AMINOGLYCOSIDE ANTIBIOTICS. XV

CHEMICAL CONVERSION OF NEOMYCIN B TO PAROMOMYCIN I, 6'''-DEAMINO-6'''-HYDROXYNEOMYCIN B AND 6'''-DEAMINO-6'''-HYDROXY-PAROMOMYCIN I

SOICHIRO TODA, SUSUMU NAKAGAWA, TAKAYUKI NAITO and HIROSHI KAWAGUCHI

Bristol-Banyu Research Institute, Ltd.
Shimo-meguro, Meguro-ku, Tokyo, 153, Japan

(Received for publication October 6, 1982)

Since the introduction of semisynthetic aminoglycoside antibiotics dibekacin[1] and amikacin[2] into clinical medicine, many attempts have been made to modify the structure of naturally occurring aminoglycoside antibiotics, mostly by deoxygenation, *N*-acylation and *N*-alkylation. Efforts were also directed to the amino→hydroxyl transformation of aminoglycoside antibiotics. In a previous paper[3] we reported the chemical conversion of kanamycin B to kanamycin C by diazotization of the 6'-amino group of kanamycin B followed by hydrolysis. A similar transformation of kanamycin B to kanamycin C was independently reported by KONDO *et al*[4]. IGARASHI[5] described the preparation of 1-deamino-1-hydroxyl analog of tobramycin through oxidation of the 1-amino group of tobramycin to a carbonyl function followed by reduction, which was accompanied by co-production of the 1-*epi*-hydroxyl derivative. In a similar manner, the 1- or 3-amino group of gentamicin and sisomicin was converted to a hydroxyl group by BOXLER and coworkers[6].

This paper reports the deamination of 6'- and/or 6'''-amino groups of neomycin B[7,8] *via* diazotization to paromomycin I[9,10], 6'''-deamino-6'''-hydroxyneomycin B and 6'''-deamino-6'''-hydroxyparomomycin I. Also described are antibacterial activity and acute toxicity of the 6'''-hydroxyl derivatives.

Neomycin B (**1**) has two NH_2CH_2- groups at the 6'- and 6'''-positions and four $NH_2CH<$ groups at the 1-, 3-, 2'- and 2'''-positions, the former two amino groups being sterically less hindered than the rest. The reaction of **1** with an equimolar amount of *N*-benzyloxycarbonyloxysuccinimide (in 40% aqueous tetrahydrofuran, 6~7°C, 2 hours) gave a mixture of 6'-*N*-benzyloxycarbonyl (Cbz) neomycin B (**3**) and 6'''-*N*-Cbz-neomycin B (**4**) in 31% yield, which could not be separated ($C_{31}H_{52}N_6O_{15}\cdot\frac{1}{2}H_2O$*1; mp 145~153°C; TLC*2 a single spot at Rf 0.12), along with 6',6'''-di-*N*-Cbz-neomycin B (**5**) in 30% yield ($C_{39}H_{58}N_6O_{17}\cdot\frac{1}{2}H_2O$; mp 170~172°C; TLC*2 Rf 0.25). The mixture of **3** and **4** was treated with ethyl chloroformate to protect the remaining free amino groups with ethoxycarbonyl (Cbe) groups, affording a mixture of penta-Cbe derivatives, **6** and **7**, in 96% yield ($C_{46}H_{72}N_6O_{25}\cdot H_2O$; mp 138~147°C; TLC*3 Rf 0.31). The mixture of **6** and **7** was subjected to catalytic hydrogenation (10% Pd/C in 50% aqueous EtOH) to give a mixture of the 6'-NH_2 (**8**) and 6'''-NH_2 (**9**) derivatives, which showed ninhydrin-positive spots at Rf 0.33 and Rf 0.41 on TLC*2. The mixture of **8** and **9** was diazotized in dilute H_2SO_4 with $NaNO_2$ (5°C, 2.5 hours) and subsequently passed through Amberlite IR-120 (H^+) and IRA-410 (OH^-) columns, successively, to give a mixture of deaminated products, **10** and **11**, which showed negative ninhydrin and positive anthrone reactions at Rf 0.34 and Rf 0.43 on TLC*4. The mixture was hydrolyzed by heating under reflux with $Ba(OH)_2$ for 5 hours. After being neutralized with $(NH_4)_2CO_3$ and resulting precipitate removed, the hydrolysate was chromatographed on Amberlite CG-50 column (NH_4^+) to separate the final products, **12** and **13**, in 11.5% and 7.4% yield, respectively, from the mixture of **6** and **7**.

In a similar manner, the di-*N*-Cbz derivative (**5**) was converted to the 6',6'''-bis-deaminated compound (**17**) *via* the tetra-*N*-Cbe derivative **14** (90 % yield; $C_{51}H_{74}N_6O_{25}\cdot H_2O$: mp 138~139°C; TLC*3 Rf 0.35), the 6',6'''-diamino derivative **15** (98% yield, mp 188~192°C, TLC*2 Rf 0.28) and the *N*-blocked deaminated derivative **16** (deblocked without purification).

Physico-chemical properties of **12**, **13** and **17** are shown in Table 1. Compound **12** was identified as paromomycin I (**2**) by direct comparison (mp, TLC, $[\alpha]_D$, ^1H NMR, ^{13}C NMR and acid hydrolysis). The structures of **13** and **17** were established to be 6'''-deamino-6'''-hydroxyneomycin B and 6'''-deamino-6'''-hydroxyparomo-

*1 Satisfactory microanalyses (C, H, N) were obtained for a molecular formula indicated.

*2 Plate: silica gel, Merck $60F_{254}$, Solvent: $CHCl_3$ - EtOH - 28 % NH_4OH (1:1:1), lower layer.

*3 Solvent: $CHCl_3$ - MeOH (7:1)

*4 Solvent: $CHCl_3$ - MeOH (5:1)

Table 1. Physico-chemical properties of **12**, **13** and **17**.

	12		**13**		**17**
mp (°C)	180~185		170~175		168~170
Elemental analysis	$C_{23}H_{45}N_5O_{14}\cdot 3H_2O$		$C_{23}H_{45}N_5O_{14}\cdot 2H_2O$		$C_{23}H_{44}N_4O_{15}\cdot 1.5H_2O$
$[\alpha]_D^{23}$	+66° (*c* 0.54, H_2O)		+61° (*c* 0.53, H_2O)		+54° (*c* 0.51, H_2O)[d]
Chemical shift of anomeric protons[a] (δ, ppm)	5.84 (1H, d, J=3.5Hz) 5.44 (1H, d, J=1.5Hz) 5.36 (1H, d, J=1.0Hz)		6.15 (1H, d, J=3.5Hz) 5.53 (1H, d, J=1.5Hz) 5.34 (1H, d, J=1.0Hz)		5.78 (1H, d, J=3.5Hz) 5.41 (1H, d, J=1.5Hz) 5.28 (1H, d, J=1.0Hz)
Rf value in TLC[b]	Paromomycin I		Neomycin B		
Solvent A	0.30	0.30	0.33	0.23	0.37
Solvent B	0.51	0.51	0.53	0.41	
Solvent C	0.30	0.30	0.33	0.21	0.45[d]
TLC[b] of acid hydrolysate[c]	Paromamine		Neamine		
Solvent D	0.13, 0.16	0.13	0.11, 0.26	0.11	0.13, 0.26
Solvent E	0.54, 0.78	0.54	0.33, 0.92	0.33	
Solvent F	0.36, 0.45	0.36	0.18, 0.73	0.18	0.36, 0.73

a) ^1H NMR was run in D_2O (pD<2) and chemical shifts were determined by using the HOD signal (4.8 ppm) as a reference line.

b) Plate: silica gel, Merck $60F_{254}$ Detection: ninhydrin, anthrone
Solvent A : $CHCl_3$ - MeOH - 28 % NH_4OH (1:3:2).
Solvent B : $CHCl_3$ - MeOH - 17 % NH_4OH (2:1:1), upper layer.
Solvent C : $CHCl_3$ - MeOH - 11 N NH_4OH-H_2O (1:4:2:1).
Solvent D : $CHCl_3$ - MeOH - 28 % NH_4OH (1:2:1).
Solvent E : Acetone - AcOH - H_2O (20:6:74).
Solvent F : 10 % aq. NH_4OAc - MeOH - 17 % NH_4OH (9:10:1).

c) A 2-mg sample in 0.2 ml of 1 N HCl, 100°C, 3 hours.

d) Data in literature 12) : $[\alpha]_D$ +54.1° (*c* 1, 0.1 N H_2SO_4), Rf 0.45.

Table 2. ^{13}C NMR data[a] of neomycin B (**1**) and 6‴-deamino-6‴-hydroxyneomycin B (**13**) [20 MHz in D_2O, internal reference dioxane (δ 67.4 ppm)].

Carbon No.	**1**			**13**		
	δ (ppm from TMS)		$\Delta\delta$[c] (ppm)	δ (ppm from TMS)		$\Delta\delta$[c] (ppm)
	Base[b]	pD<2		Base	pD<2	
3‴	71.5	68.1	3.4[d]	71.4	67.4	4.0[d]
4‴	69.4	68.5	0.9	68.9	68.5	0.4
5‴	76.6	71.1	5.5[e]	76.2	76.1	0.1
6‴	41.9	41.2	0.7	62.1	62.1	0

a) Chemical shifts of carbons of **13** other than those shown in this table agree with those of the corresponding carbons of **1** within ±0.4 ppm (mostly within ±0.2 ppm).

b) Chemical shifts of all carbons of **1** accord with those of published data[13] within ±0.2 ppm, except for a difference in C-5‴ (Δ 0.4 ppm), and a reversed assignment for C-5 and C-4‴ [15].

c) $\Delta\delta=\delta$(Base)$-\delta$(pD<2)

d) β-Carbon shift by protonation of 2‴-NH_2.

e) β-Carbon shift by protonation of 6‴-NH_2.

mycin I, respectively, by acid hydrolysis and comparison of ^{13}C NMR (CMR) spectra. Acid hydrolysis (1 N HCl, 100°C, 3 hours) of **13** afforded neamine, while **17** gave paromamine. The second component contained in the hydrolysate of **13** was the same as that present in the hydrolysate of **17**. The ^{13}C-signals of **13** agreed with those of neomycin (**1**) within ±0.4 ppm except for four carbons (C-3‴, C-4‴, C-5‴ and C-6‴). As shown in Table 2, the CMR spectrum of **13** showed an additional peak at 62.1 ppm due to CH_2OH and a lack of the signal of 6‴-CH_2NH_2 of **1** at 41~42 ppm. In addition, the β-carbon shift by protonation[11] in the C-5‴ signal of **1** (76.6 ppm→71.1 ppm) was not observed with **13**. This is also the case when the CMR spectra of paromomycin I (**2**) and **17** are compared (Table 3). These results indicated that **13** and **17** were

Table 3. ^{13}C NMR data[a] of paromomycin I (**2**) and 6‴-deamino-6‴-hydroxyparomomycin I (**17**) [20 MHz in D_2O, internal reference dioxane (δ 67.4 ppm)].

Carbon No.	**2**			**17**		
	δ (ppm from TMS)		Δδ[c] (ppm)	δ (ppm from TMS)		Δδ[c] (ppm)
	Base[b]	pD<2		Base	pD<2	
3‴	71.6	68.1	3.5[d]	71.4	67.2	4.2[d]
4‴	69.4	68.5	0.9	68.9	68.5	0.4
5‴	76.6	71.0	5.6[e]	76.2	76.3	−0.1
6‴	42.0	41.3	0.7	62.1	62.0	0.1

a) Chemical shifts of carbons of **17** other than those shown in this table agree with those of the corresponding carbons of **2** within ±0.4 ppm (mostly within ±0.2 ppm).

b) Chemical shifts of all carbons of **2** accord with those of published data[14] within ±0.1 ppm, except for differences in C-1 (Δ 1 ppm), C-3 (Δ 1 ppm) and C-5‴ (Δ 0.4 ppm) and a reversed assignment for C-5 and C-4‴ [15].

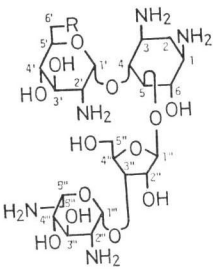
c) $\Delta\delta = \delta$ (Base) − δ (pD<2)

d) β-Carbon shift by protonation of 2‴-NH_2.

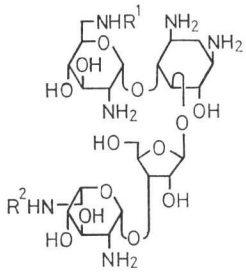
e) β-Carbon shift by protonation of 6‴-NH_2.

Table 4. Antibacterial activity of neomycin B (**1**), 6‴-deamino-6‴-hydroxyneomycin B (**13**), paromomycin I (**2**) and 6‴-deamino-6‴-hydroxyparomomycin I (**17**) (Müeller-Hinton agar).

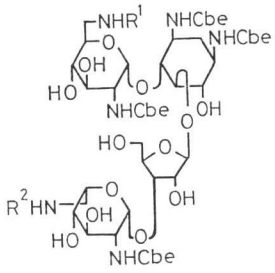
Organism	MIC (μg/ml)			
	1	**13**	**2**	**17**
Escherichia coli Juhl	1.6	3.1	6.3	50
〃 〃 K-12 NR79/W677	>100	>100	>100	>100
〃 〃 K-12 JR35/C600	>100	>100	>100	>100
Klebsiella pneumoniae D11	0.2	0.4	0.4	3.1
〃 〃 22-3038	>100	>100	>100	>100
Serratia marcescens A20019	0.8	6.3	1.6	25
Proteus vulgaris A9436	0.4	0.4	0.8	6.3
Providentia stuartii A20894	6.3	50	50	>100
Pseudomonas aeruginosa A9930	3.1	25	12.5	>100
〃 〃 GN-315	50	>100	>100	>100
Staphylococcus aureus Smith	0.2	0.8	0.8	12.5
〃 〃 A20239	25	>100	>100	>100
Bacillus subtilis PCI 219	<0.05	0.2	0.2	6.3
Geometric mean of MIC against 13 strains of neomycin-sensitive organisms (MIC≦6.3 μg/ml)	0.78	3.67	2.52	32.7



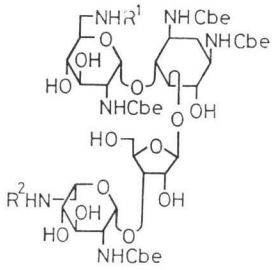
1 Neomycin B R=NH_2
2 Paromomycin I R=OH



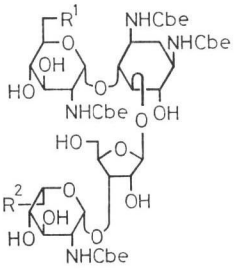
3 R^1=Cbz R^2=H
4 R^1=H R^2=Cbz
5 R^1=Cbz R^2=Cbz



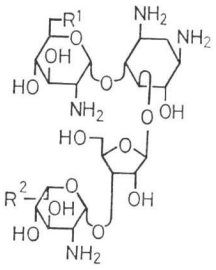
6 R^1=Cbz R^2=Cbe
7 R^1=Cbe R^2=Cbz
14 R^1=R^2=Cbz



8 R^1=H R^2=Cbe
9 R^1=Cbe R^2=H
15 R^1=R^2=H



10 R^1=OH R^2=NHCbe
11 R^1=NHCbe R^2=OH
16 R^1=R^2=OH



12 R^1=OH R^2=NH_2
13 R^1=NH_2 R^2=OH
17 R^1=R^2=OH

Table 5. Acute toxicity (mice, i.v.) of neomycin B(**1**), paromomycin I (**2**), 6‴-deamino-6‴-hydroxyneomycin B (**13**) and 6‴-deamino-6‴-hydroxyparomomycin I (**17**).

Compound	LD_{50} (mg/kg) (95 % confidence limits)	Relative toxicity*
1	28 (15~53)	1.00
2	106 (62~221)	0.26
13	141 (76~263)	0.20
17	>200**	<0.14

* Toxicity relative to **1**.
** Non-toxic at 200 mg/kg.

the 6‴-OH analogs of **1** and **2**, respectively.

According to a recent publication of AUTISSIER and coworkers[12], biosynthetic intermediates of neomycin and paromomycin were isolated from the fermentation broth of a neomycin-producing organism and of a blocked mutant of a paromomycin-producing strain. Their structures were proposed to be 6‴-deamino-6‴-hydroxyneomycins B*[1] and C, and 6‴-deamino-6‴-hydroxyparomomycins I*[2] and II, although structural verification was described only for 6‴-deamino-6‴-hydroxyparomomycin II (compound X_2 in reference 12).

The minimum inhibitory concentrations (MIC) of **1**, **2**, **13** and **17** were determined by the two-fold agar dilution method using Müeller-Hinton agar. The MIC values presented in Table 4 are representative of those obtained from a larger group of test organisms. The 6‴-hydroxyl derivatives **13** and **17** showed a similar antibacterial spectrum to that of the corresponding 6‴-amino derivatives **1** and **2**, while the intrinsic activity was 2 to 16 times lower for the 6‴-hydroxyl congeners. The last line in Table 4 shows the geometric mean of MIC against 13 strains of neomycin-sensitive organisms (MIC of **1**, $\leqq$6.3 μg/ml) employed in the present study, indicating that the antibacterial activity of the aminoglycoside derivatives described above increases in the order

*[1] Direct comparison with **13** has not yet been made due to non-availability of the biosynthetic sample (P. BARTHELEMY, Personal communication).

*[2] The sample received from P. BARTHELEMY was identical with **17** by direct comparison.

of the number of amino groups in the molecule: neomycin B (**1**) having six amino groups was the most active, followed by **2** and **13** possessing five amino groups which were about one-fourth as active as **1**. The tetra-amino derivative **17** was the least active, being about one-tenth as active as the penta-amino derivatives. Conversion of an amino group of **1** to a hydroxyl group at the 6‴-position (**1**→**13**) reduced the intrinsic activity to a greater extent than the deamination at the 6′-position (**1**→**2**).

The acute toxicities of **1**, **2**, **13** and **17** determined by intravenous injection are shown in Table 5. In terms of LD_{50}, **2** and **13** were 1/4 and 1/5 as toxic as **1**, respectively, and **17** was the least toxic of the four compounds tested, indicating that the acute toxicity is inversely proportional to the antimicrobial activity in this series of aminoglycoside derivatives.

References

1) UMEZAWA, H.; S. UMEZAWA, T. TSUCHIYA & Y. OKAZAKI: 3′,4′-Dideoxykanamycin B active against kanamycin-resistant *Escherichia coli* and *Pseudomonas aeruginosa*. J. Antibiotics 24: 485～487, 1971
2) KAWAGUCHI, H.; T. NAITO, S. NAKAGAWA & K. FUJISAWA: BB-K 8, a new semisynthetic aminoglycoside antibiotic. J. Antibiotics 25: 695～708, 1972
3) TODA, S.; S. NAKAGAWA & T. NAITO: Aminoglycoside antibiotics. X. Chemical conversion of kanamycin B to kanamycin C and 6′-deoxykanamycin C. J. Antibiotics 30: 1002～1003, 1977
4) KONDO, S.; T. MIYASAKA, K. YOSHIDA, K. IINUMA & H. UMEZAWA: Synthesis and properties of kanamycin C derivatives active against resistant bacteria. J. Antibiotics 30: 1150～1152, 1977
5) IGARASHI, K.: Chemical modification of tobramycin. Jap. J. Antibiotics 32 Suppl: S-187～S-194, 1979
6) BOXLER, D. L.; R. BRAMBILLA, D. H. DAVIES, A. K. MALLAMS, S. W. MCCOMBIE, J. B. MORTON, P. REICHERT & H. F. VERNAY: Semisynthetic aminoglycoside antibiotics. 9. Synthesis of novel 1- and 3-substituted and 1- and 3-epi-substituted derivatives of sisomicin and gentamicin from the 1- and 3-oxo-derivatives. J. Chem. Soc., Perkin I 1981: 2168～2185, 1981
7) WAKSMAN, S. A. & H. A. LECHEVALIER: Neomycin, a new antibiotic active against streptomycin-resistant bacteria, including tuberculosis organisms. Science 109: 305～307, 1949
8) REGNA, P. P. & F. X. MURPHY: The isolation of neomycin B. J. Am. Chem. Soc. 72: 1045～1046, 1950
9) COFFEY, G. L.; L. E. ANDERSON, M. W. FISCHER, M. M. GALBRAITH, A. B. HILLEGAS, D. L. KOHBERGER, P. E. THOMPSON, K. S. WESTON & J. EHRLICH: Biological studies of paromomycin. Antibiot. Chemother. 9: 730～738, 1959
10) HASKELL, T. H.; J. C. FRENCH & Q. R. BARTZ: Paromomycin. I. Paromamine, a glycoside of D-glucosamine. J. Am. Chem. Soc. 81: 3480～3481, 1959
11) NAITO, T.; S. TODA, S. NAKAGAWA & H. KAWAGUCHI: Carbon-13 NMR spectra of aminoglycoside antibiotics. *In* K. L. REINHART, Jr. & T. SUAMI (*ed*), "Aminocyclitol Antibiotics", ACS Symposium series 125, pp. 257～294, American Chemical Society, Washington, D. C. 1980 and references therein.
12) AUTISSIER, D.; P. BARTHELEMY, N. MAZIERES, M. PEYRE & L. PENASSE: 6‴-Deamino-6‴-hydroxy derivatives, as intermediates in the biosynthesis of neomycin and paromomycin. J. Antibiotics 34: 536～543, 1981
13) HANESSIAN, S.; T. TAKAMOTO, R. MASSÉ & G. PATIL: Aminoglycoside antibiotics: Chemical conversion of neomycin B, paromomycin and lividomycin B into bioactive pseudosaccharides. Can. J. Chem. 56: 1482～1491, 1978
14) HANESSIAN, S.; R. MASSÉ & (in part) G. EKBORG: Aminoglycoside antibiotics: The formation and characterization of dihydrooxazine derivatives in the paromomycin series. Can. J. Chem. 56: 1492～1499, 1978
15) TODA, S.; T. NAITO & H. KAWAGUCHI: Chemical conversion of neomycin B to paromomycin I and its 6‴-OH isomer. Nippon Kagaku Kaishi 1982: 1713～1720, 1982