Supporting Information

Post-modification of Triazole-linked Analogues of DNA for Positively Charged Variants

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Table of Contents

1. General

IR spectra were recorded on Nicolet iS10 FT-IR equipped with an attenuated total reflection (ATR) and were reported as wave numbers (ν) in cm^{-1}. Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on JEOL JNM-ECS 400 (^1H: 400 MHz; ^{13}C: 100 MHz) and JNM-LA 400 (^1H: 400 MHz; ^{13}C: 100 MHz). Methyl (CH_3), methylene (CH_2) and methyne (CH) signals in ^{13}C NMR spectra were assigned by DEPT spectra. High resolution mass spectra were obtained on a JEOL JMS-T100CS instrument (ESI-TOF MS) with reserpine (1 ng/μL) and a mixture of polyethylene glycol (PEG200 20 ng, PEG600 20 ng, PEG1000 30 ng, PEG2000 60 ng in 1 μL) as an internal standard. Thermal melting curves and a mixing curve were obtained on a UV-visible spectrometer (JASCO, V-670) equipped with a

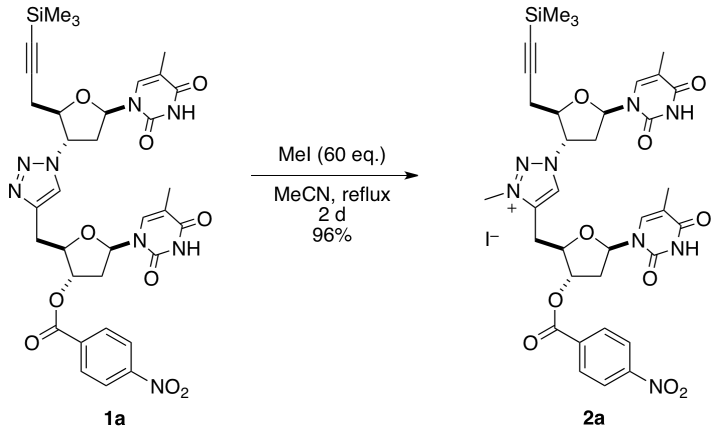
water-circulated temperature-controlled cell holder (JASCO, ETC-717) and a UV-visible spectrometer (JASCO, V-630Bio) equipped with a water-circulated temperature-controlled cell holder (JASCO, ETCS-761). The melting temperature was determined using a melting program (JASCO, VWTP-780). CD spectra were obtained on a spectropolarimeter (JASCO, J-820) equipped with a water-circulated temperature controlled cell holder (JASCO, PTC-423L).

2. Materials

Water was purified by Milli-Q ultrapure water system (Millipore). Other solvents were purified by distillation and dried over 4-Å molecular sieves. Natural oligoadenine DNA, $(dA)_{20}$, was obtained from Nihon Gene Research Laboratories Inc.

3. Synthesis and physical data

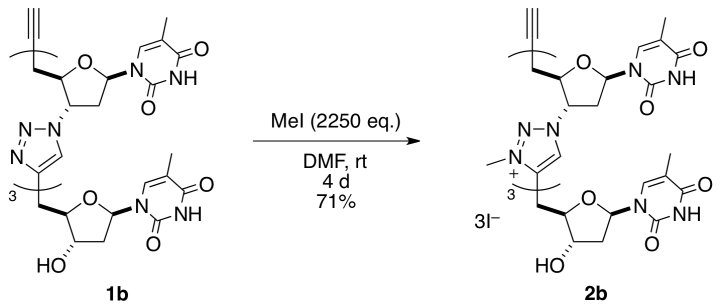
Synthesis of dimer ^{TL}DNA+ 2a



A solution of dimer ^{TL}DNA **1a** (153 mg, 205 μmol) and iodomethane (766 μL, 12.3 mmol) in acetonitrile (10 mL) was stirred at reflux temperature for 2 d under light shielding condition. After removal of volatile materials in vacuo, the crude product was

washed with chloroform to give the dimer ^{TL}DNA+ **2a** (175 mg, 197 μmol, 96%) as a yellowish-white solid. Physical data of **2a**: IR (powder) 3050, 1686, 1271, 1081, 844, 758 cm^{-1}; ^{1}H NMR (400 MHz, CD_3CN) δ 0.15 (s, 9H), 1.85 (d, *J* = 1.2 Hz, 3H), 1.89 (d, *J* = 1.2 Hz, 3H), 2.57 (ddd, *J* = 4.8, 7.6, 14.4 Hz, 1H), 2.70-2.97 (overlapped m, 5H), 3.47 (dd, *J* = 5.6, 16.0 Hz, 1H), 3.52 (dd, *J* = 4.8, 16.0 Hz, 1H), 4.18 (s, 3H), 4.45 (ddd, *J* = 4.4, 5.2, 5.6 Hz, 1H), 4.61 (ddd, *J* = 3.6, 4.8, 5.2 Hz, 1H), 5.48 (ddd, *J* = 4.8, 5.2, 8.4 Hz, 1H), 5.54 (ddd, *J* = 3.6, 3.6, 8.8 Hz, 1H), 5.96 (dd, *J* = 6.4, 7.6 Hz, 1H), 6.36 (dd, *J* = 6.4, 8.0 Hz, 1H), 7.35 (d, *J* = 1.2 Hz, 1H), 7.55 (d, *J* = 1.2 Hz, 1H), 8.25-8.30 (m, 2H), 8.31-8.37 (m, 2H), 8.61 (s, 1H), 9.20 (s, 1H), 9.31 (s, 1H); ^{13}C NMR (100 MHz, CD_3CN) δ 0.0 (CH_3), 12.4 (CH_3), 12.8 (CH_3), 25.4 (CH_2), 27.2 (CH_2), 36.0 (CH_2), 38.5 (CH_2), 39.6 (CH_3), 66.8 (CH), 76.8 (CH), 81.2 (CH), 81.9 (CH), 86.0 (CH), 88.7 (CH), 89.0, 102.5, 111.5, 111.9, 124.7 (CH), 130.8 (CH), 131.9 (CH), 135.9, 136.6 (CH), 139.6 (CH), 141.6, 151.4, 151.5, 151.8, 164.8, 165.1, 165.5; HRMS (ESI-TOF) calcd for $C_{35}H_{41}N_8O_{10}Si$ $[M - I]^+$ 761.2715, found 761.2720.

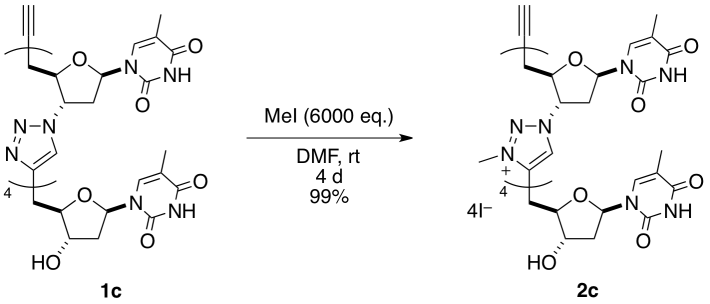
Synthesis of tetramer ^{TL}DNA+ 2b



A solution of tetramer ^{TL}DNA **1b** (12.6 mg, 11.7 μmol) and iodomethane (1.64 mL, 26.3 mmol) in DMF (0.40 mL) was stirred at ambient temperature for 4 d under light shielding condition. After removal of volatile materials in vacuo, the crude

material was washed with dichloromethane and acetonitrile to give tetramer ^{TL}DNA+ **2b** (12.5 mg, 8.32 μmol, 71%) as a white solid. Physical data of **2b**: IR (powder) 1699, 1684, 1275, 1077, 779 cm^{-1}; ^{1}H NMR (400 MHz, 20% $CD_3OD/CDCl_3$) δ 1.76 (d, *J* = 1.2 Hz, 3H), 1.78 (d, *J* = 1.2 Hz, 3H), 1.80 (d, *J* = 1.2 Hz, 3H), 1.81 (d, *J* = 1.2 Hz, 3H), 1.87-1.92 (m, 1H), 2.12 (ddd, *J* = 6.0, 7.2, 14.4 Hz, 1H), 2.21 (dd, *J* = 2.0, 2.4 Hz, 1H), 2.41 (ddd, *J* = 6.4, 7.2, 14.4 Hz, 1H), 2.56-2.74 (m, 1H), 2.72-2.76 (overlapped m, 2H), 2.90-3.01 (overlapped m, 4H), 3.02-3.19 (overlapped m, 2H), 3.44 (dd, *J* = 6.8, 16.0 Hz, 1H), 3.50-3.58 (m, 1H), 4.12 (s, 3H), 4.12 (s, 3H), 4.13-4.18 (m, 1H), 4.19 (s, 3H), 4.27 (ddd, *J* = 6.4, 6.4, 8.4 Hz, 1H), 4.57 (ddd, *J* = 4.4, 4.8, 4.8 Hz, 1H), 4.85-4.93 (m, 1H), 4.98 (ddd, *J* = 5.2, 5.2, 6.4 Hz, 1H), 5.59-5.70 (overlapped m, 3H), 5.87 (ddd, *J* = 5.6, 7.2, 8.4 Hz, 1H), 5.94 (dd, *J* = 4.4, 8.0 Hz, 1H), 6.14 (dd, *J* = 6.0, 7.2 Hz, 1H), 6.24 (dd, *J* = 6.0, 8.0 Hz, 1H), 7.06 (d, *J* = 1.2 Hz, 1H), 7.39 (d, *J* = 1.2 Hz, 1H), 7.46 (d, *J* = 1.2 Hz, 1H), 9.00 (s, 1H), 9.24 (s, 1H), 9.40 (s, 1H), three protons overlapped with solvent signals; HRMS (ESI-TOF) calcd for $C_{51}H_{62}I_2N_{17}O_{13}$ $[M - I]^+$ 1374.2802, found 1374.2865.

Synthesis of pentamer ^{TL}DNA+ 2c



1c **2c**

A solution of pentamer ^{TL}DNA **1c** (11.4 mg, 8.41 μmol) and iodomethane (3.14 mL, 50.5 mmol) in DMF (3.2 mL) was stirred at ambient temperature for 4 d

under light shielding condition. After removal of volatile materials in vacuo, the crude material was washed with hexane and acetonitrile to give pentamer ^{TL}DNA+ **2c** with iodide counteranion (15.9 mg, 8.29 μmol, 99%) as a white solid. Physical data of **2c**: IR (powder) 1692, 1469, 1272, 1073, 774 cm^{-1}; ^{1}H NMR (400 MHz, 20% CD_3OD/CD_3CN) δ 2.04 (d, *J* = 1.2 Hz, 3H), 2.06 (d, *J* = 1.2 Hz, 3H), 2.05-2.08 (overlapped m, 9H), 2.39 (ddd, *J* = 5.2, 7.6, 14.0 Hz, 1H), 2.58-2.67 (m, 1H), 2.65 (dd, *J* = 2.4, 2.4 Hz, 1H), 2.96-3.08 (overlapped m, 4H), 3.08-3.26 (overlapped m, 5H), 3.43-3.52 (overlapped m, 2H), 3.56 (dd, *J* = 4.4, 16.0 Hz, 1H), 3.74-3.78 (overlapped m, 6H), 4.32 (ddd, *J* = 4.8, 4.8, 7.6 Hz, 1H), 4.34 (s, 3H), 4.39 (s, 3H), 4.41 (s, 3H), 4.41 (s, 3H), 4.59 (ddd, *J* = 4.8, 5.2, 7.6 Hz, 1H), 4.78 (ddd, *J* = 5.2, 5.2, 5.2 Hz, 1H), 4.98-5.11 (m, 3H), 5.76 (ddd, *J* = 4.4, 4.8, 9.2 Hz, 1H), 5.81-5.97 (m, 3H), 6.28 (dd, *J* = 7.2, 7.2 Hz, 1H), 6.33 (dd, *J* = 5.2, 8.0 Hz, 1H), 6.38 (dd, *J* = 2.4, 6.4 Hz, 1H), 6.40 (dd, *J* = 2.4, 6.0 Hz, 1H), 6.57 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.52 (d, *J* = 1.2 Hz, 1H), 7.65 (d, *J* = 1.2 Hz, 1H), 7.68 (d, *J* = 1.2 Hz, 1H), 7.70 (d, *J* = 1.2 Hz, 1H), 7.77 (d, *J* = 1.2 Hz, 1H), 9.08 (s, 1H), 9.12 (s, 1H), 9.32 (s, 1H), 9.34 (s, 1H); HRMS (ESI-TOF) calcd for $C_{64}H_{78}I_3N_{22}O_{16}$ $[M - I]^+$ 1791.3100, found 1791.3179.

The iodide counteranion was exchanged to hexafluorophosphate counteranion as follows: A mixture of pentamer ^{TL}DNA+ **2c** with iodide counteranion (1.66 mg, 0.865 μmol) and a saturated solution of ammonium hexafluorophosphate in methanol (600 μL) was stirred at ambient temperature for 4 h. After removal of supernatant, the crude material was washed with methanol (200 μL × 3) to give pentamer ^{TL}DNA+ **2c** with hexafluorophosphate counteranion (1.52 mg, 0.823 μmol, 95%) as a white solid. Physical data of **2c** with hexafluorophosphate counteranion: IR (powder) 1700, 1473, 1278, 1078, 843 cm^{-1}; ^{1}H NMR (400 MHz, 20% CD_3OD/CD_3CN) δ 1.87 (s, 3H), 1.89

(s, 3H), 1.89 (s, 3H), 1.89 (s, 3H), 1.91 (s, 3H), 2.16-2.30 (m, 1H), 2.42-2.52 (m, 2H), 2.75-2.90 (overlapped m, 3H), 2.90-3.06 (overlapped m, 4H), 3.34-3.53 (overlapped m, 9H), 4.03-4.10 (m, 1H), 4.18 (s, 3H), 4.18 (s, 3H), 4.19 (s, 3H), 4.21 (s, 3H), 4.35 (ddd, J = 5.2, 5.6, 7.2 Hz, 1H), 4.55-4.64 (m, 1H), 4.64-4.80 (overlapped m, 3H), 5.44-5.61 (overlapped m, 4H), 6.02-6.14 (overlapped m, 4H), 6.38 (dd, J = 6.0, 7.2 Hz, 1H), 7.27 (s, 1H), 7.35 (s, 1H), 7.35 (s, 1H), 7.36 (s, 1H), 7.58 (s, 1H), 8.58 (s, 1H), 8.60 (s, 1H), 8.60 (s, 1H), 8.65 (s, 1H), two protons overlapped with solvent signals; HRMS (ESI-TOF) calcd for $C_{64}H_{78}F_{18}N_{22}O_{16}P_3$ $[M - PF_6]^+$ 1845.4892, found 1845.4829.

4. Spectral analysis

4-1. Analysis of solubility of ^{TL}DNA 1c and ^{TL}DNA+ 2c

Maximum aqueous solubility of pentamers (neutral ^{TL}DNA **1c** or cationic ^{TL}DNA+ **2c** with iodide counteranion was estimated by UV-visible spectra of a saturated pentamer in SSPE buffer [10 mM sodium phosphate (pH 7.0), 100 mM sodium chloride, 0.10 mM ethylenediamine tetraacetic acid] (Figure S1). The absorbance of the saturated solution of **1c** (ε_{260} = 4.21×10^4 $M^{-1}\bullet cm^{-1}$)[1] at 260 nm recorded 0.609, and the saturated concentration was determined as 14.5 μM. The absorbance of the saturated solution of **2c** (ε_{260} = 4.11×10^4 $M^{-1}\bullet cm^{-1}$)[2] at 260 nm

1. The molar absorption coefficient was estimated by nearest-neighbor approximation (Puglisi, J. D.; Tinoco Jr., I. *Methods in Enzymology*; Dahlberg, J. E.; Abelson, J. N., Eds.; Academic Press: San Diego, 1989, *180*, pp. 304–325) using the molar absorption coefficient of monomer ^{TL}DNA (ε_{260} = 8.72×10^3 $M^{-1}\bullet cm^{-1}$) and dimer ^{TL}DNA (ε_{260} = 1.71×10^4 $M^{-1}\bullet cm^{-1}$). See also: Isobe, H.; Fujino, T.; Yamazaki, N.; Guillot-Nieckowski, M.; Nakamura, E. *Org. Lett.* **2008**, *10*, 3729–3732.
2. The molar absorption coefficient at 260 nm for **2c** was determined by measuring the absorption of **2c** solution (5.02 μM) in SSPE buffer (A_{260} = 0.206).

exceeded the measurement limit. We therefore diluted the solution 10^3 times and recorded the absorbance of 0.485 (260 nm) to determine the saturated concentration of **2c** as 11.8 mM.

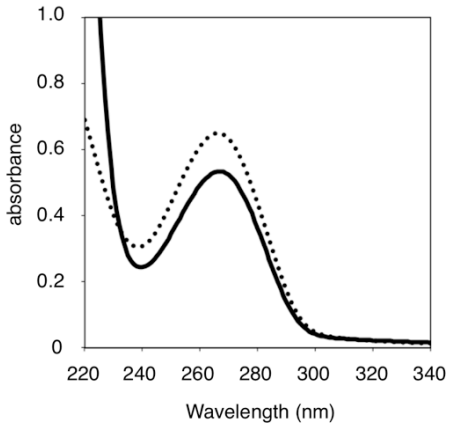


Figure S1. UV spectra of pentamers for the solubility analysis. Dashed line: **1c**. Solid line: **2c**.

4-2. Analysis of triplex of 2c and $(dA)_{20}$ with UV spectra

Triplex formation with ^{TL}DNA+ **2c** with hexafluorophosphate counteranion and DNA $(dA)_{20}$ was analyzed by UV-visible spectra. The binding stoichiometry between thymine bases (T) of **2c** and adenine bases (A) of $(dA)_{20}$ for the optimum complex was determined by Job plot analysis (Figure 1a). Thus, a solution of a mixture of **2c** and $(dA)_{20}$ was prepared by mixing a solution of **2c** (7.2 μM; 36 μM of T) in sodium phosphate buffer (40 mM, pH 7.0) and a solution $(dA)_{20}$ (1.8 μM; 36 μM of A) in sodium phosphate buffer (40 mM, pH 7.0). The total base concentration was maintained at 36 μM with the variations in the molar ratio of T and A. Before the measurement of the absorbance at 260 nm at 0 °C, each solution was heated at 75 °C for 2 min, allowed to cool to 45 °C over a period of 6 min and to 0 °C over several hours to form the complex. A plot of the absorbance against T/(T+A) is shown in

Figure S2a. In order to clarify the optimal point, we also converted the same data into a plot of the *Δ*%absorbance in Figure S2b and 1a.[3]

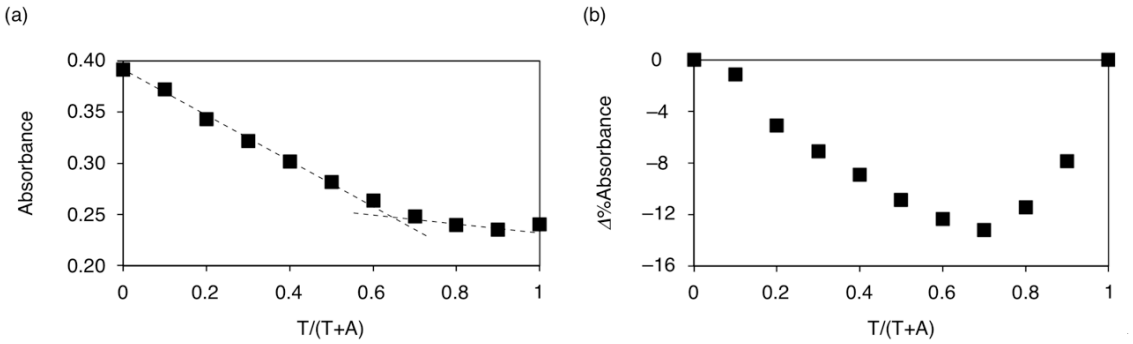


Figure S2. Job plot analysis. (a) Plot of absorbance against T/(T+A). (b) Plot of *Δ*%absorbance against T/(T+A).

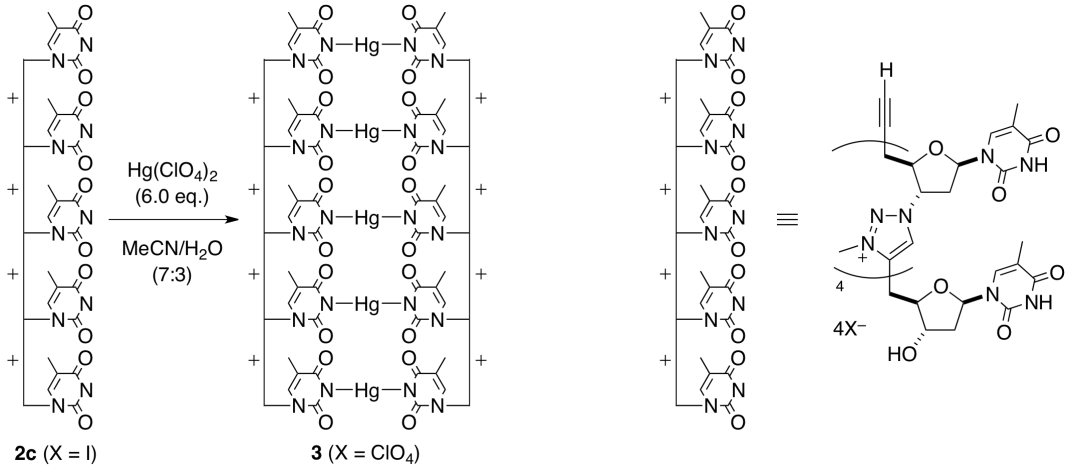
A thermal denaturation curve for the triplex between ^{TL}DNA+ **2c** and DNA $(dA)_{20}$ in sodium phosphate buffer (40 mM, pH 7.0) was obtained by variable-temperature measurement of the absorption at 260 nm under nitrogen atmosphere. A mixture of **2c** with hexafluorophosphate counteranion (5.33 μM; 26.7 μM of T) and $(dA)_{20}$ (0.667 μM; 13.3 μM of A) in a 700 μL cell with a 1 cm light length was heated at 75 °C for 2 min, allowed to cool to 45 °C over a period of 6 min and to –5 °C over a period of 250 min. The melting curve of the triplex, *i.e.*, the absorbance change at 260 nm, was obtained, as the temperature was increased with the heating rate of 0.2 °C/min. The result is shown in Figure 1b.

3. For the *Δ*%absorbance, see: Linkletter, B. A.; Szabo, I. E.; Bruice, T. C. *J. Am. Chem. Soc.* **1999**, *121*, 3888–3896. In brief, *Δ*%absorbance is obtained from the following equation, *Δ*%absorbance = 100 × [A – A_{sum}]/A_{sum} where A is the measrured absorbance under influence of the hypochromic effect and A_{sum} is the theoretical absorbance without the hypochromic effect.

4-3. Analysis of triplex of 2c and $(dA)_{20}$ with CD spectra

A mixture of **2c** (5.33 μM; 26.7 μM of T) and $(dA)_{20}$ (0.667 μM; 13.3 μM of A) in sodium phosphate buffer (40 mM, pH 7.0) was prepared, and the triplex was formed at 0 °C under the optimal conditions described in the preceding section for the Job plot analysis. The solution containing the triplex was analyzed by CD spectroscopy, and the spectrum is shown in Figure 1c.

5. Mercury-mediated duplex 3



The mercury-mediated duplex was prepared by the method reported previously.[4] To a solution of ^{TL}DNA+ **2c** with iodide counteranion in 70% v/v acetonitrile/water (212 μM, 46.6 μL) was added a solution of mercury perchlorate in 70% v/v acetonitrile/water (17.7 mM, 3.39 μL) at ambient temperature to give mercury-mediated duplex **3** (200 μM). The solution was analyzed by ESI MS, and the spectrum is shown in Figure 2a, S3 and S4. The duplex **3** was detected as a trivalent cation (MS calcd for $C_{128}H_{216}N_{44}O_{87}Cl_5Hg_5$ $[M - 3(ClO_4) + 35H_2O]^{3+}$ 1648.8, found

4. Isobe, H.; Yamazaki, N.; Asano, A.; Fujino, T.; Nakanishi, W.; Seki, S. *Chem. Lett.* **2011**, *40*, 318–319.

1648.7) and a tetravalent cation (MS calcd for $C_{128}H_{216}N_{44}O_{83}Cl_4Hg_5$ [M – 4(ClO_4) + 35H_2O]$^{4+}$ 1211.8, found 1211.8).

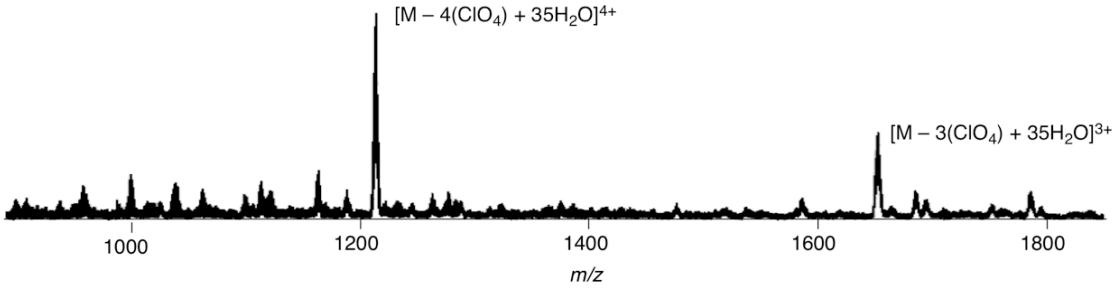


Figure S3. ESI MS spectrum of duplex **3** for whole range.

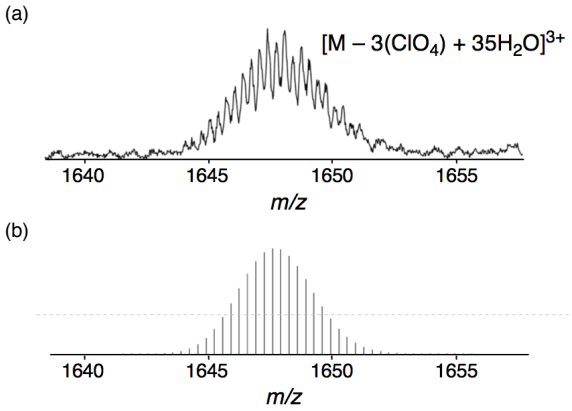


Figure S4. (a) A mass spectrum of mercury-mediated duplex **3**. (b) A simulated spectrum of duplex **3** as trivalent cation ($C_{128}H_{216}N_{44}O_{87}Cl_5Hg_5$, [M – 3($ClO_4$) + 35$H_2O$]$^{3+}$). The spectrum was obtained using MassCenter software (v.1.30) from JEOL.

Analysis with CD spectroscopy revealed the mercury-mediated duplex formation of **2c**, as previously reported for neutral ^{TL}DNA.[4] CD spectra were measured at the variable ratio of **2c** and Hg with the maintenance of the final concentration of **2c** at 20 μM. As shown in Figure S5, the ellipticity changed dramatically upon formation of the duplex. The plot of the ellipticity at 260 and 280 nm in Figure S6 showed the saturation point at the molar ratio of 1:2 for Hg:T, which shows the formation of the

duplex.

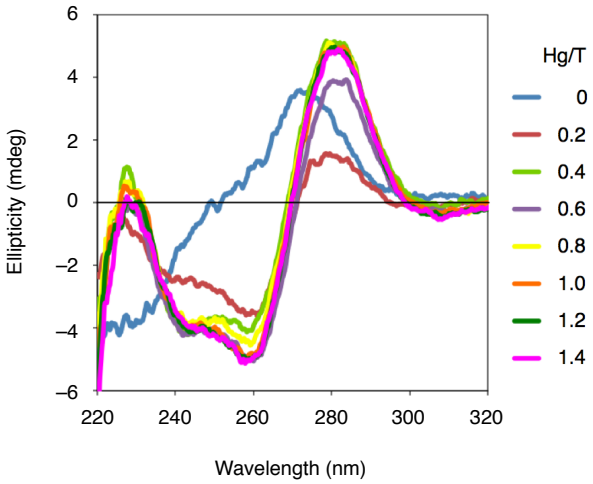


Figure S5. CD spectra of mercury-mediated duplex **3** at 20 °C. The molar ratio of mercury to thymine bases was varied from 0:1 to 1.4:1.

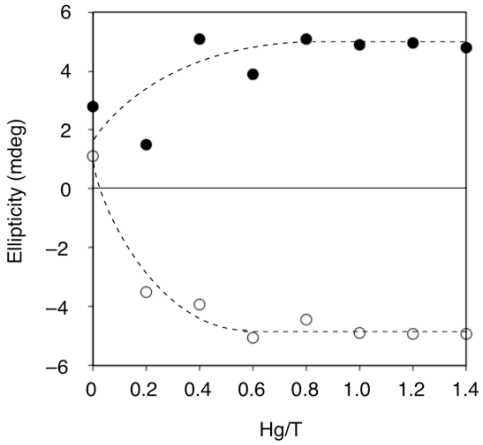


Figure S6. Change of CD spectra of self-duplex **3** in the ellipticity upon addition of mercury perchlorate. Open circles: CD intensity at 260 nm. Filled circles: CD intensity at 280 nm.

6. Charts of NMR spectra

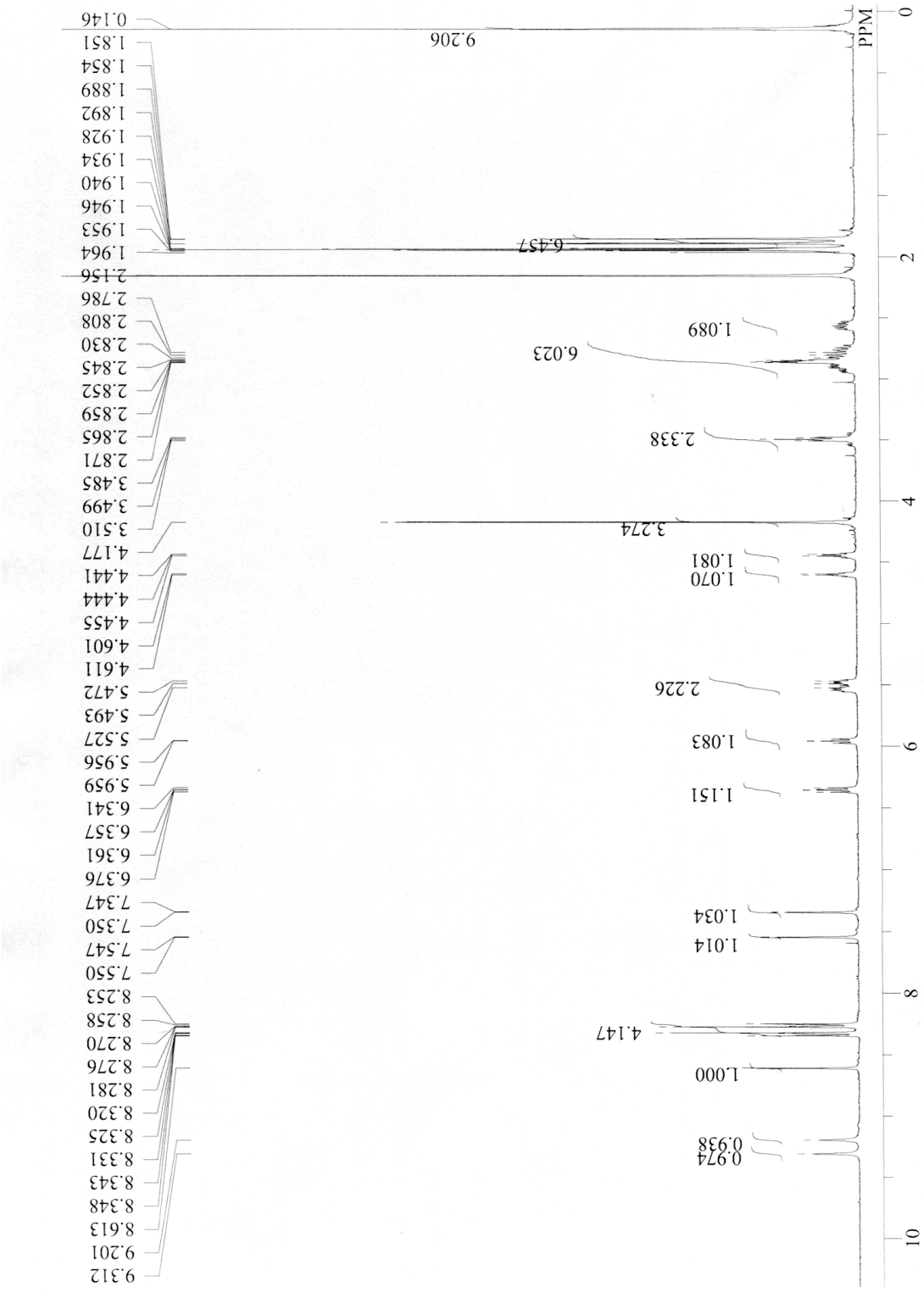


Figure S7. ^{1}H NMR spectrum of dimer ^{TL}DNA+ **2a** in CD_3CN.

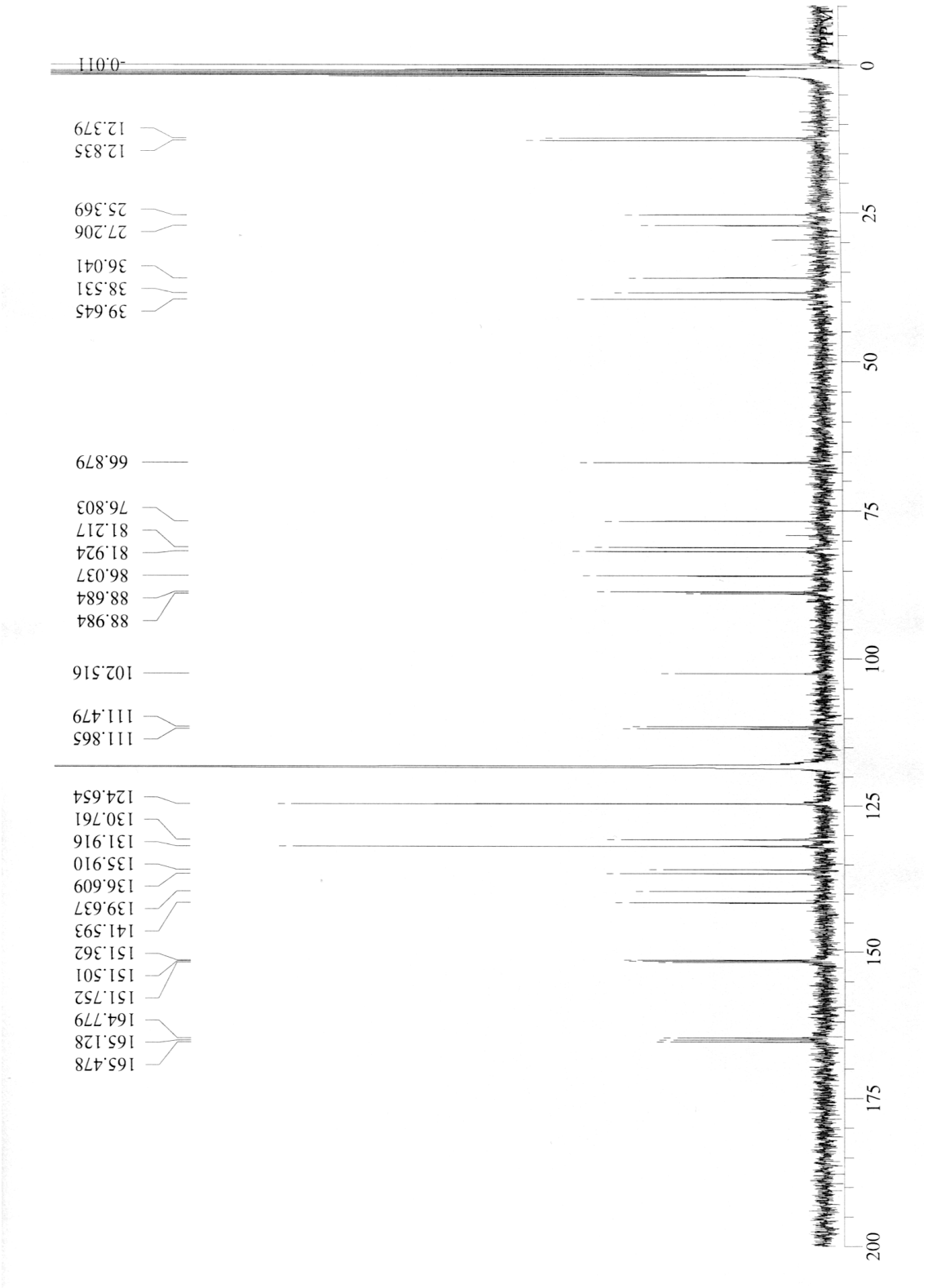


Figure S8. ^{13}C NMR spectrum of dimer ^{TL}DNA+ **2a** in CD_3CN.

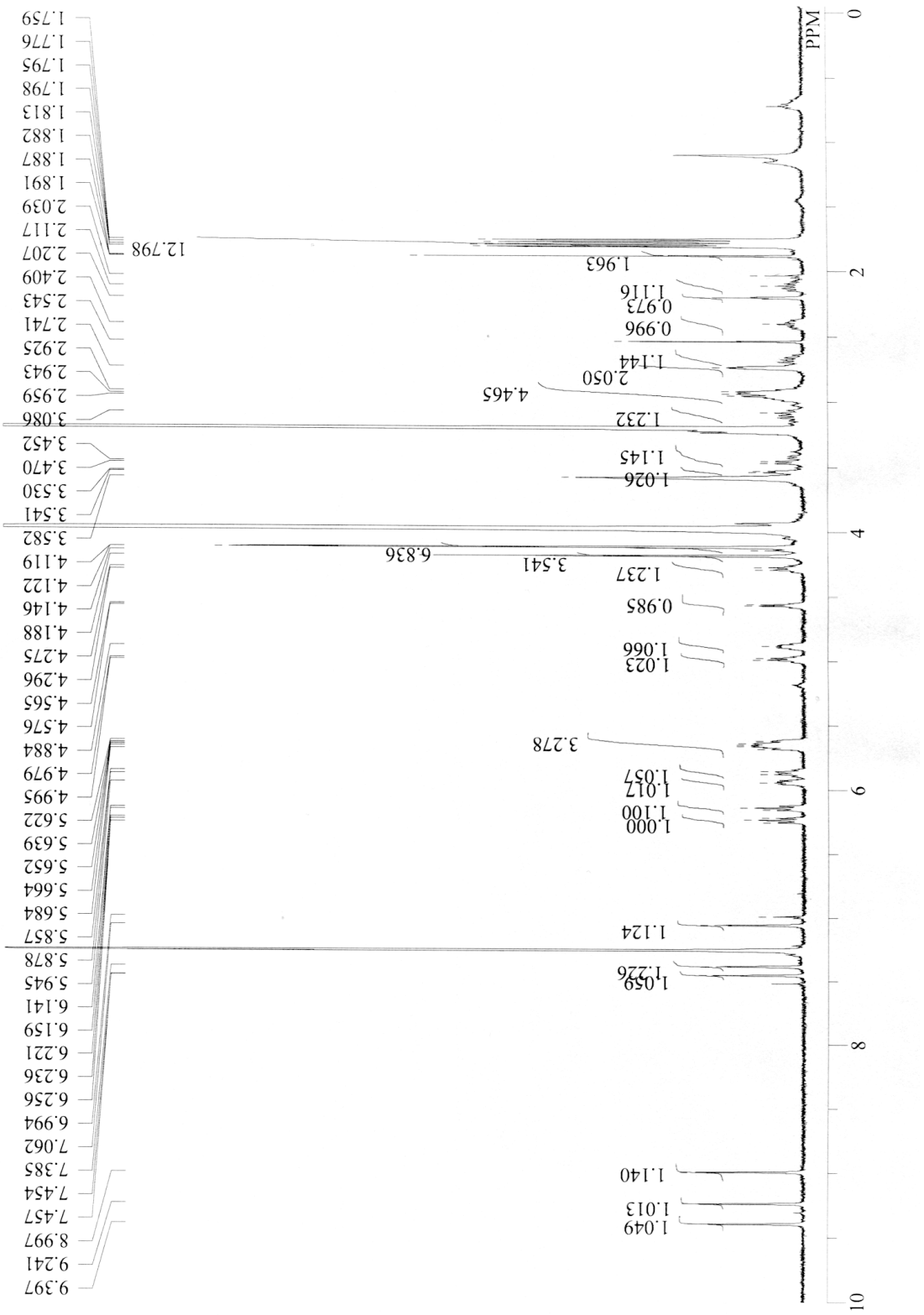


Figure S9. ^{1}H NMR spectrum of tetramer ^{TL}DNA+ **2b** in 20% $CD_3OD/CDCl_3$.

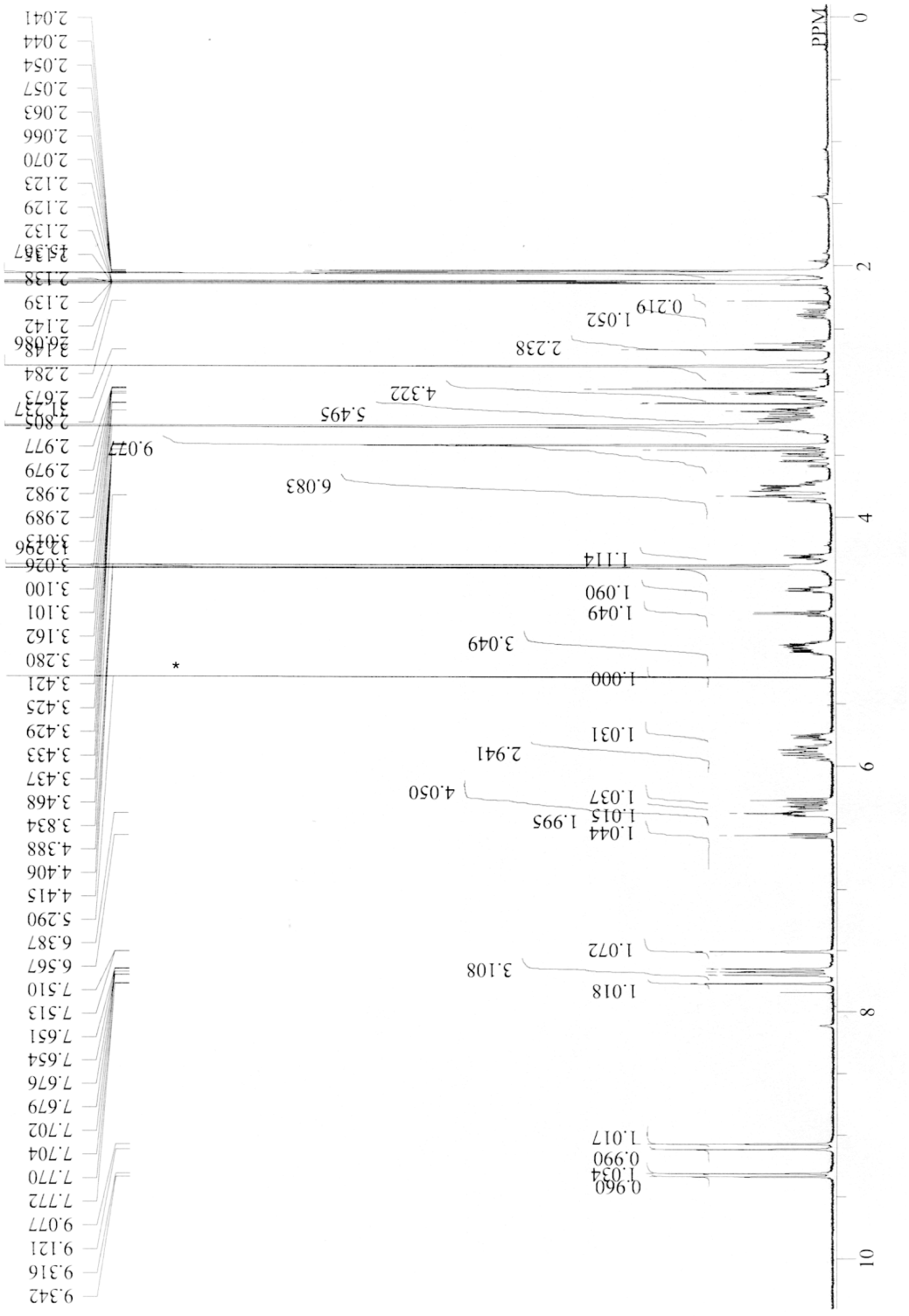


Figure S10. ^{1}H NMR spectrum of pantamer ^{TL}DNA+ **2c** with iodide counteranion in 20% CD_3OD/CD_3CN. Asterisk in the spectrum shows the peak of CH_2Br_2 used for internal standard.

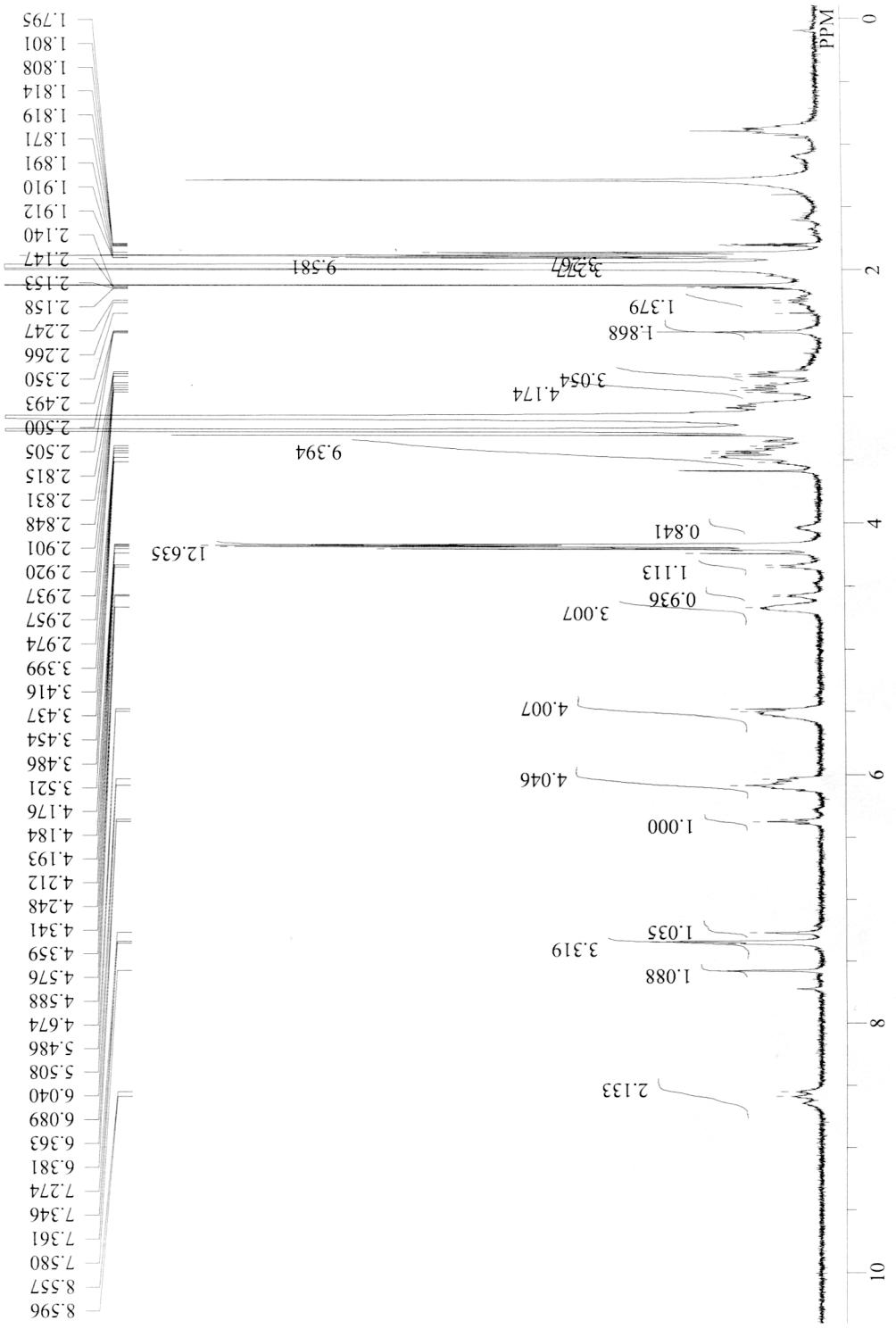


Figure S11. ^{1}H NMR spectrum of pantamer ^{TL}DNA+ **2c** with hexafluorophosphate counteranion in 20% CD_3OD/CD_3CN.