SYNTHESES AND PROPERTIES OF THE 6''-DEOXY OR 4'',6''-DIDEOXY DERIVATIVES OF THE KANAMYCIN ANTIBIOTICS

Sir:

Chemical modification of kanamycin antibiotics on the basis of the biochemical mechanism of resistance has afforded derivatives which are active against resistant bacteria including *Pseudomonas aeruginosa*. These analogs, which include 3',4'-dideoxykanamycin B (dibekacin)[1] and 1-N-[(*S*)-4-amino-2-hydroxybutyryl]kanamycin (amikacin)[2] have been widely used in treatment of resistant infections. Enzymes involved in these resistance mechanisms transfer the phosphoryl or adenylyl group from ATP to a hydroxyl group of the aminoglycoside or the acetyl group from acetyl CoA to an amino group. Therefore, these enzymes should have two binding sites, the one to the adenosine moiety of ATP or acetyl CoA and the other to aminoglycosides. It suggests that these enzymes should have close evolutional relationships and in the future, new enzymes which transfer phosphoryl or adenylyl groups to the 2'-, 4''- and 6''-hydroxyl groups of kanamycin and its derivatives may appear. Considering this possibility, the authors were interested in the structure of the active derivative which has the least number of hydroxyl groups. In order to reach this objective we have synthesized polydeoxy compounds of the kanamycin antibiotics starting from dibekacin (**1**), 1-N-[(*S*)-4-amino-2-hydroxybutyryl] dibekacin[3] (**2**), amikacin (**3**), and 3',4'-dideoxyamikacin[4] (**4**). We wish to report the preparation of the following polydeoxy analogs: 4'', 6''-dideoxydibekacin (3',4',4'',6''-tetradeoxykanamycin B, **5**), 6''-deoxydibekacin (3',4',6''-trideoxykanamycin B, **6**), 1-N-[(*S*)-4-amino-2-hydroxybutyryl]-4'',6''-dideoxydibekacin (**7**), 1-N-[(*S*)-4-amino-2-hydroxybutyryl]-6''-deoxydibekacin (**8**), 4'',6''-dideoxyamikacin (**9**), 6''-deoxyamikacin (**10**), 3',4',4'',6''-tetradeoxyamikacin (**11**) and 3',4',6''-trideoxyamikacin (**12**). All of them showed a good activity against Gram-positive and negative bacteria except against *Pseudomonas aeruginosa*.

The free amino groups of **1** were protected with the *tert*-butoxycarbonyl (Boc) group by reaction with *tert*-butyl S-4,6-dimethylpyrimid-2-ylthiocarbonate in a mixture of methanol, water and triethylamine at 60°C for 5 hours to afford 1,3,2',6',3''-penta-N-Boc-dibekacin (**13**) in 75% yield. Compound **13** was treated with 1,1-dimethoxycyclohexane in N,N-dimethylformamide in the presence of *p*-toluenesulfonic acid at room temperature for 16 hours to yield 1,3,2',6',3''-penta-N-Boc-4'',6''-O-cyclohexylidenedibekacin (**14**) in quantitative yield.

Benzoylation of **14** with benzoyl chloride in pyridine overnight at 60°C gave 2''-O-benzoyl-1,3,2',6',3''-penta-N-Boc-4'',6''-O-cyclohexylidenedibekacin (**15**, 95% yield). Hydrolysis of the O-cyclohexylidene group in **15** with a mixture of acetic acid, methanol and water (2:1:1) at 40~50°C for 4 hours followed by chloroform extraction afforded 2''-O-benzoyl-1,3,2',6',3''-penta-N-Boc-dibekacin (**16**, in quantitative yield), mp 148~156°C (decomp.), $[\alpha]_D^{23}+84°$ (*c* 0.5, $CHCl_3$).

Mesylation of **16** with 2.4 equivalents of methanesulfonyl chloride in pyridine overnight at 30°C followed by silica gel column chromatography (chloroform-ethanol, 50:1) afforded the 4'', 6''-di-O-mesyl derivative **17** in 20% yield and the 6''-O-mesyl derivative **18** in 47% yield. Compound **17** was also obtained in 88% yield by mesylation of **16** with 5 equivalents of methanesulfonyl chloride at 40~50°C.

Compound **17** was treated with an excess of sodium iodide in N,N-dimethylformamide at 95°C for 5 hours under argon atmosphere to yield the 4'',6''-di-iodo derivative **19** (57% yield), which was purified by preparative TLC on silica gel plates (chloroform-ethanol, 20:1). Dehalogenation of **19** by catalytic hydrogenation with RANEY-Ni in dioxane in a PARR apparatus (3.6 kg/cm^2), debenzolyation with 12% ammonia in methanol, removal of the Boc groups with 90% trifluoroacetic acid, followed by column chromatography on Amberlite CG-50 (NH_4^+) resin and elution with 0.4 N ammonia gave 4'',6''-dideoxydibekacin (**5**) as the sesquicarbonate salt in 52% yield from **19**.

Halogenation of **18** with sodium iodide, dehalogenation of the 6''-iodide **20** by catalytic hydrogenation, removal of the O- and N-protective groups by hydrolysis with 12% ammonia in methanol and then with 90% trifluoroacetic acid, followed by column chromatography on Amberlite CG-50 resin gave 6''-deoxydibekacin (**6**) as the sesquicarbonate salt in 75% yield from **18**.

By the similar synthetic route, the 1-N-[(*S*)-4-

amino-2-hydroxybutyryl] derivatives (**7** and **8**) of compounds **5** and **6** were synthesized from 1-N-[(*S*)-4-amino-2-hydroxybutyryl] dibekacin[3] (**2**) through its 2'', 2'''-di-O-benzoyl-3, 2', 6', 3'', 4'''-penta-N-Boc derivative (**21**), mp 128~138°C (decomp.), $[\alpha]_D^{22}$ +48° (*c* 1, $CHCl_3$).

3, 6', 3'', 4'''-Tetra-N-Boc-amikacin (**22**) prepared from amikacin (**3**, 78% yield) was treated with an excess of 2,2-dimethoxypropane in N,N-dimethylformamide in the presence of *p*-toluenesulfonic acid at room temperature for 22 hours to yield the 4'',6''-O-isopropylidene derivative **23** (66% yield), which was purified by silica gel column chromatography (chloroform-ethanol, 10: 1). Acetylation of **23** with acetic anhydride in pyridine at room temperature for 43 hours gave the 2',3',4',2'',2'''-penta-O-acetyl derivative **24** in 97% yield. Hydrolysis of the O-isopropylidene group in **24** with a mixture of acetic acid, methanol and water (3: 3: 1) at 50°C for 5 hours and then at room temperature for 16 hours, followed by chloroform extraction yielded 2',3',4',2'',2'''-penta-O-acetyl-3,6', 3'',4'''-tetra-N-Boc-amikacin (**25**, 98% yield), mp 123~129°C (decomp.), $[\alpha]_D^{24}$ +69° (*c* 1, $CHCl_3$).

Mesylation of **25** with 6 equivalents of methanesulfonyl chloride in pyridine at room temperature for 17 hours afforded the 4'',6''-di-O-mesyl derivative **26** in 98% yield. Halogenation of **26** with an excess of sodium iodide in N,N-dimethylformamide at 90°C for 17.5 hours followed by silica gel column chromatography (dichloromethane - ethanol, 80: 1) gave the 4'', 6''-di-iodo derivative **27** in 73% yield. Dehalogenation of **27** by catalytic hydrogenation with RANEY-Ni in dioxane in a PARR apparatus (4.3 kg/cm²) for 20 hours, deacetylation with 12% ammonia in methanol, removal of the Boc groups with 90% trifluoroacetic acid, followed by column chromatography on Amberlite CG-50 (NH_4^+) resin and elution with 0.65 N ammonia gave 4'',6''-dideoxyamikacin (**9**) as the dicarbonate salt in 40% yield from **27**.

Tosylation of **25** with 5 equivalents of *p*-toluenesulfonyl chloride in pyridine at room temperature for 18 hours followed by silica gel column chromatography (dichloromethane - ethanol, 50: 1) gave the 6''-O-tosyl derivative **28** in 75% yield. Halogenation of **28** with an excess of sodium iodide in N,N-dimethylformamide at 90°C for 20 hours followed by chloroform extraction gave the 6''-iodide **29** in 83% yield. Dehalogenation of **29** with RANEY-Ni in dioxane in a PARR apparatus (4.3 kg/cm²) for 20 hours, treatment with 12% ammonia in methanol and then with 90% trifluoroacetic acid, followed by column chromatography on Amberlite CG-50 (NH_4^+) resin afforded 6''-deoxyamikacin (**10**) as the dicarbonate salt in 45% yield from **29**.

By the similar synthetic method, 3',4',4'',6''-tetradeoxyamikacin (**11**) and 3',4',6''-trideoxyamikacin (**12**) were synthesized from 3',4'-

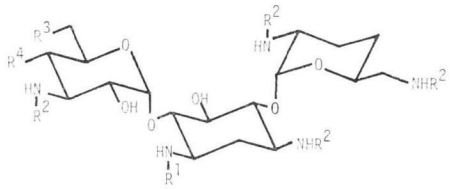
Table 1. Properties of the 6''-deoxy and 4'',6''-dideoxy derivatives.

Compound	mp (decomp.)	$[\alpha]_D$ in H_2O	Molecular formula*	MS *m/e*		Rf on TLC***	
				M^+	Fragments**	Solvent A	Solvent B
5	*ca.* 129°C	+126° at 23°	$C_{18}H_{37}N_5O_6 \cdot \frac{3}{2}H_2CO_3$	419	130, 129	0.45	
6	*ca.* 131°C	+101° at 26°	$C_{18}H_{37}N_5O_7 \cdot \frac{3}{2}H_2CO_3$	435	146, 129	0.38	
7	142~147°C	+84° at 24°	$C_{22}H_{42}N_6O_8 \cdot \frac{3}{2}H_2CO_3$		130, 129	0.21	
8	132~139°C	+73° at 23°	$C_{22}H_{42}N_6O_9 \cdot \frac{3}{2}H_2CO_3$		146, 129	0.10	
9	127~130°C	+90° at 21°	$C_{22}H_{43}N_5O_{11} \cdot 2H_2CO_3$		162, 130	0.10	0.29
10	155~160°C	+80° at 21°	$C_{22}H_{43}N_5O_{12} \cdot 2H_2CO_3$		162, 146	0.07	0.24
11	153~160°C	+66° at 23°	$C_{22}H_{43}N_5O_9 \cdot 2H_2CO_3$		130	0.29	0.43
12	142~149°C	+77° at 22°	$C_{22}H_{43}N_5O_{10} \cdot H_2CO_3$		146, 130	0.16	0.36

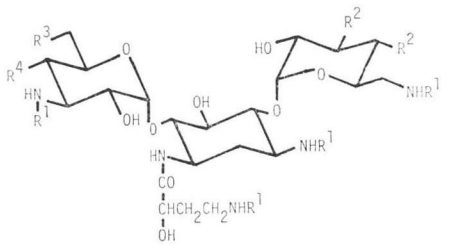
* Satisfactory elemental analyses were obtained for all the compounds.

** Fragments of aminohexoses, *m/e* 162: monoamino-monodeoxy, *m/e* 146: monoamino-dideoxy, *m/e* 130: monoamino-trideoxy, *m/e* 129: diamino-tetradeoxy.

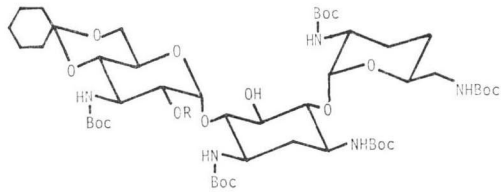
*** TLC on Silica gel G (Merck, Art 5721) using solvent A; butanol - ethanol - chloroform - 17% ammonia (4: 5: 2: 5 in volume) and solvent B; chloroform - methanol - 28% ammonia - water (1: 4: 2: 1 in volume).

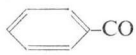


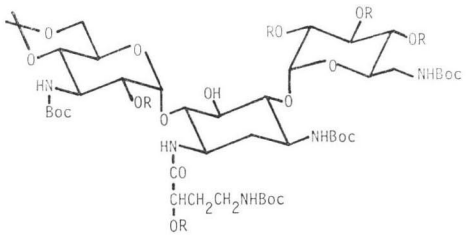
1: R^1, R^2=H, R^3, R^4=OH
2: R^1=$NH_2CH_2CH_2CH(OH)CO$, R^2=H, R^3, R^4=OH
5: R^1, R^2, R^3, R^4=H
6: R^1, R^2, R^3=H, R^4=OH
7: R^1=$NH_2CH_2CH_2CH(OH)CO$, R^2, R^3, R^4=H
8: R^1=$NH_2CH_2CH_2CH(OH)CO$, R^2, R^3=H, R^4=OH
13: R^1, R^2=Boc, R^3, R^4=OH



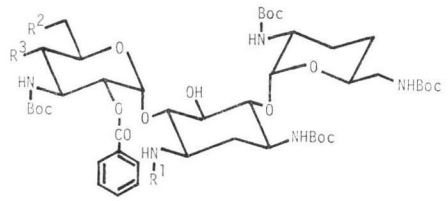
3: R^1=H, R^2, R^3, R^4=OH
4: R^1, R^2=H, R^3, R^4=OH
9: R^1, R^3, R^4=H, R^2=OH
10: R^1, R^3=H, R^2, R^4=OH
11: R^1, R^2, R^3, R^4=H
12: R^1, R^2, R^3=H, R^4=OH
22: R^1=Boc, R^2, R^3, R^4=OH



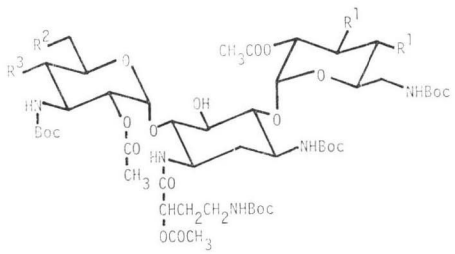
14: R=H
15: R= 



23: R=H
24: R=CH_3CO



16: R^1=Boc, R^2, R^3=OH
17: R^1=Boc, R^2, R^3=CH_3SO_3
18: R^1=Boc, R^2=CH_3SO_3, R^3=OH
19: R^1=Boc, R^2, R^3=I
20: R^1=Boc, R^2=I, R^3=OH
21: R^1=BocNHCH$_2$CH$_2$CH(OCO- )CO, R^2, R^3=OH



25: R^1=CH_3COO, R^2, R^3=OH
26: R^1=CH_3COO, R^2, R^3=CH_3SO_3
27: R^1=CH_3COO, R^2, R^3=I
28: R^1=CH_3COO, R^2=CH_3--SO_3, R^3=OH
29: R^1=CH_3COO, R^2=I, R^3=OH
30: R^1=H, R^2, R^3=OH

dideoxyamikacin[4] (**4**) through its 2′,2″,2‴-tri-O-acetyl-3,6′,3″,4‴-tetra-N-Boc derivative **30**, mp 130~145°C (decomp.), $[\alpha]_D^{21}$ +56° (*c* 1, $CHCl_3$).

The properties of the eight compounds described above are shown in Table 1. Although only two compounds (**5** and **6**) showed M^+ ions in MS spectra, the structures of all compounds were confirmed by analysis of fragmentations of the 2-deoxystreptamine moiety[5] (*m/e* 191, 163 and 145) and the aminohexose moieties (Table 1).

The minimum inhibitory concentrations of the polydeoxy derivatives (**5**~**12**) are shown in Table 2 in comparison with those of dibekacin (**1**) and amikacin (**3**). All polydeoxy derivatives showed antibacterial activities similar to each parent compound and inhibited the growth of resistant

Table 2. Minimum inhibitory concentrations (μg/ml) of the 6''-deoxy and 4'',6''-dideoxy derivatives.

Test organism	1	5	6	7	8	3	9	10	11	12
Staph. aureus FDA209P	0.78	0.39	0.78	0.78	1.56	1.56	0.78	1.56	1.56	1.56
Staph. aureus Smith	<0.20	<0.20	<0.20	<0.20	<0.20	0.20	<0.20	0.39	<0.20	<0.20
Staph. aureus Ap0l[a]	1.56	3.13	3.13	1.56	1.56	1.56	3.13	3.13	1.56	1.56
Staph. epidermidis 109[a]	1.56	3.13	1.56	1.56	1.56	1.56	3.13	3.13	1.56	1.56
Micrococcus flavus FDA16	100	50	25	1.56	1.56	3.13	6.25	6.25	6.25	25
Sarcina lutea PCI1001	100	50	25	3.13	3.13	3.13	6.25	6.25	6.25	6.25
B. anthracis	<0.20	<0.20	0.39	<0.20	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	<0.20	<0.20	<0.20	<0.20
B. subtilis NRRL B-558	<0.20	<0.20	<0.20	<0.20	<0.20	0.39	<0.20	0.20	<0.20	<0.20
B. cereus ATCC10702	3.13	6.25	6.25	1.56	3.13	1.56	1.56	1.56	1.56	1.56
Corynebact. bovis 1810	50	50	25	6.25	3.13	3.13	12.5	6.25	12.5	12.5
Myco. smegmatis ATCC607	1.56	1.56	0.78	0.39	0.39	0.78	0.39	0.78	0.78	0.78
E. coli NIHJ	1.56	1.56	1.56	1.56	1.56	3.13	1.56	1.56	1.56	1.56
E. coli K-12	1.56	1.56	1.56	1.56	1.56	0.78	1.56	0.78	1.56	0.78
E. coli K-12 R5[b]	>100	>100	100	100	50	100	>100	100	>100	100
E. coli K-12 R388	1.56	1.56	1.56	0.78	0.78	0.78	0.78	0.78	1.56	0.78
E. coli K-12 J5R11-2[c]	3.13	3.13	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56
E. coli K-12 ML1629[c]	3.13	3.13	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56
E. coli K-12 ML1630	3.13	3.13	3.13	1.56	3.13	3.13	3.13	3.13	1.56	1.56
E. coli K-12 ML1410	1.56	1.56	3.13	1.56	3.13	6.25	3.13	6.25	0.78	1.56
E. coli K-12 ML1410 R81[c]	1.56	1.56	1.56	1.56	1.56	3.13	1.56	1.56	1.56	1.56
E. coli K-12 LA290 R55[d]	100	100	>100	1.56	3.13	3.13	3.13	3.13	1.56	1.56
E. coli K-12 LA290 R56	25	50	25	1.56	1.56	1.56	1.56	0.78	1.56	1.56
E. coli K-12 LA290 R64	25	25	25	0.78	1.56	1.56	1.56	1.56	1.56	0.78
E. coli W677	1.56	0.78	1.56	1.56	1.56	3.13	1.56	1.56	0.78	0.78
E. coli JR66/W677[d,e]	100	100	100	3.13	3.13	6.25	6.25	3.13	1.56	3.13
E. coli K-12 C600 R135[f]	1.56	50	3.13	0.78	1.56	1.56	1.56	0.78	1.56	0.78
E. coli JR225[f]	>100	100	>100	1.56	1.56	1.56	1.56	1.56	0.78	0.78

Kl. pneumoniae PCI602	0.78	0.78	1.56	0.78	1.56	0.78	0.78	0.78	0.78	1.56
Kl. pneumoniae 22#3038[d,e]	100	100	100	3.13	3.13	3.13	3.13	6.25	3.13	3.13
Sh. dysenteriae JS11910	3.13	3.13	3.13	3.13	6.25	6.25	6.25	3.13	6.25	3.13
Sh. flexneri 4b JS11811	3.13	3.13	3.13	6.25	6.25	6.25	6.25	6.25	3.13	3.13
Sh. sonnei JS11746	6.25	3.13	3.13	3.13	6.25	6.25	6.25	6.25	3.13	3.13
Sal. typhi T-63	0.78	0.78	0.39	0.39	0.39	0.78	0.78	0.78	1.56	0.78
Sal. enteritidis 1891	3.13	6.25	3.13	6.25	3.13	1.56	3.13	3.13	3.13	1.56
Proteus vulgaris OX19	0.78	0.78	0.39	0.39	0.78	1.56	0.78	1.56	0.78	0.39
Proteus rettgeri GN311	6.25	3.13	6.25	25	50	12.5	12.5	25	12.5	25
Proteus rettgeri GN466	1.56	1.56	3.13	3.13	12.5	6.25	6.25	12.5	6.25	3.13
Serratia marcescens	100	100	100	100	50	12.5	12.5	12.5	25	25
Serratia sp. SOU	>100	>100	>100	>100	100	50	100	50	>100	>100
Serratia sp. 4[d]	100	100	100	6.25	6.25	12.5	25	12.5	50	25
Providencia sp. Pv16[g]	>100	>100	>100	25	25	12.5	25	50	6.25	12.5
Providencia sp. 2991[g]	>100	>100	>100	100	100	12.5	12.5	12.5	25	25
Ps. aeruginosa A3	3.13	3.13	1.56	1.56	3.13	3.13	6.25	6.25	6.25	3.13
Ps. aeruginosa No. 12	6.25	12.5	6.25	12.5	25	6.25	25	25	100	25
Ps. aeruginosa H9[e]	6.25	100	12.5	6.25	6.25	6.25	50	25	50	12.5
Ps. aerugionsa H11	12.5	100	12.5	50	50	25	50	50	100	100
Ps. aeruginosa TI-13[c]	6.25	100	12.5	25	6.25	6.25	12.5	25	50	12.5
Ps. aeruginosa GN315[b]	>100	>100	100	100	50	>100	>100	>100	>100	50
Ps. aeruginosa 99[f]	50	>100	12.5	12.5	12.5	12.5	25	25	100	50
Ps. aeruginosa B-13[c,e]	6.25	>100	50	12.5	25	12.5	50	50	100	100
Ps. aeruginosa 21-75[h]	>100	>100	>100	>100	100	12.5	100	50	>100	>100
Ps. aeruginosa PSTI[f]	>100	>100	>100	25	50	25	100	50	100	100
Ps. aeruginosa ROS134/PU21[f]	>100	>100	>100	>100	>100	100	>100	100	>100	>100
Ps. aeruginosa K-Ps102[i]	6.25	25	6.25	12.5	12.5	12.5	25	12.5	100	12.5
Ps. maltophilia GN907[i]	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

Resistance mechanisms: 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.

organisms. However the deoxygenation at the 4″ or 6″ position was believed to cause the reduction of the anti-pseudomonas activity.

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