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Application of Glycodiversification: Expedient Synthesis and Antibacterial Evaluation of a Library of Kanamycin B Analogues

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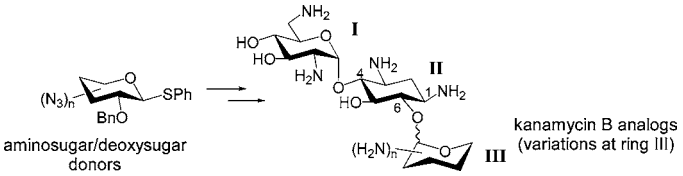
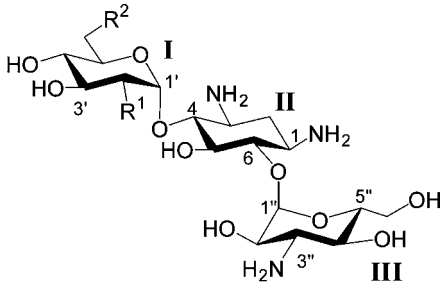
ABSTRACT



The expedient synthesis of a library of kanamycin B analogues is reported. The revealed SAR will guide future designs in developing kanamycin-type aminoglycoside antibiotics against drug-resistant bacteria.

Kanamycin belongs to a group of aminoglycoside antibiotics with 4,6-di-O-substituted 2-deoxystreptamine (Figure 1).[1] Kanamycin shows prominent antibacterial activity against both gram-positive and gram-negative susceptible strains of bacteria. However, the emergence of aminoglycoside-resistant bacteria has rendered kanamycin obsolete for clinical uses.[2] As one of the antidotes against the problem of drug resistance, numerous chemical modifications of kanamycin have been reported with the goal of reviving its activity toward resistant bacteria.[3] Except for a few publications,[4] the majority of derivatives use carbamates as protecting groups for the amino groups of kanamycin, resulting in the production of kanamycin with polycarbamate groups. Two drawbacks were often encountered with these molecules. First, the poor solubility of polycarbamate molecules results in difficulties in purification and characterization of these compounds. Second, the kanamycin scaffold imposes limited options for structural modifications.

Kanamycin B consists of neamine (rings I and II) and 3-amino-3-deoxyglucopyranose (ring III), with ring III being linked to the O-6 of neamine via an α-glycosidic bond. Neamine has been proven to be the essential component for the antibacterial activity of neamine-containing antibiotics, and modifications on neamine often result in a loss or



	R^1	R^2
Kanamycin A	OH	NH_2
Kanamycin B	NH_2	NH_2
Kanamycin C	NH_2	OH

Figure 1. Structure of kanamycin.

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decrease in antibacterial activity.[1] Therefore, we reason that ring III should be the optimal component where a glycodiversification approach could be employed leading to the synthesis of novel kanamycin B analogues with, perhaps, increased antibacterial activity.

Our laboratory has recently developed and reported general methodologies for the preparation of a library of unusual sugar donors that favor the formation of both α- and *β*-glycosidic bonds.[5] The α-glycosidic bond between rings II and III is important, as kanamycin analogues with a *β*-glycosidic bond manifest much weaker antibacterial activity.[6] However, unlike the synthesis of *β*-glycosidic derivatives, control of the stereoselective formation of an α-glycosidic bond is challenging since no neighboring group assistance can be utilized. Nevertheless, we have discovered optimal conditions for making the α-glycosidic bond. In combination with our previous synthetic work with unusual sugar donors, we have developed a library of unusual sugar donors that can satisfy needs in stereoselective incorporation of carbohydrate moieties. Herein, we wish to report a much-improved convenient and practical method for the preparation of a library of kanamycin analogues.

A library of phenylthioglucopyranose-based unusual sugar donors was constructed with procedures analogous to those in our previous work (Figure 2).[5] These donors have benzyl

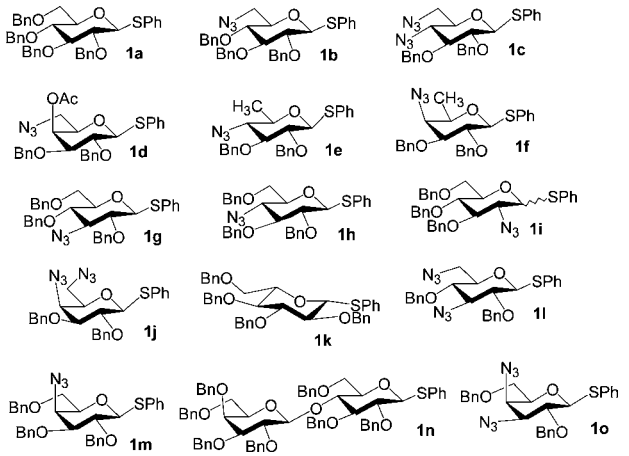
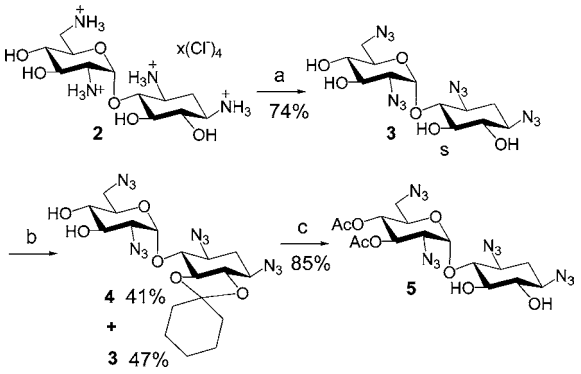


Figure 2. Unusual sugar donors for α-linked glycosidic bond.

or azido groups at the C-2 position, which favor the formation of an α-glycosidic bond under the influence of anomeric and solvent effects.[9] The neamine acceptor was prepared from neamine (Scheme 1). The amino groups of neamine were converted into azido groups producing **3**, which is quite

Scheme 1[a]



[a] Conditions: (a) TfN_3, $CuSO_4$, H_2O, CH_2Cl_2. (b) Cyclohexanone dimethyl ketal, TsOH−H_2O, CH_3CN. (c) (i) Ac_2O, Et_3N, CH_2Cl_2, DMAP; (ii) dioxane, H_2O, HOAc.

soluble in organic solvents, thus allowing for smooth purification and characterization.[7] Compound **5** was obtained from **3** via selective protection of the O-5 and O-6 diols of neamine with cyclohexanone dimethyl ketal, followed by acetylation of the O-3′ and O-4′ diols, and deprotection of the cyclohexylidene group. We were pleased to discover that the neamine acceptor, **5**, undergoes regiospecific glycosylation at O-6 position, resulting in the desired 4,6-disubstituted 2-deoxystreptamine motif (Scheme 2).[8] The optimal stereoselectivity for the formation of an α-glycosidic bond can be accomplished by operating the reaction in a solution of Et_2O and CH_2Cl_2 in a 3:1 ratio.[9] A further increase in the content of Et_2O has no effect in the stereoselectivity; however, decreasing the Et_2O content results in lower stereoselectivity. The glycosylated compounds were often

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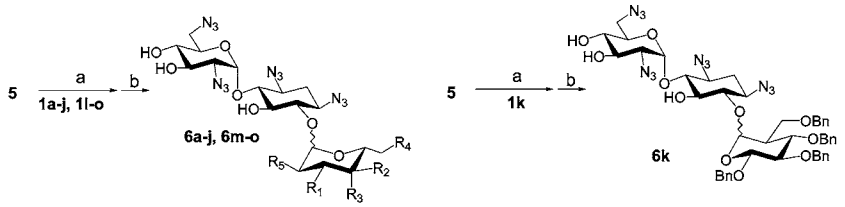
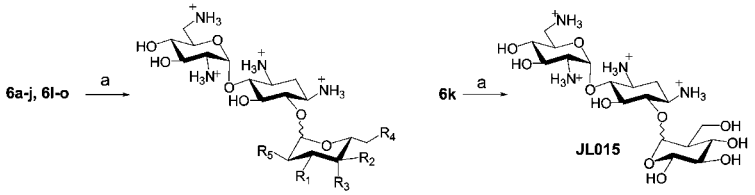
Scheme 2



a. NIS, TfOH, Et_2O:CH_2Cl_2 (3:1). b. NaOMe, MeOH:THF (5:1).

Compound	R_1	R_2	R_3	R_4	R_5	Yield (%)	α : β
6a	OBn	OBn	H	OBn	OBn	35	8:1
6b	OBn	OBn	H	N_3	OBn	32	11:1
6c	OBn	N_3	H	N_3	OBn	33	Only α
6d	OBn	H	OH	N_3	OBn	44	Only α
6e	OBn	N_3	H	H	OBn	61	7 : 1
6f	OBn	H	N_3	H	OBn	45	Only α
6g	N_3	OBn	H	OBn	OBn	62	10:1
6h	OBn	N_3	H	OBn	OBn	40	Only α
6i	OBn	OBn	H	OBn	N_3	54	2.5 : 1
6j	OBn	H	N_3	N_3	OBn	36	Only α
6k	-	-	-	-	-	62	10 : 1
6l	N_3	OBn	H	N_3	OBn	48	10:1
6m	OBn	H	N_3	OBn	OBn	54	Only α
6n	OBn	Tetrabenzly-β-D-Galactopyranosyl	H	OBn	OBn	23	Only α
6o	N_3	H	N_3	OBn	OBn	45	Only α

Scheme 3



a. i. PMe_3, NaOH, THF, ii. $Pd(OH)_2$/C, HOAc, H_2O, iii. Dowex 1X8-200 (Cl^- form).

Compound	R_1	R_2	R_3	R_4	R_5	Yield (%)	α : β	Binding score[b]	MIC (μM)[a]	
									E. coli	*S. aureus*
Kanamycin B	$^+NH_3$	OH	H	OH	OH	-	-	-394.12	1.4	0.5
JL001	OH	OH	H	OH	OH	14	10:1	-355.52	50	8
JL002	OH	OH	H	$^+NH_3$	OH	22	11:1	-396.82	Inactive	30
JL003	OH	$^+NH_3$	H	$^+NH_3$	OH	55	Only α	-398.31	22	29
JL004	OH	H	OH	$^+NH_3$	OH	54	Only α	-395.45	22	8
JL005	OH	$^+NH_3$	H	H	OH	39	Only α	-400.00	12	2
JL006	OH	H	$^+NH_3$	H	OH	89	Only α	-399.24	Inactive	16
JL007	$^+NH_3$	OH	H	OH	OH	76	20:1	-394.12	6	1
JL012	OH	$^+NH_3$	H	OH	OH	50	Only α	-403.48	23	4
JL013	OH	OH	H	OH	$^+NH_3$	39	2.5 : 1	-401.28	23	4
JL014	OH	H	$^+NH_3$	$^+NH_3$	OH	68	Only α	-398.82	Inactive	57
JL015	-	-	-	-	-	32	10 : 1	-316.90	Inactive	Inactive
JL016	$^+NH_3$	OH	H	$^+NH_3$	OH	49	10:1	-465.32	22	2
JL018	OH	H	$^+NH_3$	OH	OH	85	Only α	-398.73	Inactive	16
JL019	OH	β-D-Gal	H	OH	OH	59	Only α	-332.78	Inactive	Inactive
JL024	$^+NH_3$	H	$^+NH_3$	OH	OH	83	Only α	-464.84	-	4

[a] MIC: minimum inhibitory concentration. [b]These values were obtained from molecular modeling calculation. The lower the values, the better the binding affinity (ref 11).

mixed with inseparable impurities. Nevertheless, after hydrolysis of the acetyl groups, the triols can be afforded in good purity and improved α:*β* ratio. The final products were synthesized as chloride salts via the Staudinger reaction followed by hydrogenation and ion-exchange (Scheme 3).

The kanamycin analogues were tested against *Escherichia coli* (ATCC 25922), and *Staphylococcus aureus* (ATCC 25923) using kanamycin B as the control.[10] We also carried out molecular modeling for evaluating the binding of kanamycin analogues toward the target site of 16S rRNA. The minimum inhibitory concentrations of kanamycin ana-

(10) *Methods for Dilution Antimicrobial Susceptibility Testing for Bacteria that Grow Aerobically*. Approved standard M7-A5, and *Performance Standards for Antimicrobial Disk Susceptibility Tests*. Approved standard M2-A7; National Committee for Clinical Laboratory Standards, Wayne, PA.

logues were compared with the binding capability calculated from molecular modeling.[11] Similar to a reported study,[9] we also noticed that the binding capability of kanamycin analogues cannot be correlated to their antibacterial activity. Analogous to previous results from our laboratory,[12] an increase in the number of amino groups did not increase the antibacterial activity, albeit the binding affinity was enhanced (**JL016** vs **JL007**). Compound **JL007** is designed to be identical to kanamycin B for ensuring the regioselective glycosylation. Although both ^{1}H and ^{13}C NMR spectra of **JL007** are almost the same as those from commercially available kanamycin B, the antibacterial activity of **JL007** is about 2−4-fold lower than kanamycin B, which is probably due to the presence of the 1″-epimer of **JL007**. Nevertheless, we discovered that the amino group at the equatorial position of C-3″ is superior in terms of activity in comparison to C-6″ (**JL002**) or the equatorial position of C-4″ (**JL012**). In fact, an amino group at the C-6″ position reduced the activity of some analogues (**JL002** vs **JL001** and **JL016** vs **JL007**). Incorporating an amino group at the axial position dramatically reduces the activity (**JL012** vs **JL018** and **JL003** vs **JL014**), while an axial OH at C-4″ increases the activity (**JL004** vs **JL002**). Further introduction of a larger functional group at the C-4″ position such as galactose in **JL019** completely abolished the antibacterial activity. The amino group at the C-2″ position may produce better antibacterial activity. However, it is difficult to offer accurate evaluation due to the lower stereoselectivity in glycosylation (α: β = 2.5:1). Interestingly, **JL005** with a CH_3 group at the C-6″ position showed good activity among the analogues we prepared except for **JL007**, although its binding score is lower in ranking. Molecular modeling of **JL005** binding to the targeted RNA site did not reveal any plausible interaction that may explain such an unusual observation.[13] We have also tested these analogues against resistant strains of bacteria.[14] No significant activity was observed.

Unlike the structure activity relationship (SAR) of ring III of pyranmycins from our previous report,[12] the observed SAR from these kanamycin analogues is not completely consistent between the two different strains of bacteria that we tested. For example, **JL003** has similar activity for both strains, while **JL016** manifests a 10-fold increase in activity against *S. aureus*. On the other hand, **JL007** shows only a 6-fold increase. Since the targeted A-site of 16S rRNA is highly conserved,[2b] the presence of an outer membrane in *E. coli* (gram-negative) could be the major factor that contributes to the observed variation in the increasing antibacterial activity of kanamycin analogues against gram-negative and gram-positive bacteria. Pyranmycin contains a 4,5-disubstituted 2-deoxystreptamine core, while kanamycin contains a 4,6-disubstituted 2-deoxystreptamine core. We believe that the mechanisms involved in the importation of aminoglycoside antibiotics could have various efficiencies in transporting structurally different aminoglycosides. Although it is difficult to study such membrane-associated importation mechanisms, our SAR and binding results suggest that more research for the uptake of aminoglycosides is needed to augment the binding affinity studies between aminoglycosides and rRNA. The summary of the preliminary SAR is shown in Figure 3.

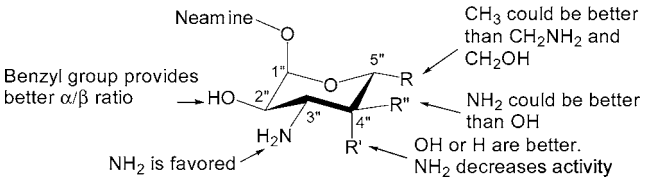


Figure 3. Summary of the SAR of ring III of kanamycin B analogues.

In conclusion, we have independently developed a convenient methodology for the construction of a library of kanamycin analogues. In contrast to our previous experience in the pyranmycin library, the kanamycin analogues manifest several leads (for example, **JL007** and **JL005**). With the revealed SAR, we are currently focusing on the introduction of more modifications with the goal of increasing activity against resistant bacteria.

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

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(11) Score function was based on Amber 96 force-field as implemented in HyperChem 7.0 and the solvent-accessible surface methodology to account for the hydration effects. The program was developed for drug design, not specifically for the calculation of absolute binding affinity. Therefore, we look for the tendency in binding rather than the meaning interpreted from the absolute numbers of binding. Nevertheless, the calculated binding scores have tendencies consistent with the compounds that have known binding affinity from the in vitro experiment. More details can be found in Supporting Information.

(12) Chang, C.-W. T.; Hui, Y.; Elchert, B.; Wang, J.; Li, J.; Rai, R. *Org. Lett.* **2002**, *4*, 4603−4606.

(13) Please refer to Supporting Information for figures from molecular modeling.

(14) *E. coli* (TG1) (APH3′-I) and *E. coli* (TG1) (AAC6′/APH2″).