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Introduction of a substituent at the 5″-position of *N*-Boc neomycin B under Mitsunobu reaction conditions†

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Because of the peculiar reactivity of the idose part of *N*-Boc neomycin B 3, special care must be exercised to introduce a substituent at the 5″-position of the antibiotic when using Mitsunobu reaction conditions.

Binding of small molecules to microbial ribosomal RNA is an important means of therapeutic intervention since such an interaction might interfere with protein biosynthesis.[1] Among the most active molecules used for the eradication of bacterial pathogens, aminoglycoside antibiotics represent the best characterized class of compounds.[2] They recognise a conserved sequence of rRNA in the vicinity of the codon–anticodon recognition site in the aminoacyl-tRNA site (A site).[3]

Fig. 1 shows the structures of two major representative aminoglycoside antibiotics: paromomycin **1** and neomycin B **2**. Both molecules possess a 2-deoxystreptamine core (ring I), the 4- and 5-hydroxyls of which are glycosylated with an aminosugar (ring II) and together with a common neobiosamine unit (rings III and IV).

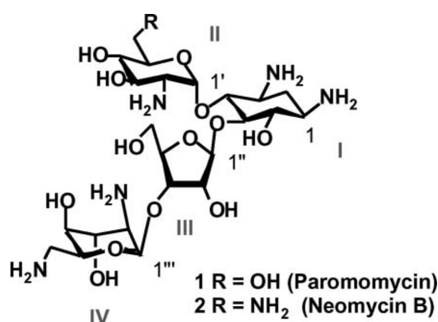

Fig. 1 Structure of aminoglycoside antibiotics.

At present, pathogen resistance and host-pathogen selectivity are two important issues. Overcoming these might contribute to the further development of aminoglycoside antibiotics in medicine.[4] Nowadays, to address these problems a new generation of such molecules is sorely needed. Because of the remarkable affinity of aminoglycosides for RNA and their role in protein synthesis inhibition, it is desirable to explore the effects of new chemical manipulations in the aminoglycoside series.

At the outset of this work we noticed that the hydroxyl located at the 5″-position of neomycin B **2** is not conserved among the functional groups of the aminoglycoside that contacts the RNA.[5] Accordingly, it might be a site of interest for various types of chemical modification. For this reason, we proposed to attach various residues to the 5″-position of neomycin B **2**, expecting that the modified glycosides might manifest an enhanced affinity for the pathogen RNA, compared to the human one, and a decreased microbial resistance.[4]

Our first efforts along this line consisted of the introduction of thymine (or uracil), which, hopefully, might show an ability to make additional contacts with the target RNA. Starting from *N*-Boc-protected neomycin B **3**,[6] we first envisaged a short route (Scheme 1) by which N^3-benzoylthymine **4** (N^3-BzT)[7] would be conjugated to the 5″-position of neomycin B using a Mitsunobu reaction.[8]

When, diisopropyl azodicarboxylate (DIAD) (10 equiv.) was slowly added to a cooled dioxane solution containing *N*-Boc neomycin B **3** (1 equiv.), **4** (N^3-BzT) (5 equiv.) and triphenylphosphine (12 equiv.), a major reaction product was isolated in 78% yield. Its characterization by ESI^+ mass spectrometry did not confirm the expected mass value $M = 1426$. Instead, the ESI^+ mass spectrum showed peaks at m/z 1431 and 724, probably corresponding to $M + Na^+$ and $(M + K^+ + H^+)/2$ ions, respectively, suggesting $M = 1408$ for the new product **7**. Upon acetylation the latter yielded a derivative **8** whose mass spectrum showed peaks at m/z 1599 and 811, which we proposed to ascribe to ions $M + Na^+$ and $(M + 2Na^+)/2$, with $M = 1576$ for the molecular weight of the new acetylated compound. However, the ^{1}H NMR spectrum of this acetate showed only four acetyl methyl signals whereas under the same acetylation conditions *N*-Boc neomycin B **3** provided a hepta-acetate. At this stage, these data could not lead to a straightforward structural assignation for the Mitsunobu reaction product.

To resolve this structural problem, on the basis of an unambiguous physical data comparison, we designed an univocal route to obtain the desired compound **12** (R = N^3-BzT, R′ = OH). As shown in Scheme 1 the first step was the selective silylation of the 5″-hydroxyl of *N*-Boc neomycin B **3** which was accomplished using *t*-butyldimethylsilyl chloride to give **9** (69%) which was per-acetylated to provide the hexa-acetate **10** (83%). Removal of the silyl group from the 5″-hydroxyl using various classical fluoride anion-based protocols proved to be problematic. Fortunately, the deprotection could be achieved conveniently by means of cerium ammonium nitrate (CAN) in methanol[9] to give **11** in 71% yield. The latter compound served to introduce N^3-benzoylthymine (N^3-BzT) **4** under the Mitsunobu reaction conditions to give compound **12** in 75% yield. The ESI^+ mass spectrum of this compound showed, as expected, a peak at m/z 1701 corresponding to $M + Na^+$.

The structural characterization of compounds **8** and **12** was achieved through the combined analyses of 1D and 2D NMR data. Interpretation of ^{1}H–^{1}H COSY, HSQC, TOCSY and HMBC spectra allowed the determination of the exact sugar sequences of each carbohydrate, together with their linkage positions and substitution mode.

Analysis of the ^{1}H–^{1}H COSY and 2D TOCSY spectra starting from the δ 5.67 anomeric proton signal H1′ allowed the sequential assignments of the signals of the four cycles. On the HMBC spectrum, the ribose anomeric proton (ring III) of both compounds showed a long-range correlation with the signal at δ 83.46 for **8** and 83.02 for **12** attributed to the C5 of the ring I unit, the proton of which at δ 3.88 showed a $^3J_{C-H}$ correlation

† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectroscopic data for **8** and **12**. See http://www.rsc.org/suppdata/ob/b5/b505280g/

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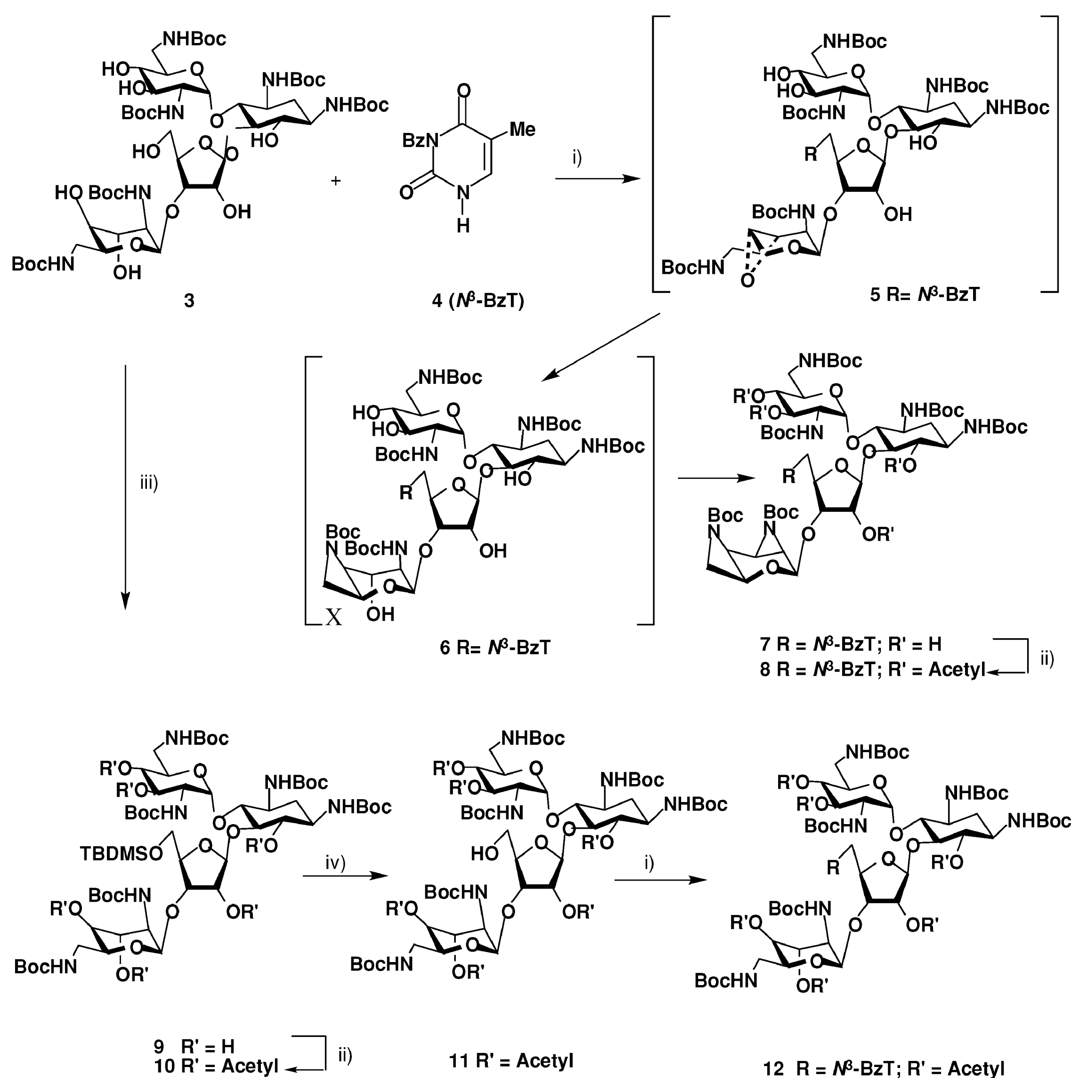

Scheme 1 *Reactions and conditions*: i) DIAD, PPh_3, dioxane, rt, 18 h; ii) Ac_2O, pyridine, rt, 18 h; iii) TBDMS chloride, imidazole, DMF, rt, 18 h; iv) CAN, methanol, rt, 18 h.

with C1″ of ring III at δ 110.15 (**8**) and 109.89 (**12**), respectively. An HMBC correlation from the anomeric proton H1‴of ring IV at δ 4.31 (**8**) and 4.74 (**12**) to C3″ of ring II at δ 76.98 (**8**) and 78.36 (**12**), respectively, was also noted.

A careful comparison of the ^{1}H and ^{13}C NMR data† of **8** with those of **12** showed that the two structures are very similar, except that **8** exhibited a proton signal at δ 3.15. The high-field resonance of this proton suggested that a nitrogen atom was attached at the corresponding carbon. Moreover, TOCSY and COSY data established that the most significant changes occurred exclusively within ring IV. In the COSY spectrum of **8** the signal of the anomeric proton H1‴ at δ 4.31 is only connected to H2‴ at δ 3.90 which is itself connected to H3‴at δ 3.15. The latter proton is coupled with the proton giving a signal at δ 3.35 that is due to H4‴. Finally, the signal at δ 4.00 arising from H5‴ is connected to the H6‴ protons (at δ 3.35) and to H4‴ (at δ 3.35). In the ^{13}C spectrum of **8**, the signals of C3‴ and C4‴ (ring IV) were shifted to a higher field than those corresponding to C3‴ and C4‴ of **12** by 17.7 and 15.6 ppm, respectively. Comparison of the ^{1}H and ^{13}C NMR data of **8** with those of **12** led to the conclusion that two cyclization reactions did occur within ring IV in the case of **8**. Clearly, these cyclizations resulted from the formation of a three-membered ring between the NHBoc at position 2‴ and C3‴ and of a four-membered ring between the NHBoc at position 6‴ and C4‴, respectively. This interpretation is confirmed by the correlations, observed in the HMBC spectrum of **8**, between H4‴ and H6‴ and the carbonyl of the *N*-Boc protecting group at 158.94 ppm as well as the correlations between H3‴ and H2‴ and the other carbonyl at 157.00 ppm. Moreover, by viewing the correlations between the methyl groups of the acetates with their corresponding carbonyls, it is very clear that six correlations are observed in the case of **12** (hexa-acetate) whereas only four correlations are observed in the case of **8** (tetra-acetate).

Compound **12** was completely unprotected in two steps consisting of an ammonia treatment, to remove both the benzoate and the acetates, followed by a trifluoroacetic acid treatment to free the amine functions. The resulting compound has been fully characterised.

Finally, a mechanistic sequence can be proposed to explain the formation of **7**. It involves the epoxide **5** to be a preliminary intermediate[10] that might rearrange to give the azetidine **6**, a tentative immediate precursor of **7**, by way of a second Mitsunobu step responsible for the formation of the 2‴,3‴-aziridine.

In conclusion, we have developed a route to introduce thymine (or uracil) at the 5″-position of neomycin B. A shorter route to these compounds was also explored in which Mitsunobu reaction conditions were applied to *N*-Boc neomycin B **3**. However, in this case we observed a double cyclization reaction involving exclusively the amino functions of ring IV introducing, on the idosopyranose ring, both a 2‴,3‴-aziridine and a 4‴,6‴-azetidine.[11]

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11 We ascribe the fact that we have been unable to obtain a correct ESI mass spectrum for compound **7** and its derivatives to the high susceptibility of its 2‴,3‴-aziridine three-membered ring to adventitious hydrolysis, presumably occurring under the mass measurment conditions.