Supporting Information: Selective Detection and Quantification of Oxidized Abasic Lesions in DNA

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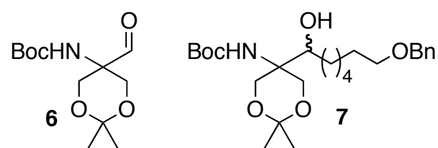
General Methods. Oligonucleotides were synthesized using standard cycles on an Applied Biosystems model 394 DNA/RNA synthesizer using reagents purchased from Glen Research. Oligonucleotides containing **C4-AP**, **DOB**, or **L** were previously prepared in this laboratory.[1-3] MALDI Mass spectra were collected on a Kratos Analytical KOMPACT SEQ. Fluorescence spectra were collected on a Varian Cary Eclipse fluorescence spectrophotometer equipped with a microplate reader. γ-Radiolysis experiments were carried out using a Shepherd Mark I ^{137}Cs irradiator. HRMS (FAB) spectra were collected on a VG70S magnetic sector mass spectrometer.

^1H, ^{13}C spectra were collected on a Bruker Avance 400 MHz FT-NMR spectrometer. IR spectra were collected on a Mattson Instruments 4030 Galaxy Series FT-IR spectrophotometer. T4 polynucleotide kinase, terminal deoxytransferase, and UDG were obtained from New England Biolabs. γ-^{32}P-ATP was from Perkin Elmer and α-^{32}P-ddATP was from GE Healthcare. C_{18}-Sep Pak cartridges were obtained from Waters. Quantification of radiolabeled oligonucleotides was carried out using a Molecular Dynamics Phosphorimager equipped with ImageQuant Version 5.1 software. All photolyses of oligonucleotides were carried out in eppendorf tubes in a Rayonet photoreactor (RPR-100) fitted with 16 lamps having an output maximum at 350 nm. Protamine sulfate and Tween 20 were purchased from Sigma (Cat. No. P 4020 and P 9416). SuperBlock/PBS was purchased from Pierce (Cat. No. 37515ZZ). 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). Ninety-six well flat-bottom assay plates (black) were purchased from Corning (Fisher, Cat. No. 07200509).

Precautions when handling ^{32}P. All manipulations were carried out behind a Lucite (3/4 inch thick) shield. Latex gloves, lab coat, and personal dosimeter were worn at all times when working with ^{32}P. Two sets of gloves were worn while labeling DNA substrates.

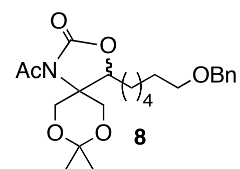
Precautions when handling peplomycin. Gloves were worn at all times when handling peplomycin. Solutions containing peplomycin were passed through activated carbon after usage.

Preparation of 7. Freshly activated Mg (398 mg, 16.5 mmol) granules (by washing Mg granules with dilute HCl

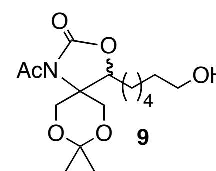


and drying in the oven overnight) was placed in a two-necked flask. The flask was heated gradually under vacuum. While the flask was hot, argon gas was introduced and the flask was cooled to room temperature. A small piece of iodine was introduced. Anhydrous THF (1 mL) was added and the mixture was heated to 40 °C for 5 min, at which time a THF solution (5 mL) of $Br(CH_2)_6OBn$[4] (271 mg, 16.6 mmol) was added with vigorous stirring. The reaction mixture started refluxing immediately and the iodine color disappeared within 15 min. The reaction mixture was stirred for 1.5 h at 40 °C and for an additional 1 h at room temperature. The mixture was cooled to -20 °C and a THF solution of **6**[5] (1.432 g, 5.53 mmol) was added over a period of 10 min. The mixture was stirred at this temperature for 6 h and an additional 1 h at room temperature. A saturated solution of NH_4Cl was added to the mixture and it was extracted with ether. The organic layer was washed with water, followed by brine. The organic extracts were dried over dry $MgSO_4$ and filtered. Ether was evaporated and the colorless oil obtained was purified by silica gel flash chromatography (35% ethyl acetate in hexanes). The residue obtained was purified by flash chromatography (40% ethyl acetate in hexanes) to yield **7** (0.5 gm, 20%) and considerable starting material. The yield with respect to unrecovered starting material was 55%. IR (KBr): 3289, 2931, 1756, 1454, 1382, 1251, 1197, 1081, 831, 700, 520 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37-7.27 (m, 5H), 5.7545 (s, 1H), 4.49 (s, 2H), 4.13 (dd, J = 10.2, 2.7 Hz, 1H), 3.98 (d, J = 11.9 Hz, 1H), 3.98-3.96 (m, 2H), 3.73-3.67 (m, 2H), 3.48-3.39 (m, 25 H); ^{13}C NMR ($CDCl_3$) δ 158.0, 138.5, 129.1, 128.6, 128.3, 127.6, 127.4, 98.7, 81.6, 72.8, 70.2, 67.2, 63.2, 55.8, 31.8, 29.6, 29.5, 29.4, 28.9, 28.2, 25.9, 25.8, 24.3, 22.6, 22.2; HRMS-FAB: ($M-C_4H_9O$) calc'd. 378.2281, found 378.2282.

Preparation of 8. Compound **7** (450 mg, 1.03 mmol) and DMAP (15 mg, 0.12 mm ol) were taken up in pyridine (1 mL). Excess acetic anhydride

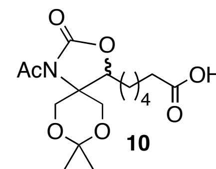


(1.21 g, 11.8 mmol) was added and the mixture was heated at 40 °C for 4 h. After evaporating the pyridine, saturated $NaHCO_3$ was added to the residue and extracted with CH_2Cl_2. The organic layer was washed with brine and the CH_2Cl_2 layer was dried over anhydrous $MgSO_4$. The solvent was evaporated and the residue obtained was purified by flash chromatography (15% ethyl acetate in hexanes) to yield **8** as a white solid (m.p. 79 °C) in 95.2% yield (399 mg). IR (KBr): 2927, 1778, 1698, 1448, 1371, 1272, 1193, 1097, 823, 730, 615 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36-7.27 (m, 5H), 4.9 (d, J = 11.8 Hz, 1H), 4.73 (d, J = 11.2 Hz, 1H), 4.61 (dd, J = 10.9, 2.3 Hz, 1H), 4.50 (s, 2H), 2.49 (s, 3H), 2.05-2.04 (m, 1H), 1.63-1.37 (m, 15 H); ^{13}C NMR ($CDCl_3$) δ 172.0, 153.6, 138.6, 128.3, 127.6, 127.4, 98.7, 82.1, 72.8, 70.3, 62.9, 59.6, 58.5, 30.3, 29.6, 29.3, 28.9, 28.5, 25.9, 25.6, 25.5, 22.7, 18.7; HRMS-FAB (M - H) calc'd. 418.2230, found 418.2224.

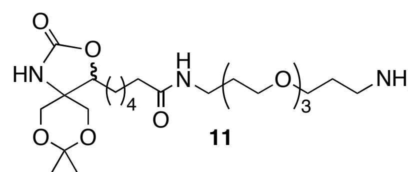


Preparation of 9. Compound **8** (400 mg, 0.95 mmol) was taken up in EtOH (4 mL) a nd 60 mg of 5% Pd/C was added. The mixture was stirred at room temperature under 50 psi H_2 pressure for 12 h. The reaction mixture was then filtered through a pad of celite, after which the EtOH was evaporated. The residue was purified by flash chromatography (30% ethyl acetate in diethyl ether) to produce **9** in 77 % (240 mg) yield. IR (KBr): 3419, 2927, 1779, 1702, 1375, 1282, 1193, 1097, 825, 759, 620 cm^{-1};^1H NMR ($CDCl_3$) δ, 4.95 (d, J = 11.8 Hz, 1H), 4.73 (d, J = 11.2 Hz, 1H), 4.61 (dd, J = 10.9, 2.3 Hz, 1H), 3.75 (dd, J = 11.8, 2.5 Hz, 1H), 3.67 (t, J = 6.52 Hz, 2H), 3.53 (dd, J = 11.2, 2.5 Hz, 1H), 2.49 (s, 3H), 2.06 (s, 1H), 1.67-1.37 (m, 15 H); ^{13}C NMR ($CDCl_3$) δ 172.0, 153.6, 98.7, 82.1, 62.9, 62.8, 59.6, 58.5, 32.6, 30.2, 28.9, 28.5, 25.6, 25.5, 25.4, 18.7; HRMS-FAB (M + H) calc′d. 330.1917, found 330.1916.

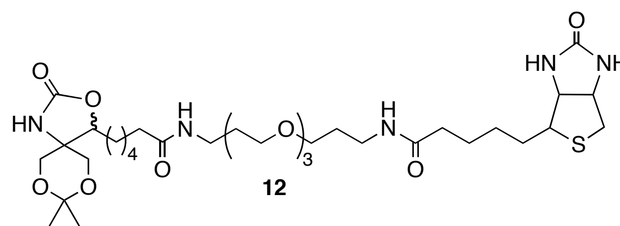
Preparation of 10. PDC (1 g, 2.6 mmol) was added to a solution of **9** (200 mg, 0.61 mmol) in DMF (1.6 mL), and the mixture was stirred for 9 h at room temperature. The reaction mixture was diluted with water (10 mL) and extracted with diethyl ether. The ether layer was dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography (40% ethyl acetate in hexanes) produce **10** in 40% yield (84 mg). IR (KBr): 3207, 2931, 2564, 1781, 1700, 1375, 1274, 1098, 1001, 825, 730, 625 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.94 (d, J = 11.9 Hz, 1H), 4.73 (d, J = 11.2 Hz, 1H), 4.60 (dd, J = 10.9, 2.3 Hz, 1H), 3.74 (dd, J = 11.8, 2.4 Hz, 1H), 3.53 (dd, J = 11.2, 2.4 Hz, 1H), 2.49 (s, 3H), 2.39 (t, J = 7.3 Hz, 2H), 2.04 (s, 1H), 1.70-1.37 (m, 13H); ^{13}C NMR ($CDCl_3$) δ 179.4, 172.1, 153.5, 98.7, 82.1, 62.8, 59.6, 58.5, 33.8, 30.1, 28.6, 28.5, 25.5, 25.2, 24.4, 18.7; HRMS-FAB (M + H) calc'd. 344.1709, found 330.1705.



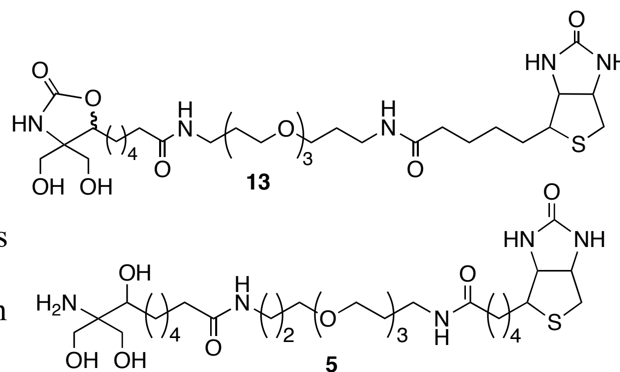
Preparation of 11. A solution of 4,7,10-trioxa-1,13-tridecanediamine (194 mg, 0.88 m mol) in CH_2Cl_2 (0.5 mL) was treated with a mixture of **10** (76 mg, 0.22 mm ol), DCC (68 mg, 0.33 mmol), and HOBT (45 mg, 0.33 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred at room temperature for 5 h. The solvent was removed and the resulting yellow oil was purified by silica gel flash chromatography (20% MeOH in $CHCl_3$) to produce **11** in 71.6% yield (120 mg). IR (KBr): 3716, 3473, 3228, 2923, 1741, 1630, 1376, 1252, 1072, 825 cm^{-1}; ^1H NMR (CD_3OD) δ 4.34 (dd, J = 10.7, 2.6 Hz, 1H), 3.99 (d, J = 12.2 Hz, 1H), 3.79-3.49 (m, 13H), 3.26 (t, J = 6.9 Hz, 2H), 3.11 (t, J = 6.4 Hz, 2H), 2.22 (t, J = 7.3 Hz, 2H), 1.94-1.37 (m, 20H); ^{13}C NMR (CD_3OD) δ 176.9, 161.2, 155.4, 100.6, 84.9, 72.3, 71.9, 70.6, 64.6, 57.7, 53.3, 41.0, 38.5, 37.8, 34.7, 31.6, 31.4, 30.9, 30.7, 28.9, 27.7, 27.1, 26.9, 25.6, 23.6; HRMS-FAB (M + H) calc'd. 504.3285, found 504.3273.



Preparation of 12. To a solution of **11** (50 mg, 0.09 mm ol) in DMF (0.5 mL) was added a DMF solution (0.5 mL) containing D-biotin (20 mg, 0.08 mmol) and DCC (28 mg, 0.13 mmol). The mixture was stirred at room temperature for 24 h. The DMF was then removed under vacuum and the residue was purified by flash chromatography (10% MeOH in CH_2Cl_2). The biotinylated product (**12**) was isolated with 76% yield (52 mg). IR (KBr): ν_{max} 3284, 2923, 1745, 1691, 1644, 1457, 1371, 1251, 1197, 1081, 830, 728 cm^{-1}; ^1H NMR (CD_3OD) δ 4.49 (dd, *J* = 7.5, 4.4 Hz, 1H), 4.34-4.28 (m, 2H), 3.99 (d, *J* = 11.6 Hz, 1H), 3.83-3.49 (m, 15H), 3.26-3.18 (m, 5H), 2.94 (dd, *J* = 12.8, 4.9 Hz, 1H), 2.71 (d, *J* = 12.7 Hz, 1H), 2.21 (t, *J* = 7.3 Hz, 4H), 1.79-1.37 (m, 24H); ^{13}C NMR (CD_3OD) δ 176.9, 176.8, 161.2, 100.6, 84.8, 72.4, 72.1, 70.9, 70.8, 69.1, 64.6, 64.2, 62.5, 57.9, 57.7, 41.9, 38.7, 38.6, 37.9, 37.7, 34.7, 31.6, 31.3, 30.7, 30.6, 30.4, 27.8, 27.7, 27.6, 27.1, 26.9, 25.5, 23.7, 19.2; HRMS-FAB (M + H) calc'd. 730.4061, found 730.4062.



Preparation of 13. To a solution of **12** (29 mg, 0.04 mmol) in MeOH (1 mL) was added TFA (1 mL) at 0 °C. The mixture was stirred for 24 h at room temperature. The solvent was evaporated and the residue was dissolved in water and washed with CH_2Cl_2. The water layer was concentrated to dryness, and **13** was purified by flash chromatography (20% MeOH in CH_2Cl_2) in 88% yield (24 mg). IR (KBr): 3315, 2933, 1685, 1469, 1203, 1131, 802, 721 cm^{-1}; ^1H NMR (CD_3OD) δ 4.46 (dd, J = 7.6, 5.1 Hz, 1H), 4.39 (dd, J = 10.7, 2.6 Hz, 1H), 4.27 (dd, J =



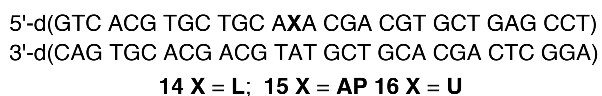
7.7, 4.4 Hz, 1H), 3.62-3.45 (m, 16H), 3.25-3.13 (m, 5H), 2.89 (dd, J = 12.8, 4.9 Hz, 1H), 2.67 (d, J = 12.7 Hz, 1H), 2.17-2.12 (m, 4H), 1.79-1.36 (m, 18H); ^{13}C NMR (CD_3OD) δ 176.9, 176.8, 168.9, 162.3, 84.2, 72.3, 72.0, 70.8, 70.7, 66.2, 65.6, 65.1, 64.2, 62.9, 62.4, 57.8, 41.9, 38.6, 38.5, 37.8, 37.7, 31.2, 31.0, 30.8, 30.6, 30.3, 28.2, 27.7, 27.6, 50.5, 49.2; HRMS-FAB (M + H) calc'd. 690.3748, found 690.3804.

Preparation of 5. Compound **13** (21 mg, 0.035 mmol) was taken up in 1 M KOH in 2:1 EtOH/H_2O (2 mL) and stirred at 60 °C for 2 h. To the reaction mixture 1 M HCl was added dropwise to neutralize the mixture, after which the solvents were evaporated. The residue was dissolved in water and washed with CH_2Cl_2. The aqueous layer was lyophilized and the residue was purified by flash chromatography (25-30% MeOH in $CHCl_3$). The fractions containing **5** were concentrated. The product was taken up in water and filterd through a nylon membrane filter to produce **5** in 35% yield (7.0 mg). IR (KBr): 3289, 2927, 2867, 1681, 1547, 1457, 1263, 1191, 1070, 719 cm^{-1}; ^{1}H NMR (CD_3OD) δ 4.52 (dd, *J* = 7.3, 4.4 Hz, 1H), 4.33 (dd, *J* = 7.8, 4.4 Hz, 1H), 3.80-3.49 (m, 17H), 3.27-3.18 (m, 5H); 2.95 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.72 (d, *J* = 12.7 Hz, 1H), 2.22-2.17 (m, 4H), 1.79-1.28 (m, 18H); ^{13}C NMR (CD_3OD) δ 176.9, 176.8, 72.3, 72.2, 71.9, 71.8, 71.7, 71.6, 71.2, 70.7, 65.8, 64.2, 62.5, 62.3, 60.9, 57.8, 41.9, 38.6, 38.5, 37.9, 37.7, 32.5, 31.3, 31.2, 30.8, 30.6, 30.4, 28.0, 27.8, 27.7; HRMS-FAB (M + H) calc'd. 664.3955, found 664.3991.

Preparation of oligonucleotide complexes containing C4-AP (1) and DOB (2). The oligonucleotide (10 pmol) containing the precursor to **C4-AP** and **DOB** was radiolabeled using standard methods (T4 polynucleotide kinase-γ-^{32}P-ATP for 5-labeling of **C4-AP** and terminal deoxynucleotidyl transferase, α-^{32}P-ddATP for 3'-labeling of **DOB**).[6] The oligonucleotides were separated from unincorporated nucleotide triphosphates using C18-Sep pak cartridges. The

cartridges were equilibrated using ammonium acetate (pH 7.2, 10 mM). After loading the samples and washing with H_2O (3 × 10 mL), the product was eluted with MeOH:H_2O (60:40, 3 mL). The duplex containing **C4-AP** (**1**) was prepared by hybridizing the radiolabeled oligonucleotide with the complementary strand in PBS (10 mM potassium hydrogen phosphate, pH 8.2, 100 mM NaCl) at 90 °C for 5 min and slowly cooling to room temperature prior to photolysis. The ternary complex containing **DOB** (**2**) was synthesized by hybridizing 3'-labeled oligonucleotide with the complementary and the 5'-fragment in PBS at 55 °C for 5 min, followed by slowly cooling to room temperature. A ternary complex containing **DOB** was created in order to most closely mimic the environment surrounding the lesion when it is produced in DNA. Photolysis (λ, 350 nm) was carried out in PBS at room temperature for 45 min.

Preparation of duplexes containing AP (14) and L (15). Duplex **14** containing **AP** was prepared by reacting 5'-^{32}P-**16** (10 pmol) containing 2'-deoxyuridine hybridized to its complement in 2.5 μL UDG buffer-1.5 μL H_2O with UDG (2 Units) at 37 °C for an hour. Duplex **15** was prepared by photolyzing the respective ^{32}P-labeled oligonucleotide containing the lactone precursor for 1 h in H_2O (50 μL), after which the sample was evaporated to dryness. Hybridization of the lesion containing oligonucleotide and complementary strand (1.0 eq.) was carried out in 25 μL (200 mM NaCl). Hybridization was carried out at 55 °C for 10 min, followed by cooling to 25 °C and then placed in a 4°C refrigerator overnight.



Determination of optimum conditions for tagging of C4-AP and DOB by 5. C4-AP (**1**) or **DOB** (**2**) (20 nM) were incubated with **5** (1, 5, or 10 mM) in sodium phosphate buffer (10 mM, pH 8.2) and 100 mM NaCl at 55°C for 4 h. Adventitious cleavage was determined by analyzing

the extent of cleavage without treatment with **5**. The extent of photo conversion of the precursor to the lesion was determined by treating with 1 M NaOH at 37 °C for 30 min, followed by neutralization with 1 M HCl. With **C4-AP** (**1**), the yields of adduct with 1, 5 and 10 mM **5** were 76.5 ± 1.3 %, 81.5 ± 2.6 %, and 88.5 ± 1.0 %, respectively. Adduct yields with **DOB** (**2**) with 1, 5 and 10 mM probe were 57.2 ± 2.8 %, 72.1 ± 4.9 %, and 76.1 ± 1.2 %, respectively. Note: Each yield is the average of 3 measurements. Analytical oligonucleotide separations were carried out using 20 % polyacrylamide denaturing gel [5% crosslink, 45% urea (w/w)].

Analysis of reactions of 5 with C4-AP (1), DOB (2) in the presence of Pol β. DNA (**1**, **2**) containing lesions (20 nM) were incubated with **5** (5 mM) in sodium phosphate buffer (10 mM, pH 8.2), 50 mM $MgCl_2$, 100 mM DTT and Pol β (100 nM) at 37°C for 1 h. Excess enzyme was removed by phenol extraction (50 μL), followed by precipitation (0.2 M NaCl and EtOH). After removal of supernatant and drying, the pellet was resuspended in sodium phosphate buffer and reacted with **5** (5 mM) at 55°C for 4 h (10 μL). Adventitious cleavage was determined by analyzing the extent of cleavage without any further treatment and the extent of photo conversion of the precursor to the lesion was determined by treating with 1 M NaOH at 37 °C for 30 min, followed by neutralization with 1 M HCl. For DNA containing **DOB** (**2**), a portion of the photolyzed solution was incubated with 500 mM $NaBH_4$ at room temperature for 30 min to reduce (and stabilize) any remaining **DOB** lesion. Adduct formation between the lesion and **5** (5 mM) was analyzed using 20% polyacrylamide denaturing gel [5% crosslink, 45 % urea (w/w)]. The yield (average of 3 independent measurements) of adduct with **C4-AP** was 82.3 ± 0.1 %, and **DOB** was 74.8 ± 0.7 %. These yields were used for translating fluorescence intensities into adduct quantities (see below).

MALDI-TOF MS analysis of reaction of 5 and DNA containing C4-AP or DOB. Oligonucleotides containing the precursor to **C4-AP** (**17**) or **DOB** (**18**) (2.6 nmol) were photolyzed for 1.5 h in PBS (100 μL, 10 mM sodium phosphate-100 mM NaCl, pH 8.2). The

5'-d(GAA GAC CT**X** GGC GCC)
17 X = C4-AP
5'-d(**X**CC GTA ATG CAG TCT)
18 X = DOB

photolysate were treated with **5** (5 mM) at 55 °C for 4 h. The DNA was precipitated from NH_4OAc and EtOH. 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, 20 μM) 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 ($\varepsilon_{260} = 20\ g^{-1}\bullet cm^{-1}\bullet L$) and the quality of PCR fragment was determined by agrose gel (3%). Sequences of the three primers and the plasmid template region were the following.

Primer 1: 5'-d(CAC TGA ATC ATGGTC ATA GCT GTT)

Primer 2: 5'-biotin-(CAC TGA ATC ATGGTC ATA GCT GTT)

Primer 3: 5'-d(GGT GAA GGG CAA TCA GCT GTT)

Template region: 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)

Generation of C4-AP from activated peplomycin. The PCR fragment (5 pmol) was treated with various concentrations of activated peplomycin (2, 4, 6, 8 and 10 μM) at 37 °C for 1 h in sodium phosphate buffer (10 mM, pH 8.2).[7] Activated peplomycin (100 μM) was formed by incubating peplomycin with equimolar $[Fe(SO_4)_2(NH_4)_2]$ in H_2O at room temperature for 10 min immediately prior to reaction with DNA. The DNA was immediately subjected to the HRP assay as described below in order to quantify the lesions.

Detection of C4-AP and DOB in a 287 nt PCR fragment subjected to γ-radiolysis. Phosphate (10 mM, pH 8.2) solutions (50 μL) of 287 nt PCR fragment DNA (25 pmol) in Pyrex glass tubes were treated with γ-radiolysis at different doses (50-300 Gy, 25 Gy/min). For experiments using anaerobic environment, DNA samples were subjected to three freeze-pump-thaw degas cycles, and sealed in Pyrex glass tubes prior to irradiation. After γ-radiolysis, DNA samples were transferred to eppendorf tubes. One half of the sample was incubated in sodium phosphate buffer (10 mM, pH 8.2), 50 mM $MgCl_2$, 100 mM DTT, and 300 nM Pol β at 37°C for 1 h. Excess enzyme was removed by phenol extraction (50 μL) followed by precipitation (0.2 M NaCl and EtOH). After removal of supernatant and drying, the pellet was resuspended in PBS and reacted

with **5** (5 mM) at 55°C for 4 h (10 μL). The half of the sample (25 μL) not treated with Pol β was evaporated to dryness, and then resuspended and treated with **5** as above. For all samples excess probe was removed by one phenol extraction and three EtOH precipitations, after which the DNA was subjected to the HRP assay (below). DNA sample concentrations were determined using UV (ε_{260} = 20 g^{-1}•cm^{-1}•L). The DNA used in the HRP 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'-biotinylated 287 nt PCR fragment and non-biotinylated 287 nt PCR fragment to make a final DNA amount of 2.5 pmol. The amount of biotinylated 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 C4-AP and DOB using the HRP 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 **C4-AP** and **DOB**, the following equations were used:

Without Pol β: $$\frac{(Yield)_{C4-AP}}{100} \times M_{C4-AP} + \frac{(Yield)_{DOB}}{100} \times M_{DOB} = M_{Lesion}$$

$$\frac{(Yield)_{C4-AP}}{100} \times \frac{M_{C4-AP}}{Dose} + \frac{(Yield)_{DOB}}{100} \times \frac{M_{DOB}}{Dose} = \frac{M_{Lesion}}{Dose} \equiv (SLOPE)_{Lesion}$$

With Pol β: $$\frac{(Yield)_{C4-AP}}{100} \times M_{C4-AP} = M_{Lesion}$$

$$\frac{(Yield)_{C4-AP}}{100} \times \frac{M_{C4-AP}}{Dose} = \frac{M_{Lesion}}{Dose} \equiv (SLOPE)_{Lesion}$$

$(Yield)_{C4-AP}$: The percentage yield of **C4-AP** (82.3) adduct determined by PAGE using oligonucleotide containing **C4-AP**.

$(Yield)_{DOB}$: The percentage yield of **DOB** (74.8) adduct determined by PAGE using oligonucleotide containing **DOB**.

$(SLOPE)_{Lesion}$: The slope of detected lesions (pmol) as a function of dose with or without Pol β treatment.

M_{C4-AP}: The amount of **C4-AP** (pmol) in DNA.

M_{DOB}: The amount of **DOB** (pmol) in DNA.

M_{Lesion}: The total amount of lesions (pmol) detected with or without Pol β treatment.

References:

(1) Kim, J.; Gil, J. M.; Greenberg, M. M. *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 5882-5885.

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

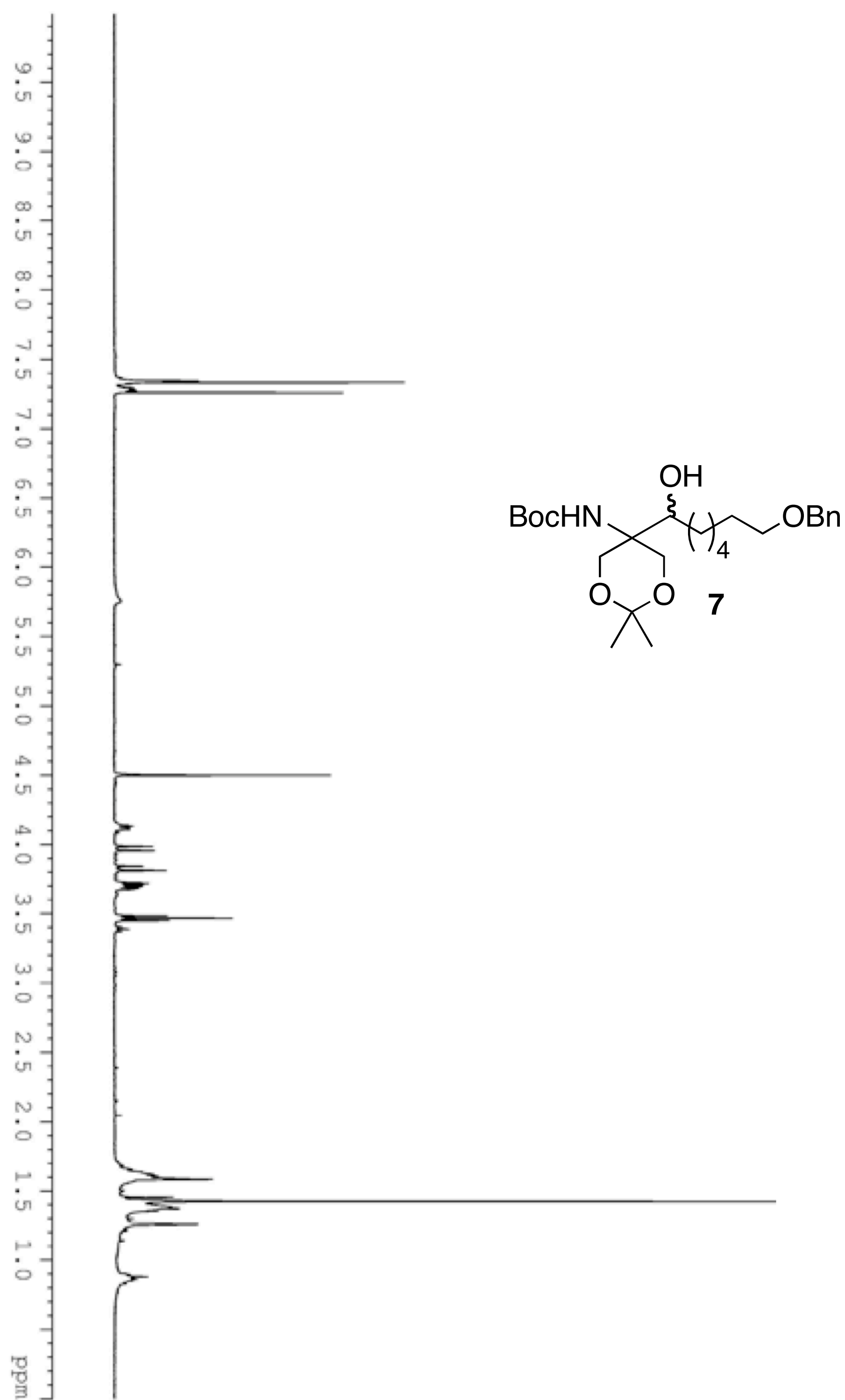
(3) Tronche, C.; Goodman, B. K.; Greenberg, M. M. *Chem. Biol.* **1998**, *5*, 263-271.

(4) Jew, S. S.; Kim, H. O.; Jeong, B. S.; Park, H. G. *Tet. Asymm.* **1997**, *8*, 1187-1192.

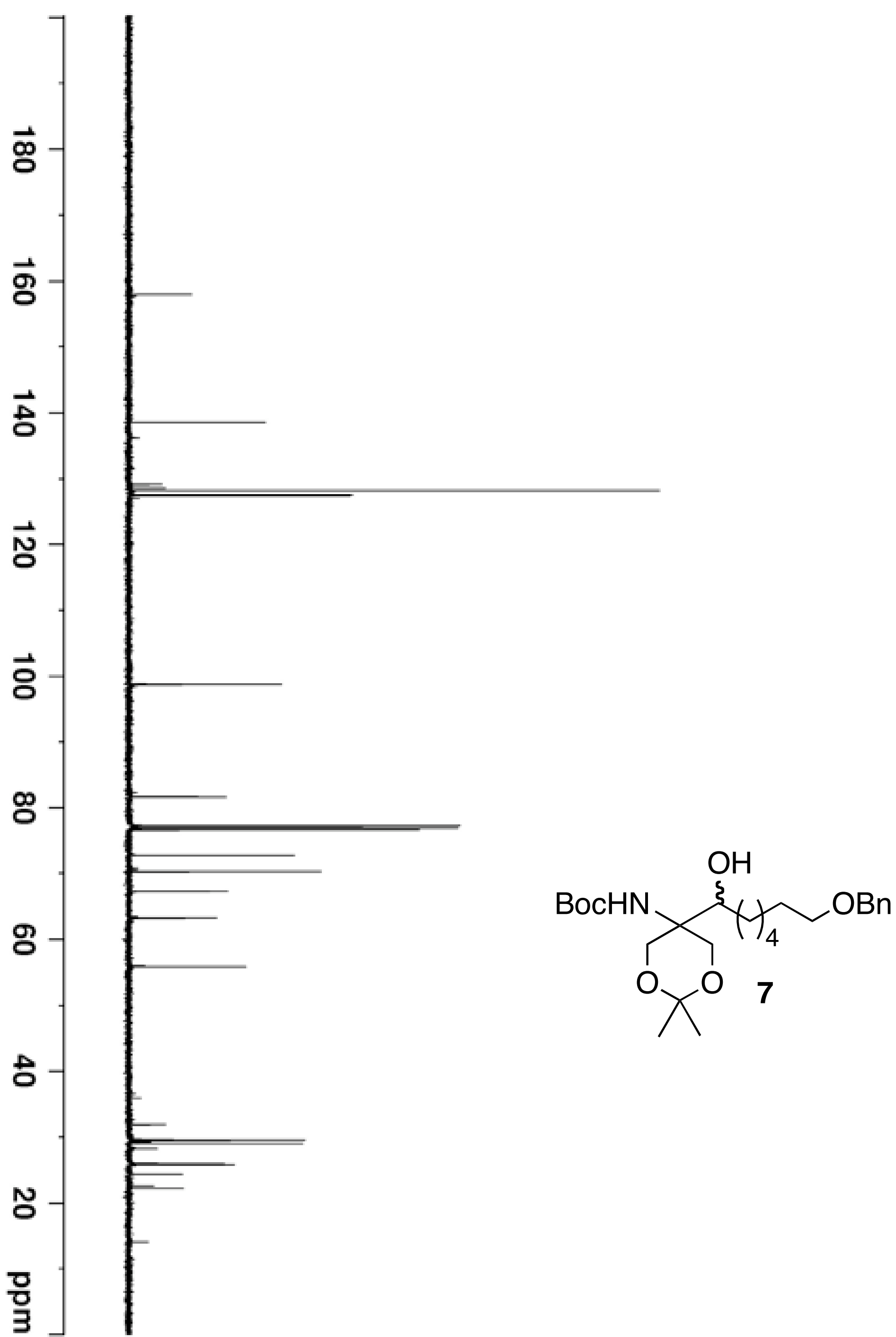
(5) Kang, J. H.; Chung, H. E.; Kim, S. Y.; Kim, Y.; Lee, J.; Lewin, N. E.; Pearce, L. V.; Blumberg, P. M.; Marquez, V. E. *Bioorg. & Med. Chem. Lett.* **2003**, *11*, 2529-2539.

(6) Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning*; Cold Spring Harbor Laboratory, Cold Spring Harbor, NY., 1982.

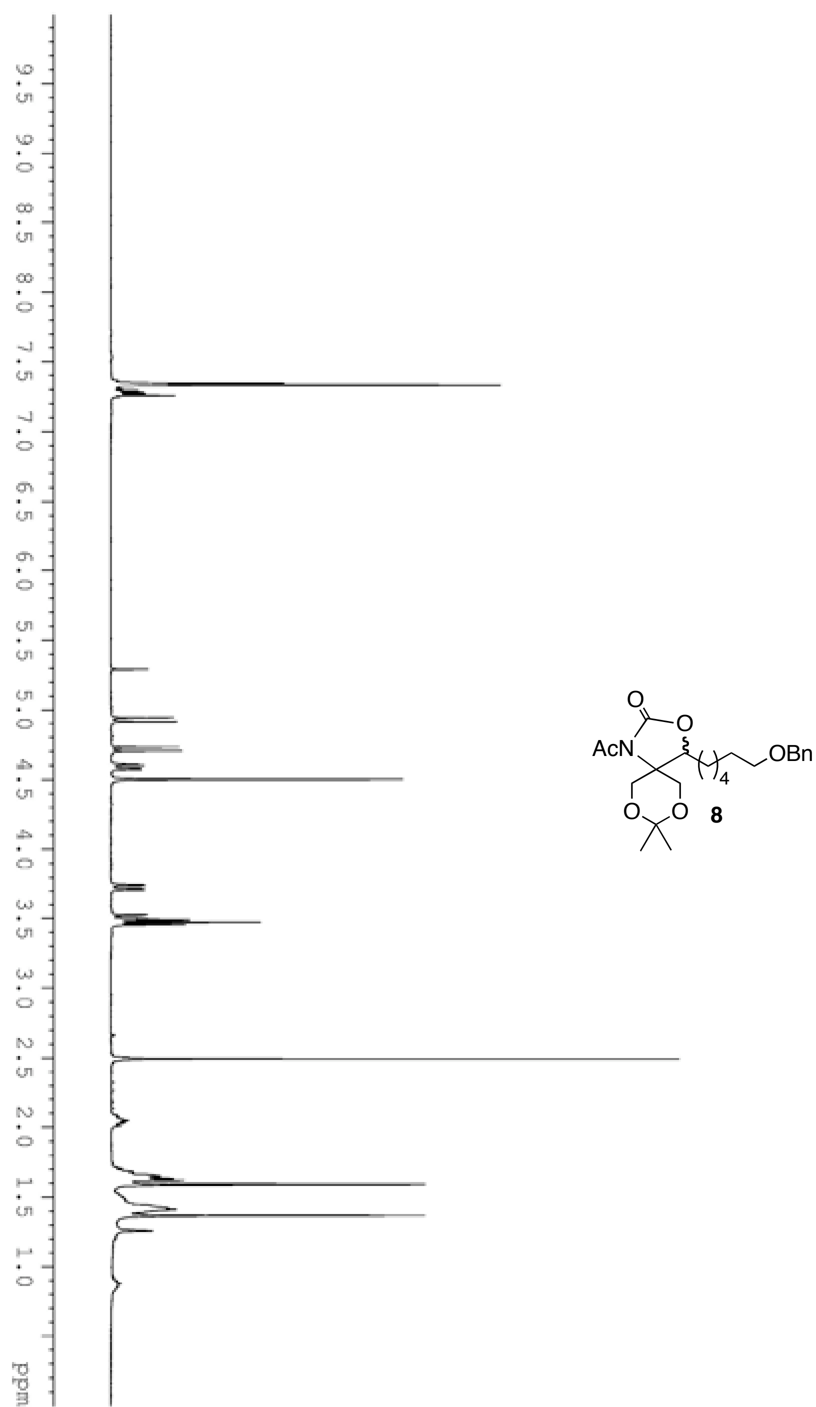
(7) Sugiyama, H.; Ohmori, K.; Saito, I. *J. Am. Chem Soc.* **1994**, *116*, 10326-7.



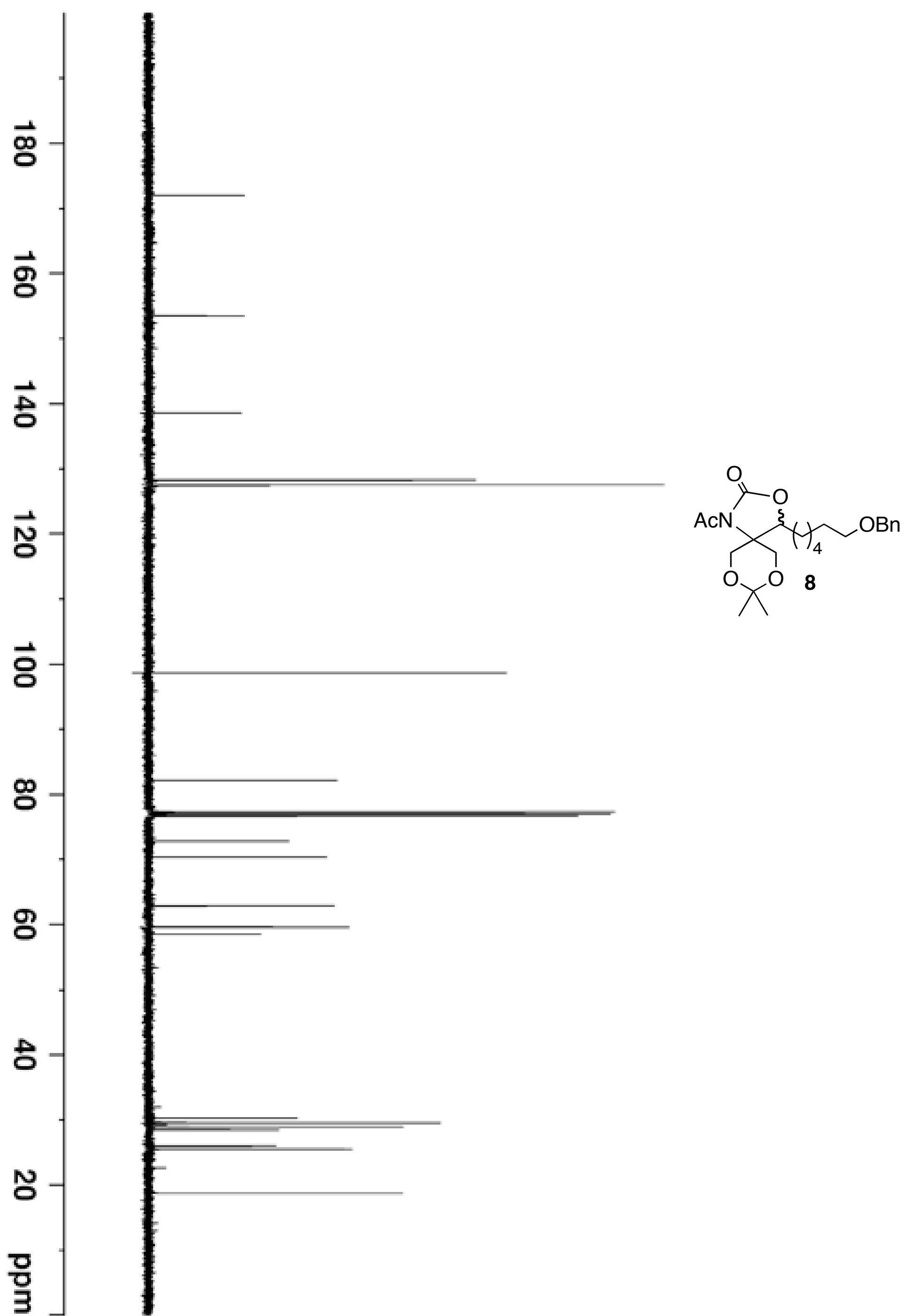
Supporting Information Figure 1. ^{1}H NMR of **7**.



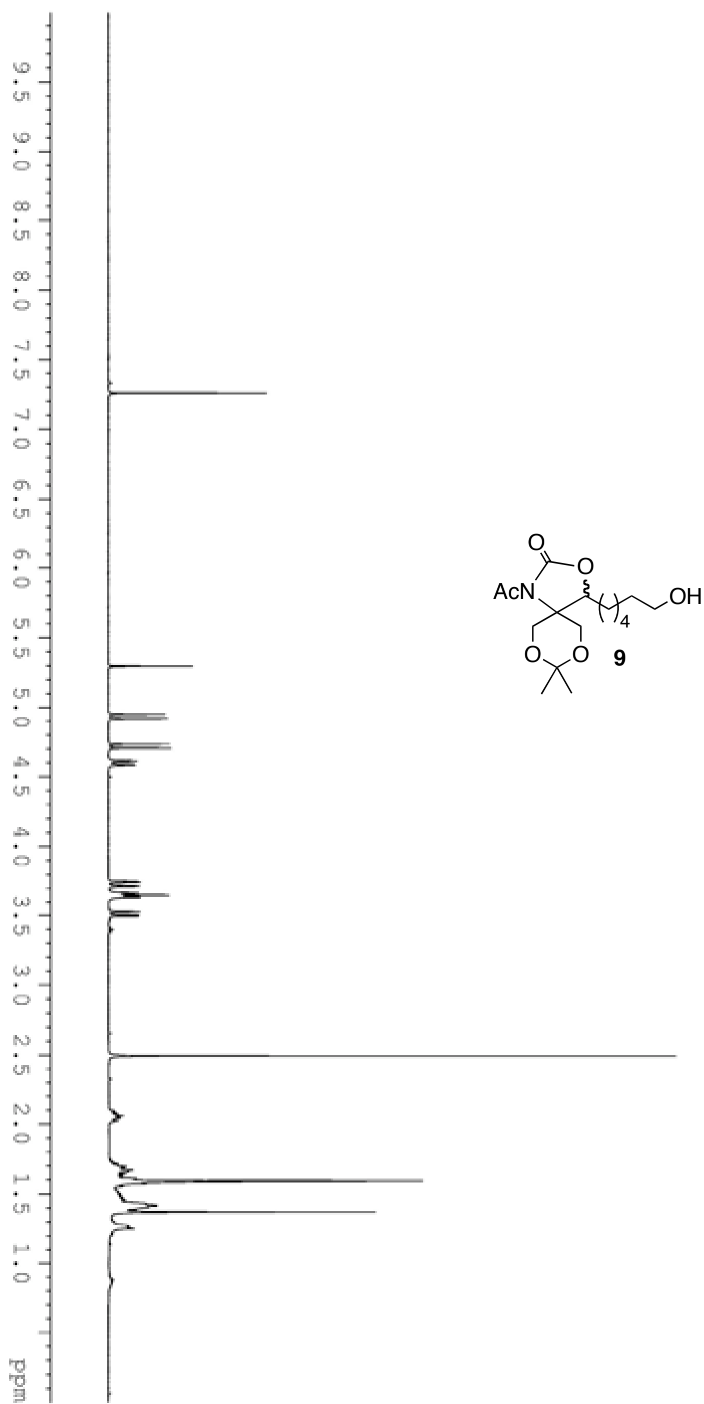
Supporting Information Figure 2. ^{13}C NMR of **7**.



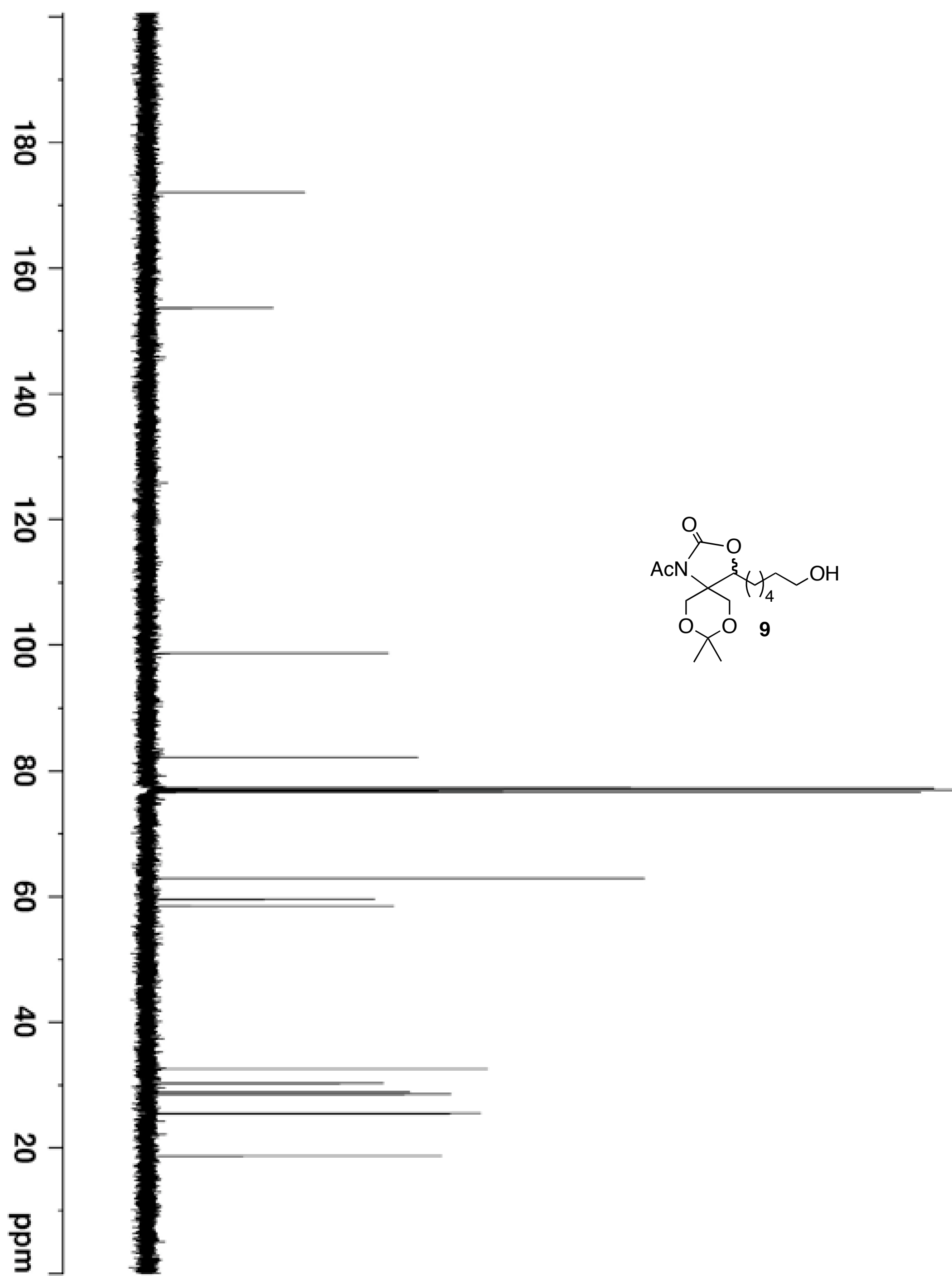
Supporting Information Figure 3. ^{1}H NMR of **8**.



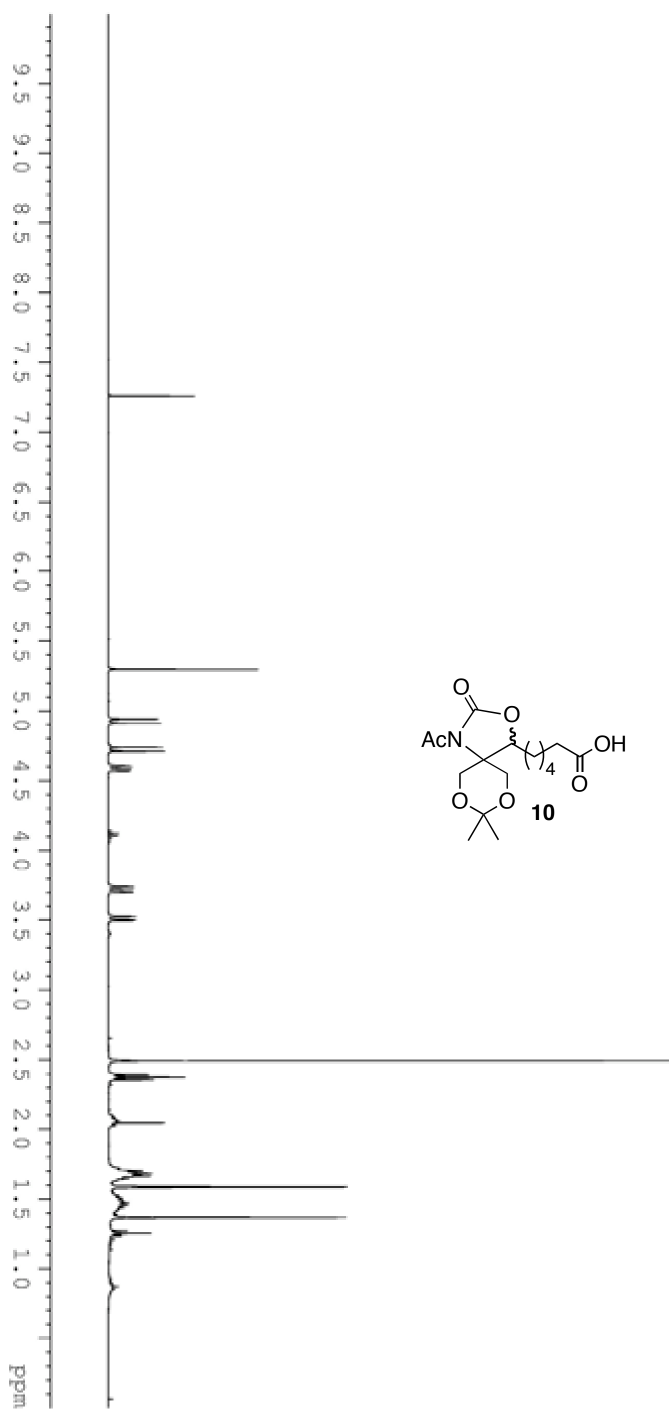
Supporting Information Figure 4. ^{13}C NMR of **8**.



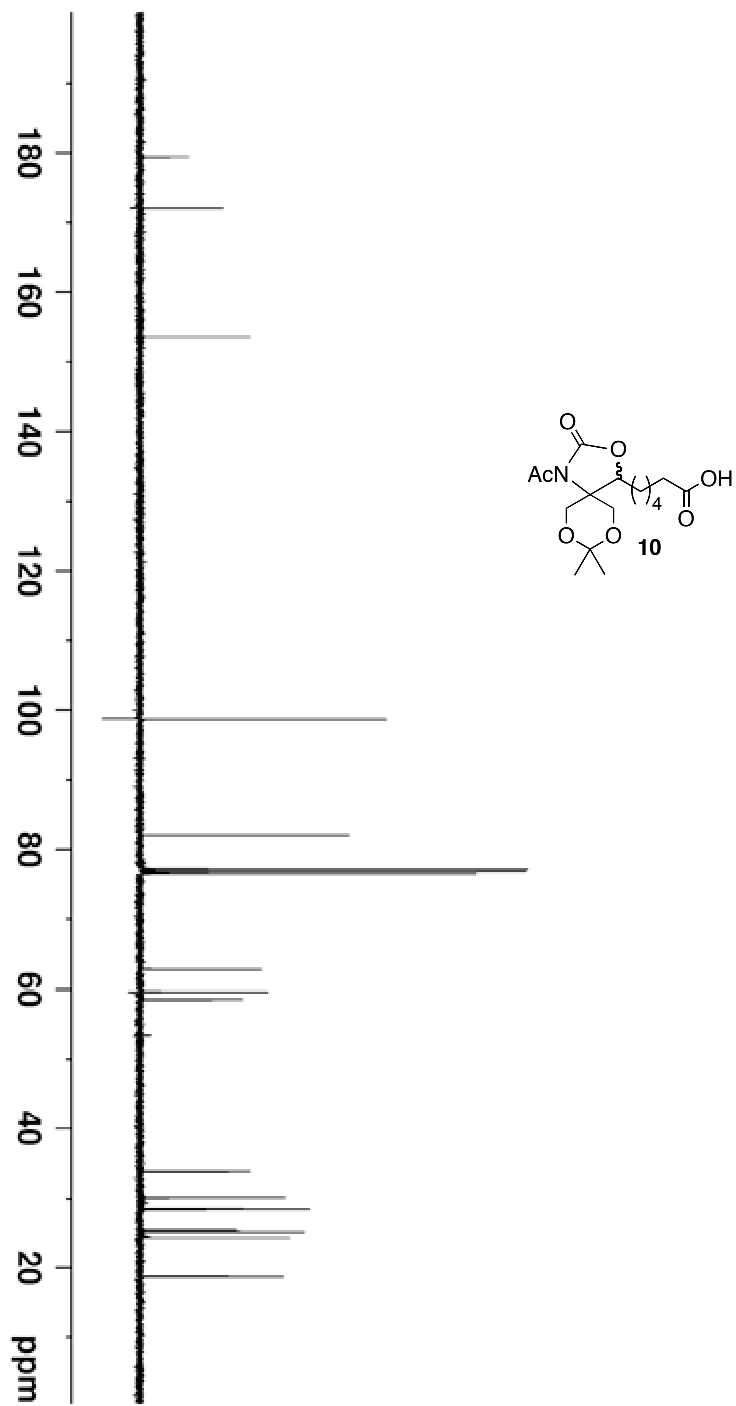
Supporting Information Figure 5. ^{1}H NMR of **9**.



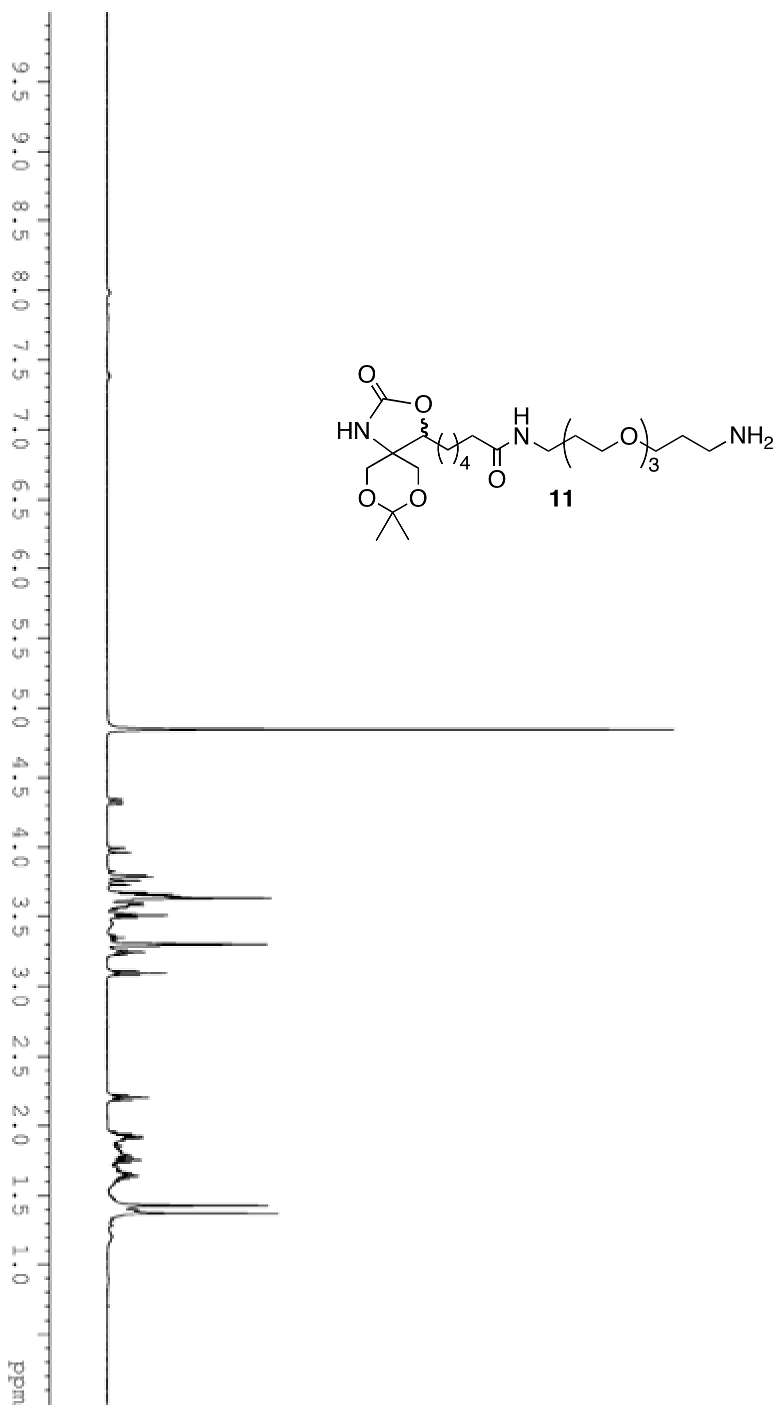
Supporting Information Figure 6. ^{13}C NMR of **9**.



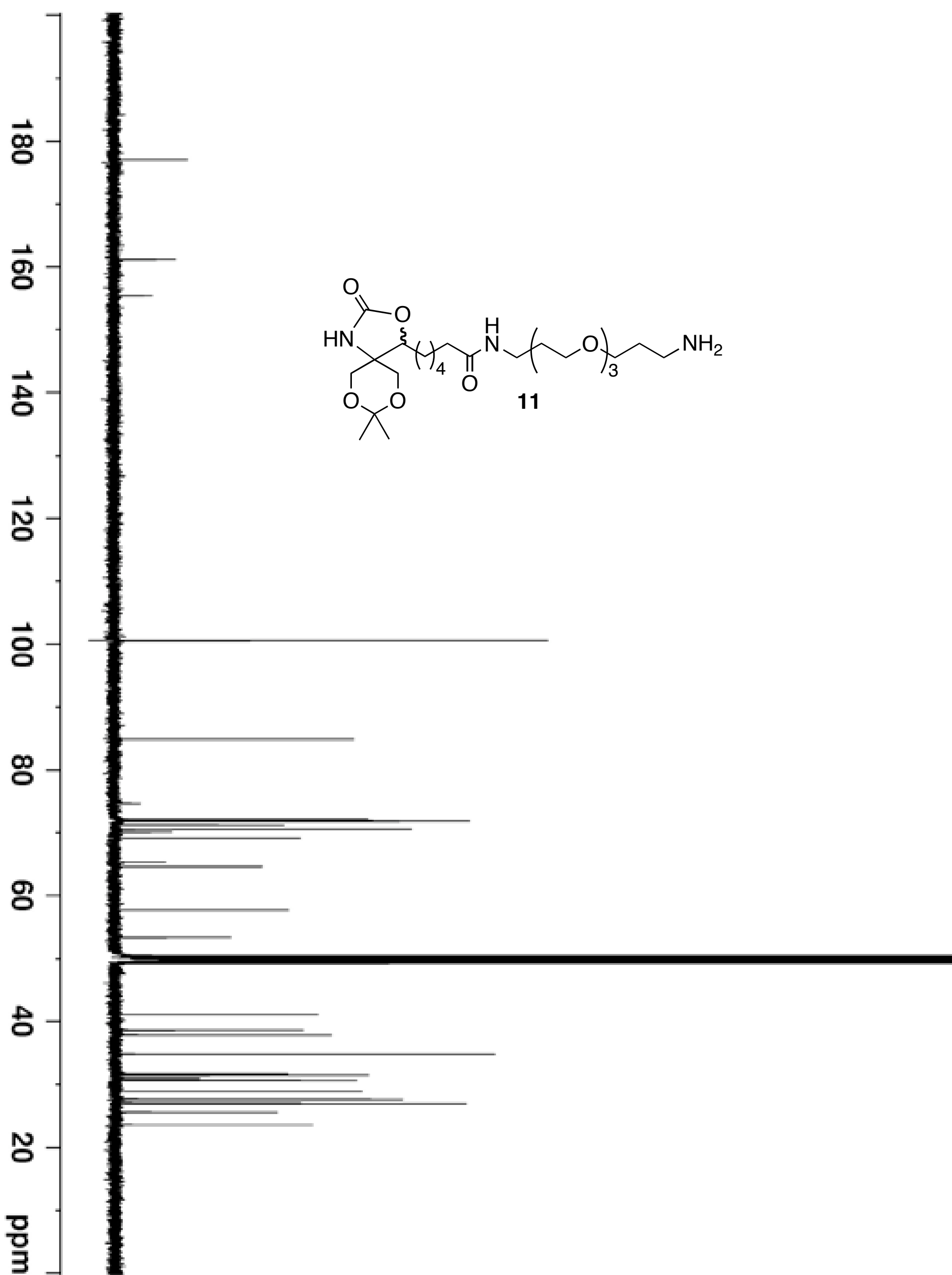
Supporting Information Figure 7. ^{1}H NMR of **10**.



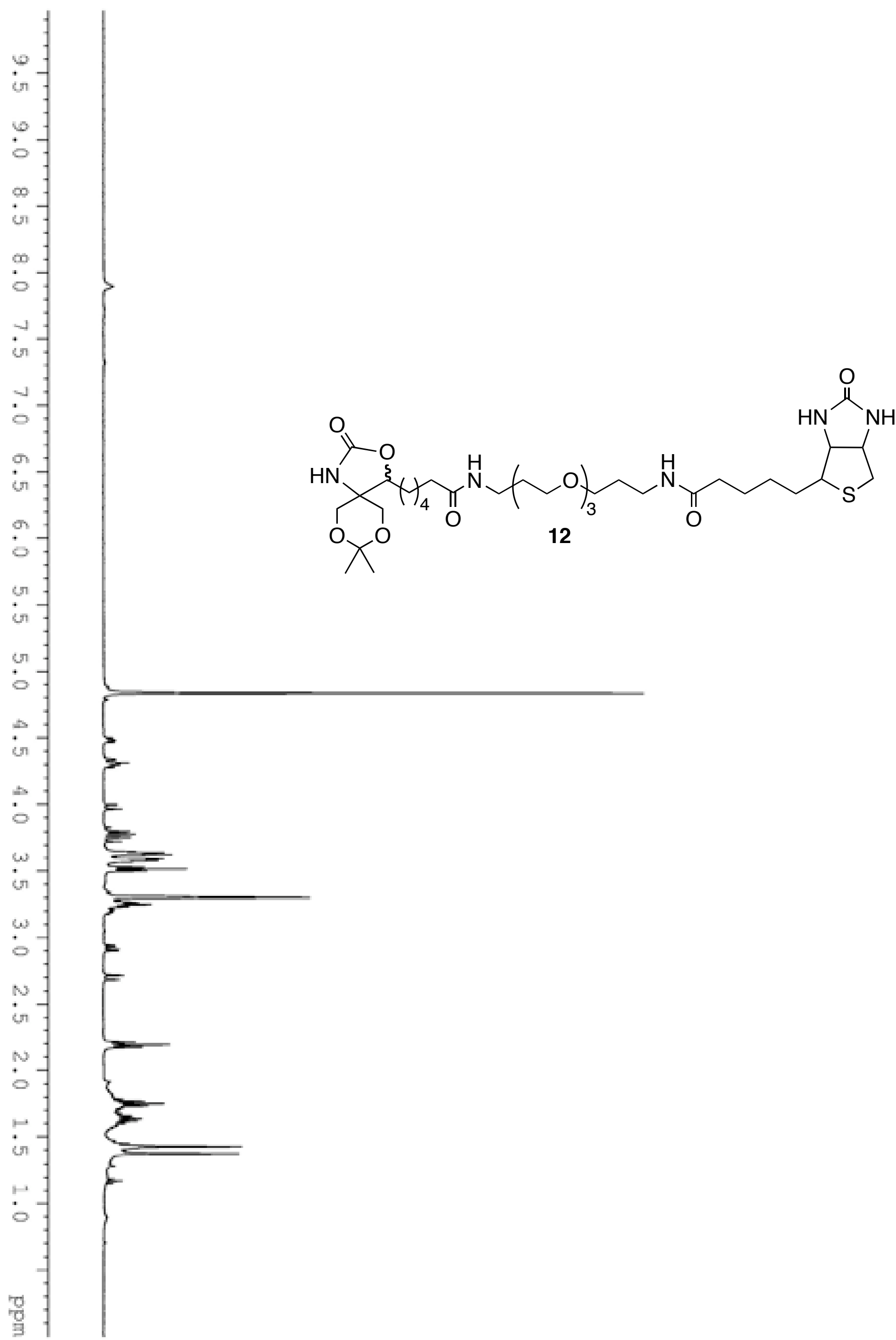
Supporting Information Figure 8. ^{13}C NMR of **10**.



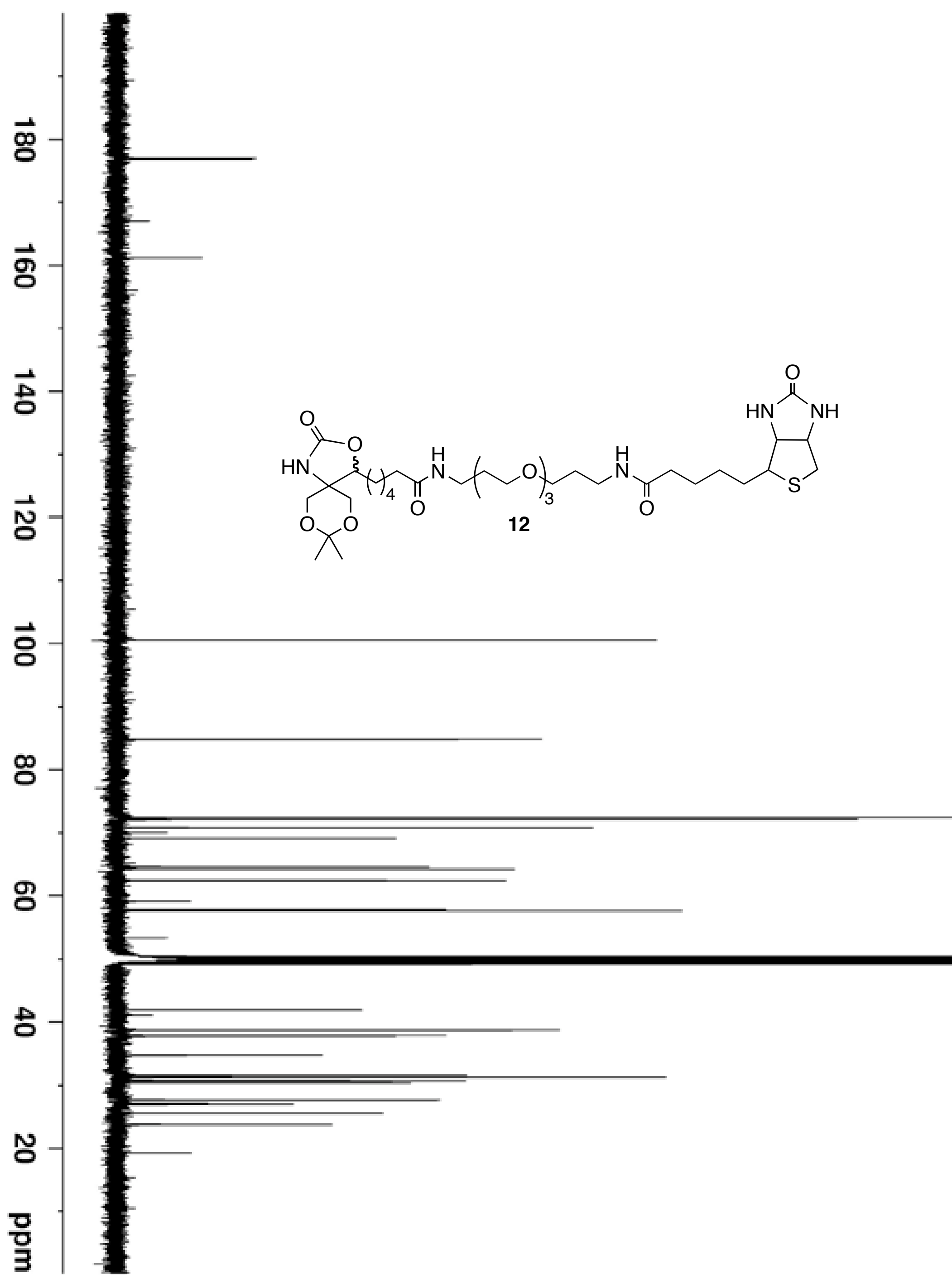
Supporting Information Figure 9. ^{1}H NMR of **11**.



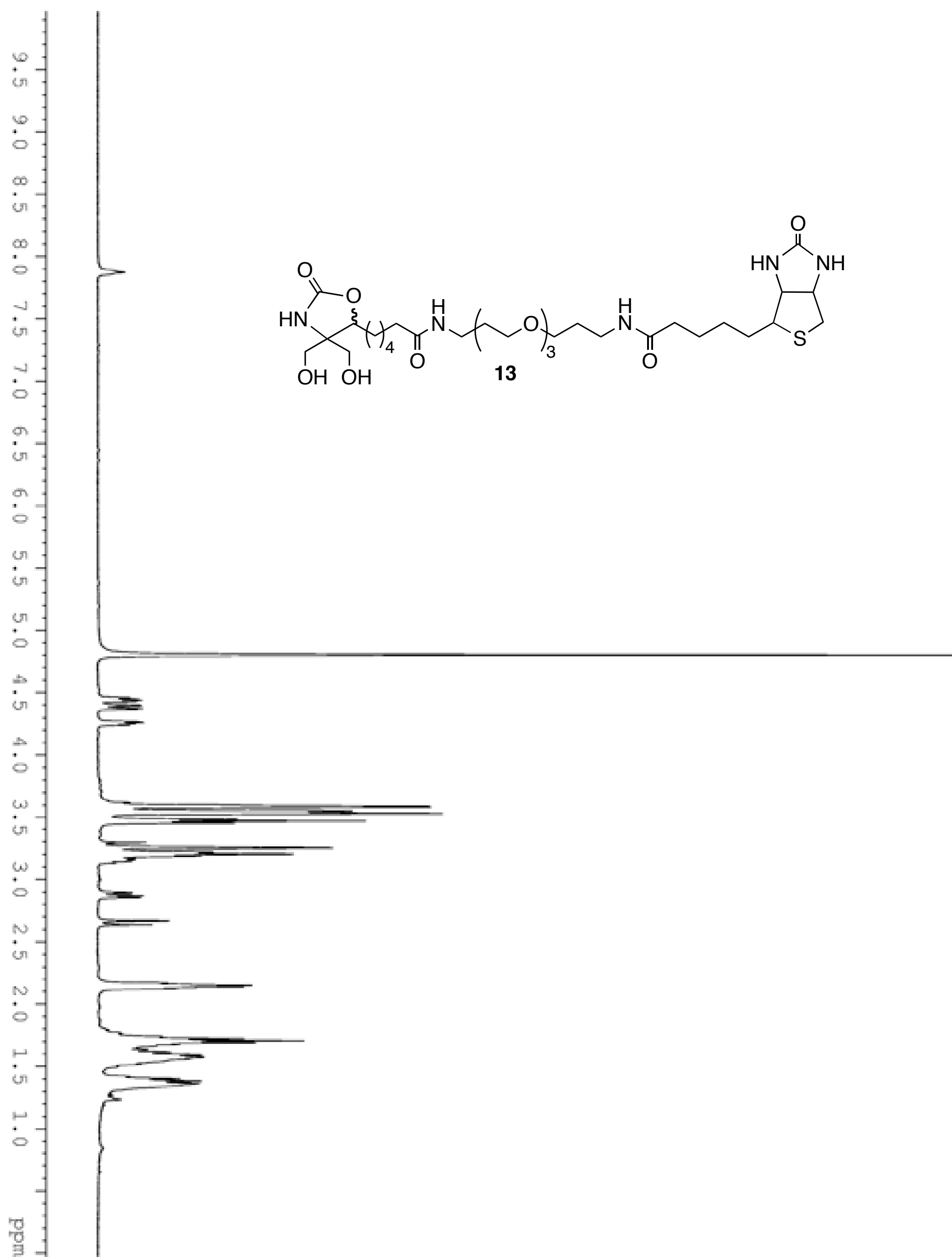
Supporting Information Figure 10. ^{13}C NMR of **11**.



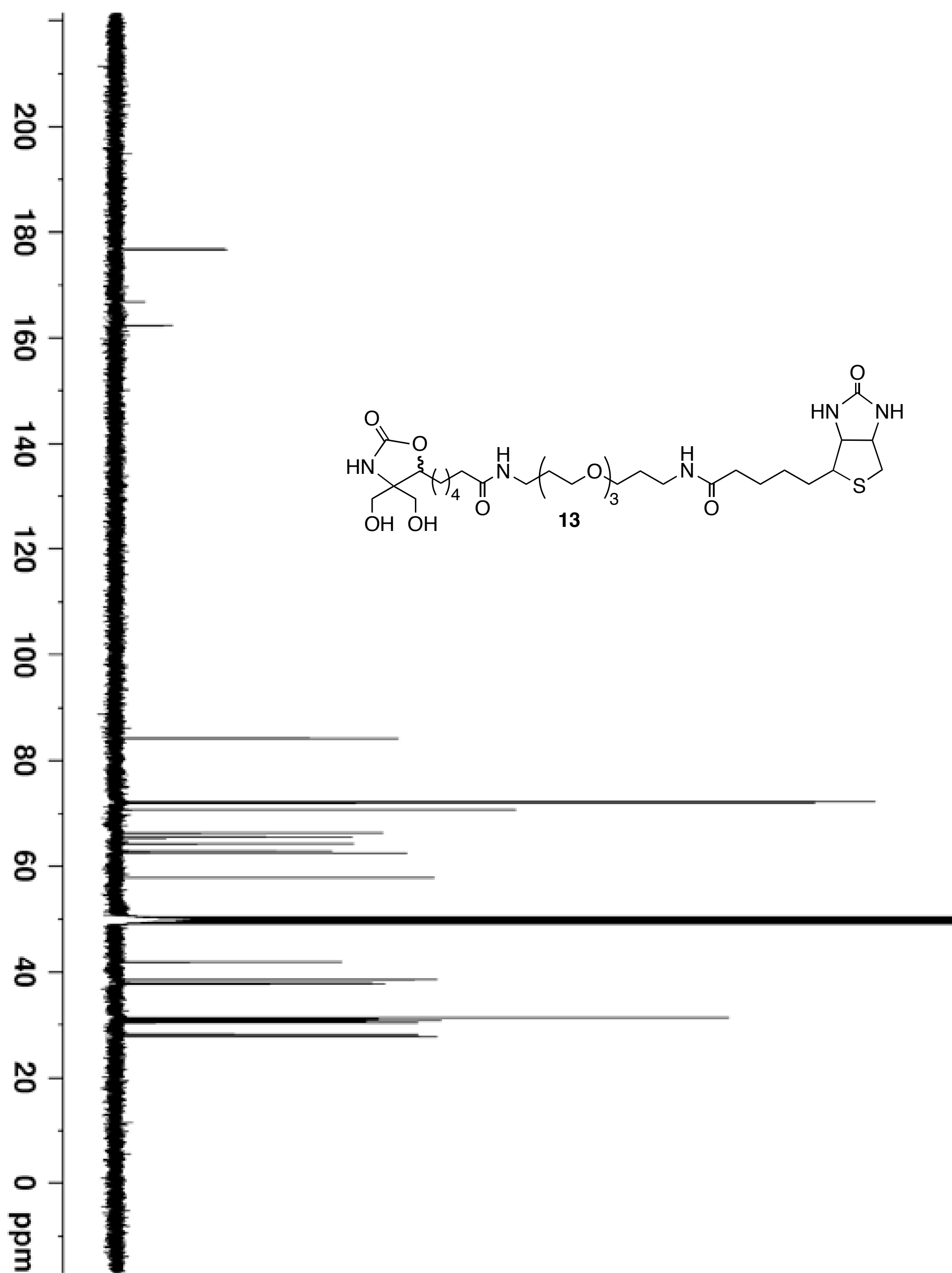
Supporting Information Figure 11. ^{1}H NMR of **12**.



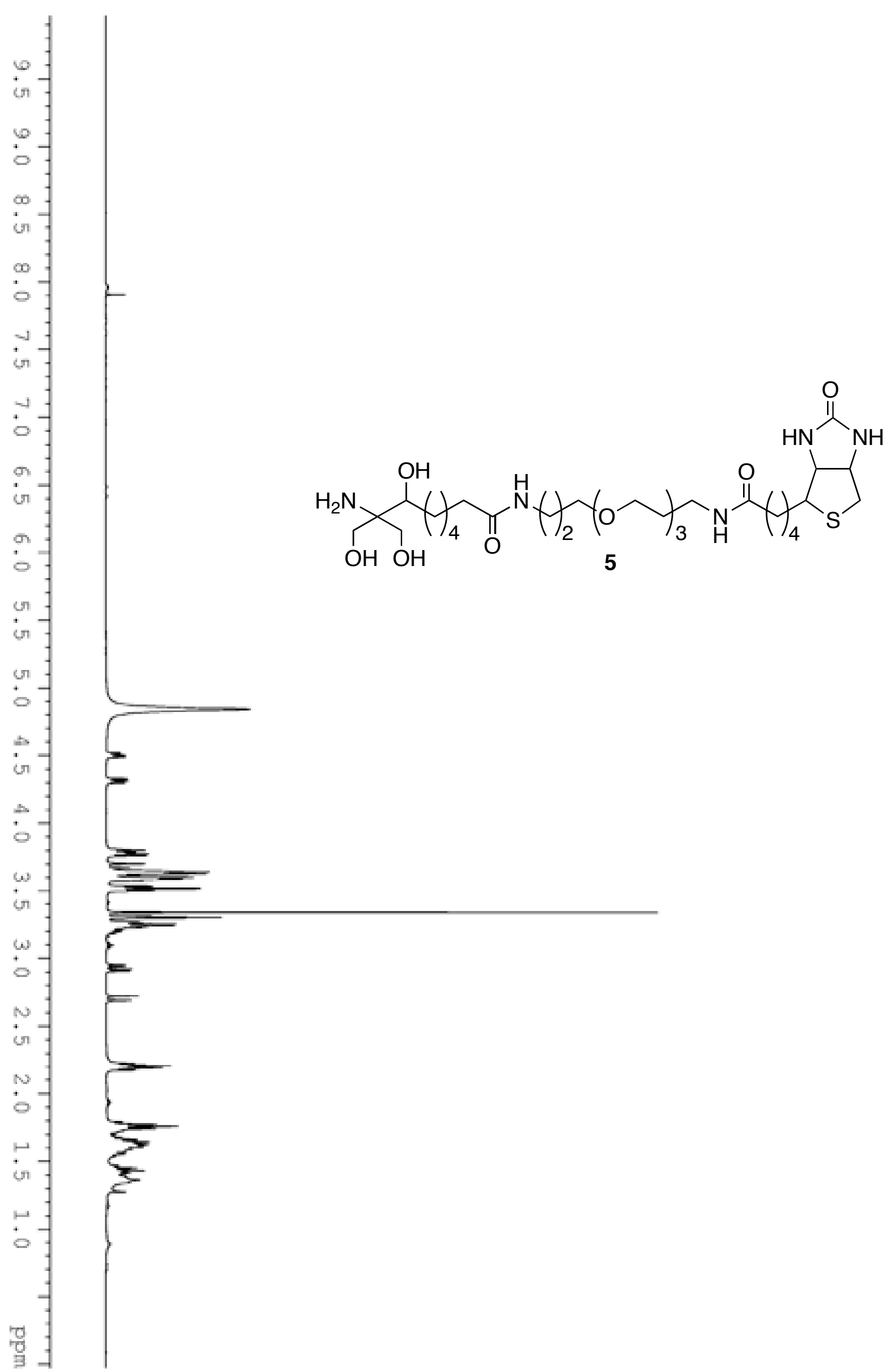
Supporting Information Figure 12. ^{13}C NMR of **12**.



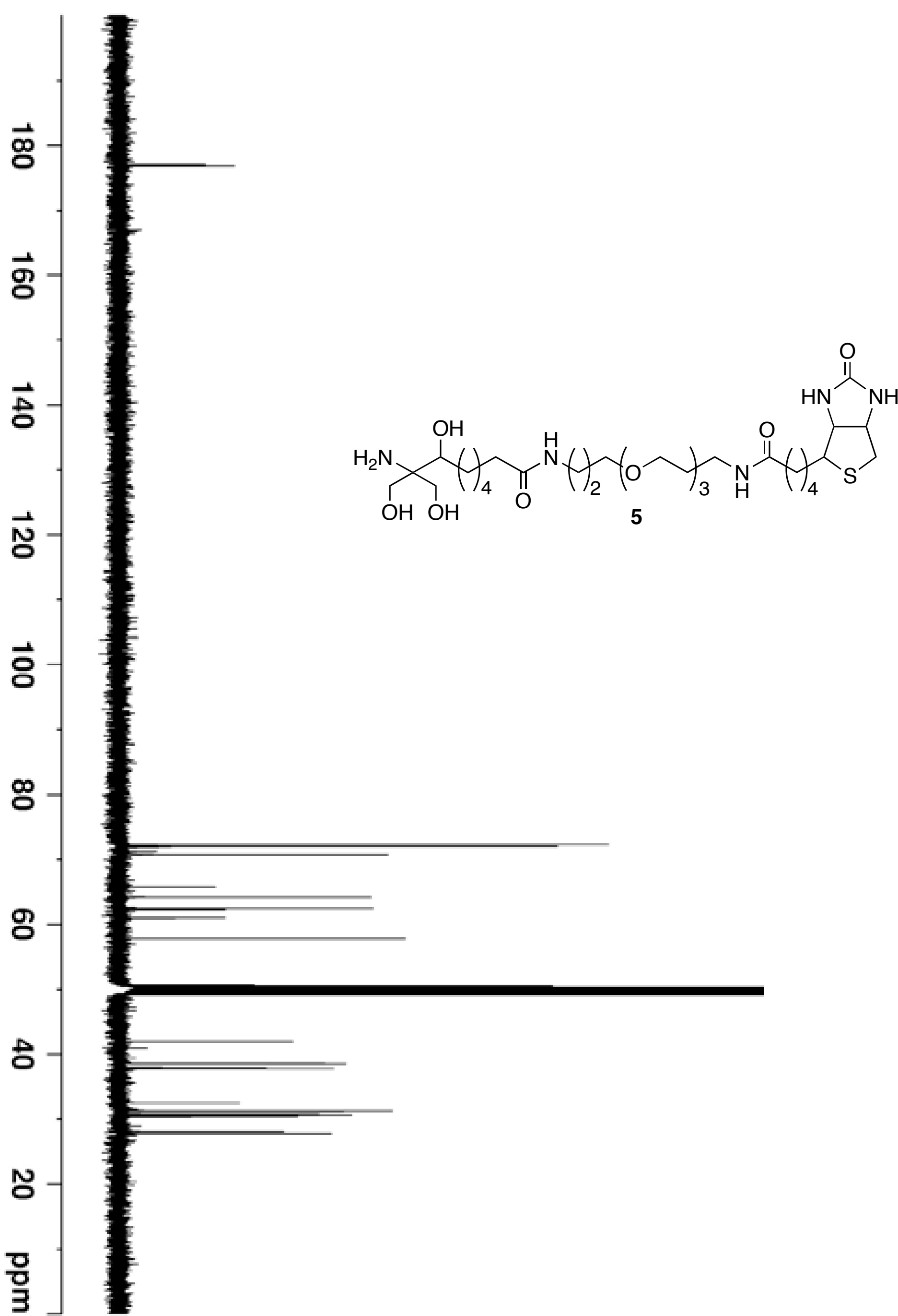
Supporting Information Figure 13. ^{1}H NMR of **13**.



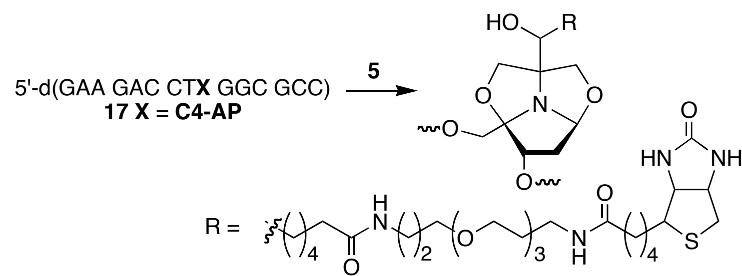
Supporting Information Figure 14. ^{13}C NMR of **13**.

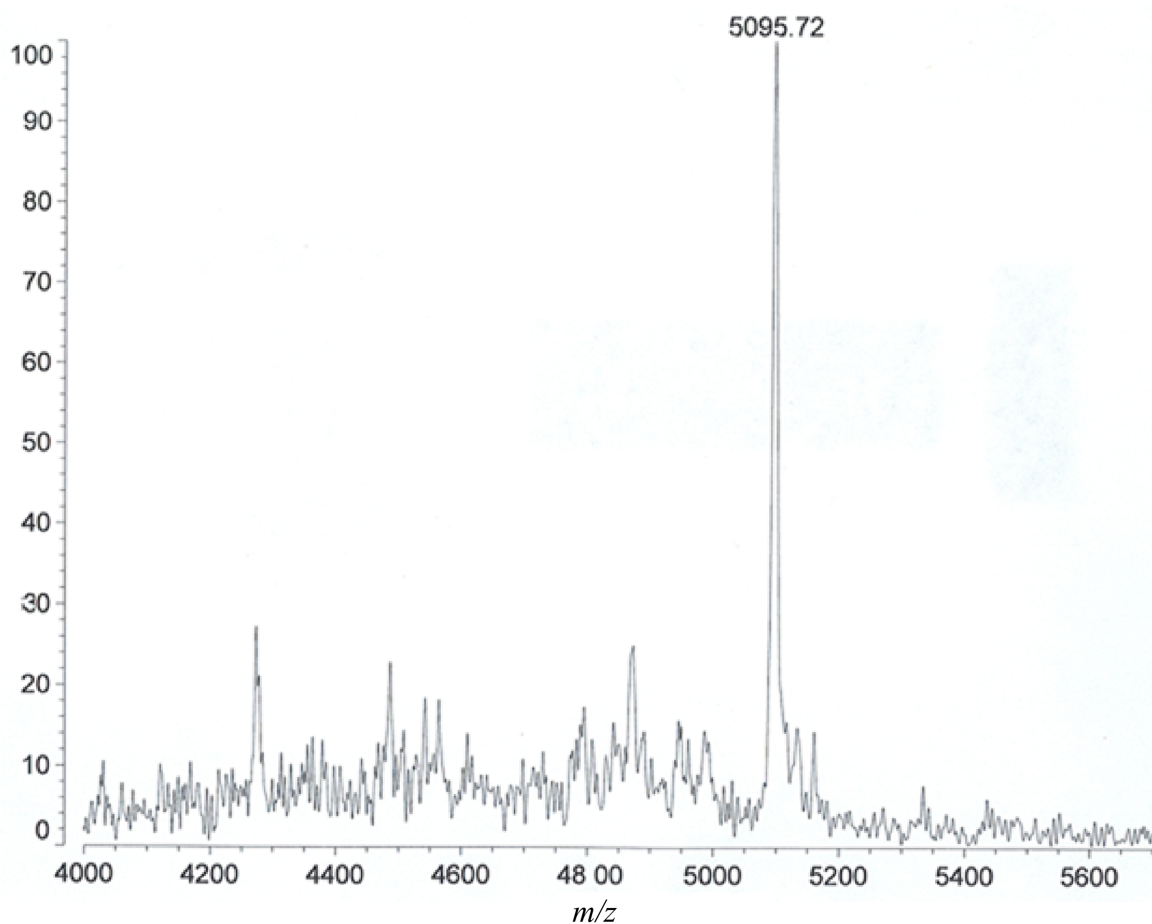


Supporting Information Figure 15. ^{1}H NMR of **5**.

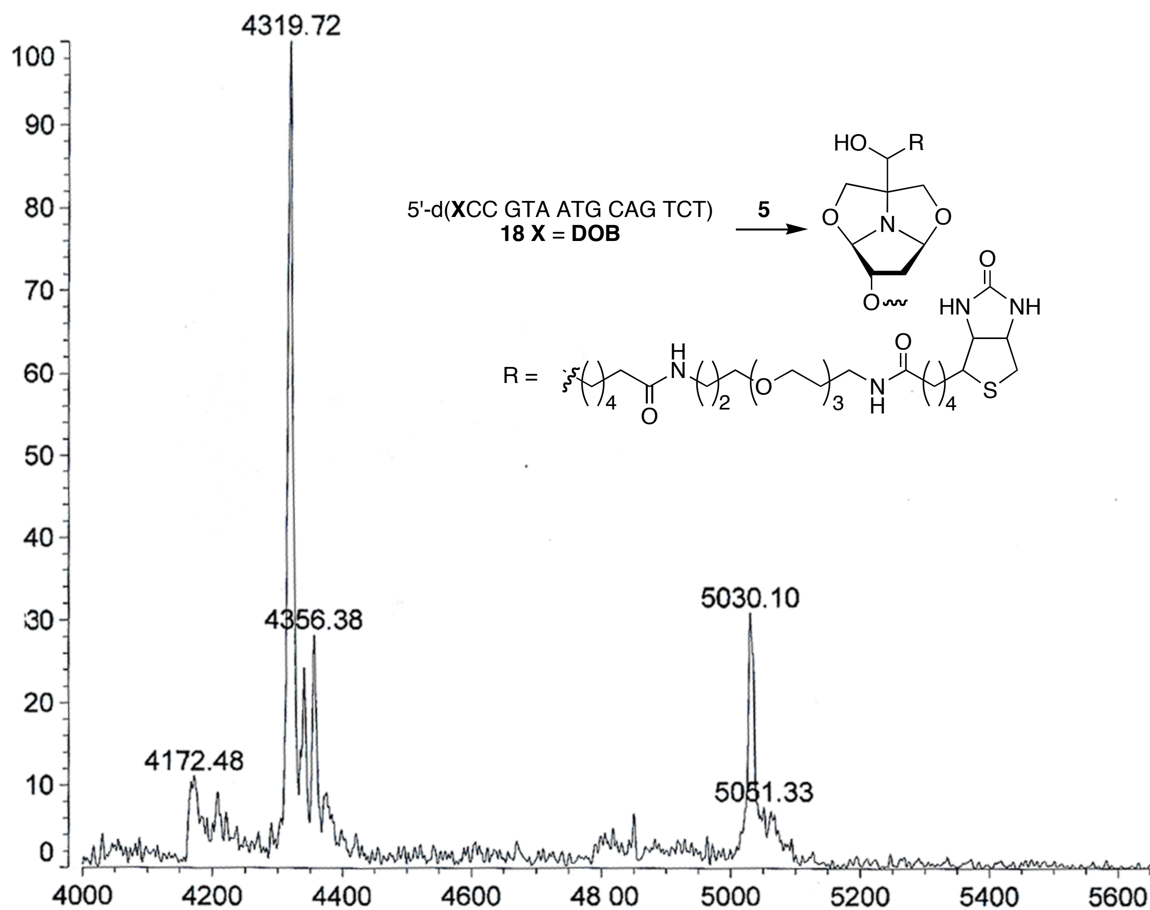


Supporting Information Figure 16. ^{13}C NMR of **5**.

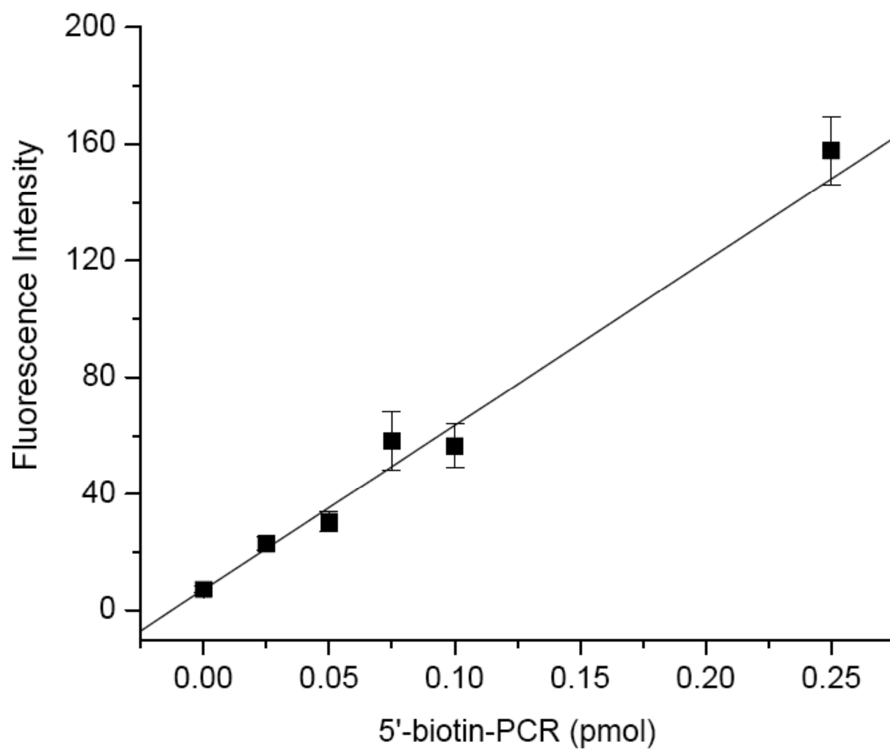




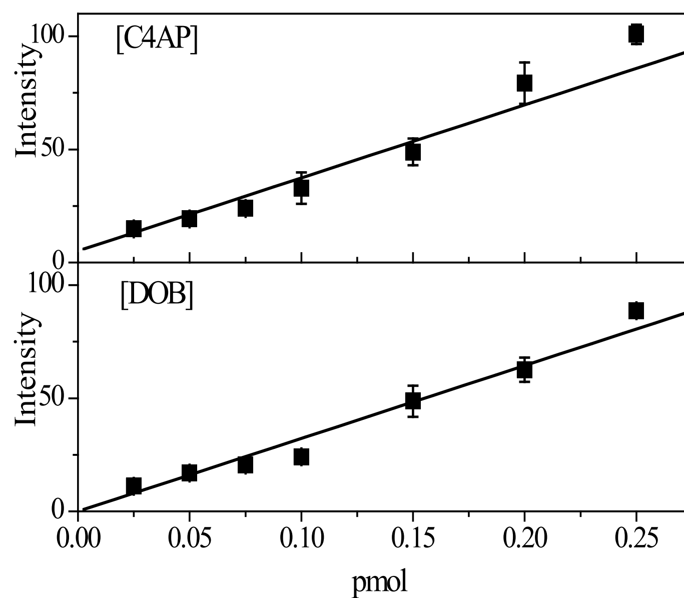
Supporting Information Figure 17. MALDI-TOF MS of the adduct formed between **5** and **C4-AP** in **17** (calc'd. m/z = 5095.32).



Supporting Information Figure 18. MALDI-TOF MS of the adduct formed between **5** and **DOB** in **18** (calc'd. *m/z* = 5029.15). The ion with *m/z* = 4319.72 is unreacted oligonucleotide.



Supporting Information Figure 19. Sample fluorescence calibration curve for biotinylated adducts.



Supporting Information Figure 20. Fluorescence intensity as a function of adduct quantity (A) **C4-AP** (**1**), (B) **DOB** (**2**).