# Structural studies of phenylhydrazine integration in carbohydrate derivatives

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## Abstract

Kanamycin is an aminoglycoside antibiotic with multiple therapeutic applications. A previous step of their synthesis is the formation of 3-amino-3-deoxy-D-glucose from commercial available products. Integration of phenylhydrazine with a dialdehyde in water, as a key step, was described in literature as an elegant and easy synthesis. However, a low reproducibility of this method makes synthetic chemists reluctant to its use. We have revisited this process as the aim to improve the described reaction conditions and to analyze, mostly by NMR, the structure of the involved compounds in an effort to contribute to elucidate its mechanism.

## Keywords

Carbohydrates; phenylhydrazine; biological activity; green chemistry; NMR.

## Introduction

Today the aminoglycosides are still the most commonly used antibiotics worldwide thanks to the combination of their high efficacy with low cost. The interest of aminoglycoside antibiotics is due to their use in treatment of wide variety infections. These compounds act on gram-positive and gram-negative bacteria as protein synthesis inhibitors.[1] However, they are nephrotoxic and ototoxic in some cases, thus limiting its use. Kanamycin is an aminoglycoside antibiotic with multiple therapeutic applications. In general, the fluorination process is an important method in medicinal chemistry.[2] Adding to the bioavailability issue, the fluorination of bioactive molecules not only

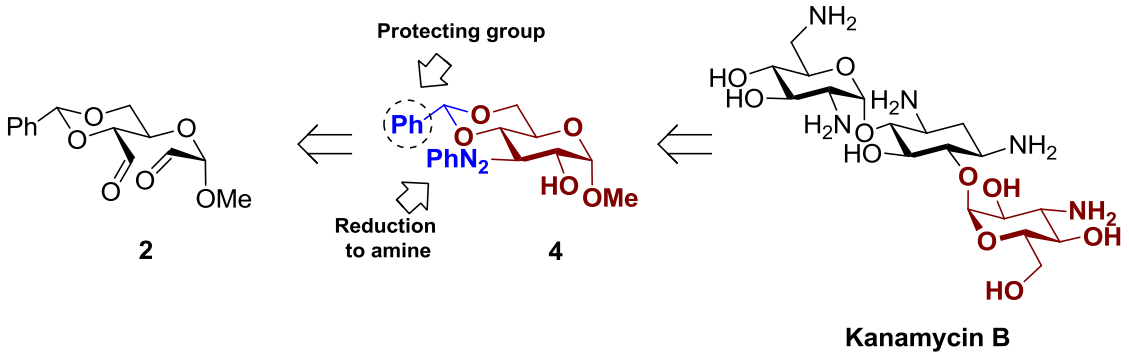
[1] a) Li, J.; Chang. T., *Anti-Infec. Agents in Med. Chem*. **2006**, *5*, 255.; b) Corey, E. J.; Czakó, B.; Kürti, L., *Molecules and Medicine*. John Wiley & Sons, New Jersey, **2007**
[2] Kirk, K. L. *Organic Process Research & Development*. **2008**, *12*, 305

increases frequently their biological power, but also usually improves their lipidic permeability and metabolic stability.

It is known that the presence of fluorine at the carbohydrate moiety contained in aminoglycoside antibiotics reduce their when the fluorine atom is adjacent to amino group. We modify these properties with a fluorinating reagent, such as diethylaminosulfur trifluoride (DAST).[3,4]

## Results and discussion

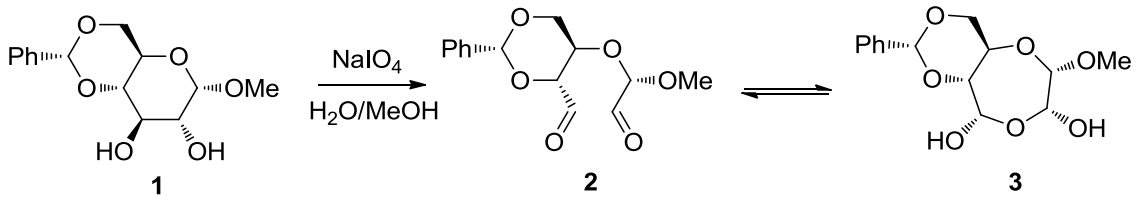
A previous step of Kanamycin synthesis is the formation of 3-amino-3-deoxy-D-glucose from commercial available products. Integration of phenylhydrazine with a sugar derived dialdehyde (**2**) in water, as a key step, has been used[5] as an elegant and easy strategy synthesis. (Scheme 1)




**Scheme 1.** Synthetic route toward Kanamycin

We have revisited this process and we report herein new NMR and IR data of compounds **2** and **4** that confirm the structures proposed in literature. In addition, we describe novel different conditions for their synthesis that reduce the time of reactions, particularly in case of equilibrium between the dialdehyde **2** and hemialdal **3,** obtained from periodate oxidation of methyl 4,6-O-benzylidene-α-D-glucoside. (Scheme 2)

[3](a) Ferret, H. et al. *Arkivoc*, **2010**, (*viii*), 126
[4] Vera-Ayoso, Y., Borrachero, P. Cabrera-Escribano, F., Carmona-Asenjo, A., Gómez-Guillén, M. *Tetrahedron: Asymmetry*. **2004**. *15*. 429
[5] Patroni, J.; Stick, R. *Aust. J. Chem*, **1985,** *38*, 947 and reference therein.




**Scheme 2.** Reaction to obtain dialdehyde **2** and equilibrium proposed

To verify that this equilibrium exit, we design a NMR experiment set. In the first place, we carried out $^{1}$H- and $^{13}$C-NMR of the obtained compound in DMSO-$d_6$. In these spectra, the aldehyde functional group was not observed (Figure 1). When we used $D_2O$ as solvent, we observed the presence of aldehyde in both $^{1}$H- and $^{13}$C-NMR spectra (not showed).

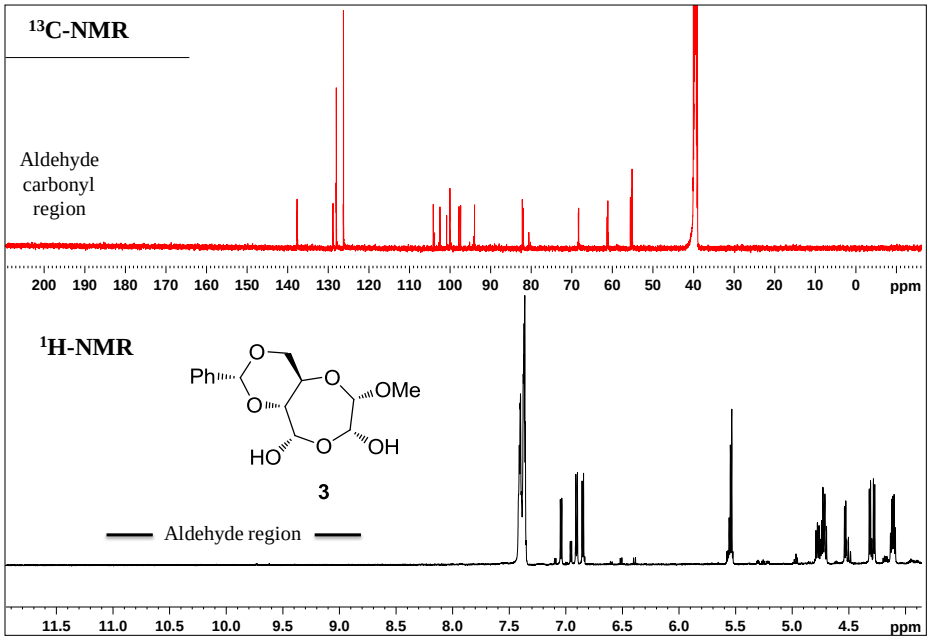
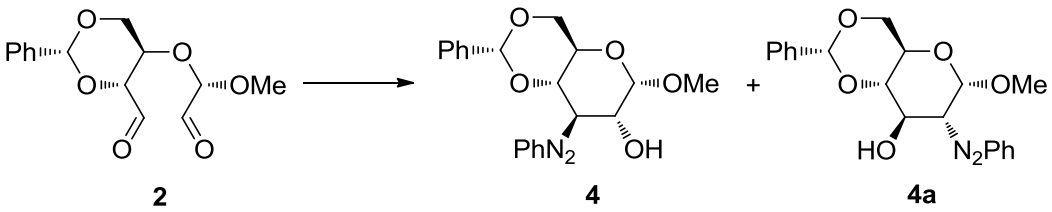


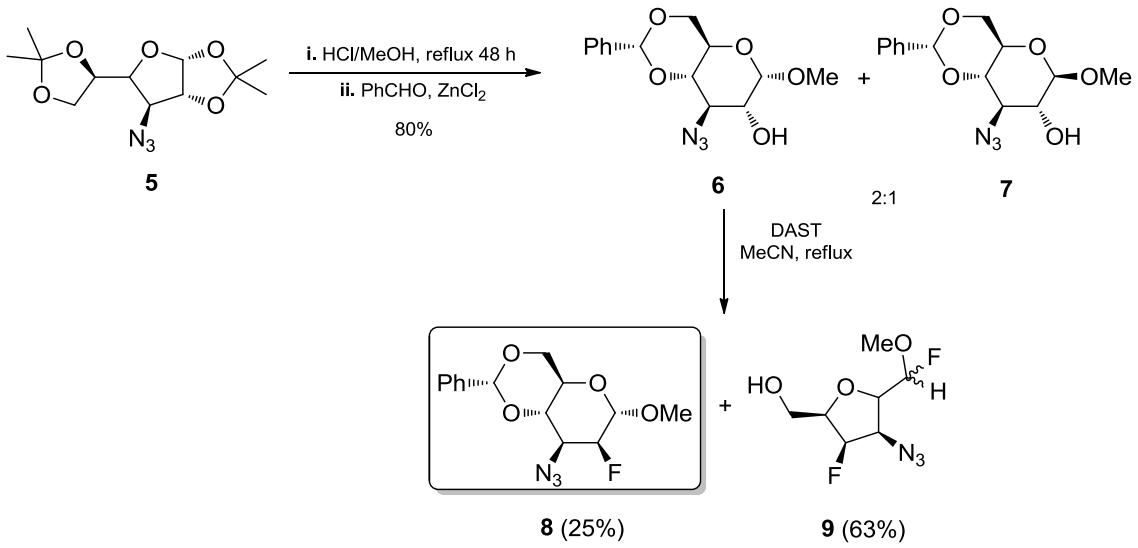

**Figure 1.** $^{1}$H and $^{13}$C-NMR spectrum of hemialdal (**3**) in DMSO-$d_6$

The integration of phenylhydrazine with dialdehyde **2** to obtain compound **4** is not reproducible (Scheme 3). Moreover, we detected by NMR a mixture of isomers **4**/**4a** in a 3:1 ratio.




**Scheme 3.** Reaction to obtain phenylazo derivative **4** from 2 and phenylhydrazine

All this, despite the fact that the above mentioned integration reaction can be considered as a representative example of green chemistry.[6] However, considering that our aim was to obtain the kanamycin precursor, the low reproducibility of this method makes us move to consider the synthesis of 3-amino-3-deoxy-D-glucose by another way.[7] (Scheme 4)




**Scheme 4.** Alternative synthetic route to obtain 3-amino-3-deoxy-D-glucose

The azide group could be easily reduced by catalytic hydrogenation in the presence of Pd/C or by Staudinger reduction with triphenylphosphine. On the other hand, the benzylidene group can be hydrolyzed in a mixture AcOH/$H_2O$ (7:3)[8] or *p*-toluensulfonic acid.[9]

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[6] Anastas, Y.; Warner, J.C. *Green Chemistry: theory and practice.* Oxford University Press, New York, **1998**

[7] Faghih, R.; Cabrera-Escribano, F. et al, *J. Org. Chem.* **1986**, *51*, 4558

[8] Baer, H.H.; Gan Y. *Carbohydr. Res.* **1991**, *210*, 233-245

[9] Vera-Ayoso, Y., Borrachero, P. Cabrera-Escribano, F.; Gómez-Guillén, M.; Caner, J. and Farrás, J. *Synlett.* **2010**, *2*, 271-275

## Experimental

All new compounds were characterized by their IR, $^{1}$H-NMR (500 MHz), $^{13}$C-NMR (125.7 MHz), and HRMS spectral data.

*General procedure of oxidation reactions* (e.g., **2**)

To a solution of sodium periodate in water at 5ºC methyl 4,6-O-benzylidene-α-D-glucopyranoside in MeOH was added, and the mixture was stirred in dark for 20 h at room temperature. EtOH was added to precipitated iodates. The solution was filtered and vacuum dried to obtain dialdehyde **2** (98% yield).

*Phenylhydrazine integration reactions* (e.g., **4**)

To a solution of compound **2** in water phenylhydrazine or phenylhydrazine hydrochloride was added. Rapid cooling and shaken of the mixture until to obtain a yellow precipitated was carried out. The solid was washed and dried to vacuum and recrystallized from butan-1-ol.

*Synthesis of fluorine-carbohydrates* (e.g., **8**)

109 mg of methyl 3-azide-4,6-O-benzilidene-3-deoxy-α-D-glucopyranoside **6** in acetonitrile was treated with DAST at 0ºC under argon, and then the mixture was warm under reflux for 6 hours. The mixture was evaporated and the mixture was purified by chromatography column on silica gel.

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