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Supporting Information

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Yunfeng Cheng, Hanjing Peng, Weixuan Chen, Nanting Ni, Bowen Ke, Chaofeng Dai,* and Binghe Wang*[a]

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A Novel Method for the Rapid and Specific Post-synthesis Modification of DNA via a Bio-orthogonal Condensation of 1,2-Aminothiols with 2-Cyanobenzothiazole †

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Supporting Information

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1. General Information

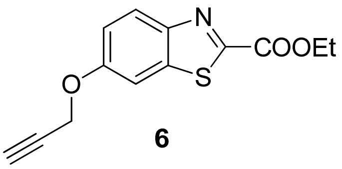
Solvents and reagents were purchased from VWR International, Oakwood Product Inc., or Sigma-Aldrich Co. and used without purification unless specified otherwise. When necessary, solid reagents were dried under high vacuum. Reactions with compounds sensitive to air or moisture were performed under argon. Solvent mixtures are indicated as volume/volume ratios. Thin layer chromatography (TLC) was run on Sorbtech

W/UV254 plates (0.25 mm thick), and visualized under UV-light or by a Ce-Mo staining solution (phosphomolybdate, 25 g; $Ce(SO_4)_2.4H_2O$, 10 g; conc. H_2SO_4, 60 mL; H_2O, 940 mL) with heating. Flash chromatography was performed using Fluka silica gel 60 (mesh size: 0.040-0.063 mm) using a weight ratio of ca. 30:1 for silica gel over crude compound. ^{1}H, ^{13}C, and ^{31}P-NMR spectra were recorded on a Bruker 400 spectrometer (400, 100, and 166 MHz, respectively) in deuterated chloroform ($CDCl_3$), methanol-*d*$_4$ (CD_3OD), and DMSO-*d*$_6$ with either tetramethylsilane (TMS) (0.00 ppm) or the NMR solvent as the internal reference. HPLC purification for CBT-TTP was carried out using a Shimadzu LC-10AT VP system with a Zobax C18 reversed-phase column (9.4 mm × 25 cm). The sample was eluted (6 mL/min) with buffer A (20 mM triethylammonium acetate, pH 6.9-7.1) and buffer B (50% acetonitrile, 20 mM triethyl ammonium acetate) with the following: 0 min 0% B; 20 min 20% B; 30 min 100% B; 38 min 100% B; 40 min 0% B; and 45 min 0% B.

DNA primers and templates were purchased from Integrated DNA Technologies. Klenow fragment (3'-5' exo), *Taq.* DNA polymerase, and Deep Vent$_R$ (exo-) DNA polymerase were purchased from New England Biolabs. KOD-XL DNA polymerase was purchased from Novagen, Darmstadt. Reaction buffers were used as provided.

2. Experimental Procedure for the Synthesis of CBT-TTP

Ethyl 6-(prop-2-ynyloxy)benzo[d]thiazole-2-carboxylate **6**



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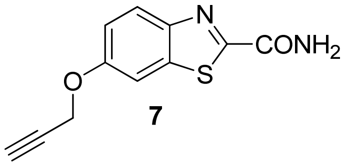
To a solution of **5** (2.23 g, 10 mmol) in DMF (30 mL) was added K_2CO_3 (2.70 g, 20 mmol) and the mixture was stirred at RT for 30 min. After addition of propargyl bromide (80% in toluene, 3.7 mL, 20 mmol), the reaction mixture was stirred at RT for 12 h before diluting with EtOAc (300 mL). Then the mixture was washed with water (50 mL × 3) and brine (50 mL), and dried over Na_2SO_4. Solvent evaporation followed by flash chromatography (Hex: EA, 4:1) gave a yellow solid (2.35 g, 90% yield). ^{1}H NMR ($CDCl_3$): 8.14 (d, *J* = 9.1 Hz, 1H), 7.49 (d, *J* = 2.3Hz, 1H), 7.24 (dd, *J* = 2.3, 9.1 Hz, 1H), 4.80 (s, 2H), 4.55 (q, *J* = 8.7 Hz, 2H), 2.58 (s, 1H), 1.48 (t, *J* =8.7 Hz); ^{13}C NMR ($CDCl_3$):

160.7, 157.4, 156.4, 148.3, 138.4, 126.3, 117.9, 105.1, 63.0, 56.4, 14.3. HRMS (ESI) for $C_{13}H_{12}NO_3S$ $[M+H]^+$ Calcd. 262.0545, Found 262.0544.

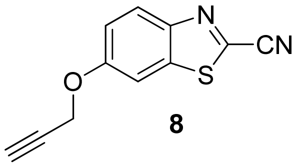
6-(Prop-2-ynyloxy)benzo[d]thiazole-2-carboxamide **7**



7

To a solution of ester **6** (2.35 g, 9 mmol) in EtOH 100 mL was added ammonium hydroxide 40 mL. The reaction mixture was heated to 75 °C for 5 h and then cooled to RT. Solvent was removed using a rota-evaporator, and the resulting precipitate was filtered, washed with H_2O, EtOH and EtOAc to give a white solid (1.54 g, yield 73%). ^{1}H NMR ($CDCl_3$): 8.41 (s, 1H), 8.03 (d, *J* = 9.1 Hz, 1H), 8.01 (s, 1H), 7.82 (d, *J* = 2.3Hz, 1H), 7.26 (dd, *J* = 2.3, 9.1 Hz, 1H), 4.92 (d, *J* = 2.2 Hz, 2H,), 3.64 (t, *J* = 2.2 Hz, 1H); ^{13}C NMR ($CDCl_3$): 163.7, 162.4, 157.3, 148.7, 138.9, 125.7, 118.3, 107.3, 79.8, 79.6, 57.1. IR film 3424.7, 3301.3, 1696.0, 1600.8, 1502.0, 1399.5, 1256.1, 1230.5; HRMS (ESI) for $C_{11}H_9N_2O_2S$ $[M+H]^+$ Calcd 233.0380; found 233.0387.

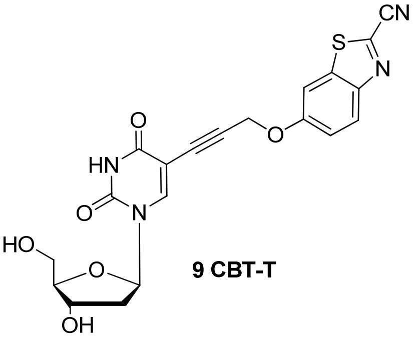
6-(Prop-2-ynyloxy)benzo[d]thiazole-2-carbonitrile **8**



8

To a suspension of amide **7** (1.1 g, 4.7 mmol) in anhydrous pyridine (25 mL) was added a solution of phosphorous oxychloride (0.85 mL, 9.4 mmol) in CH_2Cl_2 (5 mL) dropwise at 0 °C. After stirring at 0 °C for 1 h, the reaction was slowly warmed to ambient temperature and the resulting solution was stirred for an additional 12 h. CH_2Cl_2 (200 mL) was added, and the reaction was quenched by addition of H_2O 20 mL. The organic phase was separated, washed with H_2O (20 mL × 2), brine (20 mL), and dried over Na_2SO_4. Solvent evaporation followed by flash chromatography (Hex: EtOAc, 6:1) afforded compound **8** as a light yellow solid (900 mg, yield 82%). ^{1}H NMR ($CDCl_3$): 8.14 (d, *J* = 9.1 Hz, 2H), 7.51 (d, *J* = 2.5 Hz, 1H), 7.25 (dd, *J* = 2.5, 9.1 Hz, 1H), 4.83 (d, *J* = 2.4 Hz, 2H), 2.61 (t, *J* = 2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$): 158.2, 147.4, 137.2, 134.0, 126.0, 118.8, 113.1, 104.7, 56.5; IR film 3255.3, 2232.2, 2126.5, 1606.5, 1551.5, 1470.1, 1256.8, 1230.3, 1132.0, 1049.9, 127.7. HRMS (ESI) for $C_{11}H_7N_2OS$ $[M+H]^+$ Cacld. 215.0279, Found 215.0289

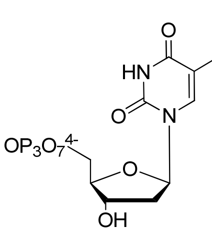
CBT-T **9**



9 CBT-T

To a mixture of 5-iodo-deoxyuridine (708 mg, 2 mmol), $Pd(PPh_3)_4$ (230 mg, 0.2 mmol), and CuI (76 mg, 0.4 mmol) in anhydrous DMF (12 mL) under argon was added DIPEA (0.62 mL, 4 mmol) followed by a solution of compound **8** (700 mg, 3.0 mg) in DMF (4 mL). After stirring at RT overnight, DMF was evaporated, and the remaining dark gum was purified by flash chromatography (DCM: MeOH, 20 : 1) to afford compound **9** as a pale yellow solid (630 mg, yield 72%). ^{1}H NMR (CD_3OD): 8.39 (s, 1H), 8.08 (d, *J* = 9.2 Hz, 2H), 7.81 (d, *J* = 2.0 Hz, 1H), 7.34 (dd, *J* = 2.0, 9.2 Hz, 1H), 6.22 (t, J = 6.4 Hz), 5.07 (s, 2H), 4.39 (m, 1H), 3.94 (d, J = 3.2 Hz), 3.83 (m, 1H), 3.23 (s, 1H), 2.30 (m, 1H), 2.20 (m, 1H); ^{13}C NMR (CD_3OD): 164.3, 159.9, 151.1, 148.4, 146.4, 138.8, 135.6, 126.5, 120.3, 114.2, 106.4, 99.2, 89.2, 88.4, 87.2, 80.6, 71.8, 62.5, 58.1, 55.8, 41.8; IR film 3413.7, 2229.5, 1725.8, 1667.6, 1616.6, 1600.8, 1475.2, 1372.7, 1278.4, 1229.3, 1129.9, 1091.8, 1001.6; HRMS (ESI) for $C_{20}H_{16}N_4O_6SNa$ $[M+Na]^+$ Cacld 463.0683; Found 463.0674.

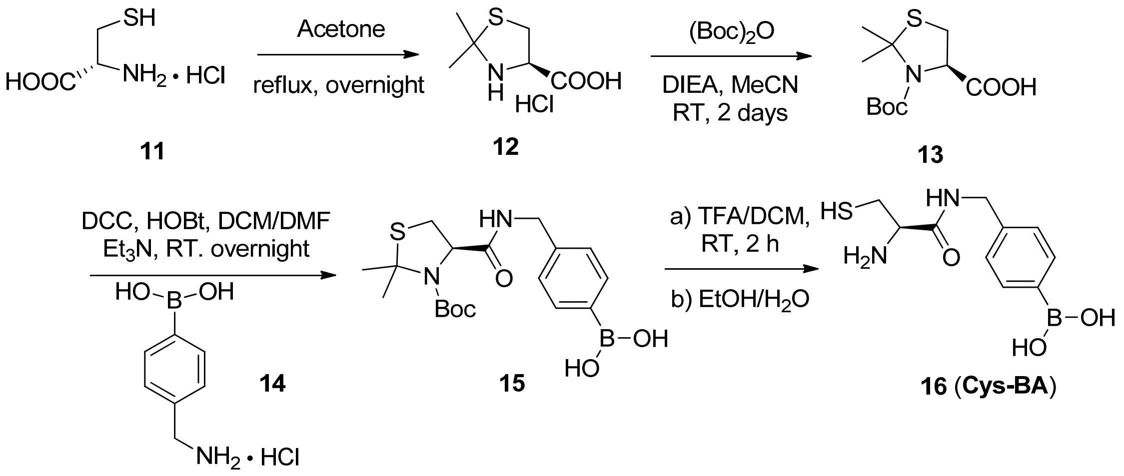
CBT-TTP **10**



10 CBT-TTP

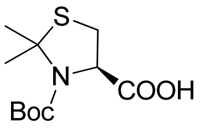
To a solution of CBT-T **9** (70 mg, 0.16 mmol) and proton sponge (42 mg, 0.19 mmol, pre-dried in vacuum over P_2O_5 overnight) in anhydrous trimethylphosphate (0.6 mL) was added freshly distilled $POCl_3$ (18 μL, 0.19 mmol) dissolved in anhydrous trimethylphosphate (0.2 mL) dropwise via a syringe with stirring under argon at 0 °C. The reaction mixture was further stirred in an ice-bath for 2 h and then a solution of tributylammonium pyrophosphate (226 mg, 0.48 mmol) and tri-*n*-butylamine (0.4 mL,) in 1.0 mL of anhydrous DMF was added in one portion. The mixture was stirred at RT for 10 min and then triethylammonium bicarbonate solution (0.1 M, pH 8, 11 mL) was added. After stirring at RT for an additional hour, the resulting reaction mixture was transferred to a 50-mL centrifuge tube. Then EtOH (33 mL) was added followed by 3 M NaCl solution (1.0 mL). After vortexing for 10 sec, the centrifuge tube was placed at – 80 ^{o}C for 1 h, and then centrifuged at 5000 rpm for 20 min. After removing the supernatant, the resulting pellet was purified by HPLC and lyophilized to give a pale yellow powder (9 mg, 10%). ^{1}H NMR (D_2O): 8.57 (s, 1H), 8.05 (d, *J* = 6.8 Hz, 2H), 7.65 (s, 1H), 7.27 (d, *J* = 9.2 Hz, 1H), 6.11 (t, *J* = 6.4 Hz), 5.00 (s, 2H), 4.11-4.13(m, 3H), 2.27-2.31 (m, 2H); ^{13}C NMR (D_2O): 158.5, 151.5, 147.1, 146.0, 138.1, 135.7, 125.7, 119.9, 113.9, 106.2, 99.2, 88.9, 86.5, 86.4, 86.3, 79.5, 70.8, 65.8, 58.1, 58.0, 39.3, 17.5; ^{31}P NMR (D_2O) -10.9, -11.5, -23.4. HRMS (ESI) for $C_{20}H_{18}N_4O_{15}P_3S$ $[M-H]^-$ Cacld. 678.9702; Found 678.9683.

3. Experimental Procedure for the Synthesis of Cys-BA



Scheme S1. Synthesis of Compound **16** (**Cys-BA**)

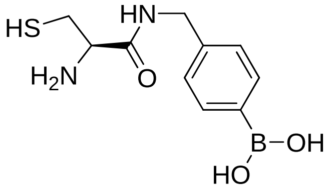
L-(-)-(*tert*-Butoxycarbonyl)-2,2-dimethylthiazolidine-4-carboxylic acid **13**



13

Compound **13** was prepared starting from L-cysteine hydrochloride (**11**) in two steps following literature procedures.[1]

Cys-BA (**16**)

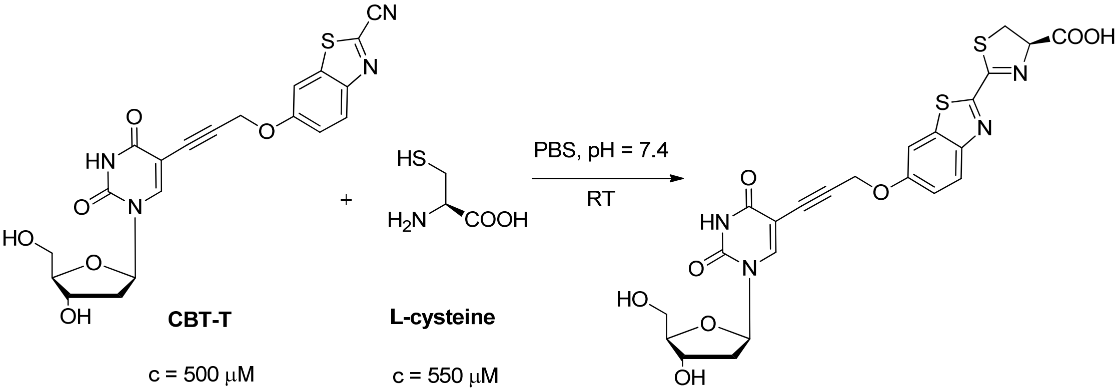


16 (**Cys-BA**)

To a mixture of compound **13** (836 mg, 3.2 mmol), **14** (500 mg, 2.7 mmol), and HOBt (397 mg, 2.9 mmol) in anhydrous DCM (20 mL) and DMF (20 mL) under ice-bath, was added Et_3N (0.7 mL, 5.3 mmol) followed DCC (607 mg, 2.9 mmol) in one portion. The mixture was stirred at RT overnight and the resulting solid was filtered off. The filtrate was concentrated under vacuum and the residue was purified by flash chromatography (DCM: MeOH, 30:1) to afford intermediate **15**. ^1H NMR ($CDCl_3$): 7.83 (d, J = 6.8 Hz, 2H), 7.25 (d, *J* = 6.8 Hz, 2H), 4.82 (brs, 1H), 4.48 (s, 2H), 3.25 (m, 2H), 1.82 (s, 3H), 1.77 (s, 3H), 1.50 (s, 6H).

Compound **15** was stirred in DCM/TFA (20 mL/20 mL) at RT for another 2 h. Solvent was evaporated under vacuum and the residue was treated with diethyl ether (100 mL). The resulting precipitate was collected through filtration. After washing three times with diethyl ether, the collected precipitate was dissolved in EtOH/H_2O (300 mL/300 mL) with stirring for 30 mins. Evaporation of the solvent yielded compound **16** as a white solid (497 mg, 73% in two steps). ^{1}H NMR (CD_3OD): 7.67 (d, *J* = 4.0 Hz, 2H), 7.25 (d, *J* = 4.0 Hz, 2H), 4.39 (m, 1H), 4.08 (m, 1H), 3.00 (m, 2H); ^{13}C NMR (CD_3OD): 168.2, 140.7, 134.9, 127.7, 55.8, 44.2, 26.1; HRMS (ESI) for $C_{10}H_{15}BN_2O_3S$ $[M+H]^+$ Cacld 255.0969; Found 255.0965.

4. Kinetic studies of the reaction between CBT-T and 1, 2-aminothiol (L-cysteine)



Scheme S2. Reaction scheme of CBT-T with L-cysteine

To a solution of CBT-T **9** in acetonitrile was added a solution of L-cysteine in PBS buffer (pH = 7.4) to afford the final concentration of CBT-T (500 μM), L-cysteine (550 μM) in a mixture of acetonitrile/PBS buffer (v/v: 1/4). The resulting mixture was stirred at room temperature and monitored by HPLC at different time points in a similar way as literature reported.[2] Applying similar kinetic analysis reported in the references, 1/[CBT-T] vs reaction time was plotted and the linear regression analysis of 1/[CBT-T] vs. time gave the second-order rate constant as 22 $M^{-1.}$ s^{-1}.

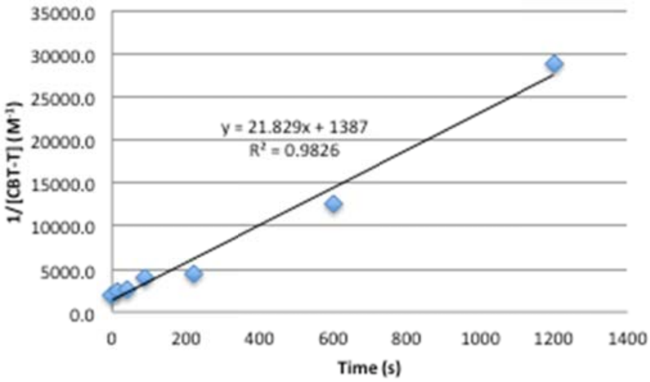


Figure S1. Linear regression analysis of 1/[CBT-T] vs. time of the CBT-T condensation reaction

5. MALDI-TOF spectra of DNA products

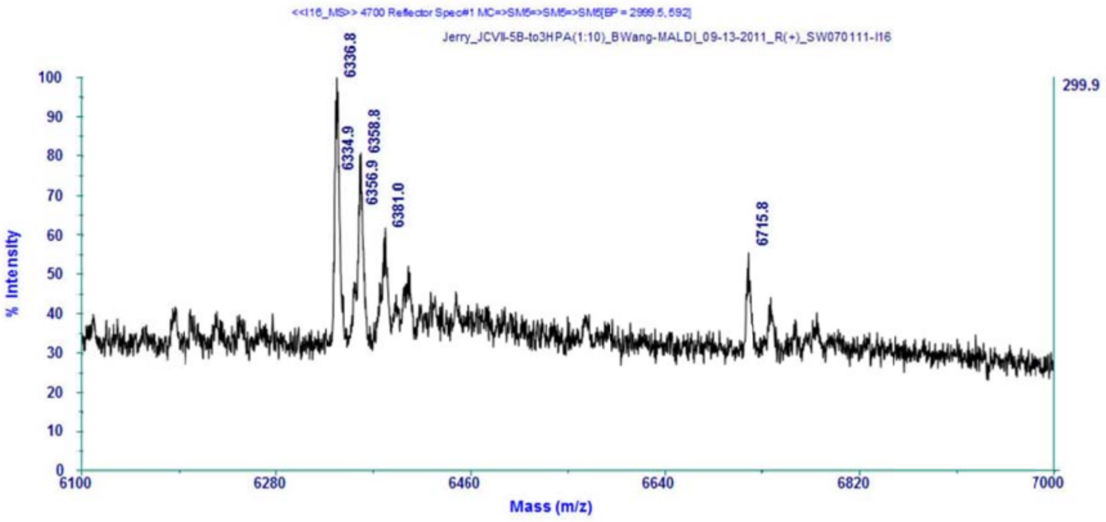


Figure S2. MALDI-TOF spectra of CBT-DNA$_{21}$ (Template-1)

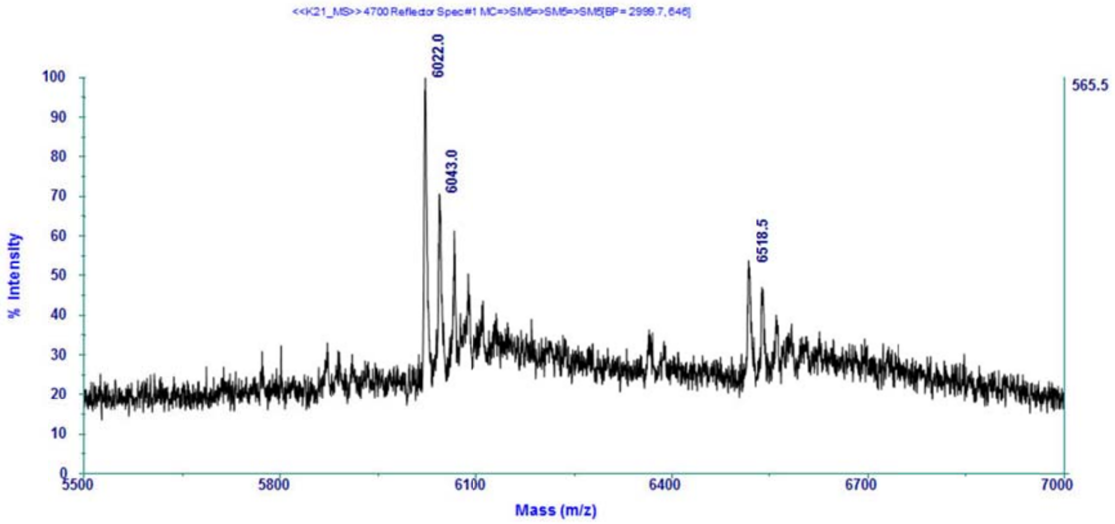


Figure S3. MALDI-TOF spectra of dNTP-DNA_{21} (Template-1)

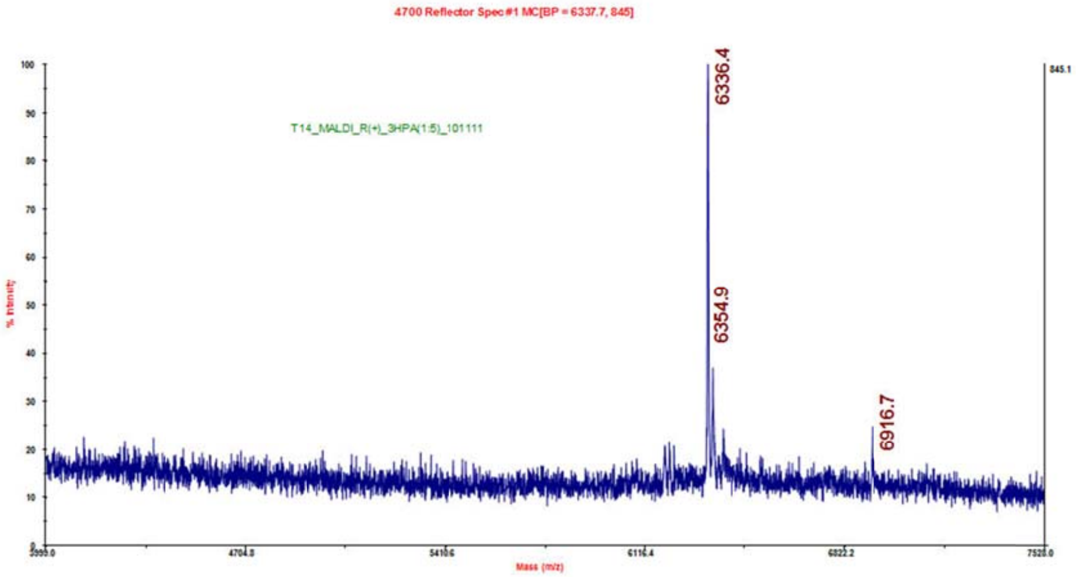


Figure S4. MALDI-TOF spectra of click product of CBT-DNA_{21} and Cys-BA (Template-1)

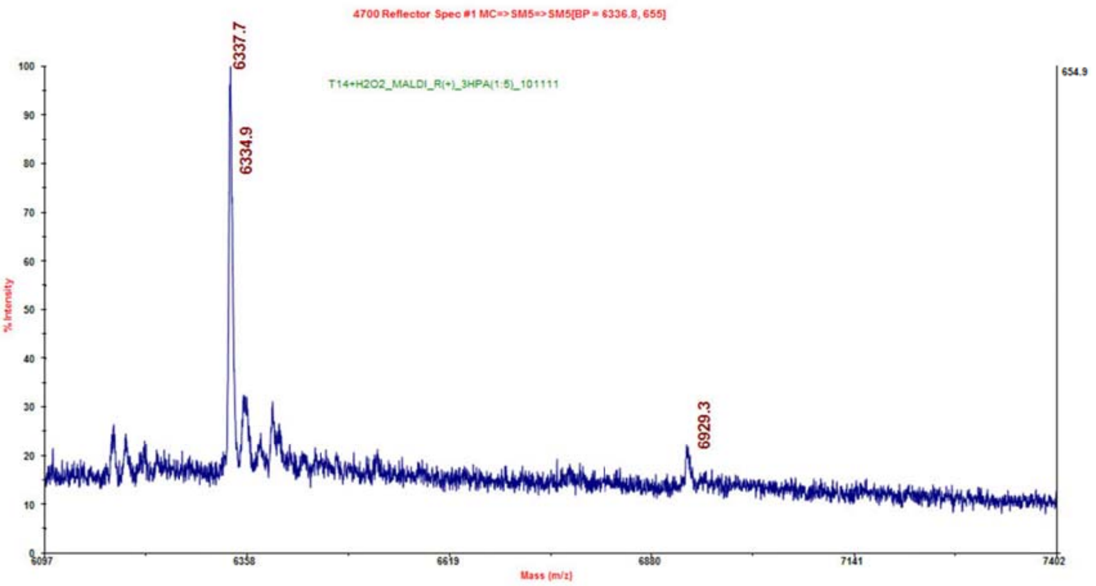


Figure S5. MALDI-TOF spectra of H_2O_2 treated click product of CBT-DNA$_{21}$ (Template-1)

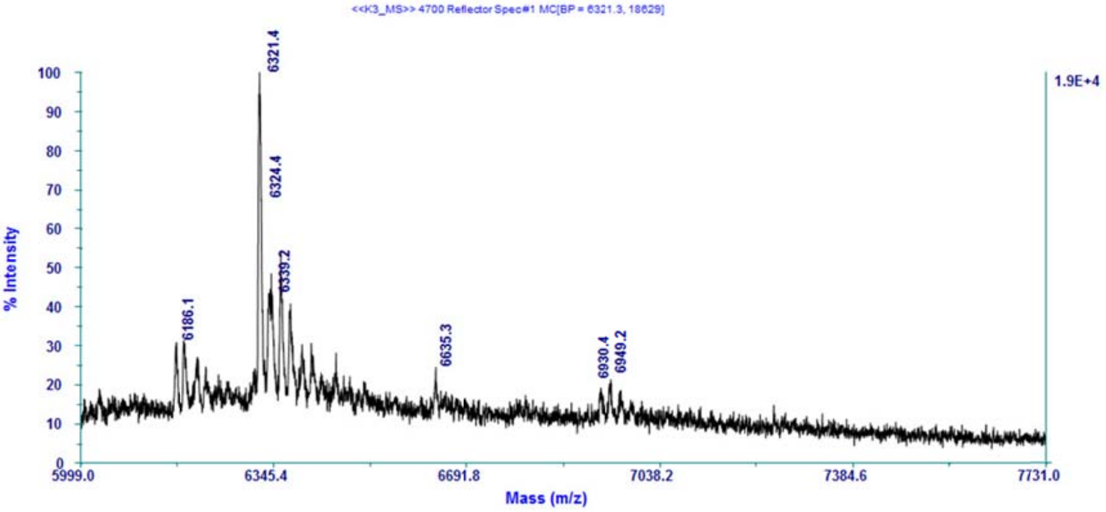


Figure S6. MALDI-TOF spectra of CBT-DNA$_{21}$ (Template-2)

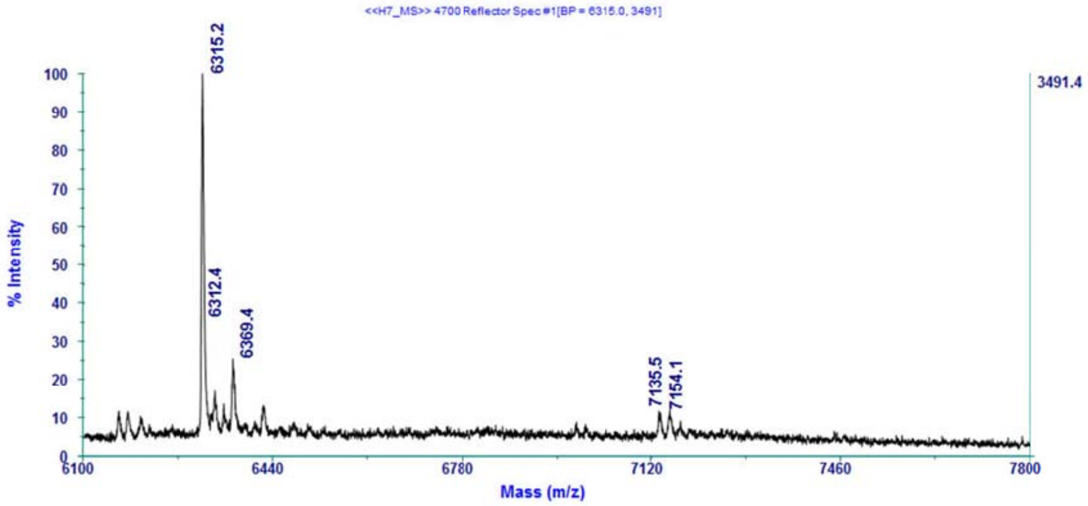


Figure S7. MALDI-TOF spectra of CBT-DNA_{21} (Template-3)

6. Thermodenaturation of duplex DNAs

The UV-melting temperature studies were carried out by Cary 300 UV–Vis Spectrophotometer and a temperature control module. Duplex DNA samples were prepared through Klenow fragment primer extension reactions with dTTP or CBT-TTP. Post-synthetic sample was prepared by click reaction of CBT-DNA_{21} with Cys-BA. All samples were cleaned using Millipore Amicon 10 kDa spin column. The samples (1 µM DNA duplexes) were dissolved in PBS buffer (pH 7.4). The samples were heated to 80 °C and cooled to 6 °C at a rate of 0.5 °C per min. 4 Rounds of melting were performed for each sample. The melting temperature data and curves are presented in Table S1 and Figure S7.

Table S1. Melting temperatures (T_m) of DNA dueplexes

DNA/Rounds	1	2	3	4	Average
dNTPs-DNA_{21}	72.97	73.02	73.02	72.97	73.00 ± 0.03
CBT-DNA_{21}	70.94	70.97	70.97	70.97	70.96 ± 0.02
CBT-DNA_{21} + Cys-BA	69.87	70.92	69.92	70.92	70.24 ± 0.59

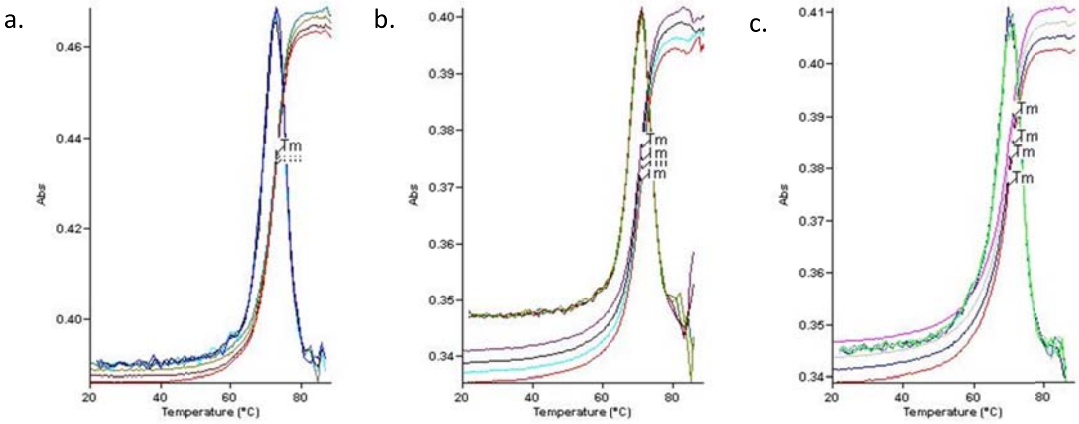


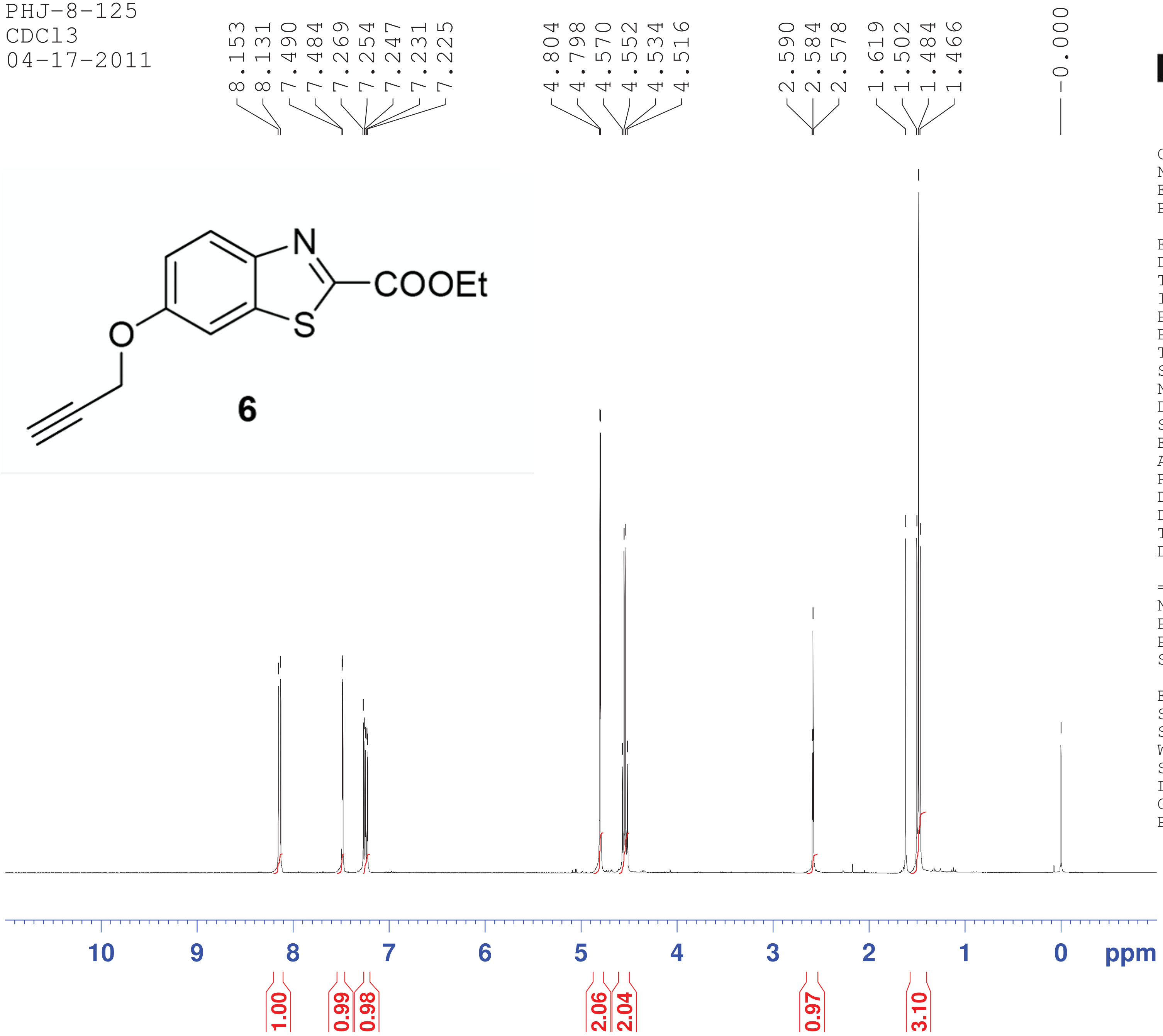
Figure S8. UV-melting curves of DNA duplexes, a. dNTPs-DNA$_{21}$, b. CBT-DNA$_{21}$, c. dNTPs-DNA$_{21}$ + Cys-BA.

References:

[1] D. S. Kemp and R. I. Carey, *J. Org. Chem* **1989**, *54*, 3640-3646.
[2] a) G. L. Liang, H. J. Ren and J. H. Rao, *Nat. Chem.* **2010**, *2*, 54-60; b) J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli and C. R. Bertozzi, *P. Natl. Acad. Sci. USA.* **2007**, *104*, 16793-16797.

7. NMR spectrum of synthesized compounds (Please see next page)

PHJ-8-125
CDCl3
04-17-2011



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RG                       161
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DE                      6.50 usec
TE                     296.6 K
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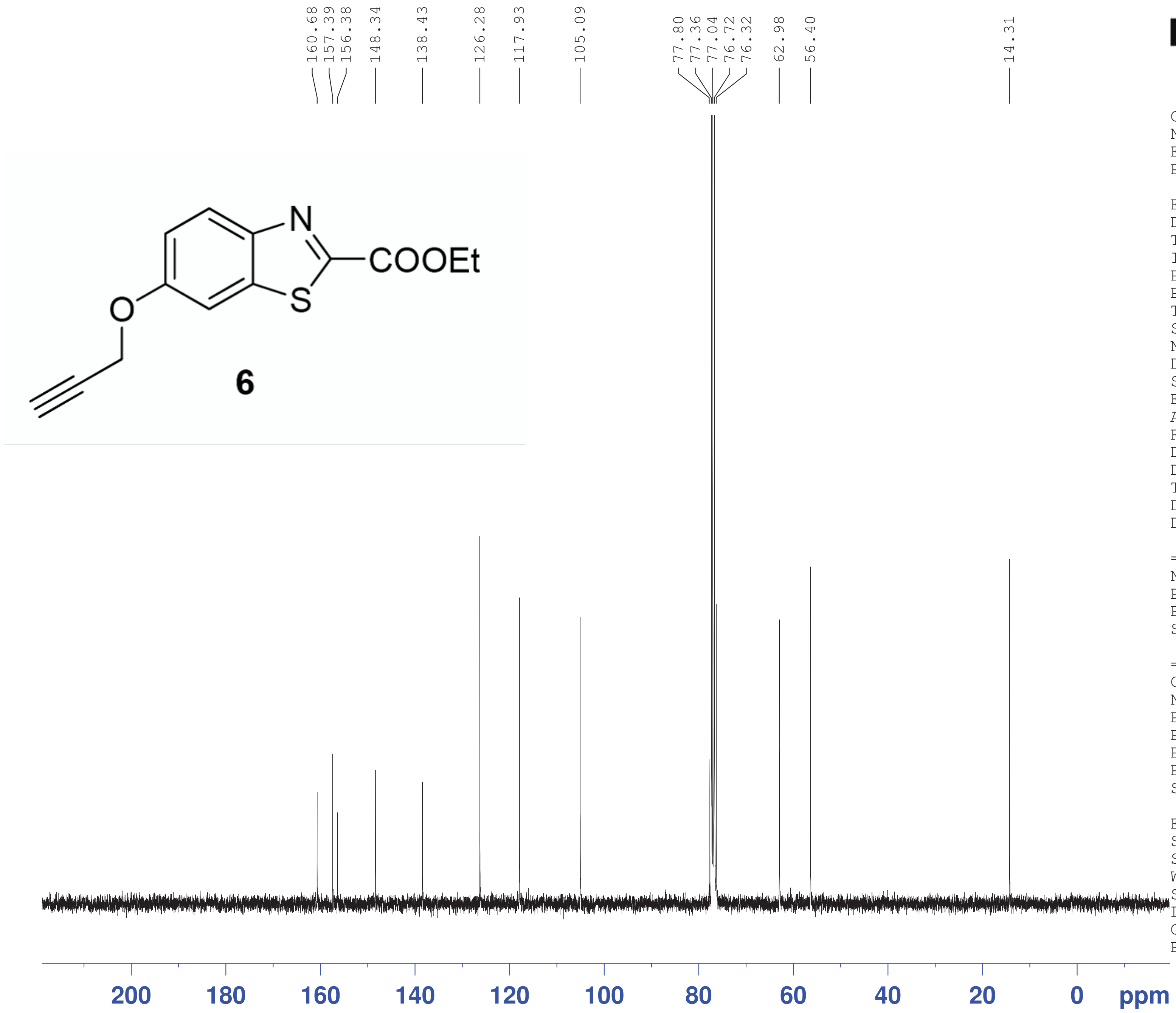

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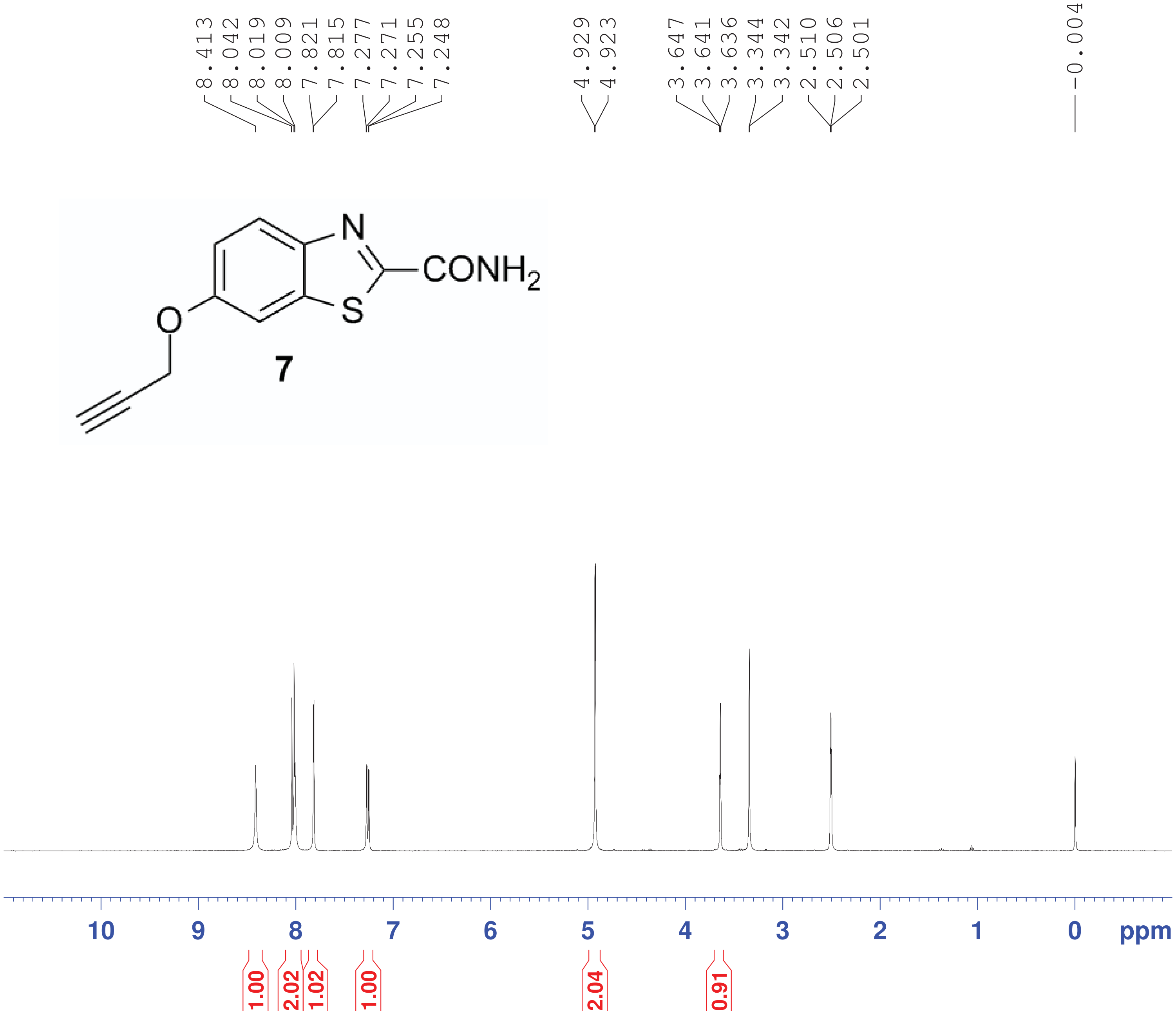

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RG                     203
DW                  60.800 usec
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TE                   295.1 K
D1              1.00000000 sec

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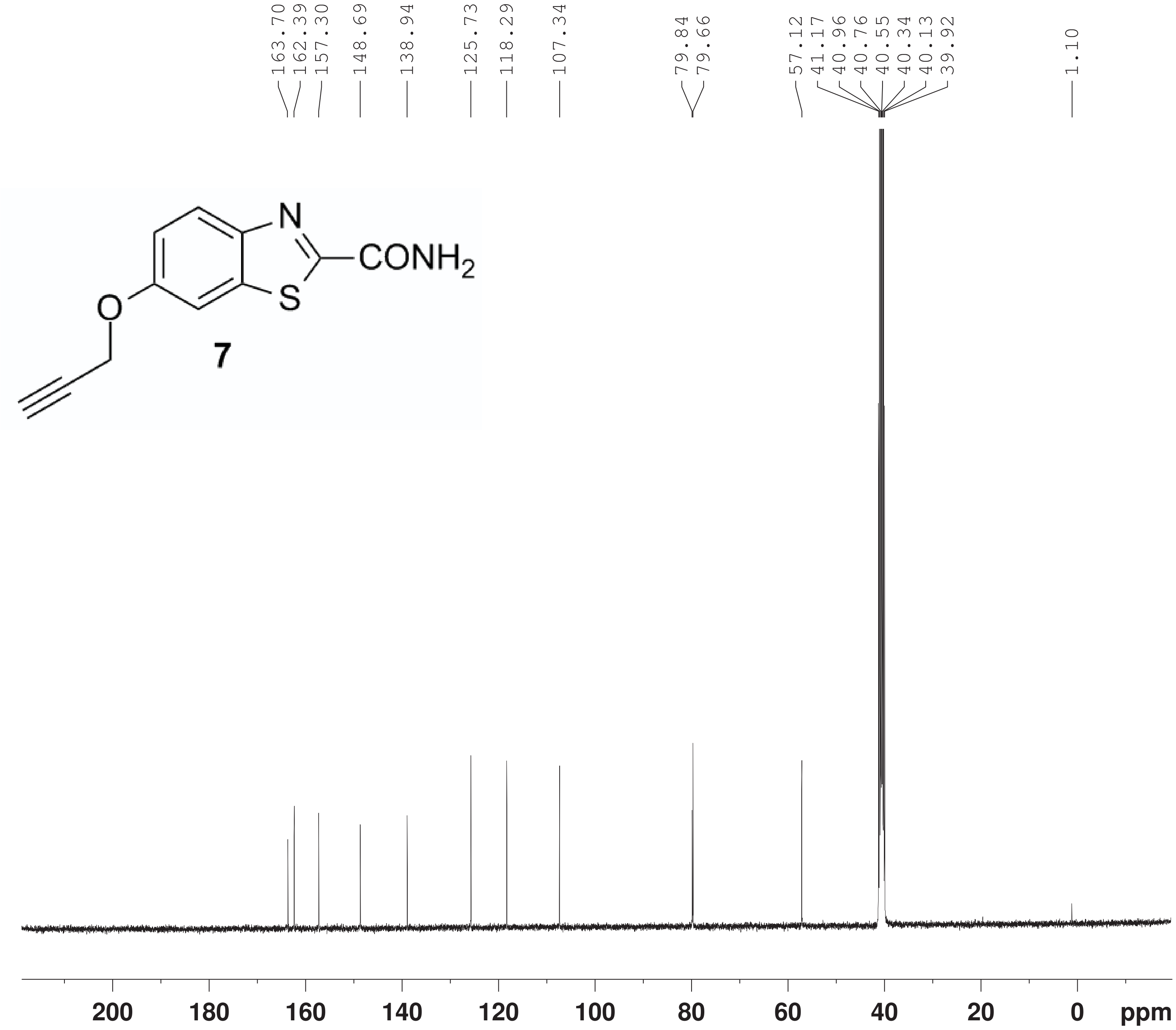

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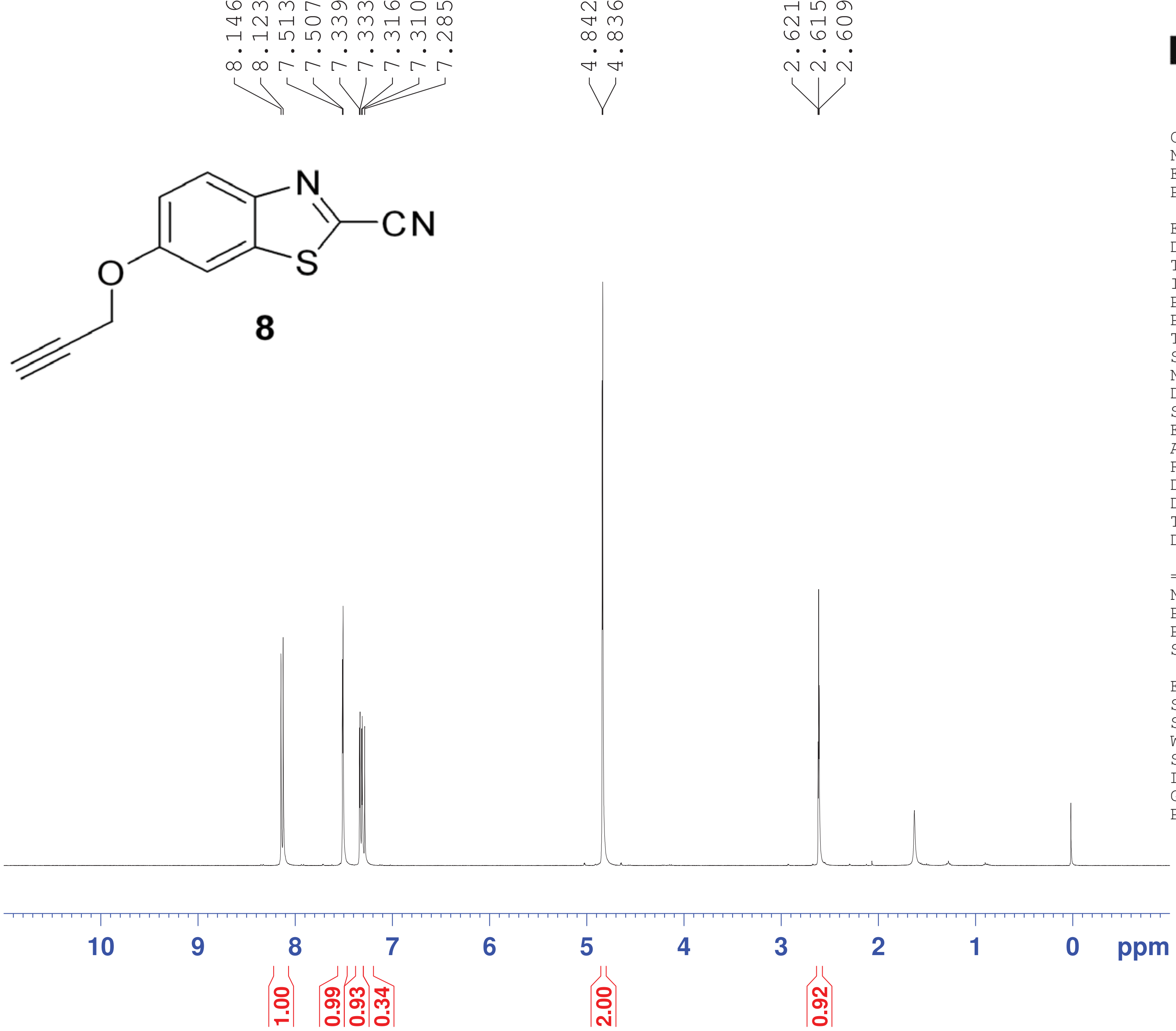
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG            zgpg30
TD                  65536
SOLVENT              DMSO
NS                  10000
DS                      4
SWH             24038.461 Hz
FIDRES           0.366798 Hz
AQ              1.3631988 sec
RG                    203
DW                 20.800 usec
DE                   6.50 usec
TE                  297.3 K
D1             2.00000000 sec
D11            0.03000000 sec

======== CHANNEL f1 ========
NUC1                  13C
P1                   9.00 usec
PLW1          53.48799896 W
SFO1          100.6253441 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                   1H
PCPD2               90.00 usec
PLW2          12.22599983 W
PLW12          0.28864691 W
PLW13          0.14466980 W
SFO2          400.1416006 MHz

F2 - Processing parameters
SI                  32768
SF            100.6152848 MHz
WDW                    EM
SSB        0
LB                   1.00 Hz
GB         0
PC                   1.40
```

DCF-IV-17-H1



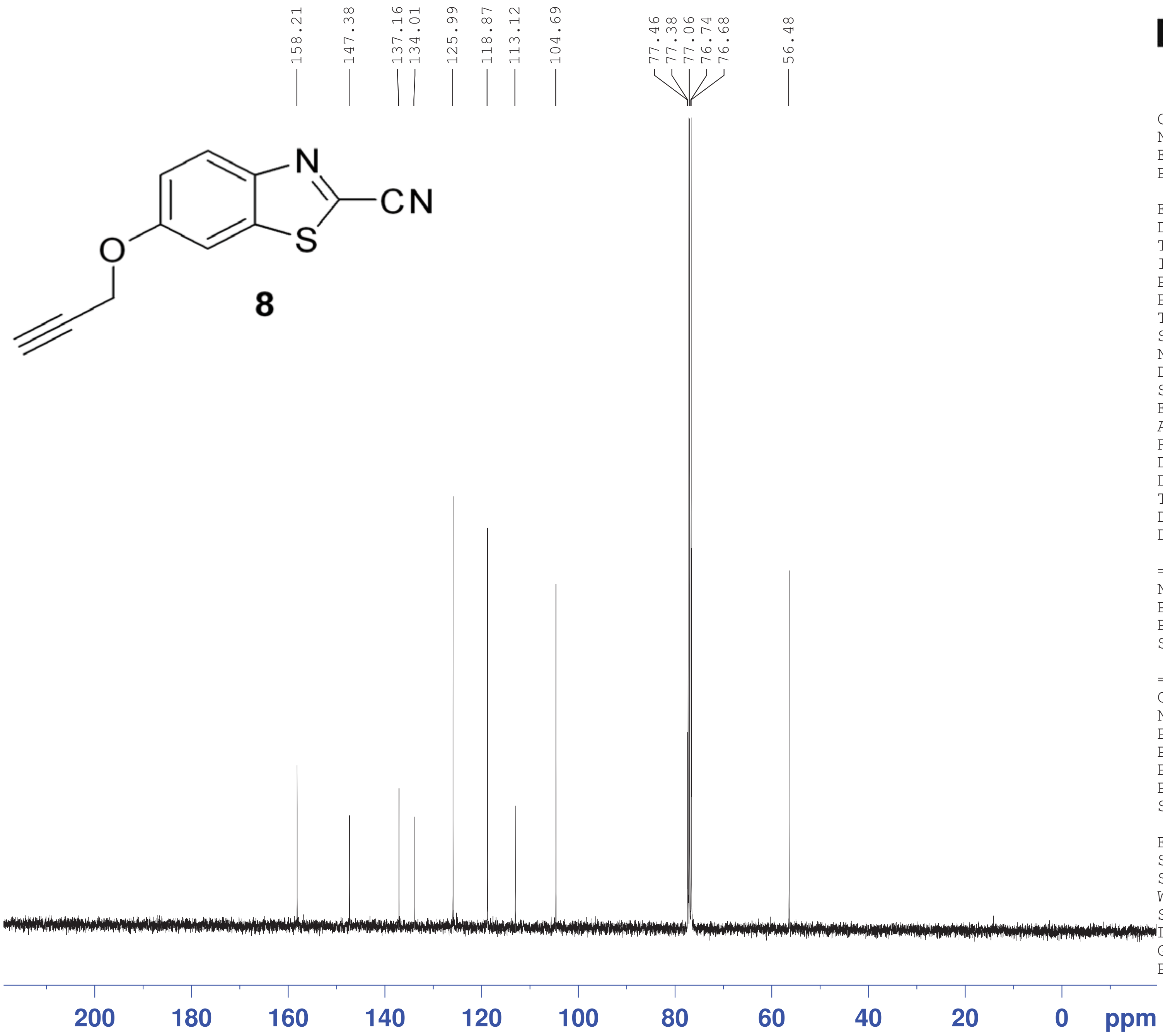
```
Current Data Parameters
NAME               DCF-IV-17
EXPNO                      2
PROCNO                     1

F2 - Acquisition Parameters
Date_               20110210
Time                   18.41
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG                 zg30
TD                     65536
SOLVENT                CDCl3
NS                        16
DS                         2
SWH                 8223.685 Hz
FIDRES              0.125483 Hz
AQ                 3.9846387 sec
RG                       181
DW                    60.800 usec
DE                      6.50 usec
TE                     295.3 K
D1                1.00000000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                     14.00 usec
PLW1             12.22599983 W
SFO1             400.1424710 MHz

F2 - Processing parameters
SI                     65536
SF               400.1400000 MHz
WDW                       EM
SSB      0
LB                      0.30 Hz
GB       0
PC                      1.00
```

DCF-IV-17b-C13



8

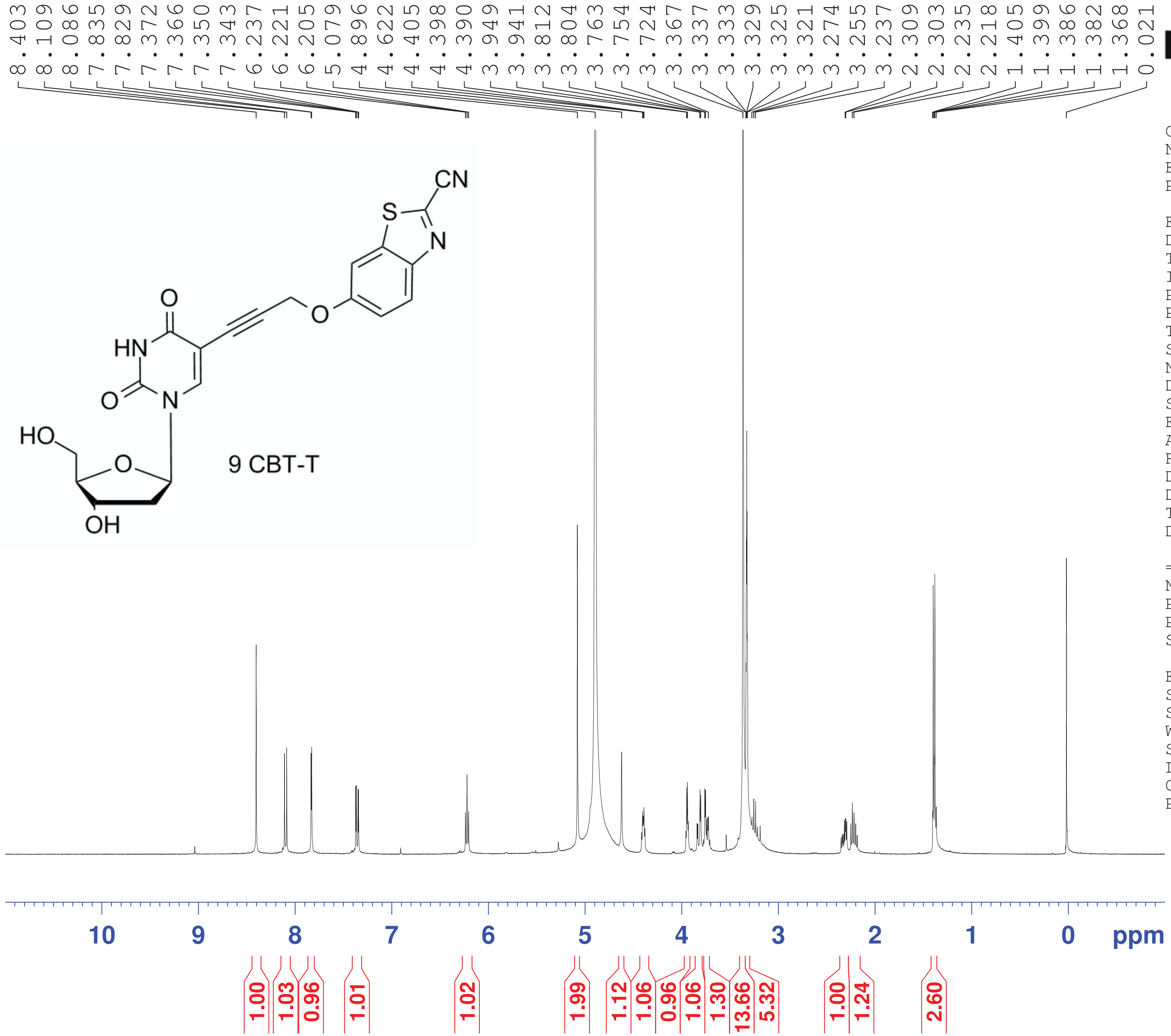
```
Current Data Parameters
NAME              DCF-IV-17b
EXPNO                      2
PROCNO                     1

F2 - Acquisition Parameters
Date_               20110213
Time                   15.36
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG              zgpg30
TD                     65536
SOLVENT                CDCl3
NS                       388
DS                         4
SWH              24038.461 Hz
FIDRES            0.366798 Hz
AQ               1.3631988 sec
RG                       203
DW                    20.800 usec
DE                      6.50 usec
TE                     295.9 K
D1               2.00000000 sec
D11              0.03000000 sec

======== CHANNEL f1 ========
NUC1                     13C
P1                      9.00 usec
PLW1            53.48799896 W
SFO1            100.6253441 MHz

======== CHANNEL f2 ========
CPDPRG2              waltz16
NUC2                      1H
PCPD2                  90.00 usec
PLW2            12.22599983 W
PLW12            0.28864691 W
PLW13            0.14466980 W
SFO2            400.1416006 MHz

F2 - Processing parameters
SI                     32768
SF              100.6152890 MHz
WDW                       EM
SSB       0
LB                      1.00 Hz
GB        0
PC                      1.40
```



BRUKER

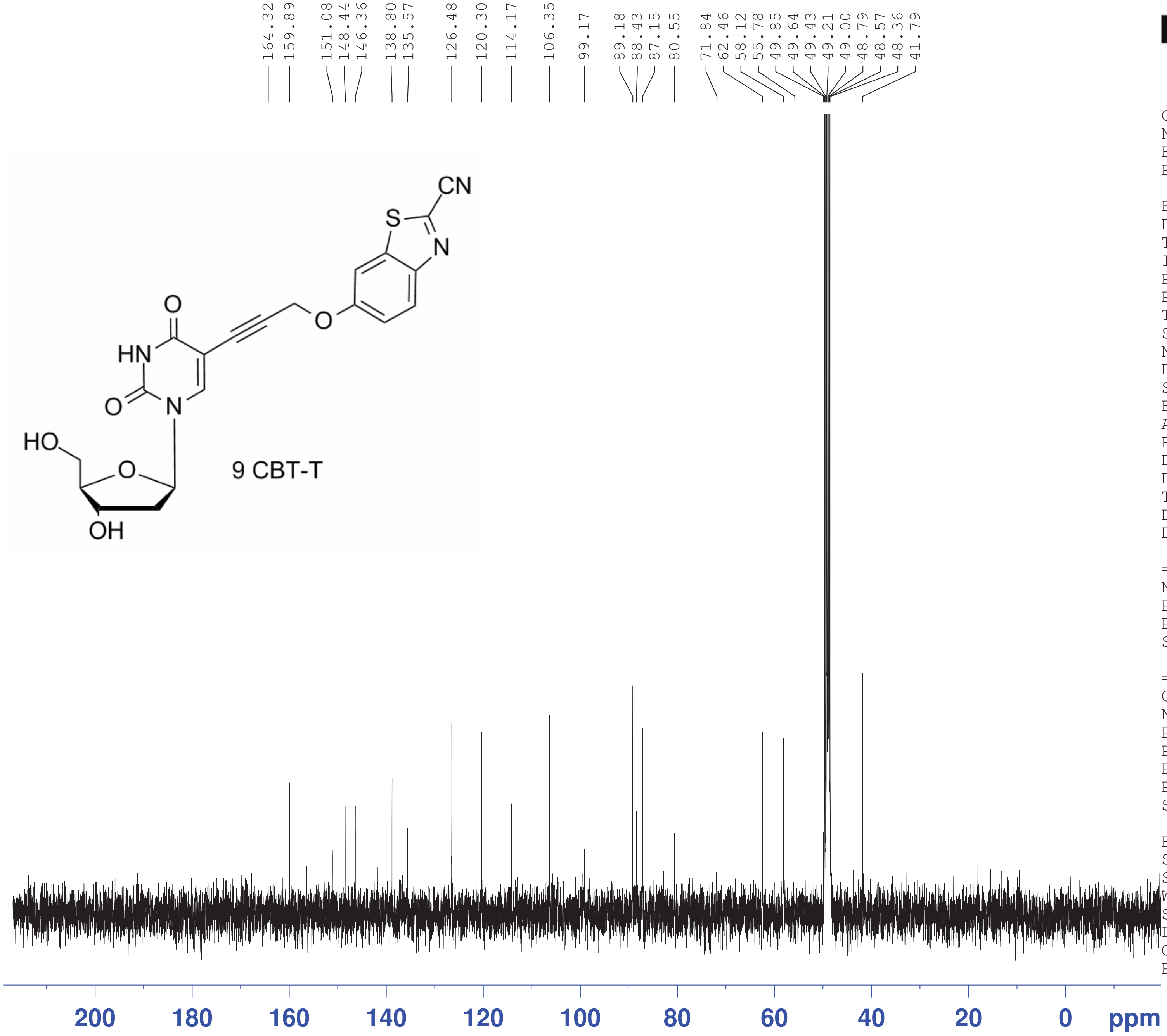
```
Current Data Parameters
NAME             DCF-IV-21
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20110219
Time                 15.14
INSTRUM              spect
PROBHD    5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT               MeOD
NS                      16
DS                       2
SWH               8223.685 Hz
FIDRES            0.125483 Hz
AQ               3.9846387 sec
RG                      32
DW                  60.800 usec
DE                    6.50 usec
TE                   296.1 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   14.00 usec
PLW1           12.22599983 W
SFO1           400.1424710 MHz

F2 - Processing parameters
SI                   65536
SF             400.1400000 MHz
WDW                     EM
SSB      0
LB                    0.30 Hz
GB       0
PC                    1.00
```

dcf-IV-21-C13



9 CBT-T

```
Current Data Parameters
NAME       dcf-IV-21-C13
EXPNO                  2
PROCNO                 1

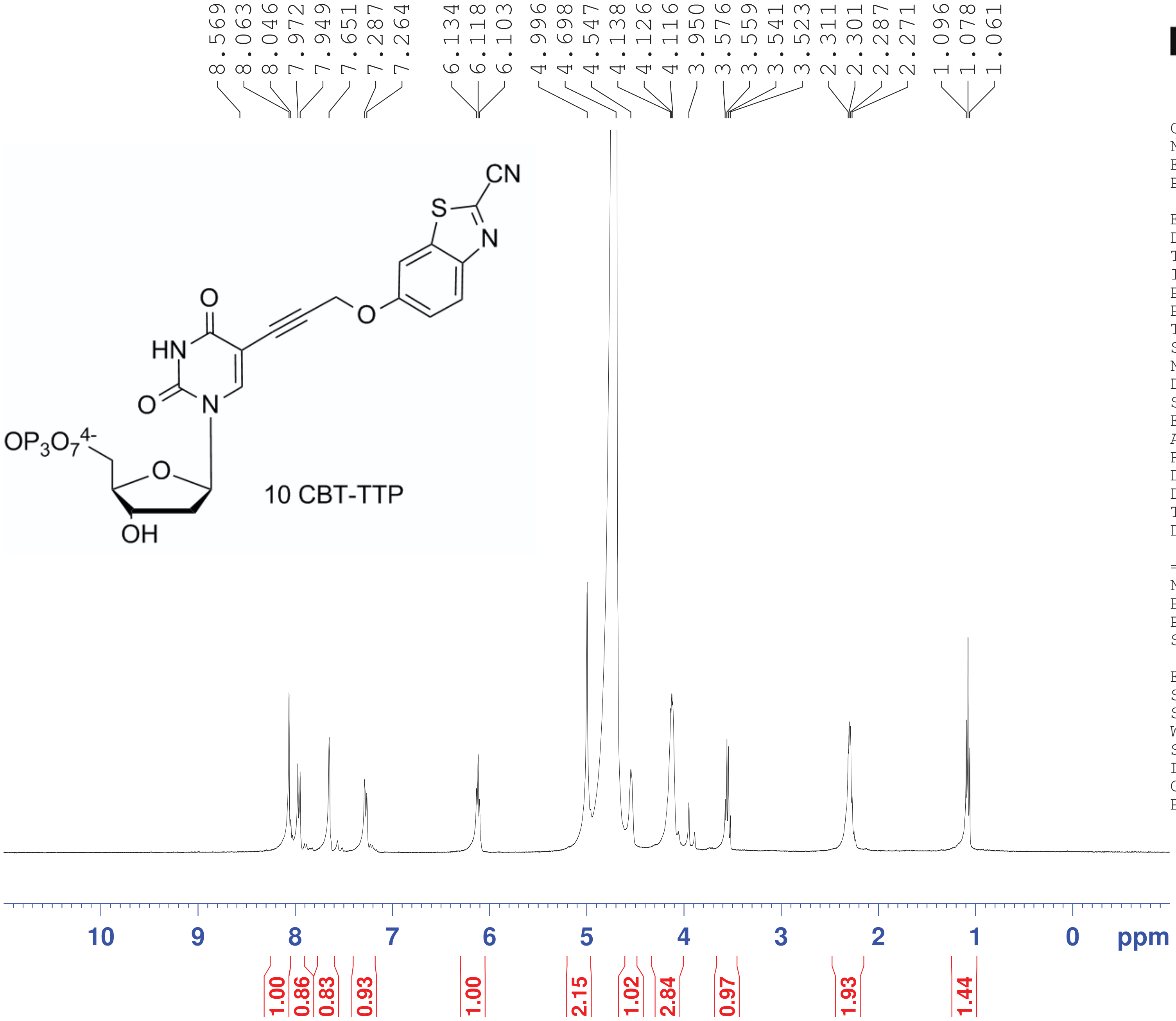
F2 - Acquisition Parameters
Date_           20120124
Time               10.05
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                 65536
SOLVENT            CDCl3
NS                   423
DS                     4
SWH            24038.461 Hz
FIDRES          0.366798 Hz
AQ             1.3631988 sec
RG                   203
DW                20.800 usec
DE                  6.50 usec
TE                 298.3 K
D1            2.00000000 sec
D11           0.03000000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                  9.00 usec
PLW1         62.00000000 W
SFO1         100.6253441 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2              90.00 usec
PLW2         16.00000000 W
PLW12         0.36000001 W
PLW13         0.29159999 W
SFO2         400.1416006 MHz

F2 - Processing parameters
SI                 32768
SF           100.6155389 MHz
WDW                   EM
SSB      0
LB                  1.00 Hz
GB       0
PC                  1.40
```

dcf-IV-45-pure-H1



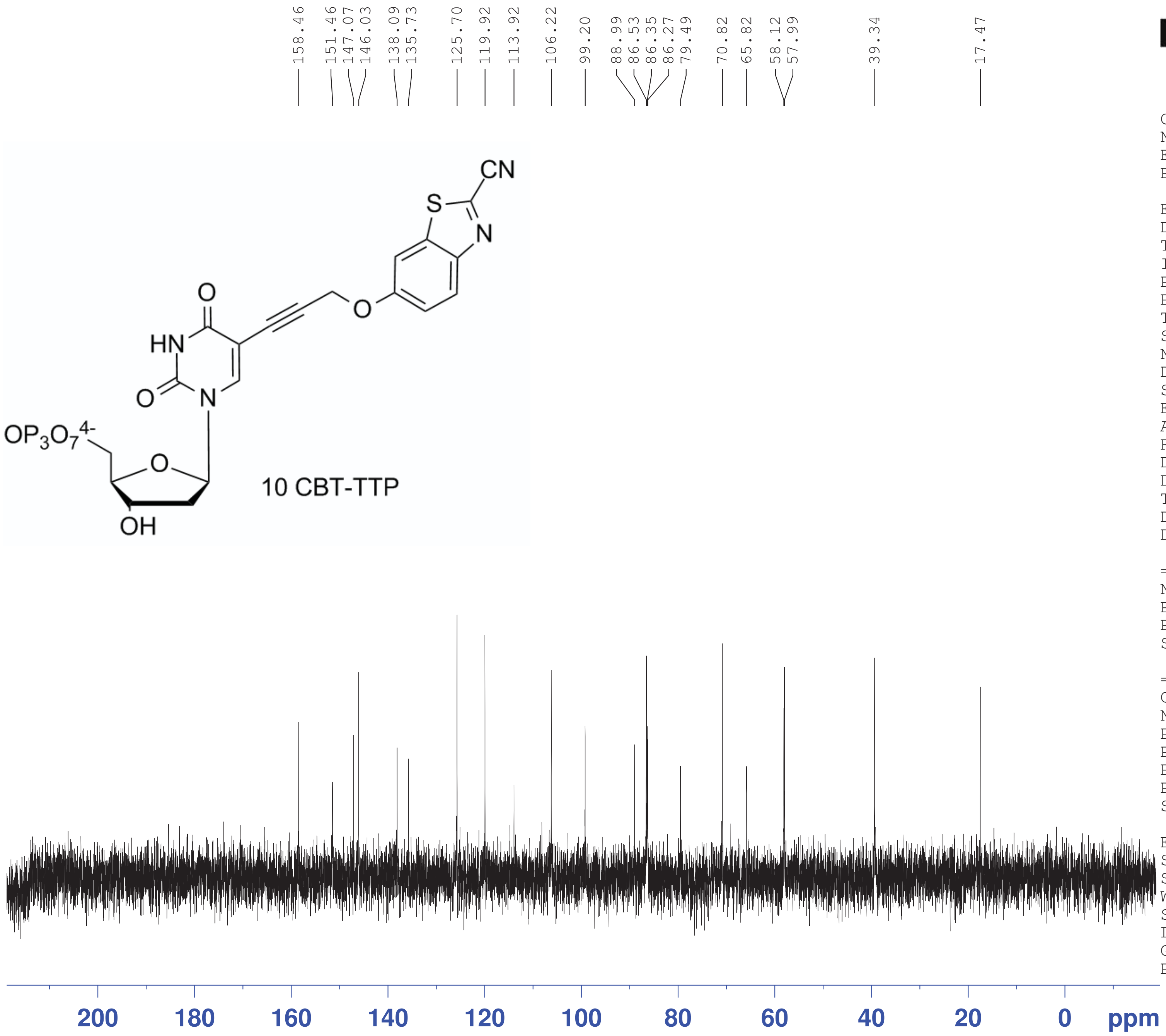
```
Current Data Parameters
NAME      dcf-IV-45-pure
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20110523
Time               18.59
INSTRUM            spect
PROBHD    5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT              D2O
NS                   100
DS                     2
SWH             8223.685 Hz
FIDRES          0.125483 Hz
AQ             3.9846387 sec
RG                   128
DW                60.800 usec
DE                  6.50 usec
TE                 296.8 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 13.50 usec
PLW1         16.00000000 W
SFO1         400.1424710 MHz

F2 - Processing parameters
SI                 65536
SF           400.1400000 MHz
WDW                   EM
SSB      0
LB                  0.30 Hz
GB       0
PC                  1.00
```

dcf-IV-45-C13



10 CBT-TTP

```
Current Data Parameters
NAME       dcf-IV-45-C13
EXPNO                  1
PROCNO                 1

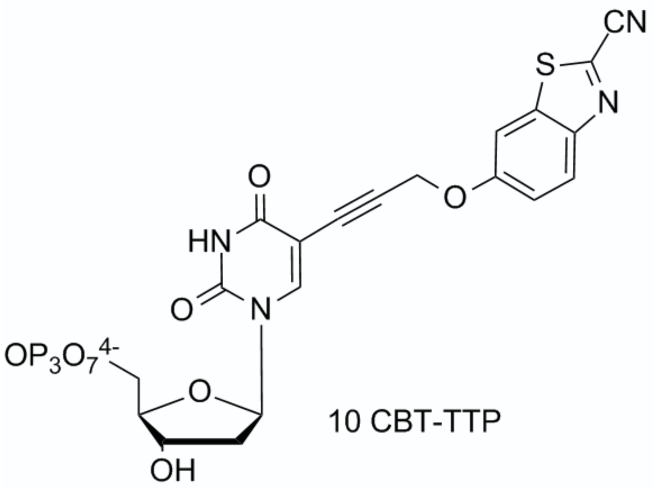
F2 - Acquisition Parameters
Date_           20110523
Time               21.33
INSTRUM            spect
PROBHD    5 mm PABBO BB-
PULPROG          zgpg30
TD                 65536
SOLVENT              D2O
NS                 11000
DS                     4
SWH            24038.461 Hz
FIDRES          0.366798 Hz
AQ             1.3631988 sec
RG                   203
DW                20.800 usec
DE                  6.50 usec
TE                 297.3 K
D1            2.00000000 sec
D11           0.03000000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                  9.00 usec
PLW1         62.00000000 W
SFO1         100.6253445 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2              90.00 usec
PLW2         16.00000000 W
PLW12         0.36000001 W
PLW13         0.29159999 W
SFO2         400.1416006 MHz

F2 - Processing parameters
SI                 32768
SF           100.6152207 MHz
WDW                   EM
SSB      0
LB                  1.00 Hz
GB       0
PC                  1.40
```

dcf-IV-45-P31



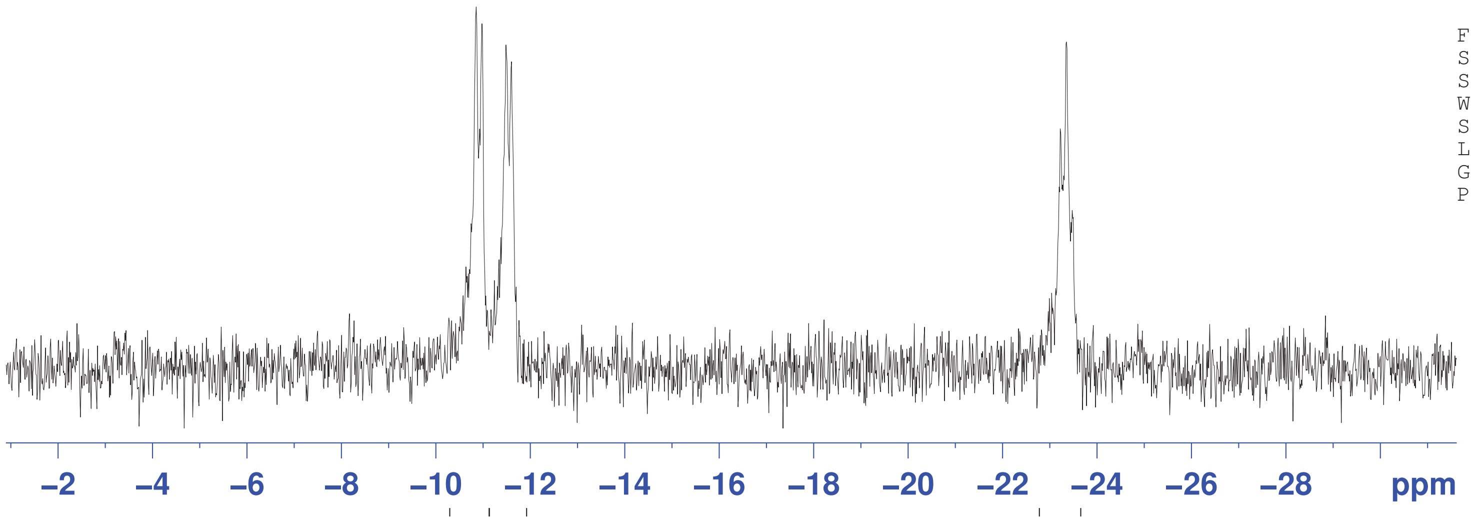
10 CBT-TTP

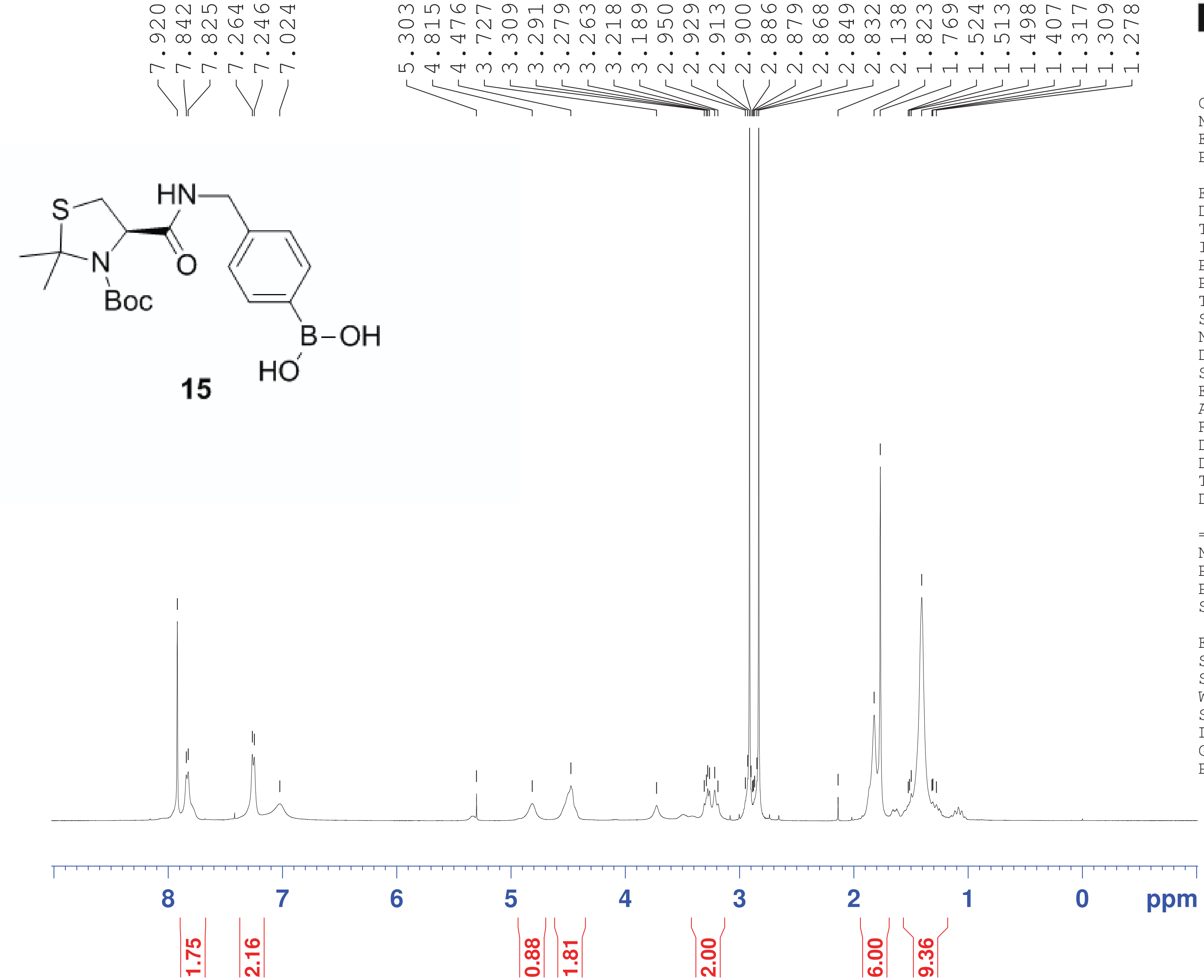
```
Current Data Parameters
NAME             dcf-IV-45
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_             20110522
Time                 14.47
INSTRUM              spect
PROBHD     5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT                D2O
NS                      32
DS                       4
SWH              64102.563 Hz
FIDRES            0.978127 Hz
AQ               0.5112308 sec
RG                     203
DW                   7.800 usec
DE                    6.50 usec
TE                   296.5 K
D1              2.00000000 sec

======== CHANNEL f1 ========
NUC1                   31P
P1                   14.00 usec
PLW1           12.50000000 W
SFO1           161.9816417 MHz

F2 - Processing parameters
SI                   32768
SF             161.9796410 MHz
WDW                     EM
SSB      0
LB                    1.00 Hz
GB       0
PC                    1.40
```



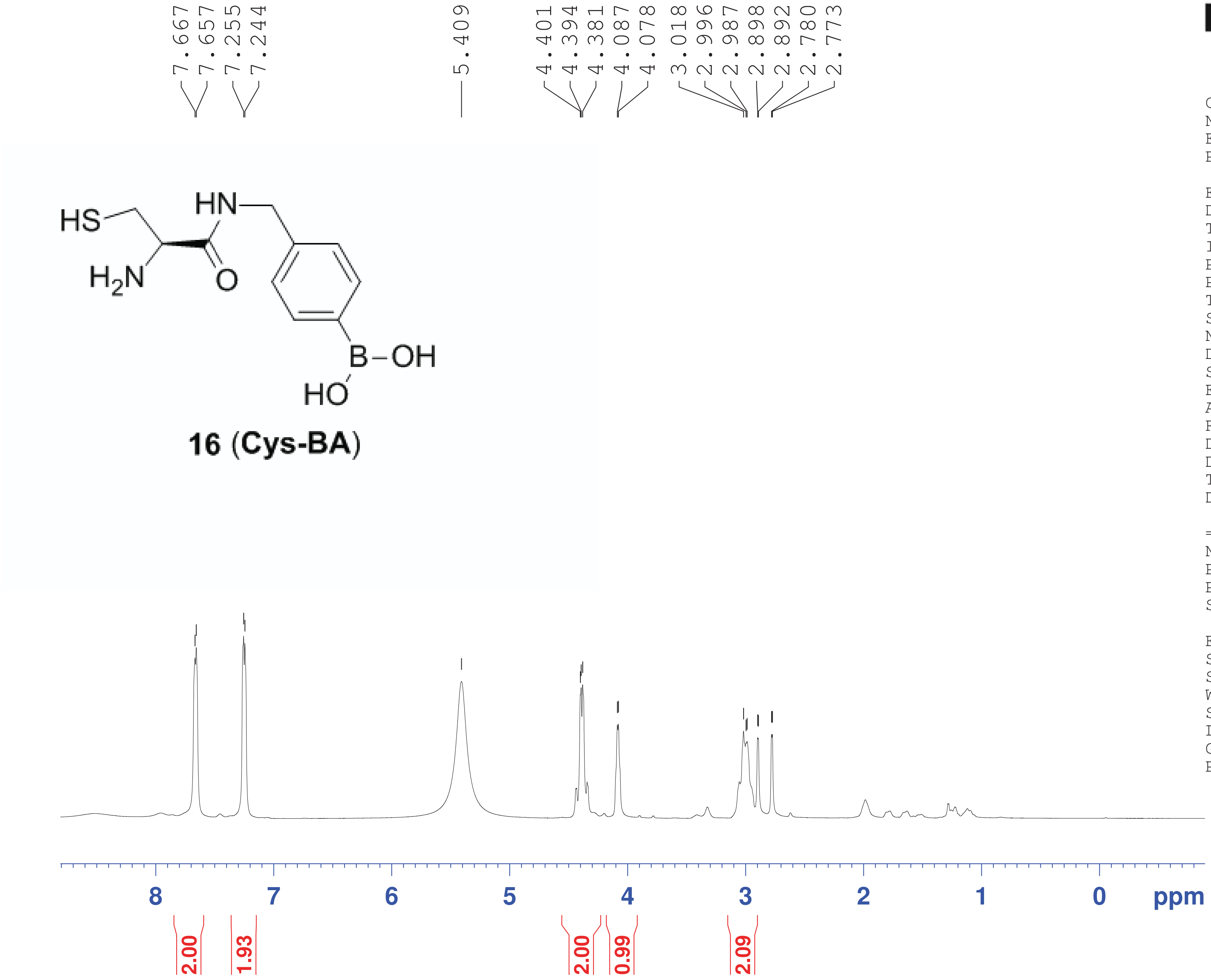


```
Current Data Parameters
NAME             JC-VI-83
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20110413
Time                16.18
INSTRUM             spect
PROBHD    5 mm PABBO BB-
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                      2
DS                      2
SWH              8223.685 Hz
FIDRES           0.125483 Hz
AQ              3.9846387 sec
RG                     10
DW                 60.800 usec
DE                   6.50 usec
TE                  296.3 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  13.50 usec
PLW1          16.00000000 W
SFO1          400.1420007 MHz

F2 - Processing parameters
SI                  65536
SF            400.1399452 MHz
WDW                    EM
SSB      0
LB                   0.30 Hz
GB       0
PC                   1.00
```



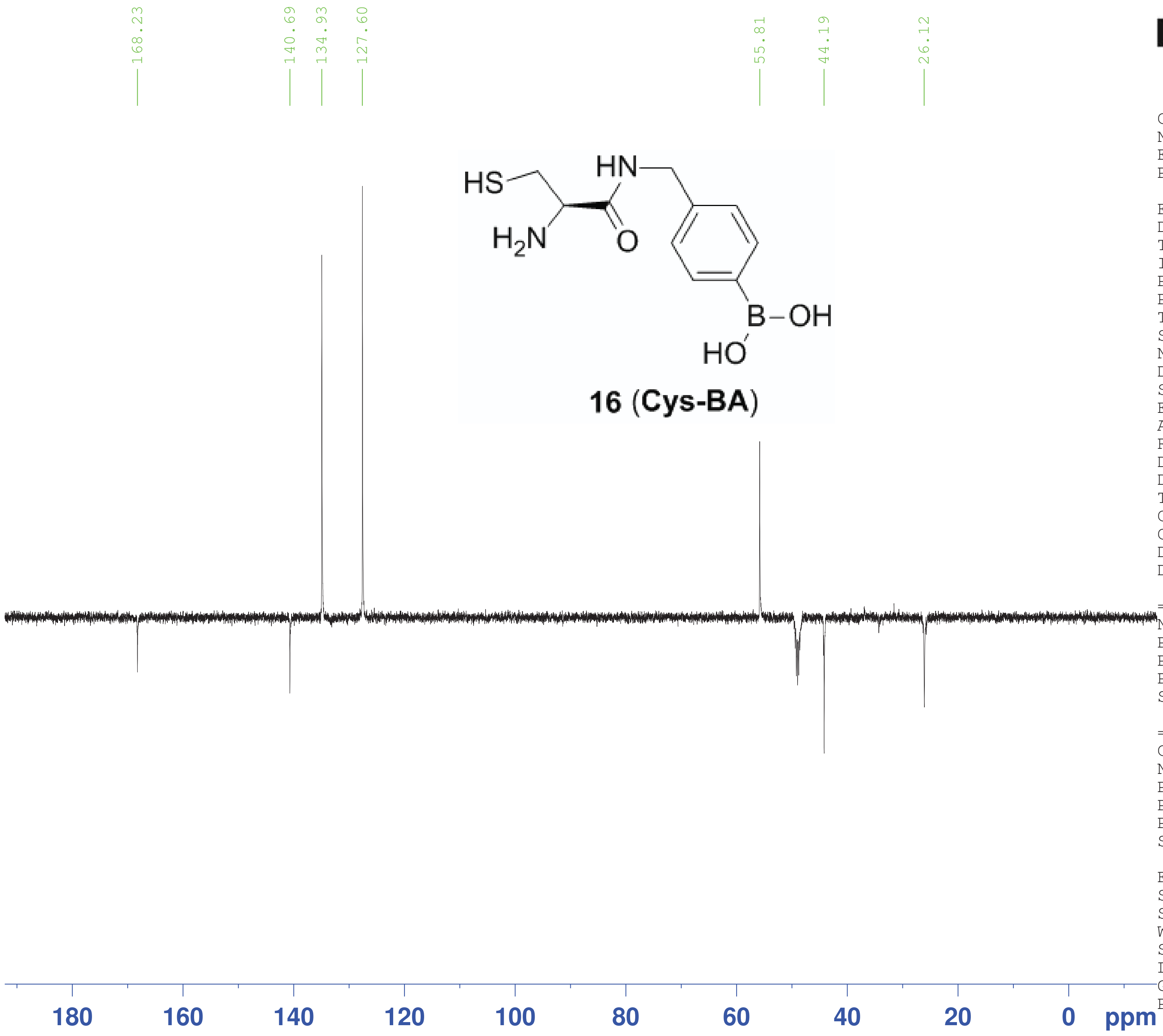
16 (Cys-BA)

```
Current Data Parameters
NAME              JC-VI-83-3
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_               20110418
Time                   17.03
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG                 zg30
TD                     65536
SOLVENT                 MeOD
NS                        16
DS                         2
SWH                 8223.685 Hz
FIDRES              0.125483 Hz
AQ                 3.9846387 sec
RG                        16
DW                    60.800 usec
DE                      6.50 usec
TE                     297.0 K
D1                1.00000000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                     13.50 usec
PLW1             16.00000000 W
SFO1             400.1424008 MHz

F2 - Processing parameters
SI                     65536
SF               400.1400000 MHz
WDW                       EM
SSB       0
LB                      0.30 Hz
GB        0
PC                      1.00
```



16 (Cys-BA)

```
Current Data Parameters
NAME          JC-VI-83-3
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20110418
Time               17.57
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             jmod
TD                 65536
SOLVENT             MeOD
NS                    33
DS                     4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631988 sec
RG                   203
DW                20.800 usec
DE                  6.50 usec
TE                 297.2 K
CNST2        145.0000000
CNST11         1.0000000
D1            2.00000000 sec
D20           0.00689655 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                  9.00 usec
P2                 18.00 usec
PLW1         62.00000000 W
SFO1         100.6238353 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2              90.00 usec
PLW2         16.00000000 W
PLW12         0.36000001 W
SFO2         400.1416006 MHz

F2 - Processing parameters
SI                 32768
SF           100.6151715 MHz
WDW                   EM
SSB         0
LB                  1.00 Hz
GB          0
PC                  1.40
```