AMINOGLYCOSIDE ANTIBIOTICS: SYNTHESIS OF 5″-AMINO-5″-DEOXYNEOMYCIN AND 5″-AMINO-5″-DEOXYPAROMOMYCIN*

Sir:

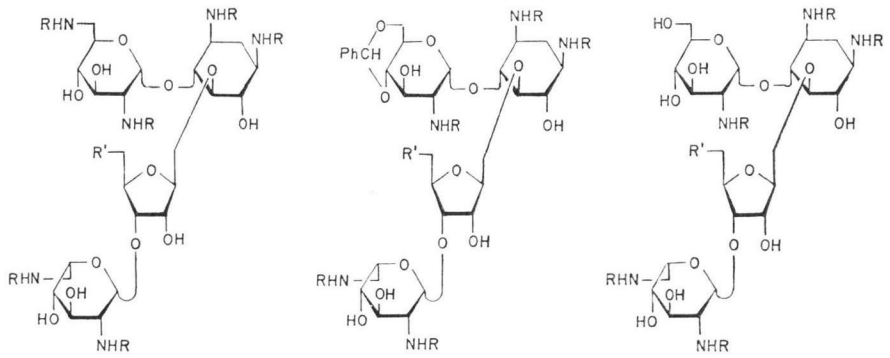
The chemical modification of aminoglycoside antibiotics by the selective and systematic introduction or removal of functional groups at strategic sites, continues to be an important facet in research programs concerned with their chemistry and biochemistry[1,2]. In view of the highly multifunctional and polar nature of the aminoglycoside antibiotics, progress in their peripheral modifications has been hampered at times, and practical methods for selective transformations are in constant demand.

We describe herein, the preparation of the title compounds from the respective *N*-protected parent antibiotics by an effective procedure[3] involving the selective and sequential conversion of a primary hydroxyl group into the corresponding bromodeoxy and aminodeoxy functions.

Treatment of penta-*N*-benzyloxycarbonylneomycin (**1**)[2] (0.52 g, 0.36 mmole) in 20 ml of dry HMPT** containing triphenylphosphine (0.24 g, 0.92 mmole), with *N*-bromosuccinimide (NBS) (0.161 g, 0.90 mmole) and heating the mixture at 55°C for 4 hours,*** followed by addition of methanol (1 ml), then excess sodium azide, and further heating under the same conditions, gave, after chromatography on silica gel ($CHCl_3$ - EtOAc - MeOH, 20 : 5 : 2), the corresponding 5″-azido-5″-deoxy derivative **2**, as a colorless amorphous solid (73%), mp 120~121°C; $[\alpha]_D^{25}$ +30° (*c* 1.09, $CHCl_3$)**** (Scheme 1).

Alternatively, treatment of **1** with NBS-Ph_3P as described above, followed by chromatography, gave the 5″-bromo-5″-deoxy derivative **3** as the dihydrate in 91% yield, mp 124~125°C; $[\alpha]_D^{25}$ +22.9° (*c* 0.52, $CHCl_3$). Subsequent nucleophilic displacement with sodium azide, as described above, gave the same 5″-azido-5″-deoxy derivative **2** in 82.5% yield. Hydrogenolysis of **2** (1.98 g) in a 2: 1 mixture of dioxane-methanol (75 ml), containing 10 ml of 1 N HCl, in the pre-

Scheme 1.



1 R = Cbz; R' = OH
2 R = Cbz; R' = N_3
3 R = Cbz; R' = Br
4 R = H; R' = NH_2

5 R = Cbz; R' = OH
6 R = Cbz; R' = N_3
7 R = Cbz; R' = Br

8 R = Cbz; R' = N_3
9 R = H; R' = NH_2

* Reported in part at the 57th meeting of the Chemical Institute of Canada, Merck Award Lecture, Regina, Saskatchewan, June 1974; taken from the Ph.D. thesis of R. MASSÉ, University of Montreal, 1975.

For a previous paper in this series: see HANESSIAN, S.; T. TAKAMOTO & R. MASSÉ: Aminoglycoside antibiotics: Oxidative degradations leading to novel biochemical probes and synthetic intermediates. J. Antibiotics 28: 835~837, 1975, and references cited therein.

** It is important to work with dry solvents and reagents. Since aminoglycoside derivatives tend to occlude water, samples were initially dissolved in dry HMPT and the solvent was distilled, thereby eliminating traces of water as an HMPT-water azeotrope. The cooled solutions were then treated with the halogenation reagents.

*** The reaction could also be conducted at 25°C, but in the present 5~6 molar excess of reagents.

**** New compounds gave microanalytical data that were consistent with their structures.

Table 1. Antibacterial activity of 5″-amino-5″-deoxyneomycin (**4**), 5″-amino-5″-deoxyparomomycin (**9**), neomycin and paromomycin.

Organism*	Minimal inhibitory concentration (mcg/ml)			
	4	Neomycin	**9**	Paromomycin
Staphylococcus aureus B 352	0.1	0.2	0.2	0.2
Staphylococcus aureus 353	0.1	0.2	0.4	0.2
Streptococcus faecalis	0.8	0.8	6.4	1.6
E. coli 198 (ATCC 1/229)	0.8	0.8	6.4	0.4
E. coli B 989**	1.6	1.6	12.5	100
E. coli B 1002***	3.2	50	—	—
Aerobacter aerogenes 357	0.8	0.8	1.6	0.2
Salmonella pullorum	0.4	0.2	0.8	0.2
P. aeruginosa 359	1.6	0.8	12.5	0.8
P. aeruginosa 87442	0.8	0.2	—	—
P. aeruginosa 377	1.6	0.4	—	—
Proteus mirabilis 360	0.8	0.4	1.6	0.2
Proteus vulgaris 361	0.8	0.2	1.6	0.2
Klebsiella pneumoniae 855 (ATCC 1068)	0.4	0.2	0.8	0.2
Sarcina marcescens 854 (ATCC 6103)	0.4	0.2	0.8	0.4

* Test organisms: Ayerst Laboratories, Montreal. We thank Dr. H. BAKER for these tests. Serial dilution method (37°C, 24 hours).

** Resistant to kanamycin (MIC, 100 mcg/ml).

*** Resistant to kanamycin and neomycin.

sence of 20% palladium hydroxide-on-charcoal[4], gave a syrup that was dissolved in 5 ml of water and the solution was diluted with ethanol to give 5″-amino-5″-deoxyneomycin (**4**), as a colorless amorphous hydrochloride salt, mp 205~210°C (dec.); $[\alpha]_D^{25}+44.7°$ (*c* 0.94, H_2O); paper chromatographic data (1-PrOH - pyridine - AcOH - H_2O, 15: 10: 3: 12): for **4**, migration distance 9.2 cm; for neomycin, 5.2 cm; elution time, 21 hours; t.l.c. data (silica gel, $CHCl_3$ - MeOH - 28% NH_4OH, 1: 3: 2); for **4**, Rf 0.16; for neomycin Rf 0.20; mass spectral data on the corresponding hepta-*N*-acetyl-per-*O*-trimethylsilyl derivative;* for a fragment $C_{16}H_{33}N_2O_5Si_2$: *m/e* calcd. 389.1928; measured, 389.1939; for $C_{16}H_{33}N_2O_4Si_2$ *m/e* calcd. 373.1979; measured, 373.2004; ^{13}C.m.r. data (ppm)**: C-5″, 43.0; C-4″, 84.9 (pH 11); C-5″, 42.8; C-4″, 78.2 (pH 1); for neomycin[5]: C-5″, 61.4; C-4″, 85.7 (pH 11).

Compound **4** could also be obtained from penta-*N*-benzyloxycarbonylparomomycin[2] by application of the above described procedure to give 5″,6′-diazido-5″,6′-dideoxy-penta-*N*-benzyloxycarbonylparomomycin in 80% yield, mp 97~100°C; $[\alpha]_D^{25}+43.9°$ ($CHCl_3$), and hydrogenation. In this case, the bromination reaction was, as expected, selective for the two primary hydroxyl groups in the parent *N*-protected antibiotic.

Starting with the readily available 4′,6′-*O*-benzylidene-penta-*N*-benzyloxycarbonylparomomycin (**5**), and applying the halogenation and azide displacement procedure, gave the corresponding 5″-azido-5″-deoxy derivative **6**, in 76% yield, mp 123~125°C; $[\alpha]_D^{25}+37°$ (*c* 1.0, $CHCl_3$). The corresponding 5″-bromo-5″-deoxy analog **7** could also be prepared as described for the neomycin derivative, in 81% yield, mp 120~123°C; $[\alpha]_D^{25}+36.9°$ (*c* 1.3, $CHCl_3$). Acid hydrolysis (80% aq.AcOH) of **6** gave the 5″-azido-5″-deoxy derivative **8** as an amorphous, colorless solid, in 80% yield, mp 118~120°C; $[\alpha]_D^{25}+40°$ (*c* 1.0, $CHCl_3$). Hydrogenolysis as previously described, gave 5″-amino-5″-deoxyparomomycin (**9**), as an amorphous hydrochlo-

* For methodology, see DE JONGH, D. C.; J. D. HRIBAR, S. HANESSIAN & P. W. K. WOO: J. Amer. Chem. Soc. 89: 1469~1470, 1967; DE JONGH, D. C.; E. B. HILLS, J. D. HRIBAR, S. HANESSIAN & T. CHANG: Tetrahedron 29: 2707~2713, 1973.

** Recorded on a Bruker-WH instrument at 22.6 MHz, (decoupling frequency at 90 MHz) in D_2O with dioxane as the internal standard.

ride salt, mp 200~202°C: $[\alpha]_D^{25}$ +42.2° (*c* 0.57, H_2O); paper chromatographic data: for **9**, migration distance, 10.4 cm; for paromomycin, 15.1 cm; elution time, 21 hours; t.l.c. data (see above); for **9**, Rf 0.26; for paromomycin, Rf 0.32. The structure was confirmed by spectroscopic data and by chemical degradation; mass spectral data on the corresponding hexa-*N*-acetyl-per-*O*-trimethylsilyl derivative, for a fragment $C_{17}H_{38}NO_5Si_2$: *m/e* calcd. 420.2058; measured, 420.2071; for $C_{16}H_{33}N_2O_5Si_2$: *m/e* calcd. 389.1928; measured, 389.1932. ^{13}C.m.r. data (ppm): C-5″, 43.0; C-4″, 84.4 (pH~11); C-5″, 42.6; C-4″, 78.3 (pH~1); for paromomycin, C-5″, 61.3; C-4″, 85.4 (pH~11).

Both 5″-amino-5″-deoxyneomycin and 5″-amino-5″-deoxyparomomycin exhibited antibacterial activities comparable to the parent antibiotics (Table 1). In addition, they were more active against certain strains of *E. coli* that were resistant to kanamycin and neomycin.

Thus, in spite of the plethora of amino and hydroxyl groups in neomycin and paromomycin, it is evident that the mere substitution of the primary hydroxyl group by an amino group at C-5″ is causing aberrant recognition *vis-a-vis* the phosphotransferases, resulting in an enhancement in the antibacterial activity. In support of this, it has been found that 5″-amino-5″-deoxyneomycin has only one-third the substrate activity of neomycin, toward phosphorylation by NPT_I and NPT_{II}*. It is also of interest that whereas replacement of the C-5″ hydroxyl group in butirosin by an amino group led to an active product[6], analogous modification of lividomycin A, led to a generally weaker antibiotic[7]. Substitution of the primary hydroxyl groups in lividomycins A and B by amino groups, led however, to bioactive substances[8].

The C-5″ position in aminoglycoside antibiotics containing a 5-β-D-ribofuranosyl 2-deoxystreptamine moiety, continues to be a promising site for further chemical modification, particularly that in some cases, it is also the site of enzymatic phosphorylation[9]. In view of this, and other considerations pertaining to structure-activity relationships[10], the successful extension of the NBS-Ph_3P halogenation reaction to the complex aminoglycosides and the subsequent facile amination, has important synthetic consequences. Previously, such functionalization of primary hydroxyl groups in this series were done *via* sulfonylation, and subsequent displacement with azide ion. However, yields of the sulfonate esters have, for the most part, been moderate to low and the entire procedure has been somewhat lengthy. The selective halogenation described in this Communication, circumvents such difficulties and provides access to the preparatively versatile primary halodeoxy derivatives in high yields and with much manipulative ease. Furthermore, the one-flask, sequential halogenation and azide displacement reactions, as exemplified by the preparation of the title compounds, should prove useful in other cases as well.

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STEPHEN HANESSIAN
ROBERT MASSÉ
MARIE-LOUISE CAPMEAU*

Department of Chemistry
University of Montreal
C.P. 6210, Station A
Montreal, Que. H3C 3V1
Canada

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