**Supporting Information** for**:**

# Efficient and Rapid Template-Directed Nucleic Acid Copying using 2′-amino-2′, 3′-dideoxyribonucleoside-5′-phosphorimidazolide Monomers

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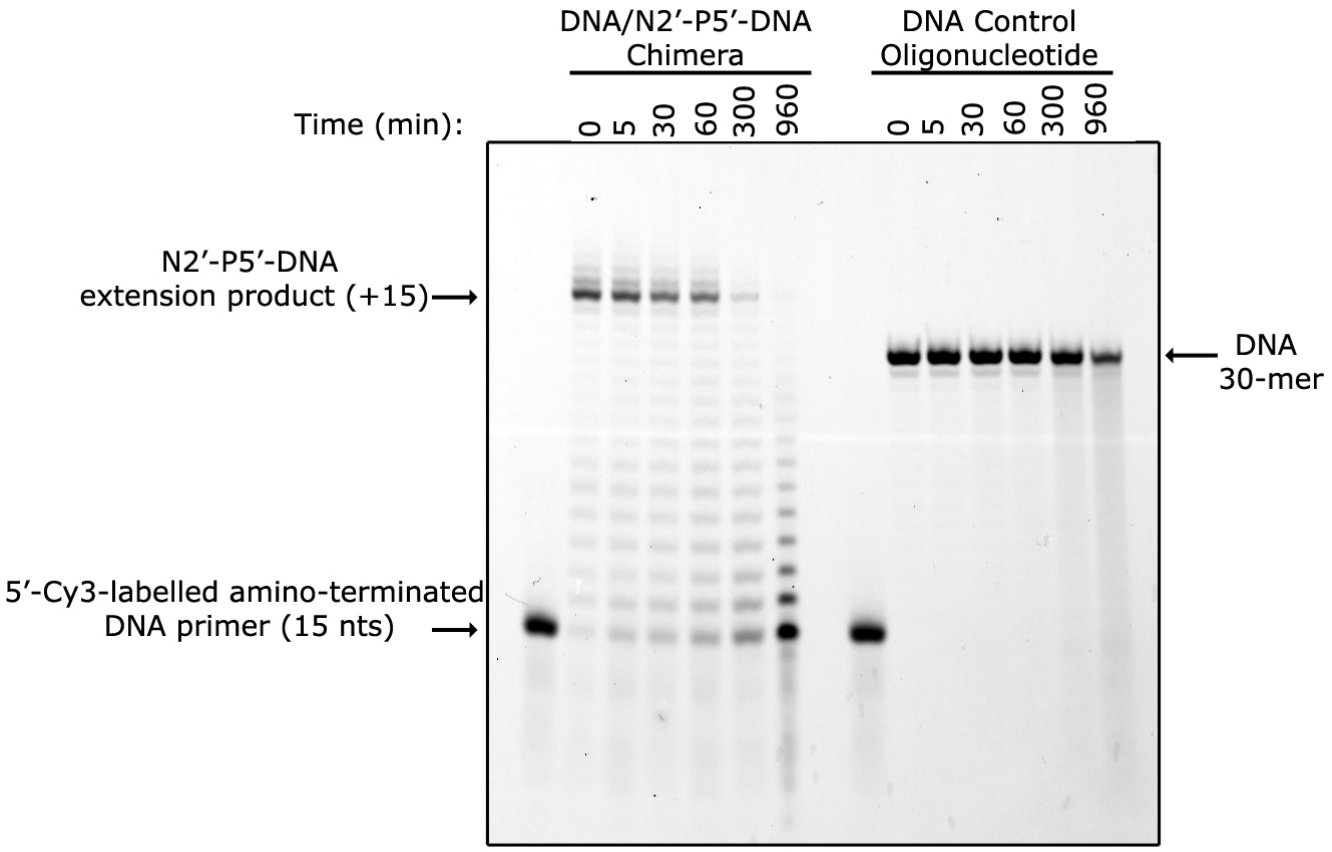


**Figure S1**. Acid sensitivity of N2′→P5′-DNA primer-extension product. 0.1μM Cy3-labelled amino-terminated primer was extended on a $dC_{15}$ template with 5mM 2′-$NH_2$-ImpddG as previously described. The primer-extension reaction was ethanol precipitated to remove buffer components and excess imidazolide and subjected to 80% acetic acid hydrolysis according to the time course as indicated. For comparison, a DNA 30-mer (5′-Cy3-GCGTAGACTGACTGGAAAAAAAAAAAAAA) was also subjected to 80% acetic acid for the given time course.

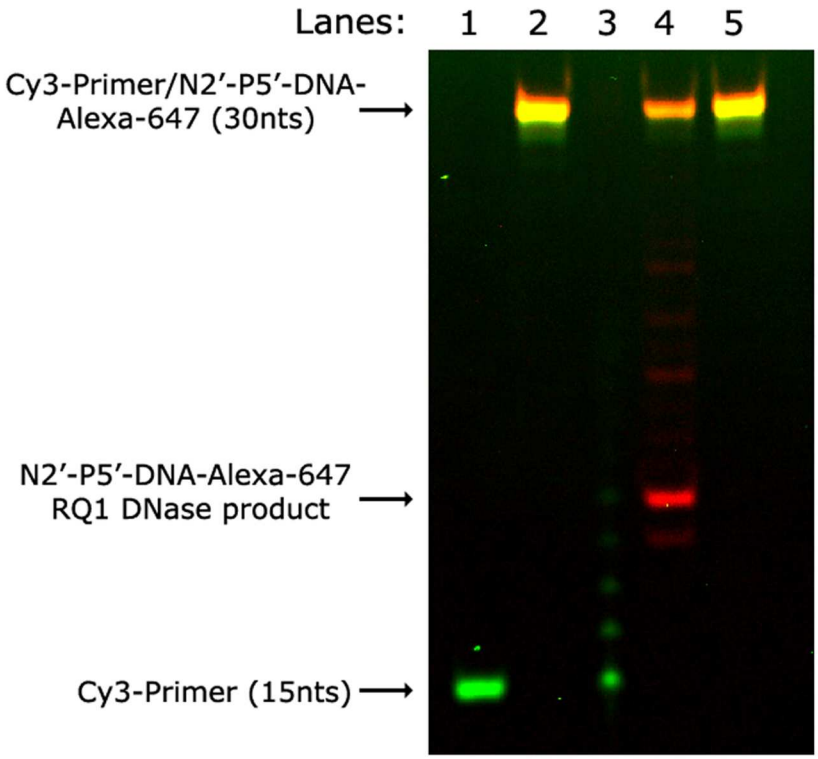


**Figure S2**. High resolution gel electrophoresis analysis of N2′→P5′-DNA-$G_{15}$ primer-extension product chemical and enzymatic digestion reactions. 0.1μM Cy3-labelled amino-terminated primer was extended on a $dC_{15}$ template with 5mM 2′-$NH_2$-ImpddG as previously described. The primer-extension reaction was ethanol precipitated and the new 2′-amino-terminus was reacted with the Alexa-647-succinimidyl ester. The resulting 5′-Cy3-DNA/N2′→P5′-DNA-2′-Alexa-647 double labeled oligonucleotide was polyacrylamide gel purified. Lanes: 1) primer alone; 2) 5′-Cy3-DNA/N2′→P5′-DNA-2′-Alexa-647 double labeled oligonucleotide; 3) 80% acetic acid hydrolysis; 4) RQ1 DNase digestion; 5) RNase $I_f$ digestion. Enzymatic digestion reactions are described in the experimental section.

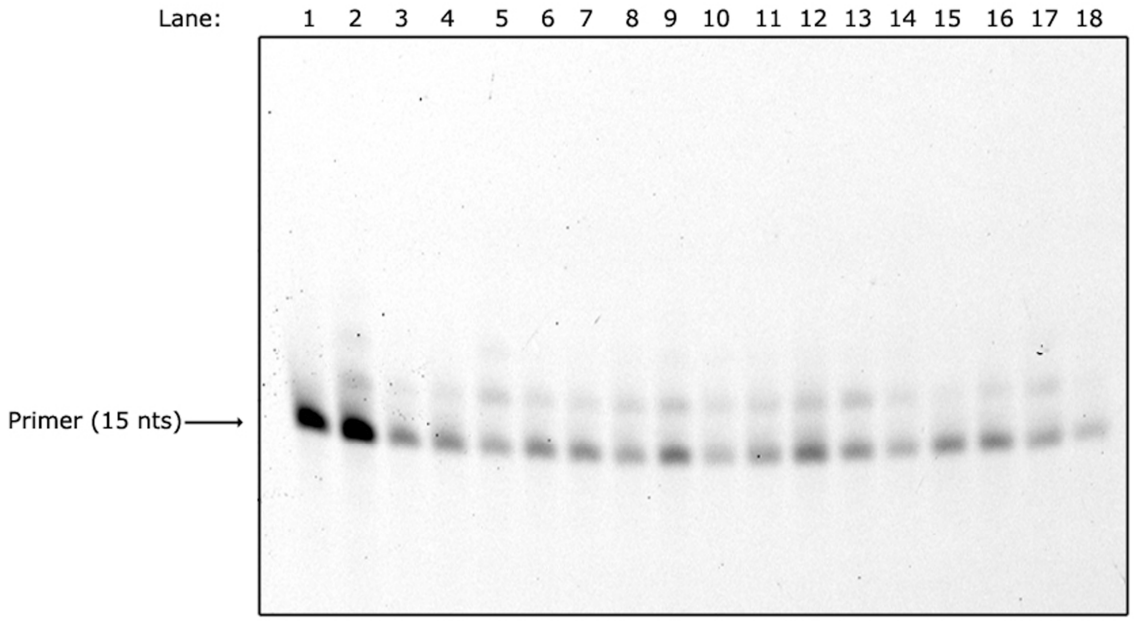


**Figure S3**. High resolution gel electrophoresis analysis of 2′-$NH_2$-ImpddG non-complementary template copying reactions. Primer-extension reactions contained 0.1μM Cy3-labeled-2′-amino-terminated DNA primer, 0.5μM non-complementary template, 100mM MES-CAPS-HEPES, pH 7.5, 100mM 1-(2-hydroxyethyl)-imidazole and 5mM 2′-$NH_2$-ImpddG. The reaction was completed as previously described and incubated at 4°C for 3 hours.

| Lane | Template | Sequence |
|---|---|---|
| 1 | primer alone | |
| 2 | no template | |
| 3 | DNA | 5′ - C**AAAA**GCAGTCAGTCTACGC |
| 4 | DNA | 5′ - C**DDDD**GCAGTCAGTCTACGC |
| 5 | DNA | 5′ - T**GGGG**GCAGTCAGTCTACGC |
| 6 | DNA | 5′ - A**TTTT**GCAGTCAGTCTACGC |
| 7 | DNA | 5′ - C**U$^{P}$U$^{P}$U$^{P}$U$^{P}$**GCAGTCAGTCTACGC |
| 8 | RNA | 5′ - C**AAAA**GCAGUCAGUCUACGC |
| 9 | RNA | 5′ - C**DDDD**GCAGUCAGUCUACGC |
| 10 | RNA | 5′ - U**GGGG**GCAGUCAGUCUACGC |
| 11 | RNA | 5′ - A**UUUU**GCAGUCAGUCUACGC |
| 12 | RNA | 5′ - A**U$^{P}$U$^{P}$U$^{P}$U$^{P}$**GCAGUCAGUCUACG |
| 13 | LNA (underlined) | 5′ - C**AAAA**GCAGTCAGTCTACGC |
| 14 | LNA (underlined) | 5′ - T**GGGG**GCAGTCAGTCTACGC |
| 15 | LNA (underlined) | 5′ - A**TTTT**GCAGTCAGTCTACGC |
| 16 | O2′→P5′-DNA (underlined) | 5′ - C**AAAA**GCAGTCAGTCTACGC |
| 17 | O2′→P5′-DNA (underlined) | 5′ - T**GGGG**GCAGTCAGTCTACGC |
| 18 | O2′→P5′-DNA (underlined) | 5′ - A**TTTT**GCAGTCAGTCTACGC |

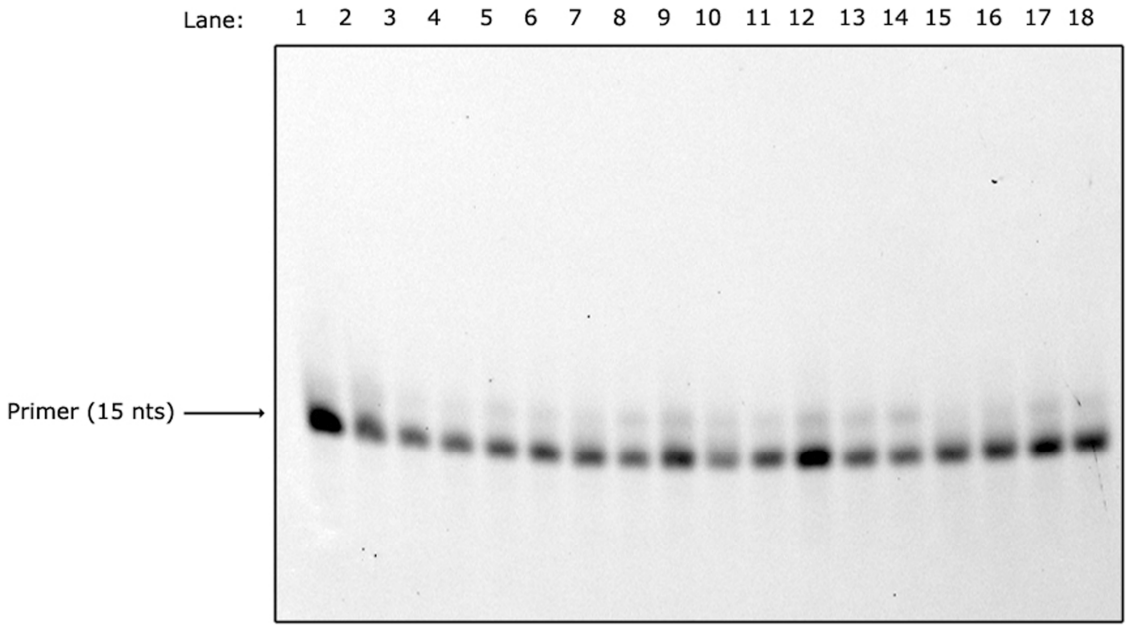


**Figure S4**. High resolution gel electrophoresis analysis of 2′-$NH_2$-ImpddC non-complementary template copying reactions. Primer-extension reactions were run as previously described with 0.5μM non-complementary template and 5mM 2′-$NH_2$-ImpddC. The reaction was completed as previously described and incubated at 4°C for 3 hours.

| Lane | Template | Sequence |
|---|---|---|
| 1 | primer alone | |
| 2 | no template | |
| 3 | DNA | 5′ - C**AAAA**GCAGTCAGTCTACGC |
| 4 | DNA | 5′ - C**DDDD**GCAGTCAGTCTACGC |
| 5 | DNA | 5′ - A**CCCC**GCAGTCAGTCTACGC |
| 6 | DNA | 5′ - A**TTTT**GCAGTCAGTCTACGC |
| 7 | DNA | 5′ - C**U$^{p}$U$^{p}$U$^{p}$U$^{p}$**GCAGTCAGTCTACGC |
| 8 | RNA | 5′ - C**AAAA**GCAGUCAGUCUACGC |
| 9 | RNA | 5′ - C**DDDD**GCAGUCAGUCUACGC |
| 10 | RNA | 5′ - A**CCCC**GCAGUCAGUCUACGC |
| 11 | RNA | 5′ - A**UUUU**GCAGUCAGUCUACGC |
| 12 | RNA | 5′ - A**U$^{p}$U$^{p}$U$^{p}$U$^{p}$**GCAGUCAGUCUACG |
| 13 | LNA (underlined) | 5′ - C**AAAA**GCAGTCAGTCTACGC |
| 14 | LNA (underlined) | 5′ - A**CCCC**GCAGTCAGTCTACGC |
| 15 | LNA (underlined) | 5′ - A**TTTT**GCAGTCAGTCTACGC |
| 16 | O2′→P5′-DNA (underlined) | 5′ - C**AAAA**GCAGTCAGTCTACGC |
| 17 | O2′→P5′-DNA (underlined) | 5′ - A**CCCC**GCAGTCAGTCTACGC |
| 18 | O2′→P5′-DNA (underlined) | 5′ - A**TTTT**GCAGTCAGTCTACGC |

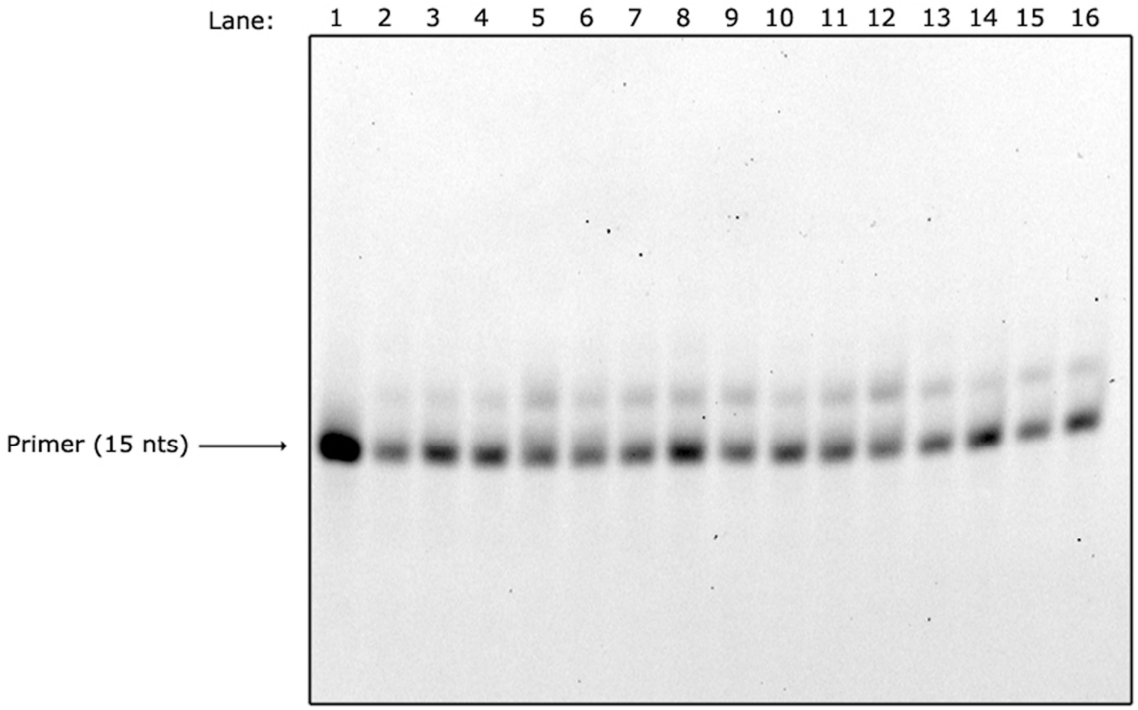


**Figure S5**. High resolution gel electrophoresis analysis of 2′-$NH_2$-ImpddD non-complementary template copying reactions. Primer-extension reactions were run as previously described with 0.5μM non-complementary template and 5mM 2′-$NH_2$-ImpddD. The reaction was completed as previously described and incubated at 4°C for 3 hours.

| Lane | Template | Sequence |
|---|---|---|
| 1 | primer alone | |
| 2 | no template | |
| 3 | DNA | 5′ - C**AAAA**GCAGTCAGTCTACGC |
| 4 | DNA | 5′ - C**DDDD**GCAGTCAGTCTACGC |
| 5 | DNA | 5′ - A**CCCC**GCAGTCAGTCTACGC |
| 6 | DNA | 5′ - T**GGGG**GCAGTCAGTCTACGC |
| 7 | RNA | 5′ - C**AAAA**GCAGUCAGUCUACGC |
| 8 | RNA | 5′ - C**DDDD**GCAGUCAGUCUACGC |
| 9 | RNA | 5′ - A**CCCC**GCAGUCAGUCUACGC |
| 10 | RNA | 5′ - U**GGGG**GCAGUCAGUCUACGC |
| 11 | LNA (underlined) | 5′ - C**AAAA**GCAGTCAGTCTACGC |
| 12 | LNA (underlined) | 5′ - A**CCCC**GCAGTCAGTCTACGC |
| 13 | LNA (underlined) | 5′ - T**GGGG**GCAGTCAGTCTACGC |
| 14 | O2′→P5′-DNA (underlined) | 5′ - C**AAAA**GCAGTCAGTCTACGC |
| 15 | O2′→P5′-DNA (underlined) | 5′ - A**CCCC**GCAGTCAGTCTACGC |
| 16 | O2′→P5′-DNA (underlined) | 5′ - T**GGGG**GCAGTCAGTCTACGC |

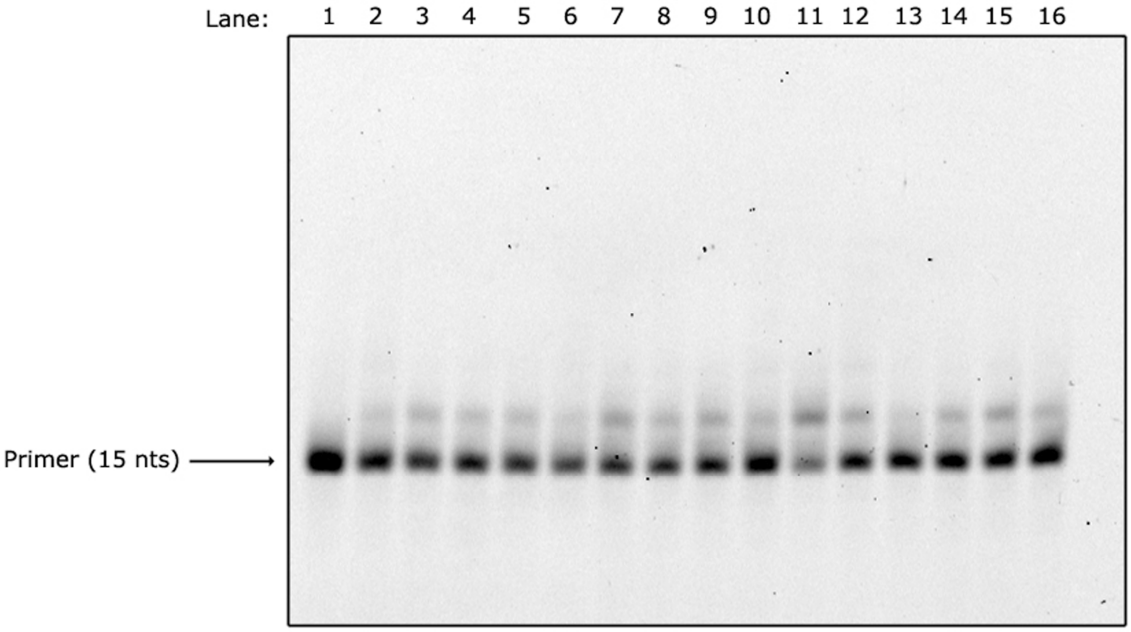


**Figure S6**. High resolution gel electrophoresis analysis of 2′-$NH_2$-ImpddU$^p$ non-complementary template copying reactions. Primer-extension reactions were run as previously described with 0.5μM non-complementary template and 5mM 2′-$NH_2$-ImpddU$^p$. The reaction was completed as previously described and incubated at 4°C for 3 hours.

| Lane | Template | Sequence |
|---|---|---|
| 1 | primer alone | |
| 2 | no template | |
| 3 | DNA | 5′ - A**CCCC**GCAGTCAGTCTACGC |
| 4 | DNA | 5′ - T**GGGG**GCAGTCAGTCTACGC |
| 5 | DNA | 5′ - A**TTTT**GCAGTCAGTCTACGC |
| 6 | DNA | 5′ - C**U$^p$U$^p$U$^p$U$^p$**GCAGTCAGTCTACGC |
| 7 | RNA | 5′ - A**CCCC**GCAGUCAGUCUACGC |
| 8 | RNA | 5′ - U**GGGG**GCAGUCAGUCUACGC |
| 9 | RNA | 5′ - A**UUUU**GCAGUCAGUCUACGC |
| 10 | RNA | 5′ - A**U$^p$U$^p$U$^p$U$^p$**GCAGUCAGUCUACG |
| 11 | LNA (underlined) | 5′ - A**CCCC**GCAGTCAGTCTACGC |
| 12 | LNA (underlined) | 5′ - T**GGGG**GCAGTCAGTCTACGC |
| 13 | LNA (underlined) | 5′ - A**TTTT**GCAGTCAGTCTACGC |
| 14 | O2′→P5′-DNA (underlined) | 5′ - A**CCCC**GCAGTCAGTCTACGC |
| 15 | O2′→P5′-DNA (underlined) | 5′ - T**GGGG**GCAGTCAGTCTACGC |
| 16 | O2′→P5′-DNA (underlined) | 5′ - A**TTTT**GCAGTCAGTCTACGC |

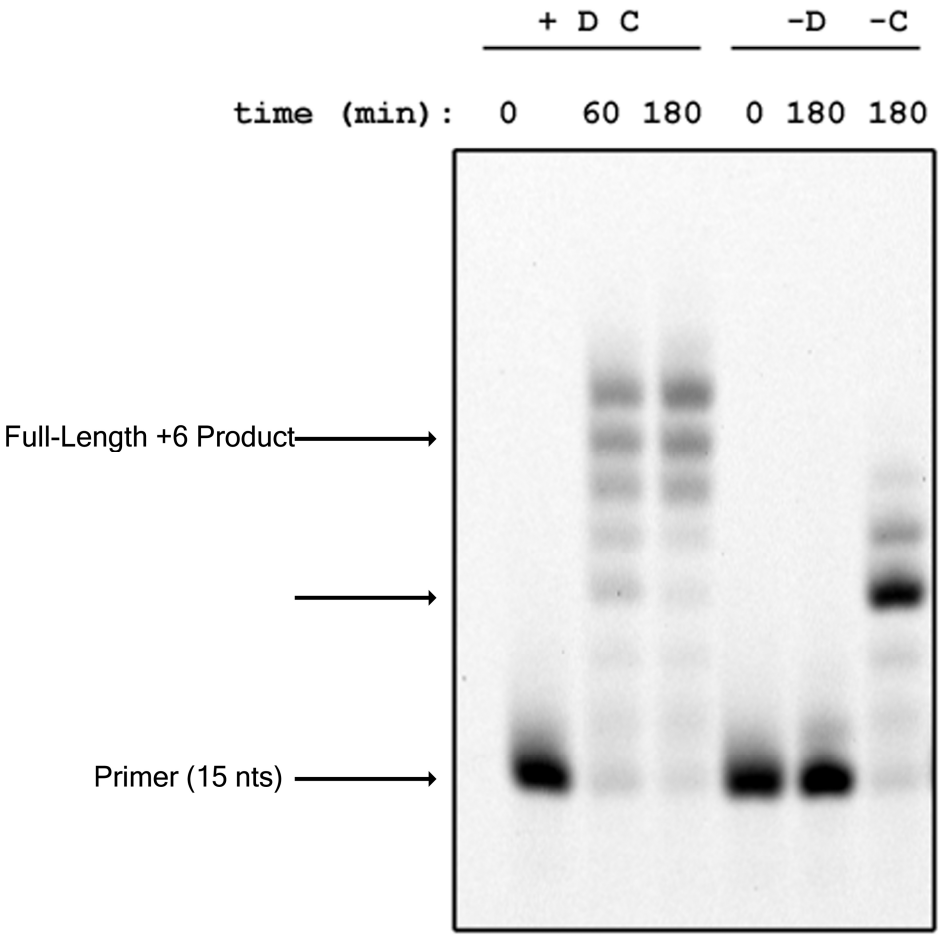


**Figure S7**. High resolution polyacrylamide gel analysis of mixed sequence RNA template copying reaction. Primer-extension reaction contained 0.1μM Cy3-labeled-2′-amino-terminated DNA primer, 0.5μM template (5′- GGGU$^{p}$U$^{p}$U$^{p}$ -3′-primer binding site), 100mM MES-CAPS-HEPES, pH 7.5, 100mM 1-(2-hydroxyethyl)-imidazole, 1mM 2′-$NH_2$-ImpddC and 5mM 2′-$NH_2$-ImpddD. The reaction was completed as previously described and incubated at 4°C for the indicated time. Arrows indicate primer, full-length product and stalled products.

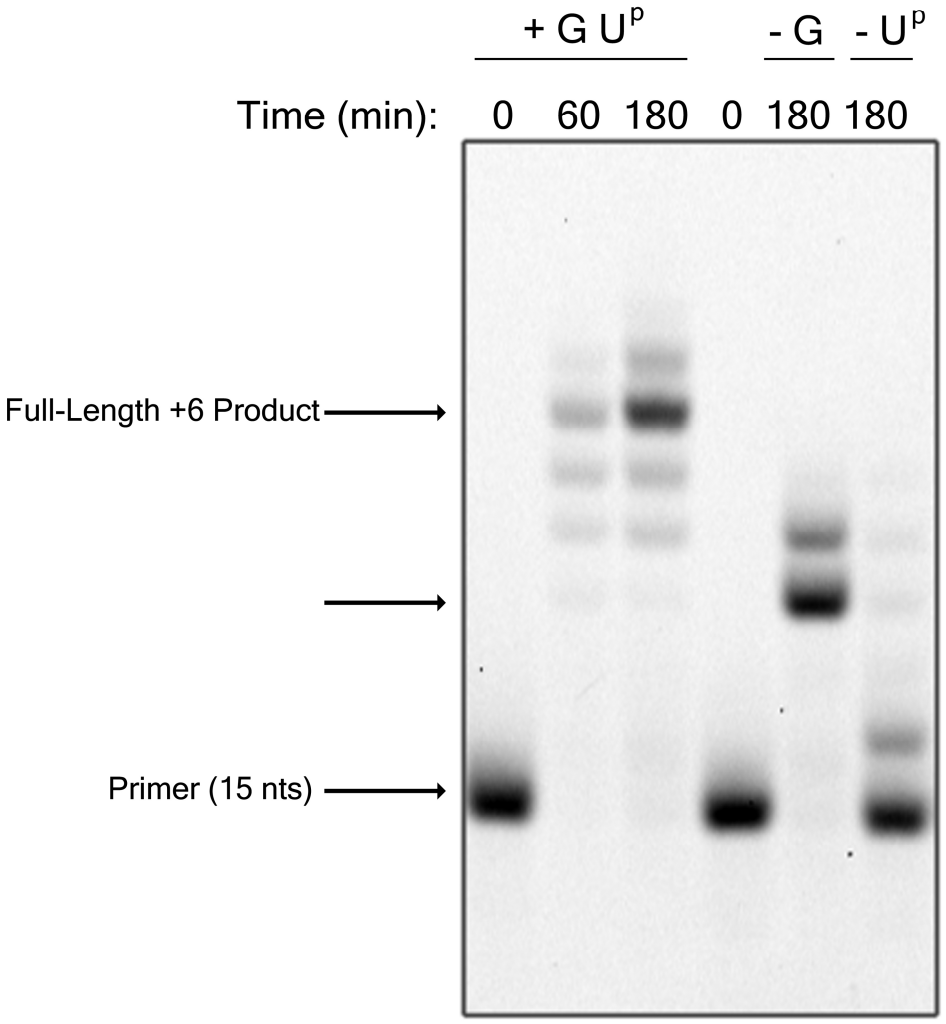


**Figure S8**. High resolution polyacrylamide gel analysis of mixed sequence RNA template copying reaction. Primer-extension reaction contained 0.1μM Cy3-labeled-2′-amino-terminated DNA primer, 0.5μM template (5′-CCCDDD-3′-primer binding site), 100mM MES-CAPS-HEPES, pH 7.5, 100mM 1-(2-hydroxyethyl)-imidazole, 1mM 2′-$NH_2$-ImpddG and 5mM 2′-$NH_2$-Impdd$U^p$. The reaction was completed as previously described and incubated at 4°C for the indicated time. Arrows indicate primer, full-length product and stalled products.

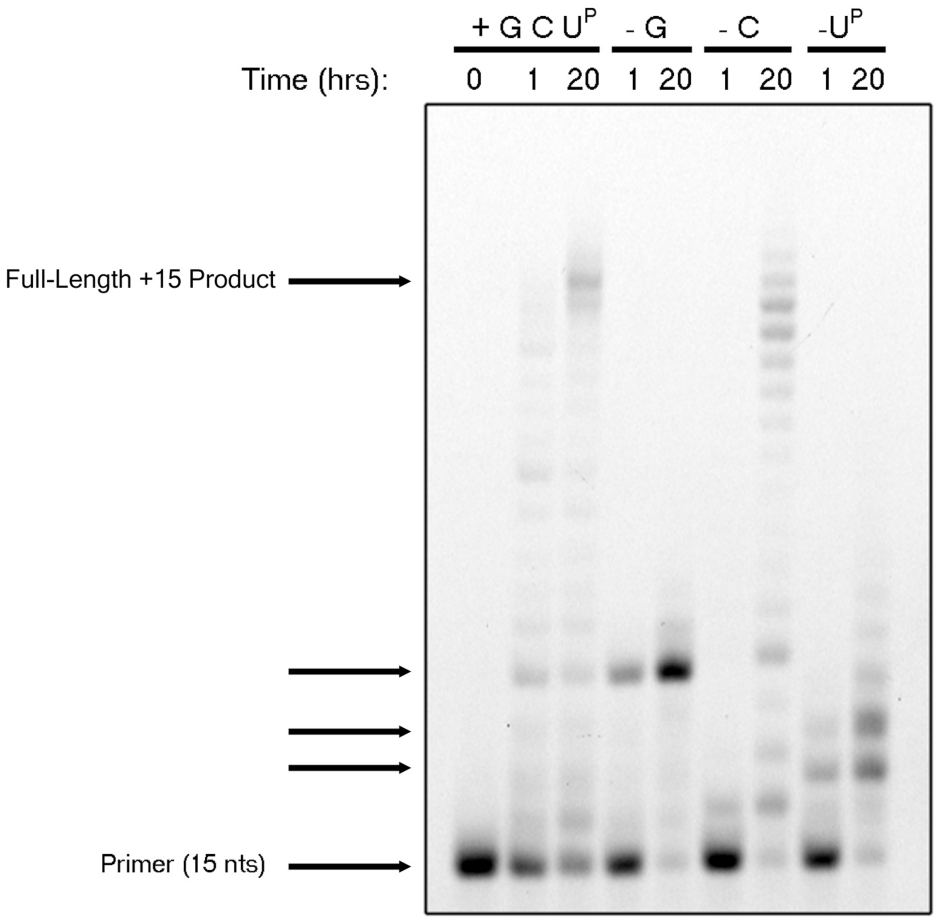


**Figure S9**. High resolution polyacrylamide gel analysis of mixed sequence RNA template copying reaction. Primer-extension reaction contained 0.1μM Cy3-labeled-2′-amino-terminated DNA primer, 0.5μM template (5′- GGDDCCDDCCCDDGG-3′-primer binding site), 100mM MES-CAPS-HEPES, pH 7.5, 100mM 1-(2-hydroxyethyl)-imidazole, 5mM 2′-$NH_2$-ImpddC, 5mM 2′-$NH_2$-ImpddG and 5mM 2′-$NH_2$-Impdd$U^p$. The reaction was completed as previously described and incubated at 4°C for the indicated time. Arrows indicate primer, full-length product and stalled products.

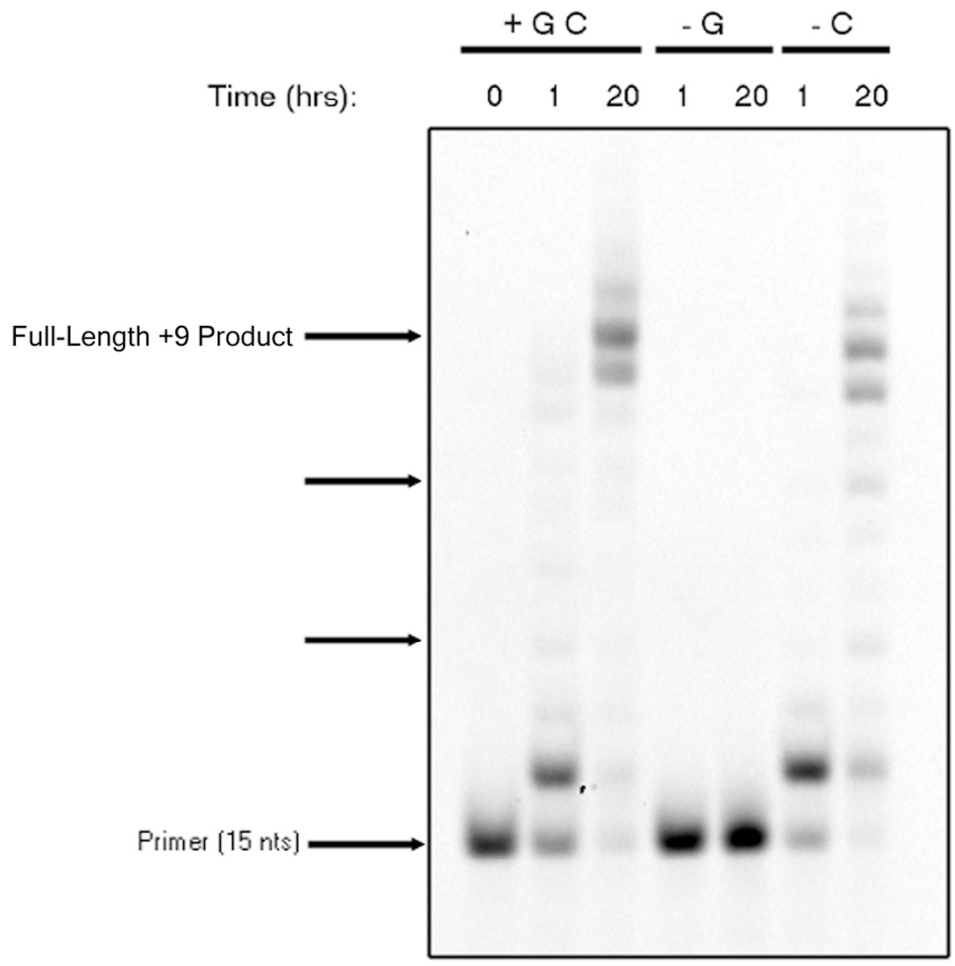


**Figure S10**. High resolution polyacrylamide gel analysis of mixed sequence RNA template copying reaction. Primer-extension reaction contained 0.1μM Cy3-labeled-2′-amino-terminated DNA primer, 0.5μM template (5′-AGCCGCCGCC-3′-primer binding site), 100mM MES-CAPS-HEPES, pH 7.5, 100mM 1-(2-hydroxyethyl)-imidazole, 5mM 2′-$NH_2$-ImpddC and 5mM 2′-$NH_2$-ImpddG. The reaction was completed as previously described and incubated at 4°C for the indicated time. Arrows indicate primer, full-length product and stalled products.

**Supporting Information Experimental Section**

**Phosphoramidate DNA Sensitivity to Acid, DNase, and RNase.**

Phosphoramidate extension product acid lability was demonstrated by extending 0.1μM Cy3-labelled 2′-amino-terminated primer on 0.5 μM $dC_{15}$ DNA template oligonucleotide in 150 mM NaCl, 100 mM 1-(2-hydroxyethyl)-imidazole, 100 mM Na-MES-CAPS-HEPES, pH 7.5 with 5mM 2′-amino-2′,3′-dideoxyguanosine-5′-phosphorimidazolide at 4 °C. The extension reaction mixture was ethanol precipitated and hydrolyzed with 80% acetic acid at room temperature for various time points. For comparison, a DNA control oligonucleotide was subjected to 80% acetic acid treatment. Hydrolysis products were compared and analyzed by polyacrylamide gel electrophoresis as previously described. N2′→P5′-phosphoramidate DNA enzymatic resistance to RQ1 DNase and RNase $I_f$ was shown by extending 0.1 μM Cy3-labelled 2′-amino-terminated DNA primer on 0.5 μM $dC_{15}$ DNA template oligonucleotide with 2′-amino-2′,3′-dideoxynucleoside-5′-phosphorimidazolide as described, reacting the new 2′-amino-terminus with the Alexa 647-succinimidyl ester (Invitrogen), polyacrylamide gel purified the doubled labeled oligonucleotide (5′-Cy3-GCGTAGACTGACTG<u>GGGGGGGGGGGGGGGG</u>-2′-Alexa 647; phosphoramidate linkages underlined), and treated with RQ1 DNase or RNase $I_f$ for 30 minutes at 37 °C. Digestion products were analyzed by polyacrylamide gel electrophoresis shown in figure S2.