Semisynthetic Aminoglycoside Antibacterials. Part 11.[1] Solution Conformations of Semisynthetic and Naturally Occurring Aminoglycoside Antibiotics

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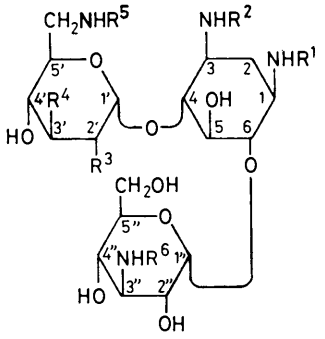
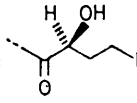
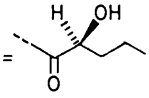
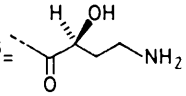
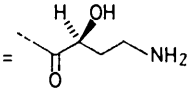
A critical analysis of the ^{13}C n.m.r. spectral data for a wide range of naturally occurring aminoglycoside antibiotics, as well as for a diverse assortment of semisynthetic aminoglycoside antibacterials, has revealed new insights into the solution conformations of these clinically important drugs. Correlation of the $\Delta\delta_C$ values for C-4 and C-6 determined in going from deoxystreptamine to the *pseudo* di- or tri-saccharides, as well as changes in the chemical shifts of C-1′ and C-1″, has revealed that these molecules adopt a wide range of well defined conformations in solution, that are dependent not only on the structure, but also the pH. The limitations of the 'Nagabhushan–Daniels Rule,' which has been reported to break down when applied to some classes of aminoglycosides, are discussed in the light of these new observations.

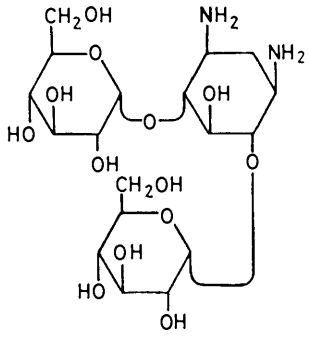
AMINOGLYCOSIDE antibiotics [2] represent a clinically important group of drugs that have achieved widespread therapeutic use throughout the world. The discovery that bacterial resistance was associated with specific chemical transformations in the molecule gave a tremendous impetus to the search for novel semisynthetic aminoglycoside derivatives that would not be capable of such enzymic modification.[3-5] This has resulted in the synthesis of a wide range of novel semisynthetic aminoglycosides in these and other laboratories over the last decade. During the course of these studies and during the course of the structural elucidation of naturally occurring aminoglycosides, a considerable body of ^{13}C n.m.r data was accumulated. It was recognized that this data if carefully analysed could yield useful information about the solution conformations of these important antibiotics.

Lemieux, in a pioneering study, defined the conformational properties of glycosidic linkages in simple alkyl and cyclohexyl glycosides.[6] He also stressed the importance of the *exo*-anomeric affect in defining the conformational properties of glycosidic linkages.[6-11] The solution conformations of the kanamycins and related synthetic analogues were first defined by ^1H n.m.r. studies.[12] It was concluded that in these compounds the 4-*O*-glycoside projects towards *C*-3 while the 6-*O*-glycoside projects towards C-5 in the free base in solution. These conclusions were in good agreement with the conformation of kanamycin A (1) in the solid state as determined by *X*-ray crystallography.[13] Kanamycin A (1), which does not contain a 2′-amino-group, was found to exhibit a pronounced deshielding of 1′-H on protonation of the amino-groups at acidic pH.[12] This deshielding was less pronounced in the case of 4,6-di-*O*-(α-D-glucopyranosyl)-2-deoxystreptamine (11) [12] and in both cases it was attributed to a change in the conformation of the 4-*O*-glycoside about the O–C-4 glycosidic bond. It was implied that protonation of the 3-amino-group was increasing the H-bonding with the 5′-ring oxygen resulting in a conformational change about the O–C-4 glycosidic bond, although the nature of this change was not specified. The chemical shift of the anomeric proton of the 6-*O*-glycoside, 1″-H, was independent of pH indicating that no significant conformational changes were occurring about the O–C-6 glycosidic bond in these molecules at acidic pH.[12] Subsequent ^{13}C n.m.r. studies carried out on the free bases led to similar conclusions regarding the general orientation of the glycosyl units about the O–C-4 and O–C-6 glycosidic bonds in gentamicin C_{1a} (12),[14] gentamicin C_2 (13),[14] gentamicin C_1 (14),[14] gentamine C_{1a} (25),[14] gentamine C_2 (26),[14] gentamine C_1 (27),[14] sisomicin (29),[14] antibiotic 66-40 B (49),[15] and antibiotic 66-40 D (50).[15] In all cases in going from 2-deoxystreptamine (51) to the *pseudo* di- or tri-saccharide, shielding at C-3 by the 4-*O*-glycoside, and at C-5 by the 6-*O*-glycoside, was observed supporting these conclusions. Independent ^{13}C n.m.r. studies on tobramycin (2) [16] and kanamycin B (3) [16] came to similar conclusions about the solution conformations of these molecules. Koch and Wenkert [16] in addition concluded from the absence of any pronounced shielding at C-1′ and C-1″ in these molecules relative to simple methyl glycosides, that in the free bases the preferred rotamer about the O–C-4 glycosidic bond was *a*, while that about the O–C-6 glycosidic bond was *b*. In both rotamers the C-4–H-4 and C-1′–H-1′ bonds, as well as the C-6–H-6 and C-1″–H-1″ bonds, are parallel, or nearly so. Subsequent ^{13}C n.m.r. studies on a wide range of 4-*O*-α-D-glycosyl derivatives of garamine (67) [17-20] and 6-*O*-α-D-glycosyl derivatives of gentamine C_{1a} (25) and gentamine C_1 (27) [21,22] have confirmed these findings for the free bases. In addition 4-*O*-β-D-glycosyl derivatives of garamine (67) [19,20] have been shown to adopt rotamer *c* about the O–C-4 glycosidic bond, while 6-*O*-β-D-glycosyl derivatives of gentamine C_1 (27) [21,22] have been shown to adopt rotamer *d* about the O–C-6 glycosidic bond for the free bases.

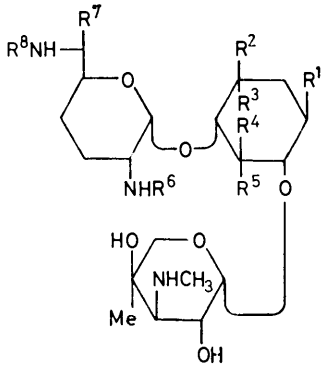
Recently Nagabhushan and Daniels [23] have exploited the fact that the chemical shifts of C-4, C-1′ and C-6, C-1″ are effected substantially differently on protonation of the amino-groups, to determine the anomeric absolute configuration of a 4-*O*-, or 6-*O*-axially linked deoxystreptamine

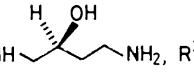


(1) $R^1 = R^2 = R^5 = R^6 = H$, $R^3 = R^4 = OH$ (kanamycin A)
(2) $R^1 = R^2 = R^4 = R^5 = R^6 = H$, $R^3 = NH_2$ (tobramycin)
(3) $R^1 = R^2 = R^5 = R^6 = H$, $R^3 = NH_2$, $R^4 = OH$ (kanamycin B)
(4) $R^1 =$  $R^2 = R^5 = R^6 = H$, $R^3 = R^4 = OH$ (amikacin)
(5) $R^1 = Ac$, $R^2 = R^5 = R^6 = H$, $R^3 = R^4 = OH$
(6) $R^1 = R^5 = R^6 = H$, $R^2 =$  $R^3 = R^4 = OH$
(7) $R^1 = R^2 = R^6 = H$, $R^3 = R^4 = OH$, $R^5 = Ac$
(8) $R^1 = R^2 = R^6 = H$, $R^3 = R^4 = OH$, $R^5 =$ 
(9) $R^1 = CO_2CH_2CH_2NH_2$, $R^2 = R^5 = R^6 = H$, $R^3 = R^4 = OH$
(10) $R^1 = R^2 = R^5 = H$, $R^3 = R^4 = OH$, $R^6 =$ 

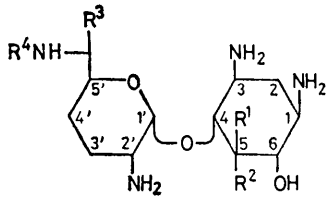


(11)

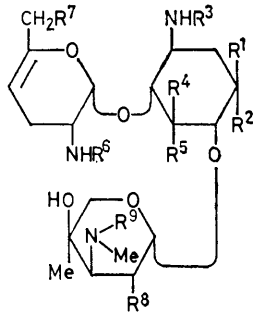


(12) $R^1 = R^2 = NH_2$, $R^3 = R^5 = R^6 = R^7 = R^8 = H$, $R^4 = OH$ (gentamicin C_{1a})
(13) $R^1 = R^2 = NH_2$, $R^3 = R^5 = R^6 = R^8 = H$, $R^4 = OH$, $R^7 = Me$ (gentamicin C_2)
(14) $R^1 = R^2 = NH_2$, $R^3 = R^5 = R^6 = H$, $R^4 = OH$, $R^7 = R^8 = Me$ (gentamicin C_1)
(15) $R^1 = NH_2$, $R^2 = R^4 = OH$, $R^3 = R^5 = R^6 = R^7 = R^8 = H$
(16) $R^1 = NH_2$, $R^2 = R^5 = R^6 = R^7 = R^8 = H$, $R^3 = R^4 = OH$
(17) $R^1 = R^3 = NH_2$, $R^2 = R^5 = R^6 = R^7 = R^8 = H$, $R^4 = OH$
(18) $R^1 = R^2 = NH_2$, $R^3 = R^4 = R^6 = R^7 = R^8 = H$, $R^5 = OH$
(19) $R^1 = R^2 = NH_2$, $R^3 = R^4 = R^6 = H$, $R^5 = OH$, $R^7 = R^8 = Me$
(20) $R^1 = R^2 = R^5 = NH_2$, $R^3 = R^4 = R^6 = H$, $R^7 = R^8 = Me$
(21) $R^1 = R^2 = NH_2$, $R^3 = R^4 = R^5 = R^6 = R^8 = H$, $R^7 = CH_3$
(22) $R^1 = R^2 = NH_2$, $R^3 = R^5 = R^8 = H$, $R^4 = OH$, $R^6 = Ac$ $R^7 = Me$
(23) $R^1 = R^4 = OH$, $R^2 = NH_2$, $R^3 = R^5 = R^6 = H$, $R^7 = R^8 = Me$
(24) $R^1 =$  $R^2 = NH_2$, $R^3 = R^5 = R^6 = H$, $R^4 = OH$, $R^7 = R^8 = Me$

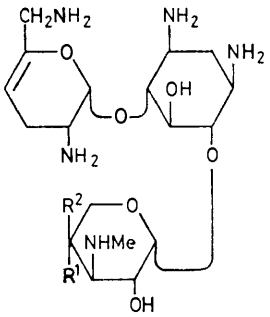
glycopyranoside. These differences were thought to arise mainly from differences in the stereochemistry about the two glycosidic oxygen atoms.[23] The above empirical rule has been successfully used to determine the absolute configuration of xylose in gentamicin A_2 (70).[24] Similar considerations have been used by Lemieux in assigning the position of attachment of the deoxystreptamine moiety in apramycin.[25] Tori [26] has recently used the glycosidation shifts in the ^{13}C n.m.r. spectra of variety of α- and β-D-glucopyranosides to determine the absolute configuration of the hydroxy-group in chiral secondary alcohols. It was evident from work carried



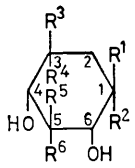
(25) R^1 = OH, R^2 = R^3 = R^4 = H (gentamine C_{1a})
(26) R^1 OH, R^2 = R^4 = H, R^3 = Me (gentamine C_2)
(27) R^1 = OH, R^2 = H, R^3 = R^4 = Me (gentamine C_1)
(28) R^1 = H, R^2 = OH, R^3 = R^4 = Me



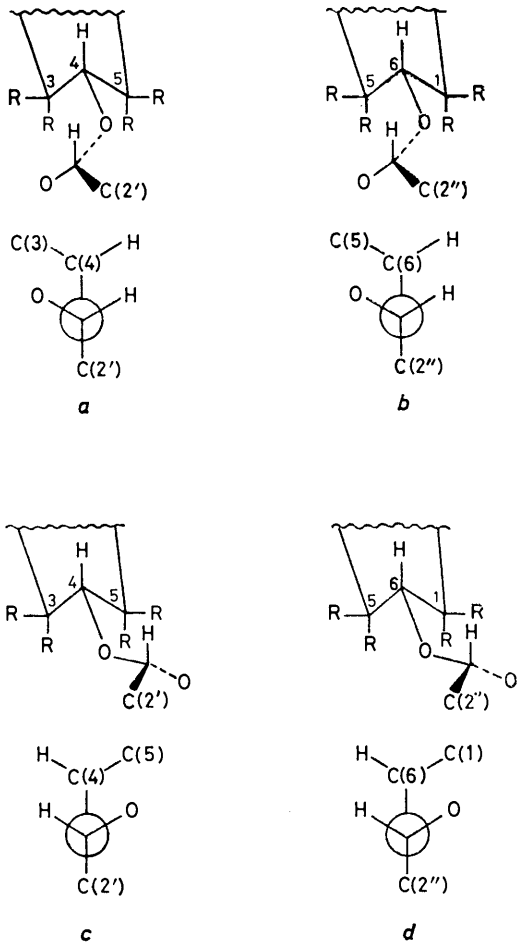
(29) R^1 = R^7 = NH_2, R^2 = R^3 = R^5 = R^6 = R^9 = H, R^4 = R^8 = OH (sisomicin)
(30) R^1 = R^7 = NH_2, R^2 = R^5 = R^6 = R^9 = H, R^3 = Ac, R^4 = R^8 = OH
(31) R^1 = NH_2, R^2 = R^3 = R^5 = R^6 = R^9 = H, R^4 = R^8 = OH, R^7 = NHAc
(32) R^1 = NH_2, R^2 = R^3 = R^5 = R^6 = R^9 = H, R^4 = R^7 = R^8 = OH
(33) R^1 = R^7 = NH_2, R^2 = R^3 = R^4 = R^6 = R^9 = H, R^5 = R^8 = OH
(34) R^1 = R^7 = NH_2, R^2 = R^3 = R^4 = R^5 = R^6 = R^9 = H, R^8 = OH
(35) R^1 = R^4 = R^7 = NH_2, R^2 = R^3 = R^5 = R^6 = R^9 = H, R^8 = OH
(36) R^1 = R^5 = R^7 = NH_2, R^2 = R^3 = R^4 = R^6 = R^9 = H, R^8 = OH
(37) R^1 = R^7 = NH_2, R^2 = R^3 = R^5 = R^9 = H, R^4 = R^8 = OH, R^6 = Ac
(38) R^1 = R^4 = R^8 = OH, R^2 = R^3 = R^5 = R^6 = R^9 = H, R^7 = NH_2
(39) R^1 = R^3 = R^5 = R^6 = R^9 = H, R^2 = R^4 = R^8 = OH, R^7 = NH_2
(40) R^1 = R^3 = R^5 = R^6 = R^9 = H, R^2 = R^7 = NH_2, R^4 = R^8 = OH
(41) R^1 = NHEt, R^2 = R^3 = R^5 = R^6 = R^9 = H, R^4 = R^8 = OH, R^7 = NH_2
(42) R^1 = R^3 = R^5 = R^6 = R^9 = H, R^2 = NHEt, R^4 = R^8 = OH, R^7 = NH_2
(43) R^1 = NHAc, R^2 = R^3 = R^5 = R^6 = R^9 = H, R^4 = R^8 = OH, R^7 = NH_2
(44) R^1 = $NHCO_2CH_2CH_2NH_2$, R^2 = R^3 = R^5 = R^6 = R^9 = H, R^4 = R^8 = OH, R^7 = NH_2
(45) R^1 = $NHCO_2Me$, R^2 = R^3 = R^5 = R^6 = R^9 = H, R^4 = R^8 = OH, R^7 = NH_2
(46) R^1 = $NHCO_2CH_2Me$, R^2 = R^3 = R^5 = R^6 = R^9 = H, R^4 = R^8 = OH, R^7 = NH_2
(47) R^1 = R^7 = NH_2, R^2 = R^3 = R^5 = R^6 = R^8 = R^9 = H, R^4 = OH
(48) R^1 = R^7 = NH_2, R^2 = R^3 = R^5 = R^6 = H, R^4 = R^8 = OH, R^7 = NH_2, R^9 = Ac



(49) R^1 = OH, R^2 = H (antibiotic 66-40B)
(50) R^1 = H, R^2 = OH (antibiotic 66-40D)



(51) R^1 = R^3 = NH_2, R^2 = R^4 = R^6 = H, R^5 = OH (2-deoxystreptamine)
(52) R^1 = NH_2, R^2 = R^4 = R^6 = H, R^3 = R^5 = OH
(53) R^1 = NH_2, R^2 = R^3 = R^6 = H, R^4 = R^5 = OH
(54) R^1 = R^4 = NH_2, R^2 = R^3 = R^6 = H, R^5 = OH
(55) R^1 = NH_2, R^2 = R^4 = R^6 = H, R^3 = NHAc, R^5 = OH
(56) R^1 = R^3 = NH_2, R^2 = R^4 = R^5 = H, R^6 = OH
(57) R^1 = R^3 = R^6 = NH_2, R^2 = R^4 = R^5 = H
(58) R^1 = R^3 = NH_2, R^2 = R^4 = R^5 = R^6 = H
(59) R^1 = R^5 = OH, R^2 = R^4 = R^6 = H, R^3 = NH_2
(60) R^1 = NHAc, R^2 = R^4 = R^6 = H, R^3 = NH_2, R^5 = OH
(61) R^1 = $NHCO_2CH_2CH_2NH_2$, R^2 = R^4 = R^6 = H, R^3 = NH_2, R^5 = OH
(62) R^1 = R^3 = R^5 = NH_2, R^2 = R^4 = R^6 = H
(63) R^1 = R^4 = R^6 = H, R^2 = R^5 = OH, R^3 = NH_2
(64) R^1 = R^4 = R^6 = H, R^2 = R^3 = NH_2, R^5 = OH
(65) R^1 = NHEt, R^2 = R^4 = R^6 = H, R^3 = NH_2, R^5 = OH
(66) R^1 = R^4 = R^6 = H, R^2 = NHEt, R^3 = NH_2, R^5 = OH

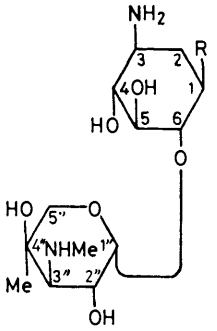


out in these laboratories that sisomicin (29),[27] antibiotic 66-40 B (49),[15] antibiotic 66-40 D (50),[15] 5-*epi*-aminoglycosides,[28,29], 1-*epi*-aminoglycosides,[30] 1-deamino-1-hydroxyaminoglycosides,[30] 1-deamino-1-*epi*-hydroxyaminoglycosides,[30] 3-deamino-3-hydroxyaminoglycosides,[30] 3-deamino-3-*epi*-hydroxyaminglycosides,[30] 1-NHCOR-substituted aminoglycosides,[1] 3-NHCOR-substituted aminoglycosides, and 6′-NHCOR-substituted aminoglycosides gave appreciably different protonation shifts from those of the empirical 'Nagabhushan-Daniels Rule.'[23] Naito[31] recently showed that the 'Nagabhushan–Daniels Rule'[23] could not be successfully applied to 1-*N*-(*S*)-HABA (4) and 1-*N*-acetyl (5) derivatives of kanamycin A and the anomalies in these and other 1-*N*-acyl derivatives were erroneously ascribed to δ effects produced by acylation of the amino-groups. From the chemical-shift values reported for kanamycin A (1)[31] it is evident that the sample was partially carbonated * which renders the $\Delta\delta_C$ values unreliable.

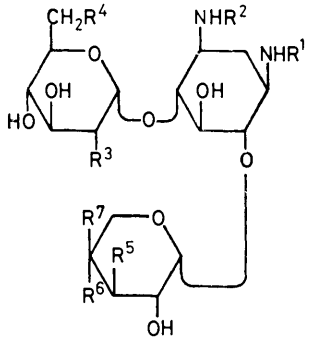
* Aminoglycoside antibiotics, owing to their strongly basic character, readily absorb carbon dioxide from the atmosphere to give carbonated species. Throughout this conformational study considerable care has been exercised to ensure that all free-base data were recorded on fully decarbonated samples as the chemical shifts of C-1′, C-4, and C-6 are all highly susceptible to carbonation shifts.

Nagabhusan has published the ^{13}C n.m.r. data for 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] (HABA) (75) and 1-*N*-[(*S*)-3-amino-2-hydroxypropionyl] (HAPA) derivatives (76) of gentamicin B,[32] but it should be noted that both samples of gentamicin B (77) and kanamycin A (1) were partially carbonated and owing to the similarity in the chemical shifts for the anomeric signals for C-1′ and C-1″ in (75) and (76), these could not be unambiguously assigned with the data available at that time, and were in fact misassigned.[1]

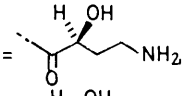
A detailed analysis of the wealth of ^{13}C n.m.r. data available to us in these laboratories has enabled us to explain the above anomalies as well as gain new insights into the solution conformations of aminoglycoside antibiotics at both basic and acidic pH where important differences are observed. Throughout the discussions that follow it should be bourne in mind that in all cases we expect the *exo*-anomeric effect to define the normal torsion angles about the O–C-1′ and O–C-1″ bonds.[6-11] We shall concern ourselves solely with changes that are occurring in the torsion angles about the O–C-4 and O–C-6 bonds. It is obvious that precise torsion angles cannot be determined from the data that we have and all rotamer diagrams in this and previous studies represent only approximations that satisfactorily account for the observed C^{13} n.m.r. data. In order to analyse the data successfully we have avoided using the protonation shifts [$\Delta\delta_C$ (Base ⟶ H^+)] in going from the *pseudo* di- or trisaccharide bases to pH 1,[23,24] as we felt that there was already sufficient evidence to suggest that the conformations were different at acidic pH.[12] We therefore analysed separately the $\Delta\delta_C$ (deoxystreptamine ⟶ trisaccharide) values for the free bases and also the $\Delta\delta_C$ (deoxystreptamine ⟶ trisaccharide values at *ca.* pH 1). In so doing we were able to eliminate all β-protonation effects at C-4 and C-6 leaving us free to observe clearly the changes induced by rotation of the glycoside units about the O–C-4 and O–C-6 glycosidic bonds. In studying the chemical shifts of C-1′ we were only able to determine the $\Delta\delta_C$ values in going from the free base to pH 1 and these values therefore reflect rotational changes that occur about the O–C-4 glycosidic bond, as well as protonation shifts in those aminoglycosides that have 2′-amino-groups. The protonation shifts at C-1′ for 2′-amino-derivatives and for 6′-amino-derivatives (α-D, $^4C_1$ conformation) were determined from model monosaccharides and when subtracted from the $\Delta\delta_C$ values above, gave the rotational contributions at C-1′ accompanying protonation. In those instances where the 4-*O*-glycoside contained no basic groups capable of being protonated, the $\Delta\delta_C$ values for C-1′ reflect the rotational changes about the O–C-4 glycosidic bond following protonation of the molecule. Similarly in studying the chemical shifts of C-1″ we were only able to determine the $\Delta\delta_C$ values in going from the free base to pH 1 and these values therefore reflect rotational changes that occur about the O–C-6 glycosidic bond, as well as protonation shifts resulting from the presence of the 3″-amino-group. The protonation shifts at C-1″ accompanying proton-

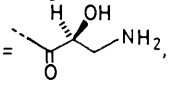


(67) R = NH_2 (garamine)
(68) R = OH
(69) R = $NHCO_2CH_2CH_2NH_2$



(70) $R^1 = R^2 = R^7 = H$, $R^3 = NH_2$, $R^4 = R^5 = R^6 = OH$ (gentamicin A_2)
(71) $R^1 = R^2 = R^7 = H$, $R^3 = NH_2$, $R^4 = R^6 = OH$, $R^5 = NHMe$ (gentamicin A)
(72) $R^1 = R^2 = R^6 = H$, $R^3 = NH_2$, $R^4 = R^7 = OH$, $R^5 = NHMe$ (gentamicin A_1)
(73) $R^1 = R^2 = R^7 = H$ $R^3 = NH_2$, $R^4 = R^6 = OH$, $R^5 = N(Me)CHO$ (gentamicin A_4)
(74) $R^1 = R^2 = R^6 = H$, $R^3 = R^7 = OH$, $R^4 = NH_2$, $R^5 = NHMe$ (gentamicin A_3)

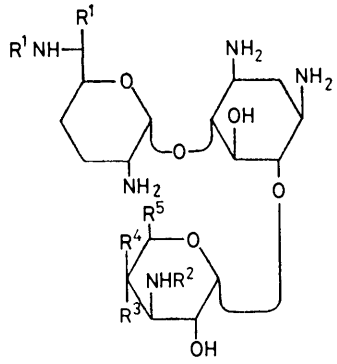
(75) $R^1 =$  $R^2 = H$, $R^3 = R^7 = OH$, $R^4 = NH_2$, $R^5 = NHMe$, $R^6 = Me$

(76) $R^1 =$  $R^2 = H$, $R^3 = R^7 = OH$, $R^4 = NH_2$, $R^5 = NHMe$, $R^6 = Me$

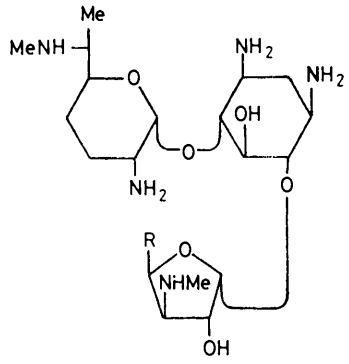
(77) $R^1 = R^2 = H$, $R^3 = R^7 = OH$, $R^4 = NH_2$, $R^5 = NHMe$, $R^6 = Me$ (gentamicin B)
(78) $R^1 = CO_2CH_2CH_2NH_2$, $R^2 = H$, $R^3 = R^7 = OH$, $R^4 = NH_2$, $R^5 = NHMe$, $R^6 = Me$

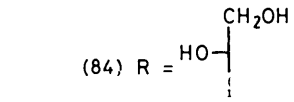
ation of the 3″-amino-group were determined from model monosaccharides and when subtracted from the above $\Delta\delta_C$ values, gave the rotational contributions at C-1″. The ^{13}C n.m.r. parameters for compounds not previously reported in the literature are given in Tables 1 and 2. The $\Delta\delta_C$ values in going from deoxystreptamine to the di- or tri-saccharides at basic and acidic pH are given in Table 3 along with the δ_C and $\Delta\delta_C$ values for C-1′ and C-1″. The rotational and in some cases protonation shifts for various structural types of aminoglycosides are summarized for C-4 and C-1′ in Table 4 and for C-6 and C-1″ in Table 5. For comparison purposes the δ_C and $\Delta\delta_C$ values for several cyclohexyl α- and β-D-glucopyranosides are summarized in Table 6.

Methylation of *trans*-4-t-butylcyclohexanol to give (112) results in deshielding of C-1 by + 8.9 (Table 6). On the other hand glycosylation of cyclohexanol (axial α-D-glycoside; $^4C_1$ conformation) results in varying deshielding of C-1 depending on the presence, or absence of substituents at C-2 and, or C-6 (Table 6).[6,33,34] These $\Delta\delta_C$ values for C-1 reflect a deshielding (+ve) contribution due to the glycosylation effect, coupled with a varying shielding effect (−ve) due to interaction between the C-1′–O-5′ and C-1–H-1 bonds [35–38] as the rotamer about the O–C-1 glycosidic bond changes as substituents are introduced at C-2 and C-6. In the unsubstituted cyclohexyl glycoside (104) a rotamer intermediate between *e* and *f* is adopted which causes a moderate shielding component C-1′–O-5′/C-1–H-1 interaction) to be introduced, resulting in a net deshielding of +7.3 at C-1 upon glycosylation. Introduction of an equatorial methyl substituent at C-2 in (105) results in a steric interaction which forces the glycoside to rotate counterclockwise about the O–C-1 bond thereby reducing the shielding component (C-1′–O-5/C-1–H-1 interaction) and producing a greater net deshielding of +9.0. In this case the rotamer about the O–C-1 bond is close to that represented by *e*. Introduction of an equatorial methyl group at C-6 as in (106) results in a marked clockwise rotation about the O–C-1 glycosidic bond to a point where a considerable shielding component (C-1′–O-5′/C-1–H-1 interaction) is introduced resulting in a greatly reduced deshielding at C-1 of only +3.9. In this instance the rotamer about the O–C-1 glycosidic bond is approaching that represented by rotamer *f*. Lemieux [6] did not publish sufficient data to analyse the 2,6-dimethyl-substituted derivative (107), but it is clear from Tori's data [26] on similar disubstituted neutral glycosides that the predominant steric effect would be expected to arise from the 2-methyl substituent, not the



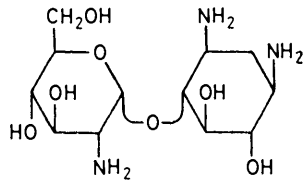
(79) $R^1 = R^2 = Me$, $R^3 = OH$, $R^4 = H$, $R^5 = CH_2OH$
(80) $R^1 = R^2 = R^4 = H$, $R^3 = OH$, $R^5 = CH_2OH$
(81) $R^1 = R^2 = Me$, $R^3 = OH$, $R^4 = R^5 = OH$
(82) $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = OH$,
(83) $R^1 = R^3 = R^5 = H$, $R^2 = Me$, $R^4 = OH$



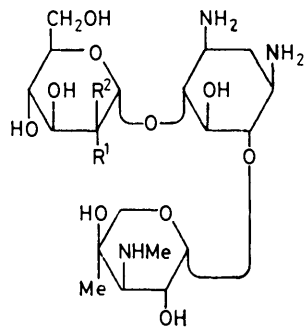


(84) R =

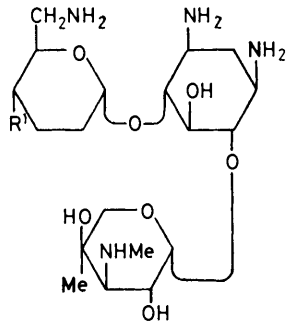
(85) $R = CH_2OH$



(86)



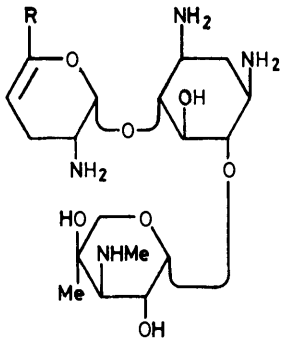
(87) $R^1 = H$, $R^2 = NH_2$
(88) $R^1 = OH$, $R^2 = H$



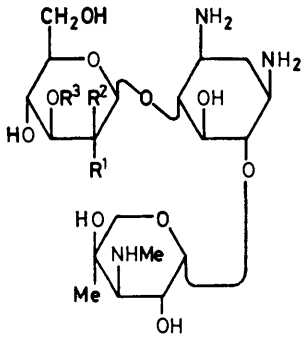
(89) $R^1 = OH$
(90) $R^1 = H$

6-methyl substituent in cases where both were present, leading to a rotamer of the type represented by *e*.

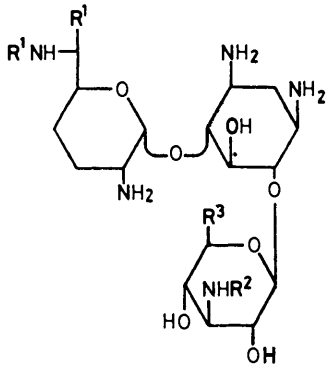
The β-cyclohexyl glycosides (equatorial β-D-glycoside; $^4C_1$ conformation) on the other hand adopt a different set of rotamers (Table 6).[6,33,34] Both the unsubstituted derivative (108) and the 6-equatorial methyl-substituted derivative (110) show a large deshielding on glycosylation (+9.3 and +10.3 respectively) suggesting that they exist as rotamers close to that represented by *g*. Both (110) and, to a greater extent, (108) appear to exist as rotamers in which slight counterclockwise rotation about the O–C-1 glycosidic bond has occurred relative to rotamer *g* as evidenced by a slight shielding component at C-1. Introduction of an equatorial methyl group at C-2 results in a steric interaction with the glycoside which produces a moderate counterclockwise rotation about the



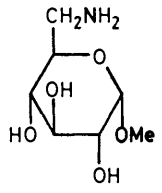
(91) R = CHO
(92) R = $CONH_2$



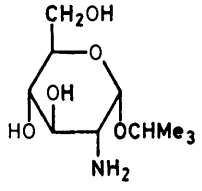
(93) $R^1 = NH_2$, $R^2 = R^3 = H$
(94) $R^1 = H$, $R^2 = NH_2$, $R^3 = Me$



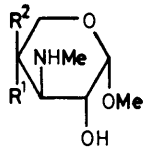
(95) $R^1 = Me$, $R^2 = H$, $R^3 = CH_2OH$
(96) $R^1 = R^2 = R^3 = H$
(97) $R^1 = R^2 = Me$, $R^3 = H$



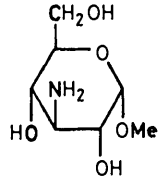
(98)



(99)



(100) $R^1 = Me$, $R^2 = OH$
(101) $R^1 = OH$, $R^2 = H$
(102) $R^1 = H$, $R^2 = OH$



(103)

O–C-1 glycosidic bond in (109) relative to rotamer *g*, which leads to the introduction of a moderate shielding component (C-1′–O-5′/C-1–H-1 interaction) which results in a net reduction of the deshielding at C-1 to +7.0. Once again complete data are not available for the 2,6-dimethylcyclohexyl glycoside (111).[6]

We shall now turn our attention to the aminoglycoside antibiotics which represent a more complex situation than encountered with the neutral glycosides discussed above.[6,26] These molecules invariably contain polar functional groups (hydroxy- and amino-groups) at C-3, C-5, C-6, C-2′, C-6′, and C-2″. We therefore have different steric interactions to consider relative to those of methyl substituted glycosides.[6,26] We also have to

Table 1

^{13}C N.m.r. chemical shifts (δ_C p.p.m. downfield from tetramethylsilane in D_2O)

Carbon	(12)H+ [a]	(25)H+	(67)H+ [b]	(14)H+ [a]	(27)H+	(88) [c]	(88)H+ [c]	(18) [d]	(18)H+ [d]	(19) [d]	(19)H+ [d]	(28) [d]	(28)H+ [d]	(20) [d]	(20)H+ [d]	(21) [e]	(21)H+ [e]
C-1	50.5	50.7	50.9	50.4	50.7	51.5	50.6	48.2	48.3	48.1	48.2	48.5	49.1	48.0	48.4	53.6	52.1
C-2	28.5	29.0	28.5	28.4	29.0	36.4	28.8	36.7	28.7	36.6	28.7	36.8	28.9	36.8	28.5	36.8	29.1
C-3	49.4	49.6	50.7	49.4	49.7	49.8	49.2	47.4	47.8	47.4	47.7	47.7	48.0	47.3	47.4	52.7	51.8
C-4	77.3	77.4	73.3	77.3	77.6	88.8	81.6	79.7	73.1	79.8	73.2	79.9	73.3	79.0	70.9	78.1	70.7
C-5	75.2	76.0	74.6	75.2	76.1	74.9	73.7	68.6	65.7	68.5	65.7	69.2	66.8	50.6	50.4	35.0	32.8
C-6	84.3	73.3	84.0	84.3	73.3	87.3	83.8	85.8	80.8	85.9	81.0	76.3	70.7	85.8	77.4	83.8	78.4
C-1′	95.6	95.5		95.7	95.9	100.5	99.5	96.3	90.4	96.7	90.8	96.9	90.8	95.9	91.9	96.8	90.7
C-2′	49.5	49.6		49.5	49.7	76.6	72.0	50.3	49.0	50.2	49.0	50.3	49.1	50.4	49.0	50.1	49.0
C-3′	21.2	21.3		21.3	21.4	73.9	73.9	27.3	21.7	27.2	21.7	26.9	21.7	27.3	21.7	27.0	22.0
C-4′	26.2	26.4		23.3	23.4	70.4	70.4	28.3	26.4	25.6	23.4	25.6	23.5	25.7	23.1	25.8	23.7
C-5′	66.8	66.7		70.0	70.0	73.5	73.4	71.2	66.6	72.7	70.1	72.5	70.0	72.8	70.2	74.0	69.5
C-6′	43.5	43.6		58.5	58.6	61.5	61.5	45.8	43.6	57.9	58.5	57.9	58.5	57.8	58.4	49.8	50.6
C-7′				10.9	11.0					14.4	10.5	14.2	10.5	14.7	10.8	18.5	13.3
6′-NCH_3				32.1	32.1					33.3	32.0	33.2	32.0	33.5	32.2		
C-1″	101.9		101.8	101.9		101.3	101.8	102.5	101.2	102.6	101.4			102.3	102.2	101.9	100.4
C-2″	67.1		67.1	67.0		70.2	67.2	70.2	67.0	70.3	66.9			70.1	66.5	70.1	67.1
C-3″	64.1		64.3	64.1		64.2	64.2	64.0	64.5	64.0	64.5			64.1	63.8	64.2	64.7
C-4″	70.8		70.8	70.8		73.2	70.8	73.2	70.8	73.2	70.7			73.2	70.6	73.2	70.8
C-5″	68.7		68.5	68.7		68.5	68.5	68.6	68.5	68.7	68.5			68.6	69.2	68.3	68.0
3″-NCH_3	35.5		35.4	35.5		37.7	35.5	37.8	36.0	37.7	35.7			37.8	35.5	37.8	36.0
4″-CH_3	21.9		21.8	21.9		22.5	21.8	22.5	21.7	22.3	21.7			22.5	22.0	22.4	22.0

Carbon	(89) [c]	(89)H+ [c]	(90) [c]	(90)H+ [c]	(22) [c]	(22)H+ [c]	(24) [a]	(24)H+ [a]	(49)H+ [f]	(30) [e]	(30)H+ [e]	(31) [e]	(31)H+ [e]	(32) [b]	(32)H+ [b]
C-1	51.6	50.9	51.6	51.0	51.2	50.7	50.1	49.3	50.7	51.6	51.3	51.7	50.7	51.7	50.7
C-2	36.4	28.4	36.5	29.7	36.4	28.5	35.3	31.5	30.5	34.7	30.3	36.3	28.3	35.8	28.3
C-3	50.1	49.5	50.1	49.7	50.8	50.0	50.3	49.7	49.1	48.8	47.8	50.3	49.2	50.1	49.2
C-4	86.1	79.3	86.1	79.5	89.4	78.5	87.4	78.2	79.8	80.4	78.9	85.8	80.3	84.9	80.1
C-5	75.6	75.1	75.6	75.2	75.1	75.2	75.6	75.5	74.3	75.9	75.7	75.3	74.2	75.3	74.2
C-6	88.0	84.7	88.0	84.9	88.1	84.5	80.4	80.5	84.0	87.4	84.6	87.8	83.9	87.5	83.9
C-1′	98.8	98.2	100.2	99.3	100.3	97.8	102.6	96.1	97.9	99.6	94.9	101.0	97.8	100.4	97.5
C-2′	26.7	25.9	28.3	27.5	50.0	49.0	50.7	49.8	47.0	47.4	47.2	47.3	47.2	47.3	47.3
C-3′	29.3	29.3	17.6	16.7	23.9	23.8	26.8	23.2	24.1	25.2	21.1	25.5	23.8	25.3	23.5
C-4′	67.7	67.2	30.3	29.7	25.2	22.9	25.6	21.4	101.5	96.7	101.6	98.4	95.6	99.5	98.0
C-5′	75.7	71.9	72.1	67.8	74.8	69.8	72.6	70.4	144.1	150.2	143.2	146.3	147.9	148.8	149.8
C-6′	42.6	41.1	46.1	43.8	50.5	50.0	58.1	58.6	41.5	43.4	41.8	41.9	41.3	62.1	61.8
C-7′					18.6	13.8	14.3	10.8							
6′-NCH_3							33.2	32.0							
C-1″	101.6	101.9	101.6	101.9	101.2	101.8	99.3	98.8	101.5	101.5	101.9	101.4	101.9	101.3	101.8
C-2″	70.2	67.0	70.2	67.8	70.0	67.1	69.8	66.8	67.1	70.2	67.2	70.1	67.2	69.6	67.2
C-3″	64.2	64.3	64.2	64.3	64.2	64.1	64.2	65.0	61.5	64.2	64.2	64.2	64.2	64.3	64.2
C-4″	73.2	70.8	73.2	70.8	73.1	70.8	73.2	70.7	64.3	73.2	70.8	73.2	70.8	72.8	70.8
C-5″	68.5	68.5	68.5	68.5	68.6	68.6	68.7	67.9	63.1	68.5	68.6	68.5	68.6	68.4	68.6
3″-NCH_3	37.7	35.4	37.7	35.4	37.8	35.5	38.0	35.8	30.5	37.7	35.4	37.8	35.5	37.4	35.5
4″-CH_3	22.4	21.7	22.4	21.8	22.6	21.9	22.4	21.8		22.3	21.8	22.6	21.9	22.4	21.9
C-1‴					174.0	174.4	177.4	176.4		174.2	174.6	174.6	175.1		
C-2‴					22.9	22.6	70.7	70.1		22.9	22.9	22.8	22.9		
C-3‴							36.5	32.0							
C-4‴							37.7	35.8							

Carbon	(33) [d]	(33)H+ [d]	(34) [d]	(34)H+ [d]	(35) [d]	(35)H+ [d]	(36) [d]	(36)H+ [d]	(37) [e]	(37)H+ [e]	(43) [e]	(43)H+ [e]	(47) [e]	(47)H+ [e]	(48)	(48)H+	(113) [b]	(113)H+ [b]
C-1	48.1	48.2	53.5	52.1	52.0	50.2	47.9	48.2	51.5	50.8	50.1	49.3	51.4	49.9	51.7	50.9	51.3	50.8
C-2	36.4	30.2	36.5	28.9	36.9	28.1	36.5	28.2	36.3	28.2	35.0	31.0	36.4	28.3	36.4	28.3	36.5	28.7
C-3	47.2	47.2	52.3	51.3	50.6	49.7	46.9	46.7	50.2	49.4	49.9	49.4	50.2	49.0	50.2	49.1	50.3	49.4
C-4	80.9	77.3	78.9	73.5	86.4	77.1	80.3	73.1	86.3	79.4	84.8	80.6	85.3	80.0	85.3	79.8	85.6	80.1
C-5	69.7	68.0	35.5	33.9	57.1	55.9	51.5	51.9	75.2	74.5	75.6	74.6	75.6	74.4	75.5	74.5	77.0	75.2
C-6	85.8	81.6	83.6	78.3	89.6	80.0	85.8	77.1	88.0	83.9	81.9	80.8	86.7	81.9	87.9	83.8	78.7	72.9
C-1′	97.1	94.1	97.4	93.3	100.6	98.2	97.0	95.3	98.4	99.1	100.9	97.9	100.8	97.9	100.8	97.9	100.8	97.2
C-2′	47.1	46.9	46.9	46.9	47.5	46.5	47.2	47.3	46.9	46.5	47.4	47.0	47.4	47.0	47.4	47.0	47.4	47.3
C-3′	25.6	23.9	25.5	23.2	26.2	25.3	25.7	24.9	23.0	25.2	25.4	24.0	25.5	23.9	25.5	24.0	25.5	23.3
C-4′	97.1	101.5	97.2	100.6	97.2	102.8	96.5	102.5	96.2	102.9	96.8	101.5	97.0	101.5	96.8	101.5	99.5	98.0
C-5′	150.3	144.1	149.7		149.8	144.6	150.4	144.5	150.8	143.5	150.7	144.3	150.0	144.0	150.2	144.1	148.8	149.7
C-6′	43.2	41.4	43.1	41.4	43.1	41.4	43.3	41.4	43.4	41.9	43.3	41.5	43.2	41.5	43.3	41.5	62.1	61.8
C-1″	102.5	101.5	101.9	101.4	101.9	101.1	102.4	102.4	101.2	101.7	99.9	99.3	100.2	98.7	101.6	102.8		
C-2″	70.3	67.1	70.1	67.1	70.0	66.1	70.2	66.5	70.1	67.4	69.8	66.8	31.2 [g]	28.6 [g]	69.7	70.4		
C-3″	64.0	64.7	64.2	64.7	64.2	63.5	64.1	63.9	64.2	64.2	64.2	64.9	57.8	58.4	56.7	56.8		
C-4″	73.3	70.8	73.2	70.8	73.0	70.1	73.2	70.7	73.2	70.9	73.1	70.8	70.4	68.3	74.8	74.2		
C-5″	68.5	68.3	68.2	68.0	68.8	69.1	68.7	69.1	68.6	68.5	68.7	67.8	69.2	67.9	65.7	65.6		
3″-NCH_3	37.7	35.8	37.7	35.8	37.6	35.2	37.7	35.6	37.8	35.7	37.7	35.8	33.4 [g]	31.5 [g]	32.9	33.1		
4″-CH_3	22.4	21.8	22.3	21.7	22.5	21.9	22.5	21.8	22.6	22.0	22.6	21.8	22.5	21.8	21.6	21.4		
C-1‴									174.5	175.9	174.8	175.1			177.2	177.7		
C-2‴									22.9	22.3	23.0	23.1			22.3	22.1		

[a] P. J. L. Daniels, unpublished observations. [b] T. L. Nagabhushan, unpublished observations. [c] Ref. 40. [d] D. F. Rane and P. J. L. Daniels, unpublished observations. [e] S. W. McCombie, unpublished observations. [f] Data published earlier at pH 4 (ref. 15) indicated that the material was not fully protonated at that pH. Data were therefore recorded at pH 1 and are cited above. The assignments for C-1′ and C-4′ in(49) and (50) used for Table 1 were the corrected values (ref. 15; D. H. Davies, A. K. Mallams, J. McGlotten, and R. W. Tkach, *J. Chem. Soc., Perkin Trans. 1*, 1977, 1407). [g] Values may be interchanged in any vertical column.

Table 2

^{13}C N.m.r. chemical shifts (δ_C p.p.m. downfield from tetramethylsilane in D_2O)

Carbon	(51) [a]	(51)H+	(52) [b]	(52)H+ [b]	(53) [c]	(53)H+ [c]	(54) [d]	(54)H+ [d]	(55) [e]	(55)H+ [e]	(56) [f]	(56)H+ [f]	(57) [f]	(57)H+ [f]	(58) [f]	(58)H+ [f]	(62) [f]	(62)H+ [f]
C-1	51.6	51.0	50.3	50.8	49.0	50.5	50.4	48.2	51.1	51.5	48.6	49.4	48.2	49.0	54.5	53.8	52.2	51.6
C-2	37.0	29.0	36.8	33.2	35.5	32.0	33.9	26.9	35.2	31.1	36.7	28.9	37.3	28.5	37.2	29.3	37.0	28.7
C-3	51.6	51.0	70.3	69.5	69.3	68.3	49.8	50.5	50.8	50.1	48.6	49.4	48.2	49.0	54.5	53.8	52.2	51.6
C-4	78.5	73.2	77.8	77.0	74.7	74.1	76.1	69.2	75.5	74.6	76.0	70.7	76.2	67.0	73.8	68.4	78.8	69.5
C-5	76.6	75.5	75.8	75.2	73.9	73.7	72.1	72.2	76.7	76.1	73.9	72.3	56.2	56.4	40.3	39.6	58.4	57.9
C-6	78.5	73.2	78.0	73.7	78.9	73.9	74.8	68.9	78.1	73.5	76.0	70.7	76.2	67.0	73.8	68.4	78.8	69.5
C-1′									174.7	174.9								
C-2′									22.9	22.9								

Carbon	(98)	(98)H+	(99)	(99)H+	(100) [g]	(100)H+ [g]	(101) [h]	(101)H+	(102) [h]	(102)H+
C-1	100.0	100.1	98.3	94.2	100.5	99.4	99.9	99.1	100.2	99.5
C-2	72.2	72.2	55.8	54.8	69.8	66.8	70.3	67.0	68.2	65.8
C-3	73.9	73.5	75.2	70.8	64.3	65.3	62.7	62.4	59.2	59.6
C-4	72.1	71.8	70.9	70.4	73.1	70.8	68.4	64.8	63.9	63.1
C-5	72.8	68.3	72.9	72.8	67.9	67.0	62.3	61.8	65.3	63.4
C-6	42.3	41.4	61.6	61.2						
C-1′			71.9	72.2						
C-2′			21.7/23.4	21.6/23.3						
3-$NHCH_3$					37.9	36.0	34.1	31.1	32.6	30.9
4-CH_3					22.4	21.9				
1-OCH_3	55.8	56.3			56.0	56.2	56.0	56.1	56.0	56.1

[a] Ref. 14. [b] Values derived from (59) (ref. 30). [c] Values derived from (63) (ref. 30). [d] Values derived from (64) (ref. 30). [e] Values derived from (±)-form of (60) (ref. 1). [f] D. F. Rane and P. J. L. Daniels, unpublished observations. [g] Ref. 40. [h] Values for freshly decarbonated samples (ref. 15).

Table 3

$\Delta\delta_C$ Values (D_2O)

Carbon $\Delta\delta_C$	Gentamicin C_{1a} (12) [a]		Gentamine C_{1a} (25) [b]		Garamine (67) [b]		Gentamicin C_1 (14) [a]		Gentamine C_1 (27) [b]		*O*-(3-Deoxy-3-methylamino-α-D-glucopyranosyl)-(1→6)-gentamine C_1 (79) [a]		*O*-(3-Deoxy-3-amino-α-D-glucopyranosyl)-(1→6)-gentamine C_{1a} (80) [a]		*O*-(3-Deoxy-3-methylamino-α-D-xylopyranosyl)-(1→6)-gentamine C_1 (81) [a]	
	Base [14]	H^+	Base [14]	H^+	Base [17]	H^+	Base [14]	H^+	Base [14]	H^+	Base [21]	H^+ [21]	Base [21]	H^+ [21]	Base [23]	H^+ [23]
C-1	+0.1	−0.5	−0.3	−0.3	+0.1	−0.1	+0.2	−0.6	−0.3	−0.3	−0.4	−0.6	−0.5	−0.5	−0.1	−0.6
C-2	−0.3	−0.5	−0.2	0	−0.4	−0.5	−0.2	−0.6	−0.2	0	−0.5	−0.5	−0.7	−0.5	−0.5	−0.6
C-3	−1.0	−1.6	−1.1	−1.4	−0.2	−0.3	−0.7	−1.6	−0.8	−1.3	−1.1	−1.6	−1.0	−1.5	−1.1	−1.5
C-4	+9.8	+4.1	+9.6	+4.2	+0.3	+0.1	+10.1	+4.1	+10.3	+4.4	+9.8	+4.2	+10.5	+4.2	+9.8	+4.1
C-5	−1.2	−0.3	+0.2	+0.5	−1.5	−0.9	−1.2	−0.3	+0.2	+0.6	−1.3	−0.3	−1.2	−0.3	−1.5	−0.3
C-6	+9.3	+11.1	−0.2	+0.1	+9.4	+10.8	+9.4	+11.1	−0.1	+0.1	+10.3	+11.4	+8.8	+11.3	+9.5	+11.4
δ_C C-1′	102.2	95.6	102.1	95.5			102.6	95.7	102.8	95.9	102.7	96.0	101.6	95.7	102.6	95.9
C-1′ [u]		−6.6		−6.6				−6.9		−6.9		−6.7		−5.9		−6.7
δ_C C-1″	101.3	101.9			101.4	101.8	101.4	101.9			100.9	101.5	100.8	101.4	100.8	101.6
C-1″ [v]		+0.6				+0.4		+0.5				+0.6		+0.6		+0.8

Carbon $\Delta\delta_C$	*O*-(3-Deoxy-3-amino-α-D-xylopyranosyl)-(1→6)-gentamine C_{1a} (82) [a]		*O*-(3-Deoxy-3-methylamino-β-L-arabinopyranosyl)-(1→6)-gentamine C_{1a} (83) [a]		*O*-(3-Deoxy-3-methylamino-α-D-glucofuranosyl)-(1→6)-gentamine C_1 (84) [a]		*O*-(3-Deoxy-3-methylamino-α-*D*-xylofuranosyl (1→6)-gentamine C_1 (85) [a]		Gentamicin A (71) [a]		Gentamicin A_1 (72) [a]		Gentamicin A_2 (70) [a]		Gentamicin A_4 (73) [a]	
	Base [23]	H^+ [23]	Base [23]	H^+ [23]	Base [21]	H^+ [21]	Base [23]	H^+ [23]	Base [w]	H^+ [w]	Base [w]	H^+ [w]	Base [24]	H^+ [24]	Base [w]	H^+ [w]
C-1	−0.2	−0.5	−0.2	−0.6	−0.9	−1.0	−0.8	−1.0	−0.1	−0.7	−0.1	−0.2	−0.2	−0.4	−0.1	−0.6
C-2	−0.5	−0.6	−0.6	−0.6	−0.2	−0.4	−0.3	−0.3	−0.5	−0.5	−0.6	+0.5	−0.8	+0.4	−0.5	−0.4
C-3	−1.0	−1.6	−1.0	−1.5	−1.2	−1.6	−1.1	−1.5	−1.3	−1.4	−1.3	−1.1	−1.4	−1.3	−1.4	−1.4
C-4	+9.7	+4.1	+10.2	+4.4	+9.6	+4.3	+9.5	+4.1	+10.1	+7.7	+10.0	+7.7	+9.6	+8.7	+10.0	+7.9
C-5	−1.4	−0.4	−1.3	−0.4	−1.2	−0.4	−1.1	−0.4	−1.5	−1.0	−1.4	−1.1	−1.6	−0.8	−1.5	−1.0
C-6	+9.1	+11.3	+9.3	+11.2	+9.4	+9.1	+9.1	+8.9	+9.4	+11.0	+9.1	+10.6	+9.4	+11.2	+9.5	+11.0
δ_C C-1′	101.7	95.6	101.9	95.8	102.7	96.1	102.4	95.9	101.7	97.7	101.7	97.9	101.5	97.3	101.6	97.8
C-1′		−6.1		−6.1		−6.6		−6.5		−4.0		−3.8		−4.2		−3.8
δ_C C-1″	100.6	101.4	101.1	102.1	104.6	103.1	103.8	103.0	100.8	101.4	101.2	101.8	101.4	101.8	100.7	101.8
C-1″		+0.8		+1.0		−1.5		−0.8		+0.6		+0.6		+0.4		+1.1

Carbon $\Delta\delta_C$	Paromamine (86) [b]		*O*-(2-Deoxy-2-amino-α-D-mannopyranosyl)-(1→4)-garamine (87) [a]		*O*-(α-D-glucopyranosyl)-(1→4)-garamine (88) [a]		Gentamicin A_3 (74) [a]		Kanamycin A (1) [a]		Gentamicin B (77) [a]		3-Deamino-3-hydroxy-gentamicin C_{1a} (15) [c]		3-Deamino-3-*epi*-hydroxy-gentamicin C_{1a} (16) [d]	
	Base [w]	H^+ [w]	Base [18]	H^+ [18]	Base [40]	H^+ [40]	Base [w]	H^+ [w]	Base [1]	H^+ [1]	Base [1]	H^+ [23]	Base [30]	H^+ [30]	Base [30]	H^+ [30]
C-1	−0.5	−0.3	−0.1	−0.4	−0.1	−0.4	−0.2	−0.3	−0.3	−0.4	−0.1	−0.4	+0.2	−0.4	+0.2	−0.4
C-2	−0.3	+0.3	−0.7	−0.6	−0.6	−0.2	−0.8	−0.6	−0.8	−0.8	−0.4	−0.7	+0.2	0	+0.1	−0.4
C-3	−1.3	−1.1	−1.5	−1.6	−1.8	−1.8	−1.8	−2.4	−1.8	−2.6	−1.7	−2.6	−1.1	−3.7	−0.8	−2.2
C-4	+10.3	+8.2	+9.2	+7.8	+10.3	+8.4	+9.2	+5.9	+10.1	+5.6	+10.2	+5.8	+8.0	+5.5	+8.2	+6.2
C-5	−0.2	+0.1	−1.4	+1.2	−1.7	−2.1	−1.6	−2.5	−1.7	−2.1	−1.8	−2.4	−1.2	−0.5	−1.8	−1.1
C-6	−0.2	+1.3	+9.2	+10.9	+8.8	+10.6	+9.6	+11.5	+9.6	+11.3	+9.0	+11.4	+9.4	+11.1	+9.3	+11.3
δ_C C-1′	102.0	97.9	103.4	97.7	100.5	99.5	100.4	96.6	100.3	96.4	100.4	96.6	101.3	96.0	102.6	97.1
C-1′		−4.1		−5.7		−1.0		−3.8		−3.9		−3.8		−5.3		−5.5
δ_C C-1″			101.5	102.0	101.3	101.8	101.1	102.0	100.8	101.2	101.0	102.0	101.3	101.6	101.4	101.6
C-1″				+0.5		+0.5		+0.9		+0.4		+1.0		+0.3		+0.2

Carbon $\Delta\delta_C$	3-*epi*-Gentamicin C_{1a} (17) [e]		3-*N*-(*S*)-HABA-kanamycin A (6) [f]		6′-*N*-Acetyl-kanamycin A (7) [a]		6′-*N*-(*S*)-HABA-kanamycin A (8) [a]		5-*epi*-Gentamicin C_{1a} (18) [g]		5-*epi*-Gentamicin C_1 (19) [g]		5-*epi*-Gentamine C_1 (28) [h]		5-*epi*-amino-5-deoxy-gentamicin C_1 (20) [i]	
	Base [30]	H^+ [30]	Base [21]	H^+ [21]	Base [21]	H^+ [21]	Base [21]	H^+ [21]	Base	H^+	Base	H^+	Base	H^+	Base	H^+
C-1	−0.1	−0.2	+0.1	−0.3	−0.4	−0.4	−0.4	−0.4	−0.4	−1.1	−0.5	−1.2	−0.1	−0.3	−0.2	−0.6
C-2	+0.9	+0.7	−0.3	−0.4	−0.6	−0.5	−0.7	−0.5	0	−0.2	−0.1	−0.2	+0.1	0	−0.5	0
C-3	−1.3	+1.4	−2.2	−2.0	−1.4	−1.8	−1.4	−1.8	−1.2	−1.6	−1.2	−1.7	−0.9	−1.4	−0.9	−1.6
C-4	+8.0	+5.6	+6.1	+4.3	+11.0	+7.1	+10.5	+7.0	+3.7	+2.4	+3.8	+2.5	+3.9	+2.6	+2.8	+3.9
C-5	−0.1	+0.4	−0.6	−0.4	−1.5	−1.8	−1.5	−1.8	−5.3	−6.6	−5.4	−6.6	−4.7	−5.5	−5.6	−6.0
C-6	+12.2	+15.1	+9.9	+11.1	+9.9	+11.0	+9.9	+11.0	+9.8	+10.1	+9.9	+10.3	+0.3	0	+9.6	+10.4
δ_C C-1′	102.3	96.8	99.5	98.6	101.4	98.8	101.1	98.8	96.3	90.4	96.7	90.8	96.9	90.8	95.9	91.9
C-1′		−5.5		−0.9		−2.6		−2.3		−5.9		−5.9		−6.1		−4.0
δ_C C-1″	101.3	101.7	100.8	101.1	100.6	101.1	100.7	101.1	102.5	101.2	102.6	101.4			102.3	102.2
C-1″		+0.4		+0.3		+0.5		+0.4		−1.3		−1.2				−0.1

Carbon $\Delta\delta_C$	5-Deoxygentamicin C_2 (21) [j]		2′,3′-Dideoxy-gentamicin B (89) [a]		2′-Deamino-gentamicin C_{1a} (90) [a]		2′-*N*-Acetyl gentamicin C_2 (22) [a]		1-Deamino-1-hydroxygentamicin C_1 (23) [k]		1-Deamino-1-hydroxygaramine (68) [l]		1-*N*-Acetyl-kanamycin A (5) [m]		1-*N*-(2-Amino-ethoxycarbonyl)-gentamicin B (78) [n]	
	Base	H^+	Base	H^+	Base	H^+	Base	H^+	Base [30]	H^+ [30]	Base [30]	H^+ [30]	Base [21]	H^+ [21]	Base [1]	H^+ [1]
C-1	−0.9	−1.7	0	−0.1	0	0	−0.4	−0.3	+0.5	+0.2	+0.7	+0.6	−0.7	−0.8	−0.5	−0.5
C-2	−0.4	−0.2	−0.6	−0.6	−0.5	+0.7	−0.6	−0.5	+0.1	+0.1	+0.3	+0.3	−0.1	−0.2	+0.2	0
C-3	−1.8	−2.0	−1.5	−1.5	−1.5	−1.3	−0.8	−1.0	−1.0	−1.2	−0.2	−0.2	−1.7	−2.7	−1.6	−2.7
C-4	+4.3	+2.3	+7.6	+6.1	+7.6	+6.3	+10.9	+5.3	+9.6	+4.6	+0.4	+0.1	+9.5	+6.5	+10.1	+6.2
C-5	−5.3	−6.8	−1.1	−0.4	−1.0	−0.3	−1.5	−0.3	−1.0	−0.7	−1.3	−1.4	−1.5	−3.0	−1.6	−3.0
C-6	+10.0	+10.0	+9.5	+11.5	+9.5	+11.7	+9.6	+11.3	+7.5	+6.5	+7.5	+6.1	+7.2	+7.7	+5.6	+6.7
δ_CC-1′	96.8	90.7	98.8	98.2	100.2	99.3	100.3	97.8	102.5	95.9			100.2	96.2	100.9	96.4
C-1′		−6.1		−0.6		−0.9		−2.5		−6.6				−4.0		−4.5
δ_CC-1″	101.9	100.4	101.6	101.9	101.6	101.9	101.2	101.8	100.5	99.2	100.4	99.0	99.5	99.0	99.6	99.3
C-1″		−1.5		+0.3		+0.3		+0.6		−1.3		−1.4		−0.5		−0.3

Carbon $\Delta\delta_C$	1-*N*-(2-Amino-ethoxycarbonyl)-garamine (69) [n]		1-*N*-(2-Amino-ethoxycarbonyl)-kanamycin A (9) [n]		1-*N*-(*S*)-HABA-gentamicin B (75) [m]		1-*N*-(*S*)-HAPA-gentamicin B (76) [m]		1-*N*-(*S*)-HABA-kanamycin A (4) [m]		1-*N*-(*S*)-HABA-gentamicin C_1 (24) [m]		3″-*N*-(*S*)-HABA-kanamycin A (10) [a]		Sisomicin (29) [a]	
	Base [1]	H^+ [1]	Base [1]	H^+ [1]	Base [32]	H^+ [32]	Base [32]	H^+ [32]	Base [31]	H^+ [31]	Base	H^+ [1]	Base [21]	H^+ [21]	Base [14]	H^+ [z]
C-1	−0.3	−0.1	−0.6	−0.6	−0.6	−0.6	−0.6	−0.7	−0.4	−0.6	−0.7	−0.8	−0.4	−0.3	+0.1	−0.3
C-2	+0.6	+0.3	+0.1	−0.1	−0.2	−0.2	0	−0.3	−0.2	−0.3	+0.1	+0.4	−0.6	−0.7	−0.6	−0.7
C-3	−0.2	−0.3	−1.6	−2.3	−1.7	−2.7	−1.7	−2.7	−1.7	−2.9	−0.8	−1.8	−1.8	−2.5	−1.3	−2.0
C-4	+0.2	+0.2	+9.6	+6.1	+8.7	+6.4	+9.4	+6.3	+9.6	+6.4	+9.3	+4.7	+9.6	+5.5	+6.7	+6.7
C-5	−1.4	−1.3	−1.4	−2.9	−1.5	−2.7	−1.4	−2.8	−1.3	−2.9	−1.1	−0.6	−1.7	−1.6	−1.3	−1.1
C-6	+5.9	+6.0	+6.6	+7.4	+5.2	+6.1	+5.1	+6.3	+5.8	+6.6	+4.9	+5.9	+9.7	+11.3	+9.2	+10.7
δ_CC-1′			100.3	96.1	100.0	96.6	100.3	96.6	100.4	96.3	102.6	96.1	100.3	96.2	100.6	97.8
C-1′				−4.2		−3.4		−3.7		−4.1		−6.5		−4.1		−2.8
δ_CC-1″	99.7	99.2	99.5	99.1	99.3	98.9	99.7	99.2	99.2	98.8	99.3	98.8	100.7	101.6	101.5	101.7
C-1″		−0.5		−0.4		−0.4		−0.5		−0.4		−0.5		+0.9		+0.2

TABLE 3 (*continued*)

Carbon	Antibiotic 66-40 B (49) [a]		Antibiotic 66-40 D (50) [a]		3-*N*-Acetylsisomicin (30) [f]		6′-*N*-Acetylsisomicin (31) [a]		Antibiotic 66-40 C (91) [a]		6′-Oxosisomicin (92) [a]		6′-Deamino-6′-hydroxysisomicin (32) [a]		6′-Deamino-6-hydroxysisamine (113) [a]	
Δδ$_C$	Base [15, x]	H$^+$	Base [15, x]	H$^+$ [15, x]	Base	H$^+$	Base	H$^+$	Base	H$^+$ [z]	Base [x]	H$^+$ [x]	Base	H$^+$	Base	H$^+$
C-1	+0.1	−0.3	0	−0.2	+0.5	−0.2	+0.1	−0.3		−0.4	+0.1	−0.3	+0.1	−0.3	−0.3	−0.2
C-2	−0.8	+1.5	−0.7	−0.7	−0.5	−0.8	−0.7	−0.7		−0.7	−0.8	−0.7	−1.2	−0.7	−0.5	−0.3
C-3	−1.4	−1.9	−1.4	−1.9	−2.0	−2.3	−1.3	−1.8		−2.0	−1.5	−1.9	−1.5	−1.8	−1.3	−1.6
C-4	+6.9	+6.6	+7.0	+6.7	+4.9	+4.3	+7.3	+7.1		+7.4	+6.3	+6.4	+6.4	+6.9	+7.1	+6.9
C-5	−1.3	−1.2	−1.2	−1.1	−0.8	−0.4	−1.3	−1.3		−0.9	−1.1	−0.9	−1.3	−1.3	+0.4	−0.3
C-6	+9.6	+10.8	+9.5	+10.8	+9.3	+11.1	+9.5	+10.7		+10.7	+9.2	+10.9	+9.9	+10.7	+0.2	−0.3
δ$_C$C-1′	100.9	97.9	100.9	97.9	99.6	94.9	101.0	97.8		97.7	100.5	97.5	100.4	97.5	100.8	97.2
C-1′		−3.0		−3.0		−4.7		−3.2				−3.0		−2.9		−3.6
δ$_C$C-1″	100.9	101.5	101.3	101.5	101.5	101.9	101.4	101.9		102.0	101.4	101.9	101.3	101.8		
C-1″		+0.6		+0.2		+0.4		+0.5				+0.5		+0.5		

Carbon	5-*epi*-Sisomicin (33) [g]		5-Deoxysisomicin (34) [j]		5-Amino-5-deoxysisomicin (35) [o]		5-*epi*-Amino-5-deoxysisomicin (36) [i]		2′-*N*-Acetylsisomicin (37) [a]		1-Deamino-1-hydroxysisomicin (38) [k]		1-Deamino-1-*epi*-hydroxysisomicin (39) [p]		1-*epi*-Sisomicin (40) [q]	
δΔ$_C$	Base	H$^+$	Base	H$^+$	Base	H$^+$	Base	H$^+$	Base	H$^+$	Base [30]	H$^+$ [30]	Base [30]	H$^+$ [30]	Base [30]	H$^+$ [30]
C-1	−0.5	−1.2	−1.0	−1.7	−0.2	−1.4	−0.3	−0.8	−0.1	−0.2	+0.7	+1.3	−4.0	−3.3	−3.0	−0.3
C-2	−0.3	+1.3	−0.7	−0.4	−0.1	−0.6	−0.8	−0.3	−0.7	−0.8	−0.2	0	−1.0	−0.9	+0.2	+0.2
C-3	−1.4	−2.2	−2.2	−2.5	−1.6	−1.9	−1.3	−2.3	−1.4	−1.6	−1.3	−2.0	−1.2	−2.0	−2.1	−2.6
C-4	+4.9	+6.6	+5.1	+5.1	+7.6	+7.6	+4.1	+6.1	+7.8	+6.2	+6.7	+6.8	+6.8	+6.8	+9.2	+10.6
C-5	−4.2	−4.3	−4.8	−5.7	−1.3	−2.0	−4.7	−4.5	−1.4	−1.0	−1.0	−1.5	−1.0	−1.6	+0.6	−0.6
C-6	+9.8	+10.9	+9.8	+9.9	+10.8	+10.5	+9.6	+10.1	+9.5	+10.7	+7.4	+5.9	+5.1	+4.9	+4.1	+8.1
δ$_C$C-1′	97.1	94.1	97.4	93.3	100.6	98.2	97.0	95.3	98.4	99.1	100.8	97.7	101.0	97.8	100.7	97.9 [w]
C-1′		−3.0		−4.1		−2.4		−1.7		+0.7		−3.1		−3.2		−2.8
δ$_C$C-1″	102.5	101.5	101.9	101.4	101.9	101.1	102.4	102.4	101.2	101.7	100.5	99.1	96.8	96.1	95.8	98.1
C-1″		−1.0		−0.5		−0.8		0		+0.5		−1.4		−0.7		+2.3

Carbon	Netilmicin (41) [r]		1-*epi*-N-Ethylsisomicin (42) [s]		1-*N*-Acetylsisomicin (43) [m]		1-*N*-(2-Aminoethoxycarbonyl)-sisomicin (44) [n]		1-*N*-Methoxycarbonylsisomicin (45) [n]		1-*N*-Ethoxycarbonylsisomicin (46) [n]		2″-Deoxysisomicin (47) [a]		3″-*N*-Acetylsisomicin (48) [a]	
Δδ$_C$	Base [30, 40]	H$^+$ [30, 40]	Base [30]	H$^+$ [30]	Base	H$^+$	Base [1]	H$^+$ [1]	Base [1]	H$^+$ [1]	Base [1]	H$^+$ [1]	Base	H$^+$	Base	H$^+$ [1]
C-1	+0.5	+0.5	−4.3	−1.3	−0.7	−0.8	+0.4	−0.3	−0.3	−0.4	−0.4	−0.5	−0.2	−1.1	+0.1	−0.1
C-2	−1.1	−0.7	+0.5	−0.3	−0.2	−0.1	0	−0.2	0	0	+0.1	0	−0.6	−0.7	−0.6	−0.7
C-3	−1.1	−1.9	−1.6	−1.9	−1.2	−2.1	−1.2	−1.9	−1.2	−2.0	−1.2	−2.1	−1.4	−2.0	−1.4	−1.9
C-4	+6.9	+6.7	+8.2	+7.0	+6.7	+7.1	+6.6	+6.9	+6.6	+7.0	+6.5	+7.0	+6.8	+6.8	+6.8	+6.6
C-5	−1.2	−1.3	+0.2	−1.3	−1.1	−1.5	−1.0	−1.6	−1.0	−1.5	−1.0	−1.5	−1.0	−1.1	−1.1	−1.0
C-6	+10.4	+11.1	+4.5	+7.0	+6.4	+6.2	+5.9	+5.9	+5.9	+5.6	+5.8	+5.6	+8.2	+8.7	+9.4	+10.6
δ$_C$C-1′	100.8	97.8	100.9	97.9	100.9	97.9	100.8	97.9	100.8	97.9	100.7	97.8	100.8	97.9	100.8	97.9
C-1′		−3.0		−3.0		−3.0		−2.9		−2.9		−2.9		−2.9		−2.9
δ$_C$C-1″	102.2	102.2	95.8	98.2	99.9	99.3	99.8	99.3	99.9	99.2	99.7	99.1	100.2	98.7	101.6	102.8
C-1″		+0.1		+2.4		−0.6		−0.5		−0.7		−0.6		−1.5		+1.2

Carbon	*O*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-(1→4)-garamine (93) [a]		*O*-(2-Amino-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→4)-garamine (94) [a]		*O*-(3-Amino-3-deoxy-β-D-glucopyranosyl)-(1→6)-gentamine C$_1$ (95) [a]		*O*-(3-Amino-3-deoxy-β-D-xylopyranosyl)-(1→6)-gentamine C$_{1a}$ (96) [a]		*O*-(3-Deoxy-3-methylamino-β-D-xylopyranosyl)-(1→6)-gentamine C$_1$ (97) [a]	
Δδ$_C$	Base [19]	H$^+$ [19]	Base [19]	H$^+$ [19]	Base [21]	H$^+$ [21]	Base [22]	H$^+$ [22]	Base [22]	H$^+$ [22]
C-1	−0.1	−0.7	0	+0.8	−2.0	−1.4	−1.9	−1.9	−2.0	−1.6
C-2	−0.9	−0.6	−0.7	+1.2	−0.7	−0.5	−0.7	−0.4	−0.7	−0.4
C-3	−1.2	−1.3	−1.3	−1.2	−0.9	−1.8	−1.0	−1.5	−1.4	−1.4
C-4	+10.1	+4.5	+10.8	+10.0	+9.4	+3.7	+9.8	+3.8	+9.7	+4.1
C-5	−3.5	−2.8	−3.2	−2.9	−0.1	+0.1	0	−0.2	−0.1	−0.1
C-6	+8.9	+11.3	+8.8	+11.5	+9.4	+7.2	+8.9	+7.4	+9.2	+7.4
δ$_C$C-1′	103.8	96.4	102.0	97.3	102.7	95.7	102.1	95.5	102.5	96.1
C-1′		−7.4		−4.7		−7.0		−6.6		−6.4
δ$_C$C-1″	101.3	101.8	101.3	101.7	104.6	103.6	105.3	104.2	105.4	104.4
C-1″		+0.5		+0.4		−1.0		−1.1		−1.0

[a] Δδ$_C$ 2-Deoxystreptamine→trisaccharide. [b] Δδ$_C$ 2-Deoxystreptamine→disaccharide. [c] Δδ$_C$ 2-Deoxy-3-deamino-3-hydroxystreptamine→trisaccharide. [d] Δδ$_C$ 2-Deoxy-3-deamino-3-*epi*-hydroxystreptamine→trisaccharide. [e] Δδ$_C$ 2-Deoxy-3-*epi*-streptamine→trisaccharide. [f] Δδ$_C$ 3-*N*-Acetyl-2-deoxystreptamine→trisaccharide. [g] Δδ$_C$ 2-Deoxy-5-*epi*-streptamine→trisaccharide. [h] Δδ$_C$ 2-Deoxy-5-*epi*-streptamine→disaccharide. [i] Δδ$_C$ 5-*epi*-Amino-2,5-dideoxystreptamine→trisaccharide. [j] Δδ$_C$ 2,5-Dideoxystreptamine→trisaccharide. [k] Δδ$_C$ 2-Deoxy-1-deamino-1-hydroxystreptamine→trisaccharide. [l] Δδ$_C$ 2-Deoxy-1-deamino-1-hydroxystreptamine→disaccharide. [m] Δδ$_C$ 1-*N*-Acetyl-2-deoxystreptamine→trisaccharide. [n] Δδ$_C$ 1-*N*-(2-Aminoethoxycarbonyl)-2-deoxystreptamine→trisaccharide. [o] Δδ$_C$ 5-Amino-2,5-dideoxystreptamine→trisaccharide. [p] Δδ$_C$ 2-Deoxy-1-deamino-1-*epi*-hydroxystreptamine→trisaccharide. [q] Δδ$_C$ 1-*epi*-2-Deoxystreptamine→trisaccharide. [r] Δδ$_C$ 2-Deoxy-1-*N*-ethylstreptamine→trisaccharide. [s] Δδ$_C$ 2-Deoxy-1-*epi*-*N*-ethylstreptamine→trisaccharide. [t] The original ^{13}C n.m.r. data may be found in the papers cited as references in each column. Where no reference is cited the data may be found in Table 1. [u] Δδ$_C$ For C-1′ in going from the free base to the protonated species. [v] Δδ$_C$ for C-1″ in going from the free base to the protonated species. [w] T. L. Nagabhushan, W. N. Turner, P. J. L. Daniels, and J. B. Morton, *J. Org. Chem.*, 1975, *40*, 2830. [x] D. H. Davies, A. K. Mallams, J. McGlotten, and R. W. Tkach, *J. Chem. Soc., Perkin Trans. 1*, 1977, 1407.

consider possible dipolar repulsions between substituents at C-5 and the 4-O-glycosidic oxygen, as well as between substituents at C-6 and the 6-*O*-glycosidic oxygen. Repulsion between protonated amino-groups at acidic pH, as well as possible hydrogen bonding between substituents on the deoxystreptamine ring and the glycosyl units, also have to be considered in these molecules. The data presented in Tables 3—5 suggest that the balancing of these effects plays an important role in defining the observed rotamer populations about the O–C-4 and O–C-6 glycosidic bonds, which ultimately defines the solution conformations of these antibiotics.

The magnitude of typical protonation shifts (α-D-glycoside; $^4C_1$ conformation) at the anomeric carbon C-1′ in those compounds containing 6′-amino- and/or 2′-amino-groups was determined by recording the ^{13}C n.m.r. data for methyl 6-amino-6-deoxy-α-D-glucopyranoside (98) and for isopropyl 2-amino-2-deoxy-α-D-glucopyranoside (99) for the free bases and at pH 1 (Table 2). It is evident from these data that protonation of the 6-amino-group produces a negligible shift (+0.1) at the anomeric carbon in (98). Protonation of the 2-amino-group in (99), on the other hand, produces as anticipated a significant β-shift at C-1 amounting to −4.1. These values were then used in assessing the rotational contributions at C-1′ in those aminoglycosides that have 4-*O*-D-glycosides with a $^4C_1$ conformation (Table 4).

We shall consider first the rotamer populations of the 4-*O*-glycosyl unit about the O–C-4 glycosidic bond. From the data in Tables 3 and 4 it is evident that in the

Table 4

Summary of selected $\Delta\delta_C$ values for C-4 and C-1′ for different structural types

Antibiotic type	Compound	Key substituents C-3	C-5	C-2′	C-5′	C-4 $\Delta\delta_C$(DOS→Trisaccharide) [a] Base	C-4 $\Delta\delta_C$(DOS→Trisaccharide) [a] H⁺	C-4 Rotational contribution [b]	C-1′ $\Delta\delta_C$ Base →H⁺ [c]	C-1′ Protonation shift [d]	C-1′ Rotational contribution [e]
Gentamicin/ kanamycin	(12)	$NH_2(eq)$	$OH(eq)$	$NH_2(eq)$	CH_2NH_2	+9.8	+4.1	−5.7	−6.6	−4.1	−2.5
	(14)	$NH_2(eq)$	$OH(eq)$	$NH_2(eq)$	$CHMeNHCH_3$	+10.1	+4.1	−6.0	−6.9	−4.1	−2.8
	(71)	$NH_2(eq)$	$OH(eq)$	$NH_2(eq)$	CH_2OH	+10.1	+7.7	−2.4	−4.0	−4.1	+0.1
	(87)	$NH_2(eq)$	$OH(eq)$	$NH_2(ax)$	CH_2OH	+9.2	+7.8	−1.4	−5.7		
	(88)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	CH_2OH	+10.3	+8.4	−1.9	−1.0	0	−1.0
	(77)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	CH_2NH_2	+10.2	+5.8	−4.4	−3.8	+0.1	−3.9
	(15)	$OH(eq)$	$OH(eq)$	$NH_2(eq)$	CH_2NH_2	+8.0	+5.5	−2.5	−5.3	−4.1	−1.2
	(16)	$OH(ax)$	$OH(eq)$	$NH_2(eq)$	CH_2NH_2	+8.2	+6.2	−2.0	−5.5	−4.1	−1.4
	(17)	$NH_2(ax)$	$OH(eq)$	$NH_2(eq)$	CH_2NH_2	+8.0	+5.6	−2.4	−5.5	−4.1	−1.4
	(6)	NH-HABA(eq)	$OH(eq)$	$OH(eq)$	CH_2NH_2	+6.1	+4.3	−1.8	−0.9	+0.1	−1.0
	(7)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	CH_2NHAc	+11.0	+7.1	−3.9	−2.6	0	−2.6
	(8)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	CH_2NH-HABA	+10.5	+7.0	−3.5	−2.3	0	−2.3
	(18)	$NH_2(eq)$	$OH(ax)$	$NH_2(eq)$	CH_2NH_2	+3.7	+2.4	−1.3	−5.9	−4.1	−1.8
	(19)	$NH_2(eq)$	$OH(ax)$	$NH_2(eq)$	$CHMeNHCH_3$	+3.8	+2.5	−1.3	−5.9	−4.1	−1.8
	(20)	$NH_2(eq)$	$NH_2(ax)$	$NH_2(eq)$	$CHMeNHCH_3$	+2.8	+3.9	+1.1	−4.0	−4.1	+0.1
	(21)	$NH_2(eq)$	H	$NH_2(eq)$	$CHMeNH_2$	+4.3	+2.3	−2.0	−6.1	−4.1	−2.0
	(89)	$NH_2(eq)$	$OH(eq)$	H	CH_2NH_2	+7.6	+6.1	−1.5	−0.6	+0.1	−0.7
	(90)	$NH_2(eq)$	$OH(eq)$	H	CH_2NH_2	+7.6	+6.3	−1.3	−0.9	+0.1	−1.0
	(22)	$NH_2(eq)$	$OH(eq)$	$NHAc(eq)$	$CHMeNH_2$	+10.9	+5.3	−5.6	−2.5	0	−2.5
	(93)	$NH_2(eq)$	$OH(eq)$	$NH_2(eq)$	CH_2OH	+10.1	+4.5	−5.6	−7.4		
	(94)	$NH_2(eq)$	$OH(eq)$	$NH_2(ax)$	CH_2OH	+10.8	+10.0	−0.8	−4.7		
Sisomicin	(29)	$NH_2(eq)$	$OH(eq)$	NH_2	CH_2NH_2	+6.7	+6.7	0	−2.8		
	(30)	$NHAc(eq)$	$OH(eq)$	NH_2	CH_2NH_2	+4.9	+4.3	−0.6	−4.7		
	(31)	$NH_2(eq)$	$OH(eq)$	NH_2	CH_2NHAc	+7.3	+7.1	−0.2	−3.2		
	(92)	$NH_2(eq)$	$OH(eq)$	NH_2	$CONH_2$	+6.3	+6.4	+0.1	−3.0		
	(32)	$NH_2(eq)$	$OH(eq)$	NH_2	CH_2OH	+6.4	+6.9	+0.5	−2.9		
	(33)	$NH_2(eq)$	$OH(ax)$	NH_2	CH_2NH_2	+4.9	+6.6	+1.7	−3.0		
	(34)	$NH_2(eq)$	H	NH_2	CH_2NH_2	+5.1	+5.1	0	−4.1		
	(35)	$NH_2(eq)$	$NH_2(eq)$	NH_2	CH_2NH_2	+7.6	+7.6	0	−2.4		
	(36)	$NH_2(eq)$	$NH_2(ax)$	NH_2	CH_2NH_2	+4.1	+6.1	+2.0	−1.7		
	(37)	$NH_2(eq)$	$OH(eq)$	$NHAc$	CH_2NH_2	+7.8	+6.2	−1.6	+0.7		

[a] These $\Delta\delta_C$ values reflect both glycosylation and conformational effects. [b] Rotational contribution accompanying protonation of the amino-groups in the molecule as derived from $\Delta\delta_C$(DOS→Trisaccharide) for the protonated species relative to the free bases. [c] The $\Delta\delta_C$[trisaccharide(Base)→trisaccharide (H^+)]values reflect conformational effects as well as protonation effects in those substrates having a 2′-amino-group. [d] Estimated protonation shifts for compounds containing 2′- and/or 6′-amino-groups derived from data obtained for (98) and (99). [e] Estimated rotational contribution after correcting for the protonation effects.

Table 5

Summary of selected $\Delta\delta_C$ values for C-6 and C-1″ for different structural types

Antibiotic type	Compound	Key substituents C-1	C-5	C-2″	C-6 $\Delta\delta_C$(DOS→trisaccharide) [a] Base	C-6 $\Delta\delta_C$(DOS→trisaccharide) [a] H	C-6 Rotational contribution [b]	C-1″ $\Delta\delta_C$(Base →H⁺) [c]	C-1″ Protonation shift [d]	C-1″ Rotational contribution [e]
Gentamicin/ Kanamycin	(12)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.3	+11.1	+1.8	+0.6	−1.1	+1.7
	(79)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+10.3	+11.4	+1.1	+0.6		
	(81)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.5	+11.4	+1.9	+0.8	−0.8	+1.6
	(83)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.5	+11.2	+1.9	+1.0	−0.7	+1.7
	(84)	$NH_2(eq)$	$OH(eq)$	OH	+9.4	+9.1	−0.3	−1.5		
	(85)	$NH_2(eq)$	$OH(eq)$	OH	+9.1	+8.9	−0.2	−0.8		
	(71)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.4	+11.0	+1.6	+0.6	−0.8	+1.4
	(70)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.4	+11.2	+1.8	+0.4	0	+0.4
	(73)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.5	+11.0	+1.5	+1.1	−0.7	+1.8
	(1)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.6	+11.3	+1.7	+0.4	+0.8	+1.2
	(18)	$NH_2(eq)$	$OH(ax)$	$OH(eq)$	+9.8	+10.1	+0.3	−1.3	−1.1	−0.2
	(20)	$NH_2(eq)$	$NH_2(ax)$	$OH(eq)$	+9.6	+10.4	+0.8	−0.1	−1.1	+1.0
	(21)	$NH_2(eq)$	H	$OH(eq)$	+10.0	+10.0	0	−1.5	−1.1	−0.4
	(23)	$OH(eq)$	$OH(eq)$	$OH(eq)$	+7.5	+6.5	−1.0	−1.3	−1.1	−0.2
	(5)	$NHAc(eq)$	$OH(eq)$	$OH(eq)$	+7.2	+7.7	+0.5	−0.5	−0.8	+0.3
	(78)	$NHAEC(eq)$	$OH(eq)$	$OH(eq)$	+5.6	+6.7	+1.1	−0.3	−1.1	+0.8
	(75)	NH-HABA(eq)	$OH(eq)$	$OH(eq)$	+5.2	+6.1	+0.9	−0.4	−1.1	+0.7
	(10)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.7	+11.3	+1.6	+0.9	−0.8	+1.7
	(95)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.4	+7.2	−2.2	−1.0	−1.1	+0.1
	(96)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+8.9	+7.4	−1.5	−1.1	−1.1	0
Sisomicin	(29)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.2	+10.7	+1.5	+0.2	−1.1	+1.3
	(49)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.6	+10.8	+1.2	+0.6	−0.8	+1.4
	(50)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.5	+10.8	+1.3	+0.2	−0.7	+0.9
	(33)	$NH_2(eq)$	$OH(ax)$	$OH(eq)$	+9.8	+10.9	+1.1	−1.0	−1.1	+0.1
	(34)	$NH_2(eq)$	H	$OH(eq)$	+9.8	+9.9	+0.1	−0.5	−1.1	+0.6
	(35)	$NH_2(eq)$	$NH_2(eq)$	$OH(eq)$	+10.8	+10.5	−0.3	−0.8	−1.1	+0.3
	(36)	$NH_2(eq)$	$NH_2(ax)$	$OH(eq)$	+9.6	+10.1	+0.5	0	−1.1	+1.1
	(38)	$OH(eq)$	$OH(eq)$	$OH(eq)$	+7.4	+5.9	−1.5	−1.4	−1.1	−0.3
	(39)	$OH(ax)$	$OH(eq)$	$OH(eq)$	+5.1	+4.9	−0.2	−0.7	−1.1	+0.4
	(40)	$NH_2(ax)$	$OH(eq)$	$OH(eq)$	+4.1	+8.1	+4.0	+2.3	−1.1	+3.4
	(41)	$NHEt(eq)$	$OH(eq)$	$OH(eq)$	+10.4	+11.1	+0.7	+0.1	−1.1	+1.2
	(42)	$NHEt(ax)$	$OH(eq)$	$OH(eq)$	+4.5	+7.0	+2.5	+2.4	−1.1	+3.5
	(43)	$NHAc(eq)$	$OH(eq)$	$OH(eq)$	+6.4	+6.2	−0.2	−0.6	−1.1	+0.5
	(44)	$NHAEC(eq)$	$OH(eq)$	$OH(eq)$	+5.9	+5.9	0	−0.5	−1.1	+0.6
	(45)	$NHCO_2Me$	$OH(eq)$	$OH(eq)$	+5.9	+5.6	−0.3	−0.7	−1.1	+0.4
	(47)	$NH_2(eq)$	$OH(eq)$	H	+8.2	+8.7	+0.5	−1.5	−1.1	−0.4
	(48)	$NH_2(eq)$	$OH(eq)$	$OH(eq)$	+9.4	+10.6	+1.2	+1.2	0	+1.2

[a] The $\Delta\delta_C$ values reflect both glycosylation and conformational effects. [b] Rotational contribution accompanying protonation of the amino-groups in the molecule as derived from $\Delta\delta_C$(DOS→trisaccharide) for the protonated species relative to the free bases. [c] The $\Delta\delta_C$[trisaccharide (base)→trisaccharide (H^+)] values reflect conformational effects as well as protonation effects in those substrates having a 3″-amino-group. [d] Estimated protonation shift at C-1″ for compounds having a 3″-amino-group derived from data obtained for (100)—(102) and for (103) (ref. 16). [e] Estimated rotational contribution after correcting for the protonation effects.

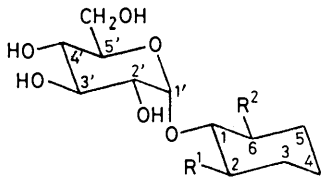
TABLE 6

δ_C and $\Delta\delta_C$ Values for cyclohexyl α- and β-D-glucopyranosides [6,33,34]

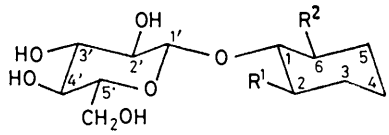
Substituents				δ_C(C-1)		
Compound	R^1	R^2	δ_C(C-1′)	Cyclohexanol	Cyclohexyl glycoside	$\Delta\delta_C$ R-OH → R-*O*-Glycoside
(104)	H	H	96.9	69.8	77.1	+7.3
(105)	Me	H	100.6	76.9	85.9	+9.0
(106)	H	Me	94.5	76.9	80.8	+3.9
(107)	Me	Me	99.1	*a*	91.0	*a*
(108)	H	H	100.9	69.8	79.1	+9.3
(109)	Me	H	100.1	76.9	83.9	+7.0
(110)	H	Me	104.0	76.9	87.2	+10.3
(111)	Me	Me	102.7	*a*	91.2	*a*
(112)				71.0	79.9	+8.9

[a] Chemical shift not available in the literature.

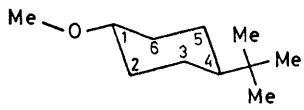
cases of the gentamicins C_{1a} (12), and C_1 (14) and gentamines C_{1a} (25) and C_1 (27), as well as the semisynthetic gentamine derivatives (79)—(85), all of which contain the 2,6-diamino-purpurosaminyl units at C-4, the $\Delta\delta_C$ values for C-4 for the bases range between +9.5 and +10.5. This indicates that the 4-*O*-glycoside adopts rotamer *a* about the O–C-4 glycosidic bond in these bond (Tables 3 and 4). This presumably arises from the interaction between 1′*eq*-H and C-5 which is present in rotamer *h* and which would be expected to result in shielding of C-1′.[35-38] In the protonated molecules it is likely that the principal repulsion is occurring between the 6′- and 3-amino-groups. In those aminoglycosides that contain a 2′-amino-group and a 6′-hydroxy-group



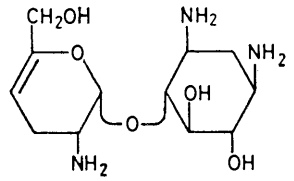
(104) $R^1 = R^2 = H$
(105) R^1 = Me, R^2 = H
(106) R^1 = H, R^2 = Me
(107) $R^1 = R^2$ = Me



(108) $R^1 = R^2 = H$
(109) R^1 = Me, R^2 = H
(110) R^1 = H, R^2 = Me
(111) $R^1 = R^2$ = Me

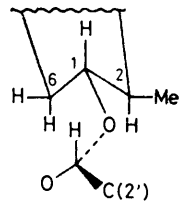
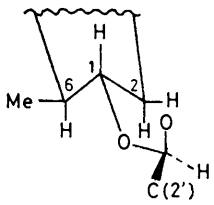
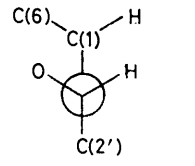
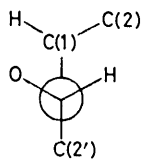


(112)

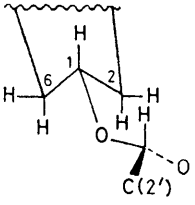
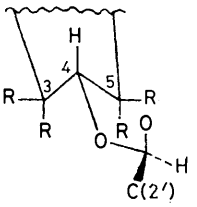
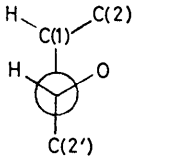
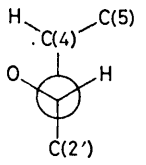


(113)

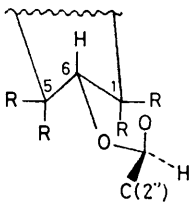
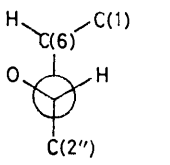
molecules. Upon protonation we see a dramatic difference in the $\Delta\delta_C$ values for C-4 for these compounds, namely +4.1 to +4.4. Repulsion between the protonated amino-groups in the molecule results in a clockwise rotation of the 4-*O*-glycoside about the O–C-4 bond to give a rotamer approaching that represented by *h*. As the sugar rotates in a clockwise direction about the O–C-4 glycosidic bond, increasing interaction between C-1′–O-5′ and C-4–H-4 would introduce a strong shielding component [35-38] at C-4, which is what is observed. If we consider the $\Delta\delta_C$(base ⟶ H^+) for C-1′ in these molecules we also see in addition to the shielding from the β-protonation shift of the 2′-amino-groups, additional shielding due to rotational changes about the O–C-4 glycosidic such as gentamicins A (71), A_1 (72), A_2 (70), and A_4 (73) and paromamine (86), we again observe rotamer *a* in the free bases with $\Delta\delta_C$ for C-4 varying from +9.6 to +10.3. Protonation of these molecules which lack the 6′-amino-group produces only a modest clockwise rotation of the 4-*O*-glycoside about the O–C-4 glycosidic bond resulting in the introduction of a modest shielding component at C-4 thus reducing the observed deshielding at C-4 to +7.7 to +8.7. The shielding observed at C-1′ in the protonated species arises solely from protonation of the 2′-amino-group in each case. In these molecules the 4-*O*-glycoside adopts a rotamer about the O–C-4 glycosidic bond in which a modest clockwise rotation has occurred relative to rotamer *a*. A similar rotamer was

e *f*

g *h*

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observed for the mannosaminyl derivative (87), which contains an axial 2′-amino-group. In the glycosyl derivative (88), which contains no amino-groups in the 4-*O*-glycoside, the free base was found to adopt rotamer *a* about the O–C-4 glycosidic bond. Once again protonation produced only a modest clockwise rotation of the glucosyl unit about the O–C-4 glycosidic bond, resulting in the introduction of a modest shielding component at C-4 which produced a net deshielding of +8.4 in this instance. Slight shielding was also observed at C-1′ in this case upon protonation. These values indicate a modest clockwise rotation of the 4-*O*-glycoside about the O–C-4 glycosidic bond on protonation relative to rotamer *a*. It is possible that this rotation is induced by the increased steric bulk of the protonated 3-amino-group, or possibly by changes in the hydrogen bonding between that group and the 5′-ring oxygen. In those aminoglycosides that contain a 6′ amino-group and a 2′-hydroxy-group such as gentamicin A_3 (74), kanamycin A (1), and gentamicin B (77) we again see a net deshielding at C-4 of +9.2 to +10.2 for the free bases indicating that these molecules also adopt rotamer *a* about the O–C-4 glycosidic bond. On protonation these molecules undergo a marked clockwise rotation about the O–C-4 glycosidic bond leading to a reduced net deshielding at C-4 to +5.6 to +5.9. This represents less of a rotation than that observed for the 2′, 6′-diamino-derivatives discussed earlier and can be attributed to the absence of the 2′-amino-group in these molecules. Shielding was also observed at C-1′ in these derivatives on protonation for the same reasons as stated earlier for gentamicin C_{1a} (12).

When the 3-amino-group was replaced by an equatorial hydroxy-group as in (15), or by an axial hydroxy-group as in (16), reduced deshielding was observed at C-4 in the free bases indicating a modest clockwise rotation about the O–C-4 glycosidic bond relative to rotamer *a*. In the protonated species further clockwise rotation occurs resulting in reduced deshielding at C-4 to +5.5 and +6.2 respectively. After correcting for the β-protonation effect, some shielding is also observed at C-1′ (15). It is possible that the initial rotation observed in the free bases is induced by changes in the hydrogen bonding between the 3-hydroxy-group and the 5′-ring oxygen, and that the change on protonation results from increased hydrogen bonding between the 2′-amino-group and the 5-hydroxy-groups. Similar arguments might be invoked in the case of the protonated species of (71)—(73) and (86).

When the 3-amino-group was epimerized as in 3-*epi*-gentamicin C_{1a} (17) reduced deshielding was again observed at C-4 relative to gentamicin C_{1a} (12) resulting in a net deshielding of +8.0, which is similar to that observed in (15) and (16) for the free bases. In the protonated species of (17), further clockwise rotation of the 4-*O*-glycoside about the O–C-4 glycosidic bond occurred relative to rotamer *a* resulting in a net deshielding of +5.6 at C-4, which is similar to that observed for (15) and (16). After correcting for the β-protonation shift, some shielding is also observed at C-1′ in (17). It is evident from the data in Table 3 that the 4-*O*-glycoside in (17) rotates less upon protonation than that in (12), which suggests that in the former case, reduced repulsion between the axial 3-amino-group and the 6′-amino-group is occurring. The reason for the observed shielding at C-3 (Table 3) for the protonated species (17) is not obvious. Acylation of the 3-amino-group as in (6) results in decreased deshielding of C-4 for the free bases to +6.1 indicating a rotamer intermediate between

a and *h* about the O–C-4 glycosidic bond. This is also evident from the shielding observed in δ_C for C-1′ in this derivative (Table 3). Protonation of these 3-acyl derivatives results in a further clockwise rotation in the direction of rotamer *h* leading to a net deshielding at C-4 of +4.3. Some shielding is also observed at C-1′ in the protonated derivative. The origin of the initial rotation in the free base (6) relative to the 3-amino-analogue (1) is presumably steric. Acylation of the 6′-amino-group on the other hand as in (7) and (8) produced no such clockwise rotation about the O–C-4 glycosidic bonds as evidenced by the large deshielding observed at C-4 of +10.5 to +11.0 and the absence of any shielding at C-1′ in the free bases. The 4-*O*-glycoside obviously adopts rotamer *a* in these derivatives. On protonation of the 6′-*N*-acyl derivatives we observe only a moderate clockwise rotation about the O–C-4 glycosidic bond relative to rotamer *a* similar to that observed in the case of the 6′-hydroxy-derivatives discussed earlier. The absence of a protonatable 6′-substituent removes the repulsive interaction with the protonated 3-amino-group relative to the parent antibiotic (1). Some shielding is also observed at C-1′ in the protonated species due to rotation.

Epimerization of the 5-hydroxy-group as in the derivatives (18), (19), and (28) resulted in a large reduction in the deshielding observed at C-4 in these compounds for the free bases. Thus $\Delta\delta_C$ for C-4 ranged between +3.7 and +3.9 indicating a large shielding component from the C-1′–O-5′ and C-4–H-4 interaction [35-38] present in a rotamer approaching that represented by *h*. Protonation of these derivatives resulted only in a slight clockwise rotation about the O–C-4 glycosidic bond relative to the free bases leading to a net deshielding at C-4 of +2.4 to + 2.6. The shielding in these instances appears to be at a maximum and would indicate that the molecules exist as rotamer *h* about the O–C-4 glycosidic bond in which C-1′–O-5′ and C-4–H-4 are eclipsed. In both the free bases and protonated species the presence of an equatorial hydrogen at C-5 would result in a non bonded interaction [39] of the type shown in Figure 1, between 5*eq*-H and 1′*eq*-H resulting in the observed shielding at C-5 and C-1′ (Table 3). Thus C-5 is shielded in the free bases by −4.7 to −5.3 and in the protonated species by −5.5 to −6.6, which agrees well with the shielding values published earlier by Beierbeck and Saunders [39] for such interactions. The anomeric carbons C-1′ in the derivatives are also markedly shielded occurring at 96.3—96.9 in the free bases which was used earlier as diagnostic proof of a change in rotamer population about the O–C-4 glycosidic bond in these and related derivatives.[28] Protonation causes a further shielding to 90.4—90.8 due to protonation of the 2′-amino-group and due to the modest clockwise rotation about the O–C-4 glycosidic bond which accompanies protonation of these derivatives. 5-Deoxy-5-*epi*-aminogentamicin C_1 (20) which contains an axial amino-group at C-5 exhibited a net deshielding of C-4 of +2.8 indicating that the 4-*O*-glycoside adopts rotamer *h* about the O–C-4 glycosidic bond in the free base. Pronounced shielding was again observed at C-5 and C-1′ for the reasons discussed above. Protonation of (20) led to a modest counterclockwise rotation about the O–C-4 glycosidic bond as evidenced by the increased deshielding of C-4 to +3.9. Shielding was once again evident at C-5 and C-1′. Repulsion between the protonated 2′- and 5-*epi*-amino-groups is probably responsible for the observed counterclockwise rotation that accompanies protonation. When the 5-substituent is removed completely as in 5-deoxygentamicin C_2 (21) we observe a similar pair of rotamers to those described for the 5-*epi*-hydroxy-derivatives (18), (19), and (28). In all the above 5-*epi*- and 5-deoxy-derivatives it is evident that removal of the 5-equatorial substituent removes a critical steric interaction with the 4-*O*- glycoside unit thereby allowing the sugar to rotate in a clockwise direction about the O–C-4 glycosidic bond. It is also likely that there is a dipolar repulsion between the 5-equatorial hydroxy-group and the glycoside oxygen at C-4 in the parent molecules which contributes to the factors resulting in the adoption of rotamer *a* in these compounds. Removal of the equatorial 5-hydroxy-group, or epimerization of the group would remove this interaction and contribute to the observed adoption of rotamer *h* by these molecules.

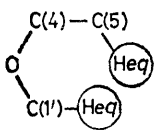

FIGURE 1 Non-bonded hydrogen interaction

It is interesting to note that in contrast to the derivatives just described, the 2′-unsubstituted derivatives (89) and (90) only undergo a modest clockwise rotation of the 4-*O*-glycoside about the O–C-4 glycosidic bond in the free bases relative to rotamer *a*. Thus C-4 exhibits a net deshielding of +7.6 in these derivatives and C-1′ is only moderately shielded. Protonation of (89) and (90) results in only a further modest clockwise rotation relative to the free bases resulting in a net deshielding of C-4 of +6.1 to +6.3. Slight shielding also occurs at C-1′ upon protonation. When the 2′-amino-group is acetylated as in (22) the free base adopts rotamer *a* about the O–C-4 glycosidic bond. Protonation results in a clockwise rotation of the 4-*O*-glycoside about the O–C-4 glycosidic bond resulting in a net deshielding at C-4 of +5.3. Shielding is also observed at C-1′ in (22) upon protonation, which is arising solely from rotational changes. It is evident that the 4-*O*-glycoside in (22) does not rotate as far as in gentimicin C_{1a} (12) or C_1 (14).

It is of interest to compare the above rotamers for α-D-glycosides at C-4 with the results obtained for two semisynthetic β-D-glycosides derived from garamine. Thus *O*-(2 amino-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-garamine (93) shows a $\Delta\delta_C$ at C-4 for the free base of +10.8. We have shown previously [19] that the 4-*O*-glycoside in (93) adopts rotamer *c* about the O–C-4 glycosidic bond. Protonation of (93) results in a counterclockwise rotation of the 4-*O*-glycoside about the O–C-4

glycosidic bond owing presumably to repulsion between the equatorial 2′-amino-group and the 3-amino-group. The net result is an increased shielding interaction between C-1′–O-5′ and C-4–H-4 resulting in a decrease in the net deshielding observed at C-4 to +4.5. It is also evident from the data in Tables 3 and 4 that shielding is also occurring at C-1′ in addition to that expected from protonation of the 2′-amino-group in (93) as reflected by the $\Delta\delta_0$ (base $\longrightarrow$ H^+) value of −7.4 for C-1′. It is interesting to compare these results with those obtained for *O*-(2-amino-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1 $\longrightarrow$ 4)-garamine (94) in which the only significant change is in having an axial 2′-amino-group instead of an equatorial one in the molecule. In the free base (94), as well as in the protonated species, we see a deshielding of C-4 of +10.8 and +10.0 respectively indicating that both adopt rotamer *c* about the O–C-4 glycosidic bond. The only shielding observed at C-1′ on protonation (−4.7) may be attributed to protonation of the 2′-amino-group. It is evident from Dreiding models that the axial 2′-amino-group in (94) is much further removed from the equatorial 3-amino-group than is the equatorial 2′-amino-group in (93). The amino-groups are indeed far enough apart so as to cause no rotational changes about the O–C-4 bond on protonation.

We shall next consider the preferred rotamers for the sisosamine unit in sisomicin (29) and related derivatives. There is currently no information available on the precise conformation of the unsaturated sisosamine moiety. We also do not know what the exact β-protonation shift is at C-1′ due to protonation of the 2′-amino-group in sisomicin (29), as no model monosaccharides were available from which to obtain this data. However, examination of the data in Tables 3 and 4 suggests that the β-protonation shift at C-1′ amounts to about −3.0 which is less than that observed for α-D-glycosides in the $^4C_1$ conformation. In sisomicin (29), 66—40 B (49), and 66—40 D (50) we observe a $\Delta\delta_0$ for C-4 of +6.7 to 7.0 for the free bases and of +6.6 to +7.4 for the protonated species. These values are in marked contrast to those observed for gentamicin C_{1a} (12) which is simply the 4′,5′-dihydro-analogue of sisomicin (29). It is evident that the sisosamine moiety is adopting a slightly different rotamer about the O–C-4 glycosidic bond in which moderate clockwise rotation has occurred relative to rotamer *a*, which is present in gentamicin C_{1a} (12). The fact that the rotamer population remains the same on protonation of the sisomicin derivatives is also noteworthy in spite of the fact that these compounds all contain 3- and 6′-amino-groups. We feel that in these molecules the different ring conformation of the sisosamine moiety is responsible for the reduced interaction of this sugar with the 5-hydroxy-group which leads to the observed rotamer in the case of the free bases. Upon protonation of the amino-groups in these derivatives the 3- and 6′-amino-groups would be further apart owing to the clockwise rotation about the O–C-4 glycosidic bond, but further repulsion would have been expected leading to greater clockwise rotation which was not observed. We feel that the reason this is so, is that the charge from the protonated 6′-amino-group is being essentially neutralized by delocalization over the vinyl ether system present in sisosamine (Figure 2) resulting in essentially no repulsion with the protonated 3-amino-group. Upon protonation, these compounds exhibit substantial deshielding at C-4′ and also substantial shielding at C-5′ ,which does not occur in the corresponding 6′-deamino-6′-hydroxy-analogues (32) and (113). This is borne out by the fact that in 66—40 C (protonated species only) (91), 6′-oxosisomicin (92), 6′-deamino-6′-hydroxysisomicin (32), 6′-deamino-6′-hydroxysisamine (113), and 6′-*N*-acetylsisomicin (31), where no protonatable amino-functionality is present at the 6′-position, similar deshieldings are

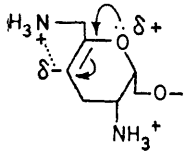

FIGURE 2 Mechanism of charge delocalization

observed at C-4 of +6.3 to +7.3 for the free bases and of +6.4 to +7.4 for the protonated species. These derivatives therefore have the same solution conformations as sisomicin (29), 66—40 B (49), and 66—40 D (50) about the O–C-4 glycosidic bond. When the 3-amino-group is acetylated as in 3-*N*-acetylsisomicin (30) the sisosamine undergoes a modest clockwise rotation about the O–C-4 glycosidic bond relative to sisomicin (29) as evidenced in the decreased net deshielding observed at C-4 to +4.9 for the free base. As expected, no change occurred on protonation. As in the case of 3-*N*-(*S*)-HABA-kanamycin A (6) discussed earlier, this effect is presumably steric in origin and is less pronounced in (30) than in (6).

When the 5-hydroxy-group of sisomicin was epimerized as in (33) the sisosamine moiety underwent a clockwise rotation about the O–C-4 bond relative to sisomicin (29), leading to an observed net deshielding of +4.9 for C-4 for the free base. Protonation resulted in this instance in a slight counter-clockwise rotation about the O–C-4 bond leading to a net deshielding in (33) of +6.6 for C-4, suggesting that at acidic pH the rotamer was similar to that observed for sisosamine in sisomicin (29) at basic pH. In (33) at both basic and acidic pHs, shielding was observed at both C-5 and C-1′ for the reasons given earlier,[39] although it was less pronounced than that observed in the case of 5-*epi*-gentamicin C_{1a} (18). This may well be due to the different ring conformation of sisosamine relative to purpurosamine A. When the 5-hydroxy-group was absent, as in 5-deoxysisomicin (34), we again observe a net deshielding at C-4 of +5.1 for both the free base and for the protonated species. Shielding was again observed at C-5 and C-1′ in (34). We feel that in both (33) and (34) the removal of the equatorial 5-hydroxy-group results in the observed clockwise rotation of the sisosamine unit about the O–C-4 glycoside bond leading to a rotamer approaching the type *h*. Replacement of the equatorial 5-hydroxy-group with an

equatorial 5-amino-group as in (35) produced a net deshielding at C-4 of +7.6 at both basic and acidic pH. This suggests that the rotamer adopted by sisosamine in (35) is one in which the sugar has rotated only slightly in a counterclockwise direction about the O–C-4 glycosidic bond relative to sisomicin (29). The 2′- and 5-amino-groups appear to be sufficiently far apart so as not to cause the rotamer to change upon protonation of the amino-groups. When the 5-amino-group is epimerized as in (36) we again see the adoption of a rotamer approaching *h* by the sisosamine, leading to a net deshielding for C-4 of +4.1 for the free base. Shielding is also evident at C-1′ and C-5.[39] Upon protonation of (36), a modest counterclockwise rotation occurs resulting in a net deshielding of +6.1 at C-4. Shielding is still observed at C-5. However, the shielding observed at C-1 in (36) on protonation has been reduced due to the counterclockwise rotation about the O–C-4 bond similar to that observed in (33) relative to (34). Acetylation of the 2′-amino-group in sisomicin to give (37) produced essentially no change in the rotamer population about the O–C-4 glycosidic bond relative to that observed for sisomicin (29).

When the 1-amino-group was epimerized as in 1-*epi*-sisomicin (40) enhanced net deshielding was observed at C-4 in both the free base and protonated species (Table 3). It was apparent from the chemical shift of C-1′ in (40) (Table 3) that this was not arising from rotation of the 4-*O*-glycoside about the O–C-4 glycosidic bond, and indeed no change in the usual sisosamine rotamer that is present in sisomicin (29) would be expected in this instance as the epimerized 1-amino-group is remote from this sugar in (40). The origin of this effect is not obvious.

We shall now turn our attention to the preferred rotamers adopted by the 6-*O*-glycoside about the O–C-6 glycosidic bond (Tables 3 and 5). The γ-protonation shifts at C-1″ associated with protonation of the 3″-amino-group were first assessed by considering the ^{13}C n.m.r. data for methyl β-L-garosaminide (100), methyl α-D-gentosaminide (101), and methyl 3-deoxy-3-methylamino-β-L-arabinopyranoside (102), which is given in Table 2. Data for methyl 3-amino-3-deoxy-α-D-glucopyranoside (103) were obtained from the literature.[16] It is evident from these model monosaccharides that protonation of the 3″-amino-group in an aminoglycoside would be expected to result in shielding of C-1″ amounting to −1.1 where (100) was present, to −0.8 where (101) was present, to −0.7 where (102) was present, and to −0.8 where (103) was present. The above protonation shifts were used in Table 5 to determine the rotational contributions at C-1″ accompanying protonation of the aminoglycosides.

It is evident from the data presented in Tables 3 and 5 that all the aminoglycosides that contain the 3″-amino-sugar units (100)—(103) in the molecule, namely the gentamicins C_{1a} (12), C_1 (14), A (71), A_1 (72), A_3 (74), and B (77), garamine (67), the semisynthetic derivatives (79)—(83), (87)—(90), (15)—(16), (6), (7), (8), and (22), kanamycin A (1), sisomicin (29), 66—40 B (49), 66—40 D (50), 66–40 C (91), and the sisomicin derivatives (30)—(32), (92), and (37) all adopt the same preferred rotamers about the O–C-6 glycosidic bond. Thus in the free bases a net deshielding of C-6 of +8.8 to +10.3 was observed, while at acidic pH the net deshielding increases to +10.6 to +11.7. In the protonated species the 6-*O*-glycoside adopts rotamer *b* about the O–C-6 glycosidic bond, while in the free bases the 6-*O*-glycoside appears to be slightly rotated in a clockwise direction about the O–C-6 glycosidic bond relative to *b* as evidenced by the slightly reduced deshielding at C-6.

In general a modest deshielding is also observed at C-1″ upon protonation. This deshielding is actually partly masked by the γ-protonation shielding effect at C-1″ and is actually numerically larger than it appears to be at first glance. The actual rotational contributions for C-1″ are given in Table 5 and they are in general similar to those observed at C-6 upon protonation of these molecules. In the case of 3-*epi*-gentamicin C_{1a} (17) the net deshielding observed at C-6 was greater than anticipated (Table 3). No rotation of the garosamine unit would be expected in (17) relative to gentamicin C_{1a} (12) and the chemical shift of C-1′ in (17) confirms this. Similar enhanced deshielding was observed at C-4 in 1-*epi*-sisomicin (40) and appears to be characteristic of the 1- and 3-*epi*-amino-derivatives. The 3″-methylamino-furanosyl derivatives of gentamine C_1 (84) and (85) both exhibited net deshielding of C-6 of +9.1 to +9.4 for the free bases indicating the presence of the same rotamer about the O–C-6 glycosidic bond as occurs in gentamicin C_{1a} (12) and the other 3″-aminopyranosyl-containing aminoglycosides. In this instance no change in rotamer was observed on protonation as evidenced by the absence of any change in the net deshielding at C-6. The observed shielding at C-1″ on protonation of (84) and (85) almost certainly arises from protonation of the 3″-methylamino-group, although this could not be verified as the appropriate models were not available. In gentamicin A_2 (70) which contains a 3″-hydroxy-group the same rotamers were observed for the free base and at acidic pH, as were observed for gentamicin A (71) which contains a 3″-methylamino-group. Similar rotamers were also observed for gentamicin A_4 (73) and 3″-*N*-acetylsisomicin (48) in which the 3″-methylamino-group is *N*-formylated and *N*-acetylated respectively.

When the equatorial 5-hydroxy-group of an aminoglycoside is either epimerized as in 5-*epi*-gentamicin C_{1a} (18), 5-*epi*-gentamicin C_1 (19), and 5-*epi*-sisomicin (33); or removed as in 5-deoxygentamicin C_2 (21) and 5-deoxysisomicin (34); or replaced by an equatorial amino-group as in 5-amino-5-deoxysisomicin (35); or replaced by an axial amino-group as in 5-*epi*-amino-5-deoxygentamicin C_1 (20) and 5-*epi*-amino-5-deoxysisomicin (36), we again observe a net deshielding at C-6 of +9.6 to +10.8 for the free bases and of +9.9 to +10.9 for the protonated species. These compounds therefore adopt a rotamer similar to that represented by *b* as does gentamicin C_{1a} (12) in the free-base form. The absence of slight coun-

terclockwise rotation upon protonation in these 5-modified derivatives relative to that observed for gentamicin C_{1a} (12) is also evident from the absence of any deshielding component at C-1'' which results in a net observed shielding of the anomeric carbon which arises from protonation of the 3''-amino-group (Table 5).

When modifications are effected at C-1 of an aminoglycoside we see some interesting changes in the rotamer population about the O–C-6 glycosidic bond [1,30] indicating that the substituent at C-1 is critical in determining the rotamer adopted by the 6-*O*-glycoside in an amino glycoside antibiotic, whereas that at C-5 has little effect on this sugar. When the equatorial 1-amino-group is replaced by an equatorial 1-hydroxy-group as in (23), (68), and (38) we observe a net deshielding at C-6 of +7.4 to +7.5 for the free bases. This reduction in the deshielding at C-6 indicates that the 6-*O*-glycoside has undergone a moderate clockwise rotation about the O–C-6 glycosidic bond in these derivatives resulting in an increase in the shielding component arising from increasing interaction between C-1''–O-5'' and C-6–H-6.[35-38] Some shielding is also evident at C-1'' in these molecules (Table 3) owing to interaction between 1''*eq*-H and C-1. On protonation of these compounds, a further decrease in the net deshielding observed at C-6 occurs to +5.9 to +6.5. Additional shielding is also evident at C-1'' at acidic pH (Table 3). This indicates that at acidic pH a further modest clockwise rotation of the 6-*O*-glycoside is occurring about the O–C-6 glycosidic bond relative to the free bases. When the equatorial 1-amino-group is replaced by an axial 1-hydroxy-group as in the sisomicin derivative (39) we observe a net deshielding at C-6 of +5.1 for the free base and of +4.9 for the protonated species. The reduction in deshielding is again occurring due to the introduction of a pronounced shielding interaction between the C-1''–O-5'' and C-6–H-6 bonds.[35-38] We also observe a pronounced shielding of both C-1 and C-1'' in these derivatives at both basic and acidic pH due to the introduction of a non-bonded interaction between 1*eq*-H and 1''*eq*-H as shown in Figure 3.[39] The slight

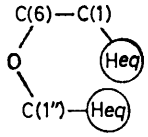

FIGURE 3 Non-bonded hydrogen interaction

deshielding observed at C-1'' upon protonation arises from protonation of the 3''-amino-group. It follows that the 6-*O*-glycoside in (39) adopts a rotamer closely resembling that represented by *i*. Epimerization of the amino-groups as in 1-*epi*-sisomicin (40) resulted in a marked reduction in the net deshielding at C-6 to +4.1 in the free base indicating that the 6-*O*-glycoside has adopted a rotamer represented by *i*.[35-38] Pronounced shielding is also evident at C-1 and C-1'' due to the interaction shown in Figure 2.[39] Protonation of (40) results in a substantial counterclockwise rotation of the 6-*O*-glycoside about the O–C-6 glycosidic bond resulting in a decrease in the shielding interaction between C-1''–O-5'' and C-6–H-6, which results in a net deshielding at C-6 of +8.1. Reduced shielding is also observed at C-1 relative to the free base (Table 3) and C-1'' is now deshielded (+2.3) relative to the free base. This is consistent with the observed counterclockwise rotation of the 6-*O*-glycoside about the O–C-6 glycosidic bond in the protonated species.

We shall next consider the effect of *N*-acylation of the 1-amino-group on the rotamer populations of the 6-*O*-glycoside about the O–C-6 glycosidic bond.[1] A variety of 1-*N*-acyl derivatives, namely (5), (78), (69), (9), (75), (76), (4), (24), and (43)—(46), all exhibit a marked decrease in the net deshielding at C-6 of +4.9 to +7.2 for the free bases and of +5.6 to +7.7 for the protonated species. A strong shielding component is thus evident at C-6 indicating that in all of these 1-*N*-acyl derivatives the 6-*O*-glycoside adopts a rotamer about the O–C-6 glycosidic bond that lies somewhere between rotamers *b* and *i* in each case.[1] Slight shielding is also observed at C-1'' in these derivatives (Table 3) due to protonation of the 3''-amino-group. In contrast, alkylation of the 1-amino-group as in netilmicin (41) [30,40] produces essentially no change in the rotamer population observed for the 6-*O*-glycoside about the O–C-6 glycosidic bond relative to that observed for sisomicin (29) (Tables 3 and 5). However, when the 1-*N*-ethylamino-group is epimerized as in 1-*epi*-netilmicin (42) [30] a marked reduction in deshielding is observed at C-6 to +4.5 in the free base, indicating that the 6-*O*-glycoside has adopted a rotamer represented by *i*.[35-38] Strong shielding is also evident at C-1 and C-1'' due to the interaction illustrated in Figure 3.[39] Protonation of (42) results in a substantial counterclockwise rotation of the 6-*O*-glycoside about the O–C-6 glycosidic bond resulting in a decrease in the shielding interaction between C-1''–O-5'' and C-6–H-6, which produces a net deshielding of +7.0 for C-6. The shielding observed at C-1 in the free base is also greatly reduced and C-1'' is now deshielded (+2.4) relative to the free base, consistent with the observed counterclockwise rotation of the 6-*O*-glycoside about the O–C-6 glycosidic bond. When the equatorial 2''-hydroxy-group was removed as in 2''-deoxysisomicin (47), a moderate clockwise rotation of the 6-*O*-glycoside about the O–C-6 glycosidic bond was observed relative to rotamer *b*. Thus a net deshielding at C-6 of +8.2 was observed for the free base (47). No change was observed on protonation which produced a slight deshielding at C-1'' as expected. It is evident from this result that the critical interaction that defines the rotamer adopted by the 6-*O*-glycoside is between the glycoside and the 1-substituent, rather than between the 2''- and 1-substituents.

It is of interest to compare the above rotamers for 6-*O*-axial glycosides with the results obtained for three 6-*O*-β-D-glycosides derived from gentamine.[21,22] Thus the 6-*O*-β-D-glycosides (95), (96), and (97) all exhibit a rotamer approximating that represented by *d* for the free bases.[21,22] This results in an observed net deshield-

ing at C-6 of +8.9 to +9.4 for the free bases. Upon protonation the deshielding at C-6 is reduced to +7.2 to 7.4 indicating that the 6-*O*-glycoside has undergone a modest counterclockwise rotation about the O–C-6 glycosidic bond relative to rotamer *d*. Slight shielding is also observed at C-1″ in these derivatives following protonation.

In conclusion it is obvious from the data presented in this study that the solution conformations of the aminoglycoside antibiotics are far more complex than had previously been appreciated. The preferred rotamers observed about the O–C-4 and O–C-6 glycosidic bonds are defined not only by the chirality of C-4, C-1′, C-6, and C-1″, but also by the nature of the substituents present at C-3, C-5, C-2′, and C-6′ in the case of the 4-*O*-glycoside, and by C-5, C-1, and C-2″ in the case of the 6-*O*-glycoside. Steric effects, charge repulsion effects, and possibly dipolar and hydrogen-bonding effects all appear to play an important role and it is the final balance between all these factors that ultimately defines the solution conformations of these aminoglycoside antibiotics. From the data presented above it is apparent that the basic principles of the ' Nagabhushan–Daniels Rule ' can still be successfully applied to the determination of the absolute stereochemistry of the glycosyl units of an aminoglycoside antibiotic provided that the rotamer populations are first established by the methods described here. It is also evident from this study that the $\Delta\delta_C$ values for C-4 and C-6 are much more sensitive to changes in the rotamer populations about the O–C-4 and O–C-6 bonds respectively, than are the $\Delta\delta_C$ values at C-3, C-5, and C-1. Great care must also be taken to ensure that the free bases are freshly and fully decarbonated, or the data will be meaningless.

Recent nuclear Overhauser effect (n.O.e) studies carried out by Lemieux [41] on kanamycin A (1) in D_2O solution where 1′-H and 1″-H were saturated, resulted in similar signal enhancement at 4-H, 2′-H, 6-H, and 2″-H for the free base and for the protonated species. The ^{13}J (C-1′–4-H) and ^{13}J (C-1″–6-H) values were also reported to be similar for both the free base and for the protonated species. It was concluded that very slight changes only were occurring in the glycoside rotamers about the C-1′–O and C-1″–O bonds in going from the free base to the protonated species. Lemieux [42] recently reported similar results for the Lewis human blood-group determinants in an elegant study and he concluded that protonation of the amino-groups produced only minor changes in torsion angle about the glycosidic bonds. The observed chemical-shift changes were attributed to changes in the hybridization of the atoms about the glycosidic bonds, which resulted in changes in the valence angles and an increase in the ϕ^H angle upon protonation. In our analysis of the data, which is purely qualitative, we have chosen to maintain the *exo*-anomeric effect and hence keep ϕ^H constant, while changing the ψ^H torsion angle. We offer the qualitative hypotheses outlined in this paper as an alternative to explain the observed ^{13}C n.m.r. data and the protonation shifts, fully realizing that further research is needed to either prove, or disprove, these proposals.

We thank Dr. R. Brambilla and Mr. S. Mittleman for running the ^{13}C n.m.r. spectra. One of us (A.K.M.) also gratefully acknowledges Drs. Daniels, McCombie, Nagabhushan, Rane, and Wright for permission to draw on much of their unpublished data for this study. Without the co-operation this comprehensive study could never have been carried out.

[0/1622 *Received*, 21*st October*, 1980]

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