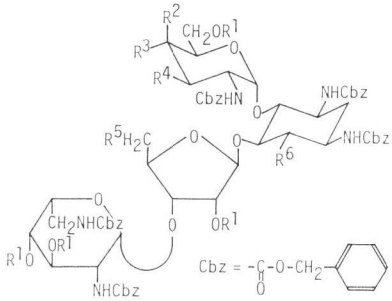
SEMISYNTHETIC AMINOGLYCOSIDE ANTIBIOTICS. IV 3′,4′-DIDEOXYPAROMOMYCIN AND ANALOGUES[1]

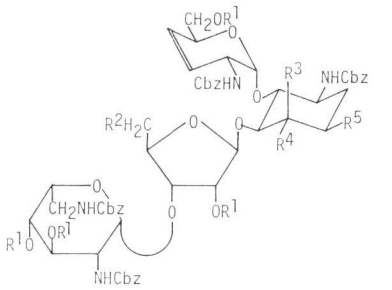
Sir:

Bacterial resistance to paromomycin is mainly due to enzymatic phosphorylation[2] of the hydroxyl groups at positions 3′ and 5″, and less frequently to a nucleotidylation at position 4″[3]. Since modifications at such positions can afford compounds with a larger spectrum of activity, we pursued deoxygenation, epimerization or substitution of the involved hydroxyl groups. In a previous paper[1] we reported the synthesis of a first series of paromomycin derivatives in which the essential feature was deoxygenation at position 4′. The present communication deals with the synthesis and the study of the biological activity of paromomycin derivatives in which both positions 3′ and 4′ are modified, namely: 3′,4′-dideoxyparomomycin (**11a**), 5″-amino-3′,4′,5″-trideoxyparomomycin (**11b**), 3′-deoxy-4′-*epi*-paromomycin (**12a**), 5″-amino-3′,5″-dideoxy-4′-*epi*-paromomycin (**12b**), 1-deamino-6,3′,4′-trideoxy, 1,6-β-epiminoparomomycin (**13**), along with 4′-amino-4′-deoxyparomomycin (**14**).*

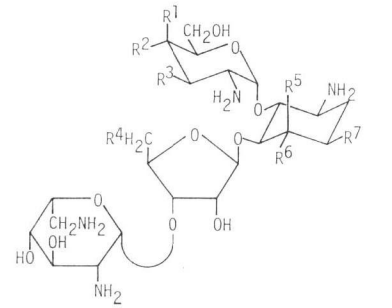
Starting materials were 6,3′,2″,5″,3‴, 4‴-hexa-*O*-acetyl-6′-*O*-benzoyl-penta-*N*-benzyloxycarbonylparomomycin[1] and 6,3′,2″,3‴,4‴-



1a : $R^1=R^3=H$, R^2-R^4=-O-, $R^5=R^6=OH$
1b : $R^1=R^3=H$, R^2-R^4=-O-, $R^5=N_3$, $R^6=OH$
2a : $R^1=Bz$, $R^3=H$, R^2-R^4=-O-, $R^5=R^6=OBz$
2b : $R^1=Bz$, $R^3=H$, R^2-R^4=-O-, $R^5=N_3$, $R^6=OBz$
3a : $R^1=Bz$, $R^2=H$, $R^3=I$, $R^4=OH$, $R^5=R^6=OBz$
3b : $R^1=Bz$, $R^2=H$, $R^3=I$, $R^4=OH$, $R^5=N_3$, $R^6=OBz$
4a : $R^1=R^3=R^4=H$, $R^2=R^5=R^6=OH$
4b : $R^1=R^3=R^4=H$, $R^2=R^6=OH$, $R^5=N_3$
5 : $R^1=R^2=H$, $R^3=N_3$, $R^4=R^5=R^6=OH$
6 : $R^1=Bz$, $R^3=H$, R^2-R^4=-O-, $R^5=OBz$, $R^6=OH$
7 : $R^1=Bz$, $R^2=H$, $R^3=I$, $R^4=R^6=OH$, $R^5=OBz$



8a : $R^1=Bz$, $R^2=R^4=OBz$, $R^3=H$, $R^5=NHCbz$
8b : $R^1=Bz$, $R^2=N_3$, $R^3=H$, $R^4=OBz$, $R^5=NHCbz$
9 : $R^1=Bz$, $R^2=OBz$, $R^3=H$, $R^4=OMs$, $R^5=NHCbz$
10 : $R^1=R^4=H$, $R^2=OH$, R^3-R^5=-N(Cbz)-



11a : $R^1=R^2=R^3=R^5=H$, $R^4=R^6=OH$, $R^7=NH_2$
11b : $R^1=R^2=R^3=R^5=H$, $R^4=R^7=NH_2$, $R^6=OH$
12a : $R^1=R^4=R^6=OH$, $R^2=R^3=R^5=H$, $R^7=NH_2$
12b : $R^1=R^6=OH$, $R^2=R^3=R^5=H$, $R^4=R^7=NH_2$
13 : $R^1=R^2=R^3=R^6=H$, $R^4=OH$, R^5-R^7=-NH-
14 : $R^1=R^5=H$, $R^2=R^7=NH_2$, $R^3=R^4=R^6=OH$

* All new compounds gave correct microanalyses and exibited ^1H, ^{13}C NMR and mass spectral characteristics that are in agreement with their structures.

penta-*O*-acetyl-5‴-azido-6′-*O*-benzoyl-5″-deoxy-penta-*N*-benzyloxycarbonylparomomycin.[1] *O*-Mesylation of such compounds at the 4′ position and subsequent treatment with sodium methoxide in chloroform gave the versatile 3′,4′-β-epoxy intermediates **1a** (60% yield, m.p. 126~128°C, $[\alpha]_D^{20}$+30.6° (*c* 1.04, $CHCl_3$)) and **1b** (80% yield) respectively. Protection of the free hydroxyl groups of **1a** by exaustive benzoylation (benzoyl chloride, pyridine, 20 hours, room temperature) afforded **2a**. Opening of the oxirane ring with sodium iodide in acetone (in the presence of AcONa-AcOH, 7 hours, reflux) afforded the iodohydrin **3a**. Reaction with methanesulfonyl chloride in pyridine at reflux temperature[4,5] gave the 3′-ene derivative **8a**: m.p. 110°C, $[\alpha]_D^{20}$+13.3° (*c* 0.9, $CHCl_3$) (33% yield from **1a**). Transfer catalytic hydrogenation (cyclohexene, 10% Pd-C, in 80% aqueous ethanol, 10 minutes, reflux) on conventionally deacylated **8a**, provided the removal of *N*-protecting groups and saturation of the double bond, affording 3′,4′-dideoxyparomomycin (**11a**) (sulfate: $[\alpha]_D^{20}$+44° (*c* 0.95, H_2O), Rf 0.35**) in 30% yield from **8a**.

The same reaction sequence was applied to the 5″-azido-5″-deoxy analog (**b** series). The previously described hydrogenation step provided, in this case, at the same time the removal of *N*-protecting groups, the saturation of the carbon-carbon double bond and the conversion of the azido group into the amino group giving 5″-amino-3′,4′,5″-trideoxyparomomycin (**11b**) (sulfate: $[\alpha]_D^{20}$+37° (*c* 0.82, H_2O), Rf 0.33**) in 10% yield from **1b**.

The synthetic versatility of the key intermediates **1a** and **1b** was also proved by opening the oxirane ring with other nucleophiles. In this respect, treatment of **1a** with lithium borohydride[6] gave as a major product the compound **4a** (Rf 0.28***) distinguishable from the isomeric 4′-deoxy-penta-*N*-benzyloxycarbonylparomomycin[1]. The final hydrogenation step afforded 3′-deoxy-4′-*epi*-paromomycin (**12a**) (Rf 0.39**). Analogously 5″-amino-3′,5″-dideoxy-4′-*epi*-paromomycin (**12b**) (Rf 0.30**) was obtained from **1b**.

Nucleophilic attack to the oxirane moiety of **1a** by azide anion (NaN_3, NH_4Cl, EtOH, H_2O, 8 hours, reflux)[7] led to 4′-azido-4′-deoxy-penta-

Table 1. *In vitro* biological activity of the new paromomycin analogues.

		11a (sulfate)	**11b** (sulfate)	**12a**	**12b**	**14** (sulfate)	Paromomycin (sulfate)	Lividomycin A (sulfate)
Test organism		Antibacterial activity: MIC (μg/ml)*1						
S. aureus 209 P		1.56	3.12	6.25	50	3.12	1.56	—
S. epidermidis FK 109*2		3.1	6.25	12.5	100	6.25	>200	—
E. coli K 12		12.5	100	25	100	12.5	6.25	6.25
E. coli K 12 (R 148)*3		25	100	50	>100	200	>200	25
Ribosomes		Inhibition of protein synthesis (μM concentration)*4						
70 S	IC_{25}	23	22	26	23	16	30	29
	IC_{50}	52	45	56	51	27	80	90
80 S	IC_{25}	47	80	>400	190	36	72	>400
	IC_{50}	220	>400	>400	>400	130	>400	>400

*1 The MICs (μg/ml) were determined *in vitro* with the twofold dilution technique in Antibiotic Medium No. 3 (Difco). The inoculum was about 10^6 cells/ml and the cultures were incubated at 37°C for 24 hours.

*2 AAD (4′) producer.

*3 APH (3′) producer.

*4 IC_{25} and IC_{50} (μM) were determined as the concentrations able to inhibit 25% and 50% respectively of the polyphenylalanine synthesis, directed by poly U in the presence of ribosomes from *Escherichia coli* (70 S) or from *Saccharomyces cerevisiae* (80 S). Elongation factors and phenylalanine tRNA were prepared from the same microorganisms.

** Silica gel TLC, $CHCl_3$-MeOH-32% aqueous ammonia (1:3:2, v/v/v).

*** Silica gel TLC, $CHCl_3$-AcOEt-MeOH (40:25:9, v/v/v)

N-benzyloxycarbonylparomomycin (**5**) in 40% yield. The usual hydrogenation procedure gave 4′-amino-4′-deoxyparomomycin (**14**) in 65% yield ($[\alpha]_D^{20}$ +59° (*c* 0.96, H_2O), Rf 0.29**).

A modification on the cyclitol ring was also achieved, taking advantage of a defective benzoylation of the epoxy derivative **1a**; mild conditions (benzoyl chloride, pyridine, 2 hours, 0°C) gave in fact compound **6** (90% yield), still bearing a free hydroxyl group at position 6. Conversion into the corresponding iodohydrin (**7**) (by treatment with NaI, AcONa, AcOH in acetone), followed by heating with methanesulfonyl chloride in pyridine, provided both the formation of the 3′-ene moiety and *O*-mesylation at position 6 (compound **9**) in 44% yield from **6**. Usual alkaline deacylation (MeONa in MeOH) caused also displacement of the methanesulfonate anion by the vicinal nitrogen atom with the formation of an aziridine ring at positions 1 and 6 (compound **10**, 66% yield). Finally transfer catalytic hydrogenation afforded 1-deamino-6,3′,4′-trideoxy-1,6-β-epiminoparomomycin (**13**) (Rf 0.53**) in 55% yield.

Compound **9** could be useful also to obtain 6-*epi* or 6-deoxyparomomycin derivatives, by displacement of the methansulfonate anion with a proper nucleophile.

The chemical modification of paromomycin at positions 3′,4′ and 5″ affects the biological properties of the antibiotic as shown in Table 1. All the new compounds, as expected, were more active than paromomycin against *Staphylococcus epidermidis* FK 109, producer of aminoglycoside-4′-adenyltransferase [AAD(4′)][3], and against *E. coli* K 12 (R 148), which is known to produce 3′-phosphotransferase II [APH(3′)-II]. On the contrary, a general reduction of potency has been observed with sensitive strains, particularly *E. coli*, and the decrease of activity seems to be mainly related to 5″-amino substitution on 3′-deoxygenated analogues, as previously observed in lividomycin[8], and to 4′-epimerization. All the new analogues were more effective than paromomycin in the inhibition of protein synthesis carried out by 70S ribosomes, while only compounds **11a** and **14** displayed higher activity on the 80S ribosome system. The lack of correlation between antibacterial activity and inhibition of protein synthesis *in vitro* suggests that the reduced efficacy on the intact growing bacteria could be related to impaired intracellular uptake. Similar results were previously obtained with other paromomycin derivatives prepared in our laboratories[9].

Acknowledgements

We thank Prof. O. Ciferri and G. Di Pasquale (University of Pavia-Italy) for the *in vitro* ribosomal tests and Prof. S. Hanessian (University of Montreal, Canada) for useful discussions.

Carlo Battistini
Giovanni Franceschi
Franco Zarini
Giuseppe Cassinelli
Federico Arcamone

Farmitalia Carlo Erba, S.p.A.
Ricerca & Sviluppo Chimico
Via dei Gracchi, 35
Milan 20146, Italy

Aurora Sanfilippo

Farmitalia Carlo Erba, S.p.A.
Ricerca & Sviluppo Biologico
20014 Nerviano (Milan), Italy

(Received October 19, 1981)

References

1) For the previous paper in the series see: Battistini C.; G. Cassinelli, G. Franceschi, F. Arcamone & R. Mazzoleni: Semisynthetic aminoglycoside antibiotics. III. New deoxy derivatives of paromomycin. J. Antibiotics 34: 240~242, 1981
2) Price, K. E.; J. C. Godfrey & H. Kawaguchi: Effect of structural modifications on the biological properties of aminoglycoside antibiotics containing 2-deoxystreptamine. *In* "Structure Activity Relationships among the Semisynthetic Antibiotics" (D. Perlman, *Ed.*) pp. 239~355. Academic Press, New York, 1977
3) Santanam, P. & F. H. Kayser: Purification and characterization of an aminoglycoside inactivating enzyme from *Staphylococcus epidermidis* FK-109 that nucleotidylates the 4′ and 4″-hydroxyl groups of the aminoglycoside antibiotics. J. Antibiotics 31: 343~351, 1978
4) Lemieux, R. U.; E. Fraga & K. A. Watanabe: Preparation of unsaturated carbohydrates. A facile synthesis of methyl 4,6-*O*-benzylidene-D-hex-2-enopyranosides. Can. J. Chem. 46: 61~69, 1968
5) Yoneta, T.; S. Shibahara, T. Matsuno, S.

TOHMA, S. FUKATSU, S. SEKI & H. UMEZAWA: An improved synthesis of 3′,4′-dideoxykanamycin B. Bull. Chem. Soc. Japan 52: 1131～1134, 1979

6) PFEIFFER, F. R.; S. J. SCHMIDT, C. M. KINZIG, J. R. E. HOOVER & J. A. WEISBACH: 3′ and 4′-axial and equatorial amino and hydroxy derivatives of neamine. Carbohydr. Res. 72: 119～137, 1979

7) ALI, Y. & A. C. RICHARDSON: The reduction of azides with sodium borohydride: a convenient synthesis of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-allopyranoside. Carbohydr. Res. 5: 441～448, 1967

8) KONDO, S.; H. YAMAMOTO, K. IINUMA, K. MAEDA & H. UMEZAWA: A synthesis of 6′,5″, 6‴-triamino-6′,5″, 6‴-trideoxylividomycin A and 6′,5″-diamino-6′,5″-dideoxylividomycin B. J. Antibiotics 29: 1134～1136, 1976

9) ARCAMONE, F. M.; G. CASSINELLI, P. JULITA, U. ROSSI, G. SCHIOPPACASSI, R. MAZZOLENI, A. SANFILIPPO & O. CIFERRI: Nuovi derivati semisintetici dell'amminosidina. Giorn. It. Chemiot. 24: 77～81, 1977