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A method for preparing *N*-alkylated kanosamines from diacetone D-glucose

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ABSTRACT:

The aminoglycoside (AG) antibiotics have seen a resurgence in their clinical use given the increase in multi drug resistant bacterial infections. Campaigns to generate novel analogs show promise that structural modification can lead to compounds with improved pharmacological properties. The results described herein include a new method to synthesize mono-, di-, and mixed *N*-alkylated kanosamine sugars and their elaboration into novel glycosides that inhibit bacterial protein synthesis in vitro.

Keywords: amino sugar, synthesis, reductive amination, diversity

Recent years have witnessed a dramatic increase in multi drug resistant (MDR) Gram-negative bacterial infections.[1,2] These infections pose an ever-increasing threat to human health, and a search for potential therapeutics has prompted research into the underlying resistance mechanisms, and new chemical entities to combat them.[3,4] In clinical practice, aminoglycoside (AG) antibiotics, which had not been considered an option for some time, have seen a resurgence in their utilization given the emergence of infections caused by MDR bacteria. Efforts to develop derivatives of AGs that improve their pharmacological properties have also been given new life in light of MDR infections and clinical AG use.[5] Issues with current treatments include the frequent occurrence of nephrotoxicity and ototoxicity.[6] While AGs remain active against many clinical isolates of MDR bacteria, toxicity limits their utility. The observed retention of activity gives promise that these compounds hold potential for new generations with improved pharmacodynamic properties to alleviate such issues.[6]

Aminoglycosides, including kanamycins A and B (Figure 1), are a potent class of antibiotics that act via protein synthesis inhibition. This mechanism of action requires binding to the ribosome as the key factor to their potency. Just as the structures of AGs vary, so too do the specifics of their binding interactions. In general, AGs derive their activity by interacting with 16s rRNA of the decoding A-site of the bacterial ribosome.[7] Crystal structures of various AGs in complex with oligonucleotides mimicking the A-site topology show that AG binding forces A1492 and A1493 to take up a "bulged-out" conformation that leads to a loss in translational fidelity. Due to their polycationic nature, AGs also owe much of their binding properties to electrostatic interactions.[8] While several studies on aminoglycoside derivatives have been conducted (vide infra), one using *N*-alkylated kanosamines has yet to be reported. Here we present a methodology for accessing various mono, di-, and mixed *N*-alkyl kanosamine derivatives, and their incorporation into kanamycin analogs.

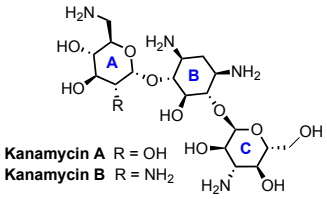
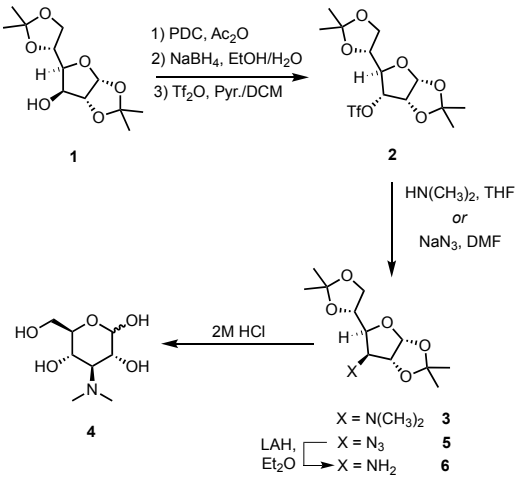


Figure 1. Kanamycins A and B.

Efforts to develop new AG analogs have focused on circumventing resistance mechanisms. Resistance primarily involves aminoglycoside modifying enzymes (AMEs)[4] that render AGs inactive by acetylation, phosphorylation, or nucleotide addition reactions. In one study, designed kanamycin B derivatives bearing *N*-alkylated and *N*-acylated modifications at the 4' position of the neamine A ring replaced its 4' hydroxyl group.[9] Antibiotic activity was observed for the series including improved activity against some MDR bacteria from clinical isolates. In a different approach, conformationally constrained kanamycin A derivatives were studied.[10] Bridging the 2' hydroxyl of the neamine ring (A) with the 5' hydroxyl of the central deoxystreptamine ring (B) constrained the rotation between the two rings. The modification also masked these sites from deactivation by AMEs. MIC values for the series varied with the length of the linker between the two rings, though no analog performed better than kanamycin A itself. In addition to the neamine core, the kanamycins bear a 3-deoxy-3-amino glucose unit (kanosamine, ring C in Figure 1). This ring aides in RNA

recognition via electrostatic and hydrogen bonding interactions,[8] is integral to binding the

Scheme 1. Synthesis of *N*-alkyl kanosamines from diacetone D-glucose

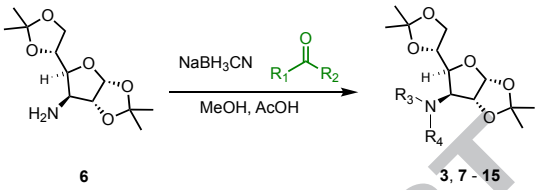


ribosome, and is also acetylated by AMEs.[4] Modification of this ring was the central aspect of work employing a glycodiversification approach.[11] A library of kanamycin B derivatives varying the placement and number of the amino groups on the C ring reinforced the placement of the amino group at the natural C3 position. The preparation of glycosylated small molecule compound libraries was essential to these studies. For antibiotics in particular, where the presence of glycosylated compounds is prevalent, synthesis presents unique challenges.

Leading methods for the preparation of glycosylated small molecule library include enzymatic glycorandomization and chemoselective neo-glycorandomization.[12,13] Both methods have been useful, though onerous in their execution. Previous work in our lab on glycosylated small molecule library synthesis leveraged a strategy where simple glycosides containing functionalized aglycones were elaborated in chemoselective reactions. We dubbed the approach post glycosylation diversification, or PGD.[14] We used desosamine, the amino sugar that anchors erythromycin in the ribosome exit tunnel, to prepare glycosides containing aglycones with amino, azido, or aryl halide moieties. Small libraries prepared from the PGD precursors were shown to inhibit protein synthesis in an in vitro assay and similarly inhibit bacterial growth.[15] In the course of that study, we became interested in changing the sugar itself, which led us to the synthesis of alkylated kanosamines and their glycosides described herein.

Kanosamine analogs were attractive targets because of their additional functionality relative to desosamine. We envisioned starting with *N,N*-dimethyl kanosamine, whose structure formally adds a hydroxyl group at C6 relative to desosamine. The C6 hydroxyl group could ultimately be an additional site of diversification. The initial objective, however, was a synthetic route that provided access to the *N,N*-dimethyl compound, as well as other *N*-substituted kanosamines. To that end, we recognized the utility of known triflate **2**, itself obtained from the epimerized product of **1** (Scheme 1) as a useful intermediate. Treatment of **2** with an excess of dimethylamine in a pressure vessel, followed by acid-catalyzed acetonide deprotection, gave dimethyl kanosamine **4** in 96% yield

Table 1. Reductive aminations with amine 5



	R_1	R_2	R_3	R_4	prod.	yield (%)
1	H	H	Me	Me	3	97
2	Me	H	Et	Et	7	95
3	Et	H	*n*-Pr	H	8	93
4	*n*-Pr	H	*n*-Bu	H	9	92
5	*n*-Pr	H	*n*-Bu	*n*-But	10	33[a]
6	Ph	H	Bn	H	11	96
7	(3-OMe,4-OH)Ph	H	(3-OMe,4-OH)Bn	H	12	94
8	Me	Me	*i*-Pr	H	13	91
9	Ph	Me	1-PhEt	H	14	96[b]
10	Cyclohexanone		Cylohexyl	H	15	92

[a]Isolated as sole product.

[b]Combined yield of separable diastereomers

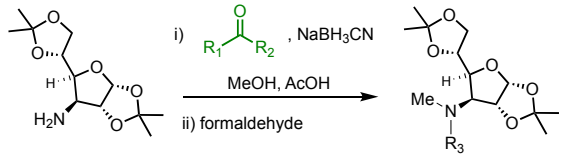
over two steps. Optimization of the amine equivalents, triflate concentration, and volume of the pressure vessel were all required to obtain this yield. The challenges encountered when using other amine partners required a different synthetic route if additional analogs were to be obtained.

We turned, therefore, to reductive amination in place of triflate displacement. Reductive amination using **6** as the amine presented itself as a robust and facile method. Synthesis of **6** followed a previously established synthetic route to kanosamine.[16] Epimerization of diacetone D-glucose **1**, through an oxidation-reduction sequence, gave the C3-epimerized *allo*-configured product in 86% yield over two steps. Treatment with triflic anhydride and pyridine then converted it to triflate **2**. Without further purification, this compound was then subjected to nucleophilic displacement using sodium azide to give intermediate **5** (96% over two steps) followed by reduction with $LiAlH_4$ yielding amine **6**, diacetone D-kanosamine, as a single diastereomer in 86% yield. To test the reductive amination, amine **6** was reacted with three equivalents of both formaldehyde and sodium cyanoborohydride. TLC indicated complete consumption of the starting material after two hours. Workup and purification of the crude product via column chromatography gave *N,N*-dimethyl kanosamine derivative **3** in near quantitative yield.

Following on the heels of the successful preparation of **3** by reductive amination, we aimed to extend the methodology to other *N*-alkylated derivatives. The scope was tested using as series of aldehydes and ketones (Table 1). To our delight, the reactions proceeded in very good yields (91-97%), similar to the initial reaction with formaldehyde. Di-alkylation products were observed in the case of small aldehydes such as

formaldehyde and acetaldehyde (entries 1-2), which gave products **3** and **7**. Longer chain aldehydes primarily gave mono-alkylation products. Propionaldehyde (entry 3) gave **8** whereas

Table 2. Formation of *N,N*-dialkyl kanosamines via reductive aminations



6 16 - 21

	R_1	R_2	R_3	prod.	yield (%)
1	Et	H	*n*-Prop	16	97
2	*n*-Prop	H	*n*-But	17	95
3	Ph	H	Bn	18	93
4	3-OMe,4-OH)Ph	H	(3-OMe,4-OH)Bn	19	92
5	Me	Me	*i*-Prop	20	91
6	Cyclohexanone		Cylohex	21	92

7 - 21 4, 22 - 37

butyraldehyde (entries 4-5) gave both mono- (**9**) and di-alkylated (**10**) products in separate runs, where di-alkylated product **10** was the only exception to the trend. The methodology was further extended to aromatic aldehydes such as benzaldehyde or vanillin, yielding products **11** and **12**. Reactivity with ketones proceeded to give mono-alkylation products (entries 8-10), giving products **13-15**. The observed mono alkylations are most likely due to steric interactions between the substrate and aldehydes/ketones that prevents di-alkylation.

We next considered exploiting the mono-alkylation selectivity to prepare disubstituted analogs containing two different alkyl groups. The logic was to do serial alkylations in one pot - first with a large aldehyde or ketone, and then a small one to give the mixed di-alkyl product. To test the approach, **6** was reacted with 1.5 equivalents of propionaldehyde under the established reaction conditions, monitored by TLC. Upon consumption of **6** and formation of a less polar spot, excess formaldehyde was added. After a short period, a new spot appeared on TLC, accompanied with the disappearance of the mono-alkylated product. Work up and purification gave *N*-methyl,*N*-propyl derivative **16** in high yield (97%) with no evidence of *N,N*-dimethyl or *N*-propyl side products (Table 2). The scope of this methodology to form mixed alkyl derivatives was then extended as summarized in Table 2. Other carbonyl partners such as butyraldehyde, benzladehyde, vanillin, cyclohexanone, and acetone were utilized in the reaction sequence with formaldehyde to deliver products **17-21** in 91-97% yield (entries 2-6). Deprotection of purified alkylated acetonide intermediates (**3**, **7-21**) was accomplished by treatment with 2M HCl. Evaporation of the acidic aqueous solution upon completion gave rise to the corresponding hydrochloride salts **4** and **22-37** in quantitative yields (Eq. 1).

A telescoped sequence would eliminate the need for isolating the protected acetonide intermediate, and facilitate the formation of a pyranose suitable for further manipulation, starting from our key intermediate **6**. To illustrate the strategy, **6** was alkylated with vanillin, then acetaldehyde, to form intermediate **38** (Scheme 2). Addition of HCl to the reaction

Scheme 2. One pot modification to *N,N*-dialkyl kanosamine peracetates

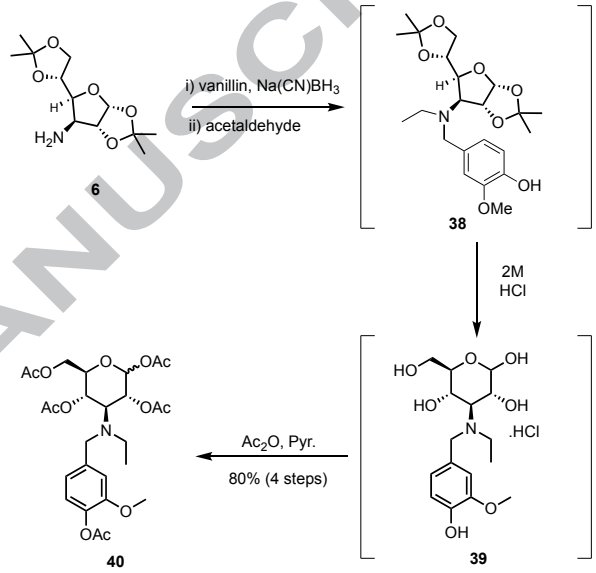


Table 3. Glycosylations using compound 41 as donor

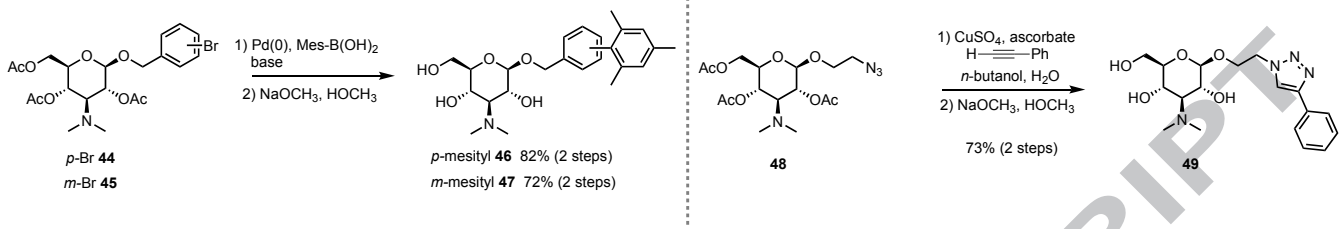
41 42 - 45

	alcohol (ROH)	conditions	prod. (β:α)	yield (%)
1	allyl	Ag_2CO_3, 4Å MS	**42** (6:1)	49
2	Cl-ethyl	Ag_2CO_3, 4Å MS	**43** (3:1)	46
3	4-Br-benzyl	Ag_2CO_3, I_2, 4Å MS	**44** (>20:1)	55
4	3-Br-benzyl	Ag_2CO_3, I_2, 4Å MS	**45** (20:1)	48

mixture at this point served two purposes. It quenched the residual borohydride from the reductive amination and it also facilitated acetonide deprotection, giving lactol **39**, which was not isolated. Workup to remove unreacted aldehyde was followed by acetylation and purification by chromatography to deliver **40** in 80% yield. Remarkably, the sequence included four reactions with only one chromatography.

Having secured a route to *N*-alkylated kanosamines, we endeavored to incorporate one into a Kanamycin B analog. Initial efforts were focused on glycosylation onto a neomycin-derived acceptor.[11] A per-*O*-acetylated thiopyrimidine donor was prepared starting from *N,N*-dimethyl kanosamine **4** and conditions were screened for its attachment onto a Neamine acceptor, but without success.[17] It was likely that the combination of basic amine functionality and acetate protecting

Scheme 3. Post glycosylation diversification of kanosamine analogs



groups deactivate the donor, preventing glycosylation. These effects were compounded by a weakly nucleophilic acceptor. *N,N*- dimethyl kanosamine **4** was alternatively converted to anomeric bromide **41**using HBr in acetic acid. As shown in Table 3, **41** served as a viable donor, delivering glycosides **42**-**45** in moderate yields (46-55%). The new glycosides contained latent reactivity handles that were exploited in post glycosylation diversification reactions. As a proof of concept, we conducted Suzuki couplings on bromobenzyl glycosides **44** and **45** to prepare, after de-acetylation, compounds **46** and **47** in 82 and 72% yields, respectively (Scheme 3). Chloroethyl glycoside **43** was converted to the azido ethyl compound **48** by substitution, and was then subjected to a click reaction with phenyl acetylene to give, after de-acetylation, compound **49** in 72% yield over two steps. A cell free, in vitro transcription-translation assay that monitors the fluorescence of EmGFP was used to evaluate new compounds **46**, **47**, and **49**.[14] Compounds **46** and **49** exhibited inhibition of approximately 70% relative to negative control of protein synthesis in the absence of inhibitor. The *m*-mesityl compound **47** reduced protein synthesis by ~40%.[17] These results show that the PGD methodology identified new protein synthesis inhibitors that are amenable to modification to improve pharmacological properties.

In conclusion, we have presented a method for the preparation of alkylated kanosamine sugars from diacetone D-glucose. The robust and versatile method allows mono, di- and mixed alkyl patterns to be accessed in a single pot fashion. In addition, the method allows taking the diacetonide starting material to a peracetylated pyranose, with a single purification, over a four-step process. While efforts to incorporate the sugars yielded from this process into Kanamycin B derivatives fell short, the PGD platform successfully generated compounds that inhibited bacterial protein synthesis.

Supplementary data

Supplementary data for this article is available free of charge at URL. The data include experimental details, characterization data, and spectra for new compounds.

Acknowledgements

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