Selective modification of the 3’-amino group of Kanamycin prevents significant loss of activity in resistant bacterial strains


[a] CSIC. Department of Bioorganic Chemistry, c/ Juan de la Cierva, 3. 28006-Madrid. Spain. Fax: (+) 34-915 644 853
Figure S1. (A) Schematic representation of some of the potential benefits promoted by \( N \)-guanidinylation in RNA- and protein-binding polycationic molecules. (B) Example of contribution of guanidine groups to biological activity of RNA ligands: antibiotic streptomycin de-guanidinylation produces a total loss of antibiotic activity. Streptomycin modifying resistance enzymes are highlighted in blue. (C) Example of effect of amine substitution by a guanidine: Increase of potency against a viral sialidase (Zanamivir®)

- Increased pKa
- Additional contacts
- Protection against resistance enzymes.

**Streptomycin**

Active

MIC (\( E.\) coli BL21(DE3)) = 5 µg/mL

De-guanidinylated Streptomycin

Inactive

MIC (\( E.\) coli BL21(DE3)) > 100 µg/mL

\( K_i (\mu M) \)

\( 4 \times 10^{-2} \)

\( 3 \times 10^{-3} \)

\( \text{Zanamivir®} \)
Figure S2. Effect of N-1 modification of 4,6-disubstituted-2-deoxystreptamine aminoglycosides.

\[
\begin{align*}
\text{MIC (µg/mL)}^{[a]} & \quad \text{Strain: } S. \text{ aureus} \\
R = \text{H} & \quad (\text{Dibekacin}) \quad 50 \\
R = \text{NH}_2 & \quad >100 \\
R = \text{NH} - \text{NH}_2 & \quad >100 \\
\end{align*}
\]

\[
\begin{align*}
\text{MIC (µg/mL)}^{[a]} & \\
R = \text{H} & \quad (\text{Arbekacin}) \quad 1-2 \\
R = \text{NH} & \quad 1-2 \\
\end{align*}
\]
Figure S3. (a) Selected signals of hetero-nuclear HSQC experiments in DMSO-d6 of kanamycin A 1 (top) and of compound 13 (bottom), corresponding to the position N-6. For both compounds similar chemical shifts of C-13 have been observed. However, a clear shift is observed in the corresponding proton signals of these positions, moving from 2.7 and 3.0 ppm for compound 1 (in red) to 3.41 and 3.67 for compound 13 (in blue). Structurally relevant carbon signals (position 6') are shown on the left and have been linked to these ¹³C HSQC signals through dashed lines. The corresponding ¹H-signals that correlate with these carbons have been marked with arrows. These data are in consonance with a regioselective guanidinylation of position N-6'. (b) Sectors of hetero-nuclear HSQC experiments in D₂O of kanamycin A 1 (top) and 3-azido-kanamycin A 14 (bottom). In comparison to the HSQC spectrum of kanamycin A 1, the corresponding J(C3-H) coupling for compound 14 shows a remarkable shift to lower field proving the regioselective introduction of the azide moiety at this position.
Figure S4. Schematic representation of the combinatorial approach developed by our group for the determination the rRNA preferences for punctual kanamycin amine modification. Interestingly, the N-3’’ position in the aminoglycoside constitutes the most common N-modificated position found in natural aminoglycosides.

![Diagram showing reductive amination leading to 243 possible derivatives.]

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Natural aminoglycosides

Figure S5. (a) Schematic representation of natural kanamycins A and B aminoglycosides. Interaction between position N-3’’ and G 1405 residue from the rRNA is shown. (b) Schematic representation of several compounds with structural modifications on N-3’’ and O-2’’ (deamination, substitution of the amino function by a hydroxyl group and displacement of the amino function to O-2’’ position respectively). The effect of these modifications in the interaction with G1405 residue and in biological activity is indicated.

G1405

Kanamycin A

Deamination in N-3’’

>100-fold decrease in biological activity

Substitution of the N-3’’ amino group by an hydroxyl

Kanamycin B

Substitution of 0-2’’ by N-3’’ and N-3’’ by 0-3’’ groups

35-fold decrease in biological activity

20-fold decrease in biological activity

Table S1.- Tm values of the enzymes ANT(4'), AAC(6') APH(3') in presence of 1 and with compound 7.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Control buffer</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tm (°C)</td>
<td>1</td>
</tr>
<tr>
<td>ANT(4')</td>
<td>56±0.1</td>
<td>60.5±0.1</td>
</tr>
<tr>
<td>APH(3')</td>
<td>49±0.1</td>
<td>62±0.12</td>
</tr>
<tr>
<td>AAC(6')</td>
<td>42±0.2</td>
<td>46±0.15</td>
</tr>
</tbody>
</table>

Figure S6. (a) X-ray structure of ANT(4') enzyme with two kanamycin A molecules. The close proximity between the two sugar units and specifically of the amine structural motifs at N-3''-position (blue) prevents an optimal fit of the guanidine-ligands to the receptor binding pocket. (b) Docking studies support the idea that the incorporation of N-3'' modifications disrupts the proper orientation of the aminoglycoside in the active site of the APH(3') enzyme, (c) the ANT(4') enzyme and (d) the AAC(6') enzyme.
$^1$H and $^{13}$C NMR spectrums of the compounds 3-7, 10, 11, 13, 14, 16 and 17.
Purity determination of compound 7 by NMR titration, using DMSO as internal standard.