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AMINOGLYCOSIDE ANTIBIOTICS - A METHOD FOR SELECTIVE N-ACYLATION BASED ON THE TEMPORARY PROTECTION OF AMINO ALCOHOL FUNCTIONS AS COPPER CHELATES

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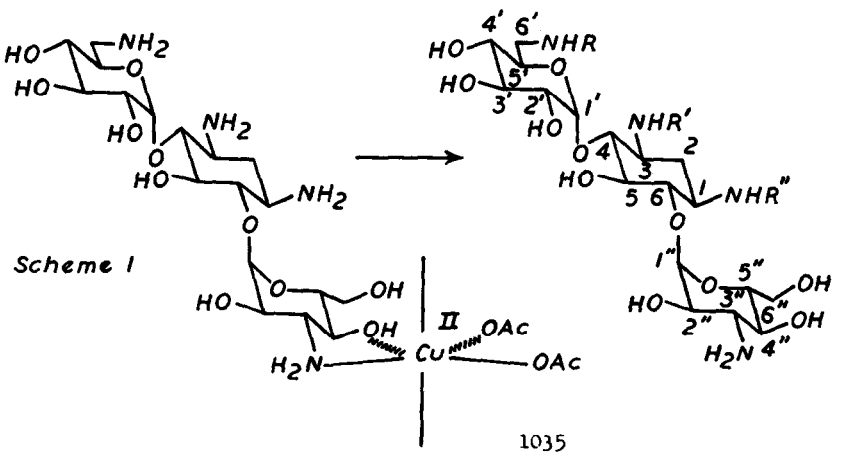
The phenomenon of bacterial resistance to aminoglycoside antibiotics [1] has fostered many new developments in the area of semi-synthesis and chemical modification [2] leading to second and third generation aminoglycosides. The latter class comprises N-acylated and N-alkylated analogs that exhibit much broader antibacterial activities compared to the parent antibiotics [3]. Methods for the selective N-acylation of aminoglycosides are therefore in demand, as current procedures are confined mostly to the more accessible sites [4] or, based on relative basicities [5]. We describe in this paper, a method for the selective acylation of various amino groups of certain aminoglycoside antibiotics, based on the temporary protection of suitably disposed vicinal amino alcohol functions as copper(II) chelates, and subsequent acylation of the unbound amino group(s) with a variety of acylating agents (Scheme 1) [6]. The readily available kanamycin A was chosen as a substrate because of the clinical importance of its derivatives [7], and the manipulative difficulties associated with selective N-acylations in this and related series [5].



Scheme I

1 R=Ac; R',R''=H
2 R=Cbz; R',R''=H
3 R,R',R''=Ac
4 R,R',R''=Cbz
5 R,R''=Ac; R'=H
6 R,R'=Ac; R''=H

Treatment of a mixture of kanamycin A free base (1 equiv.) and $Cu(OAc)_2 \cdot H_2O$ (0.75-1 equiv.) in aq. THF with p-nitrophenylacetate (1-2 equiv., 23 h) gave after decomposition of the chelate with aq. ammonia and column chromatography, 6'-N-acetylkanamycin A 1 (82%), mp 213-215°, $[\alpha]_D^{25}$ + 94.8° (H_2O). A similar reaction with benzyl p-nitrophenylcarbonate gave the known [7] 6'-N-benzyloxycarbonylkanamycin A 2 (73%), mp 204-212° (dec.) [8]; $[\alpha]_D$ + 115.6° (H_2O); (compare, 45% after countercurrent distribution and column chromatography) [7]. Acylation of kanamycin A under the same conditions but in the absence of the chelating agent led to mixtures (t.l.c). Acetylation of the chelate (30 equiv. of $CuSO_4$) [9] in aq. $NaHCO_3$ (10 equiv.) with acetic anhydride (20 equiv.) followed by addition of 2,4-pentanedione, processing of the solution and chromatography, gave 1,3,6'-tri-N-acetylkanamycin A 3 (82.5%), mp 235-237°; $[\alpha]_D$ + 88.7° (H_2O) [8]. With acetic anhydride in aq. solution, a mixture was obtained consisting of tetra-N-acetylkanamycin A as the major component. Interestingly, acetylation of a 1:1 chelate with N-acetoxy-5-norbornene-2,3-dicarboxamide [10] (4 equiv.) led to the following products [8]: 3 (44.2%); 5, mp 228-231°, $[\alpha]_D^{25}$ + 92.1° (H_2O) (24.6%); 6, mp 206-208°, $[\alpha]_D^{25}$ + 91.8° (H_2O) (18.9%). In the absence of copper sulfate, a mixture of 3 and tetra-N-acetylkanamycin A (major) was obtained.

Treatment of a mixture of kanamycin A (1 equiv.) and $Cu(OAc)_2 \cdot H_2O$ (10 equiv.) in aq. $NaHCO_3$ - THF, with N-(benzyloxycarbonyloxy)succinimide [7] (5 equiv., 10 h) followed by precipitation of the N-acylated chelate with acetone and chromatography, gave 1,3,6'-tri-N-benzyloxycarbonylkanamycin A 4 (85.8%), mp 258-263°; $[\alpha]_D^{25}$ + 83.9° (60% aq. THF); M^+ 1054 (on the corresponding per N,O-methyl derivative) [8]; reported [11] mp 258-263° (isolated as a minor component from a mixture).

A 1:1 stoichiometry was indicated for the chelate by continuous variation studies with kanamycin A and $Cu(OAc)_2 \cdot H_2O$ at two wave lengths, and by circular dichroism. ^{13}C n.m.r studies have shown the existence of an interaction between copper(II) ions and the amino alcohol system comprising C-2", C-3" and C-4" in kanamycin A [12]. The strongly negative and positive Cotton effects (Fig. 1) at low wave length of a solution of the chelate (pH ~ 7.2

and 8.8) cannot be exclusively associated to δ and λ chelates [13] respectively, since the individual contributions of such chelates in kanamycin A are not known. The changes in signs and magnitudes of CD bands with small variations in pH, could also be due to the dissociation of protons from coordinated amino alcohols, and the existence of several species in solution [14]. Moreover, it is known that the 6'-amino group (but not the C-4' hydroxyl group) is a secondary site for binding [12], and it is possible that it occupies one of the apical ligand sites in the chelate at neutral pH, giving rise to a cyclic structure, and contributing to the shape of the spectrum [15].

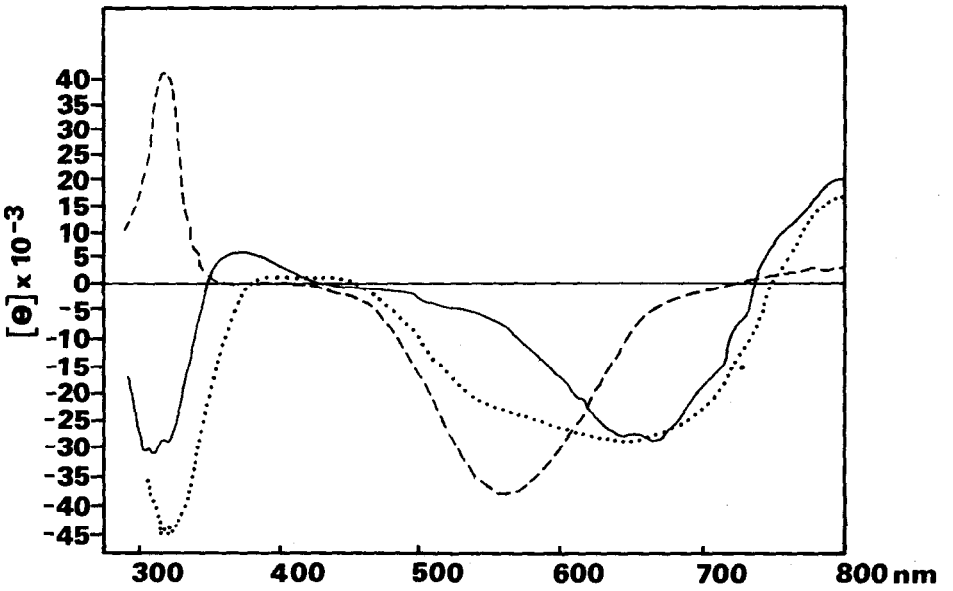


Fig. 1. CD spectra of kanamycin A with $Cu(OAc)_2 \cdot H_2O$ in aq. solution (.....) 1:1 chelate at pH ~ 7.2; (---) 2:1 chelate at pH 8.6, 1:1 chelate at pH ~ 8.8 (NaOH); (——) 1:2 chelate at pH ~ 6.8.

Complex salts of kanamycin A have been described in the patent literature [16]; and in another report [17], in which the 2-deoxystreptamine is suggested as the site of chelation based on i.r. studies. Our ^{13}C n.m.r [12] and synthetic studies show that the actual primary site of chelation over a wide range of Cu^{++} concentration, involves the kanosamine portion and that the 2-deoxystreptamine portion is in fact the least affected.

It is evident from these results that chelation of specific amino alcohol and related functions in the aminoglycoside series [18] can be utilized as a means of effecting one-pot N-acylations, with important subsequent applications. In fact, 2 and related derivatives in other series, prepared by the presently described procedure, have been subjected to various N-acylations, either directly or via the corresponding chelates [19]. It should also be noted that 2, obtained in high yield by our procedure, is a key intermediate in the manufacture of amikacin from kanamycin A [7].

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References and Notes

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8. All new structures were confirmed by ^{13}C n.m.r spectral studies, as well as by mass spectral data; see also D.C. De Jongh, M.S.B. Nayar, G. Patil and S. Hanessian, Tetrahedron (in press).
9. Acetic acid liberated in the acetylation has a tendency to break up the chelate, hence the need for excess $CuSO_4$ and $NaHCO_3$, in some reactions.
10. We thank Dr.P.Kurath (Abbott Labs., North Chicago, Ill.) for a sample of this compound.
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15. The 1:1 chelate from 1 and $Cu(OAc)_2$ gave a positive Cotton effect, similar to that obtained at pH ~ 8.8 with kanamycin A (Fig. 1).
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