N_2O_{16}\cdot 2H_2O$: C 62.97, H 7.97, N 2.58.
Found: C 62.92, H 7.63, N 2.98.

4‴-*N*-Acetyl-4‴-de-*N*-methylspiramycin I (**8**)

To a solution of **4** (549 mg) in MeOH (141 ml), acetic anhydride (0.14 ml) was added and held for 3 hours at room temp. The reaction mixture was evaporated to give an oily residue, which was dissolved in $CHCl_3$ (30 ml) and washed with H_2O (30 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 - MeOH, 3: 2, giving a colorless powder, 156 mg (27.0%). TLC Rf 0.26; $[\alpha]_D^{18}$ −72.0° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 234 (25,100).

Anal Calcd for $C_{44}H_{74}N_2O_{15}\cdot 3H_2O$: C 57.13, H 8.72, N 3.02.
Found: C 57.52, H 8.34, N 2.76.

4‴-*N*-(*t*-Butoxycarbonylglycyl)-4‴-de-*N*-methylspiramycin I (**9**)

To a solution of **4** (352 mg) and *t*-butoxycarbonylglycine (85 mg) in dichloromethane (4.8 ml), dicyclohexylcarbodiimide (100 mg) was added and stirred for 1 hour at room temp. After an addition of a drop of H_2O, a colorless precipitate was filtered off. The solution was evaporated, and chromatographed on a silica gel column with C_6H_6 - MeOH, 2: 1, to give a colorless powder, 159 mg (37.9%). TLC Rf 0.43; $[\alpha]_D^{16}$ −58.9° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 242 (39,000).

Anal Calcd for $C_{49}H_{83}N_3O_{17}\cdot H_2O$: C 58.61, H 8.53, N 4.18.
Found: C 58.37, H 8.38, N 3.76.

4‴-*N*-(9-Fluorenylmethoxycarbonylglycyl)-4‴-de-*N*-methylspiramycin I (**10**)

4 (800 mg) was treated with 9-fluorenylmethoxycarbonylglycine (316 mg) and dicyclohexylcarbodiimide (220 mg) in dichloromethane in a similar way described in a preparation of **9**, to afford a colorless powder of **10**, 478 mg (43.1%). TLC Rf 0.35; $[\alpha]_D^{18}$ −61.4° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 234 (44,500), 255 (35,400), 264 (38,000), 289 (10,500) and 299 (12,500).

Anal Calcd for $C_{59}H_{84}N_3O_{16}\cdot 3H_2O$: C 61.87, H 7.92, N 3.67.
Found: C 61.85, H 7.57, N 3.50.

4‴-*N*-Glycyl-4‴-de-*N*-methylspiramycin I (**11**)

A solution of **10** (247 mg) in 1% solution of 1,5-diazabicyclo[4.3.0]-5-nonene in THF (2.8 ml) was held for 35 minutes at room temp. The reaction mixture was diluted with $CHCl_3$ (30 ml) and washed with H_2O. The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to give a

solid, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH, 19: 1, giving a colorless powder, 105 mg (56.0%). TLC Rf 0.29; $[\alpha]_D^{16}$ −44.5° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 232 (30,000).

Anal Calcd for $C_{44}H_{75}N_3O_{15}$: C 59.64, H 8.53, N 4.74.
Found: C 60.35, H 8.31, N 4.88.

2′-*O*-Acetyl-4‴-*N*-benzyl-4″-*O*-*t*-butyldimethylsilyl-4‴-de-*N*-methylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**12**)

To a solution of **2** (630 mg) in *N*,*N*-dimethylformamide (15.8 ml), silver oxide (1.5 g) and benzyl bromide (2 ml) were added and stirred vigorously for 30 minutes at room temp. After an addition of H_2O (2 ml), a solid was filtered off. The filtrate was diluted with $CHCl_3$ (70 ml) and washed with a saturated solution of sodium hydrogen carbonate and H_2O. 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, 7: 1, giving a colorless powder, 270 mg (39.6%). TLC Rf 0.86; $[\alpha]_D^{23}$ −54.5° (*c* 0.1, $CHCl_3$).

4‴-*N*-Benzyl-4‴-de-*N*-methylspiramycin I (**13**)

A solution of **12** (360 mg) in methanol (14.5 ml) was heated at 50°C for 27 hours. The reaction mixture was evaporated to give a powder, which was dissolved in 1 M tetrabutylammonium fluoride - THF solution (0.32 ml) and held for 6 hours at room temp. The reaction mixture was diluted with $CHCl_3$ (30 ml) and washed with a saturated solution of sodium hydrogen carbonate and H_2O. The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to afford an oily residue, which was chromatographed on a silica gel column with C_6H_6 - MeOH, 3: 1, to give a colorless powder, 156 mg (56.0%). TLC Rf 0.32; $[\alpha]_D^{25}$ −45.2° (*c* 1.0, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 238 (27,400).

Anal Calcd for $C_{49}H_{78}N_2O_{14}$: C 64.03, H 8.55, N 3.05.
Found: C 63.93, H 8.60, N 2.83.

4″-*O*-*t*-Butyldimethylsilyl-4‴-de-*N*-methyl-4‴-*p*-nitrophenoxycarbonylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**14**)

To a solution of 4″-*O*-*t*-butyldimethylsilyl-4‴-de-*N*-methylspiramycin I 3,18-(*O*-*t*-butyldimethylsilyl)acetal (250 mg), which was prepared by methanolysis of **2**, in pyridine (5 ml), *p*-nitrophenoxycarbonyl chloride (138 mg) was added and stirred for 1.5 hours at room temp. The reaction mixture was diluted with $CHCl_3$ (25 ml) and washed with a saturated solution of sodium hydrogen carbonate (25 ml×6). The $CHCl_3$ solution was dried over anhydrous sodium sulfate, evaporated and chromatographed on a silica gel column with C_6H_6 - EtOAc, 2: 1, to give a pale yellow powder, 180 mg (62.3%). TLC Rf 0.71; $[\alpha]_D^{23}$ −15.0° (*c* 1.0, $CHCl_3$).

4″-*O*-*t*-Butyldimethylsilyl-4‴-*N*-dimethylcarbamoyl-4‴-de-*N*-methylspiramycin I 3,18-(*O*-*t*-Butyldimethylsilyl)acetal (**15**)

To a solution of **14** (100 mg) in THF (4.5 ml), 50% solution of dimethylamine in H_2O (2.5 ml) was added and stirred at 50°C for 14 hours. The reaction mixture was diluted with EtOAc (10 ml) and washed with a saturated solution of sodium hydrogen carbonate (10 ml×10). The EtOAc solution was dried over anhydrous sodium sulfate and evaporated to give a residual powder which was chromatographed on a silica gel column with C_6H_6 - MeOH, 4: 1, to afford a colorless powder, 50 mg (54.2%). TLC Rf 0.59; $[\alpha]_D^{23}$ −21.2° (*c* 0.17, $CHCl_3$).

4‴-*N*-Dimethylcarbamoyl-4‴-de-*N*-methylspiramycin I (**16**)

A solution of **15** (47 mg) in 1 M solution of tetrabutylammonium fluoride in THF (0.03 ml) was held for 1.5 hours at room temp. The reaction mixture was diluted with $CHCl_3$ (5 ml) and washed with H_2O. The $CHCl_3$ solution was dried orver anhydrous sodium sulfate and evaporated to give a brownish oil, which was chromatographed on a silica gel column with C_6H_6 - MeOH, 3: 1, giving a colorless powder, 9 mg (24.0%). TLC Rf 0.29; $[\alpha]_D^{25}$ −33.0° (*c* 0.2, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 230 (20,100).

Anal Calcd for $C_{45}H_{77}N_3O_{17}$: C 60.05, H 8.62, N 4.67.
Found: C 60.75, H 9.28, N 4.13.

Acknowledgment

The authors wish to thank 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. The authors also thank to Messrs. Y. IGA, K. HIRATA and K. MAEBASHI for their technical assistance.

References

1) PINNERT-SINDICO, S.; L. NINET, J. PREUD HOMME & C. COSAN: A new antibiotic-spiramycin. Antibiot. Annu. 1954/1955: 724~727, 1955
2) ŌMURA, S.; A. NAKAGAWA, M. OTANI, T. HATA, H. OGURA & K. FURUHATA: Structure of the spiramycins (foromacidins) and their relationship with the leucomycins and carbomycins (magnamycins). J. Am. Chem. Soc. 91: 3401~3404, 1969
3) ŌMURA, S. & H. SAKAKIBARA: Chemical modification of 16-membered macrolide antibiotics. Hakko to Kogyo 37: 1171~1186, 1979 (in Japanese)
4) NAKAGAWA, A.; K. SUZUKI, K. IWASAKI, T. HATA & S. ŌMURA: Chemistry of leucomycins. XI. Chemical transformation of a basic macrolide to a neutral macrolide. Chem. Pharm. Bull. 22: 1426~1428, 1974
5) SANO, H.; T. SUNAZUKA, H. TANAKA, K. YAMASHITA, R. OKACHI & S. ŌMURA: Chemical modification of spiramycins. III. Synthesis and antibacterial activities of 4''-sulfonates and 4''-alkylethers of spiramycin I. J. Antibiotics 37: 750~759, 1984
6) SANO, H.; M. INOUE & S. ŌMURA: Chemical modification of spiramycins. II. Synthesis and antimicrobial activity of 4'-deoxy derivatives of neospiramycin I and their 12-(*Z*)-isomers. J. Antibiotics 37: 738~749, 1984
7) ŌMURA, S.; H. TANAKA, J. INOKOSHI, H. SAKAKIBARA & T. FUJIWARA: Binding of [^{3}H]tetrahydroleucomycin A_3 to *Escherichia coli* ribosomes of leucomycins on the binding. J. Antibiotics 35: 491~496, 1982
8) 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
9) MOUROT, D.; B. DELÉPINE, J. BOISSEANU & G. GAYOT: Reversed-phase high-pressure liquid chromatography of spiramycin. J. Chromatogr. 161: 386~388, 1978