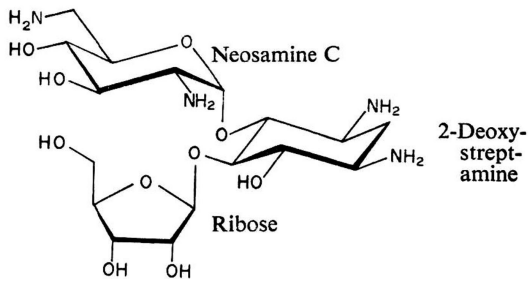
TRANSAMINATION STEREOCHEMISTRY IN THE FORMATION OF NEOSAMINE C OF RIBOSTAMYCIN

Sir:

The biosynthesis of ribostamycin produced from *Streptomyces ribosidificus* was studied by us using D-[6,6-$^2H_2$]-, D-(6*R*)-[6-$^2H_1$]- and D-(6*S*)-[6-$^2H_1$]glucose.[1,2] Our results showed that the *pro S* hydrogen of the C-6 position of D-glucose is removed and the *pro R* hydrogen is retained during the formation of neosamine C and suggested a possible D-glucos-6-ulosamine intermediate which was subsequently transaminated to form neosamine C as shown in Scheme 1.[1,2]

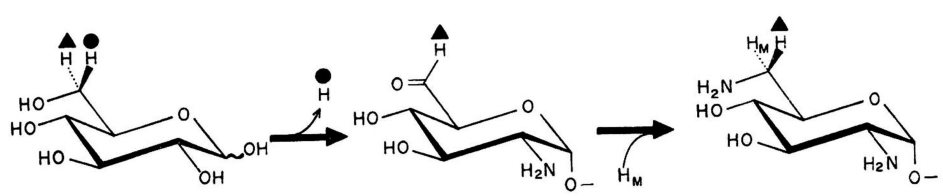


Ribostamycin

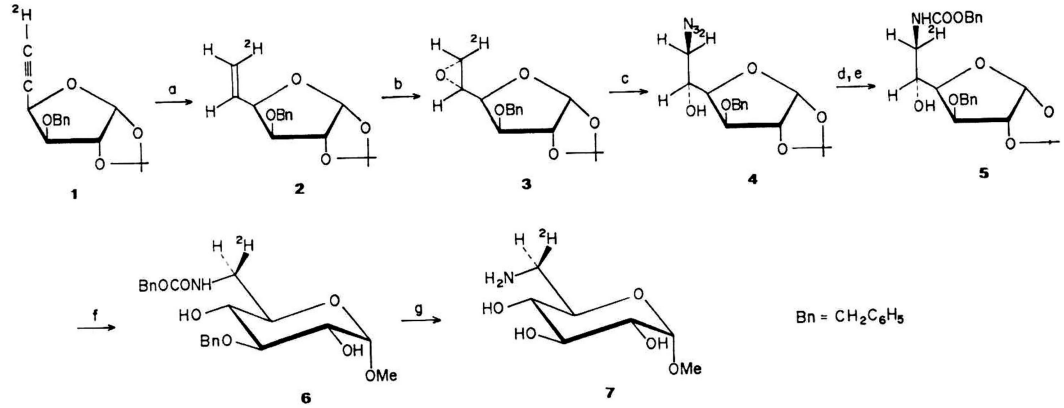
Comparison of the 400 MHz ^1H NMR spectrum of unlabeled ribostamycin with those of ^2H-labeled and unlabeled D-glucose implied that the conformation around the C-5 and C-6 bond of neosamine C is similar to that of α-D-glucopyranose, since a downfield signal due to a proton of a methylene group either of neosamine C or of α-D-glucopyranose has a small coupling constant ($^3J_{H-5,H-6}$ 2~3 Hz) whereas an upfield signal shows a large coupling constant ($^3J_{H-5,H-6}$ 6~7 Hz). If this was the case, ^2H-labeled neosamine C prepared by feeding experiments with ^2H-labeled D-glucose would have *S* configuration at the C-6 position, because the downfield signal of α-D-glucopyranose was clearly assigned to the *pro S* hydrogen of the hydroxymethyl group and because the C-6 deuterium of labeled neosamine C resonating at 3.3 ppm in the ^2H NMR spectrum could correspond to a signal at 3.05 ppm (dd, J=3 and 14 Hz) in the ^1H NMR spectrum of ribostamycin, but not to a signal at 2.88 ppm (dd, J=7 and 14 Hz).

To elucidate the stereochemistry of the above-mentioned transamination, a synthetic study of methyl (6*S*)-[6-$^2H_1$]-6-amino-6-deoxy-α-D-glucopyranoside as a model for ^1H and ^2H NMR analyses was undertaken. Scheme 2 illustrates the route employed. A key intermediate was an epoxide **3** which was prepared as described previously.[2,3] The following points are to be noted: 1) partial isomerization of the ^2H-substituted double bond took place during hydrogenation of the acetylene **1** to **2**; and 2) the deuterium enrichment of **3** was 69% estimated by mass spectrometry and ^1H NMR spectroscopy. These were advantageous because ^1H and ^2H NMR assignments of the aminomethyl hydrogens of the final product **7** were unambiguously made on the basis of the signal intensities. A nitrogen functionality was then introduced with inversion of configuration to give **4**, which was further transformed *via* **5** and **6** to yield the desired α-pyranoside **7**, ^1H NMR (400 MHz, ^2H_2O solvent, H^2HO signal as a reference at 4.80 ppm): 3.45 ppm (s, OMe), 4.85 ppm (d, J=4 Hz, H-1), 3.60 ppm (dd, J=4 and 10 Hz, H-2), 3.68 ppm (dd, J=10 and 9 Hz, H-3), 3.33 ppm (dd, J=9 and 10 Hz, H-4), 3.75 ppm (dd, J=10 and 9 Hz, H-5) and 3.01 ppm (br. d, J=9 Hz, *pro R* H-6). The *pro S* proton of the C-6 was observed at 3.13 ppm as a weak signal. Trends of chemical shifts and coupling constants discussed above were also confirmed for an aminomethyl group of **7**. The 61.48 MHz ^2H NMR spectrum of **7** clearly showed a strong signal of the *pro S* deuterium of C-6 at 3.25 ppm (H_2O solvent, ^2HHO signal as a reference at

Scheme 1. The biosynthetic pathway of neosamine C.



Scheme 2. Synthesis of methyl (6*S*)-[6-$^2H_1$]-6-amino-6-deoxy-α-D-glucopyranoside.



a) H_2/Lindlar, quinoline, b) MCPBA, c) NaN_3, NH_4Cl, d) $LiAlH_4$, e) BnOCOCl/Na_2CO_3, f) HCl - MeOH, g) H_2/Pd-C.

4.80 ppm) and a weak signal at 2.96 ppm due to the *pro R* deuterium at C-6 of a diastereoisomer formed from coproduced (*E*)-deuteroolefin *vide supra*.

As a result, we can now assign the ^2H NMR signal observed at 3.3 ppm of ^2H-labeled ribostamycin to the *pro S* deuterium at the C-6 aminomethyl group of neosamine C based on the chemical shift. Thus, the net transformation from D-glucose to neosamine C proceeds stereospecifically with inversion of configuration at the C-6 position as shown in Scheme 1 and this result is consistent with that reported recently by AKHTAR and coworkers using a completely different approach.[4]

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