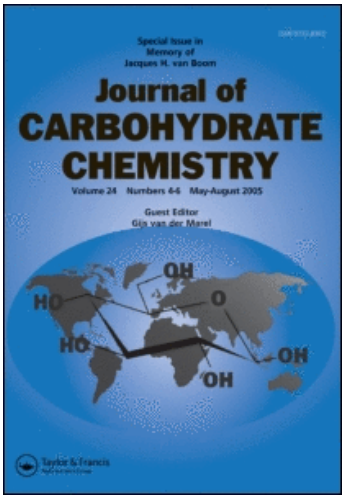

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SYNTHESIS AND CONFORMATIONAL PROPERTIES OF SOME 2′-UNSUBSTITUTED AMINOGLYCOSIDE ANTIBIOTICS

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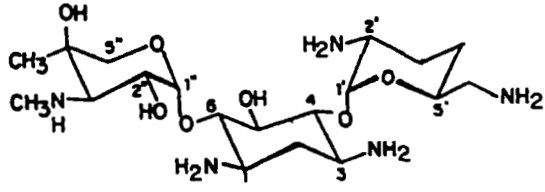
ABSTRACT

2′- Deoxygentamicin B, 2′,3′-dideoxygentamicin B, 2′-desaminogentamicin C_{1a} and 2′-desaminosisomicin were synthesised by glycosylation of the ψ-disaccharide garamine and found to be potent antibacterial agents. The solution conformations of members of this series and of their diastereoisomers have been studied by ^{13}C NMR. Using D- and L-glycosides, shielding effects on deoxystreptamine carbons are shown to provide a reliable method of assigning absolute stereochemistry to related glycosides.

INTRODUCTION

Resistance to aminoglycosides is most commonly the result of enzymic modification of amino or hydroxyl groups of the antibiotic to produce biologically inactive species. One approach to the design of more effective antibiotics lies in

the chemical modification of those functional groups. The 2′-N-alkyl[1] and 6′-N-alkyl[2] derivatives have good activity against organisms that modify these antibiotics at the 2′ or 6′ positions. This is in accord with results obtained in the kanamycin series.[3] Some antibiotics occur naturally without functional groups in positions that are common sites of inactivation and are consequently not susceptible to that mechanism of resistance. For example, gentamicin C_{1a} (1) and sisomicin, (2) both of which lack a 3′-OH group, are active against organisms that produce 3′-phosphotransferases. Removal of another site of inactivation, the 2′′-OH group of kanamycin and gentamicin led in contrast, to analogs of greatly reduced potency.[4,5] Herein are reported the first synthesis of 2′-unsubstituted members of this family of antibiotics, a series not as yet found in nature. These compounds are found to be at least as potent as the parent antibiotics and to possess improved activity against



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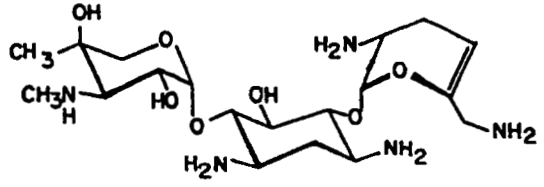

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resistant strains. This is of particular interest, as before the initial report of this work.[6] it was assumed that optimal potency required the presence of a 2′-amino group.

The most direct route, in concept, to 2′-unsubstituted glycosides, is from acid-catalysed addition of alcohols to glycals such as triacetylglucal (3). This approach has limited value in general, because of the propensity of such compounds to undergo olefin migration with loss of the group at position 3 during glycoside formation.[7] Reasoning that this unwanted reaction course should be less favored with a less avid leaving group than acetate in the allylic position of the starting glucal, a benzyl blocking group was employed to protect the hydroxyl group at that position. With the initial target compound being 2′-deoxygentamicin B (4) (FIG. 1), the glycal chosen was 6-acetamido-3,4-di-O-benzyl-6-

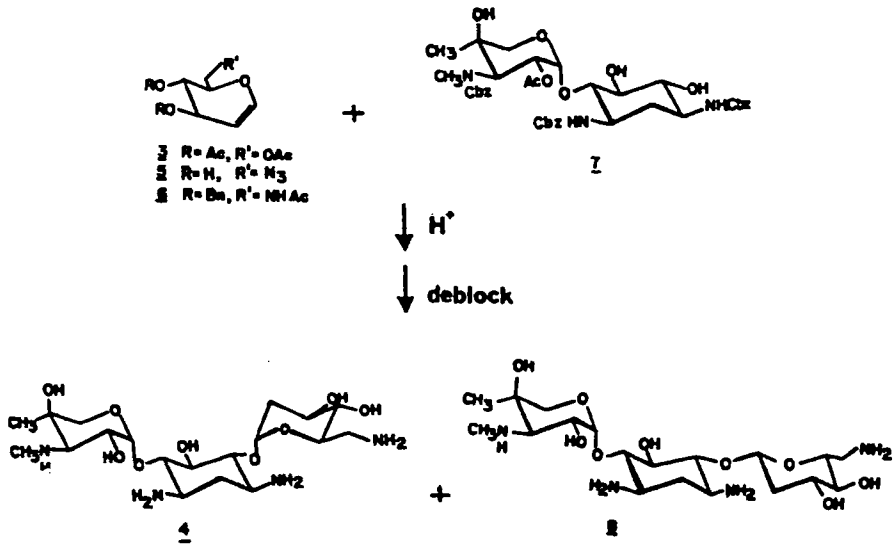


FIG. 1. Synthesis of 2'-deoxygentamicin B and its β-D isomer

deoxy-D-glucal (6), prepared from 6-azido-6-deoxy-D-glucal[10] (5) by standard procedures.

RESULTS AND DISCUSSION

The aglycone employed in the early condensation reactions was the protected garamine derivative (7) in which the amine groups were protected by carbobenzyloxy blocking groups and the reactive hydroxyl group at position 2′ was blocked as its acetate. Based on precedent,[11] reaction was expected to take place largely, if not exclusively, at position 4. Condensation of 6 with 7 was effected in dry benzene at 45° in the presence of a catalytic quantity of p-toluenesulphonic acid. Two products were obtained after removal of the blocking groups, the major being the α-D-glycoside (4) and the minor, the β-D-glycoside (8). The expected (M+1) ion at m/e 467 was prominent in the mass spectrum of both compounds. Fragment ions corresponding to the appropriate disaccharide moieties[12] were also present in both spectra.

That the major product was the α-D-glycoside was evident from the ^{1}H NMR spectrum in which the anomeric hydrogen of the newly introduced sugar appeared at δ5.03 (J = 4 Hz). The presence of disaccharide ions in the mass spectrum established that glycosylation had taken place on the deoxystreptamine moiety and the possibility that glycoside formation had occurred at position 5 and not at position 4 of the blocked garamine precursor was excluded by circular dichroism (CD) measurements of the cuprammonium complex (TACu) in solution.[13] The value obtained for $[\theta]_{288}$ of -6,360° is that expected for a 4,6-linked product. A 4,5-linked product would have possessed an extra vicinal

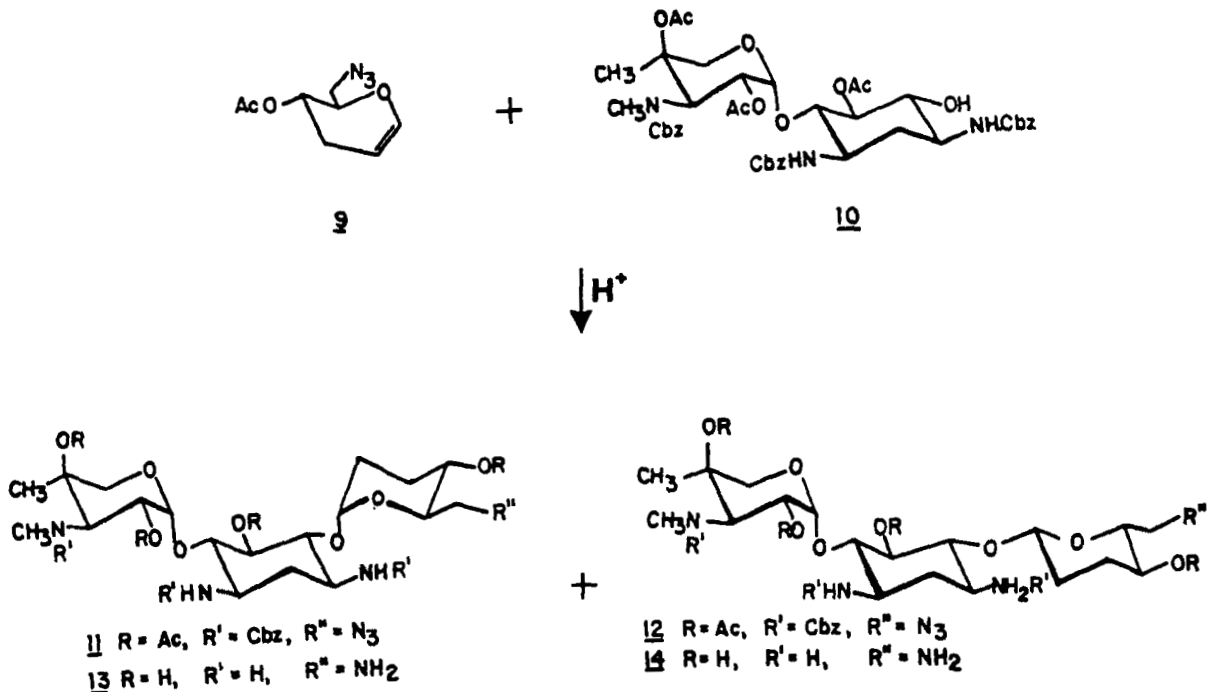
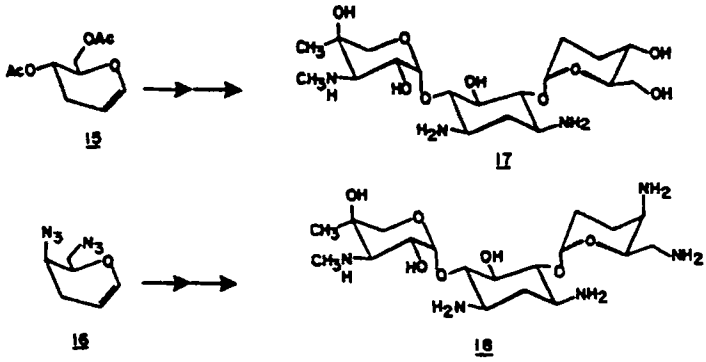


FIG. 2. Synthesis of 2',3'-deoxygentamicin B and its β-D isomer

aminoalcohol group and would have given a value of approximately $-10,000^{o}$. The structure of 4 was confirmed by its ^{13}C NMR spectrum.

The stereochemistry of the glycosidic linkage of the minor component (8) was revealed by its ^{1}H NMR spectrum in which the anomeric hydrogen appeared at $\delta 4.85$ (broad singlet). The value obtained for $[\theta]_{280}$ (TACu) of $-5,700^{o}$, established C-4 as the site of glycosylation in this case also. Compound (8) was therefore the β-D-isomer of 2′-deoxygentamicin B. It appeared that the use of the benzyl blocking group to protect the 3-hydroxyl group did allow the reaction to proceed along the desired pathway. Most importantly, the major product, 2′-deoxygentamicin B, proved to be a superior antibiotic to the parent, gentamicin B, and this finding stimulated the synthesis of a number of related compounds. One of these, 2′,3′-dideoxygentamicin B (13), was prepared from 6-azido-4-O-acetyl-3,6-dideoxy-D-glucal (9) and the fully blocked garamine precursor, 10 (FIG. 2). Reaction of 9 with 10 under the conditions previously used, gave after chromatography, the two ψ-trisaccharides 11 and 12 in 60% and 15% isolated yields respectively. Both adducts were separately submitted to catalytic hydrogenation in order to reduce the azide group and to remove the carbobenzyloxy groups. In each case the product mixture, after base-catalysed hydrolysis, was chromatographed. The major adduct gave 2′,3′-dideoxygentamicin B (13) and the minor product gave the β-isomer (14). The structures were elucidated as described above for 2′-deoxygentamicin B. In the same way, condensation of 10 with 4,6-di-O-acetyl-3-deoxy-D-glucal (15) and the novel sugar, 4,6-diazido-1,2,3,4,6-pentadeoxy-D-threo-hex-1-enopyranose (16) led to 17 and 18 respectively.



15 17

16 18

We have previously demonstrated[4] that 4,6-di-O-acetyl-3-acetamido-3-deoxy-D-glucal added in high yield to a complex alcohol, namely protected gentamine C_1, to give the 3-acetamido-2,3-dideoxy glycoside and that only the axial α-D-glycoside was obtained. Participation of the 3-acetamido group was proposed to account for the high degree of stereoselectivity. The possibility that an acetamido group at the 6-position might also direct α-glycoside formation was examined using model reactions involving glucal 9 and its 6-acetamido analog. With both compounds however, acid-catalysed condensation with isopropanol gave a 3:1 mixture of anomers in which the axial α-glycoside predominated, precluding such participation.

Aminoglycoside antibiotics possessing a 4′,5′-olefinic linkage e.g. sisomicin[14] and verdamicin[15] are more potent than their saturated counterparts and it was therefore of interest to prepare 2′-desaminosisomicin (19). The method chosen was a modification of a procedure employed in a monosaccharide[16] example and involved photolysis of the azide 11 to give the aldehyde 20 via the intermediate imine (FIG. 3). Attempted chromatography of 20 led to partial elimination to the unsaturated aldehyde 21 and it was found

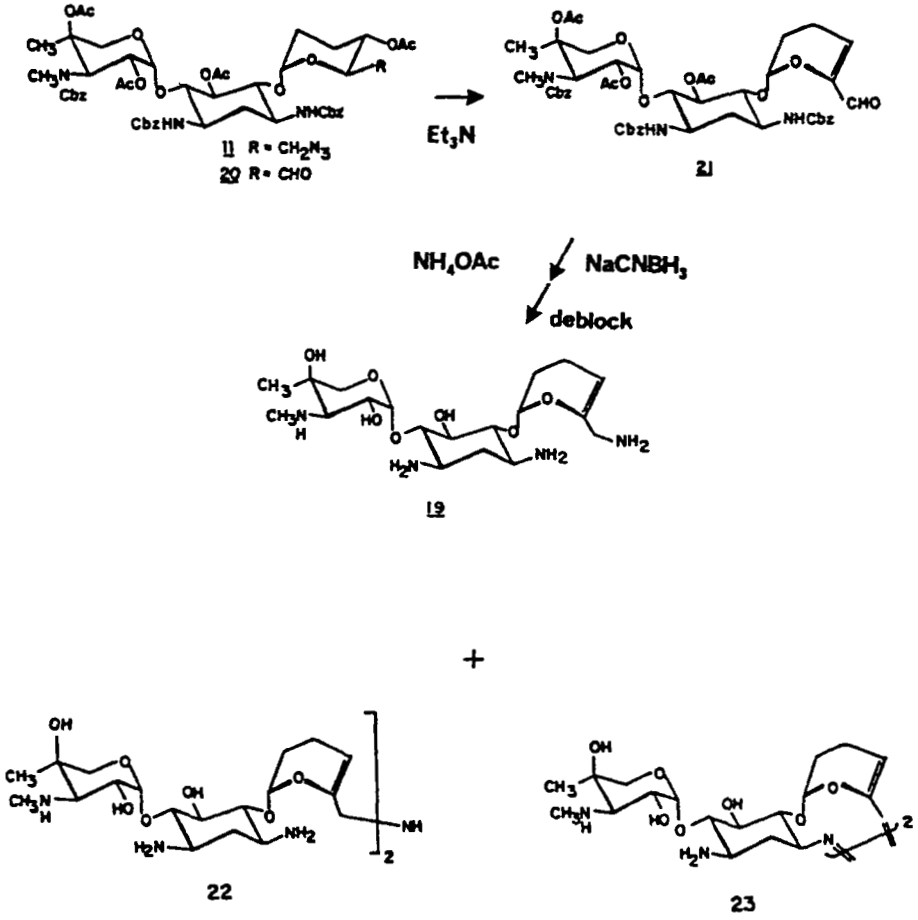


FIG. 3. Synthesis of 2'-desaminosisomicin and related compounds

more convenient to treat the crude photolysis product mixture with ethanolic triethylamine. A clean elimination was effected and chromatography gave 21 in good yield. Reductive amination of 21 using sodium cyanoborohydride in the presence of a large excess of ammonium acetate, gave a mixture of products that were not readily separable. Of several procedures applied to deblock this mixture, the only con-

ditions to prove effective were finely divided potassium hydroxide in aqueous dimethyl sulphoxide at room temperature for two days. Partition chromatography on silica gel gave three products, the major component of which was the desired 2′-desaminosisomicin.(19) The mass spectrum of this compound contained an ion at m/e 347 resulting from a retro Diels-Alder fragmentation. This ion is also present in the mass spectrum, of sisomicin and serves to locate the position of the olefinic bond.[12] The olefinic hydrogen resonated at δ4.92 in the ^{1}H NMR spectrum and the newly introduced anomeric hydrogen occurred at δ 5.46. The corresponding absorptions are found at δ4.83 and δ5.30 in the ^{1}H NMR spectrum of sisomicin.[14]

Also isolated by partition chromatography was the dimeric product (22) which resulted from the reductive amination of the unsaturated aldehyde (21) with the amine produced by the initial reductive amination reaction. That this was a significant side reaction was surprising in view of the large excess of ammonium acetate present. Compound 22 produced a weak molecular ion in the mass spectrometer (m/e 847.4902) and its ^{1}H NMR spectrum was essentially indistinguishable from that of 2′-desaminosisomicin (19). The ^{13}C NMR spectrum of 22 had nineteen absorptions as befitted its symmetry properties. Carbon 6′ in the spectrum of 22 occurred at 50.1 ppm, 6.7 ppm downfield from the corresponding carbon in 19. Carbon 5′ occurred at 148.1 ppm, 1.9 ppm upfield from its corresponding carbon. Both these differences were of the magnitude and direction expected for β and λ chemical shift increments respectively.

A third and very minor component also isolated from the chromatography was 23, a dimeric Schiff's base formed by

combination of two aldehyde molecules. The structure was deduced from its obvious similarity to the 2′,2′-diamino analog, antibiotic 66-40C, isolated from natural sources, whose structure has been established[17] and which has been synthesised.[18] The olefinic hydrogen and the imine hydrogen occur at δ5.6 and δ7.45 ppm respectively in the ^{1}H NMR spectrum of 23 (δ5.48 and δ7.56 in 66-40C). Compound 23 presumably arose from the deblocking of residual starting material, although it was possibly derived from oxidation in the presence of air during the base-catalysed hydrolysis of some 6′-alcohol formed in the reductive amination step.

The remaining series of gentamicin analogs, in which there was an interest is exemplified by 2′-desaminogentamicin C_{1a} (24). In order to prepare this compound, the racaemic azidomethyl-dihydropyran (25) was prepared from acrolein dimer by reduction to the alcohol, mesylation and displacement with azide ion. Condensation of 10 with 25 and subsequent chromatography give three of the four possible diastereoisomers. These were deblocked and shown to be 2′-desaminogentamicin C_{1a} (α-D) (24) and its α-L (26) and β-L (27) isomers (FIG. 4). There was a particular interested in the α-L and β-L isomers as these types of analogs had not been made in this class of antibiotic before and their biological properties were therefore unknown. It was readily apparent from the ^{1}H NMR spectrum of 24 and 26 that these were both axially oriented glycosides, the newly introduced anomeric hydrogens appearing at δ5.30 (ω1/2= 5.5 Hz) and δ5.14(ω1/2 = 5.5 Hz), respectively. The relative magnitude of the values obtained for the optical rotation of 24 and 26 (+166° and +83.5° respectively) strongly suggested that the structures were as assigned. This was confirmed by a study

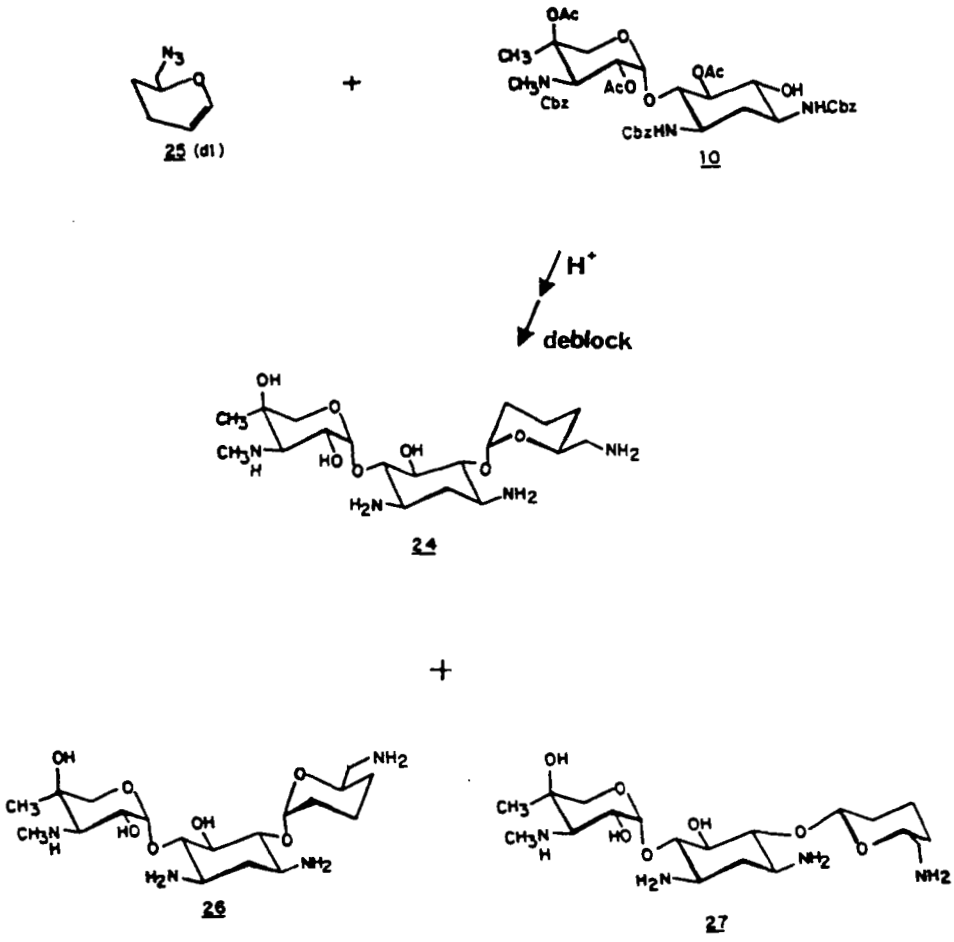


FIG. 4. Synthesis of 2'-desaminogentamicin C_{1a} and its α-L and β-L isomers.

of the ^{13}C NMR spectra of these two compounds (Table 1) and of the spectra of 4-O- α-D- and 4-O-α-L-glucopyranosyl-garamines (28) and (29). The spectrum of 24 closely resembled that of the natural antibiotics, whereas the spectrum of 26 was significantly different. These aspects will be discussed in more detail in connection with their conformational properties (vide infra).

Table 1

^{13}C NMR CHEMICAL SHIFTS[a]

Carbon	β-L Isomer of 24 (27)		D-Glucosyl Garamine (28)		L-Glucosyl Garamine (29)		R-THP Garamine (31)		S-THP Garamine (32)		(22)	(25)
	Base	H^+	Base	H^+	Base	H^+	Base	H^+	Base	H^+	Base	Base
C-1	51.5	51.2	51.5	50.6	51.8	50.9	51.6	50.9	51.6	50.9	51.7	51.6
C-2	36.4	30.9	36.4	28.8	36.3	28.2	36.4	29.3	36.3	29.4	36.2	36.4
C-3	49.8	49.7	49.8	49.2	51.5	50.9	50.0	49.6	50.6	49.7	50.1	50.0
C-4	87.2	79.4	88.8	81.6	89.2	84.1	86.9	81.1	88.8	81.9	85.1	86.1
C-5	75.1	74.7	74.9	73.7	73.5	73.5	75.2	74.2	73.6	73.3	75.5	75.5
C-6	87.7	84.5	87.3	83.8	87.7	84.1	87.8	84.4	87.6	84.5	87.9	87.9
C-1′	105.0	104.1	100.5	99.5	101.4	102.3	103.7	104.3	103.4	102.4	101.1	99.6
C-2′	28.1	27.6	72.6	72.0	72.6	72.6	25.3[b]	25.1[b]	25.1[b]	25.0[b]	26.5	25.6[b]
C-3′	22.1	21.8	73.9	73.9	73.9	73.7	21.4[b]	21.9[b]	21.2[b]	21.1[b]	16.7	24.2[b]
C-4′	31.5	31.3	70.4	70.4	70.2	70.0	31.5	31.4	31.5	31.4	99.8	45.8
C-5′	79.8	73.8	73.5	73.4	73.2	73.5	66.2	67.3	66.4	66.9	148.1	73.2
C-6′	45.9	44.0	61.5	61.5	61.0	61.2	-	-	-	-	50.1	42.6
C-1′′	101.5	101.4	101.3	101.8	101.4	102.0	101.5	101.7	101.4	101.7	101.6	101.6
C-2′′	70.2	67.4	70.2	67.2	70.2	67.3	70.1	67.3	70.2	67.0	70.2	70.1
C-3′′	64.2	64.8	64.2	64.2	64.2	64.2	64.2	64.4	64.2	64.4	64.2	64.2
C-4′′	73.2	70.9	73.2	70.8	73.2	70.8	73.2	70.8	73.2	70.8	73.2	73.6
C-5′′	68.5	68.1	68.5	68.5	68.5	68.5	68.5	68.4	68.6	68.4	68.5	68.5
$N-CH_3$	37.7	35.7	37.7	35.5	37.8	35.4	37.7	35.5	37.7	35.5	37.7	37.7
$C-CH_3$	22.4	21.5	22.5	21.8	22.5	21.8	22.5	21.9	22.4	21.7	22.4	22.4

Carbon	Garamine (30)[d]		2′, 3′-Dideoxy Gentamicin B (13)		β Isomer of 13 (14)		2′-Desamino Sisomicin (19)		2′-Desamino Gentamicin C_{1a} (24)		α-L Isomer of 24 (26)	
	Base	H^+	Base	H^{+c}	Base	H^+	Base	H^+	Base	H^+	Base	H^+
C-1	51.7	50.9	51.6	50.9	51.5	50.9	51.7	50.9	51.6	51.0	51.6	50.9
C-2	36.6	28.5	36.4	28.4	36.5	29.6	36.2	28.2	36.5	29.7	36.4	29.5
C-3	51.4	50.7	50.1	49.5	50.4	50.0	50.0	49.3	50.1	49.7	51.0	50.8
C-4	78.8	73.3	86.1	79.3	88.7	81.0	84.7	79.2	86.1	79.5	88.1	81.2
C-5	75.1	74.6	75.6	75.1	73.3	73.1	75.5	74.6	75.6	75.2	73.9	73.9
C-6	87.9	84.0	88.0	84.7	87.5	84.6	87.8	83.9	88.0	84.9	87.9	85.4
C-1′			98.8	98.2	103.5	102.6	99.6	101.9	100.2	99.3	100.4	100.1
C-2′			26.7	25.9	30.6	30.4	26.4	26.7	28.3	27.5	28.1	27.5
C-3′			29.3	29.3	30.8	30.4	16.5	18.7	17.6	16.7	17.6	17.3
C-4′			67.7	67.2	67.4	67.2	99.5	105.1	30.3	29.7	30.1	30.0
C-5′			75.7	71.9	81.2	76.8	150.0	144.0	72.1	67.8	72.1	68.6
C-6′			42.6	41.1	42.6	41.4	43.4	42.1	46.1	43.8	45.9	44.0
C-1′′	101.4		101.6	101.9	101.3	101.7	101.6	102.0	101.6	101.9	101.4	101.9
C-2′′	70.0		70.2	67.0	70.2	67.2	70.1	67.2	70.2	67.8	70.3	68.4
C-3′′	64.3		64.2	64.3	64.2	64.4	64.2	64.3	64.2	64.3	64.2	64.3
C-4′′	73.2		73.2	70.8	73.2	70.8	73.2	70.8	73.2	70.8	73.2	70.8
C-5′′	68.5		68.5	68.5	68.5	68.4	68.5	68.5	68.5	68.5	68.5	68.5
$N-CH_3$	38.0		37.7	35.4	37.7	35.5	37.6	35.3	37.7	35.4	37.7	35.3
$C-CH_3$	22.9		22.4	21.7	22.4	21.8	22.4	21.8	22.4	21.8	22.4	21.7

a. $_c$ ppm downfield from TMS in D_2O. b. Values may be interchanged within the vertical column.
c. pH of acidified solution was between 5 and 6. d. reference[20]

The remaining isomer isolated (27) was assigned the β-L rather than the β-D configuration on the basis of its ^{13}C NMR spectrum (vide infra). This was confirmed by its synthesis from 2'-desaminosisomicin (19) by catalytic hydrogenation (FIG. 5). It is known that hydrogenation of sisomicin proceeds from the face remote to the glycosidic linkage to generate exclusively the 5'-epi-gentamicin C_{1a}.[14] Hydrogenation of 2'-desaminosisomicin (19) proceeded in an analogous way to produce the equatorial β-L isomer (27), identical in all respects to the material isolated from the glucal addition reaction, confirming the initial stereochemical assignment. (Hydrogenation from the opposite face would have given the axial α-D-glycoside).

Solution Conformation Studies A knowledge of the solution conformations of aminoglycoside antibiotics is of importance to the understanding of structure-activity relationships and these have been studied by several groups

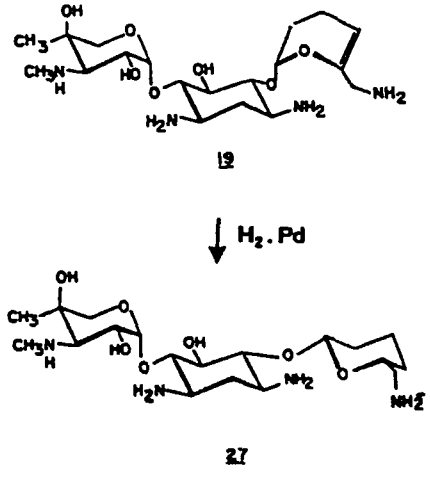
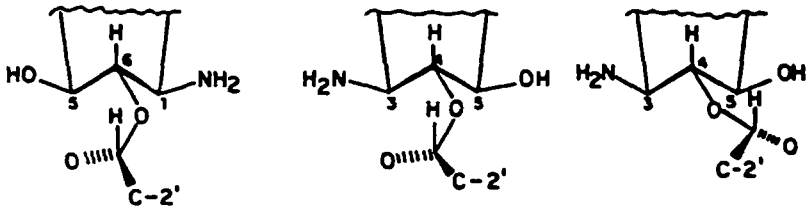


FIG. 5. Hydrogenation of 2'desaminosisomicin



(a) (b) (c)

using ^{13}C NMR spectrosopy.[19,20] It has been concluded that shielding effects on the carbon atoms of the deoxystreptamine ring β to the glycosylated hydroxyl group are indicative of conformational preferences about the aglycone carbon-glycosidic oxygen bond (O,C-4 or O,C-6 bonds). The garamine ψ-disaccharide moiety (30) is common to all of the compounds in the series under discussion and the conformation of the garosaminyl linkage at C-6 is the same in these compounds as it is in the natural gentamicin antibiotics.[21] This is inferred from the observation that the chemical shift of C-1 in these and the natural compounds is not significantly different from that in deoxystreptamine itself (51.6 ppm), whilst C-5 is shielded by an average of 1.5 ppm. This has led to the assignment of rotamer (a) to best describe the preferred conformation around the C-6 glycosidic linkage.[20] It is assumed that all preferred conformations will satisfy the exo-anomeric effect. (See reference 22.) Compounds in the 2′-unsubstituted series that have the α-D configuration, that is 13,19,22,24 and 25, all appear from their ^{13}C NMR spectra, to have the same preferred conformation about the newly formed C-4 glycosidic linkage as other 4-O- α-D-glycosides in the natural gentamicin-kanamycin series. In all cases (see Table 2) C-5 of garamine (30) shows relatively minor chemical shift changes upon formation of the glycosidic

linkage at C-4, whereas C-3 experiences a shielding effect averaging 1.3 ppm. These effects are in accord with a preferred conformation (b) which would be expected to lead to shielding of C-3 by steric compression.

The 4-O-β-L diastereoisomer (27) of 2′-desamino-gentamicin C_{1a} is linked by an equatorially oriented glycosidic linkage to the C-4 hydroxyl group of the deoxysteptamine ring. In this case also, C-3 is shielded by 1.6 ppm whilst C-5 is unchanged relative to garamine (30) (See Table 2). The conformation of the glycosidic linkage is therefore, also best described by rotamer (b).

The α-L diastereoisomer (26), in which the chirality of C-1′ is opposite to that of the natural antibiotics (and 27), has the opposite orientation as evidenced by shielding of C-5 by 1.2 ppm and only a small shielding effect (0.4 ppm) at C-3. The preferred conformation is best described by conformer (c) which is the same as that previously ascribed[23] to β-D glycosides at that position. Thus the absolute stereochemistry at the anomeric centre of the glycoside attached to deoxysteptamine, determines the preferred conformation described by the rotamers (b) or (c), irrespective of whether the glycoside is axially or equatorially linked. This is consistent with previous work, as Lemieux and Koto[22] found that in the case of cyclohexyl and β-D glycopyranosides, the pro-R methylene group of the aglycone was more shielded than the pro-S methylene group in the α-D glycoside whereas the opposite was the case for the β-D glycoside. Similar results have been obtained by Tori and co-workers using chiral aglycones and these observations are the basis of a recent technique[24] useful in the assignment of chirality to secondary alcohols. The glycoside

TABLE 2

4-O-GLYCOSIDATION SHIELDING EFFECTS

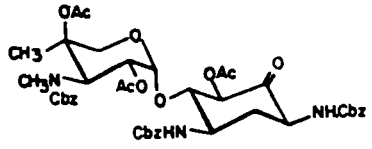
ON DEOXYSTREPTAMINE CARBONS ($\Delta\delta_c$)[a]

Compound	C-3	C-5
α-D or β-L Configuration		
13	1.3	-0.5
19	1.4	-0.4
22	1.3	-0.4
24	1.3	-0.5
25	1.4	-0.4
27	1.6	0
28	1.6	0.2
31	1.4	-0.1
α-L or β-D Configuration		
14	1.0	1.8
26	0.4	1.2
29	-0.1	1.6
32	0.8	1.5

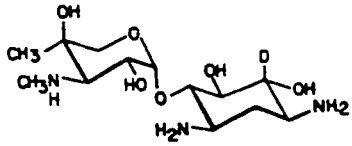
a. (δ_c garamine - δ_c compound)

conformations and therefore the shielding effects are dependent on the substitution at the beta positions of the aglycone.[20] Our results confirm that in cases in which the cyclohexane ring is substituted at both positions by equatorial substituents, as in deoxystreptamine, the shielding pattern is opposite to that in the case of an unsubstituted cyclohexane ring, in that it is the aglycone carbon syn to the ring oxygen in the preferred conformation that is most shielded.

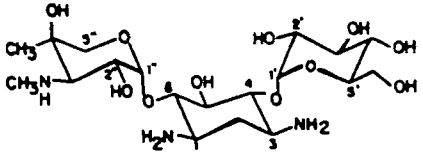
In the case of deoxysteptamine-containing antibiotics it should be possible to determine the absolute stereochemistry of glycosides (whose relative stereochemistry is known) attached to position 4 (or 6) of the deoxysteptamine ring, by using such shifts. In part to test the generality of this approach, we prepared 4-O-α-D-glucopyranosylgaramine (28), 4-O-α-L-glucopyranosylgaramine (29) and the tetrahydropyranyl ethers 31 and 32. The isomeric pair 28 and 29 were prepared by the Lemieux-Nagabhushan procedure[25] using the nitrosyl chloride adducts of D-glucal triacetate and L-glucal tri-acetate respectively. The fully blocked garamine derivative used was labelled at carbon 4 with deuterium in order to unambiguously distinguish between carbons 4 and 6 in the ^{13}C NMR spectra of the products. Oxidation of 10 to the 4-keto derivative (33) with acetic anhydride in dimethyl sulphoxide followed a procedure developed by Tanabe.[26] Reduction with sodium borodeuteride gave the 4-deuterio derivative of 10 as the major product. Side-products derived from trans-acylation could be minimized by keeping the reaction mixture at 0°-5°. Part of this material was deblocked to give 4-deuteriogaramine whose ^{13}C NMR spectrum was compared with that of garamine itself and as



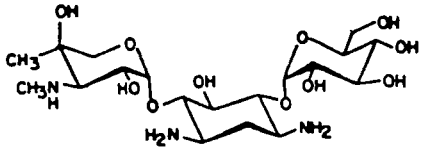
33



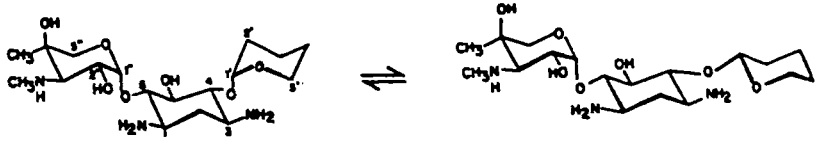
30



28



29



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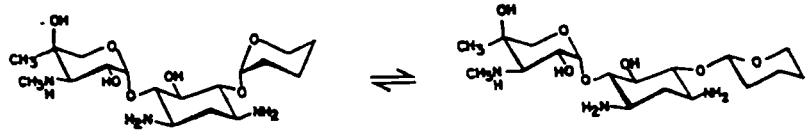

32

expected, C-4 of the former was greatly reduced in intensity. Thus the location of the deuterium atom at C-4 was established and the possibility of inversion of stereochemistry at positions 3,4 or 5 during the reaction sequence was eliminated. Deuterated 10 was then diluted with unlabelled material to achieve a final deuterium content of 71%.

Both 4-O-α-D-glucopyranosylgaramine (28) and 4-O-α-L-glucopyranosylgaramine (29) were prepared according to literature procedures. The absorption at C-4 in the ^{13}C NMR spectra of each compound (Table 1) was approximately 30% of the intensity of undeuterated material enabling a clear distinction to be made between carbons 4 and 6 in both protonated and unprotonated samples. It can be seen from Table 2 that the shielding effects at C-3 and C-5 of the bases are also in accord with the known stereochemistry at C-1'. That is, carbon 3 is shielded for the glycoside having the natural α-D configuration (28) whilst carbon 5 is shielded for its α-L isomer (29).

Preparation of the tetrahydropyranyl ethers proceeded as previously described[27] to give the two derivatives 31 ($[\alpha]_D$ + 165°) and 32 ($[\alpha]_D$ + 112°). The ^1H NMR and ^{13}C NMR spectra reflect, for each compound, approximately equal contributions of the two chair conformers of the tetrahydropyranyl ring. The ^{13}C NMR spectra (Table 2) also reveal that the chemical shifts at C-3 and C-5 of the more dextrorotatory isomer (31) are in the range expected for the α-D, β-L group of glycosidic diastereoisomers at C-4. In contrast the other isomer (32) clearly belongs to the α-L, β-D group of diasterioisomers in which C-1' has the unnatural configuration. The shielding effects on the deoxystreptamine

ring thus allows the chirality at C-1′ to be determined even in the case of the tetrahydropyranyl ethers.

The shielding effect on C-3 (Table 2) in the cases of compounds 14 and 32 is also relatively large. This has been observed before for glycosides of the β-D configuration[20] attached at this site and seems to be characteristic of this stereochemistry.

Anomalous chemical shift changes of the C-1′ and C-4 resonances on acidification of 4,6-linked aminoglycoside antibiotics form the basis of a method for the determination of the absolute sterochemistry of sugar constituents of these antibiotics.[28] The anomeric carbon of the α-D-glycoside attached to C-4 of deoxysteptamine is shifted upfield by 3 to 5 ppm on acidification, and C-4 is also shifted 3 to 7 ppm beyond the expected increment arising from protonation of the C-3 amine group. The origin of these effects is unknown although proposals for a conformational shift about the C-4 glycosidic linkage induced by protonation of the amine groups (particularly that at C-3) have been advanced.[29] The absence of such anomalous shifts was, conversely, held to be diagnostic[27] for the α-L-configuration of a glycoside attached at that site, although no such examples were known at that time.[28]

Our ^{13}C NMR protonation studies on 2′-unsubstituted aminoglycosides (Table 1) reveal that this procedure is not applicable in this series. It is applicable to the substituted case in that the chemical shift change on protonation of C-4 in 4-O- α-D-glucopyranosylgaramine (28) is much greater than that in 4-O- α-L-glucopyranosylgaramine (29) as predicted, but in 2′-desaminogentamicin C_{1a}, (24) and its α-L isomer (26), the chemical shift change of C-4 on

protonation is very similar (6.6 and 6.9 ppm), and is in each case much greater than the shift of C-6. Also, the chemical shift changes of C-1′ on protonation for the compounds in Table 1 are unexpectedly small. In particular the small protonation shift of C-1′ in 28 indicates that a 6′-amine group is required for the anomalous shift of C-1′ to be observed.

The 2′-unsubstituted analogs were at least as potent in in vitro antibacterial assays as the parent compounds and were more active against resistant strains. These examples constitute the first case in this class of antibiotic in which removal of functional groups to give a substitution pattern not produced in nature, provides compounds of superior biological properties.

EXPERIMENTAL SECTION

General Methods. Nuclear magnetic resonance spectra (^{1}H NMR spectra) were obtained at 100 MHz using a XL 100-15 spectrometer unless otherwise indicated. Chemical shifts in deuteriochloroform are reported in δ units (ppm downfield from internal tetramethylsilane). Infrared spectra were recorded with either a Perkin-Elmer 221 or 137 spectrometer. Mass spectra were recorded with a Varian CH5 spectrometer. CD spectra were recorded on a Cary 61 spectromer.

Preparation of 6-Azido-6-deoxy-3,4-di-O-benzyl-D-glucal. To sodium hydride (5.16 g) suspended in dry N,N-dimethylformamide (50 mL) at 0 °C was added benzyl chloride (12.4mL), followed by 6-azido-6-deoxy-D-glucal (4.65 g) in N,N-dimethylformamide (17 mL) dropwise with stirring. After 4 h ethanol (15 mL) was added, and after a further ten minutes,

the mixture was poured on ice, and the whole extracted with chloroform. The chloroform extracts were washed well with water, dried and evaporated under reduced pressure to give a residue that was chromatographed on a silica gel to give the title compound (4.7 g) with the following constants: $[\alpha]_D^{26}$+34.3° (c 0.5, ethanol); ^{1}H NMR ($CDCl_3$, 60 MHz) δ3.52 (2H, m, CH_2N_3), 4.6 (4H, m, Ar-CH_2), 6.38 (1H, dd, J = 1.5, 6 Hz, H-1); IR ($CHCl_3$) 2100,1640 cm^{-1}; mass spectrum, m/e 351 (M^+).

Anal. Calcd for $C_{20}H_{21}N_3O_3$: C, 68.36; H, 6.02; N, 11.96. Found: C, 68.30, H, 6.14; N,12.07.

6-Acetamido-6-deoxy-3,4-di-O-benzyl-D-glucal (6) 6-Azido-6-deoxy-3,4-di-O-benzyl-D-glucal (2.98 g) in tetrahydrofuran (30 mL) was added dropwise to a stirred suspension of lithium aluminum hydride (760 mg) in tetrahydrofuran (50 mL). The mixture was heated under reflux for 4 h and worked up by the addition of wet ether, followed by filtration to obtain, after evaporation, a residue (2.56 g) that was treated with acetic anhydride (3 mL) and distilled pyridine (10 mL). After 2 h the solvent was removed under reduced pressure, and the product crystallized from chloroform-hexane (1.8 g): $[\alpha]_D^{26}$ -41.8° (c 0.4,ethanol); ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.86 (3H, s, $COCH_3$), 3.65 (2H, m, $-CH_2-N$), 4.6 (2H, m, Ar-CH_2), 4.75 (2H, m Ar-CH_2), 4.9 (1H, dd, J = 3, 6 Hz, H-2); 6.45 (1H, dd, J = 1.5, 6 Hz, H-1); IR 3250, 1640 cm^{-1}; mass spectrum, m/e 367 (M^+).

Anal. Calcd for $C_{22}H_{23}NO_4$: C, 71.91; H, 6.86; N, 3.81. Found: C, 71.7; H, 6.9; N, 3.5.

2'-Deoxygentamicin B. (4) 2'-O-Acetyl-1,3,3'-tri-N-benzyl-oxycarbonylgaramine (1 g) 3,4-di-O-benzyl-6-acetamido-6-deoxy-D-glucal (0.7 g) and p-toluenesulphonic

acid monohydrate (5 mg) were dissolved in dry benzene and kept at 50 °C for 5 h. The cooled benzene solution was washed with dilute aqueous sodium bicarbonate solution, dried ($MgSO_4$), and concentrated under vacuum to leave a residue which was chromatographed on silica gel to give a mixture that included the desired condensation products. The mixture was dissolved in THF (5 mL) and distilled liquid ammonia (50 mL) and sodium (0.7 g) was added in small pieces. After twenty minutes' stirring with protection from moisture, the dry ice-acetone condenser was removed, and the solvent was allowed to evaporate overnight. Sodium hydroxide (0.5 g) and water (5 mL) were added to dissolve the residue, and the resulting solution was heated under reflux for 3 h. The cooled solution was stirred with an excess of Amberlite IRC-50 (H^+) ion-exchange resin, and the resin was filtered and washed thoroughly with distilled water. The resin was put onto a column and eluted with 3% aqueous ammonium hydroxide. The aminoglycoside-containing eluant was concentrated under vacuum and lyophilized. Chromatography of the lyophilized solid on silica gel in the lower phase of a chloroform-methanol-(15%) ammonium hydroxide (2:1:1) solvent system gave the title compound after passage through Amberlite IRA-401S (OH^-) ion-exchange resin and lyophilization (45 mg): $[\alpha]_D^{26}$ + 136.7° (c 0.3, water); ^1H NMR (D_2O) δ1.18 (3H, s, C-C$\underline{H}_3$), 2.57 (3H, s, N-C$\underline{H}_3$), 5.03 (1H, d, J = 4 Hz, $\underline{H}$-1''), 5.47 (1H, broad doublet, J = 3.5 Hz); mass spectrum m/e 467 $(M+1)^+$; CD (TaCu) $[\theta]^{288}$ = -6.360°.

A minor component, 2,6-dideoxy-6-amino- β-D-glucopyranosyl-(1 ⟶4)-garamine was also isolated: ^1H NMR (D_2O) δ1.18 (3H, s, C-C$\underline{H}_3$), 2.57 (s, 3H, N-C$\underline{H}_3$), 3.75 (1H, dd, J = 4 Hz,

11 Hz, H-2''), (1H, d, J = 12.5 Hz, H-5'' eq), 4.85 (1H, d, J = 10 Hz, H-1'), 5.05 (1H, d, J = 4 Hz, H-1''); mass spectrum m/e 467 (M + 1): CD (TaCu) $[\theta]^{228} = -5{,}730^{\circ}$.

2',3'-Dideoxygentamicin B. (13) 5,2'4'-Tri-O-acetyl-1,3,3'-tri-N-benzyloxycarbonylgaramine (19.52 g), 4-O-acetyl-6-azido-3,6-dideoxy-D-glucal (7.1 g) and p-toluenesulphonic acid monohydrate (180 mg) were dissolved in dry benzene and heated at 45 °C for 5 h , and then left at room temperature overnight. The cooled solution was washed with dilute aqueous sodium bicarbonate solution, dried, ($MgSO_4$), and concentrated under vacuum to leave a residue. Chromatography of this material on silica gel in 0.25% methanol in chloroform gave a major adduct (14.2 g) and a minor adduct (3.8 g) in order of their elution. The major adduct had the following constants: $[\alpha]_D^{26}$ + 105.6° (c 0.5, methanol), IR ($CHCl_3$), 2100, 1725 cm^{-1}.

Anal. Calcd for $C_{51}H_{62}N_6O_{18}$: C, 58.50; H, 5.96; N, 8.02. Found: C, 58.19; H, 6.06; N, 7.88.

A portion of the major adduct (5 g) was dissolved in 20% aqueous dioxane (100 mL) and hydrogenated at 55 psi at room temperature in the presence of 10% palladium on charcoal (500 mg) for sixteen hours. The catalyst was removed by filtration, the reaction mixture was evaporated to dryness, and residue was heated at reflux in a solution of potassium hydroxide (3 g) in distilled water (20 mL) for eighteen h. The cooled solution was treated with Amberlite IRC-50 (H^+) ion-exchange resin, and the resin was washed with water, and poured onto a column. Elution with 3% ammonium hydroxide and evaporation of the eluant gave a residue which was chromatographed on silica gel (50 g) and eluated with the lower phase of chloroform-methanol-15% aqueous ammonium hydroxide (2:1:1)

solvent mixture to give, after passage through Amberlite IRA-401S (OH^-) ion-exchange resin and lyophilization, the title compound (0.95 g): $[\alpha]_D^{26}$ + 171° (c 0.4, water); ^1H NMR (D_2O) δ 1.15 (3H, s, C-C<u>H</u>$_3$, 2.46 (3H, s, N-C<u>H</u>$_3$), 3.75 (1H, dd, J = 4, 11 Hz, <u>H</u>-2′′), 3.96 (1H, d, J = 12.5 Hz, <u>H</u>-5′′eq), 5.02 (1H, d, J = 4 Hz, <u>H</u>-1′′), 5.27 (1H, broad s, ω1/2 = 5 Hz, <u>H</u>-1′); mass spectrum m/e 451 $(M+1)^+$; CD (TaCu) $[\theta]^{288}$ - 10,000°:

Anal. Calcd for $C_{19}H_{38}N_4O_8$: C, 50.66; H, 8.44; N, 12.44. Found: C, 50.52; H, 8.45; N, 12.30.

The minor blocked adduct had the following constants: $[\alpha]_D^{26}$ + 82° (c 0.3, methanol): IR 3400, 2100, 1725 cm^{-1}. It was deblocked and chromatographed in the same way to give 6-amino-2,3,6-tri-deoxy- β-<u>D</u>-glucopyranosyl-(1 ⟶4)- garamine (0.33 g): $[\alpha]_D^{26}$ + 119.2° (c 0.3, water): ^1H NMR (D_2O) δ1.16 (3H, s, C-C<u>H</u>$_3$), 2.48 (3H, s, N-C<u>H</u>$_3$), 3.75 (1H, dd, J = 4, 11 Hz, <u>H</u>-2′′), 4.04 (1H, d, J = 12.5 Hz, <u>H</u>-5′′ eq), 4.77 (1H, m, ω1/2 = 10 Hz, <u>H</u>-1′), 5.04 (1H, d, J = 4 Hz, <u>H</u>-1′′); mass spectrum, m/e 451 $(M + 1)^+$; CD (TaCu) $[\theta]^{287}$ = -8,500°.

<u>4,6-Diamino-2,3,4,6-tetradeoxy-2-galactopyranosyl-(1→4)-garamine.</u> (<u>18</u>) 4,6-Diazido-3,4,6-trideoxy-<u>D</u>-glucal (3.31 g), 5, 2′ 4′ -tri- <u>O</u>- acetyl- 1,3,3′-<u>N</u>-benzyloxycarbonylgaramine (1.75 g) and <u>p</u>-toluenesulphonic acid were dissolved in dry benzene and kept at 45 °C for 3 h and then overnight at room temperature. The reaction was worked up as in the foregoing, and chromatography gave the desired adduct (6.9 g): $[\alpha]_D^{26}$ + 79.5° (c 0.3, methanol); IR ($CHCl_3$) 3350, 2100, 1725 cm^{-1}.

The adduct (6.9 g) was dissolved in 20% aqueous dioxane (125 mL) to which had been added 1<u>N</u> hydrochloric acid (28 mL) and 10% palladium on charcoal, and the whole was submitted to hydrogenation at 56 psi at room temperature for sixteen h.

The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. Potassium hydroxide (4.5 g) in distilled water (30 mL) was added to the residue and the solution heated at 100 °C for 3 h, followed by sixteen hours at 80 °C. The solution was neutralized to pH 11 with dilute sulphuric acid and then stirred with Amberlite IRC-50 (H^+) ion-exchange resin. The resin was washed with water, followed by 3% aqueous ammonium hydroxide. The ammoniacal washings were combined and concentrated under reduced pressure to leave a residue which was chromatographed on silica gel in the lower phase of chloroform-methanol-15% aqueous ammonium hydroxide (2:1:1) to give, after passage through Amberlite IRA-401S (OH^{-1}) ion-exchange resin and lyophilization, the title compound (1:1 g): $[\alpha]_D^{26}$ + 141.1° (c 0.3, methanol); ^1H NMR (D_2O) δ81.13 (3H, s, C-C$\underline{H}_3$), 2.44 (3H, s, N-C$\underline{H}_3$), 3.72 (1H, dd, J = 4, 11 Hz, $\underline{H}$-2''), 3.95 (1H, d, J = 12,5 Hz, $\underline{H}$-5'' eq), 5.0 (1H, d, J = 4 Hz, $\underline{H}$-1''), 5.26 (1H, broad s, ω1/2 = 6 Hz, $\underline{H}$-1''), mass spectrum m/e 450 $(M + 1)^+$; (TACu) $[\theta]^{285}$-8,850°.

Anal. Calcd for $C_{19}H_{39}N_5O_7$: C, 50.77; H, 8.99; N, 15.59. Found: C, 50.71; H, 8.69; N, 15.38.

2'-Desaminosisomicin (19) The major blocked adduct, 4-O-acetyl-6-azido-2,3,6-trideoxy-glucopyranosyl-(1 ⟶4)-5,2',4'- tri- O- acetyl- 1,3,3'- tri- N-benzyloxycarbonylgaramine, previously described in the synthesis of 2',3'-dideoxy-gentamicin B, was photolysed in dichloromethane solution with a 450W Hg lamp using a Vycor filter. Nineteen separate runs were combined, in each of which 300 mg of adduct was photolysed for 7 h. in 250 mL of dichloromethane. The crude combined photolysis product was dissolved in 95% ethanol (170 mL) and triethylamine (6.8 mL) was added. The solution

was stirred at room temperature for 5 h, the solvent was evaporated, and the residue was chromatographed on silica gel in chloroform to give an α, β-unsaturated aldehyde (2.2 g). $[\alpha]_D^{26}$ =105.8° (c 0.5, ethanol); ^{1}H NMR ($CDCl_3$) δ1.13, 1.41 (3H, d, rotamers, C-C<u>H</u>$_3$), 2.0 (12H, m, OCOC<u>H</u>$_3$), 2.9 (3H, s, N-C<u>H</u>$_3$).

Anal. Calcd for $C_{49}H_{57}O_{17}N_3$: C, 61.31; H, 5.99; N, 4.38. Found: C, 60.93; H, 5.88; N, 4.32.

The aldehyde (1.61 g) was dissolved in tetrahydrofuran (8 mL) and added to a solution of ammonium acetate (9.6 g) in methanol (45 mL). After 30 min sodium cyanoborohydride (960 mg) was added, and the reaction was stirred at room temperature for 2.5 h. and overnight at 4 °C. Water (150 mL) was added, and the mixture was extracted with chloroform (3x100 mL), and the extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The resultant residue was dissolved in dimethylsulphoxide to which was added potassium hydroxide (2.6 g) in water (5 mL) and the mixture was stirred at room temperature for two days. The pH of the reaction mixture was adjusted to pH 11 by the addition of dilute hydrochloric acid, and the solution was stirred with IRC-50 (H^+) ion-exchange resin. The resin was filtered, washed with water and extracted with 6% aqueous ammonium hydroxide. The ammoniacal extracts were evaporated to dryness, and the residue was chromatographed on silica gel in the lower phase of chloroform-methanol-7% ammonium hydroxide (2:1:1) to give, after passage through IRA-401S (OH^-) ion-exchange resin followed by lyophilization, 2′-desaminosisomicin (120 mg): $[\alpha]_D^{26}$ + 184.3° (c 0.5, water); ^{1}H NMR (D_2O) δ1.18 (3H, s, C-C<u>H</u>$_3$); 2.49 (3H, s, N-C<u>H</u>$_3$), 3.76 (1H, dd, J = 4, 11 Hz, <u>H</u>-2′′), 4.03 (1H, d, J = 12.5 Hz, <u>H</u>-5′′ eq), 4.92 (1H, m,

H-4′), 5.05 (1H, d, J = 4 Hz, H-1′′), 5.46 (1H, distorted triplet, $\omega 1/2$ = 6 Hz, H-1′), mass spectrum, m/e 432.2559 (M^+). ($C_{19}H_{36}N_4O_7$ requires 432.2584).

Similarly obtained was 2′,2′-didesamino antibiotic 66-40C (7 mg): ^{1}H NMR (D_2O) δ 1.24 (6H, s, C-CH$_3$); 2.58 (6H, s, N-CH$_3$), 5.14 (2H, m, H-1′′), 5.6 (4H, m, H-1′ and H-4′), 7.45 (2H, s, H-6′). Also obtained was 6′6′-imino - di-(2′,6′-didesamino)-sisomicin (71 mg): $[\alpha]_D^{26}$ + 160.8° (c 0.4, water); ^{1}H NMR (D_2O) δ1.23 (6H, s, C-CH$_3$); 2.54 (6H, s, N-CH$_3$), 3.76 (2H, dd, J = 4, 11 Hz, H-2′′), 4.05 (2H, d, J = 12.5 Hz, H-5′′ eq), 4.95 (2H, broad singlet, $\omega 1/2$= 8 Hz, H-4′), 5.08 (2H, d, J = 4 Hz, H-1′′), 5.48 (2H, broad singlet, $\omega 1/2$ = 8 Hz, H-1′); mass spectrum, m/e 847.4811 (M^+). ($C_{38}H_{69}N_7O_{14}$ requires m/e 847.4902).

6-Azidomethyl-5,6-dihydropyran (25) A solution of 6-hydroxymethyl-5,6-dihydropyran (4.0 g) and triethylamine (10.5 g) in dry dichloromethane (100 mL) was cooled in ice, and a solution of methanesulphonyl chloride (5.2 g) in dry dichloromethane (20 mL) was added dropwise, with stirring. After 5 h, water was added and the mixture was partitioned between water and chloroform. The chloroform layer was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The resulting crude mesylate was dissolved in dry N,N-dimethylformamide (100 mL) and stirred at 50 °C for 60 h with sodium azide (14 g). The mixture was filtered, the filtrate was poured into ice-water, and the whole extracted with ether. The ether extracts were evaporated under reduced pressure, and the residue was taken up in hexane and washed with water. The dried organic layer was evaporated under reduced pressure, and the residue was chromatographed on silica gel in 3% acetone in hexane to give the title compound

(2.1 gm): ^{1}H NMR ($CDCl_3$ 60 MHz) δ1.19 (4H, m, ring C$\underline{H}_2$), 3.35 (2H, d, J = 5.5 Hz, C$\underline{H}_2$-N_3), 4.0 (1H, m, $\underline{H}$-5), 4.75 (1H, m, $\underline{H}$-2), 6.4 (1H, dt, J = 1.5, 1.5, 6.5 Hz, $\underline{H}$-1): IR (film) 2100, 1650, 1475, 1130 cm^{-1}.

Anal. Calcd for $C_6H_9N_3O$: C, 51.7; H, 6.5; N, 30.2. Found: C, 51.9; H, 6.5; N, 30.6.

Preparation of 2′-desaminogentamicin C_{1a} (24)

6-Azido-methyl-5,6-dihydropyran (1.54 g) 5,2′4′-tri-$\underline{O}$-acetyl-1,3,3′-tri-$\underline{N}$-benzyloxycarbonylgaramine (4.33 g) and $\underline{p}$-toluenesulphonic acid (20 mg) were dissolved in dry benzene and the solution left at 40 °C overnight. Following the usual work-up, as in the foregoing examples, the crude residue was chromatographed on silica gel in 0.25% methanol in chloroform, two fractions being collected. The less polar fraction (1.9 g) was dissolved in 50% aqueous dioxane (20 mL) to which was added 5% palladium on charcoal (200 mg) and 0.1$\underline{N}$ hydrochloric acid (5 mL), and the mixture was submitted to hydrogenation at 55 psi for 16 h at room temperature. The catalyst was removed by filtration, and the filtrate was evaporated under reduced pressure. The residue was heated at 100 °C in 20% aqueous potassim hydroxide under oxygen for 16 h. The pH of the cooled solution was adjusted to pH 11 by the addition of dilute sulphuric acid, and the solution was concentrated to a small volume and added dropwise to a vigorously stirred volume of absolute ethanol (200 mL). The inorganic salts were removed by filtration, and the filtrate was reduced to dryness, taken up in the lower phase of a chloroform-methanol-10% ammonium hydroxide solvent mixture (2:1:1) and chromatographed on silica gel in the same solvent. Two compounds were obtained, the less polar being (24) (100 mg): $[\alpha]_D^{26}$ + 166° ($\underline{c}$.4, water) ^{1}H NMR (D_2O) δ1.17 (3H, s, C-C$\underline{H}_3$), 2.48 (3H, s, N-C$\underline{H}_3$), 3.71 (1H, dd, J = 4, 11

Hz, H-2''), 4.0 (1H, d, J = 12.5 Hz, H-5'' eq), 5.04 (1H, d, J = 4 Hz, H-1''), 5.30 (1H, broad singlet, $\omega 1/2$ = 5 Hz, H-1'); mass spectrum, m/e 435.2812 $(M+1)^+$, ($C_{19}H_{39}N_4O_7$ requires m/e 435.2819); CD (TaCu) $[\theta]^{289}$ -7,760°.

The more polar product (130 mg), after deionization and lyophilization was 6'-amino-2,3,4,6-tetradeoxy-β-L-glucopyranosyl-(1→4)-garamine: (27): $[\alpha]_D^{26}$ + 138.1° (c 0.4, water); ^{1}H NMR (D_2O) δ1.17 (3H, s, C-CH$_3$), 2.48 (3H, s, N-CH$_3$), 3.75 (1H, dd, J = 4, 11 Hz, H-2''), 4.02 (1H, d, J = 12.5 Hz, H-5'' eq), 4.75 (1H, d, J = 9.5 Hz, H-1'), 5.03 (1H, d, J = 4 Hz, H-1''); mass spectrum, m/e 434.2730 (M^+). ($C_{19}H_{38}N_4O_7$ requires m/e 434.2740); CD (TACu) $[\theta]^{288}$ -7,450°.

The second fraction obtained from chromatography of the blocked adducts (0.8 g) was deblocked in the same manner as in the foregoing, and the unblocked mixture was chromatographed to yield 2'-desaminogentamicin C_{1a} (26 mg), 6'-amino-2,3,4,6-tetradeoxy-α-L-glucopyranosyl-(1→4)-garamine (23 mg) and a different isomer, less polar than either of the other two, 6'-amino-2,3,4,6-tetradeoxy-α-L-glucopyranosyl (1→4)-garamine (26) (32 mg): $[\alpha]_D^{26}$ + 83.5° (c 0.4, water); ^{1}H NMR (D_2O) δ1.17(3H, s, C-CH$_3$), 2.48 (3H, s, N-CH$_3$), 3.75 (1H, dd, J = 4, 11 Hz, H-2''), 4.03 (1H, d, J = 12.5 Hz, H-5'' eq), 5.06 (1H, d, J = 4 Hz, H-1''), 5.14 (1H, broad singlet, $\omega 1/2$ = 5.5 Hz, H-1'); mass spectrum, m/e 434.2708 (M^+). ($C_{19}H_{38}N_4O_7$ requires m/e 434.2740): CD (TACu) $[\theta]^{289}$ -7,350°.

Hydrogenation of 2'-desaminosisomicin 2'-Desaminosisomicin (50 mg) was dissolved in 50% aqueous dioxane and submitted to catalytic hydrogenation under one atmosphere of hydrogen in the presence of 30 mg 5% palladium on charcoal

for 18 h. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure to leave a residue that was dissolved in water and passed through a column of Amberlite IRA-401S (OH^-) ion-exchange resin and lyophilized. The lyophilized solid (35 mg) was chromatographically homogeneous and indistinguishable (^1H NMR, ^{13}C NMR) from 6′-amino-2,3,4,6-tetradeoxy-β-L-glucopyranosyl-(1⟶4)-garamine (27) obtained in the synthesis of 2′-desaminogentamicin C_{1a}.

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