# Supplementary data

## Design, synthesis, biological evaluation, and docking studies of novel triazolo[4,3-*b*]pyridazine derivatives as dual cMet/Pim-1 potential inhibitors with antitumor activity

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# Supplementary data

# (Biological evaluation- Molecular modelling- Chemistry)

## I- Biological evaluation

### Antiproliferative Activity

Assay Human carcinoma cell lines were obtained from the International Center for Training and Advanced Research (ICTAR), (Cairo, Egypt), purchased from the American Type Culture Collection (VA, USA). Eagle's Minimum Essential Medium was used to culture MCF-7 cells, fetal bovine serum was added to the medium at a final concentration of 10%. Culture plates and flasks were treated with penicillin (100 U/mL) and streptomycin (100 mg/mL) (SPL Life Sciences, Korea). Cells were kept at 37°C in a humidified atmosphere of 5% $CO_2$ (Thermo Electron Corporation, Forma series II, 3141, USA). An inverted microscope was used to examine the confluence of cells and proceeded with the MTT assay (Zeiss, Axiovert 40- CFL, Gottingen, Germany).

### MTT cytotoxicity assay and selectivity index (SI) calculation

Cell viability, which express the cytotoxic property of the compounds **4a** and **4g**, which was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay as mentioned previously by Somarathna et al. Cells (density 1.2–1.8, 10,000 cells/well) were placed in a 96-well plate for 24 h before the MTT assay in a volume of 100 µL complete growth medium + 100 ul of the tested compound per well. 20 mL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 3 h at 37°C. After removing the medium, 100 mL of DMSO was added to each well for dissolving the purple formazan product (Sigma-Aldrich, St. Louis, MO, USA). A 96-well plate reader was used to measure the absorbance at 570 nm and 630 nm (Bio-Rad, Hercules USA). These data were used to calculate the percentage inhibition and the $IC_{50}$, which is defined as the concentration of the test substance at which cell viability declines to 50%. The $IC_{50}$ values [the concentration required for 50% inhibition of cell viability] were calculated using sigmoidal dose-response curve-fitting models. The selectivity index (SI) was calculated as the ratio of cytotoxicity ($IC_{50}$) on normal cells (MCF10a) to cancer cells (MCF7).

### c-Met Kinase Inhibition Assay

The assay is designed to measure c-Met kinase activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent (BPS Bioscience, #79559). The assay kit comes in a convenient 96-well format.

### Pim-1 Kinase Inhibition Assay

Kinase enzymatic activities were assayed with 10 µM ATP in 384-well plates using the luminescent ADP-GloTM assay (Promega, Madison, WI, USA) according to the recommendations of the manufacturer. The transmitted signal was measured using the Envision (PerkinElmer, Waltham, MA, USA) microplate

luminometer and expressed in Relative Light Unit (RLU). To determine the half maximal inhibitory concentration ($IC_{50}$), the assays were performed in duplicate in the absence or presence of increasing doses of the tested compounds.

## Human Phosphorylated Type of PI3K ELISA Kit

We used an Enzyme-Linked Immunosorbent Assay (ELISA-MBS167579). The plate has been pre-coated with human P-PI3K antibody. P-PI3K present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human P-PI3K Antibody is added and binds to P-PI3K in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated P-PI3K antibody. After incubation, unbound Streptavidin-HRP is washed away during a washing step. The substrate solution is then added, and color develops in proportion to the amount of human P-PI3K. The reaction is terminated by the addition of an acidic stop solution and absorbance is measured at 450 nm.

## Phospho-Akt

Sandwich ELISA to measure human Akt phosphorylated at S473 in cell lysates was developed. Using DuoSet® IC ELISA: (DYC887B-2) (R&D System, USA) according to the manufacturer's introductions. An immobilized capture antibody specific for Akt1 binds both phosphorylated and unphosphorylated proteins. After washing away unbound material, a biotinylated detection antibody specific for Akt1 phosphorylated at S473 is used to detect only phosphorylated protein, utilizing a standard Streptavidin HRP format.

## Phospho-mTOR

Sandwich ELISA kit for the measurement of human phospho-mTOR and total mTOR (Abcam). An anti-pan mTOR antibody has been coated onto a 96-well plate. Samples are pipetted into the wells and mTOR present in a sample is bound to the wells by the immobilized antibody and the wells are washed. In select wells, rabbit anti-phospho mTOR (S2448) antibody is added to detect phosphorylated mTOR. In the remaining wells, the biotinylated anti-pan-mTOR antibody is used to detect pan mTOR. After washing away unbound antibodies, HRP-conjugated anti-rabbit IgG or HRP-conjugated Streptavidin is pipetted into the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of mTOR (S2448). The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

## Cell cycle analysis

Flow cytometry was used to analyze the cell cycle using ab139418 propidium iodide flow cytometry kit/BD (Abcam, Cambridge, UK), as directed by the manufacturer guidelines. MCF7 cells were treated with compound **4g** at its $IC_{50}$ concentration for 24 h. The cells were washed twice with ice-cold phosphate buffer saline (PBS) and collected by centrifugation. The cells were then fixed using ice-cold 66% (v/v) ethanol, washed with PBS, and re-suspended with 0.1 mg/mL RNase to digest cellular RNA and thus minimize stained RNA in the background. The cells were next stained with PI, a fluorescent molecule that may bind to

nucleic acid, at a concentration of 40 mg/mL. In cells, PI attaches to DNA in proportion to its amount. Because the DNA content of cells at different stages of the cell cycle differs, the fluorescence intensity can be used to assess the stage of cell growth. FacsCalibur (BD Biosciences, USA) was used to estimate cell fluorescence, which was then examined using Cell-Quest software (Becton Dickinson). Cell cycle analysis of MCF7 cells without any treatment was used as a control.

## Annexin V-FITC assay for assessing apoptosis

After treatments, apoptotic cells were measured using the annexin V-FITC Apoptosis Detection Kit (BioVision) (K101-25). In a six-well plate with a cell density of 5 x $10^5$ cells/well, MCF7 cells were incubated for 24 h at 37°C. After the incubation period, the cells were centrifuged and resuspended in 500 µl of 1X binding buffer. Then, at room temperature for 5 minutes, 5 µL of annexin V-FITC and propidium iodide (PI) (BD Bioscience) were added, followed by incubation in the dark. Flow cytometry using FITC signal (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2).

## Caspase-9 activity determination

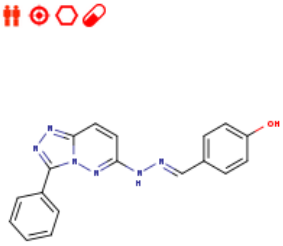
The activity of caspase-9 was determined using the Invitrogen Caspase-9 (active) Human ELISA, (USA, KHO-1091). To detect and quantify the level of human active caspase-9 protein the detailed procedure including the standard curve preparation was described in the manufacturer's instructions. All the experiments were performed in triplicate.

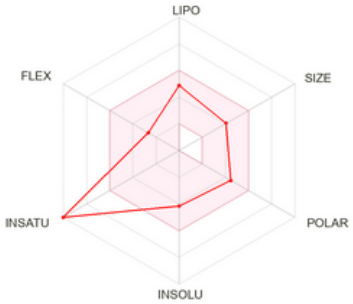
## II- Molecular modeling

### Physicochemical, pharmacokinetic, and ADME properties

Molecular properties of the most potent triazolo pyridazine derivatives **4a** and **4g** using the SwissADME website.

## 4a






SMILES Oc1ccc(cc1)/C=N/Nc1ccc2n(n1)c(nn2)c1ccccc1

| Physicochemical Properties | |
|---|---|
| Formula | C18H14N6O |
| Molecular weight | 330.34 g/mol |
| Num. heavy atoms | 25 |
| Num. arom. heavy atoms | 21 |
| Fraction Csp3 | 0.00 |
| Num. rotatable bonds | 4 |
| Num. H-bond acceptors | 5 |
| Num. H-bond donors | 2 |
| Molar Refractivity | 95.72 |
| TPSA | 87.70 Å² |

| Lipophilicity | |
|---|---|
| Log $P_{o/w}$ (iLOGP) | 1.91 |
| Log $P_{o/w}$ (XLOGP3) | 3.00 |
| Log $P_{o/w}$ (WLOGP) | 2.75 |
| Log $P_{o/w}$ (MLOGP) | 2.70 |
| Log $P_{o/w}$ (SILICOS-IT) | 1.94 |
| Consensus Log $P_{o/w}$ | 2.46 |

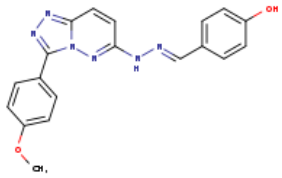
| Water Solubility | |
|---|---|
| Log *S* (ESOL) | -4.14 |
| Solubility | 2.42e-02 mg/ml ; 7.32e-05 mol/l |
| Class | Moderately soluble |
| Log *S* (Ali) | -4.51 |
| Solubility | 1.03e-02 mg/ml ; 3.12e-05 mol/l |
| Class | Moderately soluble |
| Log *S* (SILICOS-IT) | -5.92 |
| Solubility | 3.99e-04 mg/ml ; 1.21e-06 mol/l |
| Class | Moderately soluble |

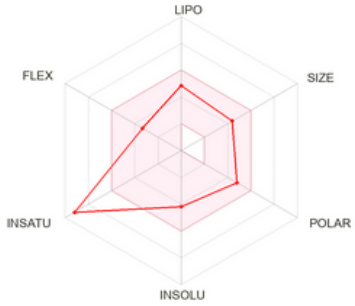
| Pharmacokinetics | |
|---|---|
| GI absorption | High |
| BBB permeant | No |
| P-gp substrate | No |
| CYP1A2 inhibitor | Yes |
| CYP2C19 inhibitor | Yes |
| CYP2C9 inhibitor | No |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | No |
| Log $K_p$ (skin permeation) | -6.19 cm/s |

| Druglikeness | |
|---|---|
| Lipinski | Yes; 0 violation |
| Ghose | Yes |
| Veber | Yes |
| Egan | Yes |
| Muegge | Yes |
| Bioavailability Score | 0.55 |

| Medicinal Chemistry | |
|---|---|
| PAINS | 1 alert: hzone_phenol_B |
| Brenk | 1 alert: imine_1 |
| Leadlikeness | Yes |
| Synthetic accessibility | 3.25 |

## 4g






SMILES COc1ccc(cc1)c1nnc2n1nc(N/N=C/c1ccc(cc1)O)cc2

| Physicochemical Properties | |
|---|---|
| Formula | C19H16N6O2 |
| Molecular weight | 360.37 g/mol |
| Num. heavy atoms | 27 |
| Num. arom. heavy atoms | 21 |
| Fraction Csp3 | 0.05 |
| Num. rotatable bonds | 5 |
| Num. H-bond acceptors | 6 |
| Num. H-bond donors | 2 |
| Molar Refractivity | 102.21 |
| TPSA | 96.93 Å² |

| Lipophilicity | |
|---|---|
| Log $P_{o/w}$ (iLOGP) | 2.22 |
| Log $P_{o/w}$ (XLOGP3) | 2.97 |
| Log $P_{o/w}$ (WLOGP) | 2.76 |
| Log $P_{o/w}$ (MLOGP) | 2.42 |
| Log $P_{o/w}$ (SILICOS-IT) | 1.98 |
| Consensus Log $P_{o/w}$ | 2.47 |

| Water Solubility | |
|---|---|
| Log *S* (ESOL) | -4.19 |
| Solubility | 2.32e-02 mg/ml ; 6.44e-05 mol/l |
| Class | Moderately soluble |
| Log *S* (Ali) | -4.67 |
| Solubility | 7.73e-03 mg/ml ; 2.14e-05 mol/l |
| Class | Moderately soluble |
| Log *S* (SILICOS-IT) | -6.03 |
| Solubility | 3.39e-04 mg/ml ; 9.42e-07 mol/l |
| Class | Poorly soluble |

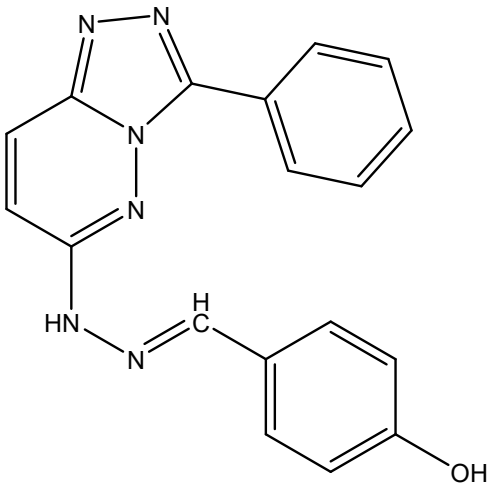
| Pharmacokinetics | |
|---|---|
| GI absorption | High |
| BBB permeant | No |
| P-gp substrate | No |
| CYP1A2 inhibitor | Yes |
| CYP2C19 inhibitor | No |
| CYP2C9 inhibitor | Yes |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | No |
| Log $K_p$ (skin permeation) | -6.39 cm/s |

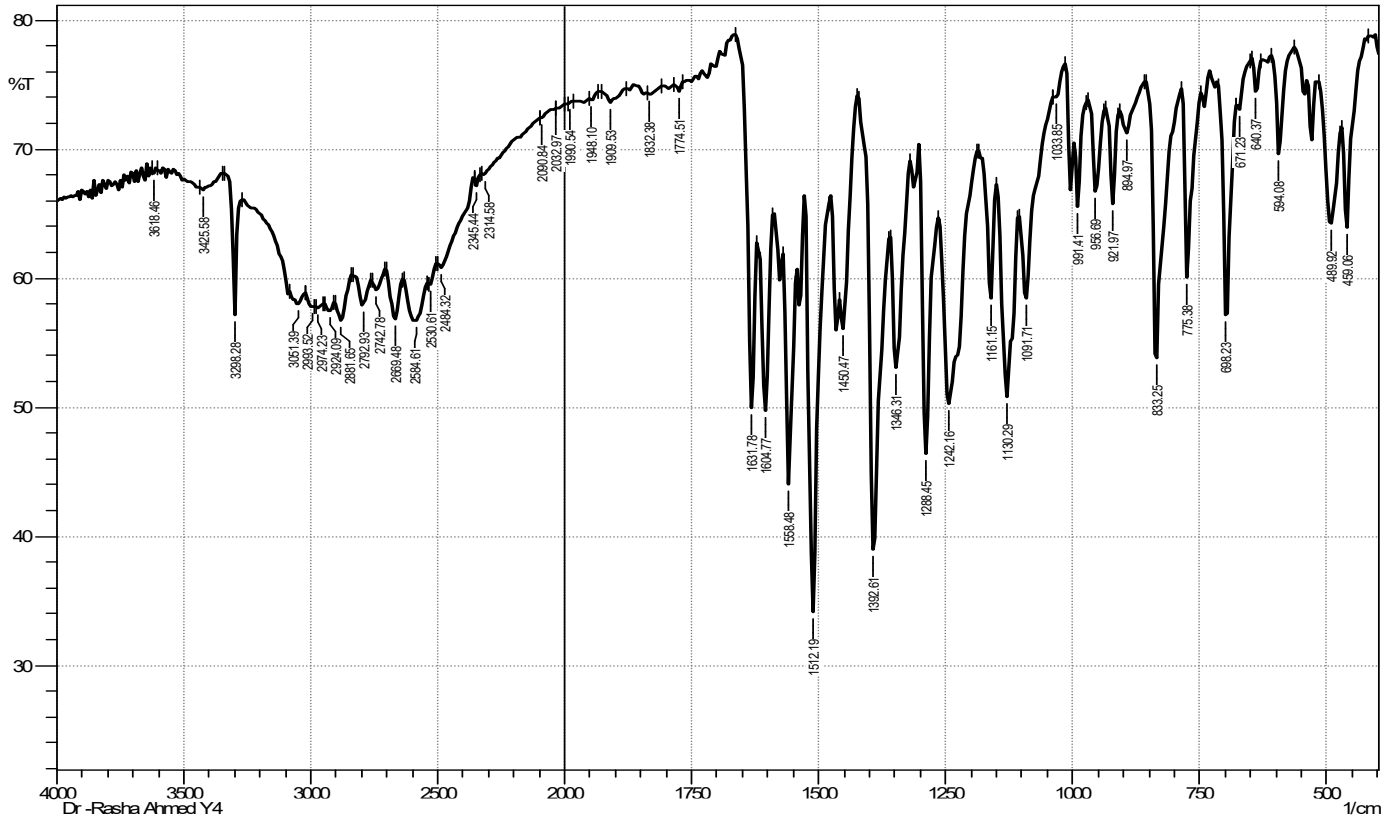
| Druglikeness | |
|---|---|
| Lipinski | Yes; 0 violation |
| Ghose | Yes |
| Veber | Yes |
| Egan | Yes |
| Muegge | Yes |
| Bioavailability Score | 0.55 |

| Medicinal Chemistry | |
|---|---|
| PAINS | 1 alert: hzone_phenol_B |
| Brenk | 1 alert: imine_1 |
| Leadlikeness | No; 1 violation: MW>350 |
| Synthetic accessibility | 3.34 |

## III- $^{1}$H NMR, $^{13}$ C NMR, and other spectral data of the new derivatives.

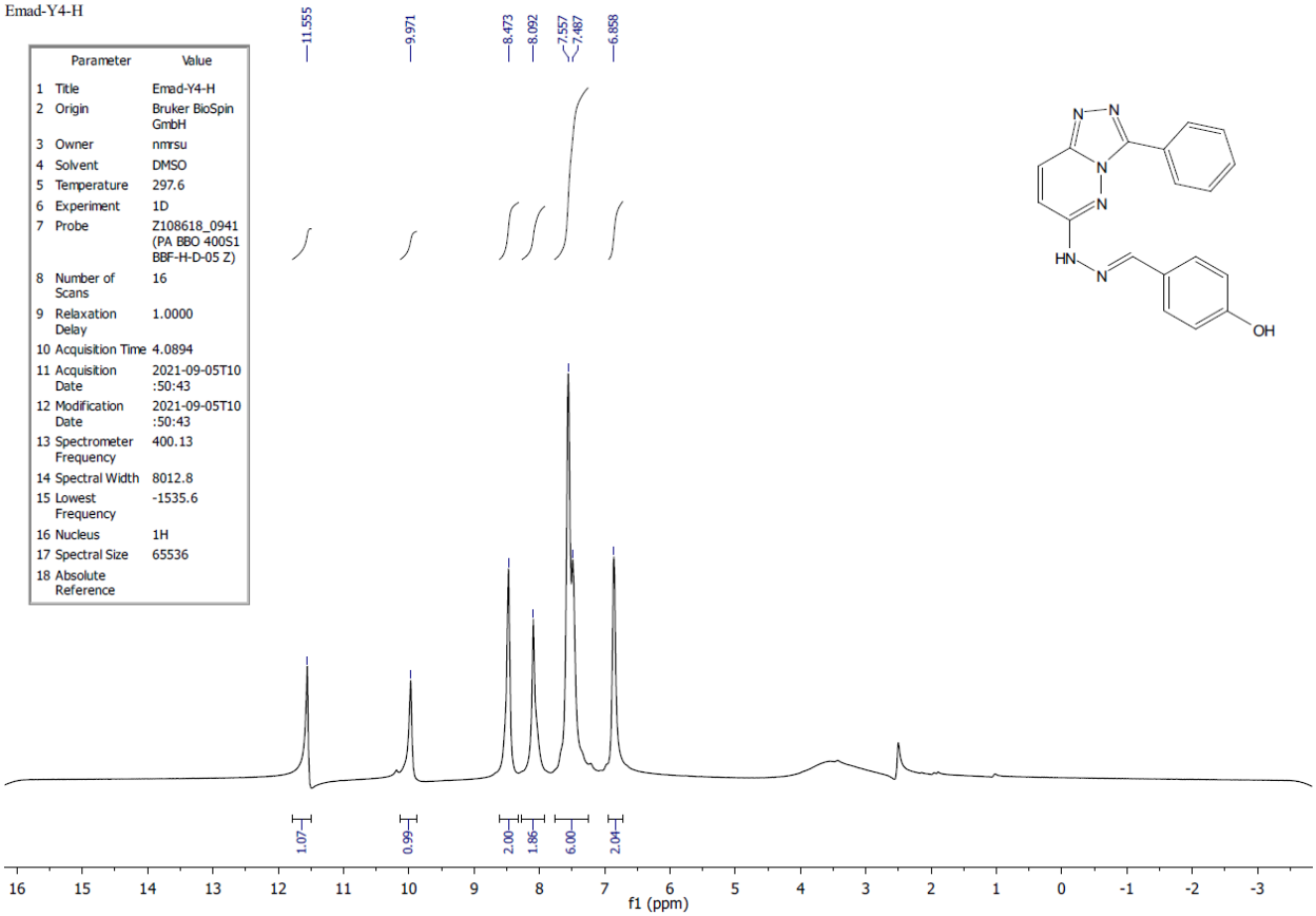
### Compound 4a



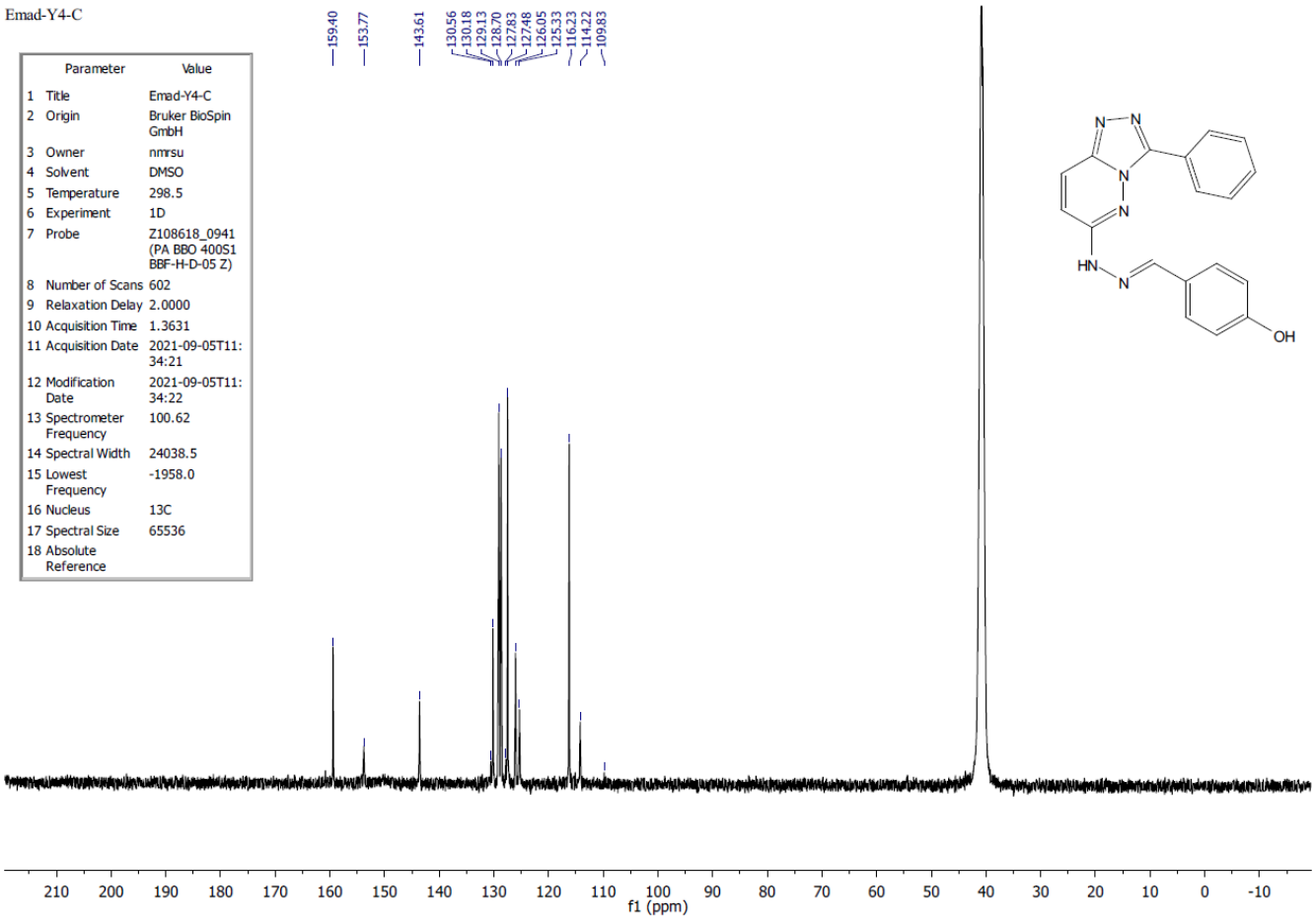
Emad-Y4-H

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y4-H |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 297.6 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 16 |
| 9 | Relaxation Delay | 1.0000 |
| 10 | Acquisition Time | 4.0894 |
| 11 | Acquisition Date | 2021-09-05T10:50:43 |
| 12 | Modification Date | 2021-09-05T10:50:43 |
| 13 | Spectrometer Frequency | 400.13 |
| 14 | Spectral Width | 8012.8 |
| 15 | Lowest Frequency | -1535.6 |
| 16 | Nucleus | 1H |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

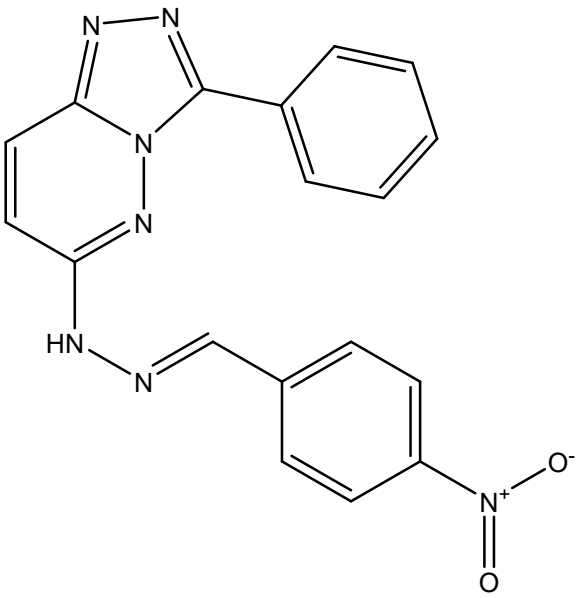



Emad-Y4-C

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y4-C |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 298.5 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 602 |
| 9 | Relaxation Delay | 2.0000 |
| 10 | Acquisition Time | 1.3631 |
| 11 | Acquisition Date | 2021-09-05T11:34:21 |
| 12 | Modification Date | 2021-09-05T11:34:22 |
| 13 | Spectrometer Frequency | 100.62 |
| 14 | Spectral Width | 24038.5 |
| 15 | Lowest Frequency | -1958.0 |
| 16 | Nucleus | 13C |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |



## Compound 4b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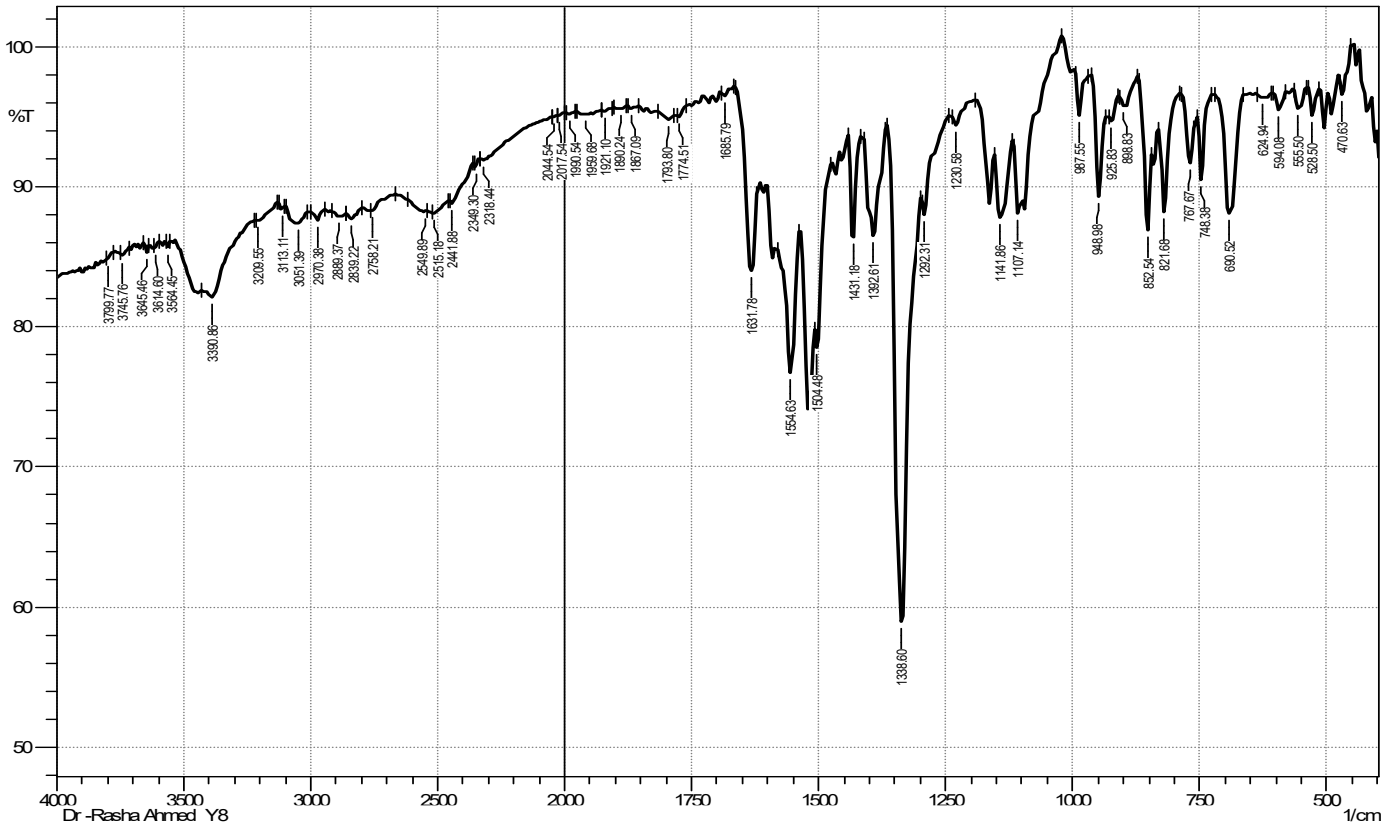
HN
N
O-
N+
O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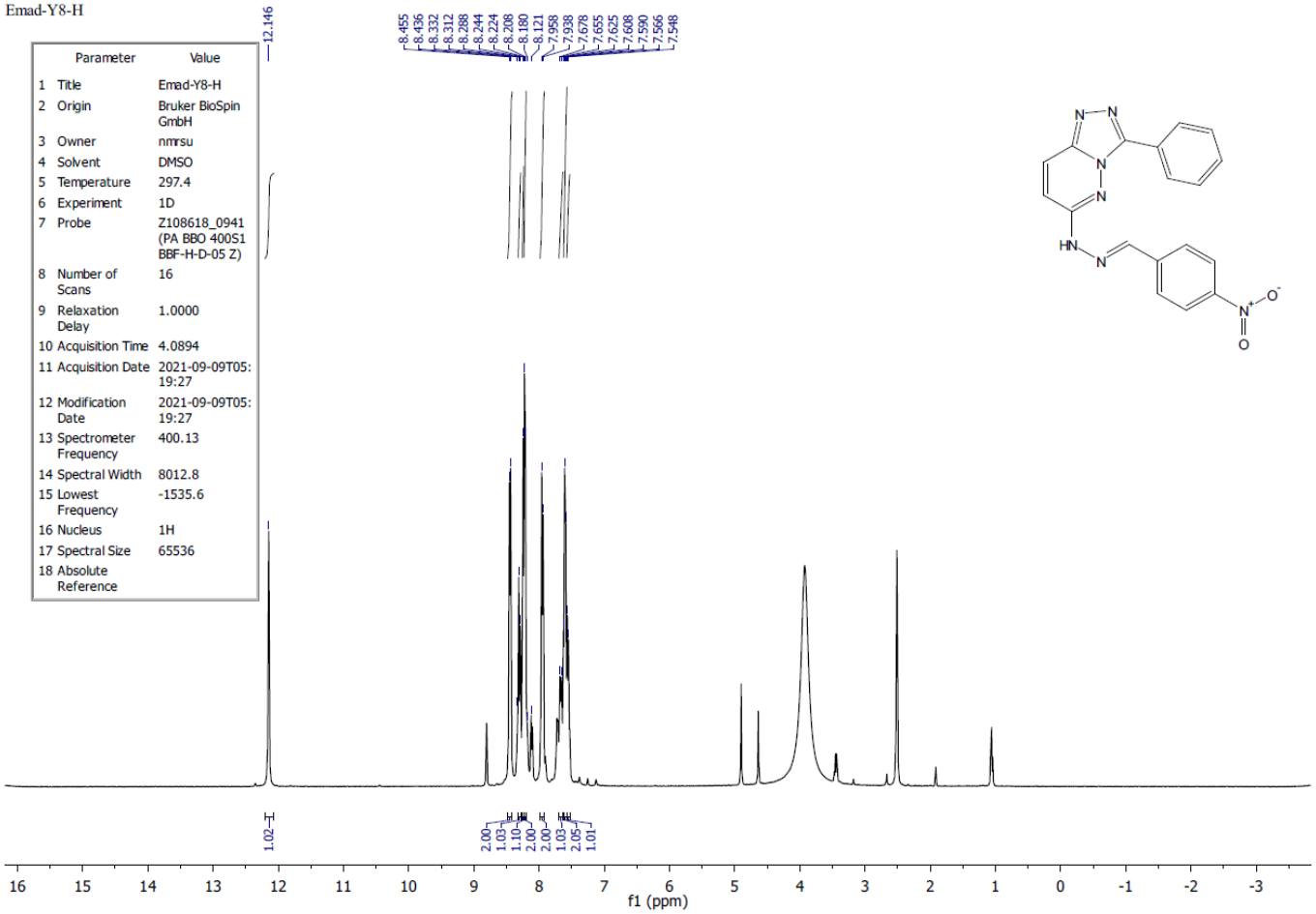
%T
90
80
70
60
50
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
Dr -Rasha Ahmed Y8
3799.77
3745.76
3645.46
3614.60
3564.45
3390.86
3209.55
3113.11
3051.39
2970.38
2888.37
2839.22
2758.21
2549.89
2515.19
2441.88
2349.30
2318.44
2044.54
2017.54
1990.56
1959.68
1921.10
1880.24
1867.09
1793.80
1774.51
1685.79
1631.78
1554.63
1504.48
1431.18
1382.61
1338.60
1292.31
1230.38
1141.86
1107.14
987.55
948.98
925.83
898.83
852.54
821.68
767.67
748.39
690.52
624.94
594.09
555.50
528.50
470.63

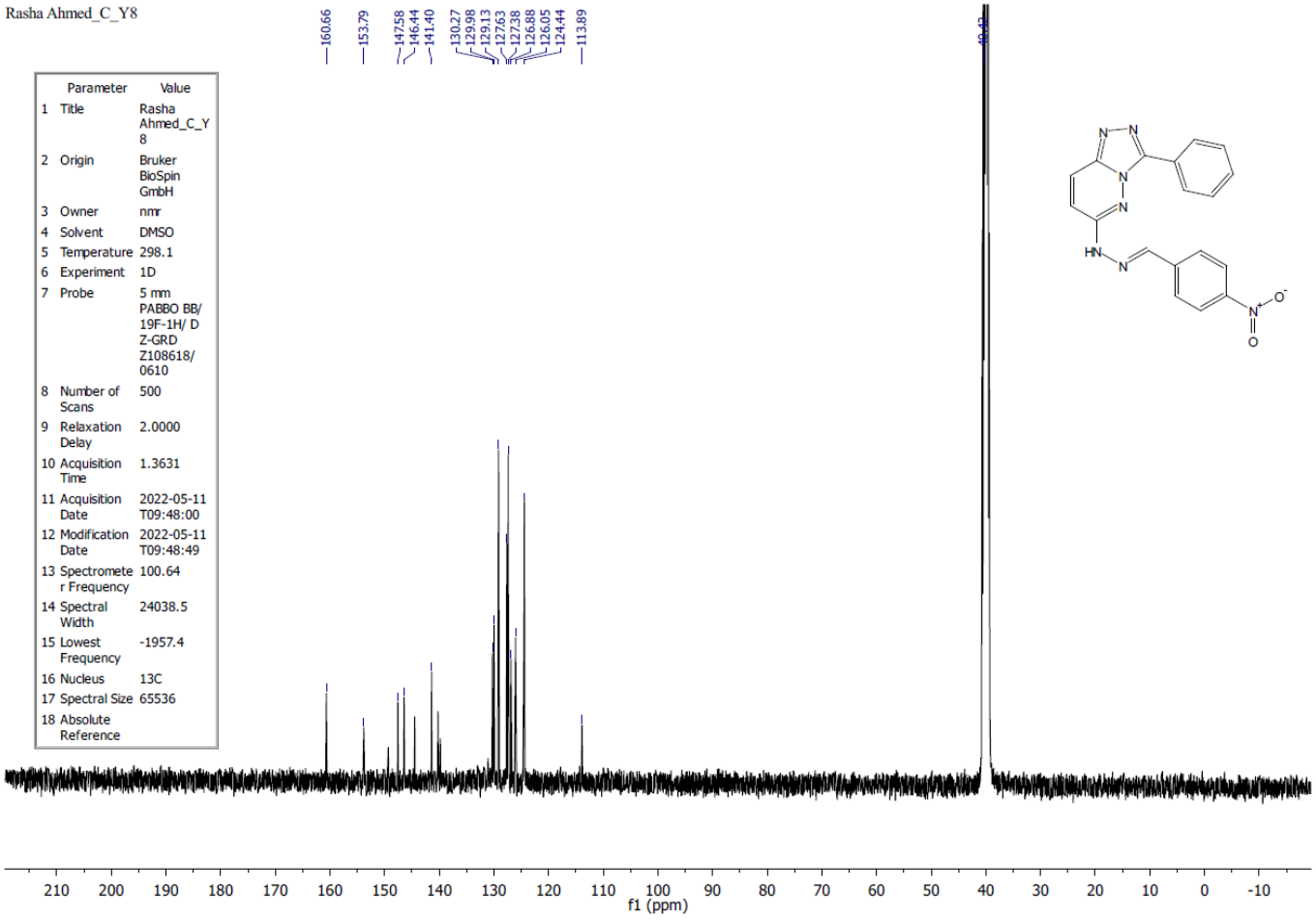
Emad-Y8-H

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y8-H |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 297.4 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 16 |
| 9 | Relaxation Delay | 1.0000 |
| 10 | Acquisition Time | 4.0894 |
| 11 | Acquisition Date | 2021-09-09T05:19:27 |
| 12 | Modification Date | 2021-09-09T05:19:27 |
| 13 | Spectrometer Frequency | 400.13 |
| 14 | Spectral Width | 8012.8 |
| 15 | Lowest Frequency | -1535.6 |
| 16 | Nucleus | 1H |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

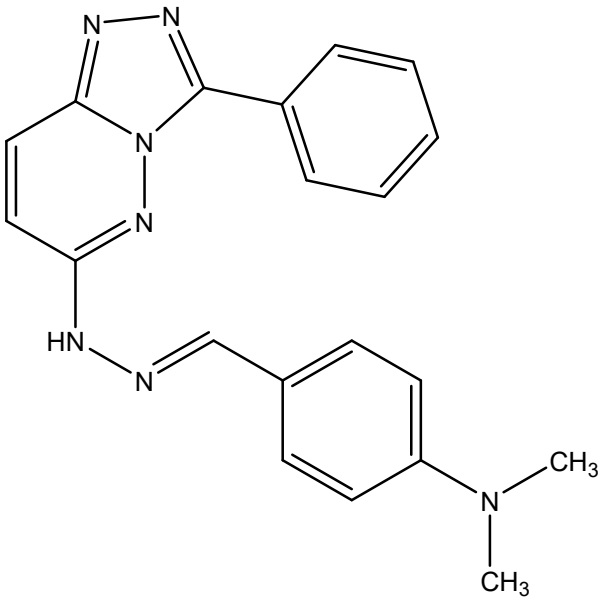



Rasha Ahmed_C_Y8

| | Parameter | Value |
|---|---|---|
| 1 | Title | Rasha Ahmed_C_Y8 |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmr |
| 4 | Solvent | DMSO |
| 5 | Temperature | 298.1 |
| 6 | Experiment | 1D |
| 7 | Probe | 5 mm PABBO BB/ 19F-1H/ D Z-GRD Z108618/ 0610 |
| 8 | Number of Scans | 500 |
| 9 | Relaxation Delay | 2.0000 |
| 10 | Acquisition Time | 1.3631 |
| 11 | Acquisition Date | 2022-05-11 T09:48:00 |
| 12 | Modification Date | 2022-05-11 T09:48:49 |
| 13 | Spectrometer Frequency | 100.64 |
| 14 | Spectral Width | 24038.5 |
| 15 | Lowest Frequency | -1957.4 |
| 16 | Nucleus | 13C |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |



## Compound 4c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HN
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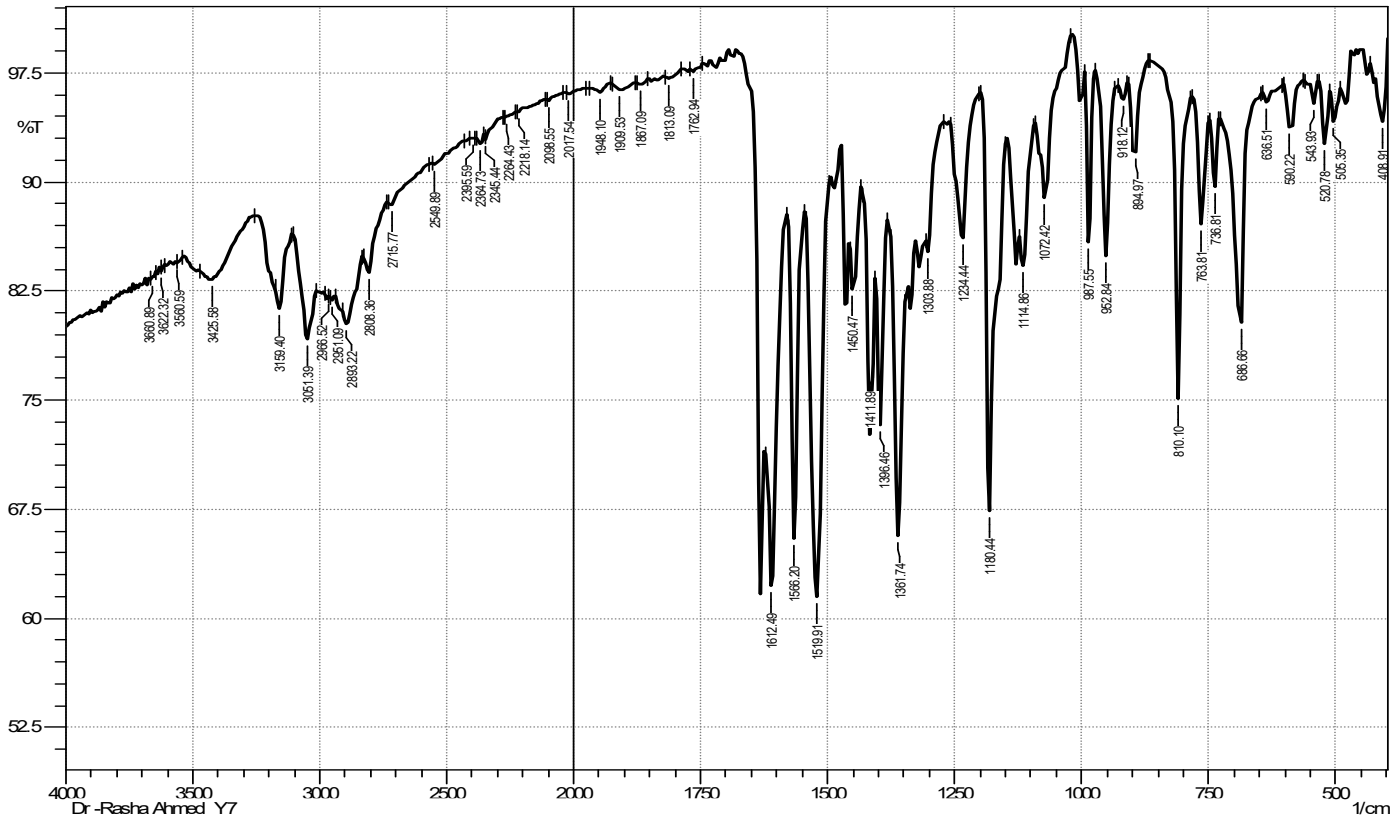
CH3
N
CH3


97.5
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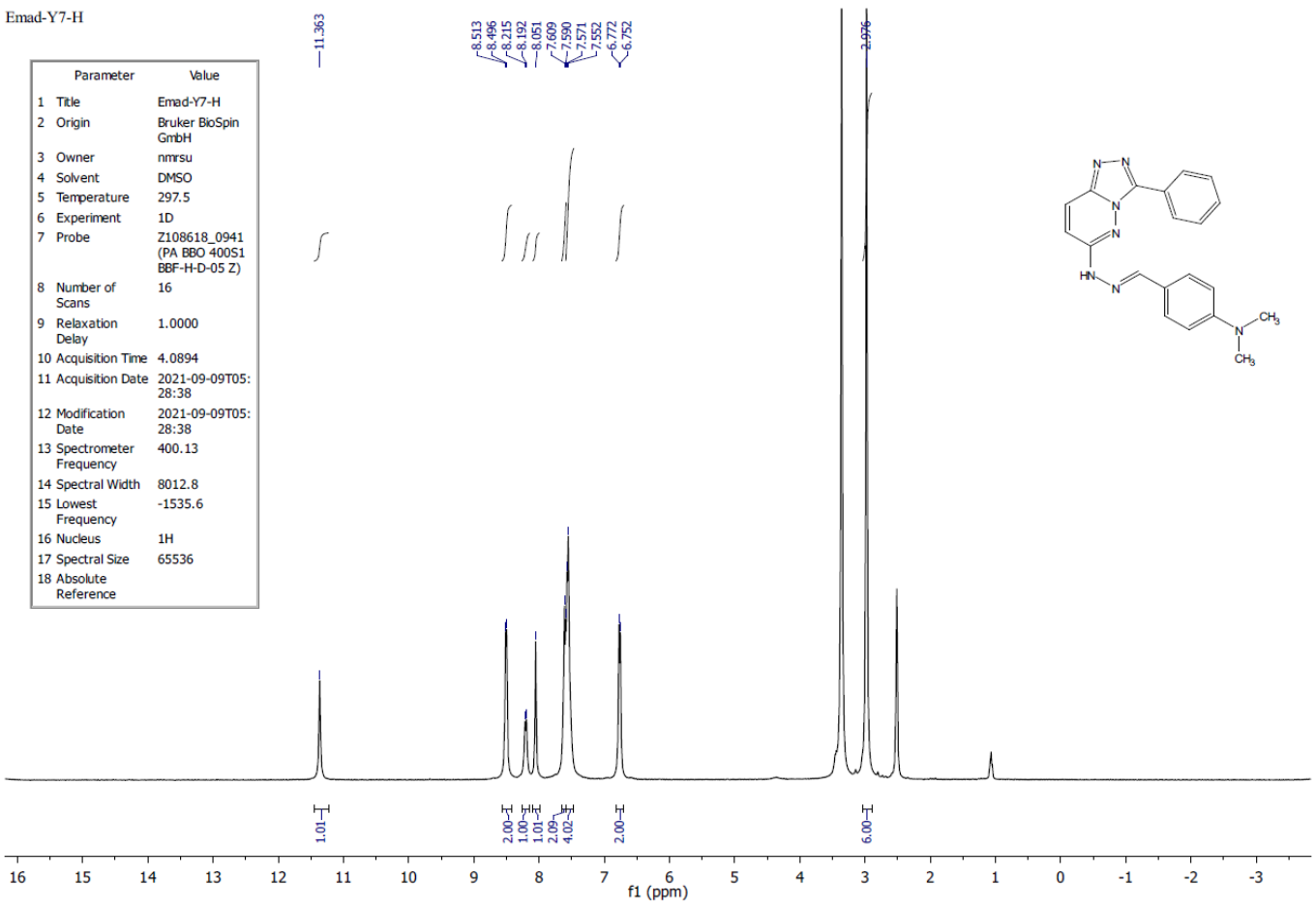
90
82.5
75
67.5
60
52.5
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
Dr -Rasha Ahmed Y7
3660.89
3622.32
3560.59
3425.58
3159.40
3051.39
2966.52
2951.09
2893.22
2808.36
2715.77
2549.89
2385.59
2364.73
2345.44
2264.43
2218.14
2098.55
2017.54
1948.10
1909.53
1867.09
1813.09
1762.94
1612.49
1566.20
1519.91
1450.47
1411.89
1396.46
1361.74
1303.88
1234.44
1180.44
1114.86
1072.42
987.55
952.84
918.12
894.97
810.10
763.81
736.81
686.66
636.51
590.22
543.99
520.78
505.35
408.91

Emad-Y7-H

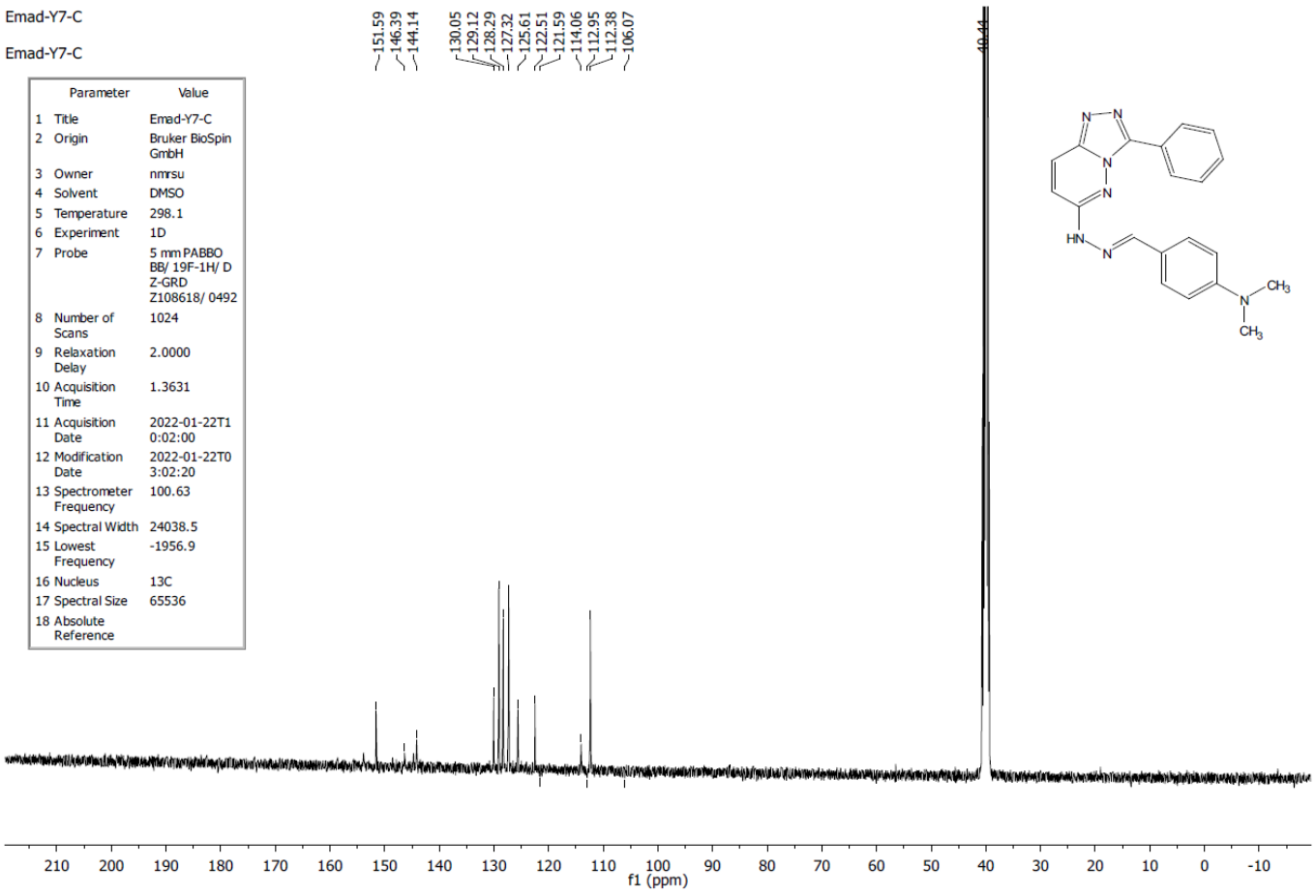
| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y7-H |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 297.5 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 16 |
| 9 | Relaxation Delay | 1.0000 |
| 10 | Acquisition Time | 4.0894 |
| 11 | Acquisition Date | 2021-09-09T05:28:38 |
| 12 | Modification Date | 2021-09-09T05:28:38 |
| 13 | Spectrometer Frequency | 400.13 |
| 14 | Spectral Width | 8012.8 |
| 15 | Lowest Frequency | -1535.6 |
| 16 | Nucleus | 1H |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |



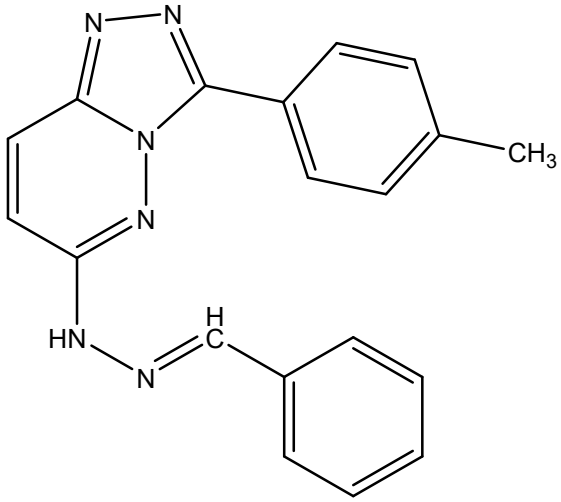

Emad-Y7-C

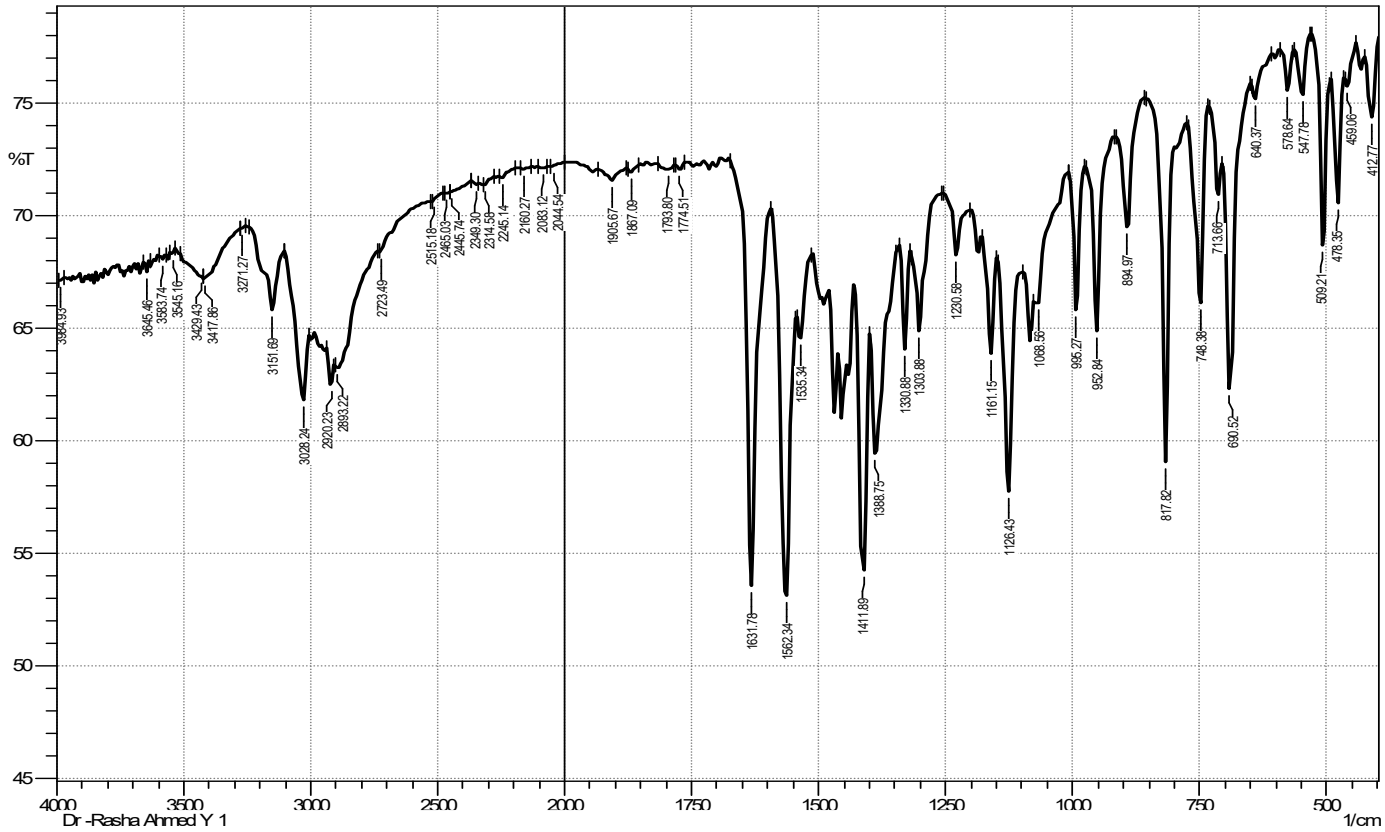
Emad-Y7-C

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y7-C |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 298.1 |
| 6 | Experiment | 1D |
| 7 | Probe | 5 mm PABBO BB/ 19F-1H/ D Z-GRD Z108618/ 0492 |
| 8 | Number of Scans | 1024 |
| 9 | Relaxation Delay | 2.0000 |
| 10 | Acquisition Time | 1.3631 |
| 11 | Acquisition Date | 2022-01-22T10:02:00 |
| 12 | Modification Date | 2022-01-22T03:02:20 |
| 13 | Spectrometer Frequency | 100.63 |
| 14 | Spectral Width | 24038.5 |
| 15 | Lowest Frequency | -1956.9 |
| 16 | Nucleus | 13C |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |



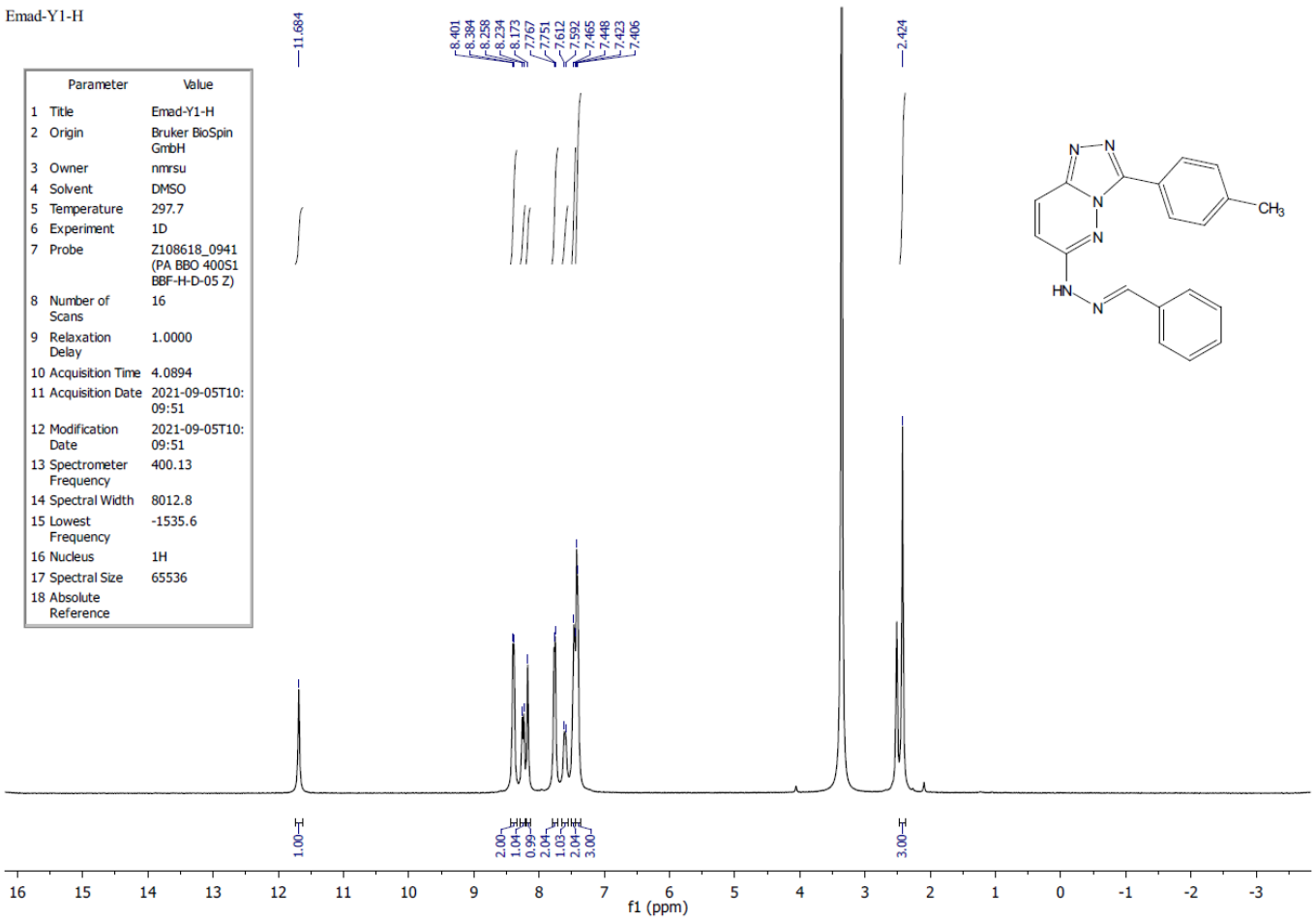
## Compound 4d


N
N
N
N
CH3
HN
N
H
C


%T
75
70
65
60
55
50
45
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
Dr -Rasha Ahmed Y 1
3894.33
3646.46
3583.74
3545.16
3429.43
3417.86
3271.27
3151.69
3028.24
2920.23
2893.22
2723.49
2515.18
2465.03
2445.74
2349.30
2314.59
2245.14
2160.27
2083.12
2044.54
1905.67
1867.08
1793.80
1774.51
1631.78
1582.34
1535.34
1411.89
1388.75
1330.88
1303.88
1230.58
1161.15
1126.43
1088.50
995.27
952.84
894.97
817.82
748.39
713.66
690.52
640.37
578.64
547.78
509.21
478.35
459.06
412.77

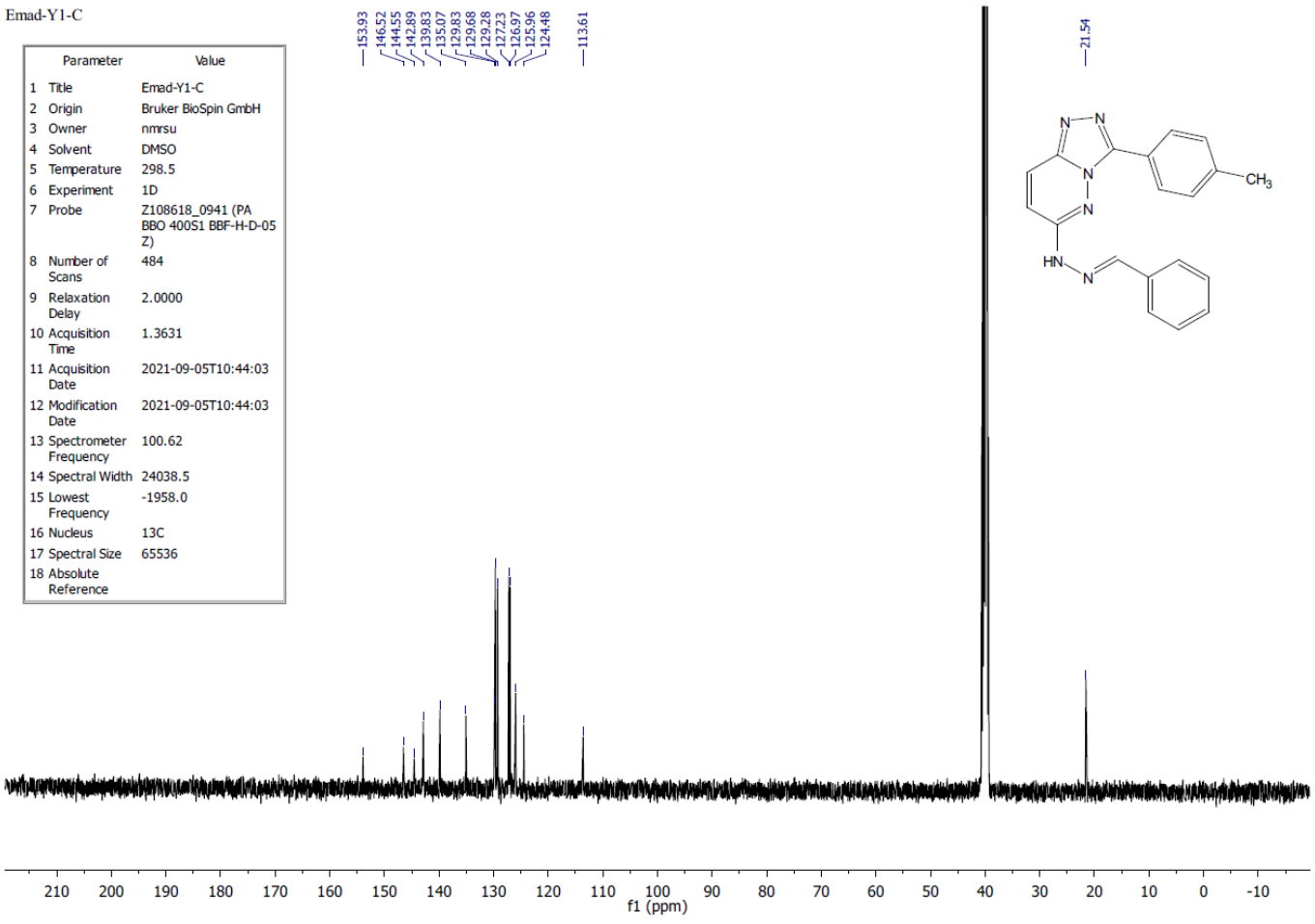
Emad-Y1-H

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y1-H |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 297.7 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 16 |
| 9 | Relaxation Delay | 1.0000 |
| 10 | Acquisition Time | 4.0894 |
| 11 | Acquisition Date | 2021-09-05T10:09:51 |
| 12 | Modification Date | 2021-09-05T10:09:51 |
| 13 | Spectrometer Frequency | 400.13 |
| 14 | Spectral Width | 8012.8 |
| 15 | Lowest Frequency | -1535.6 |
| 16 | Nucleus | 1H |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

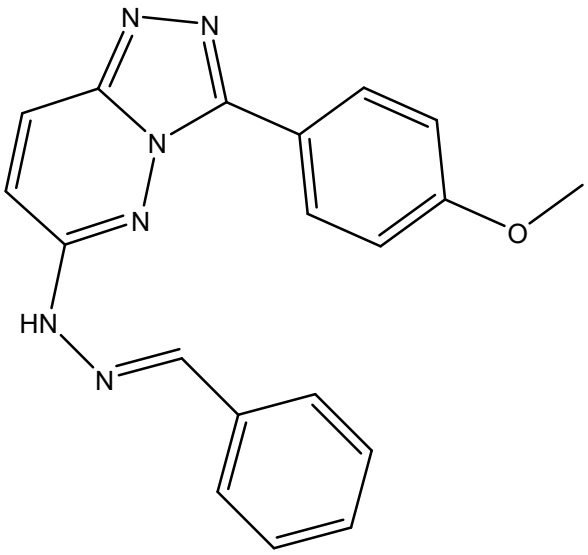


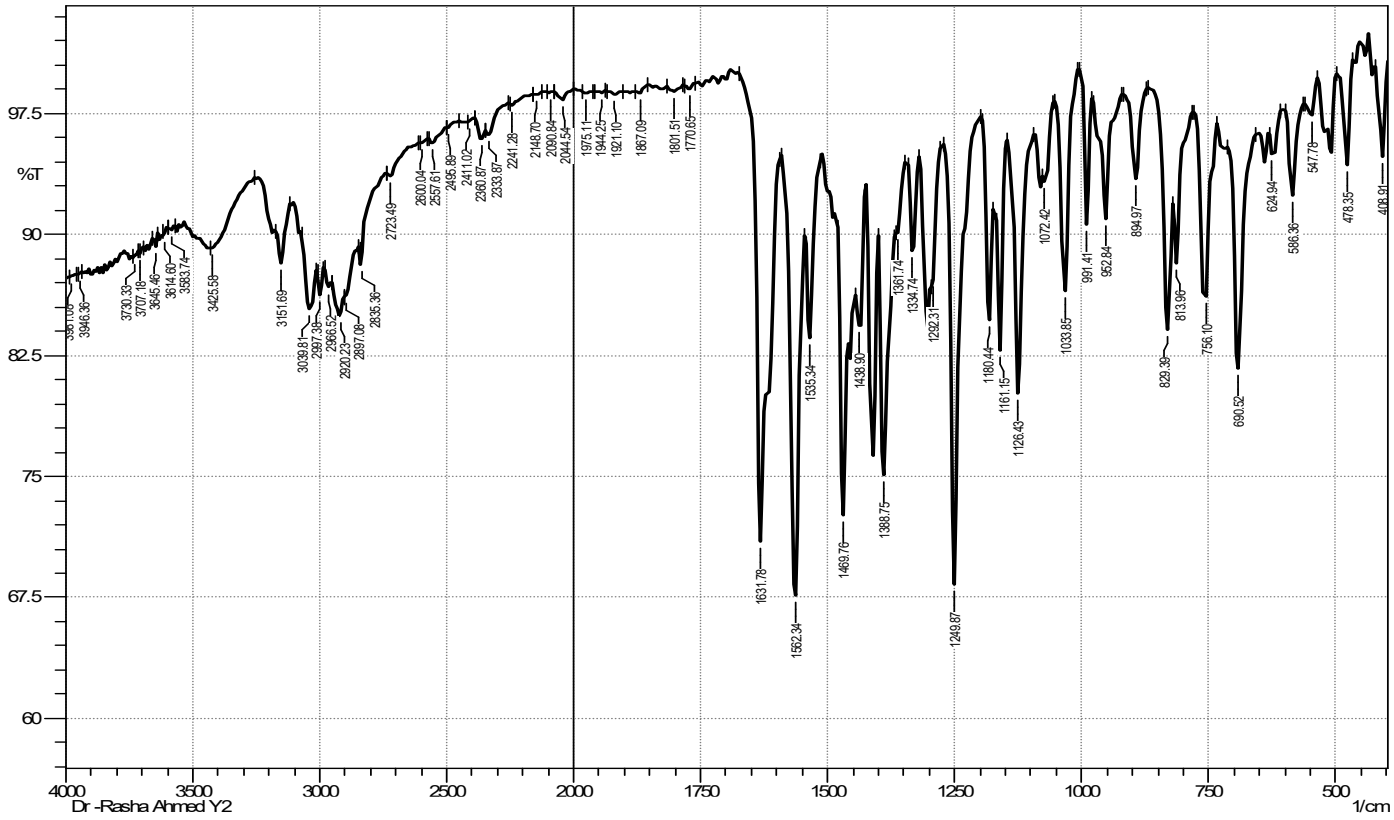

Emad-Y1-C

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y1-C |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 298.5 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 484 |
| 9 | Relaxation Delay | 2.0000 |
| 10 | Acquisition Time | 1.3631 |
| 11 | Acquisition Date | 2021-09-05T10:44:03 |
| 12 | Modification Date | 2021-09-05T10:44:03 |
| 13 | Spectrometer Frequency | 100.62 |
| 14 | Spectral Width | 24038.5 |
| 15 | Lowest Frequency | -1958.0 |
| 16 | Nucleus | 13C |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |



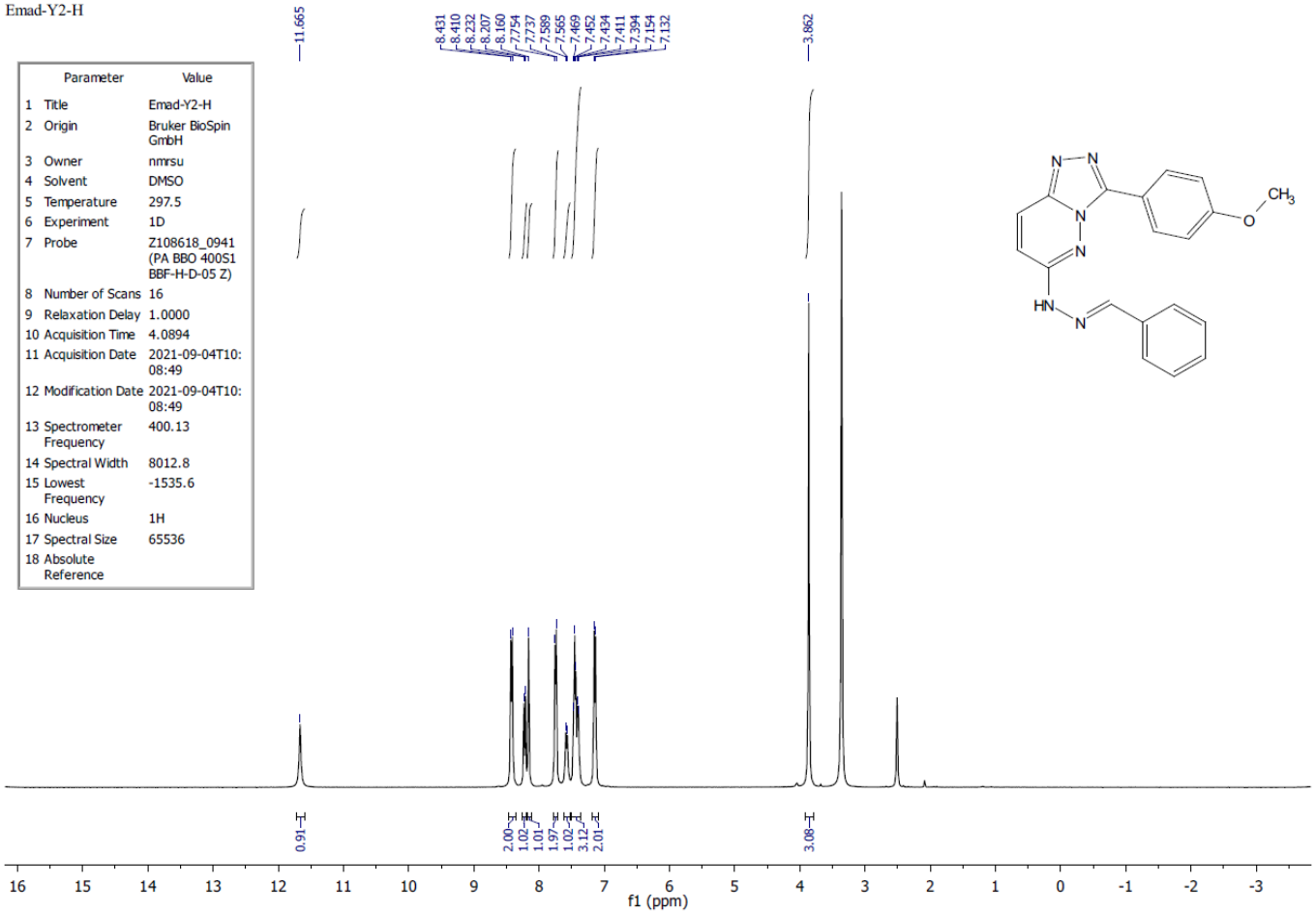
## Compound 4e


N
N
N
N
O
HN
N


97.5
%T
90
82.5
75
67.5
60
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
Dr -Rasha Ahmed Y2
3946.36
3730.33
3707.18
3645.46
3614.60
3383.74
3425.58
3151.69
3039.81
2997.39
2966.52
2920.23
2897.08
2835.36
2723.49
2600.04
2557.61
2495.89
2411.02
2360.87
2333.87
2241.28
2148.70
2090.84
2044.54
1975.11
1944.25
1921.10
1867.09
1801.51
1770.65
1631.78
1562.34
1535.34
1469.76
1438.90
1388.75
1361.74
1334.74
1292.31
1249.87
1180.44
1161.15
1126.43
1072.42
1033.85
991.41
952.84
894.97
829.39
813.96
756.10
690.52
624.94
586.36
547.78
478.35
408.91

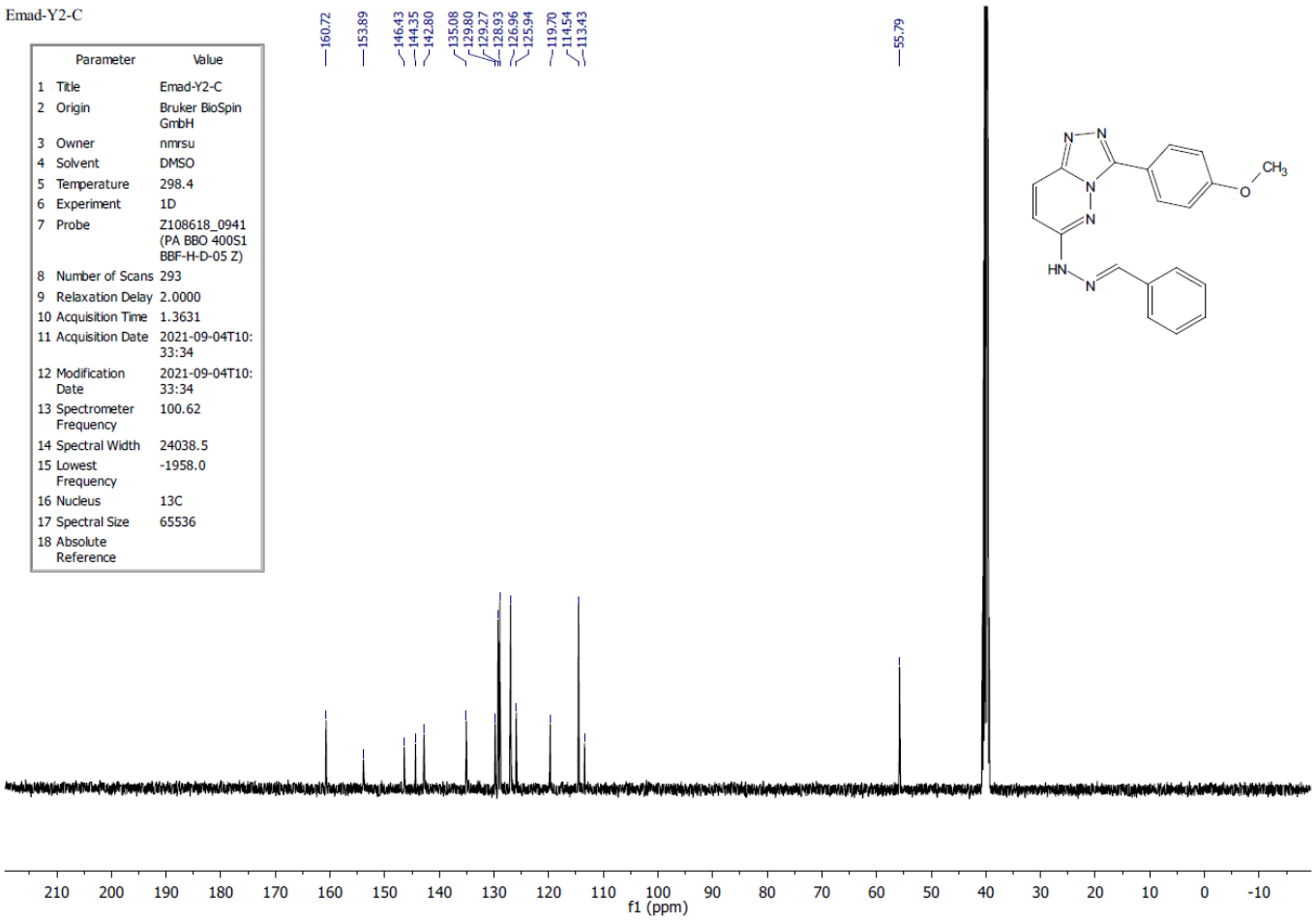
Emad-Y2-H

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y2-H |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 297.5 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 16 |
| 9 | Relaxation Delay | 1.0000 |
| 10 | Acquisition Time | 4.0894 |
| 11 | Acquisition Date | 2021-09-04T10:08:49 |
| 12 | Modification Date | 2021-09-04T10:08:49 |
| 13 | Spectrometer Frequency | 400.13 |
| 14 | Spectral Width | 8012.8 |
| 15 | Lowest Frequency | -1535.6 |
| 16 | Nucleus | 1H |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

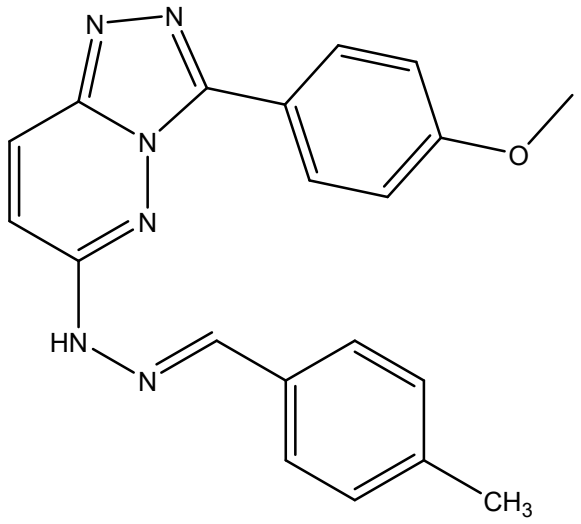


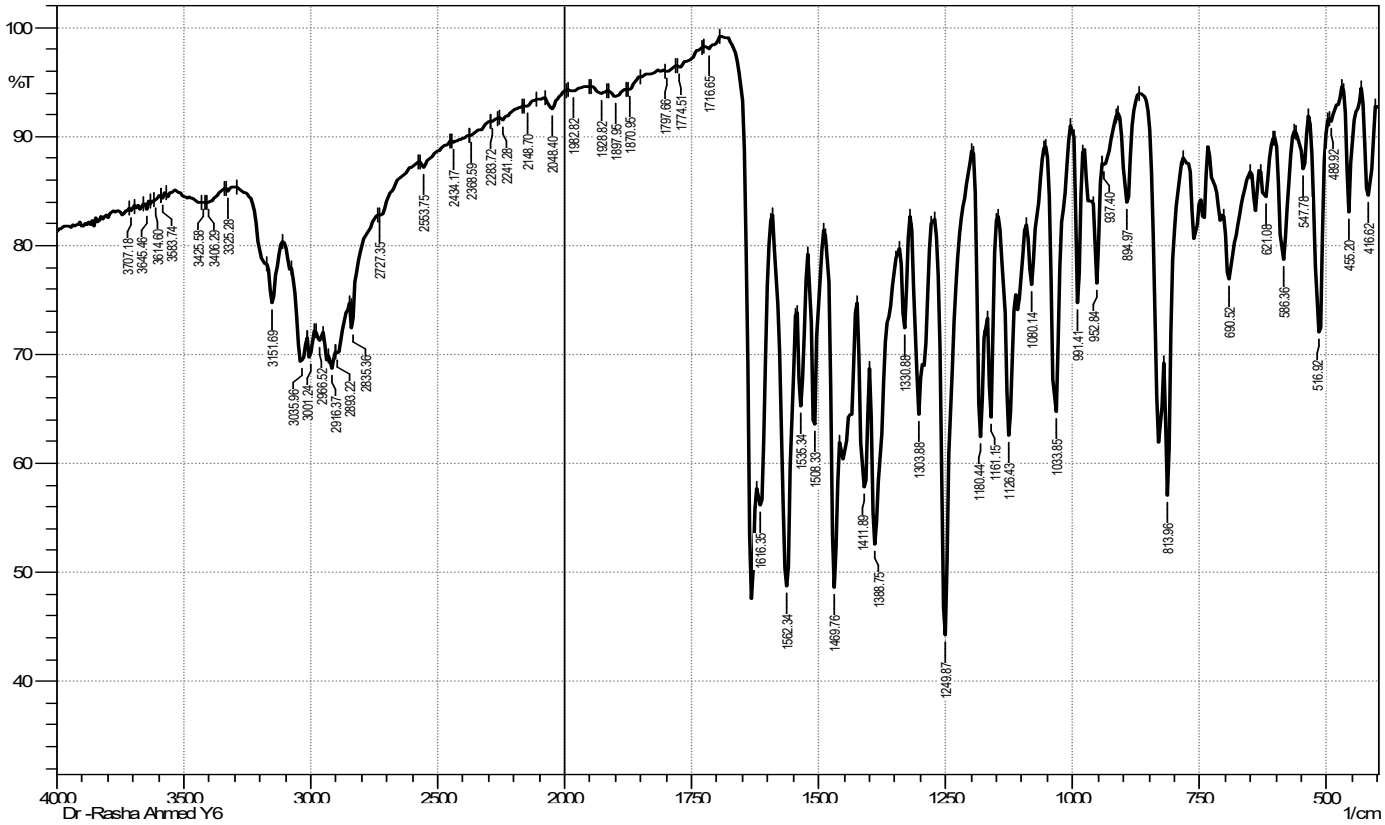

Emad-Y2-C

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y2-C |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 298.4 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 293 |
| 9 | Relaxation Delay | 2.0000 |
| 10 | Acquisition Time | 1.3631 |
| 11 | Acquisition Date | 2021-09-04T10:33:34 |
| 12 | Modification Date | 2021-09-04T10:33:34 |
| 13 | Spectrometer Frequency | 100.62 |
| 14 | Spectral Width | 24038.5 |
| 15 | Lowest Frequency | -1958.0 |
| 16 | Nucleus | 13C |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

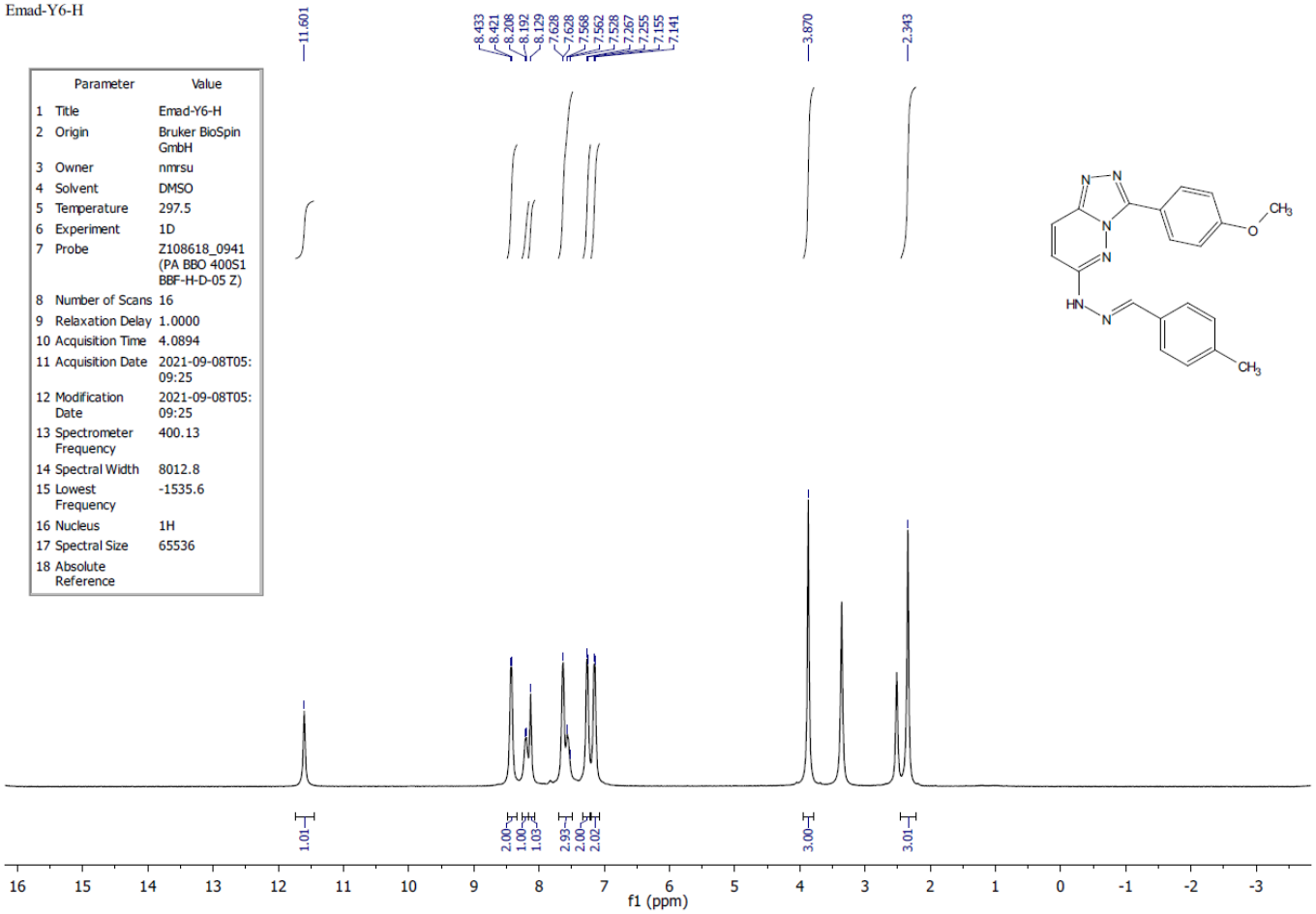


## Compound 4f


N
N
N
N
O
HN
N
CH3

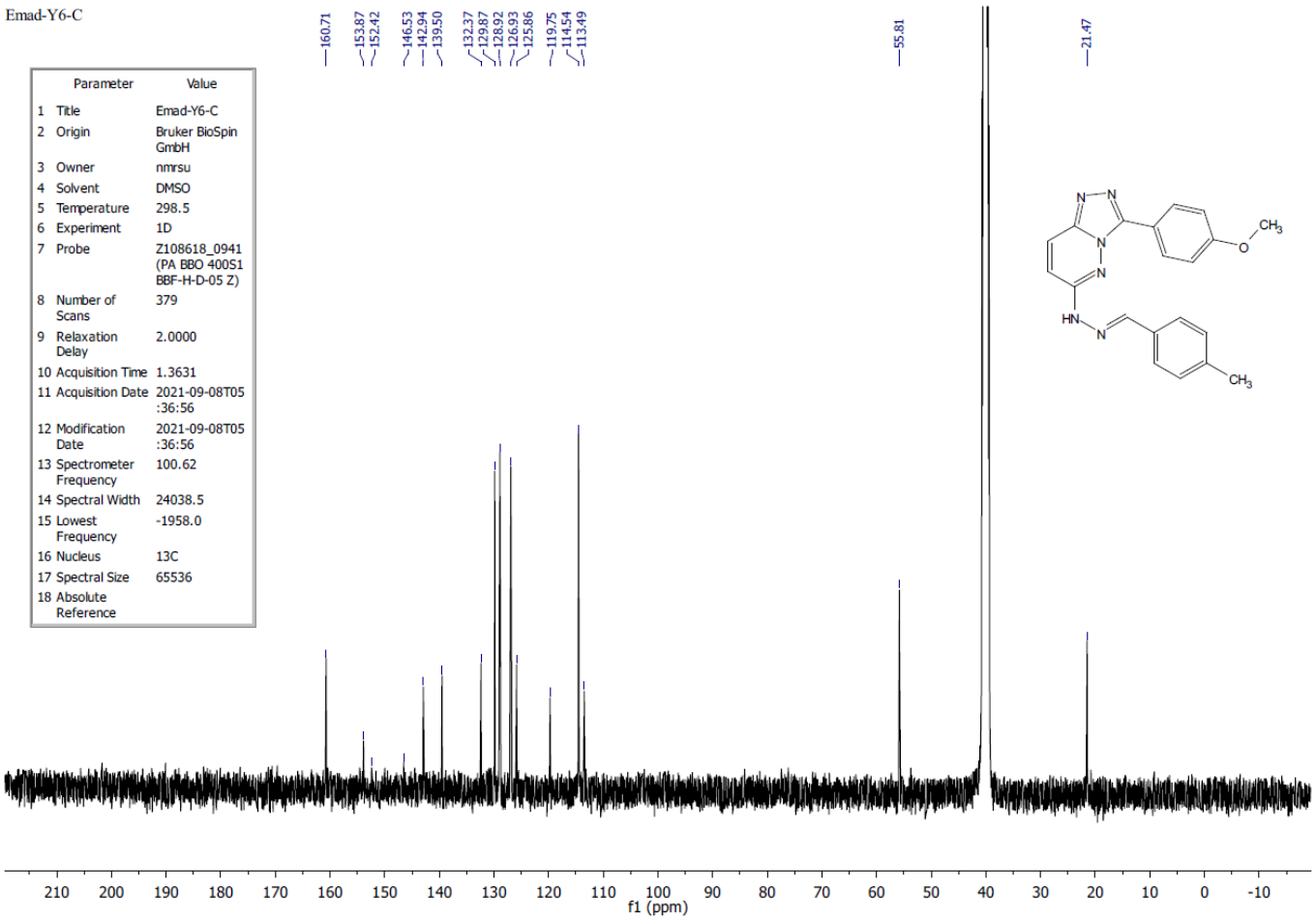

100
%T
90
80
70
60
50
40
3707.18
3645.46
3614.60
3583.74
3425.59
3406.23
3325.28
3151.69
3035.96
3001.24
2966.52
2916.37
2893.22
2835.36
2727.35
2553.75
2434.17
2368.59
2283.72
2241.28
2148.70
2048.40
1982.82
1928.82
1897.95
1870.95
1797.69
1774.51
1716.65
1616.35
1592.34
1535.34
1508.33
1469.76
1411.89
1388.75
1330.88
1303.88
1249.87
1180.44
1161.15
1126.43
1080.14
1033.85
991.41
952.84
937.40
894.97
813.96
690.52
621.09
586.36
547.78
516.92
489.92
455.20
416.62
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
Dr -Rasha Ahmed Y6

Emad-Y6-H



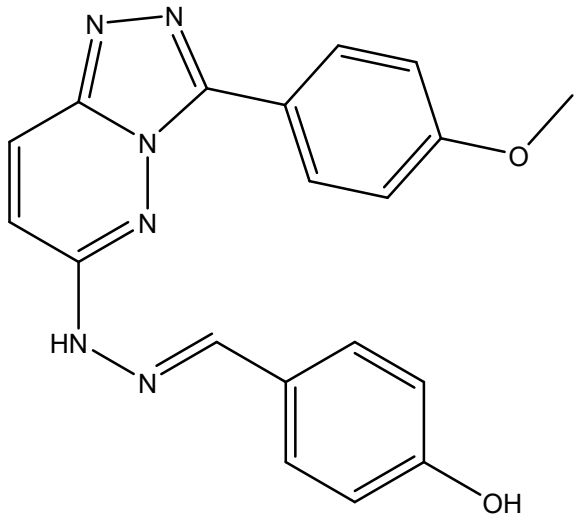

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y6-H |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 297.5 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 16 |
| 9 | Relaxation Delay | 1.0000 |
| 10 | Acquisition Time | 4.0894 |
| 11 | Acquisition Date | 2021-09-08T05:09:25 |
| 12 | Modification Date | 2021-09-08T05:09:25 |
| 13 | Spectrometer Frequency | 400.13 |
| 14 | Spectral Width | 8012.8 |
| 15 | Lowest Frequency | -1535.6 |
| 16 | Nucleus | 1H |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

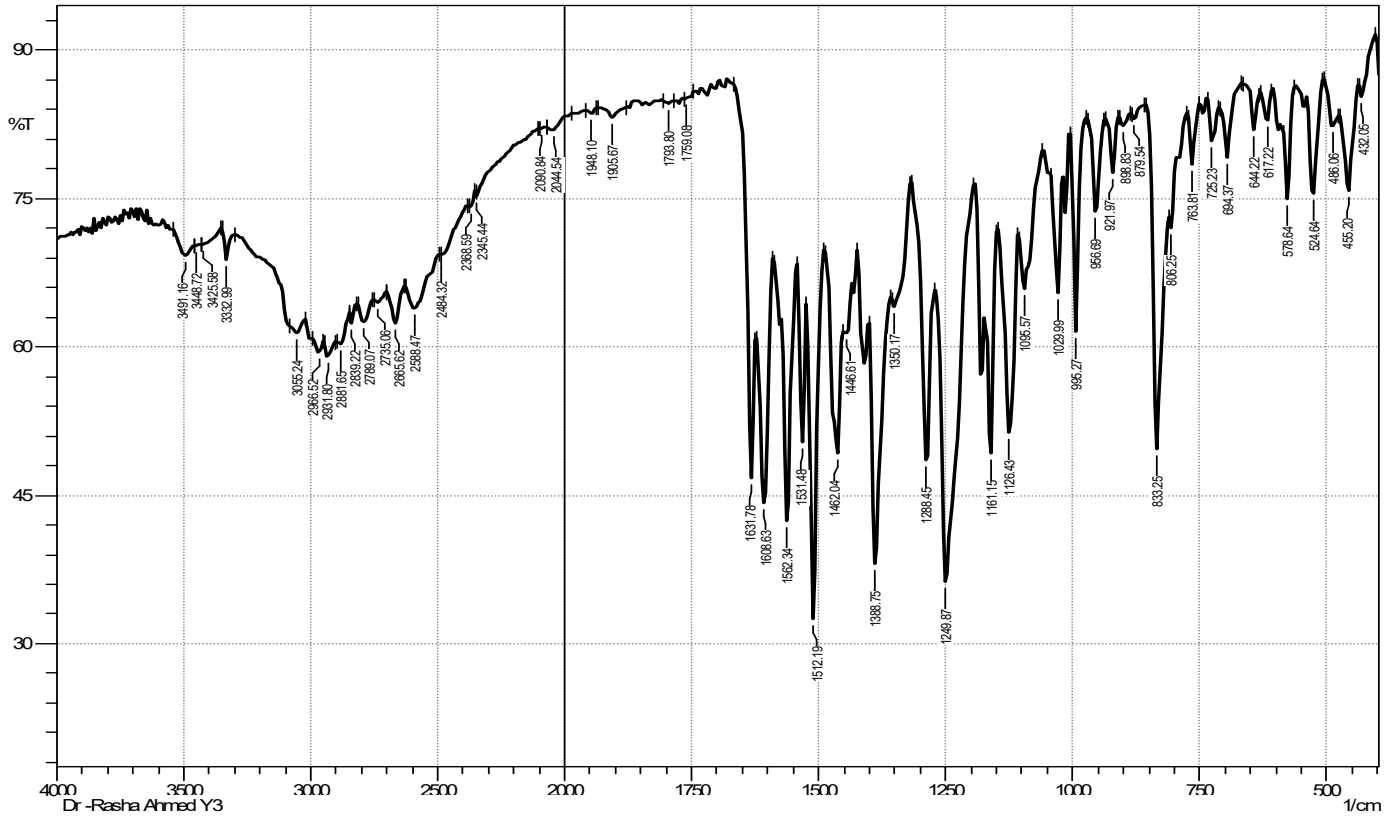
Emad-Y6-C




| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y6-C |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 298.5 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 379 |
| 9 | Relaxation Delay | 2.0000 |
| 10 | Acquisition Time | 1.3631 |
| 11 | Acquisition Date | 2021-09-08T05:36:56 |
| 12 | Modification Date | 2021-09-08T05:36:56 |
| 13 | Spectrometer Frequency | 100.62 |
| 14 | Spectral Width | 24038.5 |
| 15 | Lowest Frequency | -1958.0 |
| 16 | Nucleus | 13C |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

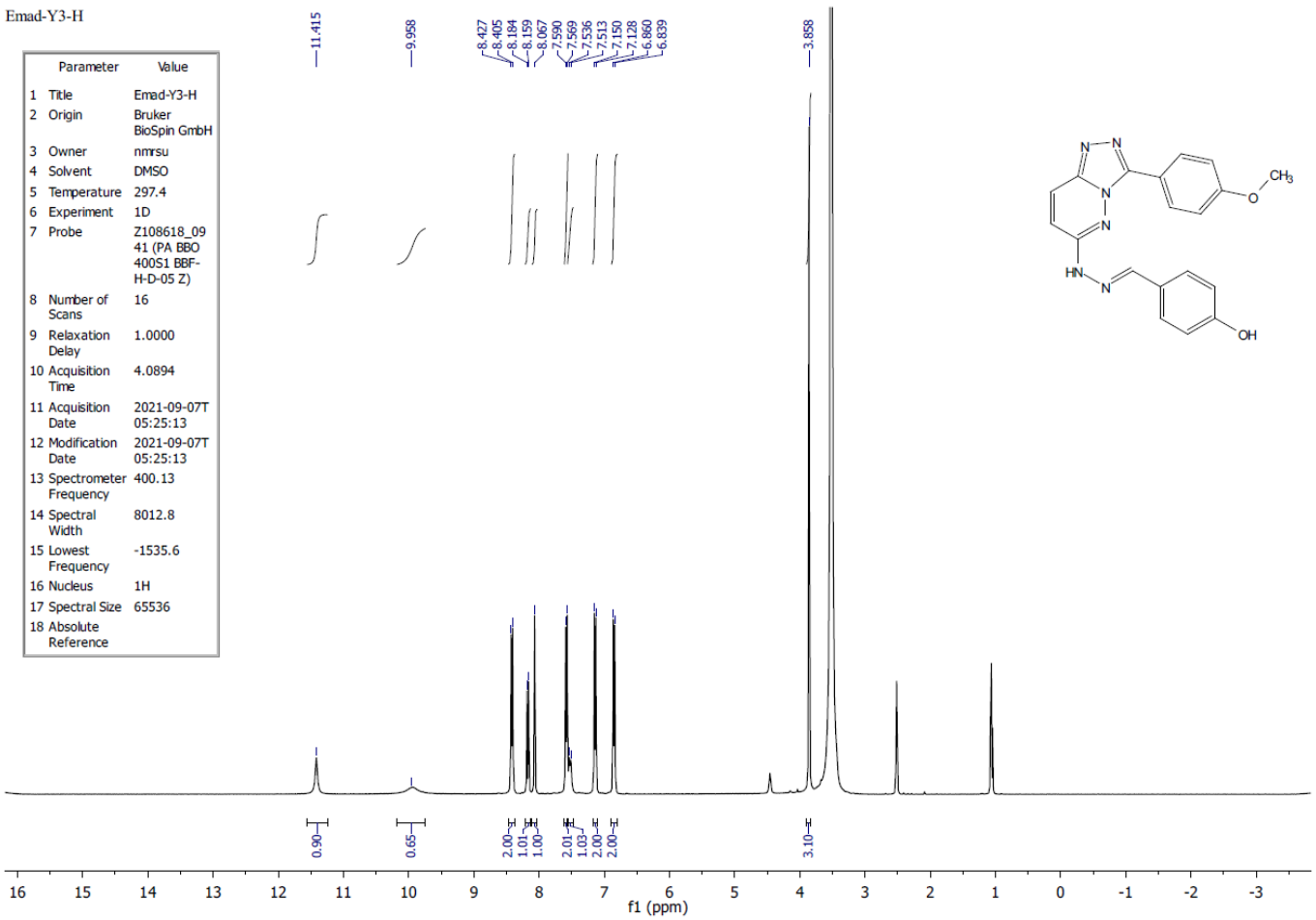
## Compound 4g


N
N
N
N
O
HN
N
OH


%T
90
75
60
45
30
3491.16
3348.72
3425.58
3332.89
3055.24
2966.52
2931.80
2881.65
2839.22
2789.07
2735.06
2665.62
2588.47
2484.32
2368.59
2345.44
2090.84
2044.54
1948.10
1905.67
1793.80
1759.08
1631.79
1608.63
1592.34
1531.45
1512.19
1462.04
1446.61
1388.75
1350.17
1289.45
1249.87
1161.15
1126.43
1095.57
1029.99
995.27
956.68
921.97
898.83
879.54
833.25
808.25
763.61
725.23
694.37
644.22
617.22
578.64
524.64
486.06
455.20
432.05
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
Dr -Rasha Ahmed Y3

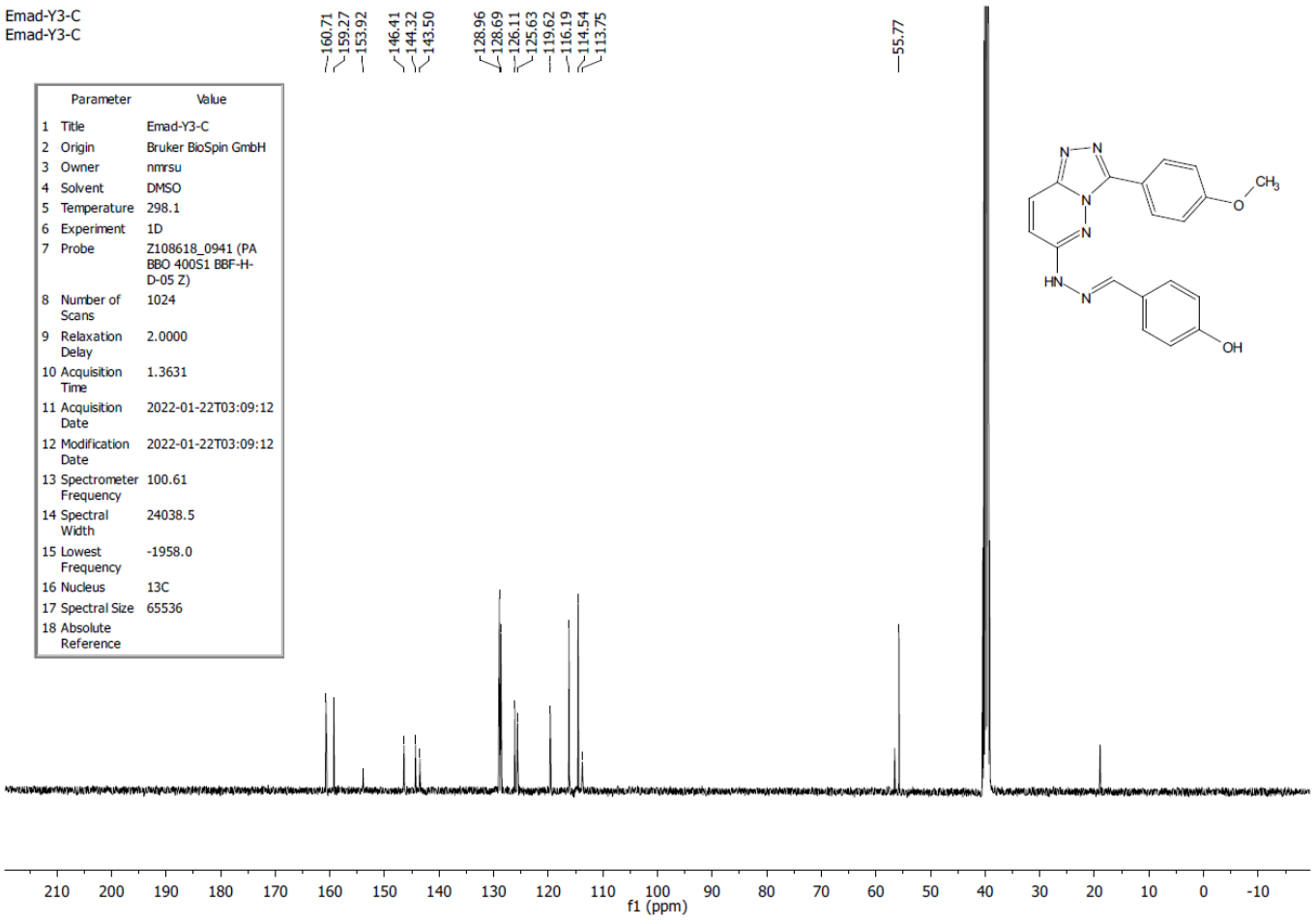
Emad-Y3-H

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y3-H |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 297.4 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 16 |
| 9 | Relaxation Delay | 1.0000 |
| 10 | Acquisition Time | 4.0894 |
| 11 | Acquisition Date | 2021-09-07T 05:25:13 |
| 12 | Modification Date | 2021-09-07T 05:25:13 |
| 13 | Spectrometer Frequency | 400.13 |
| 14 | Spectral Width | 8012.8 |
| 15 | Lowest Frequency | -1535.6 |
| 16 | Nucleus | 1H |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

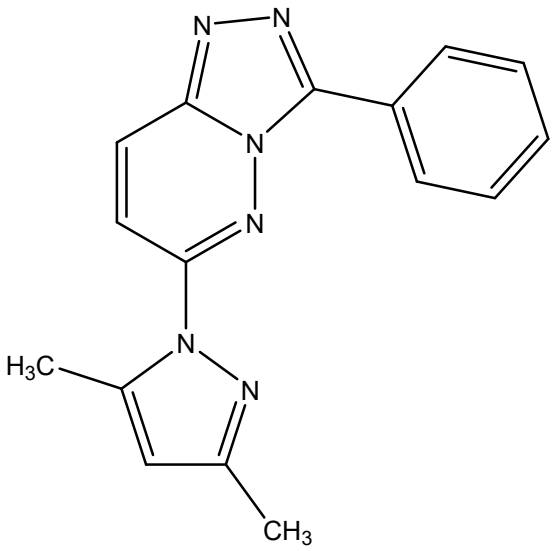


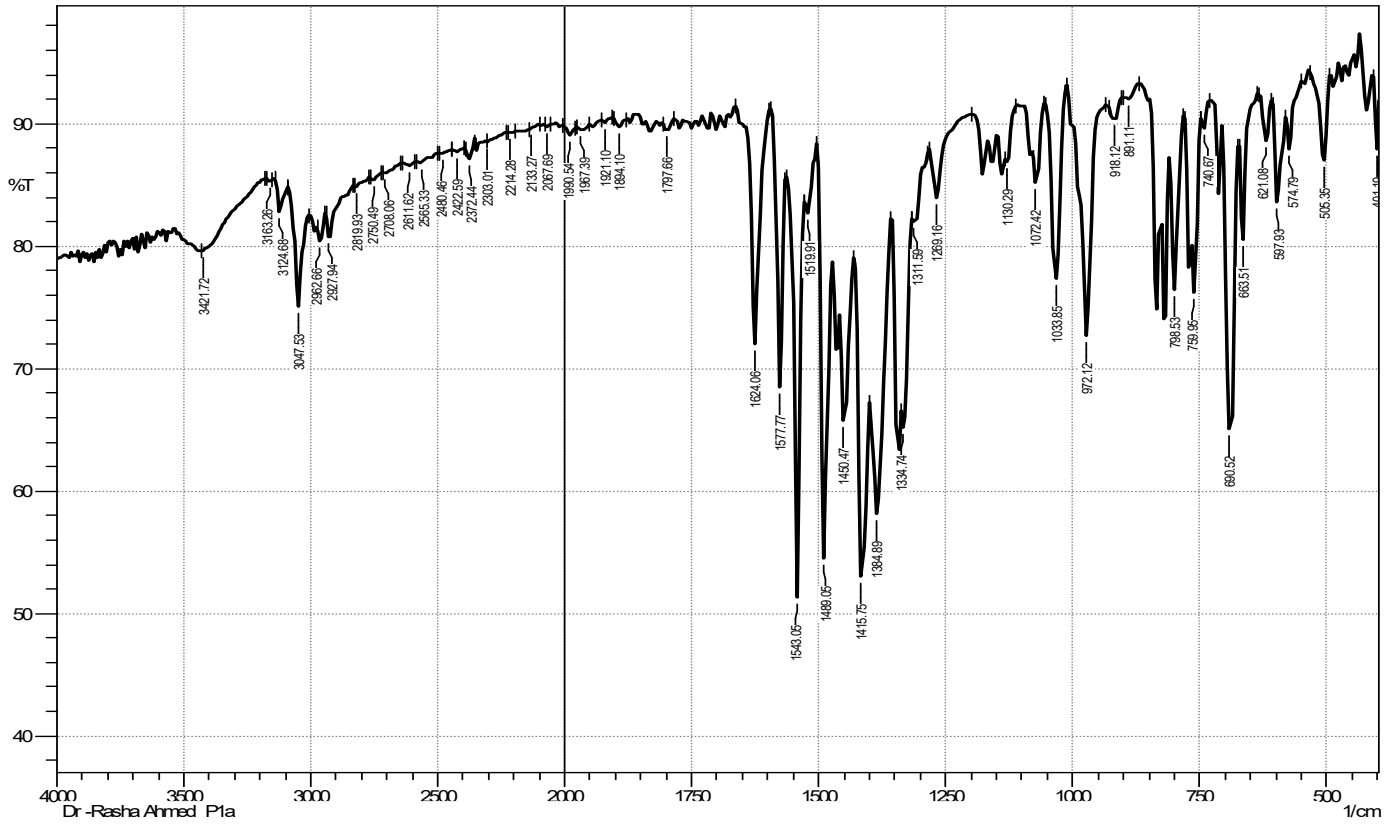

Emad-Y3-C
Emad-Y3-C

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-Y3-C |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 298.1 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 1024 |
| 9 | Relaxation Delay | 2.0000 |
| 10 | Acquisition Time | 1.3631 |
| 11 | Acquisition Date | 2022-01-22T03:09:12 |
| 12 | Modification Date | 2022-01-22T03:09:12 |
| 13 | Spectrometer Frequency | 100.61 |
| 14 | Spectral Width | 24038.5 |
| 15 | Lowest Frequency | -1958.0 |
| 16 | Nucleus | 13C |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

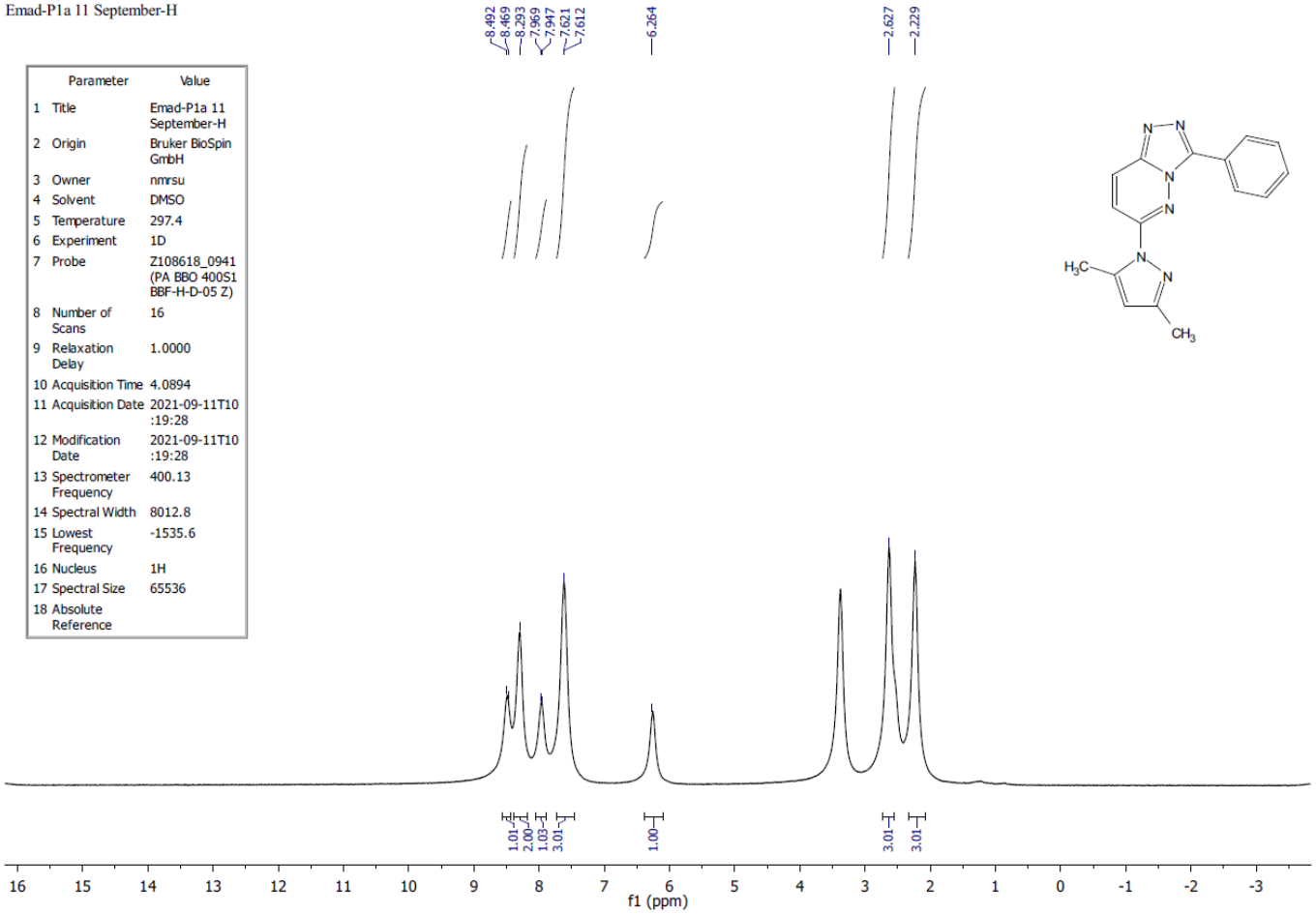


## Compound 5


N
N
N
N
N
H3C
CH3

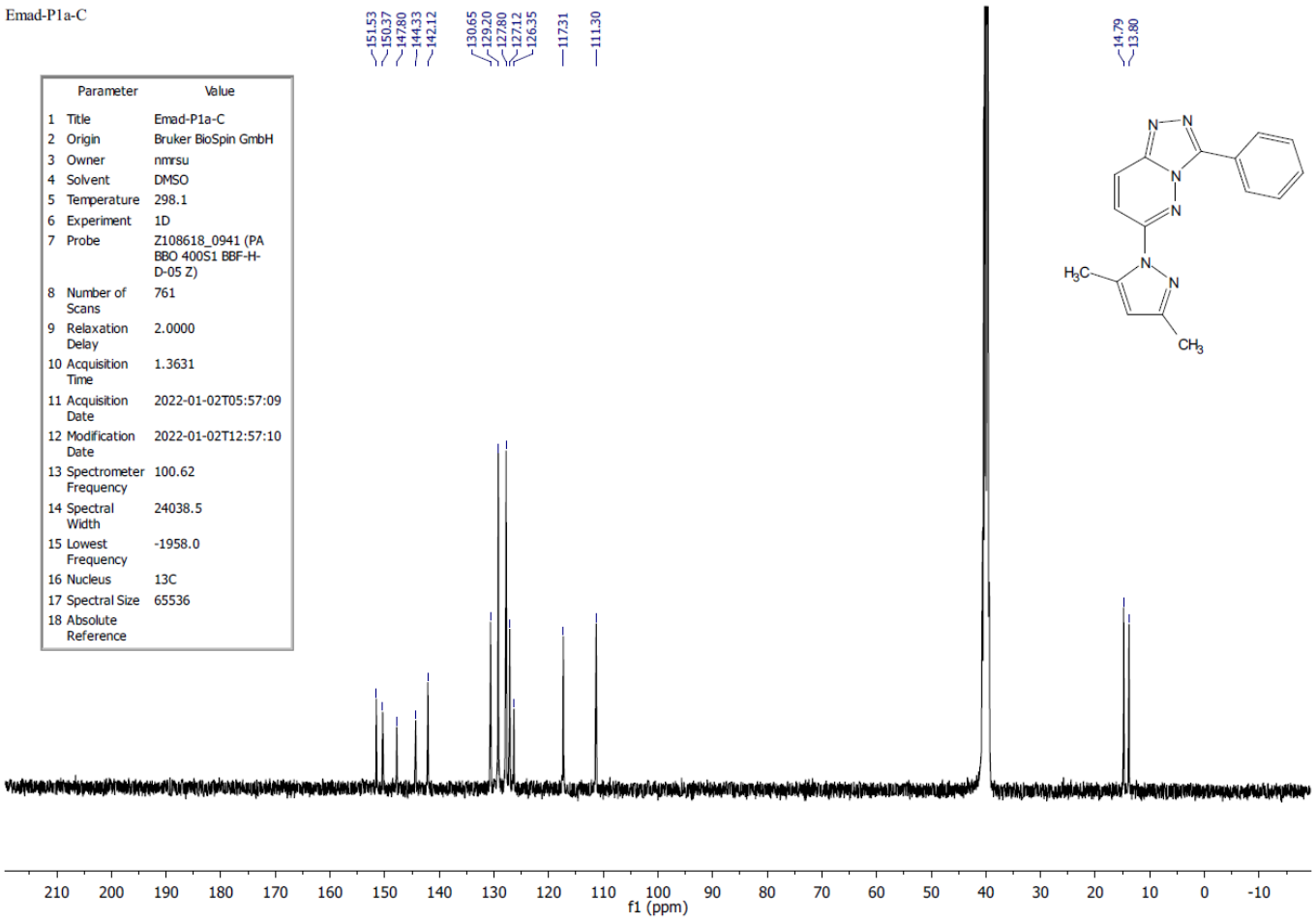

%T
90
80
70
60
50
40
3421.72
3163.36
3124.68
3047.53
2962.66
2927.94
2819.93
2750.49
2708.06
2611.62
2565.33
2480.46
2422.59
2372.44
2303.01
2214.28
2133.27
2067.69
1990.54
1967.39
1921.10
1894.10
1797.66
1624.06
1577.77
1519.91
1543.05
1489.05
1450.47
1415.75
1384.89
1334.74
1311.59
1269.16
1130.29
1072.42
1033.85
972.12
918.12
891.11
798.53
759.95
740.67
690.52
663.51
621.08
597.93
574.79
505.35
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
Dr -Rasha Ahmed P1a

Emad-P1a 11 September-H



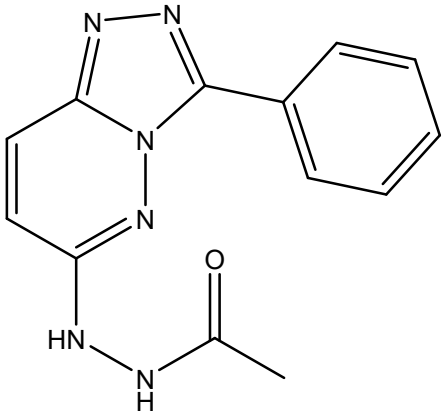

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-P1a 11 September-H |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 297.4 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 16 |
| 9 | Relaxation Delay | 1.0000 |
| 10 | Acquisition Time | 4.0894 |
| 11 | Acquisition Date | 2021-09-11T10:19:28 |
| 12 | Modification Date | 2021-09-11T10:19:28 |
| 13 | Spectrometer Frequency | 400.13 |
| 14 | Spectral Width | 8012.8 |
| 15 | Lowest Frequency | -1535.6 |
| 16 | Nucleus | 1H |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

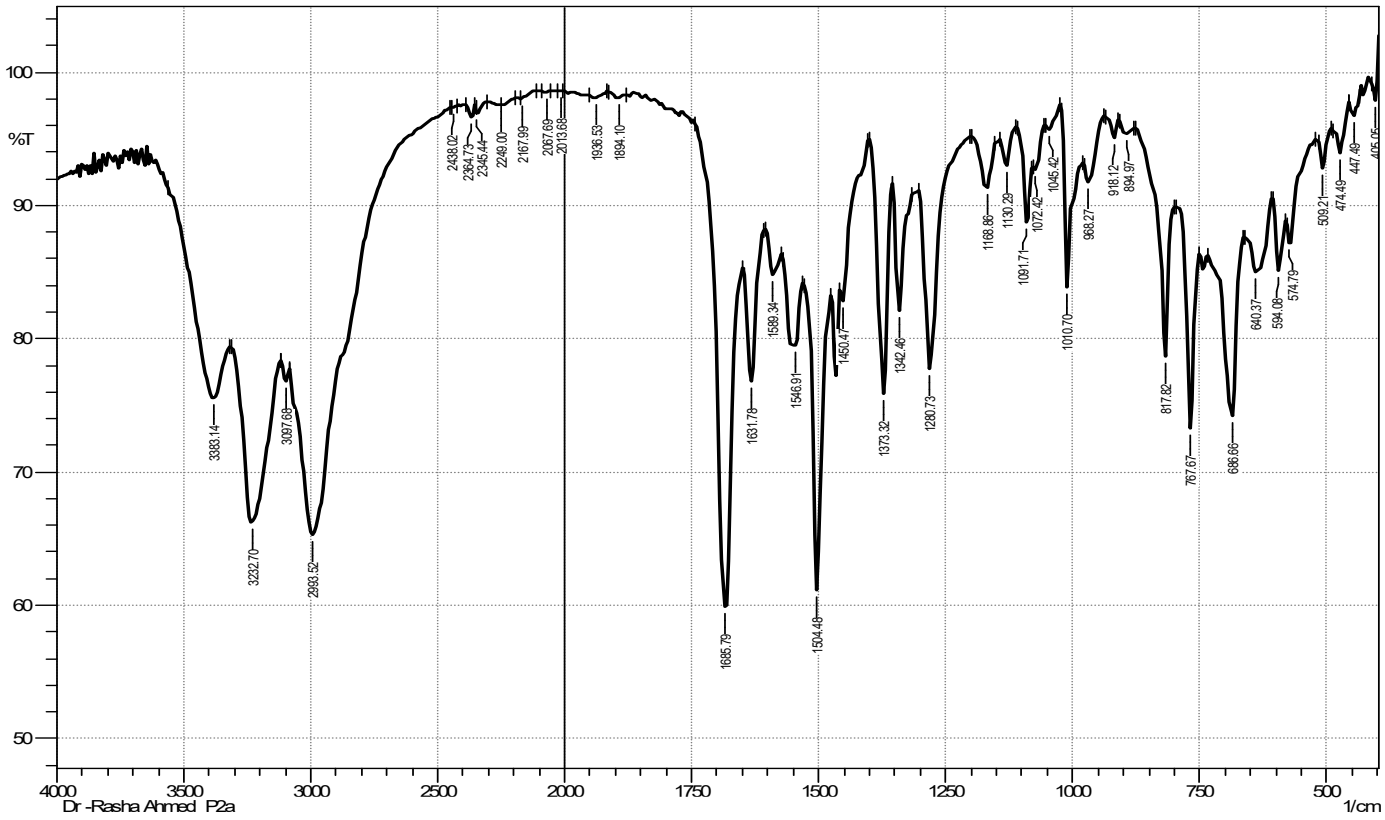
Emad-P1a-C




| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-P1a-C |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 298.1 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 761 |
| 9 | Relaxation Delay | 2.0000 |
| 10 | Acquisition Time | 1.3631 |
| 11 | Acquisition Date | 2022-01-02T05:57:09 |
| 12 | Modification Date | 2022-01-02T12:57:10 |
| 13 | Spectrometer Frequency | 100.62 |
| 14 | Spectral Width | 24038.5 |
| 15 | Lowest Frequency | -1958.0 |
| 16 | Nucleus | 13C |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

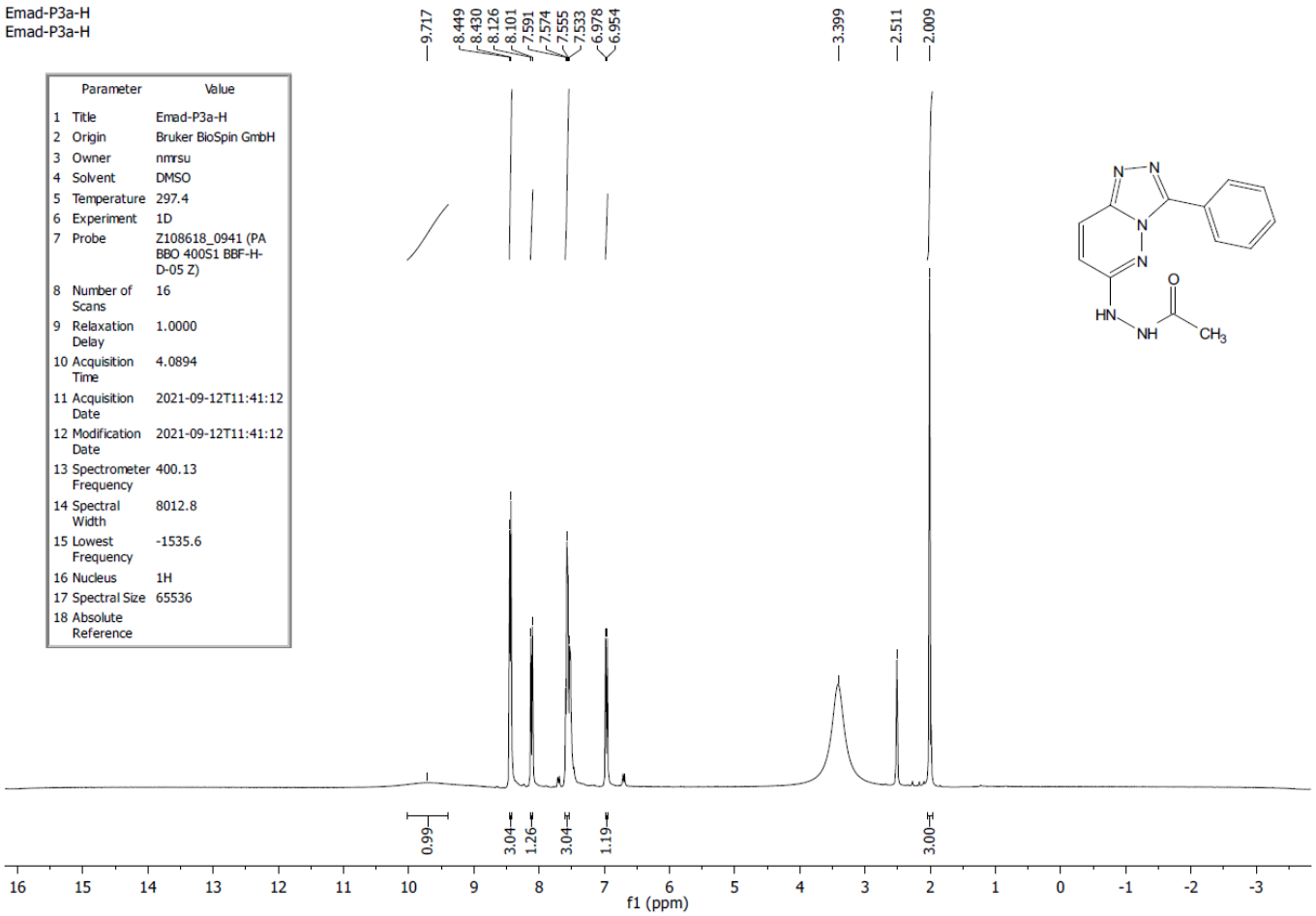
## Compound 6a


N
N
N
N
O
HN
N
H


100
%T
90
80
70
60
50
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
Dr -Rasha Ahmed P2a
3383.14
3232.70
3097.68
2993.52
2438.02
2364.73
2345.44
2249.00
2167.99
2067.69
2013.68
1936.53
1894.10
1685.79
1631.78
1598.34
1546.91
1504.48
1450.47
1373.32
1342.46
1280.73
1188.86
1130.29
1091.71
1072.42
1045.42
1010.70
968.27
918.12
894.97
817.82
767.67
686.66
640.37
594.06
574.79
509.21
474.49
447.49

Emad-P3a-H
Emad-P3a-H

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-P3a-H |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 297.4 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 16 |
| 9 | Relaxation Delay | 1.0000 |
| 10 | Acquisition Time | 4.0894 |
| 11 | Acquisition Date | 2021-09-12T11:41:12 |
| 12 | Modification Date | 2021-09-12T11:41:12 |
| 13 | Spectrometer Frequency | 400.13 |
| 14 | Spectral Width | 8012.8 |
| 15 | Lowest Frequency | -1535.6 |
| 16 | Nucleus | 1H |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |

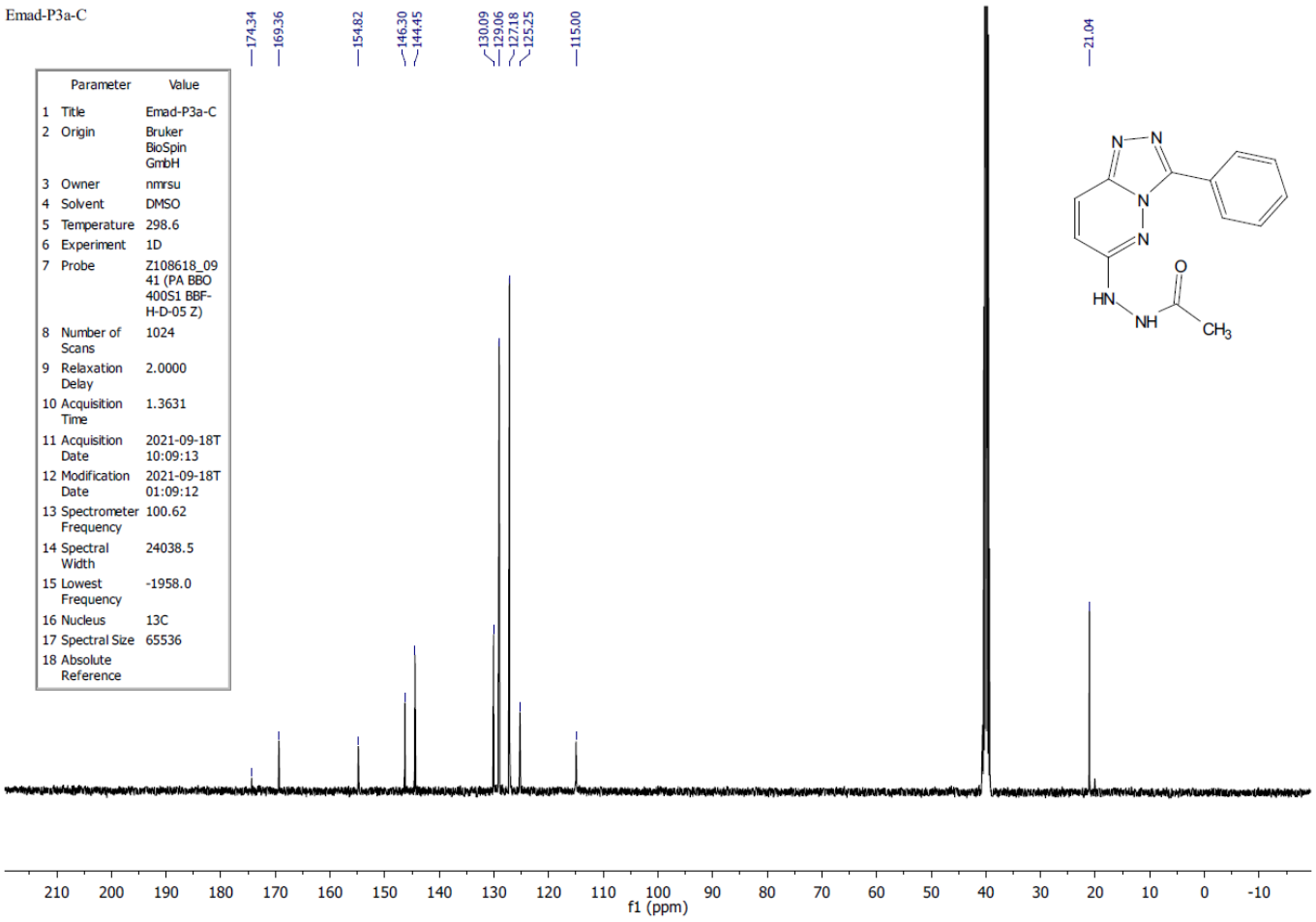




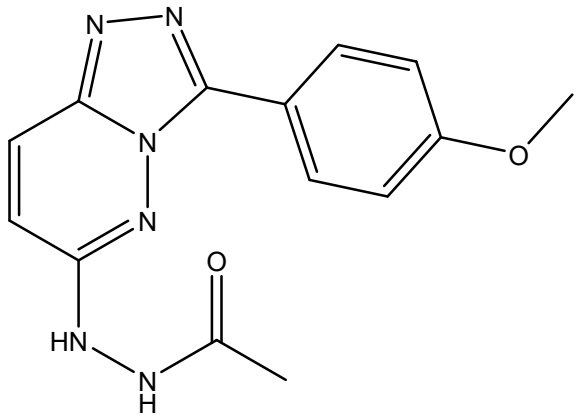


Emad-P3a-C

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-P3a-C |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 298.6 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 1024 |
| 9 | Relaxation Delay | 2.0000 |
| 10 | Acquisition Time | 1.3631 |
| 11 | Acquisition Date | 2021-09-18T10:09:13 |
| 12 | Modification Date | 2021-09-18T01:09:12 |
| 13 | Spectrometer Frequency | 100.62 |
| 14 | Spectral Width | 24038.5 |
| 15 | Lowest Frequency | -1958.0 |
| 16 | Nucleus | 13C |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |



## Compound 6b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N
N
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HN
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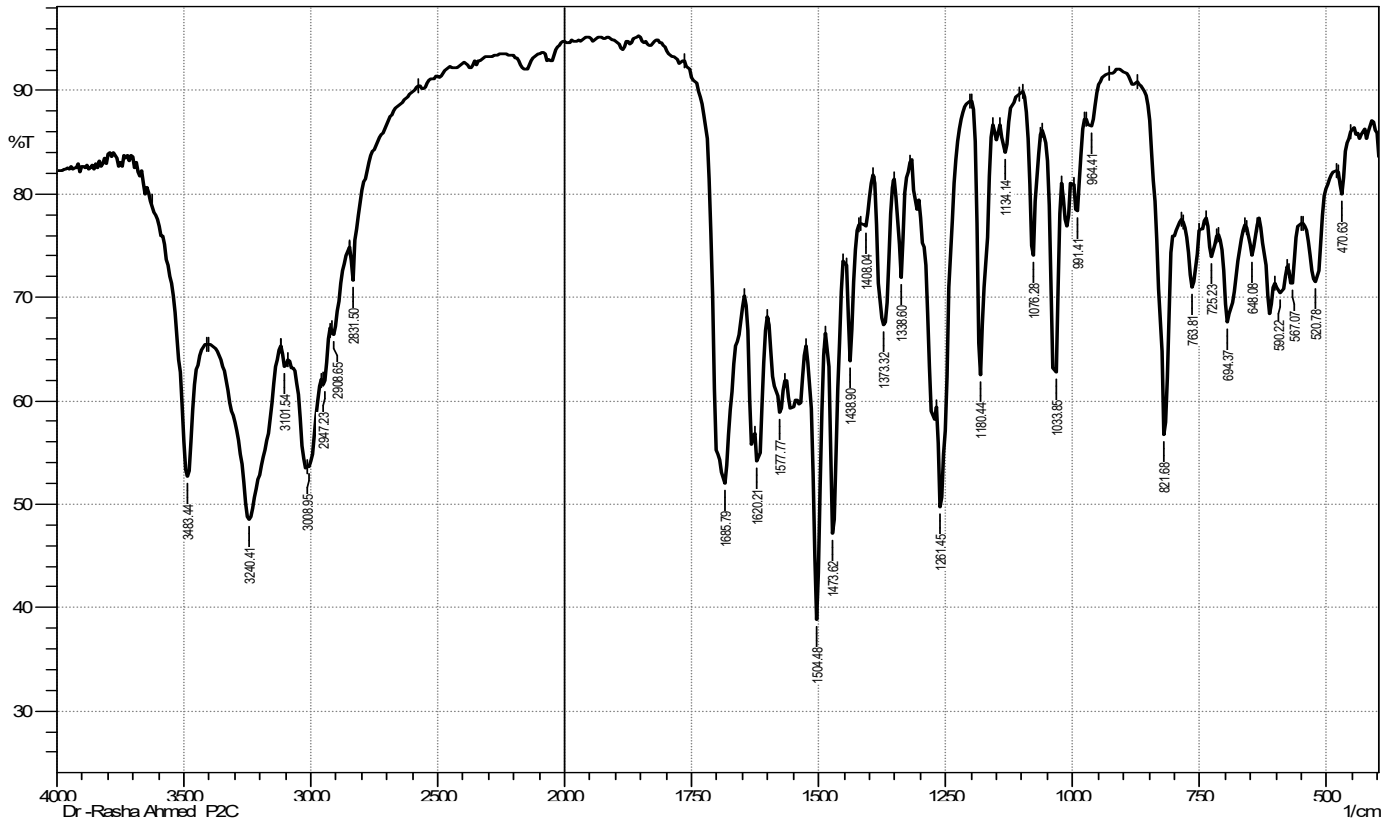
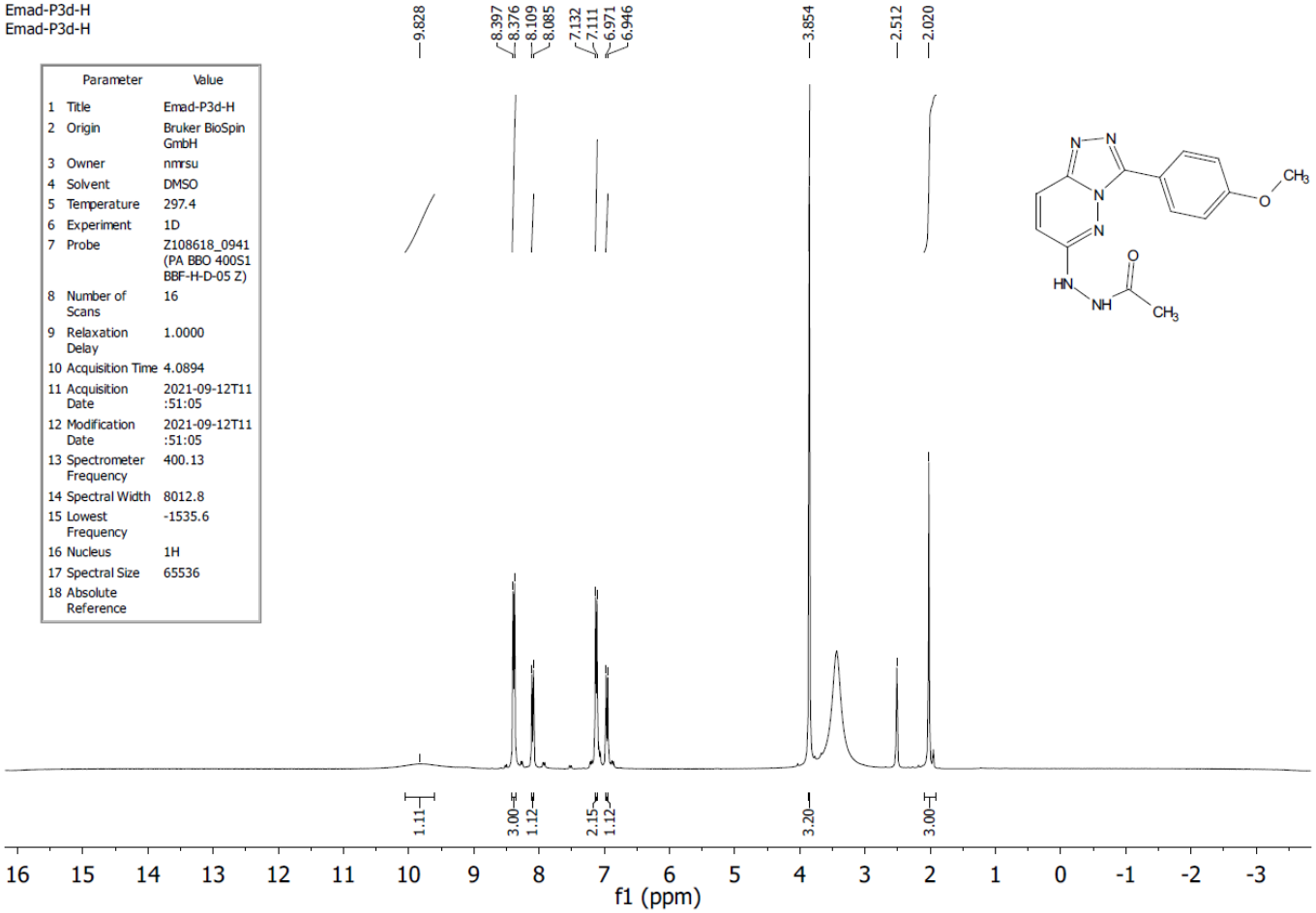

%T
90
80
70
60
50
40
30
3463.44
3240.41
3101.54
3008.95
2947.23
2908.65
2831.50
1686.79
1620.21
1577.77
1504.48
1473.62
1438.90
1408.04
1373.32
1338.60
1261.45
1180.44
1134.14
1076.28
1033.85
991.41
964.41
821.68
763.81
725.23
694.37
648.06
590.22
567.07
520.78
470.63
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
Dr -Rasha Ahmed P2C

Emad-P3d-H
Emad-P3d-H

| | Parameter | Value |
|---|---|---|
| 1 | Title | Emad-P3d-H |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmrsu |
| 4 | Solvent | DMSO |
| 5 | Temperature | 297.4 |
| 6 | Experiment | 1D |
| 7 | Probe | Z108618_0941 (PA BBO 400S1 BBF-H-D-05 Z) |
| 8 | Number of Scans | 16 |
| 9 | Relaxation Delay | 1.0000 |
| 10 | Acquisition Time | 4.0894 |
| 11 | Acquisition Date | 2021-09-12T11:51:05 |
| 12 | Modification Date | 2021-09-12T11:51:05 |
| 13 | Spectrometer Frequency | 400.13 |
| 14 | Spectral Width | 8012.8 |
| 15 | Lowest Frequency | -1535.6 |
| 16 | Nucleus | 1H |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |




Rasha Ahmed_C_P2C

| | Parameter | Value |
|---|---|---|
| 1 | Title | Rasha Ahmed_C_P2C |
| 2 | Origin | Bruker BioSpin GmbH |
| 3 | Owner | nmr |
| 4 | Solvent | DMSO |
| 5 | Temperature | 298.0 |
| 6 | Experiment | 1D |
| 7 | Probe | 5 mm PABBO BB/ 19F-1H/ D Z-GRD Z108618/ 0610 |
| 8 | Number of Scans | 1200 |
| 9 | Relaxation Delay | 2.0000 |
| 10 | Acquisition Time | 1.3631 |
| 11 | Acquisition Date | 2022-05-10T16:51:00 |
| 12 | Modification Date | 2022-05-10T16:51:57 |
| 13 | Spectrometer Frequency | 100.64 |
| 14 | Spectral Width | 24038.5 |
| 15 | Lowest Frequency | -1957.4 |
| 16 | Nucleus | 13C |
| 17 | Spectral Size | 65536 |
| 18 | Absolute Reference | |