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ORIGINAL ARTICLE

Synthesis and antibacterial activity of 4″ or 6″-alkanoylamino derivatives of arbekacin

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Arbekacin, an aminoglycoside antibiotic, is an important drug because it shows a potent efficacy against methicillin-resistant *Staphylococcus aureus*. However, resistance to arbekacin, which is caused mainly by the bifunctional aminoglycoside-modifying enzyme, has been observed, becoming a serious problem in medical practice. To create new arbekacin derivatives active against resistant bacteria, we modified the C-4″ and 6″ positions of its 3-aminosugar portion. Regioselective amination of the 6″-position gave 6″-amino-6″-deoxyarbekacin (1), and it was converted to a variety of 6″-*N*-alkanoyl derivatives (6a – z). Furthermore, regioselective modifications of the 4″-hydroxyl group were performed to give 4″-deoxy-4″-epiaminoarbekacin (2) and its 4″-*N*-alkanoyl derivatives (12 and 13). Their antibacterial activity against *S. aureus*, including arbekacin-resistant bacteria, was evaluated. It was observed that 6″-amino-6″-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-6″-deoxyarbekacin (6o) showed excellent antibacterial activity, even better than arbekacin.
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INTRODUCTION

Aminoglycoside antibiotics have been widely used in the treatment of serious infections caused by Gram-positive and/or Gram-negative bacteria. They interfere with bacterial protein synthesis by binding to the A-site decoding region of 16S rRNA in the 30S ribosomal subunit.[1–5] Arbekacin (ABK), which was synthesized from dibekacin by the attachment of the (*S*)-4-amino-2-hydoxybutyryl (AHB)[6] residue at the *N*-1 position, is efficacious for methicillin-resistant *Staphylococcus aureus* (MRSA), and is used for the treatment of infected patients.[7,8] However, the emergence of ABK-resistant MRSA, caused by bifunctional aminoglycoside-modifying enzyme AAC (6′)-APH (2″), has been observed, and causes a serious problem.[9–11] Moreover, the appearance of aminoglycoside-resistant *Pseudomonas aeruginosa* and *Acinetobacter* has recently been reported.[12] Therefore, development of a new type of ABK derivative active against resistant bacteria is urgently required to fight against the diseases caused by the pathogens.

Deoxygenation, epimerization and/or deoxyamination have frequently strengthened the antibacterial activity of aminoglycoside antibiotics.[13–15] For example, 5-deoxy-5-epi substitution[16] and 5, 4″-diepimerization[17] enhanced the antibacterial activity of ABK, including the activity against resistant bacteria. Also, it is well known that the introduction of the amino acid side-chain to the 1-amino group brings an improvement in antimicrobial activity.[18–21] Furthermore, their combined modification is also thought to be an attractive approach. 6″-Amino-6″-deoxy-arbekacin (**1**)[22] and 4″-deoxy-4″-epiamino- arbekacin (**2**),[17] which introduced an amino group in the 4″ or 6″-position of its 3-aminosugar portion, were reported to preserve the antibacterial activity. In addition, the specific mechanism of the binding affinity of the hydroxyl groups at these positions to the bacterial 16S rRNA has not been determined. These phenomena suggest that there is great potential for improvement of activity by chemical modification in this area. Therefore, we focused on the 3-aminosugar portion bearing a novel amino group, to create new ABK derivatives active against resistant bacteria, and planned a structure–activity relationship study on the *N*-acyl derivatives of the novel amino group.

In this paper, we describe the synthesis of 4″- alkanoylamino-4″-epi or 6″-alkanoylamino derivatives of ABK by regioselective amination and their antibacterial activities against *S. aureus* including ABK-resistant MRSA.

RESULTS AND DISCUSSION

Synthesis and antibacterial activity of 6″-alkanoylamino derivatives of ABK

Synthesis of **1** and its 6″-*N*-alkanoyl derivatives is shown in Scheme 1.

Penta-*N*-Boc-6″-*O*-triisopropylbenzenesulfonyl ABK (**3**) was obtained from penta-*N*-Boc ABK[17] via selective sulfonylation of the 6″-hydroxyl group with 2, 4, 6-triisopropylbenzenesulfonyl chloride (TIBS-Cl) at room temperature with 90% yield. In the ^{1}H NMR spectrum of **3** in dimethyl sulfoxide (DMSO)-d_6, the H-6″ signals of penta-*N*-Boc ABK at 3.51 and 3.58 p.p.m. shifted to 4.11 and 4.28 p.p.m., respectively, confirming the selective sulfonylation of 6″-hydroxyl group in **3** (refer to experimental section). Introduction of the leaving groups by other reagents such as *p*-toluenesulfonyl chloride, methanesulfonyl chloride or I_2 with triphenylphosphine

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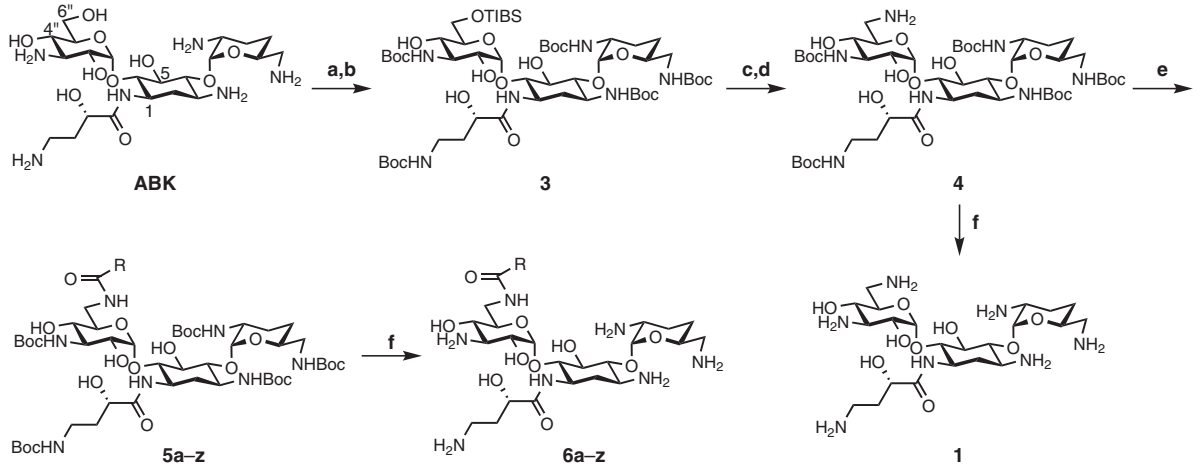


Scheme 1 Synthesis of 6"-amino-6"-deoxyarbekacin (**1**) and its 6"-*N*-alkanoyl derivatives (**6a – z**). Reagents and conditions: (**a**) Boc_2O, Et_3N, H_2O, dioxane, rt; (**b**) TIBS-Cl, pyridine, rt; (**c**) NaN_3, DMF, 100 °C; (**d**) PPh_3, H_2O, THF, 50 °C; (**e**) RCO_2H, DMT-MM or RCO-OSu; (**f**) aqueous TFA, 0 °C.

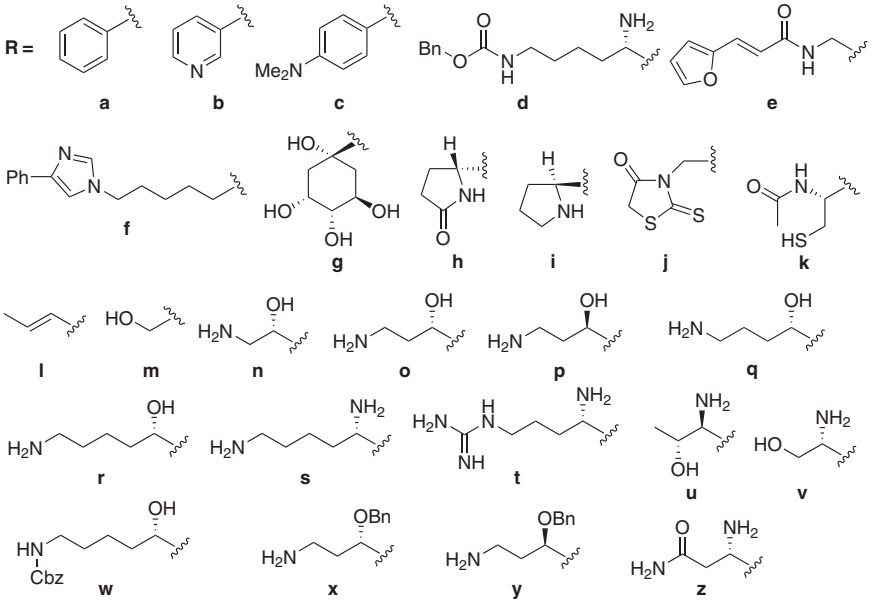


Figure 1 The structures of 6"-*N*-alkanoyl derivatives (**5a – z** and **6a – z**).

resulted in a decrease in regioselectivity. Treatment of **3** with NaN_3 followed by reduction of the resulting azide group by the Staudinger reaction[23] produced 6″-amine **4** in a high yield. Deprotection of **4** with aqueous trifluoroacetic acid provided 6″-amino-6″-deoxyarbekacin (**1**). Although the synthesis of **1** was described for a previous procedure,[22] we have established a more convenient method. On the other hand, *N*-alkanoylation of **4** with various carboxylic acids was effectively attained by using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)[24] or the activated ester method[25] to give **5a – z** with 41 – 95% yield. Removal of Boc groups afforded a variety of 6″-alkanoylamino-6″-deoxy derivatives (**6a – z**) of ABK (refer to Supplementary Information). The position of newly introduced acyl groups in **6a – z** was confirmed by HMBC correlations between the amide carbonyl and the hydrogens at C-6″. The structures of **5a – z** and **6a – z** are shown in Figure 1.

The antibacterial activities of 6″-amino-6″-deoxyarbekacin (**1**) and its 6″-*N*-alkanoyl derivatives **6a – z** are shown in Table 1.

The derivatives (**6a – j**, **w – y**) containing the aromatic or aliphatic rings in the side-chains were less active than ABK. However, **6x** and **6y**, possessing the terminal amino group, showed moderate antibacterial activity, and this result suggested that the terminal amino group in the side-chain is important for the activity. In contrast, the derivatives (**6k – v**) containing the functionalized linear chain were likely to display a relatively potent antibacterial activity. In particular, compounds **6n** and **6o**, with the (*S*)-3-amino-2-hydroxypropionyl and (*S*)-AHB residue, respectively, showed highly efficacious antibacterial activity against ABK-resistant MRSA MS16526 (the resistant mechanism is a bifunctional enzyme AAC (6′)-APH (2″)). This result indicated that the antibacterial activity is enhanced by the addition of the amino side-chain even in the case of the novel amino group at the C-6″ position. Improvement of antibacterial activity against ABK-resistant MRSA is suspected to be a likely result of the inhibition of bifunctional enzyme AAC (6′)-APH (2″). In the AHB side-chain, a comparison between **6o** and **6p** indicated that the *S*-configuration is

Table 1 MICs (μg ml^{-1}) for ABK, 6"-amino-6"-deoxyarbekacin (1) and 6"-*N*-alkanoyl derivatives (6a – z) against *Staphylococcus aureus*

	Strains						
Compounds	*FDA209P*	*Smith*	*Ap01*	*MRSA No. 5*[a]	*MRSA No. 17*[a]	*TY-04282*[a]	*MS16526*[b]
ABK	0.05	0.05	0.39	0.39	6.25	0.39	25
1	0.78	0.2	6.25	12.5	50	1.56	12.5
6a	0.78	0.2	3.13	6.25	>100	NT	NT
6b	0.78	0.39	3.13	3.13	50	NT	NT
6c	12.5	6.25	>100	12.5	>100	NT	NT
6d	3.13	0.2	1.56	6.25	100	NT	NT
6e	3.13	1.56	12.5	3.13	>100	NT	NT
6f	3.13	1.56	25	12.5	100	NT	NT
6g	1.56	0.39	6.25	12.5	100	NT	NT
6h	1.56	0.78	6.25	6.25	100	NT	NT
6i	0.2	0.1	0.78	3.13	50	NT	NT
6j	0.78	0.39	3.13	6.25	>100	NT	NT
6k	0.2	0.39	6.25	0.39	12.5	6.25	25
6l	0.1	0.2	3.13	0.39	3.13	6.25	25
6m	0.1	0.1	0.78	0.2	0.78	3.13	25
6n	0.05	0.05	0.39	0.1	0.78	0.39	0.39
6o	0.05	0.1	0.39	0.1	0.39	0.39	0.39
6p	0.2	0.1	0.78	0.2	0.78	0.78	1.56
6q	0.2	0.05	0.39	0.39	0.78	0.78	3.13
6r	0.1	0.1	0.39	0.2	1.56	1.56	0.78
6s	0.1	0.05	0.78	0.05	0.78	0.39	0.78
6t	0.1	0.05	0.78	0.1	0.78	0.39	0.78
6u	0.5	0.25	2	2	16	NT	NT
6v	0.25	0.13	1	0.5	4	NT	NT
6w	6.25	3.13	25	>100	>100	NT	NT
6x	0.39	0.2	1.56	1.56	12.5	NT	NT
6y	0.78	0.2	1.56	6.25	12.5	NT	NT
6z	0.78	0.2	12.5	12.5	>100	NT	NT

Abbreviation: ABK, Arbekacin; NT, not tested.
[a]Methicillin-resistant *Staphylococcus aureus* (MRSA).
[b]ABK-resistant MRSA.

Table 2 MICs (μg ml^{-1}) for 6o and ABK against *Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa*

	E. faecalis		*E. coli*		*P. aeruginosa*		
Compounds	*JCM5804*	*ATCC12204*	*K-12R-5*	*RJ225*	*No. 12*	*GN16145*	*H9*
ABK	6.25	3.13	6.25	0.39	6.25	12.5	6.25
6o	3.13	3.13	3.13	0.39	0.78	3.13	3.13

Abbreviation: ABK, Arbekacin.

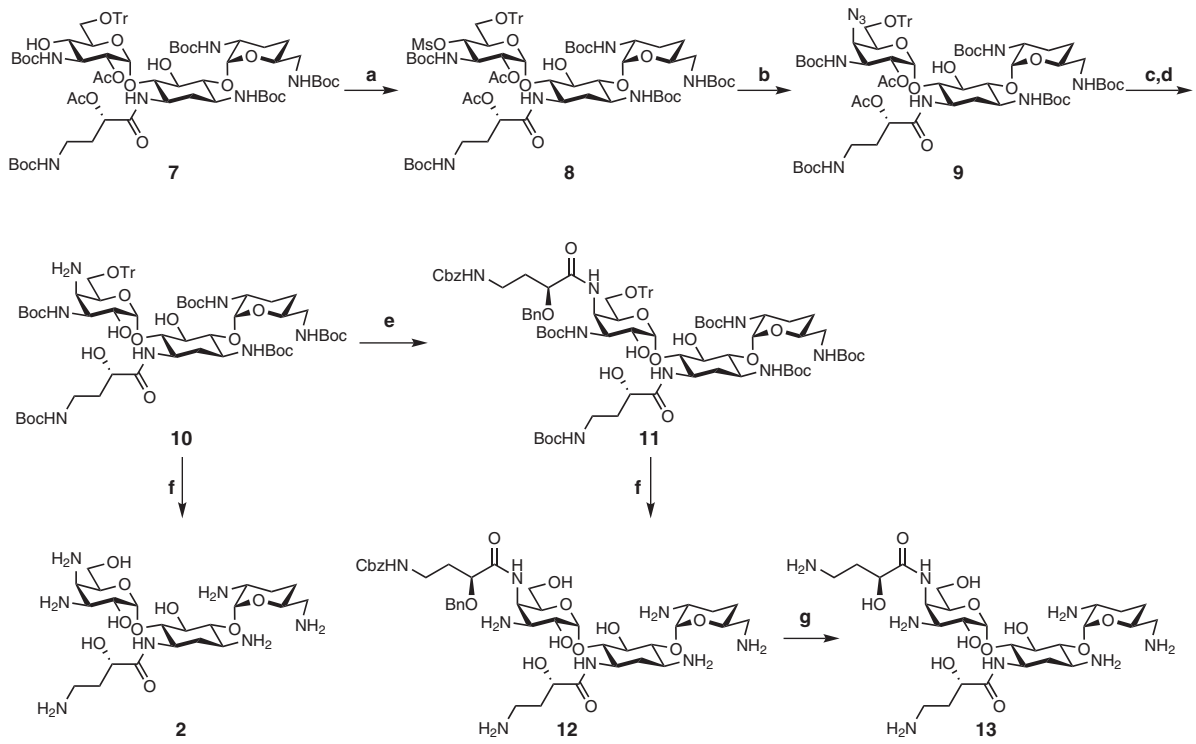
preferable over the *R*-configuration. When the carbon-chain becomes longer than that of AHB, the activity tended to decrease, and a similar phenomenon was reported in the case of 1-*N*-alkanoyl derivatives of ABK.[26] Compound **6s** demonstrated that the replacement of the hydroxyl group by an amino group at the alpha position of the linear side-chain displays excellent activity. Further, it was indicated in the compound **6t** that the replacement of the terminal amino group by the guanidine group shows similar effects in terms of antimicrobial activity. Interestingly, although **6n** and **6v** have the same functional groups in the side-chain, **6n** was more active than **6v** because of the difference in the position of the two functional groups. These results indicated the importance of the presence and location of the basic functional groups for antibacterial activity.

Moreover, we evaluated the antibacterial activity of **6o** against *Escherichia coli* and *P. aeruginosa* as Gram-negative bacteria and *Enterococcus faecalis* as Gram-positive bacteria (Table 2). On the whole, **6o** was equal or more active than ABK against these bacteria, in particular, it exhibited prominent activity against *P. aeruginosa*.

Synthesis and antibacterial activity of 4″-alkanoylamino-4″-epi derivatives of ABK

We expected that the introduction of the AHB residue into the 4″-epiamino group would potentiate the antibacterial activity, as inferred from the results of the SAR study on 6″-*N*-alkanoyl derivatives. Synthesis of 4″-deoxy-4″-epiaminoarbekacin (**2**) and its 4″-*N*-alkanoyl derivatives is shown in Scheme 2.

The reaction of 2″, 2‴-di-*O*-acetyl-3, 2′, 6′, 3″, 4‴-penta-*N*-Boc-6″-*O*-trityl ABK[17] (**7**) with methanesulfonyl chloride (MsCl) produced the 4″-*O*-mesyl derivative **8** in a high yield. Treatment of **8** with NaN_3 followed by removal of the acetyl groups and the Staudinger reaction[23]



Scheme 2 Synthesis of 4"-deoxy-4"-epiaminoarbekacin (**2**) and its 4"-*N*-alkanoyl derivatives. Reagents and conditions: (**a**) MsCl, pyridine, rt; (**b**) NaN_3, DMF, 100 °C; (**c**) NH_4OH, MeOH, rt; (**d**) PPh_3, H_2O, THF, 50 °C; (**e**) (*S*)-CbzHNCH_2CH_2CH(OBn)CO_2H, DMT-MM, THF, H_2O, rt; (**f**) aqueous TFA, 0 °C; (**g**) H_2, Pd black, rt.

Table 3 MICs (μg ml^{-1}) for ABK, 4"-deoxy-4"-epiaminoarbekacin (2) and its 4"-alkanoyl derivatives (12 and 13) against *Staphylococcus aureus*

	Strains				
Compounds	*FDA209P*	*Smith*	*Ap01*	*MRSA No. 5*[a]	*MRSA No. 17*[a]
ABK	0.06	0.06	0.5	0.5	4
2	1	0.5	4	32	128
12	128	128	>128	>128	>128
13	1	0.25	8	8	16

Abbreviation: ABK, Arbekacin.
[a]Methicillin-resistant *Staphylococcus aureus* (MRSA).

of the azide group gave the 4″-epiamine **10**. Deprotection of Boc and Tr groups under acidic conditions provided **2**. On the other hand, *N*-alkanoylation of **10** with (*S*)-2-benzyloxy-4-benzyloxycarbonylaminobutyric acid gave **11** in 86% yield. Removal of the Boc groups of **11** led to **12**, and the deprotection of the Bn and Cbz groups provided the bis-AHB derivative **13**.

The antibacterial activities of 4″-deoxy-4″-epiaminoarbekacin (**2**) and its 4″-*N*-alkanoyl derivatives (**12** and **13**) against *S. aureus* are shown in Table 3. The 4″-deoxy-4″-epiamino derivative **2** showed moderate activity against Methicillin-sensitive *S. aureus*, although its activity against MRSA strains was remarkably lower than that of ABK as reported.[17] On the other hand, compound **12**, which introduced the *N*,*O*-protected AHB residue into the *axial* amino group at the C-4″ position was not active. In contrast, the bis-AHB derivative **13** showed more potent antibacterial activity than that of **2** or **12**. This suggested that the addition of the amino side-chain to the novel *axial* amino group at the C-4″ position also enhances the antibacterial activity.

CONCLUSIONS

We synthesized the 4″ or 6″- alkanoylamino derivatives of ABK *via* the regioselective sulfonylation of the hydroxyl groups at the corresponding positions. 6″-Alkanoylamino derivatives, which introduced the (*S*)-3-amino-2-hydroxypropionyl (**6n**) or AHB (**6o**) residue, showed potent antibacterial activity against *S. aureus* including ABK-resistant MRSA, although the 4″-alkanoylamino-4″-epi derivative **13** was less active than ABK. Furthermore, **6o** was more active than ABK against Gram-negative bacteria such as *E. coli* and *P. aeruginosa*. This study can provide a basis for development of novel aminoglycoside antibiotics for clinically relevant bacteria that are recalcitrant to antibiotic treatment.

EXPERIMENTAL PROCEDURE

General information

NMR spectra were recorded on a Bruker DPX 400 or AVANCE 500 spectrometer (Bruker, Billarica, MA, USA). Chemical shifts were measured downfield from tetramethylsilane as an internal standard. Mass spectra were acquired on a Thermo Scientific LTQ XL spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) (ESI) or LTQ Orbitrap mass spectrometer (HRMS). Elemental analysis was performed on a Perkin Elmer 2400 Series II CHNS/O Elemental Analyzer (Perkin Elmer, Waltham, MA, USA). TLC was conducted with silica gel 60 F_{254} (Merck Millipore, Billerica, MA, USA) and visualized with ceric ammonium molybdate stain and/or UV. Silica gel column chromatography was carried out on Silica Gel 60 N (Kanto Chemical, Tokyo, Japan). Resin column chromatography was carried out on Amberlite CG50 Type I (NH_4^+) (Dow Chemical, Midland, MI, USA).

Synthesis

Penta-N-Boc ABK. To an aqueous solution (170 ml) of ABK (10.0 g, 2.5 H_2SO_4 salt, 12.5 mmol), Et_3N (28.5 ml, 204.7 mmol) and Boc_2O (25.1 g, 115.2 mmol) in 1,4-dioxane (170 ml) were added, and the mixture was stirred at 60 °C for 3 h. The reaction mixture was quenched with concentrated aqueous NH_3, and the quenched mixture was evaporated. The resulting residue was washed with H_2O, and then concentrated *in vacuo* to provide penta-*N*-Boc ABK (13.4 g, 98%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 500 MHz, 343 K) δ 1.32 (m, 1H, H-4′*ax*), 1.36 – 1.40 (m, 45H, 5 X C(CH_3)$_3$), 1.50 (m, 1H, H-2*ax*), 1.61 (m, 1H, H-βa), 1.62 (m, 1H, H-3′*ax*), 1.64 (m, 1H, H-4′*eq*), 1.70 (m, 1H, H-3′*eq*), 1.84 (m, 1H, H-βb), 2.02 (m, 1H, H-2*eq*), 3.09 (m, 2H, H-γ), 3.14 (m, 2H, H-6′), 3.28 (m, 1H, H-4″), 3.34 (m, 1H, H-2″), 3.38 (m, 1H, H-3), 3.41 (m, 1H, H-2′), 3.44 (m, 1H, H-4), 3.49 (m, 1H, H-3″), 3.51 (m, 1H, H-6″a), 3.53 (m, 1H, H-5), 3.58 (m, 1H, H-6″b), 3.62 (m, 1H, H-6), 3.67 (m, 1H, H-1), 3.77 (m, 1H, H-5″), 3.83 (m, 1H, H-α), 3.86 (m, 1H, H-5′), 3.98 (br, 1H, OH-2′), 4.05 (br, 1H, OH-6″), 4.51 (br, 1H, OH-4″), 4.78 (br, 1H, OH-5), 4.90 (br, 1H, OH-α), 4.92 (d, 1H, *J* = 3.1 Hz, H-1″) and 5.05 (d, 1H, *J* = 2.9 Hz, H-1′); ^{13}C NMR (DMSO-d_6, 125.8 MHz, 343 K) δ 23.8 (3′), 27.3 (4′), 28.2 (6C, C(*C*H_3)$_3$), 28.3 (6C, C(*C*H_3)$_3$), 29.0 (3C, C(*C*H_3)$_3$), 33.8 (2), 34.3 (β), 37.2 (γ), 44.6 (6′), 48.7 (1), 49.8 (3), 50.5 (2′), 55.8 (3″), 60.8 (6″), 66.9 (5′), 67.7 (4″), 69.5 (α), 70.2 (2″), 73.5 (5″), 75.4 (5), 77.5 (*C*(CH$_3$)$_3$), 77.6 *C*(CH$_3$)$_3$), 77.7 (*C*(CH$_3$)$_3$), 77.8 (2C, *C*(CH$_3$)$_3$), 81.2 (4), 81.8 (6), 98.2 (2C, 1′ and 1″), 154.8 (C = O), 154.9 (C = O), 155.5 (C = O), 155.7 (C = O), 156.5 (C = O) and 173.9 (C = O); ESI-MS calculated for $C_{47}H_{84}N_6NaO_{20}$: 1075.56; found: 1075.60 $(M+Na)^+$.

*3, 2′, 6′, 3″, 4‴-Penta-N-tert-Boc-6″-O-(2, 4, 6-triisopropylbenzenesulfonyl) arbekacin (**3**)*. To a solution of penta-*N*-Boc ABK (17.4 g, 16.5 mmol) in pyridine (348 ml), 2,4,6-triisopropylbenzenesulfonyl chloride (25.0 g, 82.5 mmol) was added, and the mixture was stirred at room temperature for 3 days. The reaction mixture was quenched with MeOH (35 ml), and the mixture was evaporated. The resulting residue was purified by silica gel column chromatography to provide **3** (19.5 g, 90%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 500 MHz, 343 K) δ 1.18 – 1.23 (m, 18H, 3X CH(CH_3)$_2$), 1.35 (m, 1H, H-4′*ax*), 1.36 – 1.40 (m, 45H, 5X C(CH_3)$_3$), 1.52 (m, 1H, H-βa), 1.54 (m, 1H, H-2*ax*), 1.57 (m, 1H, H-3′*ax*), 1.60 (m, 1H, H-4′*eq*), 1.66 (m, 1H, H-3′*eq*), 1.72 (m, 1H, H-βb), 1.80 (m, 1H, H-2*eq*), 3.03 (m, 2H, H-γ), 3.08 (m, 2H, H-6′), 3.10 – 3.13 (m, 3H, 3X C*H*(CH$_3$)$_2$), 3.29 (m, 1H, H-2″), 3.32 (m, 1H, H-4″), 3.34 (m, 1H, H-3), 3.40 (m, 1H, H-2′), 3.44 (m, 1H, H-5), 3.48 (m, 1H, H-4), 3.54 (m, 1H, H-3″), 3.61 (m, 1H, H-6), 3.68 (m, 1H, H-1), 3.81 (m, 1H, H-α), 3.85 (m, 1H, H-5′), 4.09 (m, 1H, H-5″), 4.11 (m, 1H, H-6″a), 4.17 (br, 1H, OH-2′), 4.28 (m, 1H, H-6″b), 4.71 (br, 1H, OH-5), 4.80 (br, 1H, OH-4″), 4.97 (br, 1H, OH-α), 5.01 (d, 1H, *J* = 3.1 Hz, H-1″), 5.08 (d, 1H, *J* = 2.8 Hz, H-1′) and 7.11 (s, 2H, C$_6H_2$(*i*-Pr)$_3$); ^{13}C NMR (DMSO-d_6, 125.8 MHz, 343 K) δ 23.7 (3′), 24.4 (2C, CH(*C*H$_3$)$_2$), 24.7 (4C, CH(*C*H$_3$)$_2$), 27.3 (4′), 28.2 (6C, C(*C*H$_3$)$_3$), 28.4 (6C, C(*C*H$_3$)$_3$), 29.0 (3C, C(*C*H$_3$)$_3$), 33.3 (3C, *C*H(CH$_3$)$_2$), 33.8 (2), 34.3 (β), 37.2 (γ), 44.6 (6′), 49.0 (1), 50.0 (3), 50.5 (2′), 55.8 (3″), 66.9 (5′), 67.0 (4″), 67.8 (6″), 69.5 (α), 69.7 (2″), 70.1 (5″), 75.5 (5), 80.4 (6), 81.7 (4), 98.1 (1″), 98.3 (1′), 121.0 (2C, C_6H_2(*i*-Pr)$_3$), 122.3 (2C, C_6H_2(*i*-Pr)$_3$), 123.6 (1C, C_6H_2(*i*-Pr)$_3$), 129.2 (1C, C_6H_2(*i*-Pr)$_3$), 153.4 (C = O), 154.8 (C = O), 155.5 (C = O), 155.7 (C = O), 156.6 (C = O) and 173.9 (C = O); ESI-MS calculated for $C_{62}H_{106}N_6NaO_{22}S$: 1341.70; found: 1341.72 $(M+Na)^+$.

*6″-Amino-6″-deoxy-3, 2′, 6′, 3″, 4‴-penta-N-tert-butoxycar-bonylarbekacin (**4**)*. To a solution of **3** (1.20 g, 0.91 mmol) in *N*,*N*-dimethylformamide (DMF) (24 ml), NaN_3 (296 mg, 4.55 mmol) was added, and the mixture was stirred at 100 °C for 3 h. Concentration gave a residue, which was extracted with $CHCl_3$. The organic solution was washed with water, dried over $MgSO_4$ and concentrated. The resulting residue was purified by silica gel column chromatography to provide the 6″- azide **3a** (0.89 g, 91%) as a colorless solid. ESI-MS calculated for $C_{47}H_{83}N_9NaO_{19}$: 1100.57; found: 1100.56 $(M+Na)^+$. To a solution of **3a** (795 mg, 0.74 mmol) in tetrahydrofuran (THF) – H_2O (5:1, 29 ml), PPh_3 (290 mg, 1.11 mmol) was added and the mixture was stirred at 50 °C for 4 h. Concentration gave a residue, which was purified by silica gel column chromatography to give **4** (644 mg, 89%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 500 MHz, 343 K) δ 1.32 (m, 1H, H-4′*ax*), 1.35 – 1.40 (m, 45H, 5 X C(CH_3)$_3$), 1.51 (m, 1H, H-2*ax*), 1.62 (m, 1H, H-βa), 1.63 (m, 1H, H-3′*ax*), 1.64 (m, 1H, H-4′*eq*), 1.69 (m, 1H, H-3′*eq*), 1.84 (m, 1H, H-βb), 2.02 (m, 1H, H-2*eq*), 3.07 (m, 2H, H-γ), 3.14 (m, 2H, H-6′), 3.27 (m, 1H, H-4″), 3.33 (m, 1H, H-2″), 3.38 (m, 1H, H-3), 3.42 (m, 1H, H-2′), 3.44 (m, 1H, H-4), 3.46 (m, 1H, H-6″a), 3.49 (m, 1H, H-3″), 3.53 (m, 1H, H-5), 3.55 (m, 1H, H-6″b), 3.61 (m, 1H, H-6), 3.67 (m, 1H, H-1), 3.74 (m, 1H, H-5″), 3.85 (m, 1H, H-α), 3.86 (m, 1H, H-5′), 3.95 (br, 1H, OH-2′), 4.11 (br, 1H, OH-6″), 4.51 (br, 1H, OH-4″), 4.76 (br, 1H, OH-5), 4.82 (br, 1H, OH-α), 4.92 (d, 1H, *J* = 3.2 Hz, H-1″) and 5.08 (d, 1H, *J* = 2.9 Hz, H-1′); ^{13}C NMR (DMSO-d_6, 125.8 MHz, 343 K) δ 23.7 (3′), 27.6 (4′), 28.2 (6C, C(*C*H$_3$)$_3$), 28.4 (6C, C (*C*H$_3$)$_3$), 29.0 (3C, C(*C*H$_3$)$_3$), 33.6 (2), 34.3 (β), 37.3 (γ), 44.5 (6′), 48.7 (1), 49.5 (3), 50.5 (2′), 55.7 (3″), 59.4 (6″), 66.7 (5′), 67.7 (4″), 69.5 (α), 70.1 (2″), 73.4 (5″), 75.8 (5), 77.5 (*C*(CH$_3$)$_3$), 77.6 *C*(CH$_3$)$_3$), 77.7 (*C*(CH$_3$)$_3$), 77.9 (2C, *C* (CH$_3$)$_3$), 81.2 (4), 81.5 (6), 98.2 (2C, 1′ and 1″), 154.9 (C = O), 155.0 (C = O), 155.5 (C = O), 155.8 (C = O), 156.5 (C = O) and 173.8 (C = O); ESI-MS calculated for $C_{47}H_{85}N_7NaO_{19}$: 1074.58; found: 1074.63 $(M+Na)^+$.

*6″-Amino-6″-deoxyarbekacin (**1**)*. A solution of **4** (650 mg, 0.6 mmol) in TFA – H_2O (9:1, 13 ml) was kept at 0 °C for 2 h. Concentration gave a residue that was purified by resin column chromatography to provide **1** [22] (358 mg, 94% as a carbonate) as a colorless solid.

*6″-N-Alkanoylamino-6″-deoxy-3, 2′, 6′, 3″, 4‴-penta-N-tert-butoxycarbonylarbekacin (**5a – z**)*. *Amidation by DMT-MM method* (**5a – l**, **s – v**, **x – z**). To a solution of **4** (50 – 300 mg, 0.05 – 0.29 mmol) in MeOH – THF – H_2O (15:7.5:1, 17.5 ml), a variety of carboxylic acids (1.5 eq) and DMT-MM (2 eq) were added, and the mixture was stirred at room temperature for 1 day. Concentration gave a residue that was extracted with $CHCl_3$. The organic solution was washed with saturated aqueous $NaHCO_3$ and water, dried over $MgSO_4$ and concentrated. The resulting residue was purified by silica gel column chromatography to provide **5** (41 – 90%) as a colorless solid.

Amidation by activated ester method (**5m – r**,**w**). To a solution of **4** (1.0 g, 0.95 mmol) in THF (20 ml), Et_3N (0.33 ml, 2.4 mmol) and *N*-hydroxysuccinimide ester of the corresponding amino acid (5.71 mmol) were added, and the mixture was stirred at room temperature for 19 h. The reaction mixture was quenched with 1M aqueous NH_3, and the mixture was evaporated. The residue was extracted with $CHCl_3$ and the organic solution was washed with saturated aqueous $NaHCO_3$ and water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to provide **5** (92 – 95%) as a colorless solid.

*6″-N-Alkanoylamino-6″-deoxyarbekacin (**6a – z**)*. A solution of **5** (31 – 720 mg) in TFA – H_2O (9:1, 20 v/w) was kept at 0 °C for 2 h. The solution was concentrated *in vacuo*, and then the residue was purified by resin column chromatography to provide **6** (91 – 97% as a carbonate) as a colorless solid. *Compound **6o***. ^{1}H NMR (26% $ND_3 - D_2O$, 500 MHz): δ 1.40 (m, 1H, H-4′*ax*), 1.44 (m, 1H, H-2*ax*), 1.60 (m, 1H, H-3′*ax*), 1.65 (m, 1H, H-4′*eq*), 1.73 (m, 1H, H-3′*eq*), 1.79 (m, 1H, H-β′a), 1.82 (m, 1H, H-βa), 1.87 (m, 1H, H-β′b), 1.89 (m, 1H, H-βb), 1.93 (m, 1H, H-2eq), 2.66 (m, 2H, H-6′), 2.72 (m, 2H, H-γ′), 2.74 (m, 2H, H-γ), 2.83 (m, 1H, H-2′), 2.85 (m, 1H, H-3), 2.96 (t, 1H, *J* = 9.8 Hz, H-3″), 3.12 (t, 1H, *J* = 9.8 Hz, H-4″), 3.31 (m, 1H, H-2″), 3.36 (m, 1H, H-4), 3.47 (t, 2H, *J* = 4.7 Hz, H-6″), 3.66 (t, 1H, *J* = 9.5 Hz, H-5), 3.73 (t, 1H, *J* = 9.5 Hz, H-6), 3.83 (m, 1H, H-5′), 3.96 (m, 1H, H-1), 4.12 (dd, 1H, *J* = 3.8 and 9.0 Hz, H-α′), 4.15 (dd, 1H, *J* = 3.8 and 9.2 Hz, H-α), 4.23 (m, 1H, H-5″), 5.04 (d, 1H, *J* = 3.7 Hz, H-1″) and 5.18 (d, 1H, *J* = 3.4 Hz, H-1′); ^{13}C NMR (26% $ND_3 - D_2O$, 125.8 MHz): δ 27.2 (3′), 28.1 (4′), 35.3 (2), 36.3 (β′), 37.1 (β), 38.2 (γ), 40.1 (γ′), 41.3 (6″), 45.9 (6′), 50.1 (3), 50.4 (2′), 50.2 (1), 55.8 (3″), 70.4 (α), 71.0 (α′), 71.2 (5″), 71.5 (5′), 72.1 (4″), 72.8 (2″), 75.2 (5), 81.2 (6), 87.9 (4), 99.3 (1″), 102.5 (1′), 171.0 (C = O) and 178.0 (C = O); ESI-HRMS calculated for $C_{26}H_{53}N_8O_{11}$: 653.3828; Found: 653.3825 $(M+H)^+$; Analysis calculated for $C_{26}H_{53}N_8O_{11}{\cdot}H_2CO_3{\cdot}3H_2O$: C, 42.18; H, 7.87; N, 14.57. Found: C, 41.91; H, 7.98; N, 14.59.

*2″, 2‴-Di-O-acetyl-3, 2′, 6′, 3″, 4‴-penta-N-tert-Boc 4″-O-mesyl-6″-O-tritylarbekacin (**8**)*. To a solution of **7** [17] (11.0 g, 7.97 mmol) in pyridine (220 ml) methanesulfonyl chloride (4.9 ml, 63.8 mmol) was added, and the mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with MeOH (22 ml) and was evaporated. The resulting residue was extracted with AcOEt, and the organic solution was washed with saturated aqueous NH_4Cl

and water, dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography to provide **8** (11.0 g, 95%) as a colorless solid. ESI-MS calculated for $C_{71}H_{104}N_6NaO_{24}S$: 1479.67; found: 1479.60 $(M+Na)^+$.

*2″,2‴-Di-O-acetyl-3, 2′, 6′, 3″, 4‴-penta-N-tert-Boc-4″-deoxy-4″-epiazide-6″-O-trityl- arbekacin (**9**).* To a solution of **8** (12.0 g, 8.23 mmol) in DMF (360 ml) NaN_3 (13.4 g, 206 mmol) was added, and the mixture was stirred at 100 °C for 1 day. Concentration gave a residue that was extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and concentrated. The resulting residue was purified by silica gel column chromatography to provide **9** (2.0 g, 17%) as a colorless solid. In previous study,[17] the yield of **9** was 58% from **7**. Aiming at improvement of the yield, we used 4″-*O*-Ms derivative **8** as a starting material of **9**, however, the result was a low yield because of elimination products (2″,2‴-Di-*O*-acetyl-3, 2′, 6′, 3″, 4‴-penta-*N-tert*-Boc-4″-deoxy-4″,5″-ene -6″-O-tritylarbekacin and 2″,2‴-Di-*O*-acetyl-3, 2′, 6′, 3″, 4‴-penta-*N-tert*-Boc-4″-deoxy-3″,4″-ene -6″-O-tritylarbekacin). ESI-MS calculated for $C_{70}H_{101}N_9NaO_{21}$: 1426.70; found: 1426.82 $(M+Na)^+$.

*3, 2′, 6′, 3″, 4‴-Penta-N-tert-Boc-4″-deoxy-4″-epiamino-6″-O-tritylarbekacin (**10**).* A solution of **9** (2.0 g, 1.42 mmol) in MeOH − aqueous concentrated NH_3 (2:1, 90 ml) was stirred at room temperature for 2 h. Concentration gave a 2″, 2‴-dihydroxyl derivative **9a** (1.9 g) as a solid. ESI-MS calculated for $C_{66}H_{97}N_9NaO_{19}$: 1342.68; found: 1342.78 $(M+Na)^+$. To a solution of **9a** (1.9 g, 1.44 mmol) in THF − H_2O (4:1, 30 ml) PPh_3 (1.13 g, 4.31 mmol) was added, and the mixture was stirred at 50 °C for 20 h. After concentration, the resulting residue was dissolved in pyridine (119 ml). To the solution concentrated aqueous NH_3 (90 ml) was added, and the mixture was stirred at room temperature for 3 days. The reaction mixture was concentrated, and then the residue was purified by silica gel column chromatography to provide **10** (900 mg, 49% from **9**) as a colorless solid. ESI-MS calculated for $C_{66}H_{99}N_7NaO_{19}$: 1316.69; found: 1316.82 $(M+Na)^+$.

*4″-Deoxy-4″-epiaminoarbekacin (**2**).* A solution of **10** (50.1 mg, 0.44 mmol) in TFA − H_2O (9:1, 1.0 ml) was kept at 0 °C for 2 h. Concentration gave a residue, which was purified by resin column chromatography to provide **2** (20.6 mg, 87% as a carbonate) as a colorless solid. ^1H NMR (25% ND_3-D_2O, 400 MHz) δ 1.30 (m, 1H, H-4′*ax*), 1.45 (m, 1H, H-2*ax*), 1.56 (m, 1H, H-3′*ax*), 1.60 (m, 1H, H-4′*eq*), 1.75 (m, 1H, H-3′*eq*), 1.79 (m, 1H, H-βa), 1.86 (m, 1H, H-βb), 1.98 (m, 1H, H-2*eq*), 2.62 (m, 2H, H-6′), 2.77 (m, 2H, H-γ), 2.82 (m, 1H, H-2′), 2.93 (m, 1H, H-3), 2.99 (dd, 1H, *J* = 3.7 and 7.0 Hz, H-3″), 3.07 (m, 1H, H-4″), 3.33 (t, 1H, *J* = 9.1 Hz, H-4), 3.57 (dd, 1H, *J* = 4.0 and 7.0 Hz, H-2″), 3.66 (m, 1H, H-6″a), 3.71 (m, 1H, H-6″b), 3.73 (t, 1H, *J* = 9.1 Hz, H-5), 3.76 (m, 1H, H-6), 3.82 (m, 1H, H-5′), 3.96 (m, 1H, H-1), 4.16 (m, 1H, H-5″), 4.30 (m, 1H, H-α), 5.09 (d, 1H, *J* = 4.0 Hz, H-1″) and 5.13 (d, 1H, *J* = 3.5 Hz, H-1′); ^{13}C NMR (25% ND_3-D_2O, 100.6 MHz) δ 26.9 (3′), 28.4 (4′), 35.2 (2), 37.2 (β), 38.2 (γ), 46.0 (6′), 50.1 (3), 50.4 (1), 50.8 (2′), 52.1 (3″), 52.2 (4″), 62.0 (6″), 69.9 (2″), 70.6 (5″), 71.5 (5′), 72.6 (α), 76.2 (5), 81.9 (6), 87.4 (4), 99.7 (1″), 102.4 (1′) and 177.4 (NH*C*O-1); ESI-HRMS calculated for $C_{22}H_{46}N_7O_9$: 552.3358; found: 552.3357 $(M+H)^+$.

*4″-N-[(S)-2-Benzyloxy-4-N-benzyloxycarbonylaminobutylyl]-4″-deoxy-4″-epiamino arbekacin (**12**).* To a solution of **10** (350 mg, 0.27 mmol) in MeOH − THF − H_2O (15:7.5:1, 17.5 ml) (*S*)-2-benzyloxy-4-benzyloxycarbonylaminobutyric acid (418 mg, 1.22 mmol) and DMT-MM (449 mg, 1.62 mmol) were added, and the mixture was stirred at room temperature for 16 h. The reaction mixture was evaporated, then the residue was diluted with AcOEt, and the organic solution was washed with saturated aqueous $NaHCO_3$ and water, dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography to provide **11** (376 mg, 86%) as a colorless solid. ESI-MS calculated for $C_{85}H_{118}N_8NaO_{23}$: 1641.82; found: 1641.30 $(M+Na)^+$. A solution of **11** (330 mg, 0.204 mmol) in TFA − H_2O (9:1, 6.6 ml) was kept at 0 °C for 2 h. Concentration gave a residue, which was purified by resin column chromatography to provide **12** (168 mg, 88% as a carbonate) as a colorless solid. ^1H NMR (25% ND_3-D_2O, 400 MHz) δ 1.34 (m, 1H, H-4′*ax*), 1.48 (m, 1H, H-2*ax*), 1.55 (m, 1H, H-3′*ax*), 1.60 (m, 1H, H-4′*eq*), 1.72 (m, 1H, H-3′*eq*), 1.80 (m, 1H, H-βa), 1.82 (m, 1H, H-β′a), 1.85 (m, 1H, H-βb), 1.91 (m, 1H, H-β′b), 1.99 (m, 1H, H-2*eq*), 2.60 (m, 2H, H-6′), 2.77 (m, 2H, H-γ), 2.81 (m, 1H, H-2′), 2.93 (m, 1H, H-3), 3.10 (m, 1H, H-3″), 3.19 (m, 2H, H-γ′), 3.28 (m, 1H, H-4), 3.38 (m, 1H, H-2″), 3.46 (d, 2H, *J* = 6.0 Hz, H-6″), 3.66 (m, 1H, H-5), 3.73 (m, 1H, H-6), 3.81 (m, 1H, H-5′), 3.98 (m, 1H, H-1), 4.08 (m, 1H, H-α′), 4.15 (m, 1H, H-4″), 4.23 (m, 1H, H-α), 4.40 (m, 1H, H-5″), 4.58 (m, 2H, C*H*$_2$C$_6$H$_5$), 5.01 (d, 1H, *J* = 4.0 Hz, H-1″), 5.09 (m, 2H, C*H*$_2$C$_6$H$_5$), 5.18 (d, 1H, *J* = 3.6 Hz, H-1′) and 7.31 − 7.50 (m, 10H, 2X C$_6$*H*$_5$); ^{13}C NMR (25% ND_3-D_2O, 100.6 MHz) δ 26.9 (3′), 28.4 (4′), 33.5 (β′), 35.2 (2), 37.2 (β), 37.7 (γ′), 38.2 (γ), 46.0 (6′), 50.0 (3), 50.3 (1), 50.8 (2′), 51.2 (3″), 51.3 (4″), 61.4 (6″), 67.6 (*C*H$_2$C$_6$H$_5$), 70.6 (2″), 70.7 (5″), 71.0 (5′), 71.5 (α), 73.6 (*C*H$_2$C$_6$H$_5$), 76.0 (5), 78.1 (α′), 81.8 (6), 87.4 (4), 99.5 (1″), 102.3 (1′), 128.6 (2C, *C*$_6$H$_5$), 129.2 (2C, *C*$_6$H$_5$), 129.3 (2C, *C*$_6$H$_5$), 129.4 (4C, *C*$_6$H$_5$), 129.6 (1C, *C*$_6$H$_5$), 129.8 (1C, *C*$_6$H$_5$), 158.9 (γ′-NH*C*O), 176.3 (NH*C*O-6″) and 177.4 (NH*C*O-1); ESI-HRMS calculated for $C_{41}H_{65}N_8O_{13}$: 877.4672; found: 877.4669 $(M+H)^+$.

*4″-N-[(S)-4-Amino-2-hydroxybutylyl]-4″-deoxy-4″-epi-aminoarbekacin (**13**).* A solution of **12** (93.5 mg, 0.099 mmol) in 50% aqueous AcOH (5.6 ml) was hydrogenated in the presence of Pd/C for 4 h at room temperature. After filtration, the filtrate was concentrated, and the solid was purified by resin column chromatography to provide **13** (55.7 mg, 78% as a carbonate) as a colorless solid. ^1H NMR (25% ND_3-D_2O, 400 MHz) δ 1.36 (m, 1H, H-4′*ax*), 1.50 (m, 1H, H-2*ax*), 1.58 (m, 1H, H-3′*ax*), 1.69 (m, 1H, H-4′*eq*), 1.76 (m, 1H, H-3′*eq*), 1.78 (m, 1H, H-βa), 1.81 (m, 1H, H-β′a), 1.86 (m, 1H, H-βb), 1.91 (m, 1H, H-β′b), 2.000 (m, 1H, H-2*eq*), 2.62 (m, 2H, H-6′), 2.75 (m, 1H, H-γ′ a), 2.79 (m, 2H, H-γ), 2.81 (m, 1H, H-2′), 2.92 (m, 1H, H-3), 3.14 (m, 1H, H-3″), 3.18 (m, 1H, H-γ′b), 3.37 (m, 1H, H-4), 3.48 (m, 1H, H-2″), 3.56 (m, 2H, H-6″), 3.68 (m, 1H, H-5), 3.78 (m, 1H, H-6), 3.83 (m, 1H, H-5′), 3.98 (m, 1H, H-1), 4.15 (dd, 1H, *J* = 3.6 and 5.7 Hz, H-α), 4.27 (m, 1H, H-4″), 4.31 (m, 1H, H-α), 4.48 (m, 1H, H-5″), 5.13 (d, 1H, *J* = 3.5 Hz, H-1′) and 5.15 (d, 1H, *J* = 3.9 Hz, H-1″); ^{13}C NMR (25% ND_3-D_2O, 100.6 MHz) δ 26.9 (3′), 28.4 (4′), 35.2 (2), 37.1 (β′), 37.2 (β), 37.9 (γ′), 38.0 (γ), 46.0 (6′), 50.0 (3), 50.3 (1), 50.7 (2′), 50.7 (3″), 51.3 (4″), 61.2 (6″), 70.3 (2″), 70.5 (α), 70.7 (α′), 70.9 (5″), 71.4 (5′), 76.0 (5), 81.7 (6), 87.4 (4), 99.6 (1″), 102.3 (1′), 177.3 (NH*C*O-6″) and 178.3 (NH*C*O-1); ESI-HRMS calculated for $C_{26}H_{53}N_8O_{11}$: 653.3835; found: 653.3839 $(M+H)^+$.

Antibacterial assay

The MICs were examined by the serial agar dilution method using Mueller-Hinton agar (Becton, Dickinson and Company, New Jersey, NJ, USA) for *S. aureus*, *E. coli* and *P. aeruginosa*, and using Mueller-Hinton agar supplemented with 5% defibrinated sheep blood (Nippon Biotest Laboratories Inc., Tokyo, Japan) under 5% CO_2 for *E. faecalis*. The test suspension was prepared at approximately 10^4 CFU per 5 μl using a microplanter MIT-P inculum replicating apparatus. The MIC was defined as the lowest concentration of antibiotic that inhibited development of visible growth on the agar after 18 h incubation at 37 °C for Gram-positive and Gram-negative bacteria.

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