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A HIGHLY SELECTIVE N-PROTECTION STRATEGY FOR THE PREPARATION OF 1-N-ALKYLATED KANAMYCIN ANTIBIOTICS

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Summary: An O to N acyl migration technique has been used to selectively acetylate the amino groups of the aminosugar rings of kanamycins A and B, leaving the amino groups on the 2-deoxystreptamine ring unprotected. A formylation-deformylation process then converts these N-acetylated kanamycins to their 3-N-formyl derivatives with high regioselectivity, giving key intermediates for the efficient preparation of 1-N-alkylated kanamycins.

The recent emergence of 1-N-acyl[1] and 1-N-alkyl[2,3] aminoglycoside-aminocyclitols as an important group of semisynthetic antibiotics has resulted in considerable interest in selective protection methods for the amino groups of these antibiotics, particularly with a view to large scale manufacture. Previous selective N-protection strategies[4,5] have given intermediates in which amino groups other than the 1-amino group are still unprotected, so that regiospecific 1-N-substitution cannot be achieved, and tedious chromatographic separations of positional isomers are consequently necessary. We have developed a novel sequence of reactions which allows us to convert members of the important kanamycin group of antibiotics to derivatives in which only the crucial 1-amino group is unprotected, enabling us to regioselectively prepare 1-N-alkylated antibiotics. A recent report[6] of an alternative approach to the preparation of such protected derivatives prompts us to describe our results.

Kanamycins A (1) and B (2) are tricyclic compounds in which amino sugars are glycosidically linked to the hydroxyls at C-4 and C-6 of the aglycone, 2-deoxystreptamine. The amino groups in the amino sugar rings are located in close proximity to hydroxy groups, while no such relationship exists for the amino groups in the 2-deoxystreptamine ring. We have therefore used an O to N acetyl migration technique [7,8] to selectively acetylate the amino groups in the amino sugar rings, leaving the amino groups at C-1 and C-3 of the 2-deoxystreptamine free for subsequent manipulation.

The known[9] tetra-N-benzyloxycarbonyl derivative of kanamycin A was O-acetylated using acetic anhydride (10 mol. equiv.) in pyridine at 20°C for 24 hours to give a penta-O-acetyl derivative which was isolated in 89% yield over the two steps from kanamycin A sulphate. A ^{13}C NMR study established that this material, mp 223-229° (dec), $[\alpha]_D$ + 77.4° (C=2, DMF) was the 2',3',4',4" ,6" -pentaacetate, the hindered 5-hydroxyl and 2" -hydroxyl groups remaining unacetylated. The benzyloxycarbonyl groups were removed from this material by catalytic hydrogenolysis over 5% palladium on carbon catalyst in 1:2 aqueous THF containing acetic acid (2 mol. equiv.) at 50°C and 50 p.s.i. After filtration of the catalyst the aqueous and THF phases separated, and the aqueous phase was treated with an equal volume of 15N aqueous ammonia at 20°C for 24 hours to effect the required O to N acetyl migration, and also to

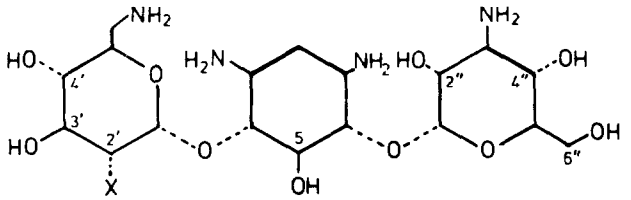
cleave the redundant 2',3' and 6" O-acetyl groups. Concentration of the reaction solution to low volume, replacement of the remaining water by isopropanol using azeotropic distillation and filtration then gave 3" ,6'-di-N-acetylkanamycin A (3), m.p. 196°-199° (dec), $[\alpha]_D$ + 118.5° (C=1, H_2O) in an overall yield of 72% from kanamycin A sulphate, without the need for chromatography.

A similar sequence of reactions on kanamycin B (2) gave 2',3" ,6'-tri-N-acetylkanamycin B (4), m.p. 191°-198° (dec), $[\alpha]_D$ + 116.0° (C=1, H_2O) in 86% overall yield from (2). This O to N acetyl migration technique which we have successfully applied to kanamycins A and B should be generally applicable to related systems. The method gives good yields, and complements the recently reported[4] approach in which suitably disposed amino-hydroxy group pairs were temporarily protected as metal chelates allowing protection of the amino groups in non-complexing positions.

The remaining two unprotected amino groups of (3) and (4) were differentiated by a formylation -deformylation process, which effected regiospecific 3-formylation. A mixture of (4) (1 equiv.) and triethylamine (4 equiv.) in aqueous THF was treated with p-nitrophenyl formate (6 equiv., 18h), the lower aqueous phase was separated and the water was replaced by isopropanol using azeotropic distillation. Filtration of the resulting solid afforded 1,3-di-N-formyl-2',3" , 6'-tri-N-acetylkanamycin B (5) (87.5%), m.p. 331°-333°C (dec.), $[\alpha]_D$ + 107.5 (C=1, H_2O). Treatment of (5) with aqueous NaOH (72h. at pH 12.5 and 20°C) then effected the series of deformylation reactions outlined in figure 1. Amberlite IR 120 (H^+ form) ion exchange resin was added to neutralise the reaction mixture and to selectively adsorb the (4) regenerated by over-reaction. The filtered solution was then percolated through a bed of Amberlite 200 (NH_4^+ form) resin, washing first with water to remove sodium formate and unreacted (5), and then with 0.15N aqueous ammonia. Concentration of the ammoniacal eluate to low volume followed by azeotropic replacement of the remaining water by isopropanol resulted in the precipitation of 3-N-formyl-2',3" ,6'-tri-N-acetylkanamycin B (6) (73%), m.p. 266°-268°C (dec), $[\alpha]_D$ + 114° (C=1, H_2O). Samples of (6) prepared in this way contained only 1% of the 1-N-formyl isomer (7)[10,11].

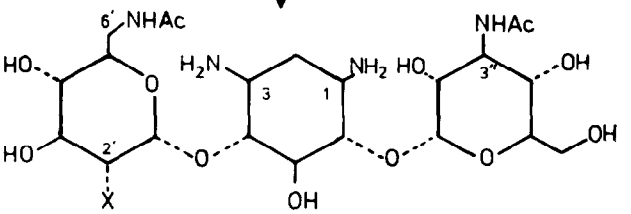
We presume that the formylamino group at C-3 of (5) hydrolyses at a slower rate than that at C—1 owing to steric interaction between the 6'-acetylamino group and the 3-formylamino group. The striking regioselectivity observed in the deformylation of (5) to (6) then results from the coupling of consecutive selective reactions: the more slowly formed (7) is relatively rapidly destroyed by over-hydrolysis to (4), while the more rapidly formed (6) is more slowly over-hydrolysed and thus accumulates in the reaction mixture.

In a similar fashion di-N-formylation of (3) followed by deformylation gave 3-N-formyl-3" ,6'-di-N-acetylkanamycin A (8) (53% from 3) m.p. 221°-224°C (dec), $[\alpha]_D$ + 111° (C=1, H_2O) which contained <2%[11] of the 1-formyl isomer. The position of the free amino groups in (3), (4), (6), (7) and (8) were readily determined by ^{13}C NMR studies at pD3 and pD11, the " β-shift" effect[12] upon deuteronation of the amino group aiding the unambiguous assignments of the spectra. Reductive alkylation of (6) or (8) followed by deprotection gave 1-N-alkylated kanamycins regioselectively, avoiding the need for any chromatographic separations of isomer mixtures. For example, reductive alkylation of (6) with 1,3-dihydroxyacetone using sodium

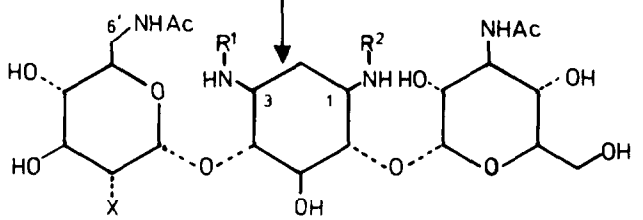


	X
(1) Kanamycin A	OH
(2) Kanamycin B	NH_2

(i) Cbz Cl/Aq Na_2CO_3
(ii) Ac_2O/pyridine
(iii) H_2/Pd-C/H_2O-THF-AcOH
(iv) Aq NH_3



	X
(3)	OH
(4)	NHAc



	R^1	R^2	X
(5)	CHO	CHO	NHAc
(6)	CHO	H	NHAc
(7)	H	CHO	NHAc
(8)	CHO	H	OH

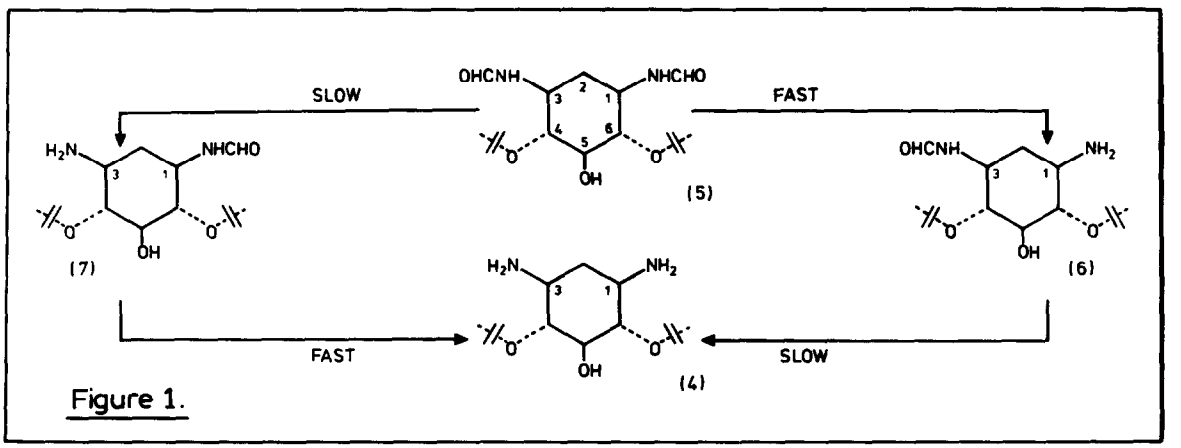
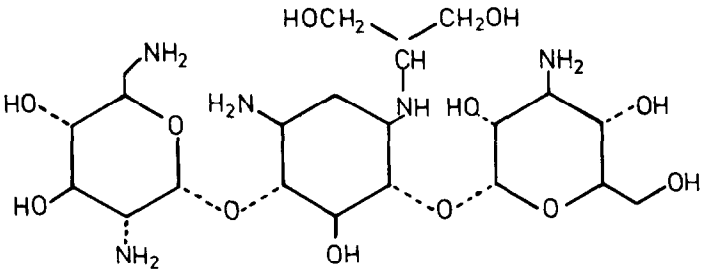


Figure 1.

cyanoborohydride in 1:4 aqueous methanol, followed by deacylation with aqueous caustic solution gave 1-N-(1,3-dihydroxy-2-propyl)kanamycin B (9) (74%) identical with authentic material[3b] in every respect.



(9)

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7. See for example: T. Yamasaki, Y. Kubota, T. Tsuchiya and S. Umezawa, Bull. Chem. Soc. Japan, 49, 3190 (1976) and references therein.
8. Following the completion of the O to N acetyl migration portion of our work [Ger. Offen., 2,716,533 (1977); Chem. Abs., 88, P51128x (1978).] the conversion of Kanamycin A to 3" ,6'-di-N-acetyl-tri-O-acetylkanamycin A using a similar reaction sequence to our own was reported by V. Kumar and W.A. Remers, J. Med. Chem., 22, 432 (1979).
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10. Partial formylation of (4) with p-nitrophenyl formate in aqueous THF gave a mixture of the two mono-N-formyl isomers (6) and (7) in a ratio of 1:3. Chromatographic purification on a column of CM Sephadex C-25 (NH_4^+ form) gave a sample of the major isomer (7), m.p. 207°-211° (dec.), $[\alpha]_D$ + 108° (C=1, H_2O).
11. These isomer levels were determined using a quantitative t.l.c. assay employing Merck plates precoated (0.25mm) with silica gel 60 F_{254} and developed with 40:40:30:1 methanol-ethyl acetate - water - 28% aqueous ammonia.
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