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Design and Synthesis of Pyrankacin: A Pyranmycin Class of Broad-Spectrum Aminoglycoside Antibiotic

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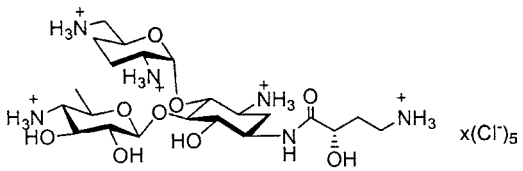
ABSTRACT



Pyrankacin

A novel broad-spectrum aminoglycoside antibiotic, pyrankacin, has been prepared. In addition to the synthetic innovation in dideoxygenation and regioselective Staudinger reduction, we have obtained prominent antibacterial activity against several clinically important pathogens in the course of this work.

Aminoglycosides have long been antibiotics of choice for their impressive antibacterial activity (Figure 1).[1] These bactericidal substances exert their antibacterial effects by interfering with the protein synthesis at the prokaryotic ribosomal RNA level.[2] Although alterations to the ribosomal binding site[3] and decreased permeability[4] into the cells have been implicated as modes of resistance toward aminoglycosides, their broad-spectrum activity has, in the main, been compromised by the prevalence of aminoglycoside modifying enzymes in resistant strains of certain pathogenic bacteria.

For some time now, our group has been working on the synthesis of novel aminoglycosides (Figure 1), and we recently came up with a neomycin class of aminoglycoside, called pyranmycin,[5] and identified a lead structure **TC005**. We then synthesized the 3′,4′-dideoxypyranmycin analogue, **RR501**, which had impressive antibacterial activity against bacteria that harbor one modifying enzyme, APH(3′)-I.[6]

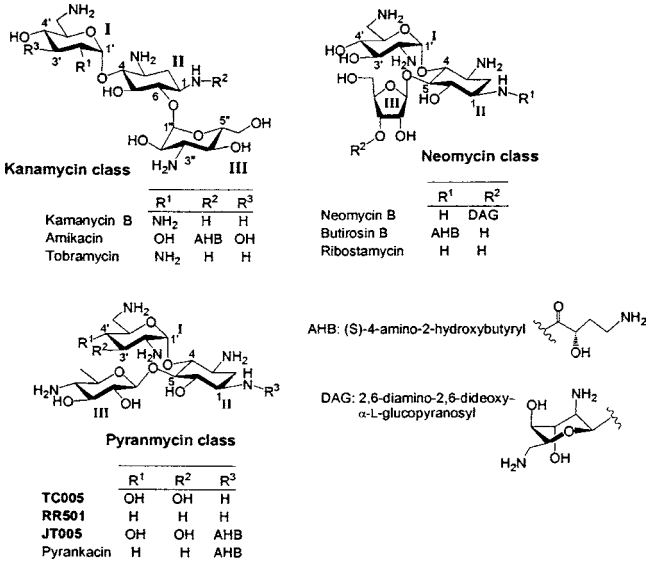


Figure 1. Structure of kanamycin and neomycin classes of aminoglycosides.

Although **TC005** and **RR501** are both not as active as neomycin against aminoglycoside susceptible bacteria, they

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have superior acid stability. In an attempt to further improve the activity of this dideoxy analogue, we have been dedicating our synthetic efforts toward attaching the (*S*)-2-hydroxy-4-aminobutyl (AHB side chain) at the N-1 position. Amikacin, a kanamycin derivative with AHB at N-1, has very impressive activity against resistant bacteria.[7] Therefore, it is expected that the pyranmycin analogue with AHB at N-1 will display similar or even improved activity against resistant bacteria while maintaining the advantageous acid stability.

There are only a few examples of an N-1 modified neomycin class of aminoglycosides,[8] and the synthetic strategies to such molecules are not suitable for modifying tetraazidoneamine, the key intermediate compound we have employed for our aminoglycoside synthesis. We have recently developed a novel method to selectively reduce the N-1 azido group of the 3′,4′-di-*O*-benzoyltetraazidoneamine, **1**, by tuning the stereoelectronic environment of the azido groups.[9] Nevertheless, we still have to extend the same methodology to dideoxyneamine **2**.[6] It has been shown that an electron-deficient azido group has greater reactivity toward the Staudinger reduction than an electron-rich one.[10] The presence of the double bond in **2** could perturb the stereoelectronic environment of 2′-N_3 and prevent the desired selective Staudinger reduction from occurring at 1-N_3. The higher chemical shift of H-2′ in **2** (3.92) as compared to **1** (3.62) confirms our speculation (Table 1). Fortuitously, by

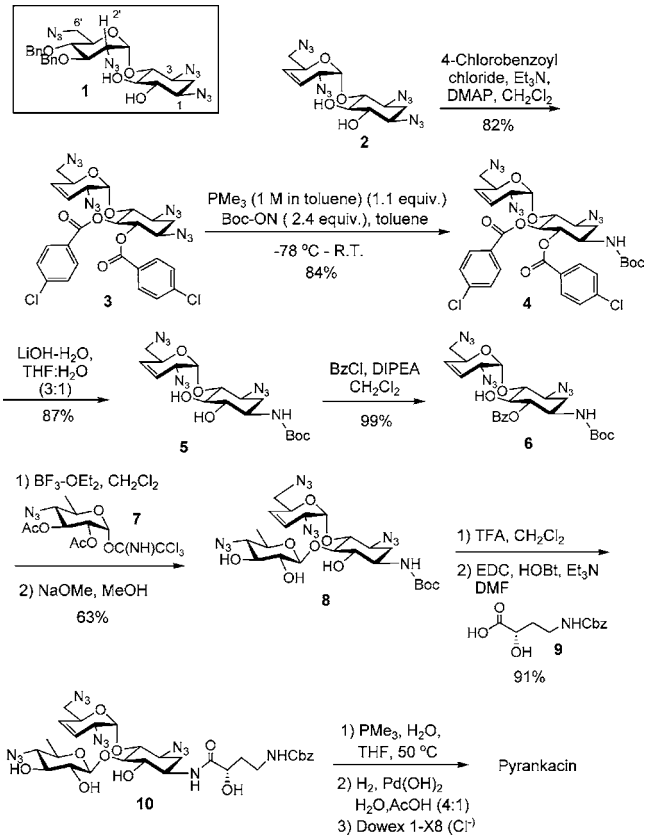
Table 1. Proton Chemical Shifts (ppm) on Acylated Neamine Derivatives

compounds	H-1	H-3	H-2′	H-6′
1	3.28	3.43	3.62	3.57/3.41
2	3.30	3.38	**3.92**	3.45/3.27
3	**3.75**	3.61	3.38	3.40/3.24
4	**4.20**	3.65	3.48	3.48/3.30

using the 4-chlorobenzoyl group on the O-5 and O-6, as in the case of **3**, the needed stereoelectronic effect can still be obtained with the N-1 (H-1) being the most reactive (electron-deficient) one. Upon further investigation, we think the observed selectivity of the Staudinger reaction is governed by a combination of both steric and stereoelectronic effects.[11]

The synthesis of pyrankacin started from the chlorobenzoylation of **2**[6] to yield **3**, which was then subjected to a selective Staudinger reaction to yield the N-1 Boc-protected compound **4** (Scheme 1). Interestingly, the obtained selectivity was even better than when 5,6-di-*O*-acyl-3′,4′-di-*O*-benzoyltetraazidoneamine was employed.[9] Hydrolysis of the ester protecting groups followed by selective benzoylation at the O-6 position gave **6**. Glycosylation of **6** with **7**[5] followed by the hydrolysis of the acyl groups offered the corresponding trisaccharide, **10**. Deprotection of the Boc group and coupling with the (*S*)-*N*-carbobenzyloxy-4-amino-2-hydroxybutyric acid yielded **10**. Global deprotection and ion exchange provided the desired final product, which we named pyrankacin.

Scheme 1. Synthesis of Pyrankacin



Pyrankacin was assayed against various strains of bacteria, and the minimum inhibitory concentration (MIC) was determined using amikacin, neomycin, butirosin, gentamicin, and kanamycin as the controls (Table 2). Aminoglycoside susceptible *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Klebsiella pneumoniae* (ATCC 13883, resistant to ampicillin, susceptible to aminoglycosides) were used as standard reference strains. *E. coli* (pSF815)

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(11) We have recently completed the synthesis of several azidoneamine analogues and studied the effect of various protecting groups on the regioselectivity of Staudinger reduction. The obtained results suggest that the regioselectivity is affected by both steric and stereoelectronic factors. Li, J.; Chang, C.-W. T. Unpublished result.

Table 2. Minimum Inhibitory Concentrations (MIC)[a]

entry	strains	amikacin	butirosin	gentamicin	neomycin	ribostamycin	kanamycin B	pyrankacin	**RR501**	**JT005**
1	*E. coli*[b]	1	2	2	4	8	2	**4**	ND	ND
2	*E. coli* (TG1)[c]	1	1	2	8	2	4	**4**	8	4
3	*E. coli* (pSF815)[d]	1	0.25	inactive[k]	2	16	inactive	**1**	4	4
4	*E. coli* (pTZ19U-3)[e]	0.5	0.5	1	inactive	32	inactive	**1**	4	4
5	*K. pneumoniae*[f]	0.5−1	0.5	8−16	inactive	inactive	inactive	**1**	2−4	1−2
6	*K. pneumoniae*[g]	1	0.5−1	1	2	4	1	**2**	2−4	2
7	*S. aureus*[h]	16	inactive	4	inactive	inactive	inactive	**8**	4	inactive
8	*S. aureus*[i]	1	2	0.5	1	8	1−2	**2**	ND	ND
9	*P. aeruginosa*[j]	0.5−1	inactive	0.5−1	inactive	inactive	inactive	**2**	inactive	inactive

[a] Unit: μg/mL. ND: not determined. [b] *Escherichia coli* (ATCC 25922). [c] *E. coli* (TG1) (aminoglycoside susceptible strain). [d] *E. coli* (TG1) (pSF815 plasmid encoded for (AAC(6′)/APH(2″)). [e] *E. coli* (TG1) (pTZ19U-3 plasmid encoded for APH(3′)-I). [f] *Klebsiella pneumoniae* (ATCC 700603). [g] *K. pneumoniae* (ATCC 13883). [h] *Staphylococcus aureus* (ATCC 33591) (MRSA). [i] *S. aureus* (ATCC 25923). [j] *Pseudomonas aeruginosa* (ATCC 27853). [k] Inactive is defined as MIC ≥ 32 μg/mL.

and *E. coli* (pTZ19U-3) are laboratory resistant strains using *E. coli* (TG1) as the host. *K. pneumoniae* (ATCC 700603)[12] is a clinical isolate that is resistant to ceftazidime, other β-lactams, and several aminoglycosides (ANT(2″)). *Pseudomonas aeruginosa* (ATCC 27853) that expresses APH(3′)-IIb manifests modest resistance toward aminoglycosides.[13] Methicillin-resistant *S. aureus* (ATCC 33591) (MRSA) is the leading cause of bacterial infections and a global scourge. Many MRSA strains contain genes encoded for APH(3′), ANT(4′), and AAC(6′)/APH(2″), which render the bacteria resistant to many aminoglycosides.[14]

From the MIC values, pyrankacin appears to be the one with the most prominent broad-spectrum antibacterial activity against all the examined strains. For example, for the clinically used gentamicin and amikacin, the former is ineffective against bacteria with the bifunctional enzyme, AAC(6′)/APH(2″) and *K. pneumoniae* (ATCC 700603) (entries 3 and 5) whereas the latter is less active against MRSA (entry 7). Pyrankacin is more active than gentamicin against *E. coli* (pSF815) and *K. pneumoniae* (ATCC 700603) (entries 3 and 5). While being less active than gentamicin against MRSA, pyrankacin is more active than amikacin against the same strain. More interestingly, even pyrankacin can be viewed as a neomycin class of aminoglycoside; it is the only active compound against *P. aeruginosa* among **JT005**,[9] neomycin, butirosin, and ribostamycin. The attachment of the AHB group at N-1 of the kanamycin class of aminoglycoside as in the case of amikacin revives the antibacterial activity, whereas the same modification on butirosin and **JT005** does not produce the same effect. This result suggests that a combination of 3′,4′-dideoxygenation and the N-1 AHB group is essential for the neomycin class of aminoglycoside to be active against *P. aeruginosa*.

Molecular modeling of pyrankacin bound to the targeted rRNA did not reveal significant conformational alteration on ring III, and rings I and II and the AHB are almost identical to the reference compound.[15] The AHB group encounters steric hindrance in the active sites of APH(3′)-III and AAC(2′). Interestingly, when pyrankacin is docked in the binding site of AAC(2′), the 6″-CH_3 of ring III that is unique to pyrankacin is pointing toward a hydrophilic area thus creating unfavorable interaction. This result may, in part, explain the difference in activity against MRSA and *P. aeruginosa*.

In conclusion, we have not only designed and synthesized a novel dideoxypyranmycin with the AHB side chain but also been able to devise a novel regioselective Staudinger reaction that can be performed in the presence of a double bond. Pyrankacin maintains the acid stability while displaying an impressive broad-spectrum antibacterial activity against several clinically important pathogens. This novel aminoglycoside can serve as the lead for further synthetic modification, pave the way to the development of a new generation of antibiotics, and tilt the fight against bacterial infections. For example, our work on pyrankacin has yielded the result that the combined modifications of 3′,4′-dideoxygenation and attaching the AHB group at N-1 are pivotal in reviving the antibacterial activity against MRSA. We are currently working on both the neomycin and kanamycin classes of aminoglycosides bearing the combination of 3′,4′-dideoxygenation and the N-1 AHB group.

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Supporting Information Available: Experimental procedures for the preparation of compounds, ^1H, ^{13}C, and COSY spectra of selected compounds, the antibacterial assay, and the molecular modeling results. This material is available free of charge via the Internet at http://pubs.acs.org.

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