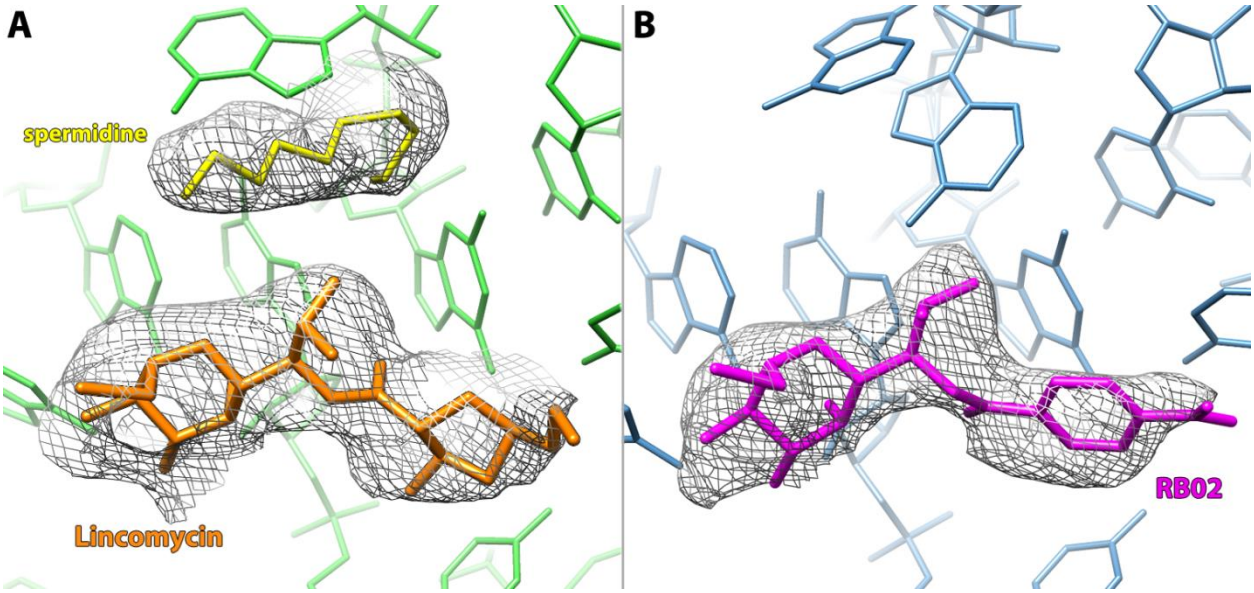

Figure S1. *2Fo-Fc* electron density maps contoured ~at 1.0 σ around the lincomycin and RB02 within their binding pockets in the SA50S-linc and SA50S-RB02 complexes respectively. A. lincomycin is colored in orange within its binding pocket (colored in green). Spermidine which is also bound at lincomycin's binding pocket and interacts with it is colored in yellow. B. RB02 is colored in magenta within its binding pocket (blue).

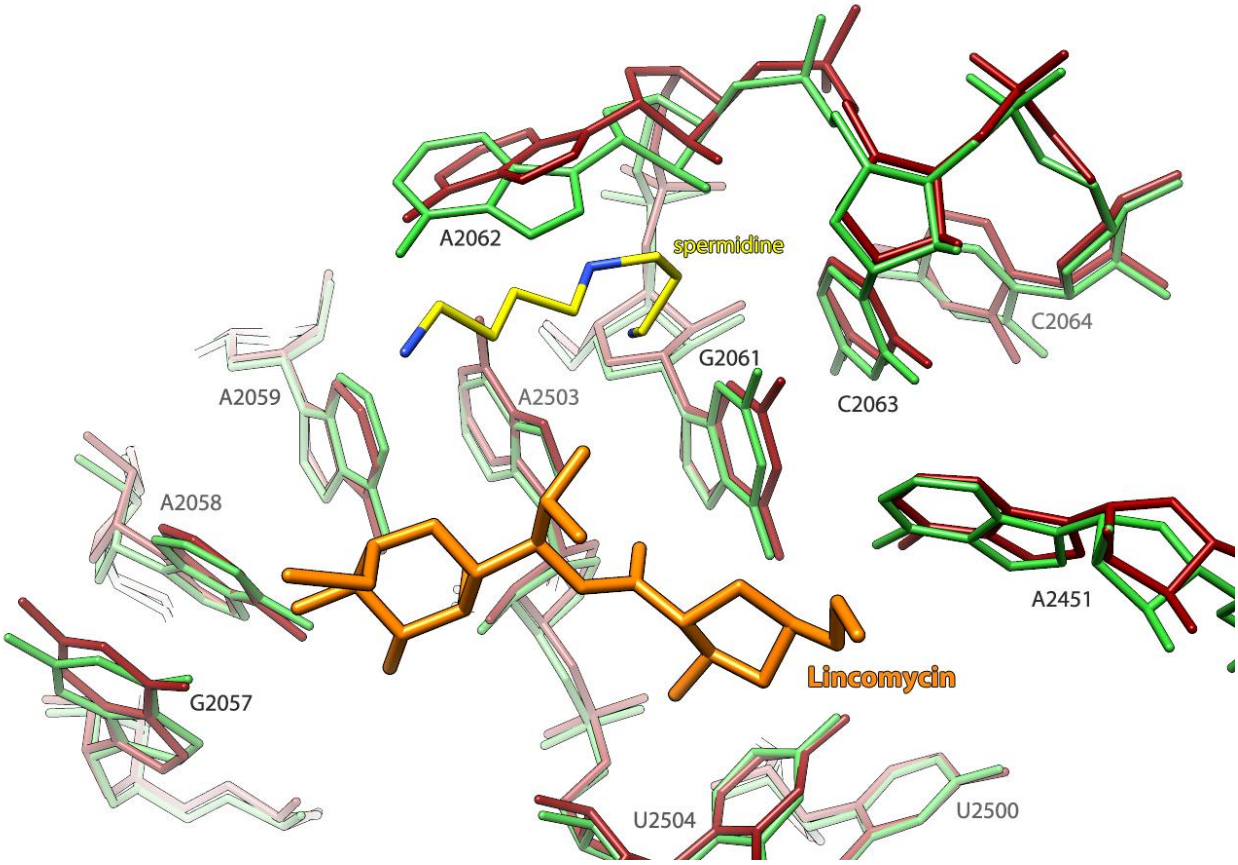

Figure S2. Comparison of the SA50S apo PTC (red) and the SA50S-linc complex. The rRNA is colored in green, lincomycin in orange and spermidine in yellow.

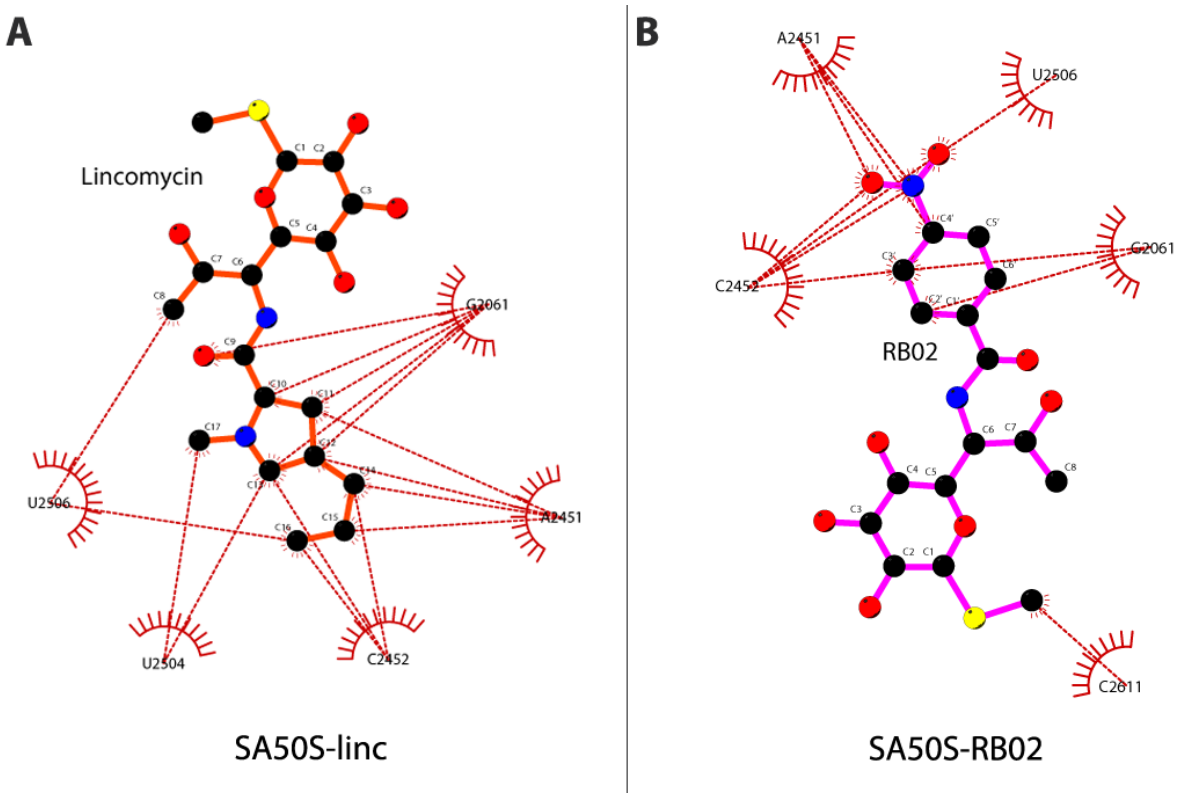

Figure S3. **Van der Waals contacts between lincosamides and the 23S rRNA.** The van der Waals contacts of **(A)** Lincomycin, colored in orange and **(B)** RB02, colored in magenta with their surrounding 23S rRNA (colored in red) calculated by ligplot (1). The length of all the contacts presented here is 2.9-3.9Å.

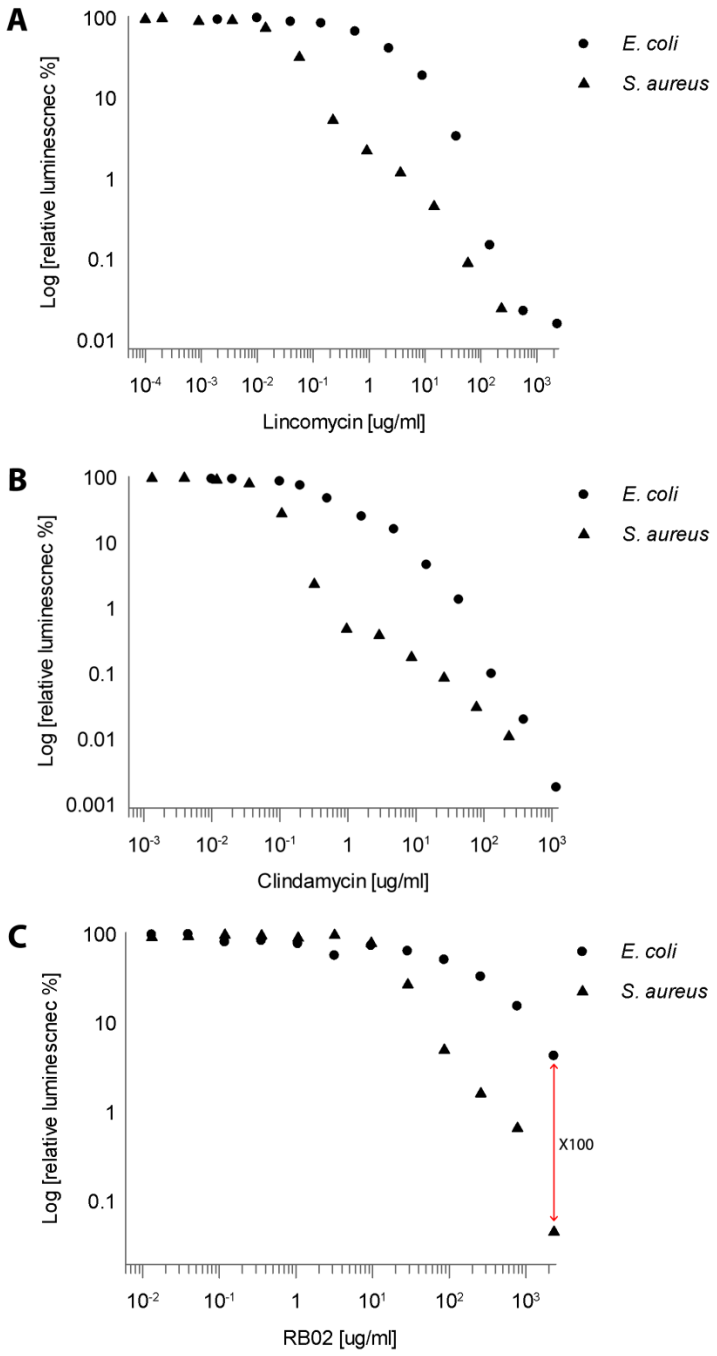

Figure S4. **Protein synthesis inhibition by lincosmides.** Inhibition results of increasing concentrations of (A) lincomycin, (B) clindamycin and (C) RB02 measured by the luminescence resulting from the translation of firefly luciferase on both *S. aureus* and *E. coli in vitro* translation systems. The luminescence is normalized relative to that measured in the absence of any inhibitor, which was assigned as 100%. To emphasize the difference of signal intensities in the SA and EC inhibitions assays, the same data shown in Fig 5 was plotted in a logarithmic scale. The red arrow (C) demonstrate the significant difference in signal intensities at the highest concentrations of RB02

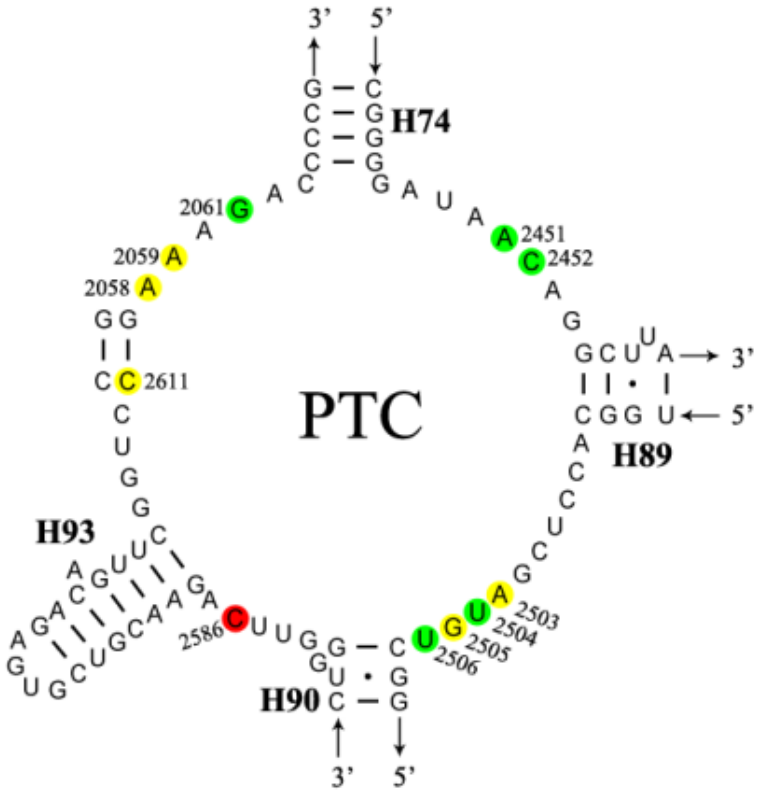

Figure S5. **2D structure diagram of the peptidyl transferase domain of *S .aureus* 23S rRNA.** Nucleotides that form hydrogen bonds with lincosamides are marked in yellow and those who form hydrophobic or van der Waals based interactions are marked in green (*E. coli* numbering). Marked in red is the only nucleotide within the lincosamide binding pocket that differs between *S. aureus* and *E. coli* (in *S. aureus* this nucleotide is cytosine whereas in *E. coli* its uracil. RNA helices are numbered with the standard Brimacombe nomenclature, prefixed by H for 23S rRNA.

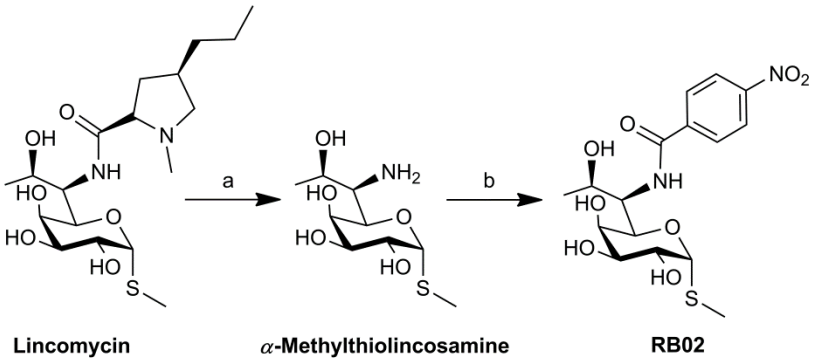

Figure S6. **Synthesis of RB02.** Reagents and conditions: a. Hydrazine hydrate, reflux (yield: 83%); b. p-Nitrobenzoic acid, HBTU, DIPEA, DMF (yield: 60%).

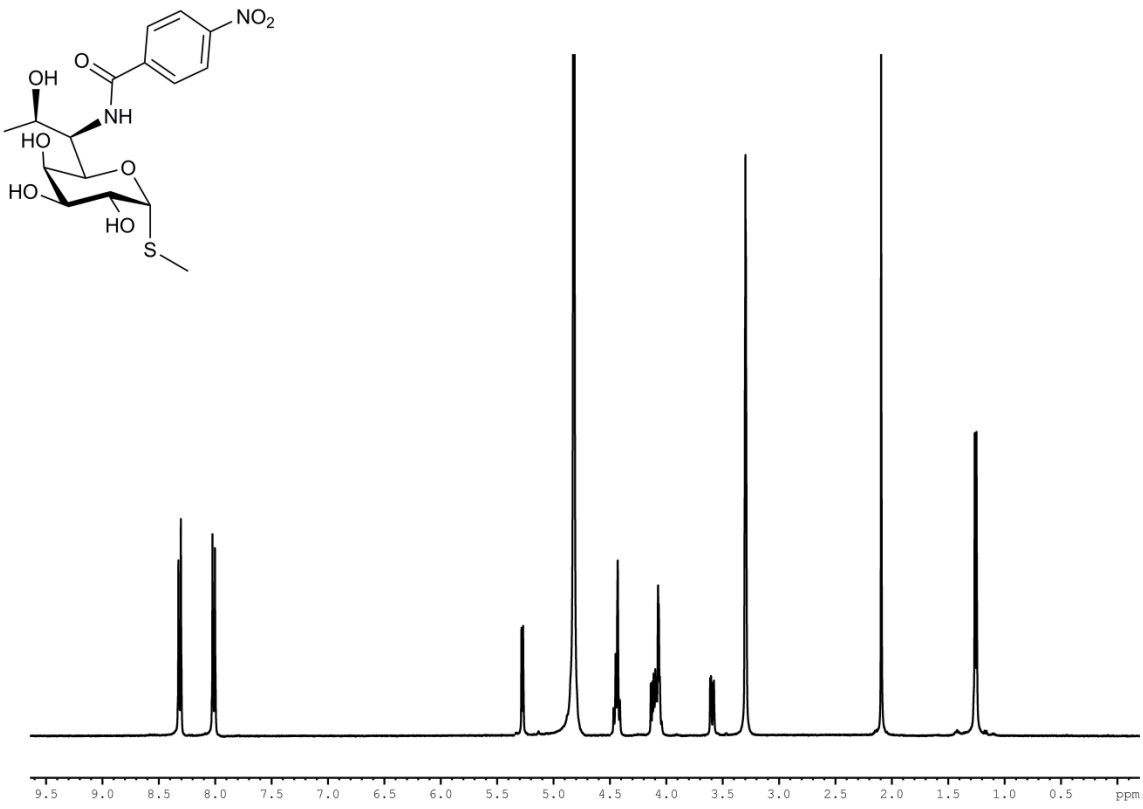

Figure S7. 400 MHz ^{1}H-NMR spectrum of **RB02** in CD_3OD at 298K

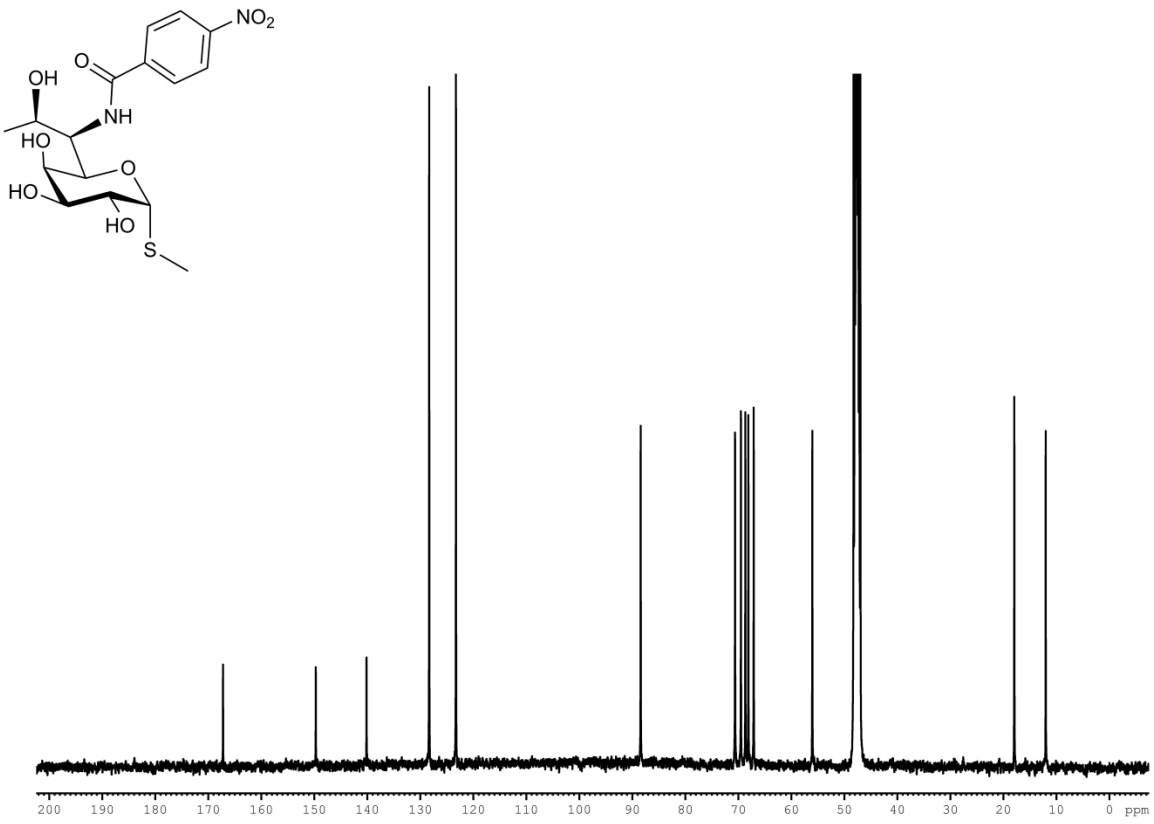

Figure S8. 100 MHz ^{13}C-NMR spectrum of **RB02** in CD_3OD at 298K

References:

1. Wallace, A.C., Laskowski, R.A. and Thornton, J.M. (1995) LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Engineering, Design and Selection*, **8**, 127-134.