Isatin-benzoazine molecular hybrids as potential antiproliferative agents: synthesis and *in vitro* pharmacological profiling

Hatem A. Abdel-Aziz[1]*, Wagdy M. Eldehna[2], Adam B. Keeton[3], Gary A. Piazza[3], Adnan A. Kadi[4], Mohamed W. Attwa[4], Ali S. Abdelhameed[4], Mohamed I. Attia[4,5]*

[1]*Department of Applied Organic Chemistry, National Research Centre, Dokki, Giza, P.O. Box 12622, Egypt*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kafrelsheikh University, Kafrelsheikh 33516, Egypt*
[3]*Department of Oncologic Sciences and Pharmacology, Drug Discovery Research Center, Mitchell Cancer Institute, University of South Alabama,1660 Springhill Avenue, Mobile, AL 36604-1405, USA*
[4]*Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*
[5]*Medicinal and Pharmaceutical Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre (ID: 60014618), El Bohooth Street, Dokki, Giza 12622, Egypt*

Pharmacological Evaluation

Antiproliferative Activity

A549, HT-29 and ZR-75 cells were harvested from 90-95% confluent 10 cm dishes, and diluted in growth media. Appropriate concentrations of cells were prepared by adding 1,250 cells in a volume of 25 µL per well in tissue culture treated Griener 384-well microplates, then incubated overnight at 37 °C. The investigational compounds **6a-f, 7a-e, 8a-f** and **9a-f** were diluted to a concentration of 30 µM in DMSO, except compounds **6b**, **6d**, **6e**, and **7d** which were diluted to 10 µM concentration due to poor solubility. Concentrated dosing solutions of twice the final concentration were prepared by dilution of the DMSO stocks 1:500 in growth media. Two-fold serial dilutions were then prepared in a medium containing 0.2% DMSO to maintain a constant concentration of vehicle throughout the concentration range of the compound. Each of these solutions was added, in equal volume (25 µL), to four wells on each cell assay plate, along with vehicle control. Top concentration and serial dilution of the positive control compound, sunitinib, was prepared exactly as the experimental compounds. Cells were incubated with the compounds for a further 72 h. At the end of the treatment period, assay plates were allowed to cool to room temperature for 10 min prior to addition of 25 µL per well Promega CellTiterGlo reagent, followed by additional 10 min incubation at room temperature. The resulting luminescence was quantitated using a Molecular Devices Spectramax Paradigm (Sunnyvale, CA, USA). Percent growth inhibition was calculated as follows, where 100 represents the viability of vehicle treated control samples:

$$100 - \frac{(100 \times (\text{sample})}{(\text{vehicle control})}$$

Thus, on average, growth inhibition of vehicle control samples equals zero. Potency of compounds was determined using the Non Linear Dose Response algorithm (4-parameter logistic fit) with Graph Pad Prism 5 software package (San Diego, CA, USA).

Apoptosis and Caspase 3/7 Activity

A549 cells were harvested from a 90% confluent 10 cm dish, and diluted in growth media. Appropriate concentrations of cells were prepared by adding 1,250 cells in a volume of 25 µL per well in tissue culture treated Corning 384-well microplates, then incubated overnight at 37 °C. A single cluster of 4-wells containing no cells was

included to serve as a background (no cell) control. Investigational compound **8c** was diluted in DMSO to concentrations 3000-fold above the IC_{50} value. Concentrated dosing solutions of twice the final concentration were prepared by dilution of the DMSO stocks 1:500 in growth media. Three-fold dilutions of each dosing medium were then prepared in medium containing 0.2% DMSO to maintain a constant concentration of vehicle throughout the concentration range of the compound. Each of these solutions was added, in equal volume (25 µL), to four wells on each cell assay plate, along with the vehicle control. Cells were incubated with the compounds for a further 48h. After 24h, a separate group of cells was treated identically for 24 h. Likewise, groups of cells were treated for durations of 16h, 8h, 4h and 2h. At the end of the treatment period, assay plates were allowed to cool to room temperature for 10 min prior to addition of 25 µL per well Promega Caspase Glo 3/7 reagent, followed by an additional 10 min incubation at room temperature. The resulting luminescence was quantitated using a Molecular Devices Spectramax Paradigm (Sunnyvale, CA, USA).

Apoptosis activity as a function of caspase 3/7 was calculated as follows:

The average mean value of the background "cell free" control sample wells were subtracted from all vehicle and test compound wells. Fold-change in caspase activity was then calculated by dividing the mean sample value for each treatment time point by the mean vehicle value for the corresponding time.

$$\text{Fold change} = \frac{(\text{sample} - \text{cell free})}{(\text{vehicle control} - \text{cell free})}$$

Thus, on average, caspase 3/7 activity of the vehicle control samples equals one. Fold-change induced by the two tested concentrations for each compound was plotted with GraphPad Prism 5 software package (San Diego, CA, USA).

Cell Cycle Effects

A549 cells were harvested from a 90% confluent 10 cm dish, and diluted in growth media. Appropriate concentrations of cells were prepared by adding 5,000 cells in a volume of 100 µL per well in tissue culture treated Perkin Elmer ViewPlate™ microplates (Perkin Elmer, CT, USA), then incubated overnight at 37 °C. The following day, experimental compound **8c** and the control compound, sunitinib, were diluted in DMSO to concentrations 1000-fold above the maximum tested concentration. Concentrated dosing solutions of twice the final concentration were prepared by dilution of the DMSO stocks 1:500 in growth media. Three-fold

dilutions of each dosing medium were then prepared in medium containing 0.2% DMSO to maintain a constant concentration of vehicle throughout the concentration range of each compound. Each of these solutions was added in equal volume (100 µL), to each well on the assay plate, along with vehicle control. One plate of cells was incubated with the compounds for a further 24h, and a separate plate was incubated with the compounds for 48 h. At the end of the respective treatment periods, 150 µL per well of neutral buffered formalin fixative (10% paraformaldehyde) was rapidly added to each plate and incubated at room temperature for 20 min. Fixative was removed by washing thrice with phosphate buffered saline (PBS). Cell membranes were permeabilized and non-specific binding sites were blocked by incubation with 0.3% Triton X-100 and 5% FBS in PBS for 1 h. Phosphorylated Rb protein (P-Rb) was detected by 1 h incubation with anti-P-Rb rabbit monoclonal antibody (Cell Signaling no. 8516) diluted 1:1600 in antibody dilution buffer (1% bovine serum albumin and 0.3% Triton X-100 in PBS). Unbound antibody was removed by washing thrice with PBS. Anti-P-Rb antibody was detected by 1 h incubation with goat anti-rabbit AlexaFluor 488 conjugate (InVitrogen) diluted 1:500 in antibody dilution buffer. The secondary antibody solution was supplemented with 1 µg/mL DAPI to quantitate double stranded DNA within each cell nucleus.

Unbound antibody was removed by washing thrice with PBS. Nine fields per well were immediately imaged in a Molecular Devices ImageXpress® MicroXL automated fluorescent microscope system (Molecular Devices, Sunnyvale, CA, USA) using a 10x magnification objective lens. DAPI and FITC filter cubes, respectively, were used to visualize nuclei and immunolabeled P-Rb residues. Identical autofocus and exposure parameters were used for every sample. Fluorescent micrographs were analyzed using Molecular Devices MetaXpress multi-wavelength cell scoring image analysis program to quantitate Rb phosphorylation. The mean nuclear average intensity of P-Rb staining (RFU) per cell was reported and has been graphed using GraphPad Prism statistical and graphing software. Where appropriate, an IC_{50} value has been reported to reflect potency of the compound effect on P-Rb. DNA content in each cell was determined by total DAPI stain intensity and assigned to the appropriate category using the MetaXpress cell cycle analysis program. Results from each treatment were reported as average cell number in each of nine fields, as well as the percentage of cells within each phase of the cell cycle. Each of these parameters was

analyzed using GraphPad Prism, and, where appropriate, IC_{50} values have been reported.

Selectivity

A-549, IEC-6, MCF-10A and 3t3 cells were harvested from 90-95% confluent cultures in 10 cm dishes, and diluted in growth media recommended by the suppliers as follows: A-549 cells were cultured in RPMI-1640 medium supplemented with 5% fetal bovine serum (FBS). IEC-6 cells were cultured in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% FBS and 0.1 unit/mL recombinant insulin. Swiss 3t3 cells were cultured in DMEM supplemented with 10% FBS. MCF-10A cells were cultured in a mixture DMEM and F12 media supplemented with 5% fetal bovine serum (FBS), 20 ng/mL recombinant epidermal growth factor, 10 µg/mL insulin, 0.5 µg/mL hydrocortisone, and 100 ng/mL cholera toxin. Appropriate concentrations of cells were prepared by adding 1,250 cells in a volume of 25 µL per well in tissue culture treated BD 384-well microplates, then incubated overnight at 37 °C.

Experimental compound **8c** and control, sunitinib, were diluted to a concentration of 25 mM in DMSO. Concentrated dosing solutions of twice the final concentration were prepared by dilution of the DMSO stocks 1:500 in growth media. Two-fold serial dilutions were then prepared in medium containing 0.2% DMSO to maintain a constant concentration of vehicle throughout the concentration range of the test compound. Each of these solutions was added, in equal volume (25 µL), to four wells on each cell assay plate, along with vehicle control. Top concentration and serial dilution of the positive control compound, sunitinib, was prepared exactly as the experimental compounds. Cells were incubated at 37 °C with the compounds for a further 72 h. At the end of the treatment period, assay plates were allowed to cool to room temperature for 10 min prior to addition of 25 µL per well Promega CellTiterGlo reagent, followed by an additional 10 min incubation at room temperature. The resulting luminescence was quantitated using a Molecular Devices Spectramax Paradigm.Percent growth inhibition was calculated as previously mentioned in the antiproliferative activity section. Potency of compounds was determined using the Non Linear Dose Response algorithm (4-parameter logistic fit) with GraphPad Prism 5 software.

Multidrug Resistant Lung Cancer Cell Line

A549 and H69AR cells were harvested from 90-95% confluent 10 cm dishes, and diluted in growth media. Appropriate concentrations of cells were prepared by adding 1,250 cells in a volume of 25 µL per well in tissue culture treated BD 384-well microplates, then incubated overnight at 37 °C. Experimental compound **8c** and the positive control, sunitinib, were diluted to a concentration of 25 mM in DMSO. Concentrated dosing solutions of twice the final concentration were prepared by dilution of the DMSO stocks 1:500 in growth media. Two-fold serial dilutions were then prepared in medium containing 0.2% DMSO to maintain a constant concentration of vehicle throughout the concentration range of the compound. Each of these solutions was added, in equal volume (25 µL), to four wells on each cell assay plate, along with vehicle control. Top concentration and serial dilution of the positive control compound, sunitinib, was prepared exactly as the experimental compound **8c**. Cells were incubated at 37 °C with the compound **8c** for a further 72 h. At the end of the treatment period, assay plates were allowed to cool to room temperature for 10 min prior to addition of 25 µL per well Promega CellTiterGlo reagent, followed by an additional 10 min incubation at room temperature. The resulting luminescence was quantitated using a Molecular Devices Spectramax Paradigm. Percent growth inhibition was calculated as previously mentioned in the antiproliferative activity section. Potency of compounds was determined using the Non Linear Dose Response algorithm (4-parameter logistic fit) with GraphPad Prism 5 software.

Metabolic Investigations

Preparation of Rat Liver Microsomes (RLM's)

The study protocol was approved by the Research Ethics Committee at the college of pharmacy, King Saud University. Animals were incapacitated by cervical dislocation and an incision was made in the peritoneal cavity and the liver was excised out. Livers were weighed and transferred into a beaker containing volume of KCl/sucrose buffer in a volume four-folds of the weighed livers. RLM's were prepared following a previously published microsomal preparation procedure.[40, 41] The content of microsomal protein was determined according to the method of Lowry *et al*.[41] using bovine serum albumin as a standard. CYP450 activity was verified *via* testing the ability of the prepared microsomes to transform phenytoin to *p*-hydroxyphenytoin according to Billings's (1983) method.[42]

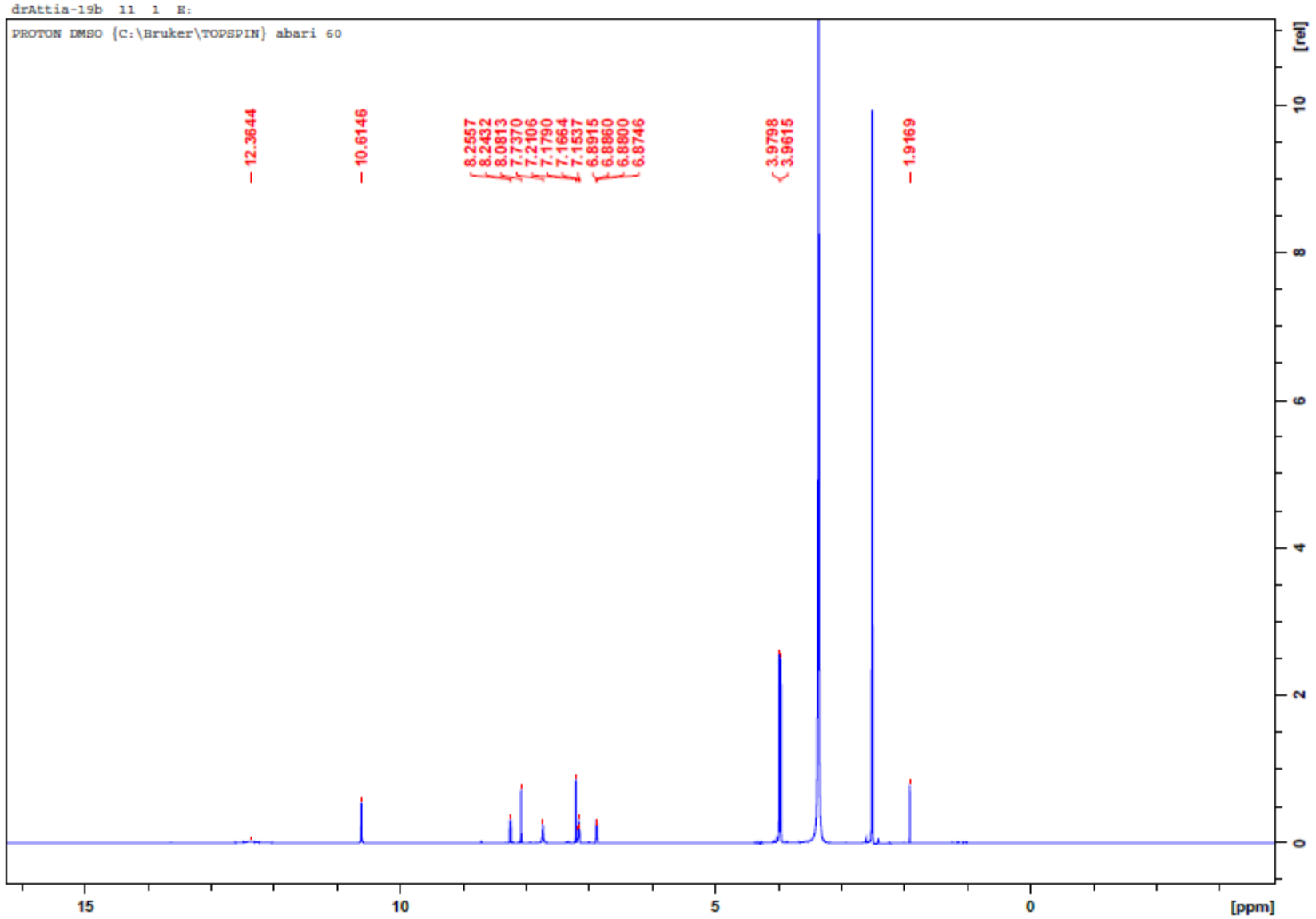
Rat Liver Microsomal Incubations

Metabolism of the test compound **8c** (1µL of 1mM stock solution) was determined with 40 µL (2 mg/mL) of microsomal protein in 0.08 M potassium phosphate buffer (0.08M KH_2PO_4/NaH_2PO_4, pH 7.4), at 37 °C for 30 min, in a final incubation volume of 1 mL with freshly prepared ice cold $MgCl_2$ solution (20.33 mg/mL). Test tubes containing the incubation mixtures were transferred to a shaking water bath and allowed to stand at a temperature of 37 °C in the water bath for 5 min. The reactions were initiated by the addition of a NADPH generating system containing 0.8 mM NADPH into the reaction mixture (8.33 mg/mL). Table 1 summarizes the amounts used for the investigation of compound **8c**. Incubation was allowed for 60 min, the reaction was terminated by the addition of 2 mL of ice cold acetonitrile. The mixture was centrifuged for 10 min at 14,000 rpm and the supernatant was transferred to a fresh tube and the solvent were evaporated under a stream of nitrogen. The residues were reconstituted in 1 mL with the mobile phase and transferred to HPLC vials for analysis.

Table 1: Volumes of the used reagents in microsomal incubations of compound **8c**.

Buffer	**$MgCl_2$**	**NADPH**	**Compound**	**Microsomes**	**Incubation type**
759µL	100µL	100µL	1µL	40µL	**8c**
760µL	100µL	100µL	0µL	40µL	**Control 1**
859µL	100µL	0µL	1µL	40µL	**Control 2**
799µL	100µL	100µL	1µL	0µL	**Control 2**

Representative examples of the NMR (^{1}H and ^{13}C) spectra of the synthesized compounds.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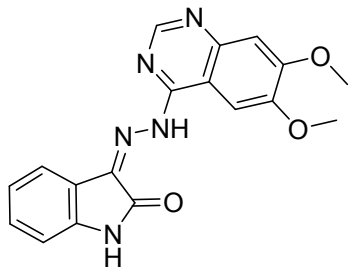

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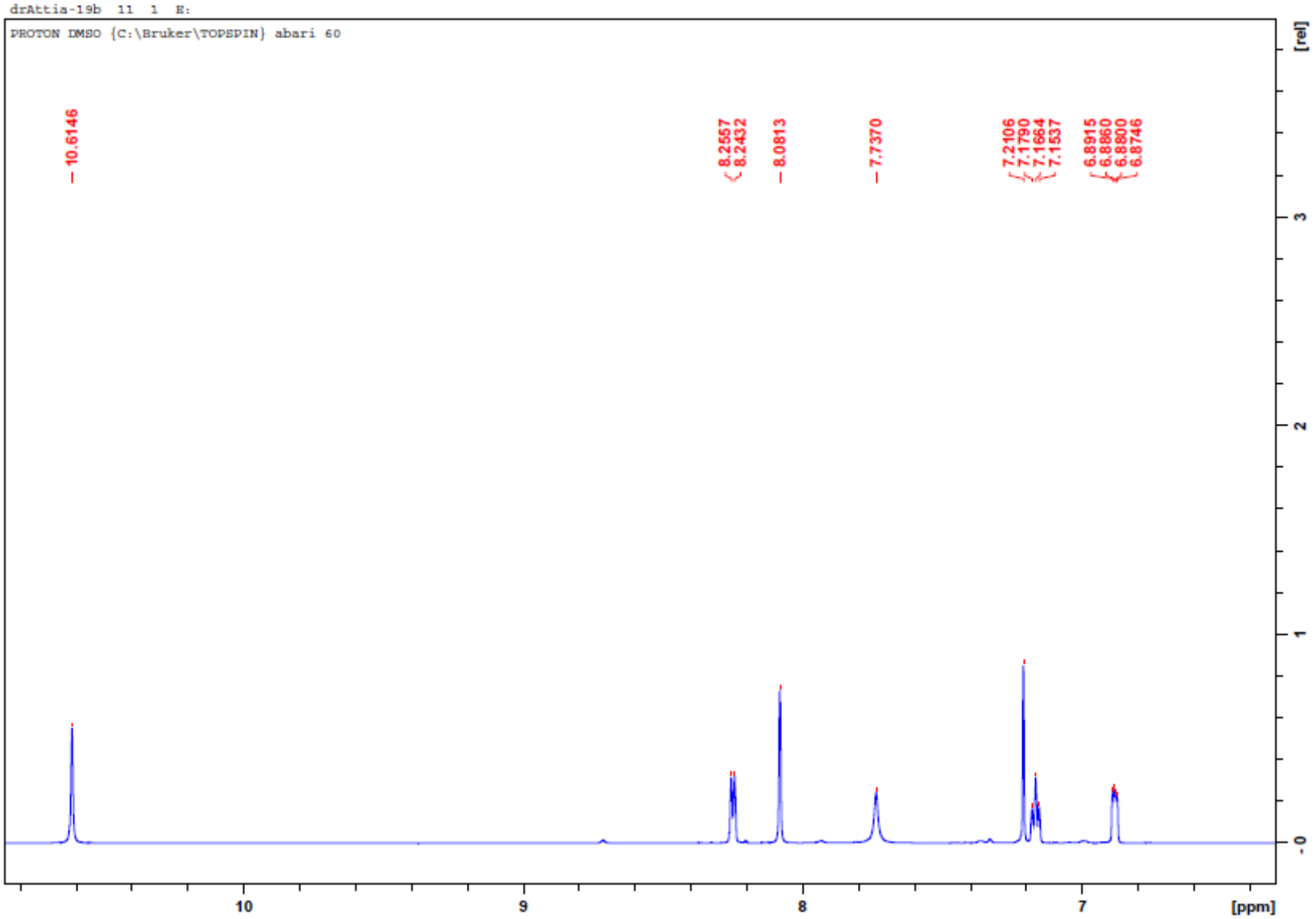
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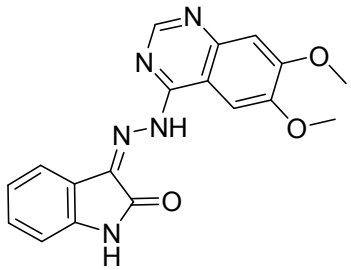
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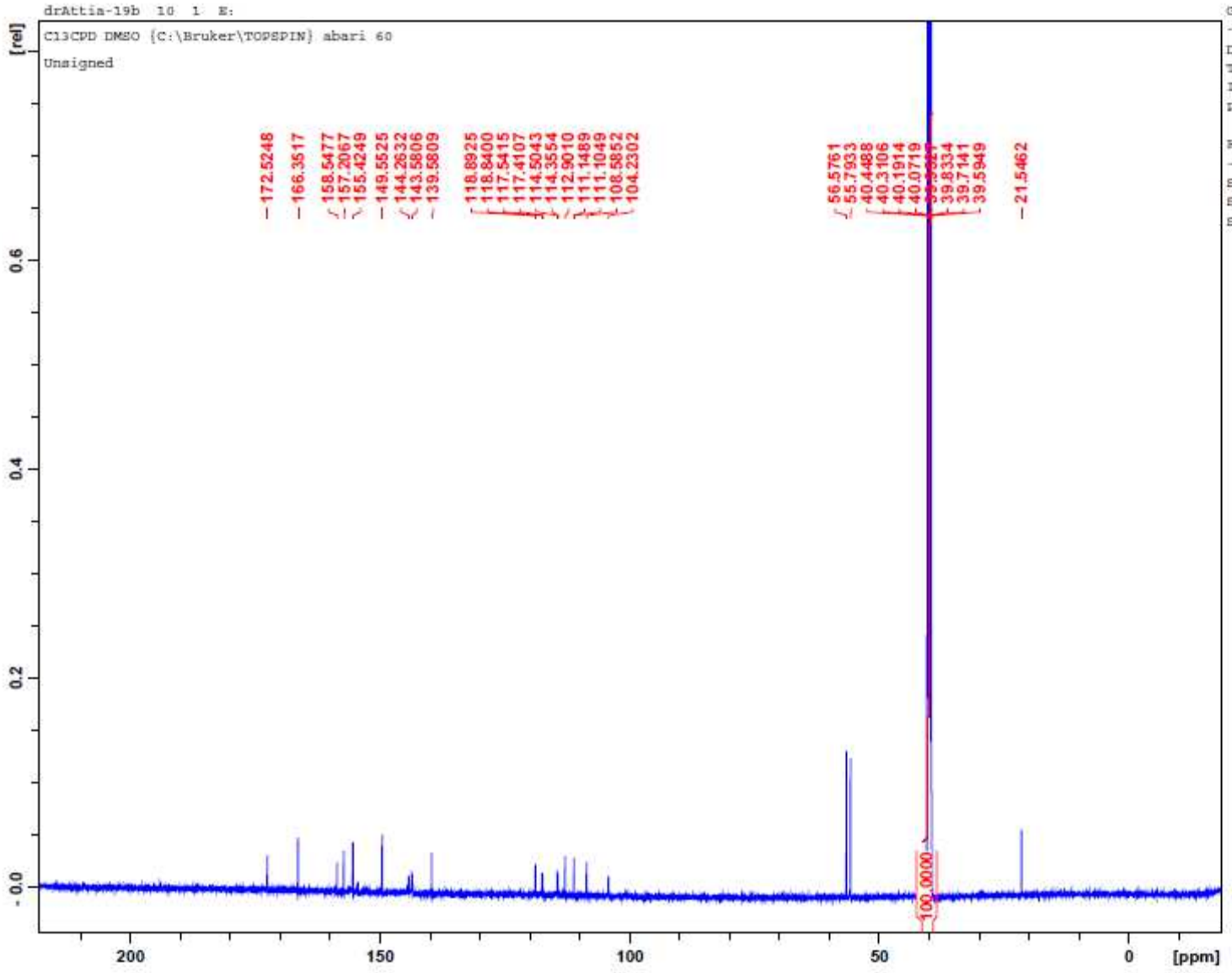

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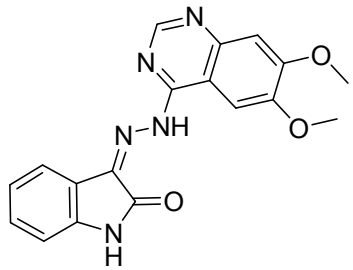

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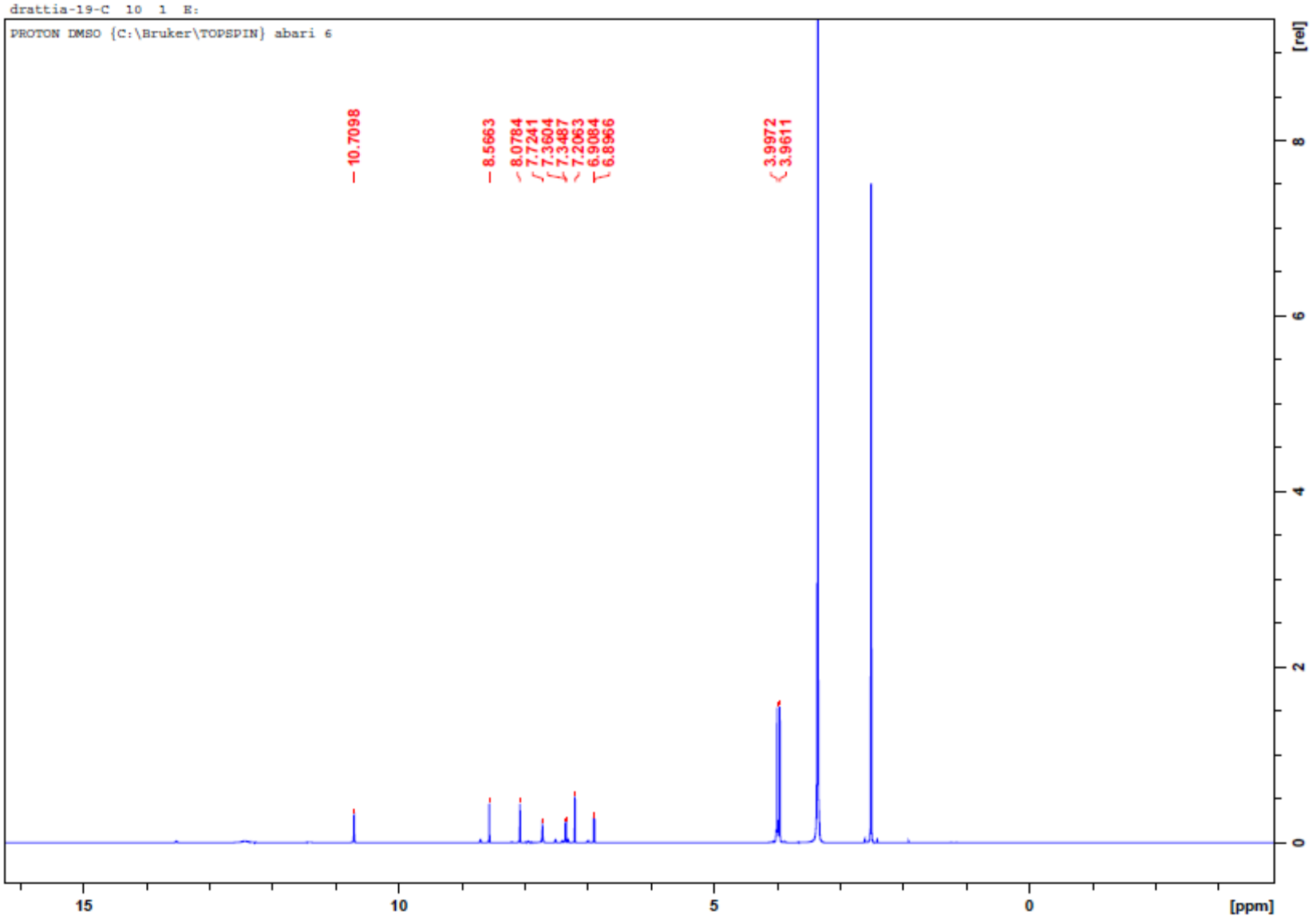
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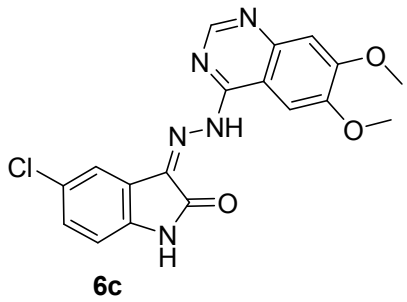
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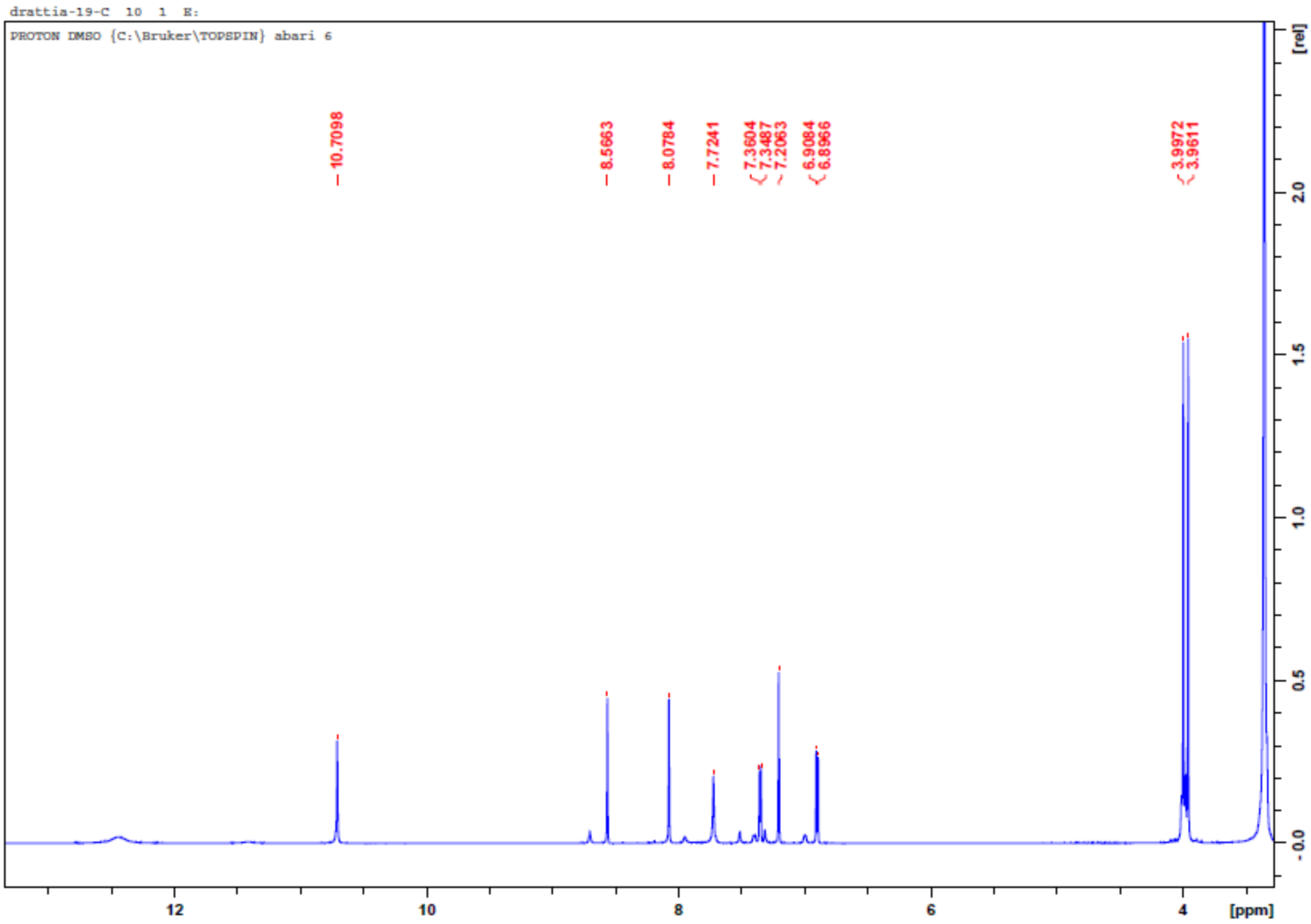
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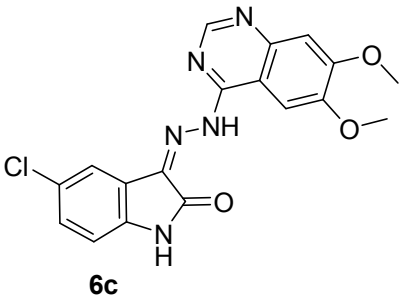
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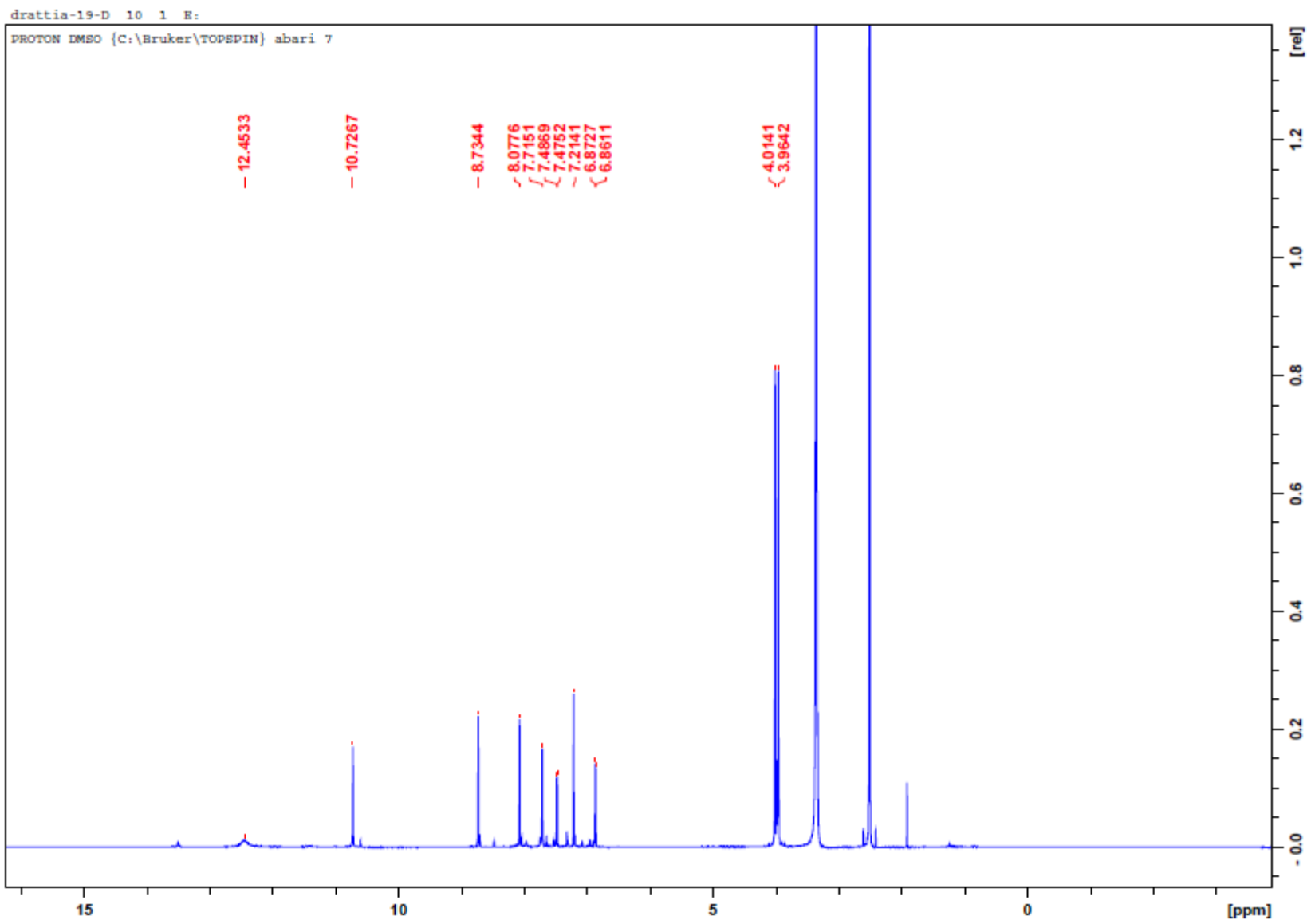
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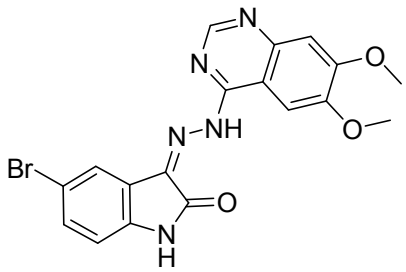
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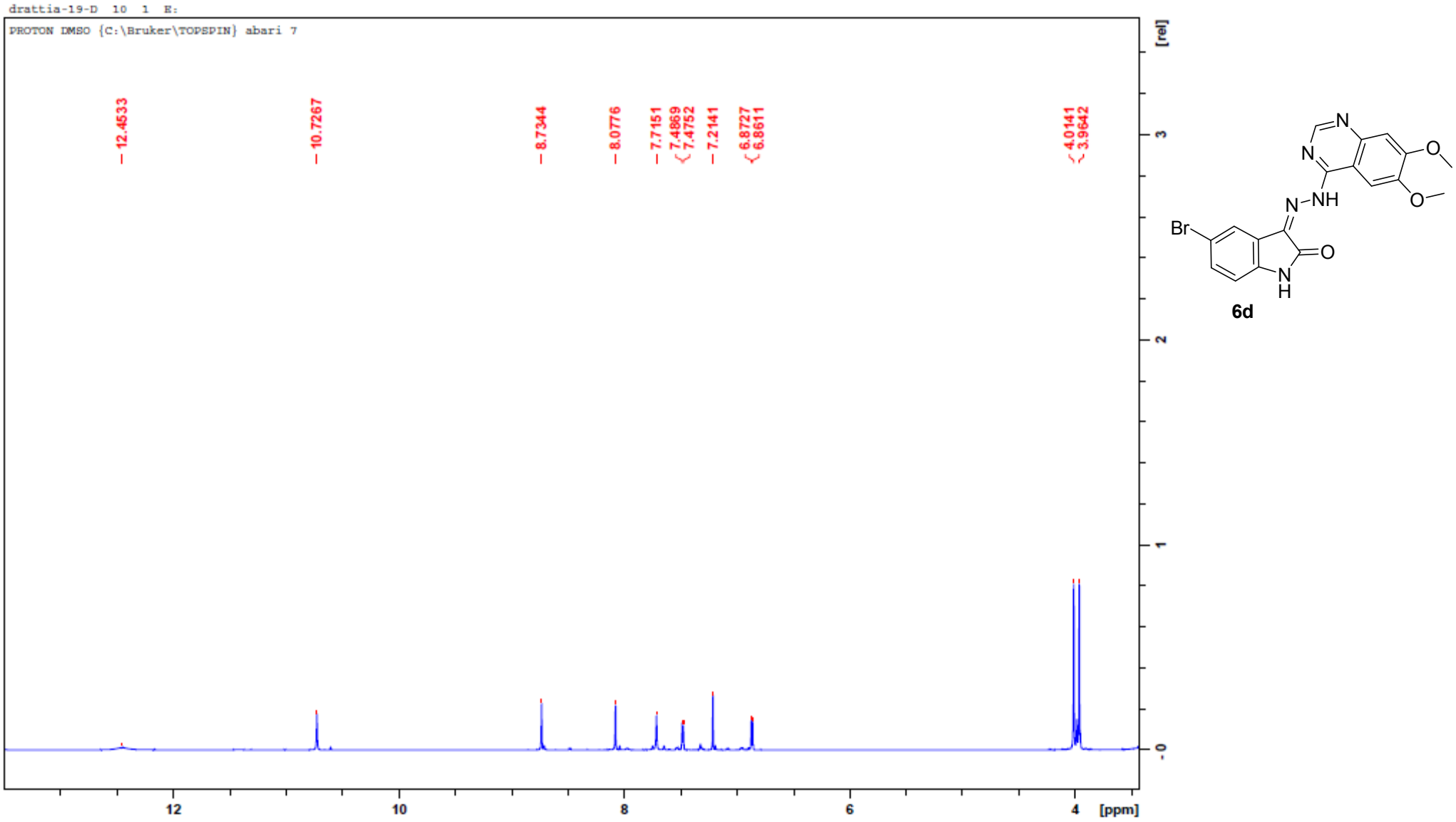
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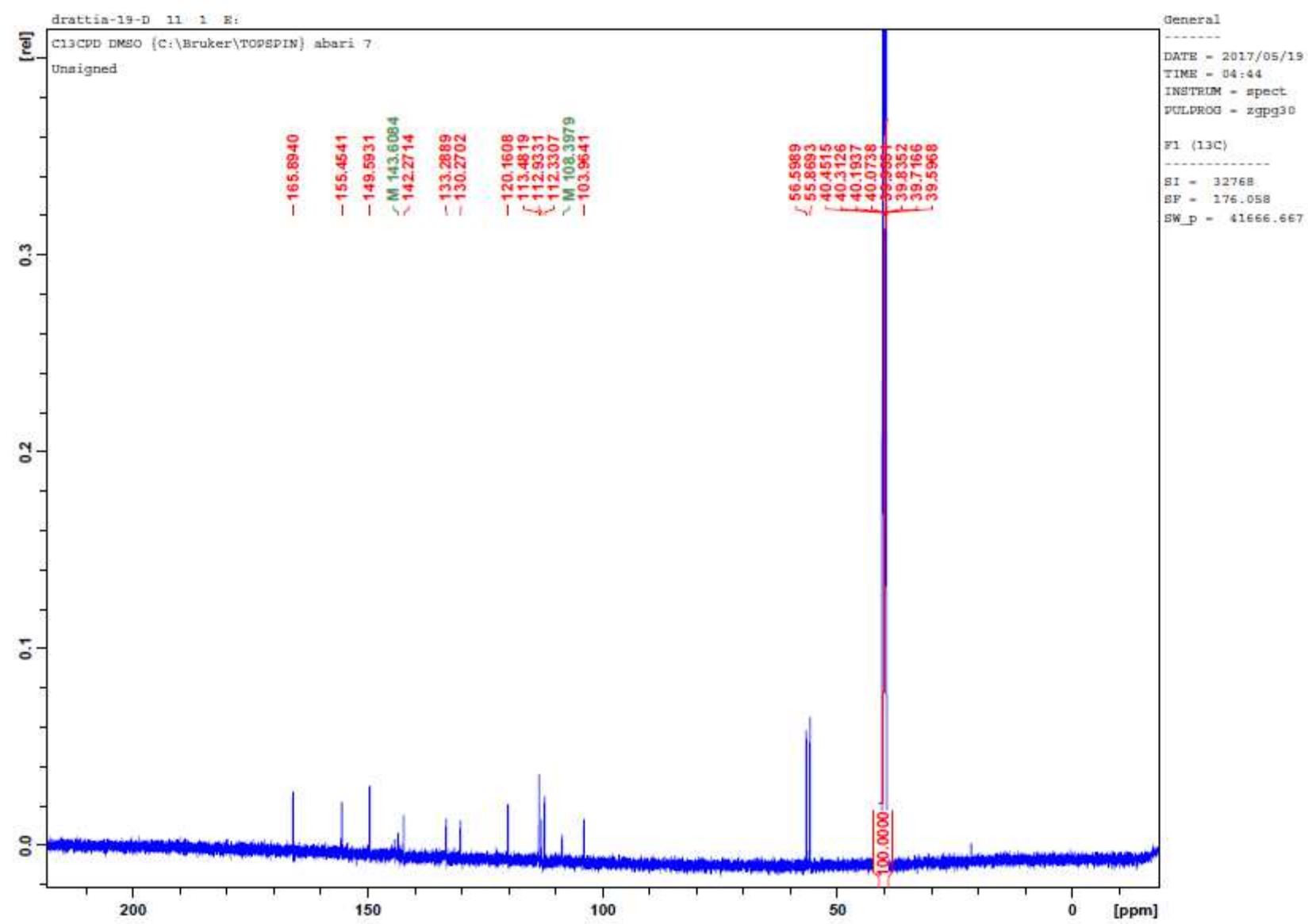
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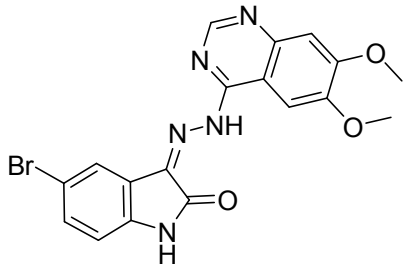
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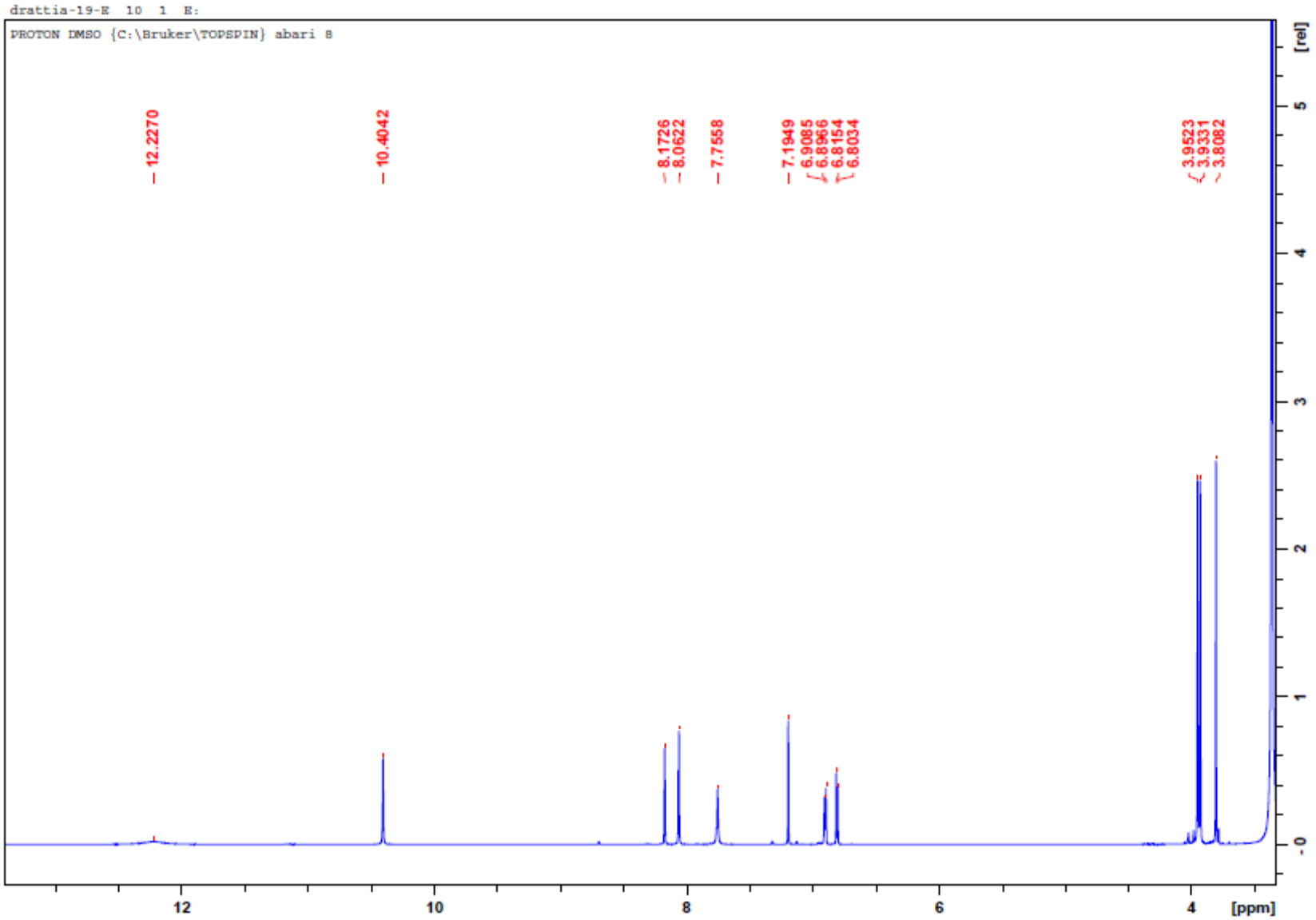
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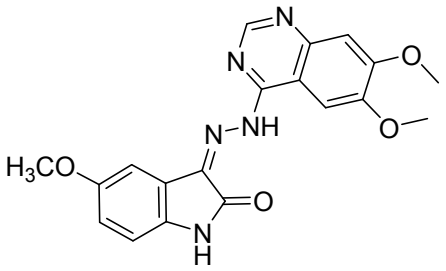
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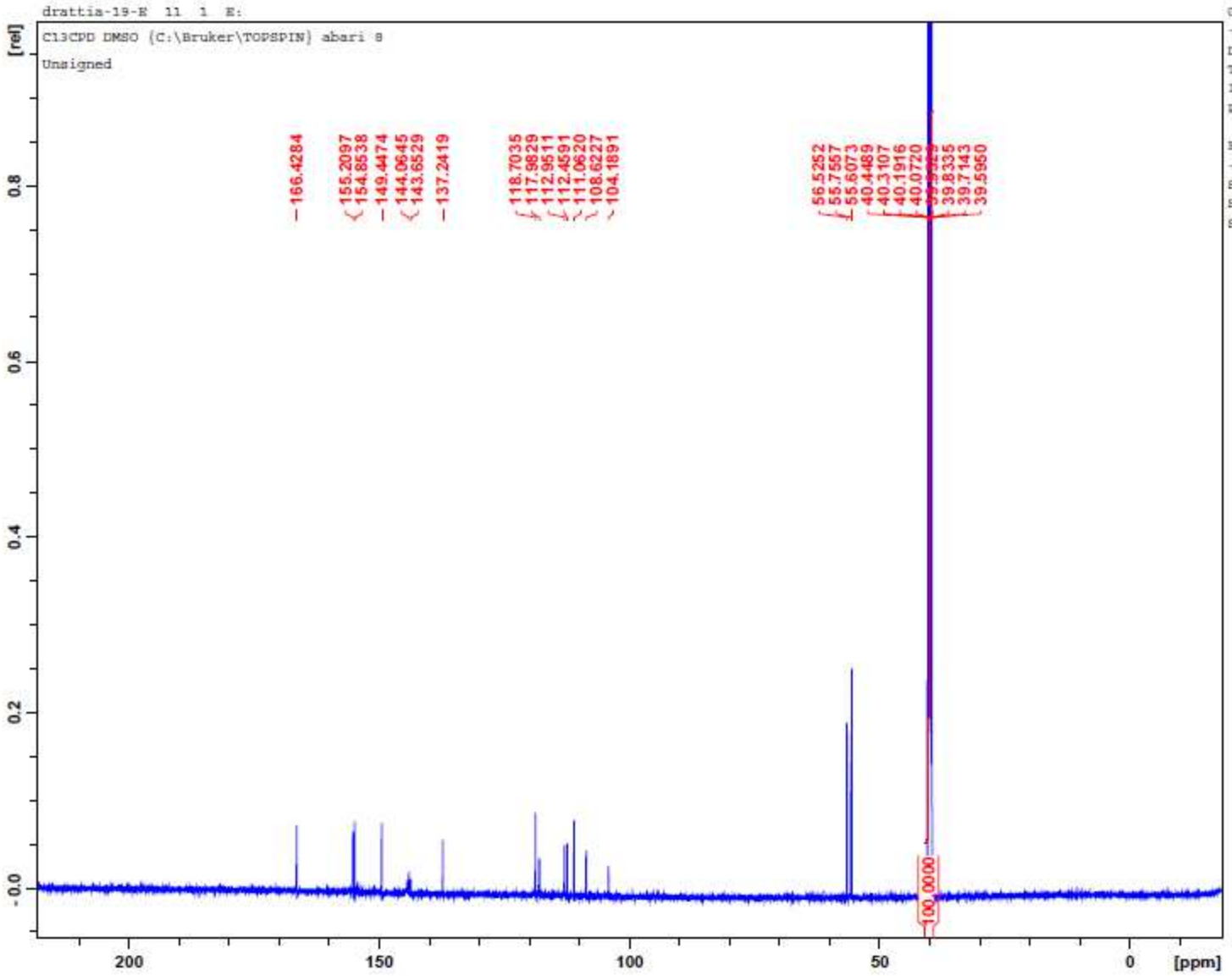


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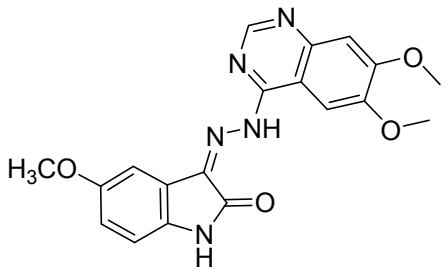

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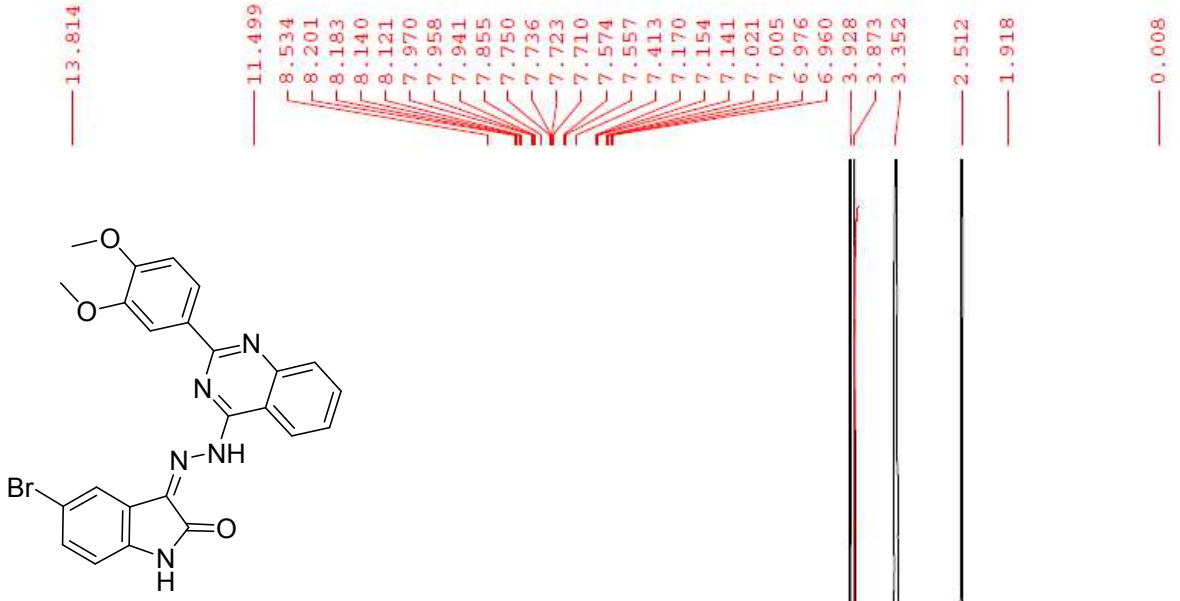

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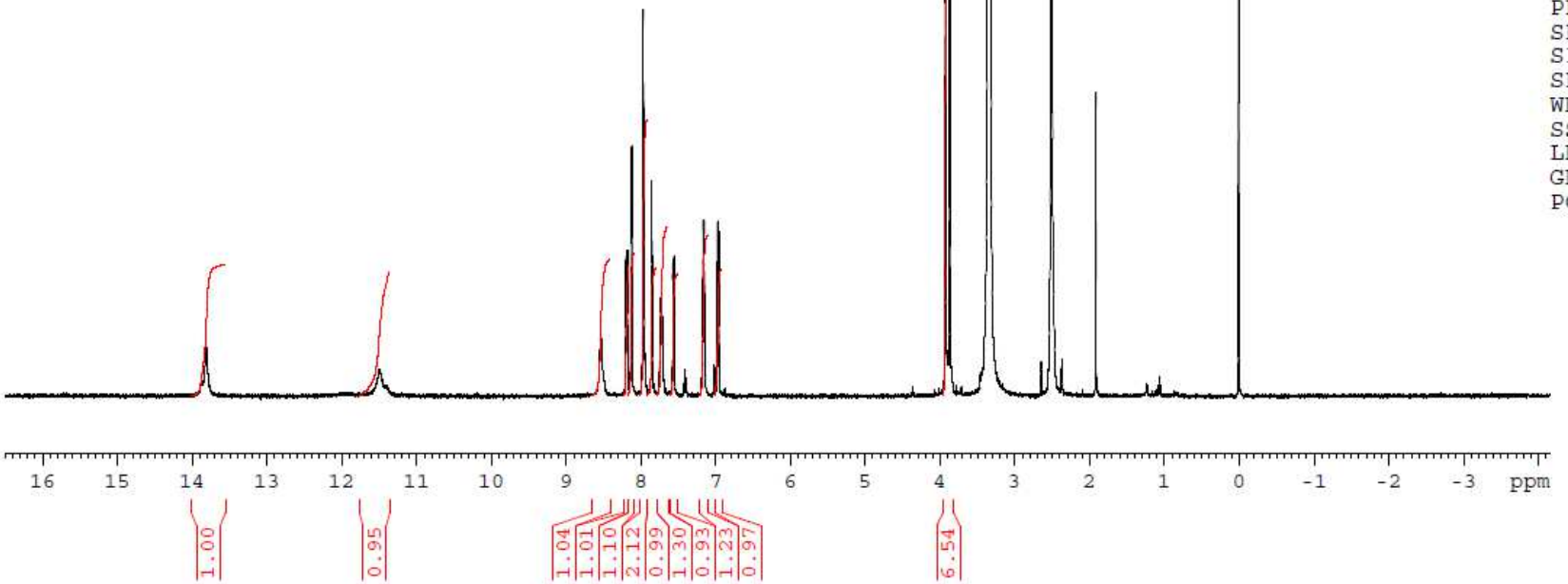

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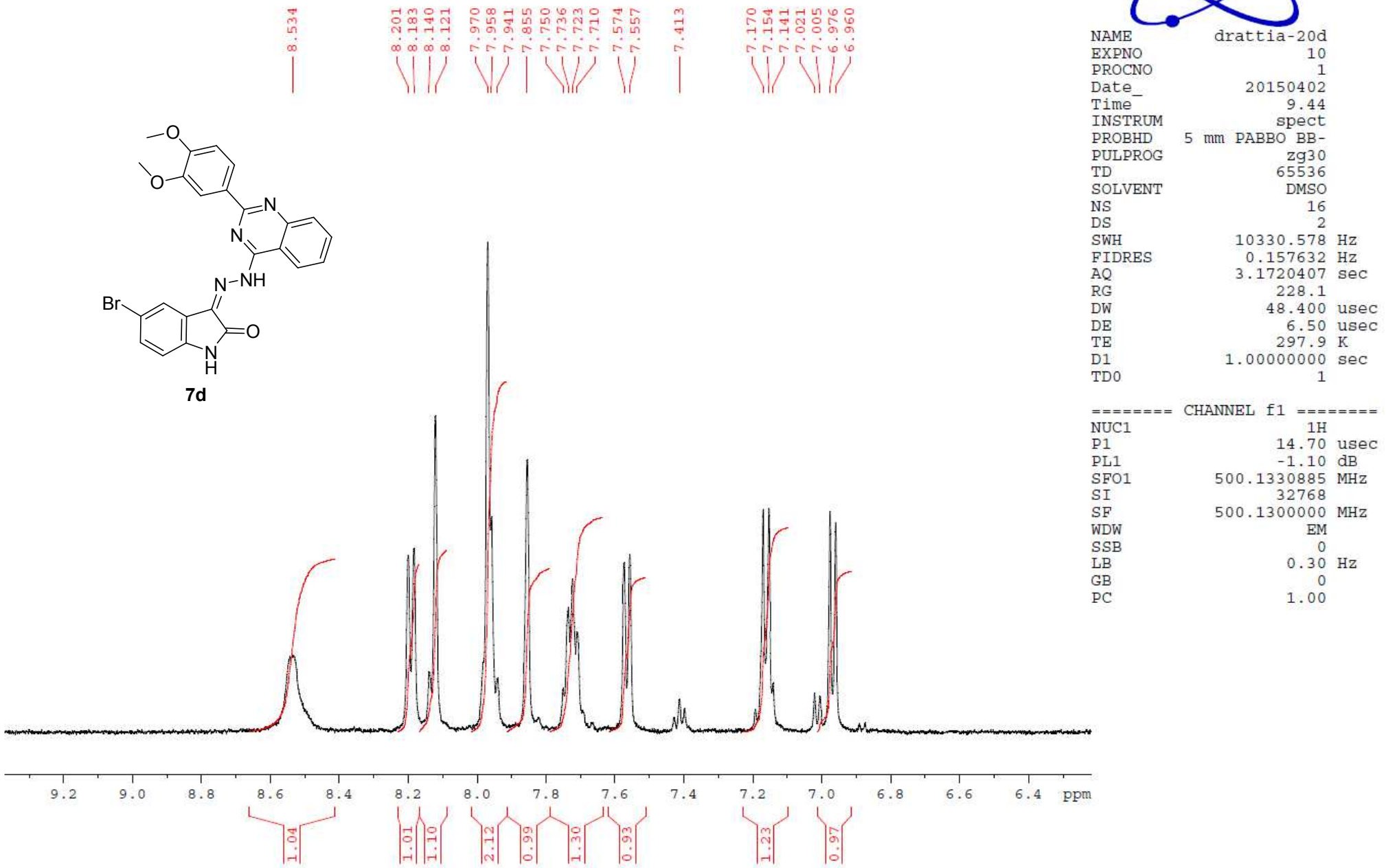
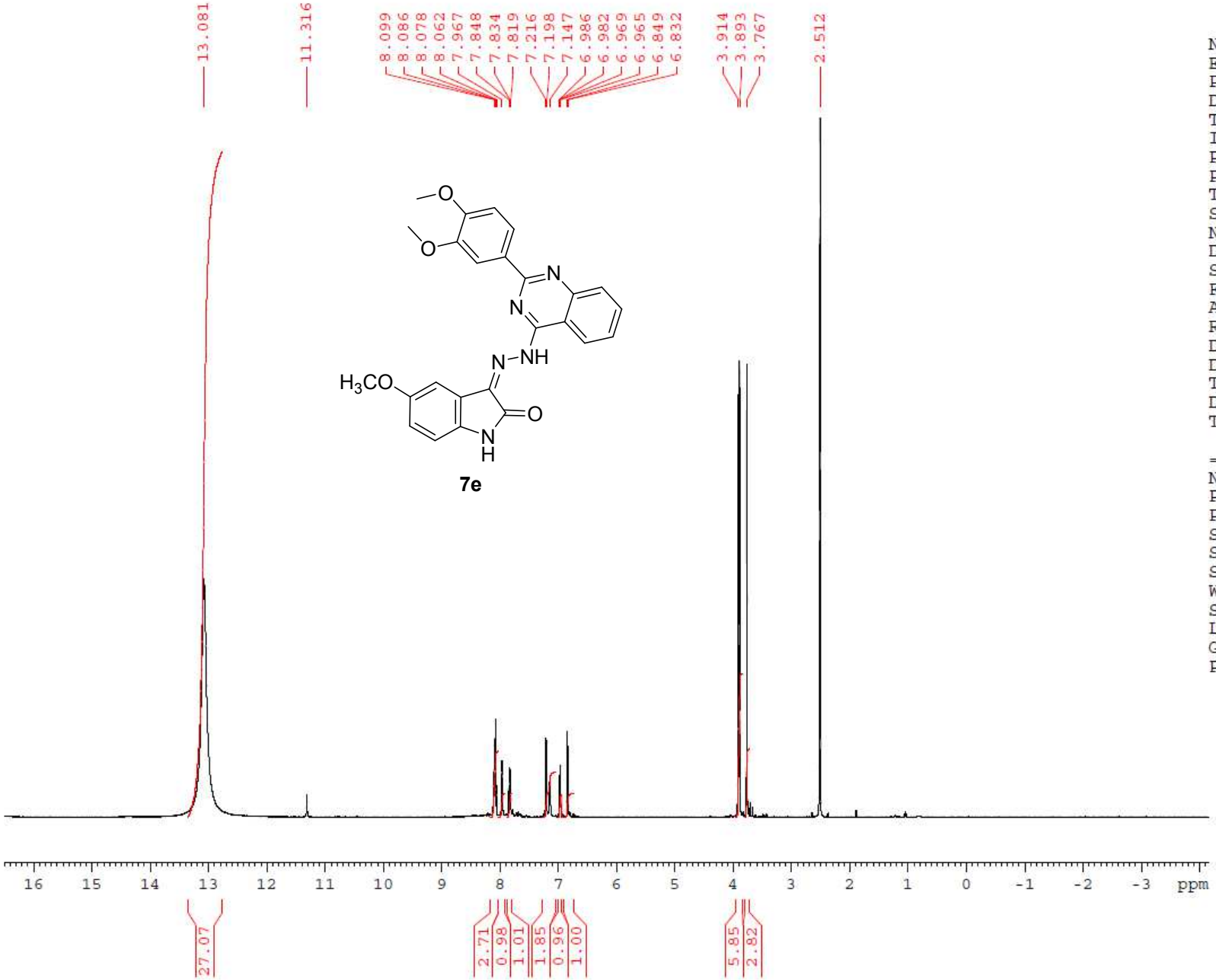
LB	0.30 Hz
GB	0
PC	1.00

)N DMSO D:\\ abar



NAME	drattia-20d
EXPNO	10
PROCNO	1
Date_	20150402
Time	9.44
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	228.1
DW	48.400 usec
DE	6.50 usec
TE	297.9 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.70 usec
PL1	-1.10 dB
SFO1	500.1330885 MHz
SI	32768
SF	500.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

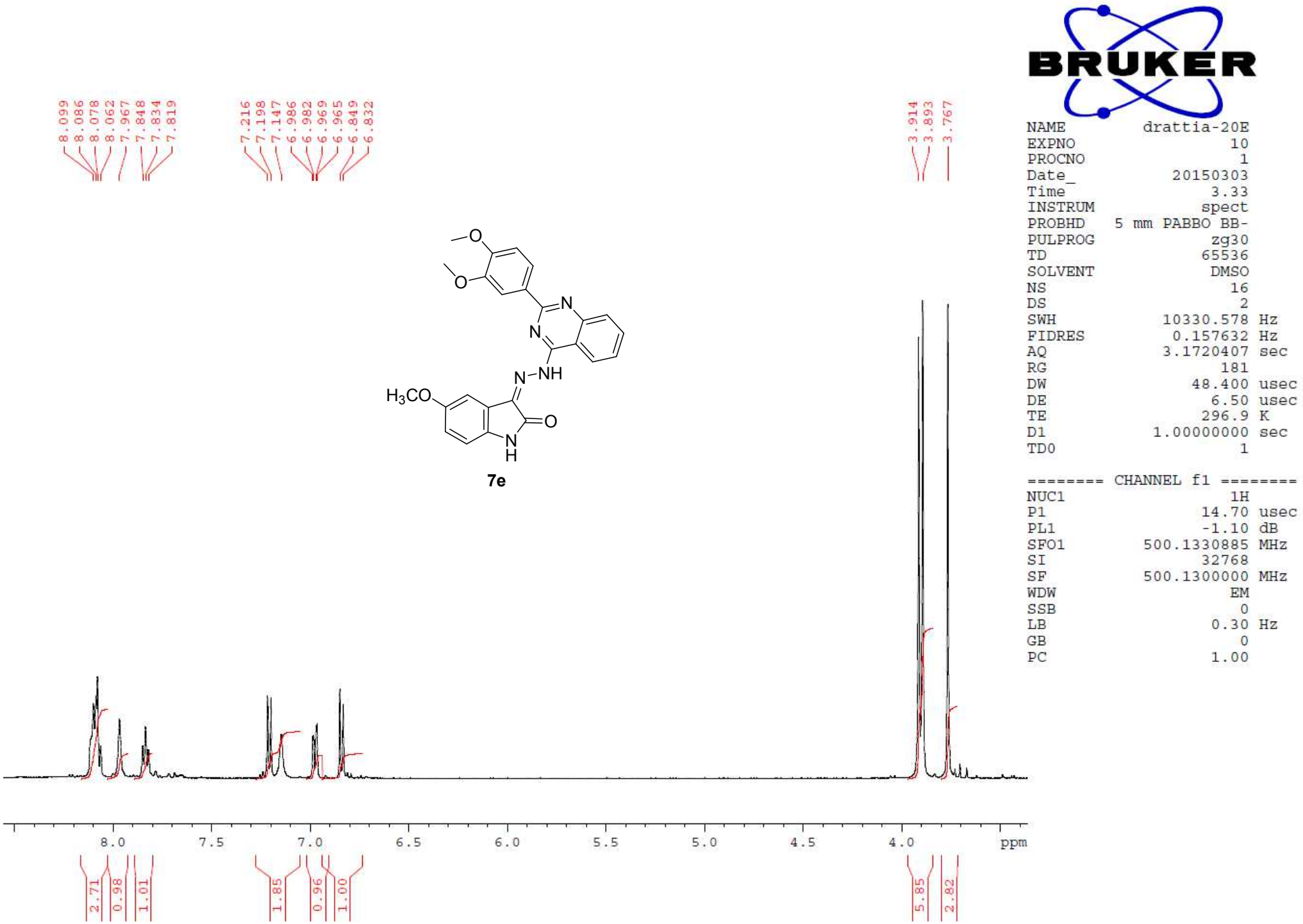
ɔN DMSO D:\\ abar



7e

NAME	drattia-20E
EXPNO	10
PROCNO	1
Date_	20150303
Time	3.33
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	181
DW	48.400 usec
DE	6.50 usec
TE	296.9 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.70 usec
PL1	-1.10 dB
SFO1	500.1330885 MHz
SI	32768
SF	500.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

ɔN DMSO D:\\ abar

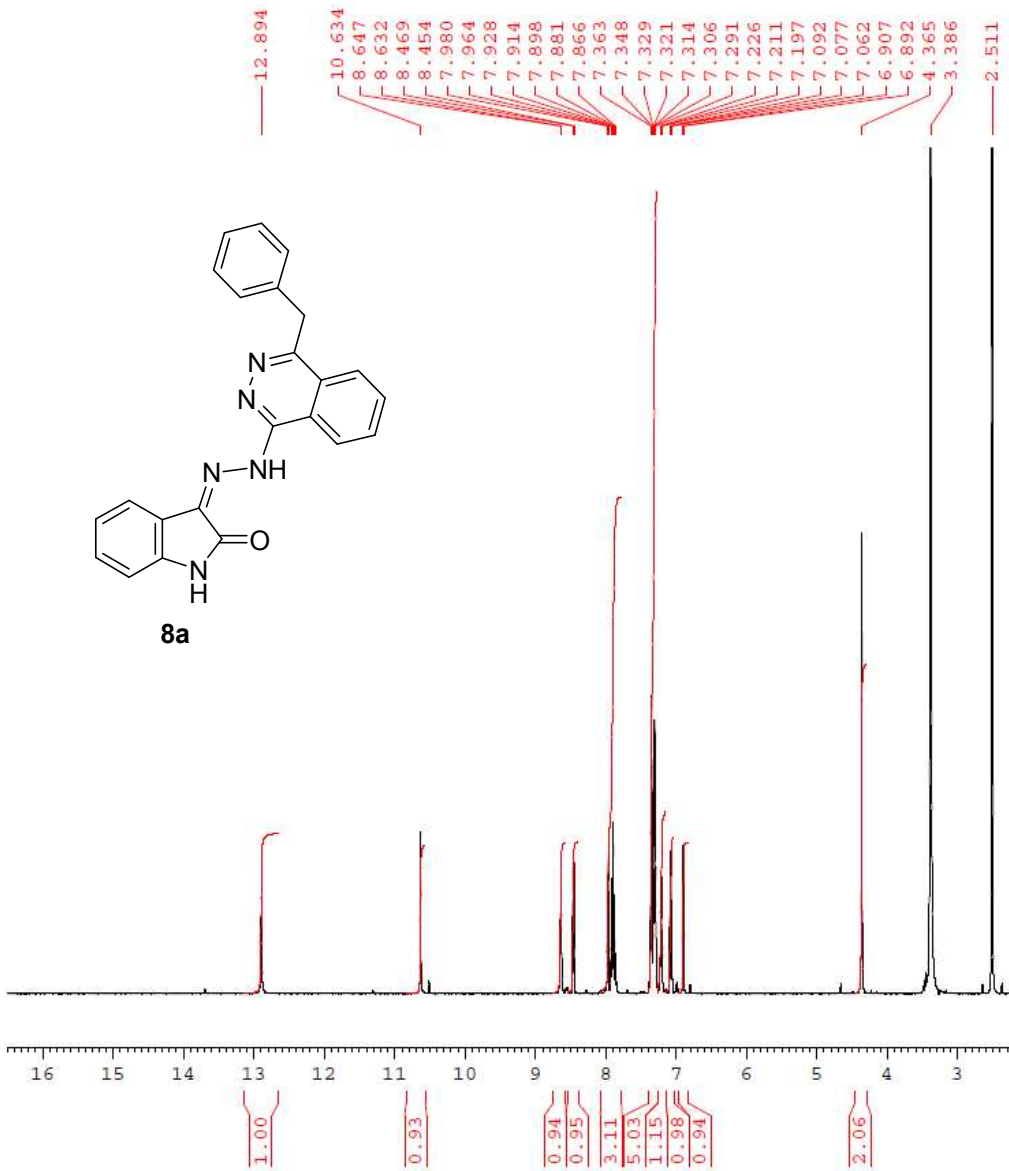


7e

BRUKER

NAME	drattia-20E
EXPNO	10
PROCNO	1
Date_	20150303
Time	3.33
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	181
DW	48.400 usec
DE	6.50 usec
TE	296.9 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.70 usec
PL1	-1.10 dB
SFO1	500.1330885 MHz
SI	32768
SF	500.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

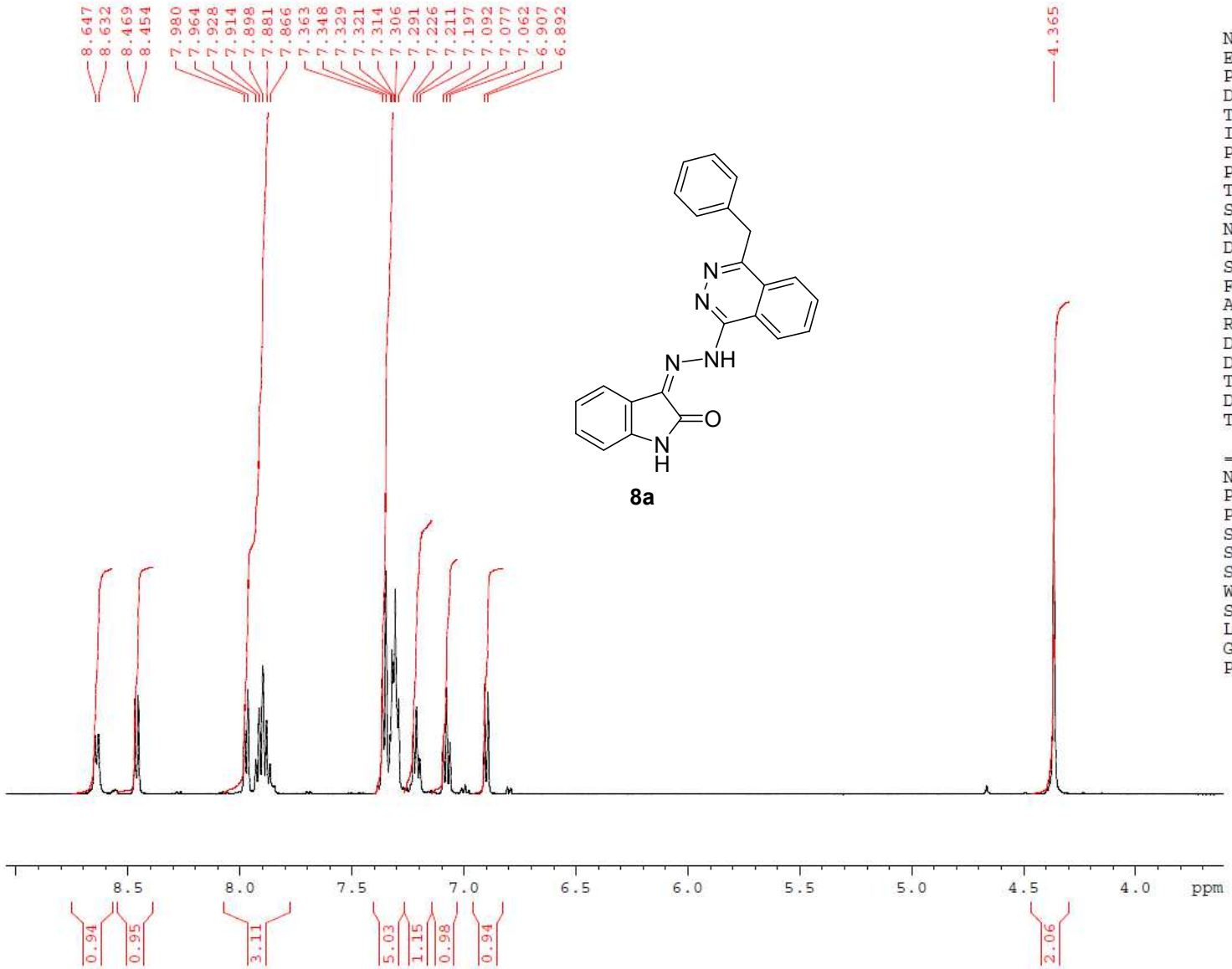
N DMSO D:\\ mmjak



BRUKER

NAME	drattia-21-a
EXPNO	10
PROCNO	1
Date_	20150312
Time	15.22
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	161.3
DW	48.400 usec
DE	6.50 usec
TE	296.8 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.70 usec
PL1	-1.10 dB
SFO1	500.1330885 MHz
SI	32768
SF	500.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

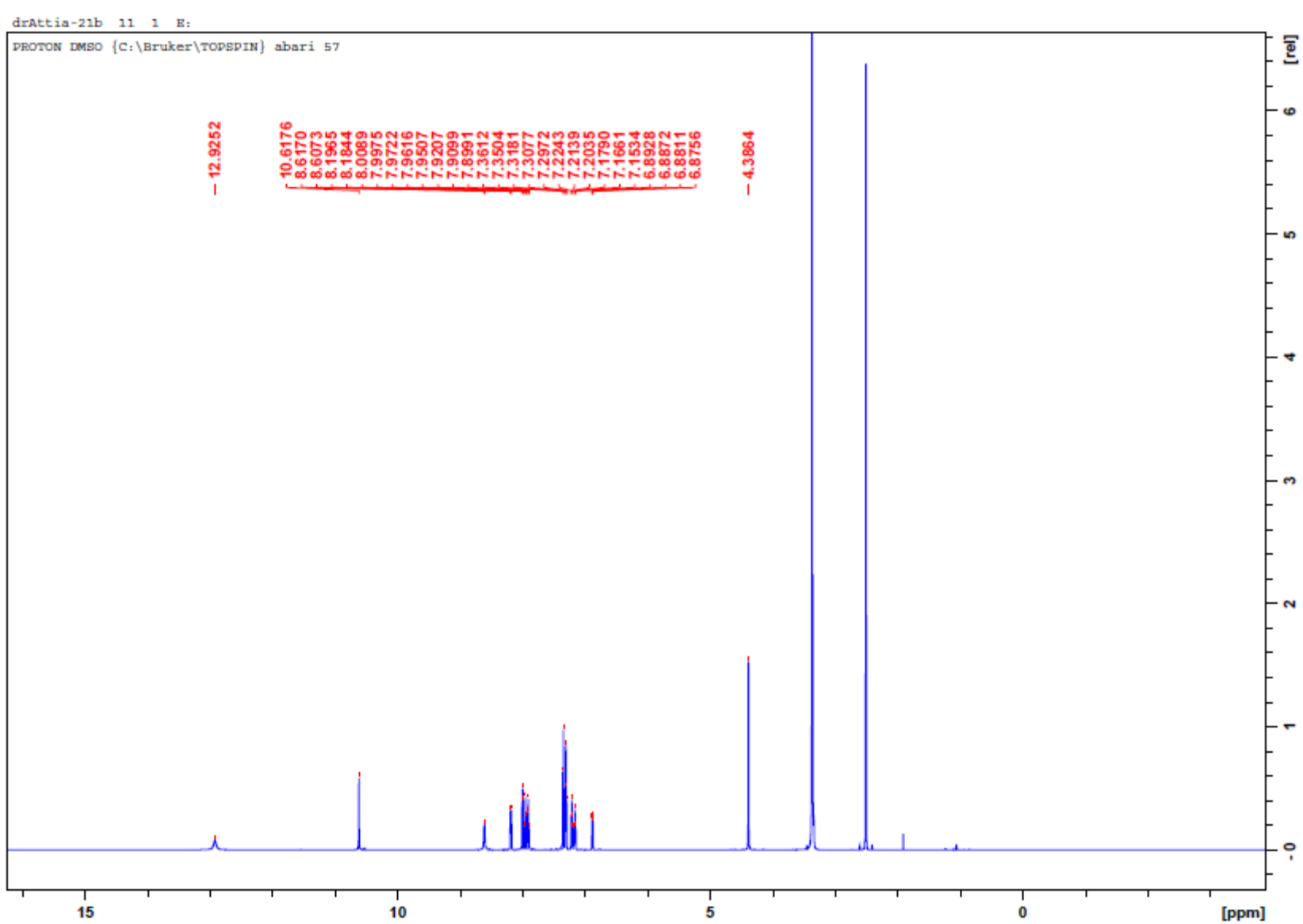
N DMSO D:\\ mmjak

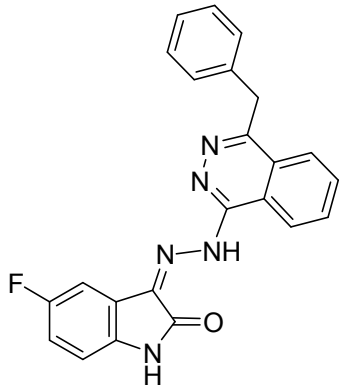


8a

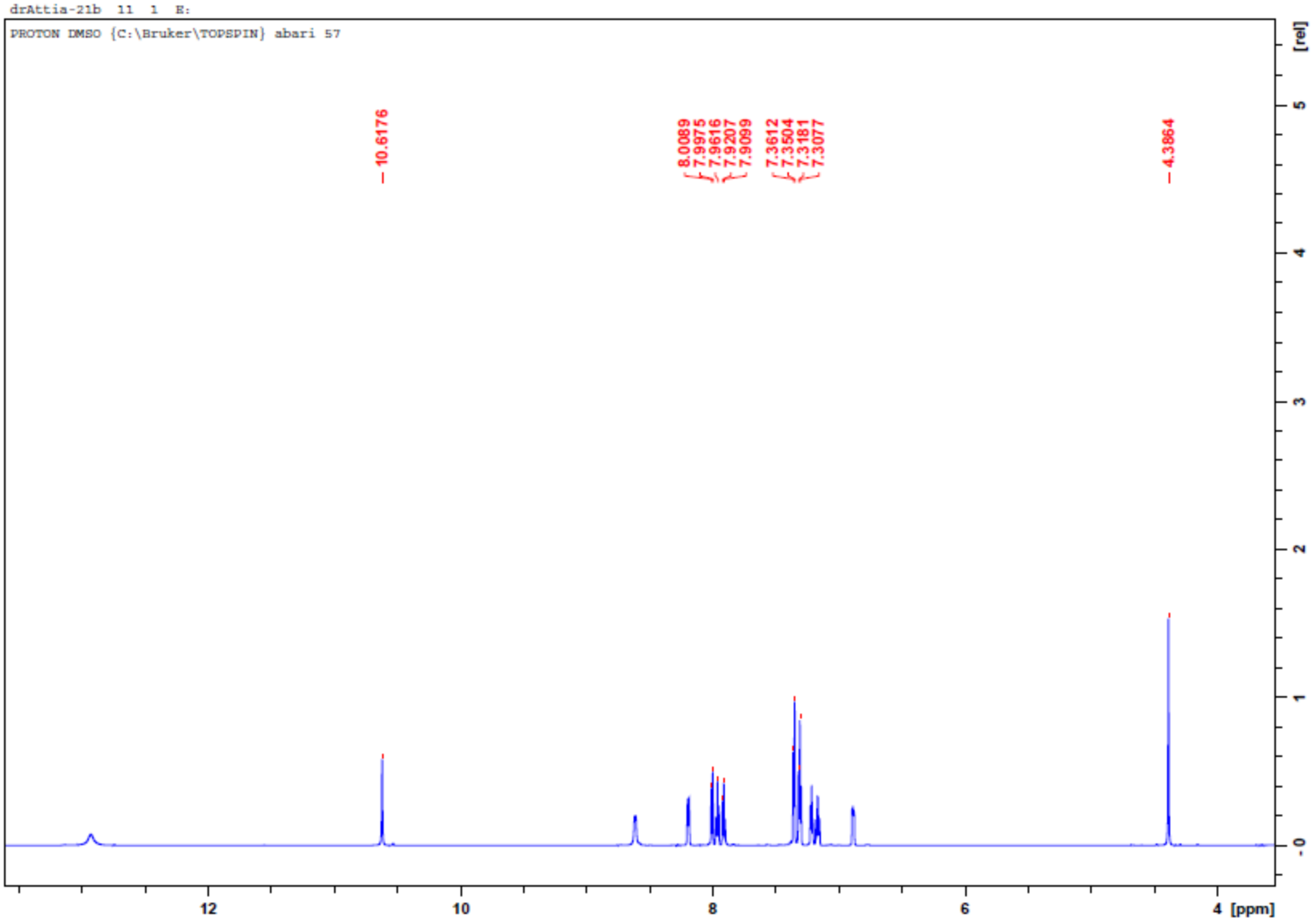
BRUKER

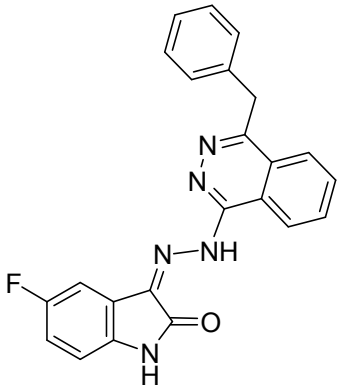
NAME	drattia-21-a
EXPNO	10
PROCNO	1
Date_	20150312
Time	15.22
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	161.3
DW	48.400 usec
DE	6.50 usec
TE	296.8 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.70 usec
PL1	-1.10 dB
SFO1	500.1330885 MHz
SI	32768
SF	500.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00


drAttia-21b 11 1 E:
PROTON DMSO {C:\Bruker\TOPSPIN} abari 57
12.9252
10.6176
8.6170
8.6073
8.1965
8.1844
8.0089
7.9975
7.9722
7.9616
7.9507
7.9207
7.9099
7.8991
7.3612
7.3504
7.3181
7.3077
7.2972
7.2243
7.2139
7.2035
7.1790
7.1661
7.1534
6.8928
6.8872
6.8811
6.8756
4.3864
[rel]
[ppm]

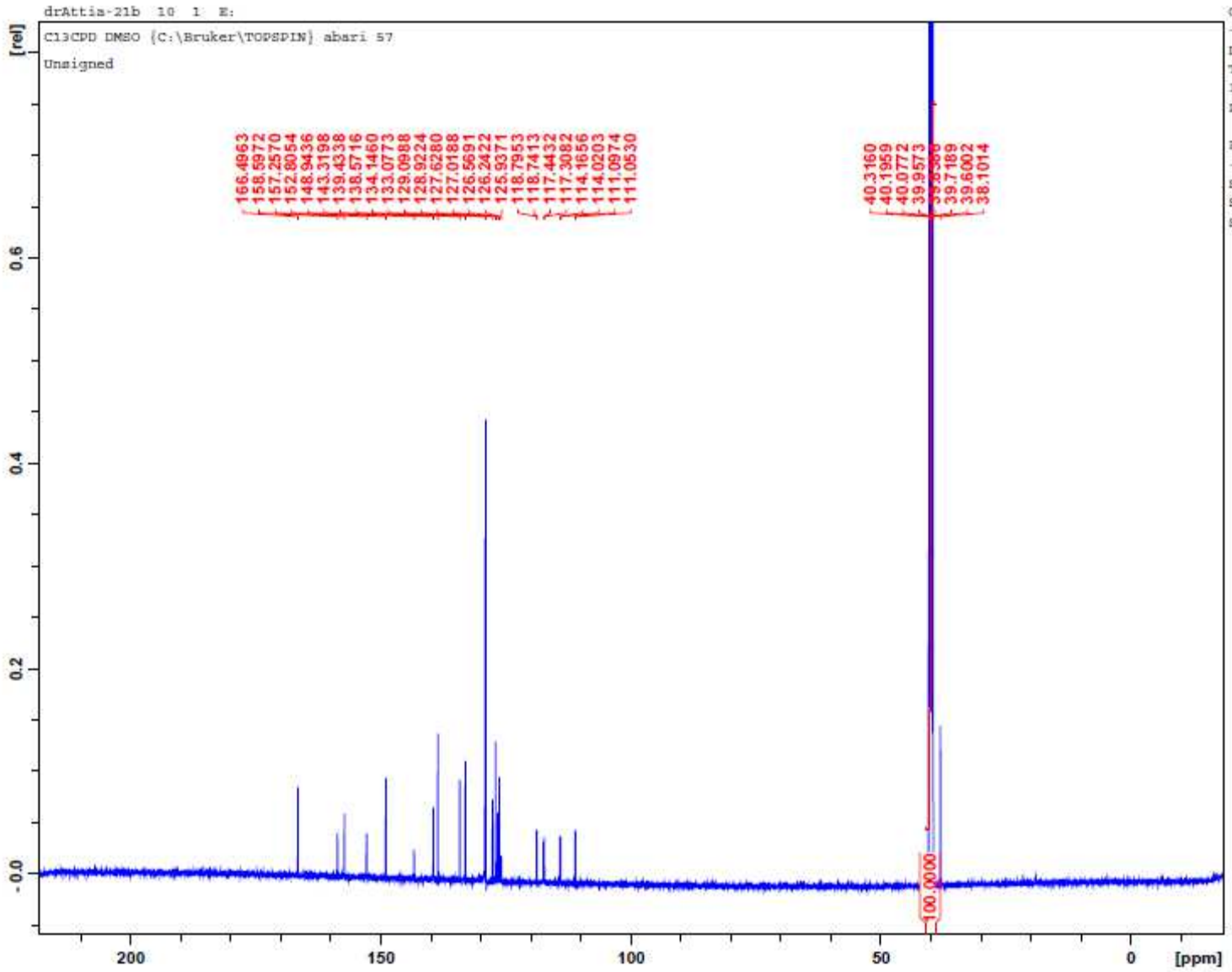

N
N
N
NH
F
O
N
H

8b


drAttia-21b 11 1 E:
PROTON DMSO {C:\Bruker\TOPSPIN} abari 57
10.6176
8.0089
7.9975
7.9616
7.9207
7.9099
7.3612
7.3504
7.3181
7.3077
4.3864
[rel]
5
4
3
2
1
-0
12
10
8
6
4 [ppm]


N
N
N–NH
F
O
N
H

8b


drAttia-21b 10 1 E:
C13CPD DMSO (C:\Bruker\TOPSPIN) abari 57
Unsigned
[rel]
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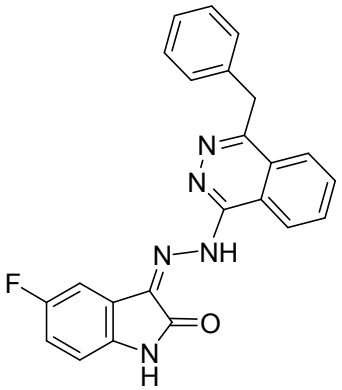
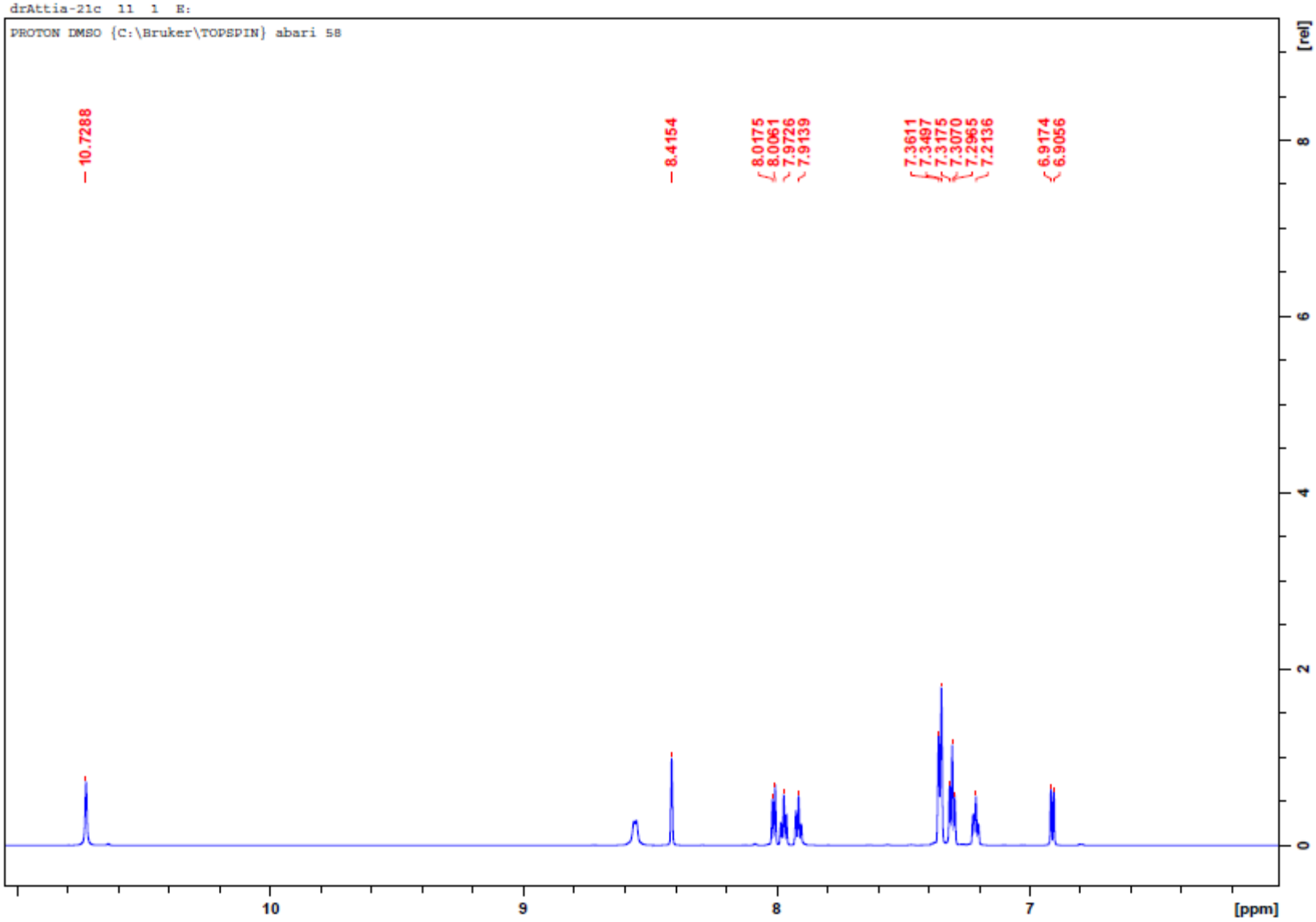
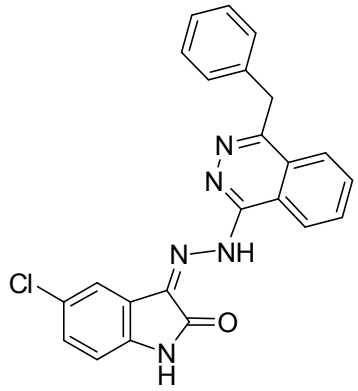
158.5972
157.2570
152.8054
148.9436
143.3198
139.4338
138.5716
134.1460
133.0773
129.0988
128.9224
127.6280
127.0188
126.5691
126.2422
125.9371
118.7953
118.7413
117.4432
117.3082
114.1656
114.0203
111.0974
111.0530
40.3160
40.1959
40.0772
39.9573
39.7189
39.6002
38.1014
100.0000
0.6
0.4
0.2
-0.0
200
150
100
50
0
[ppm]
General
DATE = 2017/05/17
TIME = 22:11
INSTRUM = spect
PULPROG = zgpg30
F1 (13C)
SI = 32768
SF = 176.058
SW_p = 41666.667

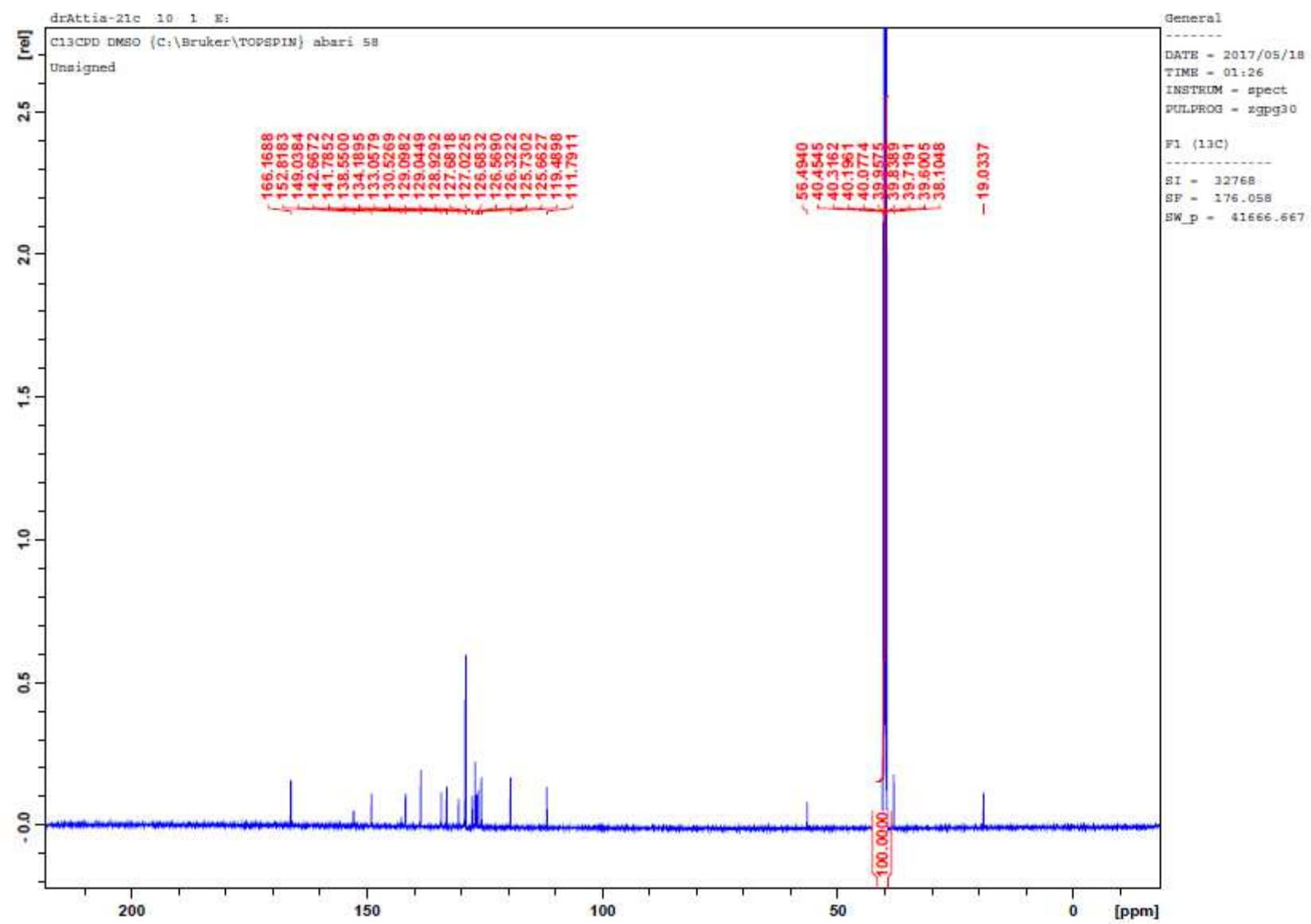

N
N
N
NH
F
O
N
H

8b


drAttia-21c 11 1 E:
PROTON DMSO {C:\Bruker\TOPSPIN} abari 58
10.7288
8.4154
8.0175
8.0061
7.9726
7.9139
7.3611
7.3497
7.3175
7.3070
7.2965
7.2136
6.9174
6.9056
[rel]
[ppm]


N
N
N—NH
Cl
O
N
H

8c


drAttia-21c 10 1 E:
C13CPD DMSO (C:\Bruker\TOPSPIN) abari 58
Unsigned
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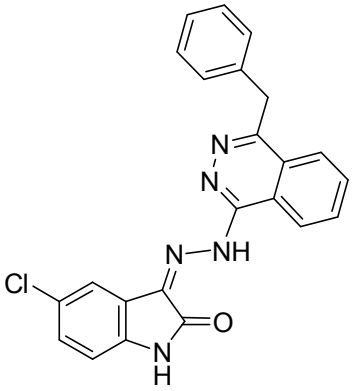
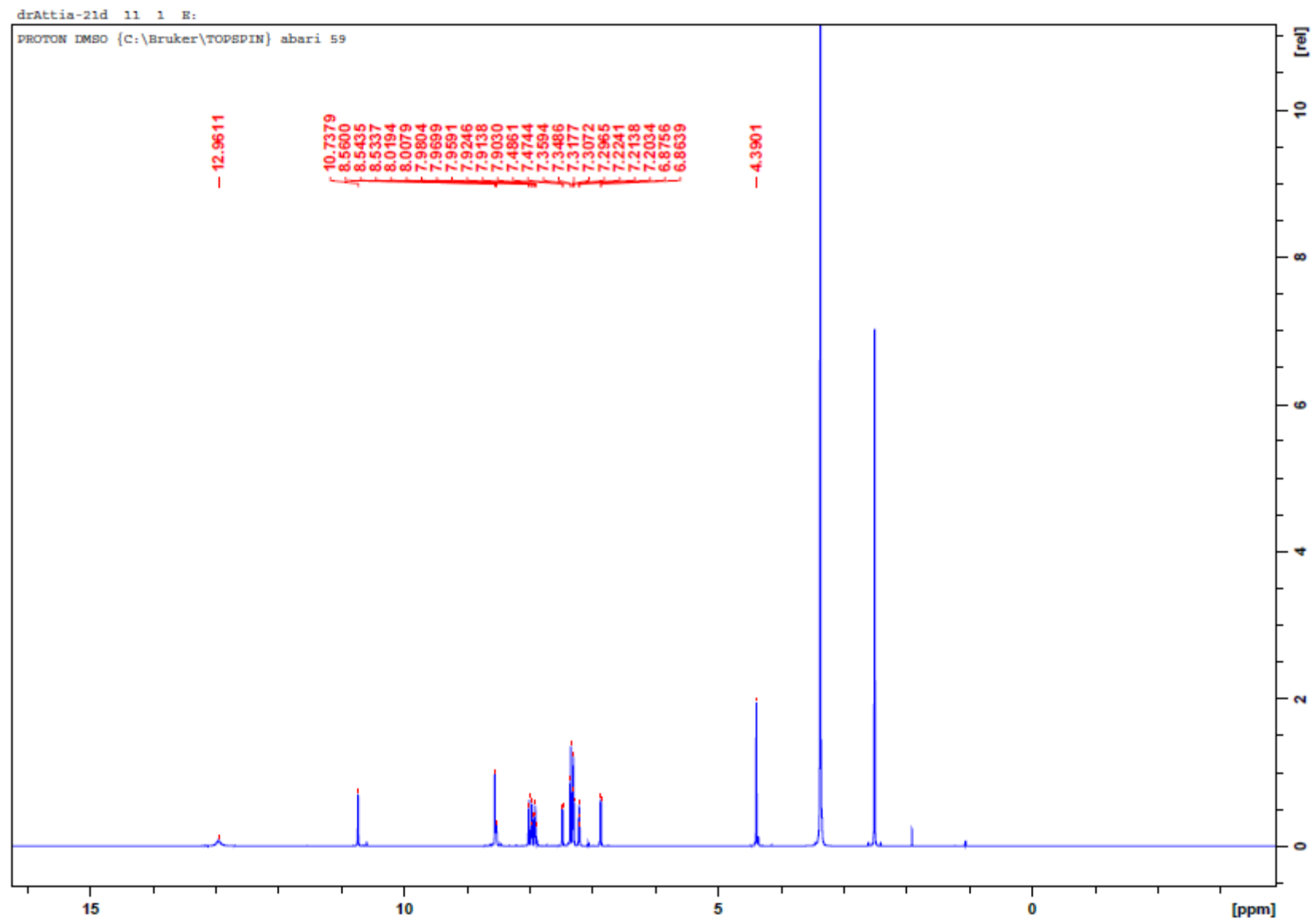
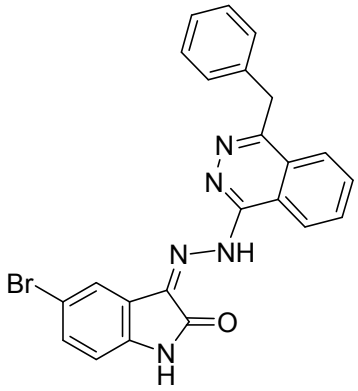
152.8183
149.0384
142.6672
141.7852
138.5500
134.1895
133.0579
130.5269
129.0982
129.0449
128.9292
127.6818
127.0225
126.6832
126.5690
126.3222
125.7302
125.6627
119.4898
111.7911
56.4940
40.4545
40.3162
40.1961
40.0774
39.9575
39.8389
39.7191
39.6005
38.1048
19.0337
100.0000
[rel]
2.5
2.0
1.5
1.0
0.5
-0.0
200
150
100
50
0
[ppm]
General
DATE = 2017/05/18
TIME = 01:26
INSTRUM = spect
PULPROG = zgpg30
F1 (13C)
SI = 32768
SF = 176.058
SW_p = 41666.667

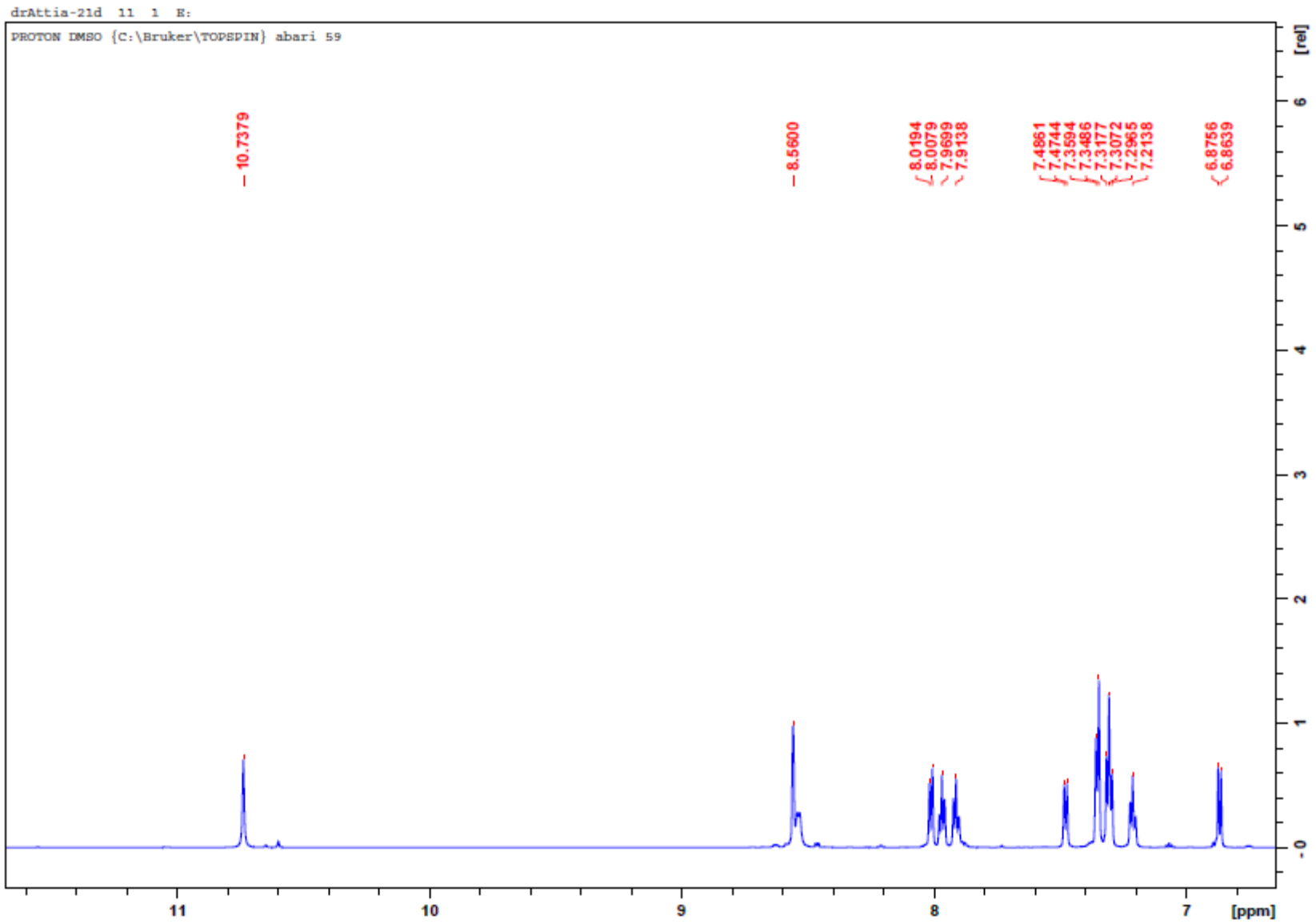

N
N
N
NH
Cl
O
N
H

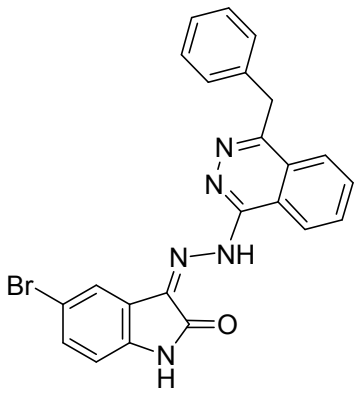
8c


drAttia-21d 11 1 E:
PROTON DMSO {C:\Bruker\TOPSPIN} abari 59
12.9611
10.7379
8.5600
8.5435
8.5337
8.0194
8.0079
7.9804
7.9699
7.9591
7.9246
7.9138
7.9030
7.4861
7.4744
7.3594
7.3486
7.3177
7.3072
7.2965
7.2241
7.2138
7.2034
6.8756
6.8639
4.3901
[rel]
10
8
6
4
2
0
15
10
5
0
[ppm]

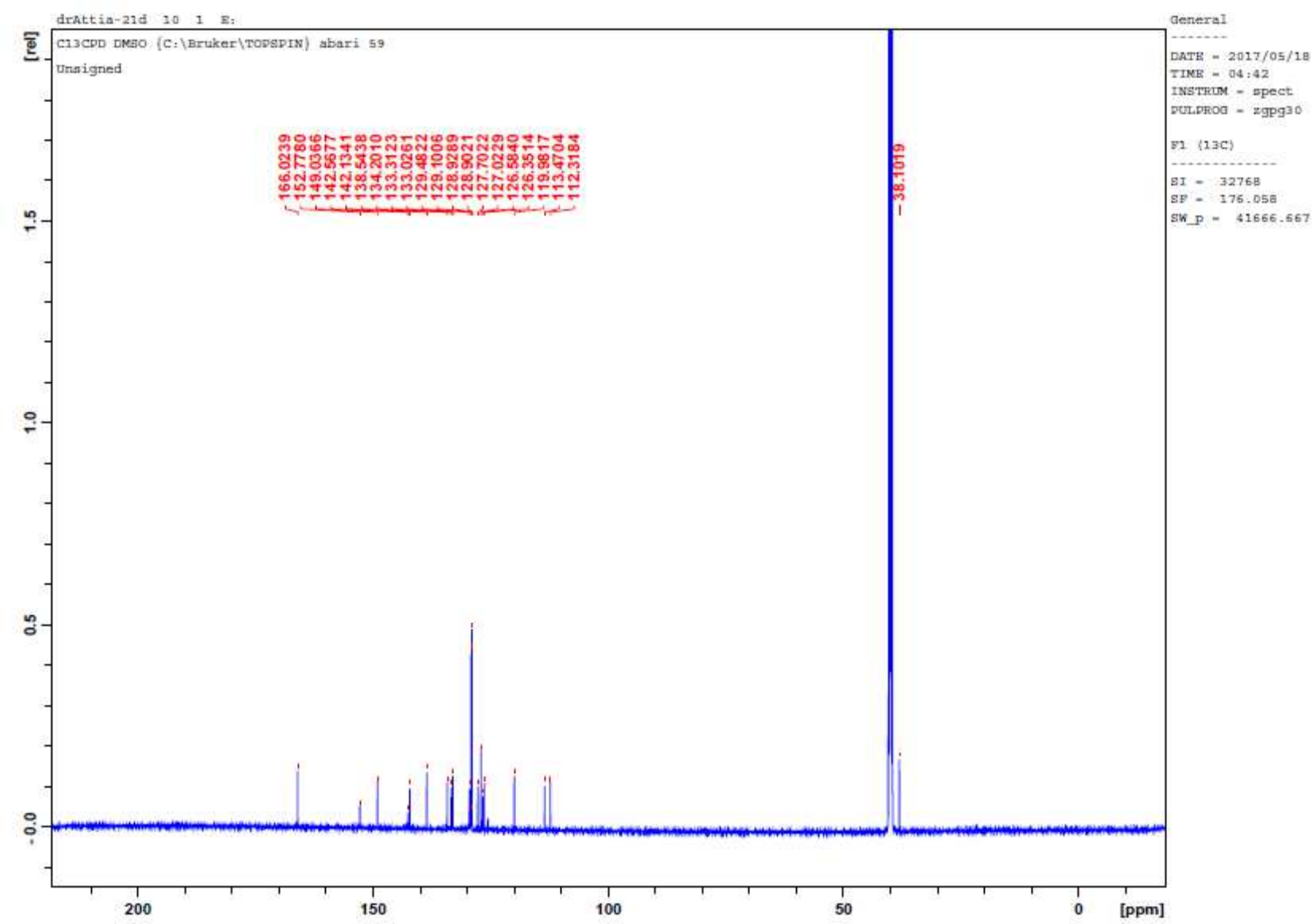

N
N
N
NH
Br
O
N
H

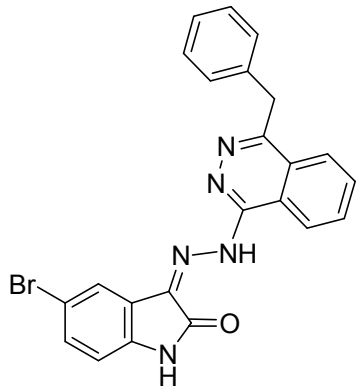
8d


drAttia-21d 11 1 E:
PROTON DMSO {C:\Bruker\TOPSPIN} abari 59
10.7379
8.5600
8.0194
8.0079
7.9699
7.9138
7.4861
7.4744
7.3594
7.3486
7.3177
7.3072
7.2965
7.2138
6.8756
6.8639
[rel]
[ppm]


N
N
N
NH
Br
O
N
H

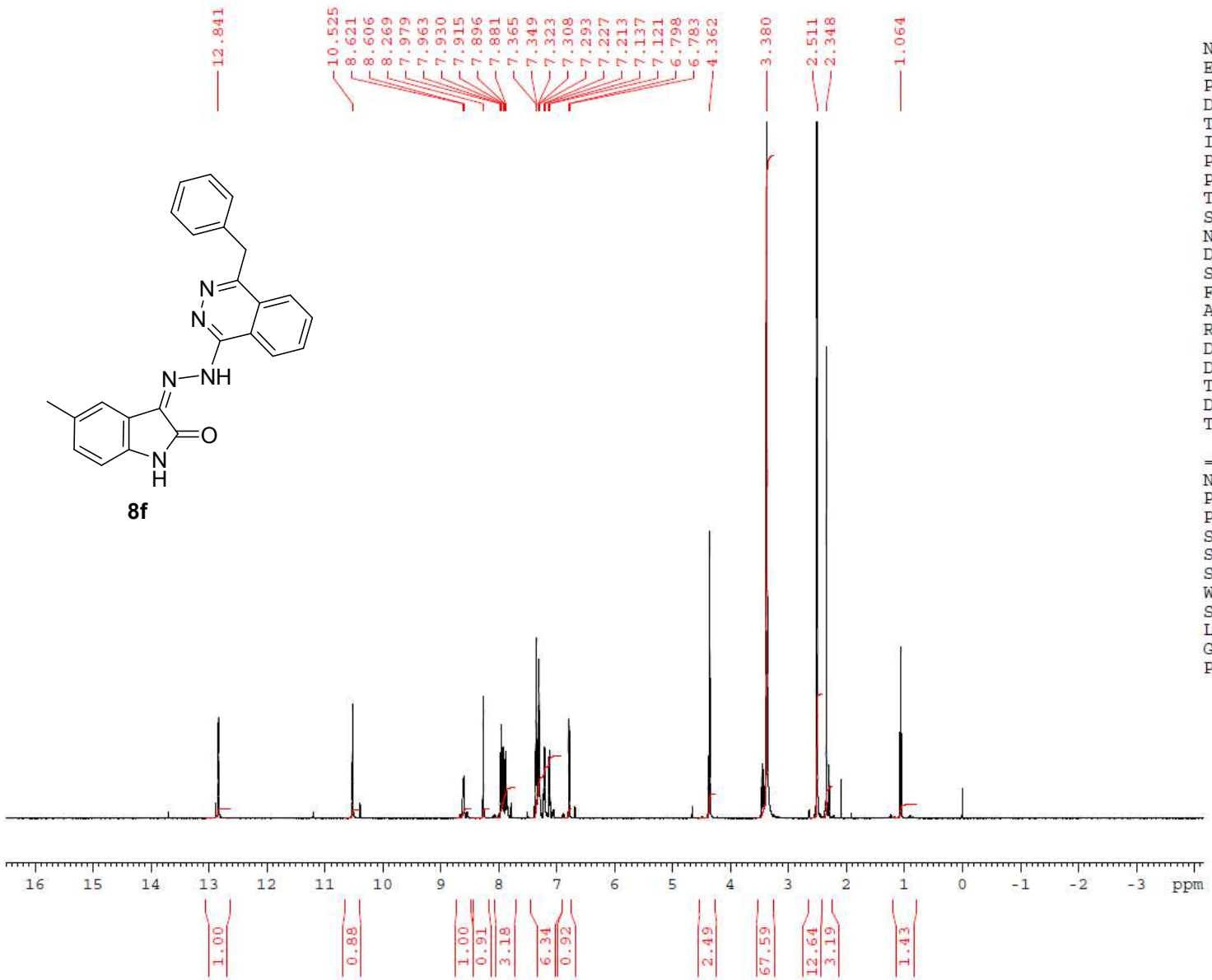
8d


drAttia-21d 10 1 E:
C13CPD DMSO (C:\Bruker\TOPSPIN) abari 59
Unsigned
166.0239
152.7780
149.0366
142.5677
142.1341
138.5438
134.2010
133.3123
133.0261
129.4822
129.1006
128.9289
128.9021
127.7022
127.0229
126.5840
126.3514
119.9817
113.4704
112.3184
38.1019
[rel]
1.5
1.0
0.5
-0.0
200
150
100
50
0
[ppm]
General
DATE = 2017/05/18
TIME = 04:42
INSTRUM = spect
PULPROG = zgpg30
F1 (13C)
SI = 32768
SF = 176.058
SW_p = 41666.667


N
N
N
NH
Br
O
N
H

8d

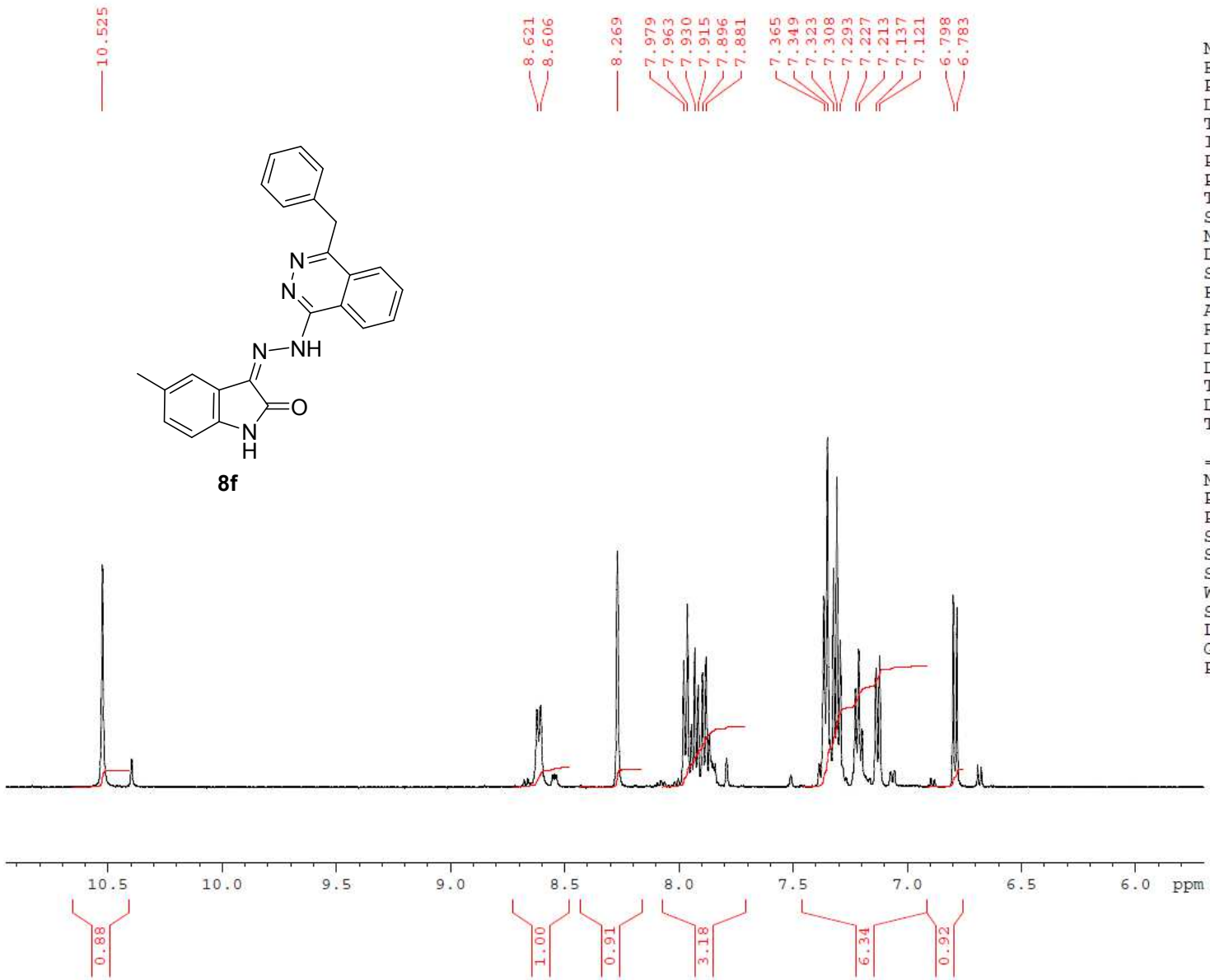
N DMSO D:\\ mmjak



BRUKER

NAME	drattia-21-f
EXPNO	10
PROCNO	1
Date_	20150313
Time	4.14
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	181
DW	48.400 usec
DE	6.50 usec
TE	296.3 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.70 usec
PL1	-1.10 dB
SFO1	500.1330885 MHz
SI	32768
SF	500.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

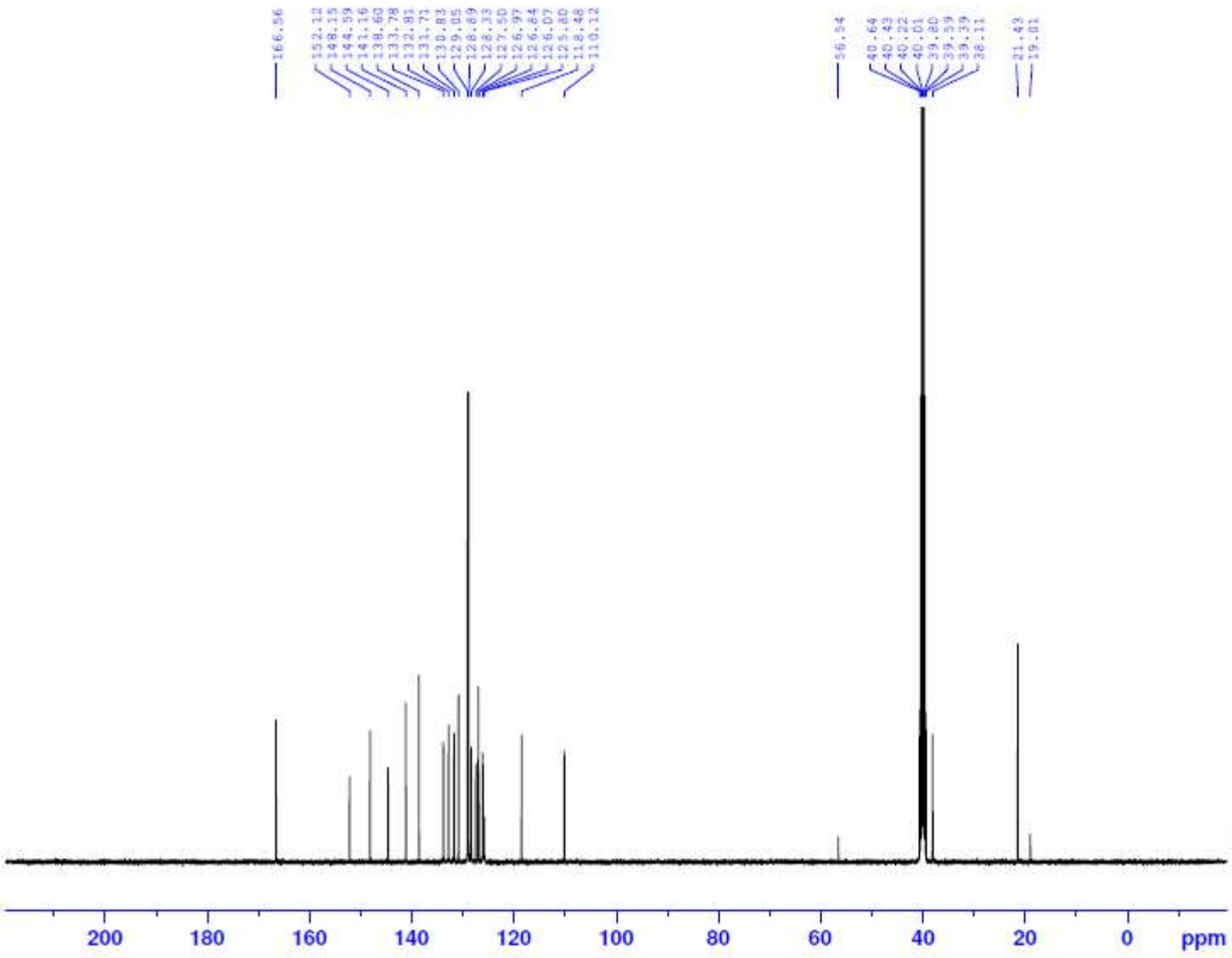
N DMSO D:\\ mmjak



8f

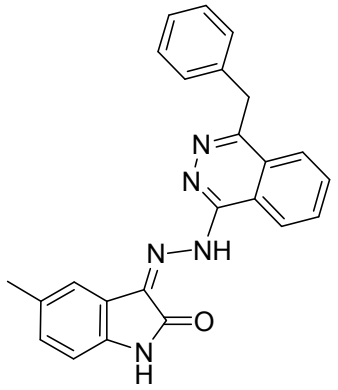
NAME	drattia-21-f
EXPNO	10
PROCNO	1
Date_	20150313
Time	4.14
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	181
DW	48.400 usec
DE	6.50 usec
TE	296.3 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.70 usec
PL1	-1.10 dB
SFO1	500.1330885 MHz
SI	32768
SF	500.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

WAGDY(21F)-13C-DMSO-2452017



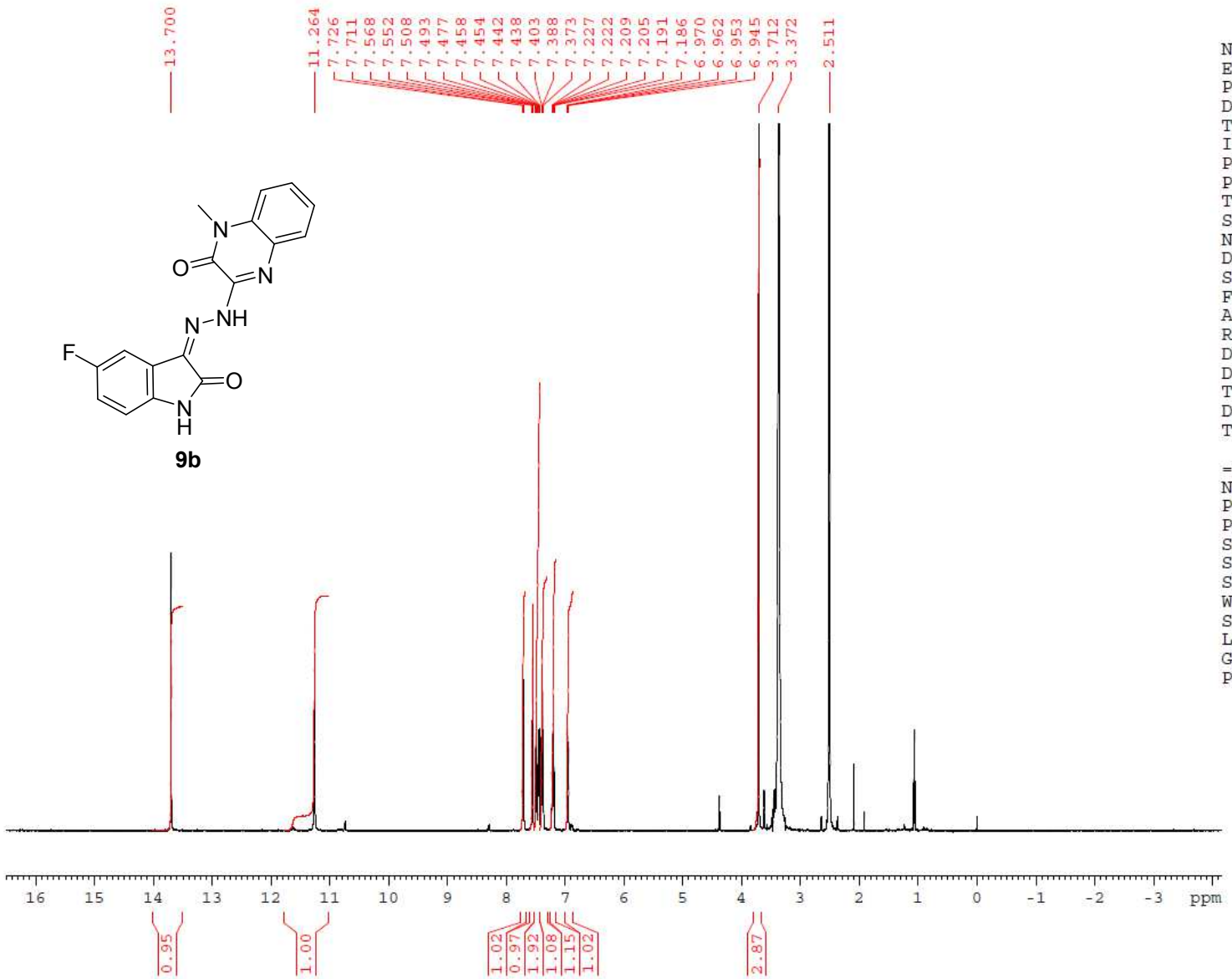
BRUKER

```
Current Data Parameters
NAME      May24-2017-nmr
EXPNO                  70
PROCNO                  1

F2 - Acquisition Parameters
Date_           20170524
Time               13.30
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                  1024
DS                     4
SWH            24038.461 Hz
FIDRES          0.366798 Hz
AQ              1.3631488 sec
RG                194.81
DW                20.800 usec
DE                 6.50 usec
TE                300.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         100.6328883 MHz
NUC1                  13C
P1                  10.00 usec
PLW1         66.00000000 W

======== CHANNEL f2 ========
SFO2         400.1716007 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2               90.00 usec
PLW2         16.50000000 W
PLW12         0.20370001 W
PLW13         0.16500001 W

F2 - Processing parameters
SI                   32768
SF           100.6228270 MHz
WDW                     EM
SSB       0
LB                   1.00 Hz
GB        0
PC                   1.40
```



8f

'ON DMSO D:\\ aba

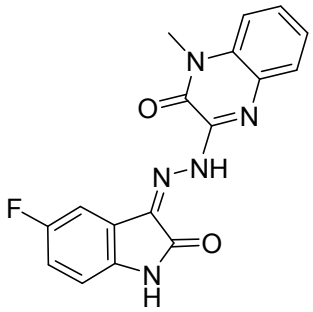


9b

BRUKER

NAME	drattia-22b
EXPNO	10
PROCNO	1
Date_	20150316
Time	3.09
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	181
DW	48.400 usec
DE	6.50 usec
TE	296.8 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.70 usec
PL1	-1.10 dB
SFO1	500.1330885 MHz
SI	32768
SF	500.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

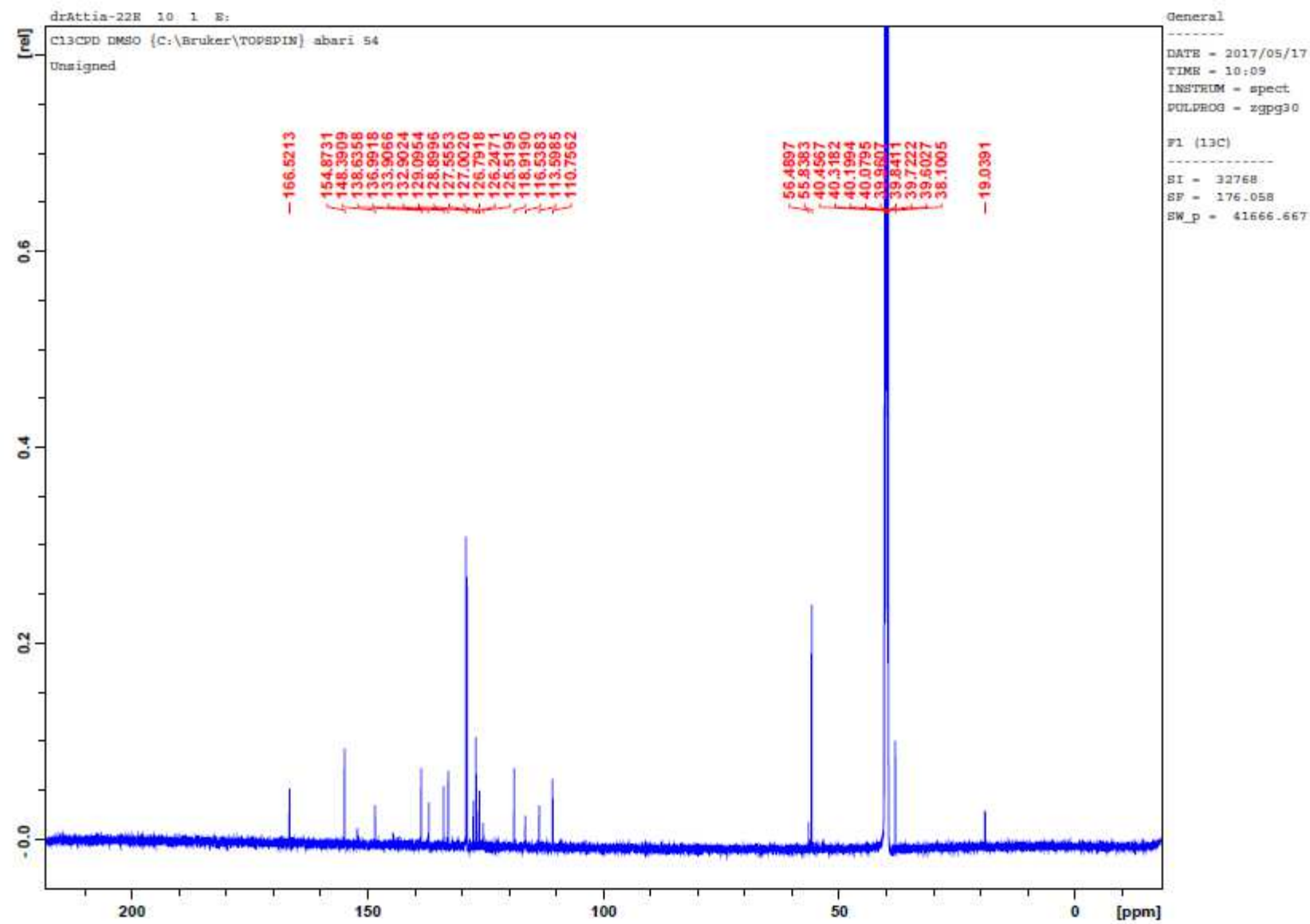
'ON DMSO D:\\ aba

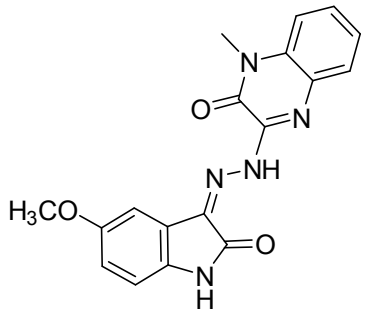


9b

BRUKER

NAME	drattia-22b
EXPNO	10
PROCNO	1
Date_	20150316
Time	3.09
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	181
DW	48.400 usec
DE	6.50 usec
TE	296.8 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.70 usec
PL1	-1.10 dB
SFO1	500.1330885 MHz
SI	32768
SF	500.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00


drAttia-22E 10 1 E:
C13CPD DMSO {C:\Bruker\TOPSPIN} abari 54
Unsigned
General
DATE = 2017/05/17
TIME = 10:09
INSTRUM = spect
PULPROG = zgpg30
F1 (13C)
SI = 32768
SF = 176.058
SW_p = 41666.667
[rel]
166.5213
154.8731
148.3909
138.6358
136.9918
133.9066
132.9024
129.0954
128.8996
127.5553
127.0020
126.7918
126.2471
125.5195
118.9190
116.5383
113.5985
110.7562
56.4897
55.8383
40.4567
40.3182
40.1994
40.0795
39.9607
39.8411
39.7222
39.6027
38.1005
19.0391
0.6
0.4
0.2
-0.0
200
150
100
50
0
[ppm]


N
O
N
N-NH
H3CO
O
N
H

9e