NISAMYCIN, A NEW MANUMYCIN GROUP ANTIBIOTIC FROM *Streptomyces* sp. K106

II. STRUCTURE DETERMINATION AND STRUCTURE-ACTIVITY RELATIONSHIPS

KEN-ICHIRO HAYASHI[†,*], MASAHIRA NAKAGAWA, TOMOYUKI FUJITA, SHINJI TANIMORI and MITSURU NAKAYAMA

Department of Agricultural Chemistry, Faculty of Agriculture, University of Osaka Prefecture, 1-1 Gakuen-cho, Sakai City, Osaka 593, Japan

(Received for publication April 25, 1994)

Nisamycin, a novel manumycin group antibiotic, was isolated from the culture broth of *Streptomyces* sp. K106. Structural elucidation of nisamycin was achieved by detailed NMR spectral analyses and comparison of the NMR data of nisamycin with those of other manumycin group antibiotics. The structure was confirmed by chromic acid oxidation. The absolute stereochemistry of nisamycin was determined to be 4*R*, 5*S* and 6*R* from the CD spectra of nisamycin and chromic oxidation of nisamycin. In addition, some structure activity-relationships were examined.

As described in the preceding paper[1], nisamycin is a new manumycin group antibiotic produced by *Streptomyces* sp. K106. In this paper, we report on the structure determination of nisamycin including absolute configuration and some structure-activity relationships of nisamycin.

Results and Discussion

Structural Elucidation

Positive FAB-MS gave ions at *m/z* 426 $(M+H)^+$ and 448 $(M+Na)^+$. The molecular formula of **1** was determined to be $C_{24}H_{27}NO_6$ by positive-ion high resolution FAB-MS (Found 426.1918, Calcd. for $C_{24}H_{28}NO_6$ 426.1917). This molecular formula was also supported by ^{13}C NMR spectral data which represented 24 signals. The IR spectrum suggested that the presence of amide (1615, 1521 cm^{-1}) and carboxyl groups (3346, 1694 cm^{-1}). The UV spectrum showed absorption maxima at 278 nm (ε 32,100) and 305 nm (ε 22,600) in MeOH, which indicated the presence of conjugated dienone and trienone structures. Structural elucidation of **1** was achieved by detailed NMR spectral analyses and the comparison of the NMR data of **1** with those of manumycin (**4**)[2], asukamycin[2], colabomycin A[3], and alisamycin (**2**)[4]. The structure of **1** was also confirmed by chromic acid oxidation of **1** to give an oxidation product (**3**) (Fig. 1).

^{13}C and ^1H NMR spectral data of **1** and their assignments are shown in Table 1. The ^{13}C NMR signals of **1** at δ_C 52.8 (C-6) and 57.3 (C-5) were attributed to an epoxide, whose protons were observed in the ^1H NMR spectrum at δ_H 3.63 (H-6) and 3.70 (H-5). A long-range "W" coupling of the olefinic proton (H-3, $J=2.1$ Hz) with the epoxide proton (H-5) was detected by ^1H-^1H COSY spectrum. The ^{13}C NMR spectrum showed the presence of a conjugated ketone carbonyl carbon (C-1), an amide carbonyl

† Present address: Research & Development Center, UNITIKA Co., Ltd., 23, Kozakura, Uji City, Kyoto, 611 Japan.

Fig. 1. Structures of nisamycin (**1**), alisamycin (**2**), oxidation product of **1** (**3**) and manumycin (**4**).

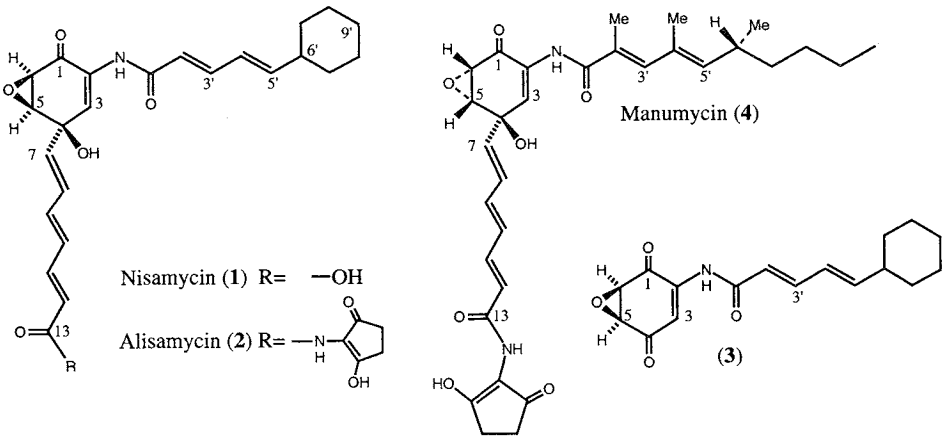


Table 1. ^{13}C and ^{1}H NMR spectral data of nisamycin.

Position	δ_C ($CDCl_3$)	δ_H ($CDCl_3$)	δ_H (C_5D_5N)
1	188.7 (s)		
2	127.8 (s)		
3	126.9 (d)	7.41 (d 2.1)	8.20 (d 2.1)
4	71.2 (s)		
5	57.3 (d)	3.70 (dd 4.0, 2.1)	4.07 (dd 3.7, 2.1)
6	52.8 (d)	3.63 (d 4.0)	3.92 (d 3.7)
7	136.6 (d)	5.82~5.93 (m)	6.17 (d 15.0)
8	131.5 (d)	6.50~6.58 (m)	7.07 (dd 15.0, 10.8)
9	139.4 (d)	6.50~6.58 (m)	6.71 (dd 14.7, 10.8)
10	131.9 (d)	6.39 (m)	6.49 (dd 14.7, 11.1)
11	145.7 (d)	7.20~7.37 (m)	7.73 (dd 15.3, 11.1)
12	121.5 (d)	5.82~5.93 (m)	6.35 (d 15.3)
13	171.2 (s)		
1′	165.4 (s)		
2′	120.8 (d)	5.82~5.93 (m)	6.54 (d 15.0)
3′	144.5 (d)	7.20~7.37 (m)	7.60 (m)
4′	125.5 (d)	6.11~6.14 (m)	6.18 (m)
5′	151.0 (d)	6.11~6.14 (m)	5.94 (dd 15.2, 6.9)
6′	41.1 (d)	2.08 (m)	1.88 (m)
7′	32.2 (t)		
8′	25.8 (t)	0.89~1.40 (m) 5H[a]	0.77~1.40 (m) 5H[a]
9′	26.0 (t)	1.75 (m) 5H[a]	1.57 (m) 5H[a]
10′	25.8 (t)		
11′	32.2 (t)		
NH		7.60 (br s)	9.66 (br s)

Chemical shifts in ppm from internal TMS. Coupling constants in J=Hz.
[a] Cyclohexyl ring protons from C-7′ to C-11′.

carbon (C-1′), an olefinic carbon (C-3), and a tertiary carbinol carbon (C-4). It also showed an olefinic carbon connected with the amide nitrogen (C-2). The NMR signals mentioned above are characteristic of manumycin group antibiotics (Table 2). 2-Amino-3-hydroxycyclopent-2-enone is also known as a component of manumycin group antibiotics. However, the NMR spectra of nisamycin did not show signals of a 2-amino-3-hydroxycyclopent-2-enone moiety.

Table 2. ^{13}C NMR signals of common structure in **1**, manumycin, asukamycin and alisamycin.

Carbon No.	**1**	Manumycin[a]	Asukamycin[a]	Alisamycin[b]
C-1	188.7[c]	189.0	189.0	188.6
C-2	127.8	128.0	128.1	128.1
C-3	126.9	126.6	126.5	126.4
C-4	71.2	71.2	70.7	71.2
C-5	57.3	57.4	57.2	57.4
C-6	52.8	52.8	52.5	52.9
C-1′	165.4	168.0	165.3	165.5

[a,b] These data were obtained from refs J. Am. Chem. Soc. 112: 3979, 1990 and J. Antibiotics 46: 1027, 1993.

[c] ppm.

Considering the chemical shifts of six carbons and eleven protons from C-6′ to C-11′, a cyclohexyl group bonded to an olefinic carbon (C-5′) was deduced. Double bond linkages from C-2′ to C-5′ and from C-7 to C-12 were established by ^1H-^1H COSY spectral data; as the result, diene (C-2′ to C-5′) and triene structures (C-7 to C-12) were suggested, which were supported by the UV spectrum. The configuration of these double bonds were determined to be all *E* by ^1H-^1H coupling constants in pyridine-d_5, because of overlapping NMR signals of olefinic protons in $CDCl_3$. A coupling behavior ($J=6.9$ Hz) between the cyclohexyl ring proton (H-6′) and the olefinic proton (H-5′) was detected by ^1H-^1H COSY spectrum.

Chromic oxidation of **1** afforded **3**. The structure of **3** was determined to be 2-(5′-cyclohexyl-pentadienoylamino)-5,6-epoxy-1,4-benzoquinone by ^1H and ^{13}C NMR spectral analyses. Furthermore, **3** was identical to the chromic oxidation product of alisamycin[5]. These data indicate that the diene moiety was connected with the amide carbonyl carbon (C-1′). All proton and carbon signals of **1** were assigned by ^1H-^1H and ^{13}C-^1H COSY spectra (Table 1).

Stereochemistry

The configuration at C-4 of **1** was determined by the exciton chirality method[6] which has been used for asukamycin, manumycin, and colabomycin A[3,7,8]. **1** satisfies all prerequisites for utilizing this method. **1** showed a medium-strong negative CD couplet in chloroform (315 nm $\Delta\varepsilon$ −14.78, 261 nm $\Delta\varepsilon$ +15.24), which is consistent only with the 4*R*-configuration. Thus, **1** was shown to possess the same stereochemistry at C-4 as manumycin and the opposite of asukamycin and colabomycin A. The configuration at C-5 and C-6 was determined by comparing the CD spectrum of **3** with those of antibiotic G7063-2 (**5**) and the chromic oxidation products of manumycin (**6**) and colabomycin A (**7**)[7,8] (Fig. 2). These compounds containing oxirane rings showed two Cotton effects for n-π^* transitions between 300 and 400 nm in their CD spectra. **3** gave two Cotton effects between 300 and 400 nm which were the same signs as those of **5** and opposite to those of **6** and **7**[3,8] (Table 3). Therefore, the absolute configuration at C-5 and C-6 of **3** was determined to be 5*S*, 6*R*. Since the degradation of **1** to **3** does not alter the chirality at C-5 and C-6 of **1**, the configuration at C-5 and C-6 of **1** was assigned as 5*S*, 6*R*. Thus, the absolute structure of **1** was determined as shown in Fig. 1.

Structure-activity Relationships

The structure of **1** lacks the 2-amino-3-hydroxycyclopent-2-enone moiety characteristic of manumycin group antibiotics. The effects of this moiety on the antimicrobial activity of manumycin group antibiotics

Fig. 2. Structures of antibiotic G7063-2 (**5**), oxidation products (**6**) and (**7**).

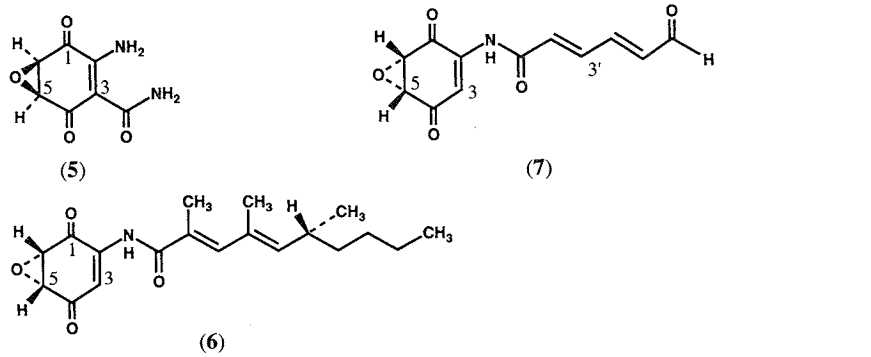


Table 3. CD values of chromic oxidation products **3**, **6** and **7** and antibiotic G7063-2 (**5**).

3 ($CHCl_3$)[c]	**5**[a] (CH_3CN)	**6**[a] ($CHCl_3$)	**7**[b] (CH_3CN)
375 (− 9.37)[d]	376 (− 6.58)	365 (+ 7.57)	370 (+15,200)[e]
328 (+16.69)	327 (+10.53)	316 (−12.50)	322 (−26,500)
266 (− 6.34)	233 (+ 4.60)	242 (+ 4.57)	283 (+10,600)
	198 (− 8.20)	219 (− 8.18)	232 (− 3,300)

[a,b] These data were obtained from refs J. Antibiotics 40: 1549, 1987 and J. Antibiotics 41: 1186, 1988.
[c] Solvent; [d] nm ($\Delta\varepsilon$); [e] nm (θ).

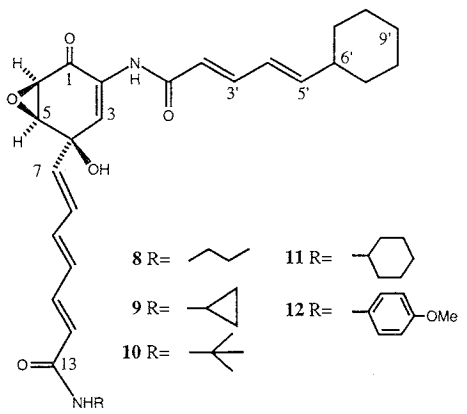
Table 4. Antimicrobial spectra of nisamycin and derivatives.

Test organism	**1**	**8**	**9**	**10**	**11**	**12**
Staphylococcus aureus IFO 3060	0.19[a]	1.3	0.75	0.75	5.0	>10
Bacillus subtilis IFO 12210	0.39	1.3	1.3	1.3	10	>10
B. cereus IFO 3514	0.19	0.75	0.75	1.3	2.5	5.0
Arthrobacter globiformis IFO 12140	0.39	1.3	1.3	1.3	10	>10
Escherichia coli K-12 IFO 3301	50	>10	>10	>10	>10	>10
Serratia marcescens IFO 12648	>100	>10	>10	>10	>10	>10

[a] MIC (μg/ml).

were not yet clear[9]. To investigate these effects, five derivatives were prepared and their antimicrobial activity were examined by the agar-dilution method on nutrient agar for Gram-positive and Gram-negative bacteria (Fig. 3, Table 4). **8**, **9**, and **10** showed antimicrobial activity against Gram-positive bacteria at concentrations of 0.75 ~ 1.3 μg/ml. These derivatives were 3-fold less active than **1**. **11** was 25-fold less active than **1**. The MIC of **12** was more than 10 μg/ml. All derivatives showed no activity against Gram-negative bacteria. These results suggested that the increase in a hydrophobicity of the

Fig. 3. Structure of nisamycin derivatives.



substituents cause the decrease in antimicrobial activity. Aromatization also caused loss of the activity. 2-Acetamino-3-hydroxycyclopent-2-enone itself has no antimicrobial activity[9]. **1** was about 6-fold more active than **2** in our experiment (data not shown). It seems that the 2-amino-3-hydroxycyclopent-2-enone moiety of manumycin group antibiotics is not essential for the antimicrobial activity of these antibiotics.

Experimental

2-(5'-Cyclohexyl-pentadienoylamino)-5,6-epoxy-1,4-benzoquinone (**3**)

Nisamycin (32 mg) in 1.5 ml of 90% acetic acid was stirred for 1 hour at room temperature with 18 mg of CrO_3 (dissolved in 1.5 ml of 60% acetic acid). 50 ml of 0.5 N HCl was added, and the solution was then extracted with ether. The organic layer was concentrated *in vacuo*. The residue was applied onto preparative silica gel TLC and developed with $CHCl_3$. The main band (Rf 0.35) was scraped off and eluted with EtOAc. The solution was evaporated *in vacuo* to give a white powder and further purified by recrystallization from $CHCl_3$ to yield 5.2 mg (23%) **3** as a colorless needles; mp 142~145°C; HR-EIMS Found 301.1332, Calcd. for $C_{17}H_{19}NO_4$ 301.1314; UV λ_{max} ($CHCl_3$) nm (ε), 270 (22,400), 336 (17,700); IR ν_{max} (KBr) cm^{-1}, 3282, 1671, 1632, 1605, 1505, 1000; $[\alpha]_D^{25}$ −50° (*c* 0.12, $CHCl_3$); CD λ extreme ($CHCl_3$) nm ($\Delta\varepsilon$), 375 (−9.37), 328 (+16.69), 266 (−6.34); ^1H NMR (270 MHz, $CDCl_3$) δ_H (J=Hz): 7.84 (NH, br s), 7.61 (1H, d, 2.1), 7.27~7.37 (1H, m), 6.09~6.22 (2H, m), 5.90 (1H, d, 15.1), 3.92 (1H, d, 3.6), 3.83 (1H, dd, 3.6, 2.1), 2.11 (1H, m), 1.75 (5H, m), 0.89~1.40 (5H, m); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ_C: 191.0 (s), 188.2 (s), 165.0 (s), 152.5 (d), 146.1 (d), 139.0 (s), 125.4 (d), 120.1 (d), 115.3 (d), 53.9 (d), 52.5 (d), 41.3 (d), 32.2 (t), 26.0 (t), 25.8 (t).

Nisamycin *n*-Propylamide (**8**)

10 mg of **1**, 2.8 mg of *n*-propylamine, and 7.7 mg of cyano phosphonic acid diethyl ester (DEPC) were dissolved in 1 ml THF. Triethyl amine (4.8 mg) was added dropwise to the mixture with stirring in an ice bath. The mixture was kept in an ice bath with stirring for 1 hour and then at room temperature for 20 minutes. The mixture was poured into 50 ml of EtOAc. The organic layer was washed with 1% $NaHCO_3$ solution and water, dried over sodium sulfate and evaporated. The residue was purified on preparative silica gel TLC ($CHCl_3$-MeOH 97:3, Rf 0.41) to yield 7.4 mg (68%) of a yellow powder **8**: FAB MS *m/z* 467 $(M+H)^+$; IR ν_{max} (KBr) cm^{-1}, 3300, 1651, 1611, 1520, 1448; ^1H NMR (270 MHz, $CDCl_3$) δ: 0.86~1.40 (8H, m), 1.52~1.75 (7H, m), 2.08 (1H, m), 3.30 (2H, m), 3.40 (OH), 3.63 (1H, d, 4.0), 3.68 (1H, dd, 4.0, 2.1), 5.70 (NH), 5.69~5.92 (3H, m), 6.05~6.20 (2H, m), 6.37 (1H, m), 6.48~6.57 (2H, m), 7.19~7.30 (2H, m), 7.43 (1H, d, 2.1), 7.57 (NH).

Nisamycin Cyclopropylamide (**9**)

5 mg of **1** on treatment with 1.3 mg of cyclopropylamine as above gave a residue which was purified on preparative silica gel TLC ($CHCl_3$-MeOH 95:5, Rf 0.38) to yield 4.2 mg (77%) of a yellow powder **9**: FAB MS *m/z* 465 $(M+H)^+$, 487 $(M+Na)^+$; IR ν_{max} (KBr) cm^{-1}, 3287, 1651, 1611, 1520, 1430; ^1H NMR (270 MHz, $CDCl_3$) δ: 0.56 (2H, m), 0.81 (2H, m), 0.87~1.40 (5H, m), 1.73 (5H, m), 2.09 (1H, m), 2.80 (1H, m), 3.38 (OH), 3.63 (1H, d, 3.9), 3.71 (1H, dd, 3.9, 2.4), 5.76 (NH), 5.78~5.92 (3H, m), 6.11~6.14 (2H, m), 6.34 (1H, m), 6.48~6.55 (2H, m), 7.18~7.35 (2H, m), 7.42 (1H, d, 2.4), 7.57 (NH).

Nisamycin *t*-Butylamide (**10**)

10 mg of **1** on treatment with 3.4 mg of *t*-butylamine as above gave a residue which was purified on preparative silica gel TLC ($CHCl_3$-MeOH 97:3, Rf 0.42) to yield 2.4 mg (21%) of a yellow powder **10**: FAB MS *m/z* 481 $(M+H)^+$; IR ν_{max} (KBr) cm^{-1}, 3331, 1667, 1613, 1521, 1451; ^1H NMR (270 MHz, $CDCl_3$) δ: 0.89~1.40 (5H, m), 1.39 (9H, s), 1.73 (5H, m), 2.09 (1H, m), 3.64 (1H, d, 4.0), 3.71 (1H, dd, 4.0, 2.5), 5.38 (NH), 5.71~5.90 (3H, m), 6.11~6.14 (2H, m), 6.38 (1H, m), 6.47~6.56 (2H, m), 7.14~7.30 (2H, m), 7.42 (1H, d, 2.5), 7.56 (NH).

Nisamycin Cyclohexylamide (**11**)

5 mg of **1** on treatment with 2.3 mg of cyclohexylamine as above gave a residue which was purified on preparative silica gel TLC ($CHCl_3$, Rf 0.52) to yield 3.2 mg (54%) of a yellow powder **11**: FAB MS *m/z* 507 $(M+H)^+$; IR ν_{max} (KBr) cm^{-1}, 3283, 1650, 1611, 1521, 1449; ^1H NMR (270 MHz, $CDCl_3$) δ: 0.89~1.44 (10H, m), 1.73~1.95 (10H, m), 2.07 (1H, m), 3.18 (OH), 3.64 (1H, d, 4.0), 3.71 (1H, dd, 4.0, 2.4), 3.64 (1H, m), 5.48 (NH), 5.77~5.89 (3H, m), 6.11~6.13 (2H, m), 6.38 (1H, m), 6.48~6.54 (2H, m), 7.17~7.35 (2H, m), 7.43 (1H, d, 2.4), 7.57 (NH).

Nisamycin *p*-Methoxyanilide (**12**)

10 mg of **1** on treatment with 5.8 mg of anisidine as above gave a residue which was purified on preparative silica gel TLC ($CHCl_3$, Rf 0.41) to yield 3.6 mg (29%) of a yellow powder **12**: FAB MS *m/z* 531 $(M+H)^+$; IR ν_{max} (KBr) cm^{-1}, 3321, 1662, 1611, 1511, 1412; ^1H NMR (270 MHz, $CDCl_3$) δ: 0.89~1.40 (5H, m), 1.74 (5H, m), 2.05 (1H, m), 3.19 (OH), 3.63 (1H, d, 4.0), 3.71 (1H, s), 3.79 (3H, s), 5.78~5.90 (2H, m), 6.03~6.14 (3H, m), 6.39 (1H, m), 6.48~6.57 (2H, m), 6.85 (2H, m), 7.20~7.34 (2H, m), 7.40~7.57 (3H, m and NH).

Acknowledgments

The authors thank Dr. KEN-ICHI HARADA (Faculty of Pharmacy, Meijyo University) for measurements of CD spectra.

References

1) HAYASHI, K.; M. NAKAGAWA & M. NAKAYAMA: Nisamycin, a new manumycin group antibiotic from *Streptomyces* sp. K106. I. Taxonomy, fermentation, isolation, physico-chemical and biological properties. J. Antibiotics 47: 1104~1109, 1994
2) THIERICKE, R.; A. ZEECK, A. NAKAGAWA, S. ŌMURA, R. E. HERROLD, S. T. S. WU, J. M. BEALE & H. G. FLOSS: Biosynthesis of the manumycin group antibiotics. J. Am. Chem. Soc. 112: 3979~3987, 1990
3) GROTE, R.; A. ZEECK & J. M. BEALE, Jr.: Metabolic products of microorganisms. 245. Colabomycins, new antibiotics of the manumycin group from *Streptomyces griseoflavus*. II. Structure of colabomycin A. J. Antibiotics 41: 1186~1195, 1988
4) CHATTERJEE, S.; E. K. S. VIJAYAKUMAR, C. M. M. FRANCO, J. BLUMBACH, B. N. GANGULI, H.-W. FEHLHABER & H. KOGLER: On the structure of alisamycin, a new member of the manumycin class of antibiotics. J. Antibiotics 46: 1027~1030, 1993
5) HAYASHI, K.; M. NAKAGAWA, T. FUJITA & M. NAKAYAMA: Absolute stereochemistry of alisamycin. Biosci. Biotech. Biochem. 58: 1332~1333, 1994
6) KOREEDA, M.; N. HARADA & K. NAKANISHI: Exciton chirality method as applied to conjugated enones, esters and lactones. J. Am. Chem. Soc. 96: 266~268, 1974
7) KAKINUMA, K.; N. IKEKAWA, A. NAKAGAWA & S. ŌMURA: The sturucture of asukamycin, a possible shunt metabolite from 3-dehydroquinic acid in the shikimate pathway. J. Am. Chem. Soc. 101: 3402~3404, 1979
8) THIERICKE, R.; M. STELLWAAG, A. ZEECK & G. SNATZKE: The structure of manumycin. III. Absolute configuration and conformational studies. J. Antibiotics 40: 1549~1554, 1987
9) ZEECK, A.; K. FROBEL, C. HEUSEL, K. SCHRÖDER & R. THIERICKE: The structure of manumycin. II. Derivatives. J. Antibiotics 40: 1541~1548, 1987