Synthesis and Anti-HIV Activity of Guanidinoglycosides

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The new guanidinylation reagent *N,N'*-diBoc-*N''*-triflylguanidine was used to efficiently convert multiamine-containing glycosides including kanamycin A and B, tobramycin, paromomycin, and neomycin B to the corresponding fully guanidinylated analogues (guanidinoglycosides). This transformation occurs in the presence of H_2O under mild conditions. Guanidinotobramycin and guanidinoneomycin B were found to inhibit the replication of the HIV virus with activities approximately 100 times greater than the parent aminoglycosides.

Introduction

Guanidine-containing sugars and sugar-like molecules have a wide range of biologically important uses such as inhibition of inappropriate mitogenic signaling,[1] therapy for bacterial infections,[2] treatment of noninsulin-dependent diabetes,[3] and inhibition of enzymes including thrombin,[4] glycosidases,[5] and nitric oxide synthases.[6] In most of these cases, the guanidino-sugars have been developed to mimic carbohydrate and peptidic molecules.

It has been documented that arginine-rich peptides have a high affinity for the Rev response element (RRE) of HIV-1.[7] The HIV-1 virally encoded sequence-specific RNA-binding protein, Rev, is an arginine-rich structure that facilitates the replication of HIV RNA.[8] Recent studies have shown that neomycin B and other aminoglycoside antibiotics competitively block the binding of the Rev protein to the RNA RRE.[9] However, these small molecules are toxic and lack site-specificity to the HIV RNA; therefore, new compounds with selective binding for the RRE site must be designed and synthesized.[10] We have discovered that conversion of aminoglycosides to their fully guanidinylated derivatives (guanidinoglycosides) enhances the affinity and selectivity of these compounds to the HIV-1 RRE as compared to the parent aminoglycosides.[11]

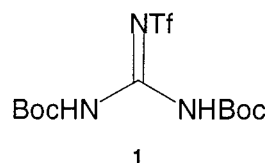

Figure 1. *N,N'*-DiBoc-*N''*-triflylguanidine (**1**).

The preparation of guanidino-sugars has been a challenge for which several methods and reagents have been developed. Most of these methods employ, however, undesirable conditions and show limited success. For example, Wessel and co-workers used 3,5-dimethylpyrazoylformamidinium nitrate (DPFN) in DMF at elevated temperatures to convert amino sugars to their guanidino derivatives in less than 50% yield.[4] Guanidinium-modified cyclodextrins were prepared by Smith and co-workers from a thiourea in one case and 1*H*-pyrazolecarboxamidine hydrochloride in another case employing DMF as the solvent with yields ranging from 33 to 74%.[1]

Additionally, the monoguanidinylation of Boc-protected aminoglycosides has been accomplished using 1*H*-pyrazolecarboxamidine;[2a] however, no yields were reported. Unprotected aminoglycosides were taken to the corresponding mono- and di(methylguanidinylated) structures with poor to moderate yields using *N,S*-dimethylthiouronium iodide with heating (100−110 °C) in DMF.[2b] Part of the difficulty with prior syntheses arises from the limited solubility of aminoglycosides in DMF. It was clear that there is a need for an improved, facile route for the conversion of multiamine-containing glycosides to guanidinoglycosides. In this paper, we report an efficient method for the simultaneous guanidinylation of unprotected aminoglycosides in aqueous media using *N,N'*-diBoc-*N''*-triflylguanidine (**1**, Figure 1).

Results and Discussion

The methodology for the guanidinylation of aminoglycosides was developed commencing with the model

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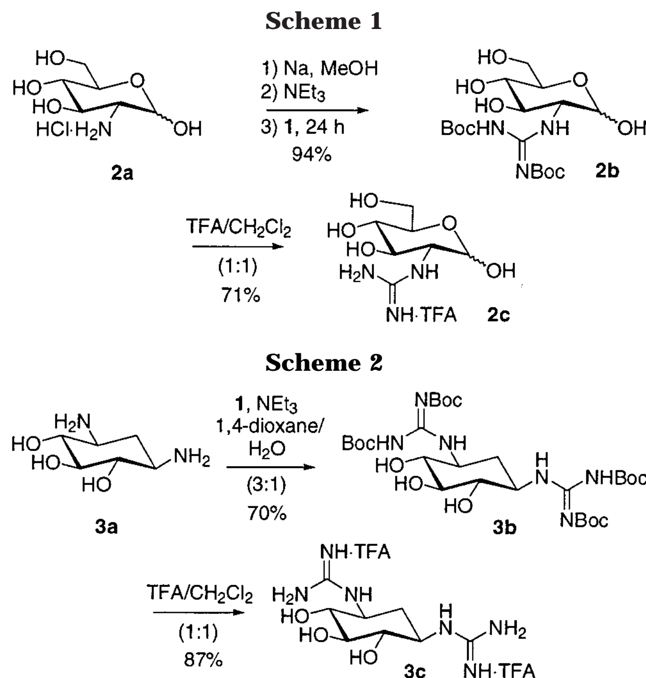
Scheme 1

Scheme 2

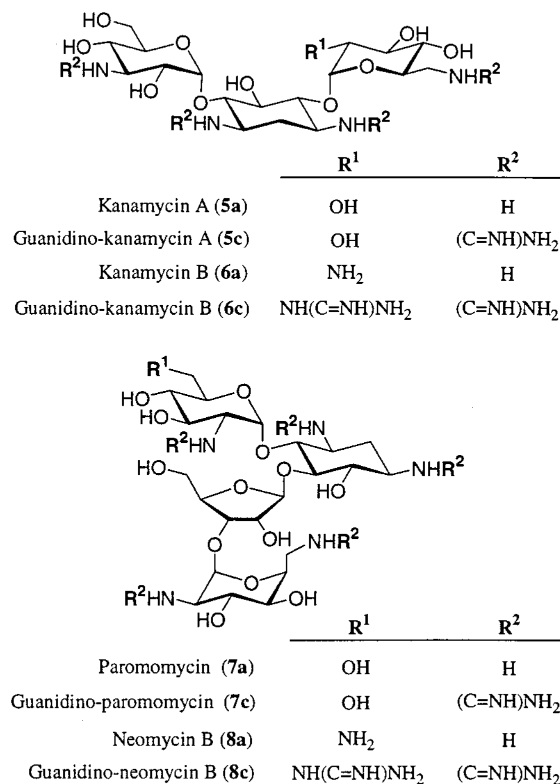


compound glucosamine hydrochloride (**2a**). Guanidinylated glucosamine (**2b**) was prepared by treatment of glucosamine hydrochloride (**2a**) with a methanolic solution of NaOMe followed by NEt_3 and *N,N*-diBoc-*N'*-triflylguanidine (**1**) in 94% yield. The Boc protecting groups were easily removed using a mixture of TFA/CH_2Cl_2 (1:1) providing compound **2c** in good yield (Scheme 1).

2-Deoxystreptamine (**3a**, 2-DOS) is the cyclitol core unit of many aminoglycoside antibiotics. When the dihydrobromide salt of 2-DOS was subjected to methanolic NaOMe, the free diamine precipitated from the solution, which prevented any reaction with reagent **1**. When the reaction mixture was heated at reflux, only diBoc-guanidine was obtained. A variety of solvents (DMSO, DMF, MeOH) were examined for the conversion of compound **3a** to compound **3b** in the presence of reagent **1** and NEt_3. The desired product was not formed.

From our previous studies, we recognized that polar solvents slow the rate of guanidinylation. We therefore searched for a relatively nonpolar solvent or solvent mixture that would dissolve both the glycoside and reagent **1**. We discovered that 1,4-dioxane fits some of these criteria but the addition of H_2O was necessary for complete solubility of the aminoglycoside. The guanidinylation of diamine **3a** with reagent **1** not only tolerated the addition of H_2O but worked efficiently yielding 70% of the target compound **3b**. Subsequent deprotection using TFA resulted in a 87% yield of compound **3c** (Scheme 2).

After the successful conversion of diamine **3a** to compound **3b**, the guanidinylation of more complex aminoglycosides was undertaken. For example, tobramycin (**4a**) was treated with excess reagent **1** in a mixture of 1,4-dioxane/H_2O followed by NEt_3. After 3 days and an aqueous workup, fully guanidinylated tobramycin (**4b**) was isolated in quantitative yield. The Boc protecting groups were removed with TFA to give compound **4c** in 99% yield (Scheme 3). Several other aminoglycosides including kanamycin A (**5a**) and B (**6a**), paromomycin (**7a**), and neomycin B (**8a**) were converted to the corresponding guanidinoglycosides **5c**, **6c**, **7c**, and **8c**, respectively (Figure 2), employing conditions similar to those for tobramycin (**4a**). These results are summarized in Table 1.

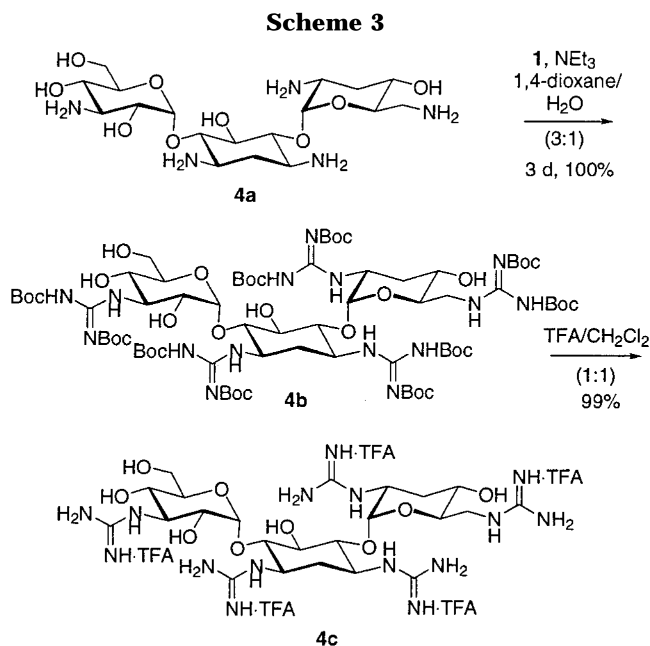


	R^1	R^2
Kanamycin A (**5a**)	OH	H
Guanidino-kanamycin A (**5c**)	OH	(C=NH)NH_2
Kanamycin B (**6a**)	NH_2	H
Guanidino-kanamycin B (**6c**)	NH(C=NH)NH_2	(C=NH)NH_2

	R^1	R^2
Paromomycin (**7a**)	OH	H
Guanidino-paromomycin (**7c**)	OH	(C=NH)NH_2
Neomycin B (**8a**)	NH_2	H
Guanidino-neomycin B (**8c**)	NH(C=NH)NH_2	(C=NH)NH_2

Figure 2. Structures of aminoglycosides kanamycin A (**5a**) and B (**6a**), paromomycin (**7a**), and neomycin B (**8a**) and their guanidino derivatives **5c**, **6c**, **7c**, and **8c**, respectively.

Scheme 3



It has been documented that guanidino-substituted sugar-like compounds have been found to inhibit HIV replication.[12] We too have found that by converting aminoglycosides into guanidinoglycosides the biological activities of these glycosides were dramatically affected. Neomycin B (**8a**) was reported to be an in vivo and in vitro inhibitor of Rev-RRE binding, as well as an effective

Table 1. Preparation of Guanidinoglycosides

aminoglycoside	guan. pdt	guan. yield[a] (%)	deprot. pdt	deprot. yield[b] (%)
tobramycin (**4a**)	**4b**	100	**4c**	99
kanamycin A (**5a**)	**5b**	91	**5c**	100
kanamycin B (**6a**)	**6b**	80	**6c**	95
paromomycin (**7a**)	**7b**	68	**7c**	92
neomycin B (**8a**)	**8b**	70	**8c**	100

[a] Reaction carried out in 1,4-dioxane/H_2O (4:1) for approximately 3-4 d with equimolar NEt_3 as **1**. [b] TFA/CH_2Cl_2 (1:1).

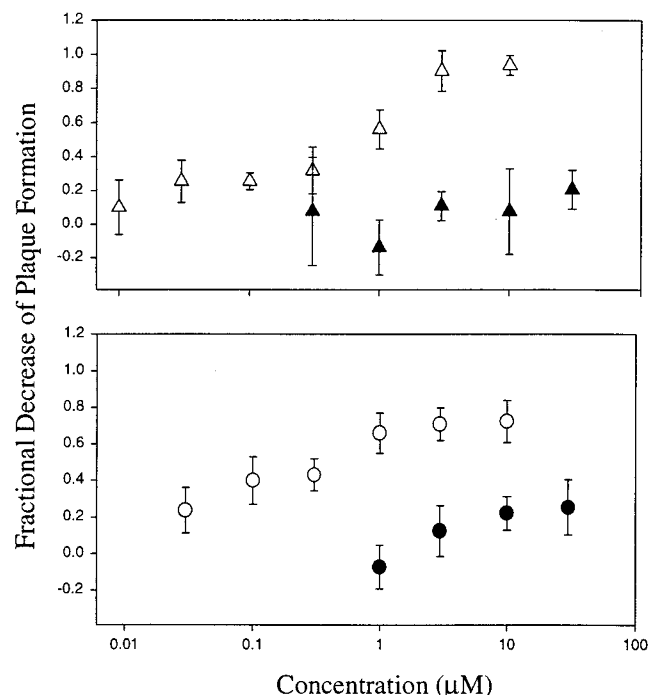


Figure 3. Inhibition of HIV-1 replication in HeLa cells. Top: tobramycin (**4a**, ▲) and guanidinotobramycin (**4c**, △). Bottom: neomycin B (**8a**, ●) and guanidinoneomycin (**8c**, ○).

inhibitor of HIV-1 replication in vivo.[9] The ability of guanidinoneomycin B (**8c**) and guanidinotobramycin (**4c**) to inhibit viral replication in HIV-1-infected CD4$^+$ HeLa cells was measured and compared to the antiviral activities of neomycin B (**8a**) and tobramycin (**4a**, Figure 3). As evidenced by the dose-dependent decrease in plaque formation, the guanidinoglycosides **4c** and **8c** inhibit HIV replication with activities approximately 100 times higher than their amino precursors **4a** and **8a** (Figure 3).[13] The antiviral activity of the guanidinoglycosides **4c** and **8c** may be related to their ability to bind the RRE with high affinity and specificity, thus preventing the HIV-1 Rev-RRE interaction and viral replication.[11]

Conclusions

We have described an efficient method for the multiguanidinylation of aminoglycosides using *N,N*′-diBoc-*N*″-triflylguanidine (**1**). This conversion is carried out under mild conditions in the presence of H_2O and at room temperature. Guanidinotobramycin (**4c**) and guanidinoneomycin B (**8c**) were found to inhibit HIV replication with activities much greater than their amino precursors **4a** and **8a**. These results illustrate the influence of guanidinylation on the biological activity of glycosides and demonstrates the therapeutic potential of the guanidinoglycosides.

Experimental Section

Materials and Methods. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. *N,N*′-DiBoc-*N*″-triflylguanidine (**1**) was prepared using the known procedure.[14] CH_2Cl_2 and $CHCl_3$ were dried first with neutral alumina and NEt_3 with KOH and then distilled from CaH_2. Analytical thin-layer chromatography was carried out on precoated silica gel plates. Flash column chromatography was performed using silica gel (230−400 mesh). All NMR spectra were obtained on a 360 or a 400 MHz spectrometer at UCSD unless otherwise noted. Mass spectra were obtained at the Scripps Research Institute, La Jolla, CA, and University of California, Riverside, CA. The purity of the new compounds was determined by NMR spectroscopy.

General Procedure A (GPA) for the Guanidinylation of Aminoglycosides. To a solution of aminoglycoside (5 amines, 0.054 mmol) in H_2O (0.5 mL) was added 1,4-dioxane (2.5 mL) and *N,N*′-diBoc-*N*″-triflylguanidine (**1**, 0.82 mmol) in alternating portions so the solution remained relatively clear. After 5 min, NEt_3 (0.82 mmol) was added at room temperature. After 3−4 days, the 1,4-dioxane was removed under reduced pressure. The remaining residue and H_2O was extracted with $CHCl_3$ (3 × 10 mL), washed with H_2O and brine, and dried ($MgSO_4$). The fully guanidinylated product can be isolated by flash column chromatography (fcc) on silica gel ($CHCl_3$/MeOH).

General Procedure B (GPB) for the Deprotection of Guanidinoglycosides. A solution of TFA/CH_2Cl_2 (1:1, 1 mL) was added to the protected guanidinoglycoside (0.041 mmol) at room temperature. After approximately 4 h, the solution was diluted with toluene, concentrated in vacuo, and dissolved in H_2O. Subsequent lyopholyzation of H_2O provided the deprotected guanidinoglycoside as a fluffy white powder.

***N*-D-Glucosamine-*N*,*N*′-bis(*tert*-butoxycarbonyl)guanidine (2b).** A solution of NaOMe was prepared from Na metal (17.4 mg, 0.76 mmol) and MeOH (6 mL) at 0 °C. Once the reaction was complete, D-glucosamine hydrochloride (**2a**, 163 mg, 0.76 mmol) was added in one portion, and the resulting mixture was allowed to warm to room temperature. After 20 min, NEt_3 (0.11 mL, 0.76 mmol) and reagent **1** (269 mg, 0.69 mmol) were added, and the mixture was allowed to stir overnight. The resulting mixture was concentrated under reduced pressure. The oily residue was purified by fcc ($CHCl_3$/MeOH 95:5) to provide compound **2b** (300 mg, 94%) as an off-white foam: ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) δ 11.43 (br s, 1H), 8.71 (d, J = 7.6 Hz, 1H), 5.27 (d, J = 3.2 Hz, 1H), 4.21 (dd, J = 8.8, 8.8 Hz, 1H), 3.90−3.87 (m, 2H), 3.82−3.78 (m, 2H), 3.59−3.55 (m, 1H), 1.46 (s, 9H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) δ 162.4, 156.9, 152.4, 91.1, 83.4, 79.7, 73.5, 71.4, 71.3, 61.7, 54.7, 28.2 (3C), 28.1 (3C); MS (FAB) *m*/*z* (rel intensity) 444 ([M + Na]$^+$, 47), 422 ([M + H]$^+$, 65); HRMS (FAB) *m*/*z* calcd for $C_{17}H_{31}N_3O_9$ (M + H)$^+$ 422.2139, found 422.2128, Δ = 2.6 ppm.

D-Glucosamine-Guanidine Trifluoroacetate (2c). According to GPB, TFA/CH_2Cl_2 (1:1, 1 mL) and compound **2b** (0.366 g, 0.869 mmol) provided **2c** (0.207 mg, 71%): ^{1}H NMR (DMSO, 400 MHz, major diastereomer) δ 8.97 (s, 1H), 8.56 (s, 1H), 8.06 (s, 2H), 5.75 (d, J = 6.0 Hz, 1H), 4.43 (br s, 4H), 4.16 (d, J = 6.4 Hz, 1H), 4.09 (d, J = 2.0 Hz, 1H), 3.67 (ddd, J = 8.4, 5.2, 2.4 Hz, 1H), 3.54 (dd, J = 8.0, 2.4 Hz, 1H), 3.52 (dd, J = 5.6, 2.0 Hz, 1H), 3.35 (dd, J = 11.2, 5.2 Hz, 1H); ^{13}C NMR (DMSO, 100 MHz, major diastereomer) δ 158.1, 88.2, 79.6, 73.1, 67.9, 66.2, 63.5; MS (ESI-positive) *m*/*z* (rel intensity) 222

(13) The same trend was observed when tobramycin (**4a**) and guanidinotobramycin (**4c**) were compared by measuring the dose-dependent decrease in viral load, as determined by standard p24 ELISA in human peripheral blood monocytes. See ref 15 for methods.

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(55), 204 (100); HRMS (FAB) *m/z* calcd for $C_7H_{15}N_3O_5$ (M + H)$^+$ 222.1084, found 222.1088, Δ = 1.8 ppm.

1,3-Di[*N,N'*-bis(*tert*-butoxycarbonyl)guanidino]-*N*-2-deoxystreptamine (3b). According to GPA, 2-DOS (**3a**, 40.0 mg, 0.25 mmol) was treated with reagent **1** (203 mg, 0.52 mmol) and NEt_3 (0.12 mL, 0.52 mmol). The fully guanidinylated product **3b** (112 mg, 70%) was isolated by fcc ($CHCl_3$/MeOH 98:2): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.37 (br s, 2H), 8.48 (br s, 2H), 4.15 (ddd, *J* = 9.4, 9.2, 4.4 Hz, 2H), 3.54 (t, *J* = 9.2 Hz, 1H), 3.40 (dd, *J* = 9.2, 9.2 Hz, 2H), 2.37 (ddd, *J* = 12.2, 4.4, 4.4 Hz, 1H), 1.48 (hidden under Boc resonance, 1H), 1.48 (s, 18H), 1.46 (s, 18H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.3 (2C), 156.8 (2C), 152.8 (2C), 83.8 (2C), 79.9 (2C), 77.5, 76.4 (2C), 50.8 (2C), 33.7, 28.2 (6C), 28.1 (6C); MS (FAB) *m/z* (rel intensity) 669 ([M + Na]$^+$, 100), 647 ([M + H]$^+$, 63); HRMS (FAB) *m/z* calcd for $C_{28}H_{50}N_6O_{11}Cs$ (M + Cs)$^+$ 779.2592, found 779.2570, Δ = 2.8 ppm.

1,3-Diguanidino-2-deoxystreptamine·2TFA (3c). According to GPB, TFA/CH_2Cl_2 (1:1, 2 mL) and compound **3b** (18.1 mg, 0.028 mmol) provided **3c** (11.6 mg, 87%): ^{1}H NMR (DMSO, 400 MHz) δ 7.87 (d, *J* = 7.6 Hz, 2H), 7.21 (br s, 6H), 5.36 (br s, 2H), 5.23 (br s, 1H), 3.31–3.23 (m, 2H), 3.15–3.02 (m, 3H), 1.89 (dd, *J* = 11.6, 1.2 Hz, 1H), 1.33 (dd, *J* = 24.0, 11.6 Hz, 1H); ^{13}C NMR (DMSO, 100 MHz) δ = 156.7 (2H), 76.2, 74.6 (2C), 51.7 (2C), 33.2; HRMS (MALDI) *m/z* calcd for $C_8H_{18}N_6O_3$ (M + H)$^+$ 247.1513, found 247.1516, Δ = 1.2 ppm.

GuanidinoBoc$_{10}$-Tobramycin (4b). According to GPA, tobramycin (**4a**, 25.4 mg, 0.054 mmol) was treated with reagent **1** (319 mg, 0.816 mmol) and NEt_3 (0.12 mL, 0.816 mmol). The fully guanidinylated product **4b** (91.2 mg, 100%) was isolated by fcc ($CHCl_3$/MeOH 98:2): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.51 (s, 1H), 11.47 (s, 1H), 11.45 (s, 1H), 11.35 (s, 1H), 11.28 (s, 1H), 8.86 (d, *J* = 3.6 Hz, 1H), 8.48–8.45 (m, 2H), 8.39 (d, *J* = 8.8 Hz, 1H), 8.15 (d, *J* = 8.4 Hz, 1H), 5.39 (d, *J* = 3.6 Hz, 1H), 5.36 (d, *J* = 3.6 Hz, 1H), 4.96 (d, *J* = 3.6 Hz, 1H), 4.45–4.25 (m, 3H), 4.05–4.01 (m, 2H), 3.91–3.80 (m, 4H), 3.70–3.62 (m, 2H), 3.56–3.29 (m, 6H), 3.18 (br d, *J* = 13.2 Hz, 1H), 3.07 (d, *J* = 11.6 Hz, 1H), 2.43–2.38 (m, 1H), 2.32–2.28 (m, 1H), 2.19–2.14 (m, 1H), 2.01 (br s, 2H), 1.54 (s, 9H), 1.51 (s, 9H), 1.49 (s, 9H), 1.47–1.46 (m, 36H), 1.44 (s, 9H), 1.43 (s, 18H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.6, 163.2, 163.0, 162.4, 161.8, 158.1, 157.1, 156.4, 155.6, 154.9, 153.08, 153.05, 152.6, 152.4, 99.4, 97.0, 86.7, 83.8 (2C), 83.7, 83.42, 83.41, 79.7, 79.5, 79.41, 79.37, 79.3, 79.2, 75.9, 73.4, 73.3, 71.7, 69.7, 63.4, 62.1, 58.3, 49.5, 48.7, 48.6, 40.9, 35.3, 31.1, 28.44 (3C), 28.39 (3C), 28.3 (3C), 28.2 (12C), 28.1 (3C), 28.1 (3C), 28.0 (3C); MS (FAB) *m/z* (rel intensity) 1811 ([M + Cs]$^+$, 100); HRMS (FAB) *m/z* calcd for $C_{73}H_{127}N_{15}O_{29}Cs$ (M + Cs)$^+$ 1810.7978, found 1810.8131, Δ = 8.5 ppm.

Guanidinotobramycin·5TFA (4c). According to GPB, TFA/CH_2Cl_2 (1:1, 1 mL) and compound **4b** (69.1 mg, 0.041 mmol) provided **4c** (50.9 mg, 99%): ^{1}H NMR (DMSO, 400 MHz) δ 8.33 (br s, 1H), 8.07 (br s, 1H), 7.85 (br s, 1H), 7.65–7.13 (m, 17H), 5.40 (d, *J* = 3.2 Hz, 1H), 5.29 (br s, 2H), 5.12 (br s, 1H), 4.95 (d, *J* = 2.8 Hz, 1H), 4.52 (br s, 3H), 4.20 (dd, *J* = 9.6, 2.4 Hz, 1H), 3.81–3.71 (m, 2H), 3.58–3.29 (m, 11H), 2.02–1.93 (m, 2H), 1.58–1.47 (m, 2H), 1.24–1.22 (m, 1H); ^{13}C NMR (DMSO, 100 MHz) δ 158.1, 157.6, 156.5, 156.4, 155.9, 97.1, 95.2, 81.2, 78.5, 74.5, 72.8, 71.4, 69.7, 67.8, 63.1, 59.9, 57.1, 50.3, 49.7, 48.9, 41.1, 33.9, 33.2; MS (ESI-positive) *m/z* (rel intensity) 750 ([M + H + 2HCl]$^+$, 19), 714 ([M + H + HCl]$^+$, 30), 678 ([M + H]$^+$, 100); HRMS (MALDI) *m/z* calcd for $C_{23}H_{47}N_{15}O_9$ (M + H)$^+$ 678.3754, found 678.3738, Δ = 2.4 ppm.

Guanidino-Boc$_8$-kanamycin A (5b). According to GPA, kanamycin A (**5a**, 27.1 mg, 0.056 mmol) was treated with reagent **1** (175 mg, 0.446 mmol) and NEt_3 (62 μL, 0.446 mmol). The fully guanidinylated product **5b** (74.0 mg, 91%) was isolated by fcc ($CHCl_3$/MeOH 98:2): ^{1}H NMR ($CDCl_3$, 360 MHz) δ 11.52 (s, 1H), 11.45 (s, 1H), 11.43 (s, 1H), 11.34 (s, 1H), 8.91 (br s, 1H), 8.43–8.39 (m, 2H), 8.29 (d, *J* = 8.3 Hz, 1H), 5.65 (br s, 1H), 5.41 (s, 1H), 5.06 (d, *J* = 2.9 Hz, 1H), 4.96 (d, *J* = 2.9 Hz, 1H), 4.49–4.41 (m, 1H), 4.32 (dd, *J* = 17.6, 7.6 Hz, 1H), 4.18 (dd, *J* = 9.0, 7.9 Hz, 1H), 4.10–4.00 (m, 3H), 3.93 (dd, *J* = 11.5, 1.8 Hz, 1H), 3.83–3.74 (m, 2H), 3.69–3.64 (m, 2H), 3.58–3.54 (m, 1H), 3.52–3.45 (m, 3H), 3.38–3.33 (m, 2H), 3.12–3.06 (m, 3H), 2.98 (dd, *J* = 13.7, 4.3 Hz, 1H), 2.74 (br s, 1H), 2.34 (ddd, *J* = 11.9, 4.0, 4.0 Hz, 1H), 1.90 (br s, 1H), 1.49 (s, 9H), 1.47 (s, 9H), 1.46 (s, 27H), 1.44 (s, 9H), 1.42 (s, 18H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 162.8, 162.1, 161.5, 158.1, 157.4, 156.3, 155.9, 153.1, 153.0, 152.5 (2C), 101.7, 97.9, 85.1, 83.91, 83.89, 83.8, 83.6, 83.5, 80.3, 80.0 (2C), 79.8, 74.8, 73.0, 72.8, 72.3, 71.8, 71.5, 70.0, 69.1, 62.2, 57.6, 49.5, 48.9, 41.0, 34.2, 28.3 (3C), 28.2 (3C), 28.14 (6C), 28.07 (9C), 28.0 (3C); HRMS (FAB) *m/z* calcd for $C_{62}H_{108}N_{12}O_{27}Cs$ (M + Cs)$^+$ 1585.6501, found 1585.6633, Δ = 8.3 ppm.

Guanidinokanamycin A·4TFA (5c). According to GPB, TFA/CH_2Cl_2 (1:1, 1 mL) and compound **5b** (49.2 mg, 0.034 mmol) provided **5c** (37.5 mg, 100%): ^{1}H NMR (DMSO, 400 MHz) δ 8.19 (d, *J* = 7.6 Hz, 1H), 8.04 (d, *J* = 4.4 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.36–7.14 (m, 17H), 5.71 (d, *J* = 6.0 Hz, 1H), 5.56 (s, 1H), 5.45 (d, *J* = 6.0 Hz, 1H), 5.40 (d, *J* = 4.8 Hz, 1H), 5.12 (d, *J* = 4.8 Hz, 1H), 5.07 (d, *J* = 2.8 Hz, 1H), 4.96 (s, 2H), 4.38 (dd, *J* = 6.8, 6.8 Hz, 1H), 3.94 (d, *J* = 9.6 Hz, 1H), 3.55–3.24 (m, 15H), 3.11–3.08 (m, 1H), 1.92 (d, *J* = 10.4 Hz, 1H), 1.50–1.41 (m, 1H); ^{13}C NMR (DMSO, 100 MHz) δ 158.1, 157.5, 156.5, 156.4, 101.1, 97.7, 83.8, 79.3, 74.5, 72.7, 72.2, 72.0, 70.4, 69.9, 69.3, 67.7, 59.7, 57.5, 51.0, 49.8, 41.2, 34.1; MS (ESI-positive) *m/z* (rel intensity) 689 ([M + H + HCl]$^+$, 24), 653 ([M + H]$^+$, 100); HRMS (MALDI) *m/z* calcd for $C_{22}H_{44}N_{12}O_{11}$ (M + H)$^+$ 653.3325, found 653.3294, Δ = 4.7 ppm.

Guanidino-Boc$_{10}$-kanamycin B (6b). According to GPA, kanamycin B (**6a**, 21.4 mg, 0.044 mmol) was treated with reagent **1** (260 mg, 0.664 mmol) and NEt_3 (90 μL, 0.664 mmol). The fully guanidinylated product **6b** (51.4 mg, 80%) was isolated by fcc ($CHCl_3$/MeOH 98:2): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.52 (s, 1H), 11.47 (s, 1H), 11.46 (s, 1H), 11.37 (br s, 2H), 8.87 (d, *J* = 4.0 Hz, 1H), 8.68 (d, *J* = 7.2 Hz, 1H), 8.50 (dd, *J* = 7.6, 4.8 Hz, 1H), 8.40 (d, *J* = 8.4 Hz, 1H), 8.15 (d, *J* = 8.8 Hz, 1H), 5.56 (d, *J* = 4.0 Hz, 1H), 5.21 (br s, 1H), 4.98 (d, *J* = 3.6 Hz, 1H), 4.46–4.28 (m, 3H), 4.07–3.98 (m, 2H), 3.91–3.78 (m, 4H), 3.76–3.54 (m 4H), 3.52–3.43 (m, 2H), 3.37 (dd, *J* = 9.6, 9.6 Hz, 1H), 3.29–3.24 (m, 2H), 3.11 (d, *J* = 10.8 Hz, 1H), 2.42–2.34 (m, 2H), 1.96 (br s, 3H), 1.56 (s, 9H), 1.52 (s, 9H), 1.50 (s, 9H), 1.48 (s, 9H), 1.47 (s, 9H), 1.46 (s, 9H), 1.44 (s, 36H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.2 (2C), 163.0, 162.5, 161.8, 158.1, 157.1 (2C), 156.4, 155.7, 153.1 (2C), 153.0, 152.7, 152.5, 99.2, 97.9, 90.6, 86.3, 84.1, 83.8 (2C), 83.5, 83.4, 79.8, 79.64, 79.57, 79.51, 79.46, 75.9, 73.4, 72.4, 71.7, 71.4, 70.5, 70.0, 69.7, 62.3, 58.2, 49.5, 48.6, 41.0, 35.2, 28.4 (3C), 28.32 (3C), 28.29 (3C), 28.27 (2C), 28.22 (9C), 28.15 (3C), 28.09 (3C), 28.06 (3C); HRMS (FAB) *m/z* calcd for $C_{73}H_{127}N_{15}O_{30}Na$ (M + Na)$^+$ 1716.8771, found 1716.8749, Δ = 1.3 ppm.

Guanidinokanamycin B·5TFA (6c). According to GPB, TFA/CH_2Cl_2 (1:1, 2 mL) and compound **6b** (15.9 mg, 9.4 μmol) provided **6c** (11.3 mg, 95%): ^{1}H NMR (DMSO, 400 MHz) δ 8.28 (br s, 1H), 8.05 (br s, 1H), 7.83 (br s, 1H), 7.62 (br s, 1H), 7.50 (br s, 2H), 7.25 (br s, 11H), 7.12 (br s, 8H), 5.60 (d, *J* = 4.4 Hz, 1H), 5.52 (d, *J* = 6.0 Hz, 1H), 5.49 (d, *J* = 5.2 Hz, 1H), 5.45 (br s, 1H), 5.29 (d, *J* = 6.8 Hz, 1H), 5.08 (d, *J* = 4.0 Hz, 1H), 4.95 (br s, 1H), 4.57 (dd, *J* = 5.6, 5.6 Hz, 1H), 3.79 (d, *J* = 9.2 Hz, 1H), 3.55–3.40 (m, 15H), 3.19–3.13 (m, 1H), 2.00–1.98 (m, 1H), 1.53–1.48 (m, 1H); ^{13}C NMR (DMSO, 100 MHz) δ 158.0, 157.6, 157.0, 156.5, 156.3, 97.1, 96.8, 81.0, 78.6, 74.5, 72.7, 71.7, 70.2, 69.72, 69.67, 67.7, 59.9, 57.1, 55.4, 50.3, 49.8, 41.2, 33.9; HRMS (MALDI) *m/z* calcd for $C_{23}H_{47}N_{15}O_{10}$ (M + H)$^+$ 694.3703, found 694.3722, Δ = 2.7 ppm.

Guanidino-Boc$_{10}$-paromomycin (7b). According to GPA, paromomycin (**7a**, 23.2 mg, 0.038 mmol) was treated with reagent **1** (221 mg, 0.565 mmol) and NEt_3 (0.10 mL, 0.565 mmol). The fully guanidinylated product **7b** (46.8 mg, 68%) was isolated by fcc ($CHCl_3$/MeOH 98:2): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.45 (s, 1H), 11.43 (s, 1H), 11.39 (s, 1H), 11.38 (s, 1H), 11.35 (s, 1H), 9.32 (br s, 1H), 8.89 (d, *J* = 8.0 Hz, 1H), 8.52 (dd, *J* = 6.0, 6.0 Hz, 1H), 8.41 (d, *J* = 6.4 Hz, 1H), 8.26 (d, *J* = 7.2 Hz, 1H), 5.69 (s, 2H), 5.30 (s, 1H), 4.98 (s, 1H), 4.51–4.48 (m, 1H), 4.41 (d, *J* = 7.6 Hz, 1H), 4.37–4.24 (m, 3H), 4.14–4.10 (m, 4H), 4.06–4.01 (m, 3H), 3.96–3.84 (m, 5H), 3.80–3.67 (m, 5H), 3.63–3.58 (m, 4H), 3.47 (s, 2H), 3.32–3.28 (m, 2H), 2.46 (d, *J* = 11.2 Hz, 1H), 1.51 (s, 9H), 1.47–1.43 (m,

82H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.0, 162.2, 162.1, 161.9, 161.4, 157.4, 157.1, 156.7, 156.4, 155.7, 153.2, 152.8, 152.6, 152.4, 151.8, 106.5, 98.0, 95.5, 85.5, 84.1, 83.9 (3C), 83.8, 81.7, 80.3, 80.2 (2C), 80.1, 79.9, 1 peak hidden under solvent peak, 75.6, 75.1, 73.9, 73.4, 73.0, 72.5, 70.9, 69.5, 65.8, 61.5, 60.7, 54.1, 52.2, 50.0, 49.4, 39.9, 33.2, 28.3 (3C), 28.2 (12C), 28.11 (3C), 28.08 (12C); HRMS (MALDI) *m/z* calcd for $C_{78}H_{135}N_{15}O_{34}Na$ (M + Na)$^+$ 1848.9194, found 1848.9173, Δ = 1.1 ppm.

Guanidinoparomomycin·5TFA (7c). According to GPB, TFA/CH_2Cl_2 (1:1, 2 mL) and compound **7b** (39.0 mg, 0.021 mmol) provided **7c** (27.4 mg, 92%): ^{1}H NMR (DMSO, 400 MHz) δ 8.07−8.03 (m, 2H), 7.71 (br s, 1H), 7.45 (br s, 4H), 7.32 (br s, 6H), 7.20 (br s, 6H), 6.88 (d, J = 9.2 Hz, 1H), 5.97 (s, 1H), 5.92 (br s, 1H), 5.66 (s, 2H), 5.38 (s, 1H), 5.27 (br s, 1H), 5.13 (br s, 1H), 5.03 (s, 1H), 5.00 (br s, 1H), 4.83 (s, 1H), 4.62−4.58 (m, 1H), 4.18 (br s, 2H), 3.91 (dd, J = 6.8, 6.8 Hz, 1H), 3.85 (s, 1H), 3.77−3.73 (m, 1H), 3.64−3.20 (m, 17H), 1.93 (d, J = 9.6 Hz, 1H), 1.55−1.48 (m, 1H); ^{13}C NMR (DMSO, 100 MHz) δ 157.3, 157.1, 157.0, 156.9, 156.4, 109.4, 97.4, 95.1, 85.3, 81.6, 76.7, 75.2, 74.1, 73.2, 72.6, 72.5, 72.1, 69.5, 69.2, 66.5, 61.4, 60.3, 55.6, 53.2, 51.4, 50.3, 41.5, 33.1; HRMS (MALDI) *m/z* calcd for $C_{28}H_{55}N_{15}O_{14}$ (M + H)$^+$ 826.4126, found 826.4135, Δ = 1.1 ppm.

Guanidino-Boc$_{12}$-neomycin B (8b). According to GPA, neomycin B (**8a**, 27.4 mg, 0.045 mmol) was treated with reagent **1** (314 mg, 0.803 mmol) and NEt_3 (0.12 mL, 0.803 mmol). The fully guanidinylated product **8b** (64.5 mg, 70%) was isolated by fcc ($CHCl_3$/MeOH 98:2): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.56 (br s, 1H), 11.44 (br s, 2H), 11.40 (br s, 3H), 9.32 (d, J = 5.6 Hz, 1H), 8.94 (d, J = 8.0 Hz, 1H), 8.59 (dd, J = 6.0, 6.0 Hz, 1H), 8.54 (dd, J = 5.6, 5.6 Hz, 1H) 8.49 (br s, 1H), 8.29 (d, J = 8.8 Hz, 1H), 5.64 (br s, 1H), 5.57 (d, J = 4.4 Hz, 1H), 5.41 (br s, 1H), 5.19 (s, 1H), 5.01 (s, 1H), 4.97−4.96 (m, 1H), 4.48−4.46 (m, 2H), 4.41−4.36 (m, 2H), 4.23−4.18 (m, 3H), 4.10−3.88 (m, 7H), 3.82−3.64 (m, 8H), 3.55 (br s, 2H), 3.49 (ddd, J = 11.6, 11.6, 5.6 Hz, 1H), 3.40−3.29 (m, 2H), 2.45 (ddd, J = 12.4, 4.0, 4.0 Hz, 1H), 1.57 (s, 18H), 1.54 (s, 18H), 1.53 (br s, 36H), 1.50 (s, 18H), 1.49 (s, 9H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 162.7 (2C), 162.4, 162.3, 162.1, 157.3, 157.0, 156.8, 156.7 (2C), 155.6, 153.1, 152.8, 152.63, 152.56, 152.3, 151.7, 108.2, 98.5, 96.6, 86.1, 83.6, 83.50, 83.45, 83.4, 83.3, 82.7, 81.8, 79.6, 79.55, 79.50, 79.46, 79.3, 77.6, 76.1, 75.0, 72.8, 72.5, 71.3, 70.3, 70.04, 70.02, 69.8, 66.2, 62.6, 54.1, 52.2, 50.1, 49.2, 40.1, 33.7, 28.30 (3C), 28.26 (3C), 28.2−28.1 (24C), 28.05 (6C); HRMS (MALDI) *m/z* calcd for $C_{89}H_{154}N_{18}O_{37}Na$ (M + Na)$^+$ 2090.0620, found 2090.0552, Δ = 3.3 ppm.

Guanidinoneomycin B·6TFA (8c). According to GPB, TFA/CH_2Cl_2 (1:1, 2 mL) and compound **8b** (22.0 mg, 0.011 mmol) provided **8c** (16.4 mg, 100%): ^{1}H NMR (DMSO, 400 MHz) δ 8.21 (br s, 1H), 8.12 (br s, 1H), 7.71 (br s, 1H), 7.50−7.10 (m, 26H), 6.87 (d, J = 8.8 Hz, 1H), 5.97 (s, 1H), 5.91 (s, 1H), 5.70 (s, 1H), 5.68 (s, 1H), 5.62 (s, 1H), 5.44 (br s, 2H), 4.99 (s, 1H), 4.95 (br s, 1H), 4.83 (s, 1H), 4.17 (br s, 2H), 3.90 (br s, 1H), 3.84 (s, 1H), 3.73 (br s, 1H), 3.64−3.28 (m, 15H), 3.15−3.11 (m, 2H), 1.94 (d, J = 9.2 Hz, 1H), 1.51 (d, J = 9.6 Hz, 1H); ^{13}C NMR (DMSO, 100 MHz) δ 157.5, 157.2, 157.0, 156.9 (2C), 156.5, 110.0, 97.4, 95.5, 85.4, 81.7, 76.5, 75.9, 74.1, 73.1, 72.0, 70.1, 69.9, 69.1, 66.4, 65.1, 62.7, 61.3, 55.4, 53.1, 51.4, 50.2, 41.3, 33.2; HRMS (MALDI) *m/z* calcd for $C_{29}H_{58}N_{18}O_{13}$ (M + H)$^+$ 867.4504, found 867.4488, Δ = 1.8 ppm.

HeLa Assay. Two independent sets of duplicate points were collected as described.[15] HT-6C cells were grown and assayed for plaque forming units (PFUs), in Dubecco's Modified Eagle's Medium containing: 10% fetal calf serum (FCS), 2 mM glutamine, and 100 μg/mL penicillin and streptomycin. Cells were seeded in 24-well Falcon plates at 2.5×10^4 cells/well and incubated overnight (37 °C in the presence of CO_2). The HIV-1 strain X794 LAI was then added such that 70 ± 10 PFUs/well were apparent after an additional 3 d incubation. Inhibitors were added 2 h following the addition of LAI and incubated (as above) for 3 d. Cells were then washed in MeOH and stained with Crystal Violet (0.5% in H_2O), and PFUs were counted and compared to no-inhibitor controls.

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Supporting Information Available: ^{1}H or ^{13}C NMR spectra for compounds **2b**,**c**, **3b**,**c**, **4b**,**c**, **5b**,**c**, **6b**,**c**, **7b**,**c**, and **8b**,**8c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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