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Synthesis and antibacterial activity of 5-deoxy-5-episubstituted arbekacin derivatives

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Abstract—5-Deoxy-5-episubstituted arbekacin derivatives have been designed and efficiently synthesized. The synthetic compounds showed potent antibacterial activity against both *Staphylococcus aureus*, including methicillin-resistant *S. aureus*, and *Pseudomonas aeruginosa*. In particular, these derivatives were superior to arbekacin against MRSA strains expressing the bifunctional aminoglycoside-modifying enzyme AAC(6′)-APH(2″). The antibacterial activity of the 5-deoxy-5-episubstituted arbekacin derivatives against *Pseudomonas aeruginosa* was markedly influenced by the efflux system of MexXY/OprM. The 6′-*N*-methyl derivative of the 5-epi arbekacin was effective against *Pseudomonas aeruginosa* expressing the aminoglycoside-modifying enzyme AAC(6′).

In recent years, infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA), including vancomycin-resistant strains, and the multidrug-resistant *Pseudomonas aeruginosa*, which has resistance against carbapenems and quinolones, has become a significant clinical problem.

The aminoglycoside antibiotics, represented by arbekacin (ABK), tobramycin, and amikacin, have been widely used as the clinically significant agents because of their potent antibacterial activities against both Gram-positive and Gram-negative bacteria.[1] However the resistance to aminoglycoside antibiotics is caused by aminoglycoside-modifying enzymes such as aminoglycoside acetyltransferase (AAC), aminoglycoside adenylyltransferase (AAD), and aminoglycoside phosphotransferase (APH)[1] has been reported. Furthermore, the MRSA strains expressing the bifunctional aminoglycoside-modifying enzyme AAC(6′)-APH(2″) have been found in clinical isolates.[2] On the other hand, efflux systems in *P. aeruginosa*, such as the tripartite resistance nodulation division (RND) efflux systems, have a role in resistance to various antibiotics. Thus, it has been recently recognized that aminoglycoside antibiotics are effluxed by the MexXY/OprM of the RND efflux system.[3]

There are several approaches to the development of a new class of aminoglycoside antibiotics that show antibacterial activity against resistant bacteria.[4] Among them, 5-deoxy-5-episubstituted aminoglycosides such as 5-epi sisomicin,[5] 5-epi isepamycin,[6] and 5-deoxy-5-epifluoro arbekacin[7] have been reported to show potent antibacterial activity against resistant Gram-positive and Gram-negative bacteria. Therefore, to overcome infection caused by MRSA and *P.aeruginosa*, we undertook synthesis of the 5-deoxy-5-episubstituted ABK derivatives **3a**–**g** and tested them for antibacterial activity, including their stability against aminoglycoside-modifying enzymes and the influence of the MexXY/OprM efflux pump.

Synthesis of 5-deoxy-5-episubstituted ABK derivatives from ABK[8] is shown in Scheme 1. The amino groups

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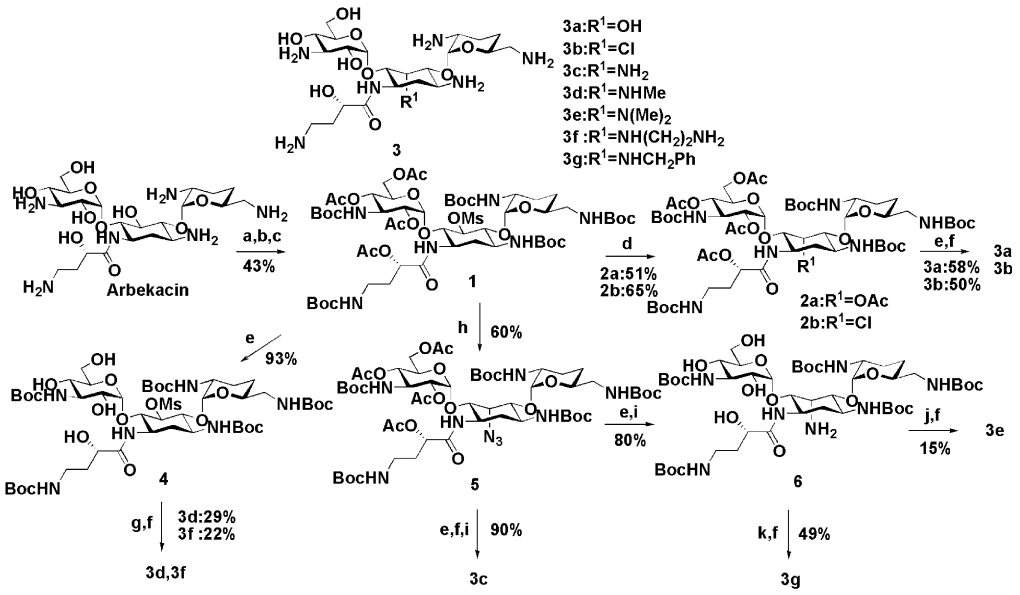


Scheme 1. Synthesis of 5-deoxy-5-epi arbekacin derivatives. Reagents and conditions: (a) $(Boc)_2O$, Et_3N, DMF, H_2O, rt; (b) Ac_2O, pyridine, rt; (c) MsCl, DMAP, CH_2Cl_2, rt; (d) CsOAc, DMF, 100 °C, (**2a**); or LiCl, DMF, 100 °C, (**2b**); (e) NaOMe, MeOH, CH_2Cl_2, rt; (f) 90% TFA, rt; (g) $MeNH_2$, MeOH, sealed tube, 60 °C, (**3d**); or $NH_2(CH_2)_2NH_2$, sealed tube, DMF, 80 °C, (**3f**); (h) NaN_3, DMF, 100 °C; (i) H_2, 10% Pd–C, H_2O, rt; (j) formaldehyde, Et_3N, MeOH, 1,4-dioxane, rt, then $NaBH_4$, MeOH, 1,4-dioxane, rt; (k) benzyl bromide, K_2CO_3, DMF, rt.

of ABK were protected with *t*-butoxycarbonyl (Boc) groups, and then the hydroxy groups, except for 5-OH, were protected with acetyl (Ac) groups to provide the penta-*N*-Boc-tetra-*O*-Ac ABK derivative, which was subsequently treated with methansulfonyl chloride (MsCl) to afford **1**. Treatment of **1** with CsOAc or LiCl, and then removal of the Ac and Boc protecting groups of the resulting compounds **2a** or **2b** gave the 5-epi ABK **3a** or 5-deoxy-5-epichloro ABK **3b**, respectively. Treatment of **1** with NaN_3 gave the 5-epiazide derivative **5**. Stepwise removal of the Ac and Boc groups of **5** and then reduction of the azide group to an amino group gave the 5-deoxy-5-epiamino ABK **3c**.

Next, we tried to introduce substituted amino groups at the C-5 position of ABK. Removal of the Ac groups of compound **1** by NaOMe in MeOH–CH_2Cl_2 gave **4**. Efficient introduction of substituted amino groups at the hindered C-5 position of **4** was achieved by treatment with $MeNH_2$ or $H_2NCH_2CH_2NH_2$ at 60 or 80 °C in a sealed tube, which followed by removal of the Boc groups gave **3d** or **3f**, respectively. Alternatively, compound **5** was transformed to compound **6** by removal of the Ac groups and reduction of the C-5 azide group to an amino group. Methylation of the 5-NH_2 of **6** with formaldehyde in the presence of $NaBH_4$, followed by removal of the Boc groups gave a mixture of the 5-deoxy-5-epidimethylamino ABK **3e**, 5-deoxy-5-epimethylamino ABK, and 5-deoxy-5-epiamino ABK. The desired product **3e** could be readily purified by column chromatography on CM Sephadex (NH_4^+ form). Treatment of **6** with benzyl bromide in the presence of K_2CO_3 and then removal of the Boc groups afforded **3g**.

The antibacterial activities of the 5-deoxy-5-episubstituted ABK derivatives are shown in Table 1.[13]

Compounds **3a–c** and **3f** were comparable with ABK in antibacterial activity against *S. aureus* 209P JC-1. Further, these compounds showed more potent antibacterial activity than ABK, against MRSA expressing the aminoglycoside-modifying enzymes AAD(4′) and

Table 1. Antibacterial activities of compounds **3a–3g**

Test organism	MIC (μg/ml)							
	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	ABK
Staphylococcus aureus 209P JC-1	0.06	0.1	0.13	0.25	1	0.13	0.13	0.06
S. aureus MF490 (MRSA)[a]	2	3.13	2	4	32	2	8	64
S. aureus MSC03571 (MRSA)[a]	2	6.25	4	16	>64	4	32	16
Escherichia coli NIH JC-2	1	1.56	2	2	16	4	4	1
Pseudomonas aeruginosa PAO1	2	2	2	4	32	2	32	2
P. aeruginosa GN315[b]	8	50	16	128	>32	64	128	16
P. aeruginosa N101(Δ*mexXY* of PAO1)	0.25	0.5	0.5	NT[c]	NT	NT	1	0.25

[a] Possessing AAD(4′) and AAC(6′)-APH(2″).
[b] Possessing AAC(6′)-Ib.
[c] NT, not tested.

AAC(6′)-APH(2″). By contrast, the 5-*N*-substituted derivatives **3e** and **3g** showed less potent activity against *S. aureus* and *P. aeruginosa* as compared with the 5-deoxy-5-epiamino ABK **3c**. The compounds **3a**–**c** and **3f** showed potent activity against *P. aeruginosa* PAO1 equal to that of ABK.

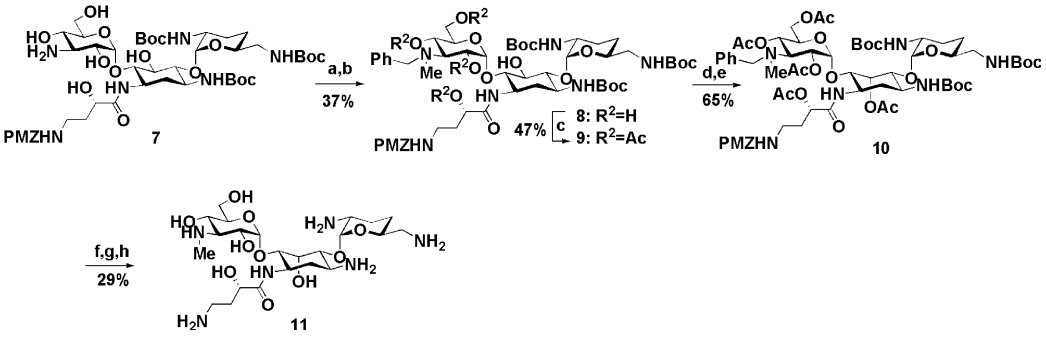
Next, we investigated the effect of the efflux system of *P. aeruginosa* by comparing the MICs of the compounds for *P. aeruginosa* PAO1 and for *P. aeruginosa* N101 (Δ mexXY/*oprM* PAO1).[9] Interestingly, **3a**–**c**, **3g**, and ABK showed increased activity (4- to 32-fold higher activity) against *P. aeruginosa* N101 than *P. aeruginosa* PAO1. It is noteworthy that, out of the derivatives, the lipophilic 5-deoxy-5-epi-*N*-benzyl derivative **3g** was the most susceptible to elimination via the MexXY/OprM tripartite efflux system of *P. aeruginosa* PAO1. These results seem to indicate that the MexXY/OprM efflux system is a major mechanism in the resistance of *P. aeruginosa* against 5-deoxy-5-episubstituted ABK derivatives and ABK.

Furthermore, to investigate 6′[10]- or 3″-*N*-substituted derivatives of the 5-deoxy-5-episubstituted ABK derivatives for antibacterial activity and stability against aminoglycoside-modifying enzymes, we focused on the 5-epi ABK **3a** that has the most potent antibacterial activity. We synthesized the 6′- and 3″-*N*-methyl derivatives of **3a** and measured their antibacterial activity. Synthesis of the 3″-*N*-methyl derivative **11** from **7**[11] is shown in Scheme 2.

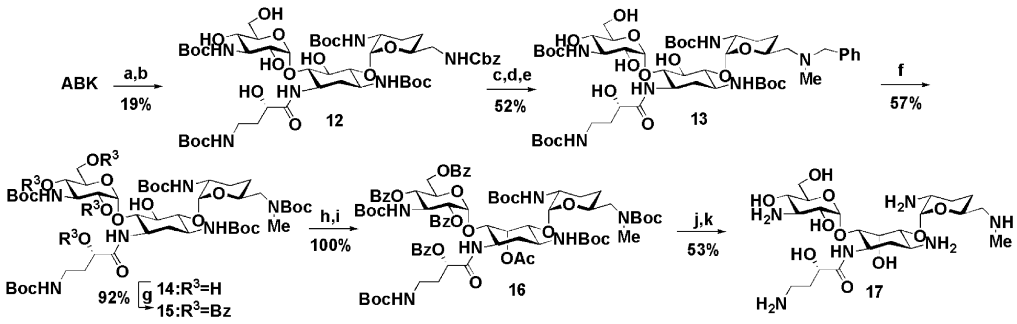
Stepwise reductive alkylations of the 3″-NH_2 of **7** with benzaldehyde and then with formaldehyde afforded **8**. Regioselective acetylation of all of the hydroxyl groups of **8**, except for 5-OH, provided **9**. Treatment of **9** with MsCl and then reaction with CsOAc gave the 5-epiacetoxy derivative **10**. Stepwise removal of the Boc, Ac, *p*-methoxybenzyloxycarbonyl (PMZ), and Benzyl protecting groups of **10** in three steps afforded **11**.

Synthesis of the 6′-*N*-methyl derivative **17** from ABK is shown in Scheme 3.

Regioselective protection[12] of the 6′-NH_2 of ABK with *N*-(benzyloxycarbonyloxy)succinimide in the presence of $Zn(OAc)_2$ gave 6′-*N*-benzyloxycarbonyl (6′-*N*-Cbz) ABK in 33% yield after column chromatography on Amberlite CG 50 (NH_4^+ form). Treatment of 6′-*N*-Cbz ABK with $(Boc)_2O$ afforded **12**. The Cbz group of **12** was removed by hydrogenolysis with 10% Pd–C to provide the 6′-NH_2 derivative. Stepwise reductive alkylations of the 6′-NH_2 with benzaldehyde and formaldehyde gave **13**. Removal of the benzyl group of **13**, followed by Boc protection of the 6′-NHMe group, afforded **14**, which was regioselectively benzoylated to



Scheme 2. Synthesis of 3″-*N*-methyl-5-epi arbekacin. Reagents and conditions: (a) benzaldehyde, Et_3N, MeOH, 1,4-dioxane, rt, then $NaBH_4$, MeOH, 1,4-dioxane, rt; (b) formaldehyde, Et_3N, MeOH, 1,4-dioxane, rt, then $NaBH(OAc)_3$, MeOH, 1,4-dioxane, rt; (c) Ac_2O, pyridine, rt; (d) MsCl, DMAP, CH_2Cl_2, rt; (e) CsOAc, DMF, 100 °C; (f) NaOMe, MeOH, CH_2Cl_2, rt; (g) 90% TFA, rt; (h) H_2, 10% Pd–C, H_2O, rt.



Scheme 3. Synthesis of 6′-*N*-methyl-5-epi arbekacin. Reagents and conditions: (a) $Zn(OAc)_2$, *N*-(benzyloxycarbonyloxy)succinimide, H_2O, rt; (b) $(Boc)_2O$, Et_3N, MeOH, 1,4-dioxane, rt; (c) H_2, 10% Pd–C, 1,4-dioxane, H_2O, rt; (d) benzaldehyde, Et_3N, MeOH, 1,4-dioxane, rt, then $NaBH_4$, MeOH, 1,4-dioxane, rt; (e) formaldehyde, Et_3N, MeOH, 1,4-dioxane, rt, then $NaBH_4$, MeOH, 1,4-dioxane, rt; (f) H_2, Pd–C, 1,4-dioxane, H_2O, rt, then $(Boc)_2O$, Et_3N, MeOH, 1,4-dioxane, H_2O, rt; (g) benzoyl chloride, pyridine, 0 °C; (h) MsCl, DMAP, CH_2Cl_2, rt; (i) CsOAc, DMF, 100 °C; (j) NaOMe, MeOH, CH_2Cl_2, rt; (k) 90% TFA, rt.

Table 2. Antibacterial activities of compounds **11** and **17**

Test organism	MIC (μg/ml)			
	11	**17**	**3a**	ABK
Staphylococcus aureus 209P JC-1	0.13	0.13	0.06	0.06
S. aureus MF490 (MRSA)[a]	4	32	2	64
S. aureus MSC03571 (MRSA)[a]	16	8	2	16
Escherichia coli NIH JC-2	2	1	1	1
Pseudomonas aeruginosa PAO1	2	4	2	2
P. aeruginosa GN315[b]	32	4	8	16

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

give **15**. Treatment of **15** with MsCl provided the 5-OMs derivative and then reaction with CsOAc gave the 5-epi-acetoxy derivative **16**. Removal of the Boc, Ac, and benzoyl groups of **16** in two steps provided the 6′-*N*-methyl derivative **17**.

The antibacterial activities of **11** and **17** are shown in Table 2.[13]

As expected, the 6′-*N*-methyl derivative **17** was slightly more active than the 6′-NH_2 derivative **3a** against *P. aeruginosa* GN315 expressing the aminoglycoside-modifying enzyme AAC(6′)-Ib. However, **17** showed less potent antibacterial activity against *S. aureus* strains MF490 and MSC03571 expressing AAC(6′)-APH(2″) and AAD(4′), as compared with **3a**. On the other hand, introduction of a methyl group at the 3″-NH_2 resulted in decreased activity against *S. aureus* and *P. aeruginosa* (compound **11**). These results indicated that introduction of a methyl group at the 6′-NH_2 of 5-deoxy-5-episubstituted derivatives led to enhanced stability against the aminoglycoside-modifying enzyme AAC(6′)-Ib.

Finally, we tested the antibacterial activity of the 5-deoxy-5-episubstituted derivatives **3a** and **3c** against 54 clinical isolates of MRSA. Against these 54 strains, the MIC_{50} and MIC_{90} of **3a** (MIC_{50}, 0.5 μg/mL, MIC_{90}, 1.0 μg/mL) and **3c** (MIC_{50}, 0.5 μg/mL, MIC_{90}, 0.5 μg/mL) indicated that these compounds were more potent than ABK (MIC_{50}, 1.0 μg/mL, MIC_{90}, 2.0 μg/mL).[13]

In summary, we have designed and synthesized several 5-deoxy-5-episubstituted ABK derivatives in order to investigate the novel aminoglycoside antibiotic agents having the antibacterial activities against aminoglycoside resistant bacteria. The compounds **3a**–**c** and **3f** showed good antibacterial activity against *S. aureus* and *P. aeruginosa*. In particular, the 5-epi ABK **3a** and the 5-deoxy-5-epiamino ABK **3c** showed potent activity against *S. aureus*, including MRSA expressing the bifunctional aminoglycoside-modifying enzyme AAC(6′)-APH(2″). Introduction of a methyl group at the 6′-NH_2 of **3a** led to enhanced activity against *P. aeruginosa* expressing the aminoglycoside-modifying enzyme AAC(6′)-Ib. The antibacterial activity of the 5-deoxy-5-episubstituted ABK derivatives and ABK was markedly reduced by the MexXY/OprM efflux system in *P. aeruginosa*. Based on these findings, further structure–activity relationship studies of this class of compounds are currently in progress.

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