Mechanism and Stereochemistry of the Elaboration of the Neosamine C Ring† in the Biosynthesis of Neomycins

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The amino-methyl group of the neosamine C ring of neomycins is formed by an oxidation–transamination process, $-CH_2-OH \rightarrow -CH{=}O \rightarrow -CH_2NH_2$.

Neomycins (**1**) belong to the aminocyclitol group of antibiotics.[1] Of the multiplicity of biochemical challenges offered by this class of antibiotics, two are particularly relevant to this and the accompanying communication. These are: (i) the mechanistic origin of the C-6 amino group of the neosamine C and B rings of neomycins (rings I and IV in **1**), and (ii) the nature of biochemical events through which a glucose-derived biological intermediate is cyclised to produce the cyclohexane ring system of the 2-deoxystreptamine moiety (ring II) present in neomycins and in at least 50 other antibiotics.[1]

In this communication we examine various possibilities for the introduction of the 6-amino group in the biosynthesis of

† The systematic name of neosamine C: 2,6-dideoxy-2,6-diamino-D-glucose.

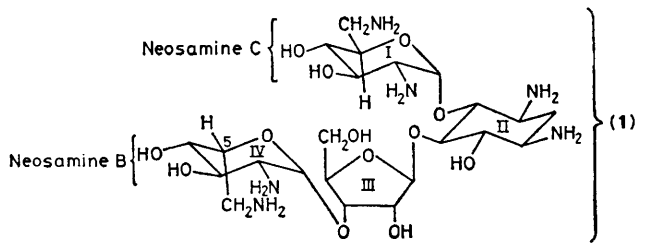
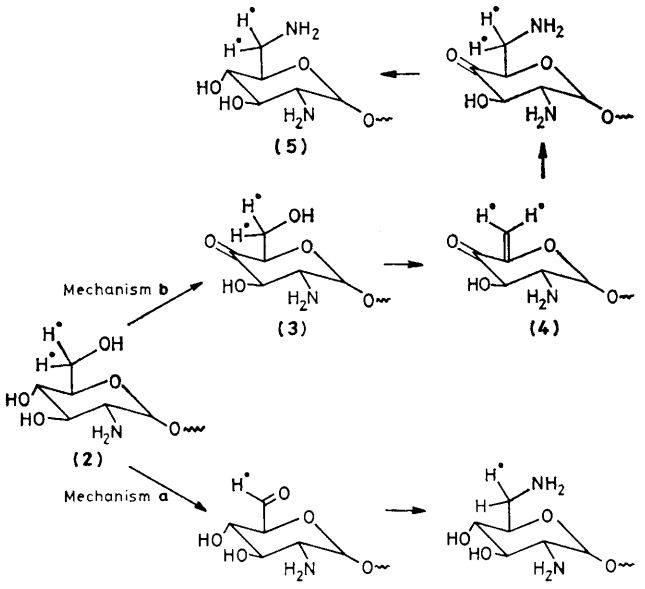


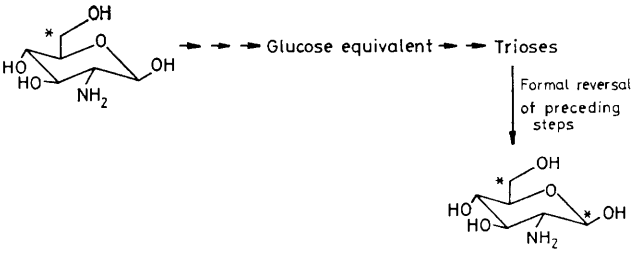
Figure 1. Neomycin B, (**1**). Neomycin C, Structure (**1**) with the stereochemistry at C-5 in ring IV that of D-glucose as in ring I.



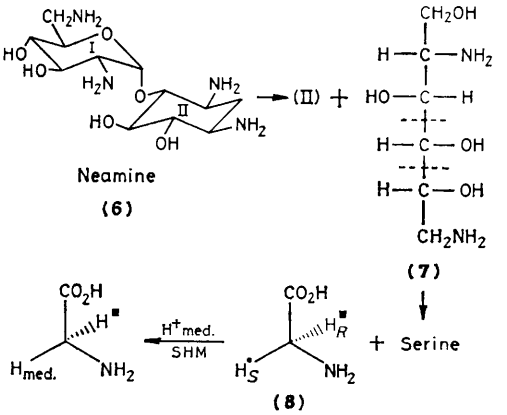
Scheme 1

the neosamine C ring (ring I in **1**) of neomycins. General enzymological considerations and precedents from the area of carbohydrate biochemistry suggest that the C-6 amination of sugars may occur by one of at least two broad mechanistic routes shown in Schemes 1a and 1b. The first, Scheme 1a, involves an initial oxidation of the 6-hydroxy group to an aldehyde that is then converted into an aminomethyl group through a transamination reaction of the type found in the conversion of α-oxo acids into the corresponding amino acid. The second mechanism, Scheme 1b, assumes that an early step in the transformation is an oxidation at C-4 to produce the keto intermediate (**3**) which upon dehydration to the α,β unsaturated ketone (**4**), followed by the Michael addition of ammonia, is converted into the desired skeleton (**5**). The two possibilities considered above may be differentiated by the fact that the mechanisms of Schemes 1a and 1b respectively require that one or none of the hydrogen atoms resident at position 6 of a suitably labelled precursor will be eliminated during its conversion into the neosamine C ring of neomycins.

The pioneering biosynthetic studies of Rinehart and his colleagues have established that all the four rings of neomycin B and C (**1**) are derived from glucose and that glucose is incorporated into the neosamine B, as well as C, rings *via* a glucosaminyl derivative.[2,3] Extending this observation we have now studied the status of hydrogen atoms at C-6 of [6-$^3H_2$]-glucosamine (**2**,-CH_2OH = -C^3H_2OH) during its conversion into ring I of neomycins. An essential prerequisite for the mechanistic work was the development of biosynthetic conditions under which the label from position 6 of glucosamine is



Scheme 2



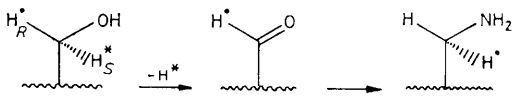
Scheme 3. The conversion of (**7**) into serine + glycine (**8**) involved: (a) acetic anhydride, (b) $NaIO_4$, (c) H^+/H_2O. SHM is serine hydroxyl methyl transferase that exchanges the H_S of glycine with the protons of the medium.

incorporated into the desired rings without the rearrangement of the carbon skeleton which may occur[2] by a metabolic interconversion commonly operational in living cells and shown in Scheme 2.

This was achieved using a protocol[4] in which 10 × 125 ml flasks containing 10 ml of a defined medium[5] were inoculated with *Streptomyces fradiae* (A.T.C.C. 10745) and the culture was grown at 28 °C until the cell density was about 90% (usually 48 h) of that expected for the stationary phase. [*U*-$^{14}C_6$,6-$^3H_2$]-D-Glucosamine (10 μmol, 4.5 × 10^6 d.p.m. of ^{14}C per flask) was then administered and the growth continued for another 12 h. After the removal of cell mass the incubation medium was subjected to chromatography on Amberlite CG-50 (NH_4^+) and the antibiotic fraction eluted with 2M NH_4OH.[6] The material was further purified by rechromatography on the same resin but the elution involved the use of a gradient from 0.05M to 0.35M NH_4OH. The combined fractions containing neomycins (the antibiotic is a mixture of about 80% neomycin B and 20% neomycin C) had 1.6 × 10^6 d.p.m. of ^{14}C (3.5%) and were processed to obtain neamine (**6**)[2] which was further hydrolysed to furnish the ring I of neomycins as neosaminol C (**7**) and the ring II as 2-deoxystreptamine. The relative distribution of ^{14}C in the two species (**7**) and 2-deoxystreptamine, revealed that, of the radioactivity associated with neamine, 75—100% (in 6 independent experiments) was present in neosaminol C (**7**), thus showing that glucosamine is the preferred precursor of the ring I of neomycins. A comparison of the ^3H:^{14}C ratio of glucosamine with neosaminol C suggested that during the biosynthesis, half of the ^3H residue at C-6 of glucosamine is lost in the elaboration of ring I. The further degradation[2] of neosaminol C (**7**), by the sequence of Scheme 3, to glycine and serine was attended by the recovery of all the ^3H in glycine (Table 1). Once again the amount of ^3H at C_α of glycine was exactly half of that originally present at C-6 of glucosamine. That the ^3H was exclusively

Table 1

Compounds	^3H: ^{14}C Ratio	^3H: ^{14}C Atomic ratio
1 [*U*-$^{14}C_6$, 6-^3H]-D-glucosamine	5.6	2:6
2 C-6 of starting [*U*-^{14}C,6-^3H]-glucosamine as formaldehyde dimedone	33.68	1.995:1
3 Neosaminol C (**7**)	2.76	0.983:6
4 Serine from (**7**)	0.226	0.026:3
5 Glycine from (**7**)	8.16	0.971:2
6 Glycine after incubation with serine hydroxymethyl transferase	0.6	0.071:2



Scheme 4

located in the H_S position of glycine was shown by an exchange reaction catalysed by serine hydroxymethyl transferase.[7] The mechanistic interpretation of these results requires the assurance that the [6-^3H]glucosamine skeleton had been incorporated into the neosamine C ring intact, and this was provided by the demonstration that, of the label from [1-^{14}C]glucosamine incorporated into the neosamine C ring, more than 98% was located at its carbon-1.

The cumulative results showing that in the construction of the aminomethyl group of the neosamine C ring, one of the ^3H from the precursor, [6-$^3H_2$]glucosamine, is lost are consistent with the basic tenets of the mechanism shown in Scheme 1a. A similar loss of one of the C-6 hydrogen atoms from [6-$^3H_2$]-glucose has recently been shown in the biosynthesis of the neosamine C ring of a related antibiotic, ribostamycin, by *Streptomyces ribosidificus*.[8] In the latter work it was also shown that it is the H_S at C-6 of the precursor, glucose, that is labilised in the biosynthesis. If it is assumed that the basic mechanism and stereochemistry of the aminomethylation process in the two antibiotics are similar then the results from the two studies may be combined to suggest that the overall process occurs with the inversion of configuration as shown in Scheme 4.

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