

# **Supporting Information**

## **High Yielding Water-Soluble Asymmetric Cyanine Dyes for Labeling Applications.**

Natalia Wolf, Louise Kersting, Christoph Herok, Cornelius Mihm, Juergen Seibel.

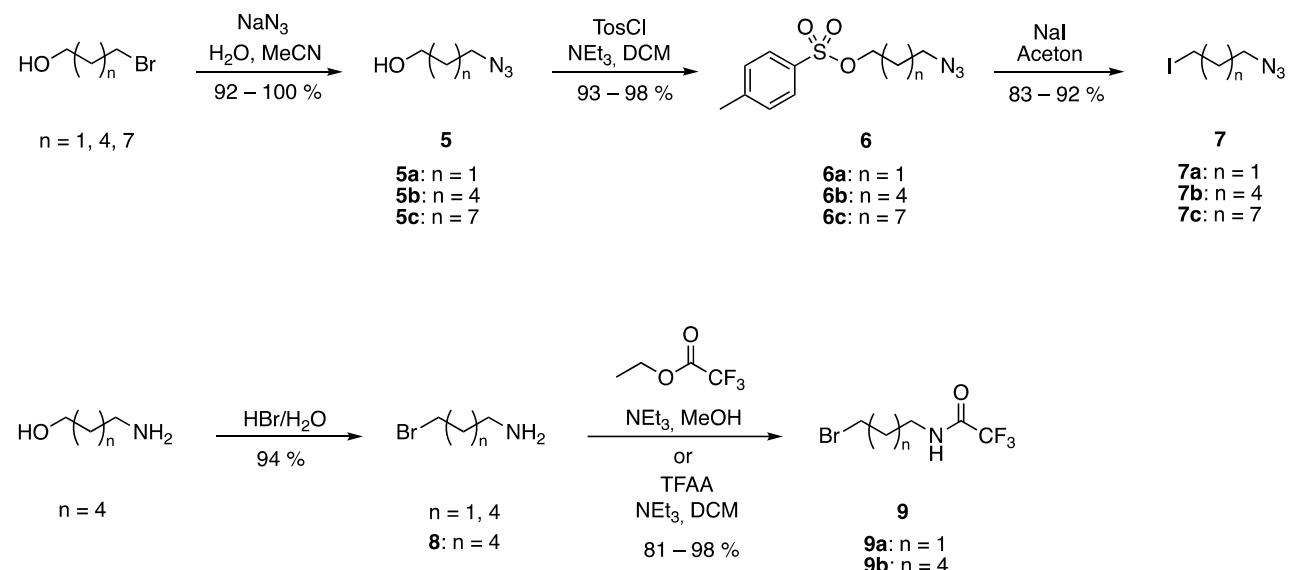
Institute of Organic Chemistry, University of Wuerzburg, 97074 Wuerzburg, Germany.

*Microwave irradiation, cyanine dyes, high yield, labeling applications, click chemistry.*

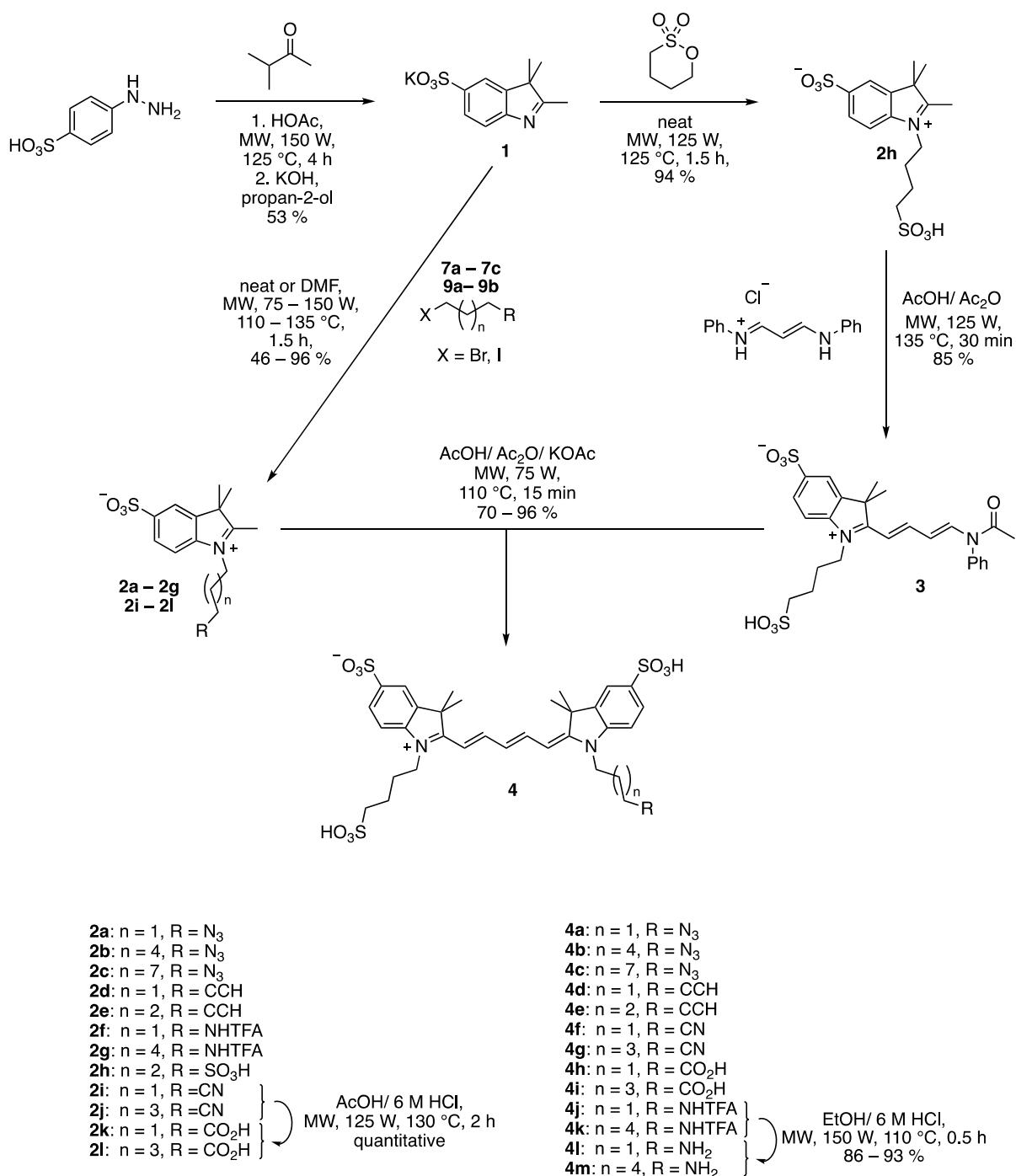
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**Scheme for Synthesized Sidechains**

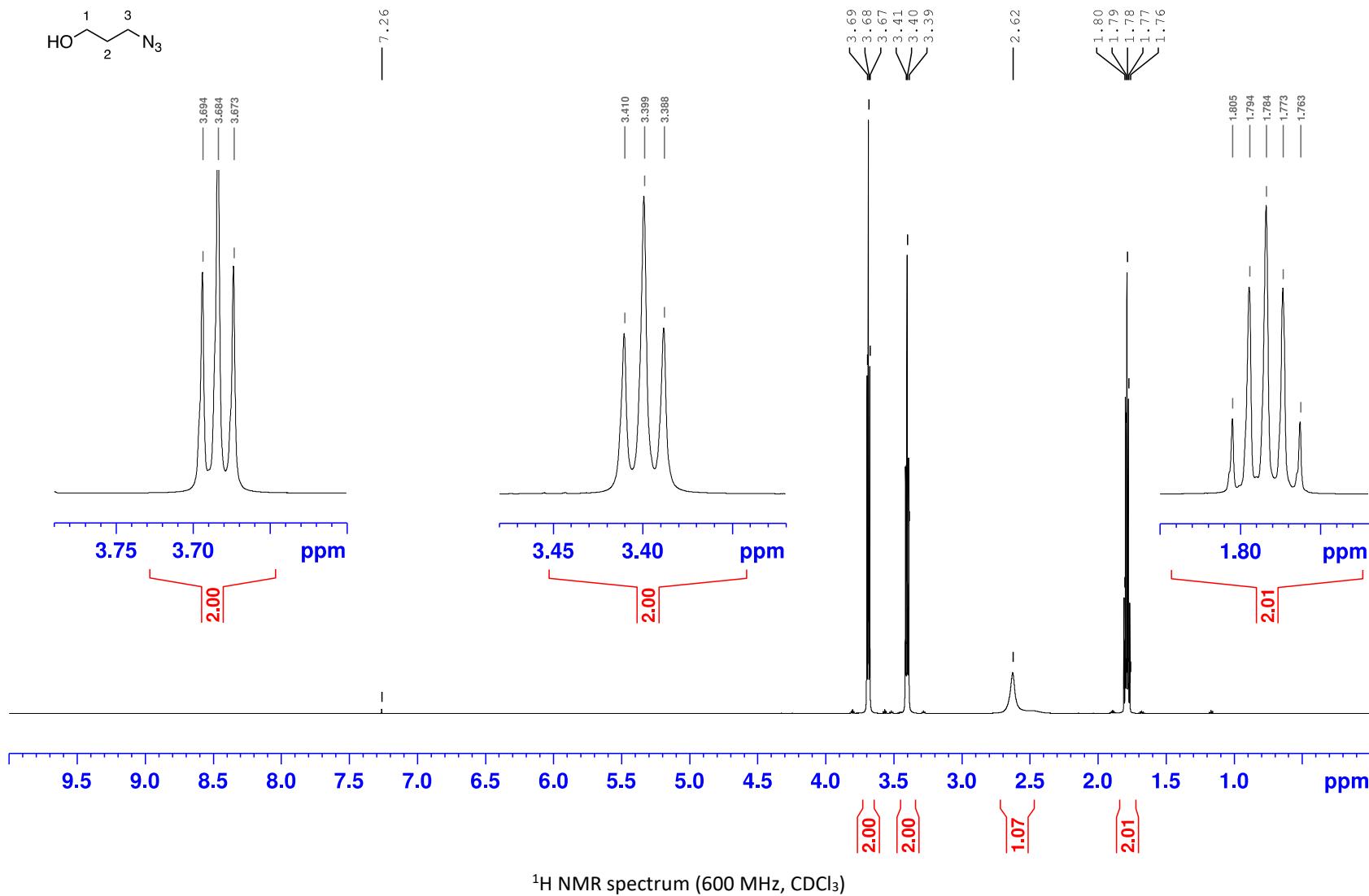


**Scheme for Synthesized Indolium Salts and Cyanines**

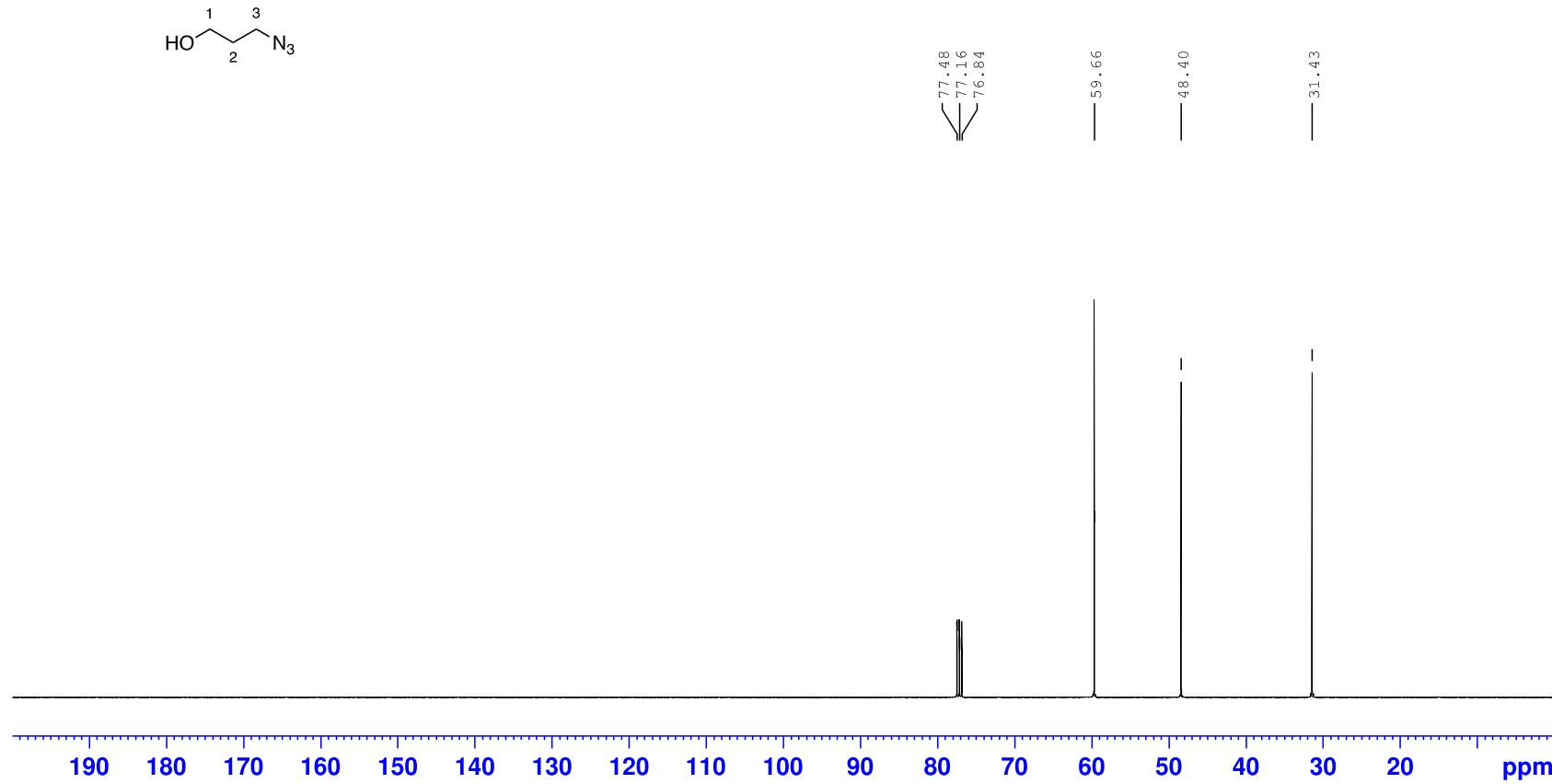


## Spectroscopic Characterization of Sidechains

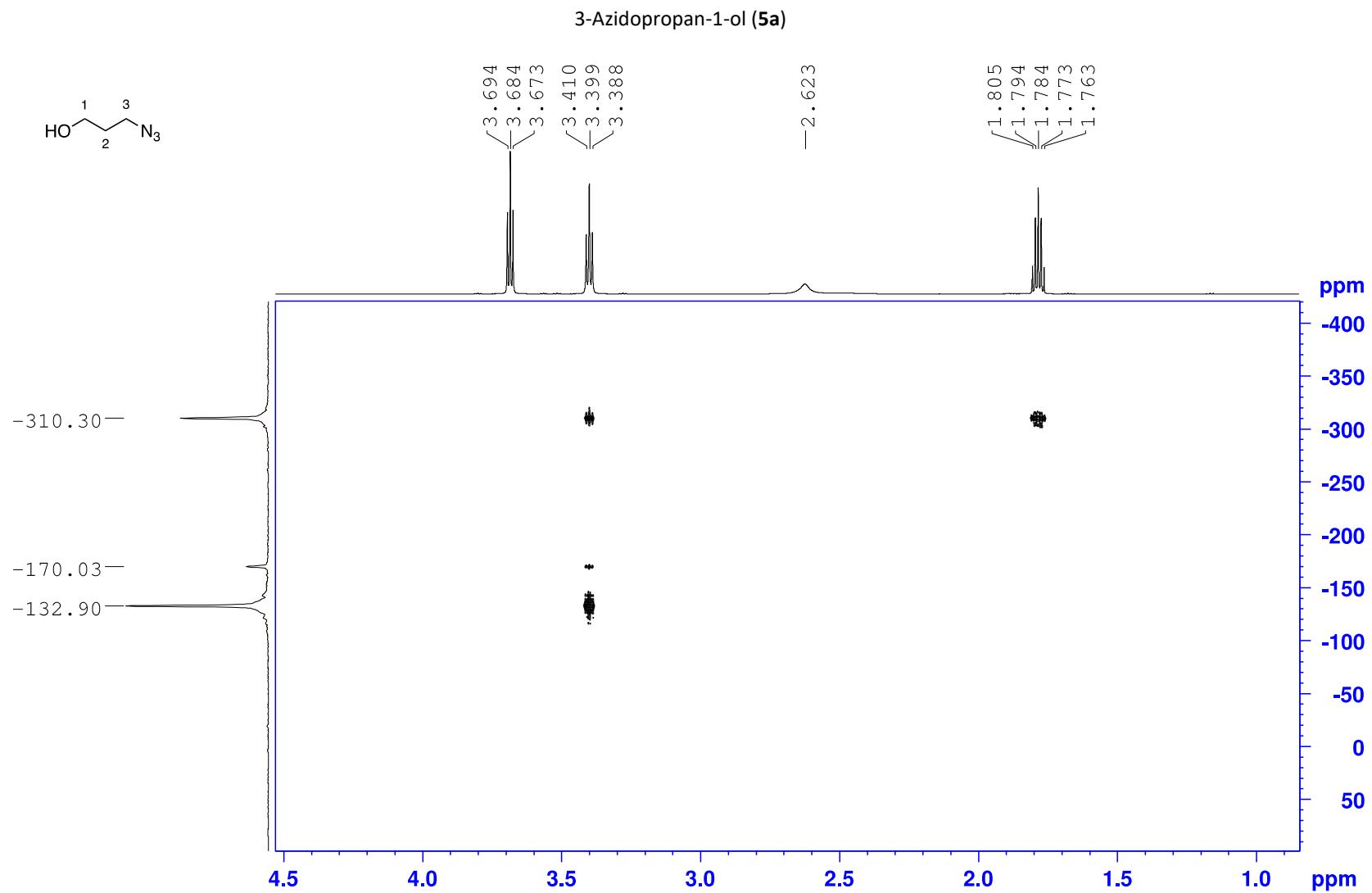
3-Azidopropan-1-ol (**5a**)



3-Azidopropan-1-ol (**5a**)

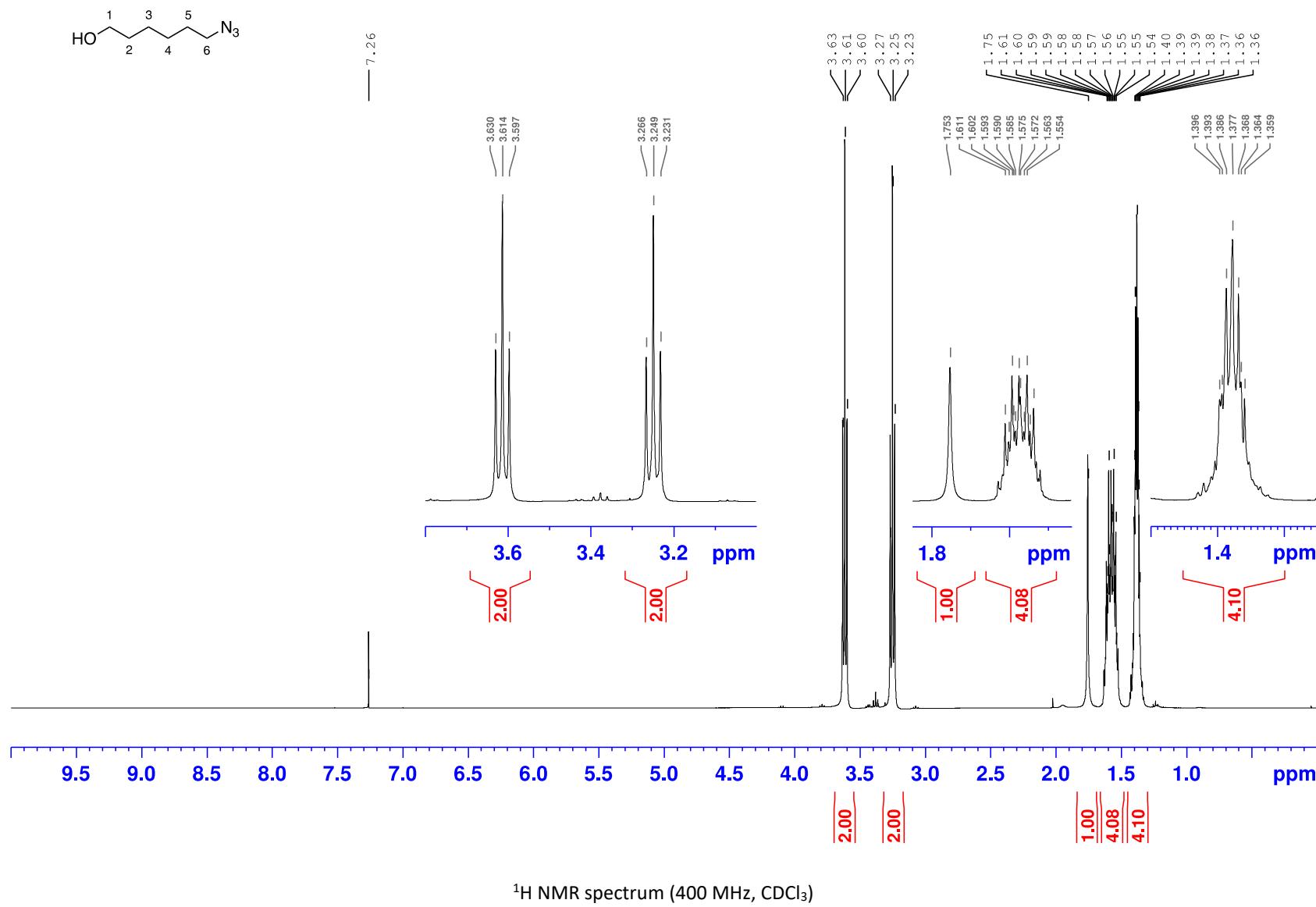


<sup>13</sup>C NMR spectrum (100 MHz, CDCl<sub>3</sub>)

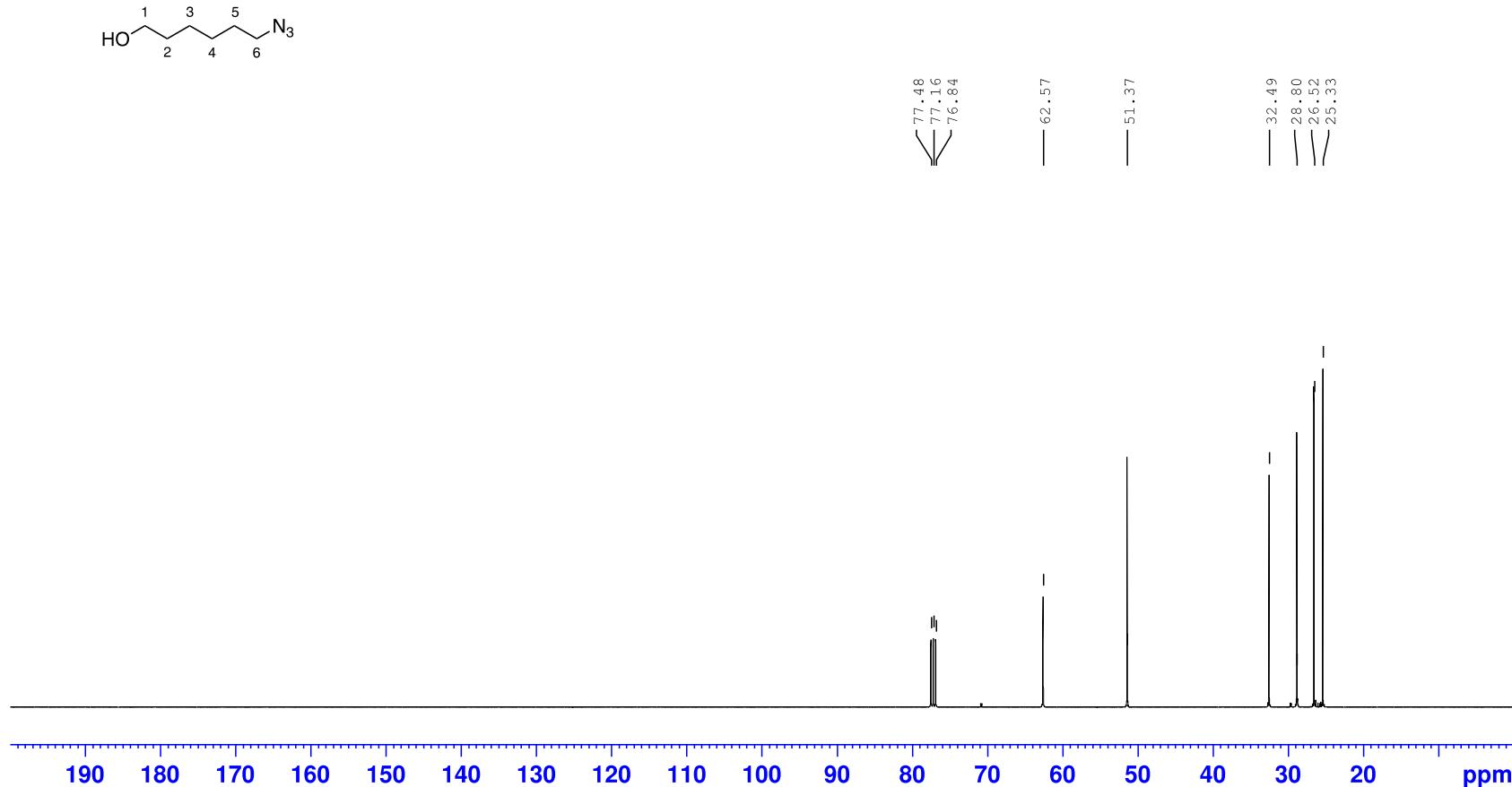


$^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum (600 MHz,  $\text{CDCl}_3$ )

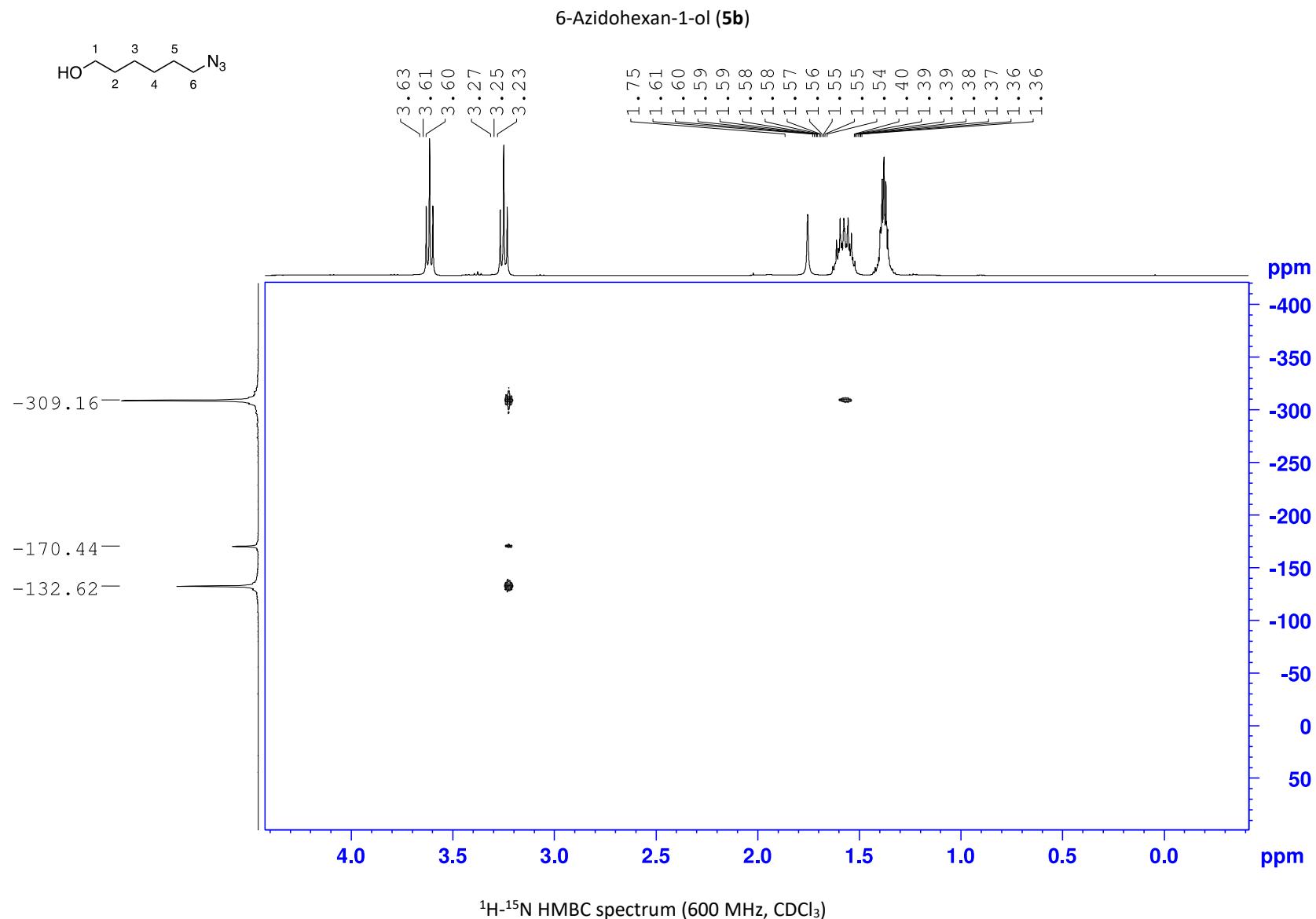
**6-Azidohexan-1-ol (**5b**)**



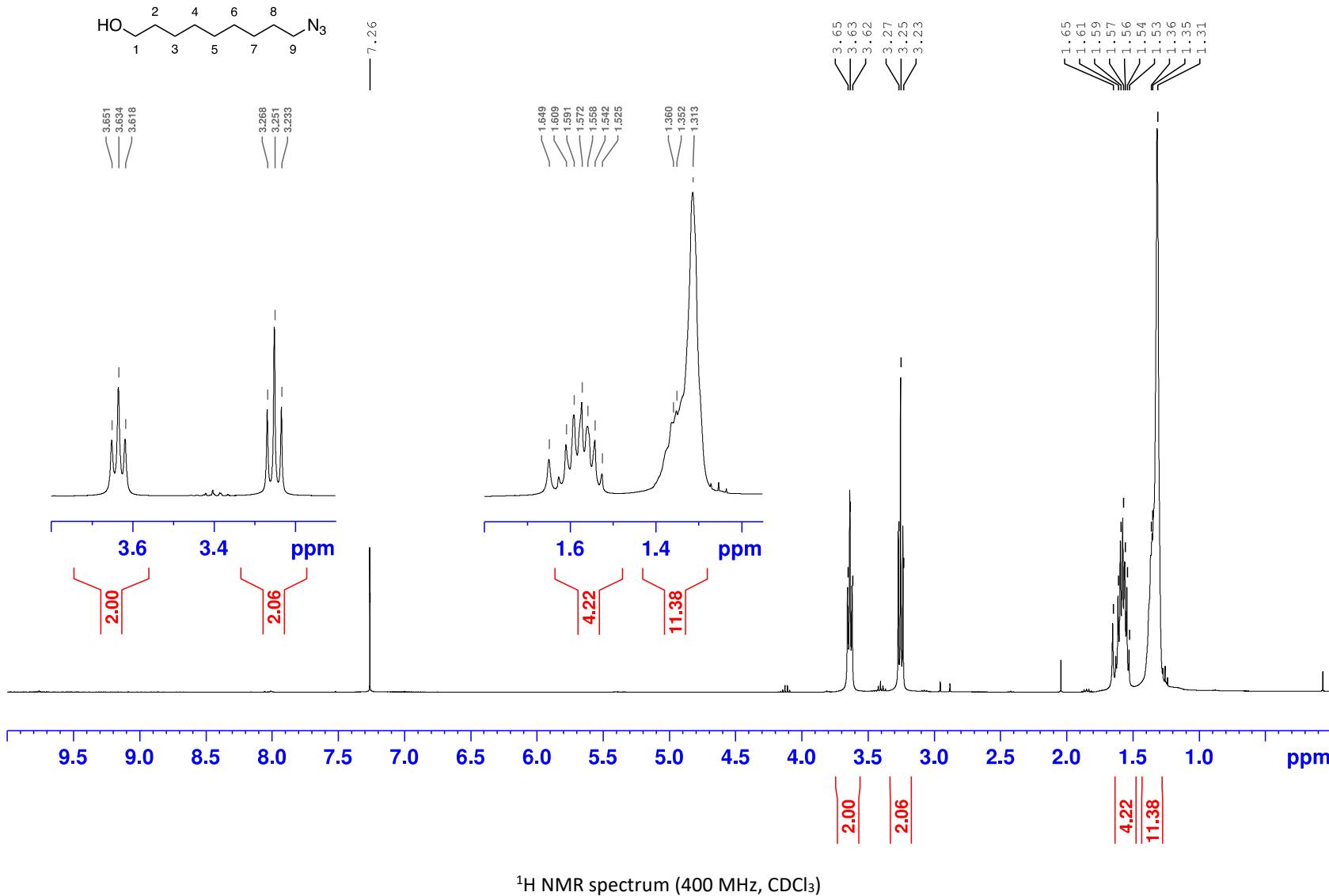
**6-Azidohexan-1-ol (**5b**)**



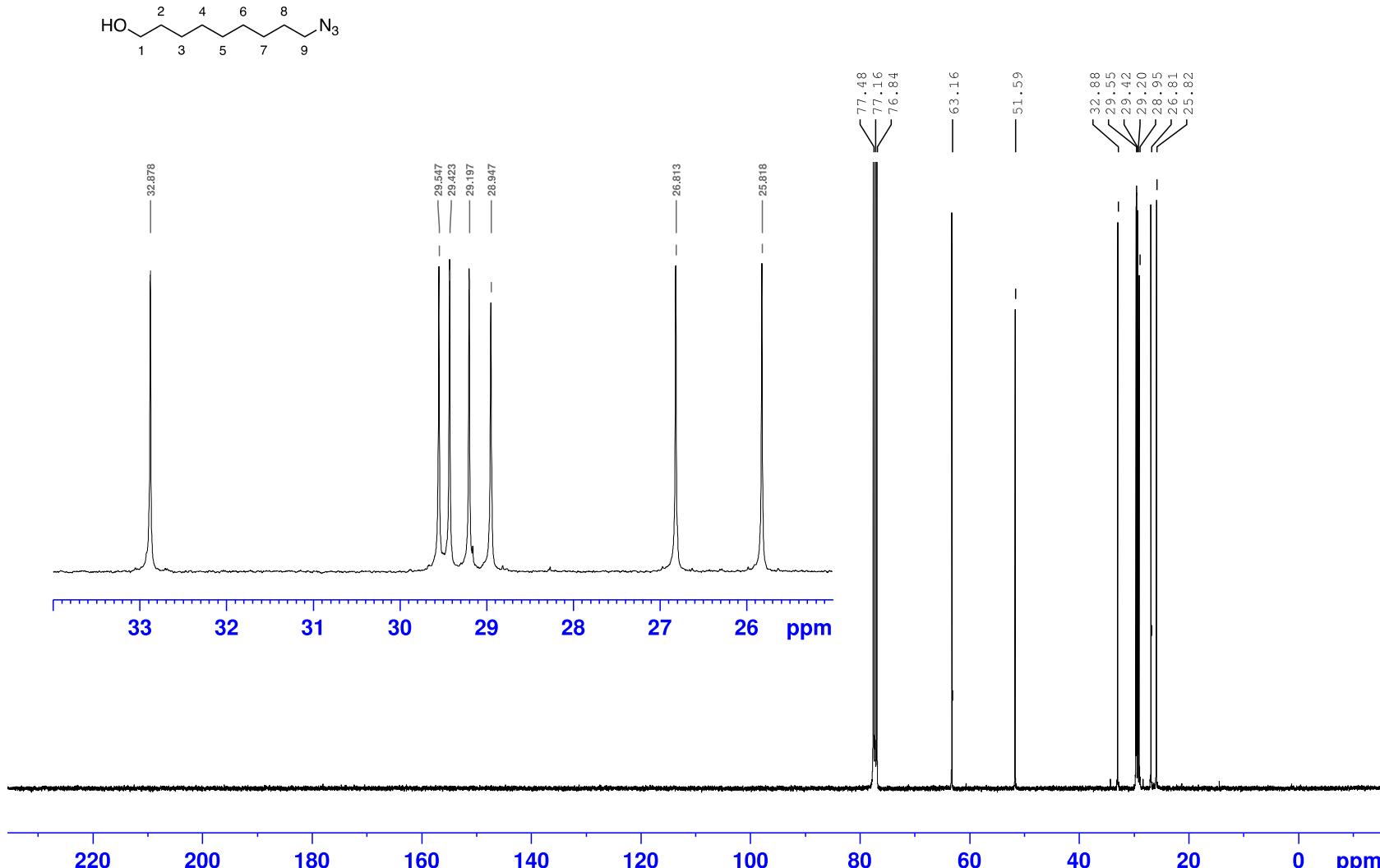
<sup>13</sup>C NMR spectrum (100 MHz, CDCl<sub>3</sub>)



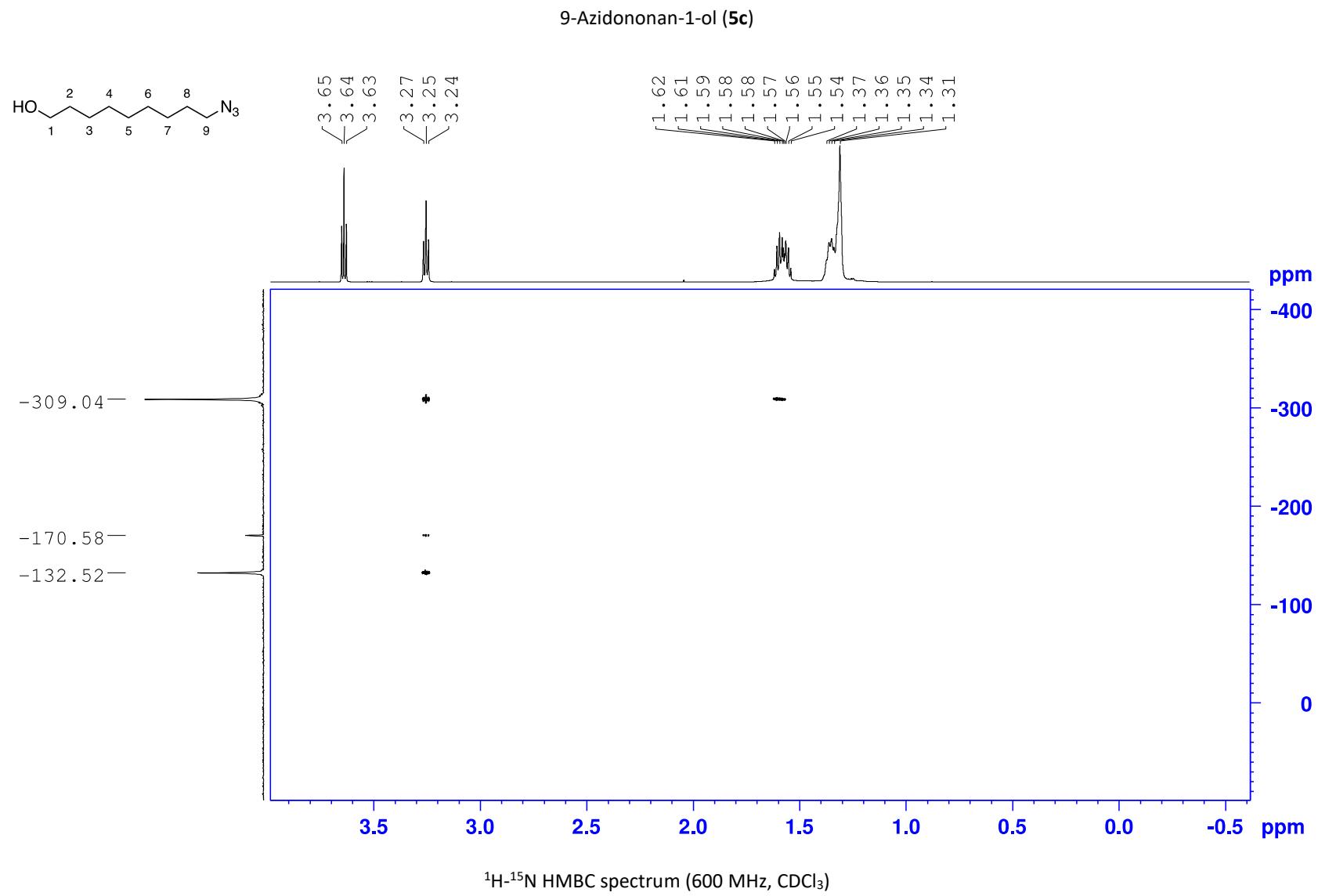
**9-Azidononan-1-ol (**5c**)**



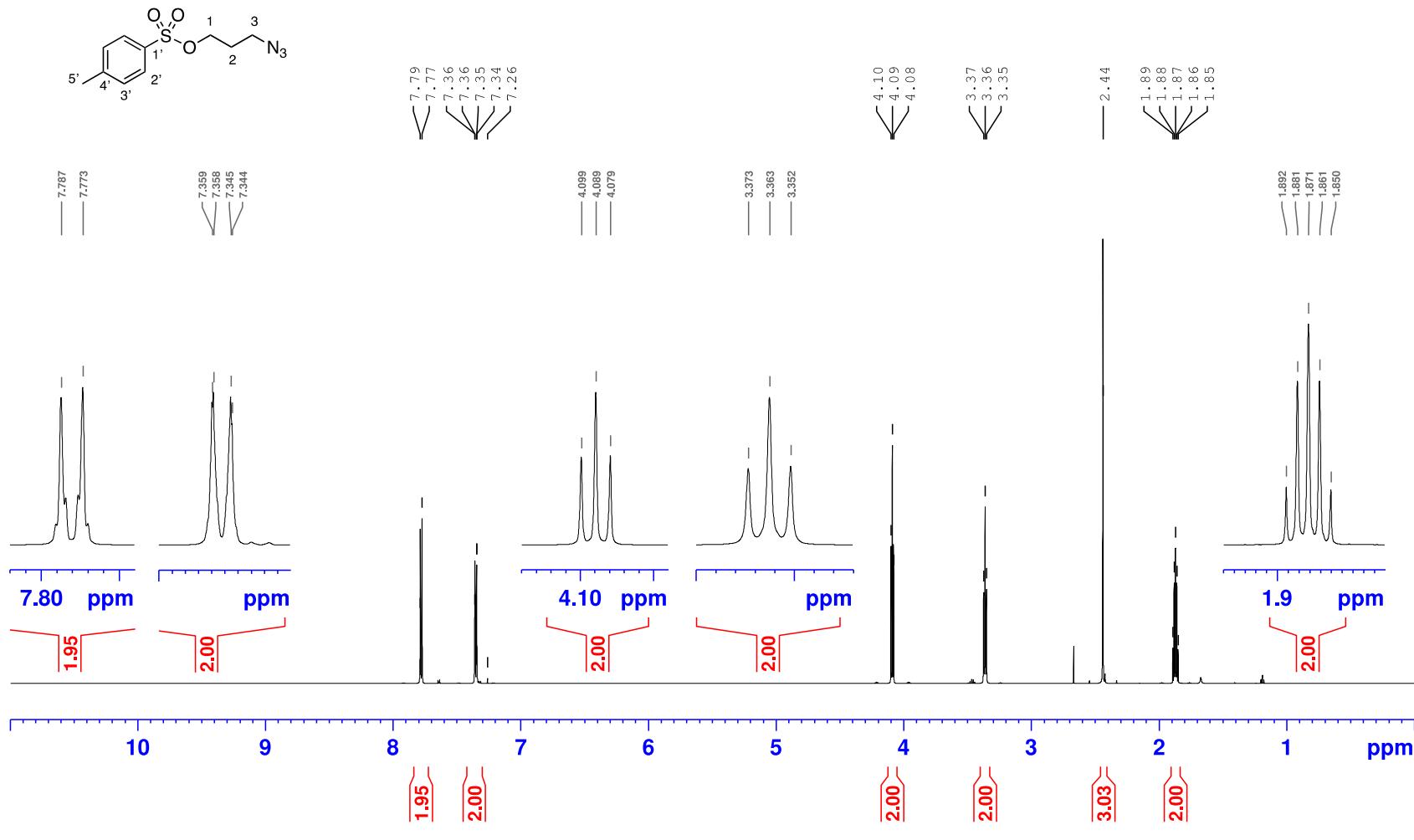
9-Azidononan-1-ol (**5c**)



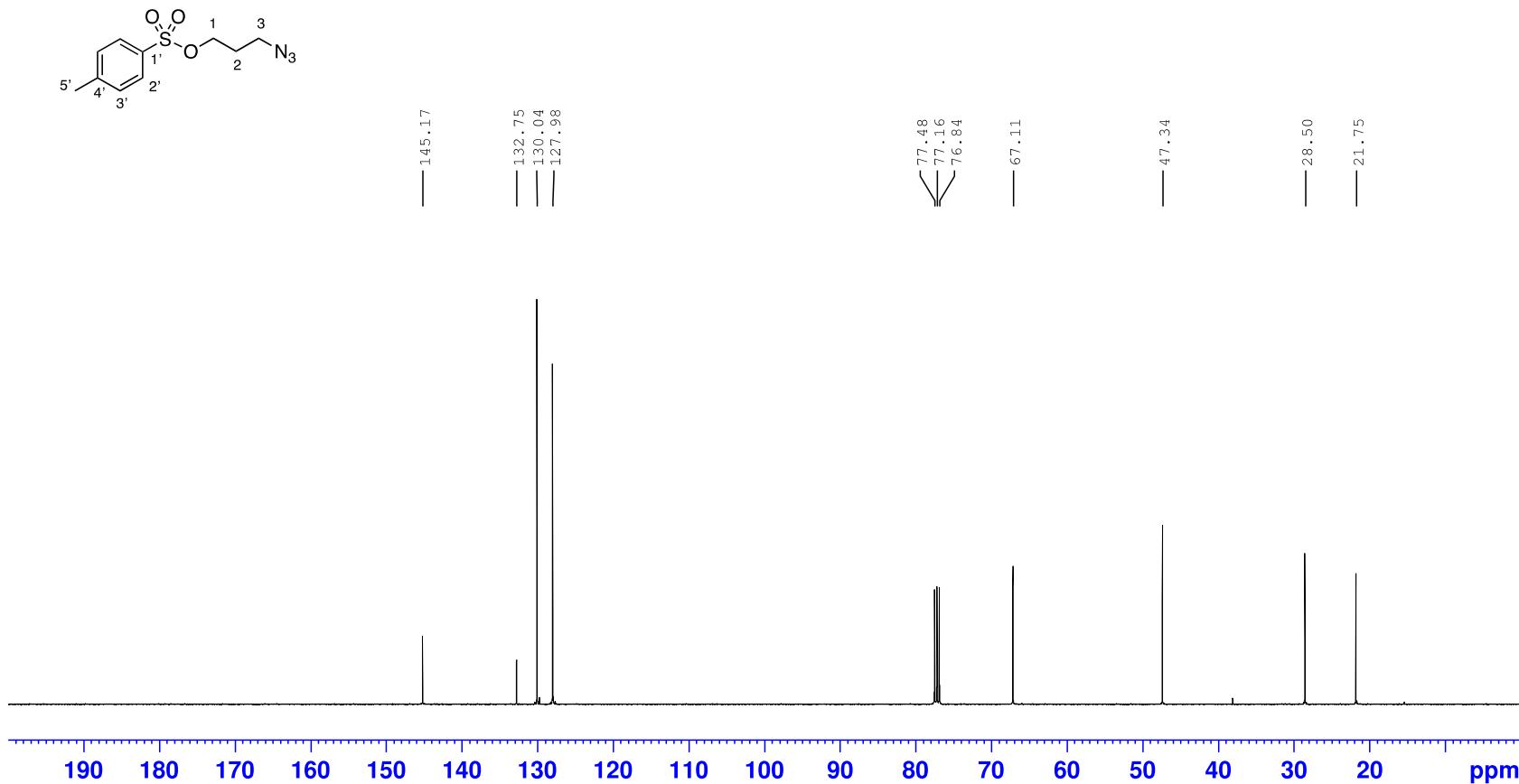
<sup>13</sup>C NMR spectrum (100 MHz, CDCl<sub>3</sub>)



3-Azidopropyl-*p*-toluenesulfonate (**6a**)

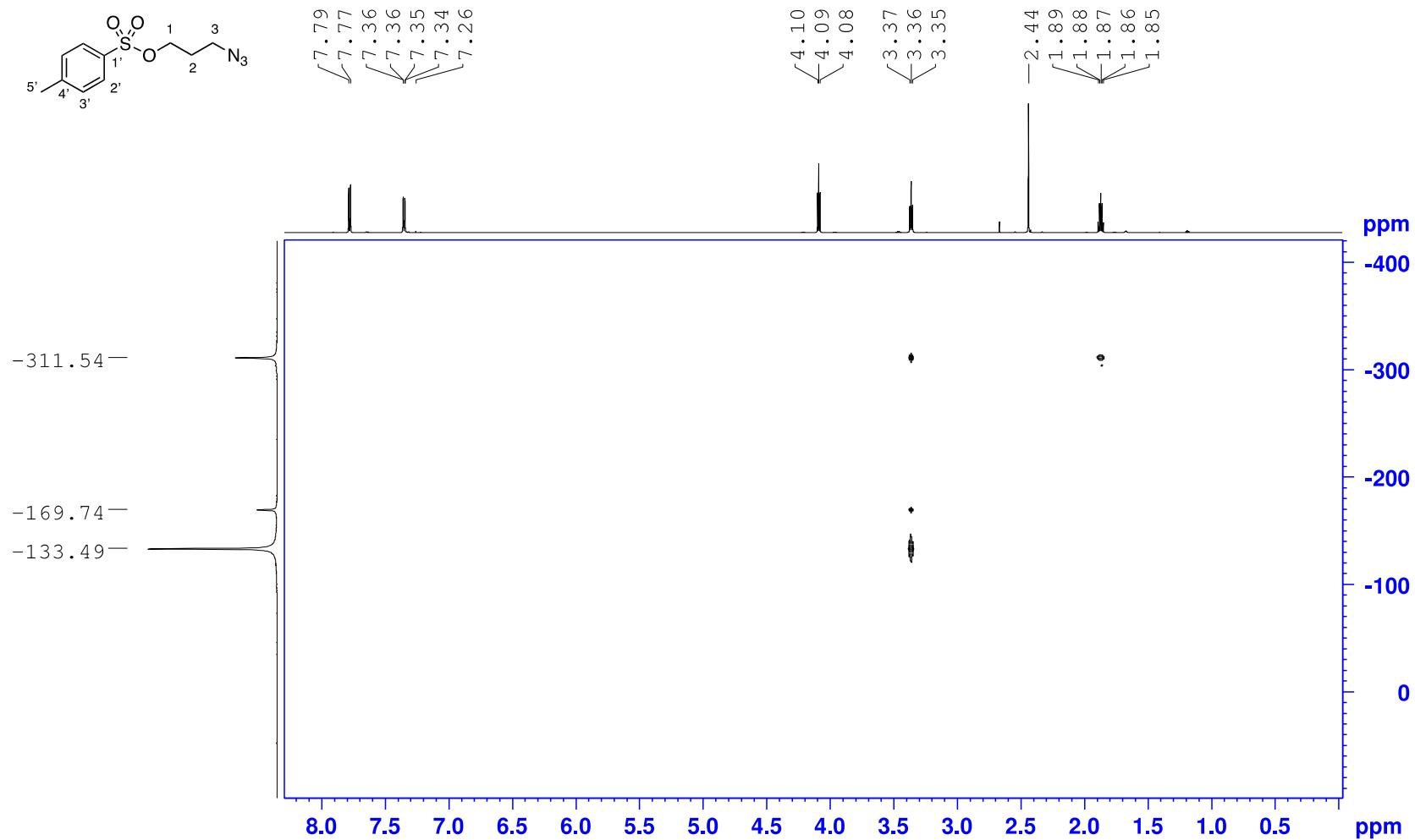


**3-Azidopropyl-*p*-toluenesulfonate (**6a**)**

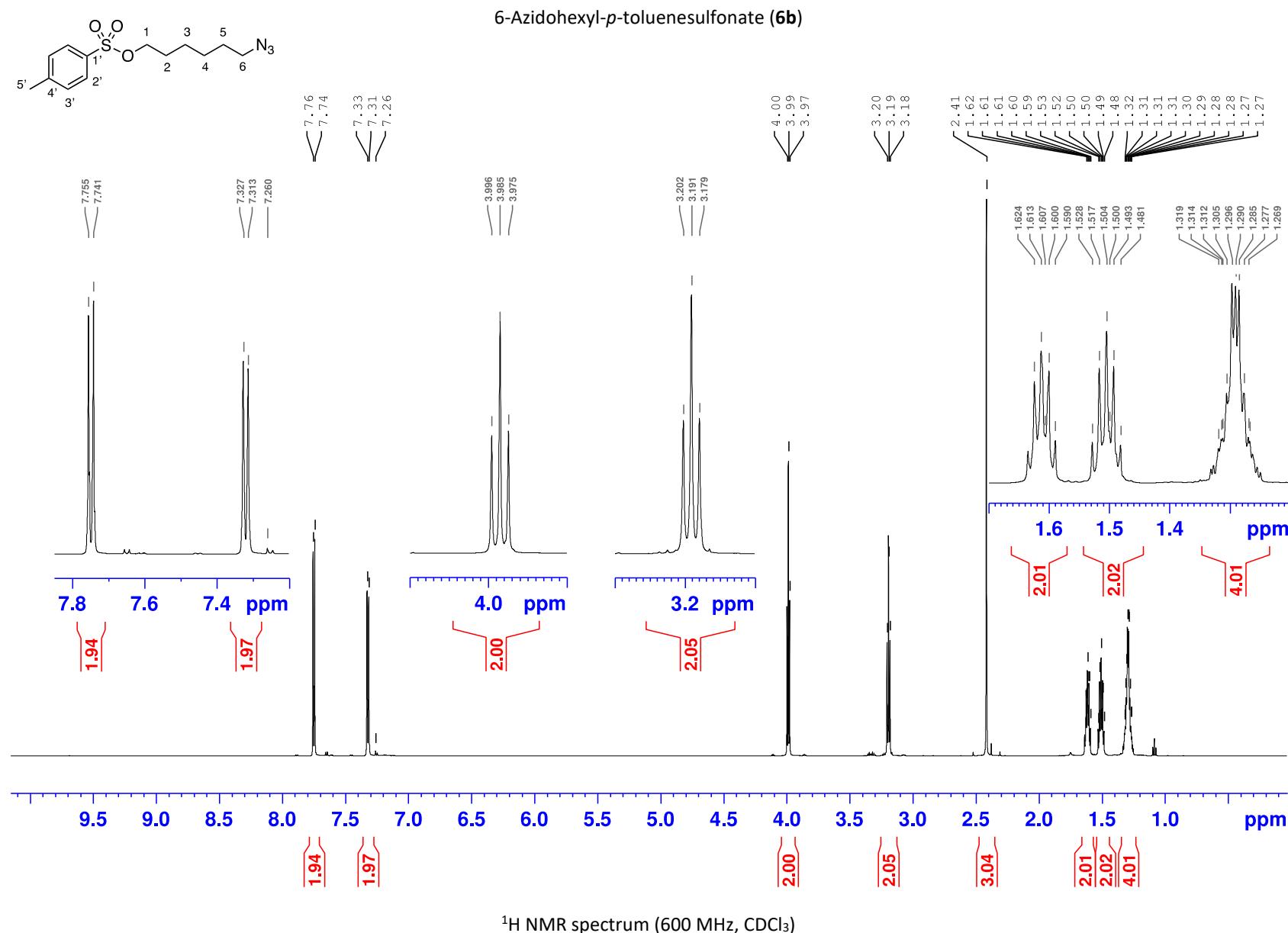


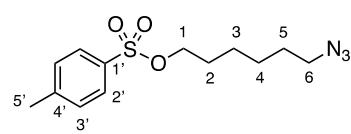
<sup>13</sup>C NMR spectrum (100 MHz, CDCl<sub>3</sub>)

3-Azidopropyl-*p*-toluenesulfonate (**6a**)

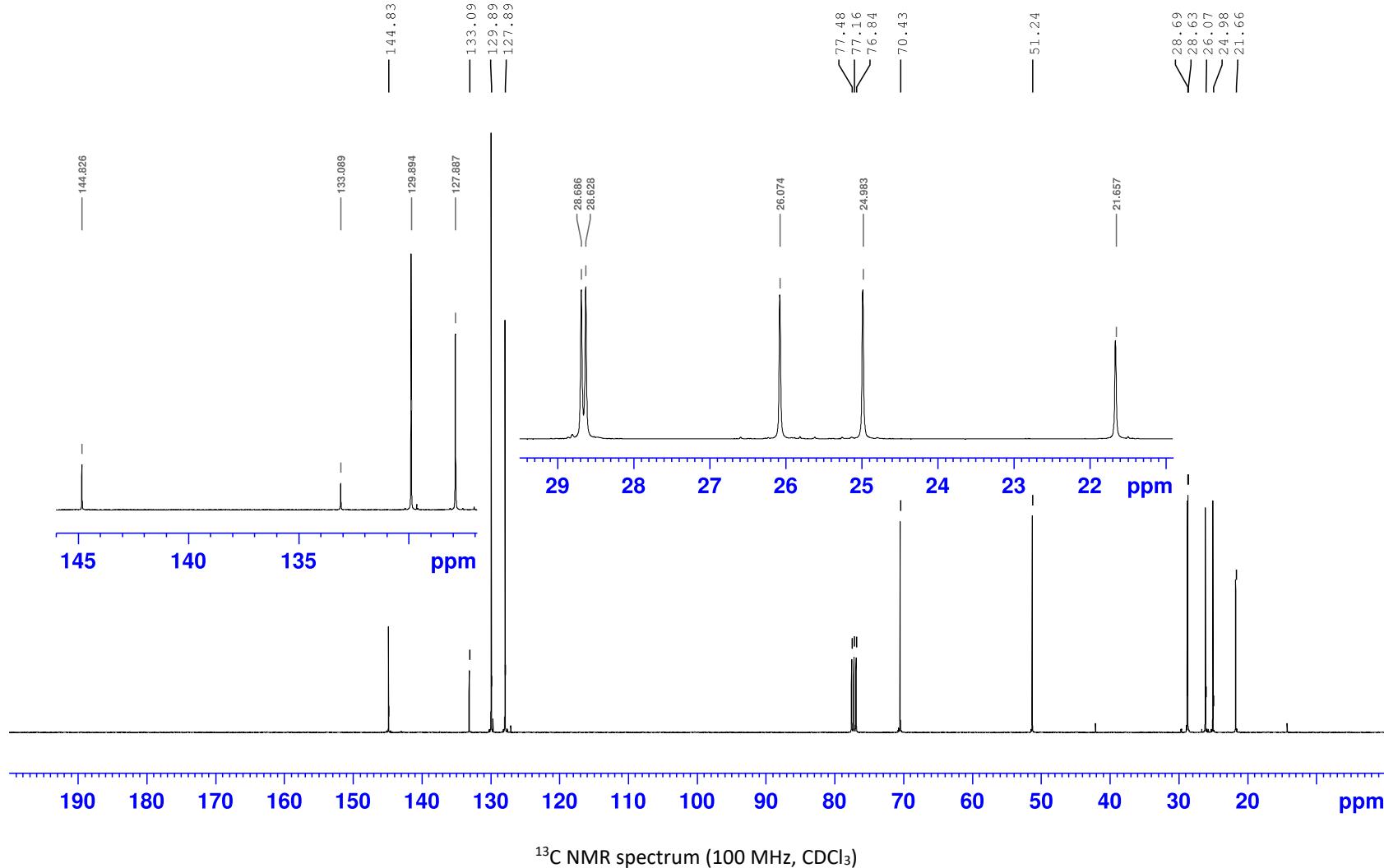


$^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum (600 MHz,  $\text{CDCl}_3$ )

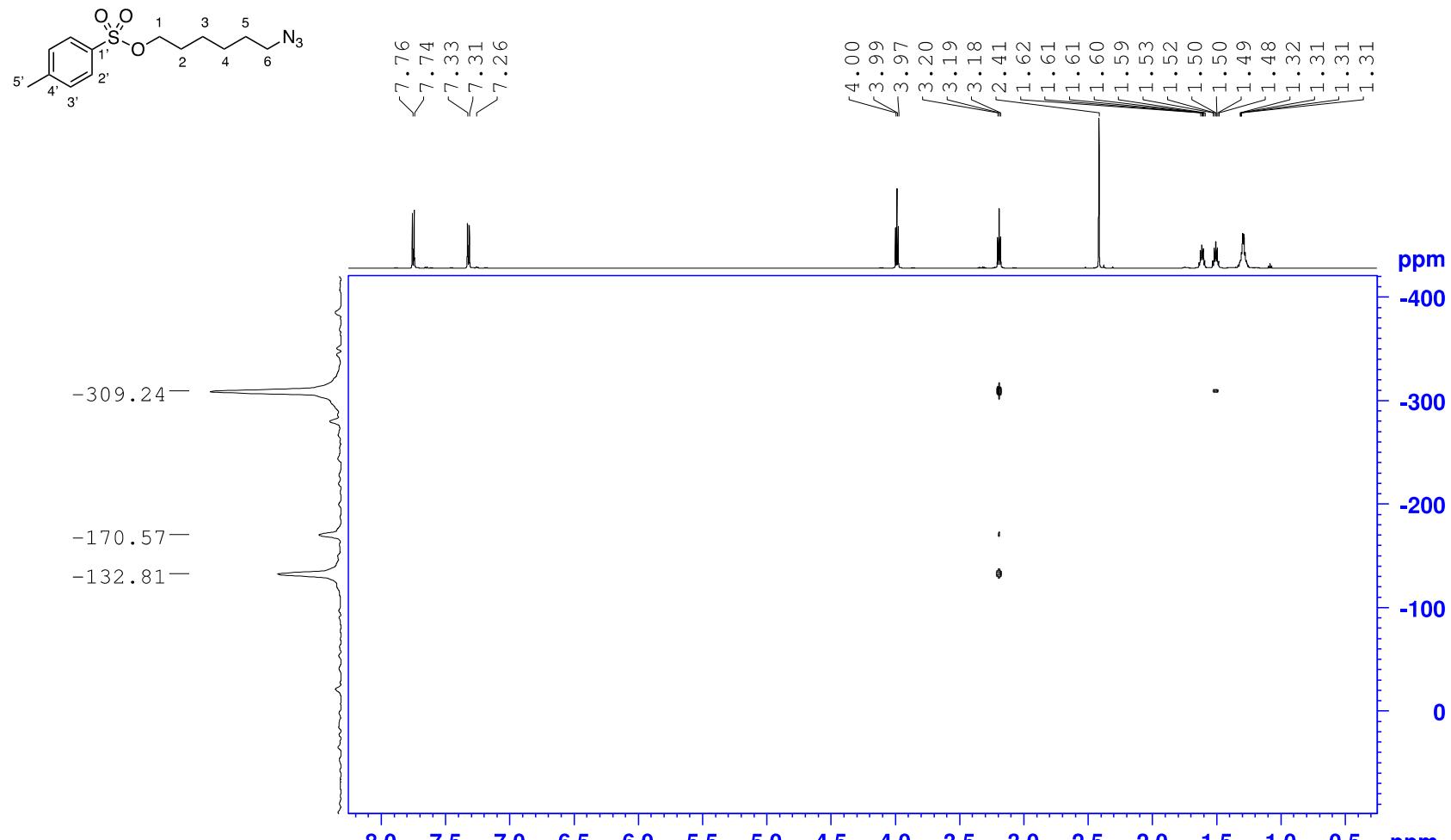


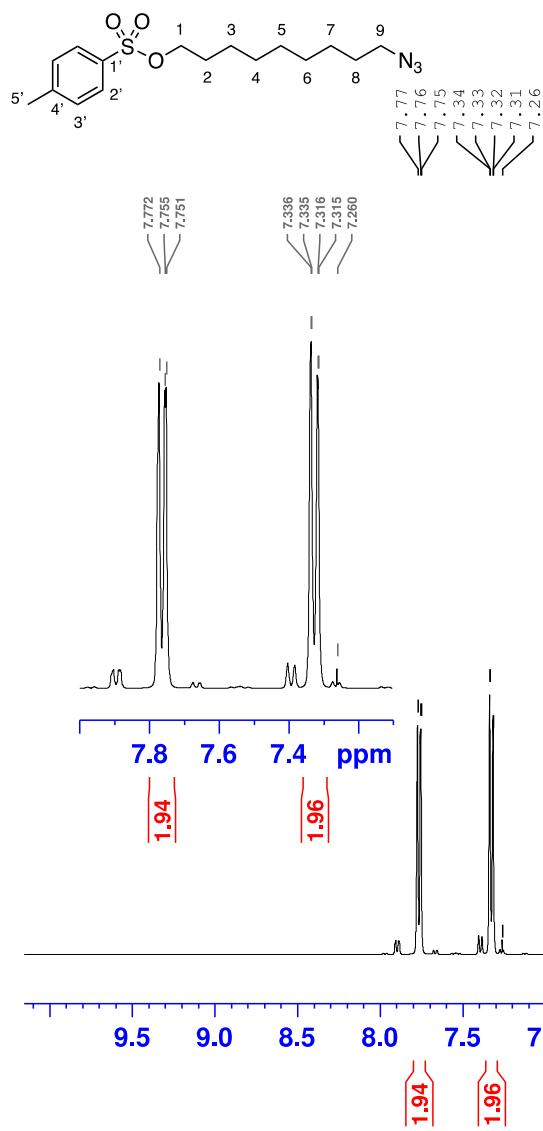


**6-Azidohexyl-p-toluenesulfonate (**6b**)**



6-Azidohexyl-*p*-toluenesulfonate (**6b**)

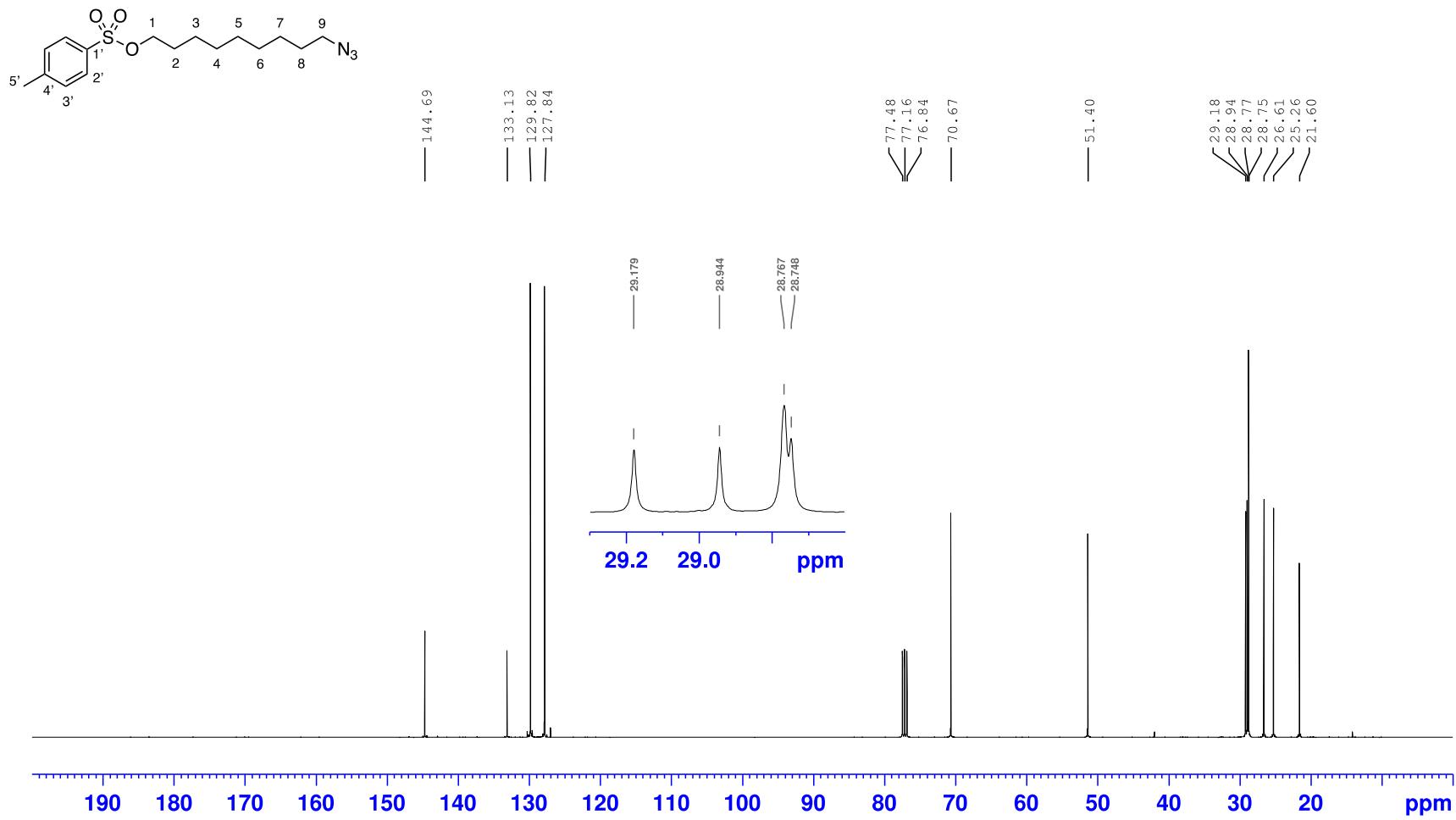




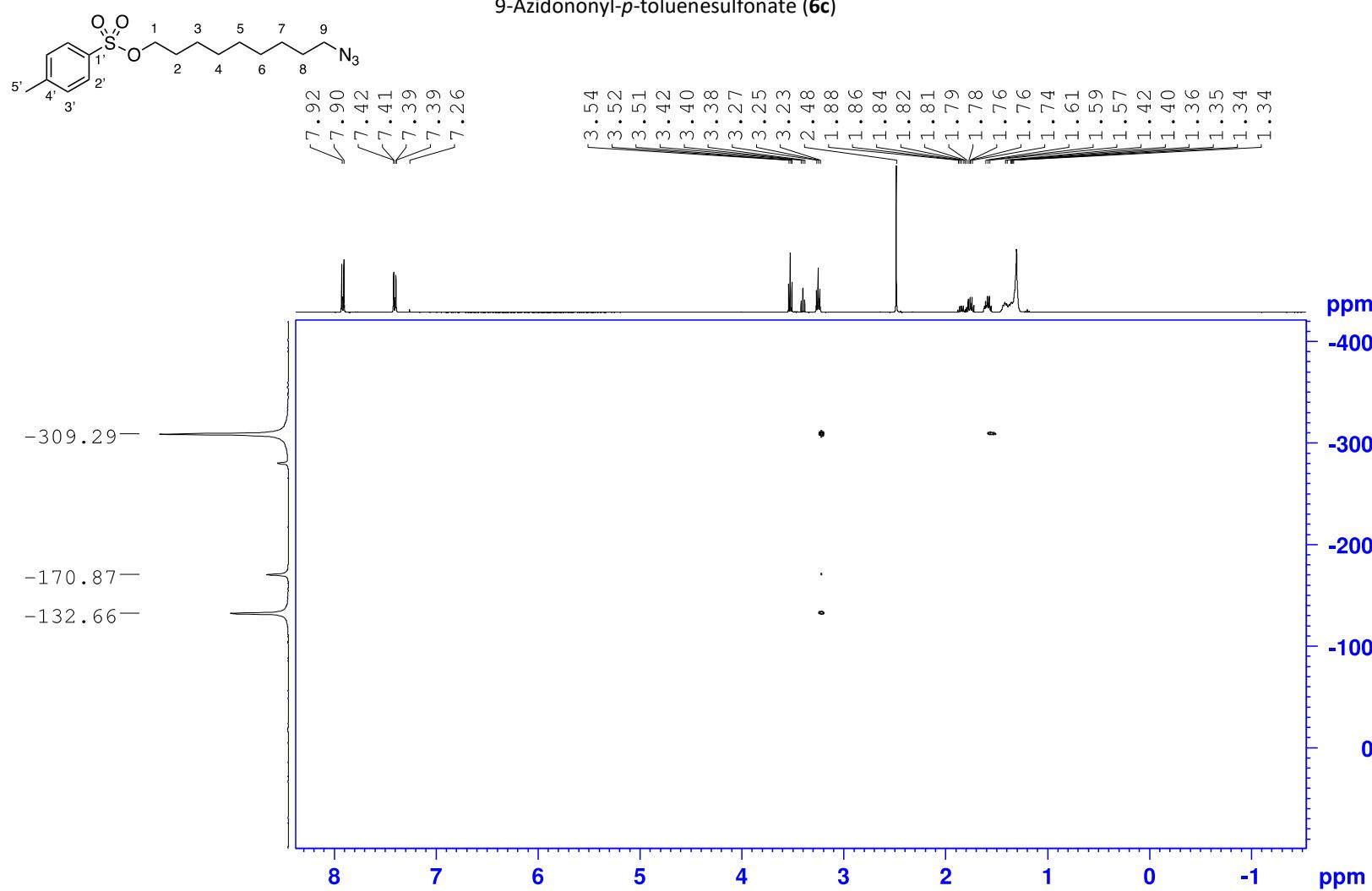
9-Azidononyl-*p*-toluenesulfonate (**6c**)

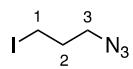
<sup>1</sup>H NMR spectrum (400 MHz, CDCl<sub>3</sub>)

**9-Azidononyl-*p*-toluenesulfonate (**6c**)**

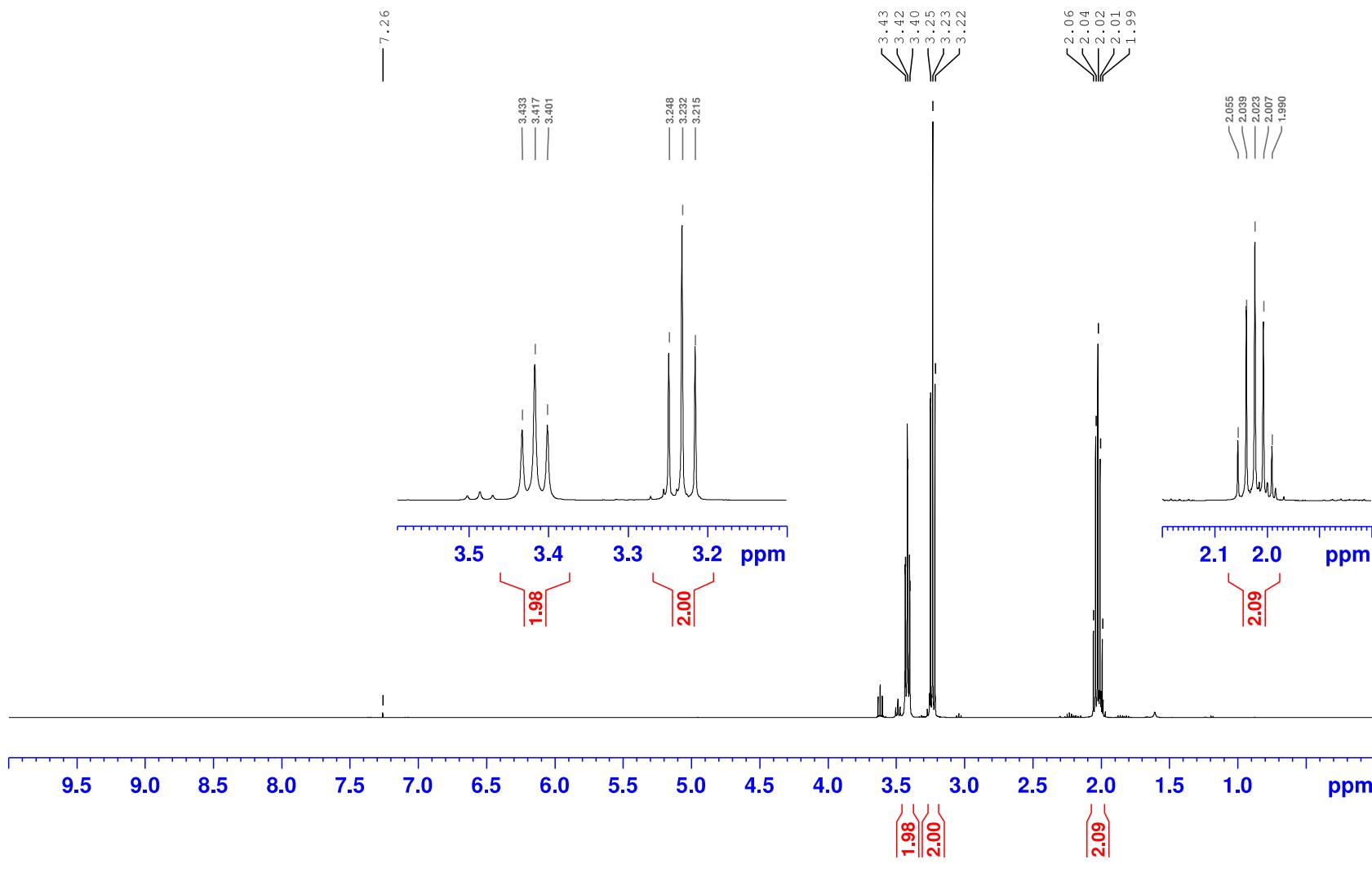


<sup>13</sup>C NMR spectrum (100 MHz, CDCl<sub>3</sub>)

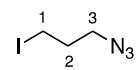




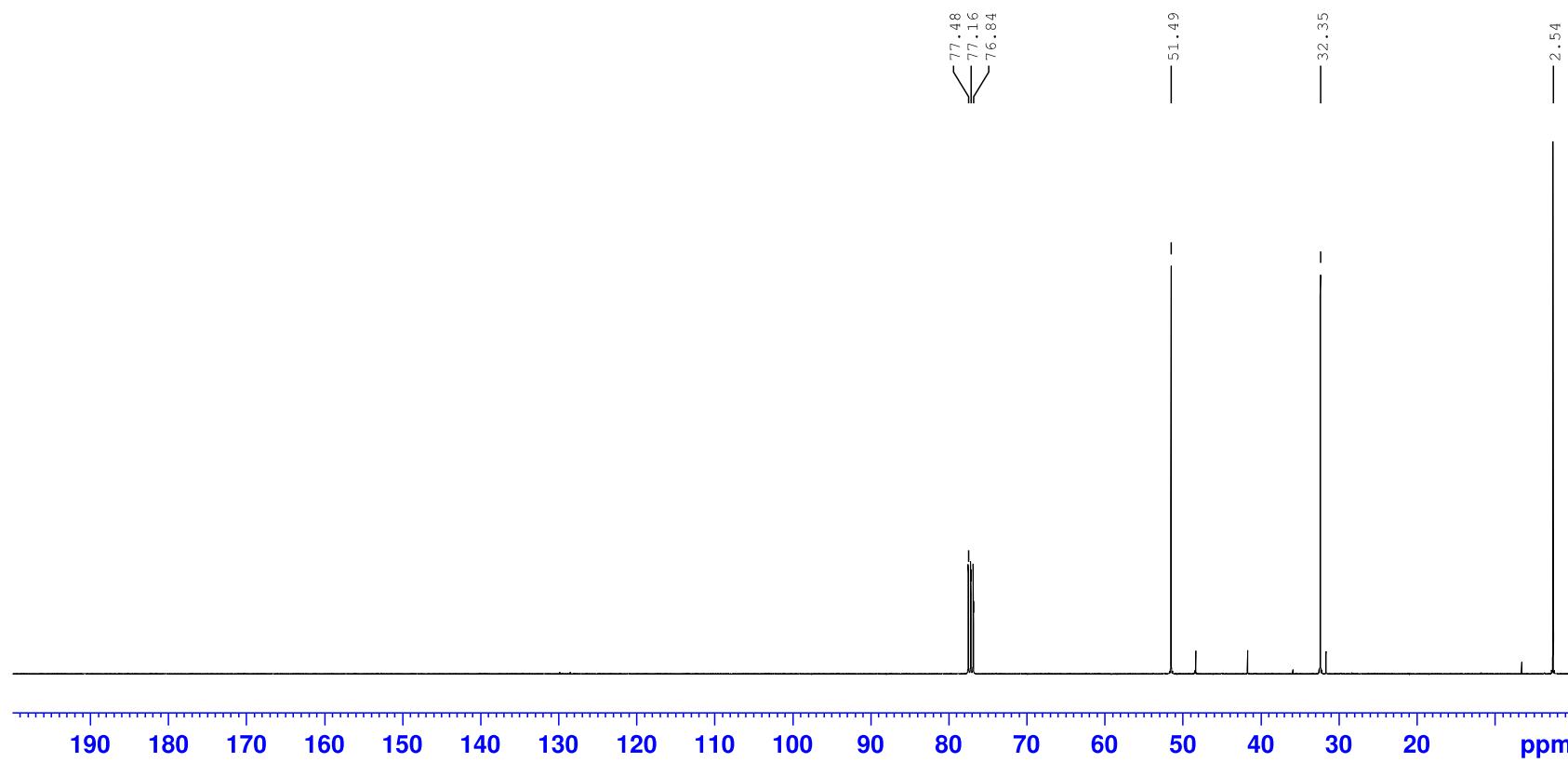
3-Azido-1-iodopropane (**7a**)



$^1\text{H}$  NMR spectrum (400 MHz,  $\text{CDCl}_3$ )

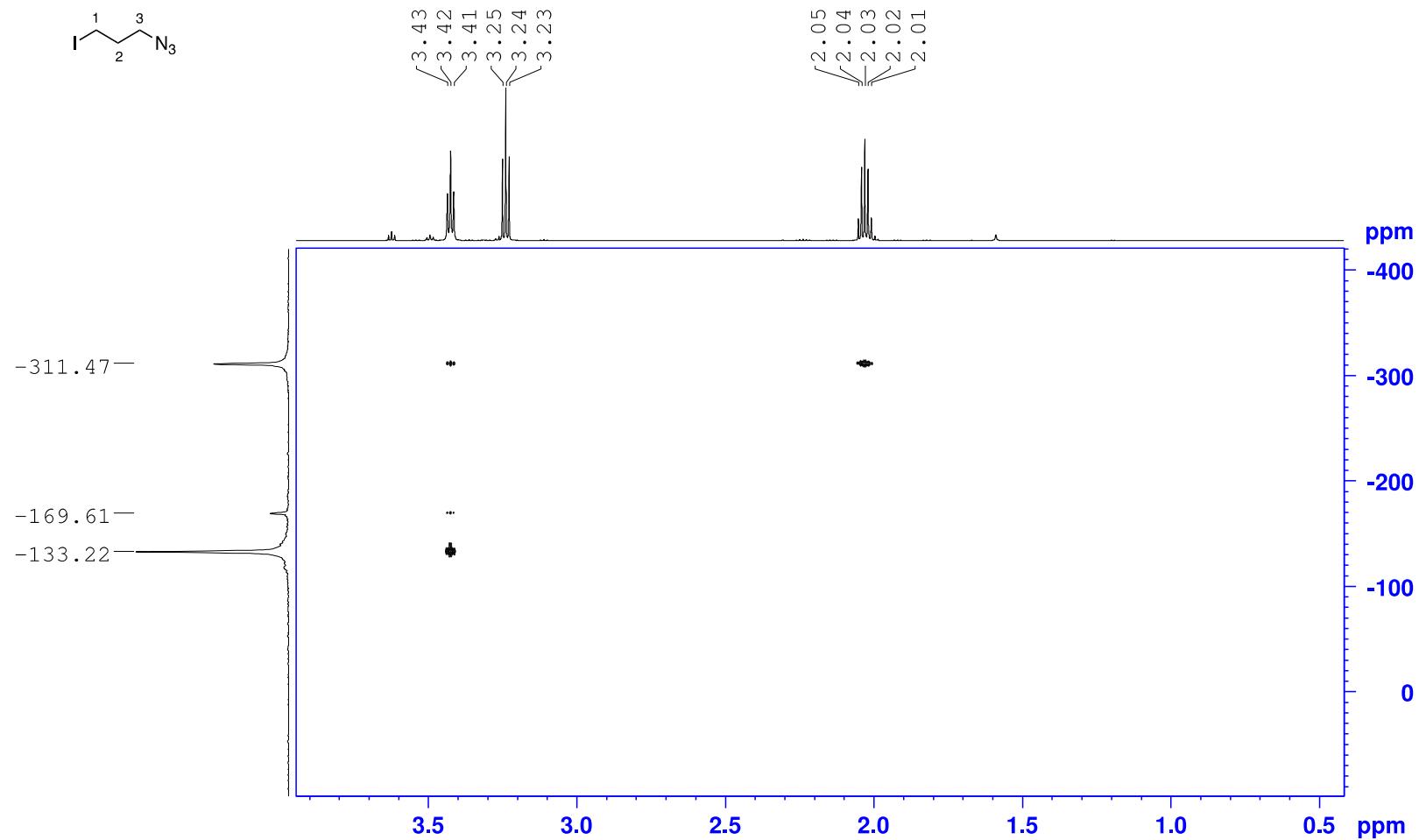


3-Azido-1-iodopropane (**7a**)

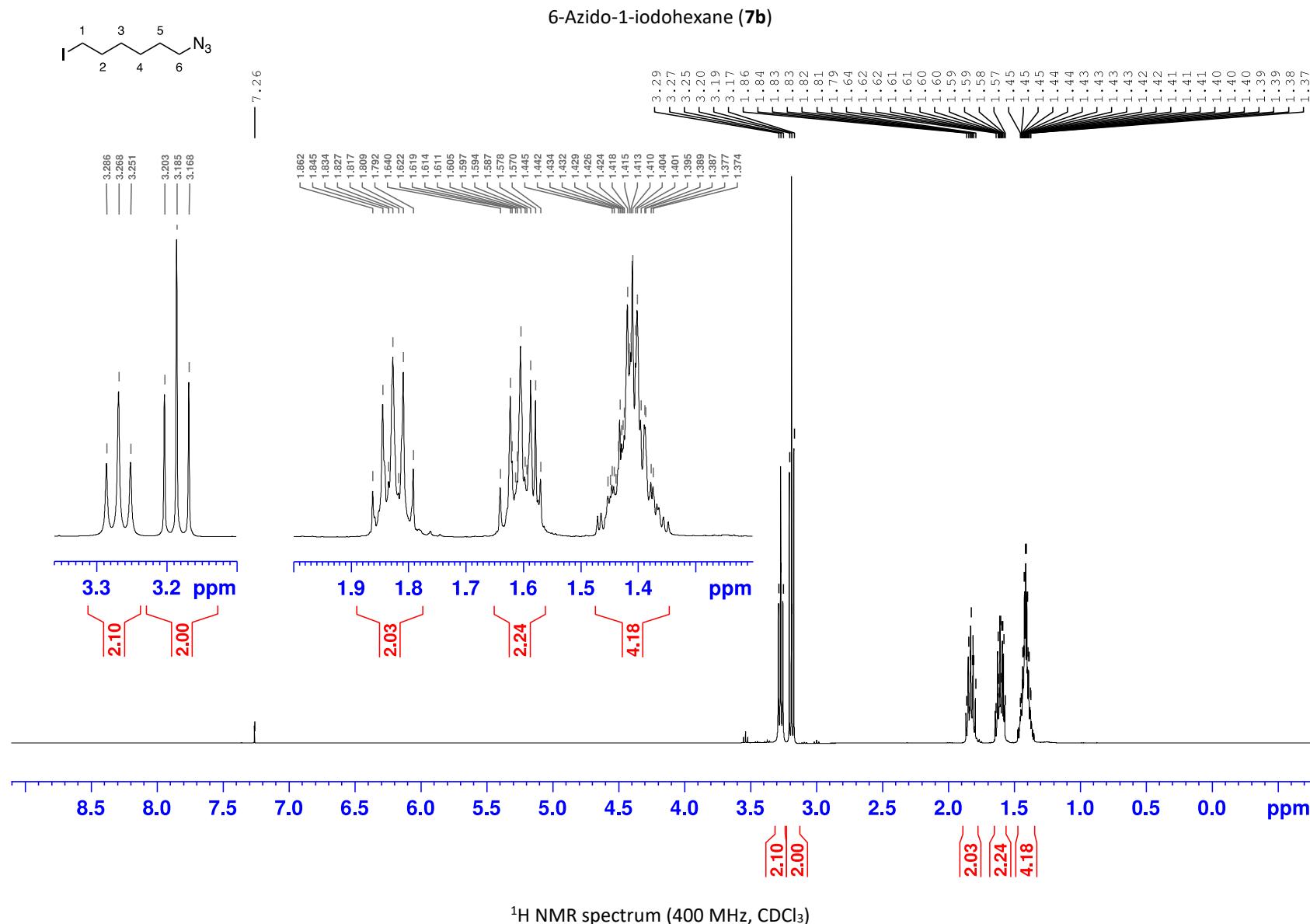


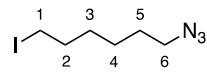
$^{13}\text{C}$  NMR spectrum (100 MHz,  $\text{CDCl}_3$ )

3-Azido-1-iodopropane (**7a**)

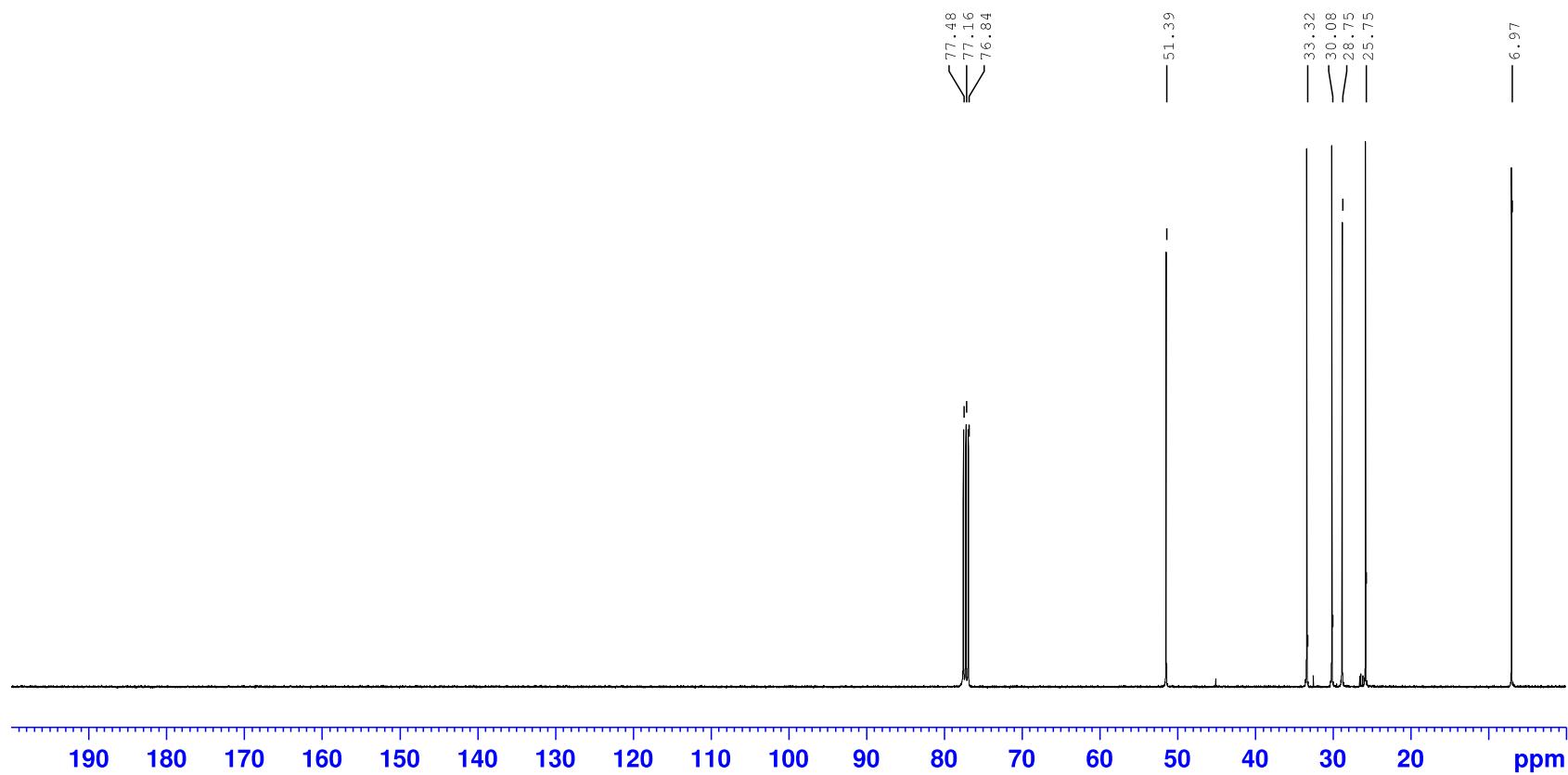


$^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum (600 MHz,  $\text{CDCl}_3$ )

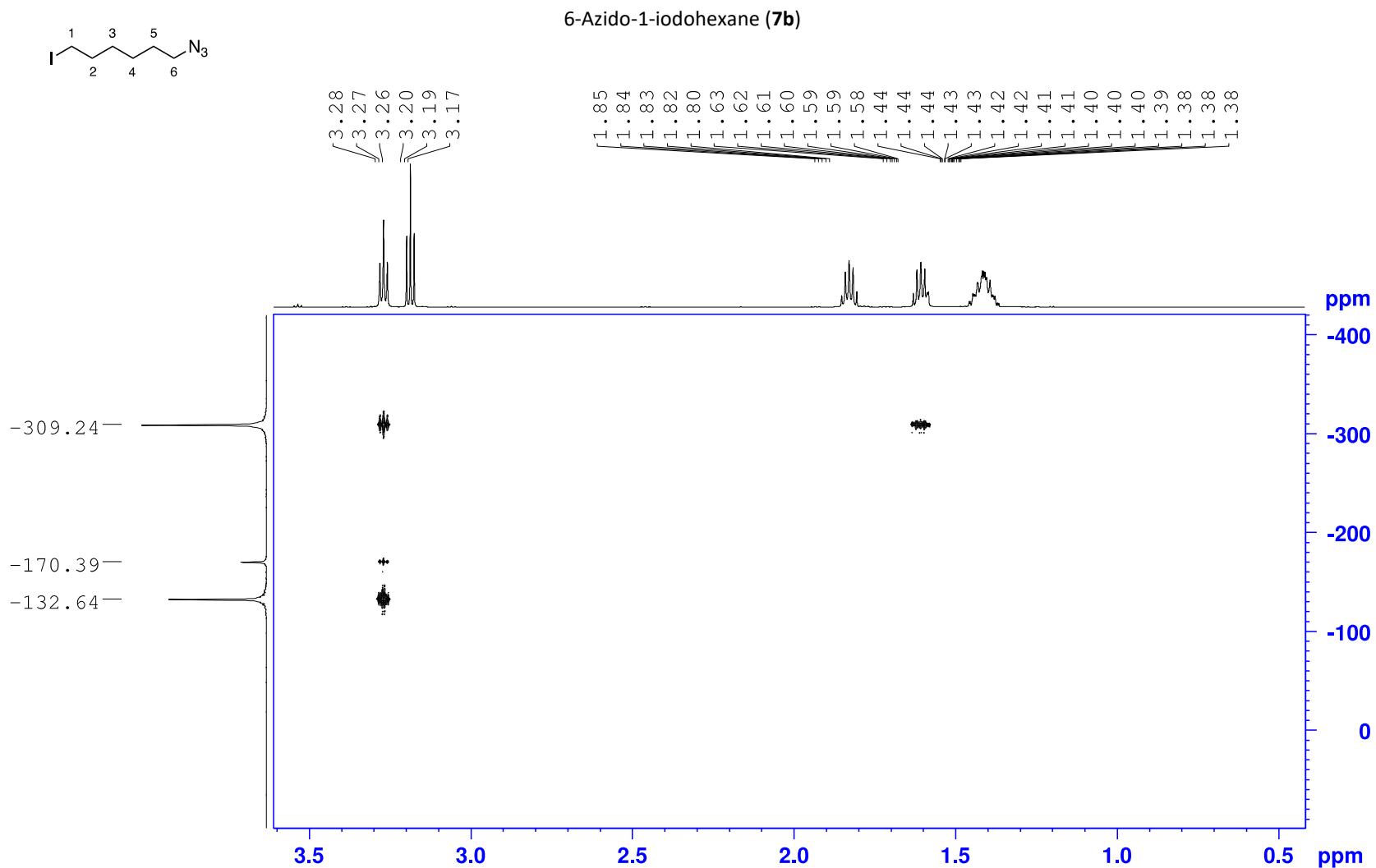




6-Azido-1-iodohexane (**7b**)

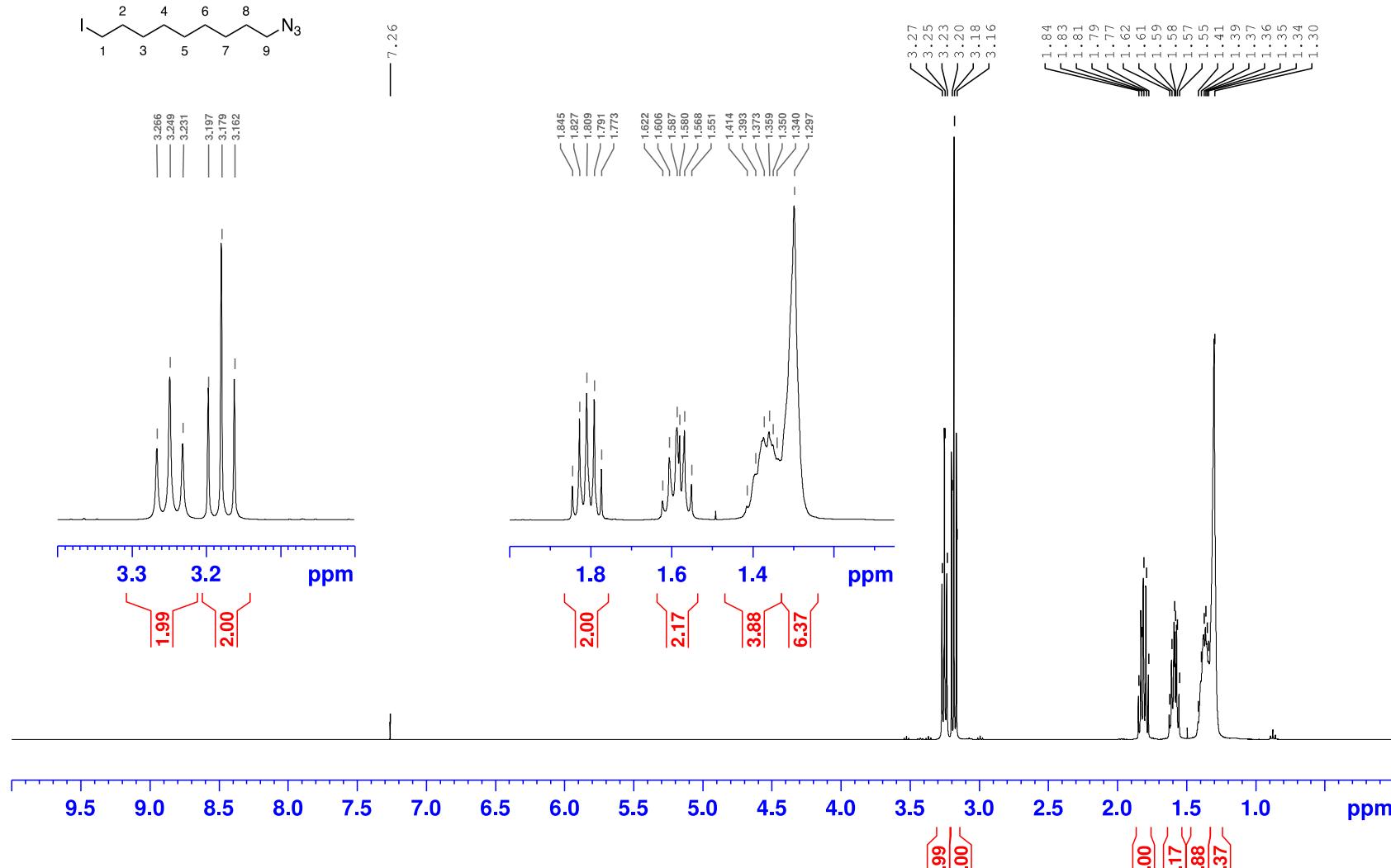


$^{13}\text{C}$  NMR spectrum (100 MHz,  $\text{CDCl}_3$ )



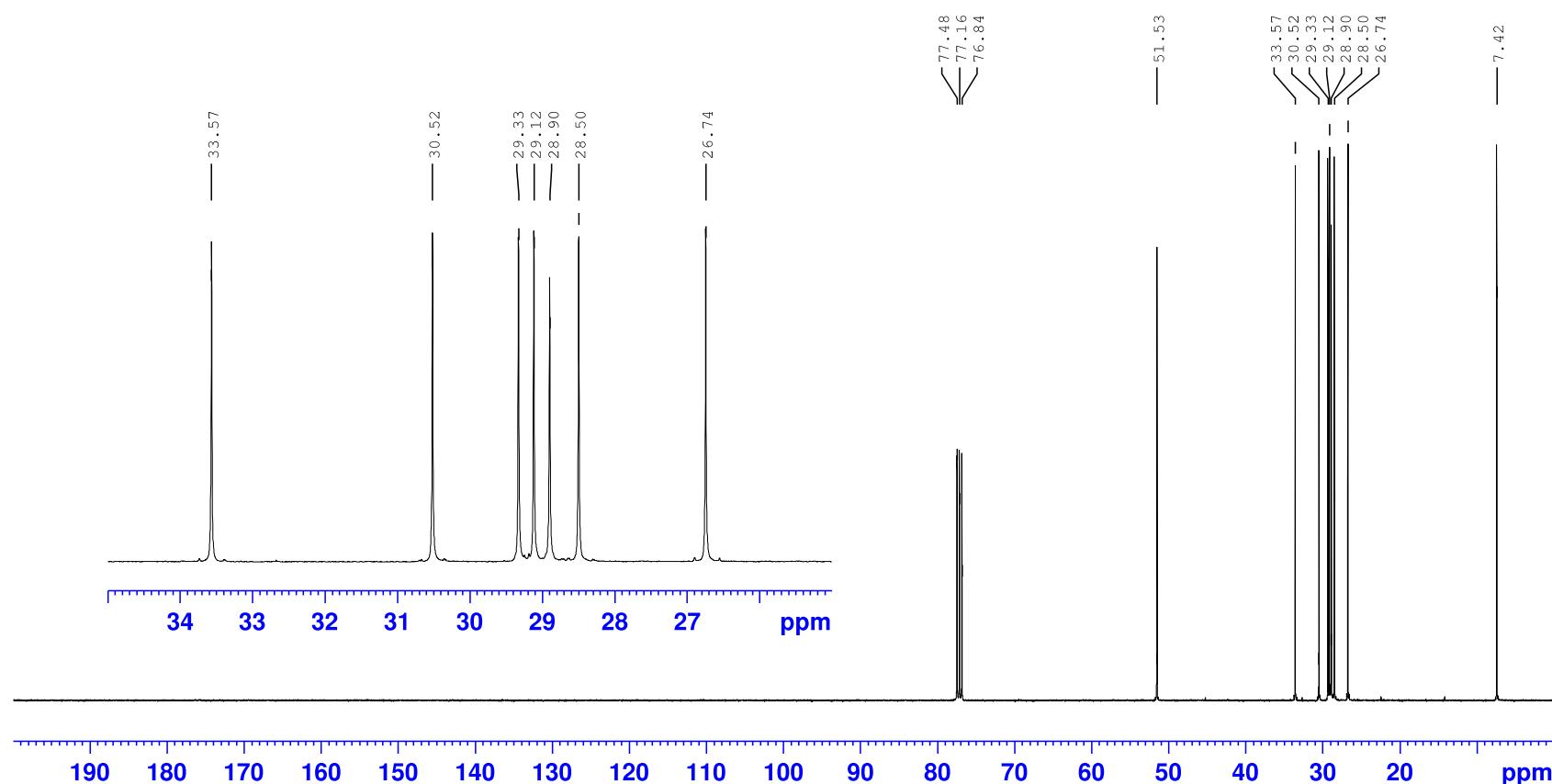
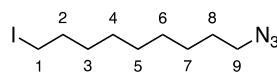
$^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum (600 MHz,  $\text{CDCl}_3$ )

**9-Azido-1-iodononane (**7c**)**



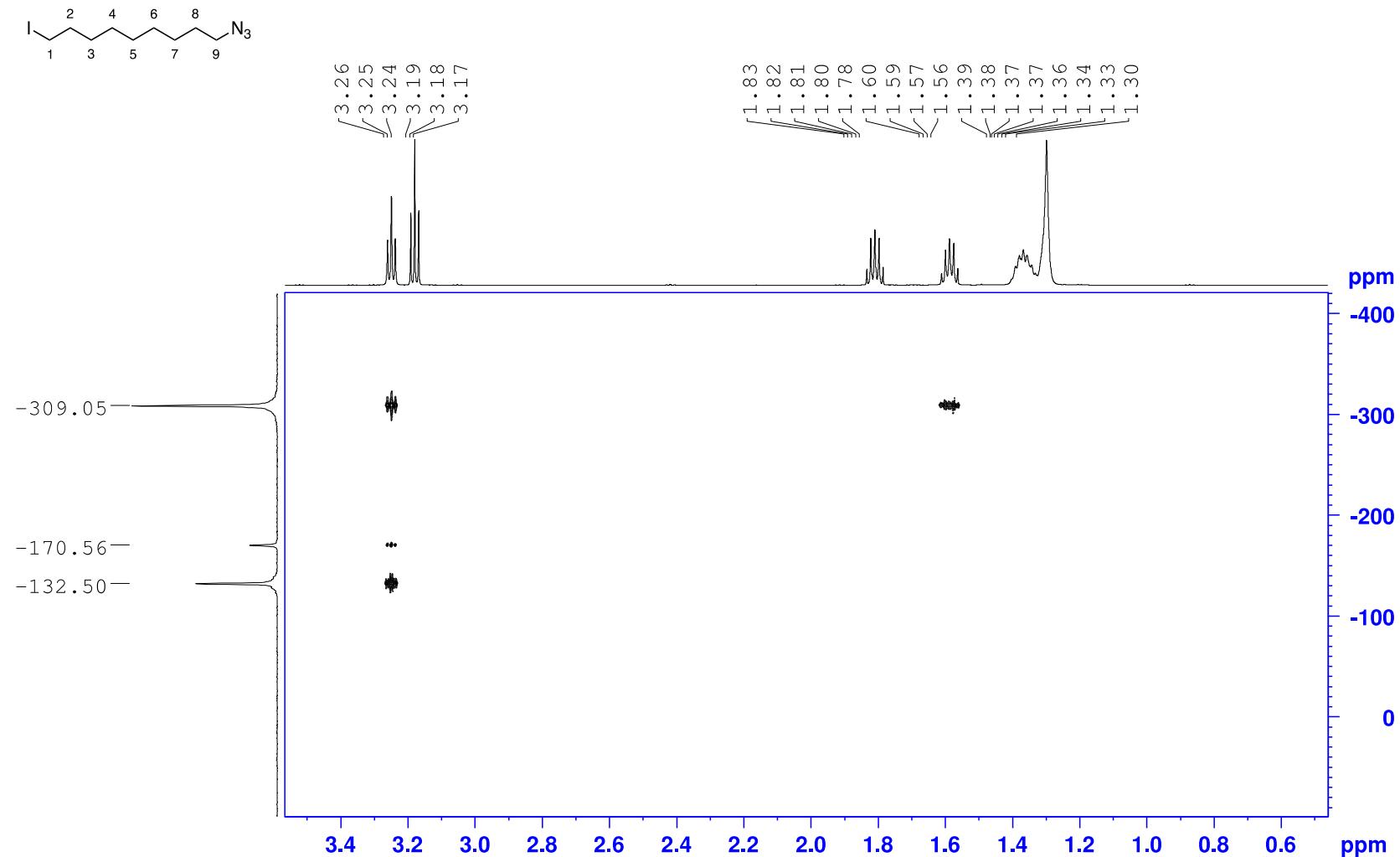
$^1\text{H}$  NMR spectrum (400 MHz,  $\text{CDCl}_3$ )

9-Azido-1-iodononane (**7c**)



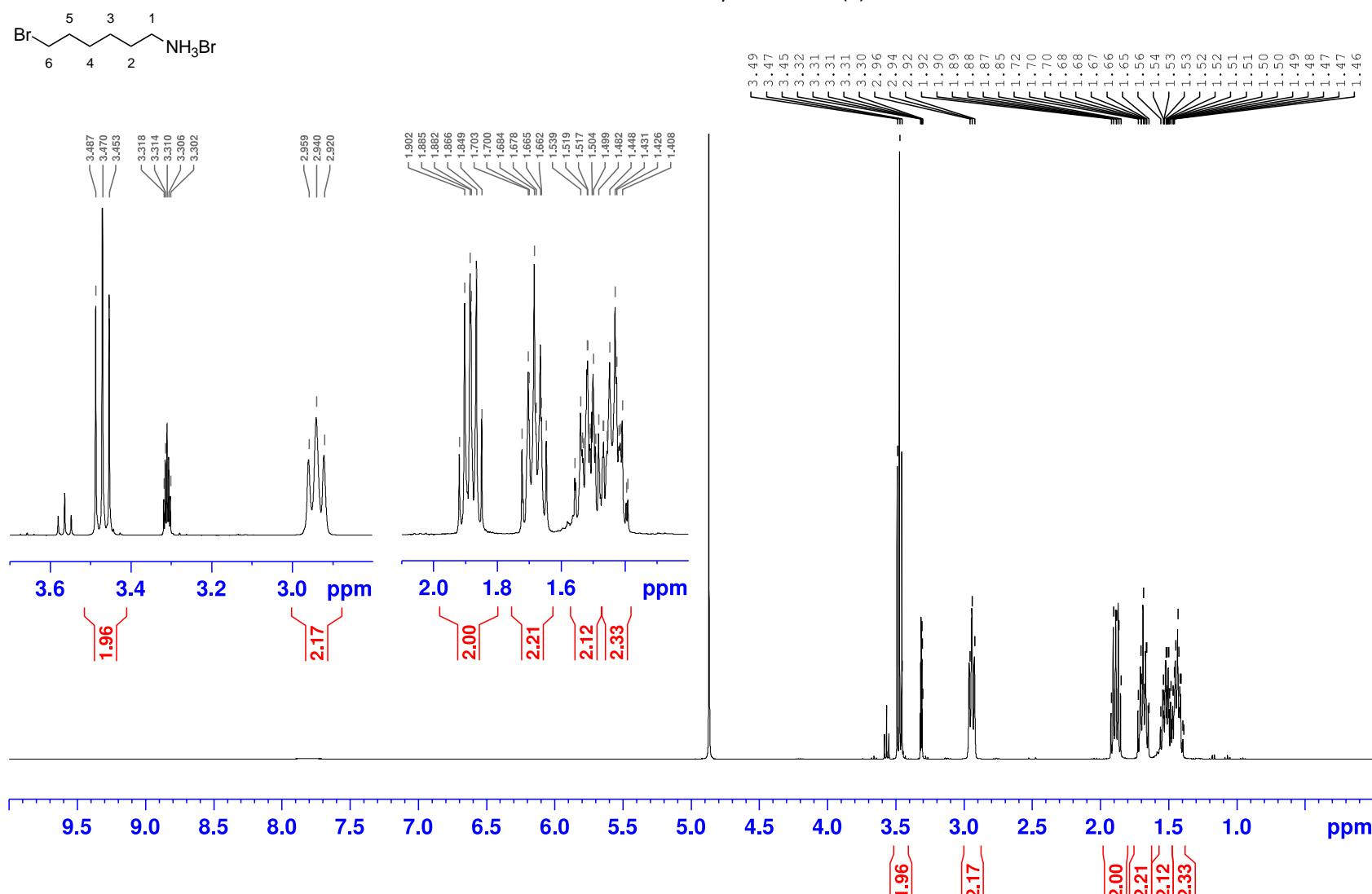
<sup>13</sup>C NMR spectrum (100 MHz, CDCl<sub>3</sub>)

9-Azido-1-iodononane (**7c**)



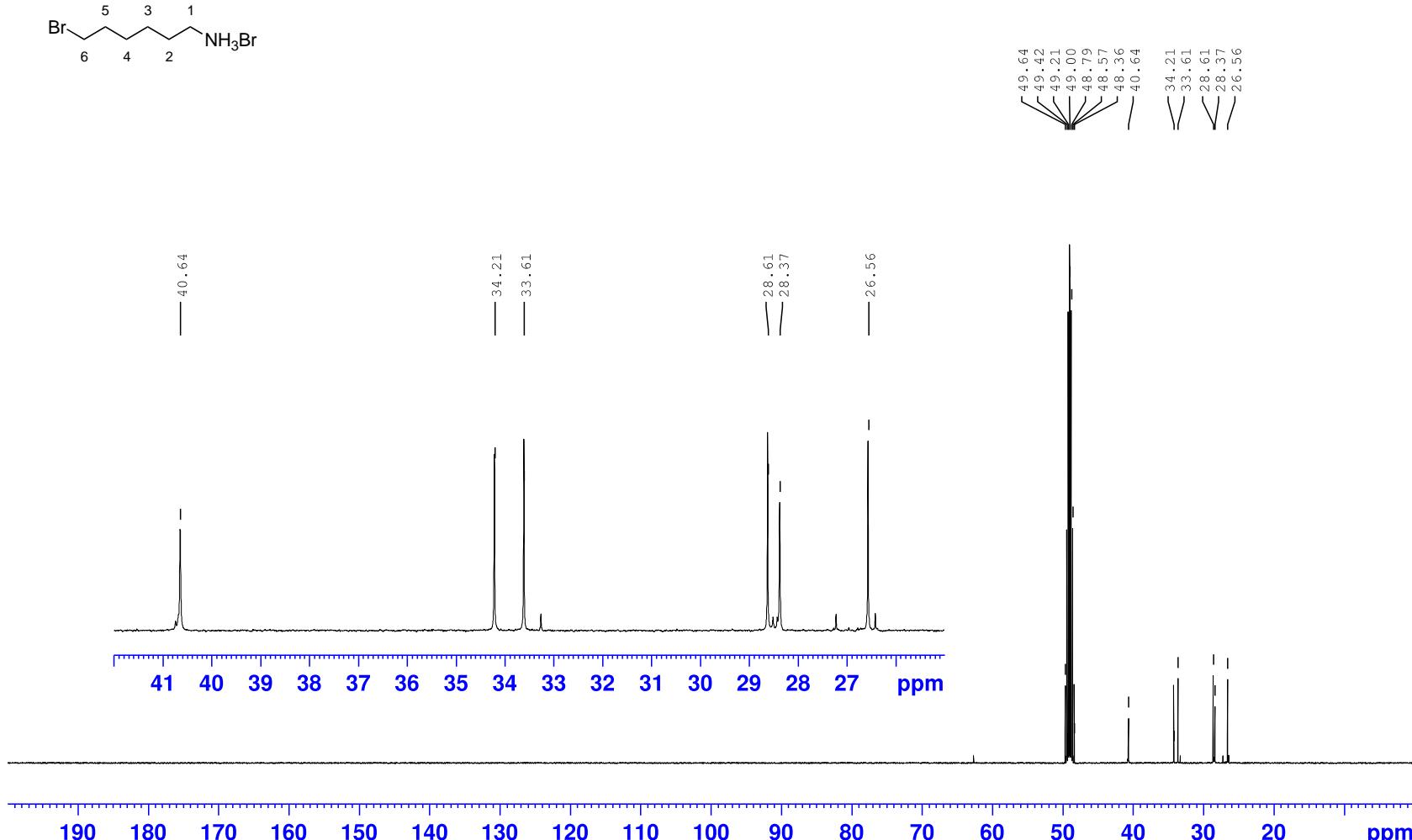
$^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum (600 MHz,  $\text{CDCl}_3$ )

6-Bromohexanamine Hydrobromide (**8**)



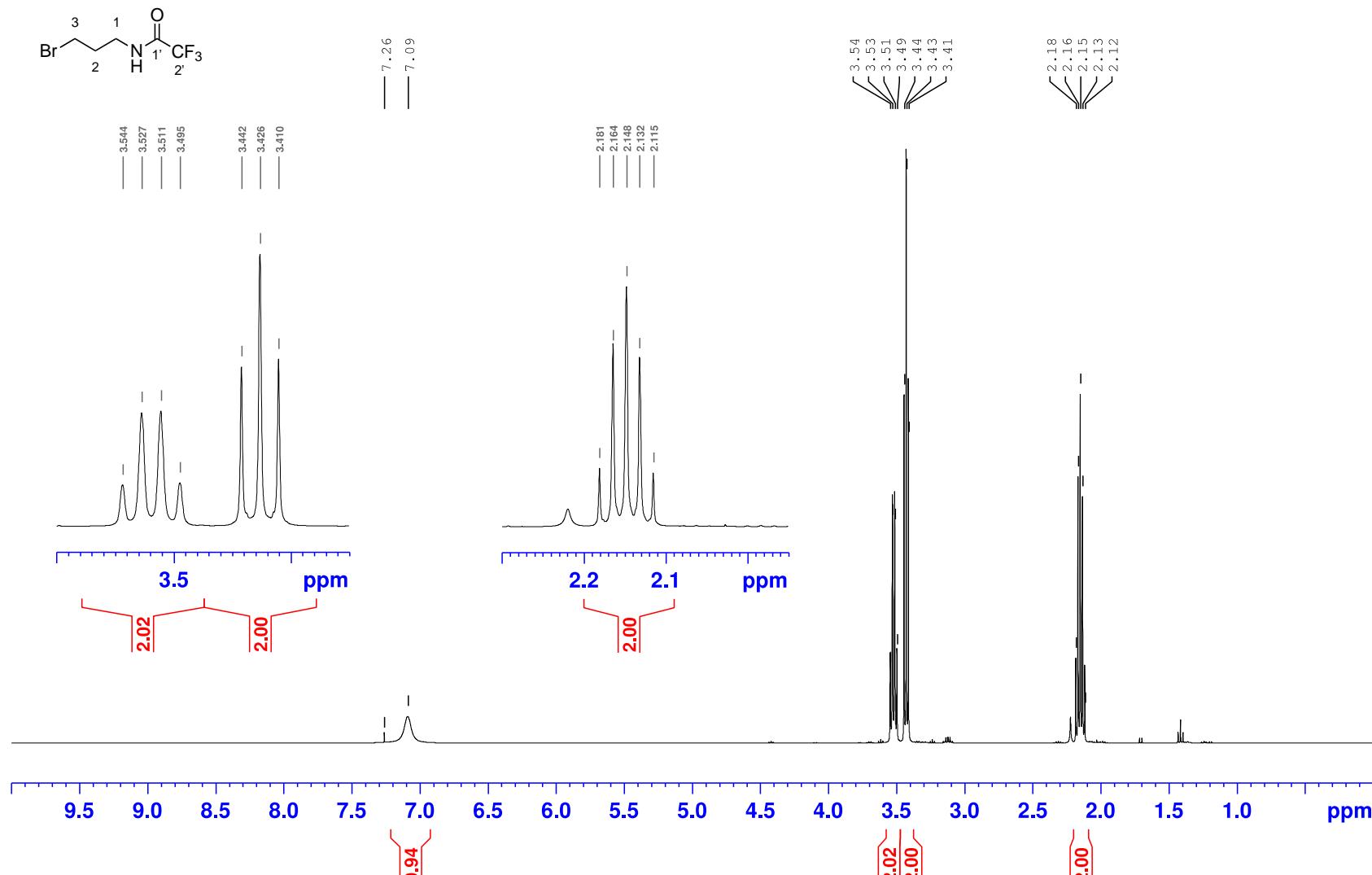
$^1\text{H}$  NMR spectrum (400 MHz, MeOD)

6-Bromohexanamine Hydrobromide (**8**)



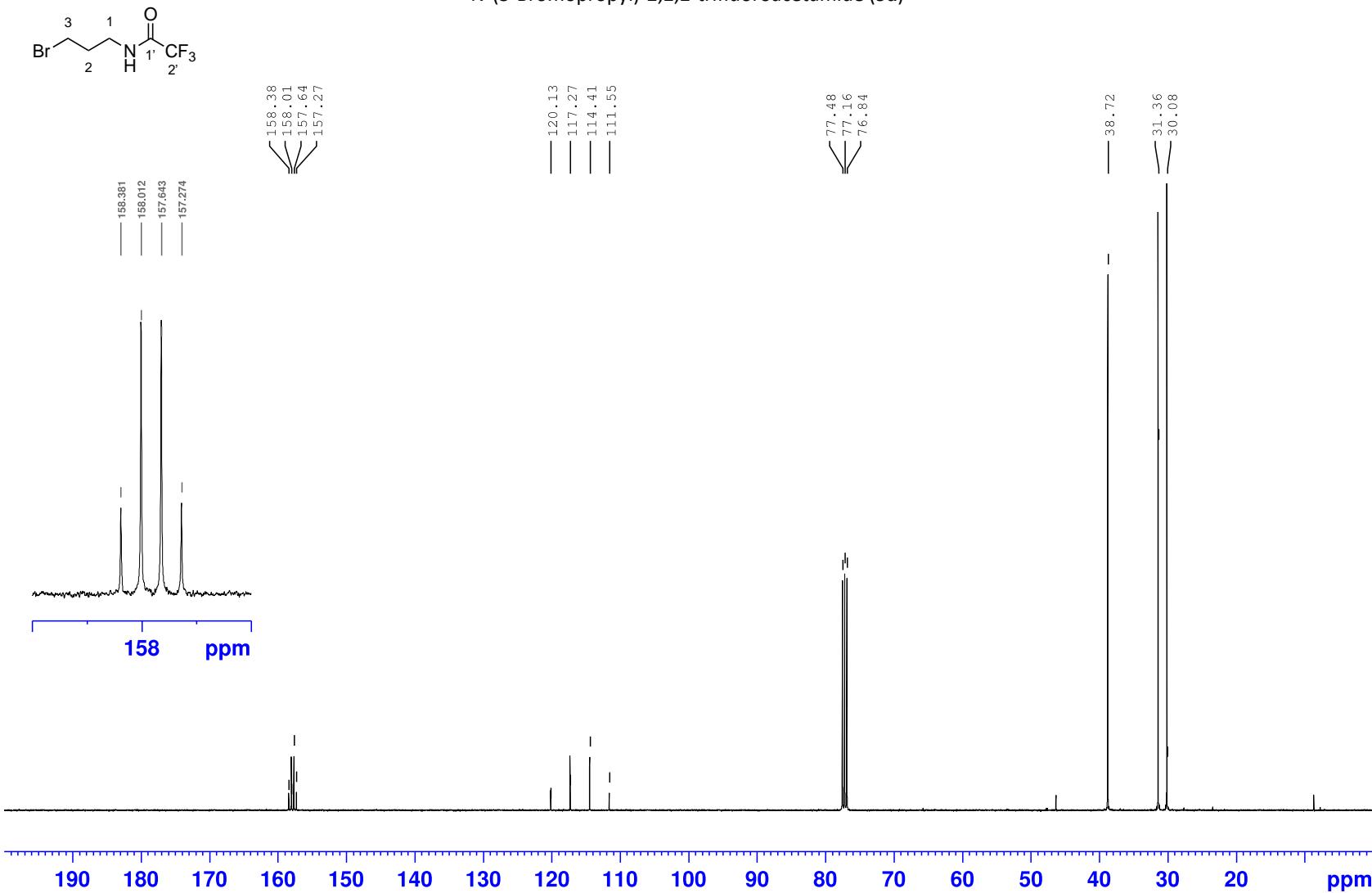
<sup>13</sup>C NMR spectrum (100 MHz, MeOD)

*N*-(3-Bromopropyl)-2,2,2-trifluoroacetamide (**9a**)

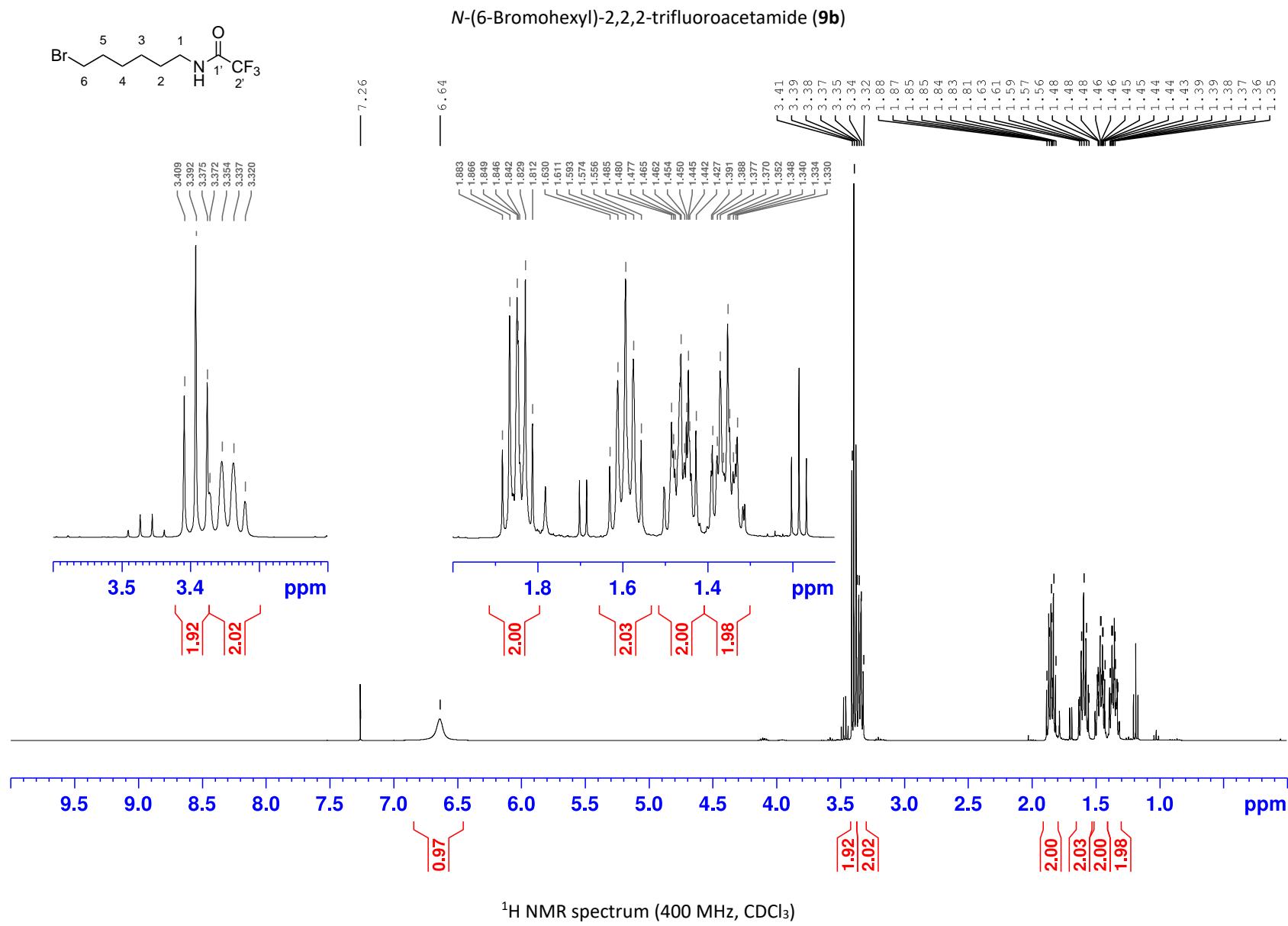


<sup>1</sup>H NMR spectrum (400 MHz, CDCl<sub>3</sub>)

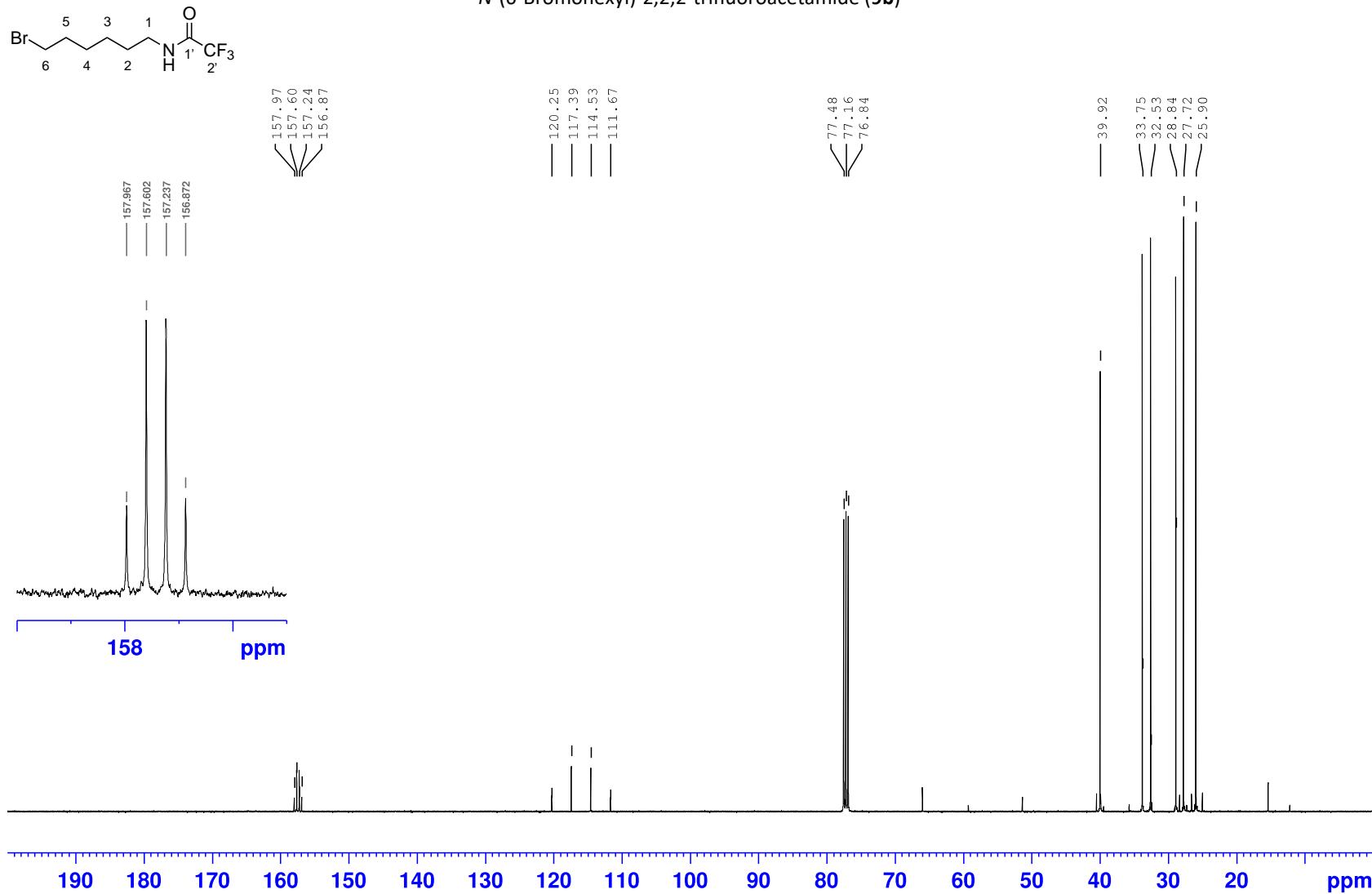
*N*-(3-Bromopropyl)-2,2,2-trifluoroacetamide (**9a**)



$^{13}\text{C}$  NMR spectrum (100 MHz,  $\text{CDCl}_3$ )

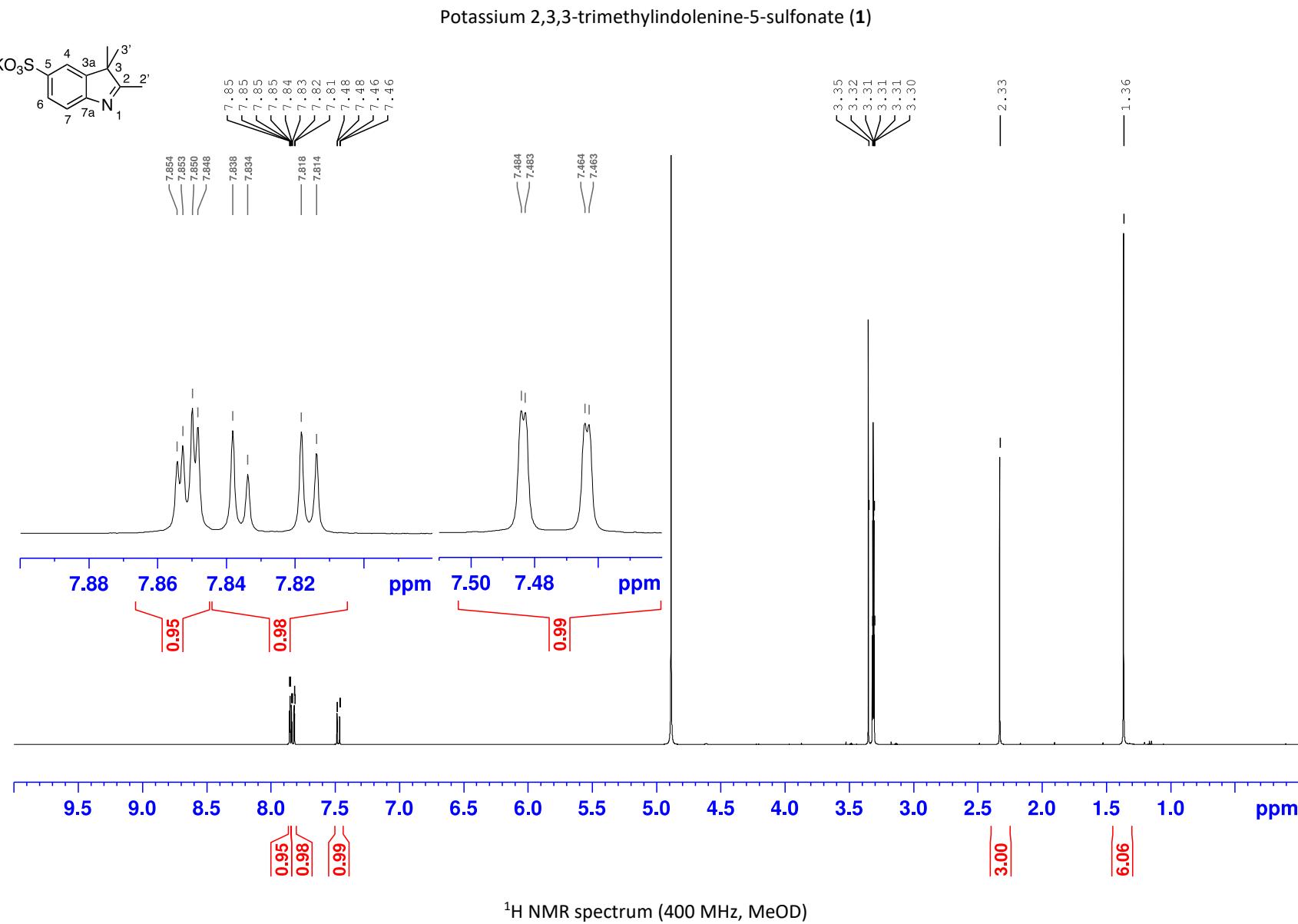


*N*-(6-Bromohexyl)-2,2,2-trifluoroacetamide (**9b**)

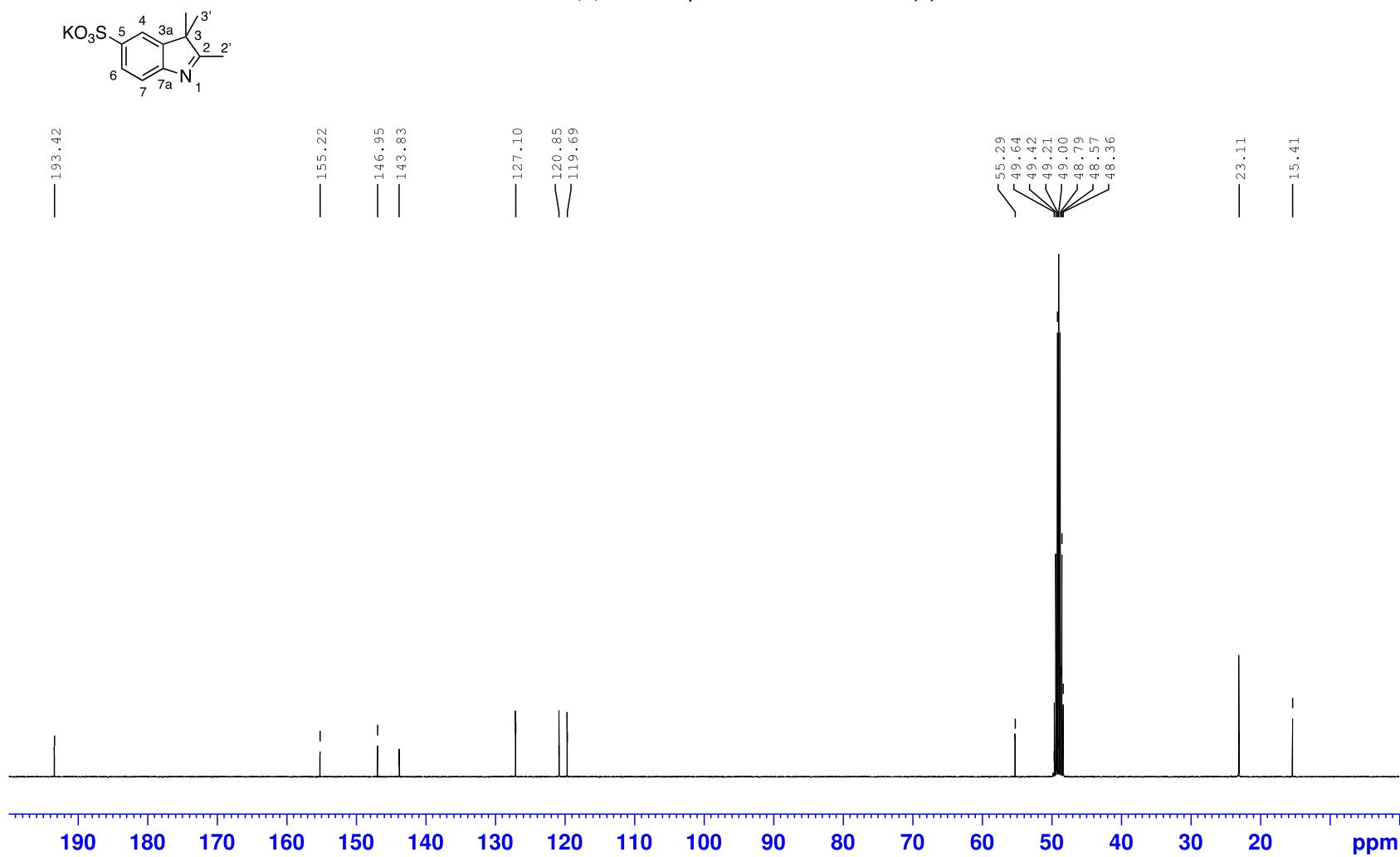


<sup>13</sup>C NMR spectrum (100 MHz, CDCl<sub>3</sub>)

## Spectroscopic Characterization of Indolium Salts

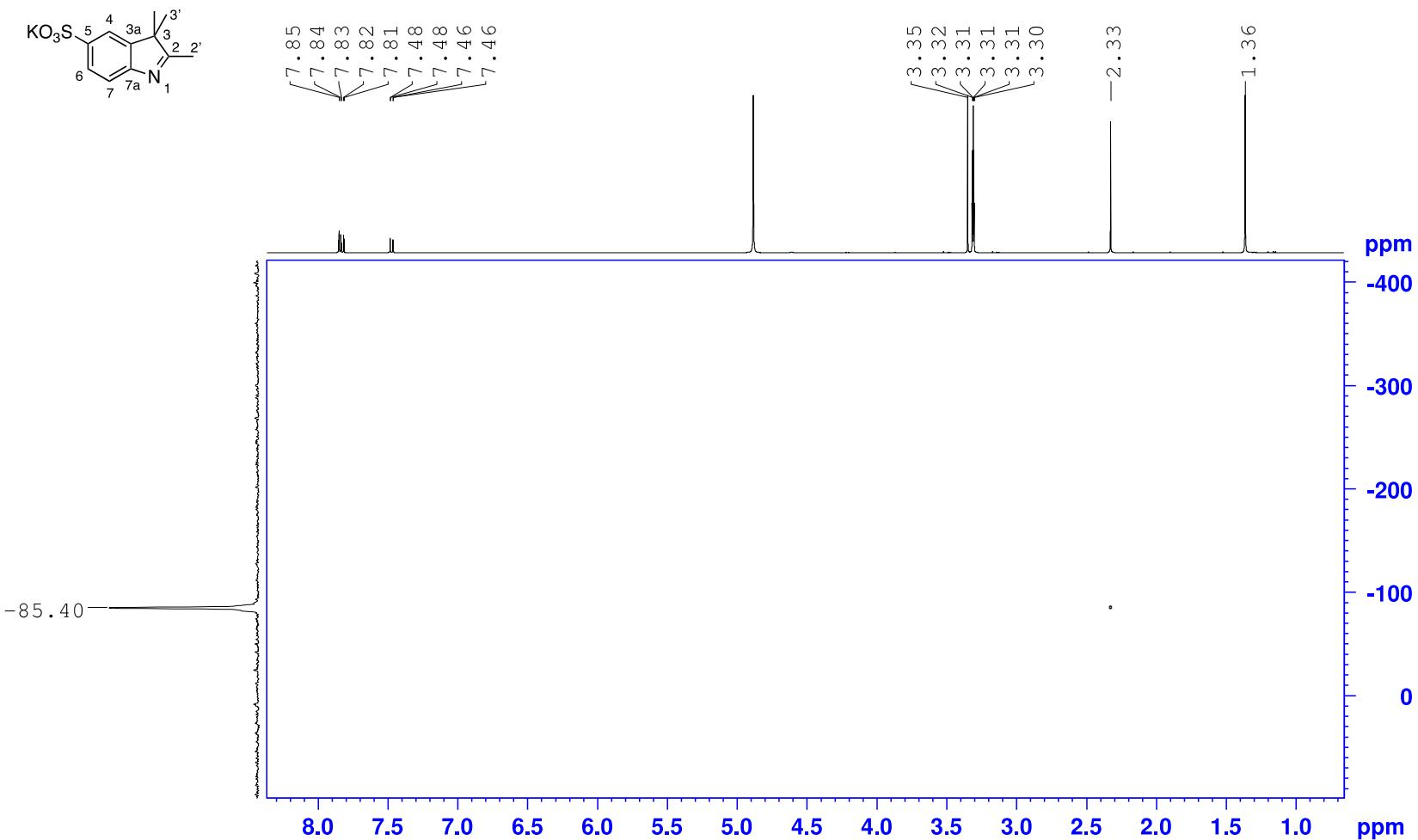


Potassium 2,3,3-trimethylindolenine-5-sulfonate (**1**)

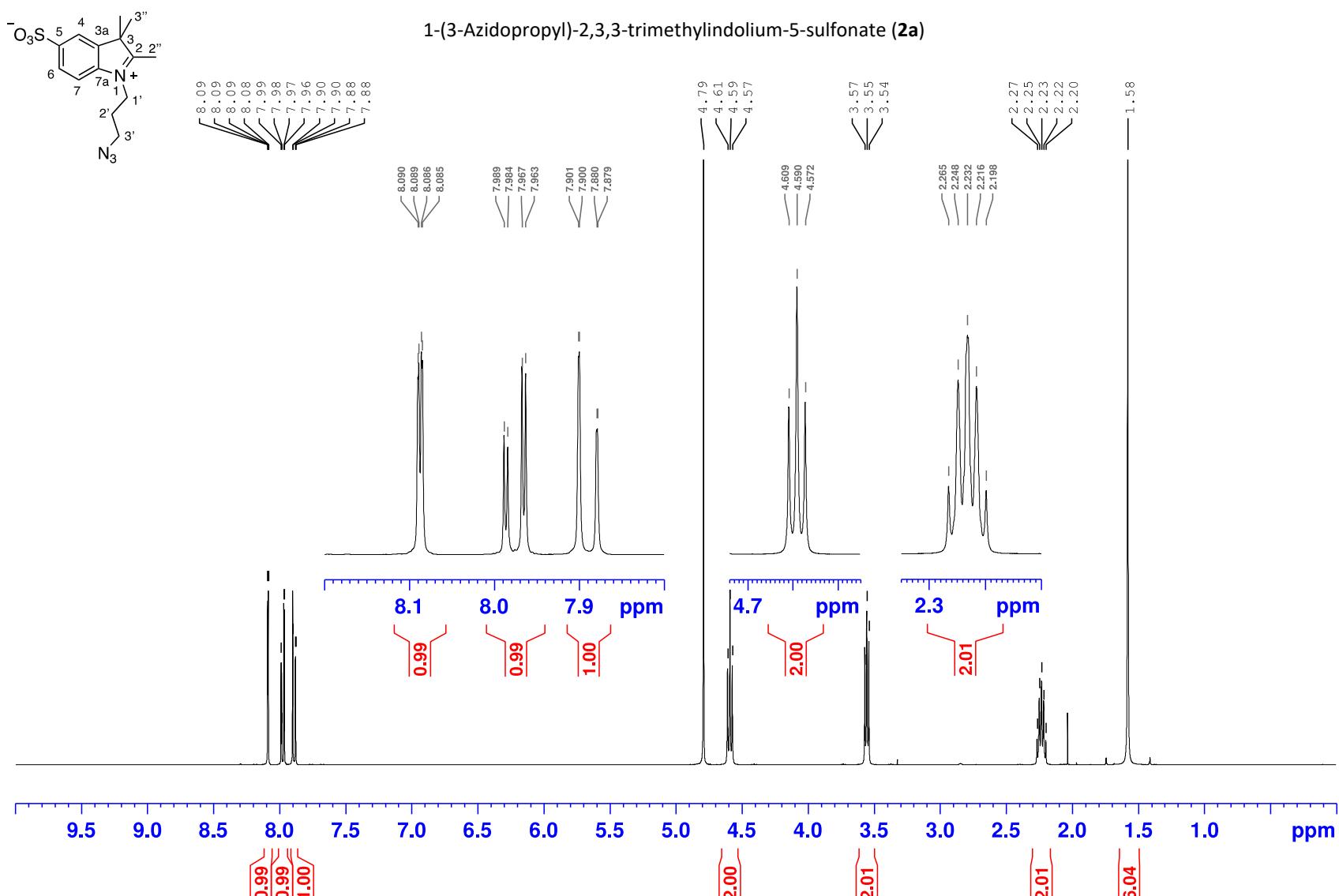


<sup>13</sup>C NMR spectrum (100 MHz, MeOD)

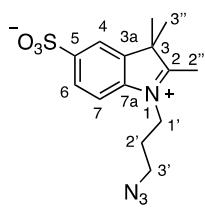
Potassium 2,3,3-trimethylindolenine-5-sulfonate (**1**)



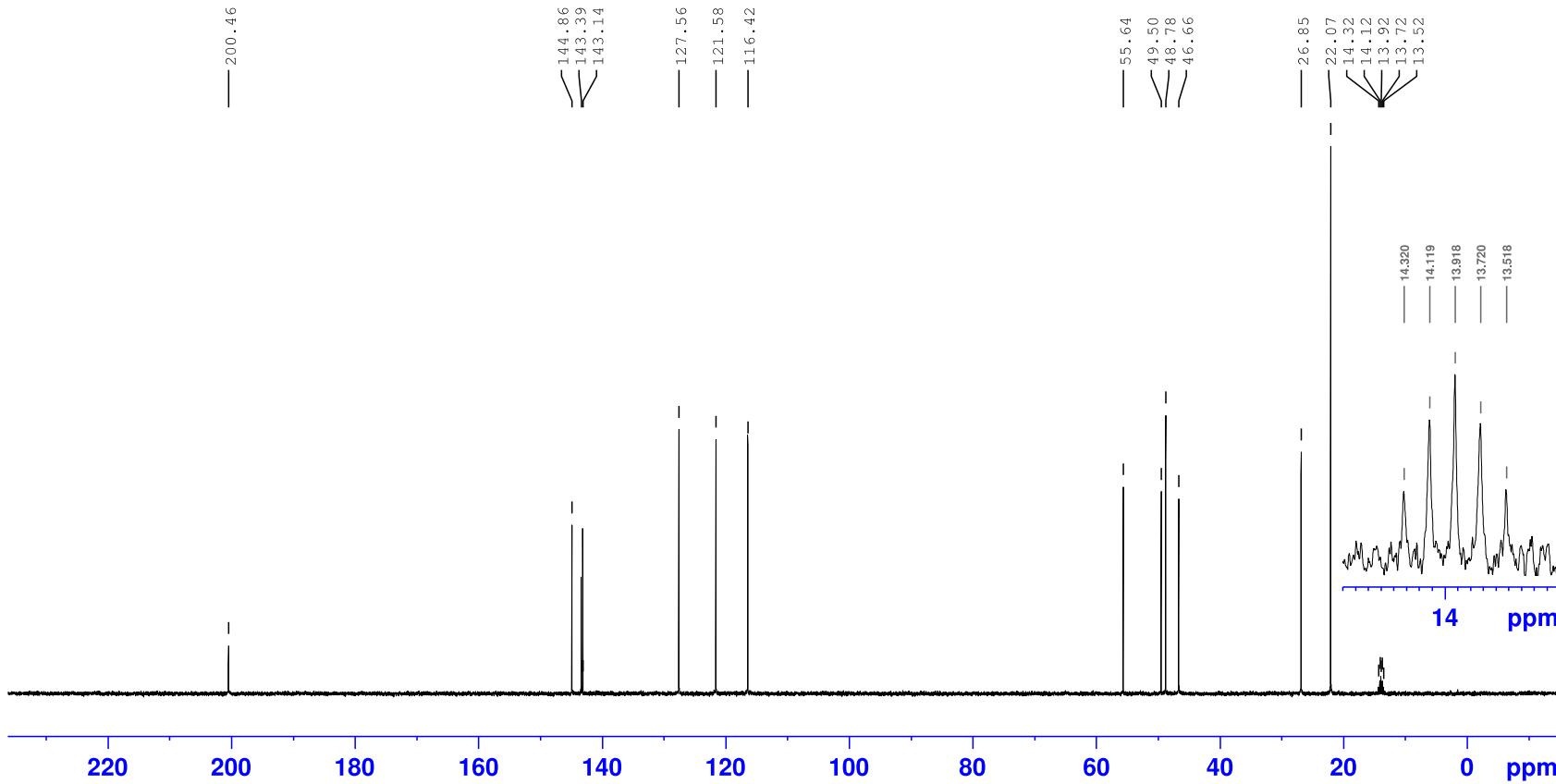
$^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum (400 MHz, MeOD)



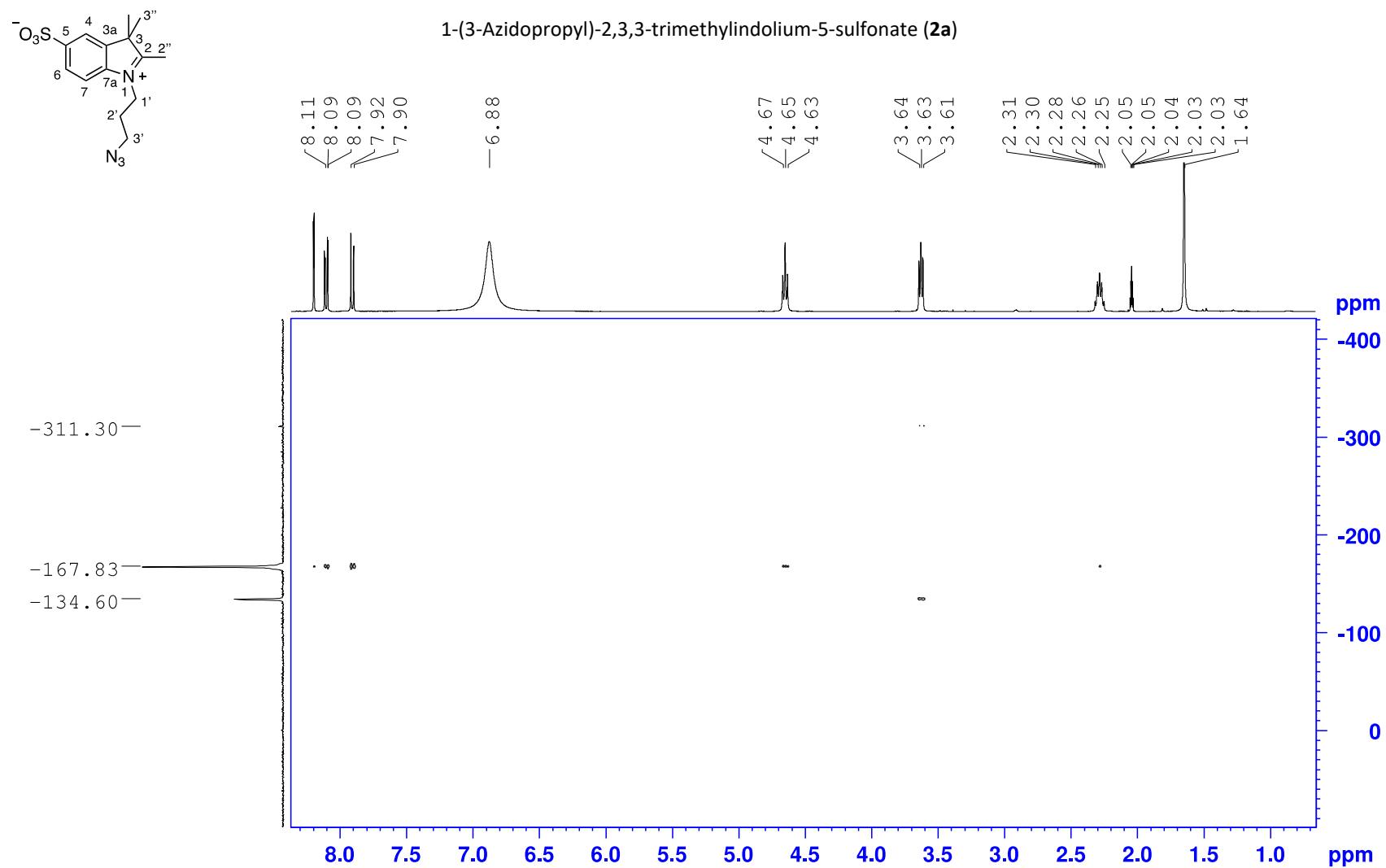
<sup>1</sup>H NMR spectrum (400 MHz, D<sub>2</sub>O)



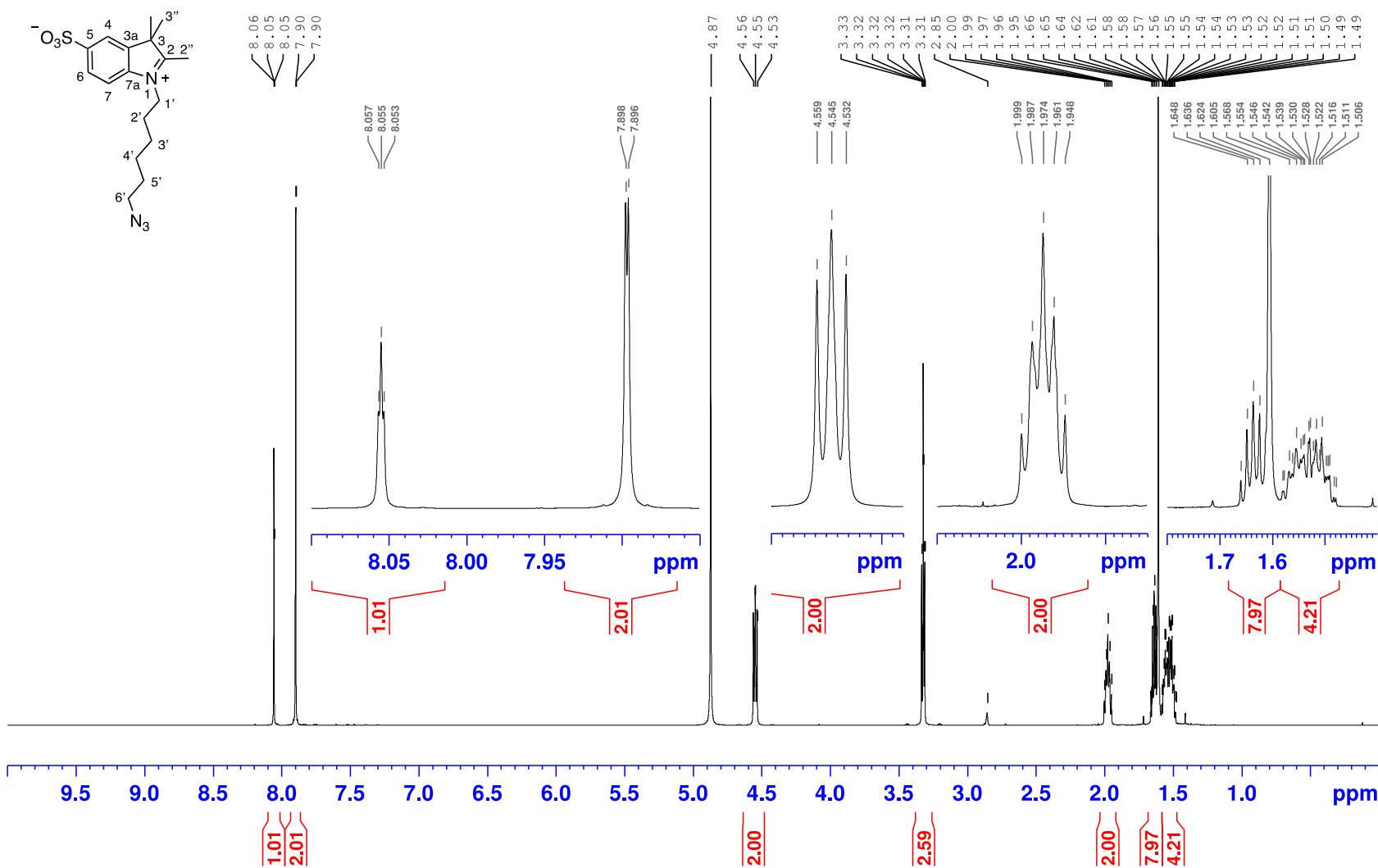
1-(3-Azidopropyl)-2,3,3-trimethylindolium-5-sulfonate (**2a**)



<sup>13</sup>C NMR spectrum (100 MHz, D<sub>2</sub>O and MeOH as internal reference)

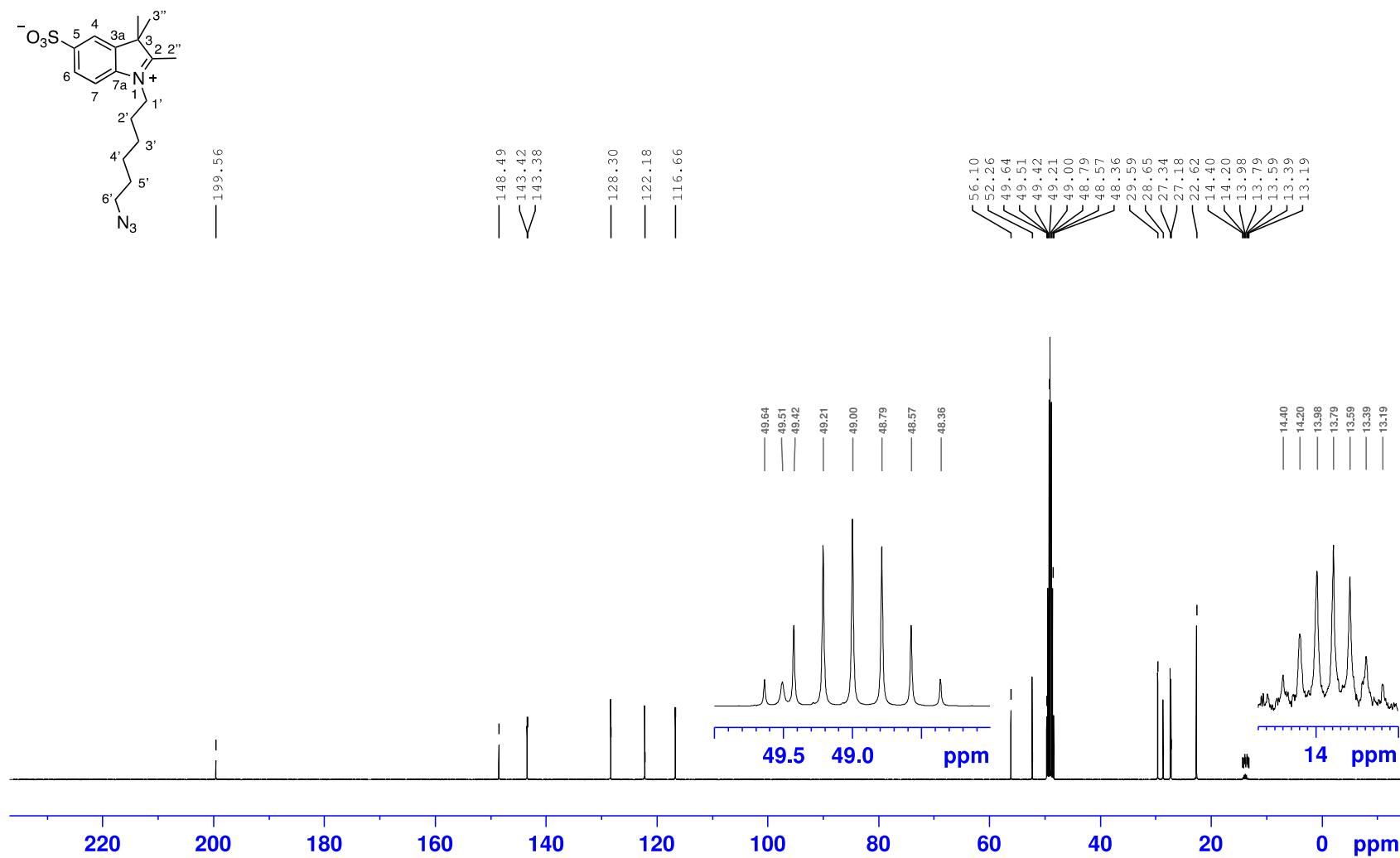


**1-(6-Azidohexyl)-2,3,3-trimethylindolium-5-sulfonate (**2b**)**



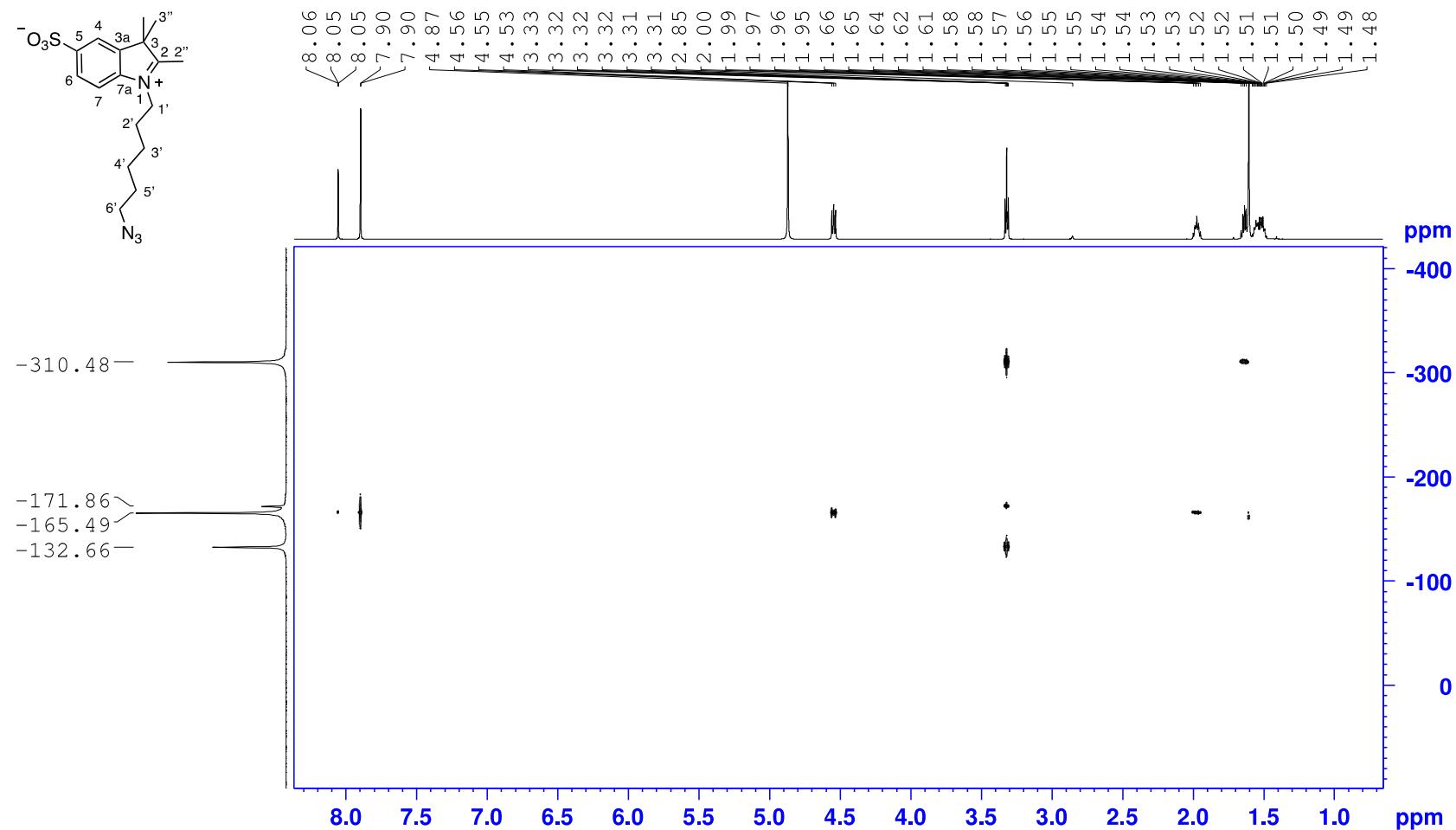
<sup>1</sup>H NMR spectrum (600 MHz, MeOD)

**1-(6-Azidohexyl)-2,3,3-trimethylindolium-5-sulfonate (**2b**)**



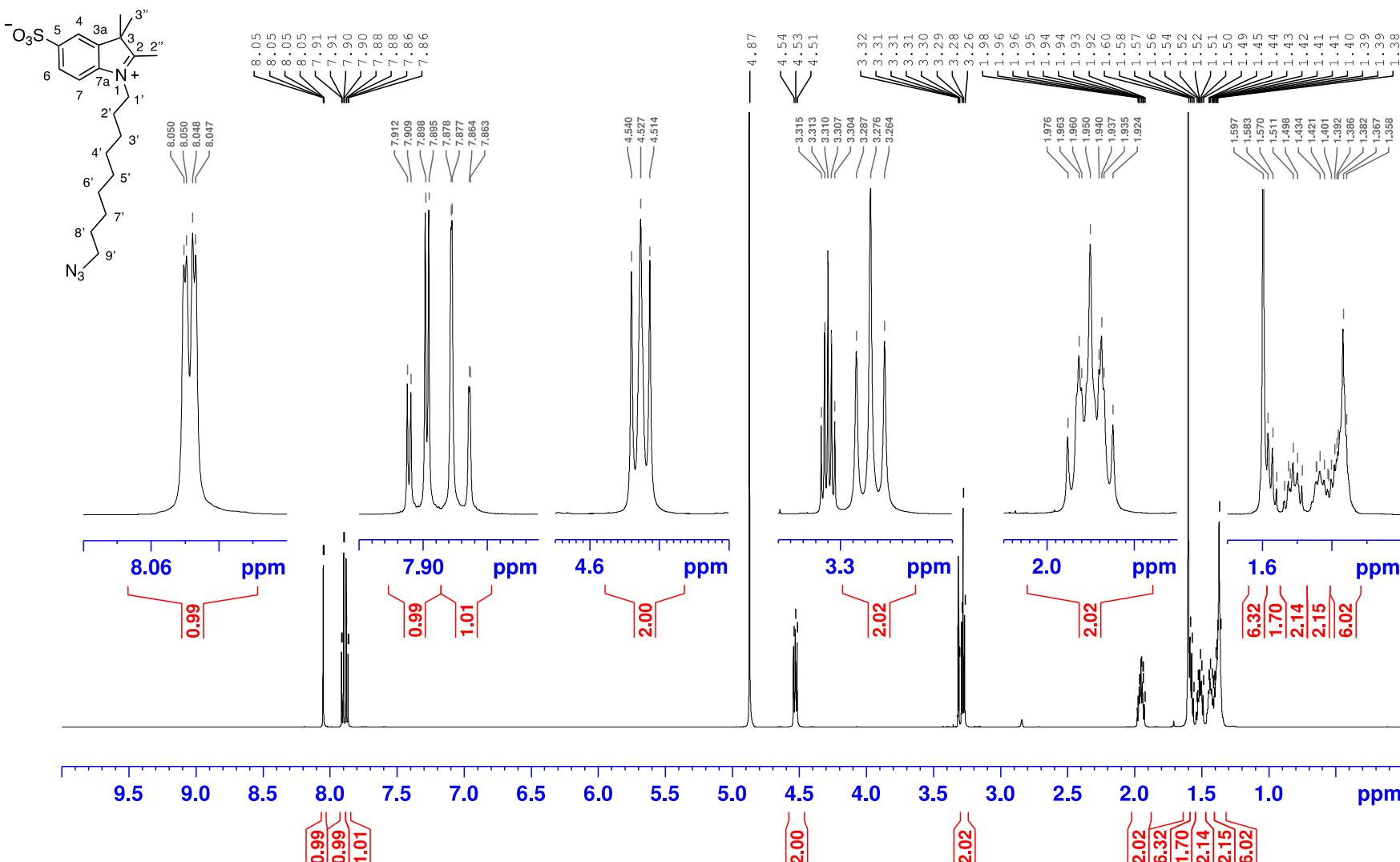
<sup>13</sup>C NMR spectrum (100 MHz, MeOD)

1-(6-Azidohexyl)-2,3,3-trimethylindolium-5-sulfonate (**2b**)



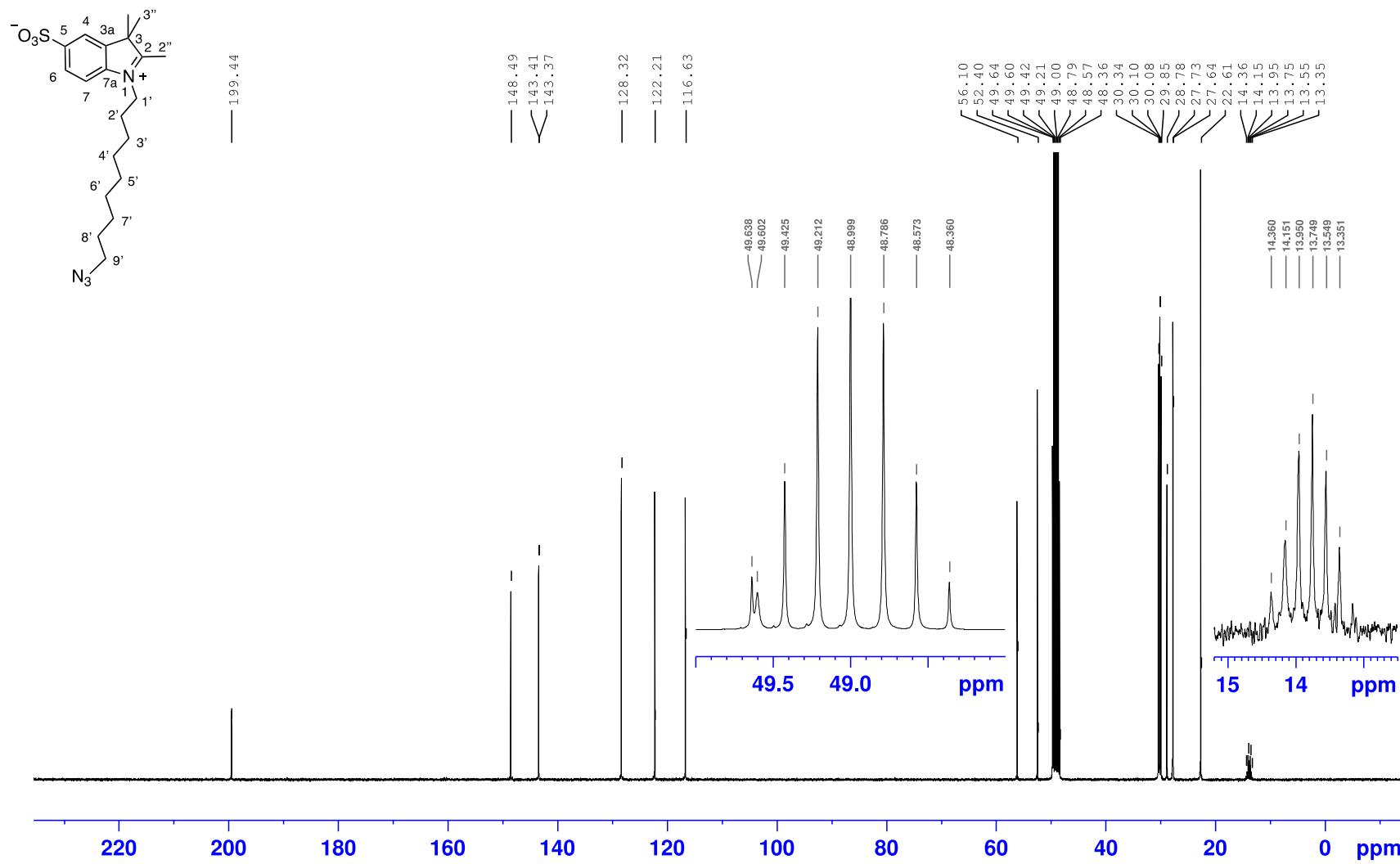
$^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum (600 MHz, MeOD)

**1-(9-Azidononyl)-2,3,3-trimethylindolium-5-sulfonate (**2c**)**

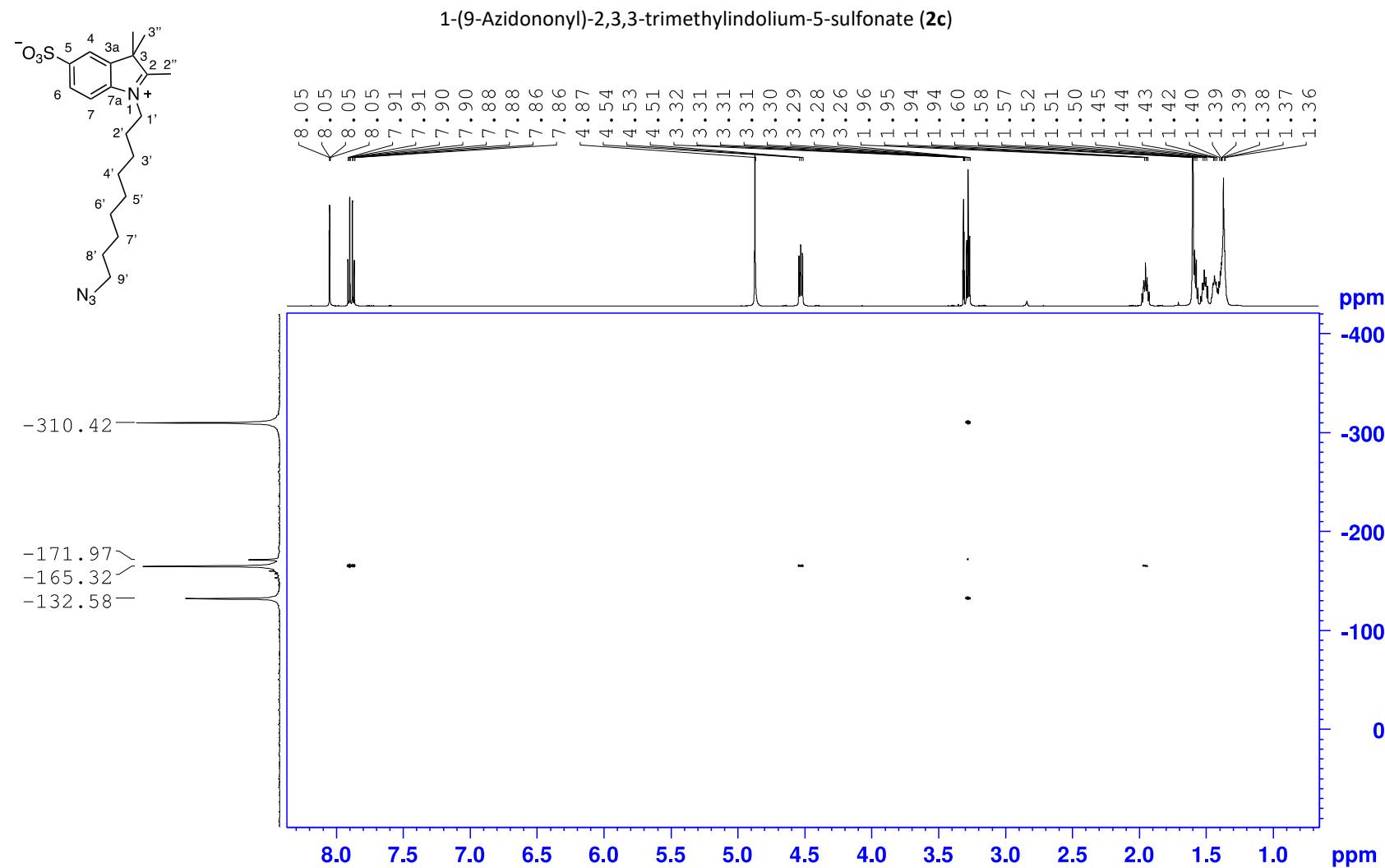


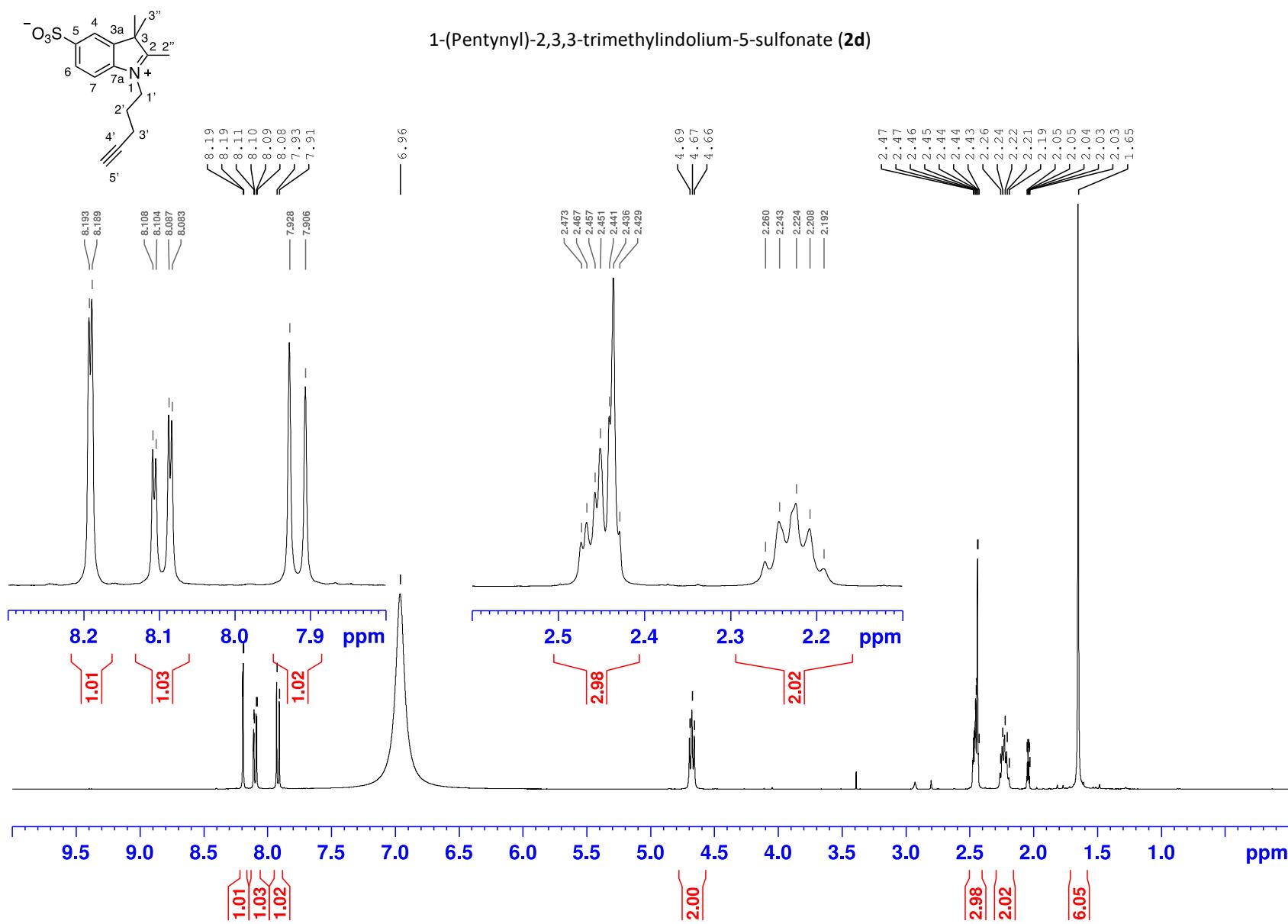
$^1\text{H}$  NMR spectrum (600 MHz, MeOD)

**1-(9-Azidononyl)-2,3,3-trimethylindolium-5-sulfonate (**2c**)**



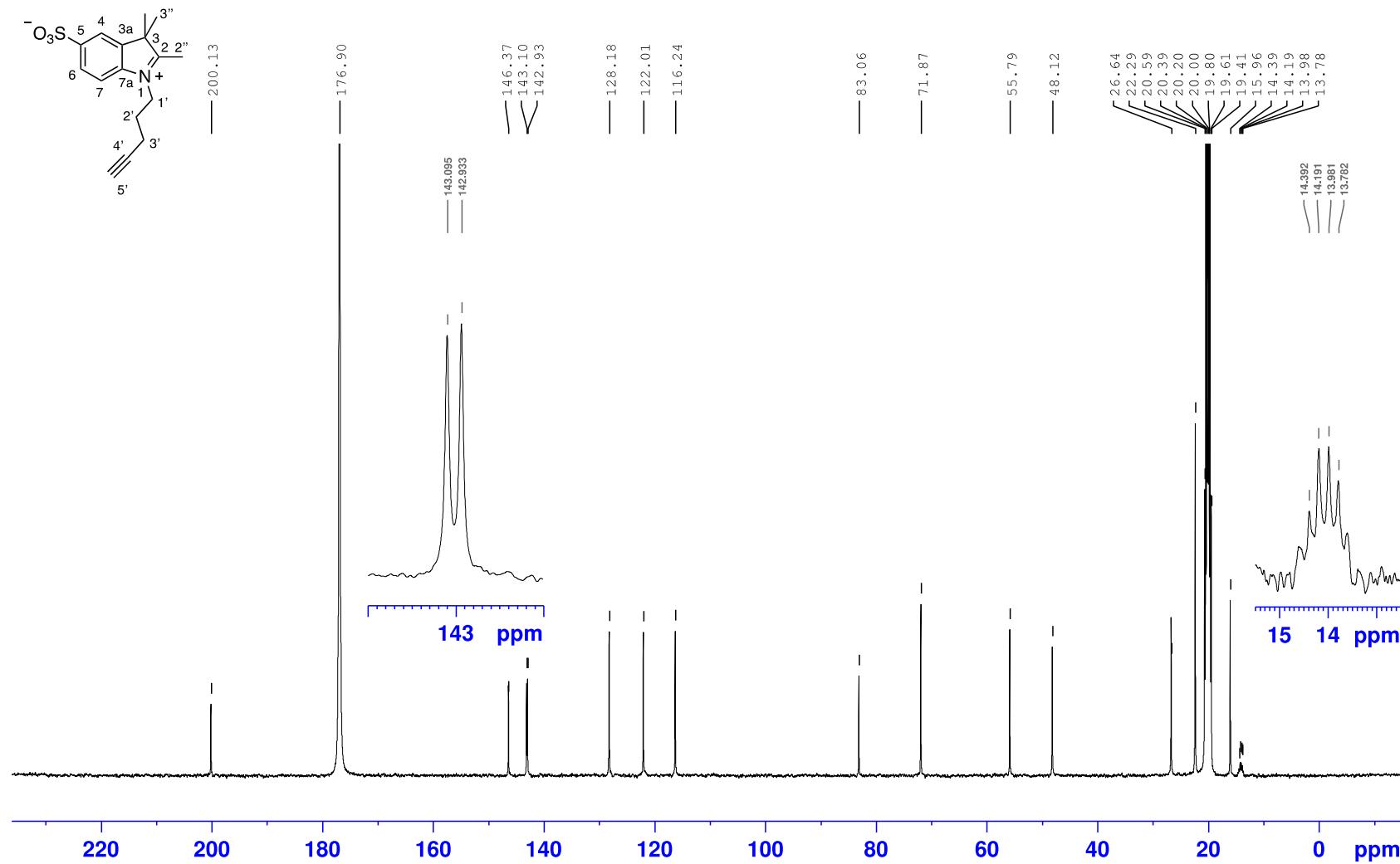
$^{13}\text{C}$  NMR spectrum (100 MHz, MeOD)



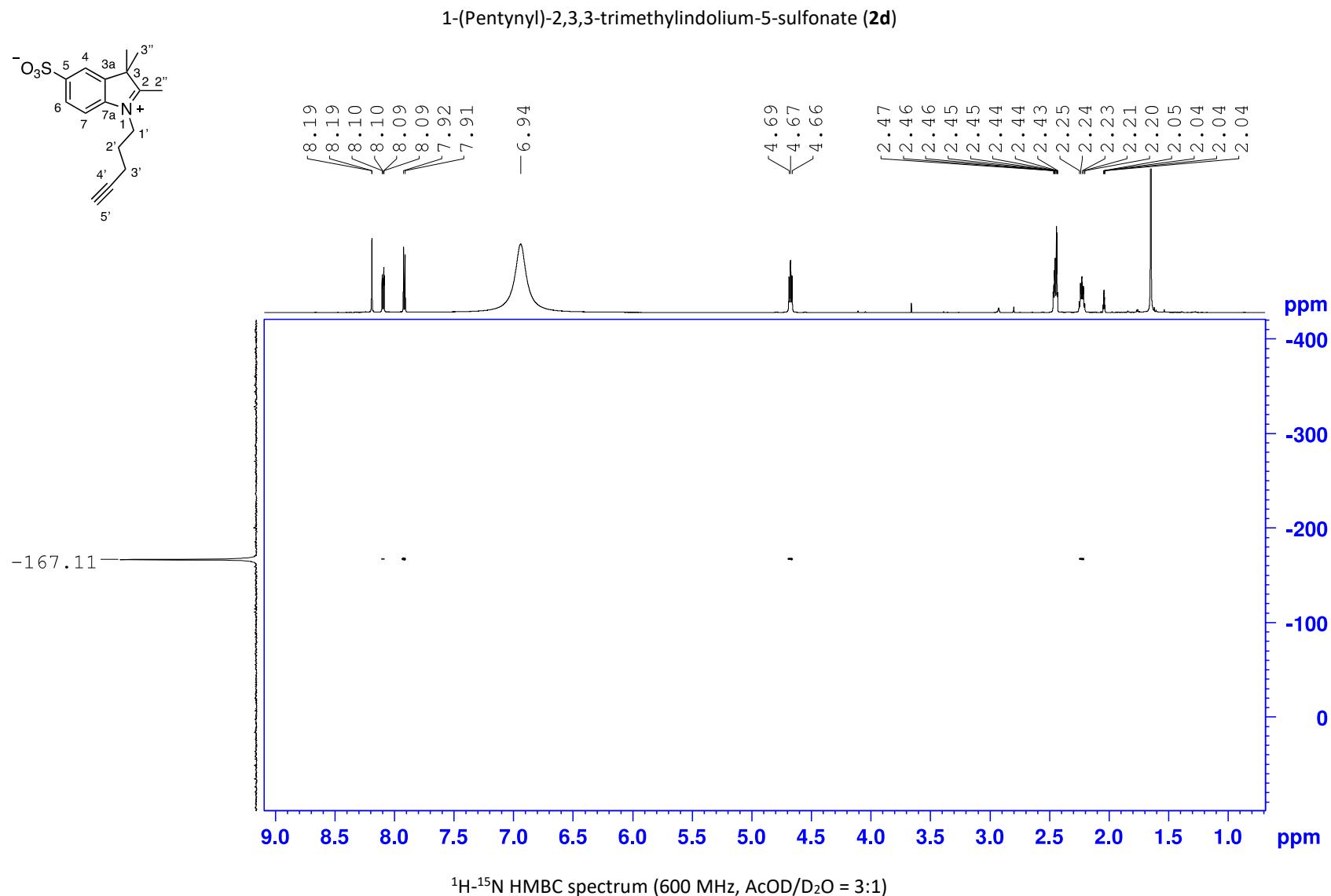


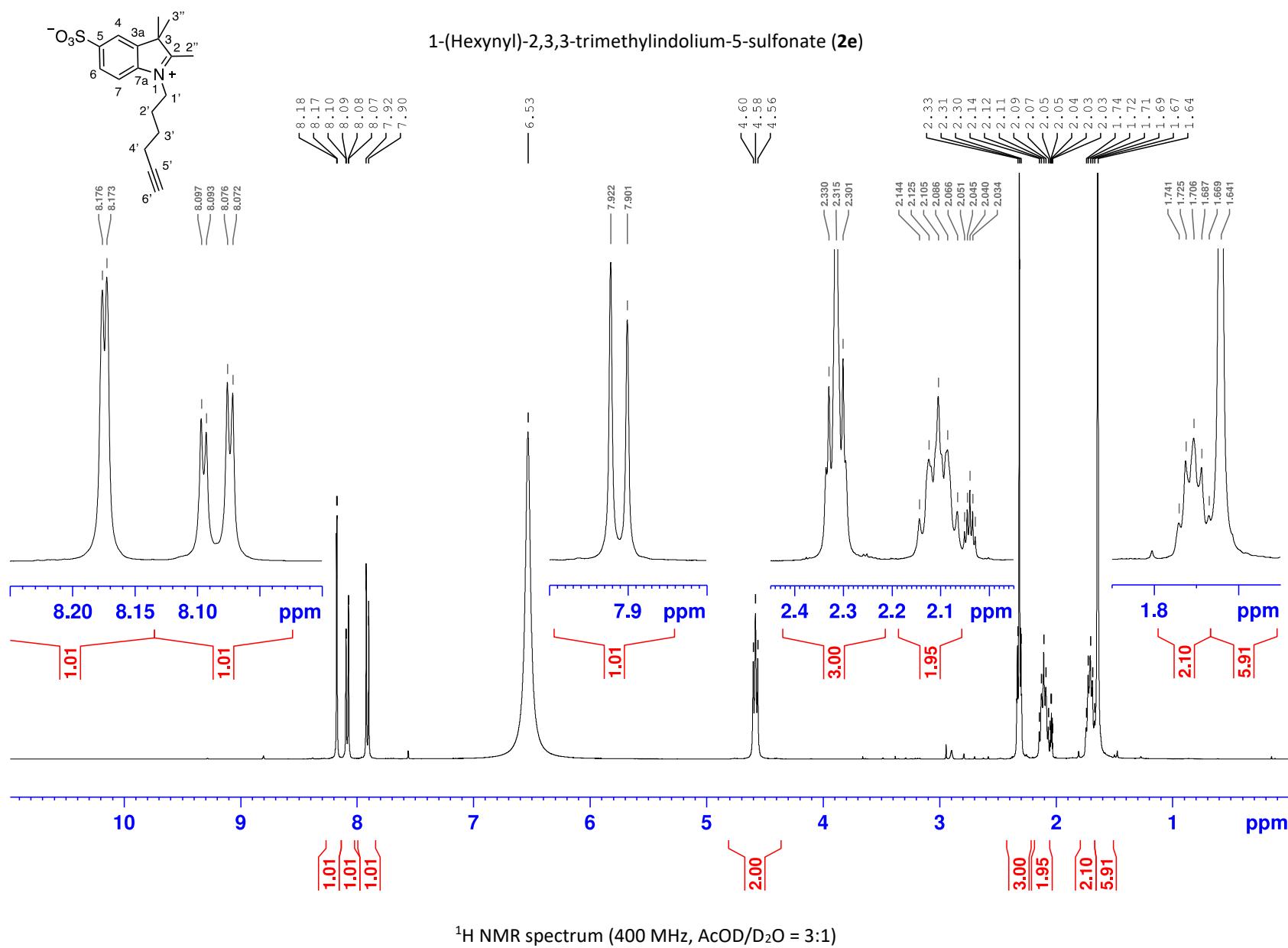
<sup>1</sup>H NMR spectrum (400 MHz, AcOD/D<sub>2</sub>O = 3:1)

**1-(Pentynyl)-2,3,3-trimethylindolium-5-sulfonate (**2d**)**

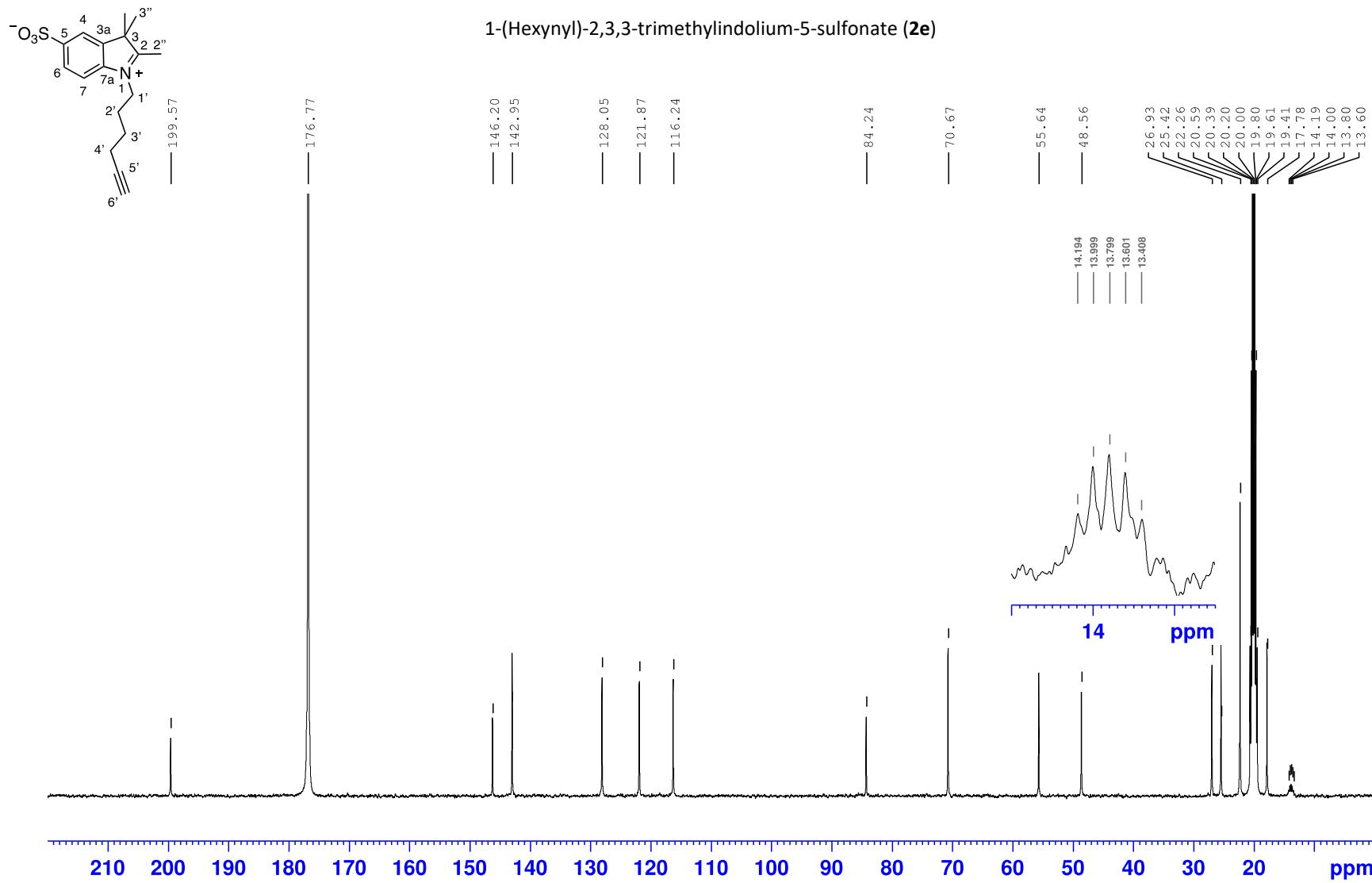


$^{13}\text{C}$ -NMR-spectrum: (100 MHz, AcOD/D<sub>2</sub>O = 3:1)

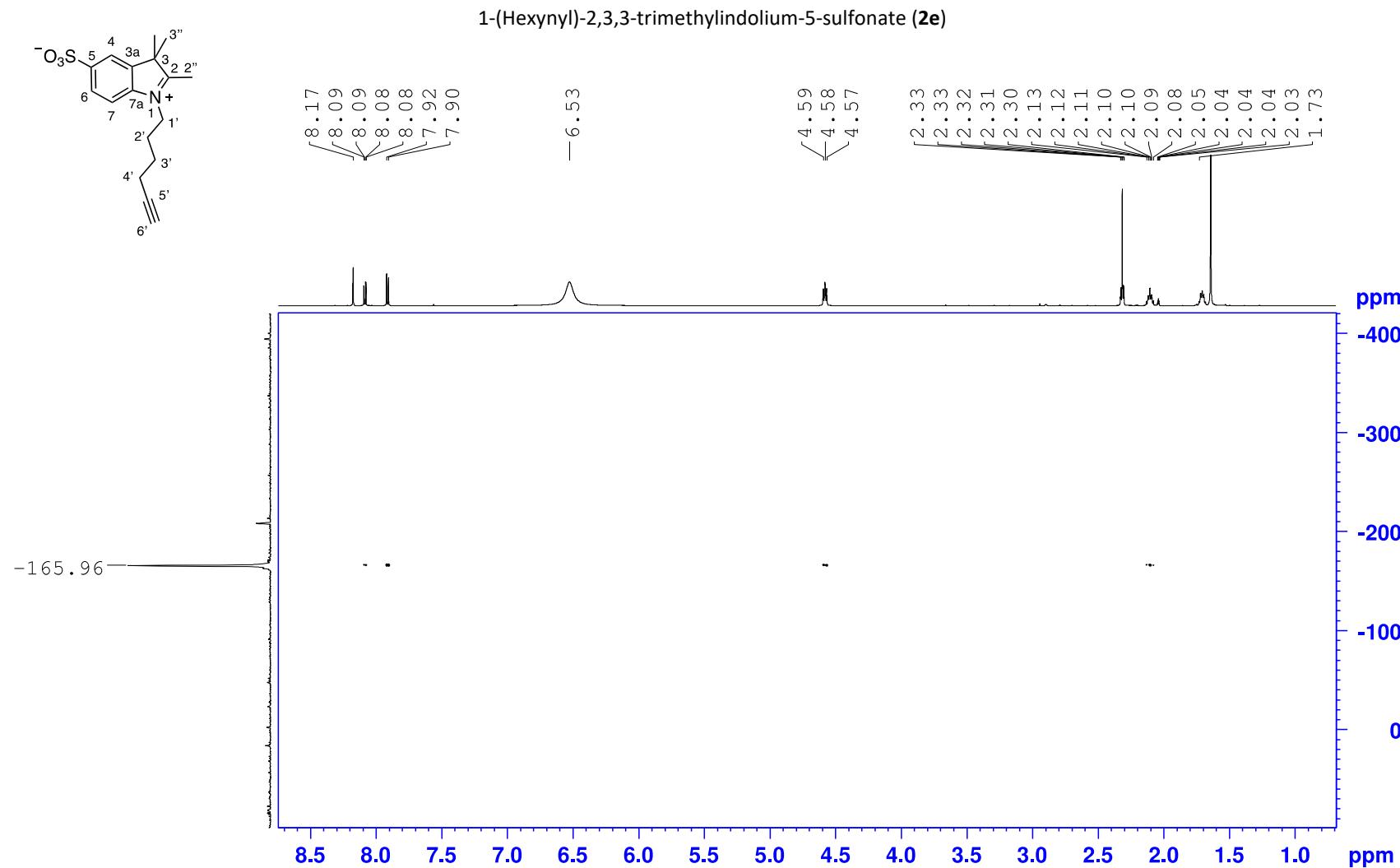




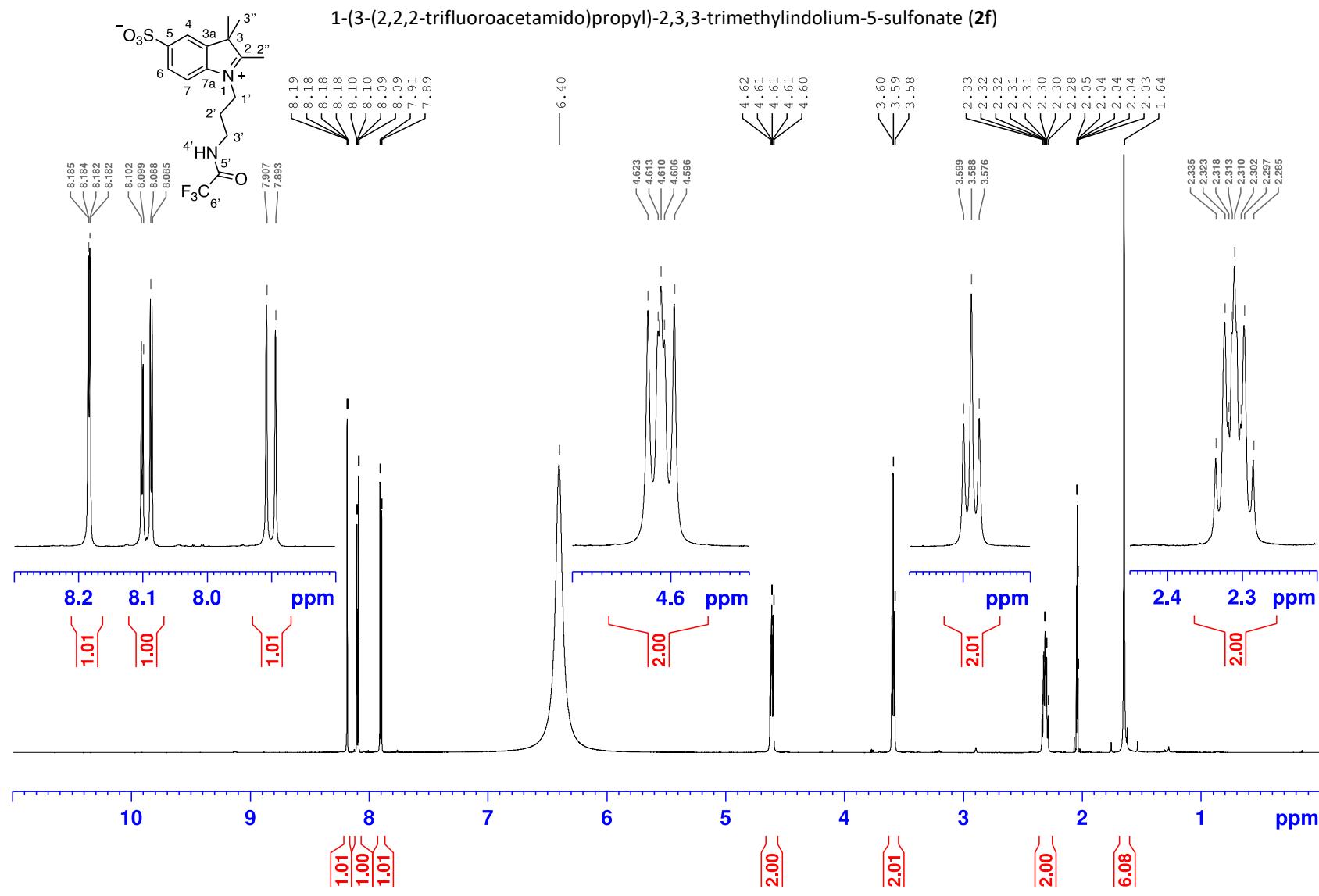
<sup>1</sup>H NMR spectrum (400 MHz, AcOD/D<sub>2</sub>O = 3:1)



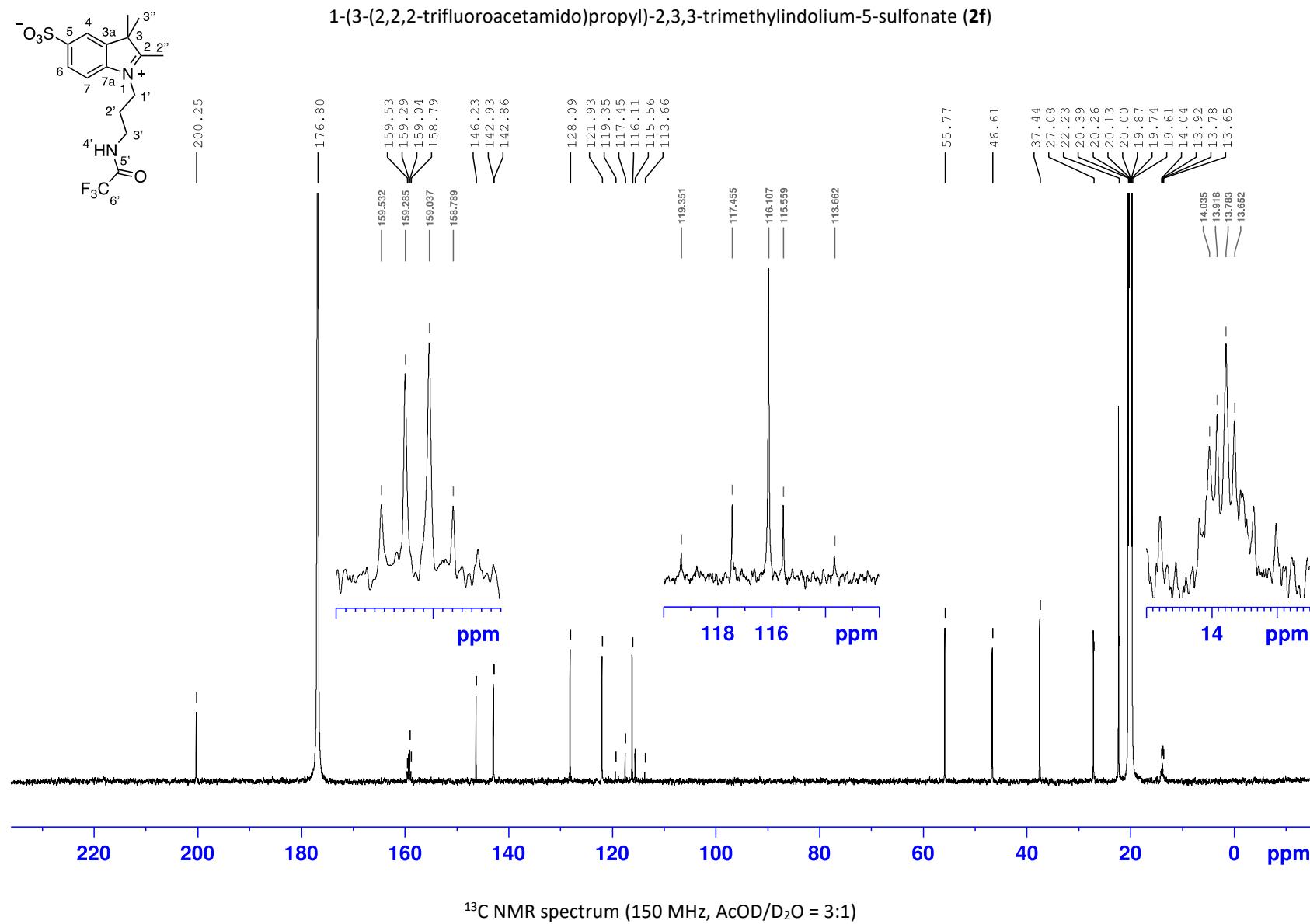
$^{13}\text{C}$  NMR spectrum (100 MHz, AcOD/D<sub>2</sub>O = 3:1)



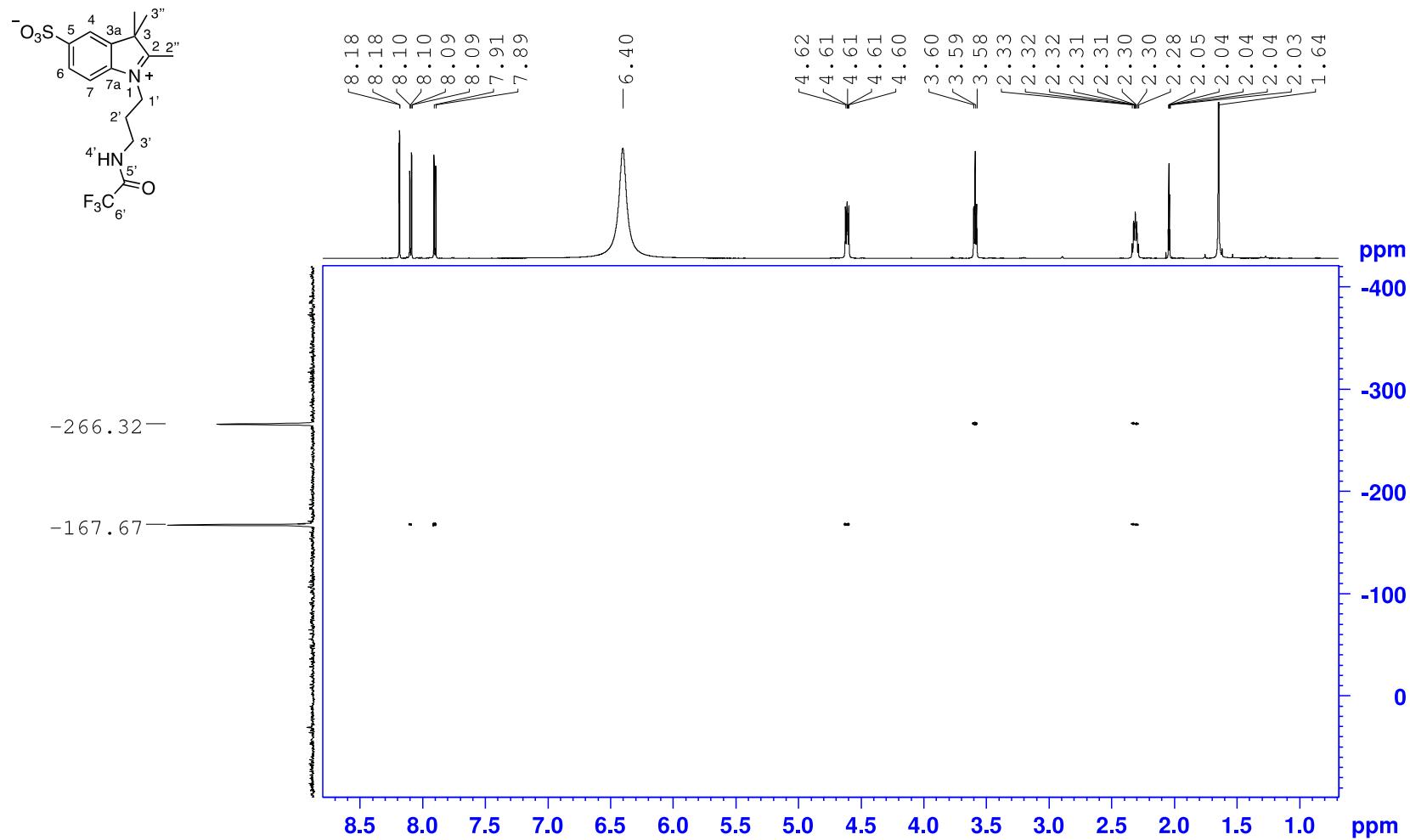
<sup>1</sup>H-<sup>15</sup>N HMBC spectrum (600 MHz, AcOD/D<sub>2</sub>O = 3:1)



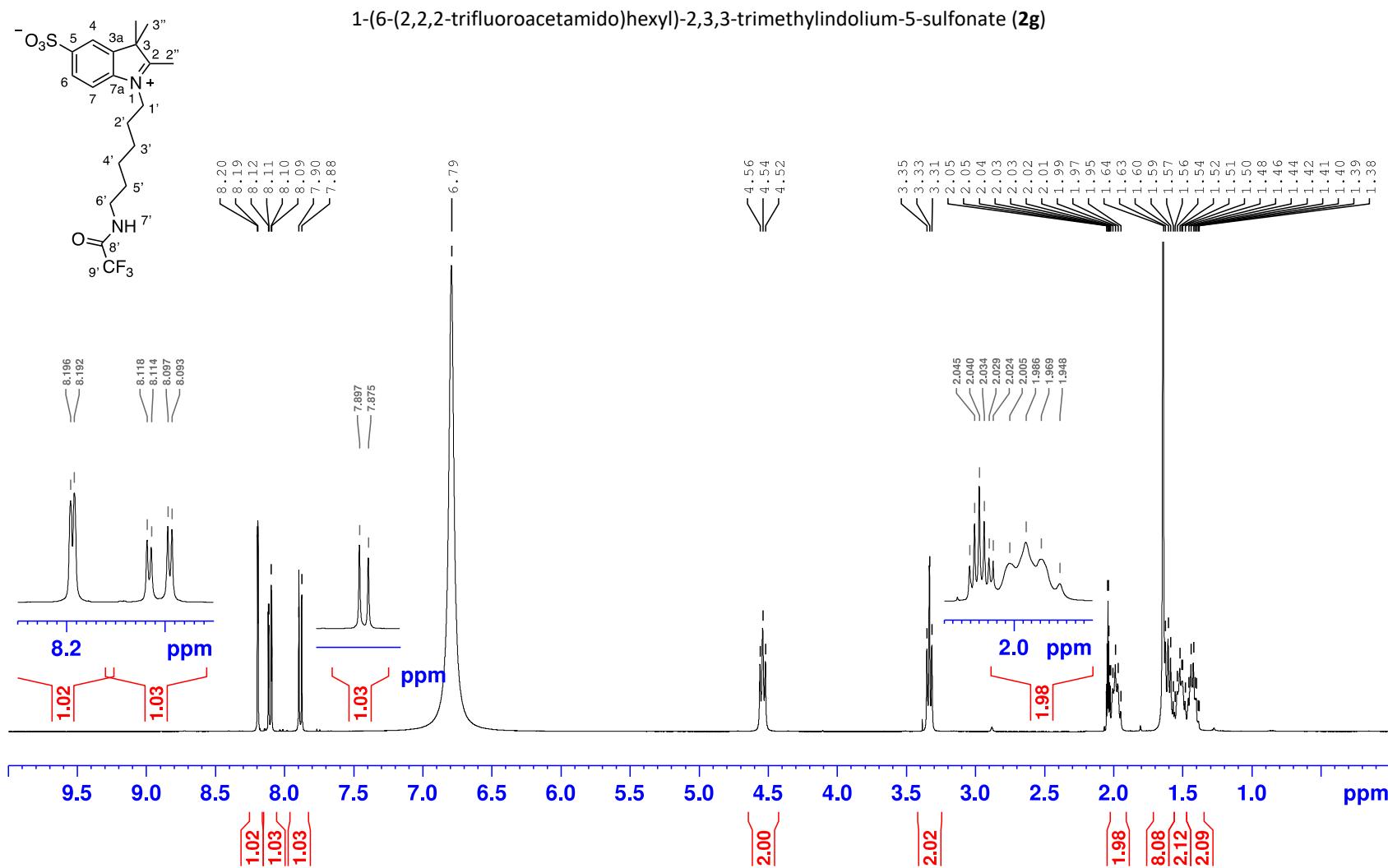
<sup>1</sup>H NMR spectrum (600 MHz, AcOD/D<sub>2</sub>O = 3:1)



**1-(3-(2,2,2-trifluoroacetamido)propyl)-2,3,3-trimethylindolium-5-sulfonate (**2f**)**

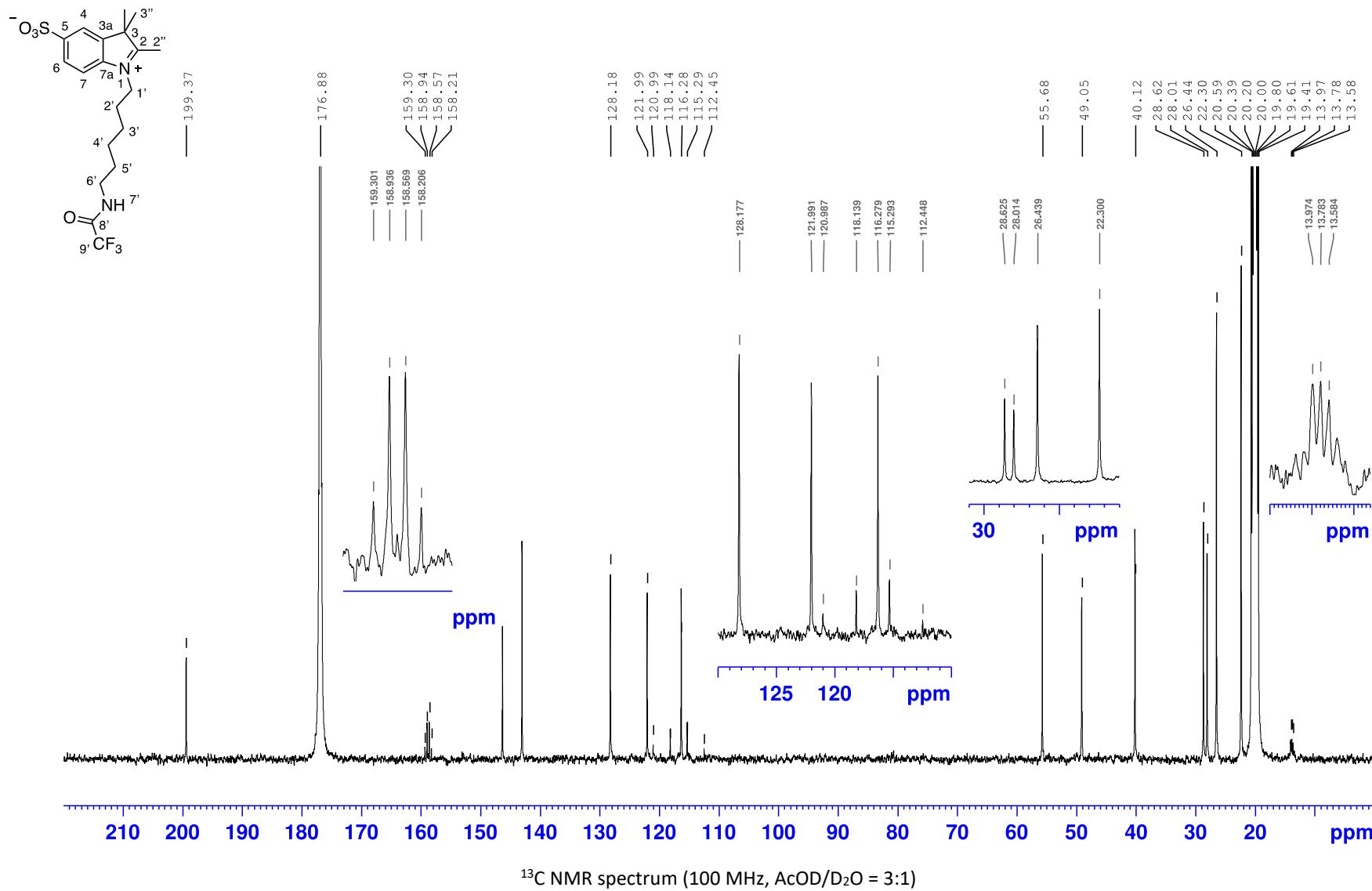


$^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum (600 MHz,  $\text{AcOD}/\text{D}_2\text{O} = 3:1$ )



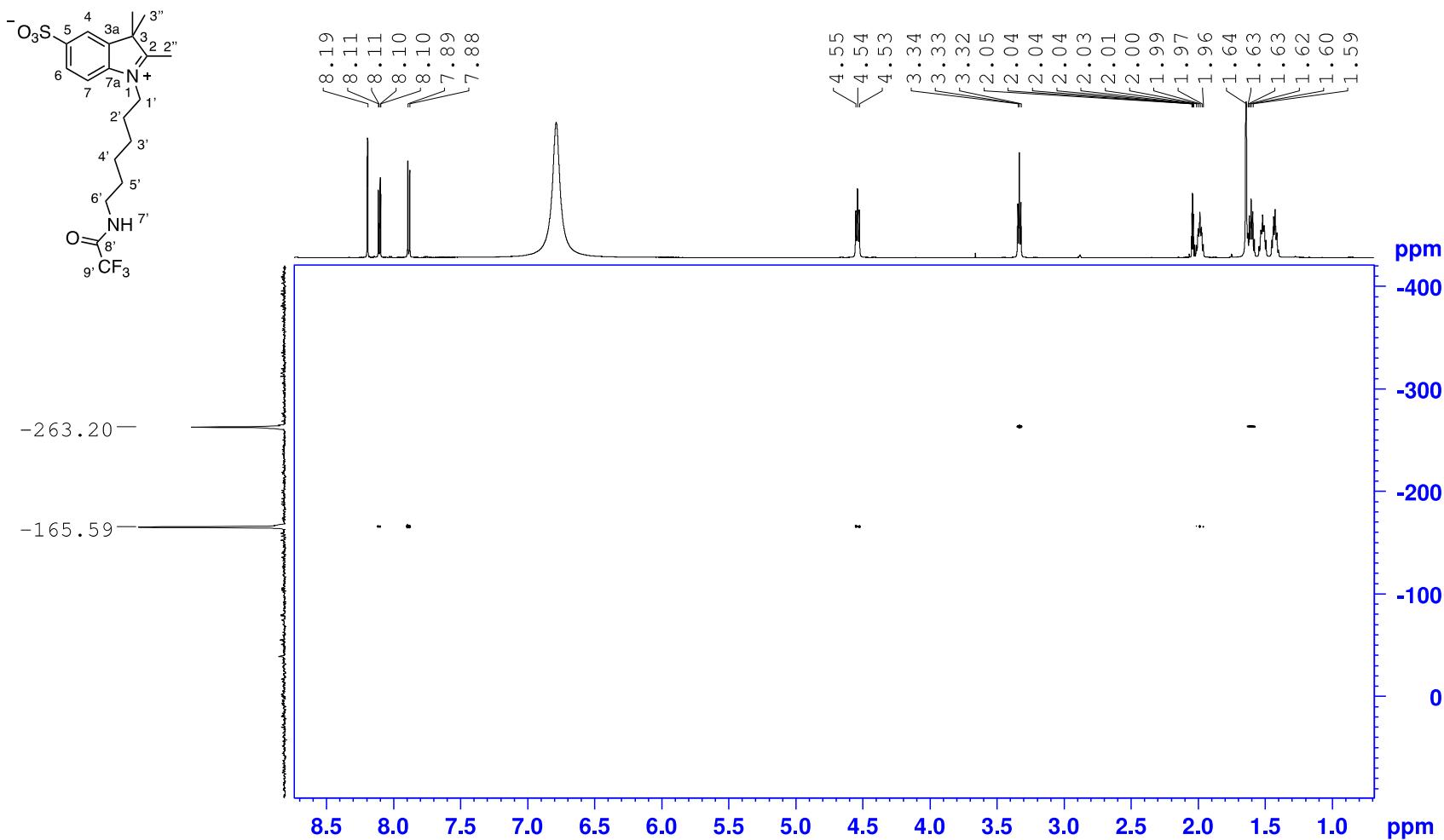
<sup>1</sup>H NMR spectrum (400 MHz, AcOD/D<sub>2</sub>O = 3:1)

**1-(6-(2,2,2-trifluoroacetamido)hexyl)-2,3,3-trimethylindolium-5-sulfonate (**2g**)**

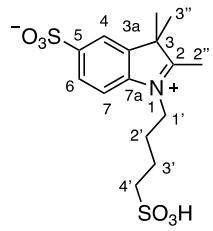


$^{13}\text{C}$  NMR spectrum (100 MHz,  $\text{AcOD}/\text{D}_2\text{O} = 3:1$ )

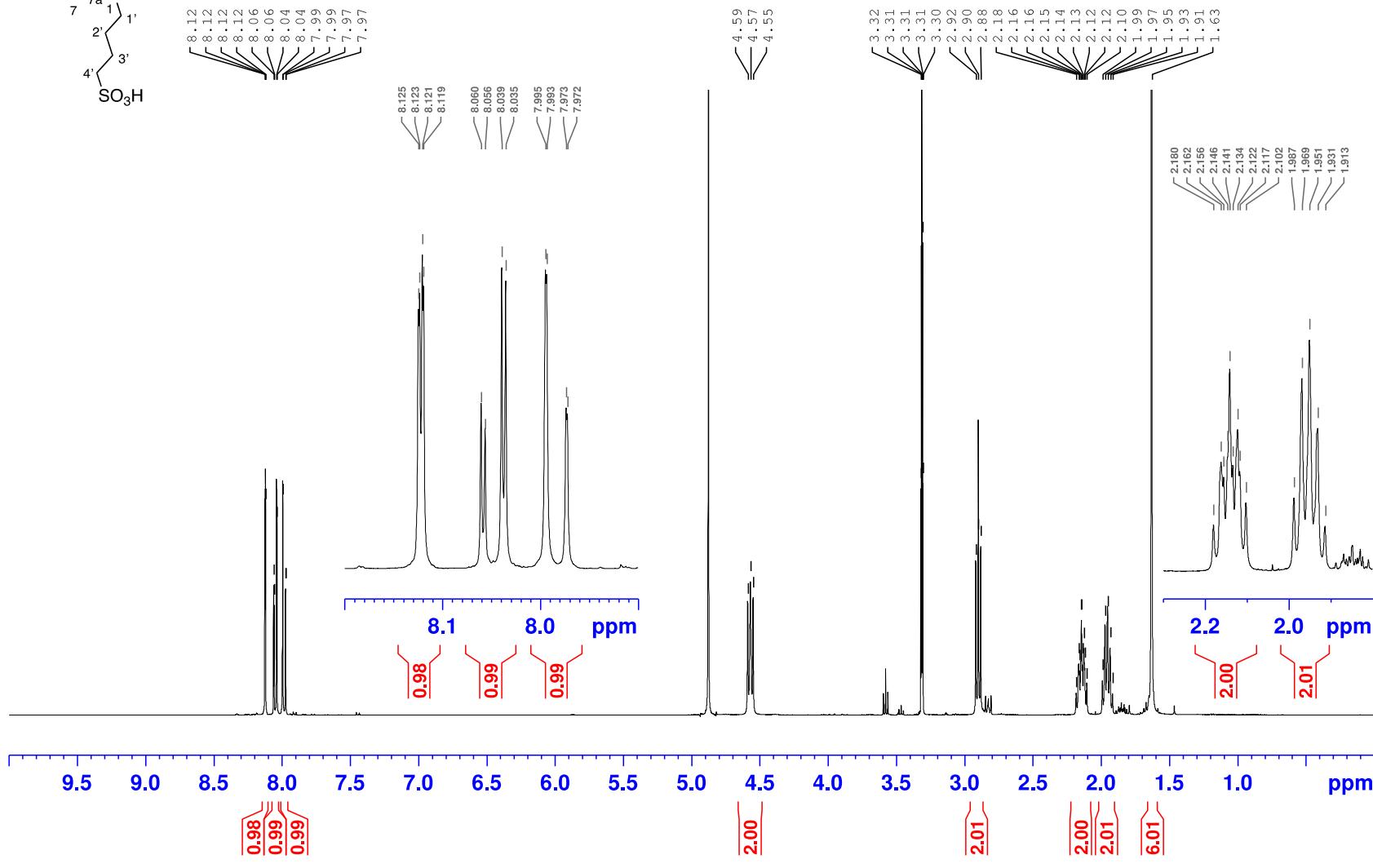
**1-(6-(2,2,2-trifluoroacetamido)hexyl)-2,3,3-trimethylindolium-5-sulfonate (**2g**)**



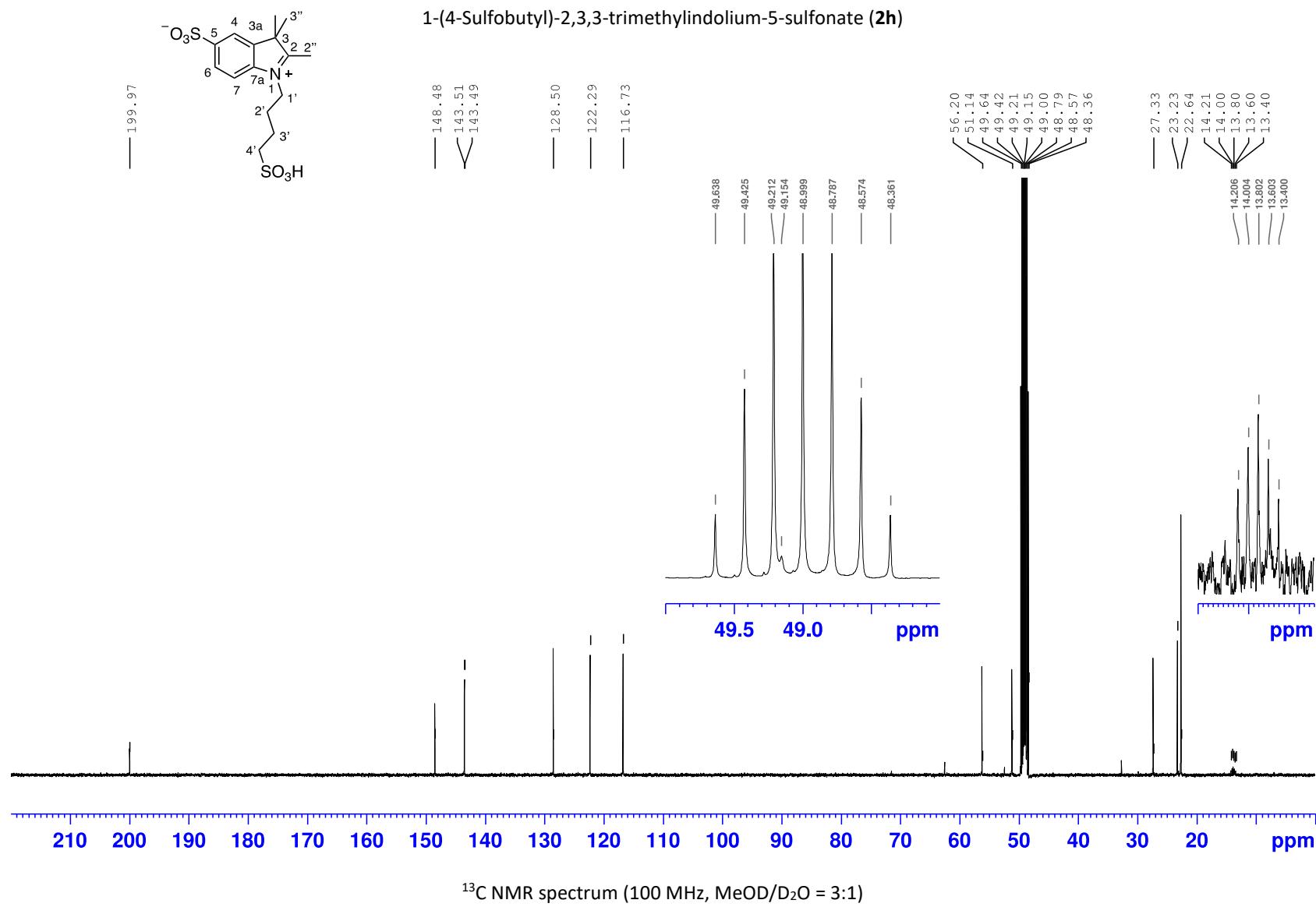
$^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum (600 MHz, AcOD/D<sub>2</sub>O = 3:1)

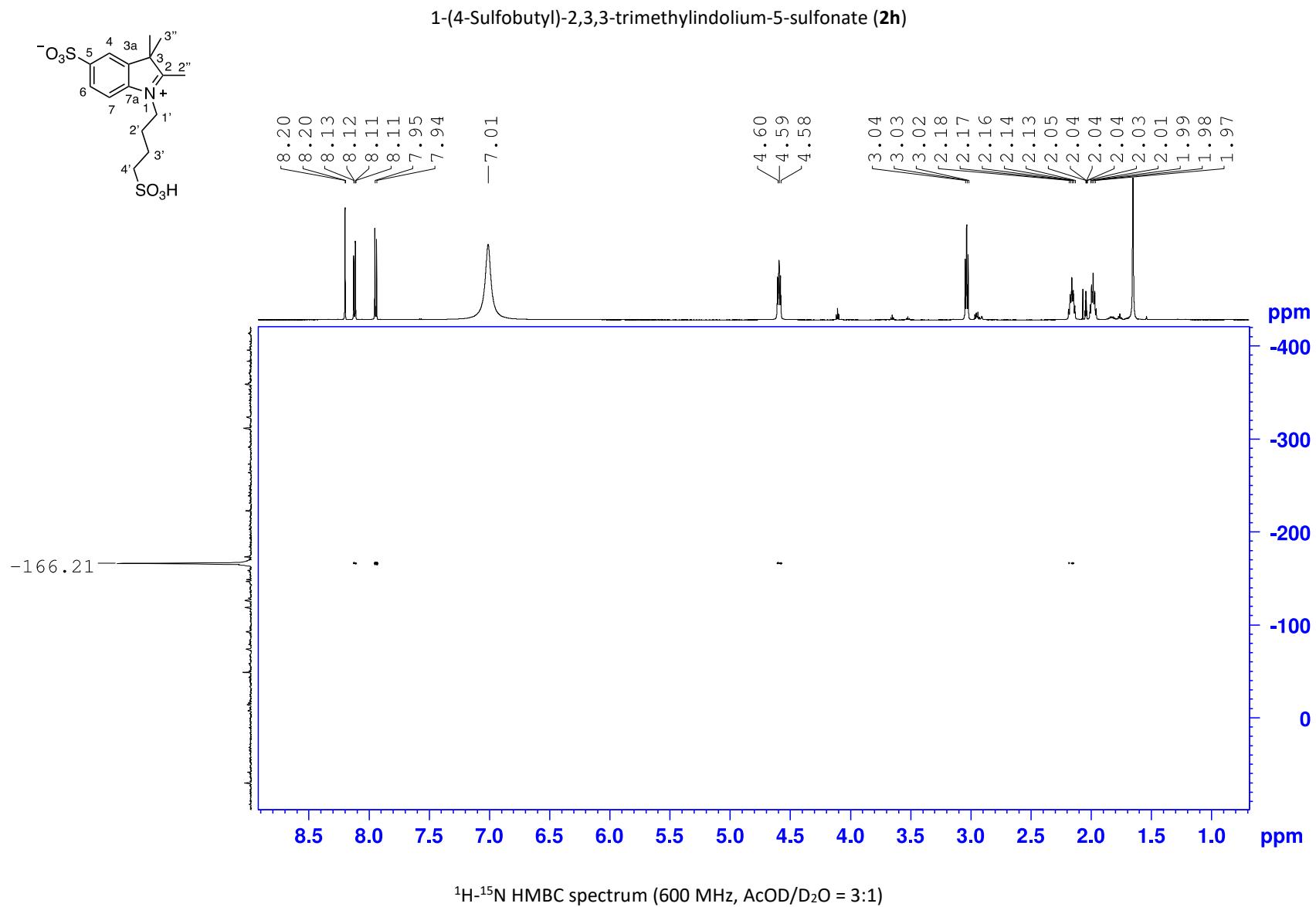


**1-(4-Sulfobutyl)-2,3,3-trimethylindolium-5-sulfonate (**2h**)**

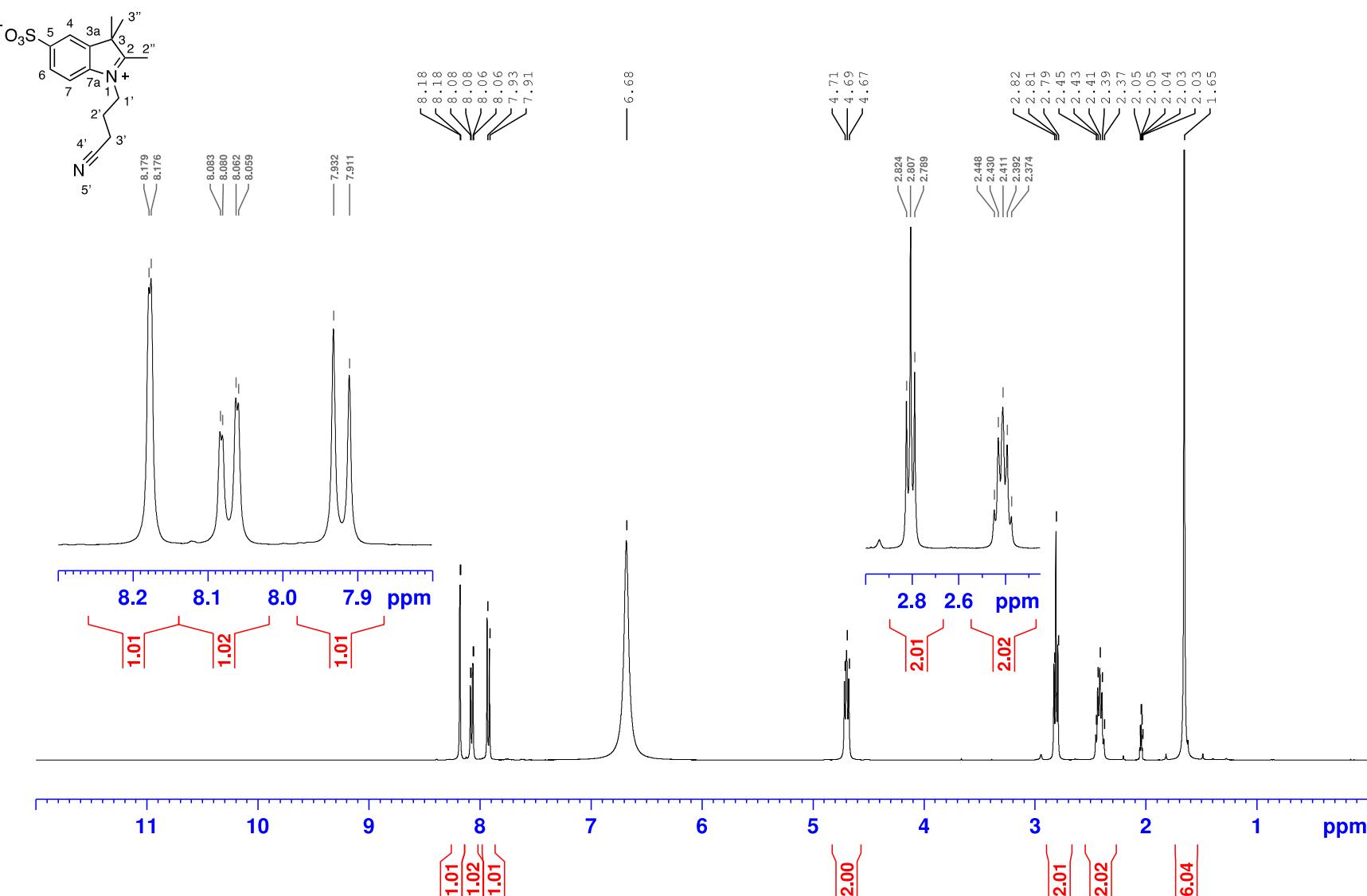


<sup>1</sup>H NMR spectrum (400 MHz, MeOD/D<sub>2</sub>O = 3:1)



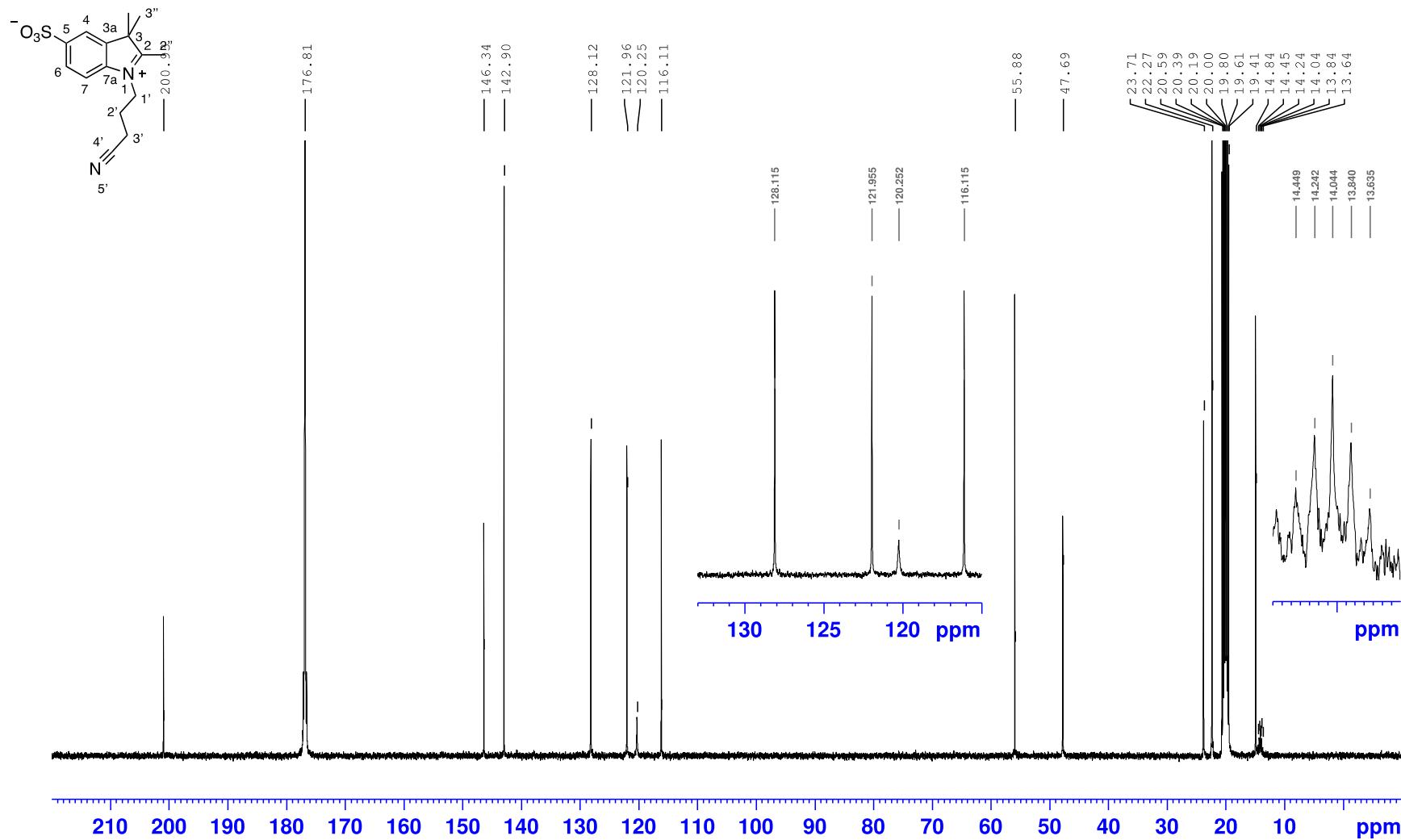


**1-(3-cyanopropyl)-2,3,3-trimethylindolium-5-sulfonate (**2i**)**



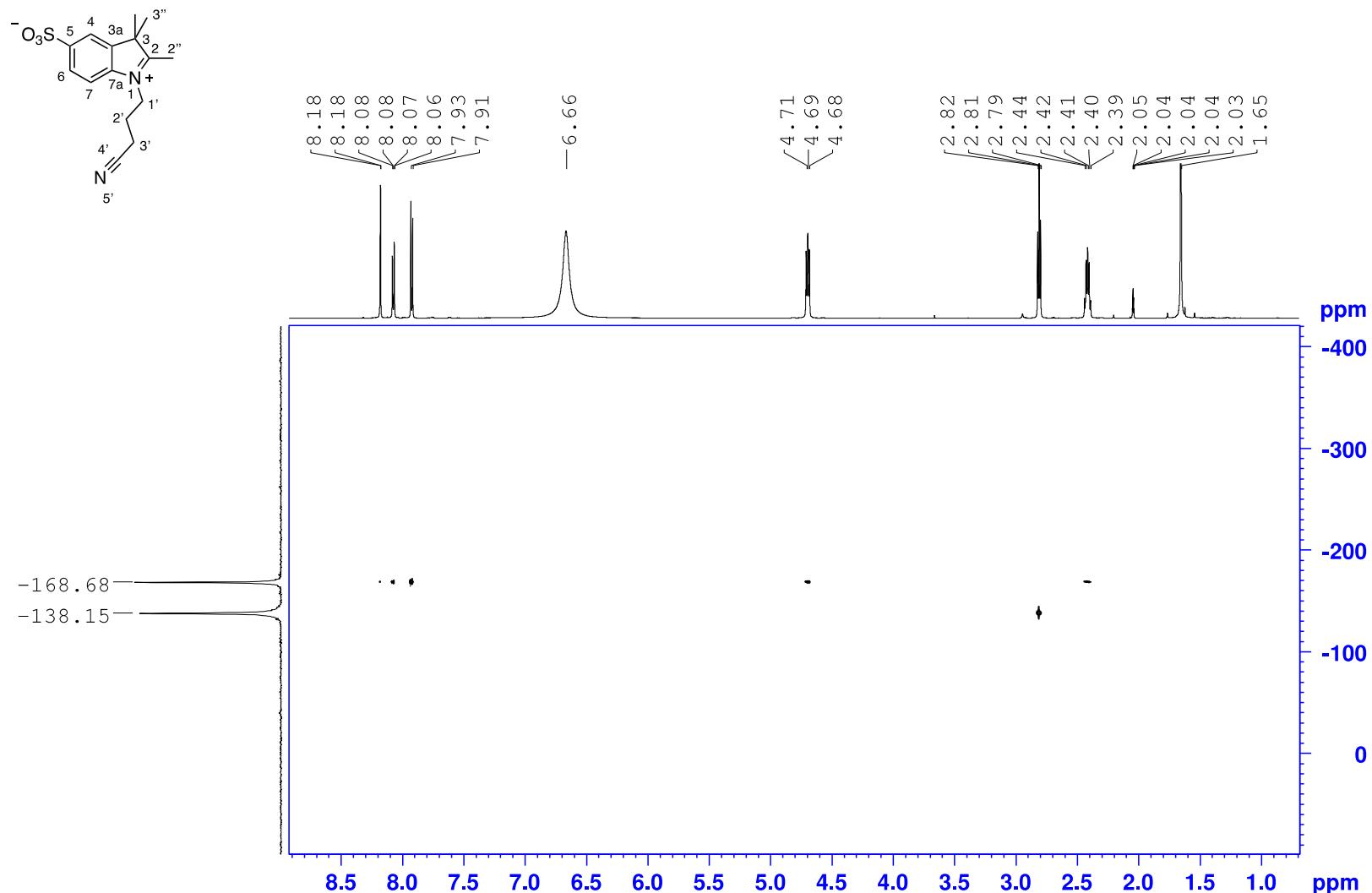
<sup>1</sup>H NMR spectrum (400 MHz, AcOD/ D<sub>2</sub>O = 3:1)

**1-(3-Cyanopropyl)-2,3,3-trimethylindolium-5-sulfonate (**2i**)**

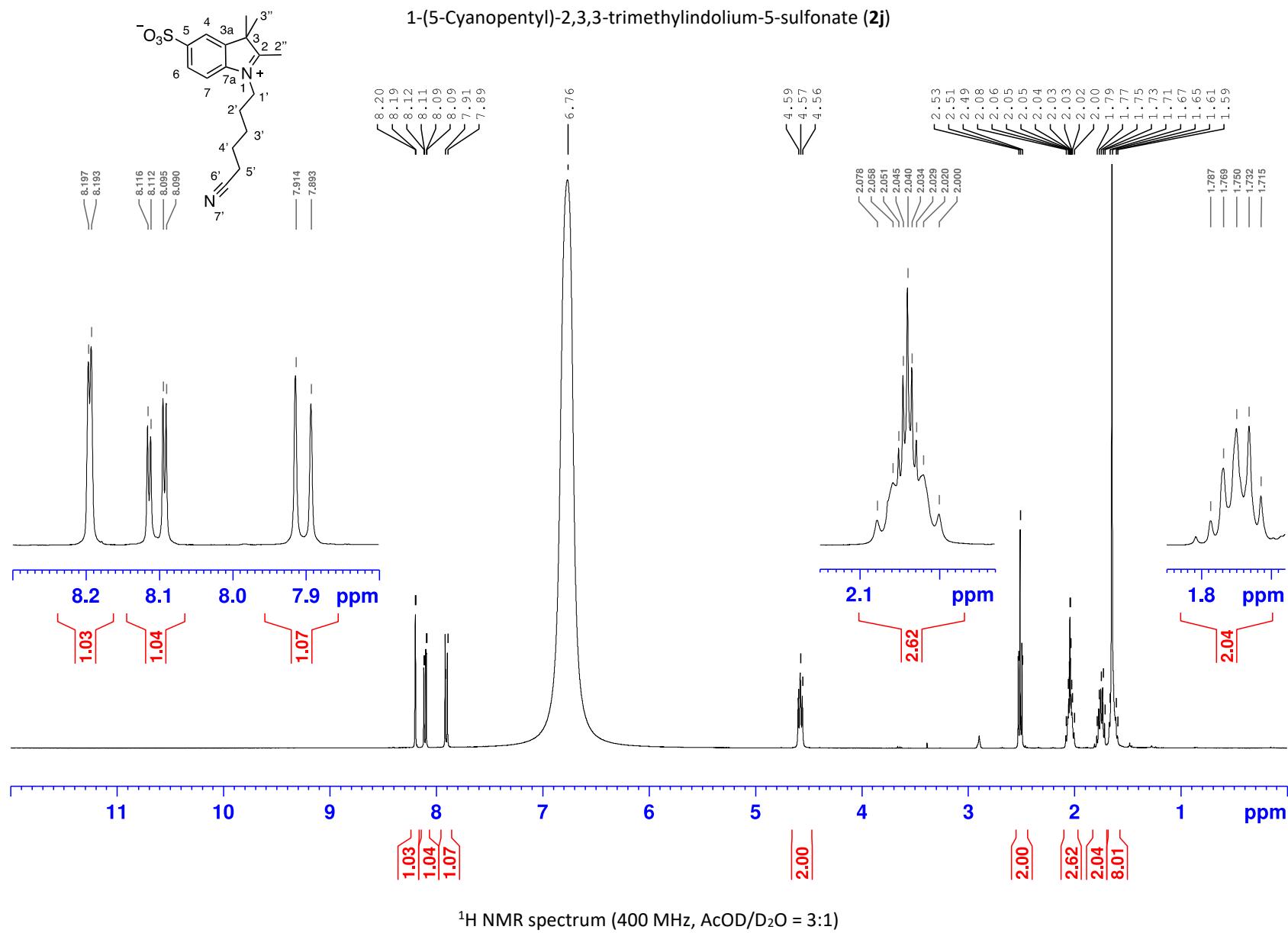


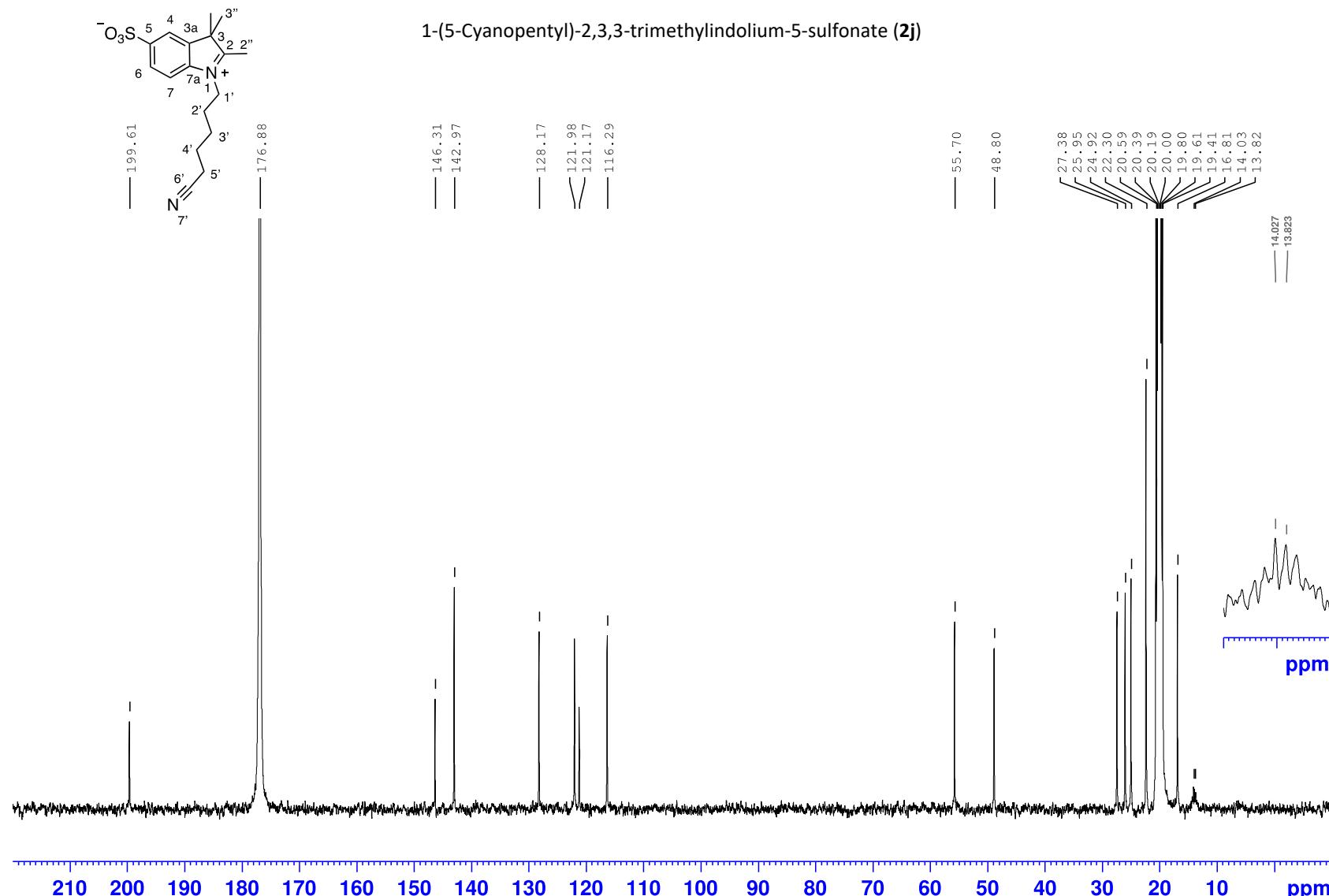
$^{13}\text{C}$  NMR spectrum (100 MHz, AcOD/D<sub>2</sub>O = 3:1)

**1-(3-Cyanopropyl)-2,3,3-trimethylindolium-5-sulfonate (**2i**)**

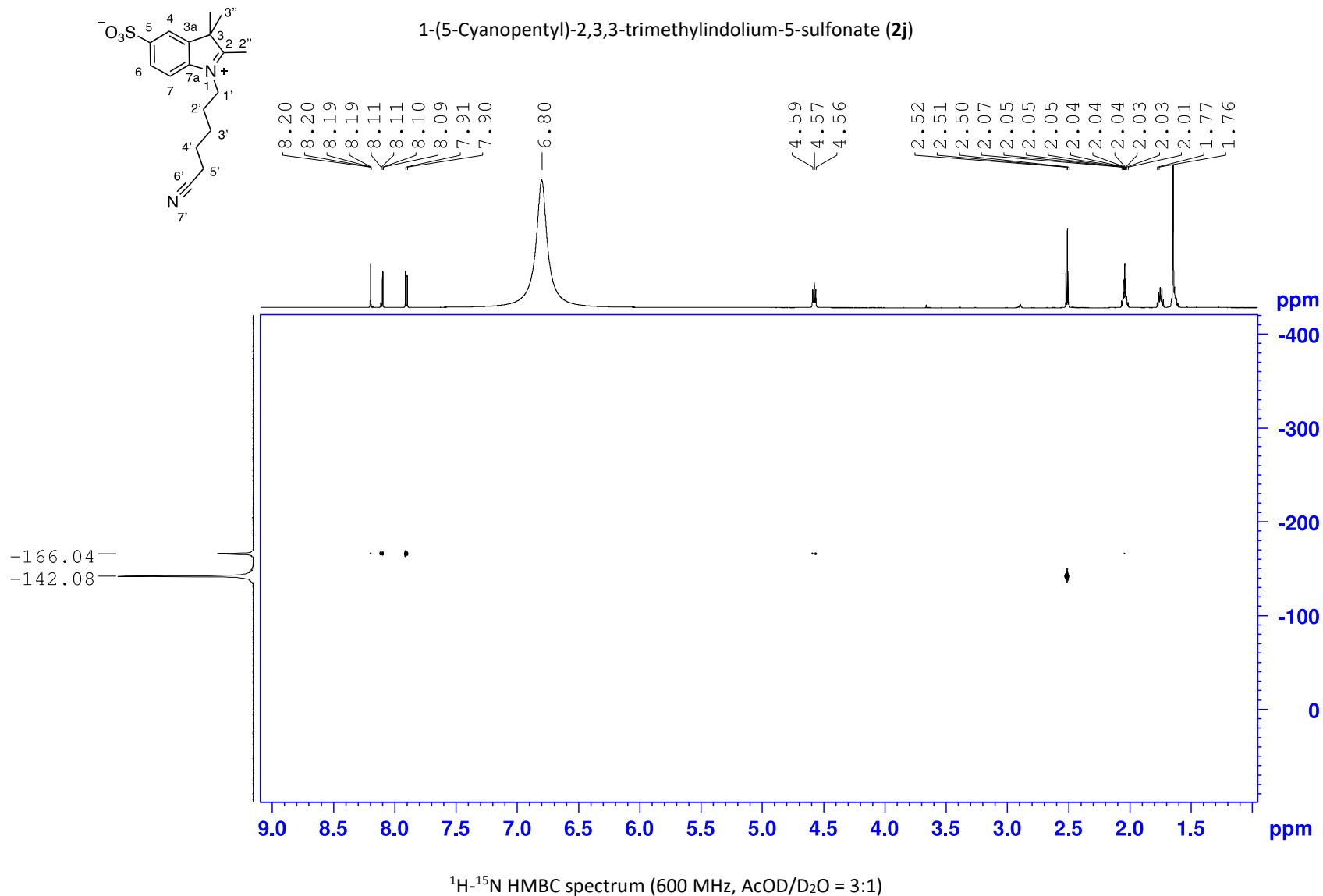


<sup>1</sup>H-<sup>15</sup>N HMBC spectrum (600 MHz, AcOD/D<sub>2</sub>O = 3:1)

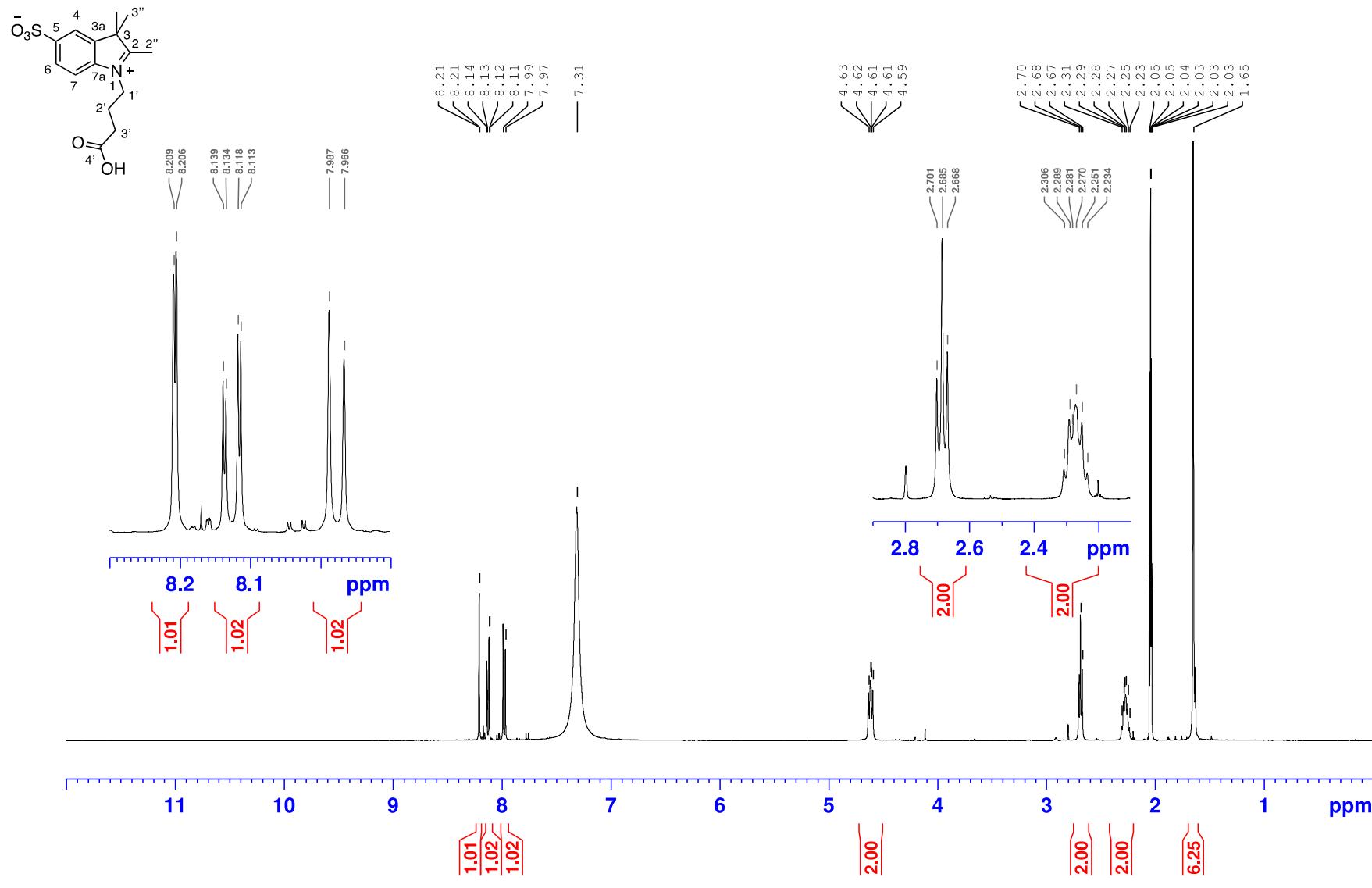




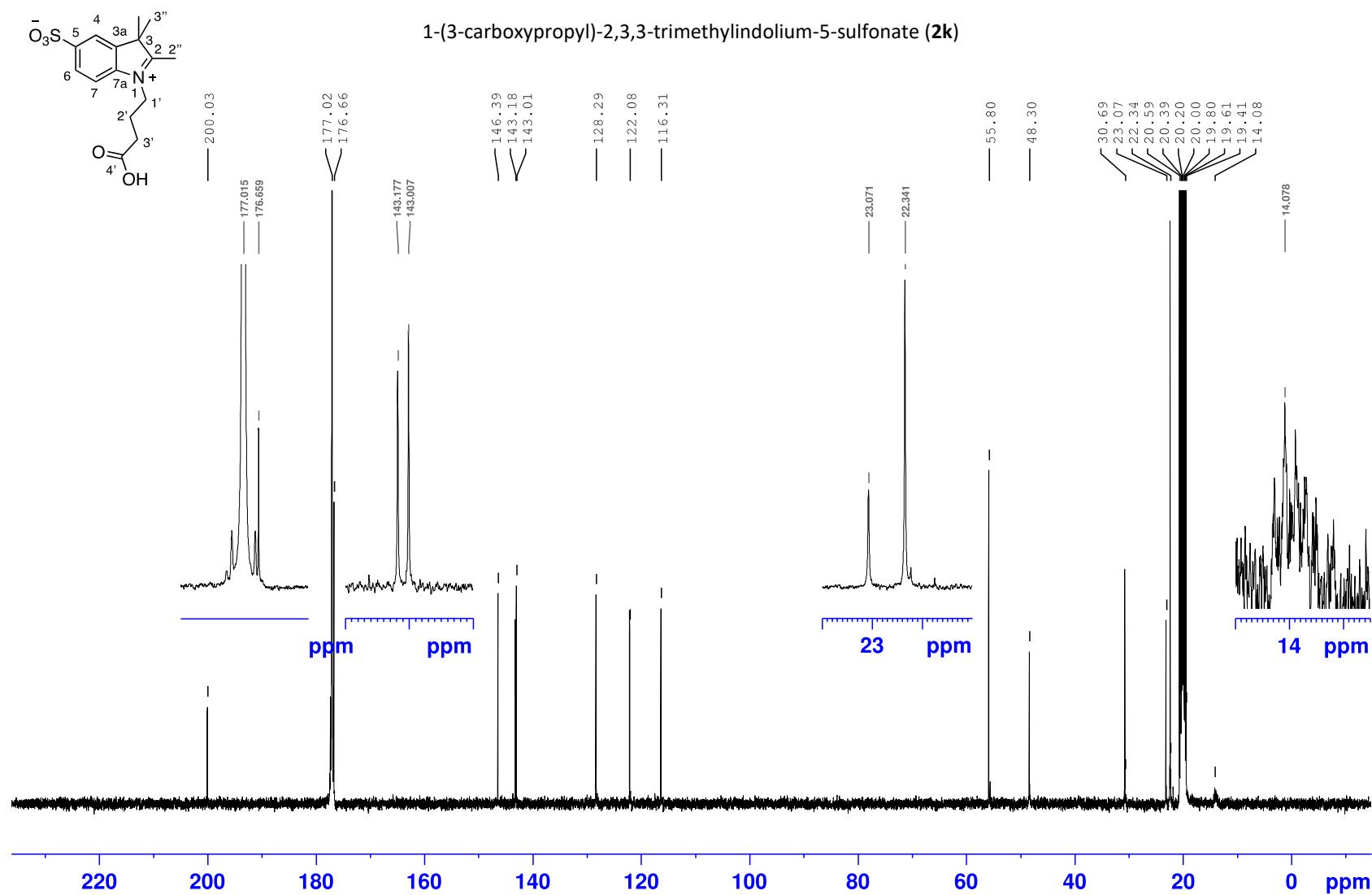
<sup>13</sup>C NMR spectrum (100 MHz, AcOD/D<sub>2</sub>O = 3:1)



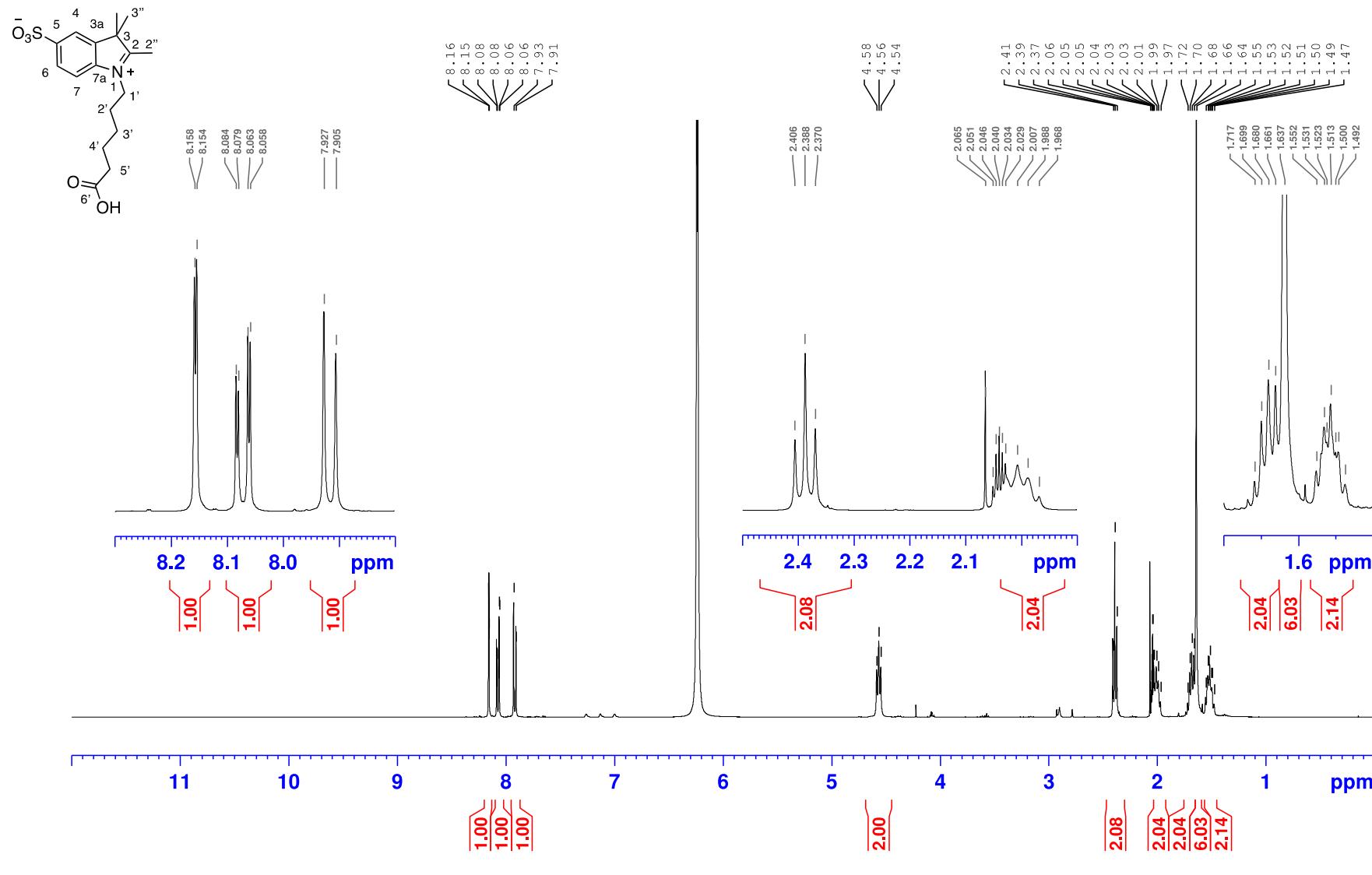
**1-(3-carboxypropyl)-2,3,3-trimethylindolium-5-sulfonate (**2k**)**



$^1\text{H}$  NMR spectrum (400 MHz,  $\text{AcOD}/\text{D}_2\text{O} = 3:1$ )

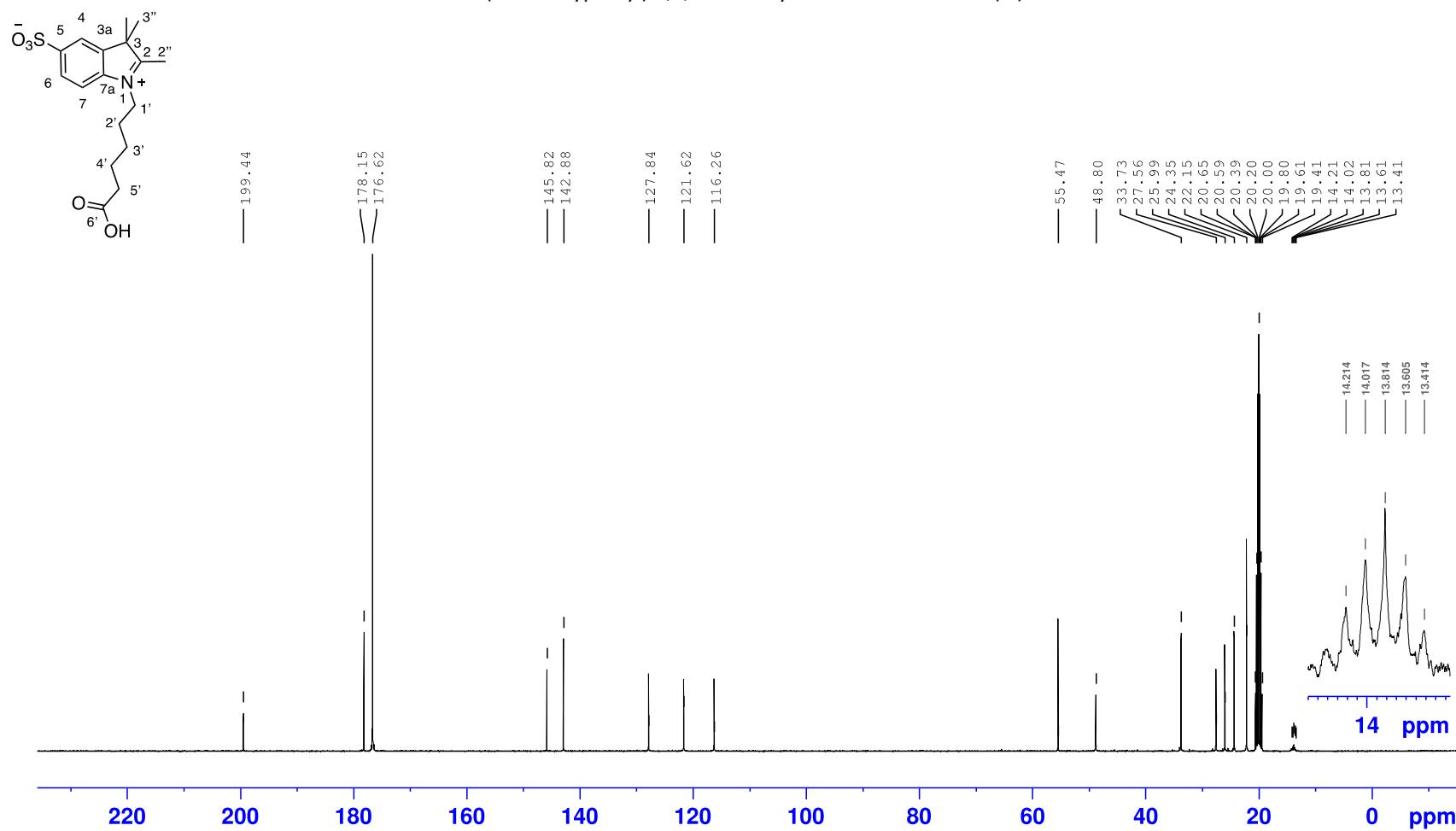


**1-(5-carboxypentyl)-2,3,3-trimethylindolium-5-sulfonate (**2l**)**

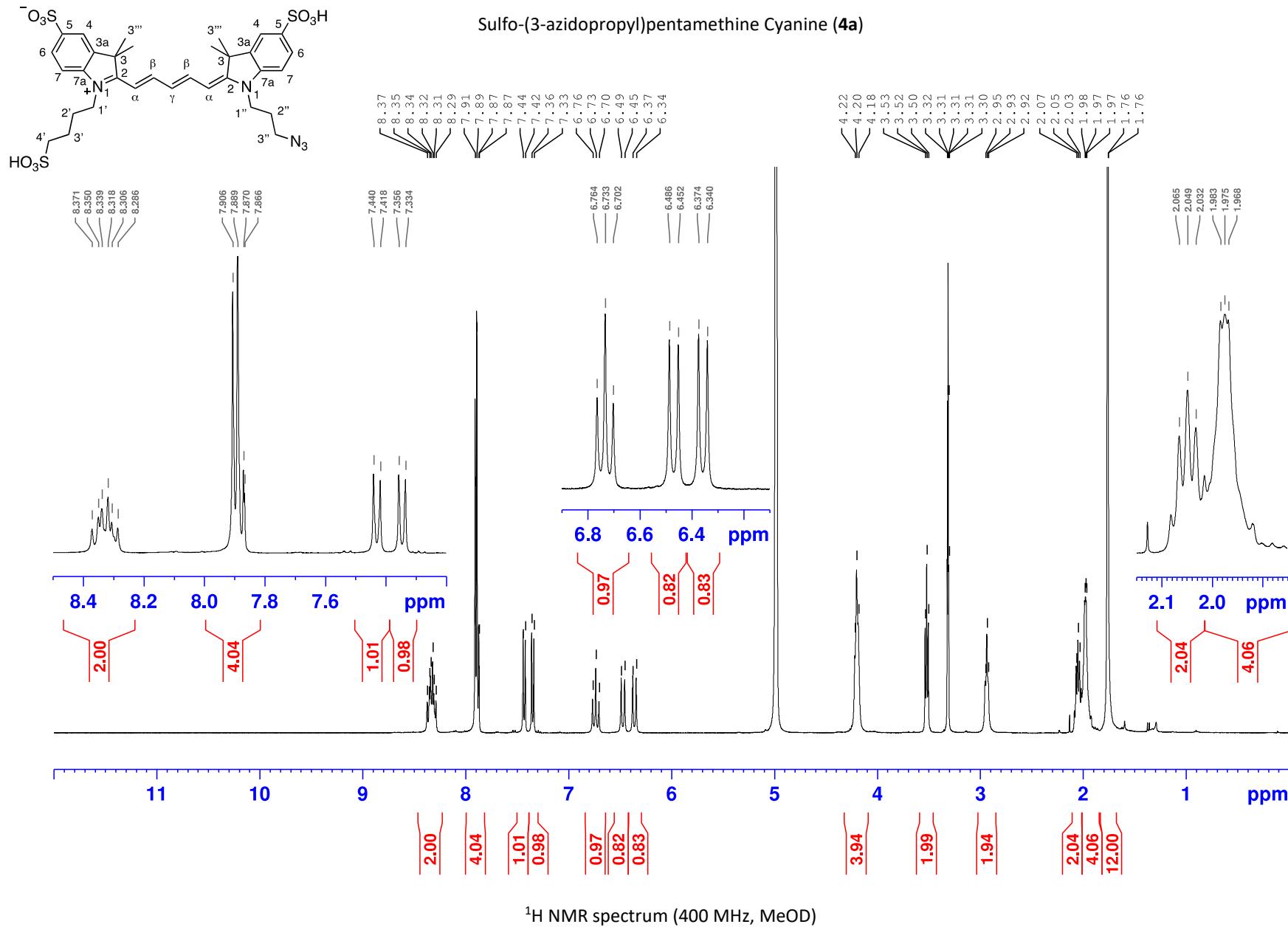


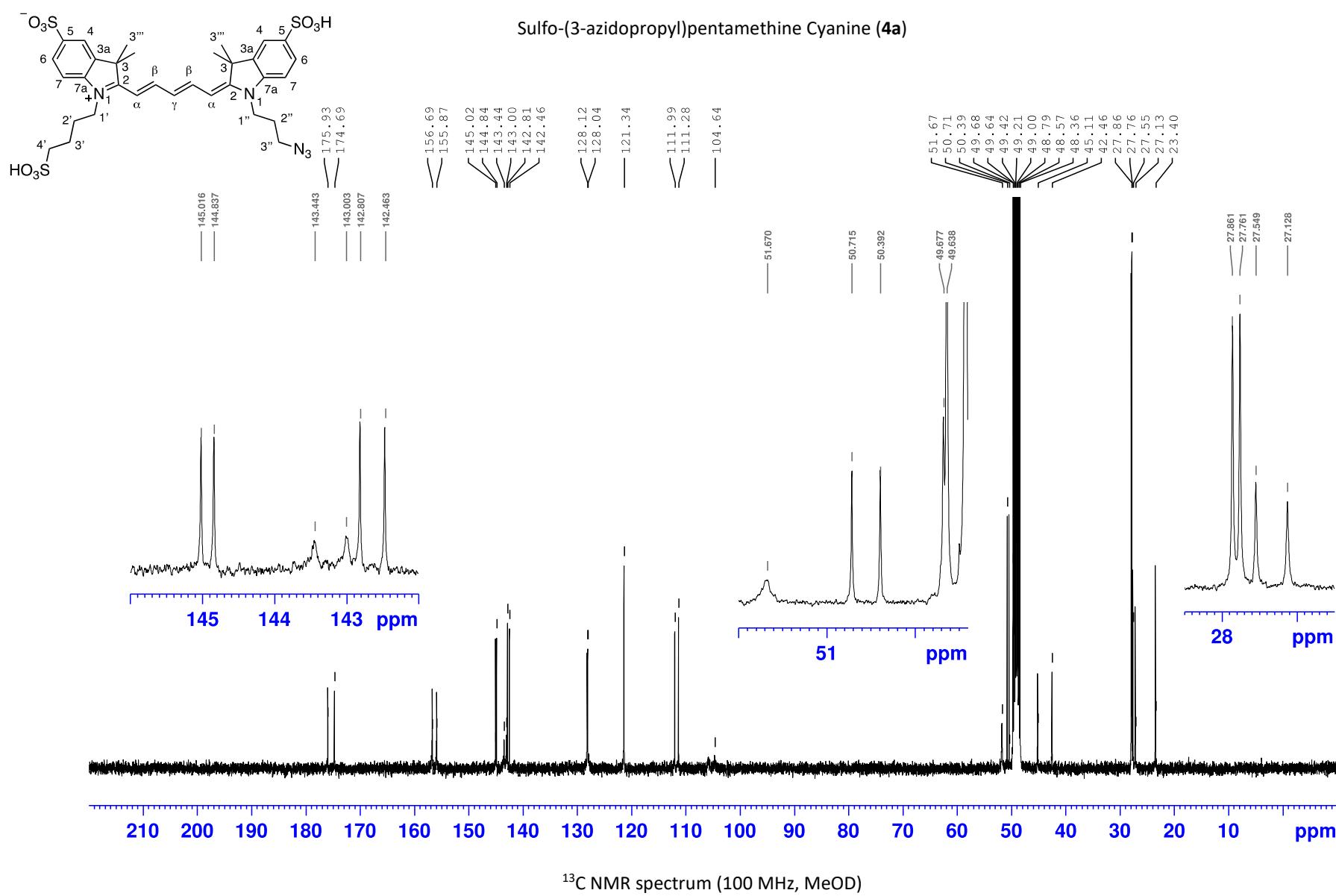
$^1\text{H}$  NMR spectrum (400 MHz,  $\text{AcOD}/\text{D}_2\text{O} = 3:1$ )

**1-(5-carboxypentyl)-2,3,3-trimethylindolium-5-sulfonate (**2I**)**

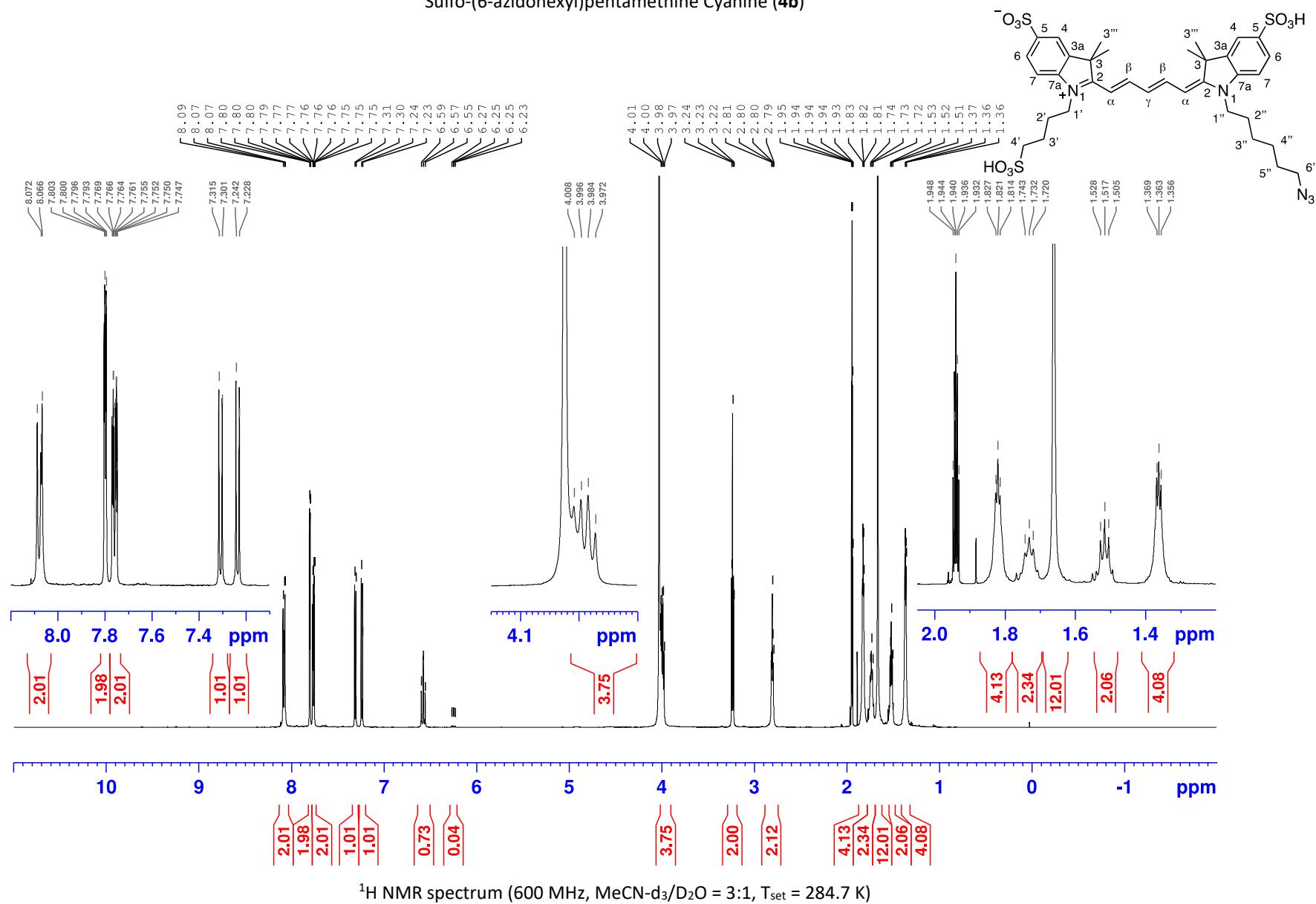


## Spectroscopic Characterization of Cyanines

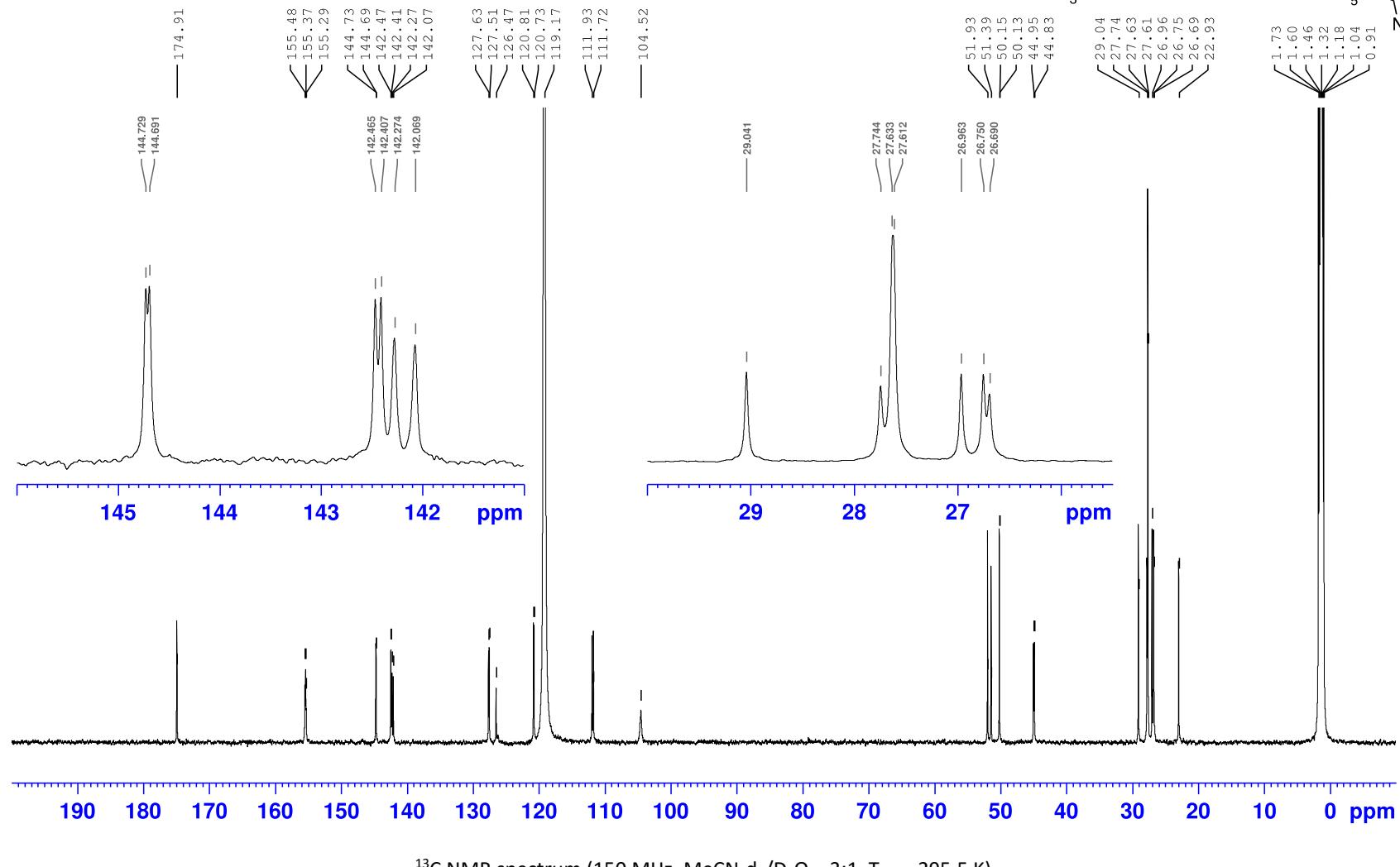


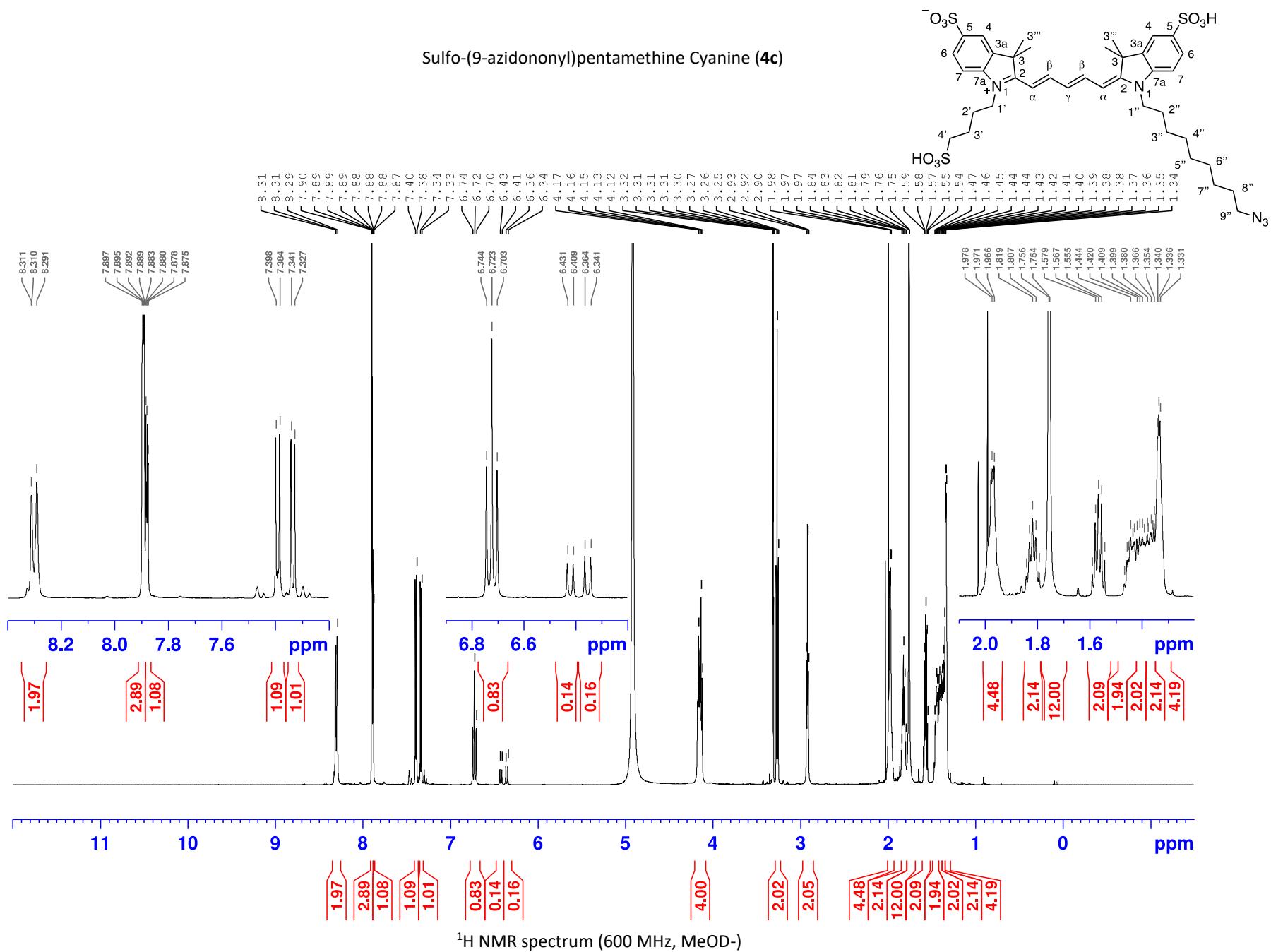


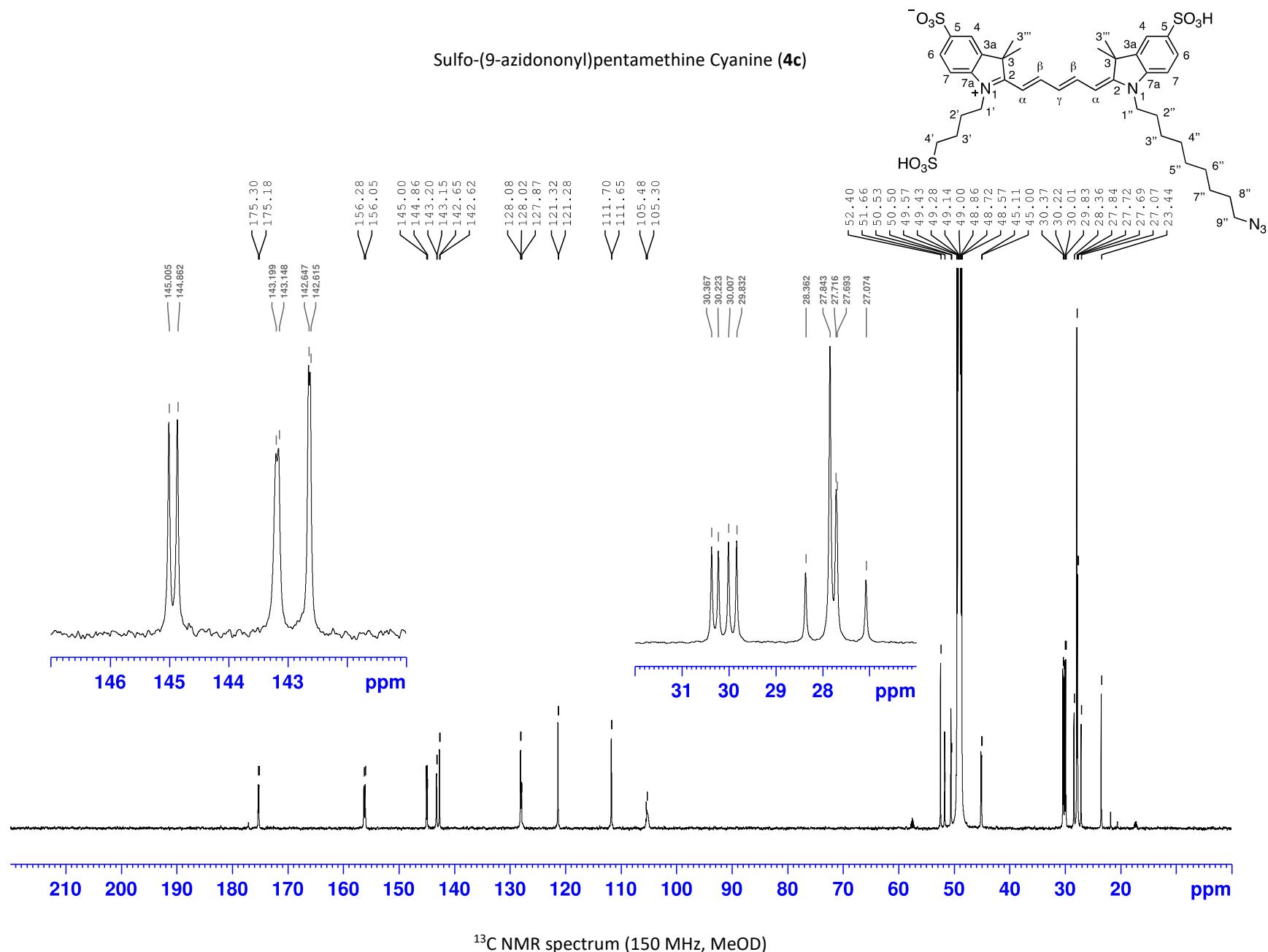
Sulfo-(6-azidohexyl)pentamethine Cyanine (**4b**)

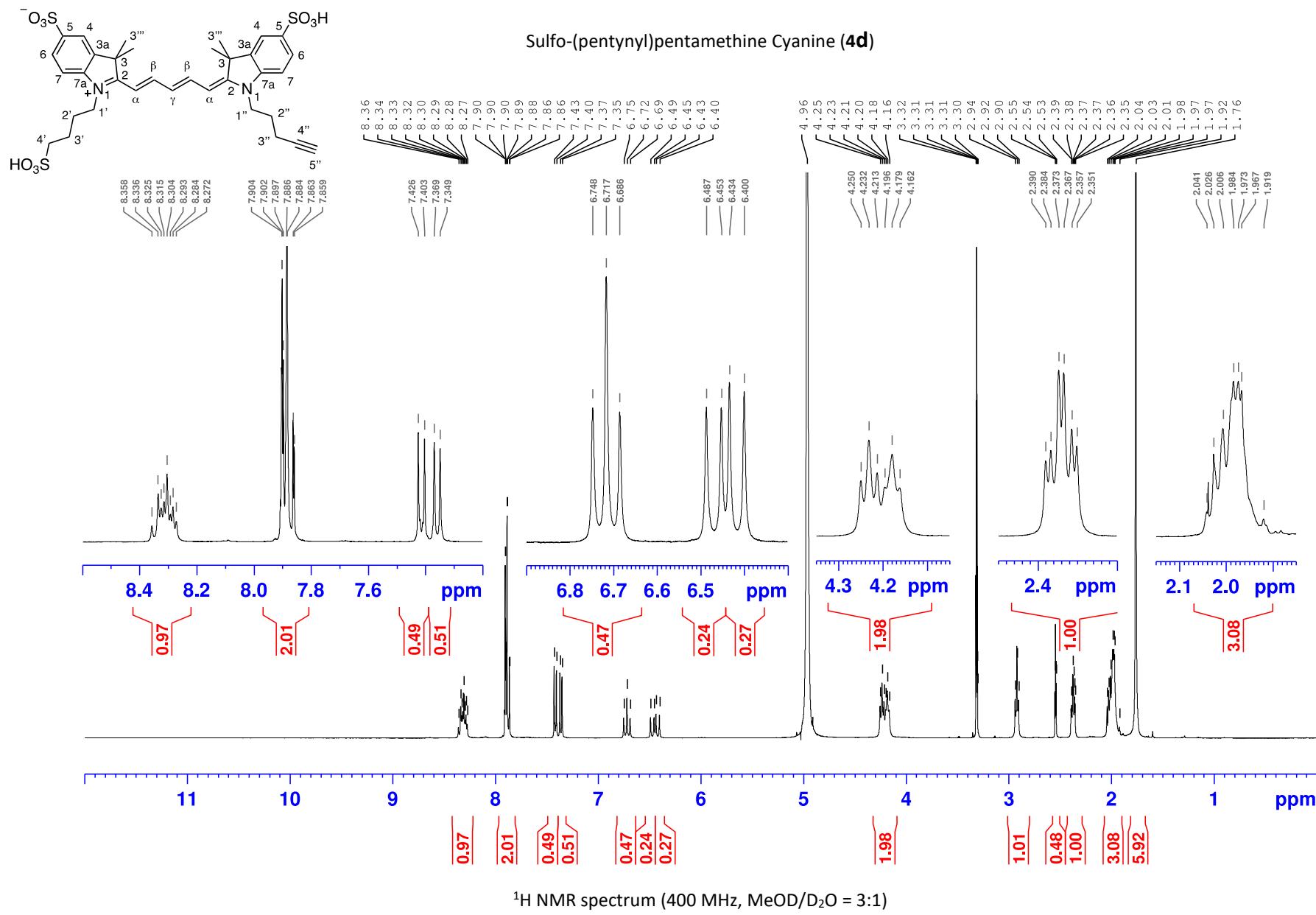


Sulfo-(6-azidohexyl)pentamethine Cyanine (**4b**)

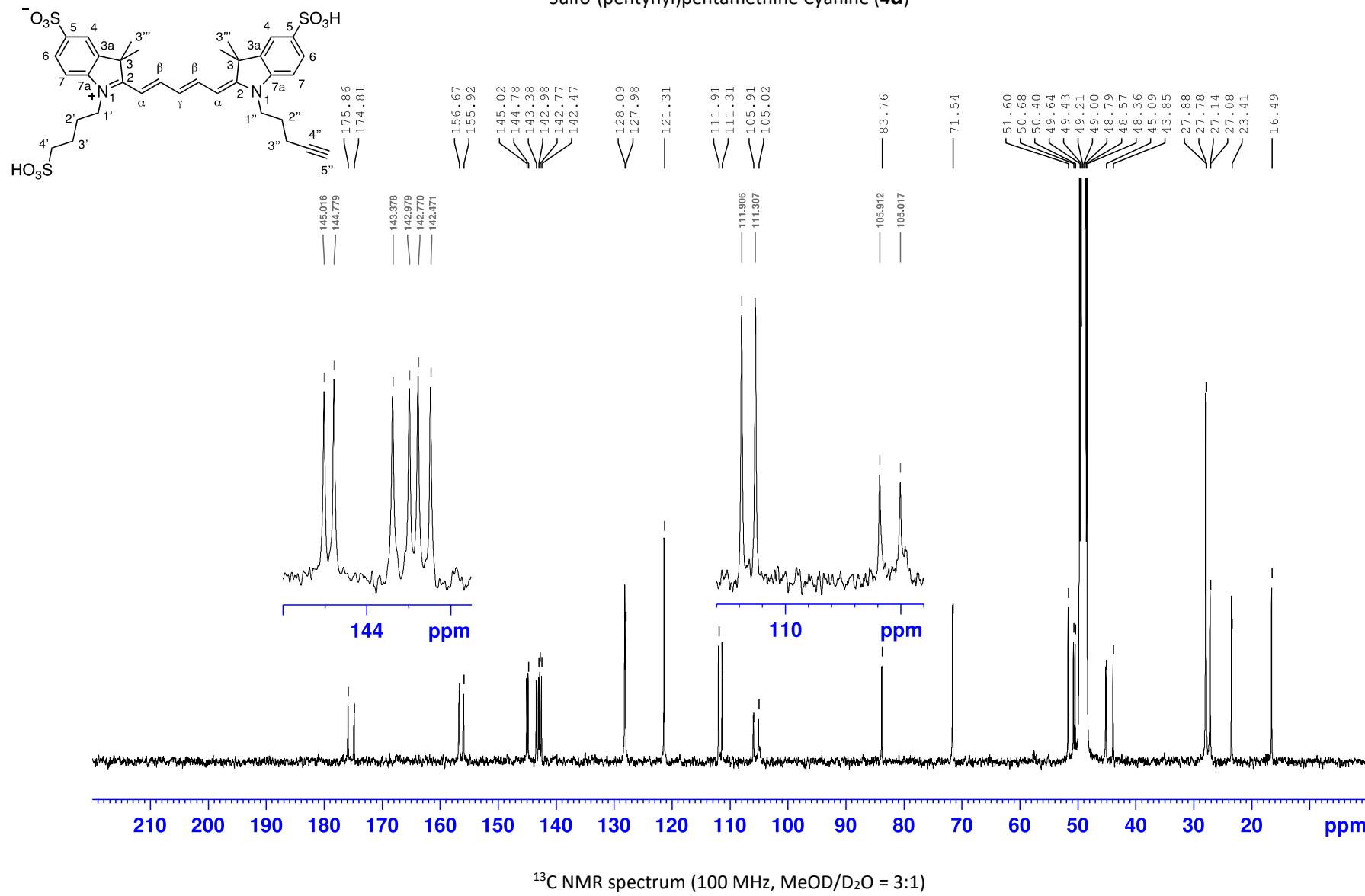


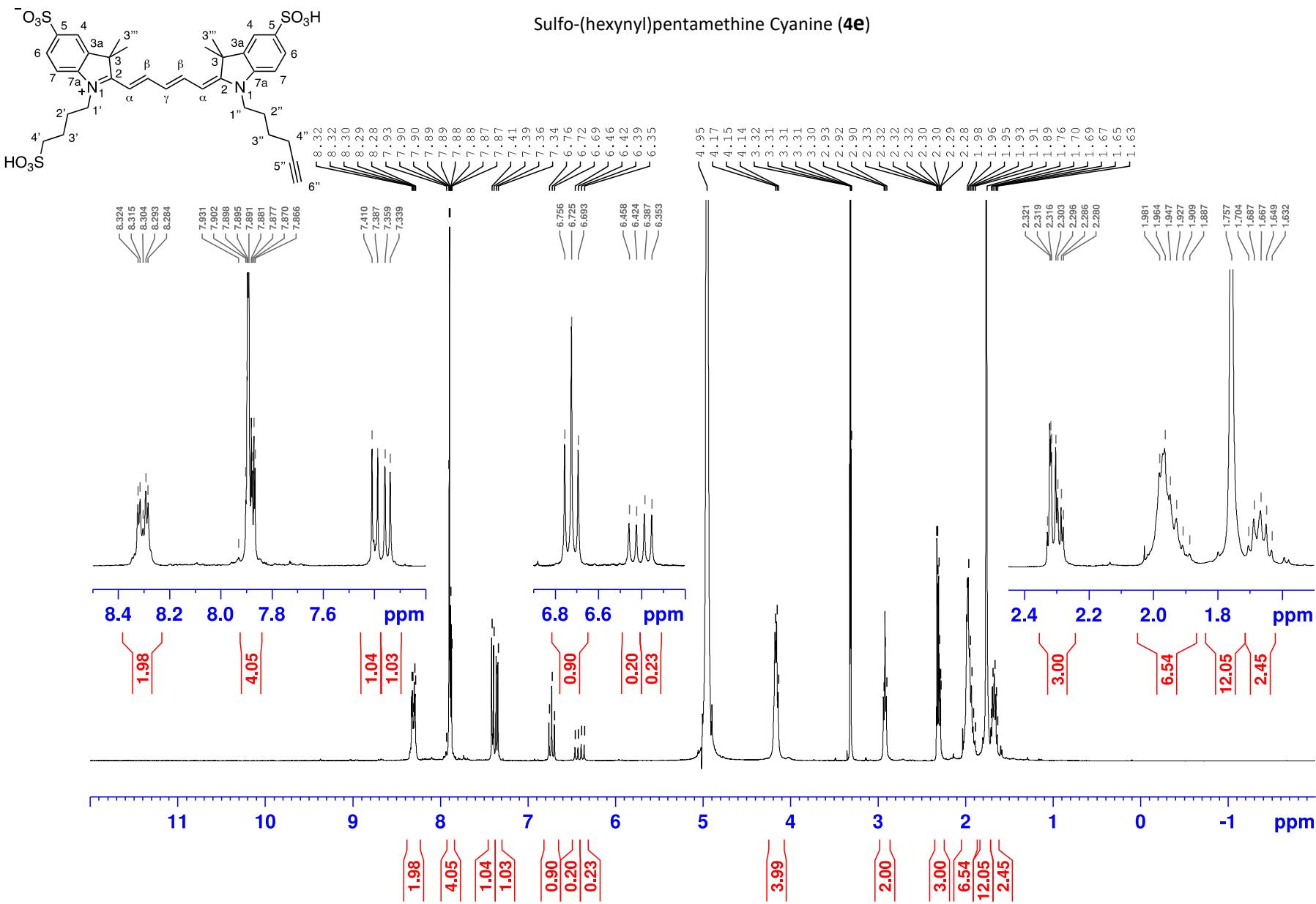






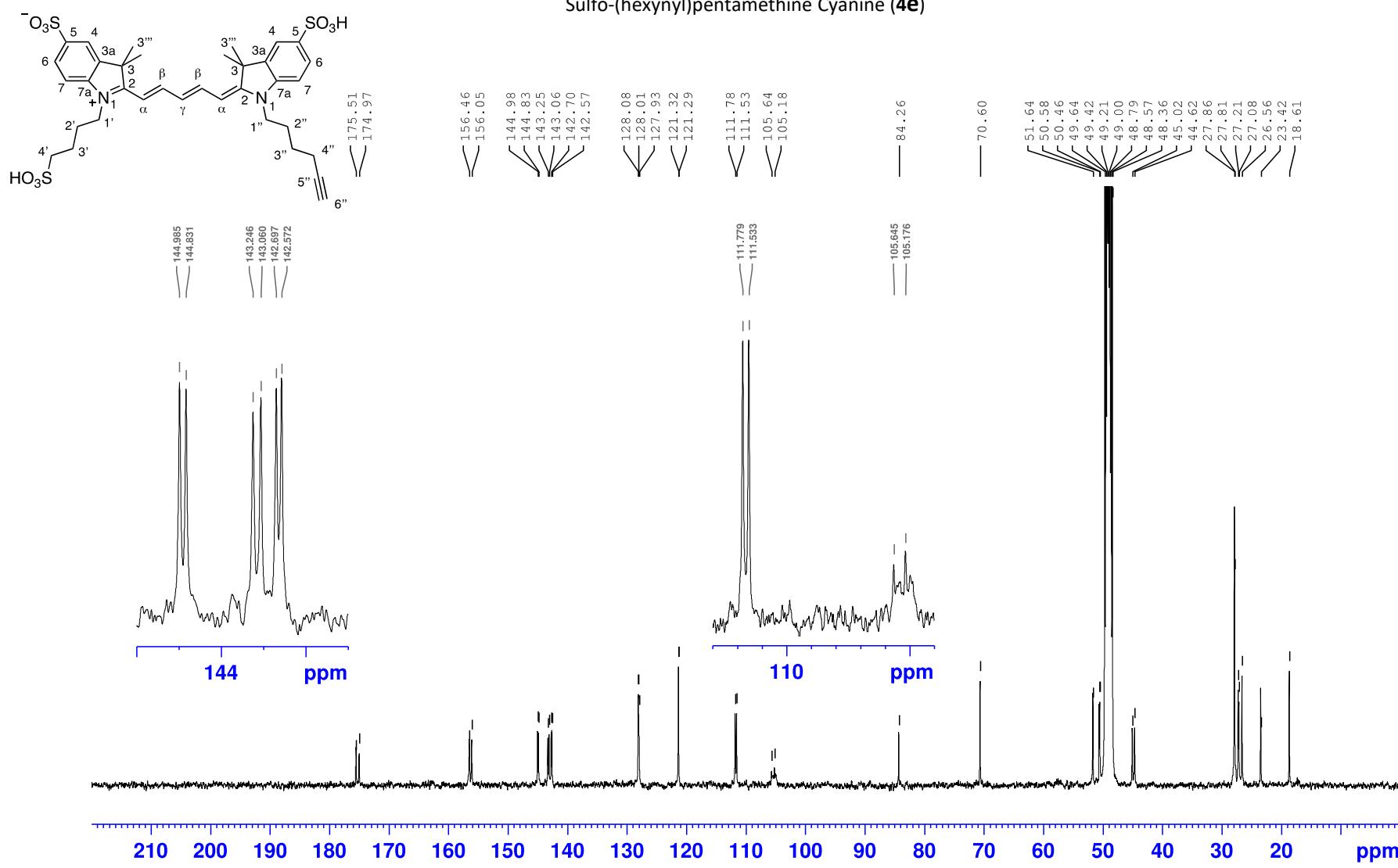
Sulfo-(pentynyl)pentamethine Cyanine (**4d**)



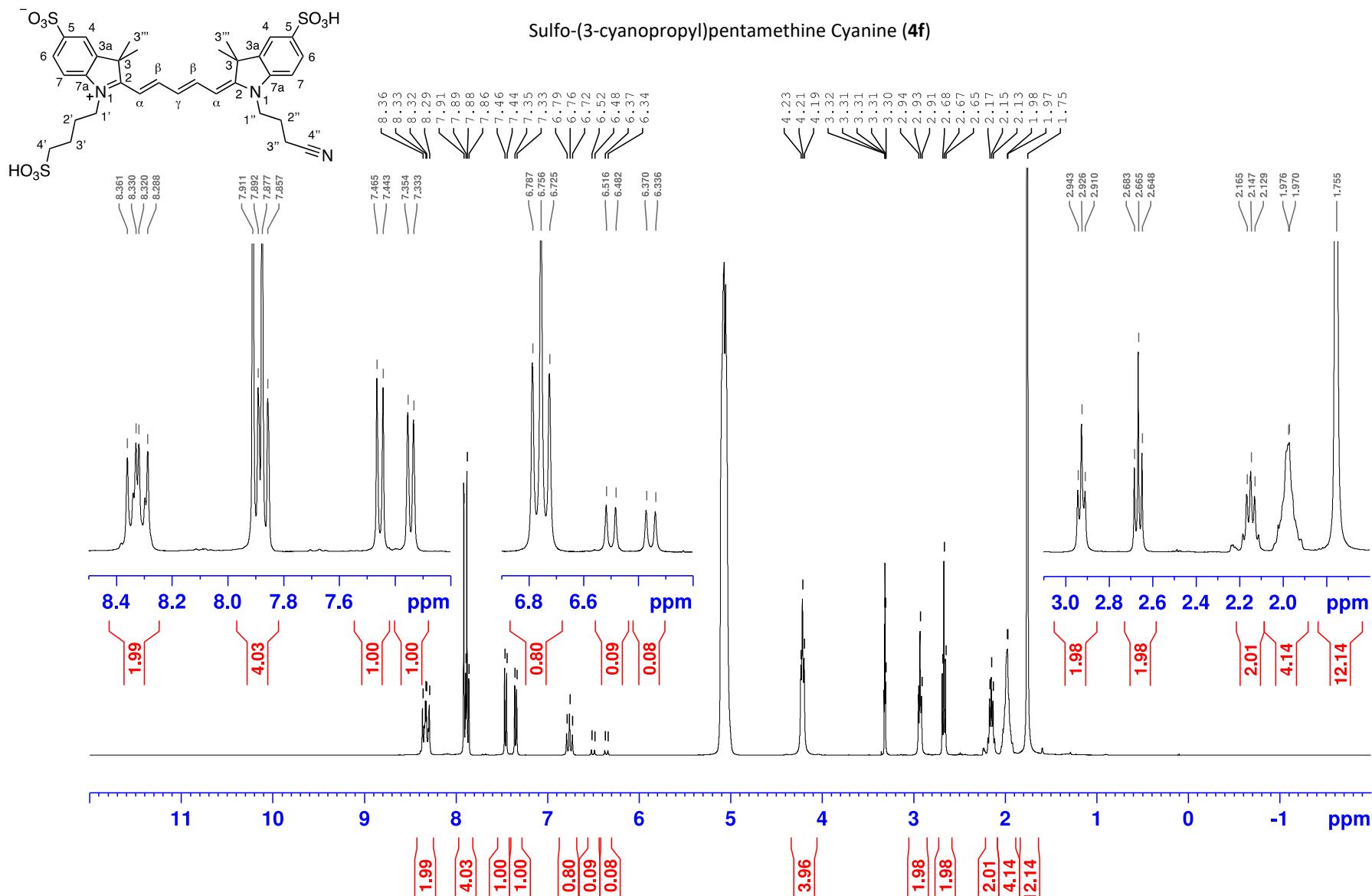


$^1\text{H}$  NMR spectrum (400 MHz, MeOD/D<sub>2</sub>O = 3:1)

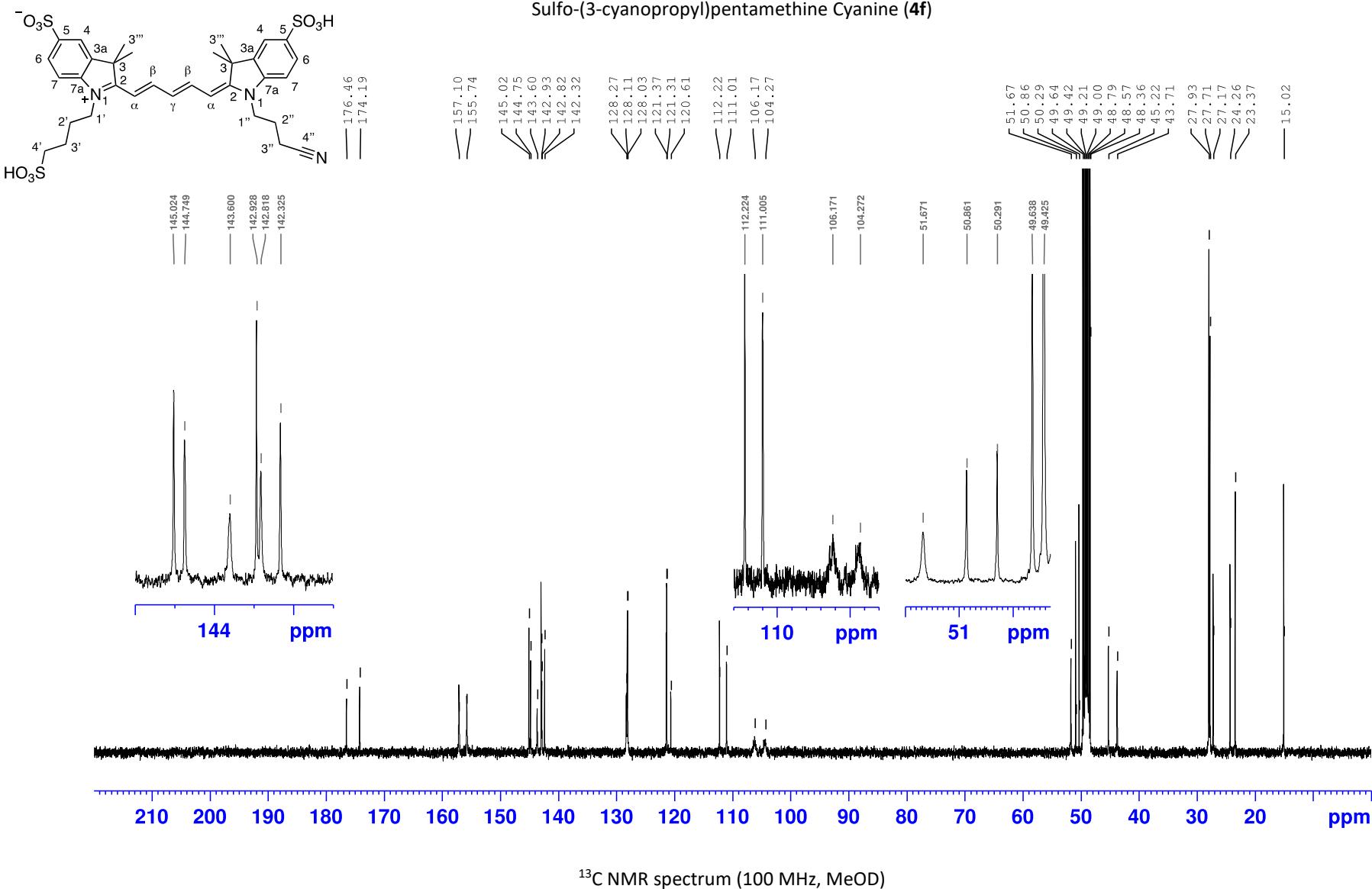
Sulfo-(hexynyl)pentamethine Cyanine (**4e**)



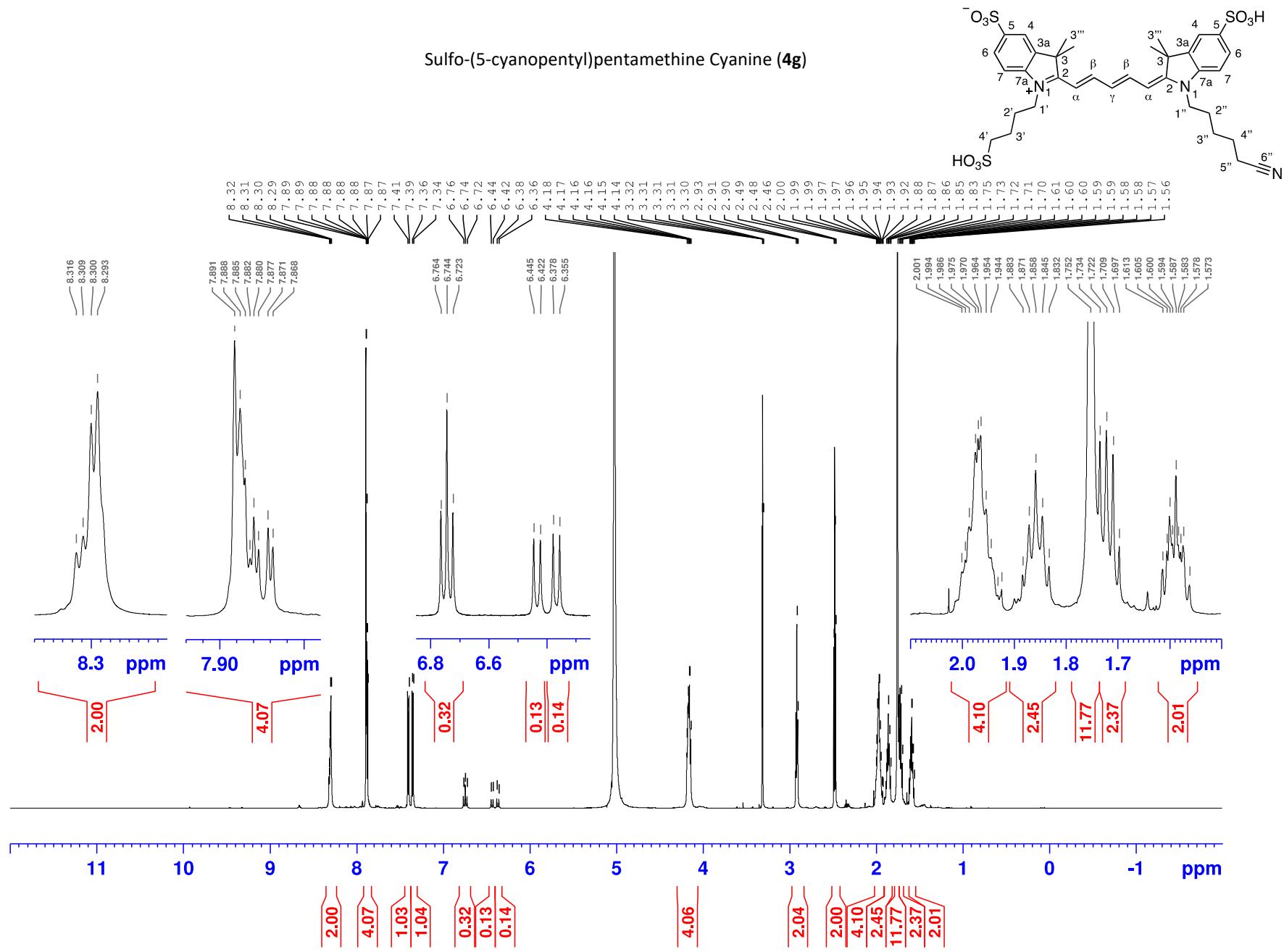
$^{13}\text{C}$  NMR spectrum (100 MHz, MeOD/D<sub>2</sub>O = 3:1)

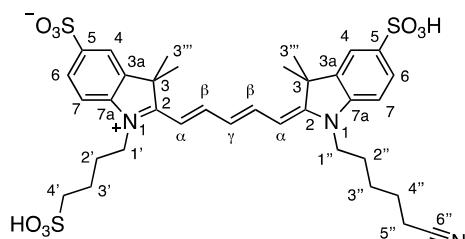


$^1\text{H}$  NMR spectrum (400 MHz, MeOD)

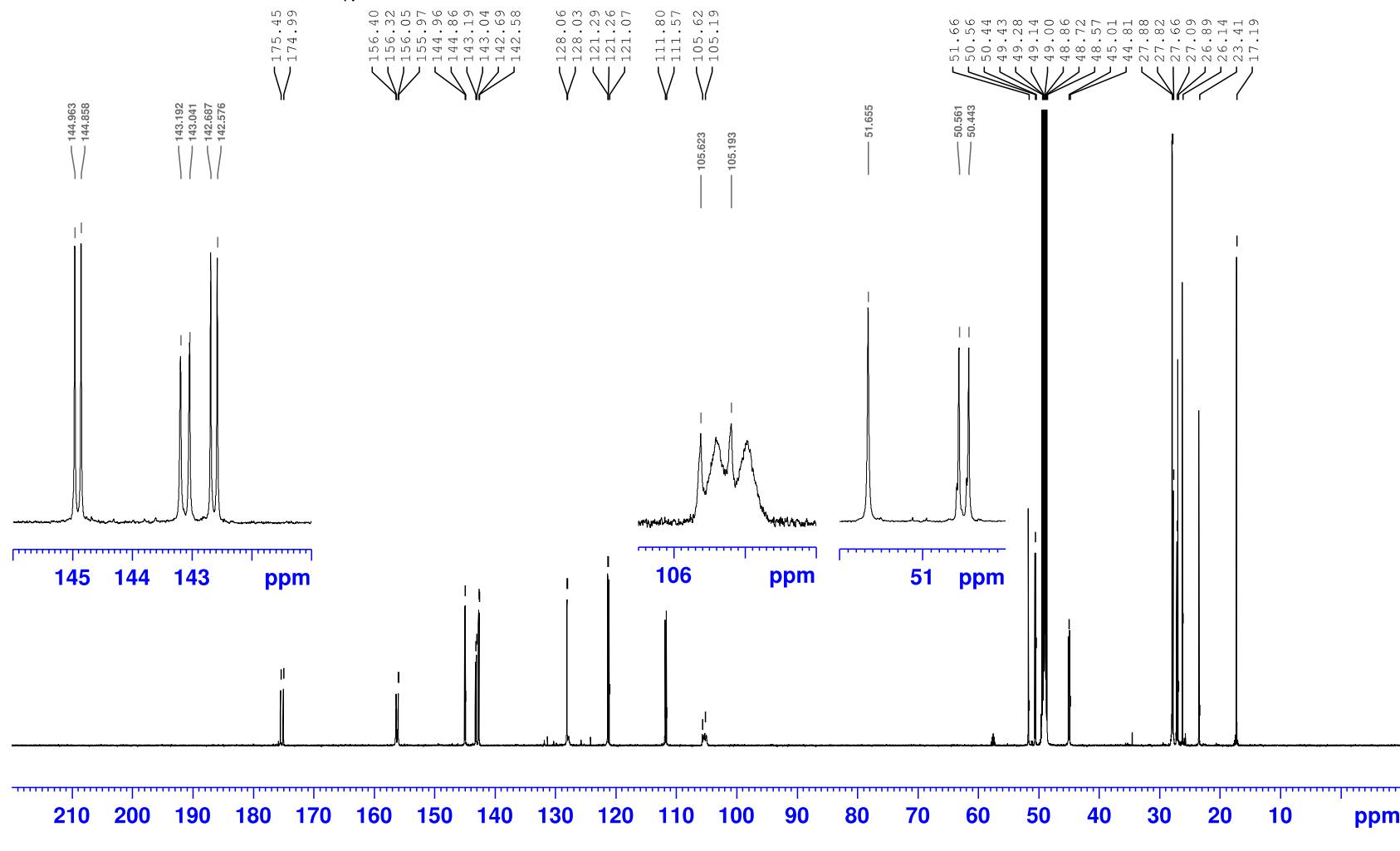


Sulfo-(5-cyanopentyl)pentamethine Cyanine (**4g**)

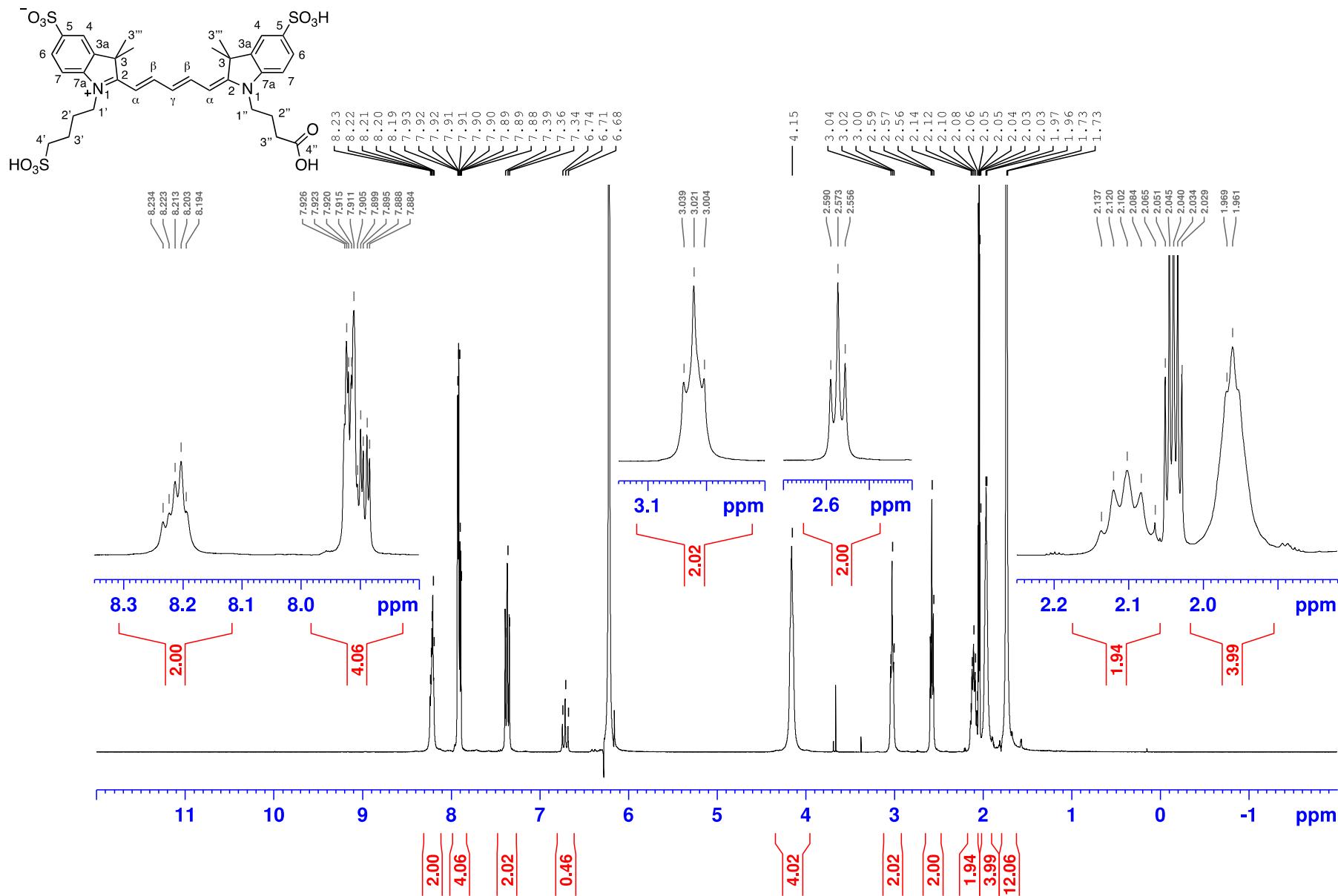


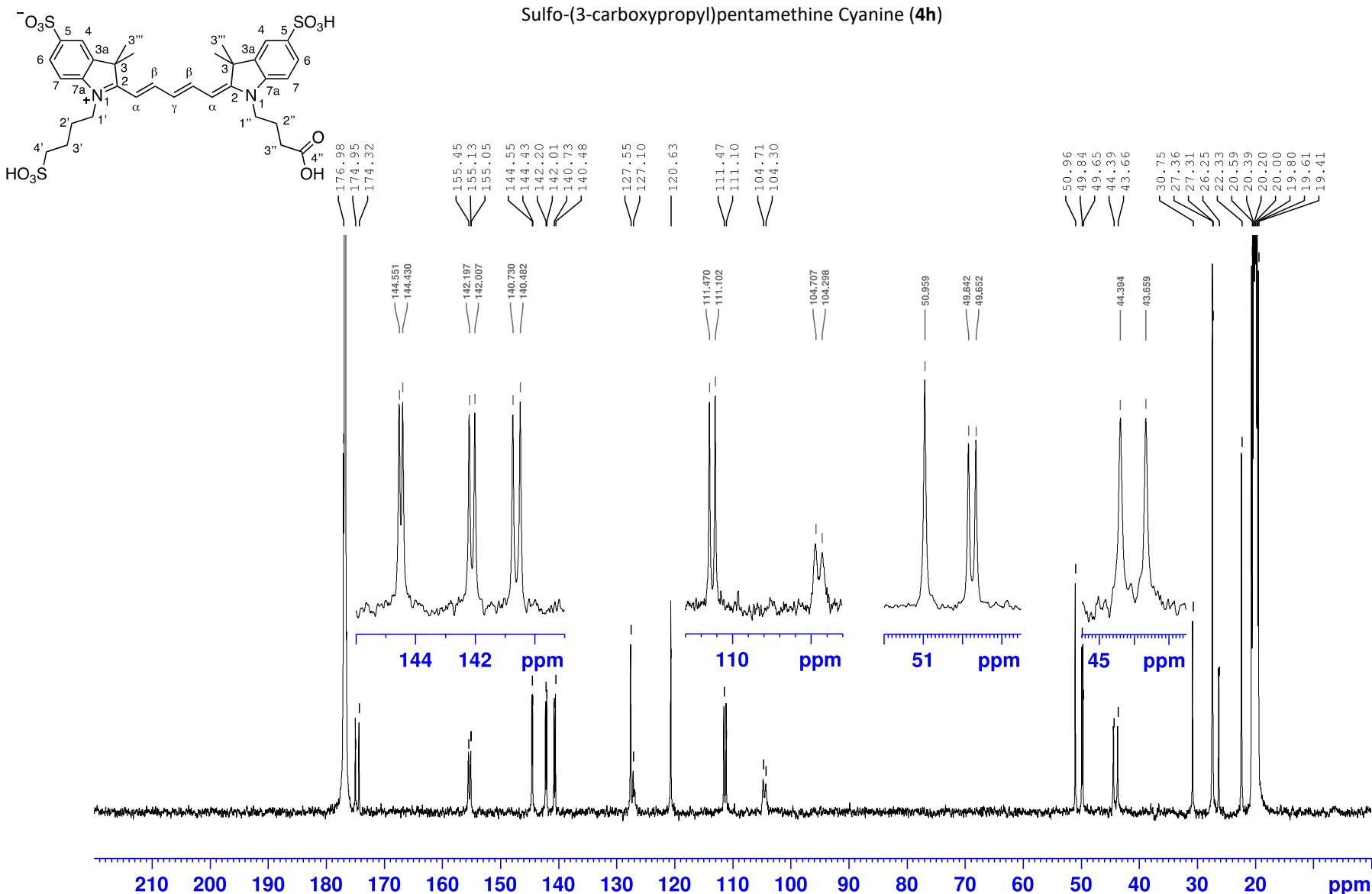


Sulfo-(5-cyanopenty)pentamethine Cyanine (**4g**)

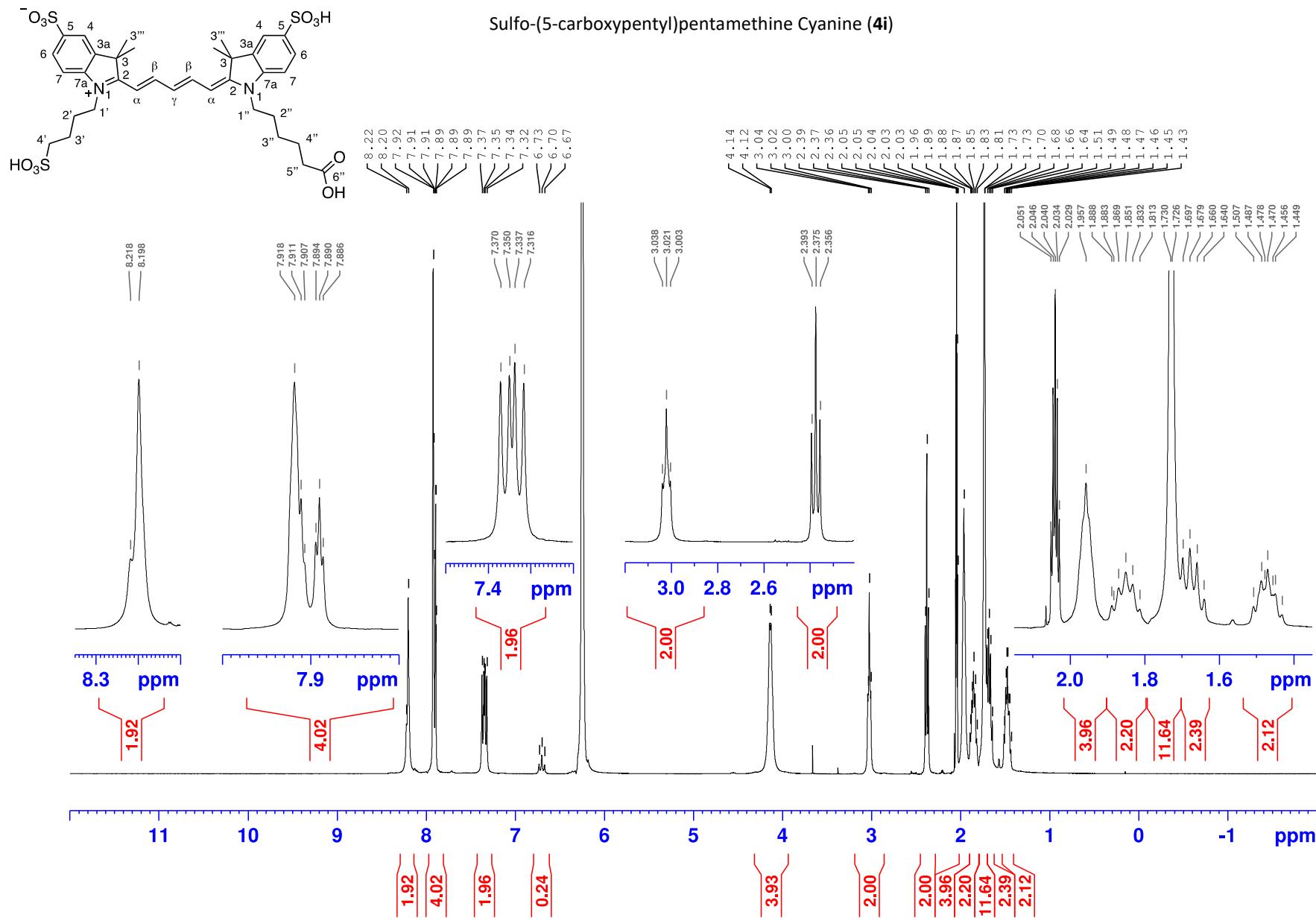


Sulfo-(3-carboxypropyl)pentamethine Cyanine (**4h**)

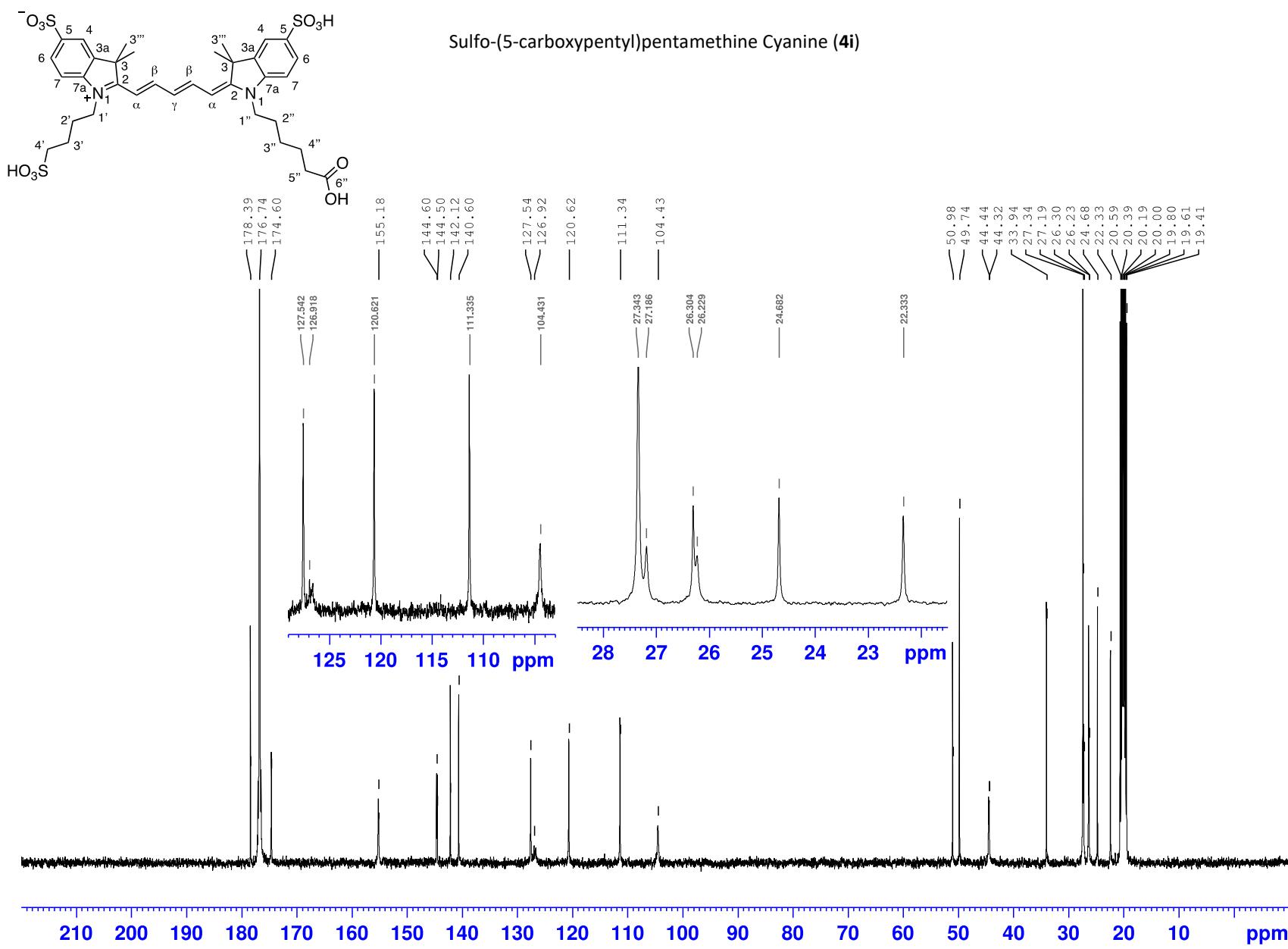


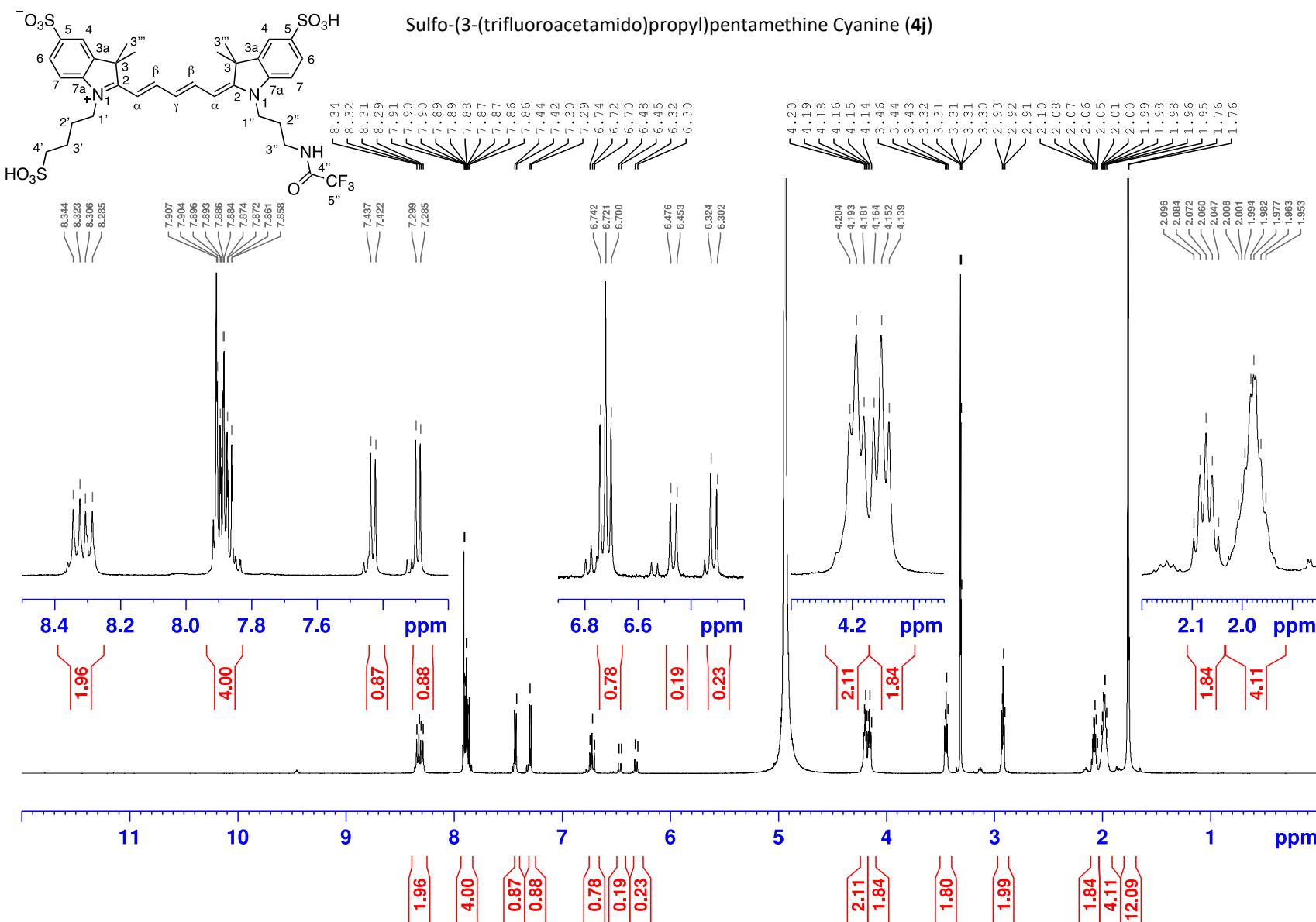


<sup>13</sup>C NMR spectrum (100 MHz, AcOD/D<sub>2</sub>O = 3:1)

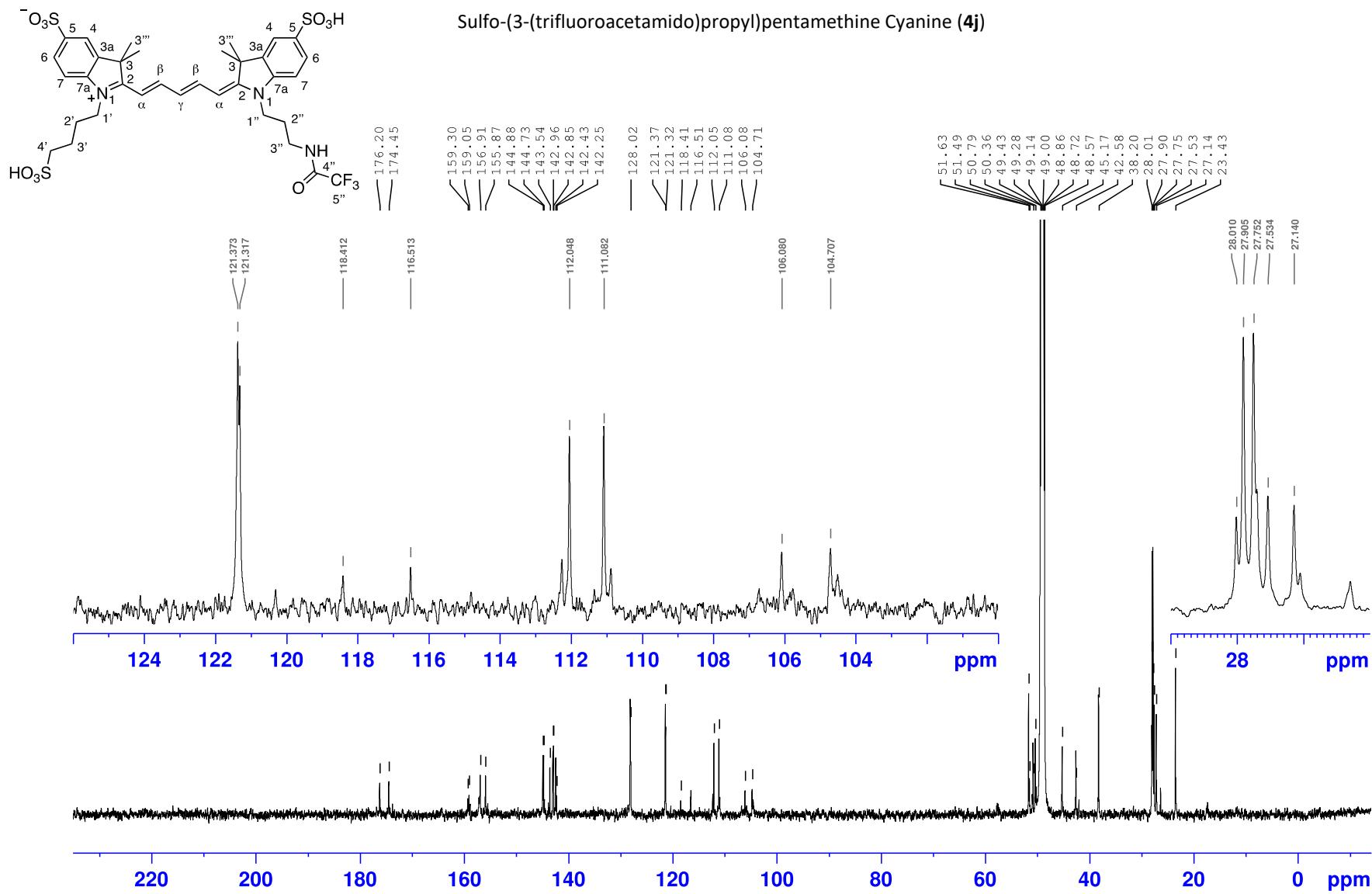


<sup>1</sup>H NMR spectrum (400 MHz, AcOD/D<sub>2</sub>O = 3:1)

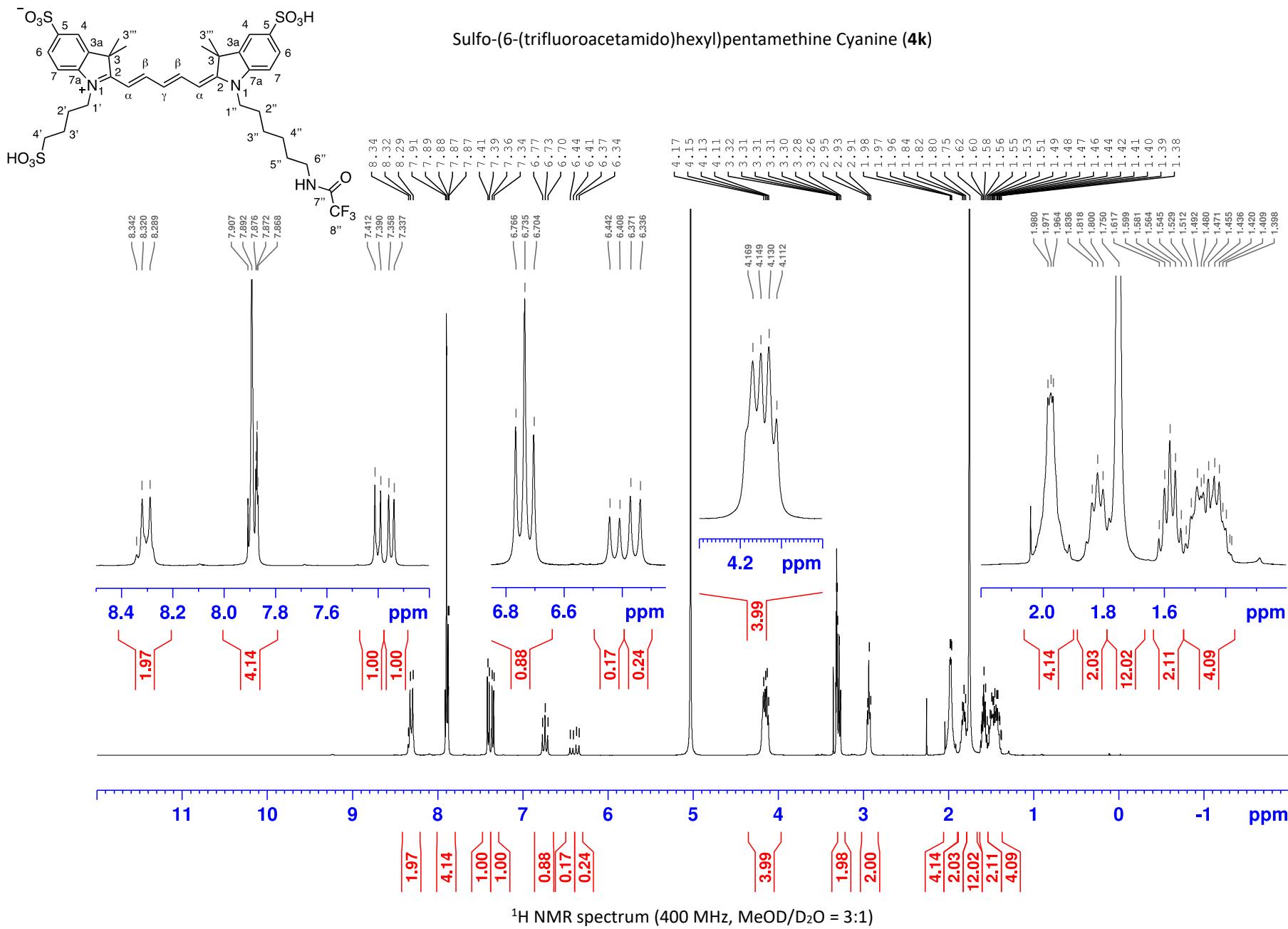




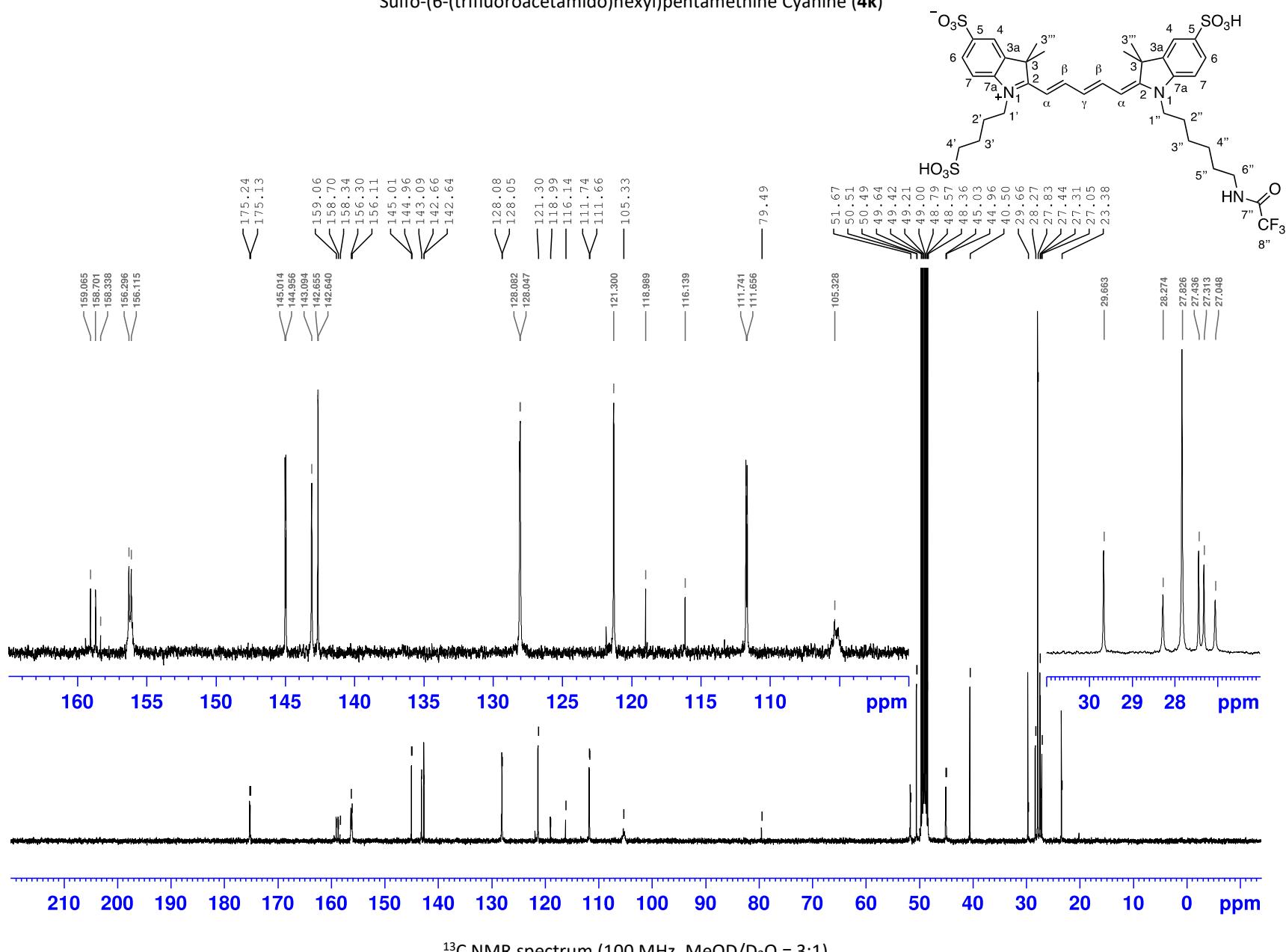
<sup>1</sup>H NMR spectrum (600 MHz, MeOD/D<sub>2</sub>O = 3:1). Double signal set due to rotamers.

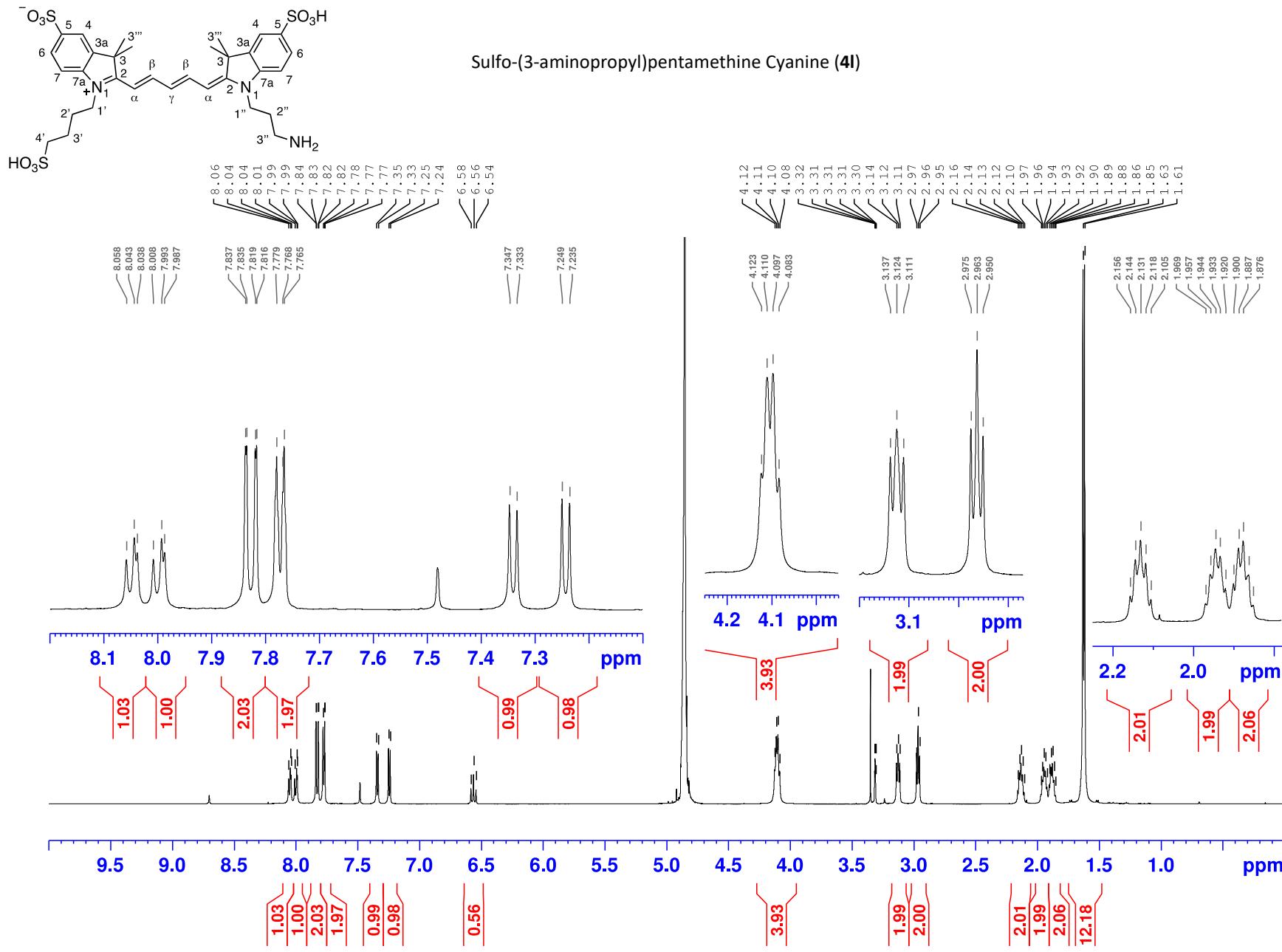


<sup>13</sup>C NMR spectrum (150 MHz, MeOD/D<sub>2</sub>O = 3:1). Double signal set due to rotamers.

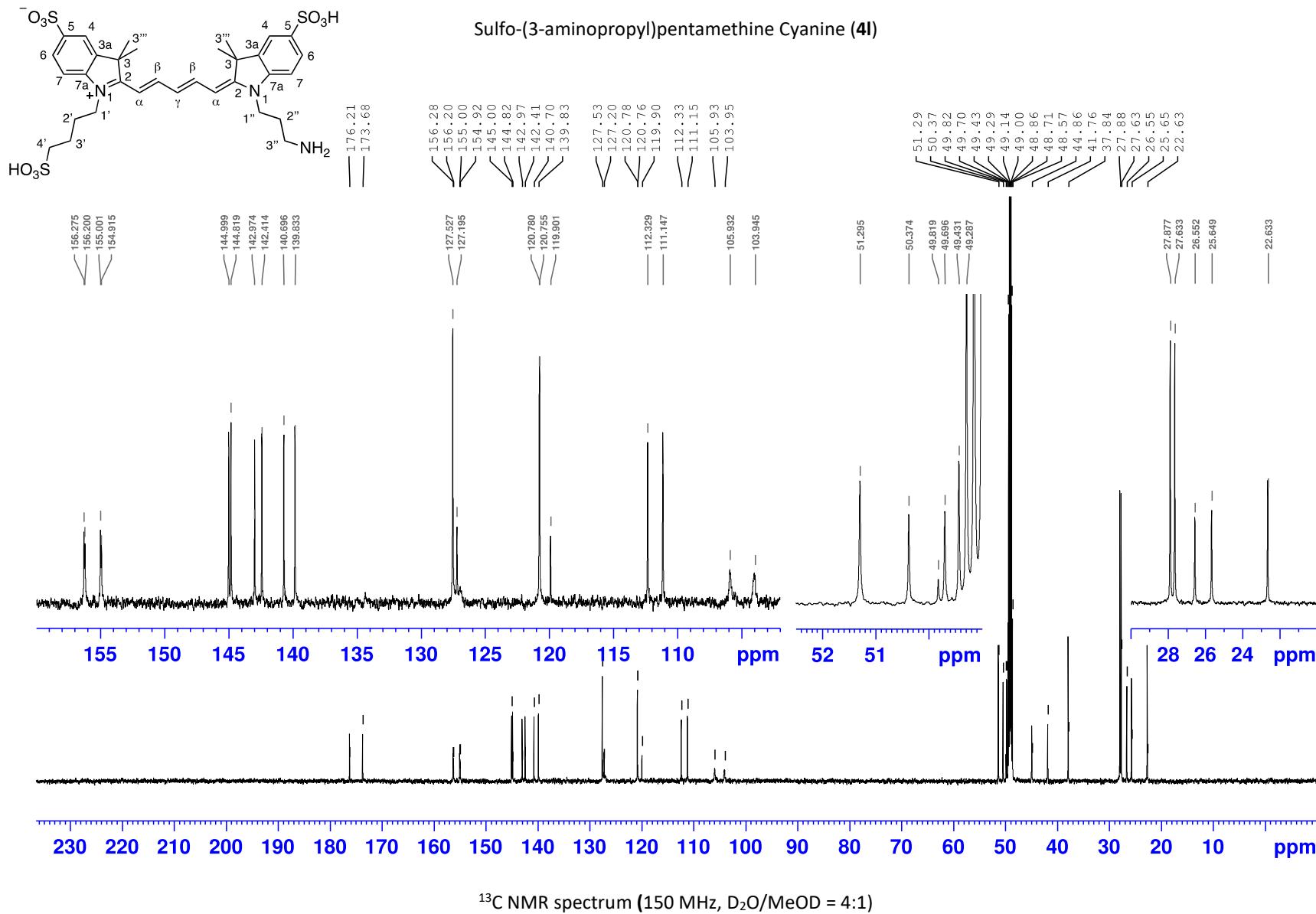


Sulfo-(6-(trifluoroacetamido)hexyl)pentamethine Cyanine (**4k**)

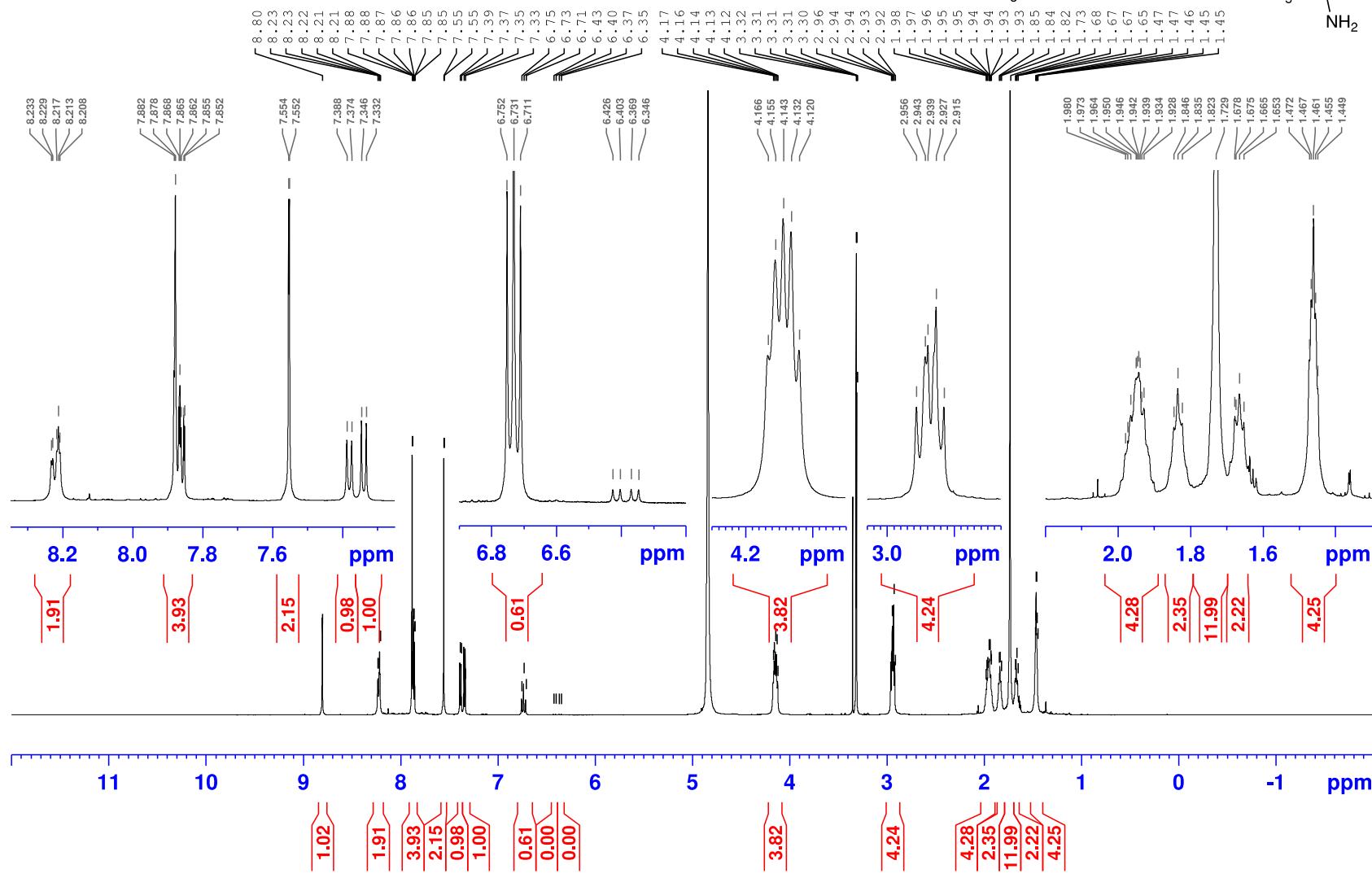
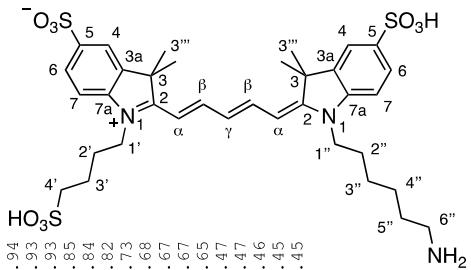




<sup>1</sup>H NMR spectrum (600 MHz, D<sub>2</sub>O/MeOD = 4:0.1)

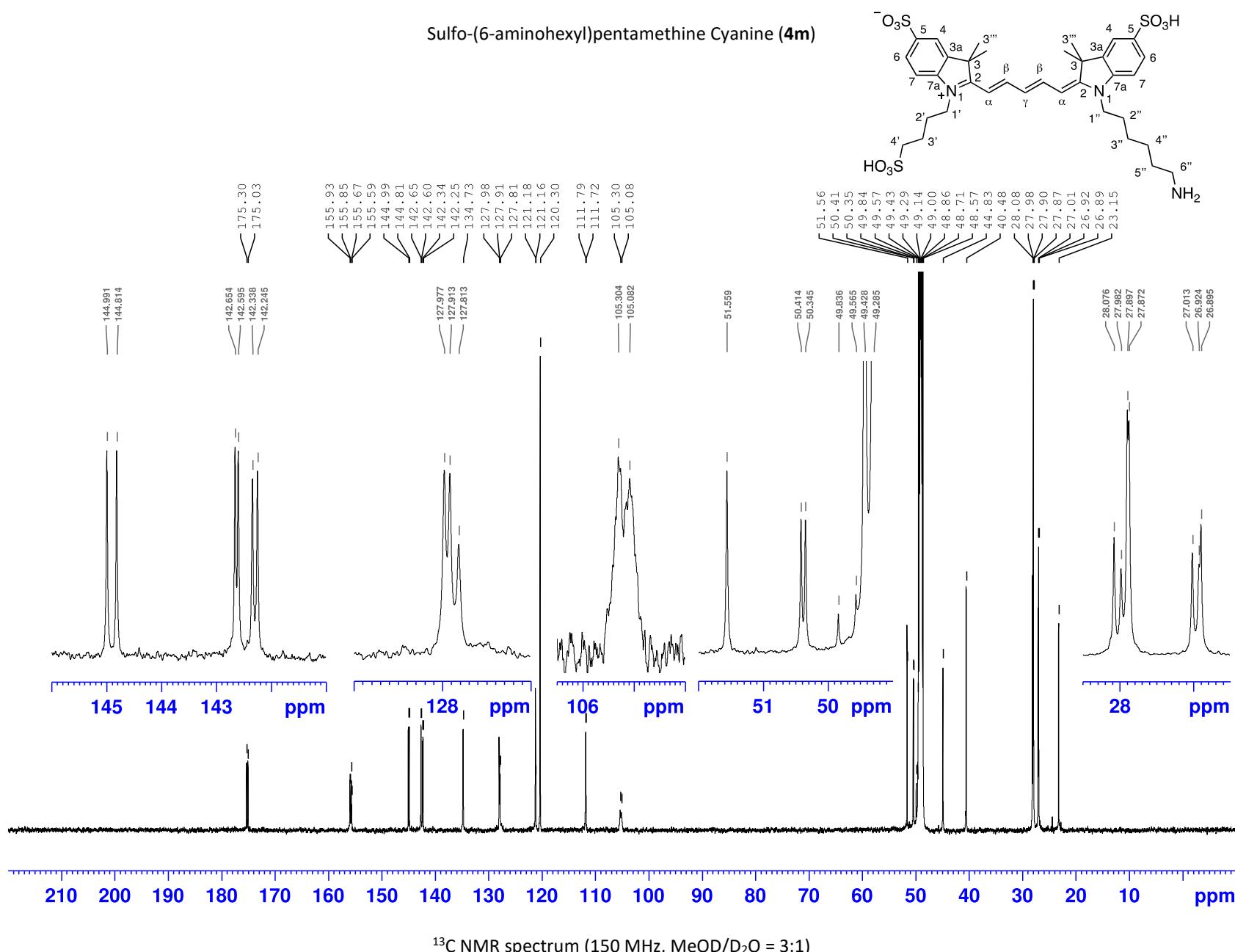


Sulfo-(6-aminohexyl)pentamethine Cyanine (**4m**)



<sup>1</sup>H NMR spectrum (600 MHz, MeOD/D<sub>2</sub>O = 3:1)

Sulfo-(6-aminohexyl)pentamethine Cyanine (**4m**)



## HRMS Spectra of Indolium Salts

Mass Spectrum of Potassium2,3,3-trimethylindolenin-5-sulfonate (**1**)

### Analysis Info

Acquisition Date 8/7/2019 12:37:21 PM

Analysis Name D:\Data\spektren2019\07082019\_NW230-A06f\_SEI\_51\_01\_4893.d

Method Automation\_esi\_tune\_neg\_low.m

Operator admin

Sample Name 07082019\_NW230-A06f\_SEI

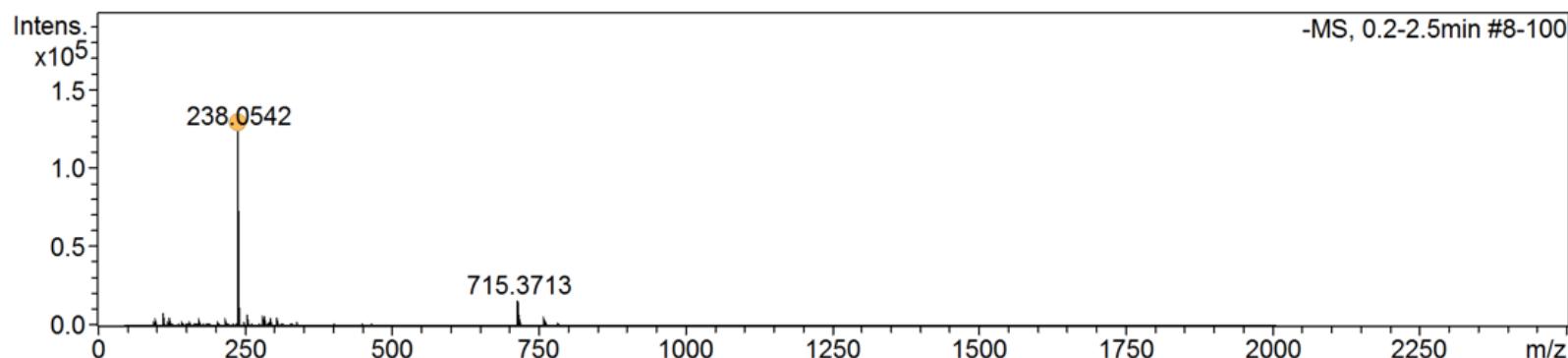
Instrument micrOTOF 213750.00088

Comment verduennt in MeOH

### Acquisition Parameter

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 50 m/z     | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 2000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

-MS, 0.2-2.5min #8-100



| Meas. m/z | # | Ion Formula | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb | e <sup>-</sup> Conf | N-Rule |
|-----------|---|-------------|----------|-----------|--------|----------|--------|-----|---------------------|--------|
| 238.0542  | 1 | C11H12NO3S  | 238.0543 | 0.5       | 7.3    | 1        | 100.00 | 6.5 | even                | ok     |

Mass Spectrum of 1-(3-Azidopropyl)-2,3,3-trimethylindolium-5-sulfonate (**2a**)

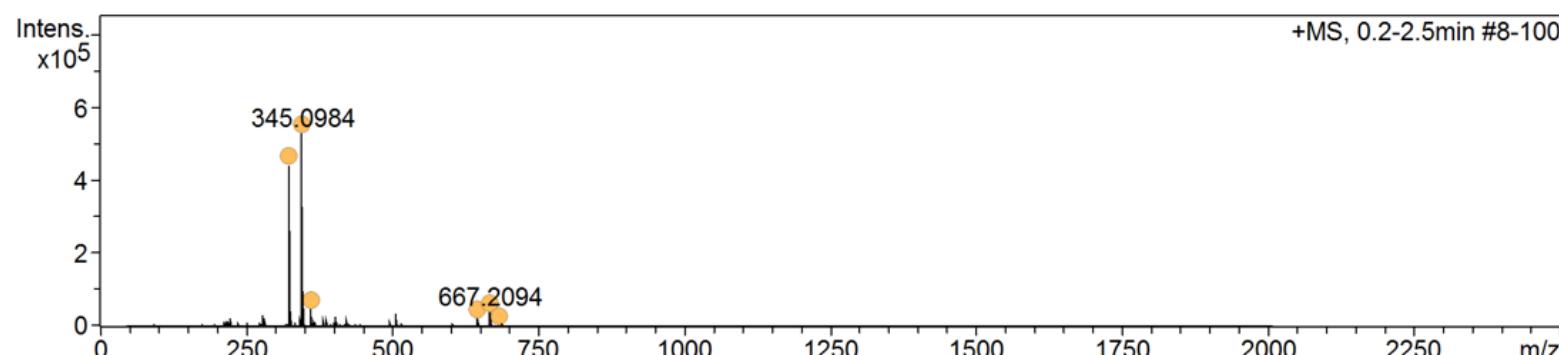
**Analysis Info**

|               |   |                  |                      |
|---------------|---|------------------|----------------------|
|               |   | Acquisition Date | 8/20/2019 5:17:29 PM |
| Analysis Name | D:\Data\spektren2019\20082019_NW395-A78-CyC3N3_SEI_53_01_5140.d |                  |                      |
| Method        | Automation_esi_tune_pos_low.m                                   | Operator         | admin                |
| Sample Name   | 20082019_NW395-A78-CyC3N3_SEI                                   | Instrument       | micrOTOF             |
| Comment       | verduennt in MeOH   |                  | 213750.00088         |

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Positive | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 4500 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 50 m/z     | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 2000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

**+MS, 0.2-2.5min #8-100**



| Meas. m/z | # | Ion Formula    | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|----------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 323.1169  | 1 | C14H19N4O3S    | 323.1172 | 1.0       | 1.8    | 1        | 100.00 | 7.5  | even                | ok     |
| 345.0984  | 1 | C14H18N4NaO3S  | 345.0992 | 2.3       | 5.4    | 1        | 100.00 | 7.5  | even                | ok     |
| 361.0732  | 1 | C14H18KN4O3S   | 361.0731 | -0.1      | 69.8   | 1        | 100.00 | 7.5  | even                | ok     |
| 645.2265  | 1 | C28H37N8O6S2   | 645.2272 | 1.1       | 8.6    | 1        | 100.00 | 14.5 | even                | ok     |
| 667.2094  | 1 | C28H36N8NaO6S2 | 667.2091 | -0.4      | 8.0    | 1        | 100.00 | 14.5 | even                | ok     |
| 683.1838  | 1 | C28H36KN8O6S2  | 683.1831 | -1.0      | 110.0  | 1        | 100.00 | 14.5 | even                | ok     |





Mass Spectrum of 1-(Pentynyl)-2,3,3-trimethylindolium-5-sulfonate (**2d**)

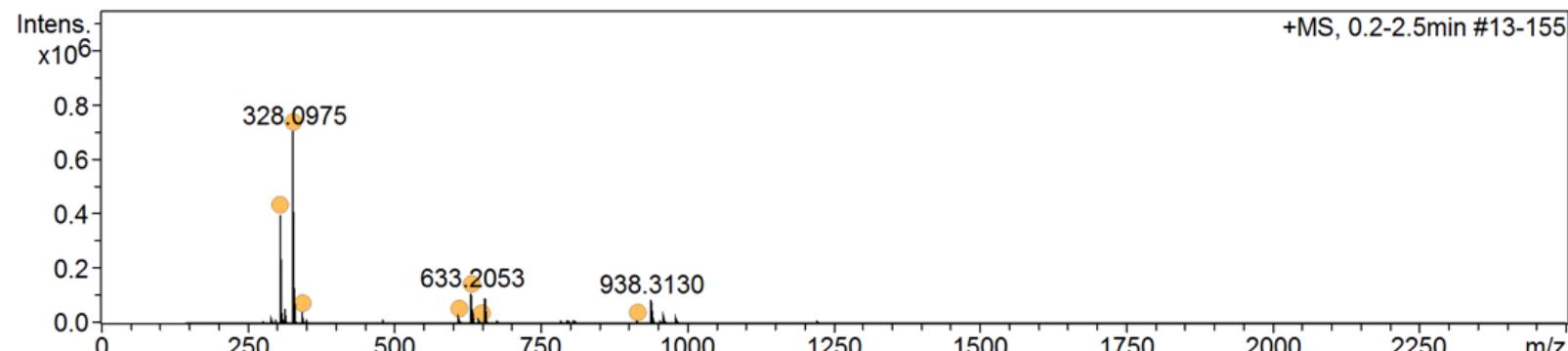
**Analysis Info**

|               |  |                  |                      |
|---------------|--|------------------|----------------------|
|               |  | Acquisition Date | 8/29/2019 1:24:11 PM |
| Analysis Name | D:\Data\spektren2019\29082019_NW320-A64_SEI_51_01_5331.d |                  |                      |
| Method        | Automation_esi_tune_pos_mid.m                            | Operator         | admin                |
| Sample Name   | 29082019_NW320-A64_SEI                                   | Instrument       | micrOTOF             |
| Comment       | verduennt in MeOH  |                  | 213750.00088         |

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Positive | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 4500 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 150 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 3500 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

**+MS, 0.2-2.5min #13-155**



| Meas. m/z | # | Ion Formula    | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e⁻ Conf | N-Rule |
|-----------|---|----------------|----------|-----------|--------|----------|--------|------|---------|--------|
| 306.1156  | 1 | C16H20NO3S     | 306.1158 | 0.9       | 2.9    | 1        | 100.00 | 7.5  | even    | ok     |
| 328.0975  | 1 | C16H19NNaO3S   | 328.0978 | 0.8       | 2.3    | 1        | 100.00 | 7.5  | even    | ok     |
| 344.0713  | 1 | C16H19KNO3S    | 344.0717 | 1.2       | 2.7    | 1        | 100.00 | 7.5  | even    | ok     |
| 611.2237  | 1 | C32H39N2O6S2   | 611.2244 | 1.2       | 8.1    | 1        | 100.00 | 14.5 | even    | ok     |
| 633.2053  | 1 | C32H38N2NaO6S2 | 633.2063 | 1.7       | 19.4   | 1        | 100.00 | 14.5 | even    | ok     |
| 649.1769  | 1 | C32H38KN2O6S2  | 649.1803 | 5.2       | 114.6  | 1        | 100.00 | 14.5 | even    | ok     |
| 916.3311  | 1 | C48H58N3O9S3   | 916.3330 | 2.1       | 50.0   | 1        | 100.00 | 21.5 | even    | ok     |

Mass Spectrum of 1-(Hexynyl)-2,3,3-trimethylindolium-5-sulfonate (**2e**)

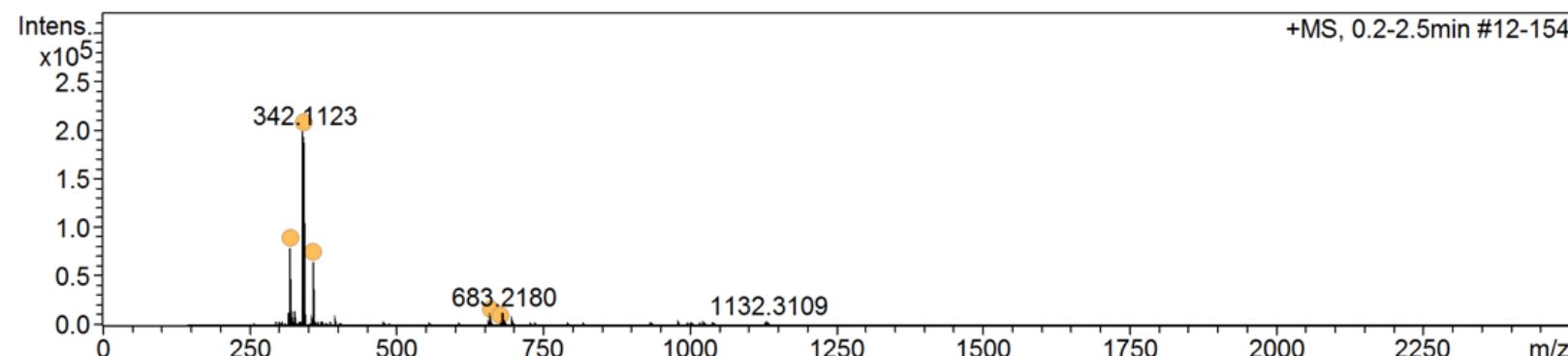
**Analysis Info**

Acquisition Date 8/29/2019 12:59:17 PM  
 Analysis Name D:\Data\spektren2019\29082019\_NW-CyC6Alkin\_SEI\_52\_01\_5328.d  
 Method Automation\_esi\_tune\_pos\_mid.m Operator admin  
 Sample Name 29082019\_NW-CyC6Alkin\_SEI Instrument micrOTOF 213750.00088  
 Comment verduennt in MeOH

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Positive | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 4500 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 150 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 3500 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

+MS, 0.2-2.5min #12-154



| Meas. m/z | # | Ion Formula    | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|----------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 320.1308  | 1 | C17H22NO3S     | 320.1315 | 2.2       | 8.5    | 1        | 100.00 | 7.5  | even                | ok     |
| 342.1123  | 1 | C17H21NNaO3S   | 342.1134 | 3.2       | 7.6    | 1        | 100.00 | 7.5  | even                | ok     |
| 358.0887  | 1 | C17H21KNO3S    | 358.0874 | -3.8      | 11.6   | 1        | 100.00 | 7.5  | even                | ok     |
| 661.2346  | 1 | C34H42N2NaO6S2 | 661.2376 | 4.6       | 13.2   | 1        | 100.00 | 14.5 | even                | ok     |
| 677.2180  | 1 | C34H42KN2O6S2  | 677.2116 | -9.4      | 147.2  | 1        | 100.00 | 14.5 | even                | ok     |

Mass Spectrum of 1-(3-(2,2,2-Trifluoroacetamido)propyl)-2,3,3-trimethylindolium-5-sulfonate (**2f**)

**Analysis Info**

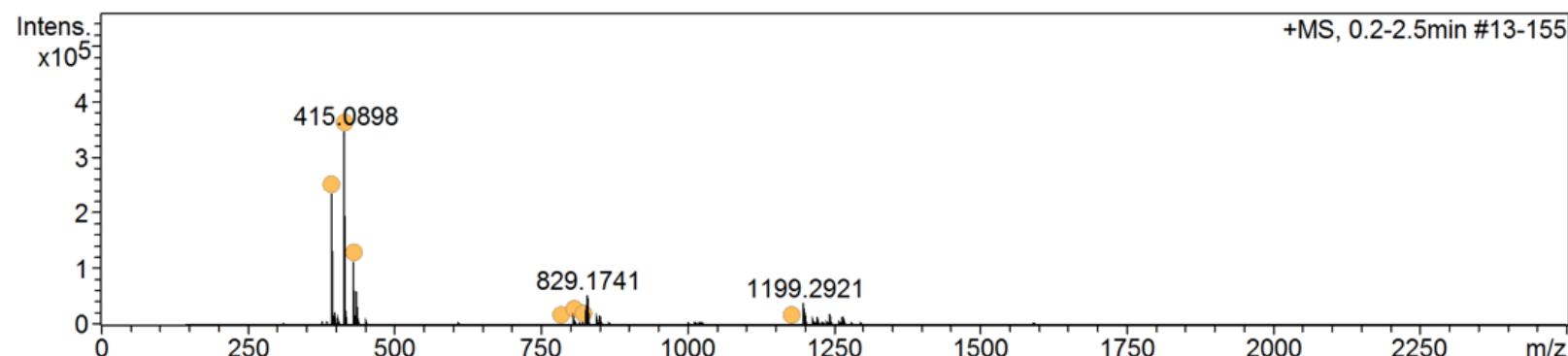
Acquisition Date 8/25/2019 4:01:57 PM

Analysis Name D:\Data\spektren2019\25082019\_NW-CyC3NHTFA\_SEI\_51\_01\_5273.d  
 Method Automation\_esi\_tune\_pos\_mid.m  
 Sample Name 25082019\_NW-CyC3NHTFA\_SEI  
 Comment verduennt in MeOH

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Positive | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 4500 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 150 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 3500 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

**+MS, 0.2-2.5min #13-155**



| Meas. m/z | # | Ion Formula      | m/z       | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|------------------|-----------|-----------|--------|----------|--------|------|---------------------|--------|
| 393.1080  | 1 | C16H20F3N2O4S    | 393.1090  | 2.6       | 0.9    | 1        | 100.00 | 6.5  | even                | ok     |
| 415.0898  | 1 | C16H19F3N2NaO4S  | 415.0910  | 2.9       | 7.6    | 1        | 100.00 | 6.5  | even                | ok     |
| 431.0637  | 1 | C16H19F3KN2O4S   | 431.0649  | 2.8       | 4.5    | 1        | 100.00 | 6.5  | even                | ok     |
| 785.2114  | 1 | C32H39F6N4O8S2   | 785.2108  | -0.8      | 27.7   | 1        | 100.00 | 12.5 | even                | ok     |
| 807.1898  | 1 | C32H38F6N4NaO8S2 | 807.1927  | 3.6       | 34.8   | 1        | 100.00 | 12.5 | even                | ok     |
| 823.1613  | 1 | C32H38F6KN4O8S2  | 823.1667  | 6.5       | 45.5   | 1        | 100.00 | 12.5 | even                | ok     |
| 1177.3096 | 1 | C48H58F9N6O12S3  | 1177.3126 | 2.5       | 57.3   | 1        | 100.00 | 18.5 | even                | ok     |

Mass Spectrum of 1-(6-(2,2,2-Trifluoroacetamido)hexyl)-2,3,3-trimethylindolium-5-sulfonate (**2g**)

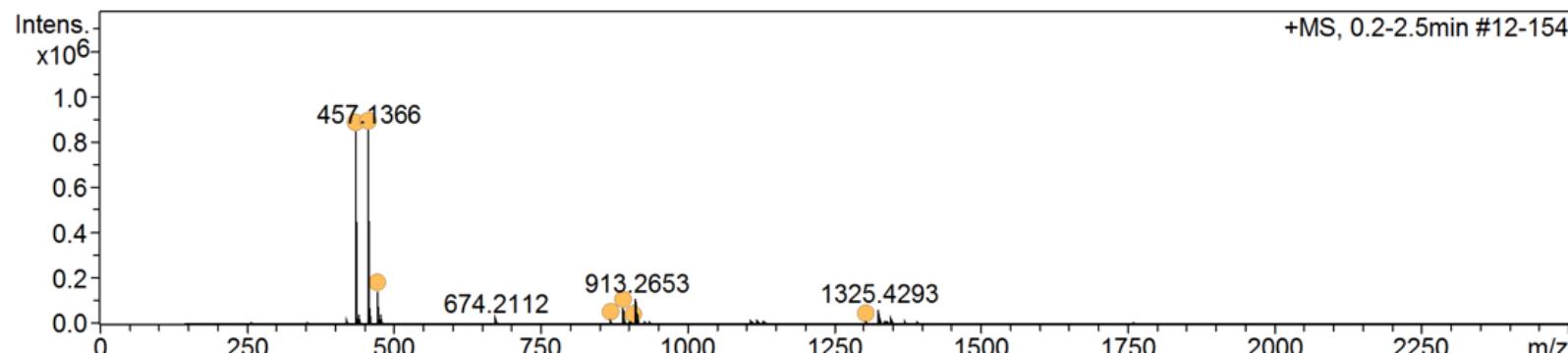
**Analysis Info**

Acquisition Date 8/24/2019 1:45:19 PM  
 Analysis Name D:\Data\spektren2019\24082019\_NW401-A111-C6NTfa\_SEI\_51\_01\_5251.d  
 Method Automation\_esi\_tune\_pos\_mid.m Operator admin  
 Sample Name 24082019\_NW401-A111-C6NTfa\_SEI Instrument micrOTOF 213750.00088  
 Comment verduennt in MeOH

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Positive | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 4500 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 150 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 3500 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

+MS, 0.2-2.5min #12-154



| Meas. m/z | # | Ion Formula      | m/z       | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|------------------|-----------|-----------|--------|----------|--------|------|---------------------|--------|
| 435.1547  | 1 | C19H26F3N2O4S    | 435.1560  | 3.0       | 15.9   | 1        | 100.00 | 6.5  | even                | ok     |
| 457.1366  | 1 | C19H25F3N2NaO4S  | 457.1379  | 3.0       | 11.6   | 1        | 100.00 | 6.5  | even                | ok     |
| 473.1098  | 1 | C19H25F3KN2O4S   | 473.1119  | 4.4       | 8.5    | 1        | 100.00 | 6.5  | even                | ok     |
| 869.3029  | 1 | C38H51F6N4O8S2   | 869.3047  | 2.0       | 8.1    | 1        | 100.00 | 12.5 | even                | ok     |
| 891.2834  | 1 | C38H50F6N4NaO8S2 | 891.2866  | 3.6       | 17.7   | 1        | 100.00 | 12.5 | even                | ok     |
| 907.2570  | 1 | C38H50F6KN4O8S2  | 907.2606  | 3.9       | 240.8  | 1        | 100.00 | 12.5 | even                | ok     |
| 1303.4477 | 1 | C57H76F9N6O12S3  | 1303.4534 | 4.4       | 24.6   | 1        | 100.00 | 18.5 | even                | ok     |

Mass Spectrum of 1-(4-sulfobutyl)-2,3,3-trimethylindolium-5-sulfonate (**2h**)

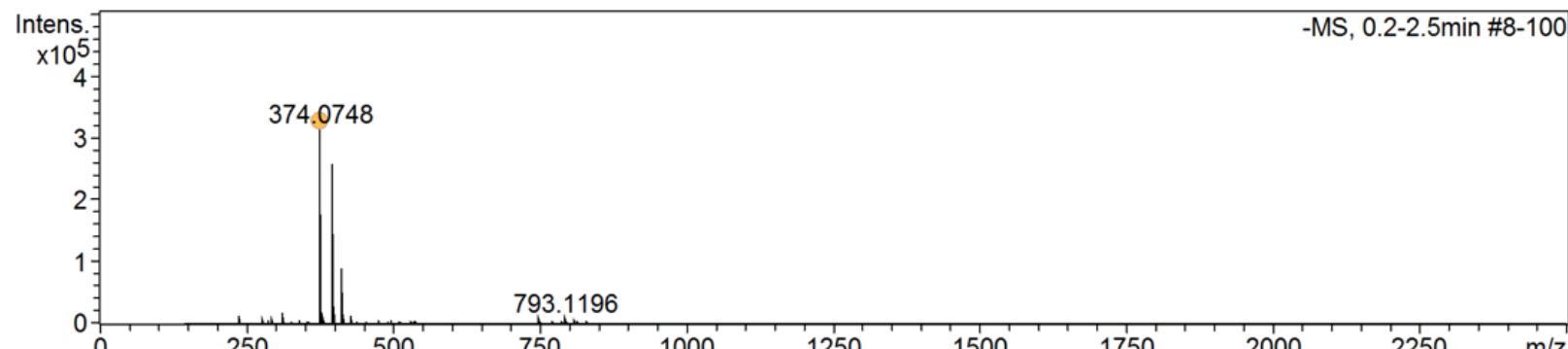
**Analysis Info**

|               |  |                  |                       |
|---------------|--|------------------|-----------------------|
| Analysis Name | D:\Data\spektren2019\17092019_NW174-A63_SEI_53_01_5715.d | Acquisition Date | 9/17/2019 12:16:38 PM |
| Method        | Automation_esi_tune_neg_mid.m                            | Operator         | admin                 |
| Sample Name   | 17092019_NW174-A63_SEI                                   | Instrument       | micrOTOF              |
| Comment       | verduennt in MeOH  |                  | 213750.00088          |

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 150 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 3500 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

-MS, 0.2-2.5min #8-100



| Meas. m/z | # | Ion Formula | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb | e <sup>-</sup> Conf | N-Rule |
|-----------|---|-------------|----------|-----------|--------|----------|--------|-----|---------------------|--------|
| 374.0748  | 1 | C15H20NO6S2 | 374.0738 | -2.8      | 9.6    | 1        | 100.00 | 6.5 | even                | ok     |

Mass Spectrum of 1-(3-Cyanopropyl)-2,3,3-trimethylindolium-5-sulfonate (**2i**)

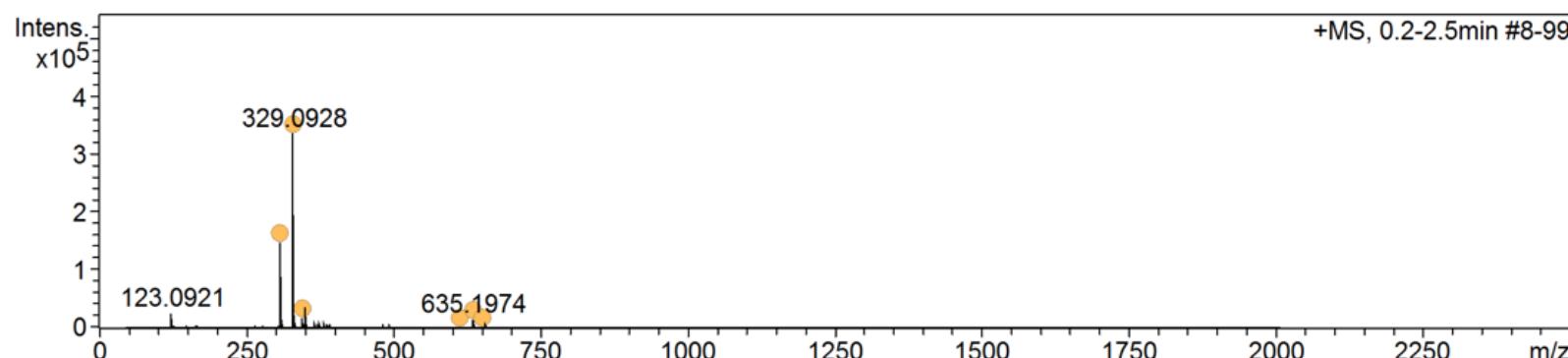
**Analysis Info**

|               |  |                  |                      |
|---------------|--|------------------|----------------------|
|               |  | Acquisition Date | 9/5/2019 11:59:24 AM |
| Analysis Name | D:\Data\spektren2019\05092019_NW304-A71-IndC3CN_SEI_51_01_5527.d |                  |                      |
| Method        | Automation_esi_tune_pos_low.m                                    | Operator         | admin                |
| Sample Name   | 05092019_NW304-A71-IndC3CN_SEI                                   | Instrument       | micrOTOF             |
| Comment       | verduennt in MeOH  |                  | 213750.00088         |

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Positive | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 4500 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 50 m/z     | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 2000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

+MS, 0.2-2.5min #8-99



| Meas. m/z | # | Ion Formula    | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|----------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 307.1112  | 1 | C15H19N2O3S    | 307.1111 | -0.3      | 9.6    | 1        | 100.00 | 7.5  | even                | ok     |
| 329.0928  | 1 | C15H18N2NaO3S  | 329.0930 | 0.6       | 16.3   | 1        | 100.00 | 7.5  | even                | ok     |
| 345.0669  | 1 | C15H18KN2O3S   | 345.0670 | 0.1       | 70.2   | 1        | 100.00 | 7.5  | even                | ok     |
| 613.2153  | 1 | C30H37N4O6S2   | 613.2149 | -0.7      | 76.7   | 1        | 100.00 | 14.5 | even                | ok     |
| 635.1974  | 1 | C30H36N4NaO6S2 | 635.1968 | -0.9      | 19.3   | 1        | 100.00 | 14.5 | even                | ok     |
| 651.1691  | 1 | C30H36KN4O6S2  | 651.1708 | 2.7       | 113.9  | 1        | 100.00 | 14.5 | even                | ok     |

Mass Spectrum of 1-(5-Cyanopentyl)-2,3,3-trimethylindolium-5-sulfonate (**2j**)

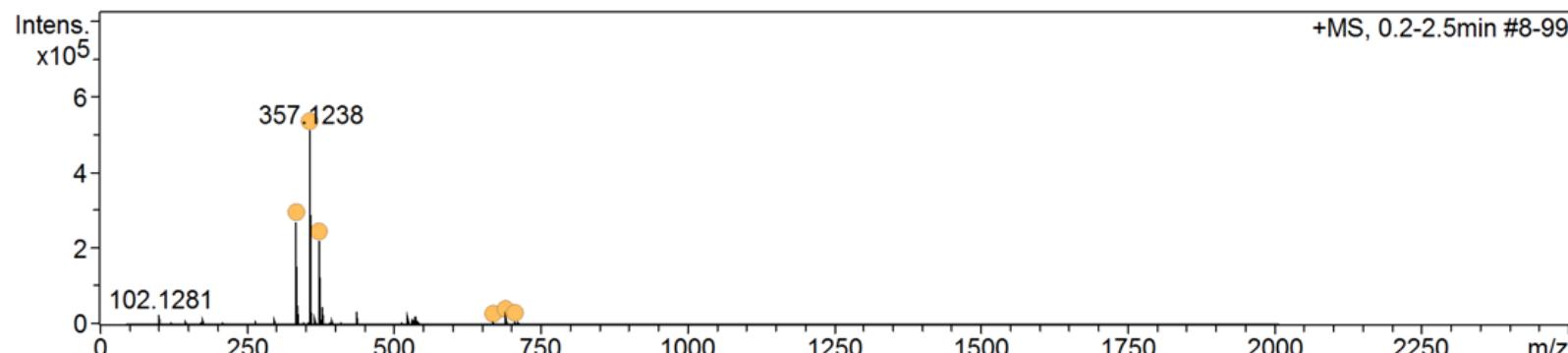
**Analysis Info**

Acquisition Date 8/26/2019 2:33:28 PM  
 Analysis Name D:\Data\spektren2019\25082019\_NW-CyC5CN\_SEI\_51\_01\_5290.d  
 Method Automation\_esi\_tune\_pos\_low.m Operator admin  
 Sample Name 25082019\_NW-CyC5CN\_SEI Instrument micrOTOF 213750.00088  
 Comment verduennt in MeOH

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Positive | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 4500 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 50 m/z     | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 2000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

+MS, 0.2-2.5min #8-99



| Meas. m/z | # | Ion Formula    | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|----------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 335.1418  | 1 | C17H23N2O3S    | 335.1424 | 1.9       | 8.8    | 1        | 100.00 | 7.5  | even                | ok     |
| 357.1238  | 1 | C17H22N2NaO3S  | 357.1243 | 1.6       | 10.3   | 1        | 100.00 | 7.5  | even                | ok     |
| 373.0975  | 1 | C17H22KN2O3S   | 373.0983 | 2.2       | 11.8   | 1        | 100.00 | 7.5  | even                | ok     |
| 669.2772  | 1 | C34H45N4O6S2   | 669.2775 | 0.5       | 23.5   | 1        | 100.00 | 14.5 | even                | ok     |
| 691.2598  | 1 | C34H44N4NaO6S2 | 691.2594 | -0.5      | 12.1   | 1        | 100.00 | 14.5 | even                | ok     |
| 707.2335  | 1 | C34H44KN4O6S2  | 707.2334 | -0.1      | 34.5   | 1        | 100.00 | 14.5 | even                | ok     |

Mass Spectrum of 1-(3-carboxypropyl)-2,3,3-trimethylindolium-5-sulfonate (**2k**)

**Analysis Info**

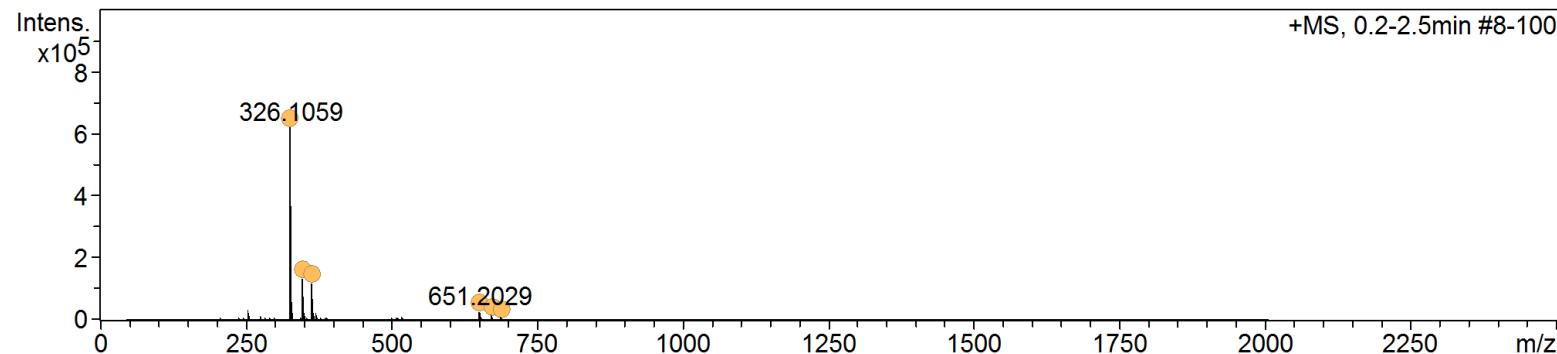
Acquisition Date 11/19/2019 11:46:15 AM

Analysis Name D:\Data\spektren2019\19112019\_NW-CyA132IndC3CO2\_SEI\_55\_01\_7083.d  
 Method Automation\_esi\_tune\_pos\_low.m  
 Sample Name 19112019\_NW-CyA132IndC3CO2\_SEI  
 Comment verduennt in MeOH  
 Operator admin  
 Instrument micrOTOF 213750.00088

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Positive | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 4500 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 50 m/z     | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 2000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

+MS, 0.2-2.5min #8-100



| Meas. m/z | # | Ion Formula     | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|-----------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 326.1059  | 1 | C15H20NO5S      | 326.1057 | -0.7      | 3.1    | 1        | 100.00 | 6.5  | even                | ok     |
| 348.0873  | 1 | C15H19NNaO5S    | 348.0876 | 0.8       | 5.2    | 1        | 100.00 | 6.5  | even                | ok     |
| 364.0608  | 1 | C15H19KNO5S     | 364.0616 | 2.1       | 4.7    | 1        | 100.00 | 6.5  | even                | ok     |
| 651.2029  | 1 | C30H39N2O10S2   | 651.2041 | 1.8       | 8.8    | 1        | 100.00 | 12.5 | even                | ok     |
| 673.1851  | 1 | C30H38N2NaO10S2 | 673.1860 | 1.3       | 22.2   | 1        | 100.00 | 12.5 | even                | ok     |
| 689.1586  | 1 | C30H38KN2O10S2  | 689.1599 | 1.9       | 27.2   | 1        | 100.00 | 12.5 | even                | ok     |

Mass Spectrum of 1-(5-carboxypentyl)-2,3,3-trimethylindolium-5-sulfonate (**2I**)

**Analysis Info**

|               |  |                  |                       |
|---------------|--|------------------|-----------------------|
| Analysis Name | D:\Data\spektren2019\14112019_NW-CyA131IndC5CO2_SEI_52_01_6972.d | Acquisition Date | 11/14/2019 2:25:22 PM |
| Method        | Automation_esi_tune_pos_mid.m                                    | Operator         | admin                 |
| Sample Name   | 14112019_NW-CyA131IndC5CO2_SEI                                   | Instrument       | micrOTOF              |
| Comment       | verduennt in MeOH  |                  | 213750.00088          |

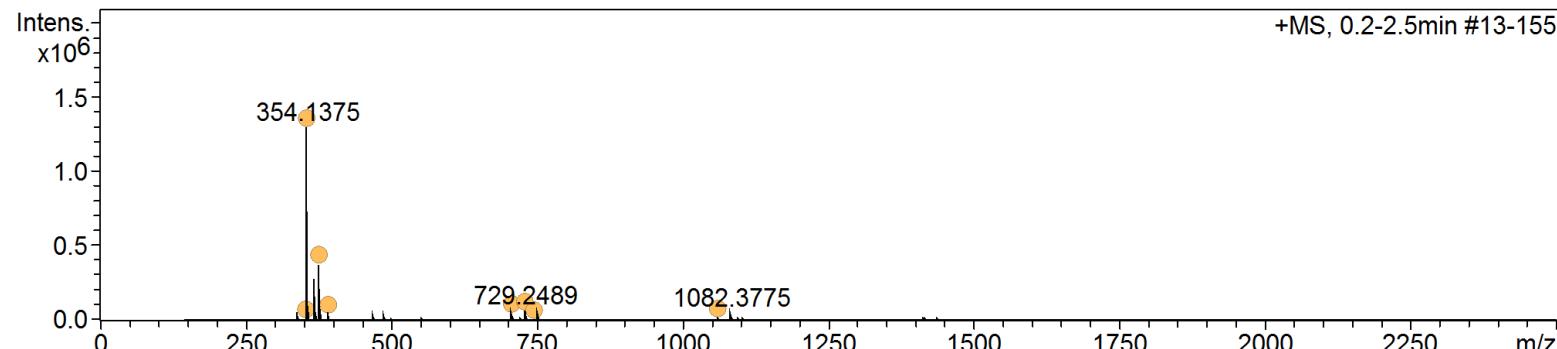
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**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Positive | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 4500 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 150 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 3500 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

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+MS, 0.2-2.5min #13-155



| Meas. m/z | # | Ion Formula     | m/z       | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|-----------------|-----------|-----------|--------|----------|--------|------|---------------------|--------|
| 353.1289  | 1 | C17H23NO5S      | 353.1291  | 0.7       | 639.2  | 1        | 100.00 | 7.0  | odd                 | ok     |
| 354.1375  | 1 | C17H24NO5S      | 354.1370  | -1.4      | 1.7    | 1        | 100.00 | 6.5  | even                | ok     |
| 376.1190  | 1 | C17H23NNaO5S    | 376.1189  | -0.1      | 7.7    | 1        | 100.00 | 6.5  | even                | ok     |
| 392.0945  | 1 | C17H23KNO5S     | 392.0929  | -4.2      | 12.9   | 1        | 100.00 | 6.5  | even                | ok     |
| 707.2662  | 1 | C34H47N2O10S2   | 707.2667  | 0.7       | 9.8    | 1        | 100.00 | 12.5 | even                | ok     |
| 729.2489  | 1 | C34H46N2NaO10S2 | 729.2486  | -0.4      | 20.5   | 1        | 100.00 | 12.5 | even                | ok     |
| 745.2252  | 1 | C34H46KN2O10S2  | 745.2225  | -3.5      | 103.3  | 1        | 100.00 | 12.5 | even                | ok     |
| 1060.3959 | 1 | C51H70N3O15S3   | 1060.3964 | 0.5       | 22.6   | 1        | 100.00 | 18.5 | even                | ok     |

# Mass Spectrum SmartFormula Report

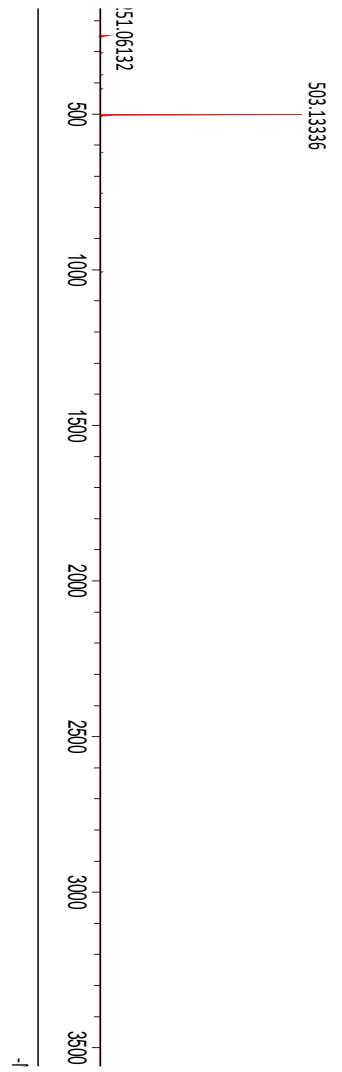
## :is Info

File Name D:\Data\Spektren\2019\2019\_2472\_SEI\_NW-A09.d  
Instrument micrOTOF-QIII  
Operator Julianne  
Date 12/12/2019  
Time 2019\_2472\_SEI\_NW-A09  
Name Wolf Natalia  
Conc NW-A09  
5 pmol/L in MeOH

## :edit Parameter

| Type | ESI      | Ion Polarity    | Negative  | Set Nebulizer    |
|------|----------|-----------------|-----------|------------------|
| sgin | Active   | Set Funnel 1 RF | 200.0 Vpp | Set Dry Heater   |
| ld   | 50 mHz   | Set Funnel 2 RF | 400.0 Vpp | Set Dry Gas      |
|      | 4000 mHz | Set Hexapole RF | 400.0 Vpp | Set Divert Valve |

2019\_2472\_SEI\_NW-A09.d:-1



| # | Ion Formula  | m/z       | err [ppm] | mSigma | #mSigma | Score  | rdB  | e <sup>-</sup> Conf | N Rule |
|---|--------------|-----------|-----------|--------|---------|--------|------|---------------------|--------|
| 1 | C24H27N2O6S2 | 503.13160 | -3.50     | 55.1   | 1       | 100.00 | 12.5 | even                | ok     |

# HRMS Spectra of Cyanines

Compass DataAnalysis 4.2

printed: 12/16/2019 10:34:23 AM by: Julianne

Mass Spectrum of Sulfo-(3-azidopropyl)pentamethine Cyanine (**4a**)

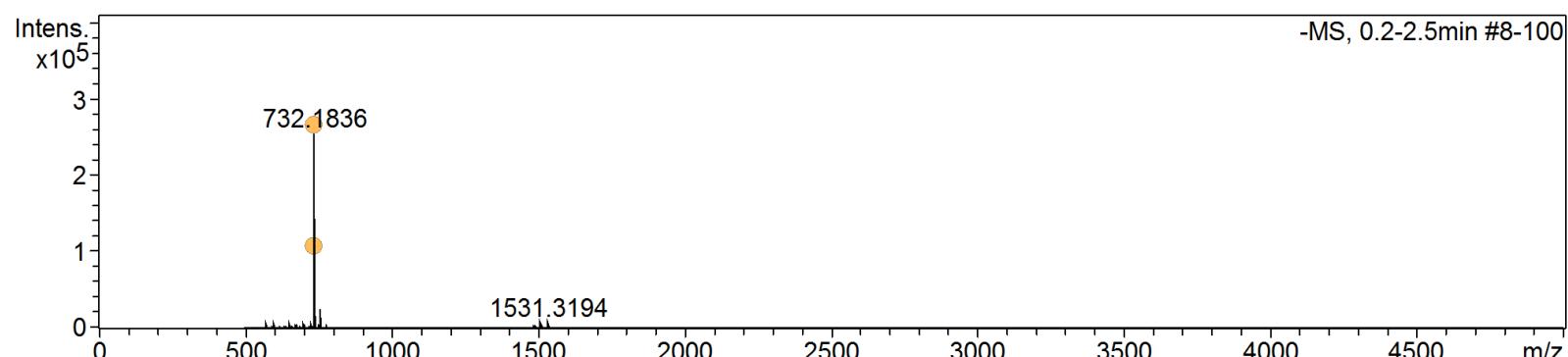
**Analysis Info**

|               |  |                  |                      |
|---------------|--|------------------|----------------------|
|               |  | Acquisition Date | 9/3/2019 10:08:06 AM |
| Analysis Name | D:\Data\spektren2019\03092019_NW333-A79_SEI_51_01_5448.d |                  |                      |
| Method        | Automation_esi_tune_neg_high.m                           | Operator         | admin                |
| Sample Name   | 03092019_NW333-A79_SEI                                   | Instrument       | micrOTOF             |
| Comment       | verduennt in MeOH  |                  | 213750.00088         |

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 500 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 5000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

**-MS, 0.2-2.5min #8-100**



| Meas. m/z | # | Ion Formula  | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|--|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 732.1836  | 1 | C <sub>32</sub> H <sub>38</sub> N <sub>5</sub> O <sub>9</sub> S <sub>3</sub> | 732.1837 | 0.2       | 23.8   | 1        | 100.00 | 16.5 | even                | ok     |
| 733.1851  | 1 | C <sub>32</sub> H <sub>39</sub> N <sub>5</sub> O <sub>9</sub> S <sub>3</sub> | 733.1915 | 8.7       | 68.7   | 1        | 100.00 | 16.0 | odd                 | ok     |

Mass Spectrum of Sulfo-(6-azidohexyl)pentamethine Cyanine (**4b**)

**Analysis Info**

|               |  |                  |                        |
|---------------|--|------------------|------------------------|
|               |  | Acquisition Date | 12/20/2019 10:56:04 AM |
| Analysis Name | D:\Data\spektren2019\20122019_NW419-A127Cy5C6N3_SEI_51_01_7601.d |                  |                        |
| Method        | Automation_esi_tune_neg_high.m                                   | Operator         | admin                  |
| Sample Name   | 20122019_NW419-A127Cy5C6N3_SEI                                   | Instrument       | micrOTOF               |
| Comment       | verduennt in MeOH  |                  | 213750.00088           |

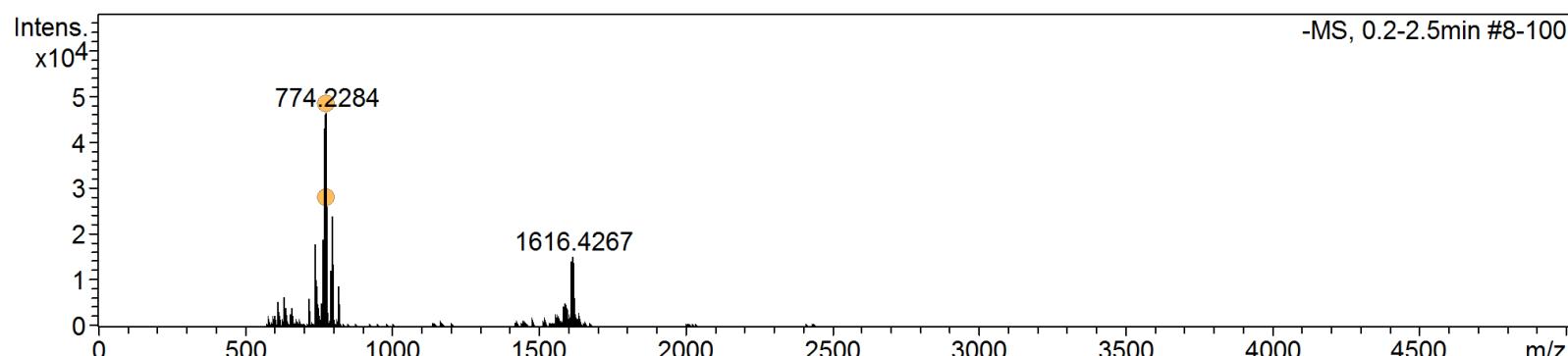
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**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 500 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 5000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

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**-MS, 0.2-2.5min #8-100**



| Meas. m/z | # | Ion Formula  | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|--------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 774.2284  | 1 | C35H44N5O9S3 | 774.2307 | 2.9       | 73.7   | 1        | 100.00 | 16.5 | even                | ok     |
| 775.2308  | 1 | C35H45N5O9S3 | 775.2385 | 10.0      | 68.2   | 1        | 100.00 | 16.0 | odd                 | ok     |

Mass Spectrum of Sulfo-(9-azidononyl)pentamethine Cyanine (**4c**)

**Analysis Info**

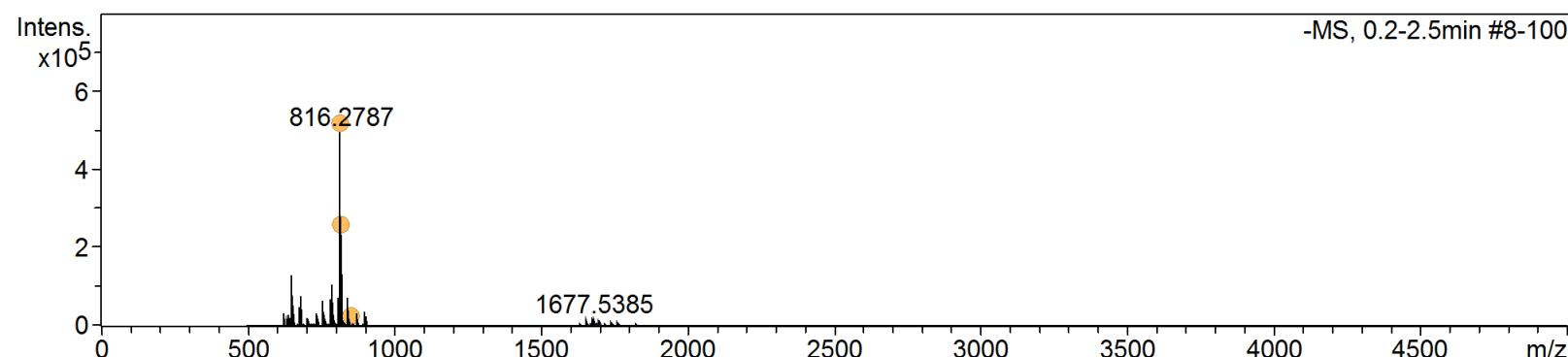
Acquisition Date 8/30/2019 3:38:15 PM

Analysis Name D:\Data\spektren2019\30082019\_NW-Cy5C9N3\_SEI\_51\_01\_5371.d  
 Method Automation\_esi\_tune\_neg\_high.m  
 Sample Name 30082019\_NW-Cy5C9N3\_SEI  
 Comment verduennt in MeOH  
 Operator admin  
 Instrument micrOTOF 213750.00088

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 500 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 5000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

**-MS, 0.2-2.5min #8-100**



| Meas. m/z | # | Ion Formula    | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|----------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 816.2787  | 1 | C38H50N5O9S3   | 816.2776 | -1.4      | 5.4    | 1        | 100.00 | 16.5 | even                | ok     |
| 817.2817  | 1 | C38H51N5O9S3   | 817.2854 | 4.6       | 53.3   | 1        | 100.00 | 16.0 | odd                 | ok     |
| 852.2471  | 1 | C38H51CIN5O9S3 | 852.2543 | 8.5       | 401.3  | 1        | 100.00 | 15.5 | even                | ok     |

Mass Spectrum of Sulfo-(pentynyl)pentamethine Cyanine (**4d**)

**Analysis Info**

|               |   |                  |                      |
|---------------|---|------------------|----------------------|
| Analysis Name | D:\Data\spektren2019\07102019_NW-Cy5Pentin_SEI_52_01_6039.d | Acquisition Date | 10/7/2019 1:52:20 PM |
| Method        | Automation_esi_tune_neg_high.m                              | Operator         | admin                |
| Sample Name   | 07102019_NW-Cy5Pentin_SEI                                   | Instrument       | micrOTOF             |
| Comment       | verduennt in MeOH   |                  | 213750.00088         |

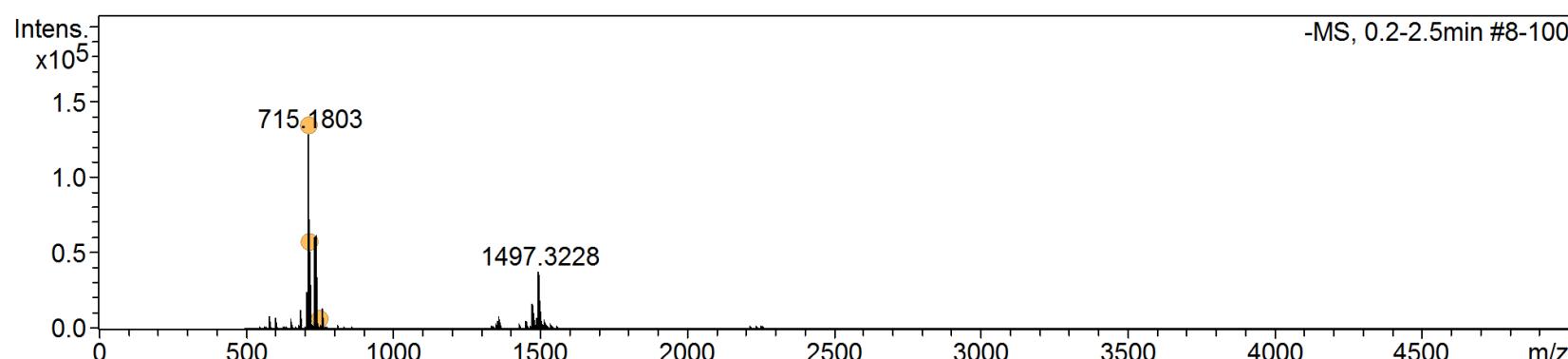
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**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 500 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 5000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

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**-MS, 0.2-2.5min #8-100**



| Meas. m/z | # | Ion Formula  | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|--|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 715.1803  | 1 | C <sub>34</sub> H <sub>39</sub> N <sub>2</sub> O <sub>9</sub> S <sub>3</sub>   | 715.1823 | 2.8       | 3.8    | 1        | 100.00 | 16.5 | even                | ok     |
| 716.1838  | 1 | C <sub>34</sub> H <sub>40</sub> N <sub>2</sub> O <sub>9</sub> S <sub>3</sub>   | 716.1901 | 8.8       | 78.6   | 1        | 100.00 | 16.0 | odd                 | ok     |
| 751.1586  | 1 | C <sub>34</sub> H <sub>40</sub> CIN <sub>2</sub> O <sub>9</sub> S <sub>3</sub> | 751.1590 | 0.5       | 260.9  | 1        | 100.00 | 15.5 | even                | ok     |

Mass Spectrum of Sulfo-(hexynyl)pentamethine Cyanine (**4e**)

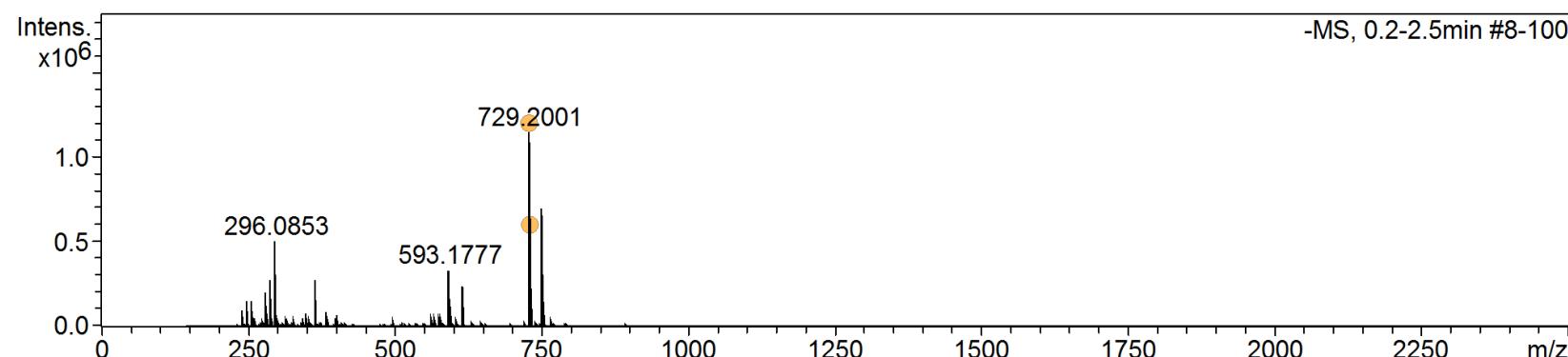
**Analysis Info**

|               |  |                  |                      |
|---------------|--|------------------|----------------------|
|               |  | Acquisition Date | 10/7/2019 4:37:19 PM |
| Analysis Name | D:\Data\spektren2019\07102019_NW-Cy5Hexin_SEI_53_01_6054.d |                  |                      |
| Method        | Automation_esi_tune_neg_mid.m                              | Operator         | admin                |
| Sample Name   | 07102019_NW-Cy5Hexin_SEI                                   | Instrument       | micrOTOF             |
| Comment       | verduennt in MeOH  |                  | 213750.00088         |

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 150 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 3500 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

**-MS, 0.2-2.5min #8-100**



| Meas. m/z | # | Ion Formula  | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|--|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 729.2001  | 1 | C <sub>35</sub> H <sub>41</sub> N <sub>2</sub> O <sub>9</sub> S <sub>3</sub> | 729.1980 | -2.9      | 29.2   | 1        | 100.00 | 16.5 | even                | ok     |
| 730.2004  | 1 | C <sub>35</sub> H <sub>42</sub> N <sub>2</sub> O <sub>9</sub> S <sub>3</sub> | 730.2058 | 7.4       | 69.4   | 1        | 100.00 | 16.0 | odd                 | ok     |

Mass Spectrum of Sulfo-(3-cyanopropyl)pentamethine Cyanine (**4f**)

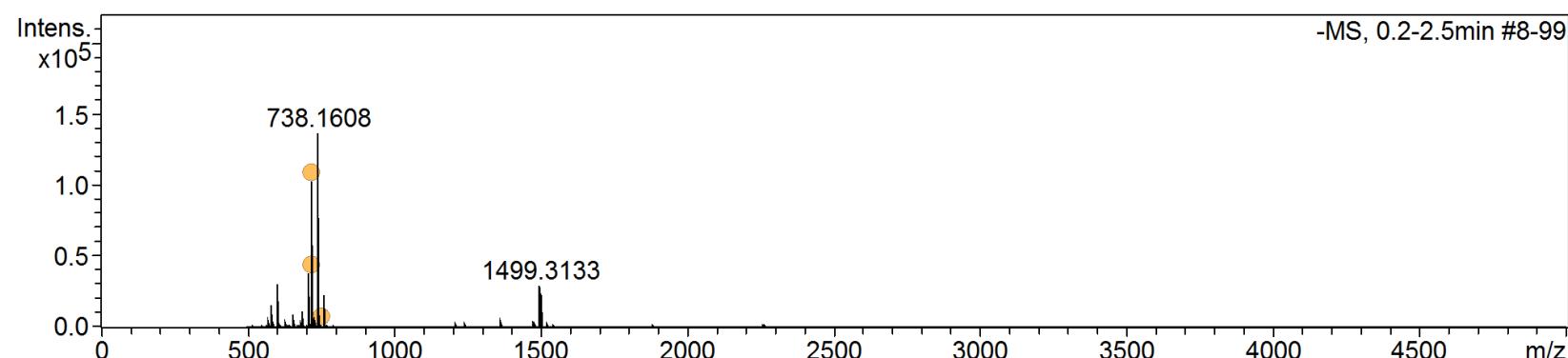
**Analysis Info**

Acquisition Date 9/12/2019 12:25:31 PM  
 Analysis Name D:\Data\spektren2019\09092019\_NW360-A96\_Cy5C3CN\_SEI\_51\_01\_5613.d  
 Method Automation\_esi\_tune\_neg\_high.m Operator admin  
 Sample Name 09092019\_NW360-A96\_Cy5C3CN\_SEI Instrument micrOTOF 213750.00088  
 Comment verduenn in MeOH

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 500 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 5000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

**-MS, 0.2-2.5min #8-99**



| Meas. m/z | # | Ion Formula    | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|----------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 716.1792  | 1 | C33H38N3O9S3   | 716.1776 | -2.3      | 19.8   | 1        | 100.00 | 16.5 | even                | ok     |
| 717.1807  | 1 | C33H39N3O9S3   | 717.1854 | 6.5       | 76.4   | 1        | 100.00 | 16.0 | odd                 | ok     |
| 752.1524  | 1 | C33H39ClN3O9S3 | 752.1542 | 2.4       | 61.2   | 1        | 100.00 | 15.5 | even                | ok     |

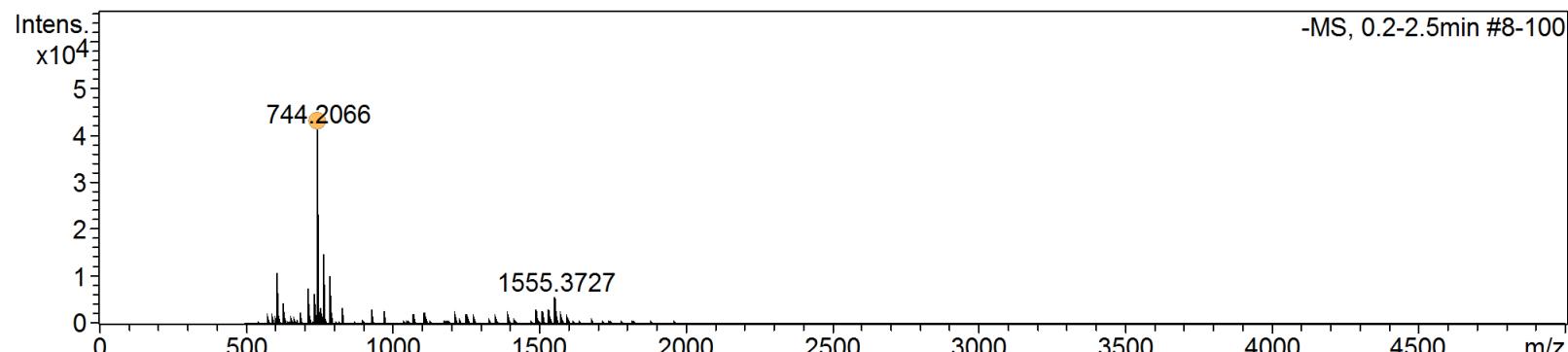
Mass Spectrum of Sulfo-(5-cyanopentyl)pentamethine Cyanine (**4g**)

| Analysis Info |   | Acquisition Date | 9/30/2019 12:24:06 PM |
|---------------|---|------------------|-----------------------|
| Analysis Name | D:\Data\spektren2019\30092019_NW-Cy5C5CN_SEI_51_01_5912.d |                  |                       |
| Method        | Automation_esi_tune_neg_high.m                            | Operator         | admin                 |
| Sample Name   | 30092019_NW-Cy5C5CN_SEI                                   | Instrument       | micrOTOF              |
| Comment       | verduennt in MeOH   |                  | 213750.00088          |

Acquisition Parameter

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 500 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 5000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

-MS, 0.2-2.5min #8-100



| Meas. m/z | # | Ion Formula  | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|--------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 744.2066  | 1 | C35H42N3O9S3 | 744.2089 | 3.1       | 8.4    | 1        | 100.00 | 16.5 | even                | ok     |

Mass Spectrum of Sulfo-(3-carboxypropyl)pentamethine Cyanine (**4h**)

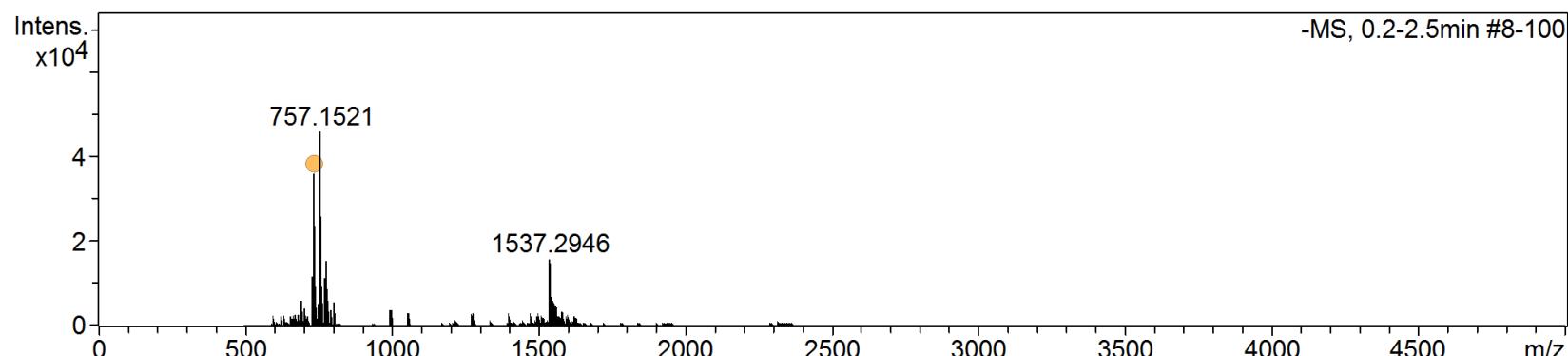
**Analysis Info**

|               |   |                  |                        |
|---------------|---|------------------|------------------------|
|               |   | Acquisition Date | 11/22/2019 12:20:14 PM |
| Analysis Name | D:\Data\spektren2019\22112019_NW-Cy5C3CO2H_SEI_52_01_7214.d |                  |                        |
| Method        | Automation_esi_tune_neg_high.m                              | Operator         | admin                  |
| Sample Name   | 22112019_NW-Cy5C3CO2H_SEI                                   | Instrument       | micrOTOF               |
| Comment       | verduennt in MeOH   |                  | 213750.00088           |

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 500 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 5000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

**-MS, 0.2-2.5min #8-100**



| Meas. m/z | # | Ion Formula   | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|---------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 735.1691  | 1 | C33H39N2O11S3 | 735.1721 | 4.2       | 12.9   | 1        | 100.00 | 15.5 | even                | ok     |

Mass Spectrum of Sulfo-(5-carboxypentyl)pentamethine Cyanine (**4i**)

**Analysis Info**

|               |   |                  |                        |
|---------------|---|------------------|------------------------|
|               |   | Acquisition Date | 11/22/2019 10:24:00 AM |
| Analysis Name | D:\Data\spektren2019\22112019_NW-Cy5C5CO2H_SEI_51_01_7202.d |                  |                        |
| Method        | Automation_esi_tune_neg_high.m                              | Operator         | admin                  |
| Sample Name   | 22112019_NW-Cy5C5CO2H_SEI                                   | Instrument       | micrOTOF               |
| Comment       | verduennt in MeOH   |                  | 213750.00088           |

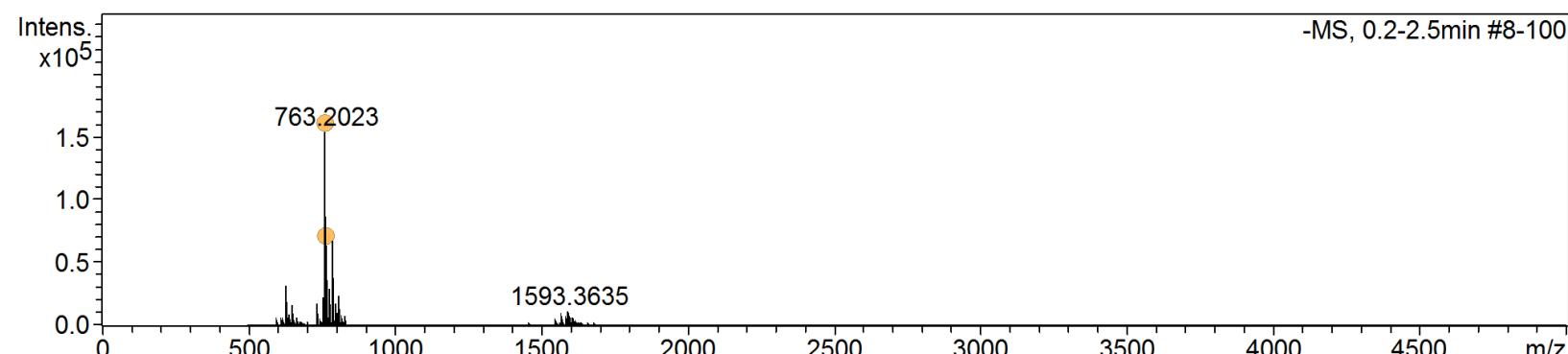
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**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 500 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 5000 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

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-MS, 0.2-2.5min #8-100



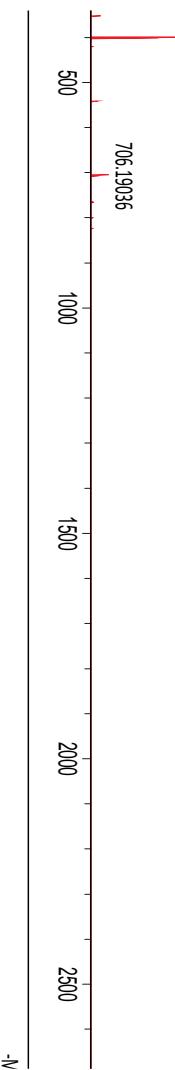
| Meas. m/z | # | Ion Formula   | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|---------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 763.2023  | 1 | C35H43N2O11S3 | 763.2034 | 1.5       | 6.4    | 1        | 100.00 | 15.5 | even                | ok     |
| 764.2052  | 1 | C35H44N2O11S3 | 764.2113 | 7.9       | 71.0   | 1        | 100.00 | 15.0 | odd                 | ok     |

# Mass Spectrum SmartFormula Report

| s info        | Acquisition Date                                  | 12/12/2016     |
|---------------|---|----------------|
| ; Name        | D:\Data\Spektren\2019\2019_2473_SEI_NW352-A62_3.d |                |
| tune_neg.mz.m |   |                |
| Name          | 2019_2473_SEI_NW352-A62_3                         |                |
| nt            | Wolf,Natalia                                      | Operator       |
|               | NW352-A62   | Instrument     |
|               | 8 pmol/µL in MeOH                                 | microTOF-Q ILL |

| ion Parameter | value      | description        |
|---------------|------------|--------------------|
| type          | ESI        | Ion Polarity       |
| jün           | Not active | Negative           |
| jin           | 250 mHz    | Set Funnel 1 RF    |
| j             | 3000 mHz   | Set Funnel 2 RF    |
|               |            | Set Hexapole RF    |
|               |            | Set Nebulizer      |
|               |            | Set Dry Heater     |
|               |            | Set Dry Gas        |
|               |            | Set Diverter Valve |

2019\_2473\_SEI\_NW352-A62\_3.d:-N



| # | Ion Formula     | m/z       | err [ppm] | mSigma | # mSigma | Score  | rdb  | e^- Conf | N-Rule |
|---|-----------------|-----------|-----------|--------|----------|--------|------|----------|--------|
| 1 | C34H38F3N3O10S3 | 400.58412 | -0.55     | 15.1   | 1        | 100.00 | 16.0 | even     | ok     |

# Mass Spectrum SmartFormula Report

## is Info

is Name D:\Data\Spektren\2019\2019\_2474\_SEI\_NW403-A13.2.d  
; Name tune\_neg\_mid.m  
; Name 2019\_2474\_SEI\_NW403-A113\_2  
; Operator Juliane  
; Date 2019\_2474\_SEI\_NW403-A113\_2  
; Instrument micrOTOF-QIII  
; Analyst Wolf Natalia  
; Sample NW403-A113  
; Concentration 8 pmol/µL in MeOH

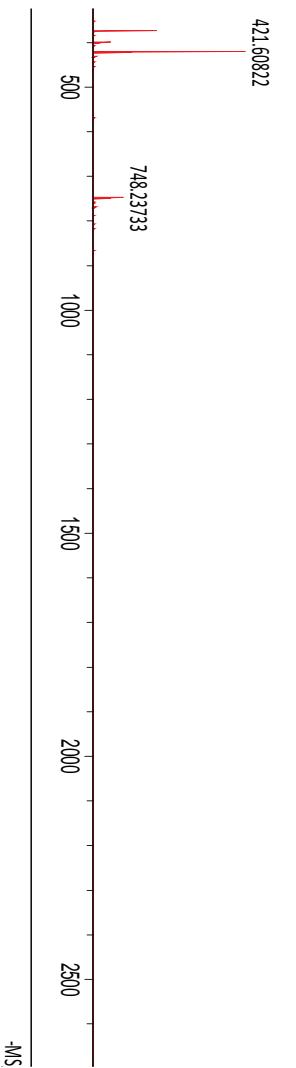
Acquisition Date 12/12/2019

Operator Juliane  
Instrument micrOTOF-QIII

## dition Parameter

| Type | ESI        | Ion Polarity    | Negative | Set Nebulizer    |
|------|------------|-----------------|----------|------------------|
| sgn  | Not active | Set Funnel 1 RF | 30.0 Vpp | Set Dry Heater   |
| rgn  | 250 mHz    | Set Funnel 2 RF | 40.0 Vpp | Set Dry Gas      |
| rd   | 3000 mHz   | Set Hexapole RF | 40.0 Vpp | Set Divert Valve |

2019\_2474\_SEI\_NW403-A113\_2.d.-MS



Mass Spectrum of Sulfo-(6-(trifluoroacetamido)hexylpentamethine Cyanine (**4k**)

| # | Ion Formula     | m/z       | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|---|-----------------|-----------|-----------|--------|----------|--------|------|---------------------|--------|
| 1 | C37H44F3N3O10S3 | 421.60759 | -1.48     | 6.2    | 1        | 100.00 | 16.0 | even                | ok     |

Mass Spectrum of Sulfo-(3-aminopropyl)pentamethine Cyanine (**4I**)

**Analysis Info**

|               |  |                  |                     |
|---------------|--|------------------|---------------------|
|               |  | Acquisition Date | 9/4/2019 7:13:24 PM |
| Analysis Name | D:\Data\spektren2019\04092019_NW351-A41Cy5C3NH2_SEI_52_01_5509.d |                  |                     |
| Method        | Automation_esi_tune_neg_mid.m                                    | Operator         | admin               |
| Sample Name   | 04092019_NW351-A41Cy5C3NH2_SEI                                   | Instrument       | micrOTOF            |
| Comment       | verduennt in MeOH  |                  | 213750.00088        |

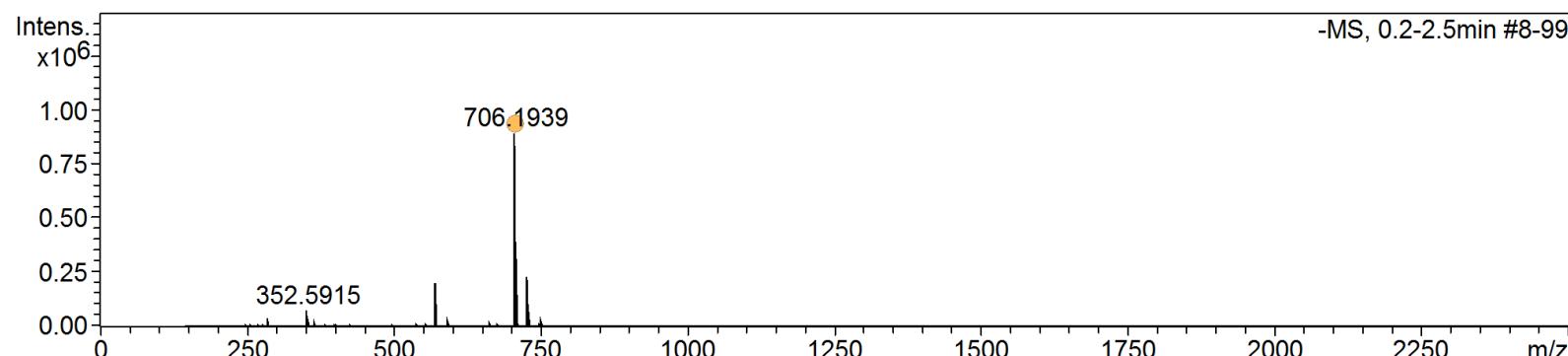
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**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 150 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 3500 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

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**-MS, 0.2-2.5min #8-99**



| Meas. m/z | # | Ion Formula  | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|--|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 706.1939  | 1 | C <sub>32</sub> H <sub>40</sub> N <sub>3</sub> O <sub>9</sub> S <sub>3</sub> | 706.1932 | -0.9      | 126.6  | 1        | 100.00 | 14.5 | even                | ok     |

Mass Spectrum of Sulfo-(6-aminohexyl)pentamethine Cyanine (**4m**)

**Analysis Info**

|               |  |                  |                     |
|---------------|--|------------------|---------------------|
|               |  | Acquisition Date | 9/4/2019 2:33:35 PM |
| Analysis Name | D:\Data\spektren2019\04092019_NW-Cy5C6NH2_SEI_51_01_5481.d |                  |                     |
| Method        | Automation_esi_tune_neg_mid.m                              | Operator         | admin               |
| Sample Name   | 04092019_NW-Cy5C6NH2_SEI                                   | Instrument       | micrOTOF            |
| Comment       | verduennt in MeOH  |                  | 213750.00088        |

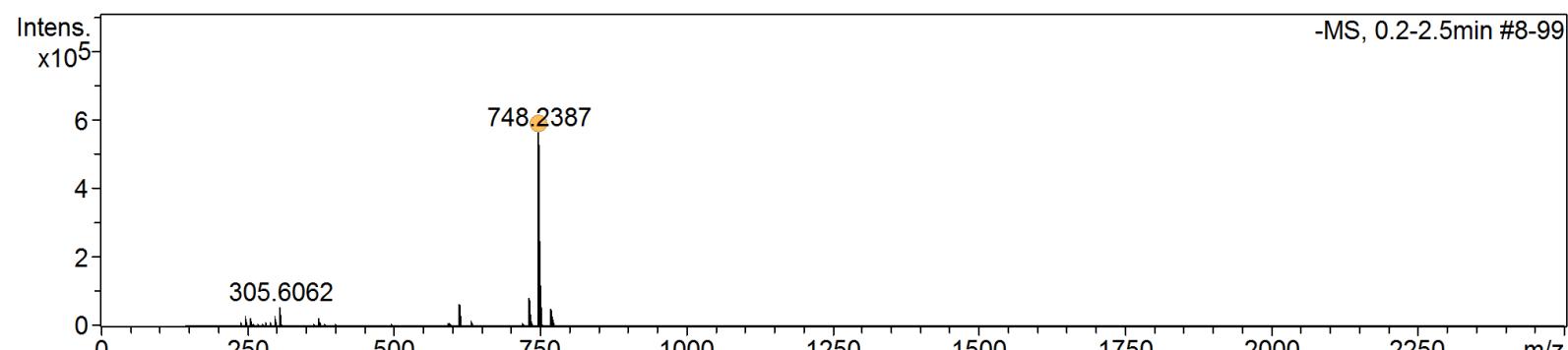
---

**Acquisition Parameter**

|             |            |                      |          |                  |           |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI        | Ion Polarity         | Negative | Set Nebulizer    | 0.7 Bar   |
| Focus       | Not active | Set Capillary        | 3200 V   | Set Dry Heater   | 200 °C    |
| Scan Begin  | 150 m/z    | Set End Plate Offset | -500 V   | Set Dry Gas      | 5.0 l/min |
| Scan End    | 3500 m/z   | n/a                  | n/a      | Set Divert Valve | Source    |

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**-MS, 0.2-2.5min #8-99**



| Meas. m/z | # | Ion Formula  | m/z      | err [ppm] | mSigma | # mSigma | Score  | rdb  | e <sup>-</sup> Conf | N-Rule |
|-----------|---|--------------|----------|-----------|--------|----------|--------|------|---------------------|--------|
| 748.2387  | 1 | C35H46N3O9S3 | 748.2402 | 1.9       | 13.8   | 1        | 100.00 | 14.5 | even                | ok     |

### **Analytical HPLC Measurements**

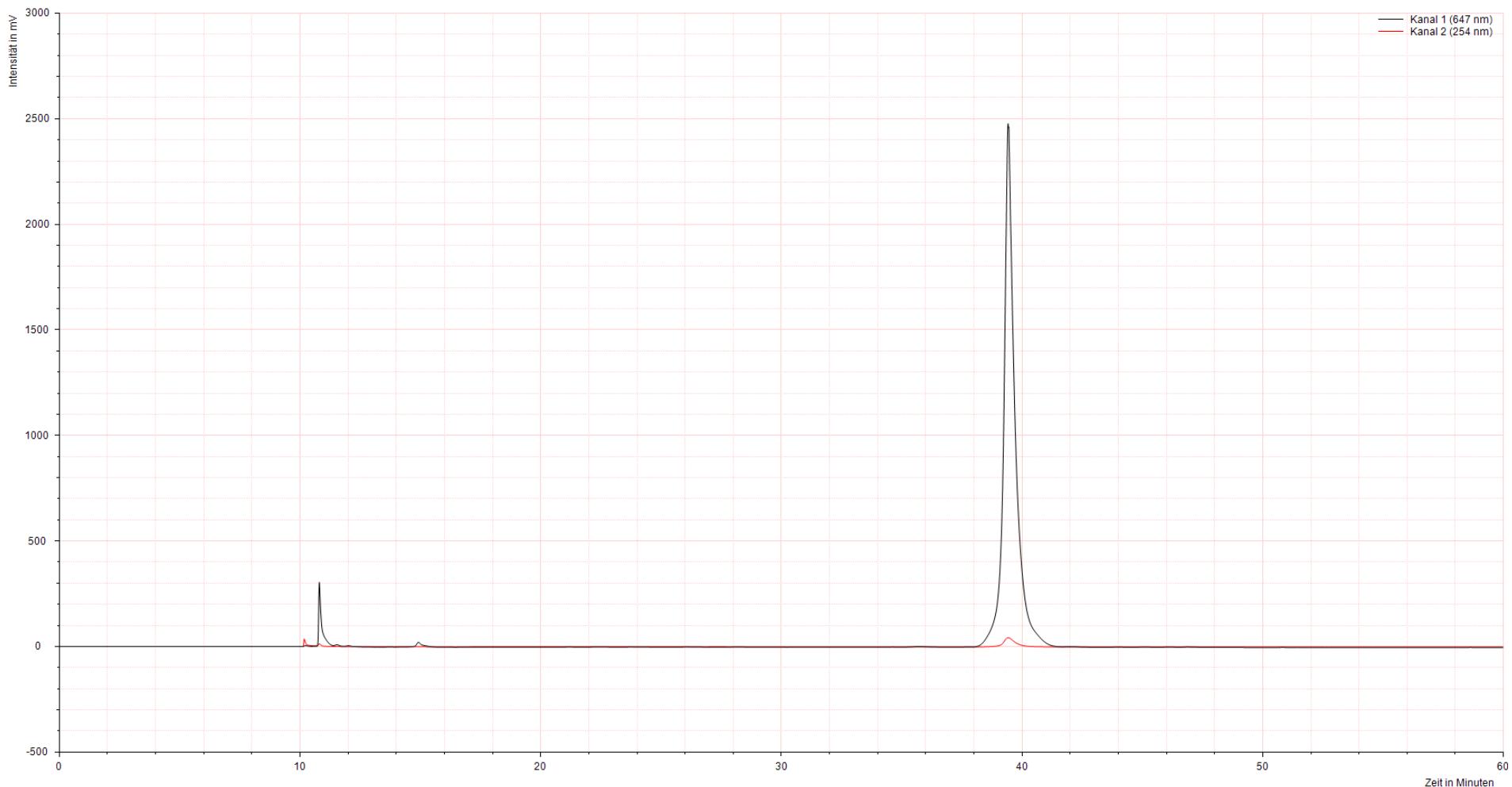
Measurements were performed on a system consisting of a quaternary pump, an injection valve and a DAD detector by SYKAM. Analyses were run on a RP-18 column from YMC (YMC-Pack ODS-AQ, 250 x 4.6 mm, S-5  $\mu$ m, 12 nm) and recorded on two wavelength channels: 254 nm and 647 nm. Different systems were applied.

System 1: Solvent A: 10 mM ammonium acetate in H<sub>2</sub>O, solvent B: 10 mM ammonium acetate in MeOH. Linear gradient from 100% A to 50% B in A in 60 min. Flow rate: 0.6 mL/min.

System 2: Solvent A: 10 mM ammonium acetate in H<sub>2</sub>O, solvent B: 10 mM ammonium acetate in MeOH. Linear gradient from 20% B in A to 80% B in A in 60 min. Flow rate: 0.6 mL/min.

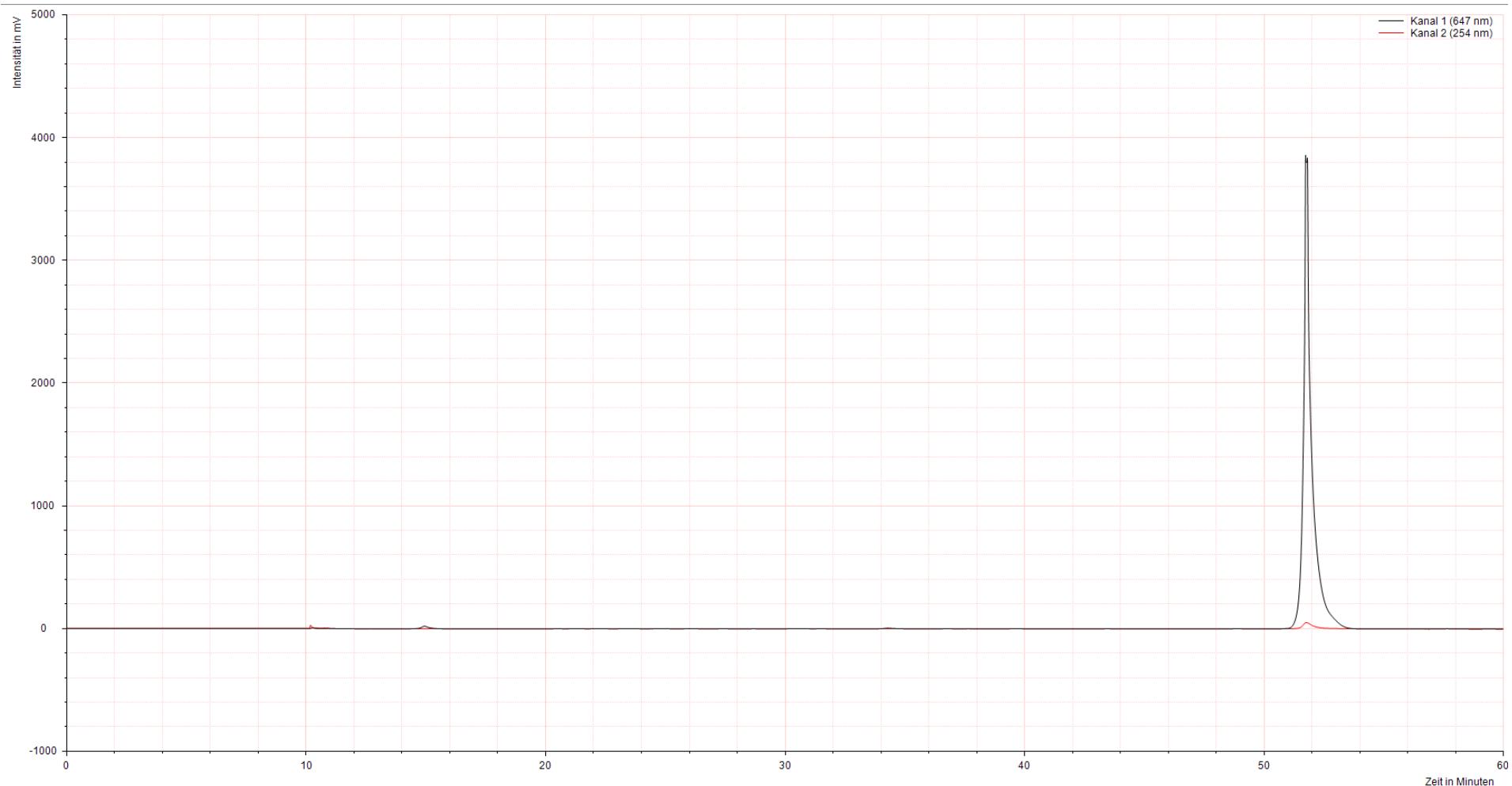
The retention time is abbreviated by  $t_R$ . The purity was calculated from the 647 nm data set through integration of all peaks.

### Sulfo-(3-azidopropyl)pentamethine Cyanine (4a)



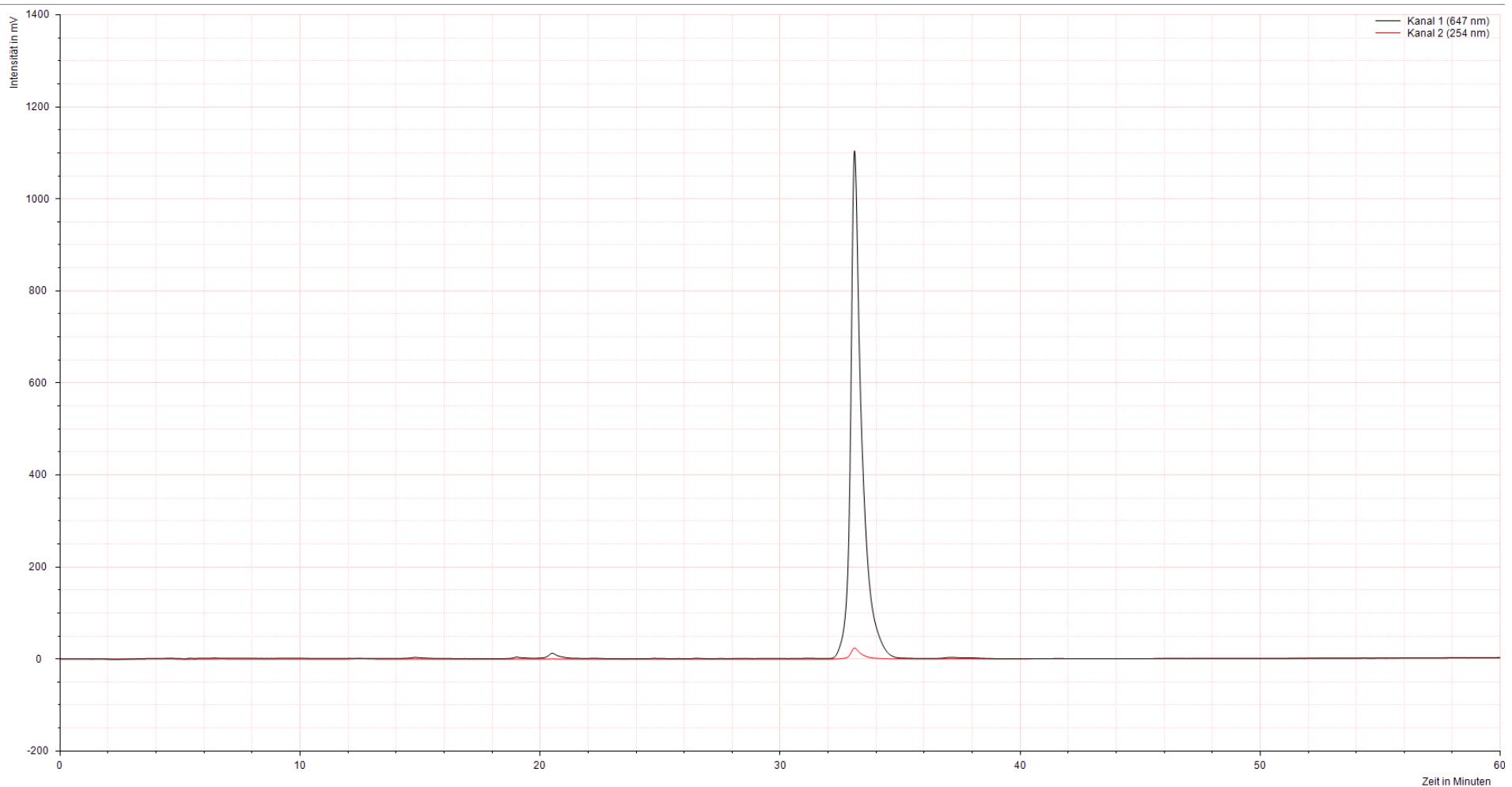
System 1 was used.  $t_R = 39.4$  min. Purity: 96%.

### Sulfo-(6-azidohexyl)pentamethine Cyanine (4b)



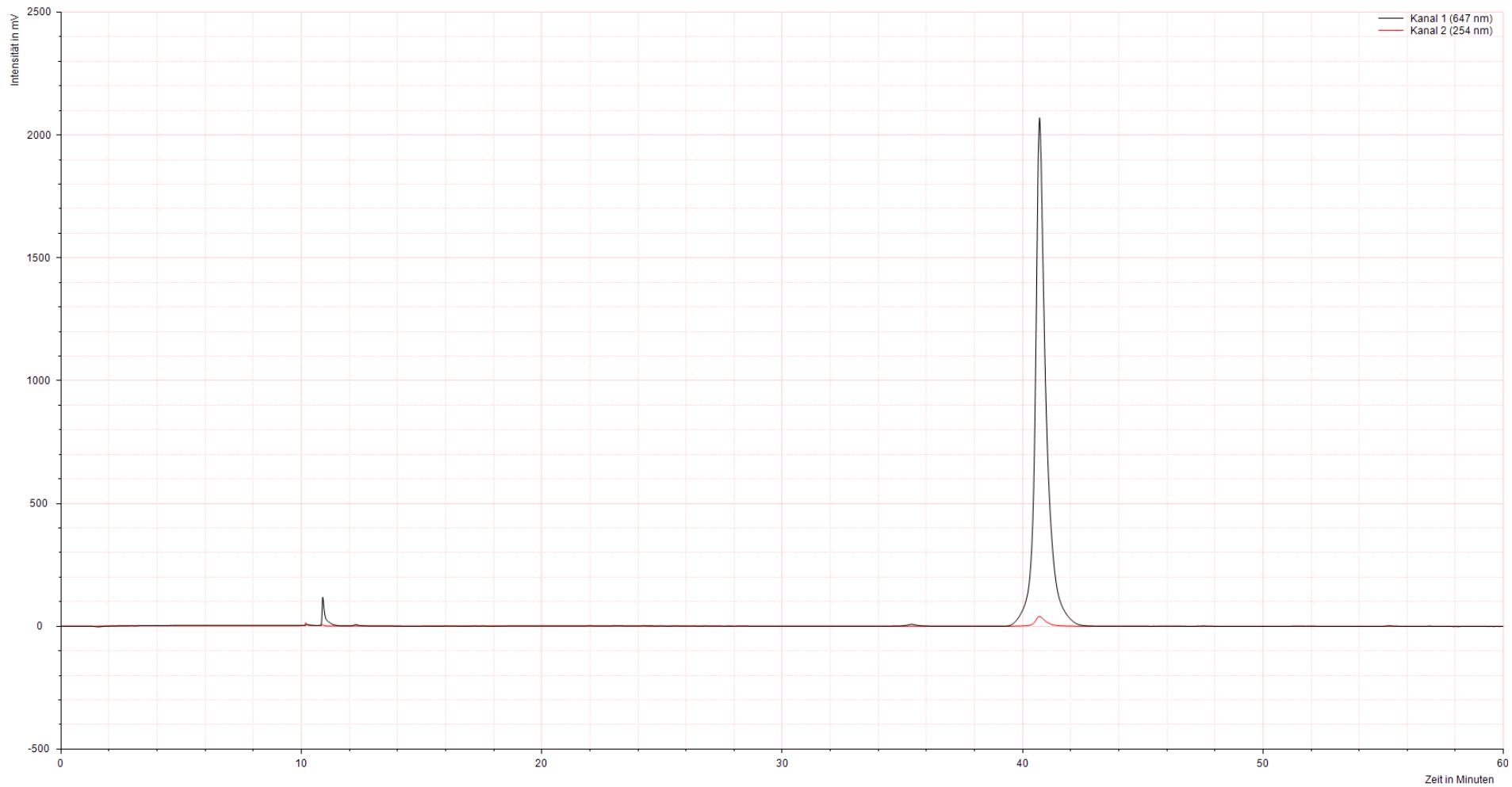
System 1 was used.  $t_R = 51.8$  min. Purity > 99%.

### Sulfo-(9-azidononyl)pentamethine Cyanine (4c)



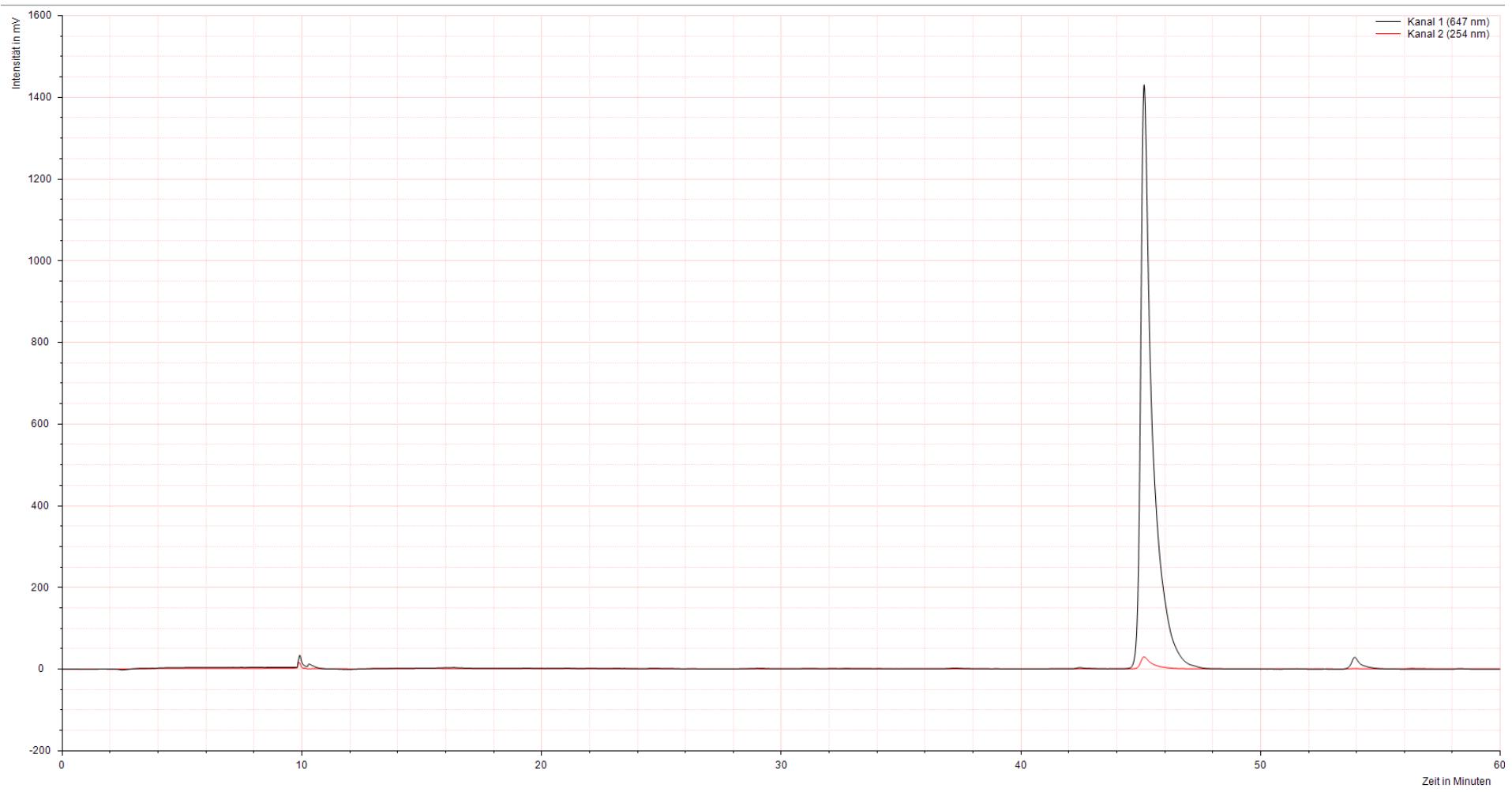
System 2 was used.  $t_R = 33.1$  min. Purity 98%.

### Sulfo-(pentynyl)pentamethine Cyanine (4d)



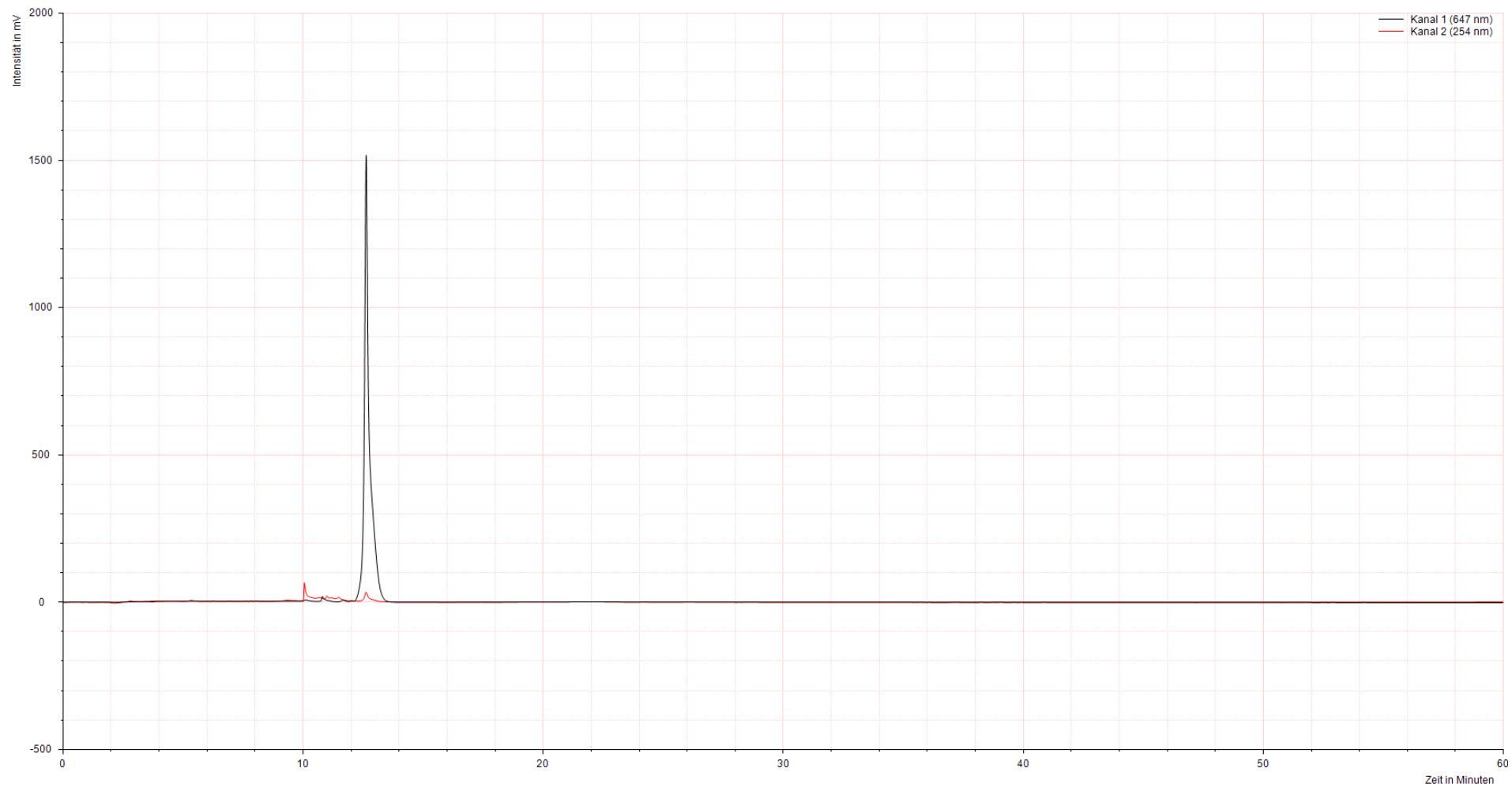
System 1 was used.  $t_R = 40.7$  min. Purity: 98%.

### Sulfo-(hexynyl)pentamethine Cyanine (4e)



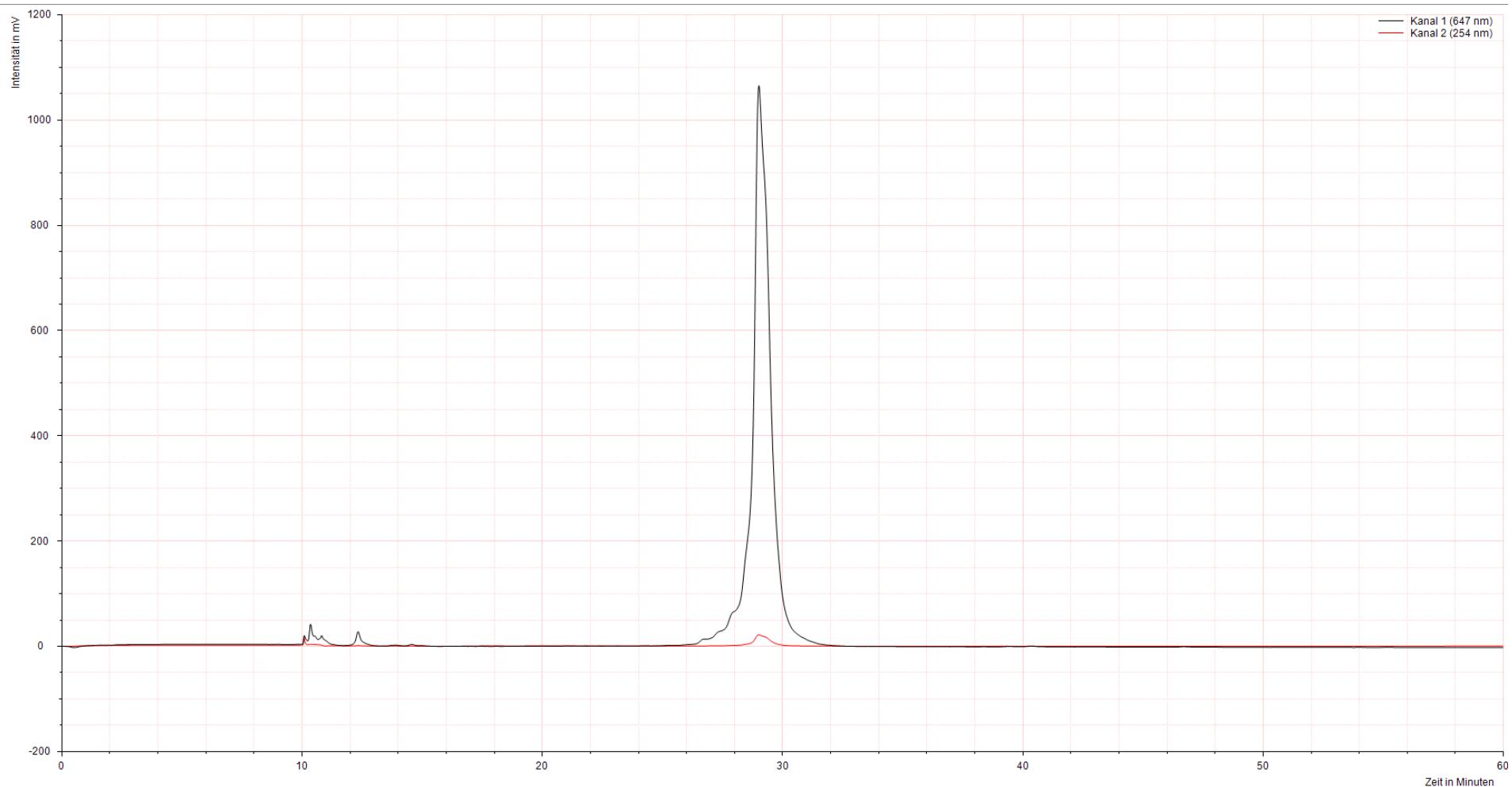
System 1 was used.  $t_R = 45.1$  min. Purity: 97%.

### Sulfo-(3-cyanopropyl)pentamethine Cyanine (4f)



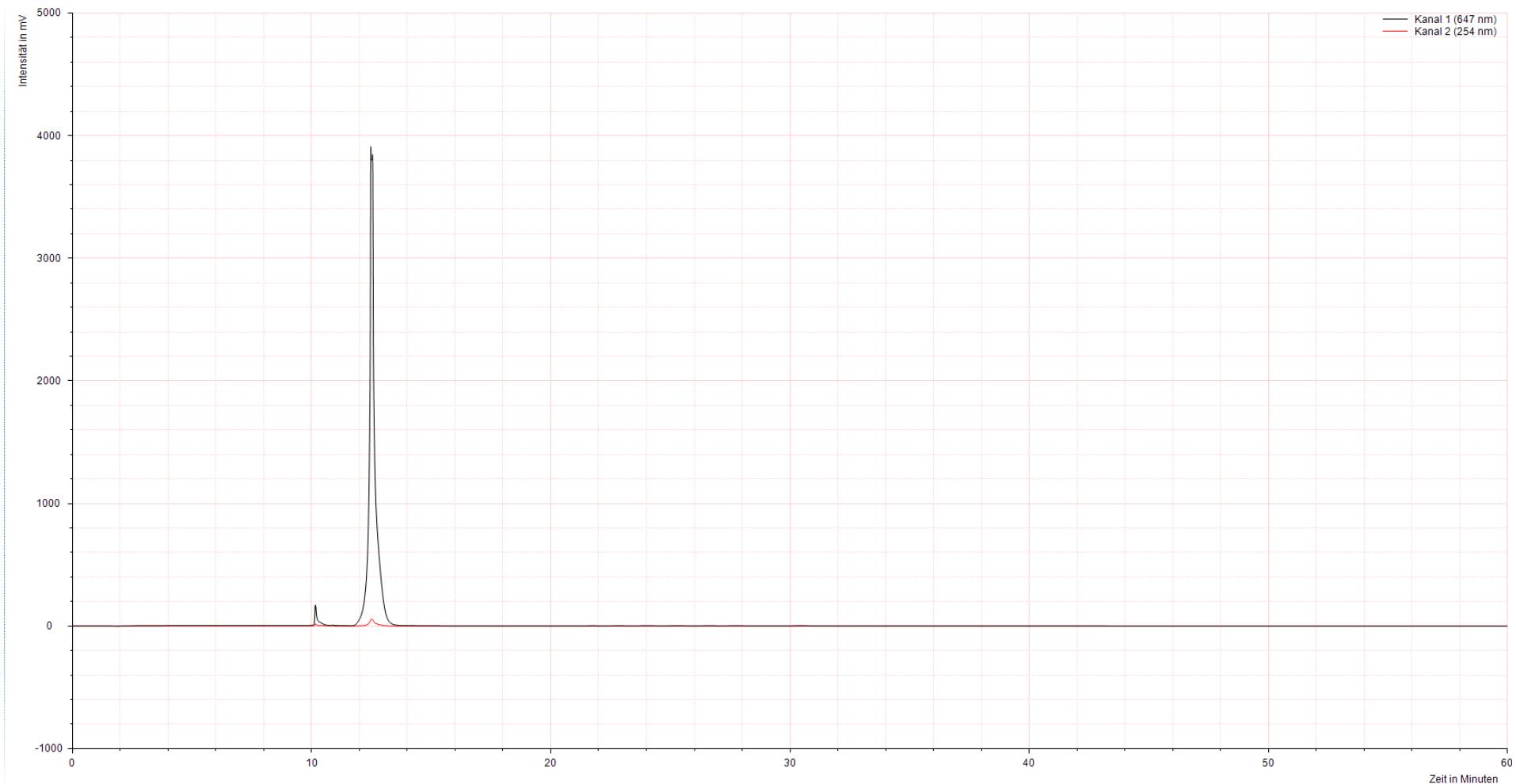
System 1 was used.  $t_R = 12.6$  min. Purity: 99%.

### Sulfo-(5-cyanopentyl)pentamethine Cyanine (4g)



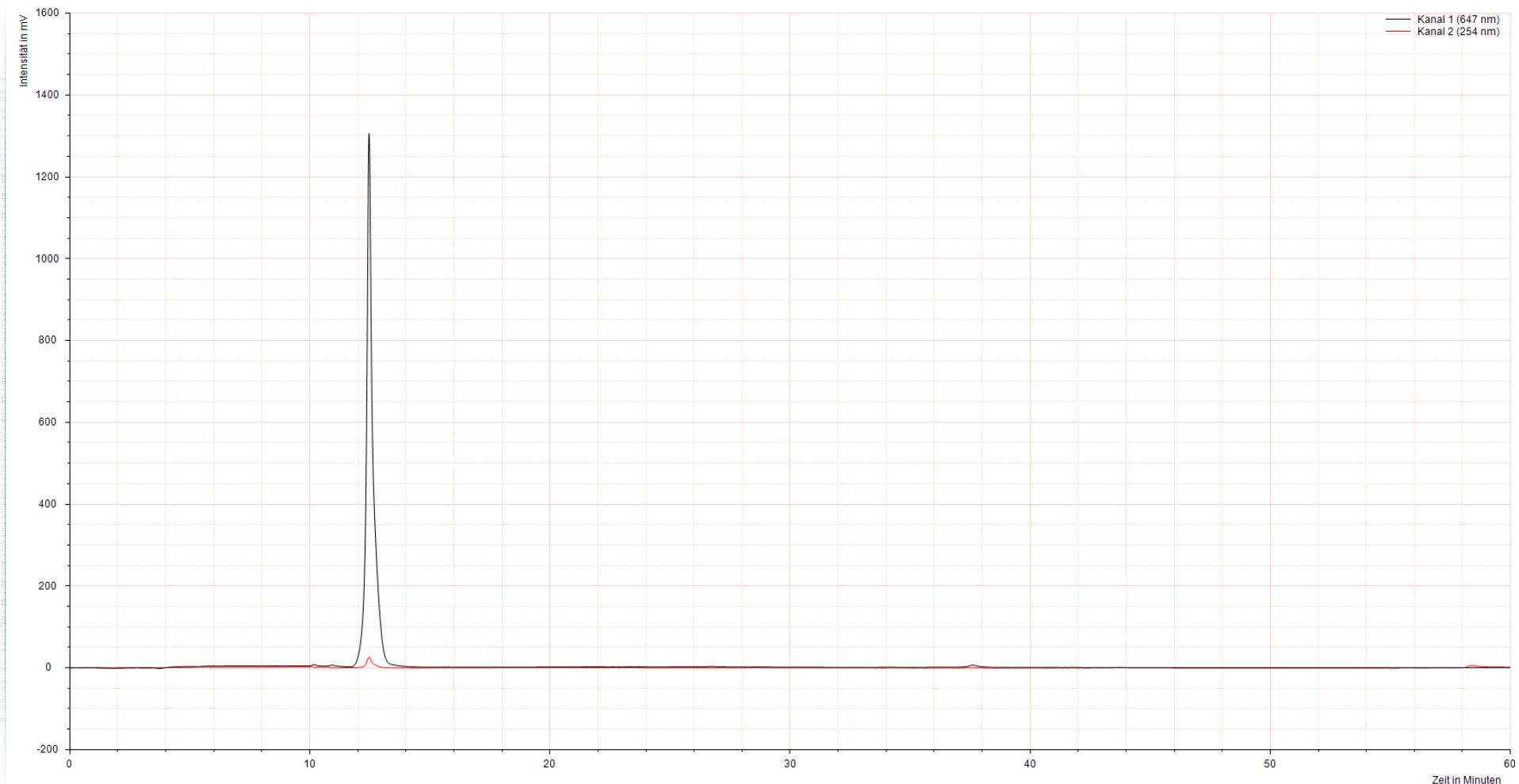
System 1 was used.  $t_R = 29.0$  min. Purity: 97%.

### Sulfo-(3-carboxypropyl)pentamethine Cyanine (4h)



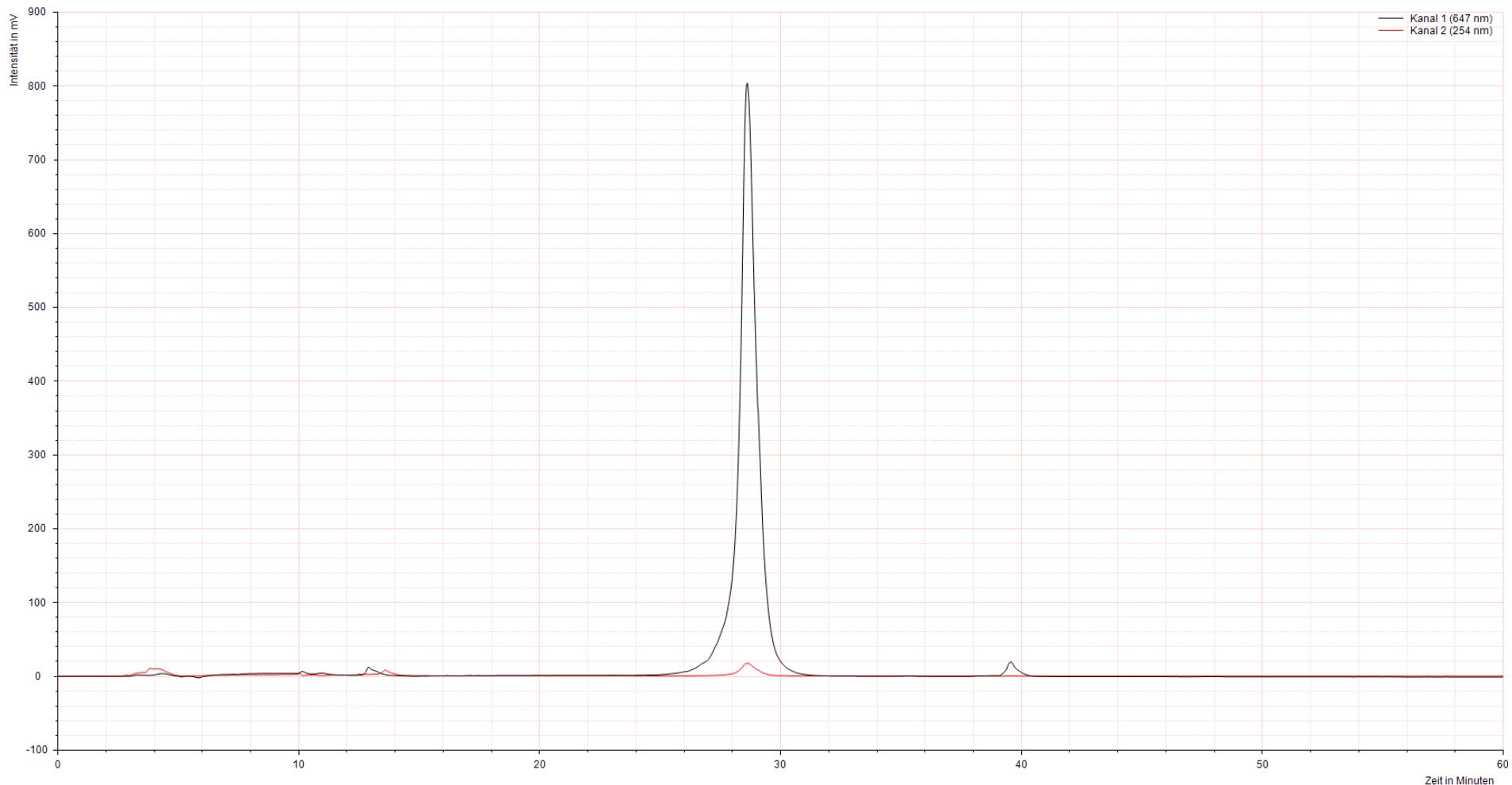
System 1 was used.  $t_R = 12.5$  min. Purity 98%.

### Sulfo-(5-carboxypentyl)pentamethine Cyanine (4i)



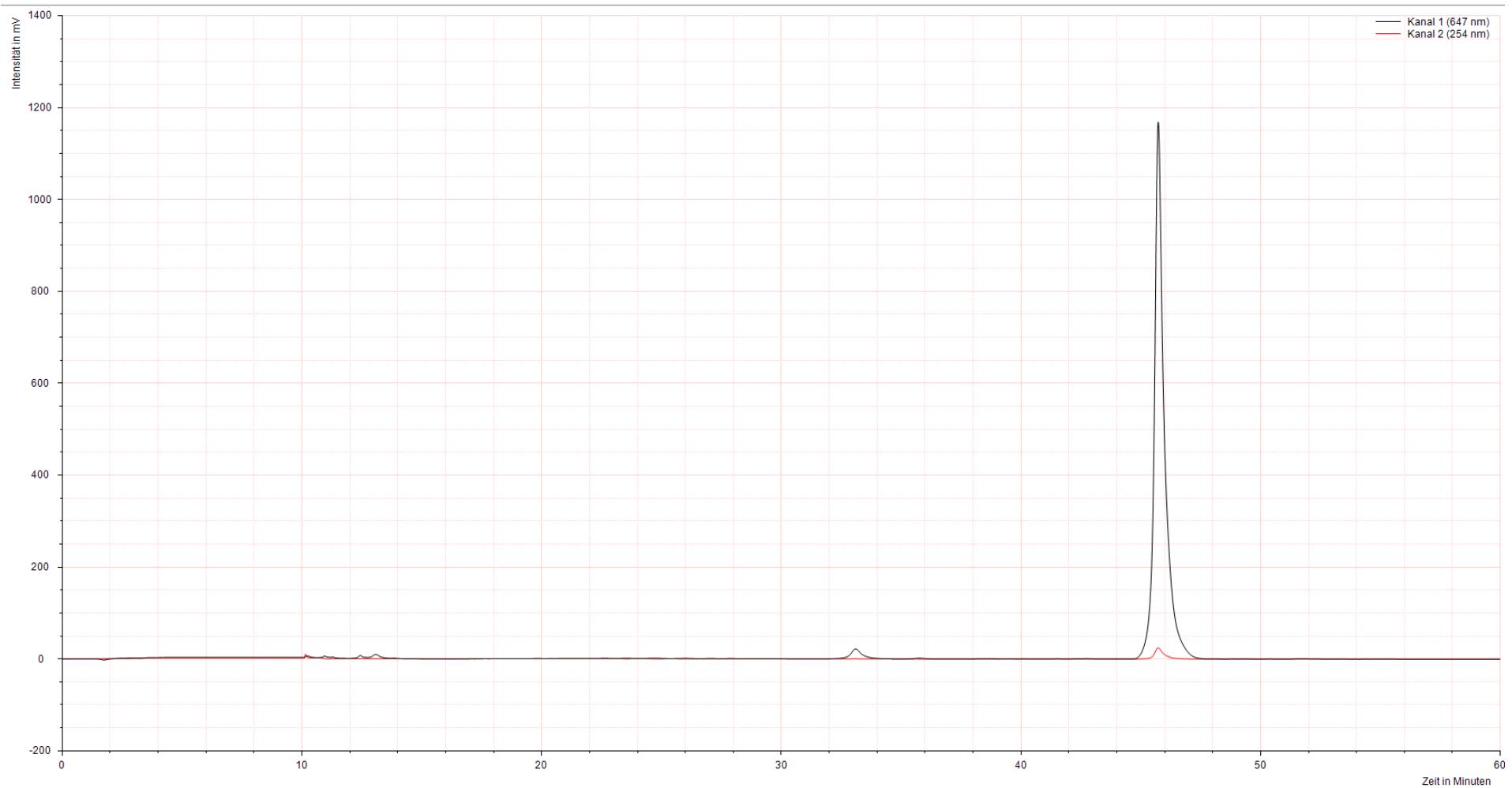
System 1 was used.  $t_R = 12.5$  min. Purity >99%.

### Sulfo-(3-(trifluoroacetamido)propyl)pentamethine Cyanine (4j)



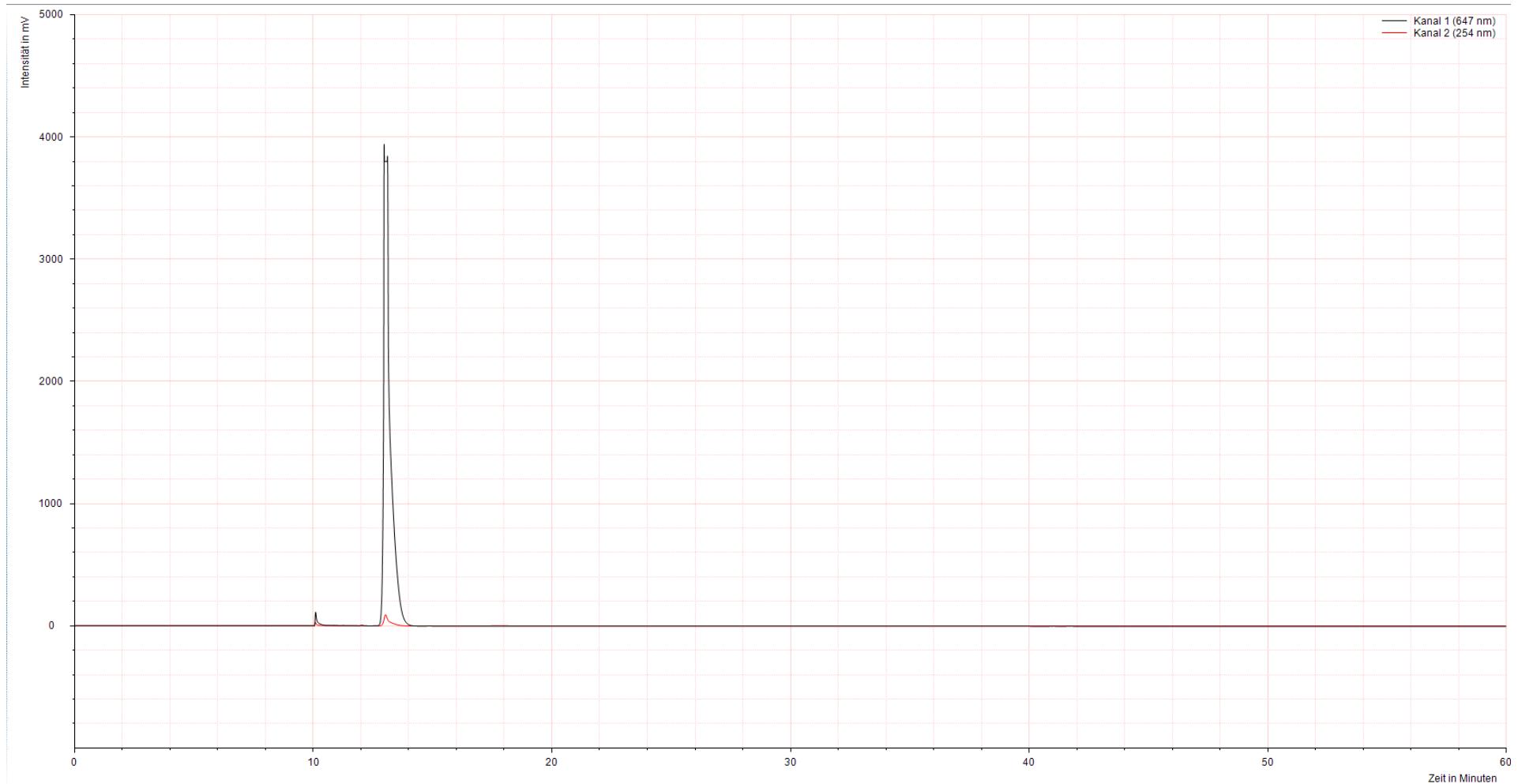
System 1 was used.  $t_R = 28.6$  min. Purity 97%.

### Sulfo-(6-(trifluoroacetamido)hexyl)pentamethine Cyanine (4k)



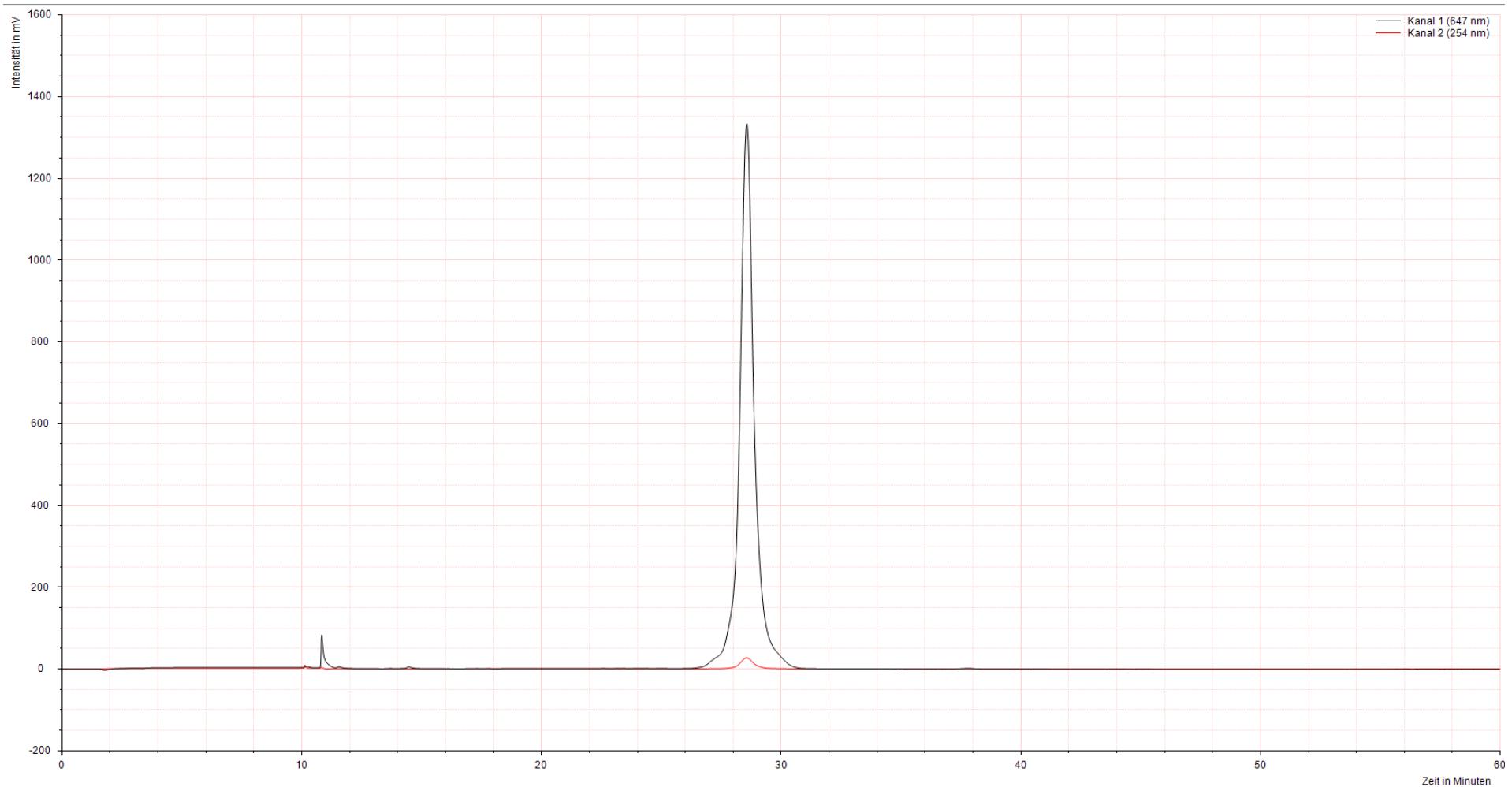
System 1 was used.  $t_R = 45.7$  min. Purity: 97%.

### Sulfo-(3-aminopropyl)pentamethine Cyanine (4l)



System 1 was used.  $t_R = 13.0$  min. Purity: > 99%.

### Sulfo-(6-aminohexyl)pentamethine Cyanine (4m)



System 1 was used.  $t_R = 28.6$  min. Purity: 98%.