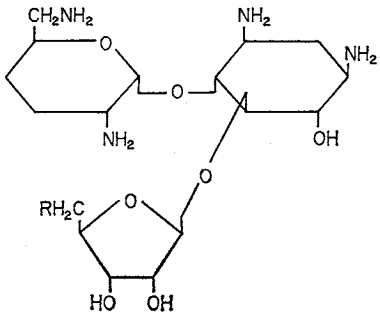
SYNTHESES OF 3′,4′-DIDEOXY AND 3′,4′,5′′-TRIDEOXYRIBOSTAMYCIN ACTIVE AGAINST KANAMYCIN-RESISTANT *E. COLI* AND *P. AERUGINOSA*

Sir:

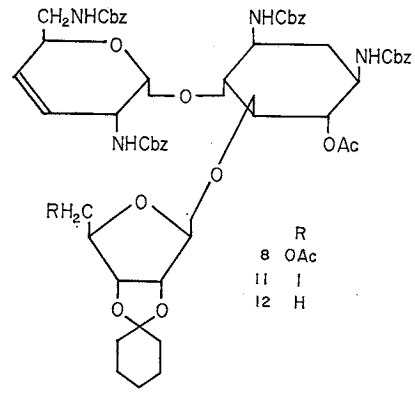
Recent studies on synthetic deoxy derivatives of aminoglycosidic antibiotics such as 3′-deoxykanamycin[1], 3′,4′-dideoxykanamycin B[2] and 3′,4′-dideoxyneamine[3] have confirmed that removal of the hydroxyl group which is phosphorylated by drug-resistant bacteria from kanamycins gives compounds active against the resistant organisms. As an extension of this work, we describe in this paper the syntheses of two deoxy-derivatives of ribostamycin[4]. If inactivation of ribostamycin by *Escherichia coli* K12-ML 1629 and K12-ML 1630 carrying R factor (see Table 1) is caused by phosphorylation

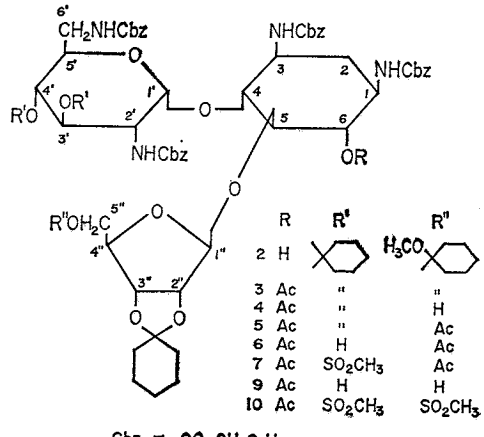


3′,4′-*Dideoxyribostamycin* R = OH
3′,4′,5′′-*Trideoxyribostamycin* R = H

at the 3′-hydroxyl group of the antibiotic, dehydroxylation at the 3′-position might give an antibiotic active against resistant organisms. Ribostamycin can be conveniently converted to 3′,4′-dideoxy and 3′,4′,5′′-trideoxyribostamycin, in which hydroxyl groups at C-4′ or C-4′ and C-5′′ are removed in addition to the 3′-hydroxyl group.

Ribostamycin was treated with benzyloxycarbonyl chloride in aqueous methanol to give tetra-N-benzyloxycarbonylribostamycin (**1**) in a yield of 89 %, $[\alpha]_D^{14}$ +32.5° (*c* 1, acetone) which was allowed to react with 1,1-dimethoxycyclohexane in DMF in the presence of *p*-toluenesulfonic acid at 50°C under reduced pressure to afford tetra-N-benzyloxycarbonyl-3′,4′; 2′′,3′′-di-O-cyclohexylidene-5′′-O-(1-methoxycyclohexyl)ribostamycin (**2**), mp 92~94°C, $[\alpha]_D^{17}$ +16.2° (*c* 2, $CHCl_3$) in a yield of 60 %. NMR (in $CDCl_3$): τ 6.83 (3H, s, OCH_3). Found: C 64.39, H 6.80, N 4.58 %; Calcd. for $C_{68}H_{86}N_4O_{19}$: C 64.65, H 6.86, N 4.43 %. Acetylation of **2** gave the 6-O-acetyl derivative (**3**), mp 95~97°C, $[\alpha]_D^{17}$ +8.7 (*c* 1, $CHCl_3$). NMR in $CDCl_3$): τ 7.94 (3H, s, OAc), 6.85 (3H, s, OCH_3). Found: C 64.39, H 6.86, N 4.43 %; Calcd. for $C_{70}H_{88}N_4O_{20}$: C 64.40, H 6.80, N 4.29 %. Selective removal of the 5′′-O-protecting group with 40 % acetic acid at room temperature gave the corresponding mono-O-acetyl-di-O-cyclohexylidene derivative (**4**), mp 109~111°C, $[\alpha]_D^{20}$ +18° (*c* 2, $CHCl_3$) in a yield of 82 %. NMR (in $CDCl_3$): τ 7.97 (3H, s, OAc). Found: C 63.62, H 6.32, N 4.88 %; Calcd. for $C_{63}H_{76}N_4O_{19}$: C 63.41, H 6.42, N 4.70 %. Further acetylation of **4** gave the 5′′,6-di-O-acetyl derivative (**5**) quantitatively, mp 95~98°C, $[\alpha]_D^{20}$ +22° (*c* 0.87, $CHCl_3$). NMR (in $CDCl_3$): τ





Cbz = $CO_2CH_2C_6H_5$

7.99 and 7.97 (each 3H, s, OAc); Found C 63.06, H 6.16, N 4.64%; Calcd. for $C_{65}H_{78}N_4O_{20}$: C 63.19, H 6.37, N 4.54%. Selective removal of a cyclohexylidene group at 3′ and 4′ with aqueous acetone-acetic acid at 50°C gave the di-O-acetyl-mono-O-cyclohexylidene derivative (**6**), mp 102~105°C, $[\alpha]_D^{20}$ +7.7° (*c* 1.8, $CHCl_3$) in a yield of 94%. Found: C 61.22, H 5.95, N 4.89%; Calcd. for $C_{59}H_{70}N_4O_{20}$: C 61.34, H 6.11, N 4.85%. Mesylation of **6** gave the 3′,4′-di-O-mesyl derivative (**7**), $[\alpha]_D^{20}$ −4.7° (*c* 1.1, $CHCl_3$) in a yield of 88%. NMR (in $CDCl_3$): τ 7.93 (6H, s, OAc), 7.18 and 6.92 (each 3H, s, Ms). Found: C 56.16, H 5.61, N 4.35, S 4.58%. Calcd. for $C_{61}H_{74}N_4O_{24}S_2$: C 55.86, H 5.69, N 4.27, S 4.89%. 3′,4′-Unsaturation of **7** was achieved as described in the previous papers[2,3] by use of sodium iodide and zinc dust in DMF at 91°C for 1 hour to give 5″,6-di-O-acetyl-tetra-N-benzyloxycarbonyl-2″, 3″-O-cyclohexylidene-3′, 4′-dideoxy-3′-enoribostamycin (**8**), mp 82~85°C, $[\alpha]_D^{20}$ −30° (*c* 2, $CHCl_3$) in a yield of 70%; NMR (in $CDCl_3$ at 60 MHz): τ 4.42 (2H slightly broadened singlet, H-3′,4′). Found: C 63.44, H 6.02, N 4.83%. Calcd. for $C_{59}H_{68}N_4O_{18}$: C 63.20, H 6.11, N 5.00%. Removal of the two acetyl groups followed by catalytic hydrogenation and removal of the cyclohexylidene group in the usual manner gave 3′,4′-dideoxyribostamycin, $[\alpha]_D^{20}$ +35° (*c* 1, H_2O) in a yield of 54% from **8**. On paper chromatogram with 1-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1) it gave $Rf_{ribostamycin}$ 1.50. Found: C 46.10, H 8.38, N 12.53%. Calcd. for $C_{17}H_{34}N_4O_8 \cdot H_2O$: C 46.35, H 8.24, N 12.72%.

Selective removal of the two cyclohexylidene groups of **3** with aqueous acetone-acetic acid at 50°C gave the 6-O-acetyl-2″, 3″-O-cyclohexylidene derivative (**9**), mp 104~107°C, $[\alpha]_D^{14}$ +1.3° (*c* 0.8, $CHCl_3$) in a yield of 98%. NMR (in $CDCl_3$): τ 8.00 (3H, s, OAc). Found: C 61.34, H 5.80, N 5.18%. Calcd. for $C_{57}H_{68}N_4O_{19}$: C 61.50, H 6.16, N 5.03%. Mesylation gave the 3′, 4′,5″-tri-O-mesyl derivative (**10**), mp 112~114°C, $[\alpha]_D^{17}$ −8.8° (*c* 2.6, $CHCl_3$) in a yield of 95%. NMR (in $CDCl_3$): τ 7.97 (3H, s, OAc), 7.18, 6.98 and 6.97 (each 3H, s, Ms). Found: C 53.74, H 5.60, N 4.29, S 7.31%; Calcd. for $C_{60}H_{74}N_4O_{25}S_3$: C 53.48, H 5.54, N 4.16, S 7.14%. Treatment of **10** with sodium iodide and zinc dust in DMF at 90°C for 1 hour gave 6-O-acetyl-tetra-N-benzyloxycarbonyl-2″, 3″-O-cyclohexylidene-3′, 4′-dideoxy-3′-eno-5″-iodoribostamycin (**11**), mp 88~90°C, $[\alpha]_D^{16}$ −30° (*c* 2, $CHCl_3$) in a yield of 73%. Found: C 57.47, H 5.38, N 4.73, I 11.08%. Calcd. for $C_{57}H_{65}N_4O_{16}I$: C 57.57, H 5.51, N 4.71, I 10.68%. Reduction of the iodo compound with RANEY nickel and hydrogen in the presence of triethylamine gave the 5″-deoxy derivative (**12**), mp 90~93°C, $[\alpha]_D^{14}$ −30° (*c* 1.3, $CHCl_3$) in a yield of 43%. NMR (in $CDCl_3$): τ 8.82 (3H, d, CH-C<u>H</u>$_3$). Found: C 64.67, H 6.17, N 5.22%. Calcd. for $C_{57}H_{66}N_4O_{16}$: C 64.39, H 6.26, N 5.27%. Removal of the acetyl group followed by catalytic hydrogenation and removal of the cyclohexylidene group in the usual manner gave 3′,4′,5″-trideoxyribostamycin, $[\alpha]_D^{14}$ +56° (*c* 0.7, H_2O) in a yield of 62% from **12**; NMR (in D_2O); τ 8.67 (3H, d, CH-C<u>H</u>$_3$). On paper-chromatogram with 1-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1) it gave $Rf_{ribostamycin}$ 1.63. Found: C 48.32, H 8.48, N 13.11%. Calcd. for $C_{17}H_{34}N_4O_7 \cdot H_2O$: C 48.10, H 8.55, N 13.21%.

The synthetic 3′,4′-dideoxyribostamycin showed antibacterial activity at the level of the parent antibiotic, ribostamycin, against most of bacteria tested and, moreover, showed activity against *Pseudomonas aeruginosa* against which ribostamycin is inactive as shown in Table 1. It is further noteworthy that, though both the parent antibiotic and its 3′,4′-dideoxy derivative are inactive against *E. coli* K12-ML 1629 and K12-ML 1630 carrying R factor, the 3′,4′-dideoxy derivative shows activity against *E. coli* JR66/W677 which is known to be resistant[5] to 3′,4′-dideoxykanamycin B and kanamycins. On the other hand, 3′,4′,5″-trideoxyribostamycin showed decreased antibacterial activity as compared with the parent antibiotic, but it did show weak activity against both resistant and sensitive strains of *E. coli*.

These results suggest that *E. coli* K12-ML 1629 and K12-ML 1630, resistant to kanamycins but sensitive to 3′-deoxykanamycin, 3′,4′-dideoxykanamycin B and 3′,4′-

Table 1. Antibacterial spectra of 3′,4′-dideoxy-, 3′,4′,5″-trideoxyribostamycin, ribostamycin and kanamycin

Test organisms*	Minimal inhibitory concentration (mcg/ml)			
	3′,4′-Dideoxy-ribostamycin	3′,4′,5″-Trideoxy-ribostamycin	Ribosta-mycin	Kanamycin
Staphylococcus aureus FDA 209P	12.5	12.5	12.5	1.56
Sarcina lutea PCI 1001	>100	50	100	1.56
Bacillus subtilis NRRL B-558	3.12	3.12	6.25	0.2
Klebsiella pneumoniae PCI 602	6.25	50	1.56	1.56
Salmonella typhosa T-63	1.56	12.5	1.56	0.78
Escherichia coli NIHJ	12.5	50	6.25	3.12
〃 K-12	6.25	12.5	3.12	1.56
〃 K-12 ML 1629	>100	50	>100	>100
〃 K-12 ML 1630	>100	50	>100	>100
〃 K-12 ML 1410	6.25	50	3.12	1.56
〃 LA 290 R55	12.5	50	3.12	100
〃 W 677	6.25	50	3.12	1.56
〃 JR66/W677	12.5	50	>100	>100
Pseudomonas aeruginosa A3	6.25	100	>100	100
〃 No. 12	25	100	>100	100
〃 GN 315	>100	>100	>100	>100
〃 TI-13	25	100	>100	>100
〃 99	50	>100	>100	>100
Proteus rettgeri GN 311	6.25	25	3.12	12.5
〃 GN 466	12.5	50	6.25	3.12
Mycobacterium smegmatis ATCC 607**	3.12	>100	6.25	0.39

* Agar dilution streak method (nutrient agar, 37℃, 18 hours).
** 48 hours.

dideoxyneamine seems to produce two enzymes inactivating ribostamycin: the one phosphorylates 3′-hydroxyl and the other phosphorylates 5″-hydroxyl. We have isolated ribostamycin 3′-phosphate[6] as the result of the enzymatic inactivation. As reported in previous papers[7,8], we obtained lividomycin 5″-phosphate after the enzymatic inactivation. The 5″-hydroxyl group of ribostamycin is considered to be involved in the antibacterial activity, because of the much lower activity of the 5″-deoxy derivative than ribostamycin against sensitive organisms. The DREIDING model of ribostamycin strongly suggests the presence of hydrogen bonding between the 5″-hydroxyl group and 2′-amino group, hindering the rotation of the ribose moiety.

E. coli JR66/W677 produces an enzyme to adenylylate 2″-hydroxyl of 3′,4′-dideoxykanamycin B[5,9]. As will be reported in a next paper[6], we have also confirmed that this resistant organism produces the other enzyme to phosphorylate 3′-hydroxyl of neamine, ribostamycin, kanamycin and butirosin A, but not the enzyme to phosphorylate 5″-hydroxyl of lividomycin. If the same enzyme is considered to be involved in phosphorylation of 5″-hydroxyl in lividomycin and ribostamycin, the sensitivity and resistance relation to 3′,4′-dideoxy and 3′,4′,5″-trideoxyribostamycin can be easily understood.

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