Supplementary Material

Site-specific synthesis of oligonucleotides containing 6-oxo-M_1dG, the genomic metabolite of M_1dG, and LC-MS/MS analysis of its *in vitro* bypass by human polymerase ι

Plamen P. Christov[‡,†], Robyn Richie-Jannetta[‡,‡], Philip J. Kingsley[‡,‡], Anoop Vemulapalli[‡], Kwangho Kim[†], Gary A. Sulikowski[†], Carmelo J. Rizzo[§], Amit Ketkar[|], Robert L. Eoff[#], Carol A. Rouzer[‡], and Lawrence J. Marnett[†,‡,]*

[†]Department of Chemistry, Vanderbilt University; Vanderbilt Institute of Chemical Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, United States

[‡]A. B. Hancock, Jr., Memorial Laboratory for Cancer Research, Departments of Biochemistry, and Pharmacology, Vanderbilt Institute of Chemical Biology, and Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, United States

[§]Departments of Chemistry and Biochemistry, Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee 37235

[|]Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205, United States

Table of Contents

Item	Page Number
Figure S1. ^{1}H NMR (DMSO-d_6) of compound **9**.	S2
Figure S2. ^{13}C NMR (DMSO-d_6) of compound **9**.	S3
Figure S3. ^{1}H NMR (DMSO-d_6) of compound **11**.	S4
Figure S4. ^{1}H-^{1}H COSY NMR (DMSO-d_6) of compound **11**.	S5
Figure S5. ^{13}C NMR (DMSO-d_6) of compound **11**.	S6
Figure S6. ^{1}H NMR (CD_3CN) of compound **12**.	S7
Figure S7. ^{31}P NMR (CD_3CN) of compound **12**.	S8
Figure S8. ^{13}C NMR (CD_3CN) of compound **12**.	S9
Figure S9. HPLC chromatogram and mass spectrum of the 6-oxo-M_1dG-containing oligonucleotides.	S10
Figure S10. ^{1}H NMR (DMSO-d_6) of compound **10**.	S11
Figure S11. ^{1}H-^{1}H COSY NMR (DMSO-d_6) of compound **10**.	S12
Figure S12. LC-MS/MS chromatogram, HPLC chromatogram, and UV spectra of individual nucleosides of an enzymatic digestion of the 6-oxo-M_1dG-containing oligonucleotide.	S13
Figure S13. Single nucleotide incorporation opposite dG (control template) and 6-oxo-M_1dG.	S14
Figure S14. Fragmentation scheme for oligonucleotides from McCluckey at al.	S15
Figure S15. Michaelis-Menten plots obtained from LC-MS/MS analyses.	S16
Table S1. Q1 and Q3 values of the SRM transitions for each oligonucleotide.	S17
Table S2. Steady state kinetic parameters for insertion of dCTP and dTTP opposite dG and 6-oxo-M_1dG.	S18

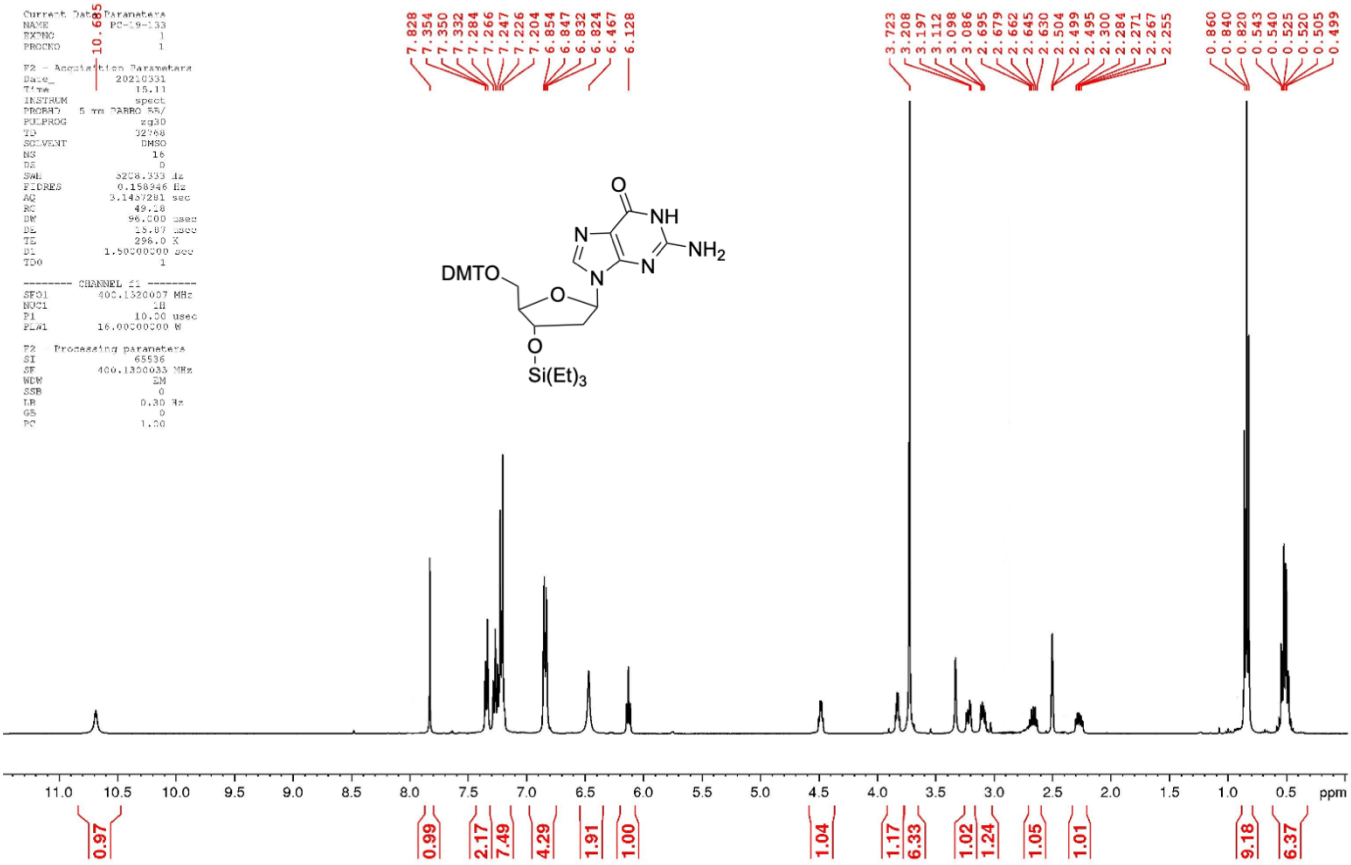


Figure S1. ^{1}H NMR (DMSO-d_6) of compound **9**.

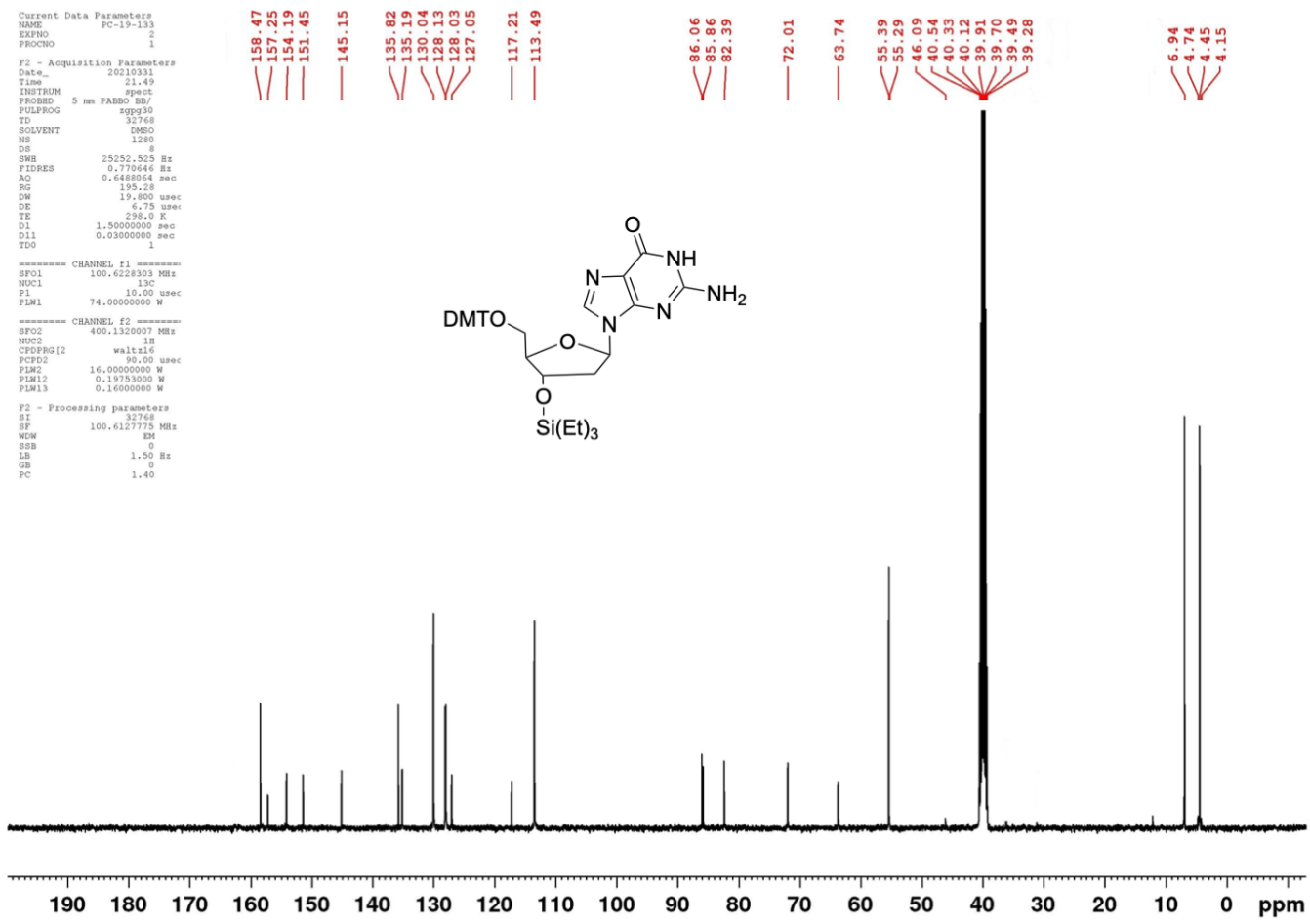


Figure S2. ^{13}C NMR (DMSO-d_6) of compound **9**.

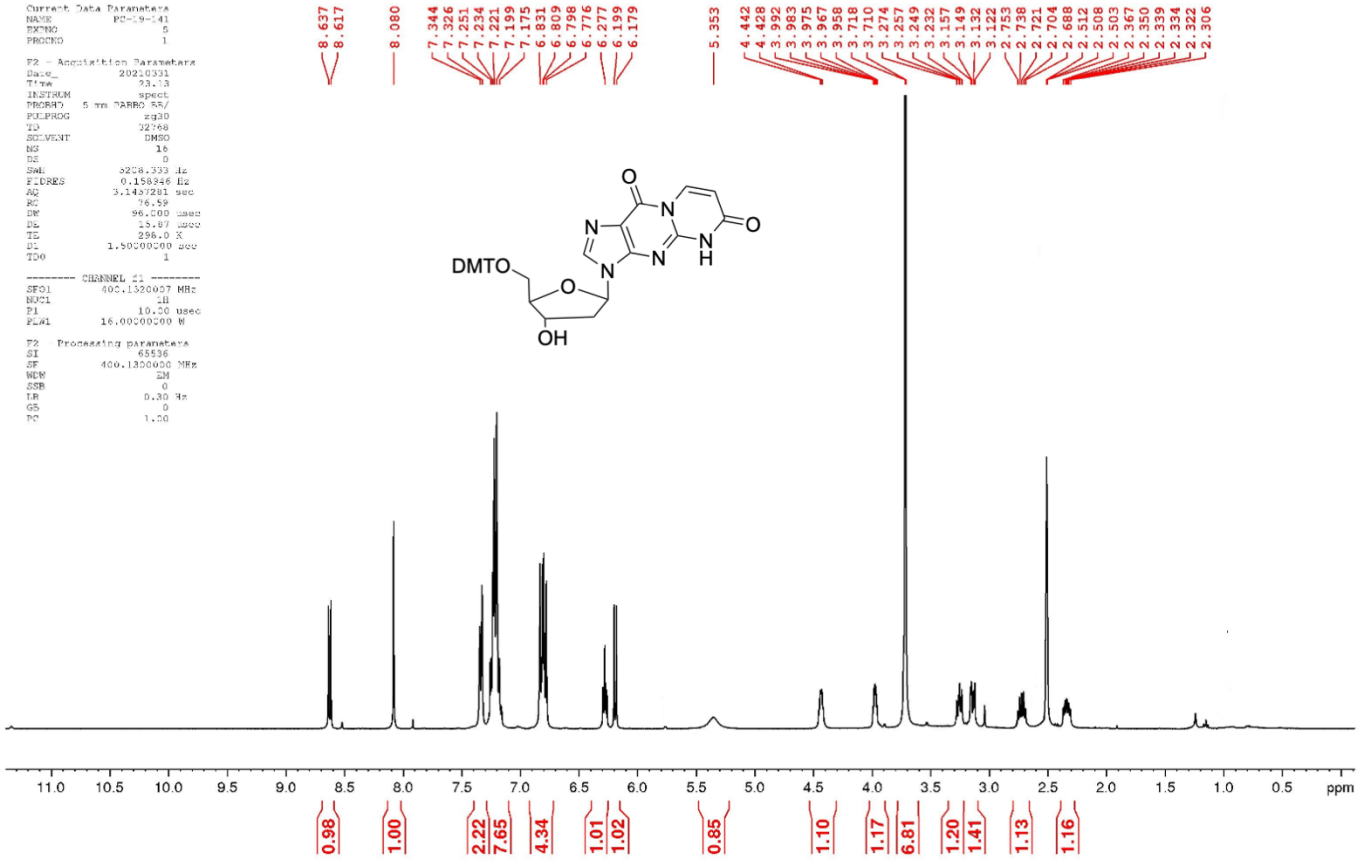


Figure S3. ^{1}H NMR (DMSO-d_6) of compound **11**.

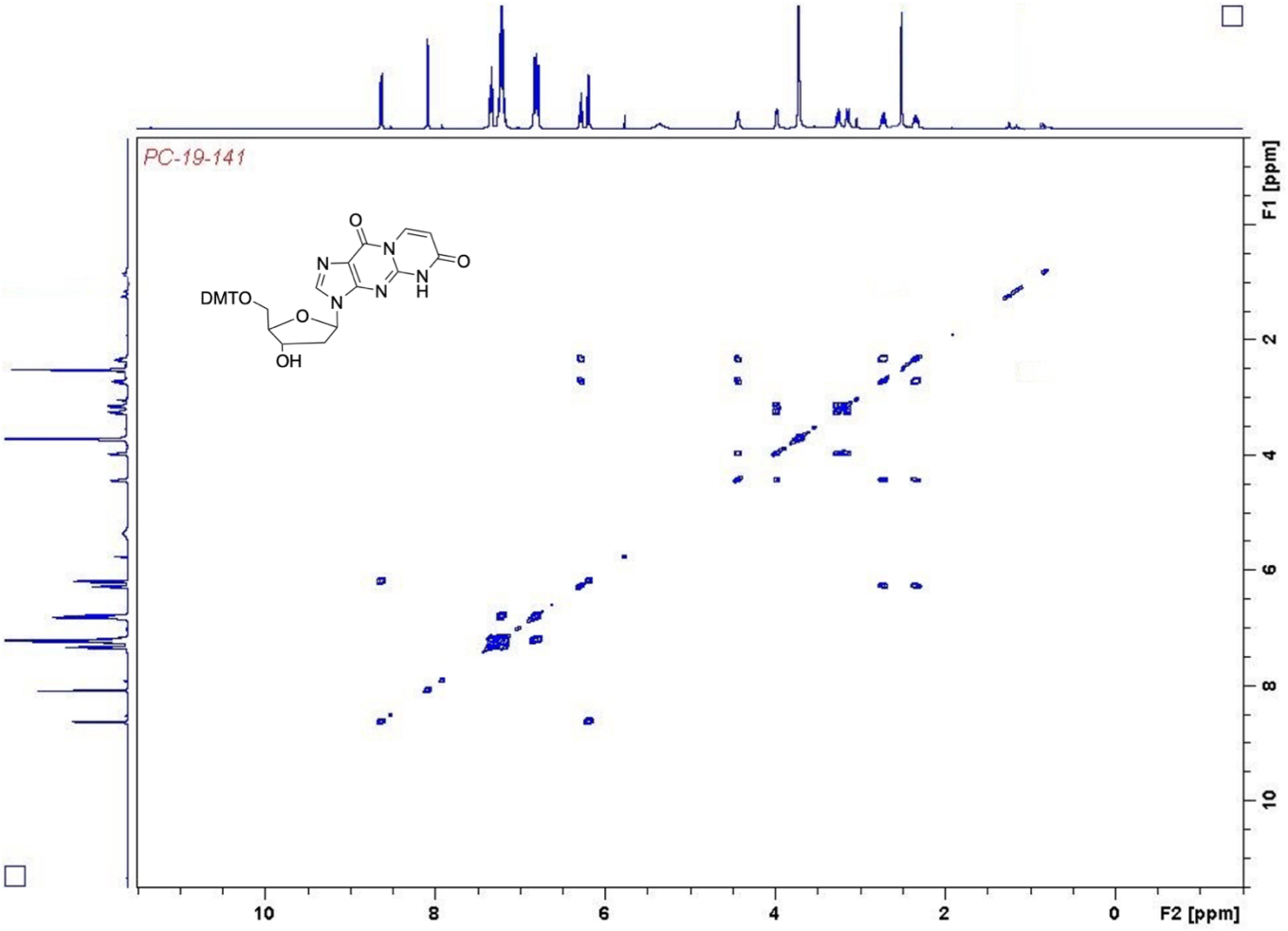


Figure S4. ^{1}H-^{1}H COSY NMR (DMSO-d_6) of compound **11**.

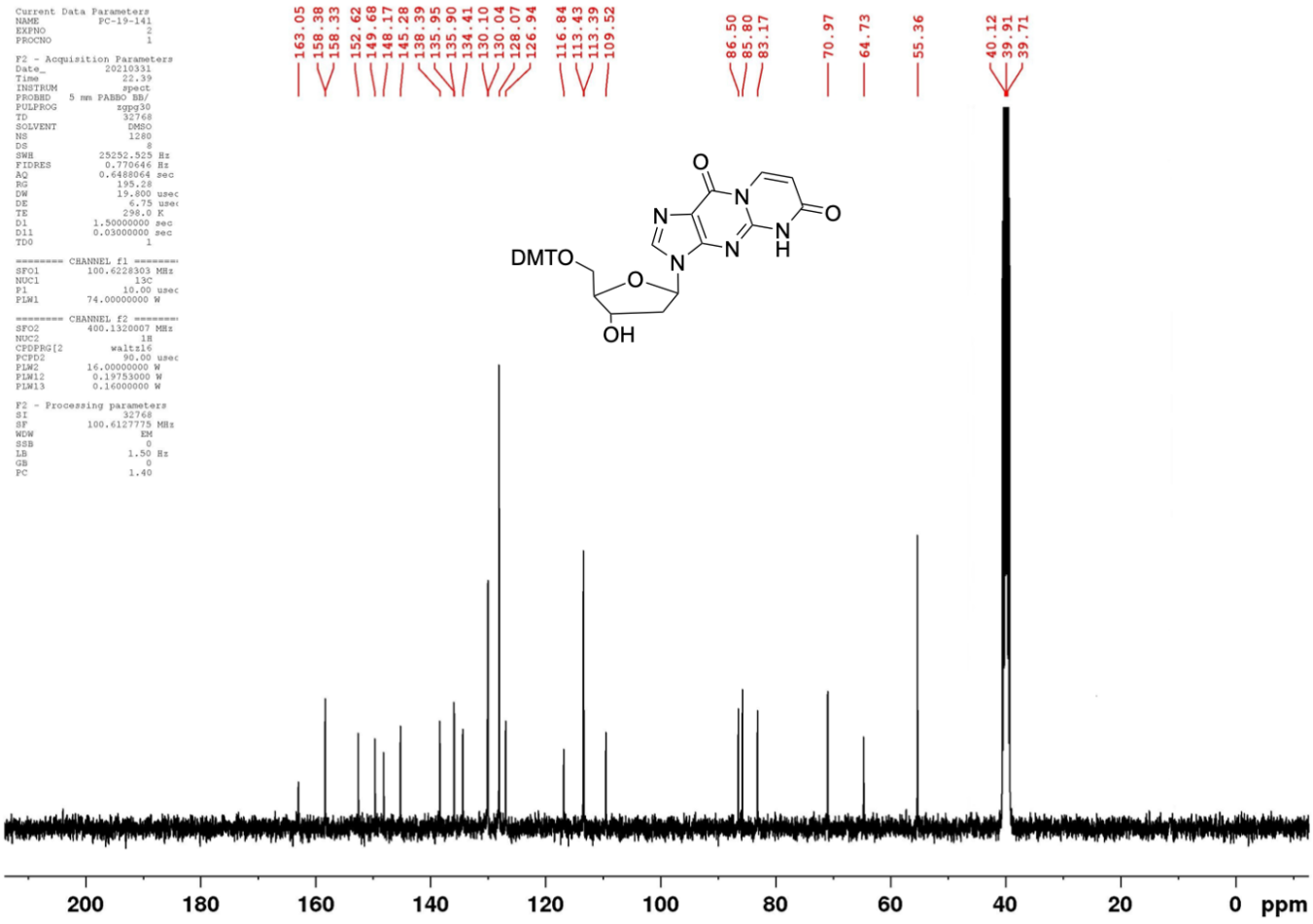


Figure S5. ^{13}C NMR (DMSO-d_6) of compound **11**.

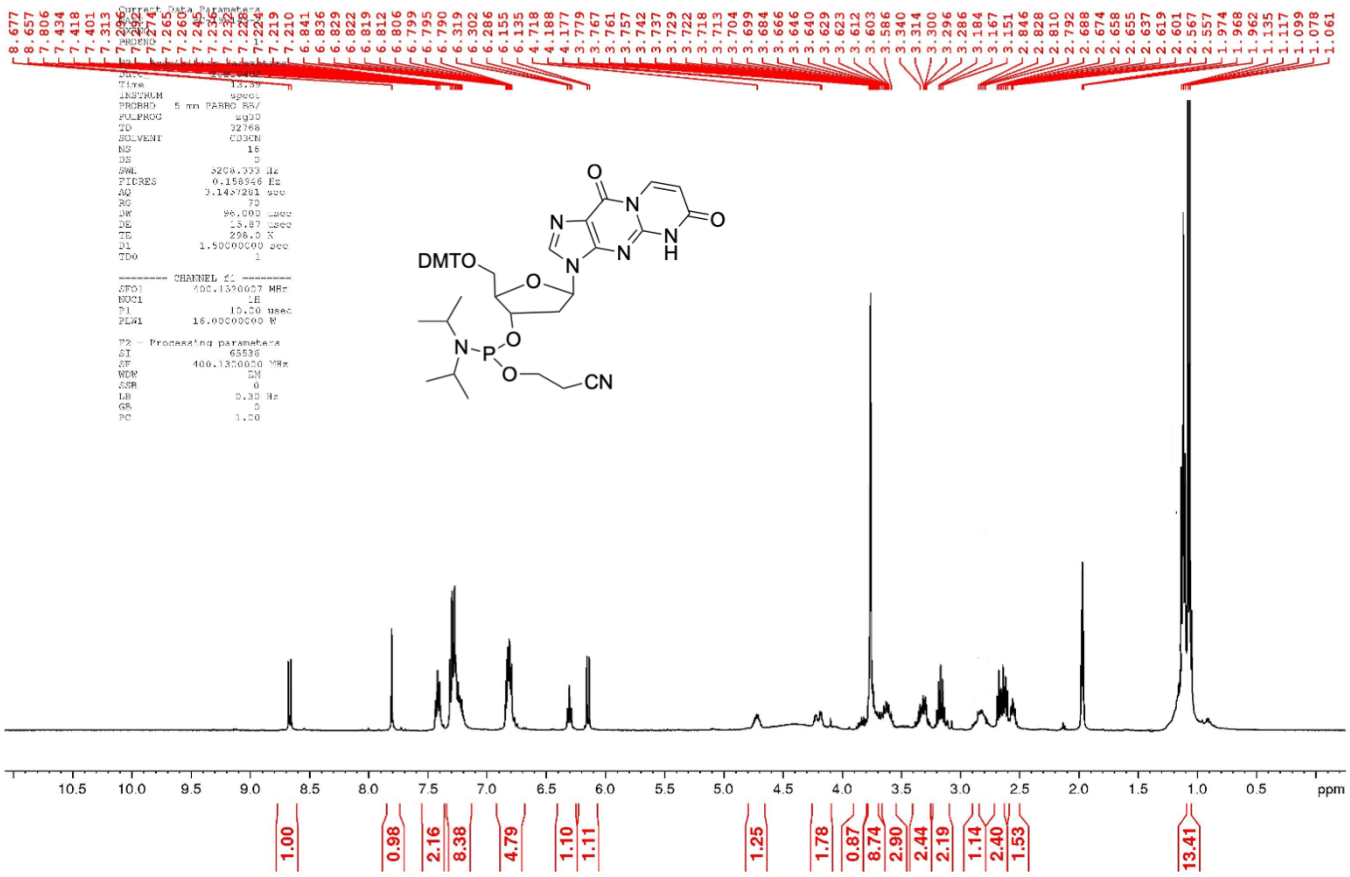
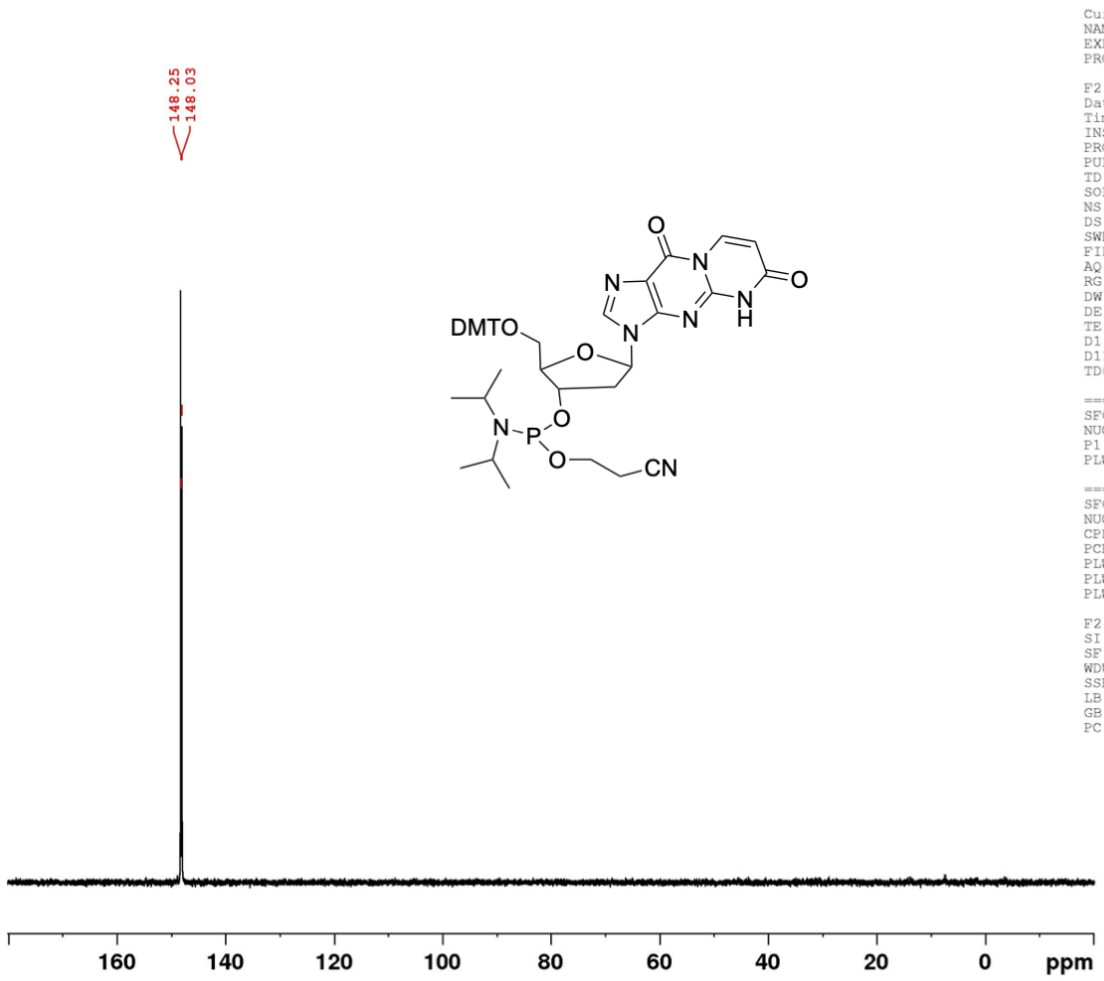


Figure S6. ^{1}H NMR (CD_3CN) of compound **12**.




```
Current Data Parameters
NAME          PC-19-145-1
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_           20210401
Time              15.11
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                32768
SOLVENT           CD3CN
NS                  256
DS                    8
SWH           32467.533 Hz
FIDRES         0.990830 Hz
AQ             0.5046272 sec
RG                195.28
DW                15.400 usec
DE                 7.20 usec
TE                298.0 K
D1           1.50000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         161.9885511 MHz
NUC1                31P
P1                 8.00 usec
PLW1        59.00000000 W

======== CHANNEL f2 ========
SFO2         400.1320007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2        16.00000000 W
PLW12        0.19753000 W
PLW13        0.16000000 W

F2 - Processing parameters
SI                32768
SF           161.9755930 MHz
WDW                 EM
SSB                  0
LB                1.50 Hz
GB                   0
PC                1.40
```

Figure S7. ^{31}P NMR (CD_3CN) of compound **12**.

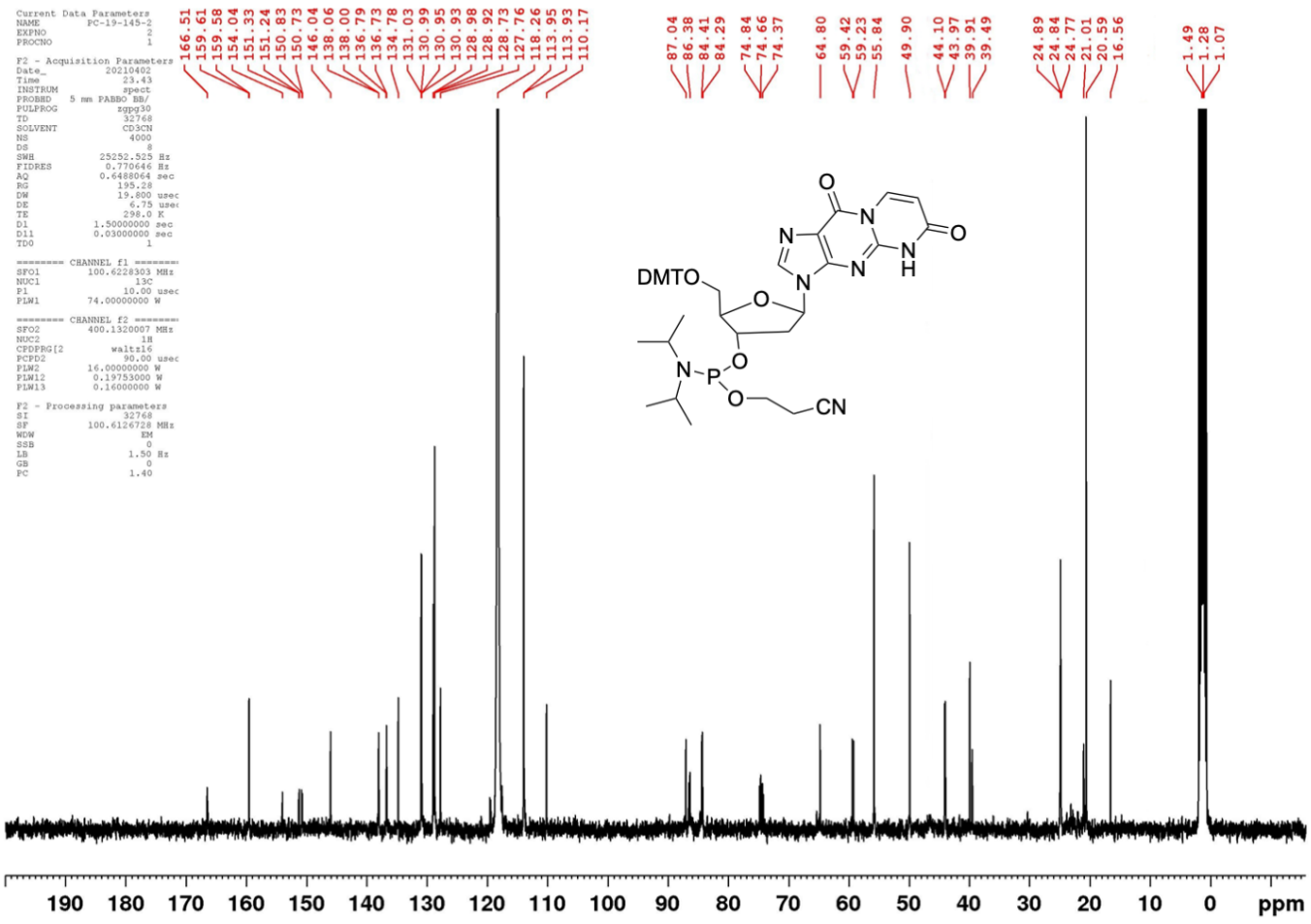


Figure S8. ^{13}C NMR (CD_3CN) of compound **12**.

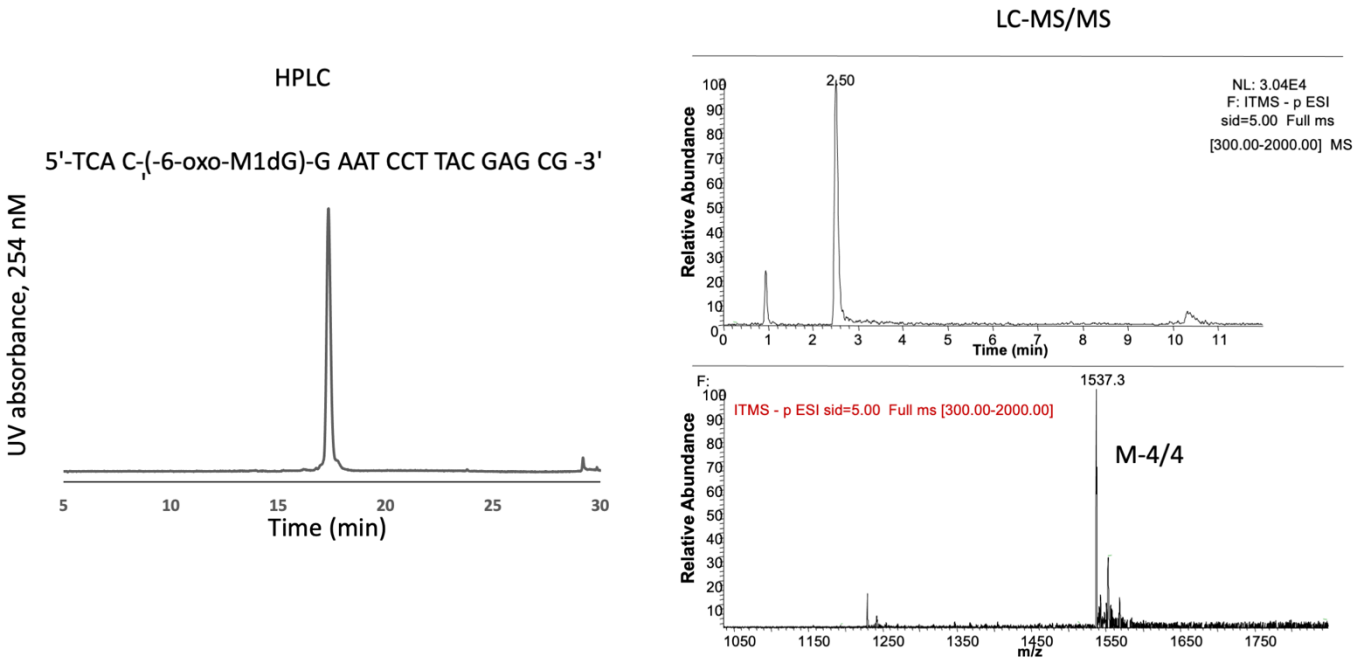


Figure S9. HPLC chromatogram and mass spectrum of the 6-oxo-M_1dG-containing oligonucleotides.

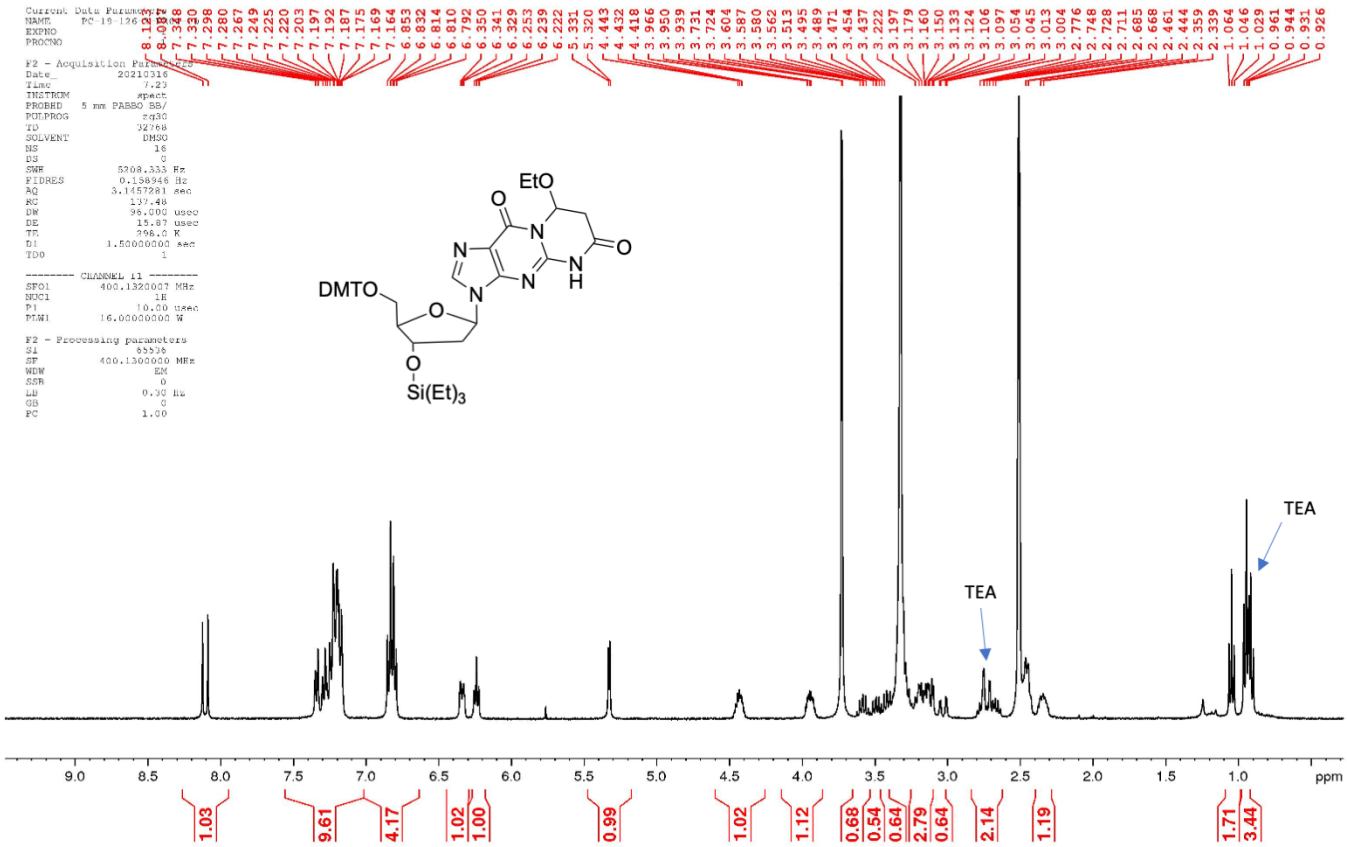


Figure S10. ^{1}H NMR (DMSO-d_6) of compound **10**.

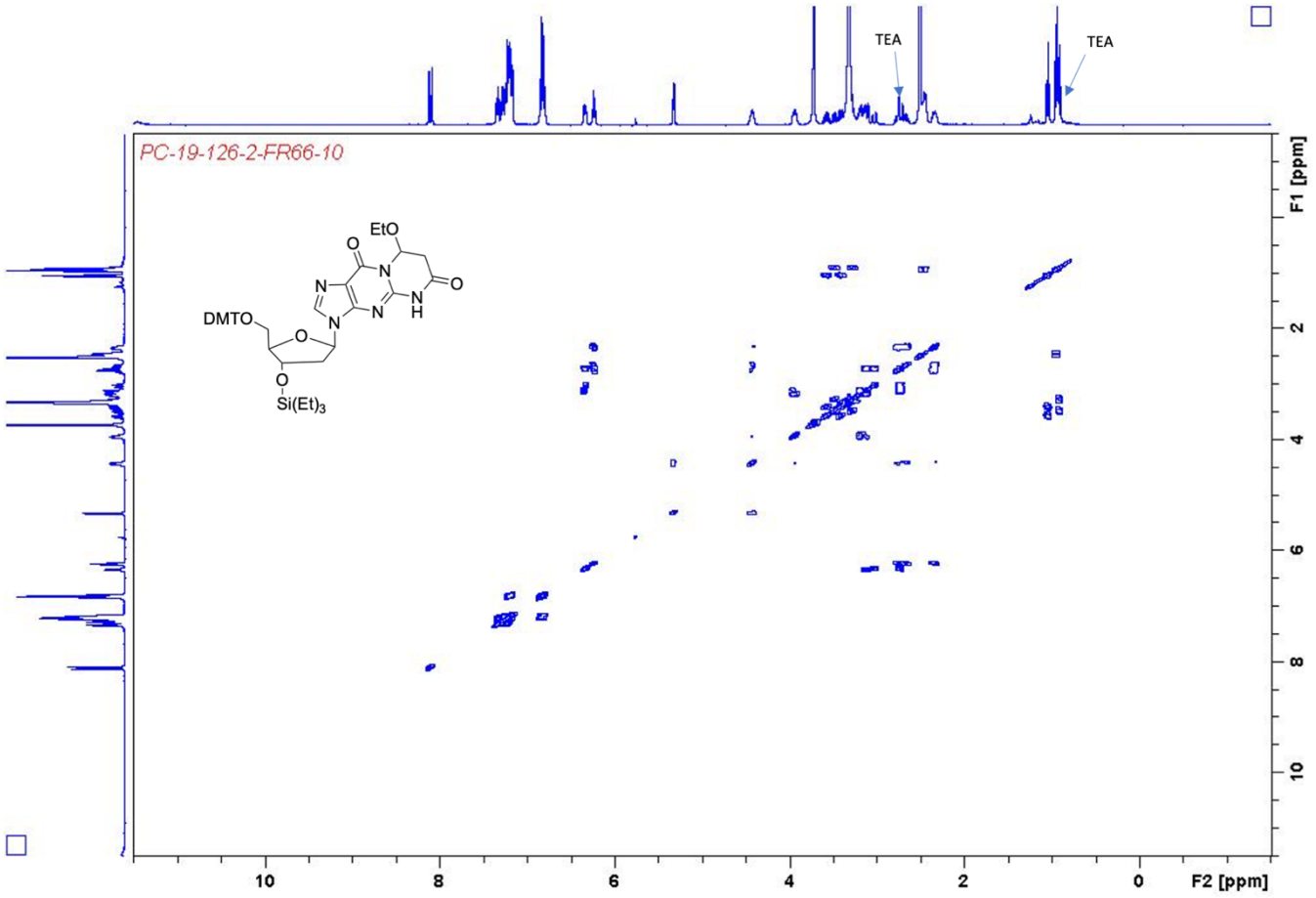


Figure S11. ^{1}H-^{1}H COSY NMR (DMSO-d_6) of compound **10**.

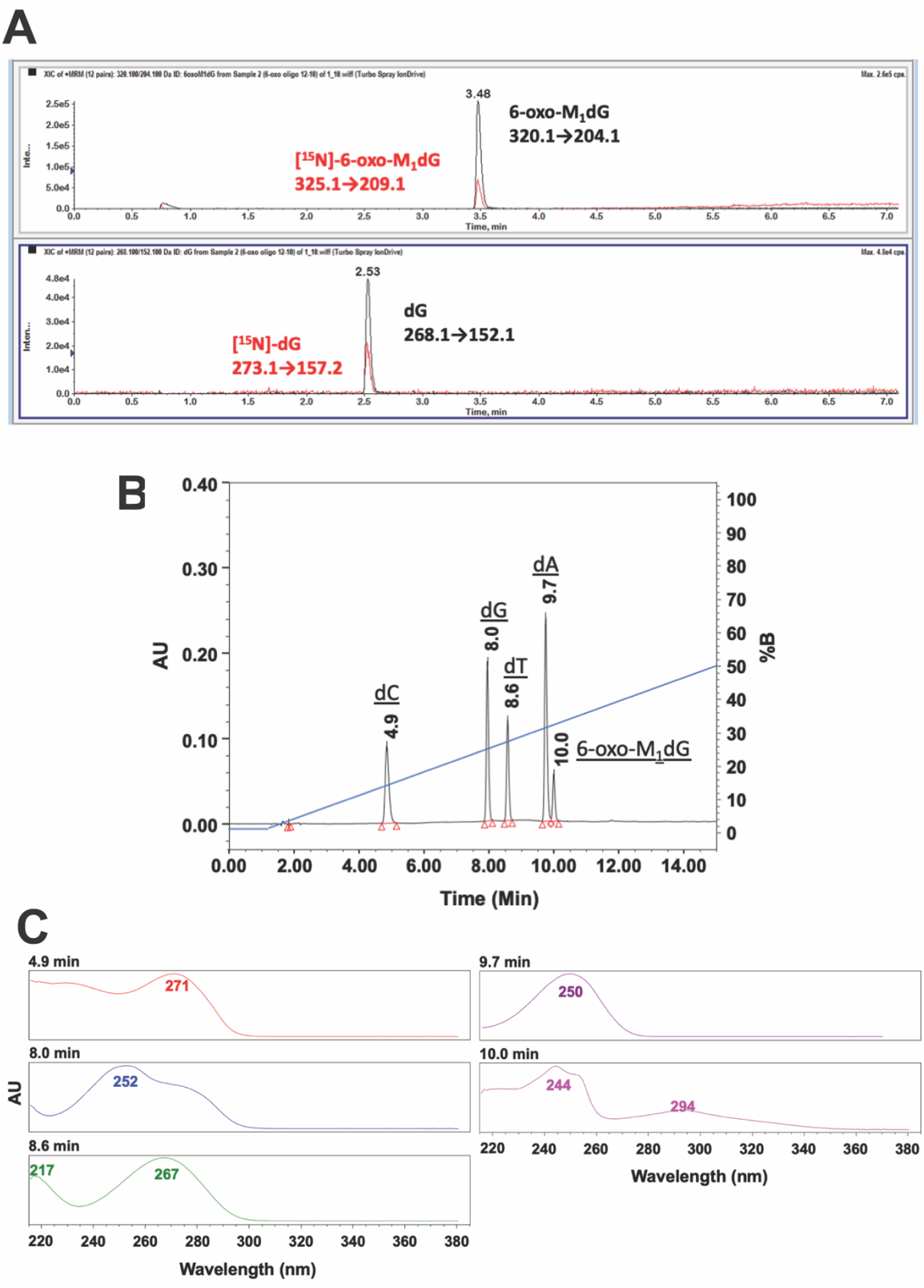


Figure S12. LC-MS/MS chromatogram (A), HPLC chromatogram (B), and UV spectra of individual nucleosides (C) of an enzymatic digestion of the 6-oxo-M_1dG-containing oligonucleotide: dC (4.9 min), dG (8.0 min), dT (8.6 min), dA (9.7 min), 6-oxo-M_1dG (10.0 min).

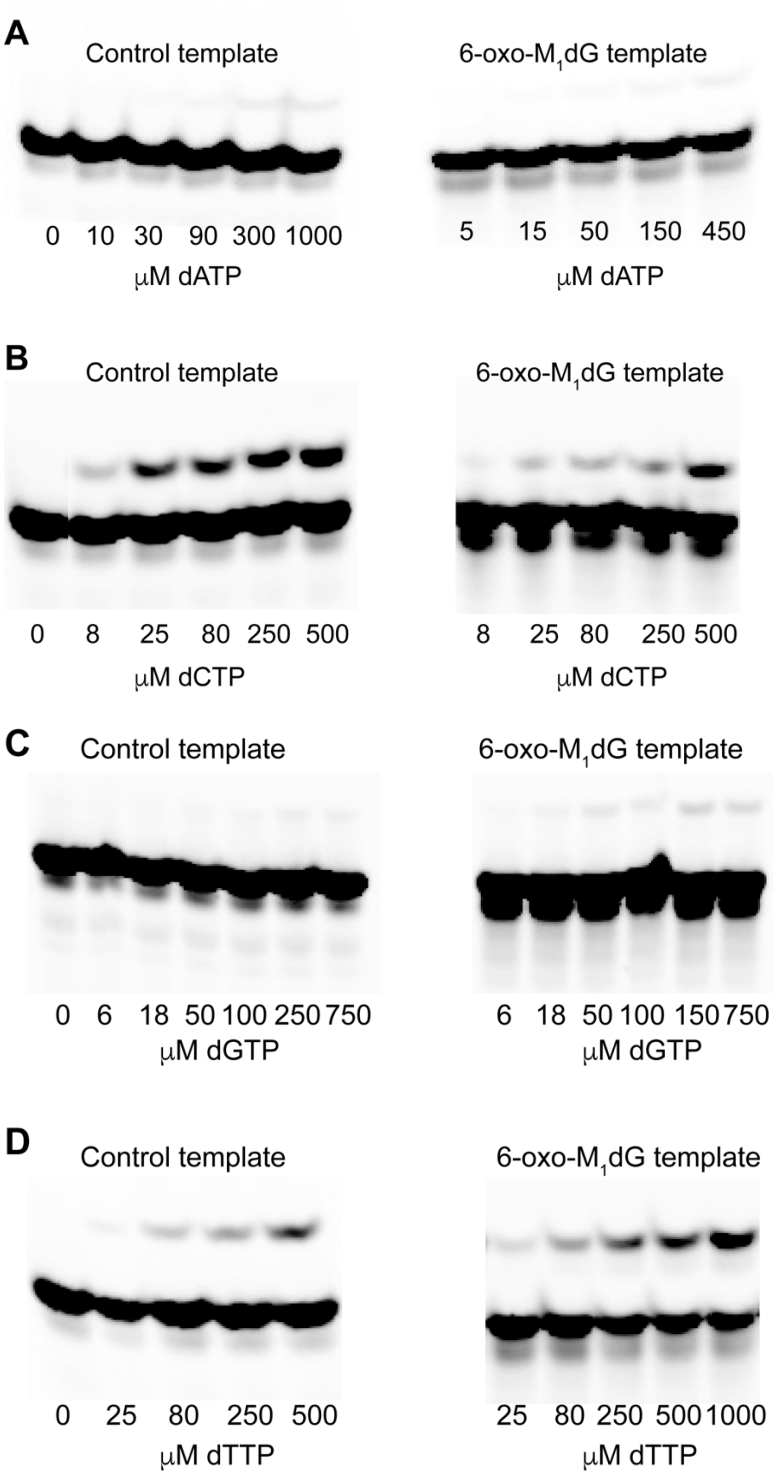


Figure S13. Single nucleotide incorporation opposite dG (control template) and 6-oxo-M_1dG. **h**Pol ι (65 nM for control duplex or 82 nM for 6-oxo-M_1dG duplex) was incubated with 5 μM DNA duplex and increasing concentrations of nucleotides. The reaction was stopped at 6 min for the control duplex and 11 min for the 6-oxo-M_1dG duplex. (A) Incorporation of dATP. (B) Incorporation of dCTP. (C) Incorporation of dGTP. (D) Incorporation of dTTP.

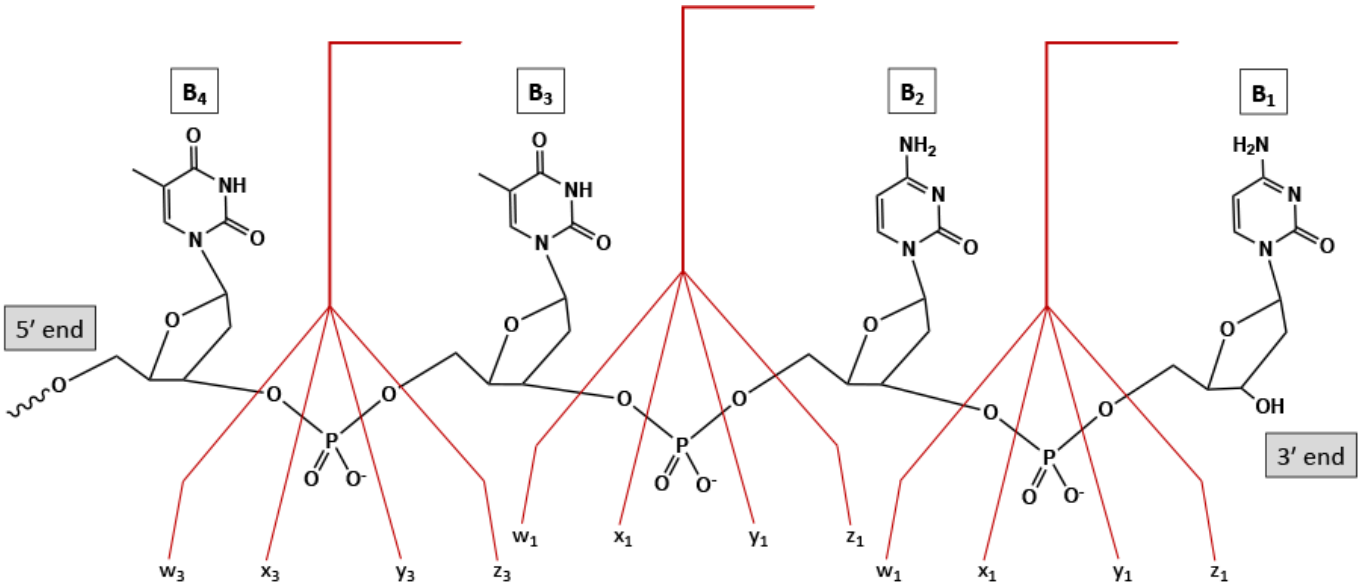


Figure S14. Fragmentation scheme for oligonucleotides from McCluckey at al. (1). Only the fragments containing the 3′ end are shown. The w- and y- ions were widely observed in our mass spectrometry system. Also, multiple charge states of larger fragments (bases 3 and up) were often seen.

Reference

1. McLuckey, S.A., Van Berkel, G.J. and Glish, G.L. (1992) Tandem mass spectrometry of small, multiply charged oligonucleotides. *J Am Soc Mass Spectrom*, **3**, 60-70.

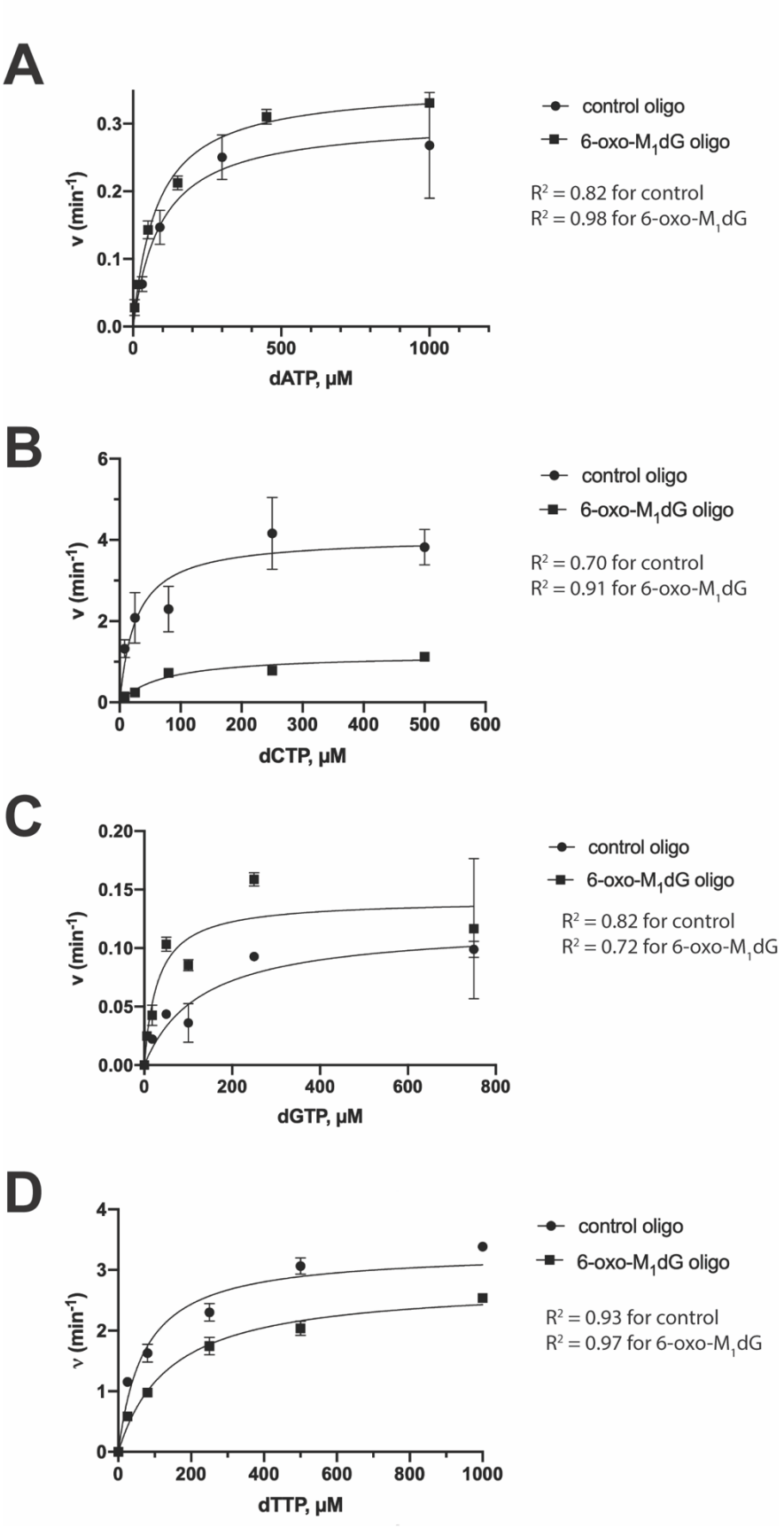


Figure S15. Michaelis-Menten plots obtained from the LC-MS/MS analysis of samples generated during the experiments described in the legend to Figure S13.

Table S1. Q1 and Q3 values of the SRM transitions for each oligonucleotide. Sequences of the oligonucleotides are provided in Supplementary Table 2.

Oligo	Q1	*z*	Q3	frag	*z*
FAM	637.0	8	306.1	w-1	1
			834.2	y-3	1
FAM+1 (16C)	673.1	8	306.1	w-1	1
			614.1	w-6	1
16T	675.1	8	321.1	w-1	1
			702.4	y-7	3
16A	600.9	9	586.1	Depur	9
			705.5	y-7	3
16G	678.1	8	661.5	Depur	8
			635.1	w-2	1
FAM+2	714.1	8	510.2	w-5	3
			765.6	w-5	2
FAM+3	752.1	8	650.2	w-2	1
			735.6	Depur	8
19C	705.2	9	346.1	w-1	1
			650.1	w-2	1
Control	761.8	8	631.1	w-6	3
			762.6	y-5	2

Table S2. Steady state kinetic parameters for insertion of dCTP and dTTP opposite dG and 6-oxo-M_1dG by human Pol ι as obtained from analyzing bands on denaturing SDS gel. For comparison, data are provided for an M_1dG template from reference 19.

Nucleotide	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1}min^{-1}$)
dCTP	4.1 ± 0.2	14 ± 4	0.29
dTTP	3.3 ± 0.2	71 ± 15	0.046
Control template			

Nucleotide	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1}min^{-1}$)
dCTP	1.4 ± 0.1	33 ± 9	0.043
dTTP	2.8 ± 0.1	140 ± 20	0.020
6-oxo-M_1dG template			

Nucleotide	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1}min^{-1}$)
dCTP	0.34 ± 0.003	2.4 ± 1.0	0.014
dTTP	0.028 ± 0.003	24 ± 14	0.001
M_1dG template			