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COMMUNICATION TO THE EDITOR

Discovery of 2-hydroxyarbekacin, a new aminoglycoside antibiotic with reduced nephrotoxicity

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The emergence and spread of bacteria with resistance to antibacterial drugs in recent years is now considered a significant threat to global public health and the world economy.[1,2] In particular, the severe bacterial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* have become serious clinical problems because of increased antimicrobial resistance, as observed for vancomycin-resistant *S. aureus* and multi-drug resistant *P. aeruginosa*.[3] Arbekacin[4] (ABK), an aminoglycoside antibiotic, is efficacious against MRSA, and is commonly used to treat infected patients in the clinical setting. This semi-synthetic antibiotic is stable toward AAC(6′)-APH(2″), a bifunctional enzyme present in MRSA, and is also effective against almost all antibiotic-resistant bacteria that produce aminoglycoside-modifying enzymes.[5] However, despite its superior antibacterial activity and stability toward aminoglycoside-modifying enzymes, treatment with ABK is limited because of its adverse effect on the kidneys.[6,7] Therefore, we have conducted synthetic studies on a number of ABK derivatives in an effort to decrease the associated toxicity.[8,9]

It is generally known that the nephrotoxicity of streptomycin is relatively low among aminoglycoside antibiotics. Streptomycin retains *N*-amidinated streptamine (streptidine) as a constituent aminocyclitol, and kanamycins such as ABK contain 2-deoxystreptamine as a constituent component (Figure 1). By comparing the structure of these two aminocyclitols, we focused on an equatorial hydroxyl group at the C-2 position and were interested in the relationship between the presence of this hydroxyl group and nephrotoxicity. Also, because the modification at the C-2 position of the ABK derivative is not known, the synthesis of 2-hydroxyarbekacin (2-OH-ABK) was also of interest from the viewpoint of its potential antibacterial activity.

As shown in Scheme 1, the synthesis of 2-OH-ABK was performed by the condensation of 1-*N*-[(*S*)-4-benzyloxycarbonylamino-2-benzyloxybutylyl]-2-benzyloxy-3,2′,6′-tris(*N*-benzyloxycarbonyl)-3′,4′-dideoxyneamine (**9**), which contains an equatorial hydroxyl group at C-2 and phenyl 4,6-di-*O*-acetyl-3-azido-2-*O*-benzyl-3-deoxy-1-thio-α-D-glucopyranoside (**16**).

Acidic hydrolysis of the natural product, 2-hydroxygentamicin C_{1a}[10], gave the pseudodisaccharide, 3′, 4′-dideoxy-2-hydroxyneamine (**1**) (85%, as the bis-carbonate). *N*-Benzyloxycarbonylation of **1** gave **2** in quantitative yield, which was then treated with 1,1-dimethoxycyclohexane in the presence of pyridinium *p*-toluenesulfonate to yield the cyclohexylidene derivative **3** (85%). Next, 2-*O*-benzylation gave **4** (83%), followed by removal of its acetal protecting group in an aqueous acidic solution to provide the 5, 6-diol **5** (93%). Treatment of **5** with NaH (6 equiv. relative to **5**) in DMF at room temperature gave the *N*,*O*-carbonyl derivative **6** (95%), which was retreated with NaH (1 equiv. relative to **6**) in aqueous dioxane at 80 °C to yield the 1-amino derivative **7** (77%). Subsequent introduction of an amino acid side-chain to **7**, which possesses a free amino group at C-1, was successfully accomplished by utilizing the active ester **8** according to conventional methodology[11] to afford the 1-*N*-acyl derivative **9** (76%), ^{1}H NMR (400 MHz, pyridine-d_5) δ 2.28 (m, 2H, H-3‴) and 5.65 (d, 1H, J = 2.9 Hz, H-1′).

Next, the glycosyl donor **16** was prepared. Acetalization of methyl 3-azido-3-deoxy-α, β-D-glucopyranoside (**10**)[12] gave the acetonide **11** (96%), followed by benzylation of the 2-hydroxyl group to yield **12** (89%). Acidic hydrolysis quantitatively gave the deacetonated derivative **13**, which was treated with acetic acid–acetic anhydride–sulfuric acid (50:50:1), thereby affording the glycosyl acetate **14** (92%). Bromination of **14** with $TiBr_4$ in CH_2Cl_2–EtOAc (9:1) gave the corresponding α-bromide **15** (97%) as a syrup, ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.43 (dd, 1H, H-2), 3.98 (t, 1H, H-3), 4.92 (t, 1H, H-4) and 6.32 (d, 1H, H-1); $J_{1,2}$ = 3.8 Hz and $J_{2,3}$ = $J_{3,4}$ = 10 Hz. Treatment of **15** with trimethyl(phenylthio)silane in the presence of trimethylsilyl trifluoromethanesulfonate in CH_2Cl_2, gave 1-α-thiophenyl glycoside **16** (76%) as crystals, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 and 2.13 (each s, 3H, Ac), 3.79 (dd, 1H, H-2), 3.86 (t, 1H, H-3), 3.96 (t, 1H, H-6a), 4.22 (dd, 1H, H-6b), 4.46 (ddd, 1H, H-5), 4.72 and 4.75 (ABq, 2H, J_{gem} = 12 Hz, CH_2Ph), 4.83 (t, 1H, H-4) and 5.59 (d, 1H, H-1); $J_{1,2}$ = 5 Hz, $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 10 Hz, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 5.5 Hz and $J_{6a,6b}$ = 12.5 Hz.

Condensation of **9** and **16** was carried out successfully in CH_2Cl_2 in the presence of *N*-iodosuccinimide, trifluoromethanesulfonic acid and 4A molecular sieves to give **17** (69% based on **9**). After removal of the acetyl group, the resulting product **18** (87%) was treated with hydrogen in the presence of palladium black to reduce the azido group and remove the benzyl and benzyloxycarbonyl groups to yield, after purification by Amberlite CG-50 resin column chromatography, a solid of 2-OH-ABK (72%) as the carbonate, $[\alpha]_D^{25}$+96 (*c* 1, H_2O); HR-MS: m/z calculation for $C_{22}H_{45}N_6O_{11}$ $(M+H)^+$ 569.3141, found 569.3151; ^{1}H NMR (500 MHz, DCl–D_2O, pD 2) δ 1.56 (m, 1H, H-4′*ax*), 2.14 (ddt, 1H, H-3‴b), 3.06 (dd, 1H, H-6′a), 3.12 (t, 2H, H-4‴), 3.21 (dd, 1H, H-6′b), 3.35 (apparent t, 1H, H-3″), 3.37 (t, 1H, H-3), 3.52

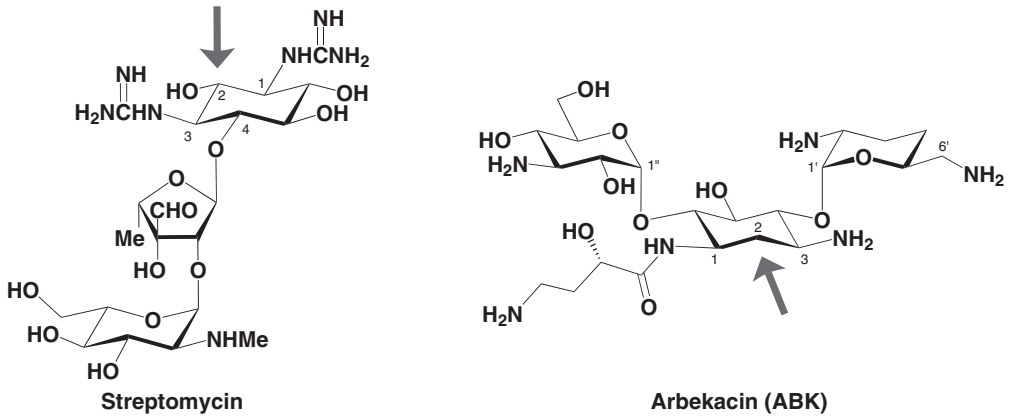
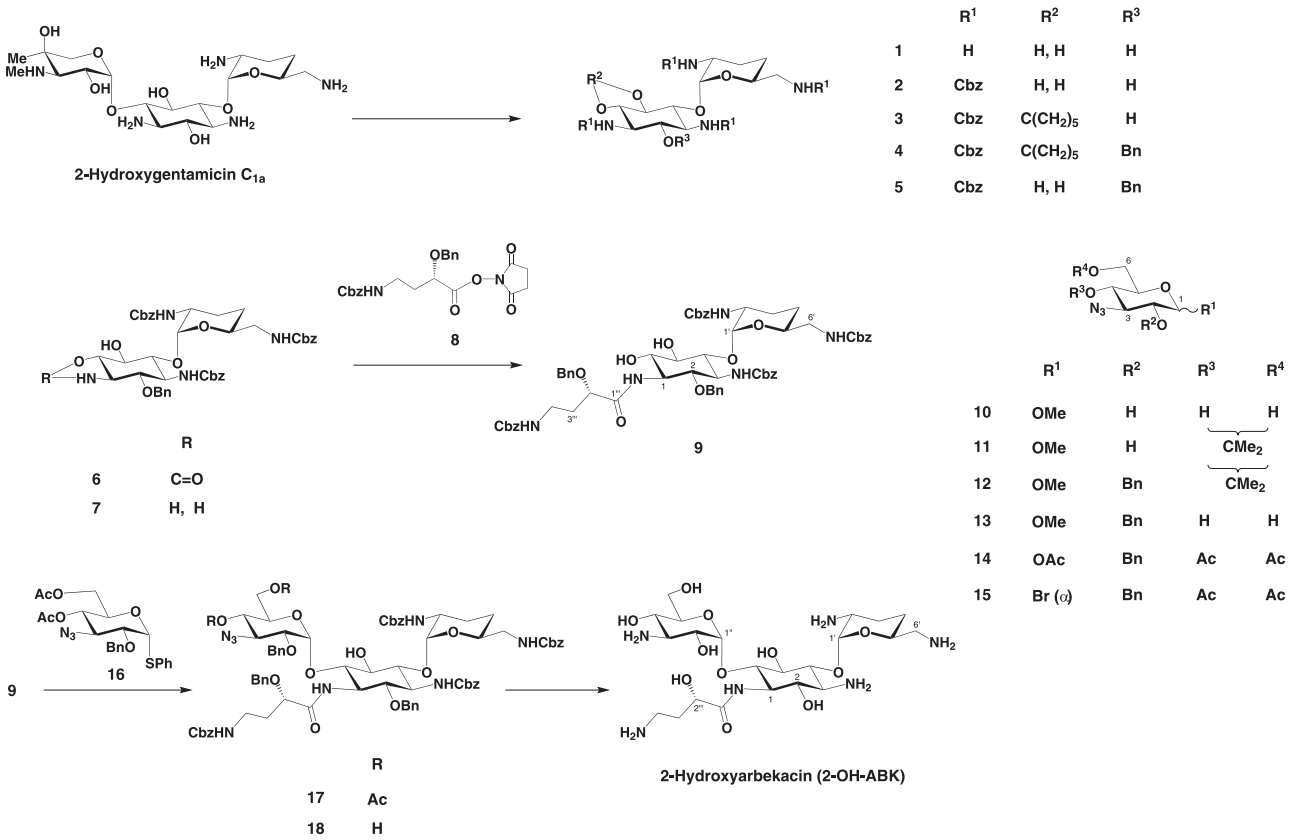


Figure 1 Structures of streptomycin and arbekacin, and the points of interest. A full colour version of this figure is available at the *Journal of Antibiotics* journal online.



	R^1	R^2	R^3
1	H	H, H	H
2	Cbz	H, H	H
3	Cbz	$C(CH_2)_5$	H
4	Cbz	$C(CH_2)_5$	Bn
5	Cbz	H, H	Bn

R	
6	C=O
7	H, H

	R^1	R^2	R^3	R^4
10	OMe	H	H	H
11	OMe	H	CMe_2	
12	OMe	Bn	CMe_2	
13	OMe	Bn	H	H
14	OAc	Bn	Ac	Ac
15	Br (α)	Bn	Ac	Ac

R	
17	Ac
18	H

Scheme 1 Synthesis of 2-hydroxyarbekacin (2-OH-ABK).

(m, 1H, H-2′), 3.63 (t, 1H, H-4″), 3.72 (dd, 1H, H-2″), 3.76 (t, 1H, H-2), 4.15 (m, 1H, H-5′), 4.28 (dd, 1H, H-2‴), 5.09 (d, 1H, H-1″) and 5.75 (d, 1H, H-1′); $J_{1,2}=J_{2,3}=J_{3,4}=10.5$ Hz, $J_{1',2'}=3.5$ Hz, $J_{5',6'a}=7.5$ Hz, $J_{5',6'b}=3.5$ Hz, $J_{6'a,6'b}=13.5$ Hz, $J_{1'',2''}=3.8$ Hz, $J_{2'',3''}=11$ Hz, $J_{3'',4''}=10$ Hz, $J_{2''',3'''a}=9.5$ Hz, $J_{2''',3'''b}=4$ Hz and $J_{3'''a,4'''}=J_{3'''b,4'''}=7.5$ Hz. Anal calcd for $C_{22}H_{44}N_6O_{11}\ 2H_2CO_3\ H_2O$: C 40.56, H 7.09, N 11.83. Found: C 40.44, H 7.10, N 11.53.

2-OH-ABK exhibited excellent antibacterial activity *in vitro* against both *S. aureus* and *P. aeruginosa*. The MICs ($\mu g\,ml^{-1}$) of 2-OH-ABK and ABK against *S. aureus* RN4220 (MSSA) were 0.5 and 0.5, against *S. aureus* RN4220/pCR1948 [AAC(6′) − APH (2″)] they were 0.5 and 2, and against clinical isolates of MRSA ($n=77$) they were 0.25–2 and 0.25–4, respectively. Furthermore, the MICs ($\mu g\,ml^{-1}$) of 2-OH-ABK and ABK against *P. aeruginosa* PAO1 were 2 and 4, and against *P. aeruginosa* PAO1/315 [AAC (6′)-I] they were 4 and 16, respectively.

The nephrotoxicity of 2-OH-ABK was examined using an *N*-acetyl-β-D-glucosaminidase (NAG) assay[13] *in vitro* and a rat *in vivo* test. As shown in Figure 2a, the amount of NAG released from LLC-PK1 cells in the presence of 2-OH-ABK was lower than that of ABK at any dose (2.5, 5 and 10 μM), indicating a tendency of reduction in nephrotoxicity for 2-OH-ABK. Furthermore,

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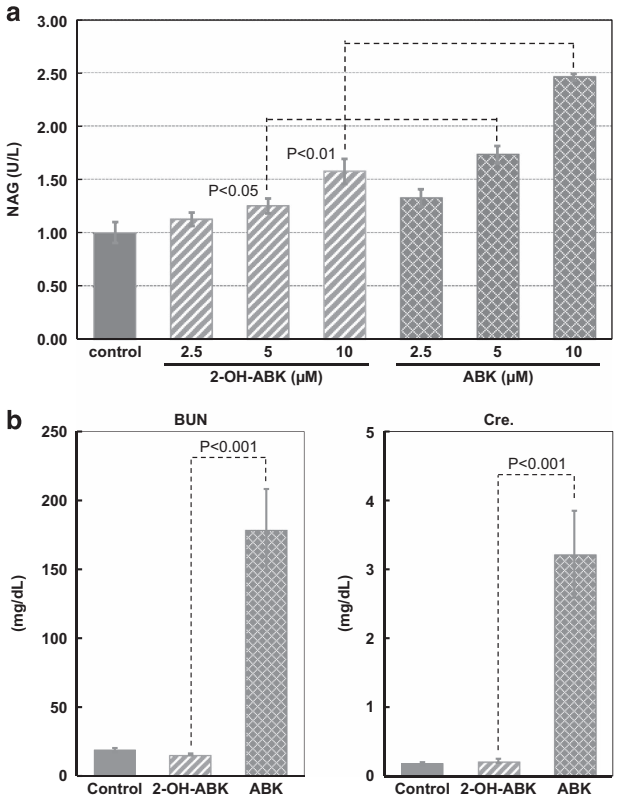

Figure 2 Nephrotoxicity of 2-hydroxyarbekacin (2-OH-ABK) *in vitro* and *in vivo*. (**a**) *N*-acetyl-β-D-glucosaminidase (NAG) assay for 2-OH-ABK. NAG release from LLC-PK1 cells, grown on porous culture inserts as individual confluent monolayers, was determined in the *in vitro* nephrotoxic screening. NAG activity in the serum-free apical conditioned medium was quantified using sodio-*m*-cresolsulfonphthaleinyl *N*-acetyl-β-D-glucosaminide as the substrate. (**b**) Nephrotoxicity of 2-OH-ABK in rats. Abbreviations: BUN, blood urea nitrogen; Cre., creatinine. A full colour version of this figure is available at the *Journal of Antibiotics* journal online.

Figure 2b shows the measurements of urea nitrogen and creatinine in the blood from the drug-treated rats. In this test, although significant increases in blood urea nitrogen and creatinine values were observed in rats treated with ABK, no such increases in these values were observed for the 2-OH-ABK-treated rats. Additionally, the histopathological renal tubular epithelial necrosis observed with ABK was not observed in the 2-OH-ABK-treated rats.

In summary, a novel aminoglycoside antibiotic, 2-OH-ABK, was created and characterized in our drug discovery program on next-generation antibiotics. Notably, its nephrotoxicity was lower than that of ABK. 2-OH-ABK also showed better antibacterial activity than that of ABK against pathogenic bacteria. 2-OH-ABK is, therefore, considered to be a promising candidate for an antibacterial medicine because of its outstanding activity and safety profile.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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