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Tuning the Regioselectivity of the Staudinger Reaction for the Facile Synthesis of Kanamycin and Neomycin Class Antibiotics with N-1 Modification

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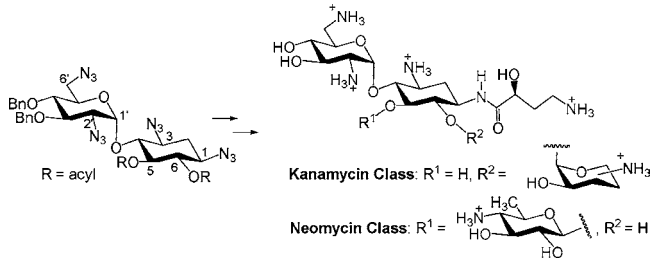
ABSTRACT



A novel method for achieving the desired regioselective reduction of the N-1 azido group on a tetraazidoneamine has been developed that leads to the synthesis of both kanamycin and neomycin class antibiotics bearing N-1 modification. Both classes of aminoglycosides are active against aminoglycoside-resistant bacteria carrying APH(3′)-I and AAC(6′)/APH(2″).

For over sixty years, aminoglycoside antibiotics have been a valuable resource against infectious diseases.[1] Nevertheless, the prevalence of aminoglycoside-resistant bacteria has significantly reduced their effectiveness.[2] With the advancements in studies of resistant mechanisms and the structural information from the binding of aminoglycosides toward the target, the A-site decoding region of 16S rRNA,[3] new strategies have been developed that aim to revive the antibacterial activity against aminoglycoside-resistant bacteria.[4] Among these strategies, attaching functionalities at the N-1 position of the 2-deoxystreptamine among kanamycin or neomycin class antibiotics is one of the most effective methods. This strategy has led to the development of semisynthetic amikacin that has an (*S*)-4-amino-2-hydroxybutyryl (AHB) group at the N-1 position (Figure 1).

The synthesis of kanamycin and neomycin class aminoglycosides with N-1 modification can be achieved via enzymatic[5] or chemical methods.[6] However, several aspects in the synthesis of kanamycin and neomycin class aminogly-

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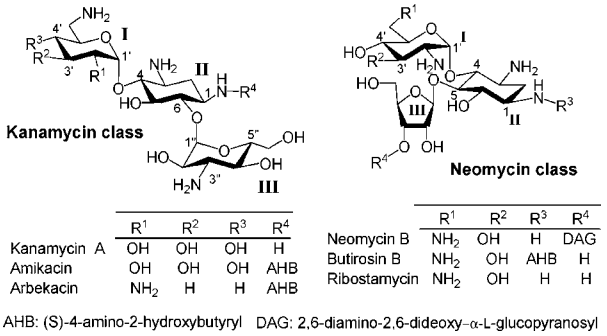


	R^1	R^2	R^3	R^4
Kanamycin A	OH	OH	OH	H
Amikacin	OH	OH	OH	AHB
Arbekacin	NH_2	H	H	AHB

	R^1	R^2	R^3	R^4
Neomycin B	NH_2	OH	H	DAG
Butirosin B	NH_2	OH	AHB	H
Ribostamycin	NH_2	OH	H	H

Figure 1. Structures of kanamycin and neomycin class aminoglycosides bearing N-1 modification.

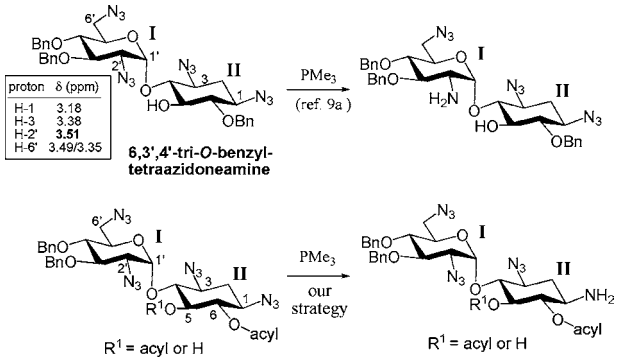


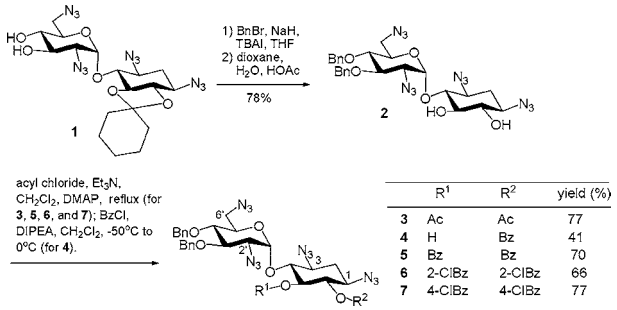
Figure 2. Strategy for regioselective reduction of adizo group.

cosides with N-1 modification have not been satisfactorily addressed. First, only a few examples of neomycin class antibiotics bearing N-1 modification have been reported.[5b,c,6b] Second, the synthetic works involved in N-1 modification on kanamycin class antibiotics are commonly achieved by using various metal chelation methods, which require certain configurations of hydroxyl and amino groups. Introduction of additional functional groups may disrupt the chelation, resulting in the loss of regioselective differentiation of the amino groups. Therefore, metal chelation methods are often limited to modifications of neamine or kanamycin. Third, many prior works employed carbamate-type protecting groups for the protection of amino groups on the aminoglycoside, resulting in the formation of polycarbamate compounds that pose difficulties in their purification and characterization. To alleviate the solubility and purification problems, Wong's group[7] and our group[8] have been using azido groups as the surrogate of amino groups for the synthesis of novel aminoglycosides. Nevertheless, there is no precedent of regioselective conversion of the N-1 azido group to an amino group.

Wong and others have reported that electron-withdrawing protecting groups will enhance the reactivity of their vicinal azido group toward the Staudinger reaction.[9a] Such an electron-withdrawing effect can be correlated with chemical shifts of the corresponding protons (H-1, H-3, H-2′, and H-6′) adjacent to the azido groups. However, the N-2′ azido group appears to be more reactive than the N-1 azido group from the experimental and spectroscopic data (Figure 2). To selectively reduce the N-1 azido group, we began to examine the possibility of tuning the stereoelectronic effect by introduction of electron-withdrawing protecting groups such as acyl groups at the O-5 and/or O-6 positions. It is expected that the acyl groups will increase the reactivity of the N-1 azido group (Figure 2).

Various acyl protecting groups were examined, including trifluoroacetyl, 2-chlorobenzoyl, 4-chlorobenzoyl, benzoyl, and acetyl groups, which have pK_a values of −0.25, 2.92, 3.98, 4.19, and 4.76 for the corresponding carboxylic acids, respectively (Scheme 1).[10] The electron-withdrawing capability of acyl groups is expected to be proportional to the pK_a of the corresponding acids. The attempt to employ trifluoroacetyl group at the O-5 and/or O-6 positions was, however, unsuccessful.

Scheme 1. Synthesis of O-5 and/or O-6 Acylated Neamine Derivatives



	R^1	R^2	yield (%)
3	Ac	Ac	77
4	H	Bz	41
5	Bz	Bz	70
6	2-ClBz	2-ClBz	66
7	4-ClBz	4-ClBz	77

By comparison to compound **2**, incorporation of acyl groups results in various degrees of deshielding of protons (Table 1). One important finding is the effect from the acyl group at the O-5 position. Although the O-6 hydroxyl group can be selectively protected, we noticed that *only* the attachment of the O-5 acyl group causes significant upfield shift for the H-2′ proton. The result suggests that a diacyl-protected azidoneamine is essential in offering desired regioselective Staudinger reaction. A possible anisotropic effect from the acyl group at the O-5 position was proposed

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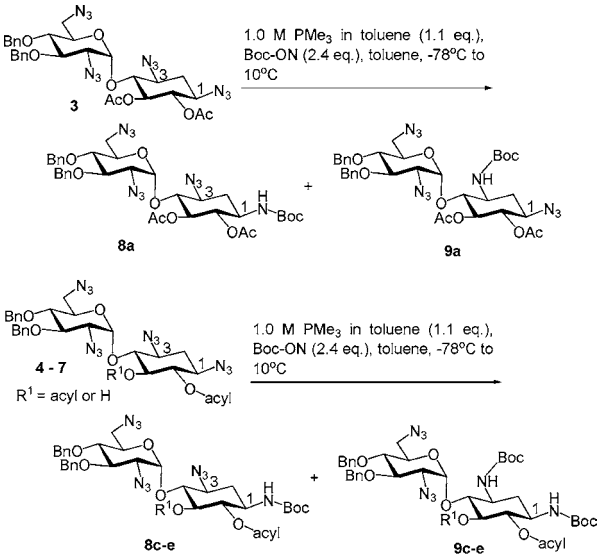
Table 1. Chemical Shifts of Protons on Acylated Neamine Derivatives

entry	compounds	H-1	H-3	H-2′	H-6′
1	**2**	3.28	3.43	3.62	3.57/3.41
2	**3**	3.65	3.46	3.35	3.62/3.42
3	**8a**	3.81	3.47	3.36	3.60/3.42
4	**4**	3.68	3.38	3.61	3.58/3.42
5	**5**	3.81	3.61	3.18	3.54/3.42
6	**8c**	4.05	3.62	3.18	3.54/3.42
7	**6**	3.78	3.61	3.25	3.54/3.42
8	**8d**	4.01	3.59	3.23	3.55/3.39
9	**7**	3.80	3.60	3.20	3.54/3.42
10	**8e**	4.05	3.61	3.19	3.54/3.42

to explain the dramatic upfield shifting of the H-2′ proton signal.[11] After the attachment of diacyl groups, a more deshielded H-1 emerges, indicating that the N-1 azido group will be more reactive toward the Staudinger reaction than N-2′ azido group (entries 2, 5, 7, and 9 in Table 1).

A one-pot azido reduction/amine protection was then employed (Scheme 2).[9b] A characteristic downfield shift (0.2

Scheme 2. Selective Reduction and Protection of the N-1 Azido Group



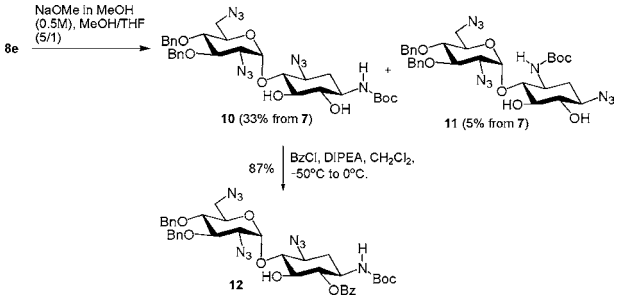
Starting material	Products (yield)	
3	**8a** (37%)	**9a** (42%)
4	[a]	[a]
5	**8c** (46%)[b]	**9c** (24%)
6	**8d** (43%)[b]	**9d** (11%)
7	**8e** (45%)[b]	**9e** (17%)

a: No identifiable product was obtained
b: **8c**, **8d**, and **8e** are mixed with inseparable *N*-3 Boc-protected neamine derivatives with the following ratios (*N*-1/*N*-3): 8/1, 10/1, and 5/1, respectively

ppm) of the proton resulting from the Boc-protected amino group and information from COSY experiment confirm the regioselectivity (entries 3, 6, 8, and 10 in Table 1).

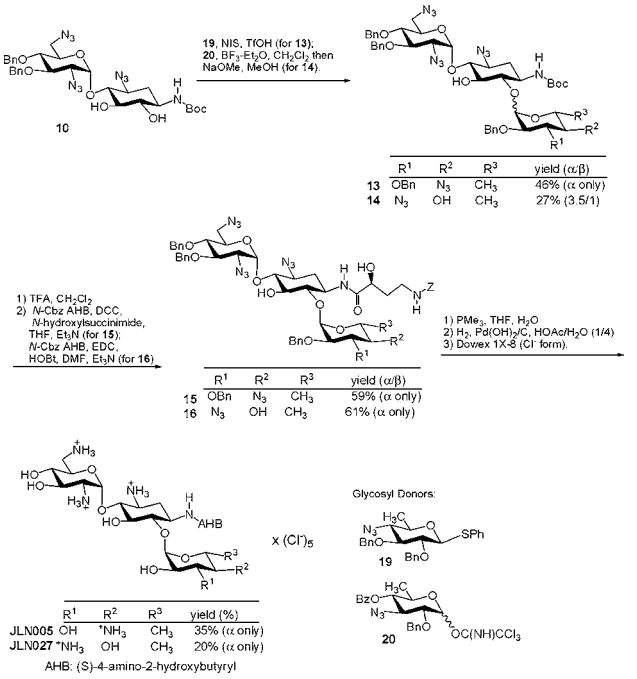
(11) We have used molecular modeling (HyperChem) to reveal the possible anisotropic effect around H-2′ caused by the O-5 carbonyl group. Please refer to Supporting Information for details.

Scheme 3. Synthesis of N-1-Modified Neamine



After one-pot Staudinger reaction/Boc protection and then hydrolysis of the acyl groups, the desired N-1-Boc-protected neamine, **10**, was synthesized in an overall yield of 33% along with 3-N-Boc-protected neamine, **11**, (5%) as the minor product (Scheme 3). Compound **10** can be directly glycosylated with the selected glycosyl donors, **19** and **20** (Scheme 4).[12] Deprotection of Boc followed by coupling with

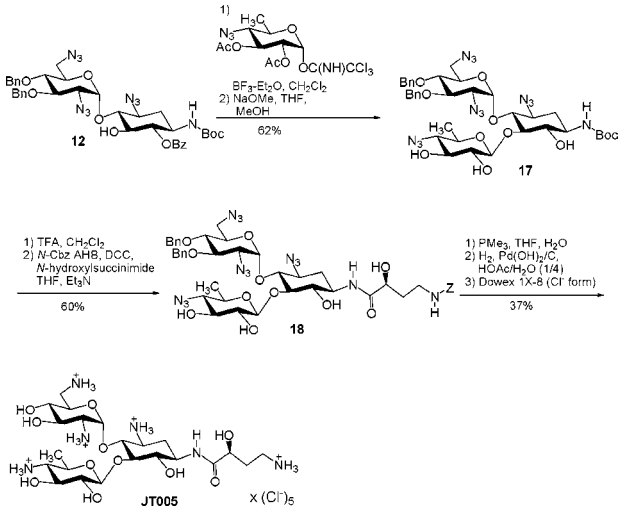
Scheme 4



the Cbz-protected AHB side chain yielded compounds, **15** and **16**. Staudinger reduction of the remaining azido groups followed by hydrogenation provided the final amikacin analogues in modest to excellent yields. Compound **10** can be selectively benzoylated at O-6 and then glycosylated at O-5 (Scheme 3). The glycosyl donor was selected on the basis of leads from our previous work (Scheme 5).[8] The glycosylated product was subjected to similar treatment to furnish the desired pyranmycin with an AHB side chain at N-1.

(12) Glycosyl donors were selected on the basis of our previous work described in ref 8 and unpublished results.

Scheme 5



The constructed amikacin analogues and N-1-modified pyranmycin were assayed against three strains of *Escherichia coli* (one susceptible and two resistant strains) using amikacin, kanamycin, ribostamycin, and butirosin as the controls. One resistant strain is equipped with the pTZ19U-3 plasmid encoded for APH(3′)-I. The other resistant strain is equipped with the pSF815 plasmid encoded for a bifunctional enzyme (AAC(6′)/APH(2″)). These enzymes are among the most prevalent modes of resistance found in aminoglycosides resistant strains. From the minimum inhibitory concentrations (MICs), all the synthesized aminoglycoside with the attachment of an AHB group at N-1 regain their activity against both resistant strains of bacteria (Table 2). One of the synthesized kanamycin analogues, **JLN027**, is even more active than amikacin (entry 5, Table 2) against APH(3′)-I. Without the attachment of an AHB group at N-1, kanamycin class antibiotics are ineffective against AAC(6′) and APH(2″), while the neomycin class antibiotics (ribostamycin and pyranmycin[8] (**TC005**, entry 10, Table 2)) seem to be modestly active against the same enzyme. Therefore, neomycin class antibiotics with the AHB group at the N-1 position may represent a better template for further modification. Nevertheless, the problem of the acid-labile glycosidic bond between rings II and III in neomycin or butirosin is an obstacle that remains to be overcome. Therefore, our design of pyranmycin that has better stability in acidic media could be valuable for designing new aminoglycosides against a broad spectrum of aminoglycoside-resistant bacteria.

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

		strains		
entry	compounds	*E. coli* TG1	*E. coli* TG1 (APH(3′)-I)	*E. coli* TG1 (AAC(6′)/APH(2″))
1	amikacin	1	0.5	1
2	kanamycin	4	inactive	inactive
3	**JLN005**	4	2	2
4	JL005[b]	8	inactive	inactive
5	**JLN027**	1	0.25	1
6	JL027[b]	2	inactive	inactive
7	butirosin	1	0.5	0.25
8	ribostamycin	2	inactive	8
9	**JT005**	4	4	4
10	TC005[c]	8	inactive	8

[a] Unit: μg/mL. [b] Related kanamycin analogues without an AHB group at N-1. [c] Related pyranmycin without an AHB group at N-1.

In conclusion, we have demonstrated that the reactivity of azido groups can be tuned by the stereoelectronic effect of protecting groups. We have developed a convenient synthesis that can be used for modification at N-1 of both kanamycin and neomycin classes of aminoglycosides. Without relying on the chelating method and carbamate-type of protecting group, novel structural entities can be easily introduced via a glycodiversification strategy. One synthesized compound, **JLN027**, manifests even better activity than the clinically used amikacin against aminoglycoside-resistant bacteria. Ongoing works that focus on further modifications based on the leads are being performed.

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

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