ORGANIC LETTERS

2002
Vol. 4, No. 26
4603−4606

Pyranmycins, a Novel Class of Aminoglycosides with Improved Acid Stability: The SAR of D-Pyranoses on Ring III of Pyranmycin

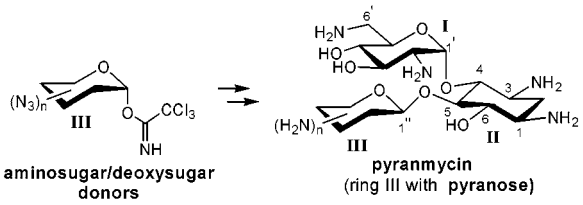
Cheng-Wei Tom Chang,* Yu Hui, Bryan Elchert, Jinhua Wang, Jie Li, and Ravi Rai

Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, Utah 84322-0300

chang@cc.usu.edu

Received September 14, 2002

ABSTRACT



The synthesis of a novel class of aminoglycosides, pyranmycins, is reported along with the structure activity relationship (SAR) of their antibacterial activity against *Escherichia coli*. Two pyranmycins show prominent activity (9 μM). Pyranmycins also manifest superior stability in acidic media. The SAR information will lead to the future designs of pyranmycin against drug resistant bacteria.

Neomycin belongs to a group of aminoglycoside antibiotics containing a 4,5-disubstituted 2-deoxystreptamine core and have been used against both gram-positive and gram-negative bacteria for more than fifty years (Figure 1).[1] Neomycin exerts its antibacterial activity by binding selectively to the A-site of 16S ribosomal RNA of bacteria, and thereby inhibits the protein synthesis of these microorganisms. Although neomycin is still widely used for the treatment of serious infections, there are two problems associated with its use. The first is the rapid emergence of drug resistance in infectious microorganisms,[2] due to the prolonged misuse and overuse of aminoglycoside antibiotics. The second is its relatively high cytotoxicity.[1]

Neomycin degrades readily in acidic media into less active neamine (rings I and II) and inactive neobiosamine (rings III and IV).[1a] Because the glycosidic bond of a furanose is more acid sensitive than that of a pyranose,[2] it is conceivable that by replacing the ring III furanose with a pyranose, superior acid stability will result, possibly leading to a reduction in the required dose for oral administration and, hopefully, a lowering of the associated cytotoxicity. To this end, we have modified neomycin by substituting the neo-

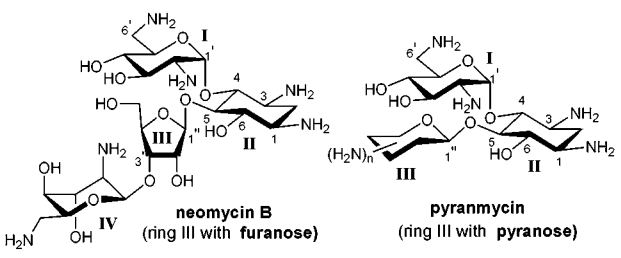


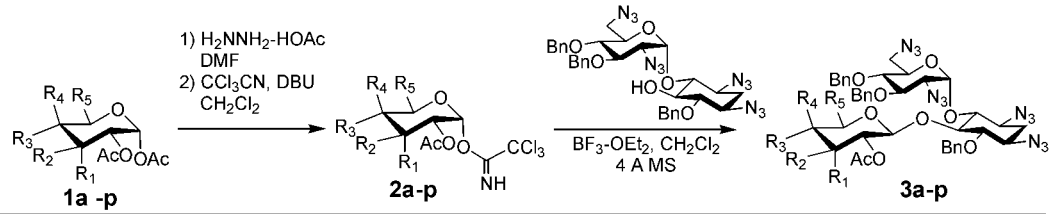
Figure 1. Structures of Neomycin and Pyranmycin.

(1) (a) Hooper, I. R. *Aminoglycoside Antibiotics*; Springer−Verlag: New York, 1982. (b) Haddad, J.; Kotra, L. P.; Mobashery, S. In *Glycochemistry Principles, Synthesis, and Applications*; Wang, P. G., Bertozzi, C. R., Eds.; Marcel Dekker: New York, 2001; p 307.

(2) Cohen, M. L. *Science* **2002**, *257*, 1050−1055. (b) Neu, H. C. *Science* **2002**, *257*, 1064−1072.

10.1021/ol0269042 CCC: $22.00
Published on Web 11/21/2002

Scheme 1. Synthesis of Glycosyl Donors and Protected Pyranmycins



Compounds (yield%)			R_1	R_2	R_3	R_4	R_5
1a[21]	**2a**[29] (70%)	**3a** (71%)	H	OAc	OAc	H	CH_2OAc
1b[16]	**2b** (69%)	**3b** (87%)	H	OAc	OAc	H	CH_2N_3
1c[16]	**2c** (67%)	**3c** (73%)	H	OAc	N_3	H	CH_2N_3
1d[22]	**2d** (67%)	**3d** (42%)	H	OAc	H	OAc	CH_2N_3
1e[16]	**2e** (65%)	**3e** (79%)	H	OAc	N_3	H	CH_3
1f[16]	**2f** (47%)	**3f** (55%)	H	OAc	H	N_3	CH_3
1g[23]	**2g** (71%)	**3g** (66%)	H	N_3	OAc	H	CH_2OAc
1h[24]	**2h** (25%)	**3h** (33%)	OAc	H	OAc	H	CH_2OAc
1i[16]	**2i** (34%)	**3i** (71%)	H	OAc	N_3	H	CH_2OAc
1j[25]	**2j** (63%)	**3j** (64%)	H	N_3	OAc	H	CH_2N_3
1k[26]	**2k** (11%)	**3k** (89%)	H	OAc	H	H	CH_3
1l[16]	**2l** (34%)	**3l** (50%)	H	OAc	H	N_3	CH_2OAc
1m[27]	**2m** (68%)	**3m** (30%)	H	OAc	Tetraacetyl-β-D-galactopyranosyl	H	CH_2OAc
1n[28]	**2n** (64%)	**3n** (61%)	H	OAc	OAc	H	CH_3
1o[27]	**2o** (44%)	**3o** (60%)	H	OAc	tetraacetyl-β-D-glucopyranosyl	H	CH_2OAc
1p[27]	**2p** (80%)	**3p** (92%)	H	OAc	OAc	H	H

biosamine component with a glycopyranose (Figure 1). We have named this novel aminoglycoside pyranmycin.

Another advantage of the pyranmycin design is that it can serve as a template for further chemical modifications. With recent advances in the structural analysis of the binding of aminoglycosides to rRNA,[4−7] these modifications can be designed to increase the binding affinity toward the targeted RNA molecules and disrupt enzyme-catalyzed inactivations,[8] which are the most prevalent mechanisms of aminoglycoside resistance from resistant bacteria. Hence, the resulting aminoglycoside entities may regain the antibacterial activity against resistant strains.

While many neomycin or ribostamycin analogues containing a ring III furanose with β linkage have been reported,[9−14] only one example uses D-glucopyranose as the ring III component via α and β linkages.[15] However, the resulting adducts were less active than neamine. With the envisioned advantages of our pyranmycin design and our recently synthesized library of glycopyranosyl donors,[16] we report a general procedure for the synthesis of pyranmycins together with the results of structure activity relationship studies targeting the ring III pyranose of pyranmycin.

Glycosyl trichloroacetimidates (glycosyl donors) were obtained from the corresponding glycosyl acetate via selective hydrolysis of anomeric acetate with hydrazine acetate in DMF, followed by treatment with trichloroacetonitrile in the presence of catalytic amount of DBU. Acid-catalyzed (BF_3-OEt_2) glycosylation of these glycosyl donors with neamine derivative[9] afforded the designed protected pyranmycins (Scheme 1). The β-glycosidic bond on ring III was

(3) (a) Bochkov, A. F.; Zaikov, G. E. *Chemistry of the O-Glycosidic Bond: Formation and Cleavage*; Pergamon Press: Elmsford, NY, 1979. (b) Shallenberger, R. S.; Birch G., G. *Sugar Chemistry*; Avi Publishing Co., Inc.: 1975.

(4) Fourmy, D.; Recht, M. I.; Puglisi, J. D. *J. Mol. Biol.* **1998**, *277*, 347−362.

(5) Fourmy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. *Science* **1996**, *274*, 1367.

(6) Fourmy, D.; Yoshizawa, S.; Puglisi, J. D. *J. Mol. Biol.* **1998**, *277*, 333−345.

(7) Ma, C.; Baker, N. A.; Joseph, S.; McCammon, J. A. *J. Am. Chem. Soc.* **2002**, *124*, 1438−1442.

(8) For example: Haddad, J.; Kotra, L. P.; Liano-Sotel, B.; Kim, C.; Azucena, E. F., Jr.; Liu, M.; Vakulenko, S. B.; Chow, C. S.; Mobashery, S. *J. Am. Chem. Soc.* **2002**, *124*, 3229−3237.

(9) Greenberg, W. A,; Priestley, E. S.; Sears, P. S.; Alper, P. B.; Rosenbohm, C.; Hendrix, M.; Hung, S.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 6527−6541.

(10) Wong, C.-H.; Hendrix, M.; Manning, D. D.; Rosenbohm, C.; Greenberg, W. A. *J. Am. Chem. Soc.* **1998**, *120*, 8319−8327.

(11) Yoshikawa, M.; Ikeda, Y.; Takenaka, K. *Chem. Lett.* **1984**, 2097−2100.

(12) Girodeau, J.-M.; Pineau, R.; Masson, M.; Le Goffic, F. *J. Antibiotics* **1984**, *37*, 150−158.

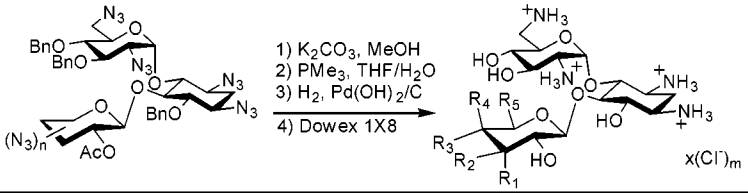
(13) Woo, P. W. K.; Haskell, T. H. *J. Antibiotics* **1982**, *35*, 692−702.

(14) (a) Kumar, V.; Remers, W. A. *J. Org. Chem.* **1978**, *43*, 3327−3331. (b) Kumar, V.; Remers, W. A. *J. Med. Chem.* **1979**, *22*, 432−436. (c) Kumar, V.; Jones, G. S., Jr.; Blacksberg, I.; Remers, W. A. *J. Med. Chem.* **1980**, *23*, 42−49. (d) Kumar, V.; Remers, W. A. *J. Org. Chem.* **1981**, *46*, 4298−4300.

(15) (a) Endo, T.; Perlman, D. *J. Antibiot.* **1972**, *25*, 681−682. (b) Suami, T.; Nishiyama, S.; Ishikawa, Y.; Katsura, S. *Carbohydr. Res.* **1977**, *56*, 414−418.

(16) Elchert, B.; Hui, Yu.; Chang, C.-W. T. Unpublished results.

Scheme 2. Synthesis of the Designed Pyranmycins



Products	R_1	R_2	R_3	R_4	R_5	Yield (%)	MIC[a] (μM)
TC001	H	OH	OH	H	CH_2OH	37	42
TC002	H	OH	OH	H	$CH_2NH_3^+$	32	16
TC003	H	OH	$^+NH_3$	H	$CH_2NH_3^+$	66	19
TC004	H	OH	H	OH	$CH_2NH_3^+$	40	25
TC005	H	OH	$^+NH_3$	H	CH_3	99	**9**
TC006	H	OH	H	$^+NH_3$	CH_3	25	**9**
TC007	H	$^+NH_3$	OH	H	CH_2OH	87	26
TC008	OH	H	OH	H	CH_2OH	66	29
TC012	H	OH	$^+NH_3$	H	CH_2OH	60	20
TC016	H	$^+NH_3$	OH	H	$CH_2NH_3^+$	99	28
TC017	H	OH	H	H	CH_3	56	45
TC018	H	OH	H	$^+NH_3$	CH_2OH	19	12
TC019	H	OH	β-D-Gal	H	CH_2OH	69	Inactive
TC020	H	OH	OH	H	CH_3	60	19
TC021	H	OH	β-D-Glc	H	CH_2OH	78	Inactive
TC022	H	OH	OH	H	H	76	Inactive

[a] MIC: minimum inhibitory concentration

formed exclusively due to the expected neighboring group assistance.[17] The protected pyranmycins were subjected to the final synthetic steps, including hydrolysis of acetyl groups with K_2CO_3 in MeOH, reduction of azido group using the Staudinger reaction, and deprotection of benzyl groups with hydrogenation (H_2, $Pd(OH)_2/C$, Degussa-type in $HOAc/H_2O$ solution). Purification was accomplished using flash chromatography, followed by an ion-exchange (Dowex 1 × 8−200, Cl^- form) (Scheme 2).

The constructed pyranmycins were assayed against *E. coli* (ATCC 25922), and the minimum inhibitory concentrations (MIC) were determined[18] using neomycin, ribostamycin, and neamine as positive controls (MIC = 2, 5, 36 μM, respectively). From the MIC values, a structure activity relationship can be elucidated. **TC001** has a MIC slightly higher than that of the neamine, which is consistent with literature results.[15] Three pyranmycins were constructed to probe the effect of monoamino substitution at different positions on the ring III pyranose, including **TC002** (6″-NH_2), **TC007** (3″-NH2), and **TC012** (4″-NH2). Among them, **TC002** (6″-NH2) is the most potent. Attempts to incorporate the glucosamine donor bearing a C″-2 amino group were, however, unsuccessful.

The diamino analogue, **TC003**, which has a novel *gluco*-1,3-diamine binding motif, is less potent than **TC002**. This result implies that simply increasing the number of amino groups may not lead to an increase in the antibacterial activity, even though the binding affinity between the constructed compounds and the RNA molecules is likely to increase in vitro.[19] We also incorporated pyranoses with *galacto*- and *allo*-configurations (**TC004**, **TC008**, and **TC018**), and found no significant differences in the activity among these scaffolds.

The most promising results came from **TC005** and **TC006**, which have an amino group at the equatorial and axial positions of C-4″, respectively, and a C-6″ CH_3 group. The potencies of **TC005** and **TC006** are similar to those of neomycin and ribostamycin, despite having weaker *trans*- and *cis*-1,2-hydroxyamine binding motifs, respectively.[20]

(17) 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 5.

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

(19) Wong and colleagues have shown in their studies (ref 9) that binding affinity and antibacterial activity cannot be correlated.

(20) Hendrix, M.; Alper, P. B.; Priestley, S.; Wong, C.-H, *Angew*. Chem., Int. Ed. Engl. **1997**, *36*, 95−98.

(21) Glucose pentaacetate was purchased from Acros Chemical Co.

(22) The glycosyl acetate was obtained from acid-catalyzed hydrolysis and peracetylation of 1:2,3:4-di-*O*-isopropylidene-D-galactopyranose (Szarek, W. A.; Jones, J. K. N. *Can. J. Chem*. **1965**, *43*, 2345).

(23) Glycosyl acetate was obtained from acid-catalyzed hydrolysis and peracetylation of 3-azido-3-deoxy-1:2,5:6-di-*O*-isopropylidene-D-galactopyranose (Knapp, S.; Gore, V. K. *Org. Lett*. **2000**, *2*, 1391−1393).

(24) Weinges, K.; Haremsa, S.; Mauer, W. *Carbohydr. Res*. **1987**, *155*, 453−458.

(25) Glycosyl acetate was obtained from selective acid-catalyzed hydrolysis (TsOH, MeOH), tosylation, azide substitution, hydrolysis, and peracetylation of 3-azido-3-deoxy-1:2,5:6-di-*O*-isopropylidene-D-galactopyranose (ref 22).

(26) (a) Banaszek, Z. *Rocz. Chem*. **1971**, *45*, 391−397. (b) Paulsen, H.; Sumfleth, B.; Redlich, H. *Chem. Ber*. **1976**, *109*, 1362−1368.

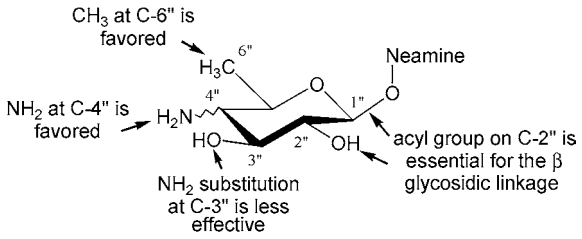


Figure 2. Summary of the SAR of Ring III of Pyranmycins.

Two possibilities may account for the unexpected high potency of **TC005** and **TC006**: the steric hindrance from C-6″ and a van der Waals interaction between the rRNA and the C-6″ methyl group. Similar trends of potency are observed between **TC001** and **TC020** and between **TC018** and **TC006**. To further study the role of the C-6″ methyl group, we prepared analogue **TC022**, with a D-xylopyranose as the ring III sugar. **TC022** was completely *inactive*, favoring the presence of a possible van der Waals interaction between the RNA and C-6″ methyl group.

The dideoxygenated analogue **TC017** showed a dramatic decrease in activity. The result manifests the importance of having either NH_2 or OH at the C-4″ position. Further modifications such as glycosylations on the 4″-OH with either glucopyranose (**TC021**) or galactopyranose (**TC019**) destroy the activity, presumably due to the steric hindrance of an overexpanded substituent on C-4″. Finally, the structure activity relationship (SAR) at the ring III D-pyranose of pyranmycin is summarized as follows (Figure 2): (1) no significant differences in the antibacterial activity are observed among the pyranoses with *allo*-, *gluco*-, and *galacto*-configurations; (2) deoxygenation at C-6″ (6″-CH_3) substantially increases the activity; (3) an NH_2 or OH group at C-4″ position is essential for activity, and deoxygenation of 4″-OH or glycosylation on 4″-OH results in a dramatic decrease in activity; and (4) amino group substitution at C-3″ has less of an effect on activity compared to substitution at C-4″ and C-6″.

We also carried out acid degradation experiments for **TC002** and **TC005**. The compounds were dissolved in D_2O, purged with anhydrous HCl (pH ca. 1), sealed in NMR tubes and incubated at 37 °C, and monitored by ^{1}H NMR. Under identical conditions, neomycin underwent a time-dependent acid degradation (20, 40, 60, and 80% degradation after 2, 6, 10, and 14 days, respectively), rendering a significant decrease in antibacterial activity (the MIC of acid-treated neomycin increases from 2 to 50 μM). On the contrary, both **TC002** and **TC005** showed no sign of degradation and maintained the same level of antibacterial activity.

In conclusion, we have reported the structure activity relationships of pyranmycins ring III D-pyranose. Pyranmycins have comparable antibacterial activity to that of neomycin, but exhibit *much-improved acid stability*. Although the effectiveness of alleviating cytotoxicity by improving acid stability will require more detailed studies, we have demonstrated that pyranmycins have great potential for development into clinically useful antibiotics. We are currently assaying pyranmycins for activity against resistant strains of bacteria. We are also studying the in vitro binding affinity of the pyranmycins with RNA constructs to validate the postulated van der Waals interaction.

Acknowledgment. We are thankful for the financial support from the Utah State University (CURI grant) and the support from Department of Chemistry and Biochemistry, Utah State University. We also thank Prof. Bradley Davidson from Utah State University for assistance on composing the manuscript, Prof. Paul Savage from Brigham Young University for reexamining the MIC value of **TC005**, and Prof. Hung-wen (Ben) Liu from University of Texas, Austin, for providing valuable discussion and suggesting the name, pyranmycin. Mass spectrometry was provided by the Washington University Mass Spectrometry Resource and the Mass Spectrometry Facilities, University of California, Riverside.

Supporting Information Available: General procedures for the preparation of compounds **2b**−**p** and **3a**−**p** and pyranmycins and the corresponding ^{1}H and ^{13}C NMR spectra and mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0269042

(27) These glycosyl acetates were prepared from peracetylation of lactose, maltose, and xylose purchased from Acros Chemical Co.

(28) Glycosyl acetate was obtained from methyl glucoside via tosylation, deoxygenation ($LiAlH_4$), and acetylation (Ac_2O, concentrated H_2SO_4).

(29) Schmidt, R. R.; Jung K.-H. In *Preparative Carbohydrate Chemistry*; Hannessian, S., Ed.; Marcel Dekker: New York, 1997; p 297.