Supplemental Material

# Substrate analogs that trap the 2'-phospho-ADP-ribosylated RNA intermediate of the Tpt1 (tRNA 2'-phosphotransferase) reaction pathway

Swathi Dantuluri, Leonora Abdullahu, Annum Munir, Adam Katolik, Masad J. Damha,
and Stewart Shuman

Supplemental Figures S1, S2, S3, S4

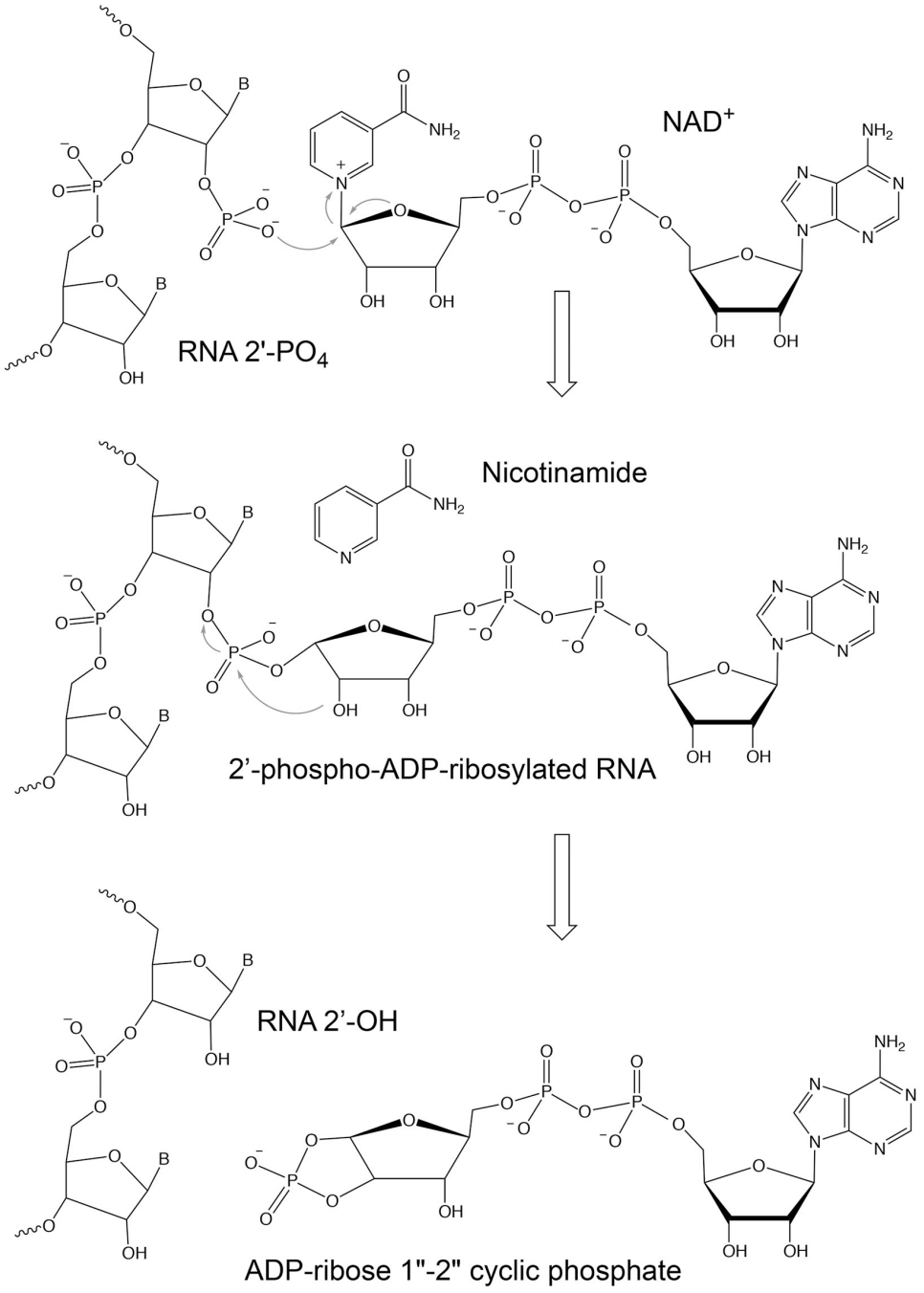


Figure S1. **Two-step Tpt1-catalyzed mechanism of 2'-$PO_4$ removal from a 2'-$PO_4$, 3'-5' phosphodiester RNA junction**. The Tpt1 reaction pathway comprises the two chemical steps shown in which: (1) the RNA 2'-$PO_4$ reacts with $NAD^+$ to expel nicotinamide and form a 2'-phospho-ADP-ribosylated RNA intermediate; and (2) transesterification of the ADP-ribose 2''-OH to the RNA 2'-$PO_4$ displaces the RNA 2'-OH and generates ADP-ribose-1'',2''-cyclic phosphate.

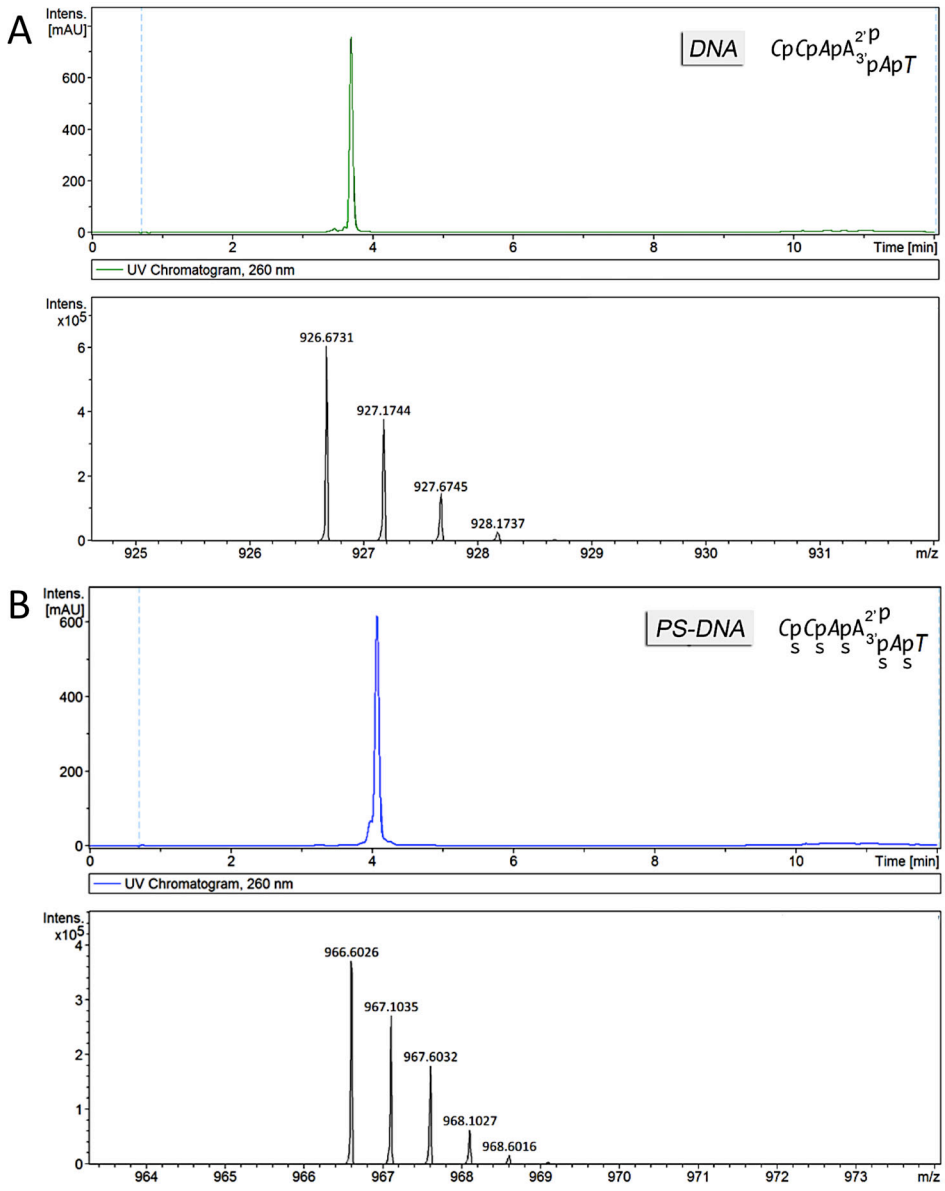


Figure S2. **LC-MS (-ve mode) chromatograms of 2'-$PO_4$ DNA and PS-DNA oligonucleotides**.

(A) DNA – mass calculated: 1854.31; mass observed: 1853.35.

(B) PS-DNA – mass calculated: 1934.32, mass observed: 1933.21.

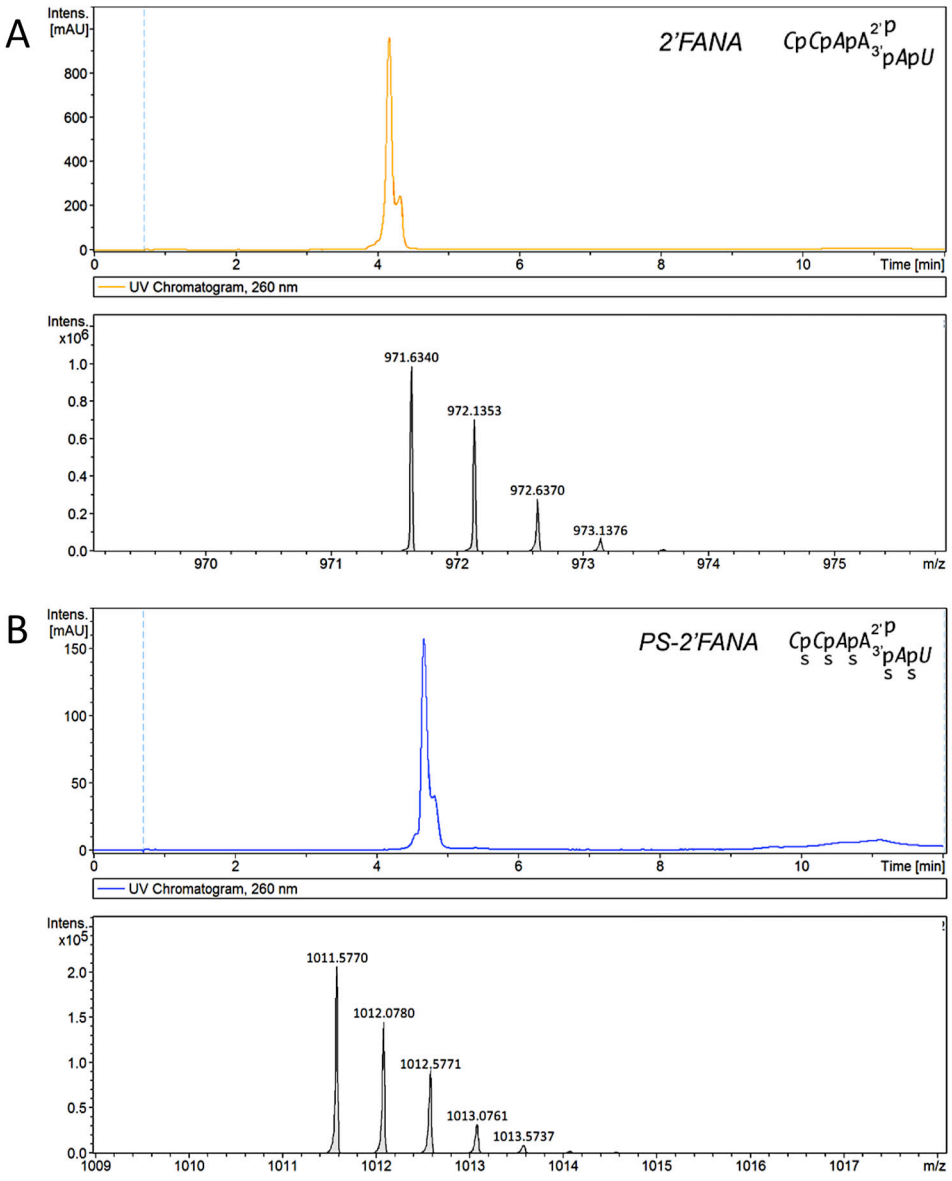


Figure S3. **LC-MS (-ve mode) chromatograms of 2'-FANA and PS-2'-FANA oligonucleotides**.

(A) 2′-FANA – mass calculated: 1942.26, mass observed: 1943.27.

(B) PS-2′-FANA – mass calculated: 2021.25, mass observed: 2023.15.

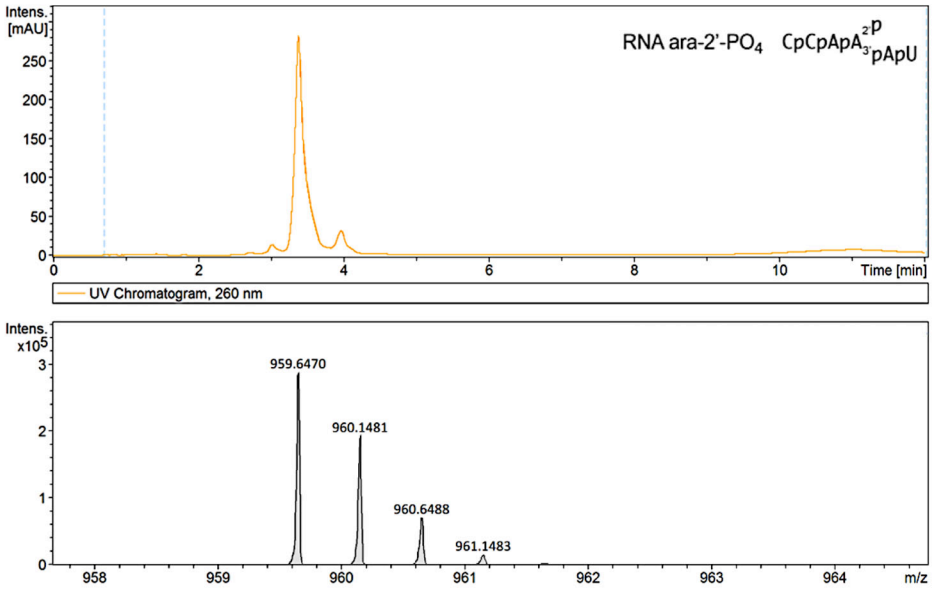


Figure S4. **LC-MS (-ve mode) chromatogram of ara-2'-$PO_4$ RNA oligonucleotide.**

Mass calculated: 1919.28, mass observed: 1919.29.