

## Supporting Information

### **Assessment of 6'- and 6'''-N-acylation of aminoglycosides as a strategy to overcome bacterial resistance**

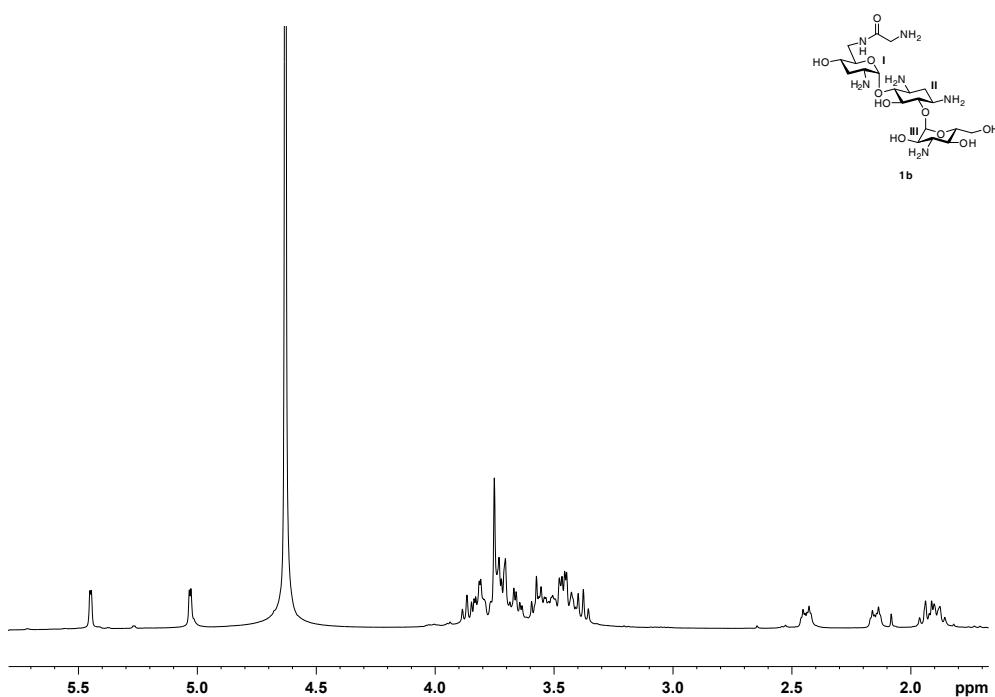
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<sup>a</sup> School of Chemistry; Raymond and Beverly Sackler, Faculty of Exact Sciences, Tel Aviv University, Tel Aviv 69978, Israel

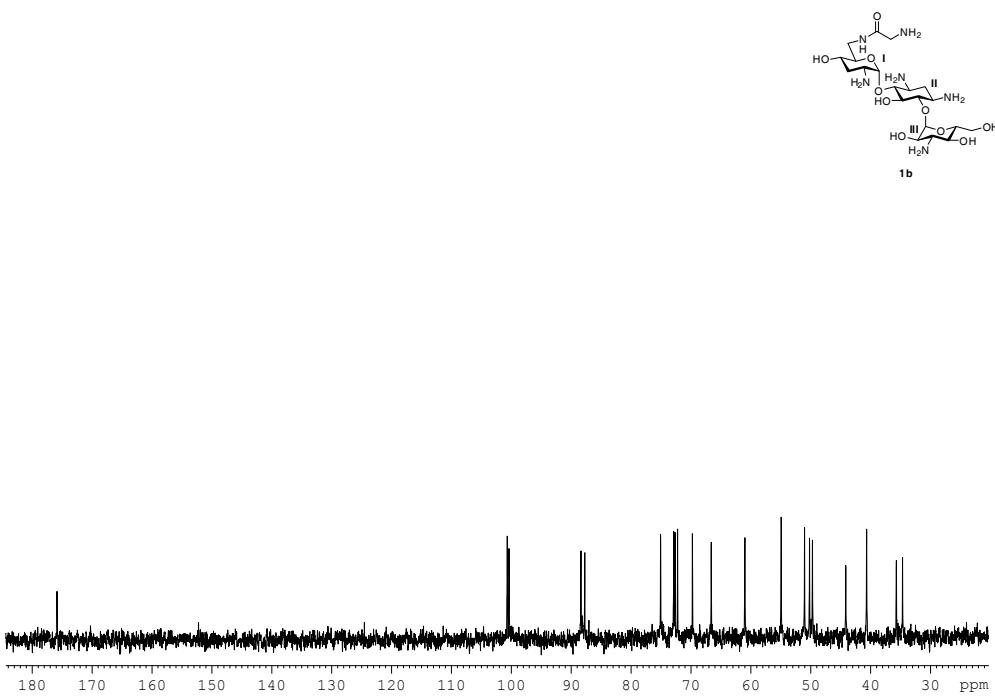
<sup>b</sup> Department of Medicinal Chemistry in the College of Pharmacy and Life Sciences Institute (210 Washtenaw Ave), University of Michigan, Ann Arbor, MI 48109-2216, U.S.A.

<sup>†</sup> Denotes equal contribution

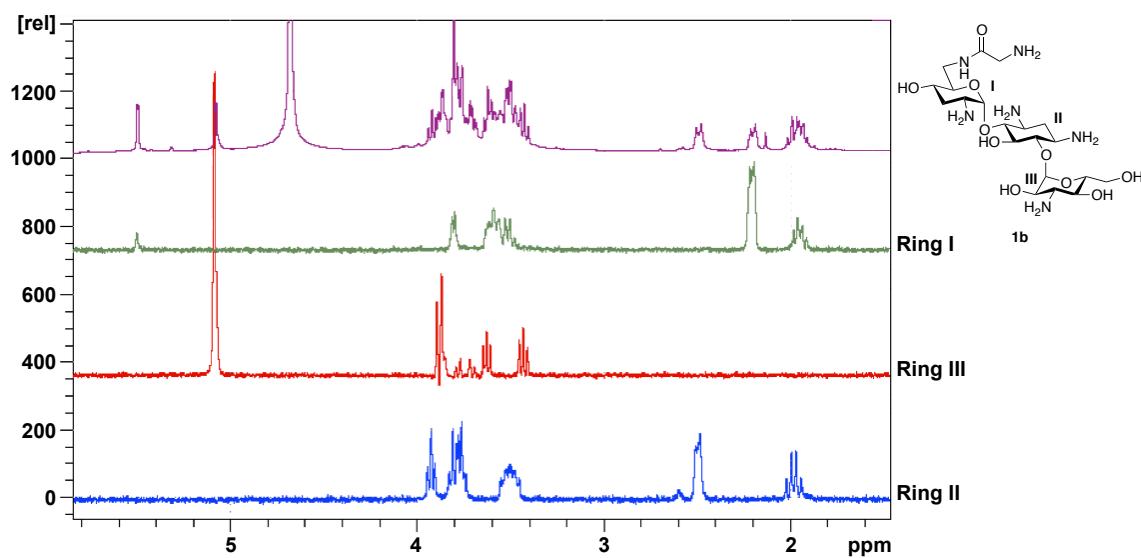
\*E-mail: sylviegt@umich.edu and mfridman@post.tau.ac.il



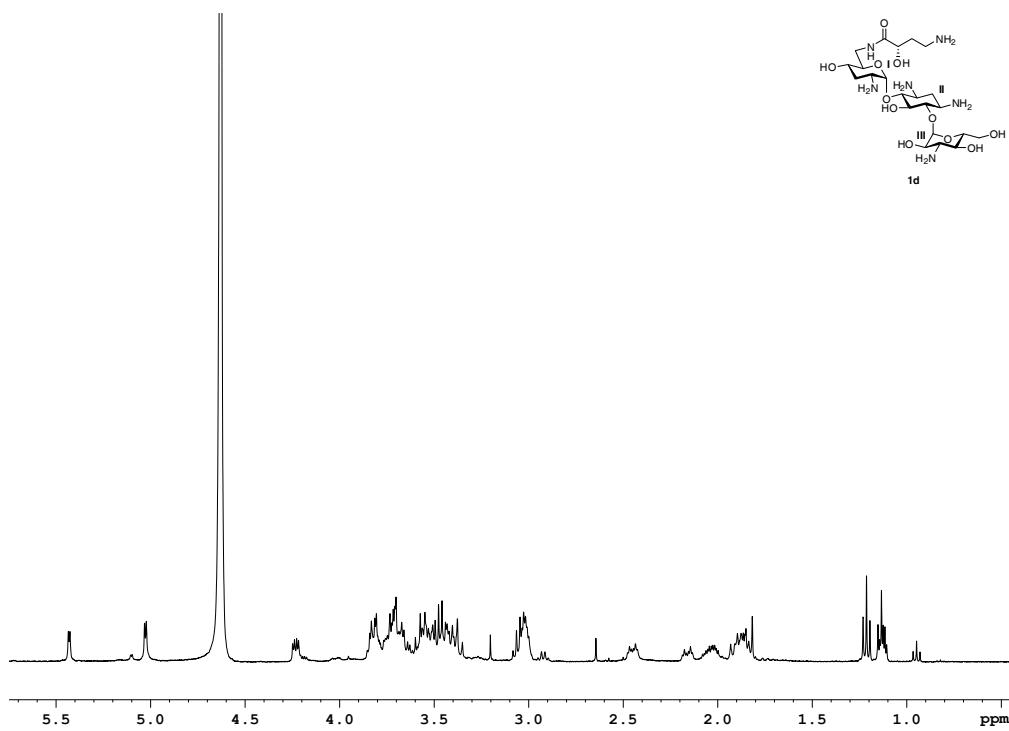
**Fig. S1.** <sup>1</sup>H NMR for the pseudo-trisaccharide **1b**.



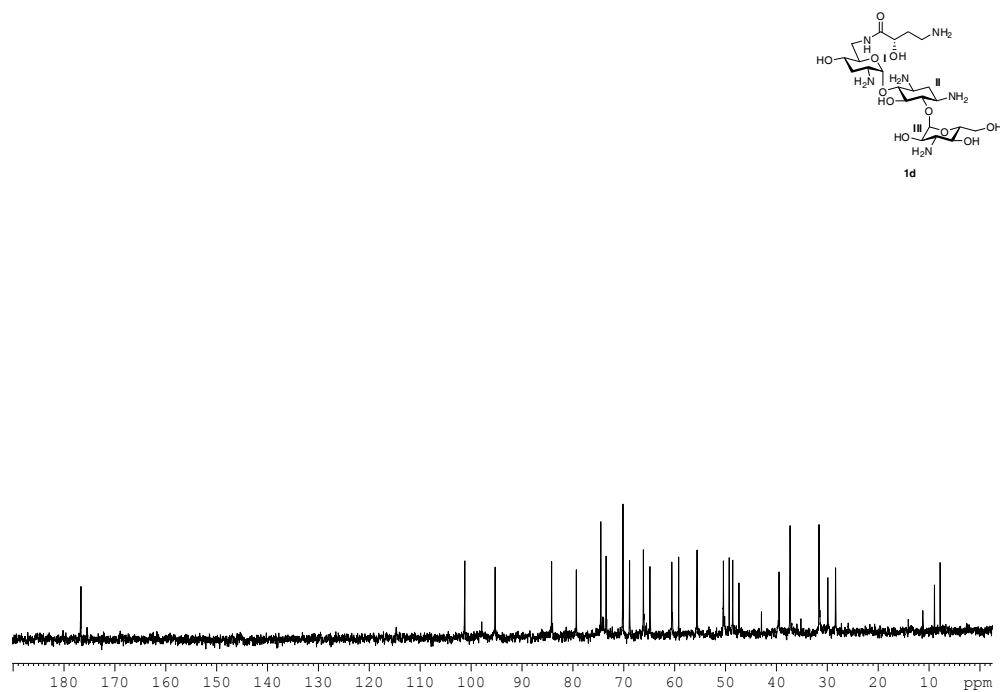
**Fig. S2.** <sup>13</sup>C NMR for the pseudo-trisaccharide **1b**.



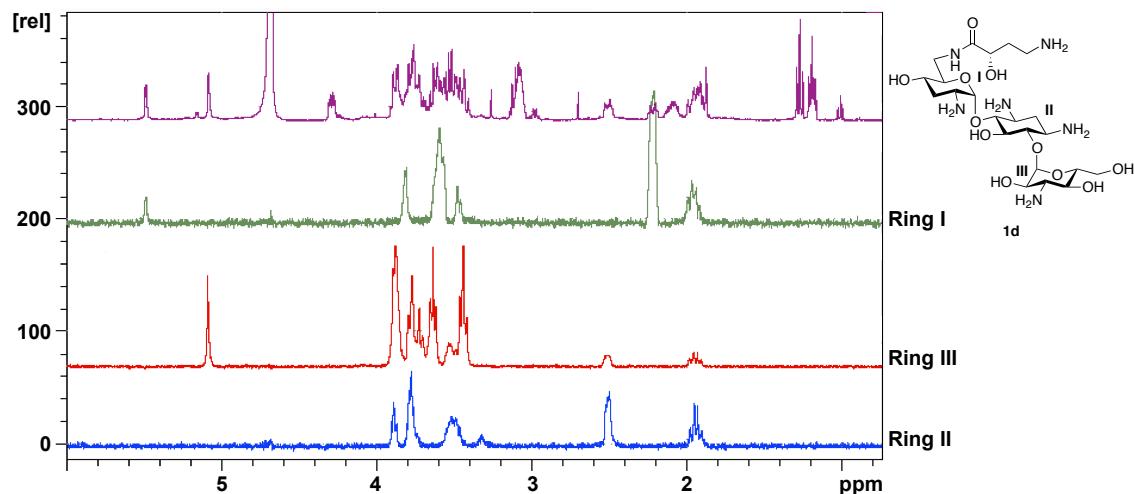
*Fig. S3.* 1D-TOCSY for the pseudo-trisaccharide **1b**.



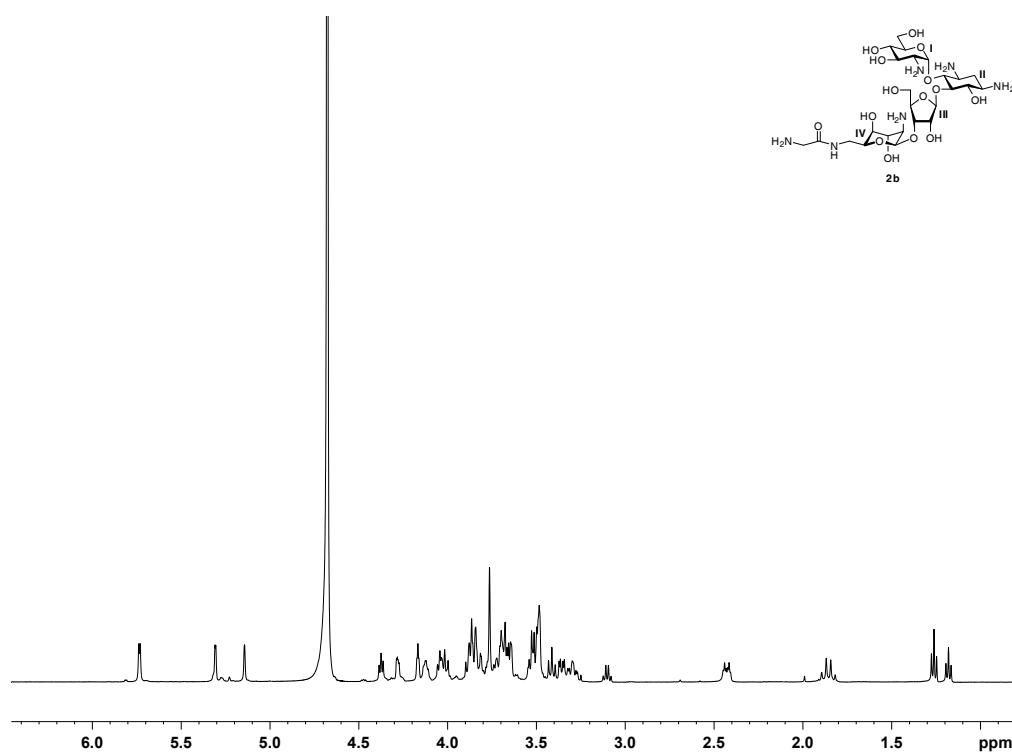
**Fig. S4.** <sup>1</sup>H NMR for the pseudo-trisaccharide **1d**.



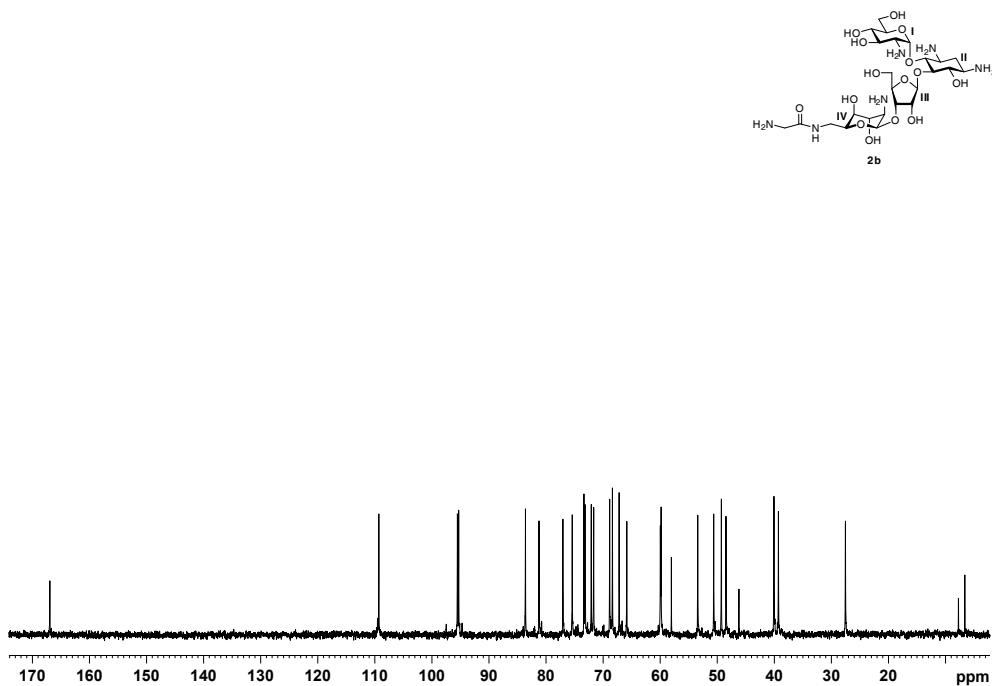
**Fig. S5.** <sup>13</sup>C NMR for the pseudo-trisaccharide **1d**.



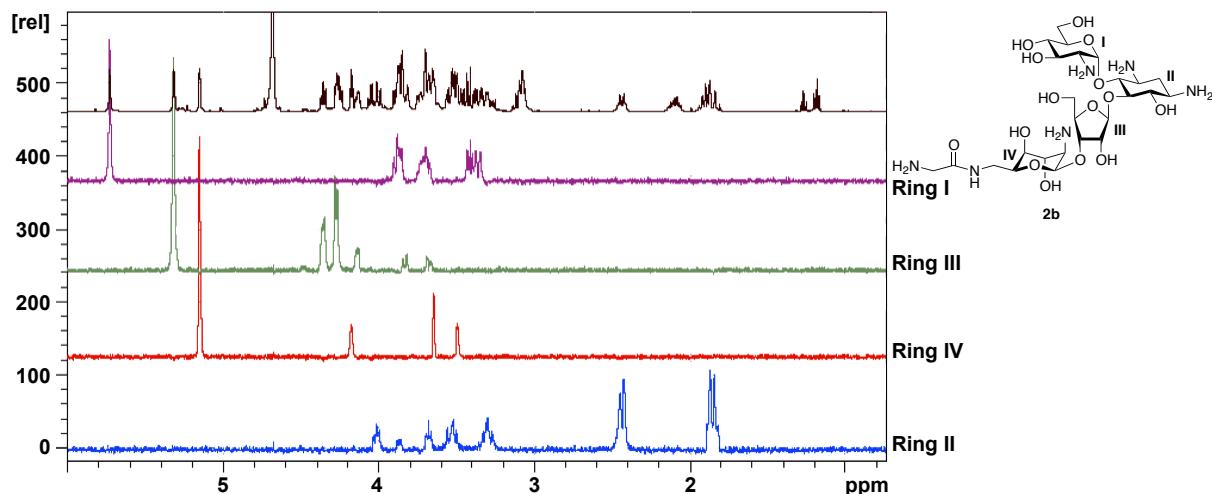
**Fig. S6.** 1D-TOCSY for the pseudo-trisaccharide **1d**.



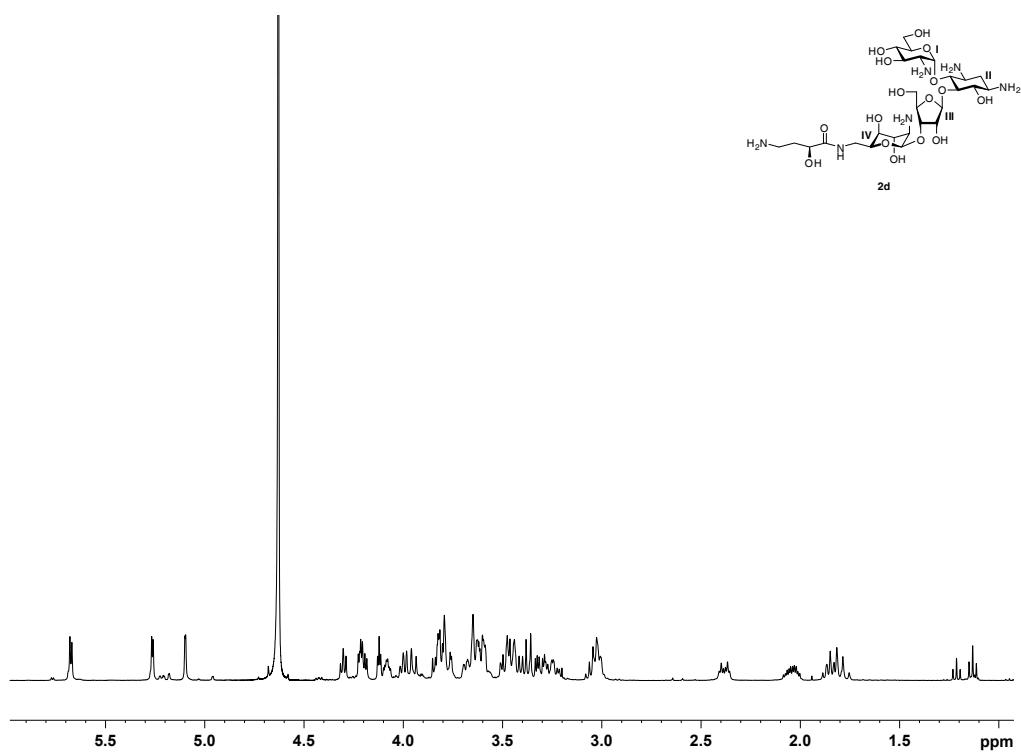
**Fig. S7.** <sup>1</sup>H NMR for the pseudo-tetrasaccharide **2b**.



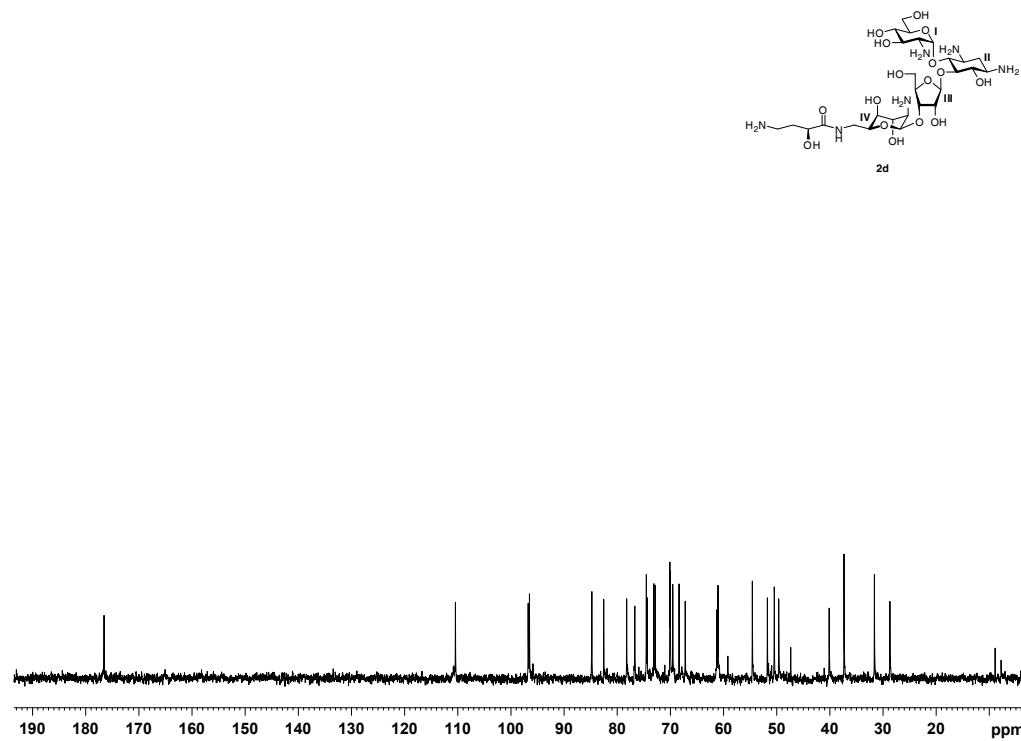
**Fig. S8.** <sup>13</sup>C NMR for the pseudo-tetrasaccharide **2b**.



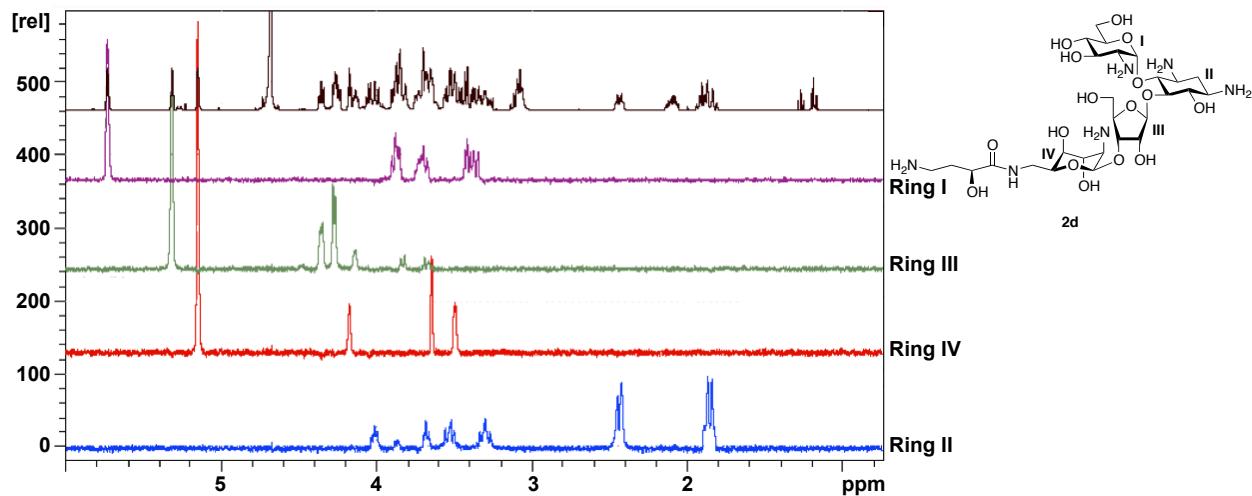
**Fig. S9.** 1D-TOCSY for the pseudo-trisaccharide **2b**.



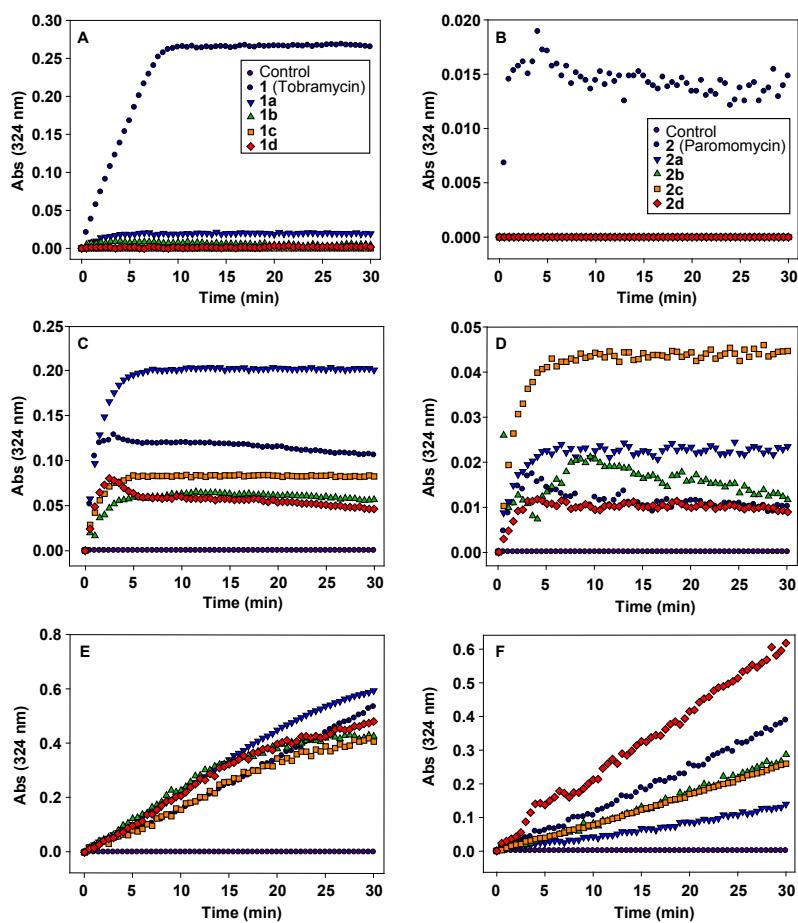
**Fig. S10.** <sup>1</sup>H NMR for the pseudo-tetrosaccharide 2d.



**Fig. S11.** <sup>13</sup>C NMR for the pseudo-tetrosaccharide 2d.



**Fig. S12.** 1D-TOCSY for the pseudo-trisaccharide **2d**.



**Fig. S13.** Spectrophotometric assay plots monitoring the acetylation reaction of aminoglycosides with acetyl-CoA using AAC(6') (**A,B**) and AAC(3)-IV (**C,D**). Spectrophotometric assay plots monitoring the phosphorylation reaction of aminoglycosides with GTP using APH(2") (**E,F**). Plots **A**, **C**, and **E** show reactions of the parent drug tobramycin (**1**) and its derivatives **1a-1d** with legend in panel **A**. Plots **B**, **E**, and **F** show reactions of the parent drug paromomycin (**2**) and its derivatives **2a-2d** with legend in panel **B**. The controls and parent compounds are represented by purple and blue circles, respectively. The different modifications are represented as follow: **a** (azidoacetyl) = blue inverted triangle, **b** (glyciny) = green triangle, **c** (CbzAHB) = orange square, and **d** (AHB) = red diamond.