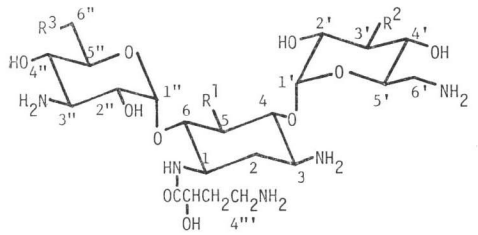
CHEMICAL MODIFICATION OF 3′-DEOXYAMIKACIN

Sir:

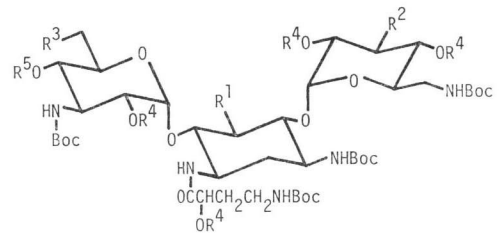
As reported previously, 3′-deoxyamikacin[1] (**2**) has better activity than amikacin[2] (**1**) in inhibiting the growth of Gram-positive and -negative bacteria, and 6″-deoxyamikacin[3] has slightly weaker activity against *Pseudomonas aeruginosa* than **1**. Furthermore, 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-5,3′,4′-trideoxykanamycin B[4] has very strong activity against Gram-positive and -negative bacteria including pseudomonas. Therefore, we synthesized 3′,6″-dideoxy (**3**), 5,3′-dideoxy (**4**) and 5,3′,6″-trideoxy (**5**) derivatives of amikacin (**1**), starting from 3′-deoxyamikacin (**2**), and measured their antibacterial activities. 5-Deoxyamikacin (**6**) was also synthesized from **1**. In this paper, we will report on the synthesis of these deoxyamikacins.



	R^1	R^2	R^3
1	OH	OH	OH
2	OH	H	OH
3	OH	H	H
4	H	H	OH
5	H	H	H
6	H	OH	OH

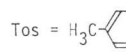
Four amino groups of **2** were protected with the *tert*-butoxycarbonyl (Boc) group by reaction with an excess of *tert*-butyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate (Kokusan Chemical Works) in a mixture of methanol, water and triethylamine (10: 10: 1) at 50°C for 3 hours to afford 3,6′,6″, 4‴-tetra-*N*-Boc-3′-deoxyamikacin (**7**) in 85% yield.

Treatment of **7** with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide in the presence of *p*-toluenesulfonic acid at room temperature for 23 hours, followed by acetylation with acetic anhydride in pyridine in the presence of 4-(*N*,*N*-dimethylamino)pyridine at room temperature for 24 hours and by column chromatography on silica gel (chloroform-methanol, 70: 1) gave 2′,4′, 2″, 2‴-tetra-*O*-acetyl-3,6′,3″,4‴-tetra-*N*-Boc-3′-deoxy-4″,6″-*O*-isopropylideneamikacin (**8**) in 59% yield. Hydrolysis of the *O*-isopropylidene group in **8** with a mixture of acetic acid, methanol and water (3: 3: 1) at 50°C for 5.5 hours afforded 2′,4′,2″,2‴-tetra-*O*-acetyl-3,6′,3″,4‴-tetra-*N*-Boc-3′-deoxyamikacin (**9**) in a quantitative yield. Tosylation of **9** with two equivalents of *p*-toluenesulfonyl chloride in pyridine at room temperature for 9 hours, followed by chloroform extraction gave the 6″-*O*-tosyl derivative **10** in 97% yield. Compound **10** was treated with an excess of sodium iodide in *N*,*N*-dimethylformamide at 90°C for 5 hours to yield the 6″-iodo derivative



	R^1	R^2	R^3	R^4	R^5
7	OH	H	OH	H	H
9	OH	H	OH	Ac	H
10	OH	H	OTos	Ac	H
11	OH	H	I	Ac	H
12	OH	H	H	H	H
13	Cl	H	OBz	Bz	Bz
14	H	H	OBz	Bz	Bz
15	H	H	OH	H	H
16	Cl	H	H	Bz	Bz
17	OH	OH	H	H	H
18	Cl	OAc	OAc	Ac	Ac

Boc = $(CH_3)_3COCO$ Ac = CH_3CO

Tos =  Bz = 

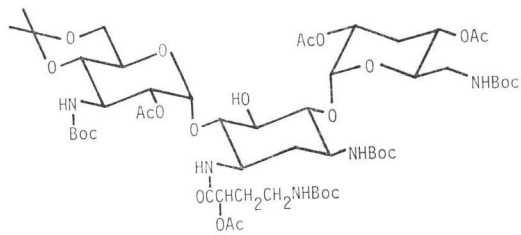


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Table 1. Minimum inhibitory concentrations (μg/ml) of 5-, 3′- and 6″-deoxy derivatives of amikacin on nutrient agar plates.

Test organism	1	2	3	4	5	6
Staphylococcus aureus 209P	1.56	<0.20	<0.20	0.39	0.39	0.78
S. aureus Smith	0.20	<0.20	<0.20	<0.20	<0.20	0.39
S. aureus Ap01[a]	1.56	1.56	3.13	6.25	12.5	6.25
S. epidermidis 109[a]	1.56	1.56	3.13	12.5	12.5	6.25
Micrococcus flavus FDA16	3.13	3.13	3.13	3.13	6.25	12.5
M. luteus PCI1001	3.13	0.78	3.13	0.78	0.78	12.5
Bacillus anthracis	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
B. subtilis PCI219	<0.20	<0.20	<0.20	<0.20	<0.20	0.20
B. subtilis NRRL B-558	0.39	<0.20	<0.20	<0.20	<0.20	0.20
B. cereus ATCC10702	1.56	1.56	0.78	1.56	1.56	3.13
Mycobacterium smegmatis ATCC607	0.78	0.39	0.78	0.39	0.78	0.78
Escherichia coli NIHJ	0.78	0.78	0.78	1.56	1.56	3.13
E. coli K-12	0.78	0.78	0.39	1.56	0.78	1.56
E. coli K-12 R5[b]	100	>100	100	>100	>100	>100
E. coli K-12 R388	0.78	0.78	0.39	1.56	0.78	1.56
E. coli K-12 J5R11-2[c]	1.56	0.78	0.78	1.56	0.78	3.13
E. coli K-12 ML1629[c]	1.56	1.56	0.78	3.13	1.56	1.56
E. coli K-12 ML1630	3.13	1.56	0.78	1.56	1.56	3.13
E. coli K-12 ML1410	6.25	0.78	1.56	1.56	1.56	3.13
E. coli K-12 ML1410 R81[c]	3.13	0.78	0.78	1.56	1.56	3.13
E. coli K-12 LA290 R55[d]	3.13	1.56	1.56	3.13	3.13	6.25
E. coli K-12 LA290 R56	1.56	0.39	0.78	0.78	0.78	3.13
E. coli K-12 LA290 R64	1.56	0.78	0.78	1.56	1.56	3.13
E. coli W677	3.13	0.78	0.78	1.56	0.78	3.13
E. coli JR66/W677[d,e]	6.25	1.56	1.56	3.13	3.13	12.5
E. coli K-12 C600 R135[f]	1.56	0.78	0.78	1.56	1.56	3.13
E. coli JR225[f]	1.56	0.39	0.78	0.78	0.78	1.56
Klebsiella pneumoniae PCI602	0.78	1.56	0.78	1.56	0.78	3.13
K. pneumoniae 22#3038[d,e]	3.13	3.13	1.56	3.13	3.13	12.5
Shigella dysenteriae JS11910	6.25	6.25	3.13	6.25	3.13	6.25
S. flexneri 4b JS11811	6.25	3.13	1.56	6.25	3.13	6.25
S. sonnei JS11746	6.25	12.5	3.13	6.25	6.25	12.5
Salmonella typhi T-63	0.78	50	0.78	6.25	6.25	3.13
S. enteritidis 1891	1.56	3.13	1.56	3.13	3.13	3.13
Proteus vulgaris OX19	1.56	0.78	0.39	0.78	0.78	1.56
P. rettgeri GN311	12.5	25	12.5	25	12.5	50
P. rettgeri GN466	6.25	3.13	3.13	3.13	6.25	12.5
Serratia marcescens	12.5	25	12.5	12.5	6.25	25
Serratia sp. SOU	50	>100	100	25	25	25
Serratia sp. 4	12.5	12.5	12.5	12.5	25	25
Providencia sp. Pv16[g]	12.5	25	12.5	25	25	25
Providencia sp. 2991[g]	12.5	12.5	6.25	25	50	12.5
Pseudomonas aeruginosa A3	3.13	0.78	1.56	1.56	3.13	3.13
P. aeruginosa No. 12	6.25	6.25	12.5	25	25	12.5
P. aeruginosa H9[e]	6.25	12.5	12.5	6.25	12.5	50
P. aeruginosa H11	25	12.5	25	25	50	25
P. aeruginosa TI-13[c]	6.25	12.5	12.5	12.5	25	12.5
P. aeruginosa GN315[b]	>100	>100	100	>100	>100	>100
P. aeruginosa 99[f]	12.5	12.5	12.5	25	50	12.5
P. aeruginosa B-13[e,e]	12.5	50	25	25	50	12.5
P. aeruginosa 21-75[h]	12.5	12.5	100	25	100	25
P. aeruginosa PST1[f]	25	12.5	25	12.5	50	>100
P. aeruginosa ROS134/PU21[f]	100	>100	100	50	>100	50
P. aeruginosa K-Ps102[i]	12.5	6.25	12.5	12.5	25	12.5
P. maltophilia GN907[i]	>100	>100	>100	>100	>100	>100

[a] AAD(4′), [b] AAC(6′), [c] APH(3′)-I, [d] AAD(2″), [e] APH(3′)-II, [f] AAC(3), [g] AAC(2′), [h] APH(3′)-III, [i] permeability.

11 (49% yield), which was purified by column chromatography on silica gel (chloroform - methanol, 60: 1), mp 135~143°C (decomp.), $[\alpha]_D^{25}$ +81° (*c* 0.2, $CHCl_3$). Dehalogenation of **11** by catalytic hydrogenation with Raney-Ni (R-200, Nikko Scientific & Chemical Ind.) in dioxane in a Parr apparatus (3.5 kg/cm²) for 19.5 hours, followed by deacetylation with 16% ammonia in methanol at room temperature for 19 hours and by column chromatography on silica gel (chloroform - methanol, 20: 1) gave 3,6′,3″,4‴-tetra-*N*-Boc-3′,6″-dideoxyamikacin (**12**) in 65% yield. The Boc groups in **12** were removed with 90% trifluoroacetic acid to yield 3′,6″-dideoxyamikacin (**3**, 78% yield), which was purified by column chromatography on Amberlite CG-50(NH_4^+) eluted with 0.8 M NH_4OH, mp 151~155°C (decomp.), $[\alpha]_D^{21}$ +62° (*c* 1, H_2O), ^1H NMR (D_2O): δ 1.69 (3H, d J=6.0 Hz, 6″-CH_3). *Anal.* Calcd. for $C_{22}H_{43}N_5O_{11}\cdot H_2CO_3\cdot H_2O$: C 43.60, H 7.48, N 11.05. Found: C 43.20, H 7.27, N 10.67.

Benzoylation of **7** with benzoyl chloride in pyridine at room temperature for 1 hour, followed by halogenation with sulfuryl chloride[5] in pyridine under ice-cooling for 1.5 hours and by column chromatography on silica gel (chloroform - methanol, 130: 1) gave 2′,4′,2″,4″,6″,2‴-hexa-*O*-benzoyl-3,6′,3″,4‴-tetra-*N*-Boc-5-chloro-5,3′-dideoxyamikacin (**13**) in 47% yield. Compound **13** was hydrogenated with Raney-Ni in dioxane in a Parr apparatus (3.5 kg/cm²) for 45 hours to yield 2′,4′,2″,4″,6″,2‴-hexa-*O*-benzoyl-3,6′,3″,4‴-tetra-*N*-Boc-5,3′-dideoxyamikacin (**14**, 97% yield), mp 117~122°C (decomp.), $[\alpha]_D^{25}$ +102° (*c* 0.5, $CHCl_3$). Hydrolysis of benzoyl groups with 16% ammonia in methanol at room temperature for 21 hours, followed by column chromatography on silica gel (chloroform - methanol, 20: 1) afforded 3,6′,3″,4‴-tetra-*N*-Boc-5,3′-dideoxyamikacin (**15**) in 52% yield. The Boc groups in **15** were removed with 90% trifluoroacetic acid at room temperature for 19 hours to yield 5,3′-dideoxyamikacin (**4**, 80% yield), which was purified by column chromatography on Amberlite CG-50(NH_4^+) eluted with 0.8 M NH_4OH, mp 154~156°C (decomp.), $[\alpha]_D^{27}$ +91° (*c* 1, H_2O). *Anal.* Calcd. for $C_{22}H_{43}N_5O_{11}\cdot 2H_2CO_3$: C 42.54, H 6.99, N 10.34. Found C 42.97, H 7.02, N 10.38.

Benzoylation of **12** with benzoyl chloride in pyridine at room temperature for 1 hour, followed by chlorination with sulfuryl chloride in pyridine under ice-cooling for 2 hours, and by column chromatography on silica gel (chloroform - methanol, 120: 1) gave 2′,4′,2″,4″,2‴-penta-*O*-benzoyl-3,6′,3″,4‴-tetra-*N*-Boc-5-chloro-5,3′,6″-trideoxyamikacin (**16**, 49% yield), mp 133~139°C (decomp.), $[\alpha]_D^{25}$ +90° (*c* 0.3, $CHCl_3$). Catalytic hydrogenation of **16** with Raney-Ni, removal of *O*- and *N*-protective groups by hydrolysis with 16% ammonia in methanol and then with 90% trifluoroacetic acid, followed by column chromatography on Amberlite CG-50 (NH_4^+) gave 5,3′,6″-trideoxyamikacin (**5**, 29% yield), mp 157~162°C (decomp.), $[\alpha]_D^{27}$ +73° (*c* 1, H_2O), ^1H NMR(D_2O): δ 1.68 (3H, d J=6.0 Hz, 6″-CH_3). *Anal.* Calcd. for $C_{22}H_{43}N_5O_{10}\cdot 2H_2CO_3\cdot H_2O$: C 42.41, H 7.26, N 10.31. Found: C 42.35, H 7.00, N 10.01.

By the similar synthetic route for the 5-deoxygenation,[5] 5-deoxyamikacin (**6**) [mp 156~159°C (decomp.), $[\alpha]_D^{23}$ +70° (*c* 0.5, H_2O). *Anal.* Calcd. for $C_{22}H_{43}N_5O_{12}\cdot 3H_2CO_3\cdot 1/2H_2O$; C 39.27, H 6.59, N 9.16. Found: C 39.42, H 6.59, N 9.28] was synthesized starting from 3,6′,3″,4‴-tetra-*N*-Boc-amikacin[3] (**17**) through 2′,3′,4′,2″,4″,6″,2‴-hepta-*O*-acetyl-3,6′,3″,4‴-tetra-*N*-Boc-5-chloro-5-deoxyamikacin [**18**, mp 126~131°C (decomp.), $[\alpha]_D^{25}$ +85° (*c* 0.2, $CHCl_3$)].

The minimum inhibitory concentrations of four new derivatives (**3**~**6**) on nutrient agar plates are shown in Table 1 in comparison with those of amikacin (**1**) and 3′-deoxyamikacin (**2**). Among these compounds, 3′,6″-dideoxyamikacin (**3**) showed the strongest activity compared with **1** and other deoxyamikacins. Intravenous administrations of compounds **3**~**6** at 200 mg/kg caused no deaths in mice.

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