Electronic Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

Click reaction between 1,2,4,5-tetrazine and cyclooctynes with tunable reaction rates

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Materials and Methods

All reagents and solvents were reagent grade or were purified by standard methods before use. Column chromatography was carried out on flash silica gel (Sorbent 230–400 mesh). TLC analysis was conducted on silica gel plates (Sorbent Silica G UV254). NMR spectra were recorded at ^{1}H (400 MHz) and ^{13}C (100 MHz) on a Bruker instrument. Chemical shifts (δ values) and coupling constants (*J* values) are given in ppm and hertz, respectively, using solvents (^{1}H NMR, ^{13}C NMR) as the internal standard. DIFO[1], methyl 1-fluorocyclooct-2-ynecarboxylate[2], compound **1**[3], compound **2**[5], compound **4**[4], and compound **9**[6] were synthesized according to literature procedures.

Preparation of Cyclooctyne 1[3]

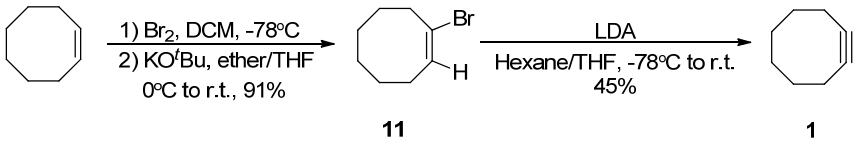


Figure S1. Reagents and conditions for the synthesis of cyclooctyne **1**

Cyclooctyne (1): ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 4H), 1.83 (s, 4H), 1.60 (s, 4H). ^{13}C NMR ($CDCl_3$) δ 94.6, 34.6, 29.8, 21.0.

Preparation of (*1R,8S,9S*)-Bicyclo[6.1.0]non-4-yn-9-ylmethanol (*endo*-4)[4]

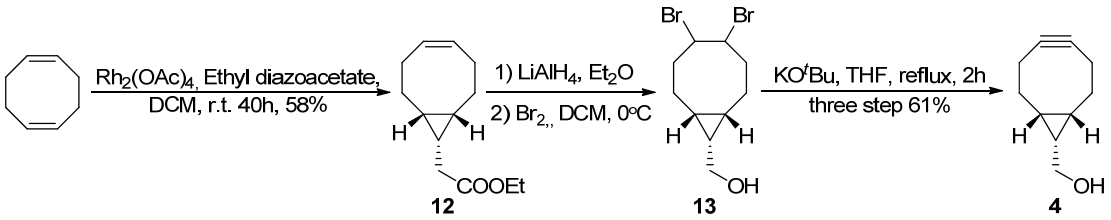
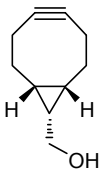
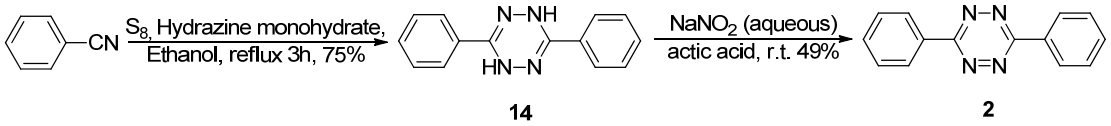


Figure S2. Reagents and conditions for the synthesis of (*1R,8S,9s*)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (*endo*-**4**)



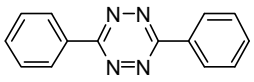
(*1R,8S,9S*)-Bicyclo[6.1.0]non-4-yn-9-ylmethanol (*endo*-4): ^{1}H NMR ($CDCl_3$): δ 3.66 (d, J = 8.0 Hz, 2H), 2.28-2.14 (m, 6H), 1.55-1.54 (m, 2H), 1.31-1.25 (m, 1H), 0.89-0.85 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 98.9, 59.7, 29.0, 21.5, 21.3, 20.0.

Preparation of 3,6-diphenyl-1,2,4,5-tetrazine 2.[5]



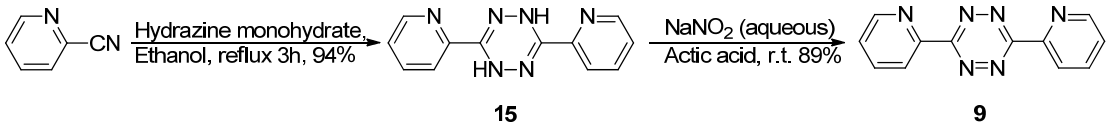
14 2

Figure S3. Reagents and conditions for the synthesis of 3,6-diphenyl-1,2,4,5-tetrazine **2**.



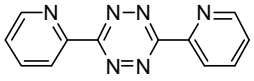
3,6-Diphenyl-1,2,4,5-tetrazine (2): ^{1}H NMR ($CDCl_3$): δ 8.65 (t, J = 6.0 Hz, 4H), 7.65-7.59 (m, 6H),. ^{13}C NMR ($CDCl_3$) δ 164.0, 132.7, 131.8, 129.3, 128.0.

Preparation of 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine 9.[6]



15 9

Figure S4. Reagents and conditions for synthesis of 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine **9**.



3,6-Di(pyridin-2-yl)-1,2,4,5-tetrazine (9): ^{1}H NMR ($CDCl_3$): δ 8.99 (d, J = 4.8 Hz, 2H), 8.76 (d, J = 8 Hz, 6H), 8.04-7.99 (m, 2H), 7.60-7.57 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 163.8, 151.0, 150.0, 137.4, 126.5, 124.5.

Preparation of *N*-isopropyl-4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin3-yl) benzamide 10.[7]

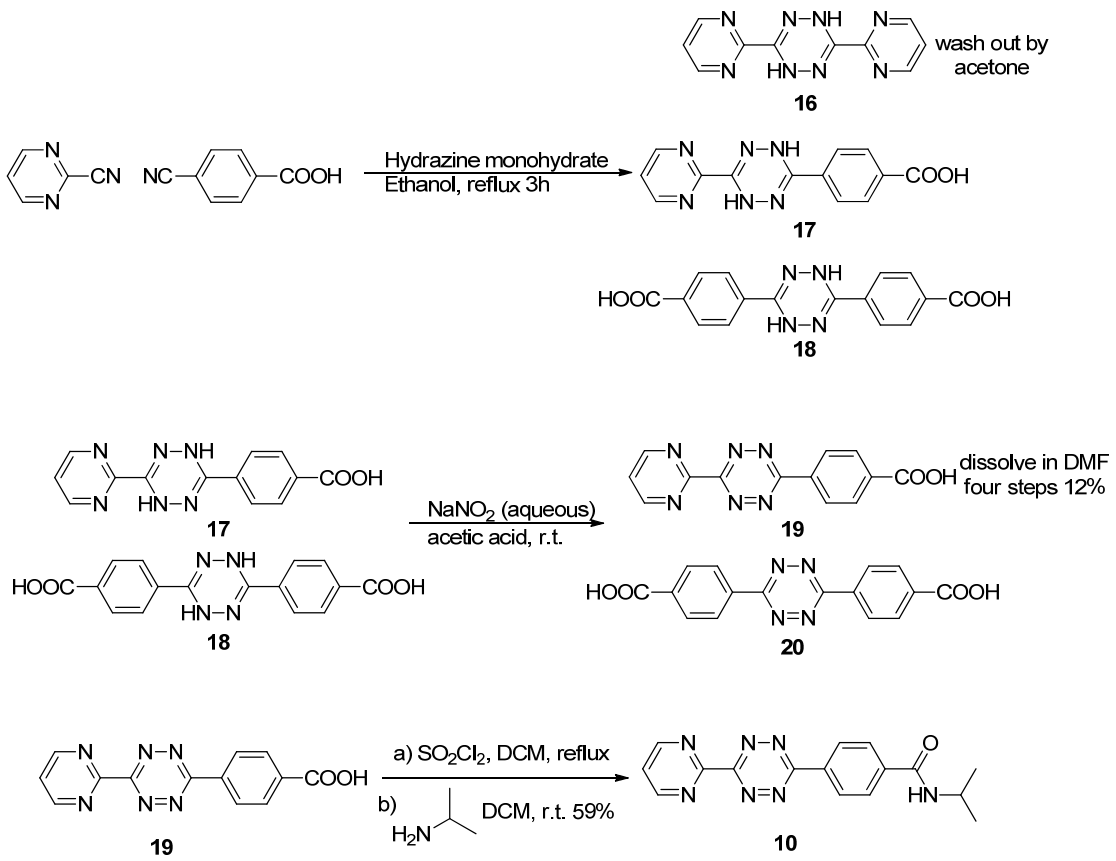
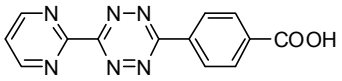
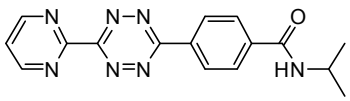


Figure S5. Reagents and conditions for the synthesis of *N*-isopropyl-4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl)benzamide **10**.



4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl)benzoic acid (19)[7]: To a solution of 2-pyrimidinecarbonitrile (935 mg, 8.9 mmol) in 30 mL ethanol, 4-cyanobenzoic acid (1.96 g 13.3 mmol) was added, followed by addition of hydrazine monohydrate (5.5 mL). Then the solution was heated under reflux for 20 h with stirring. After cooling to r.t., the solid was collected by filtration and washed with acetone (2 × 50 mL). The dihydrotetrazine without carboxyl group (**16**) went into acetone. The remaining solid was added acetic acid (10mL) followed by an aqueous solution of $NaNO_2$ (2.76g, 40 mmol) at 10 °C. The purple colored tetrazine was collected and washed with water (3 × 10 mL). The solid was added into nearly boiled DMF and kept at this temperature for another 5 min. The DMF solution was filtered while it was hot. The filtrate, which contained mono-carboxyl substituted product, was collected and dried under vacuum to give a purple product (300 mg, 12%). The benzoic acid tetrazine was used for next step without further purification.



***N*-Isopropyl-4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl)benzamide (10):** To a suspension of 4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl)benzoic acid

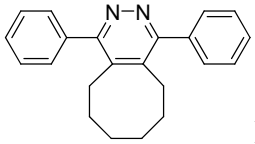
(**19,** 60 mg, 0.214 mmol) in 5 mL DCM, thionyl chloride (187 µL, 257 mmol) was added. This mixture was refluxed for 20 h with stirring, at which point TLC indicated completion of the reaction. The solution was cooled to r.t. and dried under vacuum. The residue was suspended in dry 3 mL DCM, then propan-2-amine (55 µL, 0.64 mmol) was added and the resulting mixture was stirred at r.t. for 4 h. DCM was removed in vacuum and the reaction mixture was purified by silica gel column chromatography (DCM: MeOH, 20:1) to give a purple solid product (40 mg, 59 %). ^{1}H NMR ($CDCl_3$): δ 9.14 (d, *J* = 2.0 Hz, 2H, 4-Pyr-H, 6-Pyr-H), 8.78 (d, *J* = 4.0 Hz, 2H, 2'-Ph-H, 6'-H), 8.00 (d, *J* = 4.0 Hz, 2H, 3'-Ph-H, 5'-Ph-H), 7.60 (br, 1H, 5-Pyr-H), 6.17 (d, *J* = 4.0 Hz, 1H, -NH), 4.34-4.32 (m, 1H, -CH-NH-), 1.32 (s, 3H, $-CH_3$), 1.31 (s, 3H, $-CH_3$); ^{13}C NMR ($CDCl_3$) δ 165.7, 164.0, 163.1, 159.4, 158.4, 139.2, 133.7, 128.9, 127.8, 122.6, 42.2, 22.8. MS calcd. For $C_{16}H_{15}N_7O$ $[M+H]^+$ 322.1, found 322.3

General procedure for strain-promoted inverse electron demand Diels-Alder reactions with 1,2,4,5-tetrazine.

To a solution of tetrazine (**2**, **9**, **10**) in CH_2Cl_2 (1 mL), alkyne (**1**, **4**) in CH_2Cl_2 (1 mL) was added. Reactions were stirred at room temperature for 5 to 30 min. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was directly loaded on the flash column chromatography for purification.

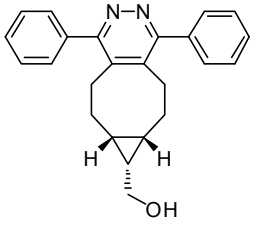
Characterization Data for cyclization products

Compound 3



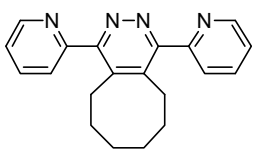
Purified by eluting with DCM:MeOH (10:1, R_f = 0.65) (white solid, 91%). ^{1}H NMR ($CDCl_3$) δ 7.55-7.47 (m, 10H, Ph-H), 2.83-2.82 (m, 4H, $-CH_2$-C=C-), 1.60 (br, 4H, $-CH_2$-), 1.43 (br, 4H, $-CH_2$-). ^{13}C NMR ($CDCl_3$) δ 161.1, 139.0, 138.2, 129.1, 128.3, 128.2, 30.3, 27.1, 25.9. MS calcd. For $C_{22}H_{22}N_2$ $[M+H]^+$ 315.2, found 315.3

Compound 5



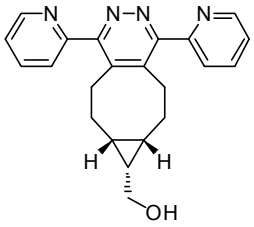
Purified by eluting DCM:MeOH (10:1, R_f = 0.55) (white solid 88%). ^{1}H NMR ($CDCl_3$) δ 7.48-7.41 (m, 10H, Ph-H), 3.63 (d, *J* = 3.6 Hz, 2H, $-CH_2$-OH), 2.93-2.85 (m, 2H, $-CH_2$-C=C-), 2.75 (br, 2H, $-CH_2$-C=C-), 2.50 (br, 1H, -CH-CH_2OH), 2.18-2.15 (br, 2H, -C-CH-C-), 1.43-1.42 (br, 2H, -CH2-), 1.13 (br, 2H, $-CH_2$-) ^{13}C NMR ($CDCl_3$) δ 160.8, 140.5, 137.9, 129.2, 128.4, 128.2, 58.8, 28.2, 24.3, 22.9, 20.4. MS calcd. For $C_{26}H_{23}NO_3$ $[M+H]^+$ 357.2, found 357.3.

Compound 21



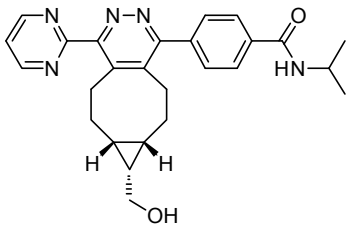
Purified by eluting DCM:MeOH (10:1, R_f = 0.54) (white solid 90%). ^{1}H NMR ($CDCl_3$) δ 8.66 (d, *J* = 2.2 Hz, 2H, 6-Py-H), 7.84-7.79 (m, 4H, 3-Py-H, 4-Py-H), 7.33-7.30 (m, 2H, 5-Py-H), 3.01-2.98 (m, 4H, -CH_2-C=C-), 1.72 (br, 4H, -CH_2-), 1.38 (br, 4H, -CH_2-). ^{13}C NMR ($CDCl_3$) δ 158.7, 156.9, 148.4, 140.9, 136.6, 124.8, 123.1, 30.3, 26.4, 25.9. MS calcd. For $C_{20}H_{20}N_4$ $[M+H]^+$ 317.2, found 317.2

Compound 22



Purified by eluting DCM:MeOH (10:1, R_f = 0.35) (white solid 89%). ^{1}H NMR ($CDCl_3$) δ 8.72 (d, *J* = 2.0 Hz, 2H, 6-Py-H), 7.95-7.85 (m, 4H, 3-Py-H, 4-Py-H), 7.39-7.36 (m, 2H, 5-Py-H), 3.72 (d, *J* = 3.6 Hz, 2H, -CH_2-C-OH), 3.06-3.05 (m, 4H, -CH_2-C=C-), 2.39-2.36 (br, 2H, -CH_2-), 1.62 (br, 2H, -CH_2-), 1.10 (br, 3H, -CH-, -CH_2-). ^{13}C NMR ($CDCl_3$) δ 159.1, 157.0, 148.7, 142.2, 136.8, 125.0, 123.3, 59.5, 27.8, 24.4, 22.7, 19.7. MS calcd. For $C_{22}H_{22}N_4O$ $[M+H]^+$ 359.2, found 359.3.

Compound 23

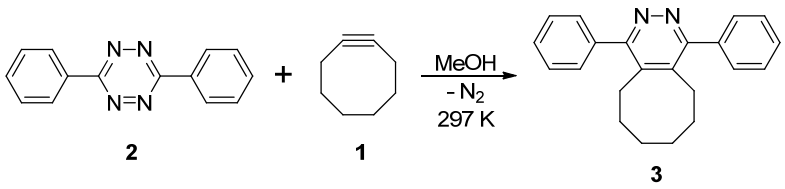


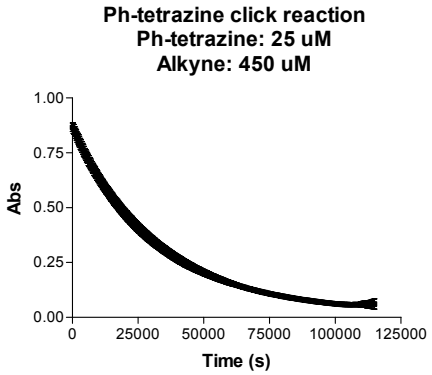
Purified by eluting DCM:MeOH (10:1, R_f= 0.32) (white solid 82%). ^{1}H NMR ($CDCl_3$) δ 8.94 (d, *J* = 2.4 Hz, 2H, 4-Pyr-H, 6-Pyr-H) 7.86 (d, *J* = 4.2 Hz, 2H, 2'-Ph-H, 6'-Ph-H), 7.53 (d, *J* = 4.0 Hz, 2H, 3'-Ph-H, 5'-Ph-H), 7.42 (t, *J*= 5.0 Hz, 1H, 5-Pyr-H), 6.25 (d, *J*=3.8 Hz, 1H, -NH), 4.35-4.26 (m, 1H, -NH-CH-), 3.72 (d, *J* = 3.6 Hz, 2H, -CH_2-C-OH), 2.99-2.89 (m, 2H, -CH_2-C=C-), 2.83-2.78 (m, 2H, -CH_2-C=C-), 2.32-2.31 (br, 1H, -CH-), 2.19-2.17 (br, 1H, -CH-), 1.84 (br, 1H, -CH-), 1.59-1.56 (br, 2H, -CH_2-), 1.29 (s, 3H, -CH_3), 1.27 (s, 3H, -CH_3), 1.15 (br, 2H, -CH_2-). ^{13}C NMR ($CDCl_3$) δ 166.4, 164.8, 161.0, 158.0, 157.2, 141.2, 141.0, 140.5, 135.2, 129.4, 127.0, 120.4, 59.2, 42.0, 28.0, 24.1, 22.8, 21.0, 19.7, MS calcd. For $C_{26}H_{29}N_5O_2$ $[M+H]^+$ 444.2, found 444.3.

Kinetics measurements of 2, 9 and 10 with 1 or 4.

UV/Vis kinetic measurements: Separate solutions of pure tetrazines **2**, **9**, **10** and pure alkynes **1**, **4** (>95-98% by 1H-NMR) were prepared in HPLC-grade MeOH at 297 K. The stability of **2**, **9**, and **10** in MeOH was examined by monitoring its absorption maximum at 295 nm. Solutions containing **2**, **9**, **10** (25 uM) and 10-18 fold excess of an alkyne (**1** or **4**) were pipetted into quartz cuvettes for UV measurements and thoroughly mixed. All kinetic runs were triple triplicates. Curve fitting was operated in Prism3 software.

A) Tetrazine **2** with alkyne **1**



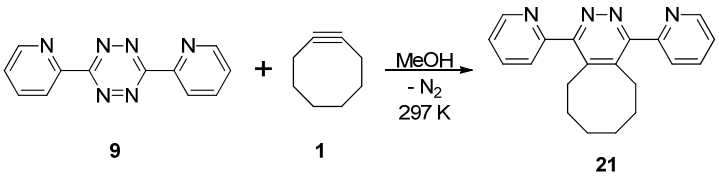


$k_1 = (3.15 \pm 0.3) \times 10^{-5}\ s^{-1}$

$k_2 = k_1 / 450\ uM = (7.0 \pm 0.7) \times 10^{-2}\ M^{-1}s^{-1}$

Figure S6. Ph-tetrazine 25 uM, alkyne 250 uM, 300 uM, 350 uM, 400 uM, 450 uM.

B) Tetrazine **9** with alkyne **1**



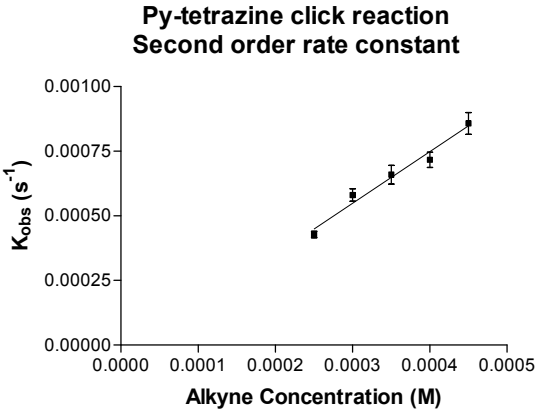
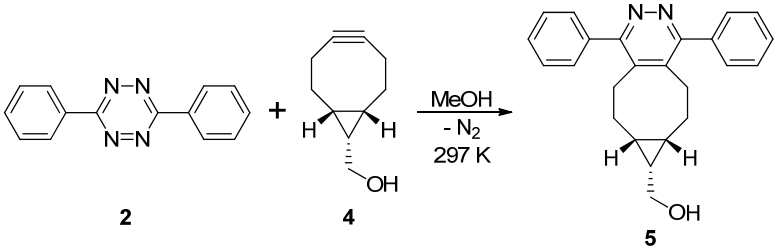


Figure S7. Py-tetrazine 25 um, alkyne 250 uM, 300 uM, 350 uM, 400 uM, 450 uM,
Second order rate constant $k_2 = 2.0 \pm 0.3\ M^{-1}s^{-1}$

C) Tetrazine **2** with alkyne **4**



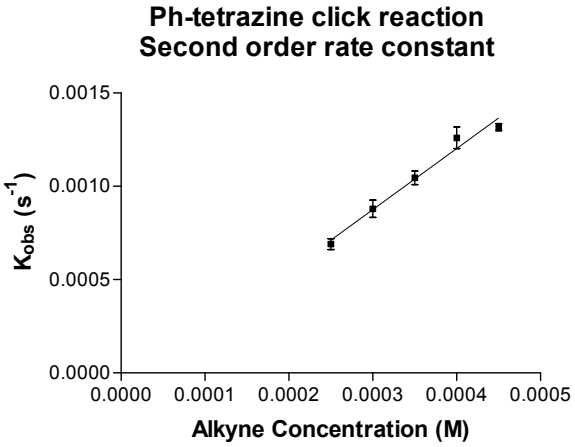
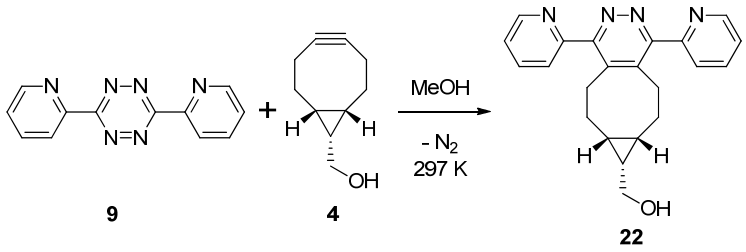


Figure S8. Ph-tetrazine 25 um, alkyne 250 uM, 300 uM, 350 uM, 400 uM, 450 uM, Second order rate constant k_2= 3.3 ± 0.4 $M^{-1}s^{-1}$

D) Tetrazine **9** with alkyne **4**



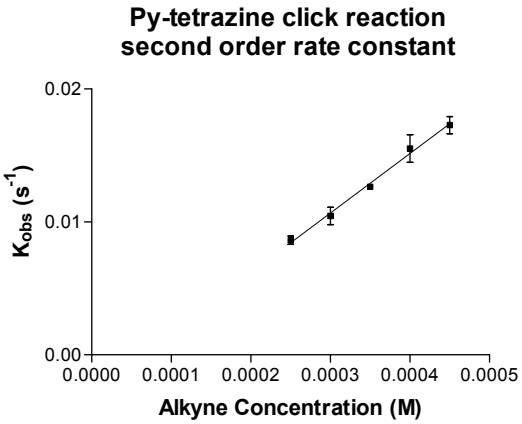
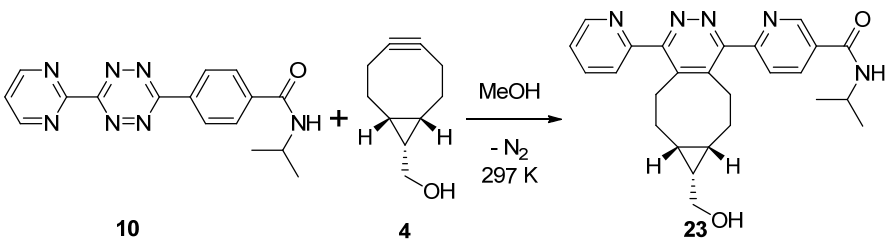


Figure S9. Py-tetrazine 25 um, alkyne 250 uM, 300 uM, 350 uM, 400 uM, 450 uM, Second order rate constant k_2= 44.8 ± 4.9 $M^{-1}s^{-1}$

E) Tetrazine **10** with alkyne **4**



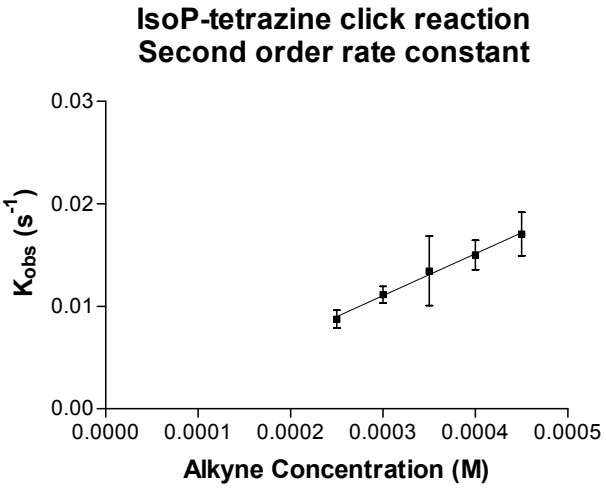
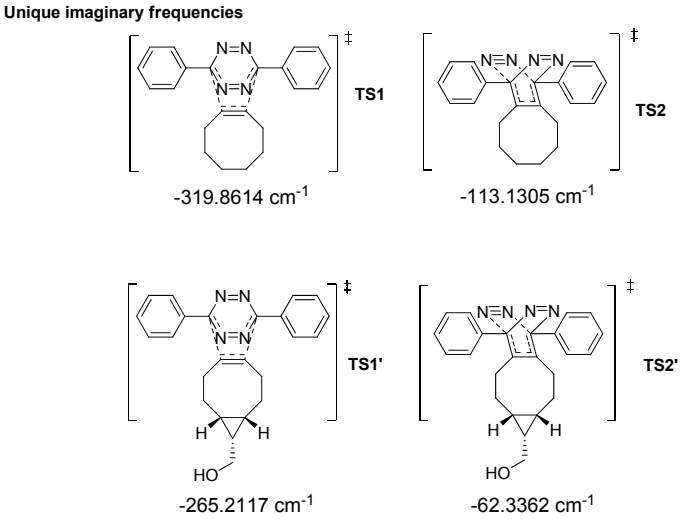


Figure 10 IsoP-tetrazine 25 uM, alkyne 250 uM, 300 uM, 350 uM, 400 uM, 450 uM,
Second order rate constant k_2= 40.9 ± 13.8 $M^{-1}s^{-1}$

Computational detail

All calculations were performed using the Gaussian 03 program.[8] Initial geometry optimizations were carried out at the AM1[9] semiempirical level. DFT calculations were carried out with use of the B3LYP[10, 11] with the standard 6-31G** basis set. The stationary points were characterized by frequency calculations to verify that TSs had one and only one imaginary frequency.



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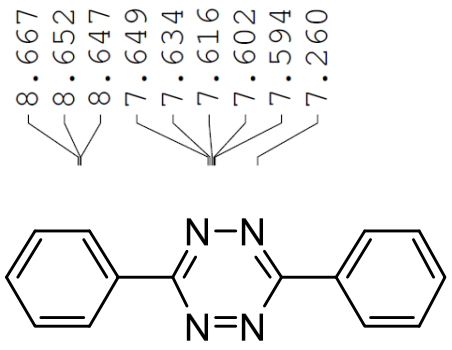
^{1}H and ^{13}C Spectra

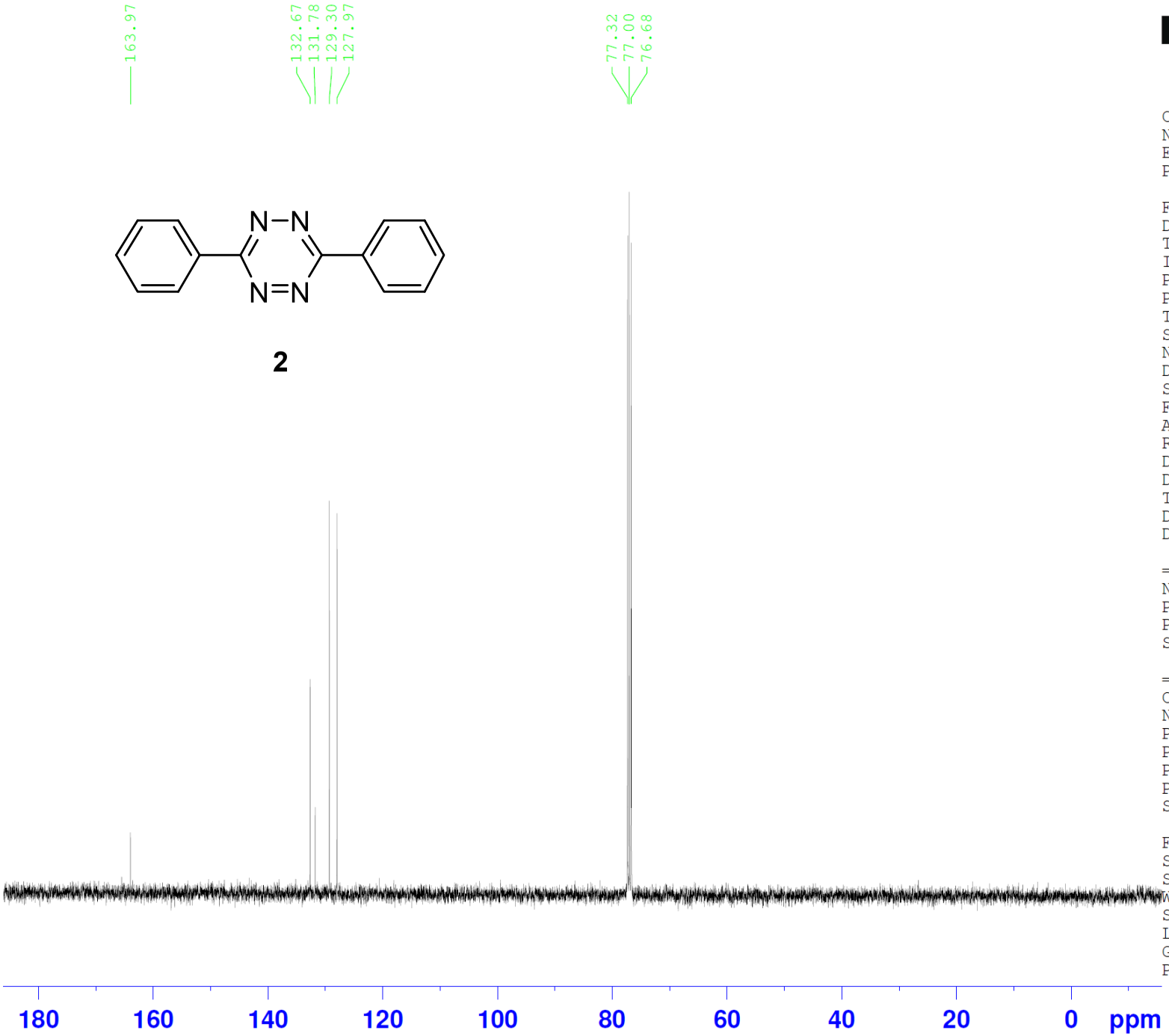
Ph-tetrazine-1H



2


```
Current Data Parameters
NAME                  DZ-II-60
EXPNO                        3
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20111115
Time                     18.46
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                        203
DW                   60.800 usec
DE                    6.50 usec
TE                    298.1 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             400.1400086 MHz
WDW                        EM
SSB          0
LB                     0.30 Hz
GB           0
PC                     1.00
```

Ph-tetrazine-C13



Current Data Parameters
NAME DZ-II-60
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20111115
Time 18.51
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 77
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631988 sec
RG 161
DW 20.800 usec
DE 6.50 usec
TE 298.3 K
D1 2.00000000 sec
D11 0.03000000 sec

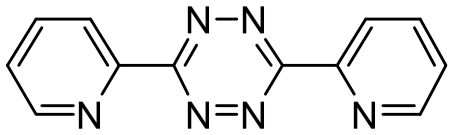
======== CHANNEL f1 ========
NUC1 13C
P1 9.00 usec
PLW1 62.00000000 W
SFO1 100.6253441 MHz

======== 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

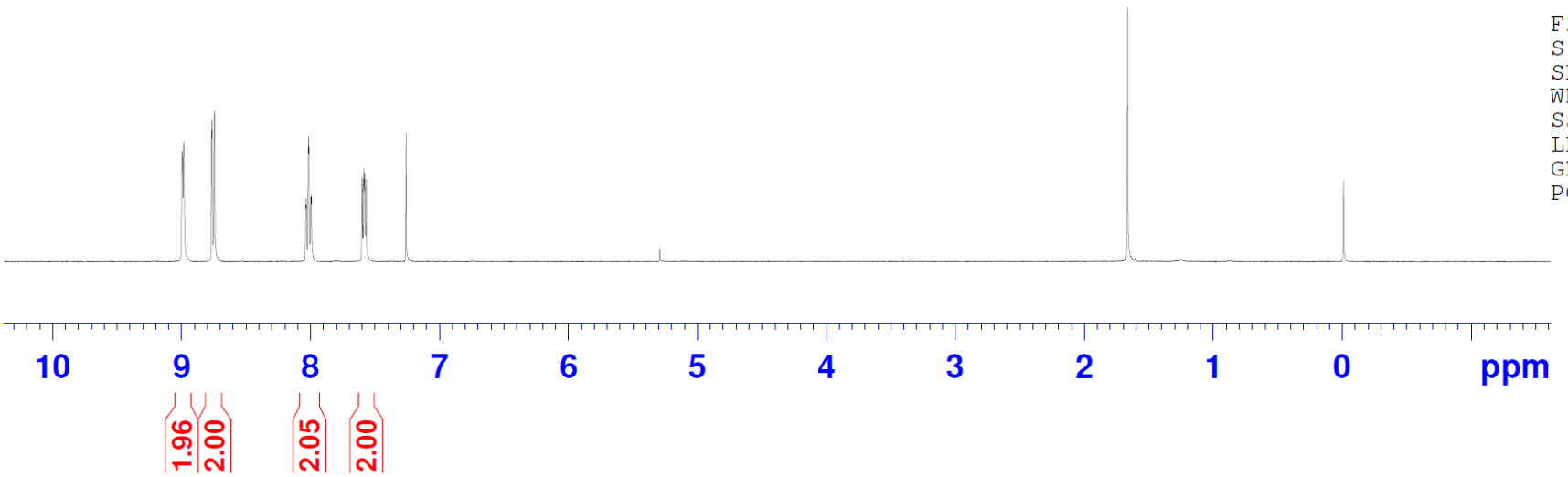
F2 - Processing parameters
SI 32768
SF 100.6152854 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

Py-tetrazine-1H

8.996
8.984
8.766
8.746
8.037
8.033
8.017
8.014
7.998
7.994
7.600
7.588
7.581
7.569
7.260

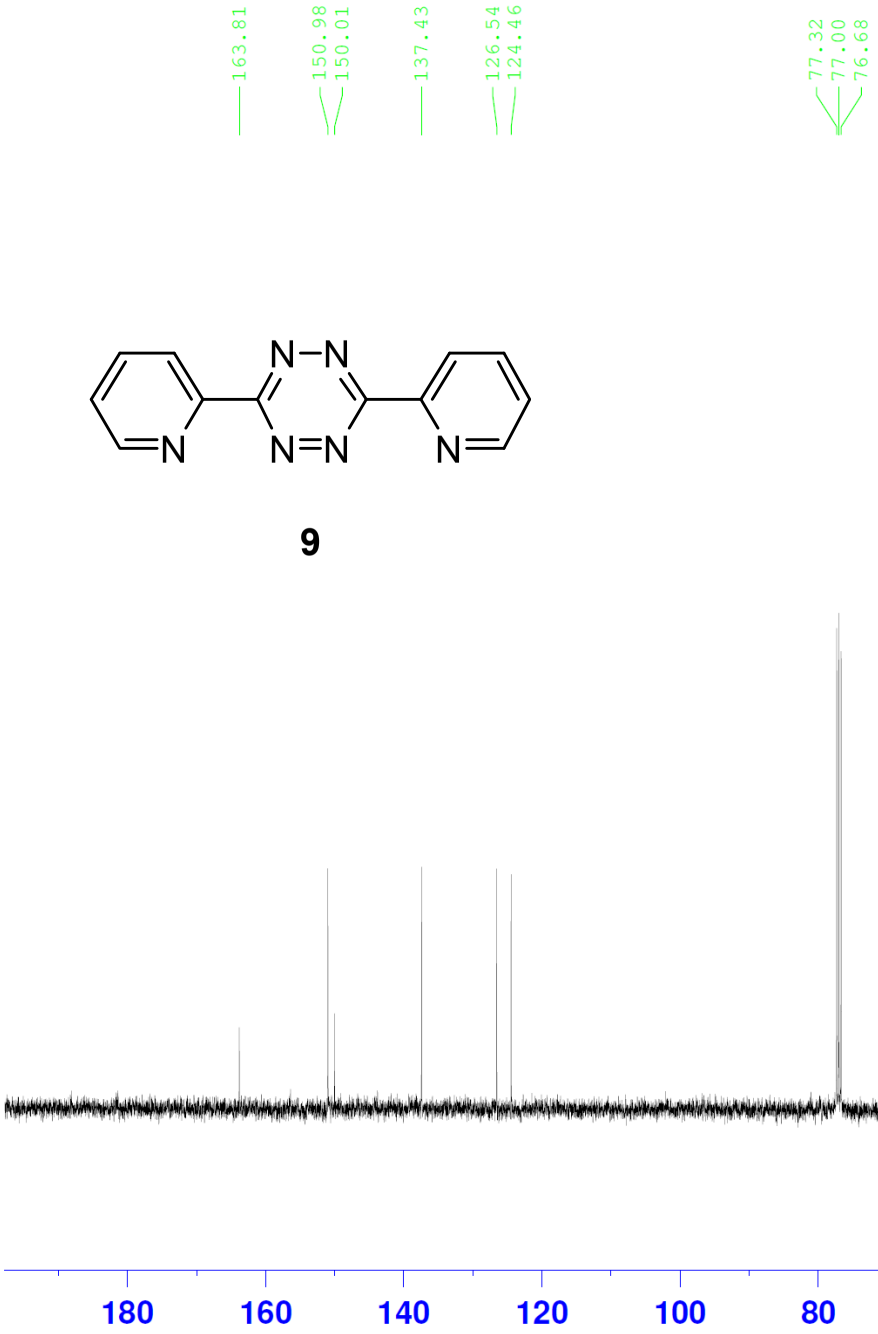


9



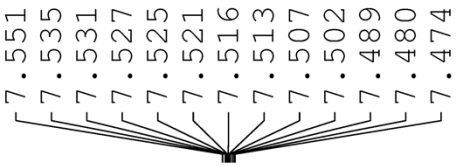

```
Current Data Parameters
NAME                  DZ-III-53
EXPNO                         7
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20111116
Time                      19.52
INSTRUM                   spect
PROBHD    5 mm PABBO BB-
PULPROG                    zg30
TD                        65536
SOLVENT                   CDCl3
NS                            3
DS                            2
SWH                    8223.685 Hz
FIDRES                 0.125483 Hz
AQ                     3.9846387 sec
RG                          203
DW                       60.800 usec
DE                        6.50 usec
TE                       298.1 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                 400.1400083 MHz
WDW                          EM
SSB             0
LB                        0.30 Hz
GB              0
PC                        1.00
```

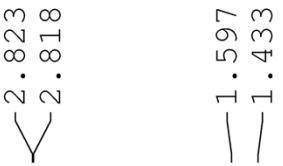
Py-tetrazine-C13

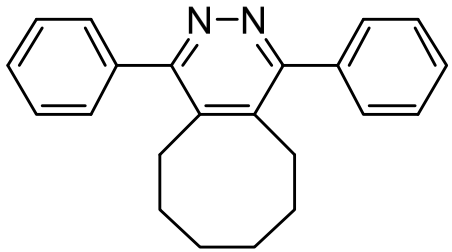



```
Current Data Parameters
NAME                 DZ-III-53
EXPNO                        6
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20111115
Time                     19.05
INSTRUM                  spect
PROBHD    5 mm PABBO BB-
PULPROG                 zgpg30
TD                       65536
SOLVENT                  CDCl3
NS                          34
DS                           4
SWH                 24038.461 Hz
FIDRES               0.366798 Hz
AQ                   1.3631988 sec
RG                         181
DW                      20.800 usec
DE                       6.50 usec
TE                      298.1 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                 100.6152892 MHz
WDW                           EM
SSB          0
LB                       1.00 Hz
GB           0
PC                       1.40
```

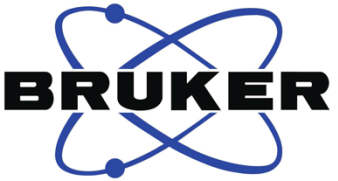
DZ-III-69-Ph-1H



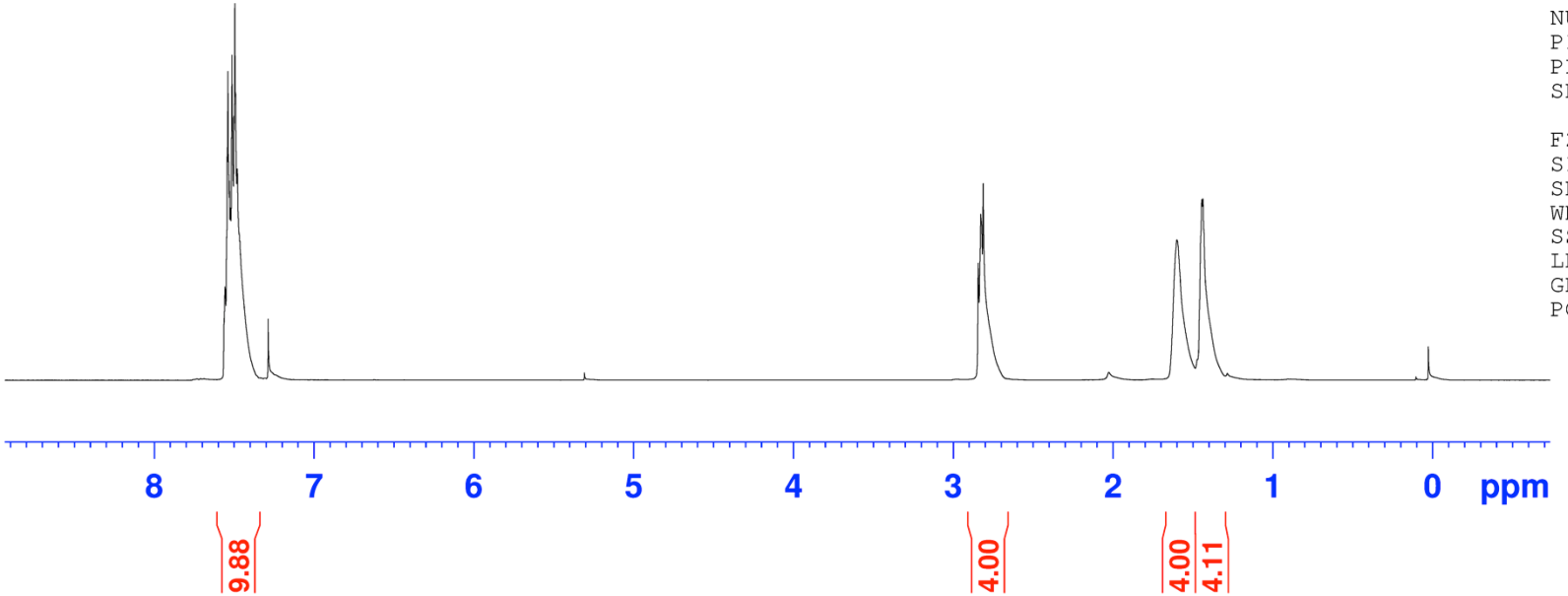




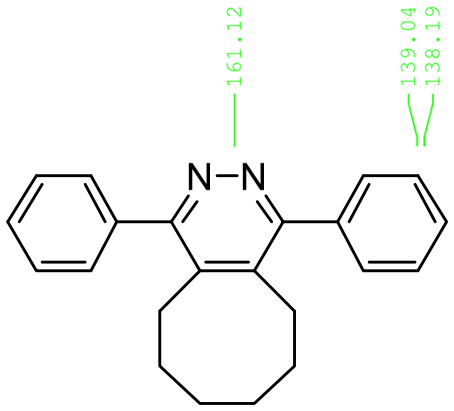
3




```
Current Data Parameters
NAME        DZ-III-69-Ph
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20110711
Time               10.15
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                  80.6
DW                60.800 usec
DE                 6.50 usec
TE        0 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
```



DZ-III-69-Ph-C13



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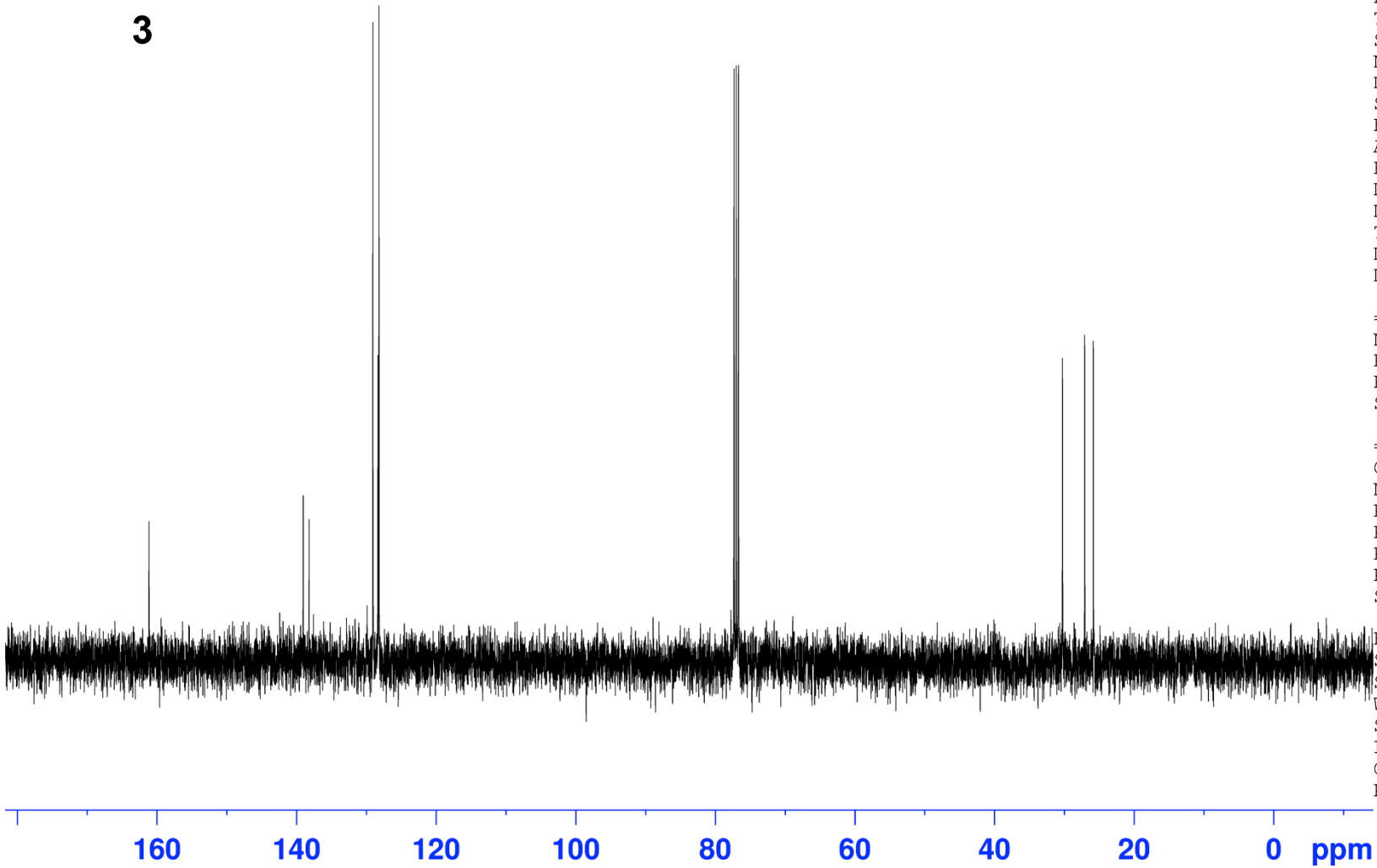
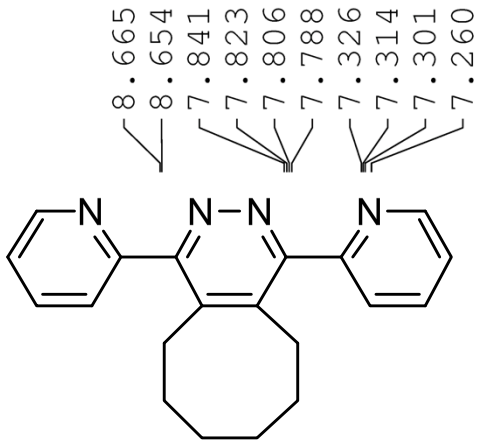
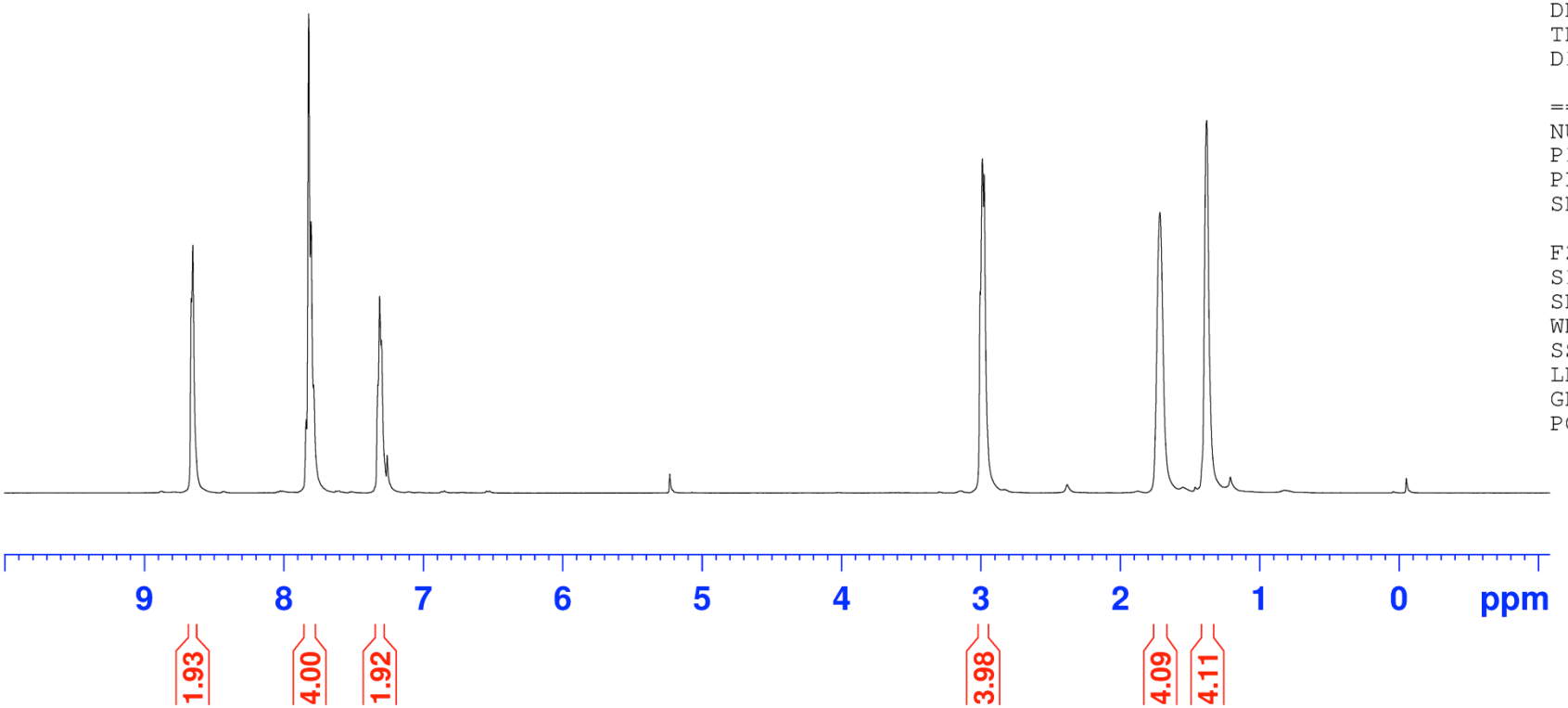



```
Current Data Parameters
NAME        DZ-III-69-Ph
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20110711
Time                9.39
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                   50
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        0 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          100.6152913 MHz
WDW                EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

DZ-III-78-Py-Tetrazine-alkyne-1H



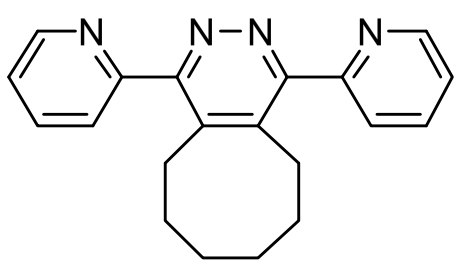
21



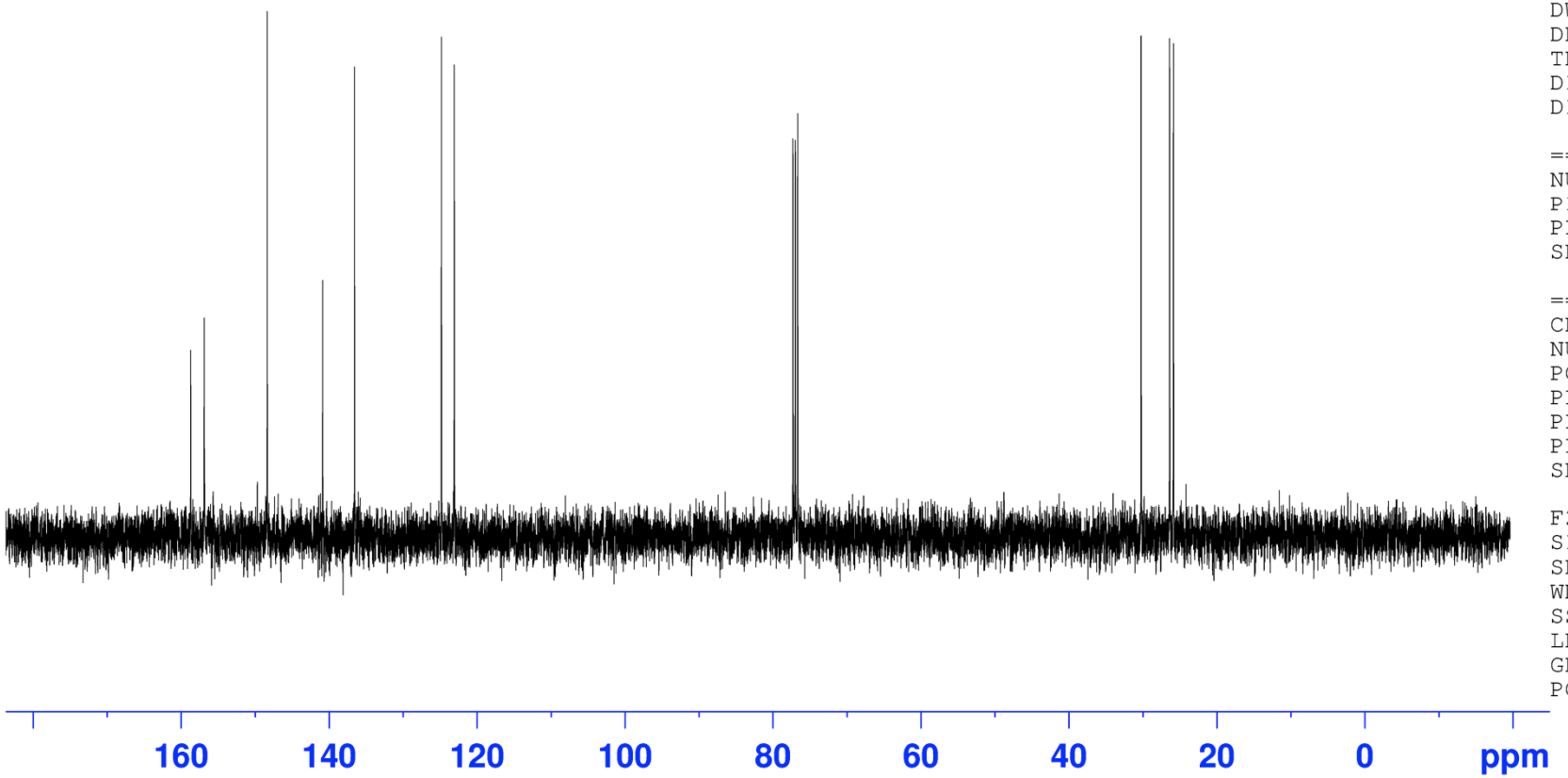

```
Current Data Parameters
NAME                  DZ-III-78
EXPNO                         1
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20110718
Time                       9.49
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                          32
DW                       60.800 usec
DE                        6.50 usec
TE                    0 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                  400.1400113 MHz
WDW                         EM
SSB                   0
LB                        0.30 Hz
GB                    0
PC                        1.00
```

DZ-III-78-Py-Tetrazine-alkyne-C13

158.71
156.89
148.37
140.88
136.57
124.81
123.08
77.32
77.00
76.68
30.25
26.40
25.88

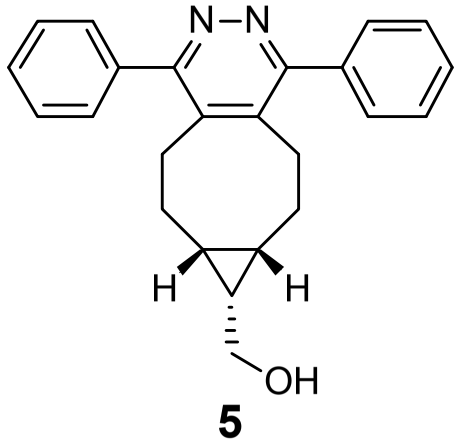


21

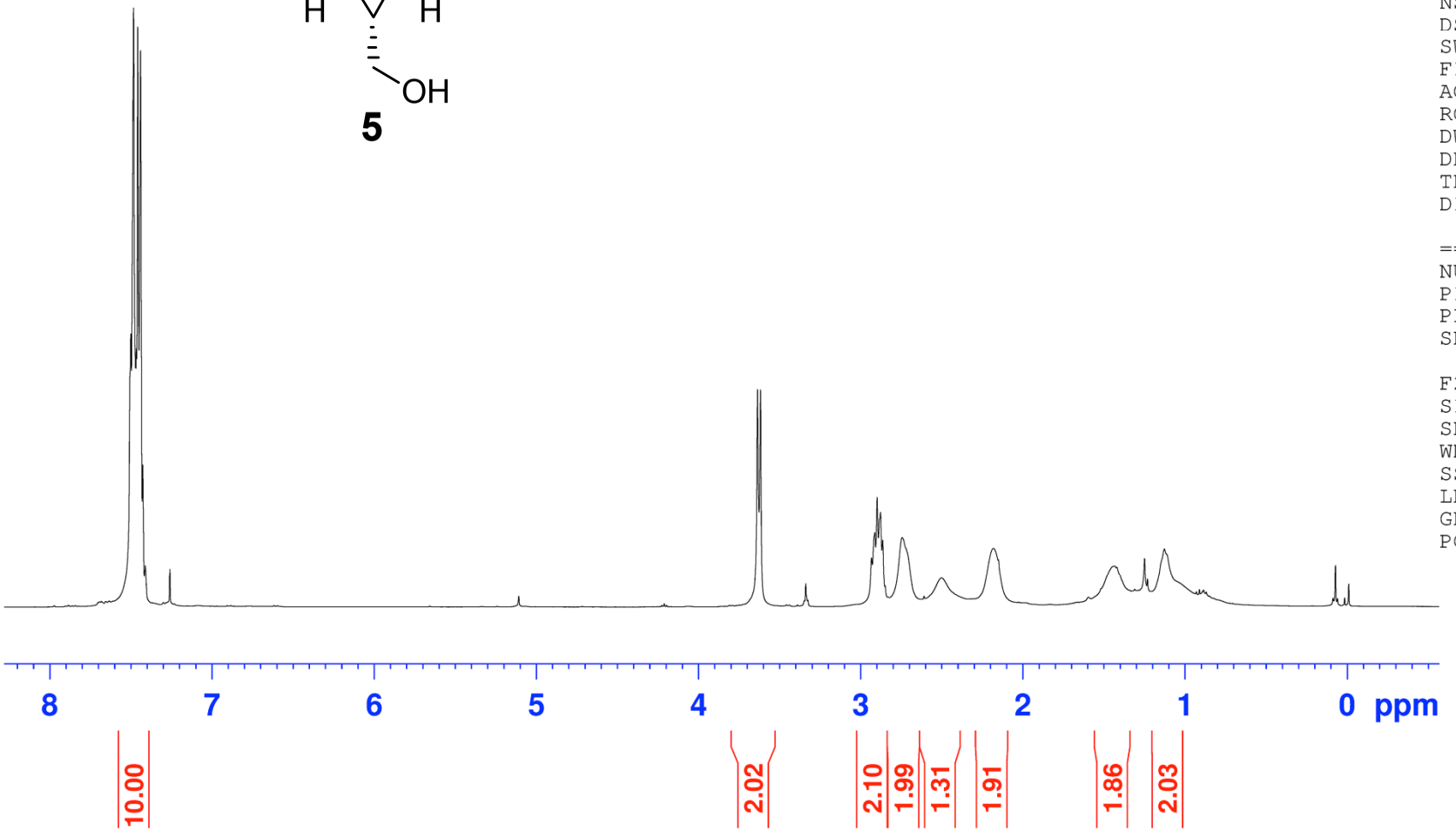



```
Current Data Parameters
NAME            DZ-III-78
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_            20110718
Time                9.53
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                    30
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        0 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          100.6153000 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

DZ-III-80-Ph-tetrazine-alkyne with 3 ring-1H

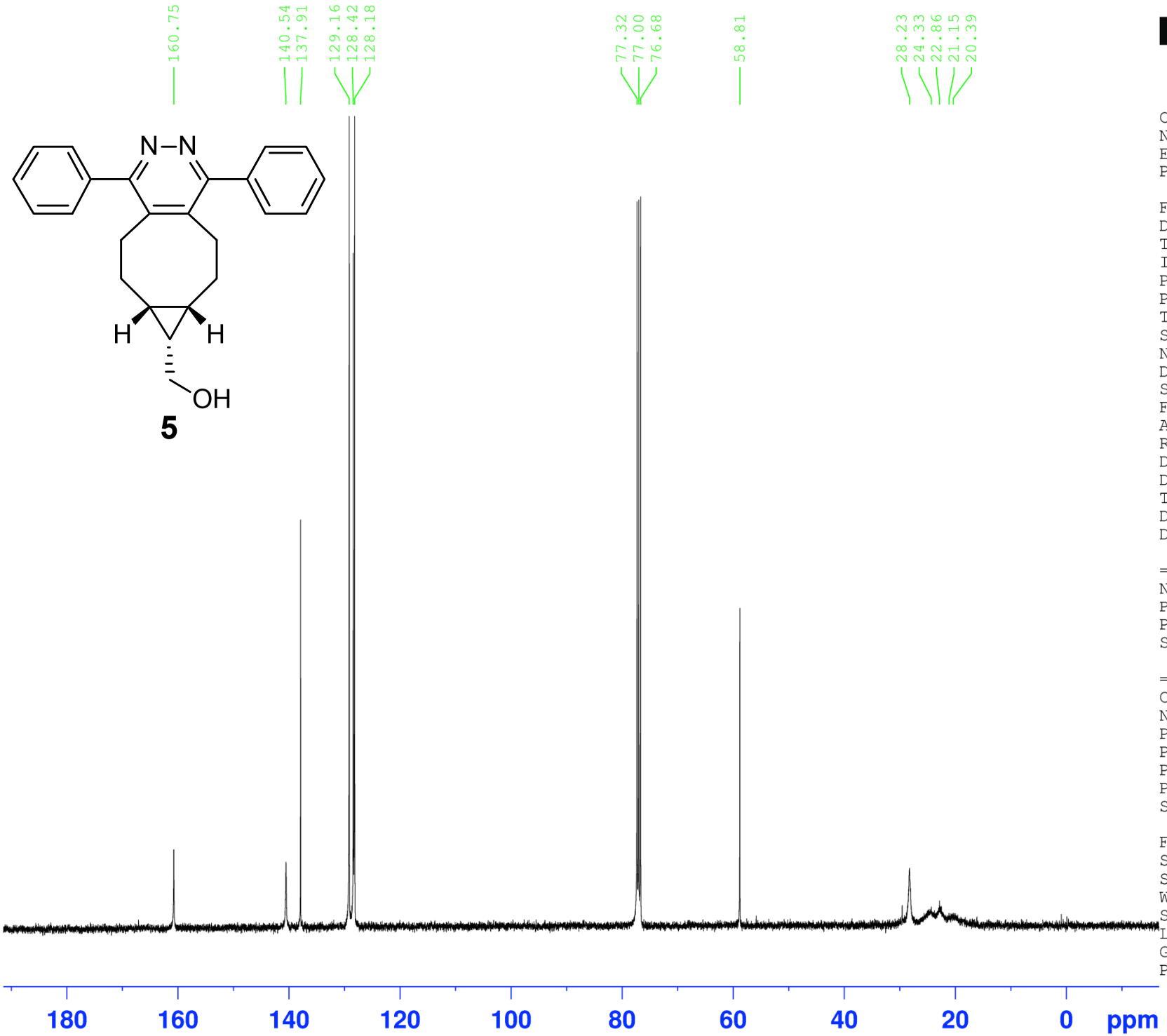


5




```
Current Data Parameters
NAME                  DZ-III-80
EXPNO                        11
PROCNO                        1

F2 - Acquisition Parameters
Date_                 20110727
Time                      9.48
INSTRUM                  spect
PROBHD    5 mm PABBO BB-
PULPROG                   zg30
TD                       65536
SOLVENT                  CDCl3
NS                          91
DS                           2
SWH                 8223.685 Hz
FIDRES              0.125483 Hz
AQ                  3.9846387 sec
RG                        50.8
DW                     60.800 usec
DE                      6.50 usec
TE                  0 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                400.1400096 MHz
WDW                         EM
SSB               0
LB                       0.30 Hz
GB                0
PC                       1.00
```

DZ-III-80-d-1H

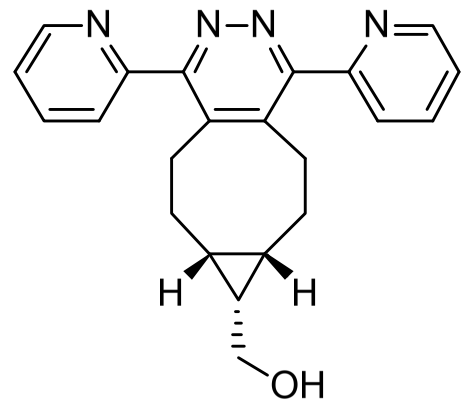



```
Current Data Parameters
NAME                  DZ-III-80
EXPNO                        20
PROCNO                        1

F2 - Acquisition Parameters
Date_                 20110728
Time                     22.02
INSTRUM                  spect
PROBHD    5 mm PABBO BB-
PULPROG                 zgpg30
TD                       65536
SOLVENT                  CDCl3
NS                        1024
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                0 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                 100.6152964 MHz
WDW                           EM
SSB               0
LB                        1.00 Hz
GB                0
PC                        1.40
```

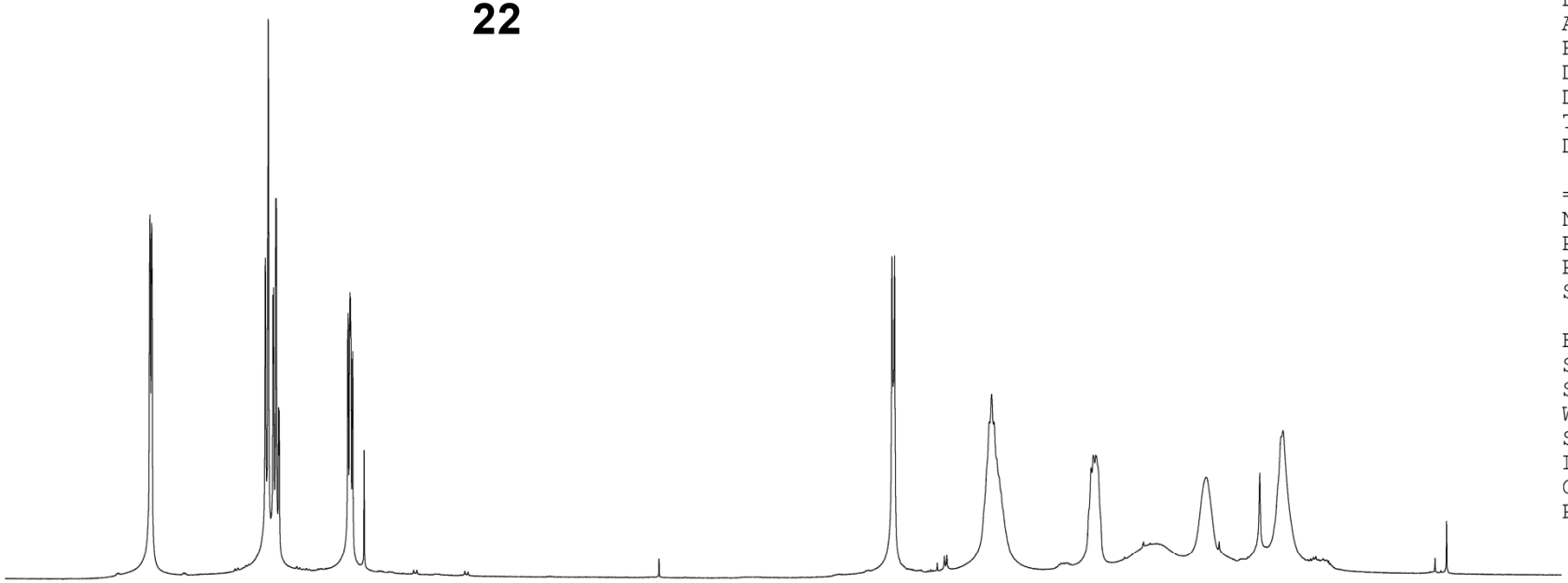
DZ-III-69-Py-H

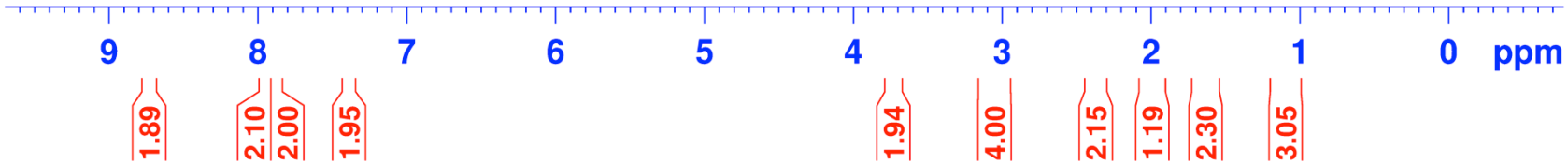
8.712 7.948 7.929 7.897 7.893 7.878 7.874 7.859 7.854 7.394 7.391 7.381 7.379 7.376 7.372 7.363 7.360 7.284

3.733 3.715 3.062 3.046 2.393 2.380 2.363 1.620 1.260 1.103 0.004



22





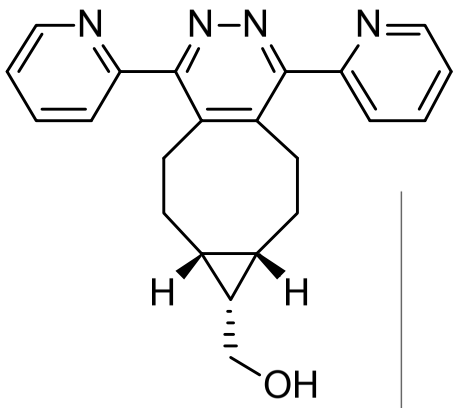
1.89 2.10 2.00 1.95 1.94 4.00 2.15 1.19 2.30 3.05

BRUKER

```
Current Data Parameters
NAME                  DZ-III-69
EXPNO                         1
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20110708
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                           32
DW                        60.800 usec
DE                         6.50 usec
TE                        297.3 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
```

DZ-III-69-Py-C13

159.07
156.96
148.65
142.24
136.81
124.96
123.25
77.40
77.08
76.76
59.48
27.81
24.38
22.74
19.56



22

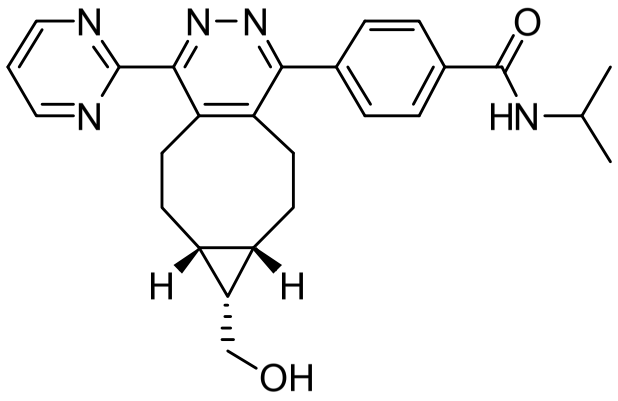
180 160 140 120 100 80 60 40 20 0 ppm

BRUKER

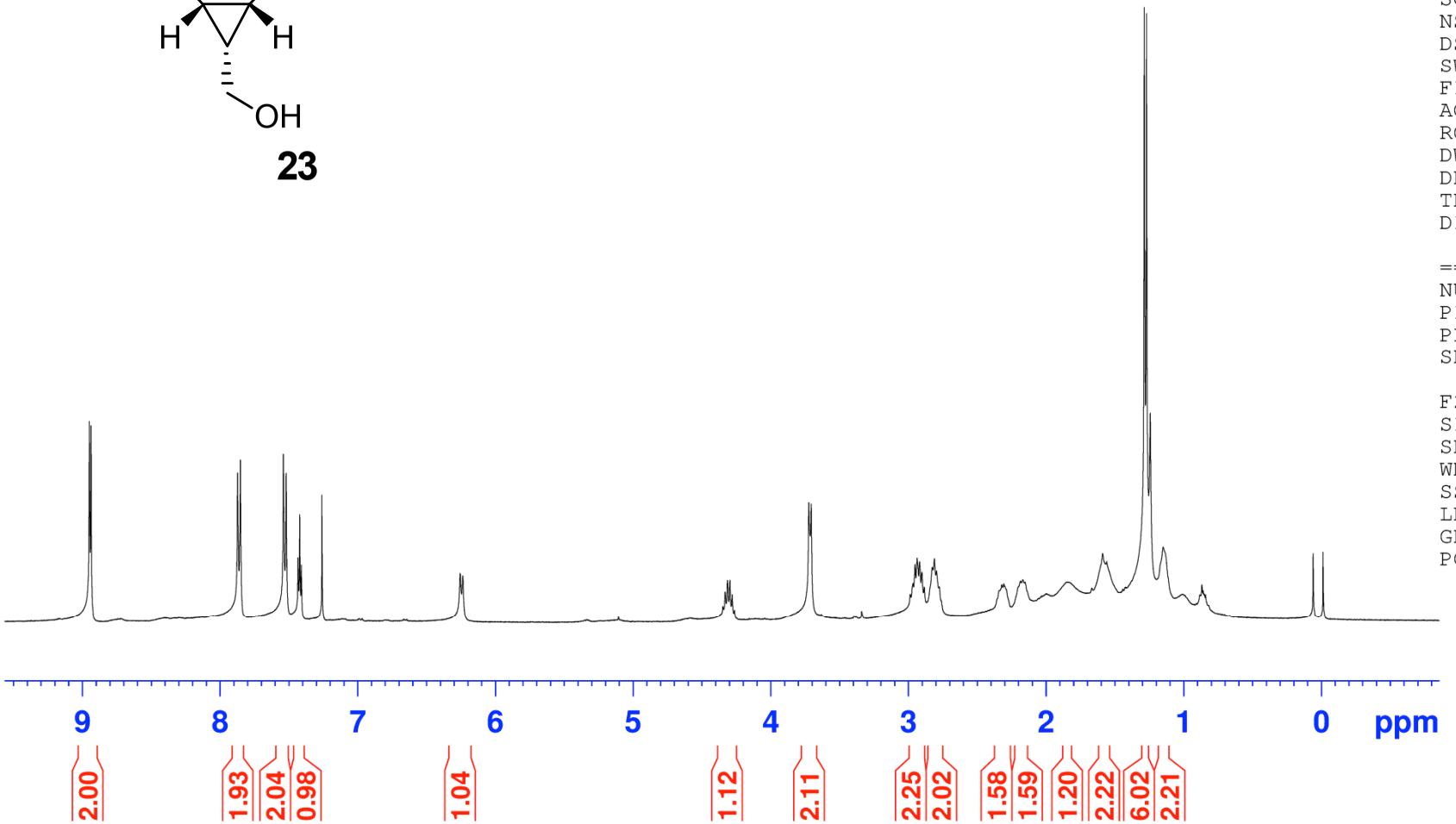
```
Current Data Parameters
NAME                 DZ-III-69
EXPNO                        3
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20110708
Time                     18.59
INSTRUM                  spect
PROBHD    5 mm PABBO BB-
PULPROG                 zgpg30
TD                       65536
SOLVENT                  CDCl3
NS                        1364
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                      297.6 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                 100.6152830 MHz
WDW                         EM
SSB                  0
LB                        1.00 Hz
GB                   0
PC                        1.40
```

DZ-III-79-isop-tetrazine-alkyne with 3 ring-1H

8.950 8.938 7.873 7.852 7.540 7.520 7.434 7.422 7.409 7.260 6.256 6.237 4.348 4.331 4.314 4.296 4.280 4.263 3.724 3.706 2.986 2.966 2.951 2.937 2.919 2.902 2.885 2.826 2.811 2.796 2.775 2.322 2.307 2.185 2.169 1.835 1.589 1.559 1.287 1.271 1.149



23



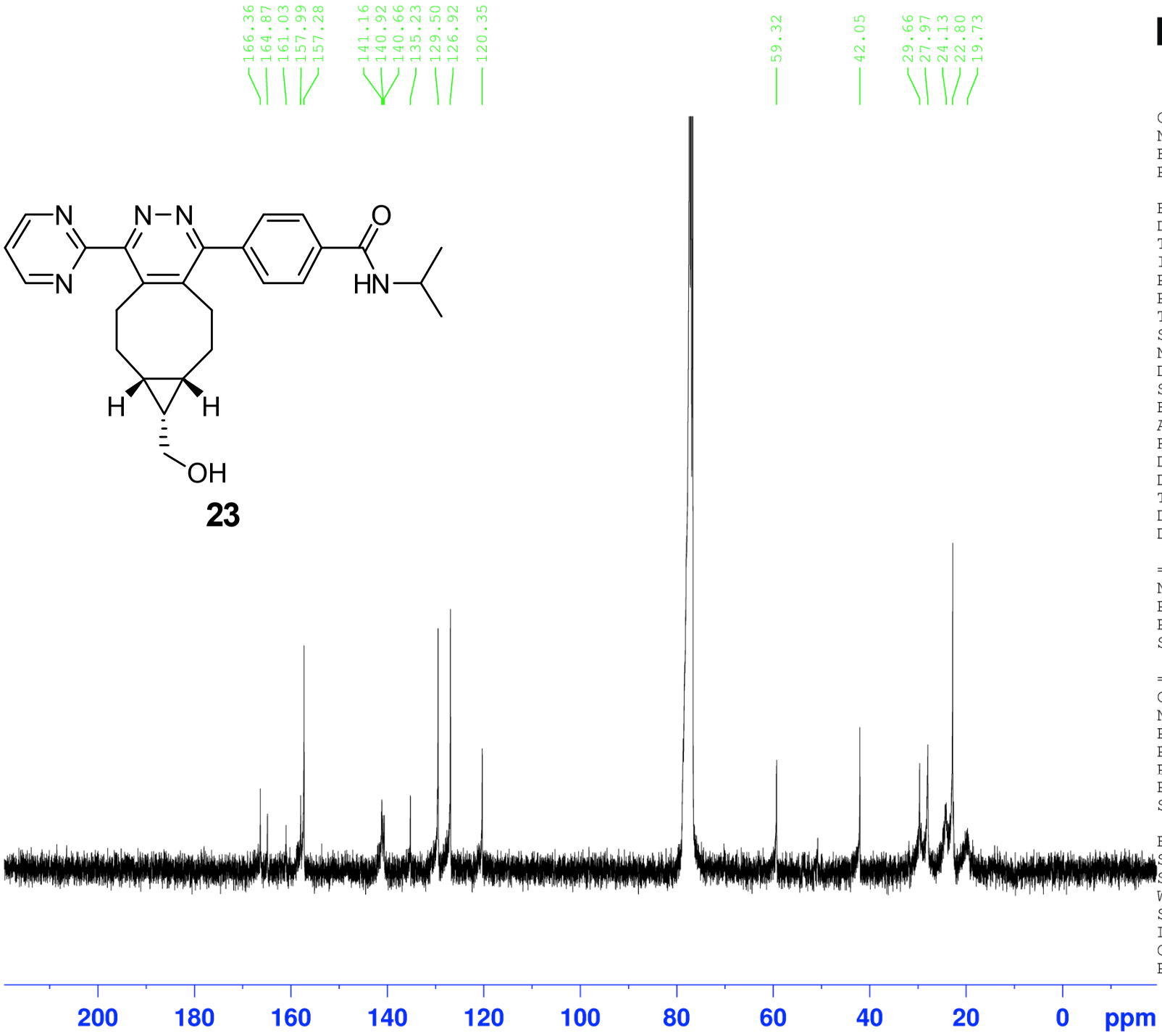
Current Data Parameters
NAME DZ-III-79
EXPNO 15
PROCNO 1

F2 - Acquisition Parameters
Date_ 20110727
Time 19.09
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 128
DW 60.800 usec
DE 6.50 usec
TE 0 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 400.1400096 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

DZ-III-79-isop-Tetrazine-alkyne with 3 ring-C13



BRUKER

```
Current Data Parameters
NAME                  DZ-III-79
EXPNO                        16
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20110727
Time                      21.36
INSTRUM                   spect
PROBHD    5 mm PABBO BB-
PULPROG                 zgpg30
TD                       65536
SOLVENT                  CDCl3
NS                       12288
DS                           4
SWH                 24038.461 Hz
FIDRES               0.366798 Hz
AQ                   1.3631988 sec
RG                         161
DW                      20.800 usec
DE                       6.50 usec
TE                  0 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                 100.6152867 MHz
WDW                           EM
SSB                0
LB                        1.00 Hz
GB                 0
PC                        1.40
```