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Exploring the Optimal Site for Modifications of Pyranmycins with the Extended Arm Approach

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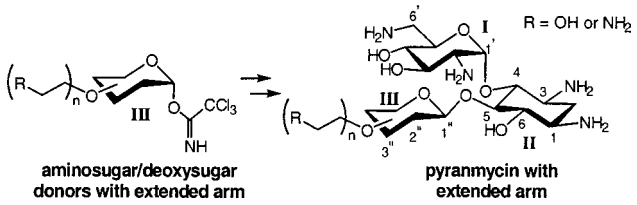
ABSTRACT



Continuing from the syntheses and the antibacterial studies of a library of pyranmycins, we further probed the proximity around ring III of pyranmycin by introducing an "extended arm" that has hydroxyethyl or aminoethyl groups at the O-2″, O-3″, or O-4″ positions. The results from the antibacterial studies reveal the optimal structural motif is the attachment of an extended arm with a terminal hydroxyl group at the O-3″ position.

Aminoglycoside antibiotics, such as neomycin, have been administered clinically for over fifty years.[1,2] Overuse and misuse of antibiotics have incurred the emergence of antibiotic resistant bacteria,[3,4] which significantly limits the usefulness of aminoglycoside antibiotics. To counteract this problem, our group has recently developed an expedited method for the preparation of a novel class of aminoglycoside antibiotics, pyranmycins,[5] based on our aminosugar library[6] and the reported X-ray and NMR structural studies (Figure 1).[7−10] Herein, we wish to report the results from further modifications of pyranmycins using the extended arm approach.

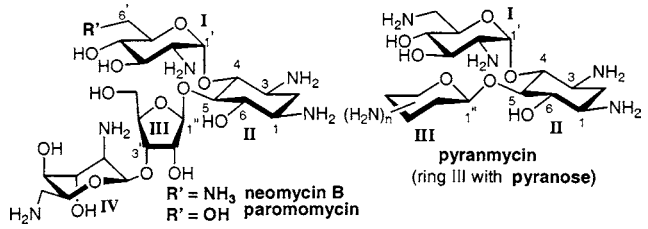


Figure 1. Neomycin-based aminoglycosides and pyranmycin.

Most of the aminoglycoside-resistant bacteria inactivate the aminoglycosides via aminoglycoside-modifying enzymes that introduce modifications on the neamine (rings I and II).[11,12] Chemical derivatization on neamine often results in

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(3) Cohen, M. L. *Science* **2002**, *257*, 1050−1055.
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a lack or a decrease in the activity.[13] To avoid the decrease in activity, we have developed a general method for the synthesis of pyranmycins, which consists of a neamine core and variable pyranoses as the ring III component. We have also identified the lead structure at ring III of pyranmycins.[5b] We wish to further explore the possible site(s) for the attachment of additional functionalities at the ring III pyranose of pyranmycin. To achieve this goal, we synthesized seven derivatives of pyranmycins by extending the amino group or hydroxyl group away from the ring III pyranose of pyranmycin (Figure 2). The identification of the optimal site(s) will allow us to introduce more complex structural components in the future.

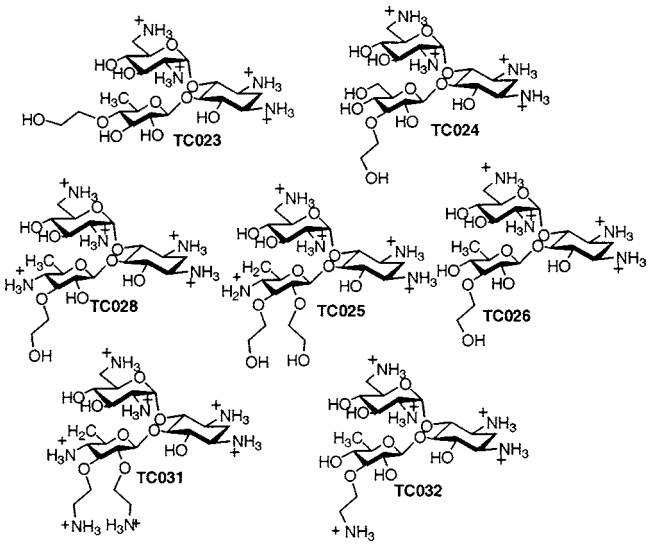


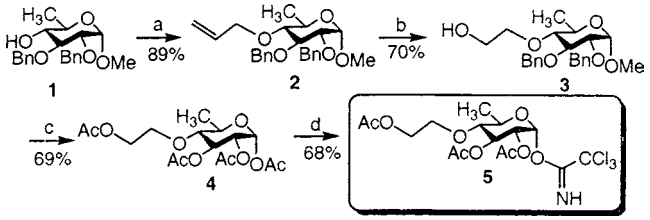
Figure 2. Structures of the designed pyranmycins.

As more diverse structural scaffolds are incorporated, the overall contour of the modified pyranmycins will deviate from their predecessors that resemble neomycin. Thus, these modified pyranmycins are expected to become poor substrates for the aminoglycoside-modifying enzymes. Therefore, if the high potency of these extended arm-modified pyranmycins can be maintained, we can generate effective new aminoglycoside antibiotics against resistant strains of bacteria.

Our previous work elucidates the importance of having a 6″-CH_3 group.[5] Therefore, all of the constructs will follow this trait except **TC024**. We began our approach by extending the 4″-OH by two carbons creating **TC023**. It is difficult to install a single extended arm at the O-2″ position while maintaining the crucial β-glycosidic bond during the glycosylation.[14] Therefore, we synthesized compounds with double extended arms at both O-2″ and O-3″ positions, such as **TC025** and **TC031**. By comparing to the single extended arm constructs (O-3″) like **TC026**, **TC028**, and **TC032**, we can evaluate the effect of the extended arm at O-2″.

The syntheses of single extended arm pyranmycins require the preparation of several glycosyl donors **5**, **13**, **17**, **22**, and **25** (Schemes 1, 2, and 3). The synthesis of **5** begins from methyl 6-deoxy-2,3-di-*O*-benzyl-α-D-glucopyranoside.[6] Allylation of 4-OH followed by ozonolysis and reductive workup provides **3** with a hydroxyethyl group at O-4 (Scheme 1). The benzyl and methyl groups were converted into acetyl groups by using Ac_2O and a catalytic amount of H_2SO_4 concomitant with the acetylation of the hydroxyl group on the extended arm. Selective deprotection of the anomeric acetyl group followed by reacting with trichloroacetonitrile and DBU gave the desired glycosyl donor, **5**.

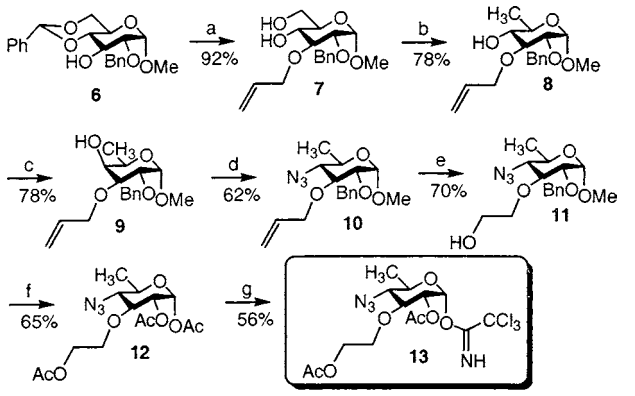
Scheme 1. Synthesis of Glycosyl Donor[a]



[a] Conditions: (a) Allyl bromide, NaH, TBAI, THF. (b) (i) O_3, CH_2Cl_2; (ii) $NaBH_4$, MeOH. (c) Ac_2O, cat. H_2SO_4. (d) (i) NH_2NH_2−HOAc, DMF; (ii) CCl_3CN, DBU, CH_2Cl_2.

The synthesis of **13** begins with **6**[15] (Scheme 2). Allylation of 3-OH followed by the deprotection of benzylidene group generated a diol, **7**. Selective tosylation of 6-OH followed by $LiAlH_4$ reduction gave the 6-deoxygenated compound,

Scheme 2. Synthesis of Glycosyl Donor[a]



[a] Conditions: (a) (i) Allyl bromide, NaH, TBAI, THF; (ii) TsOH−H_2O, MeOH. (b) (i) TsCl, py.; (ii) $LiAlH_4$, THF. (c) (i) $(COCl)_2$, DMSO; DIPEA; (ii) $NaBH_4$, MeOH. (d) (i) Tf_2O, py., CH_2Cl_2; (ii) NaN_3, DMF. (e) (i) O_3, CH_2Cl_2; (ii) $NaBH_4$, MeOH. (f) Ac_2O, cat. H_2SO_4. (g) (i) NH_2NH_2−HOAc, DMF; (ii) CCl_3CN, DBU, CH_2Cl_2.

(11) Kotra, L. P.; Haddad, J.; Mobashery, S. *Antimicrob. Agents Chemother*. **2000**, *44*, 3249−3256..
(12) Mingeot-Leclercq, M.-P.; Glupczynski, Y.; Tulkens, P. M. *Antimicrob. Agents Chemother*. **1997**, *43*, 727−737.
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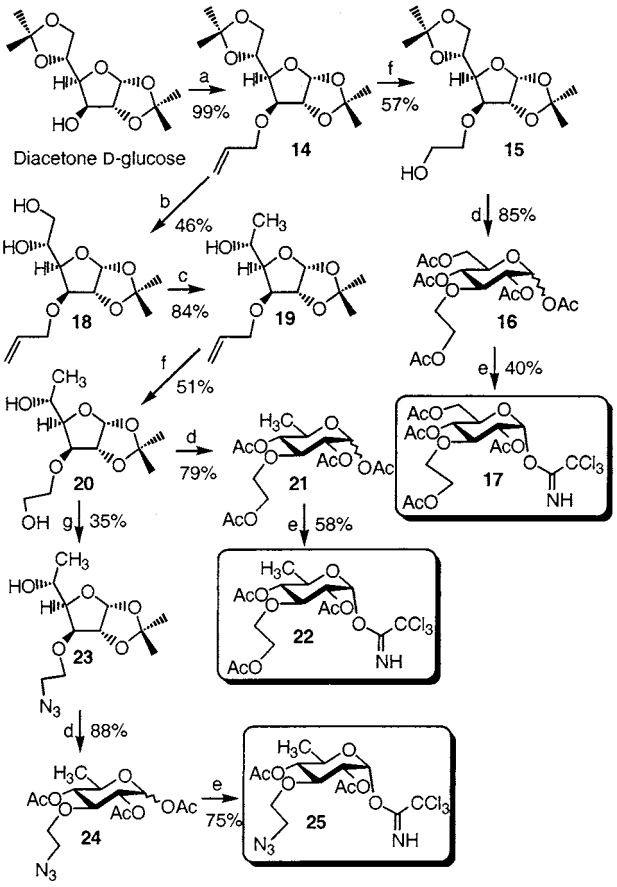
8. Epimerization of the equatorial 4-OH using Swern oxidation and $NaBH_4$ reduction allowed an S_N2 azide-substitution via treatments of Tf_2O then NaN_3. The resulting product, **10**, which decomposed gradually at room temperature, was subjected to ozonolysis and reductive workup providing **11**. Standard procedures, described in Scheme 1, were used to convert **11** into the corresponding glycosyl donor, **13**.

The syntheses of **17**, **22**, and **25** started from diacetone D-glucose through a divergent approach (Scheme 3). After

Scheme 3. Syntheses of Glycosyl Donors[a]

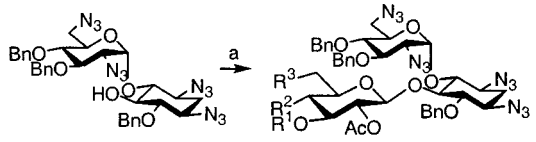


[a] Conditions: (a) Allyl bromide, NaH, $^nBu_4NI$, THF. (b) TsOH, MeOH. (c) (i) TsCl, py.; (ii) $LiAlH_4$, THF. (d) (i) HOAc, TFA, H_2O; (ii) Ac_2O, cat. H_2SO_4. (e) (i) H_2NNH_2−HOAc, DMF; (ii) CCl_3CN, DBU, CH_2Cl_2. (f) (i) O_3, CH_2Cl_2; (ii) $NaBH_4$, MeOH. (g) (i) TsCl, py.; (ii) NaN_3, DMF.

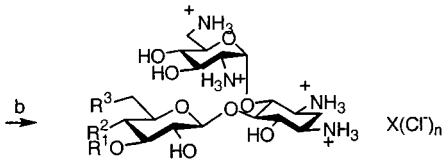
allylation at the 3-OH, the product **14** was diverted into two distinct routes. Ozonolysis and reductive workup of **14**, followed by acid hydrolysis of isoproplyidene groups and acetylation, gave **16** with a hydroxyethyl group at O-3. Standard procedures were used to convert **16** into the corresponding glycosyl donor, **17**. Selective deprotection of the 5,6-*O*-isopropylidene group of **14** allowed the deoxygenation of 6-OH, using TsCl and $LiAlH_4$ treatments. The deoxygenated product, **19**, underwent ozonolysis and reductive workup generating **20**, which led to two different routes. One route employed similar hydrolysis, acetylation, then the protocols for the synthesis of glycosyl trichloroacetimidate offering **22** with a hydroxyethyl group at O-3 and 6-deoxygenation. The other route proceeded via an azide substitution at the primary hydroxyl group on the extended arm. The product, **23**, gave rise to the glycosyl trichloroacetimidate, **25**, with the terminal azido (amino) group at the extended arm and 6-deoxygenation.

Standard procedures modified from the literature for glycosylation[16] and final syntheses[17] were used for the preparation of final products ready for antibacterial assay (Scheme 4).[4]

Scheme 4. Synthesis of the Designed Pyranmycins[a]



26, R^1 = OAc, R^2 = $(CH_2)_2OAc$, R^3 = H, 80%
27, R^1 = $(CH_2)_2OAc$, R^2 = N_3, R^3 = H, 96%
28, R^1 = $(CH_2)_2OAc$, R^2 = OAc, R^3 = OAc, 53%
29, R^1 = $(CH_2)_2OAc$, R^2 = OAc, R^3 = H, 52%
30, R^1 = $(CH_2)_2N_3$, R^2 = OAc, R^3 = H, 67%



TC023, R^1 = OH, R^2 = $(CH_2)_2OH$, R^3 = H, 36%
TC028, R^1 = $(CH_2)_2OH$, R^2 = NH_3^+, R^3 = H, 51%
TC024, R^1 = $(CH_2)_2OH$, R^2 = OH, R^3 = OH, 71%
TC026, R^1 = $(CH_2)_2OH$, R^2 = OH, R^3 = H, 75%
TC032, R^1 = $(CH_2)_2NH_3^+$, R^2 = OH, R^3 = H, 99%

[a] Conditions: (a) **5**, **13**, **17**, **22**, or **25** and BF_3−OEt_2, CH_2Cl_2, 4 A MS. (b) (i) K_2CO_3, MeOH; (ii) PMe_3, THF/H_2O; (iii) H_2, $Pd(OH)_2$/C; (iv) Dowex 1X8 (Cl^- form).

The syntheses of double extended arm pyranmycins began with the precursor we have prepared.[5] Incorporation of allyl groups, followed by ozonolysis and reductive workup, provides one of the designed diols, **33**. Further azide substitution gave the pyranmycin precursor with a double extended arm, **34**. Both compounds underwent the same final syntheses generating the desired final products (Scheme 5).

All of the final products were assayed against *Escherichia coli* with neomycin and ribostamycin as the controls (Table 1).[18] Our preliminary goal is to generate the comparable results with the pyranmycins without an extended arm

(14) The β-glycosidic bond is essential for the formation of intramolecular hydrogen bonding between 2′-NH_2 and O-5″ of the constructed pyranmycins. Please refer to the NMR study for the binding of aminoglycoside toward RNA in ref 8.

(15) Dasgupta, F.; Garegg, P. J. *Synthesis* **1994**, 1121−1123.

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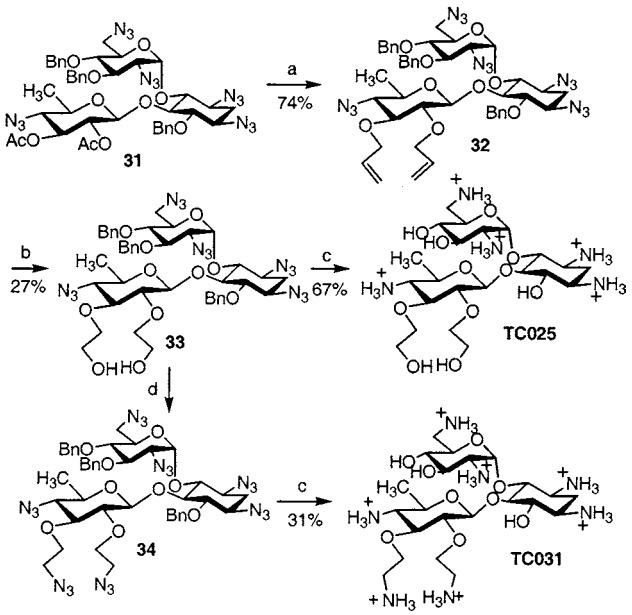
Scheme 5. Synthesis of the Designed Pyranmycins[a]



[a] Conditions: (a) (i) K_2CO_3, MeOH; (ii) allyl bromide, NaH, TBAI, THF. (b) (i) O_3, CH_2Cl_2; (ii) $NaBH_4$, MeOH. (c) (i) K_2CO_3, MeOH; (ii) PMe_3, THF/H_2O; (iii) H_2, $Pd(OH)_2/C$; (iv) Dowex 1X8 (Cl^- form). (d) (i) Tf_2O, py.; (ii) NaN_3, DMF.

(Figure 3).[5b] The structure−activity relationship (SAR) reveals that attaching an extended arm at O-2″ will completely obliterate the antibacterial activity (entries 8 and 11), while attaching the extended arm at O-4″ dramatically decreases the antibacterial activity (entries 5 vs 6). Attaching an extended arm at O-3″ also decreases the antibacterial activity (entries 4 vs 10, and entries 5 vs 9 and 12) in the scaffolds with 6-deoxypyranose (6″-CH_3) at ring III. A surprising decrease in the antibacterial activity against *E. coli* (entries 9 and 12) is noticed, as a charged ammonium group, rather than a neutral hydroxyl group, was introduced at the terminal end of the extended arm at O-3″. This finding demonstrates the value of using real molecules for the SAR studies, which is difficult to predict by computation simulation that relies on charge−charge interaction.

Table 1. MIC against *Escherichia coli*

entry	compd	MIC[a] (μM)
1	neomycin	2
2	ribostamycin	7
3	**TC001**	42
4	**TC005**	9
5	**TC020**	22
6	**TC023**	38
7	**TC024**	40
8	**TC025**	inactive
9	**TC026**	27
10	**TC028**	13
11	**TC031**	inactive
12	**TC032**	39

[a] MIC: minimum inhibitory concentration.

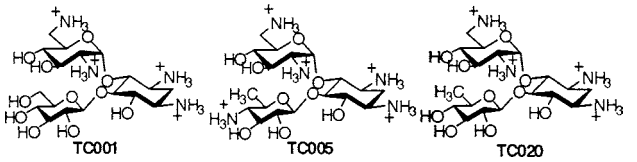


Figure 3. Pyranmycins without an extended arm.

In conclusion, we have developed a methodology to introduce functionalities for probing the perimeter of the binding sites of pyranmycins. This methodology can be applied to study interactions in other systems. From the SAR results, we are pleased to identify that the optimal site for future modification is at O-3″. As revealed by **TC028**, no severe decrease in the antibacterial activity from its unmodified counterpart, **TC005**, is observed. We are currently using solid-phase synthesis to explore more SAR information.

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Supporting Information Available: Experimental procedures for the preparation of compounds **2**−**5**, **7**−**27**, **29**, **30**, **TC023**, **TC024**, **TC025**, **TC026**, **TC028**, **TC031**, and **TC032**, and the corresponding ^1H and ^{13}C NMR spectral; mass spectral data for compounds **2**−**4**, **7**−**12**, **14**−**16**, **18**−**21**, **23**, **24**, **26**, **27**, **29**, **30**, **TC023**, **TC024**, **TC025**, **TC026**, **TC028**, **TC031**, and **TC032**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL027288C

(18) *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.