Aminoglycoside antibiotics: The formation and characterization of dihydrooxazine derivatives in the paromomycin series[1]

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Treatment of penta-*N*-benzyloxycarbonylparomomycin with benzaldehyde and excess zinc chloride gives a dibenzylidene derivative in high yield. This consists of the 4′,6′-*O*-benzylidene 4‴,6‴-*N*,*O*-benzylidene (dihydrooxazine) derivative of penta-*N*-benzyloxycarbonylparomomycin. Chemical evidence is presented to support this structure and model studies are reported for the formation of dihydrooxazine and oxazolidine derivatives of benzyloxycarbonylamino sugars containing suitably situated hydroxyl groups. The easily obtained dihydrooxazine derivative of paromomycin constitutes an interesting, preferentially blocked derivative, that is useful for the chemical modification of the parent antibiotic.

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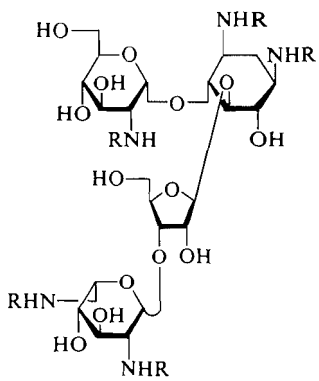
Le traitement de la penta-*N*-benzyloxycarbonylparomomycine par la benzaldehyde et un excès de chlorure de zinc donne un dérivé dibenzylidenique avec des hauts rendements. Ce dérivé implique les cycles 4′,6′-*O*-benzylidenique et 4‴,6‴-*N*,*O*-benzylidenique (dihydrooxazinique) de la penta-*N*-benzyloxycarbonylparomomycine. La structure de ce produit a été élucidée par des méthodes chimiques. Des transformations sur des subtrats modèles sont effectuées en vue d'obtenir des dérivés dihydrooxaziniques et oxazolidiniques. Les dérivés ainsi obtenue dans la série de la paromomycine sont des intermédiaires utiles, amenable à des transformations chimiques.

The knowledge that certain enzymes elaborated by bacterial strains carrying resistance transfer factors are capable of inactivating a group of aminoglycoside antibiotics (1) has been an important stimulus for intensive research work directed at the synthesis (2) and chemical modification of these substances (1*c*, 3). The planning of a synthetic sequence aimed at the selective chemical modification at a specific site in these molecules is sometimes hampered by their multifunctional character. There exists, therefore, a crucial need for the development of methods leading to preferentially blocked derivatives within a given series, by which critical portions of a given molecule are temporarily masked by appropriate functionalities, thus exposing other regions for various manipulations. The problem becomes all the more difficult if one considers that, in a large number of aminoglycosides that contain one or more sugars anchored around a 2-deoxystreptamine unit for example, the orientations and relative dispositions of amino and hydroxyl groups are, for the most part, very similar. One may be faced, for example, with the need to effect selective reactions at one vicinal *trans*-diol group in a hexopyranose unit, but not at another, located on a different sugar unit in a given aminoglycoside.

We were interested in probing the chemical reactivities of pairs of amide and alcohol functions having a 1,2- or a 1,3 disposition, in an aminoglycoside antibiotic such as paromomycin (**1**) (4) (Scheme 1) toward aldehydes and ketones, with the object of preparing oxazolidine and dihydrooxazine derivatives. Such derivatives would constitute interesting synthetic intermediates for further chemical modification work. Paromomycin (**1**) contains four sets of amine alcohol functions, two of which are situated on different rings, whereas the other two are in the 2,6-diamino sugar unit (paromose) (5). Previous work (6) has shown that it is possible to prepare a 4′,6′-*O*-benzylidene derivative in high yield by the treatment of *N*-benzyloxycarbonylparomomycin (**2**)



1 R = H, Paromomycin I
2 R = Cbz

SCHEME 1

[1]Taken, in part, from the Ph.D. Thesis submitted by R. Massé, Université de Montréal, Montréal, P.Q., 1975.

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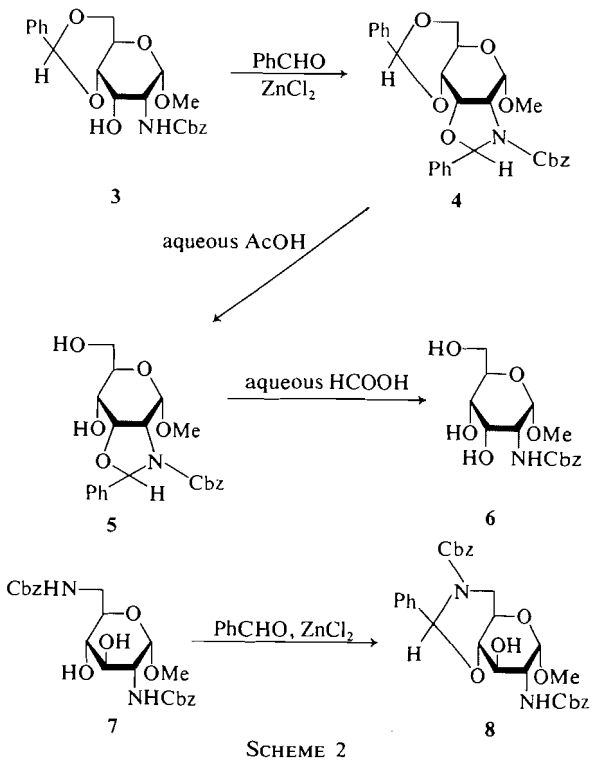
with benzaldehyde in the presence of formic acid. Using excess zinc chloride as catalyst, **2** was transformed into a product that contained two benzylidene groups. This compound isolated in over 80% yield was not oxidized by periodic acid in tetrahydrofuran. An acetal spanning a *trans*-diol group in the diamino sugar portion was excluded because of the relative acid stability of the new compound (see below). The possibility of the formation of an oxazolidine of dihydrooxazine ring, spanning favorably situated amide and hydroxyl functions, was therefore contemplated. In view of the existence of several possibilities involving such rings, we deemed it necessary to study the reaction with some model compounds which encompass some of the structural and functional features present in the various units of paromomycin.

Treatment of methyl 4,6-*O*-benzylidene-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (7) with benzaldehyde and excess zinc chloride gave back unchanged starting material, indicating the reluctance of the *trans* amide–alcohol system to form an oxazolidine derivative. It was apparent, therefore, that the newly introduced 'acetal' function in **2** did not involve any of the three sets of vicinal *trans*-oriented amide hydroxyl units. On the other hand, treatment of methyl 4,6-*O*-benzylidene-2-benzyloxycarbonylamino-2-deoxy-α-D-allopyranoside (**3**) (8) (Scheme 2) with benzaldehyde and excess zinc chloride gave the corresponding crystalline oxazolidine derivative **4**. It was of interest to study the relative stabilities of the acetal and oxazolidine rings in **4**, as it would be expected that the acetal group would be preferentially cleaved under controlled conditions. Indeed, treatment of **4** with 80% aqueous acetic acid at room temperature effected complete cleavage of the acetal function to give the oxazolidine derivative **5** in high yield. When treated with 50% aqueous formic acid, the latter compound was transformed into methyl 2-benzyloxycarbonylamino-2-deoxy-α-D-allopyranoside (**6**) (8).

Attention was next turned to the formation of *N*,*O*-benzylidene derivatives of the dihydrooxazine type, spanning three carbon atoms. Methyl 2,6-dibenzyloxycarbonylamino-2,6-dideoxy-α-D-glucopyranoside (**7**), which contains an unreactive *trans*-vicinal amide hydroxyl unit and a potentially reactive unit having a 1,3-disposition at C-4 and C-6, was treated with benzaldehyde and excess zinc chloride (Scheme 2). As anticipated, the corresponding *N*,*O*-benzylidene derivative **8** was indeed formed, although there remained some unchanged starting material. Treatment of the product with 50% aqueous formic acid gave, as in the previous case, starting compound **7** in good yield.

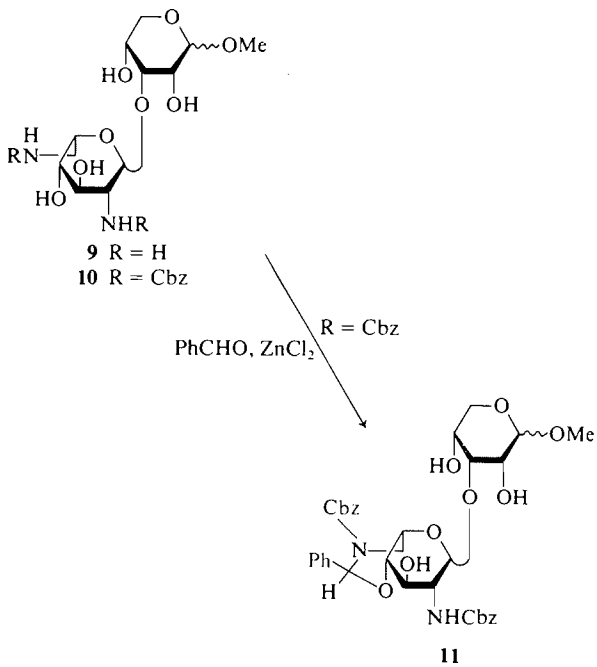


SCHEME 2

Finally, in an effort to simulate the structural and configurational requirements of the diamino sugar unit in paromomycin toward an *N*,*O*-benzylidenation reaction, a suitable degradation product of the antibiotic was sought as a model. Methanolysis of paromomycin gives, in addition to paromamine (9), the disaccharide glycoside, methyl paromobiosaminide **9** (10) (Scheme 3) in which the D-ribose portion has undergone ring expansion. Aside from this structural variation, however, the diamino sugar unit remains unchanged and should be an excellent model to simulate, as closely as possible, the dihydrooxazine ring forming reaction in the parent antibiotic derivative. Treatment of the *N*-benzyloxycarbonyl derivative of methyl paromobiosaminide (**10**) with benzaldehyde and zinc chloride gave a high yield of a new product to which the structure **11** can be assigned. This product was not oxidized with sodium metaperiodate and upon treatment with aqueous formic acid gave **10** in 70% yield.

Based on the results obtained with the model compounds, it was reasonable to assume that the new product formed in the reaction of **2** or **12** with benzaldehyde and excess zinc chloride was actually the dibenzylidene derivative **14** (Scheme 4). We then proceeded to seek direct chemical proof of this structure and to study some of its properties.

The dihydrooxazine ring formation was dependent on the concentration of zinc chloride, as a minimum



SCHEME 3

of 4 equiv. of the Lewis' acid was needed for complete reaction. With 1 or 2 equiv., the major product was the acetal derivative **12**. Treatment of **12** with benzaldehyde and zinc chloride gave the product **14** which was identical to that obtained from the direct treatment of **2**. *N,O*-Benzylidenation also took place with 4′,6′-*O*-cyclohexylidene-*N*-benzyloxycarbonylparomomycin (**13**) to give **15**. Treatment of **14** and **15** individually with aqueous acetic acid effected selective cleavage of the 4′,6′-acetal group to give the preferentially blocked paromomycin derivative **16**. Treatment with aqueous formic acid at 50°C resulted in the formation of the parent **2** but the reaction was accompanied by extensive degradation, no doubt owing to the presence of the relatively acid-labile *O*-β-D-ribofuranosyl residue. The regeneration of the parent antibiotic, paromomycin, was possible however, under conditions of hydrogenolysis, where the only detectable side product was paromamine.

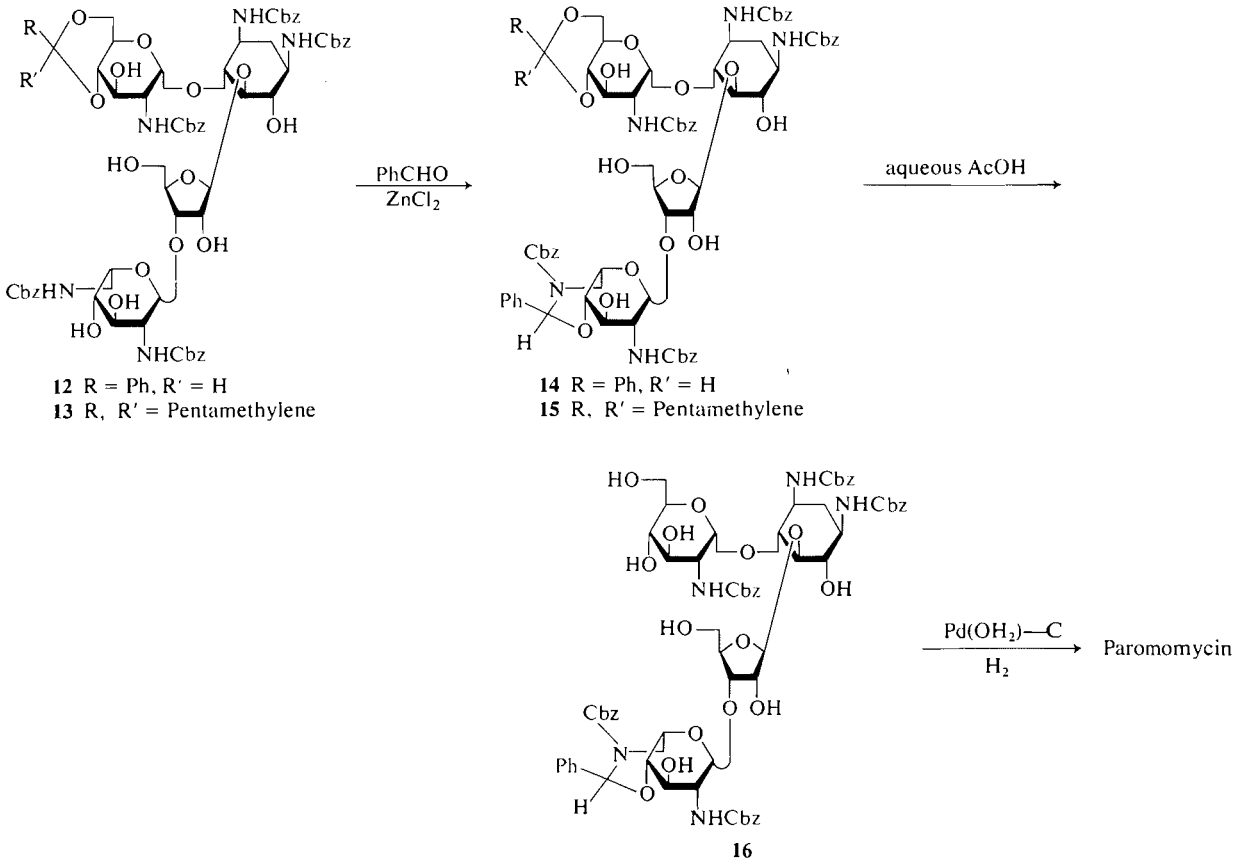
Unlike their respective precursors, compounds **14** and **15** were not susceptible to periodate oxidation owing to the lack of vicinal diol groups. In previous papers (6, 11), we described a method for the controlled degradation of amino glycoside antibiotics, based on the cleavage, with periodate, of vicinal diol groups situated on C-3,C-4 on amino sugar units, followed by treatment with mild base. This sequence provides not only useful, selectively blocked predisposed amino glycoside fragments for semisynthetic work but with the proper choice of substrates it can lead to pseudodisaccharide and pseudotrisaccharide fragments that can be used as biological probes. Treatment of **16** with 1 equiv. of periodic acid in dry tetrahydrofuran gave a dialdehyde derivative **17**, which was subjected to β-elimination in the presence of triethylamine. Based on its physical characteristics and mode of formation, the product, formed in high yield from the sequence shown in Scheme 5, was assigned structure **18**. Acid hydrolysis with 50% aqueous formic acid gave several products, among which *N*-benzyloxycarbonyl-2-deoxystreptamine and D-ribose were identified by chromatography. Hydrogenolysis in the presence of palladium-hydroxide-on-charcoal (12) gave the known (11) pseudotrisaccharide **19**.

The formation of oxazolidine derivatives in the aminoglycoside series has been demonstrated in the gentamicin group (13), and has been used as supporting evidence for the *cis* disposition of the vicinal methylamino and tertiary hydroxyl groups in the garosamine moiety (14). Thus, on gentle warming in the presence of various aromatic aldehydes in ethanol, gentamicin C_2 is converted into the corresponding oxazolidine Schiff base derivative. The benzylidene analog has been used as an intermediate in the preparation of *N*-benzyl (13) and deoxygenated derivatives (15).

An oxazolidine derivative has also been reported to arise as a by-product in the treatment of penta-*N*-ethoxycarbonylribostamycin with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in DMF at 70°C (16). It is of interest that in this case the oxazolidine ring encompasses the vicinally situated C-1 ethoxycarbonylamino and C-6 hydroxyl groups in the central 2-deoxystreptamine ring, and that these groups bear a *trans* relationship to each other. The instability of such a strained ring is evident from its hydrolytic behavior in the presence of an *O*-isopropylidene group.

Finally, an oxazolidine derivative of *N*-acetylepiinosamine-1 has been prepared in the course of ketal forming reactions, also under forcing conditions (2,2-dimethoxypropane, DMF, TsOH, at 80°C) (17). Because of the *cis* arrangement of the vicinal acetamido and hydroxyl groups, it was possible to selectively hydrolyze an *O*-isopropylidene group in the *O,O*:*N,O*-diisopropylidene derivative of *N*-acetylepiinosamine-1.

To the best of our knowledge, we are not aware of the formation of stable dihydrooxazine derivatives in the aminoglycoside series. Preliminary results have shown that the mild treatment of other aminoglycosides containing benzyloxycarbonylamino and alcohol functions in a 1,3 orientation with benzaldehyde and zinc chloride leads to the formation of the corresponding dihydrooxazine (*N,O*-benzylidene)



12 R = Ph, R′ = H
13 R, R′ = Pentamethylene

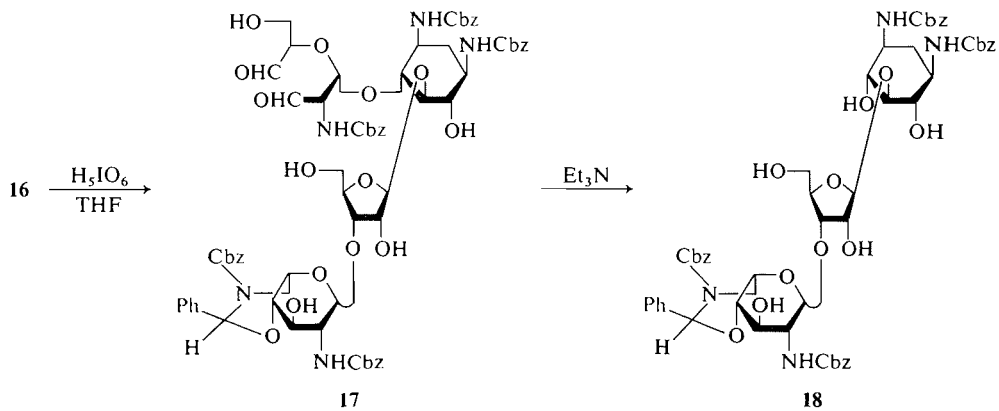
14 R = Ph, R′ = H
15 R, R′ = Pentamethylene

16

SCHEME 4

derivatives. In the paromomycin series, the reaction leads in high yield to a derivative in which the 2,6-diamino-2,6-dideoxy-L-idopyranosyl (paromose) moiety is effectively protected as the O^4,N^6-benzylidene derivative, thus paving the way for modifications at the other rings, particularly the 2-amino-2-deoxy-α-D-glucopyranosyl moiety, which is the site of enzymatic inactivation (1). By treatment with benzaldehyde and excess zinc chloride, it is thus possible to preferentially protect sets of amino alcohol groups in the multifunctional aminoglycosides, depending on their relative dispositions (1,2 or 1,3) and spatial orientations.

Finally, it may be of interest to recall the relative tendencies for the formation of oxazolidine and dihydrooxazine rings from the respective benzyloxy-



17

18

SCHEME 5

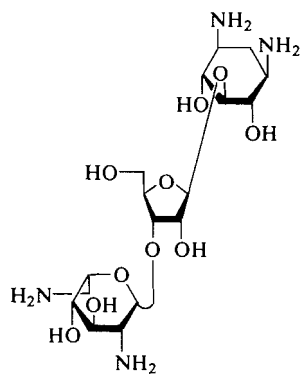
TABLE 1. ^{13}C magnetic resonance spectra of paromomycin (**1**), neamine, and methyl nebiosaminide (**9**) (ppm from TMS)[a]

Compound	Ring	Carbon atoms[b] 1	2	3	4	5	6
Paromomycin[c]	A	100.2	56.3	74.4	70.8	73.8	61.6
$pD \geq 11$	B	50.1	36.5	50.1	84.4	82.5	78.3
	C	109.1	74.4	77.0	84.9	62.3	—
	D	100.3	53.6	71.5	69.3	77.0	42.0
Neamine	A	101.9	56.4	74.7	72.5	74.3	42.9
$pD \geq 11$	B	51.5	36.8	50.5	88.5	77.1	78.6
Methyl	C	102.0	69.5	75.3	68.6	64.2	—
neobiosaminide[d]	D	99.5	53.9	71.7	69.5	76.9	42.0
$pD \geq 11$							

[a]Chemical shifts corrected to TMS scale taking δ dioxane as 67.4 ppm.
[b]For simplicity and space conservation, the carbon atoms are not primed; ring A atoms should be singly primed; ring C, doubly primed; and ring D, triply primed, as in the text.
[c]Assignments corroborated by appropriate β-shifts at acid p*D*, and by correlation with model compounds (E. Wenkert and E. W. Hagaman. Private communcation).
[d]The anomeric methoxyl group showed a signal at 56.6 ppm, corresponding to the carbon nucleus of the major α-anomer in the sample.

carbonyl amino and alcohol functions (e.g. **3** → **4**, **7** → **8**, and **10** → **11**).

The moderate yield (34%) and the recovery of unreacted starting material in the conversion of **3** → **4** can be attributed to the high energy of the conformationally biased pyranose ring, as the attachment of a five-membered oxazolidine ring is expected to introduce considerable ring strain. Molecular models show that the hexopyranose ring in **4** is forced into a half-chair conformation as a result of the presence of the oxazolidine ring. The



19

exceptionally facile formation of the dihydrooxazine ring in the disaccharide derivative **11** (89%) and in the paromomycin derivatives **14** and **15** (84%) compared to the model compound **8** (54%) is somewhat unexpected. Recent ^{13}Cmr spectroscopic studies (18) have shown that the conformations of methyl D-idopyranosides can depart significantly from the ground state *C*1(D) or 1*C*(D) forms. If the reasonable assumption is made that the 2-amino-2-deoxy-α-D-glucopyranosyl moiety in paromomycin adopts the *C*1(D) conformation, the conclusions derived from ^{13}Cmr data on paromomycin (Table 1) are that the 2,6-diamino-2,6-dideoxy-β-L-idopyranosyl portion adopts a time-averaged conformation that is substantially different than *C*1(L), particularly in the environment of C-2‴—C-4‴. Thus, we find that the chemical shifts of C-1′ and C-1‴ corresponding to the anomeric carbon atoms of the α-D-*gluco* and β-L-*ido* units, respectively, are coincident at $pD \geq 11$, while the signals due to C-2‴, C-3‴, and C-4‴ are at higher field (Δ 2.7, 2.9, and 1.5 Hz, respectively), compared to their C-2′—C-4′ counterparts, indicating quasi-axial orientations approaching a 1*C* form. Comparison of the ^{13}Cmr assignments of the carbon atoms of the diamino sugar portion in methyl neobiosaminide **9** and in neamine (19), respectively, reveal a similar upfield shift for signals associated with C-3—C-4 in the former case, indicating a different conformation (Table 1). It is of interest to recall the ^{1}Hmr studies on penta-*N*-acetylparomomycin by Rinehart *et al.* (20) who found a smaller H-1‴—H-2‴ coupling constant ($J_{1,2}$ = 1.8 Hz) in the 2,6-diamino-2,6-diamino-β-L-idopyranosyl moiety compared to that in the 2-amino-2-deoxy-α-D-glucopyranosyl moiety ($J_{1,2}$ = 3.5 Hz), reflecting a smaller dihedral angle for the vicinally disposed hydrogen atoms in the former sugar. A departure from a *C*1(L) conformation is also evident from periodate oxidation studies (refs. 11, 21, and 10*c*, pp. 29–31), as was originally proposed by Rinehart *et al.* (refs. 21 and 10*c*, pp. 29–31). Assuming that the conformation of the 2,6-diamino sugar moiety in paromomycin and methyl neobiosaminide does not change significantly in the derivatives **2** and **10**, it is not clear why the formation of the corresponding *N*,*O*-benzylidene derivatives is

more favored compared to a D-*gluco* structure (as in **9**). It is possible that in such a conformation the nucleophilicity of the C-4‴ hydroxyl group toward the benzaldehyde–$ZnCl_2$ complex is enhanced through H-bonding with the carbonyl group of the 2-benzyloxycarbonylamino group. Such an enhancement cannot be envisaged for **7** in its ground state.

Experimental

Melting points are uncorrected. Optical rotations were measured on a Perkin-Elmer automatic spectropolarimeter, model 141. ^1H magnetic resonance spectra were recorded on a JEOL instrument at 60 MHz unless otherwise stated, with tetramethylsilane as internal standard, in deuteriochloroform as solvent. Spectra were not well resolved except in certain regions but were consistent with the structures. ^{13}C magnetic resonance spectra were recorded on a Bruker WH-90 instrument at 22.6 MHz. Mass spectra were recorded on an AEI-902 mass spectrometer at low resolution. Column chromatography was done using silica gel G254, with application of moderate suction.

*Methyl 4,6-*O*-Benzylidene-2,3-*N,O*-benzylidene-2-benzyloxycarbonylamino-2-deoxy-α-*D*-allopyranoside (**4**)*

A mixture containing **3** (340 mg, 0.9 mmol) and anhydrous zinc chloride (408 mg, 3 mmol) in 3 ml of benzaldehyde was stirred at room temperature overnight. The solution was poured into aqueous 10% sodium bicarbonate (200 ml) and extracted with chloroform (2 × 50 ml). The organic phase was processed in the usual manner to give an oily residue, from which excess benzaldehyde was removed by distillation at 50°C under vacuum. The viscous residue solidified after trituration with petroleum ether to give a product that contained the title compound (tlc $CHCl_3$–EtOAc, 10:1), in addition to an appreciable amount of starting material. Separation by thick-layer chromatography gave **3** (115 mg) and the title compound (120 mg). Recrystallization of the latter from 2-propanol – petroleum ether gave 110 mg (34%) of an analytical sample; mp 180–181°C; $[\alpha]_D^{21}$ +117.5° (*c* 0.3, $CHCl_3$); ν_{max} (KBr): 1725 cm^{-1} (C=O). *Anal.* calcd. for $C_{29}H_{29}O_7N$: C 69.17, H 5.80, N 2.78; found: C 69.43, H 6.07, N 2.95.

*Methyl 2,3-*N,O*-Benzylidene-2-benzyloxycarbonylamino-2-deoxy-α-*D*-allopyranoside (**5**)*

The preceding compound (40 mg) was dissolved in 5 ml of 80% aqueous acetic acid and the solution was stirred 12 h at room temperature. Evaporation of the solvent gave a syrupy residue that was triturated with aqueous sodium bicarbonate then with water and finally with petroleum ether. The residual syrup was dissolved in 2-propanol and the solution was diluted with petroleum ether to give the title compound as a colorless amorphous solid that was homogeneous by tlc ($CHCl_3$–EtOAc–MeOH, 25:15:3); yield 28 mg (82%); mp 110–115°C; $[\alpha]_D^{25}$ +189.3° (*c* 0.14, $CHCl_3$); ν_{max} (film): 3450 cm^{-1} (C=O).

Heating a solution of the product (10 mg) in 1.5 ml of 50% formic acid at 50°C for 20 h followed by conventional work-up gave 5.5 mg of methyl 2-benzyloxycarbonylamino-2-deoxy-α-D-allopyranoside (8); mp 79–81°C; $[\alpha]_D^{25}$ +70° (*c* 0.1, MeOH), identical with an authentic sample.

*Methyl 2,6-Dibenzyloxycarbonylamino-2,6-dideoxy-α-*D*-glucopyranoside (**7**)*

A solution containing methyl 6-azido-2-benzyloxycarbonylamino-2,6-dideoxy-α-D-glucopyranoside (**22**) (235 mg, 0.67 mmol) in 40 ml of methanol and 5 ml of water was hydrogenated in the presence of 200 mg of 20% palladium-hydroxide-on-charcoal (12). After 2 h, the catalyst was filtered, the filtrate was evaporated to dryness, and the residue was dissolved in 15 ml of 3:1 aqueous methanol. The solution was treated with 1 ml of benzyloxycarbonyl chloride, 0.5 g of sodium bicarbonate, and the suspension was stirred at 0°C for 10 h. Dilution with a mixture of chloroform and petroleum ether resulted in the precipation of the product, which was filtered and dried; yield 171 mg of an amorphous colorless solid; mp 158–160°C; $[\alpha]_D^{25}$ +37.5° (*c* 0.12, $CHCl_3$).

*Methyl 4,6-*N,O*-Benzylidene-2,6-dibenzyloxycarbonylamino-2,6-dideoxy-α-*D*-glucopyranoside (**8**)*

Anhydrous zinc chloride (150 mg, 1.1 mmol) was added to a solution containing **7** (130 mg, 0.28 mmol) in 2 ml of freshly distilled benzaldehyde and the mixture was stirred at room temperature for 24 h. The solution was poured into 10% aqueous sodium bicarbonate with vigorous stirring and the resulting precipitate was collected by filtration. The filtrate was diluted with petroleum ether to give a sticky solid that was washed with the same solvent by decantation. The remaining solid consisted of a major product, contaminated with a small amount of unreacted starting material (tlc $CHCl_3$–EtOAc, 10:1). Separation by preparative thick-layer chromatography gave the title compound (61 mg, 52% based on recovered starting material). Recrystallization from 2-propanol gave an analytical product; mp 111–112°C; $[\alpha]_D^{27}$ +33° (*c* 0.10, $CHCl_3$). *Anal.* calcd. for $C_{30}H_{32}N_2O_8$: C 65.68, H 5.81, N 5.10; found: C 65.55, H 5.97, N 4.92.

Treatment of the above obtained derivative (15 mg) with 50% aqueous formic acid (50%, 20 h), followed by neutralization with Rexyn 203 (OH^-) and processing as usual, gave methyl 2,6-dibenzyloxycarbonylamino-2,6-dideoxy-α-D-glucopyranoside (7) (10 mg); mp 157–160°C; $[\alpha]_D^{27}$ +36.8° (*c* 0.1, $CHCl_3$).

*Methyl Di-*N*-benzyloxycarbonylparomobiosaminide (**10**)*

Methyl paramobiosaminide dihydrochloride (**9**) (1.2 g), obtained by methanolysis of paromomycin **1** (10), was dissolved in a mixture of water (25 ml) and methanol 10 ml, containing 2.3 g of sodium carbonate. The solution was cooled to 0°C and treated dropwise and with vigorous stirring with 1.41 g of benzyloxycarbonyl chloride. After 30 min, the mixture was left at 0°C for 10 h. Addition of cold water (200 ml) gave a sticky solid that was washed with cold water by decantation. Trituration with petroleum ether gave an amorphous solid that was dissolved in 50 ml of chloroform and the solution was filtered to eliminate a small amount of insoluble material. Evaporation of the solvent and purification by chromatography ($CHCl_3$–EtOAc–MeOH, 20:5:3) gave the title compound as a colorless solid. It was dissolved in chloroform and reprecipitated with petroleum ether; yield 490 mg; mp 230–235°C (foams with dec.); $[\alpha]_D^{27}$ +29.2° (*c* 0.51, $CHCl_3$); this chromatographically homogeneous product was used as such in the next step.

*Methyl 3-*O*-(4,6-*N,O*-Benzylidene-2,6-dibenzyloxycarbonylamino-2,6-dideoxy-β-*L*-idopyranosyl)α,β-*D*-ribopyranoside (**11**)*

A solution containing 200 mg (0.33 mmol) of the preceding compound in 5 ml of freshly distilled benzaldehyde was treated with 328 mg (2.41 mmol) of zinc chloride. After vigorous stirring for 4 h, the solution was added dropwise into 10% aqueous sodium bicarbonate and the resulting colorless precipitate was washed with water, dried, and dissolved in chloroform. The solution was dried with sodium sulfate, filtered, and the filtrate was evaporated to give a syrup that solidified from a mixture of chloroform and petroleum ether;

yield 205 mg (89%). This product was chromatographically homogeneous (benzene–EtOAc, 1:1). An analytical sample was obtained by preparative thick-layer chromatography; the colorless amorphous product softened at 95–98°C and melted at 105–110°C; $[\alpha]_D^{27}$ +29.7° (*c* 0.16, $CHCl_3$); ^{1}Hmr: 3.36 (s, OCH_3), 6.78 ppm (b, PhCH<N,O>), etc. The product was unaffected by periodic acid in tetrahydrofuran (0°C, 24 h). *Anal.* calcd. for $C_{35}H_{40}O_{12}N_2$: C 61.76, H 5.92, N 4.11; found: C 61.88, H 6.10, N 3.88.

Acetylation of the dihydrooxazine derivative **11** (94 mg) in pyridine and acetic anhydride gave 97 mg (87%) of an amorphous triacetate derivative; mp ~ 112–115°C; $[\alpha]_D^{27}$ +30.9° (*c* 0.41, $CHCl_3$). Nuclear magnetic resonance data indicated the incorporation of three *O*-acetyl groups, as judged from the relative integration of appropriate areas in the spectrum.

Acid Stability of the Dihydrooxazine Derivative ***11***

Acetic Acid

The dihydrooxazine derivative **11** (10 mg) was dissolved in 80% aqueous acetic acid and the solution was left overnight at room temperature. Thin-layer chromatographic examination indicated no change. Heating the solution at 50°C for 24 h also showed unchanged starting material.

Formic Acid

An amount of the dihydrooxazine derivative **11** (35 mg) in 5 ml of 50% aqueous formic acid was heated at 50°C for 19 h, the solution was then treated with Rexyn 203 (OH^-), and processed in the usual manner to give methyl di-*N*-benzyloxycarbonylparomobiosaminide (**10**) 21 mg (70%); $[\alpha]_D^{27}$ +30° (*c* 0.29, $CHCl_3$).

*4′,6′-O-Benzylidene-4‴,6‴-N,O-benzylidenepenta-N-benzyloxycarbonylparomomycin (**14**)*

To a suspension containing 420 mg (0.32 mmol) of penta-*N*-benzyloxycarbonylparomomycin (**2**) (6) in 10 ml of freshly distilled benzaldehyde, was added anhydrous zinc chloride (620 mg, 4.52 mmol) and the mixture was stirred overnight. The mixture was then poured into 100 ml of cold 2% aqueous sodium bicarbonate and the resulting precipitate containing zinc salts was filtered and thoroughly washed with petroleum ether (200 ml), then with methanol. The filtrate, now containing an amorphous precipitate,[3] was diluted with a further 100 ml of petroleum ether. Filtration and washing with the same solvent gave a solid that was dissolved in 100 ml of chloroform containing 3 drops of triethylamine; the solution was concentrated to about 5 ml, then it was diluted with petroleum ether. The resulting colorless precipitate was transformed into a fine powder by vigorous stirring of the mixture. The crude product 370–445 mg (87–98%) consisted of a major component when analysed by tlc ($CHCl_3$–EtOAc–MeOH, 20:5:3). Purification by chromatography on a silica gel (80 g; developed with chloroform, 100 ml, then the above stated tlc solvent) gave 385 mg (84%) of a chromatographically homogeneous solid. The product could be reprecipitated several times from chloroform – petroleum ether, with minimal loss in weight; mp 139–142°C; $[\alpha]_D^{27}$ +31.3° (*c* 4.78, $CHCl_3$). *Anal.* calcd. for $C_{77}H_{83}N_5O_{23}\cdot 1.5H_2O$: C 62.51, H 5.68, N 4.84; found: C 62.76, H 5.68, N 4.75.

The product could also be obtained from 4′,6′-*O*-benzylidenepenta-*N*-benzyloxycarbonylparomomycin (**12**) (6), using essentially the same conditions but only half the volume of benzaldehyde; yield 76%; mp 141–143°C; $[\alpha]_D^{27}$ +30.9° (*c* 3.74, $CHCl_3$).

The product **14** was unaffected when treated with periodic acid in tetrahydrofuran (0°C, 48 h) and it was recovered unchanged (98%).

Treatment with 50% aqueous formic acid (55%, 48 h), followed by neutralization with Rexyn 203 (OH^-) and tlc examination revealed the presence of **2** as the major product. Also present were traces of more polar components resulting from further degradation of the molecule. The parent penta-*N*-benzyloxycarbonylparomomycin (**2**) was subsequently recovered by preparative thick-layer chromatography; $[\alpha]_D^{27}$ +21° (*c* 0.1, $CHCl_3$) (lit. (11) $[\alpha]_D^{27}$ +22.6° ($CHCl_3$)).

*Penta-N-benzyloxycarbonyl-4′,6′-O-cyclohexylideneparomomycin (**13**)*

A solution containing 616 mg (0.48 mmol) of penta-*N*-benzyloxycarbonylparomomycin (**2**), 21 mg of *p*-toluenesulfonic acid, and 2 ml of 1,1-dimethoxycyclohexane in 20 ml of anhydrous DMF was heated at 45°C under reduced pressure (~20 Torr) for 1 h. The cooled solution was neutralized with Rexyn 253 (OH^-) and the solvent was removed by co-distillation with 1-butanol. The residue was triturated with petroleum ether to give 650 mg (~quantitative) of an amorphous solid, that consisted of a major component (tlc, $CHCl_3$–EtOAc–MeOH, 20:5:3). Purification by chromatography on silica gel, as described in the previous preparation, gave the title compound as a colorless amorphous solid. It was dissolved in chloroform and reprecipitated with hexane; yield 450 mg (68%); mp 124°C (softens), ~145°C (foams); $[\alpha]_D^{27}$ +38.1° (*c* 0.32, $CHCl_3$). *Anal.* calcd. for $C_{69}H_{83}N_5O_{24}$: C 60.65, H 6.12, N 5.12; found: C 60.18, H 6.24, N 5.48.

*4‴,6‴-N,O-Benzylidenepenta-N-benzyloxycarbonyl-4′,6′-O-cyclohexylideneparomomycin (**15**)*

A solution containing 120 mg (0.088 mmol) of **13** in 50 ml of freshly distilled benzaldehyde was treated with anhydrous zinc chloride (435 mg, 3.20 mmol) and the resulting suspension was stirred and processed as described in the preparation of **14**. Chromatography of the crude product (110 mg) gave 95 mg (80%) of the title dihydrooxazine derivative **15**; mp 142–145°C; $[\alpha]_D^{27}$ +30.6° (*c* 3.94, $CHCl_3$). *Anal.* calcd. for $C_{76}H_{87}N_5O_{24}\cdot 0.5H_2O$: C 62.37, H 5.99, N 4.78; found: C 62.44, H 5.54, N 4.58.

The product was not affected by treatment with periodic acid in tetrahydrofuran (0°C, 48 h), whereas the parent **13** was transformed to the corresponding dialdehyde derivative under the same conditions.

Treatment of the product (10 mg) with 50% aqueous formic acid (50°C, 48 h) resulted in the formation of the parent **13** in addition to several degradation products (tlc).

*4‴,6‴-N,O-Benzylidenepenta-N-benzyloxycarbonylparomomycin (**16**)*

From ***14***

A solution containing 198 mg of the dihydrooxazine derivative **14** in 10 ml of 80% acetic acid was stirred at room temperature for 30 h. Evaporation of the solvent gave a colorless syrup that solidified when triturated with cold 1% aqueous sodium bicarbonate. The solid was filtered, washed with water, dried, and redissolved in 20 ml of chloroform. Drying over sodium sulfate, filtration, and evaporation of the solvent gave an amorphous solid that showed a major spot on tlc ($CHCl_3$–EtOAc–MeOH, 20:5:3), yield 165 mg (94%). Purification by thick-layer chromatography gave the title compound as an amorphous colorless solid (from chloroform – petroleum ether); yield 138 mg (79%); mp 143–146°C; $[\alpha]_D^{27}$ +24.7° (*c* 1.04, $CHCl_3$). *Anal.* calcd. for $C_{70}H_{79}N_5O_{24}\cdot 2H_2O$: C 59.60, H 5.64, N 4.96; found: C 59.81, H 5.54, N 5.00.

[3]If a syrup is formed, it is stirred under petroleum ether until it solidifies. Decantation and trituration with cold water will also cause solidification.

From 15

The dihydrooxazine derivative **15** (77 mg) was dissolved in 4 ml of 80% acetic acid, and the solution was stirred at room temperature for 30 h. The product was isolated as described in the preceding section; yield 60 mg (81%); mp 142–146°C; $[\alpha]_D^{27}$ +25° (*c* 0.71, MeOH).

Acid hydrolysis of the product (50 mg) in 50% formic acid (50°C, 24 h), followed by neutralization with Rexyn 203 (OH^-) and chromatography gave penta-*N*-benzyloxycarbonylparomomycin (**2**) (15 mg, 32%); $[\alpha]_D^{27}$ +21° (*c* 0.15, $CHCl_3$), in addition to several degradation products (tlc).

5-O-[3-O-(4,6-N,O-Benzylidene-2,6-dibenzyloxycarbonylamino-2,6-dideoxy-α-L-idopyranosyl)-β-D-ribofuranosyl]-1,3-di-N-benzyloxycarbonyl-2-deoxystreptamine (18)

Periodic acid (5 mg, 2.9 mmol) was added to a solution of the dihydrooxazine derivative **16** (63 mg) in 5 ml of dry tetrahydrofuran. After 24 h at 0°C and 6 h at room temperature, tlc examination indicated the absence of starting material (benzene–EtOAc, 1:1) and the conversion into the dialdehyde derivative **17**; this could be detected as a major component and gave a positive test with the aniline hydrogen phthalate test for aldehydes. The solution was then neutralized with Rexyn 203 (OH^-) and was processed in the usual manner to give a syrup. The latter was dissolved in 2 ml of methanol, 1 ml of triethylamine was added, and the solution was heated at 60°C overnight. Evaporation of the solvent gave a syrup that was purified by chromatography on silica gel ($CHCl_3$, 50 ml, then $CHCl_3$–EtOAc–MeOH, 20:5:3), to give the title compound as an amorphous solid the latter was dissolved in chloroform and reprecipitated with petroleum ether; yield 42 mg (80%); mp 114–116°C; $[\alpha]_D^{27}$ +20.1° (*c* 0.42, $CHCl_3$). *Anal.* calcd. for $C_{56}H_{62}N_4O_{18}$: C 62.32, H 5.79, N 5.19; found: C 62.07, H 5.91, N 5.21.

A portion of the above product (11 mg) was subjected to acid hydrolysis in 50% formic acid (50°C, 24 h); tlc examination revealed the presence of 1,3-di-*N*-benzyloxycarbonyl-2-deoxystreptamine, ribose, and two other unidentified components.

Hydrogenolysis in the presence of 20% palladium-hydroxide-on-charcoal (13) in 1:1 aqueous methanol gave the known (12) pseudotrisaccharide **19**, in addition to a small amount (5–10%, estimated from tlc) of paromamine.

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