Supporting Information: Use of Fluorescent Sensors to Determine that 2-Deoxyribonolactone is the Major Alkali-Labile Deoxyribose Lesion Produced in Oxidatively Damaged DNA

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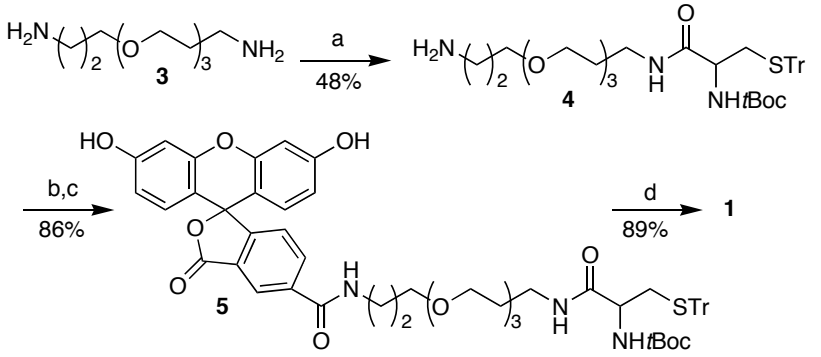
General Methods. Reactions were carried out under Argon using dry solvent, unless otherwise noted. *S*-Trityl-*N*-tBoc-cysteine was purchased from Novabiochem. Alexa Fluor® 488

hydroxylamine was purchased from Molecular Probes (Invitrogen, Cat. No. A-30629). Oligonucleotides were synthesized on an Applied Biosystems model 394 DNA/RNA synthesizer. 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 (MALDI) spectra were collected on a DE-STR mass spectrometer (UC-riverside). HRMS (FAB) spectra were collected on a VG70S magnetic sector mass spectrometer. LRMS (MALDI) spectra were collected on a Kratos analytical KOMPACT SEQ mass spectrometer. 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. T4 polynucleotide kinase and UDG were obtained from New England Biolabs. [γ-^{32}P]ATP was purchased from Amersham Pharmacia Biotech. Calf thymus DNA was purchased from Sigma-Aldrich (Cat. No. D-8661; Lot No. 085K6123). Quantification of radiolabeled oligonucleotides was carried out using a Molecular Dynamics Storm 840 Phosphorimager equipped with ImageQuant Version 5.1 software. All photolyses of oligonucleotides were carried out in Pyrex tubes in a Rayonet photoreactor (RPR-100) fitted with 16 lamps having an output maximum at 350 nm. Ninety-six well flat-bottom assay plates (black) were purchased from Corning (Fisher, Cat. No. 07200509).

Supporting Information Scheme 1.



a) S-trityl-N-*t*Boc-cysteine, EDCI, HOBt, DMF b) 5-Carboxydiacetylfluorescein, PyBOP, DIPEA, DMF c) NaOMe, MeOH d) TFA.

Preparation of 4. To a DMF solution (10 mL) of *S*-trityl-*N*-tBoc-cysteine (0.82 g, 1.76 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) (372 mg, 1.93 mmol), and 1-hydroxy-benzotrizaole (HOBt) (262 mg, 1.93 mmol), was added 4,7,10-trioxa-1,13-tridecane diamine (**3**, 3.89 g, 17.6 mmol). After stirring at room temperature for 3 h, the organic solvent was removed under vacuum and the residue was partitioned between ethyl acetate (200 mL) and water (100 mL). The organic layer was washed with H_2O (100 mL × 2), dried over anhydrous Na_2SO_4, and concentrated. Flash chromatography (CH_3OH:CH_2Cl_2:Et_3N; 1:10:0.05) yielded **4** (541 mg, 48%) as a colorless oil. R_f = 0.22 (silica gel, MeOH:CH_2Cl_2 1:5); IR (KBr): ν_{max} 3302, 3057, 2930, 2869, 1710, 1669, 1490, 1446, 1366, 1249, 1168, 1113, 1042, 864, 746, 702 cm^{-1}; ^1H NMR (CD_3OD) δ 7.25-7.49 (m, 15H), 4.13 (t, *J* = 6.0 Hz, 1H,), 3.54-3.70 (m, 14H), 3.26-3.40 (m, 2H), 2.79 (br, 2H), 2.58-2.67 (m, 2H), 1.75-1.83 (m, 4H), 1.51 (s, 9H); ^{13}C NMR (CD_3OD) δ 171.3, 155.7, 144.6, 129.4, 127.8, 126.7, 79.4, 70.3, 70.2, 70.0, 69.9, 69.1, 68.5, 66.6, 53.9, 53.8, 45.7, 38.9, 36.6, 34.3, 32.3, 28.9, 27.7; MS (HRMS-FAB) calcd. for $C_{37}H_{52}N_3O_6S$ ($M+H^+$), 666.3577 found 666.3580.

Preparation of 5. To a DMF solution (5 mL) of 5-carboxydiacetylfluorescein[1] (100 mg, 0.217 mmol), PyBOP (170 mg, 0.33 mmol), and DIPEA (61 μL, 0.37 mmol) was added **4** (145 mg, 0.217 mmol). After stirring at room temperature for 2 h, the organic solvent was removed under vacuum and the residue was partitioned between ethyl acetate (100 mL) and water (50 mL). The organic layer was washed with H_2O (50 mL × 2), dried over anhydrous Na_2SO_4, and concentrated. Flash chromatography (CH_3OH:CH_2Cl_2; 6:100) yielded the mixture of products (diacetyl, monoacetyl, and deacetylated; R_f: 0.48, 0.38, 0.32; MeOH:CH_2Cl_2, 1:10) as a yellow

foam. This mixture was dissolved in a 5 mL of CH_2Cl_2/MeOH (1:1) solution of NaOMe (46 mg, 0.85 mmol) and stirred at room temperature for 8 h. The reaction mixture was then filtrated through celite and concentrated. Flash chromatography (CH_3OH:CH_2Cl_2; 12:100) yielded the desired product as a yellow-brown foam (148 mg, 86%). R_f = 0.32 (silica gel, MeOH:CH_2Cl_2 1:10); IR (KBr): ν_{max} 3374, 2929, 1747, 1644, 1620, 1544, 1506, 1448, 1367, 1249, 1179, 1124, 841 cm^{-1}; ^1H NMR (CD_3OD) δ 8.42 (s, 1H), 8.17 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.16-7.36 (m, 16H), 6.68 (d, *J* = 2.4 Hz, 2H), 6.59 (d, *J* = 8.4 Hz, 2H), 6.51 (dd, *J* = 8.4, 2.4 Hz, 2H), 3.90 (t, *J* = 6 Hz, 1H), 3.29-3.63 (m, 16H), 2.98 (m, 2H), 2.44-2.48 (m, 2H), 1.88-1.91 (m, 2H), 1.67-1.70 (m, 2H), 1.40 (s, 9H); ^{13}C NMR (CD_3OD) δ 171.4, 166.8, 153.0, 144.5, 136.5, 133.7, 129.3, 128.9, 127.6, 126.5, 124.6, 123.7, 112.9, 109.8, 102.3, 79.5, 70.11, 70.06, 69.9, 69.8, 68.8, 68.4, 66.5, 53.8, 37.6, 36.5, 34.1, 29.0, 28.8, 27.3; HRMS (MALDI-TOF) calcd. for $C_{58}H_{60}N_3NaO_{12}S^+$ ($M+Na^+$) 1046.3907, found 1046.3868.

Preparation of 1. In a 10 mL round-bottomed flask, **5** (50 mg, 0.05 mmol) and triisopropylsilane (32 μL, 5 equiv.) were dissolved in argon-bubbled TFA (5 mL) and stirred at room temperature for 30 min. The solvent was removed under vacuum and the residue was dissolved in argon-bubbled H_2O (50 mL). The aqueous layer was washed with argon-bubbled ether (50 mL × 3). Lyophilization yielded **1** as a bright-yellow solid (35.2 mg, 89%). IR (KBr): ν_{max} 3370, 3083, 2931, 1676.4, 1638, 1602, 1544, 1459, 1390, 1281, 1203, 1135 cm^{-1}; ^1H NMR (CD_3OD) δ 8.45 (s, 1H), 8.20 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.30-7.34 (d, *J* = 8.0 Hz, 1H), 6.58-6.75 (m, 6H), 3.97 (s, 1H), 3.49-3.66 (m, 16H), 2.95-3.01 (m, 2H), 1.91-1.94 (m, 2H), 1.76-1.78 (m, 2H); ^{13}C NMR (CD_3OD) δ 165.3, 151.7, 135.1, 132.3, 127.6, 111.6, 100.7, 68.6, 68.3, 68.3,

67.3, 66.9, 53.2, 52.2, 36.0, 35.4, 27.4, 27.3, 23.3; HRMS (FAB) calcd. for $C_{34}H_{40}N_3O_{10}S^+$ ($M+H^+$) 682.24344, found 682.24322.

Analysis of reactions of 1 with DNA containing L, AP, and C4-AP by denaturing PAGE. Lesion containing DNA duplexes **6a**-**c** (0.5 pmol, 20,000 cpm adjusted by addition of 5'-[^{32}P] end-labeled oligomers during hybridization) in 20 mM phosphate buffer (pH 7.0) and 100 mM NaCl, were prepared as previously described.[2] For reactions with **1** or **2**, samples were transferred to eppendorf tubes and the aqueous solutions were removed under vacuum. The residues were redissolved in 8 μL of a mixture containing **1** (62.5 mM) and dithiothreitol (DTT, 31.25 mM), followed by addition of 375 mM NaOH (1 μL) and 500 mM N, N'-dimethylethylenediamine (DMEDA, 1 μL). The reaction mixtures were vortexed and incubated at 37°C for 2 h. The stabilities of adducts formed between **1** and a given lesion was determined by heating at 90°C for 20 min. The reactions were precipitated from NaCl (0.2 M) and EtOH at -80°C. After pelleting by centrifugation, removal of the supernatant, and drying, the residues were resuspended in formamide loading buffer. Analytical oligonucleotide separations were carried out using 20 % polyacrylamide denaturing gel [5% crosslink, 45% urea (w/w)].

The average adduct yield was determined by measuring the percentage yield of adduct in each individual reaction (3 samples) after accounting for DNA that was not converted to the lesion, and for lesion that was adventitiously cleaved. Adventitious cleavage was determined by measuring the amount of cleavage in a control lane. The extent of conversion of the precursor to the lesion was determined by treating a photolyzed sample with NaOH (0.1 M, 37°C, 20 min). The yield was determined by dividing the fraction of DNA that forms adduct by the fraction of DNA available for reaction. The latter was determined by subtracting the fraction of unconverted

material from the fraction of uncleaved material, as defined above. The corrected adduct yields were 17.5 ± 2.2% for L and 50.0 ± 6.7 % for AP, respectively.

Preparation of calf thymus DNA. Calf thymus DNA (200 μg) was treated with 0.1 M NaOH (200 μL) at 37°C for 20 min and precipitated from NaCl (0.2 M) and EtOH at -80°C. DNA was resuspended in 100 mM phosphate buffer (pH 7). The solution was heated at 90°C for 10 min, slowly cooled to room temperature, and incubated at 4°C for 16 h. The concentrations of DNA samples were determined by UV (20 g^{-1}•L•cm^{-1}).

Fluorescence detection of L and AP in CT DNA subjected to γ-radiolysis or Fe^{II}•EDTA. Samples were evaporated to dryness on a Speed-vac prior to reaction with **1** or **2**. For detection of L, the samples were treated as described above. For detection of AP, the samples were resuspended in water (5 μL) and of 1 mM **2** (5 μL). The samples containing **2** were vortexed and incubated at 37°C for 2 h. After addition of water (40 μL), the reaction mixtures were extracted with phenol (50 μL × 2 for L; 50 μL × 1 for AP) and then chloroform (50 μL × 1). The reactions were precipitated from NaCl (0.2 M) and EtOH at -80°C three times. The oligonucleotide pellets were dried and dissolved in 310 μL of PBS buffer (20 mM sodium phosphate, pH 8.0). A portion (300 μL) of the solution was used for the fluorescence measurement. Background fluorescence was eliminated by subtracting of emission intensity obtained from a DNA sample treated with fluorescent probes that had not been subjected to γ-radiolysis or Fe^{II}•EDTA treatment. The fluorescence signals were transformed into absolute amounts of lesions using calibration curves of Alexa Fluor® 488 hydroxylamine **2** for AP, and a 5'-fluorescein (Fl) labeled 24 mer oligonucleotide (5'-Fl-CACTGAATCATGGTCATAGCTGTT-3') for L. The fluorescence

measurements were correct for the adduct yields (eqn. 1) (L: 17.5 ± 2.2 %; AP: 50 ± 6.7 %) determined from denaturing PAGE experiments, as described above. The UV absorbance (260 nm) was measured for each solution (200 μL) after the fluorescence measurement was made.

To calculate the yield, the following equation was used:

$$\text{Yield}\left(\frac{pmol}{A_{260}}\right) = \frac{[(F_s - A)/B](V)(10^{-3})/(Yield\%)}{(A_{260})_s} - \frac{[(F_b - A)/B](V)(10^{-3})/(Yield\%)}{(A_{260})_b}$$

F_s: Fluorescence intensity of the reaction sample

F_b: Fluorescence intensity of the background sample

A: Y-intercept of the calibration curve

B: Slope of the calibration curve

V: Sample volume in each well (300 μL)

Yield % : The adduct yield determined by PAGE as described above.

$(A_{260})_s$: Absorbance of the reaction sample at 260 nm

$(A_{260})_b$: Absorbance of the background sample at 260 nm

γ-Radiolysis of calf thymus DNA. Calf thymus DNA (100 μg) in 100 μL of 20 mM phosphate buffer (pH 7.0) in Pyrex glass tubes was subjected to γ-radiolysis (^{137}Cs) at different doses (25-500 Gy, 25 Gy/min). For anaerobic experiments, 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 (10 μL, 10 μg DNA) were transferred to eppendorf tubes, dried under vacuum, and subjected to the fluorescence analysis described above.

Reaction of calf thymus DNA with Fe^{II}-EDTA. Ferrous solutions (20 mM) were freshly prepared using ferrous ammonium sulfate. A solution of EDTA was made up at a concentration

10 mM (pH 8.0) and stored at 4°C. Fe^{II} and EDTA were mixed together at a molar ratio 1:2 just prior to carrying out the reactions. Calf thymus DNA (50 μg) was treated with 100-500 μM of Fe^{II}•EDTA complex (Fe^{II} : EDTA 1 : 2) and 8.8 mM H_2O_2 in 50 μL of 20 mM phosphate buffer (pH 7) at room temperature for 30 min. The reactions were quenched with 1 M thiourea (5 μL). A portion of the DNA samples (11 μL, 10 μg DNA) were transferred to eppendorf tubes, precipitated from NaCl (0.2 M) and ethanol at –80°C and subjected to the fluorescence analysis described above.

Effect of mannitol on γ-radiolysis of calf thymus DNA. Calf Thymus DNA (1.5 μg) in 10 μL of 20 mM phosphate buffer (pH 7.0) in eppendorf tubes was irradiated with 500 Gray of ^{137}Cs in the presence of 0, 1 mM, or 10 mM mannitol. DNA was precipitated from NaCl (0.2 M) and ethanol at –80°C and subjected to fluorescence analysis as described above.

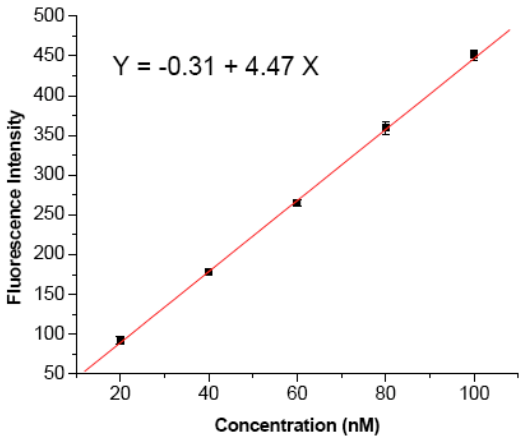
MALDI-TOF MS analysis of reaction of 1 with 2-deoxyribonolactone in DNA 7. Oligonucleotide 7 (0.5 nmol) was prepared as previously described.[2] After evaporating the sample to dryness on a Speed-Vac, the reaction was carried out on a 50 μL solution containing 1 (50 mM), DTT (25mM), NaOH (37.5 mM), and DMEDA (50 mM) at 37°C for 2 h. The reaction mixture was passed through a Sephadex G-25 column and then precipitated from NH_4OAc (2.5 M, pH 7.3) and EtOH. The MS sample was prepared by combining analyte solution (1 μL) with 2' 4' 6'-trihydroxyacetophenone matrix solution (1 μL of a 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 mass spectrometer was calibrated

with two standard oligonucleotides: 5'-d(TCGCTGT), *m/z*: 2087.4; and 5'-d(AGGCGTTCAACGGCTCTG), MW: 5515.6.

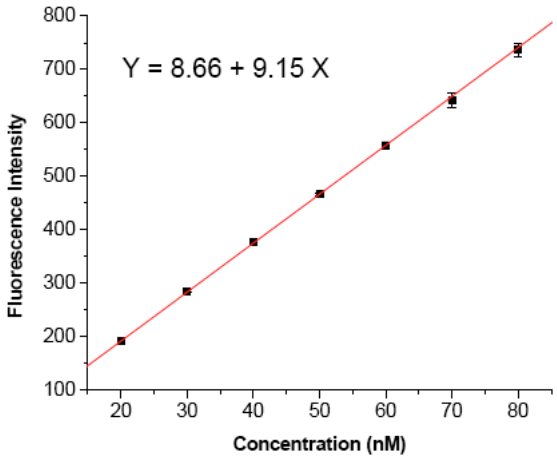
References:

[1] A. H. Coons, M. H. Kaplan, *J. Exp. Med.* **1950**, *90*, 1-13.

[2] K. Sato, M. M. Greenberg, *J. Am. Chem. Soc.* **2005**, *127*, 2806-2807.



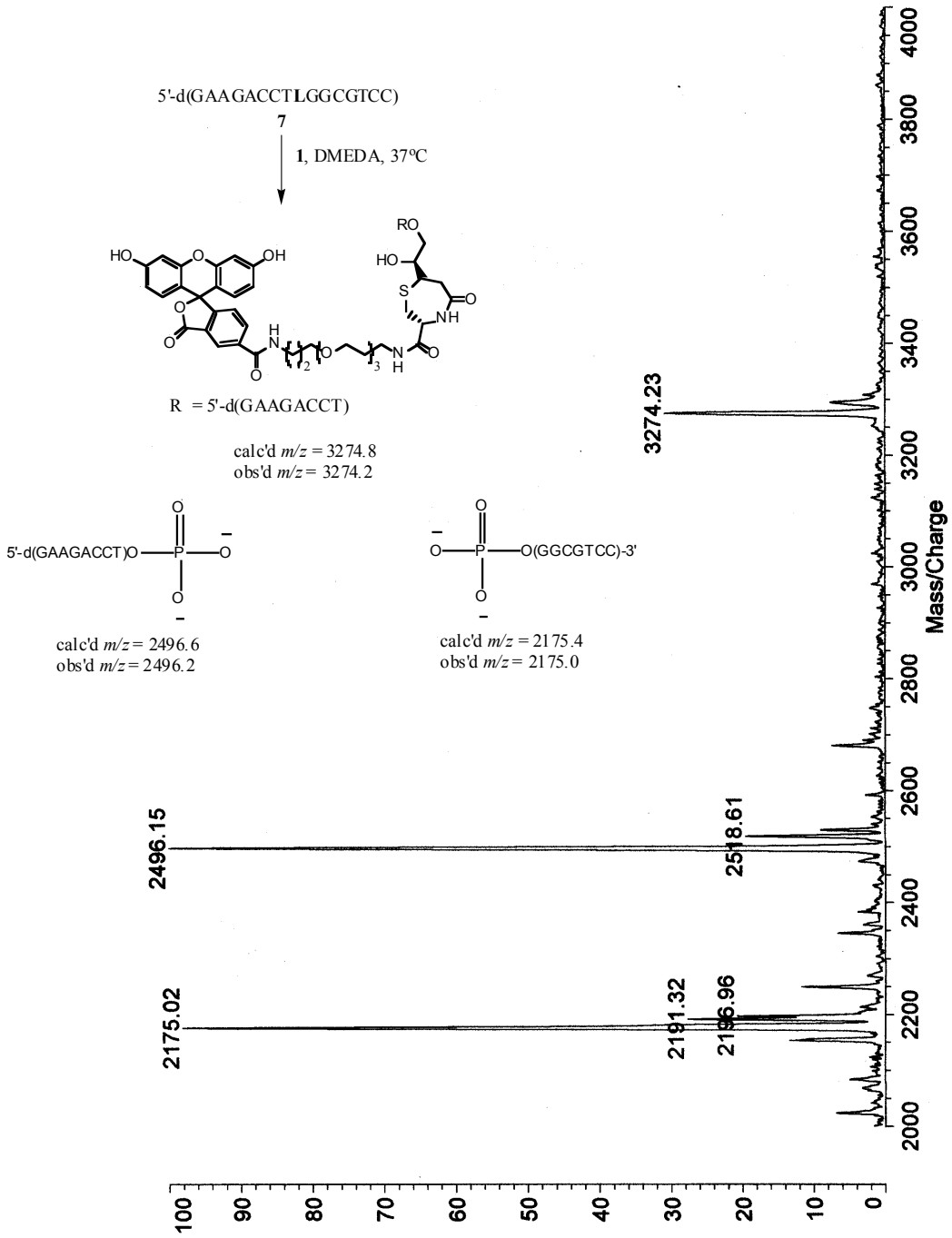
Supporting Information Figure 1. Representative calibration curve of 5'-Fl-CACTGAATCATGGTCATAGCTGTT-3' in 20 mM phosphate buffer, pH 8. Parameters for fluorescence measurement: λ_{EX} = 480 nm, λ_{EM} = 520 nm, Slit = 10 nm, PMT = 600 V.



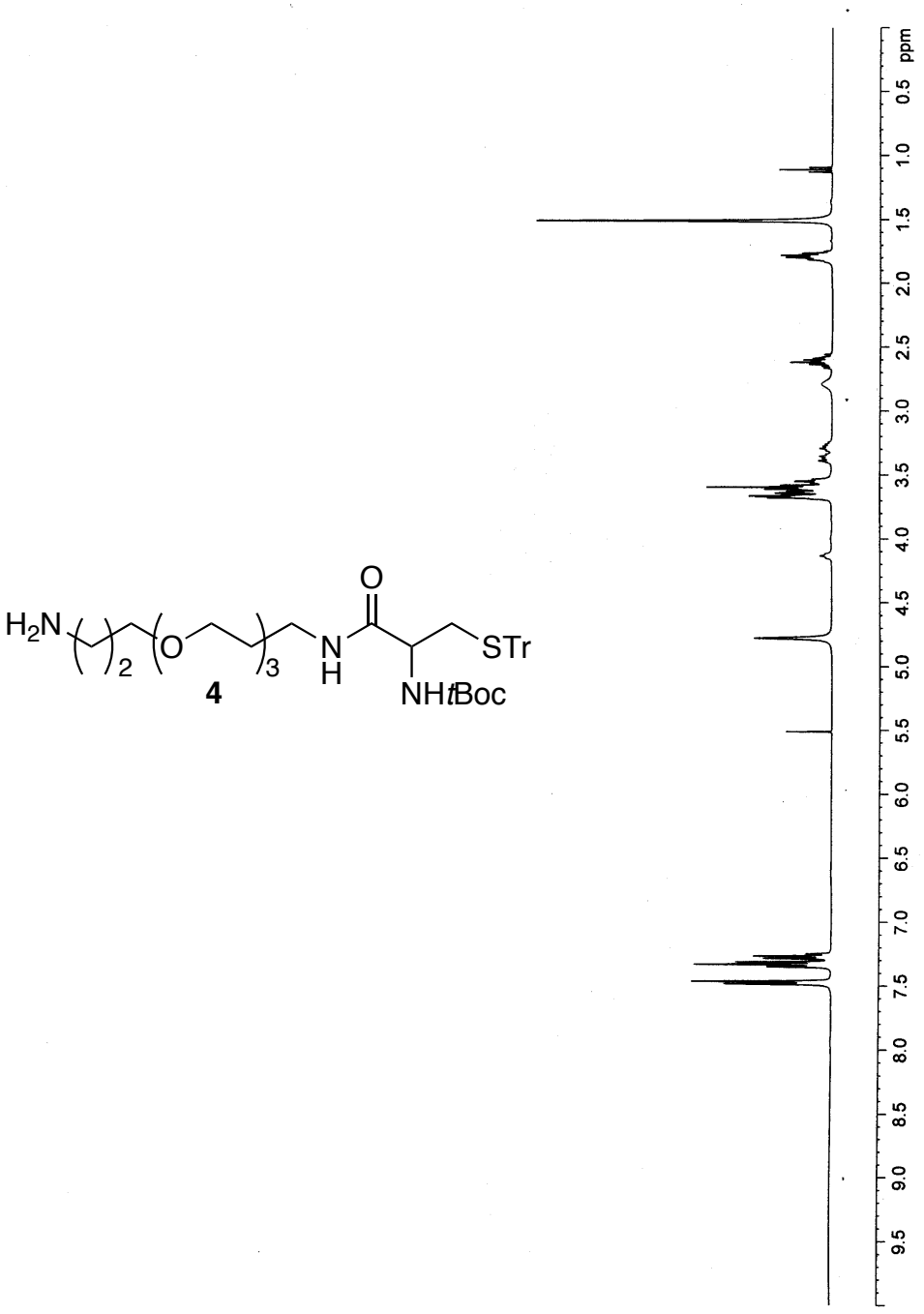
Supporting Information Figure 2. Representative calibration curve of Alexa Fluor® 488 hydroxylamine (**2**) in 20 mM phosphate buffer, pH 8. Parameters for fluorescence measurement: λ_{EX} = 485 nm, λ_{EM} = 520 nm, Slit = 10 nm, PMT = 600 V.

Supporting Information Figure 3. Representative adduct formation between **2** and AP containing DNA.

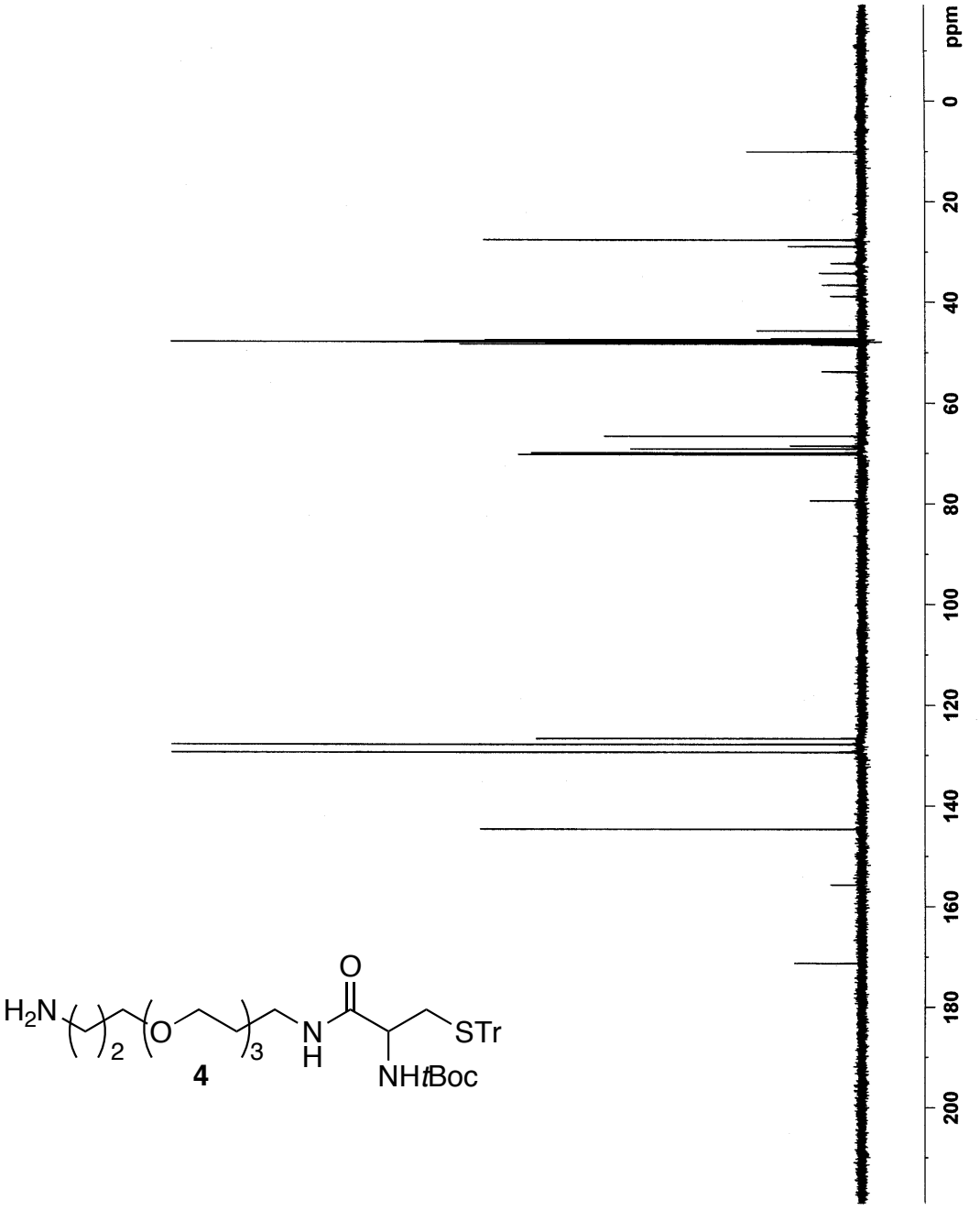
Supporting Information Figure 4. Representative adduct formation between **2** and C4-AP containing DNA.



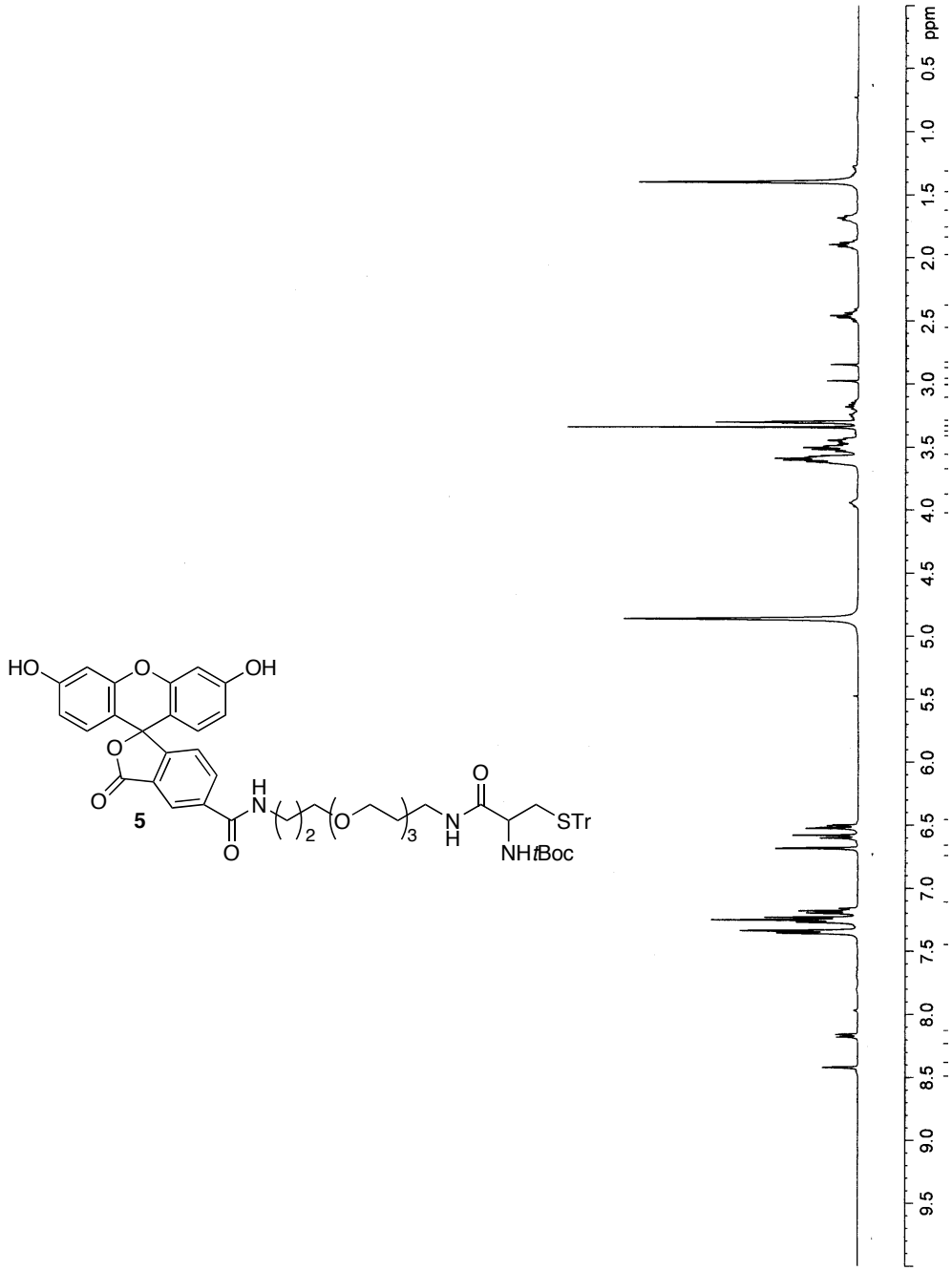
Supporting Information Figure 5. MALDI-TOF MS analysis of the reaction between **7** and **1**.



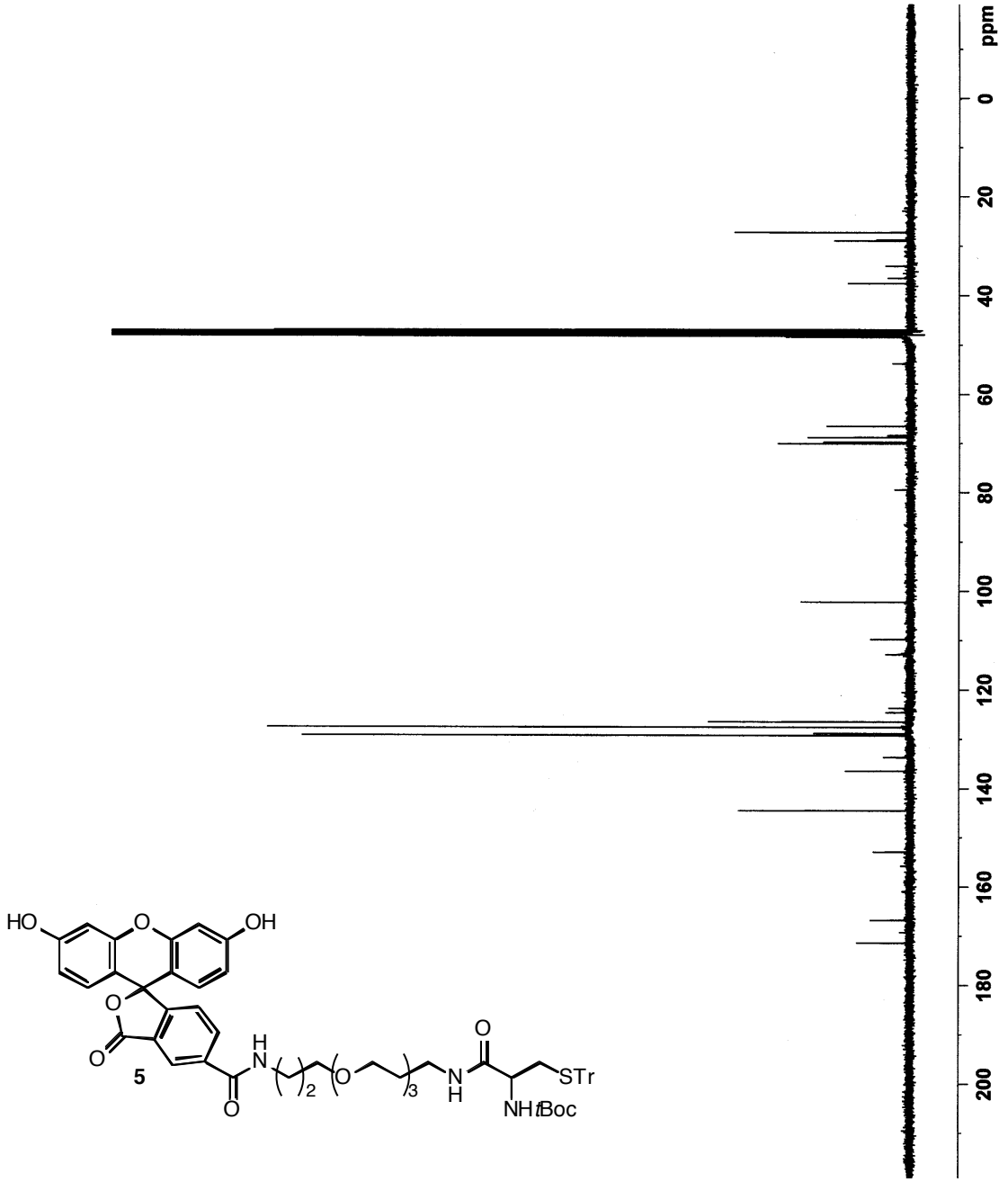
Supporting Information Figure 6. ^{1}H NMR spectrum of **4** in CD_3OD.



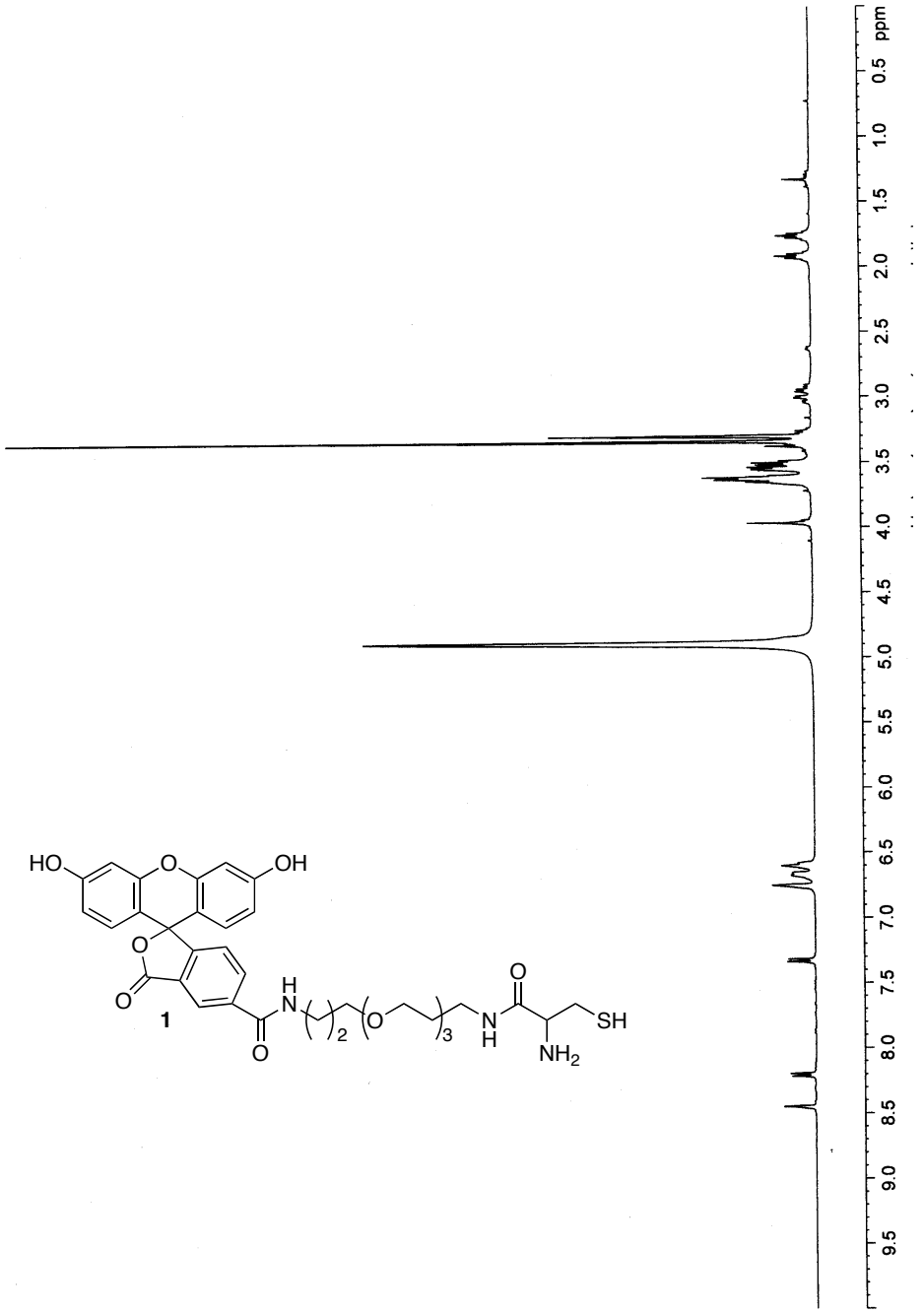
Supporting Information Figure 7. ^{13}C NMR spectrum of **4** in CD_3OD.



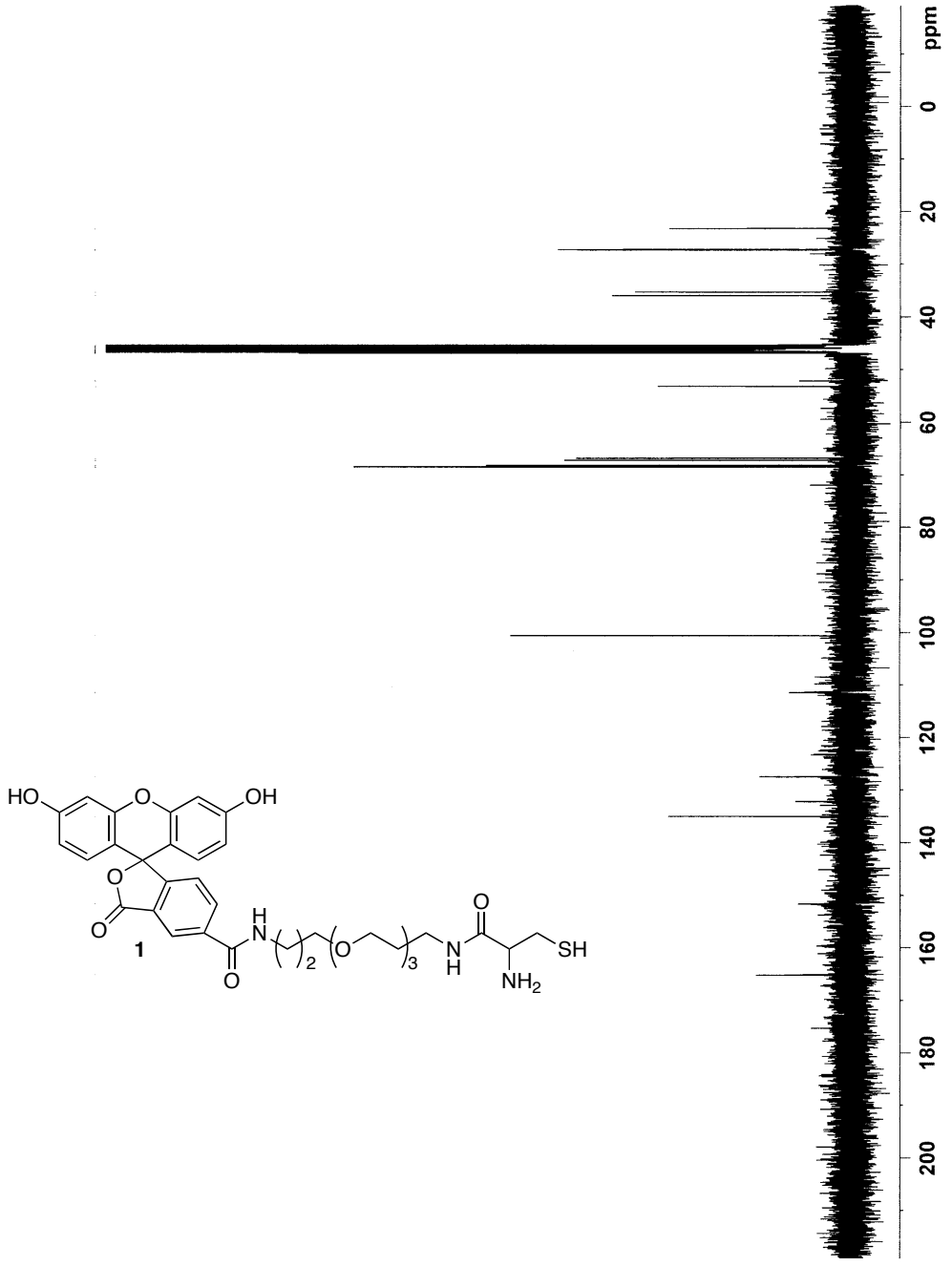
Supporting Information Figure 8. ^{1}H NMR spectrum of **5** in CD_3OD.



Supporting Information Figure 9. ^{13}C NMR spectrum of **5** in CD_3OD.



Supporting Information Figure 10. ^{1}H NMR spectrum of **1** in CD_3OD.



Supporting Information Figure 11. ^{13}C NMR spectrum of **1** in CD_3OD.