Supporting Information: Interstrand Cross-link Formation in Duplex and Triplex DNA by Modified Pyrimidines.

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Contents:

General Methods. Unless otherwise specified, chemicals were purchased from Aldrich or Fisher Scientific and enzymes was obtained from New England Biolabs. Oligonucleotides were synthesized

via standard automated DNA synthesis techniques using an Applied Biosystems model 394 instrument. The Pac-dA and ^{i}Pr-Pac-dG phosphoramidites were employed for the synthesis of oligonucleotides containing PhSedT. Pivaloyl anhydride/2,6-lutidine/THF (1:1:8) was used as capping reagent and 1 M t-butyl-hydroperoxide in toluene was used as oxidizing reagent.[1] The oxidation time is 40 s and the capping time is 25 s. Deprotection of the nucleobases and phosphate moieties as well as cleavage of the linker were carried out under mild deprotection conditions (28% aq. NH_3, room temperature, 3 h). Oligonucleotides were purified by 20% denaturing polyacrylamide gel electrophoresis and characterized by ESI-MS. Oligonucleotides containing **1** or **5** were subjected to additional purification by reversed-phase HPLC on a RP-C18 column (Varian, Microsorb-MV 100-5 C18 250 × 4.6 mm). Monitoring was carried out at 260 nm. The peak of interest was collected using the following gradient conditions: 0-5 min 0-2% B in A, 5-15 min 2-12% B in A, 15-50 min 12-25% B in A, 50-60 min 25-80% B in A, 60-65 min 80-0% B in A, 65-80 min 100% A, at a flow rate 1.0 mL/min) [A: 0.05 M (Et_3NH)OAc (pH 7.0)/MeCN 95:5; B: 0.05 M (Et_3NH)OAc (pH 7.0)/MeCN 50:50]. Radiolabeling was carried out according to the standard protocols.[2] [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. 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. ESI-MS spectra were collected using a Thermoquest LCQ-Deca Ion Trap instrument.

3',5'-bis-O-Acetyl-5-phenylselenylmethyl-N4-triazoyl-2'-deoxyuridine (7). Compound **6**[3] (2.5 g, 5.2 mmol) and 1,2,4-triazole (7.25 g, 0.105 mol) were suspended in anhydrous CH_3CN (50 mL). Et_3N (16 mL) and phosphorous oxytrichloride (2.2 mL, 24.0 mmol) were added. The reaction mixture was stirred at r.t. for one hour, diluted with ethyl acetate (100 mL) and washed with saturated $NaHCO_3$ and brine. The organic solution was dried over anhydrous Na_2SO_4, concentrated,

and the residue was submitted to flash chromatography (column 6 × 12 cm, EtOAC/Hexanes, 1:1 → 4:1) yielding a colorless foam (2.3 g, 83 %). ^{1}H NMR ($CDCl_3$): δ 1.72-1.79 (m, 1H), 2.04, 2.10 (2s, 6H), 2.71-2.76 (m, 1H), 4.10-4.28 (m, 3H), 4.40 (dd, J = 16.0, 4.0 Hz, 2H), 5.05-5.08 (m, 1H), 6.18 (dd, J = 4.0, 8.0 Hz, 1H), 7.24-7.44 (m, 5H), 7.58 (s, 1H), 8.15(s, 1H), 9.29 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 169.6, 156.3, 153.3, 152.6, 144.6, 144.5, 135.0, 128.8, 128.1, 127.8, 107.6, 86.9, 82.6, 73.2, 62.8, 38.2, 26.7, 20.3; IR: 3471, 3142, 3056, 2954, 2244, 1732, 1682, 1578, 1504, 1454, 1384, 1328, 1234, 1122 cm^{-1}; HRMS [M-H$^+$] Calc. 534.0892, found 534.0913.

5-Phenylselenylmethyl-2'-deoxycytidine (8). Compound **7** (2.2 g, 4.12 mmol) was dissolved in dioxane (45 mL). Aqueous NH_3 (28%, 45 mL) was added and the reaction mixture was stirred at r.t. overnight. After evaporation, the residue was subjected to flash chromatography (column 6 × 12 cm, CH_2Cl_2/MeOH, 9:1 → 4:1). The fractions containing the desired material were collected and evaporated to dryness to give **8** as colorless solid (1.42 g, 87%). ^{1}H NMR (CD_3OD): δ 1.58-1.66 (m, 1H), 2.10-2.18 (m, 1H), 3.54-3.68 (m, 2H), 3.79-3.90 (m, 3H), 4.12-4.18 (m, 1H), 6.11 (t, J = 6.4 Hz, 1H), 7.28-7.49 (m, 6H), 8.32 (s, 2H); ^{13}C NMR (CD_3OD): δ 165.5, 157.7, 140.6, 137.0, 130.3, 129.7, 129.3, 105.1, 88.6, 86.9, 71.7, 62.6, 42.0, 25.2; IR: 3188, 2361, 1641, 1601, 1476, 1087, 1042, 788 cm^{-1}; HRMS [M-H$^+$] Calc. 398.0619, found 398.0621.

N_4-Acetyl-5'-*O*-(4,4'-dimethoxytriphenylmethyl)-5-phenylselenylmethyl-2'-deoxycytidine (9). Into a solution of **8** (853 mg, 2.14 mmol) in anhydrous DMF (8.0 mL), acetic anhydride (240 mg, 2.35 mmol) was added dropwise. The reaction mixture was stirred at r.t. overnight. The solvent was removed under high vacuum at r.t. yielding crude product **9** as a syrup, which was co-evaporated with anhydrous pyridine (three times) and then dissolved in pyridine (8.0 mL). To this solution 4,4'-dimethoxytriphenylmethyl chloride (995 mg, 2.94 mmol) was added in four portions within 4 h, and the mixture was stirred at r.t. overnight. The reaction was quenched by addition of MeOH and the mixture was evaporated to dryness. It was dissolved in CH_2Cl_2 (5.0 mL) and subjected to flash

chromatography (column 4 × 10 cm, elution with EtOAc/Hexanes, 1:2 → 2:1) to give **9** as a colorless foam (792 mg, 50%, two steps). ^{1}H NMR ($CDCl_3$): δ 2.06-2.12 (m, 1H), 2.42 (br., 3H), 2.48-2.55 (m, 1H), 3.31-3.50 (m, 4H), 3.78 (s, 6H), 4.03 (m, 1H), 4.37 (m, 1H), 6.21 (t, J = 8.0 Hz, 1H), 6.82-6.85 (m, 4H), 7.12-7.36 (m, 15H), 7.55 (br., 1H); ^{13}C NMR ($CDCl_3$): δ 171.1, 158.4, 144.1, 135.3, 135.1, 129.8, 129.7, 127.9, 127.8, 126.8, 113.1, 86.5, 64.1, 60.2, 54.9, 30.3, 20.7, 18.8, 13.9, 13.4; IR 3300, 2934, 2361, 1649, 1508, 1252, 1177, 1034, 828 cm^{-1}; HRMS [M-Na+] calc. 764.1845, found 764.1810.

N_4-Acetyl-5'-*O*-(4,4'-dimethoxytriphenylmethyl)-5-phenylselenylmethyl-2'-deoxycytidine 3'-(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite (10). Compound **9** (730 mg, 0.99 mmol) was dissolved in anhydrous CH_2Cl_2 (10.0 mL) under Ar atmosphere. Diisopropylethylamine (313 μL, 1.80 mmol) and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (313 μL, 1.40 mmol) was added. The reaction mixture was stirred at r.t. for 30 min, diluted with CH_2Cl_2 (30 mL), washed with a 5% aqueous $NaHCO_3$ solution, followed by brine. The organic solution was dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was submitted to flash chromatography (column 4 × 10 cm, EtOAc/Hexanes, 1:2 → 2:1) yielding a colorless foam (646 mg, 70 %). ^{1}H NMR ($CDCl_3$): δ 1.05-1.12 (m, 3H), 1.17-1.25 (m, 10H), 1.27-1.63 (m, 1H), 2.08-2.22 (m, 2H), 2.42-2.49 (m, 2H), 2.55-2.68 (m, 3H), 3.25-3.41 (m, 4H), 3.50-3.64 (m, 4H), 3.77 (s, 6H), 3.70-3.86 (m, 1H), 4.16-4.20 (m, 1H), 4.58 (br., 1H), 6.23 (t, J = 8.0 Hz, 1H), 6.82-6.85 (m, 4H), 7.14-7.43 (m, 15H), 7.99 (br., 1H); ^{13}C NMR ($CDCl_3$): δ 171.0, 158.5, 144.0, 135.2, 135.1, 134.5, 130.0, 129.8, 128.9, 128.1, 127.9, 127.8, 127.0, 126.9, 117.4, 117.3, 113.1, 86.6, 85.5, 85.3, 64.1, 58.1, 57.9, 55.0, 43.1, 43.0, 30.4, 24.5, 24.4, 24.3, 20.8, 20.2, 20.0, 14.0, 13.5; IR 2966, 1707, 1654, 1509, 1366, 1252, 1179, 1034 cm^{-1}; ^{31}P NMR ($CDCl_3$): δ 149.2, 148.7; HRMS [M-Na+] calc. 964.2924, found 964.2909.

Preparation of 17 by reaction of 8 with 2'-deoxyinosine. 2'-Deoxyinosine (5.0 mg, 0.02 mmol) and **8** (12 mg, 0.03 mmol) were dissolved in 30 mM KH_2PO_4 buffer (2.0 mL). $NaIO_4$ (6.5 mg, 0.03

mmol) was added. The reaction mixture was incubated at 37 °C overnight and purified by reversed-phase HPLC (RP-18, at 260 nm) using the following gradient: 0-35 min 2-20% MeCN in H_2O, 35-38 min 20-100% MeCN in H_2O, 38-43 min 100% MeCN in H_2O, 43-45 min 100-0% MeCN in H_2O, 45-50 min 100% H_2O, flow rate 0.7 mL/min). The peak with a retention of 22 min was collected and evaporated, yielding **17** as a colorless solid (6.9 mg, 70%). ^{1}H NMR (D_2O): δ 2.26-2.31 (m, 1H), 2.45-2.47 (m, 1H), 2.61-2.63 (m, 1H), 2.83-2.89 (m, 1H), 3.62-3.86 (m, 4H), 4.05-4.06 (m, 1H), 4.17-4.19 (m, 1H), 4.38-4.40 (m, 1H), 4.65-4.67 (m, 1H), 5.15 (s, 2H), 6.21 (t, J = 6.4 Hz, 1H), 6.50 (d, J = 6.8 Hz, 1H), 7.98 (s, 1H), 8.36 (s, 1H), 8.44 (s, 1H); ^{13}C NMR (D_2O + CD_3OD): 165.7, 159.5, 158.2, 149.5, 149.0, 143.6, 142.1, 125.2, 104.2, 89.0, 88.5, 88.0, 86.2, 72.5, 71.8, 63.0, 62.5, 44.4, 41.4, 40.6 cm^{-1}. HRMS [MH^+] calc. 492.1843, found 492.1853.

Preparation of 18 by reaction of 8 with 2'-deoxyguanosine. The reaction was carried out as described above for the formation of **17**. However, we were unable to separate **18** from dG. Consequently, a mixture of **18** and dG were analyzed by ESI-MS.

Enzyme digestion of cross-linked oligonucleotides. Interstrand cross-linked oligonucleotide (38 nmol) was dissolved in 0.1 M Tris-HCl buffer, pH 8.0 (300 μL) and snake-venom phosphodiesterase (8.0 μL, 0.34 U) in a buffer of 110 mM Tris-HCl, pH 8.9, 110 mM NaCl, 15 mM $MgCl_2$, and 50% glycerol was added. The mixture was incubated at 37°C for 1 h. Then, alkaline phosphatase (8.0 μL, 80 U) in 16 μL alkaline phosphatase buffer (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$ and 1 mM dithiothreitol) was added. The reaction mixture was incubated at 37°C for another hour. The digested products were passed through a Microcon cellulose filter (10,000 molecular cutoff, Amicon Inc.) by centrifugation at 12,000 RPM. The filtrate was collected, lyophilized, redissolved in H_2O (500 μL), and analyzed by reversed-phase HPLC (RP-18, at 260 nm) using the following gradient: 0-30 min 2-20% MeCN in water, 30-35 min 20-50% MeCN in water, 35-42 min 50-0% MeCN in water, 42-50 min 0% MeCN in water, at a flow rate 1.0 mL/min.

Interstrand cross-link formation and kinetics study with duplex DNA. The ^{32}P-labelled oligonucleotide (0.5 μM) and its complementary sequence (0.75 μM) were dissolved in 100 mM NaCl and 10 mM KH_2PO_4. The solution was heated to 65 °C, allowed to cool to r.t. over the course of 2 h, and kept at 4 °C for 2 h. $NaIO_4$ (5 mM) reactions of DNA duplexes (30 nM) were carried out in 10 mM potassium phosphate (pH 7.2) and 100 mM NaCl at 37 °C for 6 h. γ-Radiolysis of DNA duplexes was performed in Pyrex tubes using a ^{137}Cs irradiator (Shepherd Mark I Irradiator, dose rate = 23.5 Gy/min). DNA samples (30 nM) were prepared in 10 mM potassium phosphate (pH 7.2) and 100 mM NaCl. After γ-radiation, samples were incubated at 37 °C in the dark for 6 h. For kinetics study, aliquots were taken at the prescribed times and immediately quenched with an equal volume of 95% formamide loading buffer, heated to 80 °C for 2 min, and stored at -80 °C until subjecting to 20% denaturing PAGE analysis.

Interstrand cross-link formation and kinetics study with triplex DNA. *Hybridization*. The ^{32}P-labelled purine strand (0.5 μM) and its complementary pyrimidine strand (0.75 μM) were dissolved in a HEPES buffer (33 mM HEPES pH 5.5, 66 mM KOAc, 100 mM NaCl, 10 mM $MgCl_2$). The solution was heated to 65 °C and allowed to cool to r.t. over the course of 2 h and kept at 4 °C for 2 h. Then, the triplex formation oligonucleotide (TFO) (2.5 μM, 5 eq.) was added. The mixture was incubated at 37 °C for 15 min, r.t. for 30 min, and 4 °C overnight.

Interstrand cross-link formation and kinetics study. $NaIO_4$ (5 mM) reactions of DNA triplexes (30 nM) were carried out in a HEPES buffer (33 mM HEPES pH 5.5, 66 mM KOAc, 100 mM NaCl, 10 mM $MgCl_2$) at r.t. for 6 h. DNA samples (30 nM) were prepared in the HEPES buffer. For kinetics studies, aliquots were taken at the prescribed times and immediately quenched with equal volumes of 95% formamide loading buffer, heated to 80 °C for 2 min, and stored at -80 °C until subjecting to 20% denaturing PAGE analysis.

Stability study of ISC product formed in triplex DNA. After the cross-link reaction, $NaIO_4$ was removed by precipitation. The reaction mixtures (50 μL) were co-precipitated with calf thymus DNA (0.5 M) in 0.3 M NaOAc in the presence of EtOH (150 μL) at -80 °C. The precipitation was repeated once. The pellets were dissolved in H_2O (50 μL) and incubated in 1.0 M piperidine (100 μL) or KH_2PO_4 (pH 7.2) (100 μL) at 90 °C for 25 min. The samples were subjected to 20% denaturing PAGE analysis.

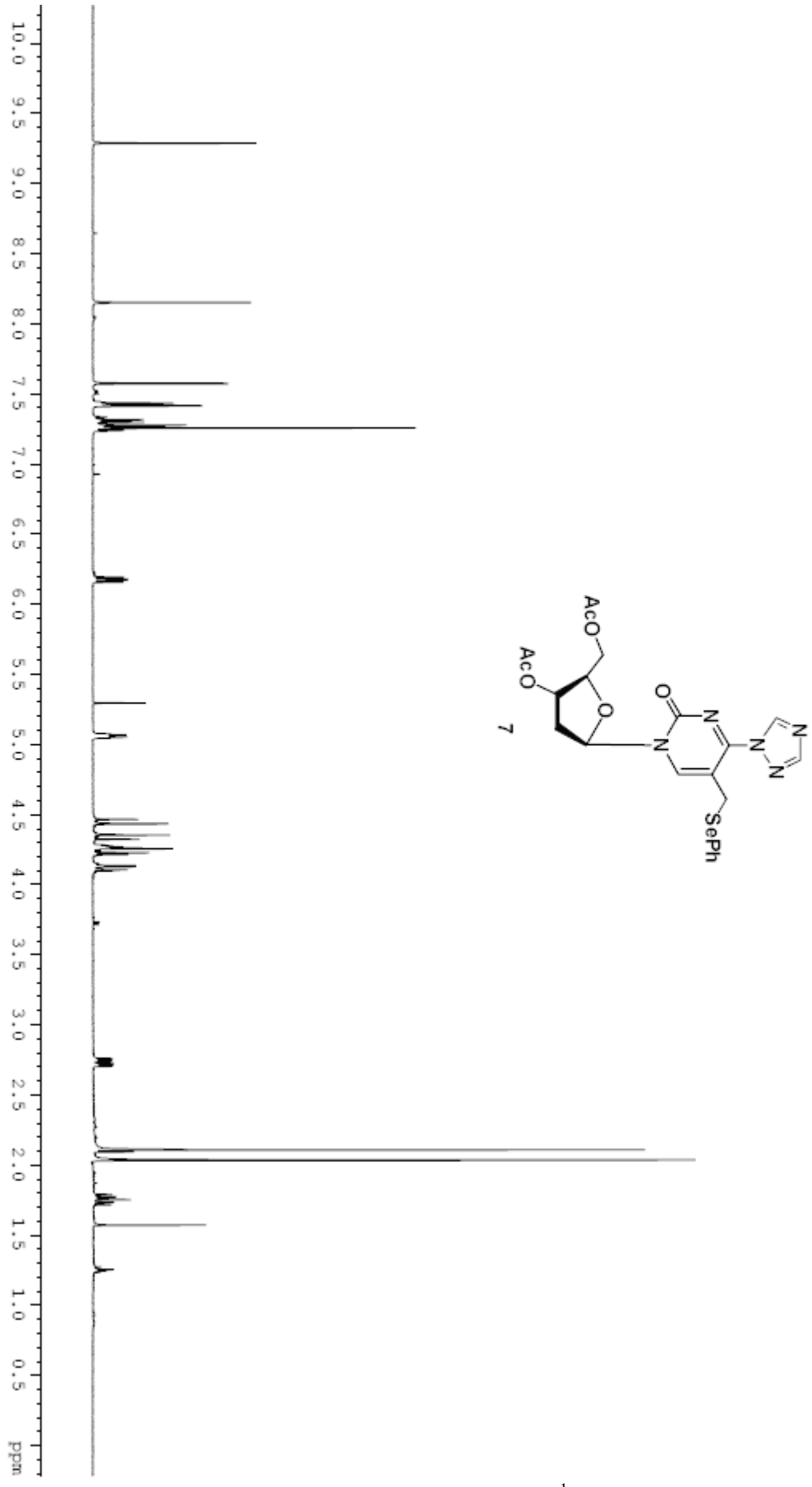
Determination of the rate constant of [2,3]-sigmatropic rearrangement. Compound **1** or **8** (50 μM, 200 μL) in potassium phosphate buffer (pH 7.2) was treated with $NaIO_4$ (2.5 mM) at 25 °C for 1.0 h. The UV absorbance at 270 nm (**2**) or at 280 nm (**12**) was recorded every 10 sec.

NMR monitoring of oxidative transformation of 8 into methide species (12). The oxidation reaction of **8** (90 μM, 540 μL) was carried out in D_2O containing 10 mM potassium phosphate (pH 7.2) and 0.8 mM $NaIO_4$ at 37 °C for 4 h. ^1H NMR was taken 10 min or 4 h after the addition of $NaIO_4$.

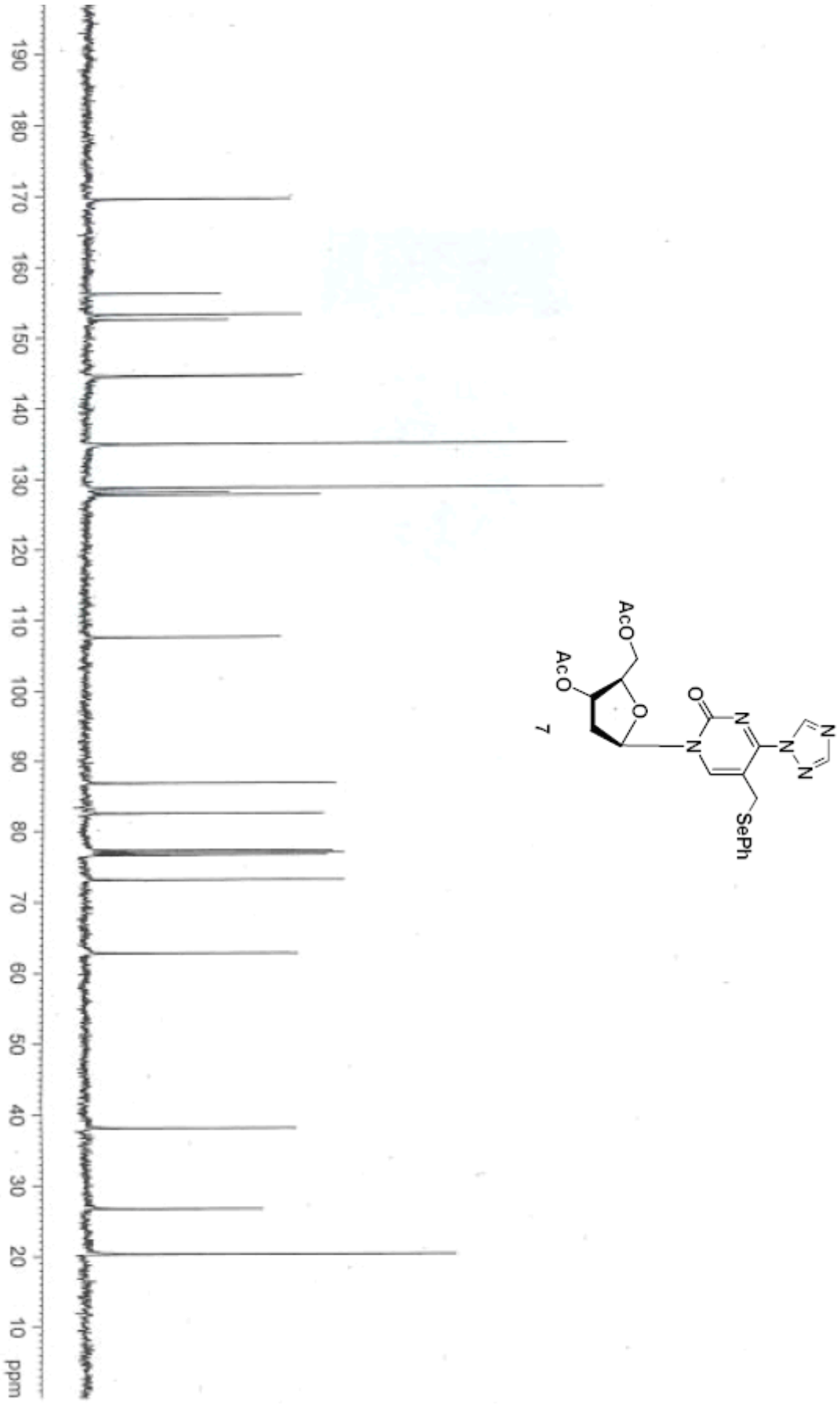
Fe(II)·EDTA analysis of cross-linked DNA. Fe(II)•EDTA cleavage reactions were carried out in 50 μM $(NH_4)_2Fe(SO_4)_2$, 100 μM EDTA, 1 mM sodium ascorbate, 10 mM H_2O_2, 10 mM NaCl, 10 mM potassium phosphate (pH 7.2), for 5 min at 25 ºC, and were quenched with 10 μL of excess thiourea (100 mM). Samples were lyophilized, suspended in 95 % formamide loading buffer and subjected to electrophoresis on a 20 % polyacrylamide gel.

References:

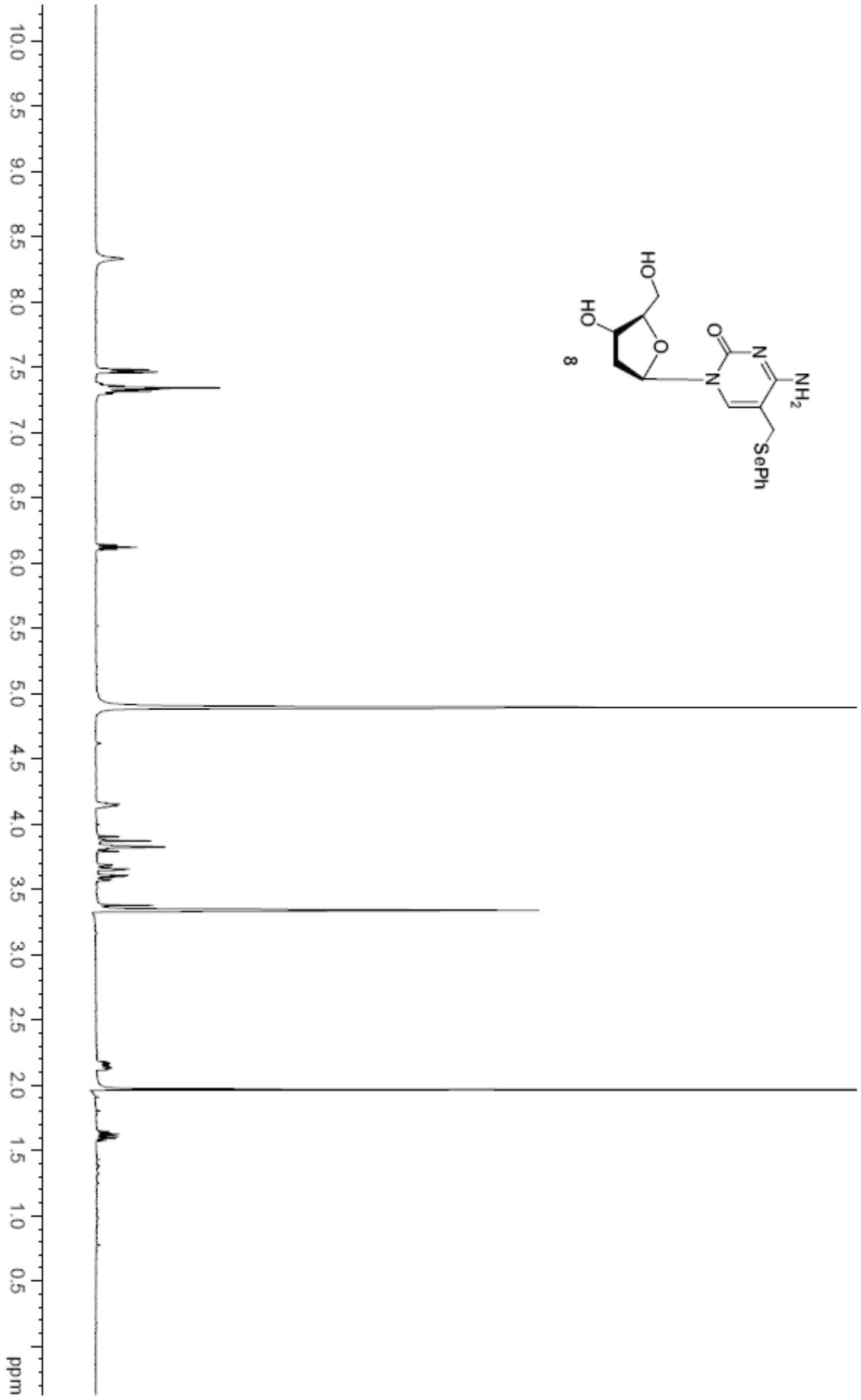
(1) Zhu, Q.; Delaney, M. O.; Greenberg, M. M. *Bioorg. & Med. Chem. Lett.* **2001**, *11*, 1105-1108.
(2) Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning*; Cold Spring Harbor Laboratory, Cold Spring Harbor, NY., 1982.
(3) Hong, I. S.; Ding, H.; Greenberg, M. M. *J. Am. Chem. Soc.* **2006**, *128*, 485-491.



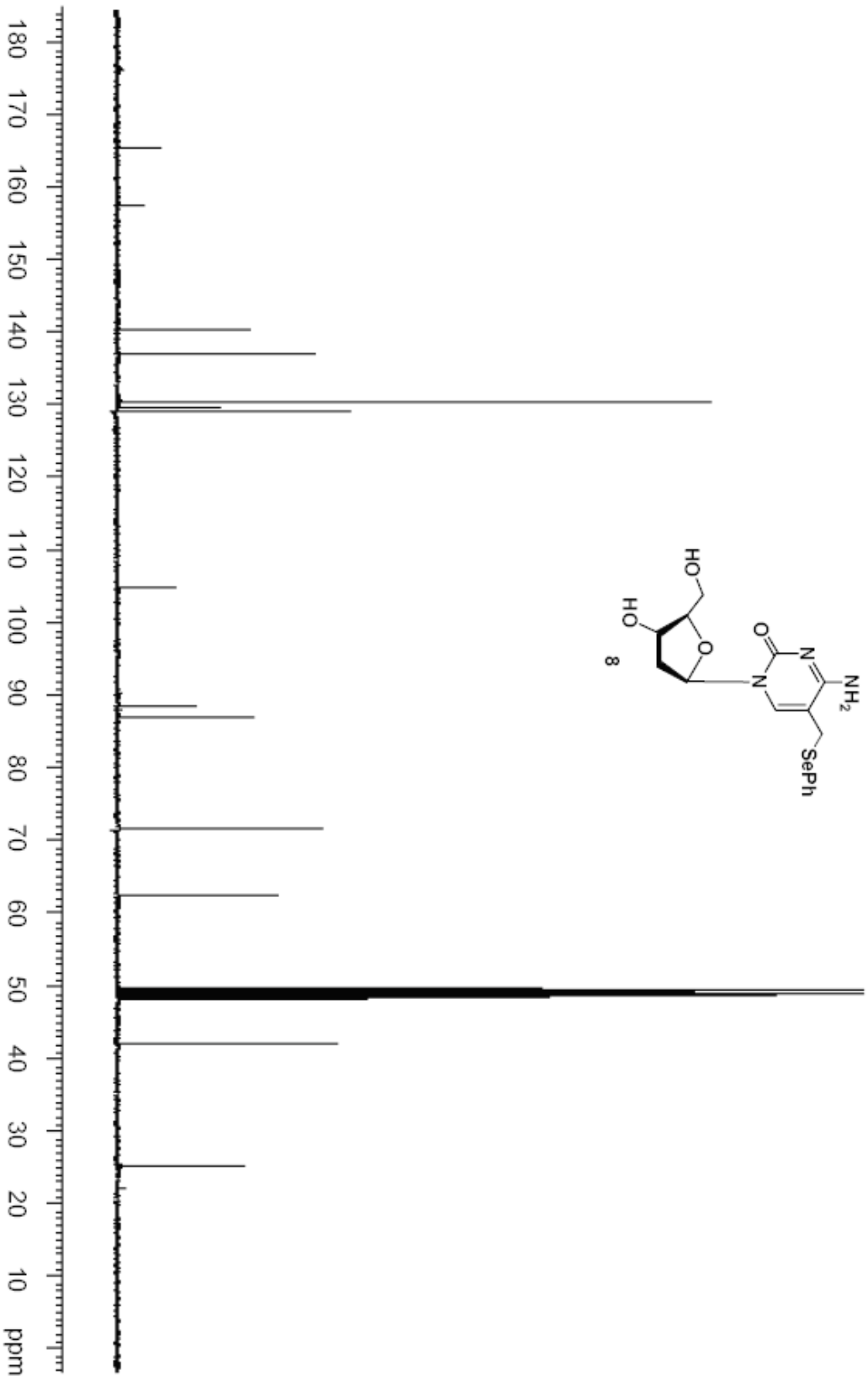
Supporting Information Figure 1. ^{1}H NMR of **7**.



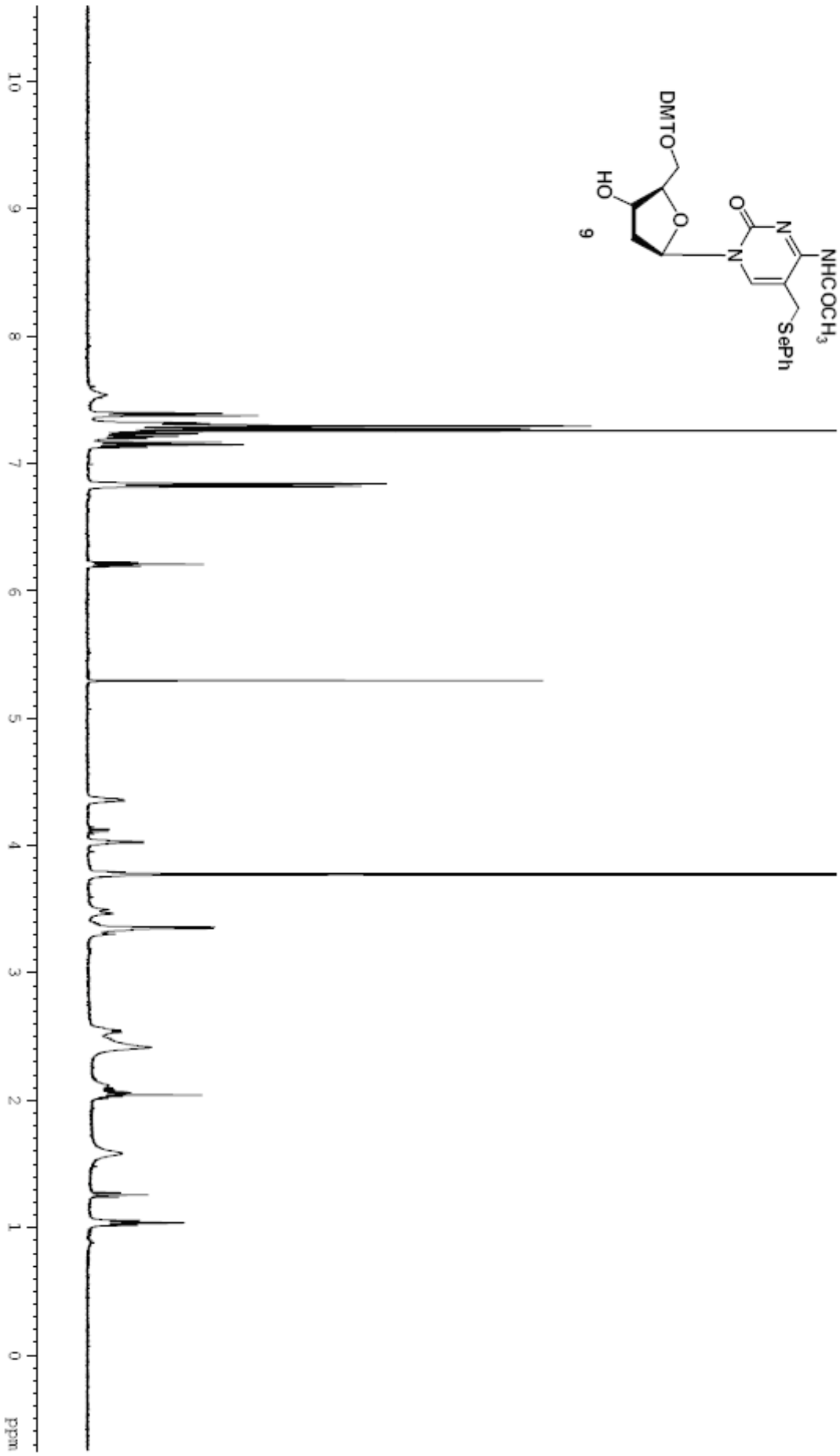
Supporting Information Figure 2. ^{13}C NMR of **7**.



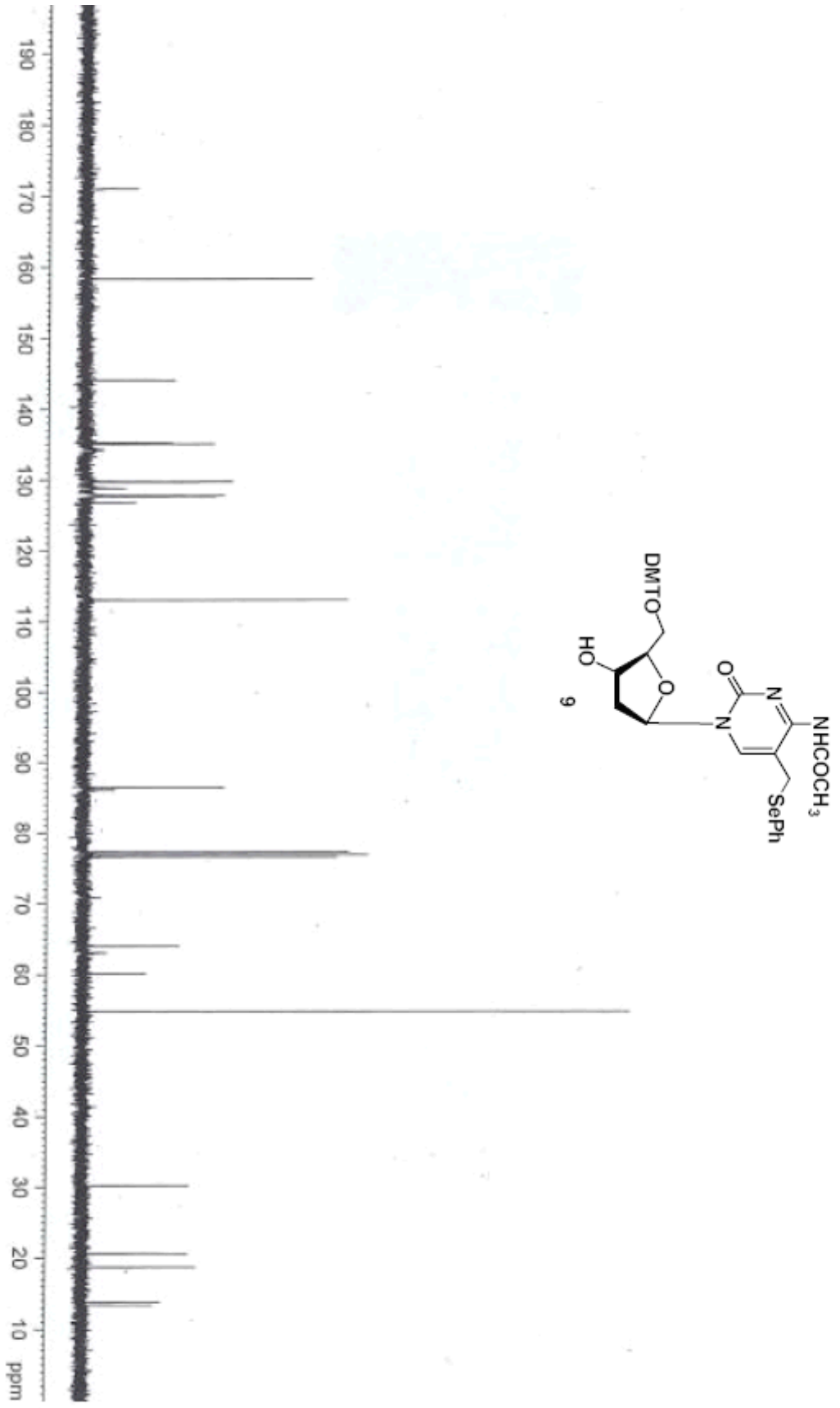
Supporting Information Figure 3. ^{1}H NMR of **8**.



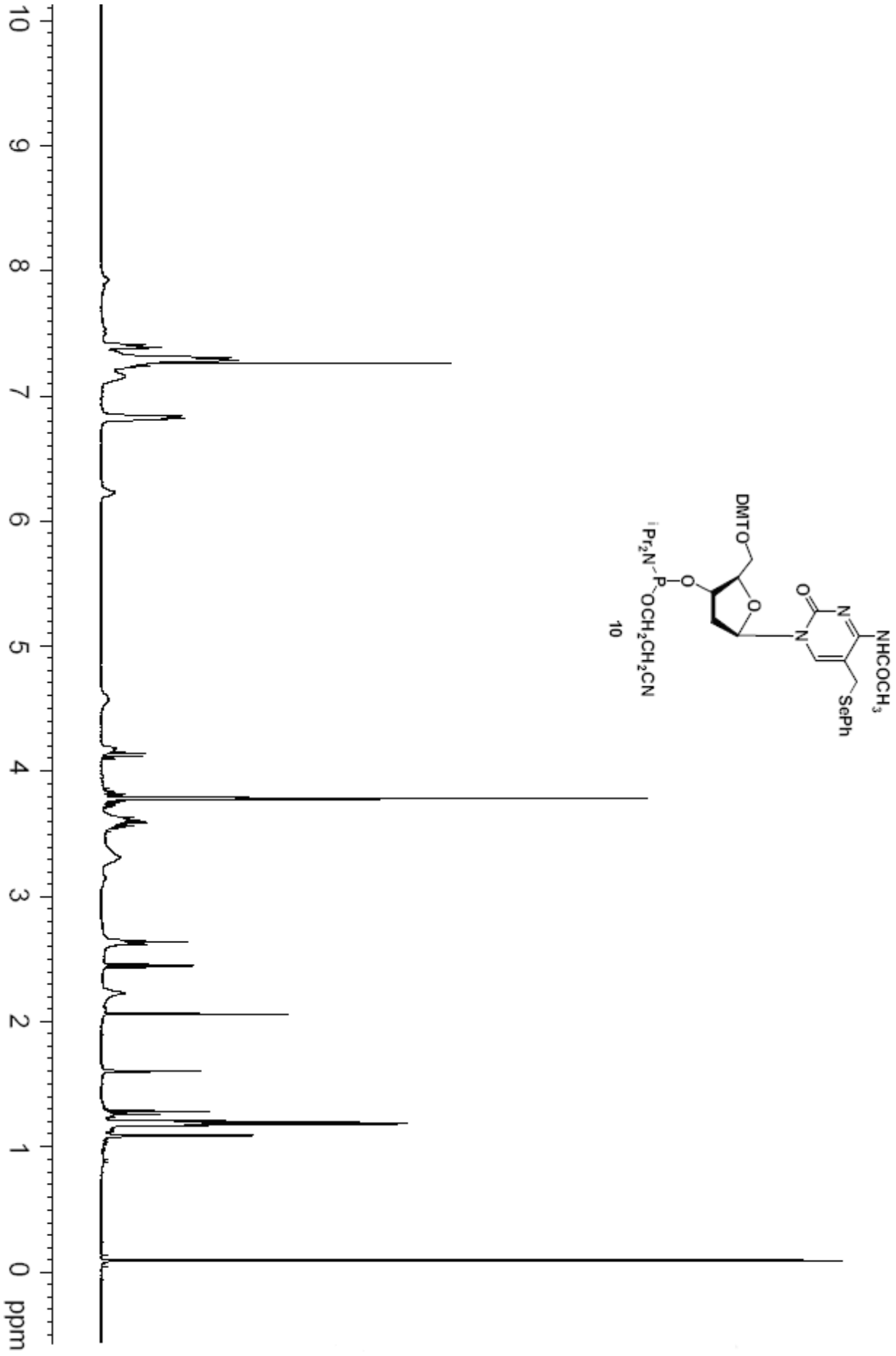
Supporting Information Figure 4. ^{13}C NMR of **8**.



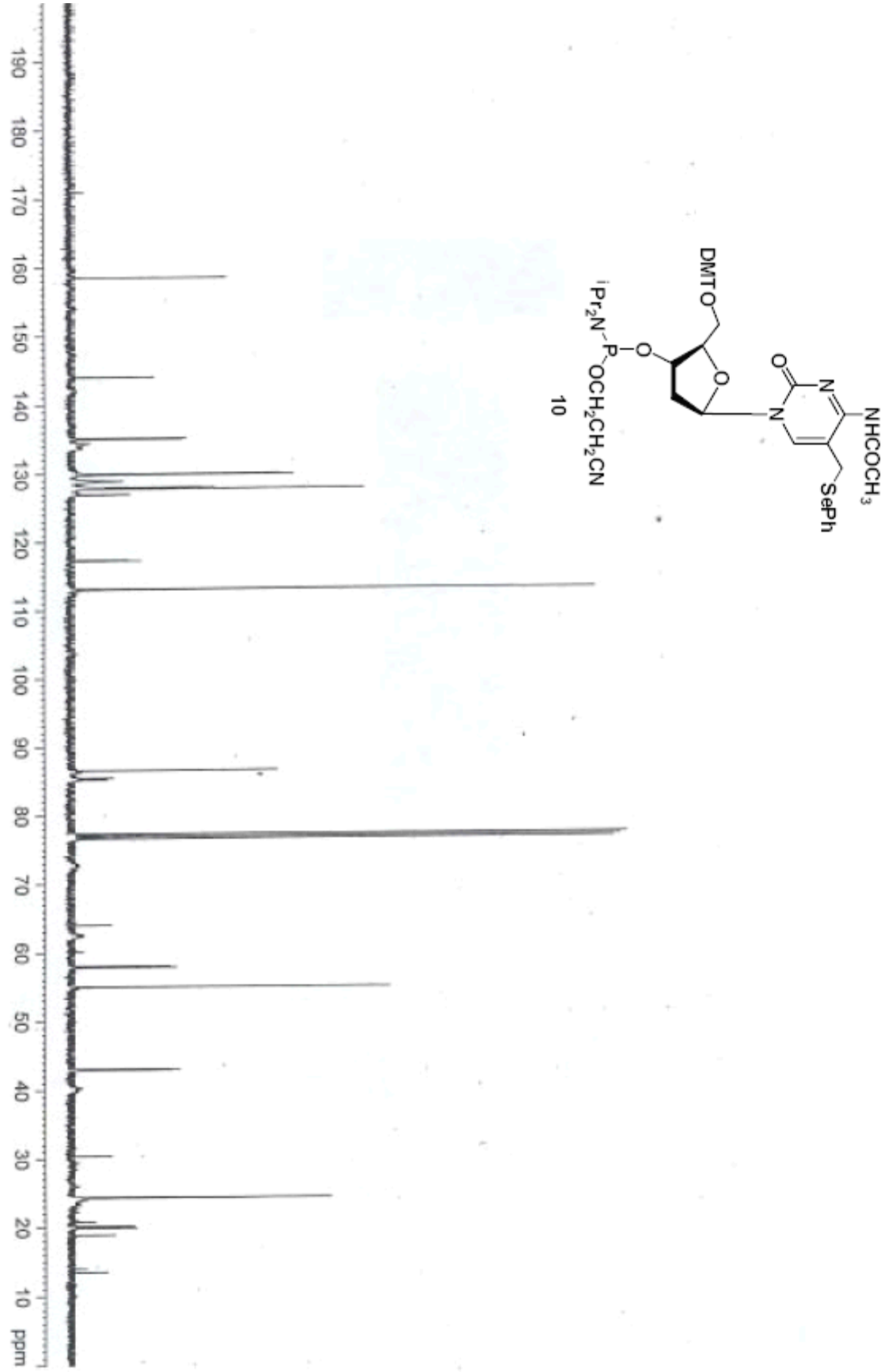
Supporting Information Figure 5. ^{1}H NMR of **9**.



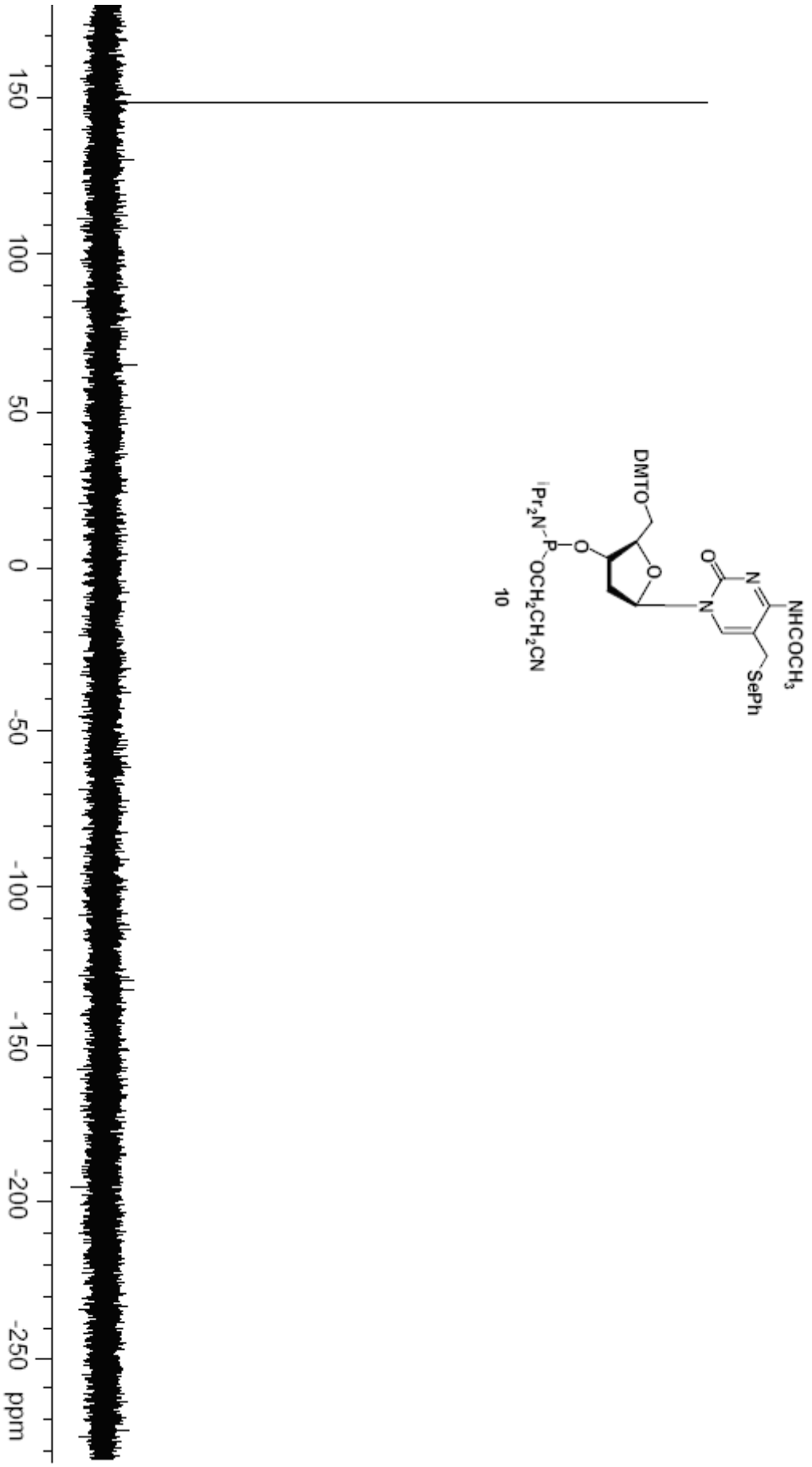
Supporting Information Figure 6. ^{13}C NMR of **9**.



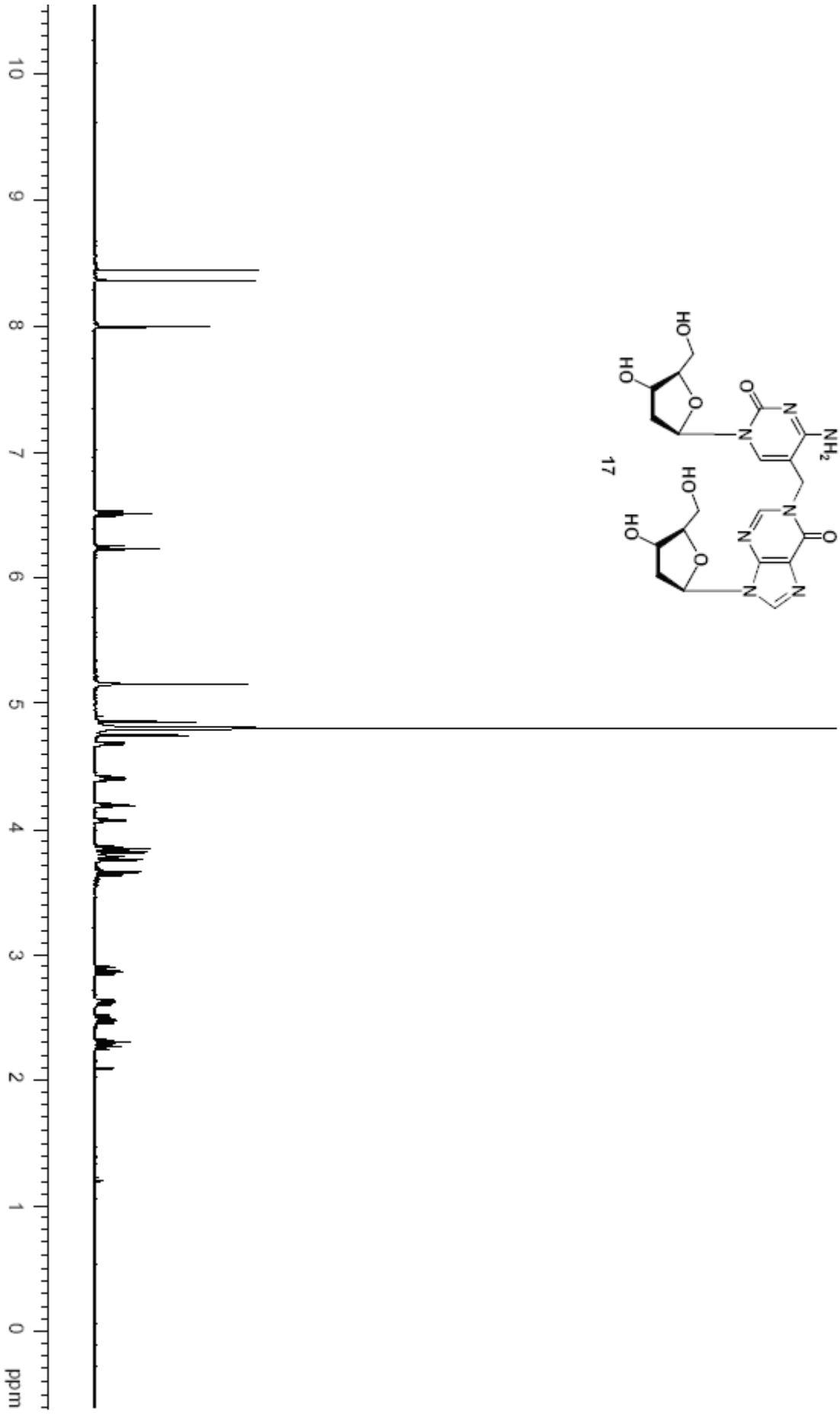
Supporting Information Figure 7. ^{1}H NMR of **10**.



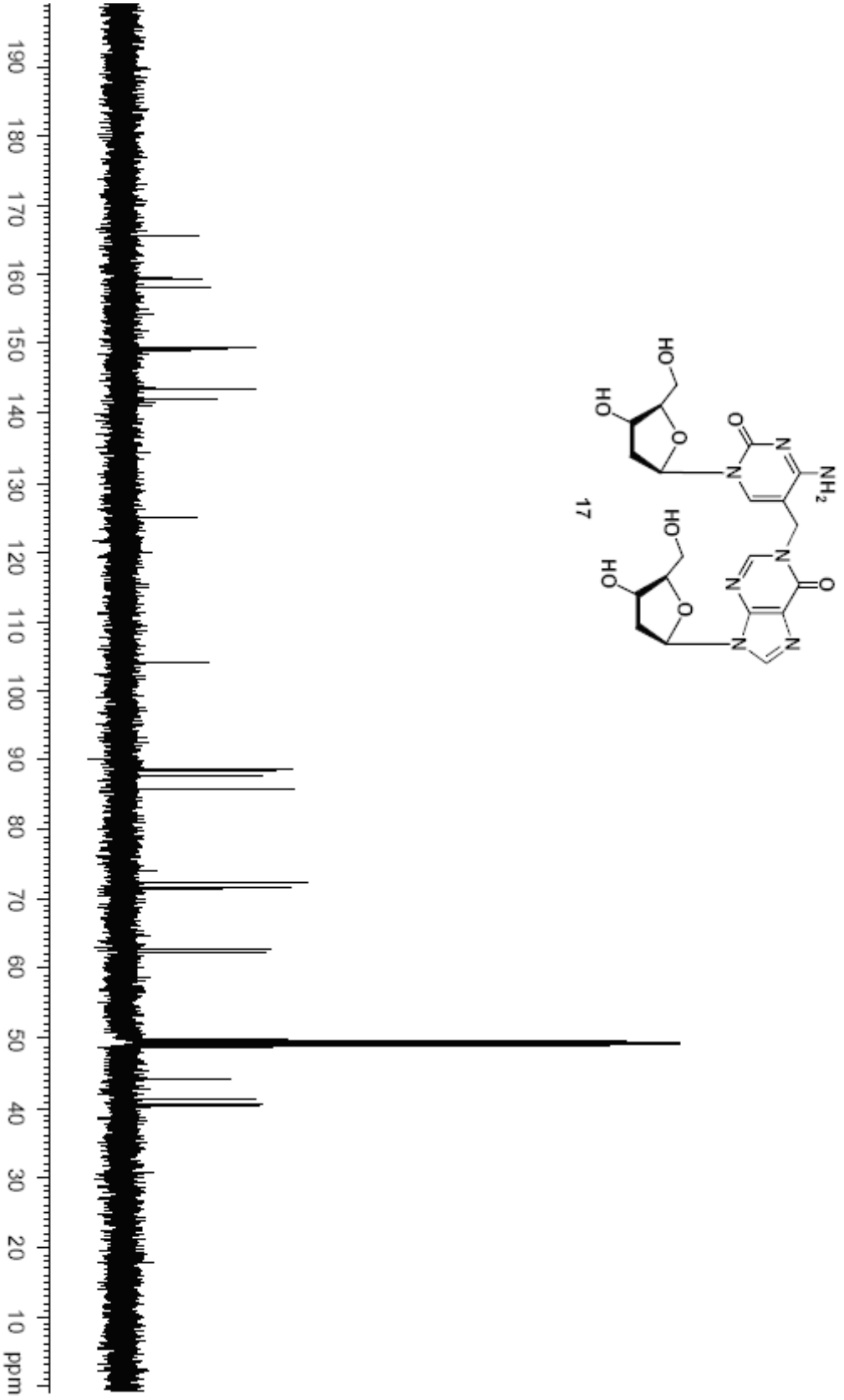
Supporting Information Figure 8. ^{13}C NMR of **10**.



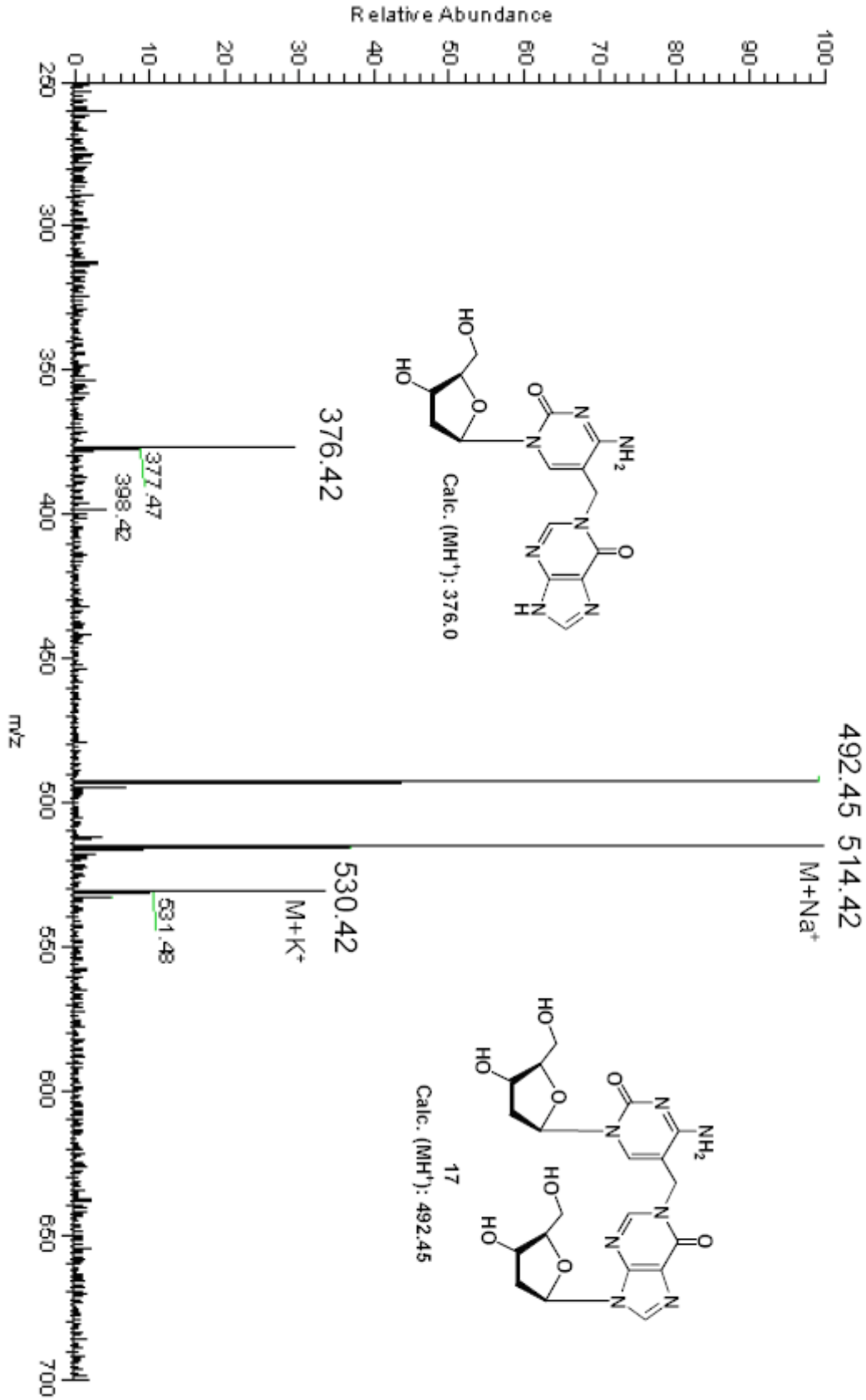
Supporting Information Figure 9. ^{31}P NMR of **10**.



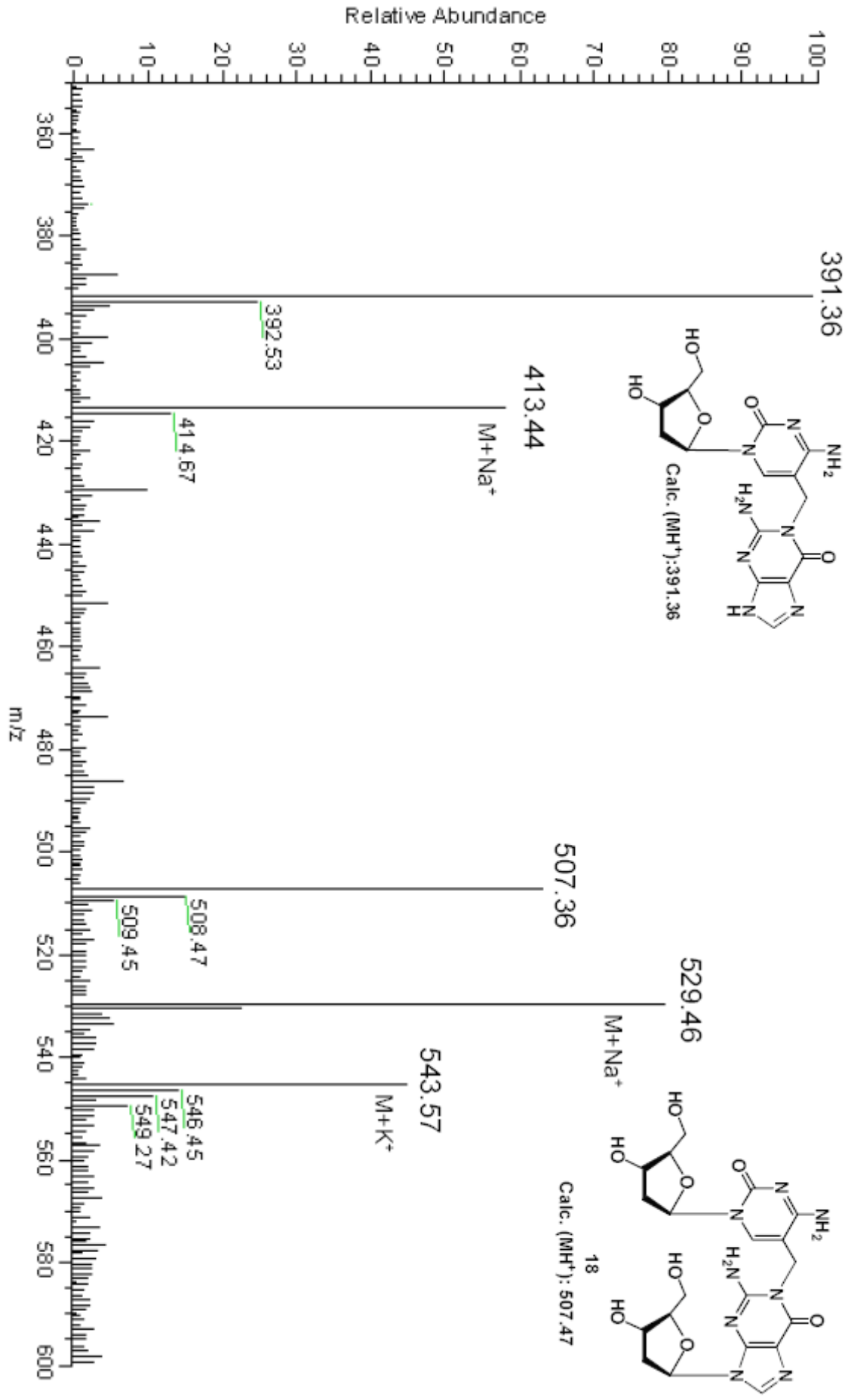
Supporting Information Figure 10. ^{1}H NMR of **17**.



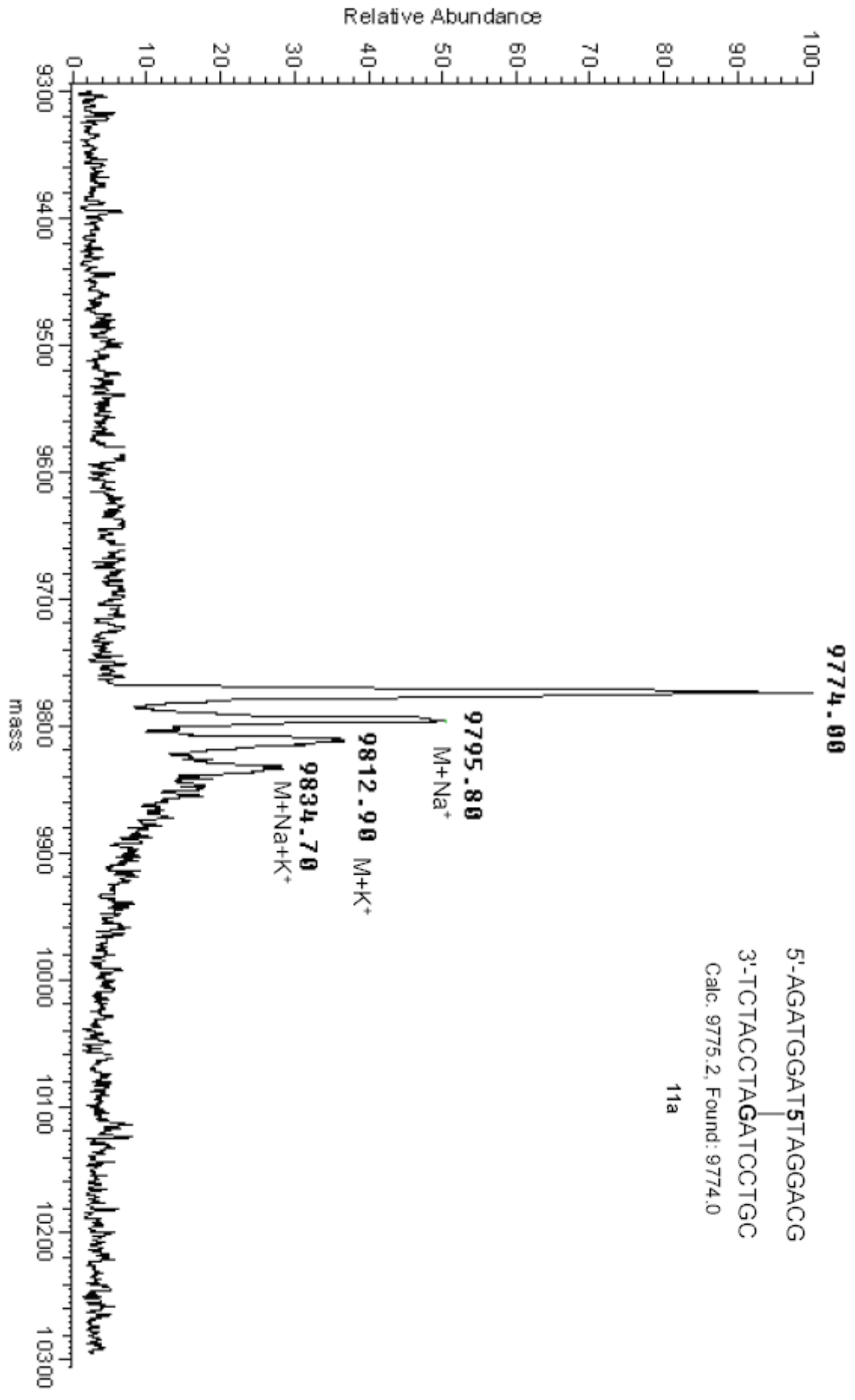
Supporting Information Figure 11. ^{13}C NMR of **17**.



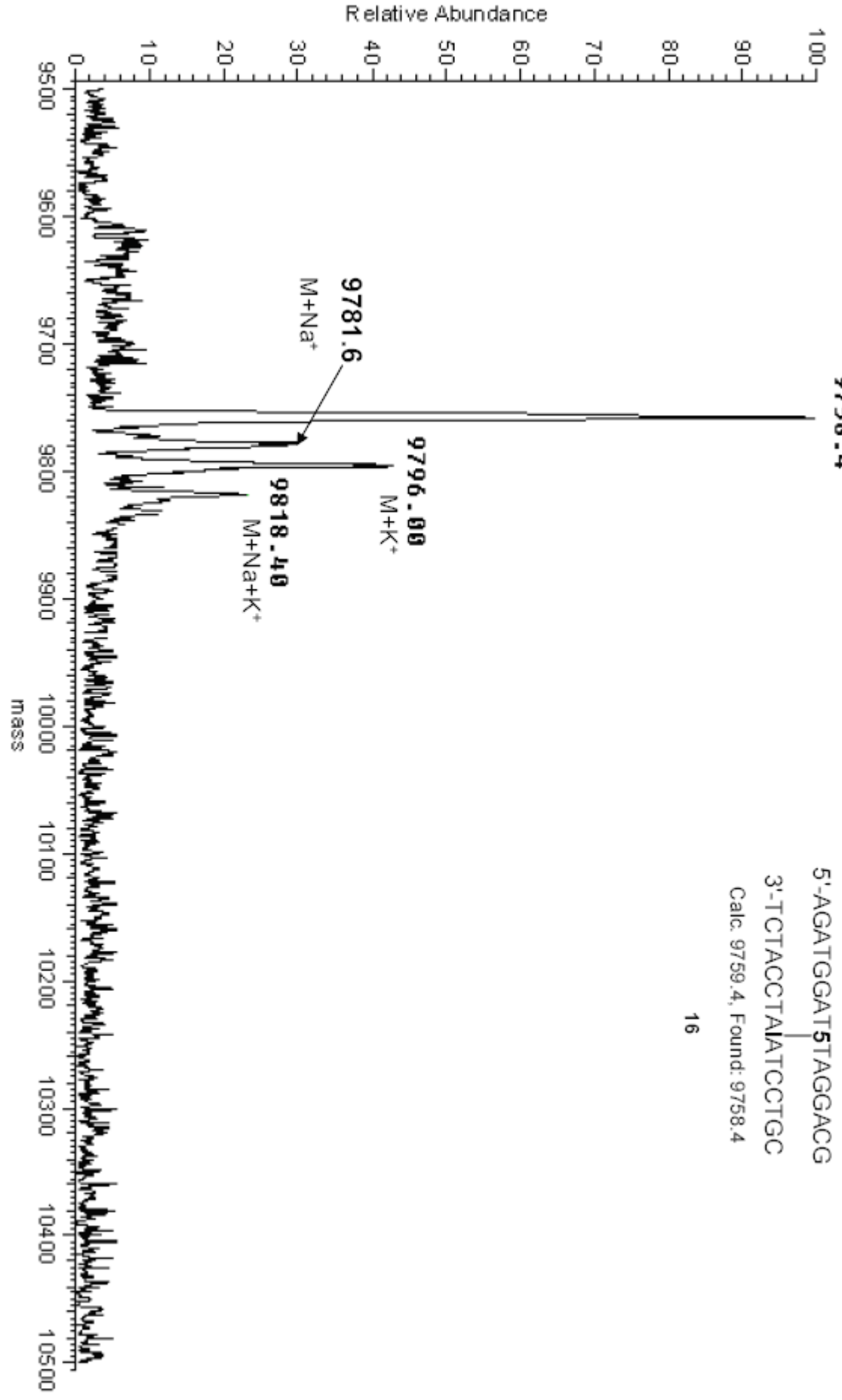
Supporting Information Figure 12. ESI-MS of **17**.



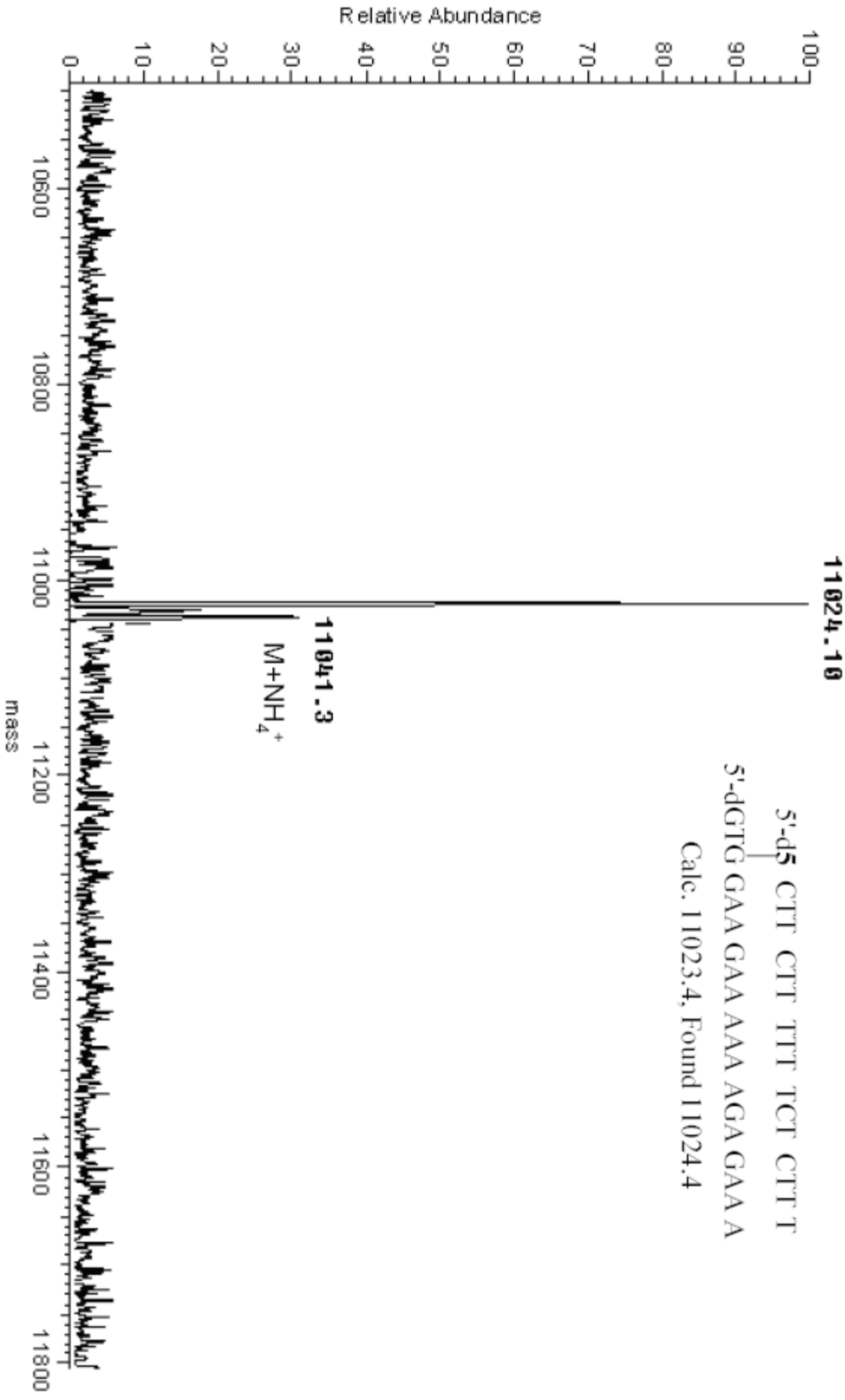
Supporting Information Figure 13. ESI-MS of **18**.



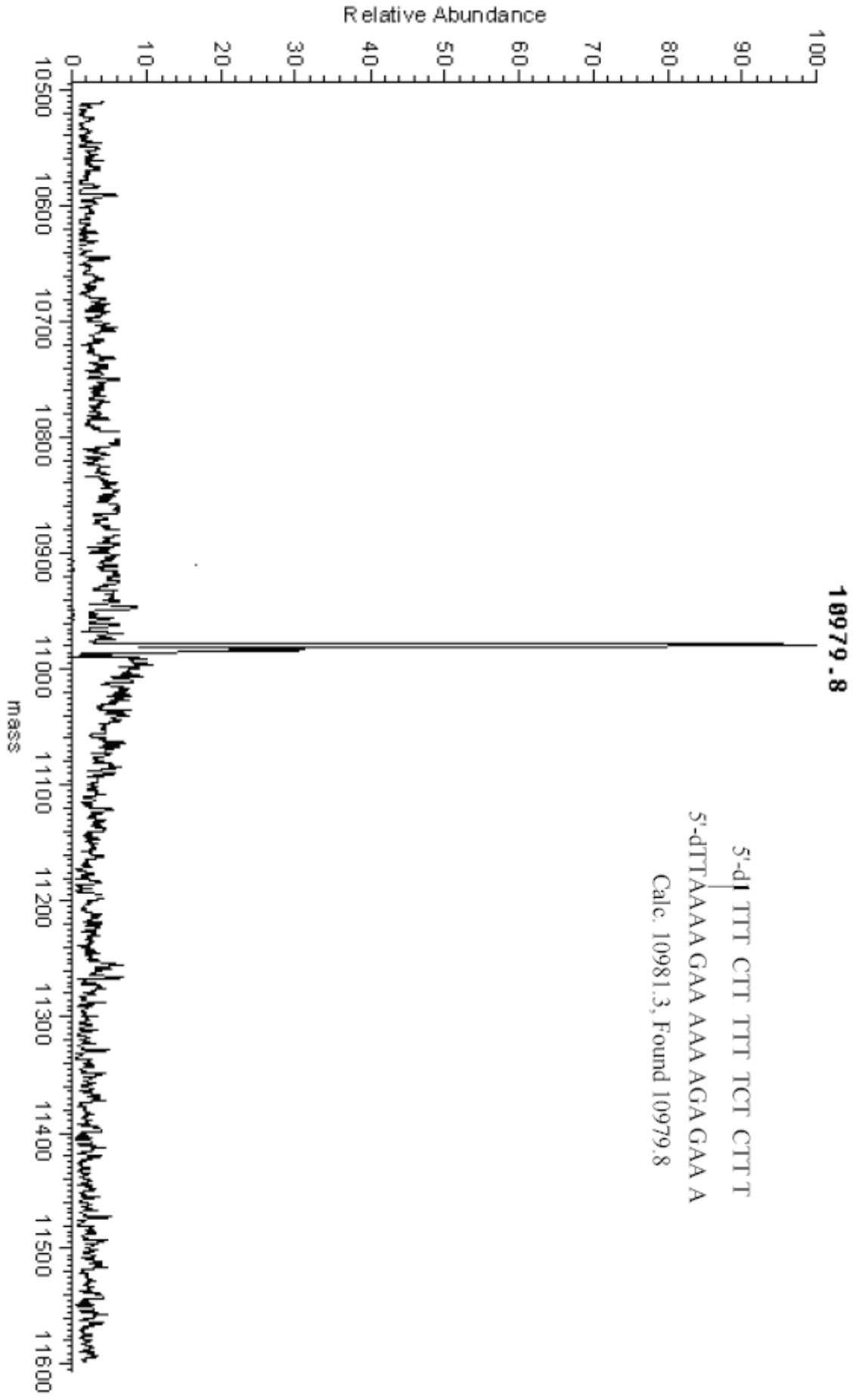
Supporting Information Figure 14. ESI-MS of the cross-linked DNA **11a**.



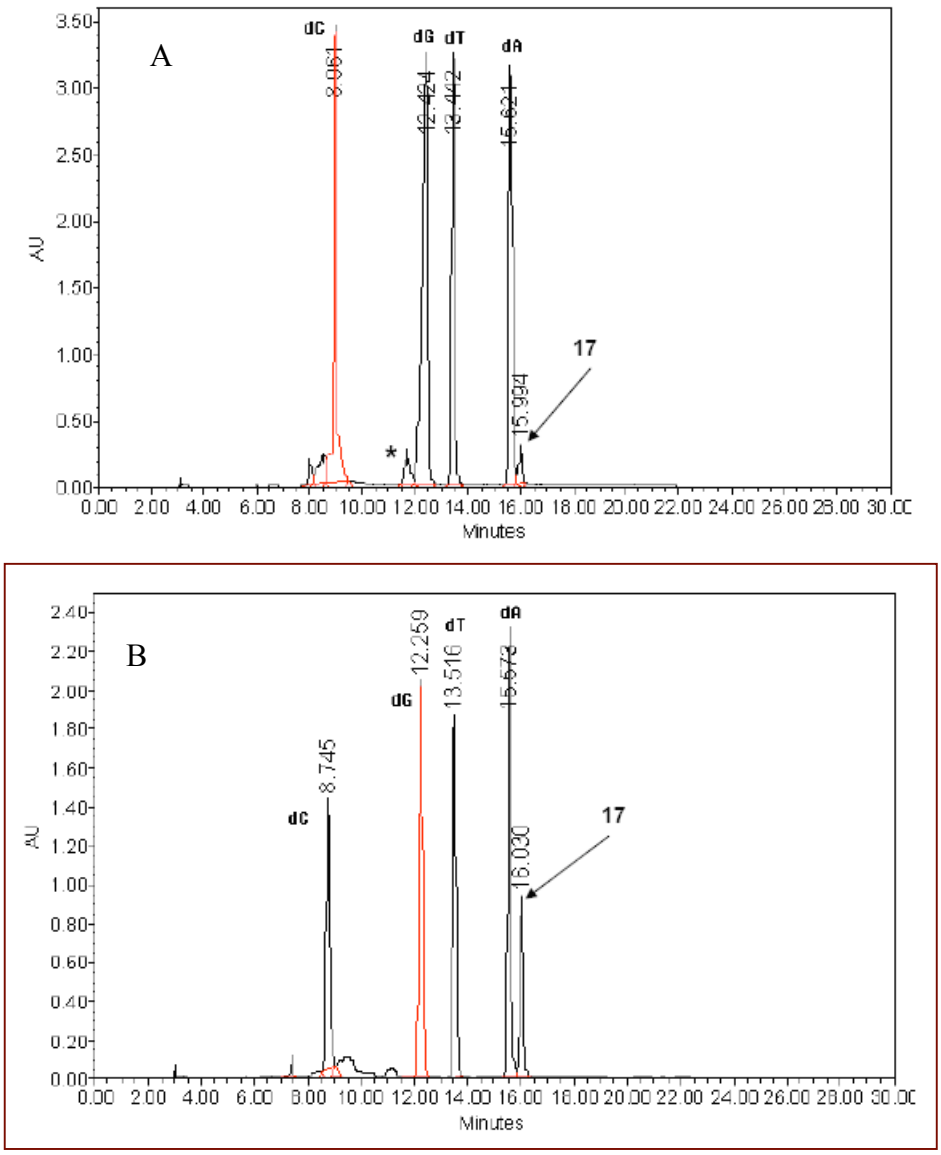
Supporting Information Figure 15. ESI-MS of the cross-linked DNA **16**.



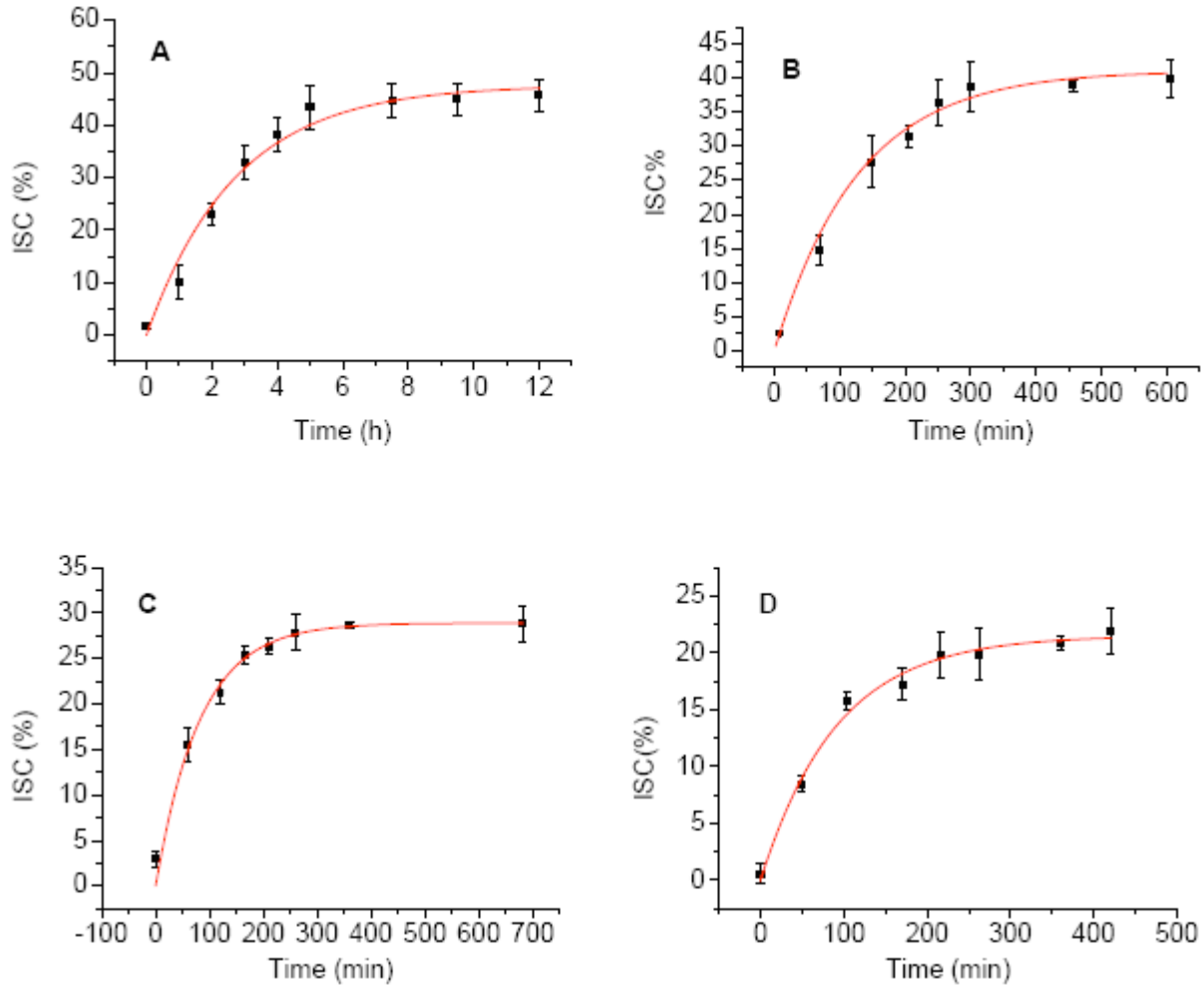
Supporting Information Figure 16. ESI-MS of the cross-linked DNA formed in triplex **29**.



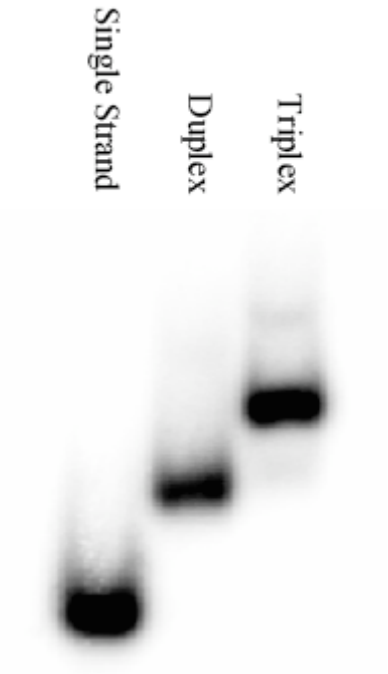
Supporting Information Figure 17. ESI-MS of the cross-linked DNA formed in triplex **31**.



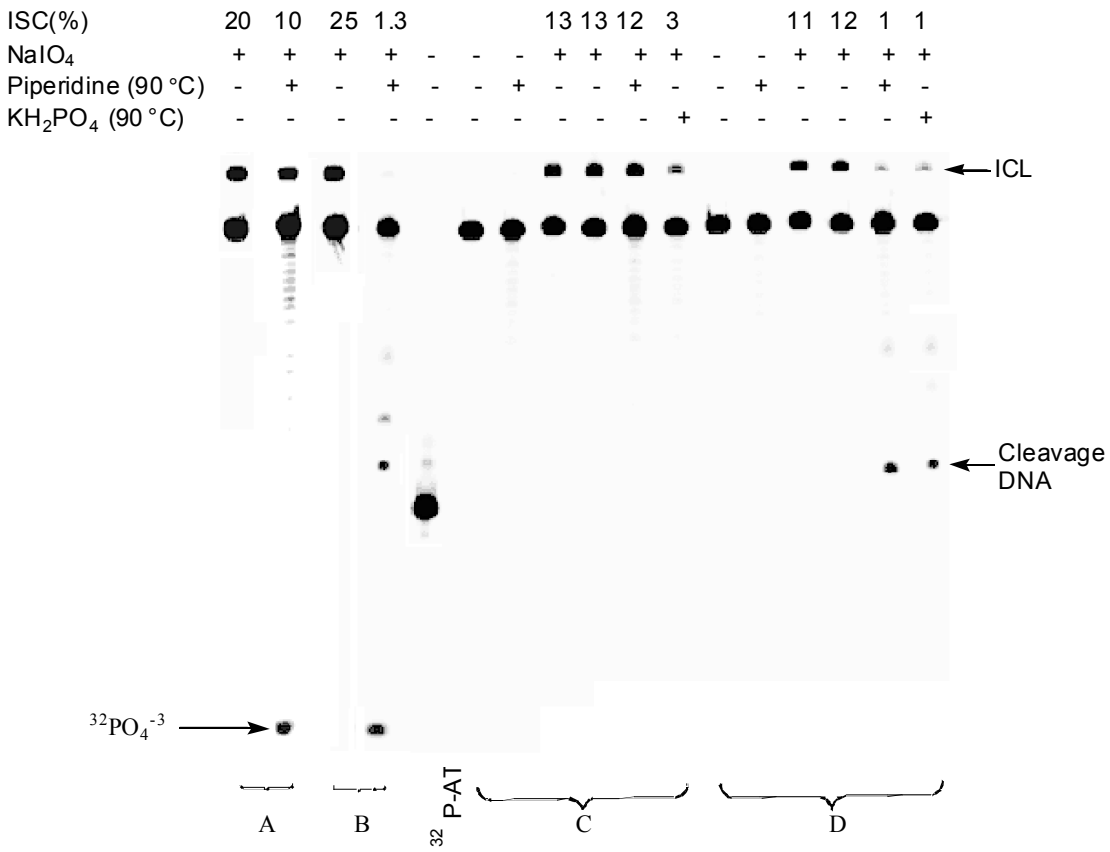
Supporting Information Figure 18. HPLC chromatogram of the enzyme digest of cross-linked DNA product obtained from oxidation of **16** (analyzed by reversed-phase HPLC (RP-18, at 260 nm) using gradient: 0-30 min 2-20% MeCN in water, 30-35 min 20-50% MeCN in water, 35-42 min 50-100% MeCN in water, 42-50 min 100% MeCN in water, at a flow rate 1.0 mL/min). (A) Digest; ∗ artifact (B) Coinjection



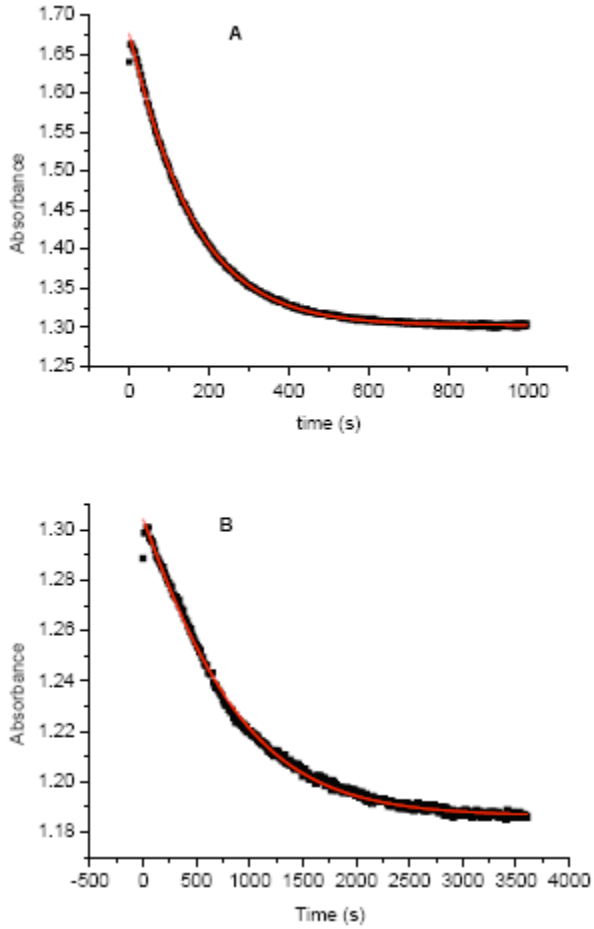
Supporting Information Figure 19. Kinetic plots for the ISC growth of **5** opposing dG (**A**: upon $NaIO_4$ treatment; **B**: upon γ-irradiation) or dI (**C**: upon $NaIO_4$ treatment; **D**: upon γ-irradiation).



Supporting Information Figure 20. Phosphorimage autoradiogram of 20% non-denaturing PAGE analysis of triplex formation from **27** performed in 10 mM Tris·acetate, 5 mM $MgCl_2$, pH 5.5.



Supporting Information Figure 21. Phosphorimage autoradiogram of 20% denaturing PAGE analysis of ISC product formed in triplexes **27** (A), **29** (B), **31** (C) and **32** (D) upon heating in 1.0 M piperidine (90 °C, 25 min) or KH_2PO_4 (pH 7.2) (90 °C, 25 min).



Supporting Information Figure 22. UV-absorption analysis of the rearrangement of selenoxides (**2**, **12**) to methide (**3**, **13**) intermediates. (A) Rearrangement of **2**. (B) Rearrrangement of **12**.