Supporting Information

Steering the Azido-Tetrazole Equilibrium of 4-Azidopyrimidines *via* substituent variation – Implications for Drug Design and Azide Alkyne Cycloadditions

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Content

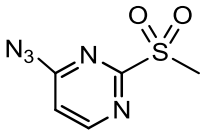
I. Experimental

All reagents used were of reagent grade or higher. ^{1}H and ^{13}C-NMR spectra were recorded on Bruker Fourier spectrometers (500/300 or 176/126/75 MHz) at ambient temperature with the chemical shifts recorded as δ values in ppm units by reference to the hydrogenated residues of deuteriated solvent as internal standard. Coupling constants (J) are given in Hz, and signal patterns are indicated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet, br, broad signal. The Surveyor LC system consisted of a pump, an autosampler, and a PDA detector. Mass spectrometry was performed on a MSQ electrospray mass spectrometer (ThermoFisher, Dreieich, Germany).The system was operated by the standard software Xcalibur. A RP C18 NUCLEODUR 100-5 (125 mm × 3 mm) column (Macherey-Nagel GmbH, Dühren, Germany) was used as the stationary phase. All solvents were HPLC grade. In a gradient run, the percentage of acetonitrile (containing 0.1% trifluoroacetic acid) was increased from an initial concentration of 0% at 0 min to 100% at 15 min and kept at 100% for 5 min. The injection volume was 10 µl, and flow rate was set to 800 µL/min. MS analysis was carried out at a spray voltage of 3800 V and a capillary temperature of 350 °C and a source CID of 10 V. Spectra were acquired in positive mode from 100 to 1000 m/z at 254 nm for the UV trace. IR Spectra were recorded on a Perkin Elmer FT-IR Spectrum 100 spectrometer equipped with an UATR accessory. High Resolution mass Spectrometry for compounds **5** and **9** were performed on a Dionex Ultimate 3000 RSLC system using a Waters BEH C18, 50 x 2.1 mm, 1.7 µm dp column by injection of two µl methanolic sample. Separation was achieved by a linear gradient with (A) H2O + 0.1 % FA to (B) ACN + 0.1 % FA at a flow rate of 600 µl/min and 45 °C. The gradient was initiated by a 0.33 min isocratic step at 5 % B, followed by an increase to 95 % B in 9 min to end up with a 1 min flush step at 95 % B before reequilibration under the initial conditions. UV and MS detection were performed simultaneously. Coupling the HPLC to the MS was supported by an Advion Triversa Nanomate nano-ESI system attached to a Thermo Scientific Orbitrap. Mass spectra were acquired in centroid mode ranging from 200 – 2000 m/z at a resolution of R = 30000. High Resolution Mass Spectrometry for compounds **1**-**4**, **6**-**8** and **10**-**12** was performed on a Dionex Ultimate 3000 RSLC system using a Waters BEH C18, 50 x 2.1 mm, 1.7 µm dp column. Separation of 1 µl sample was achieved by a linear gradient with (A) H2O + 0.1 % FA to (B) ACN + 0.1 % FA at a flow rate of 600 µl/min and 45 °C. The gradient was initiated by a 1 min isocratic step at 5 % B, followed by an increase to 95 % B in 6 min to end up with a 1.5 min step at 95 % B before reequilibration under the initial conditions. UV spectra were recorded by a DAD in the range from 200 to 600 nm. The LC flow was split to 75 µl/min before entering the maXis 4G hr-ToF mass spectrometer (Bruker Daltonics, Bremen, Germany) using the standard ESI source. Mass spectra were acquired in centroid negative mode ranging from 150 – 2000 m/z at a 2 Hz scan speed.

***Caution**: High nitrogen-content compounds are known to be instable. Although we experienced no difficulties in handling these compounds, all experiments were performed in small scale and with best safety precautions (e.g. gloves, protective eyewear, labcoat, shield)!*

a) Chemical synthesis

1: 4-azido-2-(methylsulfonyl)pyrimidine



2.0 g (12 mmol) of **3** was dissolved in EtOAc (30 ml) and 22.2g Oxone (36 mmol) dissolved in 50 ml water was added. The mixture was stirred for 3 hrs at ambient temperature. The aqueous layer was extracted 3 times with 60 ml EtOAc. The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo* to yield **1** as a pale yellow solid (2.2 g, 92%).

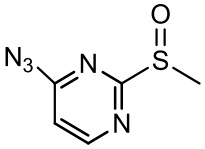
^{1}H-NMR (300 MHz, DMSO-d_6): δ = 8.90 (d, *J*=5.5 Hz, 1 H), 7.40 (d, *J*=5.5 Hz, 1 H), 3.41 ppm (s, 3 H) ppm;

^{13}C-NMR (75 MHz, DMSO-d_6): δ = 165.2, 163.4, 160.0, 113.8 ppm;

ESI-MS(+): m/z 200.0 $[M+H]^+$;

HRMS: m/z calcd. for $C_5H_6N_5O_2S^+$: 200.02367 found: 200.02353 $[M+H]^+$.

2: 4-azido-2-(methylsulfinyl)pyrimidine



70mg (0.4 mmol) of **3** was dissolved in EtOAc (5 ml) and 60mg oxone (0.2 mmol) dissolved in 5 ml water was added. The mixture was stirred for 3 hours at ambient temperature. The aqueous layer was extracted 3 times with 30 ml EtOAc. The combined organic layers were dried over $NaSO_4$ and the solvent was removed *in vacuo*. The residue was purified by flashchromatography (ethylacetate:methanol, 97.5:2.5) to yield **2** as a white solid (15 mg, 17%).

Tetrazole: ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 8.82 (d, *J* = 5.49 Hz, 1H), 7.22 (d, *J* = 5.49 Hz, 1H), 2.90 (s, 3H) ppm;

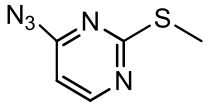
Azide: ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 8.58 - 8.68 (m, 1H), 8.37 - 8.49 (m, 1H), 3.16 (s, 3H) ppm;

^{13}C-NMR (75 MHz, DMSO-d_6): δ = 174.1, 163.4, 160.3, 112.1, 40.1 ppm

ESI-MS(+): m/z 184.1 $[M+H]^+$;

HRMS: m/z calcd. for $C_5H_6N_5OS^+$:184.02876 found: 184.02844 $[M+H]^+$.

3: 4-azido-2-(methylthio)pyrimidine



3.2 g of 4-chloro-2-(methylthio)pyrimidine (20 mmol) was dissolved in DMF (60 ml) and 4.0 g NaN_3 (60 mmol) was added. The suspension was stirred at ambient temperature for 72 hours. To the mixture was added 200 ml water and the aqueous layer was extracted 3 times with 300ml EtOAc. The combined organic layers were dried over $NaSO_4$, filtered and the solvent was removed *in vacuo* to obtain a brownish oil. Residual DMF was removed by azeotropic destillation with heptane to yield **3** as a pale yellow solid (3.3 g, 98%).

Tetratole: ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta =$, 8.37 (d, J = 6.33 Hz, 1H), 7.99 (d, J = 6.33 Hz, 1H),, 2.80 (s, 3H) ppm;

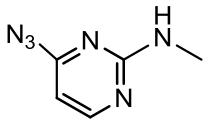
Azide: ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta =$ 8.49 (d, J = 5.40 Hz, 1H), 6.78 (d, J = 5.59 Hz, 1H), 2.52 (s, 3H) ppm;

^{13}C-NMR (75 MHz, $CDCl_3$) $\delta =$ 173.0, 161.9, 158.1, 152.9, 149.1, 145.5, 105.8, 105.0, 14.0, 13.5 ppm;

ESI-MS(+): m/z 168.1 $[M+H]^+$;

HRMS: m/z calcd. for $C_5H_6N_5S^+$: 169.03384 found: 168.03362 $[M+H]^+$.

4: 4-azido-N-methylpyrimidin-2-amine



100 mg of **1** (0.5 mmol) was dissolved in EtOH, 203 µl TEA (1.5 mmol) and 130 µl 40% aminomethyl in water (3 mmol) was added in a krimp vial. The mixture was heated to 60°C for 3 hrs. Solvent was removed *in vacuo* and the residue was purified by flashchromatography (Ethylacetate:Hexane, 1:1) to yield **4** as white needles (60 mg, 80%).

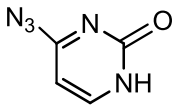
^{1}H-NMR (300 MHz, DMSO-d_6) $\delta =$ 8.90 (br. s., 1H), 8.01 (d, J = 6.24 Hz, 1H), 7.23 (d, J = 6.24 Hz, 1H), 3.06 (d, J = 3.26 Hz, 3H) ppm;

^{13}C-NMR (75 MHz, DMSO-d_6) $\delta =$ 150.3, 148.0, 145.2, 96.3, 27.8 ppm;

ESI-MS(+): m/z 151.0 $[M+H]^+$;

HRMS: m/z calcd. for $C_5H_7N_6^+$: 151.07267 found: 151.07249 $[M+H]^+$.

5: 4-azidopyrimidin-2(1H)-one



100 mg (0.5 mmol) of **1** was dissolved in Dioxane (2 ml) and 20 mg NaOH (0.5 mmol) in 3 ml Water was added. The mixture was heated to 100°C in a krimp vial for 3 hours. Solvent was removed *in vacuo* and the residue was purified by flashchromatography (ethylacetate:hexane, 7:3) yielding **5** as a white solid (30 mg, 43%).

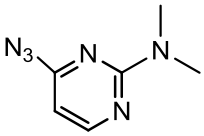
^{1}H-NMR (300 MHz, DMSO-d_6) δ = 12.44 (br. s., 1H), 7.68 (d, J = 7.36 Hz, 1H), 6.99 (d, J = 7.36 Hz, 1H) ppm;

^{13}C-NMR (75 MHz, DMSO-d_6) δ = 152.0, 143.3, 136.9, 92.7 ppm;

ESI-MS(+): m/z 138.0 $[M+H]^+$;

HRMS: m/z calcd for $C_4H_2N_5O^-$:136.0265 found: 136.0266 $[M-H]^-$.

6: 4-azido-N,N-dimethylpyrimidin-2-amine



100 mg (0.5 mmol) of **1** was dissolved in THF, 203µl TEA (1.5 mmol) and 750 µl of dimethylamine in THF (1.5mmol) was added. The mixture was heated to 60°C in a krimp vial. Solvent was removed *in vacuo* and the residue was purified by flashchromatography (ethylacetate:hexane, 2:8) to yield **6** as a colorless oil (50mg, 61%).

Tetrazole: 7.97 (d, J = 6.15 Hz, 1H), 7.29 (d, J = 6.05 Hz, 1H), 3.50 (s, 6H) ppm;

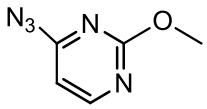
Azide: ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 8.23 (d, J = 5.22 Hz, 1H), 6.15 (d, J = 5.22 Hz, 1H), 3.11 (s, 6H) ppm;

^{13}C-NMR (75 MHz, DMSO-d_6) δ = 161.5, 161.3, 159.7, 98.2, 36.3 ppm;

ESI-MS(+): m/z 165.0 $[M+H]^+$;

HRMS: m/z calcd. for $C_6H_9N_6^+$: 165.08832 found: 165.08836 $[M+H]^+$.

7: 4-azido-2-methoxypyrimidine



100 mg (0.5 mmol) of **1** was dissolved in dry MeOH and 105 mg sodium methanolate 40% in methanol (0.75 mmol) was added. The mixture was stirred at room temperature in a krimp vial. Solvent was removed *in vacuo* and the residue was purified by flashchromatography (ethylacetate:hexane, 1:9) to yield **7** as a white solid (15 mg, 20%).

Tetrazole: ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta =$ 8.48 (d, J = 5.40 Hz, 1H), 6.72 (d, J = 5.40 Hz, 1H), 3.92 (s, 3H) ppm;

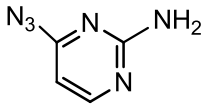
Azide: ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta =$ 8.16 (d, J = 6.43 Hz, 1H), 7.80 (d, J = 6.43 Hz, 1H), 4.29 (s, 3H) ppm;

^{13}C-NMR (126 MHz, DMSO-d_6): δ = 164.9, 163.3, 161.0, 151.7, 148.0, 146.2, 104.7, 103.4, 57.0, 54.7 ppm;

ESI-MS(+): m/z 152.0 $[M+H]^+$;

HRMS: m/z calcd. for $C_5H_6N_5O^+$:152.05669 found: 152.05643 $[M+H]^+$.

8: 4-azidopyrimidin-2-amine



100 mg (0.5 mmol) of **1** was dissolved in 2 ml MeOH, 1.3 ml of 7 N Ammonia in MeOH (1.7 mmol) and 203 µl TEA (1.5 mmol) was added. The mixture was heated to 60°C in a Krimp vial. Solvent was removed *in vacuo* and the residue was purified by flashchromatography (Ethylacetate:Hexane, 6:4) to yield **8** as a brownish solid (30 mg, 44%).

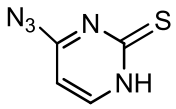
^{1}H-NMR (300 MHz, DMSO-d_6) $\delta =$ 8.54 (br. s., 2H), 7.95 (d, J = 6.24 Hz, 1H), 7.23 (d, J = 6.33 Hz, 1H) ppm;

^{13}C-NMR (75 MHz, DMSO-d_6) $\delta =$ 150.6, 148.3, 146.2, 96.6 ppm;

ESI-MS(+): m/z 137.0 $[M+H]^+$;

HRMS: m/z calcd. for $C_4H_5N_6^+$: 137.05702 found: 137.05644 $[M+H]^+$.

9: 4-azidopyrimidine-2(1H)-thione



100 mg (0.5 mmol) of **1** was given to a mixture of EtOH (2.5 ml) and 0.025M HCl_{aq} (2.5 ml). 158 mg of NaS_2O_3 (1 mmol) was added and the suspension was refluxed for 16 hours. A white precipitate formed which was filtered, washed 3 times with water and dried *in vacuo* to give **9** as a white solid (35 mg, 45%).

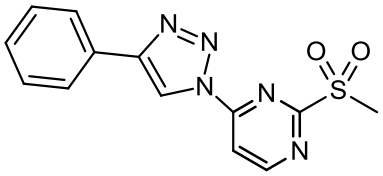
^{1}H-NMR (300 MHz, DMSO-d_6) δ = 14.15 (br. s., 1H), 7.77 (d, J = 7.26 Hz, 1H), 7.43 (d, J = 7.26 Hz, 1H) ppm;

^{13}C-NMR (75 MHz, DMSO-d_6) δ = 165.8, 148.1, 136.7, 97.9 ppm;

ESI-MS(+): m/z 154.0 $[M+H]^+$;

HRMS: m/z calcd. for $C_4H_2N_5S^-$: 152.0036 found: 152.0009 $[M-H]^-$.

10: 2-(methylsulfonyl)-4-(4-phenyl-1H-1,2,3-triazol-1-yl)pyrimidine



100 mg of **1** (0.5 mmol) and 51 mg phenylacetylene (0.5 mmol) was dissolved in 5ml *tert*BuOH:H_2O (1:1), 10 mg sodium ascorbate (0.1 mmol) and 2.5 mg $CuSO_4x5H_2O$ (0.02 mmol) was added argon atmosphere. The mixture was stirred at ambient temperature for 24 hours. The reaction mixture was extracted 3 times with EtOAc and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (Ethylacetate:Hexane, 1:1) to yield **10** as a white solid (82 mg, 55%).

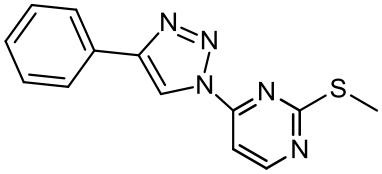
^{1}H-NMR (300 MHz, DMSO-d_6) δ = 9.60 (s, 1H), 9.29 (d, J = 5.59 Hz, 1H), 8.47 (d, J = 5.59 Hz, 1H), 7.99 - 8.19 (m, 2H), 7.48 - 7.58 (m, 2H), 7.24 - 7.48 (m, 1H), 3.60 (s, 3H) ppm;

^{13}C-NMR (75 MHz, DMSO-d_6) δ = 165.9, 162.6, 155.8, 148.4, 129.7, 129.5, 129.4, 126.3, 119.3, 113.4, 39.5 ppm;

ESI-MS(+): m/z 302 $[M+H]^+$;

HRMS: m/z calcd. for $C_{13}H_{12}N_5O_2S^+$: 302.07062 found: 302.07065 $[M+H]^+$.

11: 2-(methylthio)-4-(4-phenyl-1H-1,2,3-triazol-1-yl)pyrimidine



83 mg of **3** (0.5 mmol) and 51 mg phenylacetylene (0.5 mmol) was dissolved in 5ml *tert*BuOH:H_2O (1:1), 10 mg sodium ascorbate (0.1 mmol) and 2.5mg $CuSO_4x5H_2O$ (0.02 mmol) was added under inert atmosphere. The mixture was stirred at ambient temperature for 24 hours. The reaction mixture was extracted 3 times with EtOAc and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (ethylacetate:hexane, 3:7) to yield **11** as a brownish solid (108 mg, 81%).

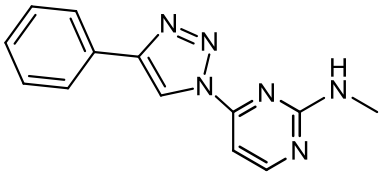
^{1}H-NMR (300 MHz, $CDCl_3$) δ = 8.78 (s, 1H), 8.71 (d, J = 5.40 Hz, 1H), 7.91 - 8.04 (m, 2H), 7.85 (d, J = 5.40 Hz, 1H), 7.44 - 7.58 (m, 2H), 7.35 - 7.44 (m, 1H), 2.66 (s, 3H) ppm;

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 173.7, 159.6, 154.9, 148.5, 129.6, 129.0, 128.9, 126.0, 116.5, 104.8, 14.3 ppm;

ESI-MS(+): m/z 270.0 $[M+H]^+$;

HRMS: m/z calcd. for $C_{13}H_{12}N_5S^+$: 270.08079 found: 270.08099 $[M+H]^+$.

12: *N*-methyl-4-(4-phenyl-1H-1,2,3-triazol-1-yl)pyrimidin-2-amine



75 mg of **1** (0.5 mmol) and 51 mg phenylacetylene (0.5 mmol) was dissolved in 5 ml *tert*BuOH:H_2O (1:1), 100 mg sodium ascorbate (0.5 mmol) and 25 mg $CuSO_4x5H_2O$ (0.1 mmol) was added. The mixture was stirred at ambient temperature for 24 hrs. The reaction mixture was extracted 3 times with EtOAc and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (ethylacetate:hexane, 1:1) to yield **12** as a brownish solid (64 mg, 51 %).

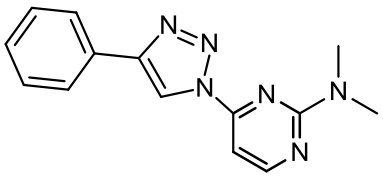
^{1}H-NMR (300 MHz, $CDCl_3$) δ = 8.73 (s, 1H), 8.47 (d, J = 5.12 Hz, 1H), 7.88 - 8.02 (m, 2H), 7.35 - 7.57 (m, 4H), 5.36 (br. s., 1H), 3.11 (d, J = 5.12 Hz, 3H) ppm;

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 162.8, 160.8, 155.8, 148.0, 129.9, 128.9, 128.6, 126.0, 116.4, 98.7, 28.5 ppm;

ESI-MS(+): m/z 253.3 $[M+H]^+$;

HRMS: m/z calcd. for $C_{13}H_{13}N_6^+$: 253.11962 found: 253.11971 $[M+H]^+$.

14: *N,N*-dimethyl-4-(4-phenyl-1H-1,2,3-triazol-1-yl)pyrimidin-2-amine



54 mg of **6** (0.3 mmol) and 34 mg phenylacetylene (0.3 mmol) was dissolved in 3 ml *t*BuOH:H_2O (1:1), 7.5 mg sodium ascorbate (0.0375 mmol) and 2 mg $CuSO_4x5H_2O$ (0.0075 mmol) was added under inert atmosphere. The mixture was stirred at ambient temperature for 24 hrs. The reaction mixture was extracted 3 times with EtOAc and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (ethylacetate:hexane 3:7,) to yield **14** as a colorless solid (64 mg, 68 %).

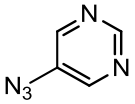
^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.72 (s, 1H), 8.50 (d, J = 5.36 Hz, 1H), 7.91 - 8.01 (m, 2H), 7.45 - 7.52 (m, 2H), 7.40 (tt, J = 1.30, 7.60 Hz, 1H), 7.34 (d, J = 5.04 Hz, 1H), 3.28 (s, 6H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) $\delta =$ 161.9, 160.4, 155.4, 147.9, 130.0, 128.9, 128.6, 126.0, 116.3, 97.0, 37.1 ppm;

ESI-MS(+): m/z 267.0 $[M+H]^+$;

HRMS: m/z calcd. for $C_{14}H_{15}N_6^+$: 267.13527 found: 267.13506 $[M+H]^+$.

15: 5-azidopyrimidine



15 was synthesized as reported before with minor modifications.[1] Briefly, to a solution of 50 mg of pyrimidine-5-boronic acid (0.4 mmol) in MeOH (5 ml) was added 60 mg sodium azide (1.0 mmol) and $CuSO_4$ (0.04 mmol). The suspension was stirred vigorously at room temperature until TLC showed complete conversion of starting material. 50 ml water was added and the aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over sodium sulfate and filtered over a double layer pad of Celite® and silica. Solvent was removed under reduced pressure to obtain the title compound as an off white solid (30 mg, 63 %). Analytical data is in good accordance with the published spectral data of Grimes et al.[1]

FT-IR (cm^{-1}): 3205, 3022, 2925, 2393, 2258, 2191, **2108**, 1727, 1574, 1557, 1437, 1415, 1354, 1296, 1191, 1167, 1097, 1036, 905, 884, 715, 693;

^{1}H-NMR (500 MHz, MeOD) $\delta =$ 8.61 (s, 2H), 8.93 (s, 1H) ppm;

^{13}C-NMR (126 MHz, MeOD) $\delta =$ 138.5, 149.2, 155.3 ppm;

ESI-MS(+): m/z 122.0 $[M+H]^+$.

b) X-Ray Crystallography

Crystallization of **3**:

100 mg of **3** was dissolved in hot chloroform. Crystals formed after 2 days at ambient temperature.

CCDC-Number: 1045217

Crystallization of **4**:

20 mg of **4** was dissolved in hot chloroform. Crystals formed after 4 days at ambient temperature.

CCDC-Number: 1045218

Crystallization of **5**:

20 mg of **5** was dissolved in hot MeOH. Crystals formed after 3 days at ambient temperature.

CCDC-Number: 1045219

X-ray structure determination of **3**-**5**

Crystals suitable for single-crystal x-ray analysis were obtained as described above. The data were collected at 133 K on a BrukerAXS X8Apex CCD diffractometer operating with graphite-monochromatized Mo Kα radiation. Frames of 0.5° oscillation were exposed; deriving data in the θ range of 2 to 30° with a completeness of ~99%. Structure solution and full least-squares refinement with anisotropic thermal parameters of all non-hydrogen atoms and rigid group refinement of the hydrogen were performed using SHELX (.[2] The final refinement result in: [1] R1= 0.026; [2] R1= 0.036; [3] R1= 0.043. Crystallographic data for the structures have been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository numbers CCDC (www.ccdc.cam.ac.uk/data_request/cif).

c) Determination of pK_a

The pKa‘s of **5** and **9** were measured using the SiriusT3 automatic titration system (Sirius Analytical Ltd, Forest Row, UK) and the software supplied with the machine for the refinement of the experimental data. Standard solutions of hydrochloric acid 0.5 M and potassium hydroxide 0.5 M in Millipore water were used as acid and base titrant, respectively. The ionic strength of the water used for dissolving the samples was adjusted adding potassium chloride obtaining a 0.15 M solution. A solution of 50 % acetonitrile - 50 % Millipore water was prepared and potassium chloride was added in it for obtaining a final

mixture 50 % acetonitrile with 0.15 M KCl. In each experiment, **5** was titrated three times in water media from high to low pH, while **9** was titrated three times in acetonitrile – water solution (first titration in 42 %, second titration in 38 % and third titration in 32 % of acetonitrile) from basic to acidic pH. Three independent experiments were performed per each compound at room temperature.

d) Molecular Modelling and Density Functional Calculations

Modelling of steric clash of the aminodimethyl group and N2 of tetrazol of compound **6**:

Based on the crystalstructure of **4** (CCDC-Number: 1045218) an additional methylgroup was introduced to the methylamine, resulting in the tetrazole tautomer of compound **6** (left figure). Steric clashes, indicated in orange, occur between methyl C and N2 of tetrazole, resulting in an energy demand of 4.6 kcal to accommodate this structure. Energy minimization *in vacuo* with AMBER 10:EHT solvation model R-field (MOE) of this structure (right figure, pink structure) yielded a conformation where the methyl group and the tetrazole evade this clash which is accompanied by a deformation of the tetrazolo[1,5-*c*]pyrimidine ring (right figure, grey structure=before energy minimization, pink structure=after energy minimization)

Modelling of compound **5** and **9** into the adenosyl bindingsite of PqsD:

To predict the binding mode of compound **5** and **9** the cocrystal structure of PqsD with anthranoyl-CoA (PDB code: 3H77) was used. The anthranoyl-CoA chain was deleted and only the adenosine moiety was kept for further experiments. The adenosine was modified using Molecular Operating Environment, Chemical Computing Group to compound **5** and **9** and the resulting structures, and the residues in 3.5Å proximity were energy minimized inside the binding site of adenosine. For energy minimization MMFF94x (Merck Forcefield) of MOE software package was used with the preset standard parameters.

Energy optimization of compounds **1**-**9** and solvation contributions:

Structures were energetically optmized at B3LYP/aug-cc-pVDZ level of theory using default parameters. Single point energies were obtained applying the COSMO solvent model[3] as implemented in NWChem (Version 6.1)[4]. The dielectric constants and solvent radii used were for water (78.0, 1.37), for DMSO (47.24, 2.455), and for $CHCl_3$ (4.8069, 2.715), respectively.

e) Calculated physicochemical properties of compounds 1-9

Physicochemical properties were calculated using ACD/Percepta version 2012 (Build 2203, 29 jan. 2013), ACD/Labs. Calculation of LogP values was done using consensus LogP model and for pKa calculation the pKa classic module was used.

Cmpd	$cLogD_{7,4}$		cLogP		cpK_a		
	T	A	T	A	T	A	Exp
1	-1.16	0.37	-1.16	0.37			
2	-1.34	0.05	-1.34	0.05			
3	-0.06	1.96	-0.06	1.96			
4	-0.79	1.49	-0.79	1.49			
5	-2.63	-0.74	-1.21	-0.72	5.9	8.7	6.8
6	-0.59	1.72	-0.59	1.72			
7	-0.22	1.21	-0.22	1.21			
8	-1.05	0.88	-1.05	0.88			
9	-0.64	-1.35	-0.15	-0.35	7.1	6.4	4.6

T=Tetrazol, A=Azide, Exp= experimentally determined.

ACD/Labs

ACD/pKa GALAS Module Report

Date: July 23, 2015 4:20 PM

Software name and version: ACD/Percepta 14.0.0 (Build 2726)

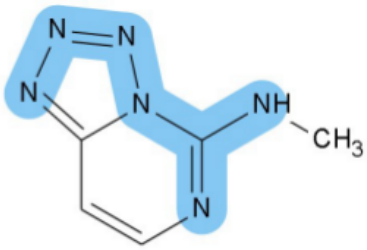
Compound name:

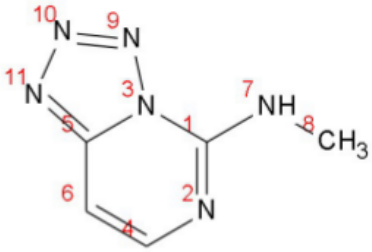
Structure:

No acid pKa

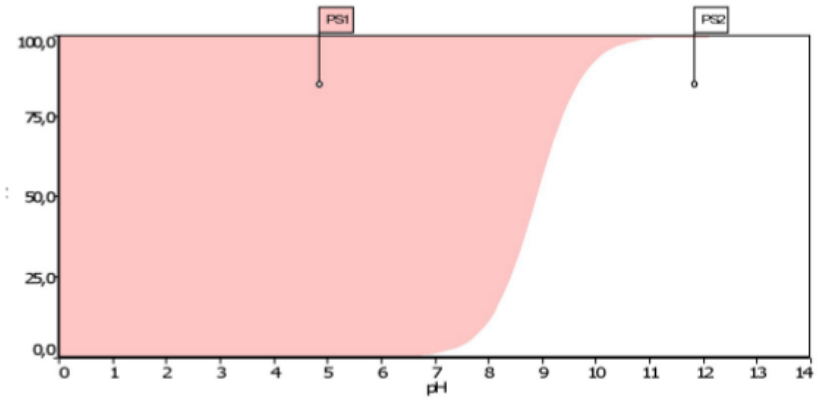
Strongest pKa(Base): 8,9 +- 2,3

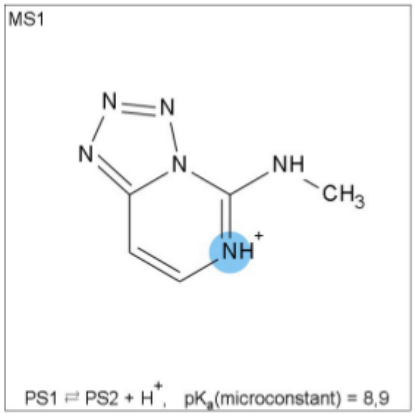
8,9 +- 2,3 (Atom number: 2), 100% MS1





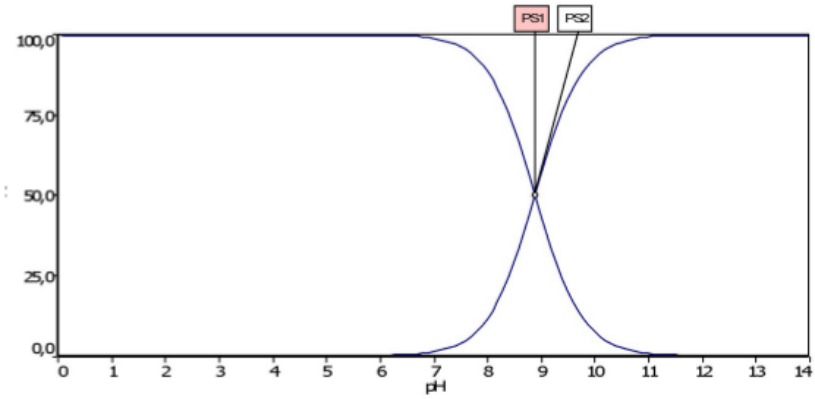
Net Charge vs pH

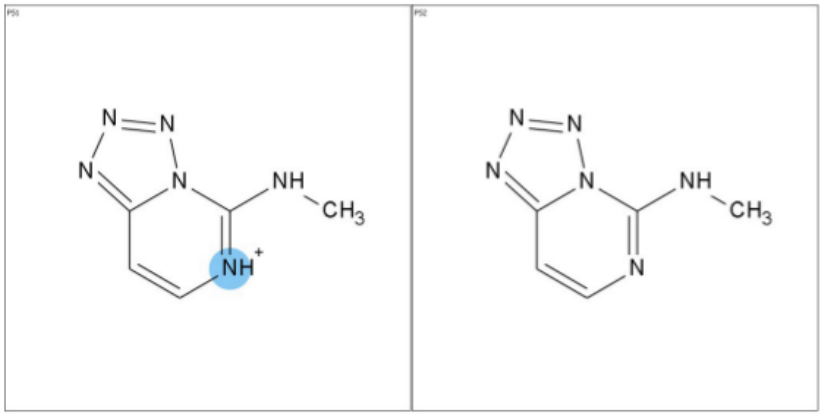




	1,7	2	3	4	4,6	5	6	6,5	7	7,4	8	9	10	11
q = 0	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,03	0,11	0,56	0,93	0,99
q = +1	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,99	0,97	0,89	0,44	0,07	0,01

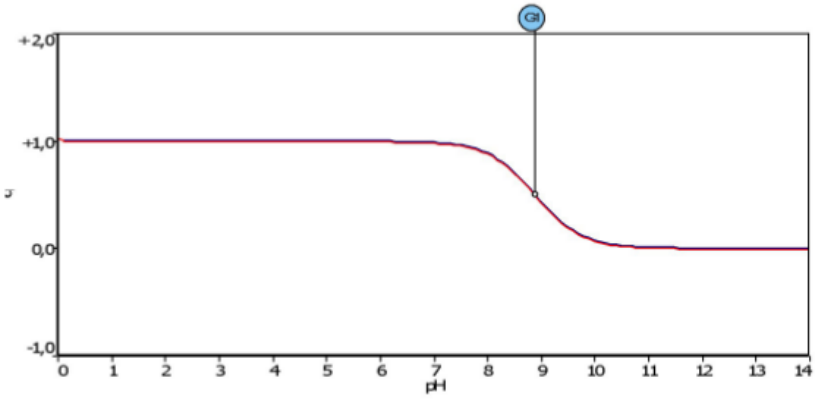
Protonation State vs pH

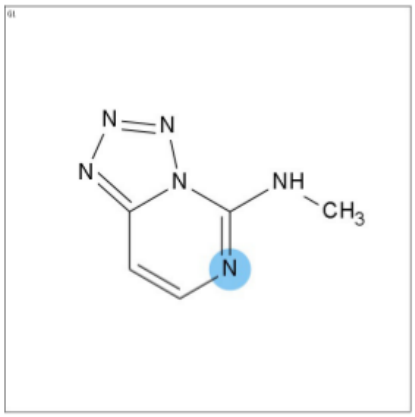




	1,7	2	3	4	4,6	5	6	6,5	7	7,4	8	9	10	11
PS1	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,99	0,97	0,89	0,44	0,07	0,01
PS2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,03	0,11	0,56	0,93	0,99

Ionogenic Group States vs pH





	1,7	2	3	4	4,6	5	6	6,5	7	7,4	8	9	10	11
G1	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,99	0,97	0,89	0,44	0,07	0,01
TC	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,99	0,97	0,89	0,44	0,07	0,01

Date: July 23, 2015 4:22 PM

Software name and version: ACD/Percepta 14.0.0 (Build 2726)

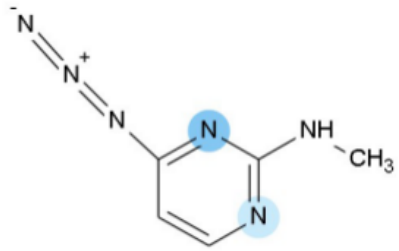
Compound name:

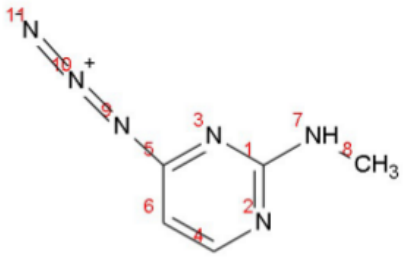
Structure:

No acid pKa

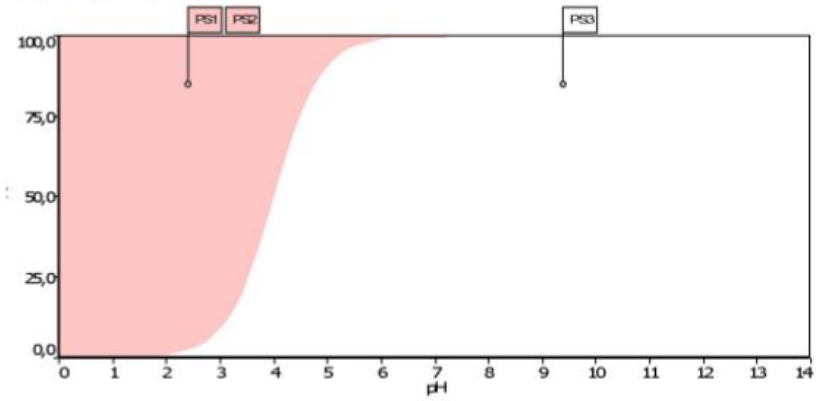
Strongest pKa(Base): 4,0 +- 0,8

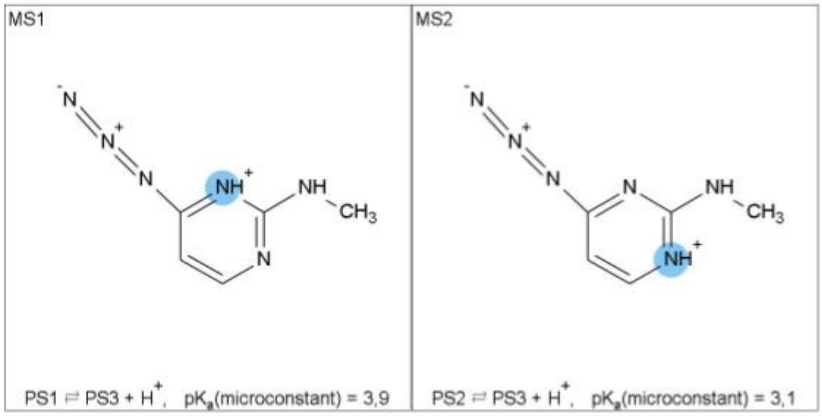
4,0 +- 0,8 (Atom number: 3,2), 88% MS1, 12% MS2





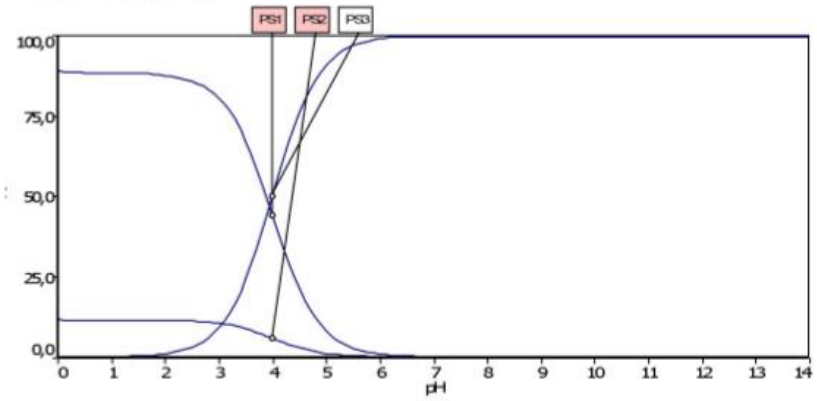
Net Charge vs pH

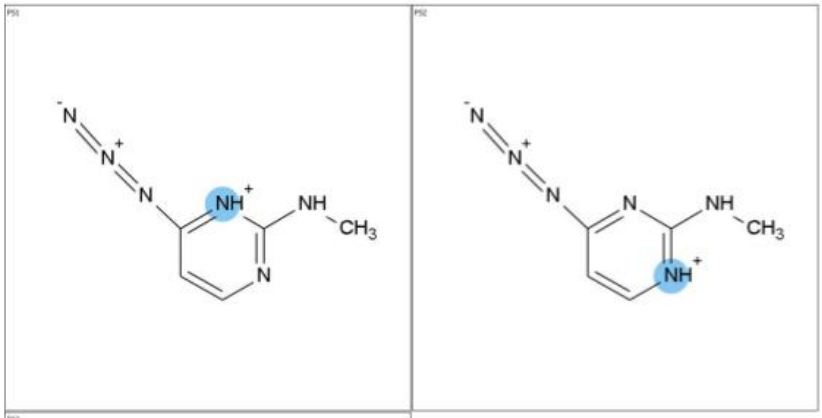


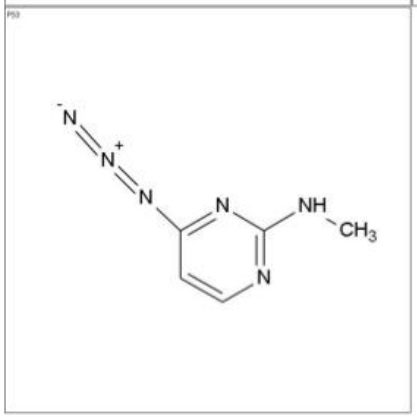


	1,7	2	3	4	4,6	5	6	6,5	7	7,4	8	9	10	11
q = 0	0,00	0,01	0,09	0,50	0,80	0,91	0,99	1,00	1,00	1,00	1,00	1,00	1,00	1,00
q = +1	1,00	0,99	0,91	0,50	0,20	0,09	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00
q = +2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

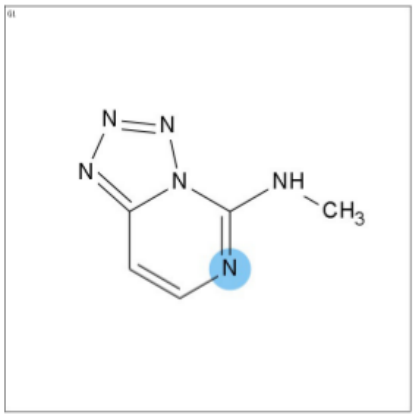
Protonation State vs pH







	1,7	2	3	4	4,6	5	6	6,5	7	7,4	8	9	10	11
PS1	0,88	0,88	0,80	0,44	0,18	0,08	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00
PS2	0,11	0,11	0,10	0,06	0,02	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00



	1,7	2	3	4	4,6	5	6	6,5	7	7,4	8	9	10	11
G1	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,99	0,97	0,89	0,44	0,07	0,01
TC	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,99	0,97	0,89	0,44	0,07	0,01

f) Calculation of Ligand Efficiency (LE) and Ligand Lipophilicity Efficiency (LLE_{Astex})

LE of compounds **5** and **9** was calculated based on their IC_{50}: [5]

$$\mathrm{LE} = 1.4 * \frac{\mathrm{pIC50}}{\mathrm{NHA}} \qquad \text{(NHA= Number of heavy atoms)}$$

LLE_{Astex} was calculated using IC_{50}'s with minor changes to the formula, based on the findings of Shultz [5,6]:

$$\mathrm{LLE_{Astex}} = 0.11 + 1.4 * \frac{\mathrm{pIC50} - \mathrm{cLogP}}{\mathrm{NHA}}$$

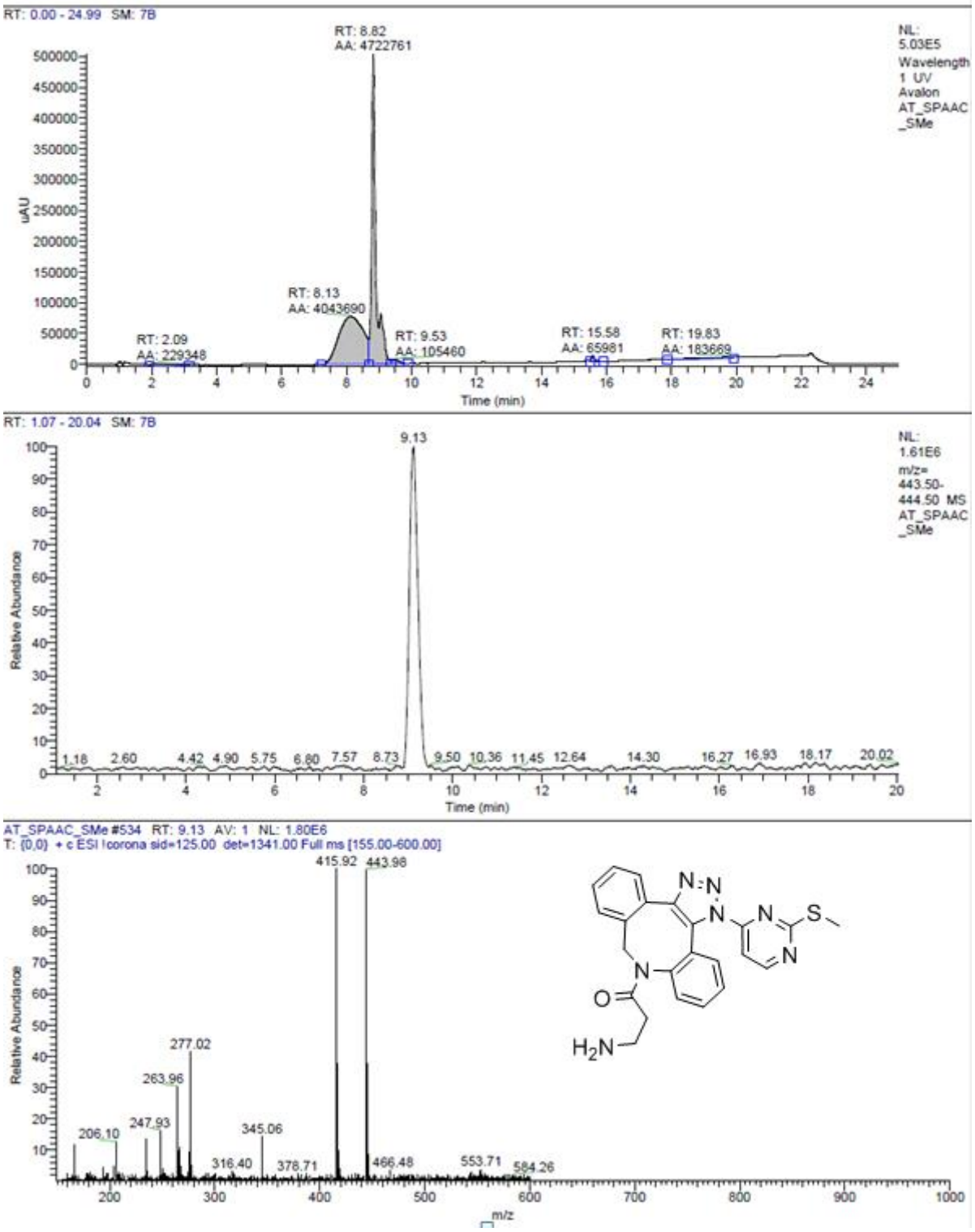
g) In vitro PqsD Inhibition Assay

PqsD In vitro Inhibition Assay: The assay was performed monitoring enzyme activity by measuring HHQ formed by condensation of anthraniloyl-CoA and β-ketodecanoic acid. The reaction mixture contained MOPS buffer (0.05 M, pH 7.0) with 0.005 % (w/v) Triton X-100, 0.1 µM of the purified enzyme, and inhibitor. The test compounds were dissolved in DMSO and diluted with buffer. The final DMSO concentration was 0.5%. After 10 min preincubation at 37 °C, the reaction was started by the addition anthraniloyl-CoA to a final concentration of 5 µM and β-ketodecanoic acid to a final concentration of 70 µM. Reactions were stopped byaddition of MeOH containing 1 µM amitriptyline as internal standardfor LC/MS-MS analysis. HHQ was quantified using a HPLC-MS/MSmass spectrometer (Thermo Fisher, Dreieich, Germany) in ESI mode.Ionization of HHQ and the internal standard amitriptyline was optimized in each case. The solvent system consisted of 10 mM ammonium acetate (A) and acetonitrile (B), both containing 0.1 % trifluoroacetic acid. The initial concentration of B in A was 45%, increasing the percentage of B to 100 % in 2.8 min and keeping it at 98% for 0.7 min with a flow of 500 µl/min. The column used was a NUCLEODUR-C18, 100-3/125-3 (Macherey & Nagel, Duehren,Germany). Control reactions without the inhibitor, but including identical amounts of DMSO, were performed in parallel, and the amount of HHQ produced was set to 100 %. All reactions were performed in triplicate except for adenine and hypoxanthine which were performed as single point measurements at 50 µM. Hypoxanthine inhibited 9% HHQ formation whereas adenine inhibited 0% HHQ formation.

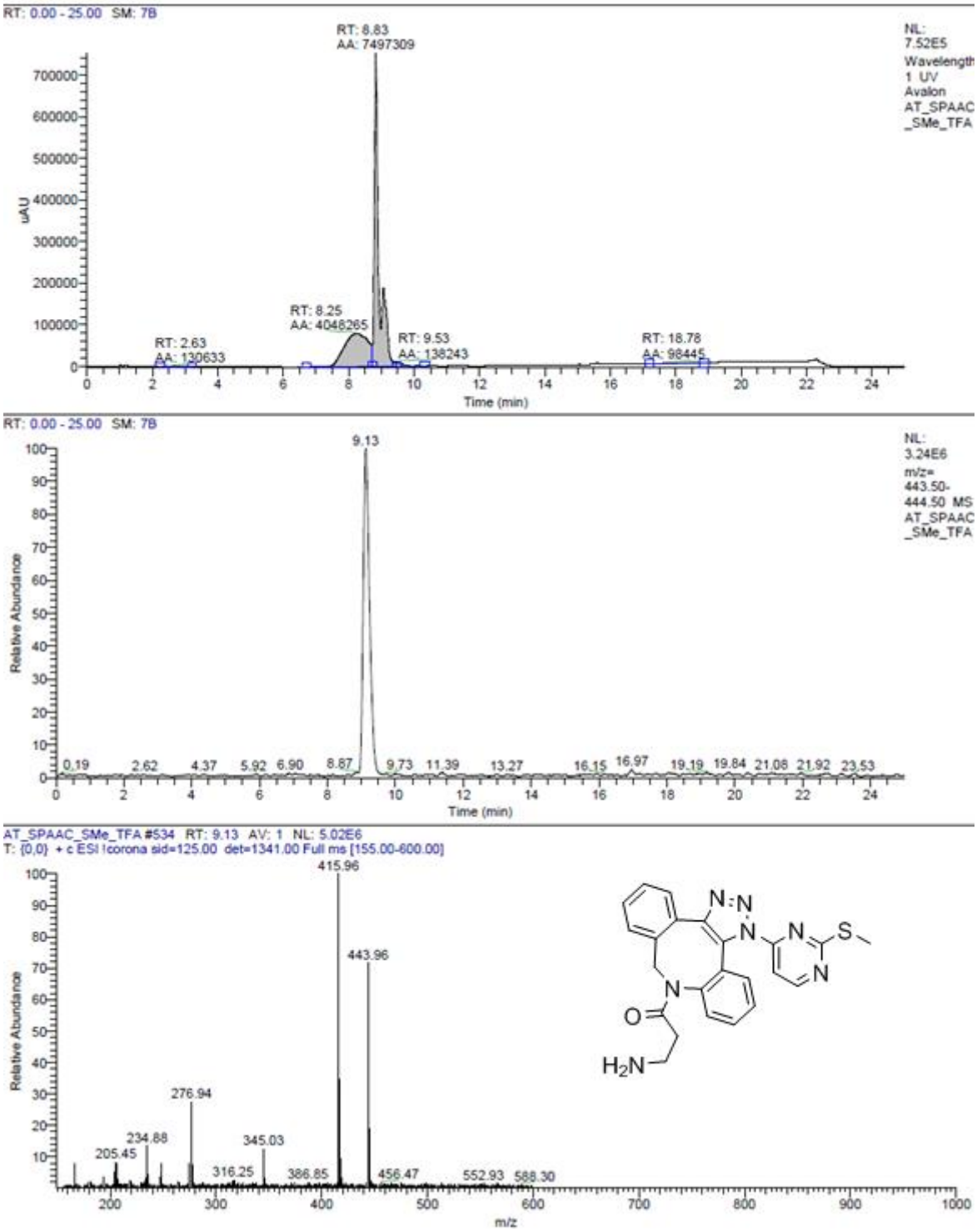
h) SPAAC reactionkinetics of 3, 4 and 5 under neutral, basic and acidic pH

10 µl of Dibenzocyclooctyne-amine (100 mM DMSO Stock) and 20 µl of the corresponding azide-compound (50 mM in *tert*BuOH:Water [1:1]) was added to 1000 µl of *tert*BuOH:Water (1:1) and shaken at room temperature for 1 hr for neutral conditions. For acidic conditions 0.2 % TFA or 0.1 M HCl was and for basic conditions 0.1 M NaOH was used in 1000 µl *tert*BuOH:Water (1:1) final. 10 µl of the reaction mixture was injected into the LC-MS System.

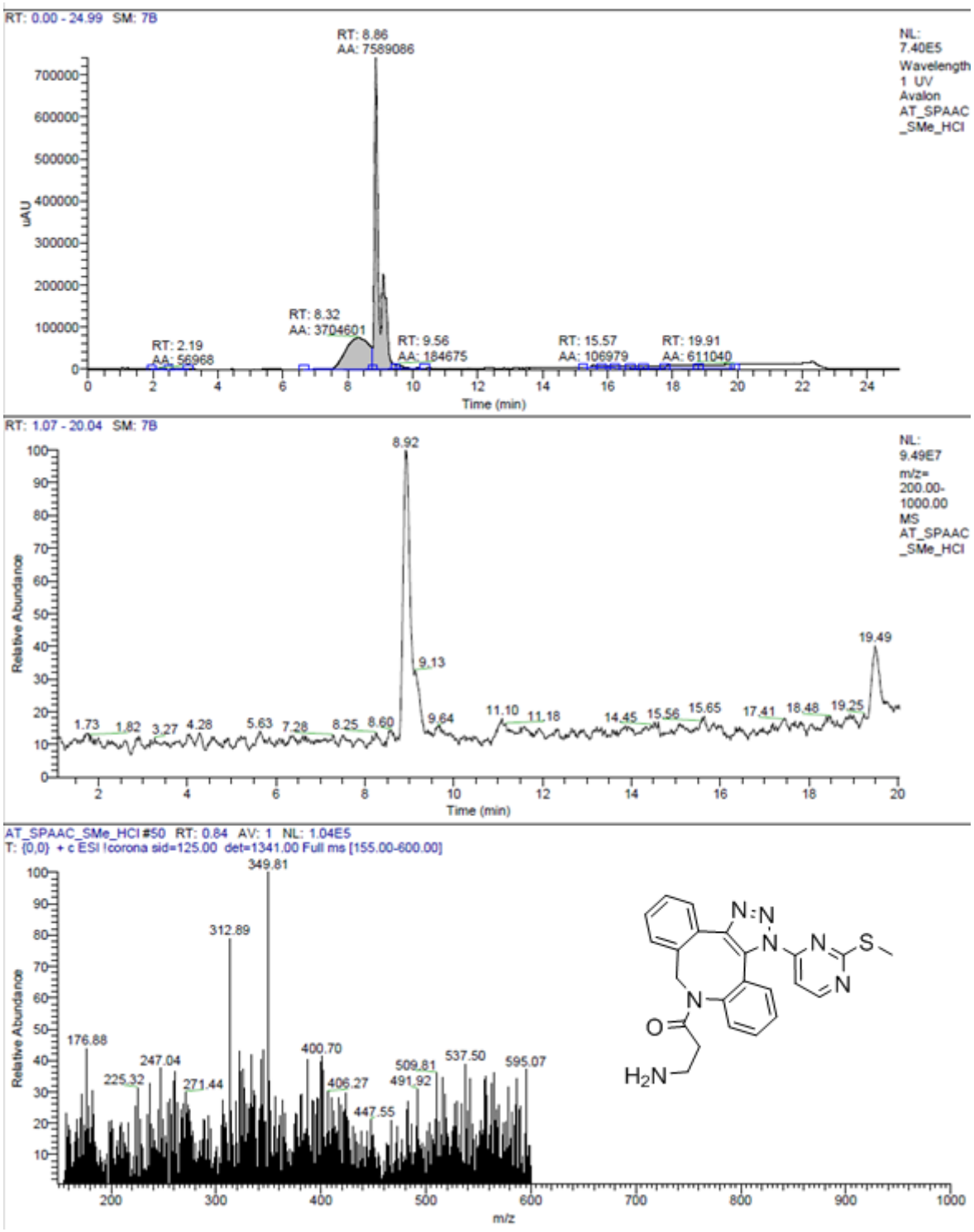
LC-MS Run of **3** under neutral conditions



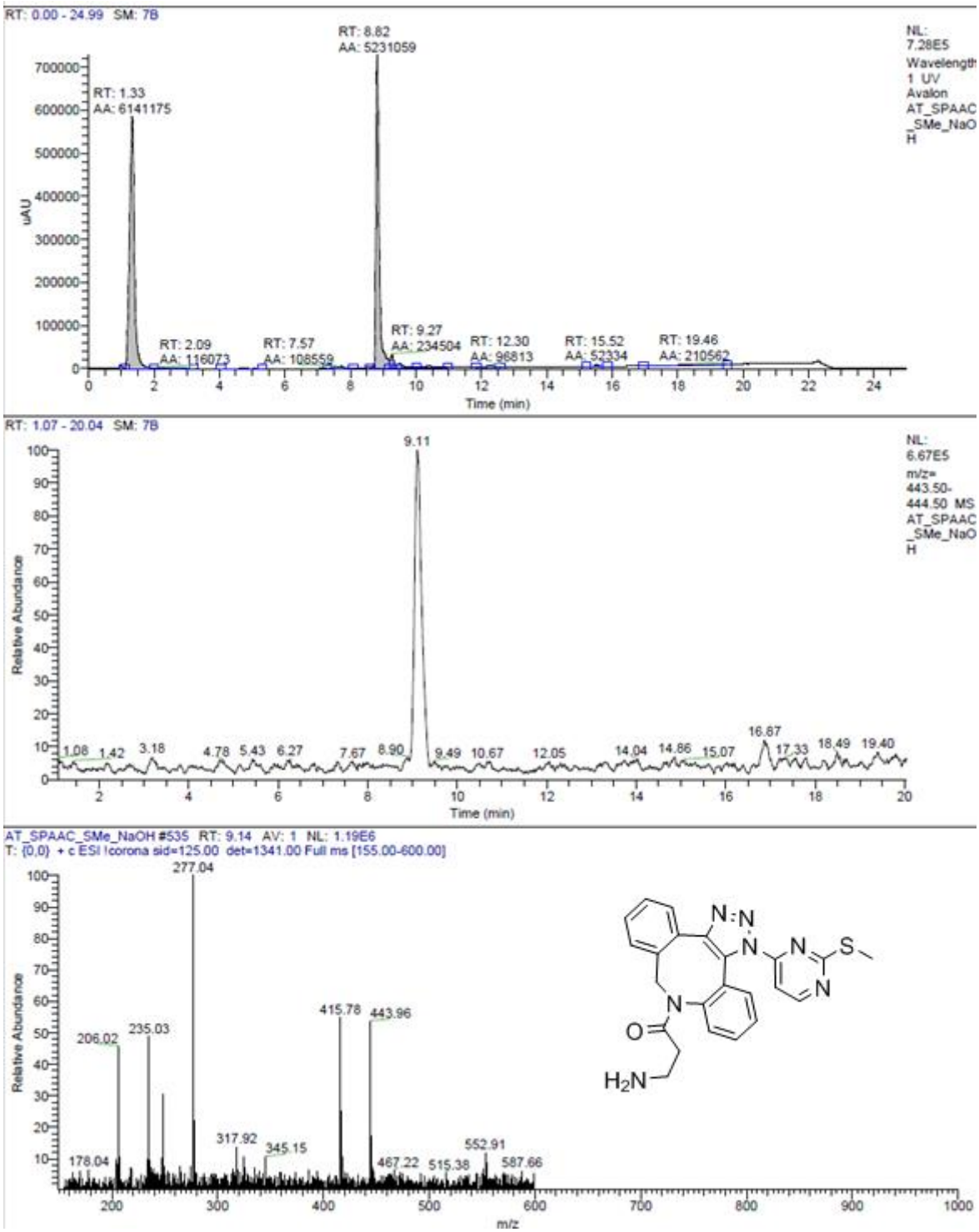
LC-MS Run of **3** under acid conditions (0.2% TFA)



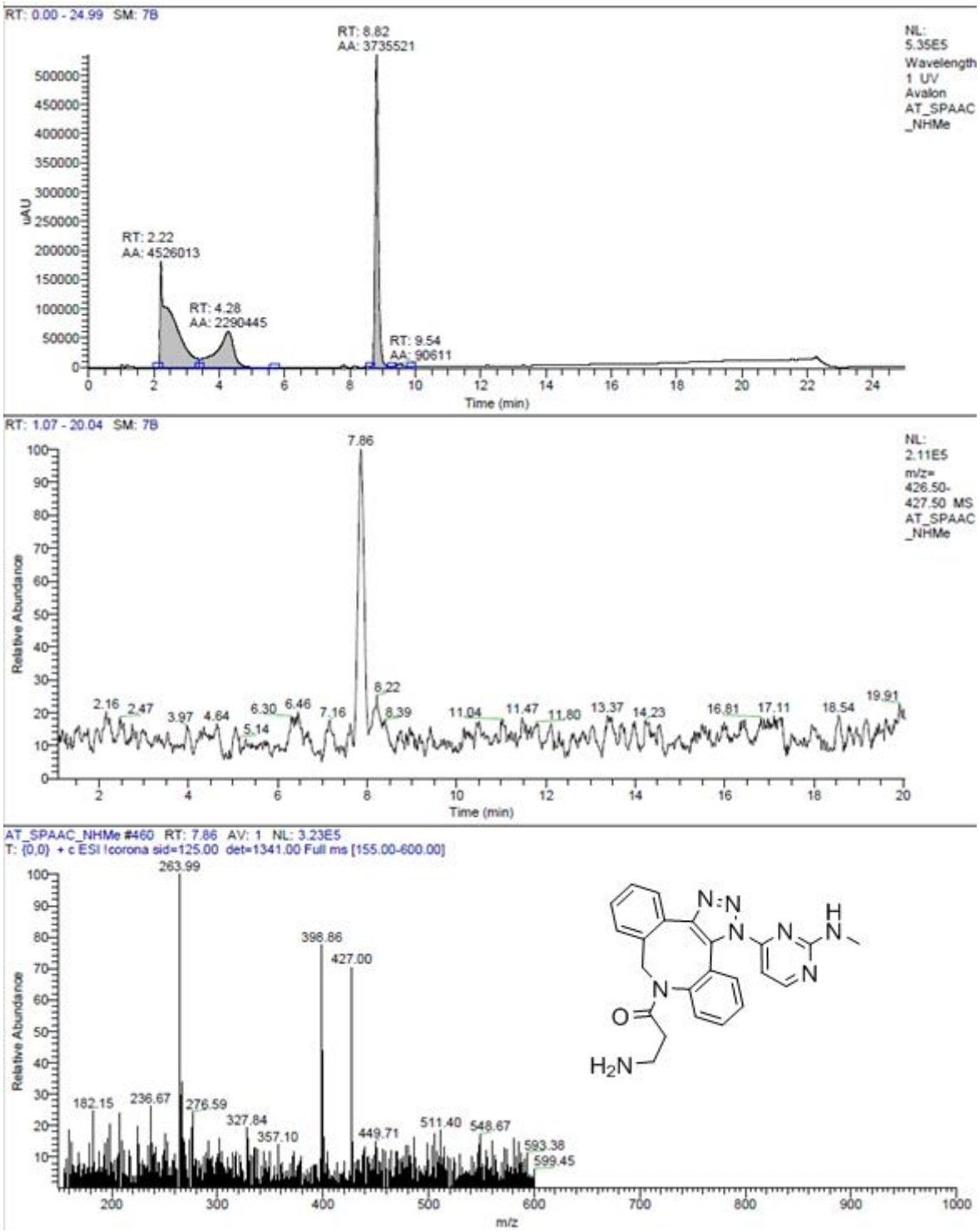
LC-MS Run of **3** under acid conditions (0.1 M HCl)



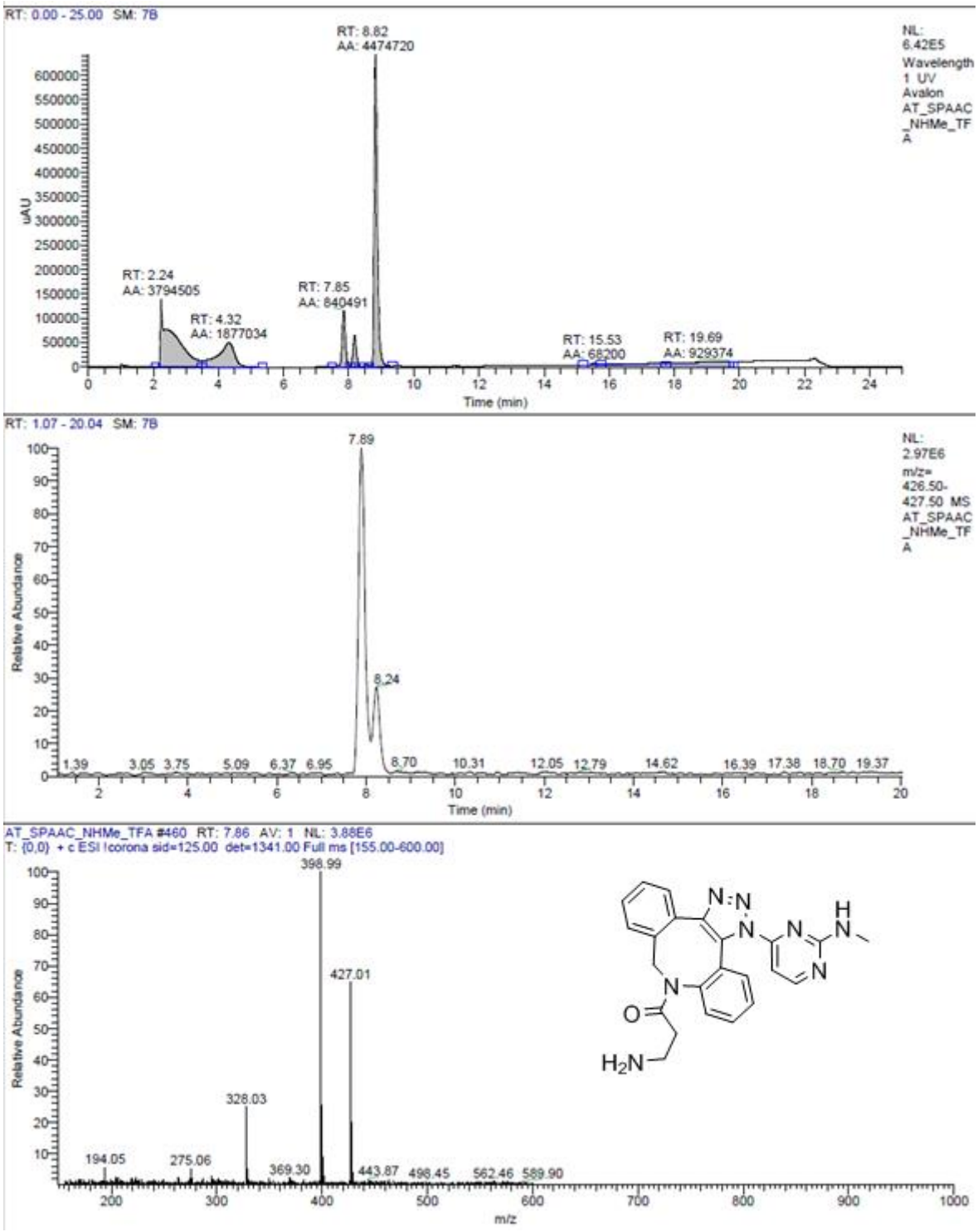
LC-MS Run of **3** under basic conditions (0.1 M NaOH)



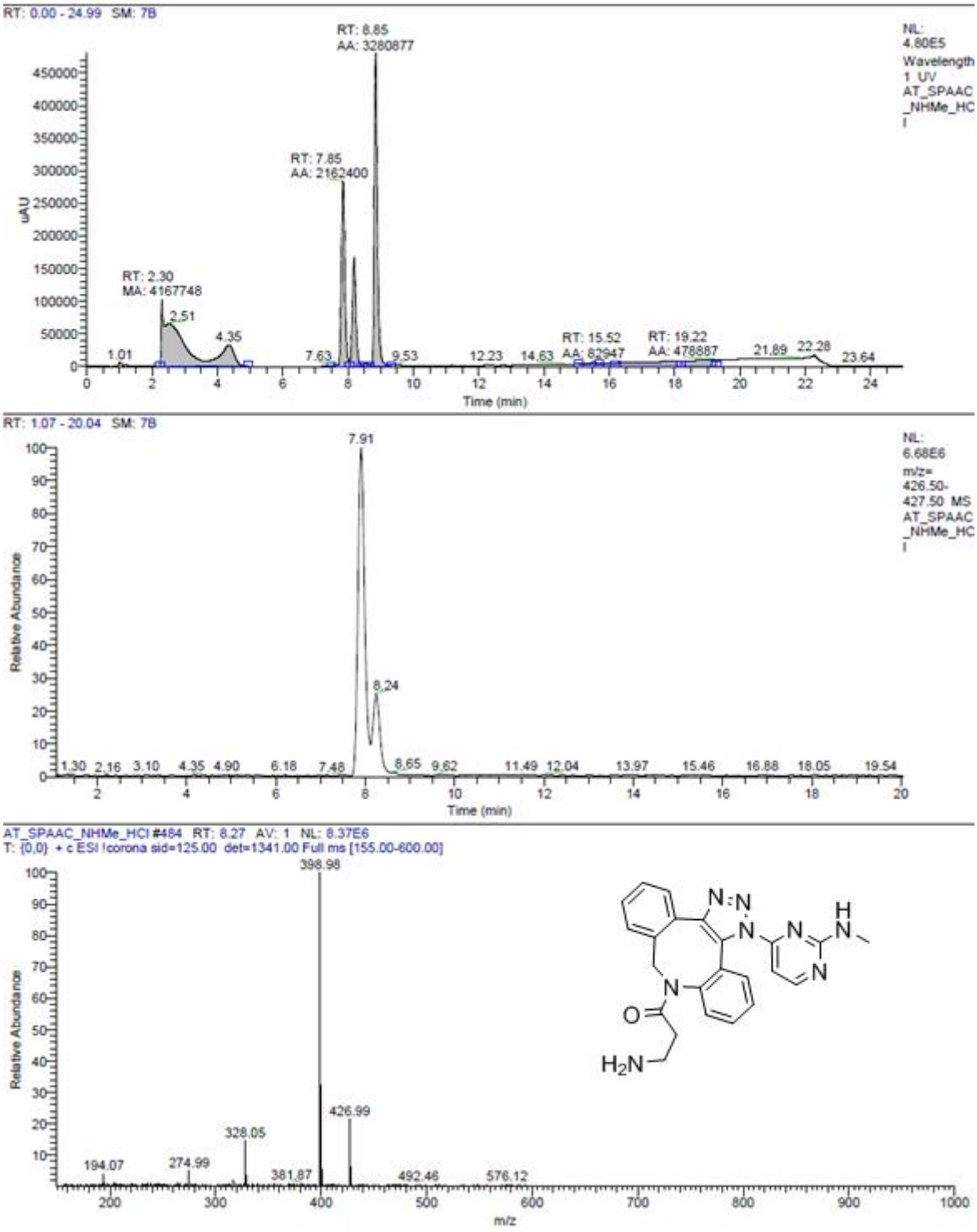
LC-MS Run of **4** under neutral conditions:



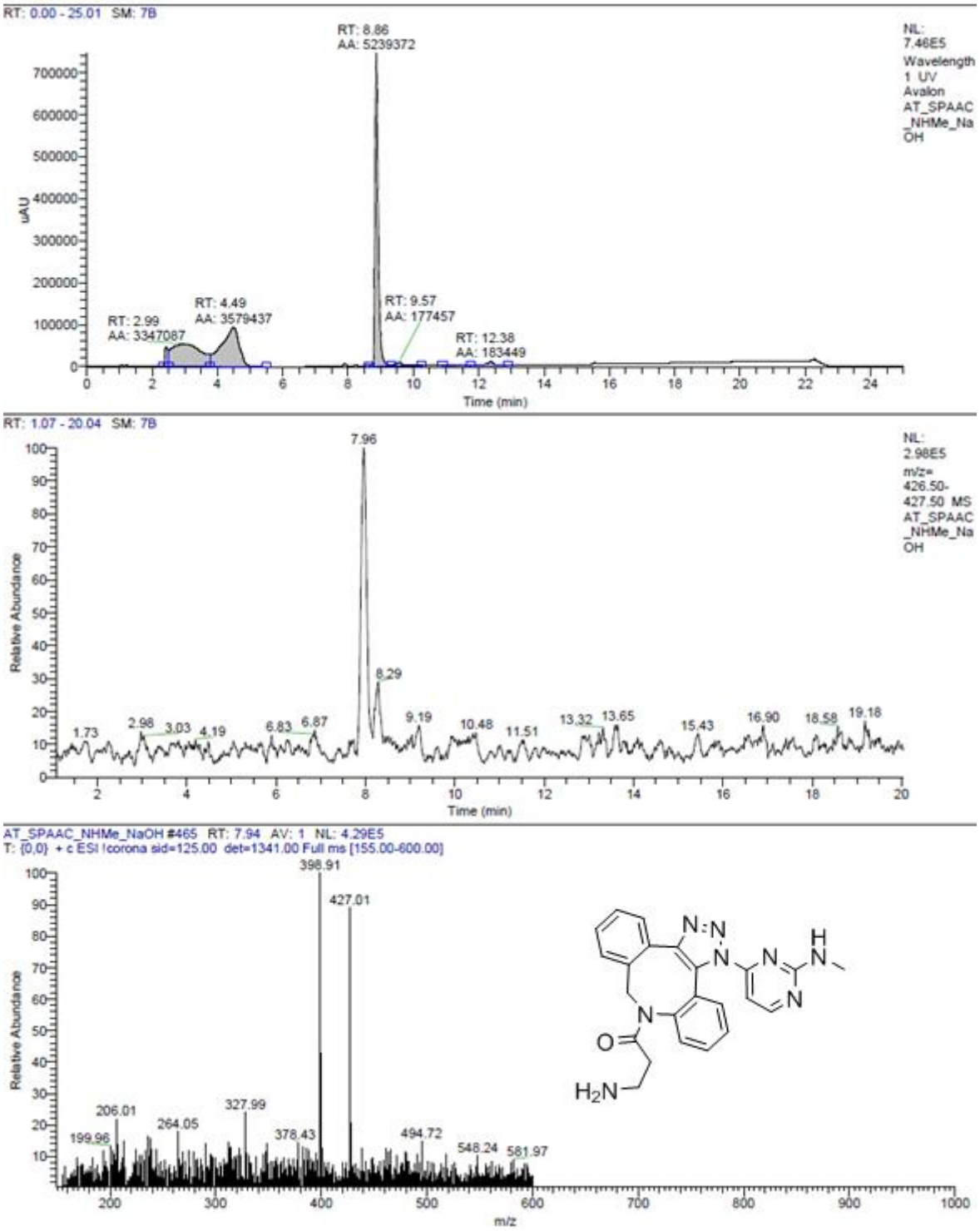
LC-MS Run of **4** under acid conditions (0.2% TFA):



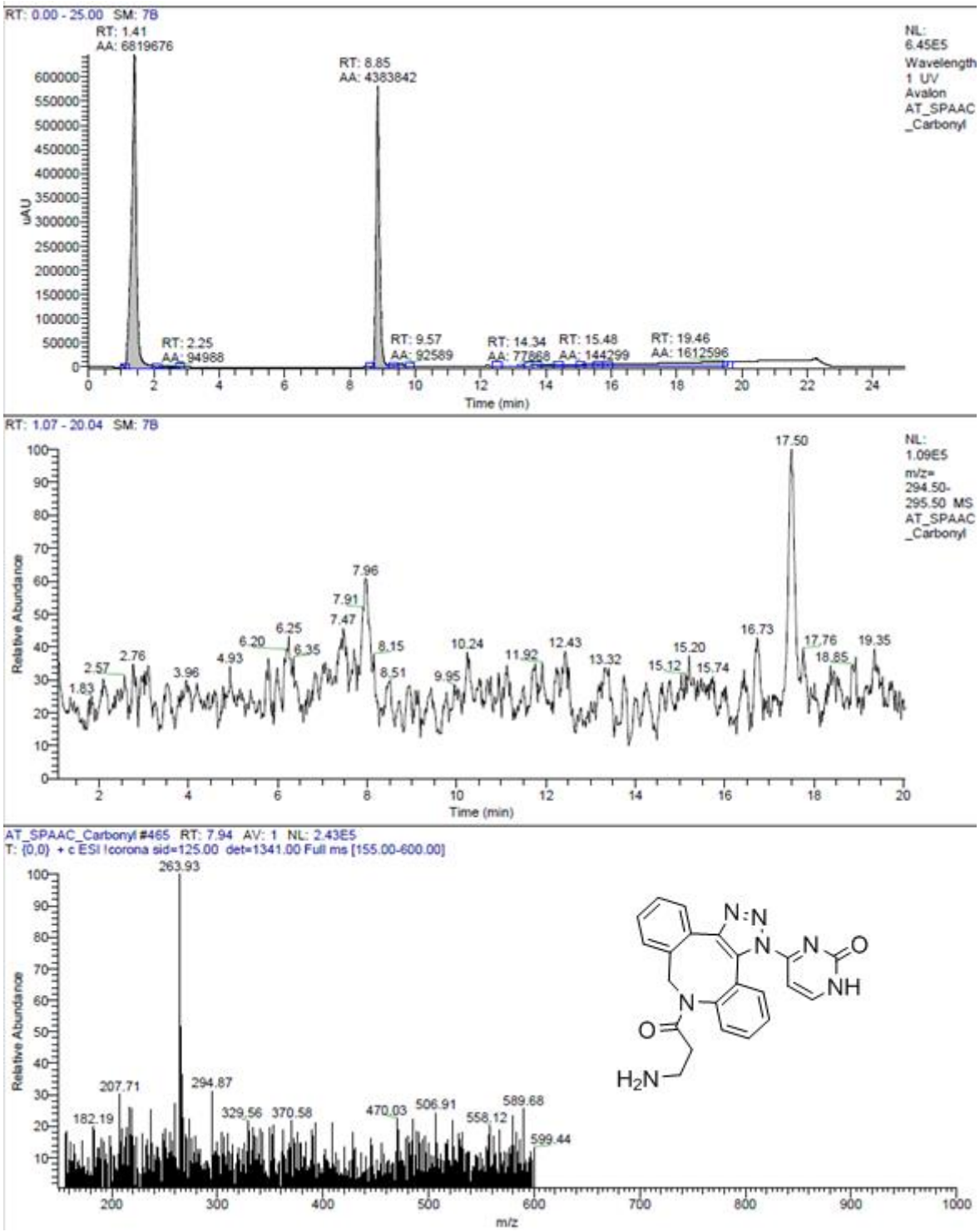
LC-MS Run of **4** under acid conditions (0.1 M HCl):



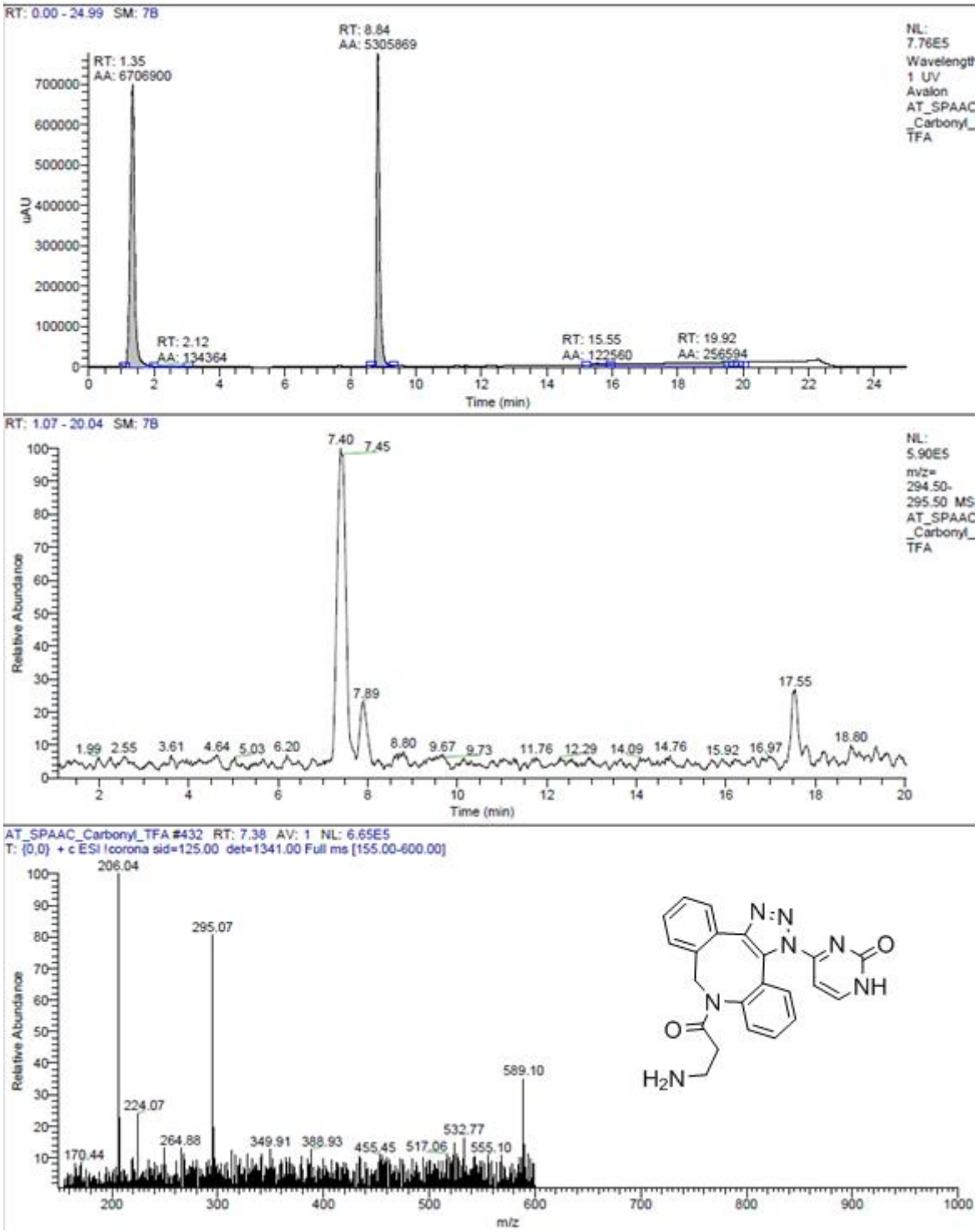
LC-MS Run of **4** under basic conditions (0.1 M NaOH):



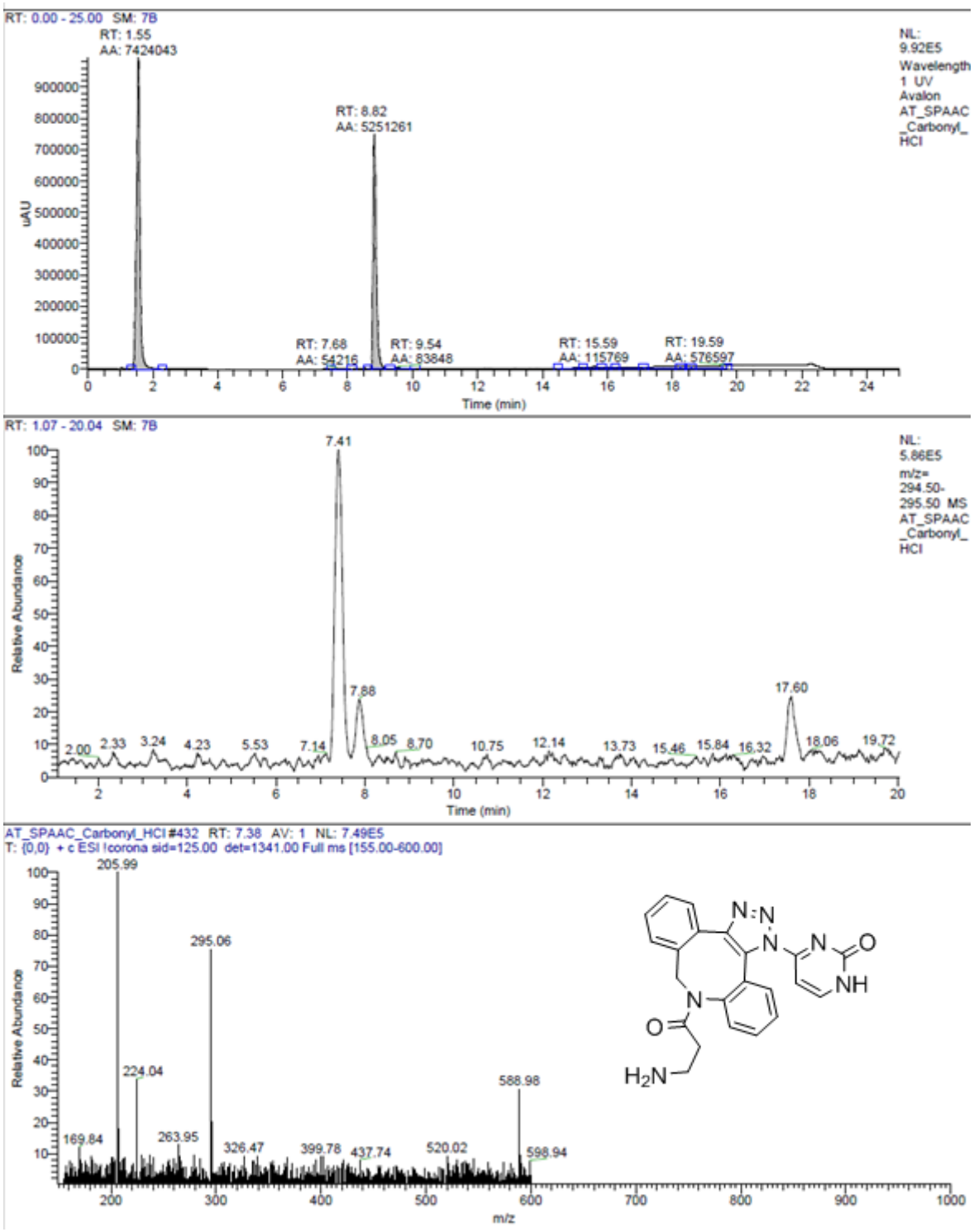
LC-MS Run of **5** under neutral conditions



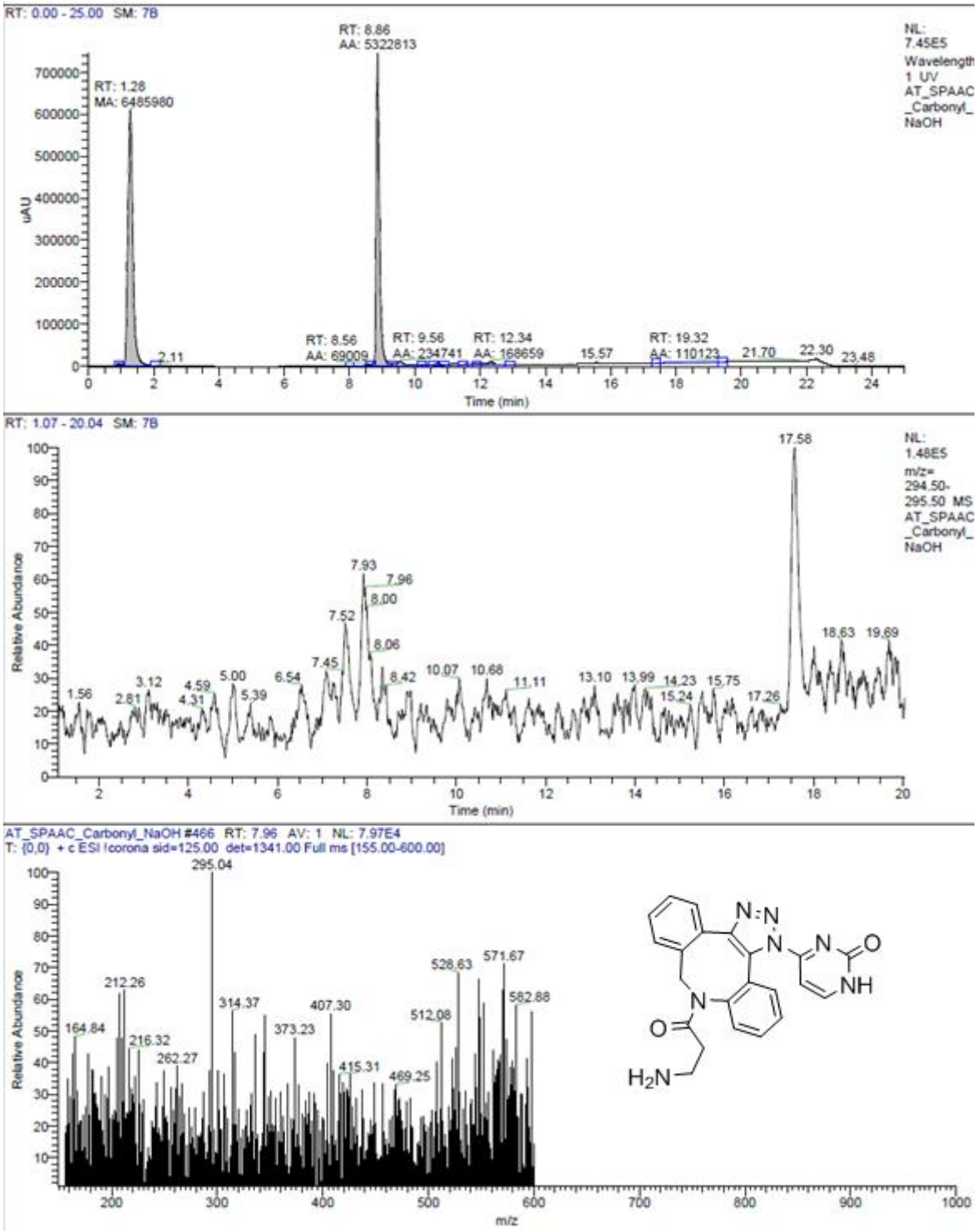
LC-MS Run of **5** under acid conditions (0.2%TFA)



LC-MS Run of **5** under acid conditions (0.1 M HCl)



LC-MS Run of **5** under basic conditions (0.1 M NaOH)



i) SPAAC reactionkinetic of 4 at pH 1.5, 2.5 and 4.1 and 15 at pH 1.4 and pH 7.0

Compound 4:

pH 1.5

Time [min]	AUC of **4** [mAU]	**4** [%]	AUC of product [mAU]	Product [%]
30	5734933	91.7	518946	8.3
60	5602499	83.6	1101498	16.4
90	5268856	77.8	1499674	22.2
120	5003182	72.2	1924425	27.8
150	4871904	67.8	2311144	32.2
180	1370705	63.7	779760	36.3

pH 2.5

Time [min]	AUC of **4** [mAU]	**4** [%]	AUC of product [mAU]	Product [%]
30	5695140	97.6	138559	2.4
60	5178777	96.1	210430	3.9
90	5230687	95.5	244025	4.5
120	5262583	94.1	329738	5.9
150	5207051	92.8	403243	7.2
180	5176146	91.9	455522	8.1

pH 4.1

Time [min]	AUC of **4** [mAU]	**4** [%]	AUC of product [mAU]	Product [%]
30	4860187	99.3	31833	0.7
60	4855123	98.5	74656	1.5
90	5065370	98.1	99397	1.9
120	5005964	97.8	114435	2.2
150	5102021	97.5	132920	2.5
180	5058522	97.5	129192	2.5

Compound 15:

pH 1.4

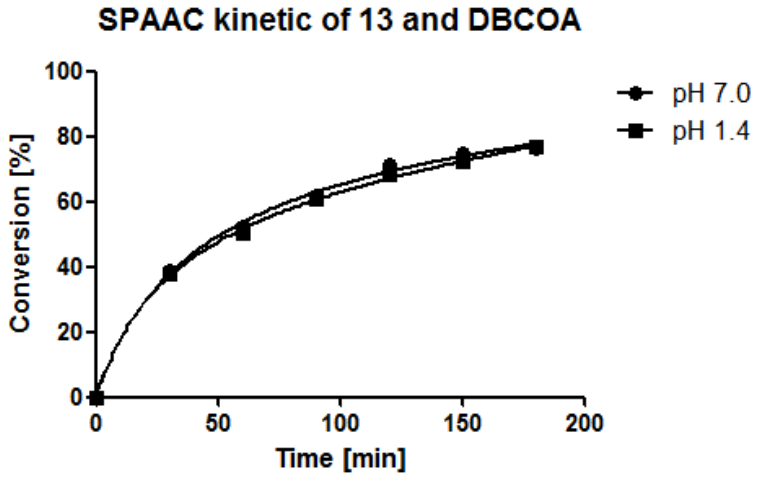
Time [min]	AUC of DBCOA[a] [mAU]	DBCOA[a] [%]	AUC of product [mAU]	Product [%]
30	3749764	62.2	2281874	37.8
60	3128959	49.9	3138413	50.1
90	2705288	39.2	4199670	60.8
120	2153357	31.7	4642839	68.3
150	2053435	27.9	5296846	72.1
180	1702508	23.4	5561344	76.6

[a]DBCOA = Dibenzocyclooctyne-amine

pH 7.0

Time [min]	AUC of DBCOA[a] [mAU]	DBCOA[a] [%]	AUC of product [mAU]	Product [%]
30	2912988	61.2	1850148	38.8
60	2577175	47.8	2812106	52.2
90	1980938	38.1	3220216	61.9
120	1698357	28.9	4181186	71.1
150	1483145	25.2	4412387	74.8
180	1387631	23.5	4528455	76.5

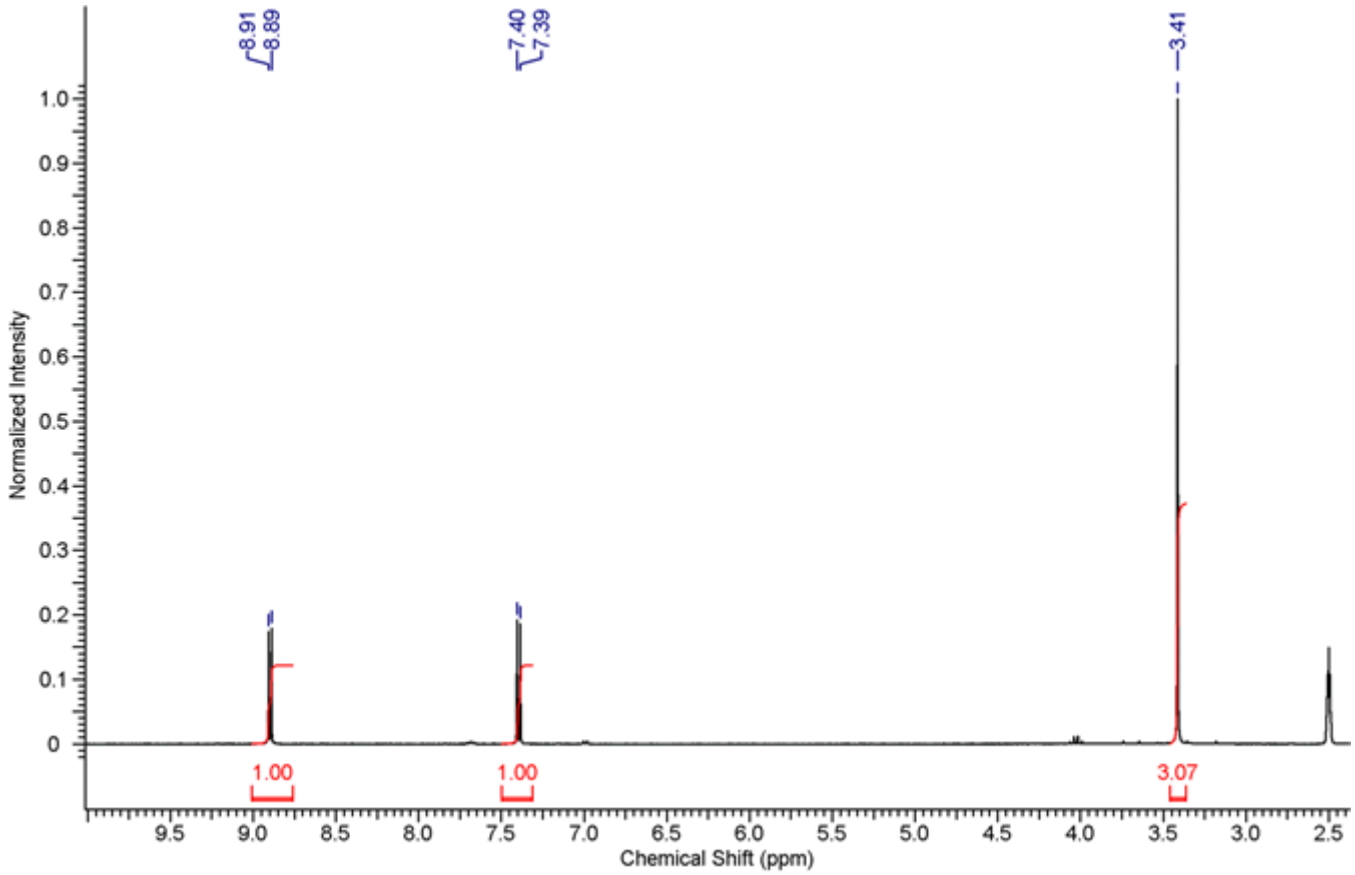
[a]DBCOA = Dibenzocyclooctyne-amine



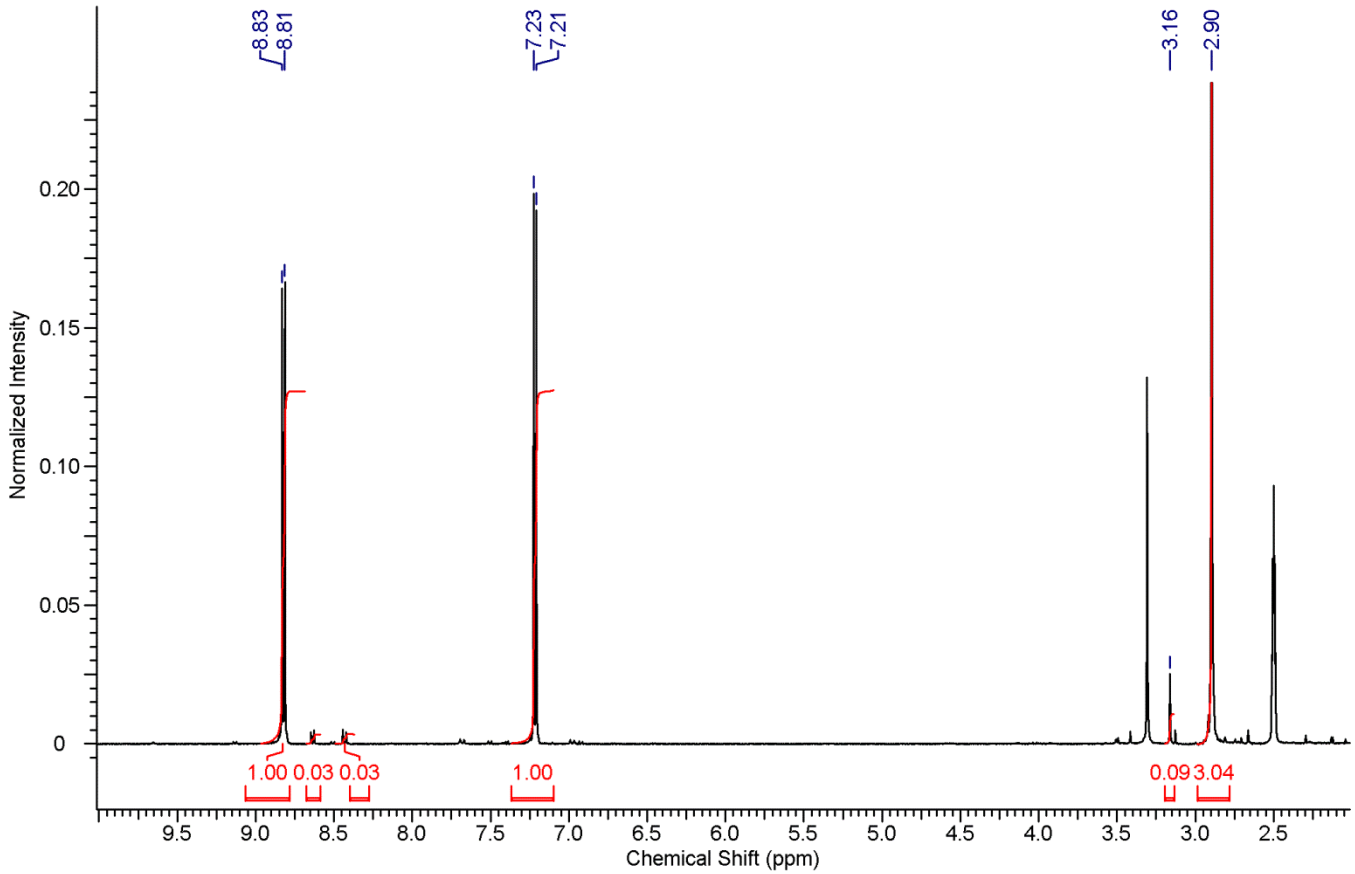
II. Spectral Data

a) ^{1}H-NMR Spectra

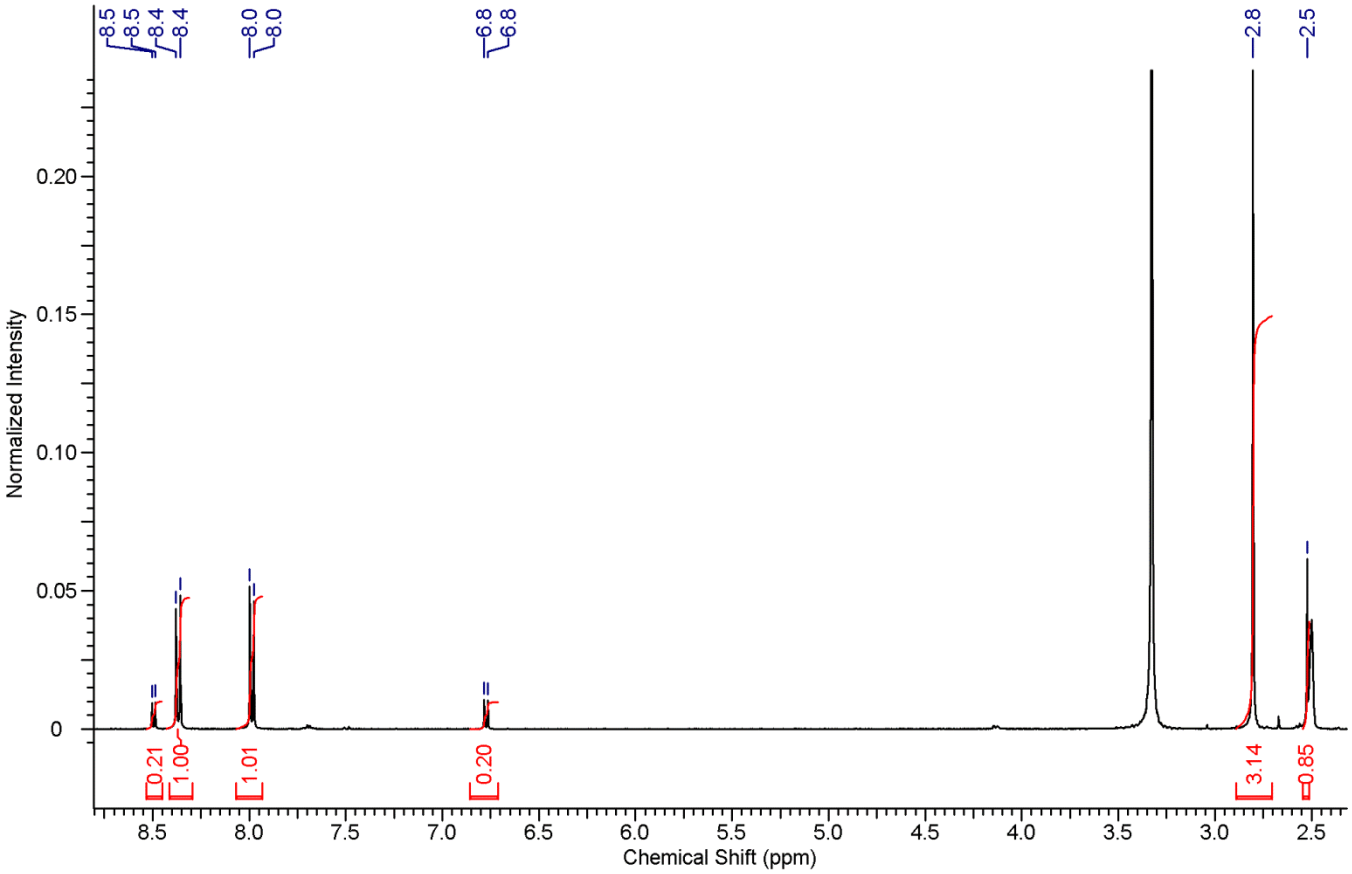
^{1}H-NMR of **1**



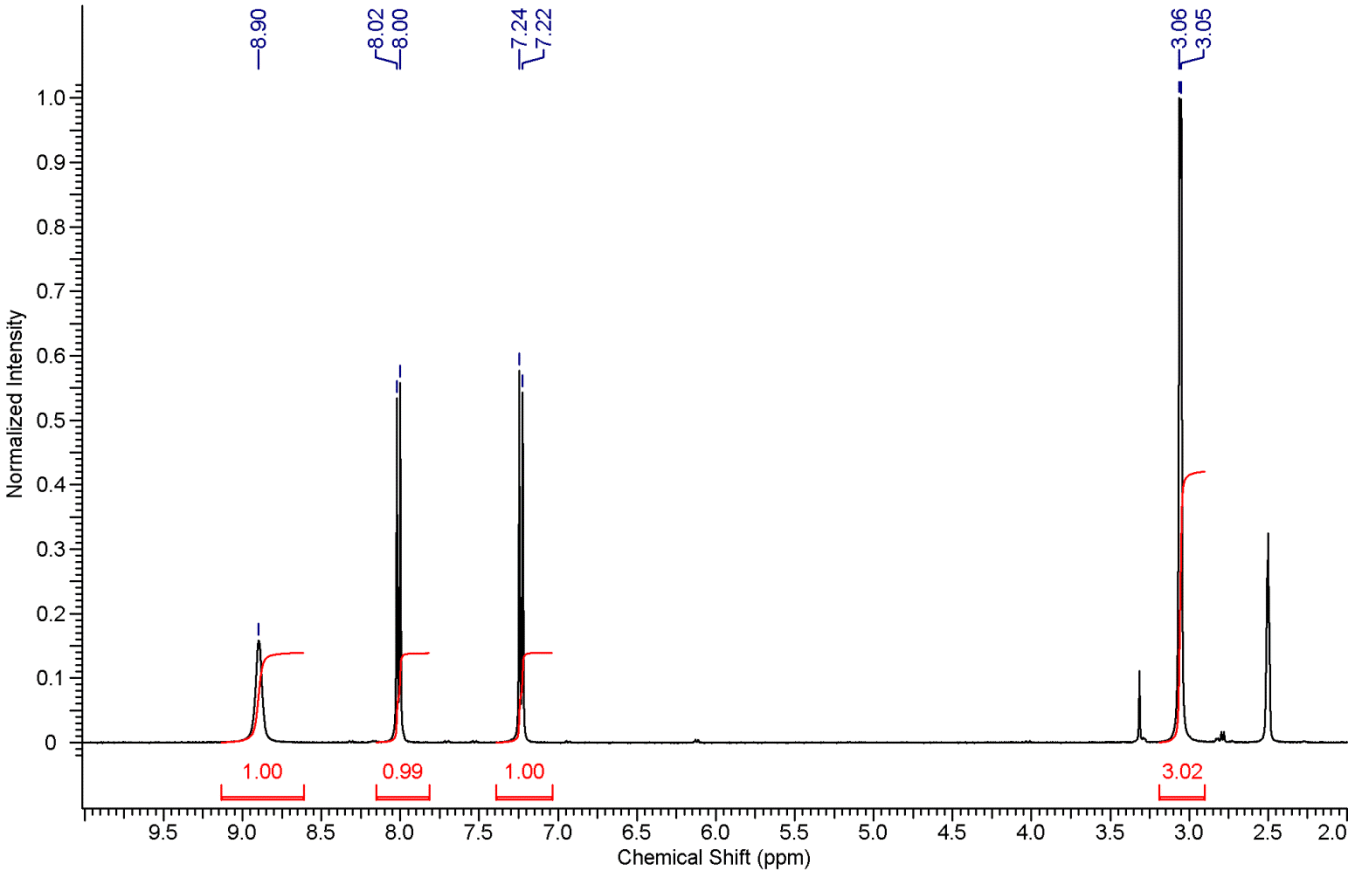
^{1}H-NMR of **2**


8.83
8.81
7.23
7.21
3.16
2.90
Normalized Intensity
0.20
0.15
0.10
0.05
0
1.00
0.03
0.03
1.00
0.09
3.04
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
Chemical Shift (ppm)

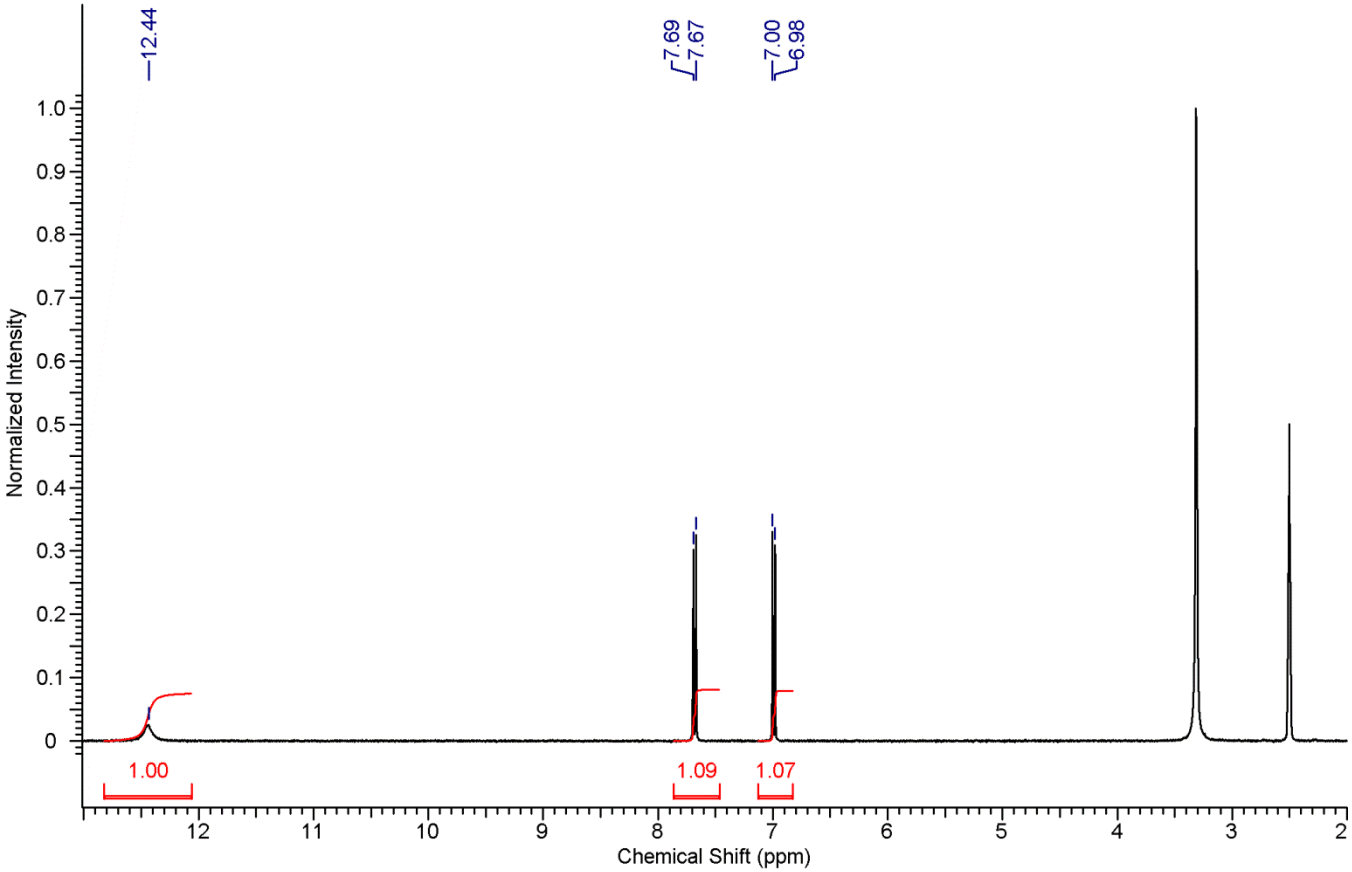
^{1}H-NMR of **3**


8.5
8.5
8.4
8.4
8.0
8.0
6.8
6.8
2.8
2.5
0.20
0.15
0.10
0.05
0
Normalized Intensity
0.21
1.00
1.01
0.20
3.14
0.85
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
Chemical Shift (ppm)

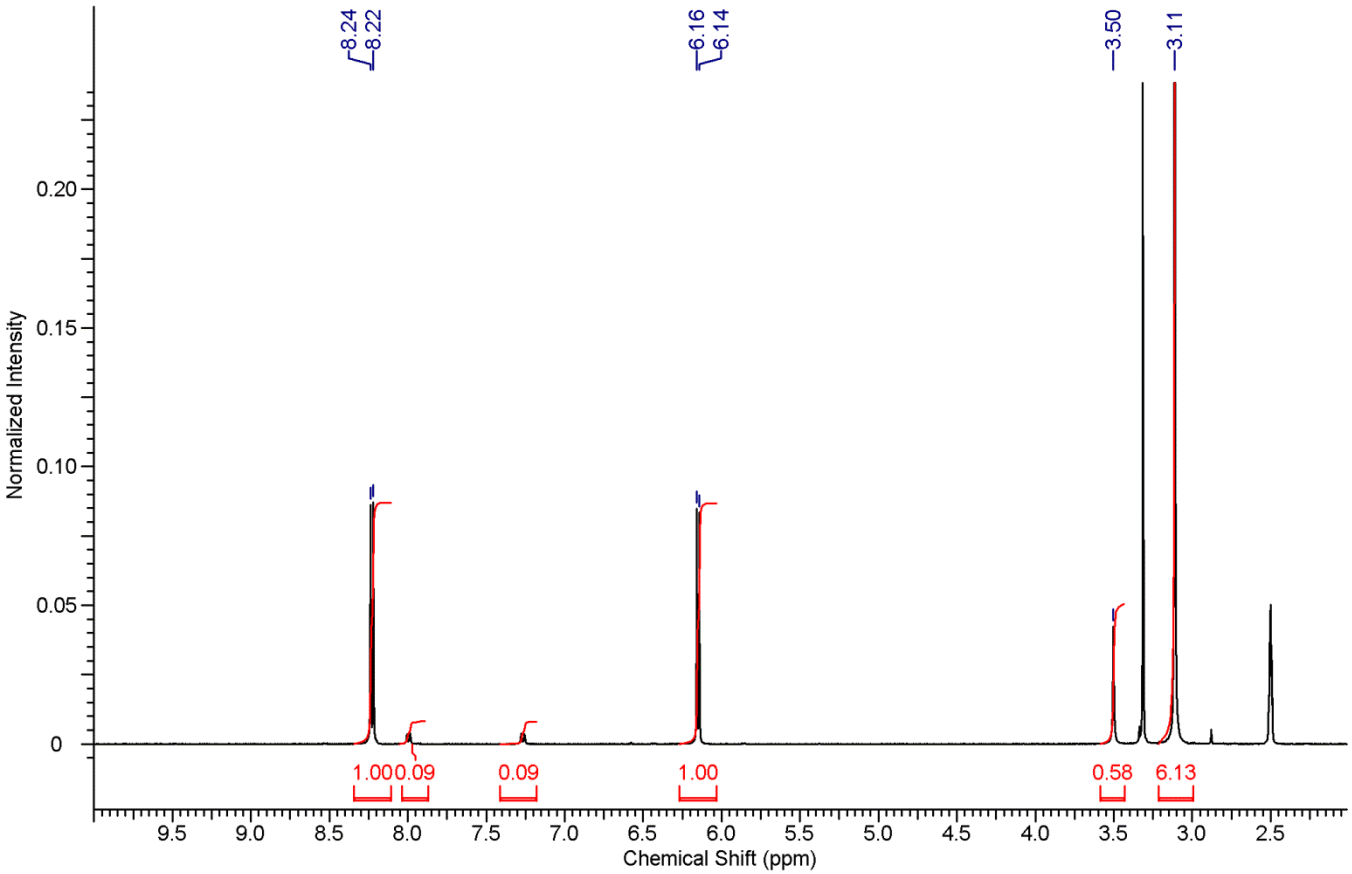
^{1}H-NMR of **4**


8.90
8.02
8.00
7.24
7.22
3.06
3.05
Normalized Intensity
1.00
0.99
1.00
3.02
Chemical Shift (ppm)

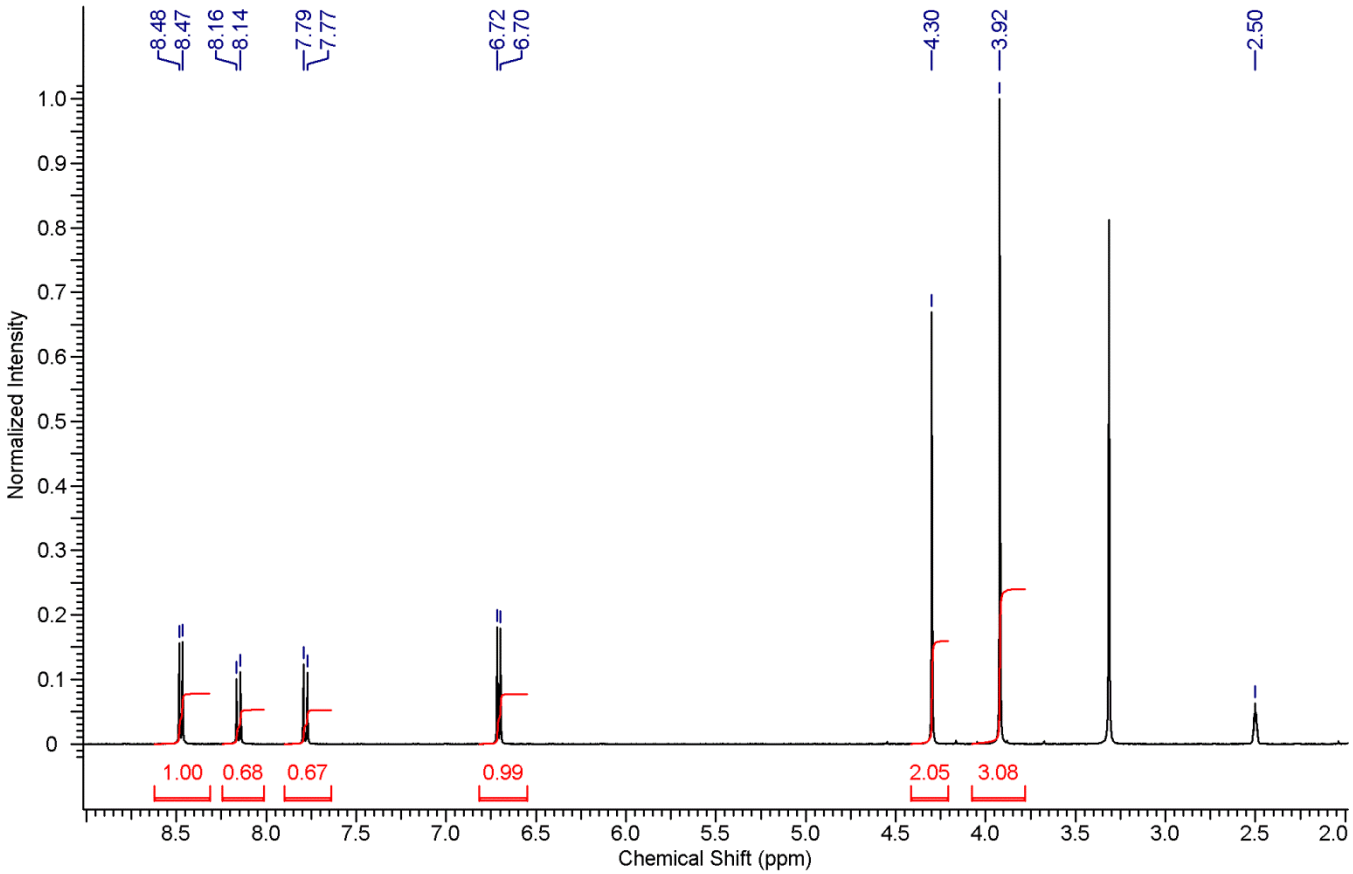
^{1}H-NMR of **5**


12.44
7.69
7.67
7.00
6.98
Normalized Intensity
1.00
1.09
1.07
Chemical Shift (ppm)

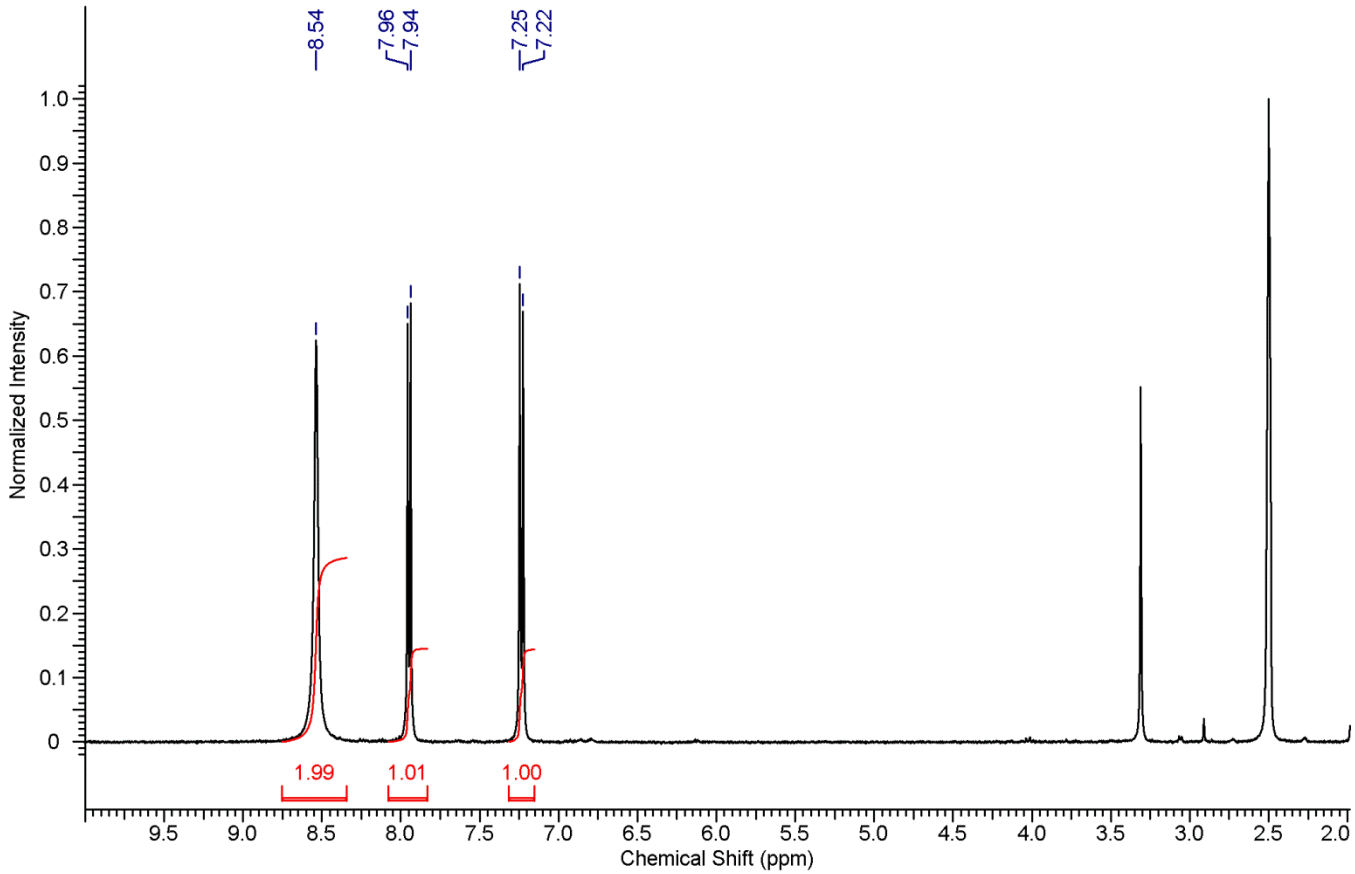
^{1}H-NMR of **6**


8.24
8.22
6.16
6.14
3.50
3.11
Normalized Intensity
0.20
0.15
0.10
0.05
0
1.00
0.09
0.09
1.00
0.58
6.13
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
Chemical Shift (ppm)

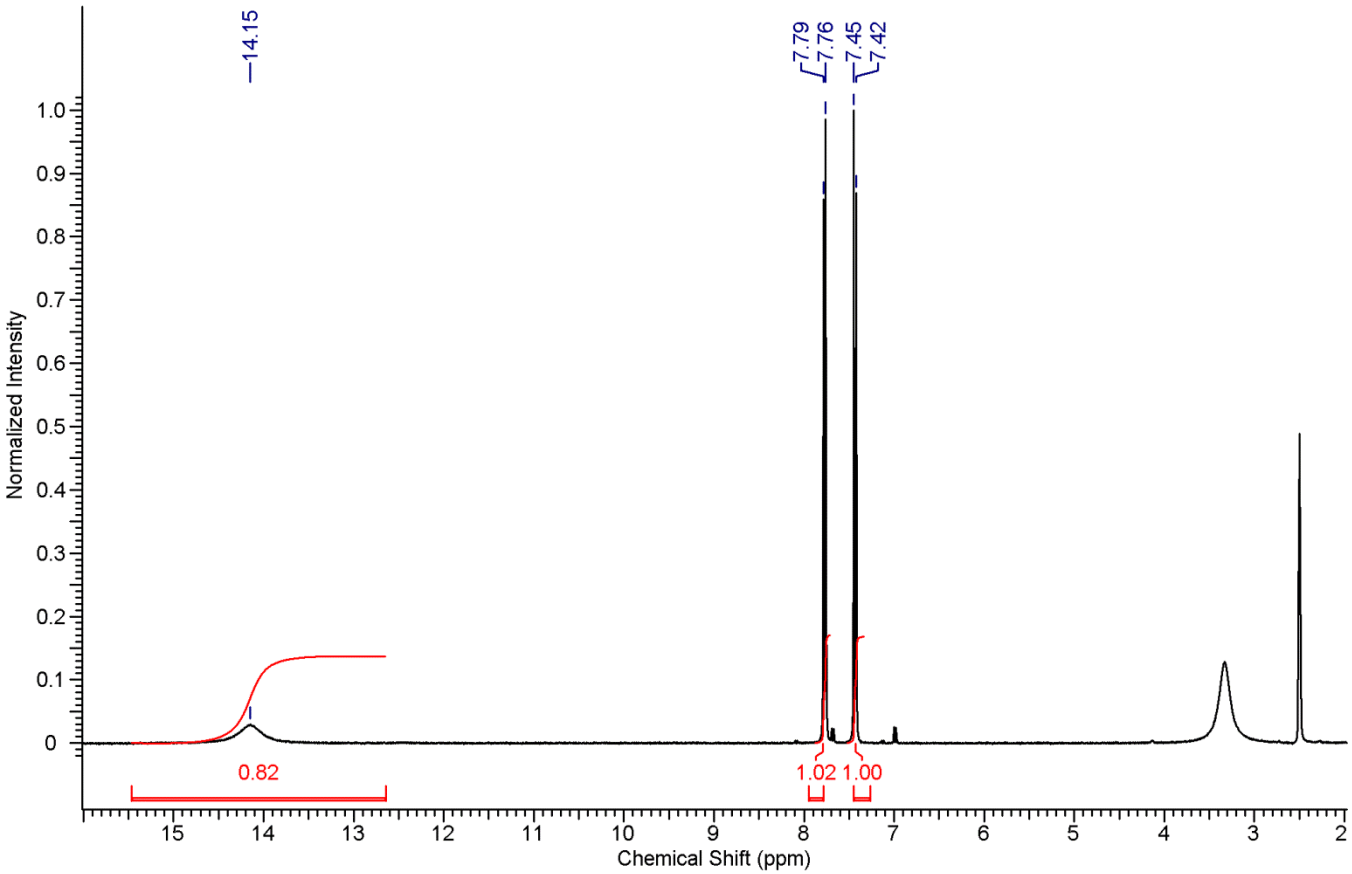
^{1}H-NMR of **7**


8.48
8.47
8.16
8.14
7.79
7.77
6.72
6.70
4.30
3.92
2.50
Normalized Intensity
1.00
0.68
0.67
0.99
2.05
3.08
Chemical Shift (ppm)

^{1}H-NMR of **8**

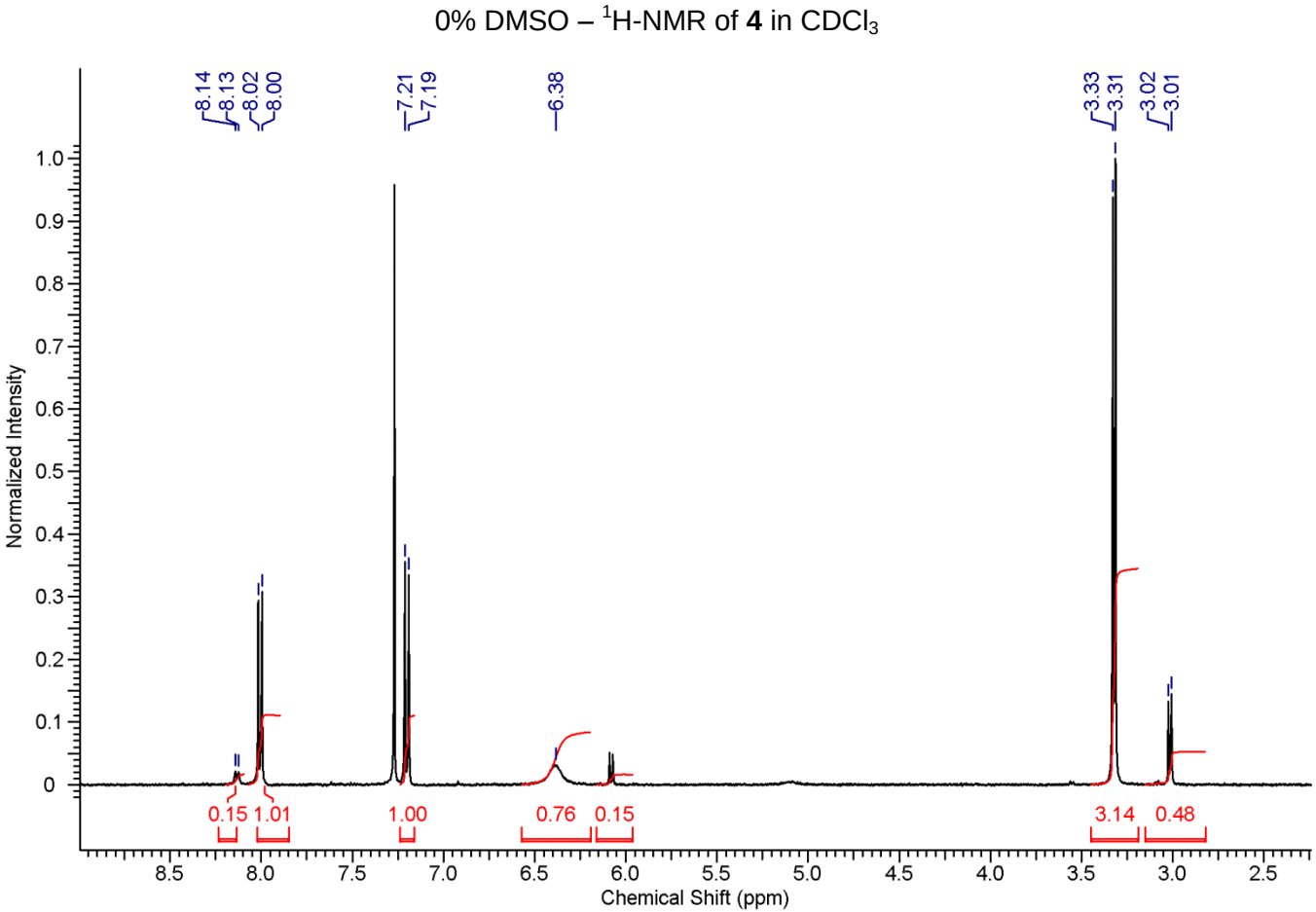

8.54
7.96
7.94
7.25
7.22
1.99
1.01
1.00
Normalized Intensity
Chemical Shift (ppm)

^{1}H-NMR of **9**

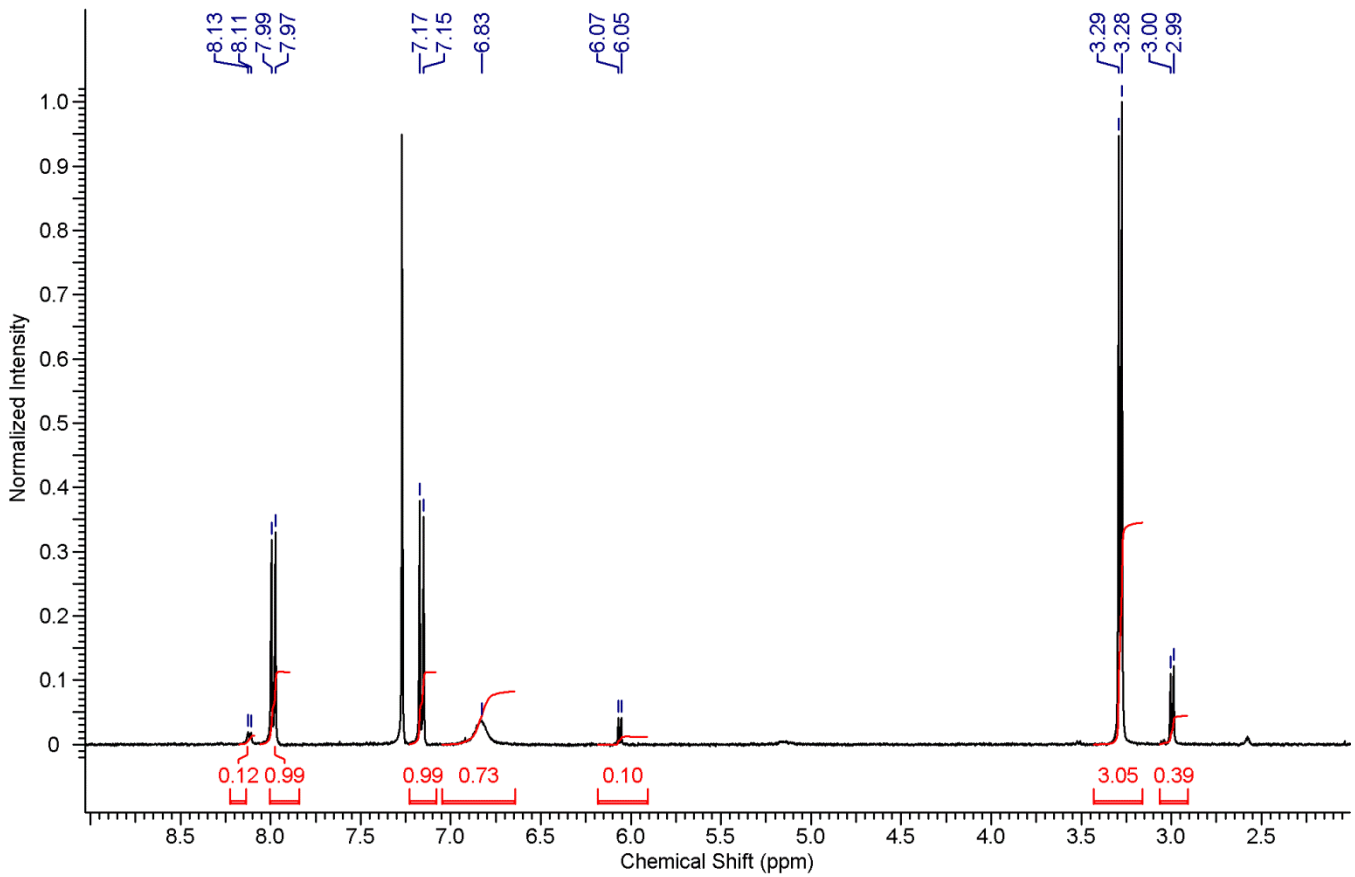


The small peaks close to the dublets at 7.77 and 7.43 are possibly due to thionyl-thiol tautomerization

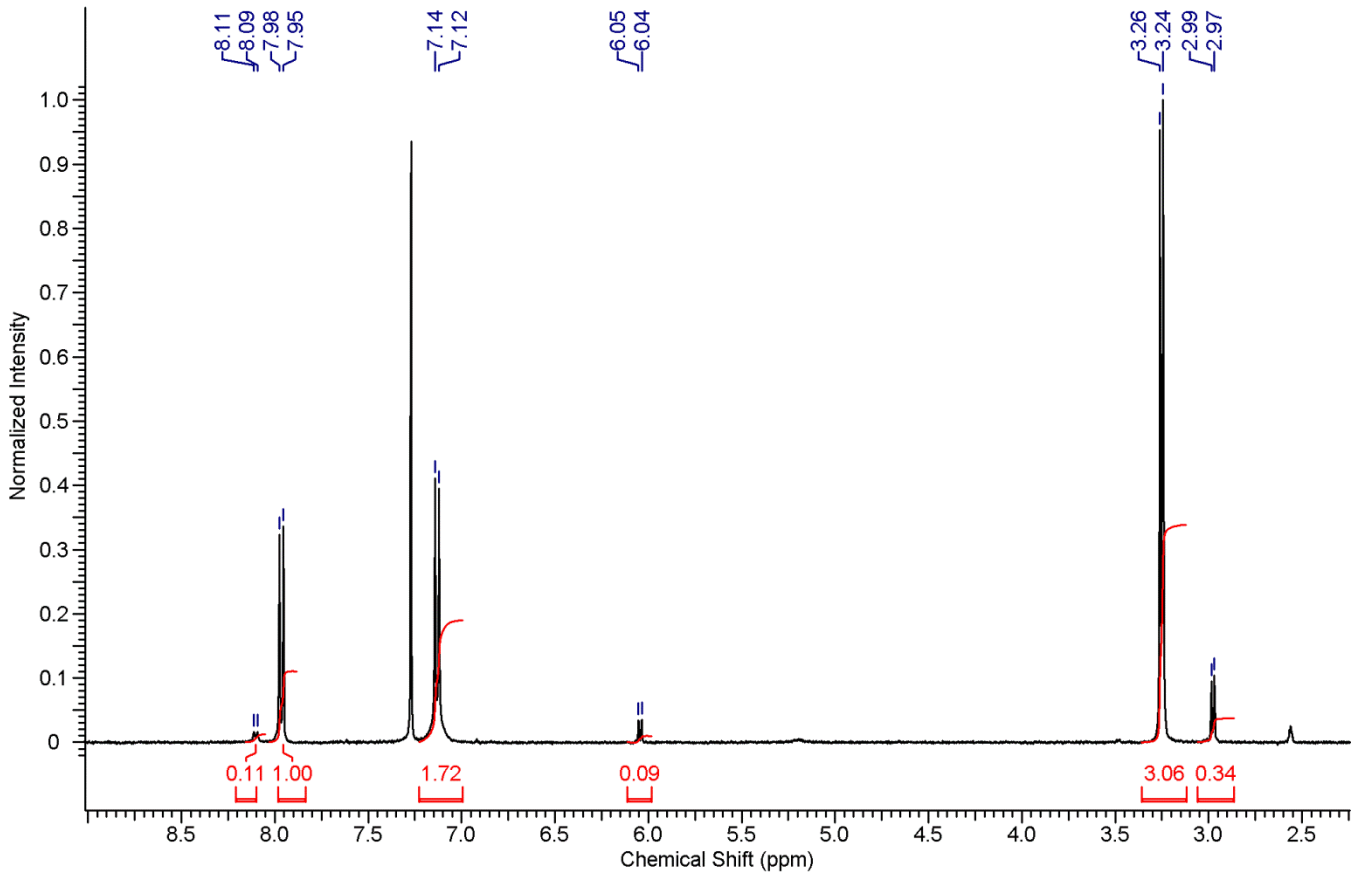
b) DMSO-d_6 titration of 4 in $CDCl_3$ monitored by ^{1}H-NMR



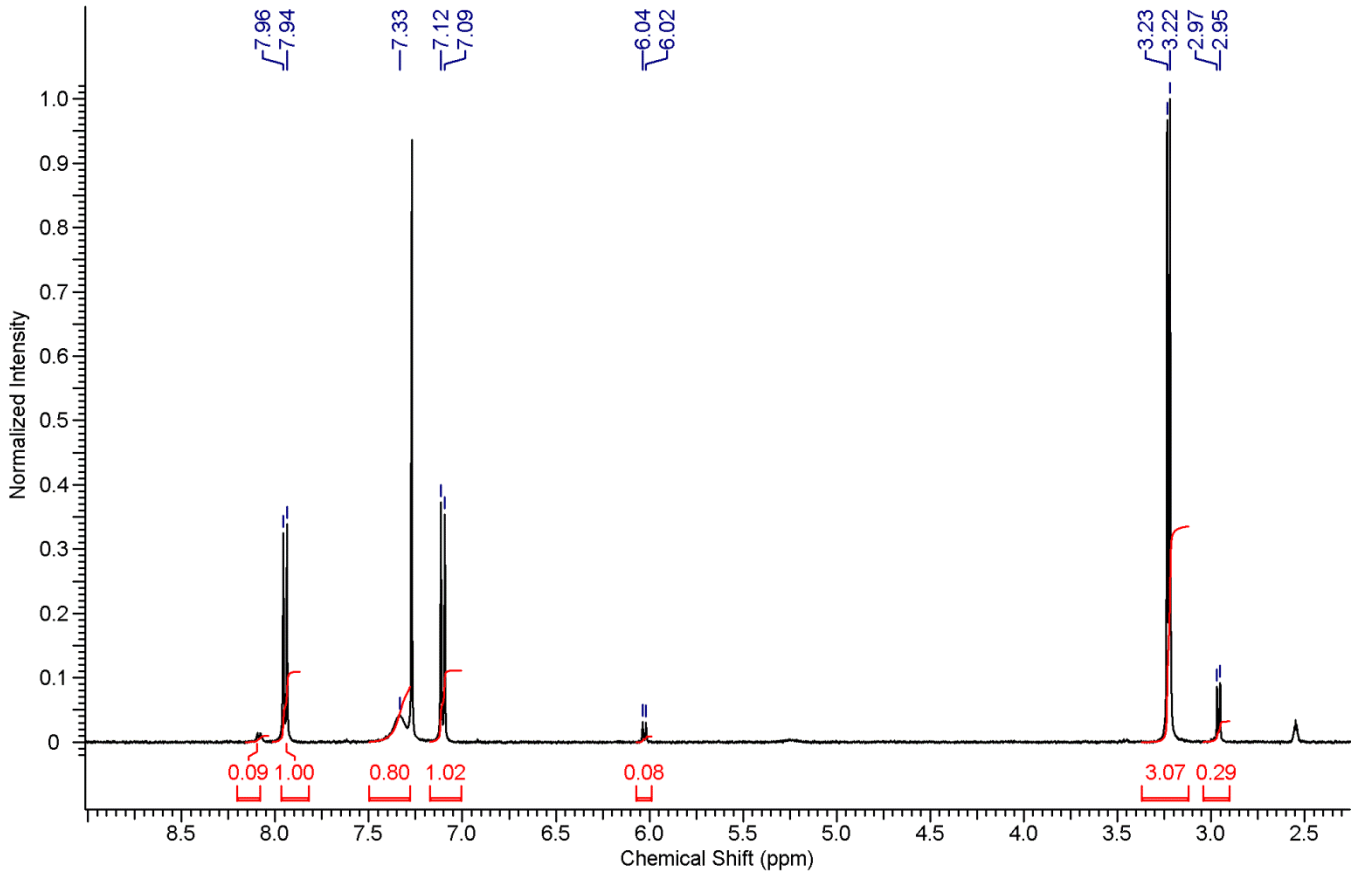
1% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



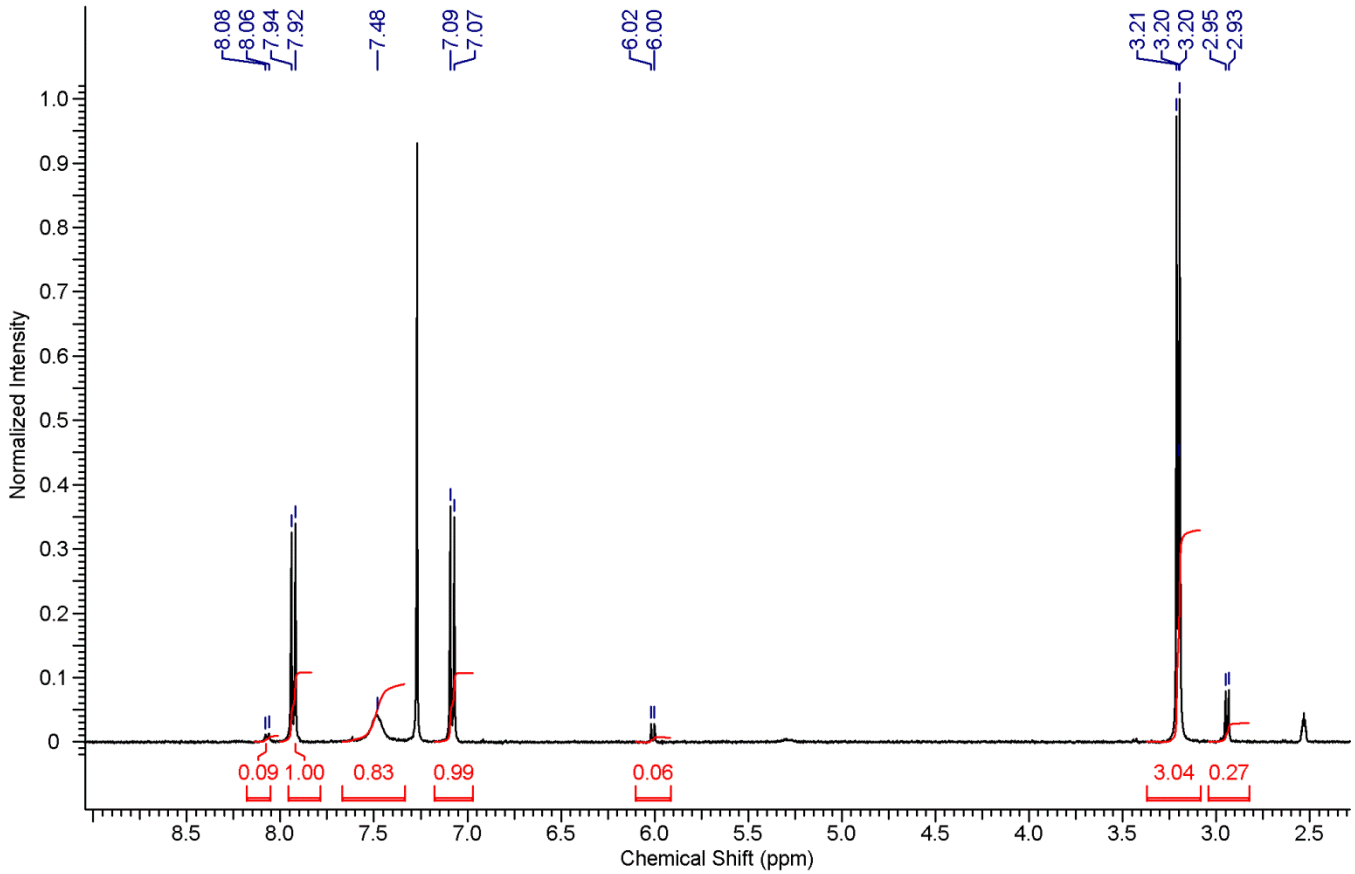
2% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



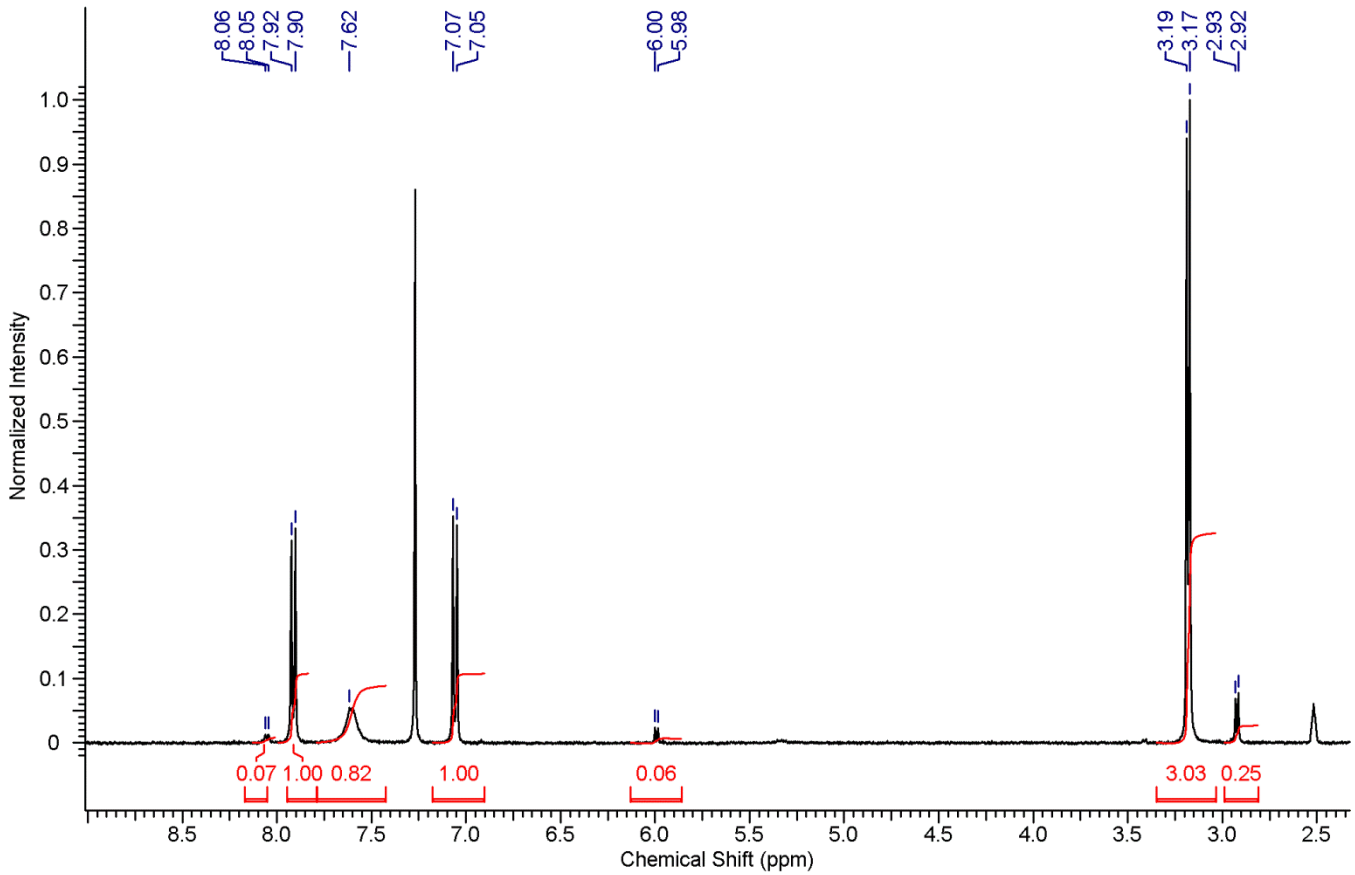
2.9% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



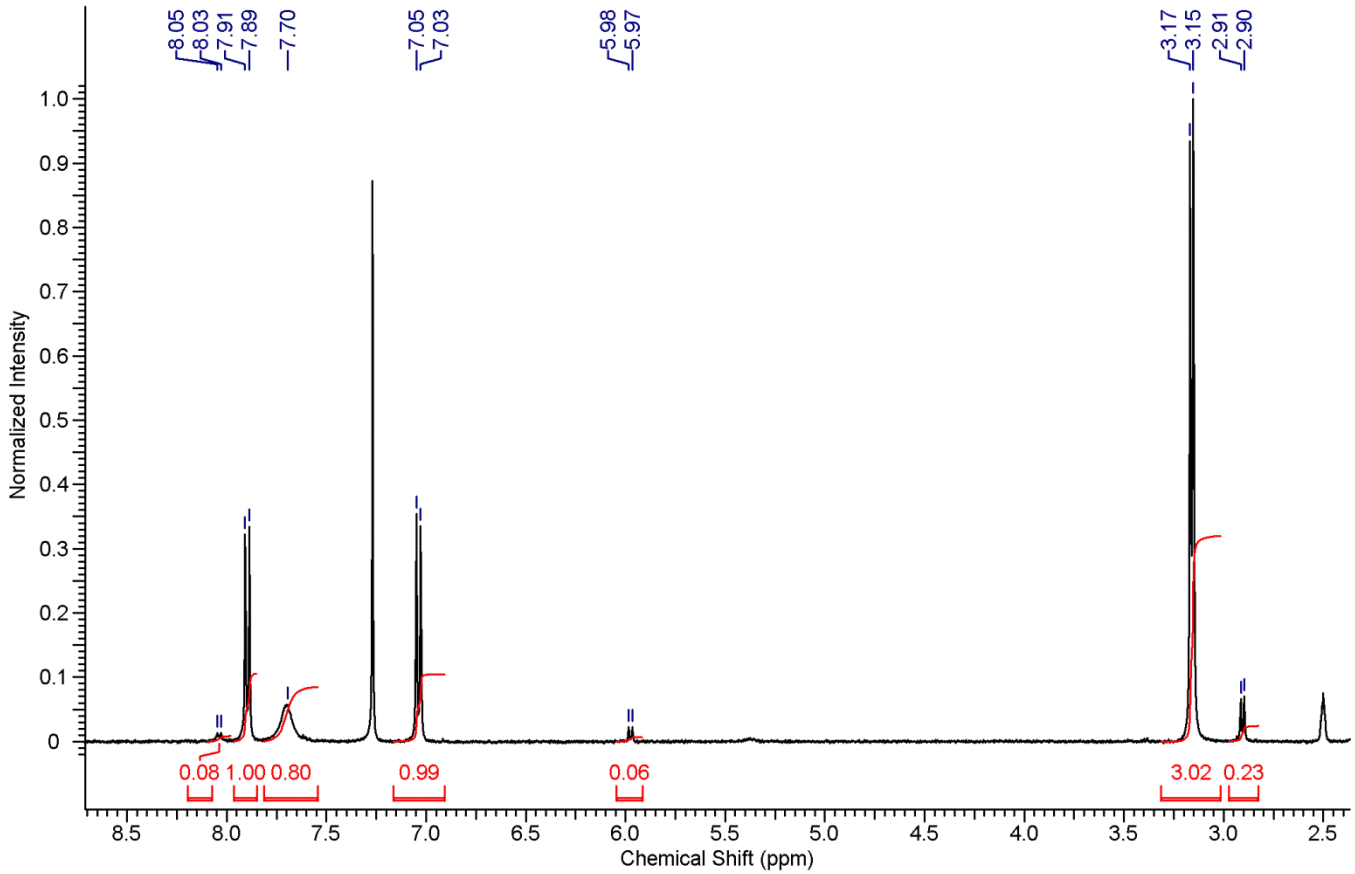
3.8% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



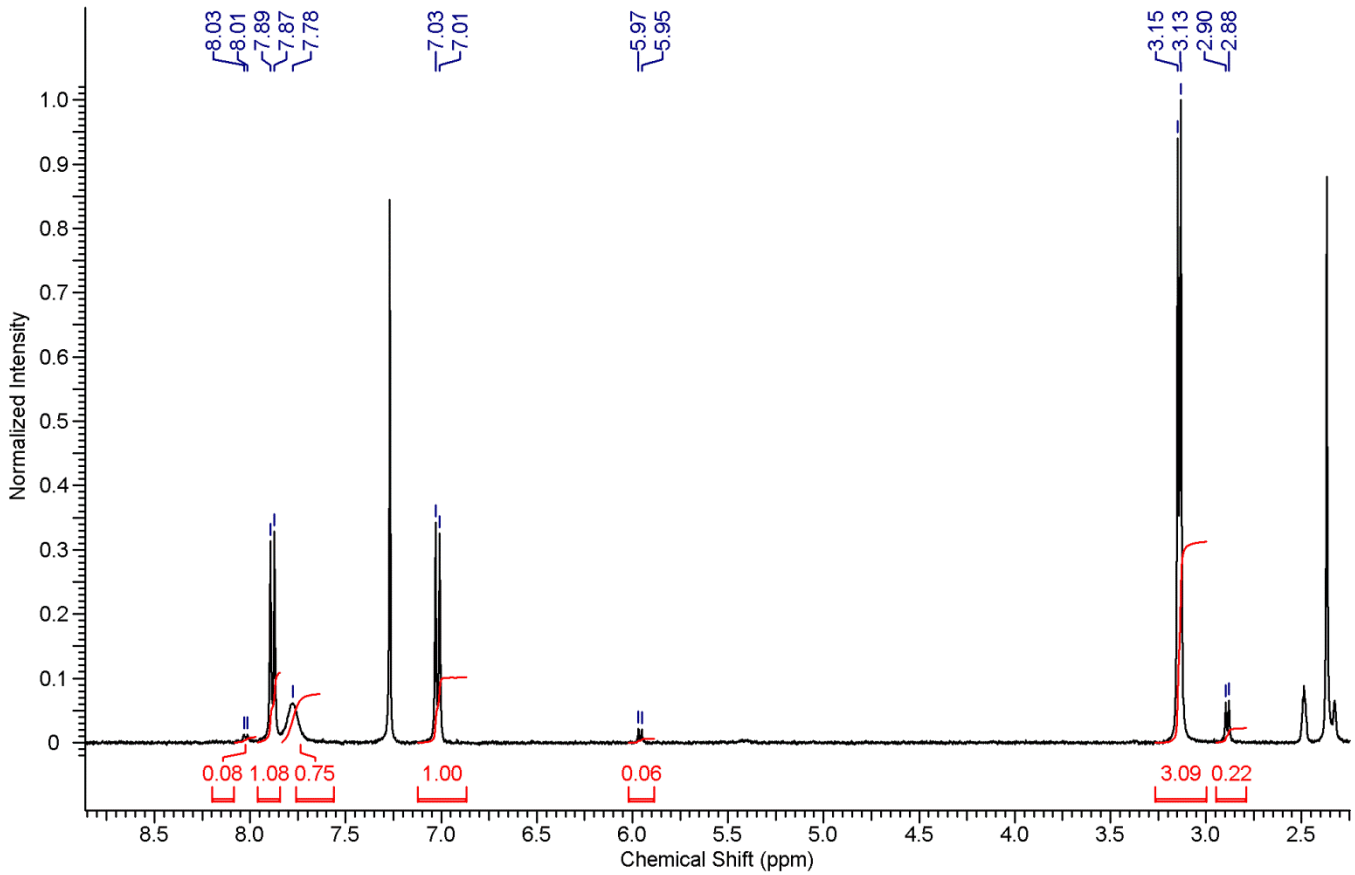
4.8% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



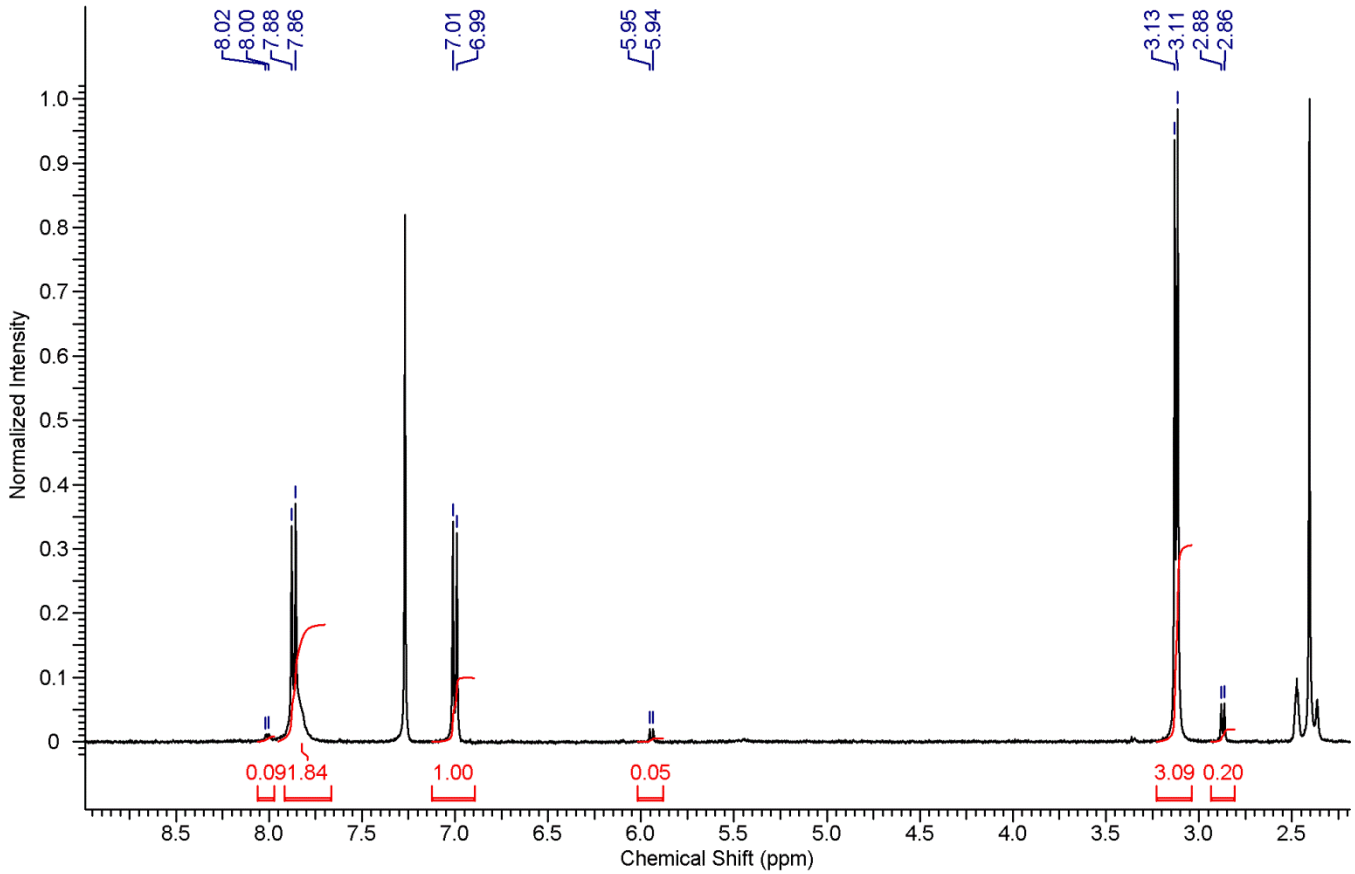
5.7% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



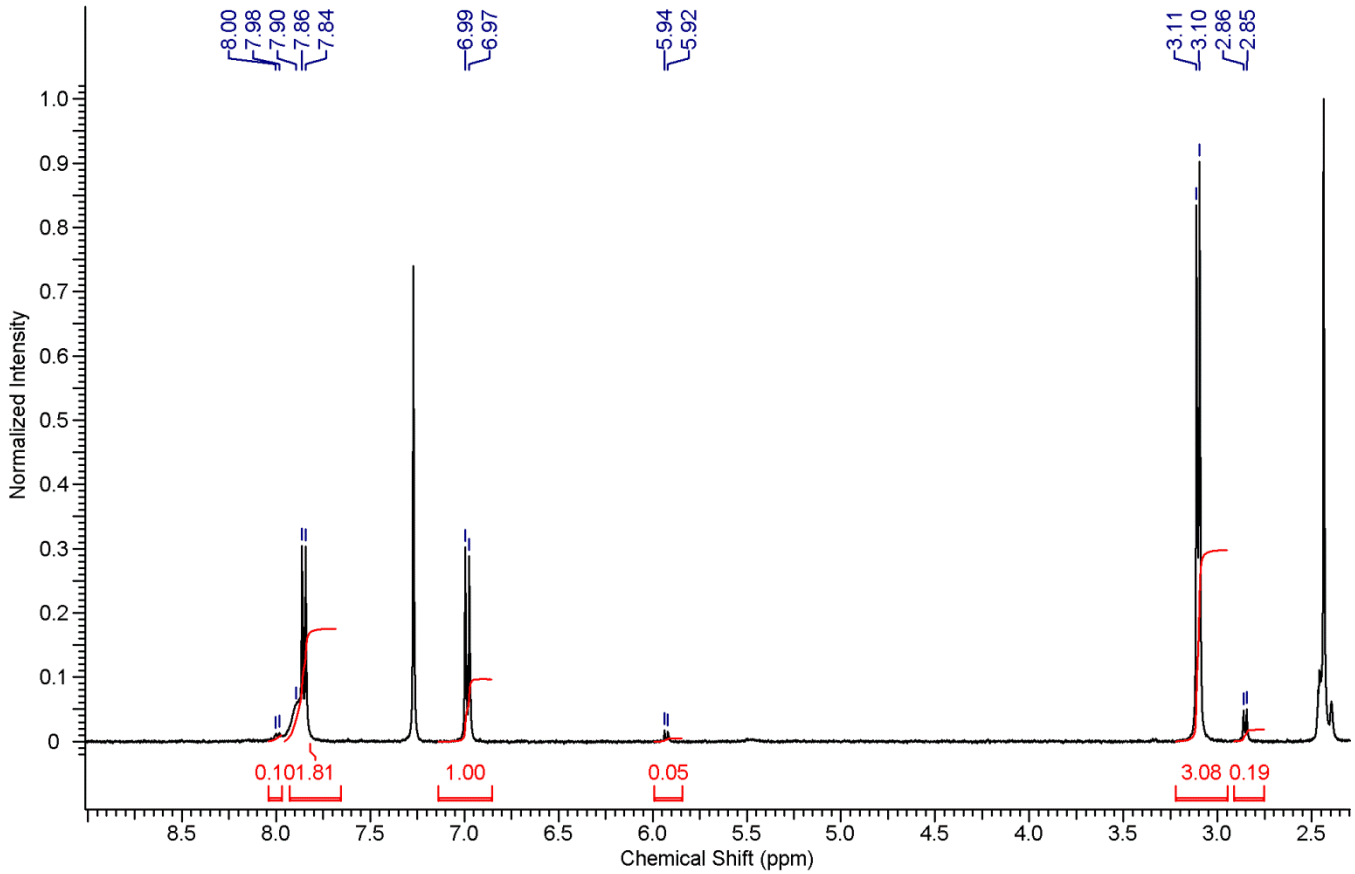
6.4% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



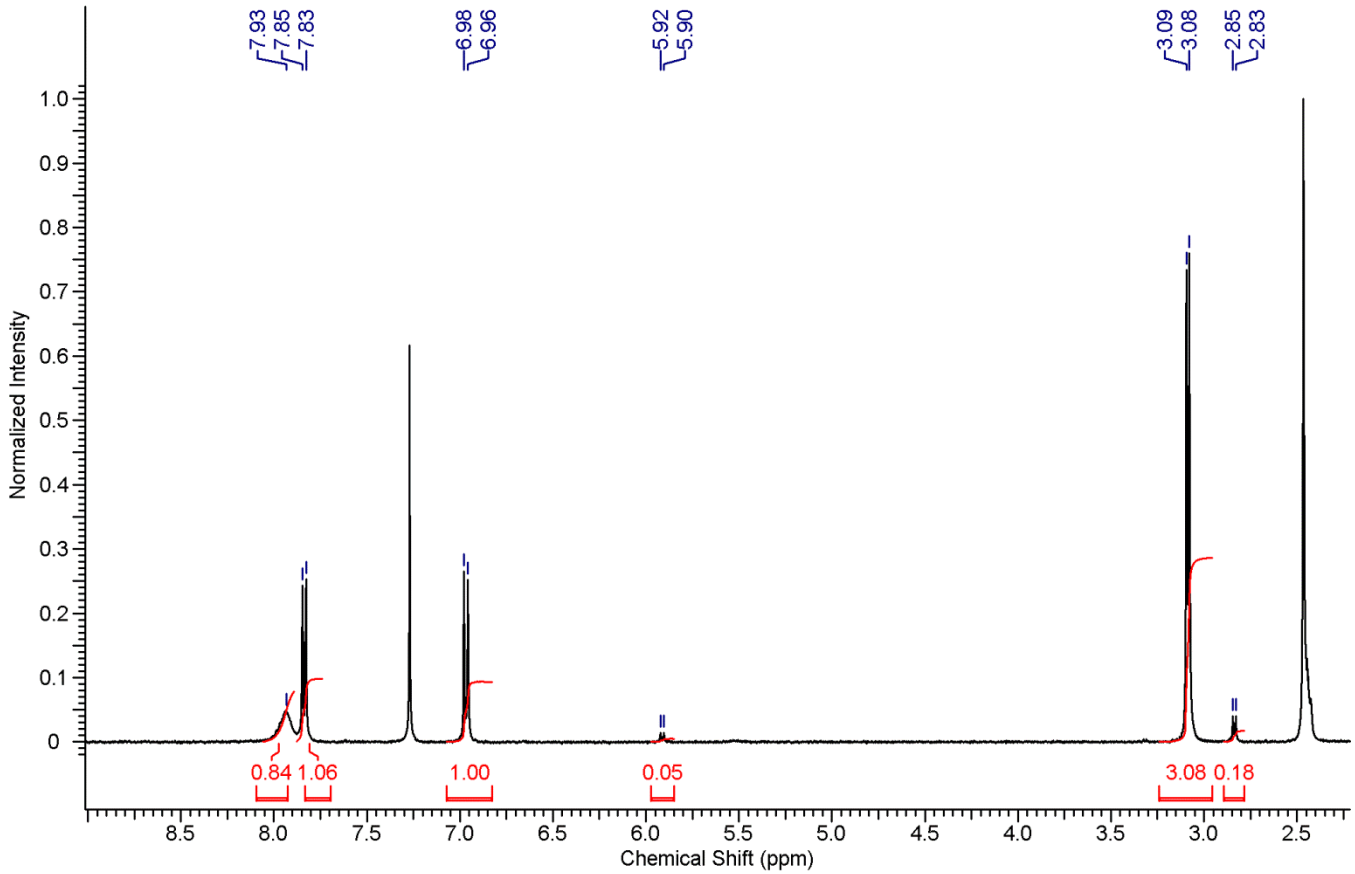
7.4DMSO – ^{1}H-NMR of **4** in $CDCl_3$



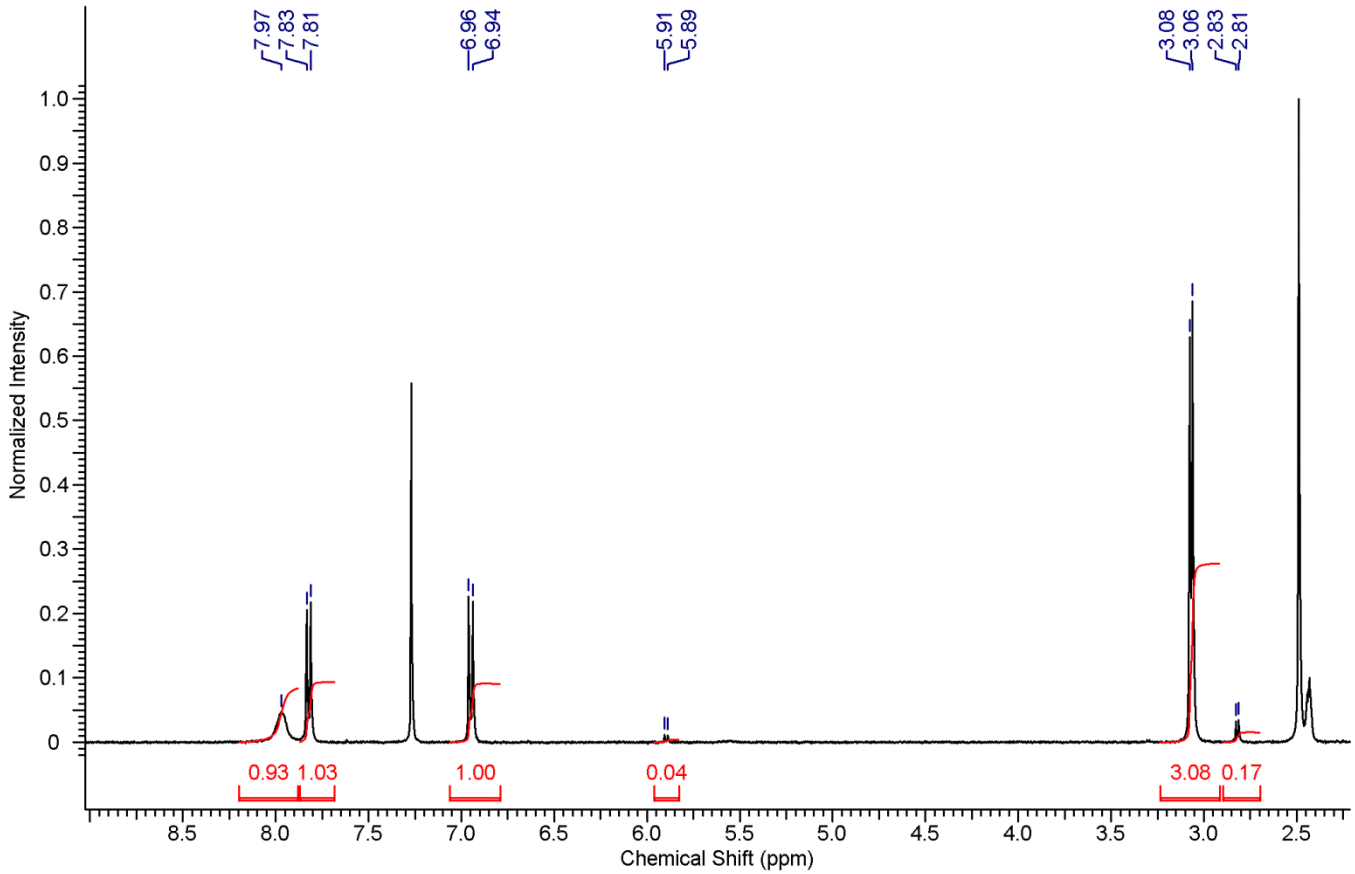
8.3% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



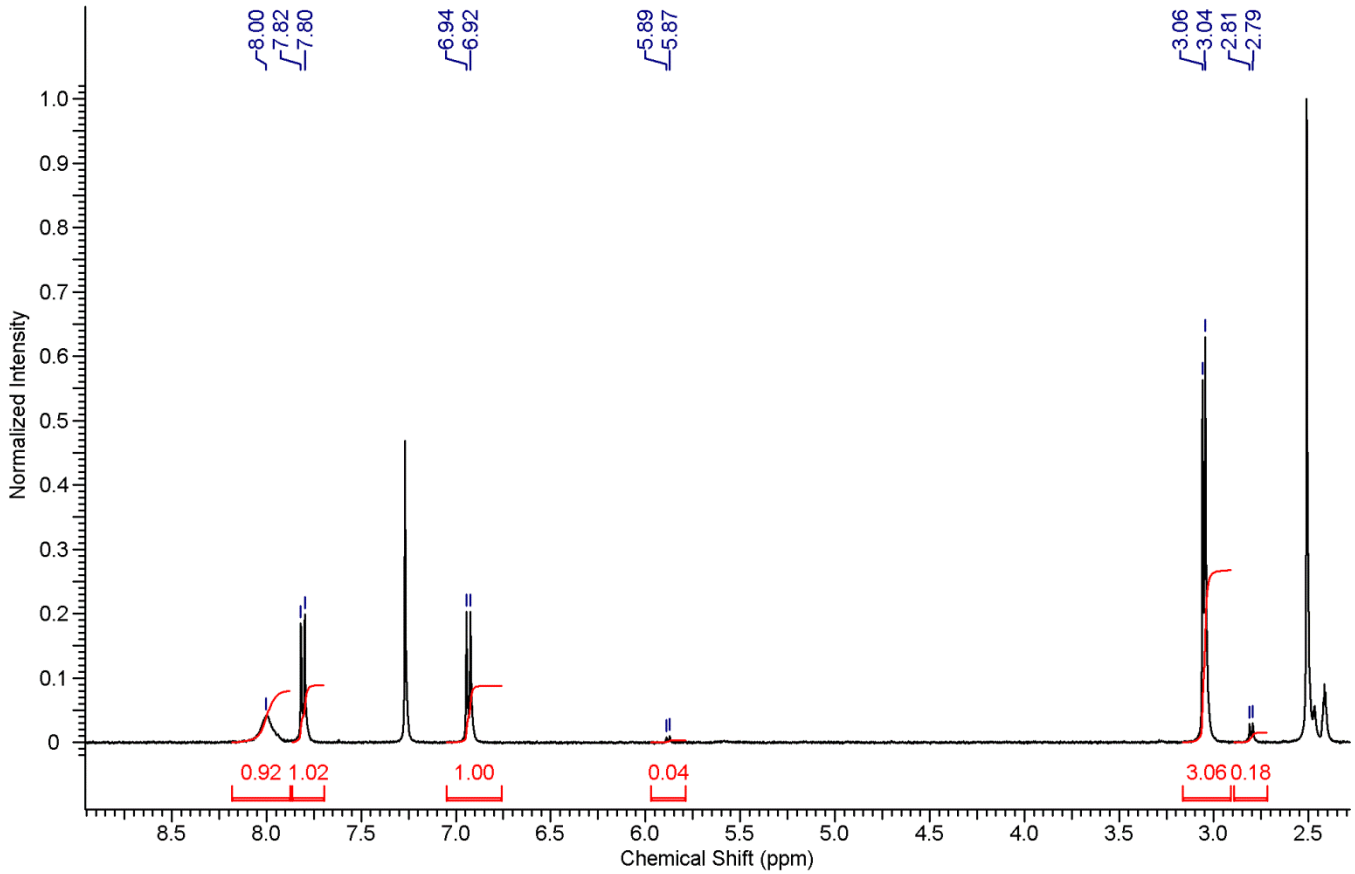
9.1% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



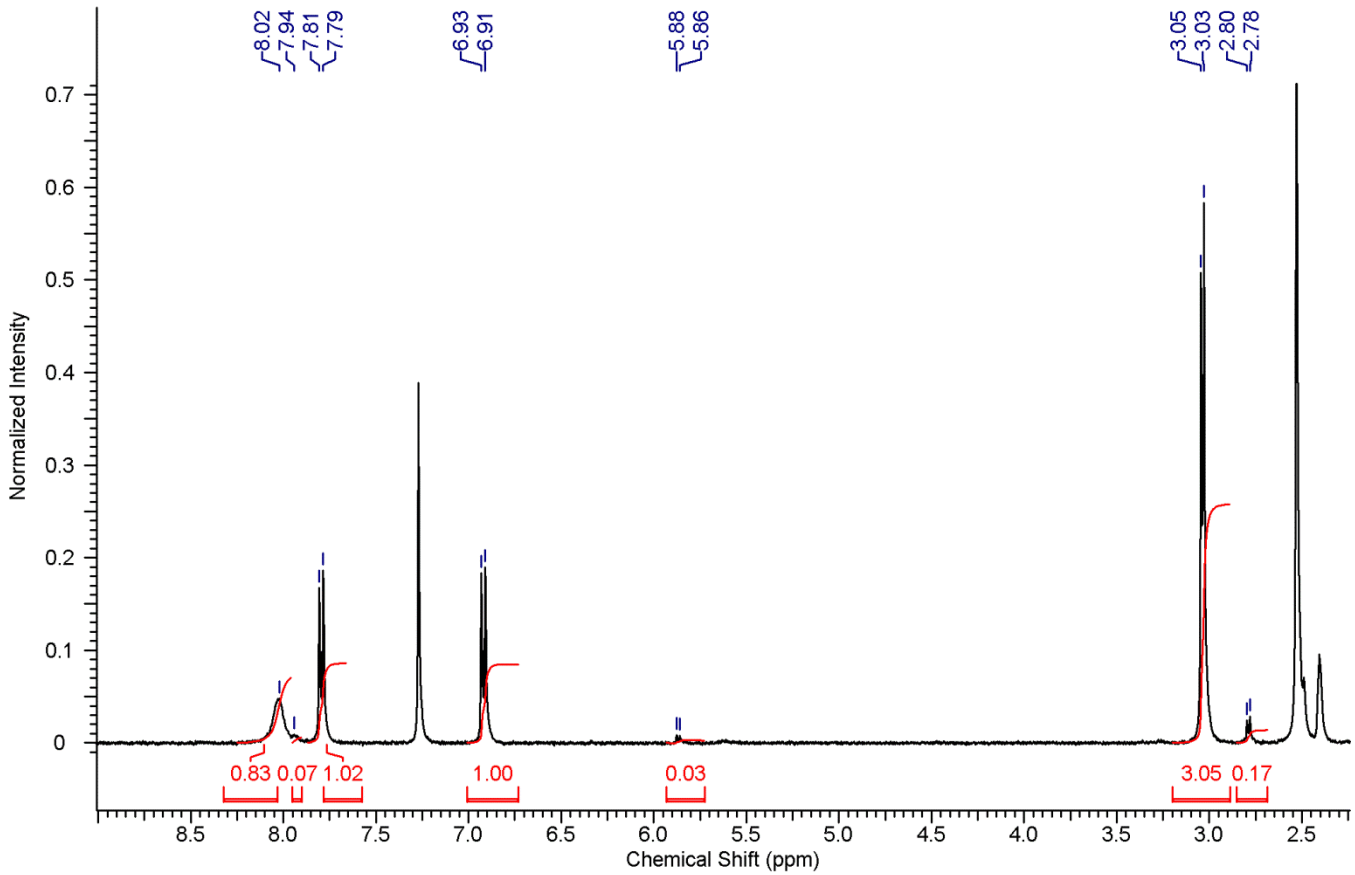
9.9% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



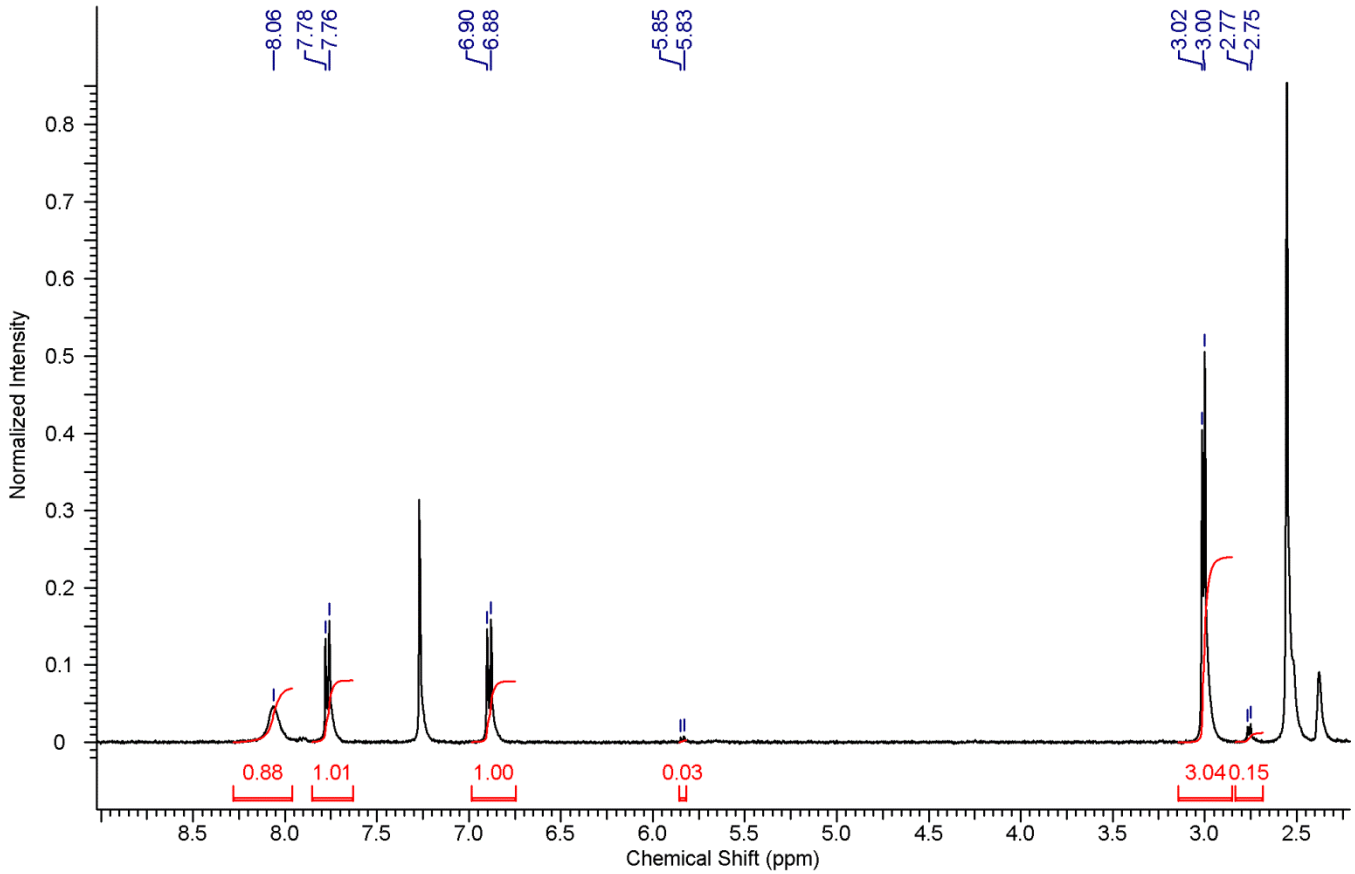
10.7% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



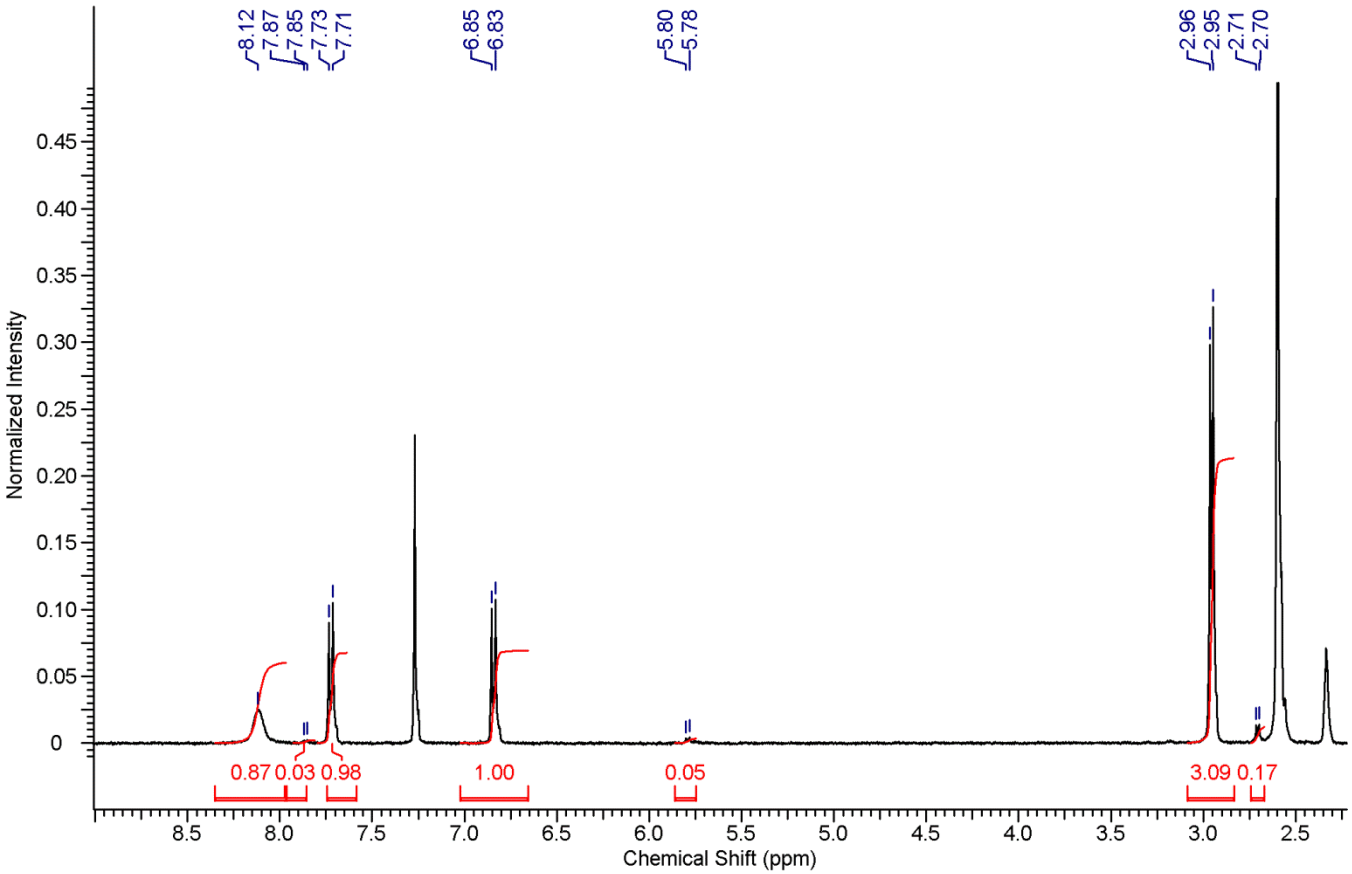
11.5% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



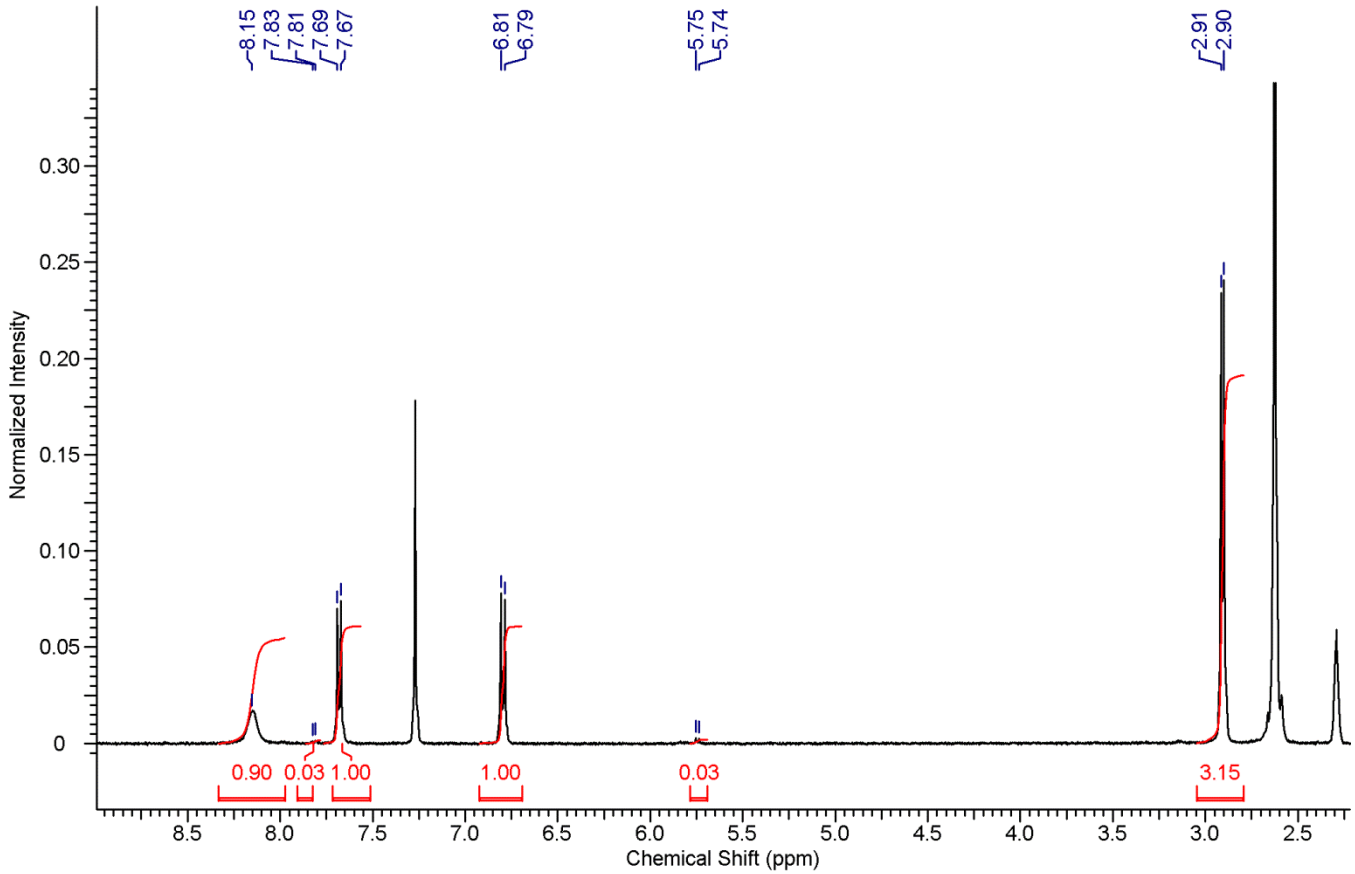
12.9% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



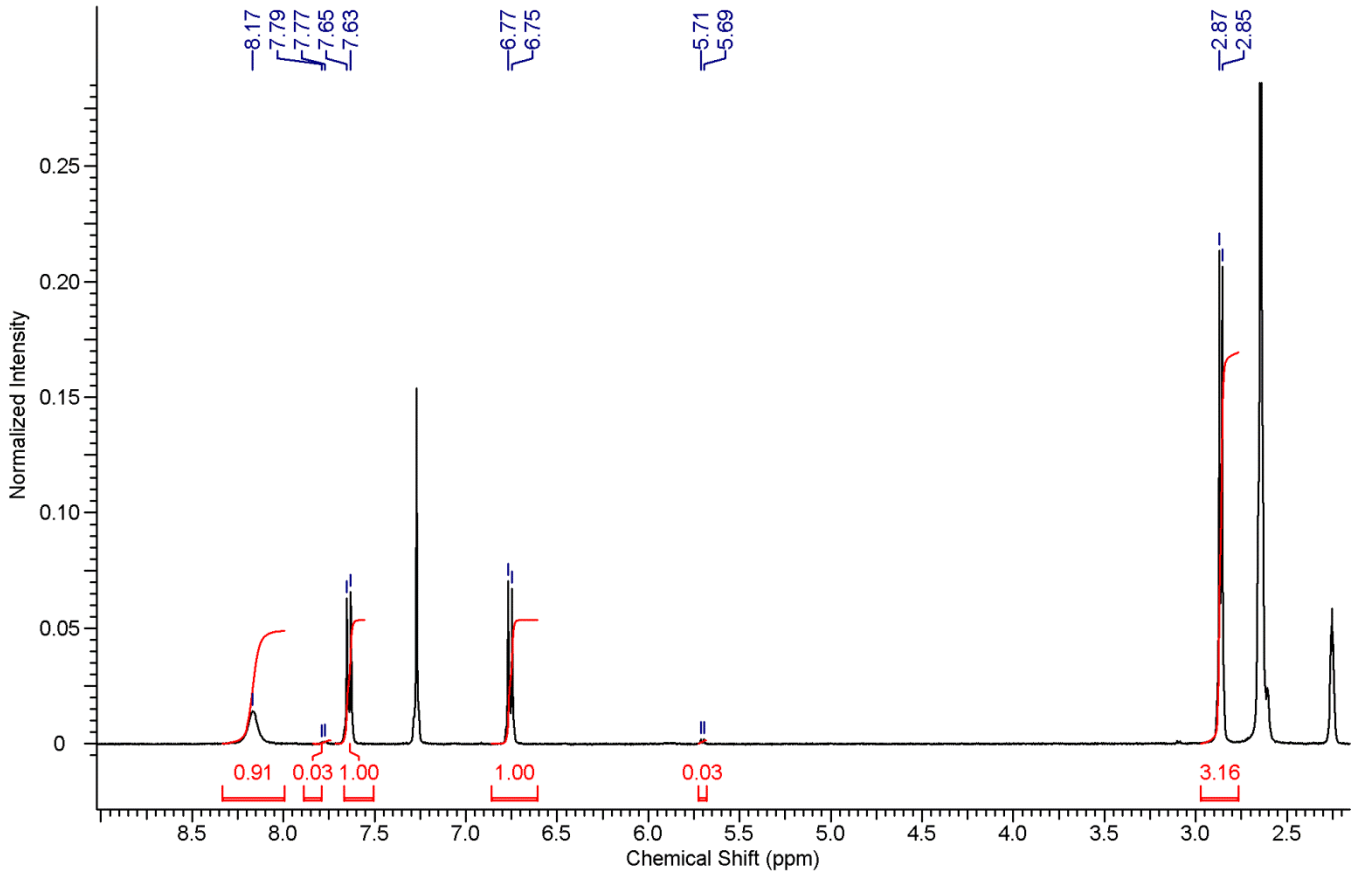
15.6% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



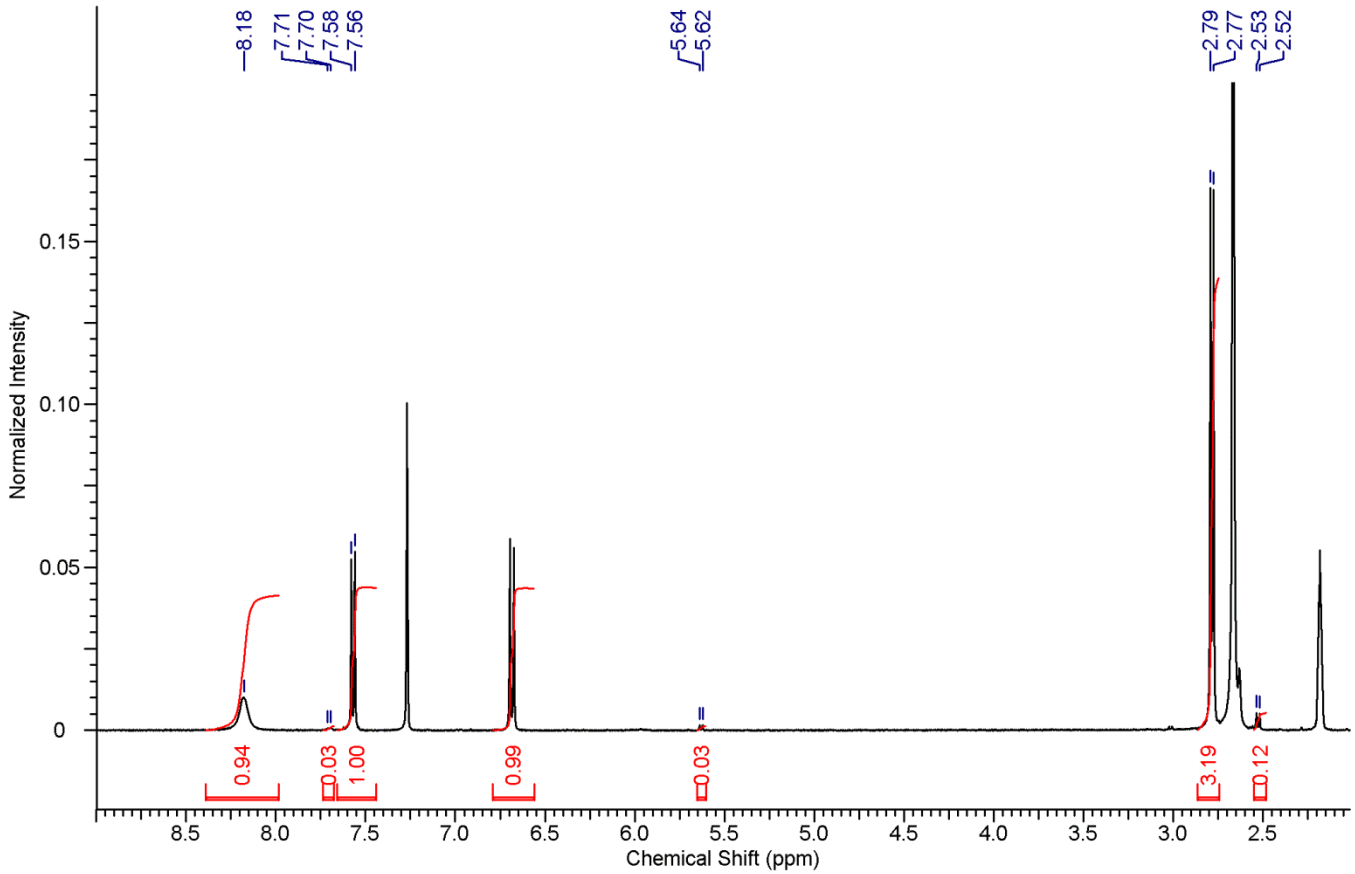
18.1´% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



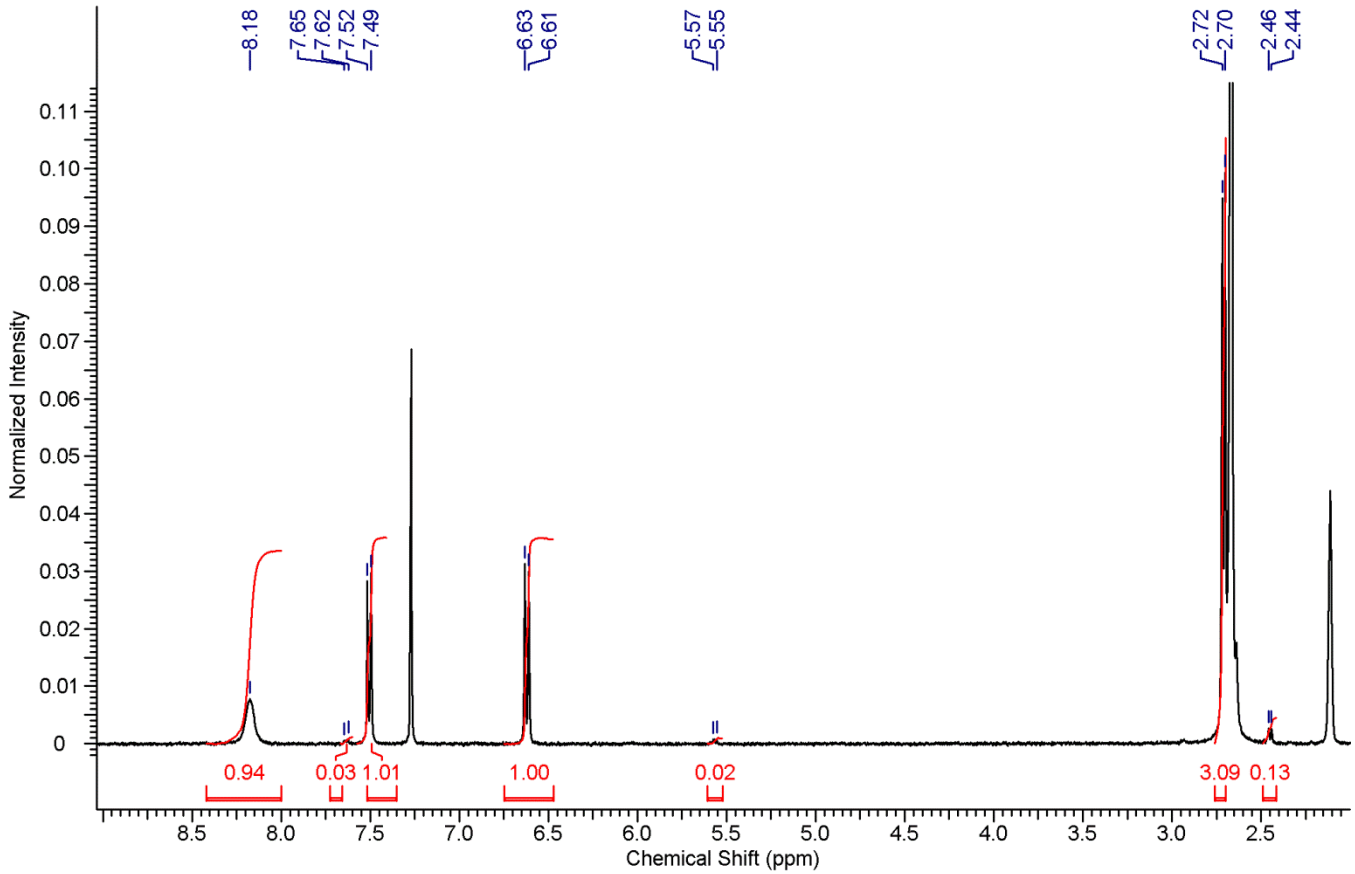
20.5% DMSO – ^{1}H-NMR of **4** in $CDCl_3$



24.8 DMSO – ^{1}H-NMR of **4** in $CDCl_3$



28.7%DMSO – ^{1}H-NMR of **4** in $CDCl_3$

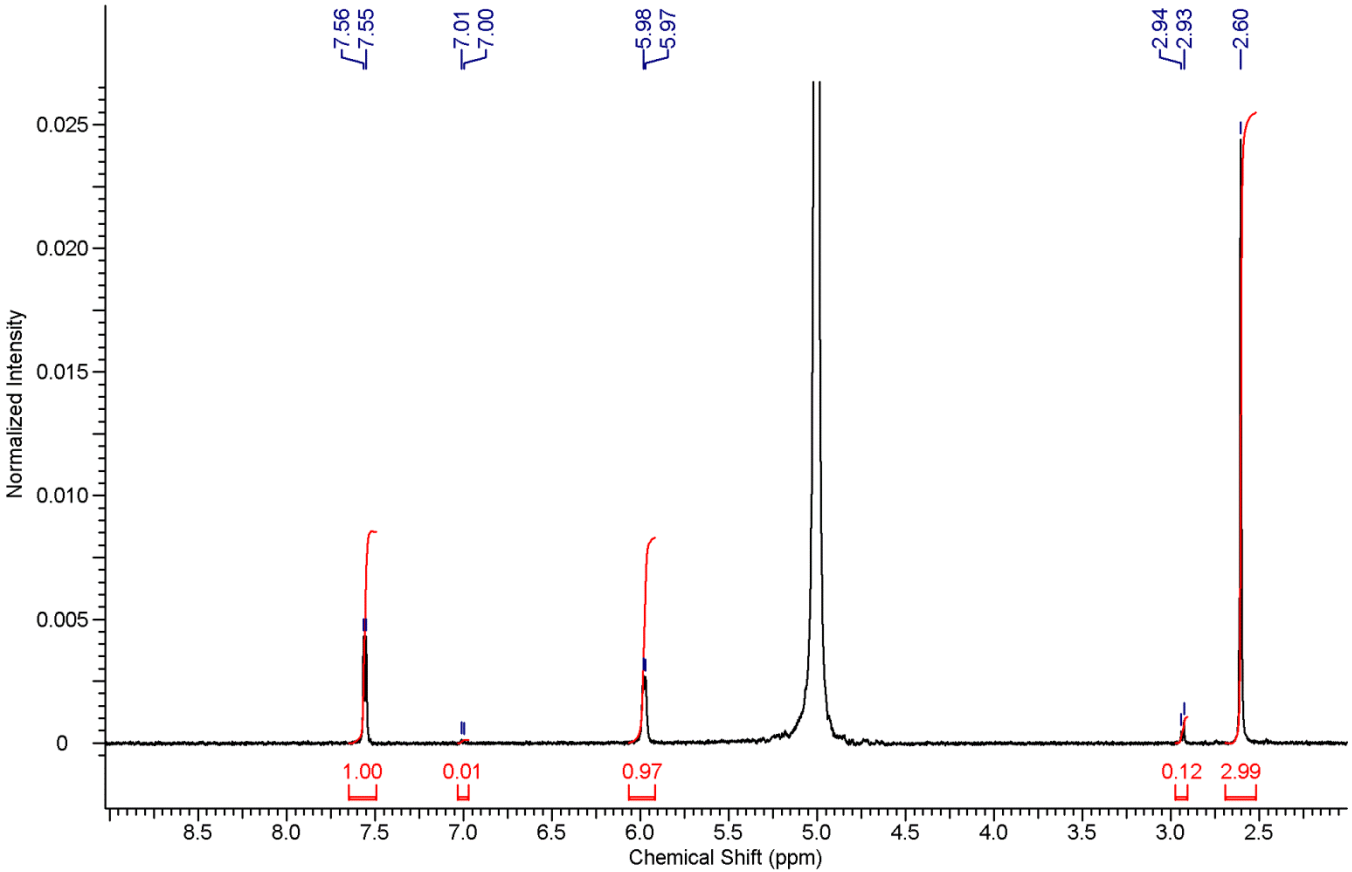


^{1}H-NMR shift of the NH-signal and percentage of azide-integrals to tetrazole-integrals of 4 under DMSO titration in $CDCl_3$

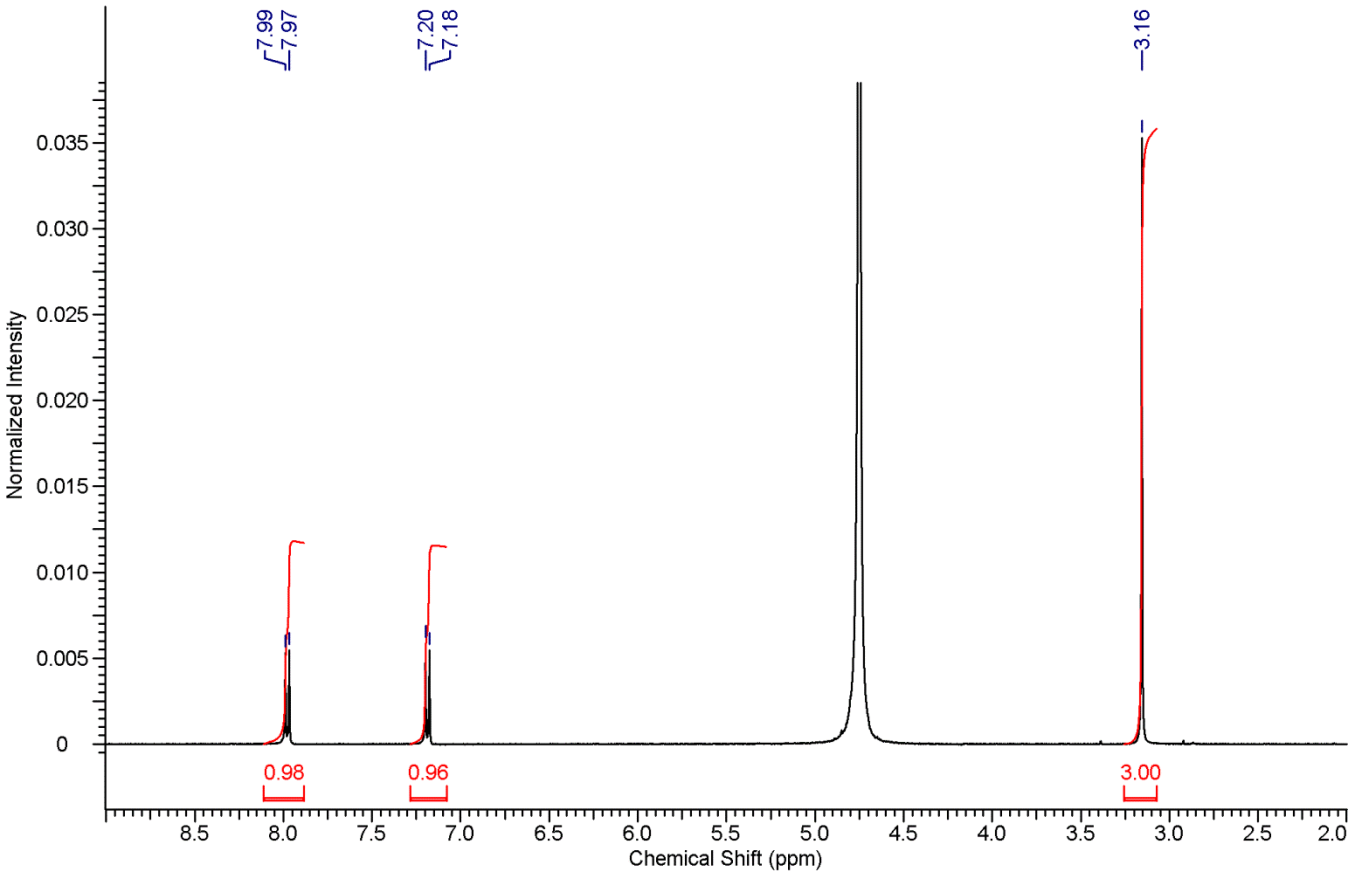
DMSO [%]	N<u>H</u>-shift [ppm]	Azide [%]	Tetrazole [%]
0.0	6.38	15.0	85.0
1.0	6.83	11.0	89.0
2.0	7.13	9.0	91.0
2.9	7.33	8.0	92.0
3.8	7.48	7.0	93.0
4.8	7.61	6.0	94.0
5.7	7.70	5.7	94.3
6.5	7.78	5.7	94.4
7.4	7.83	5.0	95.1
8.3	7.89	4.8	95.3
9.1	7.94	4.8	95.2
9.9	7.97	4.1	95.9
10.7	8.00	3.7	96.3
11.5	8.02	3.7	96.3
12.9	8.06	3.0	97.0
15.6	8.11	4.0	96.1
18.1	8.15	2.8	97.2
20.5	8.17	2.9	97.1
24.8	8.18	2.7	97.3
28.7	8.18	2.6	97.4

c) Acidic, basic and neutral ^{1}H-NMR of 4 and 5

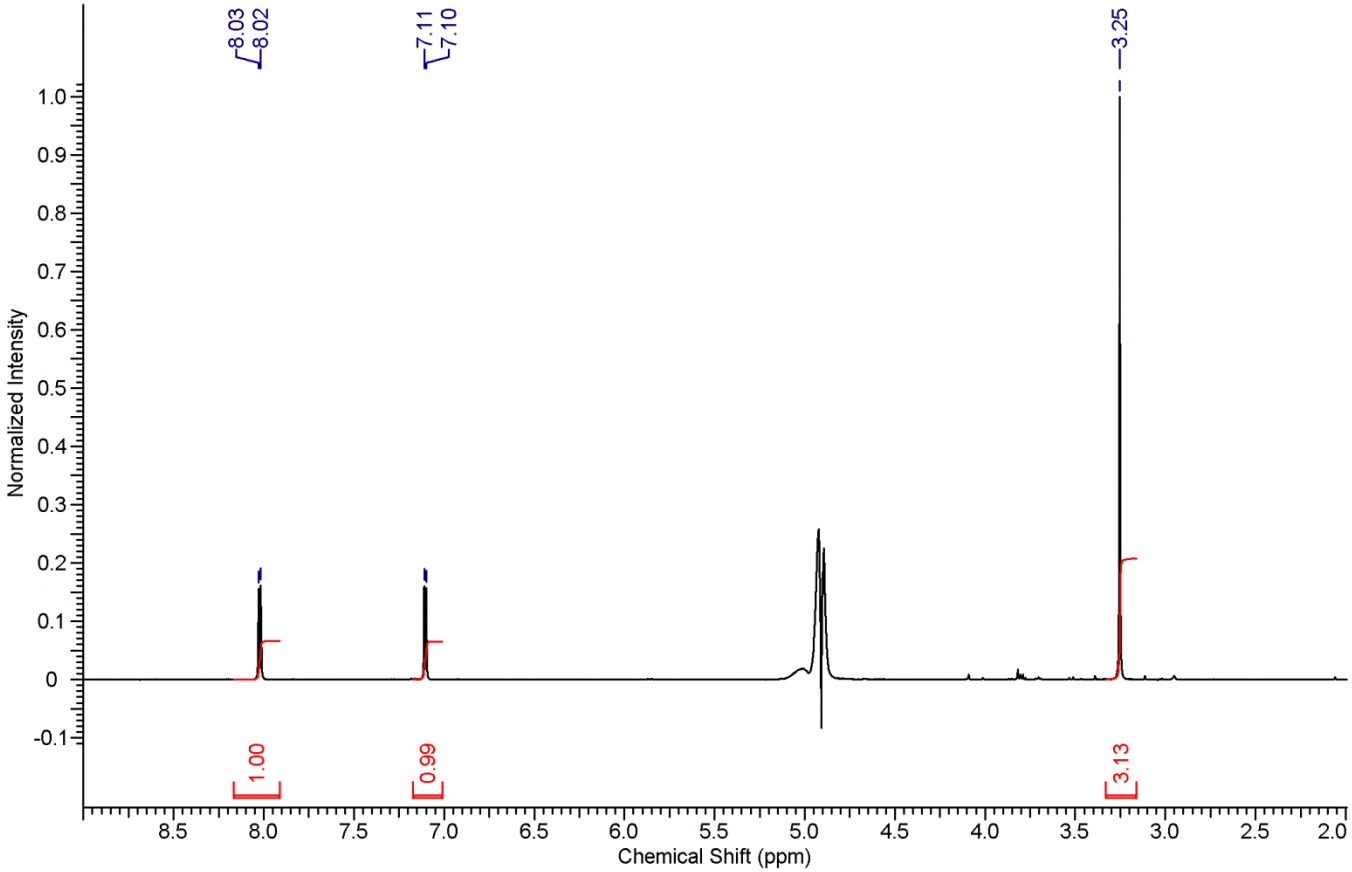
^{1}H-NMR of **4** in D_2O (acidic + TFA)



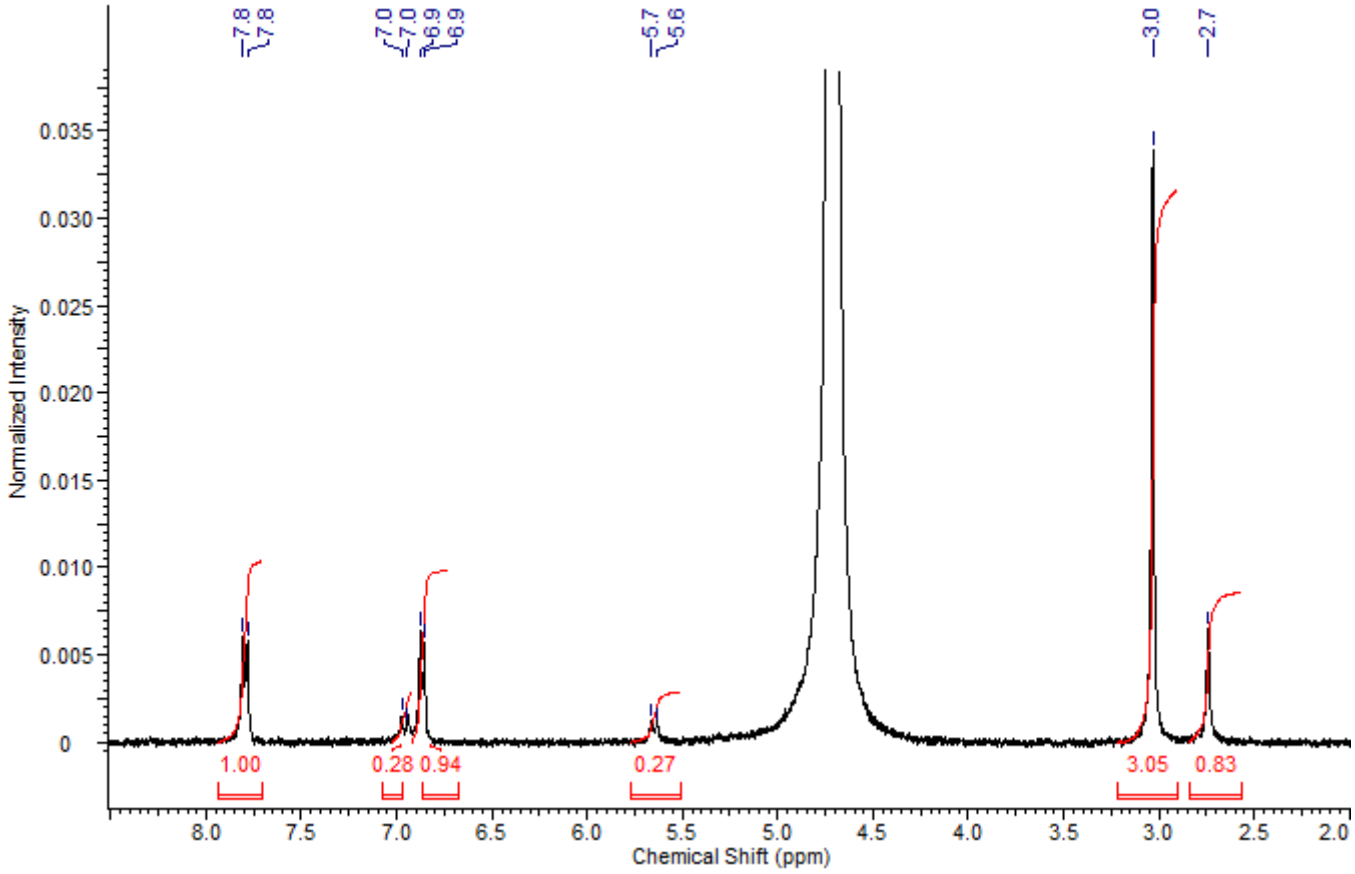
^{1}H-NMR of **4** in D_2O (neutral)



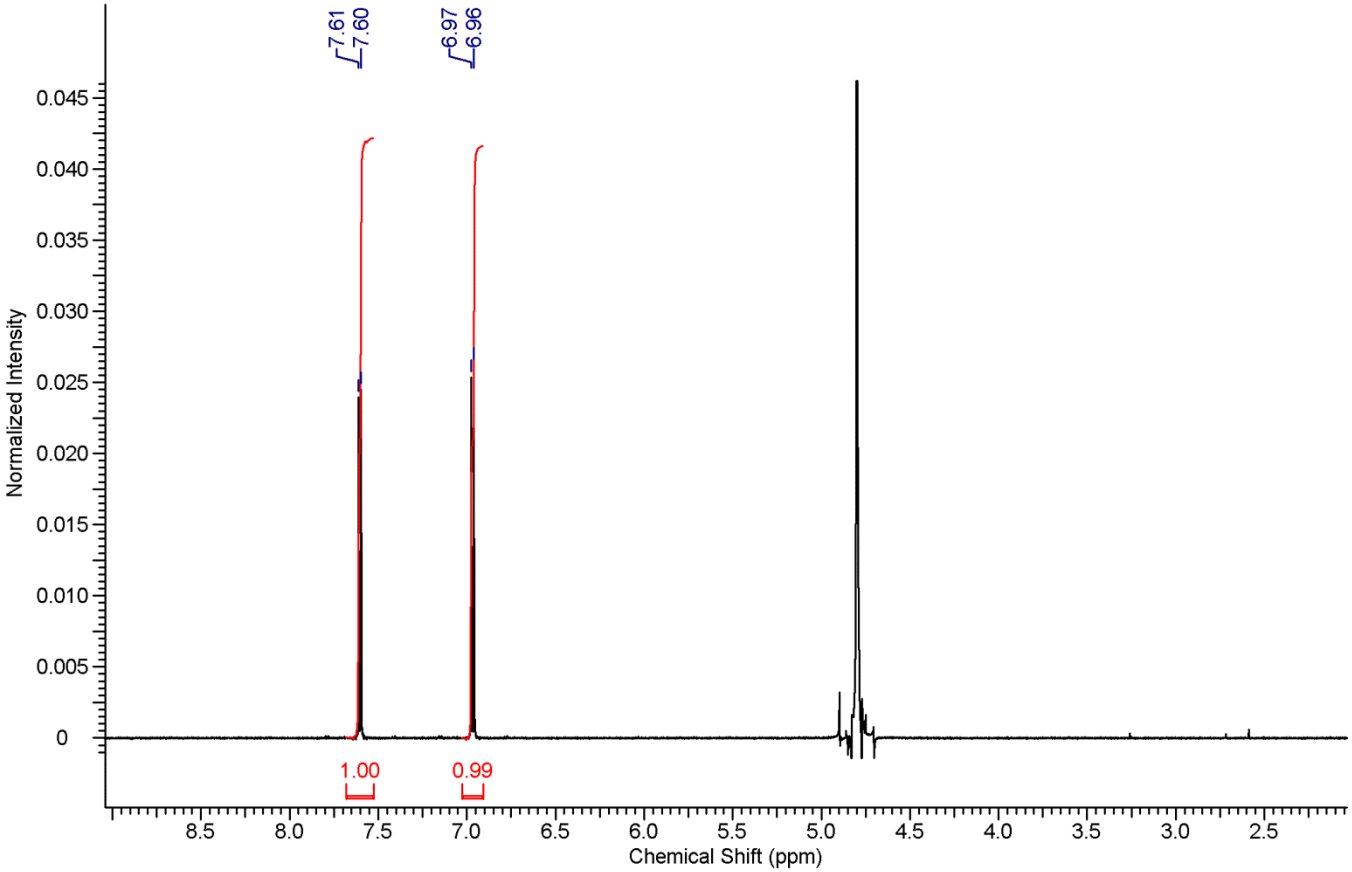
^{1}H-NMR of **4** in D_2O (basic + 0.1M NaOH)



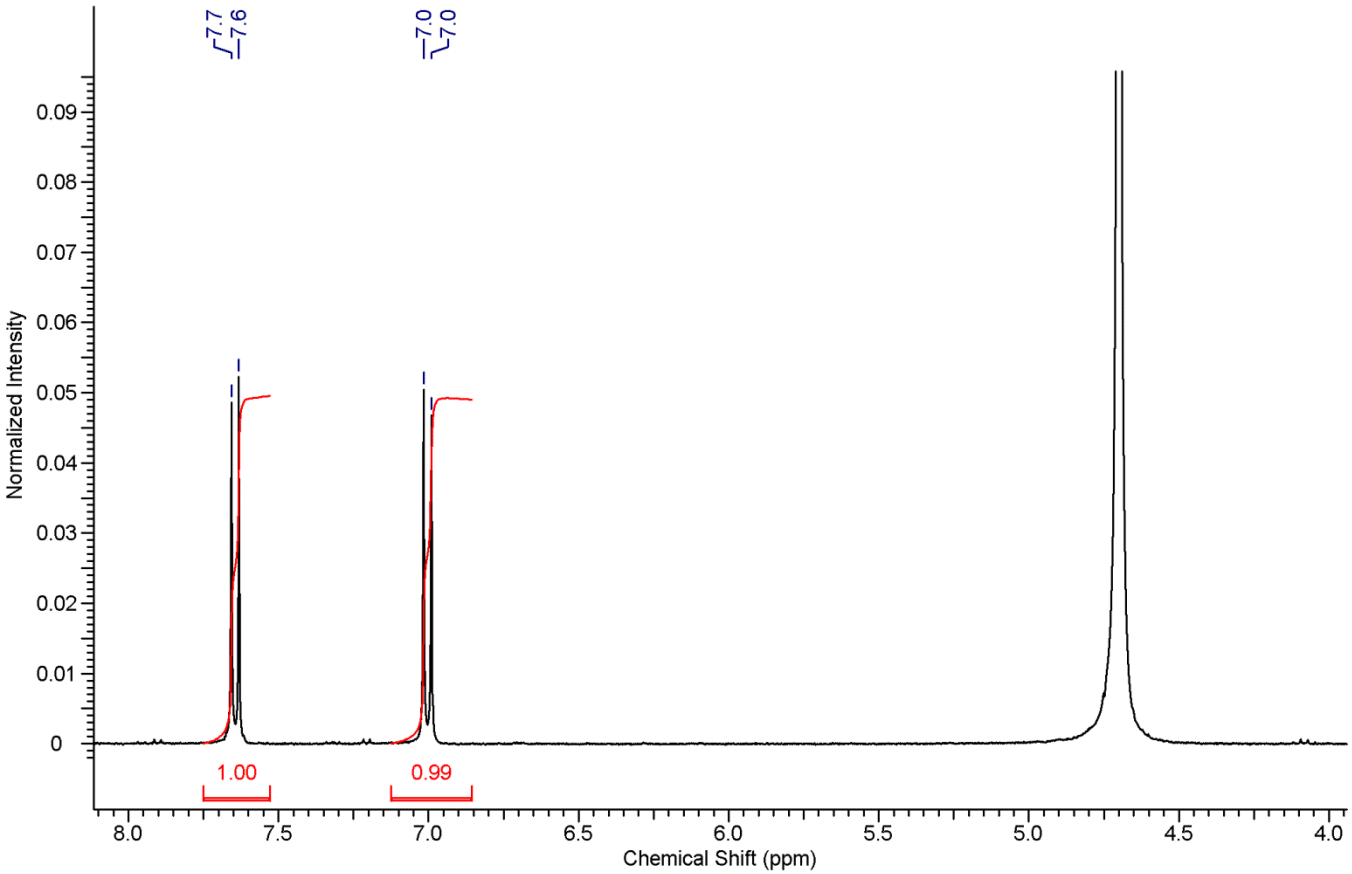
^{1}H-NMR of **4** in D_2O (basic + 1M NaOH)



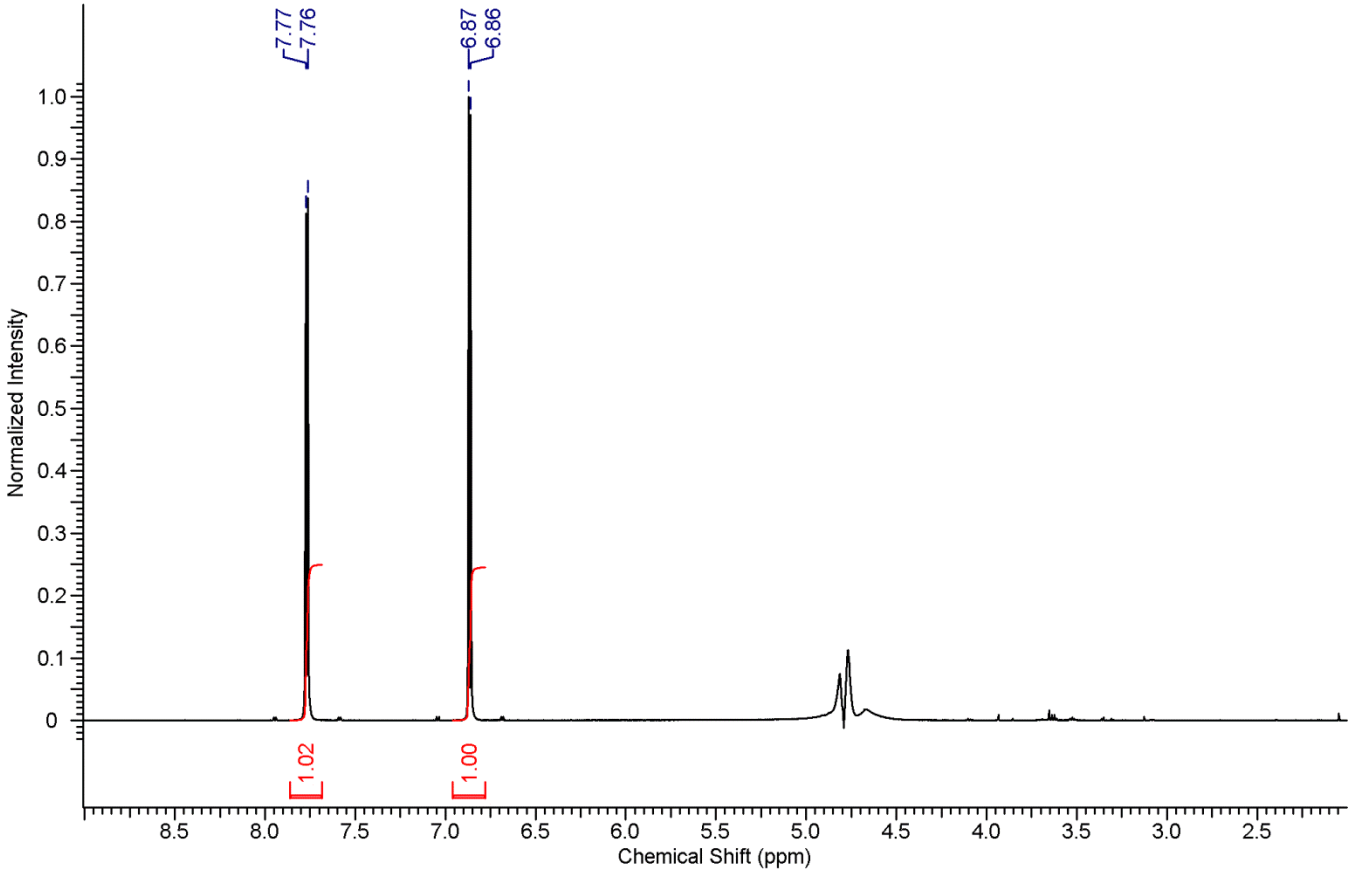
^{1}H-NMR of **5** in D_2O (acidic + TFA)



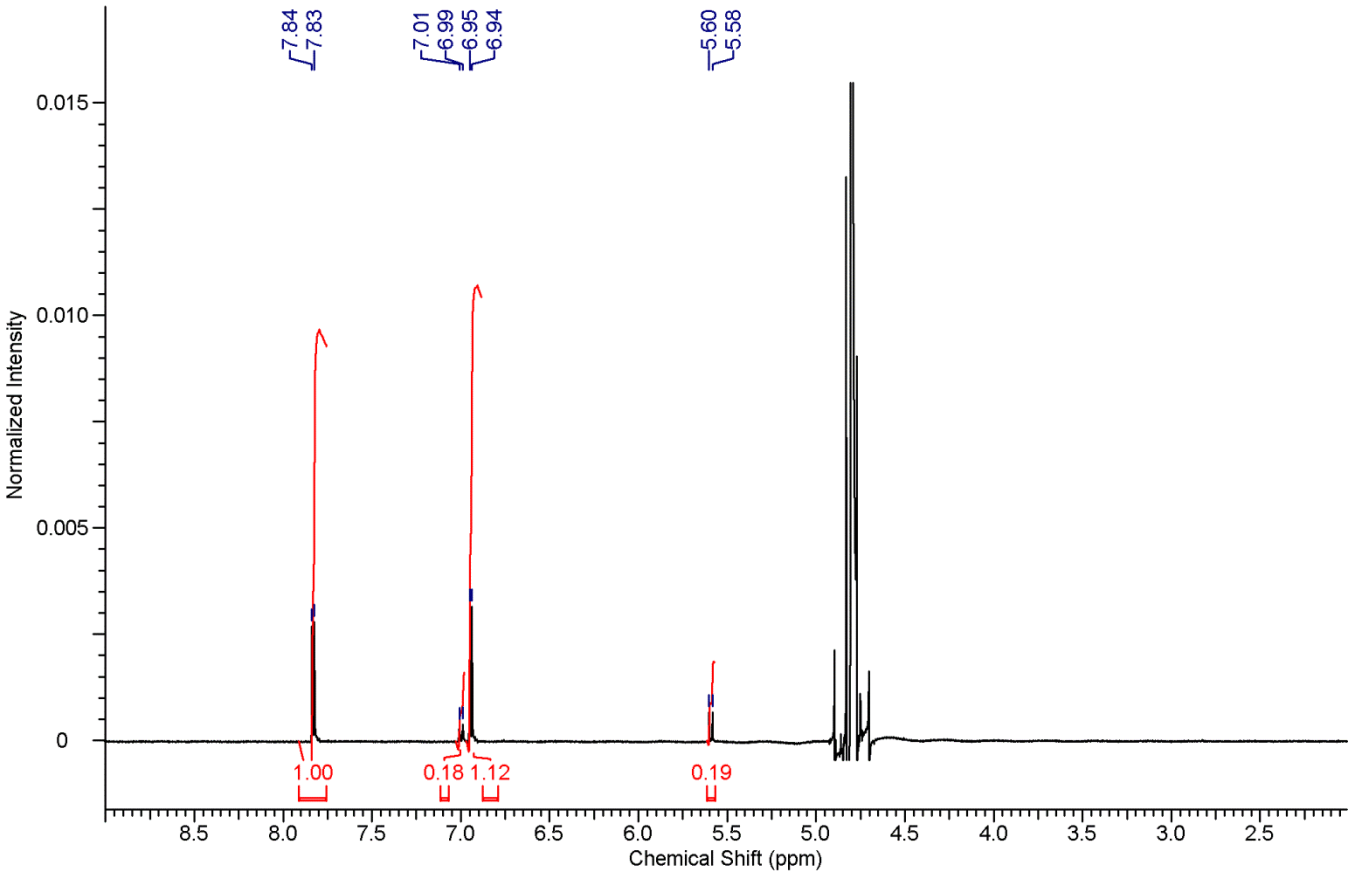
^{1}H-NMR of **5** in D_2O (neutral)



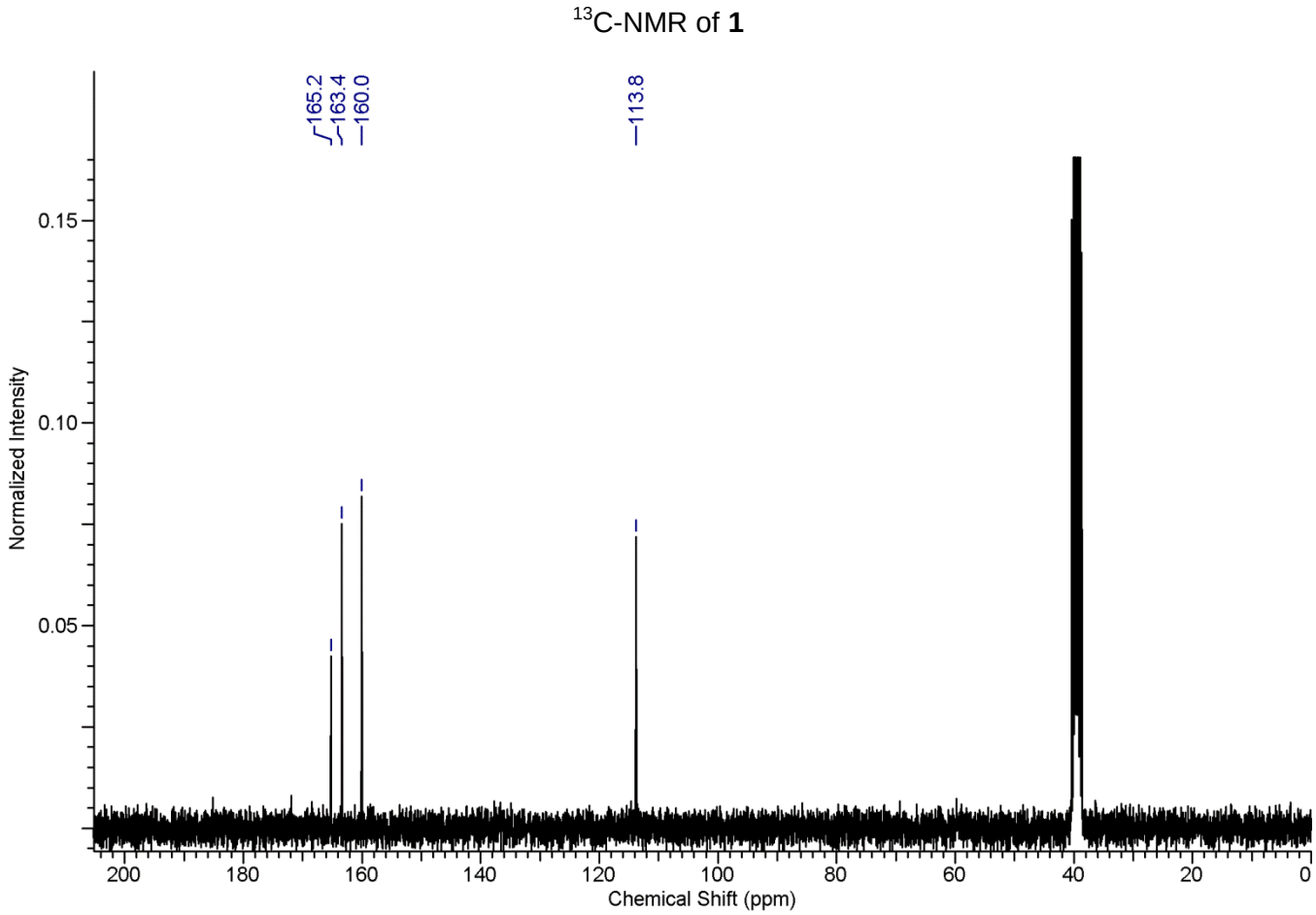
^{1}H-NMR of **5** in D_2O (basic + 0.1M NaOH)



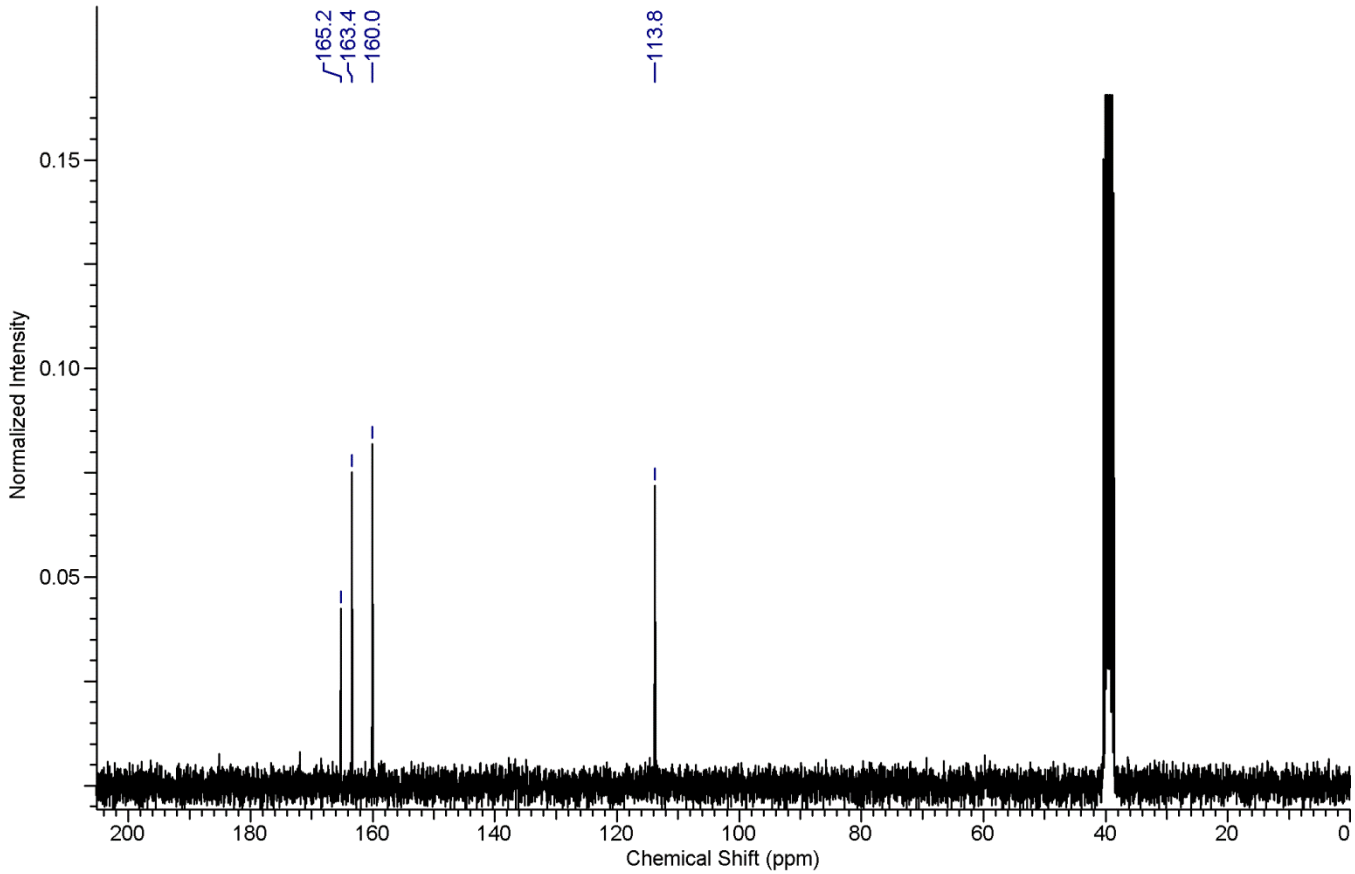
^{1}H-NMR of **5** in D_2O (basic + 1M NaOH)



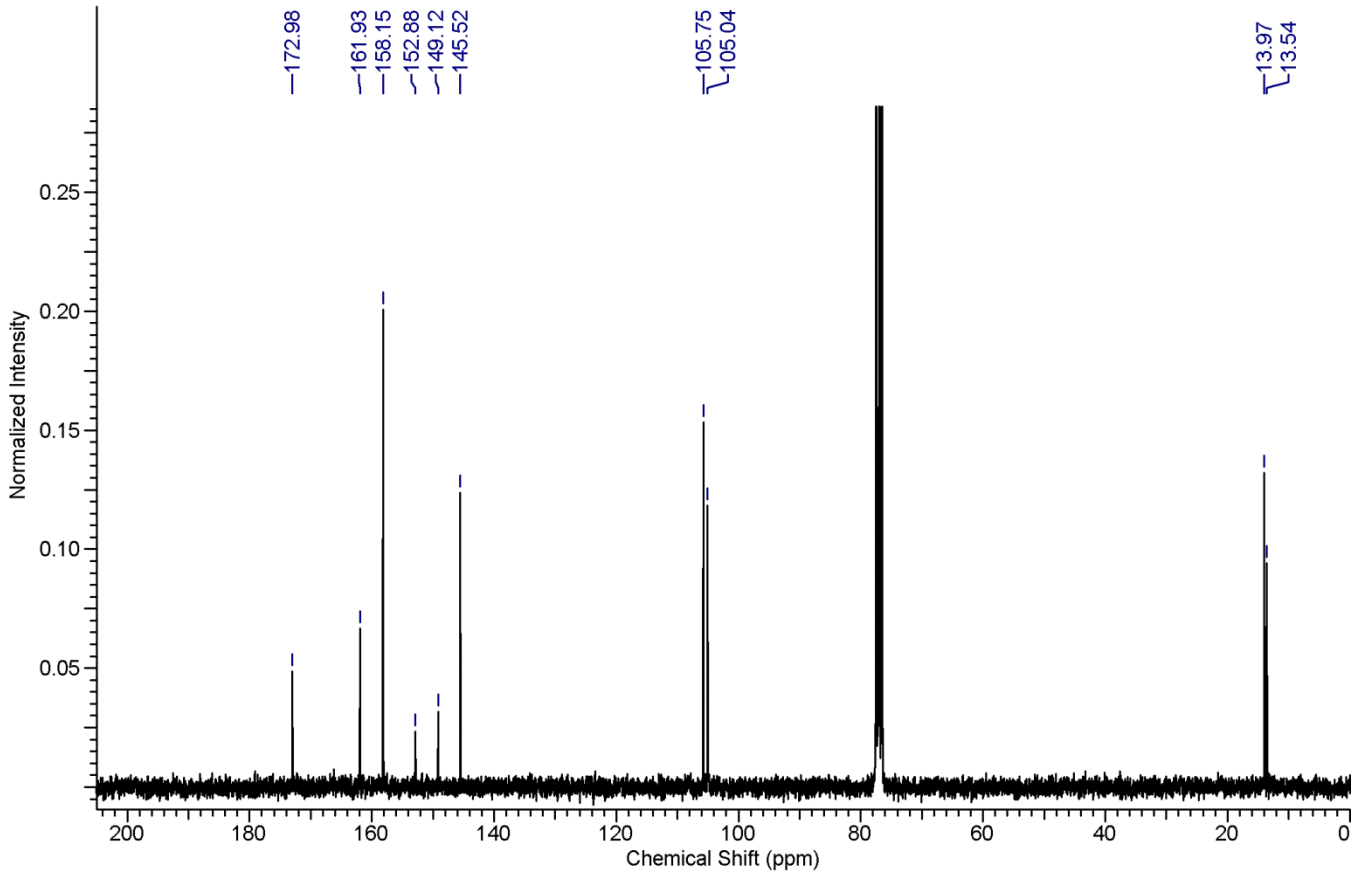
d) ***^{13}C-NMR Spectra***



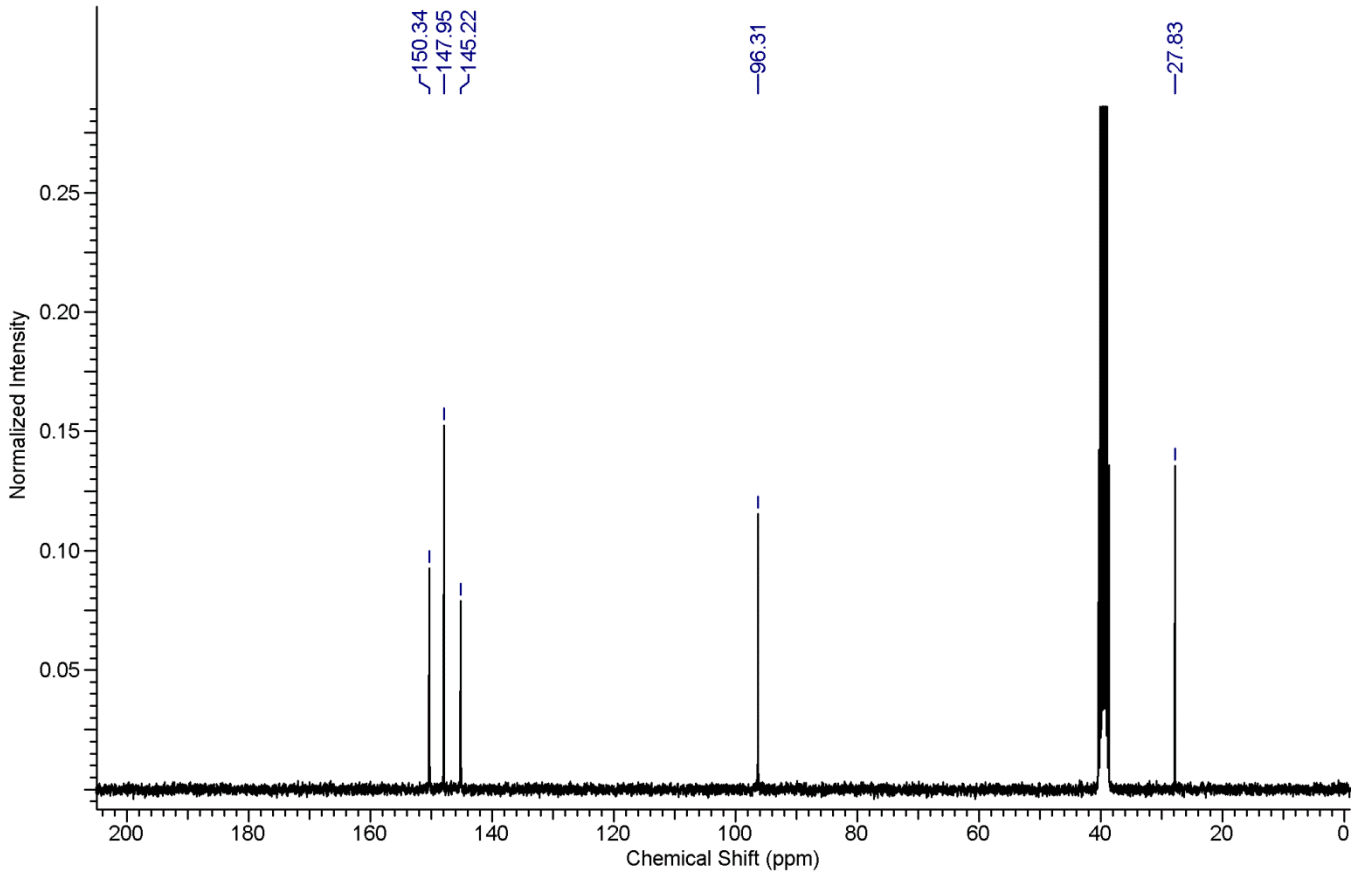
^{13}C-NMR of **2**


165.2
163.4
160.0
113.8
0.15
0.10
0.05
Normalized Intensity
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

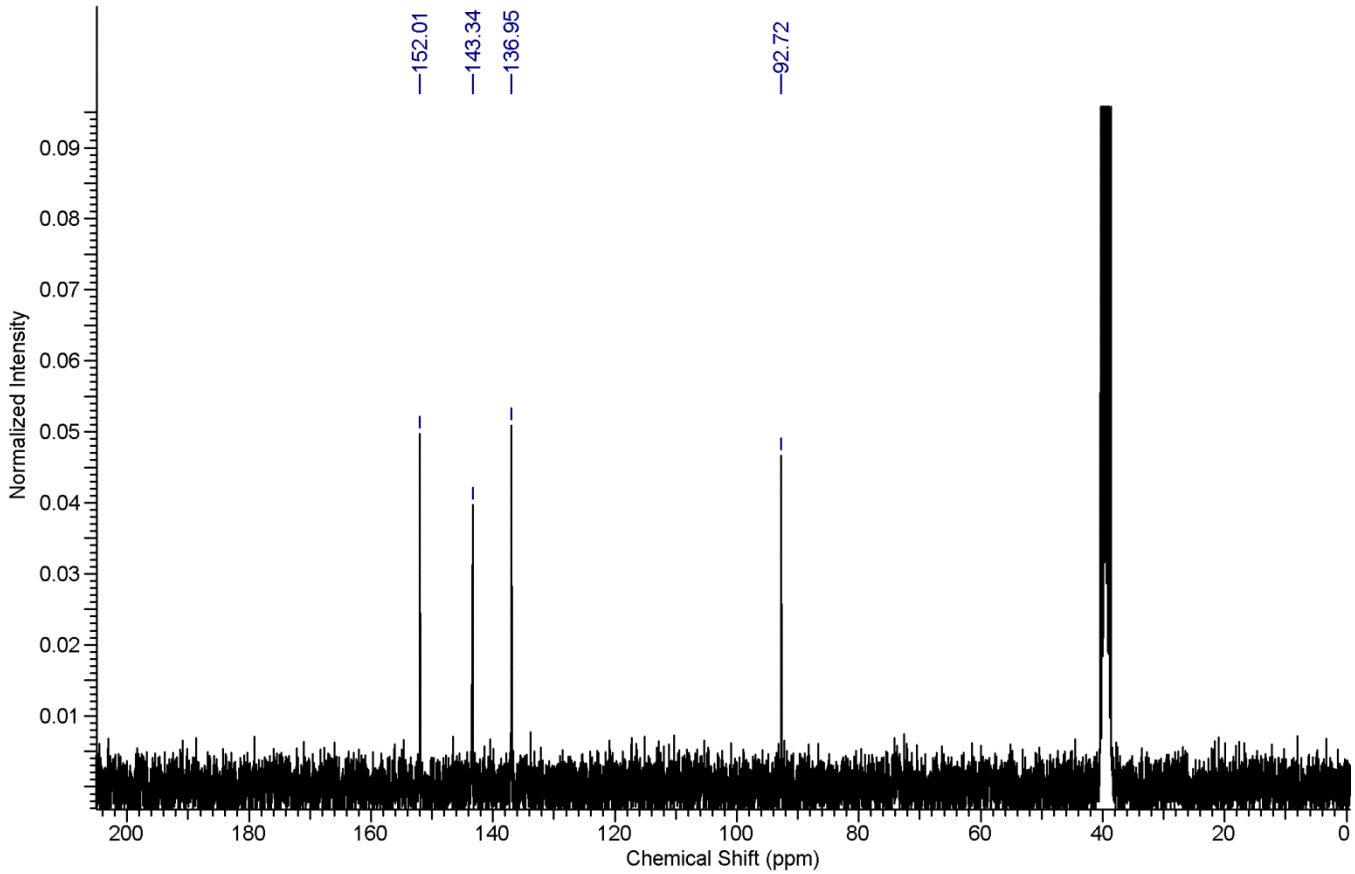
^{13}C-NMR of **3**


172.98
161.93
158.15
152.88
149.12
145.52
105.75
105.04
13.97
13.54
Normalized Intensity
0.25
0.20
0.15
0.10
0.05
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

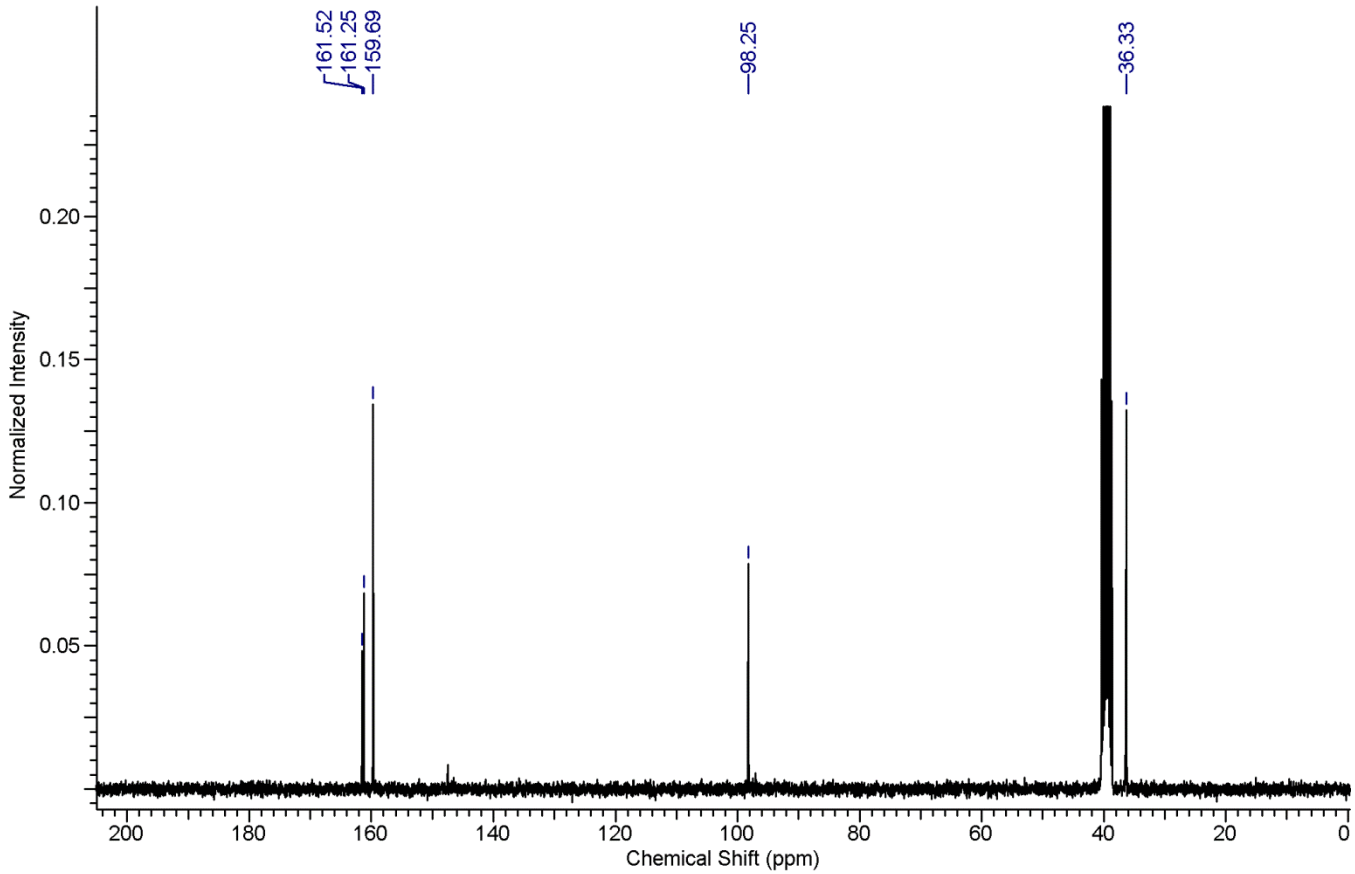
^{13}C-NMR of **4**


150.34
147.95
145.22
96.31
27.83
Normalized Intensity
0.25
0.20
0.15
0.10
0.05
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

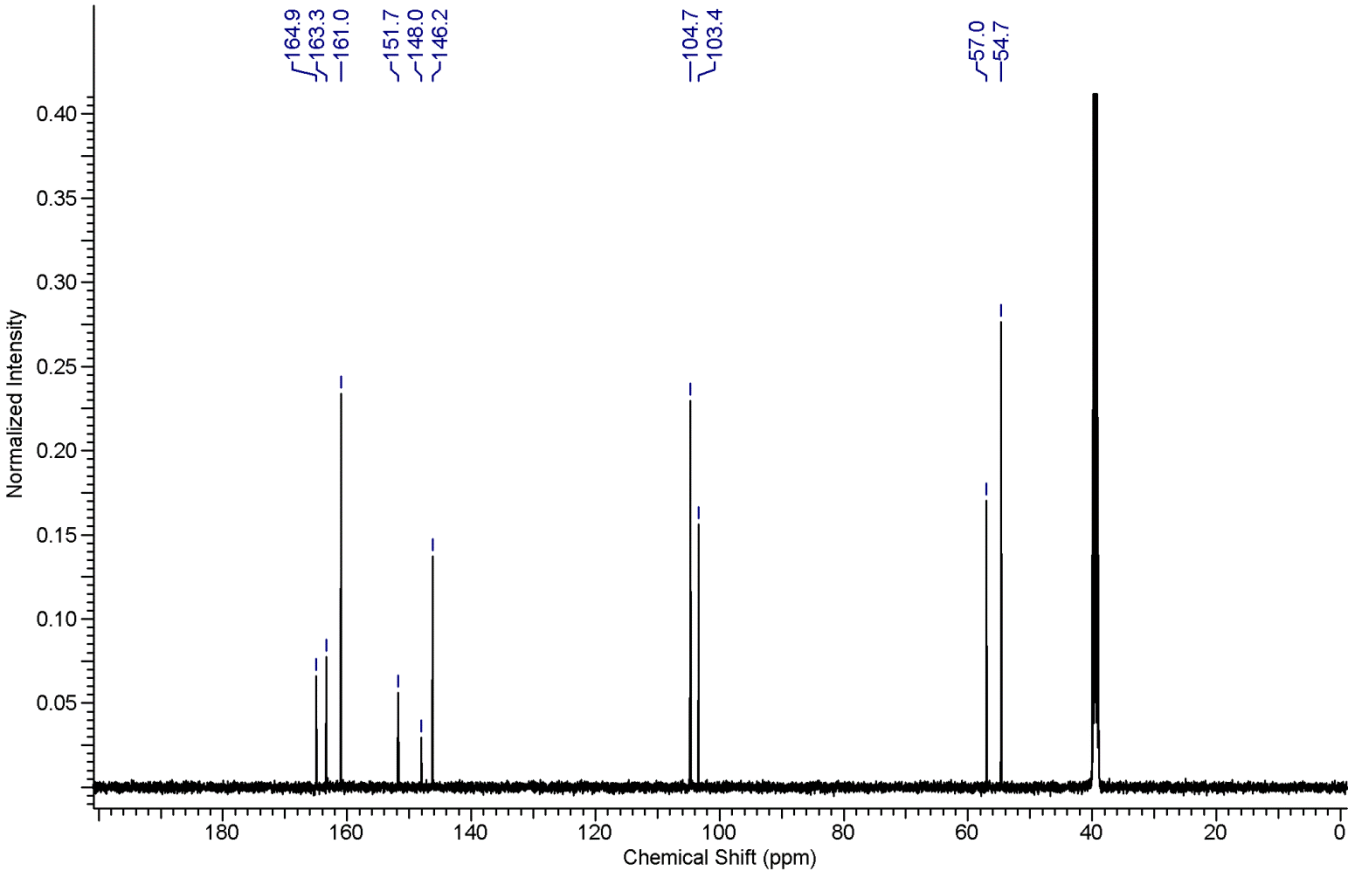
^{13}C-NMR of **5**


152.01
143.34
136.95
92.72
Normalized Intensity
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

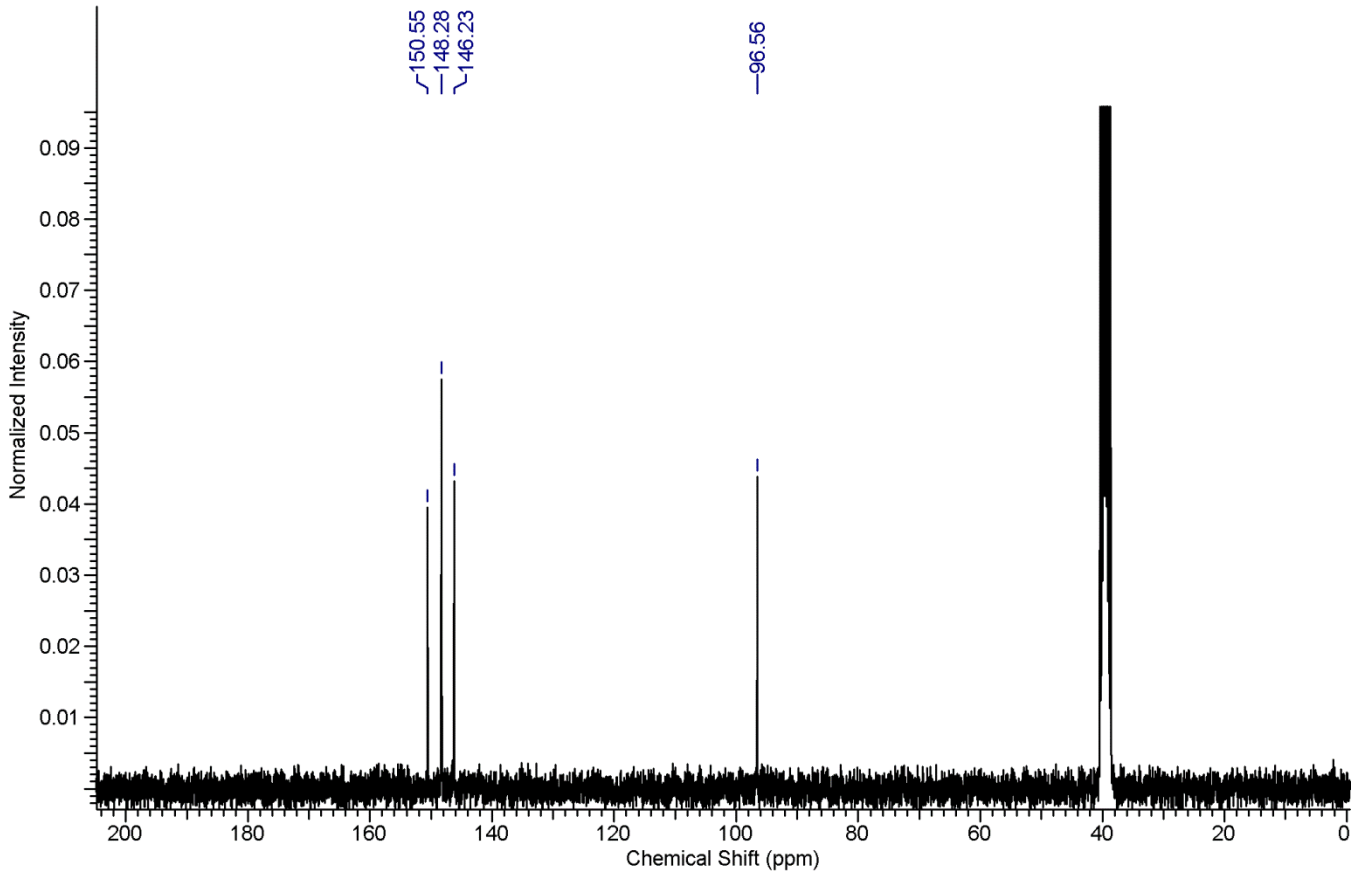
^{13}C-NMR of **6**


161.52
161.25
159.69
98.25
36.33
Normalized Intensity
0.20
0.15
0.10
0.05
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

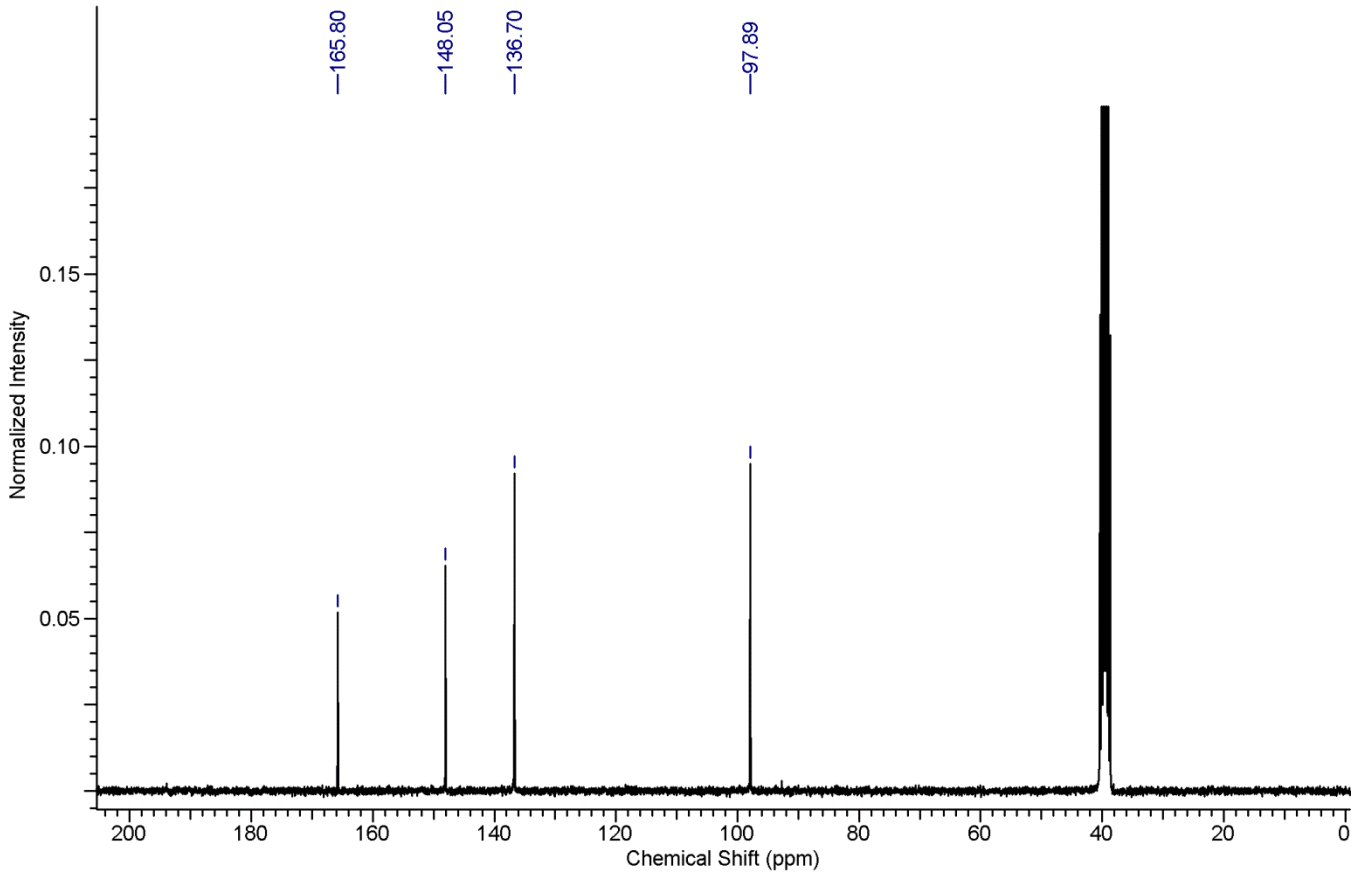
^{13}C-NMR of **7**


164.9
163.3
161.0
151.7
148.0
146.2
104.7
103.4
57.0
54.7
Normalized Intensity
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

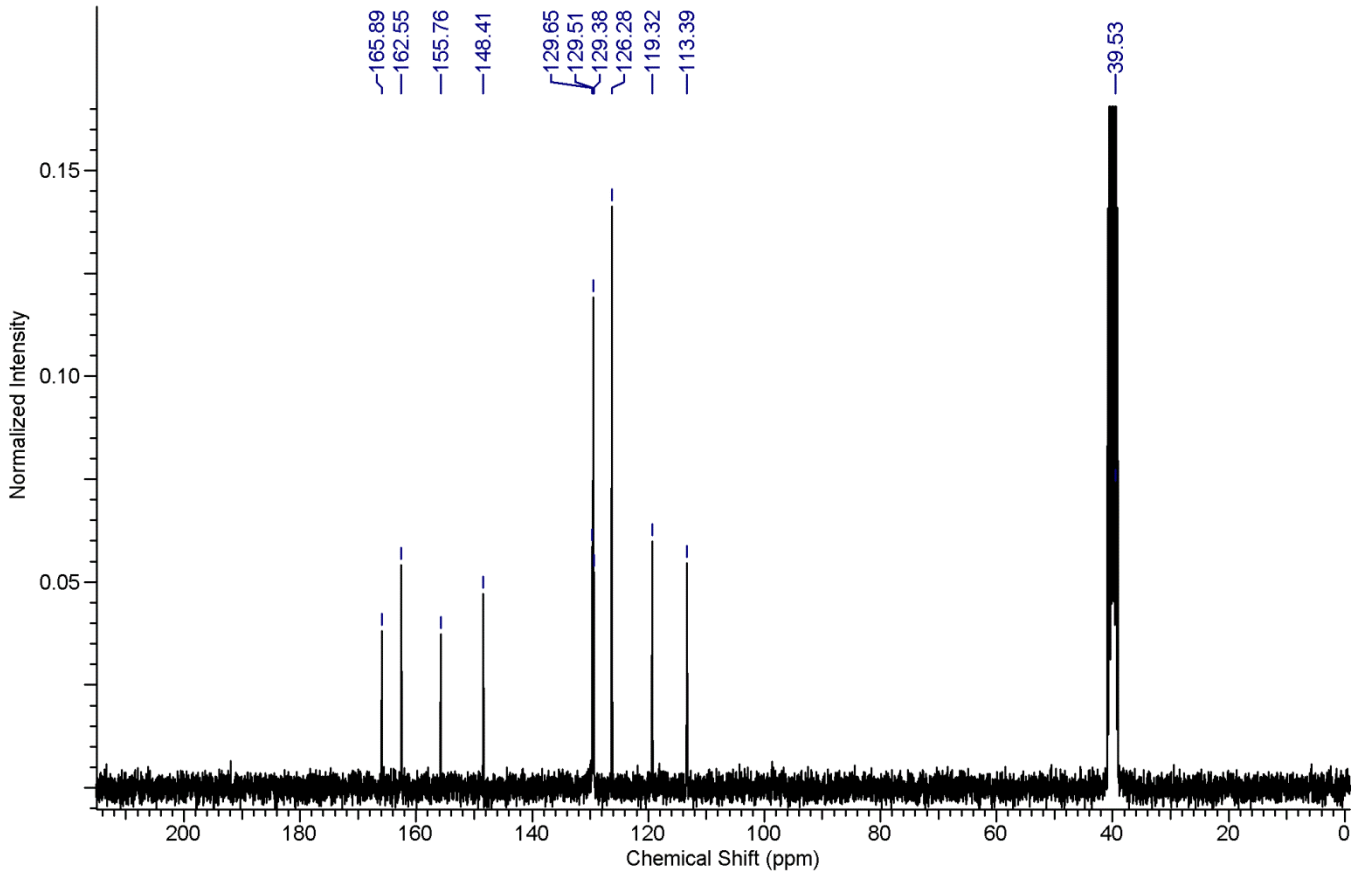
^{13}C-NMR of **8**


150.55
148.28
146.23
96.56
Normalized Intensity
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

^{13}C-NMR of **9**

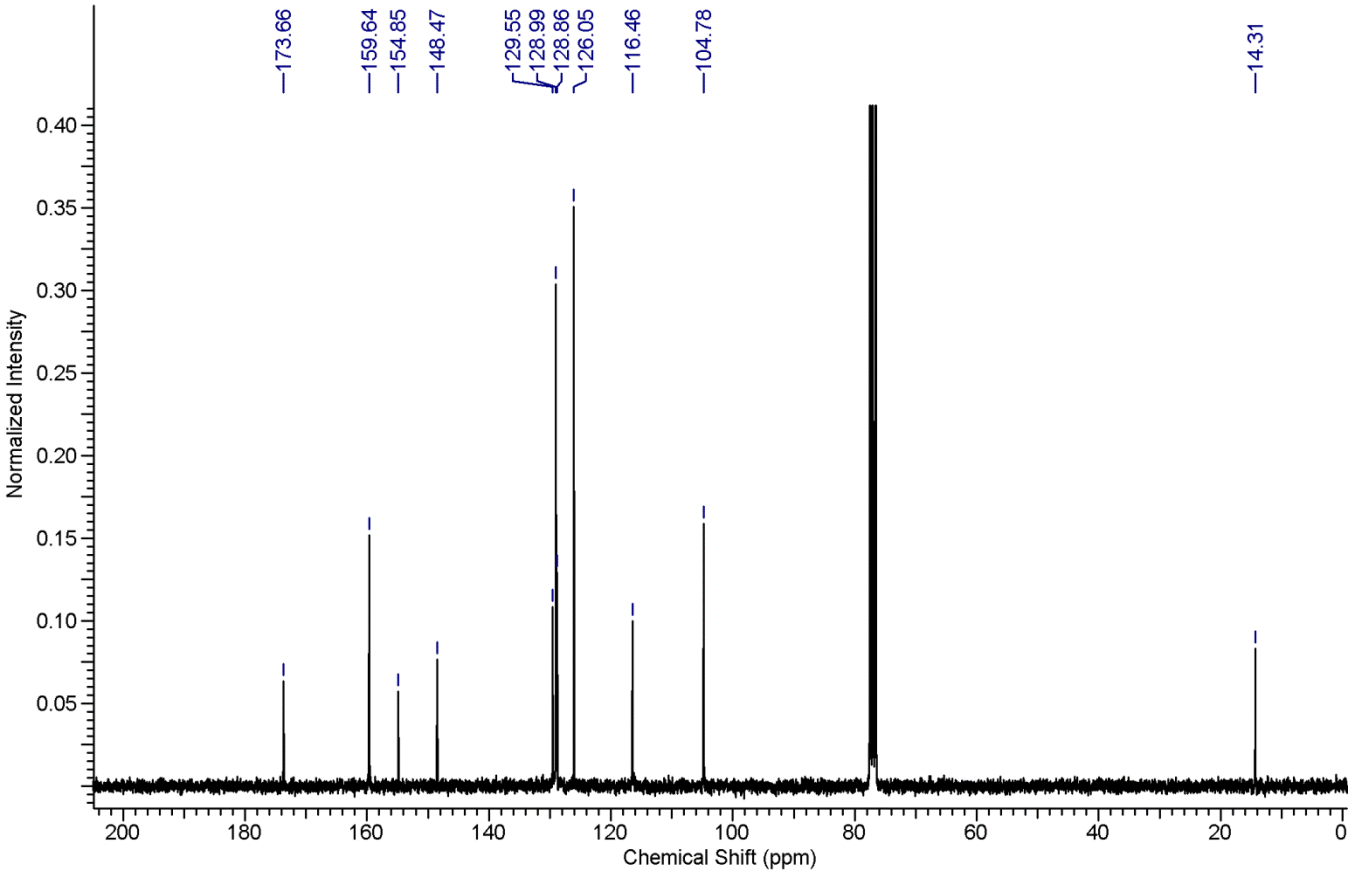

165.80
148.05
136.70
97.89
Normalized Intensity
0.15
0.10
0.05
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

^{13}C-NMR of **10**

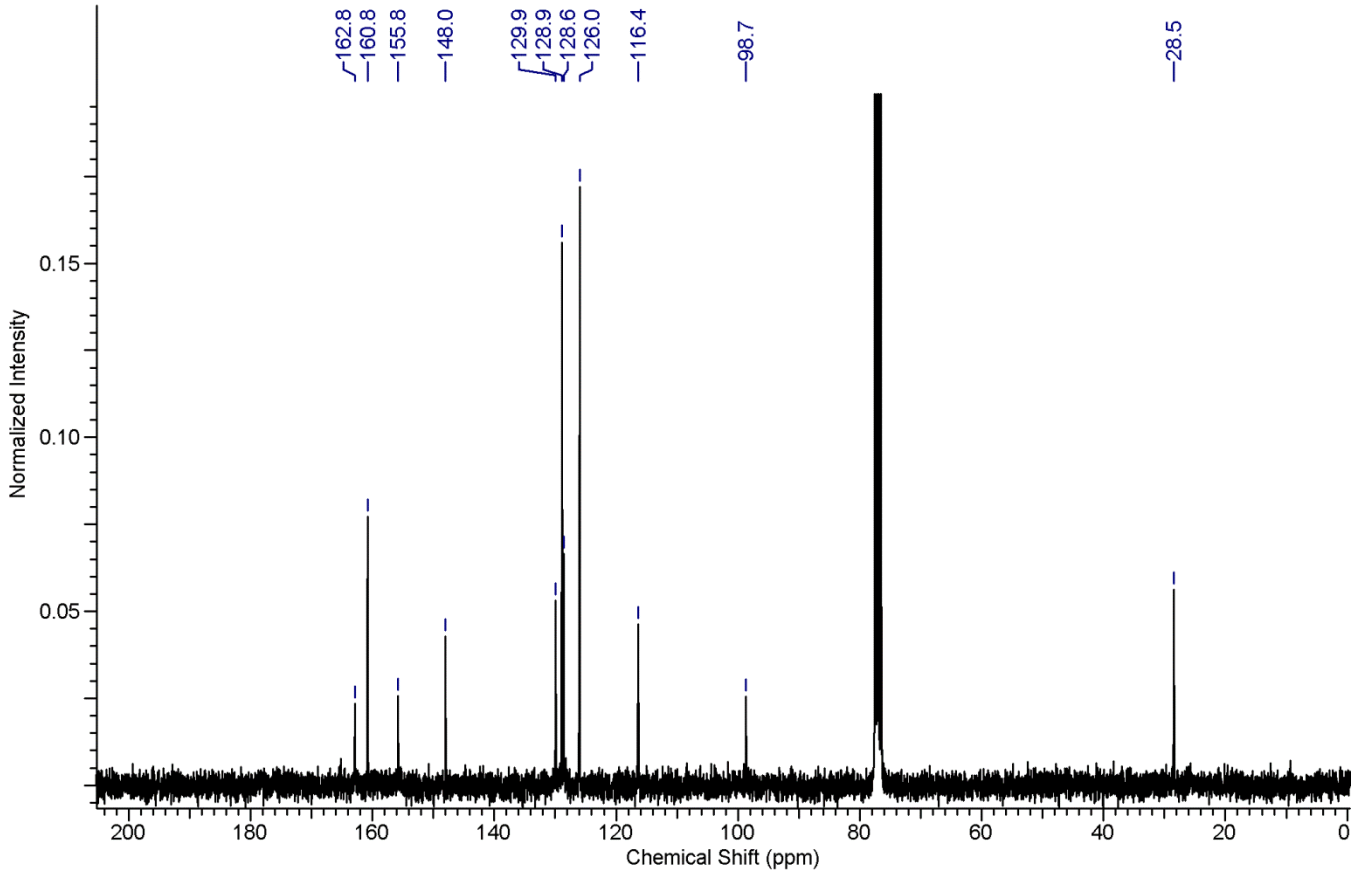


^{13}C-Signal of the methyl group at 39.53 was also detected by ^{1}H-HMQC

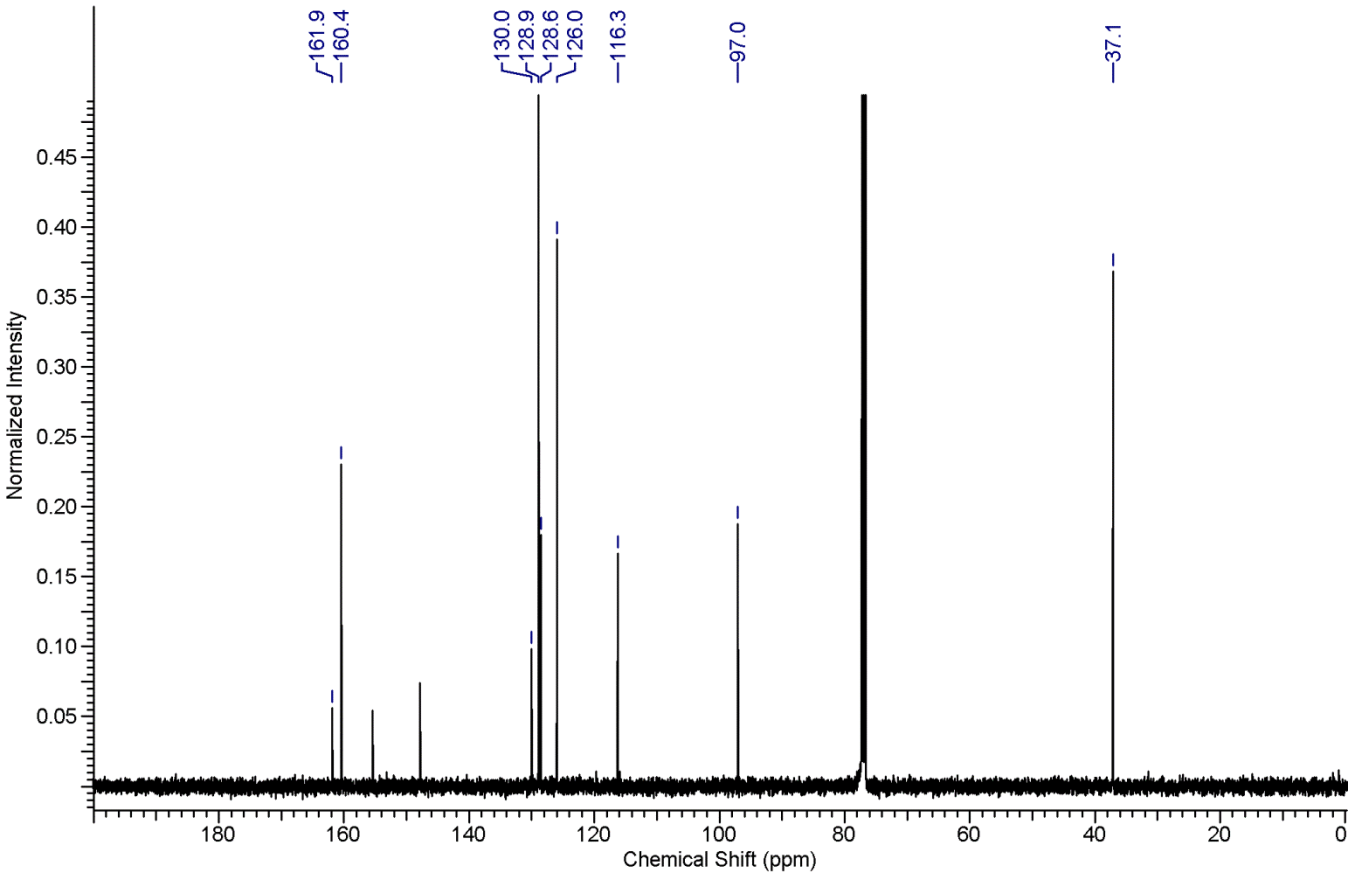
^{13}C-NMR of **11**


173.66
159.64
154.85
148.47
129.55
128.99
128.86
126.05
116.46
104.78
14.31
Normalized Intensity
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

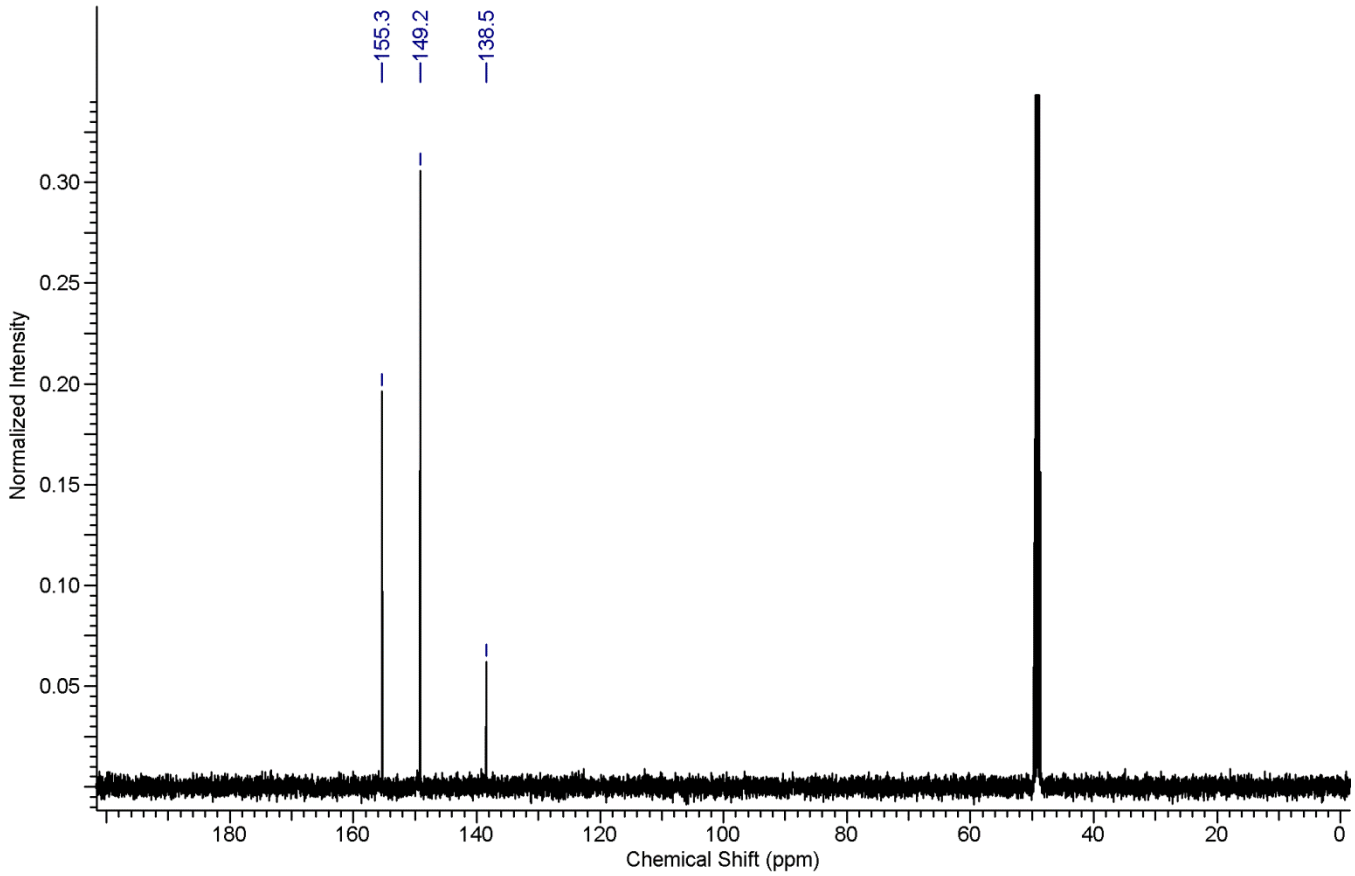
^{13}C-NMR of **12**


162.8
160.8
155.8
148.0
129.9
128.9
128.6
126.0
116.4
98.7
28.5
Normalized Intensity
0.15
0.10
0.05
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

^{13}C-NMR of **14**

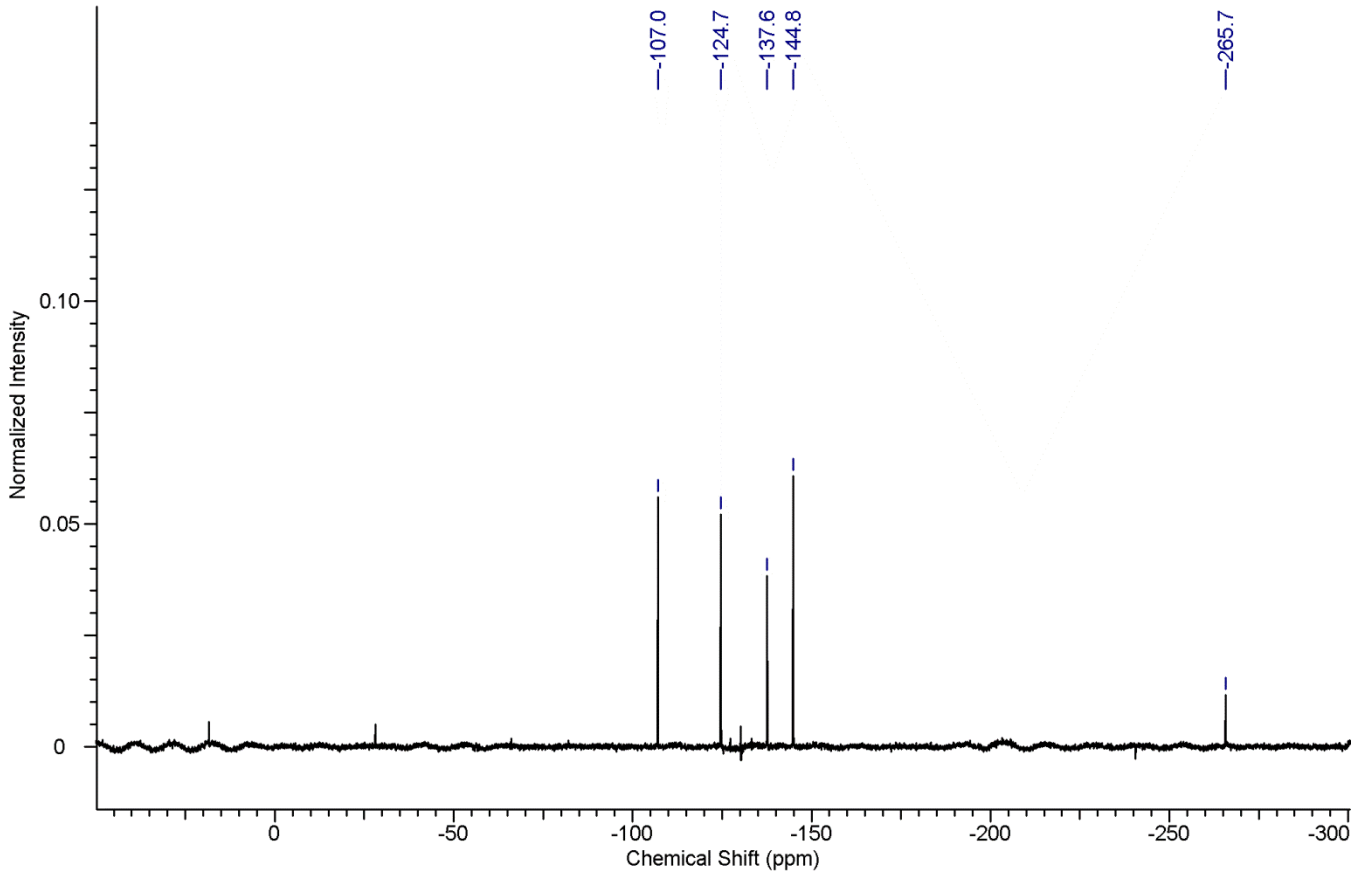

161.9
160.4
130.0
128.9
128.6
126.0
116.3
97.0
37.1
Normalized Intensity
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

^{13}C-NMR of **15**

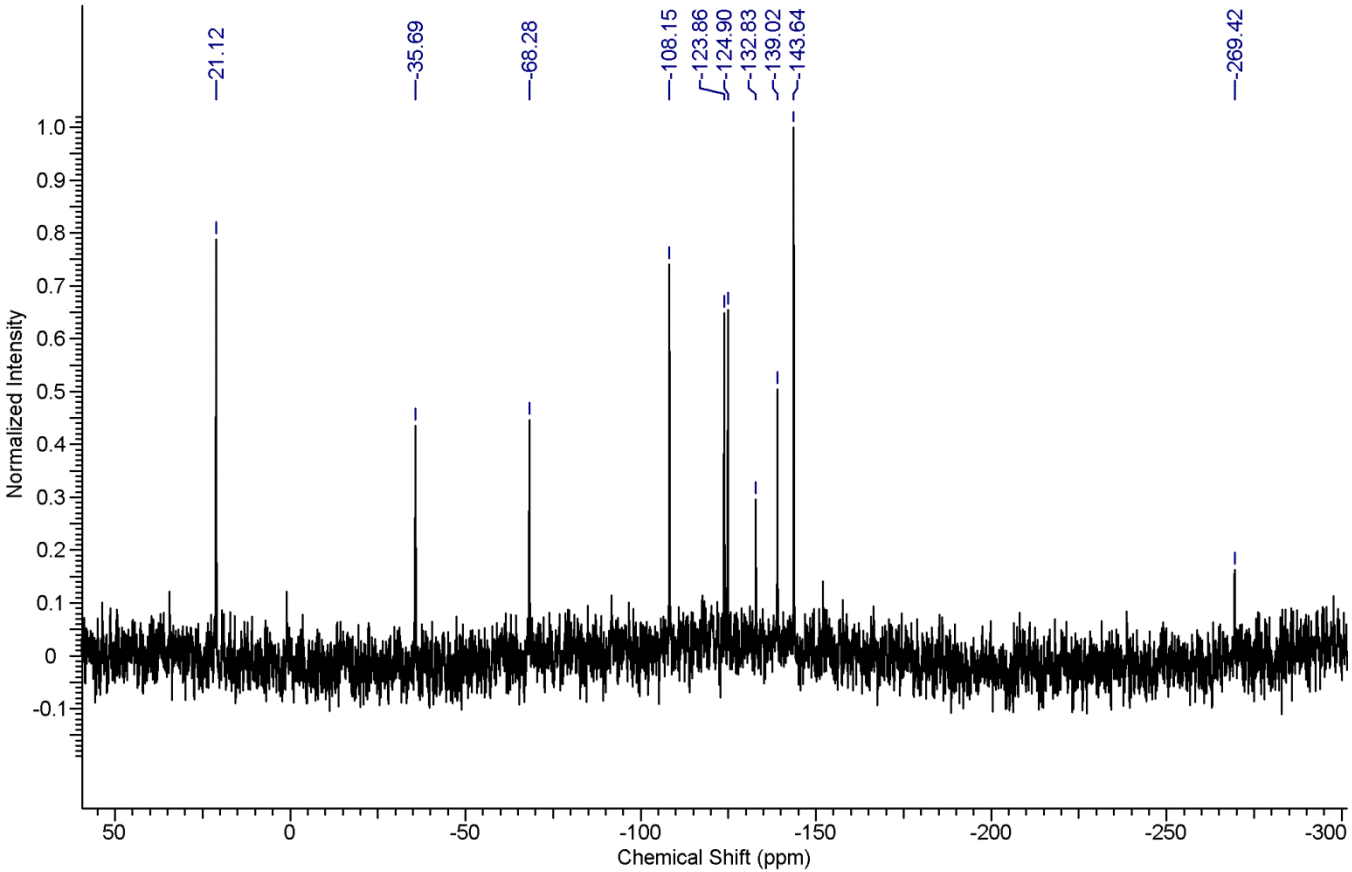

155.3
149.2
138.5
Normalized Intensity
0.30
0.25
0.20
0.15
0.10
0.05
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

e) ^{15}N-NMR Spectra

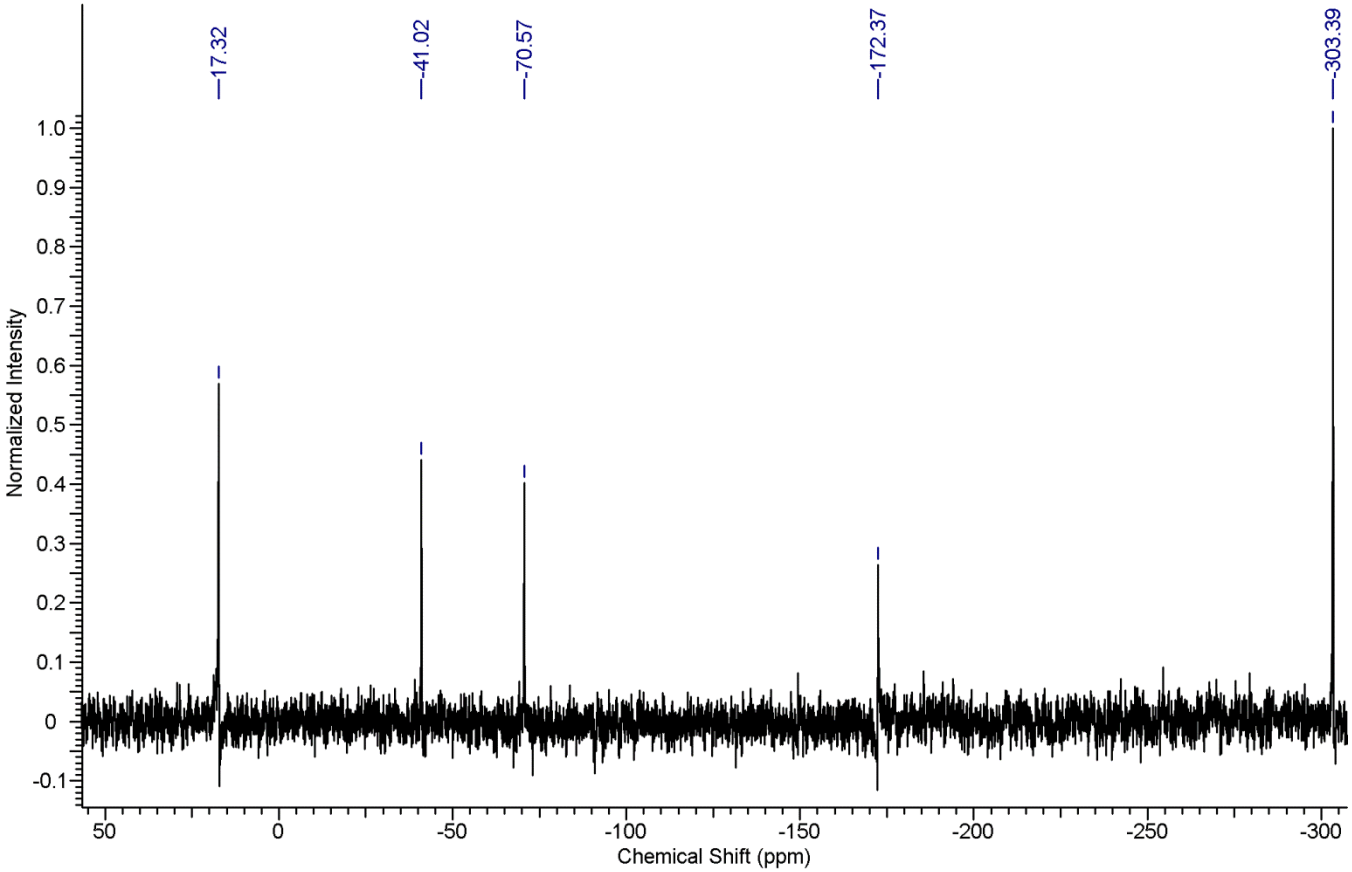
^{15}N-NMR of **1**



^{15}N-NMR of **3**

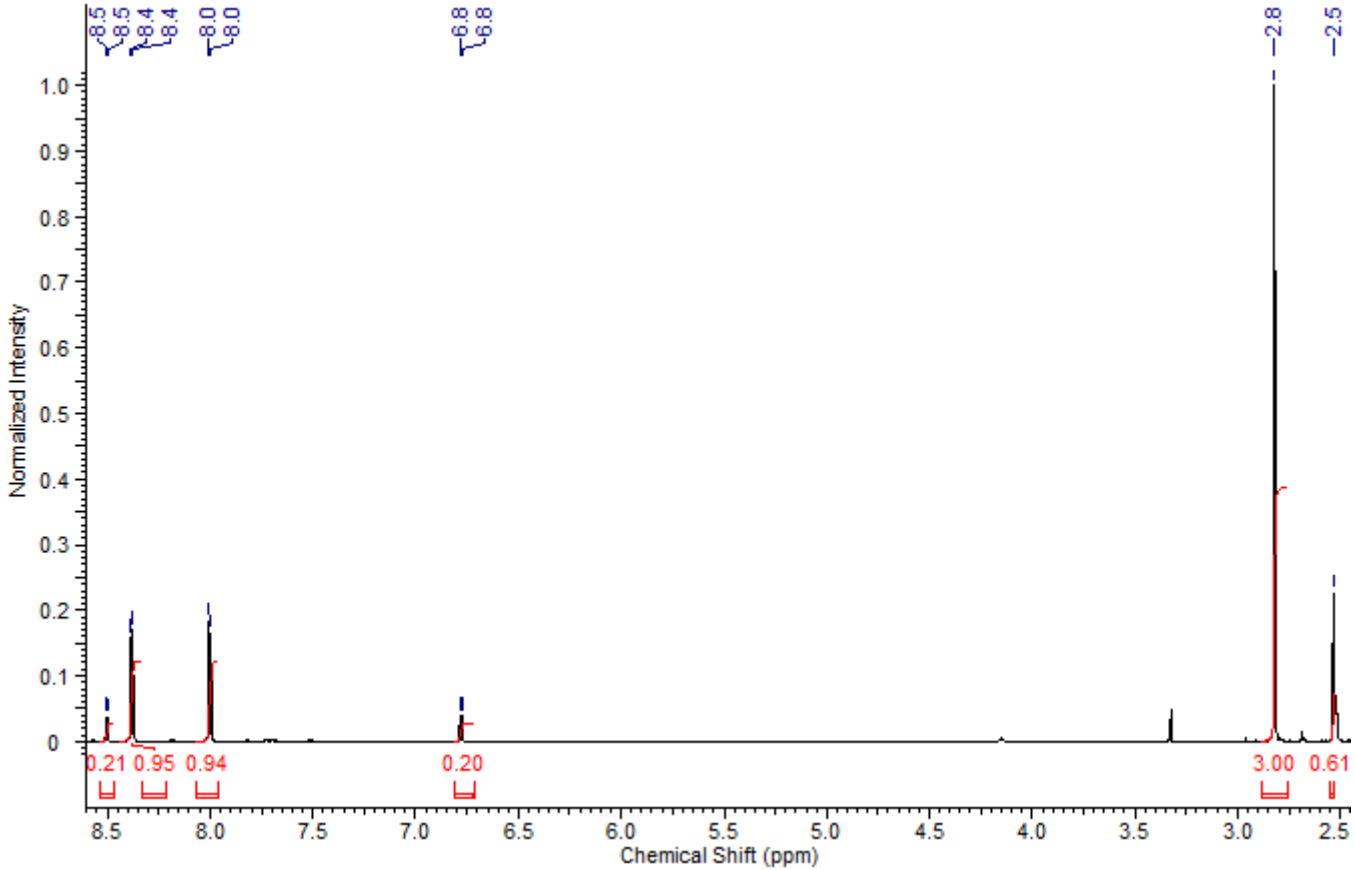

-21.12
-35.69
-68.28
-108.15
-123.86
-124.90
-132.83
-139.02
-143.64
-269.42
Normalized Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
50
0
-50
-100
-150
-200
-250
-300
Chemical Shift (ppm)

^{15}N-NMR of **4**

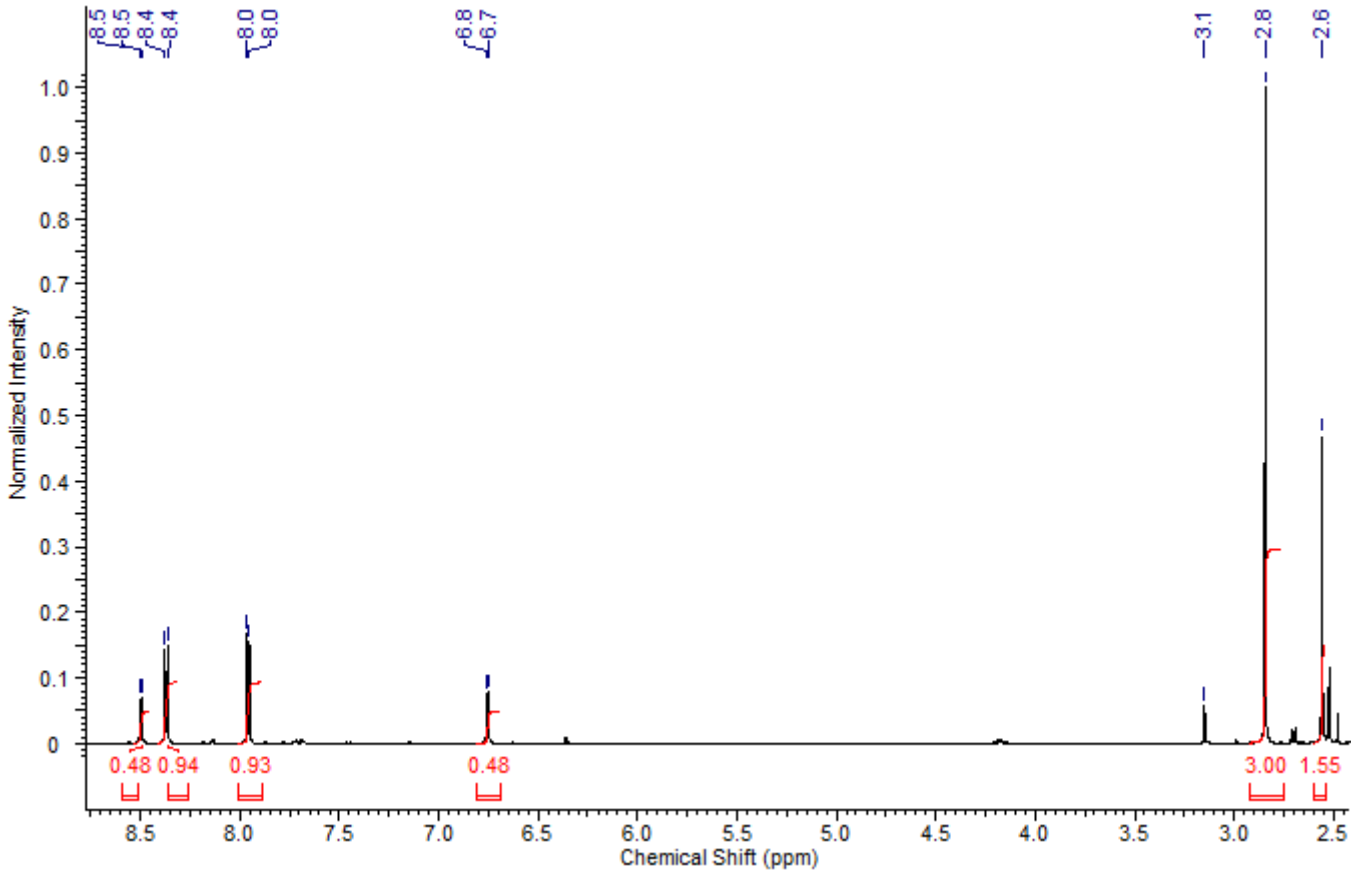

17.32
-41.02
-70.57
-172.37
-303.39
Normalized Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
50
0
-50
-100
-150
-200
-250
-300
Chemical Shift (ppm)

f) Temperature gradient ^{1}H-NMR of 3

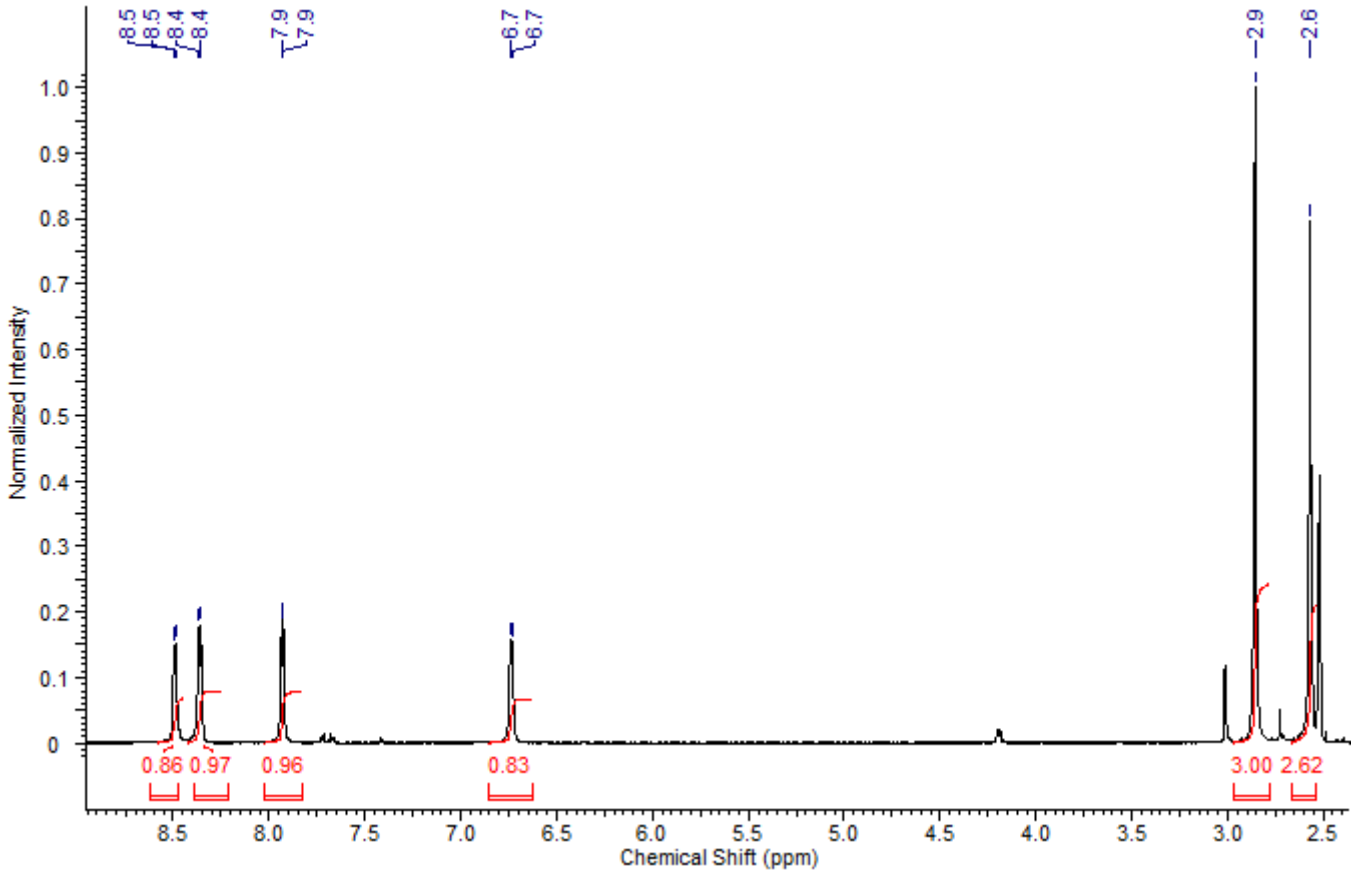
3 in DMSO at 300K



3 in DMSO at 333K

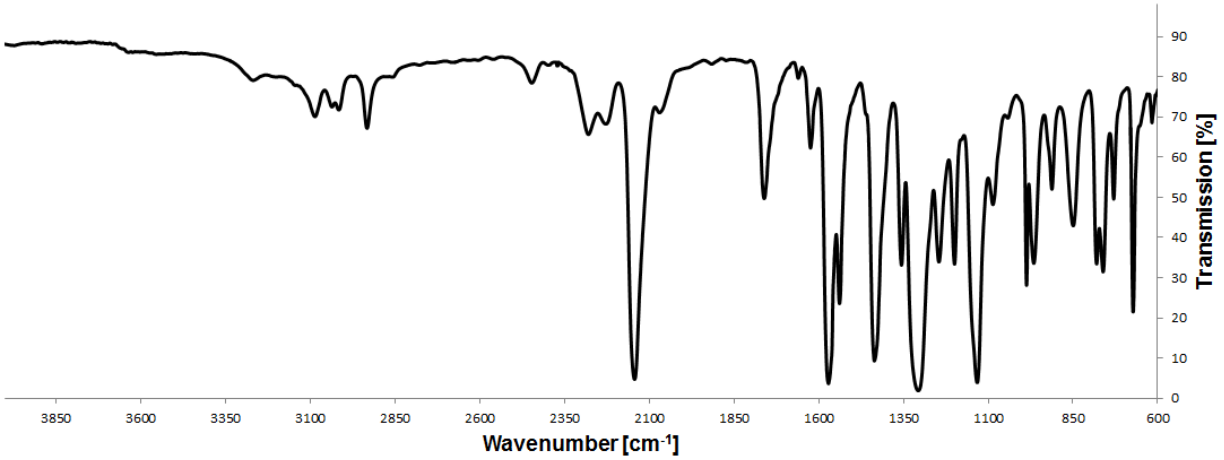


3 in DMSO at 353K



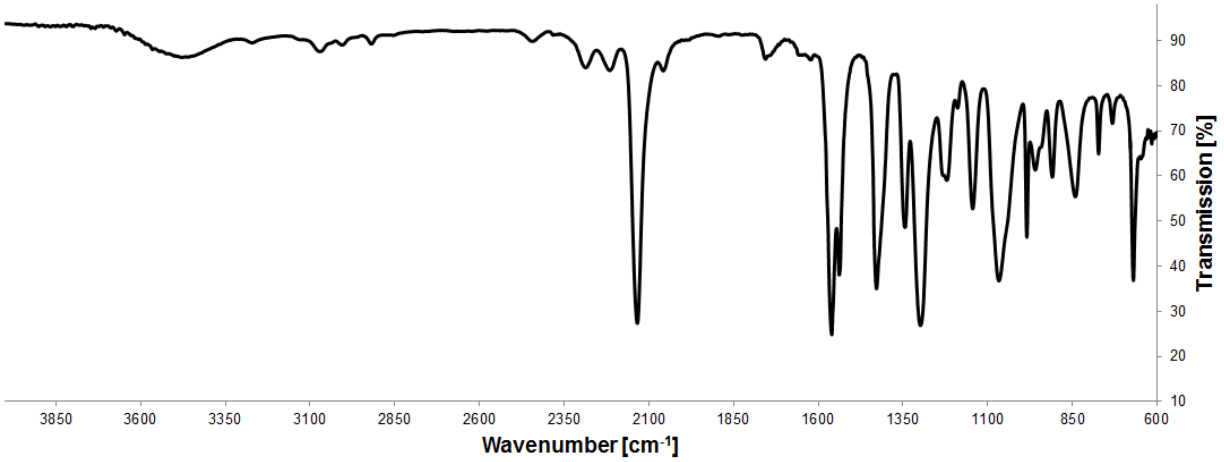
g) IR-Spectra

IR Spectrum of **1**



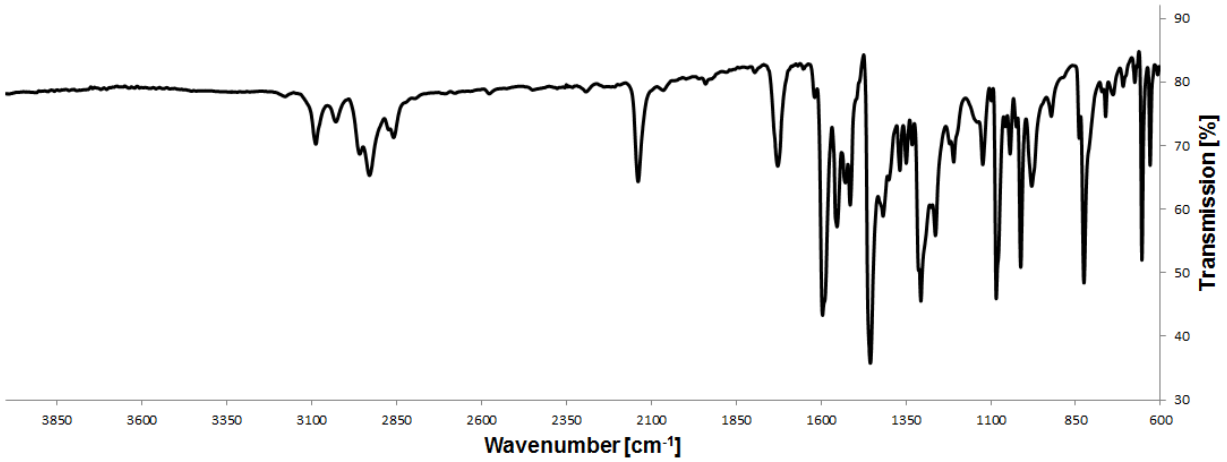
FT-IR (cm^{-1}): 3086, 3015, 2932, 2446, 2279, 2228, **2143**, 1761, 1661, 1624, 1571, 1539, 1435, 1356, 1306, 1246, 1200, 1133, 1086, 987, 966, 912, 850, 781, 761, 730, 673, 617

IR Spectrum of **2**



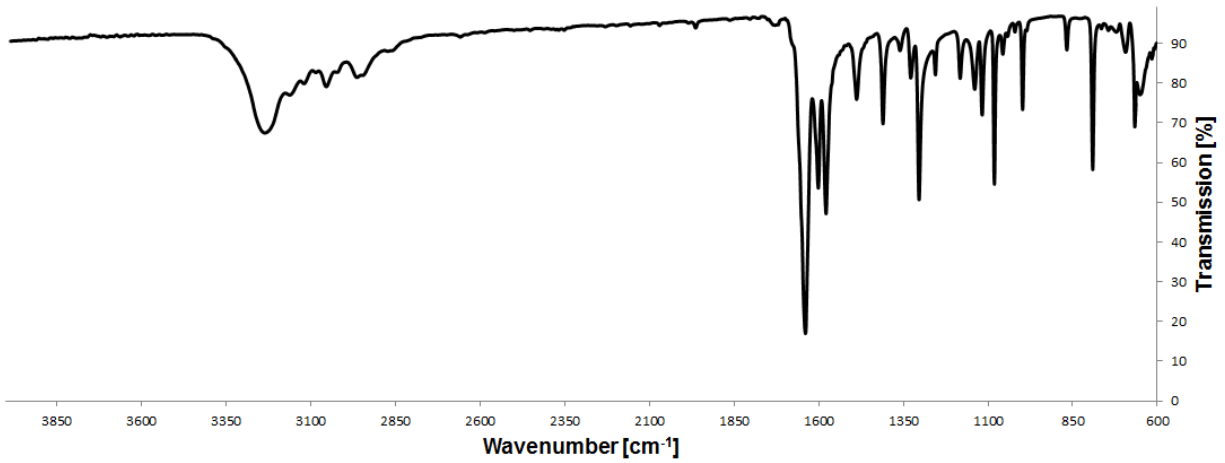
FT-IR (cm^{-1}): 3480, 3072, 2919, 2443, 2287, 2216, **2134**, 2058, 1756, 1560, 1539, 1428, 1345, 1299, 1221, 1189, 1144, 1068, 985, 960, 909, 842, 773, 732, 670, 622, 616, 585, 578, 572, 564, 553

IR Spectrum of **3**



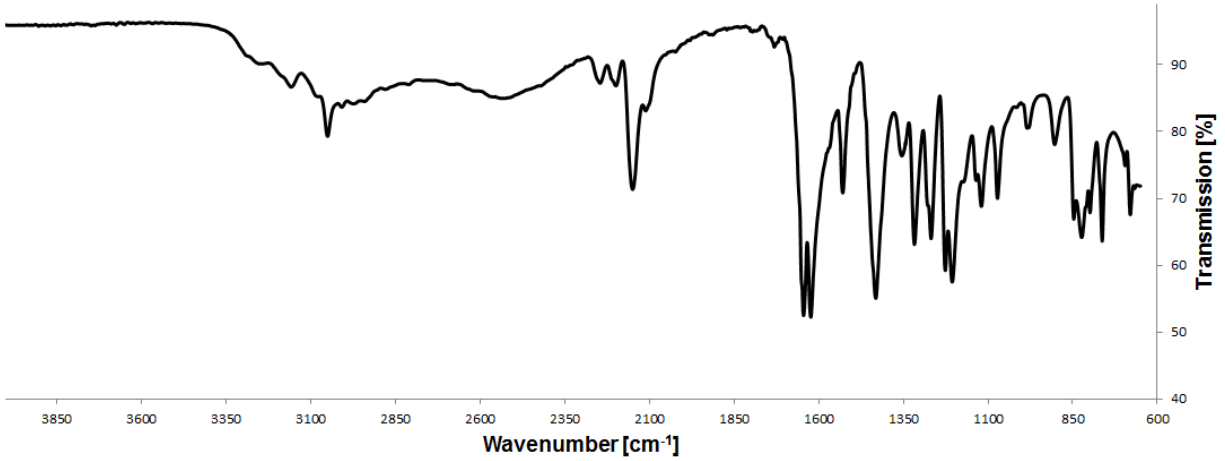
FT-IR (cm^{-1}): 3087, 3028, 2929, 2858, **2138**, 1727, 1595, 1553, 1528, 1514, 1455, 1417, 1368, 1349, 1332, 1306, 1263, 1210, 1124, 1084, 1057, 1044, 1025, 1012, 980, 922, 840, 826, 762, 740, 710, 676, 655, 631

IR Spectrum of **4**



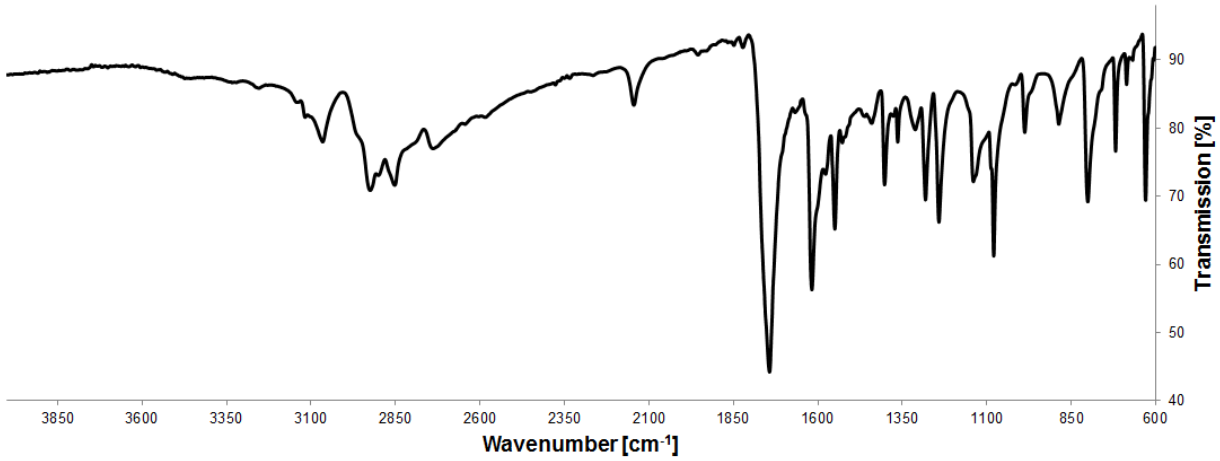
FT-IR (cm^{-1}): 3234, 3053, 2956, 1963, 1728, 1639, 1601, 1579, 1488, 1410, 1360, 1328, 1304, 1256, 1182, 1140, 1117, 1081, 1057, 1021, 998, 868, 791, 722, 694, 667, 651

IR Spectrum of **4** hydrochloric salt (lyophilized)



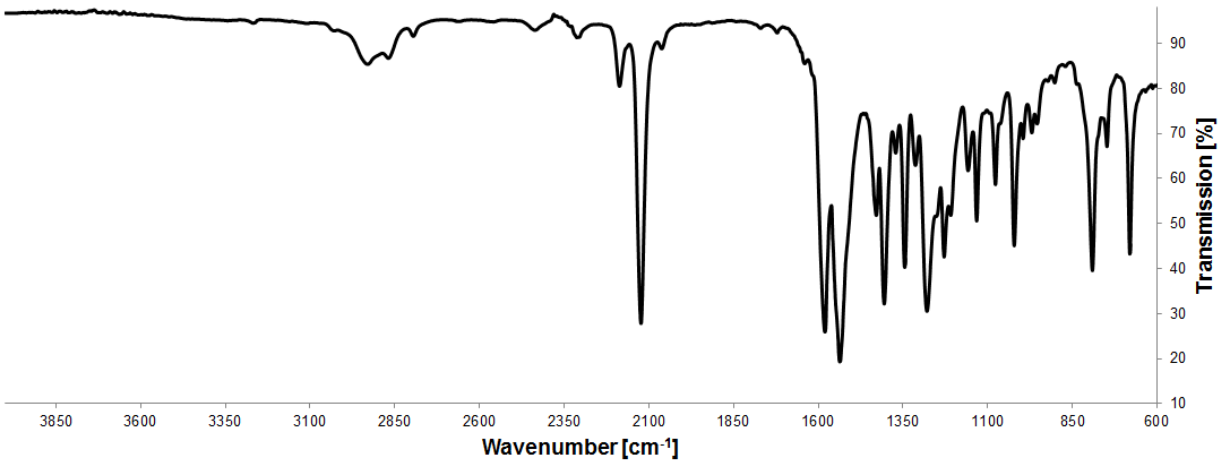
FT-IR (cm^{-1}): 3157, 3050, 2528, 2245, 2198, **2149**, 1645, 1623, 1529, 1431, 1354, 1317, 1268, 1226, 1205, 1120, 1072, 985, 903, 847, 824, 799, 763, 695, 680

IR-Spectrum of **5**



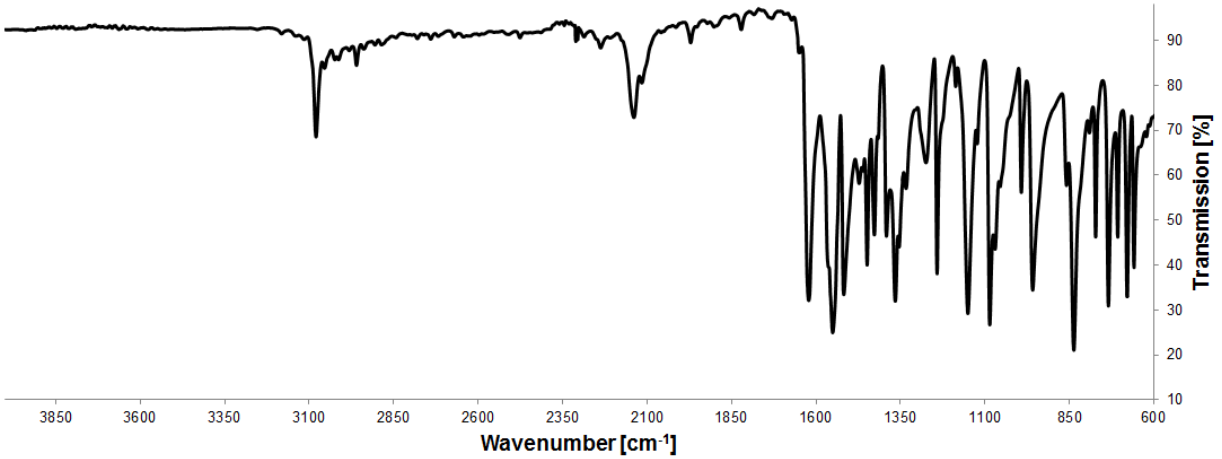
FT-IR (cm^{-1}): 3068, 2925, 2854, 2733, **2145**, 1822, 1744, 1618, 1550, 1528, 1441, 1403, 1364, 1312, 1282, 1242, 1138, 1080, 988, 888, 802, 719, 687, 631

IR-Spectrum of **6**



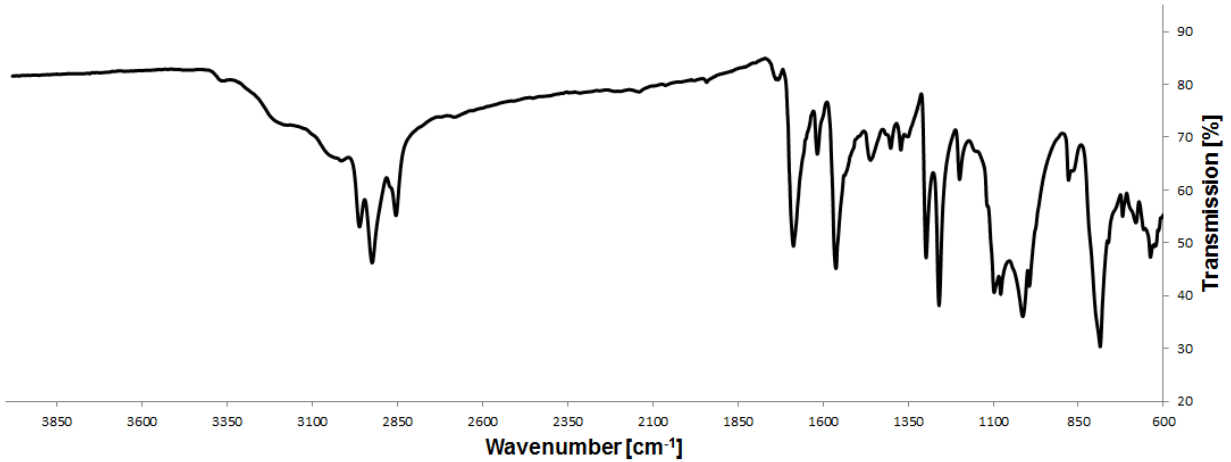
FT-IR (cm^{-1}): 2930, 2436, 2313, 2187, **2123**, 1581, 1536, 1429, 1406, 1372, 1345, 1314, 1279, 1229, 1209, 1158, 1133, 1077, 1022, 996, 970, 954, 791, 748, 681

IR-Spectrum of **7**



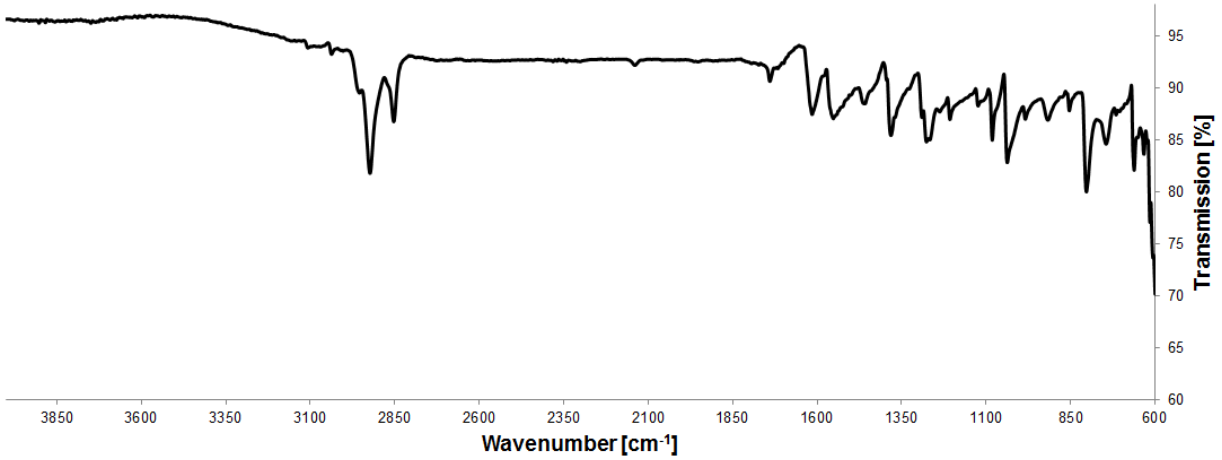
FT-IR (cm^{-1}): 3080, 3013, 2960, 2311, 2238, **2139**, 1971, 1822, 1622, 1551, 1518, 1473, 1449, 1428, 1392, 1366, 1354, 1334, 1275, 1242, 1187, 1151, 1123, 1086, 1070, 993, 959, 859, 837, 791, 773, 735, 707, 679, 659

IR-Spectrum of **8**



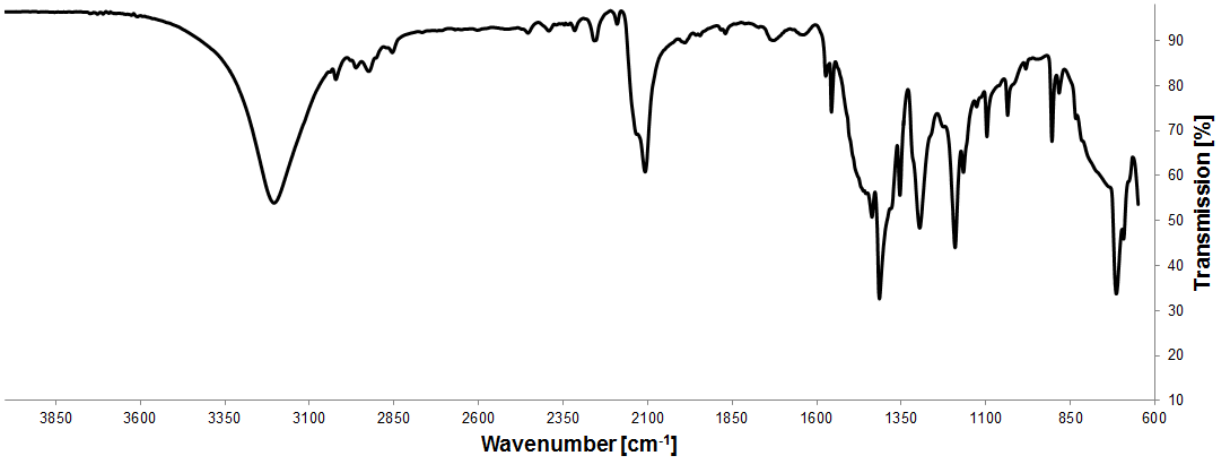
FT-IR (cm^{-1}): 2960, 2924, 2854, 1735, 1687, 1617, 1562, 1460, 1401, 1371, 1297, 1259, 1199, 1097, 1078, 1014, 994, 879, 786, 720, 682, 638

IR-Spectrum of **9**



FT-IR (cm^{-1}): 2924, 2854 ,1740, 1614, 1547, 1461, 1382, 1276, 1207, 1082, 1038, 984, 918, 854, 803, 745, 663, 633

IR-Spectrum of **15**



FT-IR (cm^{-1}): 3205, 3022, 2925, 2393, 2258, 2191, **2108**, 1727, 1574, 1557, 1437, 1415, 1354, 1296, 1191, 1167, 1097, 1036, 905, 884, 715, 693

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