Supplementary Material

A Library of Nucleotide Analogues Terminate RNA Synthesis Catalyzed by Polymerases of Coronaviruses that Cause SARS and COVID-19

Steffen Jockusch[1,2,#], Chuanjuan Tao[1,3,#], Xiaoxu Li[1,3,#], Thomas K. Anderson[5,6], Minchen Chien[1,3], Shiv Kumar[1,3], James J. Russo[1,3], Robert N. Kirchdoerfer[5,6,*], Jingyue Ju[1,3,4,*]

[1]Center for Genome Technology and Biomolecular Engineering, Departments of [2]Chemistry, [3]Chemical Engineering and [4]Pharmacology, Columbia University, New York, NY 10027; [5]Department of Biochemistry and [6]Institute of Molecular Virology, University of Wisconsin-Madison, Madison, WI 53706

[#]SJ, CT and XL contributed equally to this work.

*To whom correspondence should be addressed: dj222@columbia.edu (JJ); rnkirchdoerf@wisc.edu (RNK)

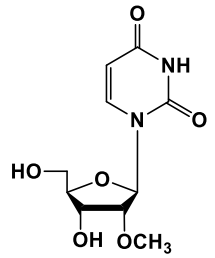
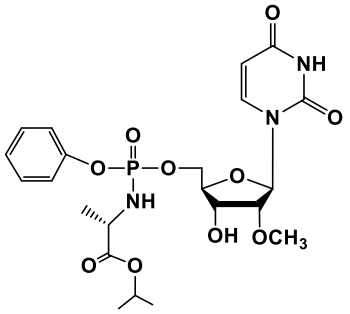
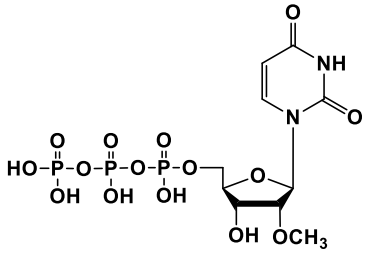
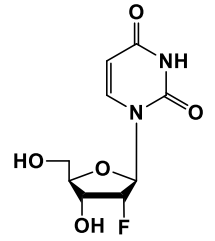
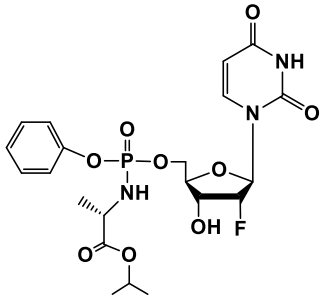
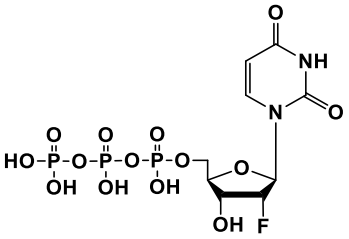
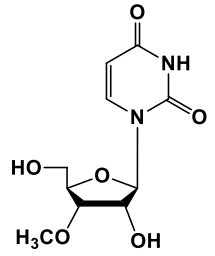
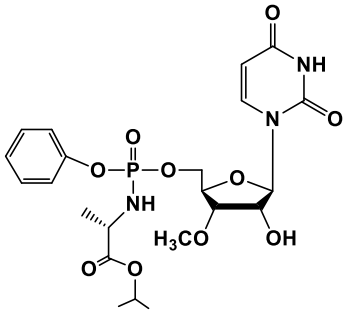
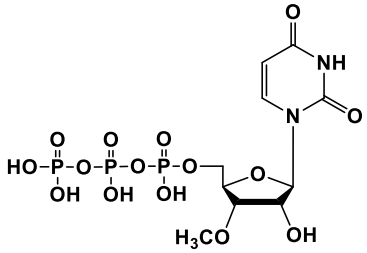
Nucleoside	Example Prodrug	Active Triphosphate
 2’-OMe-Uridine	 Phosphoramidate-2’-OMe-Uridine	 2’-OMe-Uridine-5’-Triphosphate (2’-OMe-UTP)
 2’-F-2’-Deoxyuridine	 Phosphoramidate-2’-F-2’-dU	 2’-F-2’-Deoxyuridine-5’-Triphosphate (2’-F-dUTP)
 3’-OMe-Uridine	 Phosphoramidate-3’-OMe-Uridine	 3’-OMe-Uridine-5’-Triphosphate (3’-OMe-UTP)

Fig. S1. Structures of nucleoside analogues, example prodrugs and active triphosphate forms. The nucleosides 2’-OMe-Uridine, 2’-F-2’-Deoxyuridine and 3’-OMe-Uridine (left), example prodrug forms (middle) and their active triphosphate forms (right).

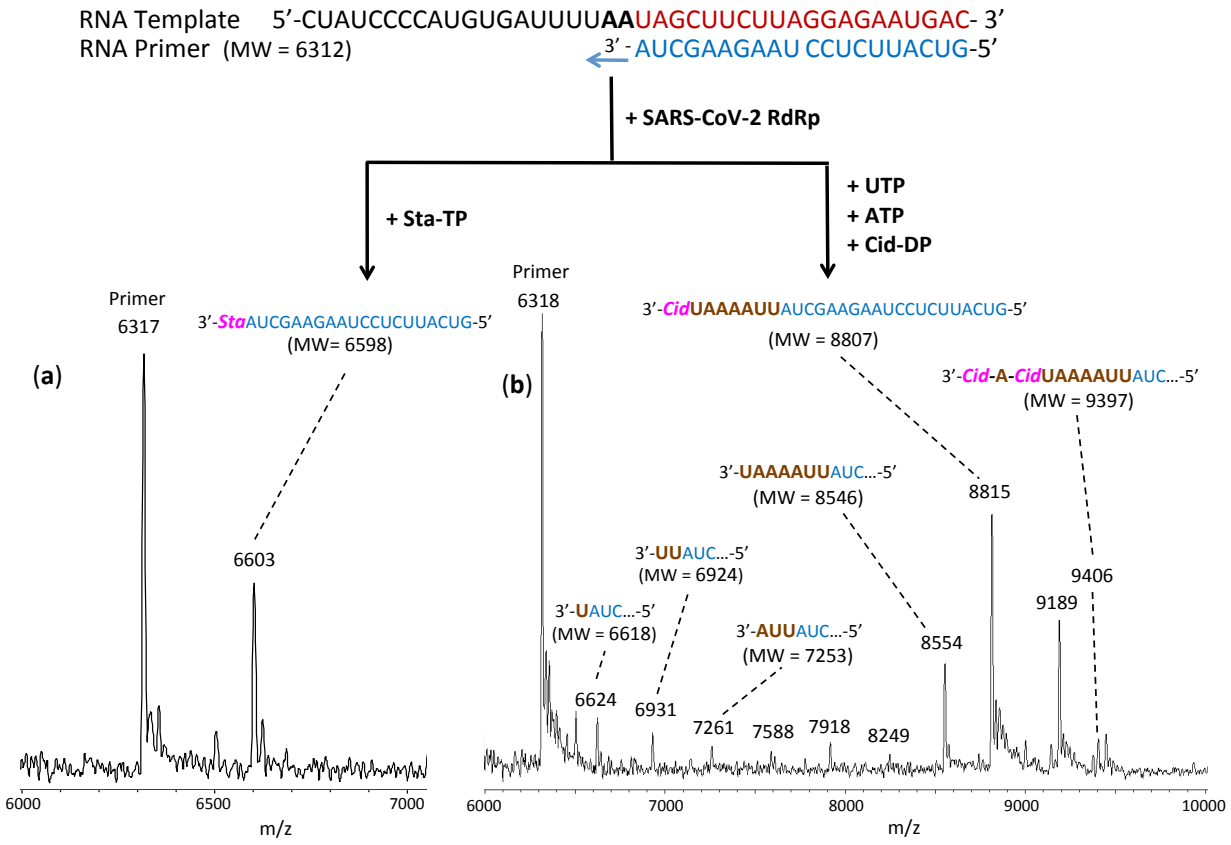


Fig. S2. Incorporation of Sta-TP and Cid-DP by SARS-CoV-2 RdRp to terminate the polymerase reaction. The sequences of the primer and template used for this extension reaction are shown at the top of the figure. Polymerase extension reactions were performed by incubating Sta-TP (50 μM) (a) and Cid-DP (50 μM), UTP and ATP (b) with pre-assembled SARS-CoV-2 polymerase (nsp12, nsp7 and nsp8), the indicated RNA template and primer and the appropriate reaction buffer, followed by detection of reaction products by MALDI-TOF MS. The progression of each nucleotide incorporation event was observable in (b). The peak at 9189 probably corresponds to the disodium adduct of the RNA extension product Primer-UUAAAAU*Cid*A (9184 Da expected). The detailed procedure is shown in the Materials and Methods section. The accuracy for m/z determination is ± 10 Da.

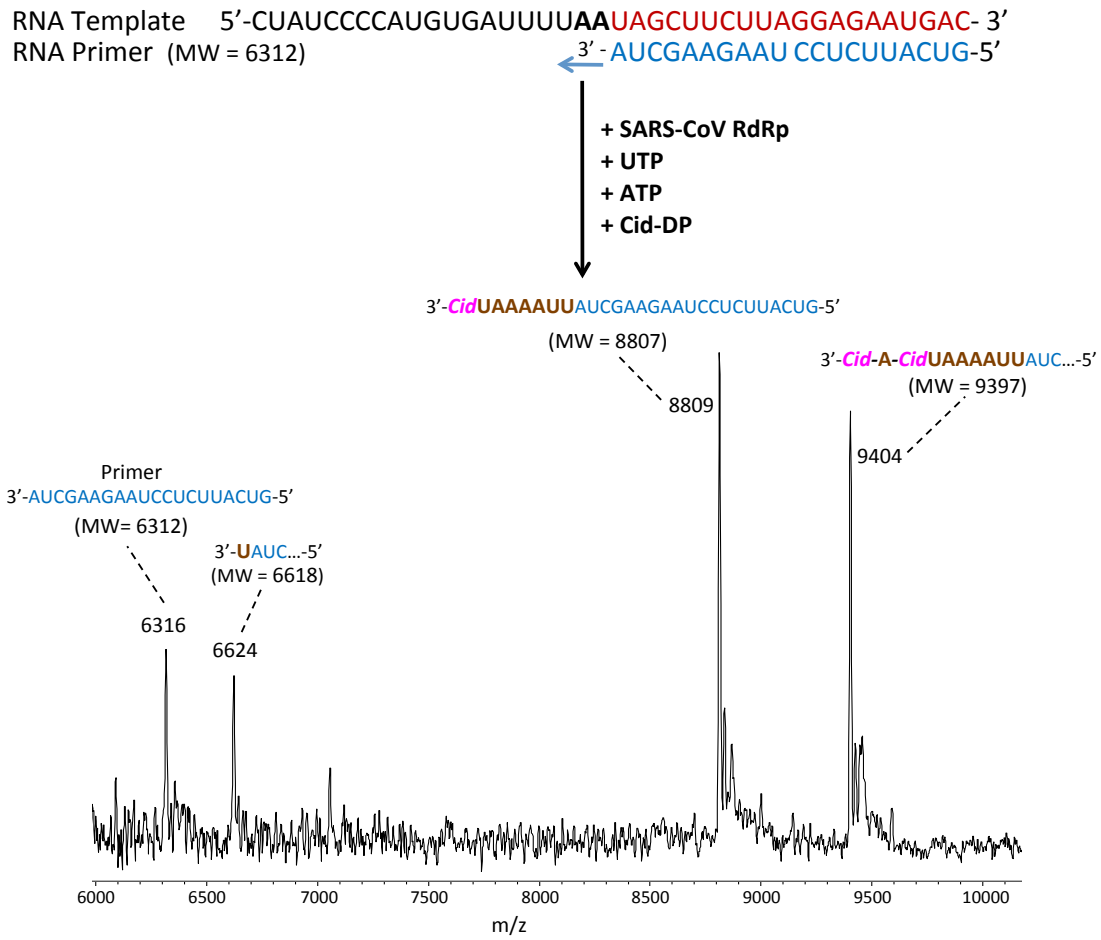


Fig. S3. Incorporation of Cid-DP by SARS-CoV RdRp to achieve delayed termination of the polymerase reaction. The sequences of the primer and template used for this extension reaction are shown at the top of the figure. The polymerase extension reaction was performed by incubating Cid-DP, UTP and ATP with pre-assembled SARS-CoV polymerase (nsp12, nsp7 and nsp8), the indicated RNA template and primer and the appropriate reaction buffer, followed by detection of reaction products by MALDI-TOF MS. The accuracy for m/z determination is ± 10 Da.

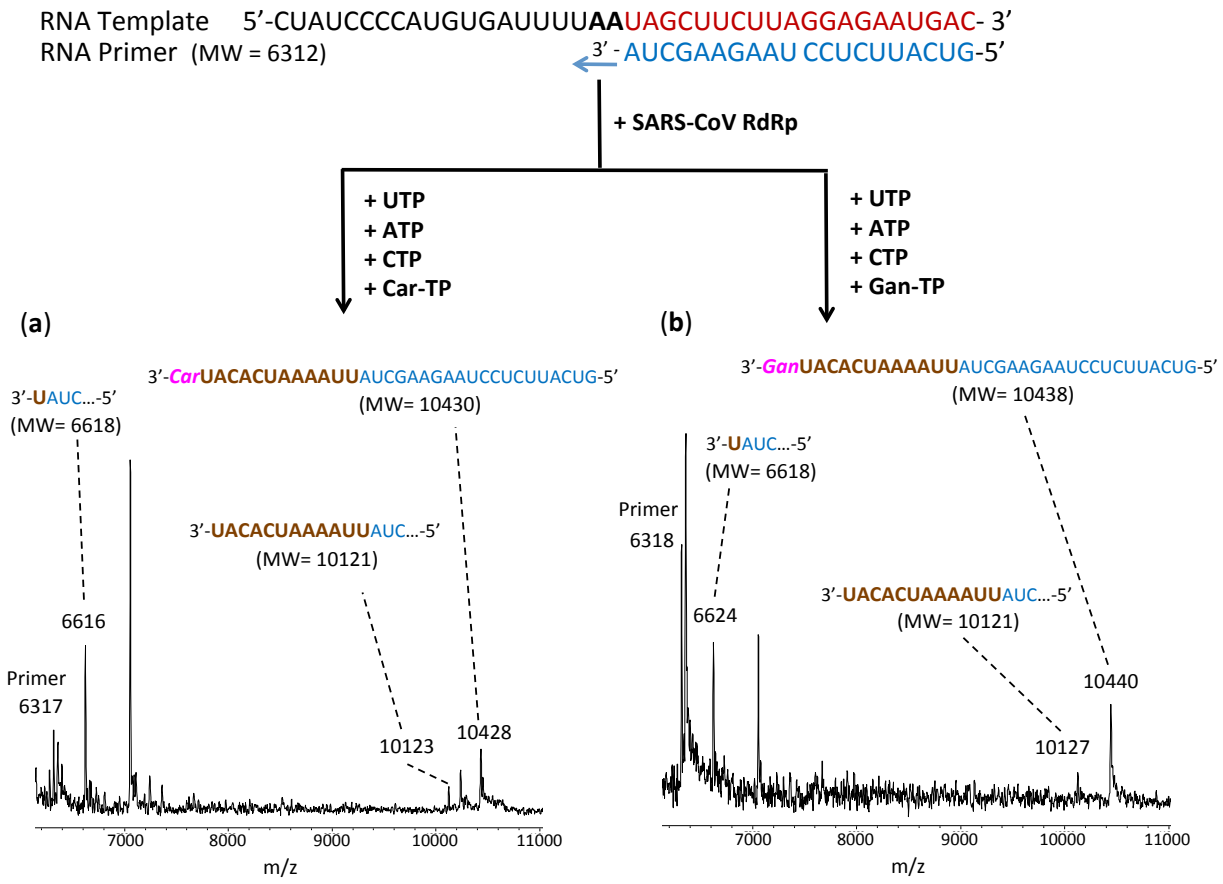


Fig. S4. Incorporation of Car-TP and Gan-TP by SARS-CoV RdRp to terminate the polymerase reaction. The sequences of the primer and template used for this extension reaction are shown at the top of the figure. Polymerase extension reactions were performed by incubating Car-TP, UTP, ATP and CTP (a) and Gan-TP, UTP, ATP and CTP (b) with pre-assembled SARS-CoV polymerase (nsp12, nsp7 and nsp8), the indicated RNA template and primer and the appropriate reaction buffer, followed by detection of reaction products by MALDI-TOF MS. The accuracy for m/z determination is ± 10 Da.

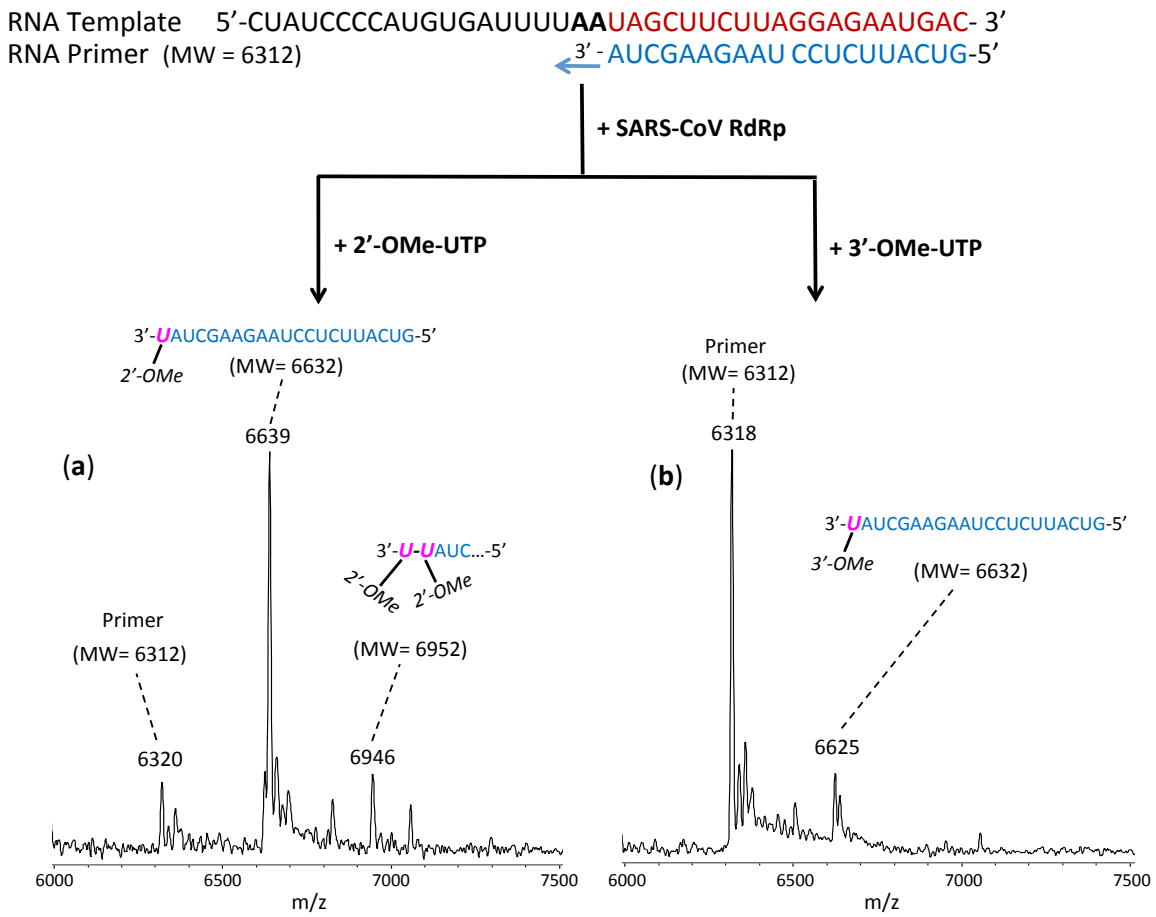


Fig. S5. Incorporation of 2'-OMe-UTP and 3'-OMe-UTP by SARS-CoV RdRp to terminate the polymerase reaction. The sequences of the primer and template used for this extension reaction are shown at the top of the figure. Polymerase extension reactions were performed by incubating 2'-OMe-UTP (a) and 3'-OMe-UTP (b) with pre-assembled SARS-CoV polymerase (nsp12, nsp7 and nsp8), the indicated RNA template and primer and the appropriate reaction buffer, followed by detection of reaction products by MALDI-TOF MS. The accuracy for m/z determination is ± 10 Da.

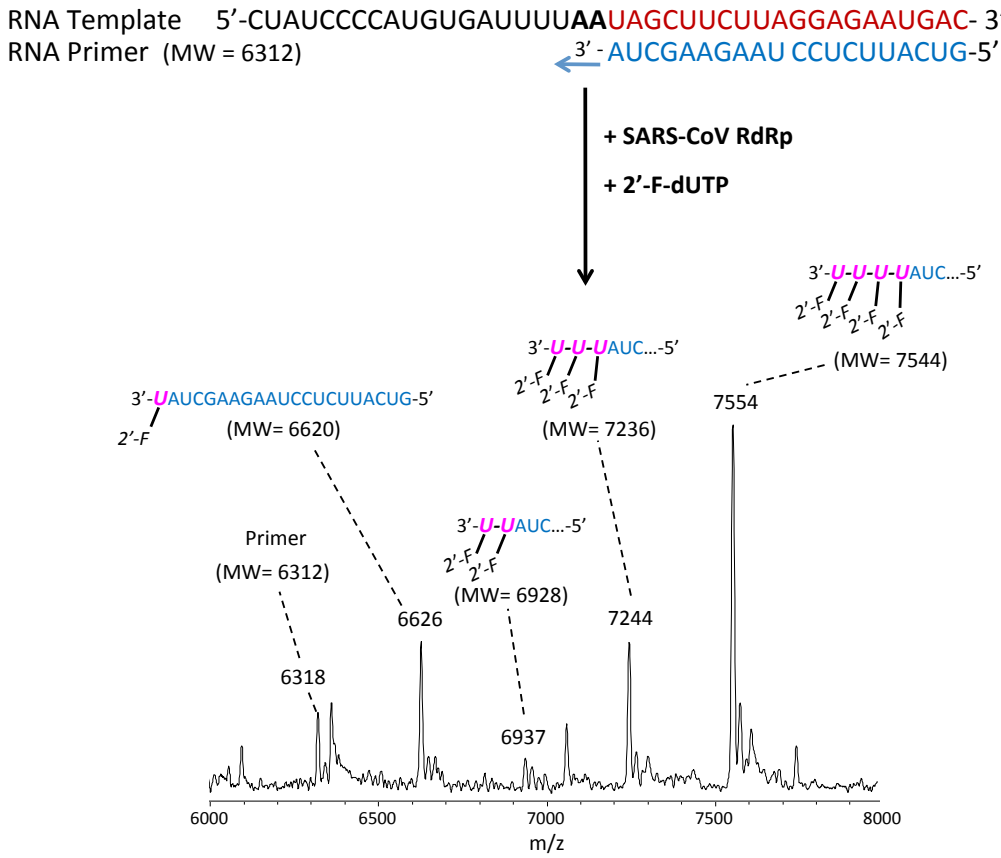


Fig. S6. Incorporation of 2'-F-dUTP by SARS-CoV RdRp catalyzed reaction. The sequences of the primer and template used for this extension reaction are shown at the top of the figure. The polymerase extension reaction was performed by incubating 2'-F-dUTP with pre-assembled SARS-CoV polymerase (nsp12, nsp7 and nsp8), the indicated RNA template and primer and the appropriate reaction buffer, followed by detection of reaction products by MALDI-TOF MS. The accuracy for m/z determination is ± 10 Da.

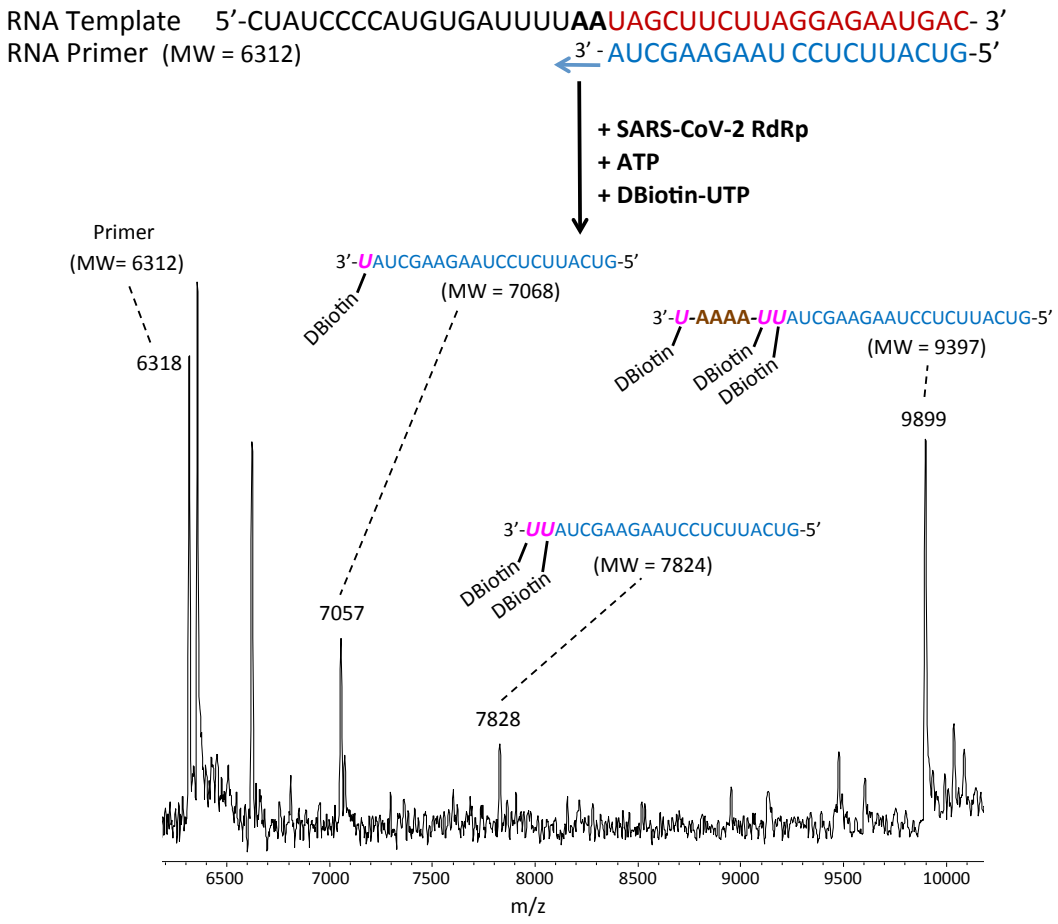


Fig. S7. Incorporation of desthiobiotin-16-UTP by SARS-CoV-2 RdRp catalyzed reaction. The sequences of the primer and template used for this extension reaction are shown at the top of the figure. The polymerase extension reaction was performed by incubating desthiobiotin-16-UTP and ATP with pre-assembled SARS-CoV-2 polymerase (nsp12, nsp7 and nsp8), the indicated RNA template and primer and the appropriate reaction buffer, followed by detection of reaction products by MALDI-TOF MS. The accuracy for m/z determination is ± 10 Da. Desthiobiotin is abbreviated DBiotin in the figure.

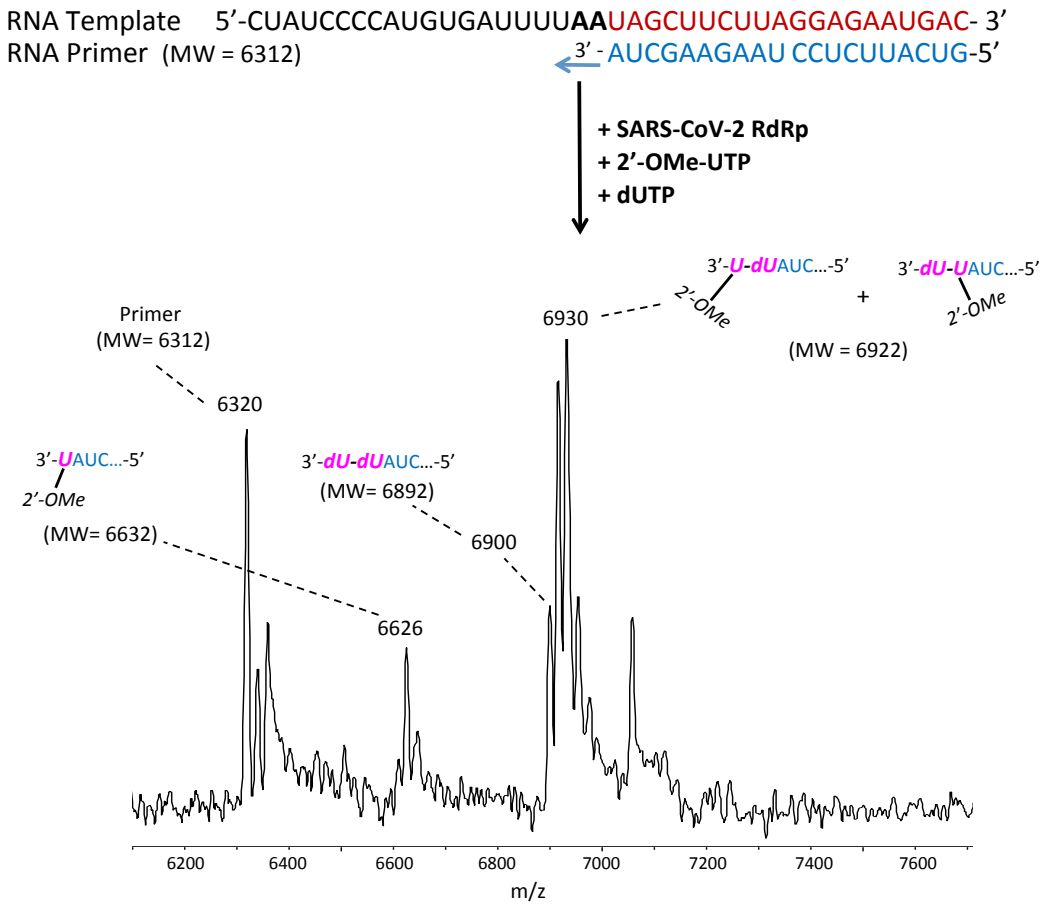


Fig. S8. Incorporation of 2'-OMe-UTP and dUTP by SARS-CoV-2 RdRp catalyzed reaction. The sequences of the primer and template used for this extension reaction are shown at the top of the figure. The polymerase extension reaction was performed by incubating 2'-OMe-UTP and dUTP with pre-assembled SARS-CoV-2 polymerase (nsp12, nsp7 and nsp8), the indicated RNA template and primer and the appropriate reaction buffer, followed by detection of reaction products by MALDI-TOF MS. The accuracy for m/z determination is ± 10 Da.

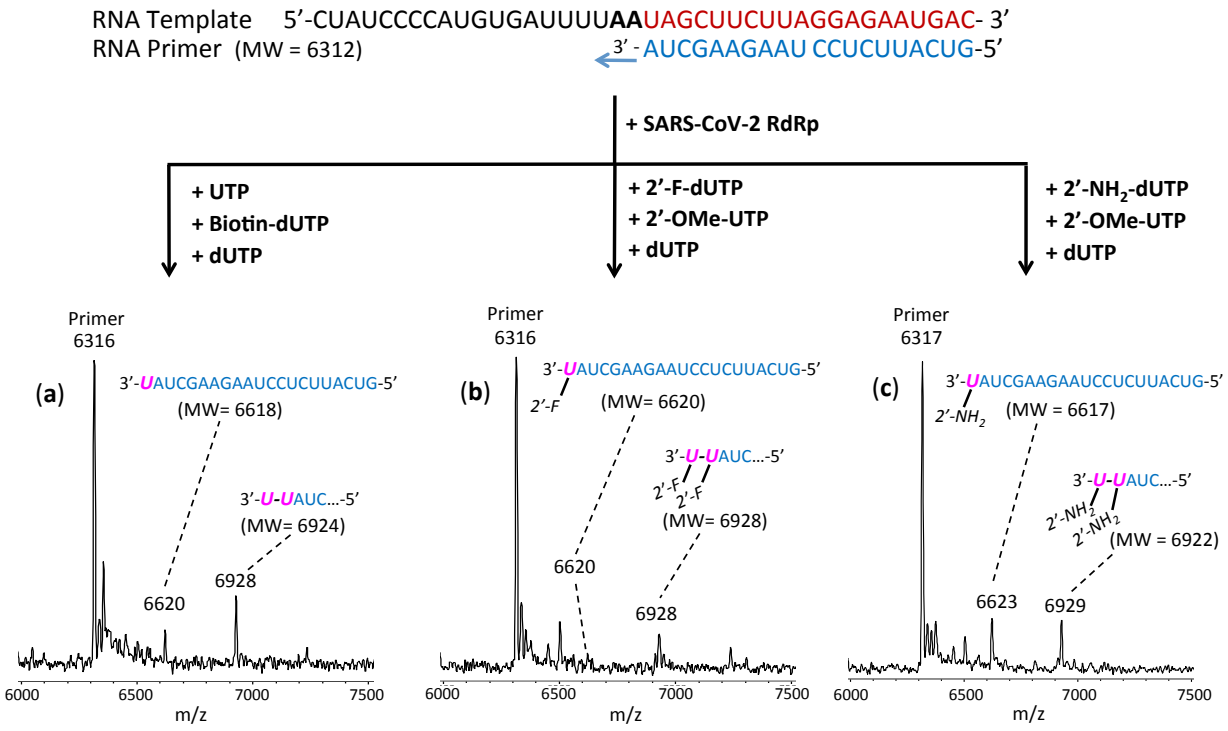


Fig. S9. Comparison of Relative Incorporation of UTP, Biotin-dUTP, dUTP; 2'-F-dUTP, 2'-OMe-UTP, dUTP; and 2'-NH_2-dUTP, 2'-OMe-UTP, dUTP by SARS-CoV-2 RdRp. The sequences of the primer and template used for this extension reaction are shown at the top of the figure. Polymerase extension reactions were performed by incubating UTP, Biotin-dUTP and dUTP (a), 2'-F-dUTP, 2'-OMe-UTP and dUTP (b) and 2'-NH_2-dUTP, 2'-OMe-UTP and dUTP (c) with pre-assembled SARS-CoV-2 polymerase (nsp12, nsp7 and nsp8), the indicated RNA template and primer and the appropriate reaction buffer, followed by detection of reaction products by MALDI-TOF MS. The accuracy for m/z determination is ± 10 Da.