

## ***Electronic Supplementary Information (ESI)***

### **Parallel-motif triplex formation via a new, bi-directional hydrogen bonding pattern incorporating a synthetic cyanuryl nucleoside into the sense chain**

**Akihiko Hatano,<sup>a\*</sup> Kei Shimazaki,<sup>a</sup> Maina Otsu,<sup>b</sup> Gota Kawai<sup>b</sup>**

<sup>a</sup>Department of Chemistry, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama, 337-8570, Japan. Tel: +81-48-687-5035, Fax: +81-48-687-5013; Email: a-hatano@sic.shibaura-it.ac.jp

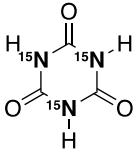
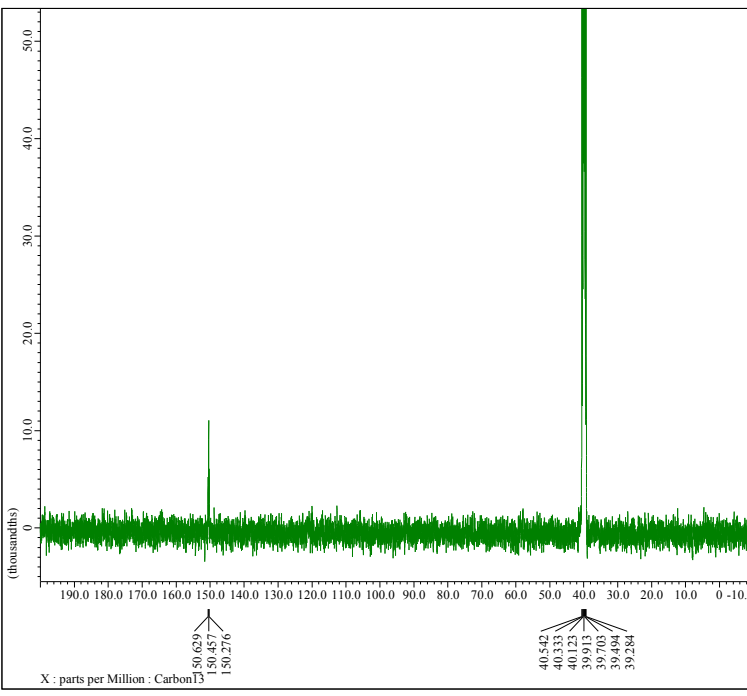

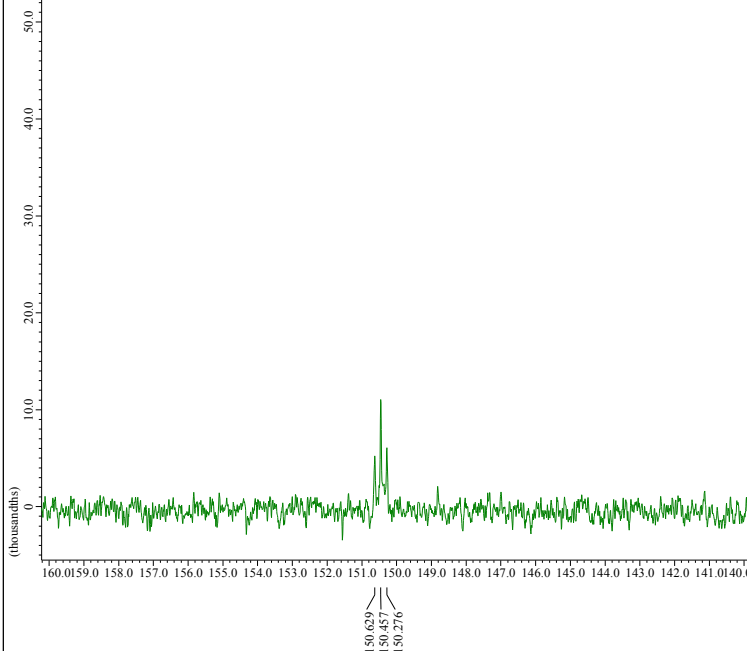

<sup>b</sup>Department of Life and Environmental Sciences, Faculty of Engineering, Chiba Institute of Technology, 2-17-1 Tsudanuma, Narashino, Chiba 275-0016, Japan.

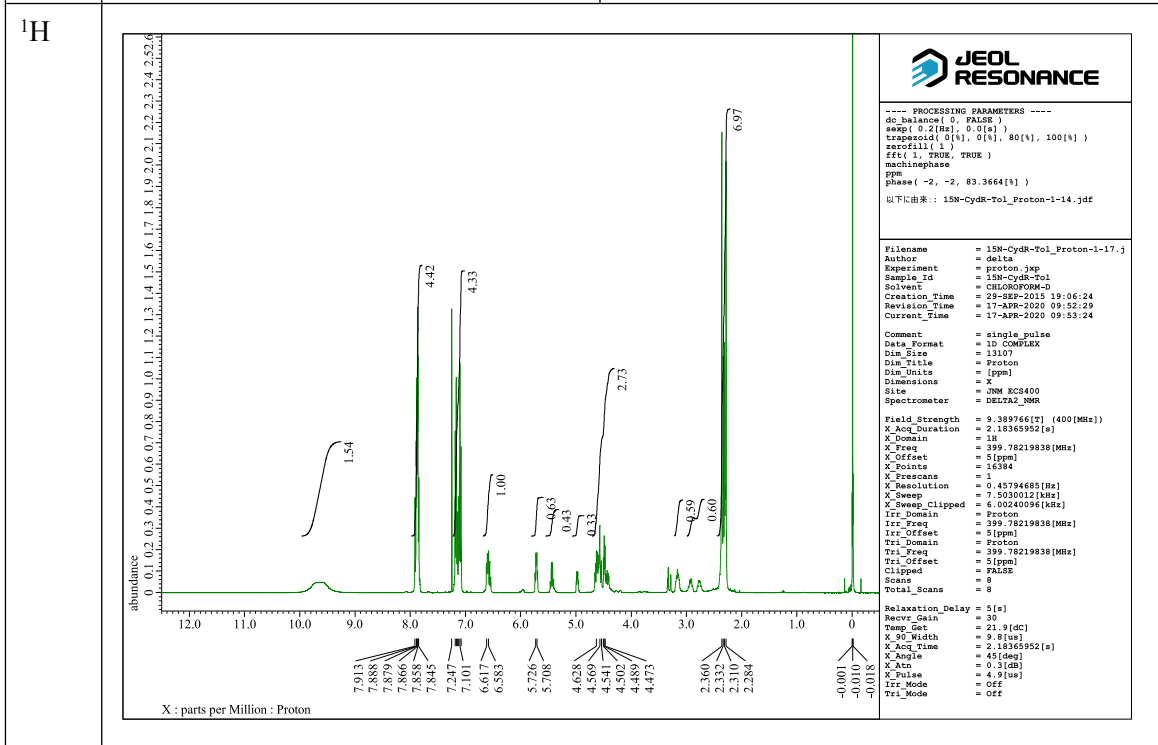
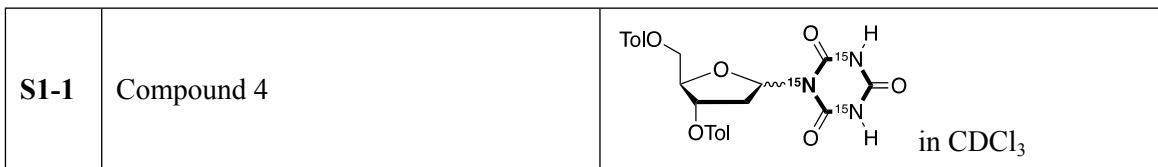
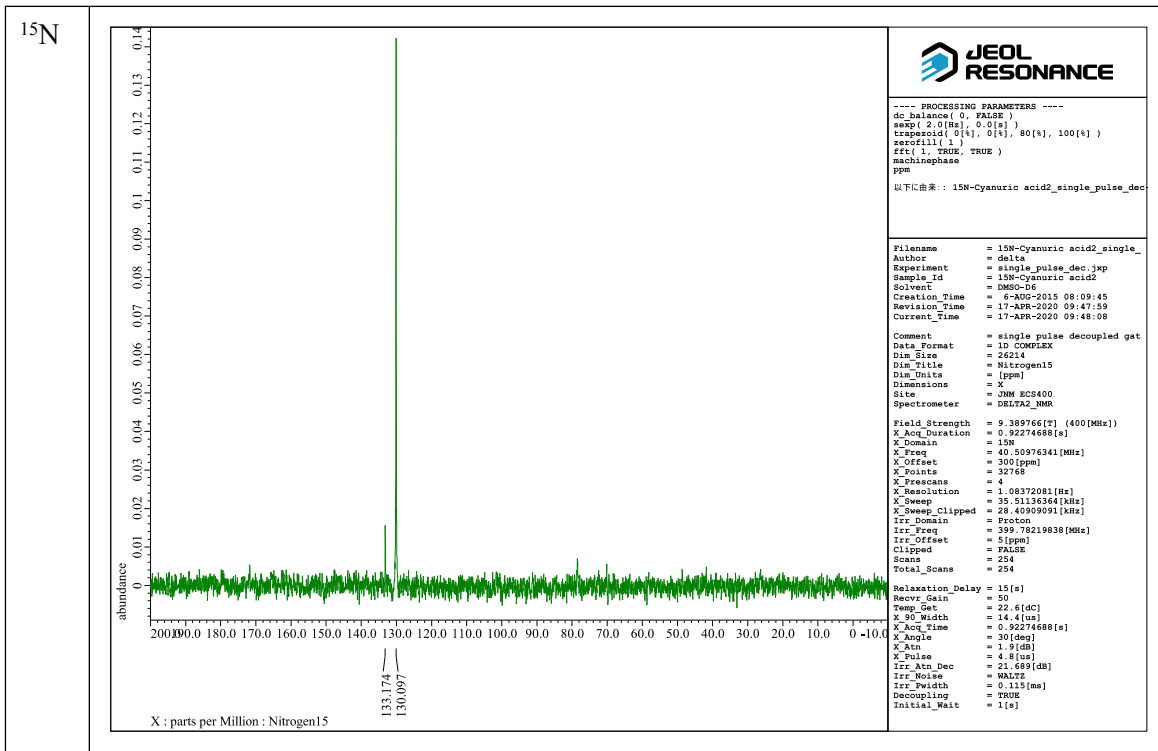
#### **S1.** NMR data for the synthetic compounds

S1-1	Compound <b>2</b>
S1-2	Compound <b>4</b>
S1-3	Compound <b>5</b>
S1-4	Compound <b>6</b>
S1-5	Compound <b>7b</b>
S1-6	Compound <b>7a</b>
S1-7	Compound <b>8</b>

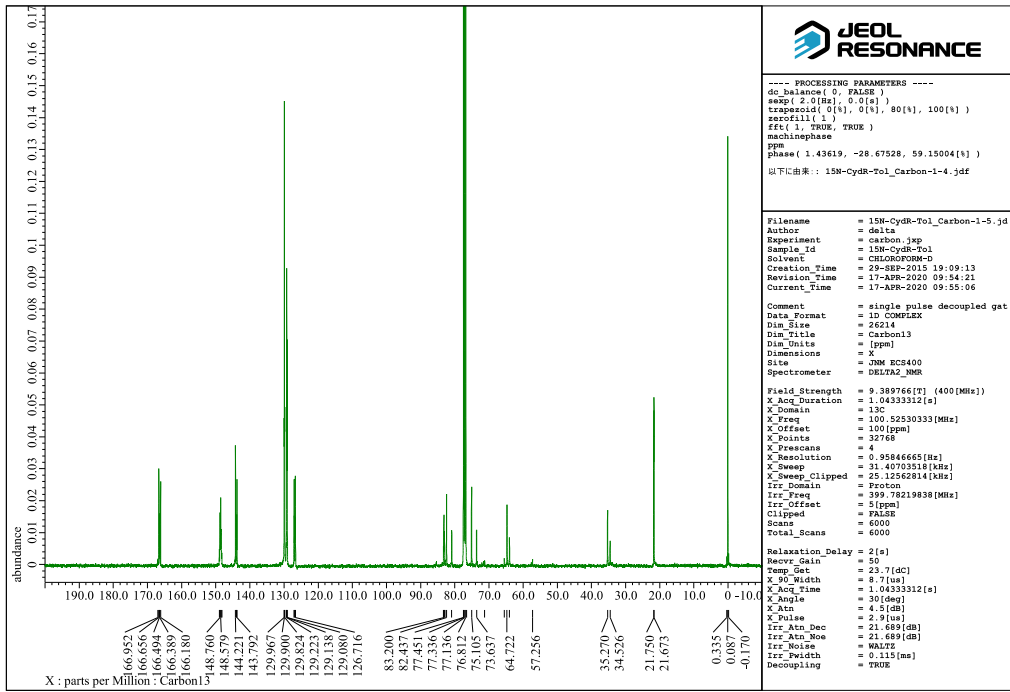
#### **S2.** Circular dichroism of duplex and triplex

# S1. NMR data for the synthetic compounds

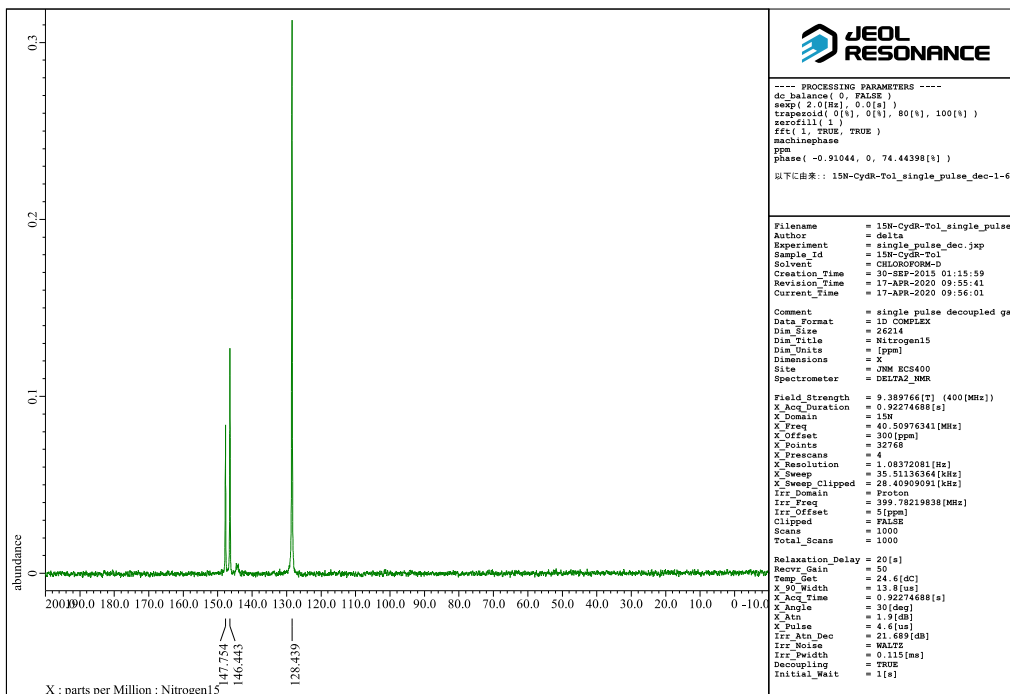
<p>S1-1</p>	<p>Compound 2</p>	 <p>in DMSO</p>
<p><sup>13</sup>C</p>		 <pre> ----- PROCESSING PARAMETERS ----- dc balance( 0, FALSE ) sexp( 2.0[Hz], 0.0[s] ) tropicacid( 0[Hz], 0[Hz], 80[Hz], 100[Hz] ) zerofill( 1 ) fft( 1, TRUE, TRUE ) machinephase ppm phase( -0.02654, 0, 74.02815[Hz] ) phase( -0.0, 0, 74.02815[Hz] ) phase( -0.0, 0, 74.02815[Hz] )  Filename = 15N-Cyanuric acid2_Carbon- Author = delta Experiment = carbon_jcp Sample_id = 15N-Cyanuric acid2 Solvent = DMSO-d6 Creation_Time = 6-AUG-2015 07:40:48 Revision_Time = 17-APR-2020 08:18:17 Current_Time = 17-APR-2020 09:38:26  Comment = single pulse decoupled gat Data_Format = 1D COMPLEX Dim_Size = 22026 Dim_Title = Carbon13 Dim_Units = [ppm] Dimensions = X Site = JNM ECS400 Spectrometer = DELTA2_NMR Field_strength = 9.389766[Hz] (400[MHz]) X_Acq_Duration = 1.0433332[s] X_Domain = 13C X_Freq = 100.52530333[MHz] X_Offset = 100[ppm] X_Points = 32768 X_Prescans = 4 X_Resolution = 0.95846665[Hz] X_Sweep = 31.40703518[kHz] X_Sweep_Clippped = 25.12562814[kHz] Irr_Domain = Proton Irr_Freq = 399.78219838[MHz] Irr_Offset = 5[ppm] Clipped = FALSE Scans = 512 Total_Scans = 512 Relaxation_Delay = 2[s] Recvr_Gain = 50 Temp_Get = 22.5[degC] X_90_Width = 8.57[us] X_Acq_Time = 1.0433332[s] X_Angle = 30[deg] X_Alt = 4.4[deg] X_Pulse = 2.8566667[us] Irr_Atn_Dec = 21.689[db] Irr_Atn_Noise = 21.689[db] Irr_Noise = WALTZ Irr_Width = 0.115[ms] Decoupling = TRUE </pre>
<p><sup>13</sup>C</p>		 <pre> ----- PROCESSING PARAMETERS ----- dc balance( 0, FALSE ) sexp( 2.0[Hz], 0.0[s] ) tropicacid( 0[Hz], 0[Hz], 80[Hz], 100[Hz] ) zerofill( 1 ) fft( 1, TRUE, TRUE ) machinephase ppm phase( -0.02654, 0, 74.02815[Hz] ) phase( -0.0, 0, 74.02815[Hz] ) phase( -0.0, 0, 74.02815[Hz] )  Filename = 15N-Cyanuric acid2_Carbon- Author = delta Experiment = carbon_jcp Sample_id = 15N-Cyanuric acid2 Solvent = DMSO-d6 Creation_Time = 6-AUG-2015 07:40:48 Revision_Time = 17-APR-2020 08:18:17 Current_Time = 17-APR-2020 09:40:14  Comment = single pulse decoupled gat Data_Format = 1D COMPLEX Dim_Size = 22026 Dim_Title = Carbon13 Dim_Units = [ppm] Dimensions = X Site = JNM ECS400 Spectrometer = DELTA2_NMR Field_strength = 9.389766[Hz] (400[MHz]) X_Acq_Duration = 1.0433332[s] X_Domain = 13C X_Freq = 100.52530333[MHz] X_Offset = 100[ppm] X_Points = 32768 X_Prescans = 4 X_Resolution = 0.95846665[Hz] X_Sweep = 31.40703518[kHz] X_Sweep_Clippped = 25.12562814[kHz] Irr_Domain = Proton Irr_Freq = 399.78219838[MHz] Irr_Offset = 5[ppm] Clipped = FALSE Scans = 512 Total_Scans = 512 Relaxation_Delay = 2[s] Recvr_Gain = 50 Temp_Get = 22.5[degC] X_90_Width = 8.57[us] X_Acq_Time = 1.0433332[s] X_Angle = 30[deg] X_Alt = 4.4[deg] X_Pulse = 2.8566667[us] Irr_Atn_Dec = 21.689[db] Irr_Atn_Noise = 21.689[db] Irr_Noise = WALTZ Irr_Width = 0.115[ms] Decoupling = TRUE </pre>



<sup>13</sup>C

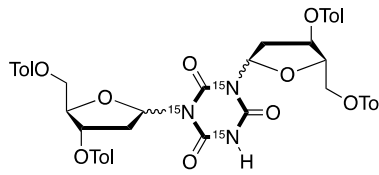


<sup>15</sup>N



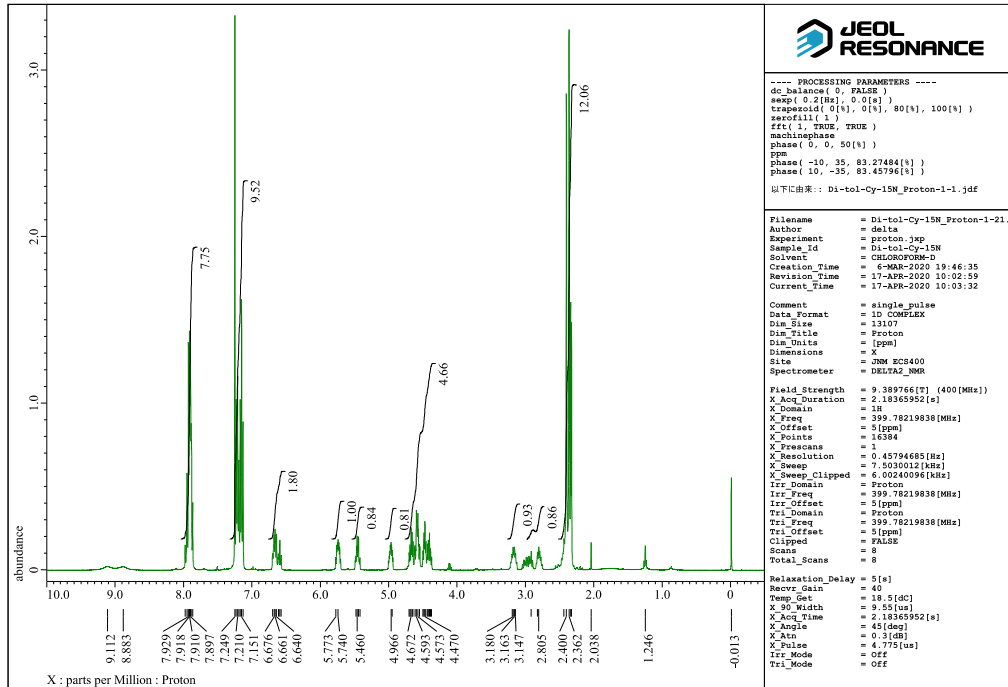
S1-3

Compound 5

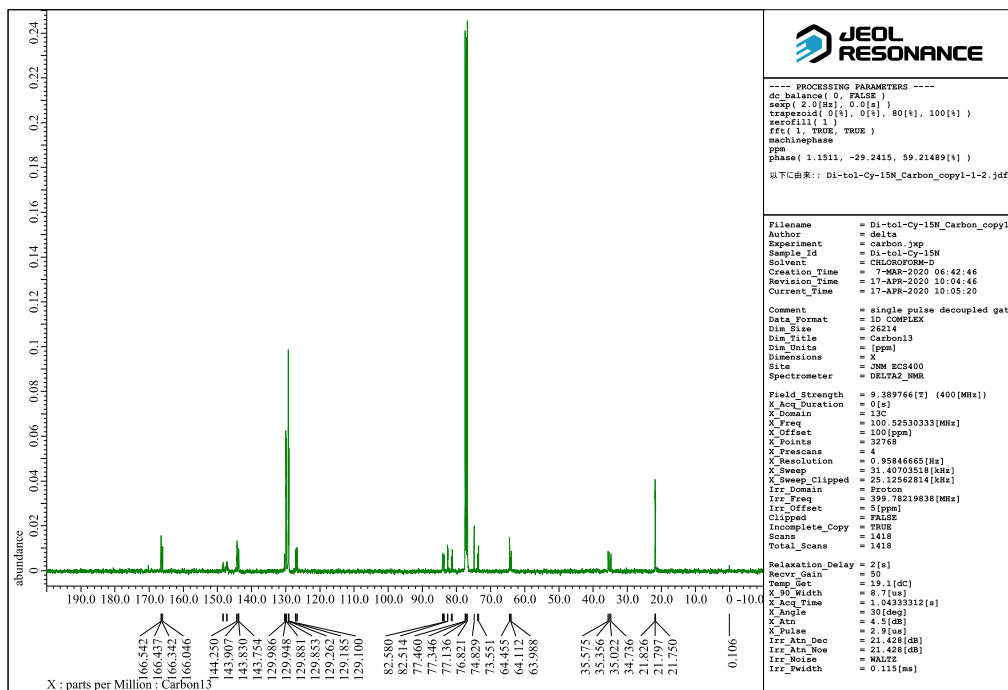


in CDCl<sub>3</sub>

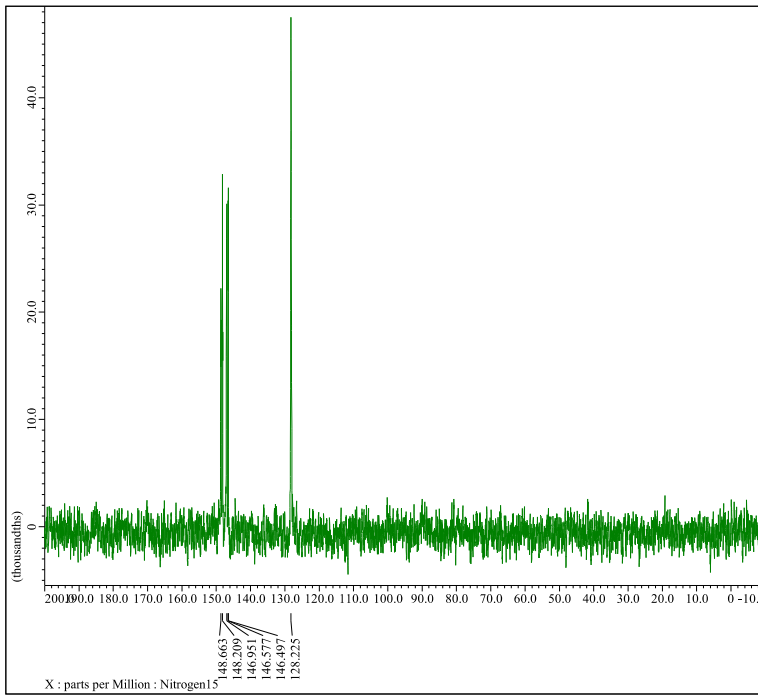
<sup>1</sup>H



<sup>13</sup>C

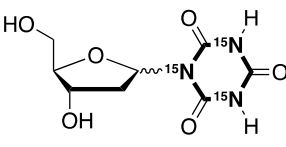
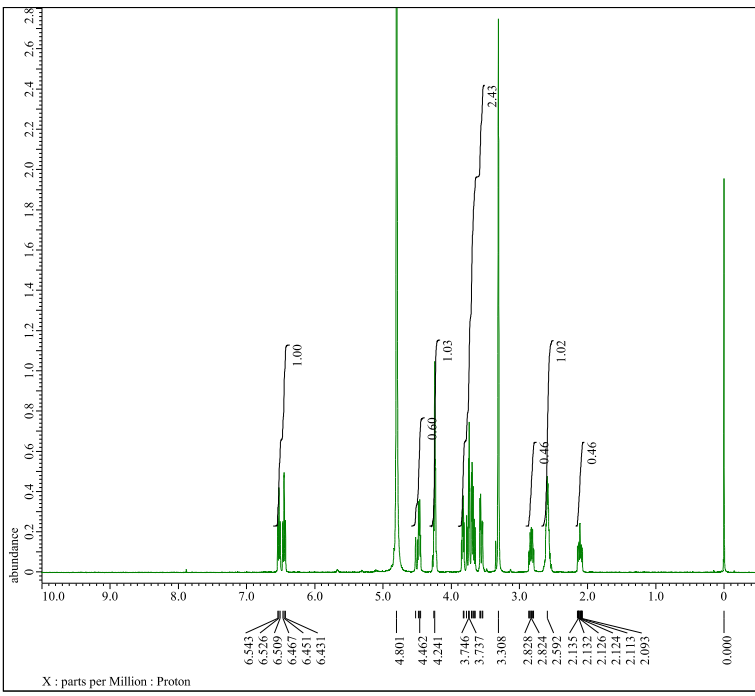
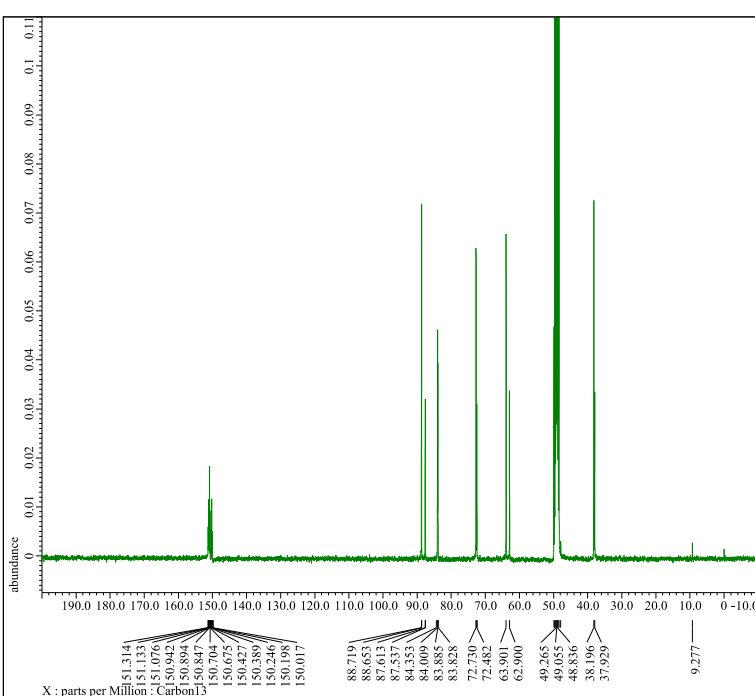


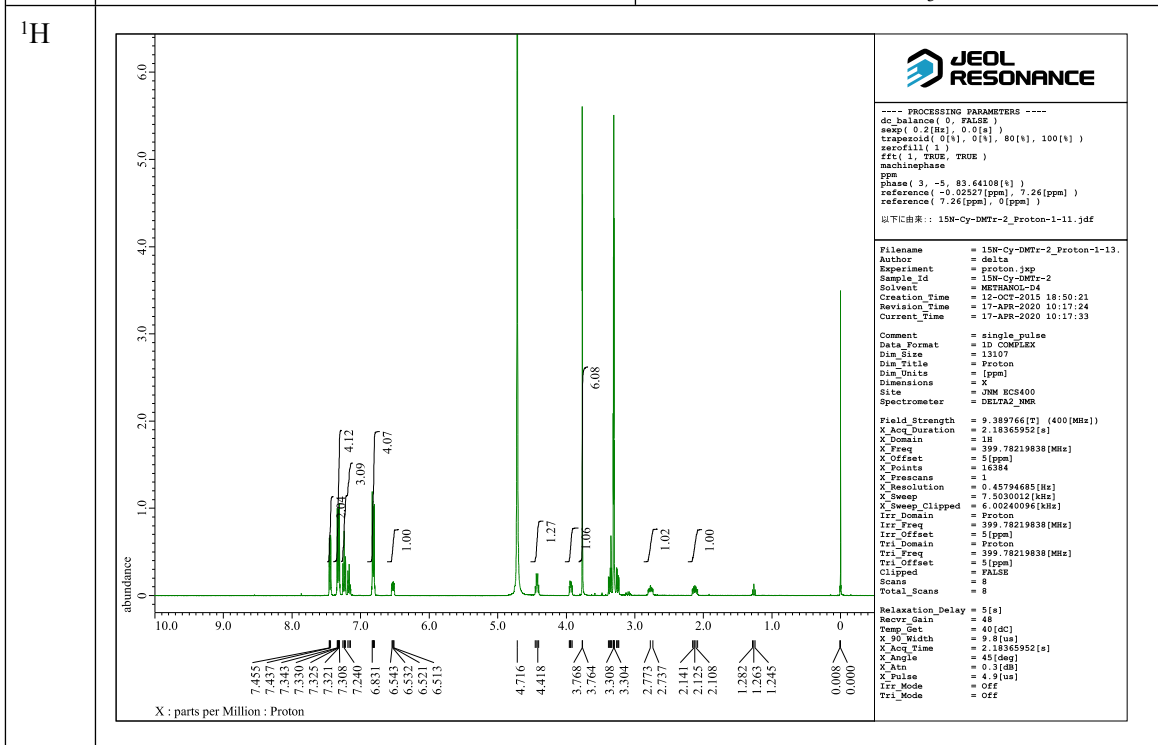
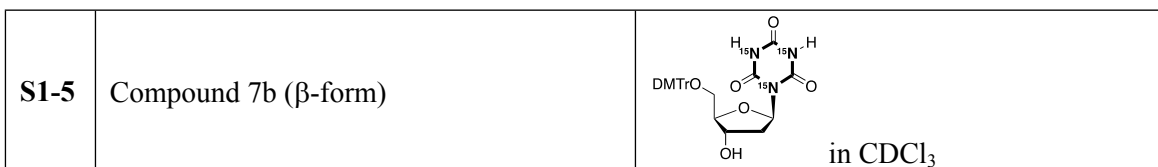
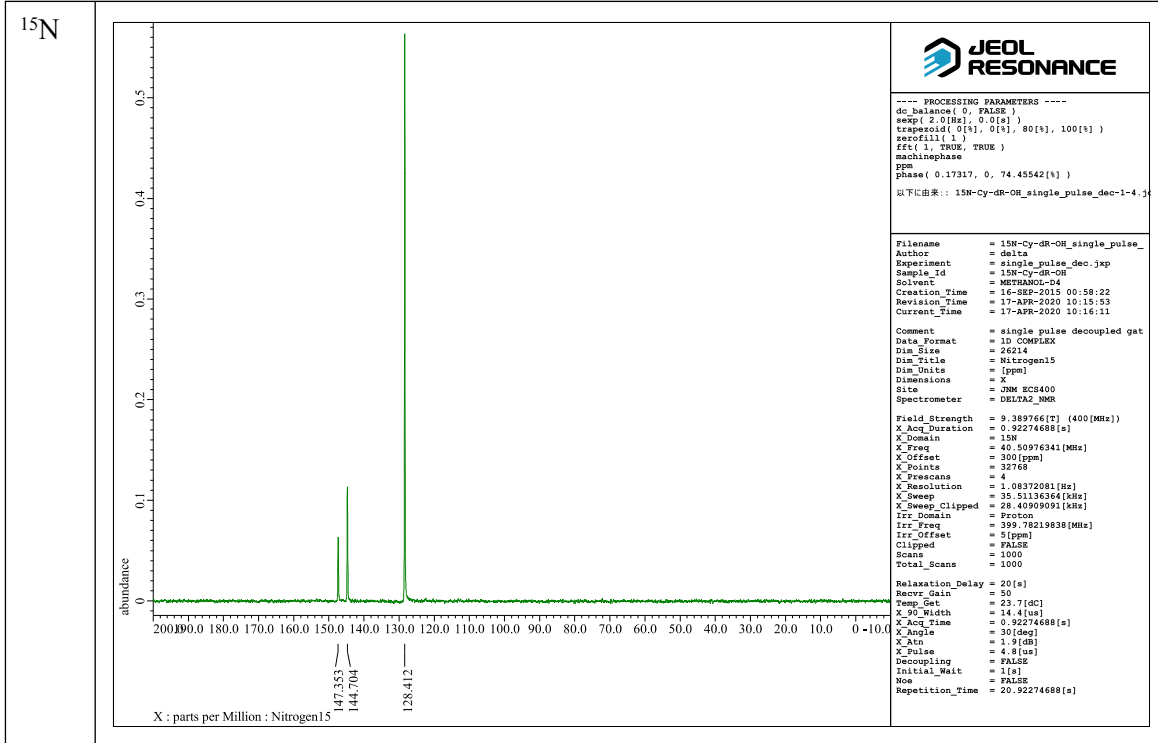
<sup>15</sup>N



---- PROCESSING PARAMETERS ----  
dc balance( 0, FALSE )  
msp( 2.0[Hz], 0.0[s] )  
trapezoid( 0[Hz], 0[Hz], 80[Hz], 100[Hz] )  
zerofill( 1 )  
fft( 1, TRUE, TRUE )  
machinephase  
ppm  
phase( 359.37302, -279.14263, 74.50883[Hz] )  
以下に由来: Di-tol-Cy-15N\_single\_pulse\_dec-1-5.3

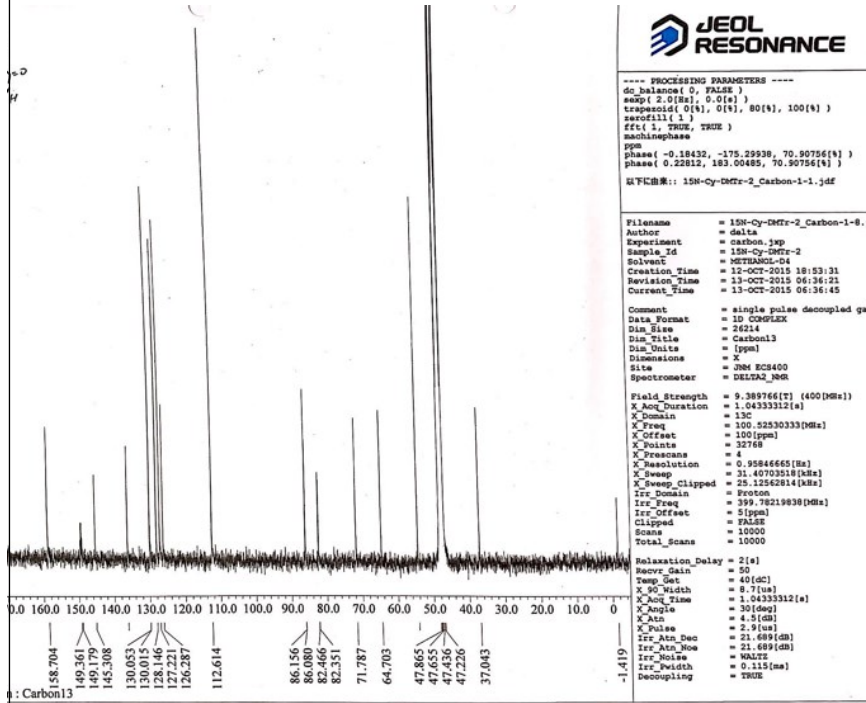
Filename = DI-tol-Cy-15N\_single\_pulse  
Author = delta  
Experiment = single\_pulse\_dec.jxp  
Sample\_Id = DI-tol-Cy-15N  
Solvent = CHLOROFORM-D  
Creation\_Time = 17-MAR-2020 08:27:23  
Revision\_Time = 17-APR-2020 10:05:58  
Current\_Time = 17-APR-2020 10:06:18  
Comment = single\_pulse decoupled gat  
Data\_Format = 1D\_COMPLEX  
Dim\_Size = 26214  
Dim\_Title = Nitrogen15  
Dim\_Units = [ppm]  
Dimensions = X  
Site = JNM ECS400  
Spectrometer = DELTA2\_NMR  
Field\_Strength = 9.389766[T] (400[MHz])  
X\_Acq\_Duration = 0.92274688[s]  
X\_Domain = 15N  
X\_Freq = 40.50976341[MHz]  
X\_Offset = 300[ppm]  
X\_Points = 32768  
X\_Freecans = 4  
X\_Resolution = 1.08372081[Hz]  
X\_Sweep = 35.51136364[kHz]  
X\_Sweep\_Clippped = 28.40300091[kHz]  
Irr\_Domain = Proton  
Irr\_Freq = 399.78219938[MHz]  
Irr\_Offset = 5[ppm]  
Clipped = FALSE  
Scans = 400  
Total\_Scans = 400  
Relaxation\_Delay = 15[s]  
Recvr\_Gain = 50  
Temp\_Gst = 13.9[dc]  
X\_50\_Width = 13.8[us]  
X\_Acq\_Time = 0.92274688[s]  
X\_Angle = 30[deg]  
X\_Atn = 1.9[db]  
X\_Pulse = 4.6[us]  
Irr\_Atn\_Dec = 21.428[db]  
Irr\_Noise = WALTZ  
Irr\_Pwidth = 0.115[ms]  
Decoupling = TRUE  
Initial\_wait = 1[s]

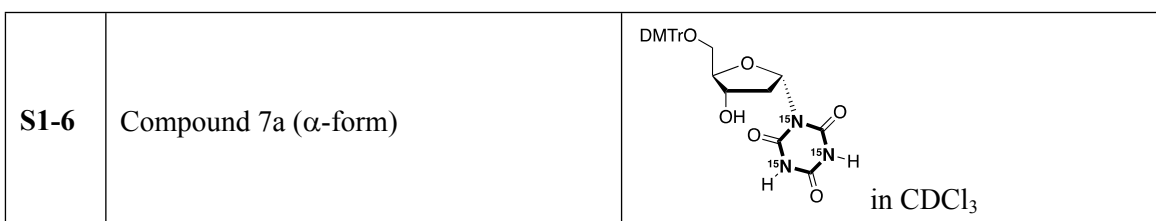
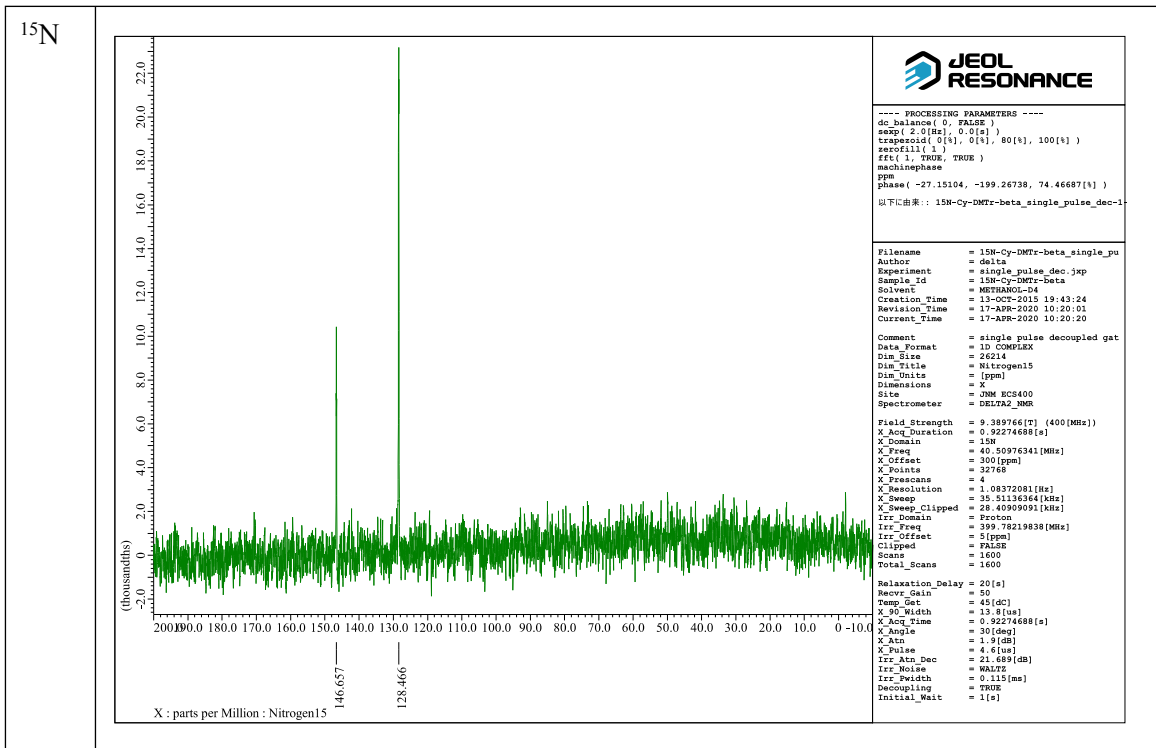
S1-4	Compound 6	 <p style="text-align: right;">in CDCl<sub>3</sub></p>
<sup>1</sup> H	 <p style="text-align: center;">X: parts per Million : Proton</p> <div style="float: right; border: 1px solid black; padding: 5px; width: 60%;"> <p style="text-align: center;"><b>JEOL RESONANCE</b></p> <p>---- PROCESSING PARAMETERS ----  dc_balance( 0, FALSE )  sqr( 0.2[Hz], 0.0[Hz] )  trapzoid( 0[Hz], 0[Hz], 80[Hz], 100[Hz] )  seofilt( 1 )  fft( 1, TRUE, TRUE )  machinephase  ppm  phase( -0.04535, -1.28873, 51.50313[Hz] )  以下に由来 : 15N-Cy-OH-3_Proton-1-8.jdf</p> <hr/> <p>Filename = 15N-Cy-OH-3_Proton-1-10.jd  Author = delta  Experiment = proton_jmp  Sample_Id = 15N-Cy-OH-3  Solvent = METHANOL-D4  Creation_Time = 6-OCT-2015 08:17:31  Revision_Time = 17-APR-2020 10:12:23  Current_Time = 17-APR-2020 10:12:31</p> <hr/> <p>Comment = single pulse  Data_Format = 1D COMPLEX  Dim_Size = 13107  Dim_Title = Proton  Dim_Units = [ppm]  Dimensions = X  Site = JNM ECS400  Spectrometer = DELTA2_NMR</p> <hr/> <p>Field_Strength = 9.389766[T] (400[MHz])  X_Acq_Duration = 2.18365952[s]  X_Domain = 38  X_Freq = 399.78219838[MHz]  X_Offset = 5[ppm]  X_Points = 14384  X_Prescans = 1  X_Resolution = 0.45784605[Hz]  X_Sweep = 7.5030012[kHz]  X_Sweep_Clippped = 6.00240096[kHz]  Irr_Domain = Proton  Irr_Freq = 399.78219838[MHz]  Irr_Offset = 5[ppm]  Tri_Domain = Proton  Tri_Freq = 399.78219838[MHz]  Tri_Offset = 5[ppm]  Clipped = FALSE  Scans = 8  Total_Scans = 8</p> <hr/> <p>Relaxation_Delay = 5[s]  Recvr_Gain = 46  Temp_Gat = 30[dc]  X_S0_Width = 9.8[us]  X_Acq_Time = 2.18365952[s]  X_Angle = 45[deg]  X_Atn = 0.3[db]  X_Pulse = 4.9[us]  Irr_Mode = Off  Tri_Mode = Off</p> </div>	
<sup>13</sup> C	 <p style="text-align: center;">X: parts per Million : Carbon13</p> <div style="float: right; border: 1px solid black; padding: 5px; width: 60%;"> <p style="text-align: center;"><b>JEOL RESONANCE</b></p> <p>---- PROCESSING PARAMETERS ----  dc_balance( 0, FALSE )  sqr( 2.0[Hz], 0.0[Hz] )  trapzoid( 0[Hz], 0[Hz], 80[Hz], 100[Hz] )  seofilt( 1 )  fft( 1, TRUE, TRUE )  machinephase  ppm  phase( 0.02305, -199.64116, 70.84271[Hz] )  以下に由来 : 15N-Cy-dR-OH_Carbon-1-9.jdf</p> <hr/> <p>Filename = 15N-Cy-dR-OH_Carbon-1-15.j  Author = delta  Experiment = carbon_jmp  Sample_Id = 15N-Cy-dR-OH  Solvent = METHANOL-D4  Creation_Time = 15-SEP-2015 18:42:39  Revision_Time = 17-APR-2020 10:15:11  Current_Time = 17-APR-2020 10:15:15</p> <hr/> <p>Comment = single pulse decoupled gat  Data_Format = 1D COMPLEX  Dim_Size = 26214  Dim_Title = Carbon13  Dim_Units = [ppm]  Dimensions = X  Site = JNM ECS400  Spectrometer = DELTA2_NMR</p> <hr/> <p>Field_Strength = 9.389766[T] (400[MHz])  X_Acq_Duration = 1.04333312[s]  X_Domain = 130  X_Freq = 100.52530333[MHz]  X_Offset = 100[ppm]  X_Points = 32768  X_Prescans = 4  X_Resolution = 0.95846605[Hz]  X_Sweep = 31.40703518[kHz]  X_Sweep_Clippped = 25.12362814[kHz]  Irr_Domain = Proton  Irr_Freq = 399.78219838[MHz]  Irr_Offset = 5[ppm]  Clipped = FALSE  Scans = 6000  Total_Scans = 6000</p> <hr/> <p>Relaxation_Delay = 2[s]  Recvr_Gain = 50  Temp_Gat = 50.1[dc]  X_S0_Width = 8.57[us]  X_Acq_Time = 1.04333312[s]  X_Angle = 30[deg]  X_Atn = 4.5[db]  X_Pulse = 8.8546667[us]  Irr_Atn_Dec = 21.689[db]  Irr_Atn_Noise = 21.689[db]  Irr_Noise = WALL2  Irr_Pwidth = 0.115[ms]  decoupling = TRUE</p> </div>	



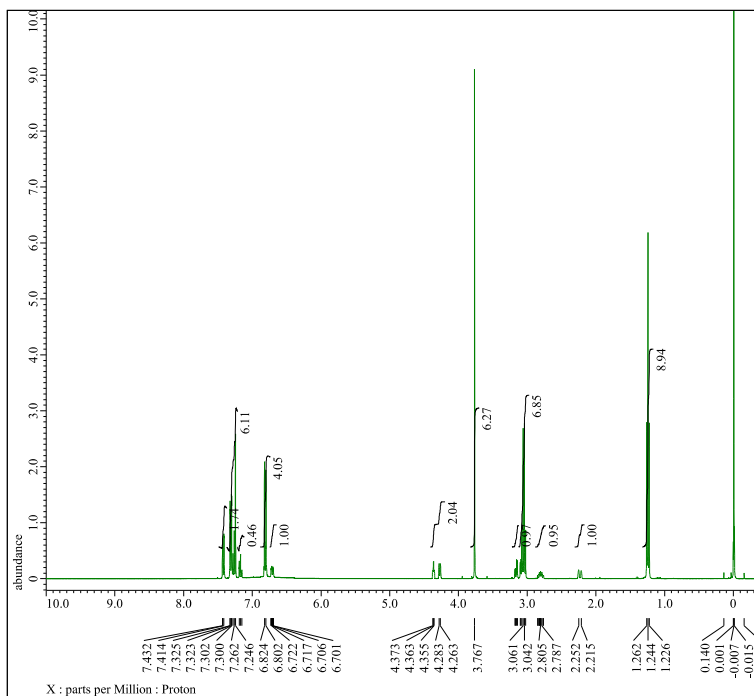


<sup>13</sup>C





<sup>1</sup>H



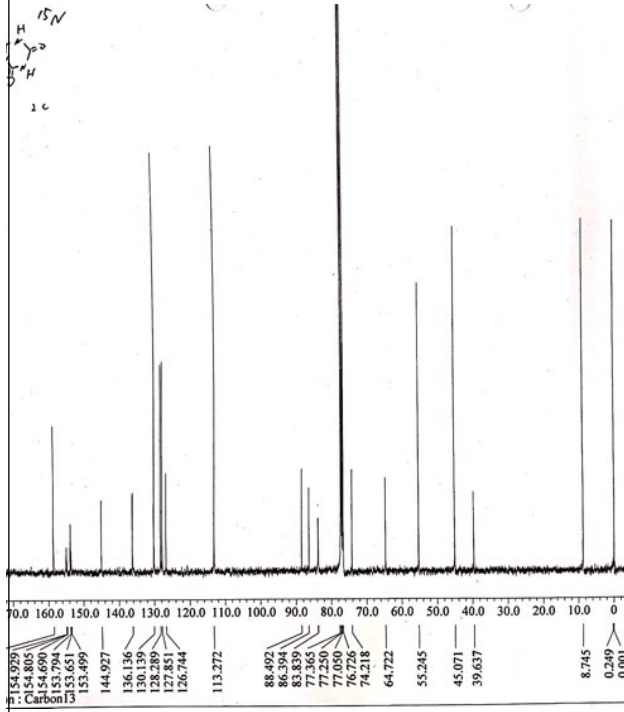
X : parts per Million : Proton



--- PROCESSING PARAMETERS ---  
dg\_balance(C, FALSE)  
sExp( 0.2 [Hz], 0.0 [s])  
trapezoid( 0 [s], 0 [s], 80 [s], 100 [s])  
zerofill( 1 )  
fft( 1, TRUE, TRUE )  
machinephase  
ppm  
Phase( 1, -0.00033, 83.35114 [°] )  
以下に由来: 15N-Cy-DMTr-alpha\_Proton-1-12.jdf

Filename = 15N-Cy-DMTr-alpha\_Proton-1  
Author = delta  
Experiment = proton\_jmp  
Sample\_Id = 15N-Cy-DMTr-alpha  
Solvent = CHLOROFORM-D  
Creation\_Time = 14-OCT-2013 06:47:58  
Revision\_Time = 17-APR-2020 10:23:29  
Current\_Time = 17-APR-2020 10:23:37  
Comment = single pulse  
Data\_Format = 1D COMPLEX  
Dim\_Size = 13107  
Dim\_Title = Proton  
Dim\_Units = [ppm]  
Dimensions = X  
Spectrometer = JNM ECS400  
Site = DELTA2\_NMR  
Field\_Strength = 9.389766 [T] (400 [MHz])  
X\_Acq\_Duration = 2.18365952 [s]  
X\_Domain = 1H  
X\_Freq = 399.78219838 [MHz]  
X\_Offset = 5 [ppm]  
X\_Points = 16384  
X\_Frequence = 1  
X\_Resolution = 0.45794685 [Hz]  
X\_Sweep = 7.5030012 [kHz]  
X\_Sweep\_Clippped = 6.00240096 [kHz]  
Irr\_Domain = Proton  
Irr\_Freq = 399.78219838 [MHz]  
Irr\_Offset = 5 [ppm]  
Tri\_Domain = Proton  
Tri\_Freq = 399.78219838 [MHz]  
Tri\_Offset = 5 [ppm]  
Clipped = FALSE  
Scans = 8  
Total\_Scans = 8  
Relaxation\_Delay = 5 [s]  
Recvr\_Gain = 38  
Temp\_Get = 45 [dC]  
X\_90\_Width = 9.8 [us]  
X\_Acq\_Time = 2.18365952 [s]  
X\_Angle = 45 [deg]  
X\_P1 = 0.2 [us]  
X\_Pulse = 4.9 [us]  
Irr\_Mode = Off  
Tri\_Mode = Off

<sup>13</sup>C



Chemical Shift (ppm)
154.959
154.805
154.690
153.794
153.651
153.499
144.927
136.136
132.789
127.851
126.744
113.272
88.492
86.392
83.839
77.365
77.250
77.050
76.726
74.218
64.722
55.245
45.071
39.637
8.745
0.249
0.001



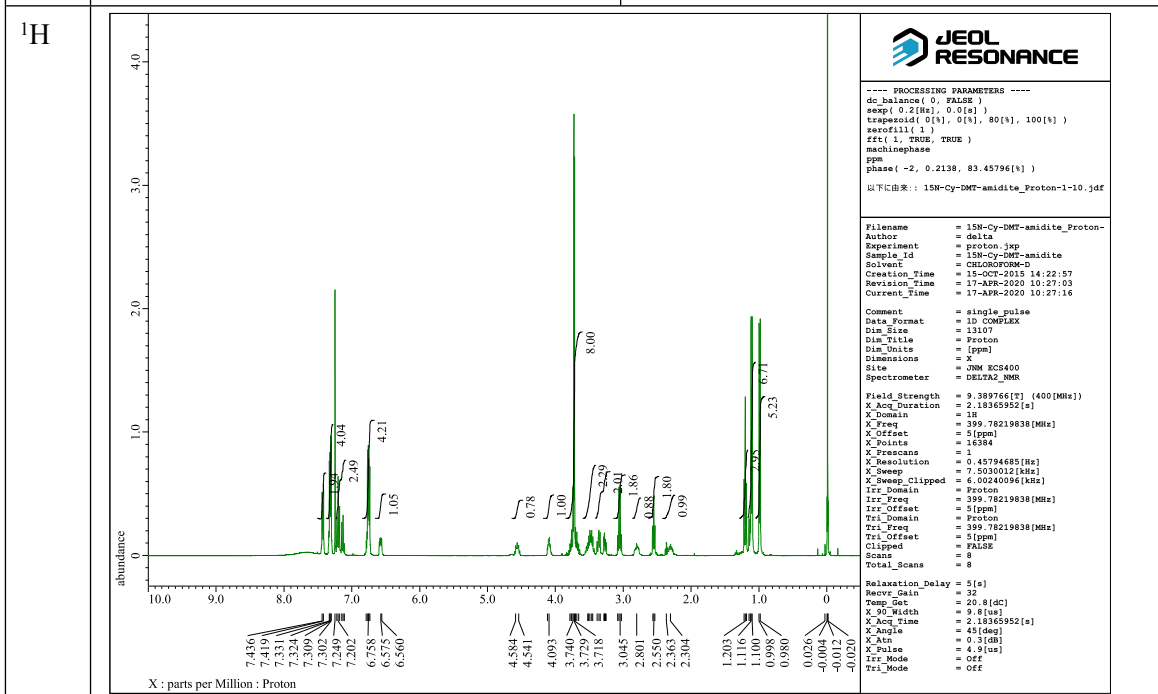
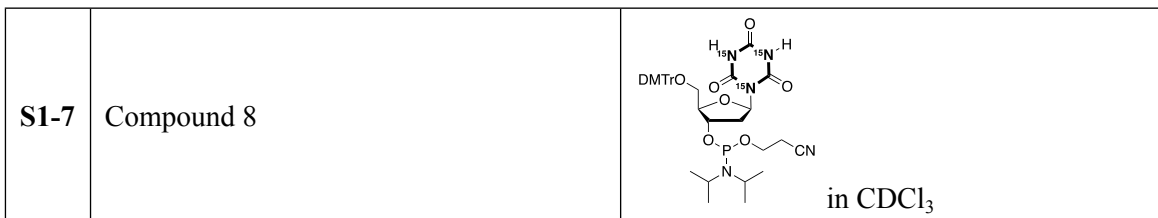
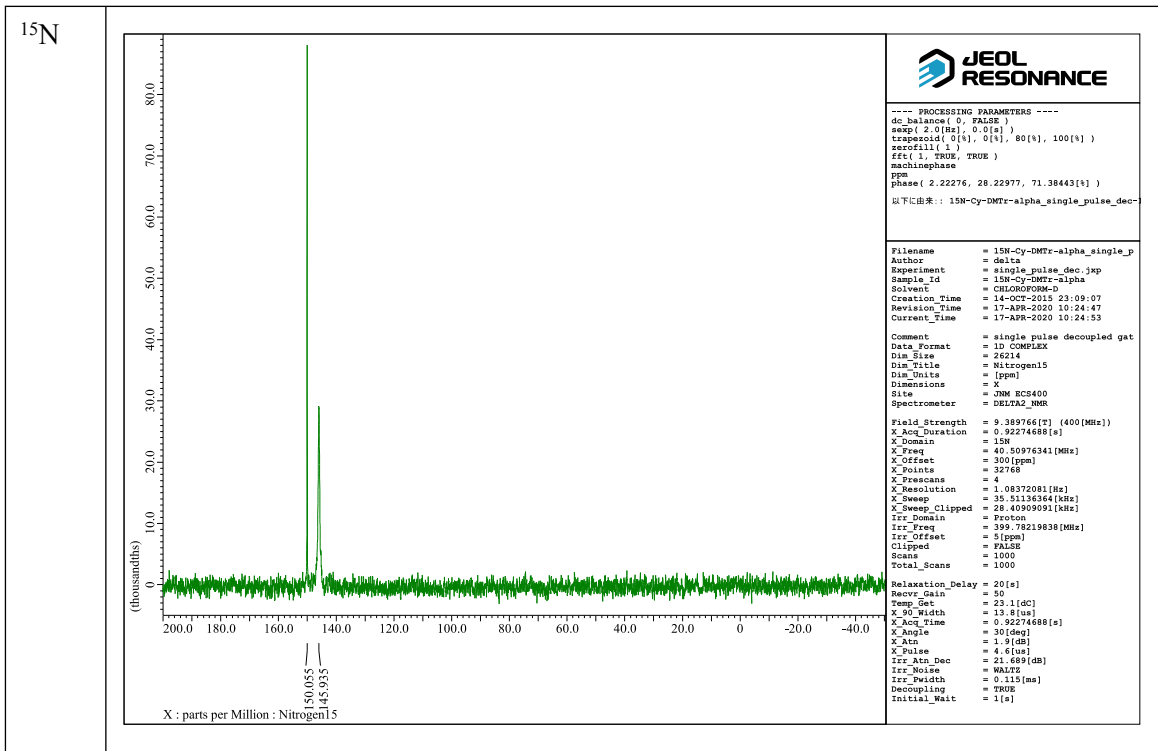
```
----- PROCESSING PARAMETERS -----
dc_balance( 0, FALSE )
snp( 2.018s, 0.01s )
trapezoid( 0%, 0%, 80%, 100% )
sinc( 1, 1 )
fft( 1, TRUE, TRUE )
magnifyphase
ppm
phase( -1.12616, 0, 89.89051(%) )
以下信息: 15N-Cy-DNtr-alpha_Carbon-1-1.jdf

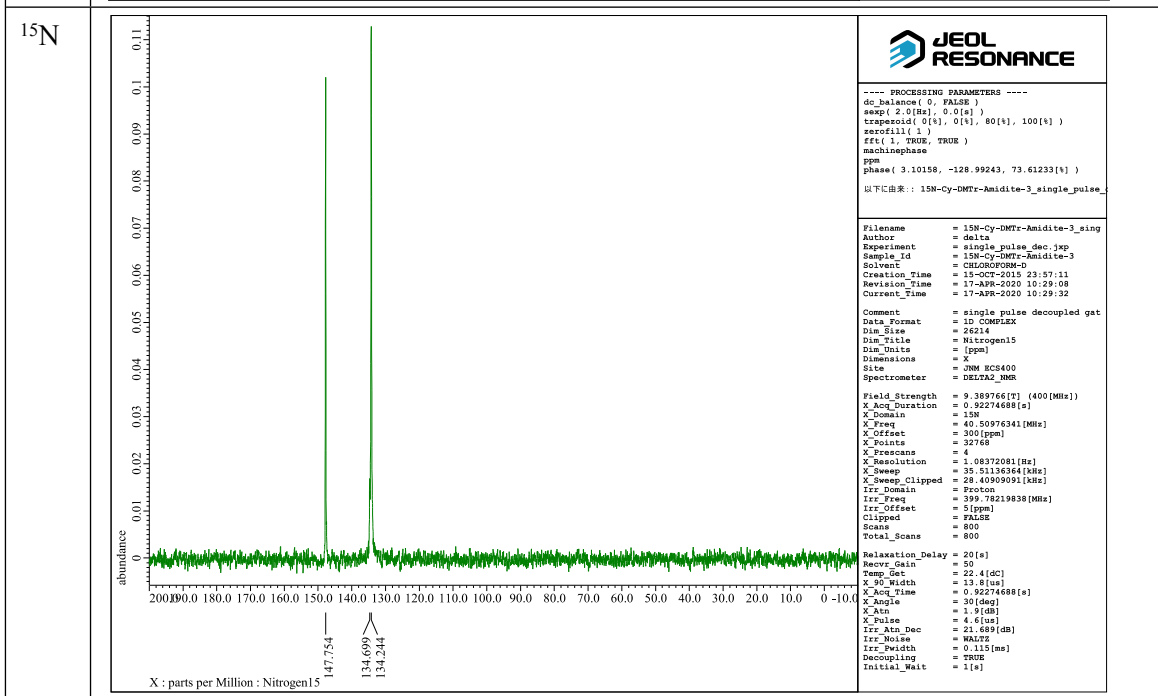
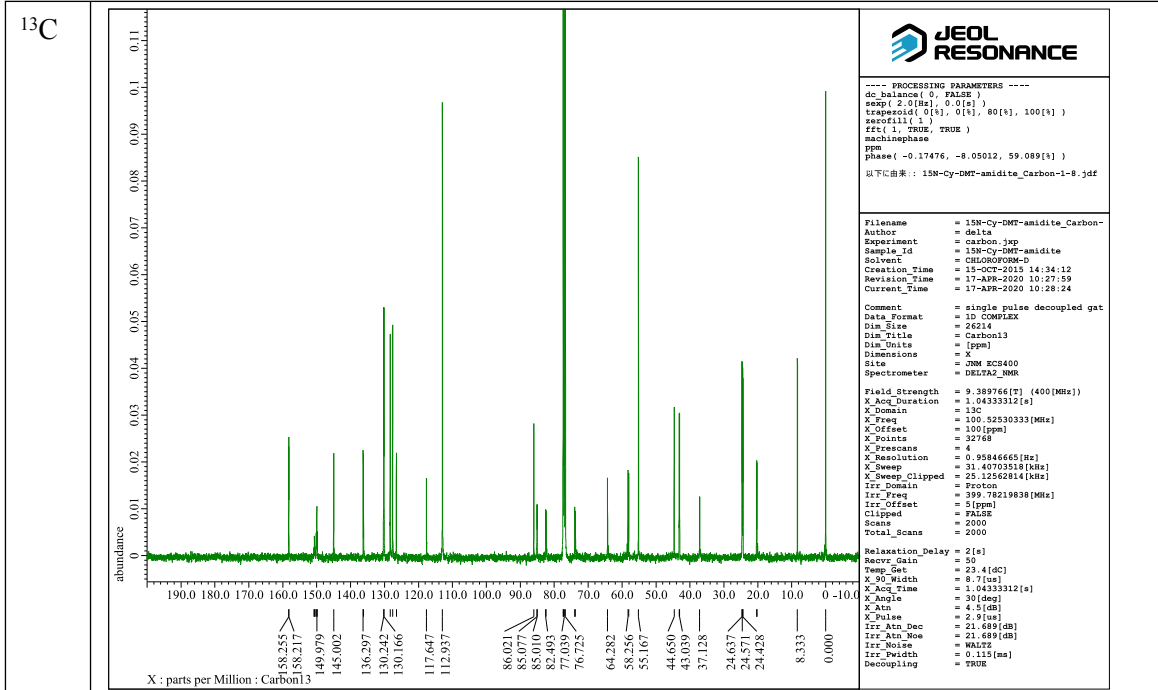
Filename      = 15N-Cy-DNtr-alpha_Carbon-1
Author        = delta
Experiment    = carbon.jxp
Sample_Id     = 15N-Cy-DNtr-alpha
Solvent       = CDCl3/DMSO-d
Creation_Time = 14-OCT-2015 06:52:17
Revision_Time = 14-OCT-2015 09:08:19
Current_Time  = 14-OCT-2015 09:08:52

Comment       = single pulse decoupled gat
Data_Format   = 1D CPMGLEX
Dir_Site      = 26214
Dir_Title     = Carbon13
Dir_Units     = [ppm]
Dimensions    = X
Site          = JNM ECX400
Spectrometer  = DELTAJ_90K

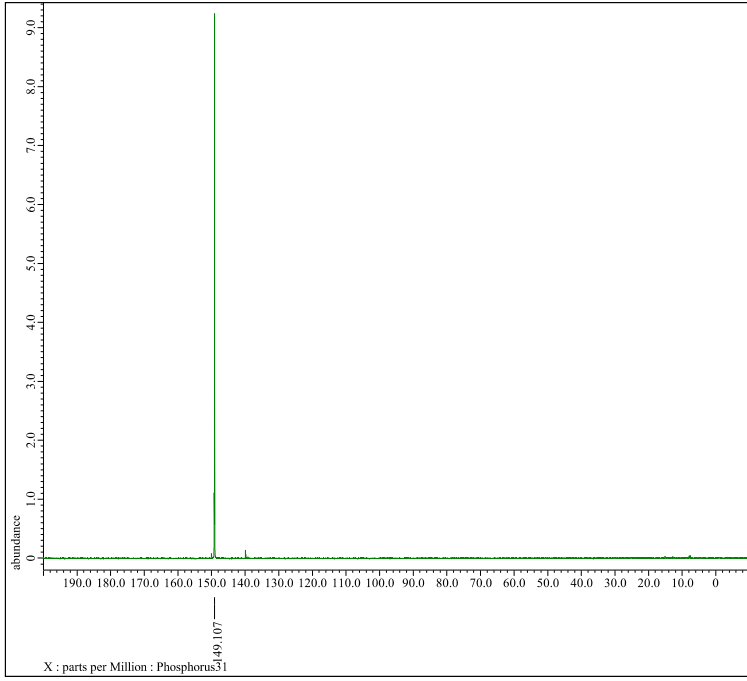
Field_Strength = 9.397766 [T] (400 [MHz])
X_Acq_Duration = 1.0433312 [s]
X_Domain       = 13C
X_Freq         = 100.6253033 [MHz]
X_Offset       = 100 [ppm]
X_Points       = 32768
X_Fscans       = 4
X_Resolution   = 0.95846665 [Hz]
X_Sweep        = 31.40703518 [kHz]
X_Sweep_Clipped = 21.12560814 [kHz]
Irr_Domain     = Proton
Irr_Freq       = 399.78219838 [MHz]
Irr_Offset     = 5 [ppm]
Clipped        = FALSE
Scans          = 2000
Total_Scans    = 2000

Relaxation_Delay = 2 [s]
Recov_Gain       = 50
Temp_Gat         = 45 [dC]
X_90_Width       = 8.7 [us]
X_Acq_Time       = 1.0433312 [s]
X_Angle          = 30 [deg]
X_Pulse          = 4.5 [dB]
X_Pulse_Prog    = 2.9 [us]
Irr_Atn_Dec     = 21.689 [dB]
Irr_Atn_Noise   = 21.689 [dB]
WALTZ           = WALTZ
Irr_Width       = 0.115 [ms]
Decoupling      = TRUE
```





31P



**JEOL RESONANCE**

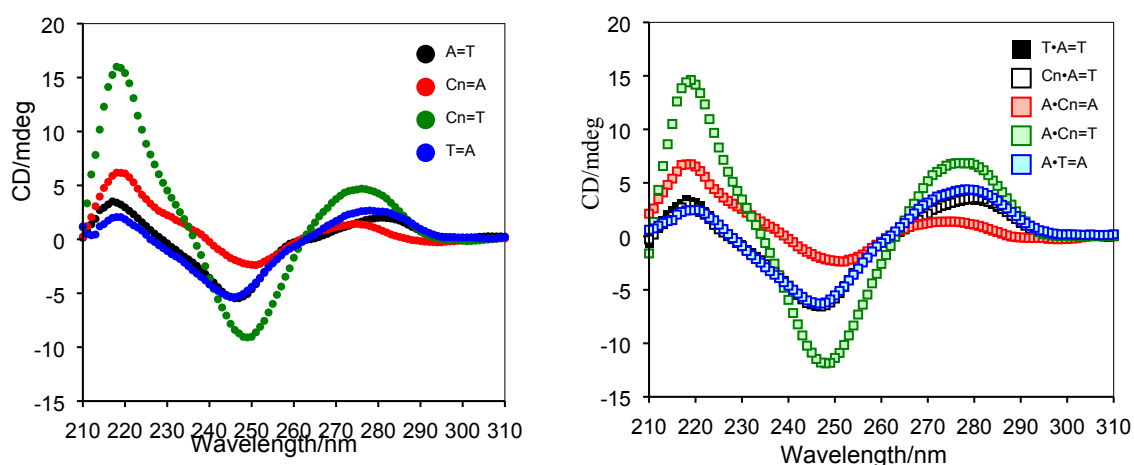
---- PROCESSING PARAMETERS ----  
dc\_balance( 0, FALSE )  
sweep( 2.0[Hz], 0.0[s] )  
trapezoid( 0[%], 0[%], 80[%], 100[%] )  
zerofill( 1 )  
fft( 1, TRUE, TRUE )  
machinephase  
ppm  
phase( -0.1569, 0, 28.88643[Hz] )  
以下に由来: 15N-Cy-DMTr-Amidite-3\_single\_pulse\_...

Filename = 15N-Cy-DMTr-Amidite-3\_sing  
Author = delta  
Experiment = single\_pulse\_dec.jxp  
Sample\_Id = 15N-Cy-DMTr-Amidite-3  
Solvent = CH2Cl2/CDCl3  
Creation\_Time = 16-OCT-2015 04:39:38  
Revision\_Time = 17-APR-2020 10:31:22  
Current\_Time = 17-APR-2020 10:31:32  
Comment = single pulse decoupled gat  
Data\_Format = 1D COMPLEX  
Dim\_Size = 26214  
Dim\_Title = Phosphorus31  
Dim\_Units = [ppm]  
Dimensions = X  
Site = JNM ECP400  
Spectrometer = DELTA2\_NMR  
Field\_Strength = 9.389766[T] (400[MHz])  
X\_Acq\_Duration = 0[s]  
X\_Domain = 31P  
X\_Freq = 161.83469309[MHz]  
X\_Offset = 0[ppm]  
X\_Points = 32768  
X\_Prescans = 4  
X\_Resolution = 4.35965402[Hz]  
X\_Sweep = 142.85714286[kHz]  
X\_Sweep\_Clippped = 114.28571429[kHz]  
Irr\_Domain = Proton  
Irr\_Freq = 399.78219838[MHz]  
Irr\_Offset = 5[ppm]  
Clipped = FALSE  
Incomplete\_Copy = TRUE  
Scans = 325  
Total\_Scans = 325  
Relaxation\_Delay = 20[s]  
Recor\_Gain = 50  
Temp\_Get = 21.6[dc]  
X\_90\_Width = 13.91[us]  
X\_Acq\_Time = 0.229376[s]  
X\_Angle = 30[deg]  
X\_Pin = 2.9[dB]  
X\_Pulse = 4.6333333[us]  
Irr\_Abs\_Dec = 21.609[dB]  
Irr\_Noise = HALTZ  
Irr\_Width = 0.115[ms]  
Decoupling = TRUE

## S2. Circular Dichroism of duplex and triplex

Circular dichroism spectra were measured on a JASCO J-710 spectropolarimeter between 350 nm and 200 nm in standard buffer containing 1.0 M NaCl, 10 mM of MacIlvaine buffer (phosphate-citric acid, pH 5.8), at 15 °C. The duplexes and triplexes concentrations were 0.8  $\mu$ M (15  $\mu$ M/base pair). Spectra were acquired every 1 nm with a bandwidth setting of 1 nm at a speed of 50 nm/min, averaging over 5 scans.

Circular dichroism spectroscopy was used to study the macroscopic helical geometry of the duplex DNA. All CD spectra for DNAs indicated by table 2 were analyzed at 15 °C. The CD spectra of triplex were indicated the same trend for each CD of duplex.



Circular dichroism of the sequences shown in Table 2 (in 10 mM McIlvaine buffer pH 5.8 and 1000 mM NaCl).