Distinct Peculiarities of In Planta Synthesis of Isoprenoid and Aromatic Cytokinins

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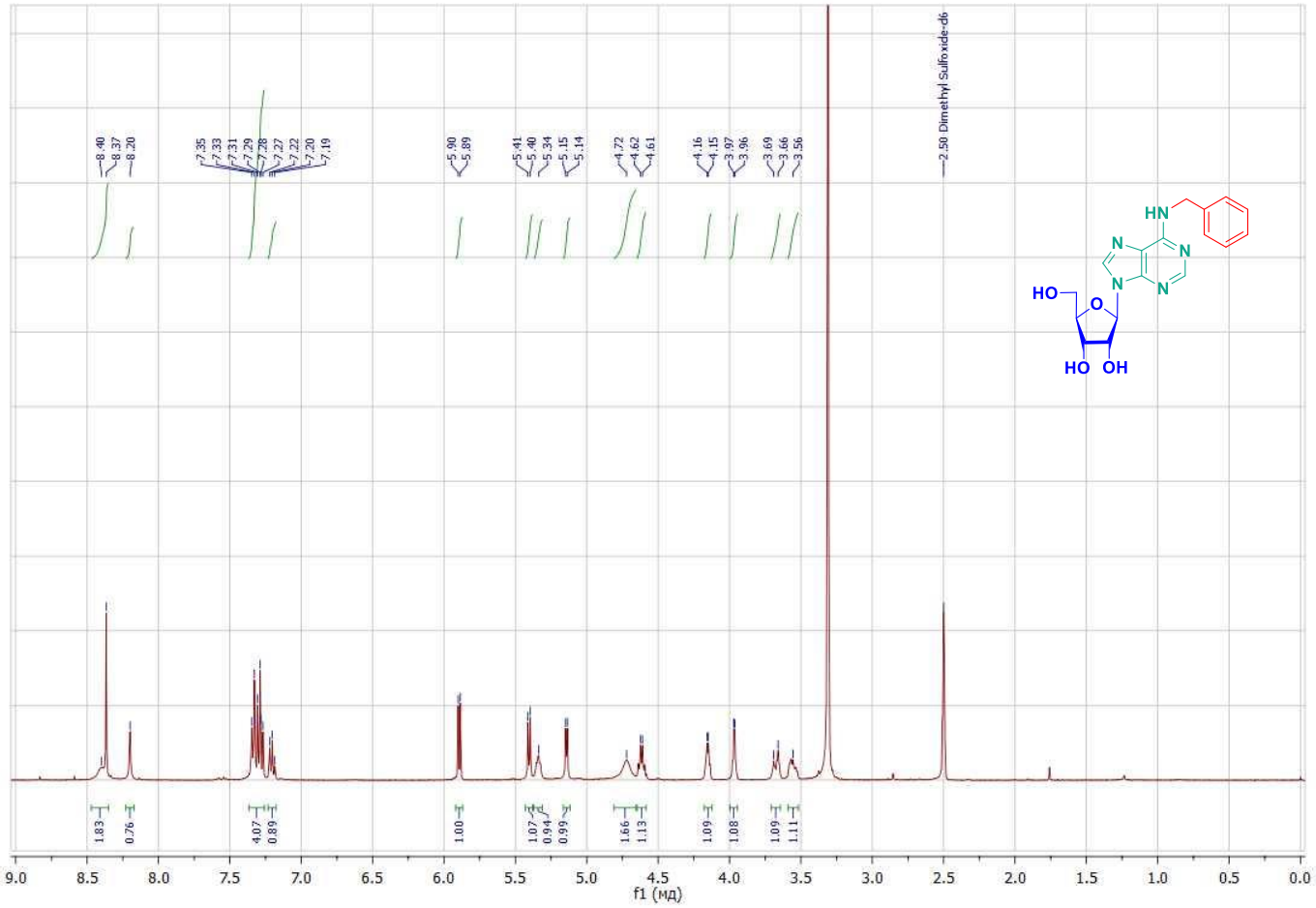
Supplementary materials

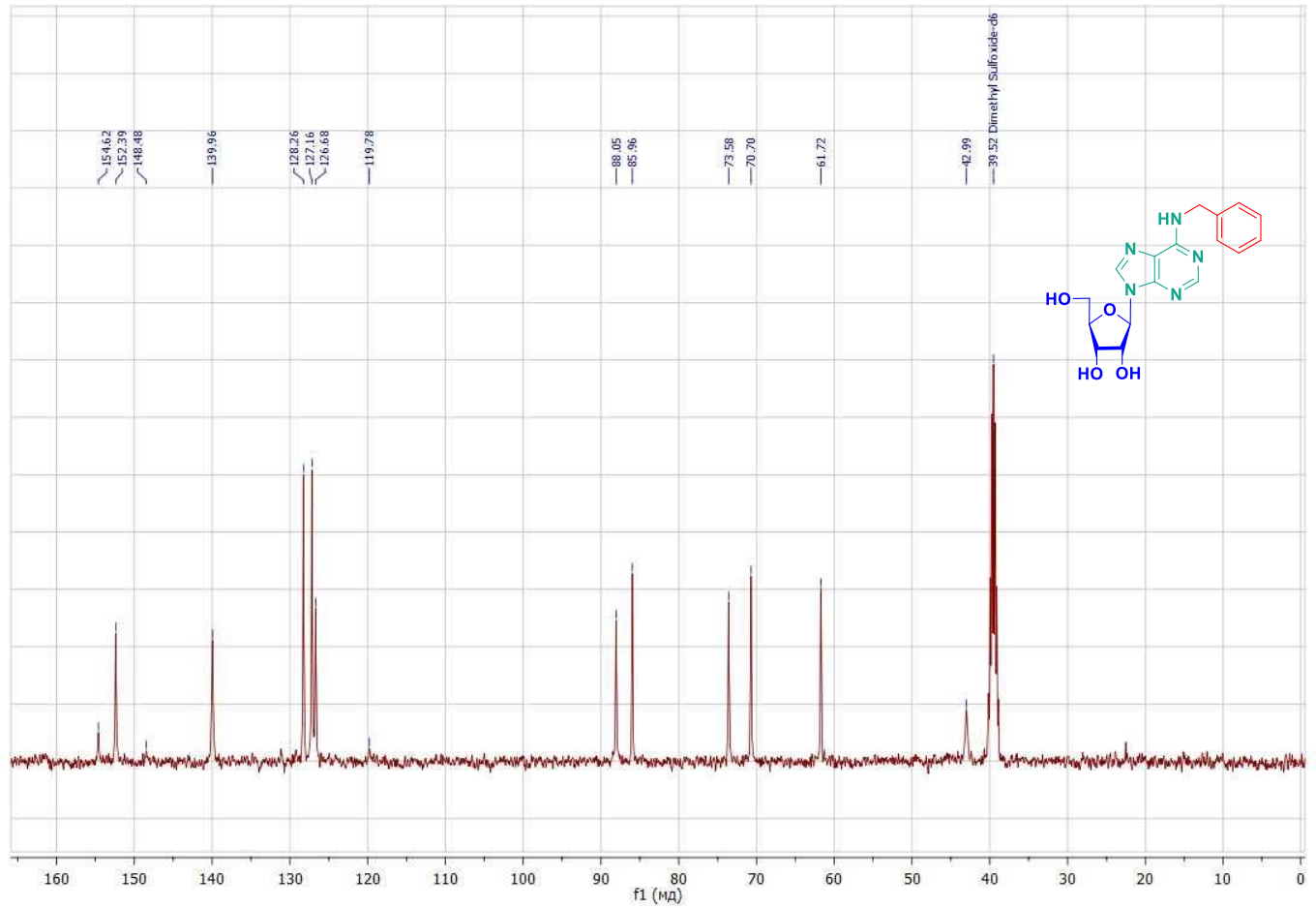
General

^{1}H and ^{13}C (with complete proton decoupling) NMR spectra were recorded on Bruker AMX 400 NMR instrument at 303 K. ^{1}H-NMR-spectra were recorded at 400 MHz and ^{13}C-NMR-spectra at 100 MHz. Chemical shifts in ppm were measured relative to the residual solvent signals as internal standards ($CDCl_3$, ^{1}H: 7.26 ppm, ^{13}C: 77.1 ppm; DMSO-d_6, ^{1}H: 2.50 ppm, ^{13}C: 39.5 ppm). Spin-spin coupling constants (J) are given in Hz. High resolution mass spectra (HRMS) were registered on a Bruker Daltonics micrOTOF-Q II instrument using electrospray ionization (ESI). The measurements were done in positive and negative ion modes. Interface capillary voltage: 4500 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 (6 l/min); interface temperature: 180°C. Samples were injected into the mass spectrometer chamber from the Agilent 1260 HPLC system equipped with an Agilent Poroshell 120 EC-C18 (3.0 × 50 mm; 2,7 µm) column; flow rate 400 µl/min; samples were injected from the acetonitrile-water (1:1) solution and the column was eluted with a gradient of concentrations of acetonitrile (A) in water (B) in the following 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. Retention times were as follows: **4** – 3.9 min; **5** – 3.9 min; **6** – 4.5 min; **7** – 3.7 min; **8** – 4.1 min; **9** – 4.1 min; **10** – 4.2 min; **11** – 3.8 min; **12** – 4.4 min; **13** – 4.3 min; **14** – 4.5 min; **15** – 4.0 min.

The following nucleosides were prepared according to the methods reported earlier: N^6-benzyladenosine (**4**), N^6-isopentenyladenosine (**5**), N^6-furfuryladenosine (**7**) [Drenichev, M.S.; Oslovsky, V.E.; Tararov, V.I.; Mikhailov, S.N. Synthesis of *N*6-substituted adenosines as cytokinin nucleosides. *Curr. Protoc. Nucleic Acid Chem.* **2018**, *72*, 14.15.1-14.15.16], N^6-(2-phenylethyl)-adenosine (**6**), N^6-benzyl-2′-deoxyadenosine (**8**), N^6-(2-phenylethyl)-2′-deoxyadenosine (**10**), N^6-benzyl-5′-deoxyadenosine (**12**), N^6-isopentenyl-5′-deoxyadenosine (**13**), N^6-(2-phenylethyl)-5′-deoxyadenosine (**14**) [Drenichev, M.S.; Oslovsky, V.E.; Sun, L.; Tijsma, A.; Kurochkin, N.N.; Tararov, V.I.; Chizhov, A.O.; Neyts, J.; Pannecouque, C.; Leyssen, P.; Mikhailov, S.N. Modification of the length and structure of the linker of *N*6-benzyladenosine modulates its selective antiviral activity against enterovirus 71. *Eur. J. Med. Chem.* **2016**, 111, 84-94].



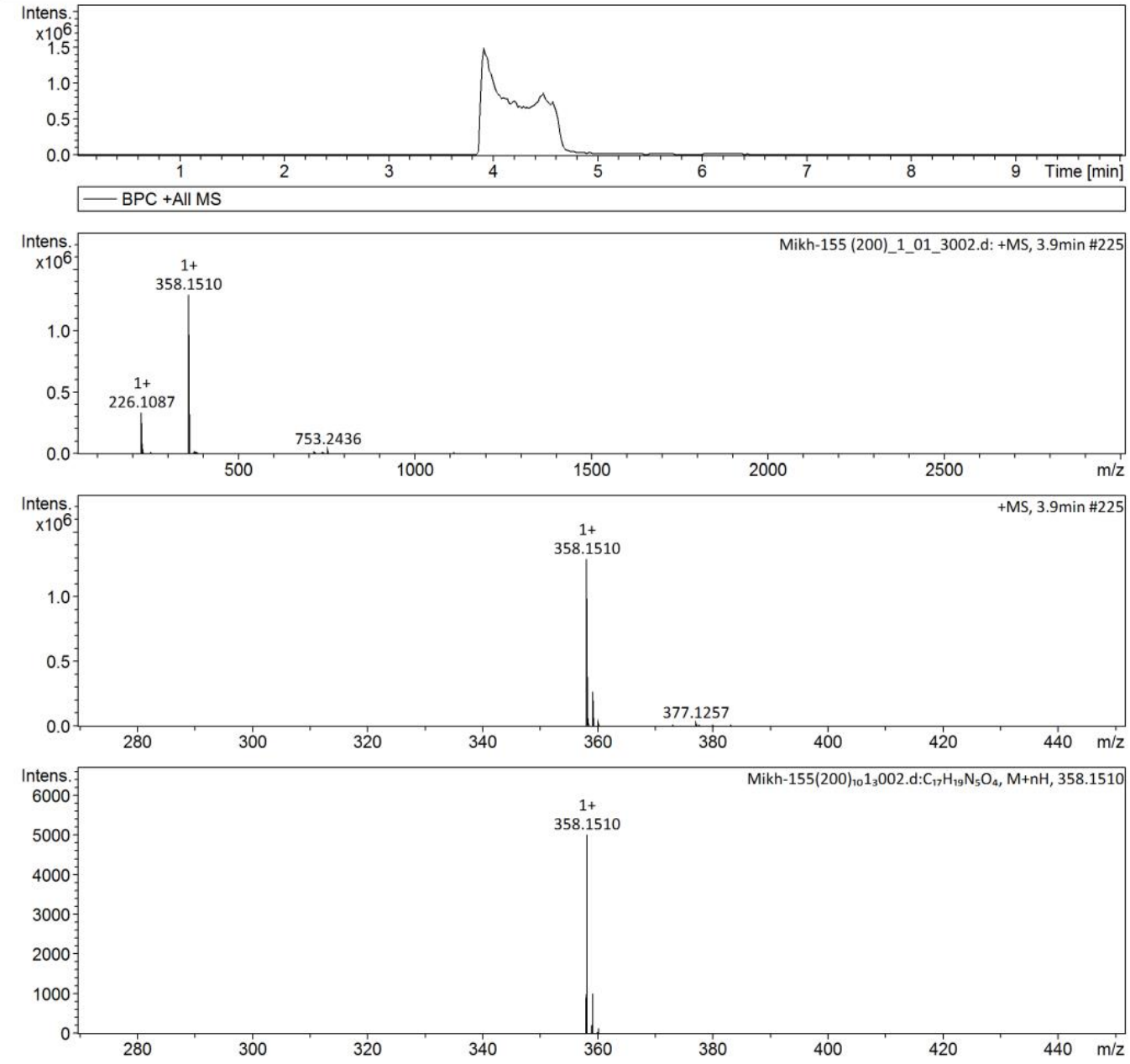
^{1}H-NMR-spectrum (400 MHz) of N^6-benzyladenosine (**4**) in DMSO-d_6 at 303 K



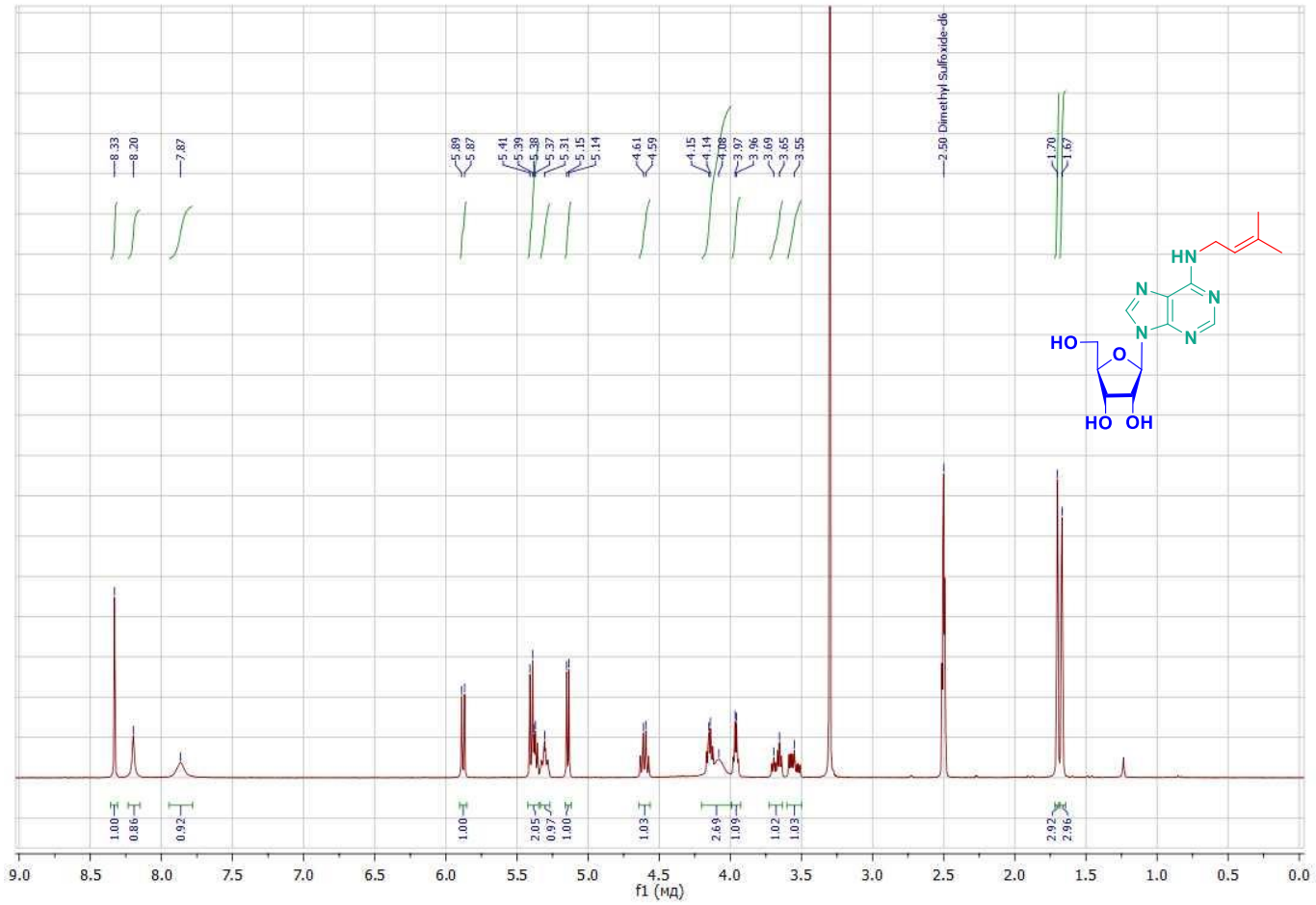
^{13}C-NMR-spectrum (100 MHz) of N^6-benzyladenosine (**4**) in DMSO-d_6 at 303 K

Acquisition Parameter

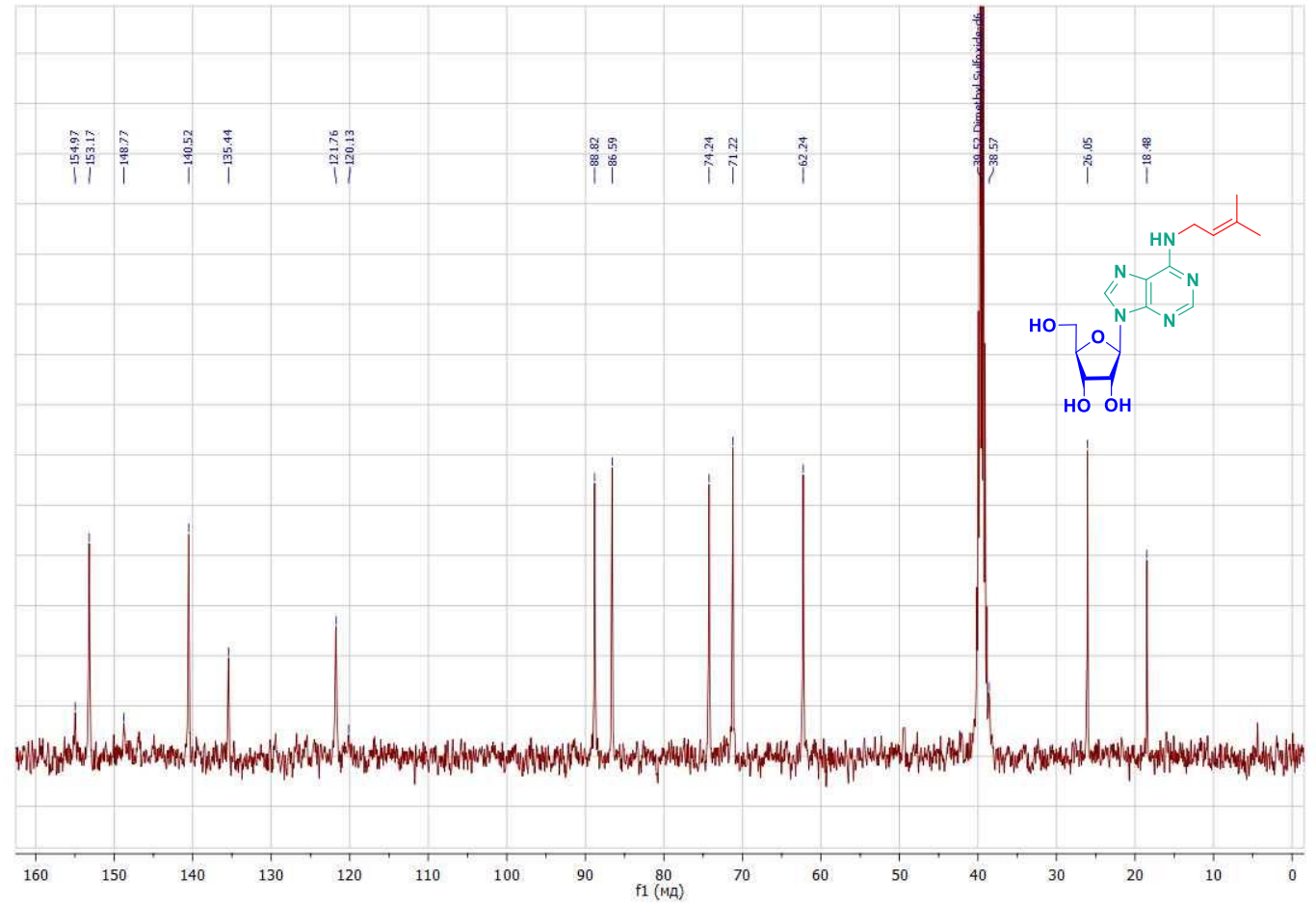
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



HPLC-HRMS spectrum of N^6-benzyladenosine (**4**)



^{1}H-NMR-spectrum (400 MHz) of N^{6}-isopentenyladenosine (**5**) in DMSO-d_6 at 303 K



^{13}C-NMR-spectrum (100 MHz) of N^{6}-isopentenyladenosine (**5**) in DMSO-d_6 at 303 K

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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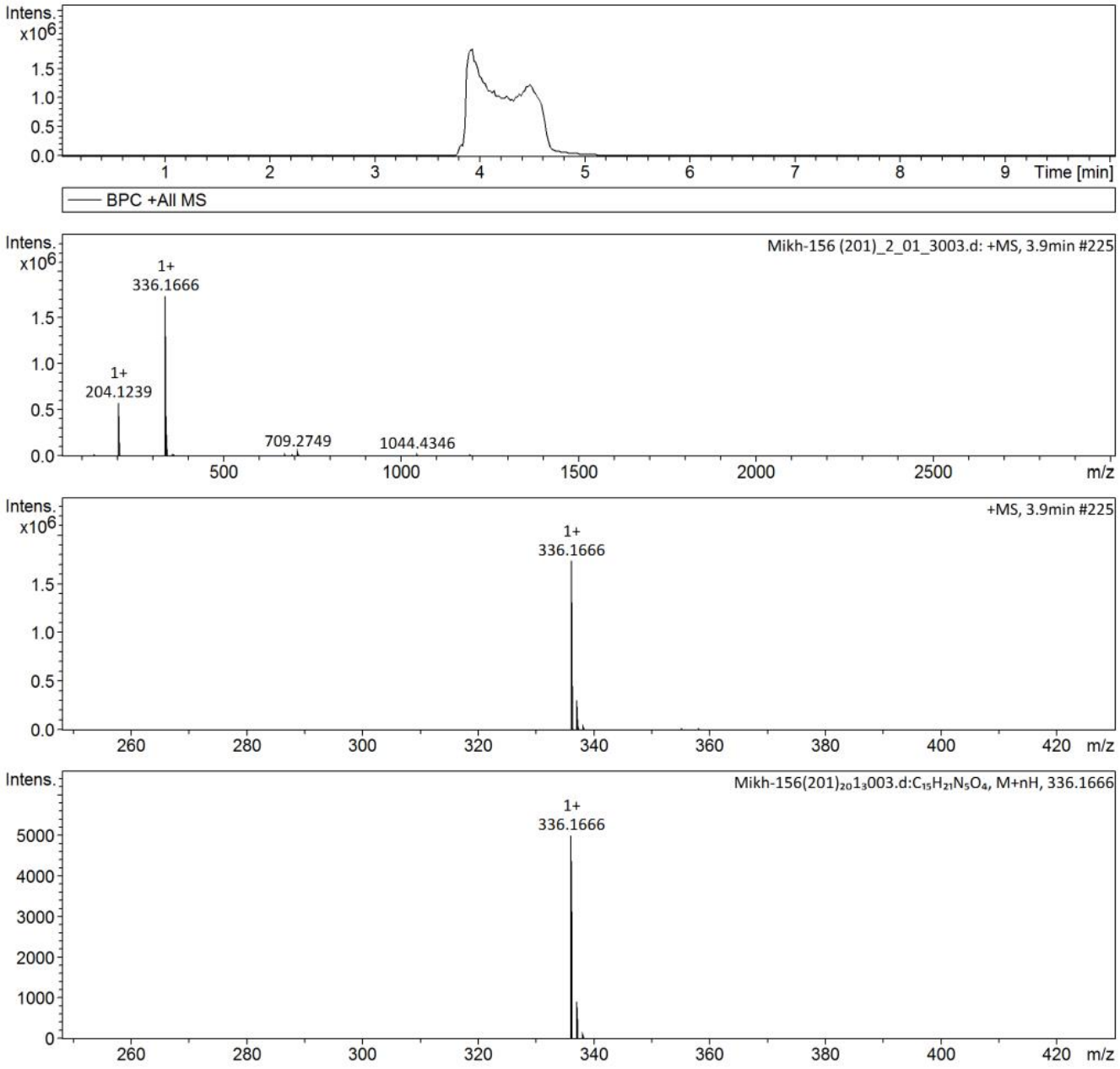
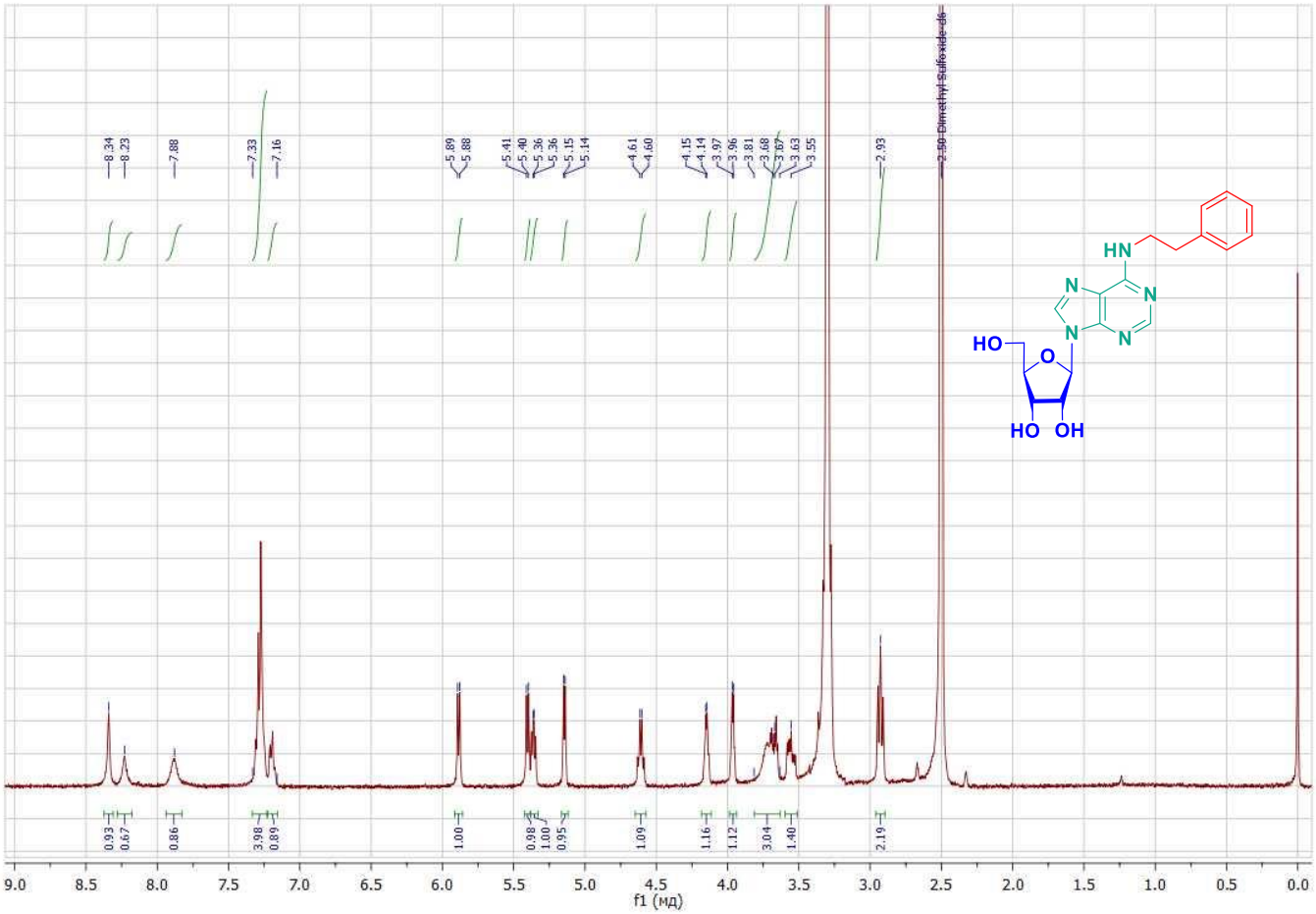
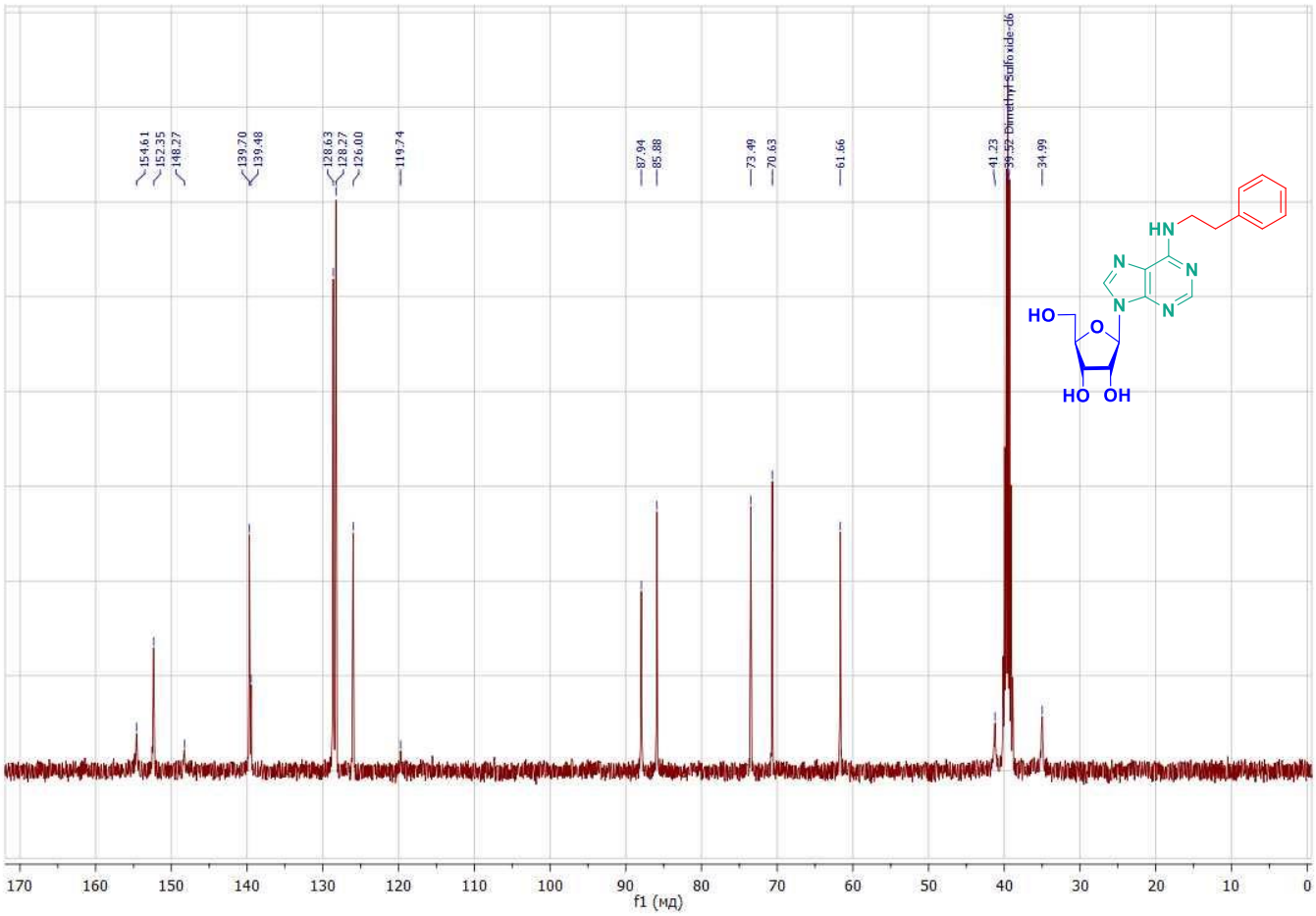


HPLC-HRMS spectrum of N^6-isopentenyladenosine (**5**)



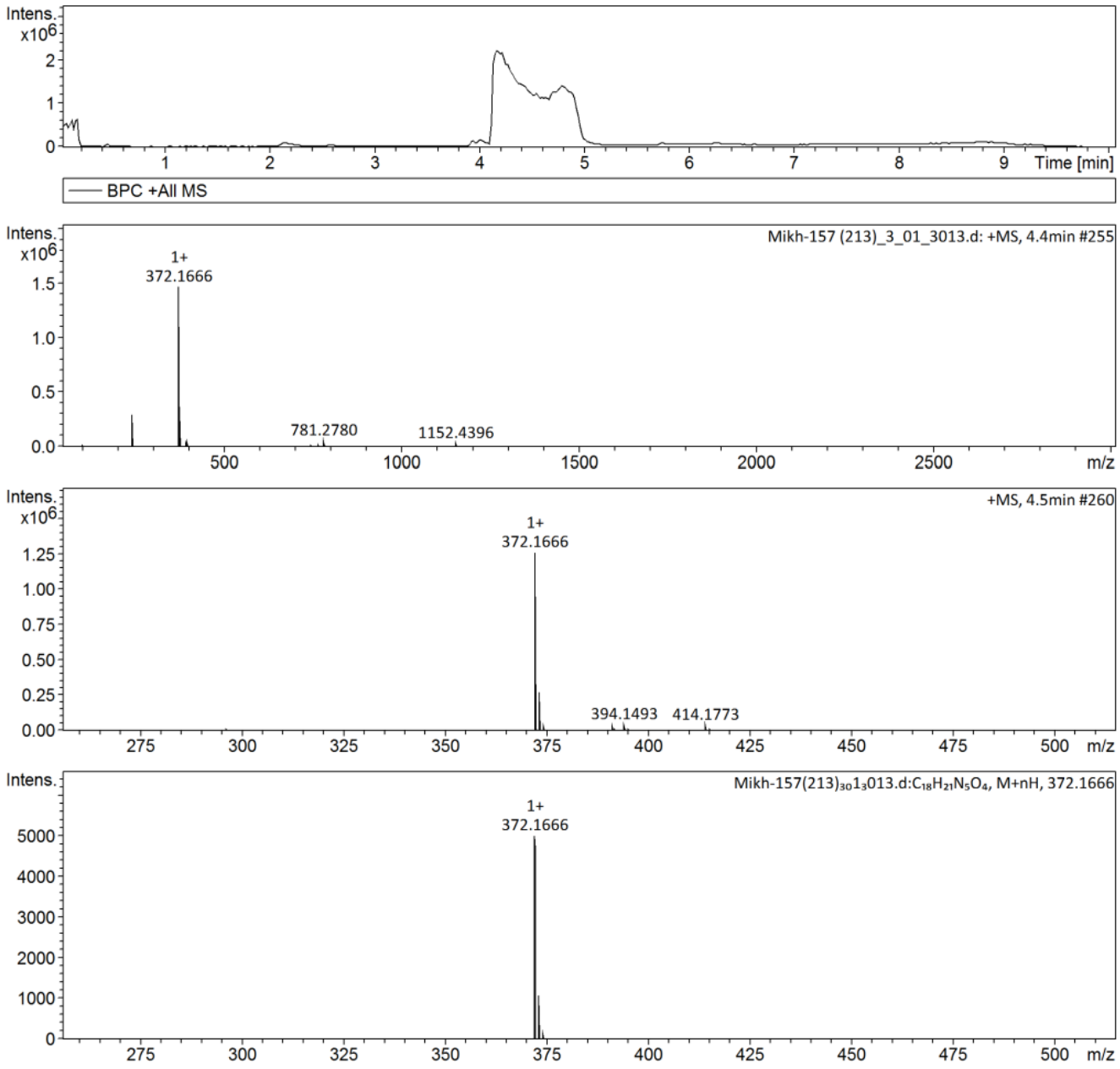
^{1}H-NMR-spectrum (400 MHz) of N^6-(2-phenylethyl)-adenosine (**6**) in DMSO-d_6 at 303 K



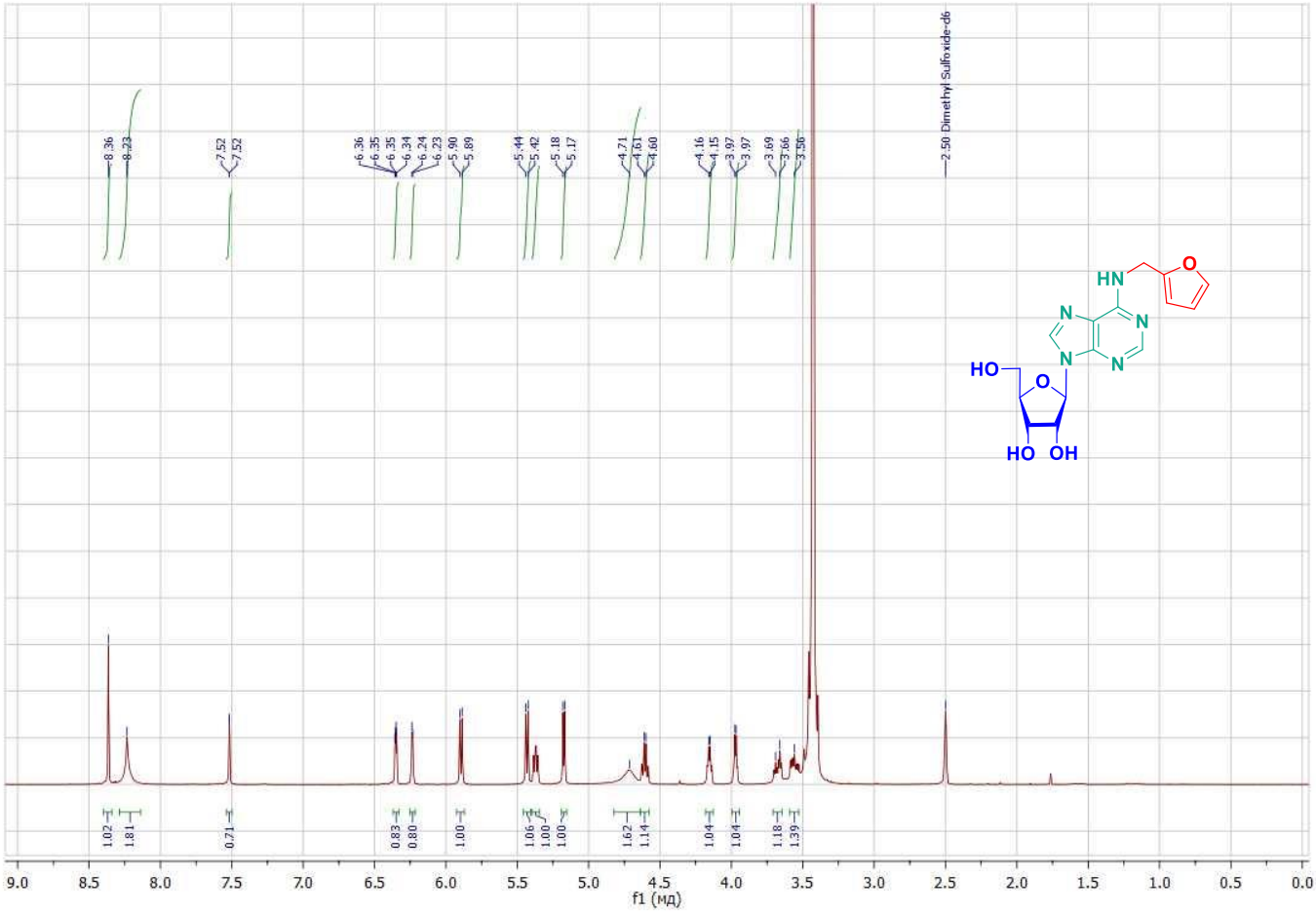
^{13}C-NMR-spectrum (100 MHz) of N^6-(2-phenylethyl)-adenosine (**6**) in DMSO-d_6 at 303 K

Acquisition Parameter

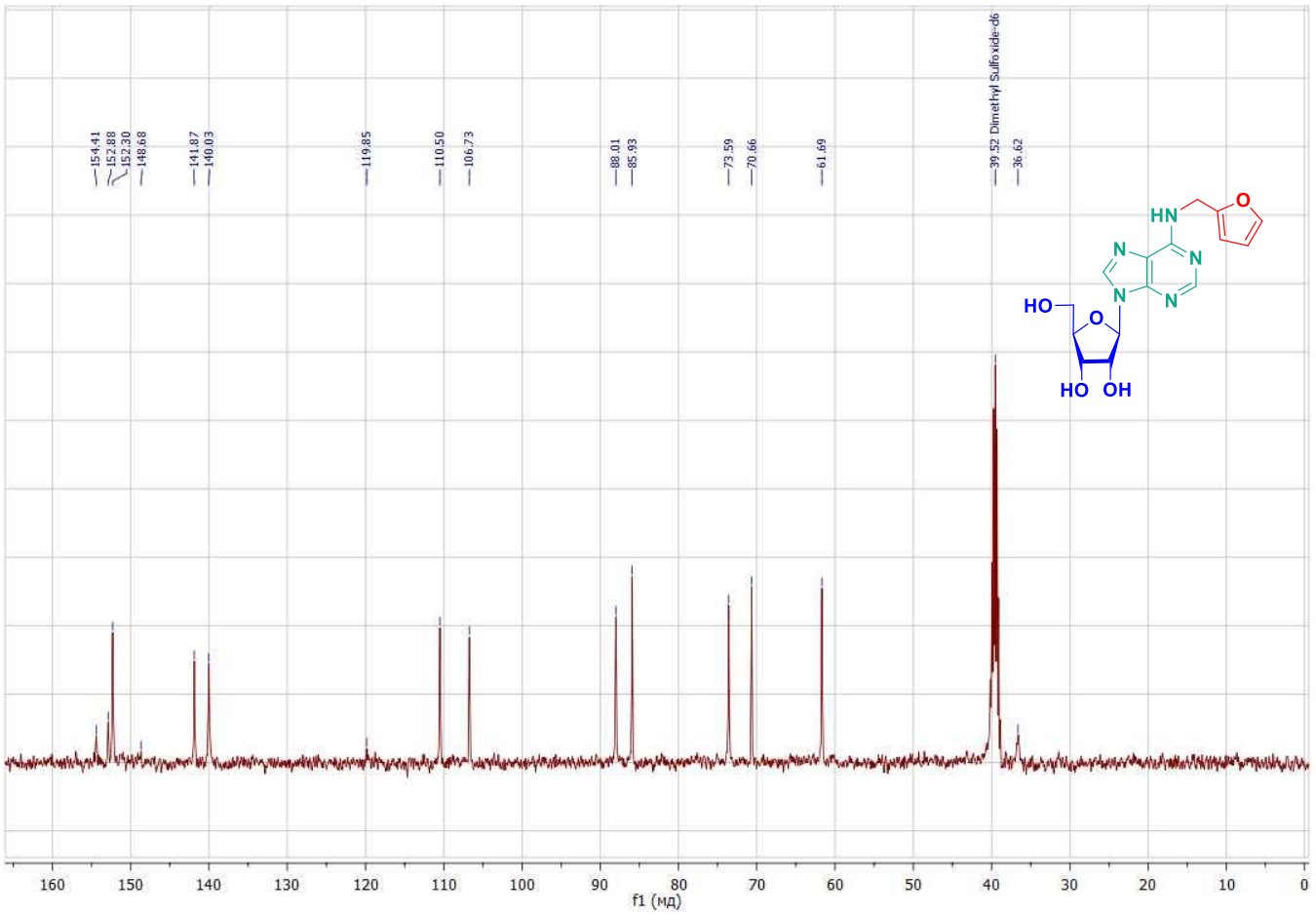
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



HPLC-HRMS spectrum of N^6-(2-phenylethyl)-adenosine (**6**)



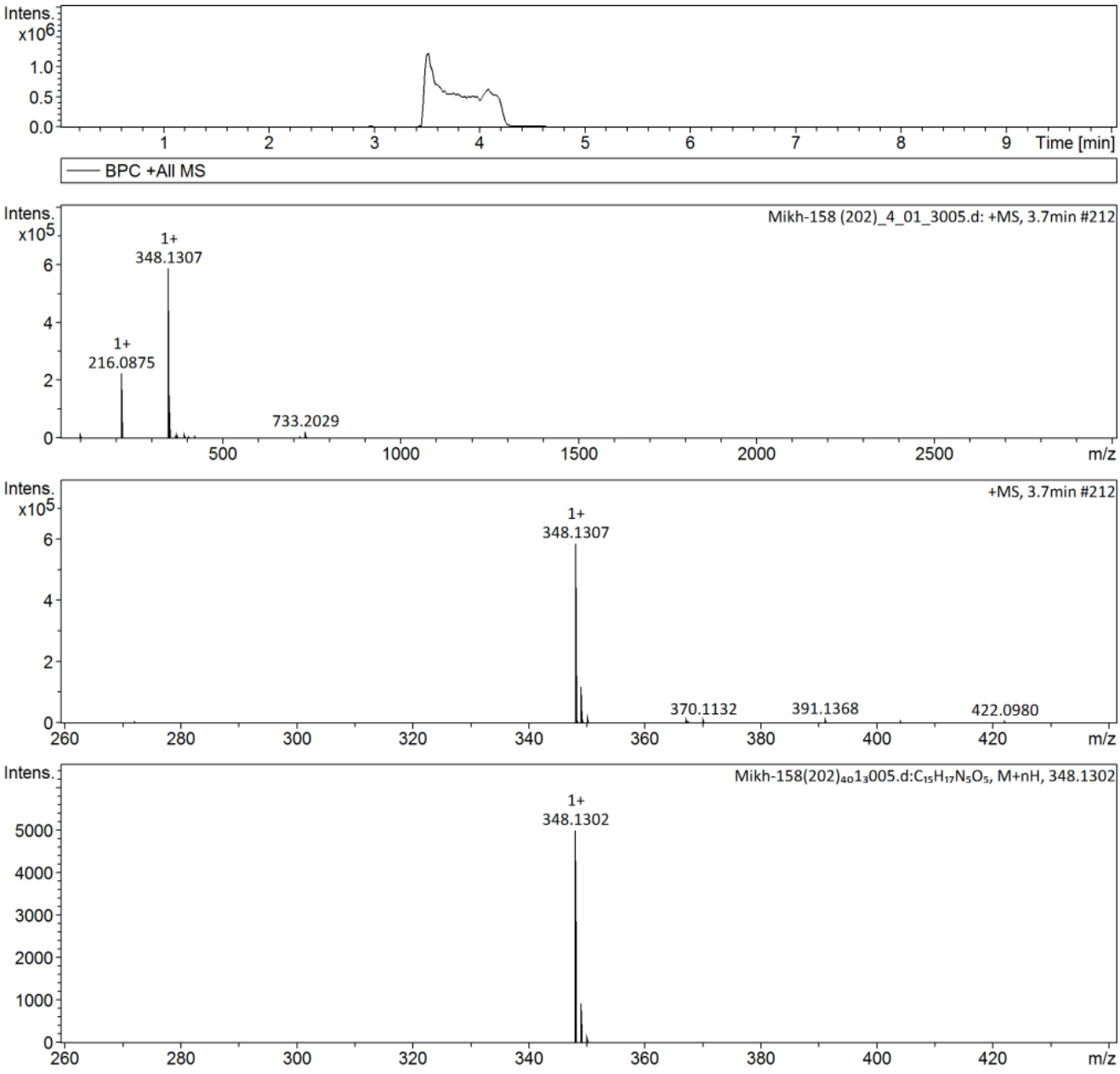
^{1}H-NMR-spectrum (400 MHz) of N^6-furfuryladenosine (**7**) in DMSO-d_6 at 303 K



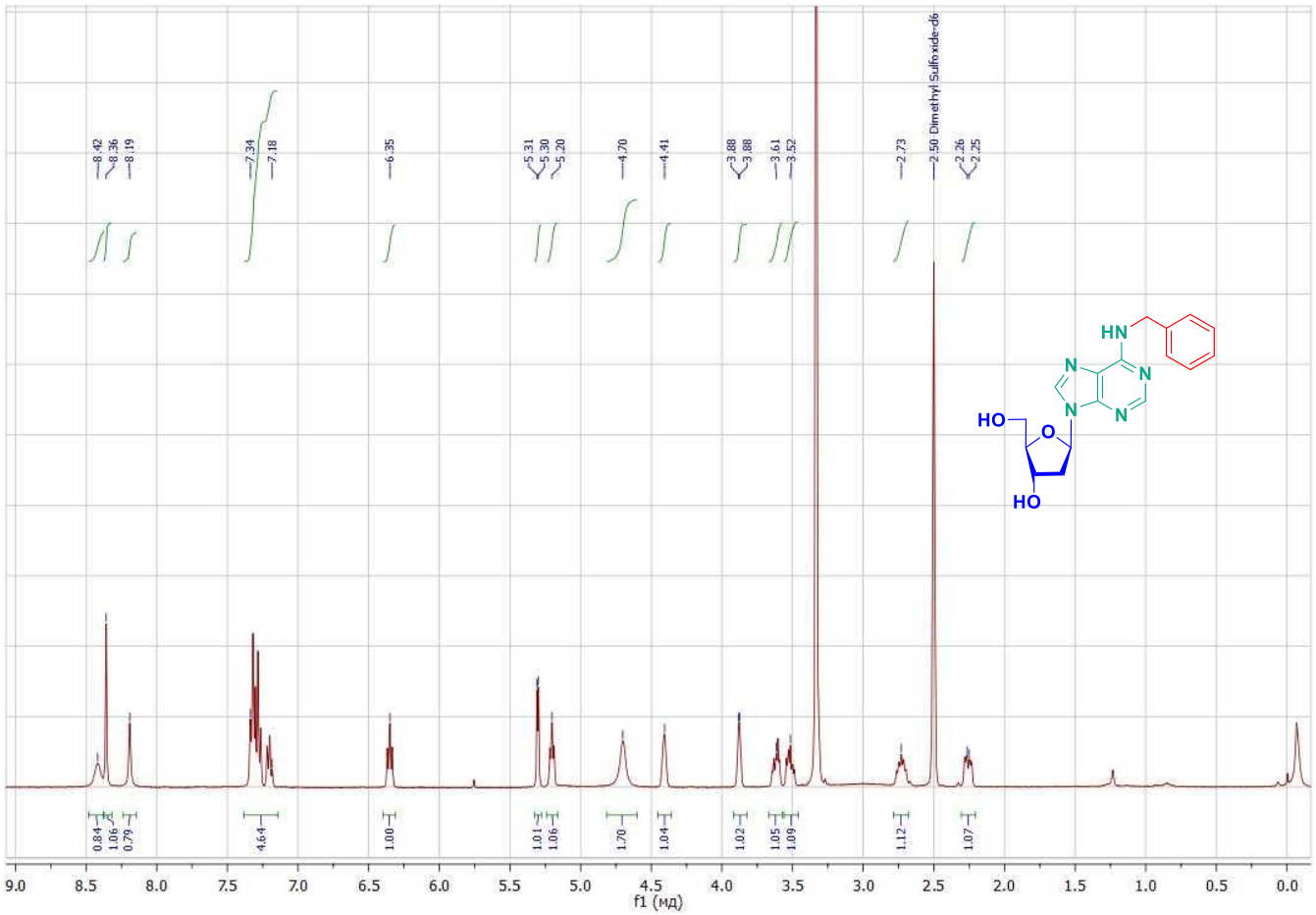
^{13}C-NMR-spectrum (100 MHz) of N^6-furfuryladenosine (**7**) in DMSO-d_6 at 303 K

Acquisition Parameter

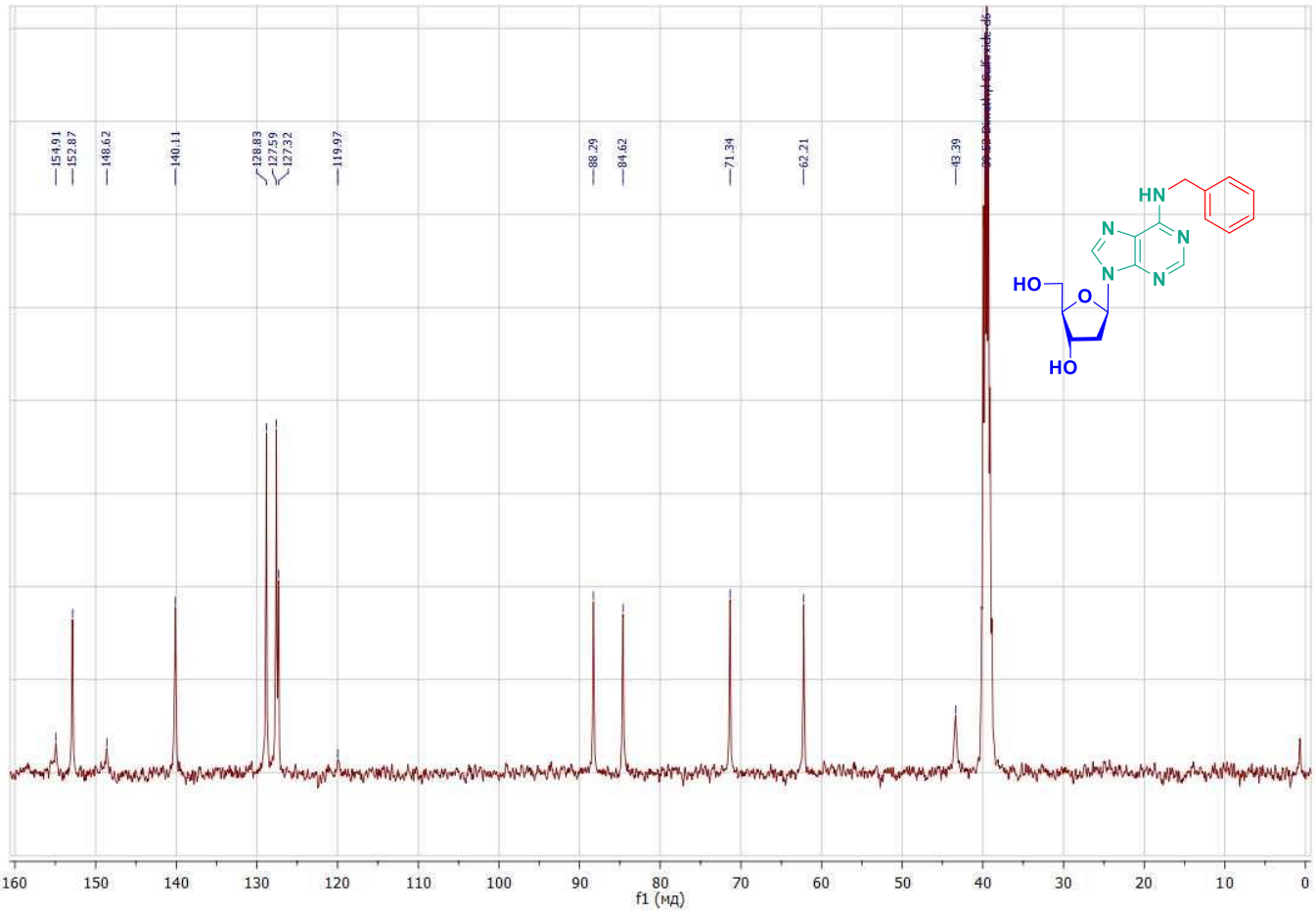
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



HPLC-HRMS spectrum of N^6-furfuryladenosine (**7**)



^{1}H-NMR-spectrum (400 MHz) of N^{6}-benzyl-2′-deoxyadenosine (**8**) in DMSO-d_6 at 303 K



^{13}C-NMR-spectrum (100 MHz) of N^{6}-benzyl-2′-deoxyadenosine (**8**) in DMSO-d_6 at 303 K

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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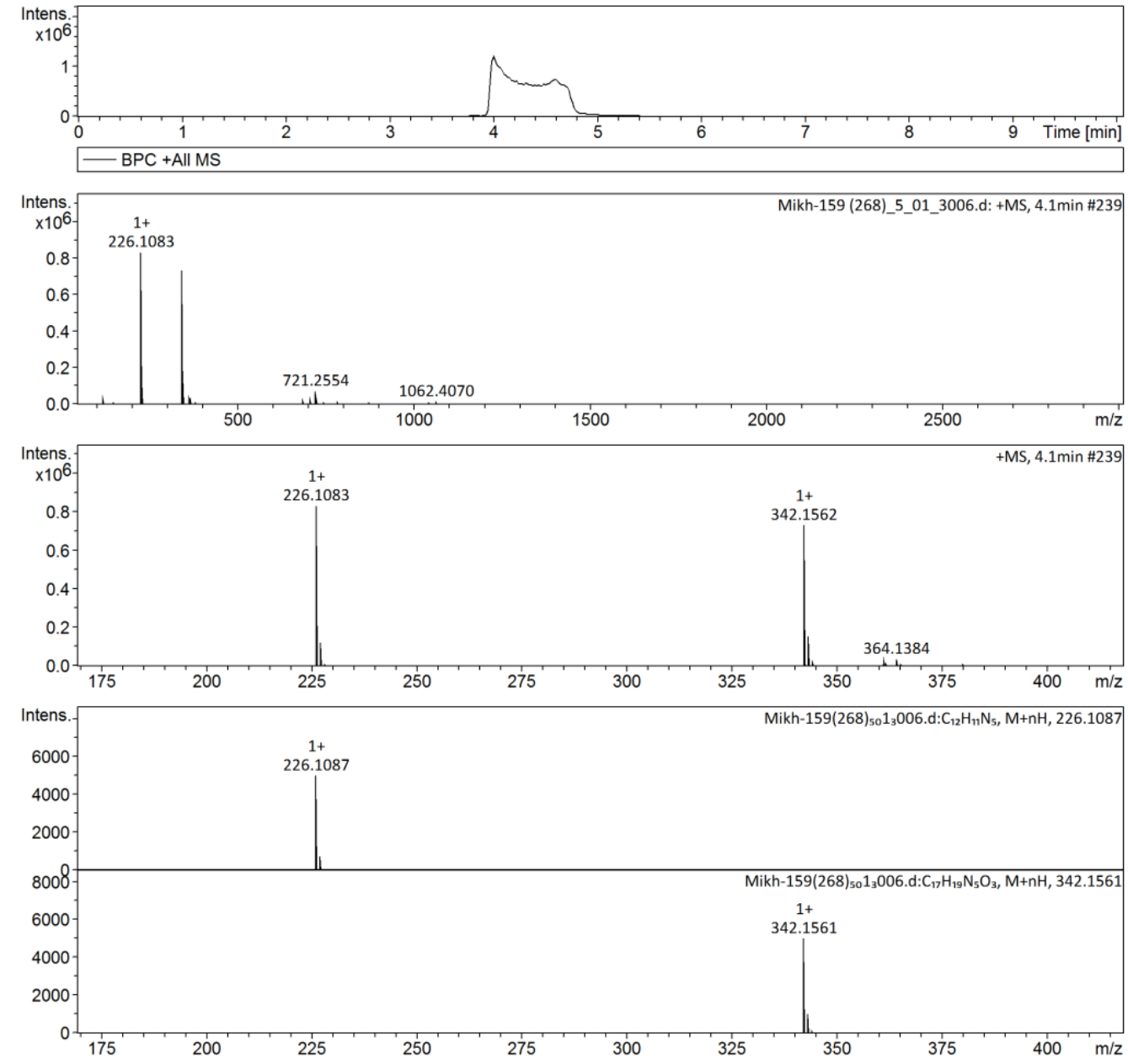
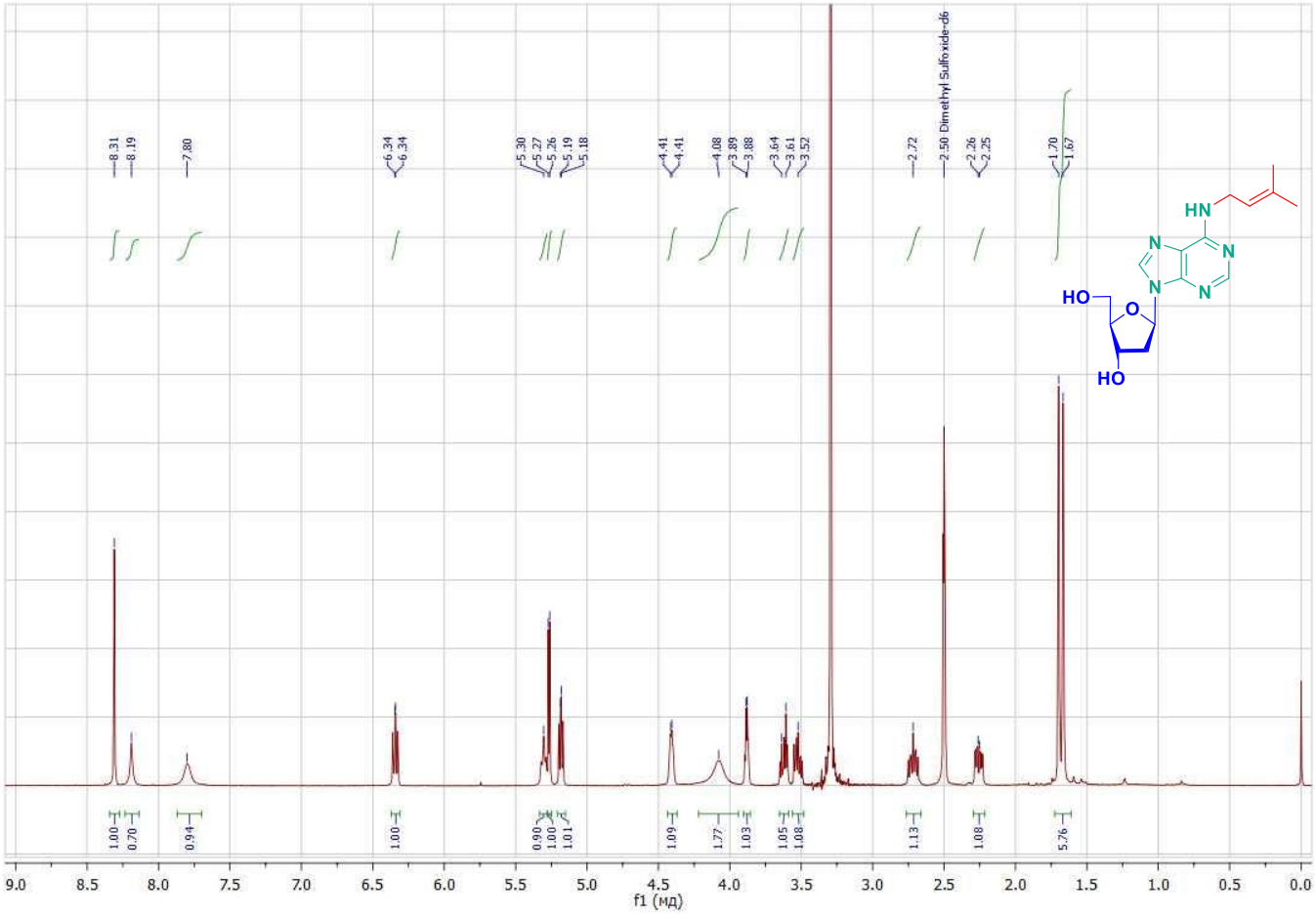
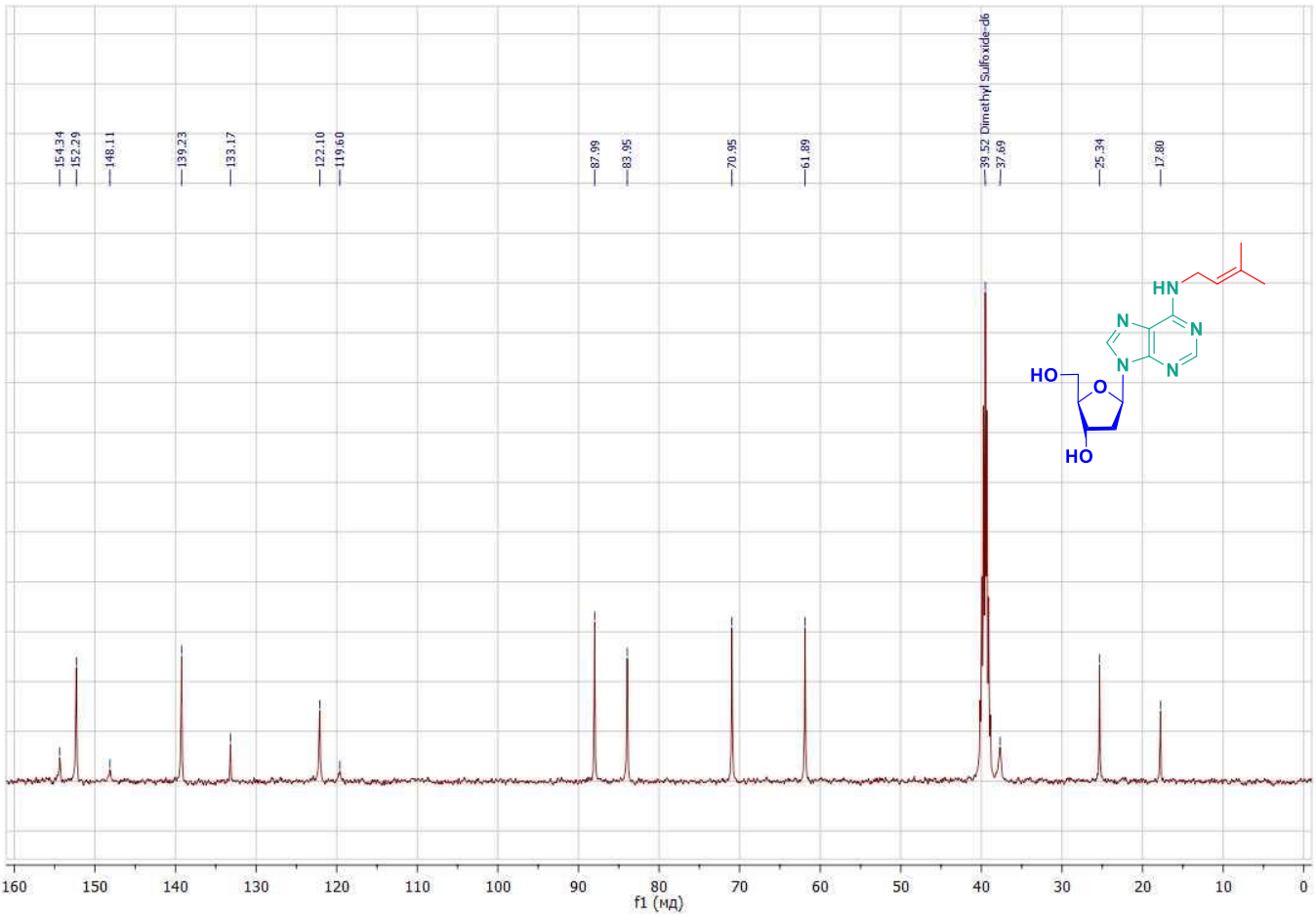


HPLC-HRMS spectrum of N^6-benzyl-2′-deoxyadenosine (**8**)



^{1}H-NMR-spectrum (400 MHz) of N^6-isopentenyl-2′-deoxyadenosine (**9**) in DMSO-d_6 at 303 K



^{13}C-NMR-spectrum (100 MHz) of N^6-isopentenyl-2′-deoxyadenosine (**9**) in DMSO-d_6 at 303 K

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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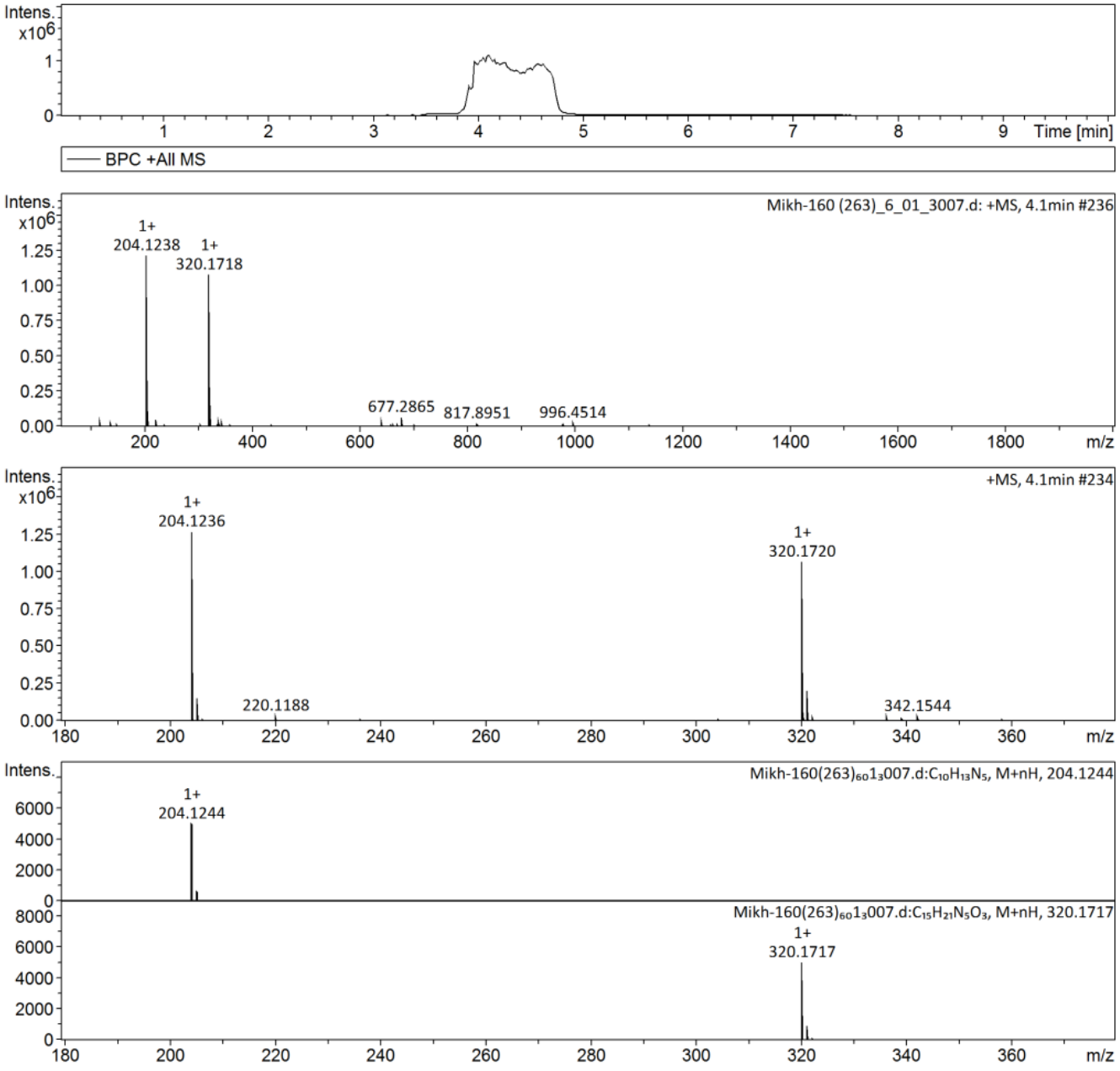
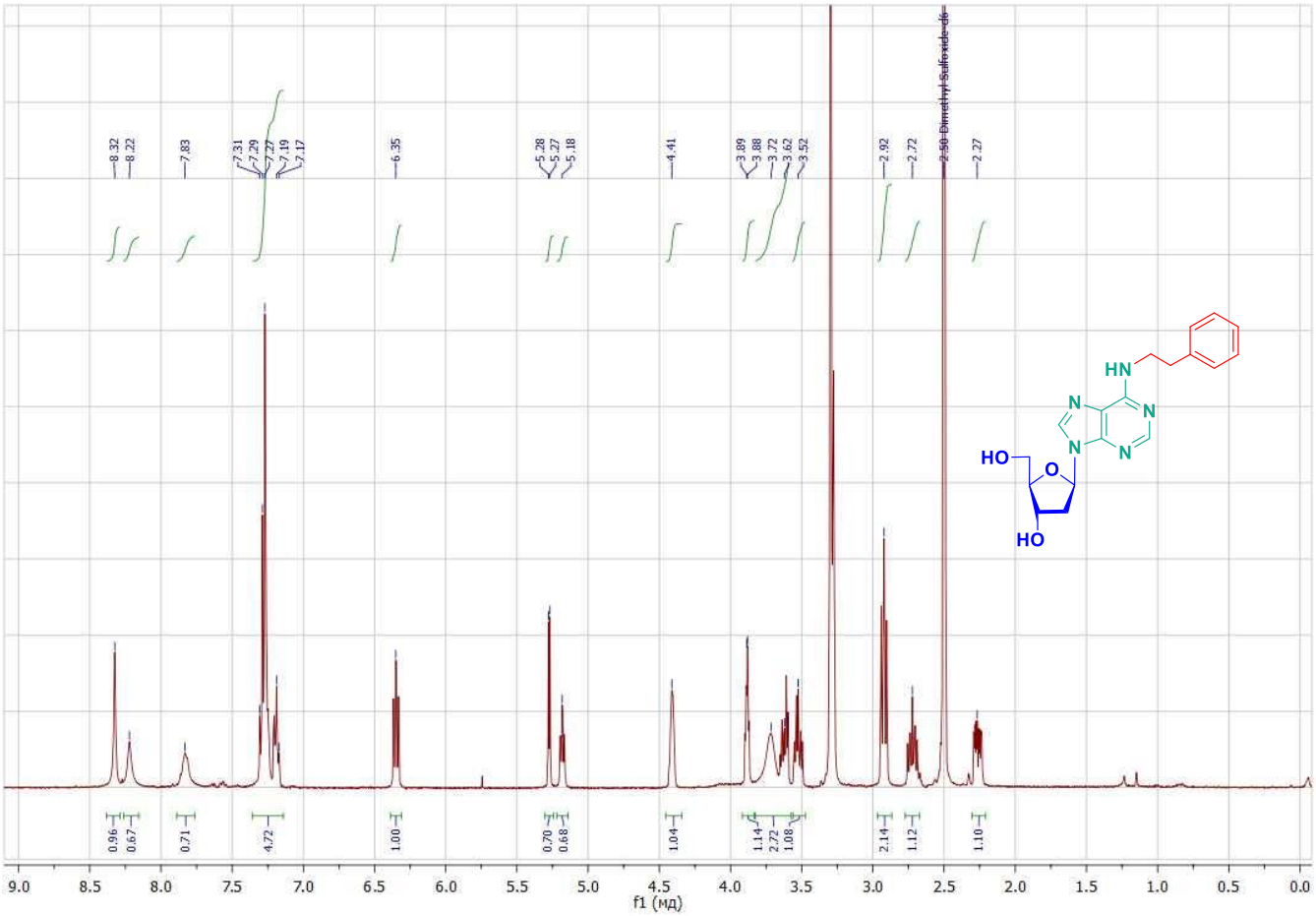
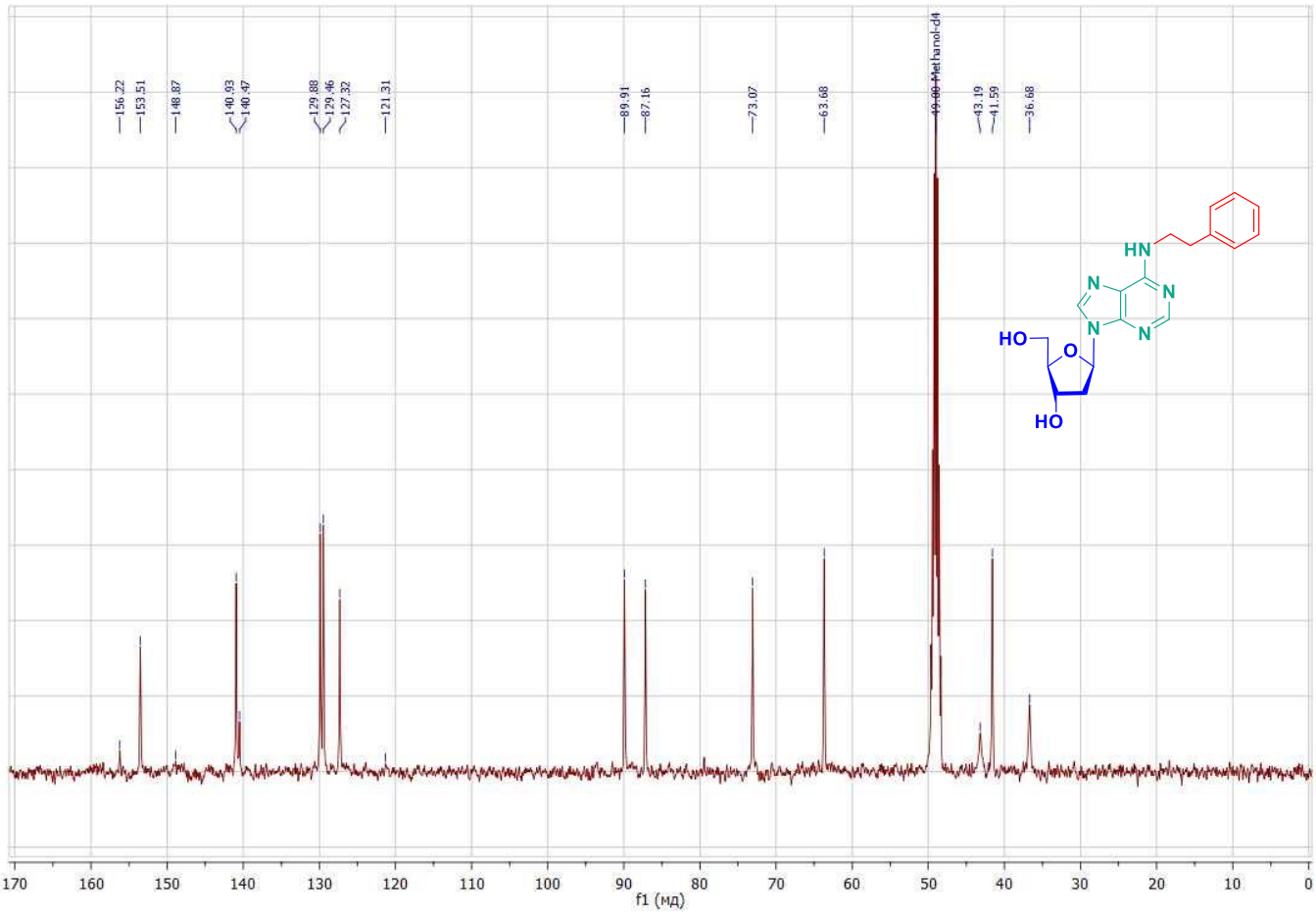


HPLC-HRMS spectrum of N^6-isopentenyl-2′-deoxyadenosine (**9**)



^{1}H-NMR-spectrum (400 MHz) of N^{6}-(2-phenylethyl)-2′-deoxyadenosine (**10**) in DMSO-d_6 at 303 K



^{13}C-NMR-spectrum (100 MHz) of N^{6}-(2-phenylethyl)-2′-deoxyadenosine (**10**) in methanol-d4 at 303 K

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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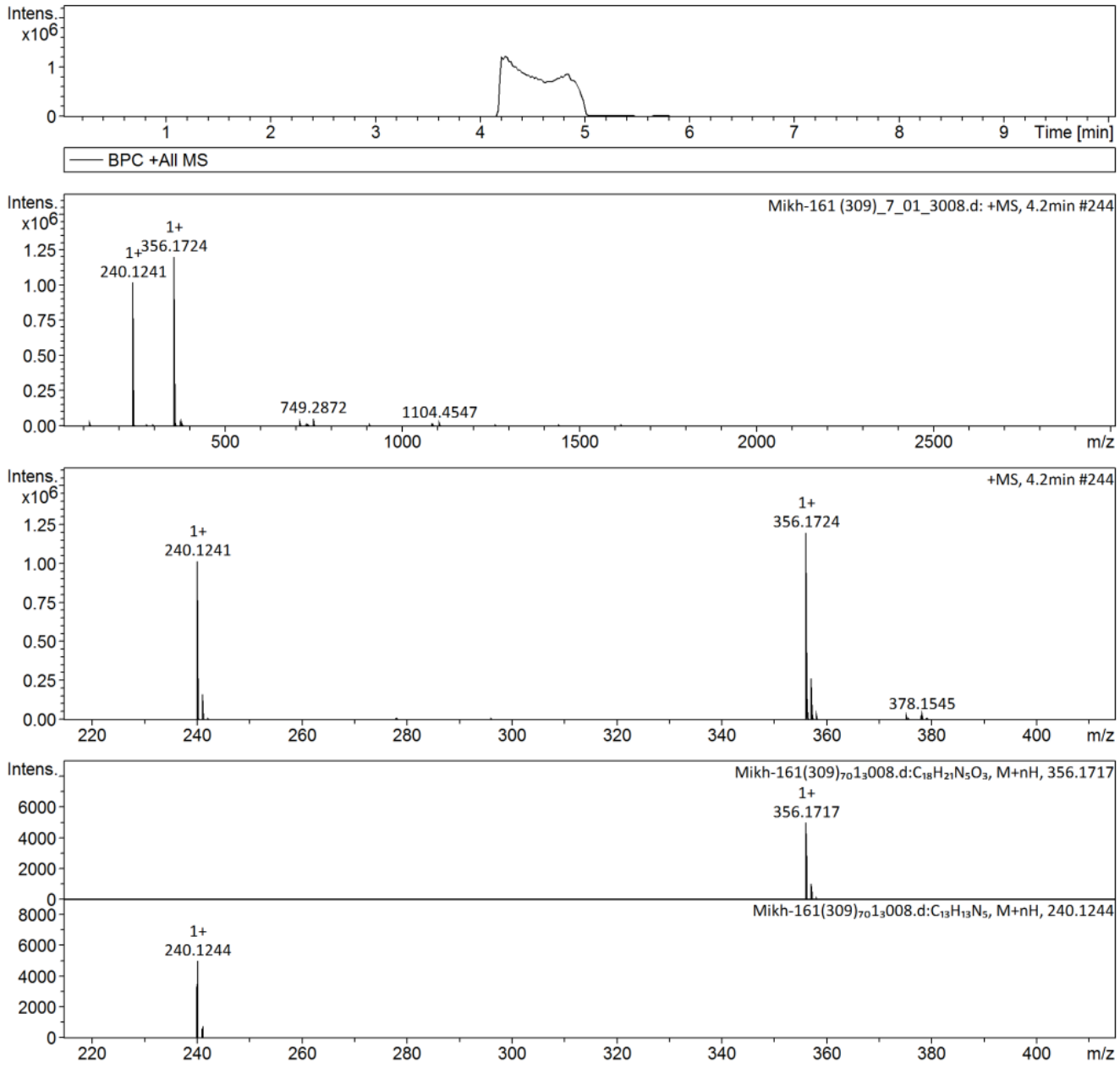
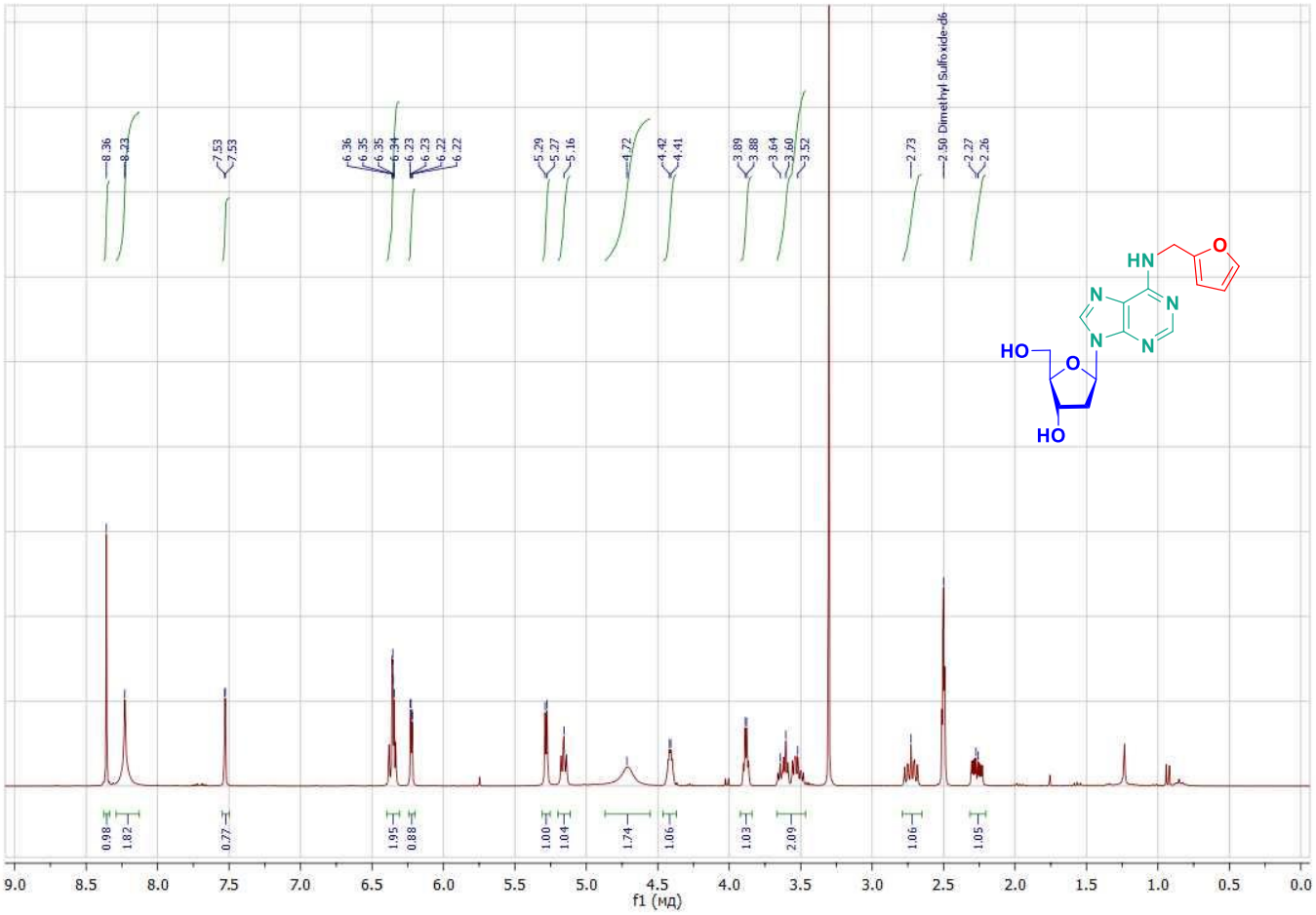
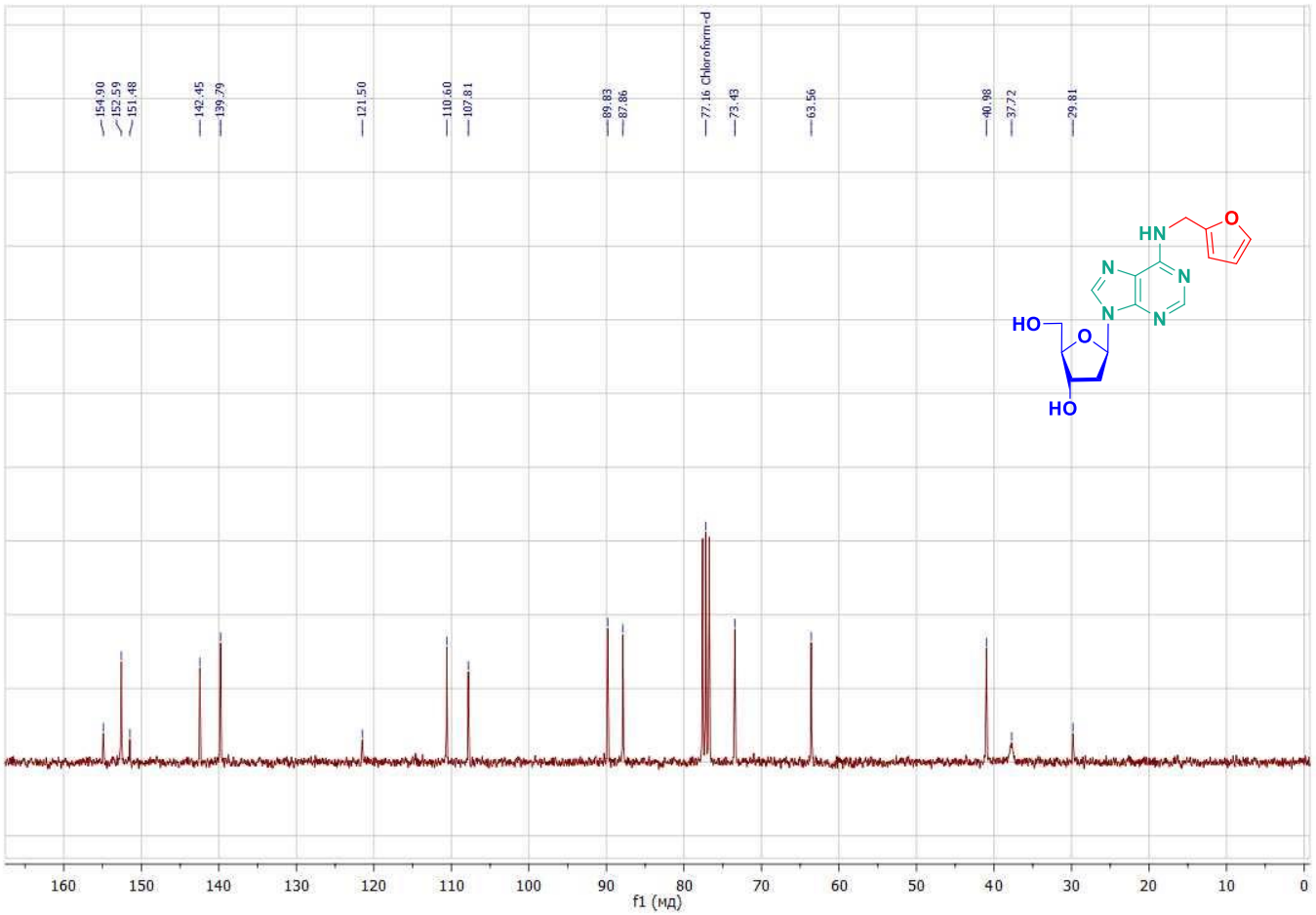


HPLC-HRMS spectrum of N^6-(2-phenylethyl)-2′-deoxyadenosine (**10**)



^{1}H-NMR-spectrum (400 MHz) of N^{6}-furfuryl-2′-deoxyadenosine (**11**) in DMSO-d_6 at 303 K



^{13}C-NMR-spectrum (100 MHz) of N^{6}-furfuryl-2′-deoxyadenosine (**11**) in $CDCl_3$ at 303 K

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
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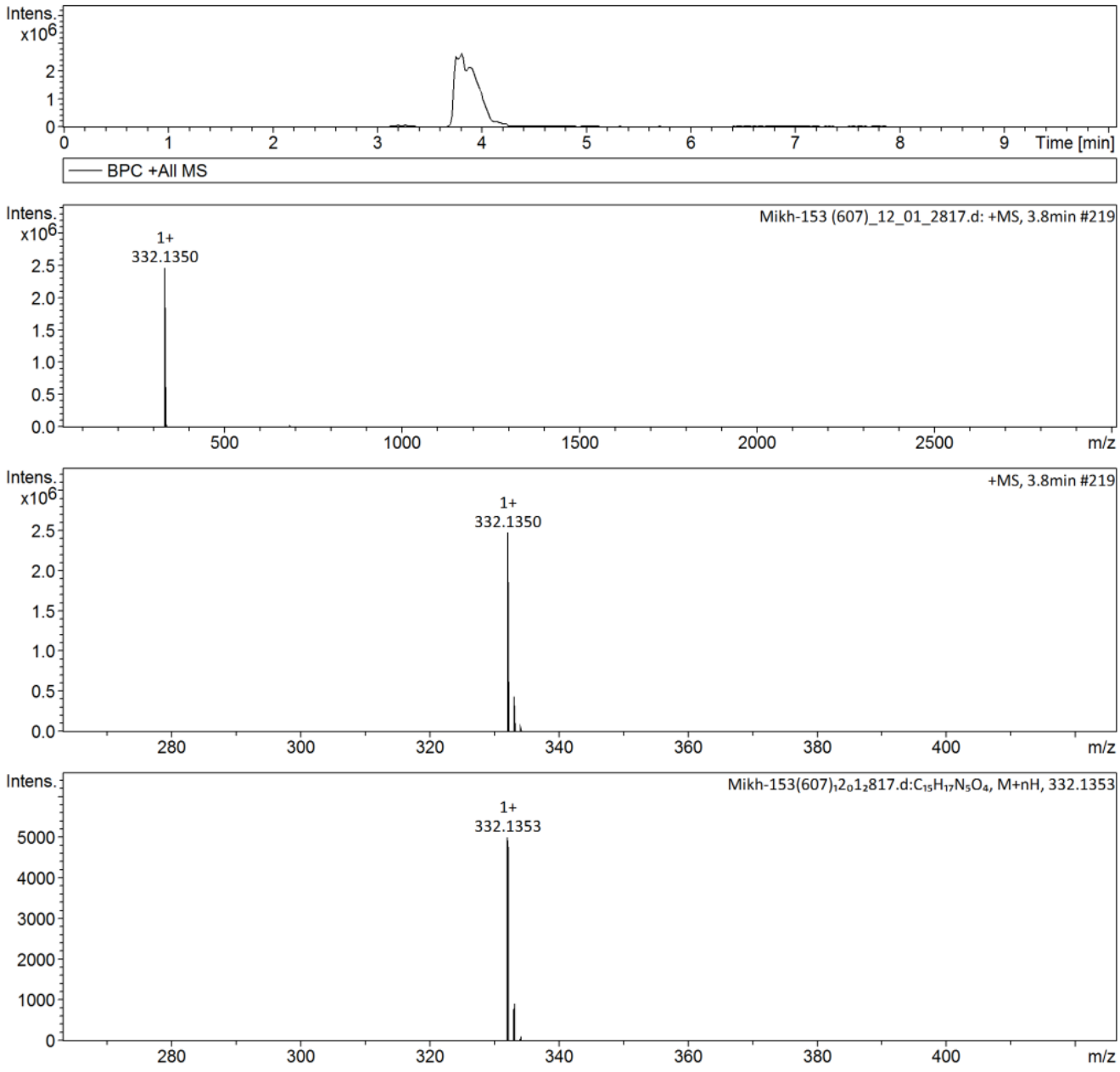
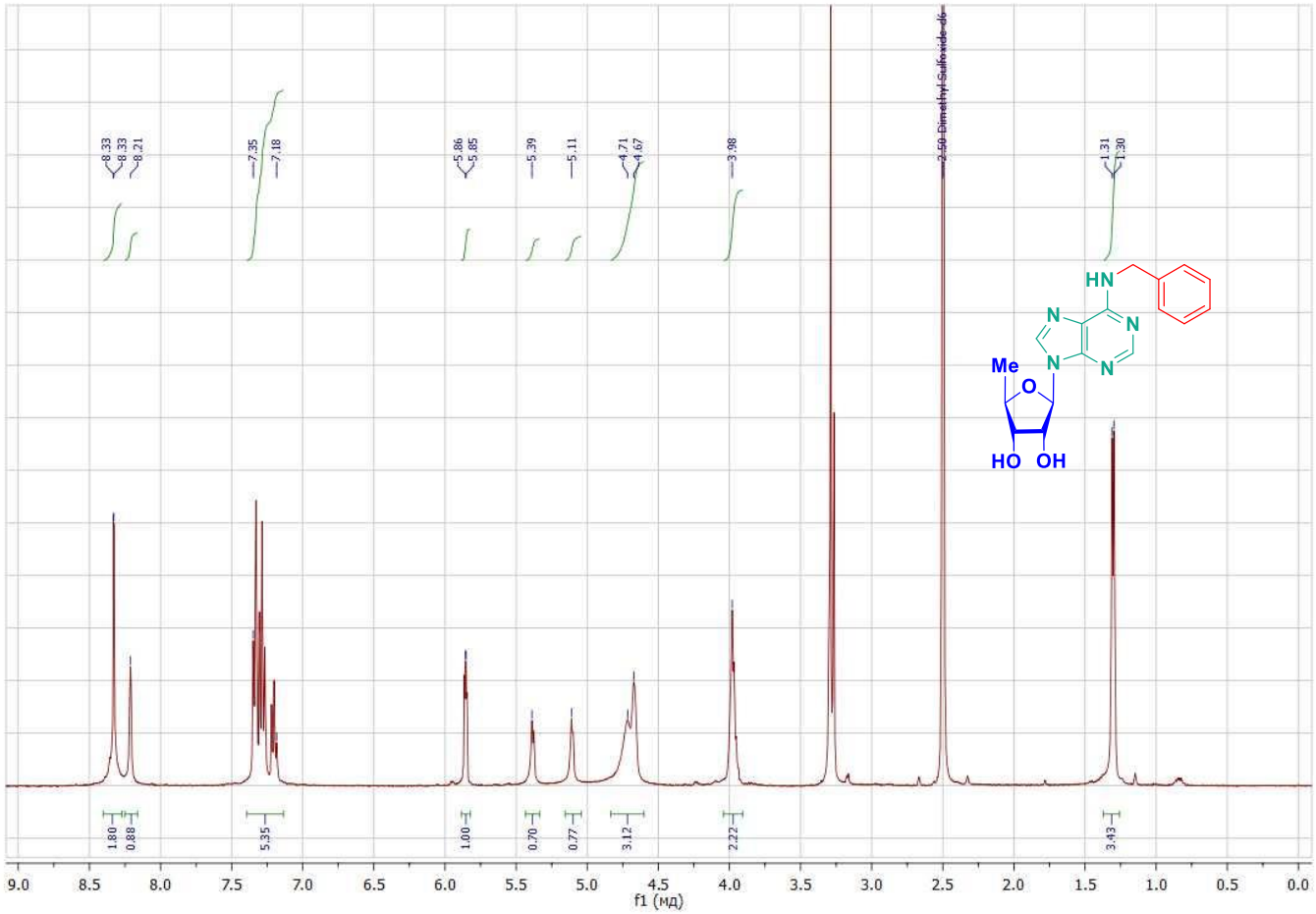
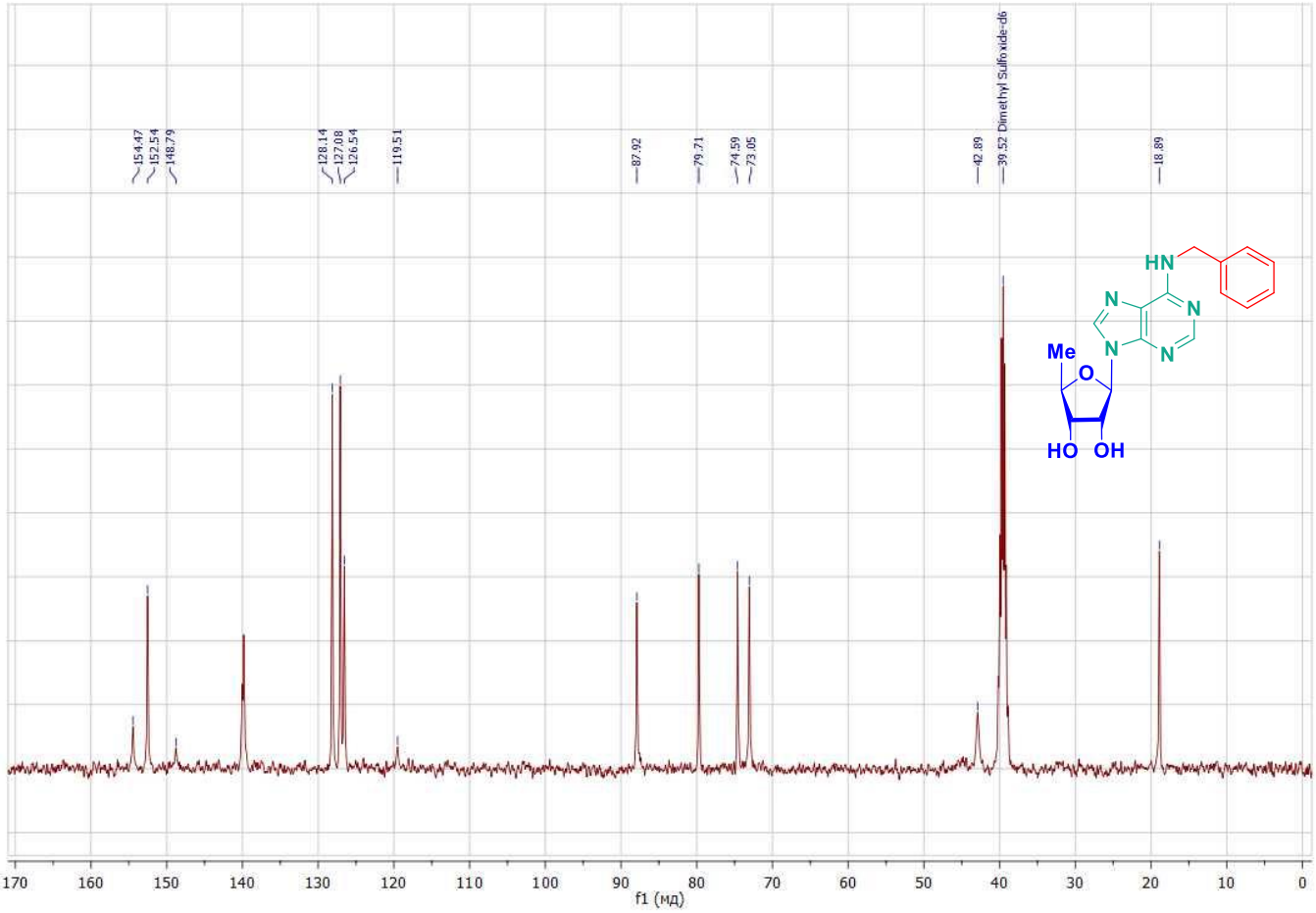
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



HPLC-HRMS spectrum of N^6-furfuryl-2′-deoxyadenosine (**11**)



^{1}H-NMR-spectrum (400 MHz) of N^{6}-benzyl-5′-deoxyadenosine (**12**) in DMSO-d_6 at 303 K



^{13}C-NMR-spectrum (100 MHz) of N^{6}-benzyl-5′-deoxyadenosine (**12**) in DMSO-d_6 at 303 K

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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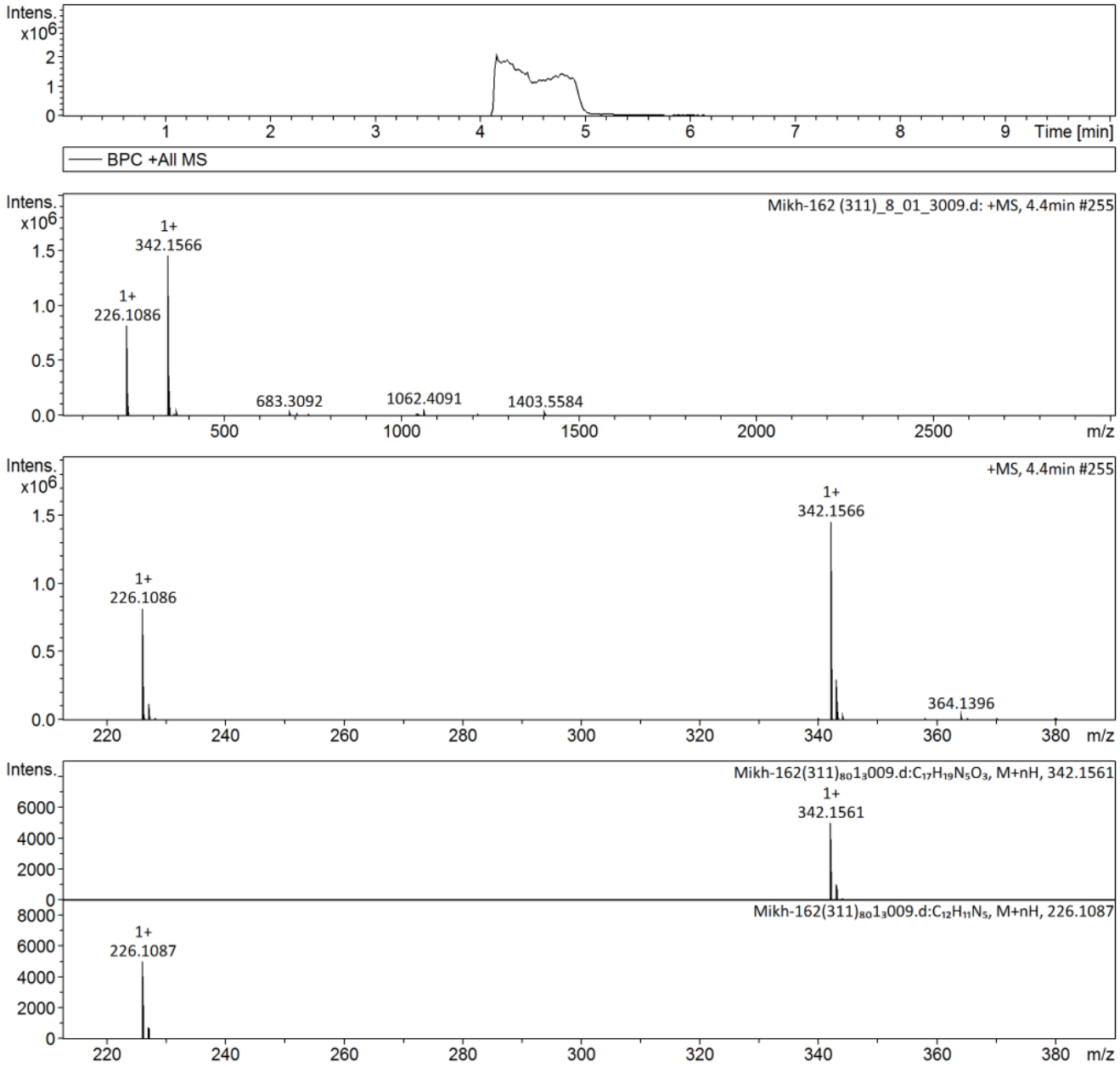
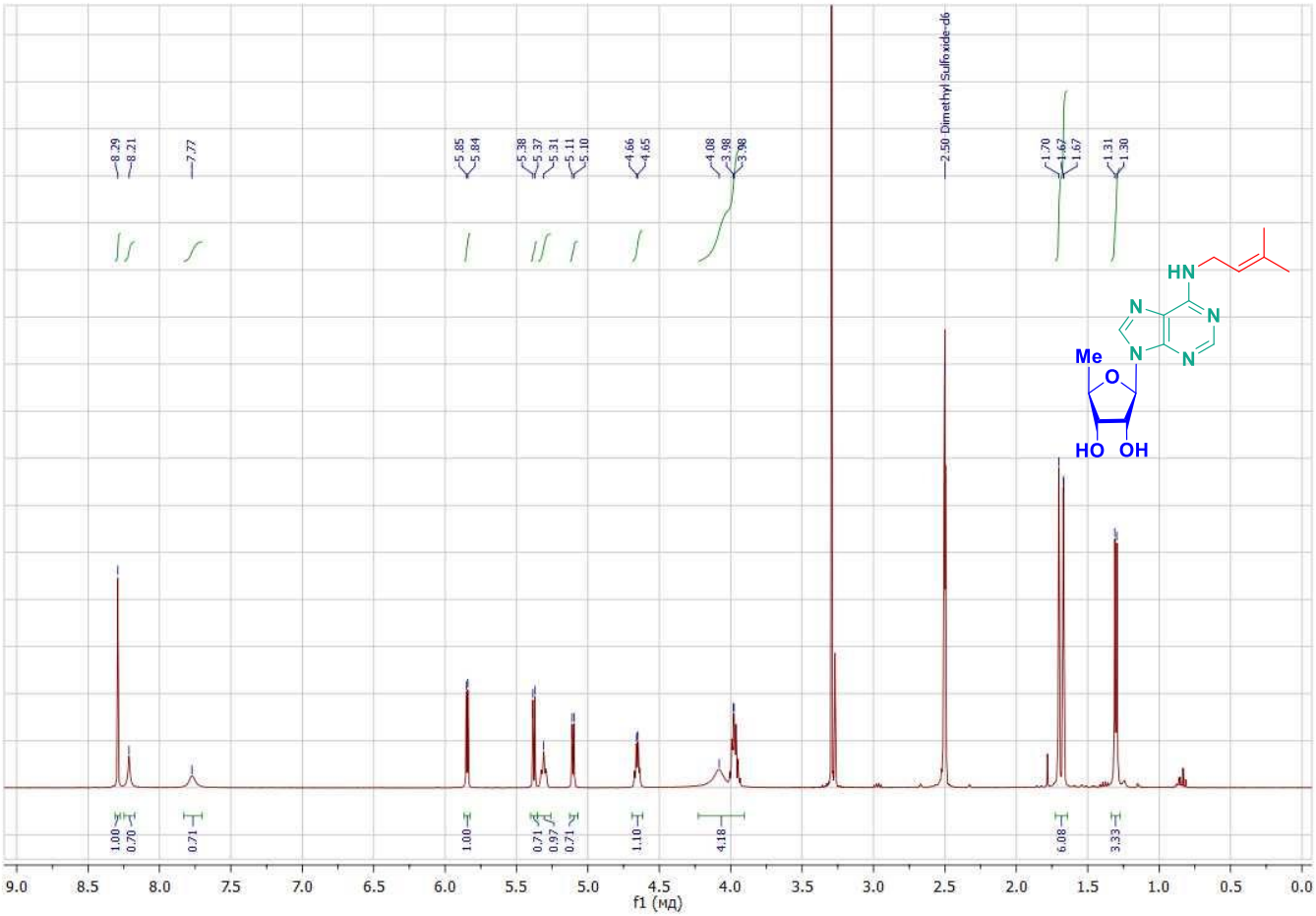
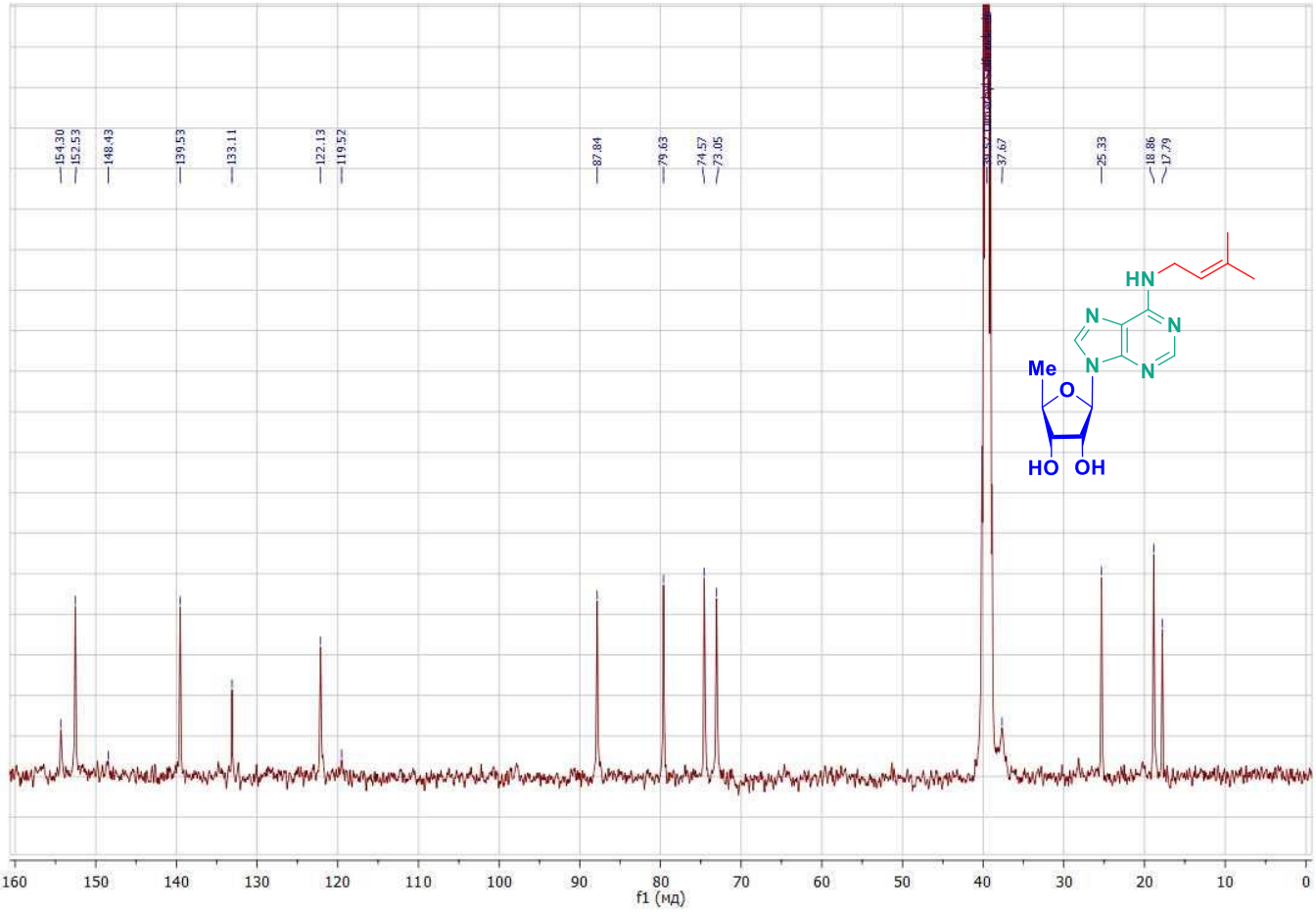


HPLC-HRMS spectrum of N^6-benzyl-5′-deoxyadenosine (**12**)



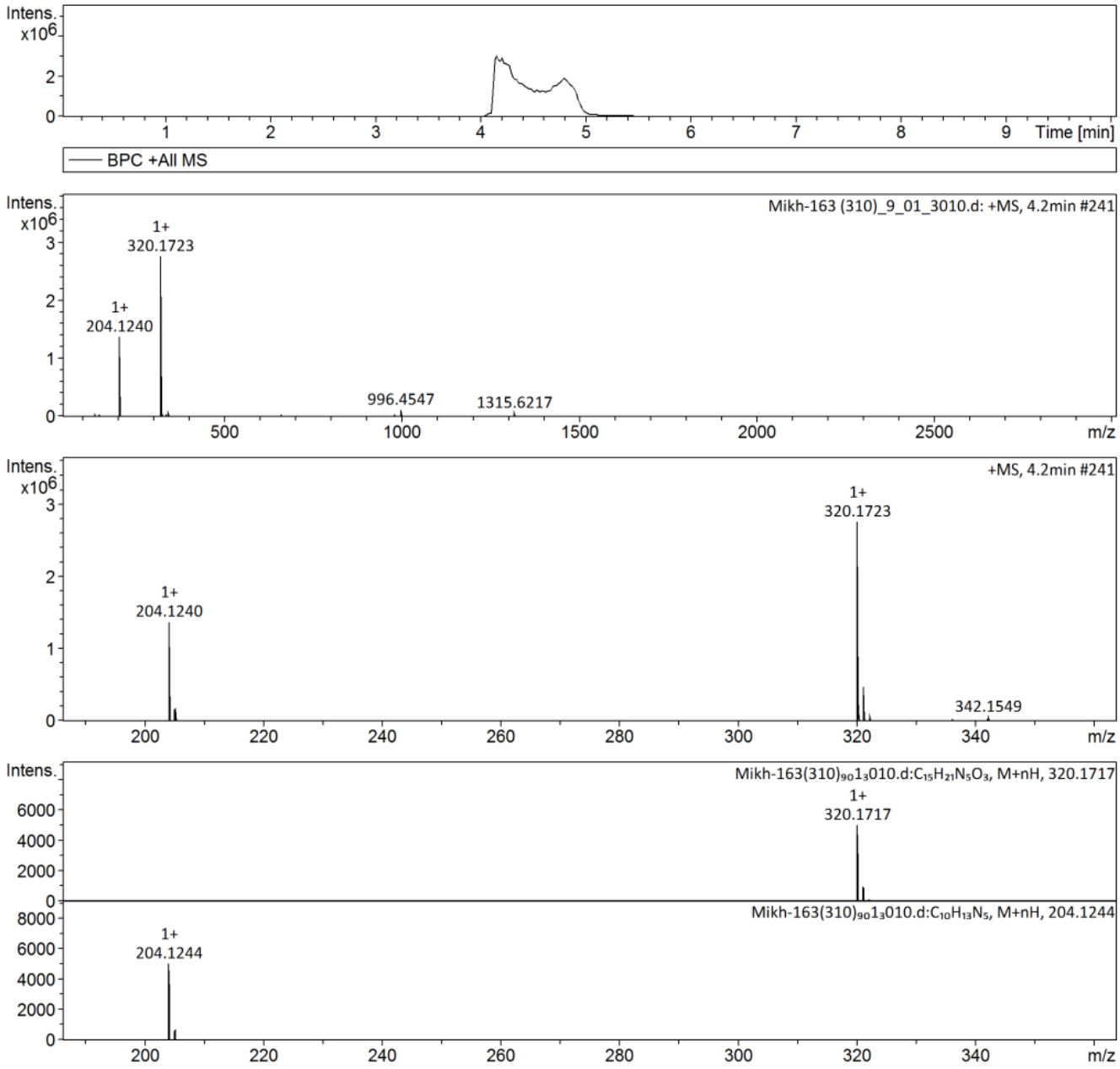
^{1}H-NMR-spectrum (400 MHz) of N^{6}-isopentenyl-5′-deoxyadenosine (**13**) in DMSO-d_6 at 303 K



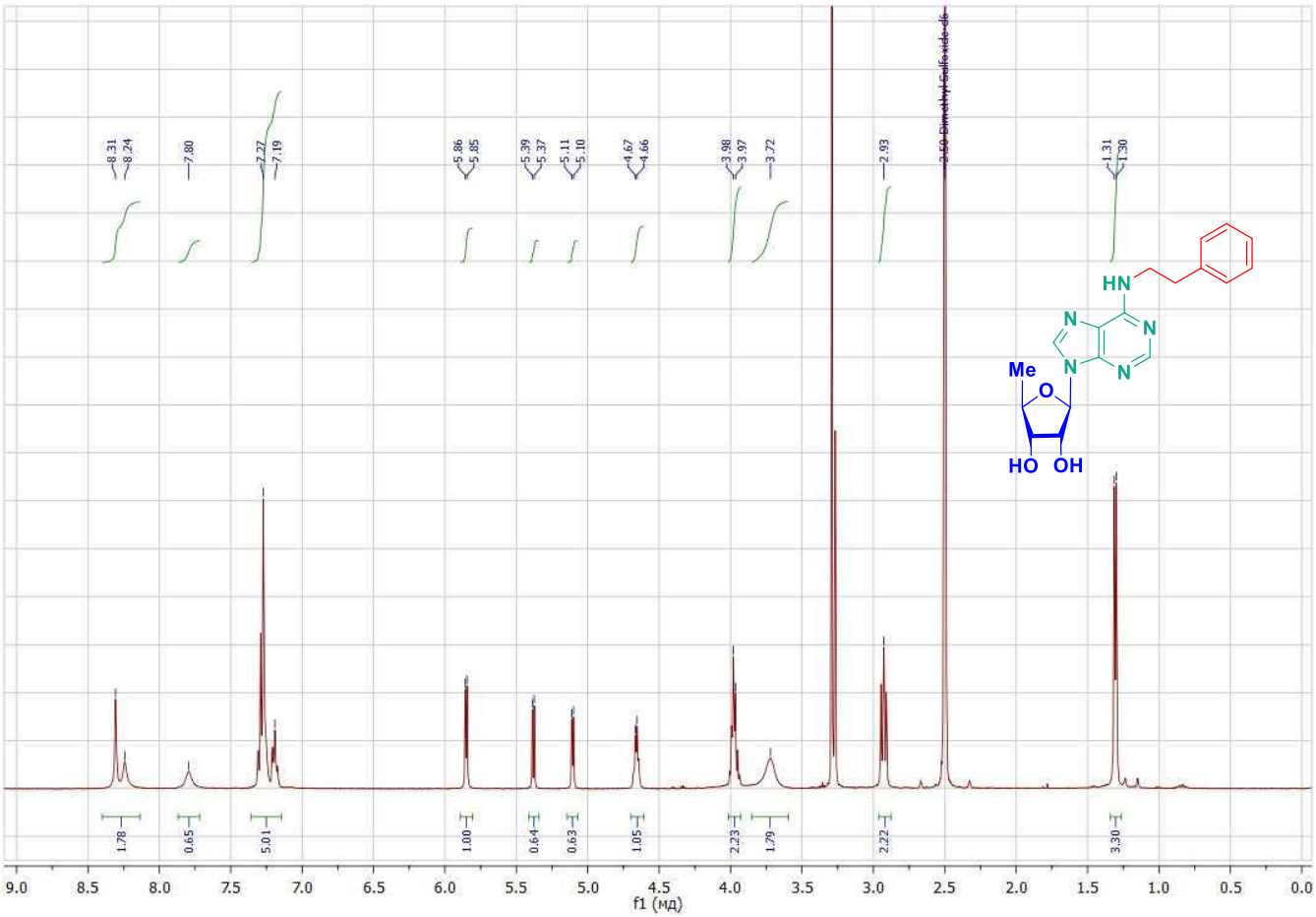
^{13}C-NMR-spectrum (100 MHz) of N^{6}-isopentenyl-5′-deoxyadenosine (**13**) in DMSO-d_6 at 303 K

Acquisition Parameter

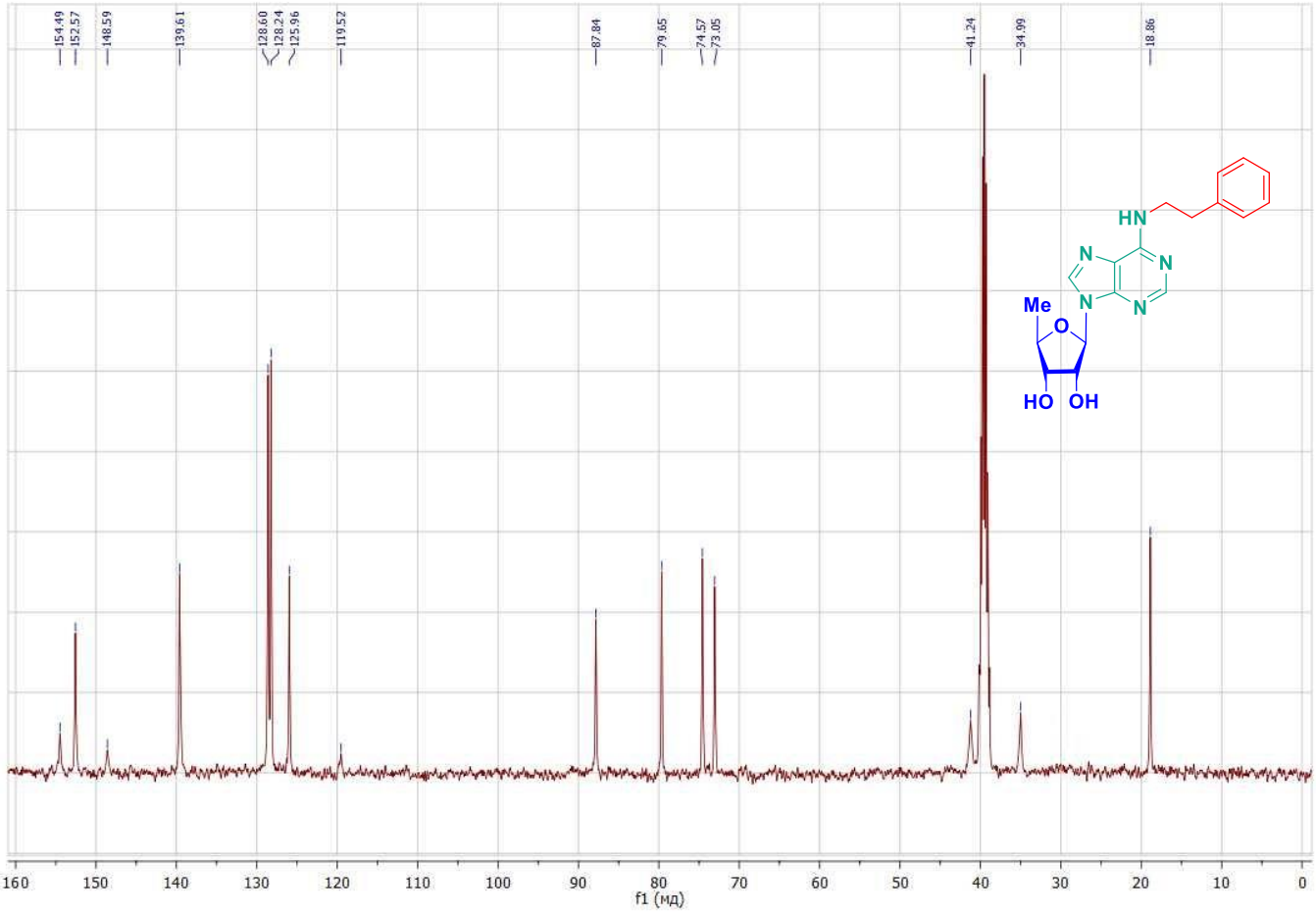
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



HPLC-HRMS spectrum of N^6-isopentenyl-5′-deoxyadenosine (**13**)



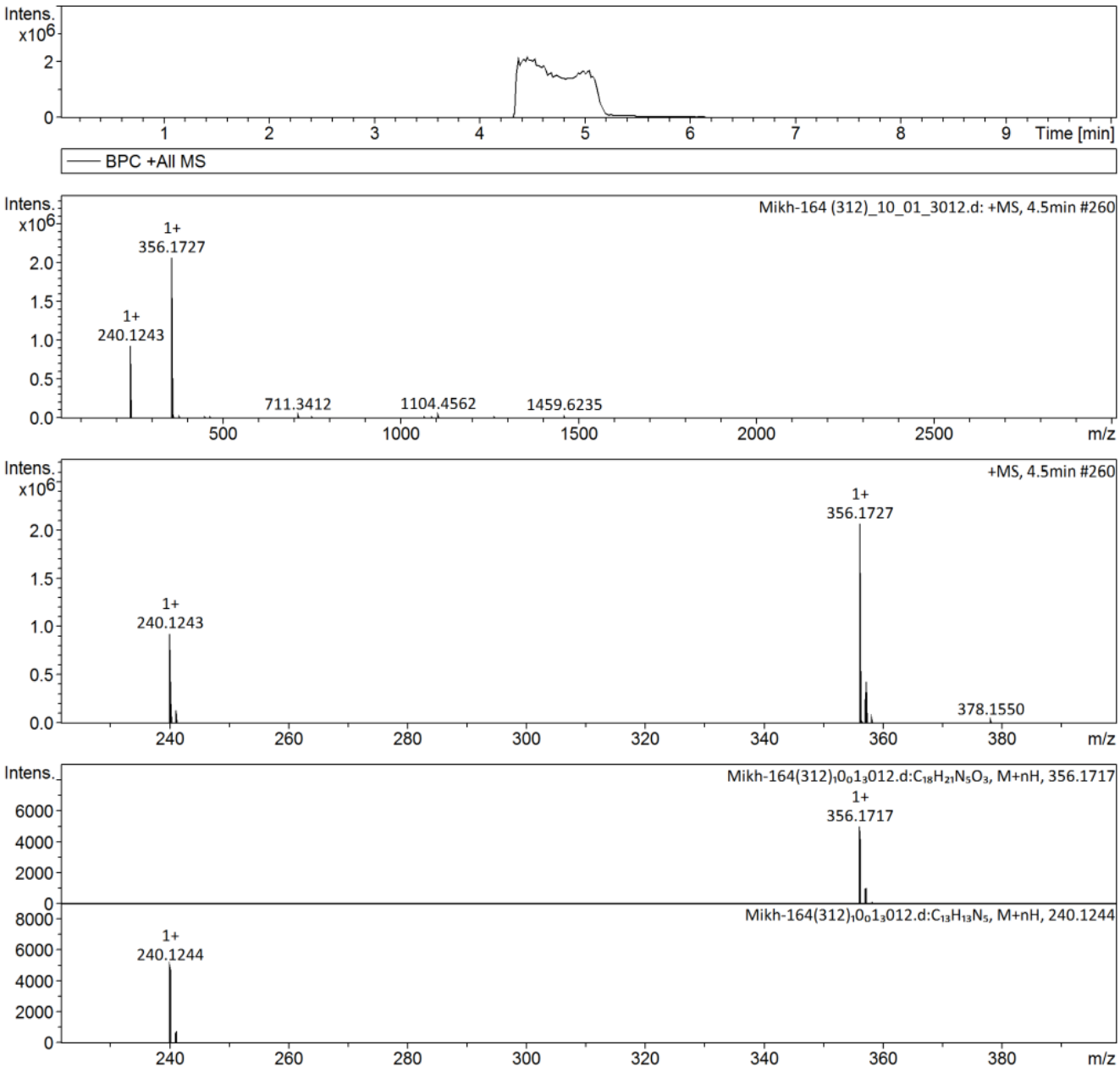
^{1}H-NMR-spectrum (400 MHz) of N^{6}-(2-phenylethyl)-5′-deoxyadenosine (**14**) in DMSO-d_6 at 303 K



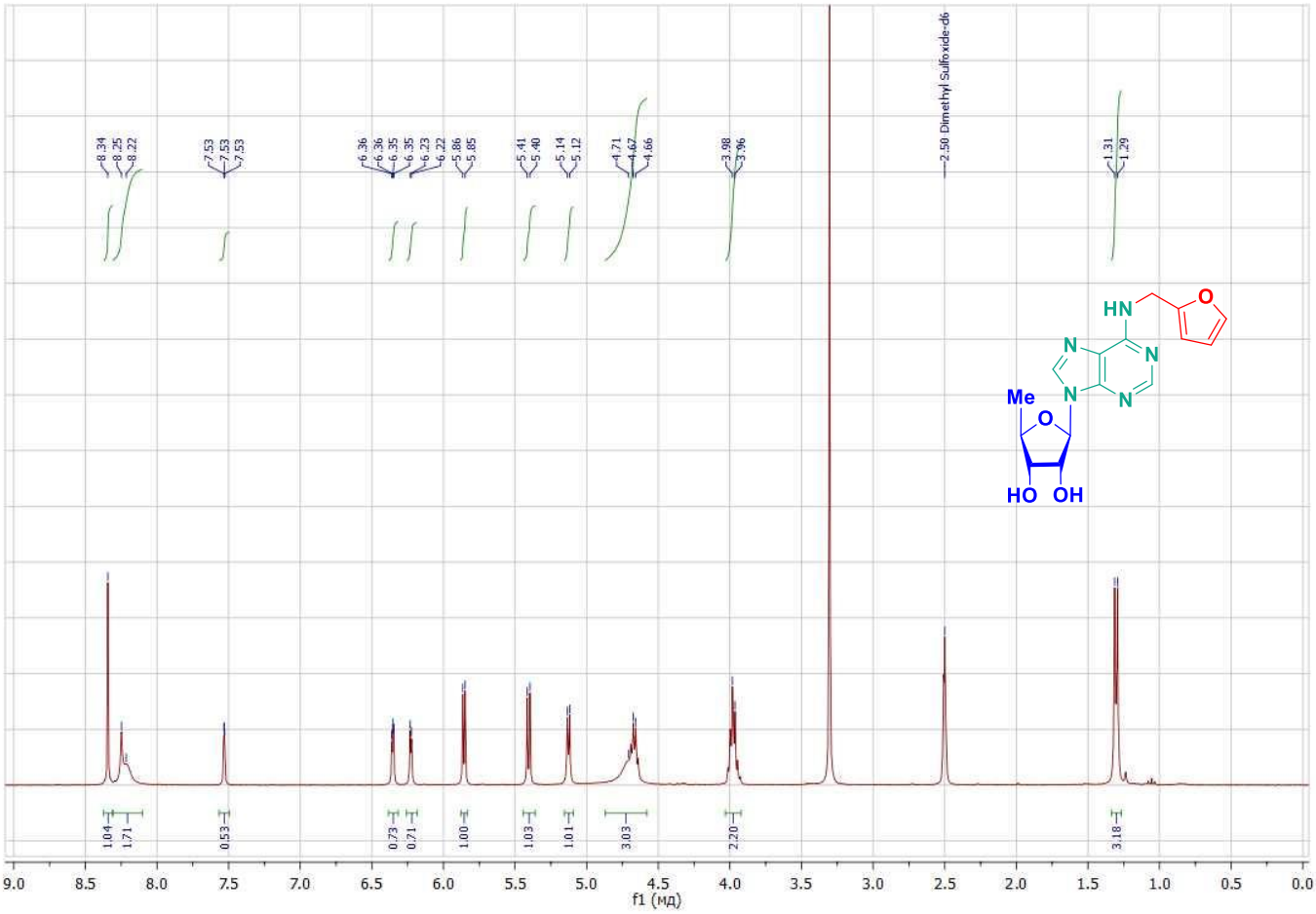
^{13}C-NMR-spectrum (100 MHz) of N^{6}-(2-phenylethyl)-5′-deoxyadenosine (**14**) in DMSO-d_6 at 303 K

Acquisition Parameter

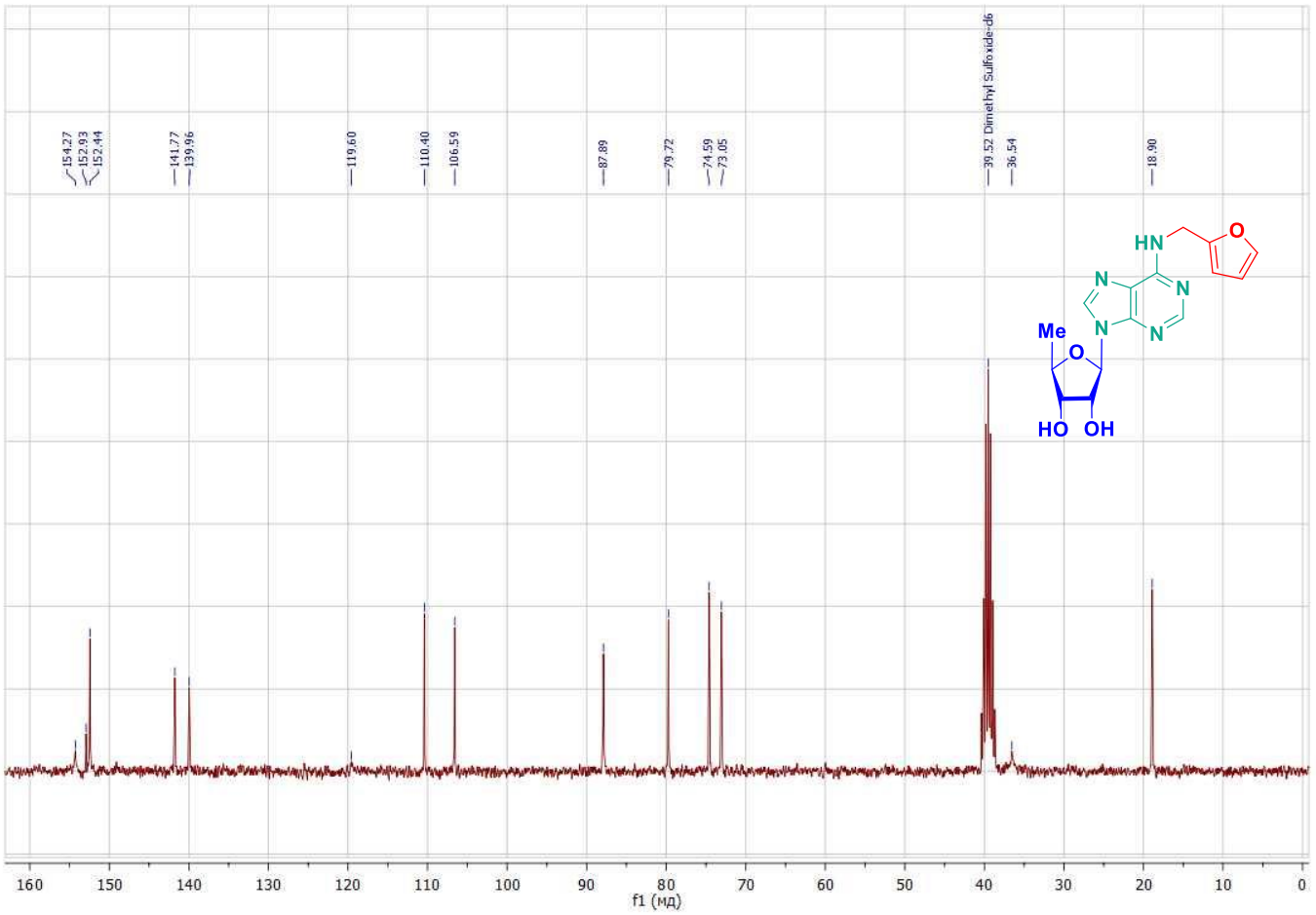
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	3000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



HPLC-HRMS spectrum of N^6-(2-phenylethyl)-5′-deoxyadenosine (**14**)



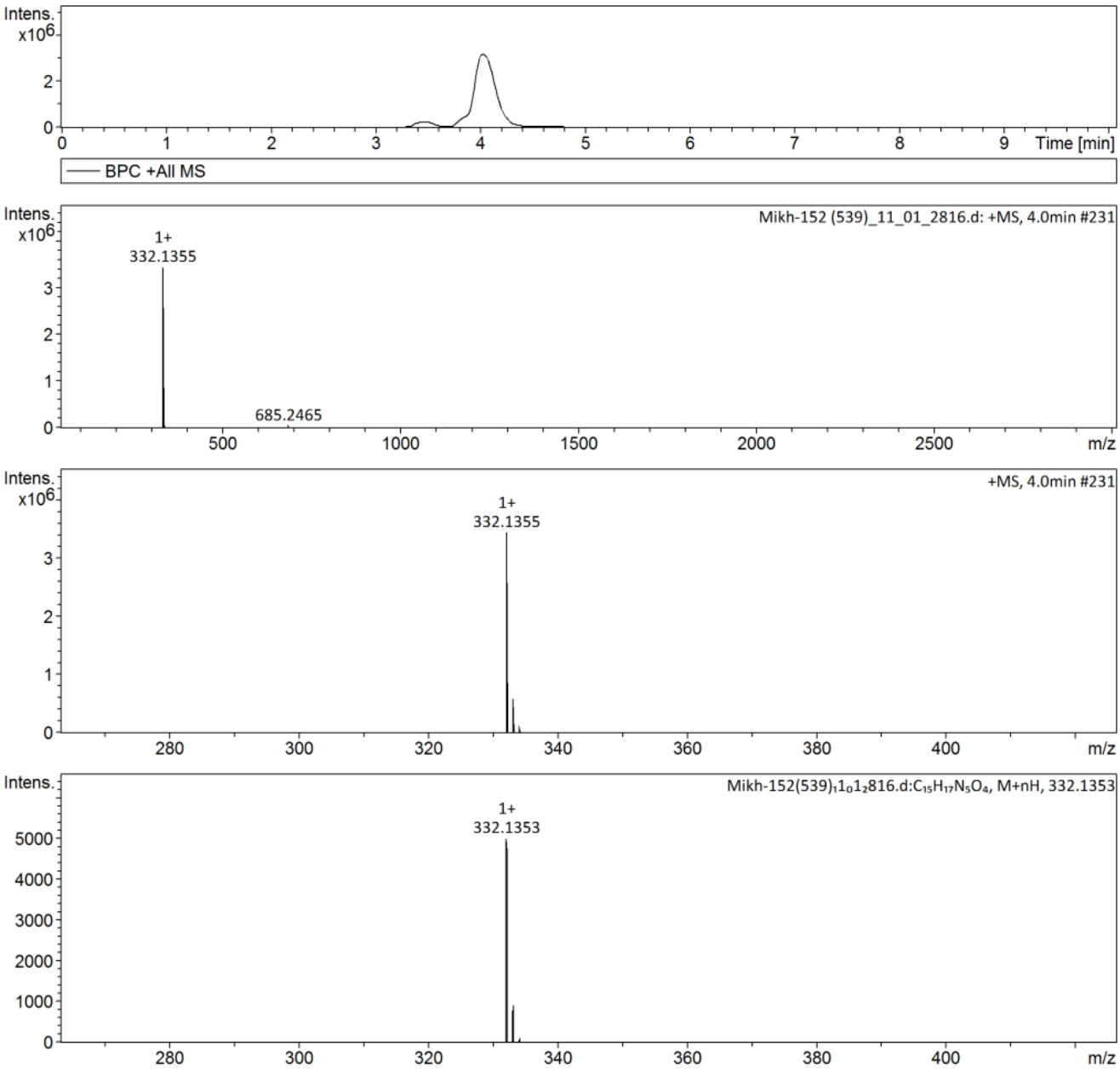
^{1}H-NMR-spectrum (400 MHz) of N^{6}-furfuryl-5′-deoxyadenosine (**15**) in DMSO-d_6 at 303 K



^{13}C-NMR-spectrum (100 MHz) of N^{6}-furfuryl-5′-deoxyadenosine (**15**) in DMSO-d_6 at 303 K

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 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



HPLC-HRMS spectrum of N^6-furfuryl-5′-deoxyadenosine (**15**)