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Effect of varying the 4″-position of arbekacin derivatives on antibacterial activity against MRSA and *Pseudomonas aeruginosa*

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Abstract—4″-Deoxy-4″-episubstituted arbekacin derivatives and 4″-epi-5-deoxy-5-episubstituted arbekacin derivatives were designed and synthesized. Arbekacin and 4″-epiarbekacin both displayed the same antibacterial activity against *Staphylococcus aureus* (including methicillin-resistant *S. aureus* (MRSA)) and *Pseudomonas aeruginosa*. The 4″-epi-5-deoxy-5-episubstituted arbekacin derivatives showed potent antibacterial activity. Among them, the antibacterial activity of 5,4″-diepiarbekacin was superior to that of arbekacin or 5-episubstituted arbekacin against Gram-positive and Gram-negative bacteria. The 6′-*N*-methyl derivative of the 5,4″-diepiarbekacin was effective against *P. aeruginosa* expressing an aminoglycoside-modifying enzyme AAC(6′)-Ib.

Infections caused by antibiotic-resistant strains of methicillin-resistant *Staphylococcus aureus* (MRSA), including vancomycin-resistant strains and the multi-drug-resistant *Pseudomonas aeruginosa*, are now a significant problem in clinical practice. The aminoglycoside antibiotics, such as arbekacin (ABK), tobramycin, amikacin and gentamicin, are widely used in the treatment of serious infection caused by Gram-positive and/or Gram-negative bacteria.[1] However, the emergence of aminoglycoside-resistant bacteria has recently been observed. Studies on clinical isolates have shown that structural modification of the aminoglycosides by aminoglycoside-modifying enzymes (AMEs), such as aminoglycoside acetyltransferase (AAC), aminoglycoside adenylyltransferase (AAD) and aminoglycoside phosphotransferase (APH), is the most common mechanism of resistance to these antibiotics.[1] MRSA is known to possess the bifunctional aminoglycoside-modifying enzyme AAC(6′)-APH (2″)[2] and AAD(4′).[3] The other common mechanism of resistance to aminoglycoside antibiotics is efflux systems of *P. aeruginosa*. The resistance nodulation division (RND) efflux systems of MexAB-OprM and MexXY/OprM are well-known examples of such an efflux system. Recently, it has been reported that aminoglycoside antibiotics are effluxed by MexXY/OprM of RND efflux system.[4]

Previously, we reported that 5-deoxy-5-episubstituted arbekacin (ABK) showed potent antibacterial activities against *S. aureus* including MRSA expressing AAC(6′)-APH (2″) and AAD(4′).[5] Interestingly, introduction of 5-axial hydroxyl group resulted in increased stability against AAC(6′)-APH (2″) and AAD (4′). AAD(4′) usually modifies both 4′-OH and 4″-OH groups of aminoglycosides.[3] In an attempt to identify novel aminoglycosides with efficient antibacterial activity, we have undertaken to study the relationship between the stereochemistry of the 4″-OH group and

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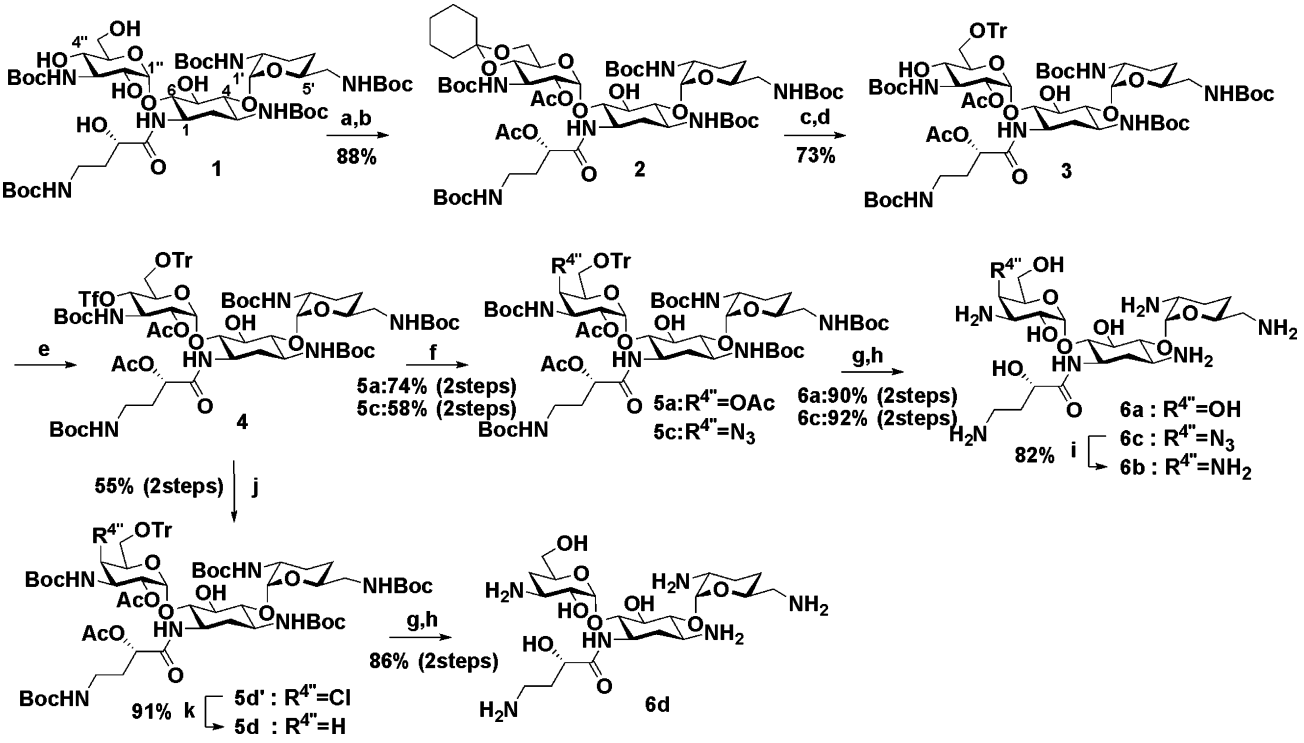
antibacterial activity. In this paper, we describe the synthesis of 4″-deoxy-4″-episubstituted ABK derivatives and 5-deoxy-5-episubstituted-4″-epi ABK derivatives, which have 5,4″-diaxial substitutions, and the corresponding antibacterial activity against *S. aureus* including MRSA and *P. aeruginosa*. Synthesis of the 4″-deoxy-4″-episubstituted ABK derivatives from penta-*N*-*tert*-butoxycarbonyl (Boc) ABK[5] is shown in Scheme 1.

Compound **2** was obtained from penta-*N*-Boc ABK **1** via selective protection of the 4″-OH and 6″-OH groups with cyclohexanone dimethyl acetal and then acetylation of the 2″-OH and 2‴-OH groups. Deprotection of cyclohexylidene group of **2** followed by selective protection of the 6″-OH group with triphenylmethyl chloride (TrCl) gave derivative **3**. Treatment of **3** with trifluoromethanesulfonic anhydride afforded **4**. Treatment of **4** with CsOAc or NaN_3, followed by removal of the Ac, Boc and Tr protecting groups, gave the 4″-epi ABK **6a** or 4″-deoxy-4″-epiazide ABK **6c**, respectively. Hydrogenolysis of the azide group of **6c** afforded 4″-deoxy-4″-epiamino ABK **6b**. Treatment of **4** with LiCl gave 4″-epichloro derivative **5d′**, followed by reduction of 4″-chloro group which gave the 4″-deoxy derivative **5d**. Stepwise removal of the Ac, Boc and Tr groups afforded 4″-deoxy ABK **6d**. The antibacterial activities of 4″-deoxy-4″-episubstituted ABK derivatives are shown in Table 1.[6]

The 4″-epi ABK **6a** showed potent antibacterial activities against *S. aureus* including MRSA and *P. aeruginosa* equivalent to that of ABK. In contrast, the antibacterial activities of 4″-deoxy-4″-epiamino ABK **6b**, 4″-deoxy-4″-epiazide ABK **6c** and 4″-deoxy ABK **6d** were



Scheme 1. Reagents and conditions: (a) cyclohexanone dimethyl acetal, *p*-toluenesulfonic acid monohydrate, DMF, 45–48 mmHg, 50 °C; (b) Ac_2O, pyridine, rt; (c) 90% TFA, MeOH, CH_2Cl_2, rt; (d) TrCl, DMAP, pyridine, 80 °C; (e) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (f) CsOAc, DMF, rt (**5a**); NaN_3, DMF, rt (**5c**); (g) NaOMe, MeOH, CH_2Cl_2, rt; (h) 90% TFA, rt; (i) H_2, 10% Pd–C, H_2O, rt; (j) LiCl, DMF, rt; (k) AIBN, Bu_3SnH, 1,4-dioxane, 80 °C.

Table 1. Minimum inhibitory concentrations (MICs) of 4″-deoxy-4″-epiderivatives of ABK

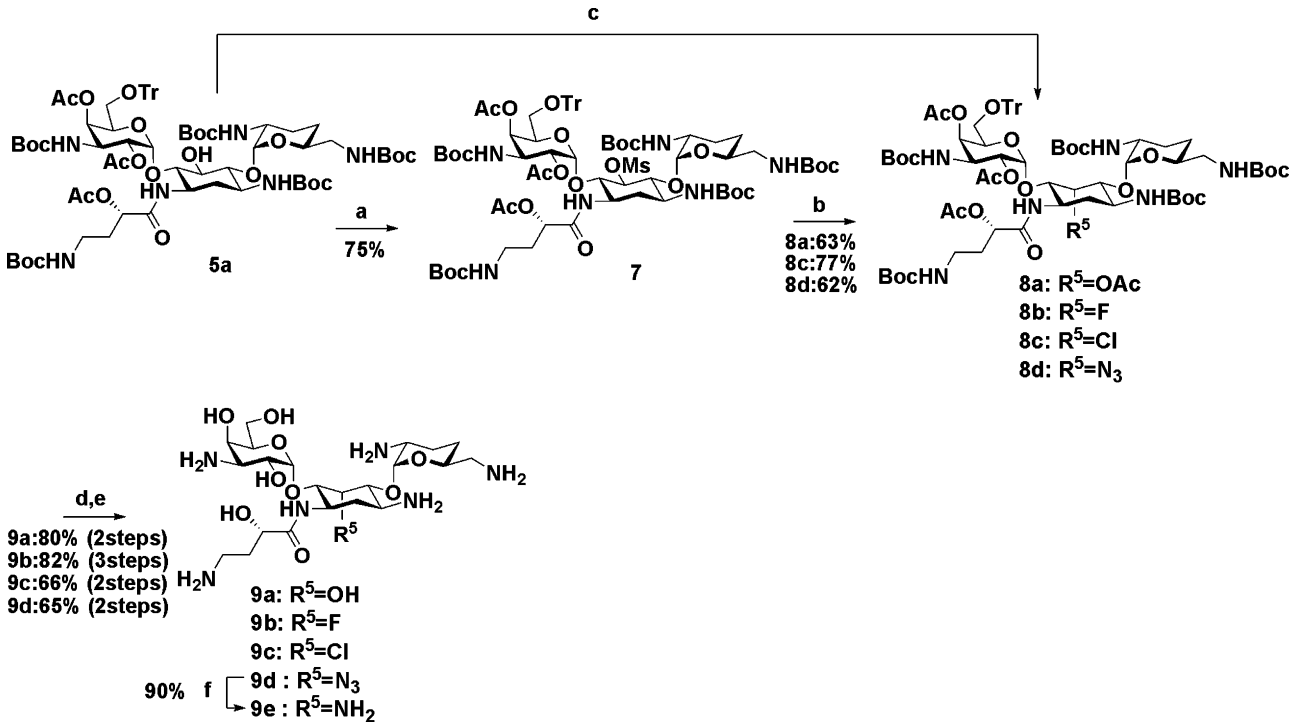
Test organism	MIC (μg/ml)				
	6a	**6b**	**6c**	**6d**	**ABK**
Staphylococcus aureus 209P JC-1	0.13	4	0.13	0.13	0.06
S. aureus MF490 (MRSA)[a]	32	>128	>128	>64	64
S. aureus MSC03571 (MRSA)[a]	16	>128	128	64	16
Escherichia coli NIH JC-2	1	16	2	2	1
Pseudomonas aeruginosa PAO1	4	64	16	4	2
P. aeruginosa N101 (Δ*mexXY* of PAO1)	0.25	8	0.5	0.5	0.25
P. aeruginosa GN315[b]	32	>128	64	32	16

[a] Possessing AAD(4′) and AAC(6′)-APH(2″).
[b] Possessing AAC(6′)-Ib.

markedly less potent. With reference to the influence of the efflux system of *P. aeruginosa*, 4″-deoxy-4″-epiazide ABK **6c** displayed more potent activity (32-fold higher activity) against *P. aeruginosa* N101 (Δ*mexXY*/*oprM*-PAO1)[7] over that of parent strain *P. aeruginosa* PAO1. These results indicated that 4″-deoxy-4″-epiazide ABK **6c** was the most sensitive substrate against the *mexXY*/*oprM* tripartite efflux system of *P. aeruginosa*. In summary, the 4″-epi ABK **6a** was superior to the other 4″-deoxy-4″-episubstituted ABK derivatives **6b**–**6d**. Based on these results, we planned the synthesis of the hybrid compounds of 5-deoxy-5-episubstituted-4″-epi ABK derivatives. Synthesis of the 5-deoxy-5-episubstituted-4″-epi ABK derivatives is shown in Scheme 2.

Treatment of **5a** with (diethylamino)sulfur trifluoride (DAST) gave 5-epifluoride derivative **8b**. Mesylation of **5a** followed by substitution of mesylate **7** with CsOAc, LiCl or NaN_3 gave 5-deoxy-5-episubstituted derivatives **8a**, **8c** and **8d**, respectively. Removal of the Ac groups, Boc groups and Tr groups of **8a**, **8b**, **8c** and **8d** afforded 5-deoxy-5-episubstituted-4″-epi ABK **9a**, **9b**, **9c** and **9d**. Further, reduction of azido group of **9d** gave **9e**. The antibacterial activities of 5-deoxy-5-episubstituted-4″-epi ABK derivatives are shown in Table 2.

These hybrid compounds showed more potent antibacterial activity than 4″-deoxy-4″-episubstituted ABK derivatives and ABK against *S. aureus* including MRSA as expected. The antibacterial activity of 5,4″-diepi ABK **9a** was superior to that of arbekacin or 5-deoxy-5-epi-substituted arbekacin against Gram-positive and Gram-negative bacteria. These results suggested that introduction of axial substituents at 5 and 4″ positions of aminoglycoside led to increased stability against the aminoglycoside-modifying enzymes AAC(6′)-APH(2″) and AAD(4′). In our previous report, we described that

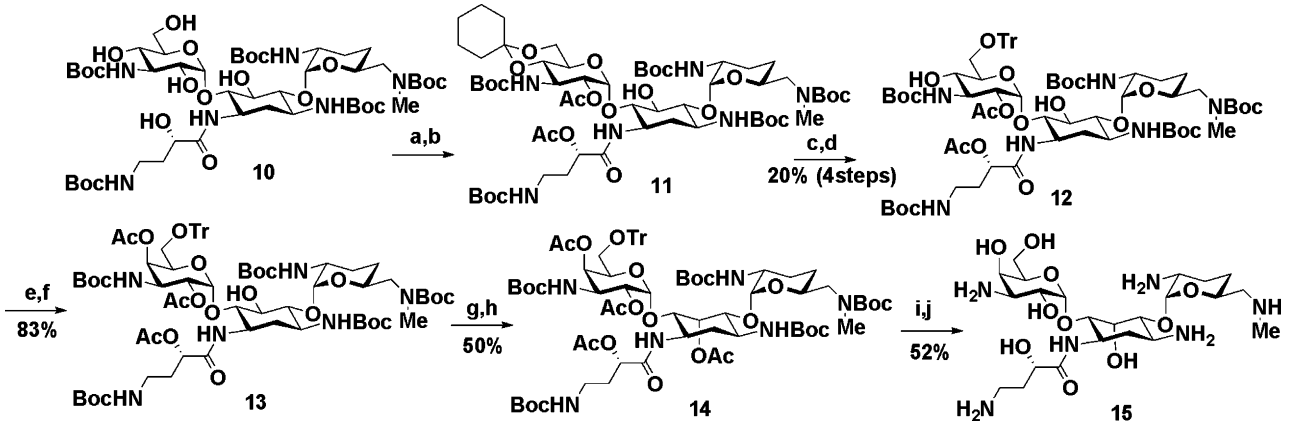


Scheme 2. Reagents and conditions: (a) MsCl, DMAP, CH_2Cl_2, rt; (b) CsOAc, DMF, 100 °C (**8a**); LiCl, DMF, 100 °C (**8c**); NaN_3, DMF, 80 °C (**8d**); (c) DAST, CH_2Cl_2, rt (**8b**); (d) NaOMe, MeOH, rt; (e) 90% TFA, rt; (f) H_2, 10% Pd–C, H_2O, rt.

Table 2. Minimum inhibitory concentrations (MICs) of 5-deoxy-5-episubstituted-4″-epiderivatives of ABK

Test organism	MIC (μg/ml)							
	9a	**9b**	**9c**	**9d**	**9e**	**15**	**ABK**	**5-epiABK**
S. aureus 209P JC-1	0.06	0.06	0.13	0.25	0.25	0.13	0.06	0.06
S. aureus MF490 (MRSA)[a]	1	4	2	4	4	4	64	2
S. aureus MSC03571 (MRSA)[a]	1	2	2	4	4	1	16	2
E. coli NIH JC-2	0.5	1	1	1	2	1	1	1
P. aeruginosa PAO1	1	2	4	8	4	2	2	2
P. aeruginosa N101 (Δ*mexXY* of PAO1)	0.25	0.5	0.5	0.5	1	0.5	0.25	0.25
P. aeruginosa GN315[b]	16	32	>32	128	>64	4	16	8

[a] Possessing AAD(4′) and AAC(6′)-APH(2″).
[b] Possessing AAC(6′)-Ib.



Scheme 3. Reagents and conditions: (a) cyclohexanone dimethyl ketal, *p*-toluenesulfonic acid monohydrate, DMF, 45–48 mmHg, 50 °C; (b) Ac_2O, pyridine, rt; (c) 90% TFA, MeOH, CH_2Cl_2, rt; (d) TrCl, DMAP, pyridine, 80 °C; (e) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (f) CsOAc, DMF, rt; (g) MsCl, DMAP, CH_2Cl_2, rt; (h) CsOAc, DMF, 100 °C; (i) NaOMe, MeOH, rt; (j) 90% TFA, rt.

the 6′-*N*-substituted derivatives showed potent antibacterial activity against *P. aeruginosa* expressing aminoglycoside-modifying enzyme AAC(6′).[5] Therefore, to investigate the antibacterial activity and the stability against aminoglycoside-modifying enzymes of the 5,4″-diepi ABK **9a**, we planned the synthesis of 6′-*N*-methyl derivatives of **9a**. Synthesis of the 5,4″-diepi-6′-*N*-methyl ABK **15** is shown in Scheme 3.

Treatment of the compound **10**[5] with cyclohexanone dimethyl acetal and then acetylation of the 2″-OH and 2‴-OH groups afforded compound **11**. Then, in a similar procedure to that given in Schemes 1 and 2, compound **14** was obtained from **11** in six steps. Removal of Ac groups, Tr groups and Boc groups of **14** afforded 5,4″-diepi-6′-*N*-methyl ABK **15** in two steps. The antibacterial activity of 5,4″-diepi-6′-*N*-methyl ABK **15** is shown in Table 2. As expected, the 5,4″-diepi-6′-*N*-methyl ABK **15** displayed potent antibacterial activity against *P. aeruginosa* GN315 expressing aminoglycoside-modifying enzyme AAC(6′)-Ib. Our findings indicate that 6′-*N*-methylation of an aminoglycoside results in increased stability against the aminoglycoside-modifying enzyme AAC(6′). Furthermore, we tested the antibacterial activity of the 5,4″-diepi ABK **9a** against 54 clinical isolates of MRSA and 54 clinical isolates of *P. aeruginosa*. Using the MRSA strains, the MIC_{50} and MIC_{90} of **9a** (MIC_{50}, 0.25 μg/mL, MIC_{90}, 0.25 μg/mL) were more potent than those of ABK (MIC_{50}, 0.5 μg/mL, MIC_{90}, 2.0 μg/mL). Against the *P. aeruginosa* strains, antibacterial activity of **9a** (MIC_{50}, 1.0 μg/mL, MIC_{90}, 4.0 μg/mL) was superior to that of ABK (MIC_{50}, 2.0 μg/mL, MIC_{90}, 16 μg/mL).

In summary, we designed and synthesized 4″-deoxy-4″-episubstituted ABK derivatives, hybrid compounds of 5-deoxy-5-episubstituted-4″-epi ABK derivatives and 6′-*N*-methyl derivative of 5,4″-diepi ABK. Among them, the 5,4″-diepi ABK showed potent antibacterial activity against both MRSA and *P. aeruginosa*. This study should provide a basis for the development of novel aminoglycosides directed against clinically relevant strains of bacteria that are recalcitrant to antibiotic treatment.

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