SYNTHESIS OF 2″-AMINO-2″-DEOXYARBEKACIN AND ITS ANALOGS HAVING POTENT ACTIVITY AGAINST METHICILLIN-RESISTANT *Staphylococcus aureus*†

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Based on our studies on the enzymatic modifications of arbekacin by methicillin-resistant *Staphylococcus aureus* (MRSA), replacement of the 2″-hydroxyl group by an amino group in arbekacin was designed to synthesize derivatives that would be active against MRSA. 2″-Amino-2″-deoxyarbekacin and five analogs were synthesized starting from dibekacin. Among them, 2″-amino-2″-deoxyarbekacin and the 5-epiamino analog showed excellent antibacterial activities against not only MRSA but also Gram-negative bacteria including *Pseudomonas*, and lower toxicities than arbekacin.

In 1967, the enzymatic inactivation of kanamycin by two aminoglycoside-modifying enzymes, AAC (6′) and APH (3′) in resistant bacteria were first found by UMEZAWA and coworkers.[1~3] Thereafter, several aminoglycoside-modifying enzymes were reported by many researchers.[3] Based on the enzymatic mechanisms of resistance, studies on the chemical modifications of kanamycin led to the synthesis of 3′,4′-dideoxykanamycin B[4] (dibekacin, **1**) and 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-3′,4′-dideoxykanamycin B[5] (arbekacin, **2**). Compound **2** was refractory to most aminoglycoside-modifying enzymes, and inhibited not only Gram-negative bacteria including pseudomonas, but also staphylococci. In 1990, **2** was launched into Japan as a useful chemotherapeutic agent for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA).

Most of clinically isolated MRSA strains were susceptible to **2**, and resistant to kanamycin, tobramycin, gentamicin and amikacin.[6] By 1993 only a few MRSA strains, which were moderately resistant to **2** (MIC, 6.25~12.5 μg/ml) were isolated clinically. As reported previously,[7] **2** was modified by reaction with excess of an enzyme preparation, APH (2″)/AAC (6′)[8] extracted from an arbekacin-resistant strain (12.5 μg/ml) of MRSA. From the enzymatic reaction mixture, arbekacin 2″-phosphate was isolated as a major inactivated product along with two minor products, 6′-*N*-acetylarbekacin and the doubly modified product of **2**.

Based on these facts, replacement of the 2″-hydroxyl group by an amino group in **1** or **2** was designed to obtain derivatives stable to an APH(2″)/AAC(6′) enzyme and active against MRSA. As reported in our communication,[9] anti-MRSA activities of 2″-amino-2″-deoxy and 2″-amino-5,2″-dideoxy derivatives

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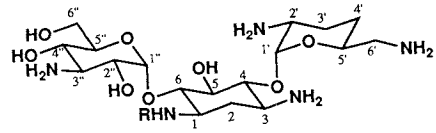
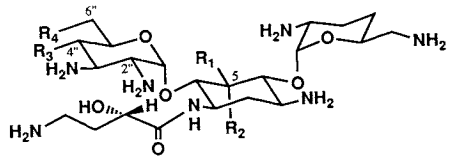
Fig. 1.



Dibekacin (**1**) R=H
Arbekacin (**2**) R=(*S*)-$H_2NCH_2CH_2CH(OH)CO$



2″-Amino-2″-deoxyarbekacin (**3**)	R_1=OH, R_2=H, R_3=OH, R_4=OH
2″-Amino-5,2″-dideoxyarbekacin (**4**)	R_1=H, R_2=H, R_3=OH, R_4=OH
2″-Amino-5,2″-dideoxy-5-epifluoroarbekacin (**5**)	R_1=H, R_2=F, R_3=OH, R_4=OH
2″-Amino-5,2″-dideoxy-5-epiaminoarbekacin (**6**)	R_1=H, R_2=NH_2, R_3=OH, R_4=OH
2″-Amino-2″,4″-dideoxyarbekacin (**7**)	R_1=OH, R_2=H, R_3=H, R_4=OH
2″-Amino-6″-chloro-2″,6″-dideoxyarbekacin (**8**)	R_1=OH, R_2=H, R_3=OH, R_4=Cl

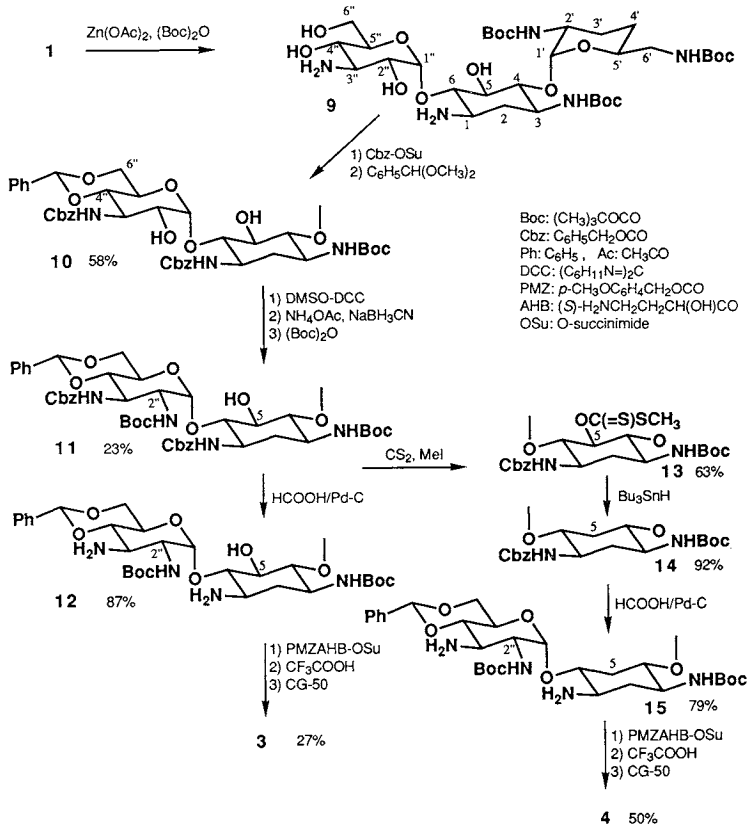
of **1** and **2** were markedly improved as expected. Both 2″-amino-2″-deoxyarbekacin (**3**) and 2″-amino-5,2″-dideoxyarbekacin (**4**) were superior to the corresponding analogs of **1** in their antibacterial activities. Therefore, we synthesized the 5-epifluoro (**5**), 5-epiamino (**6**), 4″-deoxy (**7**) and 6″-chloro (**8**) analogs of **3**. In this paper, the syntheses and antibacterial activities of the new analogs (**3**~**8**) with full experimental details are reported.

Chemistry

Compounds **3** and **4** were synthesized starting from 3,2′,6′-tris(*N*-*tert*-butoxycarbonyl)dibekacin (**9**), as shown in Scheme 1. Compound **9** was derived from **1** by the selective 3,2′,6′-*N*-protection[10,11] with *tert*-butoxycarbonyl (Boc) groups, which was established in the industrial production of **2**. Subsequent blockings of **9** with benzyloxycarbonyl (Cbz) at both 1- and 3″-amino groups and with benzylidene between the 4″- and 6″-hydroxyl groups afforded **10** in a good yield. After the 2″-hydroxyl group of **10** was oxidized to the 2″-ulose by Pfitzner-Moffatt oxidation,[12] reductive amination[13] of the ulose gave exclusively an equatorial 2″-amino compound, showing $J_{2'',3''}$=~10 Hz at 2″-H (δ 2.90) in ^{1}H NMR ($CDCl_3$-CD_3OD, 1:1). Protection of the amino group with Boc afforded **11**. Removal of the Cbz groups of **11** by hydrogenolysis with palladium-carbon gave **12**. Acylation of **12** with the *N*-hydroxysuccinimide ester of (*S*)-2-hydroxy-4-(*p*-methoxybenzyloxycarbonylamino)butyric acid in tetrahydrofuran mainly gave a 1-*N*-acylated product. Deprotection of the 1-*N*-acylated product with trifluoroacetic acid followed by column chromatography on Amberlite CG-50 (NH_4^+) resin eluted with aq ammonia provided **3**.

The deoxygenation of the 5-hydroxyl in **11** was performed by the radical reduction of the dithiocarbonate.[14,15] The 5-*O*-(methylthio)thiocarbonyl derivative **13**, which was obtained by reaction with carbon disulfide and methyl iodide in dimethyl sulfoxide, was treated with tributyltin hydride in toluene in the presence of 2,2′-azobis(isobutyronitrile) to yield the 5-deoxy derivative **14** in a good yield. The Cbz groups of **14** were deprotected by hydrogenolysis to afford **15**. By a similar 1-*N*-acylation procedure used for the preparation of **3** from **12**, compound **15** was converted to **4**, showing $J_{1'',2''}$=3.8 Hz and

Scheme 1.



$J_{2'',3''}=11$ Hz at 2″-H (δ 3.84) in the ^{1}H NMR (D_2O, pD 2).

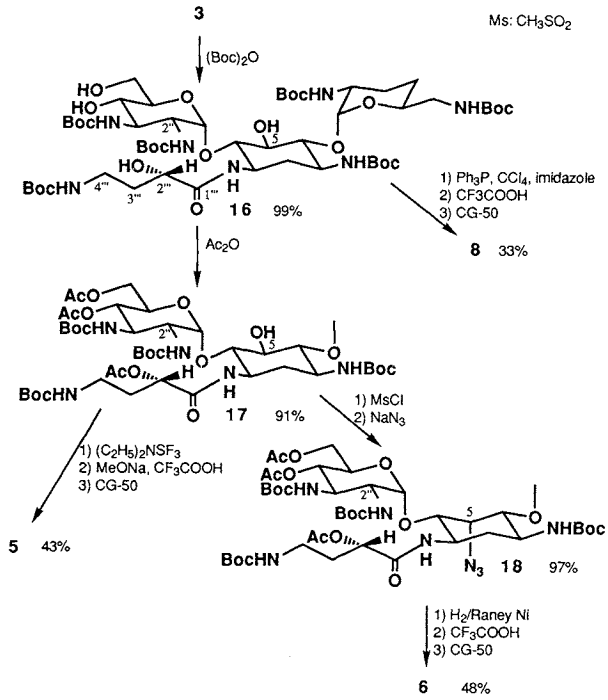
Compounds **5**, **6** and **8** were synthesized from the 3,2′,6′,2″,3″,4‴-hexakis(*N*-Boc) derivative (**16**) of **3** (Scheme 2). *O*-Acetylation of **16** with acetic anhydride in pyridine gave **17**. Treatment of the free hydroxyl group at C-5 in **17** with diethylaminosulfur trifluoride[16] in a mixture of dichloromethane and pyridine yielded the 5-epifluoro compound, which was deprotected with sodium methoxide and then with trifluoroacetic acid to give **5**, showing $J_{3,4}=10.5$ Hz and $J_{4,F}=26.5$ Hz at 4-H (δ 4.25) and $J_{5,F}=51.0$ Hz at 5-H (δ 5.69) in ^{1}H NMR (D_2O, pD 2).

Mesylation of the 5-hydroxyl group in **17** with methanesulfonyl chloride and 4-dimethylaminopyridine in dichloromethane, followed by introduction of the axial azide group with sodium azide in DMF gave **18** in a good yield. After hydrogenation of **18** with Raney Ni, deprotection with sodium methoxide and then with trifluoroacetic acid afforded **6**, showing $J_{5,6}=3.6$ Hz and $J_{1,6}=11.0$ Hz at 6-H (δ 4.62) in ^{1}H NMR (D_2O, pD 2).

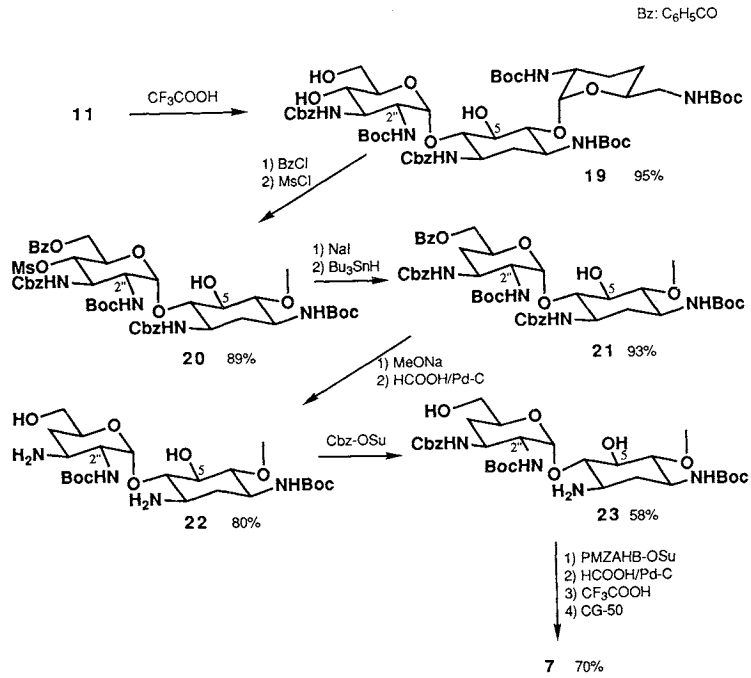
Chlorination of the primary 6″-hydroxyl group in **16** with a triphenylphosphine/tetrachloromethane/imidazole reagent,[17] followed by deprotection with trifluoroacetic acid gave **8**.

Compound **7** was synthesized from **11** as shown in Scheme 3. Selective deprotection of the benzylidene group in **11** with trifluoroacetic acid yielded **19**. Benzoylation of **19** with benzoyl chloride followed by mesylation with methanesulfonyl chloride gave the 6″-*O*-benzoyl-4″-*O*-mesyl derivative **20**. Treatment of **20** with sodium iodide gave the 4″-iodide, which was converted into the deoxy derivative **21** by reduction

Scheme 2.



Scheme 3.



with tributyltin hydride in a good yield. Deprotection of *O*-benzoyl and *N*-Cbz groups by treatment with sodium methoxide, followed by hydrogenolysis gave **22**. Selective reacylation of the 3″-amino group in **22** with *N*-(Cbz)succinimide afforded **23**. The 1-amino group of **23** was acylated by the active ester method

Table 1. ^{1}H NMR spectral data (δ, ppm) in D_2O (pD 2).

Proton	3	4	5	6	7	8
1-H	4.20 ddd	4.08 m	4.32 m	4.43 ddd	4.14 ddd	4.15 ddd
2-H_{ax}	1.98 ddd	1.79 ddd	1.87 ddd	1.99 ddd	1.93 ddd	1.95 ddd
2-H_{eq}	2.33 ddd	2.34 ddd	2.39 ddd	2.43 ddd	2.27 ddd	2.28 ddd
3-H	3.58 ddd	3.52 ddd	3.81 m	3.81 m	3.53 ddd	3.53 ddd
4-H	4.06 dd	4.06 m	4.25 br dd	4.55 dd	4.00 dd	4.02 dd
5-H_{ax}	3.95 dd	1.72 ddd			3.89 dd	3.90 dd
5-H_{eq}		3.00 ddd	5.69 br d	4.53 m		
6-H	4.13 dd	4.09 m	4.28 m	4.62 dd	4.06 dd	4.10 dd
1′-H	5.82 d	5.39 d	5.46 d	5.53 d	5.76 d	5.77 d
2′-H	3.62 ddd	3.57 ddd	3.59 m	3.71 m	3.57 ddd	3.58 ddd
3′-H_2	2.08 m	2.04 m	2.06 m	2.10 m	2.04 m	2.04 m
4′-H_{ax}	1.67 dddd	1.59 m	1.62 m	1.69 m	1.62 dddd	1.63 dddd
4′-H_{eq}	1.99 m	1.90 m	1.91 m	1.95 m	1.93 m	1.94 m
5′-H	4.24 m	4.06 m	4.11 m	4.15 m	4.18 m	4.20 dddd
6′-H_2	3.17 dd,	3.08 dd,	3.09 dd,	3.20 dd,	3.11 dd,	3.12 dd,
	3.32 dd	3.26 dd	3.26 dd	3.31 dd	3.26 dd	3.27 dd
1″-H	5.52 d	5.45 d	5.49 d	5.51 d	5.50 d	5.47 d
2″-H	3.86 m	3.84 dd	3.85 dd	3.85 dd	3.69 dd	3.86 m
3″-H	3.87 m	3.74 dd	3.75 dd	3.88 dd	4.08 ddd	3.87 m
4″-H_{ax}	3.86 m	3.69 dd	3.67 dd	3.77 dd	1.80 ddd	3.92 m
4″-H_{eq}					2.22 ddd	
5″-H	4.13 m	3.95 m	4.04 ddd	3.82 br dd	4.31 m	4.35 dt
6″-H_2	3.88 dd,	3.75 dd,	3.73 dd,	3.80 dd,	3.65 dd,	3.95 d
	3.93 dd	3.96 m	3.99 dd	4.00 br d	3.75 dd	
2‴-H	4.37 dd	4.33 dd	4.33 dd	4.35 dd	4.31 dd	4.32 dd
3‴-H_2	2.01 m,	1.94 dddd,	1.93 ddt,	1.95 ddt,	1.94 m,	1.95 ddt,
	2.22 ddt	2.19 dddd	2.20 ddt	2.19 ddt	2.16 ddt	2.17 ddt
4‴-H_2	3.22 t	3.17 m	3.17 t	3.18 t	3.17 t	3.17 t

All protons were assigned by HH COSY experiments.

with (*S*)-2-hydroxy-4-(*p*-methoxybenzyloxycarbonylamino)butyric acid, and the protective groups were removed by hydrogenolysis and acid hydrolysis, successively to yield **7**.

^{1}H and ^{13}C NMR spectra of **3**~**8** are shown in Tables 1 and 2. All protons and carbons were assigned by HH and HC COSY experiments.

Antibacterial Activity

All 2″-amino analogs (**3**~**8**) showed good antibacterial activities against staphylococci and Gram-negative bacteria including pseudomonas, as shown in Table 3. Conversion of the 2″-hydroxyl group of **2** into an amino group markedly improved the anti-MRSA activity, as expected. Anti-MRSA activities of these analogs against 50 clinically isolated strains[6] were as follows; compounds **3**, **4**, **5**, **6** and **7**: MIC_{50} 0.78 μg/ml and MIC_{90} 1.56 μg/ml, **8**: MIC_{50} 1.56 μg/ml and MIC_{90} 3.13 μg/ml, while **2**: MIC_{50} 1.56 μg/ml and MIC_{90} 6.25 μg/ml. Among 2″-amino analogs, compounds **3** and **6** exhibited not only excellent anti-MRSA activity but also low toxicity (each $LD_{50} > 150$ mg/kg, mice, iv). They will be further evaluated as new chemotherapeutic agents for treatment of the infections caused by MRSA.

Experimental

General

Melting points were obtained using an Electrothermal IA9100 digital melting point apparatus and were not corrected. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. IR spectra were

Table 2. ^{13}C NMR spectral data (δ, ppm) in D_2O (pD 2).

Carbon	3	4	5	6	7	8
1	50.1	51.6	47.4[a]	47.4	50.1	50.1
2	31.1	31.0	30.3	30.8	31.1	31.1
3	49.4	52.0	47.8[b]	47.5	49.4	49.5
4	78.2	71.2	72.7[c]	71.2	78.2	78.2
5	75.0	33.9	87.6[d]	52.1	75.1	75.0
6	78.3	77.9	78.9[e]	73.6	78.2	78.5
1′	96.0	90.7	91.1	92.1	96.0	96.0
2′	49.6	48.9	48.9	48.8	49.6	49.7
3′	21.3	21.7	21.6	21.6	21.3	21.3
4′	26.2	26.3	26.3	25.3	26.2	26.1
5′	66.8	66.4	66.7	67.9	66.7	66.9
6′	43.4	43.4	43.4	42.9	43.3	43.4
1″	93.8	96.3	97.3	96.6	94.2	93.8
2″	51.7	51.6	51.7	51.5	52.2	51.6
3″	53.0	52.9	53.1	52.8	46.8	52.7
4″	65.8	66.4	66.6	66.2	30.1	66.4
5″	72.8	73.1	73.1	74.1	69.1	71.5
6″	60.4	61.2	61.4	60.9	63.6	44.3
1‴	176.4	176.3	176.6	176.6	176.4	176.4
2‴	70.3	70.2	70.3	70.3	70.3	70.4
3‴	31.5	31.7	31.8	31.6	31.5	31.5
4‴	37.6	37.6	37.6	37.6	37.6	37.6

[a] $^3J_{CF}=4.1$ Hz, [b] $^3J_{CF}=5.4$ Hz, [c] $^2J_{CF}=17.6$ Hz, [d] $^1J_{CF}=181.7$ Hz, [e] $^2J_{CF}=19.0$ Hz. All carbons were assigned by HC COSY experiments.

Table 3. Minimum inhibitory concentrations of 2″-amino analogs of **2**.

Test organism	Aminoglycoside-modifying enzyme	MIC (μg/ml)						
		2	**3**	**4**	**5**	**6**	**7**	**8**
Staphylococcus aureus FDA209P		0.20	0.39	≤0.20	0.78	0.20	0.39	0.39
S. aureus Smith		≤0.20	≤0.20	≤0.20	≤0.20	≤0.20	≤0.20	≤0.20
S. aureus MS16502 (MRSA)	APH(2″)/AAC(6′), AAD(4′,4″)	6.25	1.56	0.78	1.56	1.56	1.56	3.13
S. aureus MS16526 (MRSA)	APH(2″)/AAC(6′), AAD(4′,4″)	12.5	1.56	1.56	1.56	0.78	3.13	3.13
Bacillus subtilis PCI219		≤0.20	0.20	≤0.20	0.39	0.20	0.39	0.39
Escherichia coli NIHJ		0.39	0.39	0.78	0.78	0.78	0.39	0.39
E. coli K-12 ML1629	APH(3′)-I	0.78	1.56	3.13	3.13	3.13	1.56	0.78
E. coli JR66/W677	APH(3′)-II	1.56	3.13	3.13	3.13	3.13	3.13	1.56
Shigella dysenteriae JS11910		1.56	3.13	3.13	3.13	3.13	3.13	3.13
Salmonella typhi T-63		0.78	0.78	1.56	0.78	0.78	1.56	1.56
Providencia sp. Pv16	AAC(2′)	1.56	1.56	12.5	1.56	0.78	3.13	3.13
Serratia marcescens		6.25	6.25	12.5	1.56	3.13	3.13	3.13
Pseudomonas aeruginosa A3		≤0.20	0.78	0.39	0.78	0.78	0.78	1.56
P. aeruginosa TI-13	APH(3′)-I	3.13	3.13	3.13	1.56	1.56	3.13	6.25
P. aeruginosa GN315	AAC(6′)-4	6.25	12.5	25	50	25	25	25
P. aeruginosa 99	AAC(3)-I	6.25	12.5	6.25	6.25	6.25	6.25	25
P. aeruginosa 21-75	APH(3′)-III	25	50	>100	6.25	12.5	100	100
P. aeruginosa PST1	AAC(3)-III	6.25	12.5	50	12.5	3.13	6.25	50

recorded on a Hitachi 260-10 spectrophotometer. ^{1}H NMR spectra were measured on a JEOL JNM-GX400 spectrometer at 400 MHz using sodium 3-(trimethylsilyl)propionate ($\delta=0$) as an internal standard and ^{13}C NMR spectra at 100 MHz were recorded with dioxane ($\delta=67.4$). MS were measured on Hitachi M-80B (FD and SI) and JEOL JMS-SX102 (FAB) mass spectrometers.

Antibacterial Activity

MICs were determined by two-fold agar dilution method with Bacto Mueller Hinton Medium (Difco) at 37°C for 18 hours, according to the method of Japan Society of Chemotherapy.

4″,6″-*O*-Benzylidene-1,3″-bis(*N*-Cbz)-3,2′,6′-tris(*N*-Boc)dibekacin (**10**)

3,2′,6′-Tris(*N*-Boc)dibekacin (**9**, 9.02 g, 12.0 mmol), which was derived from **1** by selective *N*-protection,[10,11] was dissolved in DMF (50 ml). To the solution were added pyridine (10 ml) and *N*-(Cbz) succinimide (6.28 g), and the mixture was kept at room temperature for 4 hours. After being concentrated to dryness, the residue was washed with 2% aq ammonia, water and ethyl ether, successively, to afford 1,3″-bis(*N*-Cbz)-3,2′,6′-tris(*N*-Boc)dibekacin (9.90 g, 80%) as a colorless solid; FD-MS *m/z* 1020 (MH^+). To the solution of the *N*-protected compound (4.98 g) in DMF (50 ml) were added benzaldehyde dimethylacetal (3 ml) and anhydrous *p*-toluenesulfonic acid (200 mg). The mixture was stirred at 40°C for 1 hour under reduced pressure (20 mmHg) and then $CHCl_3$ (300 ml) was added. After being washed with $NaHCO_3$-saturated aq solution (50 ml) and 10% NaCl aq solution (50 ml), the organic layer was concentrated to dryness to give **10** (3.88 g, 72%); $[\alpha]_D^{20}+50°$ (*c* 1.2, DMF).

4″,6″-*O*-Benzylidene-1,3″-bis(*N*-Cbz)-3,2′,6′-tris(*N*-Boc)-2″-(Boc)amino-2″-deoxydibekacin (**11**)

To a solution of **10** (2.95 g) and pyridinium trifluoroacetate (250 mg) in DMSO (13 ml) was added a solution of dicyclohexylcarbodiimide (1.68 g) in benzene (19 ml), and the mixture was stirred at room temperature overnight. After a solution of oxalic acid dihydrate (685 mg) in dioxane (2.5 ml) was added dropwise, the mixture was stirred at room temperature for 30 minutes. The precipitate was removed by filtration and $CHCl_3$ (180 ml) was added to the filtrate. The solution was washed with $NaHCO_3$-saturated aq solution (100 ml) and 10% NaCl aq solution (200 ml), and then concentrated to afford the 2″-ulose (3.35 g). To a solution of the 2″-ulose in MeOH (100 ml) were added NH_4OAc (3.7 g) and $NaBH_3CN$ (673 mg), and the mixture was stirred at room temperature overnight. To the reaction mixture was added $CHCl_3$ (300 ml) and the solution was washed with water, $NaHCO_3$-saturated aq solution and 10% NaCl aq solution (each 100 ml), successively. After being concentrated to 50 ml, the equatorial 2″-amino derivative (775 mg) was purified by column chromatography on silica gel (Wakogel C-300, Wako Pure Chemical Industries) eluted with $CHCl_3$-MeOH (40:1 and then 20:1); silica gel TLC (Art.5715, E. Merck, $CHCl_3$-MeOH, 20:1) Rf 0.16, ^{1}H NMR ($CDCl_3$-CD_3OD, 1:1) δ 2.90 (1H, br d, $J_{2'',3''}=\sim 10$ Hz, 2″-H). A solution of the 2″-amino derivative, triethylamine (0.1 ml) and di-*tert*-butyl dicarbonate (0.3 ml) in a mixture of THF and MeOH (each 13 ml) was kept at room temperature overnight. The reaction mixture was concentrated to dryness and purified by column chromatography on silica gel eluted with $CHCl_3$-MeOH (20:1) to afford **11** (752 mg, 23%); mp 205~215°C (dec), FD-MS *m/z* 1207 (MH^+), $[\alpha]_D^{20}+33°$ (*c* 1, $CHCl_3$).

Anal Calcd for $C_{61}H_{86}N_6O_{19}$: C 60.68, H 7.18, N 6.96.
Found: C 60.53, H 7.17, N 7.09.

4″,6″-*O*-Benzylidene-3,2′,6′-tris(*N*-Boc)-2″-(Boc)amino-2″-deoxydibekacin (**12**)

Compound **11** (730 mg) in a mixture (40 ml) of 88% HCOOH and MeOH (1:19) was hydrogenated with 10% palladium-carbon (1.45 g) as a catalyst under an argon atmosphere at room temperature for 2 hours to afford the 1,3″-diamine **12** (491 mg, 87%).

2″-Amino-2″-deoxyarbekacin (**3**)

To a solution of **12** (246 mg) in THF (6 ml) was added a solution of the active ester which was prepared from (*S*)-2-hydroxy-4-(*p*-methoxybenzyloxycarbonylamino)butyric acid (81 mg), *N*-hydroxysuccinimide (33 mg) and dicyclohexylcarbodiimide (61 mg) in THF (1.4 ml), and the mixture was stirred in the presence of triethylamine (0.035 ml) at 5°C. The temperature of the mixture was gradually rised to 20°C under

stirring overnight. The reaction mixture was concentrated to dryness and the residue was dissolved in $CHCl_3$ (6 ml). The solution was washed with $NaHCO_3$-saturated aq solution and 10% NaCl aq solution (each 2 ml), and concentrated to dryness (269 mg). The residue was purified by column chromatography on silica gel ($CHCl_3$ - MeOH, 20 : 1) to yield the 1-*N*-acyl derivative (139 mg). A solution of the 1-*N*-acyl derivative in 90% TFA aq solution (2.8 ml) was kept at room temperature for 1 hour and concentrated to dryness. After being washed with ethyl ether (9 ml), the residue was purified by column chromatography on Amberlite CG-50 (NH_4^+, 25 ml) with a gradient elution from 0.2 to 3% aq ammonia to afford **3** (38 mg, 27% from **12**); mp 155~160°C (dec), $[\alpha]_D^{20}$ +86° (*c* 0.53, H_2O), IR (KBr) 3380, 2930, 1650, 1580, 1470, 1380, 1320, 1100 and 1020 cm^{-1}. ^1H and ^{13}C NMR: Tables 1 and 2. HR-MS (FAB, positive) Found: *m/z* 552.3368 (MH^+). Calcd for $C_{22}H_{46}N_7O_9$: MH, 552.3357.

Anal Calcd for $C_{22}H_{45}N_7O_9 \cdot 1/2H_2CO_3$: C 46.38, H 7.96, N 16.83.
Found: C 46.09, H 7.58, N 16.28.

4″,6″-*O*-Benzylidene-1,3″-bis(*N*-Cbz)-3,2′,6′-tris(*N*-Boc)-2″-(Boc)amino-2″-deoxy-5-*O*-(methylthio)-thiocarbonyldibekacin (**13**)

To a solution of **11** (184 mg) in DMSO (1.6 ml) was added carbon disulfide (0.8 ml) and then dropwise 8 M NaOH (0.6 ml) under stirring at 15°C. After stirring at room temperature for 30 minutes, methyl iodide (1.6 ml) was added dropwise. The reaction mixture was stirred for further 2 hours and concentrated to dryness. A solution of the residue in $CHCl_3$ (15 ml) was washed with water (15 ml) and concentrated to dryness. Purification by column chromatography on silica gel eluted with $CHCl_3$ - MeOH (50 : 1) gave **13** (123 mg, 63%); FD-MS *m/z* 1297 (MH^+).

4″,6″-*O*-Benzylidene-1,3″-bis(*N*-Cbz)-3,2′,6′-tris(*N*-Boc)-2″-(Boc)amino-5,2″-dideoxydibekacin (**14**)

To a solution of **13** (180 mg) in toluene (4 ml) were added tributyltin hydride (0.18 ml) and 2,2′-azobis(isobutyronitrile) (5 mg), and the mixture was heated at 110°C for 50 minutes under an argon atmosphere. Addition of $CHCl_3$ and hexane gave the precipitate, which was purified by column chromatography on silica gel ($CHCl_3$ - MeOH, 50 : 1) to afford **14** (154 mg, 92%); FD-MS *m/z* 1191 (MH^+), $[\alpha]_D^{20}$ +37° (*c* 1.3, $CHCl_3$).

4″,6″-*O*-Benzylidene-3,2′,6′-tris(*N*-Boc)-2″-(Boc)amino-5,2″-dideoxydibekacin (**15**)

Compound **14** (149 mg) in a mixture (11 ml) of 88% HCOOH - MeOH (1 : 19) was hydrogenated with 10% palladium-carbon (520 mg) as a catalyst at room temperature for 2.5 hours under an argon atmosphere to give **15** (95 mg, 79%); FD-MS *m/z* 923 (MH^+).

2″-Amino-5,2″-dideoxyarbekacin (**4**)

To a solution of **15** (93 mg) in THF (3 ml) was added a solution of the active ester, which was prepared from (*S*)-2-hydroxy-4-(*p*-methoxybenzyloxycarbonylamino)butyric acid (37 mg), *N*-hydroxysuccinimide (15 mg) and dicyclohexylcarbodiimide (28 mg) in THF (1.2 ml), and the mixture was stirred in the presence of triethylamine (0.015 ml) at −15°C. The temperature of the mixture was gradually rised to 20°C under stirring overnight. The 1-*N*-acyl derivative (81 mg) in the reaction mixture was isolated by a similar procedure described in the preparation of **3** (column chromatography on silica gel eluted with $CHCl_3$ - MeOH, 30 : 1). A solution of the 1-*N*-acyl derivative in 90% TFA aq solution (1.8 ml) was kept at room temperature for 1 hour. Purification by column chromatography on Amberlite CG-50 (NH_4^+, 18 ml) gave **4** (27 mg, 50%); mp 196~198°C (dec), $[\alpha]_D^{20}$ +92° (*c* 0.74, H_2O), IR (KBr) 3400, 2940, 1650, 1580, 1460, 1390, 1340 and 1040 cm^{-1}. ^1H and ^{13}C NMR: Tables 1 and 2. HR-MS (FAB, positive) Found: *m/z* 536.3395 (MH^+). Calcd for $C_{22}H_{46}N_7O_8$: MH, 536.3408.

Anal Calcd for $C_{22}H_{45}N_7O_8 \cdot H_2CO_3$: C 46.22, H 7.93, N 16.40.
Found: C 46.46, H 7.31, N 15.61.

3,2′,6′,3″,4‴-Pentakis(*N*-Boc)-2″-(Boc)amino-2″-deoxyarbekacin (**16**)

To a solution of **3** (400 mg) in a mixture of MeOH (6 ml), dioxane (1 ml) and water (4 ml) were added di-*tert*-butyl dicarbonate (1.2 ml) and triethylamine (0.1 ml). The solution was stirred at 35°C for 26 hours and concentrated to yield **16** (830 mg, 99%).

4″,6″,2‴-Tri-*O*-acetyl-3,2′,6′,3″,4‴-pentakis(*N*-Boc)-2″-(Boc)amino-2″-deoxyarbekacin (**17**)

To a solution of **16** (830 mg) in pyridine (12 ml) was added acetic anhydride (2.4 ml), and the mixture was stirred at room temperature for 3 hours. After addition of water (0.5 ml), the solution was concentrated to dryness and the residue was dissolved in $CHCl_3$ (60 ml). The solution was washed with 5% $NaHCO_3$ (12 ml, 3 times) and 10% NaCl aq solution (12 ml), and concentrated to dryness. The residue was purified by column chromatography on silica gel eluted with $CHCl_3$-MeOH (40:1) to give **17** (840 mg, 91%); FD-MS *m/z* 1277 (M^+), $[\alpha]_D^{20}$ +41° (*c* 1.26, $CHCl_3$).

2″-Amino-5,2″-dideoxy-5-epifluoroarbekacin (**5**)

To a solution of **17** (160 mg) in dichloromethane (3 ml) was added a solution of diethylaminosulfur trifluoride (0.078 ml) in a mixture of pyridine (0.16 ml) and dichloromethane (2.4 ml) under ice-cooling, and the reaction mixture was stirred at room temperature for 2 hours. After addition of $CHCl_3$ (4 ml), the solution was washed with $NaHCO_3$-saturated aq solution (2 ml, twice), 5% $KHSO_4$ aq solution (2 ml) and water (2 ml), and concentrated to dryness. The residue was purified by column chromatography on silica gel eluted with $CHCl_3$-Me_2CO (4:1) to yield the 5-epifluoro compound (132 mg, 82%); $[\alpha]_D^{20}$ +29° (*c* 1.2, $CHCl_3$). To a solution of the compound (120 mg) in MeOH (4.8 ml) was added 1 M sodium methoxide in MeOH (0.094 ml), and the solution was stirred at room temperature for 1 hour. After being neutralized with Dowex 50W (H^+), the solution was concentrated to dryness. The residue was dissolved in 90% TFA aq solution (1.4 ml) under ice-cooling. After being stirred for 1.5 hours, the solution was concentrated to dryness and the residue was purified by column chromatography on Amberlite CG-50 (NH_4^+, 10 ml) with a gradient elution from 0.4 to 1.6% aq ammonia to yield **5** (27 mg, 53%); mp 164~171°C (dec), $[\alpha]_D^{20}$ +108° (*c* 1.01, H_2O), IR (KBr) 3400, 1660, 1600, 1490, 1400, 1350, 1130, 1060 and 860 cm^{-1}. ^1H and ^{13}C NMR: Tables 1 and 2. HR-MS (FAB, positive) Found: *m/z* 554.3304 (MH^+). Calcd for $C_{22}H_{45}N_7O_8F$: MH, 554.3314.

4″,6″,2‴-Tri-*O*-acetyl-3,2′,6′,3″,4‴-pentakis(*N*-Boc)-2″-(Boc)amino-5,2″-dideoxy-5-epiazidoarbekacin (**18**)

To a solution of **17** (235 mg) in dichloromethane (10 ml) were added 4-dimethylaminopyridine (674 mg) and methanesulfonyl chloride (0.214 ml) under ice-cooling and the solution was stirred at room temperature for 16 hours. After addition of $CHCl_3$ (15 ml), the $CHCl_3$ layer was washed with 5% $KHSO_4$ aq solution (5 ml, 4 times) and 10% NaCl aq solution (5 ml), and concentrated to give the 5-*O*-mesyl compound. The compound was dissolved in DMF (4.8 ml) and sodium azide (127 mg) was added to the solution. After being stirred at 120°C for 3 hours, the solution was concentrated to dryness and the residue was dissolved in $CHCl_3$ (25 ml). The $CHCl_3$ solution was washed with 10% NaCl aq solution (5 ml, 3 times) and concentrated to dryness. The residue was purified by column chromatography on silica gel eluted with $CHCl_3$-MeOH (20:1) to give **18** (233 mg, 97%); FD-MS *m/z* 1303 (MH^+), $[\alpha]_D^{20}$ +33° (*c* 1.05, $CHCl_3$).

2″-Amino-5,2″-dideoxy-5-epiaminoarbekacin (**6**)

Compound **18** (140 mg) in MeOH (6 ml) was hydrogenated with Raney Ni as a catalyst at room temperature for 3 hours. After removal of the catalyst, the filtrate was concentrated to dryness. The residue was purified by column chromatograhy on silica gel eluted with $CHCl_3$-Me_2CO (4:1) and then $CHCl_3$-MeOH (20:1) to yield the 5-epiamino compound (89 mg, 65%); FD-MS *m/z* 1277 (MH^+), $[\alpha]_D^{20}$ +43° (*c* 1.05, $CHCl_3$). To a solution of the compound (87 mg) in MeOH (1.8 ml) was added 1 M sodium methoxide in MeOH (0.068 ml), and the solution was stirred at room temperature for 1 hour. After being neutralized with Dowex 50W (H^+), the solution was concentrated to dryness. The residue was dissolved in 90% TFA aq solution (1 ml) under ice-cooling. After being stirred for 1.5 hours, the solution was concentrated to dryness and the residue was purified by column chromatography on Amberlite CG-50 (NH_4^+, 10 ml) with a gradient elution from 0.4 to 1.6% aq ammonia to yield **6** (28 mg, 74%); mp 192~199°C (dec), $[\alpha]_D^{20}$ +102° (*c* 1.0, H_2O), IR (KBr) 3420, 1650, 1590, 1480, 1400, 1350, 1120, 1040 and 830 cm^{-1}. ^1H and ^{13}C NMR: Tables 1 and 2. HR-MS (FAB, positive) Found: *m/z* 551.3510 (MH^+). Calcd for $C_{22}H_{47}N_8O_8$: MH, 551.3517.

Anal Calcd for $C_{22}H_{46}N_8O_8 \cdot 1/2H_2CO_3$: C 46.46, H 8.14, N 19.26.
Found: C 46.61, H 8.25, N 19.35.

1,3″-Bis(*N*-Cbz)-3,2′,6′-tris(*N*-Boc)-2″-(Boc)amino-2″-deoxydibekacin (**19**)

A solution of **11** (134 mg) in a mixture (13 ml) of 90% TFA aq solution and MeOH (1 : 50) was kept at room temperature overnight, and neutralized with $NaHCO_3$-saturated aq solution. After evaporation of MeOH, the residue was extracted with $CHCl_3$. The extract was washed with water and concentrated to give **19** (118 mg, 95%) as a colorless solid. SI-MS *m/z* 1119 (MH^+).

6″-*O*-Benzoyl-1,3″-bis(*N*-Cbz)-3,2′,6′-tris(*N*-Boc)-2″-(Boc)amino-2″-deoxy-4″-*O*-mesyldibekacin (**20**)

To a solution of **19** (100 mg) in pyridine (2 ml) was added benzoyl chloride (0.031 ml) under ice-cooling. The mixture was stirred at room temperature for 4 hours and concentrated to dryness. The residue was purified by preparative TLC on a silica gel plate (Art. 5744, E. Merck) eluted with $CHCl_3$ - MeOH (30 : 1) to give the 6″-*O*-benzoyl compound (97 mg, 89%). A solution of the compound in pyridine (2 ml) and methanesulfonyl chloride (0.0185 ml) was stirred at 40°C for 16 hours and concentrated to dryness. The residue was purified by preparative TLC on a silica gel plate eluted with $CHCl_3$ - MeOH (30 : 1) to yield **20** (92 mg, 89%).

6″-*O*-Benzoyl-1,3″-bis(*N*-Cbz)-3,2′,6′-tris(*N*-Boc)-2″-(Boc)amino-2″,4″-dideoxydibekacin (**21**)

To a solution of **20** (243 mg) in DMF (4.9 ml) was added sodium iodide (1.26 g) and the mixture was stirred at 100°C for 20 hours. After being concentrated, the residue was purified by preparative TLC on a silica gel plate eluted with toluene - Me_2CO - EtOAc (5 : 1 : 1) to give a diastereomeric mixture of the 4″-iodo compound (143 mg, 58%). To a solution of the compound (113 mg) in toluene (5.5 ml) were added tributyltin hydride (0.141 ml) and 2,2′-azobis(isobutyronitrile) (8.5 mg). The mixture was stirred at 100°C for 2 hours and concentrated to dryness. The residue was purified by preparative TLC on a silica gel plate eluted with toluene - Me_2CO - hexane (3 : 1 : 1) to give **21** (95 mg, 93%).

3,2′,6′-Tris(*N*-Boc)-2″-(Boc)amino-2″,4″-dideoxydibekacin (**22**)

To a solution of **21** (109 mg) in MeOH (2.2 ml) was added 28% sodium methoxide in MeOH (0.042 ml) and the mixture was stirred at room temperature for 17 hours. After evaporation, the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with water and concentrated to dryness. The residue was purified by preparative TLC on a silica gel plate eluted with $CHCl_3$ - MeOH (15 : 1) to give the 6″-hydroxyl compound (89 mg). The compound in a mixture (4.9 ml) of 88% HCOOH - MeOH (1 : 19) was hydrogenated with 10% palladium-carbon (162 mg) as a catalyst at room temperature for 2 hours. After removal of the catalyst, the filtrate was concentrated to give **22** (67 mg, 80%) as the diformate. SI-MS *m/z* 835 (MH^+).

3″-(*N*-Cbz)-3,2′,6′-tris(*N*-Boc)-2″-(Boc)amino-2″,4″-dideoxydibekacin (**23**)

To a solution of **22** (67 mg as the diformate) in DMF (2.1 ml) were added a solution of *N*-(Cbz) succinimide (18 mg) in THF (0.7 ml) and triethylamine (0.024 ml) under cooling at −20°C. The mixture was stirred at −20～0°C for 3 hours and concentrated to dryness. The residue was purified by preparative TLC on a silica gel plate ($CHCl_3$-MeOH, 10 : 1) to give the 3″-(*N*-Cbz) compound **23** (41 mg, 58%).

2″-Amino-2″,4″-dideoxyarbekacin (**7**)

To a solution of **23** (41 mg) in DMF (1.2 ml) was added a solution of the active ester, which was prepared from (*S*)-2-hydroxy-4-(*p*-methoxybenzyloxycarbonylamino)butyric acid (41 mg), *N*-hydroxysuccinimide (17 mg) and dicyclohexylcarbodiimide (30 mg) in THF (2 ml), under ice-cooling, and the mixture was stirred in the presence of triethylamine (0.006 ml) at room temperature for 2 hours. The 1-*N*-acyl derivative (44 mg) in the reaction mixture was isolated by preparative TLC on a silica gel plate eluted with $CHCl_3$ - MeOH (20 : 1). The 1-*N*-acyl derivative in a mixture (2.4 ml) of 88% HCOOH - MeOH (1 : 19) was hydrogenated with 10% palladium-carbon as a catalyst at room temperature for 1.5 hours. After removal of the catalyst, the filtrate was concentrated to dryness (36 mg). The residue was dissolved in 90% TFA aq solution (2 ml) under ice-cooling. After being stirred for 1.5 hours, the mixture was concentrated to dryness. Purification by column chromatography on Amberlite CG-50 (NH_4^+) gave **7** (15 mg, 70%); mp 196～198°C (dec), $[\alpha]_D^{19}$ +92° (*c* 0.39, H_2O), IR (KBr) 3430, 1640, 1580, 1490, 1390, 1350, 1120, 1050 and 1020 cm^{-1}. ^1H and ^{13}C NMR: Tables 1 and 2. HR-MS (FAB, positive) Found: *m/z*

536.3405 (MH^+). Calcd for $C_{22}H_{46}N_7O_8$: MH, 536.3408.

2″-Amino-6″-chloro-2″,6″-dideoxyarbekacin (**8**)

To a solution of **16** (295 mg) in a mixture of pyridine (4 ml) and acetonitrile (4 ml) were added triphenylphosphine (186 mg), imidazole (105 mg) and carbon tetrachloride (0.3 ml),[17] and the mixture was stirred at 65°C for 5 hours. After evaporation, the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with 10% $KHSO_4$, $NaHCO_3$-saturated and 10% NaCl aq solution, successively, and concentrated to dryness. Purification by column chromatography on silica gel ($CHCl_3$-MeOH, 40:1) yielded the 6″-chloro compound (266 mg). The compound was dissolved in 90% TFA aq solution (4 ml). After being kept at room temperature for 1.5 hours, the solution was concentrated and washed with ethyl ether. The residue was purified by column chromatography on Amberlite CG-50 (NH_4^+) eluted with 0.4, 1.0 and 1.6% aq ammonia, successively, to give **8** (48 mg, 33%); mp 130~150°C (dec), $[\alpha]_D^{25}$ +88° (*c* 0.8, H_2O), IR (KBr) 3350, 1640, 1580, 1530, 1450, 1380, 1330, 1100 and 1020 cm^{-1}. ^1H and ^{13}C NMR: Tables 1 and 2. HR-MS (FAB, positive) Found: *m/z* 570.3008 (MH^+). Calcd for $C_{22}H_{45}N_7O_8Cl$: MH, 570.3018.

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References

1) UMEZAWA, H.; M. OKANISHI, R. UTAHARA, K. MAEDA & S. KONDO: Isolation and structure of kanamycin inactivated by a cell free system of kanamycin-resistant *E. coli*. J. Antibiotics, Ser. A 20: 136~141, 1967
2) UMEZAWA, H.; M. OKANISHI, S. KONDO, K. HAMANA, R. UTAHARA, K. MAEDA & S. MITSUHASHI: Phosphorylative inactivation of aminoglycosidic antibiotics by *Escherichia coli* carrying R factor. Science 156: 1559~1561, 1967
3) UMEZAWA, H. & S. KONDO: Mechanisms of resistance to aminoglycoside antibiotics. *In* Aminoglycoside Antibiotics. *Eds*., H. UMEZAWA & I. R. HOOPER, pp. 267~292, Springer-Verlag, New York, 1982
4) UMEZAWA, H.; S. UMEZAWA, T. TSUCHIYA & Y. OKAZAKI: 3′,4′-Dideoxykanamycin B active against kanamycin-resistant *Escherichia coli* and *Pseudomonas aeruginosa*. J. Antibiotics 24: 485~487, 1971
5) KONDO, S.; K. IINUMA, H. YAMAMOTO, K. MAEDA & H. UMEZAWA: Syntheses of 1-N-{(S)-4-amino-2-hydroxybutyryl}-kanamycin B and -3′,4′-dideoxykanamycin B active against kanamycin-resistant bacteria. J. Antibiotics 26: 412~415, 1973
6) KONDO, S.; Y. IKEDA, S. HATTORI, M. HAMADA & T. TAKEUCHI: Susceptibility of methicillin-resistant *Staphylococcus aureus* to various antibiotics. Classification by aminoglycoside-modifying enzymes and antibiotics active against MRSA. Jpn. J. Antibiotics 44: 1211~1215, 1991
7) KONDO, S.; A. TAMURA, S. GOMI, Y. IKEDA, T. TAKEUCHI & S. MITSUHASHI: Structures of enzymatically modified products of arbekacin by methicillin-resistant *Staphylococcus aureus*. J. Antibiotics 46: 310~315, 1993
8) UBUKATA, K.; N. YAMASHITA, A. GOTOH & M. KONNO: Purification and characterization of aminoglycoside-modifying enzymes from *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. 25: 754~759, 1984
9) KONDO, S.; S. SHIBAHARA, T. USUI, T. KUDO, A. TAMURA, S. GOMI, Y. IKEDA, D. IKEDA & T. TAKEUCHI: New 2″-amino derivatives of arbekacin, potent aminoglycoside antibiotics against methicillin-resistant *Staphylococcus aureus*. J. Antibiotics 46: 531~534, 1993
10) TSUCHIYA, T.; Y. TAKAGI & S. UMEZAWA: 1-*N*-Acylation of aminocyclitol antibiotics via zinc chelation and regiospecific *N*-trifluoroacetylation. Tetrahedron Lett. 1979: 4951~4954, 1979
11) UMEZAWA, H.; S. UMEZAWA, T. TSUCHIYA, Y. TAKAGI & T. JIKIHARA (Microbial Research Foundation): Preparation of aminoglycoside antibiotics selectively protected the amino groups. Jpn. Pat. 1319 ('88), Jan. 12, 1988
12) PFITZNER, K. E. & J. G. MOFFATT: Sulfoxide-carbodiimide reactions. I. A facile oxidation of alcohols. J. Am. Chem. Soc. 87: 5661~5670, 1965
13) BORCH, R. F.; M. D. BERNSTEIN & H. D. DURST: The cyanohydridoborate anion as a selective reducing agent. J. Am. Chem. Soc. 93: 2897~2904, 1971
14) BARTON, D. H. R. & S. W. MCCOMBIE: A new method for the deoxygenation of secondary alcohols. J. Chem. Soc., Perkin Trans. I. 1975: 1574~1585, 1975

15) HAYASHI, T.; T. IWAOKA, N. TAKEDA & E. OHKI: Deoxysugar synthesis. IV. Deoxygenation of aminoglycoside antibiotics through reduction of their dithiocarbonates. Chem. Pharm. Bull. 26: 1786~1797, 1978
16) MIDDLETON, W. J.: New fluorinating reagents. Dialkylaminosulphur fluorides. J. Org. Chem. 40: 574~578, 1975
17) GAREGG, P. J.; R. JOHANSSON & B. SAMUELSSON: Two reagent systems for converting hydroxy compounds into chlorides using the triphenylphosphine/imidazole system. Synthesis 1984: 168~170, 1984