

## Supporting Information

### for “LC-MS/MS Identification and Yeast Polymerase $\eta$ Bypass of a Novel $\gamma$ -Irradiation-induced Intrastrand Crosslink Lesion G[8-5]C.”

by Chunang Gu and Yinsheng Wang\*

**Figure S1.** Primer extension assay for G[8-5]C-containing substrate by exonuclease-free T7 DNA polymerase and Klenow fragment of *E. coli* DNA polymerase I.

**Figure S2.** Primer extension assay for G[8-5]C-containing substrate by HIV reverse transcriptase.

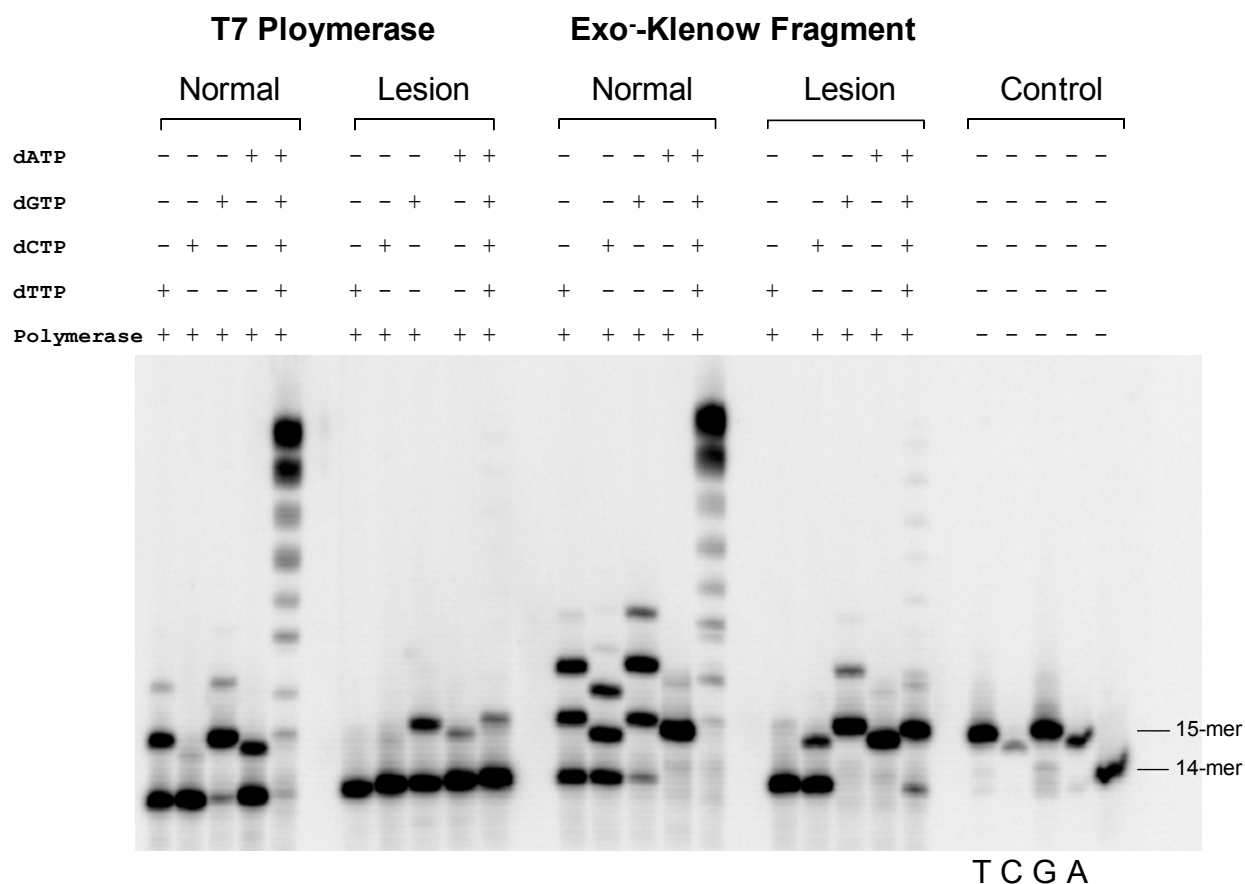
**Figure S3.** Michaelis-Menten plots for nucleotide incorporation opposite the cytosine of the undamaged substrate by yeast pol  $\eta$ .

**Figure S4.** Michaelis-Menten plots for nucleotide incorporation opposite the cytosine portion of the G[8-5]C-containing substrate by yeast pol  $\eta$ .

**Figure S5.** Michaelis-Menten plots for nucleotide incorporation opposite guanine of the undamaged substrate by yeast pol  $\eta$ .

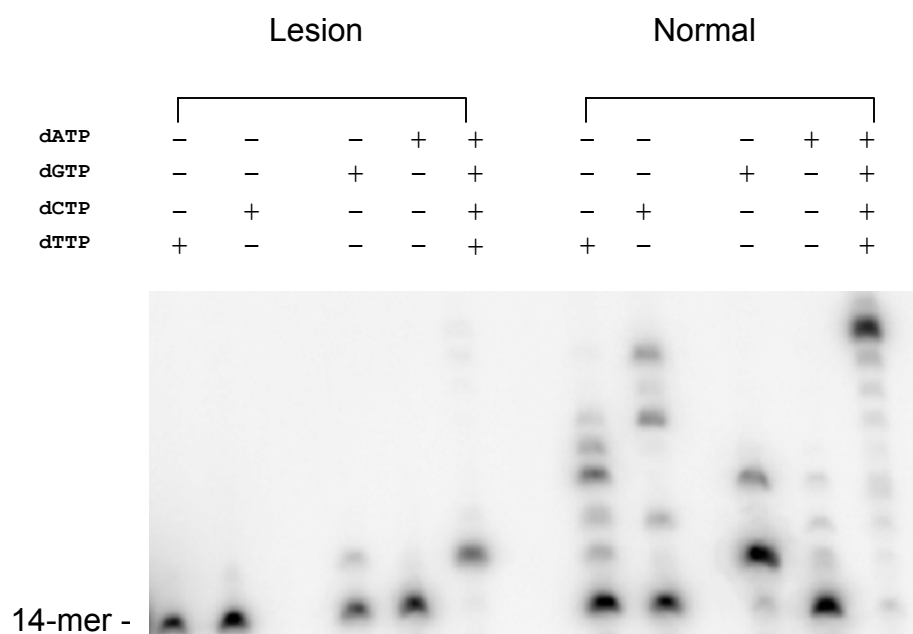
**Figure S6.** Michaelis-Menten plots for nucleotide incorporation opposite the guanine portion of G[8-5]C by yeast pol  $\eta$ .

**Figure S7.** Structure of d(G[8-5]C) as optimized by semi-empirical PM3 method.

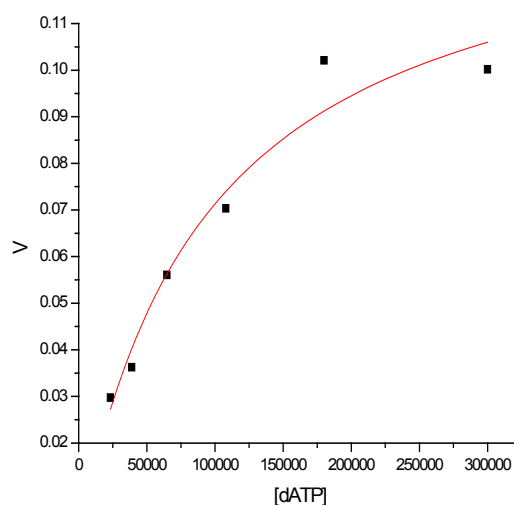
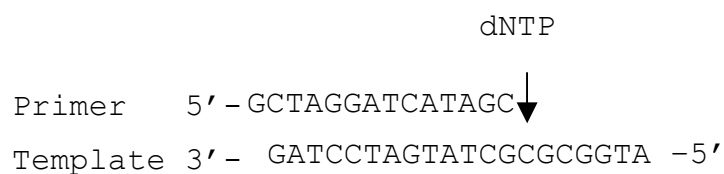


**Figure S1.** Primer extension assay for G[8-5]C-containing substrate by exonuclease-free T7 DNA polymerase and Klenow fragment of *E. coli* DNA polymerase I ([dNTP] = 100  $\mu$ M, [T7 polymerase] = 60 nM, [Klenow Fragment] = 0.05U, [primer] = 10 nM, [template] = 20 nM. The reactions were carried out at 37°C for 60 min).

## HIV-RT Polymerase

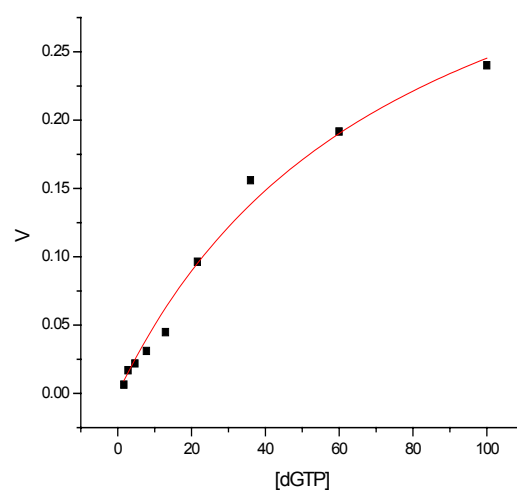


**Figure S2.** Primer extension assay for G[8-5]C-containing substrate by HIV reverse transcriptase. ([dNTP] = 100  $\mu$ M, [HIV-RT] = 4U, [primer] = 10 nM, [template] = 20 nM. The reactions were carried out at 37°C for 60 min).



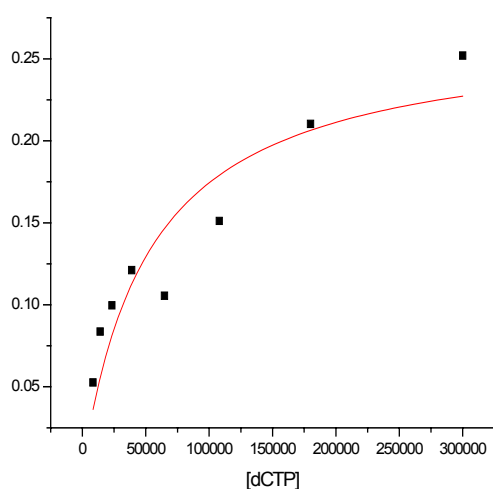
$$V_{\max} = 0.14 \text{ nM/min}$$

$$K_m = 96 \text{ uM}$$



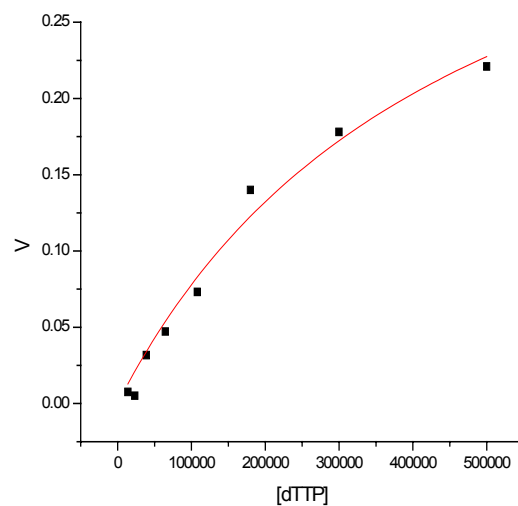
$$V_{\max} = 0.43 \text{ nM/min}$$

$$K_m = 0.077 \text{ uM}$$



$$V_{\max} = 0.27 \text{ nM/min}$$

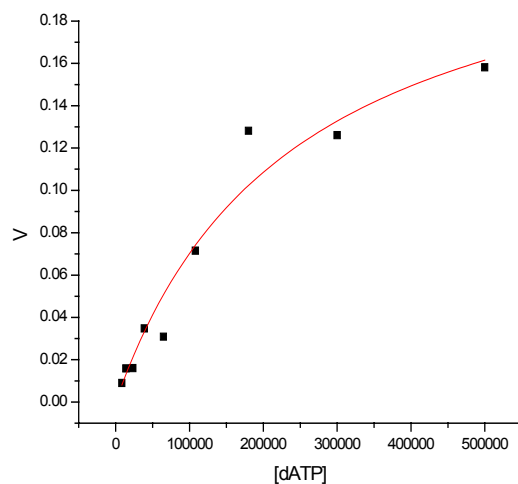
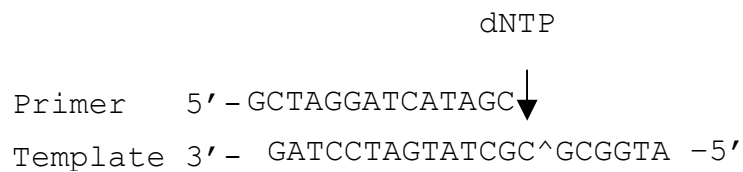
$$K_m = 53 \text{ uM}$$



$$V_{\max} = 0.44 \text{ nM/min}$$

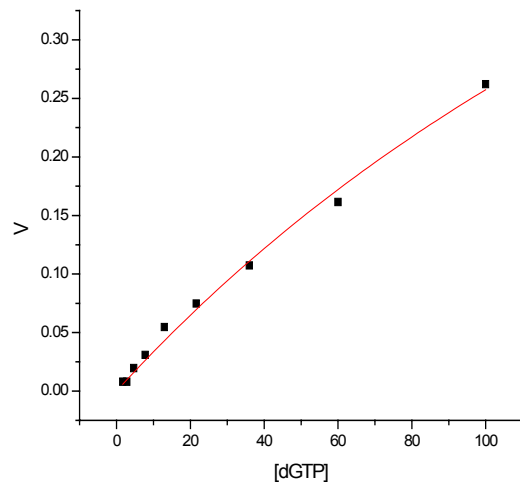
$$K_m = 460 \text{ uM}$$

**Figure S3.** Michaelis-Menten plots for nucleotide incorporation opposite the cytosine of the undamaged substrate by yeast pol  $\eta$ .



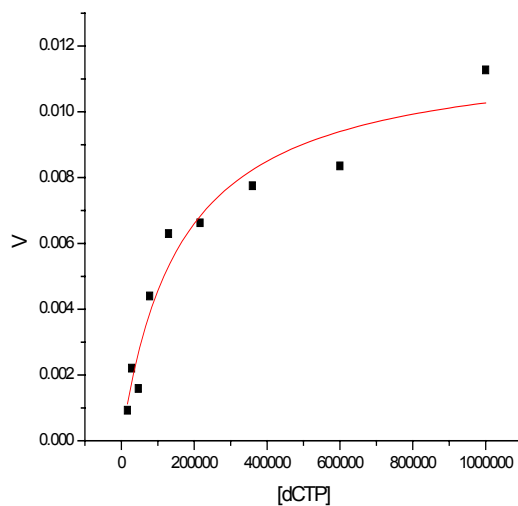
$$V_{\max} = 0.24 \text{ nM/min}$$

$$K_m = 240 \text{ uM}$$



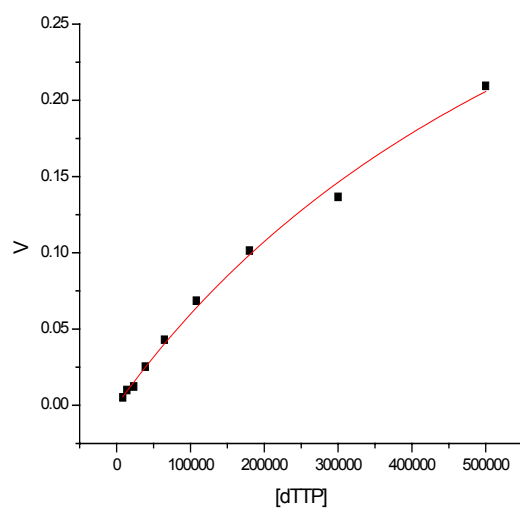
$$V_{\max} = 1.00 \text{ nM/min}$$

$$K_m = 0.29 \text{ uM}$$



$$V_{\max} = 0.012 \text{ nM/min}$$

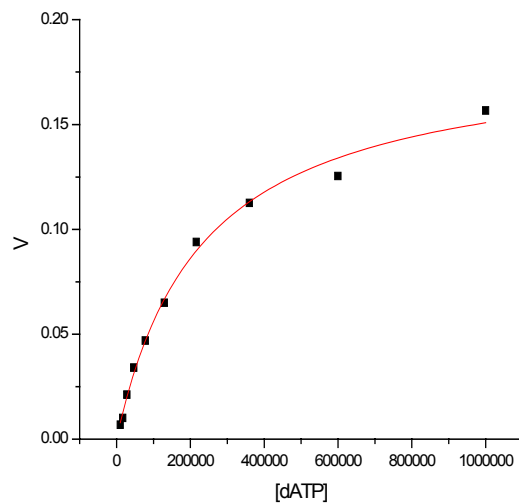
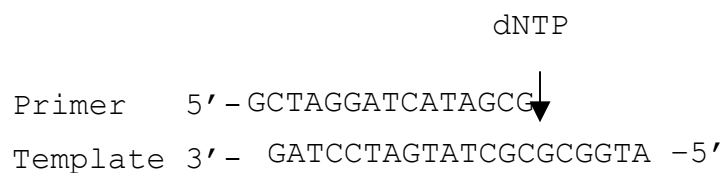
$$K_m = 160 \text{ uM}$$



$$V_{\max} = 0.53 \text{ nM/min}$$

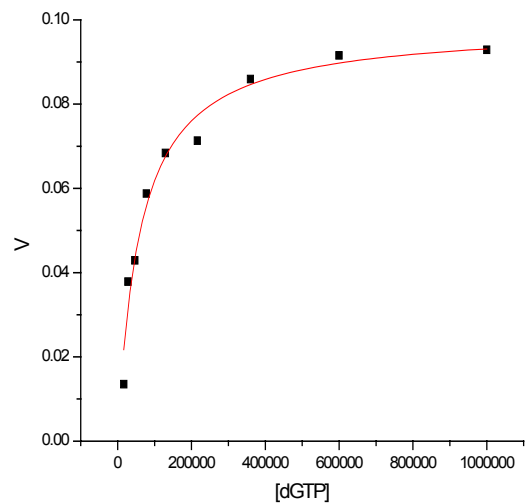
$$K_m = 790 \text{ uM}$$

**Figure S4.** Michaelis-Menten plots for nucleotide incorporation opposite the cytosine portion of the G[8-5]C-containing substrate by yeast pol  $\eta$ .



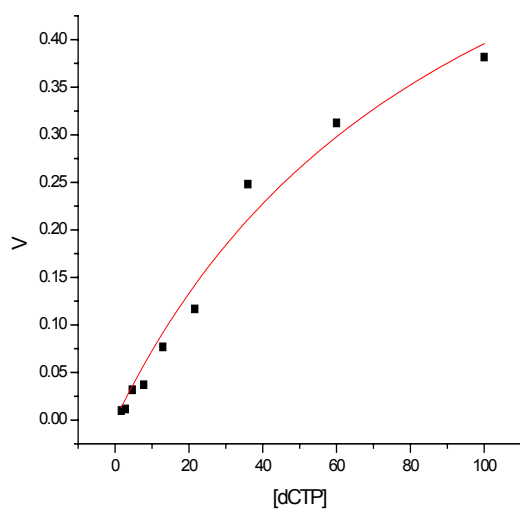
$$V_{\max} = 0.18 \text{ nM/min}$$

$$K_m = 230 \text{ uM}$$



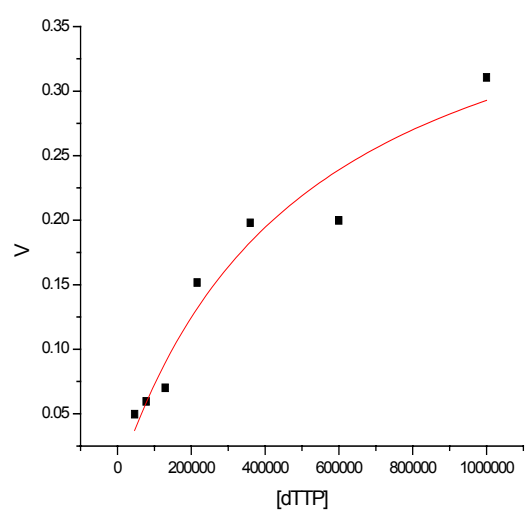
$$V_{\max} = 0.09 \text{ nM/min}$$

$$K_m = 520 \text{ uM}$$



$$V_{\max} = 0.78 \text{ nM/min}$$

$$K_m = 0.97 \text{ uM}$$



$$V_{\max} = 0.44 \text{ nM/min}$$

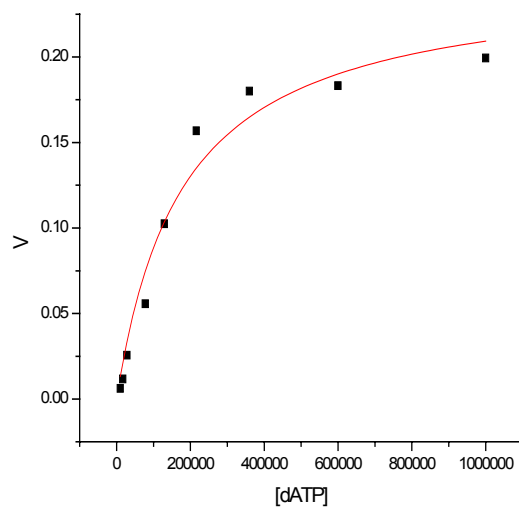
$$K_m = 510 \text{ uM}$$

**Figure S5.** Michaelis-Menten plots for nucleotide incorporation opposite guanine of the undamaged substrate by yeast pol  $\eta$ .

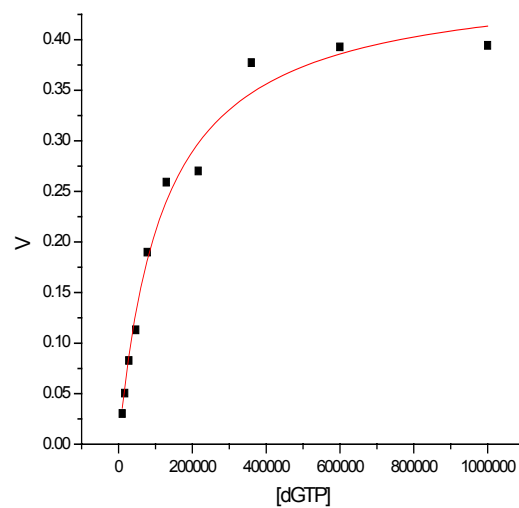
dNTP

Primer 5' - GCTAGGATCATAGCG ↓

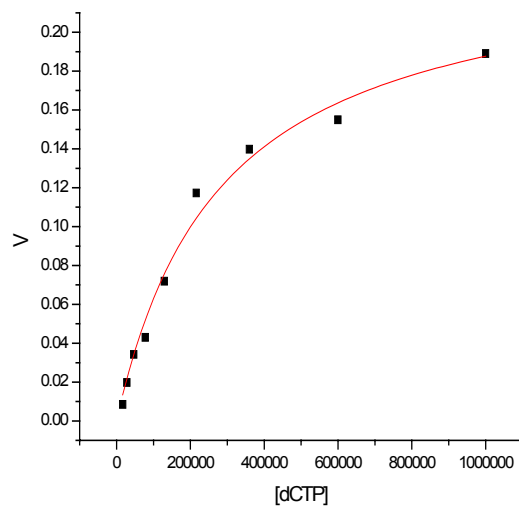
Template 3' - GATCCTAGTATCGC^GCGGTA -5'



$V_{\max} = 0.25 \text{ nM/min}$   
 $K_m = 180 \text{ uM}$



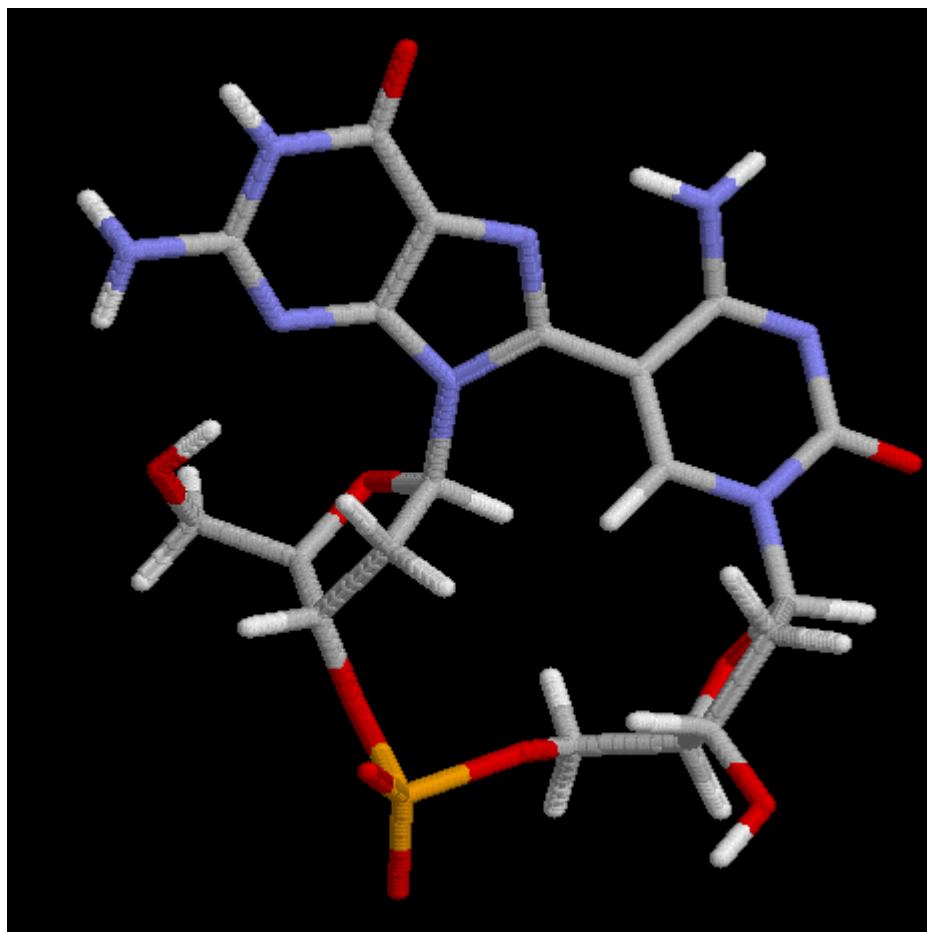
$V_{\max} = 0.46 \text{ nM/min}$   
 $K_m = 120 \text{ uM}$



$V_{\max} = 0.24 \text{ nM/min}$   
 $K_m = 280 \text{ uM}$

$V_{\max} = 0.055 \text{ nM/min}$   
 $K_m = 390 \text{ uM}$

**Figure S6.** Michaelis-Menten plots for nucleotide incorporation opposite the guanine portion of G[8-5]C by yeast pol  $\eta$ .



**Figure S7.** Structure of d(G[8-5]C) as optimized by semi-empirical PM3 method.