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Synthesis of an unusual branched-chain sugar, 5-*C*-methyl-L-idopyranose for SAR studies of pyranmycins: implication for the future design of aminoglycoside antibiotics

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Abstract—The syntheses of a challenging branched-chain sugar and several L-sugars have been accomplished. Their application in studies of the antibacterial activity of pyranmycins is reported, which could provide new strategies for the future design of aminoglycoside antibiotics.

Due to their broad spectrum antibacterial activity, aminoglycoside antibiotics have been revived as a focus for the development of new antibacterial agents to counteract the growing problem of drug-resistant infectious diseases.[1,2] Previously, we have reported the structure–activity relationship (SAR) of a novel class of aminoglycoside antibiotics, pyranmycins, which has been synthesized via a glycodiversification approach developed in our laboratory.[3] From the SAR results, we noticed that the presence of 6-deoxy-D-glucopyranose as ring III of pyranmycins is essential for antibacterial activity. On the other hand, the presence of a 6-amino-6-deoxy-L-idopyranose as ring III also manifested significant activity. Thus, we are interested in a design that combines both structural features, hypothesizing that such a structure should lead to improved antibacterial activity (Fig. 1).

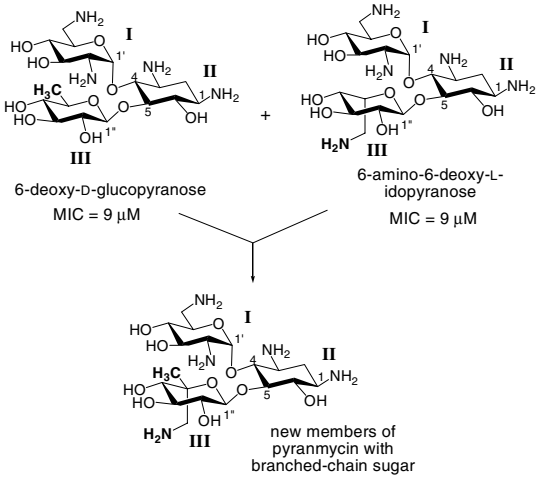

Figure 1. Strategy for the design of branched-chain sugar.

In order to examine our hypothesis, we targeted the synthesis of six pyranose donors that will be used as ring III of pyranmycins (Fig. 2). Compound **1** is designed to ensure that the steric hindrance and the quaternary carbon at *C*-5 will not hamper the antibacterial activity. Compounds **2**, **3**, and **4** are designed to demonstrate the importance of the hydroxymethyl group in L-idopyranose. Compounds **5** and **6** are branched-chain sugars that combine the desired structural components outlined above. The synthesis of **2**, **3**, and **4** began from the known compounds[4] **7**, **9**, and **11** via acid-catalyzed hydrolysis of the isopropylidene groups, acetylation, and phenylthiol substitution (Scheme 1).

Keywords: Aminoglycoside antibiotics; Branched-chain sugar; Pyranmycins; Drug development; Antibacterial.
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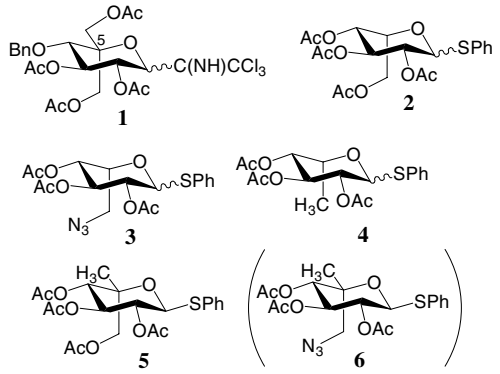
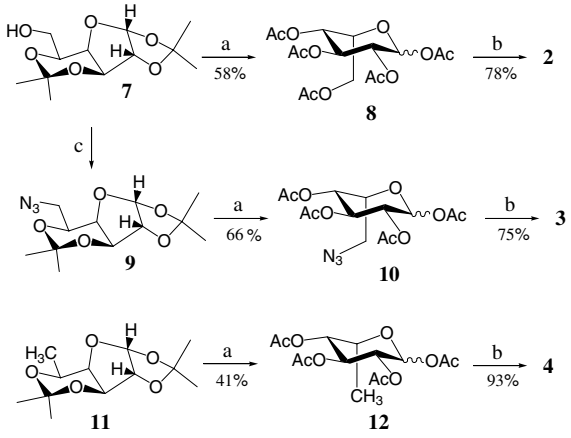
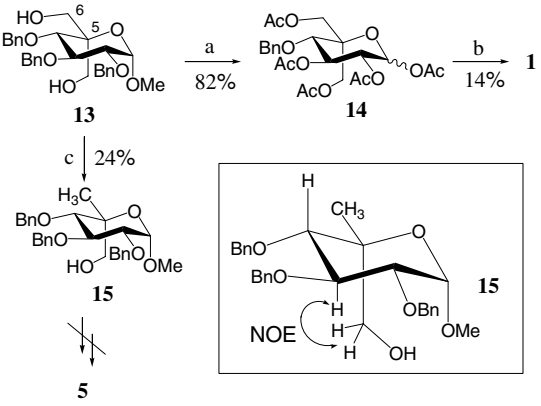


Figure 2. Proposed donors of pyranoses. Compound **6** was proposed initially. However, we were unable to synthesize it.

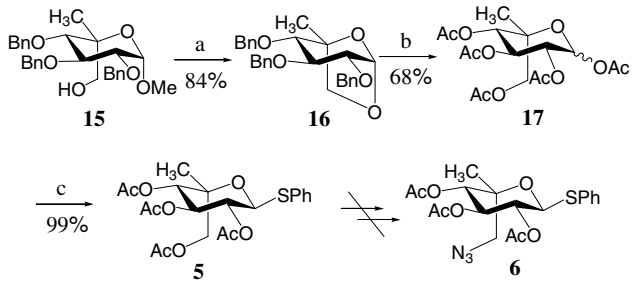


Scheme 1. Reagents: (a) (1) TFA–AcOH–H_2O, (2) Ac_2O, TFA; (b) PhSH, BF_3–OEt_2, CH_2Cl_2; (c) (1) Tf_2O, py., (2) NaN_3, DMF.

Although the synthesis of the precursor of **5**, 5-*C*-methyl-L-idopyranose, has been reported, the synthetic route is difficult to be amended for large-scale production as well as glycosylation of **5**.[5] Therefore, we decided to develop an alternative approach using compound **13**[6] as the starting material. Treatment of **13** with Ac_2O and H_2SO_4 afforded **14**, which was converted into the corresponding glycosyl trichloroacetimidate **1** (Scheme 2).



Scheme 2. Reagents: (a) Ac_2O, H_2SO_4; (b) (1) H_2NNH_2–HOAc, DMF, (2) CCl_3CN, DBU, CH_2Cl_2; (c) (1) TsCl, py., (2) $LiAlH_4$, THF.

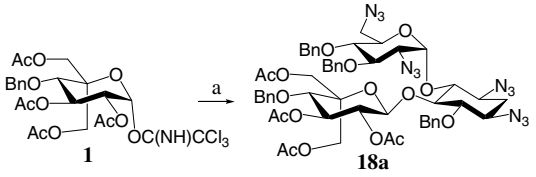


Scheme 3. Reagents: (a) TfOH–AcOH–H_2O (1/28/5); (b) (1) H_2, Pd/C, MeOH, (2) $Sc(OTf)_3$, Ac_2O, CH_2Cl_2; (c) PhSH, BF_3–OEt_2, CH_2Cl_2.

Ditosylation of **13** followed by $LiAlH_4$ reduction furnished **15** with the deoxygenation at the desired position. The 6-*O*-tosyl group (based on the nomenclature of D-glucopyranose) of **13** is accessible toward hydride attack at *C*-6, resulting in deoxygenation. However, the tosyl group on the 5-*C*-hydroxylmethyl group is sterically blocked by the anomeric methoxyl group. In this case, the hydride attack occurs at the sulfur of the tosyl group, which results in regeneration of the free hydroxyl group. Regioselectivity of the deoxygenation was confirmed by 2D NOE experiment.

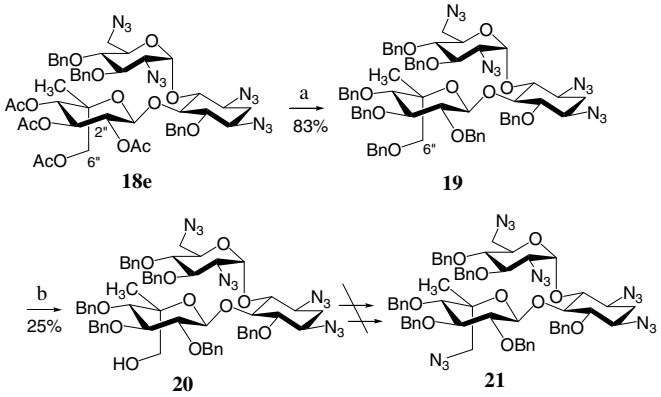
The reactions using Ac_2O and acid catalysts for converting **15** into peracetylated glycoside were unsuccessful. A 1,6-anhydro idopyranose, **16**, was often obtained (Scheme 3). Attempts at employing TMSSPh/TMSOTf[7] provided the desired phenylthio glycoside with unsatisfactory yield. Nevertheless, compound **16** was finally transformed into **17** by a recently reported method[8] using $Sc(OTf)_3$ and Ac_2O following hydrogenation. Treatment of **17** with PhSH and BF_3–OEt_2 gave the desired glycosyl donor, **5**. However, all attempts failed to generate **6**.[9]

Glycosylation of the 3′,4′,6-tri-*O*-benzyltetraazidoneamine acceptor[2e] using glycosyl trichloriacetamidate and



Products	R^1	R^2	Yield (%)
18a	-	-	56
18b	H	CH_2OAc	63
18c	H	CH_2N_3	22
18d	H	CH_3	48
18e	CH_3	CH_2OAc	78

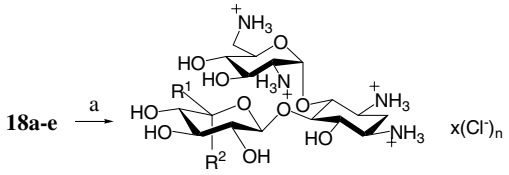
Scheme 4. Reagents: (a) 3′,4′,6-tri-*O*-benzyltetraazidoneamine, BF_3–OEt_2, CH_2Cl_2; (b) NIS–TfOH, CH_2Cl_2.



Scheme 5. Reagents: (a) (1) NaOMe, MeOH, (2) BnBr, NaH, TBAI, THF; (b) (1) TMSOTf, Ac_2O, (2) NaOMe, MeOH.

phenylthio glycosides was carried out via BF_3–OEt_2 and NIS/TfOH activation (Scheme 4). In an effort to prepare one of the designed branched-sugar-containing pyranmycins, compound **18e** was converted into **19** via hydrolysis and perbenzylation (Scheme 5). The *O*-6″ benzyl group on **20** was selectively deprotected. However, all the attempts to make **21** were unsuccessful.[10] Synthesis of final products from compounds **18a**–**e** was performed using reported procedures (Scheme 6).[3]

The synthesized new members of the pyranmycin family were assayed against *Escherichia coli* (ATCC 25922), and *Staphylococcus aureus* (ATCC 25923) using neomycin B as the control to generate the minimum inhibitory concentration (MIC) (Table 1).[11] Despite not having 5-*C*-methyl-6-aminoidopyransoe available, we have reported the synthesis and antibacterial activity of **TC020**.[3] When combining **TC020** and **TC036**, we can still evaluate the effect of branched-chain sugar as in the case of **TC054**. Compound **TC029** is active against both strains of the tested bacteria. The antibacterial activity of **TC036** is much better than the deoxygenated member, **TC037**, which is consistent with our previous finding: a C-6″ aminomethyl (or hydroxylmethyl) group is important for the activity of pyranmycins with ring III L-sugar (Table 1). However, to our surprise, **TC054**, which has a design from combining two structural features with superior antibacterial activity, individually, manifested only low activity. To provide the possible solution for this unexpected result, we evaluated the binding of **TC054** and **TC036** toward the target rRNA fragment using molecular modeling (Fig. 3).[12] From the molecular modeling structures, we did not find significant structural perturbation due to the introduction



Products	R^1	R^2	Yield (%)
TC029	CH_2OH	CH_2OH	39
TC036	H	CH_2OH	69
TC037	H	CH_3	64
TC054	CH_3	CH_2OH	50
TC020	CH_3	H	Ref. 3a
TC010	H	$CH_2NH_3^+$	62

Scheme 6. Reagents: (a) (1) K_2CO_3, MeOH, rt; (2) PMe_3, THF, 0.1 M NaOH, (3) H_2, Pd/C, HOAc–H_2O (1/1), (4) Dowex 1X8 (Cl-form).

Table 1. MIC[13] and binding scores[12] of the synthesized pyranmycins

Compounds	*E. coli*	*S. aureus*[a]	Binding score[b]
Neomycin B	2	0.3	−474.30
TC029	22	7	−314.90
TC036	11	3	−320.00
TC037	91	15	−320.40
TC054	87	27	−317.60
TC020	19	13	−314.50
TC010	9	2	−394.00

[a] Unit: μM.

[b] The tendency in binding: the lower the number, the better the binding.

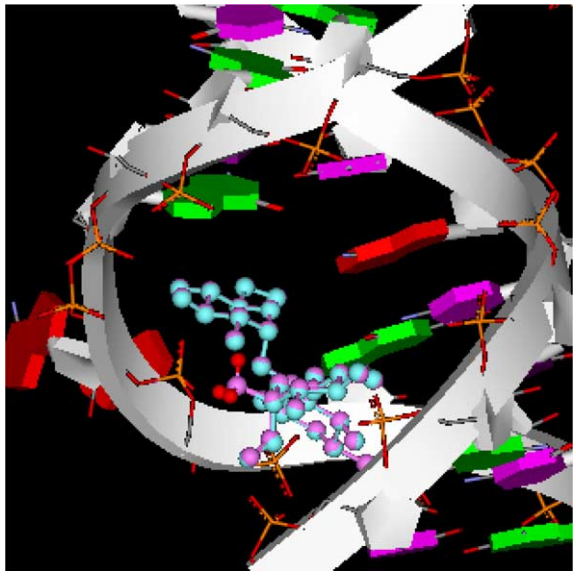


Figure 3. Binding of **TC036** and **TC054** toward the A-site of 16S rRNA (**TC036**: blue, **TC054**: pink). The hydrogen atoms are omitted for clarity except for those attached to the C-6″ of **TC054** (highlighted in red).

of the 5-*C* methyl group on **TC054**. The binding score of **TC054** also cannot explain the unexpected decreased in antibacterial activity (Table 1).

In light of the finding in the activity of the newly synthesized pyranmycins, we have learned valuable lessons. First, a traditional strategy for drug development involves identifying lead structural components (pharmacophores). Then, by combining these components on the same scaffold, it is expected that an additional effect from these components may lead to improved activity.[14] Our results demonstrate that such an addition effect may not always occur. As a result, synthetic methodologies that can furnish compounds with more structural aspects for identifying leads are essential.

Second, as indicated in our molecular modeling results, the binding (or fitting) of **TC054**, which has a ring III branched-chain sugar, toward the rRNA target, is almost identical to that of the **TC036**. Therefore, there must be other factors that could cause the dramatic decrease in antibacterial activity of **TC054**. Since aminoglycosides exert their antimicrobial activity by binding toward a cytosolic target (decoding region at A-site of the 16S rRNA), understanding the process of how aminoglycosides are recruited by bacteria is essential. Additionally, from our experience[3,15] and structures of other unusual sugars in naturally occurring antibiotics,[16–18] there are prevalent examples for the existence of methyl, methoxy, methylamino, or dimethylamino groups, which play key roles in the activity of these antibiotics. As compared to hydroxyl and amino groups, the presence of a methyl group in the forms of methoxy, methylamino, or dimethylamino groups will reduce the solvation effect that may otherwise prevent the antibiotics from entering the targeted sites. Perhaps, solvation is another factor that deserves more investigation.

In conclusion, we have overcome synthetic challenges by achieving a much more convenient synthesis of a branched-chain sugar. The syntheses of three L-sugars are reported. We have also demonstrated the practical use of these unusual sugars.[19] From our antibacterial results, new directions for the development of aminoglycoside antibiotics have also been suggested.

Acknowledgements

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9. Hydrolysis of the acetyl groups of **5**, followed by regioselective tosylation at *O*-6 and azide substitution afforded presumably an undesired 2,6-anhydro-L-idopyranose. Attempts to use phenylthio 2,3,4-tri-*O*-benzyl-L-idopyranoside were hampered by the low stereoselectivity of glycosylation.
10. We obtained a complex mixture with a degraded disaccharide as the major component when Tf_2O/pyridine was used for triflation followed by NaN_3 for azide substitution. Hydrolysis of the acetyl groups of **18e**, followed by regioselective tosylation at *O*-6″ and azide substitution afforded presumably an undesired 2″,6″-anhydro-L-idopyranose as the ring III component.
11. 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.
12. The 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: the lower the number, the better the binding.
13. The MIC values were about 2-folds higher than the reported values against *S. aureus*, for all the tested compounds including neomycin B, presumably due to the variation in the inoculated bacteria concentration.

However, these values were consistent in duplicated tests and were normalized to the reported values based on the MIC of neomycin B for clearer comparison.

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19. Selected spectroscopic information: **TC029**. ^{1}H NMR (270 MHz, D_2O) (acetate salt): δ 6.03 (d, $J=3.9$ Hz, 1H, H-1′), 5.23 (d, $J=8.2$ Hz, 1H, H-1″), 3.6–4.0 (m, 10H), 3.2–3.6 (m, 8H), 2.40 (ddd, $J=12.6$ Hz, $J=4.0$ Hz, $J=4.0$ Hz, 1H, H-2$_{eq}$), 1.93 (s, 12H, CH_3CO$_2$), 1.8 (m, 1H, H-2$_{ax}$); ^{13}C NMR (68 MHz, D_2O) (acetate salt): δ 178.1 (s, CH$_3$*C*O$_2$), 99.3 (s), 95.3 (s), 81.9 (s), 79.8 (s), 75.2 (s), 74.1 (s), 73.2 (s), 72.7 (s), 70.5 (s), 70.0 (s), 69.4 (s), 68.3 (s), 61.8 (s), 57.7 (s), 53.5 (s), 49.4 (s), 48.7 (s), 40.0 (s), 28.1 (s), 21.3 (s, *C*H$_3$CO$_2$); LRFAB *m/e* 515 ([$M^{4+}-3H^{+}$]); HRFAB calcd for $C_{19}H_{39}N_4O_{12}$ ([$M^{4+}-3H^{+}$]) *m/e* 515.2564; measure *m/e* 515.2563. **TC036**. ^{1}H NMR (270 MHz, D_2O) (chloride salt): δ 6.02 (d, $J=4.0$ Hz, 1H, H-1′), 5.28 (d, $J=7.9$ Hz, 1H, H-1″), 3.8–4.2 (m, 9H), 3.67 (dd, $J=9.2$ Hz, $J=9.2$ Hz, 1H), 3.4–3.6 (m, 6H), 3.30 (dd, $J=13.9$ Hz, $J=6.3$ Hz, 1H), 2.50 (ddd, $J=12.5$ Hz, $J=3.9$ Hz, $J=3.9$ Hz, 1H, H-2$_{eq}$), 1.92 (ddd, $J=12.5$ Hz, $J=12.9$ Hz, $J=12.9$ Hz, 1H, H-2$_{ax}$); ^{13}C NMR (100 MHz, D_2O) (chloride salt): δ 100.0 (s), 95.7 (s), 83.1 (s), 75.9 (s), 75.5 (s), 73.7 (s), 73.1 (s), 72.5 (s), 70.6 (s), 70.2 (s), 69.7 (s), 68.6 (s), 57.1 (s), 53.4 (s), 49.6 (s), 48.9 (s), 40.2 (s), 28.2 (s); LRFAB *m/e* 485 ([$M^{4+}-3H^{+}$]); HRFAB calcd for $C_{18}H_{37}N_4O_{11}$ ([$M^{4+}-3H^{+}$]) *m/e* 485.2459; measure *m/e* 485.2454. **TC037**. ^{1}H NMR (270 MHz, D_2O) (acetate salt): δ 5.86 (d, $J=3.9$ Hz, 1H, H-1′), 5.13 (d, $J=8.2$ Hz, 1H, H-1″), 4.20 (m, 1H), 4.04 (dd, $J=9.2$ Hz, $J=9.7$ Hz, 1H), 3.8–4.0 (m, 2H), 3.79 (dd, $J=9.9$ Hz, $J=9.2$ Hz, 1H), 3.68 (dd, $J=9.6$ Hz, $J=5.6$ Hz, 1H), 3.60 (dd, $J=9.6$ Hz, $J=8.9$ Hz, 1H), 3.2–3.5 (m, 2H), 3.19 (dd, $J=13.5$ Hz, $J=6.6$ Hz, 1H), 2.42 (ddd, $J=12.5$ Hz, $J=4.3$ Hz, $J=4.3$ Hz, 1H, H-2$_{eq}$), 1.94 (s, 12H,*C*H$_3$CO$_2$), 1.88 (m, 1H, H-2$_{ax}$), 1.20 (d, $J=6.9$ Hz, 3H, H-6″); ^{13}C NMR (68 MHz, D_2O) (acetate salt): δ 177.8 (s, CH$_3$*C*O$_2$), 99.0 (s), 95.9 (s), 81.7 (s), 75.8 (s), 74.1 (s), 73.0 (s), 71.7 (s, two carbons), 70.9 (s), 70.7 (s), 69.7 (s), 68.4 (s), 53.4 (s), 49.6 (s), 48.8 (s), 40.2 (s), 28.1 (s), 21.2 (s, *C*H$_3$CO$_2$), 12.1 (s); LRFAB *m/e* 469 ([$M^{4+}-3H^{+}$]); HRFAB calcd for $C_{18}H_{37}N_4O_{10}$ ([$M^{4+}-3H^{+}$]) *m/e* 469.2510; measure *m/e* 469.2501. **TC054**. ^{1}H NMR (270 MHz, D_2O) (acetate salt): δ 5.91 (d, $J=3.9$ Hz, 1H, H-1′), 5.30 (d, $J=8.3$ Hz, 1H, H-1″), 3.8–4.1 (m, 6H), 3.3–3.8 (m, 10H), 2.51 (ddd, $J=12.5$ Hz, $J=3.9$ Hz, $J=3.9$ Hz, 1H, H-2$_{eq}$), 1.92 (ddd, $J=12.5$ Hz, $J=11.9$ Hz, $J=11.9$ Hz, 1H, H-2$_{ax}$), 1.33 (s, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (acetate salt): δ 176.9 (s, CH$_3$*C*O$_2$), 99.3 (s), 96.5 (s), 82.0 (s), 79.2 (s), 76.7 (s), 76.4 (s), 74.4 (s), 73.3 (s), 72.6 (s), 70.6 (s), 69.8 (s), 68.5 (s), 59.6 (s), 53.7 (s), 49.6 (s), 49.0 (s), 40.1 (s), 28.2 (s, C-2), 22.2 (s, *C*H$_3$CO$_2$), 20.7 (s); LRFAB *m/e* 499 ([$M^{4+}-3H^{+}$]); HRFAB calcd for $C_{19}H_{39}N_4O_{11}$ ([$M^{4+}-3H^{+}$]) *m/e* 499.2615; measure *m/e* 499.2605.