Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Red-shifted and pH-Responsive Imidazole-based Azo Dyes with Potent Antimicrobial Activity

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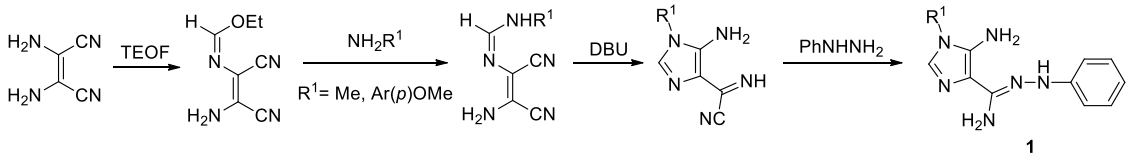
1. Materials and methods for synthesis

All commercial reagents were used without further purification. Silica gel flash chromatography was achieved using silica gel 60 (0.015-0.040 mm) for column chromatography from Millipore. The reactions were followed by thin layer chromatography (TLC) using Macherey-Nagel™ aluminum sheets UV254 that were observed directly and by UV light. The 5-amino-4-(cyanoformimidoyl)-1*H*-imidazoles were obtained by a well-known method in a three step reaction starting from commercial reagents including diaminomaleonitrile (DAMN), triethyl orthoformate (TEOF) and methylamine or *p*-anisidine.[1] Then, the 5-aminoimidazole-4-carboxamidrazones **1** were obtained by the reaction of 5-amino-4-(cyanoformimidoyl)-1*H*-imidazoles with phenyl hydrazine, accordingly to a previous method developed from the research group, with slight differences described above (SI-Figure 1).[2] The compounds **1** were used as precursors to obtain the novel azoimidazoles **2** and 2-aminoimidazoles **3**.



SI-Figure 1. General procedure to obtain 5-aminoimidazole-4-carboxamidrazones **1**.

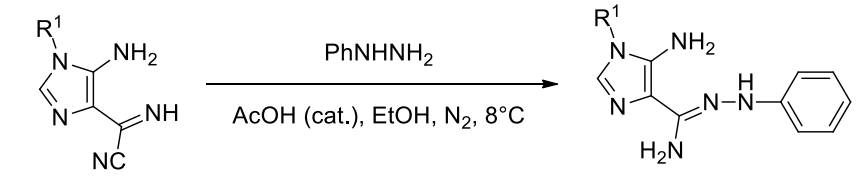
The reagents and solvents were purchased from Acros Organics, Sigma Aldrich, Chemlab, Fisher, Panreac, TCI and VWR chemicals BDH. Specifically, DAMN, dimethylamine, 1,4-dioxane, TEOF, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), trifluoroacetic acid (TFA) and triethylamine (Et_3N) were obtained from Acros Organics; acetonitrile, diatomaceus earth, *p*-anisidine, methylamine, sodium bicarbonate, anilinium chloride and silver nitrate standard solution for atomic absorption spectroscopy (AAS) (1mg mL^{-1}) were acquired from Sigma Aldrich; acetic acid from Chemlab; piperidine from Riedel-de Haen; phenylhydrazine from Fisher; Diethyl ether, *n*-hexane, silica gel 60, silver nitrate and sodium hydroxide were purchased from Panreac, deuterated DMSO from TCI and absolute ethanol from VWR chemicals BDH.

The new compounds were fully characterized by NMR (^1H, ^{13}C), including the ^1H-^{13}C correlation spectra (HMQC and HMBC) using DMSO-d_6 as solvent, FT-IR, HR-mass, DSC and TGA. The NMR spectra were performed at room temperature on a Bruker Avance 3400 (^1H: 400 MHz, ^{13}C: 100 MHz). The data are reported by chemical shifts (ppm), multiplicity (s - singlet, brs - broad singlet, d - doublet, t - triplet, dd - doublet of doublets or m - multiplet), the coupling constants (*J*) in hertz (Hz) and integration. IR spectra were recorded using Nujol mulls and NaCl or KBr cells on a FT-IR Bomem MB 104, or on a Shimadzu IR-Affinity 1 FTIR spectrophotometer with an attenuated total reflectance accessory (ATR-Dia/KRS-5). The spectra were recorded at room temperature in the range of 4000-400 cm^{-1}, at the resolution of 8 cm^{-1} and after 45 scans. The melting points were determined on a Stuart SMP3 melting

point apparatus. The silver content in samples were determined by AAS for flame using a novAA® 350 AJ Analytical Instrumentation after acid digestion. Elemental analyses were performed on a LECO CHNS-932 instrument (University of Minho). High resolution mass spectra (HR-MS) and X-Ray Crystallography were performed at the "Unidad de Espectrometria de Masas" at C.A.C.T.I., University of Vigo, Spain (MS spectra recorded on a Waters Micromass GC-TOF Bruker Daltonics instrument; and crystallography data collected on a Bruker D8 Venture diffractometer). The weight of the solids to prepare the standard solutions were measured on a Sartorius Micro micro-scale (± 0.001 mg). The solutions were prepared in volumetric flasks (10 ± 0.025) mL, and the initial volumes (Vi) were measured with a 1000 μL micro-pipette. The halochromic properties of compounds were assessed by UV-Vis spectra recorded on the SHIMADZU UV-2501PC apparatus, using 1 cm wide quartz cells.

2. Synthesis and characterization

2.1. General procedure for the synthesis of imidazole-based amidrazones 1



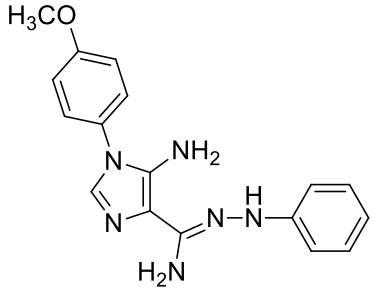
R^1= Ar(*p*)OMe, Me

R^1= Ar(*p*)OMe, Me

1

The amidrazones **1** were prepared accordingly to a previous method, developed by the research group, with slight differences.[2] Briefly, phenylhydrazine (1.5 molar equiv) and a catalytic amount of acetic acid (10 μL) were added to a suspension of the corresponding 5-amino-4-(cyanoformimidoyl)-1H-imidazole in ethanol (3-8 mL) that was previously maintained for 10 min. in an ice bath, and under magnetic stirring and nitrogen atmosphere. The suspensions were stirred at 8°C for 3h-17h, under nitrogen atmosphere, until reactions were completed by TLC. The solids were filtered and washed with ethanol and diethyl ether leading to the pure amidrazones **1** in 94-96% yield.

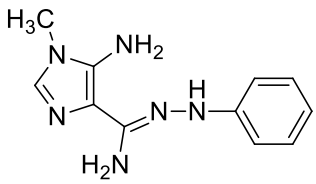
(*Z*)-5-amino-1-(4-methoxyphenyl)-*N'*-phenyl-1*H*-imidazole-4-carbohydrazonamide (1a)



To a 25 mL flask containing 5-amino-1-(4-methoxyphenyl)-1*H*-imidazole-4-carbimidoyl cyanide (2.0 g, 8.3 mmol) and acetic acid (10 μL) in ethanol (8 mL), phenylhydrazine (1.25 mL, 12.5 mmol) was added, under nitrogen atmosphere. The suspension was placed under magnetic stirring at a temperature of 8°C for 17h. The pure compound **1a** was obtained pure by filtration (2.5 g, 7.8 mmol, 94%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 3.81 (s, 3H), 5.57 (brs, 2H), 5.77 (brs, 2H), 6.59 (t, *J*= 8.0 Hz, 1H), 6.84 (d, *J*= 7.9 Hz, 2H), 7.11 (t, *J*= 7.9 Hz, 4H), 7.31 (s, 1H), 7.44 (d, *J*= 8.7 Hz, 2H), 7.92 (s, 1H).
^{13}C NMR (75 MHz, DMSO-d_6): δ 55.50, 111.62, 113.45, 114.81, 116.56, 126.29, 127.90, 128.73, 129.03, 136.93, 145.14, 147.93, 158.72 ppm.
IR (Nujol mull): ν 3414, 3360, 3267, 3217, 1608, 1554, 1512 cm^{-1}.
Anal. calcd. for $C_{17}H_{18}N_6O$: C, 63.34; H, 5.63; N, 26.07; found: C, 63.41; H, 5.62; N, 25.94.
Melting point >193.3°C (dec.).

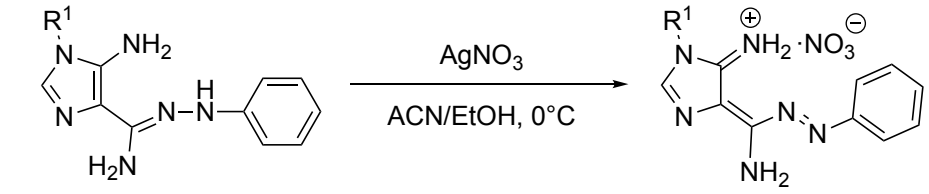
(*Z*)-5-amino-1-methyl-*N'*-phenyl-1*H*-imidazole-4-carbohydrazonamide (1b)



To a 25 mL flask containing 5-amino-1-methyl-1*H*-imidazole-4-carbimidoyl cyanide (1.1 g, 7.0 mmol) and acetic acid (10 μL) in ethanol (3 ml), phenylhydrazine (1.25 mL, 10.5 mmol) was added, under nitrogen atmosphere. The suspension was placed under magnetic stirring at a temperature of 8°C for 3h. The compound **1b** was obtained pure by filtration in (1.6 g, 6.8 mmol, 96%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 3.43 (s, 3H), 5.57 (brs, 2H), 5.65 (brs, 2H), 6.56 (t, *J*= 7.2 Hz, 1H), 6.82 (d, *J*= 8.5 Hz, 2H), 7.09 (s, 1H), 7.12 (d, *J*= 8.7 Hz, 2H), 7.82 (s, 1H).
^{13}C NMR (75 MHz, DMSO-d_6): δ 29.68, 111.63, 113.14, 116.47, 128.74, 129.34, 137.57, 145.52, 148.05 ppm.
IR (Nujol mull): ν 3448, 3344, 3282, 3107, 1651, 1616, 1597, 1530, 1554 cm^{-1}.
Anal. calcd. for $C_{11}H_{14}N_6$: C, 57.38; H, 6.13; N, 36.50; found: C, 57.16; H, 6.06; N, 36.14.
Melting point >164.7°C (dec.).

2.2. General procedure for the synthesis of the azoimidazoles 2.HNO_3

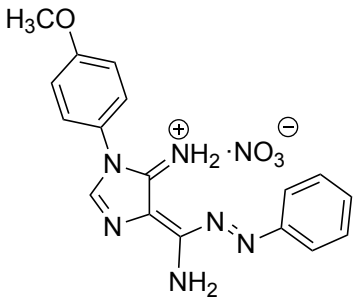


R^1= Ar(*p*)OMe, Me
1

R^1= Ar(*p*)OMe, Me
2·HNO_3

The 5-amino-*N'*-phenyl-1*H*-imidazole-4-carbohydrazonamides **1** were used to prepare azoimidazoles **2**. Fist, the silver nitrate (3.0 molar equiv) was dissolved in acetonitrile (2.5 mL) in a 50 mL flask. Then, the amidrazones **1** were suspended in an acetonitrile/ ethanol mixture (80:20) (10 mL) and the suspension was added to the silver nitrate solution under magnetic stirring at room temperature. A dark red suspension was formed and, after 15 min., the reaction was complete by TLC. The solid was filtered under vacuum and washed with cold acetonitrile and ethyl ether giving the **2.HNO_3.Ag** compounds.

(*E*)-5-amino-4-(imino(phenyldiazenyl)methyl)-1-(4-methoxyphenyl)-1*H*-imidazol-3-ium (2a.HNO_3)



In a 50 mL flask, silver nitrate (0.95 g, 5.60 mmol) was dissolved in acetonitrile (3 mL). A suspension of amidrazone **1a** (0.60 g, 1.87 mmol) in acetonitrile (12 mL) and ethanol (1.5 mL) was prepared. The dark brown solid was collected and identified as azoimidazole **2a.HNO_3** (0.45 g, 1.17 mmol, 62.6%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 3.84 (s, 3H), 7.18 (d, *J*= 8.0Hz, 2H), 7.52 (d, J= 8.0 Hz, 2H), 7.71 (t, *J*= 8.0 Hz, 2H), 7.78 (brs, 2H), 7.80 (t, *J*= 8.0 Hz, 1H), 8.02 (s, 1H), 8.09 (d; *J*= 8.0 Hz, 2H), 9.08 (brs, 1H), 9.54 (brs, 1H).

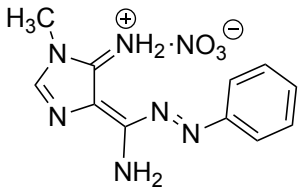
^{13}C NMR (100 MHz, DMSO-d_6): δ 55.69, 115.34, 115.33, 115.56, 124.93, 125.07, 128.02, 130.03, 135.77, 143.11, 151.16, 154.75, 159.36, 160.23 ppm.

IR (KBr cells): ν 3387, 3101, 2974, 2943, 1678, 1639, 1593, 1577, 1535, 1516, 1469, 1423 cm^{-1}.

HRMS ESI-TOF (m/z): calculated for $C_{17}H_{16}N_6O$ $[M+H]^+$: 321.1452, found 321.1458.

Melting point 146-152°C

(*E*)-5-amino-4-(imino(phenyldiazenyl)methyl)-1-methyl-1*H*-imidazol-3-ium (2b.HNO_3)



In a 50 mL flask, silver nitrate (1.12 g, 6.55 mmol) was dissolved in acetonitrile (3 mL). A suspension of amidrazone **1b** (0.51 g, 2.18 mmol) in acetonitrile (12 mL) and ethanol (1.5 mL) was prepared. The dark brown solid was collected and identified as azoimidazole **2b.HNO_3** (0.51 g, 1.75 mmol, 80.3%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 3.51 (s, 3H), 7.71 (t, *J*= 8.0 Hz, 2H), 7.80 (t, *J*= 8.0 Hz, 1H), 7.88 (s, 1H), 7.93 (brs, 2H), 8.12 (d; *J*= 8.0 Hz, 2H), 8.89 (brs, 1H), 9.28 (brs, 1H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 30.79, 116.19, 125,13, 130.01, 135.69, 144.03, 151.04, 155.34, 158.72 ppm.

IR (KBr cells): ν 3425, 3282, 3059, 1670, 1639, 1600, 1546 cm^{-1}.

HRMS ESI-TOF (m/z): calculated for $C_{11}H_{12}N_6$ $[M+H]^+$: 229.1196, found 229.1196.

Melting point 146-155°C.

Silver removal procedure

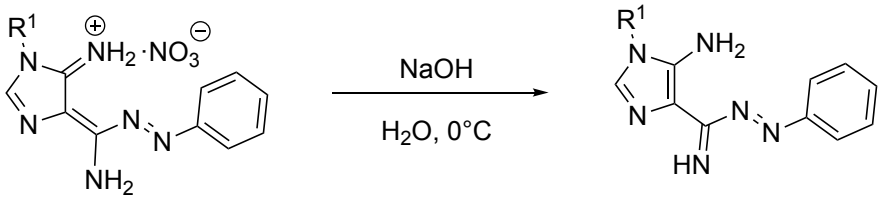
The yield of the reactions was calculated just after silver removal. Two experimental methods were tested to remove the reduced silver formed during the synthesis of azoimidazoles (**2.HNO_3.Ag**), centrifugation and flash chromatography using diatomaceous earth. In the centrifugation method, the **2.HNO_3.Ag** compounds were dissolved in water and the silver was removed at 16100 rcf for 15 min., collecting a half of supernatant in each cycle (the procedure was repeated 3 times). In the flash chromatography using diatomaceous earth method, the **2.HNO_3.Ag** compounds were also dissolved in water and the solution was purified using water as eluent. After that, the obtained solutions and control samples containing silver were digested in acidic media. For the digestion procedure, all glass material was rinsed with distilled water and soaked in a nitric acid solution (10%) for 24h. The digestion was performed by mixing the **2.HNO_3.Ag** solution with nitric acid (70%) (50:50). The mixture was heated to 95°C and kept at this temperature for 60 min. The solution was transferred to a 10 mL volumetric flask after cooling and the volume adjusted with distilled water. The silver content was determined by AAS (SI-Table 1).

Before silver removal, the silver concentration was 41.0%, indicating the reduction of the total amount of the silver nitrate added. In both methods, the concentration of the silver detected by AAE after centrifugation or filtration was in the range of 0.002-0.07 wt%. Thus, to determine the yields of the reaction, it was considered 41.0% of silver content in the final compounds **2.HNO_3.Ag**.

SI-Table 1. AAS results before and after silver removal.

Compound	Silver removal method	[Ag] (wt%)
2a.HNO_3.Ag	-	40.9
2b.HNO_3.Ag	-	41.2
2a.HNO_3	centrifugation	0.07
2b.HNO_3	centrifugation	0.07
2a.HNO_3	filtration	0.002
2b.HNO_3	filtration	0.002
3a-DMA	centrifugation	0.07
3a-DMA	filtration	0.002
3a-Pip	centrifugation	0.07
3a-Pip	filtration	0.004
3b-DMA	centrifugation	0.07
3b-DMA	filtration	0.002
3b-Pip	centrifugation	0.07
3b-Pip	filtration	0.002

2.3. General procedure for the neutralization of azoimidazoles 2.HNO_3



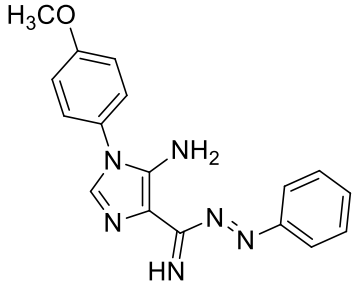
R^1= Ar(*p*)OMe, Me
2·HNO_3

R^1= Ar(*p*)OMe, Me
2

In a 25 mL flask, the azoimidazoles **2.HNO_3** were dissolved in water (4-10 mL) and placed under magnetic stirring in an ice bath. If the compound had silver, it was removed in this step by filtration or centrifugation as described in silver removal procedure.

The aqueous 3.0 or 4.0 M NaOH (3.0 molar equiv.) was added to the reaction solution and a blue solid instantly emerged. The solids were filtrated and washed with water. The pure azoimidazoles **2** were obtained in 96-97% yields.

(*E*)-4-(imino(phenyldiazenyl)methyl)-1-(4-methoxyphenyl)-1*H*-imidazol-5-amine (2a)

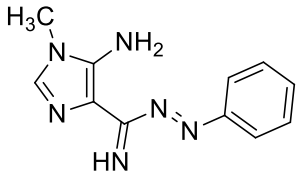


In a 25 mL flask, a solution of azoimididole **2a.HNO$_3$** (0.30 g, 0.78 mmol) was prepared in distilled water (10 mL) and placed under magnetic stirring in an ice bath. After 5 min., 3.0 M NaOH (0.8 mL, 2.34 mmol) was added to the solution. The solid that immediately precipitated was filtrated and identified as compound **2a** (0.24 g, 0.77 mmol, 96%) by ^{1}H NMR.

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 3.84 (s, 3H), 7.18 (d, *J*= 8.8 Hz, 2H), 7.52 (d, *J*= 6.8 Hz, 2H), 7.72 (brs, 3H), 8.00 (brs, 1H), 8.10 (brs, 2H), 9.22 (brs, 1H).

^{13}C NMR (100 MHz, DMSO-*d*$_6$): This sample could not be characterized by this technique due to the broadness of the spectrum.

(*E*)-4-(imino(phenyldiazenyl)methyl)-1-methyl-1*H*-imidazol-5-amine (2b)



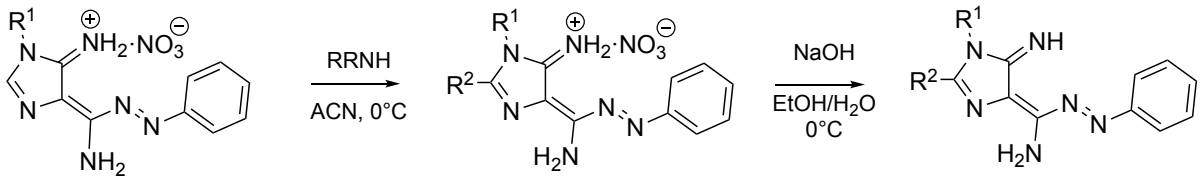
In a 25 mL flask, a solution of azoimidazole **2b.HNO$_3$** (0.10 g, 0.34 mmol) was prepared in distilled water (4 mL) and placed under magnetic stirring in an ice bath. After 5 min., 4.0 M NaOH (0.34 mL, 1.02 mmol) was added to the solution. The solid that immediately precipitated was filtrated and identified as compound **2b** (0.75 g, 0.33 mmol, 96%) by ^{1}H NMR.

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 3.44 (s, 3H), 6.43 (s, 2H), 7.10 (brs, 1H), 7,60 (brs, 3H), 7.82 (brs, 2H), 8.05 (brs, 1H).

^{13}C NMR (100 MHz, DMSO-*d*$_6$): This sample could not be characterized by this technique due to the broadness of the spectrum.

2.4. General procedure for the synthesis of 2-aminoimidazoles 3

Method A



R^1= Ar(*p*)OMe, Me
2·HNO$_3$

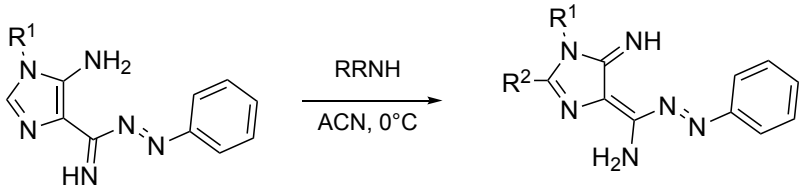
R^1 = Ar(*p*)OMe, Me
R^2 = N(CH$_3$)$_2$, N(CH$_2$)$_5$
3.HNO$_3$

R^1 = Ar(*p*)OMe, Me
R^2 = N(CH$_3$)$_2$, N(CH$_2$)$_5$
3

In this method, the 2-aminoimidazoles **3** were obtained from azoimidazoles **2.HNO$_3$** and the neutralization step was performed *in situ*. First, a suspension of azoimidazoles **2.HNO$_3$** in acetonitrile (6 mL) was prepared. The mixture was placed in an ice bath for 10 min., under

nitrogen atmosphere and magnetic stirring. Dimethylamine (DMA) or piperidine (Pip) (10.0 molar equiv.) was added to the solution. A bluish colored solution was formed and, after 1h, the reaction was purged with oxygen. After 10 min., the reaction was complete by TLC. Subsequently, the solution was evaporated to dryness using a rotary evaporator at 40°C. Ethanol (1 mL) was added into the flask and the neutralization step was performed by adding 3.0 M NaOH (2.0 molar equiv.) and water (2 mL) in an ice bath. A dark solid precipitated and it was filtered and washed with water. The obtained compounds were identified as 2-aminoimidazoles **3** in yields from 54 to 74%.

Method B



R^1 = Ar(*p*)OMe, Me

2

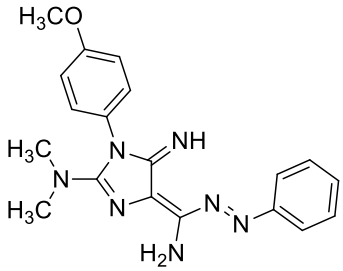
R^1 = Ar(*p*)OMe, Me
R^2 = $N(CH_3)_2$, $N(CH_2)_5$

3

A suspension of azoimidazole **2** in acetonitrile (0.5 mL) was stirred and kept in an ice bath during 10 minutes. Dimethylamine (DMA) or piperidine (Pip) (3.0 molar equiv.) was added to the reaction and a magenta/purple colored solution emerged. The reactions were complete after 30 min., as evidenced by TLC. The solution was evaporated to dryness using a rotary evaporator at 40°C. The solid obtained by precipitation with cold acetonitrile was collected and identified by NMR as being compounds **3** with yields between 34 and 58%.

After the first analysis of silver content, the reactions with secondary amines were conducted and the silver removed in different steps. In method A, the silver was removed after the reaction with the secondary amines. When the 2-aminoimidazoles **3.HNO_3** were synthesized, the compounds were totally solubilized in acetonitrile, the silver was removed and, after the acetonitrile evaporation, the compounds were neutralized using NaOH 4.0 M to obtain pure 2-aminoimidazoles **3**. In method B, the azoimidazoles **2.HNO_3** were dissolved in water, the silver was removed and then the neutralization step was carried out.

(*Z*)-4-(amino((*E*)-phenyldiazenyl)methylene)-5-imino-1-(4-methoxyphenyl)-*N*,*N*-dimethyl-4,5-dihydro-1*H*-imidazol-2-amine (3a-DMA)



Method A: A suspension of azoimidazole **2a.HNO$_3$** (0.30 g, 0.78 mmol) in acetonitrile (6 mL) was prepared in a 50 mL flask. The mixture was placed in an ice bath, under nitrogen atmosphere. Aqueous 7.89 M solution of dimethylamine (0.99 mL, 7.80 mmol) was added. The mixture was neutralized with 3.0 M NaOH (0.52 mL, 1.56 mmol). The collected solid was identified as compound **3a-DMA** (0.15 g, 0.42 mmol, 54%).

Method B: A suspension of azoimidazole **2a** (0.10 g, 0.31 mmol) in acetonitrile (0.5 mL) was prepared and placed under magnetic stirring in an ice bath. Aqueous 7.89 M solution of dimethylamine (0.12 mL, 0.93 mmol) was added. The solid obtained by precipitation with cold acetonitrile was collected and identified as being compound **3a-DMA** (0.06 g, 0.17 mmol, 55%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.88 (s, 6H), 3.79 (s, 3H), 5.37 (s, 2H), 7.03 (dt, *J*= 9.2 Hz, *J*= 3.2 Hz, 2H), 7.15 (dt, *J*= 8.8 Hz, *J*= 3.6 Hz, 2H), 7.32 (tt, *J*= 7.6 Hz, *J*= 1.2 Hz, 1H), 7.46 (t, *J*= 7.2 Hz, 2H), 7.60 (dt, *J*= 8.0 Hz, *J*= 1.6 Hz, 2H), 8.91 (s, 1H).

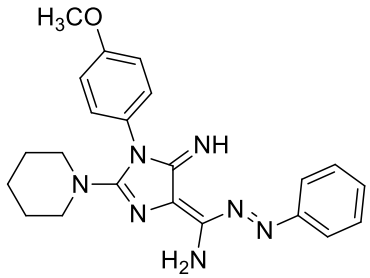
^{13}C NMR (75 MHz, DMSO-d_6): δ 39.33, 55.35, 114.36, 121.64, 127.88, 128.80, 129.20, 129.39, 129.79, 146.11, 152.87, 159.73, 159.92, 163.93 ppm.

IR (KBr cells): ν 3360, 2927, 2835, 1620, 1585, 1562, 1508, 1450, 1423, 1384 cm^{-1}.

HRMS ESI-TOF (m/z): calculated for $C_{19}H_{21}N_7O$ $[M+H]^+$: 364.1884, found 364.1880.

Melting point 163-173°C (dec.)

(*Z*)-(5-imino-1-(4-methoxyphenyl)-2-(piperidin-1-yl)-1*H*-imidazol-4(5*H*)-ylidene)((*E*)-phenyldiazenyl)methanamine (3a-Pip)

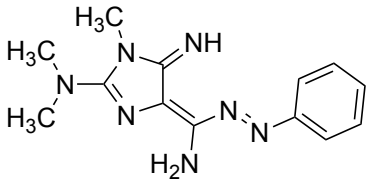


Method A: A suspension of azoimididole **2a.HNO$_3$** (0.30 g, 0.78 mmol) in acetonitrile (6 mL) was prepared in a 50 mL flask. The mixture was placed in an ice bath, under nitrogen atmosphere. Piperidine (0.77 mL, 7.80 mmol) was added to the reaction. The mixture was neutralized with 3.0 M NaOH (0.52 mL, 1.56 mmol). The collected solid was identified as compound **3a-Pip** (0.23 g; 0.58 mmol; 74%).

Method B: A suspension of azoimidazole **2a** (0.10 g of 0.31 mmol) in acetonitrile (0.5 mL) was prepared and placed under magnetic stirring in an ice bath. Piperidine (0.092 mL, 0.93 mmol) was added. The solid obtained by precipitation with cold acetonitrile was collected and identified as being compound **3a-Pip** (0.07 g, 0.18 mmol, 58%).

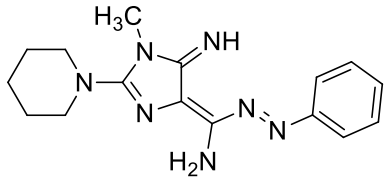
^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41-1.55 (m, 6H), 3.34-3.37 (m, 4H), 3.79 (s, 3H), 5.40 (s, 2H), 7.04 (dt, *J*= 9.2 Hz; *J*= 3.2 Hz, 2H), 7.29 (dt, *J*= 8.8 Hz; *J*= 3.2 Hz, 2H), 7.33 (tt, *J*= 7.2 Hz, *J*= 1.2 Hz, 1H), 7.46 (t, *J*= 7.6 Hz, 2H), 7.61 (dt, *J*= 8.4 Hz, *J*= 1.2 Hz, 2H), 8.90 (s, 1H).
^{13}C NMR (75 MHz, DMSO-d_6): δ 23.48, 24.92, 47.54, 55.35, 114.44, 121.7, 127.53, 128.96, 129.37, 129.41, 146.29, 152.82, 158.59, 159.35, 163.63 ppm.
IR (KBr cells): ν 3402, 3309, 2935, 2850, 1589, 1535, 1512, 1450, 1392, 1377 cm^{-1}.
HRMS ESI-TOF (m/z): calculated for $C_{22}H_{25}N_7O$ $[M+H]^+$: 404.2191, found 404.2193.
Melting point 93-103°C (dec.).

(*Z*)-4-(amino((*E*)-phenyldiazenyl)methylene)-5-imino-*N*,*N*,1-trimethyl-4,5-dihydro-1*H*-imidazol-2-amine (3b-DMA)



Method A: A suspension of azoimidazole **2b.HNO$_3$** (0.30 g, 1.03 mmol) in acetonitrile (6 mL) was prepared in a 50 mL flask. The mixture was placed in an ice bath, under nitrogen atmosphere. The aqueous 7.89 M dimethylamine solution (1.31 mL, 10.30 mmol) was added. The mixture was neutralized with NaOH 3.0 M (0.52 mL, 2.06 mmol). The collected solid was identified as compound **3b-DMA** (0.20 g, 0.73 mmol, 71%).
Method B: A suspension of azoimidazole **2b** (0.10 g, 0.44 mmol) in acetonitrile (0.5 mL) was prepared and placed under magnetic stirring in an ice bath. Aqueous 7.89 M solution of dimethylamine (0.17 mL, 1.32 mmol) was added. The solid, obtained by precipitation with cold acetonitrile, was collected and identified as being compound **3b-DMA** (0.05 g, 0.17 mmol, 39%).
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.20 (s, 6H), 3.33 (s, 3H), 5.30 (s, 2H), 7.34 (tt, *J*= 7.2 Hz, *J*= 1.2 Hz, 1H), 7.49 (tt, *J*= 7.6 Hz, *J*= 1.6 Hz, 2H), 7.62 (dt, *J*= 8.4 Hz, *J*= 1.2 Hz, 2H), 8.90 (s, 1H).
^{13}C NMR (75 MHz, DMSO-d_6): δ 30.42, 39.49, 121.66, 128.56, 128.83, 129.45, 146.14, 152.87, 162.06, 163.36 ppm.
IR (KBr cells): ν 3402, 3305, 2117, 1728, 1585, 1546, 1481, 1454, 1427, 1388, 1350, 1307 cm^{-1}.
HRMS ESI-TOF (m/z): calculated for $C_{13}H_{17}N_7$ $[M+H]^+$: 272.1615, found 272.1618.
Melting point >78°C (dec.)

(*Z*)-(5-imino-1-methyl-2-(piperidin-1-yl)-1*H*-imidazol-4(5*H*)-ylidene)((*E*)-phenyldiazenyl)methanamine (3b-Pip)



Method A: A suspension of azoimidazole **2b.HNO$_3$** (0.30 g, 1.03 mmol) in acetonitrile (6 mL) was prepared in a 50 mL flask. The mixture was placed in an ice bath, under nitrogen atmosphere. Piperidine (1.02 mL, 10.30 mmol) was added to the reaction. The mixture was neutralized with NaOH 3.0 M (0.68 mL, 2.06 mmol). The collected solid was identified as compound **3b-Pip** (0.21 g, 0.68 mmol, 66%).

Method B: A suspension of azoimidazole **2b** (0.10 g, 0.44 mmol) in acetonitrile (0.5 mL) was prepared and placed under magnetic stirring in an ice bath. Piperidine (0.13 mL, 1.32 mmol) was added. The solid obtained by precipitation with cold acetonitrile was collected and identified as being compound **3a-Pip** (0.05 g, 0.15 mmol, 34%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.64 (s, 6H), 3.25 (s, 3H), 3.57 (s, 4H), 5.35 (s, 2H), 7.36 (tt, *J*= 7.2 Hz, *J*= 1.2 Hz, 1H), 7.50 (tt, *J*= 7.6 Hz, *J*= 1.6 Hz, 2H), 7.64 (dt, *J*= 7.2 Hz, *J*= 1.2 Hz, 2H), 8.86 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): δ 23.67, 25.30, 30.75, 48.12, 121.76, 128.02, 129.09, 129.45, 146.49, 152.76, 161.80, 163.35 ppm.

IR (KBr cells): ν 3305, 2935, 2850, 1597, 1573, 1535, 1477, 1446, 1377 cm^{-1}.

HRMS ESI-TOF (m/z): calculated for $C_{16}H_{21}N_7$ $[M+H]^+$: 312.1940, found 312.1931.

Melting point 165-170°C.

3. Colorimetric properties

The compounds **3** described in the previous sections are highly colored, ranging from red, blue and purple. In order to study the colorimetric properties of these molecules, the UV-Vis spectroscopy technique was used.

3.1. Solvatochromism

Maximum absorption wavelengths (λ_{max}) in the region of visible light (between 350 and 700 nm) in different solvents (water, ethanol, acetonitrile, tetrahydrofuran and dichloromethane) were determined to study the influence of the solvent and structure on the spectra of UV-Vis. Just the absorption bands between 350 and 700 nm were considered to determine the molar absorptivity coefficients (ε), since the changes verified in this region were responsible for the color changes in the different solutions.

Molar absorptivity was determined by the Beer-Lambert Law (Equation 1), where "**A"** is the absorbance, "**ε"** is the molar absorptivity coefficient (M^{-1} cm^{-1}), **"l"** is the cell width (cm) and "**c"** is the concentration of the solution (mol L^{-1}).

$$A = \varepsilon l c$$ **Equation 1**

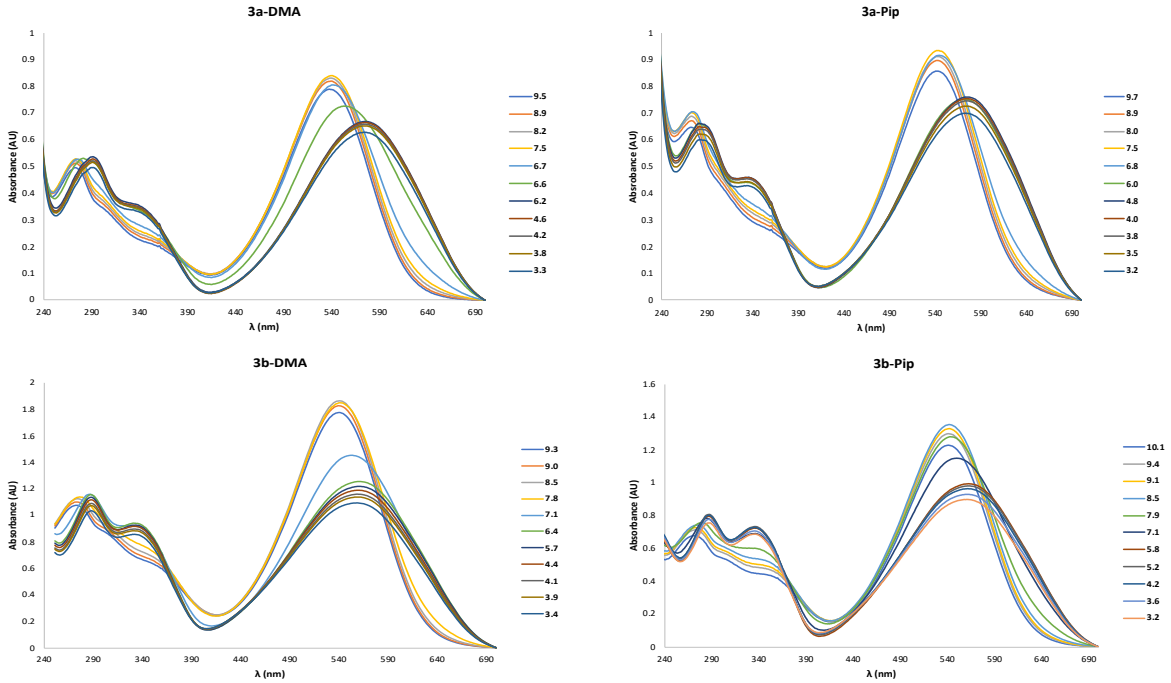
The molar absorptivity was determined as follows: i) the absorbance of standard solutions of different concentrations was analyzed at maximum wavelength; ii) the absorbance was plotted as a function of concentration; iii) the calibration line and the ε value obtained by the slope of this line (for l = 1 cm) were determined (SI-Table 2).

SI-Table 2. Maximum wavelength values and molar absorptivity coefficient for compounds **3** in different solvents.

	Ethanol		**Dichloromethane**		**Acetonitrile**		**Tetrahydrofuran**	
	$\lambda_{máx}$	**ε**	**$\lambda_{máx}$**	**ε**	**$\lambda_{máx}$**	**ε**	**$\lambda_{máx}$**	**ε**
3a-DMA	551	(17.3 ± 0.6) x10^3	554	(33 ± 1) x10^3	555	(26.4 ± 0.3) x10^3	560	(7.9 ± 0.4) x10^3
3a-Pip	553	(16.9 ± 0.2) x10^3	556	(26.9 ± 0.8) x10^3	557	(25.2 ± 0.3) x10^3	562	(25 ± 1) x10^3
3b-DMA	552	(16.4 ± 0.4) x10^3	556	(24.1 ± 0.9) x10^3	558	(22.0 ± 0.8) x10^3	560	(2.17 ± 0.06) x10^3
3b-Pip	552	(9.5 ± 0.2) x10^3	557	(22 ± 1) x10^3	558	(16.2 ± 0.5) x10^3	563	(15.1 ± 0.2) x10^3

3.2. Halochromism

The assessment of the halochromic properties of compounds **3** was carried out following the methods of Ossowski et al.[3] Solutions of the compounds were prepared in H_2O/EtOH (80:20) and the pH was modified by successive additions of 50 µL of a 0.1 M HCl solution (to study the effect of the acidic medium) or NaOH solution (to study the effect of the basic medium). After each addition, the pH was measured with a pH electrode and the corresponding UV-Vis absorbance spectrum was acquired immediately (SI-Figure 1). The pKa of each compound was calculated based on the graphical method from Vidal Salgado et al., where the spectra of the maximum and minimum pH levels were used to determine the wavelengths of maximum absorbance. Then, the absorbance *vs*. pH graph at these wavelengths was plotted. The pKa was acquired by calculating the pH of the point of intersection of the two linear curves (SI-Table 3).[4]



SI-Figure 2. UV-vis spectra of 2-aminoimidazoles **3** at different pH values**.**

SI-Table 3. Absorption spectra data of 2-aminoimidazoles **3** at different pH values**.**

3a-DMA pKa = 6.54			3a-Pip pKa = 6.25			3b-DMA pKa = 7.22			3b-Pip pKa = 6.93		
pH	**λ max (nm)**	**Abs.**	**pH**	**λ max (nm)**	**Abs.**	**pH**	**λ max (nm)**	**Abs.**	**pH**	**λ max (nm)**	**Abs.**
9.5	538	0.79	9.7	542	0.86	9.3	540	1.78	10.1	541	1.23
8.9	539	0.82	8.9	542	0.90	9	540	1.83	9.4	541	1.30
8.2	539	0.83	8	543	0.91	8.5	541	1.86	9.1	542	1.33
7.5	540	0.84	7.5	543	0.93	7.8	541	1.85	8.5	542	1.36
6.7	541	0.81	6.8	544	0.92	7.1	553	1.45	7.9	543	1.28
6.6	554	0.73	6.0	571	0.75	6.4	561	1.25	7.1	550	1.15
6.2	574	0.67	4.8	575	0.76	5.7	561	1.22	5.8	562	1.00
4.6	575	0.66	4	575	0.76	4.4	560	1.19	5.2	562	0.98
4.2	575	0.66	3.8	574	0.75	4.1	559	1.16	4.2	562	0.96
3.8	575	0.65	3.5	574	0.73	3.9	559	1.14	3.6	561	0.93
3.3	575	0.63	3.2	573	0.70	3.4	559	1.09	3.2	561	0.90

4. Materials and methods for biological experiments

4.1. Antimicrobial tests

4.1.1. Culture media

The following culture media were used: Sabouraud Dextrose Agar (SDA) from bio-Mérieux (Marcy L'Etoile, France), RPMI-1640 broth medium (containing L-glutamine and the pH indicator phenol red, but without bicarbonate) from Biochrom AG (Berlin, Germany) buffered with 3-(N-morpholino) propanesulfonic acid (MOPS) from Sigma-Aldrich (St. Louis, MO, USA) to pH 7.0, and Mueller-Hinton Agar (MHA) from BioKar Diagnostics (Allone, France).

4.1.2. Microorganisms

American Type Culture Collection (ATCC) reference strains for fungi used were *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, *Aspergillus fumigatus* ATCC 204305, *Aspergillus niger* ATCC 16404 and, for bacteria, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. A reference strain from Colección Española de Cultivos Tipo (CECT) was used: *Cryptococcus neoformans* CECT 1078. Clinical strains of yeasts were included: *C. albicans* DSY294 (fluconazole susceptible-S); *C. albicans* DSY296 (fluconazole resistant-R); *Candida glabrata* DSY562 (S); *C. glabrata* DSY565 (R) were kindly provided by D. Sanglard (University of Lausanne).

Clinical strains of filamentous fungi included: *Trichophyton rubrum* FF5 and *Trichophyton mentagrophytes* FF7; *Nannizzia gypsea* FF3; *Fusarium solani* FF125; *Scedosporium* spp..

4.1.3. Compounds solutions

Compounds were dissolved in Dimethyl sulfoxide (DMSO) from Sigma-Aldrich (St. Louis, MO, USA). For each assay, dilution series were prepared (1:2) in the suitable culture medium, with concentrations ranging between 256 and 0.5 μg mL^{-1}.

4.1.4. Susceptibility tests

Susceptibility tests for yeasts were performed based on the Clinical and Laboratory Standards Institute (CLSI), description for the broth microdilution method on the reference document M27A-3.[5] In resume, from yeasts colonies growing on SDA (24h incubation) a suspension with a final concentration of 10^3 colony forming units (CFU) mL^{-1} was prepared in RPMI-1640 broth culture media. Using 96-well plates, equal amounts (100 μL) of cell suspension and compound dilution were mixed, and incubated for 48h at 35°C.

Susceptibility tests for filamentous fungi were performed based on the CLSI description for the broth microdilution method on the reference document M38-A2.[5] In resume, from moulds cultures growing on SDA a suspension of 0.4-5 x 10^4 CFU mL^{-1} (for filamentous fungi, except dermatophytes) or 1-3 x 10^3 CFU mL^{-1} (for dermatophytes) was prepared in the same media used for yeasts. After addition of compounds, the plates were incubated 35°C for 48h (for the isolates of *Aspergillus*, *Fusarium* and *Scedosporium*) or at 25°C for 5 to 7 days for dermatophytes.

Susceptibility tests for bacteria were performed based on the CLSI description for the broth microdilution method on the reference document M07-A10.[5] In resume, a suspension of 5 x 10^5 CFU mL^{-1} was prepared in MH broth and, after mixture with the desired concentrations of compound, plates were incubated at 37°C for a period of 16 to 18h.

4.1.5. Controls and MIC determination

For all the experiments a positive control (microorganism in culture medium) representing 100% growth, a negative control (culture medium) corresponding to 0% growth, and a DMSO control (microorganism in culture medium with DMSO 1.0%; v/v) were included. In addition, a silver control using the maximum concentration found in AAS results (0.07%) was tested. Microorganisms' growth was related with the presence of turbidity and MIC referred as the lowest concentration that was able to totally inhibit the growth when comparing to control (non-treated microorganism in culture medium). All results are presented in SI-Table 4.

4.1.6. MLC determination

After MIC values determination, 20 μL of the content of wells with no visual growth detected were transferred to SDA plates and incubated respecting the same times and temperatures used for MIC determination. For bacteria, the same protocol was used with some modifications: 10 μL of wells content transferred to MH agar plates. The Minimum Lethal Concentration (MLC) corresponded to the lowest concentration having no visual growth (100% growth inhibition). All results are presented in SI-Table 4.

SI-Table 4. Antimicrobial activity (MIC and MLC) of the 2-aminoimidazoles derivatives against fungi (yeasts and filamentous fungi) and bacteria (Gram-positive and Gram-negative).

			MIC (MLC) µg/mL					
			2a	2b	3a-DMA	3a-Pip	3b-DMA	3b-Pip
Fungi	Yeasts	*Candida albicans* ATCC 10231	64 (256)	64 (128)	32 (64)	16 (32)	32 (64)	16 (32)
		Candida albicans DSY294 (S)	32 (256)	32 (128)	32 (64)	16 (32)	16 (32)	16 (32)
		Candida albicans DSY296 (R)	32 (256)	16 (128)	16 (64)	16 (32)	16 (32)	16 (32)
		Candida glabrata DSY562 (S)	64 (256)	64 (128)	64 (128)	32 (64)	32 (64)	16 (32)
		Candida glabrata DSY565 (R)	64 (256)	64 (128)	64 (128)	32 (64)	32 (64)	16 (32)
		Candida krusei ATCC 6258	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)	4 (4)
		Cryptococcus neoformans CECT1078	4 (4)	2 (4)	2 (4)	2 (2)	2 (2)	2 (2)
	Filamentous fungi	*Aspergillus fumigatus* ATCC 204305	256 (>256)	256 (>256)	128 (>256)	128 (>256)	128 (256)	64 (256)
		Aspergillus niger ATCC 16404	256 (>256)	256 (>256)	128 (>256)	128 (>256)	64 (256)	64 (256)
		Fusarium solani FF125	256 (>256)	256 (>256)	128 (256)	64 (128)	64 (128)	64 (64)
		Scedosporium spp.	256 (>256)	256 (256)	64 (128)	32 (64)	32 (64)	32 (64)
		Trichophyton rubrum FF5	64 (128)	64 (128)	64 (64)	32 (32)	16 (32)	16 (32)
		Trichophyton mentagrophytes FF7	32 (64)	64 (64)	32 (32)	32 (32)	32 (32)	16 (16)
		Nannizzia gypsea FF3	128 (≥256)	128 (256)	64 (128)	64 (128)	32 (64)	32 (64)
Bacteria	Gram-negative	*Escherichia coli* ATCC 25922	>256 (>256)	>256 (>256)	>256 (>256)	>256 (>256)	256 (256)	256 (256)
	Gram-positive	*Staphylococcus aureus* ATCC 25923	64 (≥256)	128 (>256)	32 (≥256)	32 (≥256)	32 (256)	32 (128)

MIC-minimum inhibitory concentration; MLC-minimum lethal concentration. S-Fluconazole susceptible strain; R-Fluconazole resistant strain.

4.2. Cytotoxicity

4.2.1. Materials

Reagents used in cell culture, including Dulbecco's modified Eagle's medium (DMEM) with 4.5 g L^{-1} glucose and GlutaMAX™, heat-inactivated fetal bovine serum (FBS), 0.25 % trypsin/ 1 mM ethylenediaminetetraacetic acid (EDTA), antibiotic (10,000 U mL^{-1} penicillin, 10,000 µg mL^{-1} streptomycin) and Hanks' balanced salt solution (HBSS) without calcium and magnesium [HBSS (-/-)] were acquired from Gibco™ (Thermo Fisher Scientific, Alfagene, Portugal). Trizma® base, neutral red (NR) solution and resazurin (REZ) were obtained from Sigma-Aldrich (Germany). Triton™ X-100 detergent solution and glacial acetic acid were acquired from Thermo Fisher Scientific (Waltham, MA, USA). Sulforhodamine B (SRB) was acquired from Santa Cruz Biotechnology (Dallas, TX, USA). Methanol (≥99.8%) was acquired from VWR Chemicals (Radnor, PA, USA). All the reagents used were of analytical grade or of the highest grade available.

4.2.2. HaCaT cell culture

HaCaT cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and were routinely cultured in 75 cm^2 flasks using DMEM with 4.5 g L^{-1} glucose and GlutaMAX™, supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 U mL^{-1} penicillin and 100 µg mL^{-1} streptomycin. Cells were maintained in a 5% CO_2 - 95% air atmosphere, at 37°C, and the medium was changed every 2-3 days. Cultures were passaged weekly by trypsinization (0.25% trypsin / 1mM EDTA). The cells used in all experiments were taken between the 40^{th} and 45^{th} passages.

4.2.3. Compounds cytotoxicity

The compounds cytotoxicity (0-64 µg mL^{-1}) was evaluated, 24h after exposure, by the neutral red (NR) uptake, resazurin (REZ) reduction and sulforhodamine B (SRB) assays. For that purpose, the cells were seeded in 96 well-plates at a density of 60,000 cells.cm^{-2} and, 24h after seeding, the cell culture medium was removed, and the cells exposed to the tested compounds (0-64 µg mL^{-1}). A stock solution of each compound was freshly prepared in DMSO on the day of the experiment, and further diluted in fresh cell culture medium, ensuring that DMSO did not exceed 0.1% of the exposure media. Triton™ X-100 (1%) was used as positive control.

Neutral Red uptake assay

Based on the ability of viable cells to incorporate and bind the supravital dye NR in the lysosomes, the NR uptake assay provides a quantitative estimation of the number of viable cells in a culture. The NR uptake assay was performed as previously described [5, 6]. Briefly, 24h after exposure to the tested compounds, the cell culture medium was removed and replaced by fresh cell culture medium containing 50 µg mL^{-1} NR. The cells were then incubated, at 37°C, in a humidified 5% CO_2 - 95% air atmosphere, for 90

minutes. At the end of this incubation period, the cell culture medium was removed, followed by the extraction of the dye absorbed only by viable cells with absolute ethyl alcohol/distilled water (1:1) with 5% acetic acid. The absorbance was then measured at 540 nm in a multiwell plate reader (PowerWaveX BioTek Instruments, VT, USA). The percentage of NR uptake relative to control cells (0 µM) was used as the cytotoxicity measure. Four independent experiments were performed, in triplicate.

Resazurin reduction assay

Based on the evaluation of living cells-mediated reduction of the oxidized blue dye to a fluorescent pink resorufin product, the REZ reduction assay represents a simple, rapid, sensitive, and inexpensive method to estimate the cellular metabolic capacity and, thus, to evaluate drug-induced cytotoxicity. Twenty-four hours after exposure to the tested compounds, the cell culture medium was removed and replaced by fresh cell culture medium containing 10 µg mL^{-1} REZ. The cells were then incubated, at 37°C, in a humidified 5% CO_2 - 95% air atmosphere, for 90 minutes. At the end of this incubation period, the resorufin fluorescence was measured at excitation/emission wavelengths of 560/590 nm, in a multiwell plate reader (PowerWaveX BioTek Instruments, VT, USA). The percentage of REZ reduction relative to control cells (0 µM) was used as the cytotoxicity measure. Four independent experiments were performed, in triplicate.

Sulforhodamine B assay

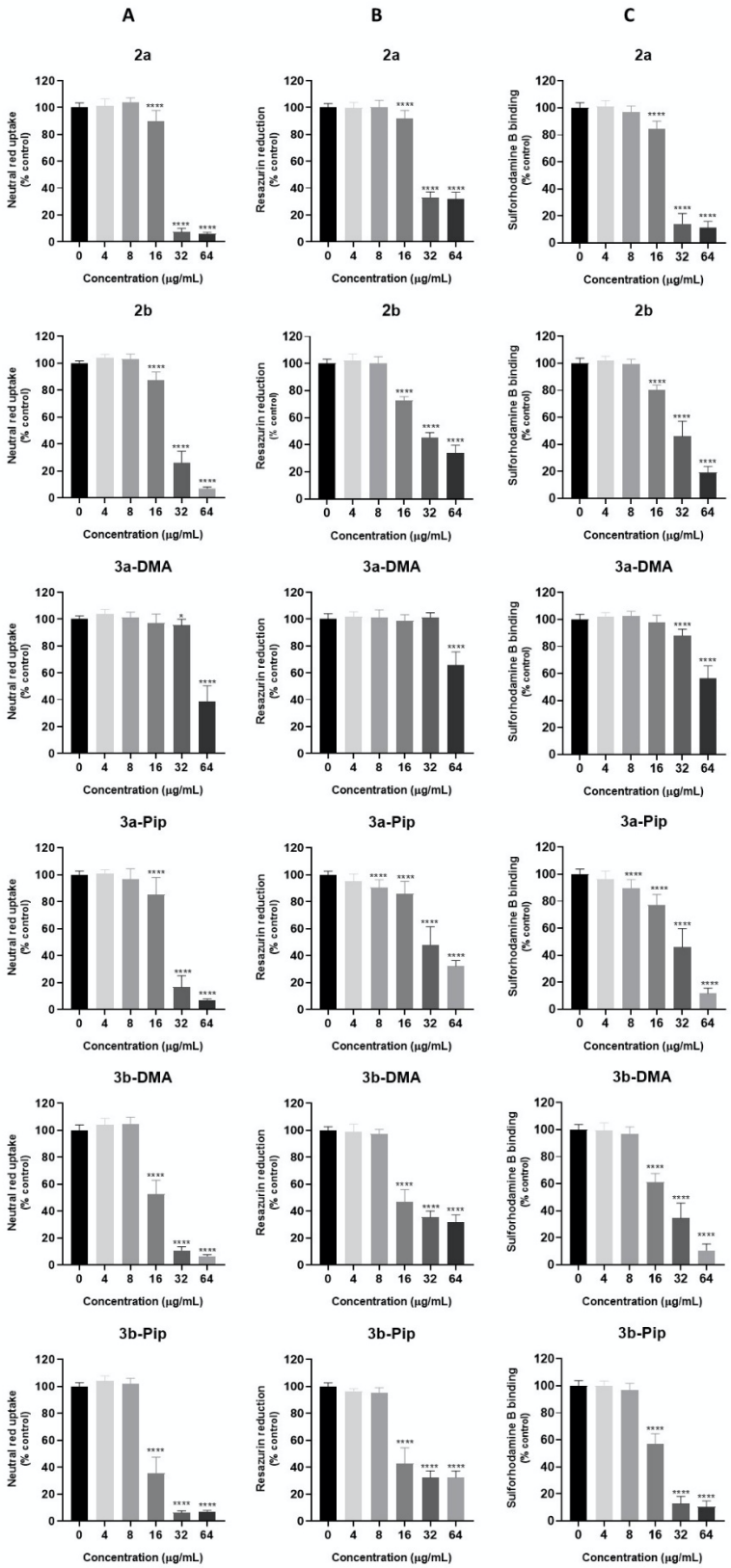
Based on the binding of the SRB dye to the basic amino acids of cellular proteins under mild acidic conditions, the SRB binding assay provides an estimation of the total protein mass, which is related to the number of cells in culture. Twenty-four hours after exposure to the tested compounds, the cell culture medium was removed, the cells washed with HBSS (+/+) and fixed overnight, at -20°C, with a methanolic solution of 1% acetic acid (v/v). Afterwards, the fixation medium was removed, and the cells incubated with a 0.05% SRB solution (prepared in 1% acetic acid) for 45 min., at 37°C. At the end of this incubation period, the SRB solution was aspirated, and the cells washed three times with 1% acetic acid (v/v) to ensure the complete removal of the unbound dye. The SRB bound to the basic amino acids of cellular proteins was then extracted with a Tris base solution (10 mM, pH 10.5). The absorbance was then measured at 540 nm in a multiwell plate reader (PowerWaveX BioTek Instruments, VT, USA). The percentage of SRB binding relative to the control cells (0 µM) was used as the cytotoxicity measure. Four independent experiments were performed, in triplicate.

SI-Table 5. Cytotoxic concentrations of the tested compounds towards HaCaT cells, evaluated by the Neutral red uptake assay, 24 h after exposure. Results were obtained from 4 independent experiences, performed in triplicate. Statistical comparisons were made using One-way ANOVA, followed by the Dunnett's multiple comparisons test (*p < 0.05; ****p < 0.0001 vs. 0 µg/mL). In all cases, p values < 0.05 were considered significant.

COMPOUND	CYTOTOXIC CONCENTRATIONS (Neutral red uptake assay)
2a	≥ 16 µg/mL****
2b	≥ 16 µg/mL****
3a-DMA	≥ 32 µg/mL*
3a-Pip	≥ 16 µg/mL****
3b-DMA	≥ 16 µg/mL****
3b-Pip	≥ 16 µg/mL***

4.2.4. Statistical analysis

All the statistical analysis were performed using the GraphPad Prism 8 for Windows (GraphPad Software, San Diego, CA, USA). The normality of the data distribution was assessed using the KS, D'Agostino & Pearson omnibus, and Shapiro-Wilk normality tests. One-way ANOVA was used to perform the statistical comparisons, followed by the Dunnett's multiple comparisons test. Detailed statistical analysis is available in the figure legend. In all cases, *p* values smaller than 0.05 were considered significant.



SI-Figure 3. Compounds cytotoxicity towards HaCaT cells, evaluated by the Neutral red uptake (A), Resazurin reduction (B) and Sulforhodamine B binding (C) assays, 24h after exposure. Results are expressed as Mean + SD from 4 independent experiences, performed in triplicate. Statistical comparisons were made using One-way ANOVA, followed by the Dunnett's multiple comparisons test (*$p < 0.05$; ****$p < 0.0001$ vs. 0 µg mL^{-1}). In all cases, p values < 0.05 were considered significant.

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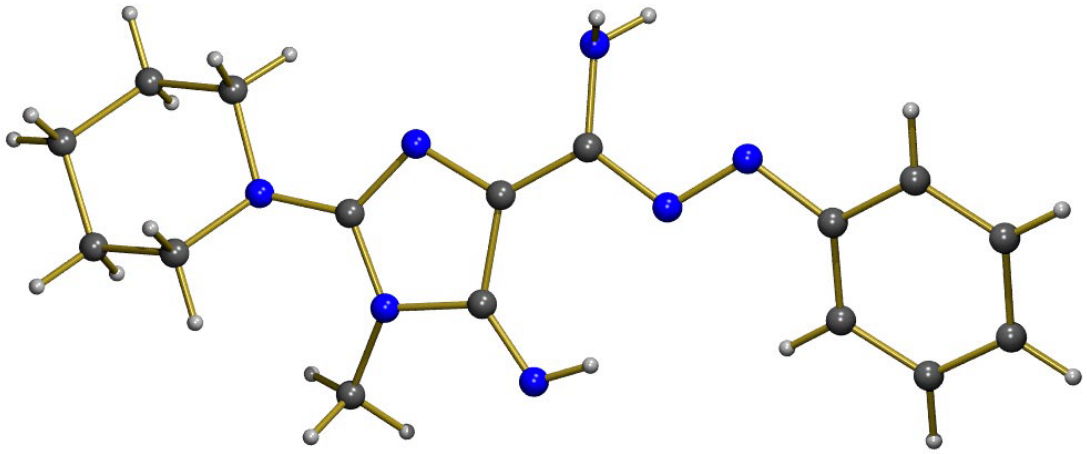
5. Crystallography Analysis

5.1. Report

A single crystal of compound **3b-Pip** was analysed by X-ray diffraction and a summary of the crystallographic data and the structure refinement parameters is reported in Table 1. Crystallographic data were collected at 150 K using a Bruker D8 Venture diffractometer with a Photon II CMOS detector and Mo-Kα _radiation (λ _= 0.71073 Å) generated by an Incoatec high brillance microfocus source equipped with Incoatec Helios multilayer optics.
The software APEX3. Drawings were produced with PLATON[7] and MERCURY[1] was used for collecting frames of data, indexing reflections, and determination of lattice parameters, SAINT[2] for integration of intensity of reflections, and SADABS[3] for scaling and empirical absorption correction. The structure was solved by dual-space algorithm using the program SHELXT.[4] All non-hydrogen atoms were refined with anisotropic displacement parameters by full-matrix least-squares calculations on F_2 using the program SHELXL[5] with OLEX2[6]. Hydrogen atoms were inserted at calculated positions and constrained with isotropic displacement; except for the hydrogen atoms of the –NH and $–NH_2$ groups, which were located from a Fourier-difference map and refined restraining the N-H distances[8].



SI-Figure 4. Ball and stick representation the molecular structure of compound **3b-Pip**.

5.2. Tables

SI-Table 6. Crystal data and structure refinement for compound **3b-Pip**.

Identification code	32081501_0m_a	
Empirical formula	$C_{16}\ H_{21}\ N_7$	
Formula weight	311.40	
Temperature	150.0 K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 1 21 1	
Unit cell dimensions	a = 10.6297(11) Å	α= 90°.
	b = 6.3019(6) Å	β= 111.388(3)°.
	c = 12.7387(12) Å	γ = 90°.
Volume	794.56(14) $Å^3$	
Z	2	
Density (calculated)	1.302 Mg/m^3	
Absorption coefficient	0.084 mm^{-1}	
F(000)	332	
Crystal size	0.093 x 0.027 x 0.021 mm^3	
Theta range for data collection	2.058 to 25.348°.	
Index ranges	-12<=h<=12, -7<=k<=7, -15<=l<=15	
Reflections collected	14603	
Independent reflections	2912 [R(int) = 0.0686]	
Completeness to theta = 25.242°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7244 and 0.6382	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2912 / 4 / 218	
Goodness-of-fit on F^2	1.170	
Final R indices [I>2sigma(I)]	R1 = 0.0785, wR2 = 0.1938	
R indices (all data)	R1 = 0.0881, wR2 = 0.1996	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.451 and -0.333 $e.Å^{-3}$	

SI-Table 7. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for compound **3b-Pip**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
N(1)	4995(5)	1787(7)	1666(4)	23(1)
N(2)	5094(5)	6274(7)	4570(4)	22(1)
N(3)	5867(4)	2824(7)	3497(4)	21(1)
N(7)	3448(5)	7160(6)	2759(4)	20(1)
N(8)	3162(5)	8720(7)	3275(4)	20(1)
N(13)	3336(5)	4357(8)	836(4)	28(1)
N(14)	6810(5)	-199(7)	2964(4)	24(1)
C(2)	5929(6)	1410(8)	2730(4)	19(1)
C(4)	4844(5)	4232(9)	2913(4)	20(1)
C(5)	4263(6)	3613(8)	1701(5)	21(1)
C(6)	4469(5)	5908(8)	3429(5)	18(1)
C(9)	2053(6)	9937(8)	2581(5)	21(1)
C(10)	1069(6)	9201(9)	1588(5)	25(1)
C(10')	1950(6)	11986(8)	2960(4)	23(1)
C(11)	9(6)	10503(10)	992(5)	29(1)
C(11')	900(6)	13312(8)	2336(5)	27(1)
C(12)	-91(6)	12546(10)	1352(5)	33(2)
C(15)	7497(7)	-706(11)	2167(5)	32(1)
C(15')	7722(6)	-475(10)	4150(5)	27(1)
C(16)	7986(7)	-2955(11)	2319(5)	35(2)
C(16')	8288(6)	-2692(9)	4345(5)	28(1)
C(17)	8963(7)	-3321(11)	3525(5)	36(2)
C(18)	4441(7)	235(9)	747(5)	30(1)

SI-Table 8. Bond lengths [Å] for compound **3b-Pip**.

N(1)-C(2)	1.378(7)	C(10')-C(11')	1.389(8)
N(1)-C(5)	1.399(7)	C(11)-H(11)	0.9500
N(1)-C(18)	1.473(7)	C(11)-C(12)	1.384(9)
N(2)-H(2A)	0.87(3)	C(11')-H(11')	0.9500
N(2)-H(2B)	0.86(3)	C(11')-C(12)	1.397(9)
N(2)-C(6)	1.380(7)	C(12)-H(12)	0.9500
N(3)-C(2)	1.341(7)	C(15)-H(15A)	0.9900
N(3)-C(4)	1.391(7)	C(15)-H(15B)	0.9900
N(7)-N(8)	1.279(6)	C(15)-C(16)	1.497(9)
N(7)-C(6)	1.361(7)	C(15')-H(15C)	0.9900
N(8)-C(9)	1.414(7)	C(15')-H(15D)	0.9900
N(13)-H(13)	0.88(3)	C(15')-C(16')	1.505(8)
N(13)-C(5)	1.270(7)	C(16)-H(16A)	0.9900
N(14)-C(2)	1.338(7)	C(16)-H(16B)	0.9900
N(14)-C(15)	1.486(7)	C(16)-C(17)	1.526(9)
N(14)-C(15')	1.478(7)	C(16')-H(16C)	0.9900
C(4)-C(5)	1.490(7)	C(16')-H(16D)	0.9900
C(4)-C(6)	1.377(7)	C(16')-C(17)	1.519(8)
C(9)-C(10)	1.394(8)	C(17)-H(17A)	0.9900
C(9)-C(10')	1.397(7)	C(17)-H(17B)	0.9900
C(10)-H(10)	0.9500	C(18)-H(18A)	0.9800
C(10)-C(11)	1.377(8)	C(18)-H(18B)	0.9800
C(10')-H(10')	0.9500		

Symmetry transformations used to generate equivalent atoms:

SI-Table 9. Bond angles [°] for compound **3b-Pip**.

C(2)-N(1)-C(5)	108.7(4)	N(14)-C(15)-H(15A)	109.6
C(2)-N(1)-C(18)	127.1(5)	N(14)-C(15)-H(15B)	109.6
C(5)-N(1)-C(18)	120.4(4)	N(14)-C(15)-C(16)	110.4(5)
H(2A)-N(2)-H(2B)	95(6)	H(15A)-C(15)-H(15B)	108.1
C(6)-N(2)-H(2A)	112(4)	C(16)-C(15)-H(15A)	109.6
C(6)-N(2)-H(2B)	121(4)	C(16)-C(15)-H(15B)	109.6
C(2)-N(3)-C(4)	105.7(4)	N(14)-C(15')-H(15C)	109.5
N(8)-N(7)-C(6)	114.1(4)	N(14)-C(15')-H(15D)	109.5
N(7)-N(8)-C(9)	113.3(4)	N(14)-C(15')-C(16')	110.6(5)
C(5)-N(13)-H(13)	109(4)	H(15C)-C(15')-H(15D)	108.1
C(2)-N(14)-C(15)	119.3(5)	C(16')-C(15')-H(15C)	109.5
C(2)-N(14)-C(15')	117.5(5)	C(16')-C(15')-H(15D)	109.5
C(15')-N(14)-C(15)	111.7(5)	C(15)-C(16)-H(16A)	109.6
N(3)-C(2)-N(1)	113.1(5)	C(15)-C(16)-H(16B)	109.6
N(14)-C(2)-N(1)	123.0(5)	C(15)-C(16)-C(17)	110.5(6)
N(14)-C(2)-N(3)	123.9(5)	H(16A)-C(16)-H(16B)	108.1
N(3)-C(4)-C(5)	109.5(5)	C(17)-C(16)-H(16A)	109.6
C(6)-C(4)-N(3)	122.7(5)	C(17)-C(16)-H(16B)	109.6
C(6)-C(4)-C(5)	127.8(5)	C(15')-C(16')-H(16C)	109.0
N(1)-C(5)-C(4)	102.9(4)	C(15')-C(16')-H(16D)	109.0
N(13)-C(5)-N(1)	122.7(5)	C(15')-C(16')-C(17)	112.9(5)
N(13)-C(5)-C(4)	134.3(5)	H(16C)-C(16')-H(16D)	107.8
N(7)-C(6)-N(2)	122.1(5)	C(17)-C(16')-H(16C)	109.0
N(7)-C(6)-C(4)	116.6(4)	C(17)-C(16')-H(16D)	109.0
C(4)-C(6)-N(2)	121.3(5)	C(16)-C(17)-H(17A)	109.8
C(10)-C(9)-N(8)	124.4(5)	C(16)-C(17)-H(17B)	109.8
C(10)-C(9)-C(10')	119.6(5)	C(16')-C(17)-C(16)	109.5(5)
C(10')-C(9)-N(8)	116.0(5)	C(16')-C(17)-H(17A)	109.8
C(9)-C(10)-H(10)	120.2	C(16')-C(17)-H(17B)	109.8
C(11)-C(10)-C(9)	119.6(5)	H(17A)-C(17)-H(17B)	108.2
C(11)-C(10)-H(10)	120.2	N(1)-C(18)-H(18A)	109.5
C(9)-C(10')-H(10')	119.8	N(1)-C(18)-H(18B)	109.5
C(11')-C(10')-C(9)	120.4(5)	N(1)-C(18)-H(18C)	109.5
C(11')-C(10')-H(10')	119.8	H(18A)-C(18)-H(18B)	109.5
C(10)-C(11)-H(11)	119.4	H(18A)-C(18)-H(18C)	109.5
C(10)-C(11)-C(12)	121.2(6)	H(18B)-C(18)-H(18C)	109.5
C(12)-C(11)-H(11)	119.4		
C(10')-C(11')-H(11')	120.3		
C(10')-C(11')-C(12)	119.4(5)		
C(12)-C(11')-H(11')	120.3		
C(11)-C(12)-C(11')	119.6(6)		
C(11)-C(12)-H(12)	120.2		
C(11')-C(12)-H(12)	120.2		

SI-Table 10. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for compound **3b-Pip**.

The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + \ldots + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
N(1)	28(3)	21(2)	21(2)	-4(2)	10(2)	3(2)
N(2)	29(3)	18(2)	20(2)	-5(2)	10(2)	-1(2)
N(3)	22(2)	25(2)	17(2)	-2(2)	9(2)	1(2)
N(7)	27(2)	15(2)	20(2)	-2(2)	11(2)	-2(2)
N(8)	21(2)	19(2)	19(2)	-2(2)	8(2)	2(2)
N(13)	33(3)	32(3)	17(2)	-2(2)	6(2)	5(2)
N(14)	28(3)	30(3)	17(2)	0(2)	11(2)	4(2)
C(2)	28(3)	19(2)	15(3)	2(2)	12(2)	-2(2)
C(4)	23(3)	20(2)	15(3)	1(2)	7(2)	-3(2)
C(5)	23(3)	22(3)	20(3)	-1(2)	9(2)	-2(2)
C(6)	21(3)	21(3)	13(3)	-2(2)	7(2)	-4(2)
C(9)	25(3)	23(3)	18(3)	2(2)	11(2)	2(2)
C(10)	30(3)	22(3)	23(3)	-3(2)	9(3)	3(2)
C(10')	33(3)	24(3)	16(3)	-2(2)	15(2)	-4(2)
C(11)	33(3)	37(3)	18(3)	3(2)	10(3)	4(3)
C(11')	40(4)	21(3)	27(3)	4(2)	21(3)	7(2)
C(12)	35(3)	36(3)	31(3)	15(3)	17(3)	13(3)
C(15)	36(3)	42(3)	23(3)	1(3)	18(3)	7(3)
C(15')	29(3)	36(3)	15(3)	2(2)	8(2)	5(3)
C(16)	45(4)	41(3)	27(3)	1(3)	23(3)	16(3)
C(16')	30(3)	30(3)	23(3)	-3(2)	9(3)	2(3)
C(17)	39(4)	39(3)	36(4)	2(3)	21(3)	14(3)
C(18)	43(4)	32(3)	17(3)	-8(2)	12(3)	6(3)

SI-Table 11. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^{2}x 10^3) for compound **3b-Pip**.

	x	y	z	U(eq)
H(2A)	5620(50)	7370(70)	4710(50)	26
H(2B)	4640(50)	6720(90)	4960(40)	26
H(13)	3080(70)	5600(60)	1000(60)	34
H(10)	1130	7809	1324	30
H(10')	2603	12475	3648	27
H(11)	-667	9989	322	35
H(11')	857	14728	2577	32
H(12)	-830	13423	932	39
H(15A)	8272	265	2301	38
H(15B)	6861	-495	1383	38
H(15C)	7220	-198	4654	33
H(15D)	8472	560	4332	33
H(16A)	8447	-3267	1787	42
H(16B)	7206	-3928	2149	42
H(16C)	7547	-3703	4271	34
H(16D)	8955	-2799	5124	34
H(17A)	9226	-4835	3632	43
H(17B)	9790	-2464	3672	43
H(18A)	4853	455	182	46
H(18B)	3460	424	396	46
H(18C)	4640	-1205	1053	46

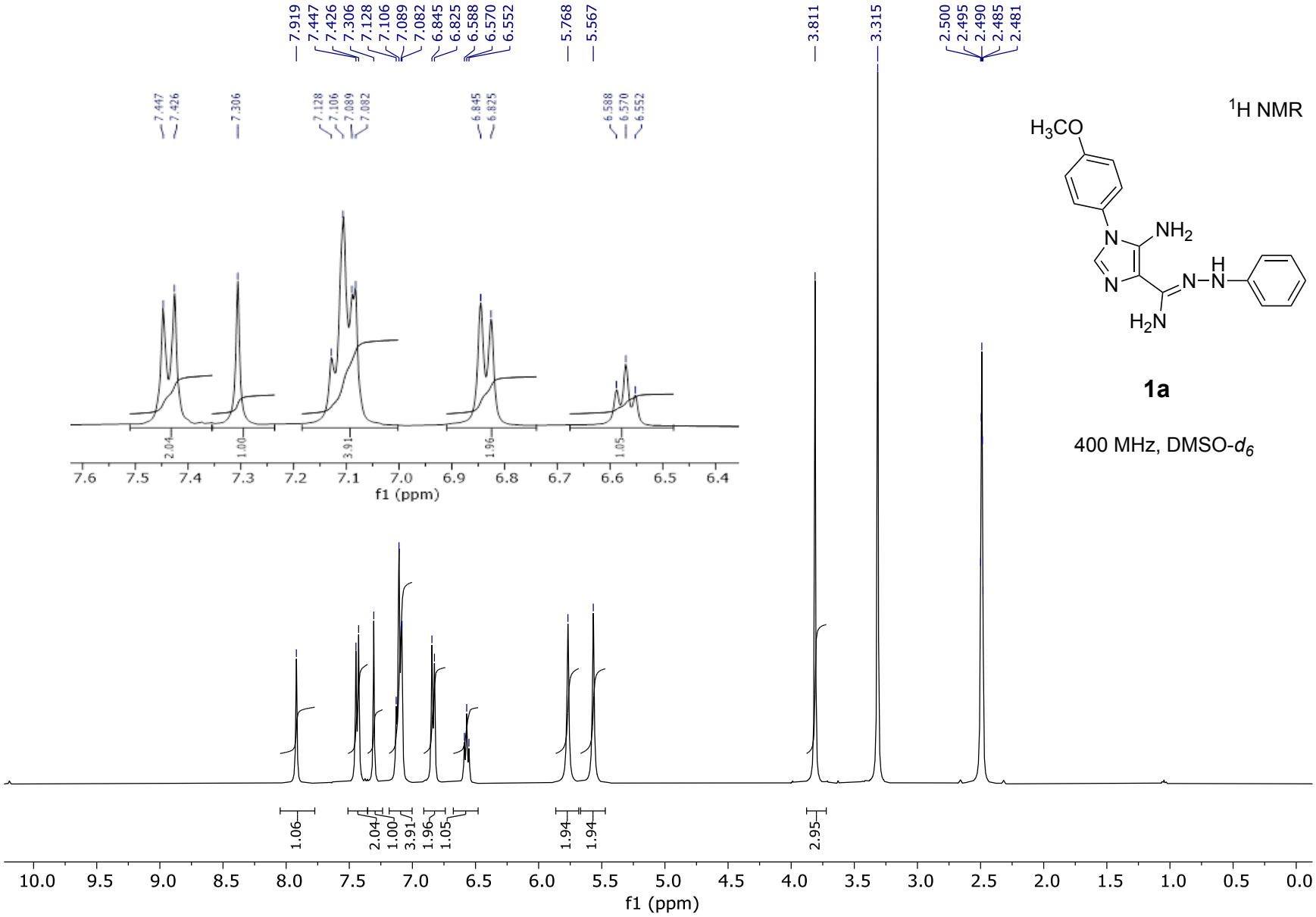
SI-Table 12. Torsion angles [°] for compound **3b-Pip**.

N(3)-C(4)-C(5)-N(1)	1.4(5)	N(3)-C(4)-C(5)-N(13)	-179.8(6)
N(3)-C(4)-C(6)-N(2)	-0.4(8)	N(3)-C(4)-C(6)-N(7)	-179.8(5)
N(7)-N(8)-C(9)-C(10)	21.4(7)	N(7)-N(8)-C(9)-C(10')	-160.4(5)
N(8)-N(7)-C(6)-N(2)	2.2(7)	N(8)-N(7)-C(6)-C(4)	-178.5(5)
N(8)-C(9)-C(10)-C(11)	178.2(5)	N(8)-C(9)-C(10')-C(11')	179.7(5)
N(14)-C(15)-C(16)-C(17)	-58.8(7)	N(14)-C(15')-C(16')-C(17)	53.8(7)
C(2)-N(1)-C(5)-N(13)	179.4(5)	C(2)-N(1)-C(5)-C(4)	-1.6(5)
C(2)-N(3)-C(4)-C(5)	-0.6(6)	C(2)-N(3)-C(4)-C(6)	179.1(5)
C(2)-N(14)-C(15)-C(16)	-157.8(5)	C(2)-N(14)-C(15')-C(16')	160.5(5)
C(4)-N(3)-C(2)-N(1)	-0.4(6)	C(4)-N(3)-C(2)-N(14)	179.1(5)
C(5)-N(1)-C(2)-N(3)	1.3(6)	C(5)-N(1)-C(2)-N(14)	-178.1(5)
C(5)-C(4)-C(6)-N(2)	179.3(5)	C(5)-C(4)-C(6)-N(7)	-0.1(8)
C(6)-N(7)-N(8)-C(9)	-176.8(4)	C(6)-C(4)-C(5)-N(1)	-178.3(5)
C(6)-C(4)-C(5)-N(13)	0.5(10)	C(9)-C(10)-C(11)-C(12)	0.9(9)
C(9)-C(10')-C(11')-C(12)	3.0(8)	C(10)-C(9)-C(10')-C(11')	-2.0(8)
C(10)-C(11)-C(12)-C(11')	0.1(9)	C(10')-C(9)-C(10)-C(11)	0.0(8)
C(10')-C(11')-C(12)-C(11)	-2.1(9)	C(15)-N(14)-C(2)-N(1)	41.9(7)
C(15)-N(14)-C(2)-N(3)	-137.5(6)	C(15)-N(14)-C(15')-C(16')	-56.3(6)
C(15)-C(16)-C(17)-C(16')	55.2(7)	C(15')-N(14)-C(2)-N(1)	-177.7(5)
C(15')-N(14)-C(2)-N(3)	2.9(8)	C(15')-N(14)-C(15)-C(16)	59.6(7)
C(15')-C(16')-C(17)-C(16)	-53.2(7)	C(18)-N(1)-C(2)-N(3)	-156.2(5)
C(18)-N(1)-C(2)-N(14)	24.4(8)	C(18)-N(1)-C(5)-N(13)	-21.3(8)

Symmetry transformations used to generate equivalent atoms:

6. NMR Spectra

6.1. ^{1}H NMR and ^{13}C NMR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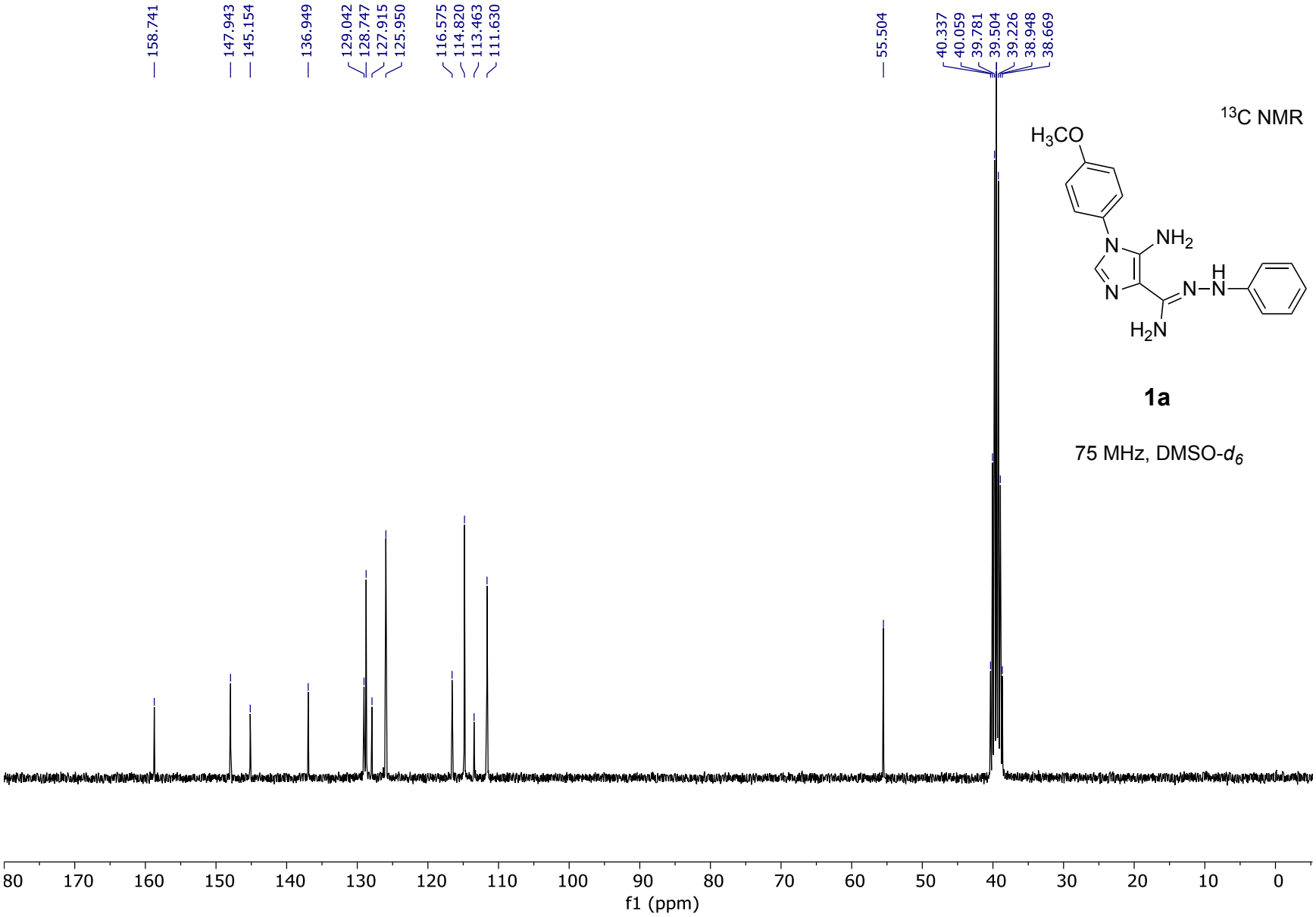
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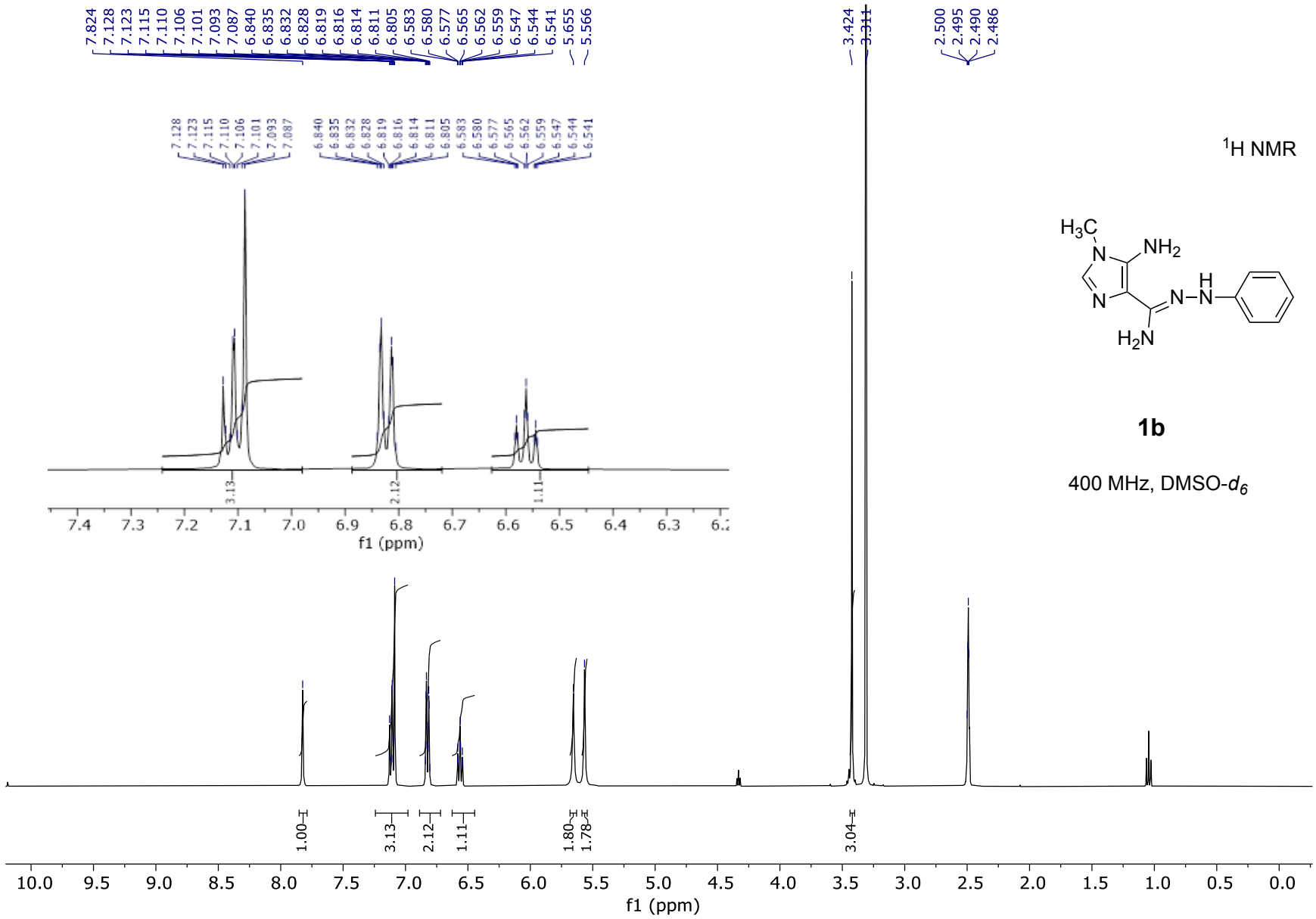
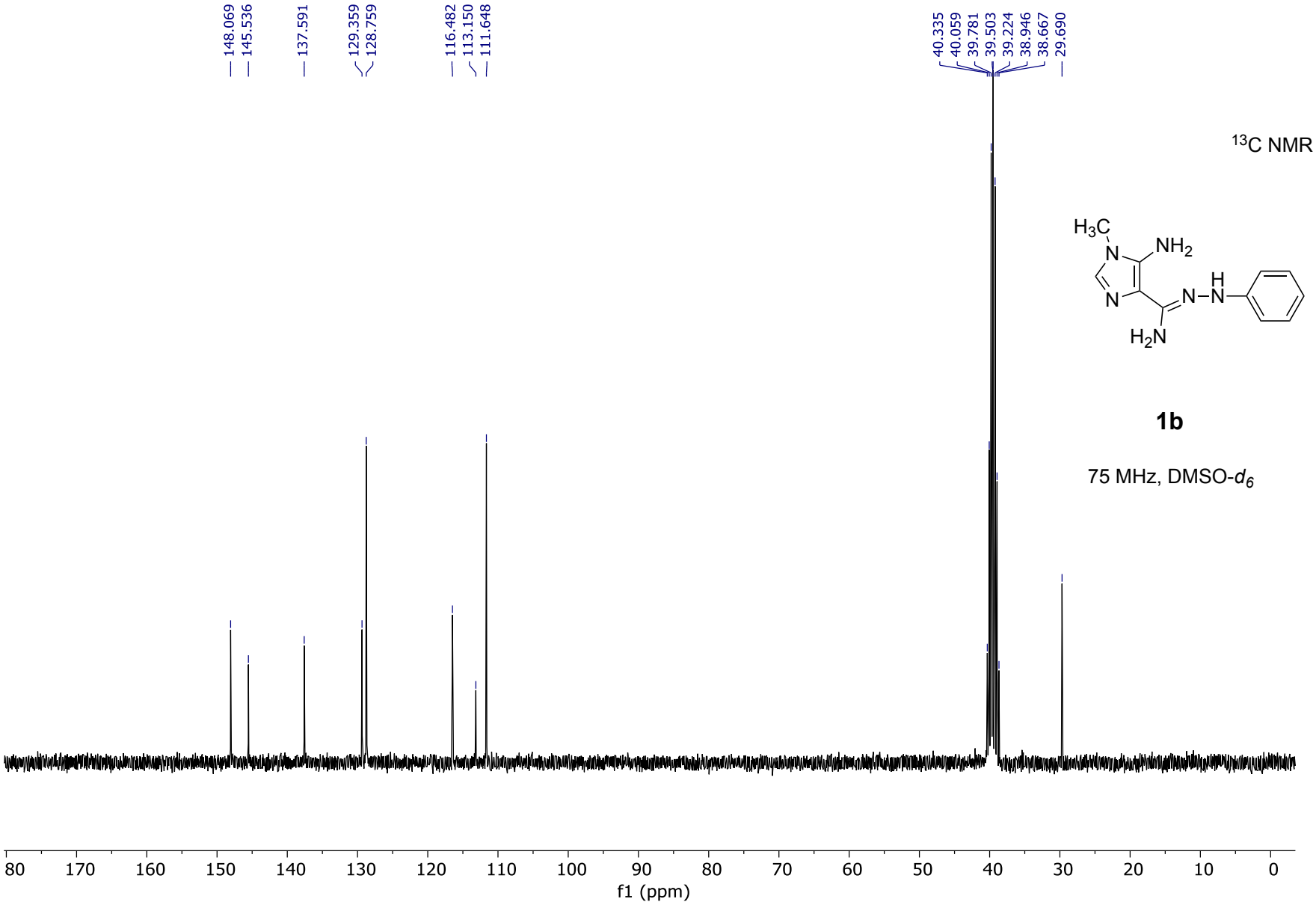
0
f1 (ppm)

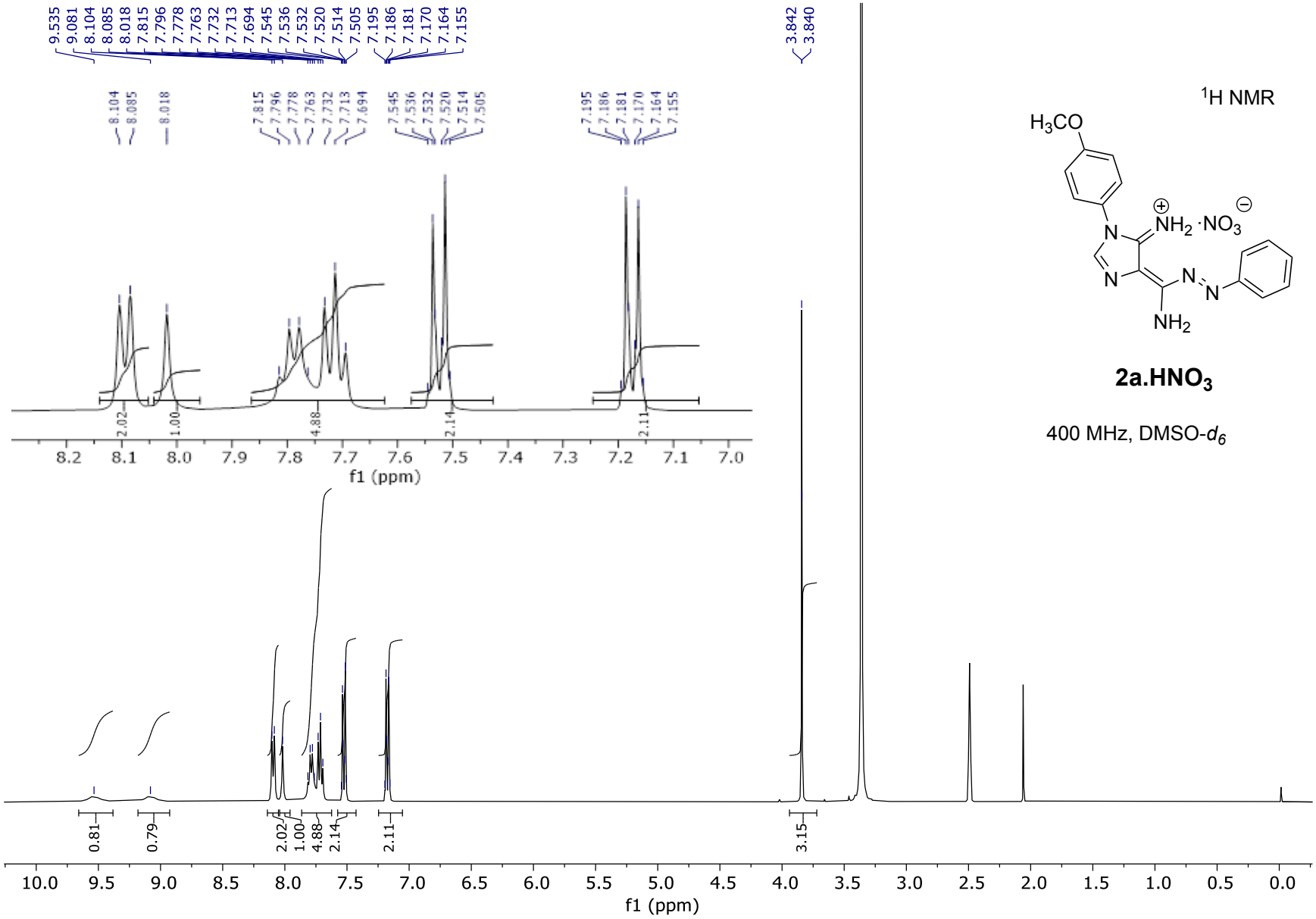


^{1}H NMR

1b

400 MHz, DMSO-d_6

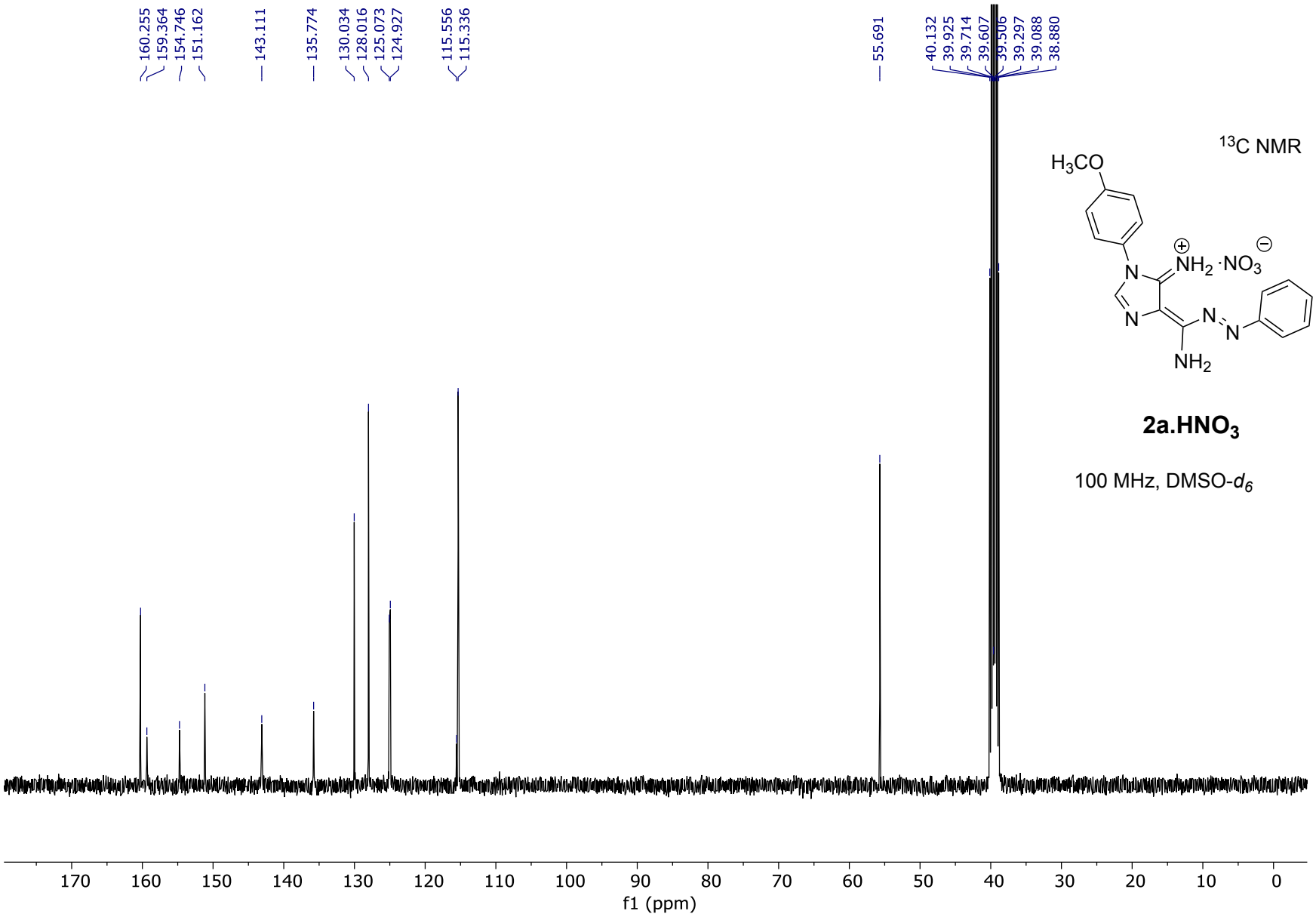

148.069
145.536
137.591
129.359
128.759
116.482
113.150
111.648
40.335
40.059
39.781
39.503
39.224
38.946
38.667
29.690
^{13}C NMR
H_3C
N
NH_2
N
H
N–N
H_2N
1b
75 MHz, DMSO-d_6
80 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



^{1}H NMR

2a.HNO_3

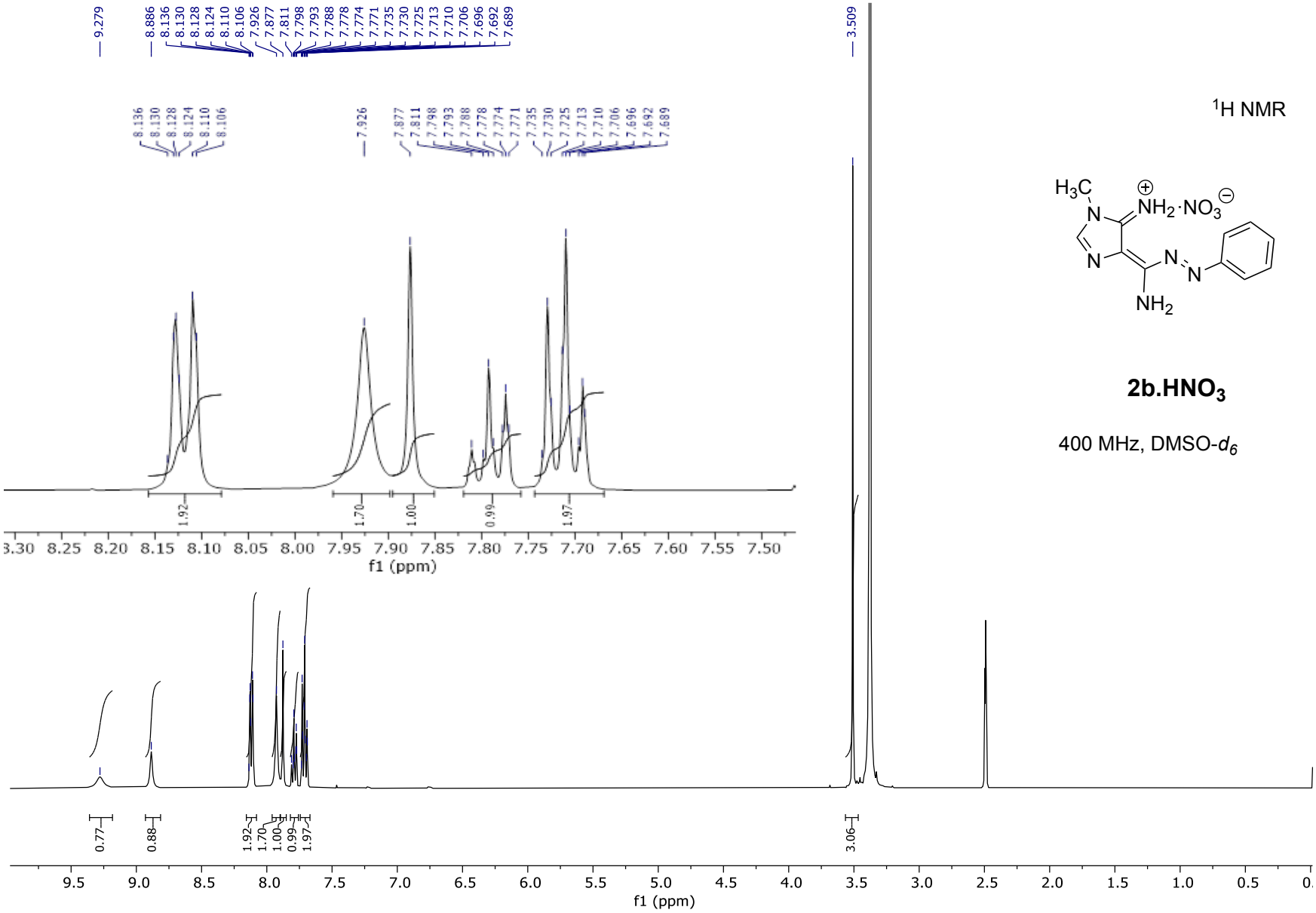
400 MHz, DMSO-d_6



^{13}C NMR

2a.HNO$_3$

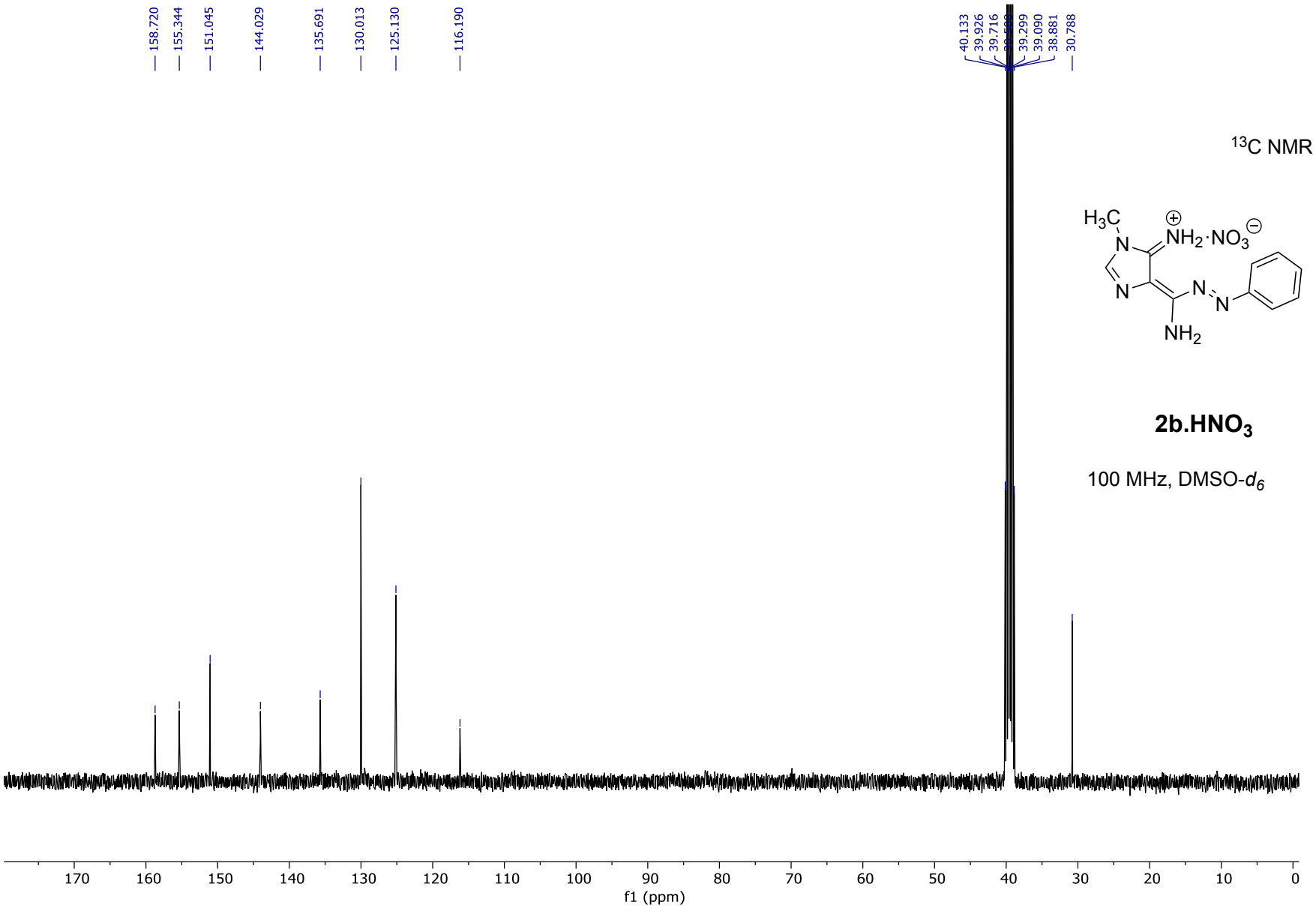
100 MHz, DMSO-d_6

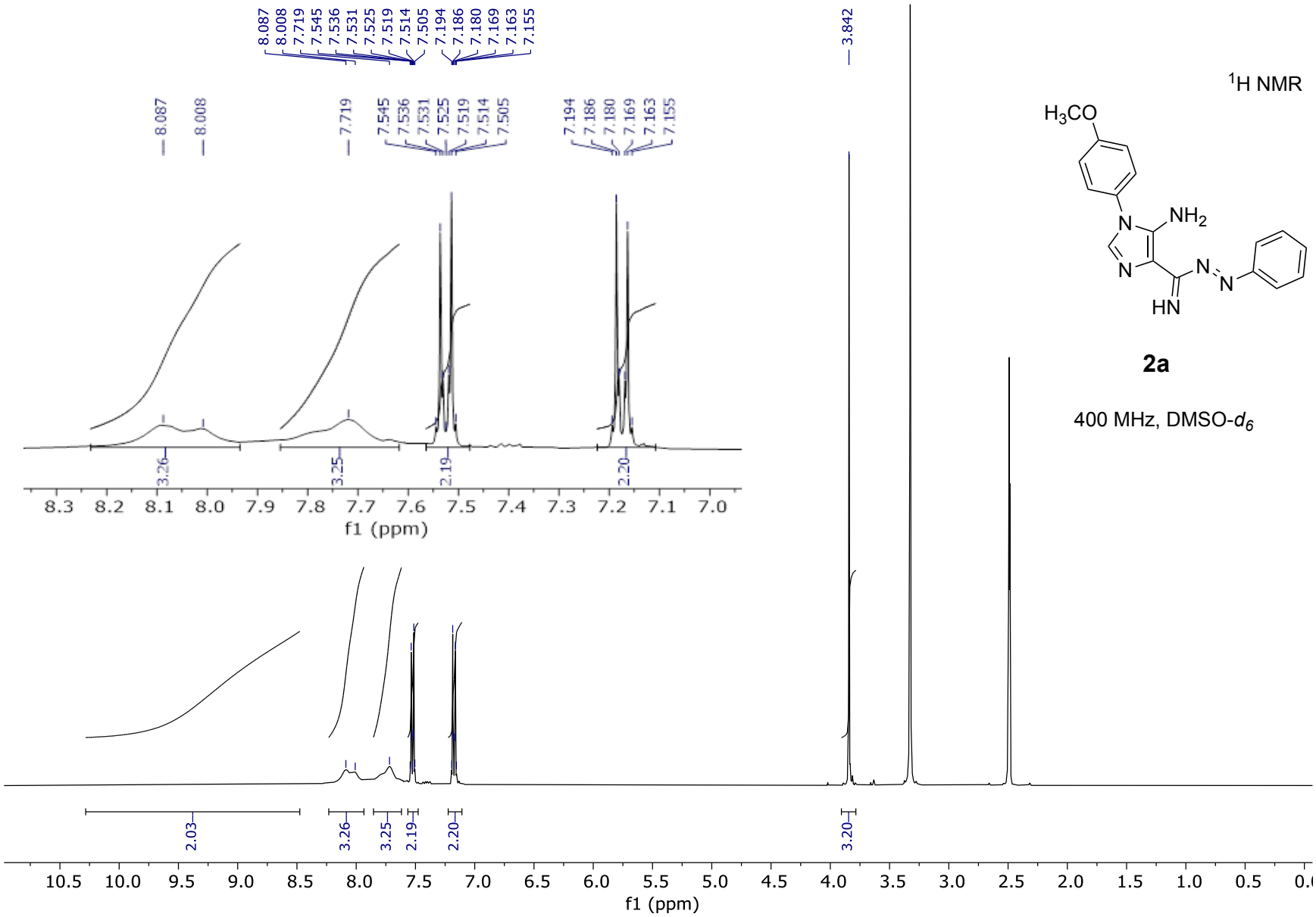


^{1}H NMR

2b.HNO$_3$

400 MHz, DMSO-*d*$_6$

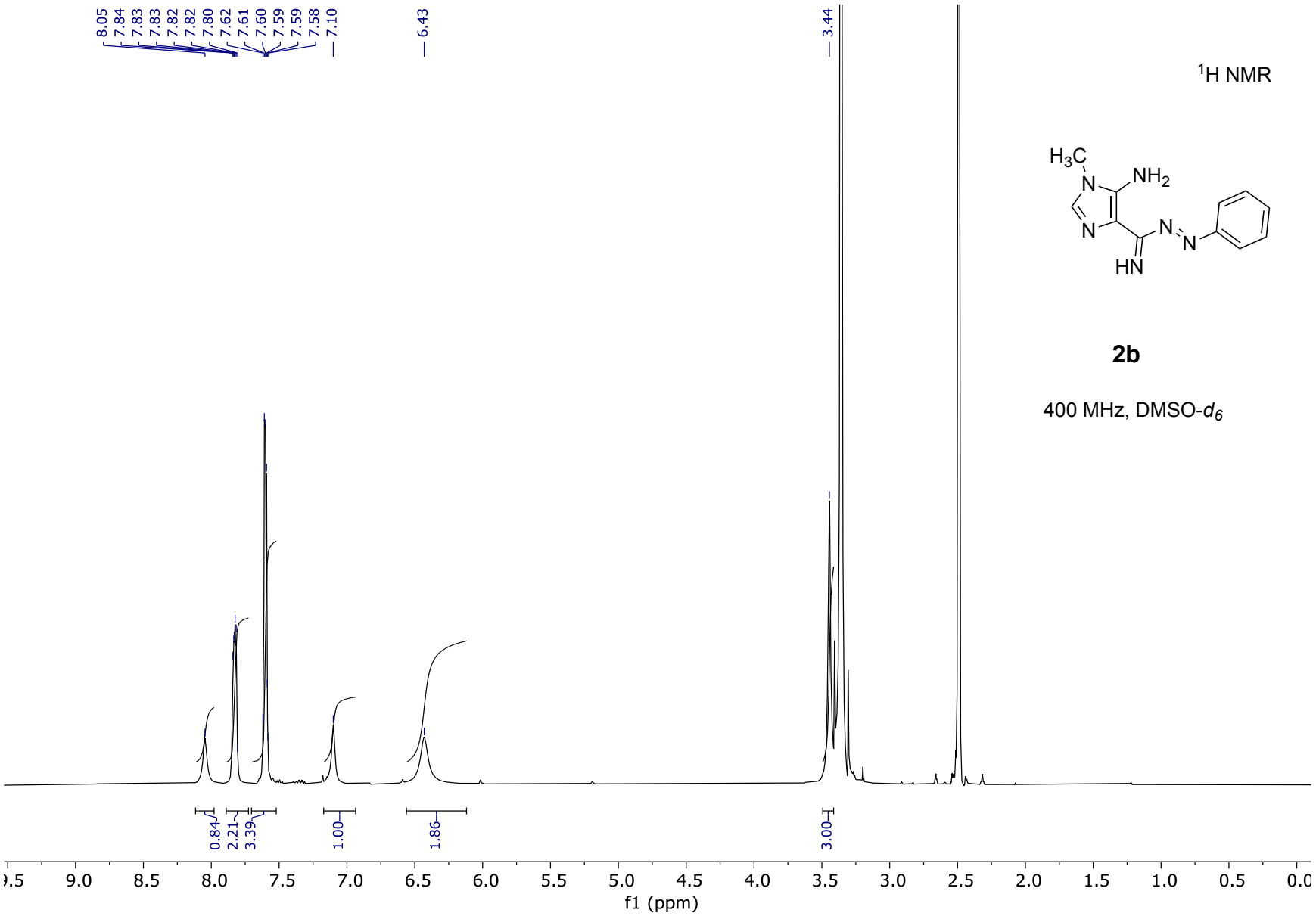

158.720
155.344
151.045
144.029
135.691
130.013
125.130
116.190
40.133
39.926
39.716
39.299
39.090
38.881
30.788
^{13}C NMR
H_3C
N
N
$NH_2 \cdot NO_3$
N
N
NH_2
2b.HNO$_3$
100 MHz, DMSO-d_6
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



^{1}H NMR

2a

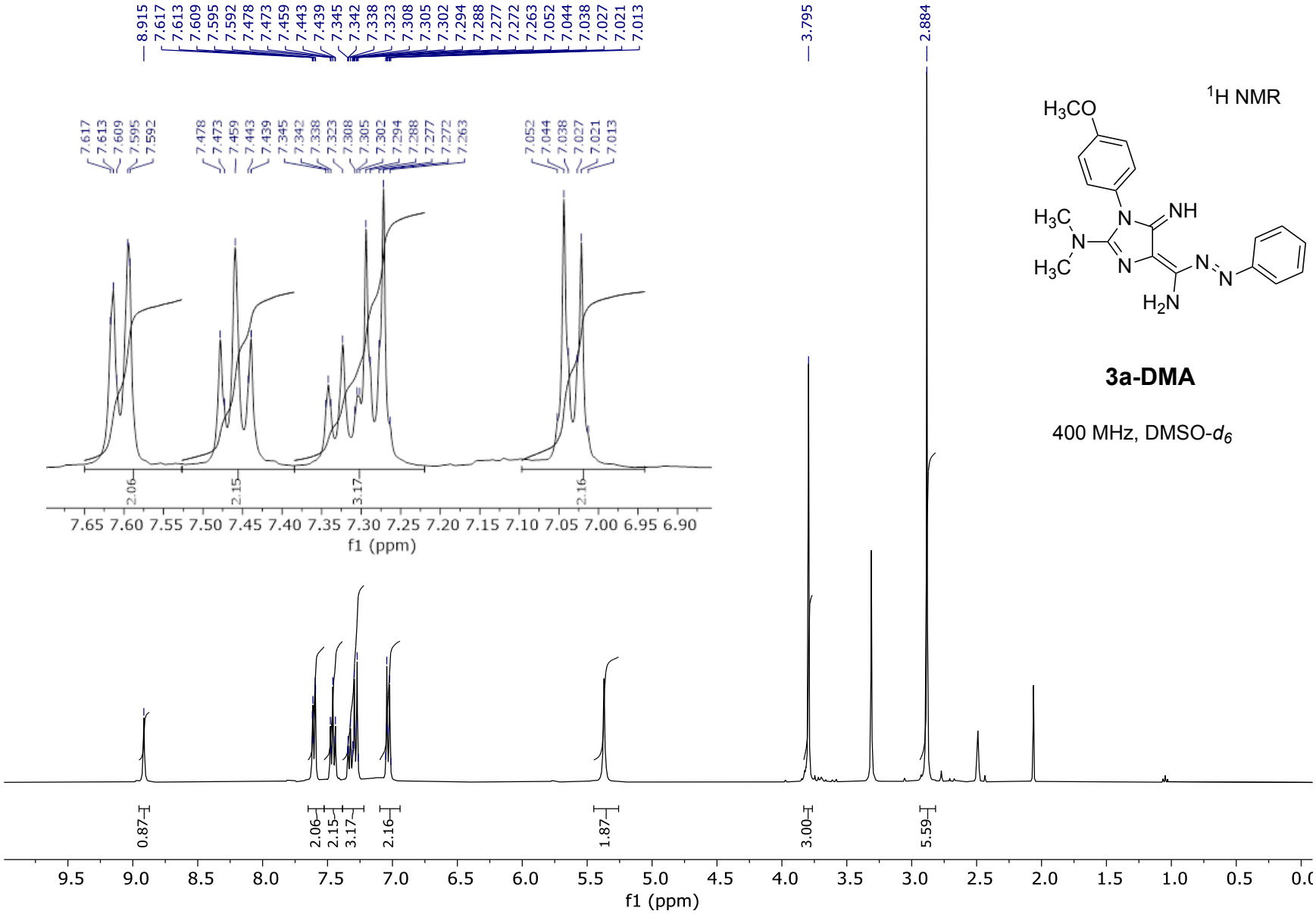
400 MHz, DMSO-d_6



^{1}H NMR

2b

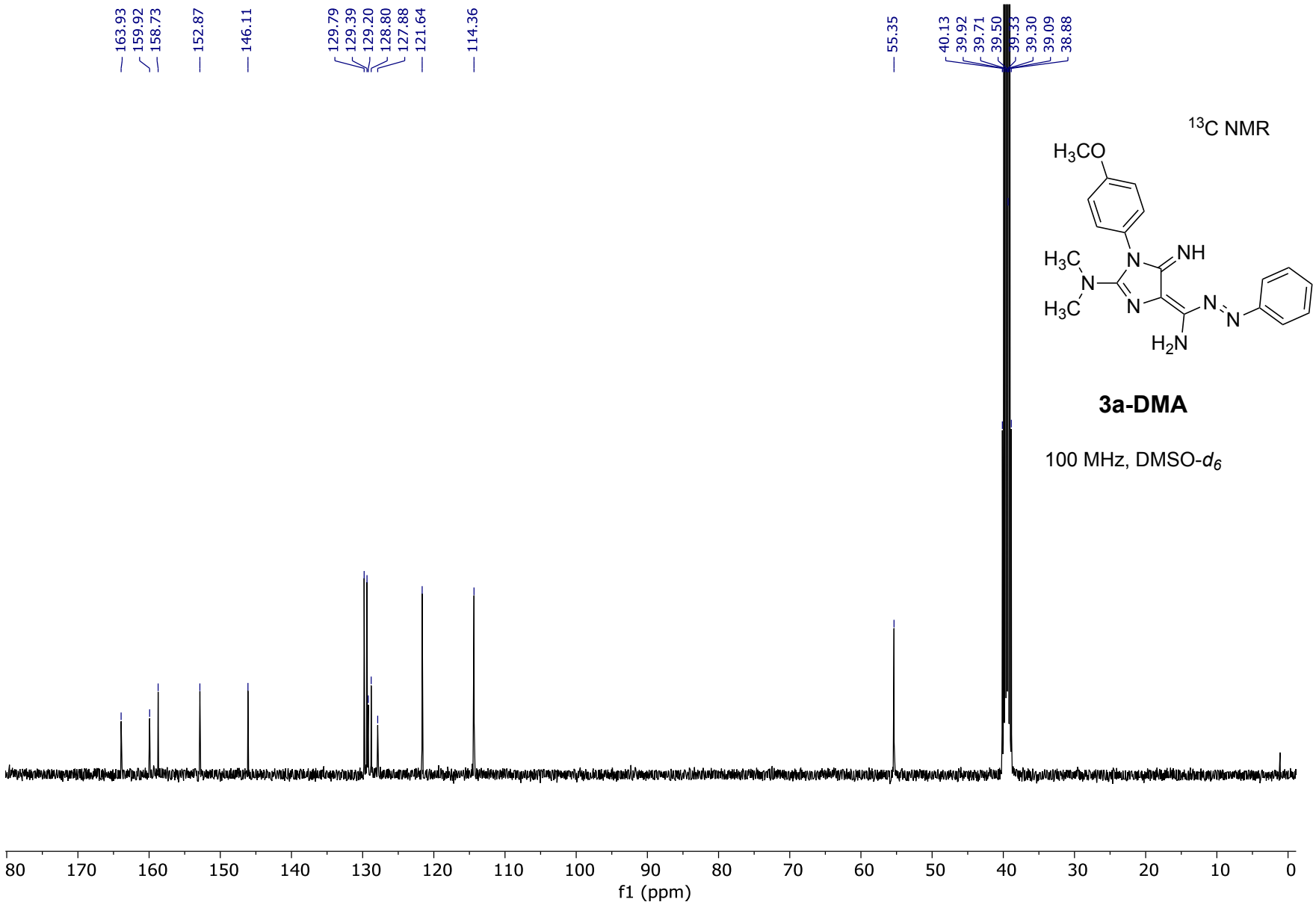
400 MHz, DMSO-d_6

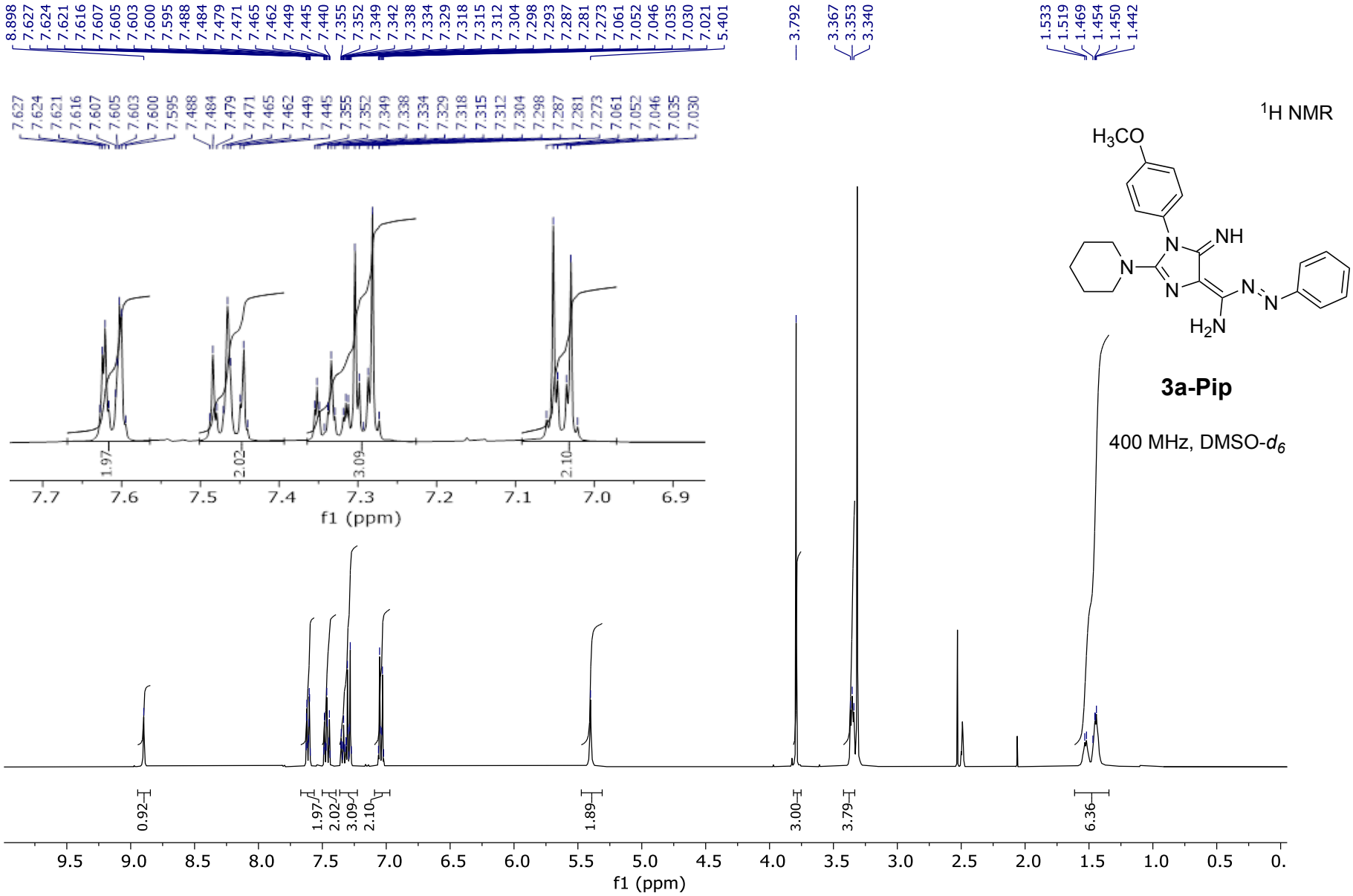


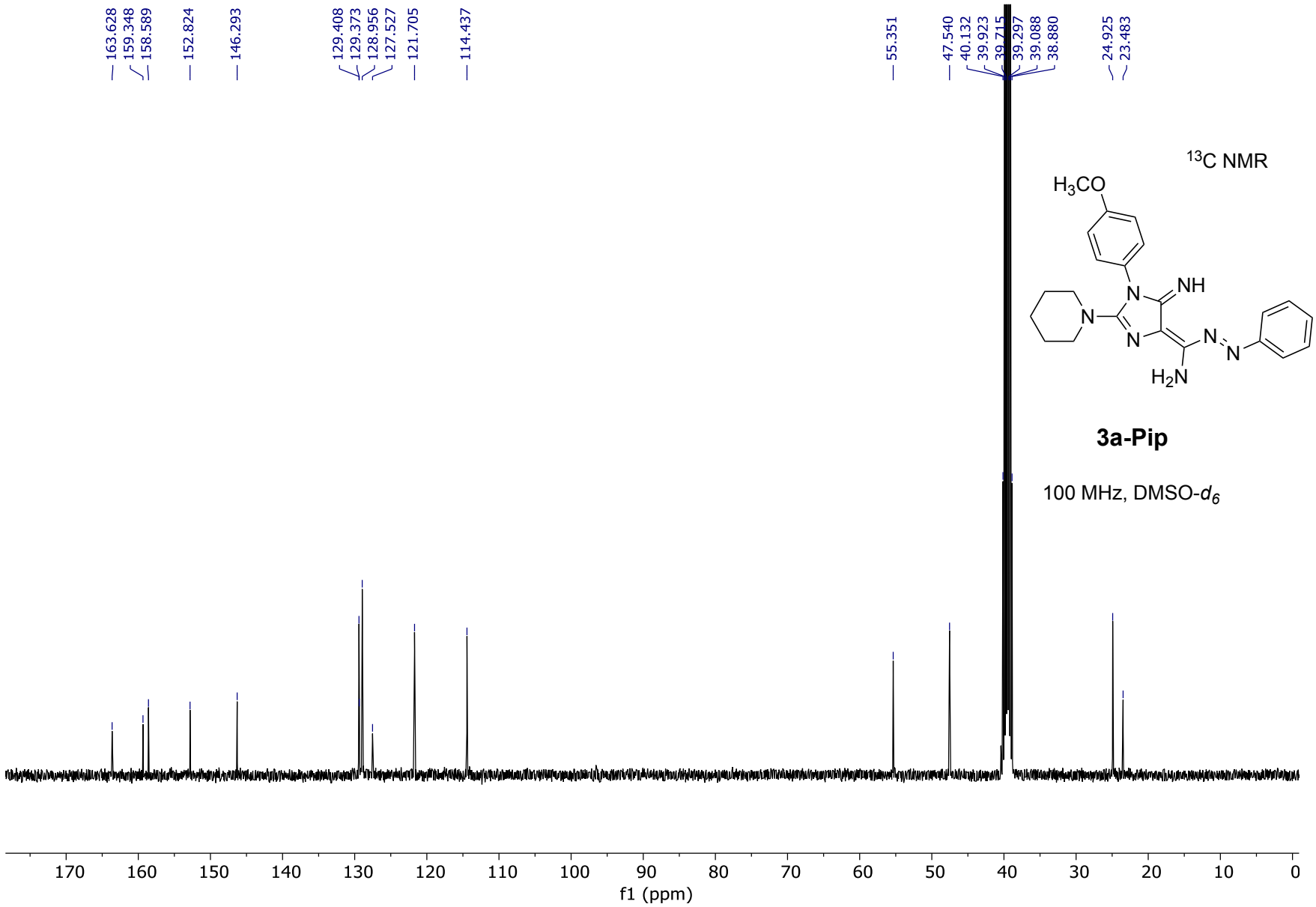
^{1}H NMR

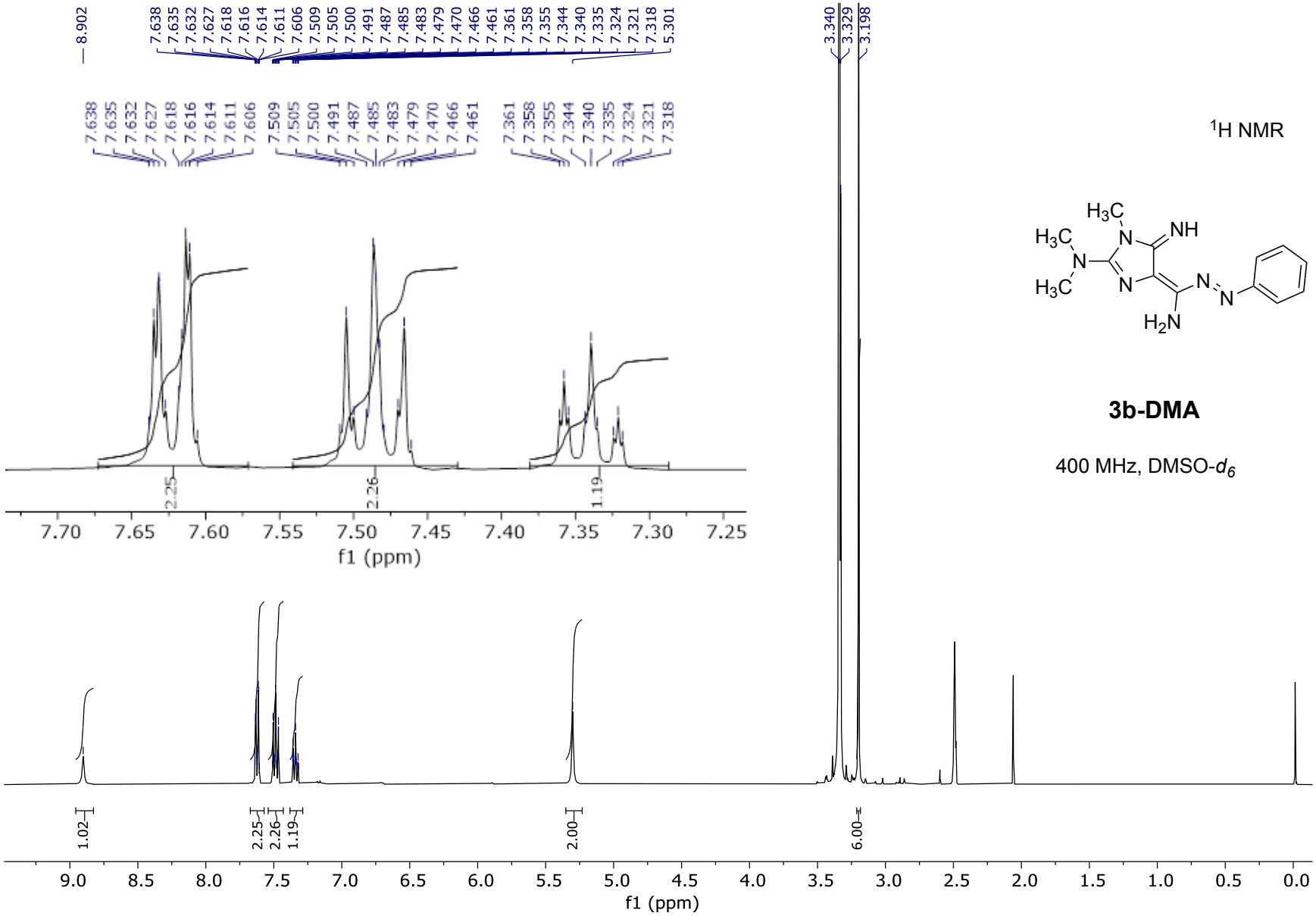
3a-DMA

400 MHz, DMSO-d_6


163.93
159.92
158.73
152.87
146.11
129.79
129.39
129.20
128.80
127.88
121.64
114.36
55.35
40.13
39.92
39.71
39.50
39.33
39.30
39.09
38.88
^{13}C NMR
H_3CO
H_3C
N
H_3C
N
N
NH
N
N
H_2N
3a-DMA
100 MHz, DMSO-d_6
80
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


^{1}H NMR
H_3CO
NH
N
N
N
N
N
H_2N
3a-Pip
400 MHz, DMSO-d_6
f1 (ppm)
f1 (ppm)

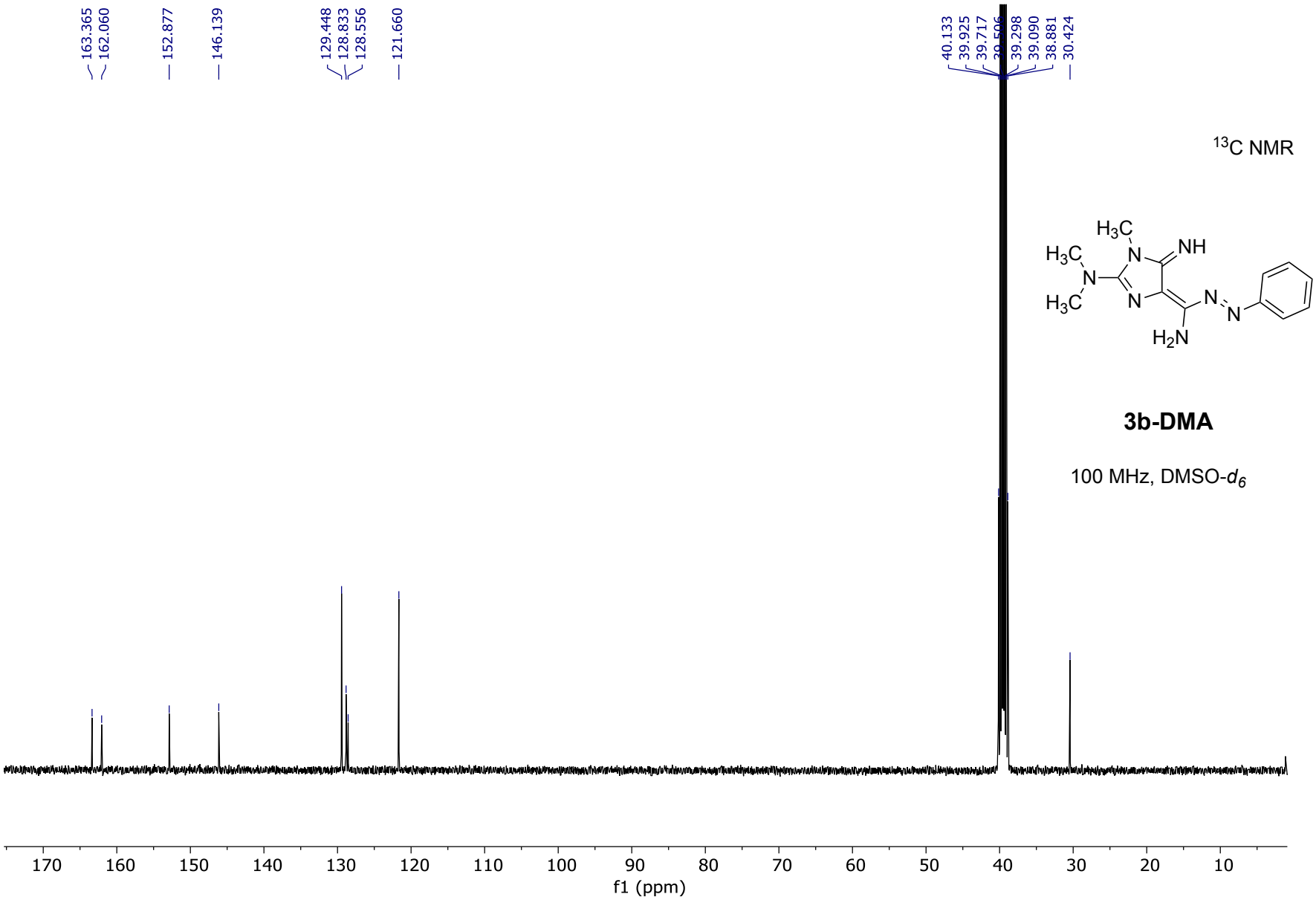

163.628
159.348
158.589
152.824
146.293
129.408
129.373
128.956
127.527
121.705
114.437
55.351
47.540
40.132
39.923
39.715
39.297
39.088
38.880
24.925
23.483
^{13}C NMR
H_3CO
NH
N
N
N
N
N
H_2N
3a-Pip
100 MHz, DMSO-d_6
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

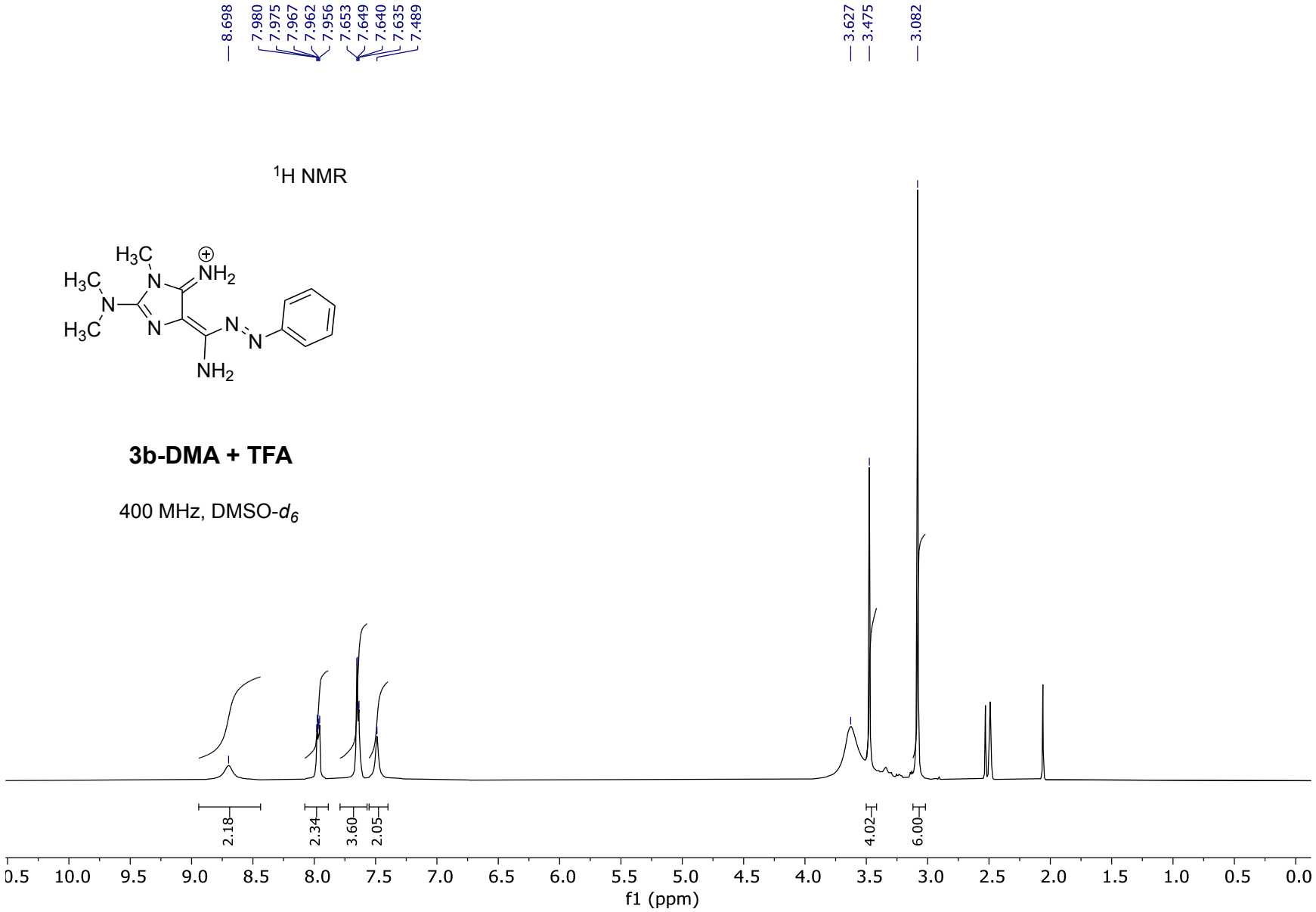


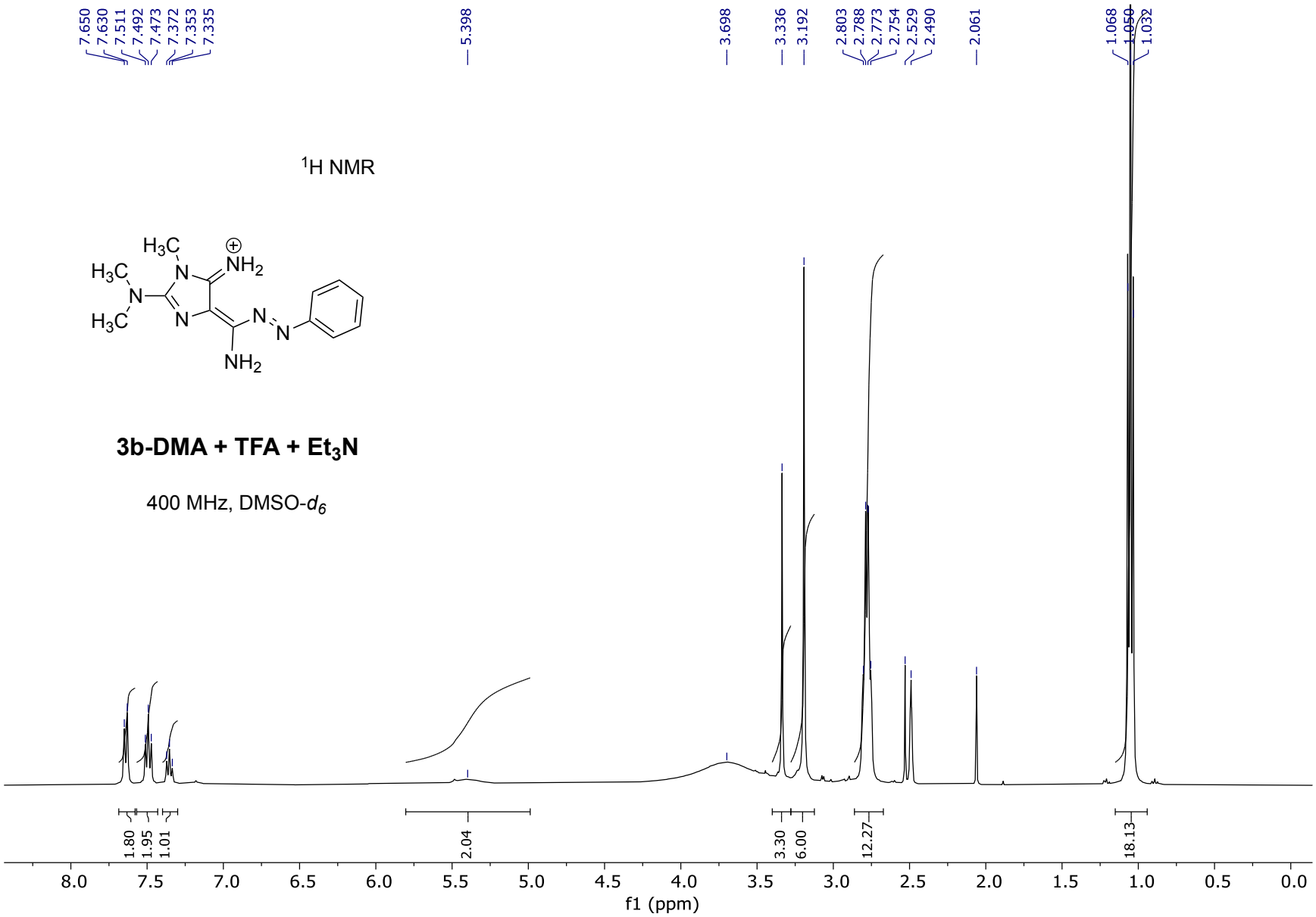
^{1}H NMR

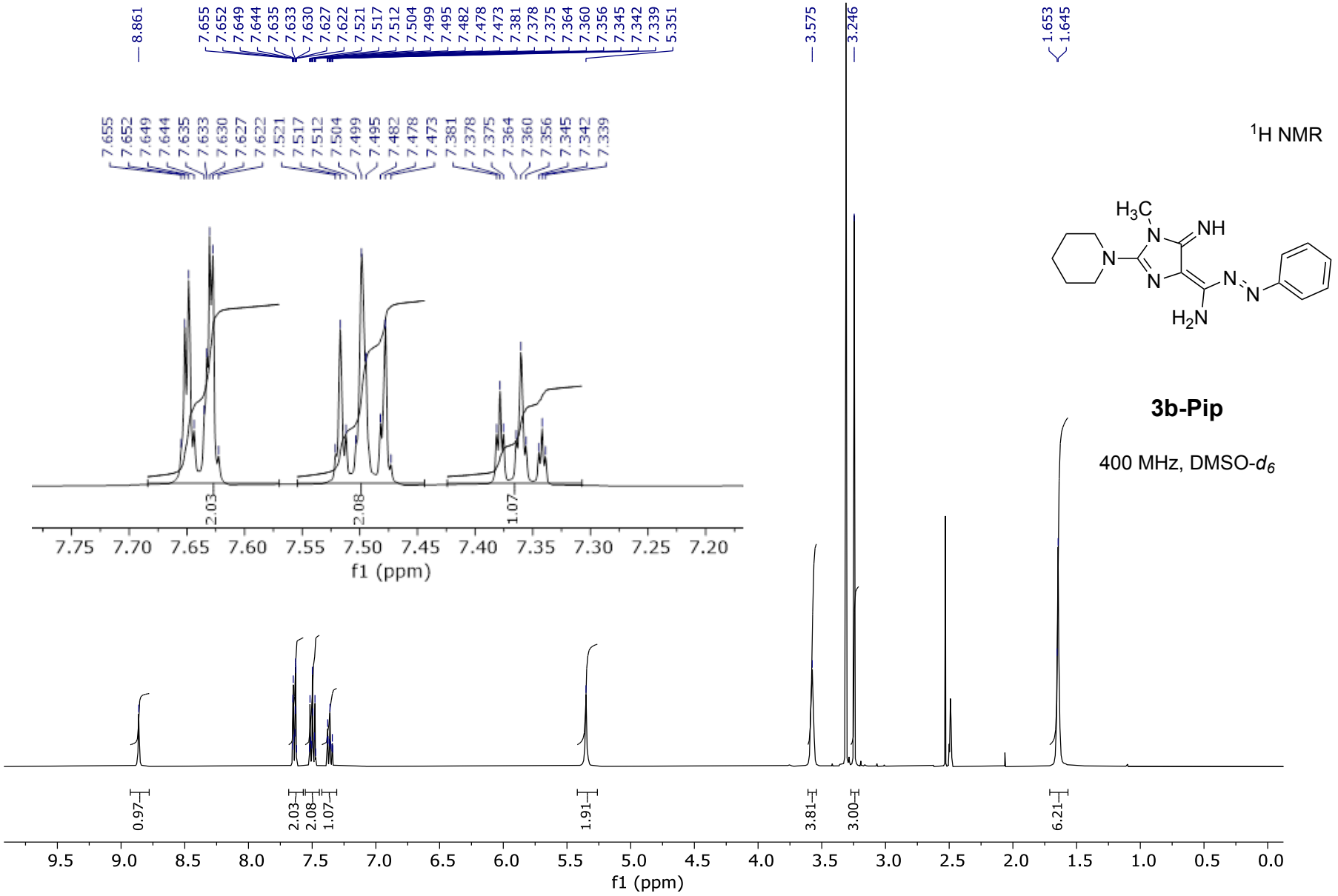
3b-DMA

400 MHz, DMSO-d_6


163.365
162.060
152.877
146.139
129.448
128.833
128.556
121.660
40.133
39.925
39.717
39.506
39.298
39.090
38.881
30.424
^{13}C NMR
H_3C
N
NH
H_3C
N
N
H_3C
N
N
H_2N
3b-DMA
100 MHz, DMSO-d_6
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


8.698
7.980
7.975
7.967
7.962
7.956
7.653
7.649
7.640
7.635
7.489
3.627
3.475
3.082
^{1}H NMR
H_3C
H_3C
H_3C
N
N
N
N
NH_2
NH_2
N
3b-DMA + TFA
400 MHz, DMSO-d_6
2.18
2.34
3.60
2.05
4.02
6.00
f1 (ppm)

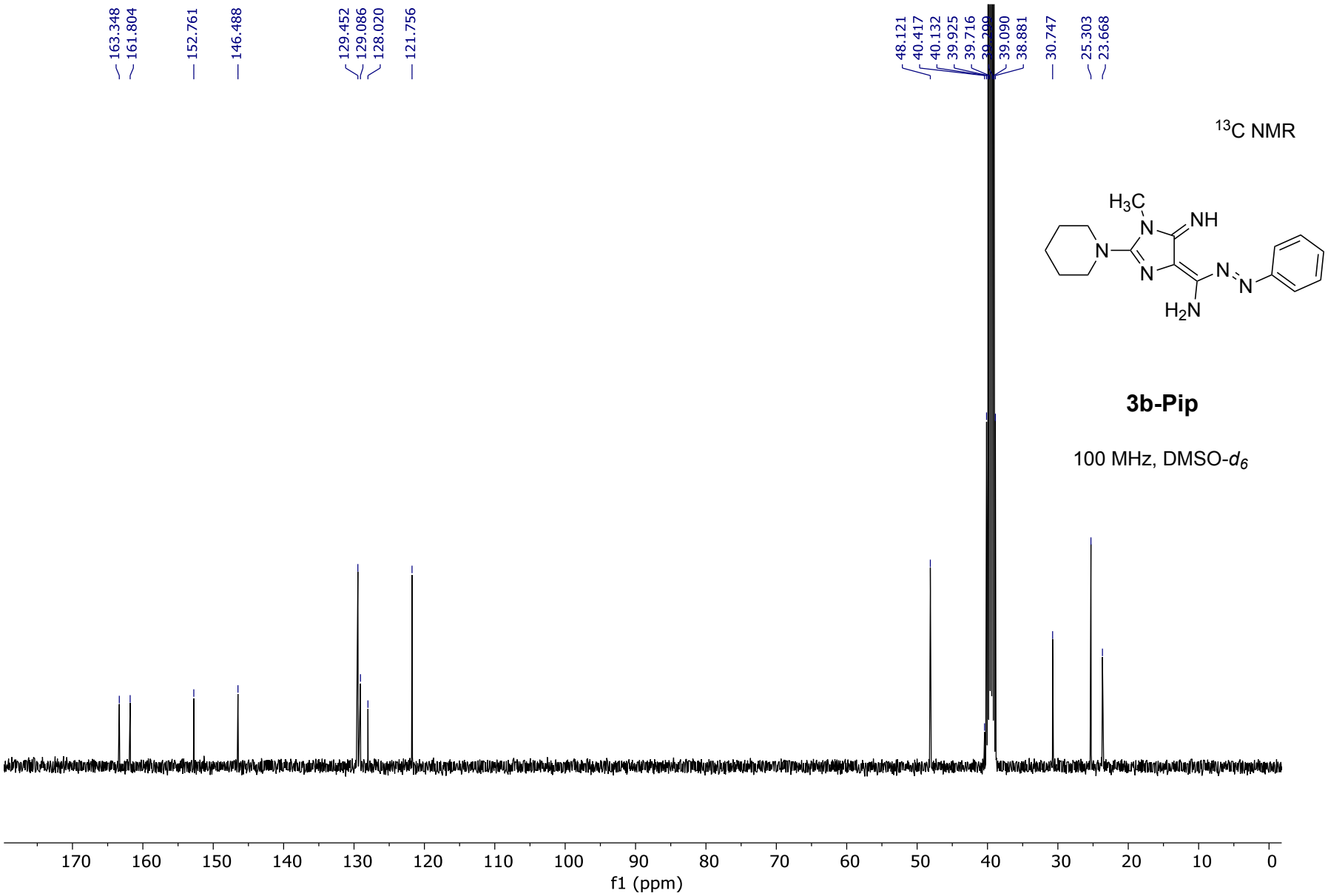

7.650
7.630
7.511
7.492
7.473
7.372
7.353
7.335
5.398
3.698
3.336
3.192
2.803
2.788
2.773
2.754
2.529
2.490
2.061
1.068
1.050
1.032
^{1}H NMR
H_3C
NH_2
H_3C
N
N
H_3C
N
N
N
NH_2
3b-DMA + TFA + Et_3N
400 MHz, DMSO-d_6
1.80
1.95
1.01
2.04
3.30
6.00
12.27
18.13
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

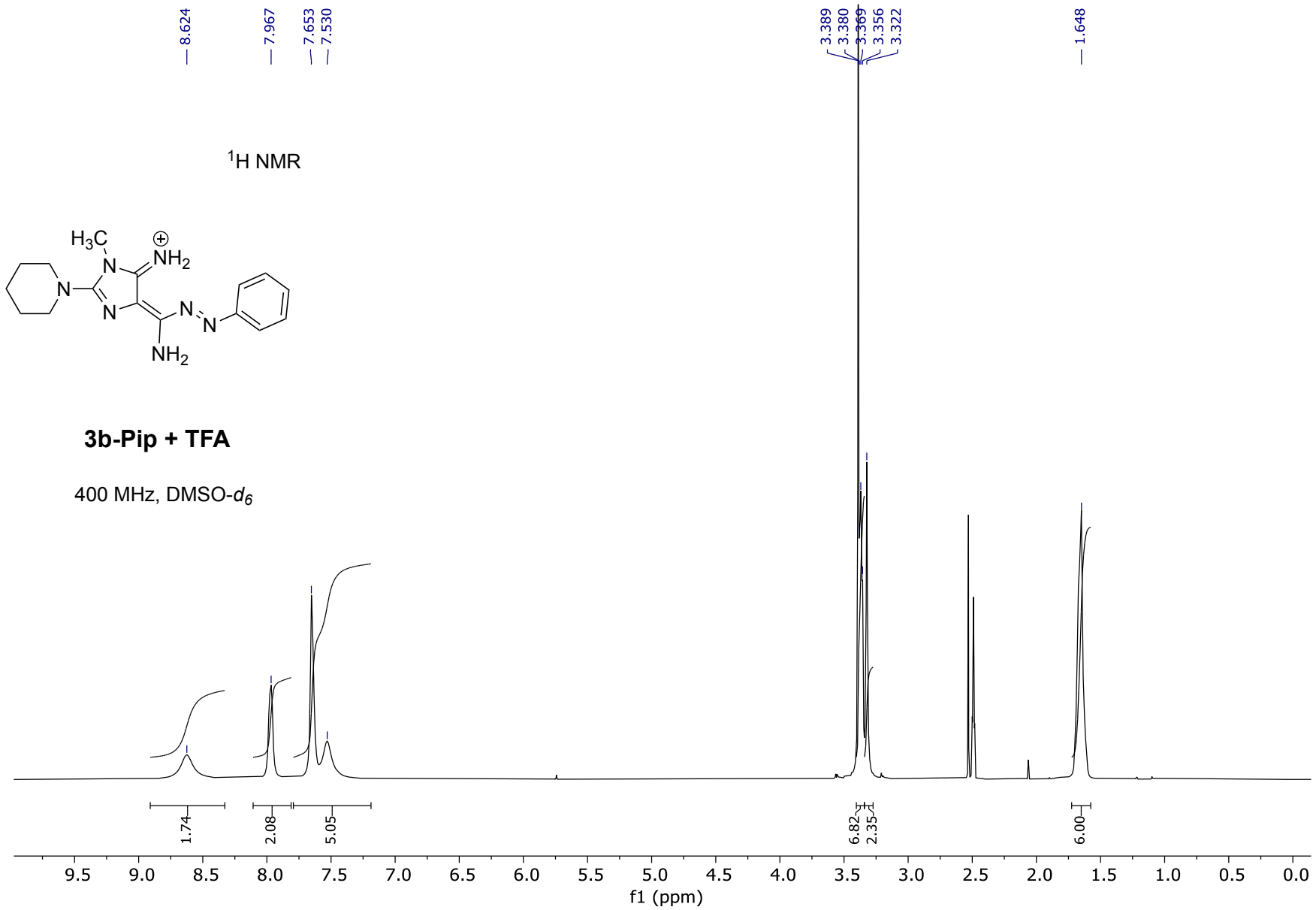


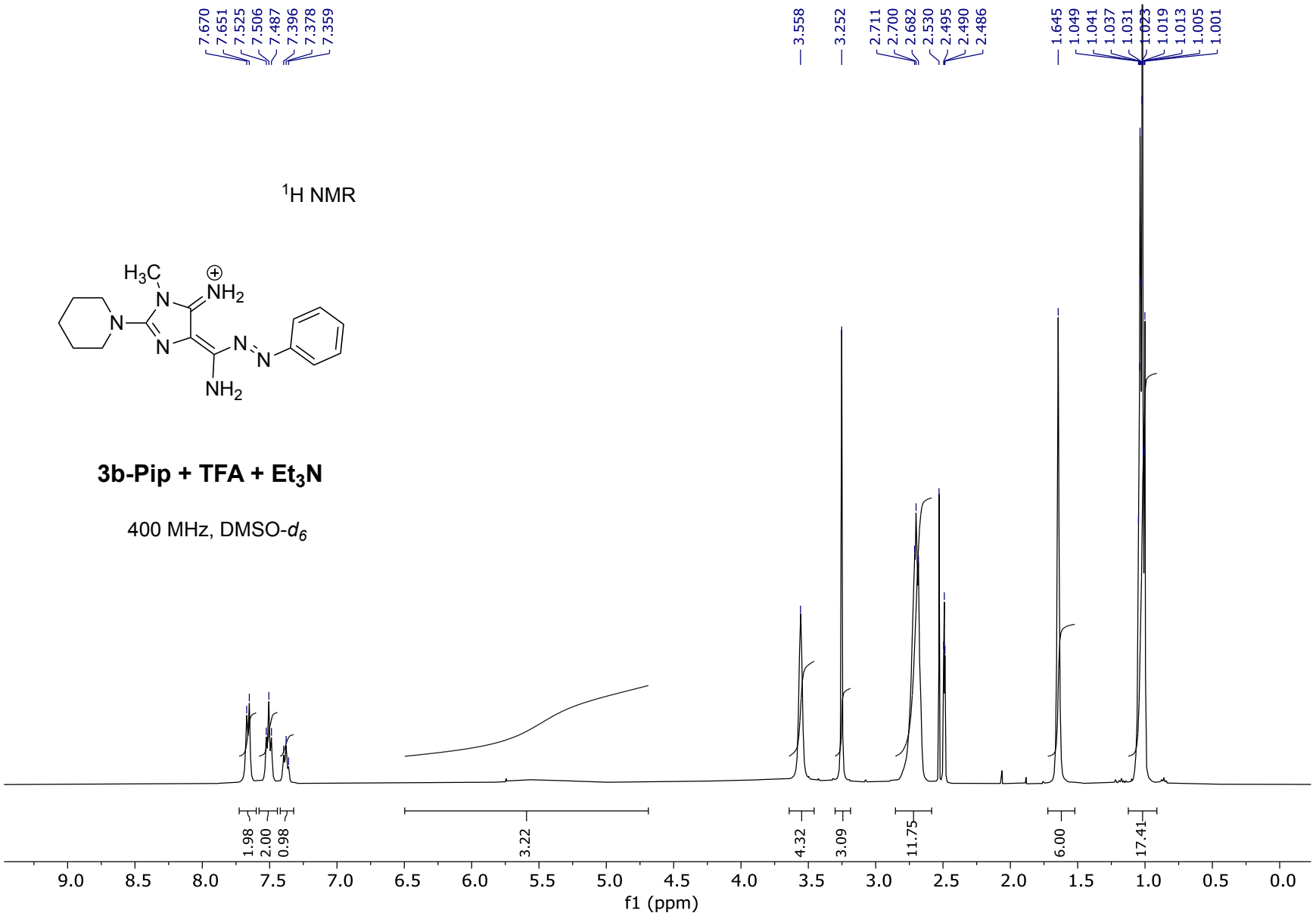
^{1}H NMR

3b-Pip

400 MHz, DMSO-d_6

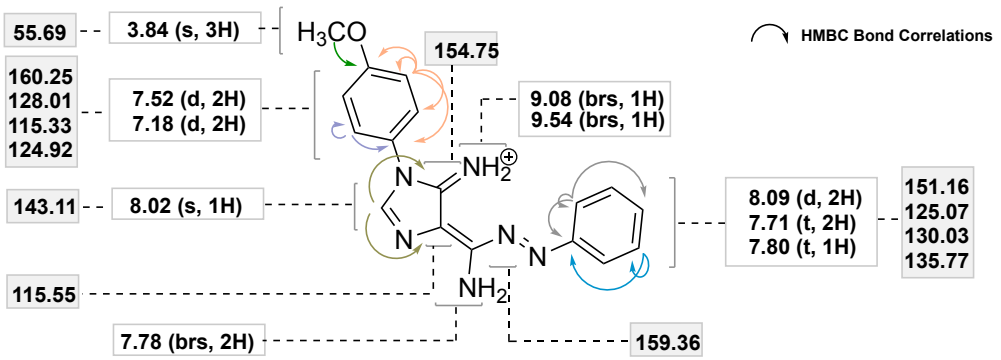

163.348
161.804
152.761
146.488
129.452
129.086
128.020
121.756
48.121
40.417
40.132
39.925
39.716
39.090
38.881
30.747
25.303
23.668
^{13}C NMR
H_3C
N
NH
N
N
N
N
H_2N
3b-Pip
100 MHz, DMSO-d_6
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

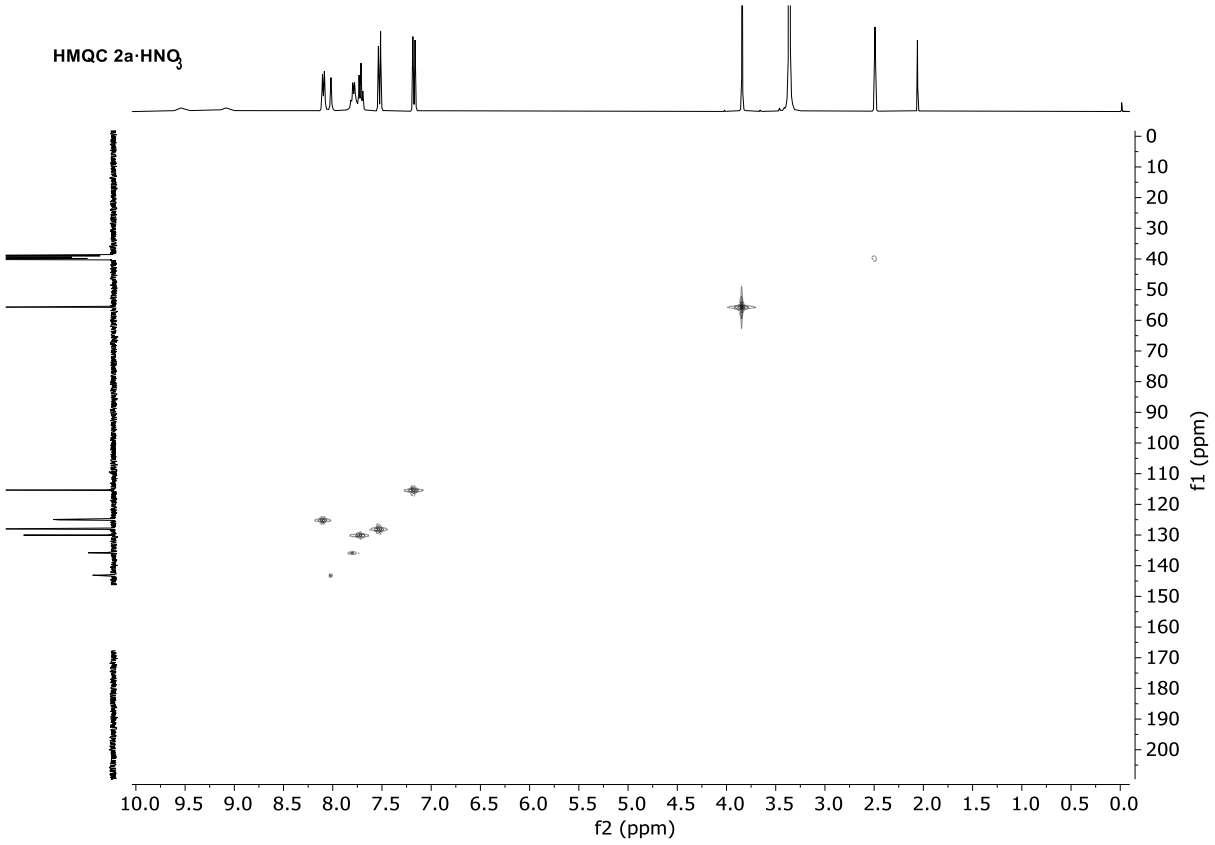

8.624
7.967
7.653
7.530
3.389
3.380
3.369
3.356
3.322
1.648
^{1}H NMR
H_3C
N
NH_2
N
N
N
N
NH_2
3b-Pip + TFA
400 MHz, DMSO-d_6
1.74
2.08
5.05
6.82
2.35
6.00
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

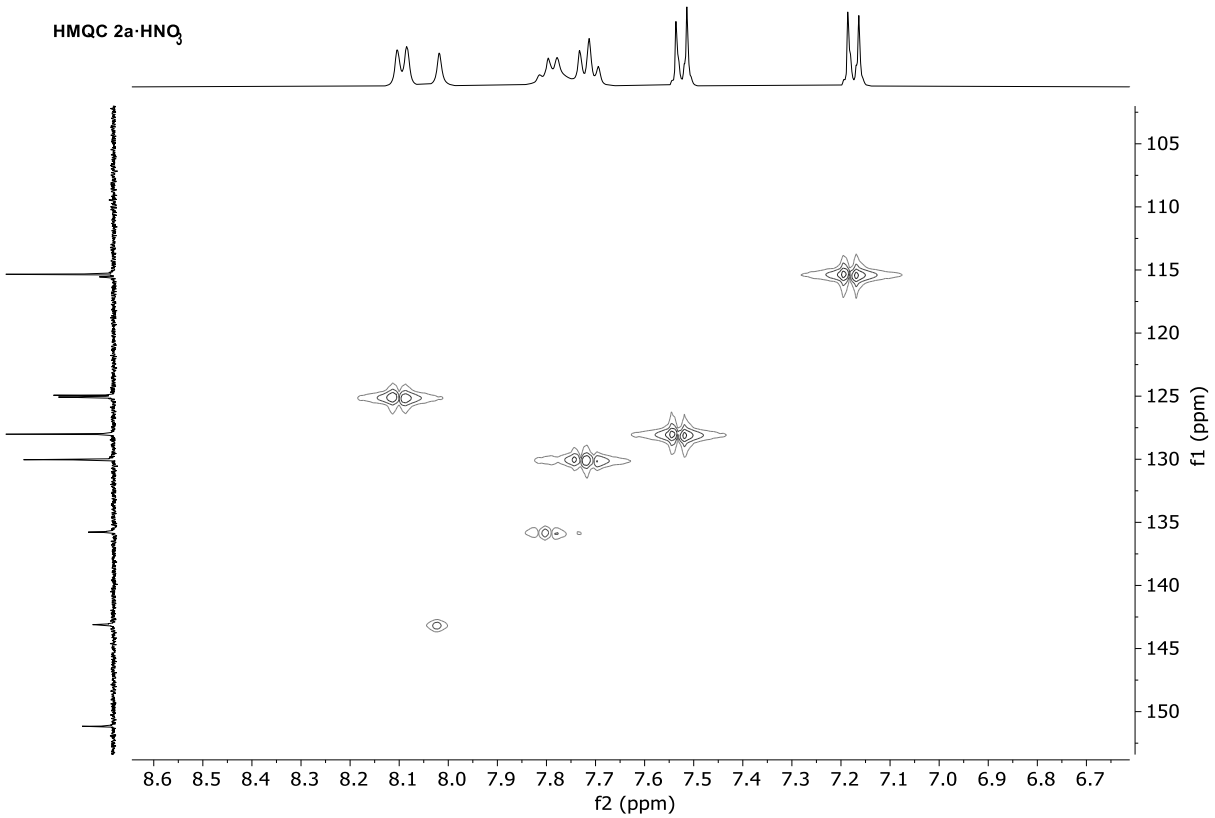

7.670
7.651
7.525
7.506
7.487
7.396
7.378
7.359
3.558
3.252
2.711
2.700
2.682
2.530
2.495
2.490
2.486
1.645
1.049
1.041
1.037
1.031
1.023
1.019
1.013
1.005
1.001
1H NMR
H3C
NH2
N
N
N
N
N
NH2
3b-Pip + TFA + Et3N
400 MHz, DMSO-d6
1.98
2.00
0.98
3.22
4.32
3.09
11.75
6.00
17.41
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

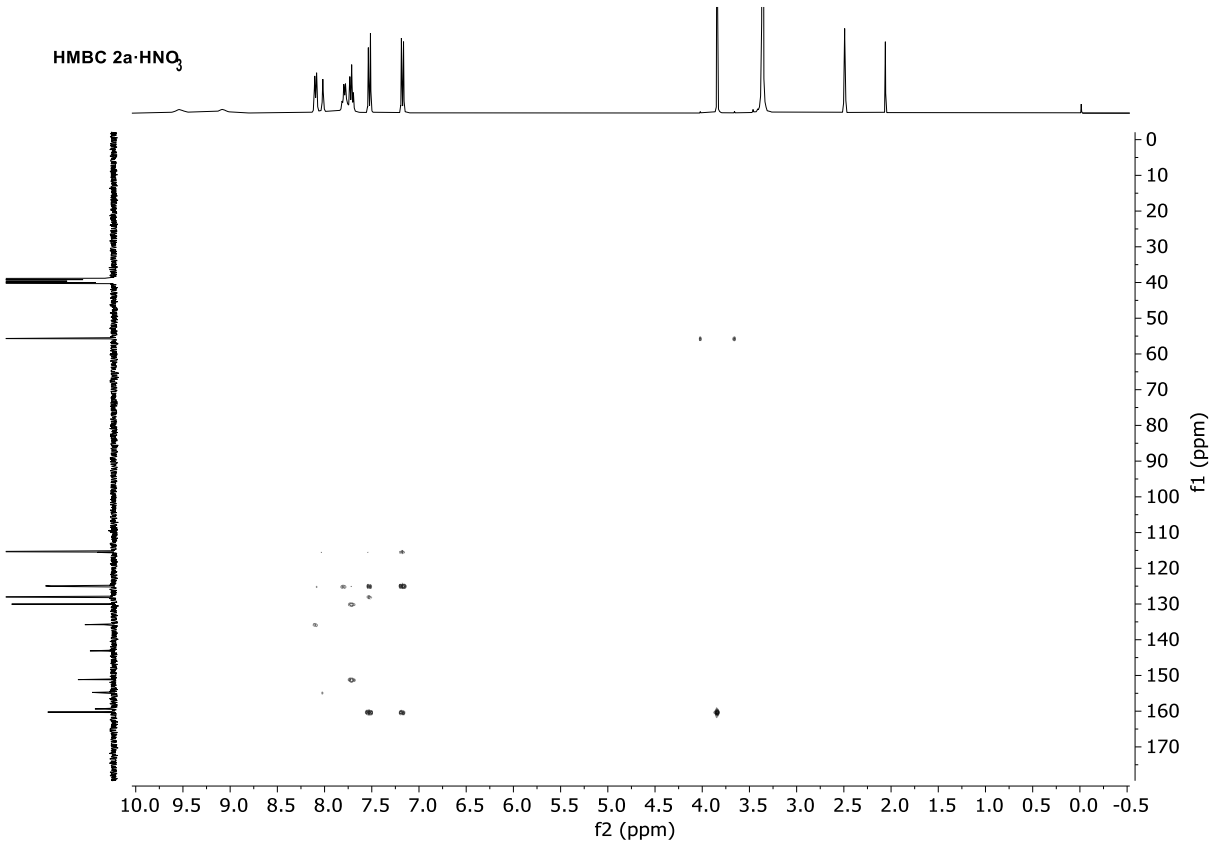
6.2. HMQC and HMBC

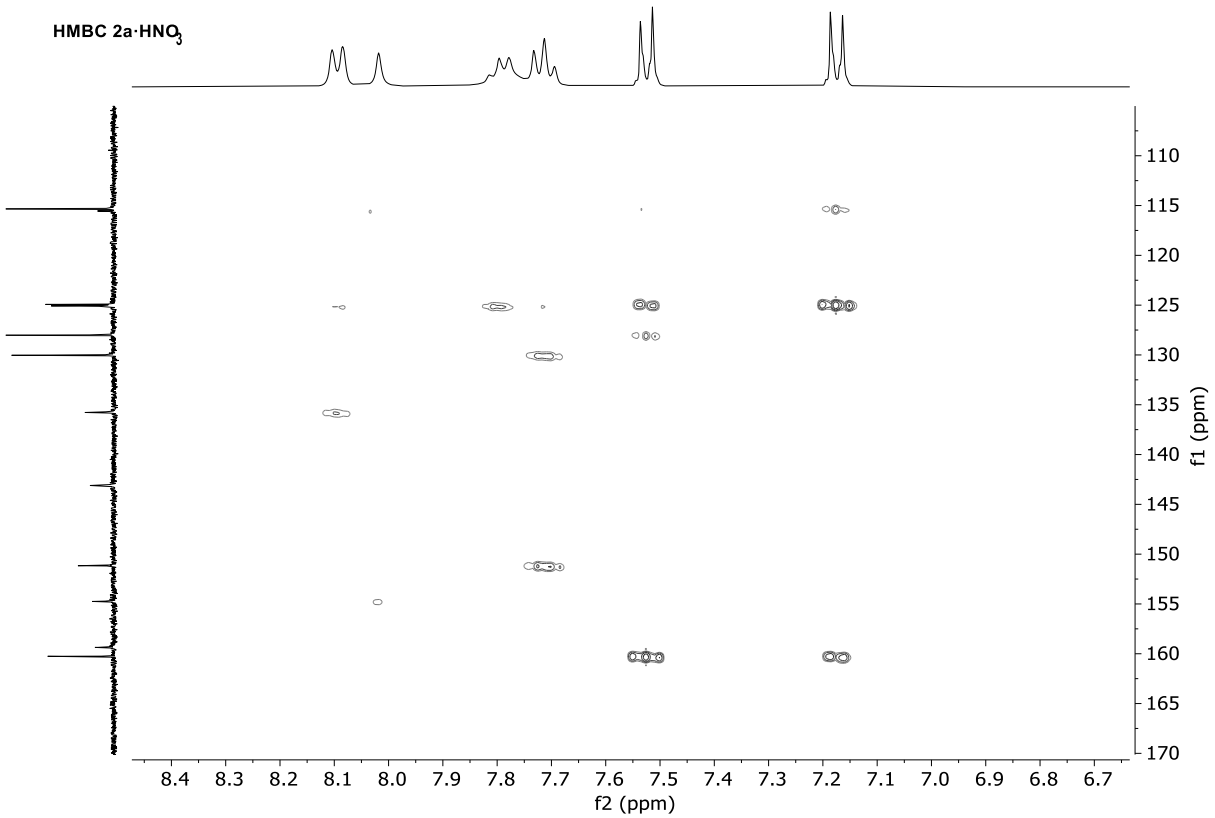
6.2.1. Compound 2a.HNO_3



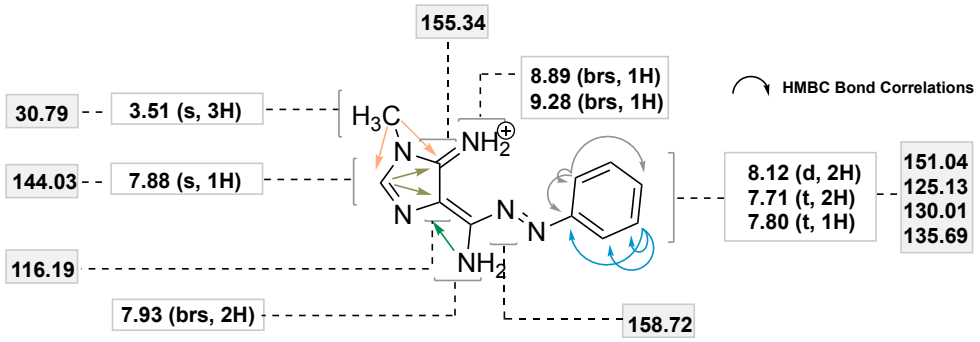


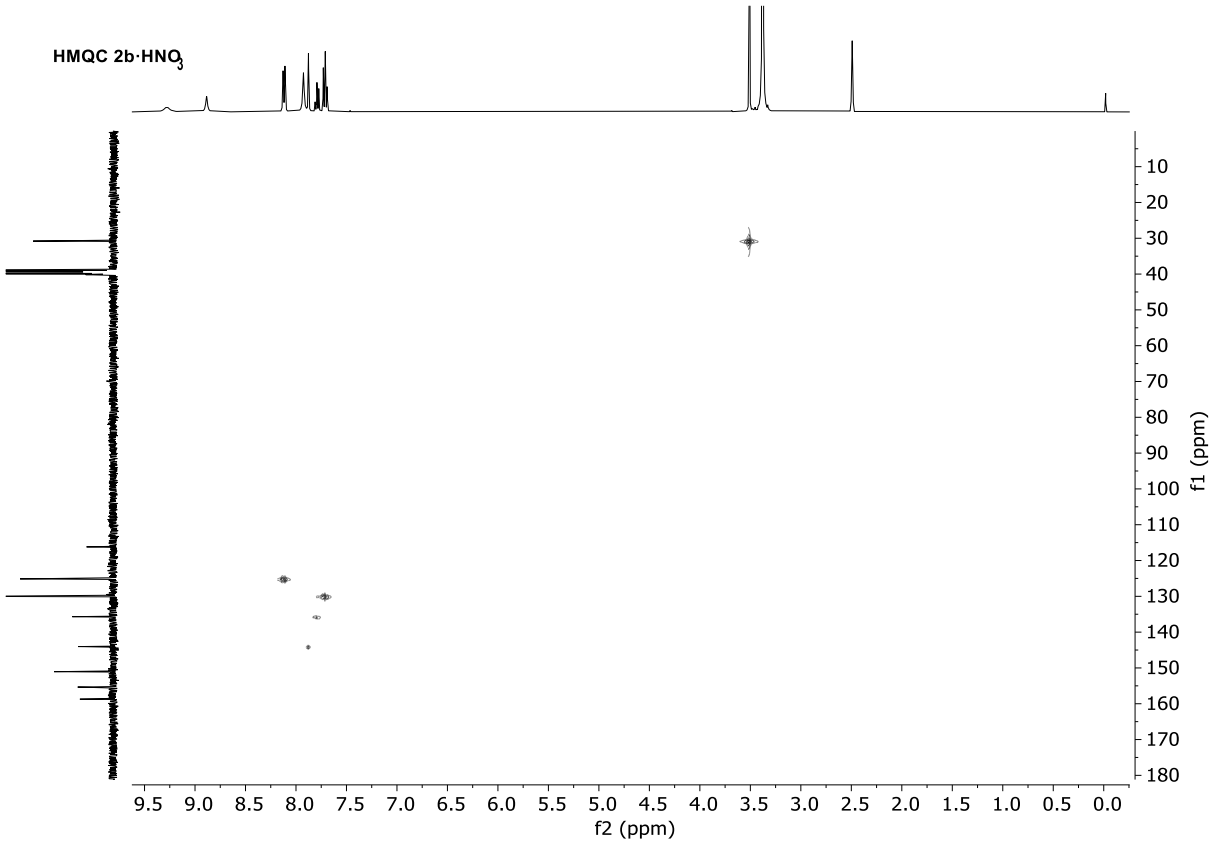

HMQC 2a·HNO3
105
110
115
120
125
130
135
140
145
150
f1 (ppm)
8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7
f2 (ppm)

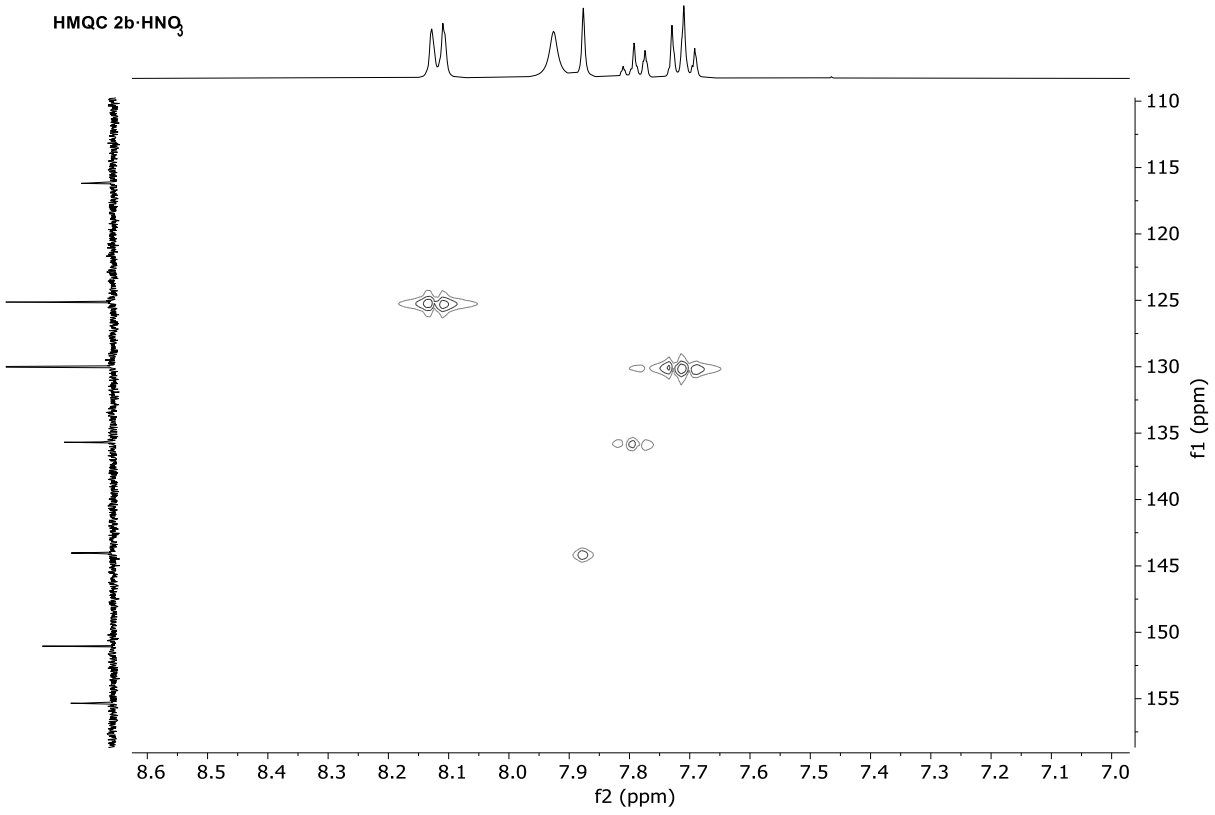

HMBC 2a·HNO3
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
f1 (ppm)
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f2 (ppm)

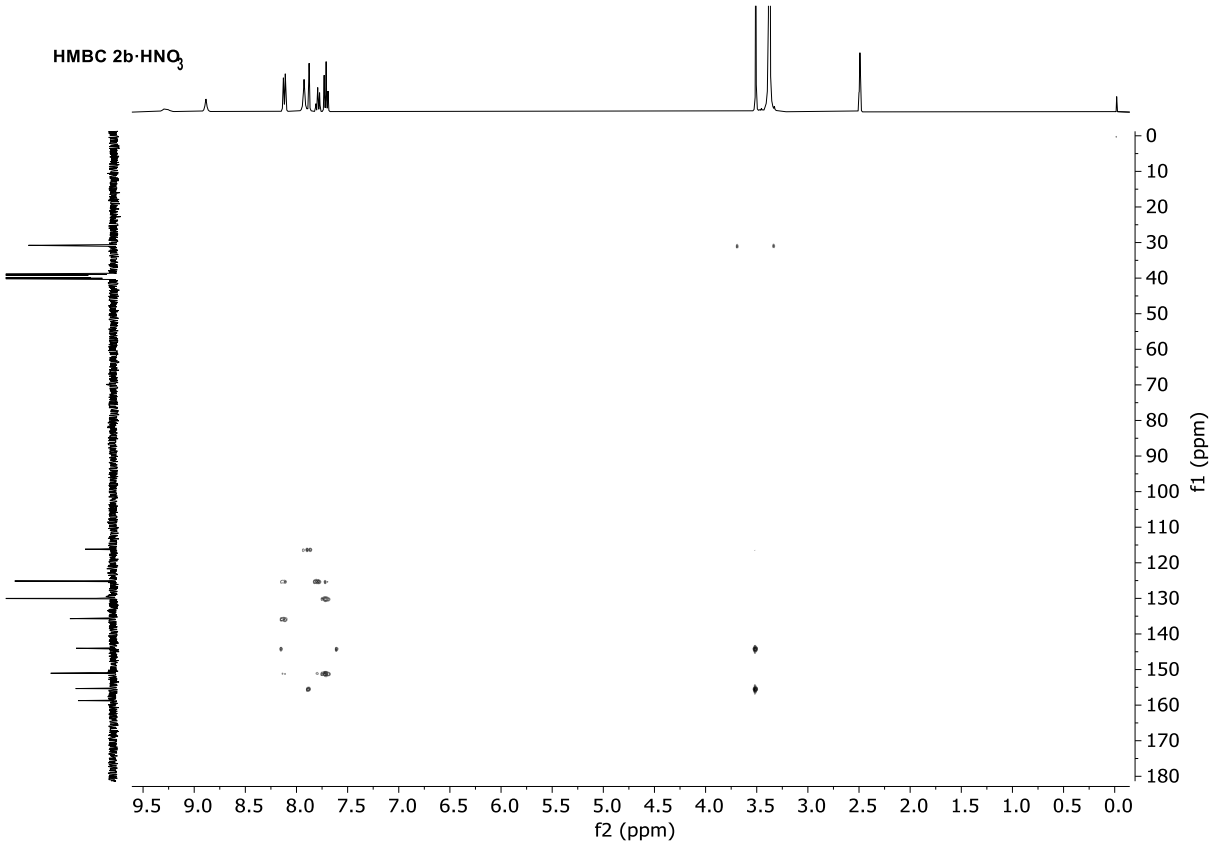


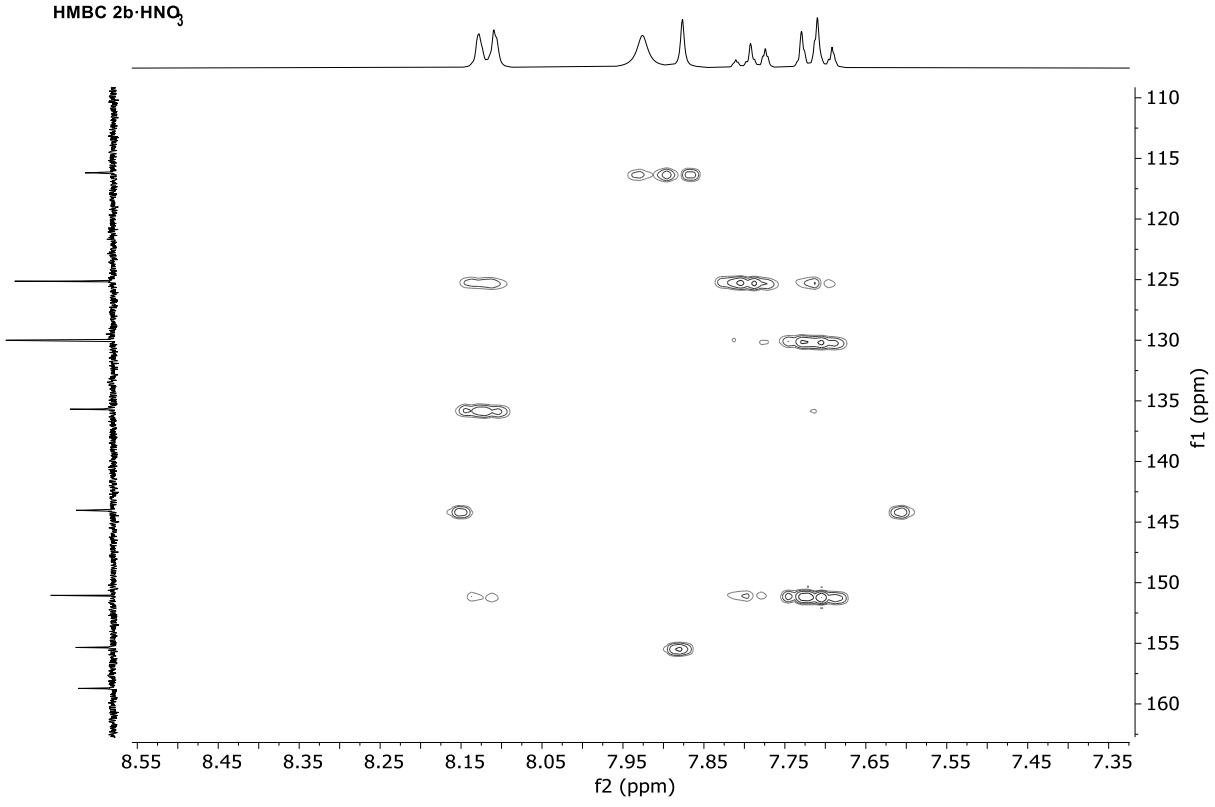
6.2.2. Compound 2b.HNO_3

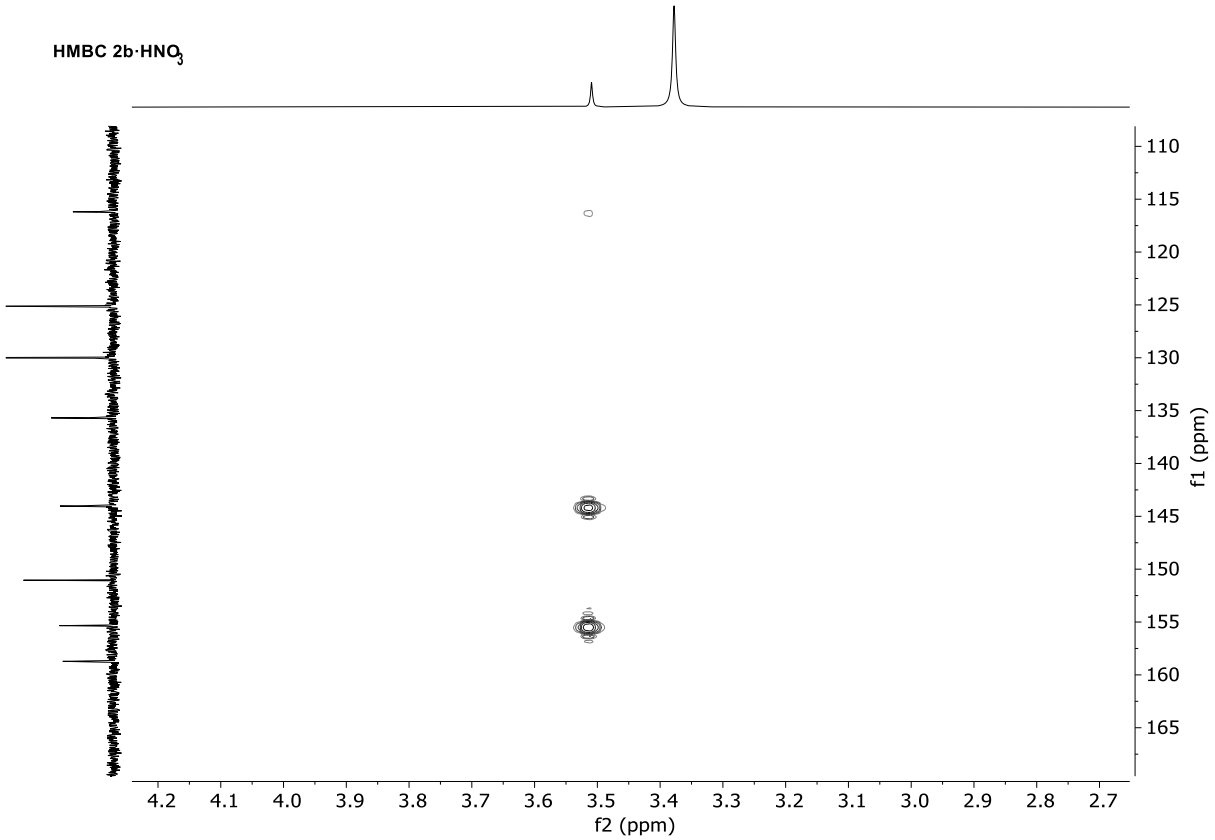



HMQC 2b·HNO3
f2 (ppm)
f1 (ppm)

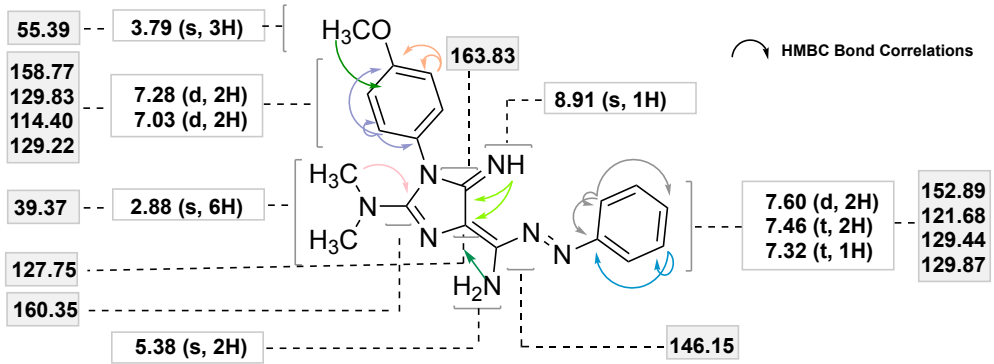

HMQC 2b·HNO3
f2 (ppm)
f1 (ppm)

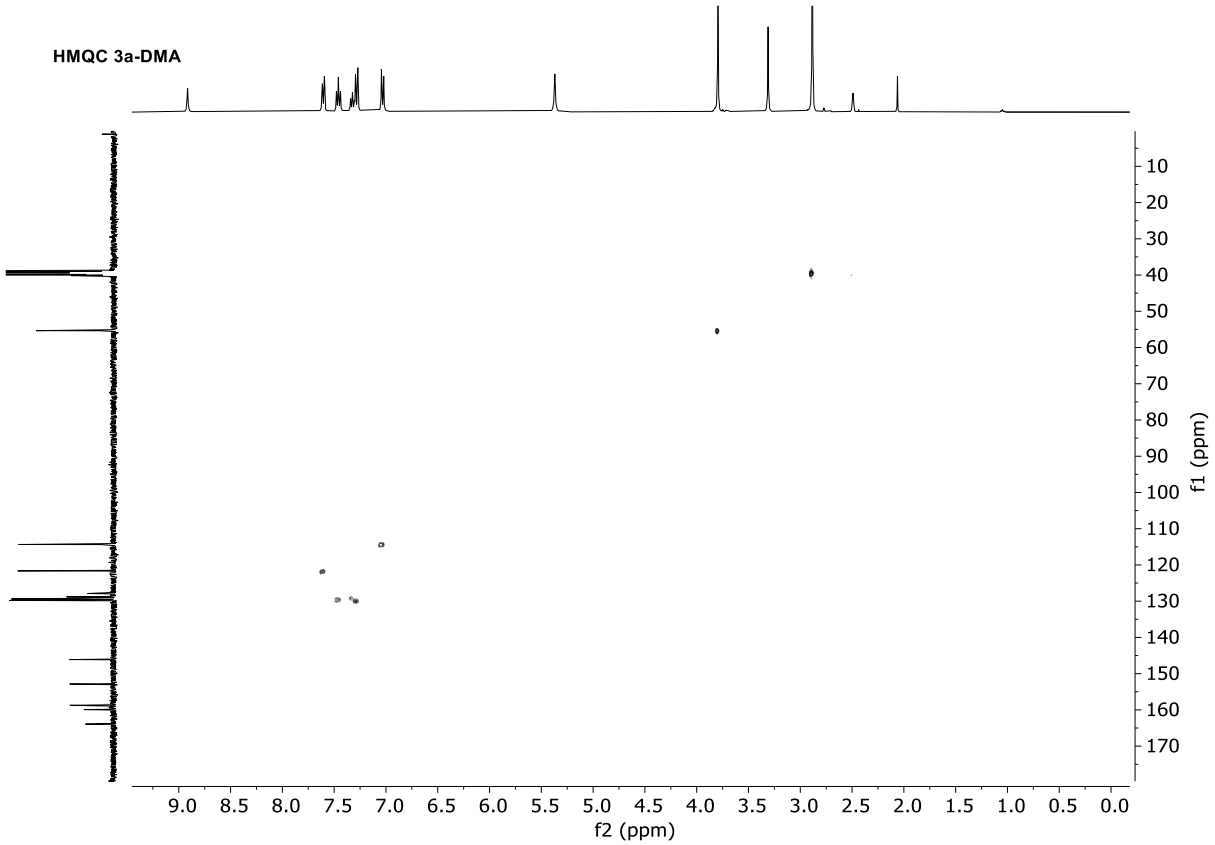

HMBC 2b·HNO3
f1 (ppm)
f2 (ppm)

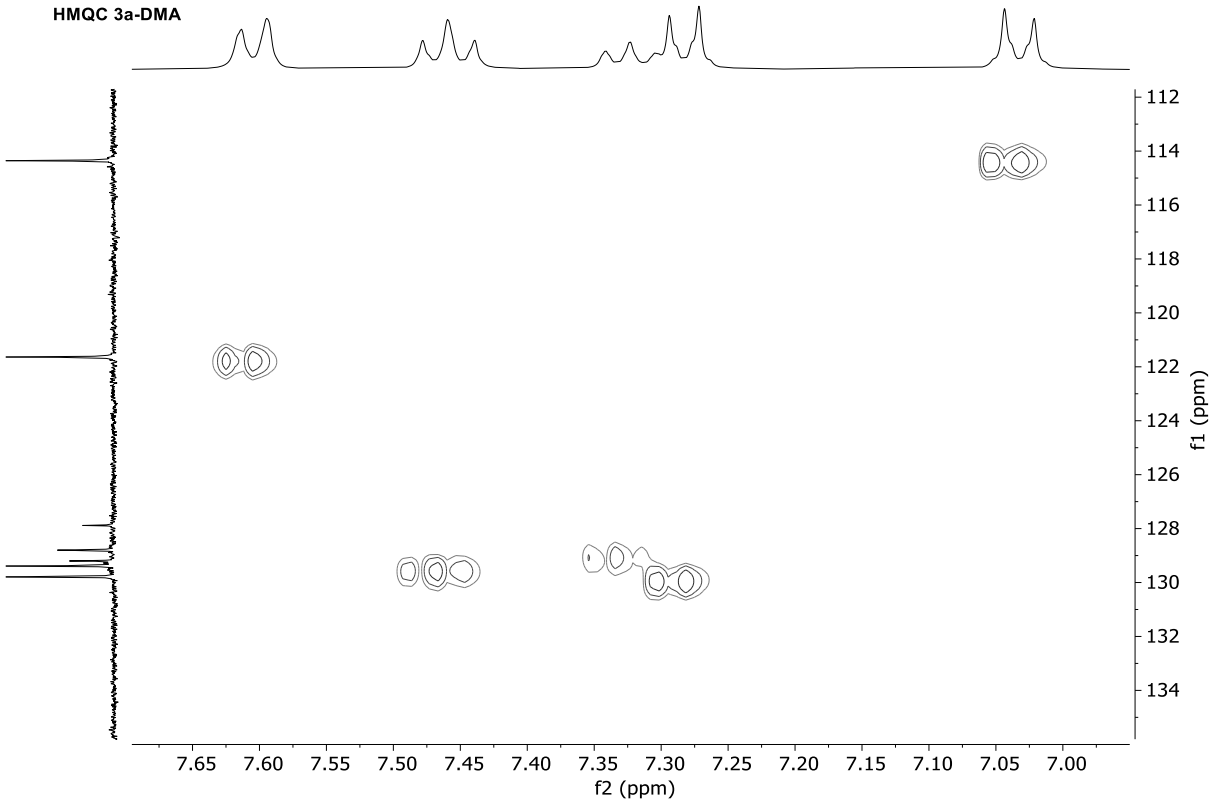

HMBC 2b·HNO3
f1 (ppm)
f2 (ppm)

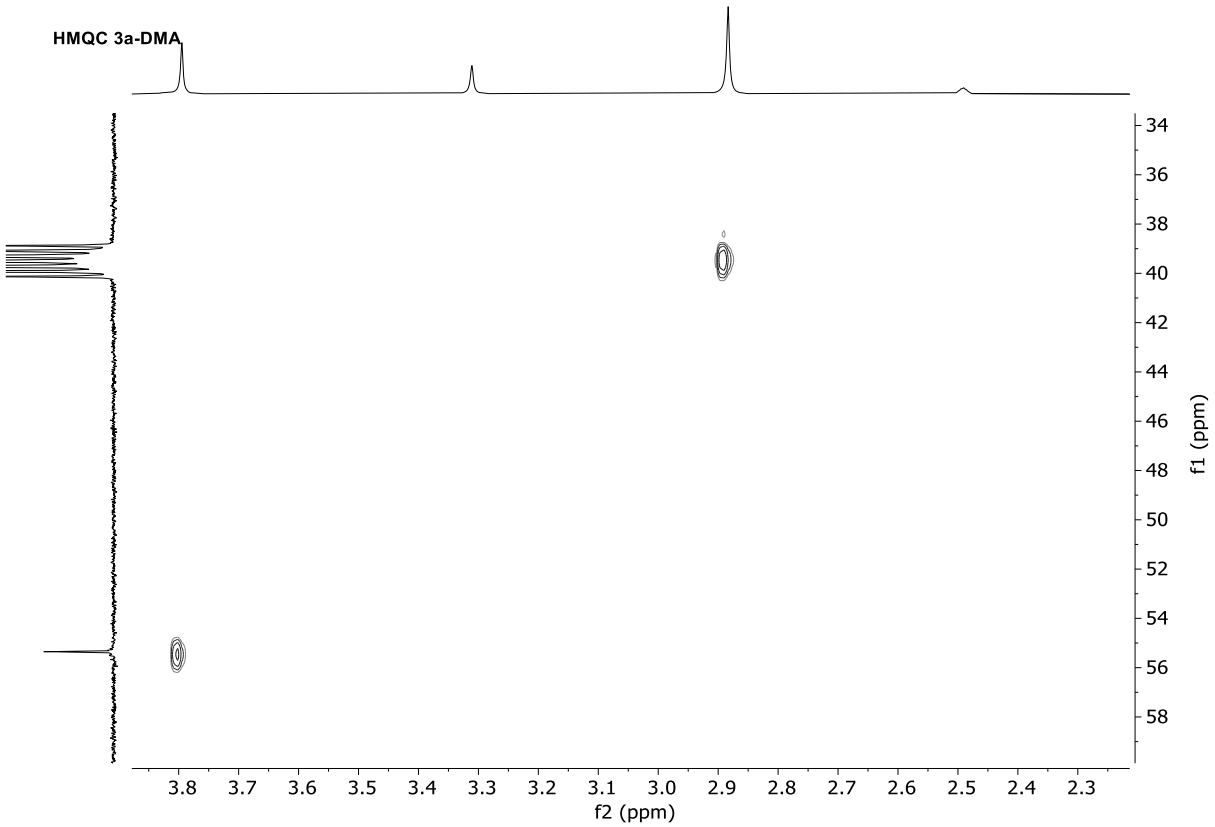


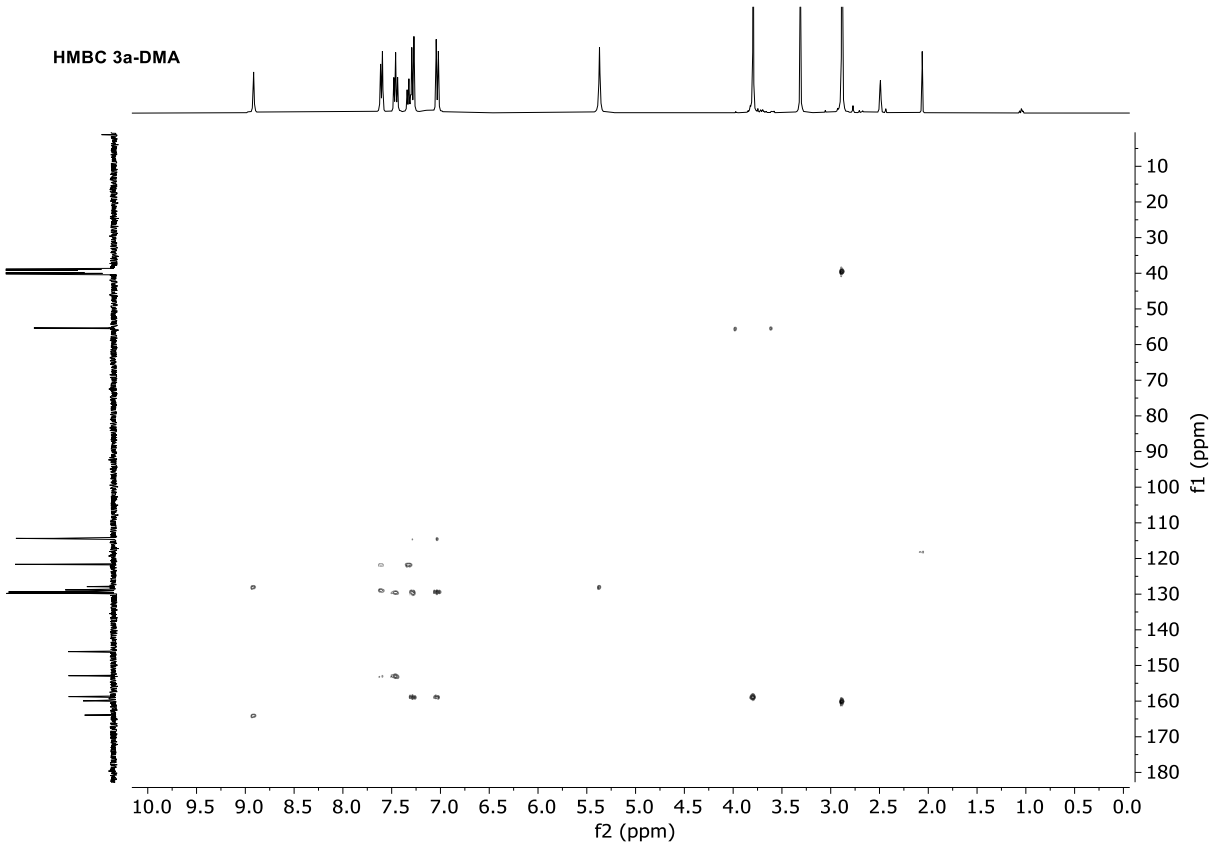
6.2.3. Compound 3a-DMA

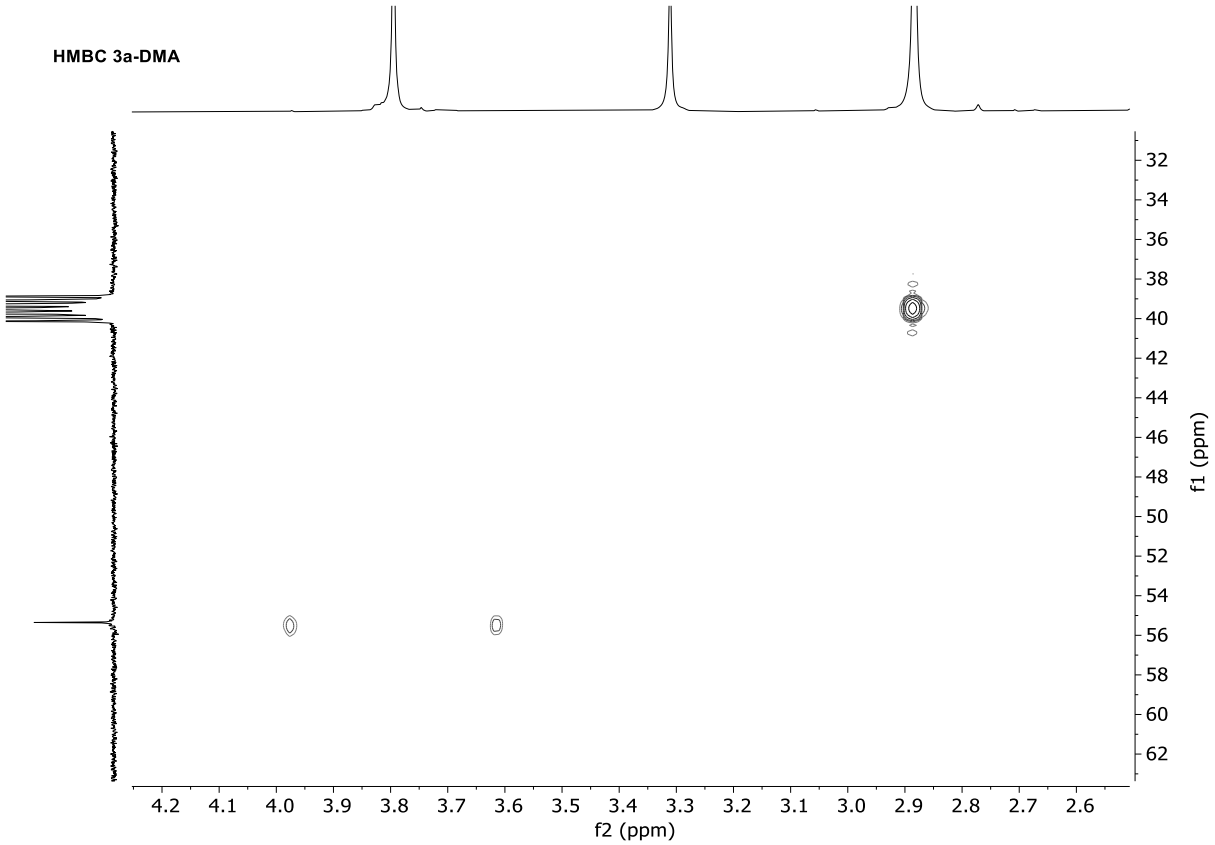


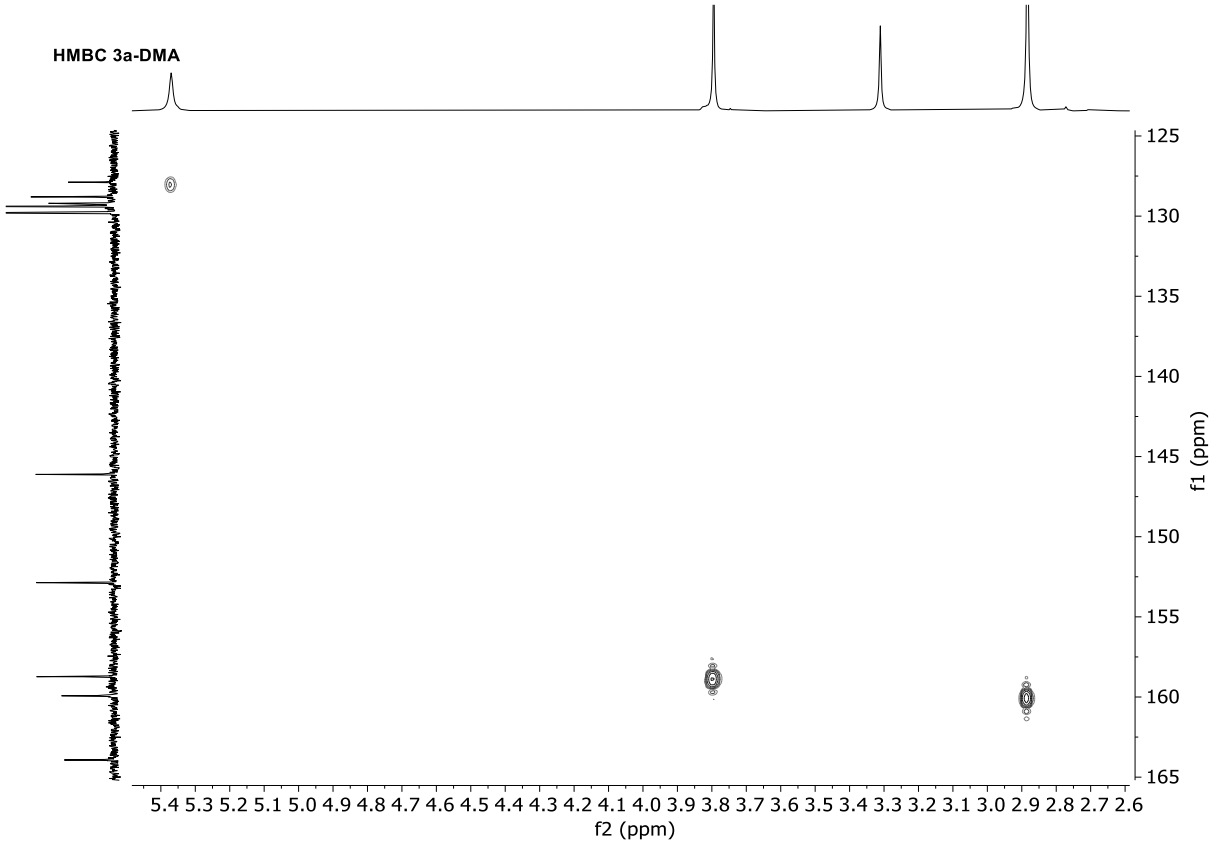

HMQC 3a-DMA
f1 (ppm)
f2 (ppm)

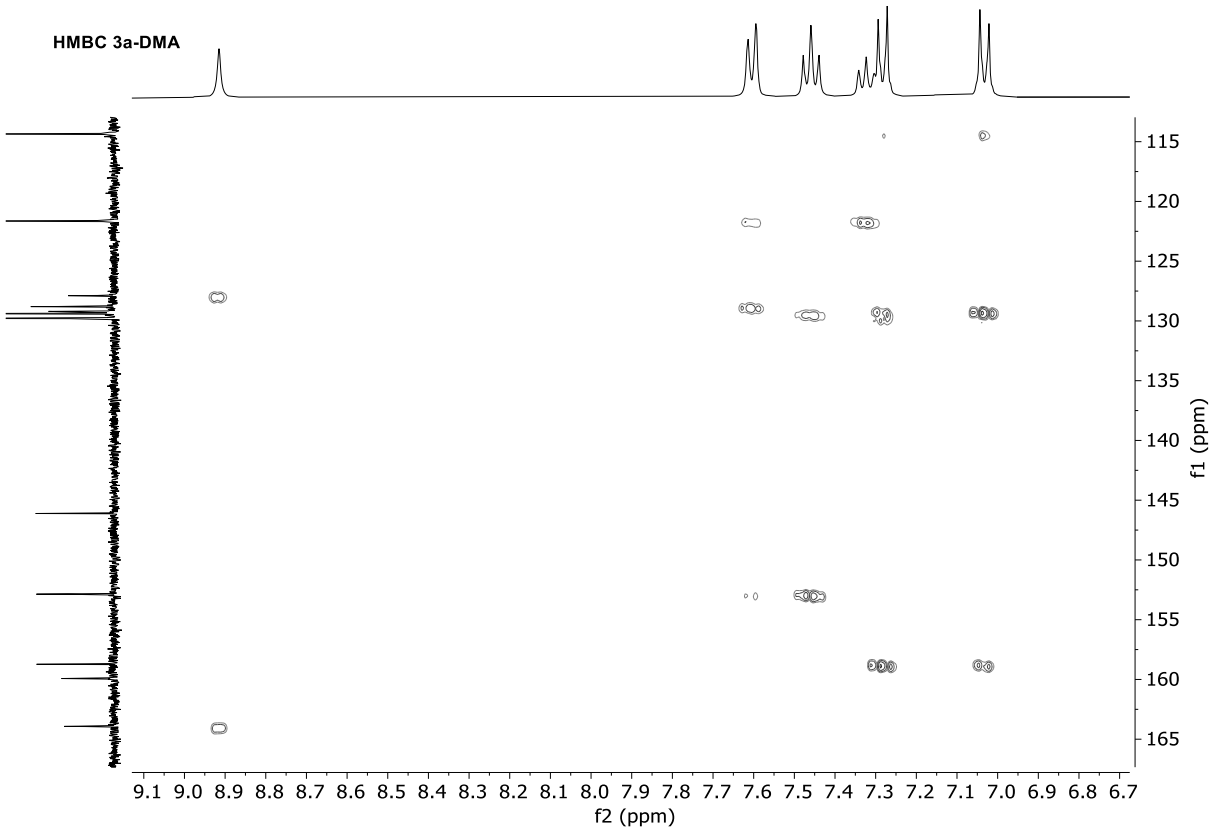

HMQC 3a-DMA
f1 (ppm)
f2 (ppm)


HMQC 3a-DMA
f1 (ppm)
f2 (ppm)

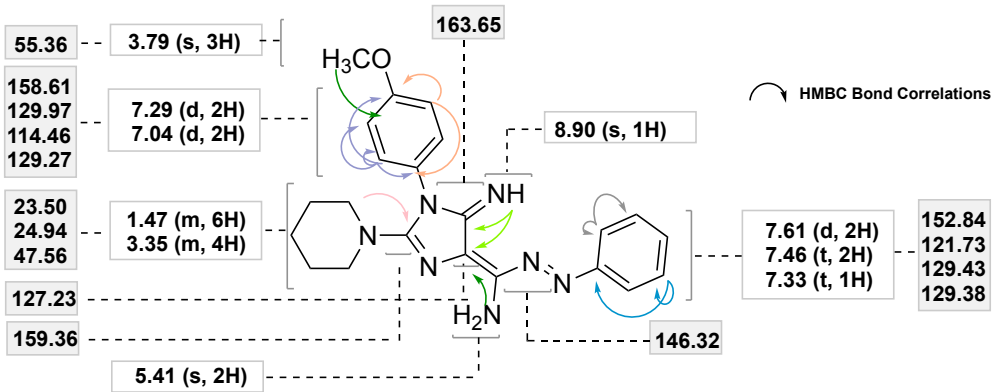

HMBC 3a-DMA
f1 (ppm)
f2 (ppm)

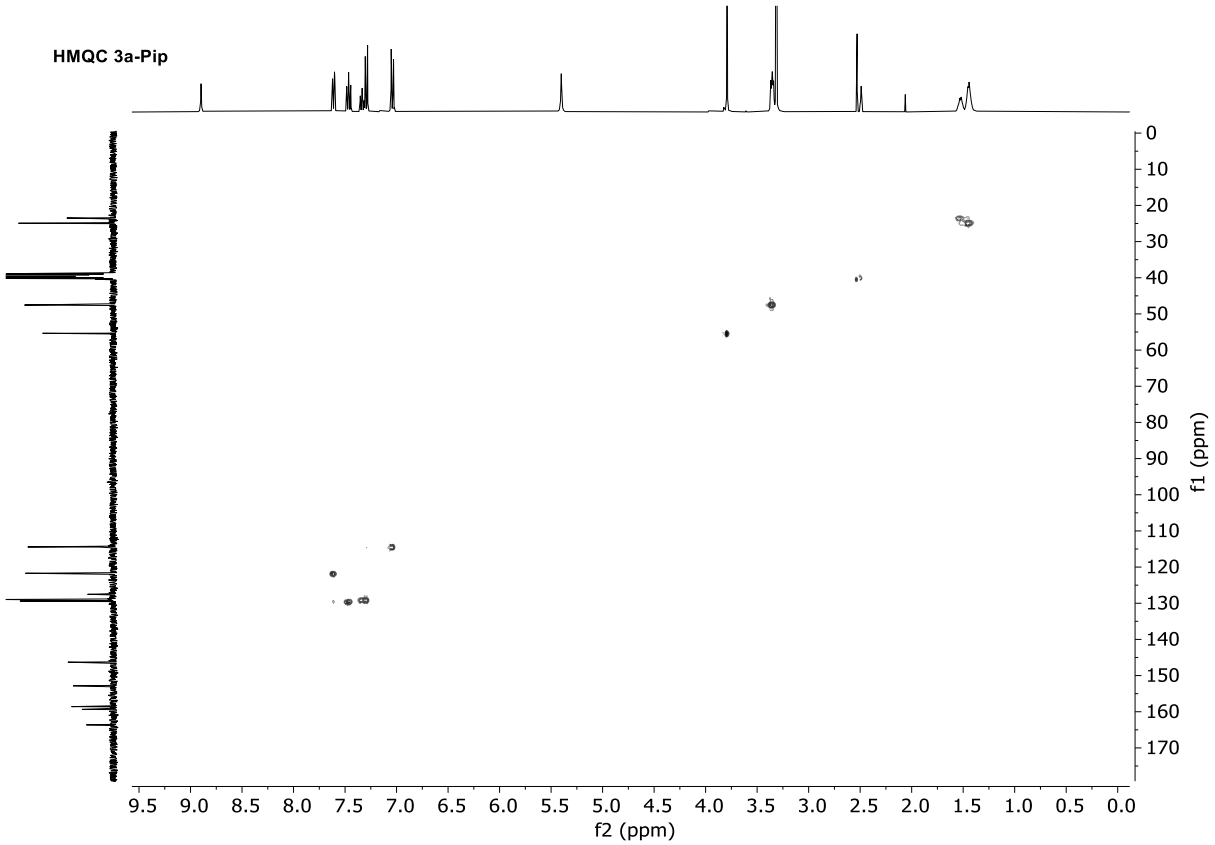

HMBC 3a-DMA
32
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
f1 (ppm)
4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6
f2 (ppm)

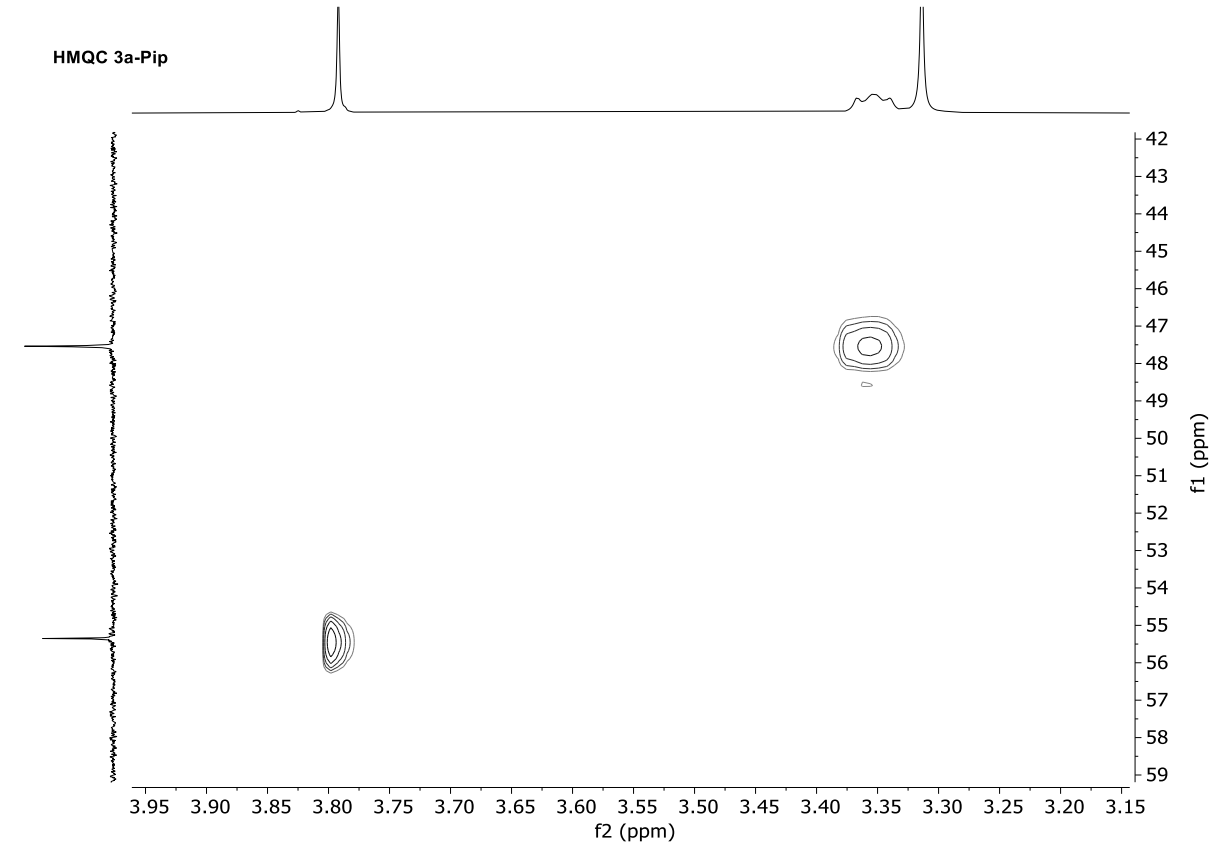

HMBC 3a-DMA
125
130
135
140
145
150
155
160
165
f1 (ppm)
5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6
f2 (ppm)

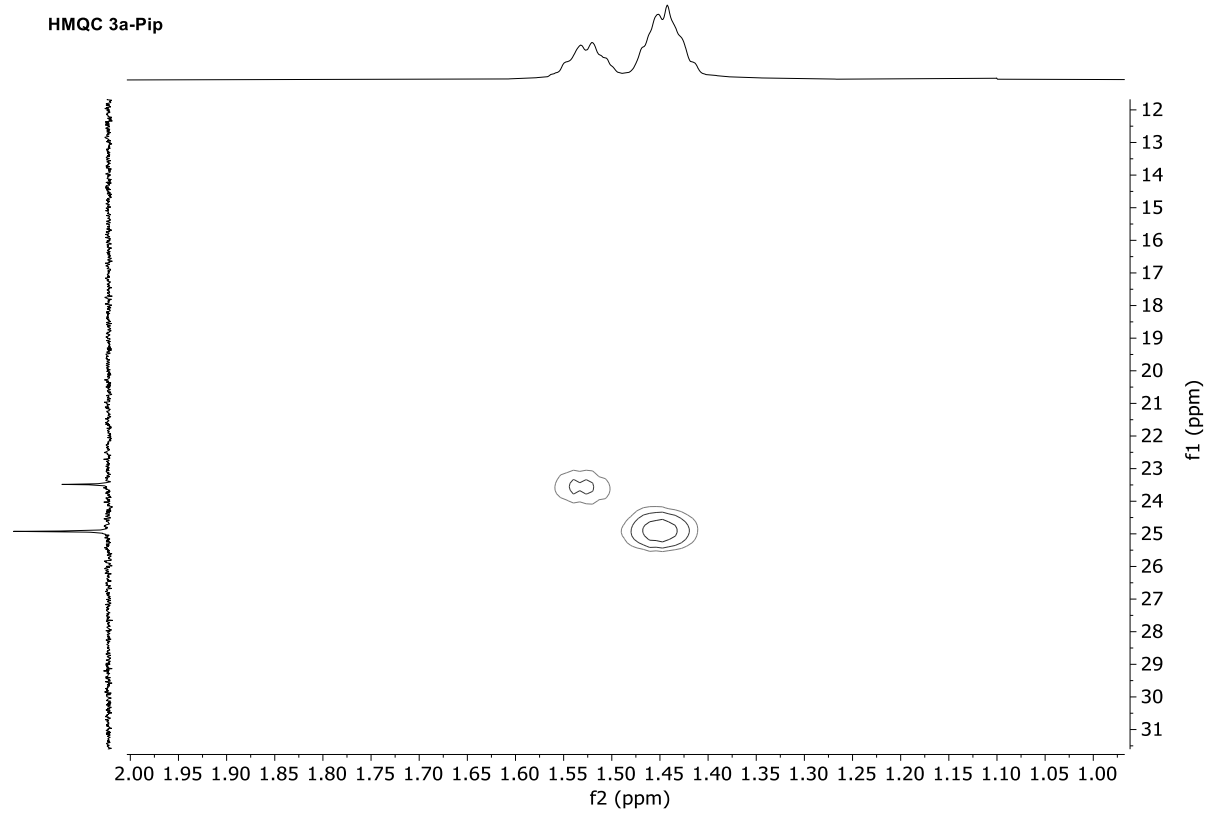


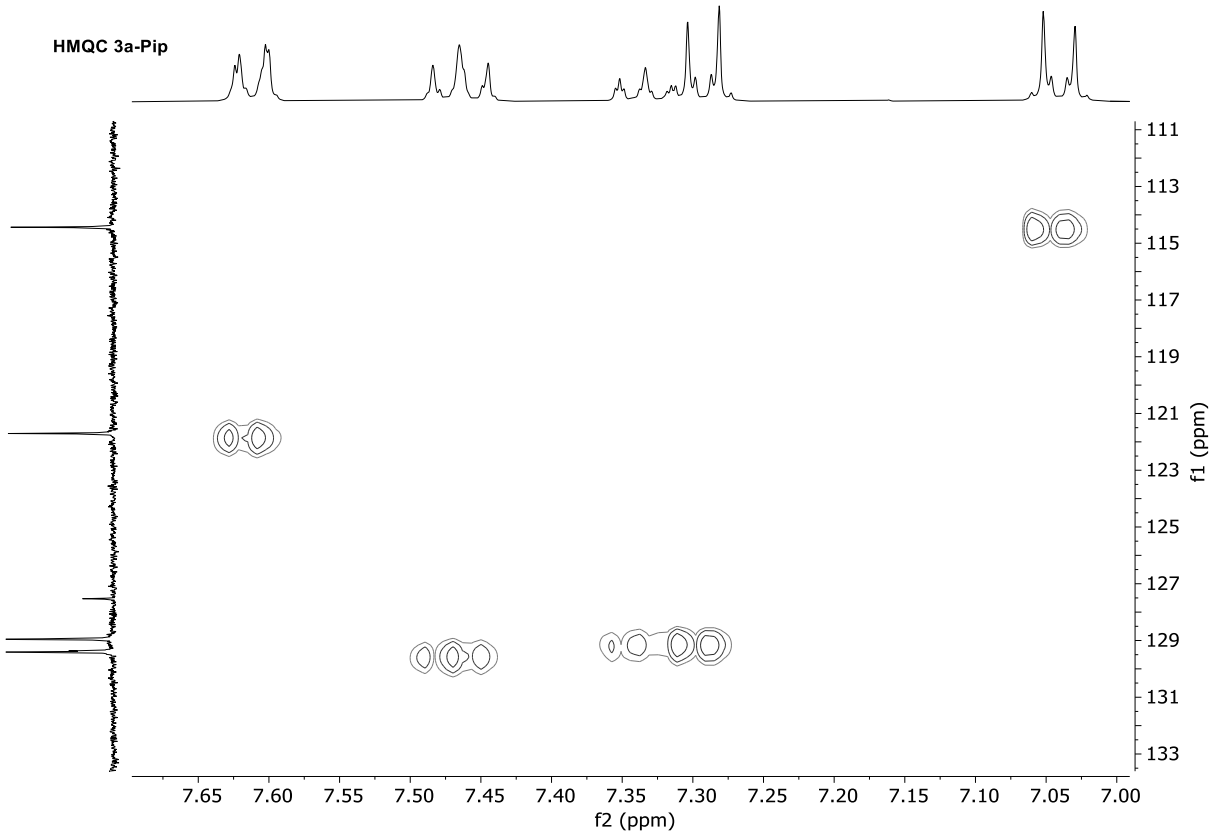
6.2.4. Compound 3a-Pip

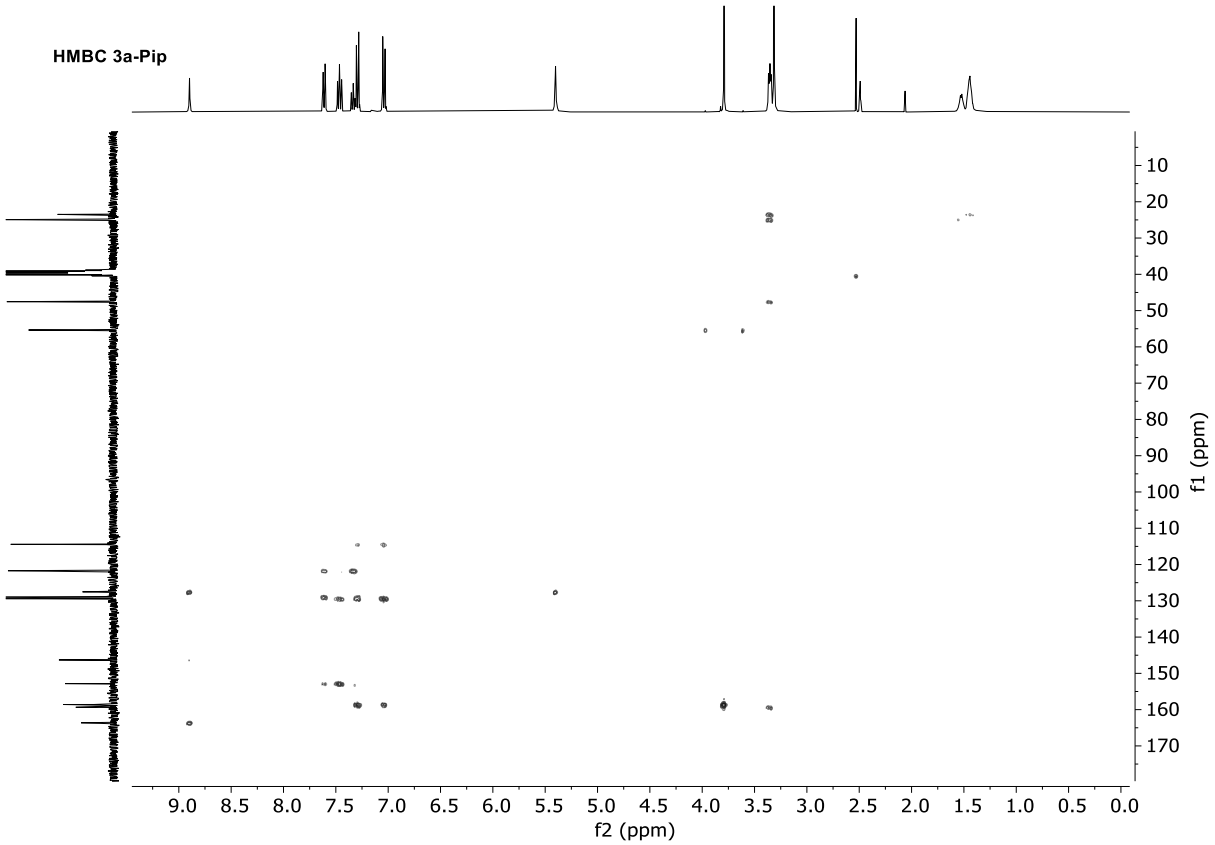


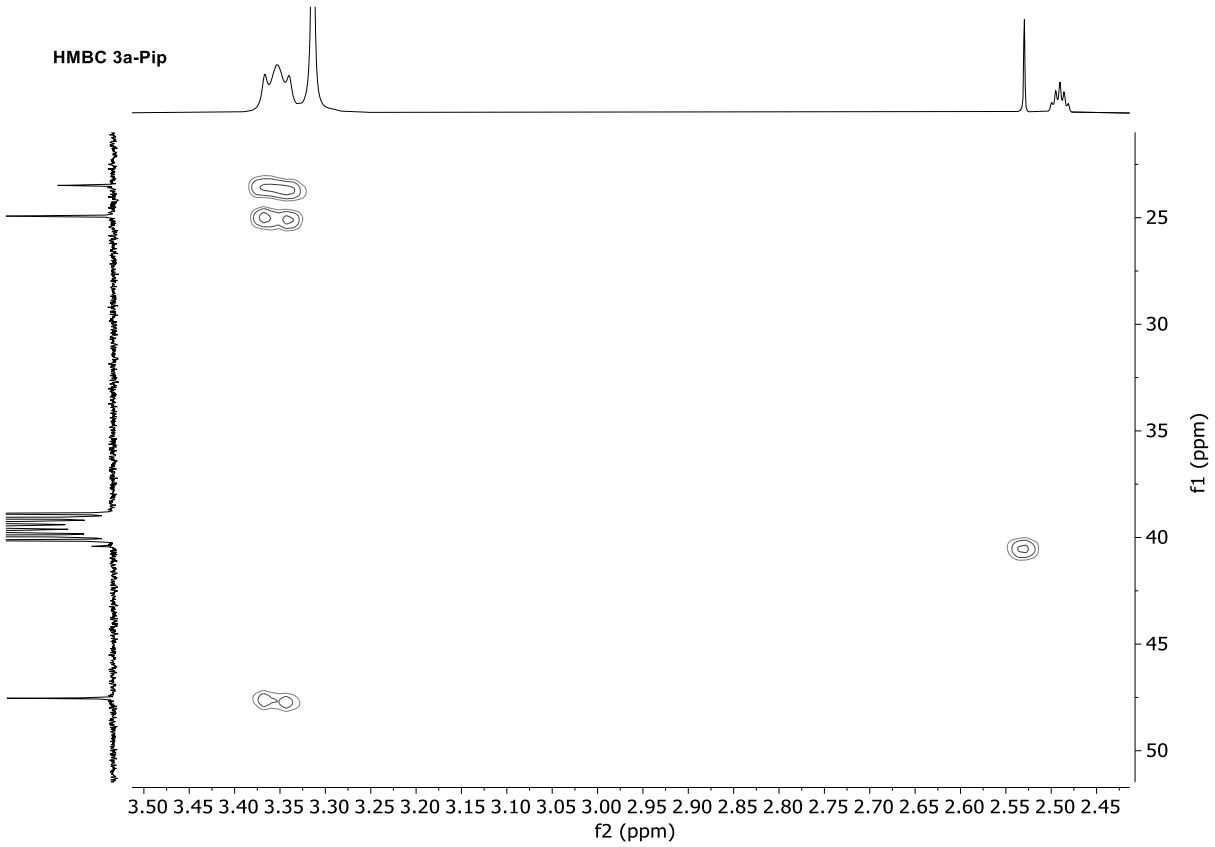

HMQC 3a-Pip
f1 (ppm)
f2 (ppm)

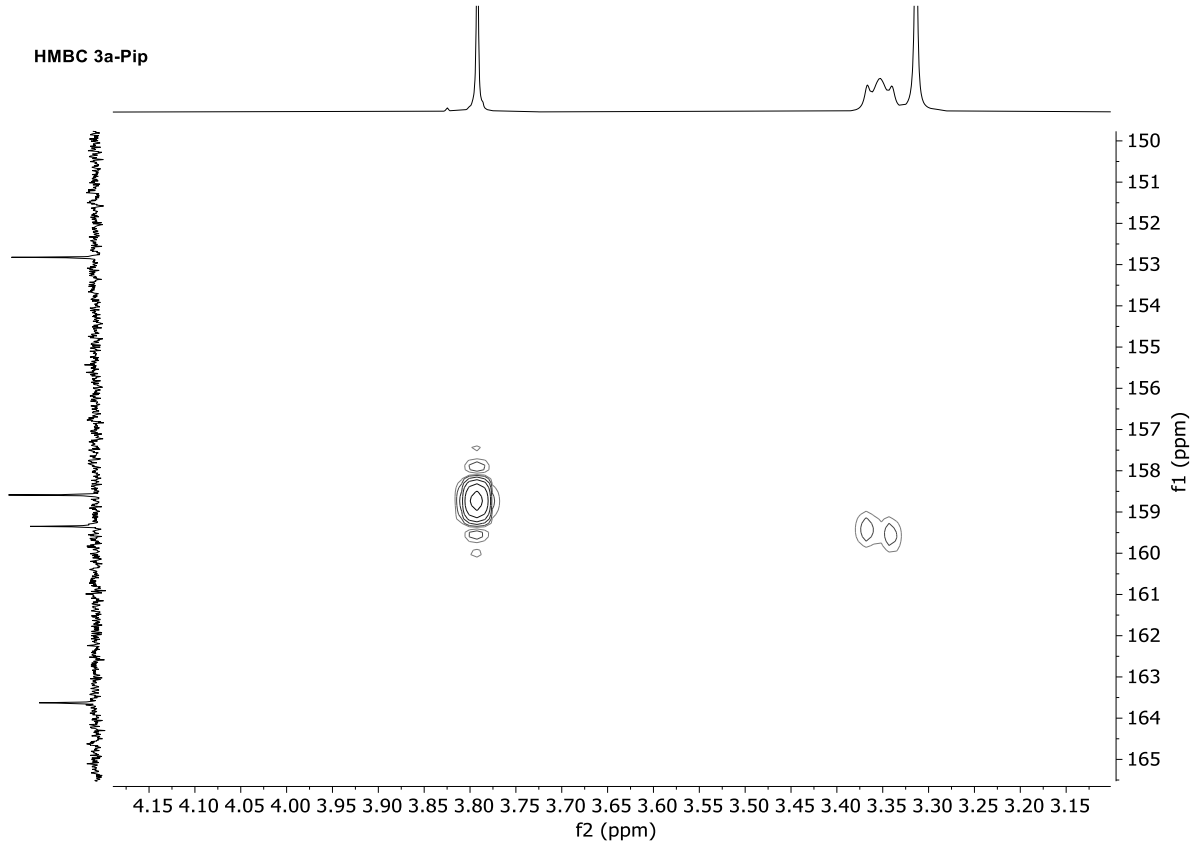

HMQC 3a-Pip
f1 (ppm)
f2 (ppm)

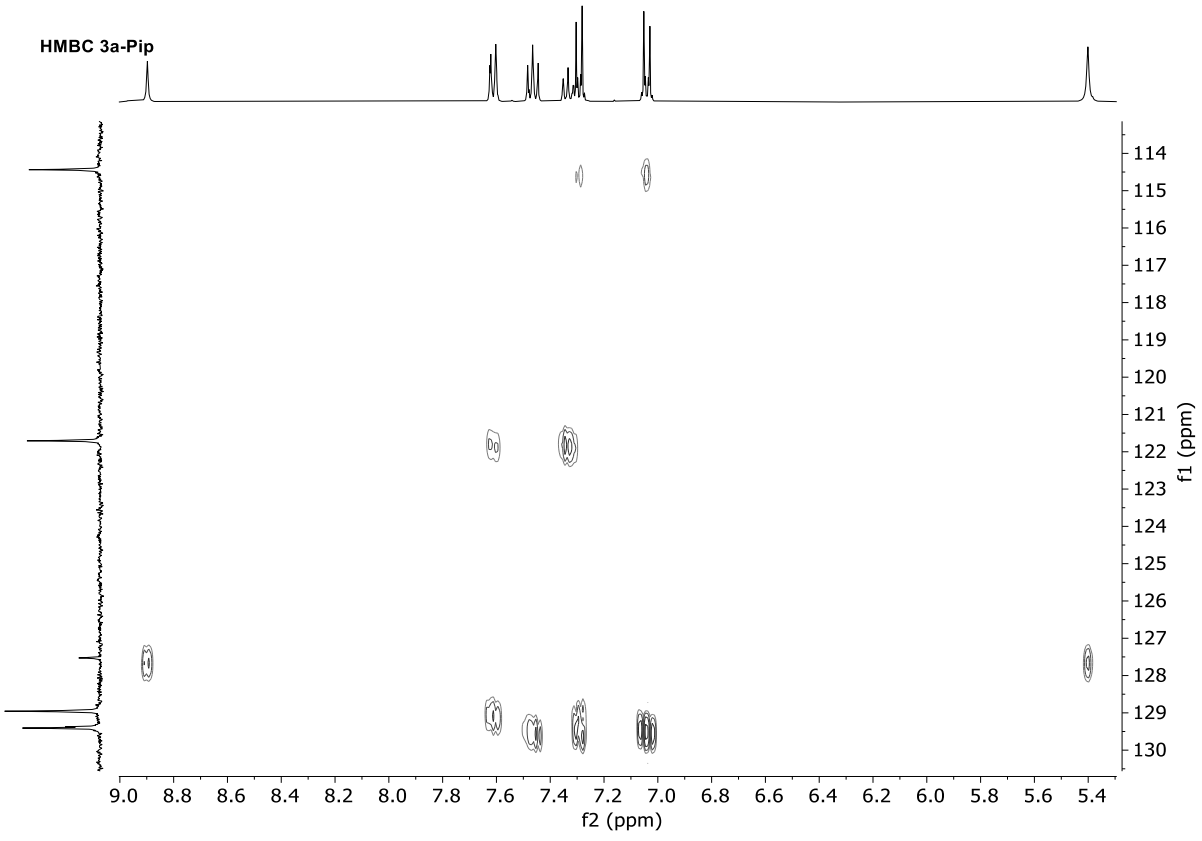

HMQC 3a-Pip
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
f1 (ppm)
2.00 1.95 1.90 1.85 1.80 1.75 1.70 1.65 1.60 1.55 1.50 1.45 1.40 1.35 1.30 1.25 1.20 1.15 1.10 1.05 1.00
f2 (ppm)

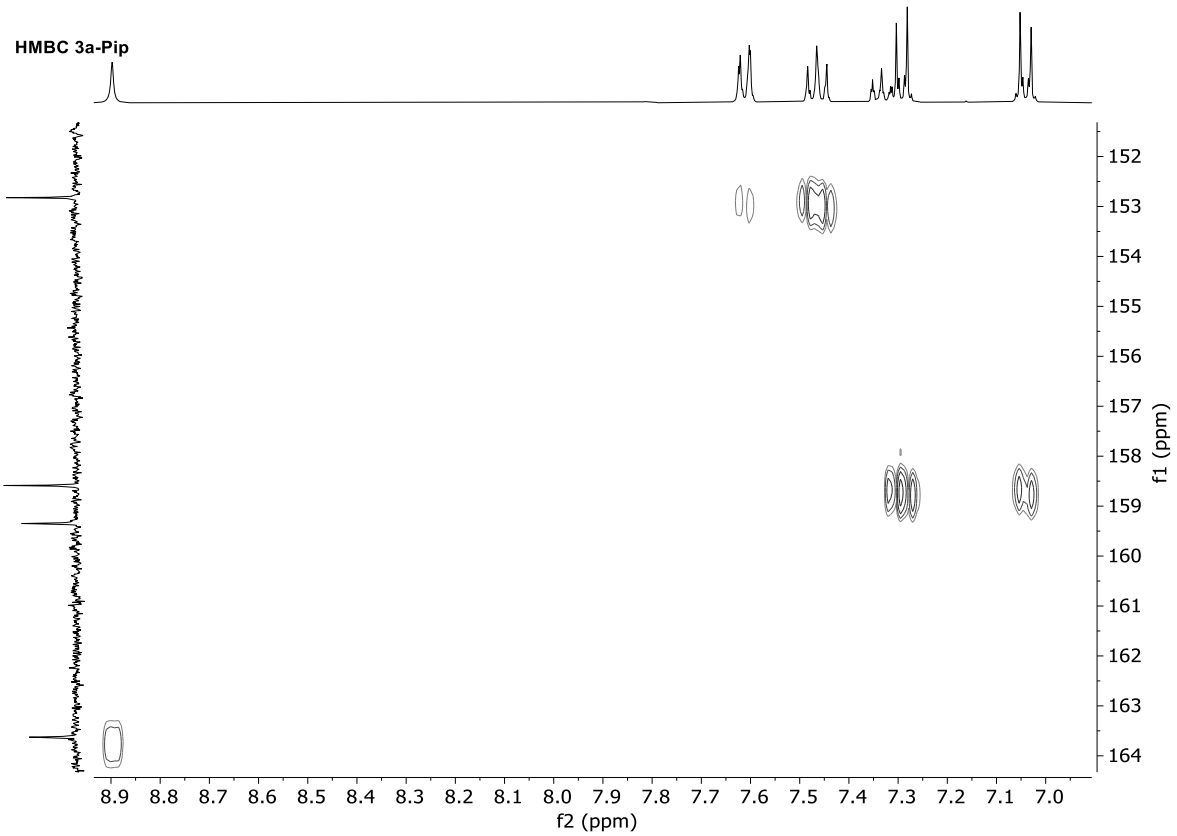

HMQC 3a-Pip
111
113
115
117
119
121
123
125
127
129
131
133
f1 (ppm)
7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20 7.15 7.10 7.05 7.00
f2 (ppm)


HMBC 3a-Pip
f1 (ppm)
f2 (ppm)

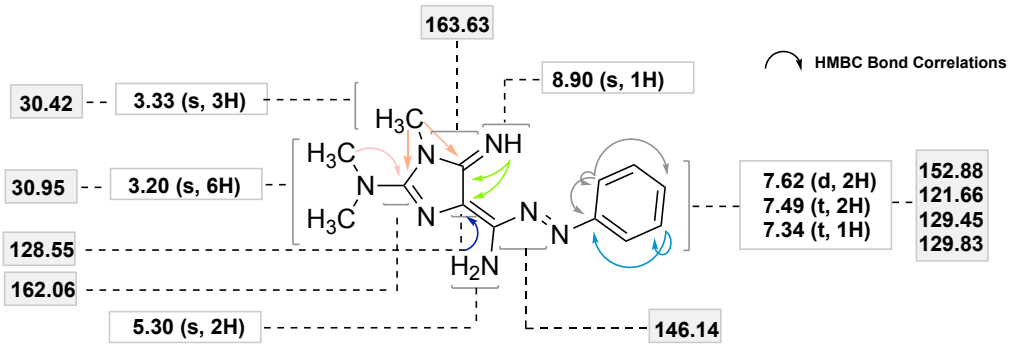

HMBC 3a-Pip
f1 (ppm)
f2 (ppm)

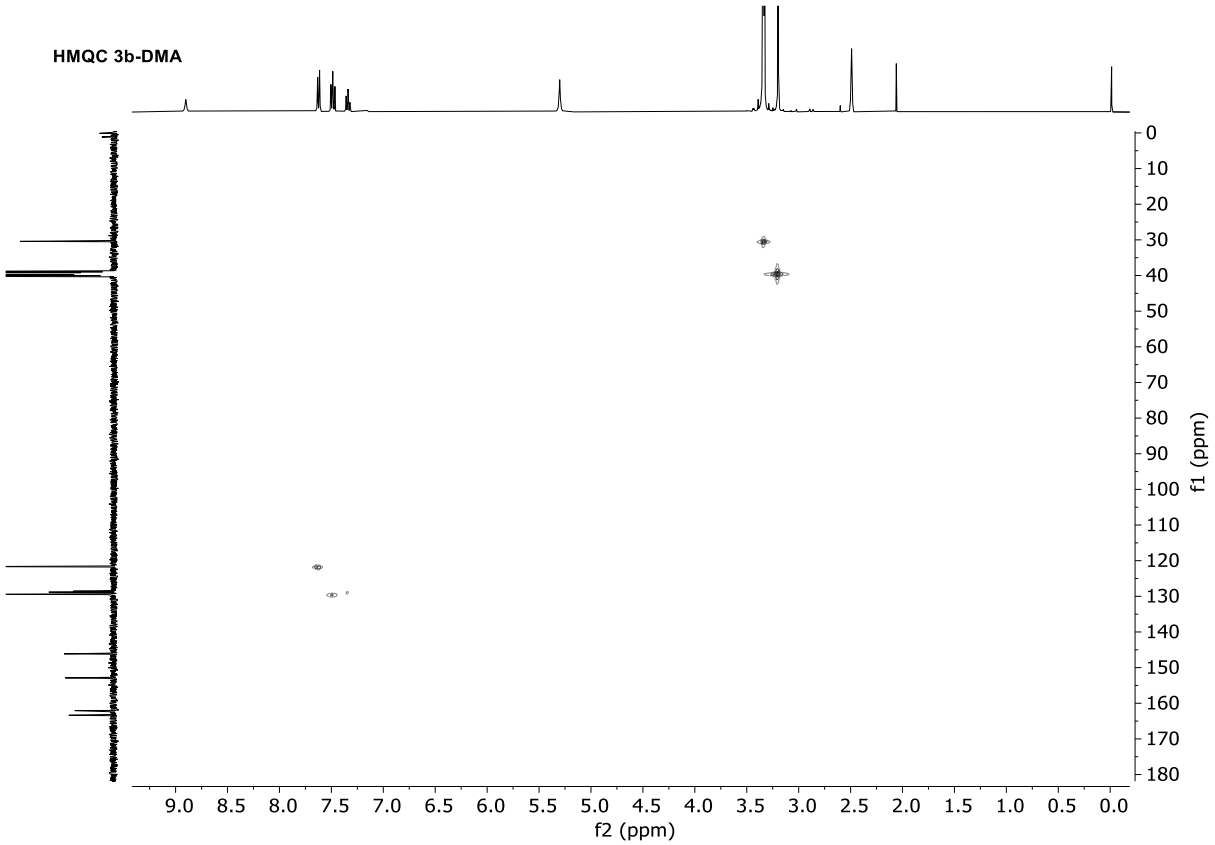

HMBC 3a-Pip
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
f1 (ppm)
4.15 4.10 4.05 4.00 3.95 3.90 3.85 3.80 3.75 3.70 3.65 3.60 3.55 3.50 3.45 3.40 3.35 3.30 3.25 3.20 3.15
f2 (ppm)

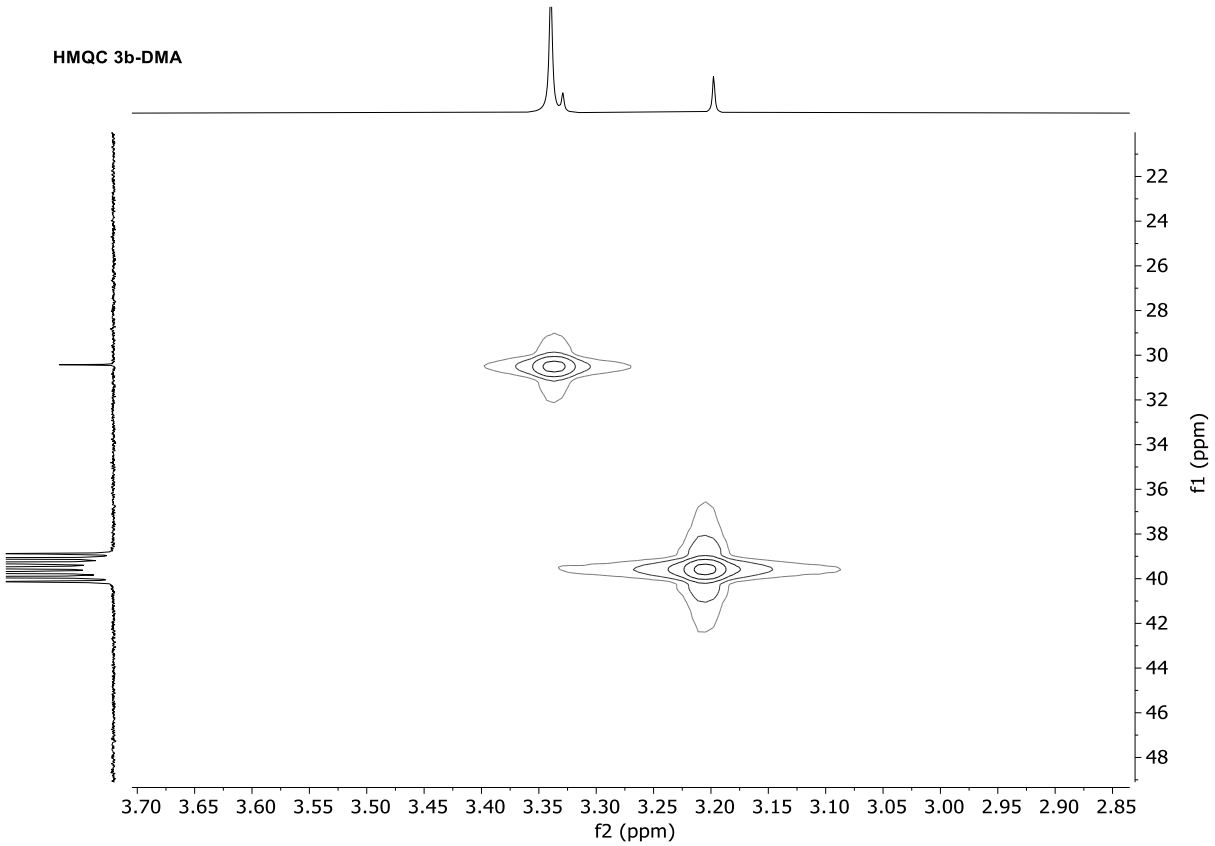

HMBC 3a-Pip
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
f1 (ppm)
9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4
f2 (ppm)

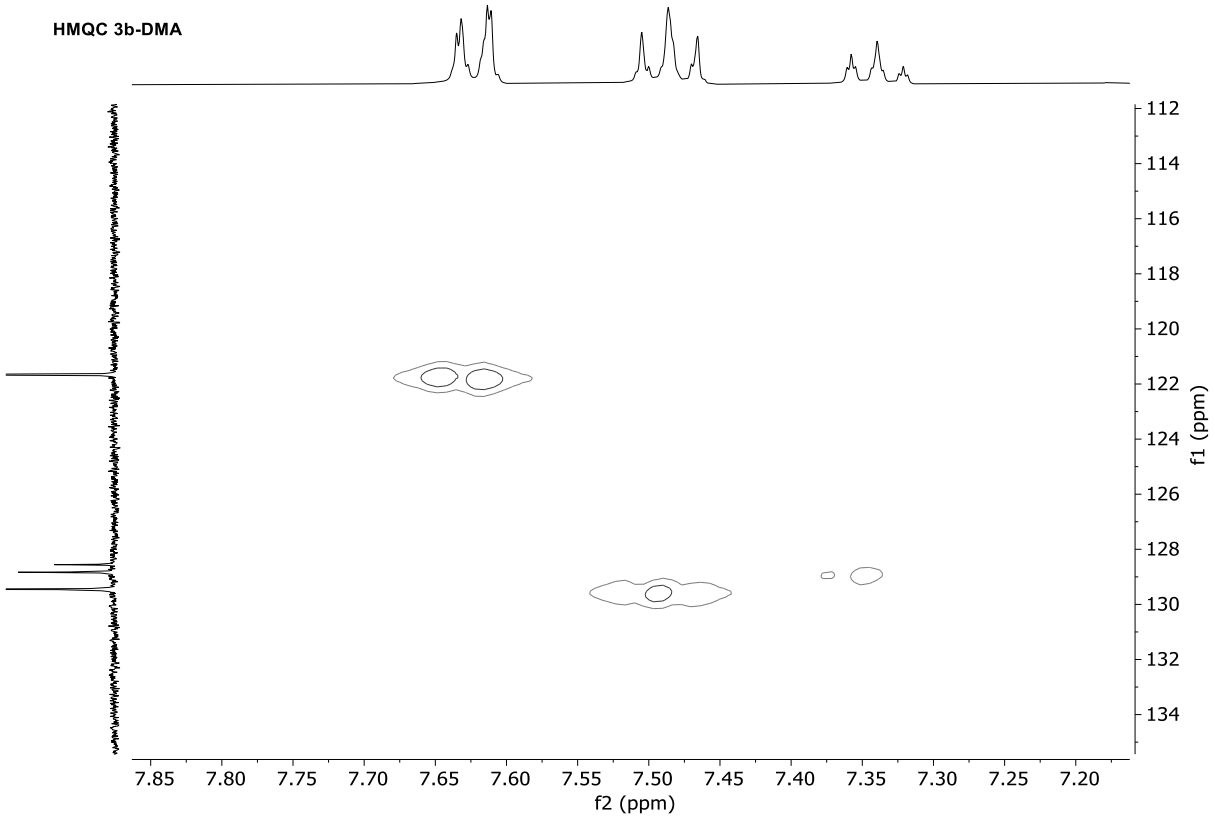


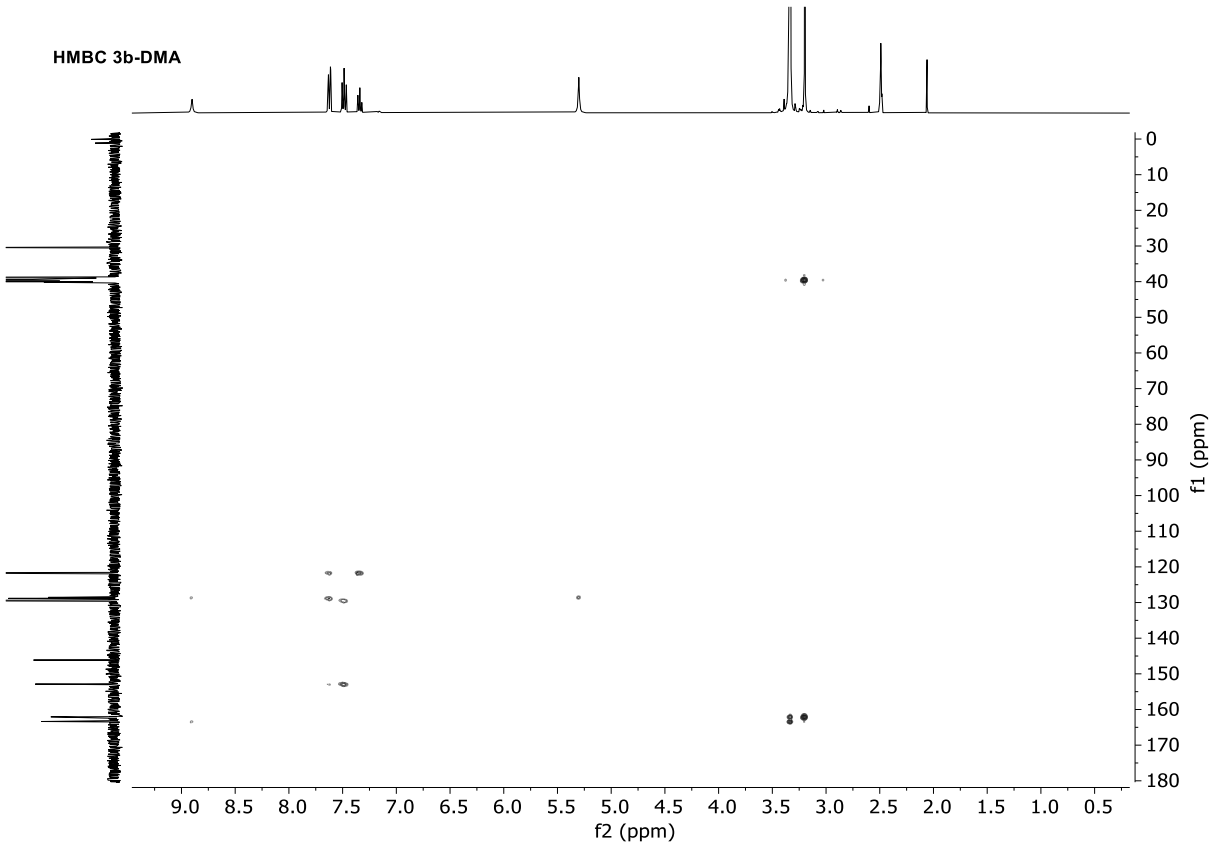
6.2.5. Compound 3b-DMA

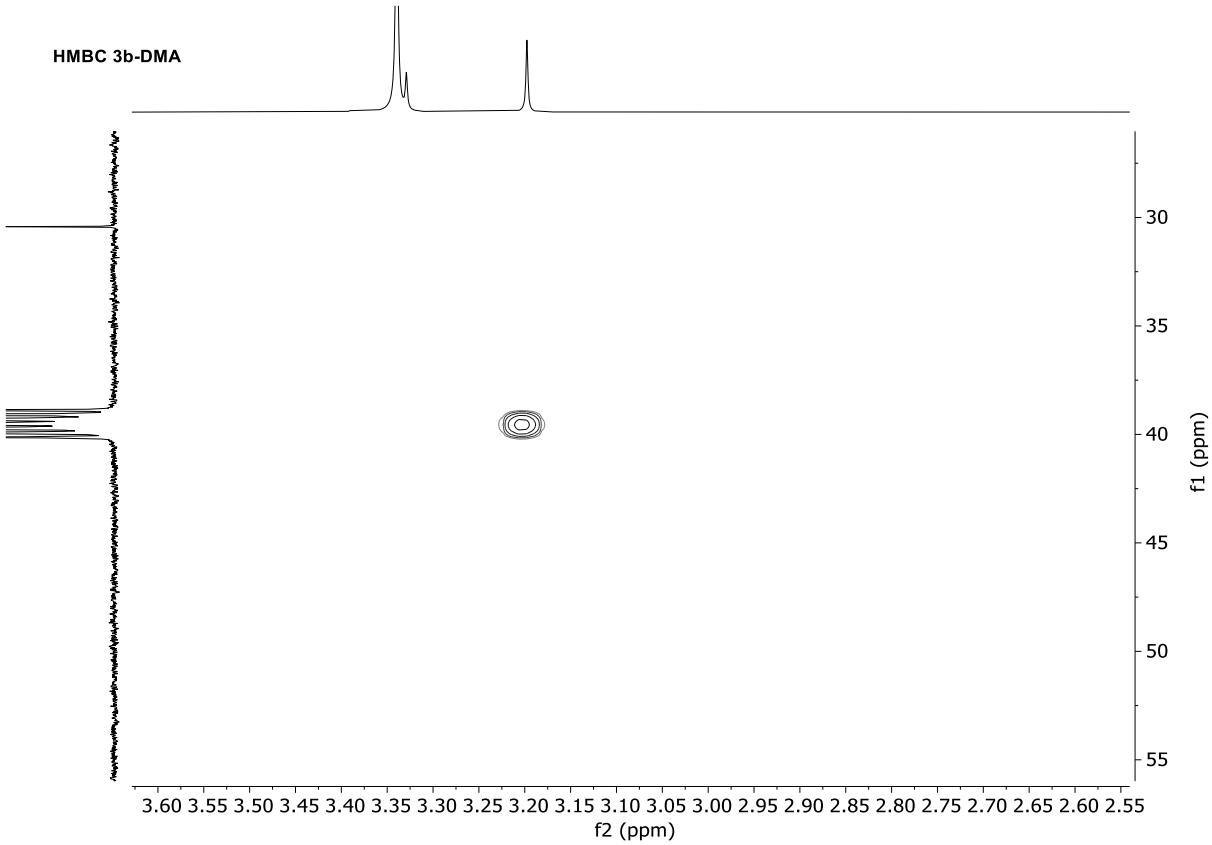


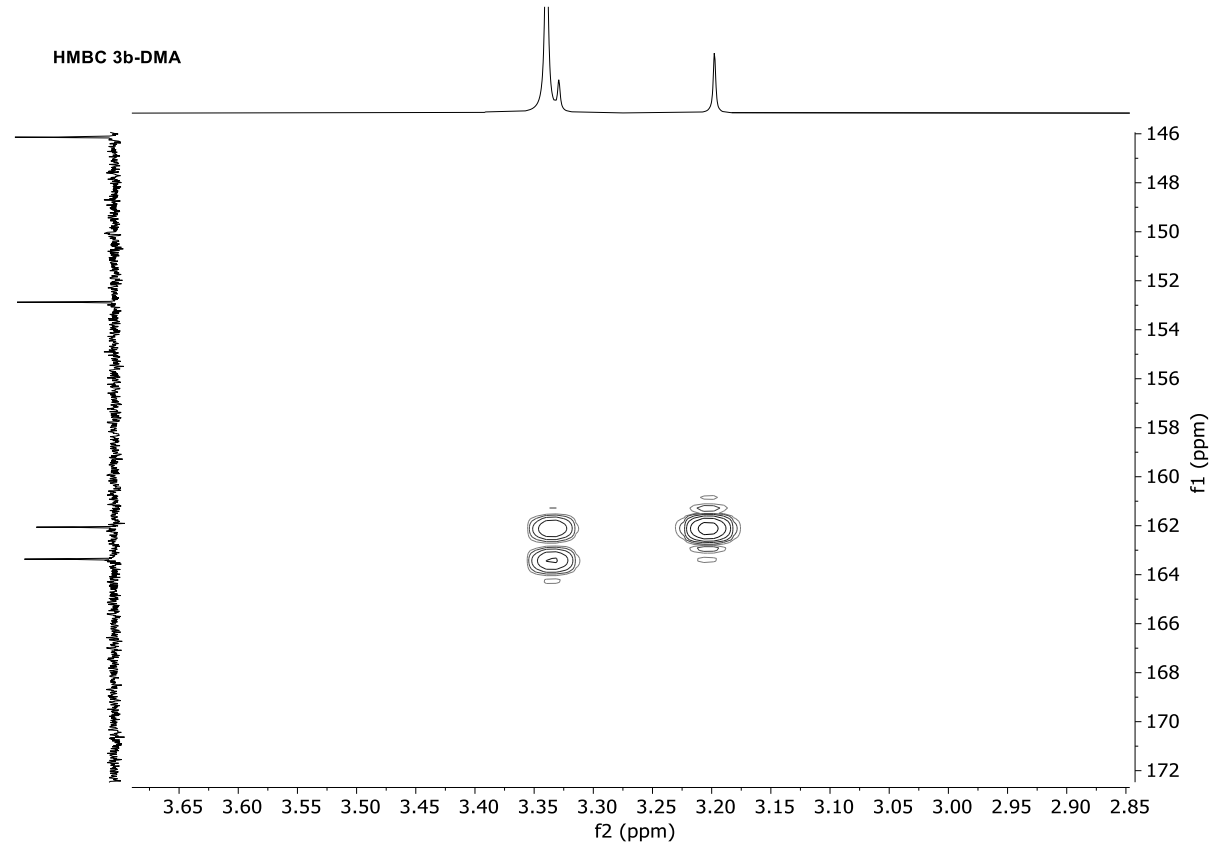

HMQC 3b-DMA
f1 (ppm)
f2 (ppm)

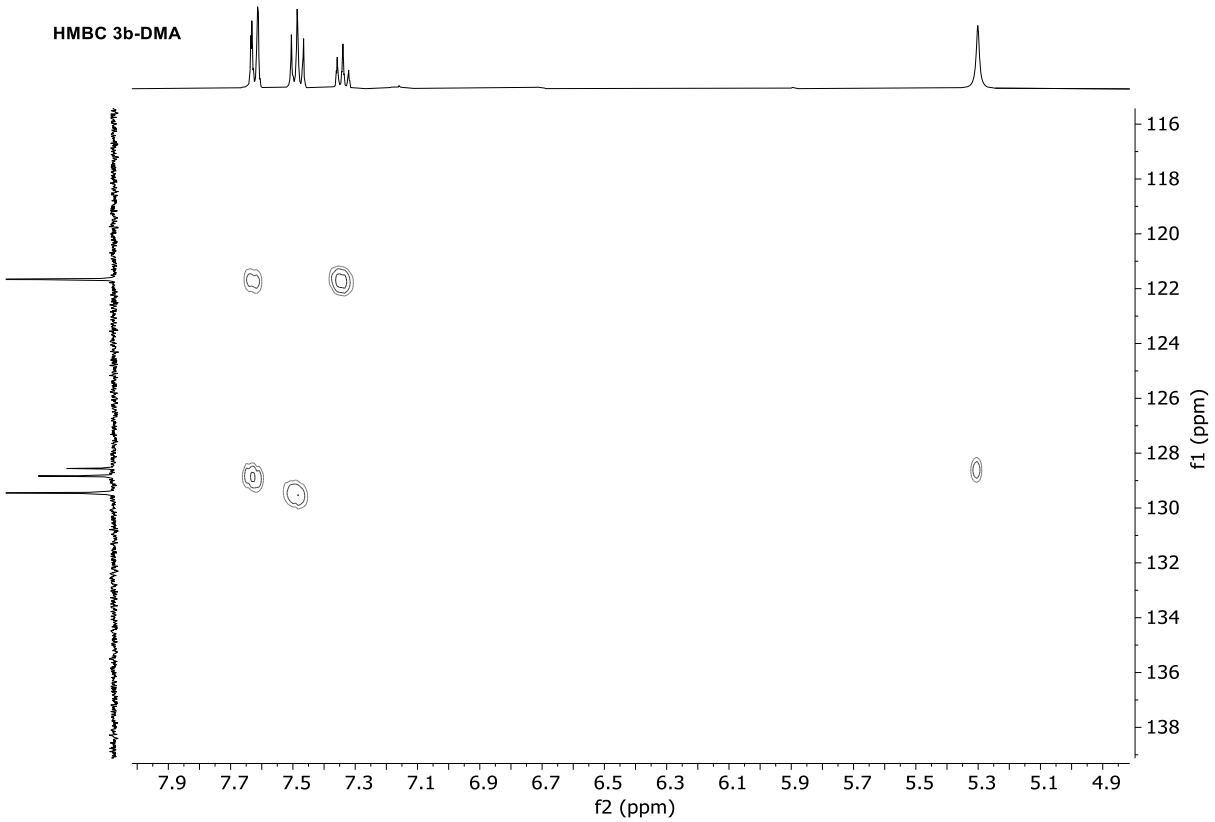

HMQC 3b-DMA
f1 (ppm)
f2 (ppm)

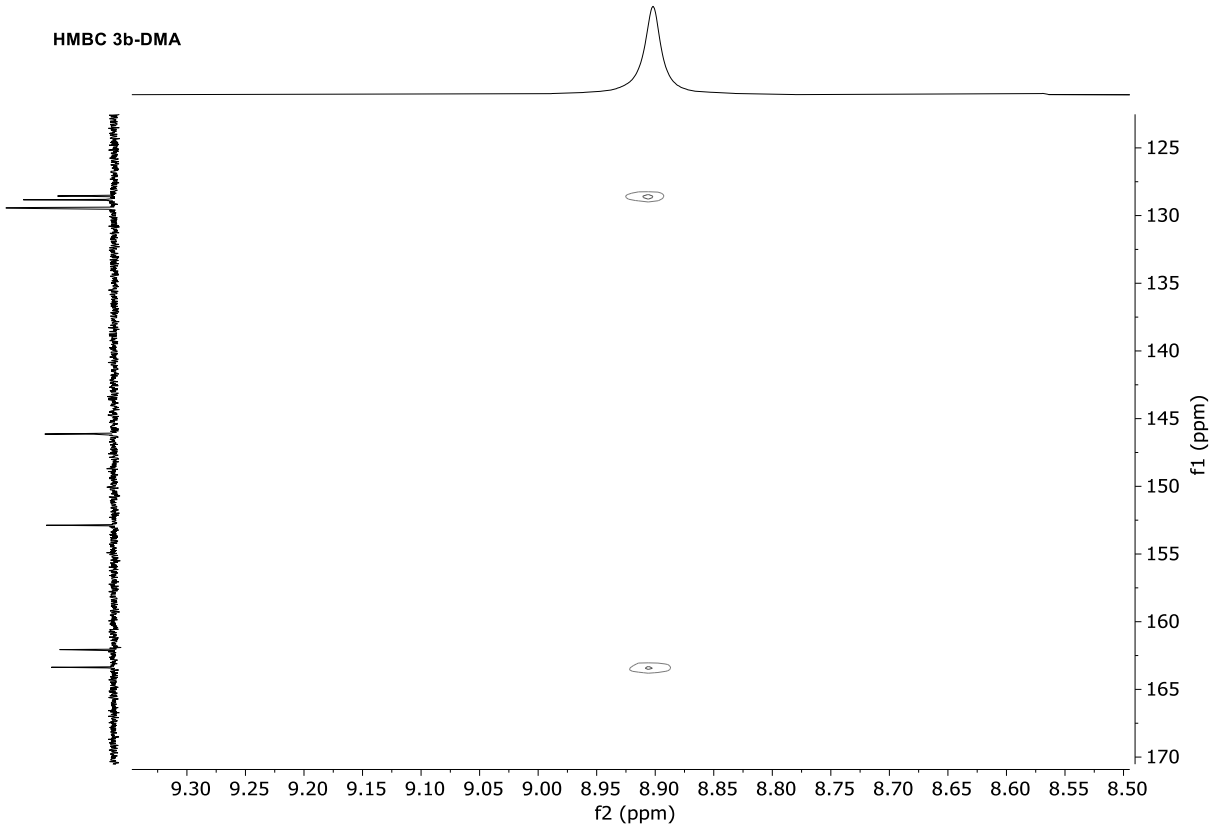

HMQC 3b-DMA
112
114
116
118
120
122
124
126
128
130
132
134
f1 (ppm)
7.85
7.80
7.75
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.30
7.25
7.20
f2 (ppm)

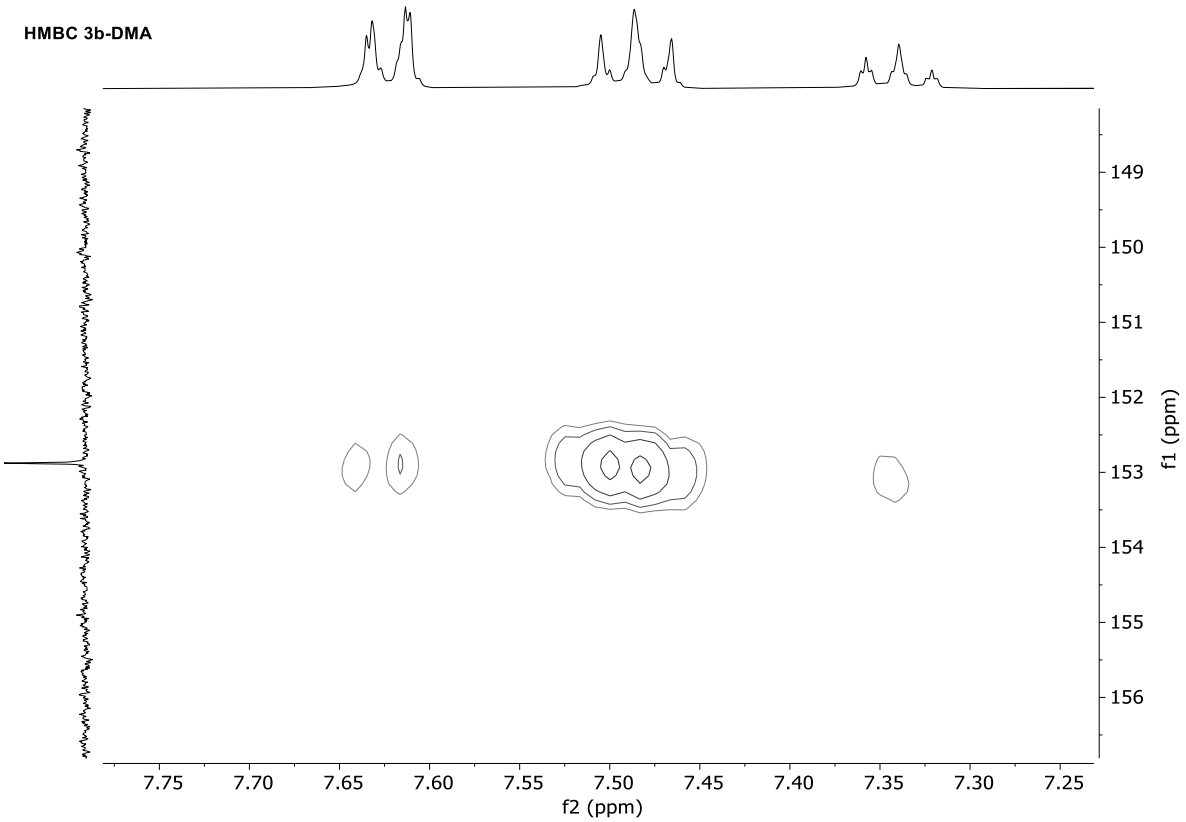

HMBC 3b-DMA
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)


HMBC 3b-DMA
30
35
40
45
50
55
f1 (ppm)
3.60 3.55 3.50 3.45 3.40 3.35 3.30 3.25 3.20 3.15 3.10 3.05 3.00 2.95 2.90 2.85 2.80 2.75 2.70 2.65 2.60 2.55
f2 (ppm)

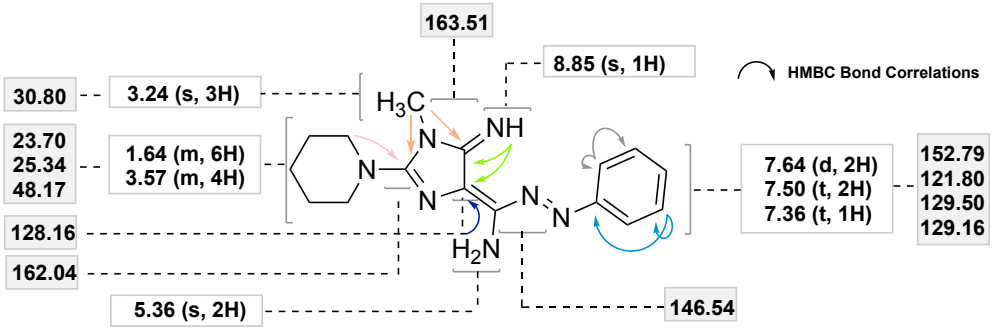

HMBC 3b-DMA
146
148
150
152
154
156
158
160
162
164
166
168
170
172
f1 (ppm)
3.65 3.60 3.55 3.50 3.45 3.40 3.35 3.30 3.25 3.20 3.15 3.10 3.05 3.00 2.95 2.90 2.85
f2 (ppm)

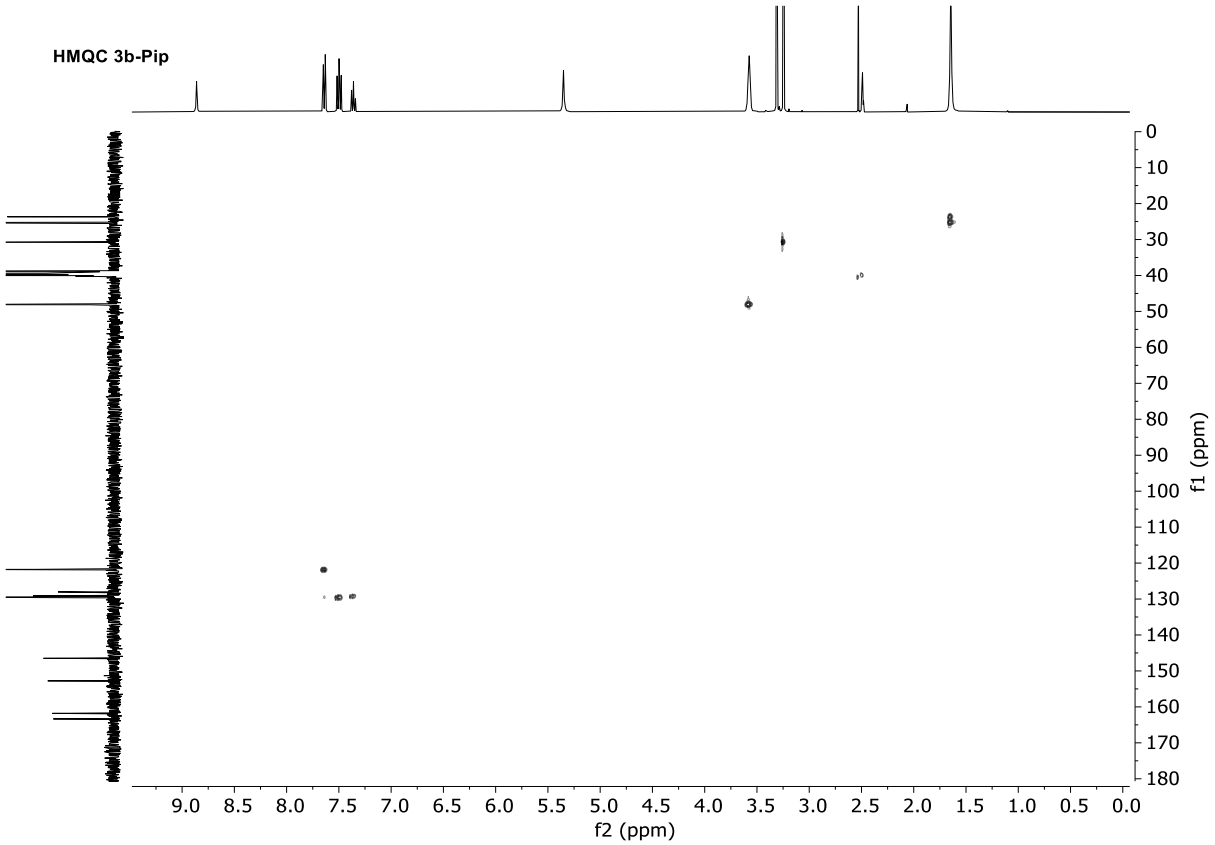

HMBC 3b-DMA
116
118
120
122
124
126
128
130
132
134
136
138
f1 (ppm)
7.9
7.7
7.5
7.3
7.1
6.9
6.7
6.5
6.3
6.1
5.9
5.7
5.5
5.3
5.1
4.9
f2 (ppm)

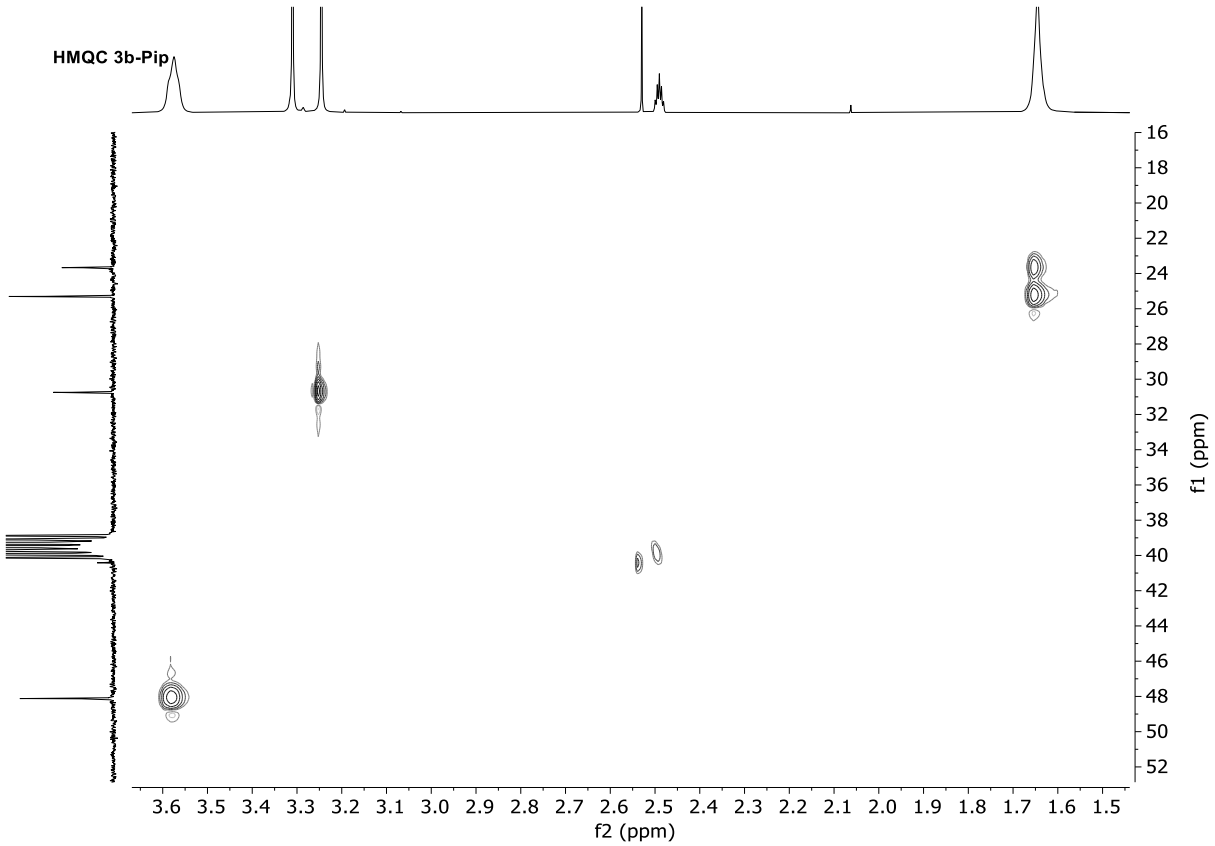

HMBC 3b-DMA
125
130
135
140
145
150
155
160
165
170
f1 (ppm)
9.30
9.25
9.20
9.15
9.10
9.05
9.00
8.95
8.90
8.85
8.80
8.75
8.70
8.65
8.60
8.55
8.50
f2 (ppm)

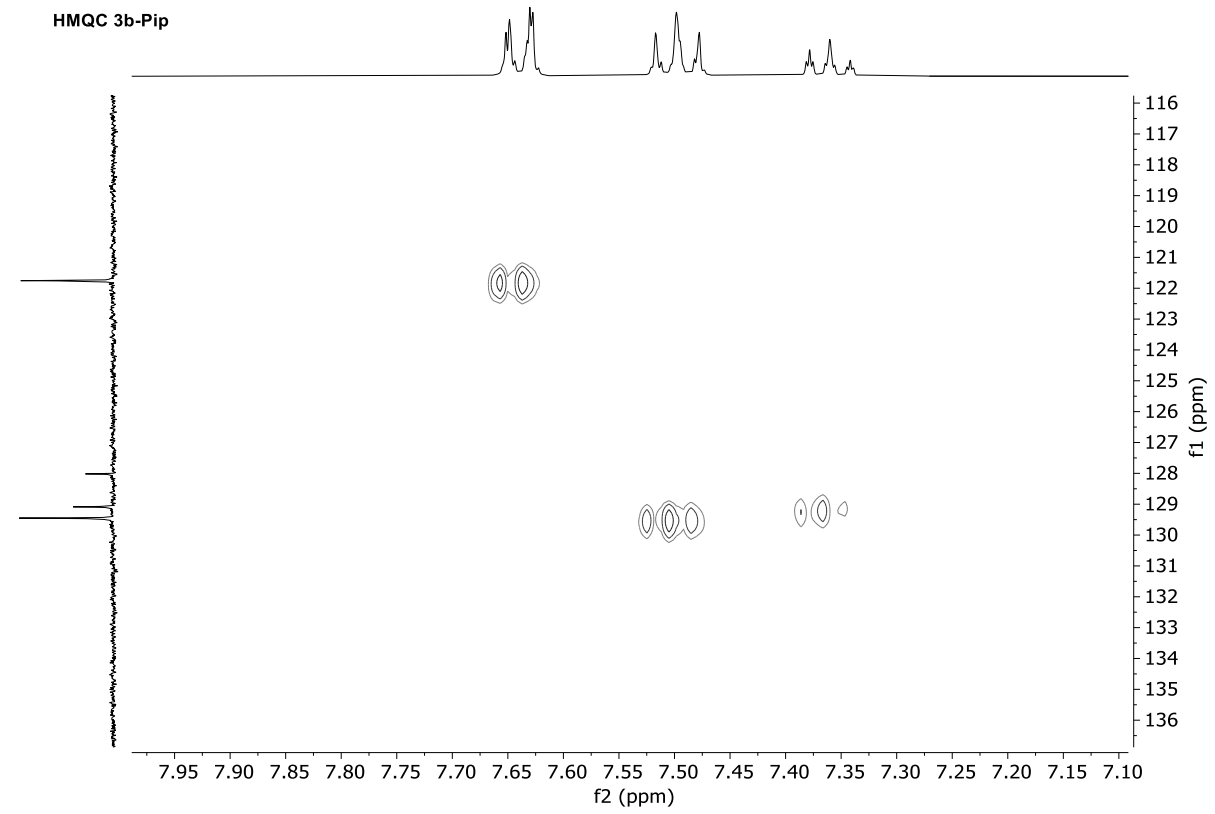


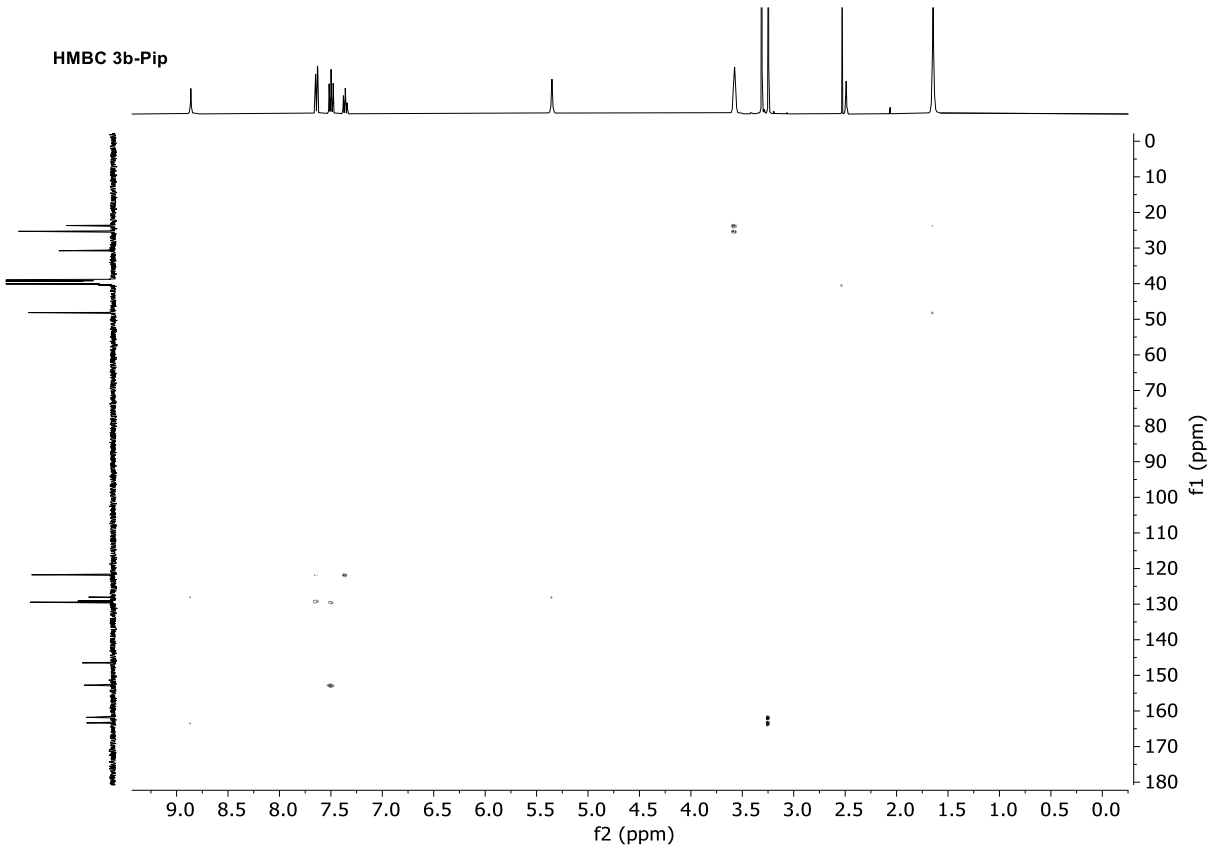
6.2.6. Compound 3b-Pip

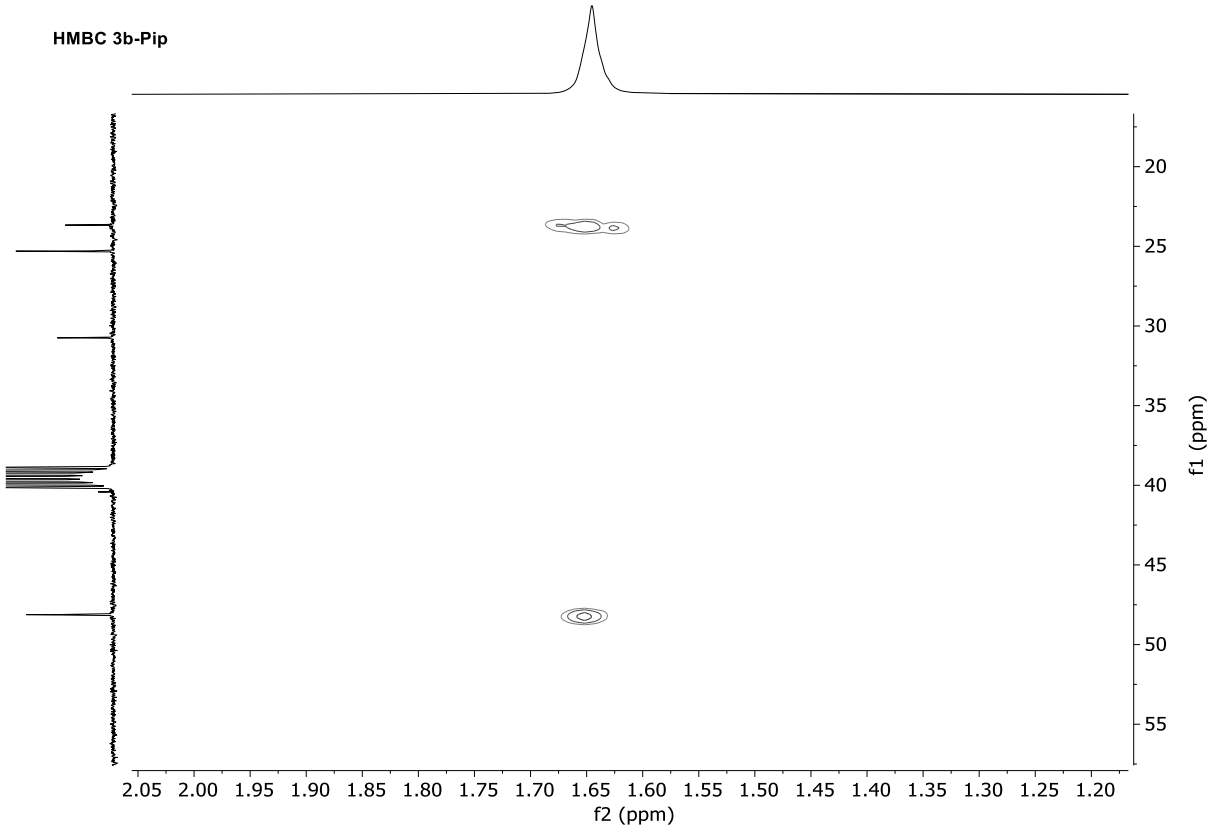


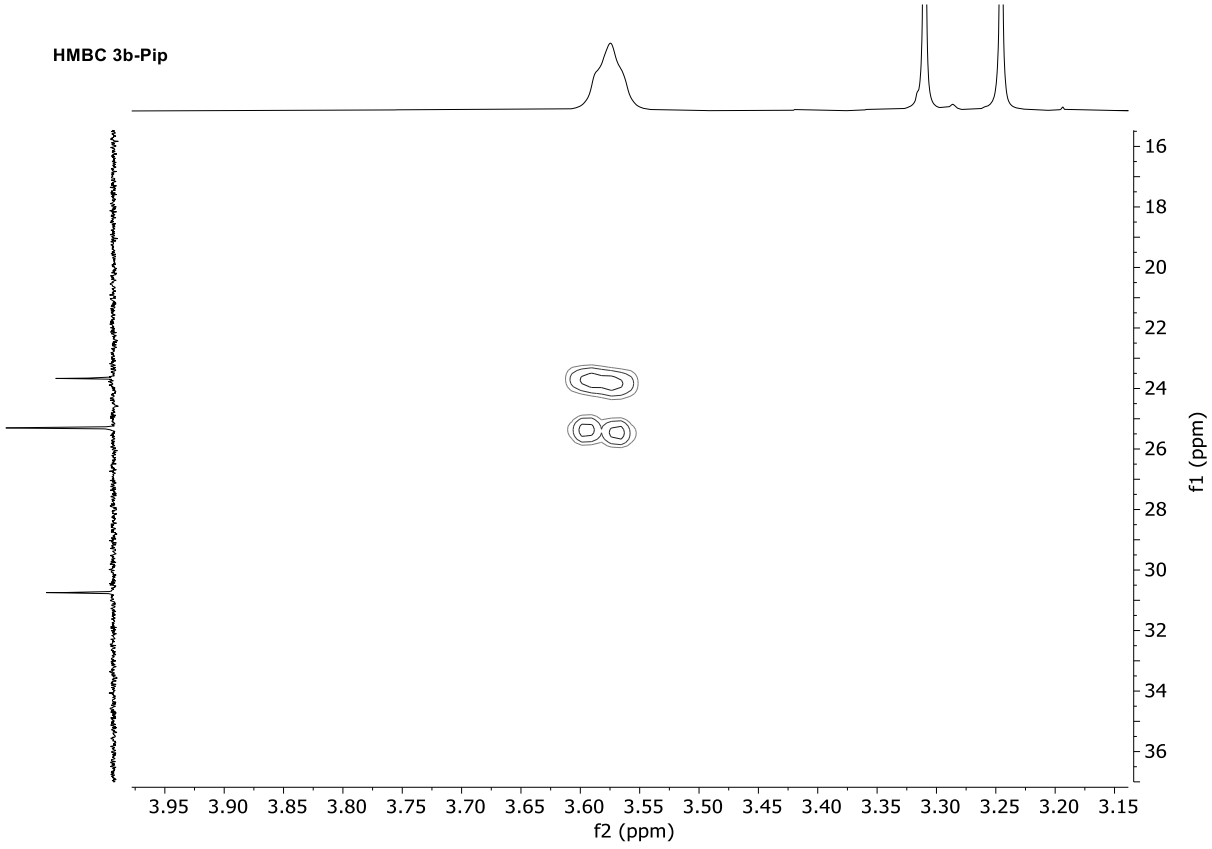

HMQC 3b-Pip
f2 (ppm)
f1 (ppm)

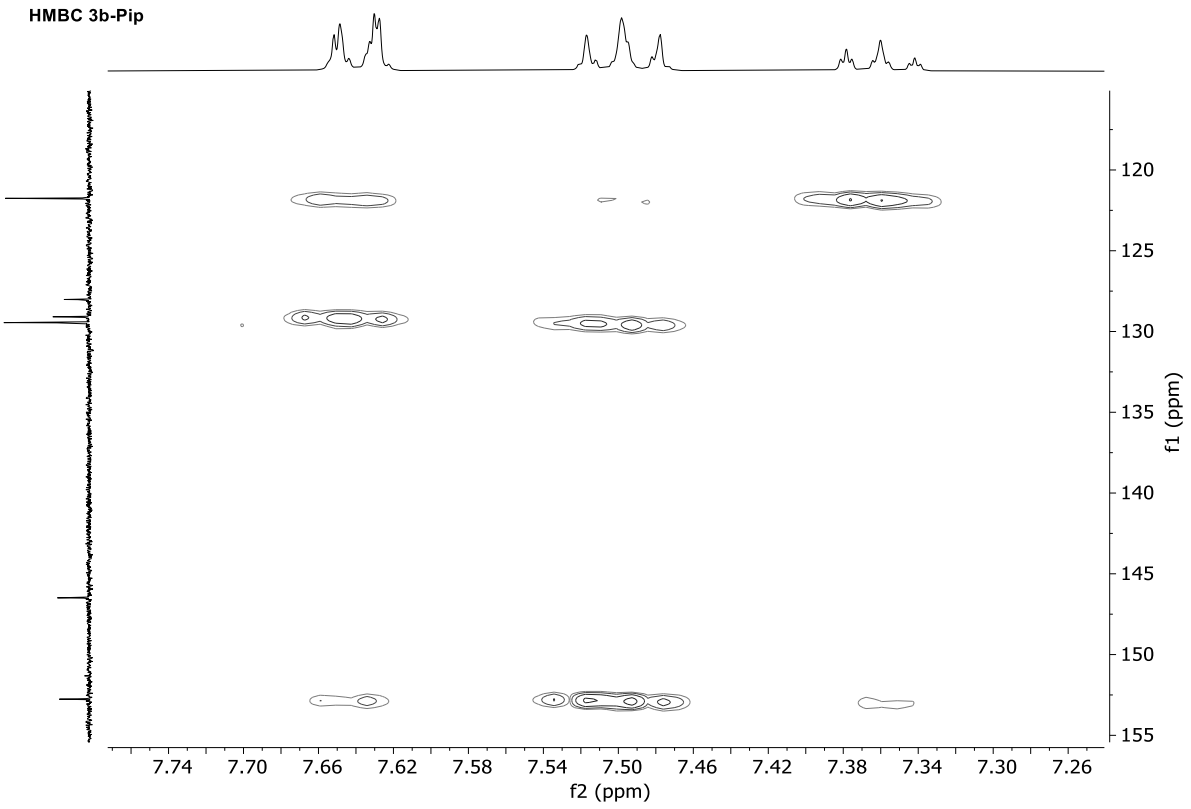

HMQC 3b-Pip
f2 (ppm)
f1 (ppm)

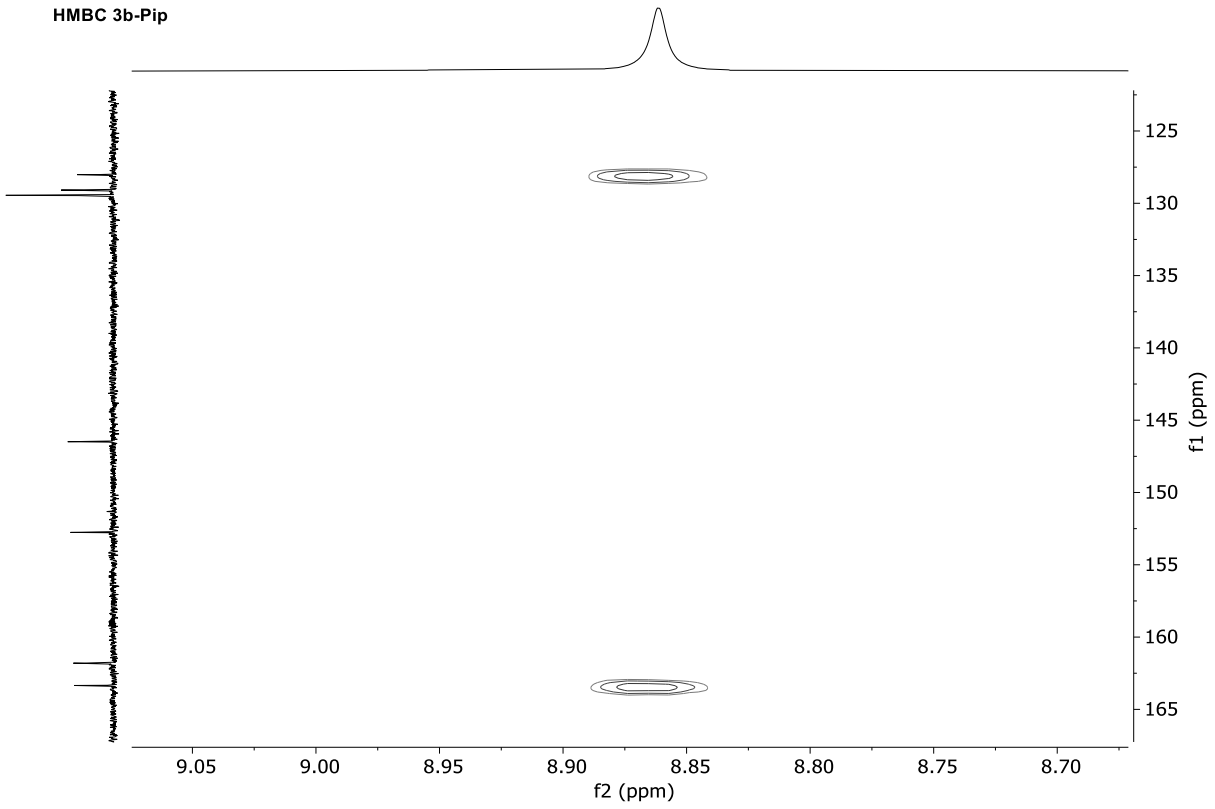

HMQC 3b-Pip
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
f1 (ppm)
7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20 7.15 7.10
f2 (ppm)

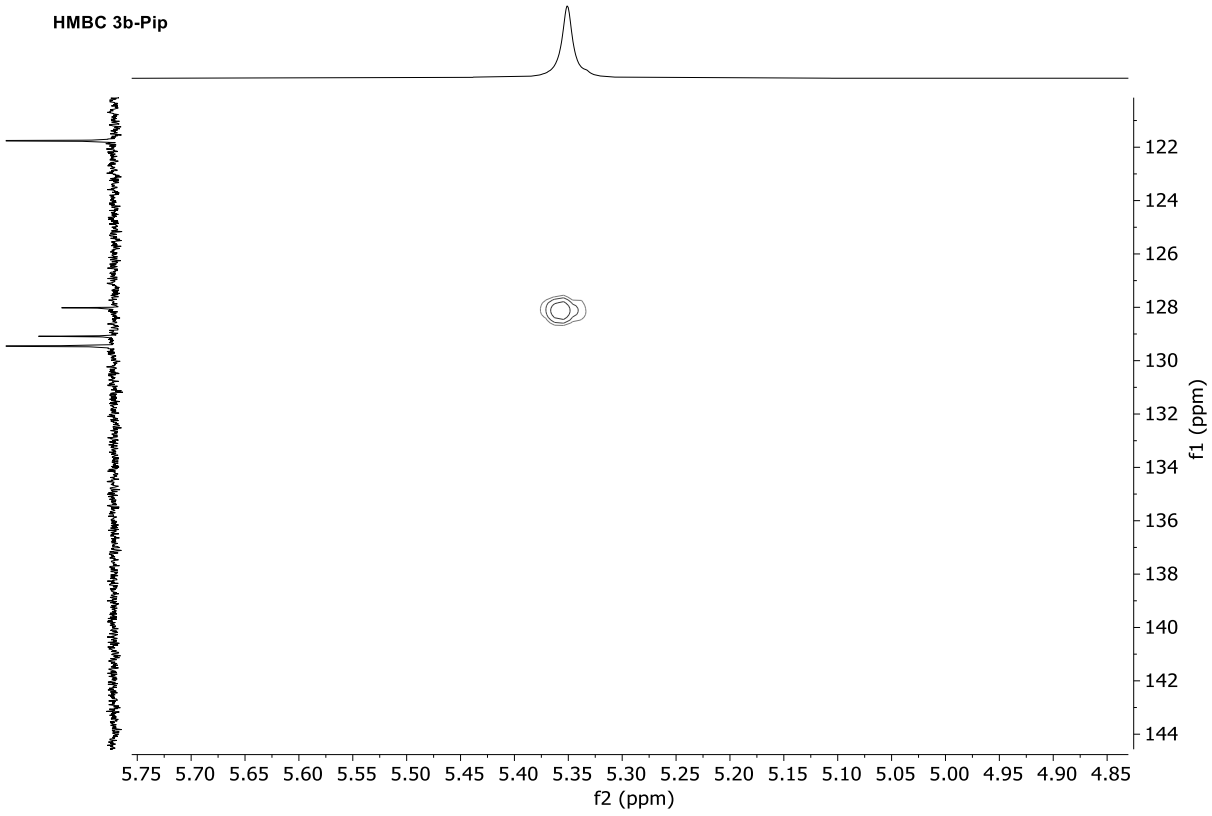

HMBC 3b-Pip
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f2 (ppm)

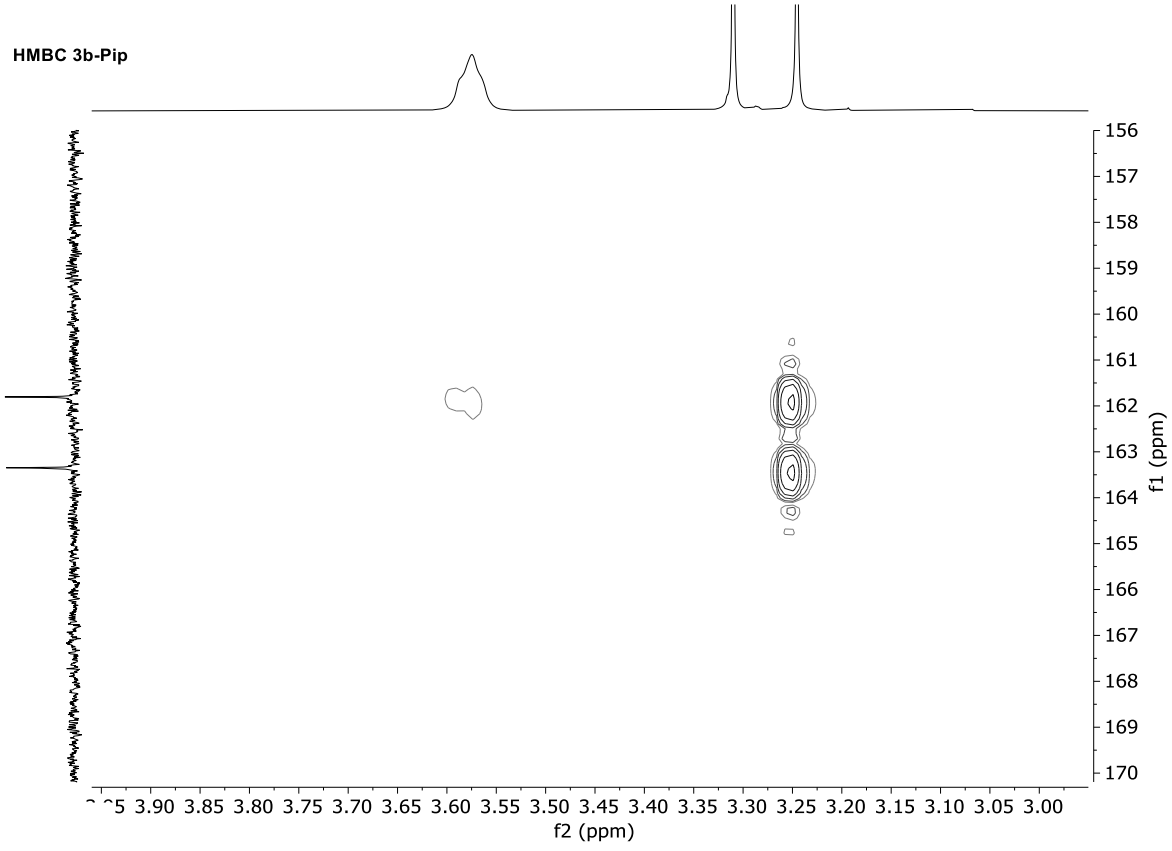

HMBC 3b-Pip
20
25
30
35
40
45
50
55
f1 (ppm)
2.05 2.00 1.95 1.90 1.85 1.80 1.75 1.70 1.65 1.60 1.55 1.50 1.45 1.40 1.35 1.30 1.25 1.20
f2 (ppm)


HMBC 3b-Pip
16
18
20
22
24
26
28
30
32
34
36
f1 (ppm)
3.95 3.90 3.85 3.80 3.75 3.70 3.65 3.60 3.55 3.50 3.45 3.40 3.35 3.30 3.25 3.20 3.15
f2 (ppm)


HMBC 3b-Pip
120
125
130
135
140
145
150
155
f1 (ppm)
7.74
7.70
7.66
7.62
7.58
7.54
7.50
7.46
7.42
7.38
7.34
7.30
7.26
f2 (ppm)


HMBC 3b-Pip
125
130
135
140
145
150
155
160
165
f1 (ppm)
9.05
9.00
8.95
8.90
8.85
8.80
8.75
8.70
f2 (ppm)


HMBC 3b-Pip
122
124
126
128
130
132
134
136
138
140
142
144
f1 (ppm)
5.75 5.70 5.65 5.60 5.55 5.50 5.45 5.40 5.35 5.30 5.25 5.20 5.15 5.10 5.05 5.00 4.95 4.90 4.85
f2 (ppm)


HMBC 3b-Pip
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
f1 (ppm)
3.90 3.85 3.80 3.75 3.70 3.65 3.60 3.55 3.50 3.45 3.40 3.35 3.30 3.25 3.20 3.15 3.10 3.05 3.00
f2 (ppm)