

**6-O-Nucleotidyltransferase: an aminoglycoside-modifying enzyme
specific for streptomycin/streptidine**

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Figure S1. Left; SDS-PAGE for the expression and purification of the ANT(6). Molecular weight markers (kDa), CFE of *E. coli* BL21(DE3) cells harbouring the pET-*aadk* plasmid; and NHis₆-tag ANT(6) purified. Right; Line 1, 2 ANT(6) Δ His and line 3 ANT(6) purified

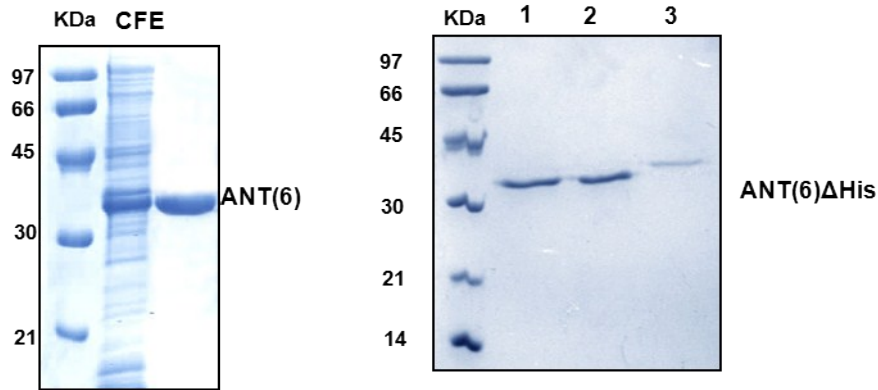


Figure S2.- Sedimentation equilibrium analysis to different concentration of MgCl₂ of the; a) ANT(6) with 0, 2.5 20mM MgCl₂ and b) ANT(6) Δ His with 0, 2.5mM MgCl₂ Mw; Apparent Molecular weight, Mw₀=Molecular weight of the monomer.

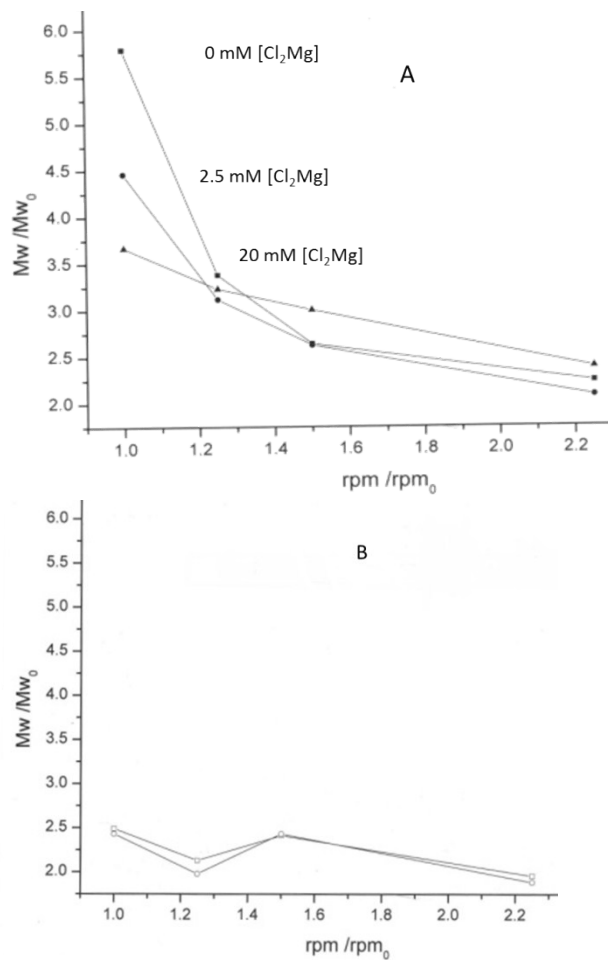


Figura S3. Effect of the pH on the activity of the ANT(6) enzyme.

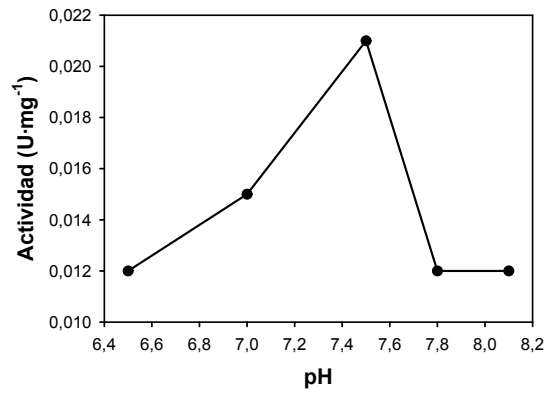
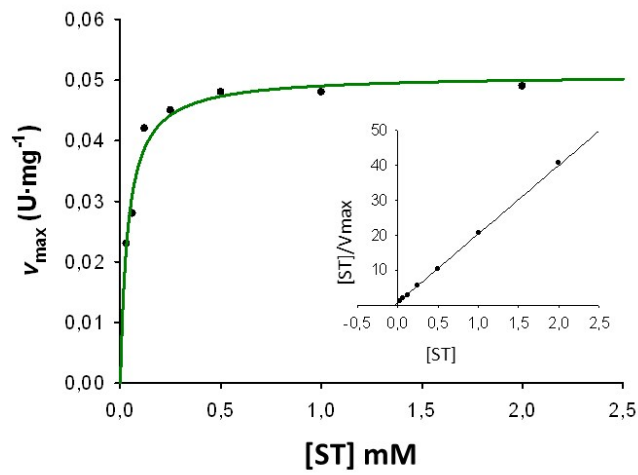


Figure S4.-Activity of the ANT(6) at different concentration of a) Streptomycin (ST) and b) adenosine-5'-triphosphate (ATP).

a)



b)

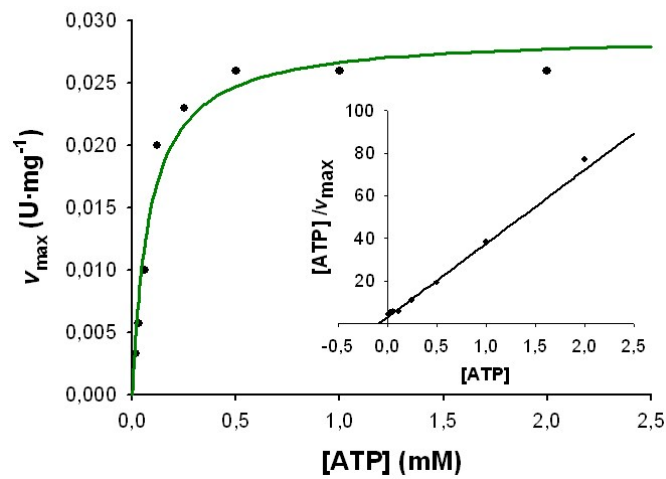
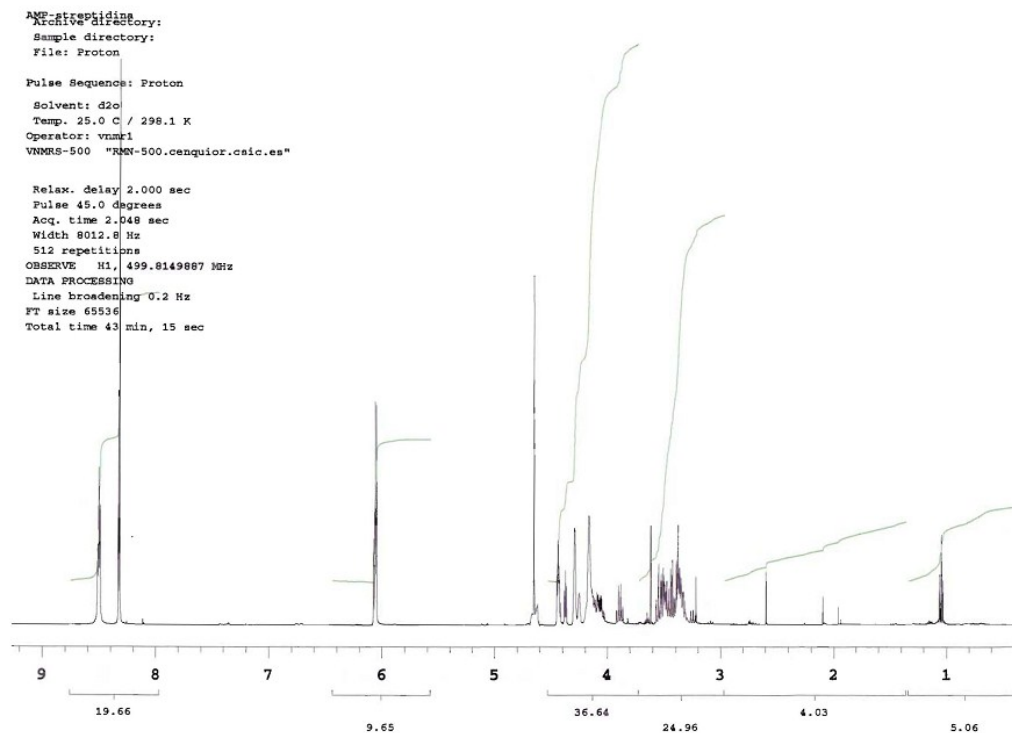


Figure S5.- Characterization of the AMP-Streptidine product.

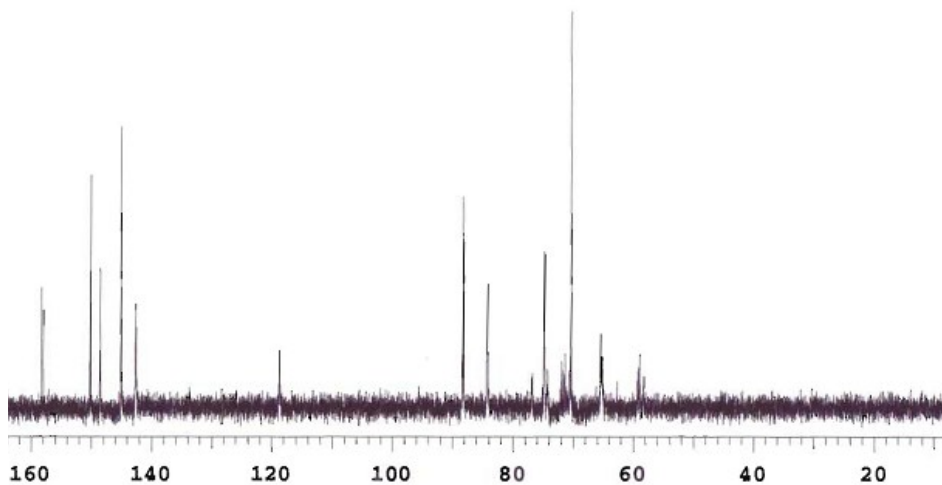
$^1\text{H-NMR}$ (D_2O at 500MHz, pH=3.0) δ = 1.06 (t, 1H, J =7.1Hz),3.45(m,5H),3.90(dd,1H,J =9.3,18.8Hz),4.09 (m, 1H), 4.18 (d, 1H, J = 1.6 Hz), 4.27 (m, 1H), 4.31 (s, 1H), 4.39 (t, 1H,J =4.56 Hz), 4.45 (t, 1H, J = 5.4 Hz), 6.07 (d, 1H,J = 5.2 Hz), 8.34 (d, 1H,J = 4.5 Hz), 8.52 (t, 1H, J =5.7Hz);

$^{13}\text{C-NMR}$ (D_2O at 125Hz) δ = 15.3, 58.2, 59.0, 65.5, 70.2, 70.6, 71.6, 74.3, 77.1, 84.3, 88.2, 145.3,158.0, 158.3.

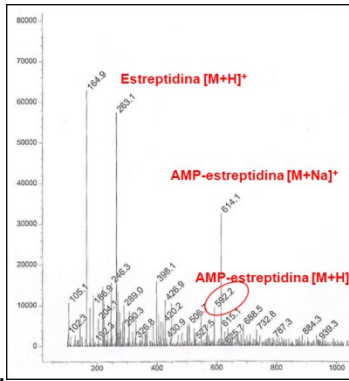
$^1\text{H-NMR}$ (D_2O at 500MHz, pH=3.0)



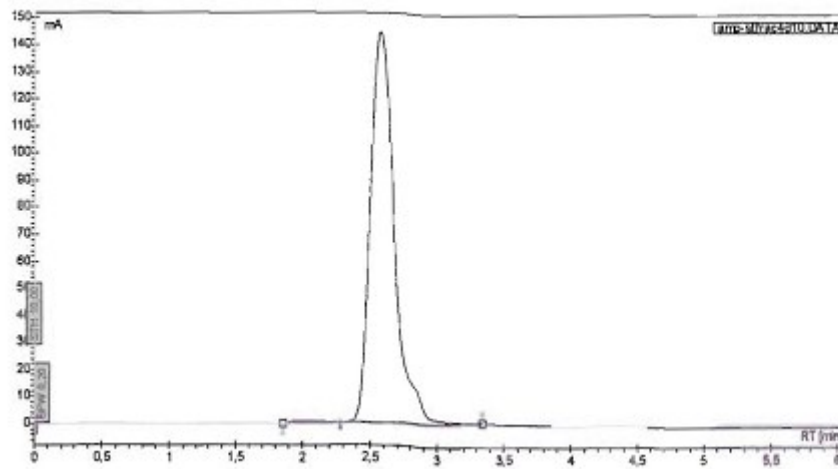
$^{13}\text{C-NMR}$ (D_2O at 125Hz)



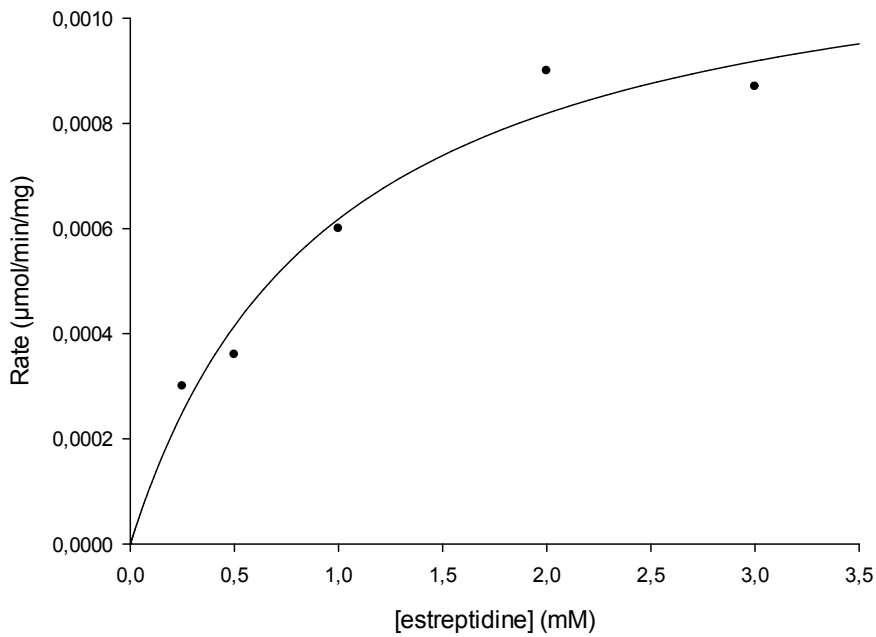
MS (ES+) m/z (%): 592.2 [M + H]⁺



HPLC chromatogram of the AMP-streptidine.



Michaelis-Menten



Activity of the ANT(6) at different concentration of Streptidine

Table S1. Activity *in vitro* of the ANT(6) with different aminoglycosides.

Aminoglycoside	Activity (U·mg ⁻¹)
Streptomycin	0.06
Streptidine	6 · 10 ⁻⁴
Neomicine B	0.3 · 10 ⁻⁴
Neamine	0.2 · 10 ⁻⁴
Spectinomycin	0
Kanamycin	0
Paromomycin	0
Ribostamycin	0

Table S2. Activity *in vitro* of the ANT(6) with different nucleotides.

Nucleotides^[a]	Activity (U·mg ⁻¹)
ATP	0.018
GTP	0.010
UTP	3 · 10 ⁻⁴
CTP	36 · 10 ⁻⁴
AMPCPP	0
GDP	2 · 10 ⁻⁴
ADP	4 · 10 ⁻⁴
AMP	0

^[a]ATP; adenosine-5'-triphosphate, GTP: guanosine-5'-triphosphate, CTP; cytidine-5'-triphosphate UTP; uridine-5'-triphosphate, ADP; adenosine-5'-diphosphate and AMP; adenosine-5'-monophosphate.