SYNTHESIS AND ACTIVITY OF BUTIROSIN DERIVATIVES WITH 5″-AMIDINO AND 5″-GUANIDINO SUBSTITUENTS

YUKIO NARITA, SHINJI MASUYOSHI, TETSURO YAMASAKI, TAKAYUKI NAITO and HIROSHI KAWAGUCHI

Bristol-Myers Squibb Research Institute,
2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

(Received for publication September 17, 1990)

The preparation and antibacterial activity of the 5″-guanidino (**6**) and 5″-amidino (**7**) derivatives of 4′-deoxybutirosin A (**1**) as well as the 5″-guanidino derivative (**8**) of butirosin A are described. The key intermediates, tetra-*N*-benzyloxycarbonyl-5″-azido derivatives were selectively reduced with $NiCl_2$-$NaBH_4$ to give the corresponding 5″-amino derivatives. Subsequent guanidination or amidination followed by deblocking afforded the final compounds **6**, **7** and **8**. The 5″-guanidino derivatives (**6** and **8**) were more active against Gram-positive and Gram-negative bacteria than the corresponding 5″-hydroxy derivatives (**1** and butirosin A). Compound **6** was also active against a variety of methicillin-resistant *Staphylococcus aureus* (MRSA).

4′-Deoxybutirosin A (**1**)[1] discovered in our screening program demonstrated broader antimicrobial spectrum than butirosin A, inhibiting some butirosin-resistant microorganisms and showing increased anti-pseudomonal activity. It also showed relatively low acute toxicity (LD_{50}, 520 mg/kg) compared to other aminoglycoside antibiotics.[2] In the course of our chemical modification of **1**, we prepared the 5″-amino derivative of **1** (**2**)[3], which exhibited more potent antimicrobial activity as compared to **1**, although it showed higher acute toxicity (LD_{50}, 170 mg/kg). Later, WOO and HASKELL[4] prepared **2** from butirosin A through a different synthetic route from ours. The good activity of *2* prompted us to introduce other aminoglycoside antibiotics[2]. In the course of our chemical modification of **1**, we prepared the and antibacterial activity of the 5″-amidino and 5″-guanidino derivatives of **1** and butirosin A.

Chemistry

In order to introduce a guanidino or amidino function selectively at the 5″-position of **1**, the *N*-benzyloxycarbonyl(*Z*)-protected 5″-azido derivative (**3**)[3] was prepared from **1** in 54% overall yield. Catalytic hydrogenation of **3** resulted in removal of all *Z* groups along with reduction of the 5″-azido group to afford the 5″-amino derivative (**2**). Selective reduction of the azido function was carried out successfully by modified borohydride reduction[5]. Treatment of **3** with $NiCl_2$-$NaBH_4$ in aqueous methanol at 0~5°C for 3 hours gave the *N*-*Z*-5″-amino derivative (**4**) in a quantitative yield. An alternative method using stannous chloride[6] required a prolonged reaction period (2 to 3 days, yield 84%). Treatment of **4** with 2-methyl-1-nitro-2-thiopseudourea[7] in DMSO gave the nitroguanidino compound (**5**) in 67% yield. The *Z* and nitro groups in **5** were removed by catalytic hydrogenation in acetic acid to give the 5″-guanidino compound (**6**) in 89% yield. Amidination of **4** with excess ethyl formimidate hydrochloride[8] in methanol at pH 7, followed by hydrogenation and chromatography on Sephadex G-10 gave the 5″-amidino compound (**7**) in 81% yield. Generally an amidino group is rather unstable in neutral or alkaline solution

Most of this work was presented at the 109th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, Apr. 6, 1989.

Table 1. Physico-chemical properties of **1**, **2**, **6**, **7** and **8**.

		1	**2**	**6**	**7**	**8**
^{1}H NMR[a]	1′-H (d)	6.23 (J=4)	6.07 (J=4)	6.23 (J=4)	6.19 (J=4)	6.07 (J=4)
δ (ppm)	1″-H (s)	5.39	5.48	5.39	5.42	5.31
	5″-H (dd)	3.83 (J=9, 12), 3.99 (J=4, 12)	3.45 (J=7, 14), 3.54 (J=4, 14)	3.59 (J=7, 14), 3.52 (J=5, 14)	3.66 (J=7, 14), 3.75 (J=6, 14)	3.45 (J=7, 14), 3.52 (J=6, 14)
	NHC*H*=NH(s)	—	—	—	7.94	—
^{13}C NMR[a]	C-4″	83.72	79.47	80.92	80.00	80.90
δ (ppm)	C-5″	61.42	39.95	41.83	41.70	41.52
	C=O	176.41	176.50	176.44	176.43	176.44
	$NH_2-\overset{NH}{\overset{\|\|}{C}}-NH-$	—	—	158.10	—	158.10
	NH=CH–NH–	—	—	—	155.88	—
FAB-MS m/z $(M+H)^+$		540	539	581	566	597
Sakaguchi test		−	−	+	−	+

[a] All spectra were determined in D_2O (≦pH 2) with Jeol GX-400. Internal standard: DOH (4.80 ppm) for ^{1}H NMR and dioxane (67.40 ppm) for ^{13}C NMR.
J=Hz.

to degradate to the corresponding formamido derivative[9,10]. This is also the case in **7**. The HPLC study of a solution of **7** in water showed that the peak of **7** (9.7 minutes) gradually decreased, and a newly generated peak (5.7 minutes) became predominant after 1 day.

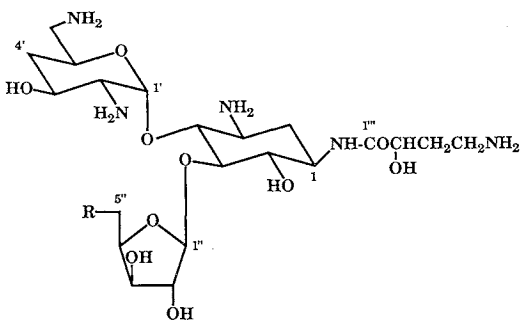
The 5″-guanidino derivative of butirosin A (**8**) was prepared from the corresponding 5″-azido compound (**9**)[11] by a procedure similar to that described above for **6**.

Physico-chemical properties of **2**, **6**, **7** and **8** are shown in Table 1 compared with those of **1**. The ^{1}H NMR spectra of **2**, **6**, **7** and **8** are similar to that of **1** except for the chemical shifts of the 5″-methylene protons. In these compounds bearing an *N*-substituent at the 5″-position, the 5″-methylene protons resonated at relatively higher field (3.45~3.75 ppm) as compared to those of the 5″-OH derivative (**1**) (3.83~3.99 ppm). This is also the case in the ^{13}C NMR spectra, in which the C-5″ of **2**, **6**, **7** and **8** (39.95~41.83 ppm) is shifted approximately 20 ppm higher than that of **1** (61.42 ppm). Compound **7** exhibited a characteristic signals due to the amidino group at 7.94 ppm due to the amidino proton in the ^{1}H NMR and at 155.88 ppm in the ^{13}C NMR spectra, while the guanidino carbon appeared at 158.10 ppm in the ^{13}C NMR of both **6** and **8**. The Sakaguchi test was positive for **6** and **8** and negative for **1**, **2** and **7**. In the FAB-MS spectra, each product showed a pseudomolecular ion peak at m/z corresponding to M+H. These data supported the structures of **2**, **6**, **7** and **8** as shown in Fig. 1.

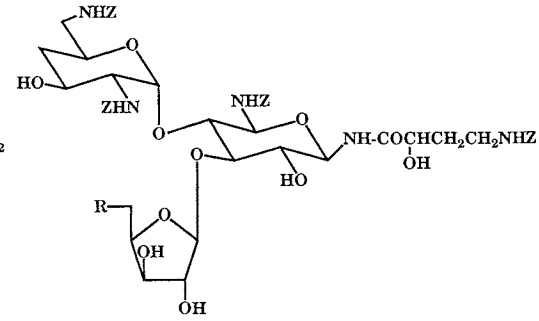
Biological Activity

The MIC values of **2**, **6**, **7** and **8** were determined by the 2-fold serial agar dilution method against both aminoglycoside-sensitive and -resistant organisms in comparison with **1**, butirosin A (BTN), kanamycin A (KM) and amikacin (AMK). The results are summarized in Table 2. Compounds **2**, **6** and **7**, which are 5″-substituted derivatives of 4′-deoxybutirosin (**1**), showed an antibacterial spectrum similar to that of **1** and were all active against *Staphylococcus aureus* A21978[12] and *Escherichia coli* JR66/W677[13], which resisted to BTN having the 4′-OH group. Replacement of the 5″-OH group with an amino group (**1**→**2**) improved the antibacterial activity of **1** against both Gram-positive and Gram-negative organisms. A much

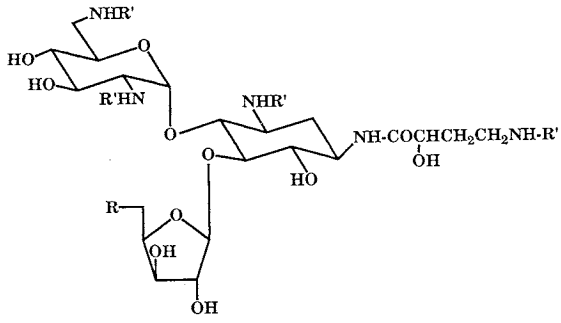
Fig. 1. Chemical structures of butirosin derivatives.



1 R=OH
2 R=NH_2
6 R=NH-C(=NH)-NH_2
7 R=NH-CH=NH



3 R=N_3
4 R=NH_2
5 R=NHC(=NH)-$NHNO_2$



8 R=NH-C(=NH)-NH_2 R'=H
9 R=N_3 R'=Z

Table 2. *In vitro* antibacterial activity of **1**, **2**, **6**, **7** and **8**.

Organism	Aminoglycoside-modifying enzyme[a]	MIC (μg/ml)							
		1	**2**	**6**	**7**	**8**	BTN	KM	AMK
Staphylococcus aureus Smith		1.6	0.4	0.2	1.6	0.4	1.6	0.8	0.8
S. aureus A21978[12]	AAD (4′)	12.5	3.1	1.6	12.5	>50	>50	>100	50
Escherichia coli Juhl		3.1	3.1	1.6	6.3	0.8	1.6	3.1	3.1
E. coli ML1630[17]	APH (3′)-I	3.1	1.6	0.8	3.1	0.8	1.6	>100	1.6
E. coli JR66/W677[13]	APH (3′)-II, AAD (2″)	12.5	3.1	1.6	25	>50	>50	>100	3.1
Proteus vulgaris A4936		0.8	0.8	0.4	1.6	0.4	0.8	0.8	0.4
Serratia marcescens A20019		3.1	1.6	0.8	3.1	0.8	3.1	1.6	1.6
Pseudomonas aeruginosa A9843A		12.5	3.1	3.1	25	6.3	25	>100	3.1
P. aeruginosa GN315[18]	AAC (6′)-IV	>50	>50	25	>50	25	>50	>100	12.5

[a] Abbreviation for aminoglycoside-modifying enzymes, see ref 19.

greater effect was observed by substitution with a more basic guanidino group (**1**→**6**). The 5″-guanidino derivative (**6**) is 2- to 8-fold more active than the corresponding 5″-OH derivative (**1**) and about two times more active than the 5″-amino derivative (**2**). As compared to AMK, **6** was more potent against most of

Table 3. *In vivo* antibacterial activity.

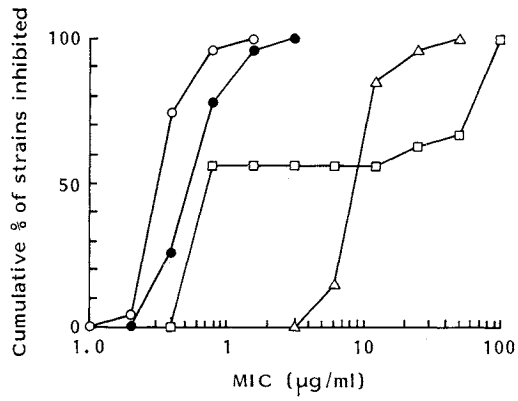
Organism	PD_{50} (mg/kg, mice, im)			
	1	**2**	**6**	AMK
Staphylococcus aureus Smith	4.9	1.5	0.85	1.6
S. aureus BX-1633	1.6	0.77	0.33	1.2
Escherichia coli Juhl	4.8	1.7	2.7	1.8
Serratia marcescens A20019	>8.1	4.3	2.7	2.9
Pseudomonas aeruginosa A9843A	12	7.7	5.2	8.2
P. aeruginosa A21509	>41	>43	25	23
P. aeruginosa A20599	8.5	2.7	1.8	3.0

Table 4. Acute toxicity (mice, iv).

Compound	LD_{50} (mg/kg)
1	520
2	170
6	98
AMK	280~330
GM	75

Fig. 2. Cumulative curve of MICs against 27 MRSA strains.

○ **6**, ● **2**, □, GM, △ AMK.



the strains tested, especially distinctly more active against *S. aureus* A21978 which inactivated AMK by 4′-*O*-adenylylation[12].

The 5″-amidino derivative (**7**), another basic derivative of **1**, did not show enhanced activity, but it was nearly as active as or somewhat less active than **1**. This is presumably due to hydrolysis of the amidino group to a non-basic formamido group as described above.

Preferable effect of the 5″-guanidino group was also observed in the BTN series. Compound **8**, the 5″-guanidino analog of BTN, is two to four times more active than BTN against the test organisms except for the two resistant strains described above.

Compound **6** was selected for further evaluation. Fig. 2 shows the cumulative curve of MICs against clinical isolates of gentamicin C (GM)-sensitive (15 strains), and GM-resistant and methicillin-resistant *Staphylococcus aureus* (MRSA) (12 strains)[14], which were kindly provided by Dr. UBUKATA of Teikyo University. Compound **6** showed the most potent activity against the MRSA strains and was followed by **2**.

Table 3 shows *in vivo* activity of **1**, **2**, **6** and AMK against seven experimental infections in mice. Their *in vivo* efficacies were related to the *in vitro* activities. Compound **6** was the most potent against most of the infections tested, especially against *S. aureus* species. Acute toxicity of **1**, **2**, **6**, AMK and GM determined at pH 7 by intravenous injection to mice is shown in Table 4. The results showed that the more active, the more toxic in this series of compounds. In terms of LD_{50}, **6** was approximately 5- and 3-fold more toxic than **1** and AMK, respectively, and comparable to GM.

Experimental

MP's were obtained on a Yanagimoto micro-hot-stage apparatus and are uncorrected. The NMR

spectra were determined on a Varian FT80A (80 MHz) or a Jeol GX400 (400 MHz). IR spectra were measured with a Jasco IRA-1. The FAB mass spectra were obtained on a Jeol JMS-AX505H mass spectrometer.

5″-Amino-4′,5″-dideoxy-2′,3,4‴,6′-tetra-*N*-benzyloxycarbonylbutirosin A (**4**)

Method A: To a chilled and stirred solution of 5″-azido-4′,5″-dideoxy-2′,3,4‴,6′-tetra-*N*-benzyloxycarbonylbutirosin A (**3**)[3] (440 mg, 0.4 mmol) in 80% aq MeOH (40 ml) were added $NiCl_2 \cdot 6H_2O$ (90 mg, 0.3 mmol) and $NaBH_4$ (30 mg, 0.8 mmol). The mixture was stirred for 3 hours at room temperature. The suspension was evaporated to a small volume under reduced pressure, diluted with EtOAc (40 ml), washed with chilled water and brine successively, dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give 430 mg (quantitative) of **4**. MP 110~111°C; IR (KBr) cm^{-1} 1700, 1558, 1543; ^1H NMR (80 MHz, DMSO-d_6) δ 7.30 (20H, s, Ph), 5.00 (8H, s, CH_2Ph).

Anal Calcd for $C_{53}H_{66}N_6O_{18} \cdot 2H_2O$: C 57.29, H 6.35, N 7.56.
Found: C 57.59, H 6.14, N 7.48.

Method B: To a stirred solution of $SnCl_2 \cdot 2H_2O$ (5.5 g, 24.5 mmol) in MeOH (50 ml) was added portionwise a solution of **3** (9 g, 8.2 mmol) in MeOH (50 ml). The mixture was stirred for 2.5 days at room temperature and worked up by a method similar to that described above to give 7.4 g (84%) of **4**, which was identical to the sample obtained by Method A.

4′,5″-Dideoxy-5″-(*N*-nitroguanidino)-2′,3,4‴,6′-tetra-*N*-benzyloxycarbonylbutirosin A (**5**)

To a stirred solution of **4** (640 mg, 0.58 mmol) in DMSO (6 ml) were added sodium methoxide (28% in MeOH, 0.11 ml) and 2-methyl-1-nitro-2-thiopseudourea (640 mg, 4.7 mmol). The mixture was heated at 60°C for 2 hours, poured into EtOAc (50 ml) and filtered. The filtrate was successively washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue was chromatographed on a silica gel column (elution with $CHCl_3$-MeOH, from 20:1 to 3:1) to give 463 mg (67%) of **5**. MP 125~130°C; IR (KBr) cm^{-1} 1700, 1558, 1543, 1538, 1533; ^1H NMR (80 MHz, DMSO-d_6) δ 7.30 (20H, s, Ph), 5.00 (8H, s, $-CH_2Ph$).

Anal Calcd for $C_{54}H_{67}N_9O_{20} \cdot 2H_2O$: C 54.13, H 5.97, N 10.52.
Found: C 54.01, H 5.63, N 10.66.

4′,5″-Dideoxy-5″-guanidinobutirosin A (**6**)

A solution of **5** (3.8 g, 3 mmol) in acetic acid (250 ml) was stirred in the presence of 10% Pd-carbon (2 g) under atmospheric pressure of hydrogen for 2.5 days at room temperature. The reaction mixture was filtered, the filtrate was concentrated under reduced pressure, and the residue was co-evaporated with water several times to give a white powder. It was dissolved in ethanol (150 ml) and the solution was acidified to pH 2 with 4 N H_2SO_4. The precipitate was collected by filtration, washed with ethanol, and dissolved in water (50 ml). The solution was adjusted to pH 5 with Dowex 1X2 (OH^-) resin and filtered. The filtrate was evaporated and lyophilized to give 2.4 g (89%) of **6**. MP >200°C (gradually dec); IR (KBr) cm^{-1} 1650, 1540, 1100; ^1H NMR, ^{13}C NMR and FAB-MS, see Table 1.

4′,5″-Dideoxy-5″-amidinobutirosin A (**7**)

To a solution of **4** (320 mg, 0.3 mmol) in dry MeOH (5 ml) was added ethylformimidate hydrochloride (300 mg, 2.7 mmol). The mixture was adjusted to pH 7 with dil sodium methoxide in MeOH and stirred overnight at room temperature. The mixture was acidified to pH 4 with dil HCl and concentrated under reduced pressure. The residue was extracted with BuOH and the BuOH layer was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in a mixture of acetic acid (10 ml) and water (3 ml) and hydrogenated in the presence of 10% Pd-carbon under atmospheric pressure of hydrogen overnight at room temperature. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a column of Sephadex G-10, eluting with water. The desired fractions were combined and concentrated under reduced pressure. The residue was dissolved in 2 N H_2SO_4 (1 ml) and the solution was poured into stirred and ice-chilled ethanol (50 ml). The resulting precipitate was collected by filtration to give 218 mg of **7** (81%). MP >200°C (gradually dec); IR (KBr) cm^{-1} 1655, 1530, 1070; ^1H NMR, ^{13}C NMR and

FAB-MS, see Table 1.

HPLC Study of **7**

A solution of **7** (1 mg) in water (1 ml) was kept to stand at room temperature. A 5-μl each of the solution was injected at an indicated time to the HPLC column (Gilson, M & S Pack C_{18}, 4.6 × 150 mm; mobile phase, MeOH - pH 2 phosphate buffer, 25 : 75) detected according to the post column procedure by WATANABE *et al.*[15].

Table 5. Stability test of **7** in water by HPLC.

Time (hours)	Relative peak area (%)	
	Peak at 5.7 minutes	Peak at 9.7 minutes (**7**)
1	24	73
4	33	66
24	54	44
48	71	27
120	81	14

5″-Deoxy-5″-guanidinobutirosin A (**8**)

By a similar procedure to that for preparing **6** from **3**, 5″-azido-5″-deoxy-2′,3,4‴,6′-tetra-*N*-benzyloxycarbonylbutirosin A (**9**, 365 mg, 0.33 mmol) was converted to **8** (30.4 mg, 12%). MP >200°C (gradually dec); IR (KBr) cm^{-1} 1640, 1540, 1110; ^1H NMR, ^{13}C NMR and FAB-MS, see Table 1.

5″-Amino-4′,5″-dideoxybutirosin A (**2**)

A solution of **3** (1.4 g, 1.3 mmol) in 0.2 N HCl - MeOH (80 ml) was stirred in the presence of 10% Pd-C (300 mg) under atmospheric pressure of hydrogen for 1 day. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified with an Amberlite CG-50 (NH_4^+) column (aq NH_4OH, 0.2 N - 1.0 N). The desired fractions were combined, concentrated under reduced pressure, and lyophilized to give 690 mg (quantitative) of **2**, which was identical to that previously prepared[3] on the basis of its spectroscopic data and anti-microbial activity.

In Vitro Antibacterial Activity

MICs were determined by the standard 2-fold agar dilution method on Mueller-Hinton agar (Difco). Overnight broth cultures served as the source of inoculum. A volume of approximately 0.005 ml of the diluted culture containing 10^6 cfu/ml was applied to the surface of the antibiotic-containing agar plates with a multi-inoculator. After incubation at 37°C (32°C in the experiment with MRSA strains) for 18 hours, the plates were examined for colony development, and the lowest concentration of antibiotic causing no visible growth was recorded as the MIC.

In Vivo Antibacterial Activity

Organisms were cultured overnight at 37°C in brain heart infusion broth and suspended in 5% hog mucin (American Laboratory, Omaha, Neb., U.S.A.). Male *dd*Y-mice, each weighing 20 ~ 26 g, were infected intraperitoneally with about one hundred times the median lethal dose of the pathogen. Five mice were individually given an antibiotic solution in pH 7.0 phosphate buffer at each of 4-fold graded levels intramuscularly immediately after the bacterial challenge. The 50% protective dose (PD_{50}, mg/kg) was calculated by the method of LITCHFIELD and WILCOXON[16], from survival rate recorded 4 days after the bacterial infection. Untreated mice died within 40 hours.

Acute Toxicity

Male *dd*Y-mice, each weighing 20 ~ 26 g, were injected *via* the tail vein with graded doses of an antibiotic solution in pH 7.0 phosphate buffer (5 mice for each dose) and observed for 4 days after the iv administration to record the mortality. The LD_{50} (mg/kg) was calculated from the mortality rate by the published method[16].

Acknowledgments

We wish to thank Dr. K. UBUKATA of Teikyo University (The Department of Clinical Pathology) for supplying methicillin-resistant strains of *S. aureus*. We also thank Dr. S. ABURAKI for his helpful suggestion, Dr. T. TSUNO and associates for the microanalytical and spectral data.

References

1) KAWAGUCHI, H.; K. TOMITA, T. HOSHIYA, T. MIYAKI, K. FUJISAWA, M. KIMEDA, K. NUMATA, M. KONISHI, H. TSUKIURA, M. HATORI & H. KOSHIYAMA: Aminoglycoside antibiotics. V. The 4'-deoxybutirosins (BU-1975C_1 and C_2), new aminoglycoside antibiotics of bacterial origin. J. Antibiotics 27: 460~470, 1974
2) FUJISAWA, K.; T. HOSHIYA & H. KAWAGUCHI: Aminoglycoside antibiotics. VII. Acute toxicity of aminoglycoside antibiotics. J. Antibiotics 27: 677~681, 1974
3) NAITO, T.; S. NAKAGAWA & S. TODA (Bristol-Banyu Res. Inst.): 5''-Amino-4',5''-dideoxybutirosin A. Jpn. Kokai 35132 ('75), Mar. 4, 1975
4) WOO, P. W. K. & T. H. HASKELL: Deoxy derivatives of butirosin A and 5''-amino-5''-deoxybutirosin A, aminoglycoside antibiotics resistant to bacterial 3'-phosphorylative enzymatic inactivation. Synthesis and NMR studies. J. Antibiotics 35: 692~702, 1982
5) KINZY, W. & R. R. SCHMIDT: Application of the trichloroacetimidate method to the synthesis of glycopeptides of the mucin type containing a β-D-Galp-(1→3)-D-gulpNAc unit. Carbohydr. Res. 164: 265~276, 1987
6) MAITI, S. N.; M. P. SINGH & R. G. MICETICH: Facile conversion of azides to amines. Tetrahedron Lett. 27: 1423~1424, 1986
7) FISHBEIN, L. & J. A. GALLAGHAN: The preparation and reactions of 2-alkyl-1(or 3)-nitro-2-thiopseudourea Part I. Reaction with amines. J. Am. Chem. Soc. 76: 1877~1879, 1954
8) OHME, R. & E. SCHMITZ: A simple synthesis of alkyl formimidates. Angew. Chem. Int. Ed. 6: 566, 1967
9) OHBA, K.; T. TSURUOKA, K. MIZUTANI, N. KATO, S. OMOTO, N. EZAKI, S. INOUYE, T. NIIDA & K. WATANABE: Studies on a new aminoglycoside antibiotic, dactimicin. II. Isolation, structure and chemical degradation J. Antibiotics 34: 1090~1100, 1981
10) YAMASAKI, T.; Y. NARITA, H. HOSHI, S. ABURAKI, H. KAMEI, T. NAITO & H. KAWAGUCHI: Synthesis and biological activity of 1-*N*-[4-(substituted)amino and guanidino-2-hydroxybutyryl]-kanamycin A and B. J. Antibiotics, to submitted
11) CULBERTSON, T. P.; D. R. WATSON & T. H. HASKELL: 5''-Amino-5''-deoxybutirosin, a new semi-synthetic aminoglycoside antibiotic. J. Antibiotics 26: 790~793, 1973
12) TODA, S.; S. NAKAGAWA, T. NAITO & H. KAWAGUCHI: Structure determination of amikacin derivatives modified by enzymes from resistant *S. aureus* strains. Tetrahedron Lett. 1978: 3917~3920, 1978
13) YAGISAWA, M.; H. YAMAMOTO, H. NAGANAWA, S. KONDO, T. TAKEUCHI & H. UMEZAWA: A new enzyme in *Escherichia coli* carrying R-factor phosphorylating 3'-hydroxyl of butirosin A, kanamycin, neamine and ribostamycin. J. Antibiotics 25: 748~750, 1972
14) KONNO, M.; S. OHONARI, N. ITOH, K. UBUKATA, Y. HASHIMOTO & S. KAWAKAMI: Trends of fluoroquinolone-resistant strains isolated from clinical materials. J. Jpn. Assoc. Infect. Dis. 62: 641~651, 1988
15) WATANABE, M.; Y. KONDOU, K. MASHIMO, T. KAWAMOTO & K. SHIMADZU: High-pressure liquid-chromatographic method for determination of aminoglycoside in serum. Chemotherapy 30: 21~24, 1982
16) LITCHFIELD, J. T. & F. WILCOXON: Simplified method of evaluating dose effect experiments. J. Pharmacol. Exp. Ther. 96: 99~113, 1949
17) OKANISHI, M.; S. KONDŌ, R. UTAHARA & H. UMEZAWA: Phosphorylation and inactivation of aminoglycosidic antibiotics by *E. coli* carrying R factor. J. Antibiotics 21: 13~21, 1968
18) KAWABE, H.; S. KONDO, H. UMEZAWA & S. MITSUHASHI: R Factor-mediated aminoglycoside antibiotiic resistance in *Pseudomonas aeruginosa*: a new aminoglycoside 6'-*N*-acetyltransferase. Antimicrob. Agents Chemother. 7: 494~499, 1975
19) MITSUHASHI, S.: Proposal for a rational nomenclature for phenotype, genotype, and aminoglycoside-aminocyclitol modifying enzymes. *In* Drug action and drug resistance in bacteria. 2. Aminoglycoside Antibiotics. *Ed.*, S. MITSUHASHI, pp. 269~275, University of Tokyo Press, 1975