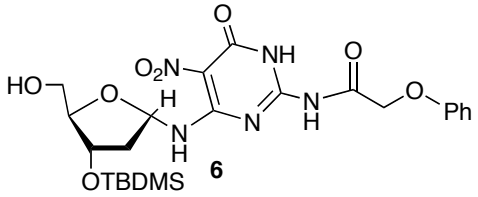
Supporting Information: Facile Quantification of Lesions Derived From 2'-Deoxyguanosine in DNA

Liang Xue and Marc M. Greenberg*
Department of Chemistry, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218

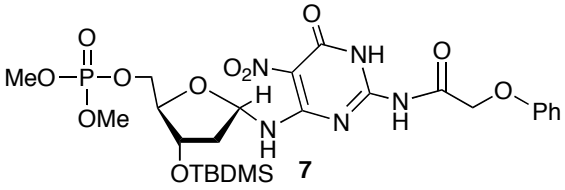
Contents:

General Methods. Unless otherwise specified, chemicals were purchased from Aldrich or Fisher Scientific and used without further purification. Reactions were carried out under Argon using

dry solvent, unless otherwise noted. Oligonucleotides containing OxodG, OxodA, 5-OHdU, or 5-OHdC were synthesized using standard cycles on an Applied Biosystems model 394 DNA/RNA synthesizer using reagents purchased from Glen Research. Oligonucleotides containing Fapy•dG or Fapy•dA were prepared previously in this laboratory.[1,2] IR spectra were collected on a Bruker Vector 33 FT-IR spectrophotometer. ^{1}H, ^{13}C spectra were collected on a Bruker Avance 400 MHz FT-NMR spectrometer. HRMS (FAB) spectra were collected on a VG Instruments VG70S magnetic sector mass spectrometer. LRMS (MALDI) spectra were collected using a Kratos analytical KOMPACT SEQ mass spectrometer. ESI-MS spectra were collected using a Thermoquest LCQ-Deca Ion Trap instrument. Fluorescence spectra were collected on a Varian Cary Eclipse fluorescence spectrophotometer equipped with a microplate reader. γ-Radiolysis experiments were carried out on a Gammacell 40 ^{137}Cs irradiator. T4 polynucleotide kinase was obtained from New England Biolabs. [γ^{32}P]ATP was purchased from Amersham Pharmacia Biotech. Quantification of radiolabeled oligonucleotides was carried out using a Molecular Dynamics Phosphorimager equipped with ImageQuant Version 5.1 software. Ninety-six well flat-bottom assay plates (black) were purchased from Corning (Fisher, Cat. No. 07200509). Protamine sulfate and Tween 20 were purchased from Sigma (Cat. No. P 4020 and P 9416). SuperBlocking buffer in PBS and BlockBSA in PBS (10 x) were purchased from Pierce (Cat. No. 37515 and 37525). ABC Elite ultra kit was purchased from Vector Laboratories (Cat. No. PK-6100). Amplex Red and Amplex Red Stop reagents were purchased from Invitrogen (Cat. No. A36006 and A33855).



6

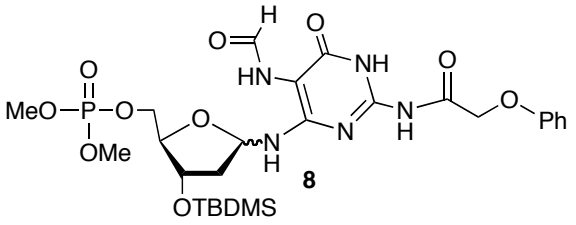


7

Synthesis of 7. In a round-bottomed flask (10

mL), **6**[1] (506 mg, 0.878 mmol) and tetrazole (369 mg, 5.27 mmol, 6 equiv.) were dissolved in THF (5 mL) at room temperature. Dimethyl N, N-diisopropylphosphoramidite (306 mg, 1.58 mmol, 1.8 equiv.) was added and the reaction mixture was stirred at room temperature for 1 h. After addition of 6 M t-BuOOH (340 μL) in decane and stirring at 0°C for 1 h, the reaction mixture was concentrated under vacuum. Flash chromatography of the residue (4% MeOH in ethyl acetate) yielded the desire product (400 mg, 66%). IR (KBr): 3240, 2959, 2855, 1692, 1626, 1579, 1504, 1429, 1251, 1176, 1025, 828, 782 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.32 (bd s, 1H), 10.22 (d, 1H, *J* = 8.2 Hz), 9.34 (bd s, 1H), 7.18 (d, 2H, *J* = 8.6 Hz), 6.89 (d, 2H, *J* = 8.6 Hz), 6.29 (t, 1H, *J* = 7.5 Hz), 4.68 (s, 2H), 4.48 (d, 1H, *J* = 5.0 Hz), 4.24 (t, 1H, *J* = 4.4 Hz), 3.93-4.08 (m, 2H), 3.77-3.81 (m, 6H), 2.87 (septet, 1H, *J* = 6.9 Hz), 2.35-2.41 (m, 1H), 2.03 (d, 1H, *J* = 13.7 Hz), 1.21-1.25 (d, 6H, *J* = 6.9 Hz), 0.92 (s, 9H), 0.18 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 171.1, 158.1, 154.6, 154.5, 149.3, 143.6, 127.8, 114.7, 113.7, 86.1, 86.0, 82.5, 73.7, 67.3, 66.9, 66.87, 54.6, 54.5, 40.3, 33.3, 25.9, 25.7, 24.0, 18.3, -4.72, -4.80; ^{31}P NMR ($CDCl_3$) δ 1.20; HRMS (FAB) calcd. for $C_{28}H_{45}N_5O_{11}PSi^+$ ($M+H^+$) 686.2623, found 686.2638.

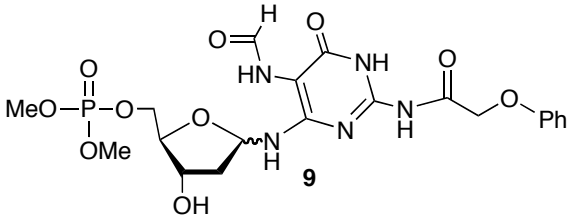
Synthesis of 8. To a solution of compound **7** (400 mg, 0.58 mmol) in 10 mL of THF, was added Pd/C (300 mg) and DIPEA (478 μL). The reaction mixture was then stirred under 80 psi hydrogen at room temperature for 2 h. The disappearance of starting material and the appearance of two products were observed by TLC (3% MeOH in CH_2Cl_2, developed twice). Pyridine (117 mg, 1.46 mmol, 114 μL) and acetic formic anhydride (100 mg, 1.13 mmol, 92 μL) were added into the reaction mixture at 0°C and stirring was continued for 60 min at the same temperature. The reaction mixture was then concentrated under vacuum. Flash chromatography of the residue



8

(4.5% MeOH in methylene chloride) yielded two isomers (**8a**: high mobility; **8b**: low mobility) (**8a**, 195 mg, 49%; **8b**, 65 mg, 16%). **8a**: IR (KBr): 3259, 2959, 2846, 1654, 1579, 1504, 1251, 1185, 1025, 838, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 11.40 (bd s, 1H), 9.73 (m, 0.9H), 9.50 (m, 0.1H), 8.21-8.22 (m, 1H), 8.00 (d, 0.1H, *J* = 11.2 Hz), 7.14 (d, 2H, *J* = 8.6 Hz), 6.86 (d, 2H, *J* = 8.6 Hz), 6.63 (d, 0.9H, *J* = 9.0 Hz), 6.04-6.08 (m, 1H), 4.61 (s, 2H), 4.35 (m, 1H), 4.13 (m, 1H), 3.93-4.04 (m, 2H), 3.67-3.75 (m, 6H), 2.80-2.87 (m, 1H), 2.27-2.34 (m, 1H), 1.92 (d, 1H, *J* = 13.4 Hz), 1.19 (d, 6H, *J* = 7.0 Hz), 0.87 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.6, 160.7, 158.3, 155.1, 154.8, 154.7, 146.7, 143.2, 127.7, 127.66, 114.69, 114.66, 96.7, 84.2, 84.1, 82.2, 73.2, 72.5, 67.1, 66.9, 54.53, 54.51, 54.48, 40.3, 33.3, 25.8, 25.7, 24.1, 18.0, 17.9, -4.72, -4.83, -4.86, -4.89; ^{31}P NMR ($CDCl_3$) δ 1.19; HRMS (FAB) calcd for $C_{29}H_{47}N_5O_{10}PSi^+$ ($M+H^+$) 684.2830, found 684.2829. **8b**: IR (KBr): 3259, 2959, 2855, 1645, 1589, 1504, 1429, 1251, 1185, 1035, 828, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 11.38 (bd s, 1H), 9.31 (bd s, 0.7H), 9.21 (bd, s 0.3H), 8.24 (m, 0.6H), 8.06 (bd s, 0.5H), 7.63 (d, 0.3 H, *J* = 11.5 Hz), 7.20 (d, 2H, *J* = 8.6 Hz), 7.02 (d, 0.7H, *J* = 9.1 Hz), 6.91 (d, 2H, *J* = 8.7 Hz), 6.18 (m, 0.3H), 6.10 (m, 0.7H), 4.64 (s, 2H), 4.40-4.43 (m, 1H), 4.06-4.09 (m, 2H); 3.99 (m, 1H), 3.74-3.79 (m, 6H), 2.89 (m, 1H), 2.19 (m, 1H), 2.04-2.09 (m, 1H), 1.23 (d, 6H, *J* = 7.0 Hz), 0.87 (s, 9H), 0.12 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.2, 160.3, 157.8, 154.6, 154.1, 146.1, 143.5, 127.8, 114.7, 98.0, 84.6, 84.5, 82.3, 72.5, 67.4, 67.2, 54.7, 54.6, 54.58, 54.5, 41.1, 33.3, 25.8, 25.7, 24.1, 18.0, -4.7, -4.9; ^{31}P NMR ($CDCl_3$) δ 0.98; HRMS (FAB) calcd for $C_{29}H_{47}N_5O_{10}PSi^+$ ($M+H^+$) 684.2830, found 684.2823.

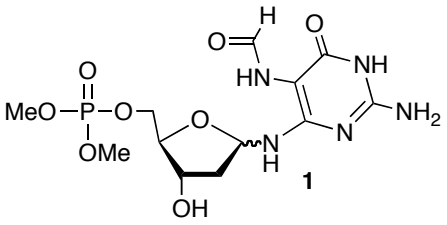
Synthesis of 9. To a solution of compound **8a** (185mg, 0.27 mmol) in THF (10 mL), was added $Et_3N•3HF$ (521 μL, 3.2 mmol, 12 equiv.). After stirring at room temperature for 8 h (TLC was



9

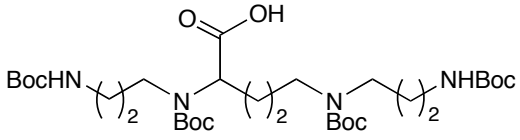
used to monitor the reaction; 1:10 MeOH:CH_2Cl_2), the reaction mixture was concentrated under vacuum. Flash chromatography of the residue (10% MeOH in CH_2Cl_2) yielded the desired product as a pale yellow solid (131 mg, 85%). IR (KBr): 3259, 2958, 1654, 1589, 1513, 1430, 1241, 1194, 1035, 847, 772 cm^{-1}; ^1H NMR (MeOD) δ 8.26 (s, 0.5H), 8.24 (s, 0.3H), 7.97 (m, 0.2 H), 7.17 (d, 2H, *J* = 8.6 Hz), 6.94 (d, 2H, *J* = 8.6 Hz), 6.14 (m, 1H), 4.74 (m, 2H), 4.30-4.36 (m, 1H), 3.98-4.16 (m, 3H), 3.74-3.82 (m, 6H), 2.82-2.89 (m, 1H), 2.39 (m, 0.6H), 2.17 (m, 0.8H), 1.98-2.03 (m, 0.6H), 1.22 (d, 6H, *J* = 6.9 Hz); ^{13}C NMR (MeOD) δ 171.9, 171.8, 162.7, 162.5, 158.1, 155.7, 148.4, 148.3, 142.4, 127.1, 114.4, 94.1, 93.9, 84.0, 83.7, 83.6, 82.2, 82.1, 71.5, 71.2, 67.8, 67.5, 67.4, 66.9, 54.1, 54.02, 54.0, 53.96, 53.93, 38.9, 38.8, 33.2, 23.2; ^{31}P NMR (MeOD) δ 0.98, 0.96; HRMS (FAB) calcd for $C_{23}H_{33}N_5O_{10}P^+$ ($M+H^+$) 570.1965, found 570.1954.

Synthesis of 1. To a solution of compound **9** (16.5mg, 0.029 mmol) in MeOH (2 mL), was added anhydrous K_2CO_3 (6.9 mg) under Ar. After stirring for 3 h at room temperature (TLC was used to monitor the reaction; 1:10 MeOH:CH_2Cl_2), the reaction mixture was neutralized with AcOH (5.7 μL) and concentrated under vacuum. Flash chromatography of the residue (H_2O, RP-18 silica gel, 5 g, 1 cm diameter column) yielded the desired product associated with acetic acid (14.5 mg, 98%). IR (KBr): 3410, 2961, 2856, 1688, 1647, 1589, 1561, 1484, 1408, 1341, 1256, 1188, 1046, 1018, 925, 854, 777 cm^{-1}; ^1H NMR (MeOD) δ 8.18-8.20 (m, 0.75 H), 7.85 (s, 0.25 H), 6.09-6.15 (m, 1H), 4.27-4.32 (m, 1H), 4.02-4.11 (m, 2.5H), 3.94 (m, 0.5H), 3.74-3.83 (m, 6H), 2.33-2.36 (m, 0.5H), 2.09-2.17 (m, 1H), 1.97-2.02 (m, 0.5H), 1.91 (s, $C\underline{H}_3COOH$); ^{13}C NMR (MeOD) δ 179.3 ($CH_3\underline{C}OOH$), 168.9, 163.4, 163.2, 160.9, 159.8, 159.6, 154.5, 89.0, 88.9, 83.7, 83.6, 83.5, 83.4, 83.3, 81.9, 81.8, 71.5, 71.1, 78.0, 67.9, 67.5,



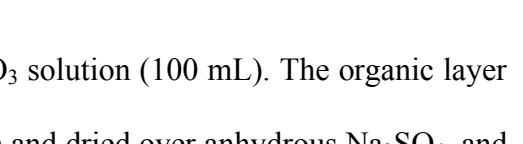
1

67.46, 54.1, 54.0, 53.97, 38.9, 22.9 ($\underline{C}H_3COOH$); ^{31}P NMR (MeOD) δ 0.97; ESI-MS calcd for $C_{12}H_{21}N_5O_8P^+$ ($M+H^+$) 394.29, found 394.35.



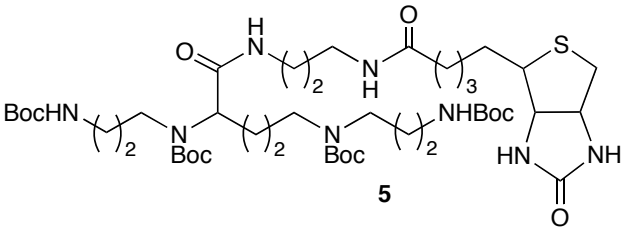
3

Synthesis of 4. To a DMF solution (5 mL) of **3**[3] (327 mg, 0.773 mmol), EDCI (106 mg, 0.85 mmol), and HOBt (75 mg, 0.85 mmol), was added a DMF solution (5 mL) of 1,3-diaminopropane (573 mg, 7.73 mmol) and stirred overnight at room temperature. The reaction mixture was concentrated under vacuum and partitioned between CH_2Cl_2 (200 mL) and saturated aqueous $NaHCO_3$ solution (100 mL). The organic layer was further washed by brine (100 mL) and H_2O (100 mL) and dried over anhydrous Na_2SO_4, and concentrated to afford colorless oil (266 mg, 48 %). This product was used in the next step without further purification. IR (KBr): 3250, 2879, 1674, 1556, 1537, 1454, 1348, 1201, 1133 cm^{-1}; ^1H NMR (MeOD) δ 4.01 (m, 1H), 3.06-3.34 (m, 9H), 3.03-3.06 (m, 3H), 2.73 (t, 2H, J = 6.6 Hz), 1.57-1.72 (m, 10H), 1.45-1.48 (m, 36H); ^{13}C NMR (MeOD) δ 173.8, 156.9, 156.4, 156.0, 79.6, 79.4, 79.1, 78.5, 56.8, 54.6, 44.1, 38.2, 38.0, 37.8, 37.6, 36.1, 34.4, 31.2, 29.4, 28.9, 28.1, 27.7, 27.6, 27.52, 27.50, 27.47, 14.6; HRMS (FAB) calcd for $C_{41}H_{80}N_6O_{12}^+$ ($M+H^+$) 849.5912, found 849.5913.



4

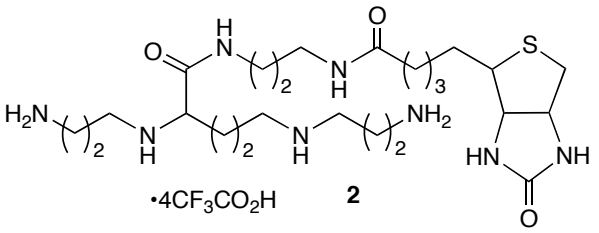
Synthesis of 5. To a DMF solution (5 mL) of biotin (88 mg, 0.361 mmol), PyBOP (282 mg, 0.54 mmol), and DIPEA (102 μL, 0.61 mmol), was added a DMF solution (5 mL) of **4** (254 mg, 0.361 mmol) and stirred overnight at room temperature. The reaction mixture was concentrated under vacuum and partitioned between CH_2Cl_2 (200 mL) and saturated aqueous



5

$NaHCO_3$ solution (100 mL). The organic layer was dried over anhydrous Na_2SO_4, and concentrated. Flash chromatography of the residue (CH_3OH:CH_2Cl_2:NH_4OH 15:100:0.1) yielded the desired product (253 mg, 76%). IR (KBr): 3308, 2983, 2933, 1688, 1525, 1452, 1362, 1289, 1167, 842 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.03 (bd s, 1H), 6.63 (bd s, 1H), 6.17 (bd s, 1H), 4.43 (s, 1H), 4.24 (s, 1H), 4.04-4.07 (m, 1H), 2.99-3.35 (m, 14H), 2.79-2.81 (m, 2H), 2.64-2.67 (m, 1H), 2.13 (m, 2H), 1.43-1.83 (m, 16H), 1.19-1.36 (m, 36H); ^{13}C NMR ($CDCl_3$) δ 173.8, 164.4, 155.9, 79.6, 78.9, 76.9, 61.8, 60.2, 55.8, 40.5, 36.5, 36.2, 36.0, 30.1, 29.2, 28.8, 28.4, 28.3, 28.0, 25.6; HRMS (FAB) calcd for $C_{41}H_{80}N_6O_{12}^+$ ($M+H^+$) 929.5754, found 929.5746.

Synthesis of 2. In a round-bottomed flask (50 mL), compound 5 (160 mg, 0.172 mmol) was dissolved in TFA (10 mL) and stirred at room temperature for 10 min. TFA was removed



2

under vacuum. The residue was redissolved in fresh TFA (10 mL) and stirred at room temperature for another 10 min. After removing TFA under vacuum, the residue was dissolved in H_2O (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The aqueous layer was concentrated under vacuum. Flash chromatography of the residue (RP-C18 silica gel, 5 g, 1 cm diameter column, CH_3OH:H_2O 1:1) yielded the desired product (140 mg, 80%). IR (KBr): 3296, 3062, 2949, 1673, 1551, 1466, 1438, 1204, 1138, 838, 800, 725 cm^{-1}; ^1H NMR (D_2O) δ 4.50 (s, 1H), 4.32 (s, 1H), 3.87 (m, 1H), 2.88-3.32 (m, 16H), 2.65-2.68 (m, 1H), 1.30-2.15 (m, 18H); ^{13}C NMR (D_2O) δ 176.7, 168.8, 162.7, 162.4, 117.7, 114.8, 62.0, 60.3, 55.3, 52.7, 46.8, 44.5, 39.6, 36.9, 36.5, 36.45, 35.4, 27.84, 27.8, 27.7, 27.6, 25.1, 23.6, 21.3; HRMS (FAB) calcd for $C_{24}H_{48}N_8O_3S^+$ ($M+H^+$) 529.3648, found 529.3627.

Analysis of reactions of 2 with DNA containing OxodA, OxodG, Fapy•dA, Fapy•dG, 5-OHdU, and 5-OHdC by PAGE. The lesion containing oligonucleotide (50 pmol), 5'-[^{32}P]-labeled material, and complementary strand (2.5 equivalents) were dissolved in a mixture (100 μL) of Tris (20 mM, pH 7.5) and NaCl (100 mM). The mixture was hybridized at 55°C for 10 min, slowly cooled to 25°C, and incubated at 4°C overnight. 5'-[^{32}P]-Labeled oligonucleotide was added such that the radiation of DNA solution was approximately 30,000 cpm/μL**.** The DNA was incubated in a mixture (10 μL) of phosphate (20 mM, pH 7.5), NaCl (100 mM), and **2** (0.5 mM) at 25°C for 30 min, followed by addition of aqueous solutions (2 μL) of Na_2IrCl_6 (0.6 mM), Na_2IrBr_6 (6 mM), or $K_3Fe(CN)_6$ (6 mM). The reaction mixtures were vortexed and incubated at 25°C for 30 min. After quenching the reaction by addition of EDTA (2 μL, 10 mM), the reactions were precipitated from NaCl (0.2 M) and EtOH at -80°C. After centrifugation, removal of the supernatant, and drying, the residues were resuspended in formamide loading buffer (5 μL). Analytical oligonucleotide separations were carried out using 20 % polyacrylamide denaturing gel [5% crosslink, 45% urea (w/w)].

The average adduct yield for a particular lesion in different DNA sequence (TXA, TXT, TXG, TXC, GGGY, or GY, where X = Fapy•dG or OxodG, Y = OxodG) was determined by measuring the sum of adduct percentage yield in each individual reaction (3 samples). The overall average adduct yield for a particular lesion was then calculated by averaging the adduct yield for each individual sequence determined by PAGE.

DNA sequences (duplex) used in PAGE experiments are listed below. The complementary sequences are not included.

TXC:	5'-d(GAAGACCTXCGCGTCC)	X = OxodG, Fapy•dG
TXA:	5'-d(GAAGACCTXAGCGTCC)	X = OxodG, Fapy•dG
TXT:	5'-d(GAAGACCTXTGCGTCC)	X = OxodG, Fapy•dG, 5-OHdU(dC)

TXG:	5'-d(GAAGACCTXGGCGTCC)	X = OxodG, Fapy•dG, OxodA
GGGX:	5'-d(GAAGAGGGXTGCGTCC)	X = OxodG
GX:	5'-d(GAAGACCGXTGCGTCC)	X = OxodG
TXT:	5'-GGACTGGCAGCTCTGCACTXTCAGC	X = Fapy•dA

MALDI-TOF MS analysis of reaction of 2 and DNA containing OxodG and Fapy•dG. The oligonucleotides containing OxodG and Fapy•dG (1 nmol) were incubated in a mixture (20 μL) of phosphate (20 mM, pH 7.5) and **2** (0.5 mM) at 25°C for 30 min, followed by addition of Na_2IrCl_6 (2.2 μL, 2 mM) and incubation at 25°C for 30 min. The reactions were precipitated from NH_4OAc (2.5 M) and EtOH at -80°C. After centrifugation, removal of the supernatant, and drying, the residues were resuspended in water (10 μL). The MS sample was prepared by combining analyte solution (2 μL) with 2,4,6-trihydroxyacetophenone matrix solution (2 μL, 10 mg of matrix in 1 mL of 22 mM ammonium citrate in 1:1 H_2O : CH_3CN) and spotted on the MALDI target plate. The MALDI plate was dried in a desiccator and then analyzed. The MALDI-TOF mass spectrometer was calibrated (externally) with two standard oligomers: 5'-d(TCGCTGT) (7 mer, MW: 2087.4) and 5'-d(AGGCGTTCAACGGCTCTG) (18 mer, MW: 5515.6).

Preparation of a 287 nt PCR fragment. A 287 nt PCR fragment was prepared from M13mp7 plasmid (10 fmol), which was amplified with primer 1 or 2 and primer 3 (250 pmol each), dNTP (0.5 mM each), Taq DNA polymerase (5 Units) in 100 μL of Taq DNA polymerase buffer (20 mM Tris, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8). PCR was performed using the following conditions: 94 °C, 30 sec for melting and 58 °C, 1 min for annealing and then 72 °C, 1 min for polymerase reaction. After repeating the cycle 60 times, the reaction solution was extracted by phenol and further purified by Microcon (MY-30) using a standard protocol. The concentration of the PCR fragment was determined by UV (ε_{260} = 20 g^-

^{1}•cm^{-1}•L) and the quality of PCR fragment was determined by agrose gel (3%). Sequences of three primers and PCR fragment: 5'-CAC TGA ATC ATGGTC ATA GCT GTT-3' (primer 1), 5'-biotin-CAC TGA ATC ATGGTC ATA GCT GTT-3' (primer 2) and 5'-GGT GAA GGG CAA TCA GCT GTT-3' (primer 3) used for primers. The sequence of the PCR fragment is 5'-GGT GAA GGG CAA TCA GCT GTT GCC CGT CTC ACT GGT GAA AAG AAA AAC CAC CCT GGC GCC CAA TAC GCA AAC CGC CTC TCC CCG CGC GTT GGC CGA TTC ATT AAT GCA GCT GGC ACG ACA GGT TTC CCG ACT GGA AAG CGG GCA GTG AGC GCA ACG CAA TTA ATG TGA GTT AGC TCA CTC ATT AGG CAC CCC AGG CTT TAC ACT TTA TGC TTC CGG CTC GTA TGT TGT GTG GAA TTG TGA GCG GAT AAC AAT TTC ACA CAG GAA ACA GCT ATG ACC ATG ATT CAG TG-3'.

Sample preparation of a PCR fragment (287 nt) treated with γ-radiolysis for assay detection. The aqueous solutions (55 μL) of 287 nt PCR fragment DNA (22 pmol) in Pyrex glass tubes were treated with γ-radiolysis at different doses (1-20 Gy, 0.67 Gy/min). After γ-radiolysis, DNA samples (25 μL, 10 pmol DNA) were transferred into eppendorf tubes, followed by addition of phosphate (2.78 μL, 200 mM, pH 7.5) and of **2** (3.1 μL, 5 mM). The samples were incubated at 25 °C for 30 min. To the reaction mixtures, was added 3.4 μL of Na_2IrCl_6 (1 mM) or $K_3Fe(CN)_6$ (10 mM) and incubated at 25°C for another 30 min. After quenching with 6.1 μL of EDTA (10 mM), the reactions were precipitated from NaCl (0.2 M) and EtOH at -80°C three times. After centrifugation, removal of the supernatant, and drying, the residues were resuspended in 200 μL of Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 7.5). The concentrations of DNA samples after precipitation were determined by UV (ε_{260} = 20 g^{-1}•cm^{-1}•L). The DNA used in the ELISA-like assay was prepared by mixing the DNA solution from

reactions (0.5 pmol) with untreated 287 nt PCR fragment (2 pmol) to make a final DNA amount of 2.5 pmol in each solution.

Sample preparation for calibration curve used in fluorescence assay detection. Each assay plate must include the samples for calibration. The DNA solutions used for calibration were prepared by mixing a 5'-biotin tagged 287 nt PCR fragment and a non-biotin tagged 287 nt PCR fragment to make a final DNA amount of 2.5 pmol. The amount of biotin-tagged DNA in each solution for calibration in this particular experiment was 0, 0.025, 0.05, 0.075, 0.1, and 0.25 pmol, respectively.

Detection of OxodG and Fapy•dG using an ELISA-like assay. A 96-well black flat-bottom plate was incubated with 0.1% protamine (200 μL) for 20 h at 4°C, followed by washing with water (3 × 250 μL). DNA (2.5 pmol, 200 μL) in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) was incubated in the pretreated assay plate in an incubator at 37°C for 2 h without shaking. After washing with TPBS buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, and 0.1% Tween 20, pH 7.5, 10 × 250 μL), the plate was incubated (2 ×) with 250 μL BSA/SuperBlock solution (1 mg/mL) for 15 min at room temperature with shaking. The plate was then washed with TPBS buffer (5 × 250 μL), followed by incubation with ABC ultra solution (50 times dilution, Vector Lab) for 30 min at room temperature. After washing with TPBS (10 × 200 μL), the plate was incubated with Amplex Ultra Red substrate solution (100 μL, 0.1 mM Amplex Ultra Red and 0.015% H_2O_2 in 50 mM sodium phosphate buffer, pH 7.5) for 30 min at room temperature with shaking, followed by addition of 20 μL Amplex ultra Red stop solution (Invitrogen). Sodium phosphate buffer (180 μL, 50 mM, pH 7.5) was added into each well to achieve the final volume (300 μL). The fluorescence of each well in the plate was then determined using a fluorescence plate reader (Excitation: 568 nm, Emission: 581 nm, PMT 400,

slit width 10 nm). The fluorescence signals were transformed into absolute amounts of lesions using a calibration curve, which was established on the same assay plate. Note: the plate washing was accomplished by inverting the plate followed by gently tapping on paper towels to remove the remaining residue.

To calculate the yield of 8-oxodG and Fapy•dG, the following equations were used:

a) $$\frac{(Yield)_{OG-Cl}}{100} \times M_{OG} + \frac{(Yield)_{FG-Cl}}{100} \times M_{FG} = M_{Lesion-Cl} \Longrightarrow$$

$$\frac{(Yield)_{OG-Cl}}{100} \times \frac{M_{OG}}{Dose} + \frac{(Yield)_{FG-Cl}}{100} \times \frac{M_{FG}}{Dose} = \frac{M_{Lesion-Cl}}{Dose} \equiv (SLOPE)_{Lesion-Cl}$$

b) $$\frac{(Yield)_{OG-CN}}{100} \times M_{OG} = M_{Lesion-CN} \Longrightarrow$$

$$\frac{(Yield)_{OG-CN}}{100} \times \frac{M_{OG}}{Dose} = \frac{M_{Lesion-CN}}{Dose} \equiv (SLOPE)_{Lesion-CN}$$

$(Yield)_{OG-Cl}$: The percentage yield of OxodG adduct (88%) determined by PAGE in the presence of Na_2IrCl_6 using oligonucleotide containing OxodG.

$(Yield)_{FG-Cl}$: The percentage yield of Fapy•dG adduct (81%) determined by PAGE in the presence of Na_2IrCl_6 using oligonucleotide containing Fapy•dG.

$(Yield)_{OG-CN}$: The percentage yield of OxodG adduct (79%) determined by PAGE in the presence of $K_3Fe(CN)_6$ using oligonucleotide containing OxodG.

$(SLOPE)_{Lesion-Cl}$: The slope of detected lesions (pmol) as a function of dose in the presence of Na_2IrCl_6.

$(SLOPE)_{Lesion-CN}$: The slope of detected lesions (pmol) as a function of dose in the presence of $K_3Fe(CN)_6$.

M_{OG}: The amount of OxodG (pmol) in DNA.

M_{FG}: The amount of Fapy•dG (pmol) in DNA.

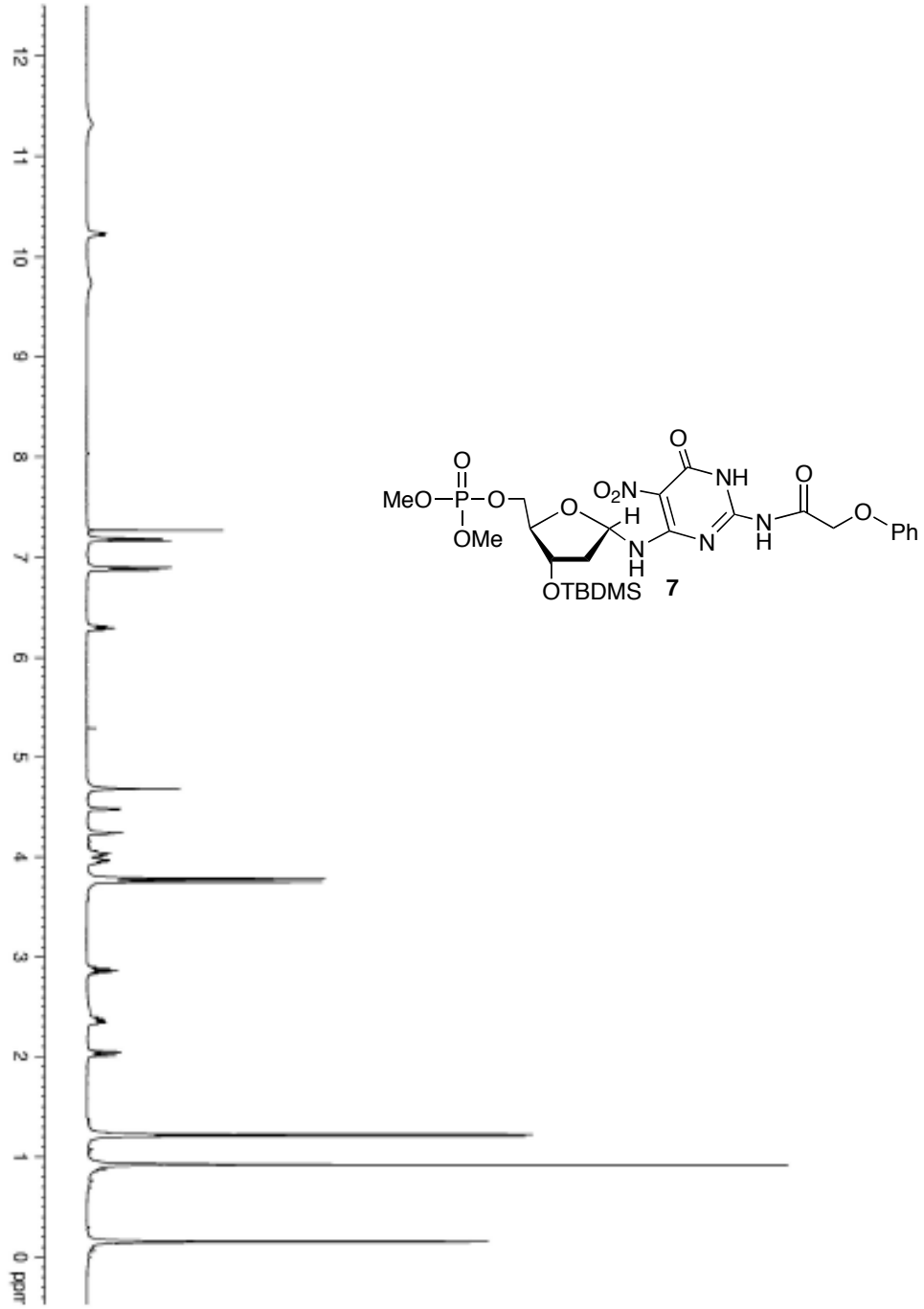
$M_{Lesion-Cl}$: The total amount of lesions (pmol) detected in the presence of Na_2IrCl_6.

$M_{Lesion-CN}$: The total amount of lesions (pmol) detected in the presence of $K_3Fe(CN)_6$.

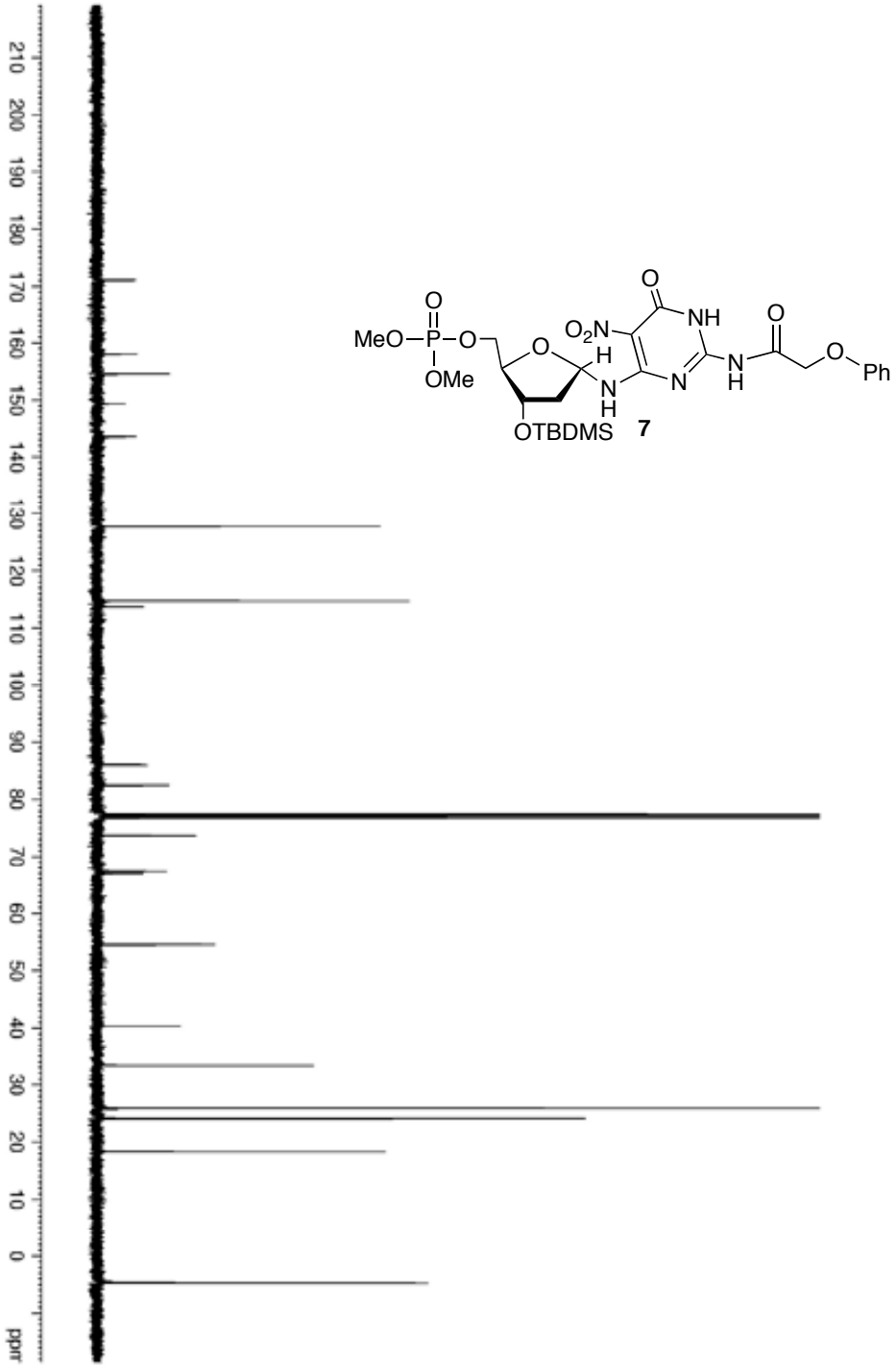
Determination of standard oxidation potential of Fapy•dG using 1. The oxidation potential of **1** (2 mM, 1 mL aqueous solution) was measured by cyclic voltammetry using KCl (0.1 M) as

a supporting electrolyte under various scan rates (100, 150, 200, 250, 300 mV/s). A three-electrode setup was used, which was comprised of a glassy carbon working electrode, a platinum wire auxiliary electrode, and a saturated silver/silver chloride (Ag/AgCl) electrode. The measured oxidation potential was plotted as a function of scan rate. The standard oxidation potential (E_0) of **1** was determined from the y-intercept of a linearly fit curve.

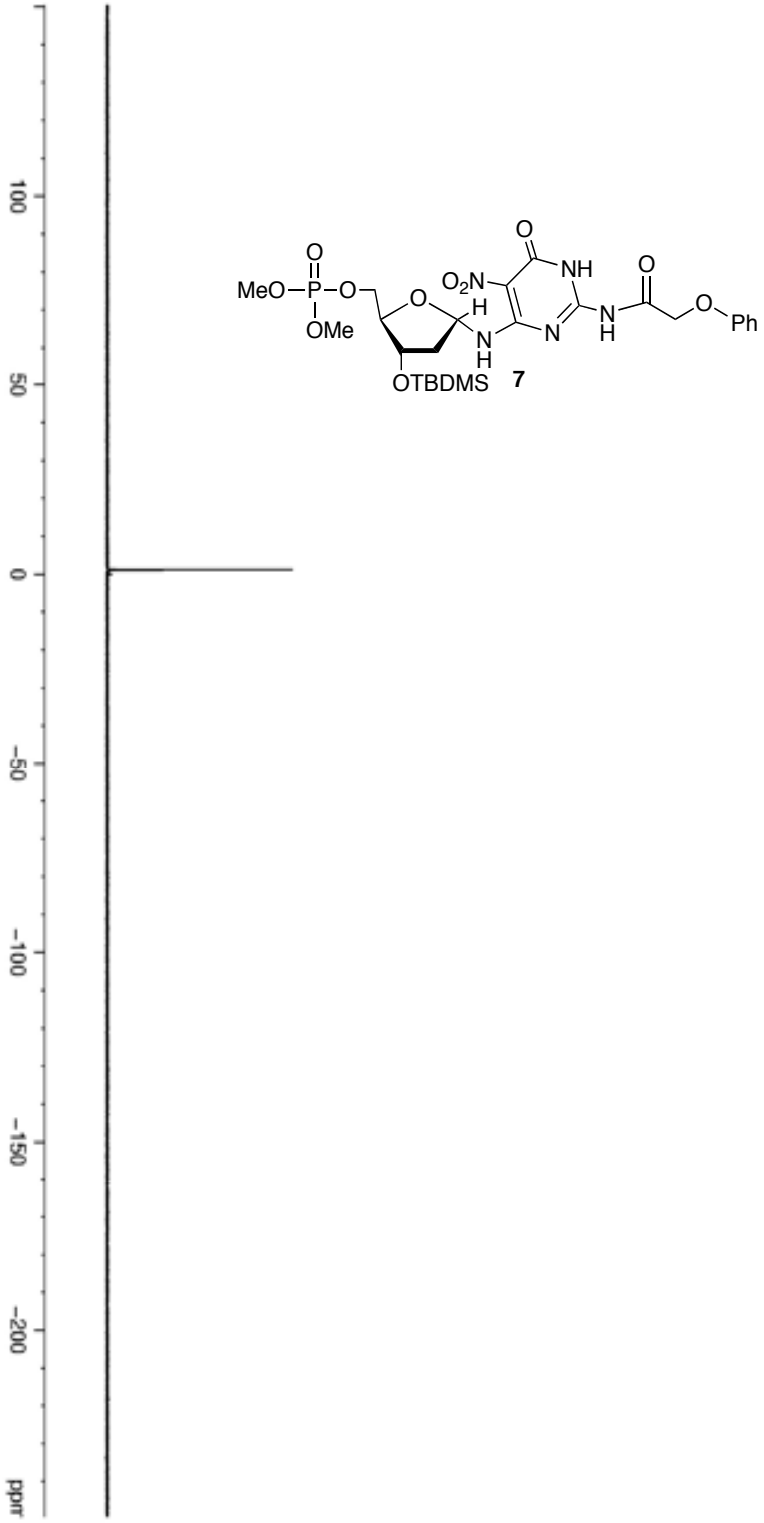
ESI MS analysis of reaction of 1 with 2. A mixture (18 μL) of **1** (6.0 mM), **2** (6.0 mM), and Na_2IrCl_6 (7 mM) was incubated at room temperature for 2 h. After diluting with water (980 μL), the reaction mixture (500 μL) was mixed with MeOH (500 μL) and analyzed using the ESI mass spectrometer in positive mode.



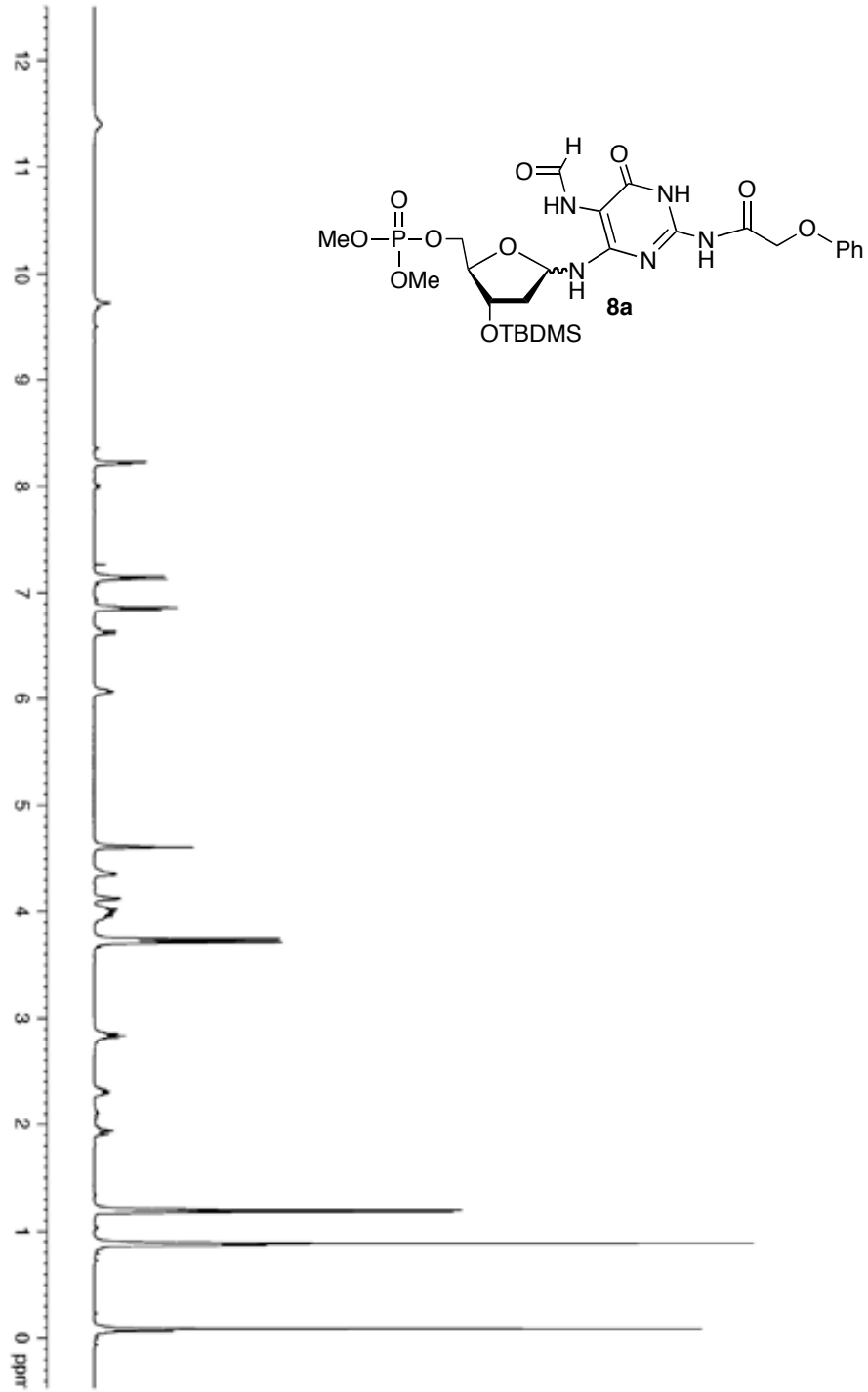
Supporting Information Figure 1. ^{1}H NMR of **7**.



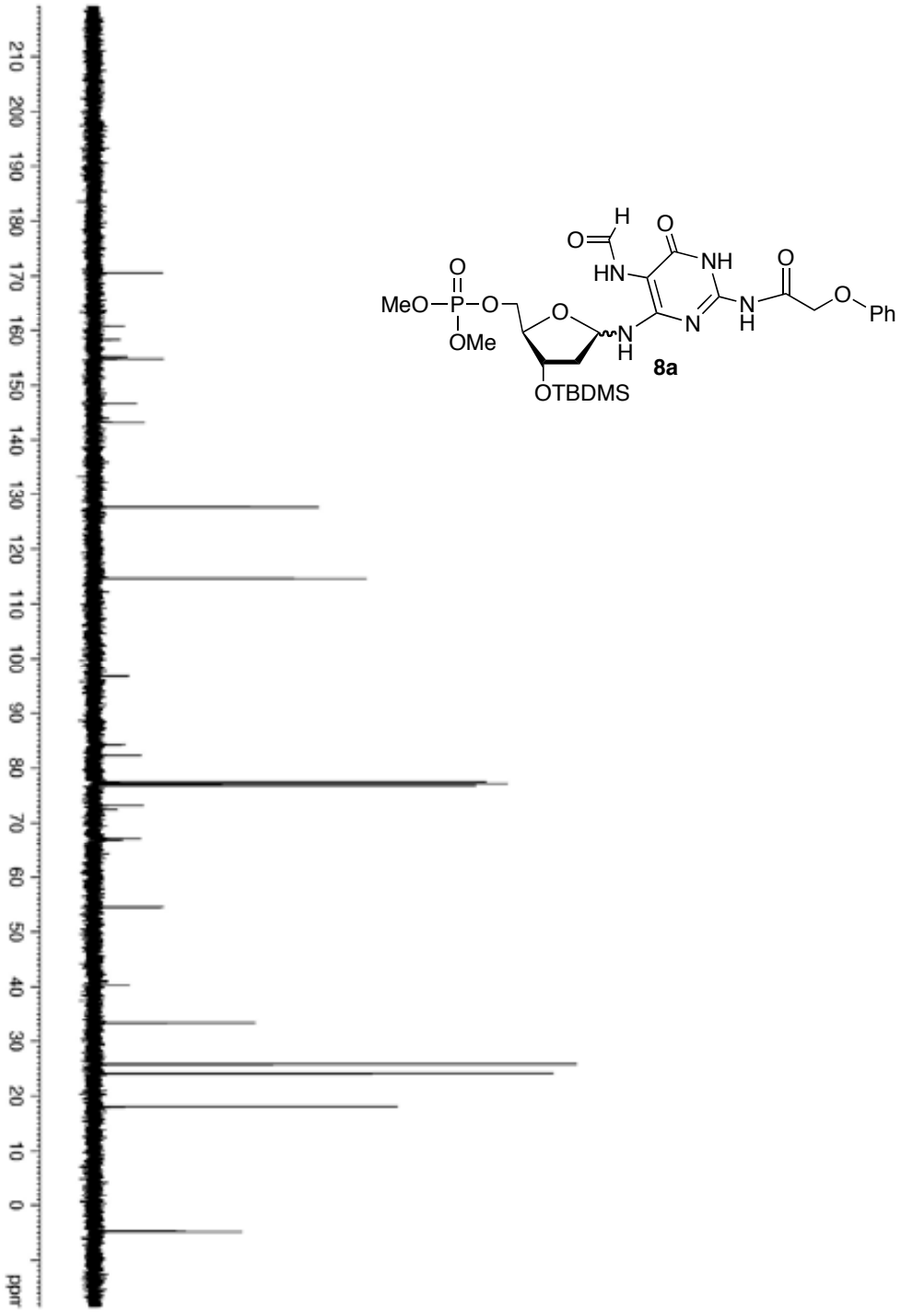
Supporting Information Figure 2. ^{13}C NMR of **7**.



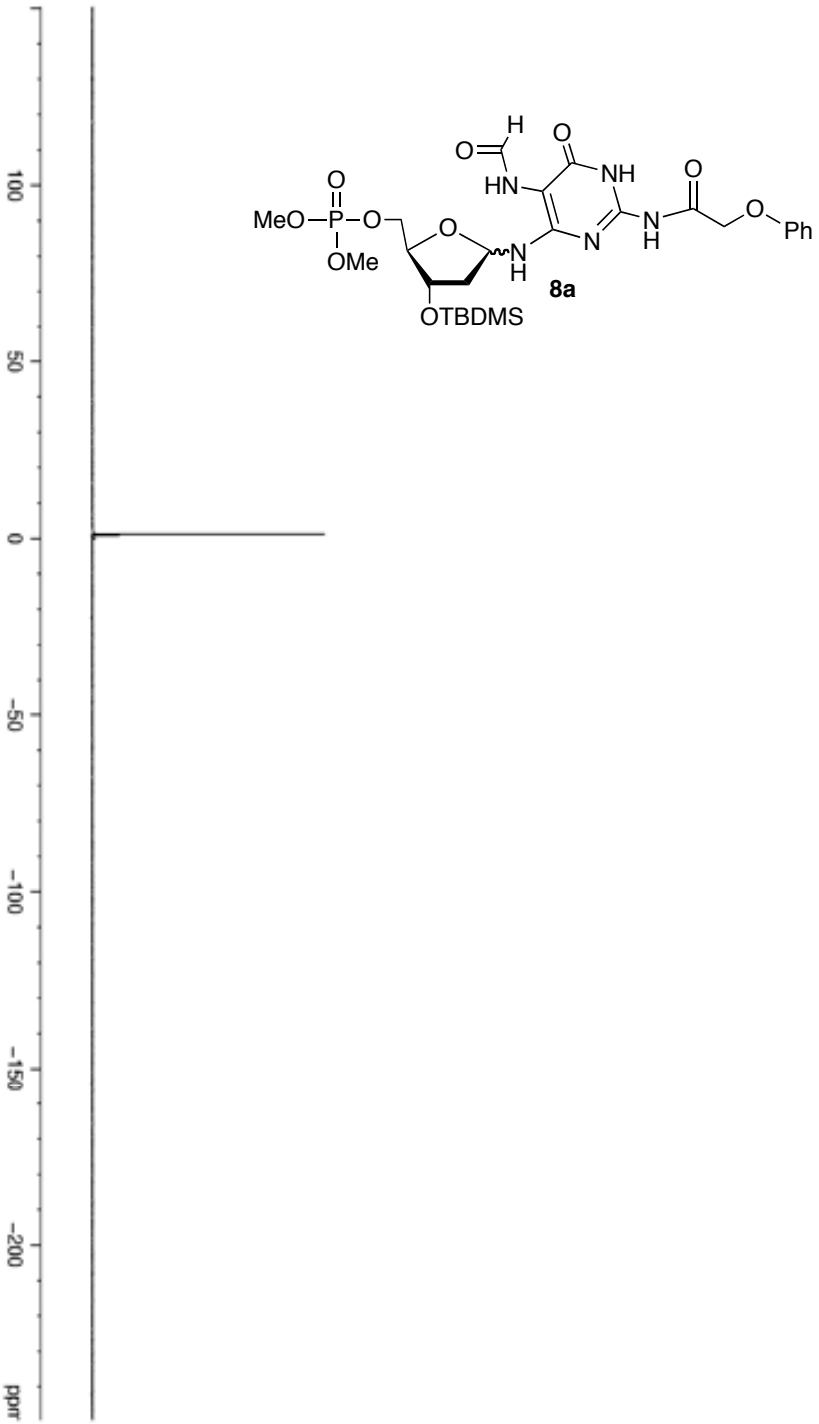
Supporting Information Figure 3. ^{31}P NMR of **7**.



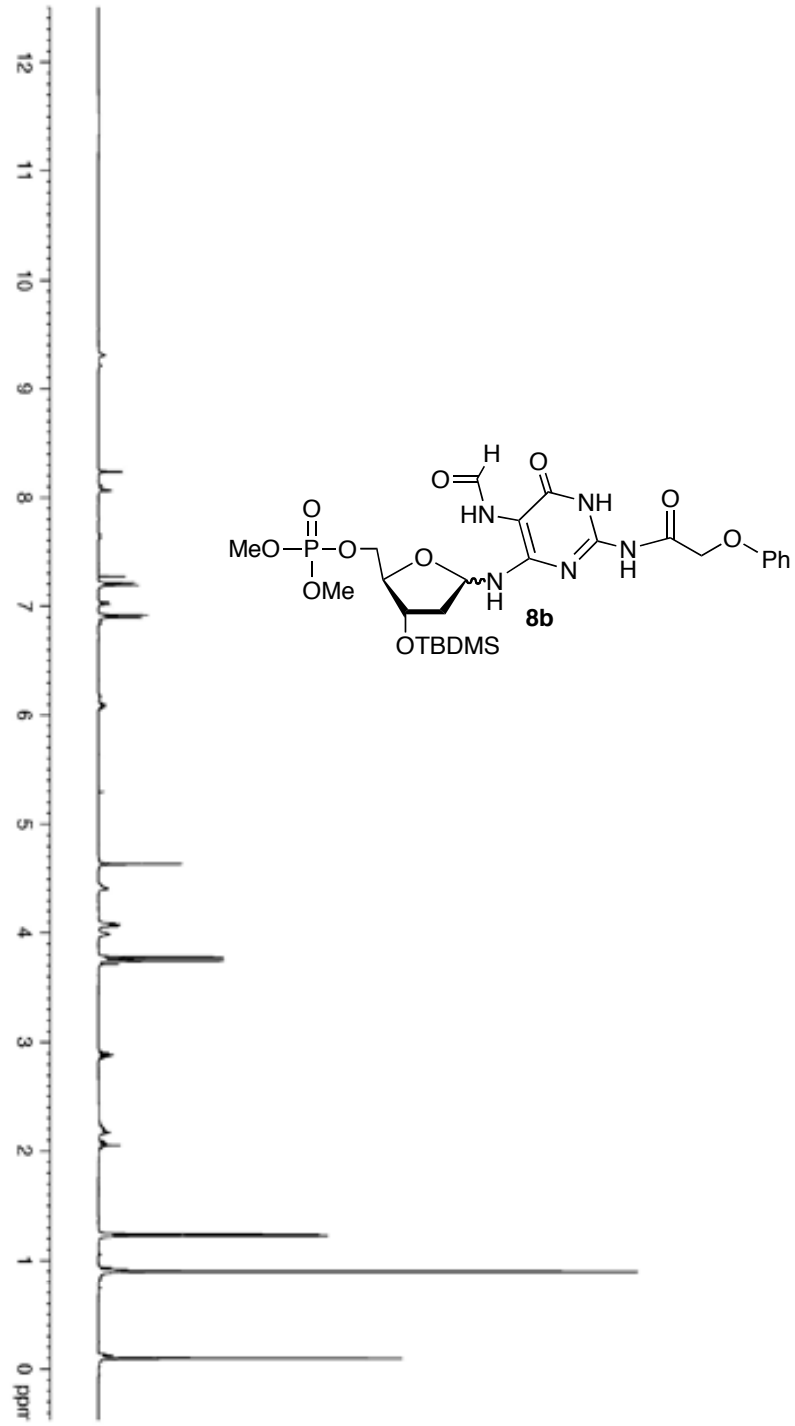
Supporting Information Figure 4. ^{1}H NMR of **8a**.



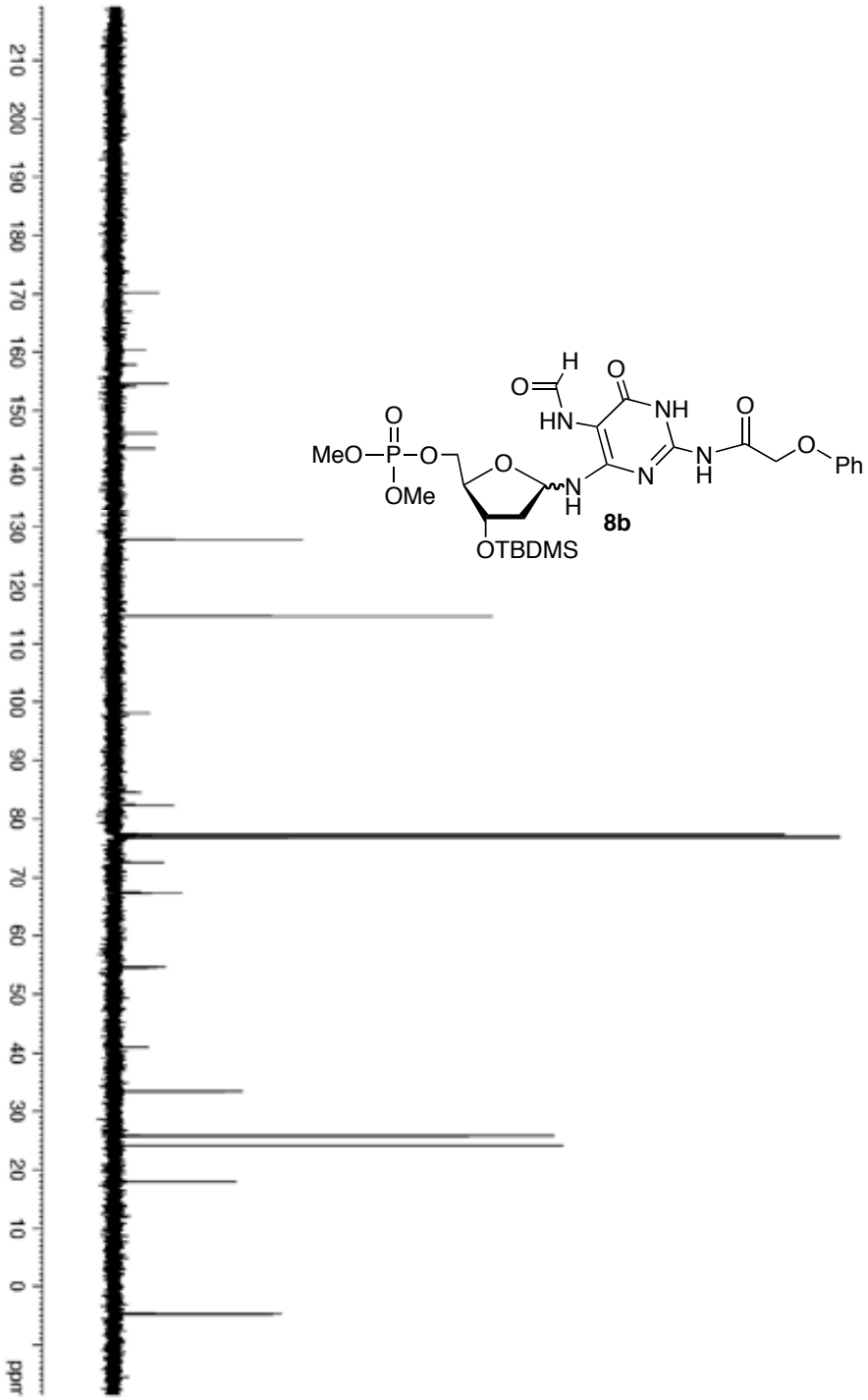
Supporting Information Figure 5. ^{13}C NMR of **8a**.



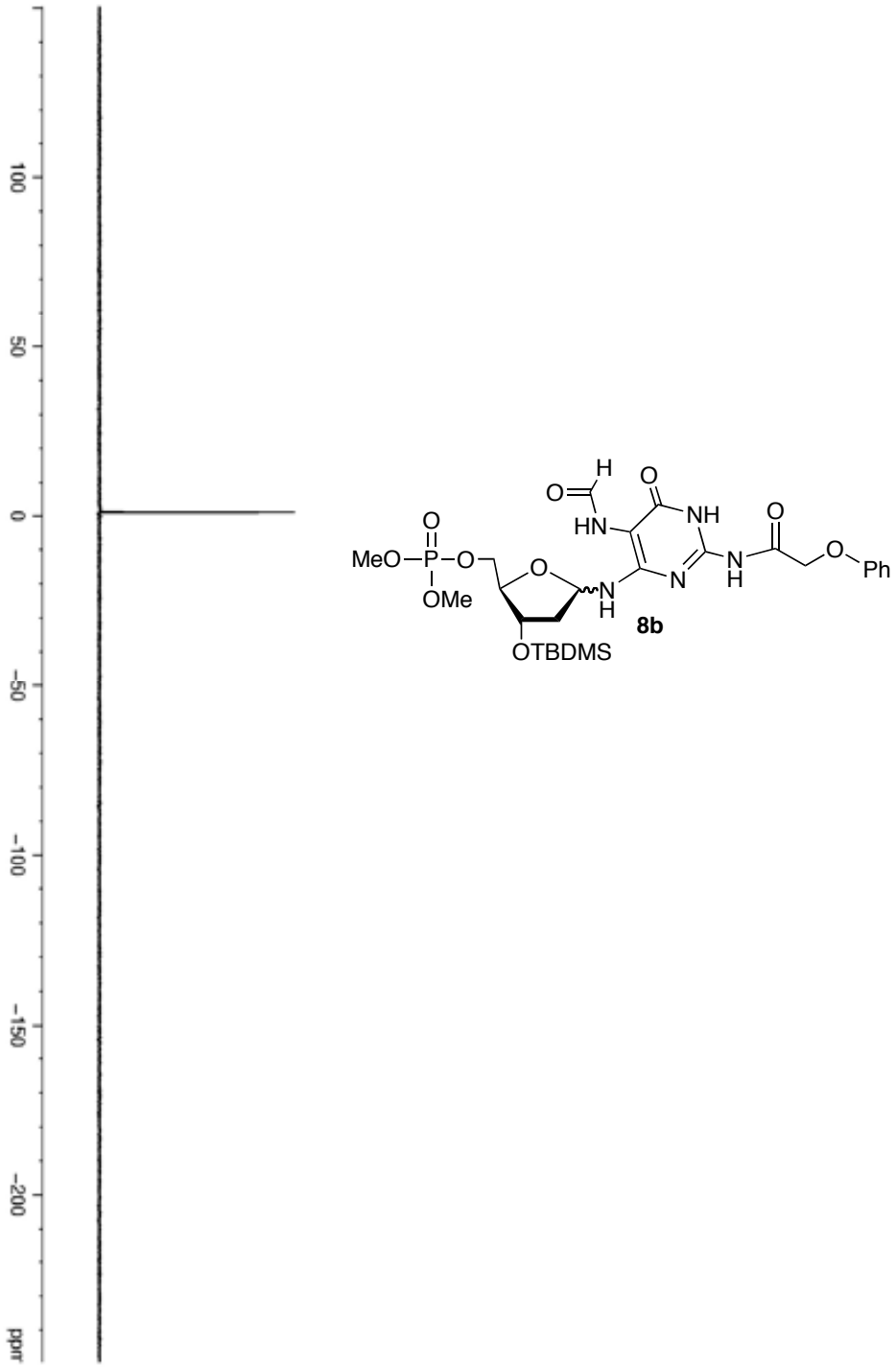
Supporting Information Figure 6. ^{31}P NMR of **8a**.



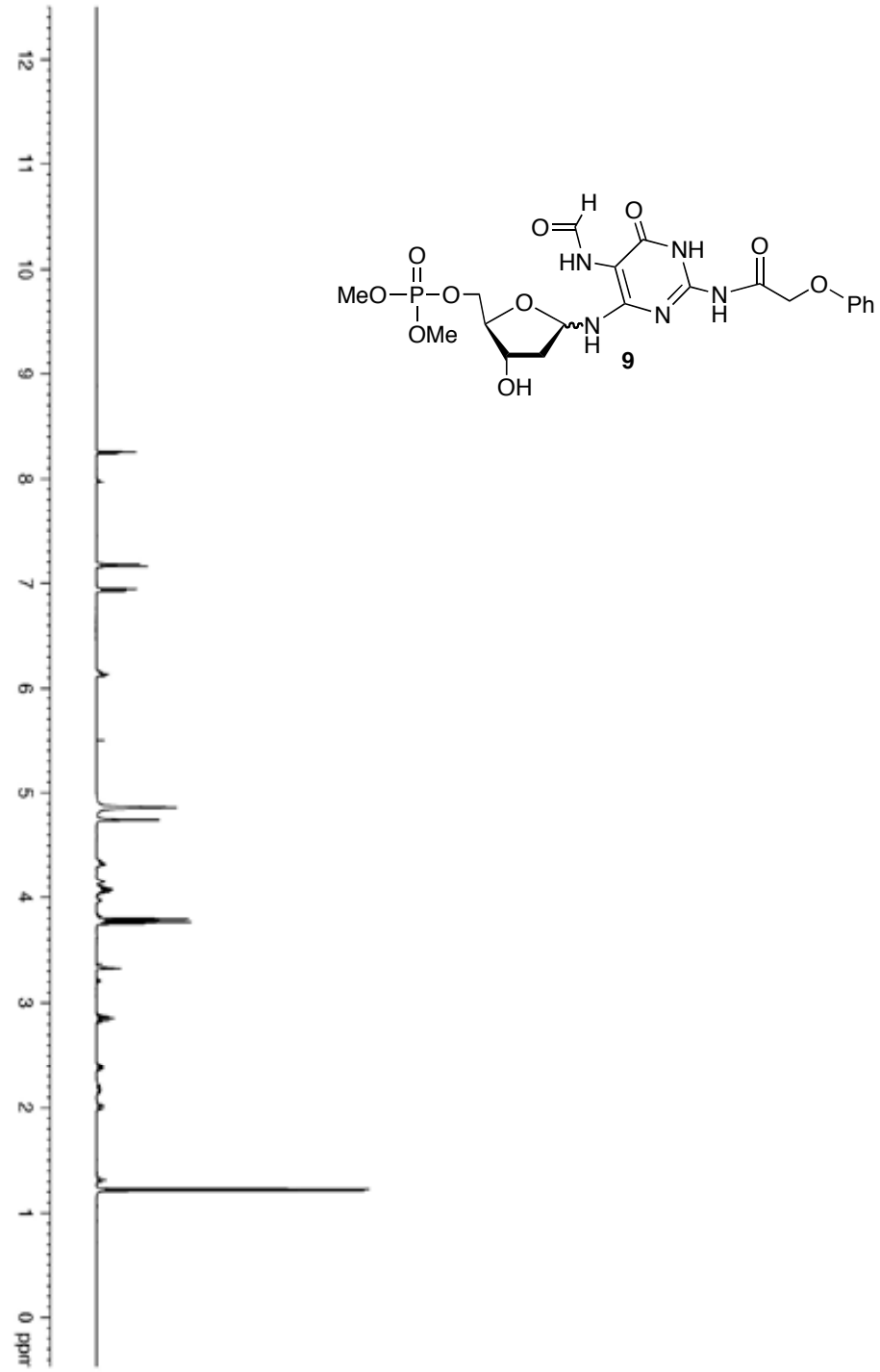
Supporting Information Figure 7. ^{1}H NMR of **8b**.



Supporting Information Figure 8. ^{13}C NMR of **8b**.

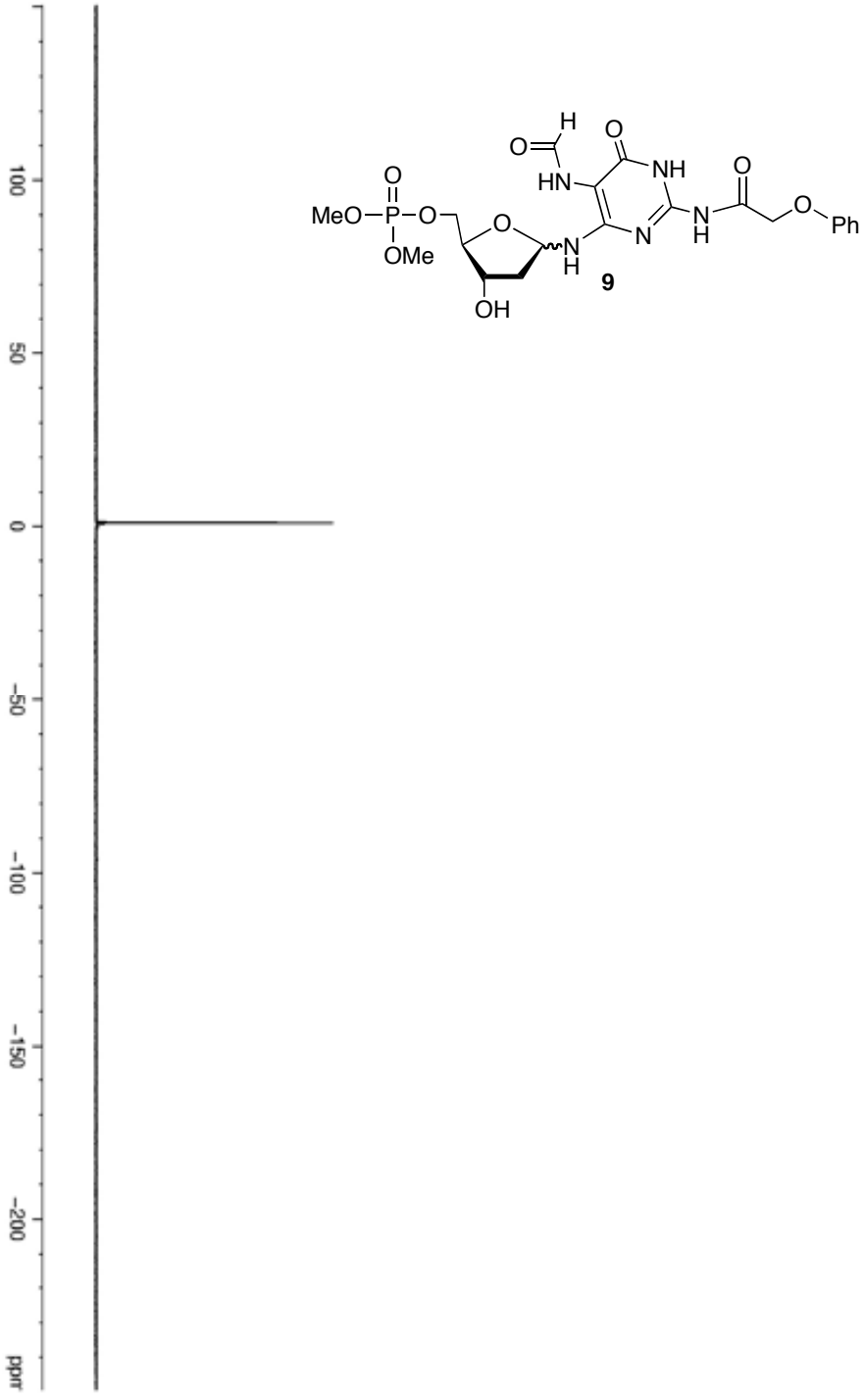


Supporting Information Figure 9. ^{31}P NMR of **8b**.

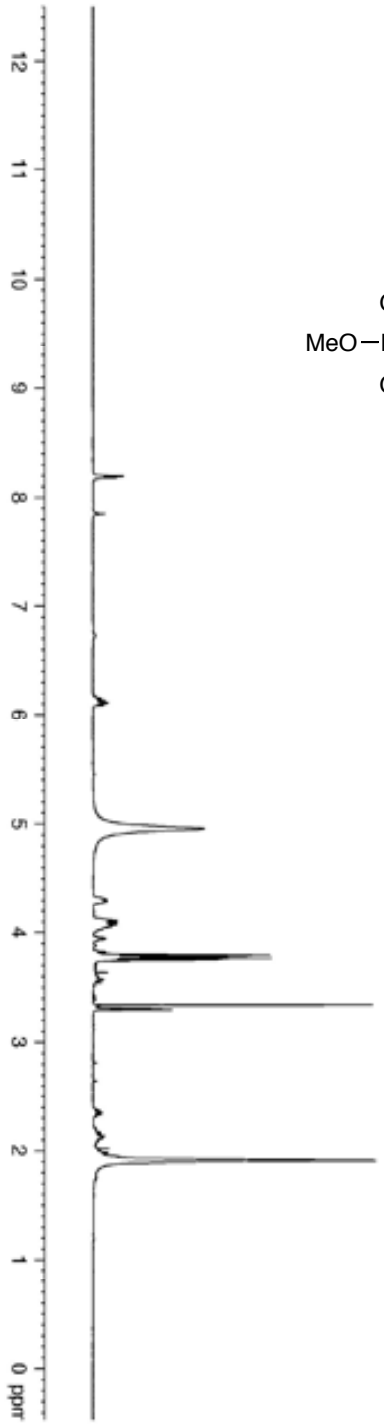


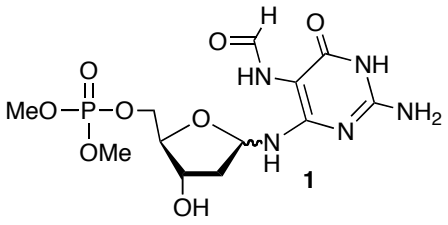
Supporting Information Figure 10. ^{1}H NMR of **9**.

Supporting Information Figure 11. ^{13}C NMR of **9**.

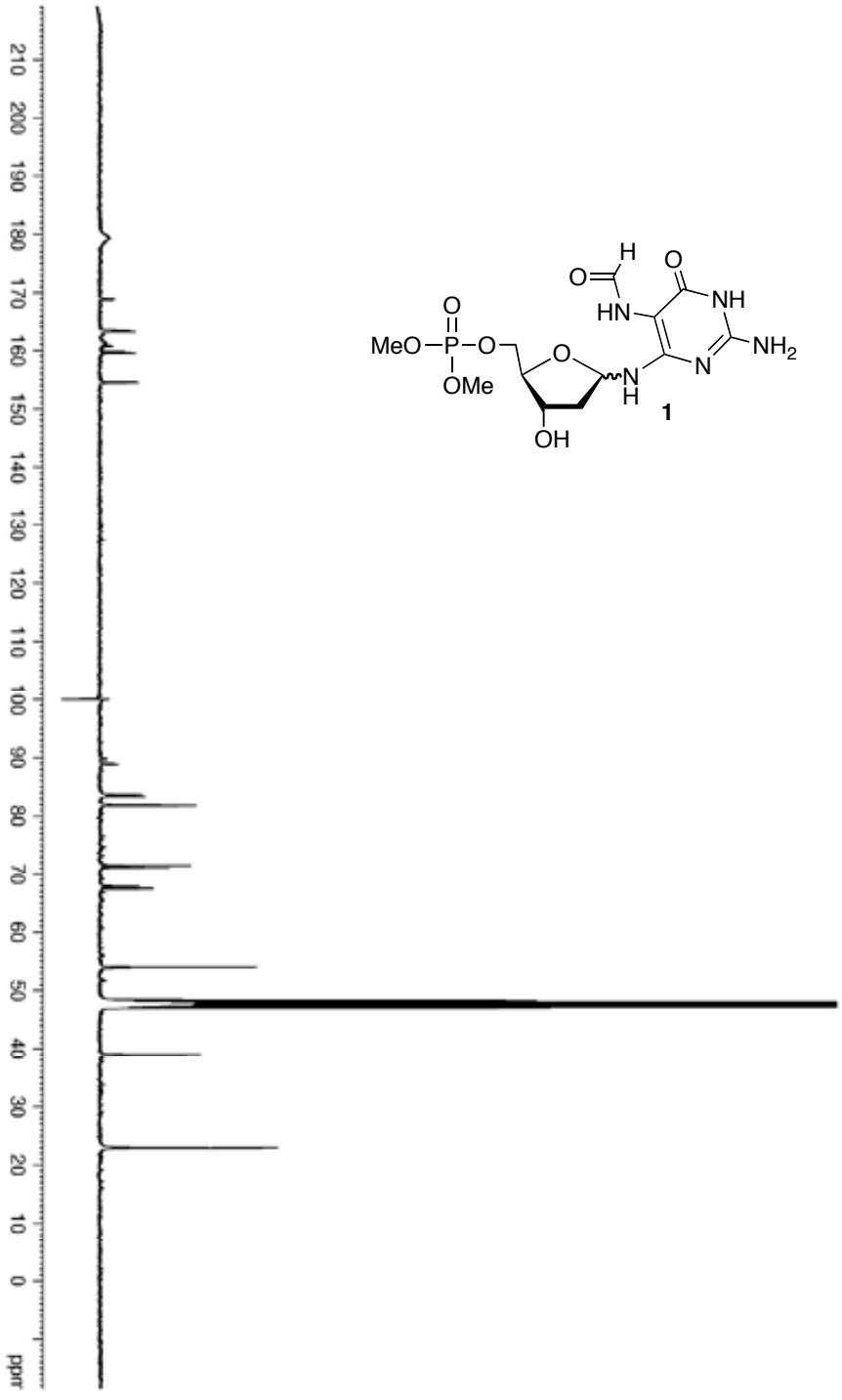


Supporting Information Figure 12. ^{31}P NMR of **9**.

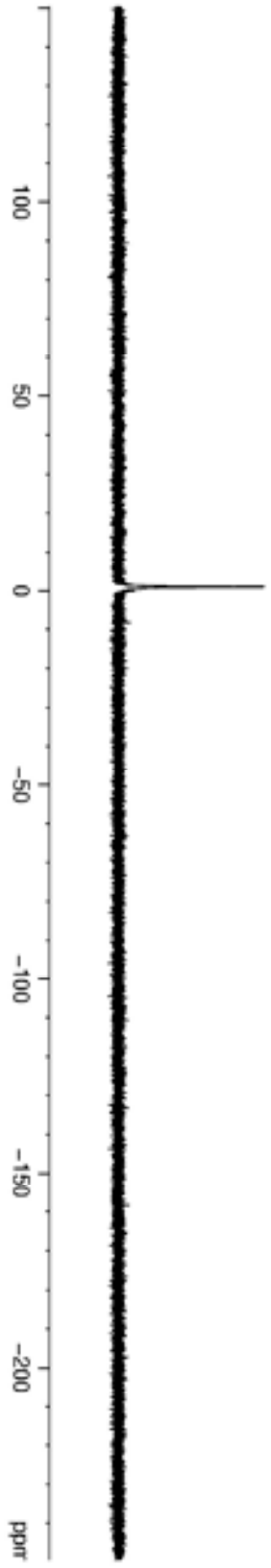


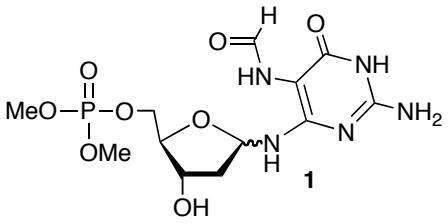


Supporting Information Figure 13. ^{1}H NMR of **1**.



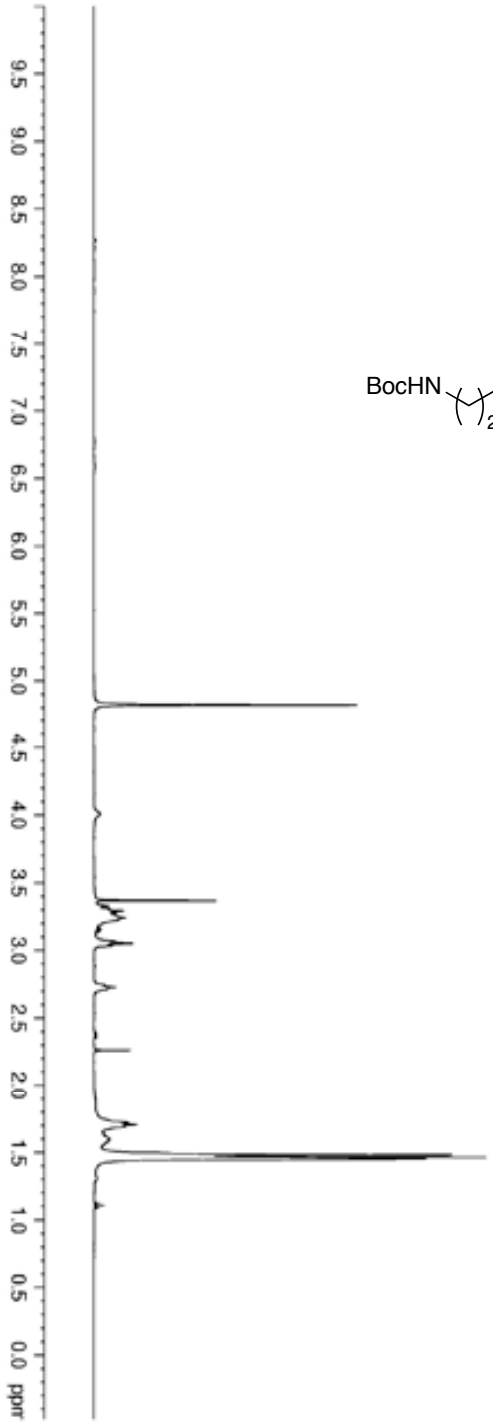
Supporting Information Figure 14. ^{13}C NMR of **1**.

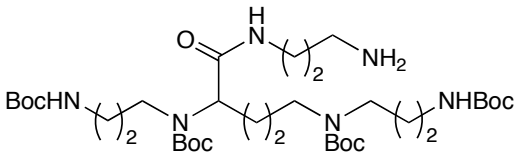




1

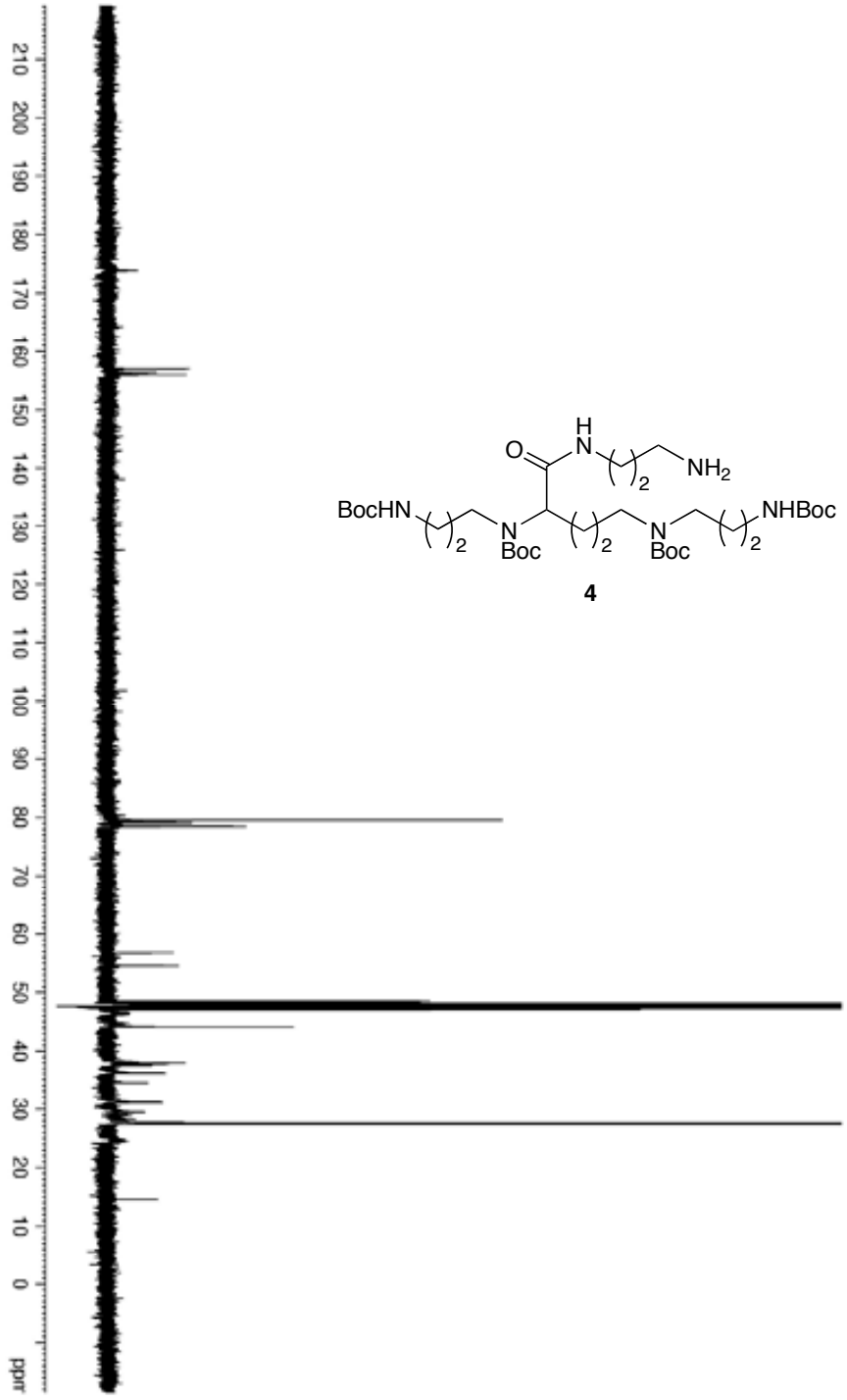
Supporting Information Figure 15. ^{31}P NMR of **1**.





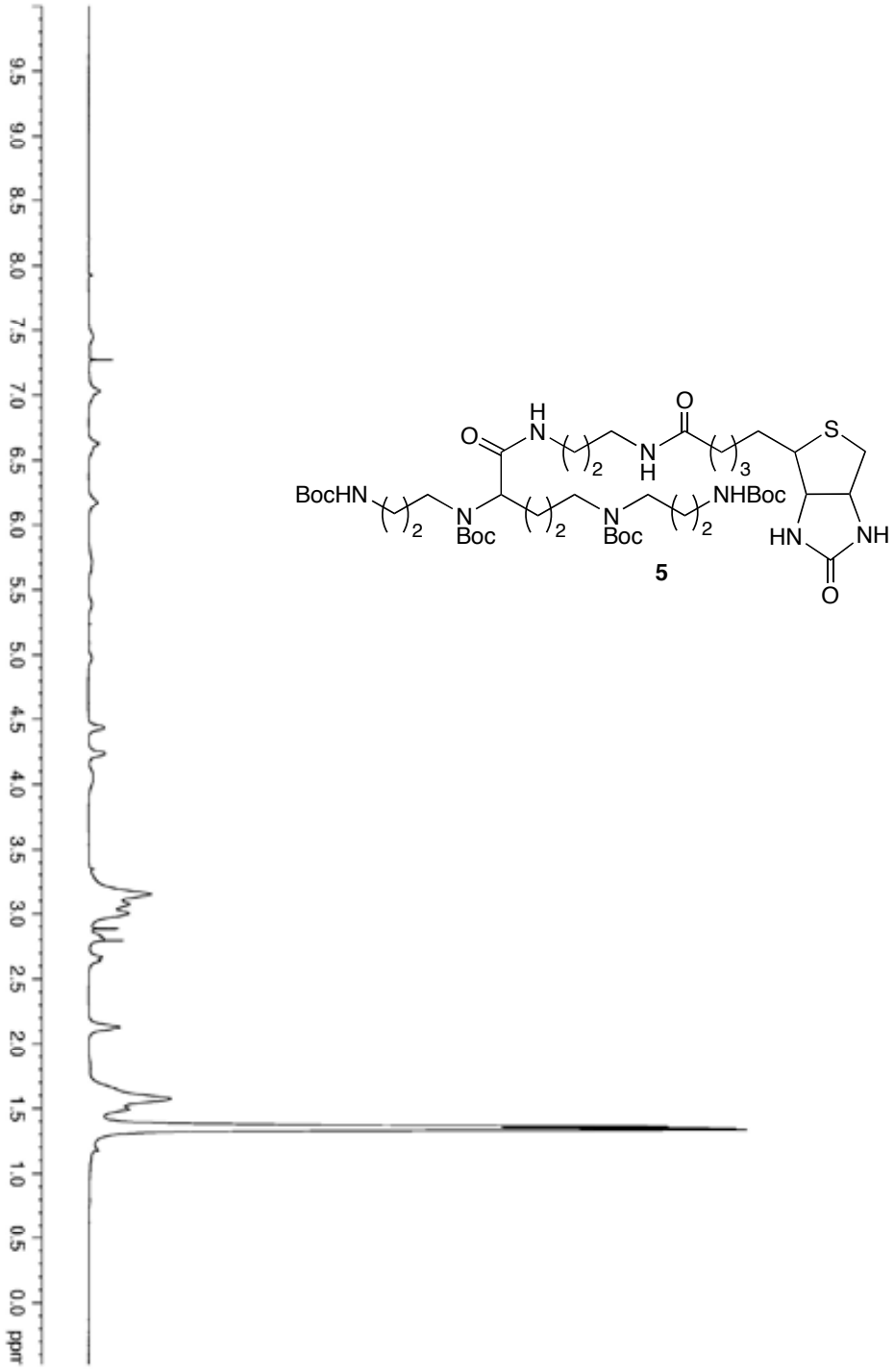
4

Supporting Information Figure 16. ^{1}H NMR of **4**.

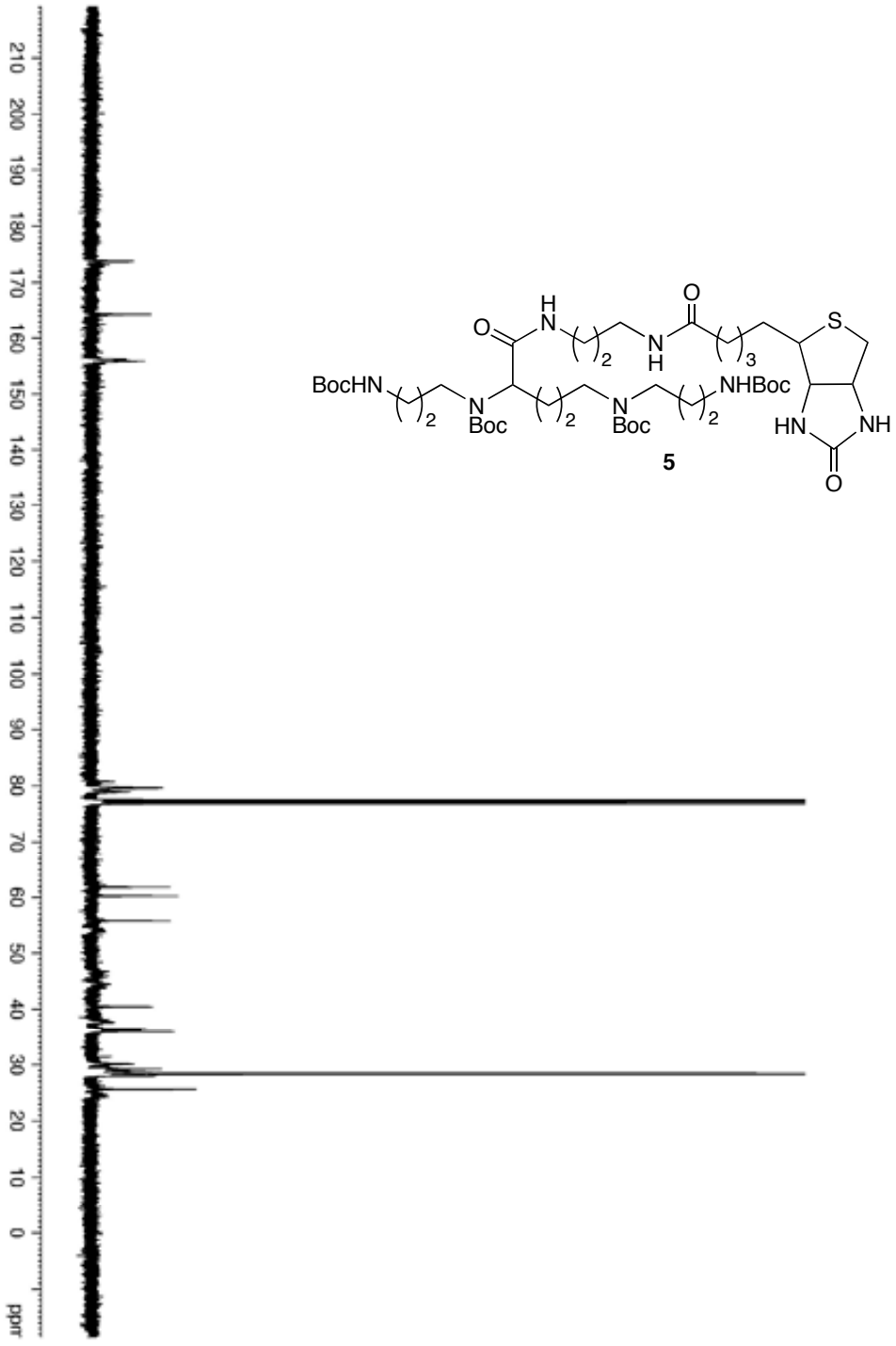


4

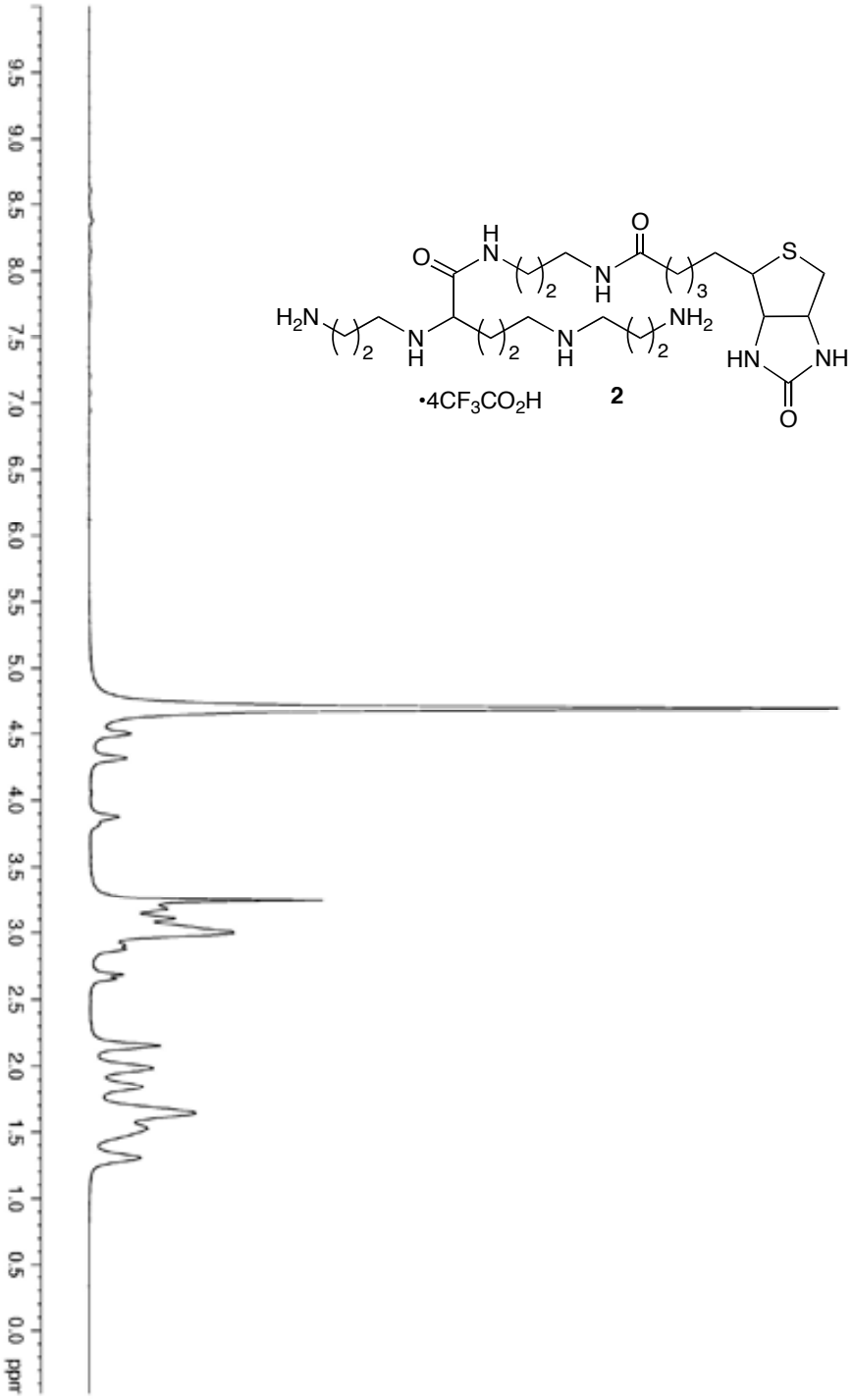
Supporting Information Figure 17. ^{13}C NMR of **4**.



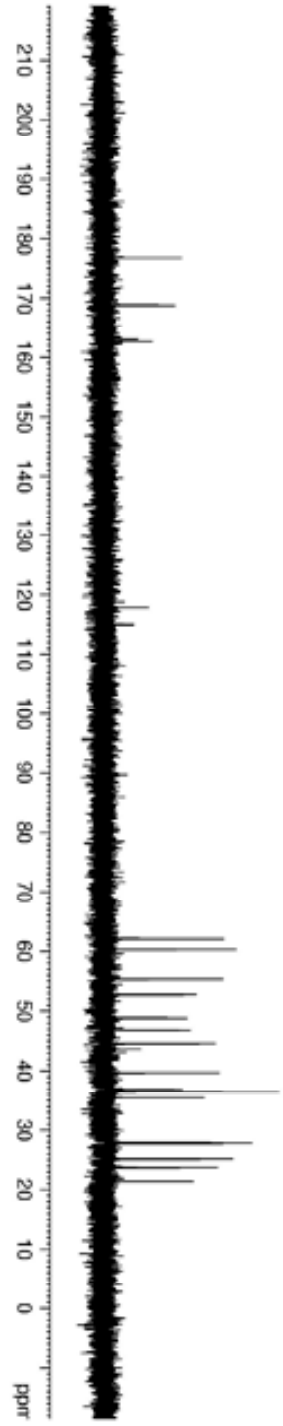
Supporting Information Figure 18. ^{1}H NMR of **5**.

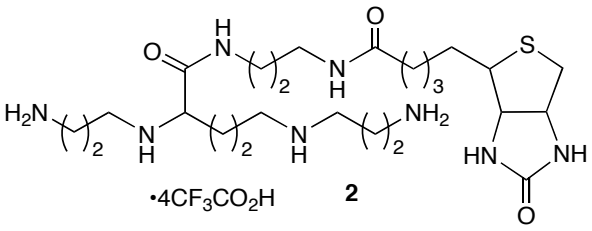


Supporting Information Figure 19. ^{13}C NMR of **5**.

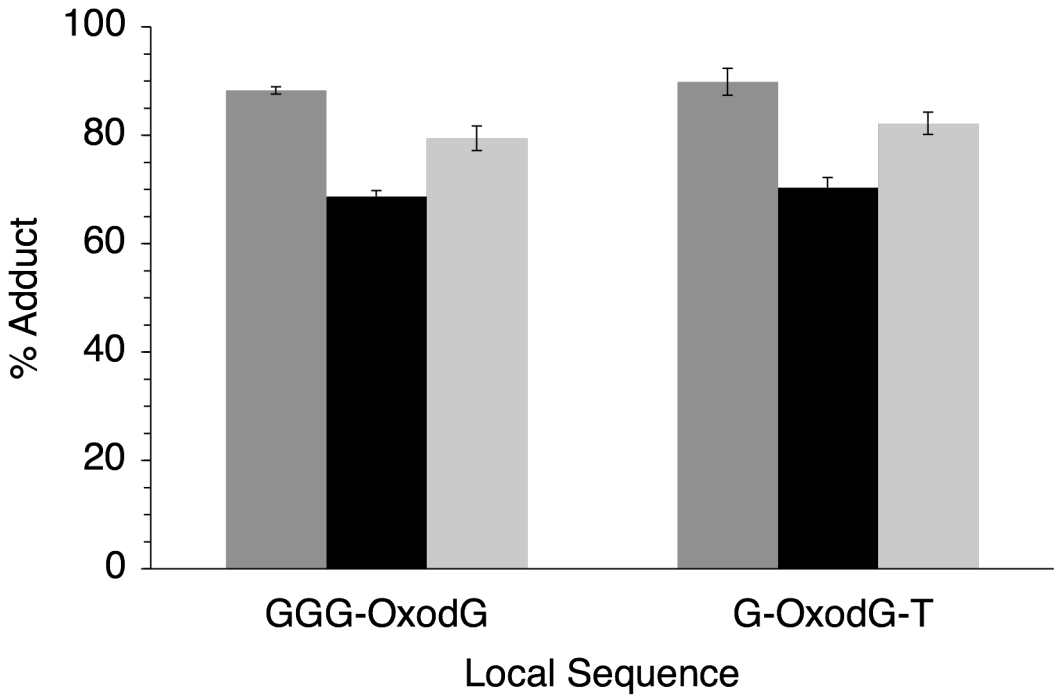


Supporting Information Figure 20. ^{1}H NMR of **2**.

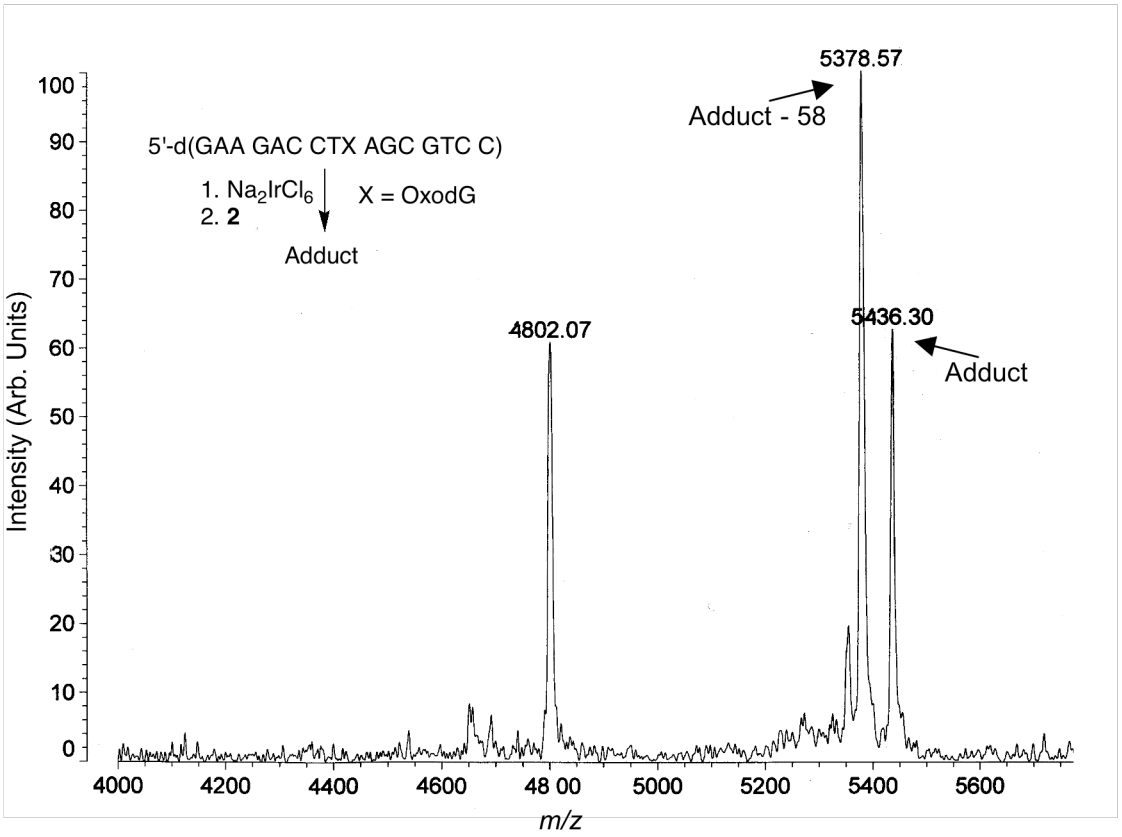




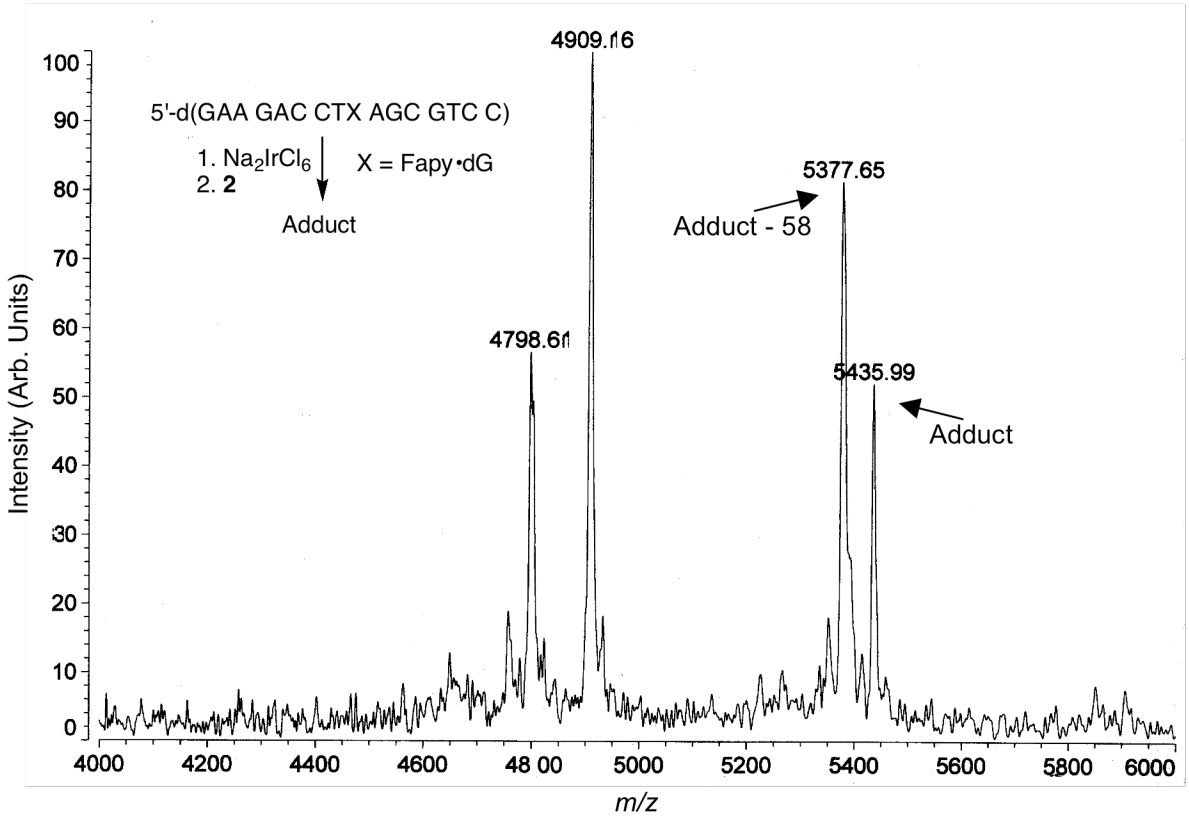
Supporting Information Figure 21. ^{13}C NMR of **2**.



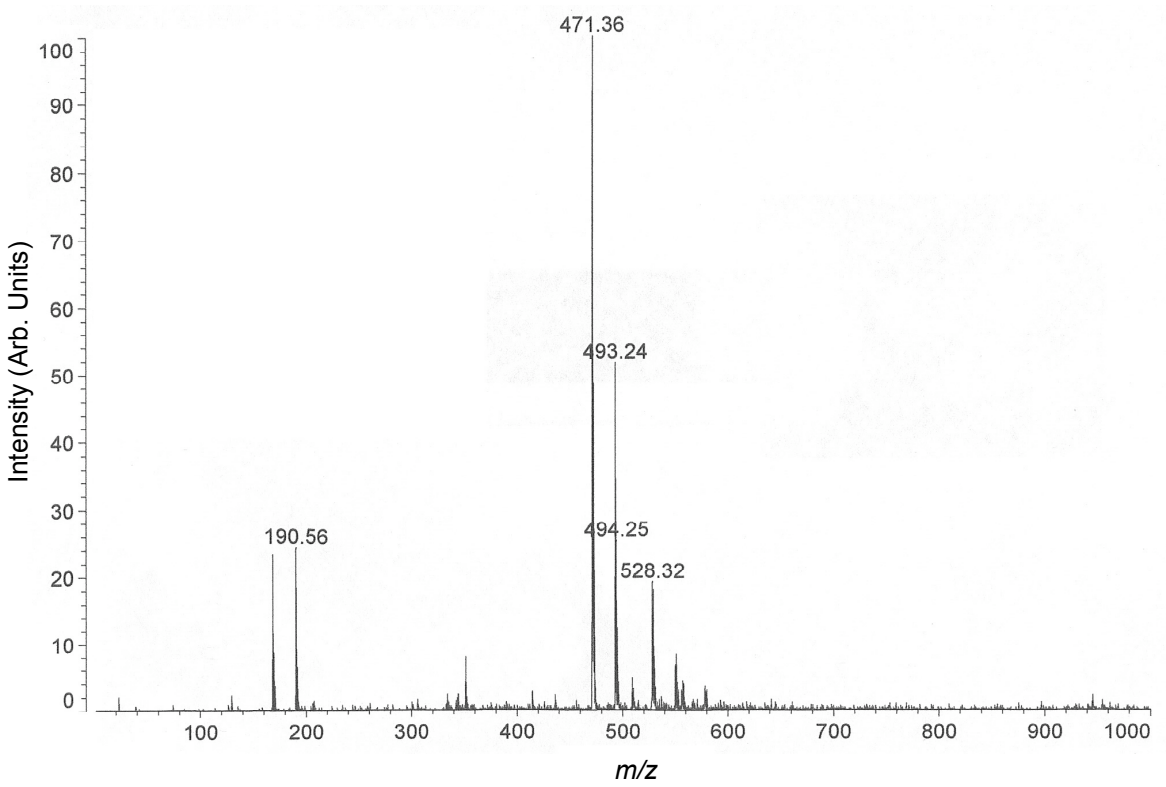
Supporting Information Figure 22. Adduct yields upon reaction of **2** with OxodG containing DNA (local sequences shown; see above for complete sequence). Dark gray: Na_2IrCl_6; Black: Na_2IrBr_6; Light gray: $K_3Fe(CN)_6$.



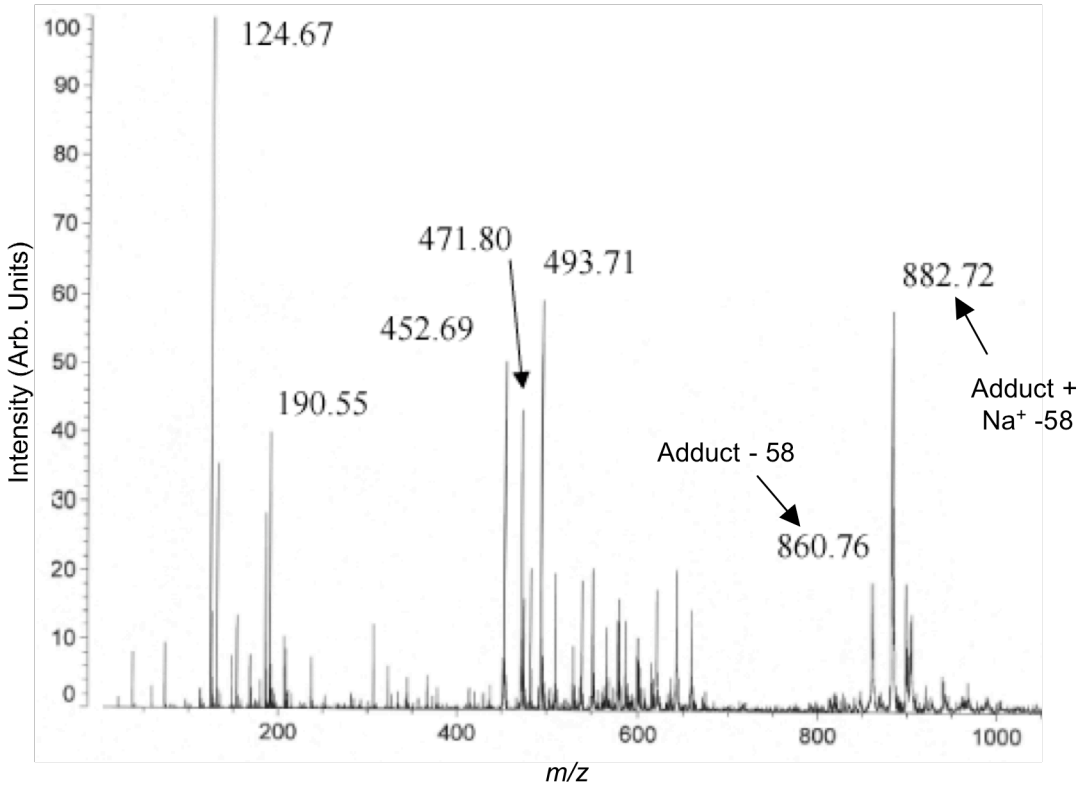
Supporting Information Figure 23. MALDI-TOF MS upon reaction of a single stranded oligonucleotide containing OxodG with Na_2IrCl_6 and **2**. The calculated MW (5433.0) is based upon the assumption that OxodG undergoes 2 e^- oxidation. The fragment corresponding to loss of *m/z* = 58 is observed in **2** as well (see Supporting Information Figure 25). The compound with *m/z* = 4802.07 is attributed to fragmentation to a urea product and is also present in the Fapy•dG sample (see Supporting Information Figure 24).[4]



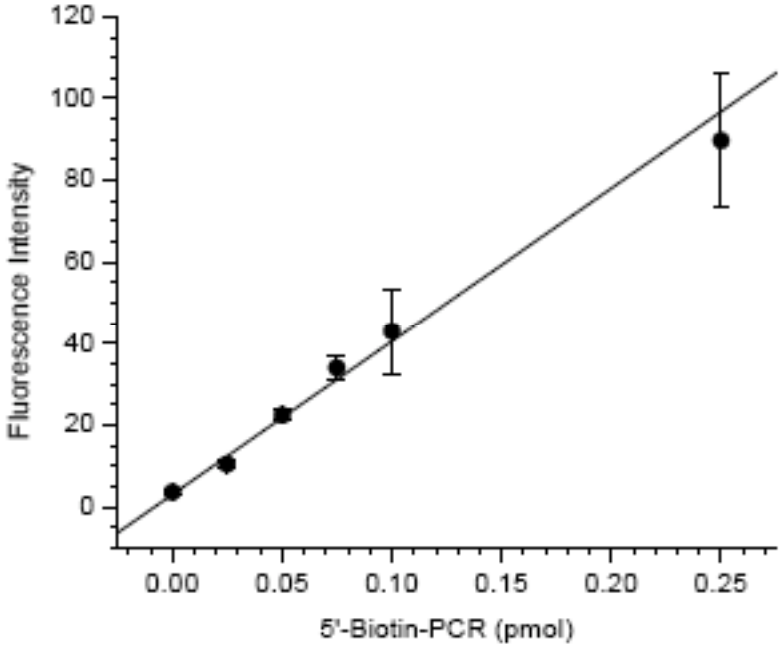
Supporting Information Figure 24. MALDI-TOF MS upon reaction of a single stranded oligonucleotide containing Fapy•dG with Na_2IrCl_6 and **2**. The calculated MW (5435.0) is based upon the assumption that Fapy•dG undergoes 2 e^- oxidation. The fragment corresponding to loss of *m/z* = 58 is observed in **2** as well (see Supporting Information Figure 25). The compound with *m/z* = 4909.16 corresponds to starting oligonucleotide. The compound with *m/z* = 4798.6 is attributed to fragmentation to a urea product and is also present in the OxodG sample (see Supporting Information Figure 23).[4]



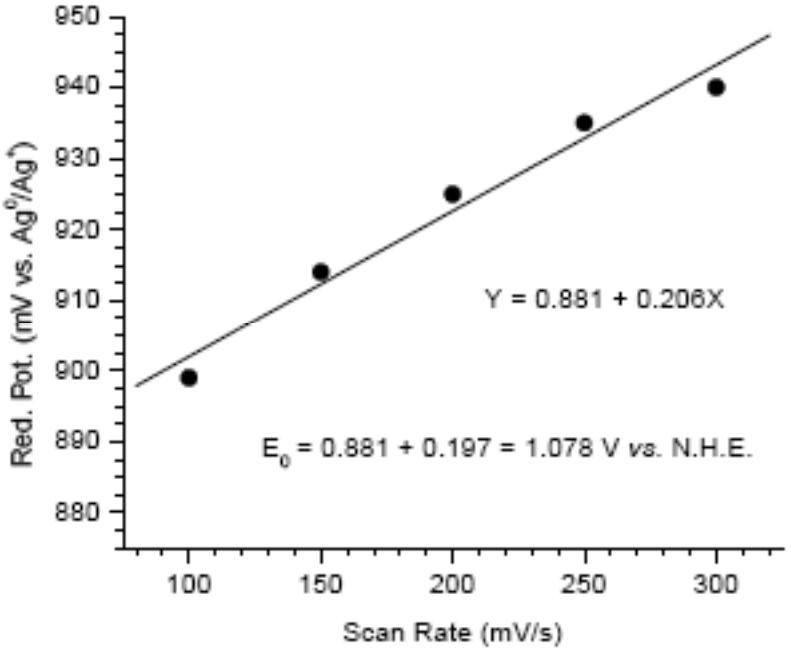
Supporting Information Figure 25. MALDI-TOF MS of **2** (Note: positive ion mode).



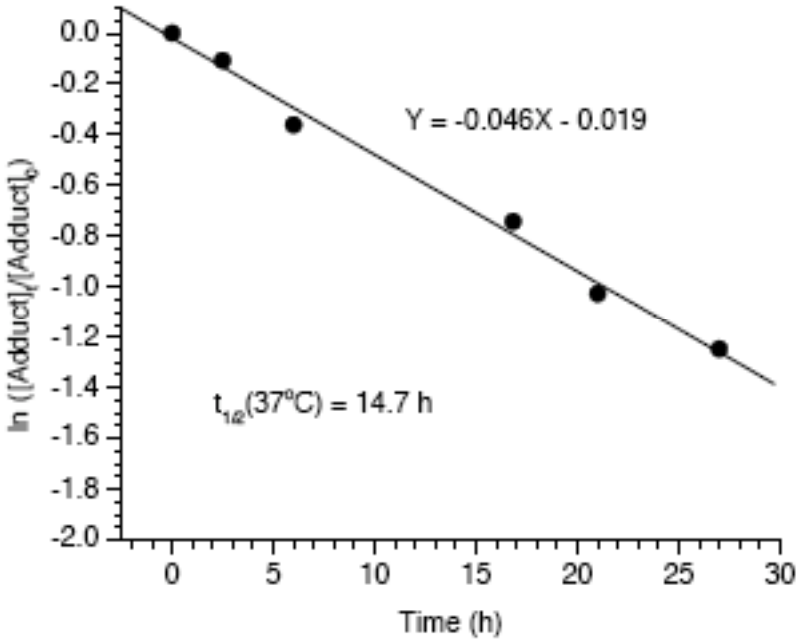
Supporting Information Figure 26. ESI- MS (positive ion mode) of the adduct formed from reaction of **1** with **2** in the presence of Na_2IrCl_6. The $[M^+ - 58]$ and $[M^+ - 58 + Na^+]$ ions are observed.



Supporting Information Figure 27. Sample fluorescence calibration curve for biotinylated adducts.



Supporting Information Figure 28. Reduction potential of **1** as a function of scan rate.



Supporting Information Figure 29. Decomposition of Fapy•dG adduct obtained from reaction of **2** with **4b**.

References:

(1) Jiang, Y. L.; Wiederholt, C. J.; Patro, J. N.; Haraguchi, K.; Greenberg, M. M. *J. Org. Chem.* **2005**, *70*, 141-147.
(2) Haraguchi, K.; Delaney, M. O.; Wiederholt, C. J.; Sambandam, A.; Hantosi, Z.; Greenberg, M. M. *J. Am. Chem. Soc.* **2002**, *124*, 3263-3269.
(3) Huber, M.; Pelletier, J. G.; Torossian, K.; Dionne, P.; Gamache, I.; Charest-Gaudreault, R.; Audette, M.; Poulin, R. *J. Biol. Chem.* **1996**, *271*, 27556-27563.
(4) Hosford, M. E.; Muller, J. G.; Burrows, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 9540-9541.