Mitsunobu dehydration of *N*-Boc neomycin B†

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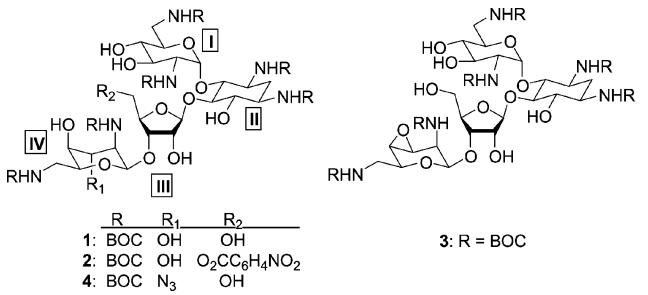
Reaction of hexa-*N*-Boc neomycin B with TPP and DIAD in toluene results in the formation of an epoxide in ring IV, not an aziridine or azetidine as previously reported.

Aminoglycosides were used as the first successful treatment of the devastating effects of tuberculosis over 60 years ago. In spite of drug resistance to these compounds, they are still an important component of the chemotherapeutic arsenal against this disease. Today, *Mycobacterium tuberculosis* continues to kill more people than any other single microorganism due primarily to its virulence in HIV-infected individuals.[1] The waxy, mycolate-laden exterior of the mycobacterium presents a formidable barrier to most drug molecules. Accordingly, we sought to modify aminoglycosides with fatty acids to improve their efficacy against this microorganism. Based on previous success with mycolic acids in the Mitsunobu esterification of the primary hydroxyl groups of trehalose, for the synthesis of cord factor analogues,[2] we set out to apply this chemistry to hexa-*N*-Boc neomycin B. This compound has a single primary hydroxyl group that should be the most reactive site for this reaction, obviating the need for selective protection. However, two recent reports have indicated that it is preferable to protect all secondary hydroxyl groups as acetates to maximise the yield of Mitsunobu displacement at the 5III-position with thioacetic acid (84%)[3] and N^3-benzoylthymine (78%),[4] respectively. Similar chemistry has been carried out on tobramycin analogues by Hanessian, *et al.*[5]



	R	R_1	R_2
1:	BOC	OH	OH
2:	BOC	OH	$O_2CC_6H_4NO_2$
4:	BOC	N_3	OH

3: R = BOC

Fig. 1 Neomycin B derivatives.

As a model reaction, we treated hexa-*N*-Boc neomycin B **1** (Fig. 1, 1 equiv.) with *p*-nitrobenzoic acid (2 equiv.), triphenylphosphine (TPP) (2 equiv.), and diisopropyl azodicarboxylate (DIAD) (2 equiv.) in various anhydrous solvents. While no significant conversion was observed in DMF or THF, reaction in toluene did produce the desired 5III-ester. In addition to the anticipated product **2** (29%), dehydrated products of this ester and of neomycin were obtained as by-products. When this reaction was attempted with lauric acid and double the amount of DIAD and TPP (4 equiv. ea.), more substantial amounts of these by-products were observed. In order to better identify these dehydration products, the Mitsunobu reaction was repeated in the absence of any carboxylic acid. Reaction of **1** with TPP (2 equiv.) and DIAD (2 equiv.) in toluene at RT for 20 h afforded a 40% yield of a major monodehydration product ($M + Na^+ = 1219.60032$, HRMS), following purification by column chromatography. Acetylation of this product produced a pentaacetate ($M + H^+ = 1407.67615$, HRMS) indicating that two hydroxyl groups of the parent neomycin had reacted under the Mitsunobu conditions, consistent with formation of an epoxide (**3**).

Rigorous characterisation of these compounds by NMR initially proved quite difficult. The spectrum for the parent compound as its peracetate (**1**-heptaacetate) in $CDCl_3$ at room temperature appears broadened by exchange processes, presumably due to restricted rotation and steric congestion. Significantly more spectral dispersion is observed in d^5-pyridine solution, however there is still significant broadening of many resonances in the spectrum and it is not readily assigned. Both **1**-heptaacate and the epoxide **3**-pentaacetate, however, gave well-resolved ^{1}H-NMR signals in d^5-pyridine at 90 °C (Fig. 2). All further ^{1}H-NMR studies on the neomycin derivatives were, therefore, undertaken using the peracetylated compounds at elevated temperatures.

Analysis of the COSY, HMQC and HMBC and 1D NOE spectra obtained for **1**-heptaacetate at 363 K enabled the complete assignment of the ^{1}H-NMR signals of the four sugar units (I–IV). The chair conformation of the *ido* ring (IV) is unchanged from the unprotected parent species,[6] as confirmed by the small values observed for the $^3J_{HH}$ vicinal coupling constants of the *ido* ring protons ($^3J_{2IV,3IV}$, $^3J_{3IV,4IV}$, $^3J_{4IV,5IV} = 2$–3 Hz). These small couplings attest to the mutually equatorial disposition of H_{2IV}, H_{3IV} and H_{4IV} in contrast to the *trans*-diaxial disposition of the H_{2I}, H_{3I}, H_{4I} sets of the *gluco* ring (I) residue ($^3J_{2I,3I}$, $^3J_{3I,4I}$, $^3J_{4I,5I} = 9.5$–11 Hz). Comparison of the COSY spectra for **1**-heptaacetate and **3**-pentaacetate confirms that the *gluco* ring, streptamine, and ribose rings remain unchanged as a result of the reaction. The resonances of the *ido* ring, however, are significantly different in the anhydro derivative **3** in comparison to **1** (Fig. 2). The shielding of ^{1}H-NMR signals at H_{3IV} and H_{4IV} in **3** is consistent with formation of an epoxide at C_{3IV} and C_{4IV} (δ $H_{3IV} = 3.48$, δ $H_{4IV} = 3.30$, δ $C_{3IV} = 52.4$, δ $C_{4IV} = 52.7$ ppm; *cf.* compound **1**: δ $H_{3IV} = 5.52$, δ $H_{4IV} = 5.24$, δ $C_{3IV} = 70.0$, δ $C_{4IV} = 67.5$ ppm). The loss of a distinctly separated AX spin system observed for the H_{6IV} protons and the inversion of resonances for the H_{2IV} and H_{5IV} protons is indicative of significant conformational change in the *ido* ring.

Further evidence in support of the epoxide structure was obtained by reaction with sodium azide. A monoazide product

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† Electronic supplementary information (ESI) available: 1D and 2D NMR data for the peracetates of **1**, **3**, **4** and **5**. See DOI: 10.1039/b513113h

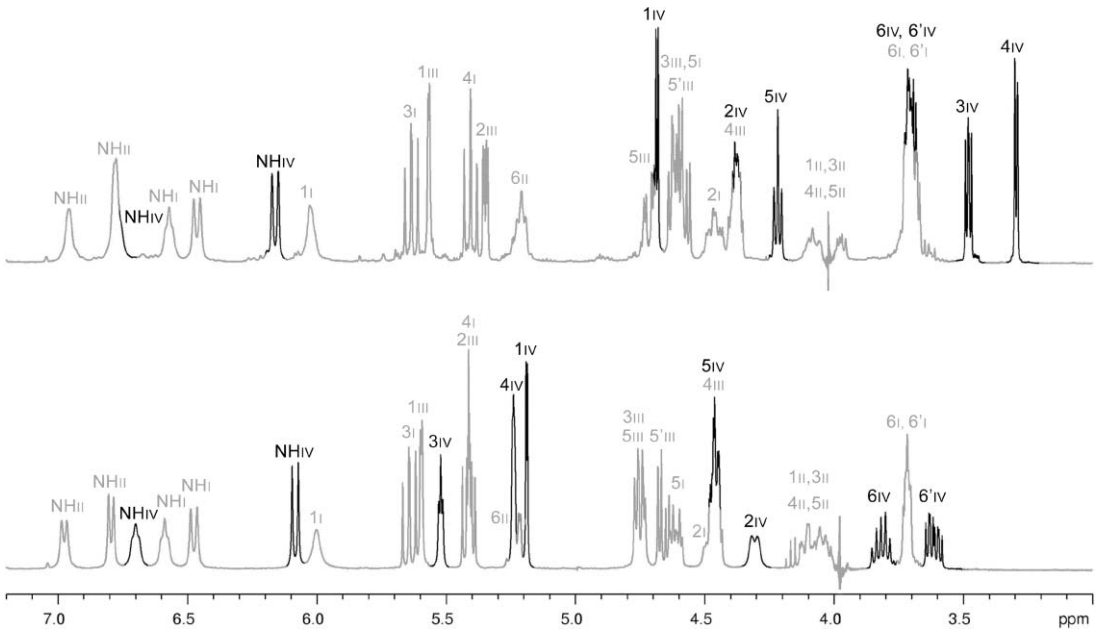

Fig. 2 Expansions of the ^{1}H NMR spectra (400 MHz, 363 K, C_5D_5N) obtained for **1**-heptaacetate (bottom) and **3**-pentaacetate (top), with suppression of residual water present in the solvent. The changes observed in the resonances assigned to ring IV are highlighted.

4 was obtained the ^{1}H-NMR data of which are consistent with the stereochemistry shown. Such an arrangement would arise from **3** through reaction at C3IV with inversion of configuration (overall retention of neomycin B configuration) by the azide anion. Formation of the (*talo*) epoxide **3** under Mitsunobu conditions is consistent with generation of the (triphenylphosphonium) leaving group on the less hindered O-3 position of the *ido* ring. Formation of the alternative (*altro*) epoxide would require generation of the (triphenylphosphonium) leaving group on the more hindered O-4 position which would be subject to a severe 1,3-diaxial interaction with the 2-*N*-Boc group. In addition, the transition state for (*altro*) epoxide formation would be subject to a dipolar repulsion[7] (field effect) between the leaving group and the 2-*N*-Boc group.

Fourrey, *et al.* have recently reported that in the Mitsunobu reaction of hexa-*N*-Boc neomycin B with N^3-benzoylthymine (a reaction analogous to the reaction reported here with *p*-nitrobenzoic acid) a highly strained tricyclic derivative of ring IV of hexa-*N*-BOC neomycin B is formed, in addition to the expected displacement at the 5III-position by N^3-benzoylthymine.[4] Their proposed structure contains both aziridine and azetidine ring systems fused to ring IV. We believe that epoxide formation is much more likely than aziridine or azetidine formation based on literature precedent and the additional evidence reported here. It is noteworthy that the mass spectral data reported by Fourrey, *et al.* is actually consistent with an epoxide, not the aziridine–azetidine structure proposed, and there is a considerable degree of agreement in the ^{1}H- and ^{13}C-NMR data reported for their compound and epoxide **3**. It is clear that their reported structure does not fit our data, for several reasons, but particularly because we observe carbamate proton (NH) resonances for both of the nitrogens at the C2IV and C6IV positions (Fig. 2, see also ref. 11). Furthermore, aziridine opening by azide in their putative compound would not generate a product in the parent conformation of the *ido* ring as was observed here.

In conclusion, we have presented evidence for the formation of a new epoxide derivative of neomycin B. Epoxide formation is favoured by the *trans*-diaxial arrangement of the vicinal diol in the *ido* ring[6] and this competes effectively with reaction at the primary hydroxyl group at C5III.[8] Although aziridines can be formed by Mitsunobu reaction of *N*-Boc–amino alcohols,[9] we have seen no evidence for aziridine or azetidine ring formation within ring IV of neomycin[10] under these reaction conditions, or those of Fourrey *et al.*[11] This epoxide may have interesting biological properties in its own right and serve as a versatile intermediate for the preparation of modified neomycins or neomycin conjugates.

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11 At the suggestion of both a referee and Professor Fourrey, we have repeated their experiment and isolated a compound identical by ^{13}C-NMR to their compound **8**. However, the ^{1}H-NMR in d^5-pyridine at 90 °C shows 6 NH peaks (compound **5**, refer to the electronic supplementary information), consistent with an epoxide, but not the aziridine–azetidine structure assigned. **Note added in Proof**. In a personal communication, Professor Fourrey has accepted our interpretation and agreed that the structure assigned to **8** in their paper should be revised.