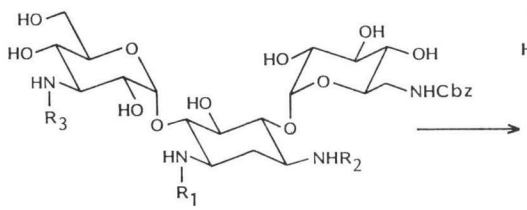
THE 6′-AMIDINE DERIVATIVES OF AMIKACIN AND DIBEKACIN

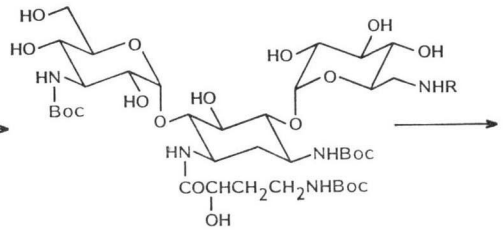
Sir:

As has previously been reported, 6′-*N*-methylation[1,2] and 6′-*C*-alkylation[3] of kanamycins leads to derivatives which have good antibacterial activity against resistant strains producing 6′-*N*-acetyltransferases [AAC(6′)]. In this paper, we report the preparation and antibacterial activity of 6′-*N*-formimidoylamikacin (**1**), 6′-*N*-acetimidoylamikacin (**2**) and 6′-*N*-formimidoyldibekacin (**3**).

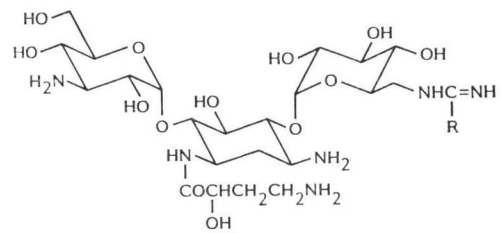
Compounds **1** and **2** were synthesized starting from 6′-*N*-benzyloxycarbonylkanamycin (**4**)[1,4] through 3, 3″,4‴-tri-*N*-*tert*-butoxycarbonylamikacin (**9**). The selective protection at the 3-amino group of **4** with *tert*-butyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate (Boc-S reagent, Kokusan Chemical Works) in DMSO by the method of zinc-chelation[5] gave 6′-*N*-benzyloxycarbonyl-3-*N*-*tert*-butoxycarbonylkanamycin (**5**) in 70% yield. Successive treatment of **5** with ethyl trifluoroacetate in DMSO for 1 hour at room temperature followed by the *N*-hydroxysuccinimide ester of (*S*)-4-*tert*-butoxycarbonylamino-2-hydroxybutyric acid in the presence of triethylamine in DMSO for 4 hours at room temperature



4 R_1, R_2, R_3 = H
5 R_1, R_3 = H, R_2 = Boc
6 R_1 = COCH(OH)CH_2CH_2NHBoc, R_2 = Boc, R_3 = $COCF_3$
7 R_1 = COCH(OH)$CH_2CH_2NH_2$, R_2, R_3 = H
8 R_1 = COCH(OH)CH_2CH_2NHBoc, R_2, R_3 = Boc

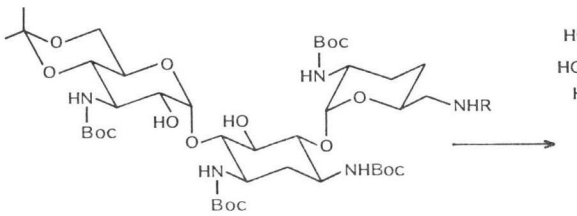


9 R = H
10 R = CH=NH
11 R = C(=NH)CH_3



1 R = H
2 R = CH_3

Cbz = $COOCH_2C_6H_5$
Boc = $COOC(CH_3)_3$



12 R = H
13 R = CH=NH

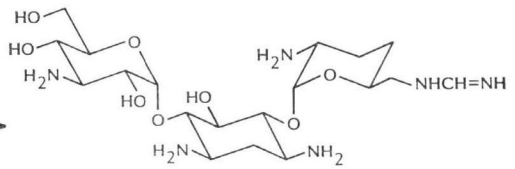


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Table 1. Minimum inhibitory concentrations on Mueller-Hinton agar plates.

Test organism	Minimum inhibitory concentrations* (μg/ml)				
	6′-*N*-Formimidoyl-amikacin (**1**)	6′-*N*-Acetimidoyl-amikacin (**2**)	Amikacin	6′-*N*-Formimidoyl-dibekacin (**3**)	Dibekacin
Staphylococcus aureus 209P	3.13	3.13	1.56	0.78	<0.20
S. aureus Smith	1.56	1.56	0.78	<0.20	<0.20
S. aureus Ap01[a]	3.13	6.25	1.56	3.13	0.78
S. epidermidis 109[a]	3.13	6.25	6.25	3.13	0.78
Micrococcus flavus FDA16	12.5	12.5	3.13	50	6.25
Micrococcus luteus PCI1001	6.25	12.5	3.13	25	6.25
Bacillus anthracis	1.56	1.56	0.78	0.39	0.39
B. subtilis PCI219	0.78	0.78	0.39	<0.20	<0.20
B. subtilis NRRL B-558	0.78	1.56	0.39	<0.20	<0.20
B. cereus ATCC 10702	3.13	12.5	3.13	6.25	1.56
Corynebacterium bovis 1810	6.25	6.25	1.56	12.5	3.13
Mycobacterium smegmatis ATCC 607	1.56	1.56	0.78	1.56	0.78
Escherichia coli NIHJ	0.78	3.13	0.78	3.13	0.39
E. coli K-12	0.78	1.56	0.39	0.78	0.39
E. coli K-12 R5[b]	12.5	12.5	100	3.13	100
E. coli K-12 P388	1.56	1.56	0.78	0.78	0.39
E. coli K-12 J5R11-2[c]	3.13	3.13	1.56	3.13	0.39
E. coli K-12 ML1629[e]	3.13	3.13	3.13	1.56	0.39
E. coli K-12 ML1630	12.5	12.5	3.13	3.13	0.78
E. coli K-12 ML1410	12.5	12.5	3.13	3.13	0.78
E. coli K-12 ML1410 R81[c]	6.25	6.25	3.13	6.25	0.39
E. coli K-12 LA290 R55[d]	6.25	3.13	1.56	25	25
E. coli K-12 LA290 R56	3.13	3.13	0.78	1.56	6.25
E. coli K-12 LA290 R64	3.13	3.13	3.13	1.56	3.13
E. coli W677	1.56	3.13	0.78	1.56	0.20
E. coli JR66/W677[d,e]	12.5	12.5	3.13	100	25
E. coli K-12 C600 R135[f]	3.13	3.13	1.56	0.78	0.39
E. coli JR225[f]	1.56	1.56	0.78	>100	50
Klebsiella pneumoniae PCI602	3.13	3.13	1.56	1.56	0.39
K. pneumoniae 22 #3038[d,e]	6.25	12.5	3.13	50	100
Shigella dysenteriae JS11910	12.5	12.5	1.56	3.13	1.56
S. flexneri 4b JS11811	12.5	12.5	1.56	3.13	0.78
S. sonnei JS11746	6.25	6.25	1.56	3.13	0.78
Salmonella typhi T-63	3.13	3.13	1.56	1.56	<0.20
S. enteritidis 1891	3.13	6.25	1.56	1.56	0.78
Proteus vulgaris OX19	1.56	1.56	1.56	0.39	<0.20
P. rettgeri GN311	1.56	1.56	0.78	<0.20	<0.20
P. rettgeri GN466	1.56	1.56	0.78	0.39	<0.20
Serratia marcescens	6.25	6.25	6.25	3.13	6.25
Serratia sp. SOU	6.25	12.5	25	3.13	50
Serratia sp. 4	25	25	3.13	6.25	0.78
Providencia sp. Pv16[g]	3.13	3.13	0.78	50	25
Providencia sp. 2991[g]	3.13	6.25	3.13	>100	100
Pseudomonas aeruginosa A3	0.78	1.56	0.20	0.39	<0.20
P. aeruginosa No. 12	25	25	6.25	6.25	1.56
P. aeruginosa H9[e]	25	25	6.25	6.25	3.13
P. aeruginosa H11	100	100	25	25	6.25
P. aeruginosa TI-13[c]	12.5	6.25	3.13	6.25	1.56
P. aeruginosa GN315[b]	25	25	50	6.25	100
P. aeruginosa 99[f]	50	50	12.5	12.5	3.13
P. aeruginosa B-13[c,e]	50	50	25	25	3.13
P. aeruginosa 21-75 [h]	50	50	12.5	>100	>100
P. aeruginosa PSTI[f]	50	50	12.5	>100	>100
P. aeruginosa ROS134/PU21[f]	>100	>100	50	>100	>100
P. aeruginosa K-Ps102[i]	25	50	6.25	12.5	3.13
P. maltophilia GN907[i]	>100	>100	>100	>100	100

Resistance: [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.

* Concentrations are shown as the free bases.

afforded **6**. Removal of the *tert*-butoxycarbonyl and trifluoroacetyl groups in **6** were achieved by treatment with 90% trifluoroacetic acid followed by adjustment of the pH to 11 with 17% ammonia to yield 6′-*N*-benzyloxycarbonylamikacin (**7**, 61% yield from **5**) which was purified by column chromatography on Amberlite CG-50 (NH_4^+) with 0.3 M ammonia.

The amino groups of **7** were protected with Boc-S reagent in methanol in the presence of triethylamine at 60°C to give 6′-*N*-benzyloxycarbonyl-3,3″,4‴-tri-*N*-*tert*-butoxycarbonylamikacin (**8**, 72% yield). Hydrogenolysis of **8** with 5% Pd on carbon in a mixture (300: 1) of 90% aqueous methanol and acetic acid under an atmospheric hydrogen stream provided 3,3″,4‴-tri-*N*-*tert*-butoxycarbonylamikacin (**9**) in 88% yield.

The 6′-amino group of **9** was amidinated by treatment with three equivalents of ethyl formimidate hydrochloride in anhydrous methanol for 16 hours at 0°C to room temperature to afford 3,3″,4‴-tri-*N*-*tert*-butoxycarbonyl-6′-*N*-formimidoylamikacin (**10**) in 81% yield. Removal of the *tert*-butoxycarbonyl groups in **10** with 90% trifluoroacetic acid at 0~5°C for 2 hours, followed by column chromatography on Amberlite IRA-400 (SO_4^{2-}) gave 6′-*N*-formimidoylamikacin (**1**) as the disulfate sesquihydrate in 74% yield, mp 209~213°C (dec), $[\alpha]_D^{23}$ +46° (*c* 1, water), SIMS (glycerol matrix) *m/z* 613 (MH^+).

Treatment of **9** with methyl acetimidate hydrochloride in anhydrous methanol for 3 hours at 60°C gave 3,3″,4‴-tri-*N*-*tert*-butoxycarbonyl-6′-*N*-acetimidoylamikacin hydrochloride (**11**, 51% yield). Further treatment of **11** as described for **10** afforded 6′-*N*-acetimidoylamikacin (**2**) as the disulfate dihydrate (77% yield), mp 204~207°C (dec), $[\alpha]_D^{21}$ +47° (*c* 1, water), SIMS (glycerol matrix) *m/z* 627 (MH^+).

6′-*N*-Formimidoyldibekacin (**3**) was synthesized from 1,3,2′,3″-tetra-*N*-*tert*-butoxycarbonyl-4″,6″-*O*-isopropylidenedibekacin (**12**)[3] through **13** by amidination with ethyl formimidate hydrochloride in anhydrous methanol for 16 hours at 0°C to room temperature, followed by treatment with 90% trifluoroacetic acid for 2 hours at 0°C and the salt-exchange on a column of Amberlite IRA-400 (SO_4^{2-}). Compound **3** was obtained as the sestersulfate monohydrate in 63% yield, mp 208~213°C (dec), $[\alpha]_D^{23}$ +77° (*c* 1, water), SIMS (glycerol matrix) *m/z* 479 (MH^+).

Minimum inhibitory concentrations of compounds **1**, **2** and **3** on Mueller-Hinton agar plates are shown in Table 1 in comparison with those of amikacin and dibekacin. All these compounds are more active than parent antibiotics against resistant strains, *Escherichia coli* K-12 R-5 and *Pseudomonas aeruginosa* GN315 which produced AAC(6′). Generally however, the 6′-*N*-amidination slightly decreased the antibacterial activity of kanamycins.

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