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Chemoenzymatic Synthesis of Cytokinins from Nucleosides: Ribose as a Blocking Group

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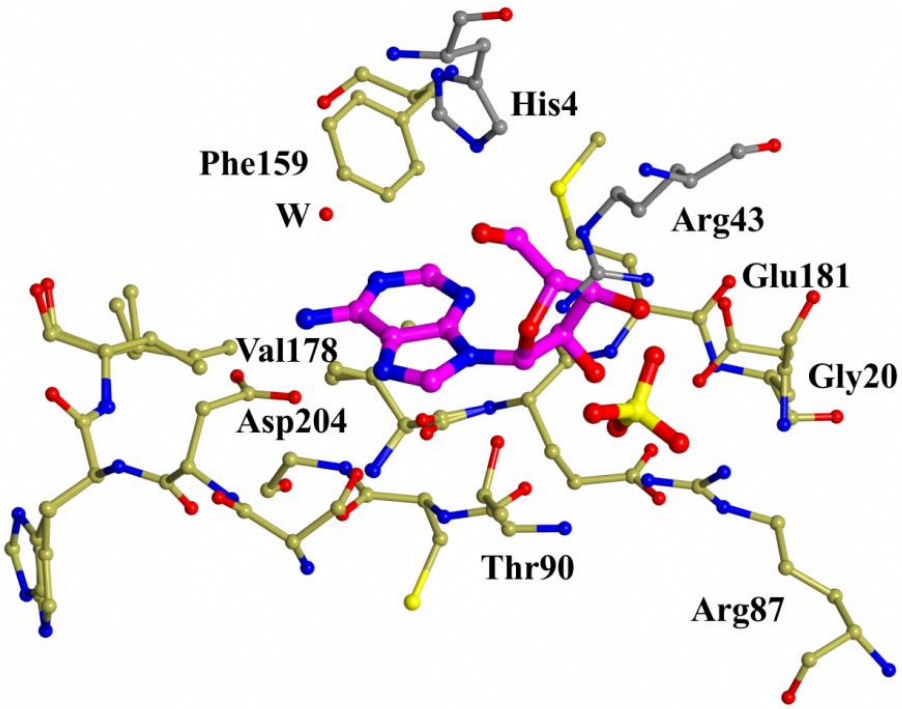
[†] The authors contributed equally.

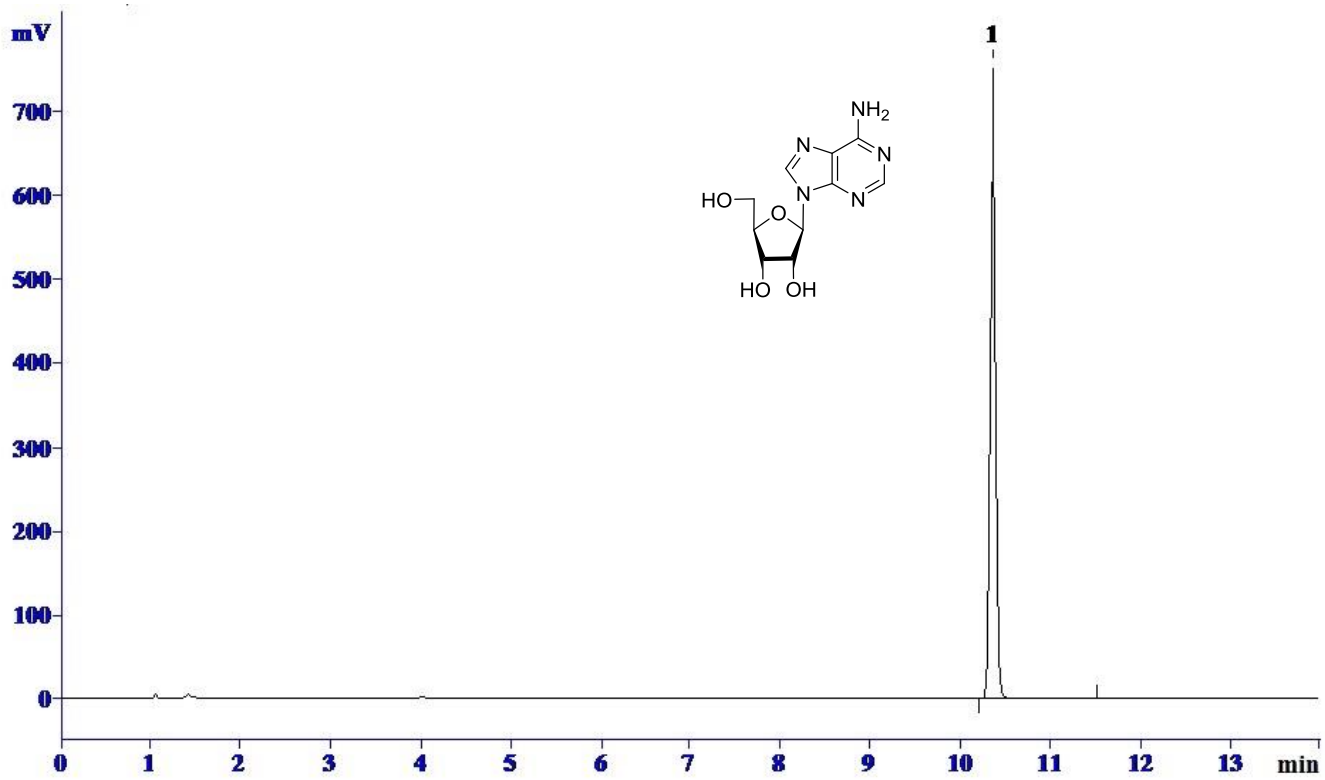
Keywords: Arsenolysis; phosphorolysis; biocatalysis; enzymatic reactions; modified purine derivatives; purine nucleoside phosphorylase.



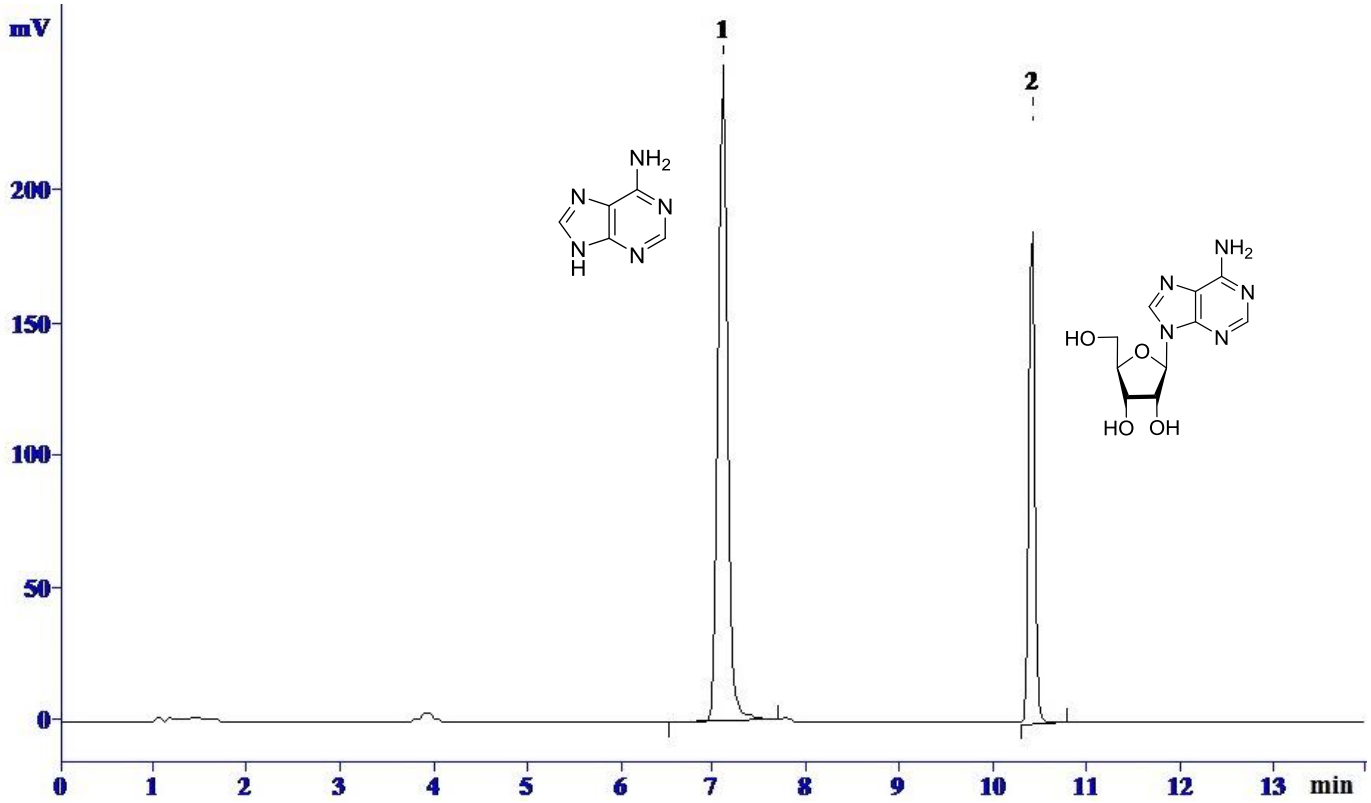
The active site structure of purine nucleoside phosphorylase from *Bacillus cereus* complex with adenosine (magenta colour) and sulfate (yellow colour) (Dessanti, P., Zhang, Y., Allegrini, S., Tozzi, M.G., Sgarrella, F., Ealick, S.E. Structural basis of the substrate specificity of Bacillus cereus adenosine phosphorylase. *Acta Crystallogr. Sect. D.* **2012**, 68, 239-248 (3UAW - PDB ID). The atoms are colored by atom type (the carbon atoms for His4 and Arg43 from the second subunits of dimer are colored by grey).

HPLC analysis of the enzymatic arsenolysis of adenosine

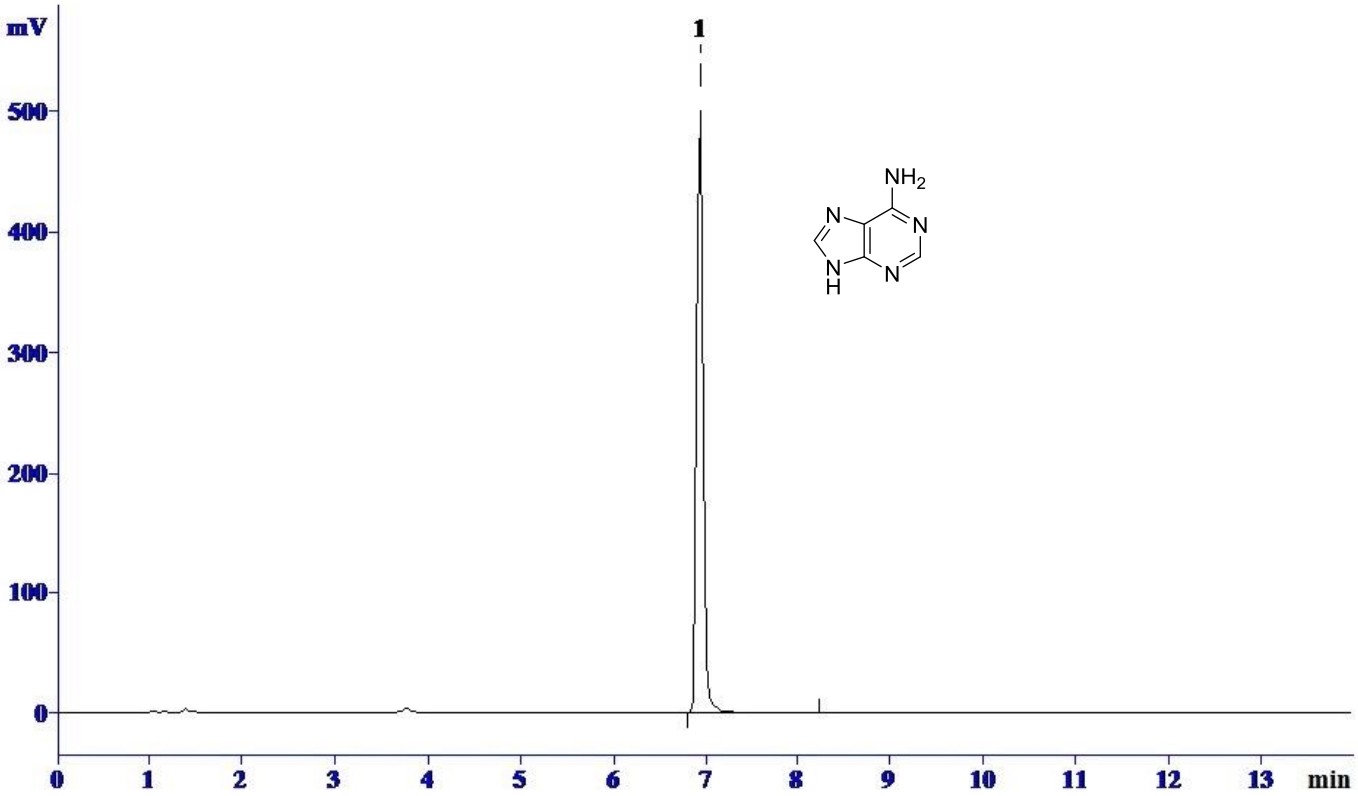
HPLC analysis of the enzymatic arsenolysis of adenosine was performed on chromatographic column 4×150mm Dr. Maisch HPLC column (5µm, Reprosil-Pur C_{18}-AQ 120 Å, Part No r15.aq.s1504, Dr. Maisch HPLC GmbH (Germany), in the linear gradient of acetonitrile in deionized water from 2 to 12% for 10 min at the flow rate of 1 ml/min with UV detection at the wavelength 260 nm.



SAMPLE	Ado 200 µM, Na_2HAsO_4×$7H_2O$ 100 µM 1:0.5, Tris-HCl, pH 7.50, H_2O, λ=260 nm, Enz. not added	
COLUMN	Size: 2.0 × 60 mm; Particle size: 5.0 µm	
REACTION TIME	0 h, Enzyme not added	
RESULTS:	1 (adenosine)	2 (adenine)
	10.36 min; 100%	0%



SAMPLE	Ado 200 µM, $Na_2HAsO_4 \times 7H_2O$ 100 µM 1:0.5, Tris-HCl, pH 7.50, H_2O, λ=260 nm, PNP 2 µl, Enz. added	
COLUMN	Size: 2.0 × 60 mm; Particle size: 5.0 µm	
REACTION TIME	2 hours	
RESULTS:	1 (adenine)	2 (adenosine)
	7.106 min; 58.91%	10.41 min; 41.09%



SAMPLE	Ado 200 µM, $Na_2HAsO_4 \times 7H_2O$ 100 µM 1:0.5, Tris-HCl, pH 7.50, H_2O, λ=260 nm, PNP 2 µl, Enz. added	
COLUMN	Size: 2.0 × 60 mm; Particle size: 5.0 µm	
REACTION TIME	24 hours	
RESULTS:	1 (adenine)	2 (adenosine)
	6.946 min; 100%	0%

HPLC analysis of the enzymatic arsenolysis of the purine nucleosides 1b, 1i-m

HPLC analysis of the test reaction was performed on a Dr. Maisch Reprosil-Pur C_{18}-AQ column (4×150mm, 5µm, 120 Å, Part No r15.aq.s1504, Dr. Maisch HPLC GmbH (Germany), in a linear gradient of acetonitrile in deionized water from 2 to 12% for 10 min at the flow rate of 1 ml/min with UV detection at wavelength 260 nm.

COMPOUND	SAMPLE	COLUMN	REACTION TIME	RESULTS
1b	Nucleoside 0.28 µmol, $Na_2HAsO_4×7H_2O$ 0.14 µmol, 50mM Tris-HCl buffer, pH 7.5 (10 ml), PNP *E.coli* 2.8 µl (0.1 activity units)	Size: 2.0×60 mm; Particle size: 5.0 µm	0 h	8.6 min, 100%
2b			24 h	8.9 min, 100%
1i	Nucleoside 0.29 µmol, $Na_2HAsO_4×7H_2O$ 0.145 µmol, 50mM Tris-HCl buffer, pH 7.5 (10 ml), PNP *E.coli* 2.8 µl (0.1 activity units)		0 h	8.6 min, 100%
2i			24 h	8.8 min, 100%
1j	Nucleoside 0.25 µmol, $Na_2HAsO_4×7H_2O$ 0.125 µmol, 50mM Tris-HCl buffer, pH 7.5 (10 ml), PNP *E.coli* 2.8 µl (0.1 activity units)		0 h	13.5 min, 100%
2j			24 h	14.9 min, 100%
1k	Nucleoside 0.26 µmol, $Na_2HAsO_4×7H_2O$ 0.13 µmol, 50mM Tris-HCl buffer, pH 7.5 (10 ml), PNP *E.coli* 2.8 µl (0.1 activity units)		0 h	10.3 min, 100%
2k			24 h	10.9 min, 100%
1l	Nucleoside 0.28 µmol, $Na_2HAsO_4×7H_2O$ 0.14 µmol, 50mM Tris-HCl buffer, pH 7.5 (10 ml), PNP *E.coli* 2.8 µl (0.1 activity units)		0 h	9.7 min, 100%
2l			24 h	10.1 min, 100%
1m	Nucleoside 0.27 µmol, $Na_2HAsO_4×7H_2O$ 0.135 µmol, 50mM Tris-HCl buffer, pH 7.5 (10 ml), PNP *E.coli* 2.8 µl (0.1 activity units)		0 h	10.3 min, 100%
2m			24 h	10.9 min, 100%
1n	Nucleoside 0.26 µmol, $Na_2HAsO_4×7H_2O$ 0.13 µmol, 50mM Tris-HCl buffer, pH 7.5 (10 ml), PNP *E.coli* 2.8 µl (0.1 activity units)		0 h	8.6 min, 100%
2n			24 h	10.1 min, 100%

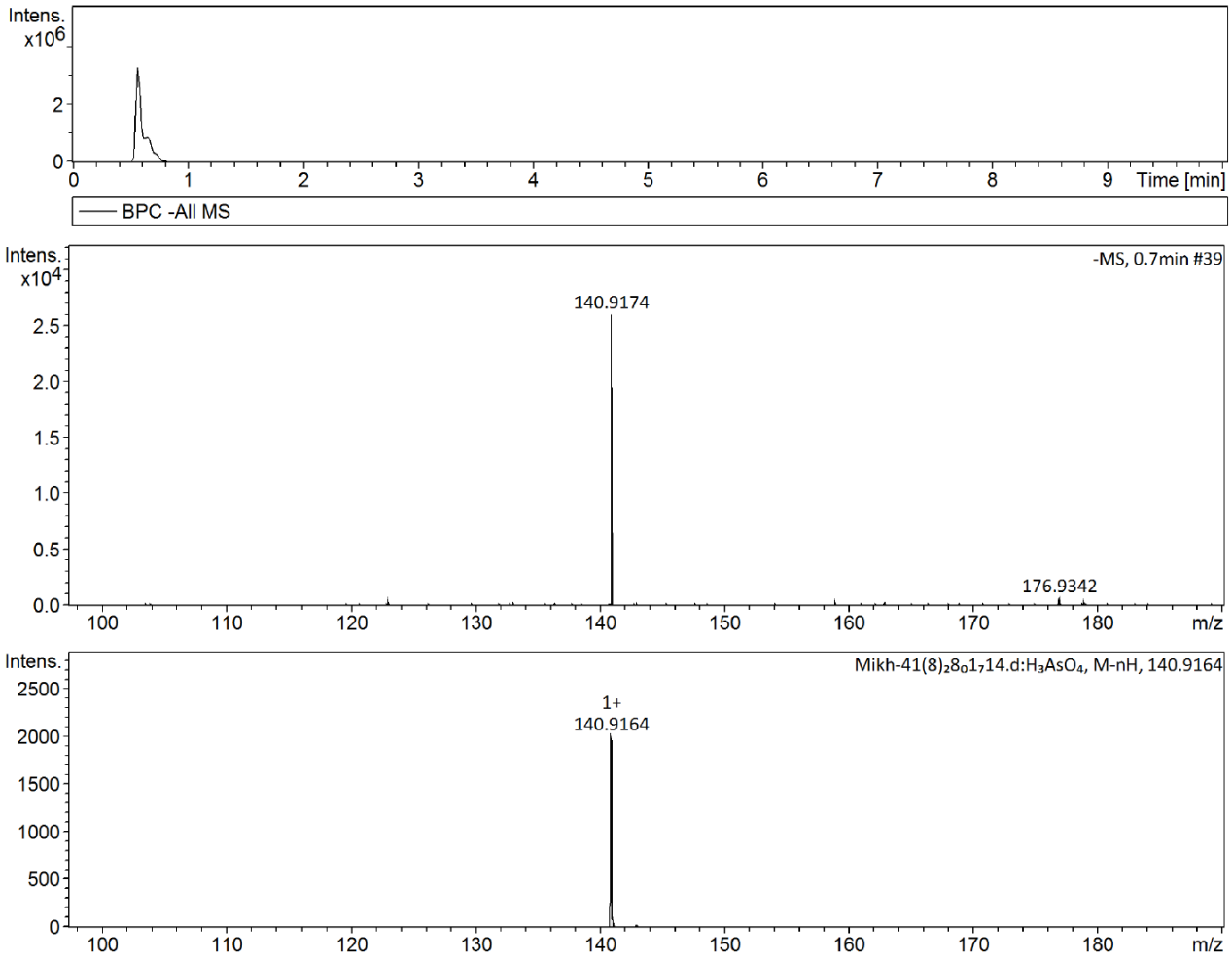
NMR and Mass-Spectrometry

^{1}H and ^{13}C (with complete proton decoupling) NMR spectra were recorded on a Bruker AMX 400 NMR instrument at 300 K relative to the residual solvent signals as internal standards ($CDCl_3$, 1H: δ = 7.26, 13C: δ = 77.16; DMSO-d_6, 1H: δ = 2.50, 13C: δ = 39.52; CD_3OD, 1H: δ = 3.31, 13C: δ = 49.00). ^{1}H-NMR-spectra were recorded at 400 MHz and ^{13}C-NMR-spectra at 100 MHz.

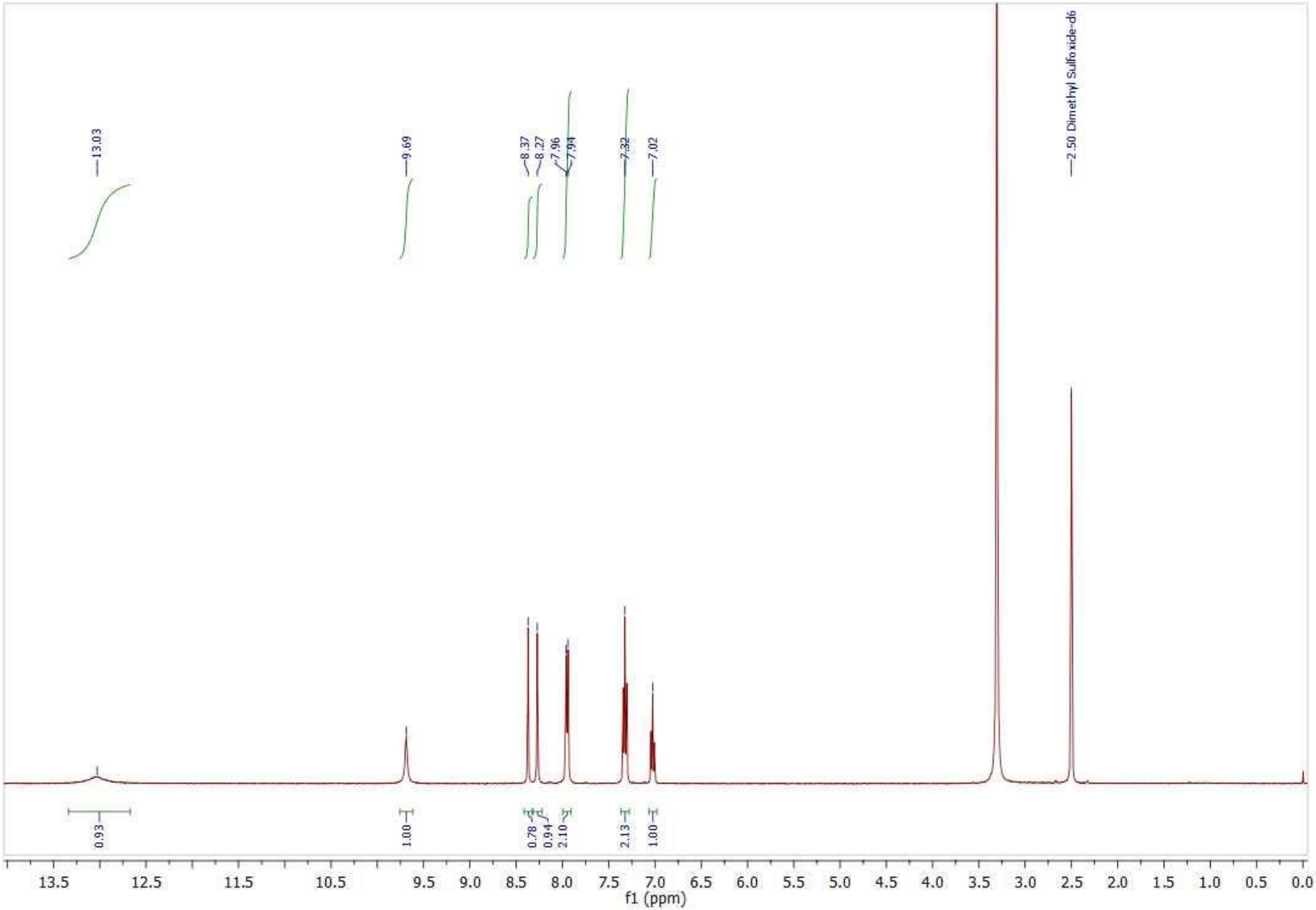
High resolution mass spectra (HRMS) were registered on a Bruker Daltonics micrOTOF-Q II instrument using electrospray ionization (ESI). The measurements were done in a negative ion mode. Interface capillary voltage: 4700 V; mass range from m/z 50 to 3000; external calibration (Electrospray Calibrant Solution, Fluka); nebulizer pressure: 0.4 Bar; flow rate: 3 µL/min; dry gas: nitrogen (4L/min); interface temperature: 200°C. Samples were injected in to the mass spectrometer chamber from the Agilent 1260 HPLC system equipped with Agilent Poroshell 120 EC-C18 column (3.0 × 50 mm; 2,7 µm) and an identically packed security guard, using an autosampler. The samples were injected from the 50% acetonitrile (LC-MS grade) in water (MilliQ ultrapure water, Merck Millipore KGaA, Germany) solution in the concentration of 0.1 mg/ml (350 µl) with 50 µl dopant solution of 5% trimethylamine. The autosampler syringe was washed before and after injection two times each with 1% formic acid in acetonitrile (wash 1) followed by 20% methanol in water (wash 2). The column temperature was 30°C and 15 µl of the sample solution was injected. The column was eluted in a gradient of concentrations of A (acetonitrile) in B (water) with the flow rate of 400 µl/min in the following gradient parameters: 0-15% A for 6.0 min, 15%-85% A for 1.5 min, 85%-0% A for 0.1 min, 0% A for 2.4 min.

Acquisition Parameter

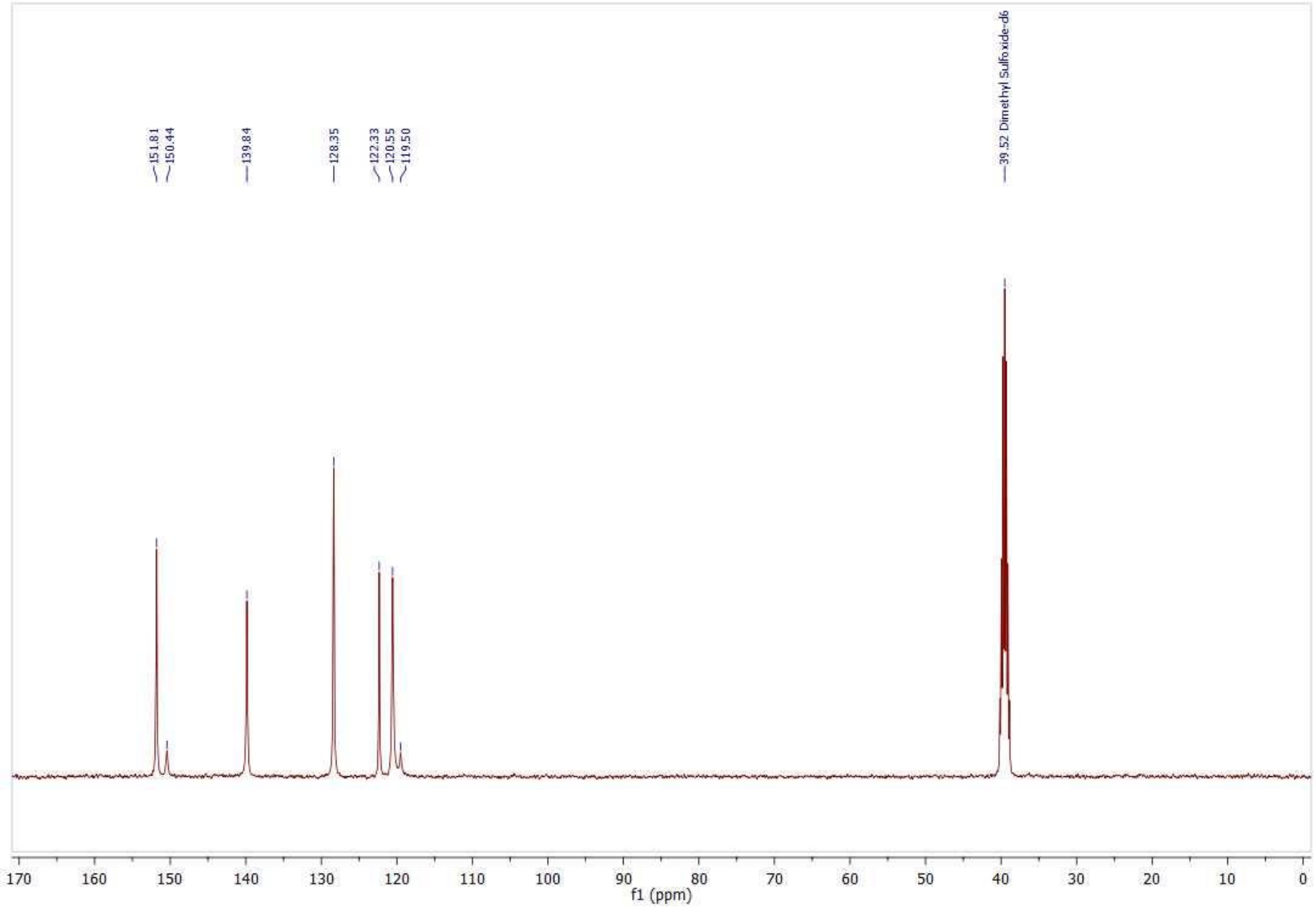
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of arsenate in the concentration of 100 µM (350 µl of the solution) with 50 µl dopant solution of 5% trimethylamine.



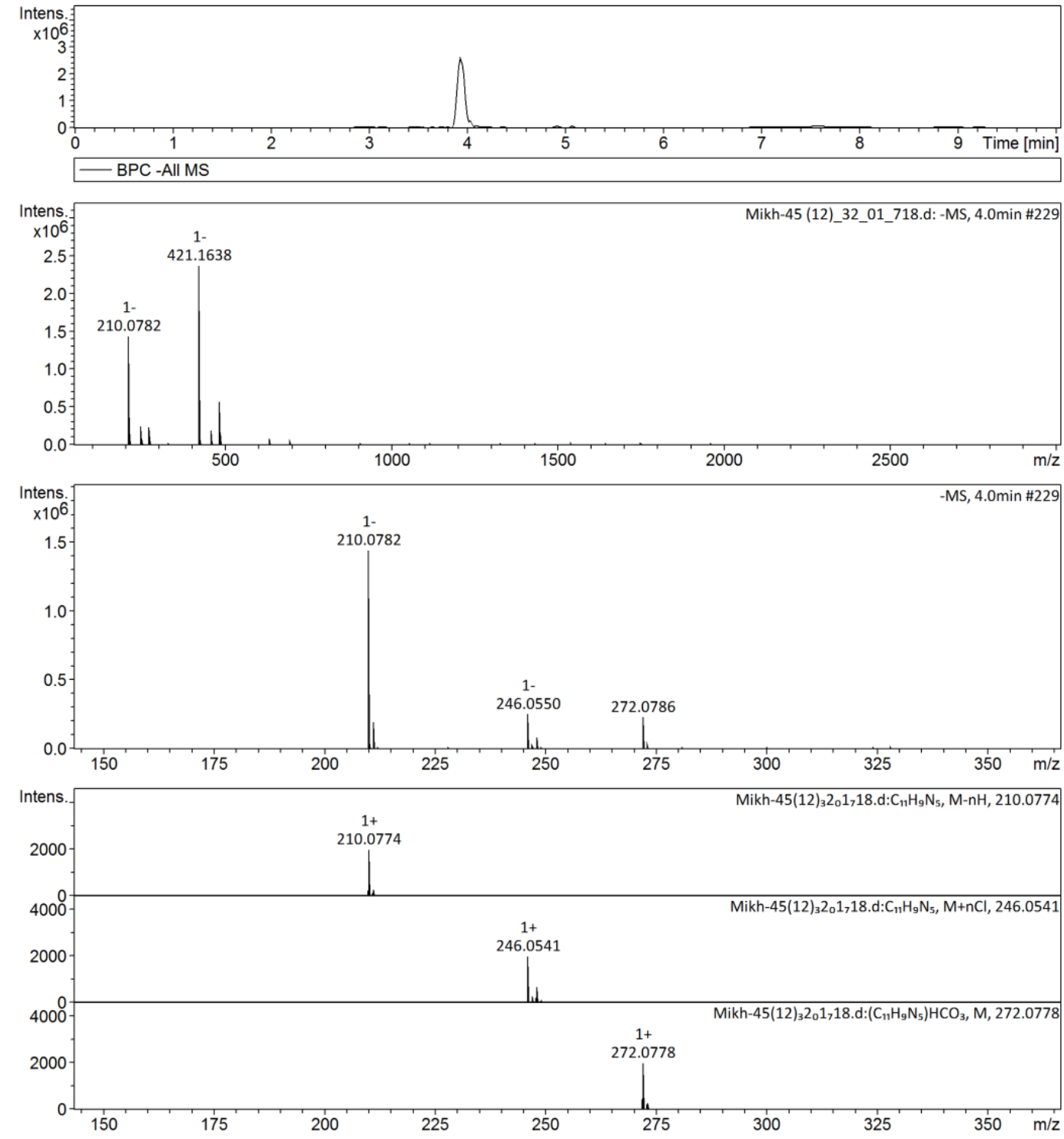
^{1}H-NMR-spectrum of N^{6}-phenyladenine (**2a**) (400 MHz) of in DMSO-d_6 at 300 K



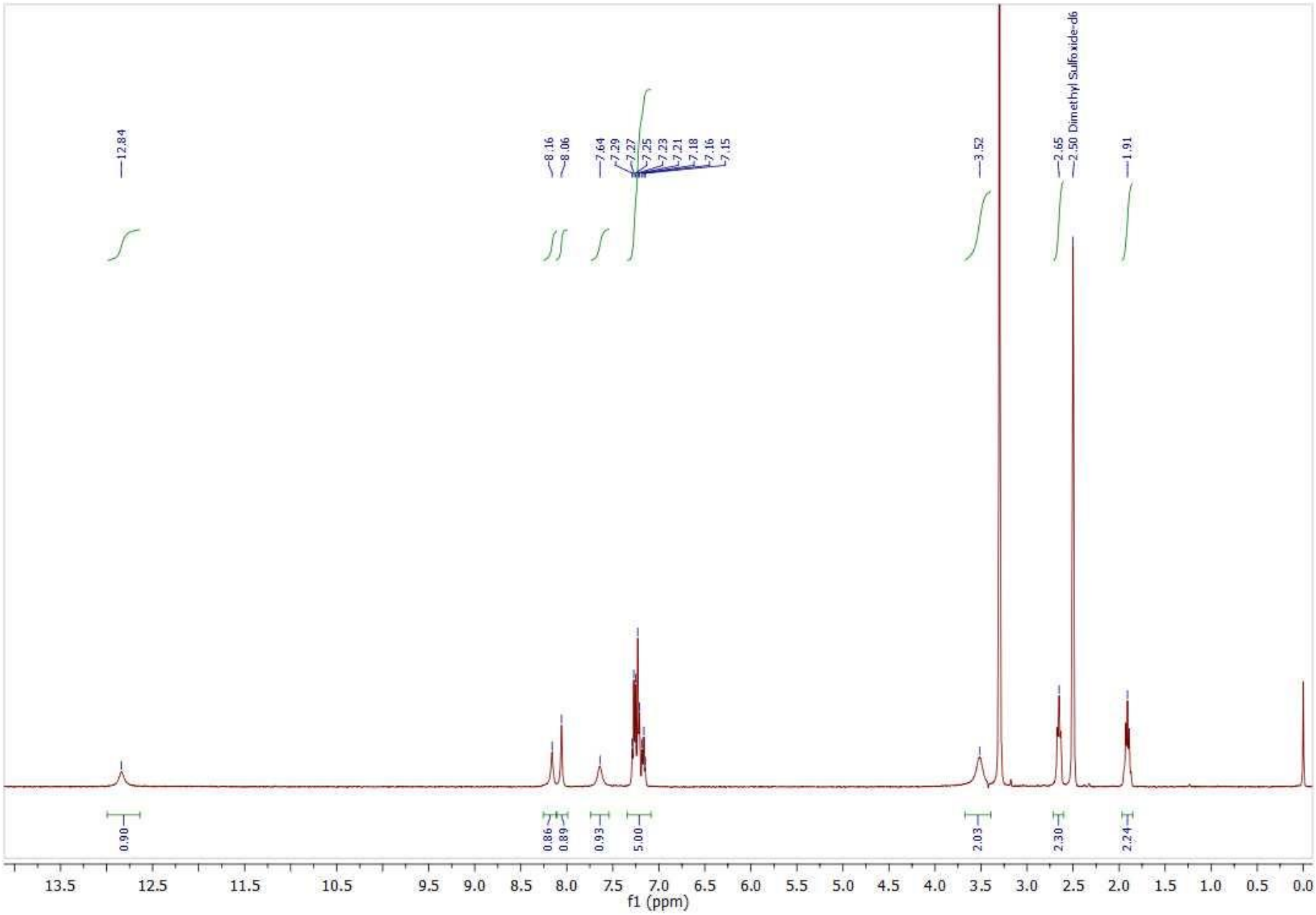
^{13}C-NMR-spectrum of N^6-phenyladenine (**2a**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

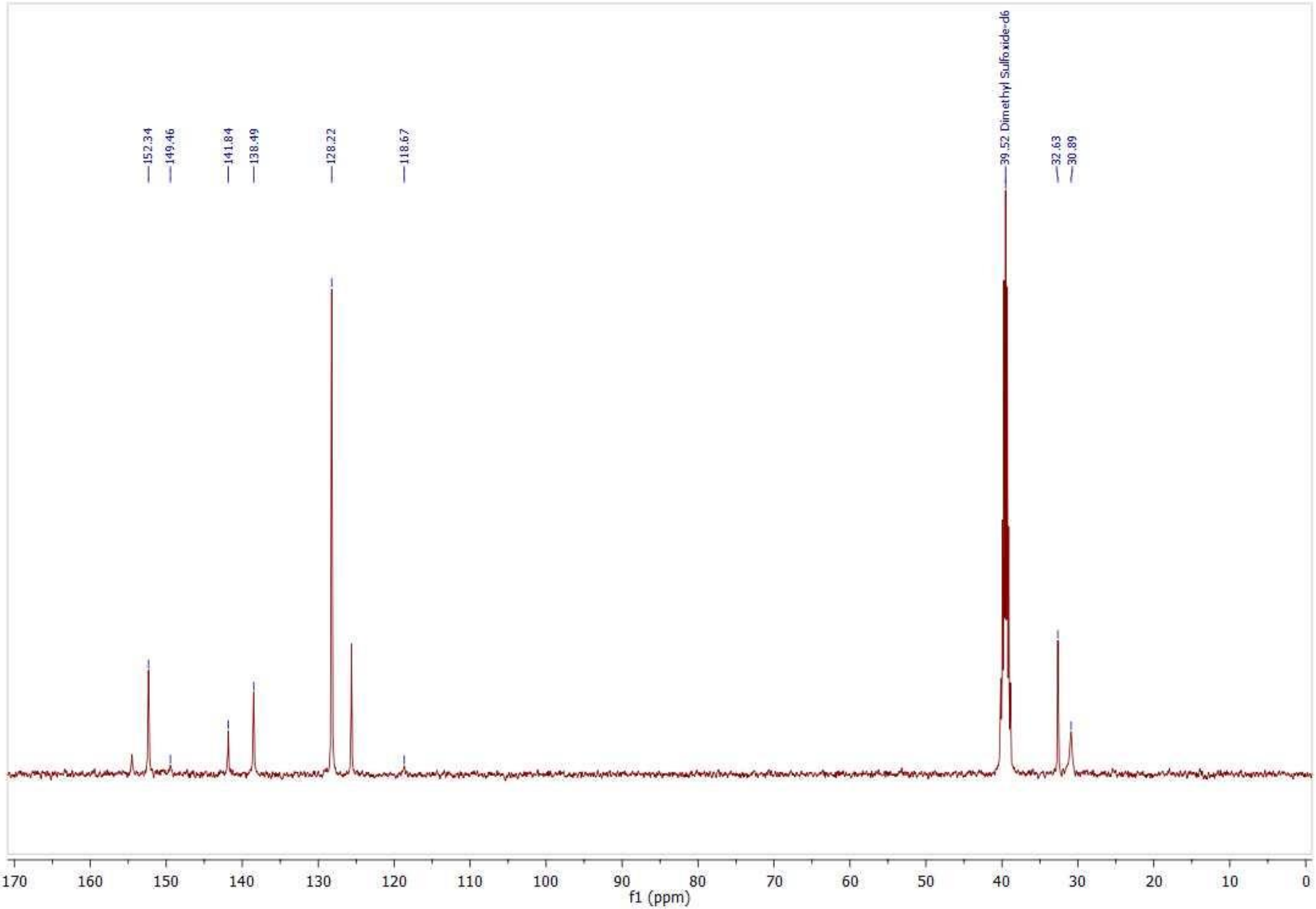
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of N^6-phenyladenine (**2a**)



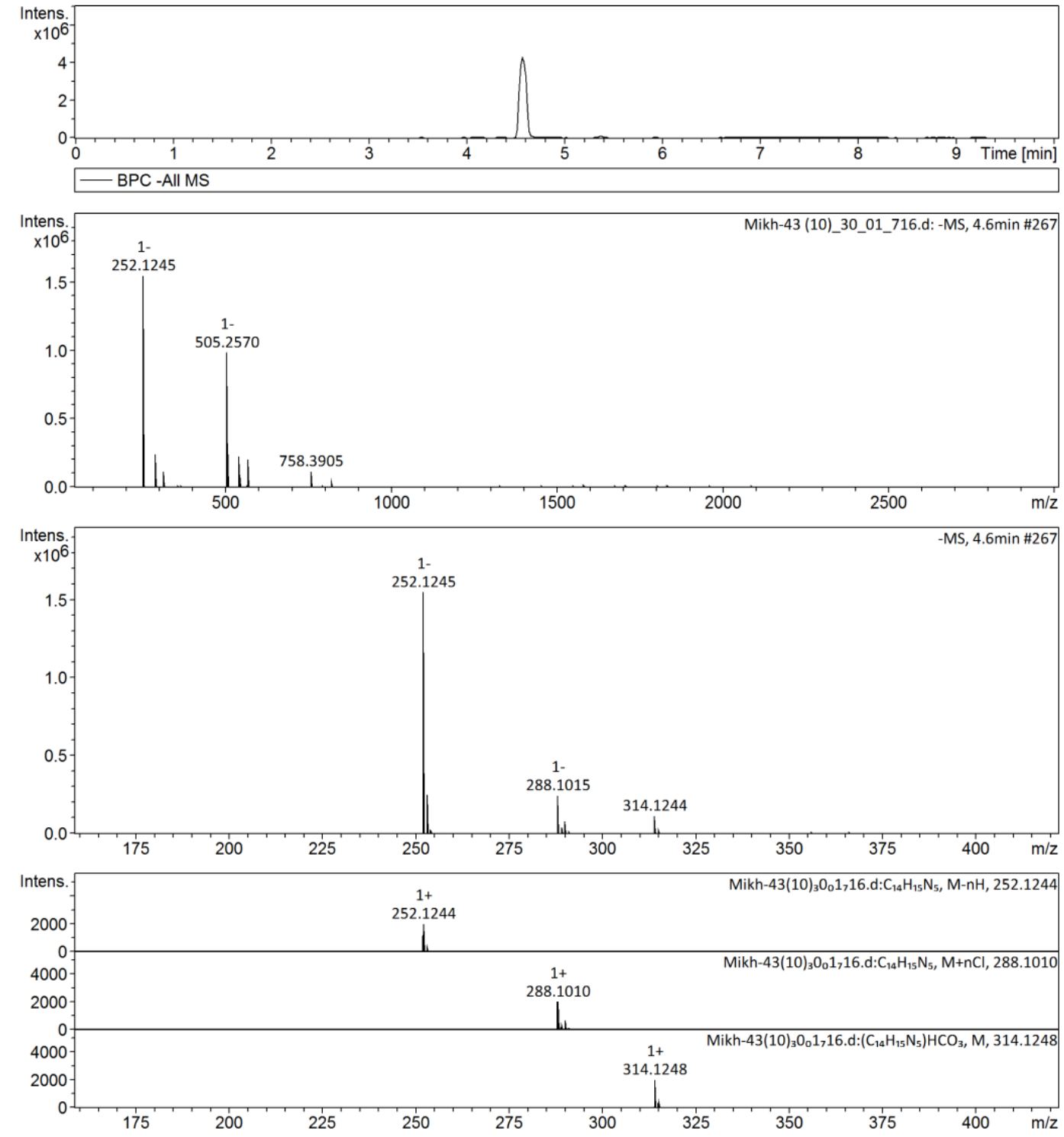
^{1}H-NMR-spectrum of N^{6}-(3-phenylpropan-1-yl)adenine (**2c**) (400 MHz) of in DMSO-d_6 at 300 K



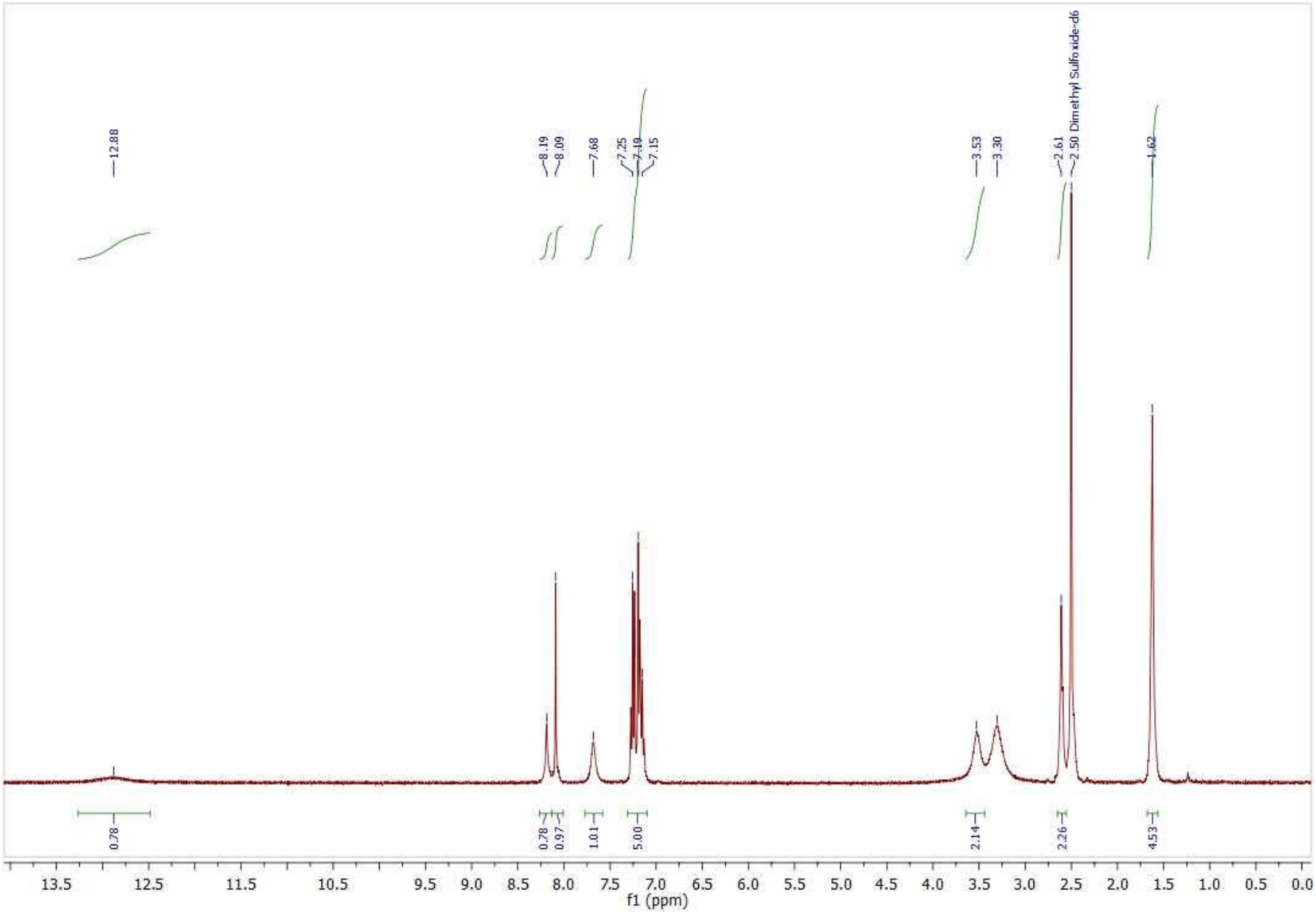
^{13}C-NMR-spectrum of N^6-(3-phenylpropan-1-yl)adenine (**2c**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

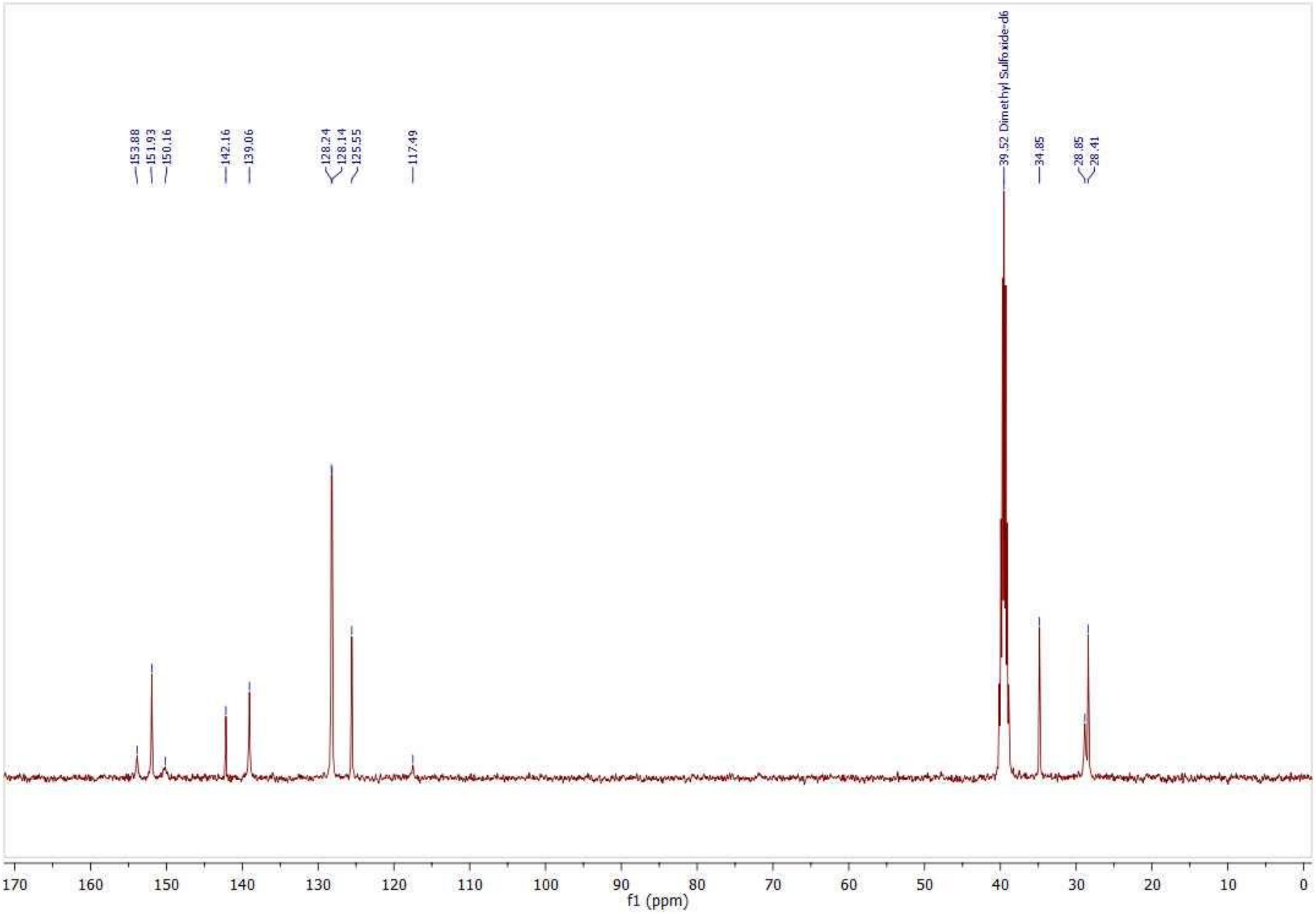
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of N^6-(3-phenylpropan-1-yl)adenine (**2c**)



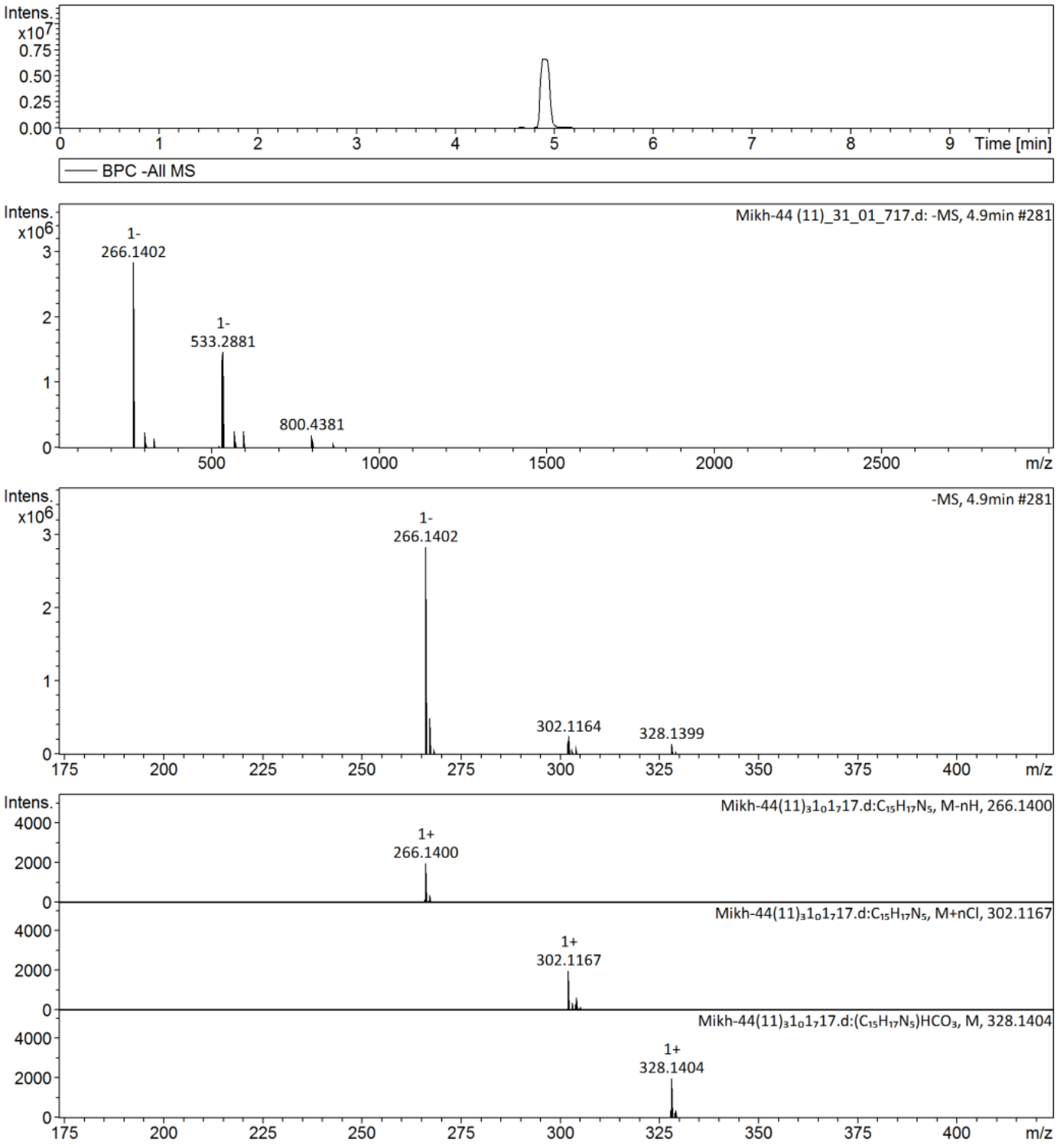
^{1}H-NMR-spectrum of N^{6}-(4-phenylbutane-1-yl)adenine (**2d**) (400 MHz) of in DMSO-d_6 at 300 K



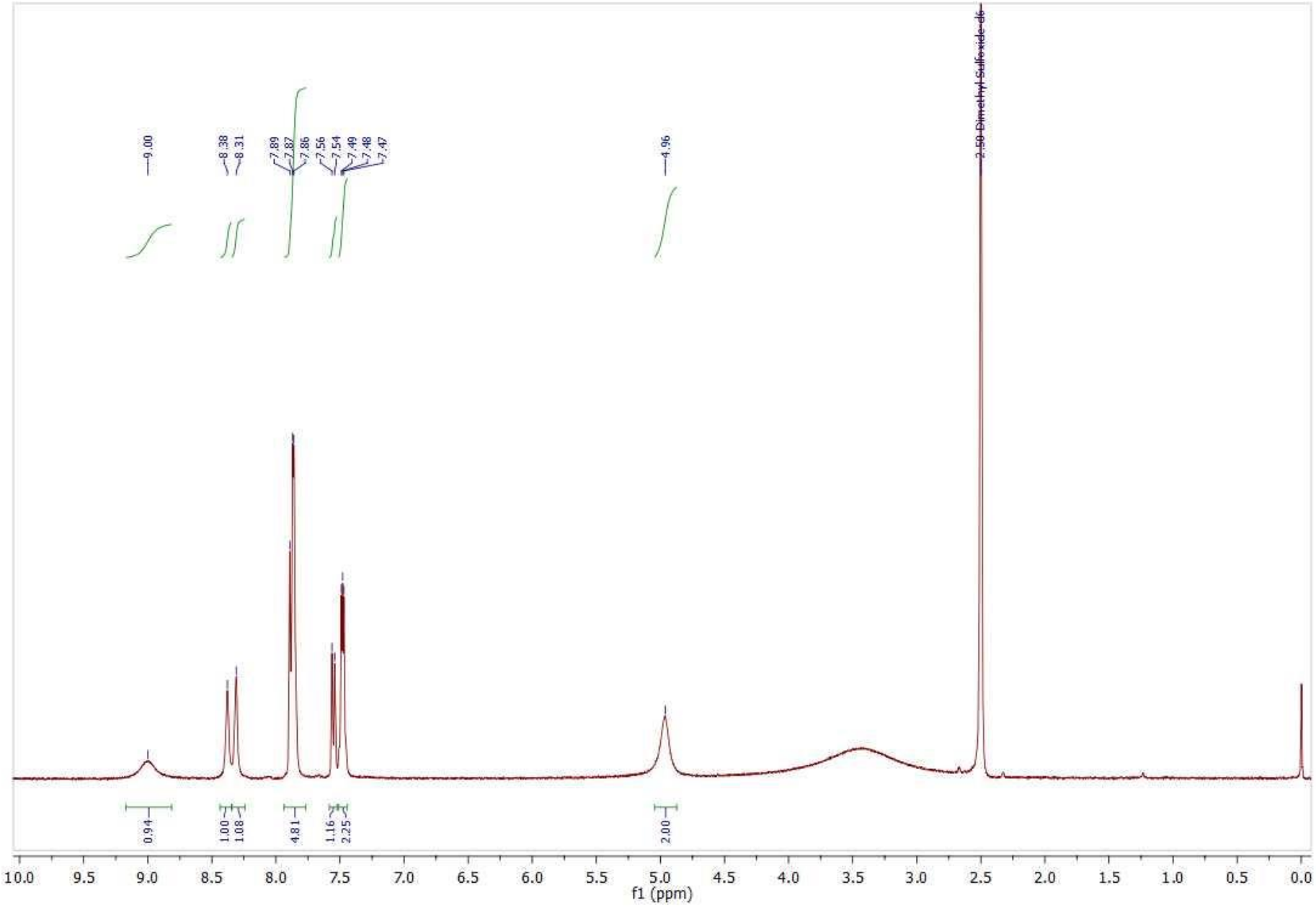
^{13}C-NMR-spectrum of N^6-(4-phenylbutane-1-yl)adenine (**2d**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

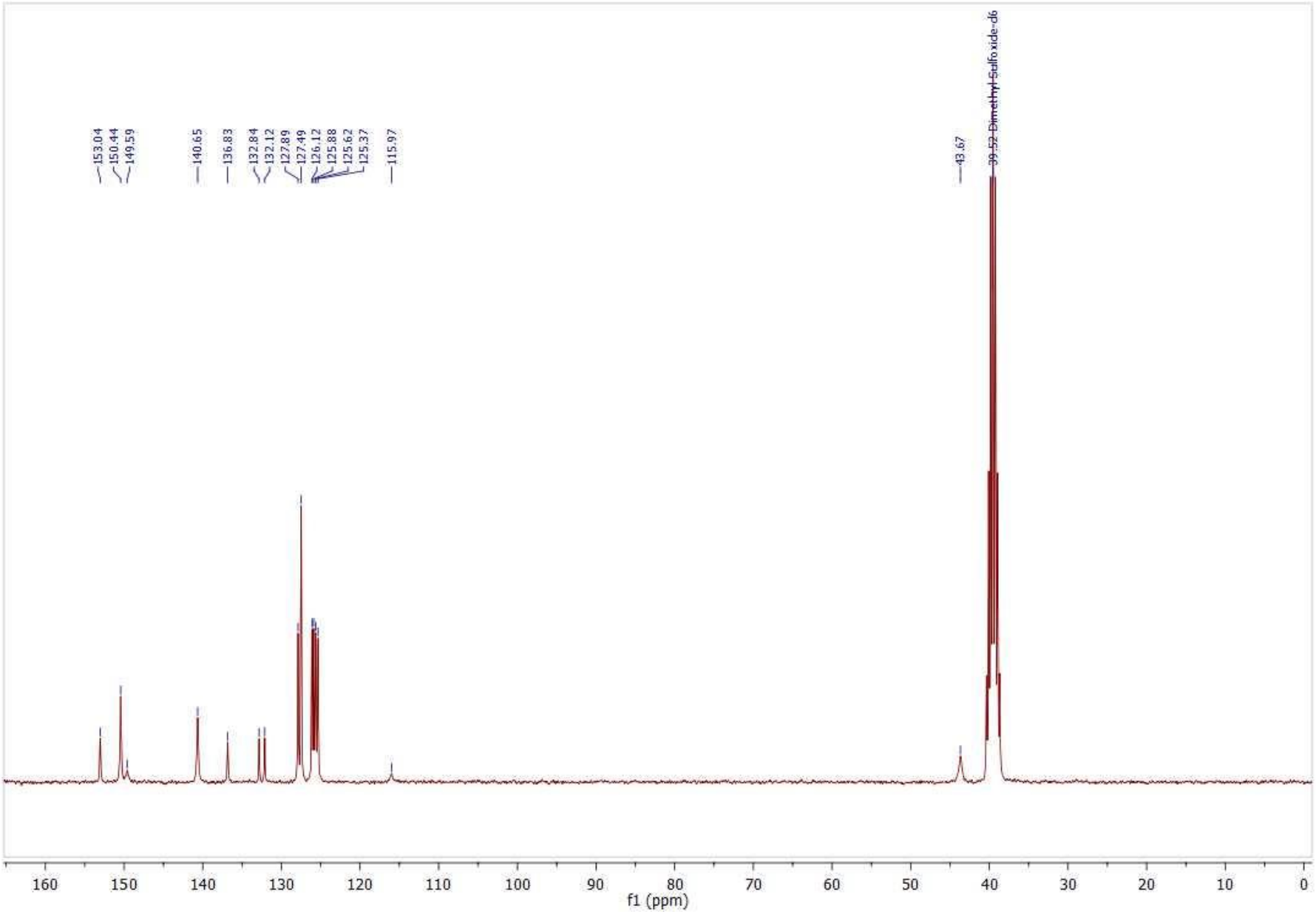
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of N^6-(4-phenylbutane-1-yl)-adenine (**2d**)



^{1}H-NMR-spectrum of N^{6}-(β-naphthylmethyl)adenine (**2e**) (400 MHz) of in DMSO-d_6 at 300 K



^{13}C-NMR-spectrum of N^6-(β-naphthylmethyl)adenine (**2e**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
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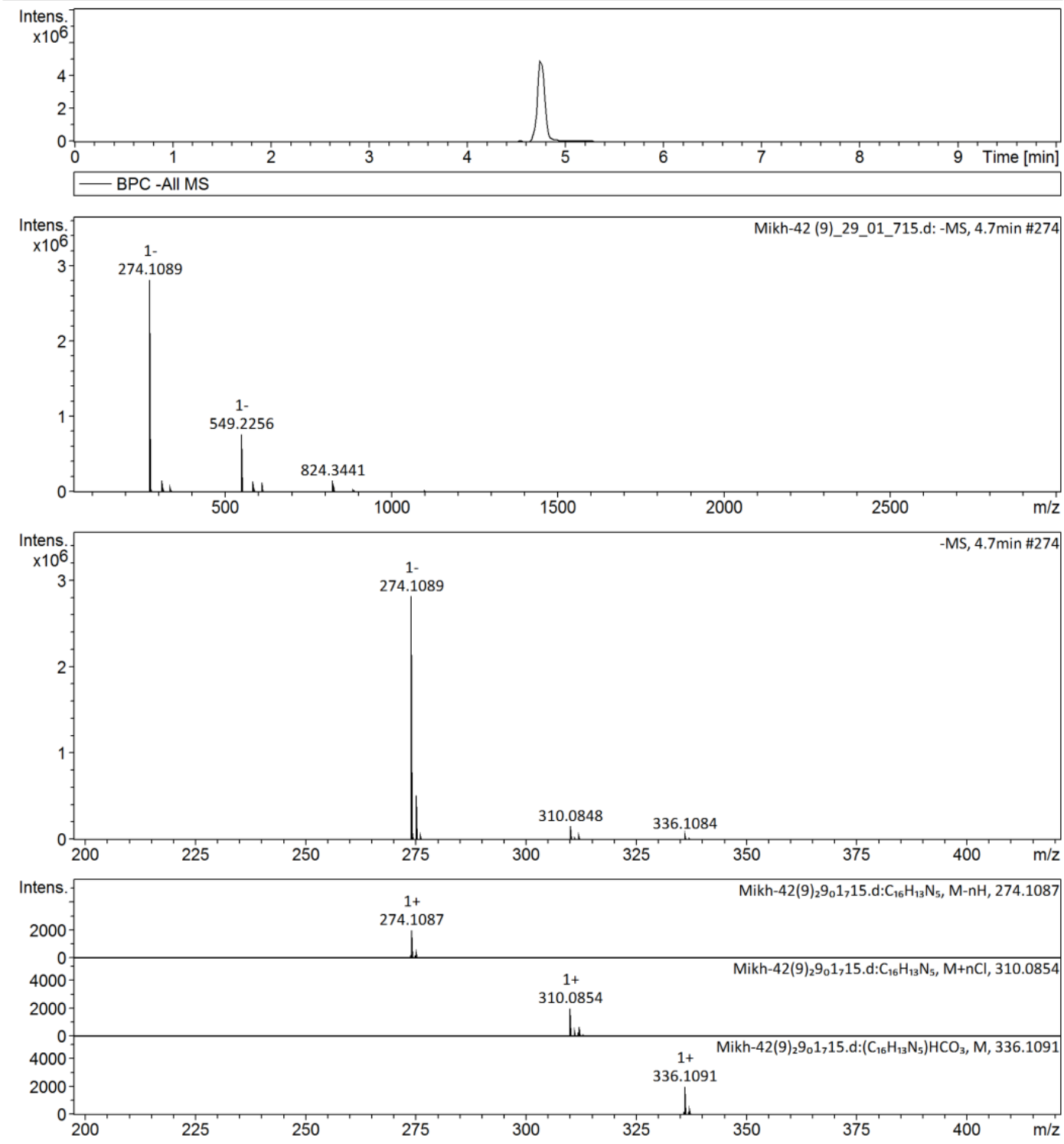
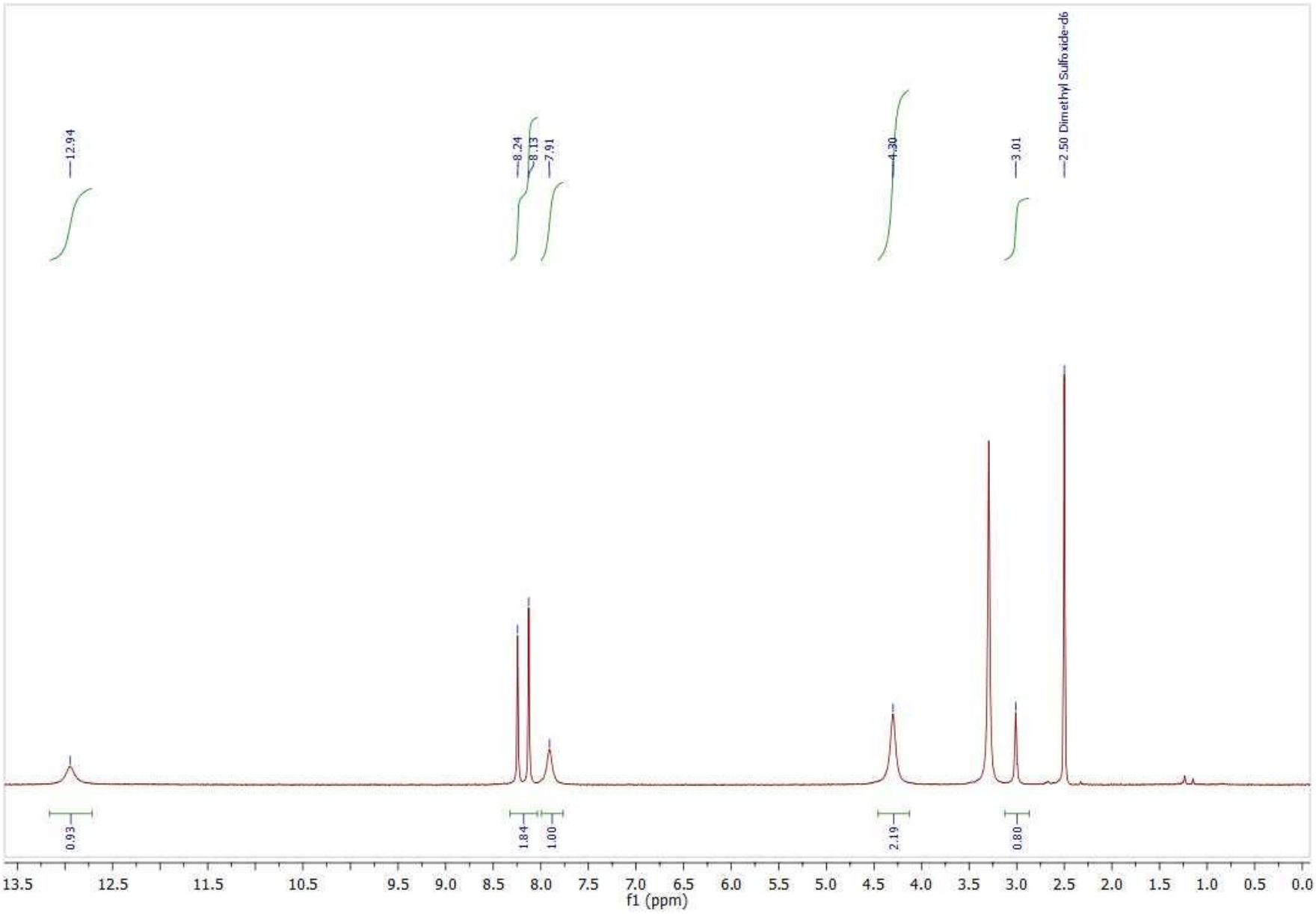
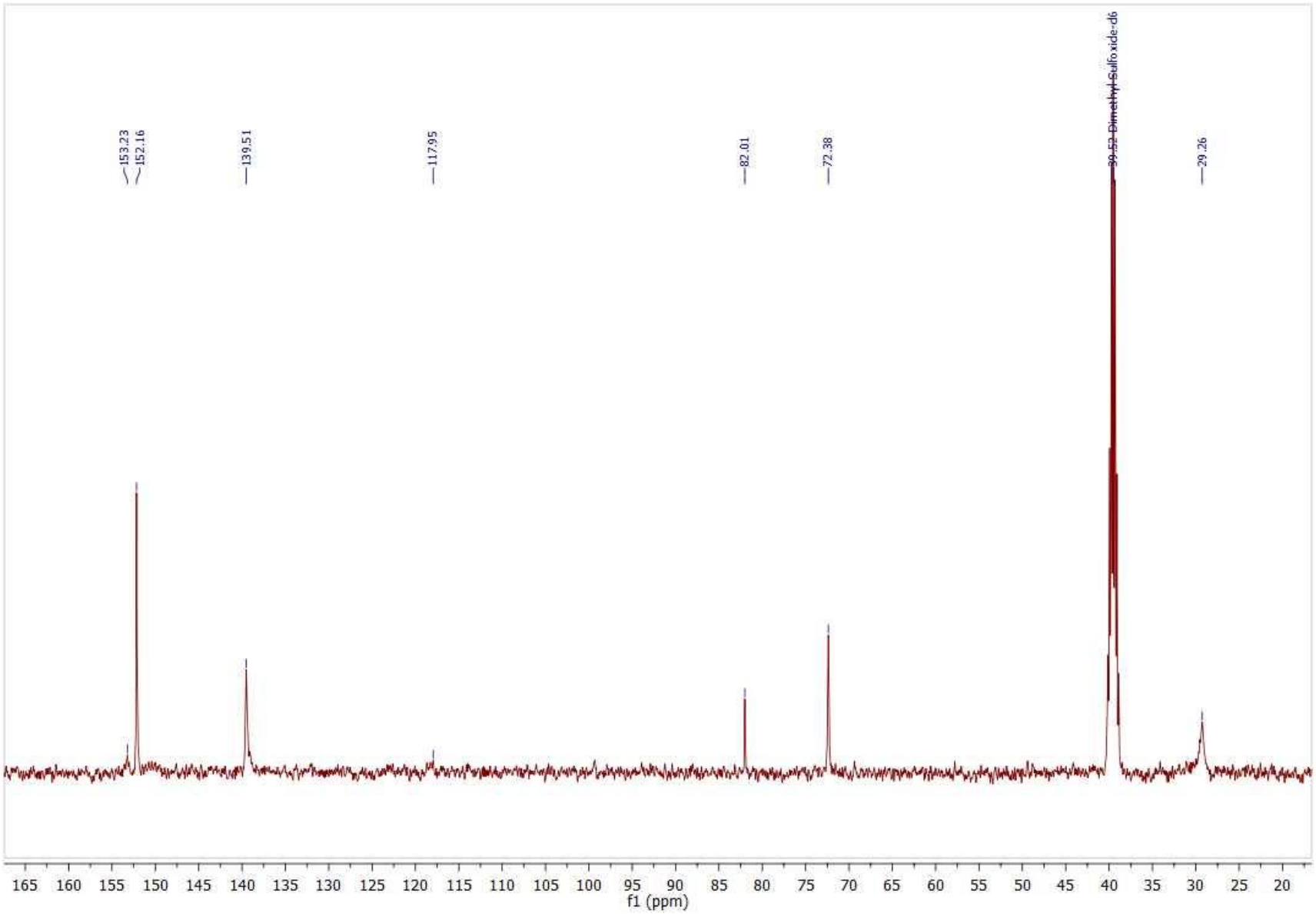


High-resolution mass spectrum (HRMS) of N^6-(β-naphthylmethyl)adenine (**2e**)



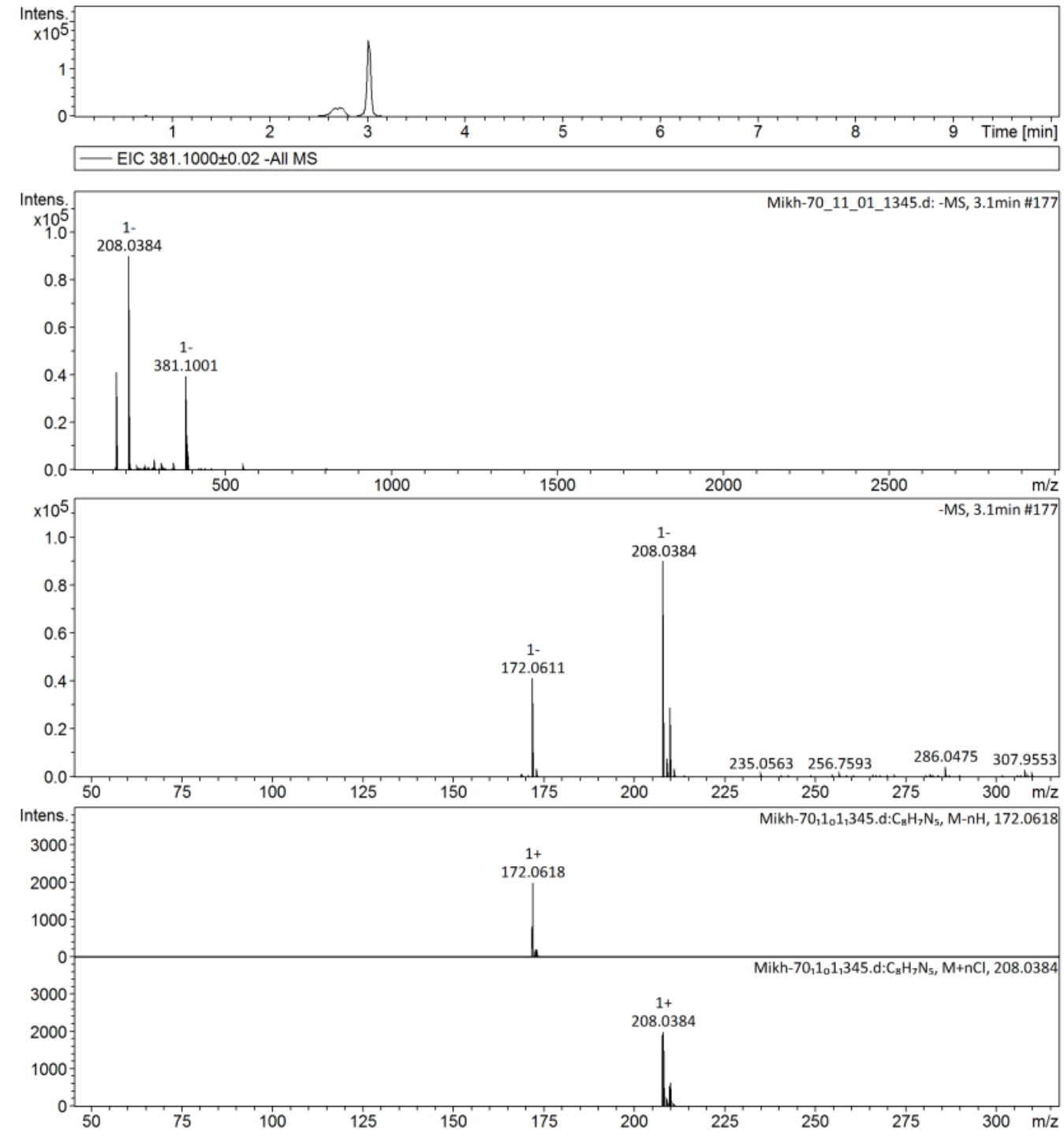
^{1}H-NMR-spectrum of N^{6}-propargyladenine (**2f**) (400 MHz) of in DMSO-d_6 at 300 K



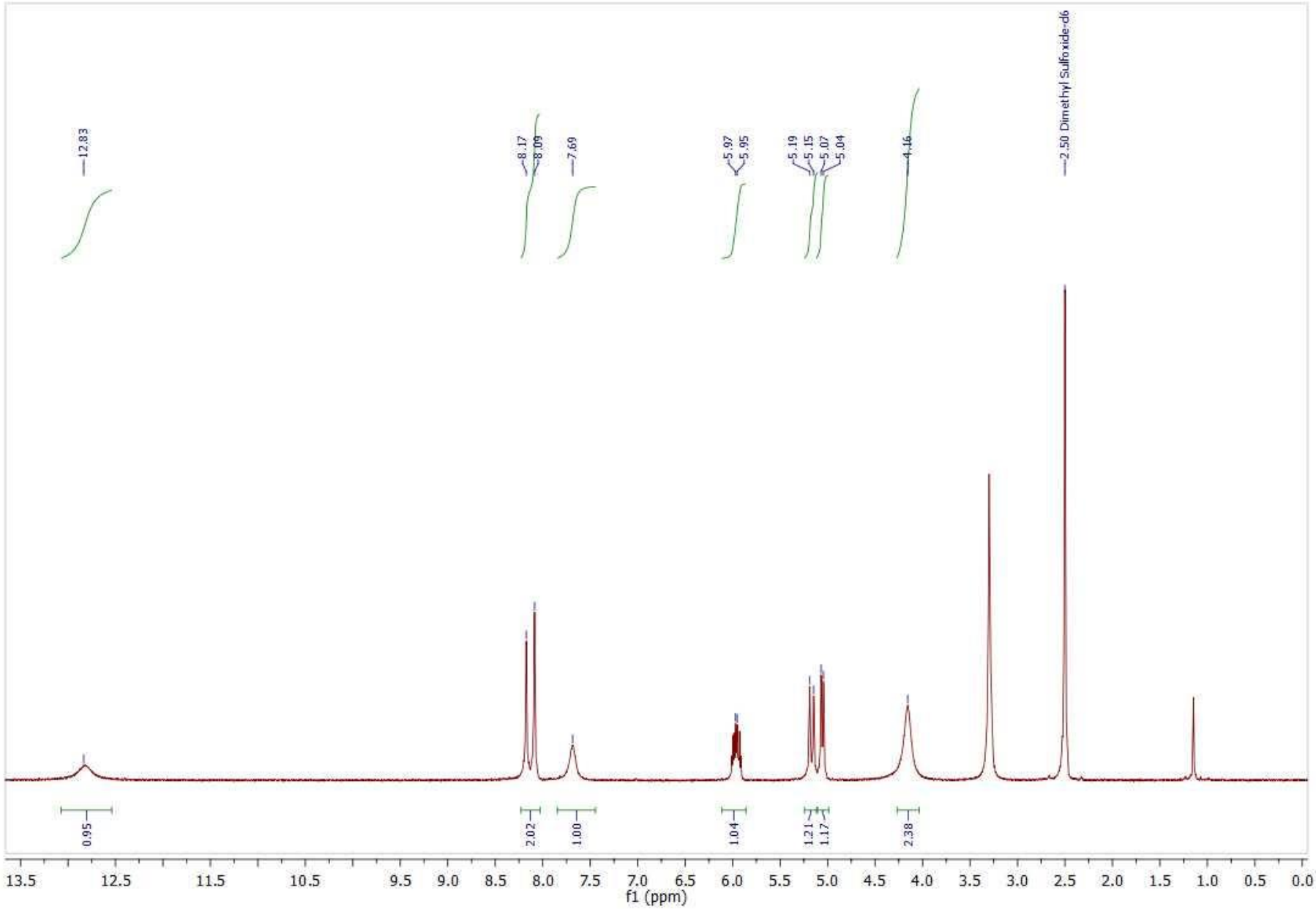
^{13}C-NMR-spectrum of N^6-propargyladenine (**2f**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

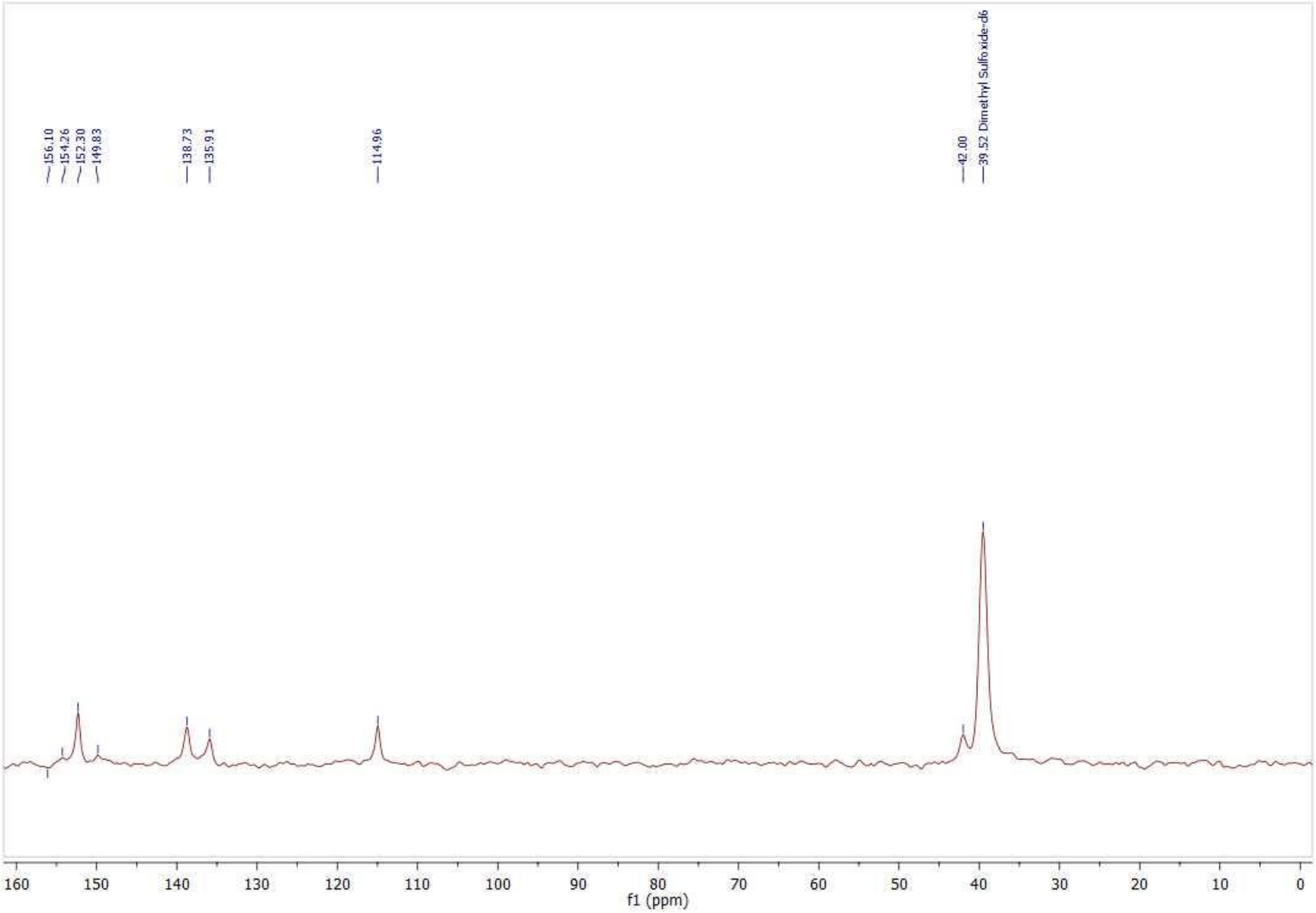
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of N^6-propargyladenine (**2f**)



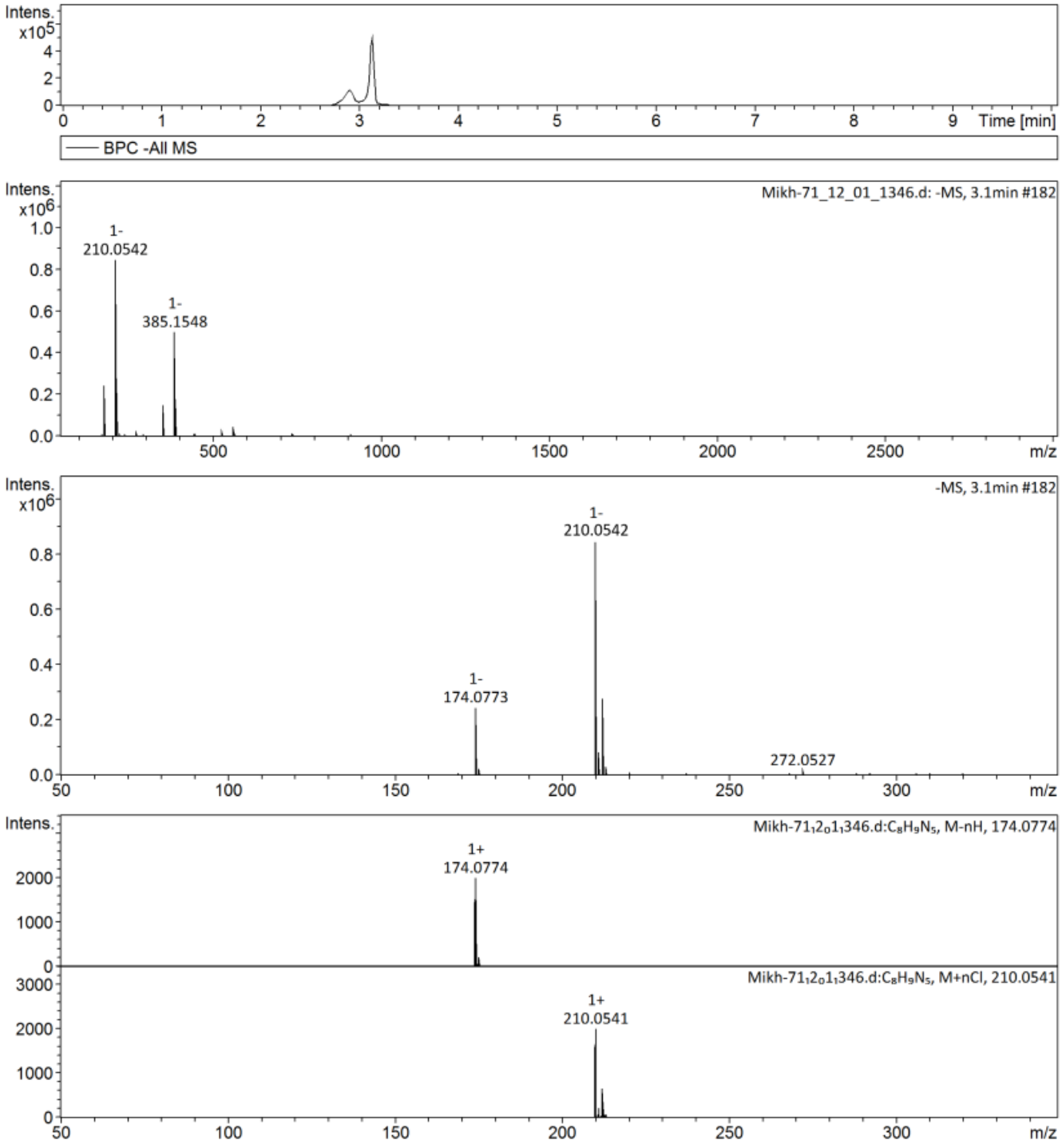
^{1}H-NMR-spectrum of N^{6}-allyladenine (**2g**) (400 MHz) of in DMSO-d_6 at 300 K



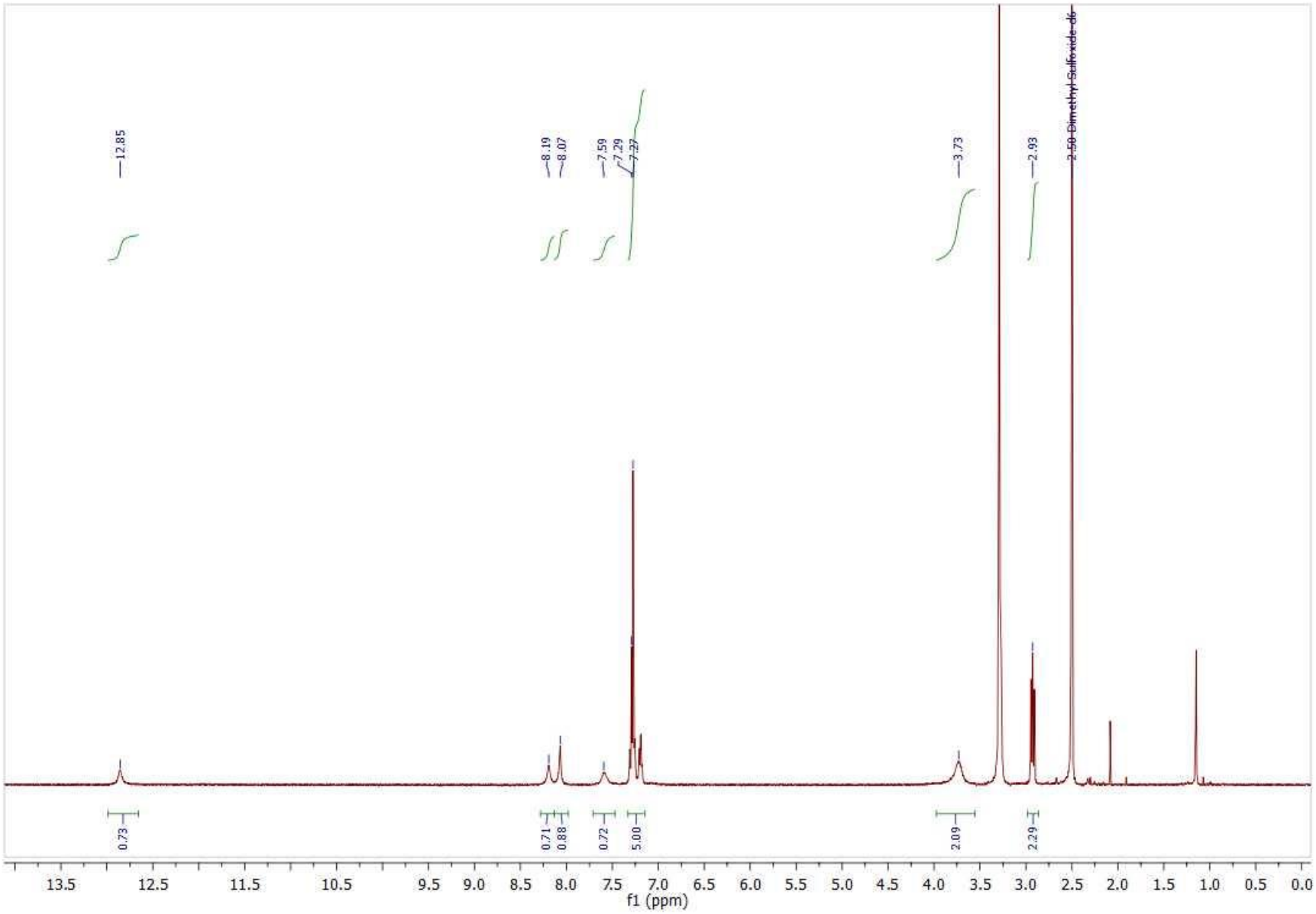
^{13}C-NMR-spectrum of N^6-allyladenine (**2g**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

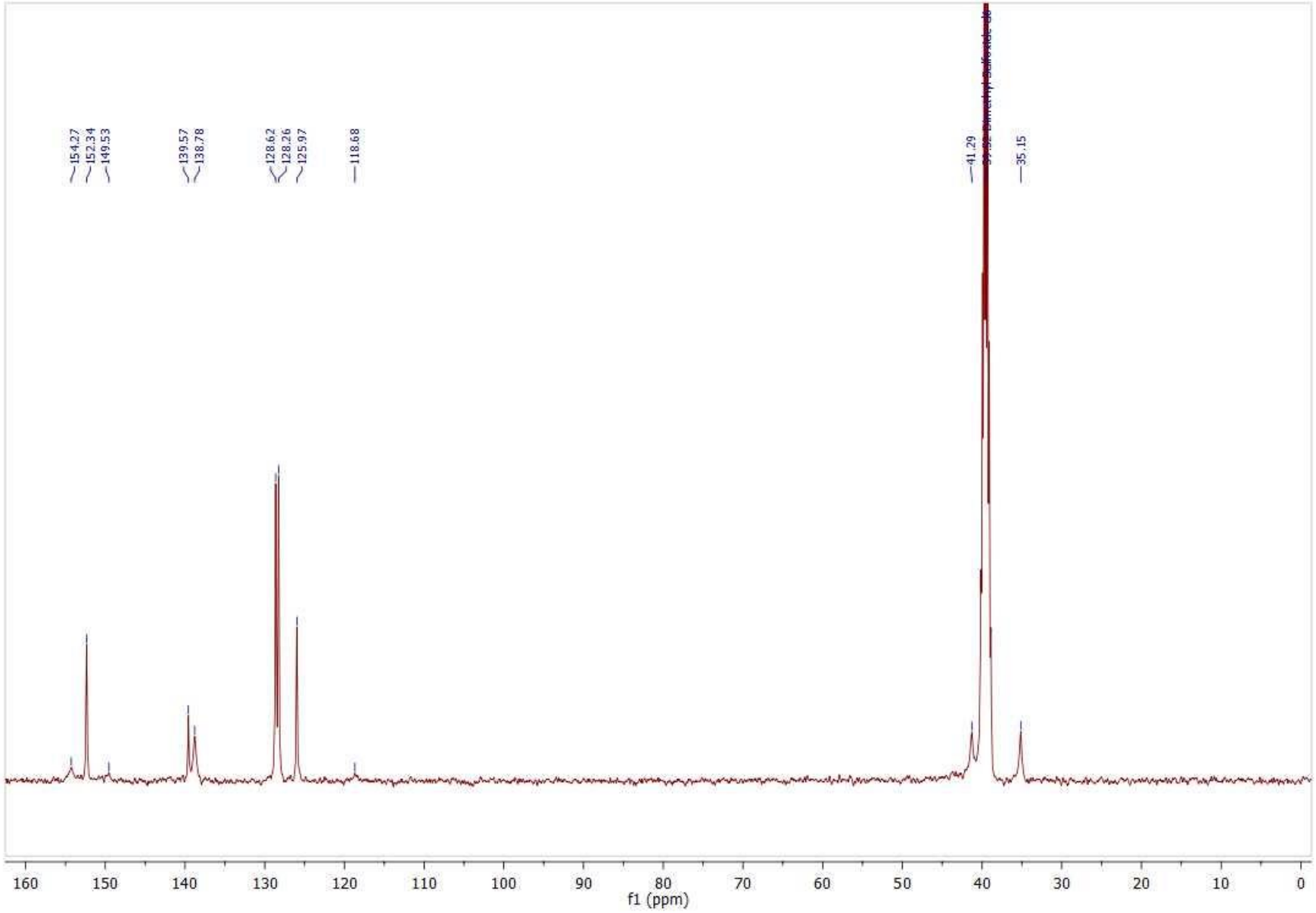
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of N^6-allyladenine (**2g**)



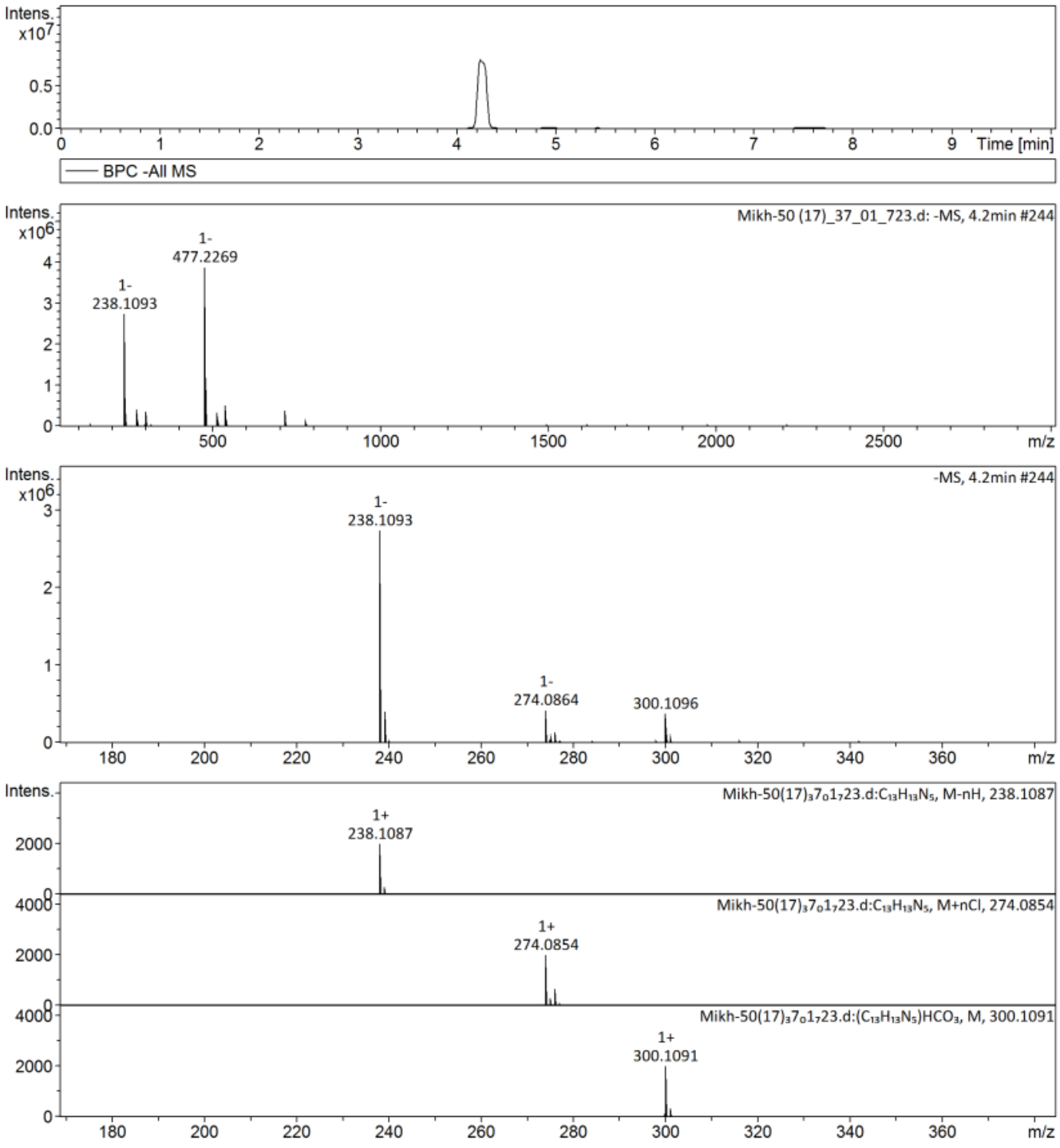
^{1}H-NMR-spectrum of N^{6}-(2-phenylethyl)adenine (**2h**) (400 MHz) of in DMSO-d_6 at 300 K



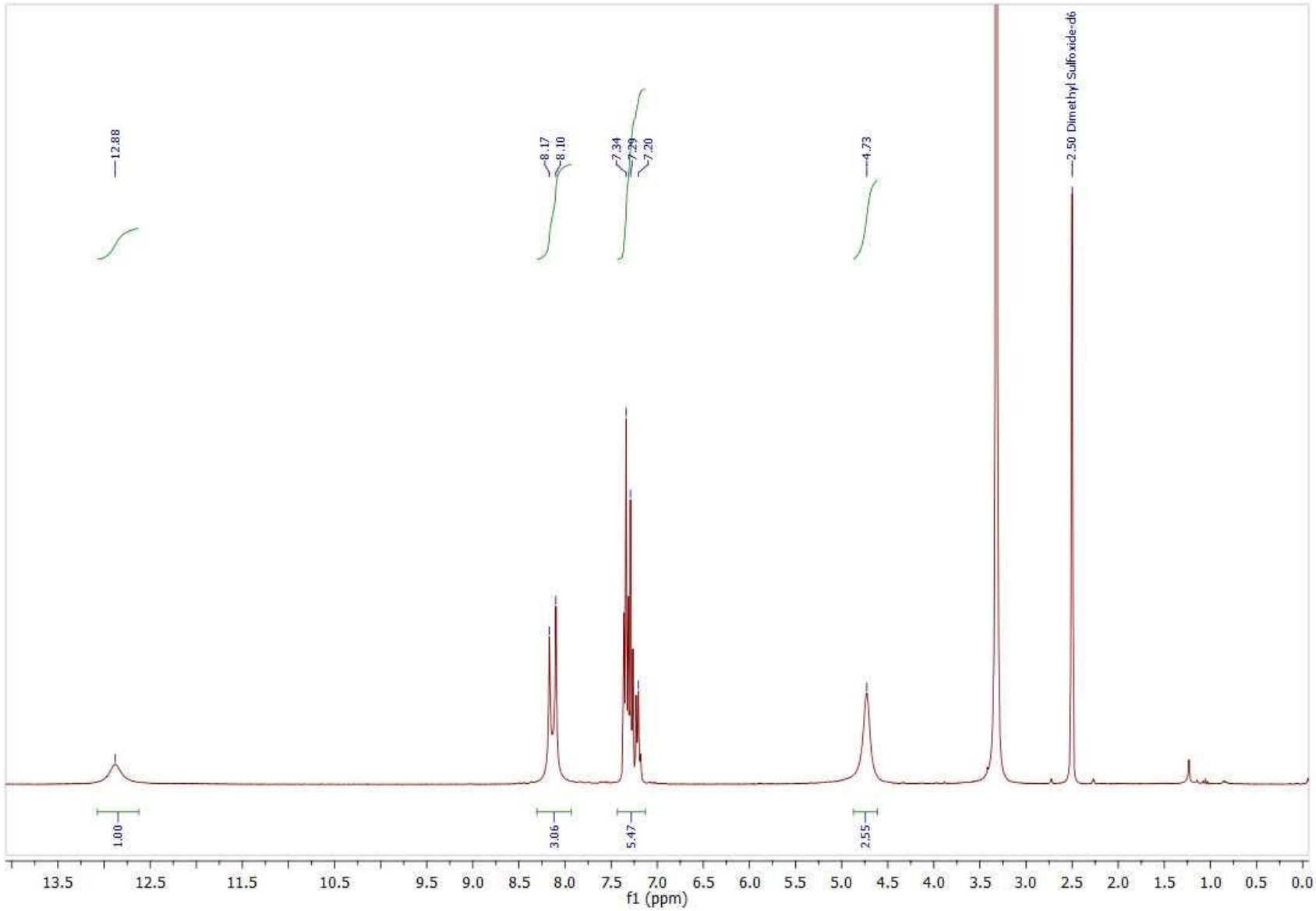
^{13}C-NMR-spectrum of N^6-(2-phenylethyl)adenine (**2h**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of N^6-(2-phenylethyl)adenine (**2h**)

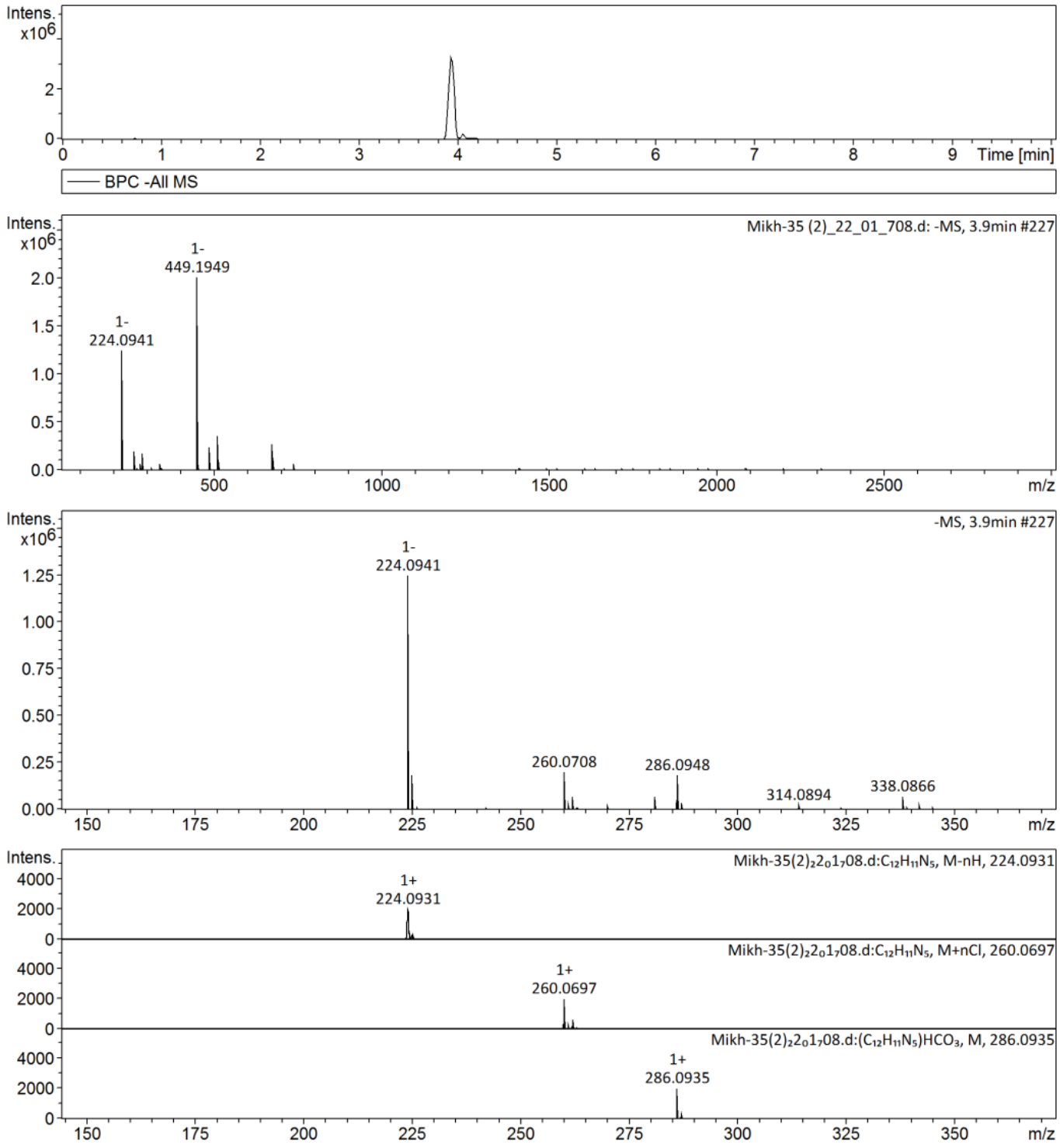


^{1}H-NMR-spectrum of N^{6}-benzyladenine (**2b**) (400 MHz) of in DMSO-d_6 at 300 K

(Prepared by the arsenolysis reaction)

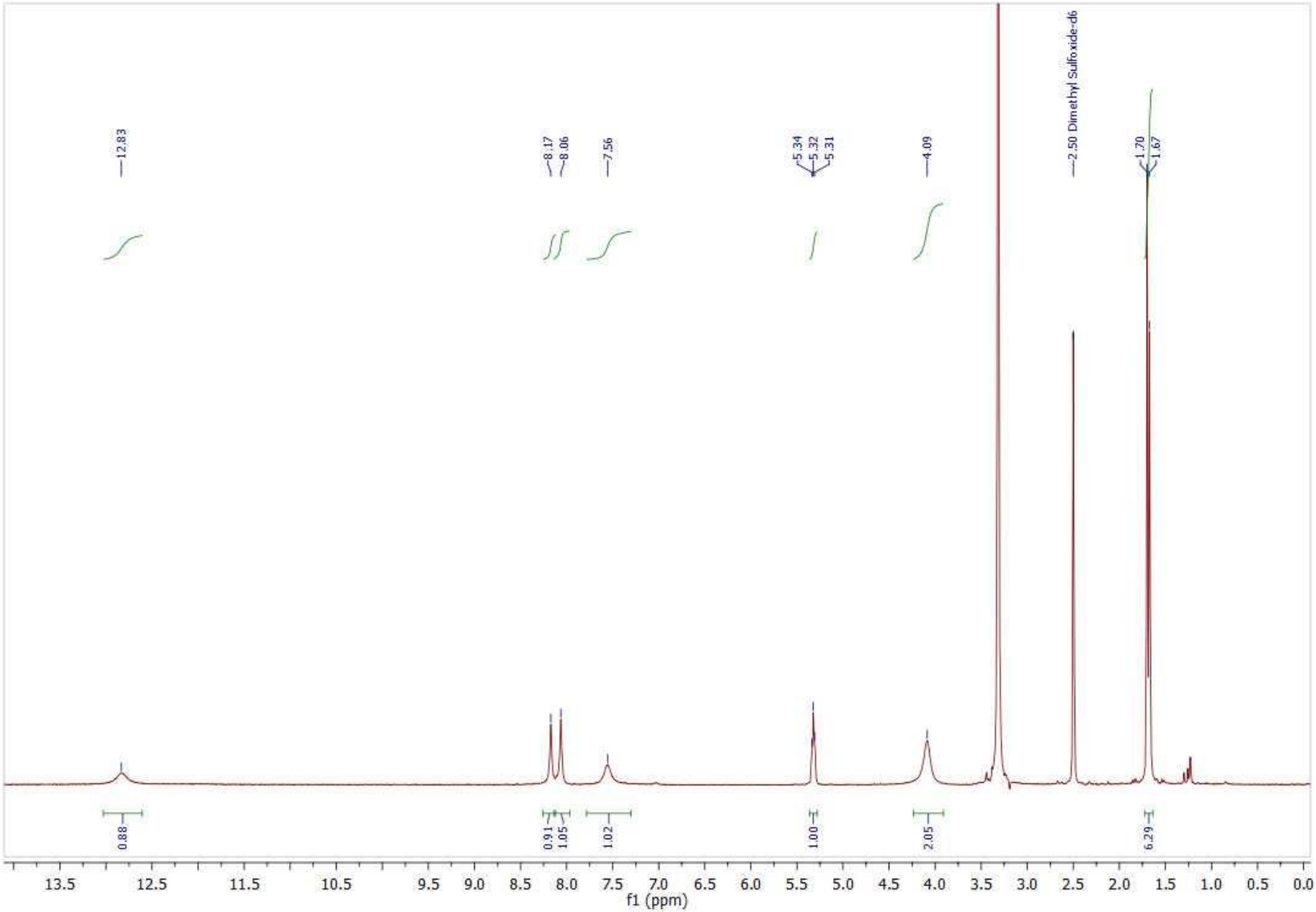
Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of N^6-benzyladenine (**2b**)

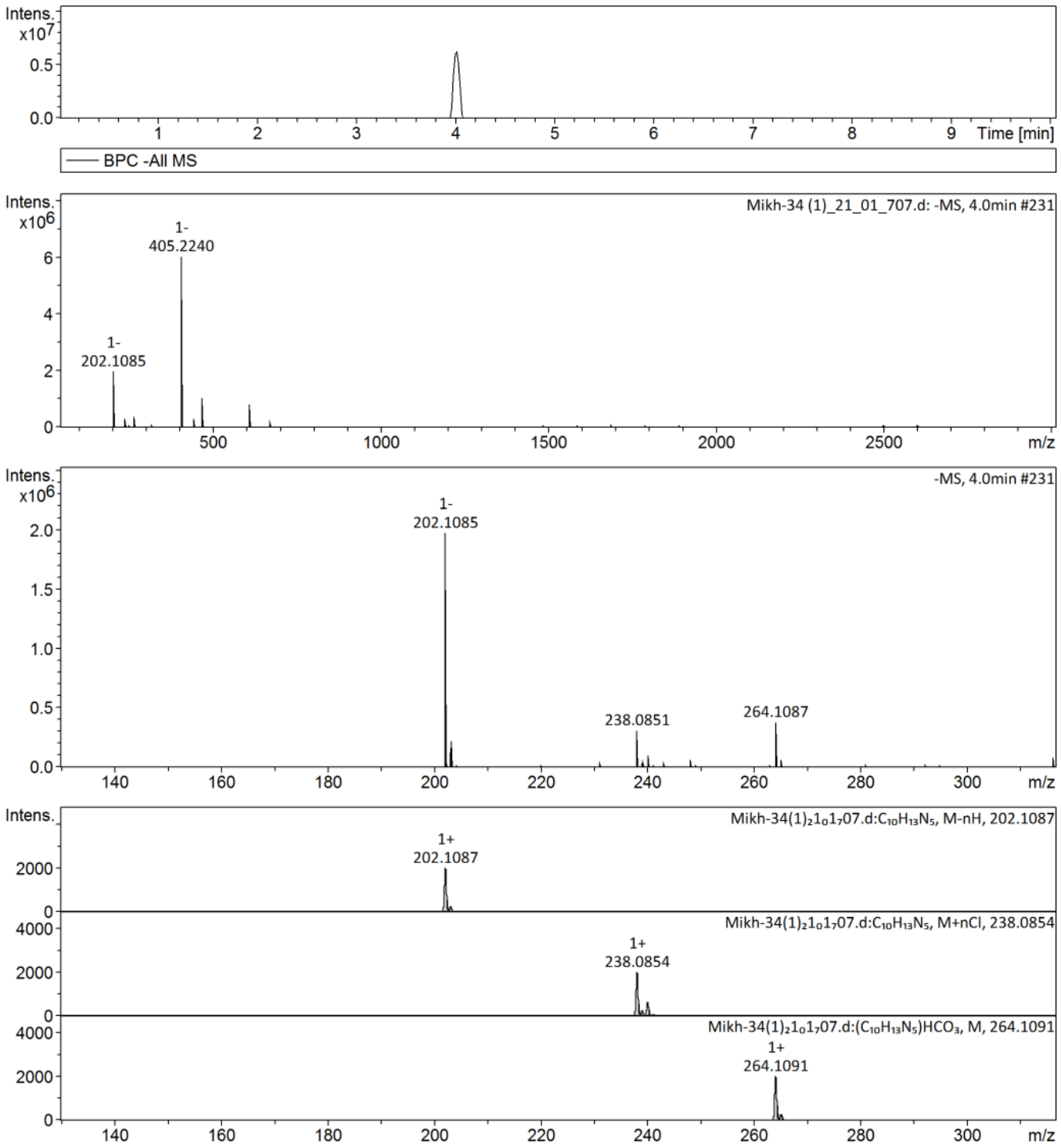
(Prepared by the arsenolysis reaction)



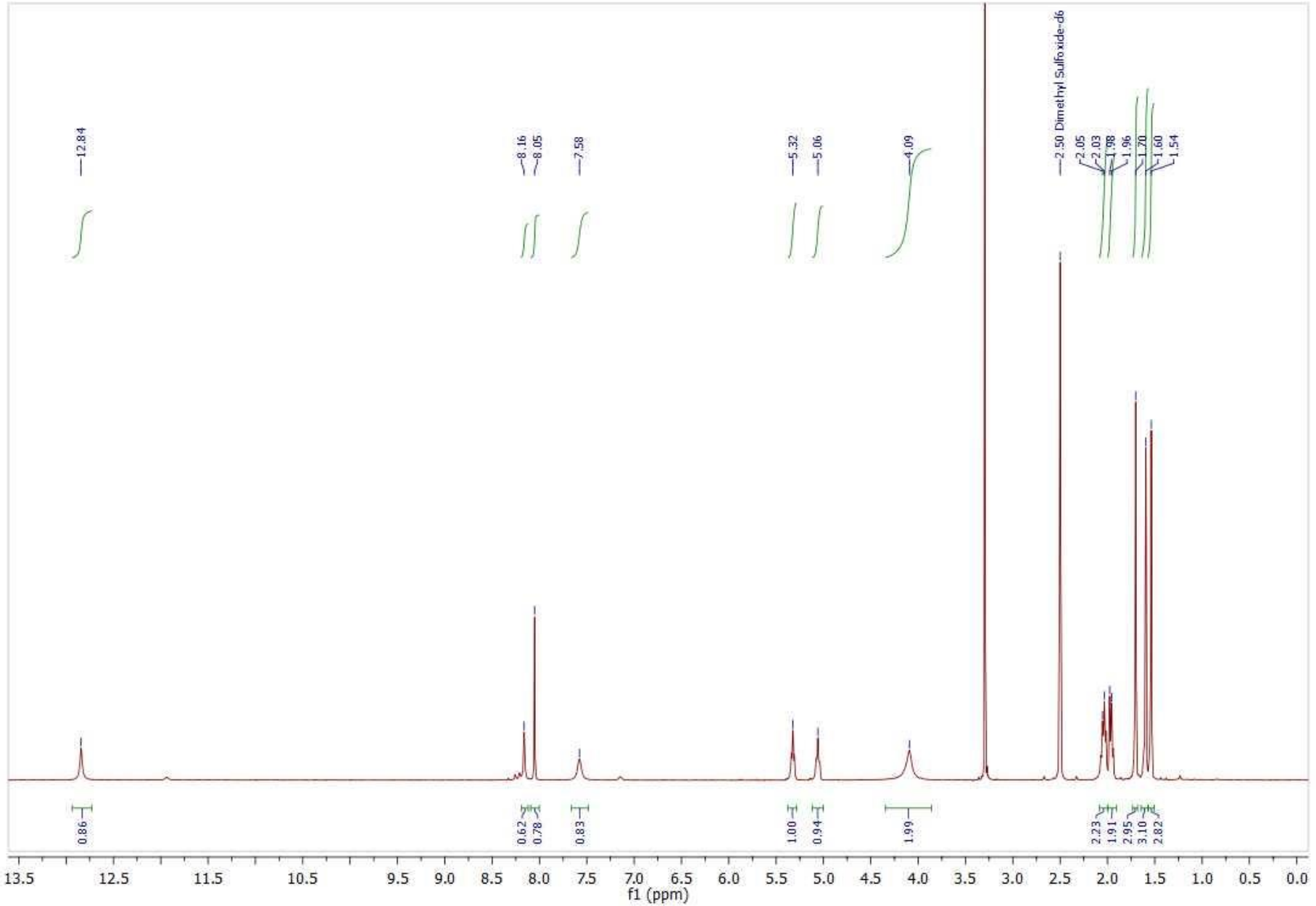
^{1}H-NMR-spectrum of N^{6}-isopentenyladenine (**2i**) (400 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

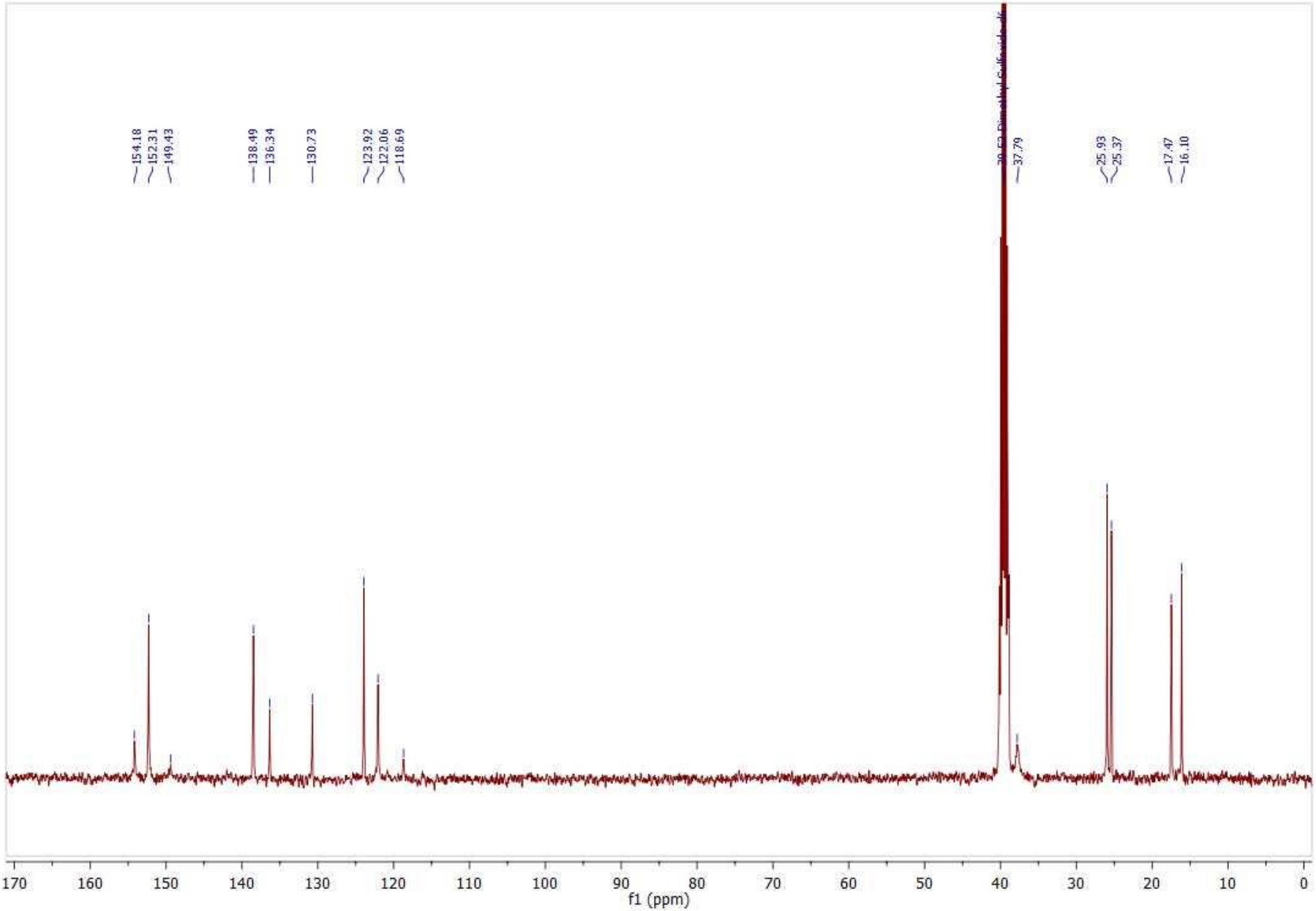
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of N^6-isopentenyladenine (**2i**)



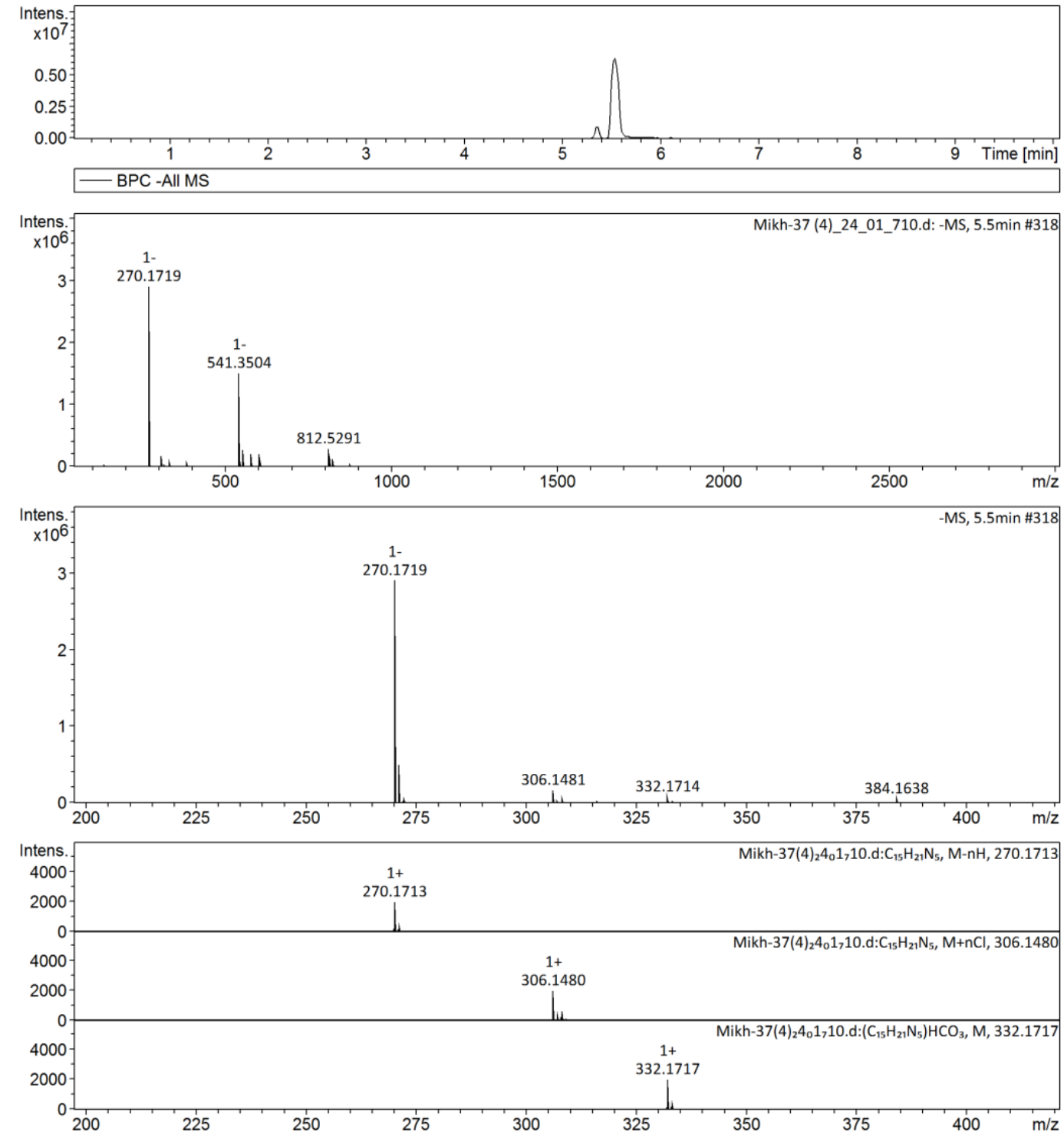
^{1}H-NMR-spectrum of N^{6}-geranyladenine (**2j**) (400 MHz) of in DMSO-d_6 at 300 K



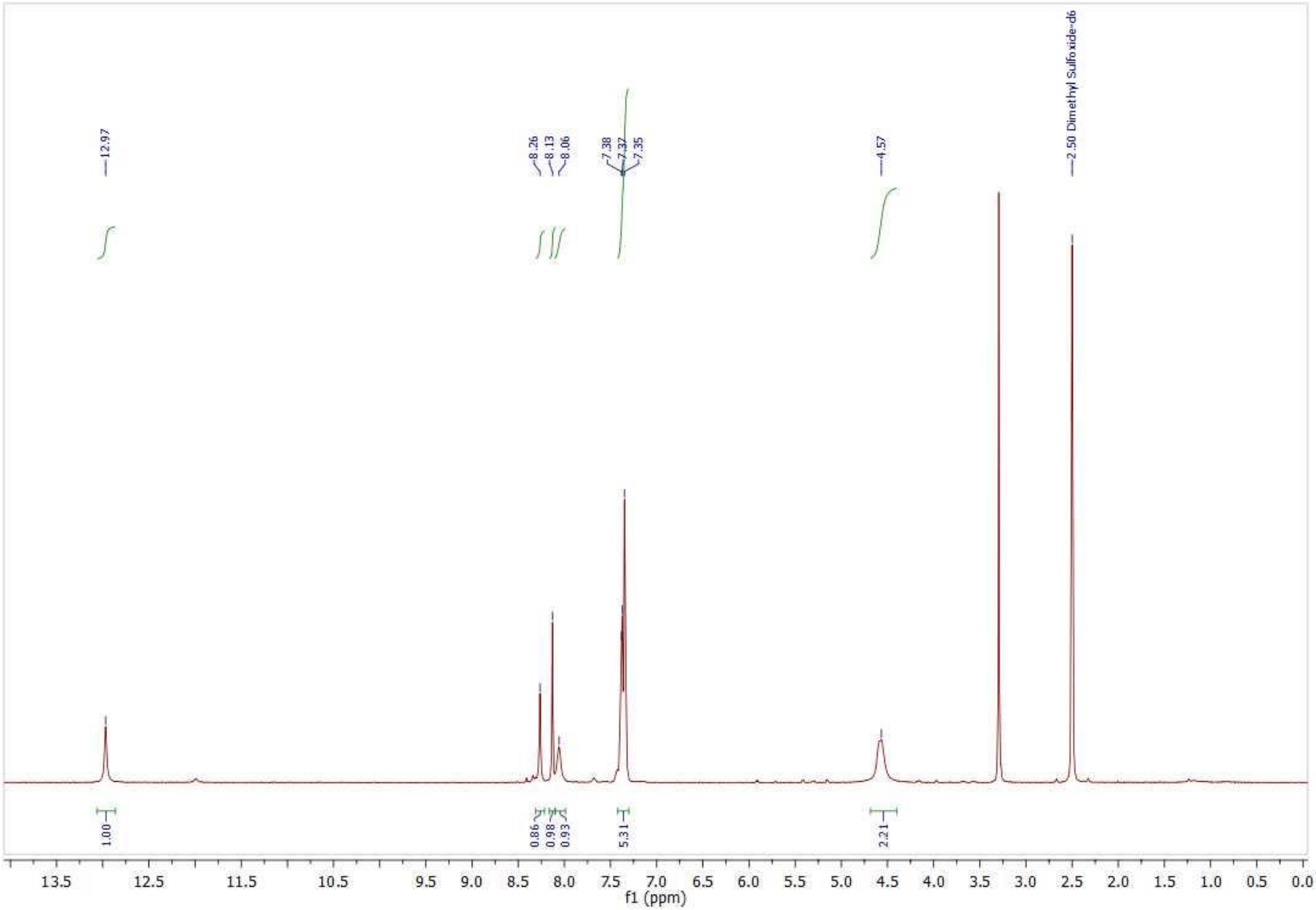
^{13}C-NMR-spectrum of N^6-geranyladenine (**2j**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

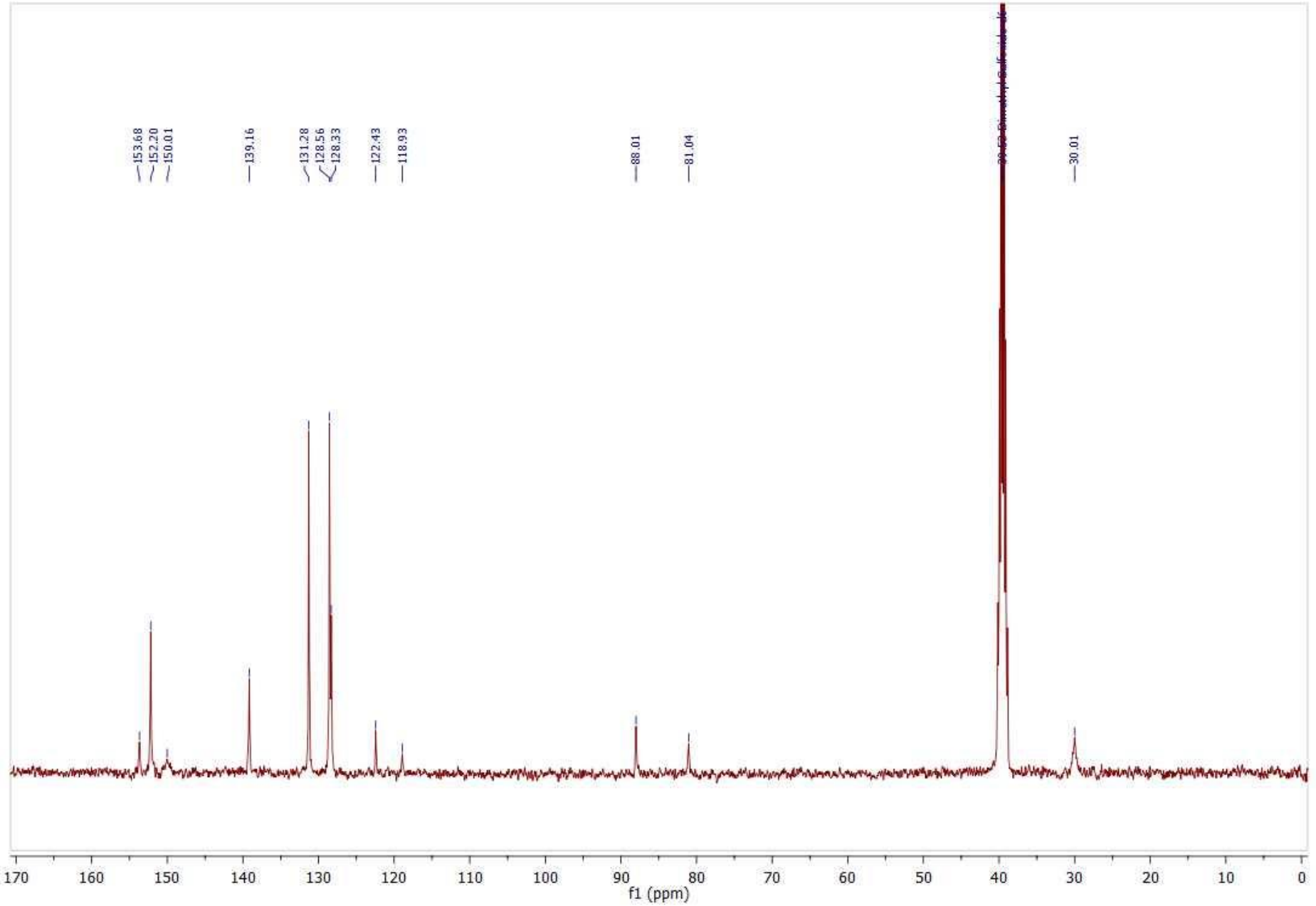
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of N^6-geranyladenine (**2j**)



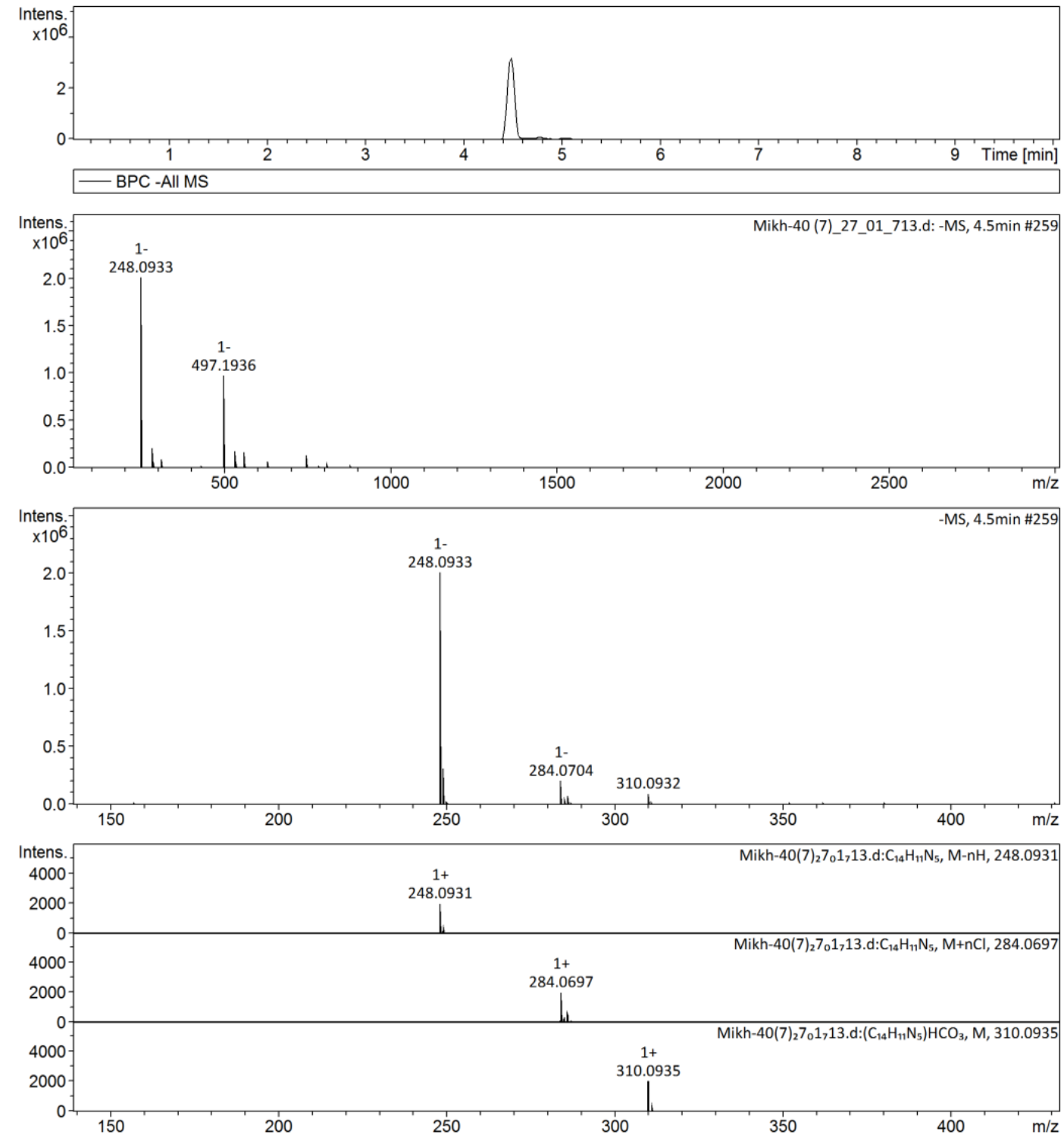
^{1}H-NMR-spectrum of N^{6}-(3-phenyl-2-propin-1-yl)adenine (**2k**) (400 MHz) of in DMSO-d_6 at 300 K



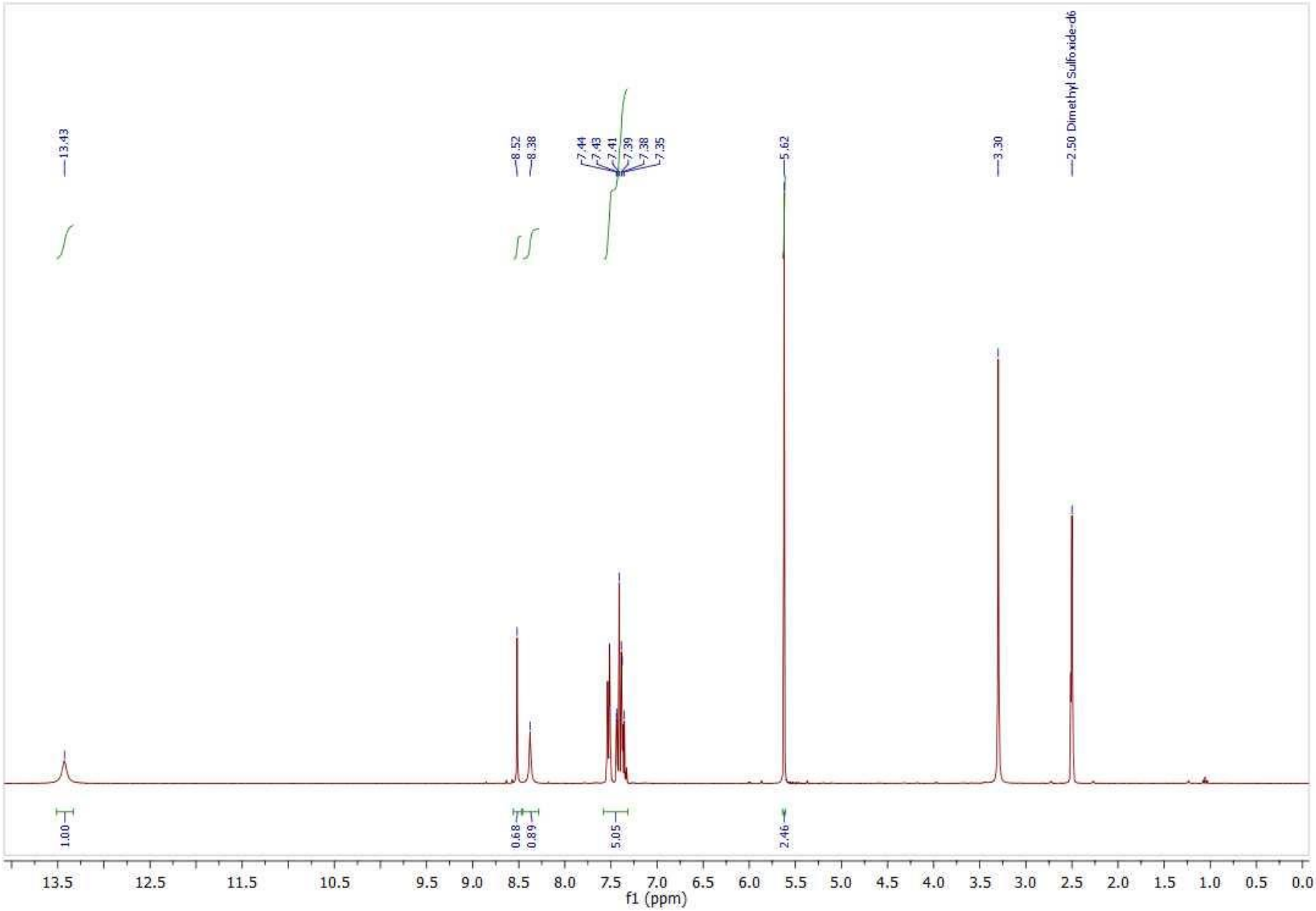
^{13}C-NMR-spectrum of N^6-(3-phenyl-2-propin-1-yl)adenine (**2k**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

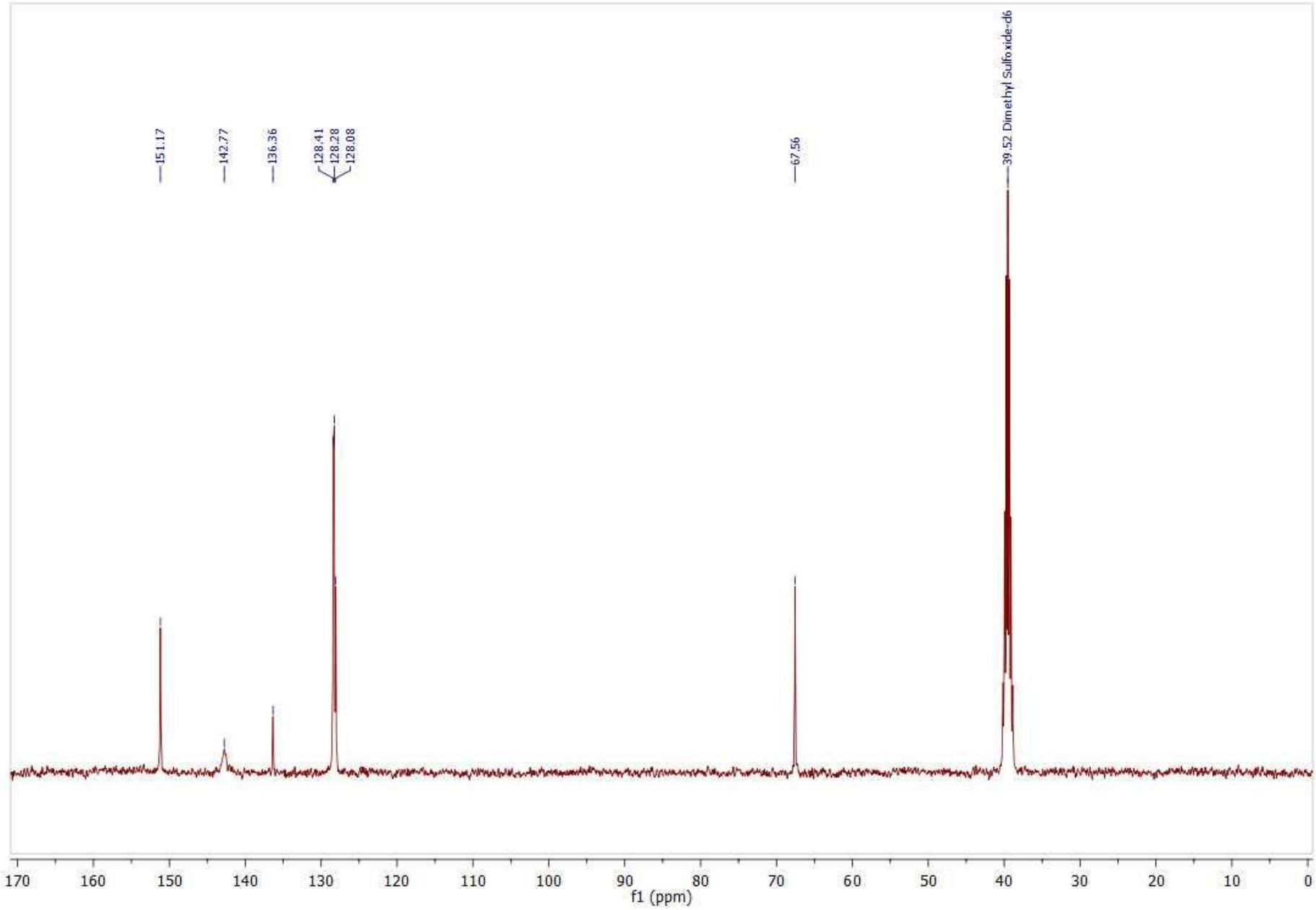
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of N^6-(3-phenyl-2-propin-1-yl)adenine (**2k**)



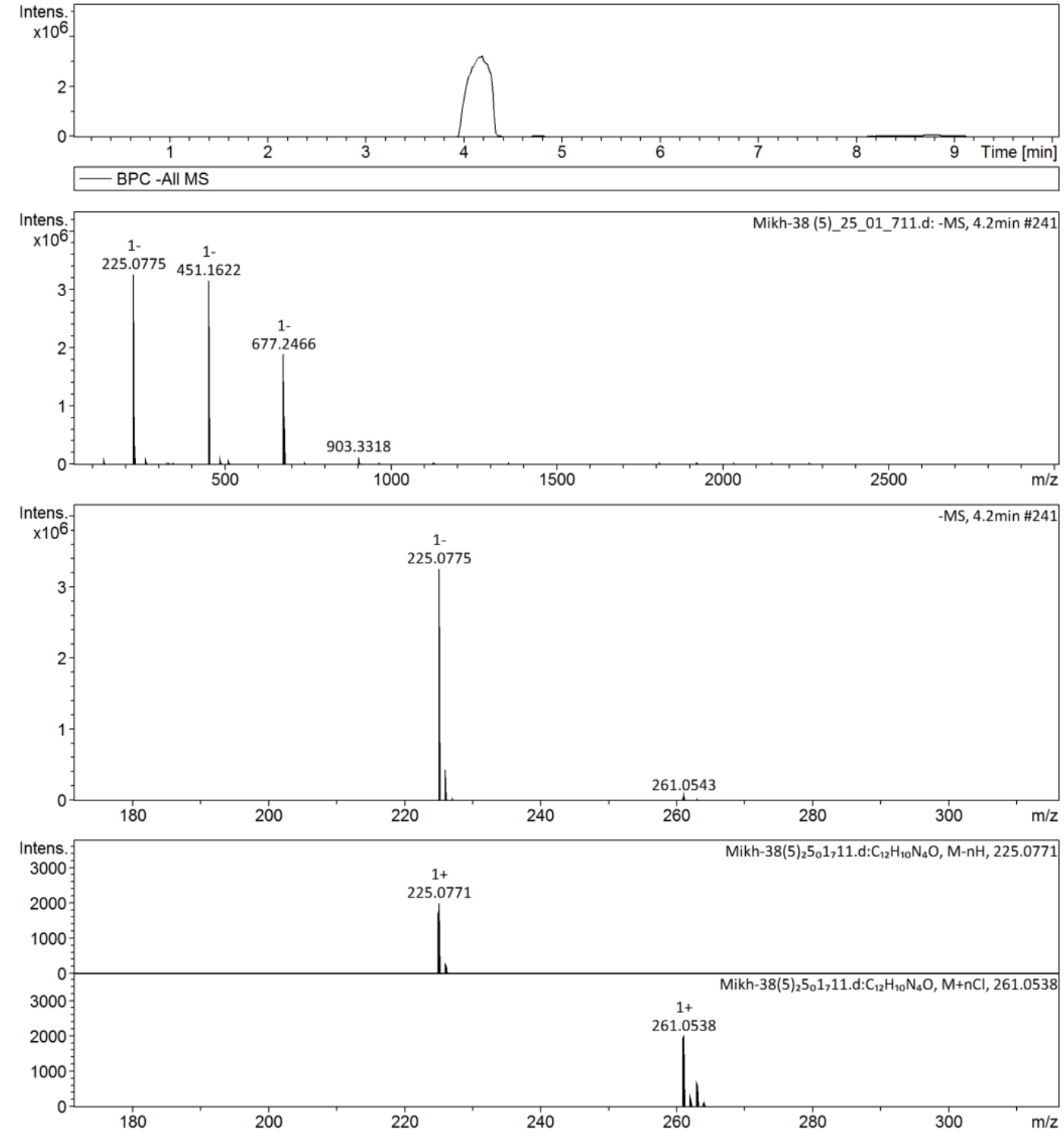
^{1}H-NMR-spectrum of O-6-benzylhypoxanthine (**2l**) (400 MHz) of in DMSO-d_6 at 300 K (Method A)



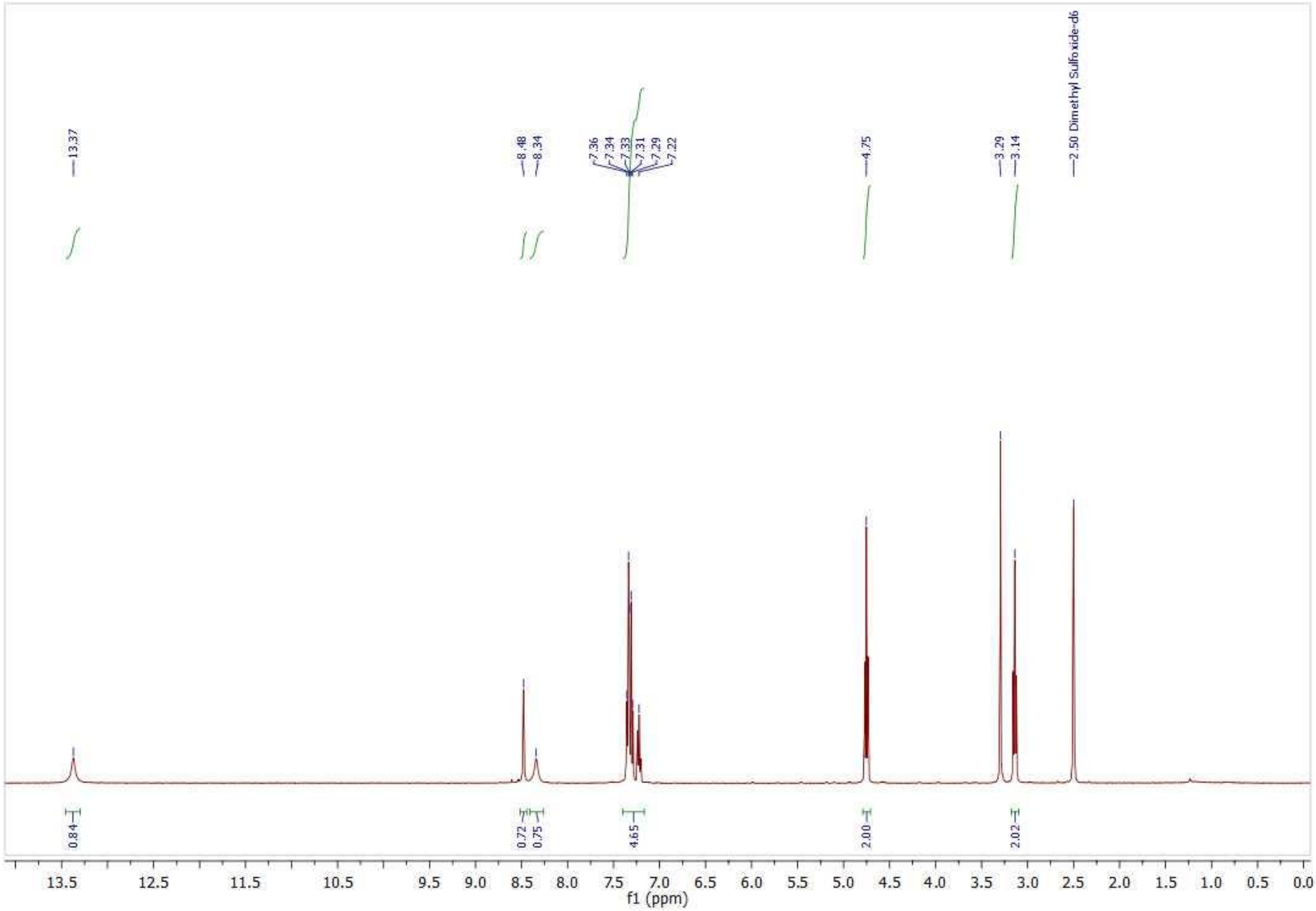
^{13}C-NMR-spectrum of O-6-benzylhypoxanthine (**2l**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

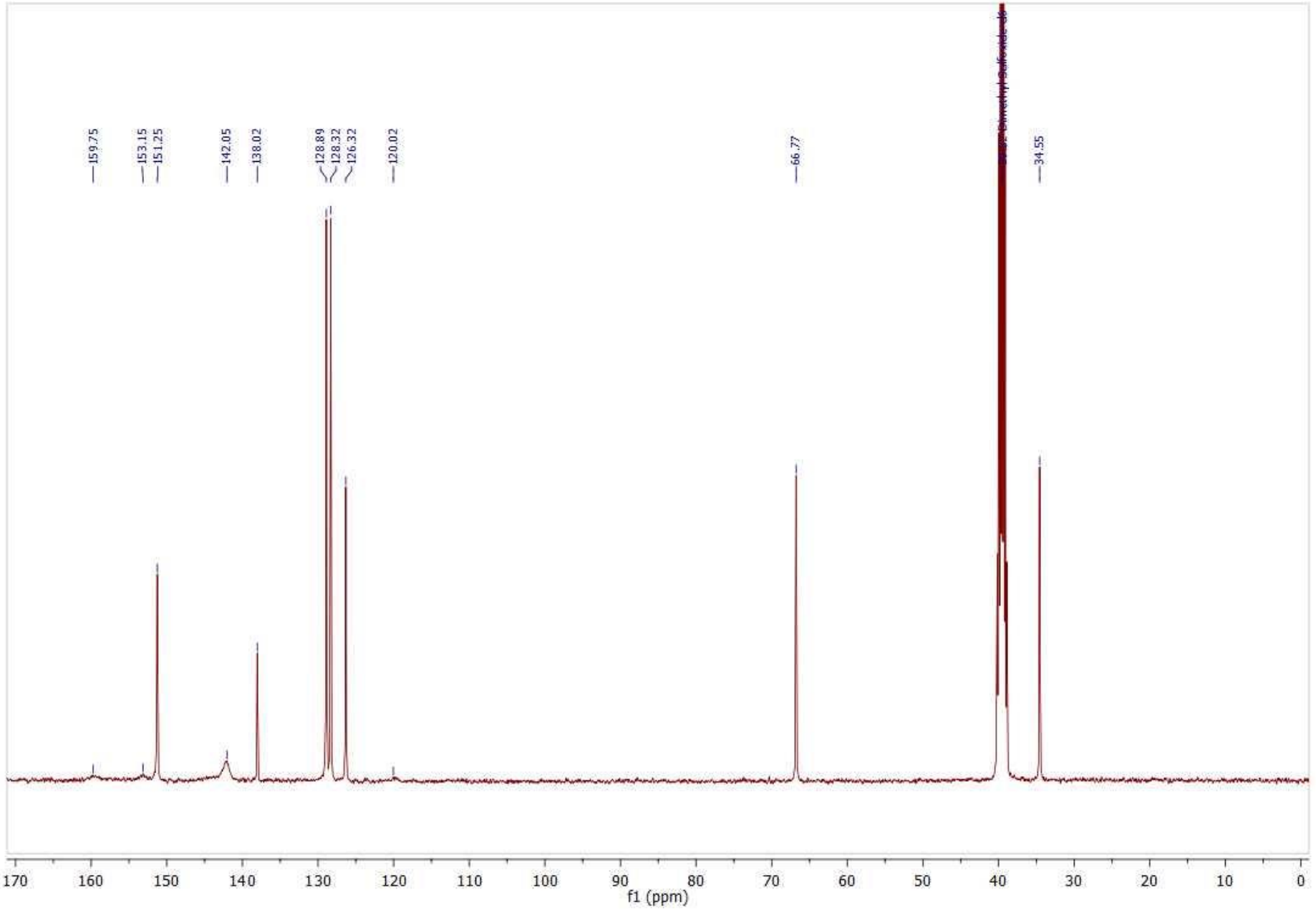
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of O-6-benzylhypoxanthine (**2l**)



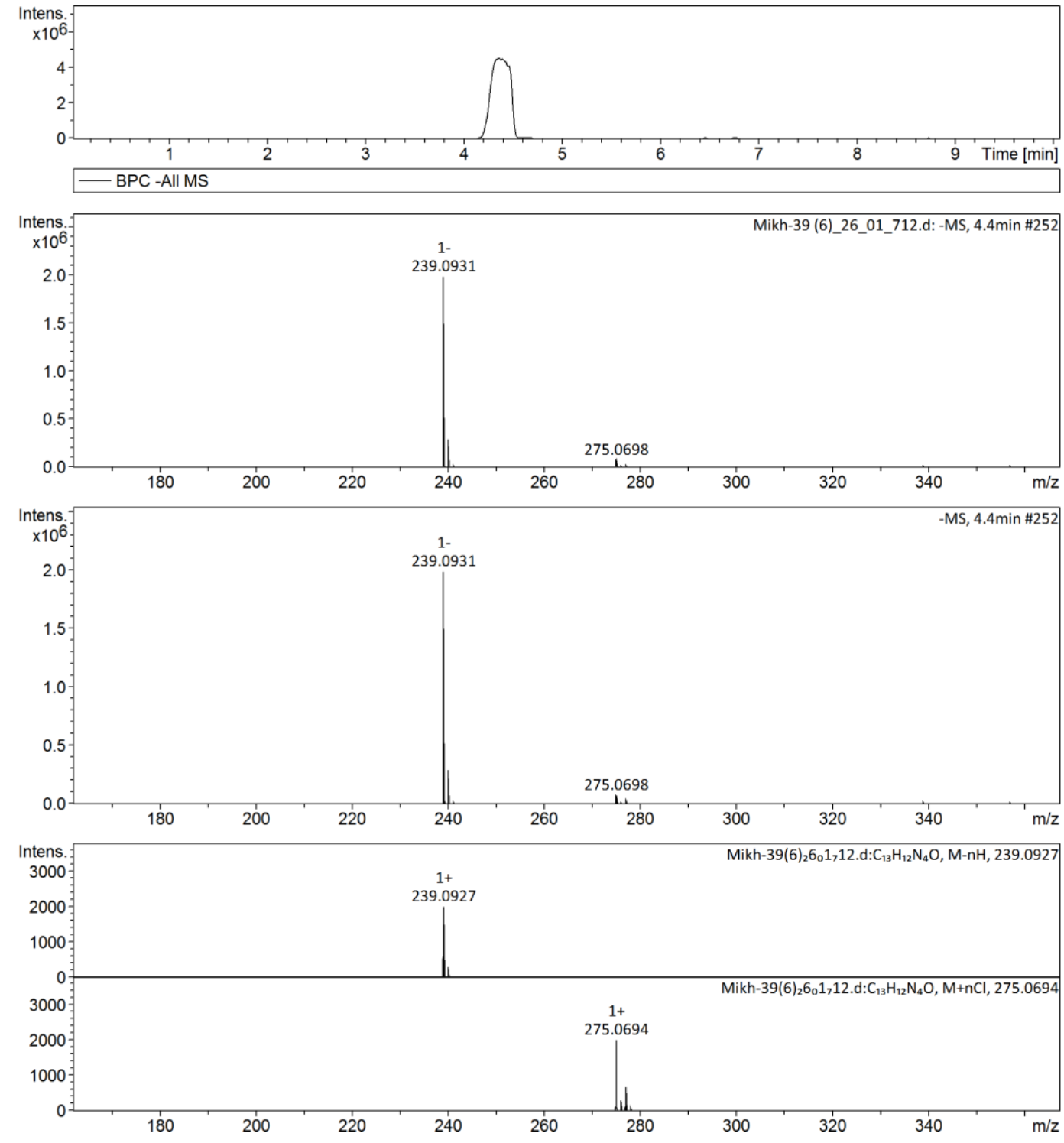
^{1}H-NMR-spectrum of O-6-(2-phenylethyl)hypoxanthine (**2m**) (400 MHz) of in DMSO-d_6 at 300 K



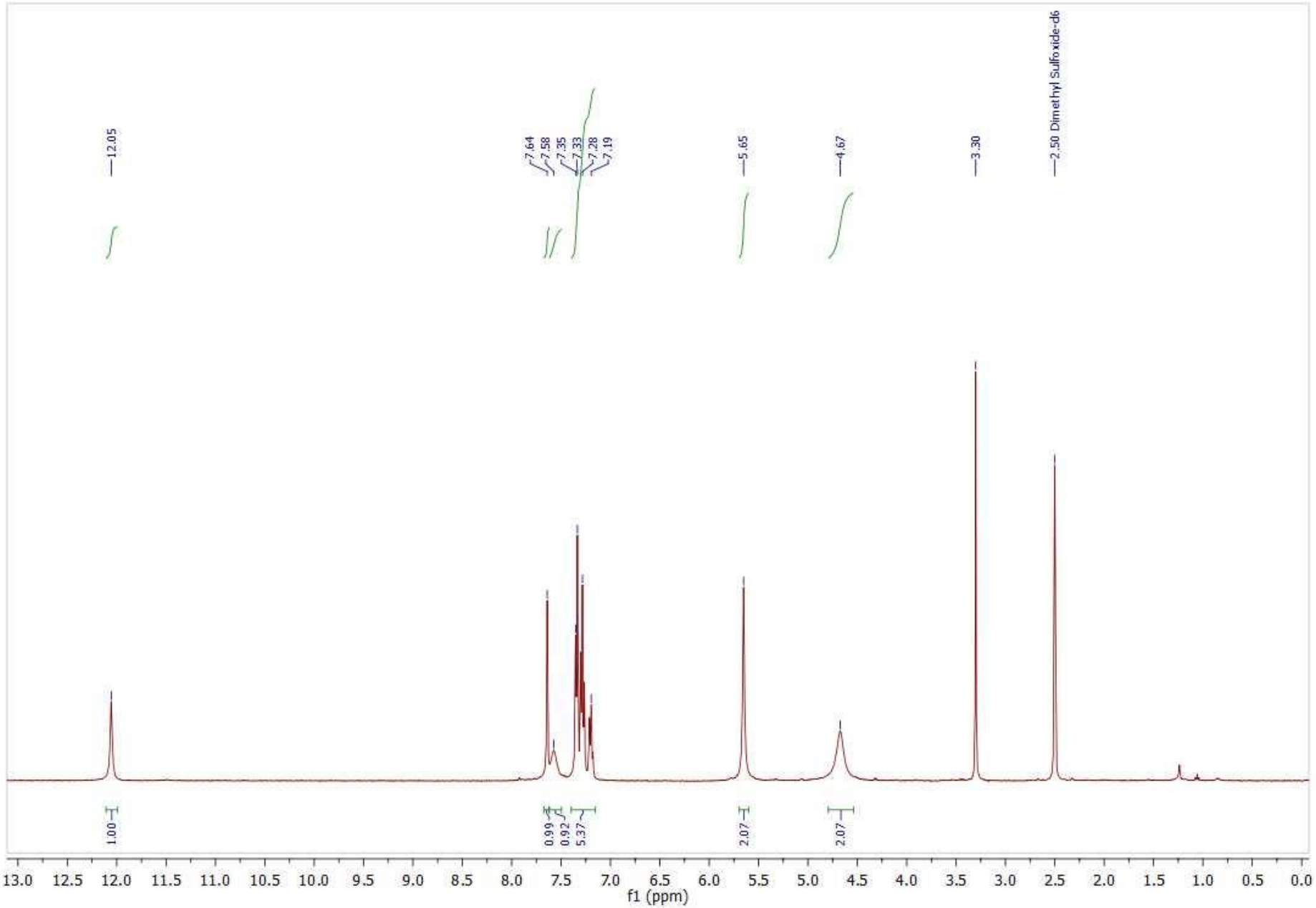
^{13}C-NMR-spectrum of O-6-(2-phenylethyl)hypoxanthine (**2m**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

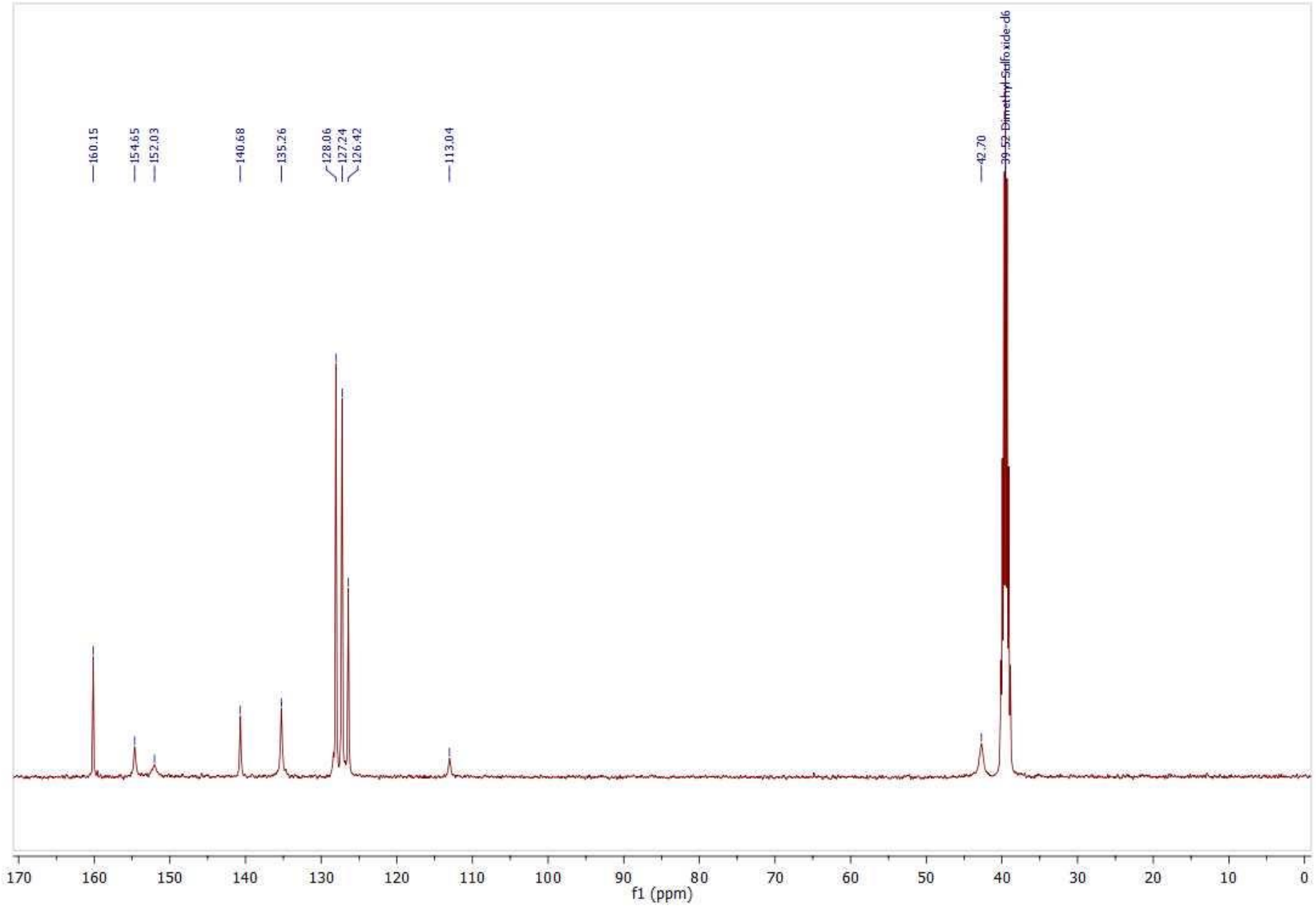
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of O-6-(2-phenylethyl)hypoxanthine (**2m**)



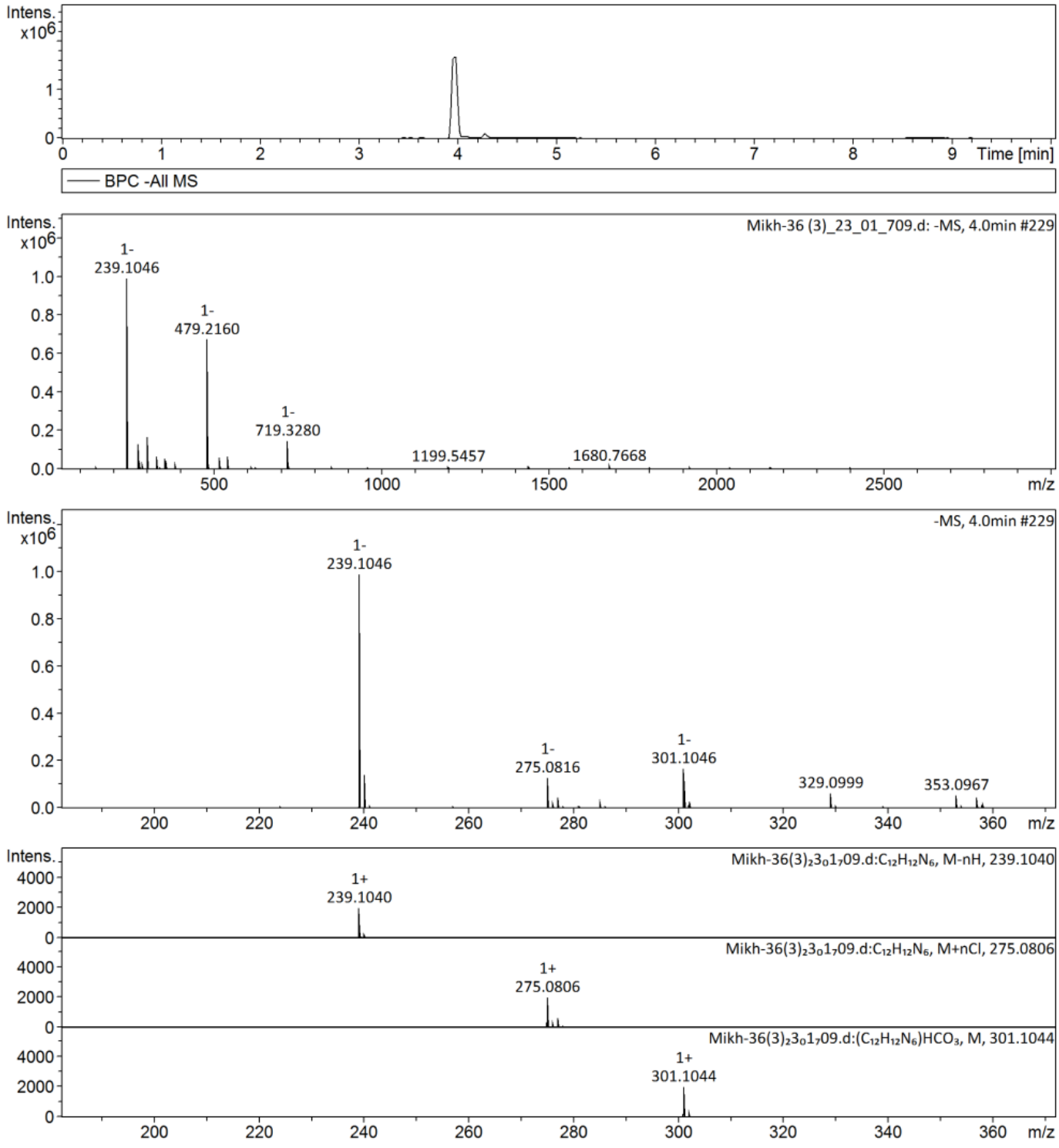
^{1}H-NMR-spectrum of 2-amino-N^{6}-benzyladenine (**2n**) (400 MHz) of in DMSO-d_6 at 300 K



^{13}C-NMR-spectrum of 2-amino-N^6-benzyladenine (**2n**) (100 MHz) of in DMSO-d_6 at 300 K

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4700 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



High-resolution mass spectrum (HRMS) of 2-amino-N^6-benzyladenine (**2n**)