Guanidinoglycosides: A Novel Family of RNA Ligands

Nathan W. Luedtke, Tracy J. Baker, Murray Goodman,* and Yitzhak Tor*

Department of Chemistry and Biochemistry
University of California, San Diego
La Jolla, California 92093-0358

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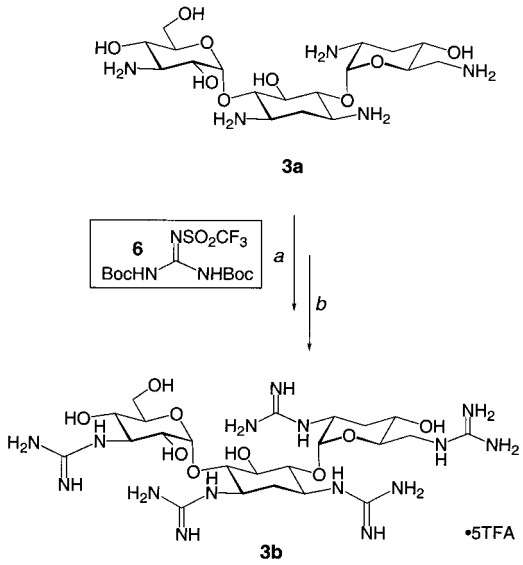
Aminoglycoside antibiotics are a family of structurally diverse polyamines that have been a central focus of small molecule−RNA recognition studies over the past decade.[1] The antibacterial activity of these compounds is believed to derive from their interaction with prokaryotic rRNA.[2] More recently, aminoglycosides have been synthetically modified in ongoing efforts to discover new antiviral and antitumor agents.[3] Aminoglycosides show effective selectivity in their preferential binding of RNA over DNA,[4] but are relatively nonselective in their differentiation between natural RNAs. Aminoglycosides are reported to bind a wide range of unrelated RNA structures; including 16S and 18S rRNAs,[2,5] mRNA transcripts,[3c] tRNA,[6] catalytic RNAs,[7] and viral RNAs.[8] This general affinity for RNA is related to the ability of aminoglycosides to bind RNA through electrostatic interactions mediated by ammonium groups.[9]

The guanidinium group plays a key role at many RNA−protein binding interfaces, including the complexes formed between transcriptional elongation factors with mRNA, tRNA synthetases with tRNAs, ribosomal proteins with rRNA, and viral regulatory proteins with their cognate RNA binding sites.[10] In contrast to ammonium groups, guanidinium groups are highly basic, planar, and exhibit directionality in their H-bonding interactions. We hypothesized that the RNA affinity *and* selectivity of aminoglycoside-based ligands can be increased by replacing the ammonium groups with guanidinium groups. In this report, we disclose a new family of RNA ligands, termed "guanidinoglycosides", in which all of the ammonium groups of the natural aminoglycoside antibiotics have been converted into guanidinium groups (Figure 1).[11]

The preparation of guanidinoglycosides has been accomplished through the treatment of aminoglycosides with *N*,*N*′-di-*tert*-butoxycarbonyl-*N*″-triflylguanidine **6**, a new guanidinylating reagent.[12] This novel reagent facilitates the guanidinylation of polyfunctional amines in aqueous media and in high yields. For example, when tobramycin (**3a**) is treated with an excess of **6** in a 1,4-dioxane/water mixture, the Boc-protected, fully guanidinylated derivative is obtained (Scheme 1, step a). Subsequent deprotection of the Boc groups affords guanidino-tobramycin (step b).[13]

Scheme 1. Example of Guanidinoglycoside Synthesis[a]



[a] Reaction conditions: (a) 15 equiv of **6** in 1,4-dioxane/H_2O (5:1), 15 equiv of NEt_3, 3d, rt. [b] TFA/CH_2Cl_2 (1:1).

The HIV-1 Rev-RRE interaction was used to examine the impact of guanidinylation upon RNA binding, and to probe the potential antiviral activity of these compounds. The binding of Rev to the RRE (Rev response element) is responsible for the export of unspliced and singly spliced HIV genomic RNA out of the host nucleus.[14] This essential protein−RNA interaction remains an important, and nonutilized, therapeutic target. The high-affinity Rev binding site on the RRE has been localized to the purine-rich bulge shown in Figure 2.[15] The binding of Rev to the RRE is governed by the arginine-rich fragment, Rev_{34-50}.[16] Key guanidinium groups make direct contacts with the RNA platform and are essential for the specific binding of Rev to the RRE.[17]

Fluorescence anisotropy has been employed to determine the affinity of the new derivatives to the RRE in solution.[13] The RRE-bound fluorescent Rev peptide has a slower Brownian tumbling motion relative to the free peptide. Upon displacement of the fluorescein-labeled Rev peptide from the RRE by an inhibitor, a decrease in the anisotropy value is observed. Table 1 (column a) compares the IC_{50} values of the guanidinoglycosides to the aminoglycosides.[18] Guanidinylation of kanamycin A, kanamycin

* Corresponding author. E-mail: ytor@ucsd.edu.

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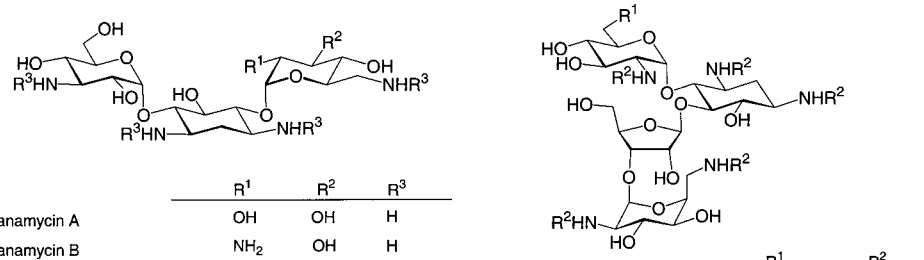

		R^1	R^2	R^3
1a	Kanamycin A	OH	OH	H
2a	Kanamycin B	NH_2	OH	H
3a	Tobramycin	NH_2	H	H
1b	Guanidino-kanamycin A	OH	OH	$(C{=}NH)NH_2$
2b	Guanidino-kanamycin B	$NH(C{=}NH)NH_2$	OH	$(C{=}NH)NH_2$
3b	Guanidino-tobramycin	$NH(C{=}NH)NH_2$	H	$(C{=}NH)NH_2$

		R^1	R^2
4a	Paromomycin	OH	H
5a	Neomycin B	NH_2	H
4b	Guanidino-paromomycin	OH	$(C{=}NH)NH_2$
5b	Guanidino-neomycin B	$NH(C{=}NH)NH_2$	$(C{=}NH)NH_2$

Figure 1. Structures of aminoglycosides (**1a**−**5a**) and guanidinoglycosides (**1b**−**5b**).

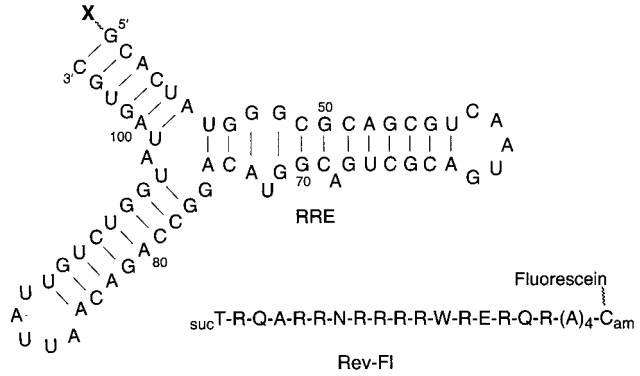

Figure 2. Minimal binding domains for Rev and RRE. For fluorescence anisotropy experiments, **X** = triphosphate. For solid-phase assay, **X** = biotin−streptavidin linkage to solid-support.[13,21]

Table 1. IC_{50} Values (μM) as Determined by Fluorescence Anisotropy and Solid-Phase Assays[a]

glycoside	(a) anisotropy	(b) solid-phase	(c) solid-phase +polyA•polyU	(d) solid-phase +DNA	(e) ratio (c)/(b)
1a	750	700	1300	750	1.9
1b	65	50	60	55	1.2
2a	80	80	160	90	2.0
2b	3.5	3.0	4.7	3.5	1.6
3a	44	45	140	50	3.1
3b	3.8	3.0	5.0	3.5	1.7
4a	65	55	110	60	2.0
4b	18	14	20	16	1.4
5a	6.0	6.0	16	8.0	2.7
5b	1.3	0.8	4.5	0.9	5.6

[a] The standard devaition of all values is less than ±25% of the reported value.[13,22]

B, and tobramycin results in a greater than 10-fold increase in inhibitory activity relative to the parent compounds.[19] A 5-fold increase in activity is observed upon guanidinylation of neomycin B and paromomycin.[20]

A novel solid-phase assembly was used to evaluate the RNA specificity of the new derivatives.[21] This assay relies on the displacement of a fluorescent Rev peptide from a solid-phase immobilized RRE and can be performed in the presence of competing nucleic acids.[22] Table 1 (columns b−d) summarizes the IC_{50} values determined by this method. By comparing IC_{50} values measured in the absence of any competitors to the values measured in the presence of DNA or RNA, a quantitative measure for the relative selectivity of each ligand can be made.[21] Aminoglycosides have a substantial affinity to poly A•poly U duplex RNA (compare column b to c, Table 1).[22] Dividing the IC_{50} values in column c by column b produces a ratio proportional to the RRE selectivity of each compound (Table 1, column e). Upon guanidinylation of kanamycin A, kanamycin B, tobramycin, and paromomycin, this ratio decreases, suggesting that these compounds are more selective for the RRE than their amino precursors.[23] For neomycin, this ratio increases upon guanidinylation, indicating a lower RRE selectivity.[24] These results demonstrate that guanidinylation impacts the RNA specificity of glycoside-based ligands. Both the aminoglycosides and the guanidinoglycosides show very little affinity for double-stranded DNA (compare column b to d, Table 1). This suggests that the core structure of the glycosides, and not the identity of the basic groups, is responsible for the RNA over DNA selectivity exhibited by both families of glycosides.[25]

The transformation of aminoglycosides into guanidinoglycosides has created a new family of compounds for the study of RNA−small molecule interactions. Our results indicate that guanidinoglycosides bind RNA preferentially over DNA, and show selectivity between various RNAs. The higher affinity and selectivity of guanidinoglycosides to the RRE implicate their potential use as antiviral agents.

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Supporting Information Available: General synthetic procedures, analytical data for all new derivatives, as well as conditions and procedures for RNA binding assays (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) IC_{50} values are reported instead of K_i since the number of binding sites is unknown.

(19) The differences in RRE affinity between kanamycin B and tobramycin are lost upon guanidinylation. Previous studies suggest that the pK_a of the ammonium at position R_1 is lowered by the hydroxyl at position R_2 (Figure 1).[9b] Consequently, kanamycin B has a lower total charge and shows lower RNA affinity, relative to tobramycin. This postulate is supported by the nearly identical activities exhibited by guanidino-tobramycin and guanidino-kanamycin B, since guanidinium groups are significantly more basic and have pK_a values that are less variable, compared to aliphatic amines.

(20) Both guanidino-tobramycin (**3b**) and guanidino-paromomycin (**4b**), have five guanidinium groups, but guanidino-tobramycin shows a significantly higher inhibitory activity. This may indicate that the spatial arrangement of the five guanidinium groups on **3b** is better suited for binding the RRE than the arrangement presented by **4b**.

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(22) Experiments with competing nucleic acids contain 15 μg/mL plasmid DNA (pGEM) or 15 μg/mL of poly A•poly U duplex RNA (~50-fold molar excess of DNA or RNA nucleotides, relative to the RRE).

(23) Similar differences in RRE selectivity were seen when a complex mixture of mature and pre-tRNAs (at 64 μg/mL) is used instead of poly A•poly U. This suggests that the changes in RRE selectivity, observed upon guanidinylation, is a general phenomenon.

(24) The lower RRE selectivity of guanidinylated neomycin is likely to reflect a general trend exibited by highly cationic compounds; as the total charge increases, the specificity for a particular RNA typically decreases.

(25) Our results indicate that both families of glycosides have a higher affinity to simple duplex RNA (poly A•poly U) than to simple duplex DNA. This suggests that the difference between A-form and B-form helical structure is an important determinant for glycoside recognition.