Supporting Information: Synergistic Effects of an Irreversible DNA Polymerase Inhibitor and DNA Damaging Agents on HeLa Cells

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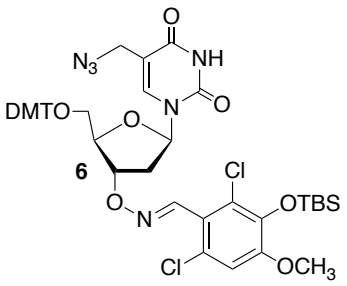
General Methods.

Preparation of DMT-azide 5. Phthalimide **4**[1] (0.6 g, 0.87 mmol) was azetropically dried (3 × 3 mL) and resuspended in dry benzene (13 mL). *N*-Bromosuccinimide (245 mg, 1.38 mmol) and AIBN (30 mg, 0.18 mmol) were added and the mixture was stirred at 85 ºC for 1 h at which time silica TLC indicated most (~80%) of **4** was consumed. The reaction mixture was concentrated under reduced pressure. The residue was dissolve in dry DMF (8 mL) and NaN_3 (350 mg, mmol) was added. The mixture was stirred at room temperature overnight. The reaction was diluted with EtOAc (80 mL), washed with sat. aq. $NaHCO_3$ solution (40 mL), brine (30 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on a silica gel column (25 × 3 cm). Elution with 1:2 → 2:3 → 1:1 ethyl acetate–hexane gave **5** (285 mg, 45%) as a colorless foam. Silica gel TLC R_f = 0.60 (1:10:10 methanol–ethyl acetate–hexane); ^{1}H NMR ($CDCl_3$) δ 8.85 (s, 1H), 7.90 – 7.67 (m, 5H), 7.37 – 7.26 (m, 3H), 7.26 – 7.14 (m, 6H), 6.81 (dd, *J* = 9.0, 1.0 Hz, 4H), 6.59 (dd, *J* = 8.5, 5.6 Hz, 1H), 5.11 (d, *J* = 6.0 Hz, 1H), 4.53 (d, *J* = 2.0 Hz, 1H), 3.78 (s, 6H), 3.58 – 3.29 (m, 4H), 2.87 (dt, *J* = 13.0, 6.0 Hz, 1H), 2.35 (ddd, *J* = 14.5, 8.5, 6.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 177.9, 163.8, 162.58, 162.57, 159.2, 158.82, 158.79, 149.8, 144.1, 138.7, 135.1, 135.0, 134.8, 130.1, 130.0, 128.7, 128.7, 128.10, 128.08, 127.3, 123.9, 113.4, 110.0, 88.0, 87.22, 87.22, 85.4, 84.9, 82.7, 63.5, 62.8, 55.2, 47.0, 46.6, 44.9, 37.5, 35.4, 31.6, 30.2, 29.6, 22.6, 21.0, 14.1; HRMS (ESI-TOF) $C_{39}H_{34}N_6O_9Na$ $(M + Na)^+$ calcd. *m/z* 753.2285, found 753.2272.

Preparation of TBS-Aldehyde 9. *N*,*N*-Dimethylaminopyridine (25 mg, 0.2 mmol) followed by TBSCl (101 mg, 0.67 mmol) were added to a solution of **8**[2] (115 mg, 0.53 mmol) in dry CH_2Cl_2 (3 mL) containing triethylamine (0.5 mL, 0.36 g, 3.59

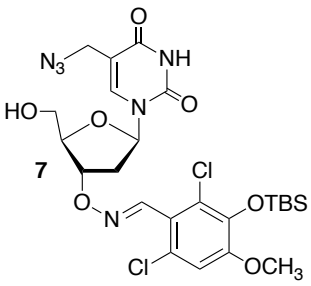
mmol) at 0 ºC and the mixture was stirred at room temperature overnight. The reaction was diluted with CH_2Cl_2 (30 mL), washed with sat. aq. NH_4Cl solution (20 mL), brine (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on a silica gel column (15 × 2 cm). Elution with 1:9 → 1:4 ethyl acetate–hexane gave **9** (162 mg, 91%) as a colorless foam. Silica gel TLC R_f = 0.64 (1:9 ethyl acetate–hexane); ^{1}H NMR ($CDCl_3$) δ 10.40 (s, 1H), 6.84 (s, 1H), 3.88 (s, 3H), 1.16 – 0.92 (m, 9H), 0.19 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 188.6, 154.8, 141.7, 130.1, 129.0, 123.2, 112.3, 55.9, 25.9, 19.0, -3.8; HRMS (EI-magnetic sector instrument) $C_{14}H_{21}N_3O_3Cl_2Si$ $(M + H)^+$ calcd. *m/z* 335.0637, found 335.0639.

Preparation of DMT-TBS-Oxime 6. An aq. solution (8%) of methylamine (0.5 mL) was added to a solution of **5** (300 mg, 0.41 mmol) in THF (6 mL) and the resulting solution was stirred at room temperature for 2 h. The reaction mixture was concentrated and the residue was coevapoarted with MeOH (3 × 5 mL). The residue was suspended in MeOH (2.4 mL) and a solution of **9** (150 mg, 0.45 mmol) in THF (0.6 mL) was added, followed by AcOH (~ 25 μL). The reaction mixture was stirred at room temperature overnight and concentrated. The residue was purified by flash column chromatography on a silica gel column (25 × 2 cm). Elution with 1:3 → 1:2 ethyl acetate–hexane gave **6** (296 mg, 78%) as a colorless foam. Silica gel TLC R_f = 0.87 (1:10:10 methanol–ethyl acetate–hexane); ^{1}H NMR ($CDCl_3$) δ 9.71 (d, J = 16.1 Hz, 1H), 8.30 (s, 1H), 7.92 (d, J = 4.7 Hz, 1H), 7.46 – 7.37 (m, 2H), 7.35 – 7.25 (m, 7H), 6.92 – 6.82 (m, 5H), 6.53 (dt, J = 14.2, 7.1 Hz, 1H), 5.15 (d, J = 6.4 Hz, 1H), 4.41 (d, J = 2.0 Hz, 1H), 3.79 (dd, J = 7.5, 4.1 Hz, 9H), 3.62 – 3.41 (m, 3H), 3.27 (t, J = 15.6 Hz, 1H), 2.80 (dd, J = 13.2, 5.8 Hz, 1H), 2.50 – 2.35 (m, 1H), 1.05 – 1.01 (m, 9H), 0.20 – 0.18 (m, 6H); ^{13}C NMR (101

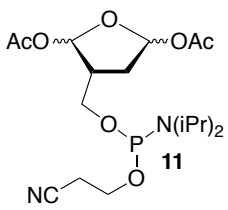


MHz, $CDCl_3$) δ 162.7, 158.8, 158.8, 151.8, 150.1, 146.9, 144.2, 141.3, 139.2, 135.3, 135.1, 130.2, 130.1, 128.2, 128.1, 127.3, 126.7, 126.1, 120.9, 113.4, 111.8, 110.0, 87.1, 85.1, 83.7, 83.6, 64.0, 55.6, 55.27, 55.26, 46.5, 38.4, 25.9, 18.9, -4.0; HRMS (ESI-TOF) $C_{45}H_{50}Cl_2N_6O_9SiNa$ $(M + Na)^+$ calcd. *m/z* 939.2683, found 939.2665.

Preparation of TBS-Oxime 7. A solution of **6** (392 mg, 0.43 mmol) in 85% aq. AcOH (15 mL) was stirred at room temperature for 1.5 h and diluted with MeOH (20 mL). The reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography on a silica gel column (20 × 2 cm). Elution with 1:3 → 1:2 → 2:3 ethyl acetate–hexane gave **7** (220 mg, 84%) as a colorless oil. Silica gel TLC R_f = 0.72 (1:10:10 methanol–ethyl acetate–hexane); ^1H NMR ($CDCl_3$) δ 8.46 (s, 1H), 8.30 (s, 1H), 7.87 (s, 1H), 6.85 (s, 1H), 6.32 (dd, *J* = 7.9, 6.0 Hz, 1H), 5.03 (d, *J* = 6.7 Hz, 1H), 4.39 (d, *J* = 2.2 Hz, 1H), 4.20 – 4.14 (m, 2H), 3.98 (dt, *J* = 11.8, 10.5 Hz, 2H), 3.83 (s, 3H), 2.67 (dd, *J* = 14.1, 4.0 Hz, 1H), 2.53 – 2.37 (m, 1H), 1.02 (s, 9H), 0.20 (d, *J* = 3.0 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 162.7, 151.9, 150.2, 146.9, 141.5, 139.7, 126.7, 126.2, 121.0, 111.9, 109.9, 87.4, 85.1, 83.1, 63.2, 55.7, 47.4, 37.7, 26.0, 19.0, -3.9; HRMS (ESI-TOF) $C_{24}H_{33}Cl_2N_6O_7Si$ $(M + H)^+$ calcd. *m/z* 615.1557, found 615.1552.

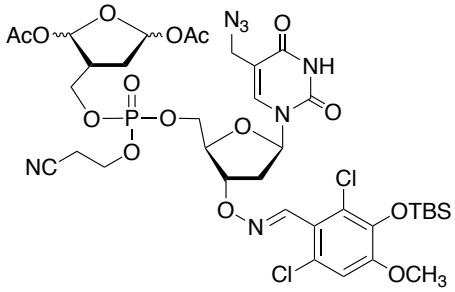


Preparation of Phosphoramidite 11. Alcohol **10**[2] (110 mg, 0.50 mmol) was dried under vacuum overnight, dissolved in anhydrous CH_2Cl_2 (4 mL), and cooled to 0 °C. *N*,*N*-Diisopropylethylamine (0.37 g, 0.5 mL, 2.89 mmol), followed by 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (159 mg, 150 μL, 0.67 mmol) were added. The mixture was stirred at 0 °C for 15 min and at room temperature for 1 h. The reaction mixture was diluted with EtOAc (50 mL) and sat. aq. $NaHCO_3$ solution (20 mL).



The organic layer was separated, washed with brine (15 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on a silica gel column (20 × 1 cm). Elution with 1:3 → 1:2 ethyl acetate–hexane gave **11** (100 mg, 48%) as a colorless foam. Silica gel TLC R_f = 0.79 (1:10:10 methanol–ethyl acetate–hexane); ^{1}H NMR ($CDCl_3$) δ 6.43 – 6.17 (m, 2H), 3.90 – 3.38 (m, 6H), 2.81 – 2.33 (m, 4H), 2.15 – 1.95 (m, 6H), 1.81 (ddt, *J* = 13.4, 11.1, 4.6 Hz, 1H), 1.12 (dt, *J* = 7.1, 3.7 Hz, 12H); ^{31}P NMR ($CDCl_3$) δ 148.3, 148.0.

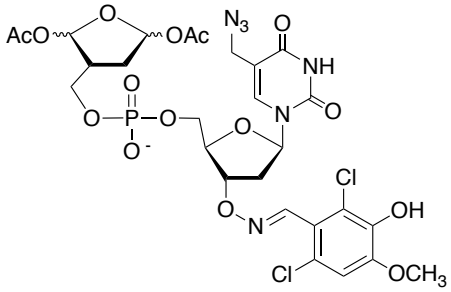
Preparation of the TBS-phosphate triester precursor to 12. Alcohol **7** (280 mg, 0.46 mmol) was azeotropically dried with pyridine (3 × 3 mL) and dissolved in the activator solution (5.5 mL, 0.25 M S-ethyltetrazole in THF, 1.4 mmol). This solution was then added to a reaction flask containing **11** (192 mg, 0.46 mmol) under argon and the mixture was stirred at room temperature for 30 min. A solution of *tert*-butyl hydroperoxide in decane (0.2 mL, 7 M, 1.4 mmol) was added and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated, diluted with EtOAc (60 mL), and washed with sat. aq. $NaHCO_3$ (15 mL), H_2O (20 mL), and brine (15 mL). The EtOAc layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on a silica gel column (25 × 2 cm). Elution with 1:2 ethyl acetate–hexane → 0.1:3:2 methanol–ethyl acetate–hexane gave the phosphate triester precursor to **12** (270 mg, 62%) as a colorless foam. Silica gel TLC R_f = 0.28 (1:10:10 methanol–ethyl acetate–hexane); ^{1}H NMR ($CDCl_3$) δ 9.77 (t, *J* = 13.0 Hz, 1H), 8.27 (s, 1H), 7.77 (s, 1H), 6.83 (s, 1H), 6.48 – 6.19 (m, 3H), 5.04 – 4.78 (m, 1H), 4.52 – 4.10 (m, 9H), 3.81 (d, *J* = 5.9 Hz, 3H), 2.98 – 2.34 (m, 5H), 2.25 (dd, *J* = 13.5, 6.1 Hz, 1H), 2.11



phosphate triester precursor to **12**

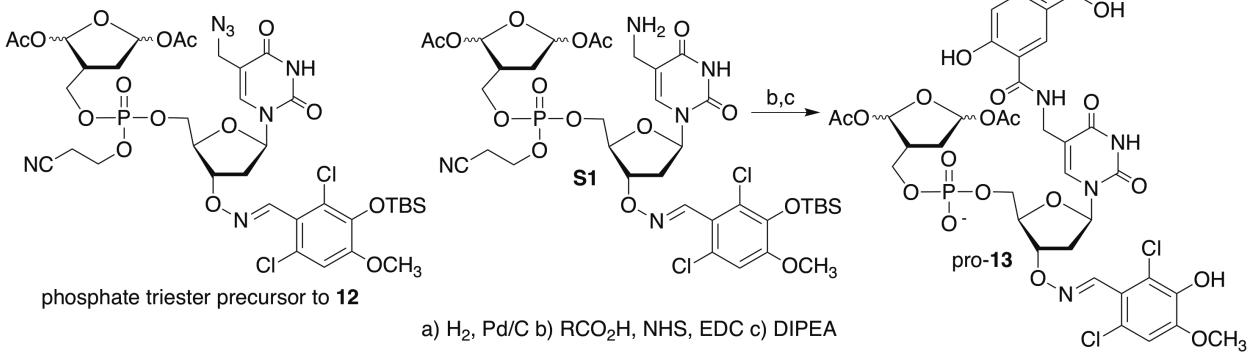
– 2.01 (m, 6H), 1.92 – 1.75 (m, 1H), 0.98 (s, 9H), 0.15 (s, 6H); ^{31}P NMR ($CDCl_3$) δ -1.8, -1.9, -2.0; HRMS (ESI-TOF) $C_{36}H_{48}N_7O_{15}SiNaPCl_2$ $(M + Na)^+$ calcd. *m/z* 970.1990, found 970.1947.

Preparation of 12. The phosphate triester precursor to **12** (22 mg, 23 μmol) was dissolved in 1:3 mixture of CH_2Cl_2–Et_3N (4 mL) and the mixture was heated to 60 ºC for 16 h. The reaction

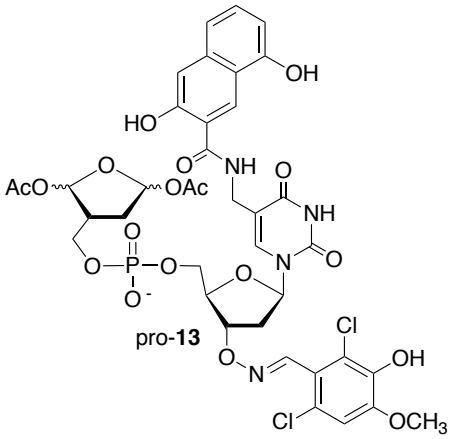


mixture was concentrated under reduced pressure and the residue was diluted with H_2O (8 mL), washed with CH_2Cl_2 (2 × 5 mL), and EtOAc (5 mL). The aqueous layer was lyophilized to obtain **12** (15 mg, 83%) as a light yellow solid. TLC R_f = 0.72 (0.1:1:9 triethylamine–methanol–dichloromethane); ^{1}H NMR (D_2O) δ 8.22 (s, 1H), 7.96 (s, 1H), 6.92 (s, 1H), 6.30 (s, 1H), 6.21 – 6.04 (m, 2H), 4.96 (s, 1H), 4.39 (s, 1H), 4.16 – 3.85 (m, 6H), 3.78 (s, 3H), 2.77 – 2.21 (m, 4H), 2.04 – 1.90 (m, 6H), 1.76 (m, 1H); ^{31}P NMR (D_2O) δ -0.2; HRMS (ESI-TOF) $C_{27}H_{30}N_6O_{15}PCl_2$ $(M - H)^-$ calcd. *m/z* 779.0841, found 779.0884.

Scheme S1. Alternative synthesis of pro-**13**.

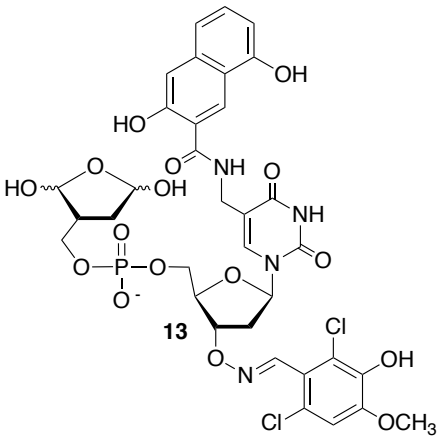


Preparation of pro-13 from the TBS-phosphate triester precursor to 12 (Scheme S1). AcOH (50 μL) and palladium on carbon (5 mg, 10% by wt.) were added to a solution containing the



phosphate triester protected precursor of **12** (9 mg, 9.5 μmol). The resulting suspension was bubbled with H_2 for 10 min and then stirred at room temperature for 2 h under H_2. The reaction mixture was filtered and concentrated to obtain protected amine (**S1**), which was used for the next step without any purification; yield 7.5 mg (85%). Dihydroxy naphthalene carboxyl acid (100 μL, 0.2 M, 20 μmol in DMF), *N*-hydroxysuccinimide (200 μL, 0.2 M, 20 μmol in DMF), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (100 μL, 0.2 M, 20 μmol in DMF) was incubated at room temperature for 2 h. The solution containing the activated acid was then treated with a solution of **S1** (7.5 mg, 8.1 μmol) in DMF (0.9 mL) and phosphate buffer (pH 7.2, 10 mM, 1 mL). The mixture was incubated at room temperature overnight. The mixture was subsequently concentrated to ~ 0.2 mL and treated with a solution of DIPEA in DMF (33% by vol., 1.5 mL). The mixture was incubated 55 ºC for 4 h, and purified by reverse phase HPLC on a C18 column, Waters Delta Pak 300 x 7.8 mm. A gradient of 5 → 40% ACN in 0.1 M aq. ammonium acetate over 15 min and a second gradient of 40 → 100% ACN in 0.1 M aq. ammonium acetate over 2 min was employed at a flow rate of 5 mL/min. The peak at 14.0 min was collected and lyophilized to obtain pro-**13** (1.5 mg, 19%) as a yellow solid. ^{1}H NMR (CD_3CN-D_2O, a few drops of D_2O) δ 8.51-8.47 (m, 1H), 8.37 – 8.17 (m, 2H), 7.93 (s, 1H), 7.54 – 7.34 (m, 1H), 7.14 – 6.80 (m, 2H), 6.32 – 6.10 (m, 4H), 4.97 (br s, 1H), 4.49 – 4.16 (m, 4H), 4.06 – 3.87 (m, 7H), 3.85 (m, 6H), 2.63 – 2.47 (m, 1H), 2.38 – 2.30 (m, 2H), 2.11 – 2.09 (m, 1H), 2.02 – 1.96 (m, 6H); ^{31}P NMR (CD_3CN-D_2O, a few drops of D_2O) δ -0.7; HRMS (ESI-TOF) $C_{38}H_{38}Cl_2N_4O_{18}P$ $(M-H)^-$ calcd. *m/z* 939.1296, found 939.1304.

Preparation of 13. A suspension of pro-**13** (1.0 mg, 0.94 μmol) in ACN containing 2% H_2O (1 mL) was treated with a solution of BF_3•Et_2O in ACN (100 μL, 0.4 M, 0.04 mmol) at room temperature for 1.0 h at which time analysis by C_{18}-HPLC analysis showed complete disappearance of pro-**13** and formation of **13**. The reaction mixture was diluted with phosphate buffer (1 mL, 10 mM, pH 7.2) and concentrated to ~ 0.5 mL. The crude mixture was purified by a C_{18} silica plug in a Pasteur pipette (2 inches). Elution with 0% → 20% ACN in H_2O gave of **13** (0.4 mg, 44%). ^{1}H NMR (DMSO-d_6) δ 8.45 – 8.20 (m, 2H), 8.03 – 7.80 (m, 1H), 7.64 – 7.23 (m, 1H), 7.98 – 6.65 (m, 3H), 6.45 – 5.98 (m, 2H), 5.35 – 4.81 (m, 3H), 4.01 – 3.65 (m, 8H), 3.15 – 2.69 (m, 3H), 2.15 – 1.77 (m, 4H); ^{31}P NMR (CD_3CN-D_2O) δ 0.45; HRMS (ESI-TOF) $C_{34}H_{34}Cl_2N_4O_{16}P$ $(M-H)^-$ *m/z* calcd. 855.1055, found 855.1030.



Deoxyribose phosphate (dRP)- aldehyde reactive probe (ARP) adduct formation. To study the reactivity of ARP towards deoxyribose phosphate (dRP), the photochemical precursor to **15** (~20,000 cpm) was diluted to 30 μL in 1 × PBS.[2] A 6 μL of solution was removed as unphotolyzed control. The remaining solution (24 μL) was photolyzed (350 nm in a Rayonet photoreactor) for 10 min at room temperature. Immediately after photolysis, two aliquots (6 μL) were removed from the reaction. One aliquot was treated with 0.1 N NaOH at 37 °C for 30 min and the other with $NaBH_4$ (0.1 M) for 1 h at 4 °C. The NaOH treated sample was then neutralized with 0.1 N HCl. The other two aliquots (6 μL each) were treated with 10 μL of ARP solution (from DNA damage quantification kit from Dojindo Molecular Technologies (DK02-12)). One of the reactions was quenched with $NaBH_4$ (0.1 M, final concentration). All of the

aliquots were then analyzed directly by 20 % denaturing PAGE (40 × 32 × 0.04 cm). The gel was run under limiting power (55 W) until the bromophenol blue band migrated to the bottom.

Cell culture. HeLa (human cervical carcinoma) cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 U mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin at 37 °C in a humidified incubator at 5% CO_2. The adherent cultures were grown as a monolayer and passaged once after 3-4 days by trypsinizing with 0.25% Trypsin-EDTA. At 90 % confluency, there are around 1.5 x 10^7 cells in one dish of 150 mm × 25 mm.

Cell viability assay. Approximately 10^6 HeLa cells were plated in each well of a 6 well culture plate (well size; 35 mm X 18 mm) in DMEM containing 10% FBS (2 mL) and kept for 24 h at 37 °C in a CO_2 incubator. After the indicated time, the medium was removed from the cell culture by aspiration, and washed once with PBS. A stock solution of bleomycin sulphate (1 mM) in DMSO was diluted in the culture medium to 2 μM and then added to the plates keeping the quantity of DMSO constant at 1% for all tests. Cells were then incubated for 2 h at 37 °C in a CO_2 incubator. The medium was then subsequently replaced with fresh DMEM-FBS and incubation was continued for an additional 1 or 2 h at 37 °C in a CO_2 incubator. After the indicated time, cells were trypsinized with 0.25% w/v Trypsin-EDTA (100 μL in each well, 2 min incubation at 37 °C) and the cell suspension was prepared in 1 mL PBS. A portion (10 μL) of the cell suspension was thoroughly mixed with 10 μL of 0.4% solution of trypan blue in PBS (pH 7.2 to 7.3), and placed on a counting slide (BIO-RAD) to count the % of live cells using a TC20 automated cell counter (BIO-RAD). A control experiment without treating with bleomycin sulphate was carried out in parallel. All the experiments were carried out at least 3 times, and each experiment consisted of 3 replicates.

Comet assay to visualize DNA damage in bleomycin treated cells. Bleomycin sulphate treatment of HeLa cells was carried out exactly in the same way as described above. The only difference was that approximately 2 x 10^7 HeLa cells were plated in each well of the 6 wells culture plate. After the required incubation steps, the medium was aspirated from the cell culture, and washed with PBS (3 ×) by adding 5 mL of PBS to each well, scratching with a cell scraper (3 cm blade) and transferring the cells to a 15 mL Falcon tube. Cells were pelleted by centrifuging at 3000 g for 10 min at room temperature. Cells were resuspended in PBS and pelleted again. For the comet assay, cells were suspended in 2 mL PBS/well and counted (generally ~ 1 x 10^7 /mL). An aliquot (10 µL) of the cell suspension was diluted to 1 × 10^5 cells/mL using PBS and used in an Oxiselect TM Comet Assay Kit (Cell Biolabs, INC. Catalog number STA-350) according to the product manual (http://www.cellbiolabs.com/sites/default/files/STA-350-comet-assay-kit.pdf). In brief, cell samples were combined with Comet Agarose at 1:10 ratio (v/v), titrurated with a pipette to mix, and immediately pipette 75 µL/well onto the OxiSelect™ Comet Slide. Ensure complete well coverage by spreading the suspension over the well with the pipette tip (Note: For multiple samples, maintain suspensions at 37 ºC in a water bath to avoid gelation). Maintaining the slide horizontally, transfer the slide to 4 ºC in the dark for 15 min. Carefully transfer the slide to a container containing pre-chilled lysis buffer (from the Kit; ~25 mL/slide). Immerse the slide in the buffer for 60 min at 4 ºC in the dark. Carefully, aspirate the lysis Buffer from the container and replace with pre-chilled alkaline solution (from the Kit), pH >13 (~25 mL/slide). Immerse the slide in the solution for 30 min at 4 ºC in the dark. Maintaining the slide horizontally, carefully transfer the slide from the alkaline solution to a horizontal electrophoresis chamber. Fill the chamber with cold alkaline electrophoresis solution (300 mM NaOH, 1 mM EDTA, pH >13)

until the buffer level covers the slide. Apply voltage to the chamber for 30 min at 1 volt/cm electrode distance. In addition, adjust the volume of alkaline electrophoresis solution to produce a current of 300 mA. Maintaining the slide horizontally, carefully transfer the slide from the electrophoresis chamber to a clean and small container containing pre-chilled H_2O (~25 mL/slide). Immerse the slide for 2 min, aspirate, and then repeat twice more. Aspirate the final water rinse and replace with cold 70% ethanol solution for 5 min. Maintaining the slide horizontally, remove the slide from the 70% ethanol solution and allow to air dry. Once the agarose and slide are completely dry, add 100 μL/well of 1 × Vista Green DNA Dye (from the Kit). Incubate at room temperature for 15 min. View slides by a fluorescence microscopy using a FITC filter. A control experiment without any bleomycin sulphate tretment was carried out in parallel. All the experiments were carried at least 3 times each consisting of 3 replicates. The relative tail lengths were determined with Open Comet software.

Clonogenic assay for cell survival. HeLa cells (2×10^5) were seeded in each well of a 24 well culture plate (well size; 15.5 mm X 18 mm) in 1 mL Dulbecco's Modified Eagle Medium (DMEM) growth medium supplemented with 10% FBS. After overnight incubation at 37 °C in a humidified atmosphere of 5% CO_2, cells were subjected to the appropriate DNA damaging conditions (or controls). For alkylation experiments, cells were incubated with MMS (0, 0.1, or 0.2 mM), with or without pro-**13** (5 μM) for 1 or 2 h. For experiments involving recovery after MMS treatment, the medium containing MMS and/or pro-**13** was replaced after 2 h with fresh medium with or without pro-**13** (5 μM) and incubation continued for 2–8 h. For BLM experiments, cells were incubated with BLM (2 μM), with or without pro-**13** (5 μM) for 2 h, at which time the medium was replaced with fresh medium containing only pro-**13** (5 μM) and incubation was continued for 2 h. The growth medium was then removed from each well and the

cells were washed with PBS (2 × 1 mL). The cells were treated for 2 min with 0.25% Trypsin-EDTA (50 μL in each well) at 37 °C to detach them from the plates and then diluted in DMEM-FBS medium (0.5 mL/well). The single cell suspensions were collected in 1.6 mL tubes and counted using a Bio-Rad TC20 Automated Cell Counter. Stock solutions of single cell suspensions were made in two groups for each concentration with 500 cells/mL (for untreated control) and 1500 cells/mL (for treated cells), respectively. These cells were seeded in 6 well plates (well size; 35 mm × 18 mm) in 2 mL of 10% DMEM-FBS. The plates were incubated in humidified atmosphere with 5% CO_2 for 7 days. After 7 days, the growth medium was discarded and the attached cells were treated with 0.2% w/v crystal violet solution. The excess dye was washed with water and the colonies were counted under a stereomicroscope. Plating efficiencies (PE) and survival fractions (SF) were calculated as follows: PE = number of colonies ÷ number of cells seeded; SF = PE ÷ PE (control).

AP/dRP site accumulation in genomic DNA of HeLa cells. HeLa cells (approximately 1 x 10^7) were plated in 150 mm x 25 mm dishes and treated with DMSO (1% final concentration), MMS (0.3 or 0.4 mM), or pro-**13** (5 μM) alone or with a combination of MMS (0.3 or 0.4 mM), and pro-**13** (5 μM) for 1 h at 37 °C. The cellular medium was then replaced with fresh DMEM-FBS and incubation was continued for 23 h at 37 °C in a CO_2 incubator in the absence or presence of pro-**13** (5 μM). Cells were then harvested with 0.25% Trypsin-EDTA, and the genomic DNA of each sample was isolated according to Dojindo Genomic DNA isolation kit (https://www.dojindo.com/TechnicalManual/Manual_GK03.pdf). The concentration of genomic DNA was measured at 260 nm and adjusted to 100 ng/μL. An aliquot of purified DNA (1 μg) was labeled with 10 μL of aldehyde reactive probe (ARP) reagent (N′-aminooxymethylcarbonylhydrazino-D-biotin), and AP/dRP sites were determined using the

DNA damage quantification kit from Dojindo Molecular Technologies (DK02-12) by measuring the absorbance at 650 nm using an ELISA microplate reader (BioRad).

Chart S1. Carboxylic acids used to prepare inhibitor candidates.

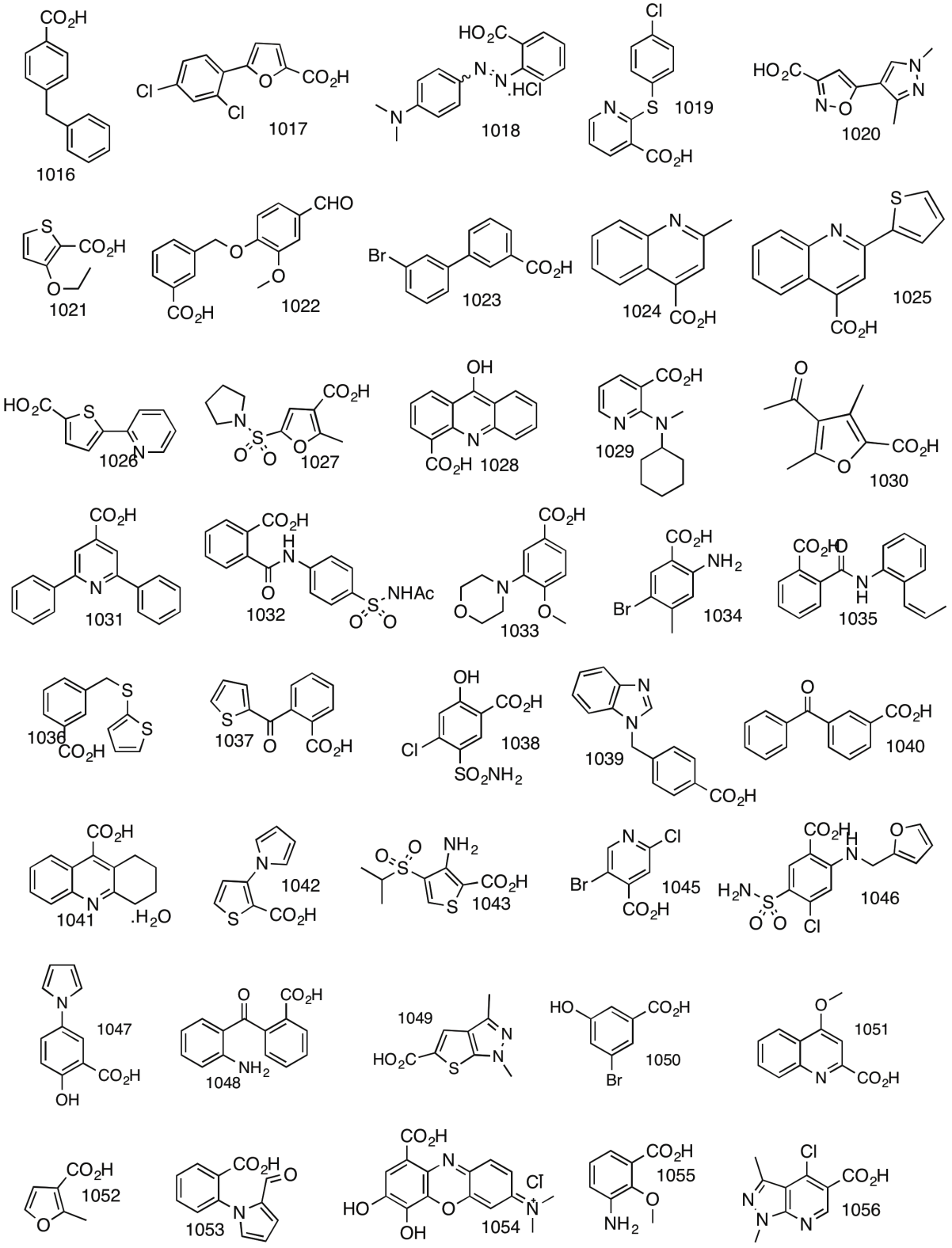


Chart S1 (continued). Carboxylic acids used to prepare inhibitor candidates.

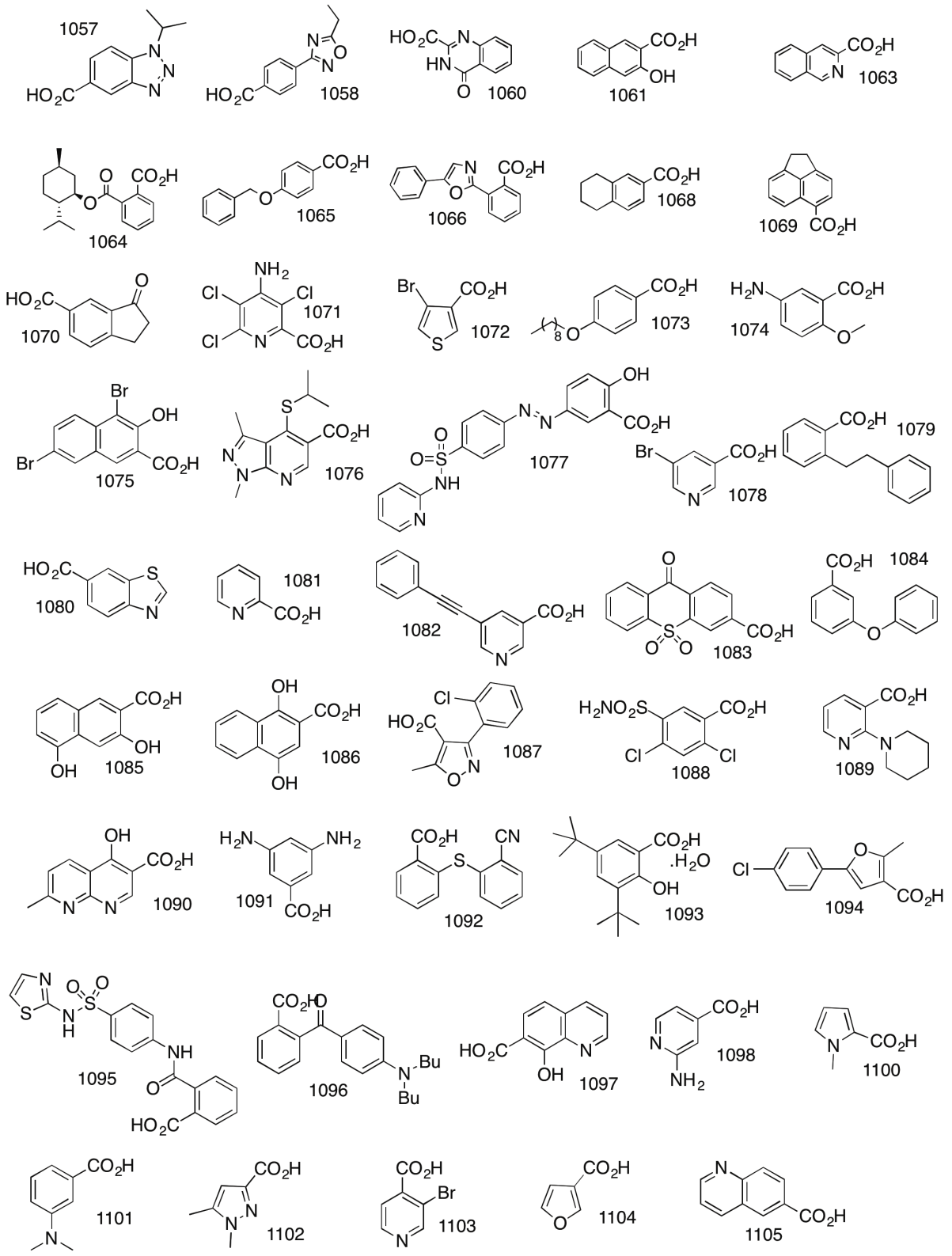
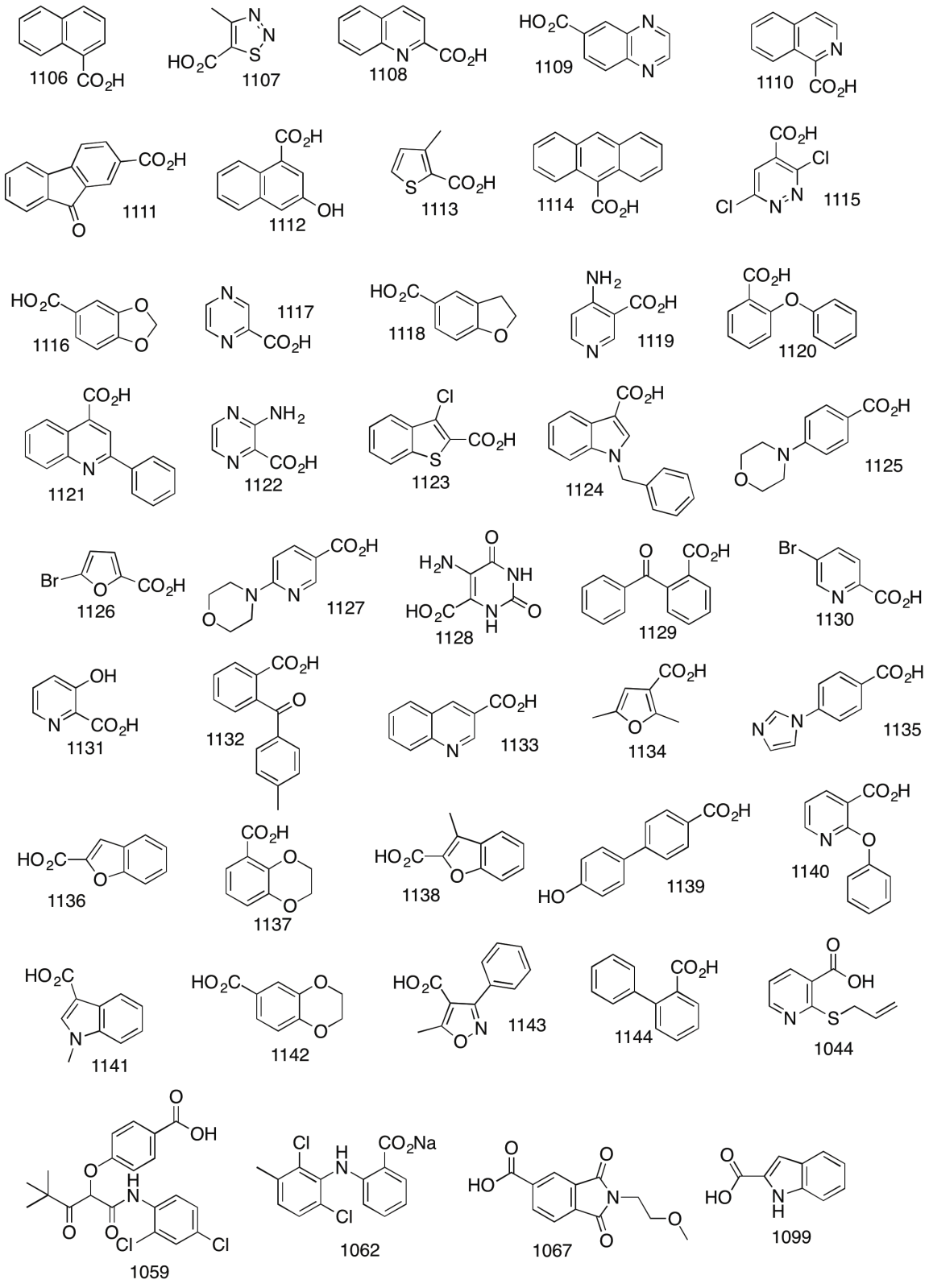
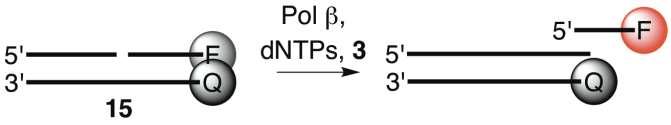


Chart S1 (continued). Carboxylic acids used to prepare inhibitor candidates.

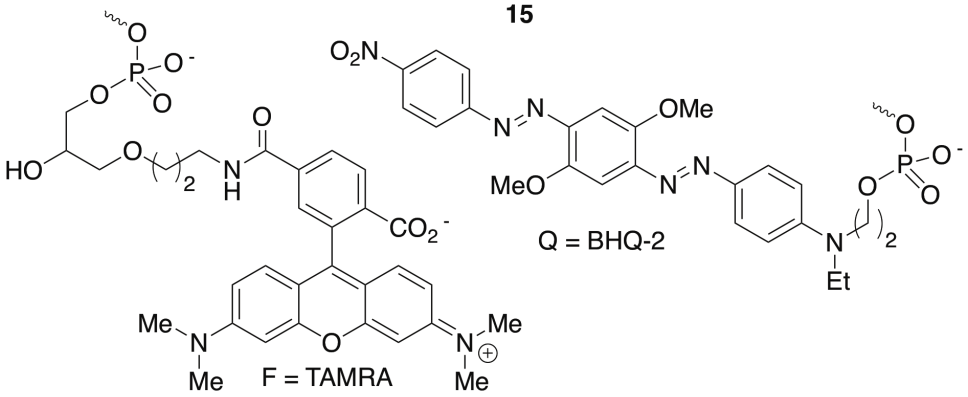


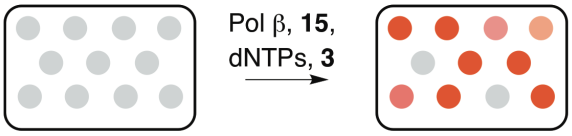
Scheme S1. Fluorescence screen for inhibitors.



5'-d(TCA CCC TCG TAC GAC TC TTT TTT TTT TGC-F)
3'-d(AGT GGG AGC ATG CTG AG AAA AAA AAA ACG-Q)

15





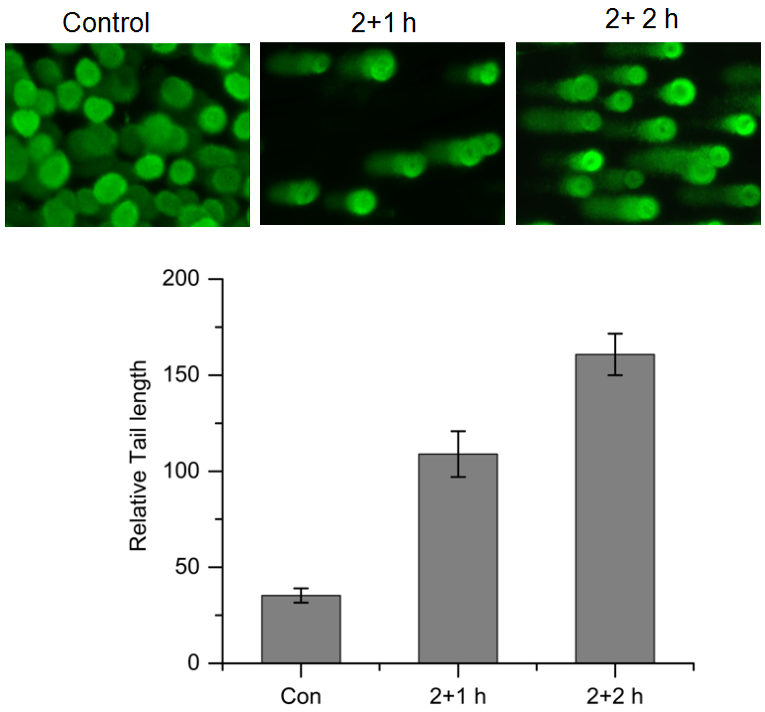


Figure S1. Detection of DNA damage in HeLa cells treated with bleomycin. (Top) A representative image showing increase in the tail length due to BLM treatment. (Bottom) Quantification of the relative tail lengths in the HeLa cells incubated with BLM (2 µM) for 2 h followed by 1 h (2+1 h) and 2 h (2+2 h) recovery. (Con: BLM untreated control).

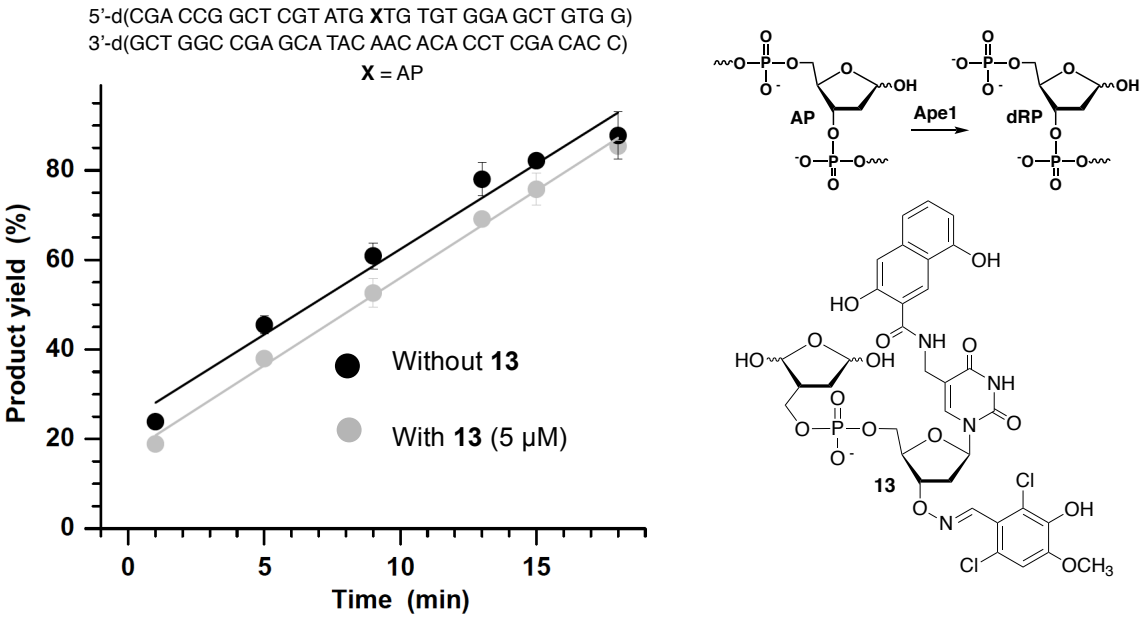


Figure S2. Effect of **13** on Ape1 activity.

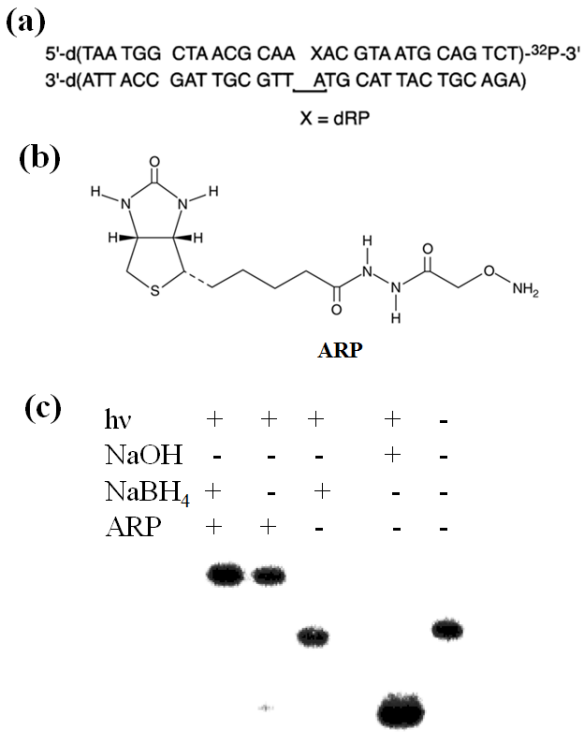
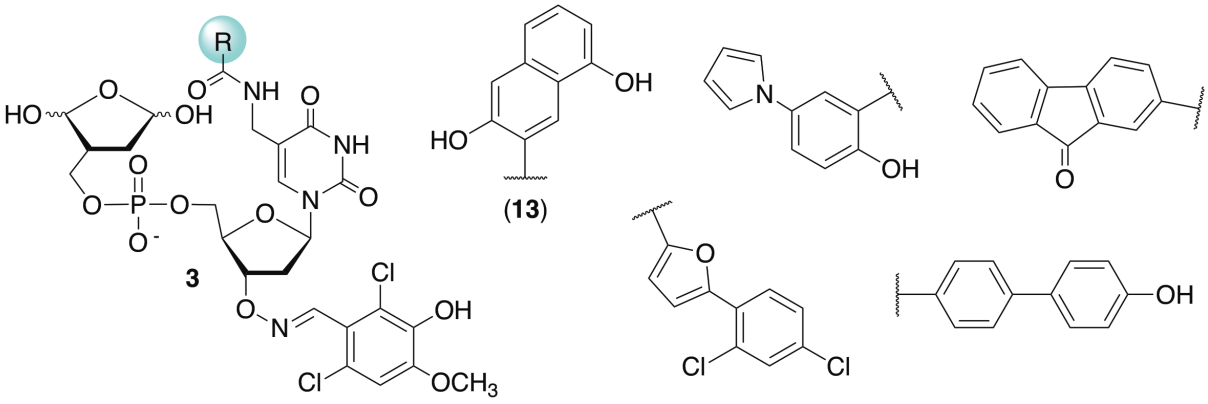


Figure S3. Demonstration that ARP reacts with dRP in **14**. (a) dRP containg 3′-^{32}P-**14**. (b) The aldehyde reactive probe (ARP) (c) 20 % Denaturing PAGE gel diagram showing the dRP-ARP adduct formation with other controls.

Chart S2. Inhibitor candidates identified from fluorescence screen.



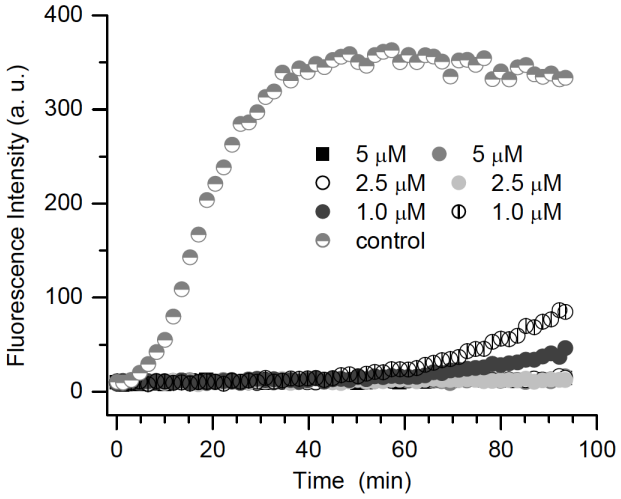


Figure S4. Concentration dependence of effect of **13** on strand displacement synthesis in **15** by Pol β measured via fluorescence.

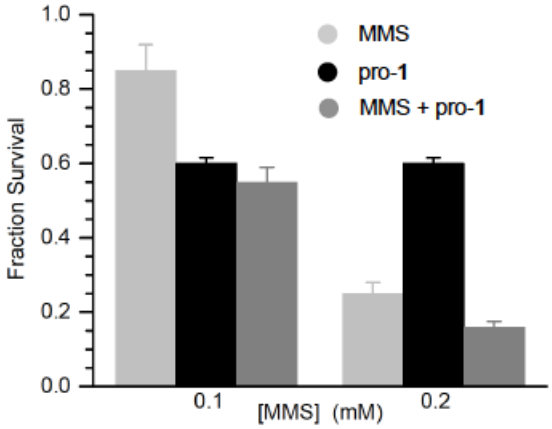


Figure S5. HeLa cell cytoxicity following treatment with MMS and/or pro-**1** (5 μM) for 2 h.

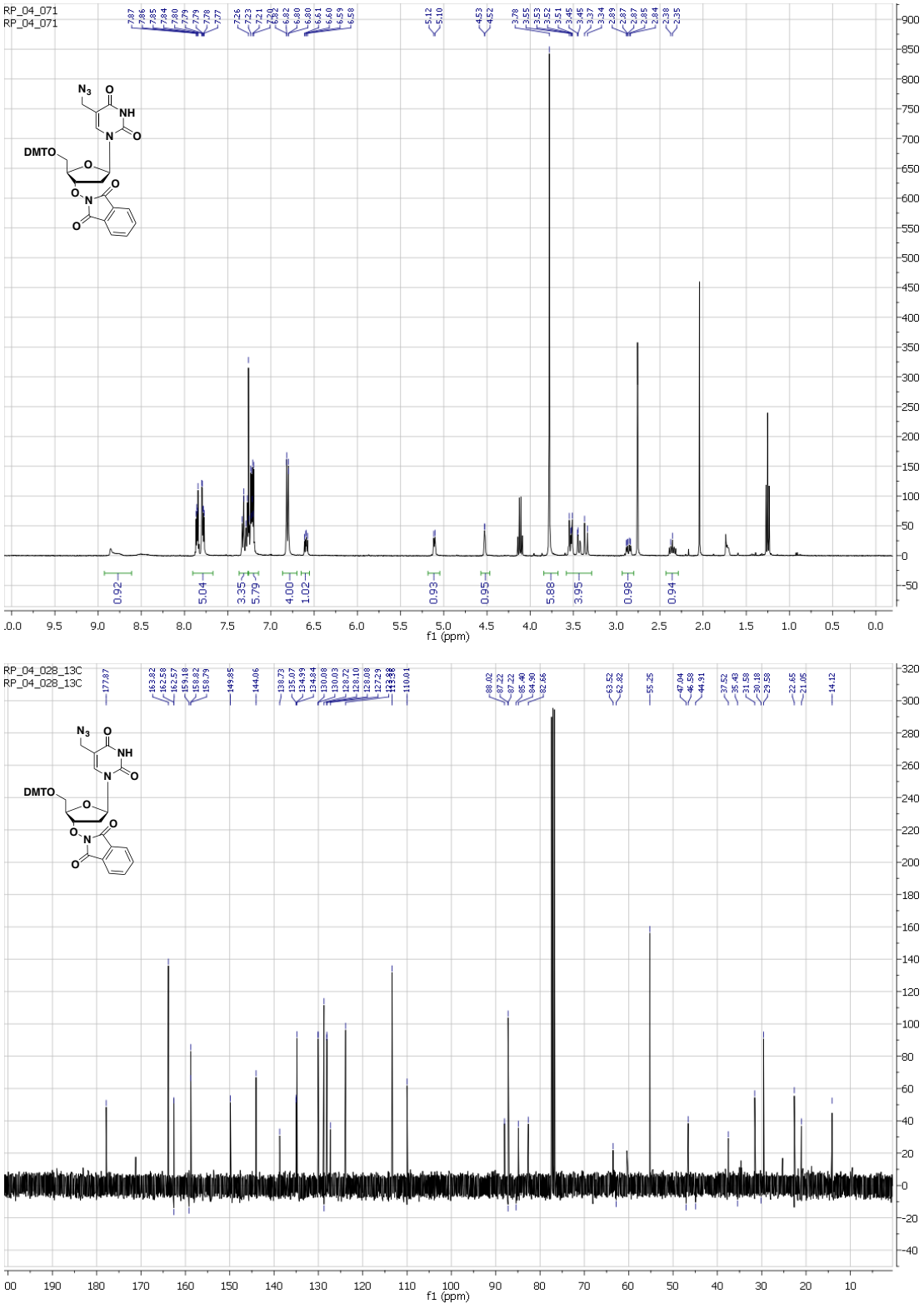


Figure S6. ^{1}H and ^{13}C NMR spectra of **5**.

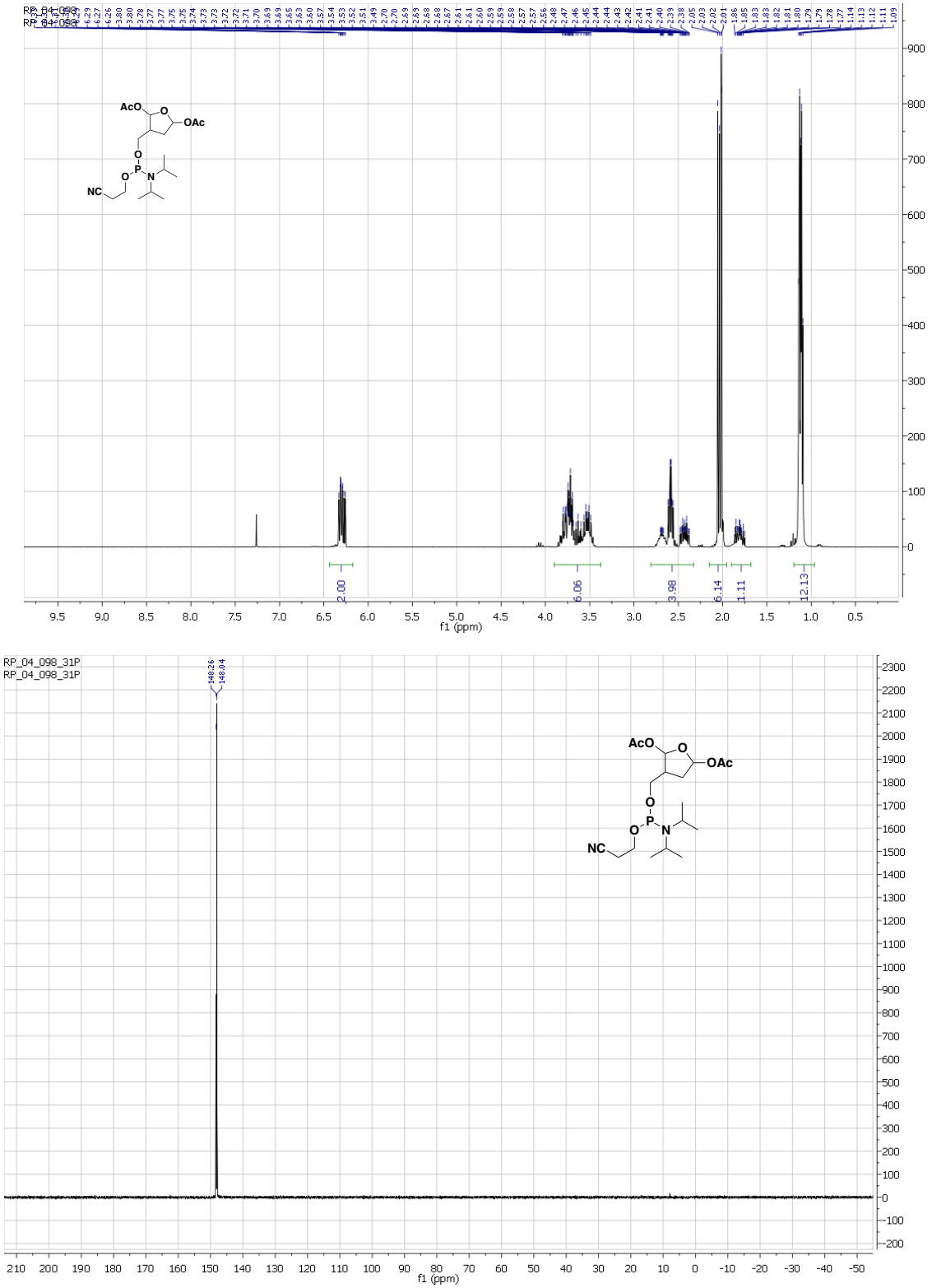


Figure S7. ^{1}H and ^{31}P NMR spectra of **11**.

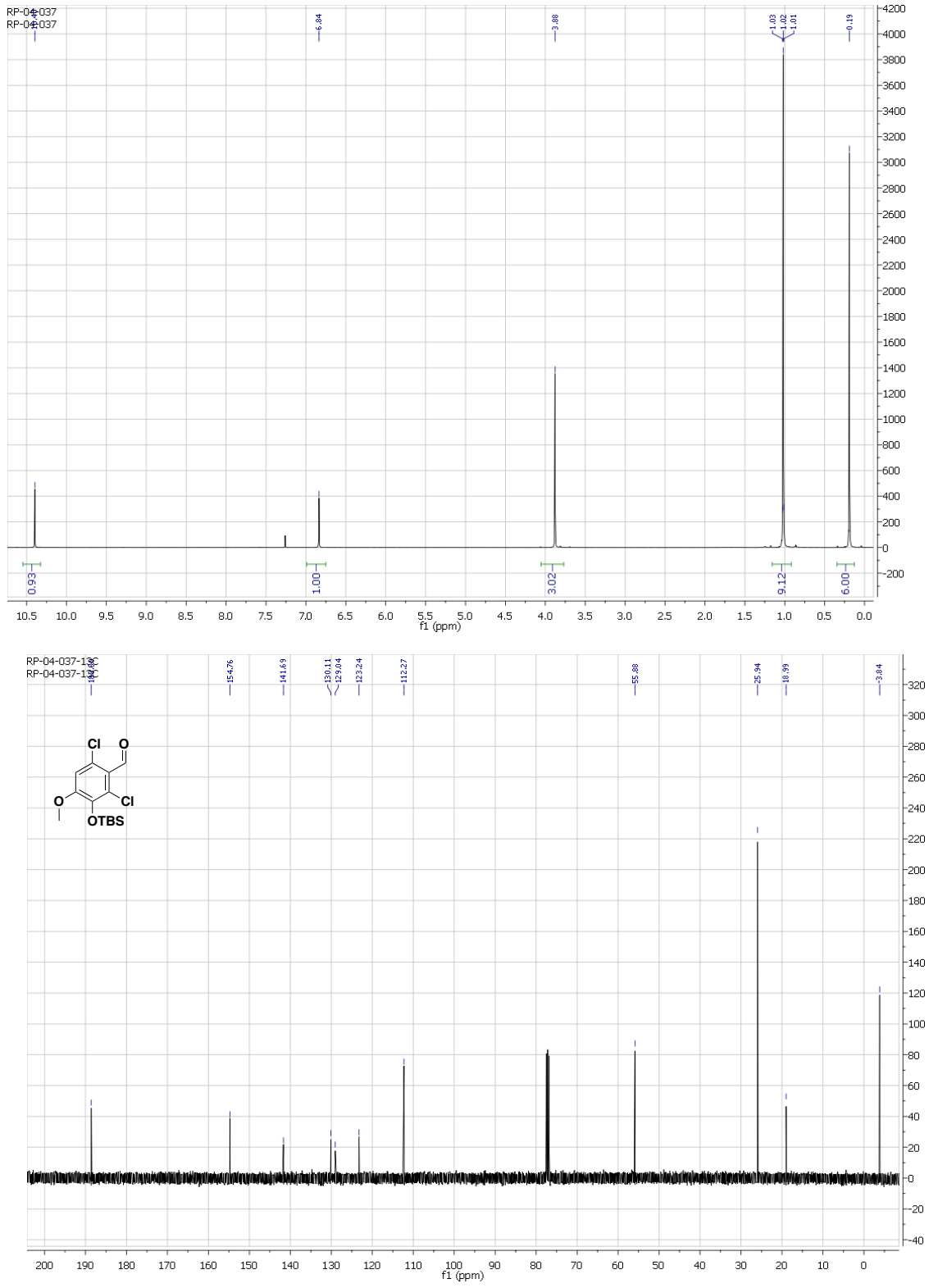


Figure S8. ^{1}H and ^{13}C NMR spectra of **9**.

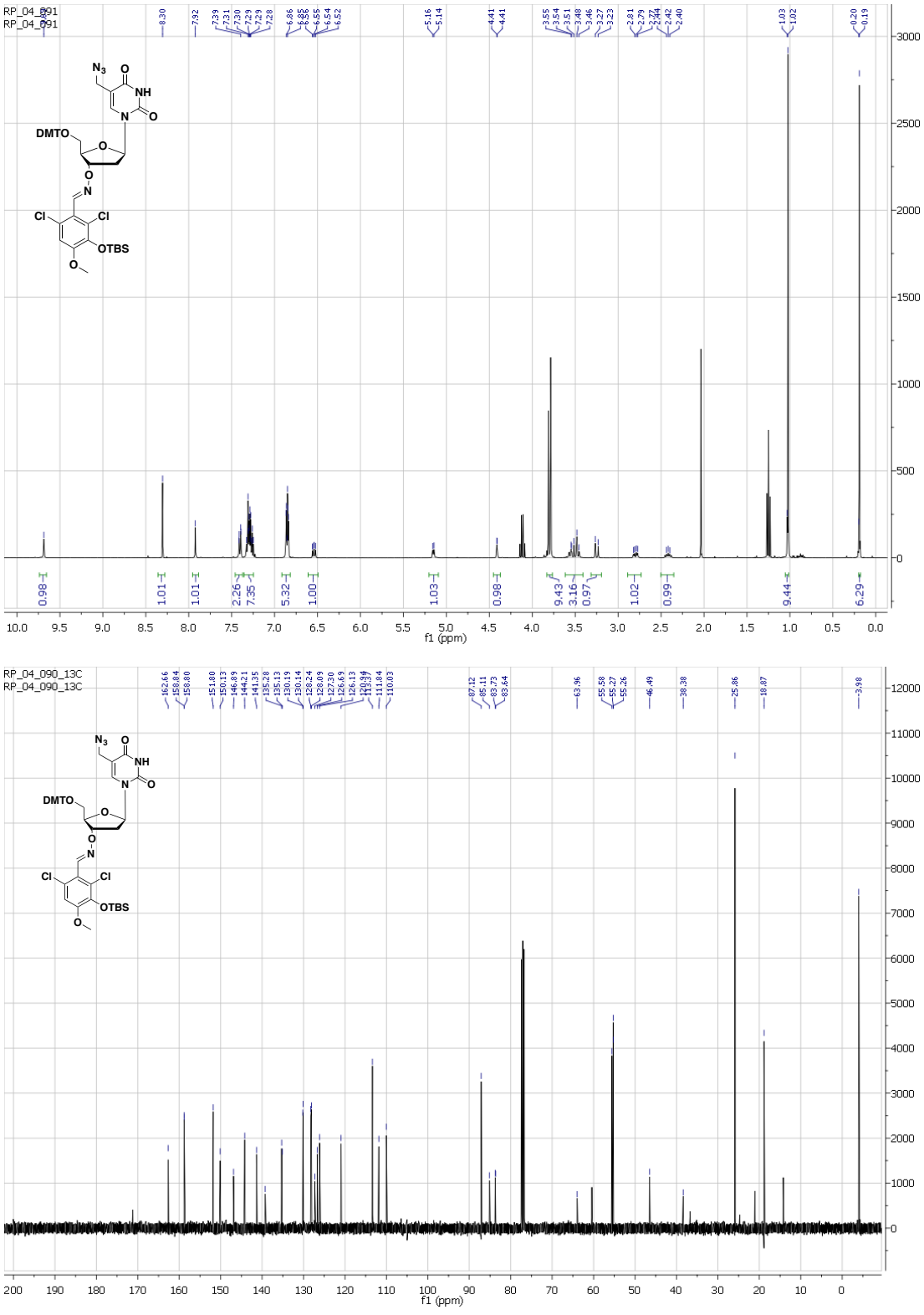


Figure S9. ^{1}H and ^{13}C NMR spectra of **6**.

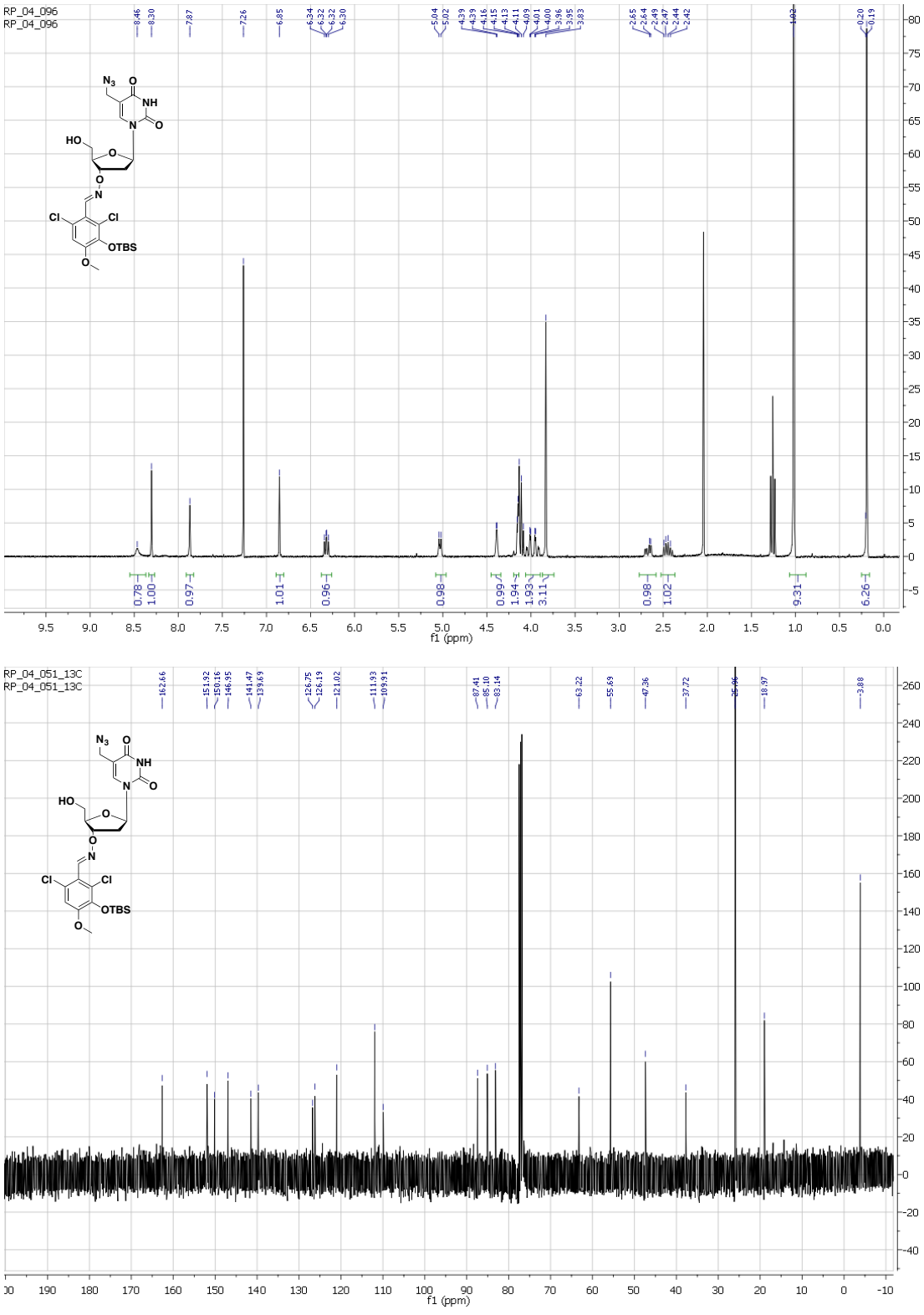


Figure S10. ^{1}H and ^{13}C NMR spectra of **7**.

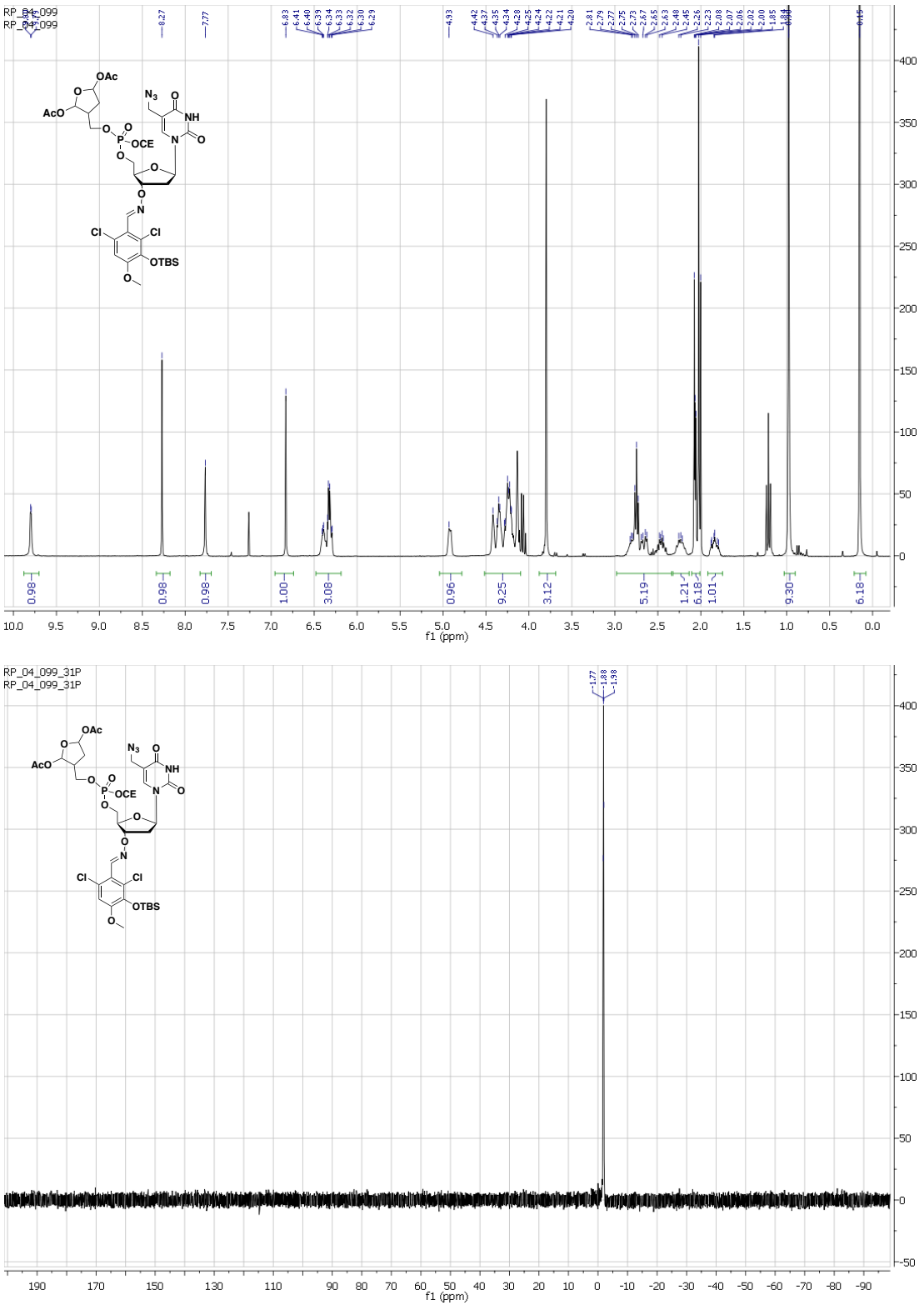


Figure S11. ^{1}H and ^{31}P NMR spectra of TBS-phosphate triester precursor to **12**.

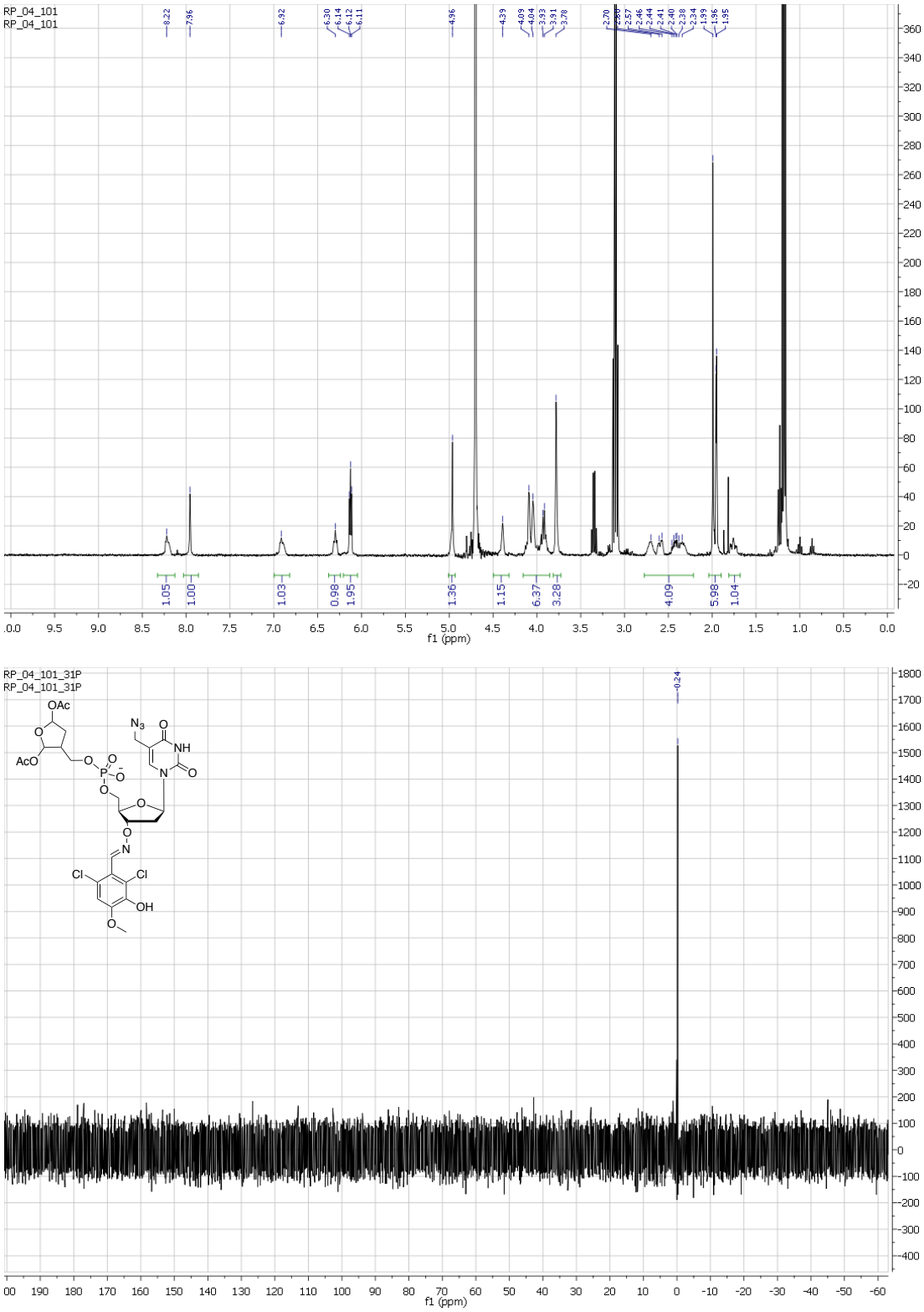


Figure S12. ^{1}H and ^{31}P NMR spectra of **12**.

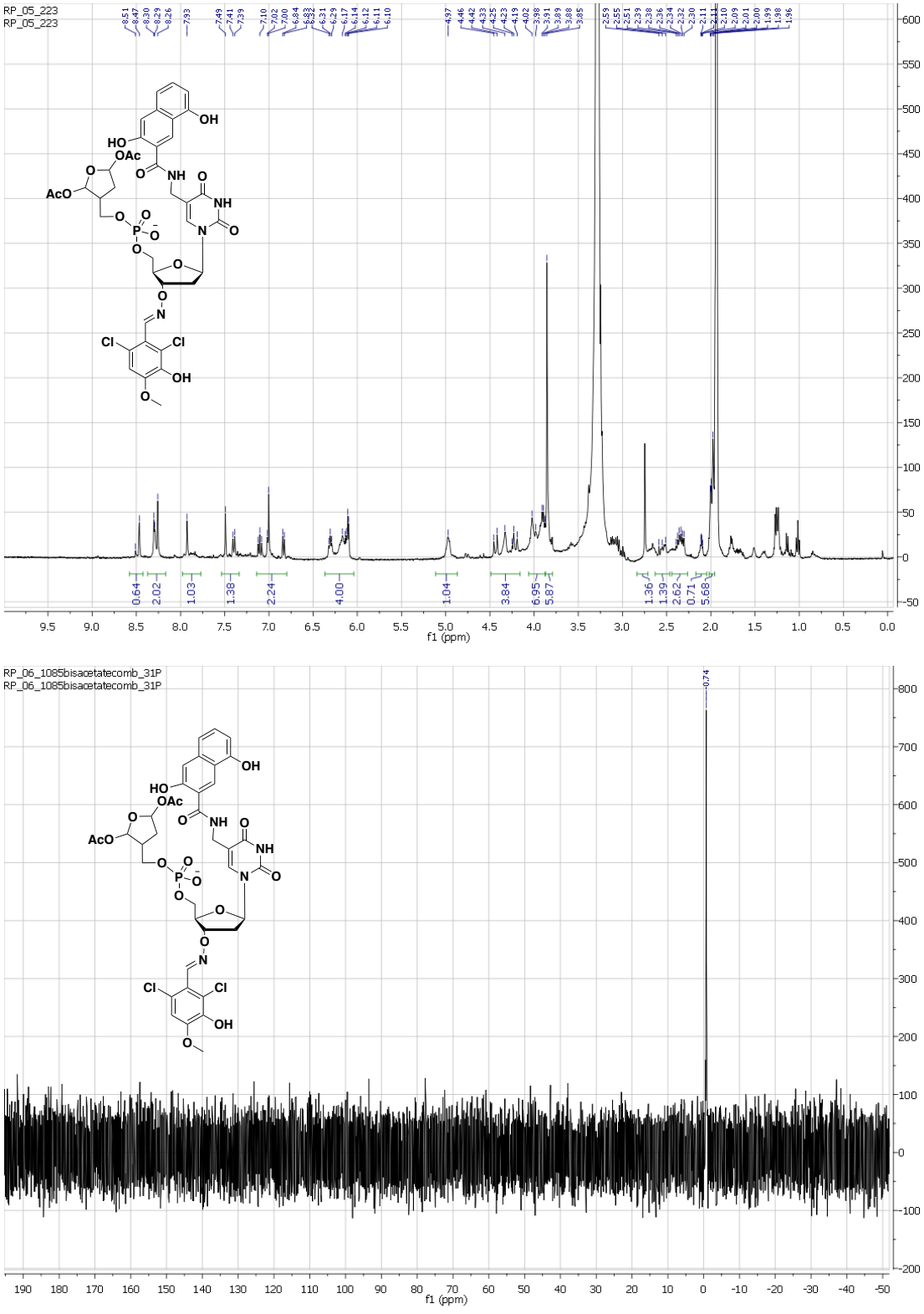


Figure S13. ^{1}H and ^{31}P NMR spectra of pro-**13**.

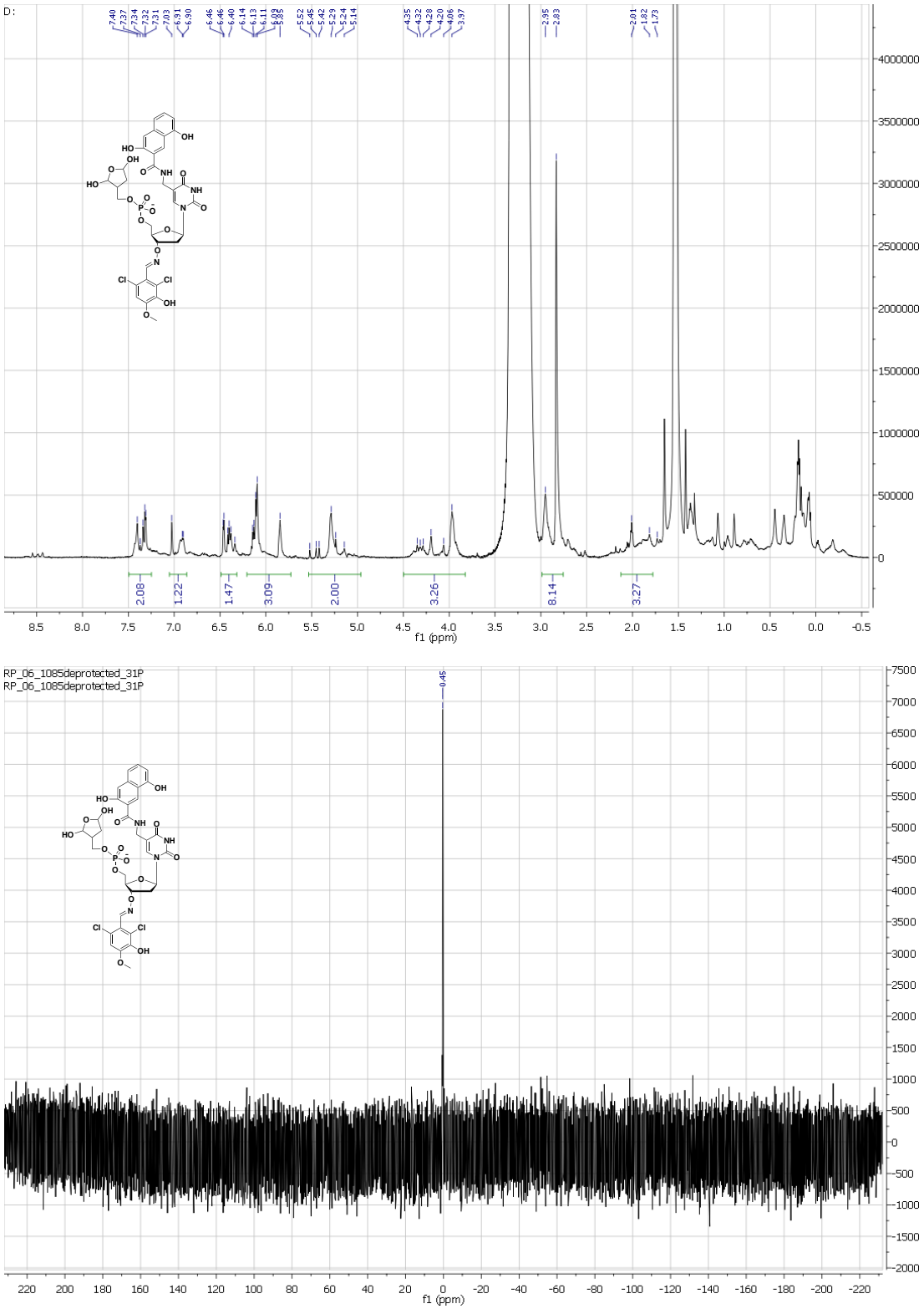


Figure S14. ^{1}H and ^{31}P NMR spectra of **13**.

1. Chen, F., Gaucher, E. A., Leal, N. A., Hutter, D., Havemann, S. A., Govindarajan, S., Ortlund, E. A., and Benner, S. A. (2010) Reconstructed evolutionary adaptive paths give polymerases accepting reversible terminators for sequencing and SNP detection, *Proc. Natl. Acad. Sci. USA 107*, 1948-1953.
2. Arian, D., Hedayati, M., Zhou, H., Bilis, Z., Chen, K., DeWeese, T. L., and Greenberg, M. M. (2014) Irreversible Inhibition of DNA Polymerase β by Small-Molecule Mimics of a DNA Lesion, *J. Am. Chem. Soc. 136*, 3176-3183.