Bifacial nucleobases for hexaplex formation in aqueous solution

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General

Oligonucleotides

All conventional phosphoramidite monomers, CPG (controlled pore glass) columns, reagents for D-*a*TNA synthesis, and Poly-Pak II cartridges were purchased from Glen Research. Other reagents for the synthesis of phosphoramidite monomers were purchased from Tokyo Chemical Industry, Wako, and Aldrich. D-*a*TNA oligomers were synthesized on an ABI 3400 DNA/RNA synthesizer using D-*a*TNA phosphoramidite monomers bearing **P**, **Y**, **M** and thymine. Phosphoramidite monomer bearing thymine was synthesized as reported previously.[1] Phosphoramidite monomers bearing **P**, **Y** and **M** were synthesized as shown in Scheme S1-3. CPG tethering **P**, **Y**, **M** or thymine, which was synthesized according to Scheme S4, was used for the synthesis of D-*a*TNA oligomer bearing **P**, **Y**, **M** or thymine at 3' terminus, respectively. 3'-(6-FAM) CPG or 3'-Dabcyl CPG (Glen Research) was used for the syntheses of oligomers bearing FAM or dabcyl at 3' terminus, respectively. 5'-Fluorescein phosphoramidite or 5'-dabcyl phosphoramidite (Glen Research) was used to synthesize oligomers tethering FAM or dabcyl at 1' terminus, respectively.

For sequences containing **M** monomer, CPG was dried *in vacuo* for 15 min after the oligomer synthesis. Deprotection of Boc group on **M** monomer was performed by adding TFA (2 mL) and CH_2Cl_2 (0.5 mL) and shaking at room temperature for 4h. For sequences containing **Y** monomer, cleavage and deprotection were performed by incubating in aqueous ammonia solution (28%) at room temperature for 2 h. For other sequences, CPG was incubated in aqueous ammonia solution (28%) at 55 ºC for 8 h. Oligomers except for those containing **M** monomer were purified via Poly-Pak and then reversed-phase HPLC. Oligomers containing **M** monomer were directly purified by HPLC after deprotection. All oligomers were characterized by MALDI TOFMS (Autoflex, Bruker Daltonics). Purities of oligomers were checked by MALDI-TOFMS and HPLC. Concentrations of oligomers were determined from absorbance of FAM or dabcyl residues.

The MALDI-TOFMS data for the *a*TNA oligomers were as follows: **f-P10**: Obsd. 3498 (Calcd. for [**f-P10**+H^+]: 3497). **f-MP9**: Obsd. 3528 (Calcd. for [**f-MP9** +H^+]: 3527). **f-MP8M**: Obsd. 3558 (Calcd. for [**f-MP8M** +H^+]: 3557). **f-T10**: Obsd. 3808 (Calcd. for [**f-T10** +H^+]: 3807). **f-Y10**: Obsd. 3838 (Calcd. for [**f-Y10** +H^+]: 3837). **MP9-F**: Obsd. 3559 (Calcd. for [**MP9-f** +H^+]: 3559). **Y10-f**: Obsd. 3868 (Calcd. for [**Y10-f** +H^+]: 3869). **d-P10**: Obsd. 3392 (Calcd. for [**d-P10** +H^+]: 3390). **d-MP9**: Obsd. 3421 (Calcd. for [**d-MP9** +H^+]: 3420). **d-T10**: Obsd. 3701 (Calcd. for [**d-T10** +H^+]: 3701). **d-Y10**: Obsd. 3731 (Calcd. for [**d-Y10** +H^+]: 3730). **MP9-d**: Obsd. 3454 (Calcd. for [**MP9-d** +H^+]: 3452). **T10-d**: Obsd. 3733 (Calcd. for [**T10-d** +H^+]: 3732). **Y10-d**: Obsd. 3763 (Calcd. for [**Y10-d** +H^+]: 3762).

Fluorescence measurements

Fluorescence spectra were measured on a JASCO model FP-6500 or FP-8500. The excitation wavelength was 488 nm. Band widths were 3 nm (FP-6500) or 2.5 nm (FP-8500) for excitation and emission. Sample solutions containing D-*a*TNA oligomers were heated at 90 °C for 4 min, then slowly cooled down to 0 °C at a rate of 2.5 °C min^{-1}. Spectra were measured at 10 °C intervals. For the analyses of aminopyrimidine oligomers and thymine oligomers, sample solutions contained 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0) and 1.0 μM aminopyrimidine strand and 2.0 μM thymine strand. For the analyses of aminopyrimidine oligomers and cyanuric acid oligomers, sample solutions contained 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0) and 1.0 μM aminopyrimidine strand and 1.0 μM cyanuric acid strand unless otherwise noted. For measurements at other pH values, MES (pH 5.5-6.0) or HEPES buffer (pH 6.5-8.0) was used.

Measurement of melting temperatures (Fluorescence change)

The melting curves were recorded by monitoring emission intensity at 517 nm with 488 nm excitation except for Figures 6a and b. In Figure 6, absorbance was monitored at 320 nm and CD at 246 nm. The melting temperature (T_m) was determined from the maximum in the first derivative of the melting curve. Sample solutions containing D-*a*TNA oligomers were heated at 90 °C for 4 min, then cooling and heating curves were measured at a rate of 1.0 °C min^{-1} unless otherwise noted.

Absorption spectra measurements

Absorption spectra were measured on a JASCO model V-530. Sample solutions containing D-*a*TNA oligomers were heated at 90 °C for 4 min, then slowly cooled down to 0 °C at a rate of 1.0 °C min^{-1}. Spectra were measured at 20 °C intervals. Sample solution contained 20 mM $MgCl_2$, 10 mM MES buffer (pH 6.0) and 5.0μM aminopyrimidine strand (**f-MP9**) and 5.0 μM cyanuric acid strand (**d-Y10**). The melting curve shown in Figure 6a was measured by monitoring 320 nm absorbance versus temperature. Both cooling and heating curves were measured and temperature ramp was 1.0 °C min^{-1}.

Circular dichroism (CD) measurement

CD spectra were measured on a JASCO model J-820 equipped with programmed temperature-controllers using 10 mm quartz cells. Sample solutions containing *a*TNA oligomers were heated at 90 °C for 4 min, then slowly cooled down to 0 °C at a rate of 1.0 °C min^{-1}. Spectra were measured at 20 °C intervals. Sample solution contained 20 mM $MgCl_2$, 10 mM MES buffer (pH 6.0) and 10.0 μM aminopyrimidine strand (**f-MP9**) and 10.0 μM cyanuric acid strand (**d-Y10**). The melting curves shown in Figure 6b were measured by monitoring CD at 246 nm versus temperature. Both cooling and heating curves were measured and temperature ramp was 1.0 °C min^{-1}.

Gel electrophoresis (Native PAGE)

The samples were cooled from 95 °C to 4 °C over 1.5 h and then loaded onto a 10% polyacrylamide gel with 10% glycerol (1× TA buffer containing 15.2 mM $MgCl_2$). The concentration of each D-*a*TNA was 1.0 μM. The temperature of the gel was kept constant at 0 °C during electrophoresis by using a water-bath holder in which iced-water was circulated. After gel electrophoresis, the gel was analyzed with a Typhoon FLA-9500 bio-imaging analyzer (Fujifilm) by monitoring the emission from FAM.

Energy minimization based on molecular mechanics

The energy minimization for the molecular structure model of MP_9/Y_{10} hexaplex was carried out using MacroModel (MacroModel, version 11.6; Schrodinger, LLC: New York, 2017) applying the OPLS3 force field. Initial structure of a right-handed hexaplex of MP_9 and Y_{10} in parallel orientation was first constructed by using a graphical program. The water solvent effects were simulated using the analytical Generalized-Born/Surface-Area (GB/SA) model. Convergence threshold was set to 0.05 kJ $Å^{-1}$ mol^{-1}.

Native mass spectrometry

The **f-MP9** and **d-Y10** oligomers (60 μM) were buffer-exchanged into 100 mM ammonium acetate, pH 7.0 by passing the oligomers through Bio-Rad Micro Bio-Spin 6 columns. The buffer-exchanged oligomers were immediately analyzed by nanoflow electrospray ionization mass spectrometry using gold-coated glass capillaries made in house (approximately 2–5 μL sample loaded per analysis). Spectra were recorded on a Waters SYNAPT G2-S*i* HDMS mass spectrometer in negative ionization mode at 1.33 kV with 150 V sampling cone voltage and source offset voltage, 4 V trap and 2 V transfer collision energies, and 5 mL/min trap gas flow. The spectra were calibrated using 1 mg/mL cesium iodide and analyzed using MassLynx software (Waters).

Size exclusion chromatography (SEC)

The hexaplex formation was analysed via SEC by using similar procedure to the literature.[2] A TSKgel G2000SW column (Tosoh) was used for SEC analysis. Flow rate was 1.0 ml/min, and chromatogram was recorded by monitoring the absorbance at 480 nm. A buffer containing 20 mM $MgCl_2$ and 10 mM MES (pH 6.0) was used as a mobile phase. Each sample was heated at 90 ˚C for 4 min, and then slowly cooled to room temperature prior to SEC measurement.

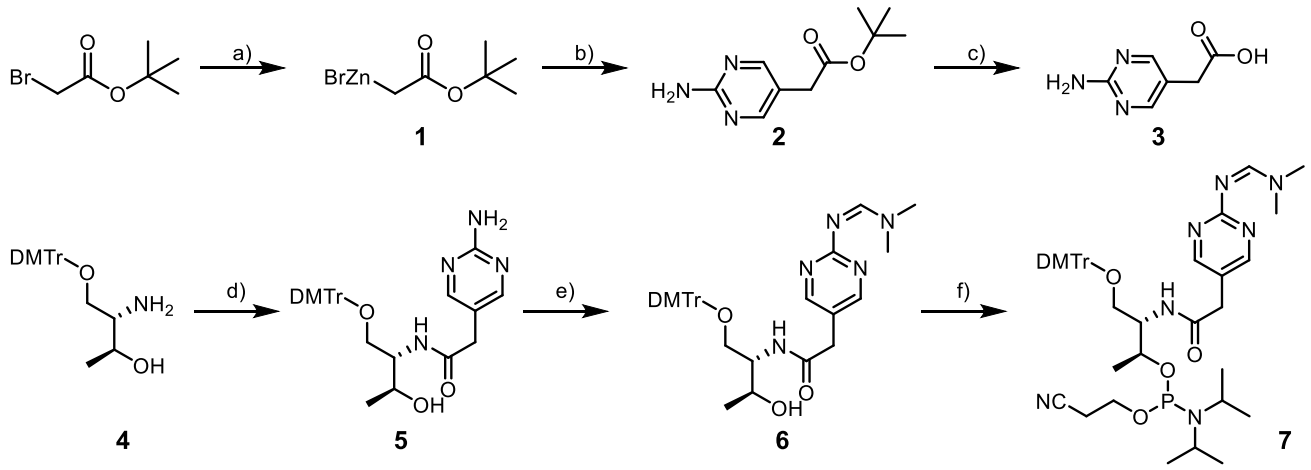
Atomic force microscopy (AFM) measurements

AFM measurements were performed with a laboratory-built high speed AFM (HS-AFM) apparatus similar to a previously reported AFM[3,4]. The detailed procedures towards HS-AFM imaging are reported

elsewhere[5]. The HS-AFM was equipped with small cantilevers (k = 0.1 – 0.2 N/m, f = 800 – 1200 kHz in water (Olympus)) and operated in tapping mode. The AFM styli were grown on each cantilever by electron beam deposition. A sample stage made of quartz glass was placed on the z-scanner, and a 1.5-mm-diameter mica disk was glued onto the sample stage. For HS-AFM measurements of the hexaplex, a freshly cleaved mica surface was treated for 3 min with 50mM $NiCl_2$. After rinsing the surface with a solution (Buffer-A) containing 10mM Tris (pH 6.0) and 5mM $MgCl_2$, a 2μL sample droplet of 1μM hexaplex was placed on the mica surface and incubated for 4 min. HS-AFM observation was performed under Buffer-A solution at room temperature.

The height of hexaplex in AFM image was analysed using SPIP image analysis software (Image Metrology) and Igor Pro (WaveMetrics).

Synthesis of aminopyrimidine monomer



Scheme S1. Synthesis of phosphoramidite tethering aminopyrimidine. Reagents and conditions: a) Zn, TMSCl, THF, reflux, 0.5 h; b) 2-amino-5-bromopyrimidine, $Pd_2(dba)_3$, X-Phos, THF, reflux, overnight, 98 %; c) TFA, CH_2Cl_2, rt, overnight, quant.; d) compound **3**, EDC, HOBt, Et_3N, DMF, rt, overnight, 50 %; e) *N*,*N*-dimethylformamide dimethyl acetal, MeOH, rt, overnight, 67 %; f) $(i\text{Pr})_2\text{NP(Cl)(OCH}_2\text{CH}_2\text{CN})$, Et_3N, CH_2Cl_2, 0 °C → rt, 1h, 74 %.

***tert*-butyl 2-(2-aminopyrimidin-5-yl)acetate** (**2**). Compound **1** was synthesized according to the previous report.[6] Compound **1** in THF (130 mL, 39.0 mmol) was added to 2-amino-5-bromopyrimidine (2.70 g, 15.6 mmol), X-Phos (744 mg, 1.56 mmol), tris(dibenzylideneacetone)dipalladium(0) (714 mg, 0.78 mmol) at room temperature under N_2 atmosphere. After vigorous stirring at 60 °C overnight, the reaction mixture was cooled to room temperature and quenched with saturated NH_4Cl solution. The aqueous layer was extracted with EtOAc (twice). The organic layers were combined, washed with brine, dried over $MgSO_4$ and the solvent was removed by evaporation, followed by silica gel column chromatography ($CHCl_3$: MeOH = 10:1, R_f = 0.24) to afford compound **2** (yellow solid, 3.11 g, yield 98 %).

^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 8.24 (s, 2H), 5.14 (s, 2H), 3.36 (s, 2H), 1.45 (s, 9H). ^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 170.0, 162.0, 158.9, 118.2, 81.8, 36.5, 28.2. HRMS(FAB) Calcd for $C_{10}H_{16}N_3O_2$ ($M+H^+$) 210.1243. Found 210.1241.

2-(2-aminopyrimidin-5-yl)acetic acid (**3**). To a stirred solution of compound **2** (3.11 g, 15.2 mmol) in CH_2Cl_2 (68 mL) was added trifluoroacetic acid (35 mL) and the mixture was stirred at room temperature. After vigorous stirring overnight, the solvent was removed by evaporation. The residue was washed with hexane. Compound **3** (yellow solid, 1.49 g, yield quant.) was used for the subsequent rection without further purification.

^{1}H-NMR [DMSO, 500 MHz] δ = 8.34 (s, 2H), 3.51 (s, 2H).

2-(2-aminopyrimidin-5-yl)-*N*-((2*S*,3*S*)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl)acetamide (**5**). Compound **4** was synthesized as reported previously.[7] To a stirred solution of compound **4** (3.89 g, 9.60 mmol) and triethylamine (17.8 mL, 128 mmol) in DMF (64 mL) were added compound **3** (980 mg, 6.40 mmol), HOBt (1.38 g, 10.2 mmol) and EDC (1.96 g, 10.2 mmol). After the stirring at room temperature overnight, DMF was removed by evaporation. Then $CHCl_3$ was added and washed with saturated $NaHCO_3$(twice). After drying over $MgSO_4$, the solvent was removed by evaporation, followed by silica gel column chromatography ($CHCl_3$: MeOH = 5:1 (3% triehtylamine was added), R_f = 0.51) to afford compound **5** (yellow solid, 1.75 g, yield 50 %).
^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 8.21 (s, 2H), 7.19-7.35 (m, 9H), 6.81-6.83 (m, 4H), 6.34 (br, 1H), 5.25 (br, 2H), 4.05-4.10 (m, 1H), 3.93-3.95 (s, 1H), 3.77 (s, 6H), 3.34-3.38 (m, 3H), 3.26 (dd, *J* = 3.5, 9.5 Hz, 1H), 1.09 (d, *J* = 6 Hz, 3H). ^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 170.3, 162.4, 158.7, 144.4, 135.5, 135.4, 130.0 (2C), 128.1, 128.0, 127.2, 118.1, 113.4, 86.8, 68.4, 64.9, 55.3, 53.9, 37.3, 20.2. HRMS(FAB) Calcd for $C_{31}H_{35}N_4O_5$ ($M+H^+$) 543.2607. Found 543.2605.

***N*-((2*S*,3*S*)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl)-2-(2-(((*Z*)-(dimethylamino)methylene)amino)pyrimidin-5-yl)acetamide** (**6**). Similar protocol to the previous report was used for the protection of amino group of aminopyrimidine.[8] To a stirred solution of compound **5** (2.71 g, 5.0 mmol) in dry MeOH (50 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (33.5 mL, 250 mmol) at room temperature under N_2 atmosphere. After vigorous stirring at room temperature overnight, the solvent was removed by evaporation, followed by silica gel column chromatography ($CHCl_3$: MeOH = 8:1 (0.5% triehtylamine was added), R_f = 0.41) to give compound **6** (yellow solid, 2.01 g, yield 67 %).
^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 8.57 (s, 1H), 8.36 (s, 2H), 7.18-7.36 (m, 9H), 6.80-6.82 (m, 4H), 6.65 (br, 1H), 4.07-4.11 (m, 1H), 3.96-3.98 (m, 1H), 3.77 (s, 6H), 3.42 (s, 2H), 3.38 (dd, *J* = 5, 9.5 Hz, 1H), 3.23 (dd, *J* = 4, 9.5 Hz, 1H), 3.10 (s, 6H), 1.09 (d, *J* = 6.5 Hz, 3H). ^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 170.1, 165.9, 158.8, 158.7, 158.6, 158.4, 144.5, 135.7, 135.5, 130.0, 128.1, 128.0, 127.1, 121.7, 113.4, 86.7, 68.1, 64.8, 55.3, 54.2, 41.2, 37.6, 35.2, 20.2.
HRMS(FAB) Calcd for $C_{34}H_{40}N_5O_5$ ($M+H^+$) 598.3029. Found 598.3022.

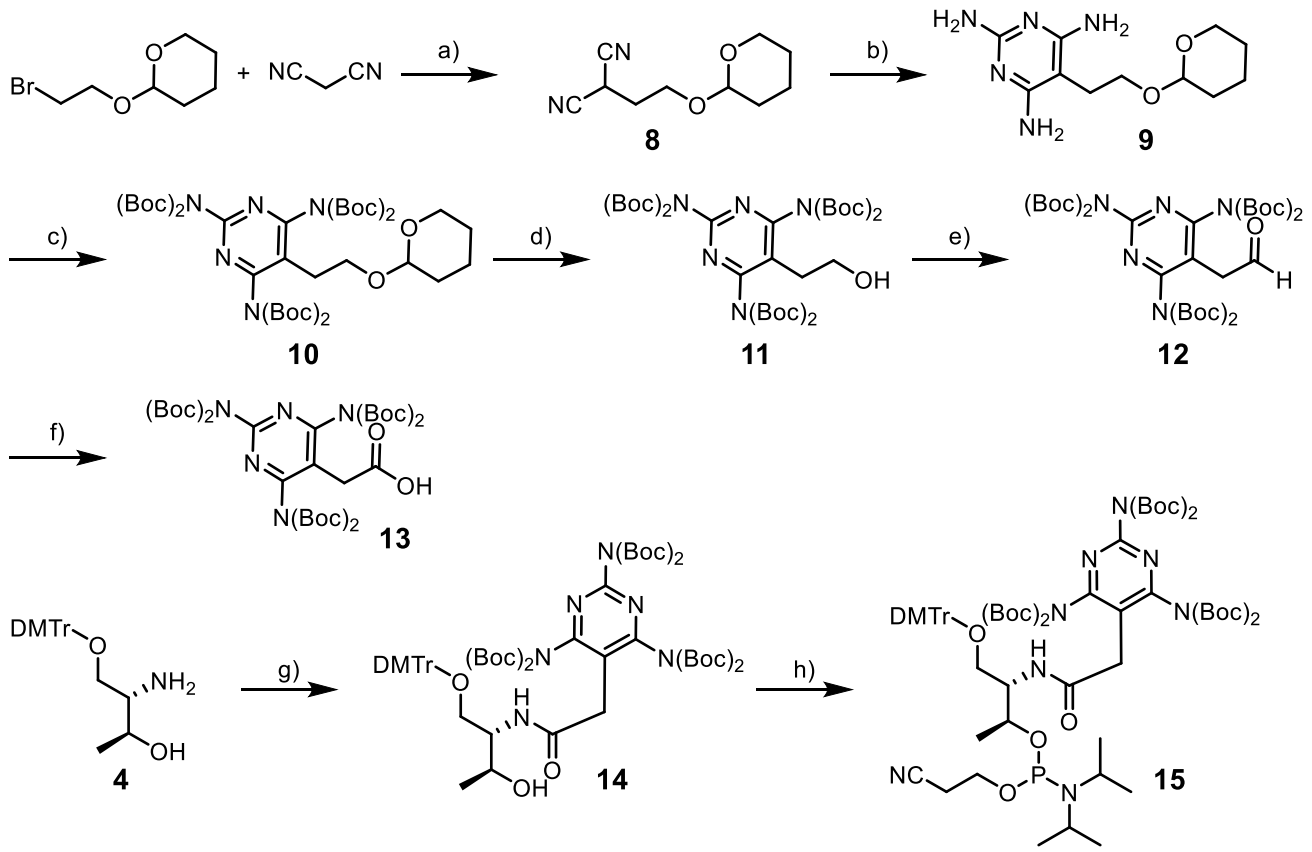
(2*S*,3*S*)-4-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-(2-(2-(((*Z*)-(dimethylamino)methylene)amino)pyrimidin-5-yl)acetamido)butan-2-yl (2-cyanoethyl) diisopropylphosphoramidite (**7**). In 20 mL dry dichloromethane under nitrogen, compound **6** (1.19 g, 2.0 mmol) and triethylamine (1.39 mL, 10.0 mmol) were reacted with 2-cyanoethyl diisopropylchlorophosphoramidite (0.89 mL, 4.0 mmol) at 0 °C. After 30 min, the reaction mixture was diluted with excess $CHCl_3$ and washed with saturated aqueous solutions of $NaHCO_3$. After drying over $MgSO_4$, the solvent was removed by evaporation, followed by silica gel

column chromatography ($CHCl_3$: Acetone = 1:3 (3% triehtylamine was added), R_f = 0.36) to afford compound **7** (white solid, 1.14 g, yield 73 %).

^{31}P-NMR [202 MHz, $CDCl_3$] δ =148.5, 147.9.

HRMS(FAB) Calcd for $C_{43}H_{57}N_7O_6P$ ($M+H^+$) 798.4108. Found 798.4129.

Synthesis of triaminopyrimidine monomer



Scheme S2. Synthesis of phosphoramidite tethering triaminopyrimidine. Reagents and conditions: a) NaH, THF, rt, overnight, 41%; b) Guanidine Hydrochloride, NaO^{t}Bu, EtOH, reflux, overnight, 94 %; c) $(Boc)_2O$, DMAP, Et_3N, THF, rt, 28 h, 72 %; d) *p*-TsOH, DMAP, MeOH, rt, 5 h, 93 %; e) Dess-Martin periodinane, CH_2Cl_2, rt, 4 h, 96 %; f) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, rt, overnight, quant.; g) compound **13**, EDC, HOBt, Et_3N, DMF, rt, overnight, 52 %; h) $(i\text{Pr})_2\text{NP(Cl)(OCH}_2\text{CH}_2\text{CN)}$, Et_3N, CH_2Cl_2, 0 ^{o}C → rt, 1h, 92 %.

2-(2-((tetrahydro-2*H*-pyran-2-yl)oxy)ethyl)malononitrile (**8**). Malononitrile (1.26 ml, 20.0 mmol) in dry THF (20 mL) was added dropwise to 60% NaH (480 mg, 12.0 mmol) and dry THF (12 mL) at room temperature under N_2 atmosphere. 2-(2-Bromoethoxy)tetrahydro-2*H*-pyran (1.53 mL, 10.0 mmol) in dry THF (5 mL) was added dropwise. After vigorous stirring at room temperature overnight, the reaction was quenched with 5 % aqueous NH_4Cl until pH~8.0. THF was removed by evaporation. The resulting material was dissolved in EtOAc and the organic layer was washed with H_2O and brine. After drying over $MgSO_4$, the solvent was removed by evaporation, followed by silica gel column chromatography (Hexane : EtOAc = 2:1, R_f = 0.29[I_2]) to afford compound **8** (colorless oil, 803 mg, yield 41 %). ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 4.53 (t, *J* = 4 Hz, 1H), 4.04 (t, *J* = 7 Hz, 1H), 3.88-3.92 (m, 1H), 3.77-3.88 (m, 1H), 3.56-3.60 (m, 1H), 3.47-3.48 (m, 1H), 2.23-2.28 (m, 2H), 1.66-1.80 (m, 2H), 1.46-1.53 (m, 4H).

^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 112.8, 112.7, 99.7, 63.0, 62.5, 31.7, 30.5, 25.3, 19.8, 19.7.
HRMS(FAB) Calcd for $C_{10}H_{15}N_2O_2$ ($M+H^+$) 195.1134. Found 195.1140.

5-(2-((tetrahydro-2*H*-pyran-2-yl)oxy)ethyl)pyrimidine-2,4,6-triamine (**9**). A mixture of Guanidine Hydrochloride (326 mg, 3.41 mmol) and NaO^{t}Bu (328 mg, 3.41 mmol) in EtOH (4 mL) were stirred at room temperature for 30 min under N_2 atmosphere. Then to the reaction mixture was added compound **8** in EtOH (3.8 mL). The reaction mixture was refluxed at 85 °C overnight. The reaction was then cooled to room temperature and quenched with 1 *M* aqueous HCl until pH~8.0. Then extracted with EtOAc (twice) and organic layer was dried over $MgSO_4$. Compound **9** (yellow solid, 740 mg, yield 94 %) was obtained without further purification. Obtained product was used for next reaction without further purification.

tri-*tert*-butyl (5-(2-((tetrahydro-2*H*-pyran-2-yl)oxy)ethyl)pyrimidine-2,4,6-triyl)tris((*tert*-butoxycarbonyl)carbamate) (**10**). Compound **9** (2.03 g, 8.01 mmol), DMAP (1.92 g, 15.7 mmol), dry THF (445 mL), Et_3N (24.1 mL, 173.0 mmol), Boc_2O (36.0 mL, 157.0 mmol) were stirred at room temperature for 28 h under N_2 atmosphere. The reaction was quenched with 5 % aqueous $NaHCO_3$ until pH~8.0. THF was removed by evaporation. The resulting material was dissolved in Et_2O and the organic layer was washed with 5 % aqueous $NaHCO_3$, H_2O and brine. After drying over $MgSO_4$, the solvent was removed by evaporation, followed by silica gel column chromatography (Hexane : EtOAc = 2:1, R_f = 0.45) to afford compound **10** (white solid, 4.45 g, yield 72 %). ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 4.57 (t, *J* = 4 Hz, 1H), 3.79-3.87 (m, 2H), 3.47-3.56 (m, 2H), 2.81-2.85 (m, 2H), 1.82-1.86 (m, 1H), 1.70-1.74 (m, 1H), 1.44-1.55 (m, 58H). ^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 162.3, 156.5, 150.6, 150.0, 125.1, 99.1, 84.1, 83.4, 64.7, 62.4, 30.7, 27.9, 27.8, 27.4, 25.4, 19.7. HRMS(FAB) Calcd for $C_{41}H_{67}N_5NaO_{14}$ ($M+Na^+$) 876.4582. Found 876.4588.

tri-*tert*-butyl (5-(2-hydroxyethyl)pyrimidine-2,4,6-triyl)tris((*tert*-butoxycarbonyl)carbamate) (**11**). Compound **10** (3.93 g, 4.60 mmol), *p*-TsOH (158 mg, 0.92 mmol) and MeOH (115 mL) were stirred at room temperature for 5 h under N_2 atmosphere. The reaction was quenched with 5 % aqueous $NaHCO_3$, and MeOH was removed by evaporation. The resulting material was dissolved in Et_2O and the organic layer was washed with H_2O and brine. The organic layer was dried over $MgSO_4$ and the solvent was removed by evaporation. Compound **11** (white solid, 3.33 g, yield 93 %) was used for next reaction without further purification.
^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 3.82-3.83 (m, 2H), 2.77 (t, *J* = 6.5 Hz, 2H), 1.44-1.45 (m, 54H).
^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 162.4, 156.7, 150.5, 150.2, 125.4, 84.5, 83.6, 60.5, 30.3, 27.9 (2C).
HRMS(FAB) Calcd for $C_{36}H_{60}N_5O_{13}$ ($M+H^+$) 770.4188. Found 770.4183.

tri-*tert*-butyl (5-(2-oxoethyl)pyrimidine-2,4,6-triyl)tris((*tert*-butoxycarbonyl)carbamate) (**12**). Similar procedures to the previous report was used for the synthesis of compound **12**.[9] Compound **11** (1.46 g, 1.89 mmol), Dess-Martin periodinane (1.60 g, 3.78 mmol), CH_2Cl_2 (73 mL) were stirred at room temperature for 4 h under N_2 atmosphere. The reaction was quenched with 5 % aqueous $NaHCO_3$, diluted with CH_2Cl_2 and washed with 5 % aqueous $NaHCO_3$ (three times) and brine. After drying over $MgSO_4$, the solvent was removed by evaporation, followed by silica gel column chromatography (Hexane : EtOAc = 2:1, R_f = 0.42) to afford compound **12** (white solid, 1.39 g, yield 96 %).
^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 9.50 (t, *J* = 2 Hz, 1H), 3.46 (d, *J* = 2 Hz, 2H), 1.45 (s, 18H), 1.44 (s, 36H). ^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 196.3, 162.5, 157.5, 150.5, 149.9, 120.8, 84.8, 83.8, 40.9, 27.9 (2C). HRMS(FAB) Calcd for $C_{36}H_{58}N_5O_{13}$ ($M+H^+$) 768.4031. Found 768.4030.

2-(2,4,6-tris(bis(*tert*-butoxycarbonyl)amino)pyrimidin-5-yl)acetic acid (**13**). $NaClO_2$ (214.0 mg, 2.37 mmol) and NaH_2PO_4 (284 mg, 2.37 mmol) in H_2O (1.82 mL) were added dropwise to compound **12** (1.39 g, 1.82 mmol), 2-methyl-2-butene (1.26 mL, 1.81 mmol) and *t*-BuOH, (27 mL) at room temperature under N_2 atmosphere. After vigorous stirring at room temperature overnight, the reaction was quenched with H_2O and extracted with EtOAc (twice). The organic layer was dried over $MgSO_4$ and the solvent was removed by evaporation. Compound **13** (white solid, 1.49 g, yield quant.) was used for next reaction without further purification.
^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 3.57 (s, 2H), 1.43-1.45 (m, 54H).
HRMS(FAB) Calcd for $C_{36}H_{57}N_5NaO_{14}$ ($M+Na^+$) 806.3800. Found 806.3798.

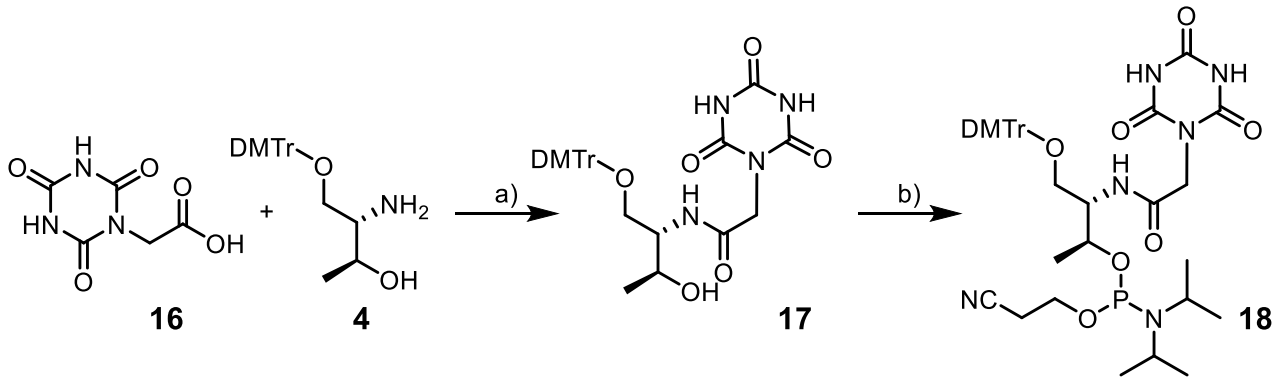
tri-*tert*-butyl(5-(2-(((2*S*,3*S*)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl)amino)-2-oxoethyl)pyrimidine-2,4,6-triyl)tris((*tert*-butoxycarbonyl)carbamate) (**14**)

To a stirred solution of compound **4** (1.89 g, 4.60 mmol) and triethylamine (3.2 mL, 23.0 mmol) in DMF (46 mL) was added compound **13** (3.61 g, 4.60 mmol), HOBt (994 mg, 7.36 mmol) and EDC (1.41 g, 7.36 mmol), and the mixture was stirred at room temperature overnight. DMF was removed by evaporation. Then $CHCl_3$ was added, and washed with brine. After drying over $MgSO_4$, solvent was removed by evaporation. Purification by silica gel column chromatography (Hexane : EtOAc = 1:1 (3% triehtylamine was added), R_f = 0.5) afforded compound **14** (white solid, 2.70 g, yield 52 %).
^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 7.37-7.39 (m, 2H), 7.25-7.28 (m, 6H), 7.18-7.21 (m, 1H), 6.79-6.81 (m, 4H), 5.94 (d, 8.5 Hz, 1H), 3.96-4.00 (m, 1H), 3.91-3.95 (m, 1H), 3.78 (s, 6H), 3.47 (s, 2H), 3.09-3.17 (m, 2H), 2.68 (d, *J* = 6 Hz, 1H) , 1.43 (s, 36H), 1.36 (s, 18H), 1.07 (d, *J* = 6.5 Hz, 3H). ^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 167.0, 162.3, 158.6, 157.1, 150.5, 150.0, 144.8, 136.0, 135.9, 130.1, 128.2, 126.9, 122.9, 113.3, 86.4, 84.8, 83.7, 77.4, 67.1, 62.9, 55.3, 55.2, 34.7, 27.9, 27.8, 20.2.

HRMS(ESI) Calcd for $C_{61}H_{84}N_6NaO_{17}$ ($M+Na^+$) 1195.5785. Found 1195.5770.

tri-*tert*-butyl (5-(2-(((2*S*,3*S*)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-(((2-cyanoethoxy)(diisopropylamino)phosphaneyl)oxy)butan-2-yl)amino)-2-oxoethyl)pyrimidine-2,4,6-triyl)tris((*tert*-butoxycarbonyl)carbamate) (**15**). In 7.5 mL dry dichloromethane under nitrogen, compound **14** (884 mg, 0.75 mmol) and triethylamine (0.52 mL, 3.75 mmol) were reacted with 2-cyanoethyl diisopropylchlorophosphoramidite (0.33 mL, 1.5 mmol) at 0 ºC. After stirring for 30 min at room temperature, the reaction mixture was diluted with excess $CHCl_3$ and washed with saturated aqueous solutions of $NaHCO_3$(twice). After drying over $MgSO_4$, the solvent was removed by evaporation, followed by silica gel column chromatography (Hexane : EtOAc = 2:1 (3% triehtylamine was added), R_f = 0.19) to afford compound **15** (white solid, 952 mg, yield 92 %).
^{31}P-NMR [202 MHz, $CDCl_3$] δ =148.8, 149.5.
HRMS(ESI) Calcd for $C_{70}H_{101}N_8NaO_{18}P$ ($M+Na^+$) 1395.6864. Found 1395.6868.

Synthesis of cyanuric acid monomer



Scheme S3. Synthesis of phosphoramidite tethering cyanuric acid. Reagents and conditions: a) EDC, HOBt, Et_3N, DMF, rt, overnight, 56 %; b) $(iPr)_2NP(Cl)(OCH_2CH_2CN)$, Et_3N, CH_2Cl_2, 0 °C →rt, 1h, 69 %.

***N*-((2*S*,3*S*)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl) -2-(2,4,6-trioxo-1,3,5-triazinan-1-yl)acetamide** (**17**). Compound **16** was synthesized according to the previous report.[10] To a stirred solution of compound **4** (3.26 g, 8.0 mmol) and triethylamine (5.6 mL, 40.0 mmol) in DMF (80 mL) was added compound **16** (1.50 g, 8.0 mmol), HOBt (1.73 g, 12.8 mmol) and EDC (2.45 g, 12.8 mmol) and the mixture was stirred at room temperature overnight. DMF was removed by evaporation. Then $CHCl_3$ was added and washed with saturated $NaHCO_3$. After drying over $MgSO_4$, the solvent was removed by evaporation, followed by silica gel column chromatography ($CHCl_3$: MeOH = 10:1 (3% triehtylamine was added), R_f = 0.14) to afford compound **17** (white solid, 2.56 g, yield 56 %).

^{1}H-NMR [DMSO, 500 MHz] δ = 11.54 (s, 2H), 7.95 (d, *J* = 8.5 Hz, 1H), 7.37-7.38 (m, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.19-7.25 (m, 5H), 6.88-6.89 (m, 4H), 4.56 (d, *J* = 5 Hz, 2H), 4.34 (s, 2H), 3.86-3.90 (m, 2H), 3.74 (s, 6H), 3.07 (dd, *J* = 5.5, 9 Hz, 1H), 2.87 (dd, *J* = 6, 8.5 Hz, 1H), 0.93 (d, *J* = 6 Hz, 3H). ^{13}C-NMR [DMSO, 126 MHz] δ = 166.4, 158.0 (2C), 149.8, 148.7, 145.1, 135.8, 129.8, 127.8, 126.5, 113.2, 85.1, 65.0 (2C), 55.0, 54.3, 42.6, 20.2. HRMS(FAB) Calcd for $C_{30}H_{32}N_4O_8$ (M^+) 576.2220. Found 576.2219.

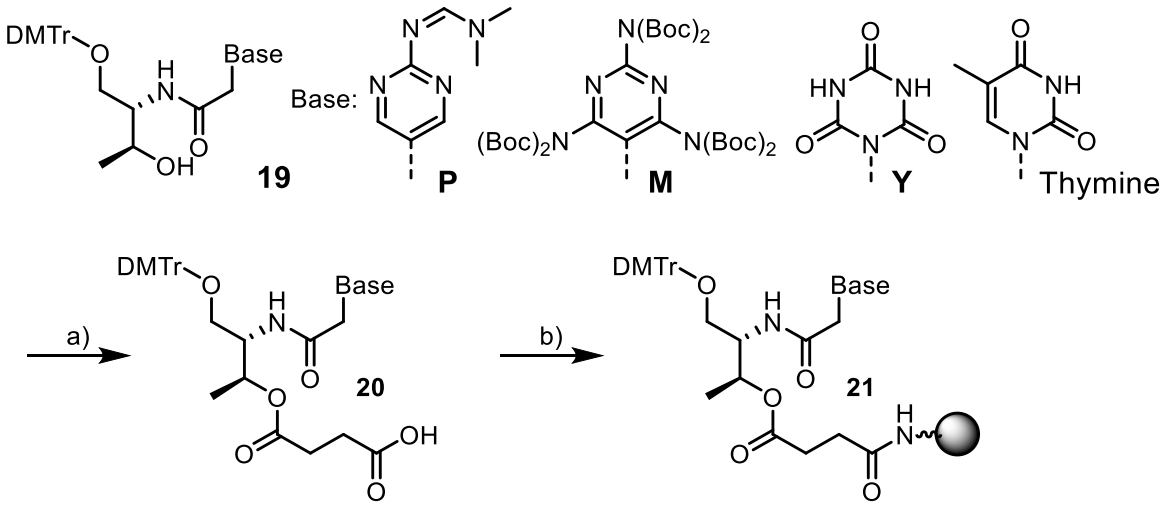
(2*S*,3*S*)-4-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-(2-(2,4,6-trioxo-1,3,5-triazinan-1-yl)acetamido)butan-2-yl (2-cyanoethyl) diisopropylphosphoramidite (**18**). In 10 mL dry dichloromethane under nitrogen, compound **17** (575 mg, 1.0 mmol) and triethylamine (0.76 mL, 5.0 mmol) were reacted with 2-cyanoethyl diisopropylchlorophosphoramidite (0.45 mL, 2.0 mmol) at 0 °C. After 30 min of stirring at room temperature, the reaction mixture was diluted with excess $CHCl_3$ and washed with saturated aqueous solutions of $NaHCO_3$(twice). After drying over $MgSO_4$, the solvent was

removed by evaporation, followed by silica gel column chromatography ($CHCl_3$: Acetone = 1:1 (3% triehtylamine was added), R_f = 0.06) to afford compound **18** (white solid, 537 mg, yield 69 %).

^{31}P-NMR [202 MHz, $CDCl_3$] δ =147.9, 148.8.

HRMS(ESI) Calcd for $C_{39}H_{50}N_6O_9P$ ($M+H^+$) 777.3371. Found 777.3377.

Synthesis of CPG modified with D-*a*TNA monomers



Scheme S4. Synthesis of CPG bearing **P**, **M**, **Y** and thymine monomers. Reagents and conditions: a) succinic anhydride, Et_3N, CH_2Cl_2, rt, overnight; b) native amino lcaa CPG, *N*, *N*'-diisopropylcarbodiimide, HOBt, pyridine, rt, overnight.

General procedures for the syntheses of CPG bearing D-*a*TNA is as follows:

Compound 20. A mixture of **19** (0.30 mmol), succinic anhydride (45.0 mg, 0.45 mmol) and Et_3N (0.13 mL, 0.91 mmol) in dry CH_2Cl_2 (3 mL) were stirred at room temperature overnight under N_2 atmosphere. The reaction was quenched with 4 % citric acid aqueous solution. Then extracted with $CHCl_3$ (three times) and the combined organic layers were removed by evaporation and drying *in vacuo*. Obtained product **20** was used for next reaction without further purification.

CPG modified with D-*a*TNA monomers (**21**). A 2 mL CH_3CN solution of native amino lcaa CPG 500 Å (500 mg, ChemGenes Corp.), HOBt (2 mg), pyridine (100 μl) and *N*, *N*'-diisopropylcarbodiimide (24 μl) in a polyethylene syringe equipped with a filter was shaken at room temperature for 30 min. Then, compound **20** (0.055 mmol) was added to the reaction mixture and further shaking overnight. After overnight, the CPG was filtered off and washed with methanol and $CHCl_3$. After drying *in vacuo*, THF (4 mL), 2,6-lutidine (0.50 mL), acetic anhydride (0.50 mL) and Cap B (16% methylimidazole in tetrahydrofuran) capping reagents (5 mL) were added to the CPG for the capping of amino groups. The reaction mixture was shaken at room temperature for 1 h. Then, the CPG was filtered off and washed with methanol and $CHCl_3$. The CPG was dried *in vacuo* and directly used for D-*a*TNA oligomer synthesis.

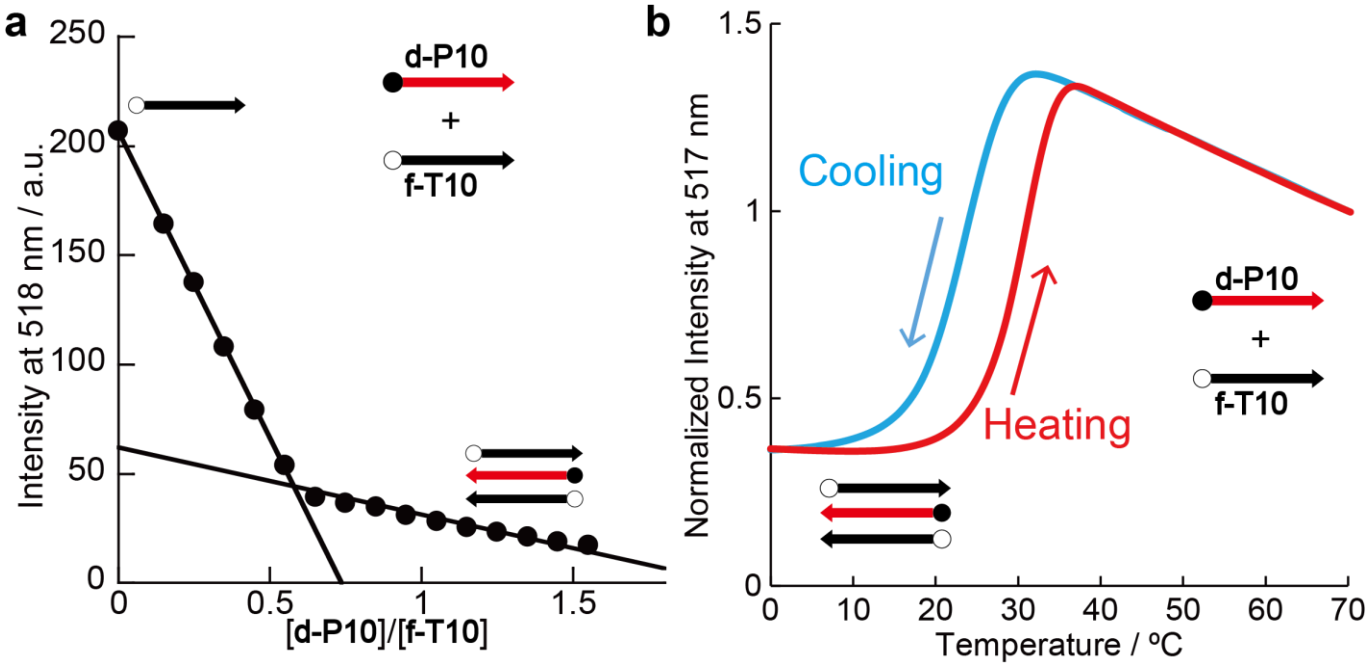


Figure S1. Triplex formation of **d-P10** and **f-T10**. (a) Titration of 1.0 µM **f-T10** with **d-P10** over the range from 0 to 1.55 µM. (b) Cooling and heating curves of **d-P10**/**f-T10** triplex monitored by emission intensity of FAM. Conditions for (a): [**f-T10**] = 1.0 µM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0). Conditions for (b): [**d-P10**] = 1.0 µM, [**f-T10**] = 2.0 µM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0).

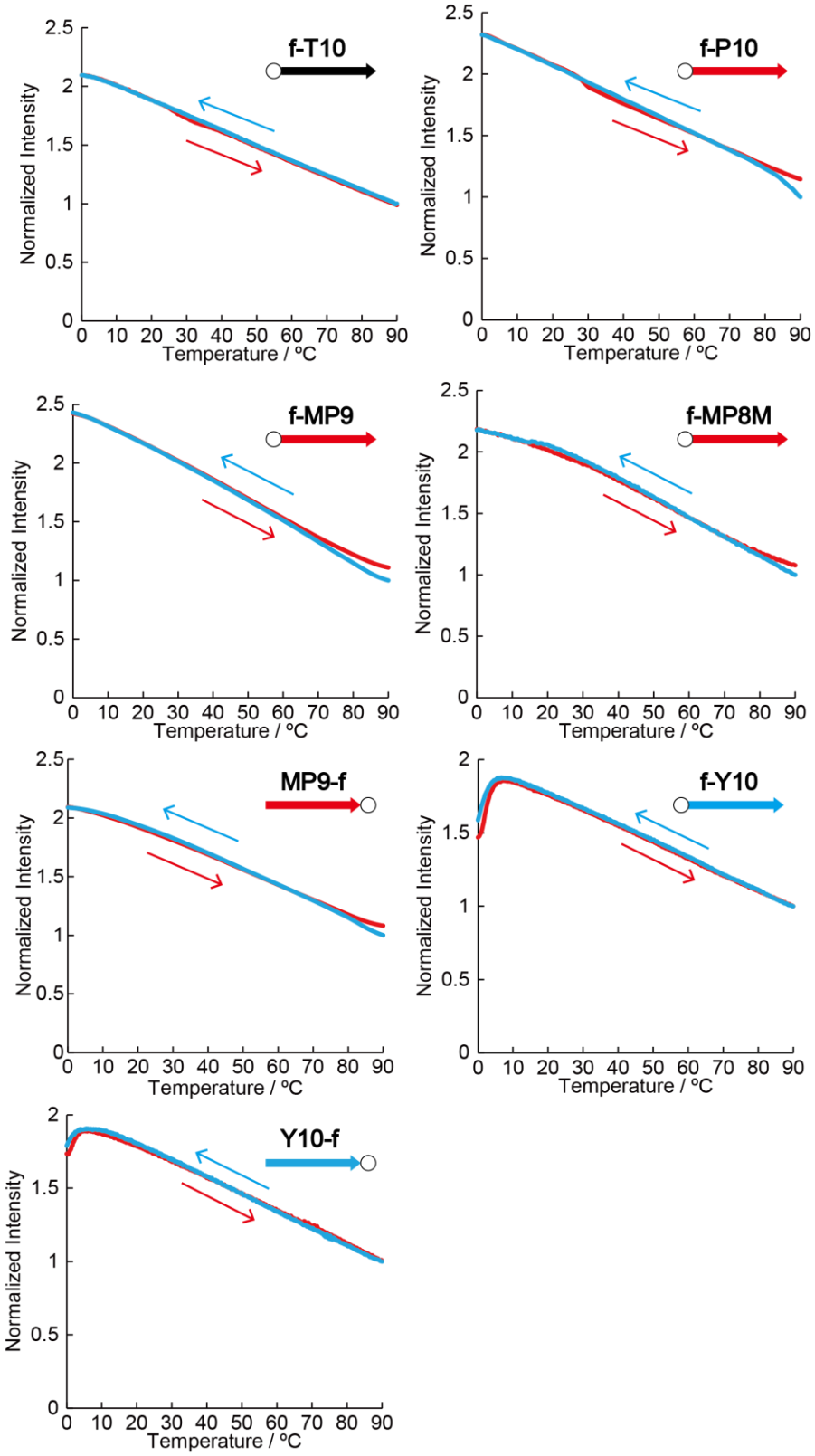


Figure S2. Cooling and heating curves of single strands. Conditions: [each strand] = 1.0 μM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0).

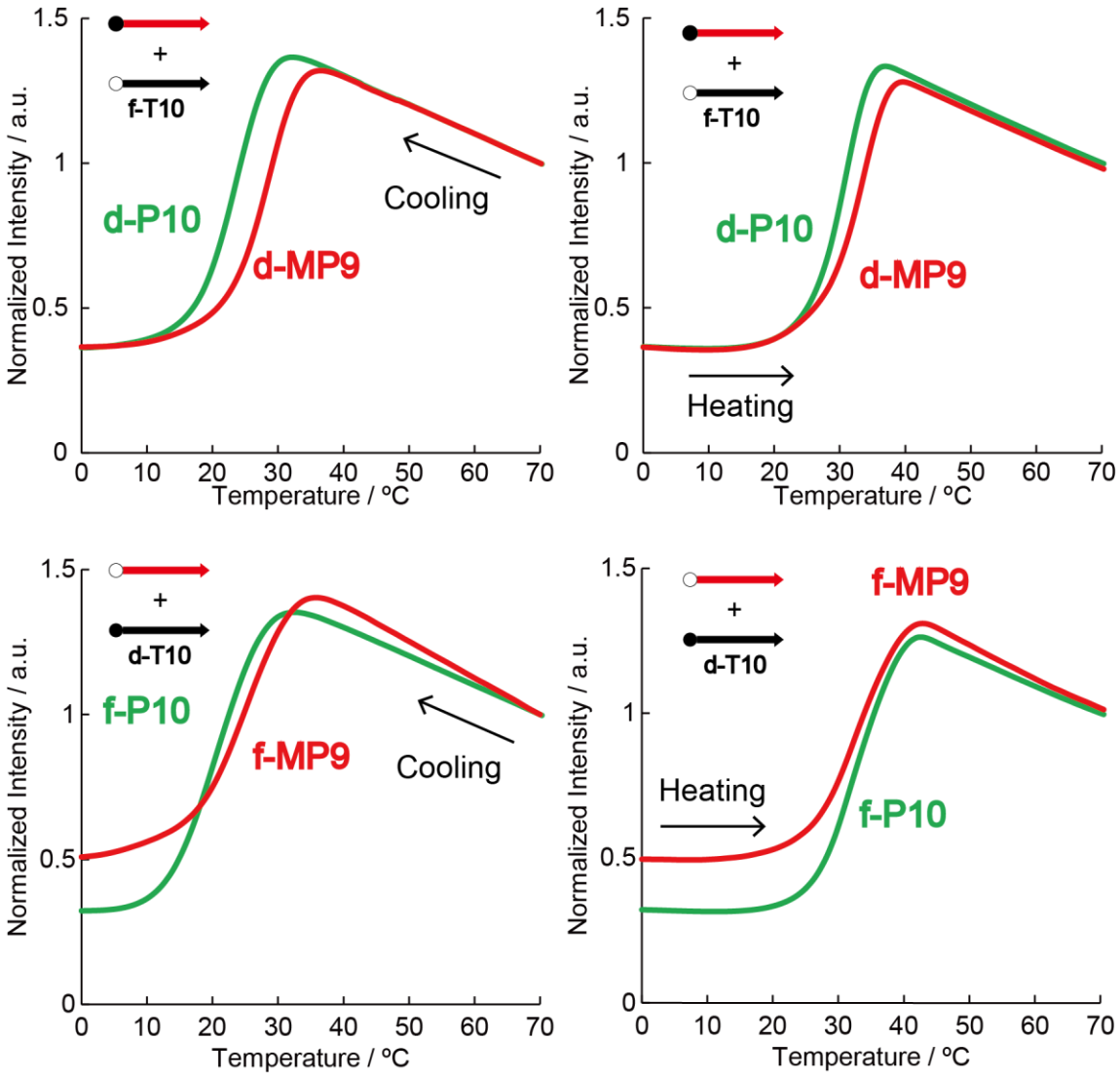


Figure S3. Effects of triaminopyrimidine moiety on the triplex stability. Both cooling (left) and heating (right) curves are shown. Top: **d-P10/f-T10** and **d-MP9/f-T10** triplexes, bottom: **d-P10/f-T10** and **d-MP9/f-T10** triplexes. Conditions: [aminopyrimidine strand] = 1.0 µM, [thymine strand] = 2.0 µM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0).

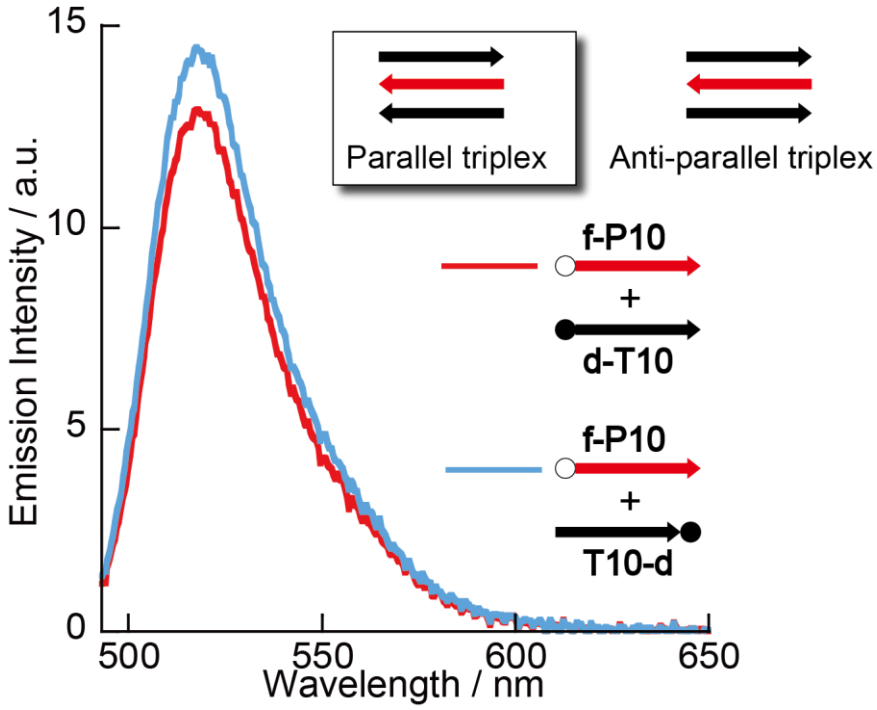


Figure S4. Emission spectra of triplexes tethering a quencher at different positions. Emission spectra of **f-P10**/**d-T10** and **f-P10**/**T10-d** triplexes at 0 ºC. Schematic illustration of parallel and anti-parallel triplexes are also shown. When the triplex is formed in "anti-parallel" orientation, i.e. two thymine strands are orientated in the same direction, quenching efficiencies of **f-P10**/**d-T10** and **f-P10**/**T10-d** should be much different with each other. The result suggested that aminopyrimidine and thymine oligomers formed a "parallel-type" triplex. Conditions: [**f-P10**] = 1.0 µM, [thymine strand] = 2.0 µM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0).

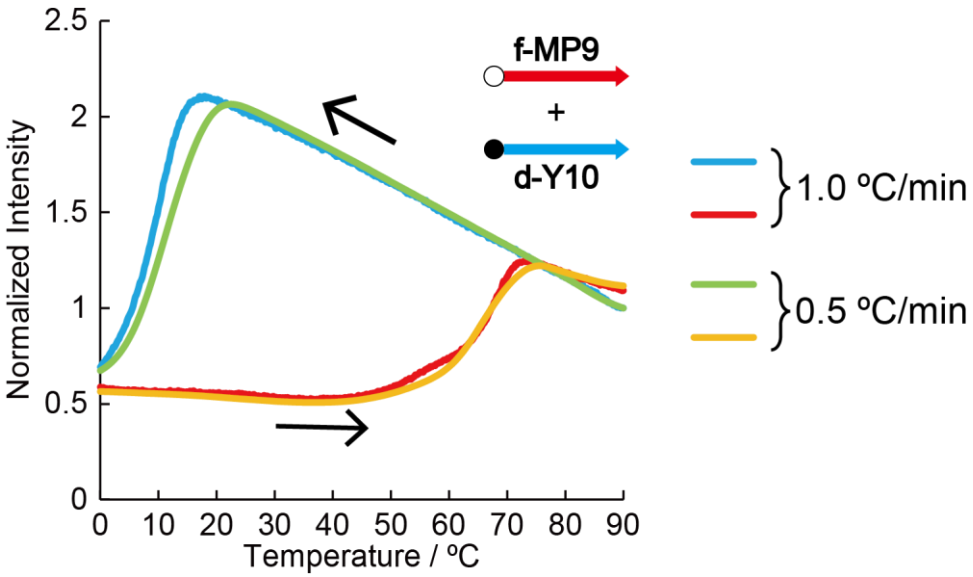


Figure S5. Effects of temperature ramp on annealing and melting behaviors of **f-MP9**/**d-Y10** hexaplex. Conditions: [**f-MP9**] = [**d-Y10**] = 1.0 μM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0).

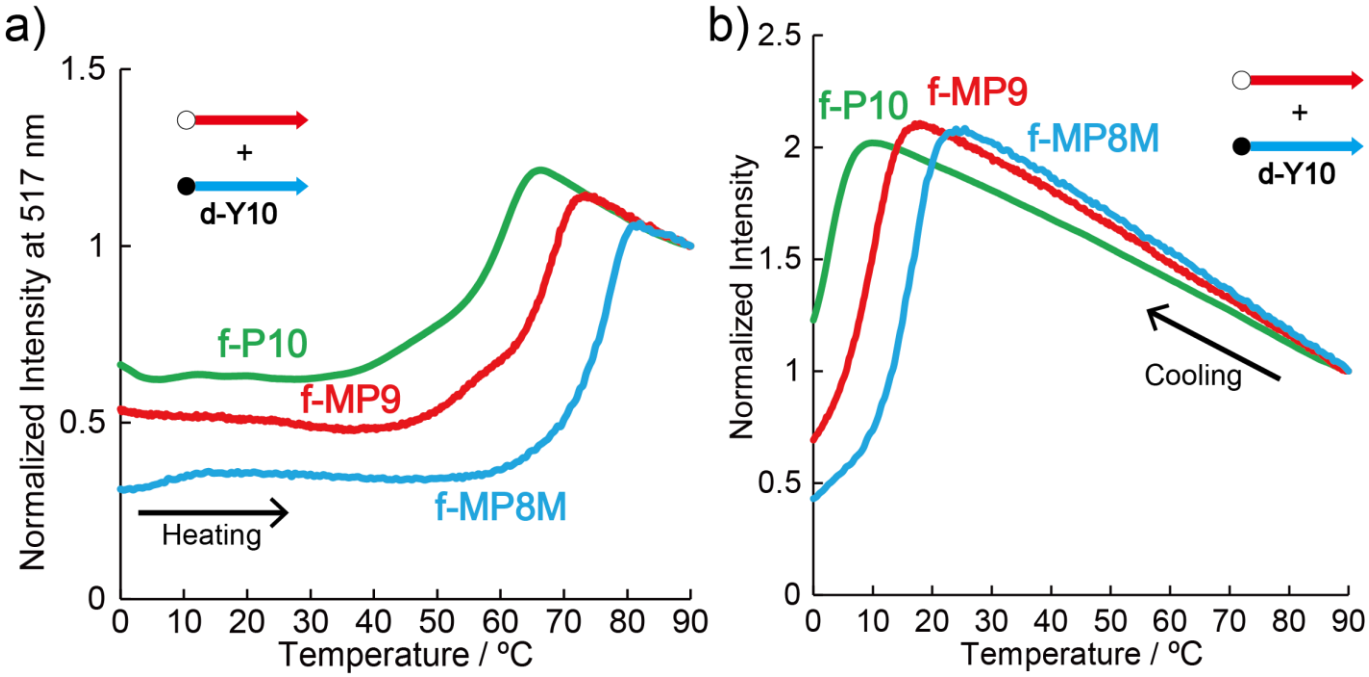
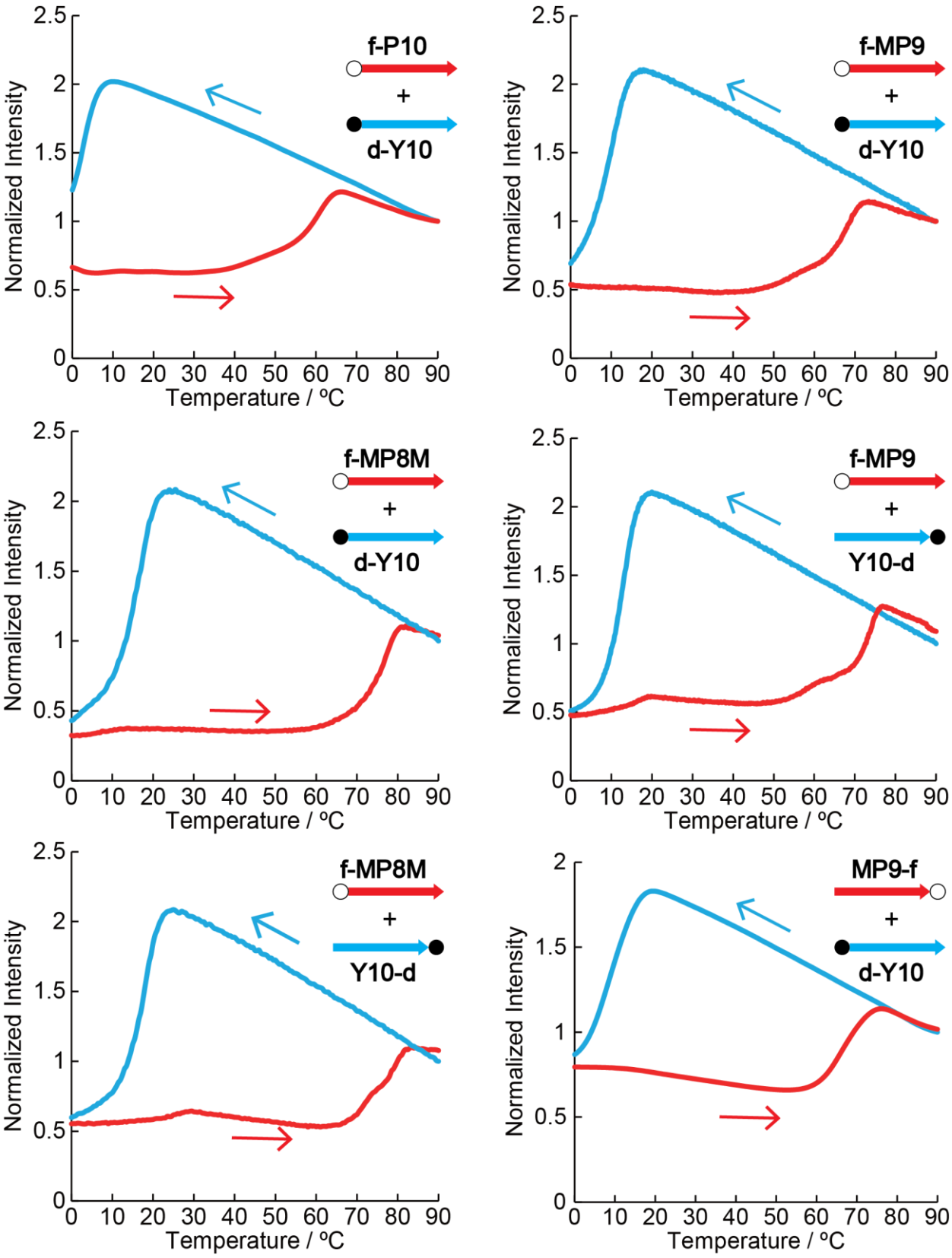


Figure S6. Effects of the number of **M** residues on the stability of the hexaplex. (a) Heating curves and (b) cooling curves of **f-MP9**/**d-Y10**, **f-P10**/**d-Y10** and **f-MP8M**/**d-Y10** hexaplexes. Conditions: [aminopyrimidine strand] = [**d-Y10**] = 1.0 μM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0).


f-P10
+
d-Y10
f-MP9
+
d-Y10
f-MP8M
+
d-Y10
f-MP9
+
Y10-d
f-MP8M
+
Y10-d
MP9-f
+
d-Y10
Normalized Intensity
Temperature / ºC

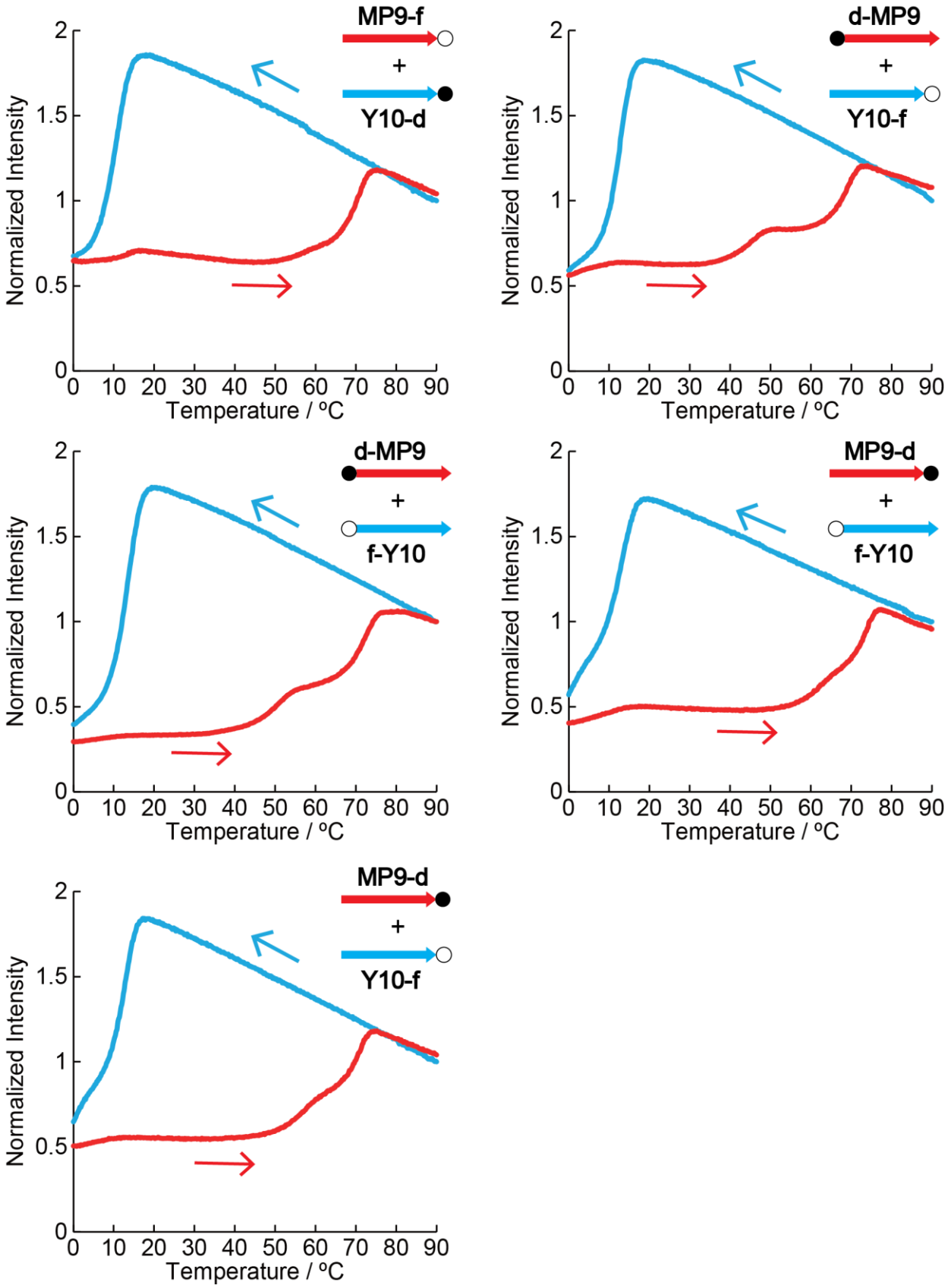


Figure S7. Cooling and heating curves of hexaplexes with various combination. Conditions: [aminopyrimidine strand] = [cyanuric acid strand] = 1.0 μM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0).

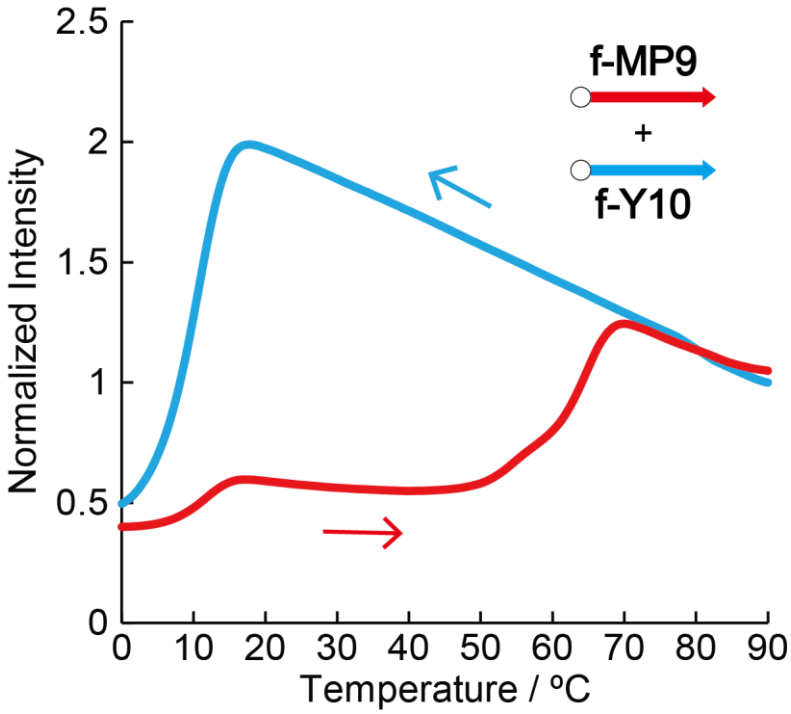


Figure S8. Self-quenching of FAM moieties upon hexaplex formation. Cooling and heating curves of **f-MP9**/**f-Y10** hexaplex are shown. Severe decrease in emission intensity below 20 ºC indicated self-quenching of FAM residues. Conditions: [**f-MP9**] = [**f-Y10**] = 1.0 µM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0).

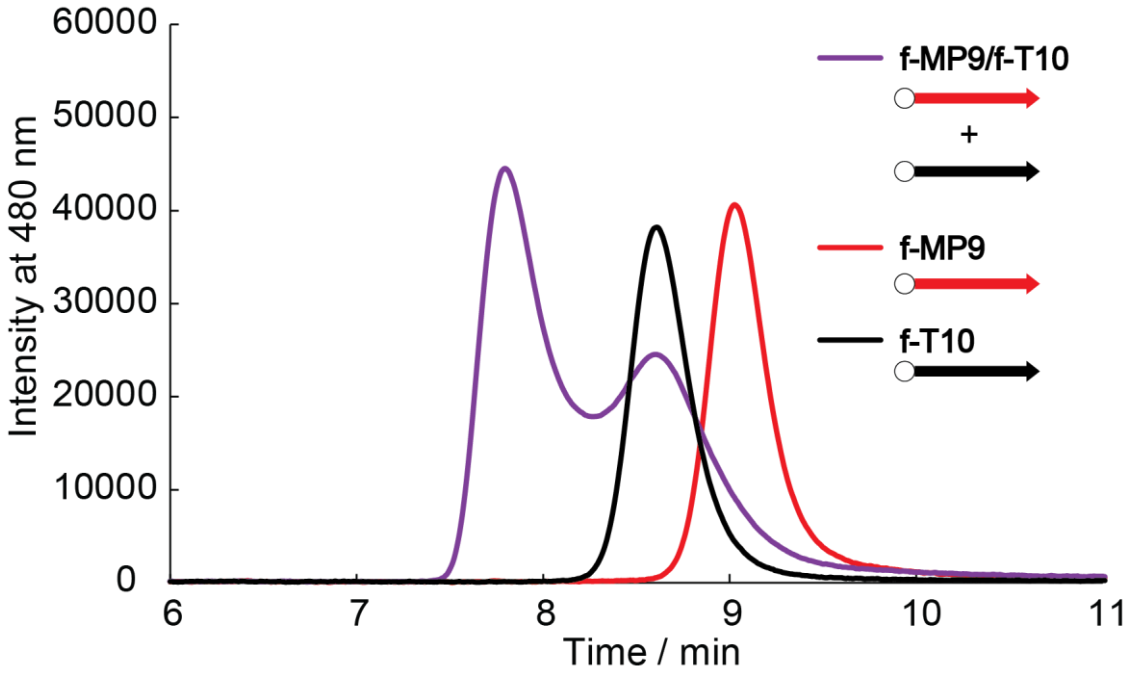


Figure S9. SEC charts of **f-MP9/f-T10** triplex. Charts of single strands are also shown. Chromatograms were recorded by monitoring the absorption maximum of FAM (480 nm).

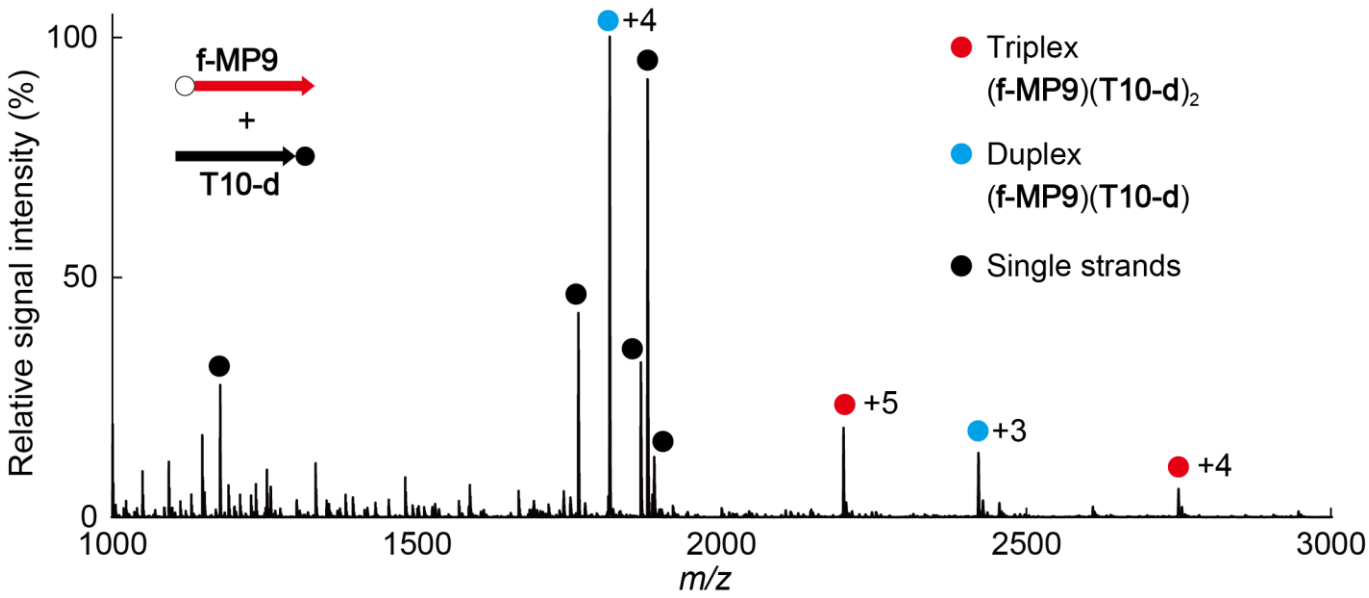


Figure S10. Mass spectrum of **f-MP9**/**T10-d** triplex under native conditions with positive ionization mode. Peaks assigned as a triplex, duplex and single strands are indicated by red, blue and black dots, respectively.

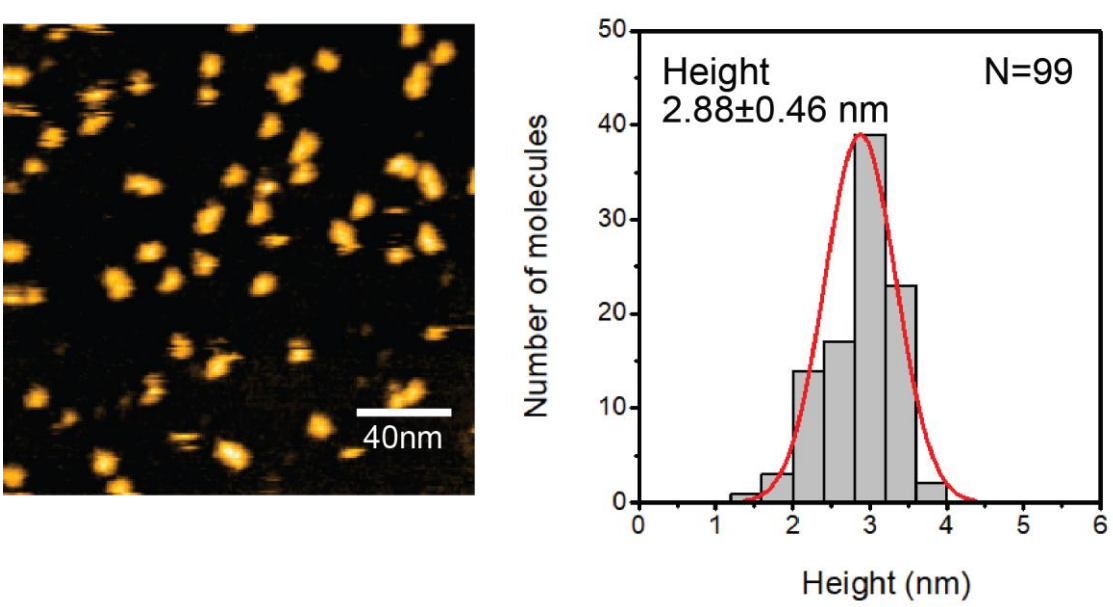


Figure S11. AFM images in solution and height statistical analysis of **f-MP9/d-Y10** hexaplex.

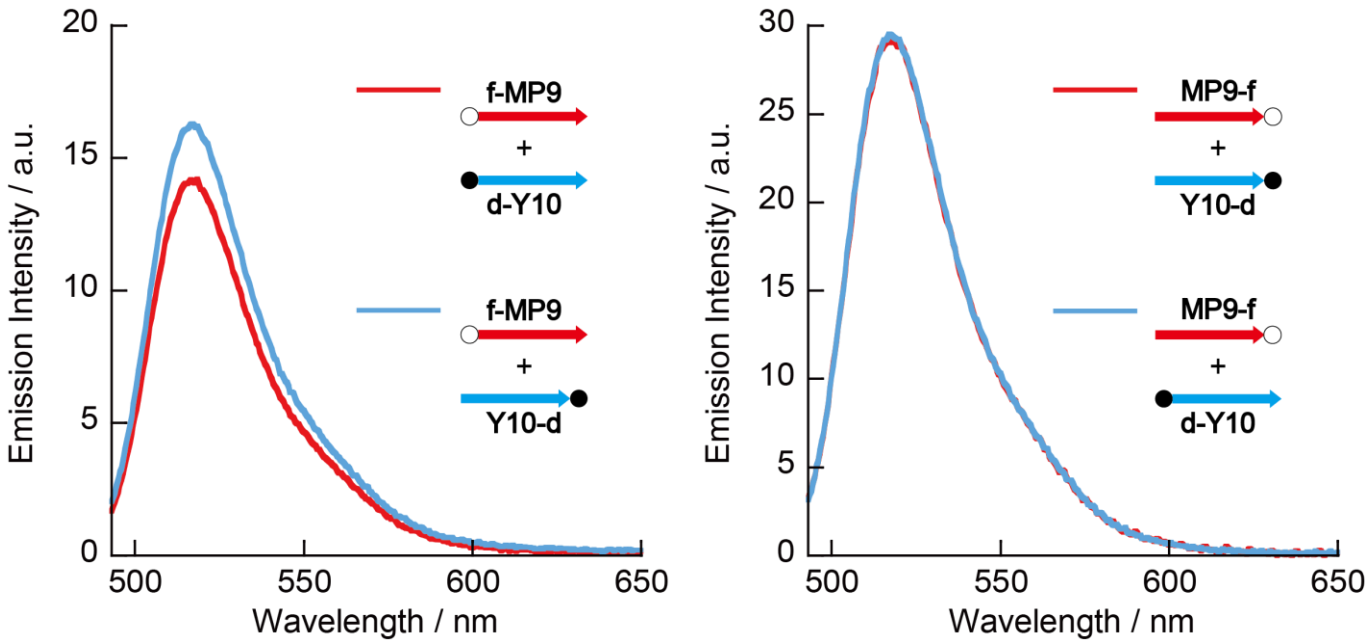


Figure S12. Comparison of fluorescence spectra of hexaplexes tethering a fluorophore and a quencher at different positions. Left: emission spectra at 0 ºC of **f-MP9**/**d-Y10** and **f-MP9**/**Y10-d**. Right: emission spectra at 0 ºC of **MP9-f**/**Y10-d** and **MP9-f**/**d-Y10** are shown. Conditions: [aminopyrimidine strand] = [cyanuric acid strand] = 1.0 µM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0).

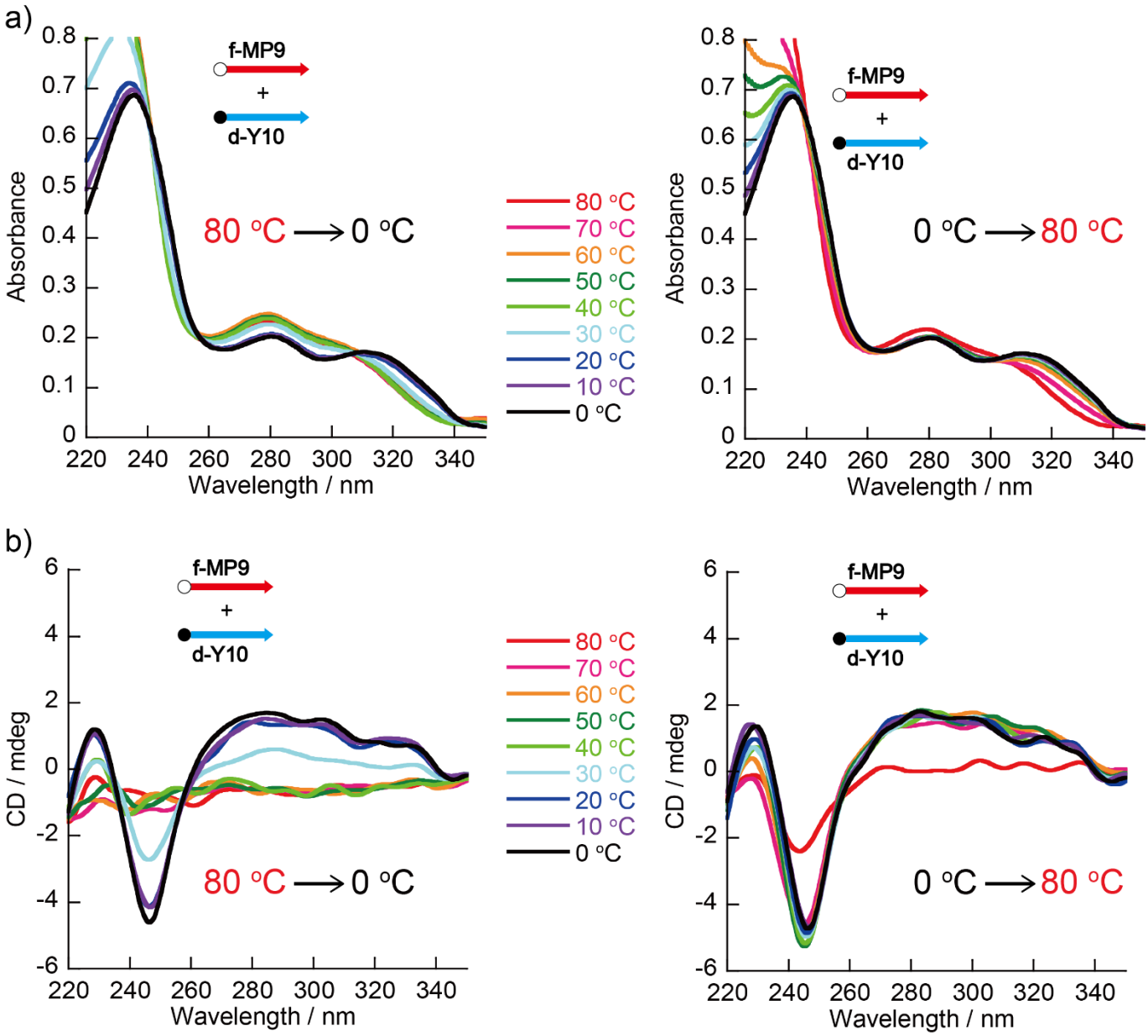


Figure S13. (a) UV-VIS and (b) CD spectra of **f-MP9**/**d-Y10** hexaplexes recorded at 10 ˚C interval. Spectra were measured in both cooling and heating processes. Conditions: [**f-MP9**] = [**d-Y10**] = 5.0 µM (for UV-VIS) or 10 µM (for CD), 20 mM $MgCl_2$, 10 mM MES buffer (pH 6.0).

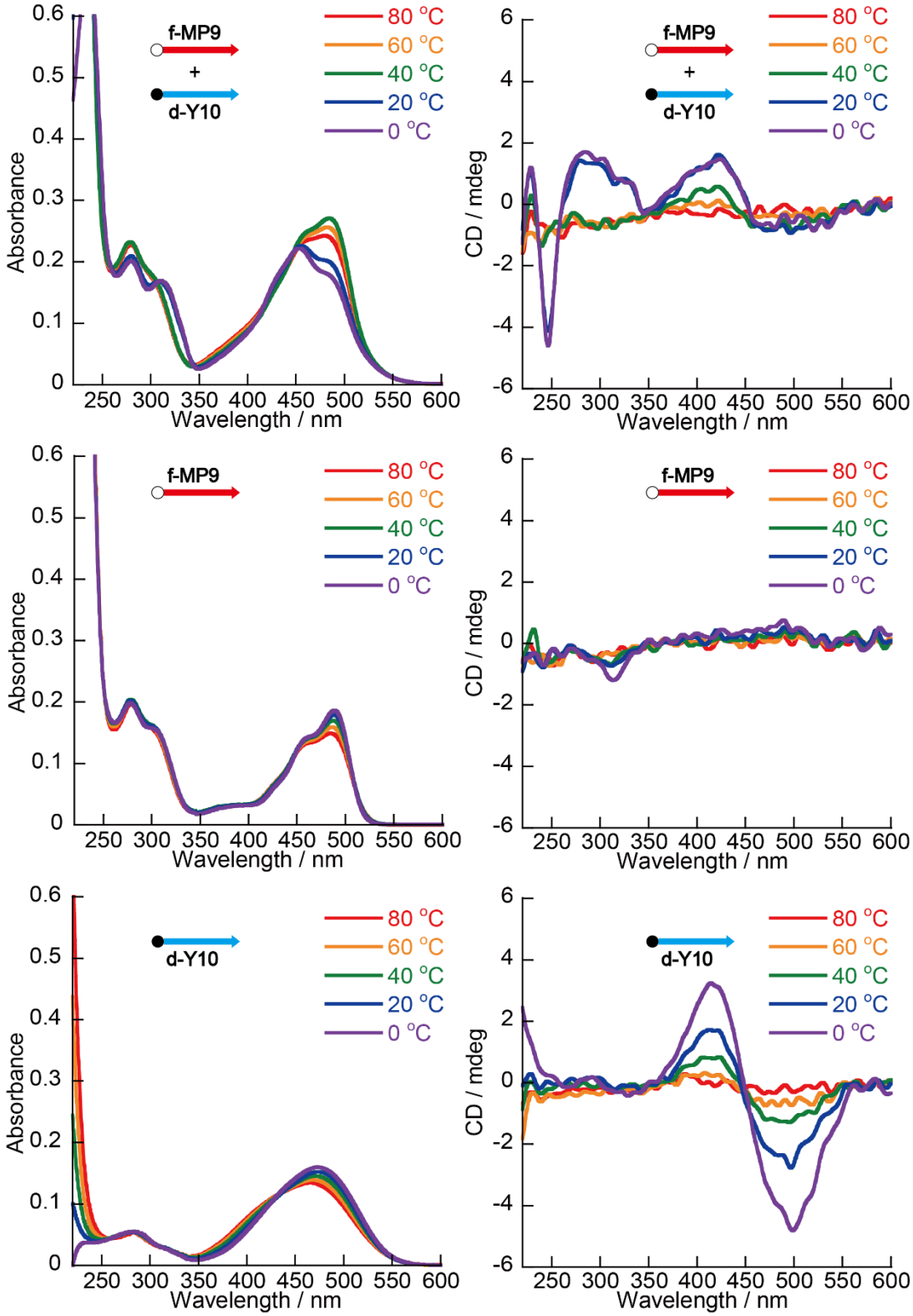


Figure S14. Spectroscopic behavior of **f-MP9**/**d-Y10** hexaplex and their single-strands. UV-VIS (left) and CD spectra (right) of **f-MP9**/**d-Y10** (top), **f-MP9** (middle) and **d-Y10** (bottom) were measured from 80 ºC to 0 ºC (annealing process). Conditions: [**f-MP9**] = [**d-Y10**] = 5.0 µM (for UV-VIS) or 10 µM (for CD), 20 mM $MgCl_2$, 10 mM MES buffer (pH 6.0).

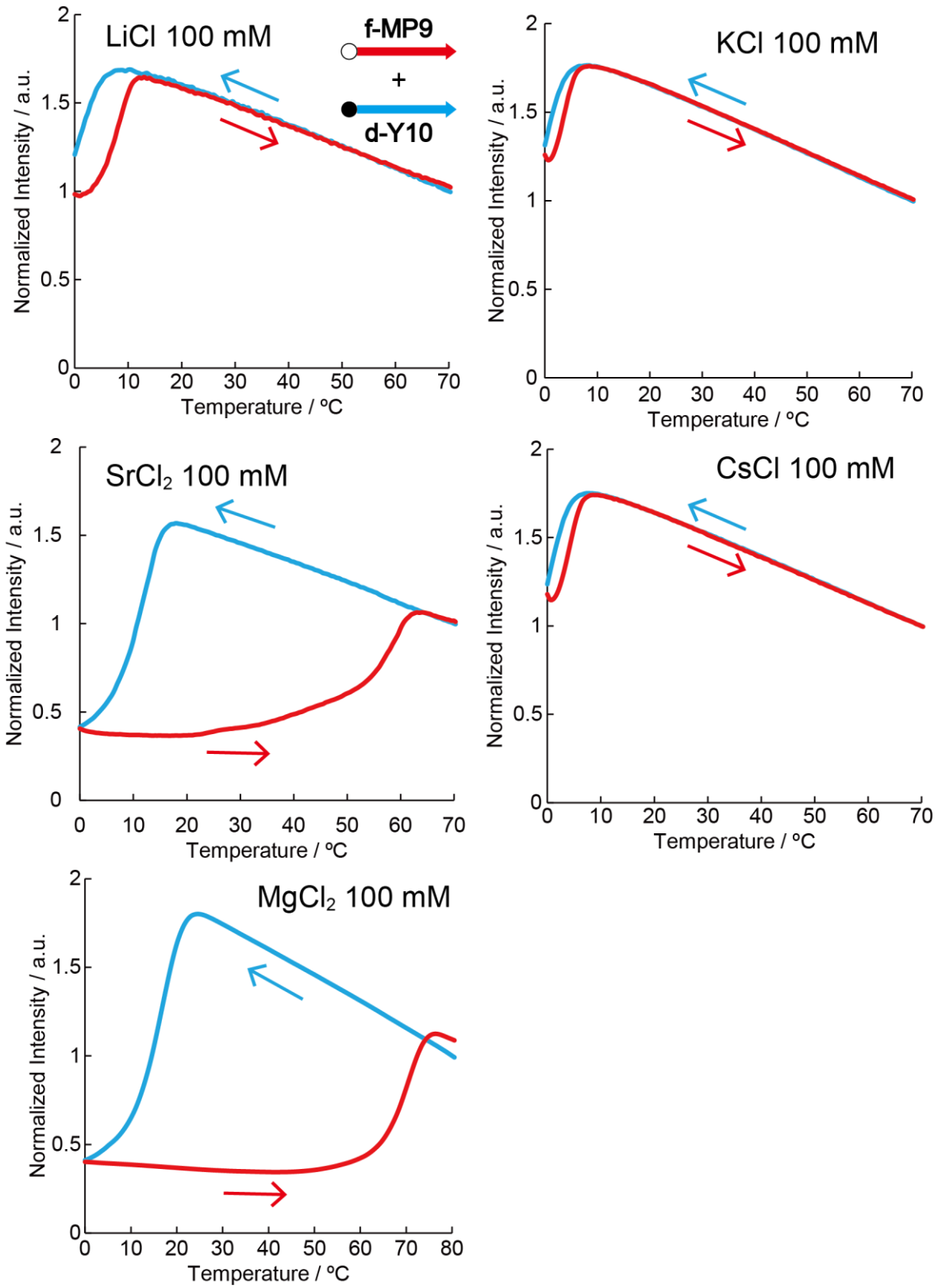


Figure S15. Effect of counter cation on the hexaplex stability. Cooling and heating curves of **f-MP9**/**d-Y10** hexaplex in the presence of 100 mM of various cations. Conditions: [**f-MP9**] = [**d-Y10**] = 1.0 μM, 100 mM MCl or MCl_2, 10 mM HEPES buffer (pH 7.0).

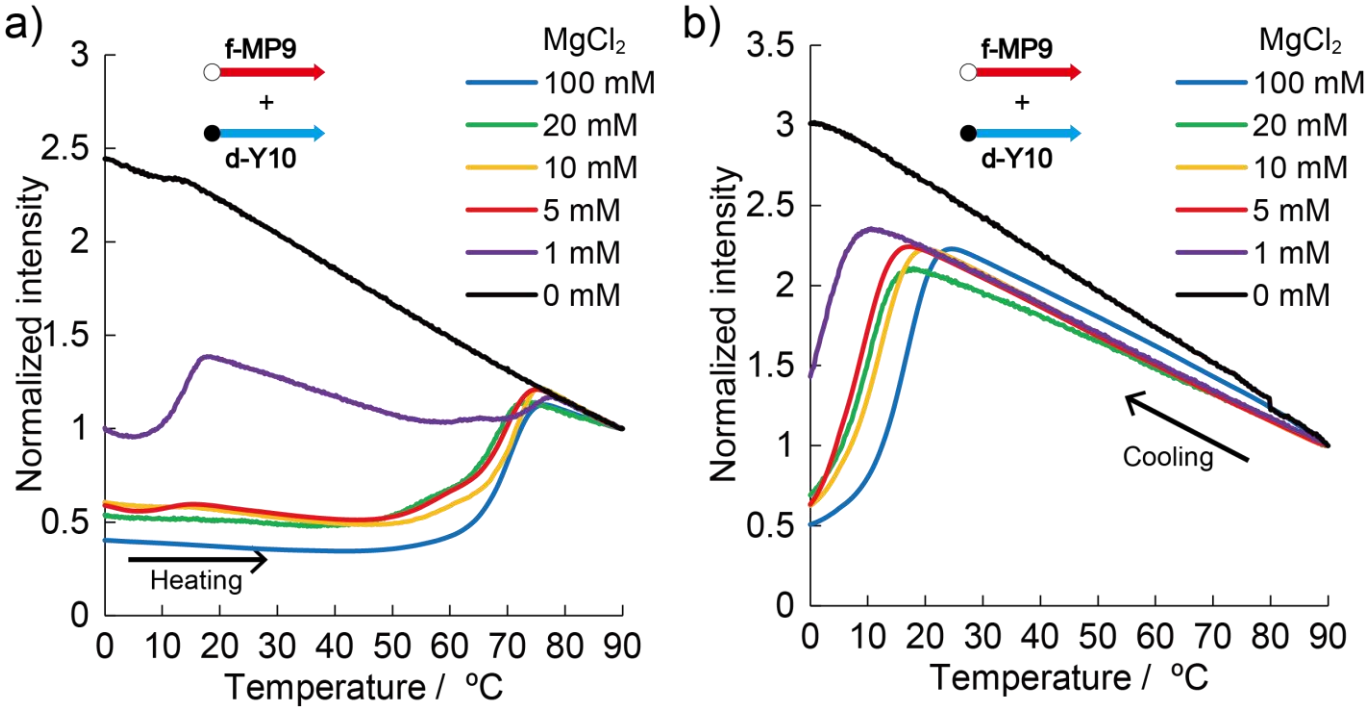


Figure S16. Effects of $MgCl_2$ concentration on the stability of **f-MP9**/**d-Y10** hexaplex. Both heating and cooling curves are shown. Conditions: [**f-MP9**] = [**d-Y10**] = 1.0 μM, 10 mM HEPES buffer (pH 7.0).

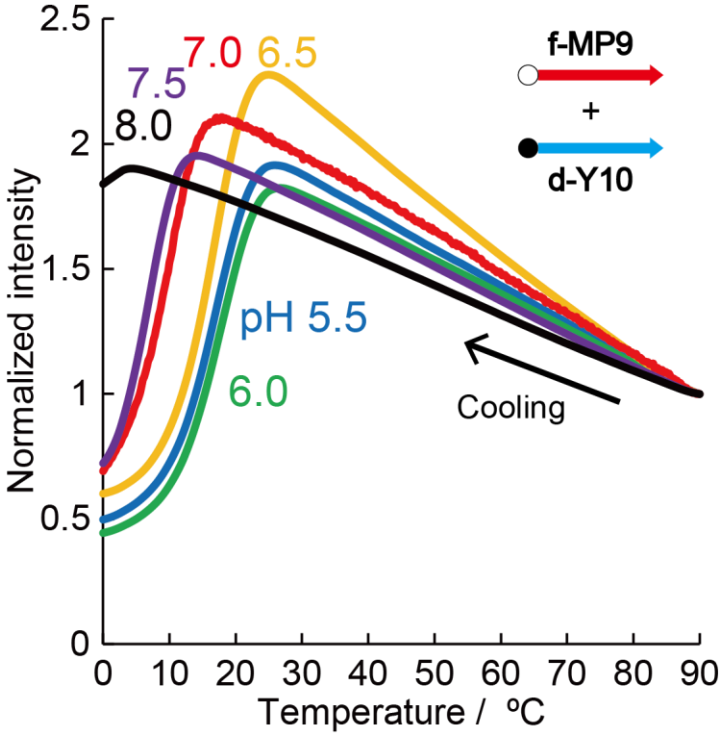


Figure S17. Effects of pH on annealing behavior of **f-MP9**/**d-Y10** hexaplex. Cooling curve of **f-MP9**/**d-Y10** at various pH. See Figure 7b for heating curves. Conditions: [**f-MP9**] = [**d-Y10**] = 1.0 µM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 6.5-8.0) or 10 mM MES buffer (pH 5.5-6.0).

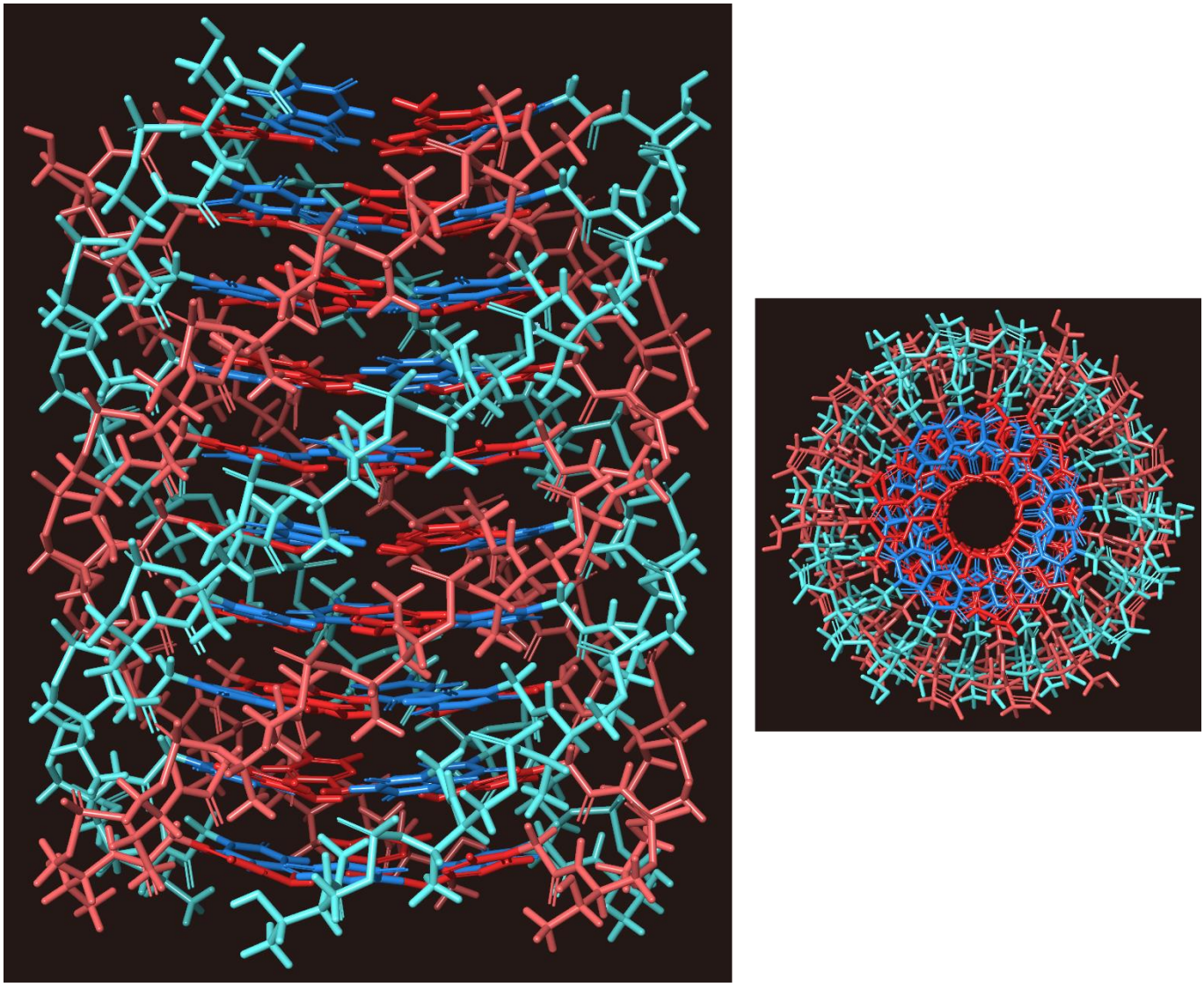


Figure S18. Energy minimized structure of MP_9/Y_{10} hexaplex shown in a stick model. Dye moieties were not included in this model. Bases of aminopyrimidine and cyanuric acid strands are coloured in red and blue whereas their backbones are in light red and light blue, respectively. Top view of the hexaplex is also shown on the right.

Table S1. Melting temperatures of triplexes determined from emission intensity.

			T_m^{cool} / °C[a]	T_m^{heat} / °C[a]	T_m / °C[a]
	**f-P10**	**d-T10**	20.7	32.0	26.4
+					
	**f-MP9**	**d-T10**	25.1	33.0	29.1
	**d-P10**	**f-T10**	23.1	30.0	26.6
+					
	**d-MP9**	**f-T10**	27.9	32.4	30.2

[a] T_m^{cool} and T_m^{cool} were melting temperatures determined from cooling and heating curves, respectively. T_m is the average of T_m^{cool} and T_m^{cool}. Conditions: [aminopyrimidine strand] = 1.0 µM, [thymine strand] = 2.0 µM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0).

Table S2. Melting temperatures of **f-MP9**/**d-Y10** under different conditions.

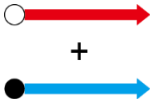
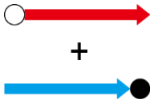
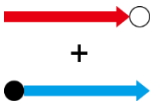
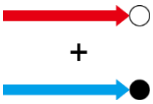
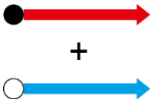
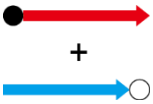
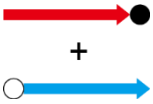
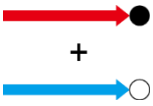
pH	Salt concentration		Concentration	Temperature ramp	T_m^{cool} / ºC[a]	T_m^{heat} / ºC[a]
5.5	$MgCl_2$	20 mM	1.0 μM	1.0 ºC / min	16.9	78.7
6.0	$MgCl_2$	20 mM	1.0 μM	1.0 ºC / min	17.6	78.5
6.5	$MgCl_2$	20 mM	1.0 μM	1.0 ºC / min	16.0	74.7
7.0	$MgCl_2$	20 mM	1.0 μM	1.0 ºC / min	10.0	67.9
7.5	$MgCl_2$	20 mM	1.0 μM	1.0 ºC / min	6.8	42.6, 53.8[b]
8.0	$MgCl_2$	20 mM	1.0 μM	1.0 ºC / min	n.d.[c]	n.d.[c]
7.0	$MgCl_2$	100 mM	1.0 μM	1.0 ºC / min	15.9	69.3
7.0	$CaCl_2$	100 mM	1.0 μM	1.0 ºC / min	11.1	57.8
7.0	$SrCl_2$	100 mM	1.0 μM	1.0 ºC / min	10.3	56.1
7.0	LiCl	100 mM	1.0 μM	1.0 ºC / min	<10	<10
7.0	NaCl	100 mM	1.0 μM	1.0 ºC / min	<10	<10
7.0	KCl	100 mM	1.0 μM	1.0 ºC / min	<10	<10
7.0	CsCl	100 mM	1.0 μM	1.0 ºC / min	<10	<10
7.0	$MgCl_2$	20 mM	1.0 μM	0.5 ºC / min	11.2	65.8
6.0	$MgCl_2$	20 mM	0.1 μM	1.0 ºC / min	<10	82.1
6.0	$MgCl_2$	20 mM	0.2 μM	1.0 ºC / min	9.1	79.4
6.0	$MgCl_2$	20 mM	0.4 μM	1.0 ºC / min	11.1	79.0
6.0	$MgCl_2$	20 mM	0.8 μM	1.0 ºC / min	15.3	78.5
6.0	$MgCl_2$	20 mM	1.6 μM	1.0 ºC / min	18.0	78.2
6.0	$MgCl_2$	20 mM	3.2 μM	1.0 ºC / min	22.6	78.6

[a] T_m^{cool} and T_m^{cool} are melting temperatures determined from cooling and heating curves, respectively. Conditions: [aminopyrimidine strand] = [cyanuric acid strand] = 1.0 μM, 10 mM HEPES buffer (for pH 6.5-8.0) or 10 mM MES buffer (pH 5.5-6.0).

[b] Two melting temperatures were observed due to biphasic transition.

[c] Sigmoidal curve was not observed.

Table S3. Melting temperatures of various combinations of hexaplexes.

			T_m^{cool} / °C[a]	T_m^{heat} / °C[a]
	**f-P10**	**d-Y10**	2.6	60.8
+	**f-MP9**	**d-Y10**	10.0	67.9
	f-MP8M	**d-Y10**	15.2	74.5
	**f-MP9**	**Y10-d**	12.9	58.3, 73.2[b]
+	**f-MP8M**	**Y10-d**	16.6	75.5
	**MP9-f**	**d-Y10**	9.0	66.7
	**MP9-f**	**Y10-d**	10.5	70.2
	**d-MP9**	**f-Y10**	13.4	50.6, 72.0[b]
	**d-MP9**	**Y10-f**	12.6	45.2, 68.7[b]
	**MP9-d**	**f-Y10**	12.9	63.0, 73.2[b]
	**MP9-d**	**Y10-f**	12.5	57.3, 70.3[b]

[a] T_m^{cool} and T_m^{cool} are melting temperatures determined from cooling and heating curves, respectively. Conditions: [aminopyrimidine strand] = [cyanuric acid strand] = 1.0 μM, 20 mM $MgCl_2$, 10 mM HEPES buffer (pH 7.0).

[b] Two melting temperatures were observed due to biphasic transition.

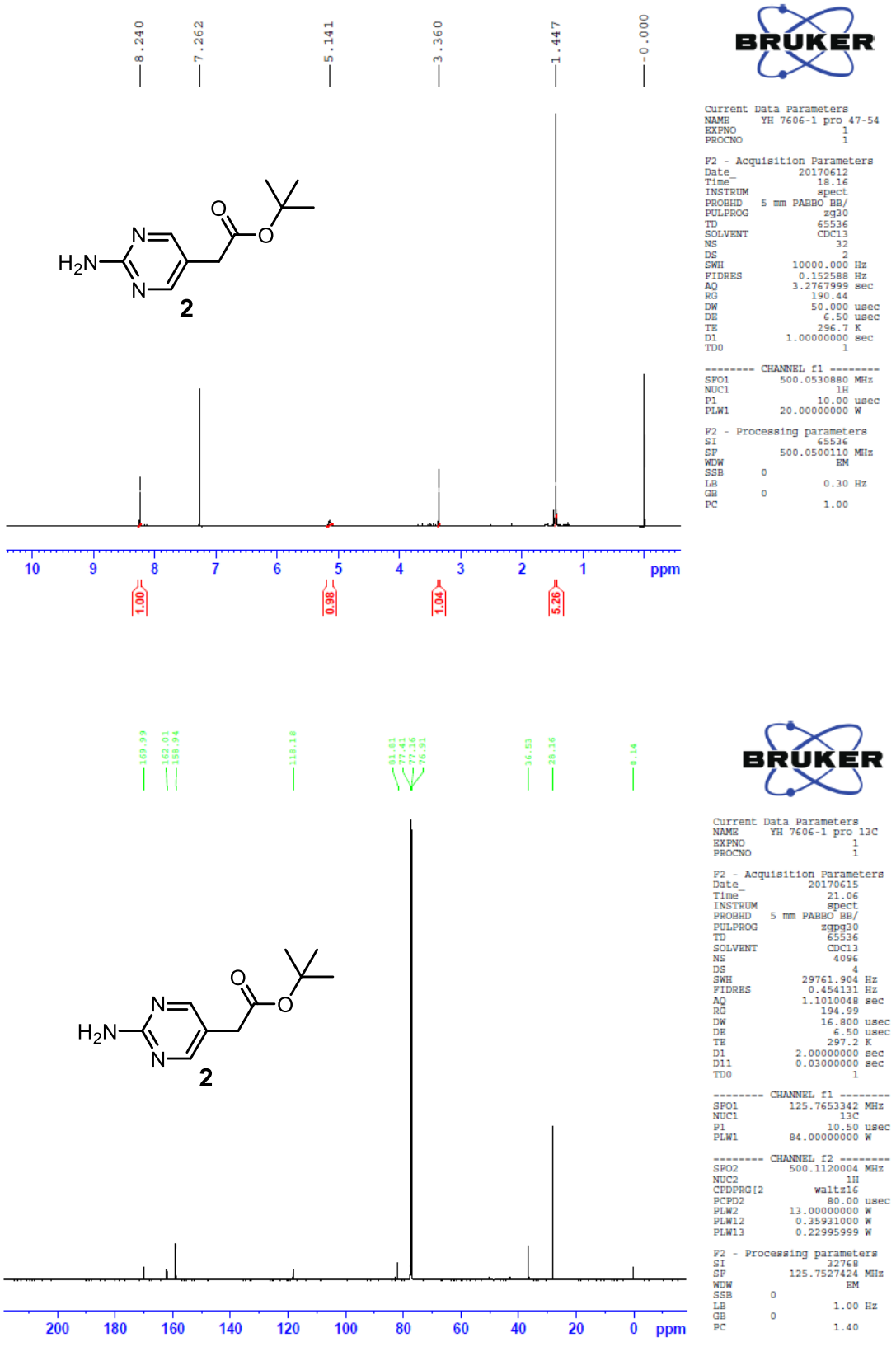


Figure S19. ^{1}H and ^{13}C NMR charts of compound **2**.

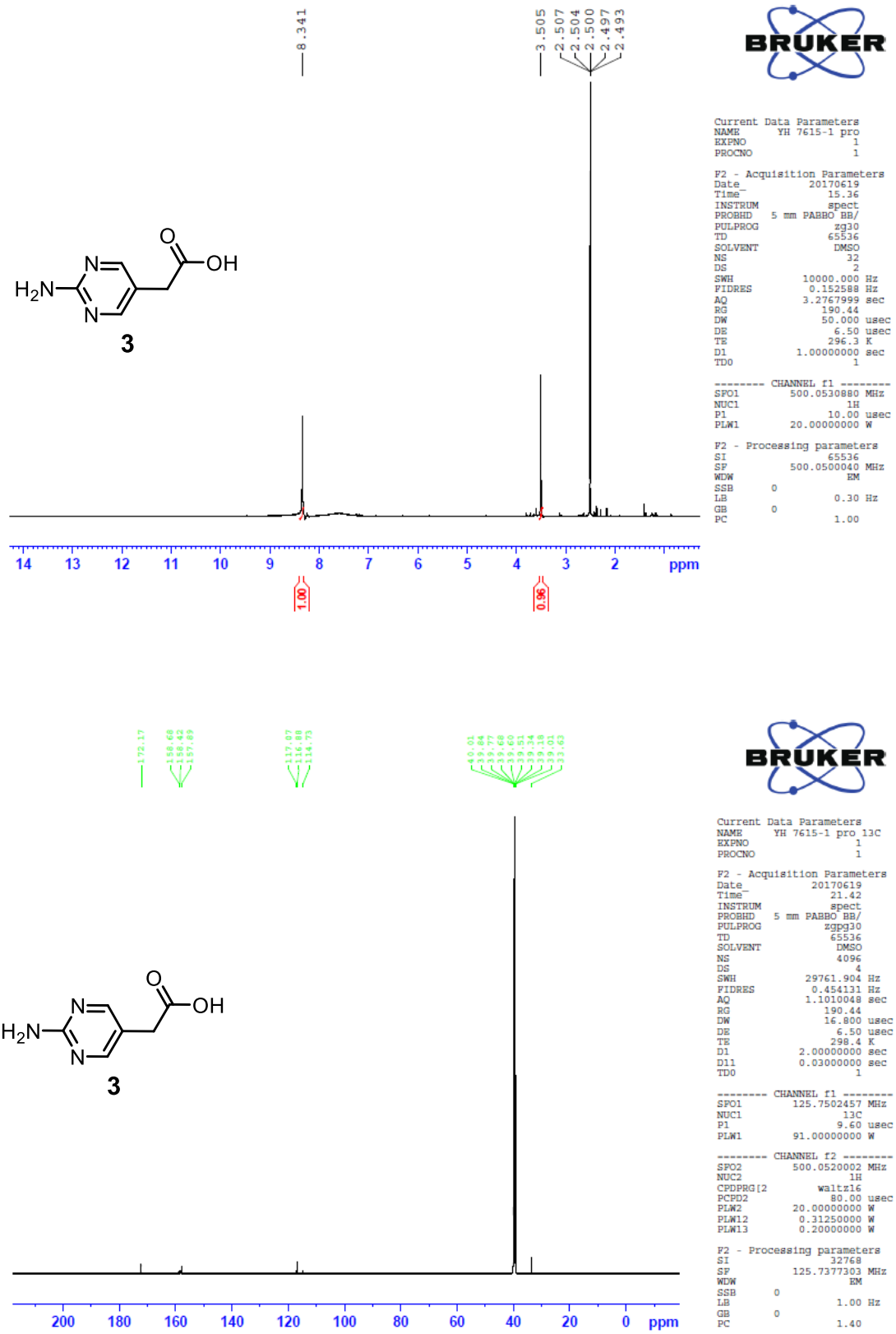


Figure S20. ^{1}H and ^{13}C NMR charts of compound **3**.

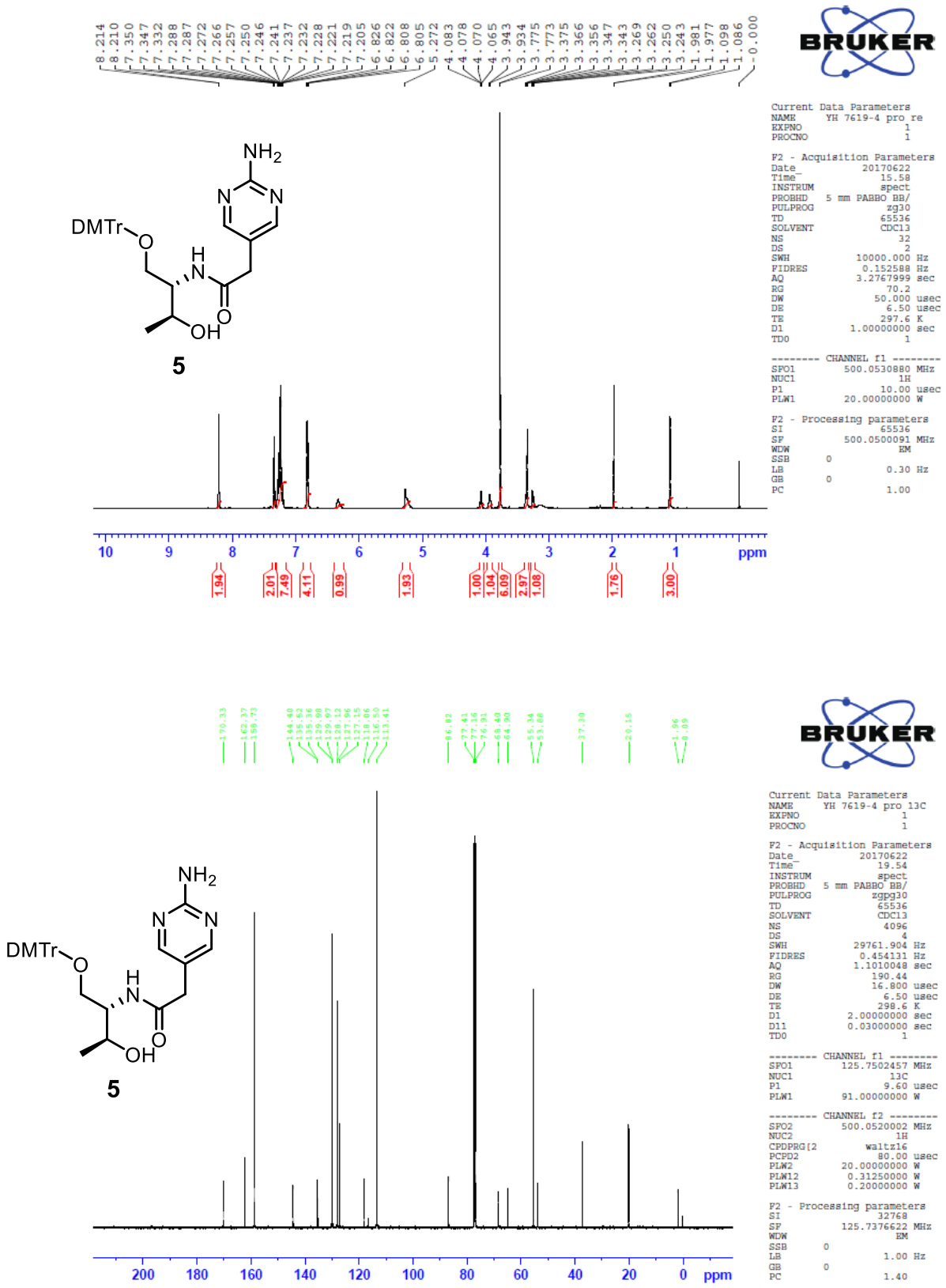


Figure S21. ^{1}H and ^{13}C NMR charts of compound **5**.

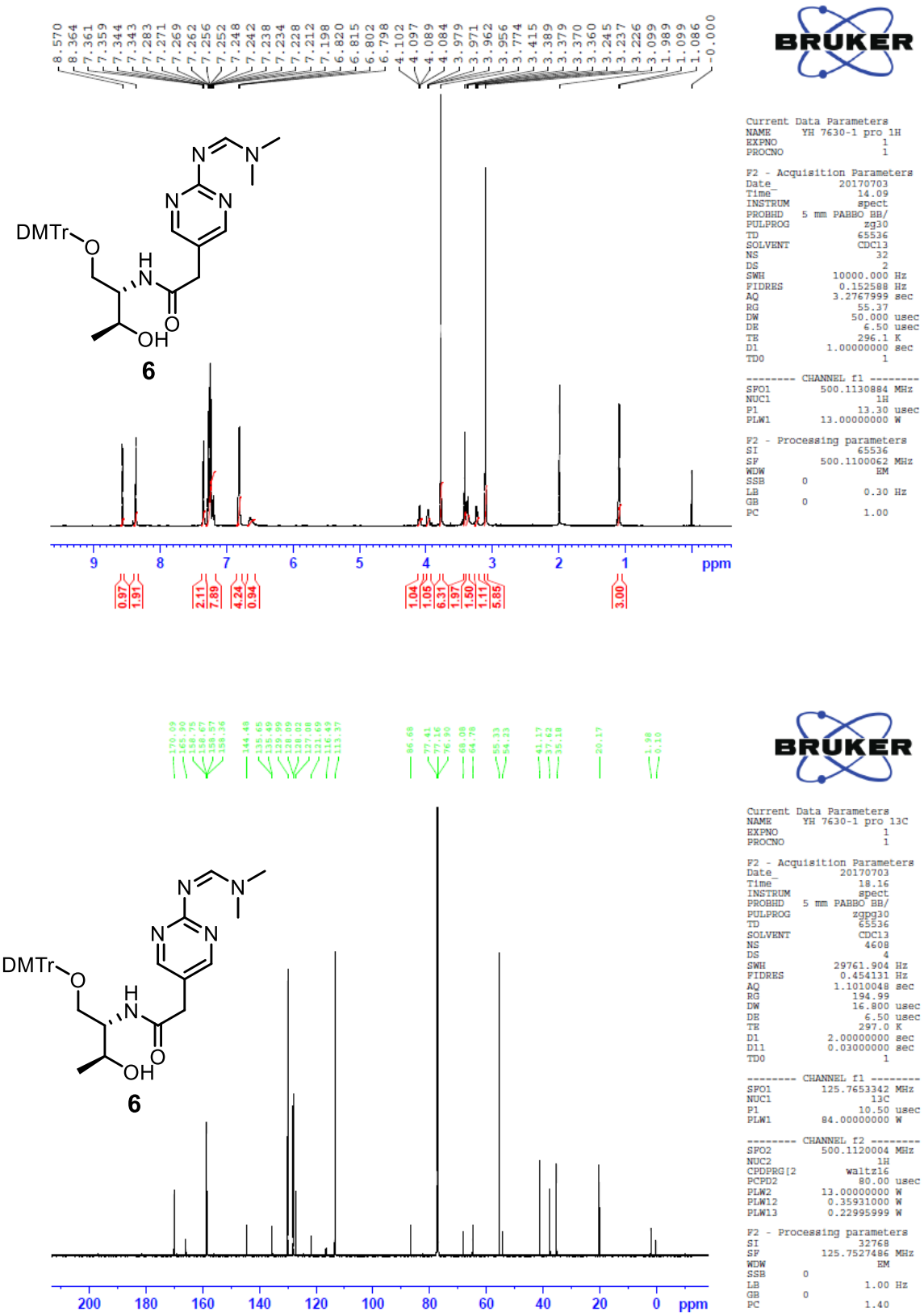


Figure S22. ^{1}H and ^{13}C NMR charts of compound **6**.

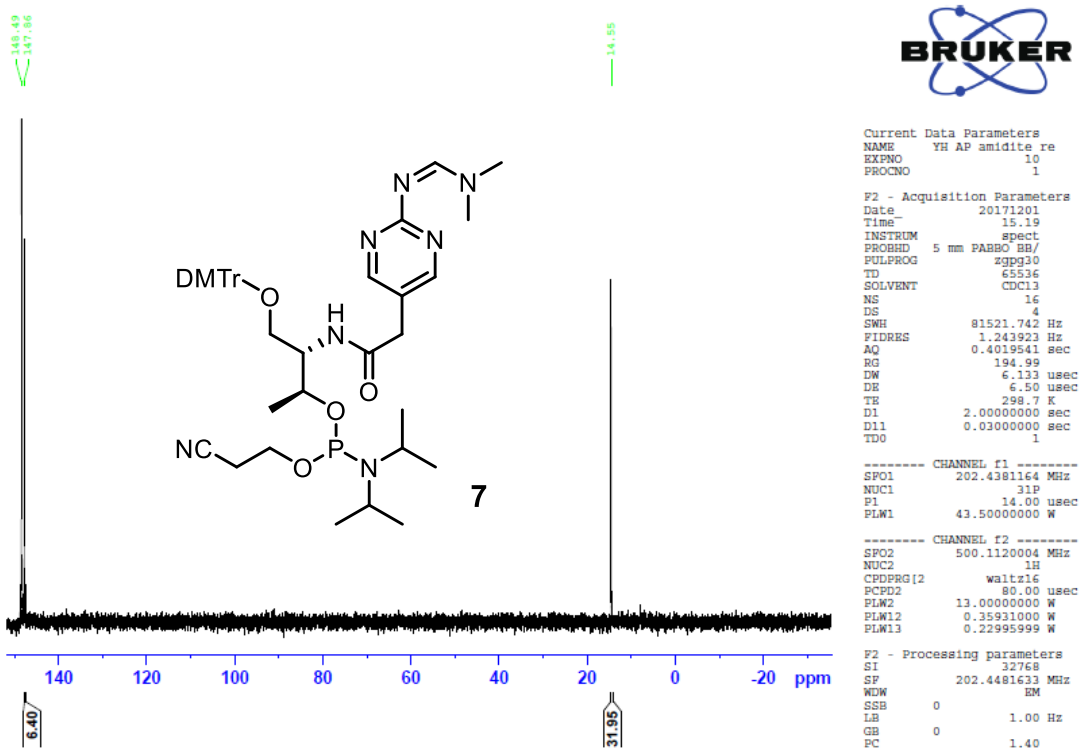


Figure S23. ^{31}P NMR chart of compound **7**.

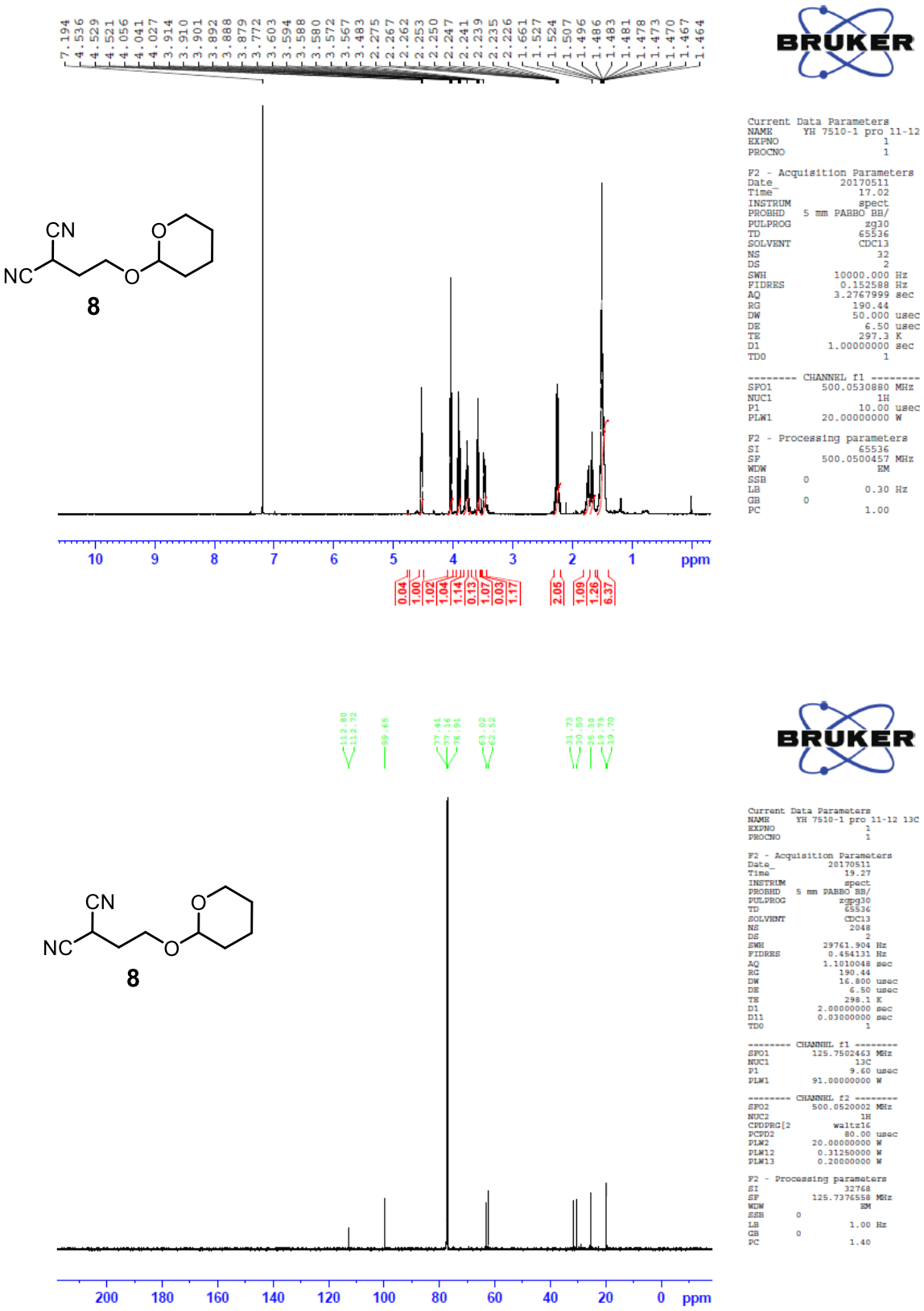


Figure S24. ^{1}H and ^{13}C NMR charts of compound **8**.

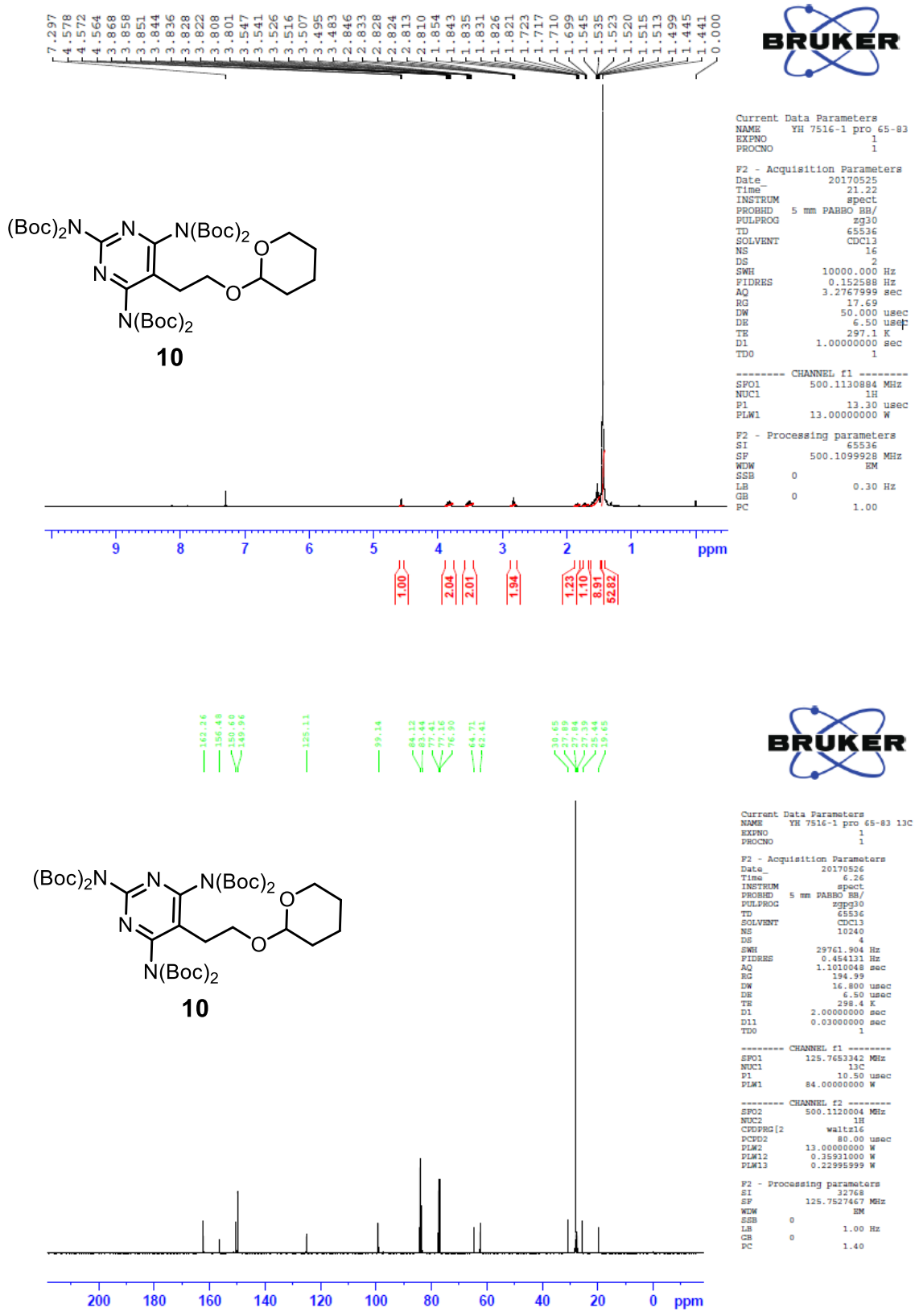


Figure S25. ^{1}H and ^{13}C NMR charts of compound **10**.

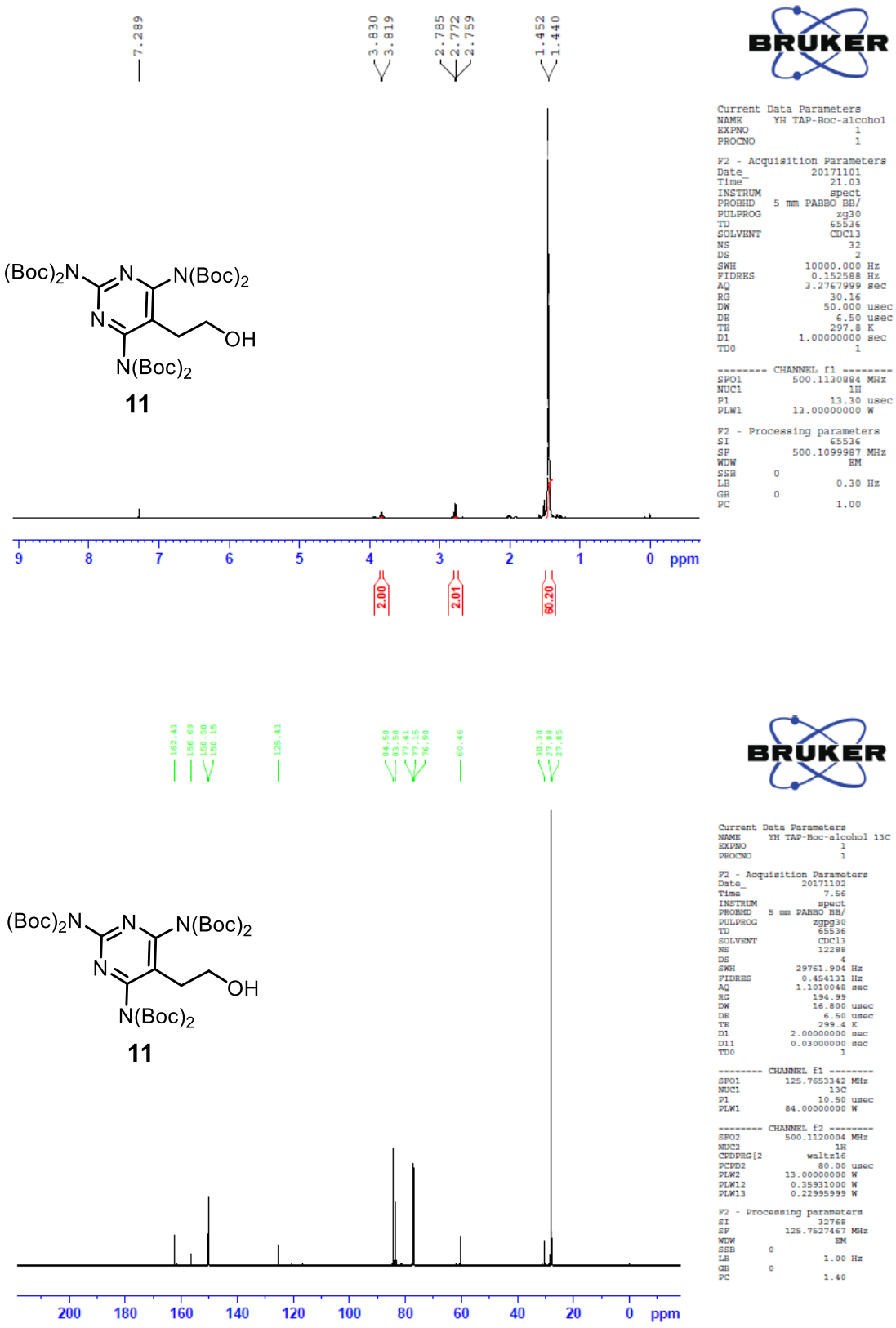


Figure S26. ^{1}H and ^{13}C NMR charts of compound **11**.

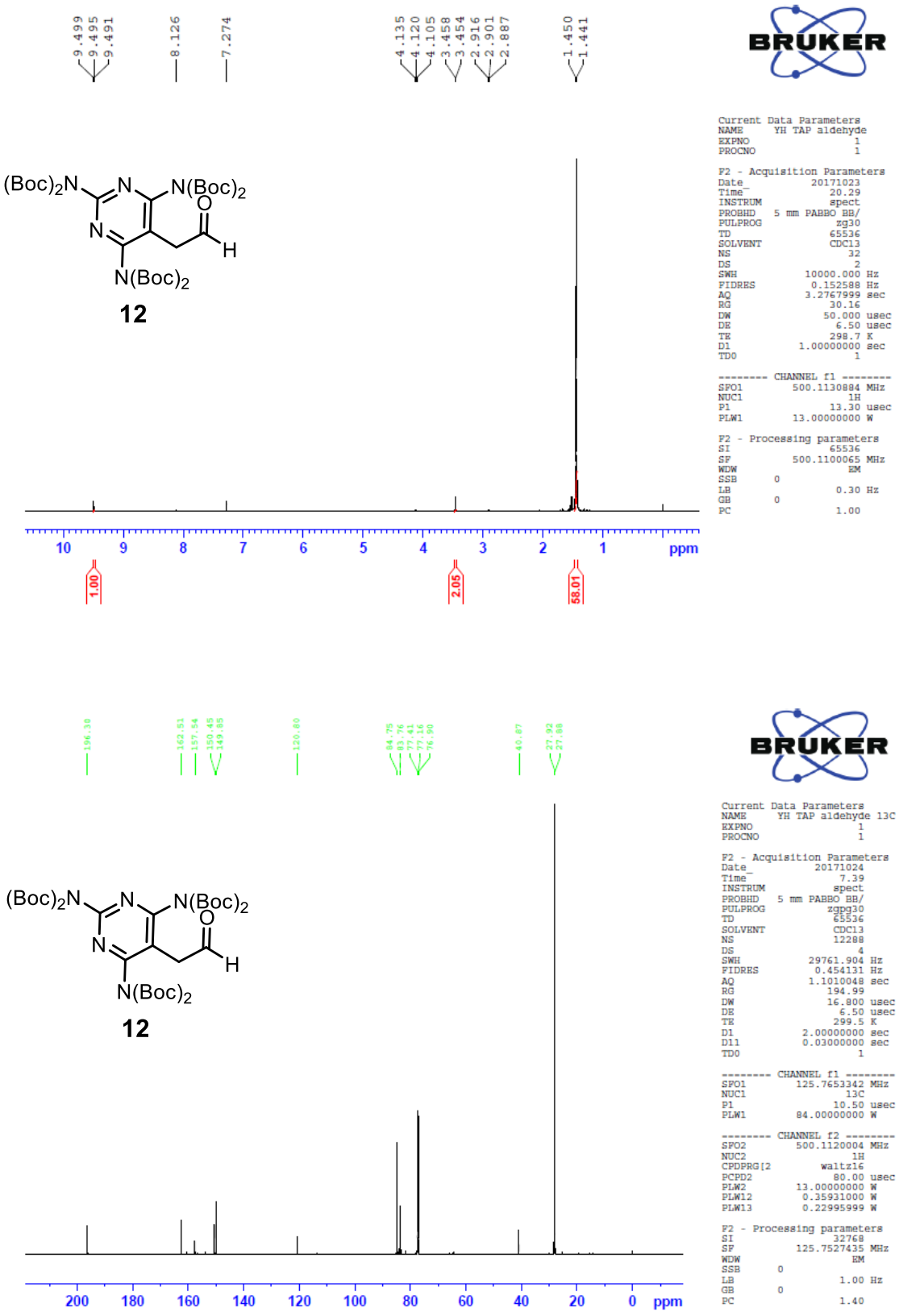


Figure S27. ^{1}H and ^{13}C NMR charts of compound **12**.

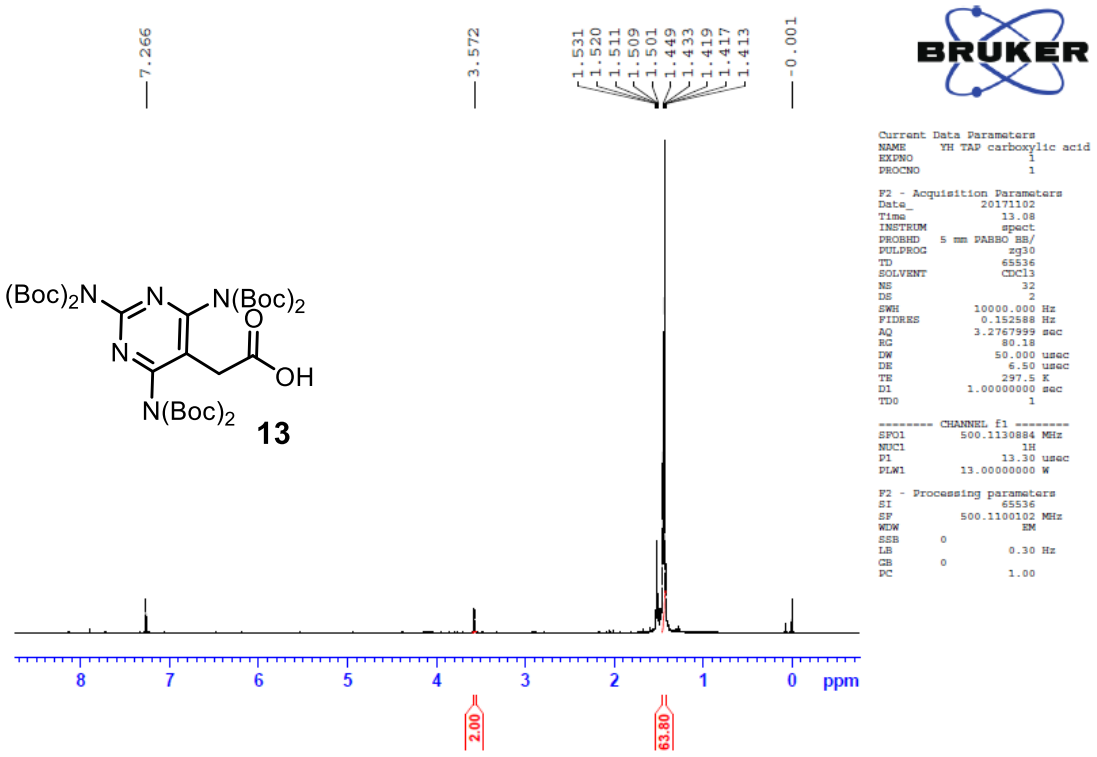


Figure S28. ^{1}H NMR chart of compound **13**.

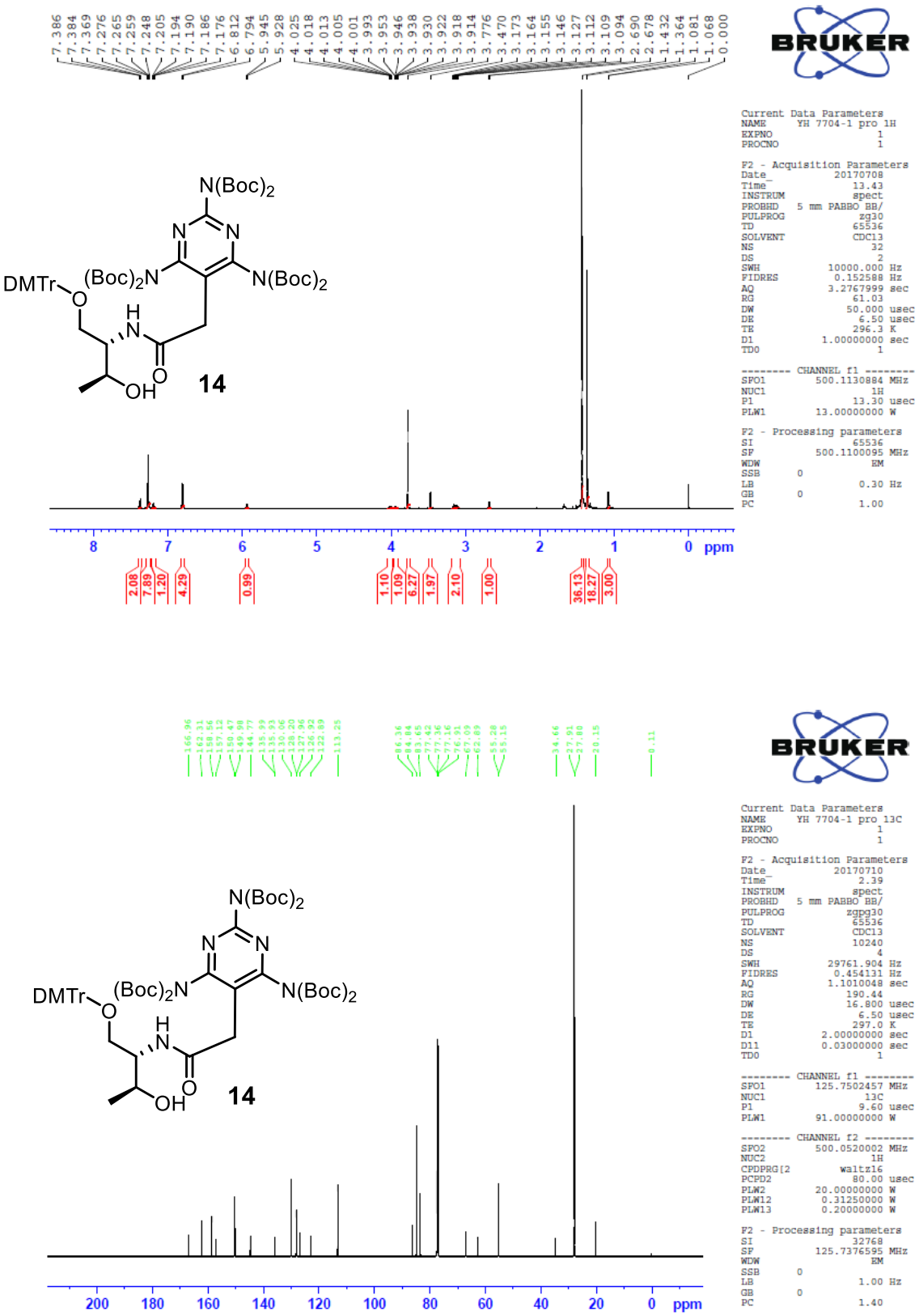


Figure S29. ^{1}H and ^{13}C NMR charts of compound **14**.

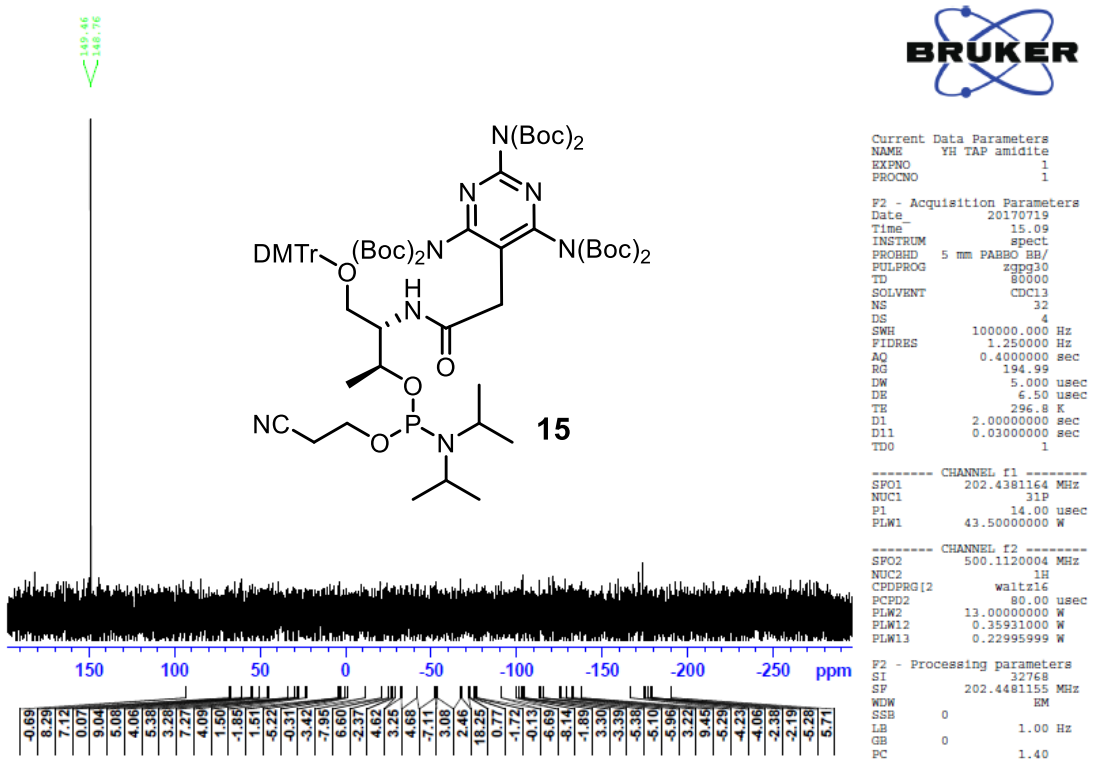


Figure S30. ^{31}P NMR chart of compound **15**.

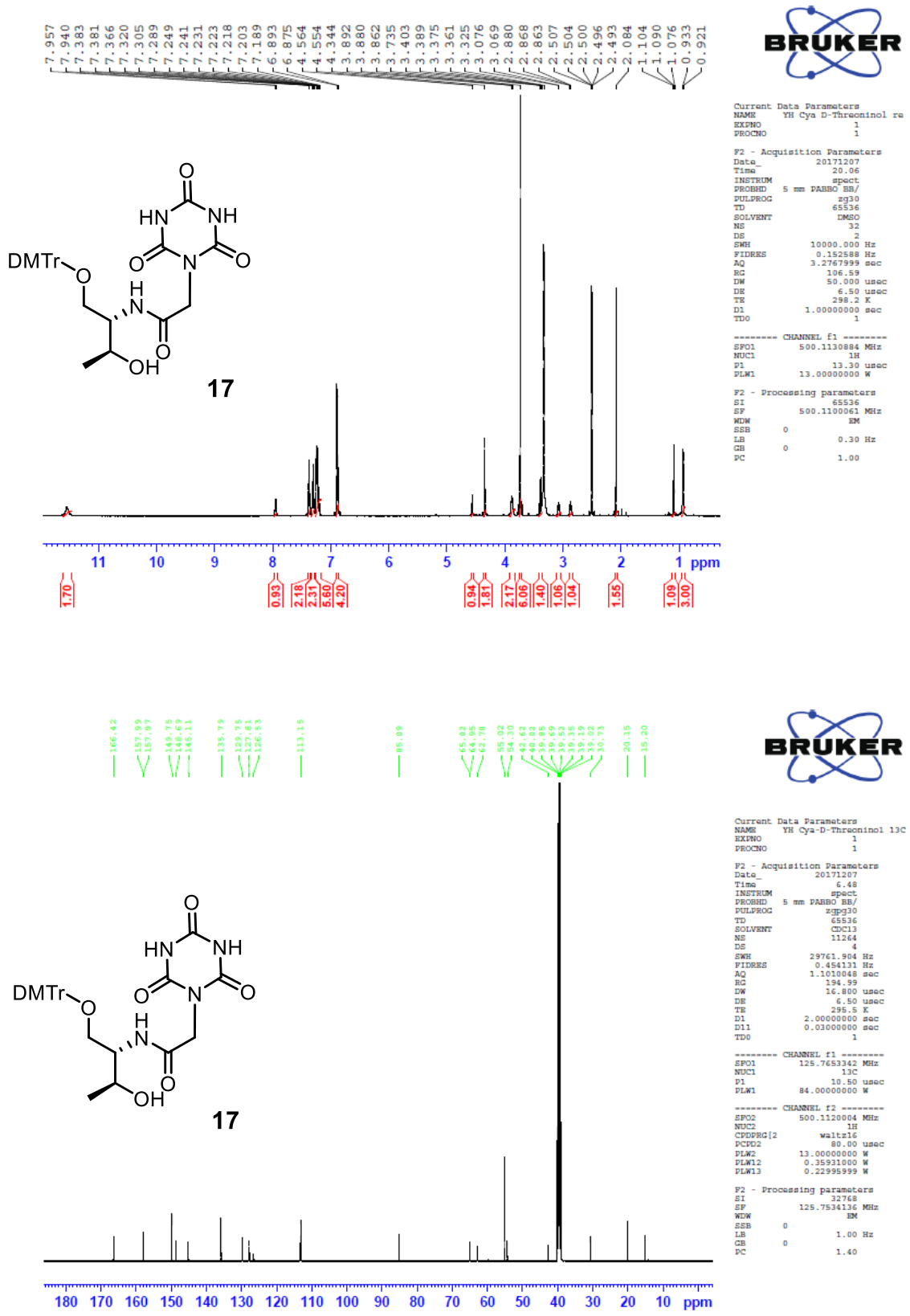


Figure S31. ^{1}H and ^{13}C NMR charts of compound **17**.

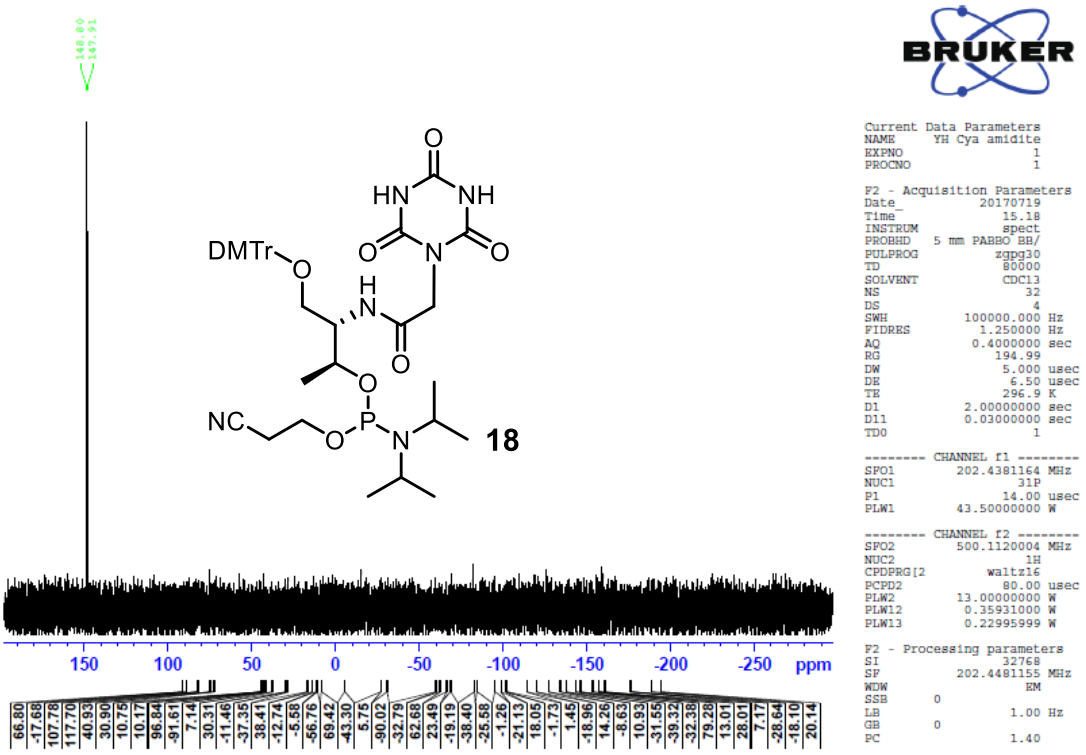


Figure S32. ^{31}P NMR chart of compound **18**.

Supplementary References

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