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Synthesis of novel guanidine incorporated aminoglycosides, guanidinopyranmycins

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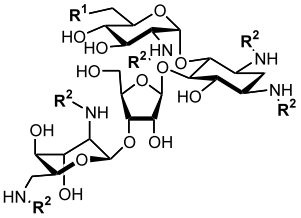
Abstract—A library of new guanidinoglycosides, guanidinopyranmycins were synthesized along with the studies of their preliminary assay against HIV-1.

Viral pathogens have long been associated with the problem of public health.[1] HIV-1, for example, has infected an estimated 40 million people worldwide as of the end of 2001.[2] Aminoglycoside antibiotics,[3,4] due to their broad spectrum of activities, have attracted attention for their antiviral applications by interacting with the viral RNA molecules, such as the RRE[5] and TAR[6] regions of HIV-1, and the human hepatitis delta virus (HDV) ribozyme[7]. Tor and co-workers have recently reported an innovative guanidinoglycoside design,[8,9] which is derived from the transformation of the amino groups of several commercially available aminoglycosides into guanidine groups (Fig. 1). They have also demonstrated that these guanidine-incorporated aminoglycosides have superior anti-HIV-1 activity than their unmodified counterparts.[8,9]

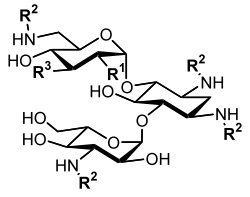
We are particularly interested in Tor's findings since our group has constructed a library of novel aminoglycoside antibiotics,[10] pyranmycins. Following the reported procedures, we converted five members of pyranmycins into the guanidine-incorporated forms. Herein, we wish to report the syntheses of these new additions of guanidinoglycosides and the preliminary result from the assay of their anti-HIV-1 activity.

Among the five guanidinopyranmycins (Fig. 2), TCV001 has no amino group on ring III while TCV002, TCV007, and TCV012 have amino groups on C-6″, C-3″, and C-4″, respectively. TCV005 was selected because its unmodified counterpart, TC005 shows very promising antibacterial activity against various strains of bacteria.[10] We also synthesized the guanidinoneamine, **1**, and used it for comparison. The *N*,*N*′-di-*tert*-butoxycarbonyl-*N*″-triflylguanidine reagent, **2**, was prepared according to the literature procedure.[11,12]

Through the neamine guanidinylation, we found that the reaction is sensitive to reaction solvent, particularly the polarity of the solvent system. When pure methyl-



R^1 = OH, R^2 = (C=NH)NH_2, guanidino-paromomycin
R^1 = NH(C=NH)NH_2, R^2 = (C=NH)NH_2, guanidino-neomycin B



R^1 = OH, R^2 = (C=NH)NH_2, R^3 = OH, guanidino-kanamycin A
R^1 = NH(C=NH)NH_2, R^2 = (C=NH)NH_2, R^3 = OH, guanidino-Kanamycin B
R^1 = NH(C=NH)NH_2, R^2 = (C=NH)NH_2, R^3 = H, guanidino-tobramycin

Figure 1. Guanidino-aminoglycosides from Tor's group.

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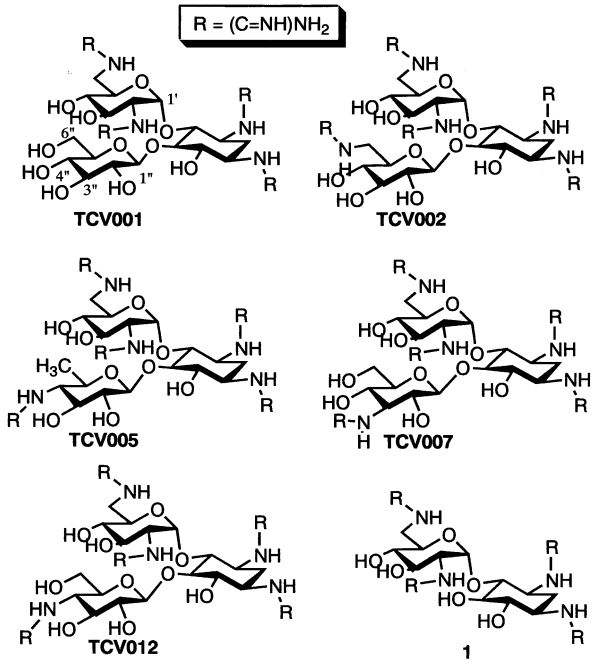


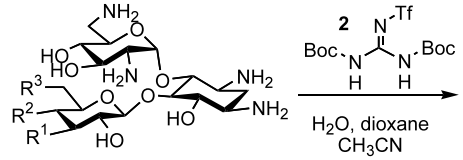
Figure 2. Structures of the designed guanidinopyranmycins.

ene chloride, acetonitrile, ordioxane were employed as solvent, no desired product was obtained. Using H_2O:dioxane (1:5) as reported, we successfully converted neamine to guanidino-neamine precursor after 3 days. We proceeded to test this reaction condition on pyranmycins. The reactions proceeded accordingly except for TC001 and TC007 (Scheme 1). Tor and co-worker have noted that the nonpolar solvent would accelerate the reaction. Therefore, we modified the solvent system for the guanidinylation to a H_2O:dioxane:CH_3CN (1:5:5) mixture. This system provided the desired products after 3–7 days. Deprotection of the *tert*-butoxycarbonyl groups with TFA/CH_2Cl_2, the final products can be obtained and characterized as trifluroracetate salt, and used for assay directly.

The constructed guanidinopyranmycins were tested for their activity in an HIV-1 cytoprotection assay as previously described[13] with minor changes. Briefly, the assays involve the killing of T4 lymphoid cells (CEM-SS cell line) by HIV-1 strain RF and inhibition of cell killing by active compounds. Compounds were diluted as appropriate and added to a 96-well microtiter plate. Cells were infected in the well at a multiplicity of infection (MOI) of approximately 0.2 and viability assayed at 6 days after infection by reduction of the CellTiter 96® Reagent (Promega, Madison, WI). No significant activity was observed in this assay. Furthermore, we are currently exploring additional assays for the HIV-1 regulatory proteins Tat and Rev.

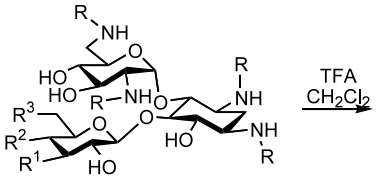
In conclusion, we have successfully synthesized a novel library of guanidine-incorporated aminoglycoside antibiotics, guanidinopyranmycins.[14] Although the preliminary results from the assay against HIV-1 are less than expected, they only represent our initial effort. We are currently constructing a larger library of guanidinopyranmycins based on the pyranmycins we have synthesized.

General procedure for guanidinylation and Boc deprotection. To a mixture of starting material in H_2O:dioxane (1:5) or H_2O:dioxane:CH_3CN (1:5:5) solution, triethyl amine (4 equiv. per amino group) and *N*,*N*′-di-*tert*-butoxycarbonyl-*N*″-triflylguanidine (3 equiv. per amino group) were added. If necessary, more H_2O or dioxane can be added to adjust the turbidity until the solution is homogenously clear. Stir the reaction at room temperature for 3–7 days. The reaction can be monitored by TLC (EtOAc:hexane = 75:25, R_f ranging from 0.1–0.8 depending on the substrate). Upon the completion of the reaction, the solvents were removed by aspirator, and the crude products were purified with a gradient chromatography (hexane:EtOAc = 90:10 to 0:100). To deprotect the *tert*-butoxycarbonyl groups, the products from previous step were dissolved in a solution of TFA:CH_2Cl_2 (1:4), and stirred for 4 h until the completion of the reaction. After removal of solvents, the final products were obtained as a yellowish salt.



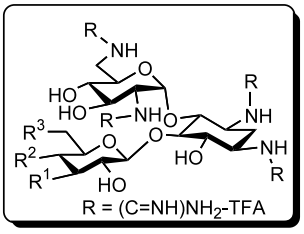
pyranmycins

TC001, R^1 = OH, R^2 = OH, R^3 = OH
TC002, R^1 = OH, R^2 = OH, R^3 = NH_2
TC007, R^1 = NH_2, R^2 = OH, R^3 = OH
TC012, R^1 = OH, R^2 = NH_2, R^3 = OH
TC005, R^1 = OH, R^2 = NH_2, R^3 = H



R = (C=NBoc)NHBoc

3, R^1 = OH, R^2 = OH, R^3 = OH
4, R^1 = OH, R^2 = OH, R^3 = NHC(=NBoc)NHBoc
5, R^1 = NHC(=NBoc)NHBoc, R^2 = OH, R^3 = OH
6, R^1 = OH, R^2 = NHC(=NBoc)NHBoc, R^3 = OH
7, R^1 = OH, R^2 = NHC(=NBoc)NHBoc, R^3 = H



R = (C=NH)NH_2-TFA

TCV001, R^1 = OH, R^2 = OH, R^3 = OH, 15% (2 steps)
TCV002, R^1 = OH, R^2 = OH, R^3 = NHC(=NH)NH_2-TFA, 49% (2 steps)
TCV007, R^1 = NHC(=NH)NH_2-TFA, R^2 = OH, R^3 = OH, 22% (2 steps)
TCV012, R^1 = OH, R^2 = NHC(=NH)NH_2-TFA, R^3 = OH, 32% (2 steps)
TCV005, R^1 = OH, R^2 = NHC(=NH)NH_2-TFA, R^3 = H, 46% (2 steps)

Scheme 1. Synthesis of the designed guanidinopyranmycins.

Acknowledgements

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14. Spectra for **1**: ^{1}H NMR (270 MHz, D_2O) δ 5.44 (d, $J=3.3$ Hz, 1H, H-1′), 3.00–4.20 (m, 11H), 2.16 (m, 1H, H-2$_{eq}$), 1.58 (q, $J=12.2$ Hz, 1H, H-2$_{ax}$); ^{13}C NMR (68 MHz, D_2O) δ 158.0 (s), 157.4 (s), 157.2 (s), 156.6 (s), 98.0 (s, C-1′), 80.9 (s), 76.1 (s), 75.2 (s), 71.5 (s), 71.4 (s), 69.8 (s), 56.0 (s), 51.9 (s), 50.7 (s), 41.9 (s), 32.5 (s). TCV001: ^{1}H NMR (270 MHz, D_2O) δ 5.64 (d, $J=3.3$ Hz, 1H, H-1′), 5.14 (d, $J=7.6$ Hz, 1H, H-1″), 3.00–4.20 (m, 17H), 2.22 (dt, $J=13.0$ Hz, $J=3.6$ Hz, 1H, H-2$_{eq}$), 1.60 (q, $J=13.0$ Hz, 1H, H-2$_{ax}$); ^{13}C NMR (68 MHz, D_2O) δ 157.3 (s), 156.8 (s), 156.7 (s), 156.1 (s), 101.8 (s, C-1″), 96.3 (s, C-1′), 80.7 (s), 77.0 (s), 76.7 (s), 75.3 (s), 71.9 (s), 71.6 (s), 70.9 (s), 69.0 (s), 67.8 (s), 59.9 (s), 59.8 (s), 55.5 (s), 51.4 (s), 50.4 (s), 41.3 (s), 31.7 (s). TCV002: ^{1}H NMR (270 MHz, D_2O) δ 5.78 (s, broad, 1H, H-1′), 5.05 (d, $J=7.9$ Hz, 1H, H-1″), 3.00–4.20 (m, 17H), 2.19 (m, 1H, H-2$_{eq}$), 1.60 (q, $J=11.9$ Hz, 1H, H-2$_{ax}$); ^{13}C NMR (68 MHz, D_2O) δ 158.0 (s), 157.5 (s), 157.4 (s), 157.3 (s), 156.6 (s), 101.8 (s, C-1″), 95.9 (s, C-1′), 80.9 (s), 76.4 (s), 76.0 (s), 75.6 (s), 73.9 (s), 73.7 (s), 72.4 (s), 71.6 (s), 70.6 (s), 69.6 (s), 56.0 (s), 52.1 (s), 50.8 (s), 42.4 (s), 41.9 (s), 32.1 (s). TCV005: ^{1}H NMR (270 MHz, D_2O) δ 5.81 (d, $J=1.0$ Hz, 1H, H-1′), 4.97 (d, $J=7.9$ Hz, 1H, H-1″), 3.00–4.20 (m, 14H), 2.21 (m, 1H, H-2$_{eq}$), 1.62 (q, $J=12.5$ Hz, 1H, H-2$_{ax}$), 1.20 (d, $J=6.3$ Hz, 3H, H-6″); ^{13}C NMR (68 MHz, D_2O) δ 158.0 (s), 157.7 (s), 157.3 (s), 157.2 (s), 156.6 (s), 102.0 (s, C-1″), 95.9 (s, C-1′), 81.2 (s), 76.6 (s), 75.9 (s), 74.5 (s), 74.3 (s), 72.4 (s), 71.5 (s), 70.9 (s), 69.6 (s), 59.7 (s), 55.9 (s), 51.9 (s), 50.9 (s), 41.9 (s), 32.2 (s), 16.7 (s). TCV007: ^{1}H NMR (270 MHz, D_2O) δ 5.64 (d, $J=2.9$ Hz, 1H, H-1′), 5.05 (d, $J=7.9$ Hz, 1H, H-1″), 3.00–4.20 (m, 17H), 2.19 (m, 1H, H-2$_{eq}$), 1.62 (q, $J=12.9$ Hz, 1H, H-2$_{ax}$); ^{13}C NMR (68 MHz, D_2O) δ 157.5 (s), 156.8 (s), 156.6 (s), 156.1 (s), 156.0 (s), 101.0 (s, C-1″), 96.5 (s, C-1′), 79.4 (s), 77.1 (s), 75.8 (s), 75.4 (s), 75.3 (s), 73.4 (s), 71.5 (s), 70.9 (s), 69.3 (s), 69.0 (s), 55.5 (s), 51.5 (s), 50.4 (s), 50.3 (s), 41.3 (s), 31.7 (s). TCV012: ^{1}H NMR (270 MHz, D_2O) δ 5.64 (d, $J=3.3$ Hz, 1H, H-1′), 5.07 (d, $J=7.9$ Hz, 1H, H-1″), 3.00–4.20 (m, 17H), 2.19 (m, 1H, H-2$_{eq}$), 1.60 (q, $J=12.5$ Hz, 1H, H-2$_{ax}$); ^{13}C NMR (68 MHz, D_2O) δ 157.5 (s), 157.2 (s), 156.8 (s), 156.6 (s), 156.1 (s), 101.5 (s, C-1″), 96.3 (s, C-1′), 80.8 (s), 77.1 (s), 75.2 (s), 74.2 (s), 73.8 (s), 73.4 (s), 71.6 (s), 71.0 (s), 69.0 (s), 59.6 (s), 55.5 (s), 53.3 (s), 51.3 (s), 50.4 (s), 41.3 (s), 31.7 (s).