

The Synthesis of L-Aminosugar and the Studies of L-Pyranoses on the Ring III of Pyranmycins

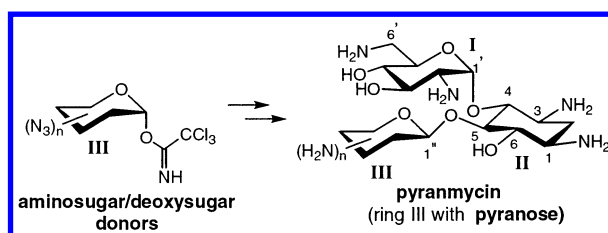
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ABSTRACT



The synthesis of a novel class of aminoglycoside, pyranmycin, and a convenient method for the preparation of 6-amino-L-idopyranosides were reported. One of the members in the reported pyranmycin families, TC010, has prominent activity against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus megaterium*. We also discovered that the ⁴C₁ chair conformation on ring III of pyranmycin is essential for the antibacterial activity.

Neomycin belongs to a group of aminoglycoside antibiotics containing a 4,5-disubstituted 2-deoxystreptamine core and has been used against both gram-positive and gram-negative bacteria for more than fifty years.^{1,2} The neomycin class of antibiotics (Figure 1) exert its antibacterial activity by binding

Although neomycin is still widely used for the treatment of serious infections, its usefulness is significantly hampered by the rapid emergence of drug resistance^{3,4} and its relatively high cytotoxicity.¹

Many neomycin analogues containing a ring III furanose, aiming to increase the antibacterial activity, compensate resistance from aminoglycoside-modifying enzymes, and reduce the cytotoxicity, have been reported.^{5–11} However,

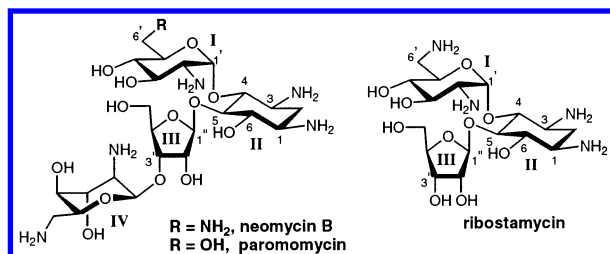


Figure 1. Structures of neomycin class antibiotics.

selectively to the A-site 16S ribosomal RNA of bacteria, and thereby inhibit the protein synthesis of these microorganisms.

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only two examples use D-glucopyranose as the ring III component via α and β linkages.^{12,13} These glucopyranose incorporated adducts are less active than neamine, and no following work has been reported since. Because the glycosidic bond of a furanose is more acid sensitive than that of a pyranose,^{14,15} replacing the furanose ring (III) with an aminopyranose, which leads to the design of pyranmycin, will have two conceivable advantages (Figure 2). First, it

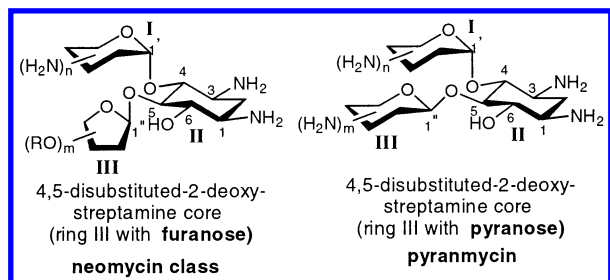


Figure 2. Structural relationship between neomycin class aminoglycoside antibiotics and pyranmycin.

may increase the acid-stability and, therefore, reduce the required orally administered dose of drug and the associated cytotoxicity. Second, from the NMR and X-ray crystallography studies,^{16–19} there is a relatively large cavity within the RNA target to accommodate the conformational change, especially on the ring IV of neomycin. This could allow the introduction of more complex structural components, such as pyranose derivatives, to enhance the antibacterial activity. The resulting artificial aminoglycosides could also become poor substrates for the resistance enzymes, and thereby regain the antibacterial activity against drug resistant bacteria as indicated by Mobashery and colleagues.²⁰

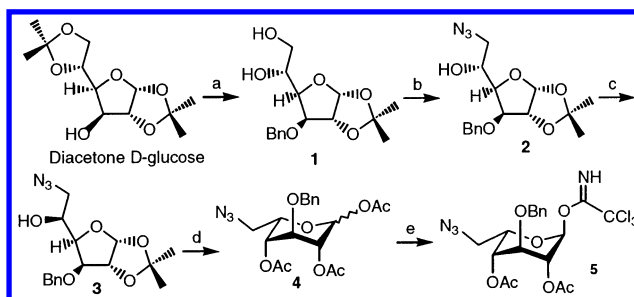
Encouraged by the advantages of our pyranmycin design and the newly developed method for the preparation of L-aminosugar generated from our laboratory, we quickly assembled several pyranmycins with various ring III L-glycopyranoses. Herein, we wish to report our findings in the synthesis of L-aminoidopyranose, and the preparation and antibacterial studies of pyranmycin with L-pyranose at the ring III.

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Wong and co-worker have reported the studies of the roles of the L-idose (ring IV) of the neomycin.⁵ From the molecular modeling of the scaffold of pyranmycin, we discovered that a β -glycosidic bond between rings II and III is crucial in orienting the necessary conformation of rings I and II. Therefore, an analogue of the ring IV idose of neomycin, 6-amino-6-deoxy-L-idose, was selected as the ring III of pyranmycin with the expectation that the presence of the 2-acetyl group instead of the amino (azido) group will favor the formation of the β -glycosidic bond.

We began our synthesis of L-aminoidose from commercially available diacetone-D-glucose (Scheme 1). Benzylation

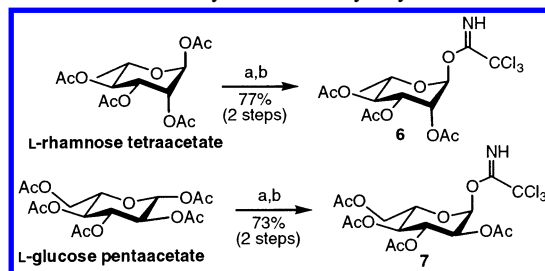
Scheme 1. Synthesis of the 6-Amino-6-deoxy-L-idopyranose Donor^a



^a Conditions: (a) (i) BnBr, NaH, ⁿBu₄NI, THF, (ii) TsOH, MeOH, 65%; (b) (i) TsCl, (ii) NaN₃, 75%; (c) (i) (COCl)₂, DMSO, DIPEA, (ii) NaBH₄, 37%; (d) (i) 60% HOAc, (ii) Ac₂O, 43%; (e) (i) H₂NNH₂–HOAc, (ii) CCl₃CN, DBU, 10%.

of the C-3 hydroxyl group, followed by the regioselective deprotection of the *O*-5,6-isopropylidene group, provided the diol, **1**. A regioselective tosylation of the C-6 hydroxyl group and an azide substitution using NaN₃ incorporated the azido at C-6. In the attempts to invert the chirality of the C-5 hydroxyl group, we were pleased to find out that the standard procedure employed in our laboratory, Swern oxidation then NaBH₄ reduction, can achieve our objective conveniently, providing compound **3**. The finding offers a practical method of making L-sugars from D-sugar in addition to the modification, mainly azide incorporation, on the C-6 position. The observed stereoselectivity of the NaBH₄ reduction can be

Scheme 2. Syntheses of Glycosyl Donors^a

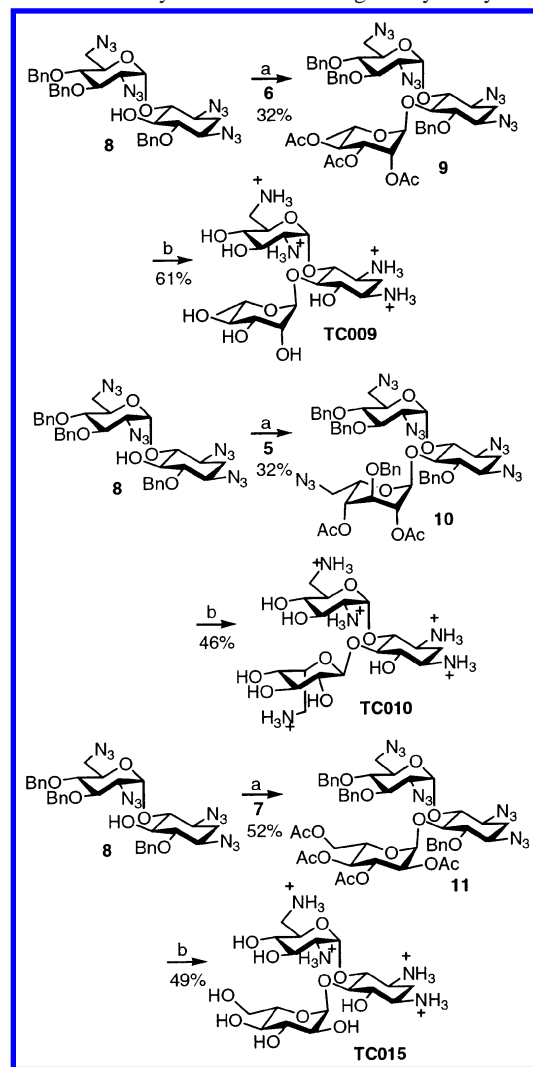


^a Conditions: (a) H₂NNH₂–HOAc, DMF; (b) CCl₃CN, DBU, CH₂Cl₂.

explained with the Felkin-Ahn model. Deprotection of the *O*-1,2-isopropylidene group using HOAc/TFA/H₂O solution, followed by peracetylation afforded the acetyl 6-azido-6-deoxy-L-idopyranose, **4**.

The acetyl 6-azido-6-deoxy-L-idopyranose, **4**, was converted into a glycosyl trichloroacetimidate after hydrolysis of the anomeric acetyl group using hydrazine acetate followed by reaction with trichloroacetonitrile. A similar procedure was used for the preparation of designed ribostamycin analogues with L-rhamnose and L-glucose as the ring III sugars (Scheme 2). Acid-catalyzed glycosylation of these three glycosyl donors with neamine acceptor⁵ afforded the designed protected pyranmycins (Scheme 3). These three

Scheme 3. Synthesis of the Designed Pyranmycins^a



^a Conditions: (a) BF₃·OEt₂, CH₂Cl₂, 4 Å MS; (b) (1) K₂CO₃, MeOH, (2) PMe₃, THF/H₂O, (3) H₂, Pd(OH)₂/C.

protected pyranmycins were subjected to the final synthesis, hydrolysis of acetyl groups, reduction of azido with Staudinger reaction, and deprotection of benzyl groups with hydrogenation. We modified the procedure for the final synthesis from the literature²¹ and obtained the final products, **TC009**,

TC010, and **TC015**, with excellent purity and with minimum effort devoted to column chromatography purification. The three analogues were assayed against *E. coli*, *Staphylococcus aureus*, and *Bacillus megaterium*, and the minimum inhibitory concentrations (MIC)²² were determined using neomycin, ribostamycin, and neamine as the control (Table 1).

Table 1. MIC of Pyranmycins

compd	MIC (μM)		
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. megaterium</i>
neomycin B	2	2	ND ^a
ribostamycin	5	12	ND
neamine	36	ND	ND
TC009	inactive	inactive	ND
TC010	9	23	2
TC015	inactive	inactive	ND

^a ND: not determined.

Among the three members in pyranmycin families with L-sugar on ring III, **TC009** contains a L-rhamnopyranose, **TC010** contains a 6-amino-6-deoxy-L-idopyranose, and **TC015** has a L-glucopyranose. **TC010** is almost as active as ribostamycin while **TC009** and **TC015** are not active at all. From the coupling constant of the ¹H NMR, we learned that all of the trichloroacetimidate glycosyl donors from L-rhamnose, L-glucose, and 6-amino-6-deoxy-L-idopyranose have a ¹C₄ chair conformation. Interestingly, after the glycosylation and final syntheses, the 6-amino-6-deoxy-L-idopyranose on **TC010** adopts a ⁴C₁ chair conformation (*J*_{1'',2''} of the L-idopyranose changes from 0.0 to 7.24 Hz) while the L-rhamnopyranose on **TC009** remains in its ¹C₄ chair conformation (*J*_{1'',2''} of the L-rhamnopyranose varies from 2.0 to 2.97 Hz).

In an effort to provide an explanation for the lack of activity of **TC009**, we carried out molecular modeling for both **TC009** and **TC010** (Figure 3). The results show that a

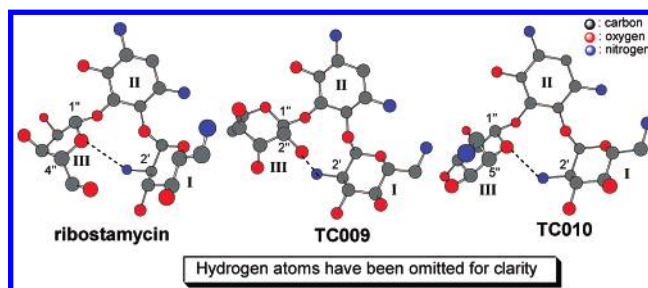


Figure 3. Optimized conformations of ribostamycin, **TC009**, and **TC010**.

⁴C₁ chair conformation will preserve the desired intramolecular hydrogen bond between 2''-OH and 2'-NH₂ in

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TC010, which has been suggested to be crucial in orienting the neamine core for binding toward the A-site of the 16S RNA.¹⁷ To maintain an intramolecular hydrogen bond between the ring III with a ¹C₄ chair conformation and the neamine core, a similar interaction between 2''-OH and 2'-NH₂ was produced. However, a rather dramatic conformational difference, a 180° flipping on the ring III of **TC009**, is observed, which may account for the lack of antibacterial activity for **TC009**.

We also carried out acid-degradation experiments for the constructed analogue using neomycin as the control. Neomycin and **TC010** were dissolved in D₂O purged with anhydrous HCl (pH ca. 1) then sealed in the NMR tubes and incubated at 37 °C. The controls were prepared from neomycin and **TC010** in neutral D₂O. All four samples were monitored with ¹H NMR. The controlled samples showed no sign of degradation during the 14-day incubation period as indicated by the signals of the anomeric H-1' and H-2 protons. However, the neomycin and **TC010** in acidified D₂O underwent a time-dependent acid degradation (20%, 40%, 60%, and 80% degradation after 2, 6, 10, and 14 days for neomycin, and 5%, 20%, 35%, and 50% for **TC010**, respectively). After the incubation, the samples in acidic D₂O were pump-dried and assayed against *E. coli*. We observed a significant decrease in the antibacterial activity of neomycin and **TC010**. The MIC of acid-treated neomycin increases from 2 μM to 50 μM, while the MIC of **TC010** increases from 9 μM to 40 μM.

In conclusion, we have reported a concise and convenient method for the preparation of L-aminoidopyranose and the synthesis of a novel class of aminoglycoside, pyranmycins. The constructed aminoglycoside has promising antibacterial activity. The ⁴C₁ chair conformation on ring III of pyran-

mycin appears to be essential for the antibacterial activity. This finding provides us a guideline for our future approach. Interestingly, the 2,6-diamino-2,6-dideoxy-L-idose (ring IV) of neomycin adopts an ¹C₄ chair conformation. Although **TC010** shows degradation in acidic media, the slower rate of degradation provides us the support of using pyranose as the substitution of furanose at the ring III of neomycin class aminoglycoside antibiotics. We believe this new addition of aminoglycoside can pave the way to the development of more potent antibiotics against drug-resistant bacteria. We have currently constructed a library of pyranmycins with both D- and L-aminosugars as the ring III component. We expect to generate more interesting structural activity relationships of the new class of aminoglycosides soon, and provide valuable information for developing a library of new antibacterial agents.

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Supporting Information Available: Experimental procedures for the preparation of compounds **2–7**, **9–11**, **TC009**, **TC010**, and **TC015** and the corresponding ¹H and ¹³C NMR spectra; mass spectra for compounds **3**, **4**, **9–11**, **TC009**, **TC010**, and **TC015**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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