A PREDOMINANT ROLE OF AMINO GROUPS IN THE ANTIBACTERIAL ACTION OF AMINOGLYCOSIDES: SYNTHESIS OF HEXA- AND HEPTADEOXYKANAMYCIN DERIVATIVES*

Sir:

As reported in a previous paper[1] we have studied polydeoxy derivatives of kanamycins and found that 5,3′,4′,4″,6″-pentadeoxykanamycin B having only one hydroxyl group at C-2″ is still active in inhibiting both the growth of bacteria and protein synthesis on pseudomonas ribosomes. Its 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] derivative had strong activity against Gram-positive and -negative bacteria including pseudomonas.[1] In order to understand the role of the 2″-hydroxyl group in the antibacterial activity of polydeoxykanamycins, we have synthesized 5,2′,3′,4′,4″,6″-hexadeoxykanamycin (**1**), 5,2′,3′,4′,2″,4″,6″-heptadeoxykanamycin (**2**), 5,2′,3′,4′,4″,6″-hexadeoxyamikacin (**3**) and 5,2′,3′,4′,2″,4″,6″-heptadeoxyamikacin (**4**), starting from 3′,4′-dideoxykanamycin B (**5**).** In this paper, we report on the synthesis of these deoxy derivatives and their antibacterial activity.

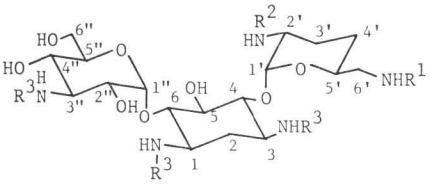
Compound **1** was synthesized by successive deoxygenation of **5** at C-5, C-4″ and C-6″, followed by deamination at C-2′. Compound **2** was obtained by deoxygenation of **1**. 6′-*N*-Cbz-3′,4′-dideoxykanamycin B[2] (**6**) prepared from **5** was acylated with an equimolar amount of *tert*-butyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate (Kokusan Chemical Works, Tokyo) in a mixture (10: 10: 1) of pyridine, water and triethylamine at room temperature for 21 hours to afford 2′-*N*-Boc-6′-*N*-Cbz-3′,4′-dideoxykanamycin B (**7**) in 28% yield. From this reaction mixture, 42% of **6** was recovered. Compound **7** was treated with 4.5 equivalents of benzyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate in a mixture of methanol and triethylamine at room temperature for 19 hours to give 2′-*N*-Boc-1,3,6′,3″-tetra-*N*-Cbz-3′,4′-dideoxykanamycin B (**8**) in 81% yield. Acetonization of **8** with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide in the presence of *p*-toluenesulfonic acid at room temperature for 20 hours followed by benzoylation of the 2″-hydroxyl group with benzoyl chloride in pyridine at room temperature for 3 hours gave the 4″,6″-*O*-isopropylidene-2″-benzoate **9** in 86% yield. The 5-deoxygenation[3] of **9** was accomplished by chlorination with sulfuryl chloride (3 equiv.) in pyridine under ice-cooling for 1 hour followed by reduction with tributylstannane in toluene under an argon atmosphere in the presence of α,α'-azobisisobutyronitrile at 100°C for 2 hours affording 2″-*O*-benzoyl-2′-*N*-Boc-1,3,6′,3″-tetra-*N*-Cbz-4″,6″-*O*-isopropylidene-5,3′,4′-trideoxykanamycin B (**10**) in 60% yield, mp 148~152°C, $[\alpha]_D^{21}$ +129° (*c* 1, $CHCl_3$).

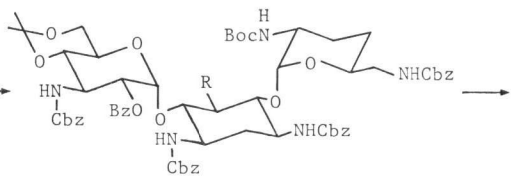
Removal of the *O*-isopropylidene group in **10** with a mixture (3: 3: 1) of acetic acid, methanol and water at 50°C for 2 hours afforded 2″-*O*-benzoyl-2′-*N*-Boc-1, 3, 6′, 3″-tetra-*N*-Cbz-5, 3′, 4′-trideoxykanamycin B (**11**) in 94% yield. The 4″,6″-dideoxygenation[4] of **11** was accomplished by mesylation with methanesulfonyl chloride (4 equiv.) in pyridine at room temperature for 3 hours affording the dimesylate **12** (96% yield), iodination with an excess of sodium iodide in *N*, *N*-dimethylformamide at 90°C for 16 hours (85% yield), catalytic hydrogenation with Raney-Ni (R-200, Nikko Scientific & Chemical Ind., Tokyo) in dioxane in a Parr apparatus (3.5 kg/cm²) for 25 hours and re-*N*-benzyloxycarbonylation (71% yield) to give 2″-*O*-benzoyl-2′-*N*-Boc-1,3,6′,3″-tetra-*N*-Cbz-5,3′,4′,4″,6″-pentadeoxykanamycin B (**13**), mp 162~165°C, $[\alpha]_D^{21}$ +106° (*c* 1, $CHCl_3$).

The 2″-*O*-benzoyl and 2′-*N*-Boc groups of **13** were removed by successive treatments with 5% sodium methylate in methanol at room temperature for 2.5 hours and with 90% trifluoroacetic acid for 1 hour to afford 1,3,6′,3″-tetra-*N*-Cbz-5,3′,4′,4″,6″-pentadeoxykanamycin B (**14**) in 59% yield, mp 189~194°C (decomp.), $[\alpha]_D^{22}$ +109° (*c* 1, DMF). Deamination of **14** at the 2′-position was carried out by the method of BARTON[5] as follows. *N*-Formylation of **14** with *p*-nitrophenyl formate in *N*,*N*-dimethylformamide at room temperature for 3 hours followed by *O*-acetylation with acetic anhydride in pyridine in the presence of 4-dimethylaminopyridine at room temperature

* Dedicated to Professor EDGAR LEDERER on the occasion of his 75th birthday.

** Compounds **1**~**4** and **7** were purified by column chromatography on Amberlite CG-50 (NH_4^+) eluted with diluted ammonia. Compounds, **9**, **10**, **13**~**19** and **21**~**23** were purified by column or thin-layer chromatography on silica gel developed with mixtures of chloroform and methanol. Reasonable NMR spectral data were obtained for all compounds cited in this report.





5 R^1, R^2, R^3 = H

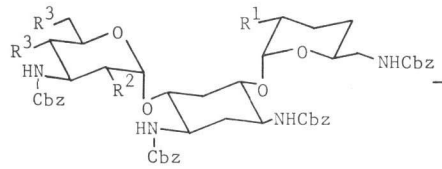
6 R^1 = Cbz, R^2, R^3 = H

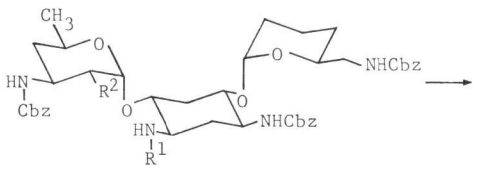
7 R^1 = Cbz, R^2 = Boc, R^3 = H

8 R^1, R^3 = Cbz, R^2 = Boc

9 R = OH

10 R = H





11 R^1 = NHBoc, R^2 = OBz, R^3 = OH

12 R^1 = NHBoc, R^2 = OBz, R^3 = OSO_2CH_3

13 R^1 = NHBoc, R^2 = OBz, R^3 = H

14 R^1 = NH_2, R^2 = OH, R^3 = H

15 R^1 = NHCHO, R^2 = $OCOCH_3$, R^3 = H

16 R^1 = -N≡C, R^2 = $OCOCH_3$, R^3 = H

17 R^1 = Cbz, R^2 = $OCOCH_3$

18 R^1 = Cbz, R^2 = OH

19 R^1 = H, R^2 = OH

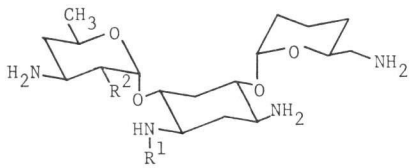
20 R^1 = $COCF_3$, R^2 = OH

21 R^1 = $COCF_3$, R^2 = OSO_2CF_3

22 R^1 = $COCF_3$, R^2 = S- 

23 R^1 = $COCF_3$, R^2 = H

24 R^1, R^2 = H



1 R^1 = H, R^2 = OH

2 R^1, R^2 = H

3 R^1 = $COCHCH_2CH_2NH_2$ (S), OH; R^2 = OH

4 R^1 = $COCHCH_2CH_2NH_2$ (S), OH; R^2 = H

Abbreviations

Cbz: Benzyloxycarbonyl

Boc: *tert*-Butoxycarbonyl

Bz: Benzoyl

for 2 hours gave the 2′-*N*-formyl-2″-*O*-acetate **15** in 72% yield. Compound **15** was heated with *p*-toluenesulfonyl chloride (1.5 equiv.) in pyridine[6] at 80°C for 4 hours to yield the 2′-isonitrile **16** in 71% yield, mp 167~171°C (decomp.), $[\alpha]_D^{21}$ +76° (*c* 0.5, DMF). Reduction of **16** with tributylstannane in toluene in the presence of α,α'-azobisisobutyronitrile at 120°C for 8 hours (**17**, 30% yield) followed by removal of the *O*-acetyl group with 16% ammonia in methanol at room temperature for 3 hours (78% yield) gave 1,3,6′, 3″-tetra-*N*-Cbz-5,2′, 3′, 4′, 4″, 6″-hexadeoxykanamycin (**18**), mp 176~181°C, $[\alpha]_D^{20}$ +55° (*c* 0.5, CH_3OH). Catalytic hydrogenation of **18** under a hydrogen stream with 5% Pd on charcoal in a mixture (1: 500: 500) of acetic acid, methanol and water at room temperature for 13 hours afforded **1** as the carbonate ($C_{18}H_{36}N_4O_5 \cdot H_2CO_3 \cdot H_2O$) in

Table 1. Minimum inhibitory concentrations (μg/ml) on Mueller-Hinton agar plates.

Test organism	Kanamycin (KM)	Hexadeoxy KM (1)	Heptadeoxy KM (2)	Amikacin (AM)	Hexadeoxy AM (3)	Heptadeoxy AM (4)
Staphylococcus aureus 209P	0.78	1.56	12.5	0.78	0.78	6.25
S. aureus Smith	0.39	0.78	6.25	0.39	1.56	3.13
S. aureus ApOl[a]	12.5	3.13	>100	1.56	3.13	25
S. epidermidis 109[a]	50	3.13	25	3.13	1.56	25
Micrococcus flavus FDA16	12.5	12.5	100	6.25	6.25	50
M. luteus PCI1001	12.5	12.5	50	3.13	6.25	50
Bacillus anthracis	0.78	1.56	12.5	0.78	1.56	3.13
B. subtilis PCI219	0.39	0.78	6.25	0.39	0.78	3.13
B. subtilis NRRL B-558	0.39	0.78	6.25	0.39	0.78	6.25
B. cereus ATCC10702	3.13	6.25	50	6.25	6.25	25
Corynebacterium bovis 1810	6.25	6.25	50	1.56	6.25	50
Mycobacterium smegmatis ATCC607	0.78	0.78	6.25	0.78	1.56	6.25
Escherichia coli NIHJ	0.78	3.13	12.5	1.56	1.56	3.13
E. coli K12	0.78	1.56	12.5	0.39	0.78	6.25
E. coli K12 R5[b]	>100	>100	>100	100	100	>100
E. coli K12 R388	0.78	1.56	6.25	0.39	0.78	3.13
E. coli K12 J5R11-2[e]	>100	25	12.5	1.56	1.56	3.13
E. coli K12 ML 1629[c]	>100	25	25	1.56	1.56	6.25
E. coli K12 ML1630	>100	25	50	3.13	3.13	12.5
E. coli K12 ML1410	3.13	3.13	25	1.56	3.13	12.5
E. coli K12 ML1410 R81[c]	>100	25	50	1.56	3.13	12.5
E. coli K12 LA290 R55[d]	50	100	100	1.56	1.56	6.25
E. coli K12 LA290 R56	6.25	12.5	12.5	0.78	1.56	6.25
E. coli K12 LA290 R64	6.25	25	50	1.56	3.13	6.25
E. coli W677	0.78	1.56	12.5	0.78	0.78	6.25
E. coli JR66/W677[d,e]	>100	>100	>100	3.13	3.13	12.5
E. coli K12 C600 R135[f]	0.78	1.56	12.5	0.78	1.56	12.5
E. coli JR225[f]	1.56	1.56	25	0.78	0.78	6.25
Klebsiella pneumoniae PCI602	1.56	1.56	12.5	0.78	1.56	6.25
K. pneumoniae 22#3038[d,e]	>100	>100	>100	3.13	3.13	25
Shigella dysenteriae JS11910	6.25	6.25	50	3.13	6.25	25
S. flexneri 4b JS11811	12.5	6.25	25	3.13	6.25	25
S. sonnei JS11746	3.13	3.13	25	3.13	3.13	25
Salmonella typhi T-63	0.39	0.78	12.5	0.39	0.78	6.25
S. enteritidis 1891	3.13	1.56	50	1.56	3.13	25
Proteus vulgaris OX19	0.78	1.56	12.5	1.56	0.78	6.25
P. rettgeri GN311	0.39	0.78	6.25	0.39	1.56	12.5
P. rettgeri GN466	0.78	1.56	3.13	0.78	1.56	1.56
P. inconstans Pv16[g]	3.13	12.5	50	1.56	3.13	12.5
P. inconstans 2991[g]	12.5	50	>100	1.56	6.25	100
Serratia marcescens	1.56	6.25	50	1.56	6.25	25
Serratia sp. SOU	>100	>100	>100	25	50	>100
Serratia sp. 4	25	25	>100	3.13	12.5	>100
Pseudomonas aeruginosa A3	6.25	3.13	12.5	<0.20	0.78	12.5
P. aeruginosa No. 12	25	50	>100	3.13	25	>100
P. aeruginosa H9[e]	>100	100	>100	6.25	50	>100
P. aeruginosa H11	100	>100	>100	12.5	100	>100
P. aeruginosa TI-13[c]	50	50	100	3.13	12.5	>100
P. aeruginosa GN315[b]	>100	>100	>100	25	>100	>100
P. aeruginosa 99[f]	>100	>100	>100	6.25	50	>100
P. aeruginosa B-13[e,e]	>100	>100	>100	6.25	50	>100
P. aeruginosa 21-75[h]	>100	>100	>100	12.5	100	>100
P. aeruginosa PST1[f]	>100	>100	>100	12.5	50	>100
P. aeruginosa ROS134/PU21[f]	>100	>100	>100	100	>100	>100
P. aeruginosa K-Ps102[i]	>100	>100	>100	6.25	50	>100
P. maltophilia GN907[i]	>100	>100	>100	>100	>100	>100

Resistance mechanisms[g] [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.

59% yield, mp 191~196°C (decomp.), $[\alpha]_D^{23}$ +103° (*c* 1, H_2O), SIMS *m/z* 389 (MH^+), TLC (silica gel) 1-butanol - ethanol - chloroform - 17% ammonia, 4: 5: 2: 5 (solvent A): Rf 0.47, chloroform - methanol - concd. ammonia - water, 1: 4: 2: 1 (solvent B): Rf 0.78.

Preferential *N*-protection[7] of **1** by chelate formation with zinc diacetate dihydrate (4 equiv.) in 90% dimethyl sulfoxide at room temperature for 24 hours and acylation with benzyl *S*-4, 6-dimethylpyrimid-2-ylthiocarbonate (4 equiv.) at 50°C for 18 hours yielded 3,6′,3″-tri-*N*-Cbz-5,2′,3′,4′,4″,6″-hexadeoxykanamycin (**19**) in 62% yield. The 1-amino group of **19** was acylated with ethyl trifluoroacetate in methanol in the presence of triethylamine at room temperature for 23 hours to yield the *N*-trifluoroacetate **20** in 83% yield, mp 183~187°C (decomp.), $[\alpha]_D^{22}$ +51° (*c* 0.2, CH_3OH). The deoxygenation[8] of the 2″-hydroxyl group in **20** was carried out by successive treatments with trifluoromethanesulfonic anhydride (2 equiv.) in pyridine in the presence of 4-dimethylaminopyridine (1 equiv.) at room temperature for 4 hours (**21**, 74% yield), followed by reaction with sodium thiophenolate in *N*,*N*-dimethylformamide at room temperature for 6 hours (**22**, 84% yield), reduction with Raney-Ni in ethanol at 80°C for 30 minutes and re-*N*-benzyloxycarbonylation (94% yield) to give 3,6′,3″-tri-*N*-Cbz-1-*N*-trifluoroacetyl-5,2′,3′,4′,2″,4″,6″-heptadeoxykanamycin (**23**). The *N*-trifluoroacetyl group in **23** was removed by treatment with 16% ammonia in methanol at room temperature for 15 hours yielding 3,6′,3″-tri-*N*-Cbz-5, 2′, 3′, 4′, 2″, 4″, 6″-heptadeoxykanamycin (**24**) in 97% yield. Removal of the *N*-Cbz groups in **24** by catalytic hydrogenation under a hydrogen stream with 5% Pd on charcoal in a mixture (1: 150: 150) of acetic acid, methanol and water gave **2** as the carbonate ($C_{18}H_{36}N_4O_4 \cdot H_2CO_3 \cdot H_2O$) in 78% yield, mp 193~197°C (decomp.), $[\alpha]_D^{23}$ +98° (*c* 0.1, H_2O), SIMS *m/z* 373 (MH^+), TLC (silica gel) solvent A: Rf 0.53, solvent B: Rf 0.81.

The 1-amino group of **19** was acylated with the *N*-hydroxysuccinimide ester of *N*-Cbz-(*S*)-4-amino-2-hydroxybutyric acid in dimethyl sulfoxide in the presence of triethylamine at room temperature for 4 hours and the Cbz groups of the acylated product were removed by catalytic hydrogenation with 5% Pd on charcoal to yield **3** as the carbonate ($C_{22}H_{43}N_5O_7 \cdot H_2CO_3 \cdot 2H_2O$) in 51% yield, mp 157~162°C (decomp.), $[\alpha]_D^{25}$ +61° (*c* 1, H_2O), SIMS *m/z* 490 (MH^+), TLC (silica gel) solvent A: Rf 0.31, solvent B: Rf 0.46.

The 1-*N*-acylation of **24** with the *N*-hydroxysuccinimide ester of *N*-Cbz-(*S*)-4-amino-2-hydroxybutyric acid in dimethyl sulfoxide in the presence of triethylamine at room temperature for 5 hours followed by catalytic hydrogenation with 5% Pd on charcoal afforded **4** as the carbonate ($C_{22}H_{43}N_5O_6 \cdot H_2CO_3 \cdot H_2O$) in 63% yield, mp 147~152°C, $[\alpha]_D^{22}$ +54° (*c* 0.2, H_2O), SIMS *m/z* 474 (MH^+), TLC (silica gel) solvent A: Rf 0.38, solvent B: Rf 0.49.

The minimum inhibitory concentration of compounds **1**~**4** on a Mueller-Hinton agar plate are shown in Table 1 in comparison with those of kanamycin and amikacin. Although compound **2** having no hydroxyl group is weakly active, **1** having only one hydroxyl group at C-2″ is very active against Gram-positive and -negative bacteria except pseudomonas and some resistant bacteria[9] producing AAC (6′) and AAD (2″). Compound **3**, the 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] derivative of **1**, shows strong activity, but is slightly less than amikacin. Thus, it can be concluded that the amino groups of the kanamycin antibiotics play a predominant role in the antibacterial activity,[10] and the 2″-hydroxyl group and the (*S*)-4-amino-2-hydroxybutyryl moiety on the 1-amino group markedly augment the activity.

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(Received May 4, 1983)

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