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^{1}H-NMR Analysis of Copper-Aminoglycoside Complexes in Solution and Its Implication for Regioselective Modification of Multifunctional Aminoglycoside Antibiotics

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Abstract: A method for analysis of complexes of the cupric ion with aminoglycoside antibiotics based on the measurement of the paramagnetic contribution of the cupric ion to T_1 relaxation time on ^{1}H-NMR spectra of the antibiotics is described. The information from the NMR experiments was supplemented by molecular modeling studies and proved valuable in predicting the reactivities of these complexes toward reagents for modification of amines, which were used in regioselective, and often regiospecific, derivatization of these important antibiotics. © 1998 Elsevier Science Ltd. All rights reserved.

Many aminoglycoside antibiotics that are being used in the clinic today are semisynthetic products that have been developed by selective modification of specific amino groups of natural aminoglycoside precursors,[1] such as sisomicin (**1**), kanamycin A (**2**) or gentamicin B (**3**). For example, netilmicin[2] (**4**) is the 1-N-ethyl-derivative of sisomicin, amikacin[3] (**5**) is 1-N-(4-amino-(2*S*)-2-hydroxybutyryl)-kanamycin A, whereas isepamicin[4] (**6**) is 1-N-(3-amino-(2*S*)-2-hydroxypropanoyl)-gentamicin B. Recent studies on the toxicity of such antibiotics have shown that the amines in these molecules play an important role in both aminoglycoside acute toxicity[5] and nephrotoxicity,[6] either due to their basic character[5] or their interactions with phospholipid bilayers of the inner cortex tissue.[6] Thus, selective modification of aminoglycoside amines has led to products with reduced toxicity.[5,6] Furthermore, bacterial resistance to aminoglycosides is widespread among pathogens. Recent efforts in mechanistic studies of the enzymes of aminoglycoside resistance have provided additional impetus for the development of additional synthetic analogues of aminoglycosides as mechanistic tools.[7,8]

This synthetic objective presents, however, challenging obstacles since aminoglycosides often possess four to six amines of comparable reactivity. The problem has been addressed in the past either by taking advantage of the regiochemical preferences of aminoglycosides, which are due to steric reasons and/or the varying basicity of aminoglycoside amino groups,[9] or by employing selective temporary protection schemes of aminoglycoside amino groups with transition-metal ions (Cu^{2+}, Zn^{2+}, Cd^{2+}, etc.).[10] This type of temporary protection is believed to proceed through intermediary chelates between proximal (vicinal or non-vicinal) amino and hydroxyl groups of the aminoglycoside molecule, thus rendering the coordinated amino group less reactive to electrophilic reagents, and allowing others to react preferentially with the reagent. Among the metal ions that have been used in such temporary protection schemes, cupric ion is the most common.[10] Although this scheme has proved invaluable for the selective modification of aminoglycoside amines, the examples that have been

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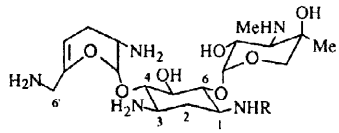
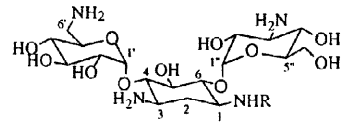
reported are limited to aminoglycosides that are not presently in clinical use, and in addition, as we will discuss below, the regioselectivity that can be achieved by solely employing this scheme is often modest.

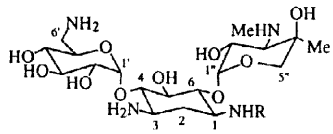
Furthermore, the mechanism of the process has not been studied and, with only two exceptions,[11] the structures of the intermediary chelates have merely been postulated. As a result, the regioselectivity that one should expect from the temporary protection scheme with the cupric ion remains today mostly unpredictable *(vide infra)*. We report herein on a ^{1}H-NMR-based method which uses T_1 proton relaxation time measurements, supplemented by molecular modeling, as a versatile tool for gaining insight into the structures of the cupric ion chelates with aminoglycosides, as well as on the mechanism for regioselective modification of aminoglycoside amines by temporary metal protection schemes. These studies allowed applications for regioselective modification of aminoglycoside amino groups, which support our structural and mechanistic studies.



1 R = H
4 R = Et



2 R = H
5 R = COC*H(OH)$CH_2CH_2NH_2$

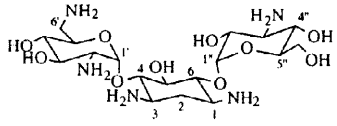


3 R = H
6 R = $COC^{*}_{2'''}H(OH)CH_2NH_2$

RESULTS AND DISCUSSION

The interactions of two aminoglycoside molecules, kanamycin A (**2**) and ribostamycin, with cupric ion have been briefly described in the past by observing the specific line broadening induced to ^{13}C-NMR resonances in the presence of a low concentration of these paramagnetic ions.[11] A strong line broadening was observed for the 3"-, 4"-, and *possibly* 2"- and 5"- carbons of kanamycin A as a result of coordination of the cupric ion to the kanosamine portion of the molecule. The ambiguity in these conclusions was due to the complexity of ^{13}C-NMR spectra of aminoglycosides, which consisted of several interfering signals that in most cases have only tentatively been assigned.[12] However, a more serious problem was encountered in the identical experiment with ribostamycin. The ^{13}C line broadening in this case was not specific enough to permit the identification of any particular chelation sites.[11] In an effort to overcome these limitations in analysis of these metal complexes, and to build a firm basis for regiospecific synthetic modifications of these important biologically active compounds, we chose to study the interactions of the cupric ion with aminoglycosides by their ^{1}H-NMR spectra, and more specifically, by measuring the relaxation induced by the cupric ion to aminoglycoside protons in the vicinity of its coordination site(s). The high natural abundance of ^{1}H nuclei would permit accurate measurements of induced relaxation and these measurements would then be applied for unambiguous identification, as well as quantification, of the interactions between the cupric ion and various sites in aminoglycosides. An obvious prerequisite for this effort was the availability of complete assignments for

aminoglycoside ^{1}H-NMR spectra, which are in general very complex. A series of selective ^{1}H-^{1}H homonuclear decoupling and NOE experiments at 500 MHz enabled us to identify the entire sets of spectra and provide full and unambiguous assignments[13] for the ^{1}H-NMR spectra of four representative aminoglycosides, kanamycin A (**2**), karamycin B (**7**), neamine (**8**) and isepamicin (**6**).



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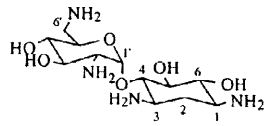

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In view of these considerations, we chose to quantify the relaxation induced by the cupric ion to protons of coordinated aminoglycosides by measuring the paramagnetic contribution to nuclear T_1 relaxation time[14] (T_1^e) for each aminoglycoside proton, at a standard low concentration of the cupric ion (*ca.* 2 x 10^{-5} M), in D_2O. We found that this low concentration of the cupric ion largely enhanced the relaxation of specific protons, whereas changes in relaxation of other protons were not of an appreciable magnitude (see Tables 1-4). This effect is due to preferential coordination of the cupric ion to amine, hydroxyl or glycosidic oxygen functions geminal to the affected protons. Chelates other than those identified by this method may potentially form only with lower affinity for the cupric ion, but those identified under the copper-deficient conditions that we have used (*ca.* 5 x 10^{-4} mole Cu^{2+}/mole of aminoglycoside) must be considered the most favored, and hence the ones that would confer protection to their amine ligands. On the other hand, there is no absolute T_1^e value that could be considered as a clear evidence for a stable chelate, mainly because T_1^e values are highly dependent on concentration of the paramagnetic ions. However, the trends that are established from published results,[7,8,10,11] as well as those discussed herein, for the outcome of reactions in the presence of the cupric ion provide support for the unambiguous protection of the 2'-amino group of neamine, 3"-amino group of kanamycins A and B, as well as 3"'-amino group of isepamicin. The range for the measured T_1^e values for protons geminal to these sites (*i.e.* $H_{2'}$ of neamine, $H_{3''}$ of kanamycins A and B, and $H3'''_{R,S}$ of isepamicin) is from 0.83 to 3.98 s (see Tables 1-4), and it is thus reasonable to assume these numbers to be the *optimal range of T_1^e values for the specific cupric ion concentration used.* Should the distances from copper (II) to amine ligands be approximately the same in the various chelates formed (*vide infra*), the T_1^e values will solely depend on the average time the cupric ion spends on a specific amino or hydroxyl group, and consequently T_1^e values lower than 3.98 s should in general be evidence of efficient coordination of the cupric ion to the corresponding sites, under the conditions employed by us. We hasten to add that the average distances of 1.9–2.1 Å that our survey of the copper complexes revealed indicates that the average bond length to either oxygen or nitrogen ligands do not vary much, hence the assumption here is not unreasonable.

In Tables 1-4 we report the proton T_1^e values measured for solutions (*ca.* 40 mM) of the free base forms of the aforementioned aminoglycosides in deuterium oxide containing the standard low concentration of copper (II) acetate.

Table 1. Paramagnetic Contribution to Nuclear T_1 Relaxation Times (T_1^e) for Protons of Kanamycin A free base (**2**), measured in the presence of 5 x 10^{-4} moles of Copper (II) Acetate / mole of Aminoglycoside in D_2O at pH 9.5, at 500 MHz.

Proton	$T_{1(dia)}$ (s)	$T_{1(para)}$ (s)	ΔT_1 (s)	T_1^e(*) (s)	Proton	$T_{1(dia)}$ (s)	$T_{1(para)}$ (s)	ΔT_1 (s)	T_1^e(*) (s)
H_1	0.80	0.78	0.02	-	$H_{5'}$	1.02	0.99	0.03	-
H_{2ax}	0.31	0.33	-0.02	-	$H_{6'R}$	0.44	0.45	-0.01	-
H_{2eq}	0.31	0.33	-0.02	-	$H_{6'S}$	0.44	0.45	-0.01	-
H_3	0.80	0.78	0.02	-	$H_{1''}$	0.81	0.62	0.19	2.65
H_4	0.77	0.75	0.02	-	$H_{2''}$	1.20	0.57	0.63	1.09
H_5	0.99	0.87	0.12	7.18	$H_{3''}$	1.56	0.54	1.02	0.83
H_6	0.75	0.72	0.03	-	$H_{4''}$	1.32	0.63	0.69	1.21
$H_{1'}$	0.81	0.80	0.01	-	$H_{5''}$	1.02	0.70	0.32	2.23
$H_{2'}$	1.3	1.27	0.03	-	$H_{6''R}$	0.45	0.41	0.04	-
$H_{3'}$	1.88	1.80	0.08	42.30	$H_{6''S}$	0.45	0.41	0.04	-
$H_{4'}$	1.37	1.29	0.08	22.09					

* T_1^e values for differences in T_1-relaxation times which fall within the experimental error ($|\Delta T_1| \leq 0.04$ s) are not shown.

Table 2. Paramagnetic Contribution to Nuclear T_1 Relaxation Times (T_1^e) for Protons of Kanamycin B free base (**7**), measured in the presence of 5 x 10^{-4} moles of Copper (II) Acetate / mole of Aminoglycoside in D_2O at pH 10.4, at 500 MHz.

Proton	$T_{1(dia)}$ (s)	$T_{1(para)}$ (s)	ΔT_1 (s)	T_1^e(*) (s)	Proton	$T_{1(dia)}$ (s)	$T_{1(para)}$ (s)	ΔT_1 (s)	T_1^e(*) (s)
H_1	0.83	0.78	0.05	12.95	$H_{5'}$	0.98	0.84	0.14	5.88
H_{2ax}	0.34	0.33	0.01	-	$H_{6'R}$	0.44	0.39	0.05	3.43
H_{2eq}	0.34	0.33	0.01	-	$H_{6'S}$	0.44	0.39	0.05	3.43
H_3	0.83	0.78	0.05	12.95	$H_{1''}$	0.81	0.72	0.09	6.48
H_4	0.78	0.66	0.12	4.29	$H_{2''}$	1.16	0.78	0.38	2.38
H_5	0.99	0.87	0.12	7.18	$H_{3''}$	1.47	0.81	0.66	1.80
H_6	0.73	0.69	0.04	-	$H_{4''}$	1.27	0.84	0.43	2.48
$H_{1'}$	0.84	0.72	0.12	5.04	$H_{5''}$	0.98	0.77	0.21	3.59
$H_{2'}$	1.09	0.87	0.22	4.31	$H_{6''R}$	0.50	0.42	0.08	2.63
$H_{3'}$	1.61	1.26	0.35	5.80	$H_{6''S}$	0.50	0.42	0.08	2.63
$H_{4'}$	1.23	1.08	0.15	8.86					

* T_1^e values for differences in T_1-relaxation times which fall within the experimental error ($|\Delta T_1| \leq 0.04$ s) are not shown.

Table 3. Paramagnetic Contribution to Nuclear T_1 Relaxation Times (T_1^e) for Protons of Neamine free base (**8**), measured in the presence of 5 x 10^{-4} moles of Copper (II) Acetate / mole of Aminoglycoside in D_2O at pH 10.2, at 500 MHz.

Proton	$T_{1(dia)}$ (s)	$T_{1(para)}$ (s)	ΔT_1 (s)	T_1^e(*) (s)	Proton	$T_{1(dia)}$ (s)	$T_{1(para)}$ (s)	ΔT_1 (s)	T_1^e(*) (s)
H_1	0.92	0.66	0.26	2.34	$H_{1'}$	0.84	0.68	0.16	3.57
H_{2ax}	0.35	0.35	0.00	-	$H_{2'}$	1.09	0.59	0.50	1.29
H_{2eq}	0.37	0.36	0.01	-	$H_{3'}$	1.68	0.94	0.74	2.13
H_3	0.86	0.74	0.12	5.29	$H_{4'}$	1.24	0.87	0.37	2.92
H_4	0.74	0.65	0.09	5.34	$H_{5'}$	1.04	0.87	0.17	5.32
H_5	1.15	0.80	0.35	2.63	$H_{6'R}$	0.45	0.46	-0.01	-
H_6	1.31	0.85	0.46	2.42	$H_{6'S}$	0.44	0.45	-0.01	-

* T_1^e values for differences in T_1-relaxation times which fall within the experimental error ($|\Delta T_1| \leq 0.04$ s) are not shown.

Table 4. Paramagnetic Contribution to Nuclear T_1 Relaxation Times (T_1^e) for Protons of Isepamicin free base (**6**), measured in the presence of 5 x 10^{-4} moles of Copper (II) Acetate / mole of Aminoglycoside in D_2O at pH 10.3, at 500 MHz.

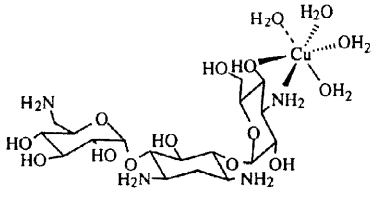
Proton	$T_{1(dia)}$ (s)	$T_{1(para)}$ (s)	ΔT_1 (s)	T_1^e(*) (s)	Proton	$T_{1(dia)}$ (s)	$T_{1(para)}$ (s)	ΔT_1 (s)	T_1^e(*) (s)
H_1	0.78	0.77	0.01	-	$H_{6'R}$	0.45	0.44	0.01	-
H_{2ax}	0.33	0.31	0.02	-	$H_{6'S}$	0.45	0.44	0.01	-
H_{2eq}	0.33	0.31	0.02	-	$H_{1''}$	0.91	0.77	0.14	5.00
H_3	0.83	0.77	0.06	10.65	$H_{2''}$	1.48	1.17	0.31	5.59
H_4	0.73	0.73	0.00	-	$H_{3''}$	0.99	0.84	0.15	5.54
H_5	0.91	0.91	0.00	-	4''-Me	0.50	0.49	0.01	-
H_6	0.88	0.81	0.07	10.18	$H_{5''ax}$	0.42	0.41	0.01	-
$H_{1'}$	0.83	0.77	0.06	10.65	$H_{5''eq}$	0.36	0.34	0.02	-
$H_{2'}$	1.22	1.22	0.00	-	$H_{2'''}$	1.72	1.20	0.52	3.97
$H_{3'}$	1.50	1.50	0.00	-	$H_{3'''R}$	0.51	0.46	0.05	3.98
$H_{4'}$	1.27	1.27	0.00	-	$H_{3'''S}$	0.51	0.46	0.05	3.98
$H_{5'}$	1.05	1.03	0.02	-	N-Me	0.95	0.88	0.07	11.94

* T_1^e values for differences in T_1-relaxation times which fall within the experimental error ($|\Delta T_1| \leq 0.04$ s) are not shown.

As shown in Table 1, relaxation of the ring protons of the kanosamine portion of kanamycin A ($H_{1''}$ to $H_{5''}$) is greatly affected by the cupric ion, with $H_{3''}$ proton suffering the most profound paramagnetic effect ($T_1^e = 0.83$ s). A careful examination of these results revealed that protons with the same approximate distance

from $H_{3''}$ have comparable T_1^e values (1.09 and 1.21 s for $H_{2''}$ and $H_{4''}$, respectively, as well as 2.65 and 2.23 s for $H_{1''}$ and $H_{5''}$, respectively). This indicates that the $H_{2''}$ and $H_{4''}$, and the $H_{1''}$ and $H_{5''}$ pairs of protons, are individually affected by complexes originating from and comprising the 3''-amine.

In the light of the NMR results and the structural requirements for an octahedral structure (see Experimental Section), we have concluded that the principal Cu^{2+} chelates of kanamycin A (**2**) are structures **9**, **10** and **11**. Interestingly, the ring oxygen of the kanosamine portion appears to take part in a chelate with the 3''-amine because of the induced relaxation effect on $H_{1''}$ and $H_{5''}$ protons. Computer modeling showed that such a coordination for Cu^{2+} requires a twisted boat conformation for this ring (see structure **11**, and the text in the Experimental Section). Energy calculations with the TRIPOS force field showed that this structure possesses a somewhat higher energy than the more favorable structures **9** and **10**, and this result is consistent with the higher T_1^e values, with respect to T_1^e values for other protons of the kanosamine ring, measured for $H_{1''}$ and $H_{5''}$ protons.[15] It is noteworthy that our results provide no evidence for the existence of a complex with the pair of the non-vicinal 1-amino and 2''-hydroxyl groups, that had been postulated previously.[10b]



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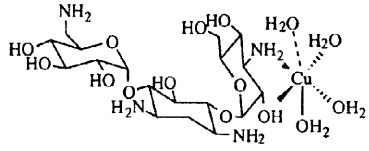

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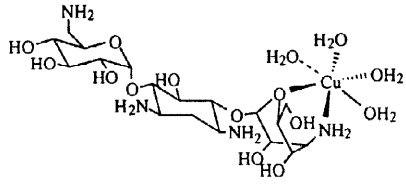

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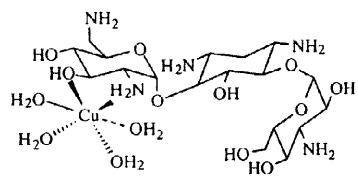
Kanamycin B (**7**) showed a weaker coordination in the kanosamine ring than did kanamycin A, as evidenced from the relatively higher T_1^e values shown in Table 2. This is most likely due to the presence in the molecule of an additional amino group which offered the cupric ion more possibilities for coordination than in the case of kanamycin A. Nevertheless, kanamycin B seems to follow the coordination pattern of kanamycin A at the kanosamine portion, whereas additional coordination at the glucosamine portion is shown with structures **12** and **13**.



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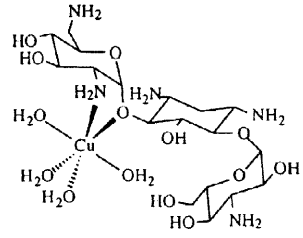

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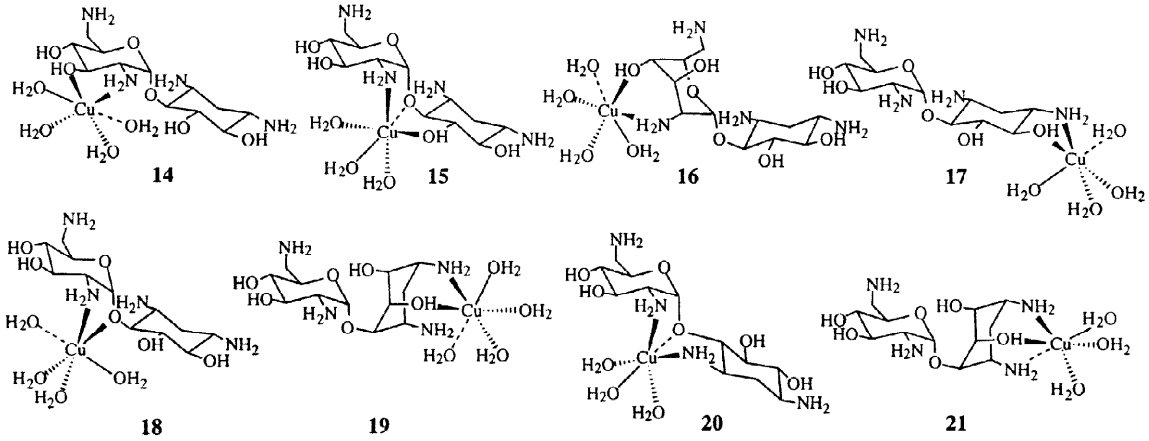
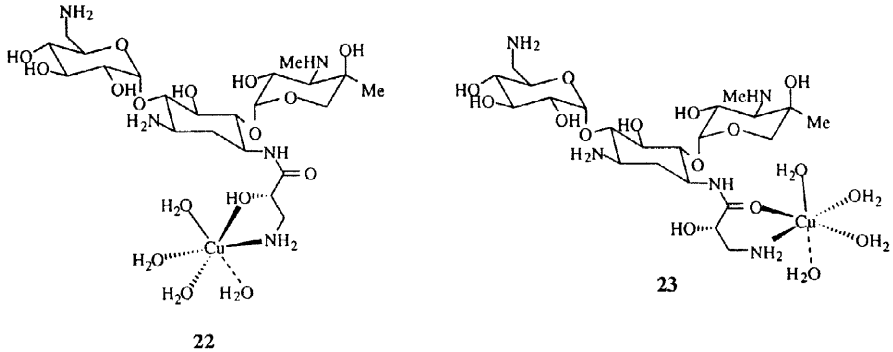
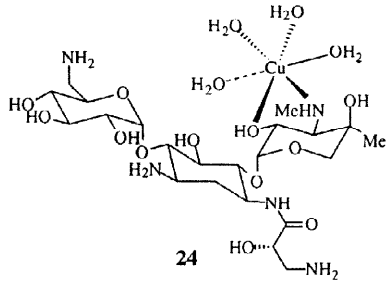
Neamine (**8**) showed a coordination pattern that was much more complicated than that of kanamycins A and B, as evidenced from the data of Table 3. Two of the four amino groups of the molecule, the 2'- and 1-amino groups, coordinated strongly with the cupric ion (T_1^e values of 1.29 and 2.34 s for $H_{2'}$ and H_1 protons, respectively) to form various plausible chelates (see structures **14-21**). On the basis of data shown in Table 3, structures **14**, **16**, **17**, and **19** appear to be predominant in the solution, whereas structures **15**, **18**, **20** and **21** seem to have a limited, and negligible, contribution to the total solution structure (i.e., T_1^e value of 5.29 s for H_3 proton).



Isepamicin (**6**) showed strong coordination at its N_1-side chain. Thus, the data presented in Table 4 revealed that structure **22** is one of the primary complexes in the complexed mixture. We thought, however, of examining the possibility of chelate **23** as well, since this coordinated species seemed plausible from modeling and the lack of hydrogen at the $C_{1'''}$ could conceal its presence in the solution by ^{1}H-NMR analysis. Thus, when the ^{13}C-spectrum of isepamicin was recorded in the presence of 3.0 x 10^{-3} moles Cu^{2+}/mole of aminoglycoside, a severe line broadening was observed for $C_{1'''}$ and $C_{3'''}$ signals (174.9 and 43.7 ppm, respectively), whereas the $C_{2'''}$ signal could not be observed due to interference with other signals. Therefore, chelate **23** would appear to be a stable coordinated species in solution. The T_1^e value of 5.59 s for $H_{2''}$ proton suggests, on the other hand, that a weak coordination of the cupric ion with the pair of 3''-methylamino and 2''-hydroxyl groups is possible (structure **24**).





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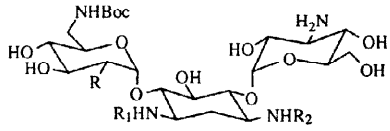
The chelates identified above explain the regioselectivity which is observed in reactions of aminoglycosides with amine-protecting reagents in the presence of the cupric ions (*vide infra*). However, it should be borne in mind that such multiple-site reaction systems are very complex and the regioselectivity of a given reaction is oftentimes the combined result of several interacting factors. Apart from the structures of the intermediary chelates, steric hindrance, intramolecular hydrogen bonding, the stoichiometry of the reactants, the nature of the solvent, as well as the pH of the reaction mixture, are factors of great importance to the regiochemistry of these reactions. However, steric hindrance and the structures of the intermediary chelates appear to be the most significant among these factors.

An observation during our synthetic studies was, for example, that these reactions proceeded in a stepwise fashion with the 6'-amino group to react with electrophilic reagents in a much faster rate than other amino groups. Thus, when the reaction of kanamycin A (**2**) with di-*t*-butyldicarbonate (2.0 equivs.) in the presence of copper (II) acetate (4.0 equivs.)[16] in DMSO[17] was monitored by tlc, a single intermediate was observed after 30 min. This intermediate gradually converted over the course of 2 h to the final product of the reaction, which turned out to be 3,6'-di-N-(*t*-Boc)-kanamycin A (**25**). In an identical experiment, the reaction was stopped after 30 min and the intermediate was isolated and was identified to be 6'-N-(*t*-Boc)-kanamycin A (**26**). The rate for the reaction at the 6'-amino group is therefore anticipated to be faster than that for the reaction at the 3-amino group due to reduced hindrance, which would account for the stepwise process. In a similar manner, when kanamycin B (**7**) was treated with copper (II) acetate (4.0 equivs.) and di-*t*-butyldicarbonate (3.0 equivs.) in DMSO, a mixture of three products was observed by tlc after 2h. The anticipated major intermediate was 6'-N-(*t*-Boc)-kanamycin B (**27**). The other products were a dicarbamoylated species and the tricarbamoylated 1,3,6'-tri-N-(*t*-Boc)-kanamycin B (**28**), which proved to be the final product. The proportion of the tricarbamoylated product in the mixture increased over time, and it (*i.e.*, **28**) was the only product after 24 h. The stepwise process hence appears to be a general one and to operate on a steric hindrance basis, with the 6'-monocarbamoylated product to be the first intermediate to form. Therefore, the introduction of a second or a third Boc group to the aminoglycoside molecule seems to take place at slower rates.[18] Another factor besides hindrance in the modifications of the secondary sites could be the extent of coordination of these sites to the metal. If poorly coordinated by the metal, the sites would have the opportunity to react with the reagent, albeit slowly.

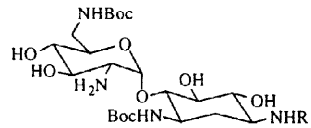
The absence of reaction for the 2'- and 3''-amino groups of kanamycin B as well as for the 3''-amino group of kanamycin A which was observed in the synthetic experiments mentioned above, are well explained by the presence in the reaction mixture of the chelates identified for these molecules from the NMR results.

However, the difference in reactivity which was observed between the 1-amino group of kanamycin B, which reacted with the reagent to give the corresponding carbamate, and the similar group of kanamycin A, which remained unaffected by the reagent, was an unexpected result, in view of the fact that we have shown above that the 1-amino groups of both kanamycins was not coordinated to the cupric ion. Although the possibility that the aminoglycoside-Cu^{2+} chelates which form in DMSO, which was used as the solvent of the reaction, are different from those identified from our NMR results in D_2O cannot be excluded, there are indications that this difference is due a hydrogen bonding between the 1-amino and the 2''-hydroxyl groups of kanamycin A.[19] Such a hydrogen bond would explain the low reactivity of the 1-amino group as this amino group would have a low nucleophilicity due either to its function as an intramolecular hydrogen bond acceptor or to steric hindrance that may result from such a hydrogen bonding. On the other hand, the presence of an additional, with respect to kanamycin A, amino group at $C_{2'}$ of kanamycin B seems to give rise to a different intramolecular hydrogen bond network[20] does not affect the 1-amino group of this molecule.



25 R = OH, R_1 = Boc, R_2 = H
26 R = OH, R_1 = R_2 = H
27 R = NH_2, R_1 = R_2 = H
28 R = NH_2, R_1 = R_2 = Boc

Other aspects of the regiochemistry of these reactions were revealed from synthetic experiments with neamine (**8**) and isepamicin (**6**). Thus, when neamine (**8**) underwent reaction with 2-3 equivs. of di-*t*-butyldicarbonate in the presence of copper (II) acetate (4.0 equivs.) in DMSO to afford an 1:1 mixture of the anticipated 3,6'-di-N-(*t*-Boc)-derivative (**29**), along with the 1,3,6'-tri-N-(*t*-Boc)-derivative (**30**).[8] Cupric ion provided an efficient protection for the 2'-amino group in this case, but failed to do so for the 1-amino group. This result most likely indicates that the simultaneous coordination of two cupric ions to the various chelation sites of a given neamine molecule is less favorable than the coordination of a single ion, a notion which was supported by our modeling studies. In addition, electrostatic repulsion of the two metal ions would also make it difficult for two such ions to coordinate with one aminoglycoside molecule. As a result, doubly coordinated neamine species should be relatively rare in solution, and therefore only the amino group which gives the most stable chelates (*i.e.*, the 2'-amino group) would be efficiently protected by the cupric ions. Nevertheless, the considerable amount of the dicarbamoylated derivative (**29**) that was formed indicated that the cupric ion did actually coordinate to the 1-amino group and lowered its reactivity.

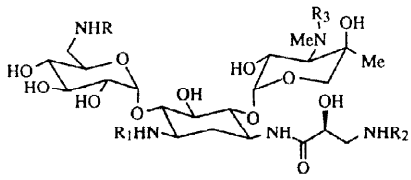


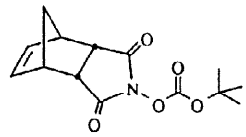
29 R = H
30 R = Boc

Isepamicin (**6**) underwent reaction with di-*t*-butyldicarbonate (4.0 equivs.) in the presence of copper (II) acetate (4.0 equivs.) in DMSO to produce a complex mixture of products, which could not be separated by

column chromatography. The ^{1}H- and ^{13}C-NMR spectra of the mixture suggested that the major products were 3,6'-di-N-Boc-isepamicin (**31**) and 3",6'-di-N-Boc-isepamicin (**32**), thus confirming the strong and weak coordination of the cupric ion to 3'''- and 3''- amino groups, respectively, as suggested by the NMR results.

The structures of the intermediary chelates, as well as steric hindrance and intramolecular hydrogen bonding are hence factors which govern the regiochemistry of these reactions. The regioselectivity that can be achieved, although satisfactory in some cases, is in general modest. We found, however, that high regioselectivity, even regiospecificity, could be achieved when temporary copper protection scheme was combined with the use of a hindered carbamoylating reagent such as N-(*t*-butoxycarbonyloxy)-5-norbornene-*endo*-2,3-dicarboximide (**33**), whose application to the regioselective protection of aminoglycoside amines we have reported earlier.[9b] Thus, when isepamicin (**6**) was stirred with copper (II) acetate (4.0 equivs.) in DMSO, and was subsequently treated with reagent **33** (1.0 equiv.), the 6'-N-(*t*-Boc) derivative (**34**) was obtained as the sole product of the reaction in 83% isolated yield. The cupric ion protected the 3'''-amino group efficiently, as mentioned above, whereas the steric hindrance of reagent **33** permitted the reaction to occur exclusively at the 6'-amine. It is noteworthy that the identical experiment in the absence of copper (II) acetate led to an inseparable mixture of 6'-N-(*t*-Boc)- (**34**) and 3'''-N-(*t*-Boc)- (**35**) derivatives of isepamicin.[9b] In a similar manner, when neamine (**8**) was stirred with copper (II) acetate (4.0 equivs.) in DMSO, and was subsequently treated with reagent **33** (3.5 equivs.), *t*-Boc group was introduced at 3- and 6'-amino groups affording the 3,6'-di-N-(*t*-Boc) derivative (**29**) as the sole product of the reaction, whereas the 1-amino group was unaffected by the reagent.





33

31 R= R_1= Boc, R_2= R_3= H
32 R= R_3= Boc, R_1= R_2= H
34 R= Boc, R_1= R_2= R_3= H
35 R= R_1= R_3= H, R_2= Boc

In conclusion, we have described the use of a versatile NMR technique in gaining insight into the nature of metal complexes of multifunctional aminoglycoside antibiotics with the cupric ion. These experiments were carried out such that at any given time only one metal ion was interacting with a given aminoglycoside. An interesting outcome of these analyses with each of the aminoglycosides studies by us was that these complexes exist in multiple forms. Nevertheless, the equilibrium mixtures of these metal complexes were useful in synthetic preparation of aminoglycoside derivatives in a regioselective, and often regiospecific manner. The generality of the utility of the NMR analysis for knowledge of the sites of coordination, and the synthetic applications should find further applications both in aminoglycoside chemistry, as well as in other systems in the future.

EXPERIMENTAL SECTION

General Procedures

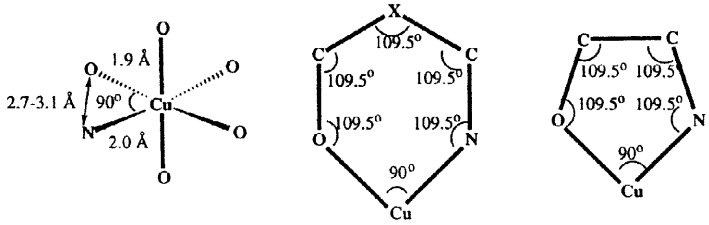
^{1}H- and ^{13}C-NMR spectra were obtained at 500- and 125-MHz, respectively, on a Varian U-500 spectrometer. Chemical shift values (δ) are given in ppm and they are referenced to the deuterium of the lock solvent. NOE experiments were carried out with a repetition rate of 4.9 s. Proton T_1 relaxation times were determined by the method of inversion recovery with a d_1 delay of

10.0 s and an experimental error of ± 0.04 s was estimated. The pH values of NMR samples in deuterium oxide were measured with a Radiometer RHM82 pH-meter and they are uncorrected for deuterium. All aminoglycosides were used in the free-base form, which were prepared from the corresponding ammonium salts by the use of Amberlite IRA 400 (OH^-) strongly basic ion-exchange resin and were used promptly afterwards. Infrared and mass spectra were recorded on a Nicolet DX and a Kratos MS 80RFA spectrometers, respectively. Melting points were taken on a Hoover UniMelt apparatus and are uncorrected. All the aminoglycoside carbamate products sintered at approximately 60 °C and charred above 120 °C with concomitant release of CO_2. Thin-layer chromatograms were made on silica gel and were visualised by spraying with a ninhydrin solution followed by heating. When ammonia was used in the solvent system the thin-layer chromatograms were briefly heated to remove ammonia prior to spraying with the ninhydrin solution. Isepamicin and kanamycin B sulfates were a generous gift from the Schering-Plough Corp. and the Meiji Seika Kaisha, respectively. Neamine hydrochloride was prepared from neomycin sulfate by methanolysis.[9b] Kanamycin A and neomycin sulfates were purchased from the Sigma Chemical Co. All other reagents, were purchased from the Aldrich Chemical Company.

Modeling and Energy-Minimization Protocols

The TRIPOS force-field parameters for copper atom were used. MOPAC MNDO ESP charges were used for the aminoglycoside cores. Among the 152,464 structures available to us from the Cambridge Structural Data Bank,[21] more than 7,000 entries possess copper. Our analysis of these structures revealed that 22% of the entries had copper ligands arranged in octahedral coordination. Furthermore, Cu^{2+} complexes of aminoglycosides have been shown previously to be octahedral structures.[22] The crystal structure of kanamycin A[18] was used for model building and in most cases, two coordination sites for copper were provided by functional groups of the aminoglycoside, two were occupied by water molecules and the remaining two were filled by two hydroxyls (to produce a neutral species with the Cu^{2+} ion). The complexes were energy minimized using the TRIPOS force field until the energy gradient was less than 0.001 kcal/(mol·Å).

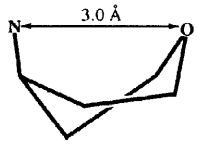
Our analysis of the structural data revealed that Cu–N and Cu–O bond lengths in such complexes are in the range of 1.9–2.1 Å. The dimensions for the octahedral unit cell are in the range of 2.7–3.1 Å for the edges, with X–Cu–X angles of 90°. These requirements favor copper to coordinate with two vicinal heterogroups (1,2-substituents) forming five-membered rings, as opposed to the less common 1,3 arrangement giving six-membered rings. Our analysis of the Cambridge Structural Data Bank supports that Cu^{2+} has higher propensity to form five-membered rings with the complexed ligands (54% of all copper containing entries, compared to 40% for six-membered rings). However, when coordinated with 1,3-substituted six-membered ring systems, the coordinated substituents are found only at axial positions. Hence, whereas in the uncomplexed ring system the positions of the ligands (*i.e.*, substituents to the ring) for the lowest energy favors all equatorial orientation, for the complexed ligand the low energy is when the same substituents are all axial.[23]

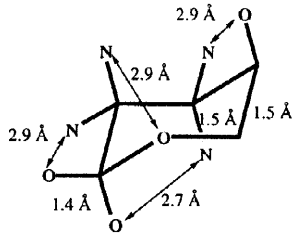


Additional possibilities arise when the ring system is a heterocycle. For example, a heteroatom such as the ring oxygen of a pyranoid ring may coordinate with the metal along with a second suitable substituent of the ring. Three such examples are Cambridge entries ANTROS01, BELDAX, and BAGZUE.[21] Structures below show modeling results for possible coordination sites of a pyranoid ring with heteroatom substituents and the distances among them. As shown, in the chair conformation of such a ring the sites that are favored for coordination with the metal are either 1,2-substituents of which at least one is equatorial or, alternatively

1,3-substituents which are both axial. Furthermore, coordination of the ring oxygen to the metal seems possible only when a second axial substituent is found three carbons away from this oxygen. Alternatively, such a coordination is equally possible when an axial ligand is found four bonds away from the oxygen, but a twisted boat conformation is required in this case.

All these possibilities for coordination during complex formation were explored with models of kanamycins A and B, neamine and isepamicin in order to explain the changes in proton T_1-relaxation times observed in the presence and absence of copper (II) acetate.





1,3,6'-tri-N-(*t*-Butoxycarbonyl)-kanamycin B acetate salt (28). Kanamycin B free base (484 mg, 1 mmol) was dissolved in 30 mL DMSO, followed by the addition of copper (II) acetate monohydrate (799 mg, 4 mmol). The blue solution was stirred overnight at room temperature, before di-*t*-butyldicarbonate (655 mg, 3 mmol) was added. The solution was stirred for an additional 24 h, by which time a tlc analysis revealed total consumption of the starting material in favor of a single product with R_f 0.62 ($CHCl_3$/MeOH/conc. ammonia, 5:3:1). The solution was poured into ethyl ether and was stirred vigorously until a dark oil separated from the solution. The ether layer was carefully decanted from the flask and the residual oil was dissolved in methanol/water (250 mL, 4:1). Sodium sulfide nonahydrate (960 mg, 4 mmol) was added to the mixture and the resultant solution was stirred for 45 min at room temperature, during which time copper sulfide precipitated. The suspension was filtered through a layer of Celite to give a clear yellow solution. The filtrate was taken to dryness *in vacuo* to afford 510 mg of a yellowish solid. The product did not dissolve well in various solvents, a difficulty which prevented us from carrying out full characterization of this compound. Nevertheless, the MS-FAB$^+$ spectrum of this product showed the requisite molecular ion of *m/z* 785, which corresponds to a tri-N-Boc derivative of kanamycin B. Furthermore, this product showed a different chromatographic behavior when compared with a sample of 3,2',6'-tri-N(*t*-Boc)-kanamycin B.[7] This difference in chromatographic behavior suggests that the resultant single product is the title compound, as the 3''-amino group has been repeatedly reported to be efficiently protected by the cupric ion,[7,8,10,11] and no reaction is therefore expected at this position.

3,6'-di-N-(*t*-Butoxycarbonyl)-neamine (29). Neamine free base (**8**) (322 mg, 1 mmol) was dissolved in DMSO (30 mL), and copper (II) acetate monohydrate (799 mg, 4 mmol) was added to the solution. The blue solution was stirred at room temperature for 30 min before N-(*t*-butoxycarbonyloxy)-5-norbornene-*endo*-2,3-dicarboximide[9h] (**33**) (837 mg, 3 mmol) was added. The resultant solution was stirred at room temperature overnight, by which time a tlc analysis ($CHCl_3$/MeOH/conc. ammonia 5:3:1) showed one major product (R_f 0.60, the title dicarbamoylated compound) accompanied by a minor one (R_f 0.40, monocarbamoylated). An additional amount of the reagent (140 mg, 0.5 mmol) was added and the solution was stirred at room temperature until the monocarbamoylated product disappeared on tlc plates (6 h). Cupric ions were removed by precipitation with sodium sulfide as described earlier for compound **28** to afford a yellowish residue. The residue was dissolved in 10 mL of dioxane/water (1:1) and was subjected to ion-exchange chromatography on an Amberlite CG-50 (NH_4^+) column (1.5 x 15 cm, packed in dioxane/water 2:1). Elution with dioxane/water (2:1) removed the N-hydroxy-5-norbornene-2,3-dicarboximide byproduct, whereas the desired product was retained in the column. Subsequently, addition of 2% conc. ammonia to the eluent permitted the elution of the title compound (228 mg). Yield 54%; IR(KBr) cm^{-1} 1689; ^1H NMR 500 MHz (D_2O, pH >12): δ 1.13 (1H, q, J =

12.5 Hz, H_{2ax}), 1.28 (18H, s, –OC(CH_3)$_3$), 1.82 (1H, dt, *J*= 4.0, 4.0 and 12.5 Hz, H_{2eq}), 2.55 (2H, overlapping multiplets, H_1 and $H_{2'}$), 2.94 (1H, t, *J*= 10.0 Hz, H_6), 3.12 (1H, t, *J*= 10.0 Hz, $H_{4'}$), 3.23 (1H, t, *J*= 10.0 Hz, H_4), 3.28 (2H, broad, $H_{6'R,S}$), 3.34 (1H, t, *J*= 10.0 Hz, H_5), 3.35 (1H, t, *J*= 10.0 Hz, $H_{3'}$), 3.39 (1H, ddd, *J*= 4.0, 10.0 and 12.5 Hz, H_3), 3.55 (1H, unresolved m, $H_{5'}$), 5.08 (1H, d, *J*= 3.5 Hz, $H_{1'}$); ^{13}C NMR 125 MHz (DMSO-d_6) : δ 28.7 and 28.8 (–OC(<u>C</u>H_3)$_3$), 36.6 (C_2), 42.2 ($C_{6'}$), 49.7 (C_3), 51.8 (C_1), 56.2 ($C_{2'}$), 70.6 ($C_{4'}$), 71.6 ($C_{5'}$), 73.9 ($C_{3'}$), 76.9 (C_5), 77.4 (C_6), 78.1 and 78.4 (–O<u>C</u>(CH_3)$_3$), 85.0 (C_4), 102.1 ($C_{1'}$), 155.6 and 156.8 (C=O); MS FAB$^+$ 523 (M+H, 18%).

6'-N-(*t*-Butoxycarbonyl)-isepamicin (34). Isepamicin free base (**6**) (485 mg, 0.85 mmol) was dissolved in DMSO (30 mL) followed by the addition of copper (II) acetate monohydrate (680 mg, 3.41 mmol) in one portion. The deep blue solution was stirred for 30 min, before N-(*t*-butoxycarbonyloxy)-5-norbornene-*endo*-2,3-dicarboximide (**33**) (237 mg, 0.85 mmol) was added to the mixture. The solution was stirred overnight at room temperature. The mixture was subsequently poured into ethyl ether (500 mL), and was stirred vigorously until a dark oil separated from the solution. Ether was carefully decanted from the flask and the residual oil was dissolved in methanol/water (250 mL, 4:1). Sodium sulfide nonahydrate (0.82 g, 3.41 mmol) was added to the mixture, and the resultant solution was stirred for 45 min at room temperature, during which time copper sulfide precipitated. The suspension was filtered through a layer of Celite to give a clear yellow filtrate, which on tlc analysis ($CHCl_3$/MeOH/conc. ammonia, 5:3:1) revealed to contain a single aminoglycoside product (R_f 0.28). The filtrate was taken to dryness *in vacuo*, the residue was redissolved in 15 mL of dioxane/water (1:1) and was further purified by ion-exchange chromatography as described earlier for compound **29** to afford 470 mg of the title product. Yield, 83%; IR (KBr) cm^{-1} 1682, 1654; ^{1}H NMR 500 MHz (D_2O, pH >12): δ 1.01 (3H, s, 4''–CH_3), 1.25 (1H, q, *J*= 13.0 Hz, H_{2ax}), 1.25 (9H, s, –OC(CH_3)$_3$), 1.75 (1H, dt, *J*= 4.5 and 13.0 Hz, H_{2eq}), 2.29 (3H, s, N–CH_3), 2.33 (1H, d, *J*= 11.0 Hz, $H_{3''}$), 2.60 (1H, dd, *J*= 6.0 and 13.0 Hz, $H_{3'''R}$), 2.68 (1H, m, H_3), 2.71 (1H, dd, *J*= 4.0 and 13.0 Hz, $H_{3'''S}$), 3.00 (1H, dd, *J*= 14.0 and 9.0 Hz, $H_{6'R}$), 3.06 (1H, d, *J*= 12.0 Hz, $H_{5''ax}$), 3.07 (1H, dd, *J*= 14.0 and 2.5 Hz, $H_{6'S}$), 3.36 and 3.54 (7 H, unresolved multiplets, $H_{2'}$, $H_{2''}$, $H_{3'}$, $H_{4'}$, H_4, H_5 and H_6), 3.61 (1H, broad t, $H_{5'}$), 3.83 (1H, ddd, *J*= 4.5, 9.0 and 13.0 Hz, H_1), 3.91 (1H, dd, *J*= 4.0 and 6.0 Hz, $H_{2'''}$), 3.94 (1H, d, *J*= 12.0, $H_{5''eq}$), 4.92 (1H, d, *J*= 3.5 Hz, $H_{1''}$), 4.96 (1H, d, *J*= 4.5 Hz, $H_{1'}$); ^{13}C NMR 125 MHz (D_2O, pH >12): δ 21.6 (4''–CH_3), 27.9 (–OC(<u>C</u>H_3)$_3$), 34.6 (C_2), 36.8 (N–CH_3), 41.1 ($C_{6'}$), 43.6 ($C_{3'''}$), 49.3 (C_3), 49.5 (C_1), 63.5 ($C_{3''}$), 67.6 ($C_{5''}$), 68.1 ($C_{5'}$), 71.1 ($C_{4'}$), 71.9 ($C_{4''}$), 72.0 ($C_{2'}$ and $C_{2''}$), 72.9 ($C_{3'}$), 74.7 ($C_{2'''}$), 79.3 (–O<u>C</u>(CH_3)$_3$), 79.4 (C_5), 81.2 (C_6), 88.1 (C_4), 98.9 ($C_{1'}$), 100.7 ($C_{1''}$), 158.4 (–NHCOO–), 174.8 ($C_{1'''}$); MS FAB$^+$ 670 (M+H, 21%).

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13. Kanamycin A (**2**) ^{1}H-NMR 500 MHz (40 mM in D_2O; native pH 9.5): δ 1.09 (1H, q, *J*= 12.5 Hz, H_{2ax}), 1.83 (1H, dt, *J*=4.0 and 12.5 Hz, H_{2eq}), 2.69 (1H, dd, *J*= 7.5 and 14.0 Hz, $H_{6'R}$), 2.77 (2H, m, H_1 and H_3), 2.87 (1H, t, *J*= 10.0 Hz, $H_{3''}$), 2.92 (1H, dd, *J*= 3.0 and 14.0 Hz, $H_{6'S}$), 3.12 (1H, t, *J*= 9.5 Hz, H_6), 3.18 (1H, t, *J*= 10.0 Hz, $H_{4'}$), 3.19 (1H, t, *J*= 10.0 Hz, $H_{4''}$), 3.20 (1H, t, *J*= 9.5 Hz, H_4), 3.37 (1H, dd, *J*= 3.5 and 10.0 Hz, $H_{2''}$), 3.45 (1H, dd, *J*= 4.0 and 10.0 Hz, $H_{2'}$), 3.53 (1H, t, *J*= 9.5 Hz, H_5), 3.57 (1H, t, *J*= 10.0 Hz, $H_{3'}$), 3.63 (2H, m, $H_{6''R}$ and $H_{6''S}$), 3.66 (1H, ddd, *J*= 3.0, 7.5, and 10.0 Hz, $H_{5'}$), 3.77 (1H, dt, *J*= 3.0 and 10.0 Hz, $H_{5''}$), 4.90 (1H, d, *J*= 3.5 Hz, $H_{1''}$), 5.22 (1H, d, *J*= 4.0 Hz, $H_{1'}$).

Kanamycin B (**7**) ^{1}H NMR 500 MHz (40 mM in D_2O; native pH 10.4): δ 1.04 (1H, q, *J*= 12.5 Hz, H_{2ax}), 1.77 (1H, dt, *J*=4.0 and 12.5 Hz, H_{2eq}), 2.59 (1H, dd, *J*= 3.5 and 10.0 Hz, $H_{2'}$), 2.60 (1H, dd, *J*= 7.5 and 14.0 Hz, $H_{6'R}$), 2.68 (1H, ddd, *J*= 4.0, 9.5, and 12.5 Hz, H_3), 2.72 (1H, ddd, *J*= 4.0, 9.5, and 12.5 Hz, H_1), 2.82 (1H, dd, *J*= 3.0 and 14.0 Hz, $H_{6'S}$), 2.83 (1H, t, *J*= 10.0, $H_{3''}$), 3.08 (1H, t, *J*= 9.5 Hz, H_6), 3.14 (2H, m, H_4 and $H_{4'}$), 3.17 (1H, t, *J*= 10.0 Hz, $H_{4''}$), 3.32 (1H, dd, *J*= 4.0 and 10.0 Hz, $H_{2''}$), 3.38 (1H, dd, *J*= 9.5 and 10.0 Hz, $H_{3'}$), 3.47 (1H, t, *J*= 9.5 Hz, H_5), 3.58 (1H, ddd, *J*= 3.0, 7.5, and 9.5 Hz, $H_{5'}$), 3.59 (2H, m, $H_{6''R}$ and $H_{6''S}$), 3.74 (1H, dt, *J*= 3.5 and 10.0 Hz, $H_{5''}$), 4.86 (1H, d, *J*= 4.0 Hz, $H_{1''}$), 5.14 (1H, d, *J*= 3.5 Hz, $H_{1'}$).

Neamine (**8**) ^{1}H NMR 500 MHz (40 mM in D2O; native pH 10.2): δ 1.04 (1H, q, *J*= 12.5 Hz, H_{2ax}), 1.81 (1H, dt, *J*= 4.0 and 12.5 Hz, H_{2eq}), 2.55 (1H, ddd, *J*= 4.0, 10.0 and 12.5 Hz, H_1), 2.63 (1H, dd, *J*= 7.5 and 13.5 Hz, $H_{6'R}$), 2.65 (1H, dd, *J*= 3.5 and 10.0 Hz, $H_{2'}$), 2.71 (1H, ddd, *J*= 4.0, 10.0 and 12.5 Hz, H_3), 2.85 (1H, dd, *J*= 2.5 and 13.5 Hz, $H_{6'S}$), 2.99 (1H, t, *J*= 10.0 Hz, H_6), 3.12 (1H, t, *J*= 10.0 Hz, H_4), 3.15 (1H, t, *J*= 10.0 Hz, $H_{4'}$), 3.36 (1H, t, *J*= 10.0 Hz, H_5), 3.42 (1H, t, *J*= 10.0 Hz, $H_{3'}$), 3.61 (1H, ddd, *J*= 2.5, 7.5, and 10.0 Hz, $H_{5'}$), 5.12 (1H, d, *J*= 3.5 Hz, $H_{1'}$).

Isepamicin (**6**) 1H NMR 500 MHz (40 mM in D2O; native pH 10.3): δ 1.04 (1H, s, 4"-Me), 1.27 (1H, q, *J*= 13.0 Hz, H_{2ax}), 1.77 (1H, dt, *J*= 4.0 and 13.0 Hz, H_{2eq}), 2.33 (1H, s, N-Me), 2.37 (1H, d, *J*= 10.5 Hz, $H_{3''}$), 2.64 (1H, dd, *J*= 8.0 and 14.0 Hz, $H_{6'R}$), 2.73 (1H, dd, *J*= 6.0 and 13.5 Hz, $H_{3'''R}$), 2.81 (1H, ddd, *J*= 4.0, 9.3, and 13.0 Hz, H_3), 2.83 (1H, dd, *J*= 3.5 and 13.5 Hz, $H_{3'''S}$), 2.86 (1H, dd, *J*= 2.5 and 14.0 Hz, $H_{6'S}$), 3.11 (1H, d, *J*= 12.5 Hz, $H_{5''ax}$), 3.17 (1H, t, *J*= 10.0 Hz, $H_{4'}$), 3.20 (1H, t, *J*= 9.3 Hz, H_4), 3.46 (1H, dd, *J*= 4.0 and 10.0 Hz, $H_{2'}$), 3.50 (1H, dd, *J*= 4.5 and 10.5 Hz, $H_{2''}$), 3.55 (1H, t, *J*= 9.3 Hz, H_6), 3.56 (1H, t, *J*= 10.0 Hz, $H_{3'}$), 3.57 (1H, t, *J*= 9.3 Hz, H_5), 3.64 (1H, ddd, *J*= 2.5, 8.0 , and 10.0 Hz, $H_{5'}$), 3.91 (1H, ddd, *J*= 4.0, 9.3, and 13.0 Hz, H_1), 3.94 (1H, d, *J*= 12.5 Hz, $H_{5''eq}$), 4.01 (1H, dd, *J*= 3.5 and 6.0 Hz, $H_{2''}$), 4.92 (1H, d, J= 4.5 Hz, $H_{1''}$), 5.20 (1H, d, *J*= 4.0 Hz, $H_{1'}$).

14. The paramagnetic contribution to nuclear T_1 relaxation time (T_1^e) gives a measure of the relaxation induced to nuclei in the proximity of a paramagnetic ion (Botto, R. E.; Coxon, B. *J. Am. Chem. Soc.* **1983**,*105*, 1021). T_1^e values

were derived from the equation $1/T_1^e = 1/T_{1(para)} - 1/T_{1(dia)}$, for which the $T_{1(para)}$ and $T_{1(dia)}$ relaxation times were determined in the presence and absence of copper (II) acetate, respectively.

15. The force-field parameters within the TRIPOS package were not developed specifically for the transition-metal complexes of aminosugars. The empirical molecular mechanic force-field methods usually give a qualitative measure of the energy contents of molecules, however, the reliability of the energy-minimized structures are high. We were compelled to use the TRIPOS force-field in light of the fact that to our knowledge this is the only force-field package that describes the parameters for coordination with cupric ion for complexes as large as the ones studied by us.
16. Use of excess of di-*t*-butyldicarbonate does not affect the regiochemistry of the reaction. Excess of copper (II) acetate (4.0 equivs.) is, however, required in all cases. Reactions of kanamycin B (**7**) with excess di-*t*-butyldicarbonate at lower copper (II) acetate ratios showed that at least 3-fold excess of copper (II) acetate is needed for achieving the described regioselectivity.
17. The solvent typically used in our synthetic experiments was DMSO. This solvent appears to be the most suitable due not only to its dipolar character that accounts for its capacity to dissolve both the intermediate chelates and the carbamoylating reagent, but also to its aprotic character that stabilizes the chelates.[10c] We found that other polar organic solvents could also be used without any effect on the regioselectivity of the reactions. For example, the reactions of neamine (**8**) and kanamycin B (**7**) with di-*t*-butyldicarbonate in the presence of the cupric ion were tried in methanol, which gave products identical to those obtained in DMSO. However, a crucial difference in the case of nucleophilic solvents is that the carbamoylating reagent is solvolized with metal ions serving as a catalyst, and a large excess of the reagent should therefore be used to bring the reaction to completion.
18. One could envision that the introduction of the first Boc group at the 6'-amino group would potentially alter the conformation of the aminoglycoside molecule, and consequently change its subsequent coordination pattern with the cupric ion. Should this coordination pattern be different from that for the parent aminoglycoside, a different regioselectivity for the reaction would be expected. However, our modeling showed that the 6'-N-Boc group was always oriented away from the rest of the molecule and that it does not affect the conformation of the various rings. The experimental proof for this notion was obtained by determining the T_1^e values for protons of 6'-N-Boc-kanamycin A (**26**). These values (data not shown) were essentially identical to those of kanamycin A, and consequently the coordination patterns of these two compounds with the cupric ion were unaltered.
19. Rotation about C_6–O–$C_{1''}$ glycosidic bonds in solution gives the 2"-hydroxyl group the opportunity to form a hydrogen bond with either the 1-amino or 5-hydroxyl groups. Hydrogen bonding between an amino and a hydroxyl group has, however, been shown to be the most beneficial to the system in terms of energy (Vanquickenborne, L.G. "Quantum Chemistry of the Hydrogen Bond" in *Intermolecular Forces*, Huyskens, P. L.; Luck, W. A. P.; Zeegers-Huyskens, T. (Eds.), Springer-Verlag Berlin Heidelberg **1991**, pp. 31-53). The pK_a of the 1-amino group of tobramycin, which has the same structure as kanamycins A and B around the C_6–O–$C_{1''}$ bonds, has been reported to be 6.2 (Dorman, D. E.; Paschal, J. W.; Merkel, K. E. *J. Am. Chem. Soc.* **1976**, *98*, 6885). The low pK_a value for this amine supports its involvement in hydrogen bonding. In addition, the crystal structure of kanamycin A[23] revealed the existence of a hydrogen bond between the 1-amino and 2"-hydroxyl groups (a distance of 3.1 Å).
20. Coordination, for example, of the cupric ion to the 2'-amino group of kanamycin B would diminish the probability of its hydrogen bonding to the 5-hydroxyl group, whereas this latter group would be more available for hydrogen bonding to 2''-hydroxyl group. In such a case the probability of a hydrogen bond between 2''-hydroxyl and the 1-amino group of kanamycin B would be diminished with respect to a similar hydrogen bond for kanamycin A.
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24. Two examples are entries CHXCUA and TACCUN of the Cambridge data bank,[21] for 1,3-diaminocyclohexane and 1,3,5-triaminocyclohexane coordinated with copper. In both cases all substituents are in the axial positions to permit coordination. Other examples for even bulkier ligand substituents at positions 1-, 3-, and 5- of cyclohexane coordinated with Fe, Co and Ni are entries HECWAN, PYALNI10, SCHXCO, YUSHID, and YUSHUP,[21] all of which have coordinated heterogroups located in bulky substituents at axial positions. In the absence of metal, these structures should prefer equatorial arrangement for the 1-, 3-, and 5-substituents. However, coordination with the metal appears to stabilize the axial orientation for the substituents of the metal coordinated species.

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