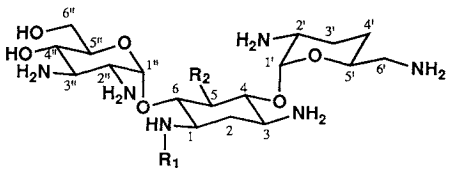
Communication to the Editor

NEW 2″-AMINO DERIVATIVES OF ARBEKACIN, POTENT AMINOGLYCOSIDE ANTIBIOTICS AGAINST METHICILLIN-RESISTANT *Staphylococcus aureus*

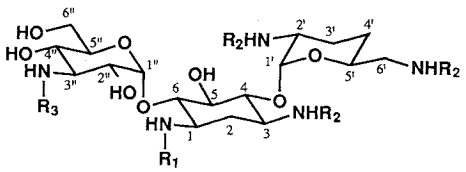
Sir:

At the end of 1990, arbekacin (ABK, 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-3′,4′-dideoxykanamycin B)[1] was launched into Japan as a useful chemotherapeutic agent for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). By 1992 only a few MRSA strains which were moderately resistant to ABK (MIC, 12.5~25 μg/ml) were clinically isolated. In a previous paper,[2] we reported that ABK was modified by reaction with excess of a crude enzyme preparation extracted from ABK-resistant MRSA (25 μg/ml) and ABK 2″-*O*-phosphate was afforded as a major inactivated product along with two minor products, 6′-*N*-acetyl-ABK and the double modified ABK. Based on these results, replacement of the 2″-hydroxyl group by amino group in ABK or in dibekacin (DKB, 3′,4′-dideoxykanamycin B)[3] was designed to obtain potent derivatives against MRSA. Among known aminoglycoside antibiotics, only seldomycin factor 5 isolated from the culture of *Streptomyces hofunensis* contains a 2,3-diamino sugar in the structure.[4] McALPINE and colleagues[5] described that 3′-deoxyseldomycin factor 5 showed a good antibacterial activity. In this communication, we report the synthesis and antibacterial activity of 2″-amino-2″-deoxy-ABK (**1**), 2″-amino-5,2″-dideoxy-ABK (**2**), 2″-amino-2″-deoxy-DKB (**3**) and 2″-amino-5,2″-dideoxy-DKB (**4**).

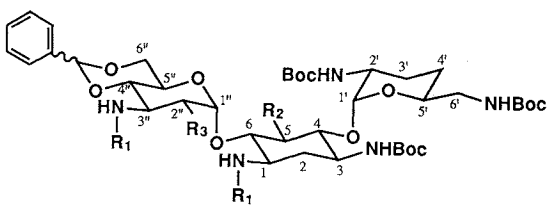
Compounds **1** and **2** were synthesized starting from 3,2′,6′-tris(*N*-*tert*-butoxycarbonyl)-DKB (**5**) which was derived by selective *N*-protection with *tert*-butoxycarbonyl (Boc) group.[6,7] Subsequent blocking of **5** with benzyloxycarbonyl (Cbz) at 1- and 3″-amino groups and with benzylidene between 4″- and 6″-hydroxyl groups afforded **6** in 58% yield. After the 2″-hydroxyl group of **6** was oxidized to 2″-ulose by PFITZNER-MOFFATT oxidation[8] (dimethyl sulfoxide, pyridinium trifluoroacetate and dicyclohexylcarbodiimide), reductive amination[9] of the ulose (ammonium acetate and sodium cyanoborohydride in methanol) exclusively gave **7** having an equatorial 2″-amino group (^{1}H NMR (400 MHz, $CDCl_3$-CD_3OD, 1:1) δ 2.90 (1H, br d, $J_{2'',3''}$ = ~10 Hz, 2″-H). TLC (silica gel, $CHCl_3$-



1 R_1=AHB, R_2=OH
2 R_1=AHB, R_2=H
3 R_1=H, R_2=OH
4 R_1,R_2=H



ABK: R_1=AHB, R_2,R_3=H
DKB: R_1,R_2,R_3=H
5 R_1,R_3=H, R_2=Boc
11 R_1,R_2,R_3=Boc



6 R_1=Cbz, R_2,R_3=OH
7 R_1=Cbz, R^2=OH, R_3=NH_2
8 R_1=Cbz, R_2=OH, R_3=BocNH
9 R_1=H, R_2=OH, R_3=BocNH
10 R_1,R_2=H, R_3=BocNH
12 R_1=Boc, R_2,R_3=OH
13 R_1=Boc, R_2=OH, R_3=NH_2
14 R_1=Boc, R_2=H, R_3=BocNH

(*S*)
AHB: $H_2NCH_2CH_2CH(OH)CO$
Boc: $(CH_3)_3COCO$
Cbz: $C_6H_5CH_2OCO$

MeOH, 20:1) Rf 0.16). The 2″-amino group of **7** was protected with Boc group to give **8** (23% from **6**), $[\alpha]_D^{20}$ +33° (*c* 1, $CHCl_3$), FD-MS *m/z* 1,207 $(M+H)^+$. Removal of the Cbz groups of **8** by hydrogenation with Pd-C afforded **9**. Acylation of **9** with *N*-hydroxysuccinimide ester of (*S*)-4-[(*p*-methoxybenzyl)oxycarbonylamino]-2-hydroxybutyric acid in THF mainly gave a 1-*N*-acylated product. Treatment of the main product with 90% trifluoroacetic acid followed by column chromatography on Amberlite CG-50 (NH_4^+) eluted with aq ammonia provided **1** (23% from **8**), mp 155~160°C (dec), $[\alpha]_D^{20}$ +86° (*c* 0.53, H_2O), SI-MS *m/z* 552 $(M+H)^+$.

The deoxygenation of the 5-hydroxyl group in **8** was performed by the radical elimination of dithiocarbonate[10] [i) carbon disulfide, methyl iodide and sodium hydroxide in dimethyl sulfoxide, ii) tributylstannane and 2,2′-azobis(isobutyronitrile) in toluene]. The Cbz groups of the 5-deoxy product were deprotected by hydrogenation to afford **10** (47% from **8**). By the similar 1-*N*-acylation procedure used for **1** from **9**, compound **10** was converted to **2** (50%), mp 196~198°C (dec), $[\alpha]_D^{20}$ +92° (*c* 0.74, H_2O), FD-MS *m/z* 536 $(M+H)^+$.

Compound **3** was synthesized by the similar route described above starting from 1,3,2′,6′,3″-pentakis-(*N*-Boc)-DKB[11] (**11**). Successive treatment of **11** by *O*-benzylidene protection (compound **12**), oxidation of the 2″-hydroxyl group of **12** and reductive amination gave **13** (43% from **11**), $[\alpha]_D^{18}$ +48° (*c* 0.65, $CHCl_3$-MeOH, 1:1), ^1H NMR (400 MHz, $CDCl_3$-CD_3OD, 1:1) δ 2.90 (1H, dd, $J_{2'',3''}$=~10 Hz, $J_{1'',2''}$=2 Hz, 2″-H), SI-MS *m/z* 1,039 $(M+H)^+$. Removal of the protective groups of **13** followed by column chromatography on Amberlite CG-50 (NH_4^+) gave **3** (99% yield), mp 129~133°C (dec), $[\alpha]_D^{20}$ +122° (*c* 0.39, H_2O), SI-MS *m/z* 451 $(M+H)^+$.

After protection of the 2″-amino group in **13** by Boc group, replacement of the 5-hydroxyl group with sulfuryl chloride in pyridine,[12] followed by reduction with tributylstannane in dioxane gave **14** (87% from **13**). Removal of the protective groups with 90% trifluoroacetic acid followed by column chromatography on Amberlite CG-50 (NH_4^+) gave

Table 1. Minimum inhibitory concentrations of derivatives.

Test organism	Modifying enzyme	MIC (μg/ml)					
		1	**2**	**3**	**4**	ABK	DKB
Staphylococcus aureus FDA209P		0.39	≦0.20	0.78	0.39	0.20	≦0.20
S. aureus Smith		≦0.20	≦0.20	0.39	≦0.20	≦0.20	≦0.20
S. aureus MS16526	APH(2″)/AAC(6′), AAD(4′,4″)	1.56	1.56	100	50	12.5	>100
S. epidermidis 109	AAD(4′,4″)	0.78	0.39	1.56	0.78	0.39	0.78
Bacillus subtilis PCI219		0.20	≦0.20	≦0.20	≦0.20	≦0.20	0.39
B. cereus ATCC 10702		3.13	0.78	3.13	1.56	1.56	1.56
Escherichia coli NIHJ		0.78	0.39	0.78	1.56	0.39	0.39
E. coli K-12 ML1629	APH(3′)-I	3.13	1.56	3.13	6.25	0.78	1.56
E. coli K-12 ML1410		1.56	1.56	1.56	3.13	0.78	0.78
E. coli K-12 LA290 R55	AAD(2″)	0.78	0.78	1.56	3.13	0.39	50
E. coli JR66/W677	APH(3′)-II, AAD(2″)	3.13	3.13	3.13	6.25	1.56	50
Klebsiella pneumoniae PCI602		1.56	0.78	3.13	3.13	0.78	0.78
Shigella dysenteriae JS11910		3.13	1.56	3.13	6.25	1.56	1.56
Salmonella typhi T-63		0.78	0.78	1.56	3.13	0.39	0.39
Proteus vulgaris OX19		1.56	0.78	1.56	1.56	0.78	0.78
Providencia rettgeri GN311		1.56	0.78	1.56	1.56	1.56	0.39
Providencia sp. Pv16	AAC(2′)	3.13	1.56	12.5	12.5	1.56	25
Serratia marcescens		3.13	6.25	12.5	25	12.5	50
Pseudomonas aeruginosa A3		1.56	0.39	1.56	0.78	0.39	0.39
P. aeruginosa H9	APH(3′)-II	6.25	3.13	6.25	12.5	3.13	1.56
P. aeruginosa TI-13	APH(3′)-I	3.13	1.56	3.13	3.13	1.56	1.56
P. aeruginosa GN315	AAC(6′)-4	12.5	50	25	>100	6.25	>100
P. aeruginosa 99	AAC(3)-I	12.5	6.25	6.25	6.25	6.25	3.13
P. aeruginosa 21-75	APH(3′)-III	25	25	>100	>100	12.5	>100
P. aeruginosa PST1	AAC(3)-III	12.5	6.25	50	100	6.25	>100

Table 2. Antibacterial activities against MRSA (50 strains).

Derivative	MIC (μg/ml)		
	Range	50%	90%
1	0.20～3.13	0.78	1.56
2	0.20～3.13	0.78	1.56
3	≦0.20～50	6.25	25
4	0.78～25	3.13	25
ABK	≦0.20～6.25	0.39	6.25
DKB	≦0.20～>100	50	>100

MICs were determined by 2-fold agar dilution method at 37°C for 18 hours using Bacto Mueller Hinton Medium (Difco). MRSA isolated clinically from a hospital at Osaka in 1986～1990 (purchased from Takeda Analytical Research Laboratories Ltd.) were used.

4 (88%), mp 129～131°C (dec), $[\alpha]_D^{20}$ +166° (*c* 0.51, H_2O), SI-MS *m/z* 435 $(M+H)^+$.

Minimum inhibitory concentrations of compounds **1**～**4** by 2-fold agar dilution method on Bacto Mueller Hinton Medium (Difco) are shown in Table 1. All 2″-amino derivatives exhibited excellent antibacterial activities and 5-deoxy derivative **2** was strongly active as similar to 5-deoxy-ABK.[13] Furthermore, activities of **1** and **2** against both APH(2″)/ACC(6′)- and AAD(4′,4″)-producing MRSA[2,14] (MS16526 strain) were markedly improved. Compounds **1** and **2** showed the most potent antibacterial activity against 50 strains of clinically isolated MRSA (Table 2). Replacement of hydroxyl group by amino group at C-2″ in DKB and in ABK enhanced anti-MRSA activity as expected. Single intravenous administration of **1** or **3** at 100 mg/kg caused no death in mice. While, LD_{50} values of **2** and **4** were 50～100 mg/kg.

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References

1) KONDO, S.; K. IINUMA, H. YAMAMOTO, K. MAEDA & H. UMEZAWA: Syntheses of 1-N-{(S)-4-amino-2-hydroxybutyryl}-kanamycin B and -3′,4′-dideoxy-kanamycin B active against kanamycin-resistant bacteria. J. Antibiotics 26: 412～415, 1973
2) 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
3) UMEZAWA, H.; S. UMEZAWA, T. TSUCHIYA & Y. OKAZAKI: 3′,4′-Dideoxy-kanamycin B active against kanamycin-resistant *Escherichia coli* and *Pseudomonas aeruginosa*. J. Antibiotics 24: 485～487, 1971
4) MCALPINE, J. B.; A. C. SINCLAIR, R. S. EGAN, R. L. DE VAULT, R. S. STANASZEK, M. CIROVIC, S. L. MUELLER, P. C. GOODLEY, R. J. MAURITZ, N. E. WIDEBURG, L. A. MITSCHER, K. SHIRAHATA, H. MATSUSHIMA, S. SATO & T. IIDA: A new aminoglycoside antibiotic complex—the seldomycins. IV. The structure of seldomycin factor 5. J. Antibiotics 30: 39～49, 1977
5) MCALPINE, J. B.; R. E. CARNEY, R. L. DEVAULT, A. C. SINCLAIR, R. S. EGAN, M. CIROVIC, R. STANASZEK & S. MUELLER: The effects of *O*-methylation on the activity of aminoglycosides. *In* Aminocyclitol Antibiotics. *Ed.*, K. L. RINEHART, Jr. & T. SUAMI, pp. 183～196, American Chemical Society, 1980
6) 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
7) UMEZAWA, H.; S. UMEZAWA, T. TSUCHIYA, Y. TAKAGI & T. JIKIHARA (Microbial Chemistry Research Foundation): Preparation of aminoglycoside antibiotics selectively protected the amino groups. Jpn. Pat. 1319 ('88), Jan. 12, 1988
8) PFITZNER, K. E. & J. G. MOFFATT: Sulfoxide-carbodiimide reactions. I. A facile oxidation of alcohols. J. Am. Chem. Soc. 87: 5661～5670, 1965
9) 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
10) 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
11) MIYASAKA, T.; D. IKEDA, S. KONDO & H. UMEZAWA: Syntheses and properties of the 6″-deoxy or 4″,6″-dideoxy derivatives of the kanamycin antibio-

tics. J. Antibiotics 33: 527~532, 1980

12) SUAMI, T.; S. NISHIYAMA, Y. ISHIKAWA & E. UMEMURA: Modification of aminocyclitol antibiotics. 6. Preparation of 5-deoxykanamycin B. Bull. Chem. Soc. Jpn. 51: 2354~2357, 1978

13) UMEZAWA, H.; T. MIYASAKA, H. IWASAWA, D. IKEDA & S. KONDO: Chemical modification of 5,3',4'-trideoxykanamycin B. J. Antibiotics 34: 1635~1640, 1981

14) 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