Supporting Information: Irreversible Inhibition of DNA Polymerase β by an Oxidized Abasic Lesion.

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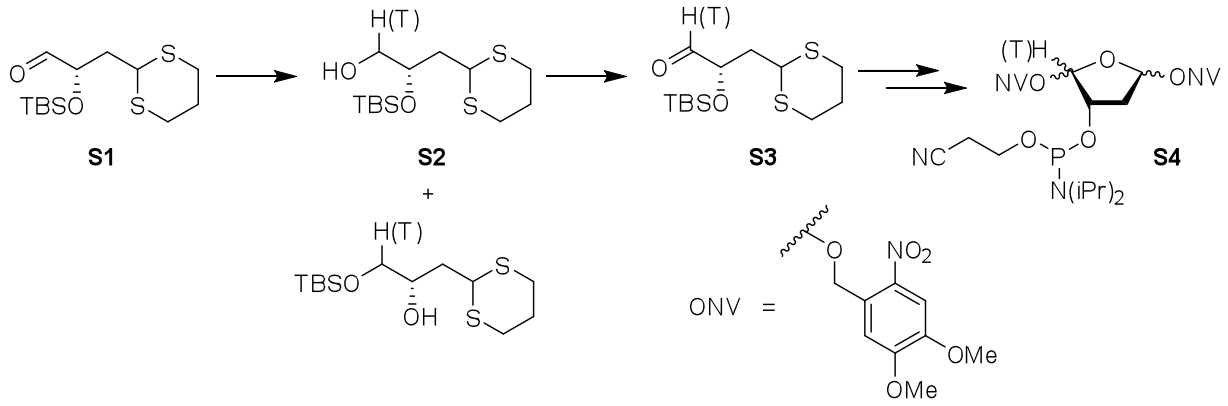
Contents:

General Methods. Oligonucleotides were synthesized on an Applied Biosystems Incorporated 394 oligonucleotide synthesizer. Oligonucleotide synthesis reagents were purchased from Glen Research (Sterling, VA). All chemicals were purchased from either Sigma-Aldrich or Acros and were used without further purification. ESI-MS analysis was carried out on a LCQ-Deca Ion Trap. All Oligonucleotides were precipitated from 1.25 M ammonium

acetate (pH 5.6) prior to MS analysis. NaB^3H_4 was from Perkin Elmer. Terminal deoxytransferase and uracil-DNA glycosylase were obtained from New England Biolabs. α-^{32}P-cordycepin 5′-triphosphate was purchased from Perkin Elmer. C_{18}-Sep-Pak cartridges were obtained from Waters. Quantification of radiolabeled oligonucleotides was carried out using a Molecular Dynamics Phosphorimager 840 equipped with ImageQuant Version 5.1 software. All photolyses of oligonucleotide were carried out in clear eppendorf tubes in a Rayonet photoreactor (RPR-100) fitted with 16 lamps having an output maximum at 350 nm. In all experiments



Phosphoramidite S4. The tritium labeled DOB precursor **S4** was synthesized from compound **S3** using established methods.[1] The ^{1}H NMR ($CDCl_3$) and ^{31}P NMR ($CDCl_3$) of **S4** matches previously reported data.[1] The synthesis of **S3** is described below.

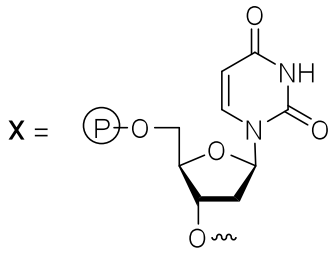
Compound S2. MeOH was refluxed with $NaBH_4$ while gently bubbling argon through it for 24 h at 30 °C. Sodium was then added and the solution was refluxed for another 24 h. MeOH was distilled and kept over 4 Å molecular sieves. Compound **S1**[1] (200 mg, 0.645 mmol) in distilled MeOH (2 mL) was added to NaB^3H_4 (8.2 Ci/mmol, 100 mCi). After stirring at 25 °C for 1 h. $NaBH_4$ (30 mg, 0.8 mmol) was added. The resulting mixture was stirred at the same

temperature for 0.5 h. The reaction mixture was diluted with diethyl ether and washed with saturated NH_4Cl, followed by brine. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography (EtOAc : hexanes = 1 : 4) to give **S2** as a 1:1 mixture of two isomers (192 mg, 95% total). The mixture contained 50 % desired product and 50 % undesired product with silyl migration to the primary alcohol. ^{1}H NMR ($CDCl_3$) δ 0.08-0.15 (3s, 6H), 0.91-0.92 (2s, 12H), 1.78-2.05 and 2.47 (m, 4H), 2.12 (m, 1H), 2.86 (m, 4H), 3.46 (m, 1H), 3.63 (m, 1H), 3.98 and 4.31 (m, 1H), 4.07 (m, 1H).

Compound S3. The mixture of **S2** (192 mg, 0.623 mmol), Dess-Martin periodinane (396 mg, 0.94 mmol), and anhydrous K_2CO_3 (138 mg, 1 mmol) in CH_2Cl_2 (6 mL) was stirred at 25 °C for 30 min. A mixture of saturated $Na_2S_2O_3$ and saturated $NaHCO_3$ (1:4) was added to the reaction mixture and stirred vigorously for 5 min. The organic phase was washed with saturated $NaHCO_3$, followed by brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (EtOAc : hexanes = 1 : 6) to give **S3** (91.8 mg, 48%, 554 Bq/nmol). ^{1}H NMR ($CDCl_3$) δ 0.11 (s, 6H), 0.95 (s, 12H), 1.97-2.08 (m, 2H), 2.21 (m, 2H), 2.77-2.80 (m, 6H), 4.08 (m, 1H), 4.26 (m, 1H), 9.68 (s, 1H).

5'-d(**X**TG TGT GGA GCT GTG GCG G)

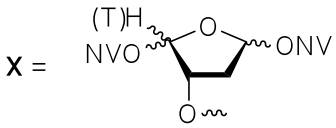
S5



Synthesis of Oligonucleotide S5 containing AP Lesion Precursor. Standard synthesis cycles (25 s coupling, 5 s capping with acetic anhydride, 15 s oxidation with 1 M iodine in THF, 95 s detritylation with 3% TCA in methylene chloride) were used to incorporate phosphoramidites including all four native phosphoramidites, 2'-deoxyuridine phosphoramidite and the chemical phosphorylation reagent (Glen Research). After AMA (conc. aq. ammonia : methylamine v/v 1:1) deprotection (65 °C, 10 min), the oligonucleotide was purified by 20% denaturing PAGE.

5'-d(**X**TC GTA ATG CAG TCT)

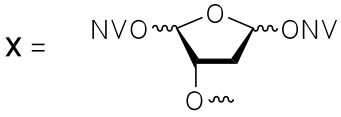
S6



Synthesis of Oligonucleotide (S6) Containing DOB Lesion Precursor. Standard synthesis cycles (25 s coupling, 5 s capping with acetic anhydride, 15 s oxidation with 0.1 M iodine in THF, 95 s detritylation with 3% TCA in methylene chloride) were used prior to incorporating the lesion precursor. The phosphoramidite of the tritium labeled lesion (**S4**) was incorporated manually with 5 min coupling, 25 s capping, 40 s oxidation using the same synthesis reagents. After AMA (conc. aq. ammonia : methylamine v/v 1:1) deprotection (65 °C, 10 min), the oligonucleotide was purified by 20% denaturing PAGE.

5'-d(TAA TGG CTA ACG CAA **X**TC GTA ATG CAG TCT)
3'-d(ATT ACC GAT TGC GTT AAG CAT TAC GTC AGA)

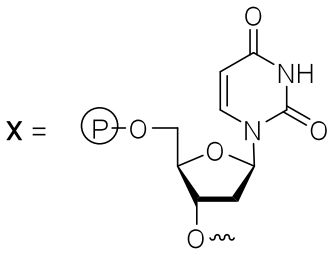
S7



The preparation of ternary complex 1. Freshly hybridized ternary complex 3'-^{32}P-**S7** (2 μM) in HEPES buffer (50 mM, 100 mM NaCl, pH 7.2) was photolyzed for 60 min at 25 °C. The resulting solution of **1** was used immediately. Passage of the photolyzed ternary complex through Sephadex G-25 prior to incubation with Pol β had not effect on inhibition.

5'-d(CCC CGA CCG GCT CGT ATG **X**TG TGT GGA GCT GTG GCG G)
3'-d(GGG GCT GGC CGA GCA TAC AAC ACA CCT CGA CAC CGC C)

S8



The preparation of ternary complex 2. The hybridized ternary complex 3'-^{32}P-**S8** (1 μM) was incubated with uracil-DNA glycosylase (UDG) (155 units) in UDG reaction buffer (100 μL, 20 mM Tris-HCl, pH 8.0, 1 mM DTT, 1 mM EDTA) at 37 °C for 10 min. The resulting solution of **2** was used immediately without further purification.

Pol β active site titration essay. Freshly prepared ternary 3'-^{32}P-**2** (500 nM) was incubated with Pol β (5 nM, 10 nM and 15 nM) in HEPES buffer (50 mM, pH 7.4, containing 5 mM $MgCl_2$) at 25 °C for 20 min. Aliquots (5 μL) was removed at the indicated time and quenched with $NaBH_4$ (2 μL, 500 mM) at 25 °C. for 1 h. The samples were mixed with formamide loading buffer (15 μL, 90%, 10 mM EDTA). An aliquot of the mixture (5 μL) was loaded on to a 20% denaturing polyacrylamide gel and the product was analyzed using a phosphorimager. The amount of product versus time was fit using the equation: $y = A_0*(1 - \exp(-a*x)) + b*x$. The percentage of active Pol β (48.3 ± 3.8 %) was calculated from A_0/[Pol β].

Pol β lyase reaction with 1 and 2. The freshly prepared ternary complex 3'-^{32}P-**1** or 3'-^{32}P-**2**

(200 nM) was incubated with Pol β (5 nM) in HEPES buffer (200 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$) at 37 °C. Aliquots (5 μL) were removed at the indicated times and quenched with $NaBH_4$ (2 μL, 500 mM) at 25 °C for 1 h. The samples were mixed with formamide loading buffer (15 μL, 90%, 10 mM EDTA). An aliquot of the mixture (5 μL) was loaded on to a 20% denaturing polyacrylamide gel and the product was analyzed using a phosphorimager.

Stepwise Inhibition of Pol β lyase reaction by DOB. Freshly prepared ternary complex 3'-^{32}P-**1** (200 nM) was incubated with Pol β (5 nM) in HEPES buffer (200 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$) at 37 °C. Aliquots (5 μL) were removed at the indicated times and quenched with $NaBH_4$ (2 μL, 500 mM) at 25 °C for 1 h. After 10 min, Pol β (10 μL, 100 nM, 1 pmol) was added to the solution. Aliquots (5 μL) were removed at the indicated times and quenched with $NaBH_4$. After another 10 min, Pol β (10 μL, 100 nM, 1 pmol) was added to the solution. Aliquots (5 μL) were removed at the indicated times and quenched with $NaBH_4$. The samples were mixed with formamide loading buffer (15 μL, 90%, 10 mM EDTA). An aliquot of the mixture (5 μL) was loaded on to a 20% denaturing polyacrylamide gel and the product was analyzed using a phosphorimager.

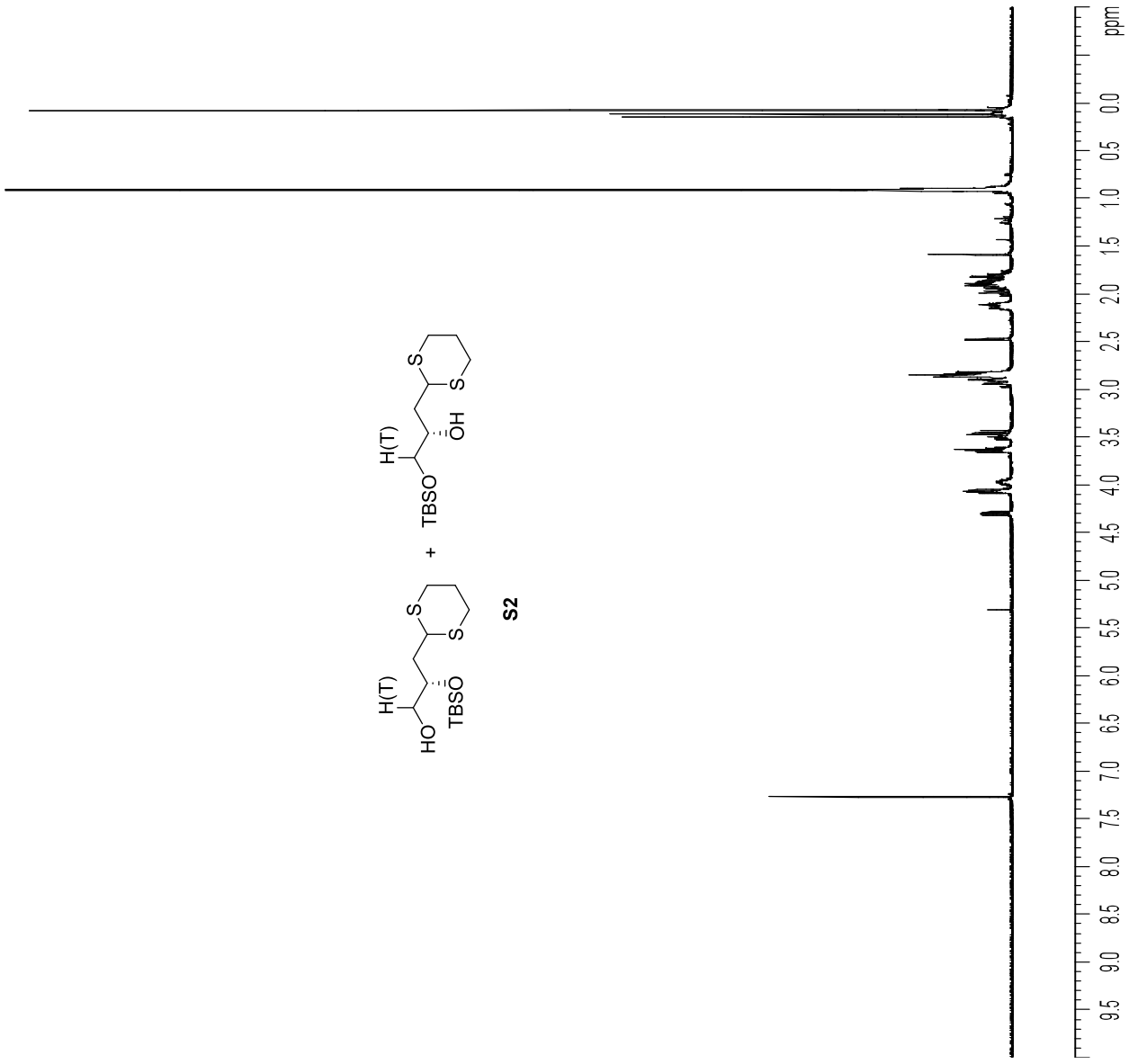
Measurement of IC_{50} of DOB. Freshly prepared ternary complex **1** (0, 10 nM, 20 nM, 40 nM, 60 nM, 100 nM, 200 nM, 400 nM, 600 nM, 1 μM) was incubated with Pol β (50 nM) in HEPES buffer (40 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$) at 25 °C for 3 min. Aliquots (5 μL) were added to 3'-^{32}P-**2** in HEPES buffer (95 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$). The resulting solutions (100 μL, Pol β: 2.5 nM; 3'-^{32}P-**2**: 100 nM; **1**: 0, 0.5 nM, 1 nM, 2 nM, 3 nM, 5 nM, 10 nM, 20 nM, 30 nM, 50 nM) were incubated at 37 °C for 10 min. After 10 min, the solution was quenched with $NaBH_4$ (10 μL, 500 mM) at 25 °C for 1 h. An aliquot of the reaction

solution (1 μL) was mixed with formamide loading buffer (4 μL, 90%, 10 mM EDTA) and loaded on to a 20% denaturing polyacrylamide gel. The product was analyzed using a phosphorimager.

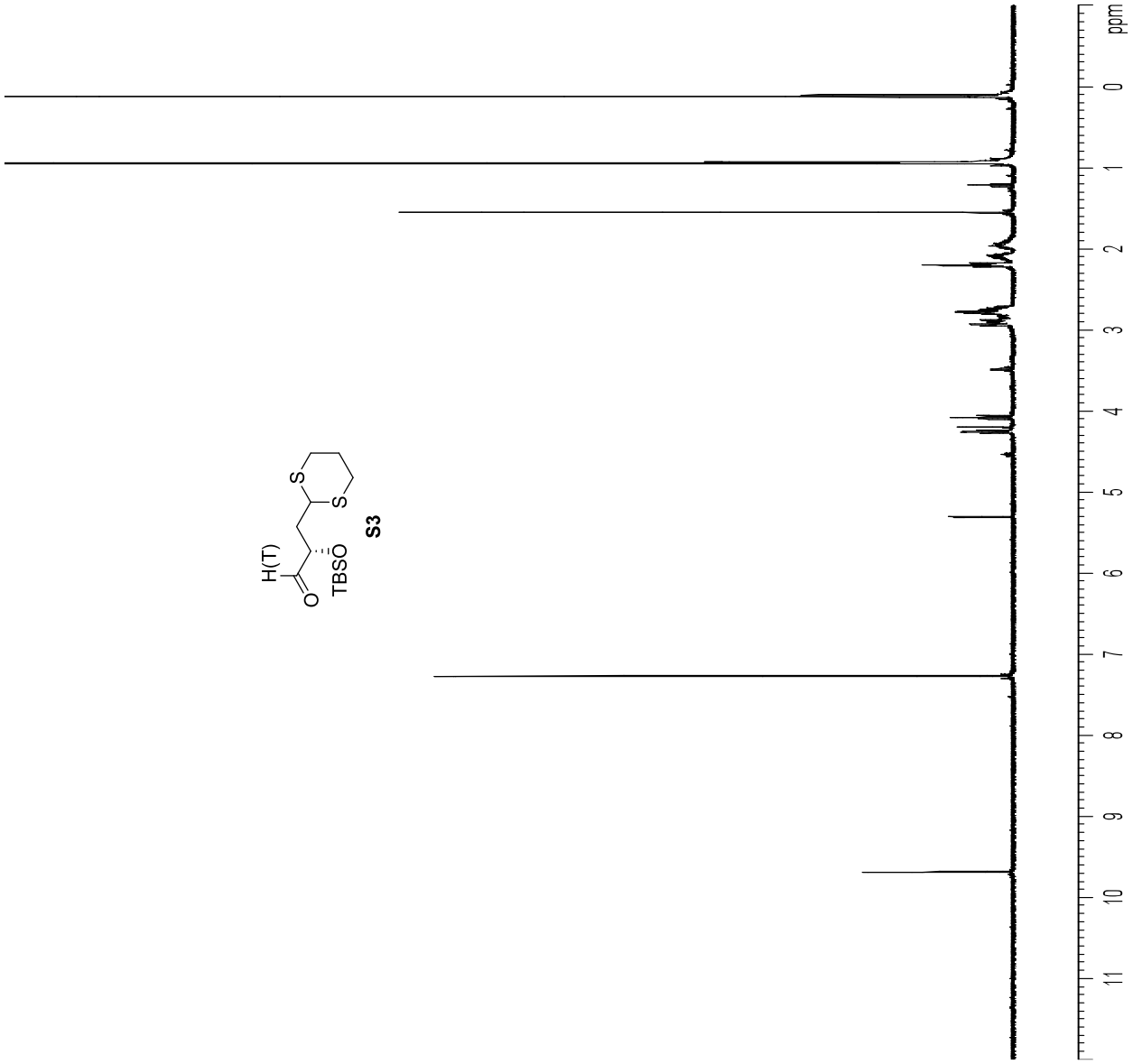
Time-dependent irreversible inhibition kinetics of Pol β. Freshly prepared ternary complex **1** with various concentrations (0 nM, 7.5 nM, 15 nM, 30 nM, 45 nM, 60 nM) were incubated with 3'-^{32}P-**2** (500 nM) and Pol β (7.5 nM) in HEPES buffer (100 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$) at 37 °C. Aliquots (10 μL) were removed at the indicated times and quenched with $NaBH_4$ (2 μL, 500 mM) at 25 °C for 1 h. The samples were mixed with formamide loading buffer (20 μL, 90%, 10 mM EDTA). An aliquot of the mixture (5 μL) was loaded on to a 20% denaturing polyacrylamide gel and the product was analyzed using a phosphorimager.

Phenol extraction assay. Ternary complex ^{3}H-**1**, 3'-^{32}P-**1** or 3'-^{32}P-**2** (125 nM) was incubated with Pol β (1.25 μM) or BSA (0.8 μM) in HEPES buffer (200 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$) at 37 °C for 2 h. Calf thymus DNA (1 μL, 10 μg) was then added and the resulting solution was incubated at 37 °C for 15 min. The solution was extracted with buffer-saturated (Tris-HCl, pH 7.5) phenol (200 μL) three times. The phenol layers were combined. The phenol layer and aqueous layer were mixed separately with liquid scintillation cocktail (16 mL) and the ^{3}H or ^{32}P in each sample was counted using a liquid scintillation counter.

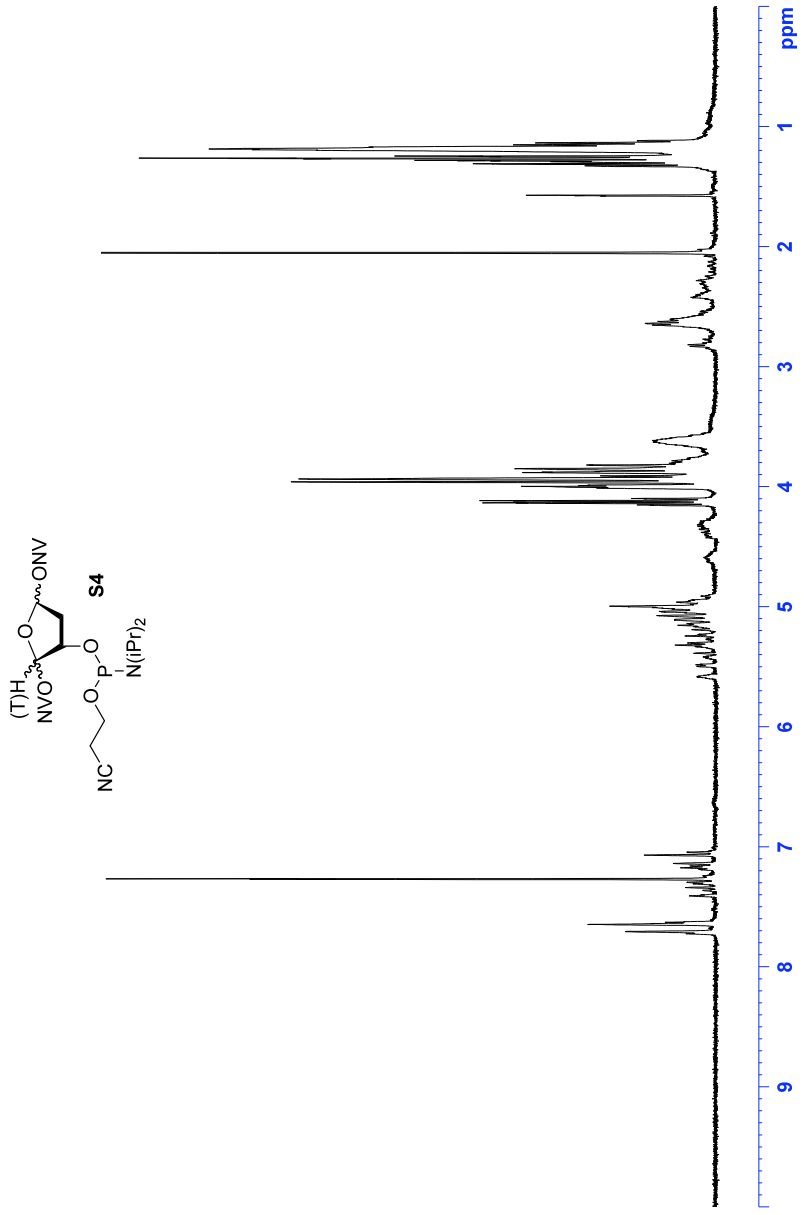
1. Kodama, T.; Greenberg, M. M. *J. Org. Chem.* **2005**, *70*, 9916-9924.



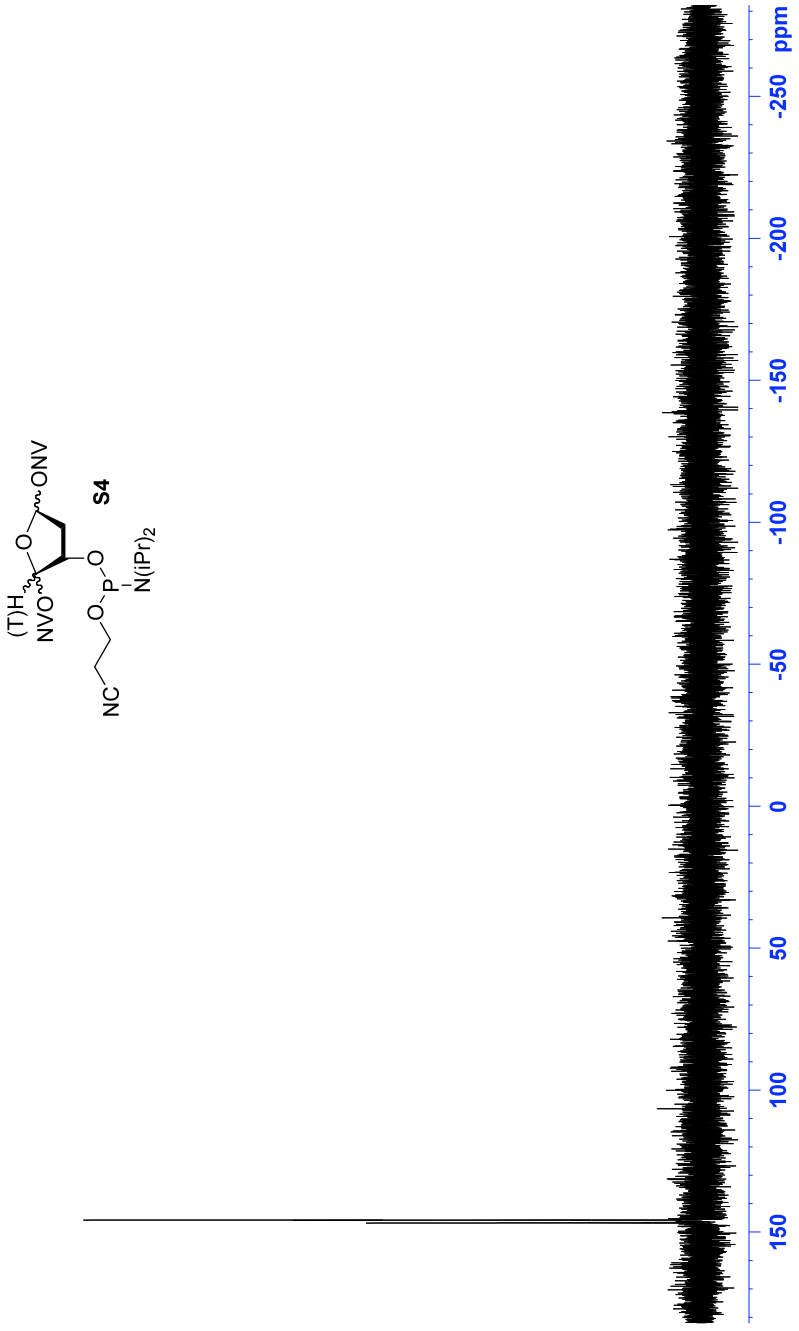
Supporting Information Figure 1. ^{1}H NMR spectrum of **S2**.



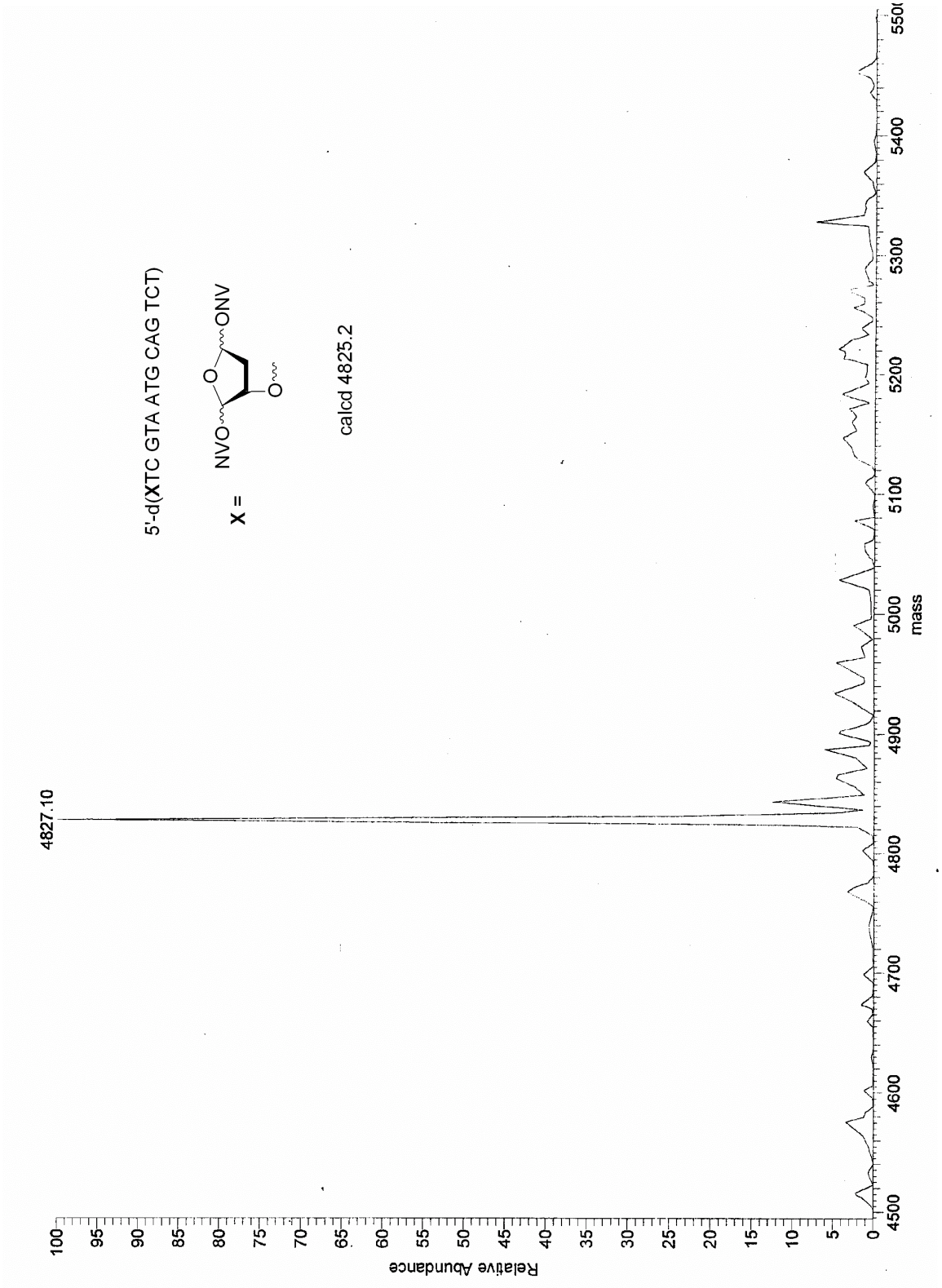
Supporting Information Figure 2. ^{1}H NMR spectrum of **S3**.



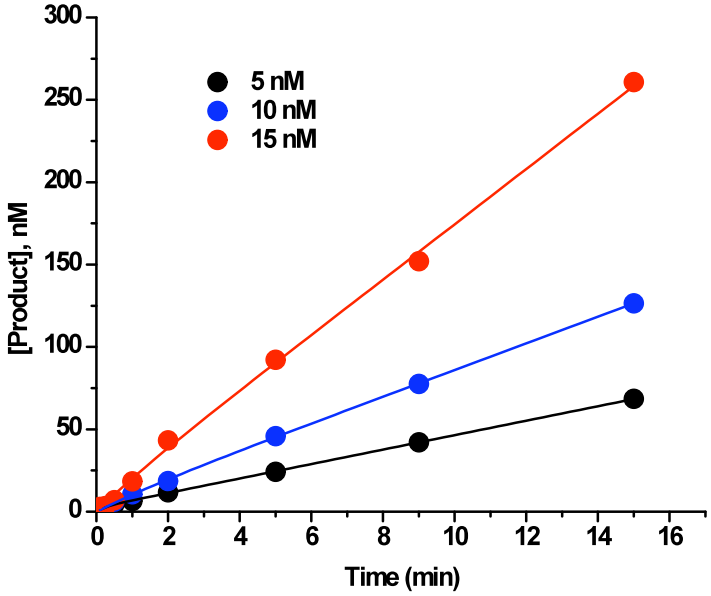
Supporting Information Figure 3. ^{1}H NMR spectrum of **S4**.



Supporting Information Figure 4. ^{31}P NMR spectrum of **S4**.

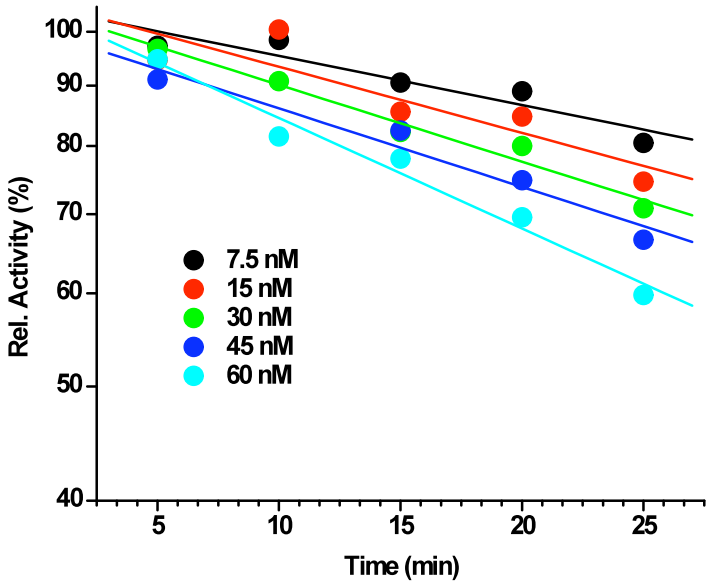


Supporting Information Figure 5. ESI-MS of oligonucleotide containing DOB precursor.

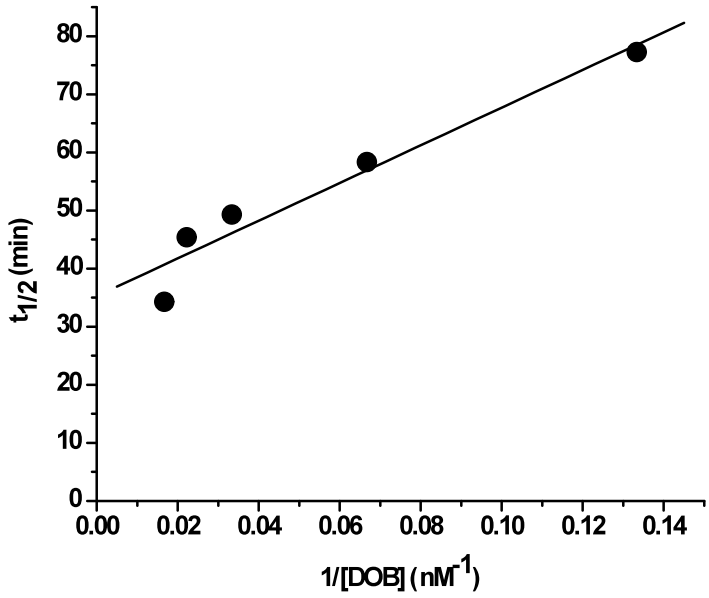


Supporting Information Figure 6. Active site titration of Pol β (5 nM, 10 nM, 15 nM) using 3'-^{32}P-**2** (500 nM) as substrate.

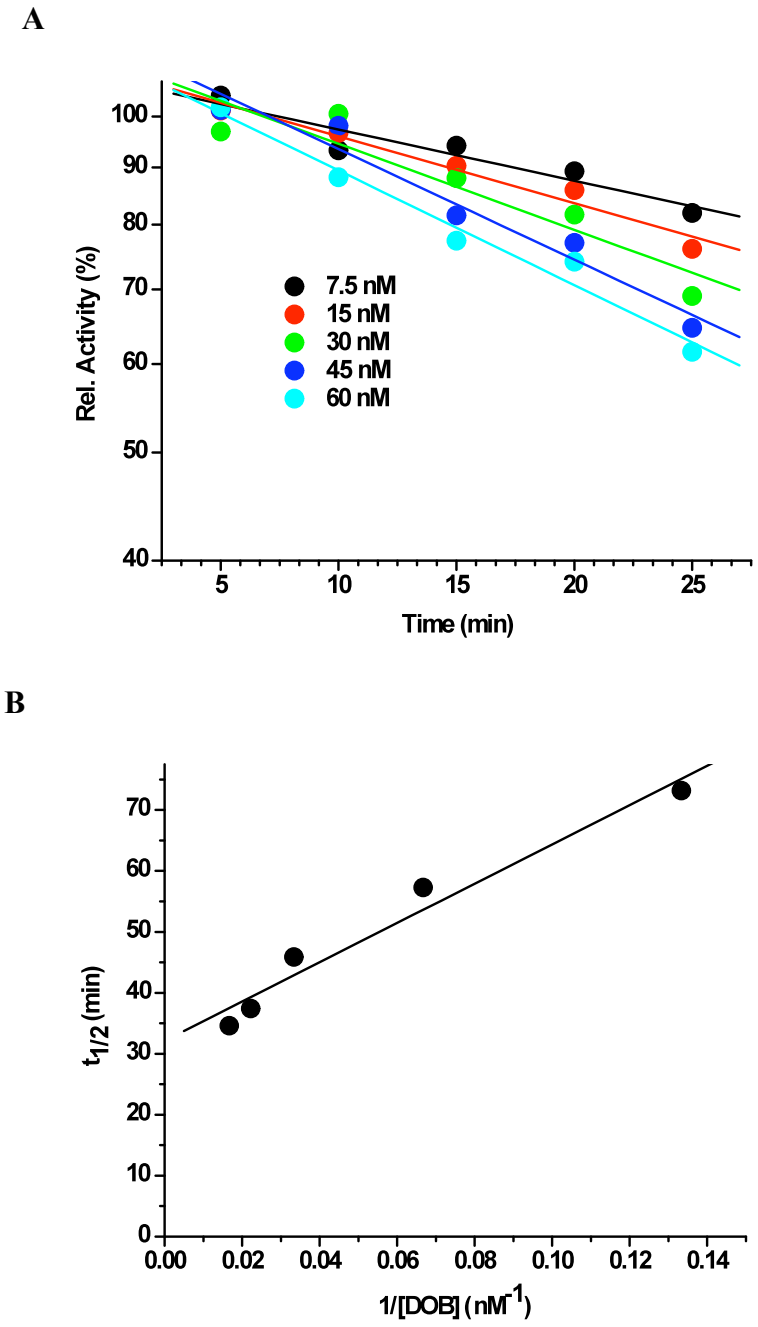
A



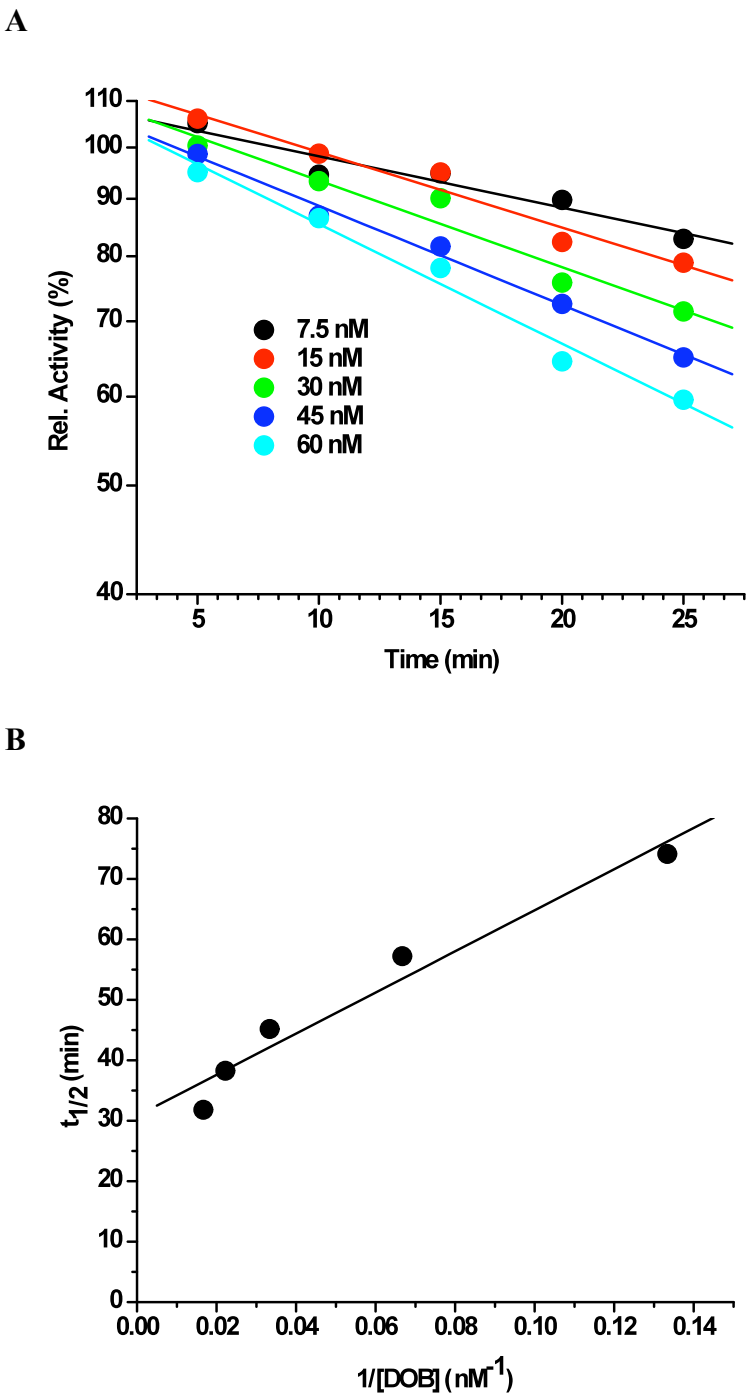
B



Supporting Information Figure 7. Kinetic analysis #1 of irreversible inhibition of Pol β by DOB (**1**). (A) Effect of increasing [DOB] (**1**) on Pol β (7.5 nM) lyase reaction of AP (3'-^{32}P-**2**, 500 nM). (B) Half-life of Pol β inactivation as a function of [DOB].

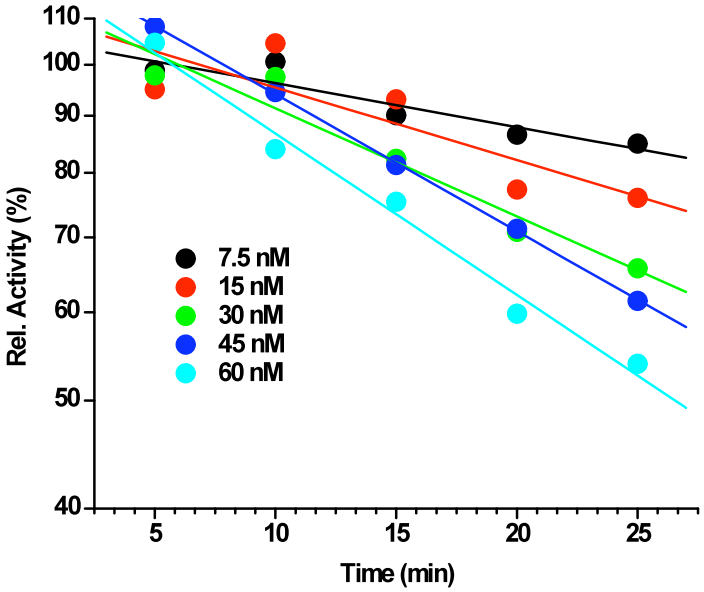


Supporting Information Figure 8. Kinetic analysis #2 of irreversible inhibition of Pol β by DOB (**1**). (A) Effect of increasing [DOB] (**1**) on Pol β (7.5 nM) lyase reaction of AP (3'-^{32}P-**2**, 500 nM). (B) Half-life of Pol β inactivation as a function of [DOB].



Supporting Information Figure 9. Kinetic analysis #3 of irreversible inhibition of Pol β by DOB (**1**). (A) Effect of increasing [DOB] (**1**) on Pol β (7.5 nM) lyase reaction of AP (3'-^{32}P-**2**, 500 nM). (B) Half-life of Pol β inactivation as a function of [DOB].

A



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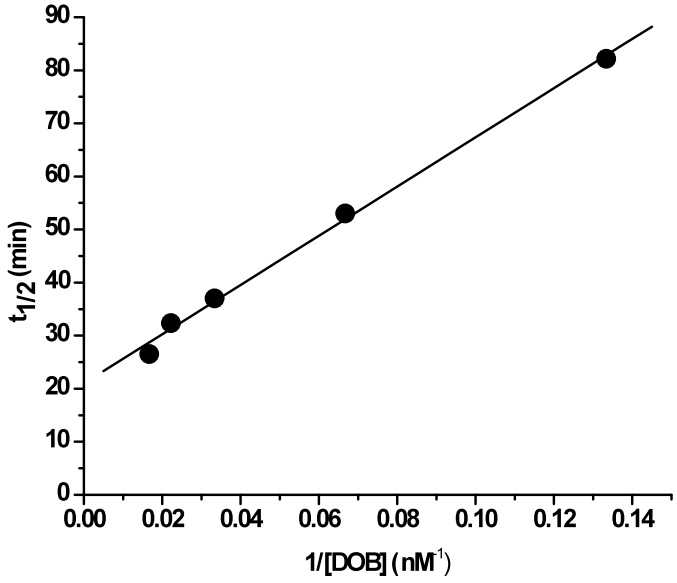
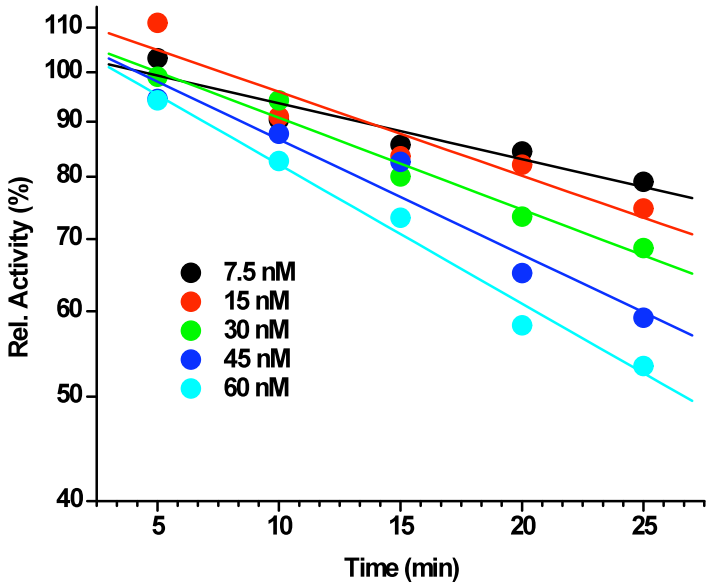


Supporting Information Figure 10. Kinetic analysis #4 of irreversible inhibition of Pol β by DOB (**1**). (A) Effect of increasing [DOB] (**1**) on Pol β (7.5 nM) lyase reaction of AP (3'-^{32}P-**2**, 500 nM). (B) Half-life of Pol β inactivation as a function of [DOB].

A



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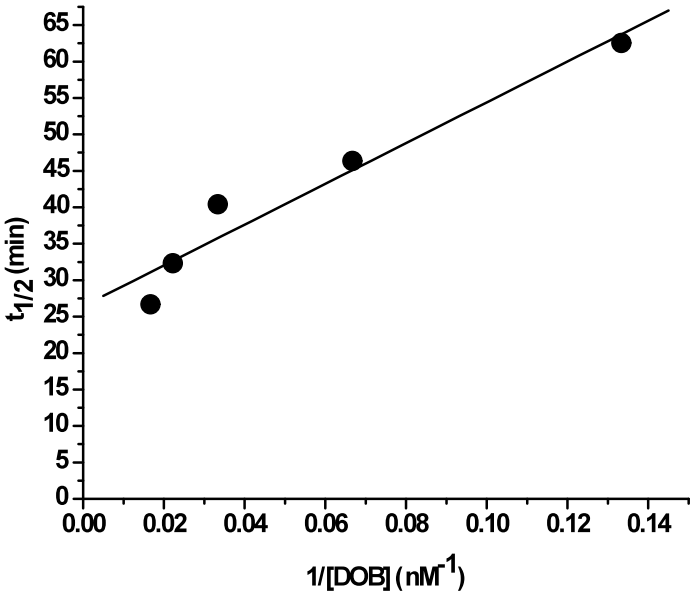
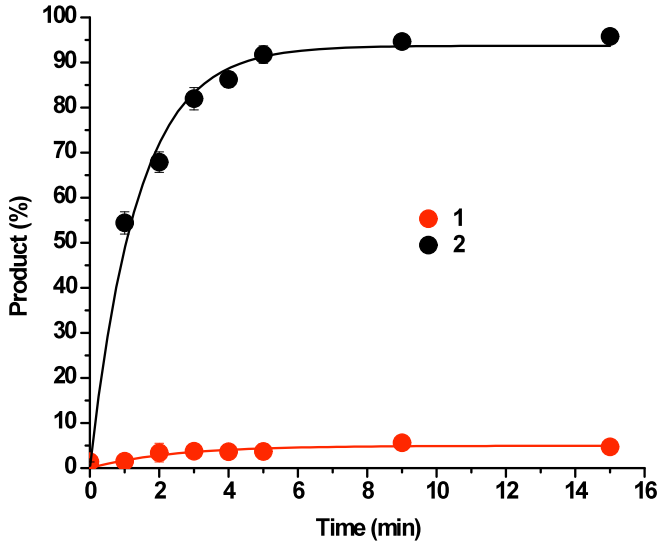


Supporting Information Figure 11. Kinetic analysis #5 of irreversible inhibition of Pol β by DOB (**1**). (A) Effect of increasing [DOB] (**1**) on Pol β (7.5 nM) lyase reaction of AP (3'-^{32}P-**2**, 500 nM). (B) Half-life of Pol β inactivation as a function of [DOB].

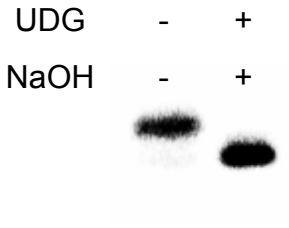
Supporting Information Table 1. Summary of individual kinetic analyses of irreversible inhibition of Pol β by DOB (**1**).

Experiment	1 (Figure S7)	2 (Figure S8)	3 (Figure S9)	4 (Figure S10)	5 (Figure S11)	6 (Figure 2)*
K_I (nM)	9.2	10.0	9.1	22.1	10.6	15.7
k_{inact} (s^{-1})	3.3×10^{-4}	3.6×10^{-4}	3.8×10^{-4}	5.5×10^{-4}	4.4×10^{-4}	5.0×10^{-4}
$K_I = 12.8 \pm 5.2$ nM, $k_{inact} = 4.2 \pm 0.9 \times 10^{-4}$ s^{-1}.						

*See main text for this kinetic plot.



Supporting Information Figure 12. Time course experiment of Pol β (5 nM) lyase reaction with DOB (3'-^{32}P-**1**, 200 nM) and AP (3'-^{32}P-**2,** 200 nM).



Supporting Information Figure 13. Demonstration of completeness of UDG preparation of **1**. 3'-^{32}P-**1** was treated with UDG and then NaOH.