

Photoactivatable Rhodamine Spiroamides and Diazoketones Decorated with “Universal Hydrophilizer” or Hydroxyl Groups

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Supporting Information

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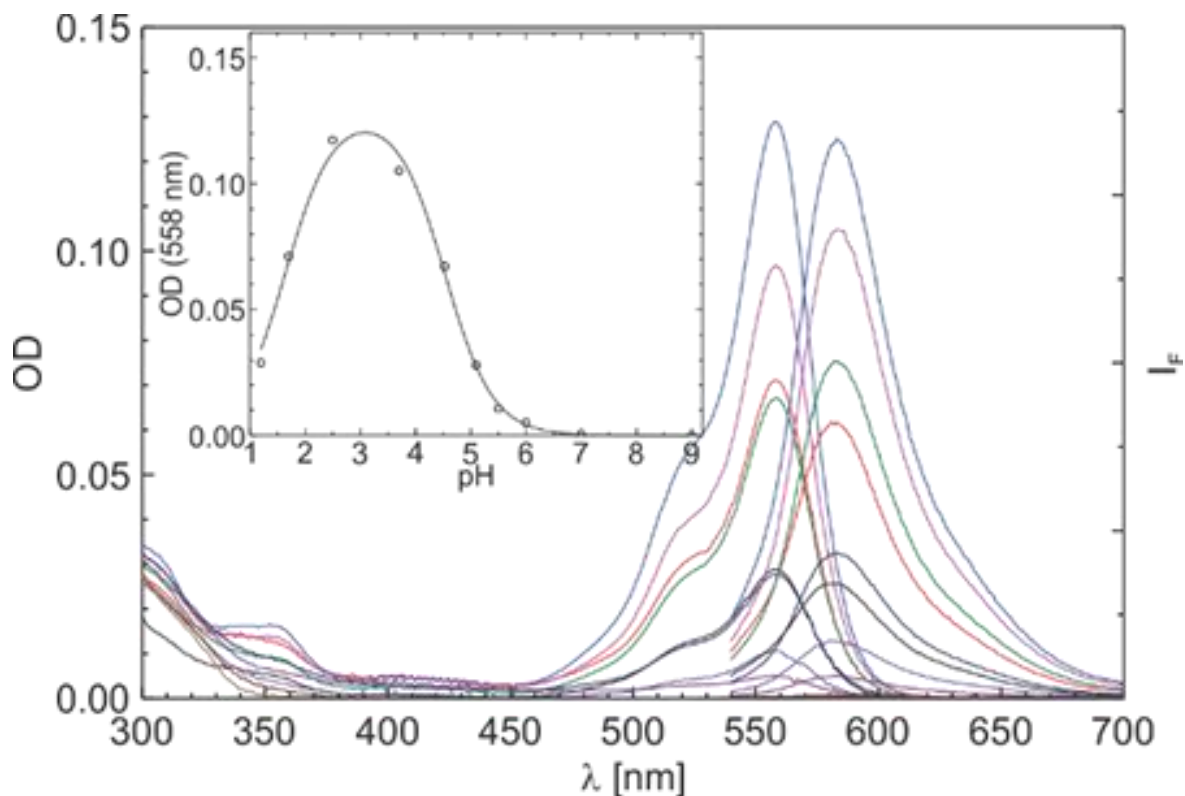


Figure S1. Absorption and emission of compound **4-H / 4-Li** in buffered solutions. The absorption intensity at the maximum (558 nm) is plot vs. the pH of the solution in the inset; data was fit with a two-equilibrium model; the plots related to compound **5-H** are given in Figure 1 (main text)

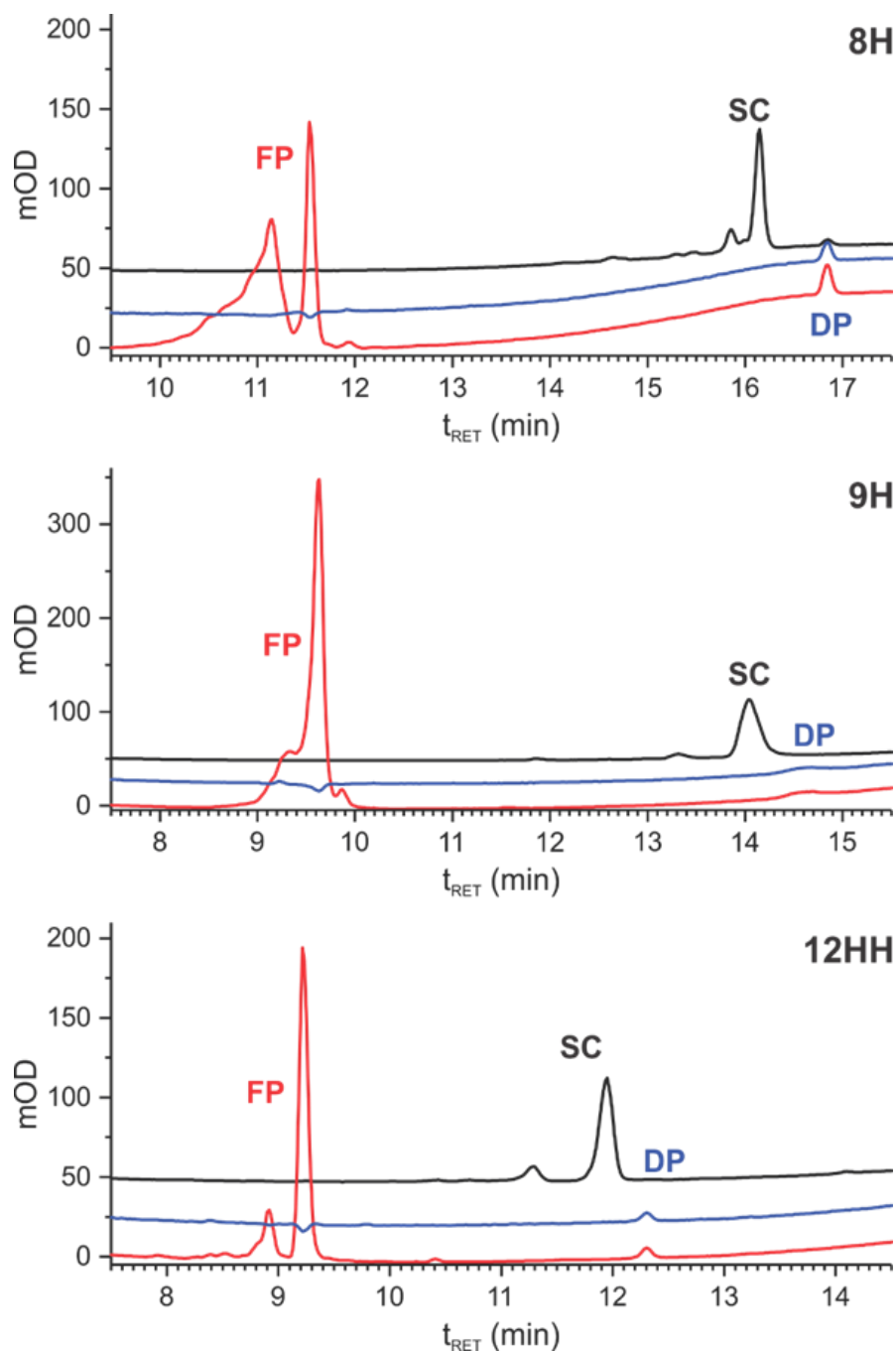


Figure S2. HPLC traces of the initial solutions of compounds **8-H**, **9-H** and **12-H,H** with detection at the absorption maxima of the starting (masked) compounds (SC; 344 nm, black curves), irradiated solutions at full conversion with detection at the absorption maxima of the fluorescent product (FP; 590-598nm, red curves), and at 660 nm (blue curves), where only the dark by-product (DP) absorbs. The contribution of the DP to the absorption at the maximum of the FP was accounted for in calculations. DP contributed less than 5% to the total absorption ($\text{Area-DP}/[\text{Area-DP}+\text{Area-FP}]$) was measured at the maximum of the FP). The negative peaks produced by the FP at 660 nm are due to fluorescence emission (diode array detector). Double peaks for the SC and the FP are attributed to the mixtures of isomers ($5'/6'$).

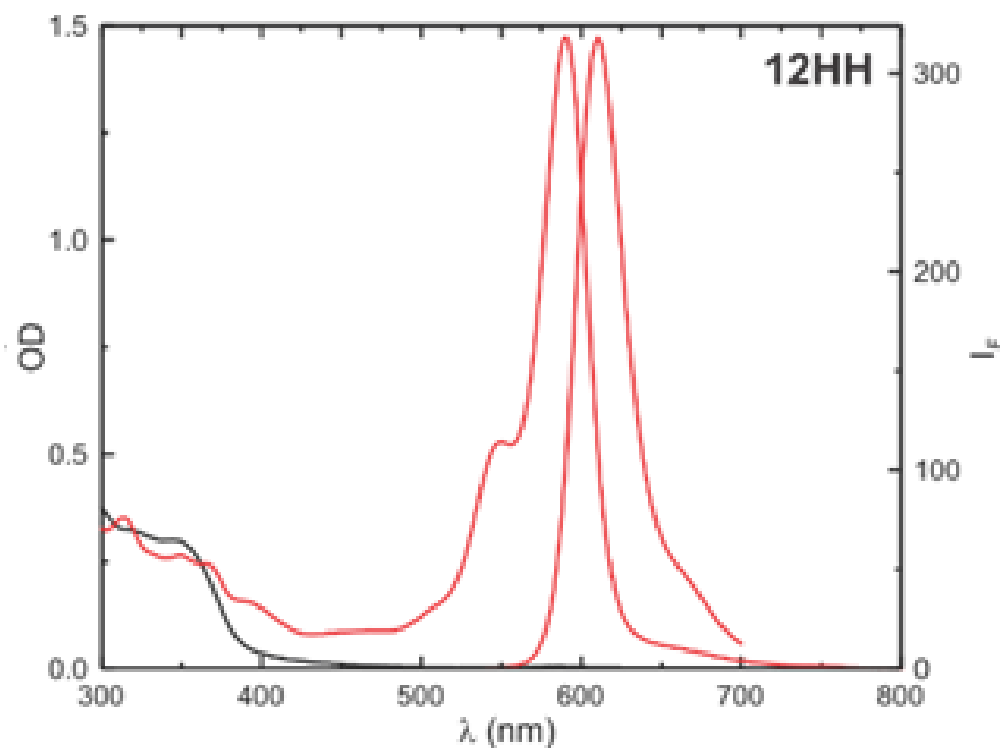


Figure S3. Absorption and emission spectra of compound **12-H,H** in the course of photoactivation with 405 nm light (solutions in methanol). Black curve: initial state (masked), red curves: absorption and emission upon full activation (unmasked).

Cloning (see Figure 4 in the main text)

The hORFeome collection was used to amplify the *KRT6A* ORF by PCR. Subsequently the PCR fragment was cloned into the Gateway donor vector pDONR223. Finally the pH-Krt6A-Halo expression plasmid was constructed by Gateway vector conversion (Invitrogen, Carlsbad, CA, USA) from the donor vector pDONR223-Krt6A and the empty destination vector pH-Gateway-Halo (Lamesch, P. *et al.* hORFeome v3.1: a resource of human open reading frames representing over 10,000 human genes. *Genomics* **89**, 307-315 (2007)).

Cell Culture (see Figure 4 in the main text)

In this study, the mammalian cell line Ptk2 was utilized for all experiments. Ptk2 cells are epithelial kidney cells derived from a male rat-kangaroo (*Potorous tridactylis*) and were cultivated in DMEM with Glutamax and 4.5g/L glucose (Invitrogen, Carlsbad, CA, USA), supplemented with 50 U/mL penicillin, 50 µg/mL streptomycin (all Biochrom, Berlin, Germany), 1 mM Na-pyruvate (Sigma-Aldrich, St.Louis, MO, USA), and 10 % (vol/vol) FCS (Invitrogen, Carlsbad, CA, USA) at 37 °C and 5 % (vol/vol) CO₂.

For transient transfection with pH-Krt6A-Halo the Nanofectin Kit (PAA, Pasching, Austria) was used according to the manufacturer's recommendations. Cells were labelled and imaged 24 – 48 h after transfection.

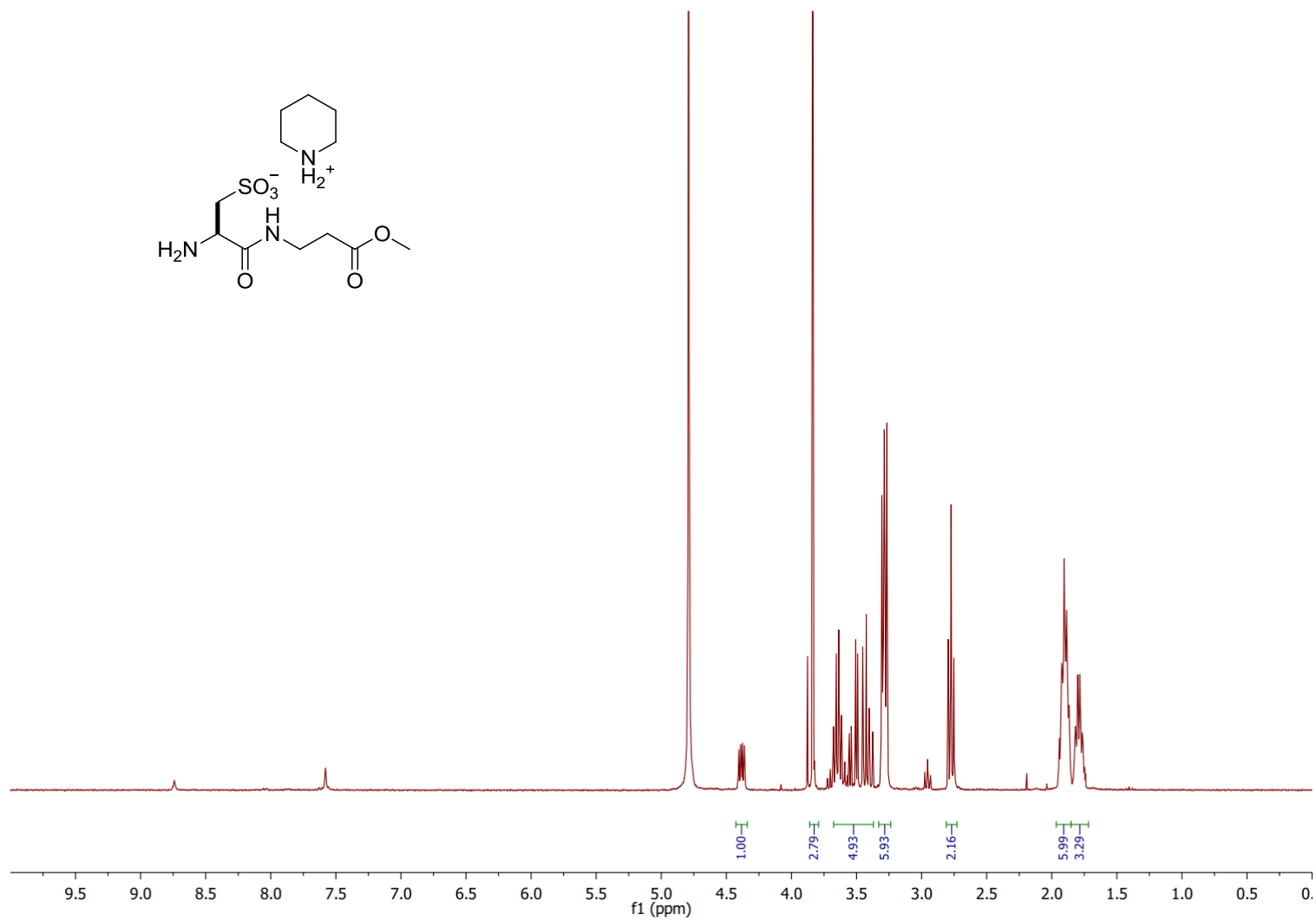
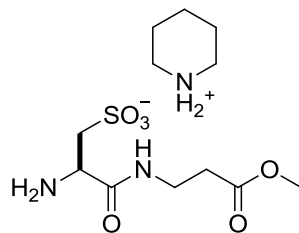
Lasers (see Figures 3 and 4 in the main text)

A 532 nm laser (LC-532-200, Shanghai Laser Century Technology Co., Shanghai, China) was used for wide-field excitation with an average intensity of 2 kW/cm² in the sample plane. For activation, a 375 nm laser (Cube 375-16C, Coherent Inc., Santa Clara, CA, USA) was kept constant at an average intensity of 0.1 W/cm² during the acquisition.

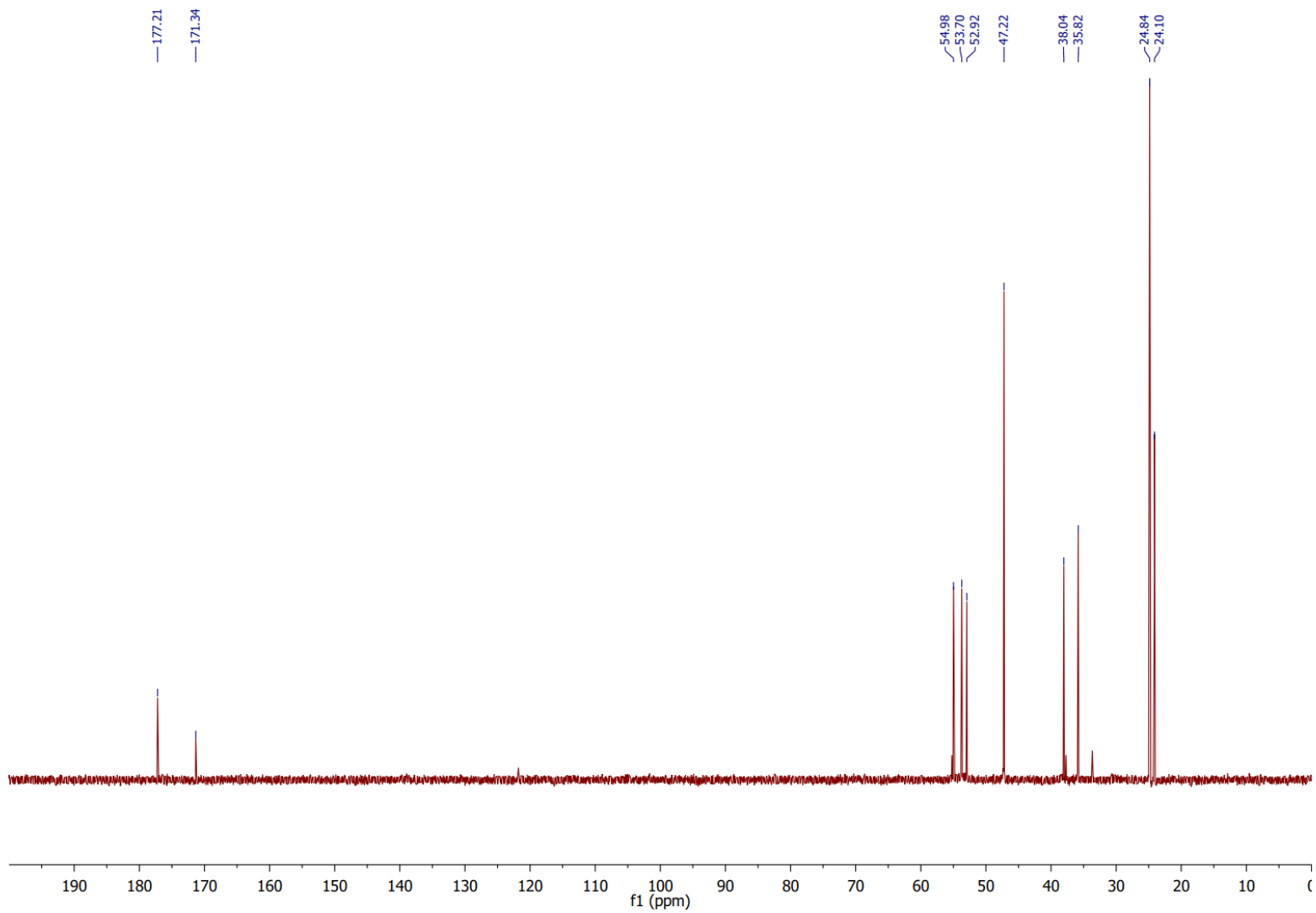
NMR and HRMS-ESI spectra

Methyl *N*-(3-sulfo-L-alanyl)-3-aminopropanoate piperidinium salt (2)

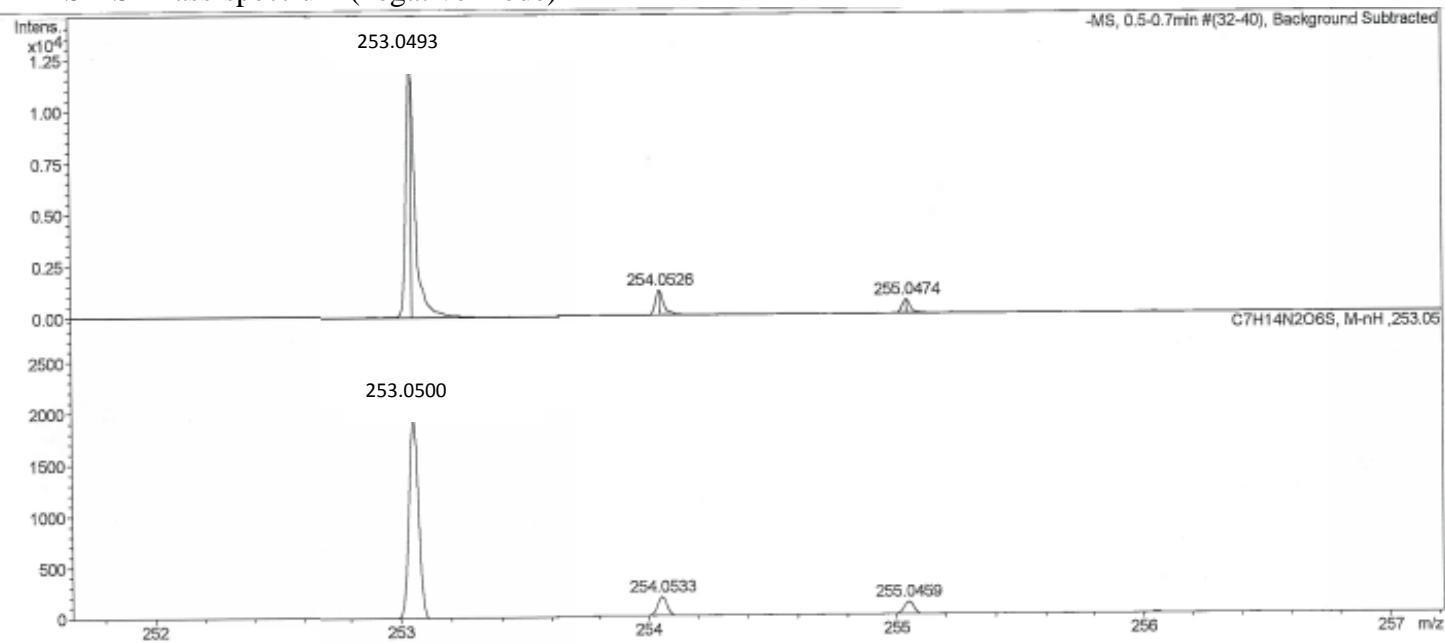
^1H NMR spectrum recorded in D_2O (300 MHz)



^{13}C NMR spectrum recorded in D_2O (125 MHz)

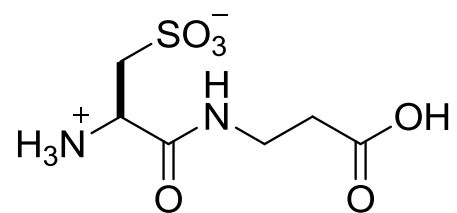


HRMS-ESI mass-spectrum (negative mode)

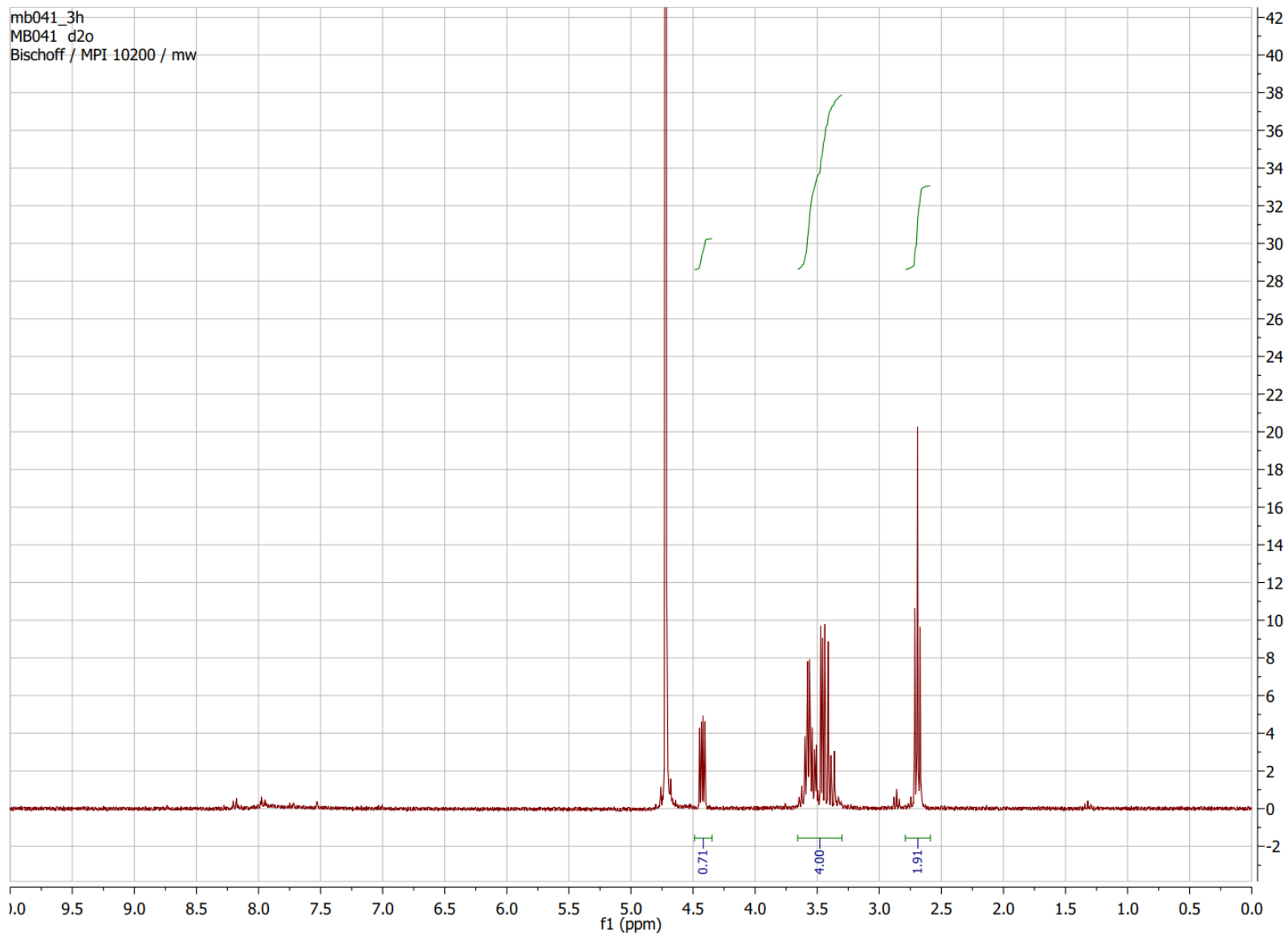


(R)-3-(2-amino-3-sulfopropanamido)propanoic acid (3)

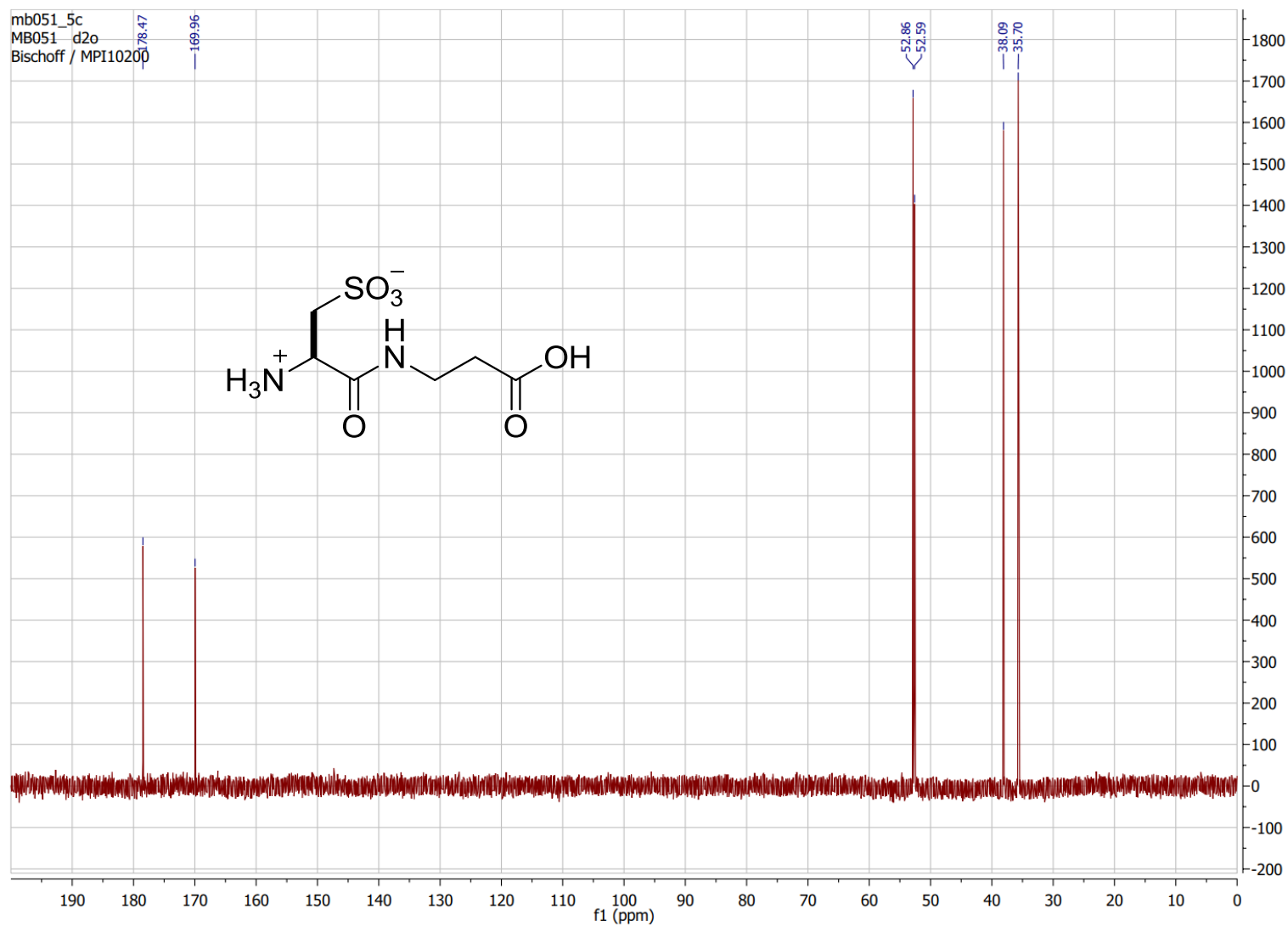
¹H NMR spectrum recorded in D₂O (300 MHz)



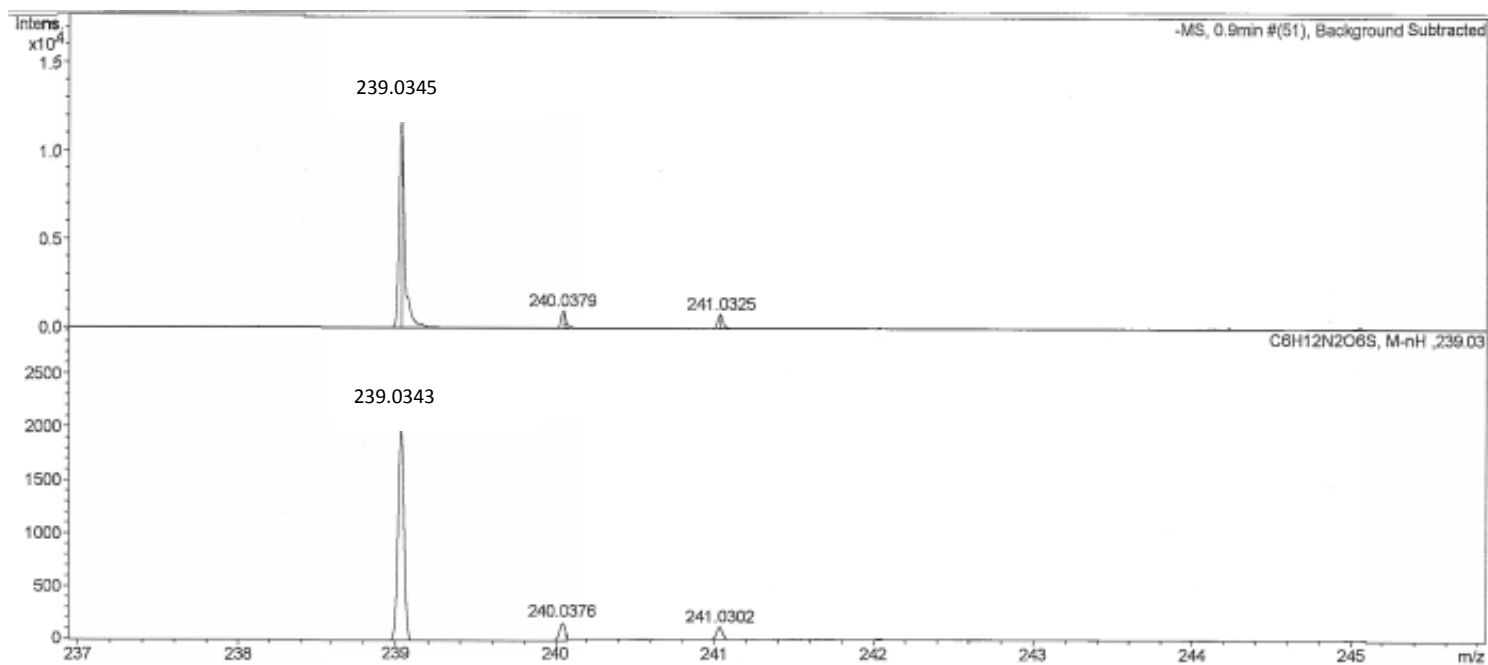
mb041_3h
MB041 d2o
Bischoff / MPI 10200 / mw



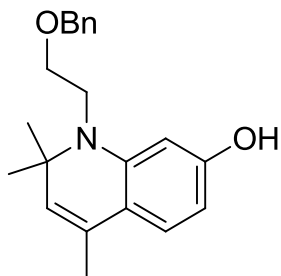
^{13}C NMR spectrum recorded in D_2O (125 MHz)



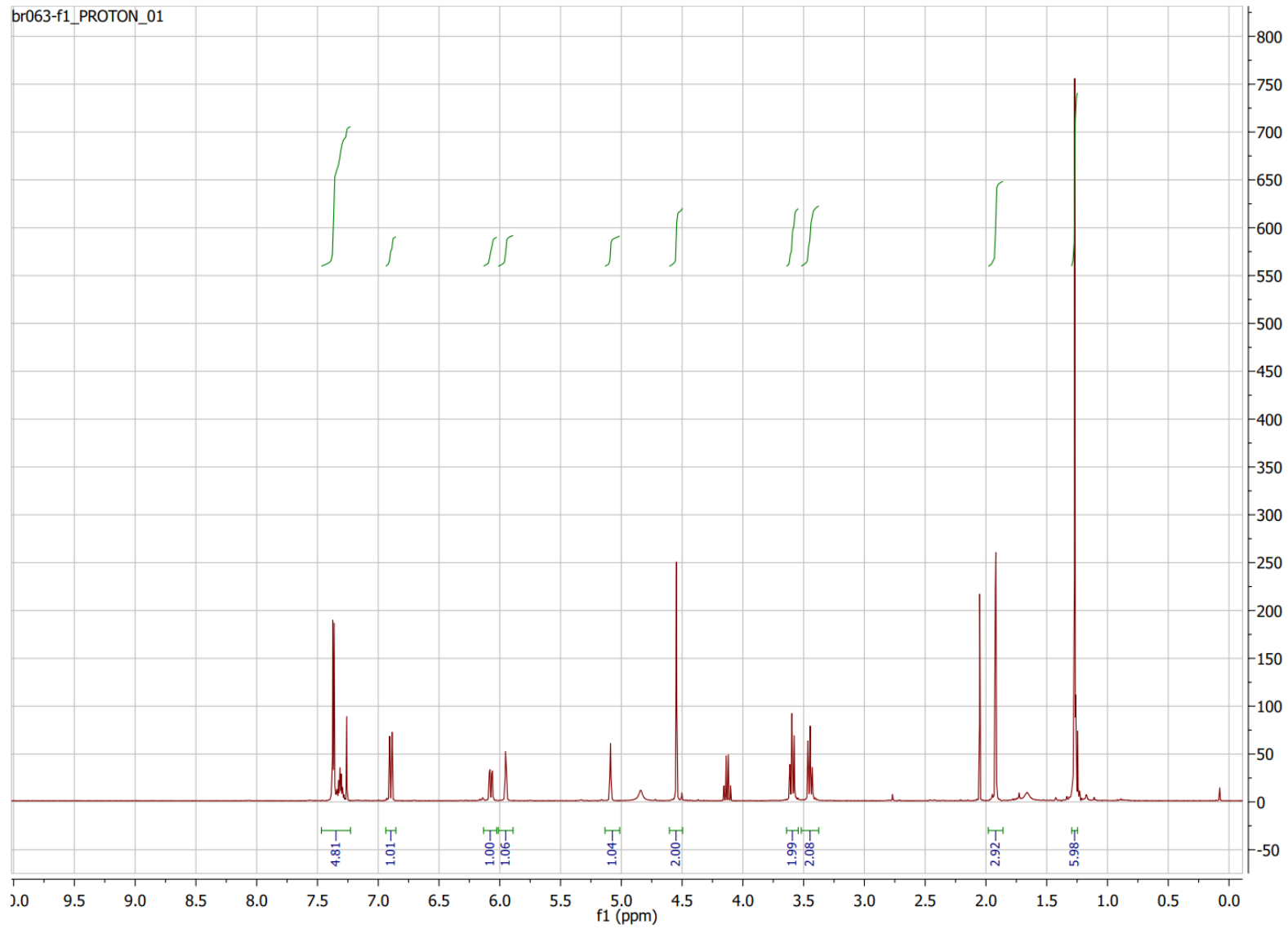
HRMS-ESI mass spectra (negative mode)



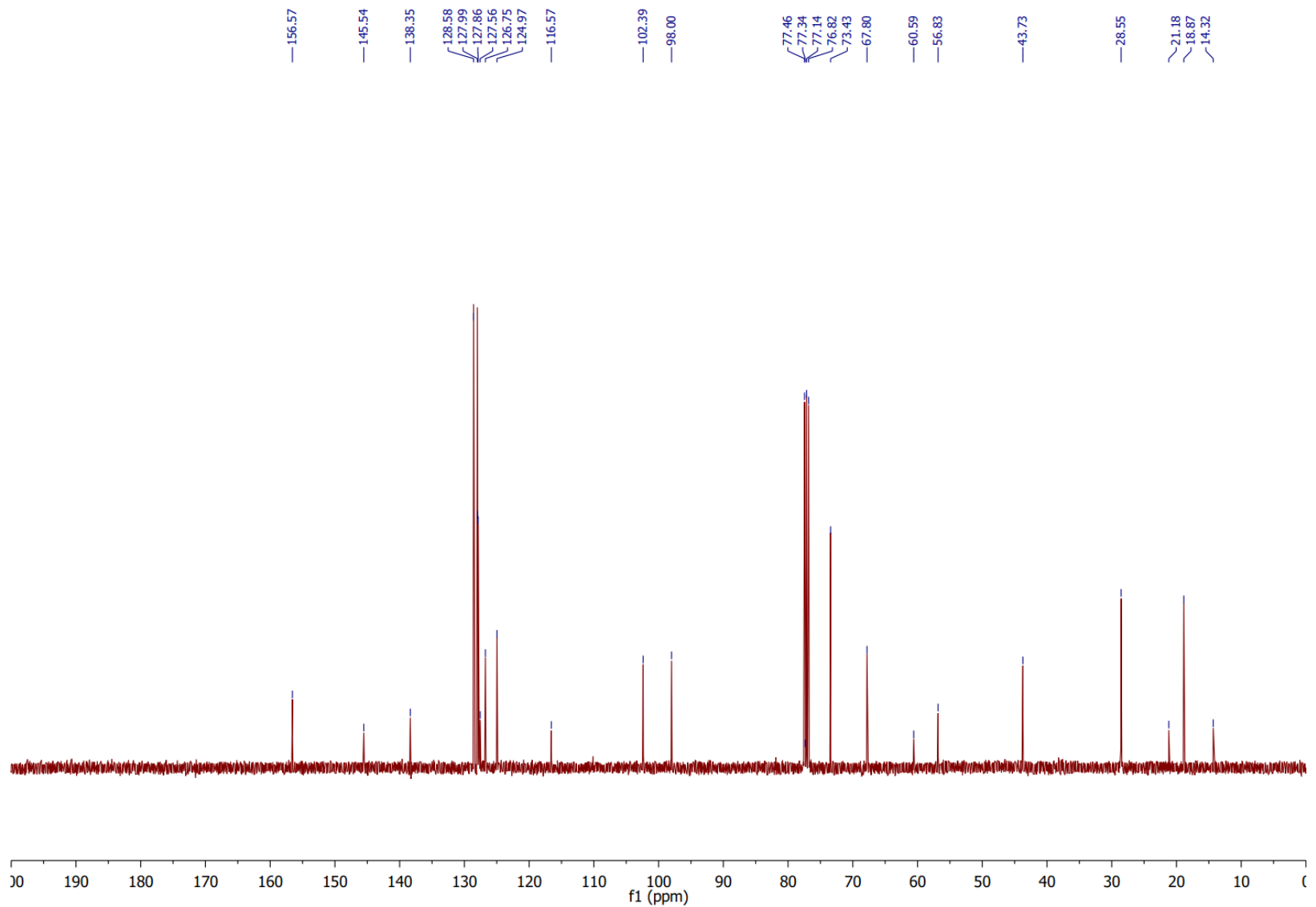
NMR and ESI spectra of 1-[2-(benzyloxy)ethyl]-2,2,4-trimethyl-1,2-dihydroquinolin-7-ol



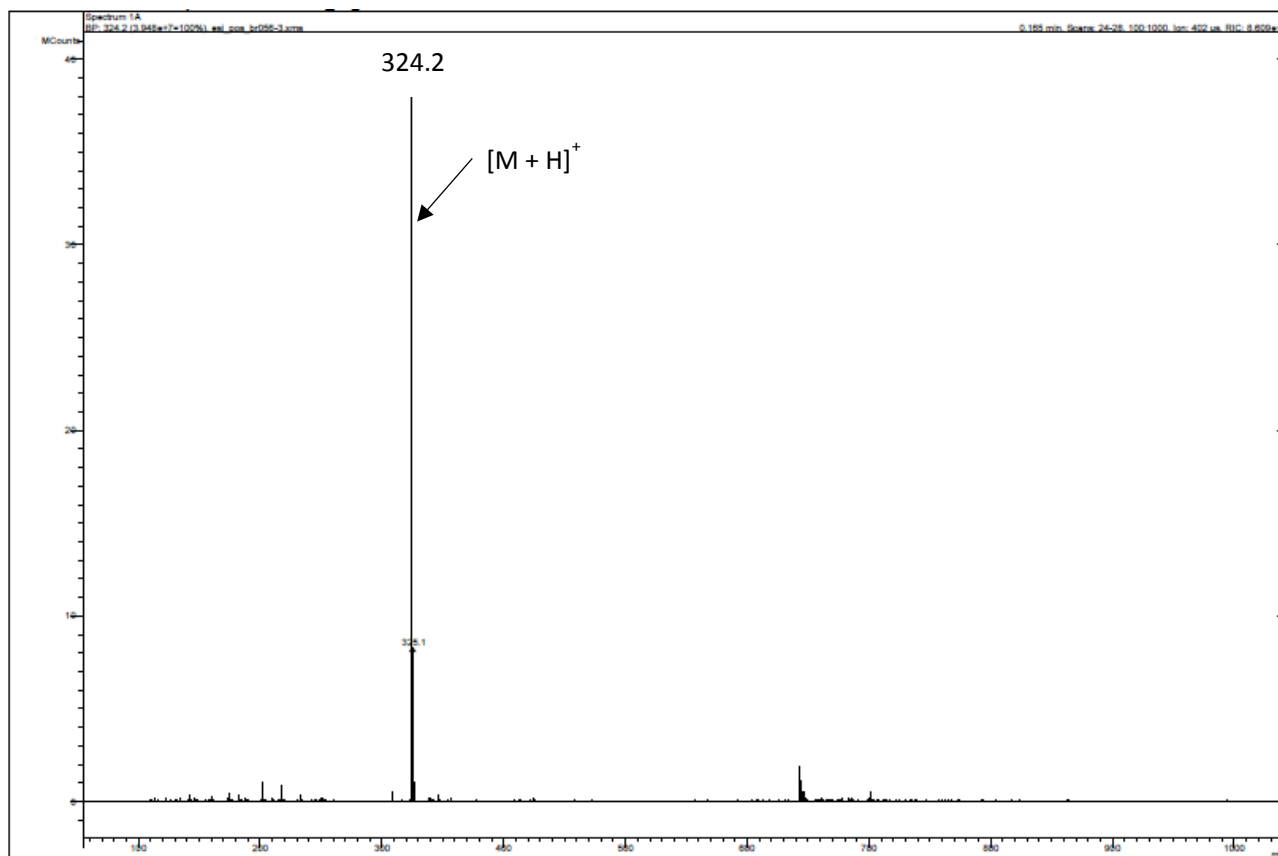
¹H NMR spectra recorded in CDCl₃ (400 MHz); residual signals of ethyl acetate are seen at 1.23, 2.10 and 4.23 ppm.



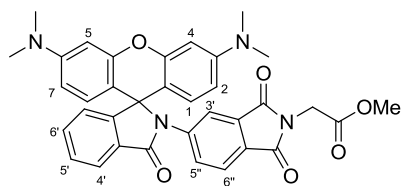
^{13}C NMR spectrum recorded in CDCl_3 (101 MHz); residual signals of ethyl acetate are seen at 14.3, 21.2 and 60.6 ppm.



ESI mass spectra (positive mode)

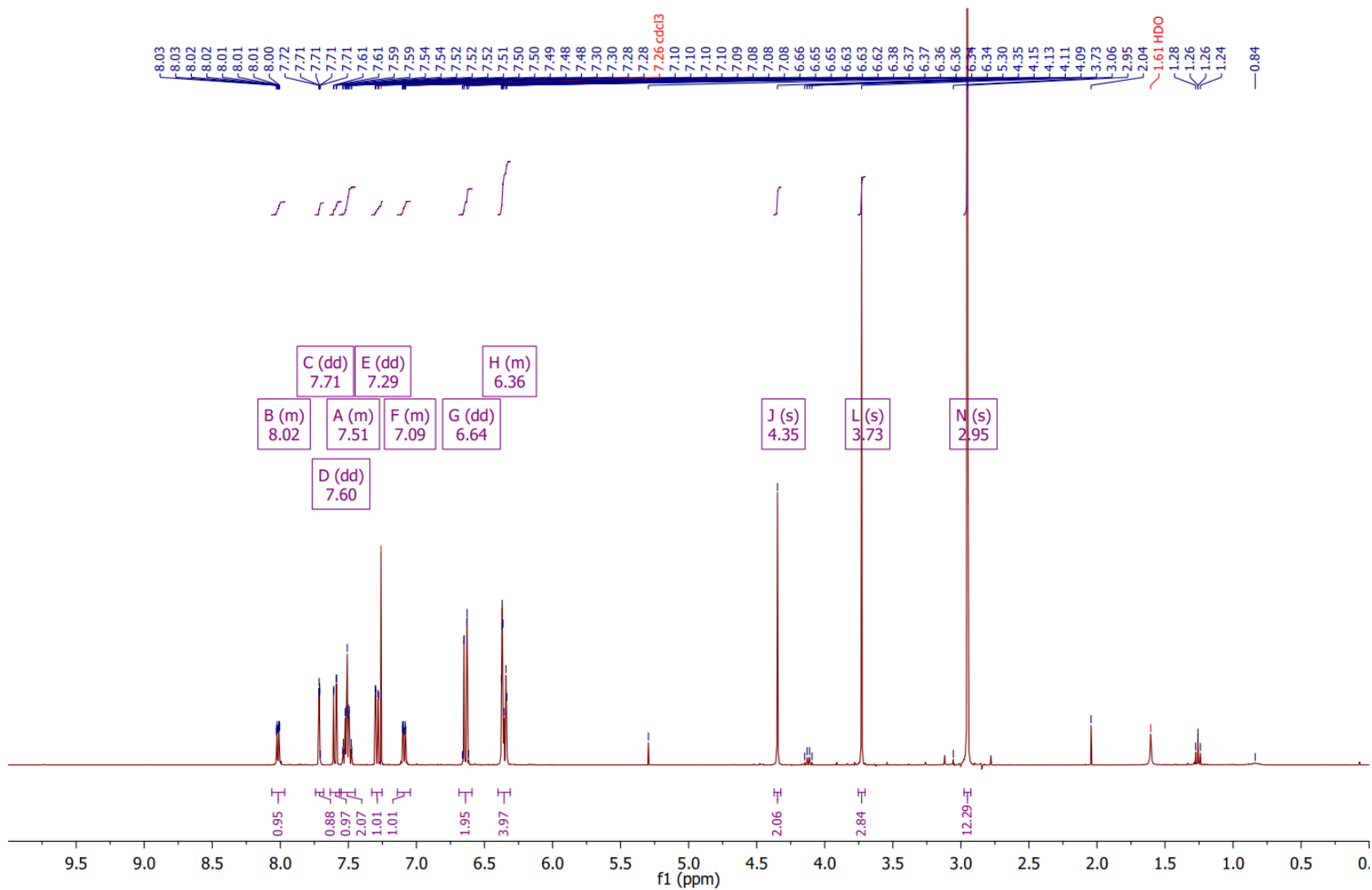


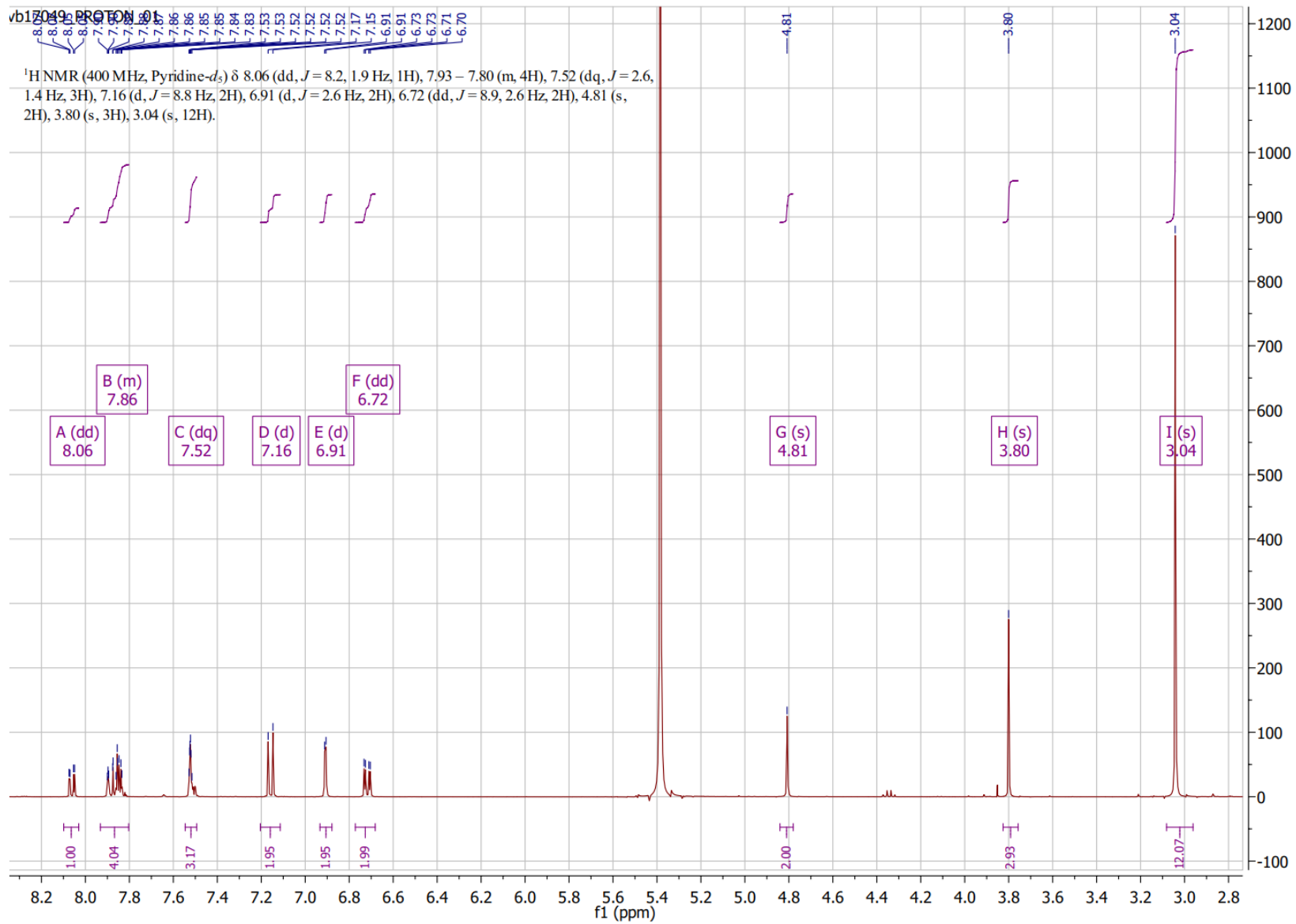
NMR and HRMS-ESI spectra of ester 4-Me

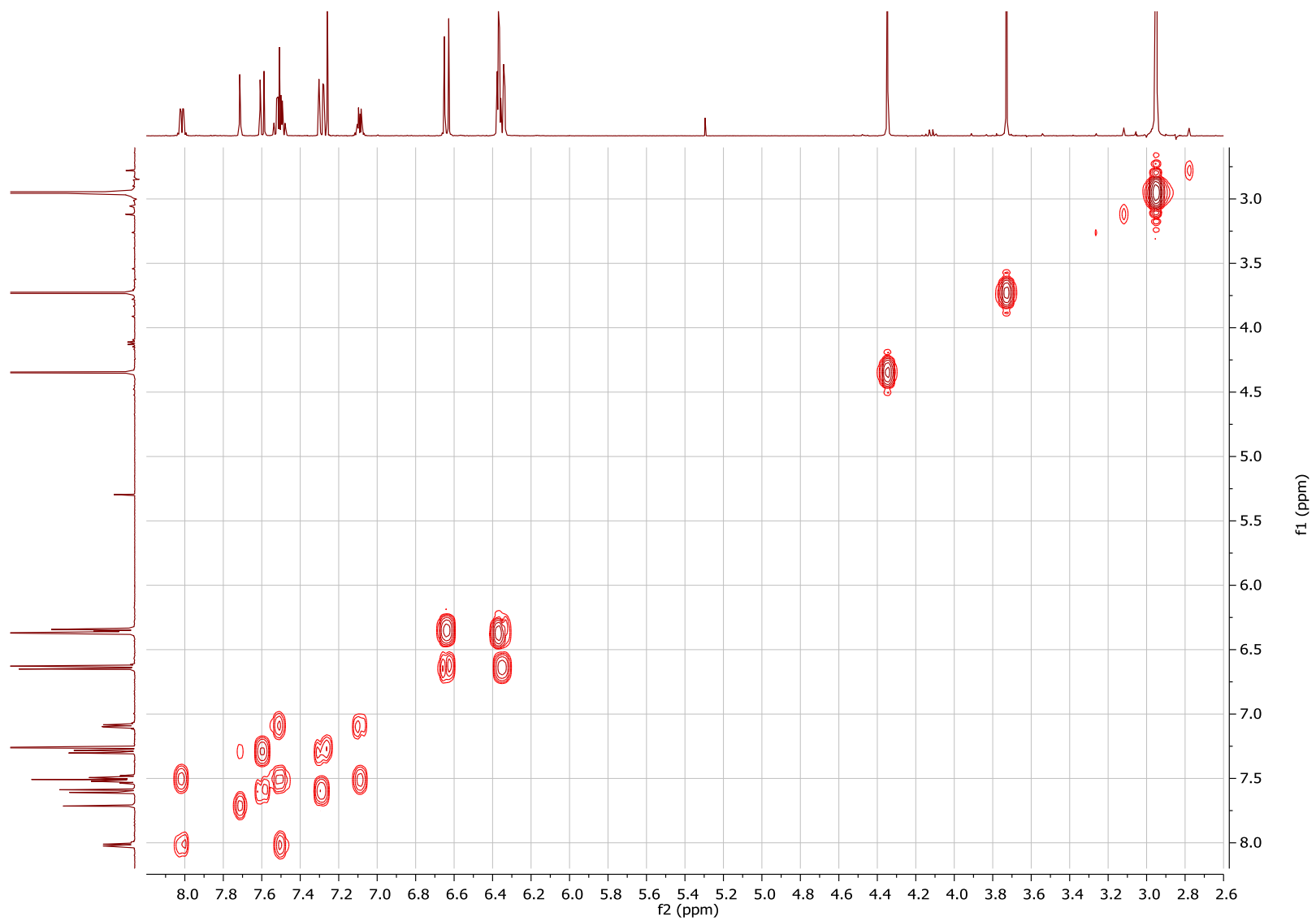


¹H (¹H-¹H COSY) NMR spectra were recorded in CDCl₃ and pyridine-*d*₅ (400 MHz), in order to unequivocally assign the signals of the aromatic protons

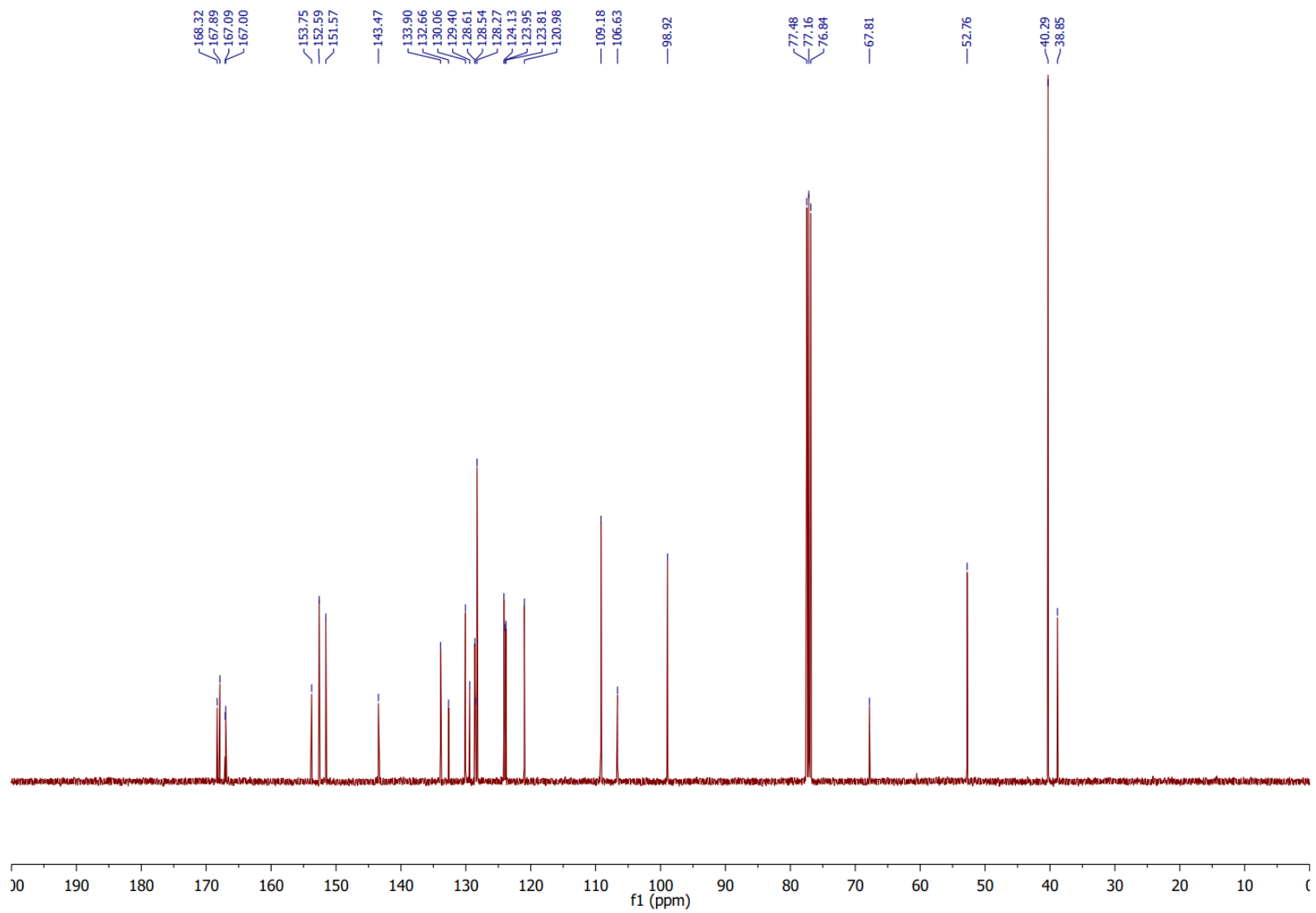
¹H NMR (400 MHz, Chloroform-*d*) δ 8.06 – 7.97 (m, 1H), 7.71 (dd, *J* = 1.9, 0.6 Hz, 1H), 7.60 (dd, *J* = 8.2, 0.6 Hz, 1H), 7.57 – 7.45 (m, 2H), 7.29 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.14 – 7.04 (m, 1H), 6.64 (dd, *J* = 8.6, 0.5 Hz, 2H), 6.40 – 6.31 (m, 4H), 4.35 (s, 2H), 3.73 (s, 3H), 2.95 (s, 12H).



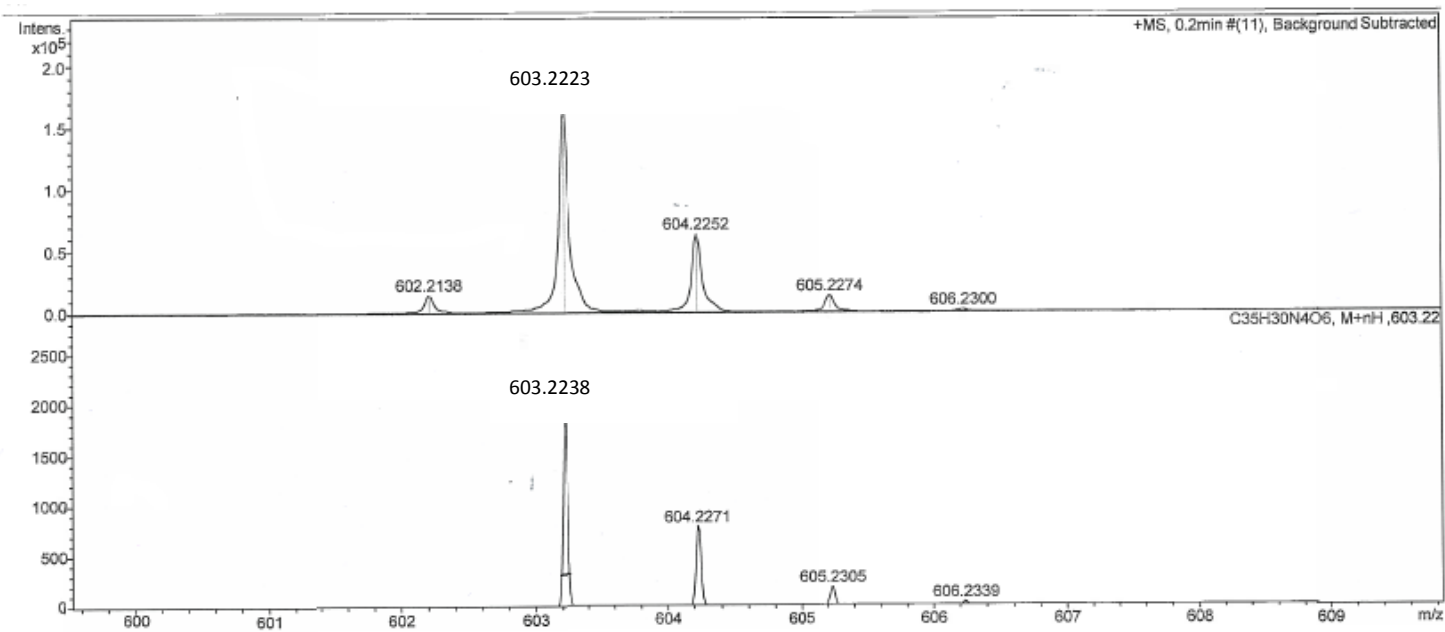




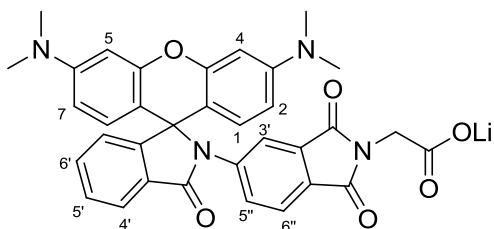
^{13}C NMR spectrum recorded in CDCl_3 (100 MHz); residual dioxane peak at 67.8 ppm



HRMS-ESI mass spectra (positive mode)

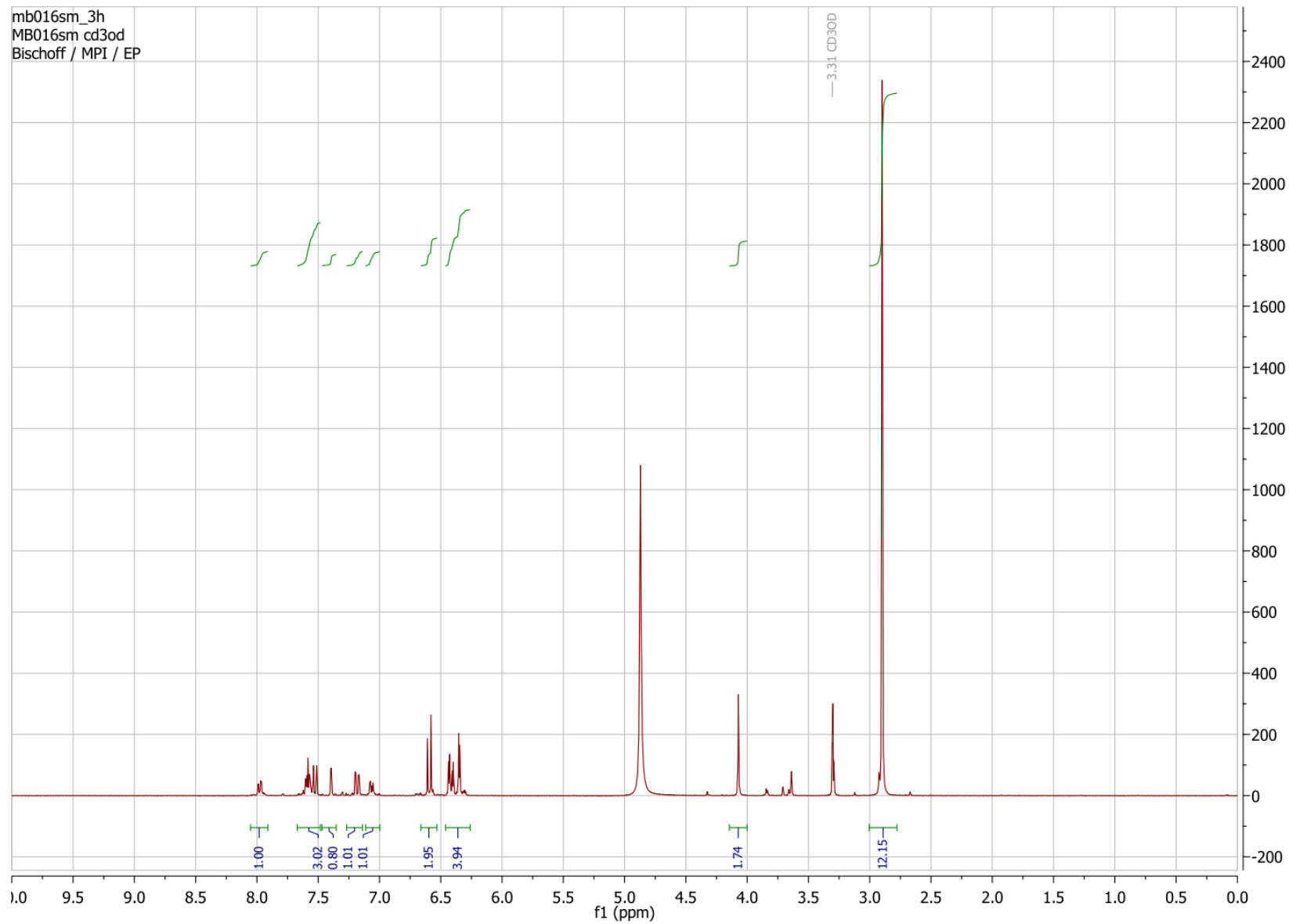


Lithium salt (4-Li)

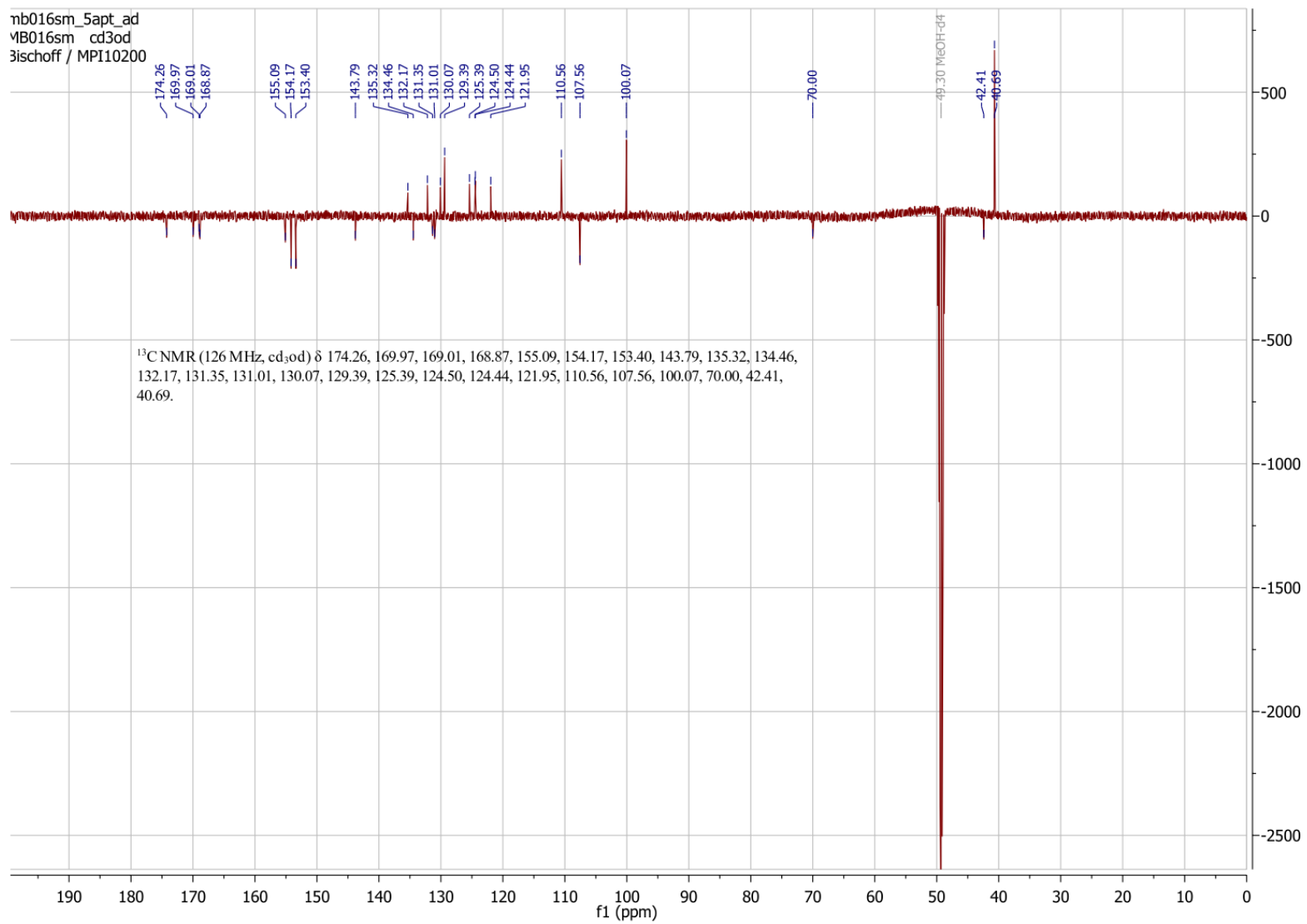


¹H-NMR spectrum recorded in MeOH-d₄ (300 MHz)

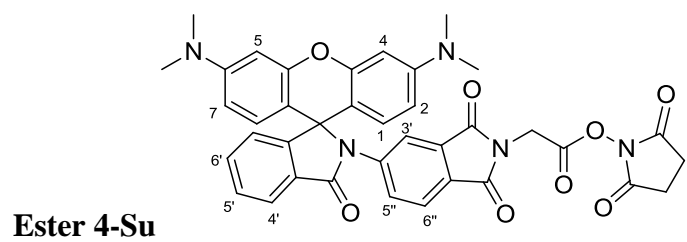
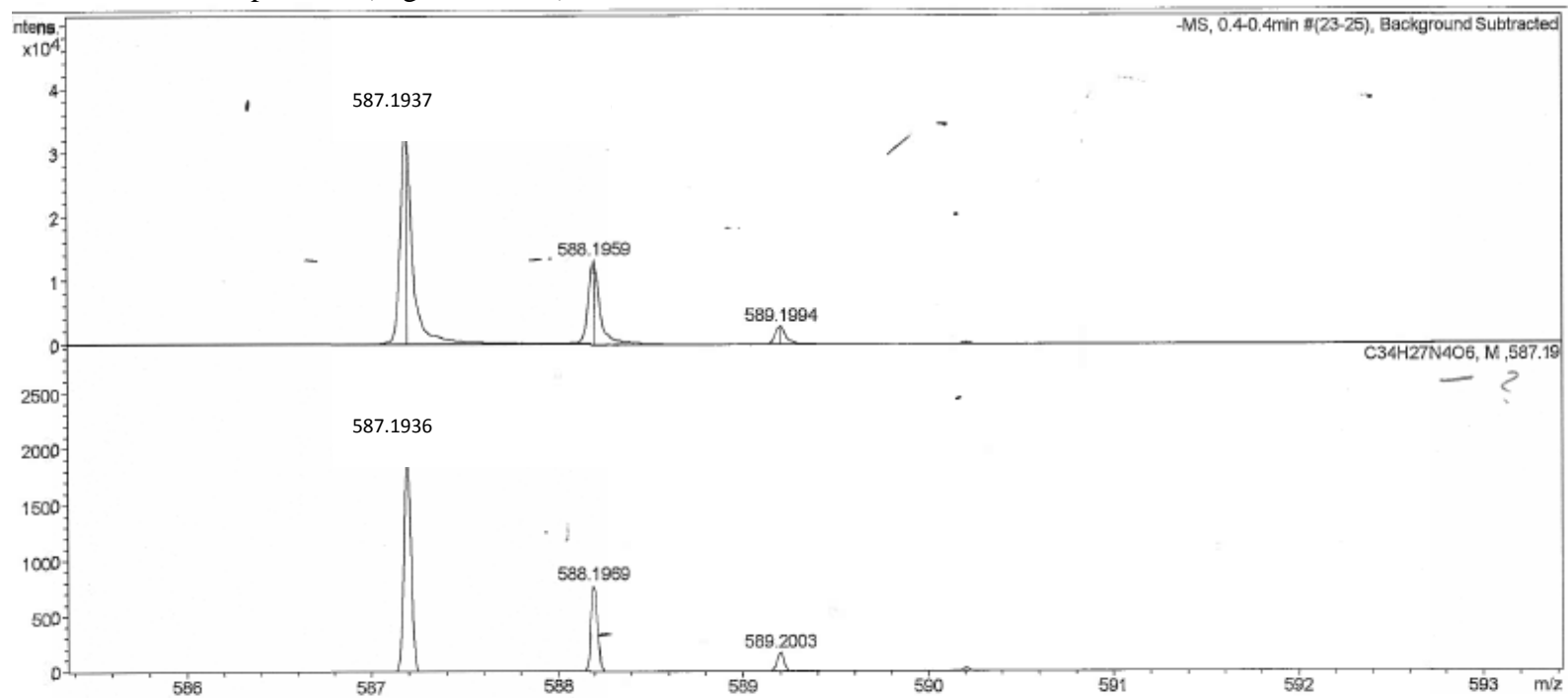
mb016sm_3h
MB016sm cd3od
Bischoff / MPI / EP



^{13}C NMR- APT spectrum (CH_3/CH – positive, CH_2/C_q – negative peaks); MeOH-d_4 (125 MHz)

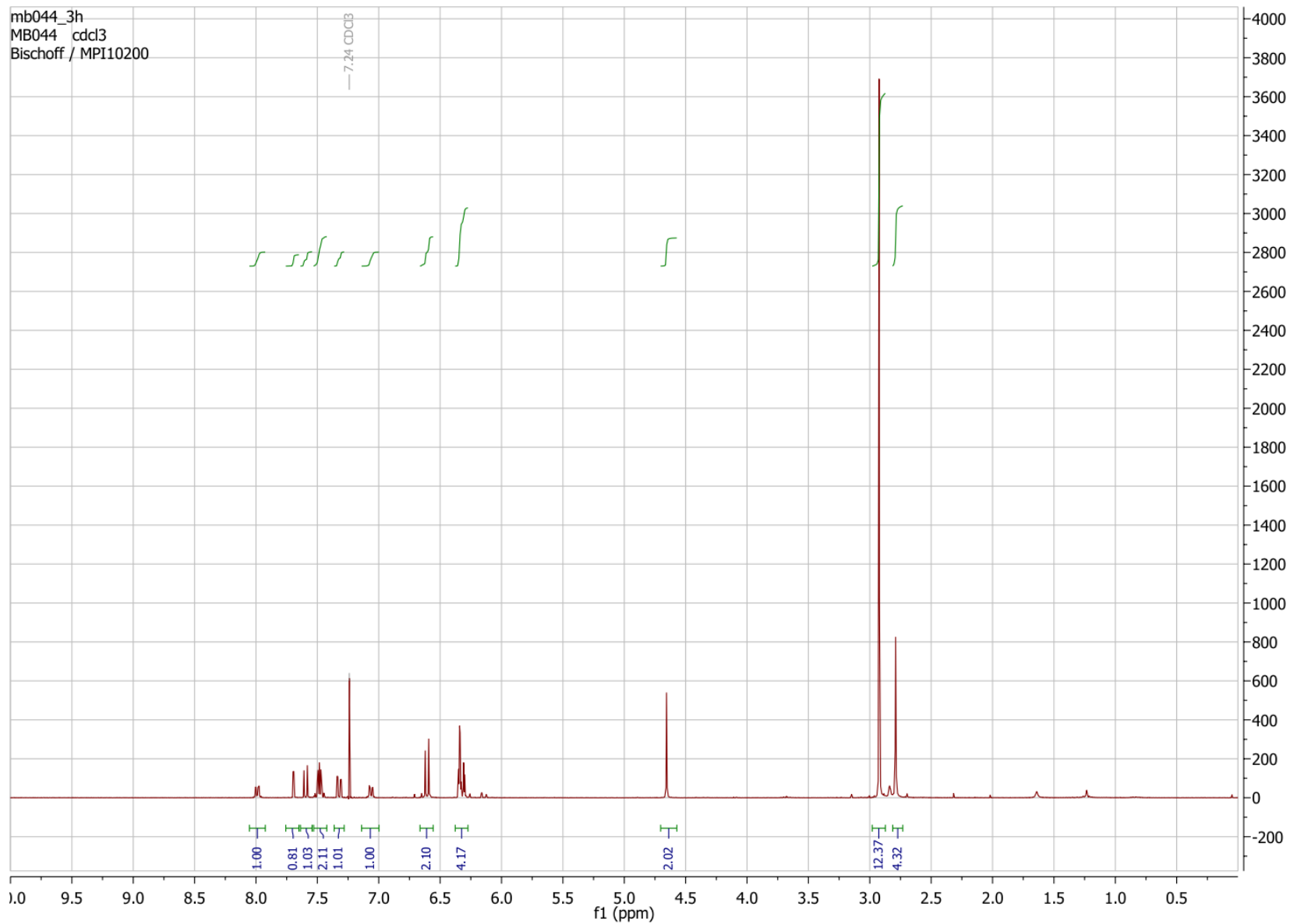


HRMS-ESI mass spectrum (negative mode)

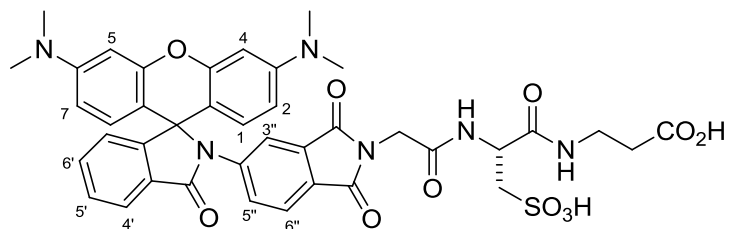
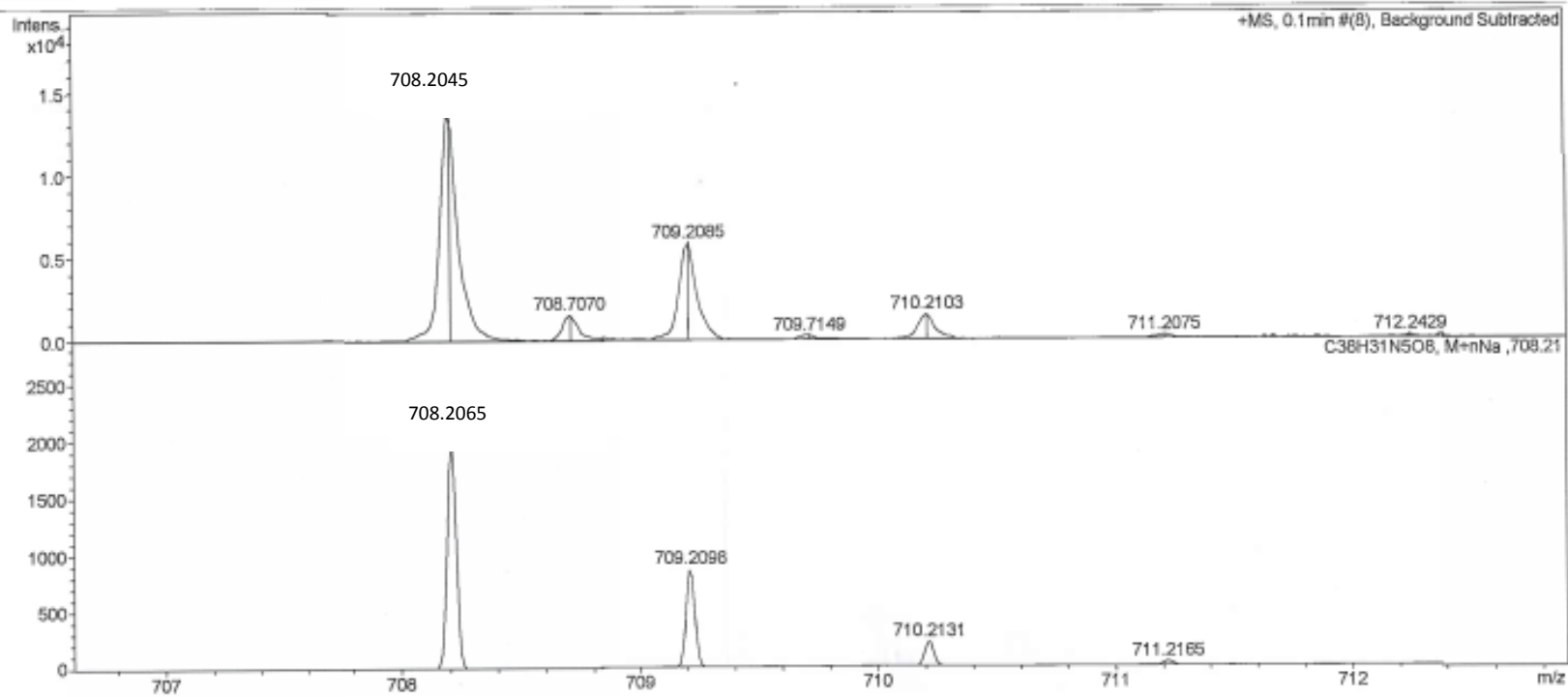


¹H-NMR spectrum (CDCl₃, 300 MHz)

mb044_3h
MB044 cdc13
Bischoff / MPI10200



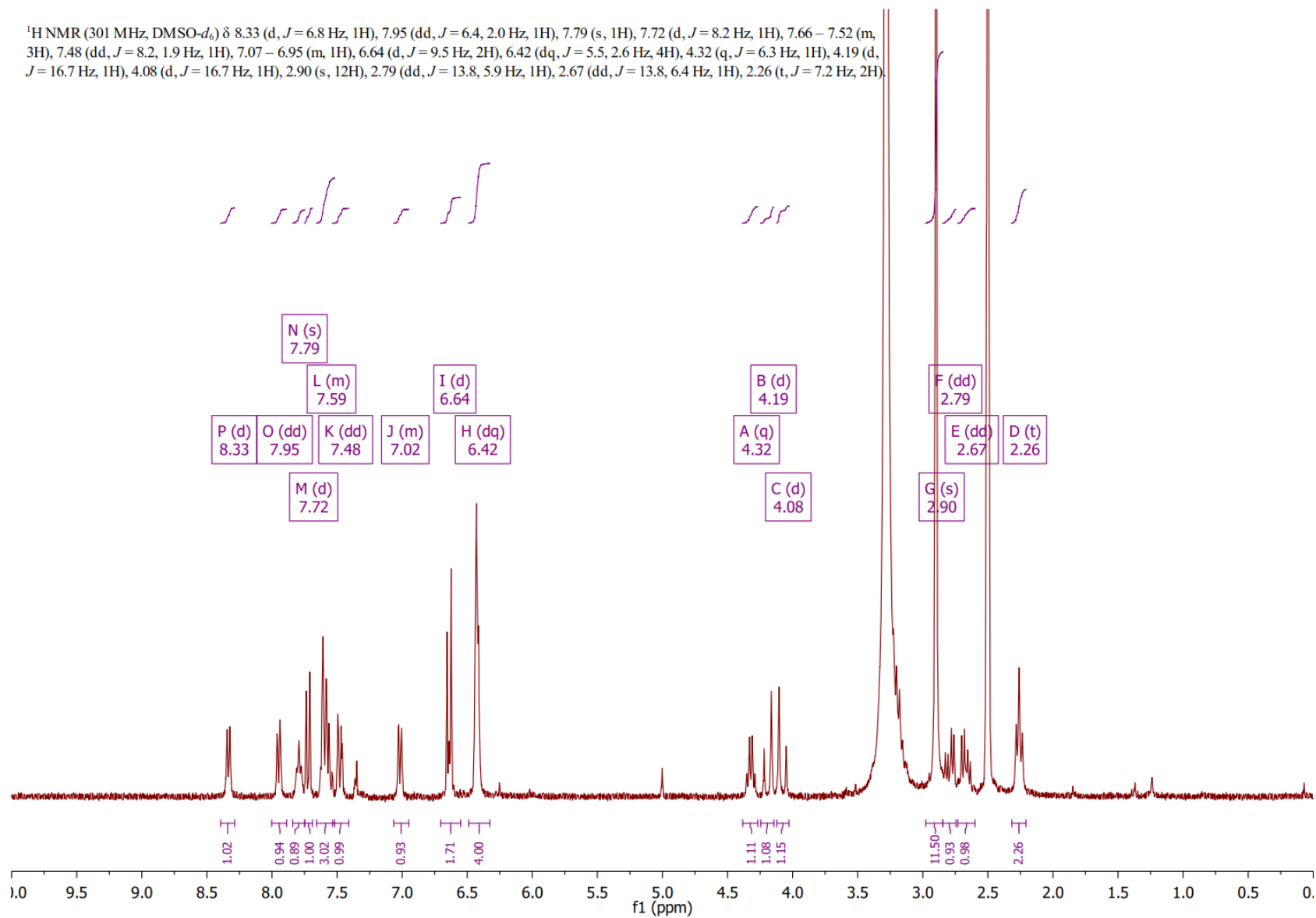
HRMS-ESI mass spectrum (positive mode)

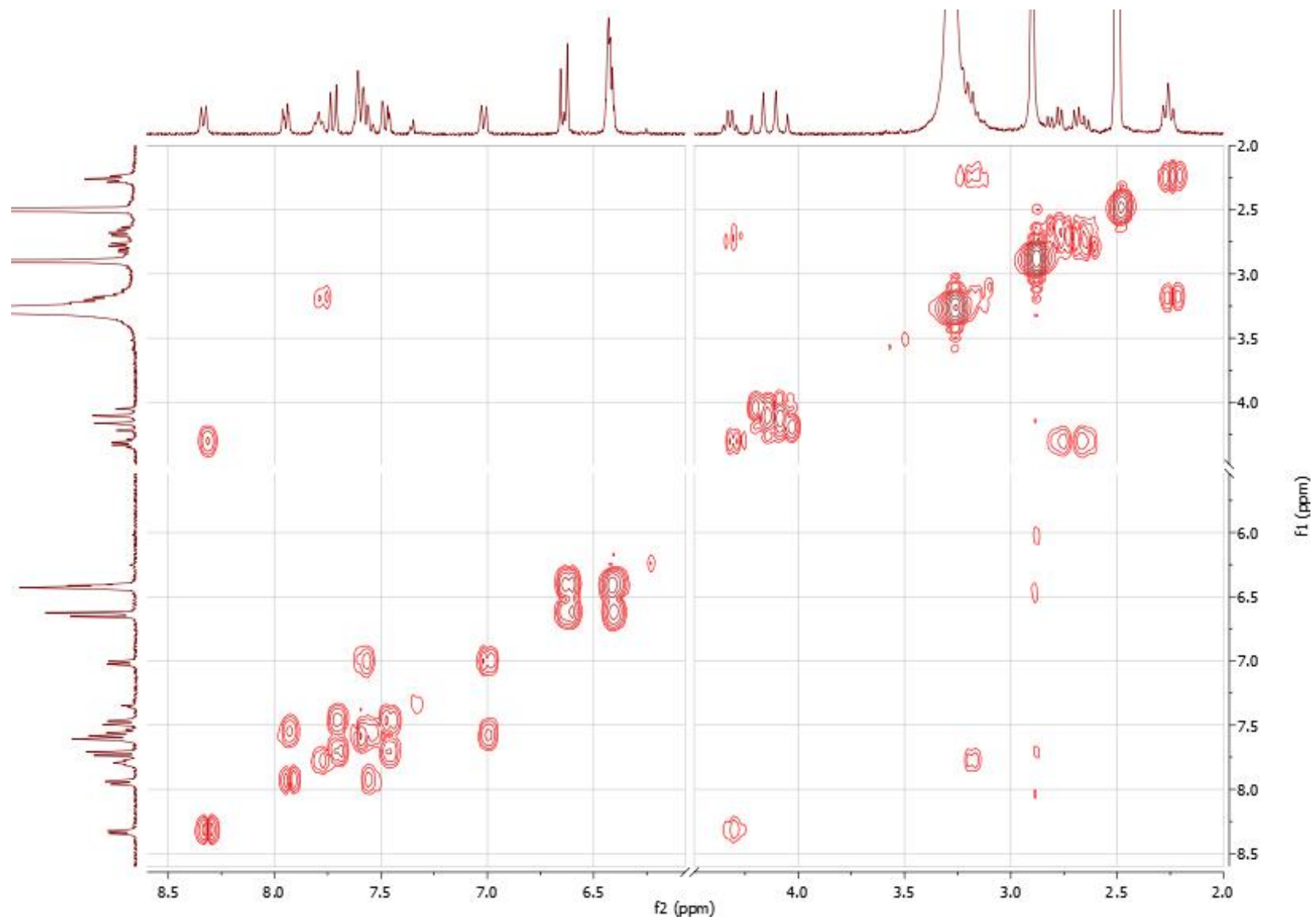


Spiroamide 5-H

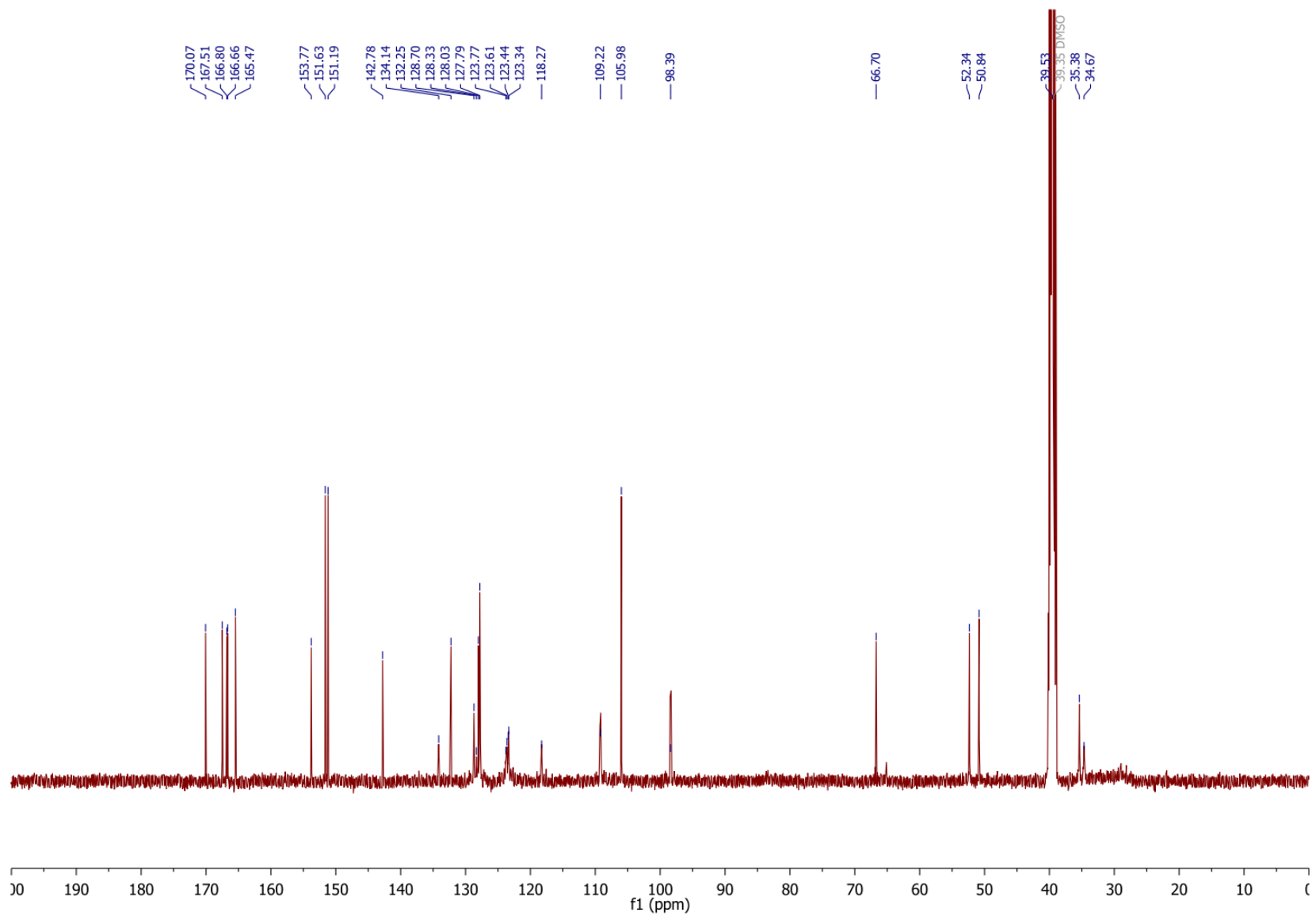
¹H- and ¹H-¹H COSY NMR spectra (DMSO-d₆, 300 MHz)

¹H NMR (301 MHz, DMSO-*d*₆) δ 8.33 (d, *J* = 6.8 Hz, 1H), 7.95 (dd, *J* = 6.4, 2.0 Hz, 1H), 7.79 (s, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.66 – 7.52 (m, 3H), 7.48 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.07 – 6.95 (m, 1H), 6.64 (d, *J* = 9.5 Hz, 2H), 6.42 (dq, *J* = 5.5, 2.6 Hz, 4H), 4.32 (q, *J* = 6.3 Hz, 1H), 4.19 (d, *J* = 16.7 Hz, 1H), 4.08 (d, *J* = 16.7 Hz, 1H), 2.90 (s, 12H), 2.79 (dd, *J* = 13.8, 5.9 Hz, 1H), 2.67 (dd, *J* = 13.8, 6.4 Hz, 1H), 2.26 (t, *J* = 7.2 Hz, 2H).

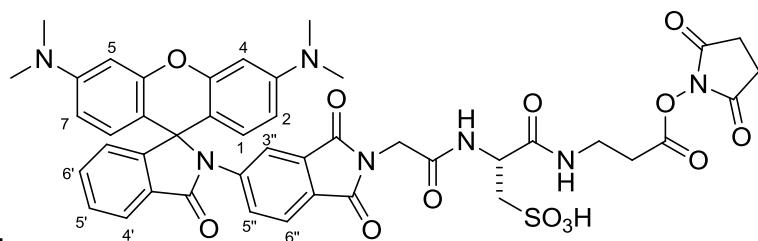
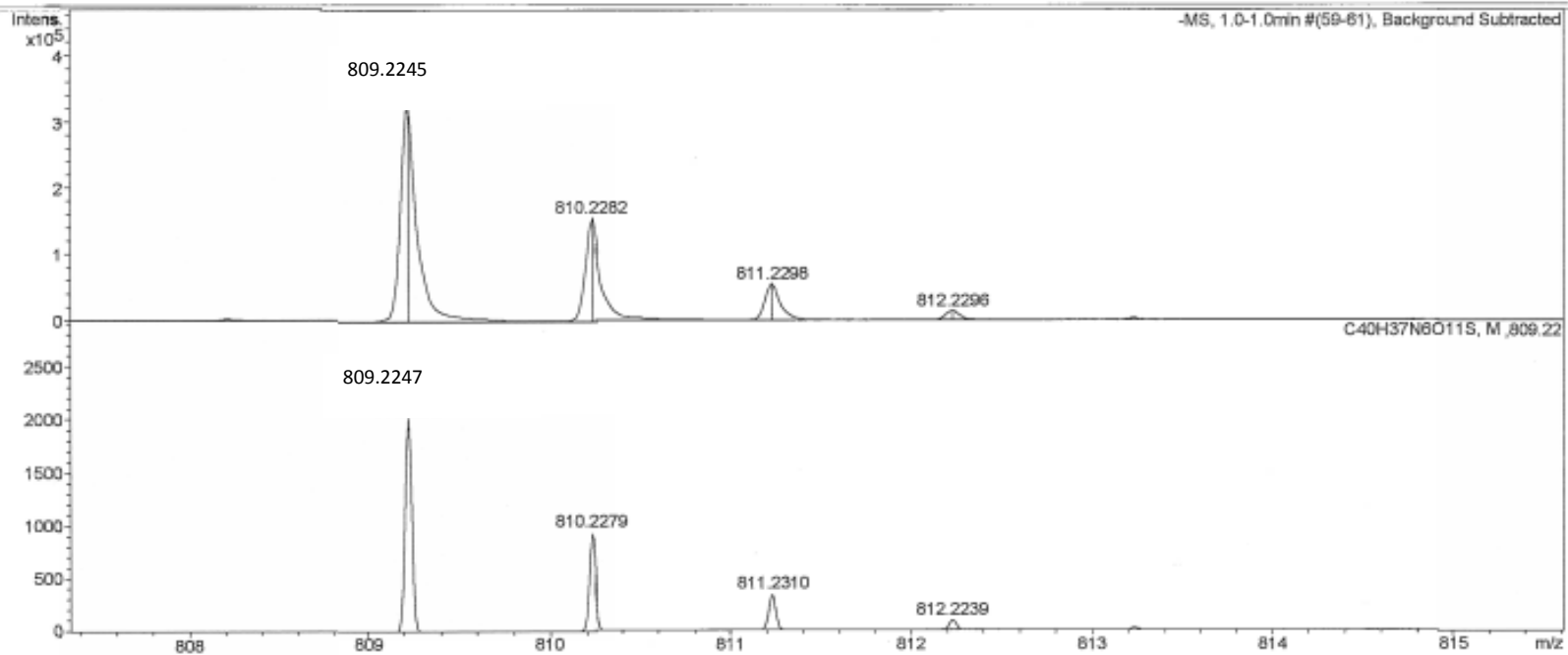




^{13}C NMR spectrum recorded in DMSO-d_6 (125 MHz)



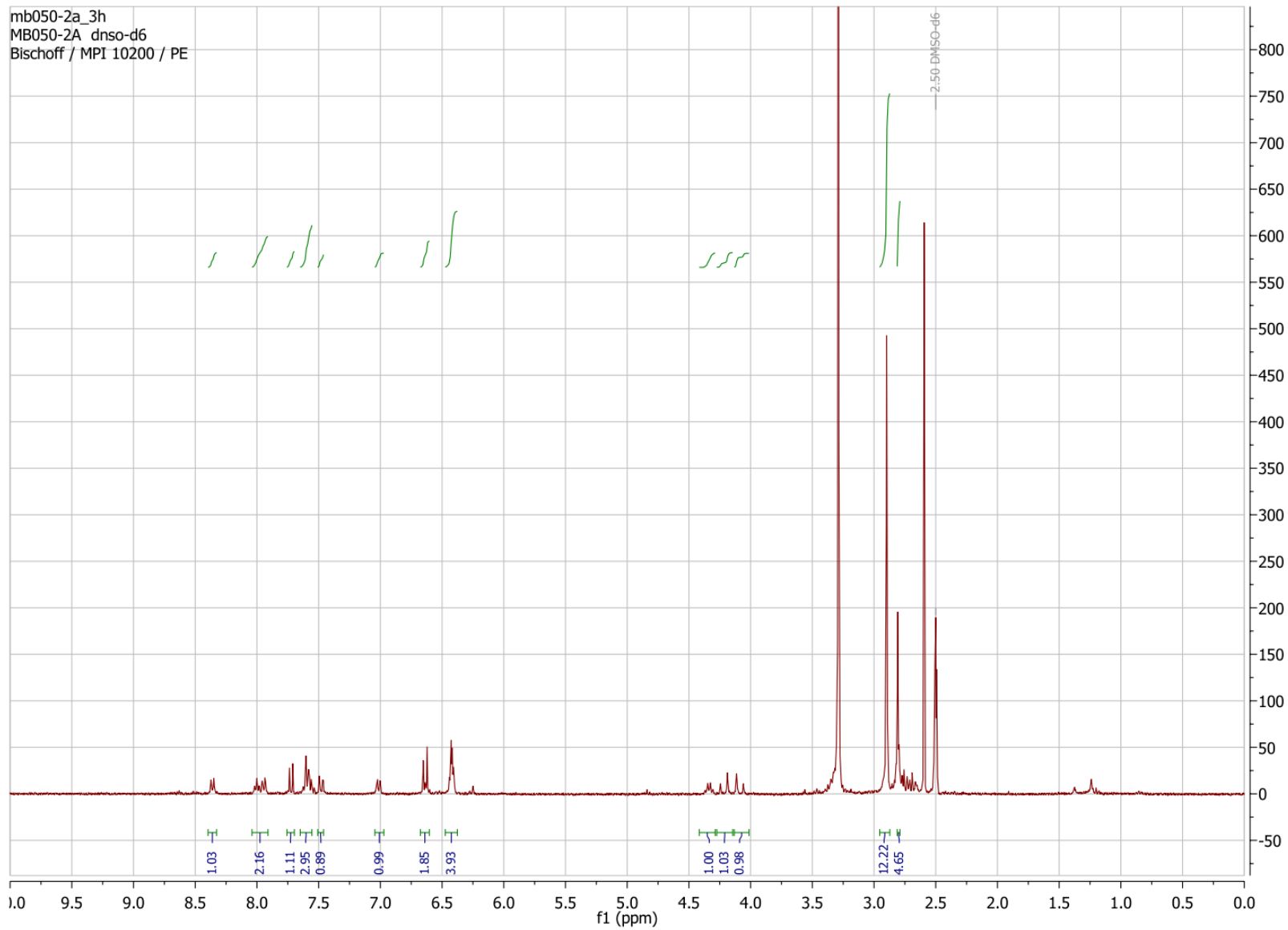
HRMS-ESI mass spectra (negative mode)

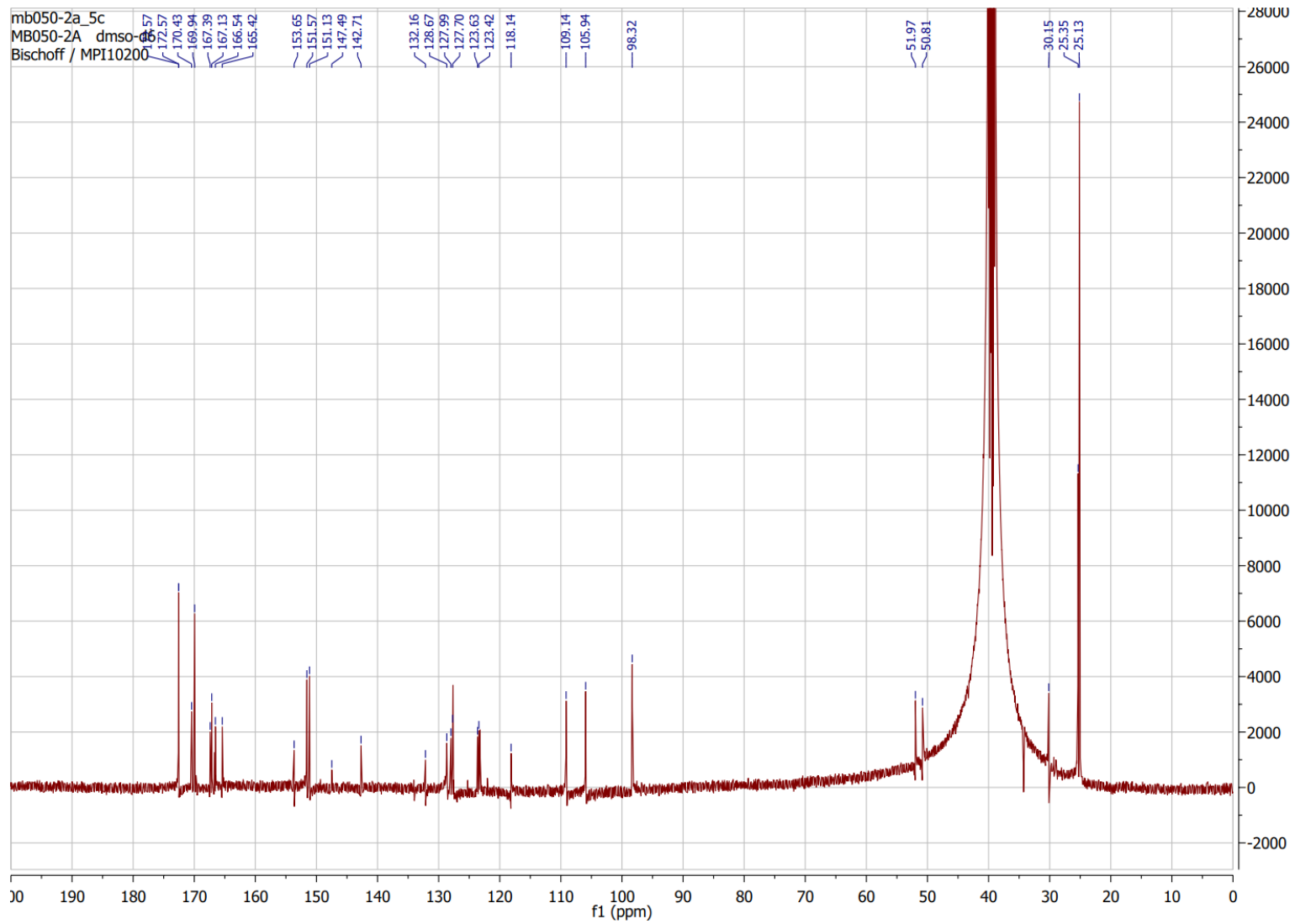


Ester 5-Su

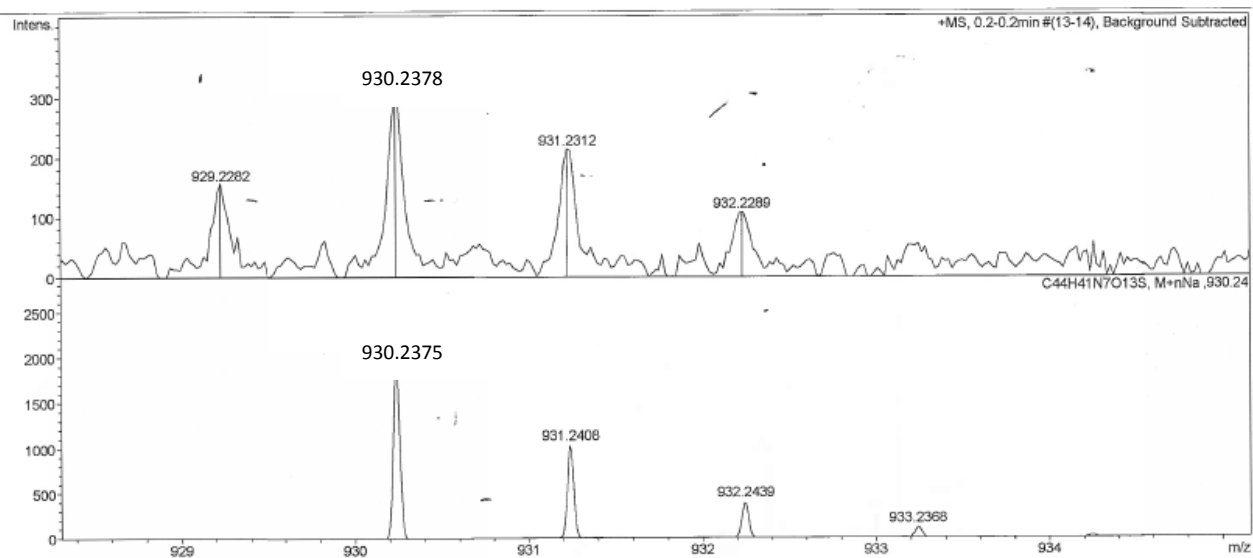
¹H (300 MHz) and ¹³C NMR (125 MHz) spectra recorded in DMSO-d₆

mb050-2a_3h
MB050-2A dns0-d6
Bischoff / MPI 10200 / PE

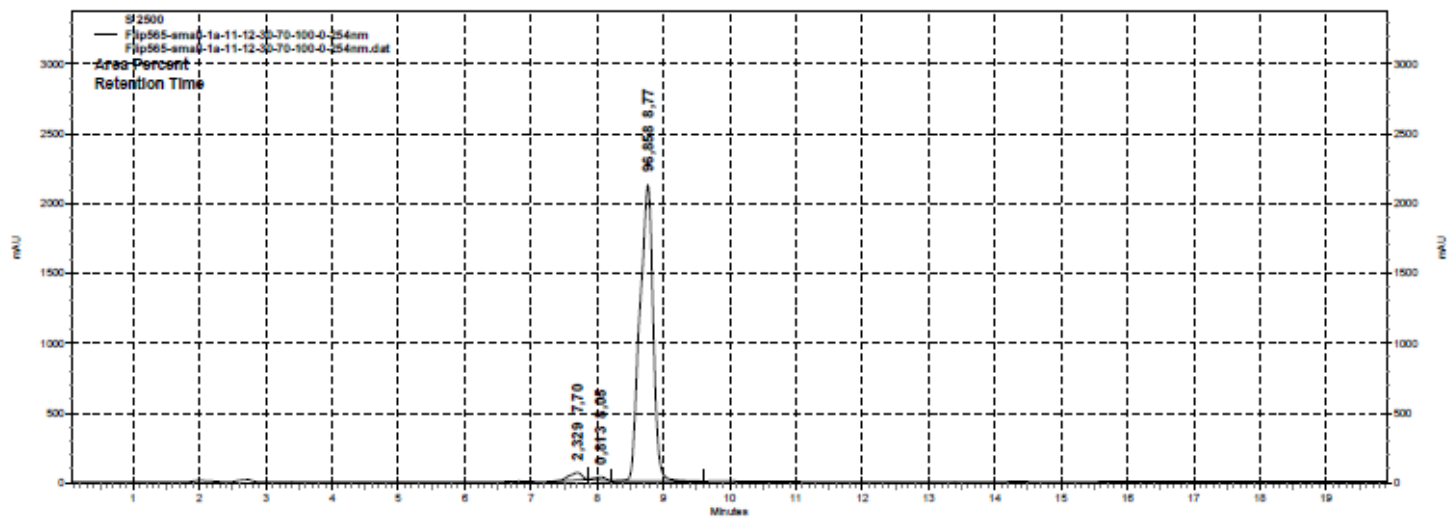


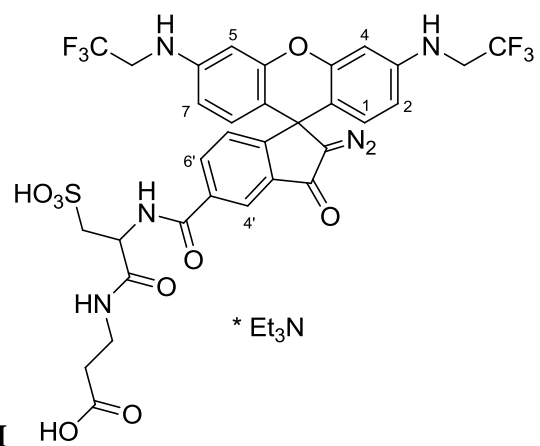


HRMS-ESI mass spectra (positive mode)

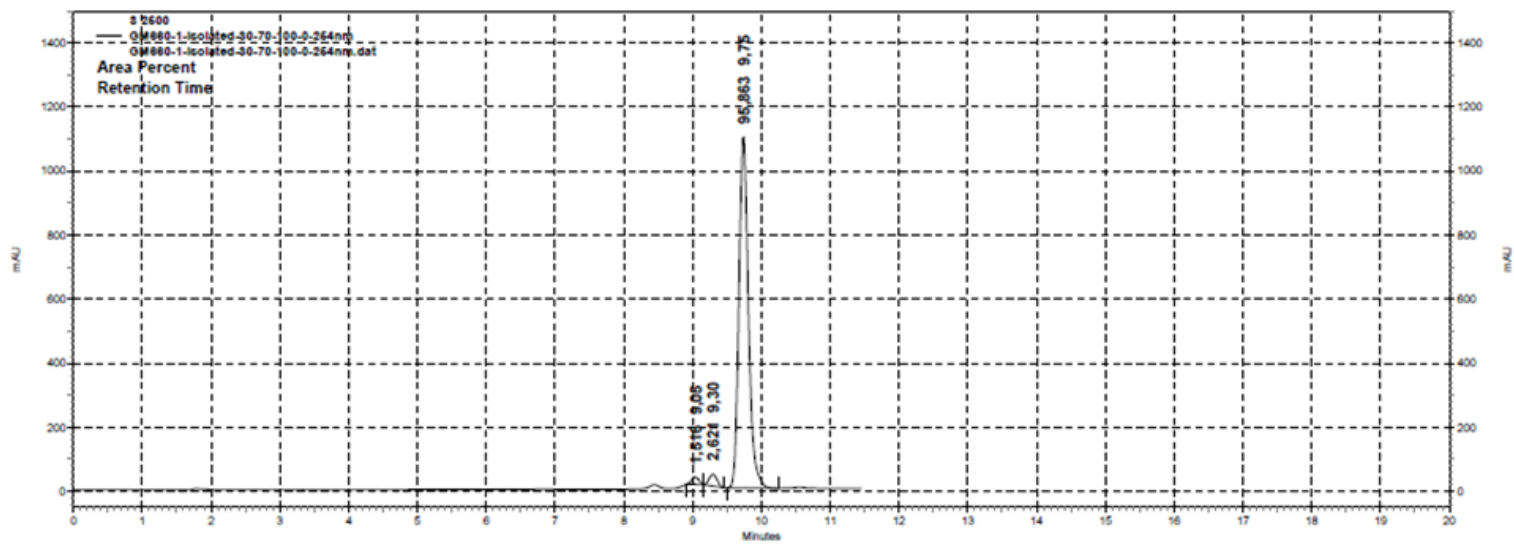


HPLC elution profile (system B)

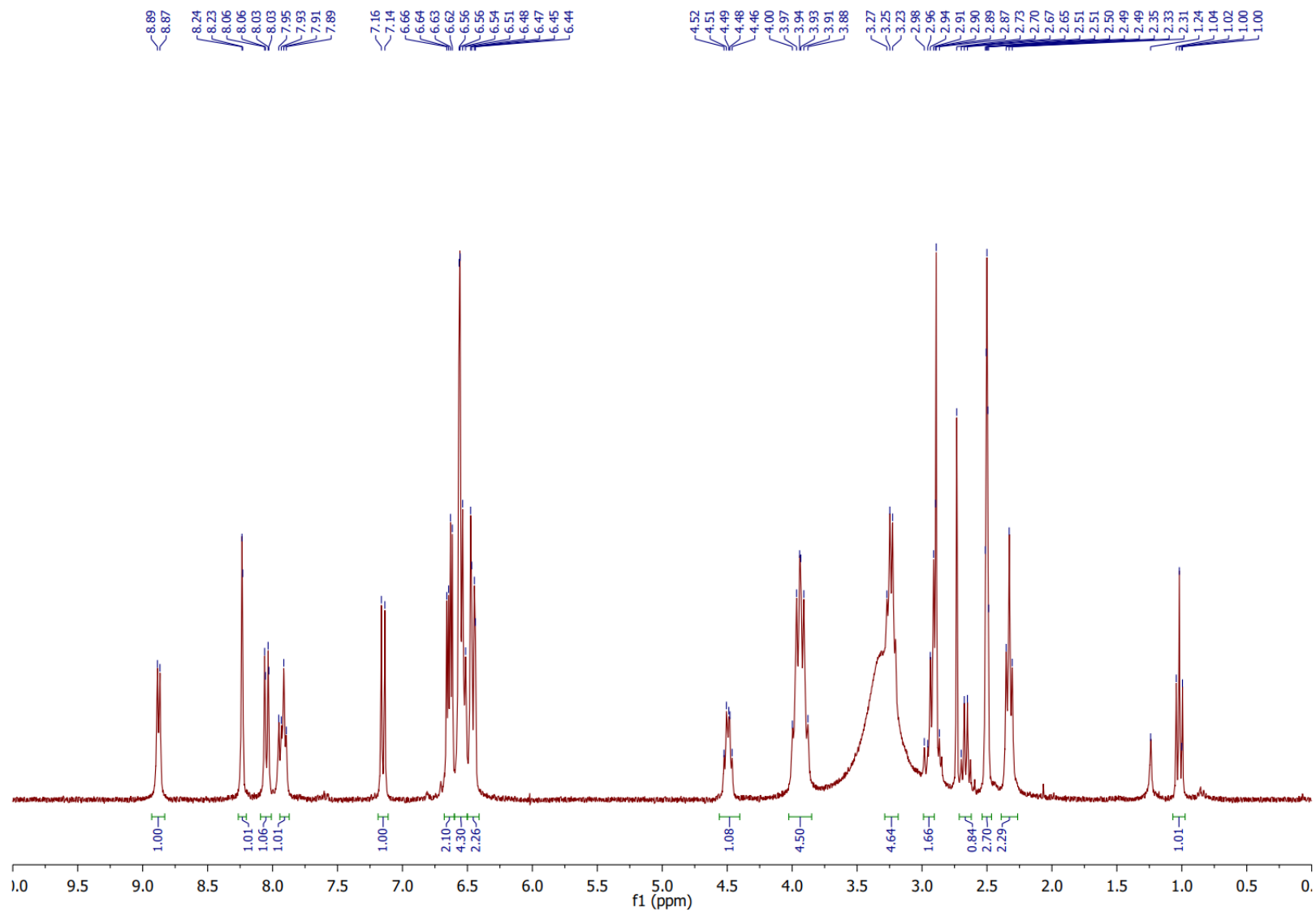


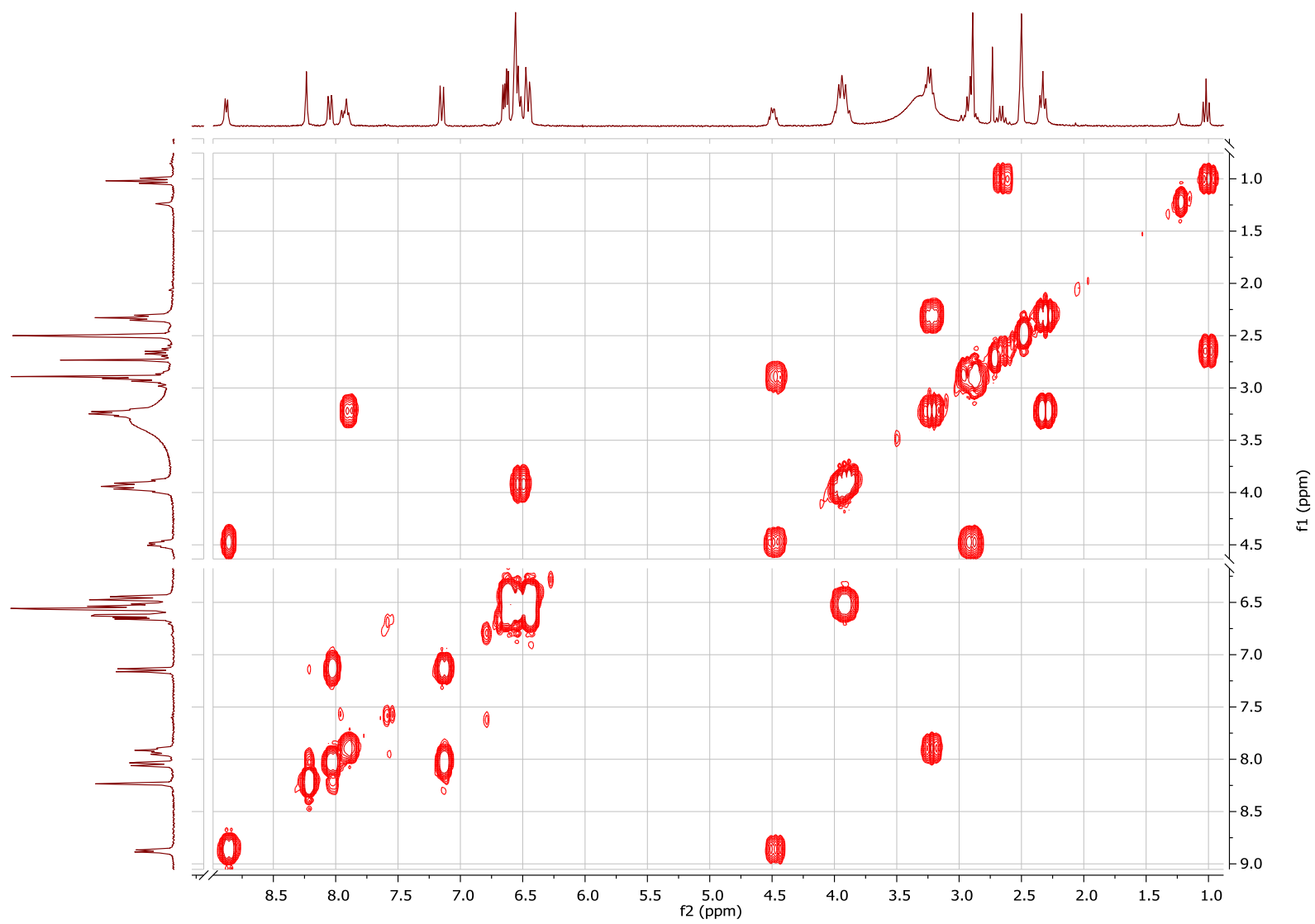


Diazoketone 7-H
HPLC Trace (system B)

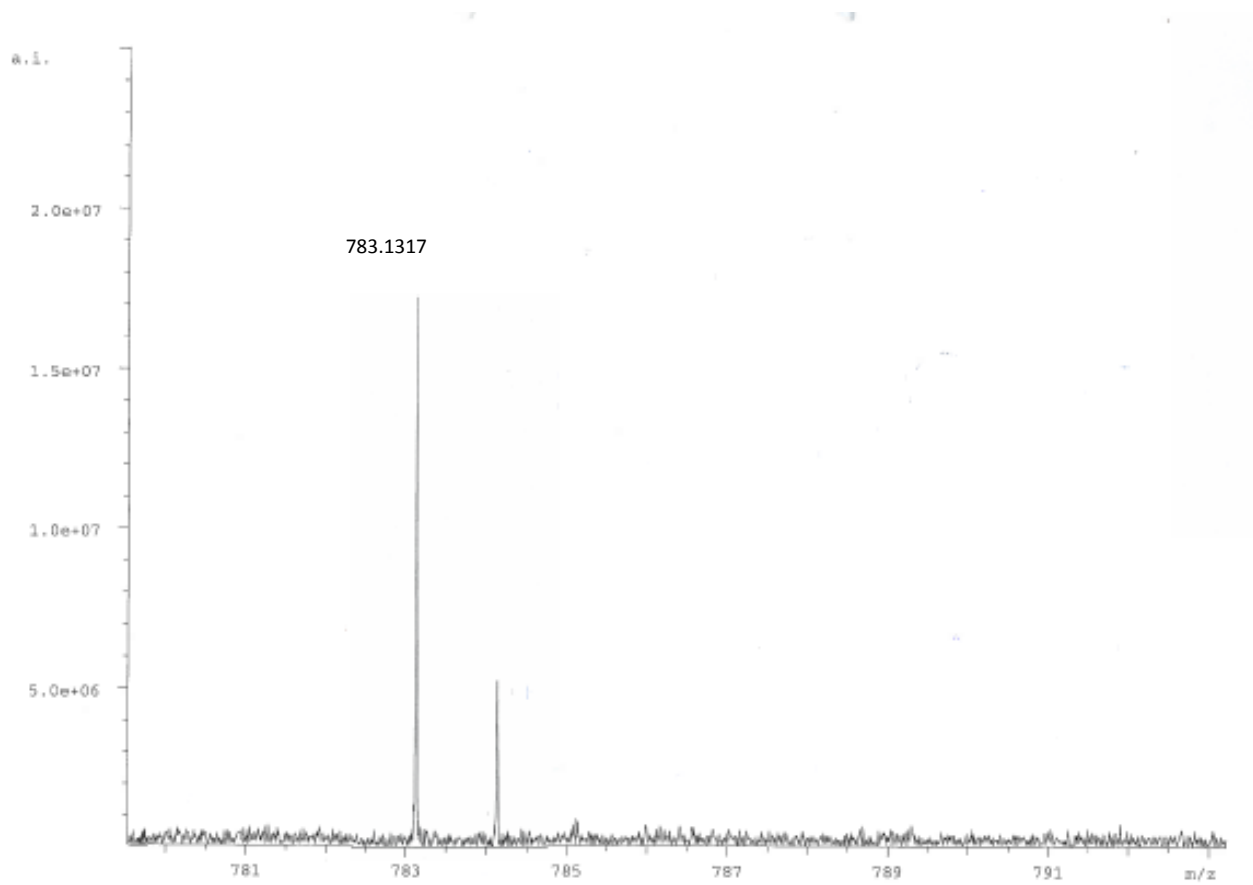


^1H and ^1H - ^1H COSY NMR spectra recorded in DMSO-d_6 (300 MHz); singlets at 2.7, 2.9 and 7.9 ppm belong to DMF



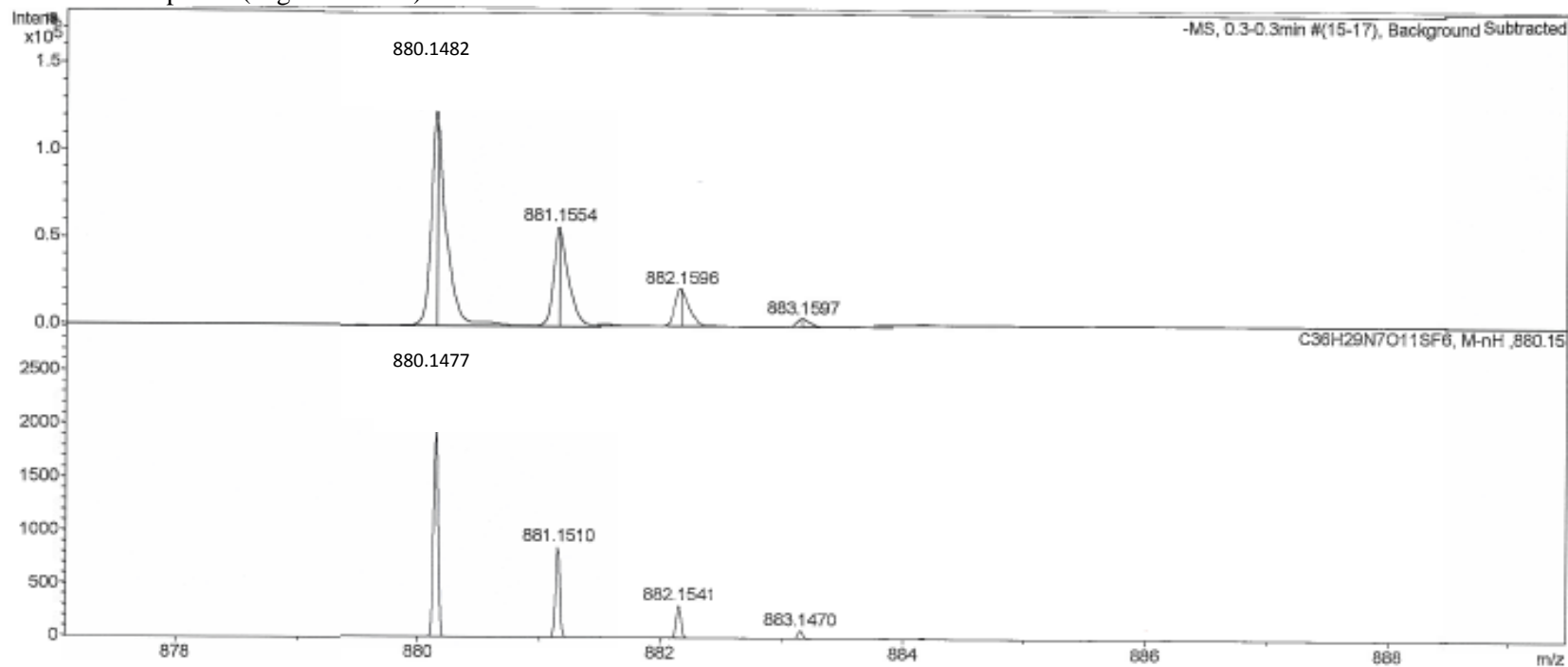


HRMS-ESI mass spectra (negative mode)

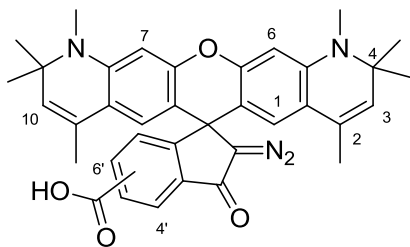


Ester 7-Su

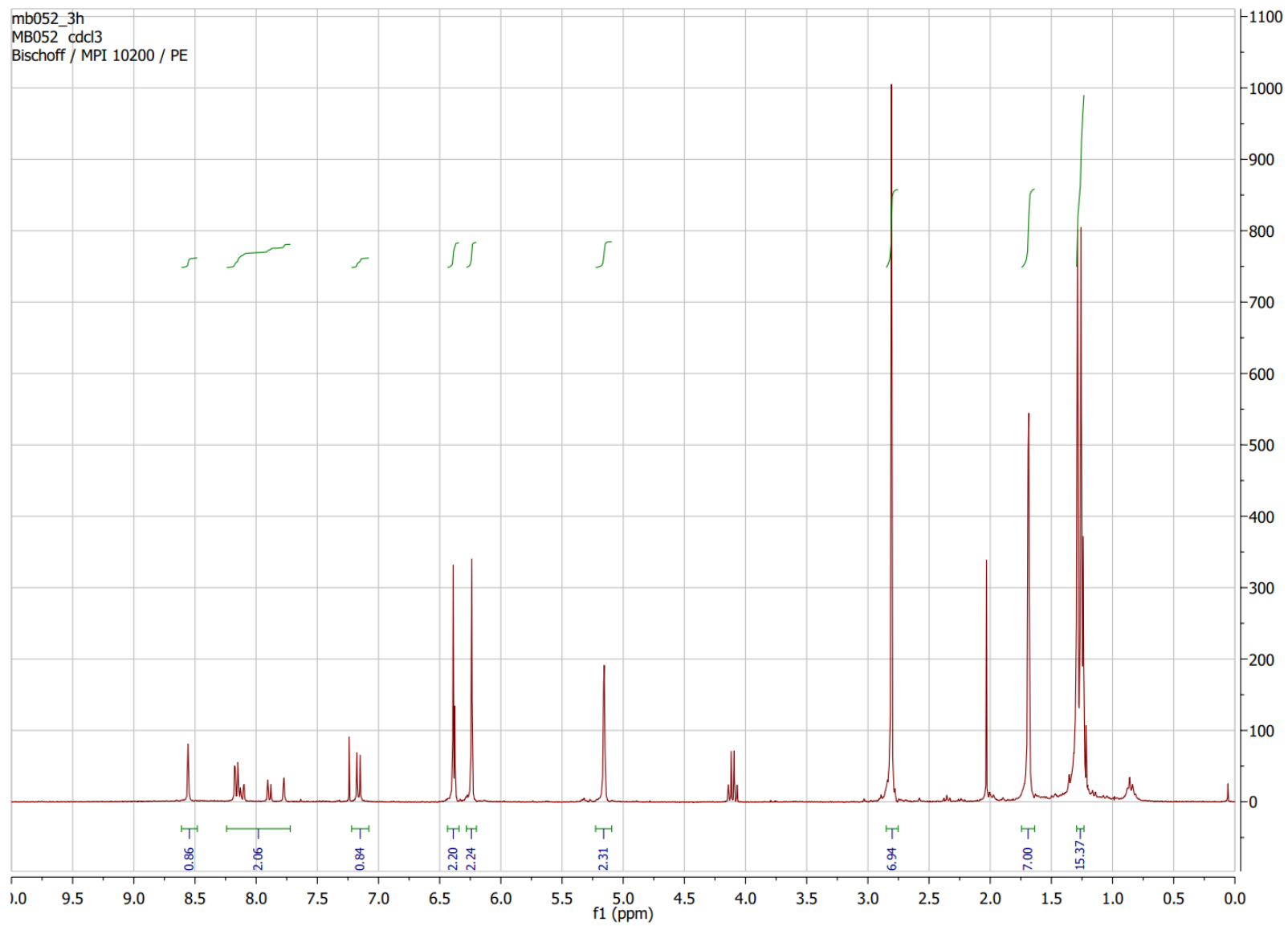
HRMS-ESI mass spectra (negative mode)



Diazoketone 8-H, mixture of 5'- and 6'-isomers (3:1). ^1H NMR (CDCl_3 , 400 MHz); signals at 1.23 t, 2.05 s and 4.15 q belong to EtOAc

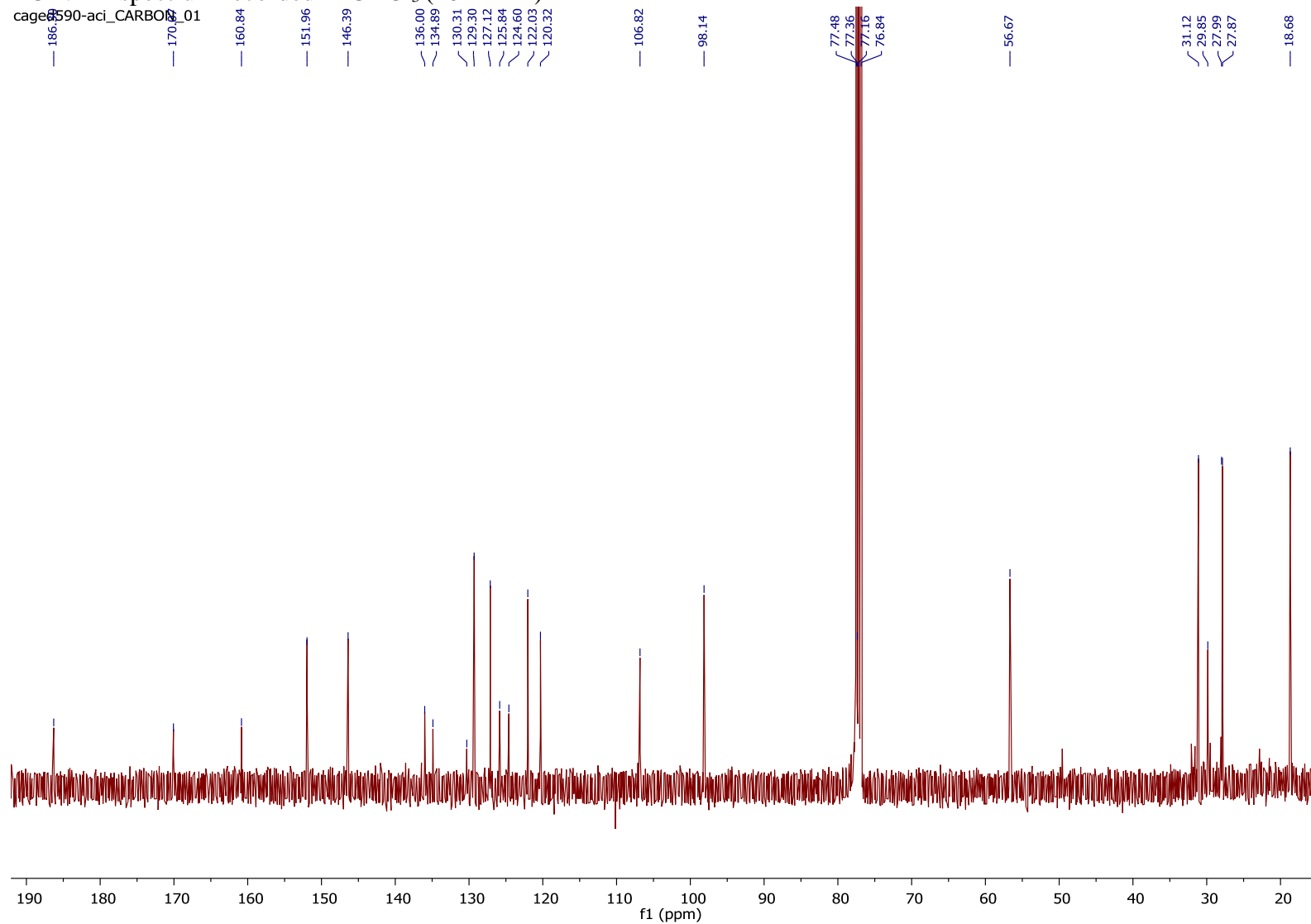


mb052_3h
MB052 ccd3
Bischoff / MPI 10200 / PE

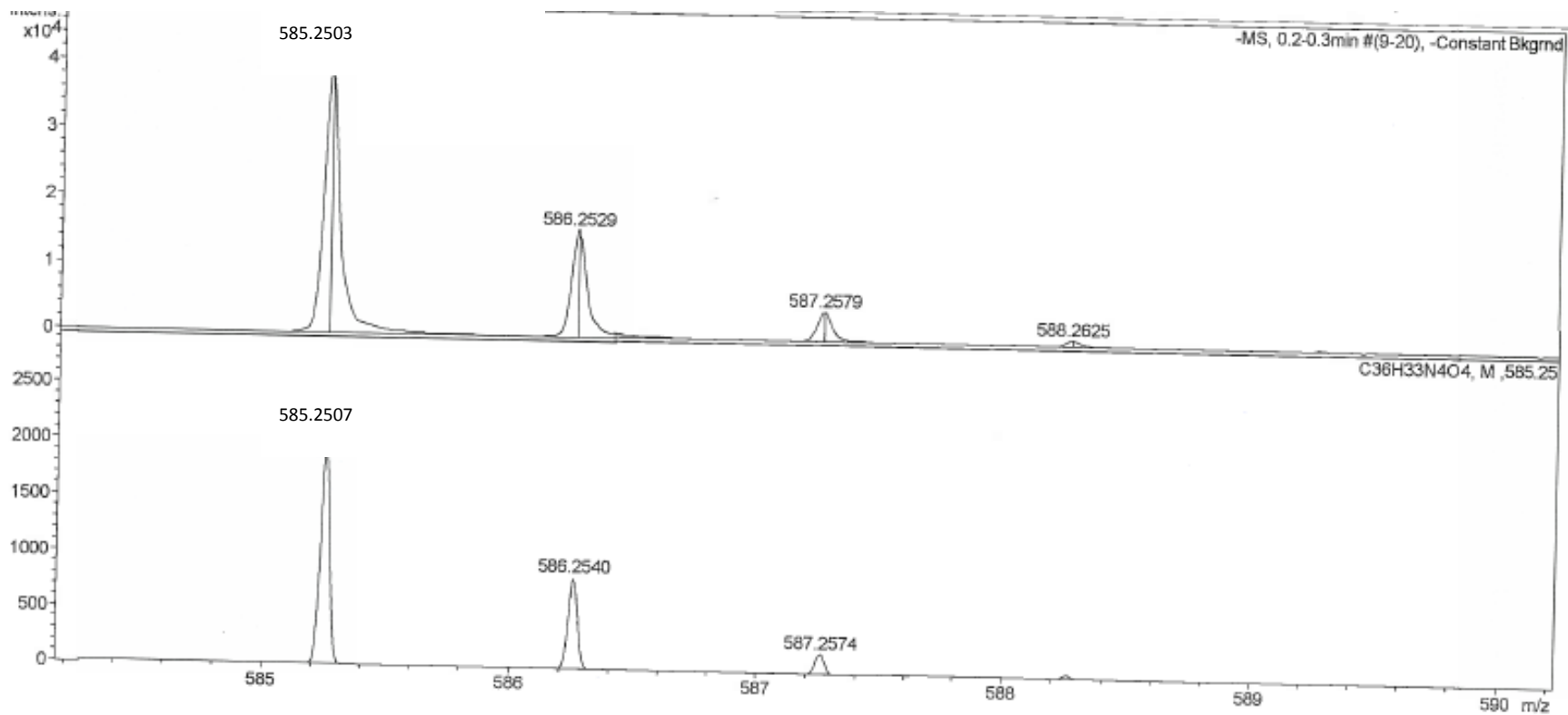


¹³C NMR spectrum recorded in CDCl₃ (101 MHz)

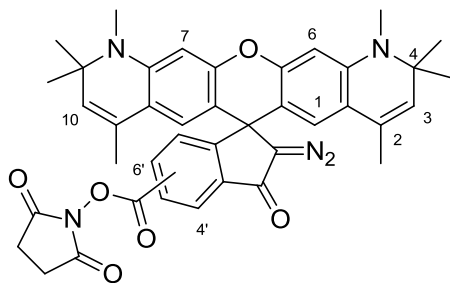
cage8590-aci_CARBON_01



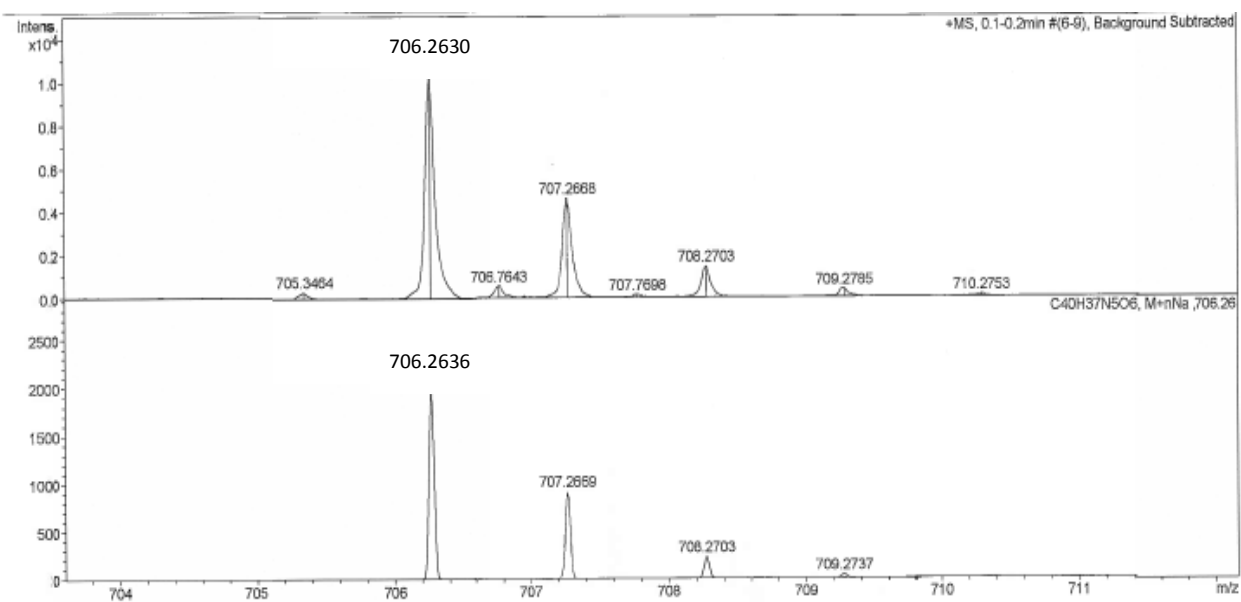
HRMS-ESI mass spectra (negative mode)



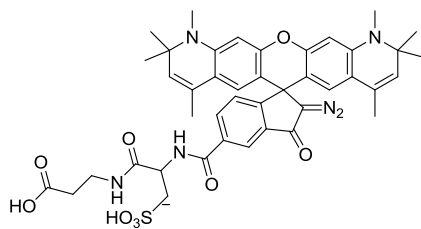
Ester 8-Su



HRMS-ESI mass spectra (positive mode)

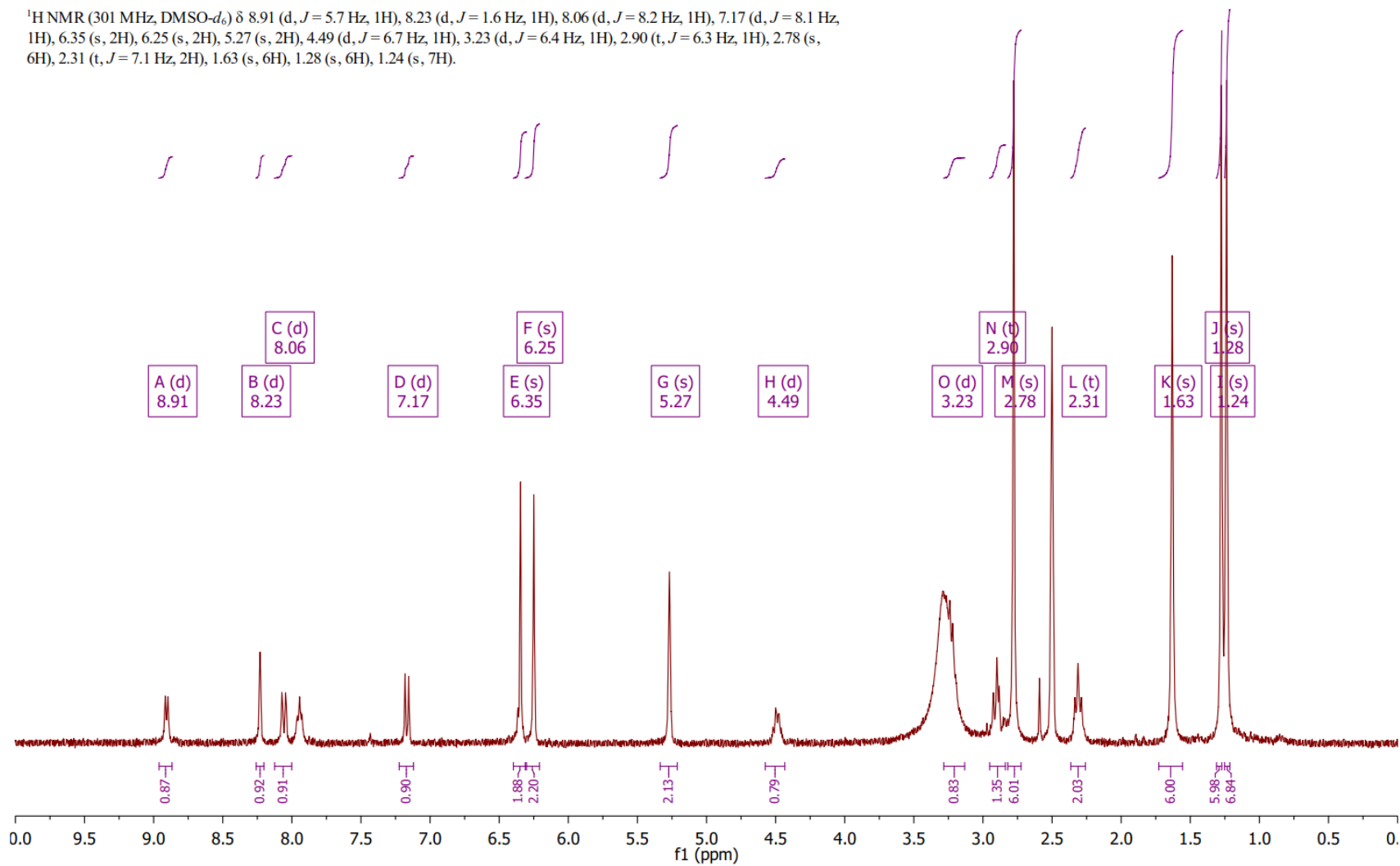


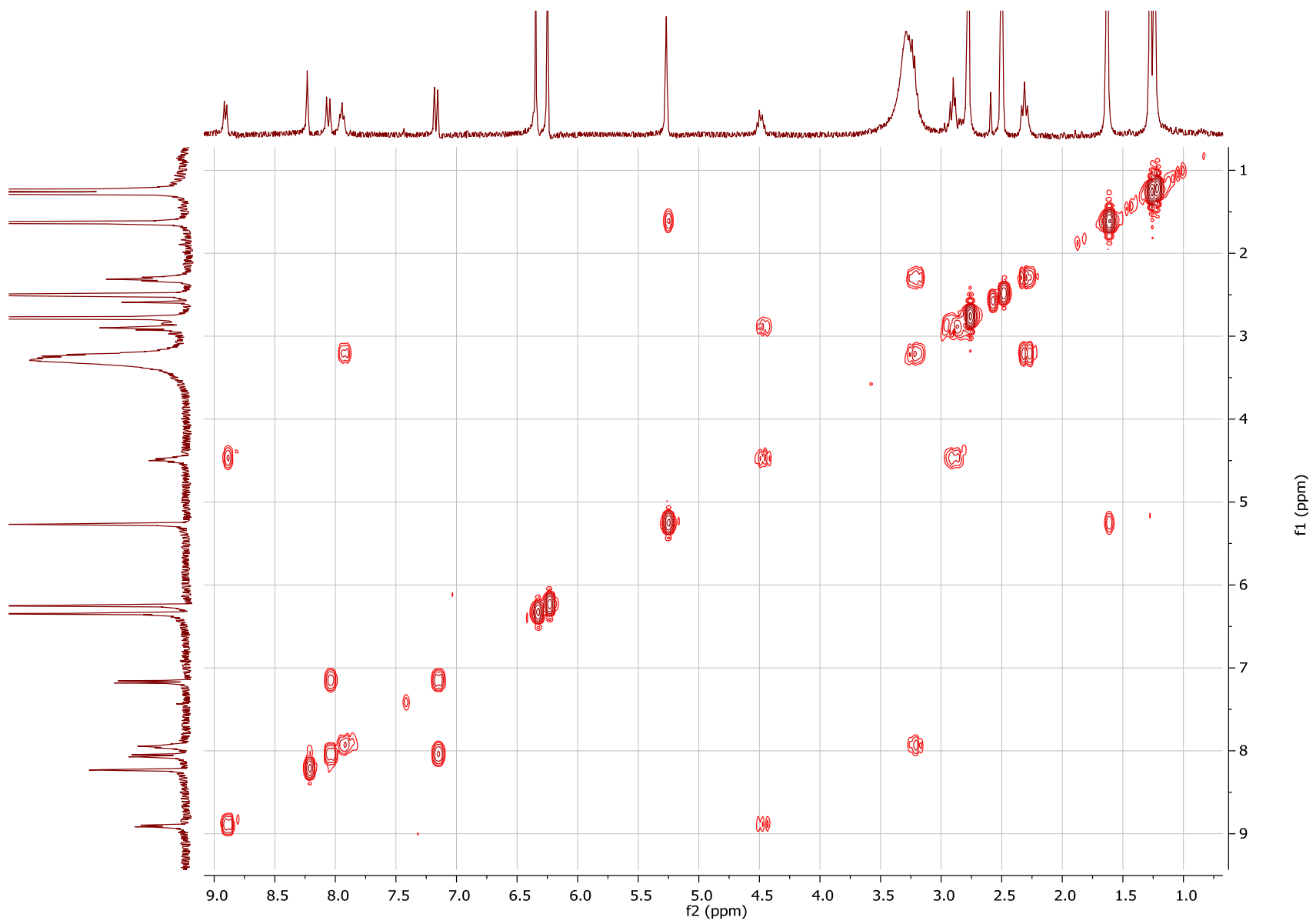
Compound 9-H



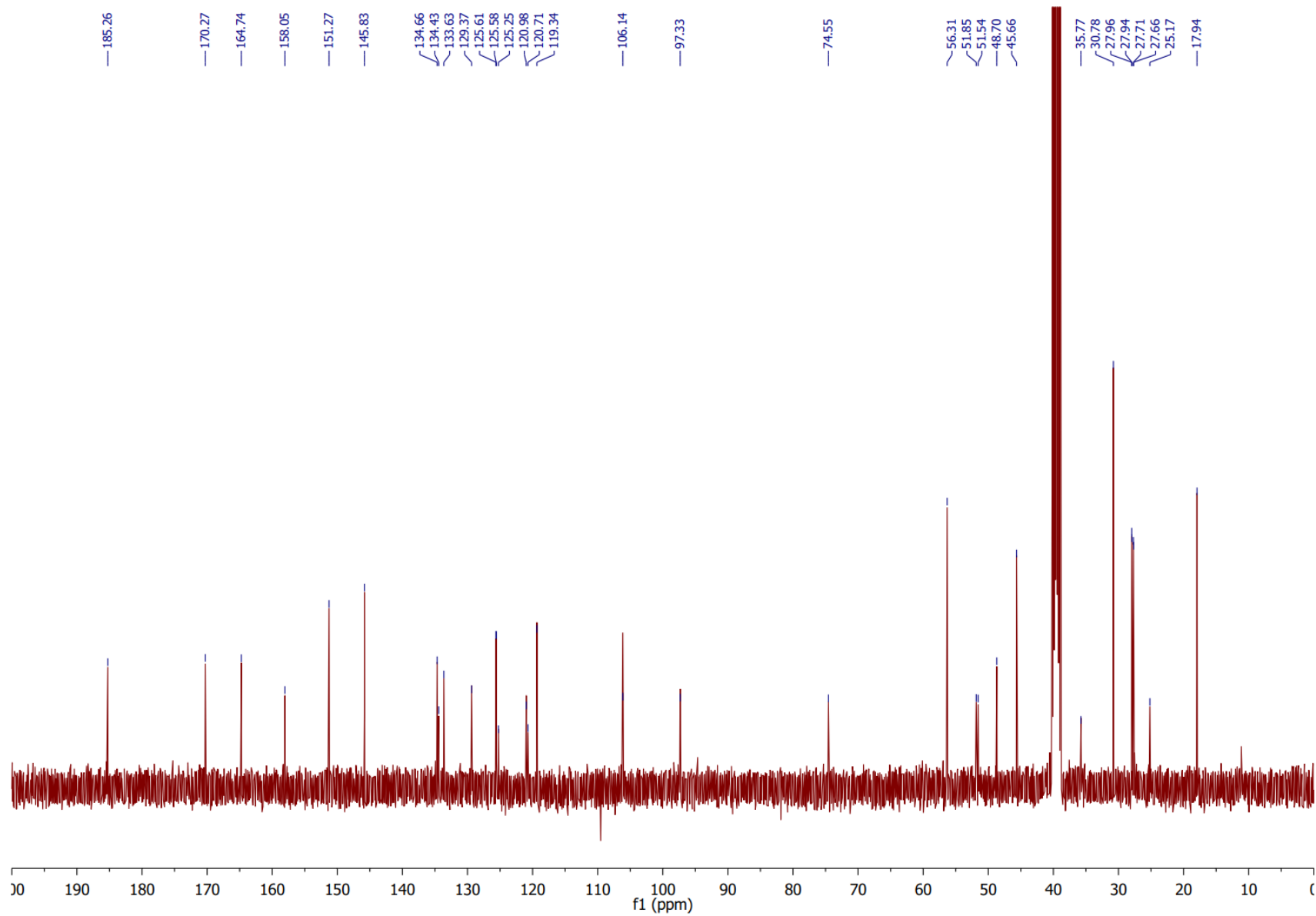
¹H & ¹H-¹H COSY NMR spectra recorded in DMSO-d₆ (300 MHz)

¹H NMR (301 MHz, DMSO-*d*₆) δ 8.91 (d, *J* = 5.7 Hz, 1H), 8.23 (d, *J* = 1.6 Hz, 1H), 8.06 (d, *J* = 8.2 Hz, 1H), 7.17 (d, *J* = 8.1 Hz, 1H), 6.35 (s, 2H), 6.25 (s, 2H), 5.27 (s, 2H), 4.49 (d, *J* = 6.7 Hz, 1H), 3.23 (d, *J* = 6.4 Hz, 1H), 2.90 (t, *J* = 6.3 Hz, 1H), 2.78 (s, 6H), 2.31 (t, *J* = 7.1 Hz, 2H), 1.63 (s, 6H), 1.28 (s, 6H), 1.24 (s, 7H).



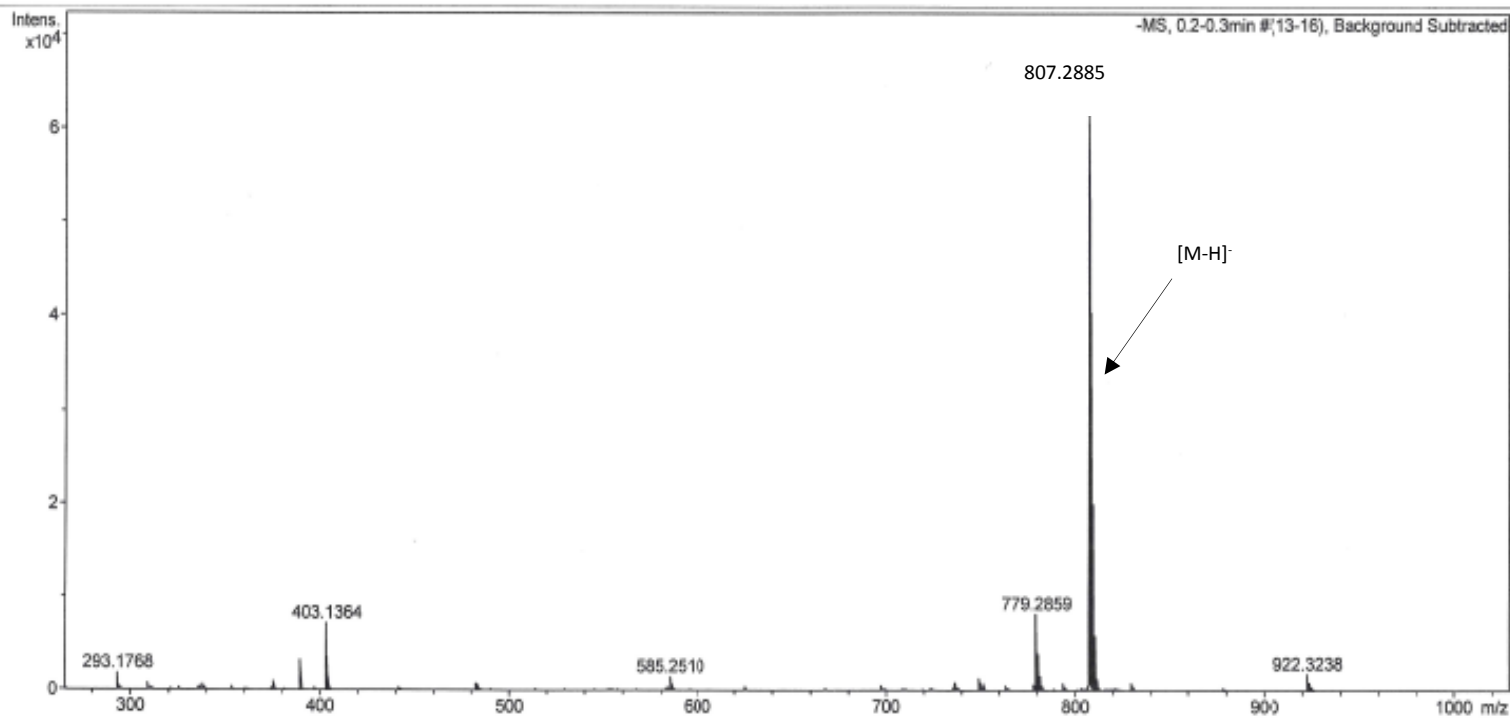


¹³C NMR spectrum recorded in DMSO-d₆ (125 MHz)

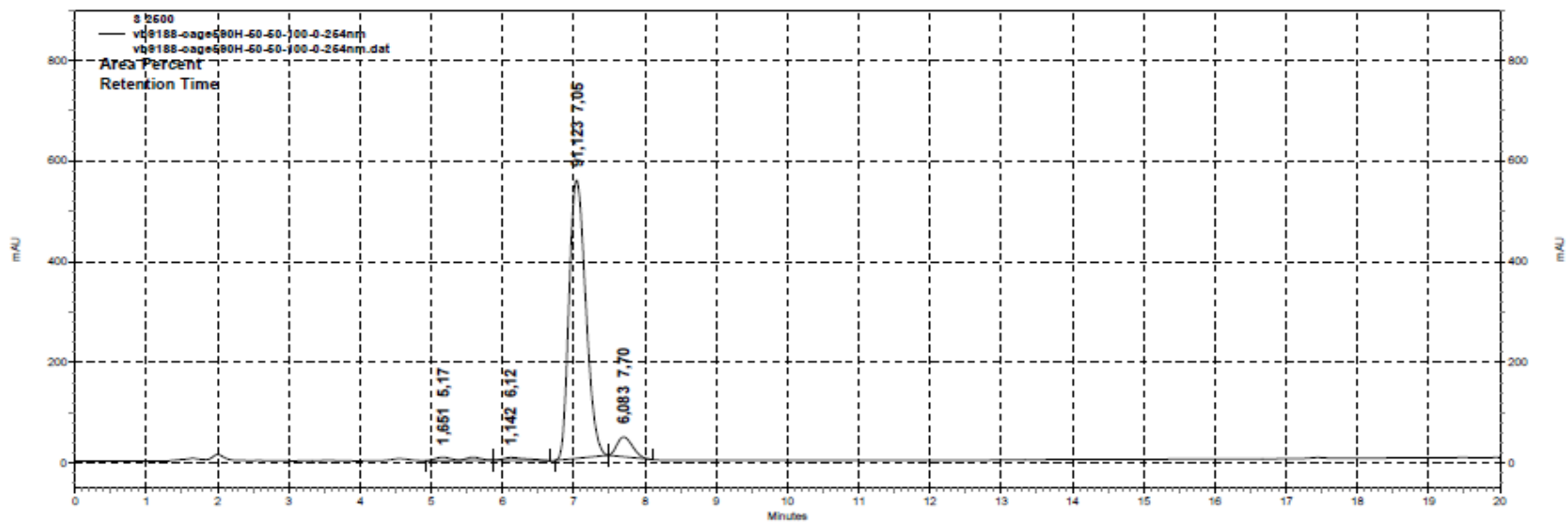


HRMS-ESI mass spectra (negative mode)

Acquisition Parameter					
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	3800 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

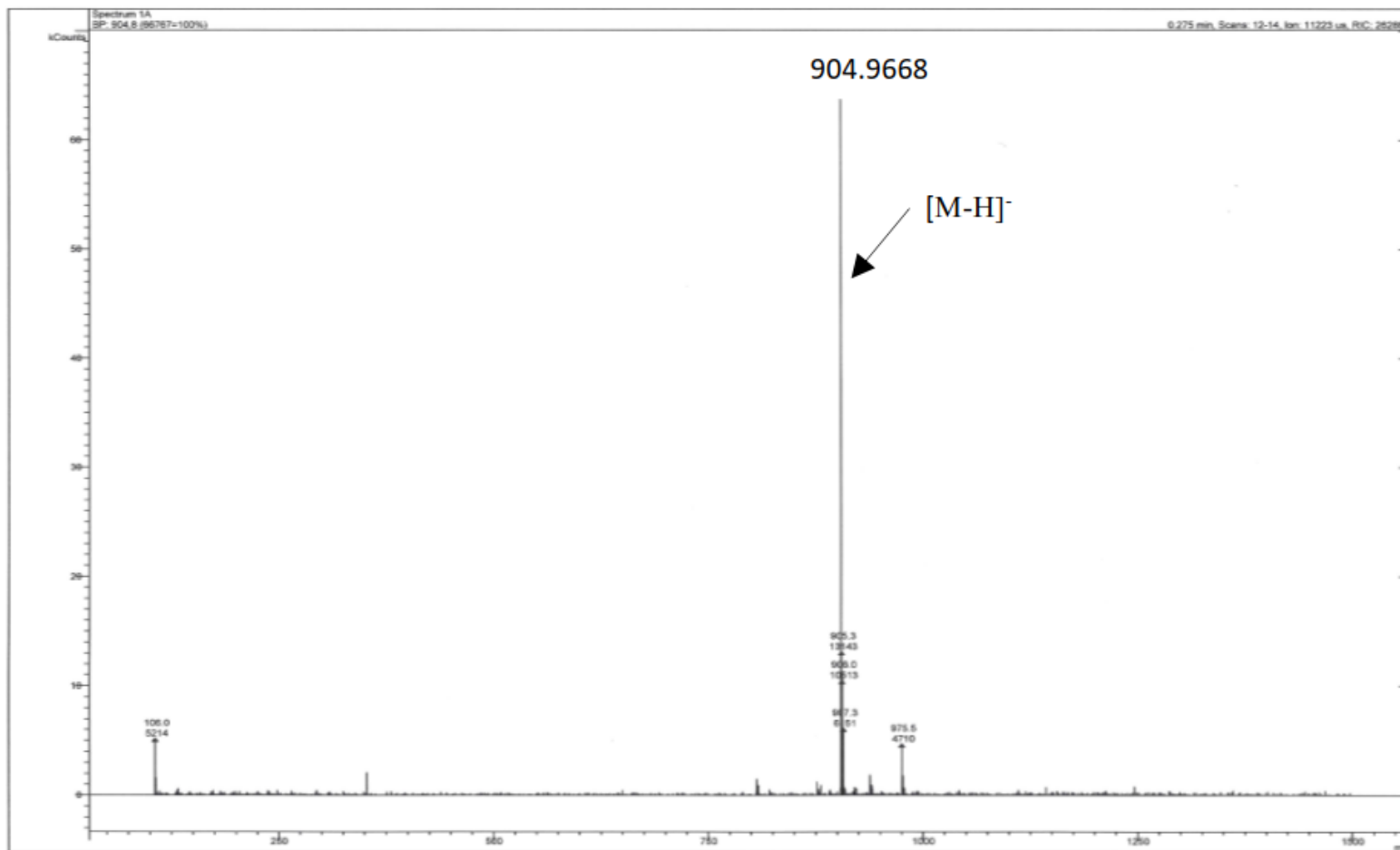


RP-HPLC elution profile (system C)

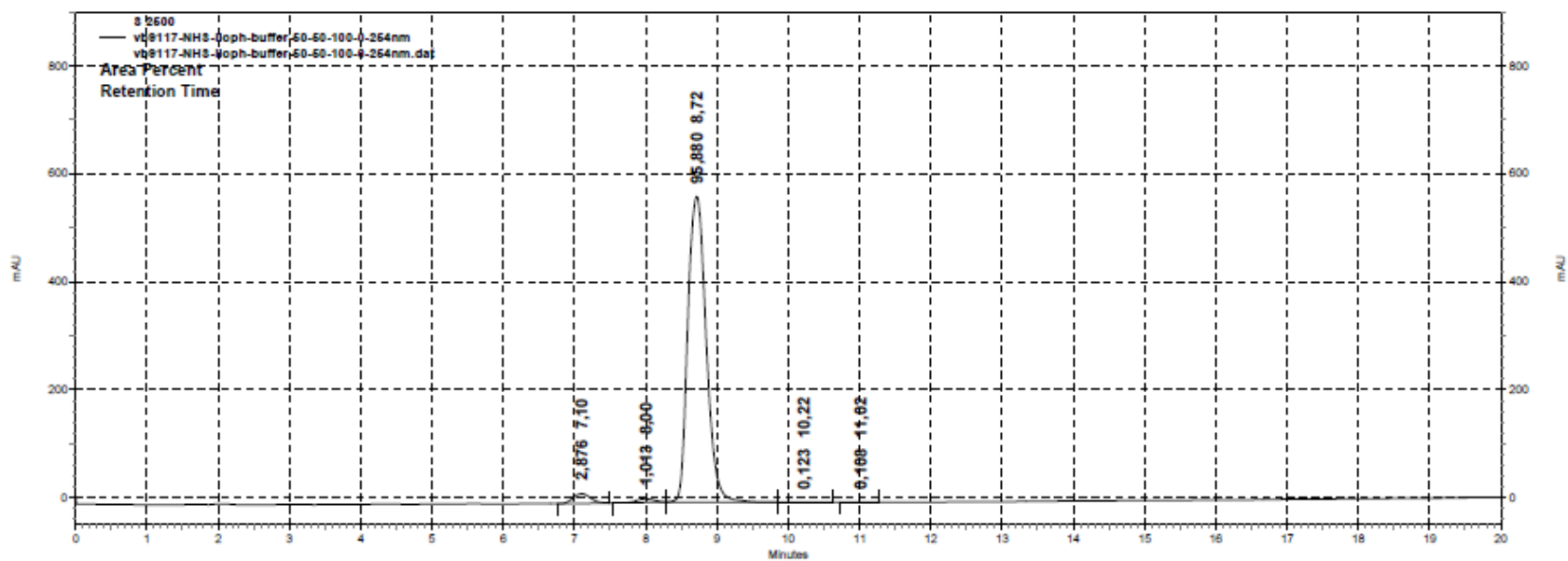


Ester 9-Su

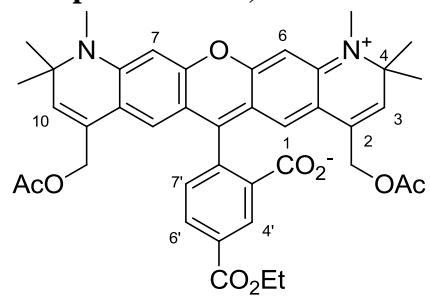
HRMS-ESI mass spectra (negative mode)



RP-HPLC elution profile (system C)

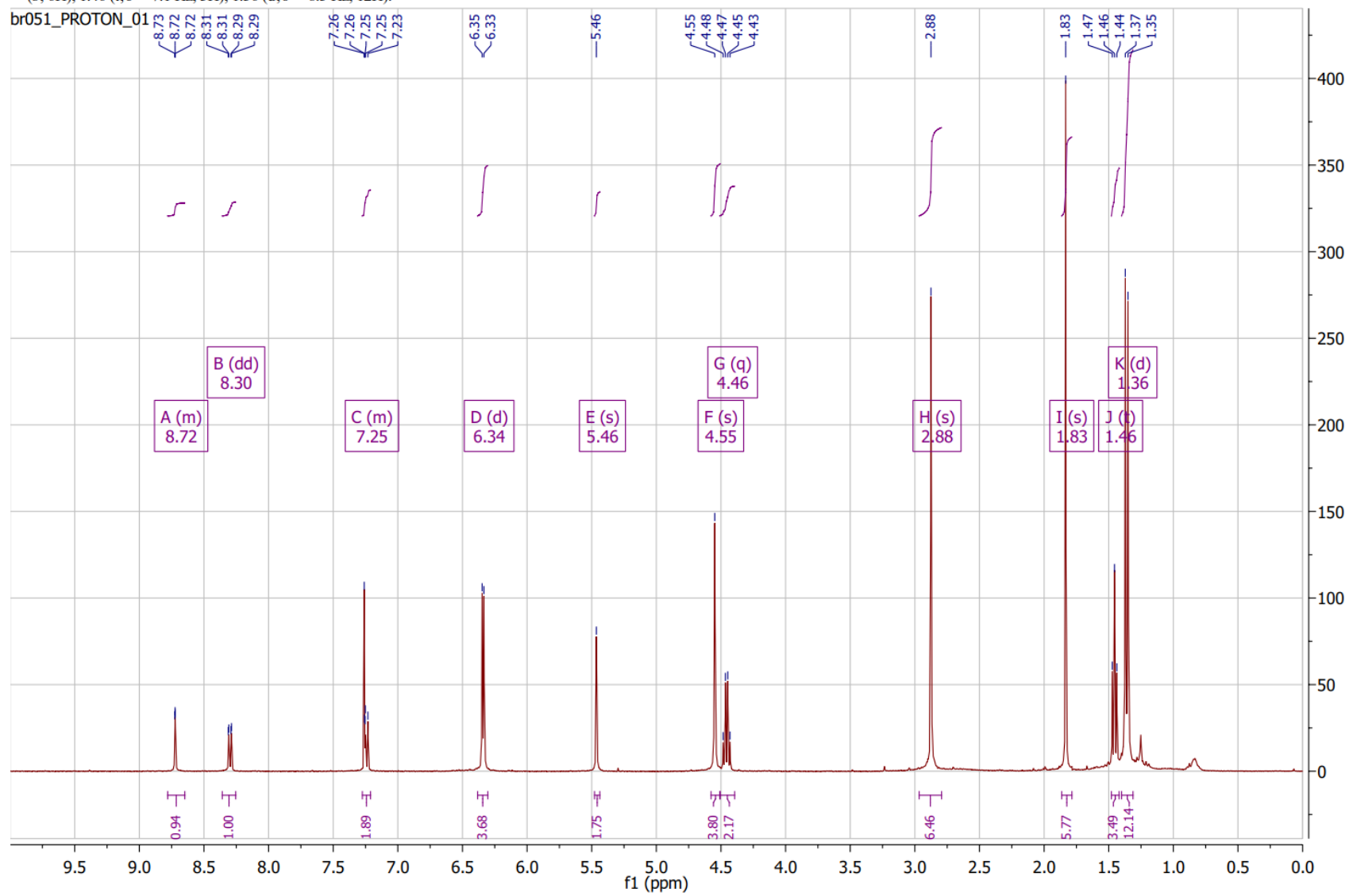


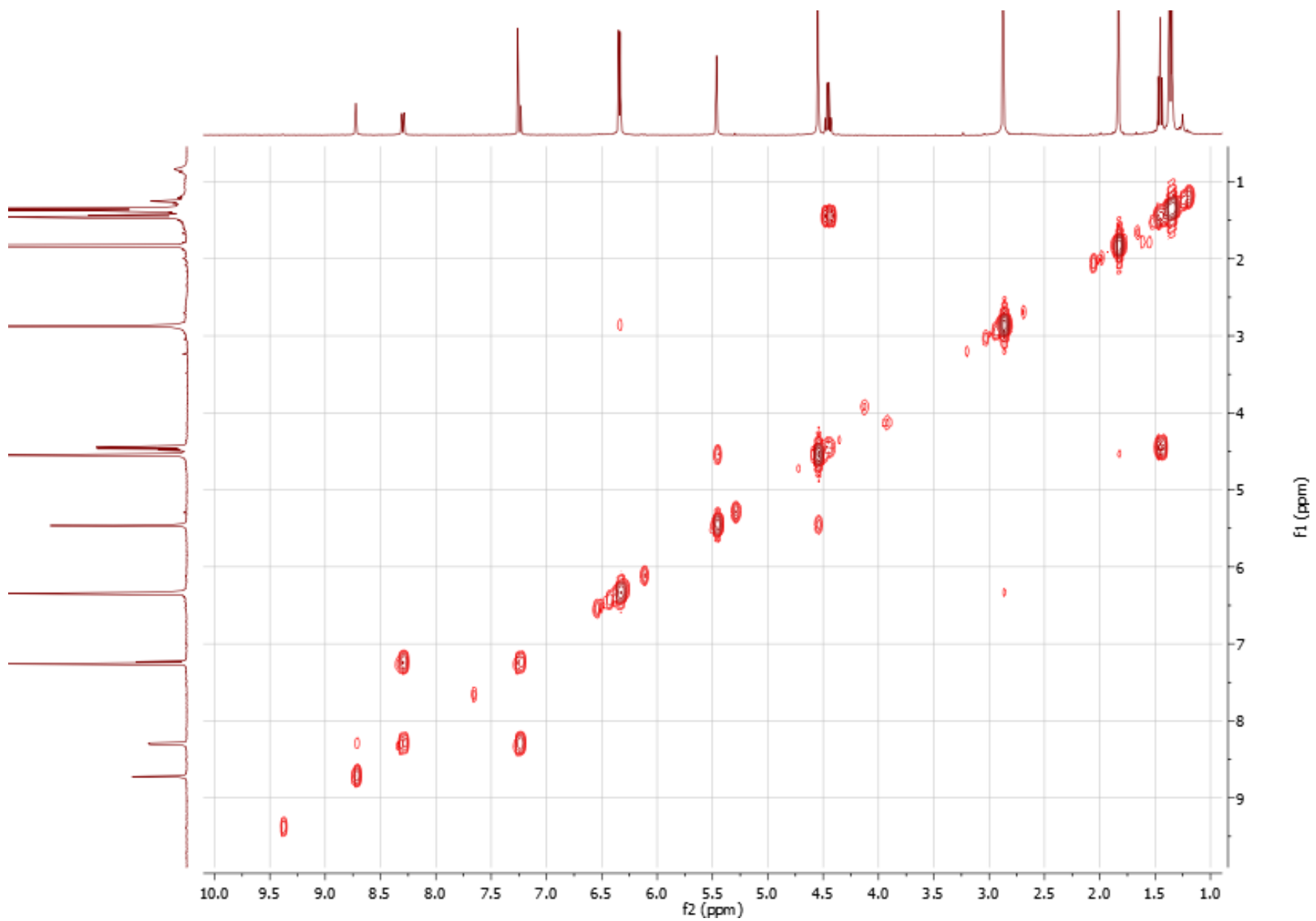
Compound 10-Ac,Et

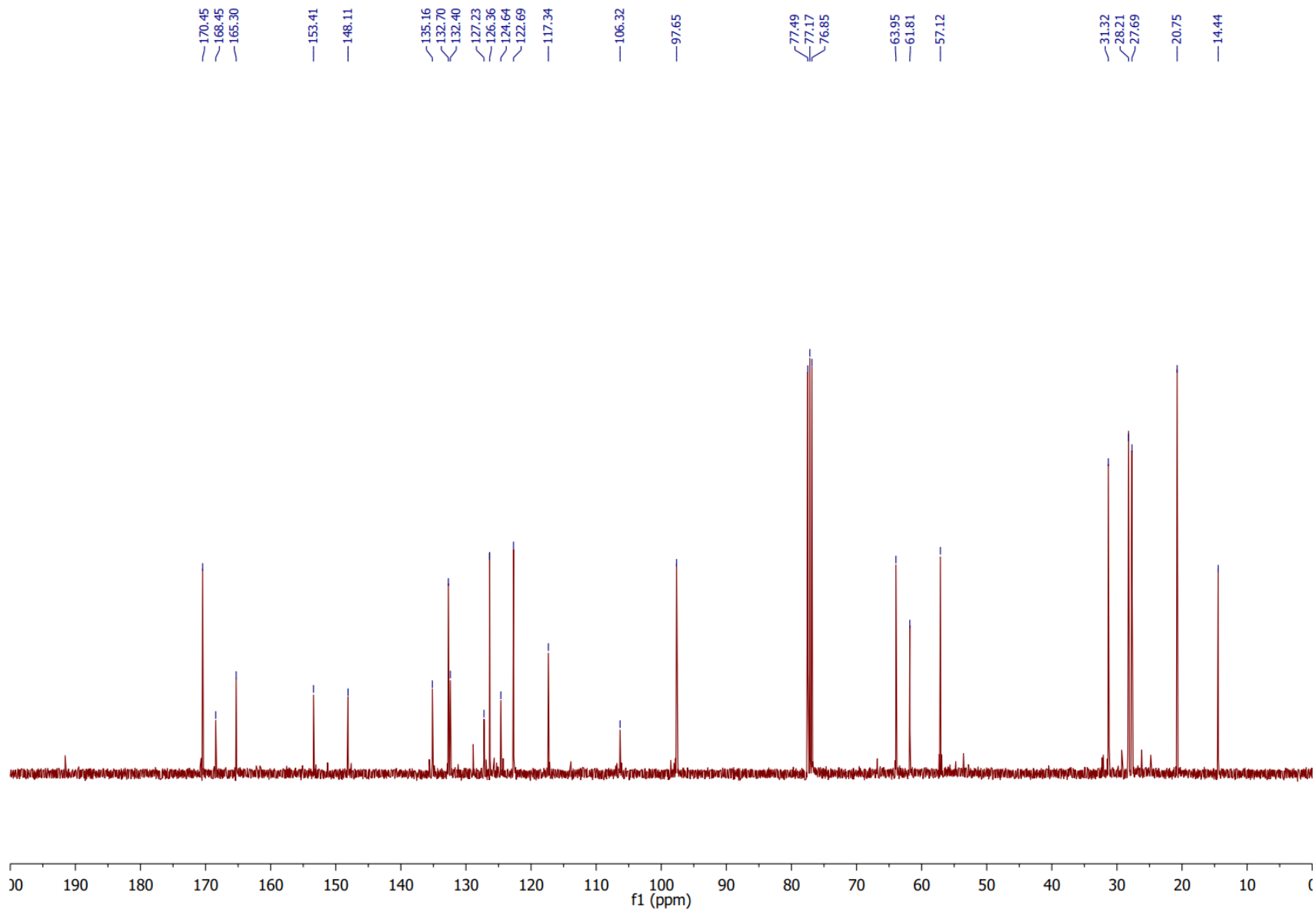


¹H, ¹H-¹H COSY and ¹³C NMR spectra recorded in CDCl₃ (400 and 101 MHz)

¹H NMR (400 MHz, Chloroform-*d*) δ 8.78 – 8.65 (m, 1H), 8.30 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.27 – 7.21 (m, 2H), 6.34 (d, *J* = 5.4 Hz, 4H), 5.46 (s, 2H), 4.55 (s, 4H), 4.46 (q, *J* = 7.1 Hz, 2H), 2.88 (s, 6H), 1.83 (s, 6H), 1.46 (t, *J* = 7.1 Hz, 3H), 1.36 (d, *J* = 8.3 Hz, 12H).



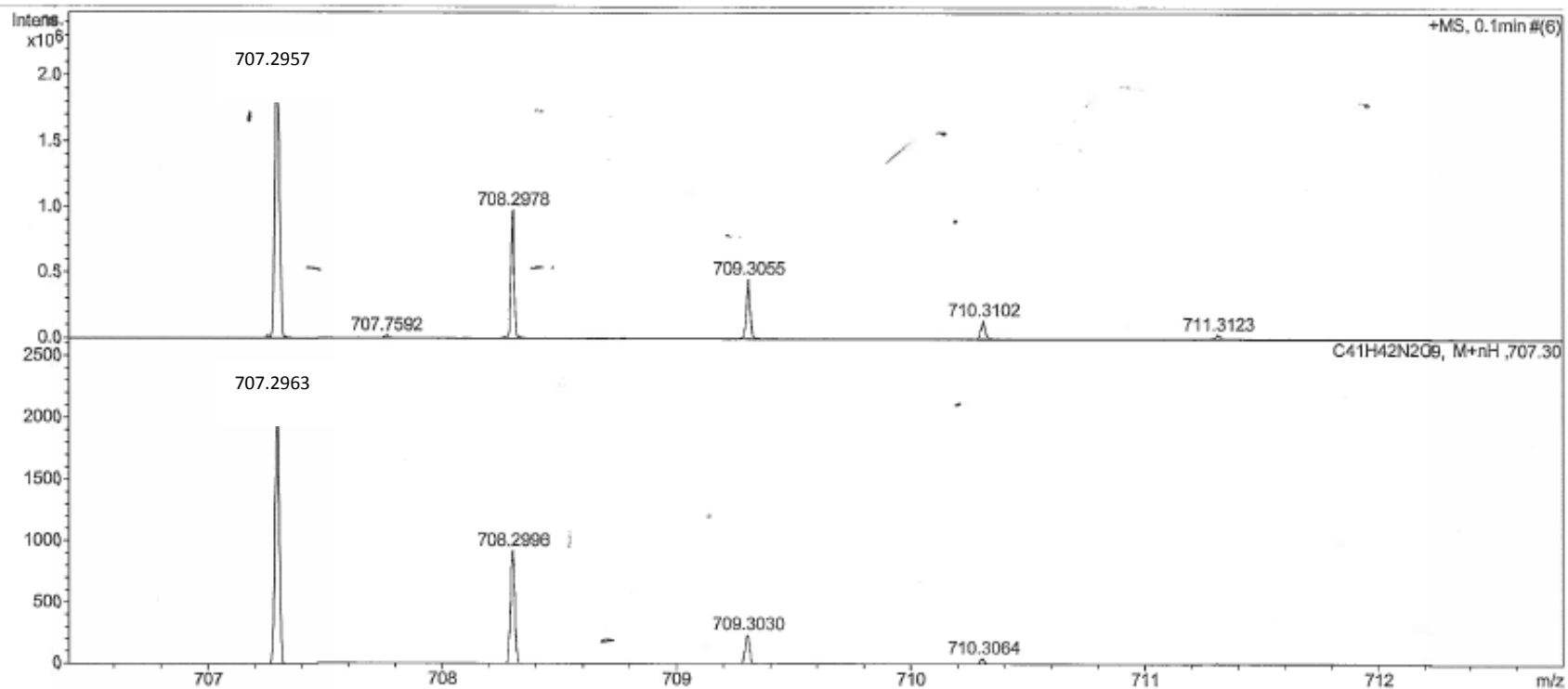


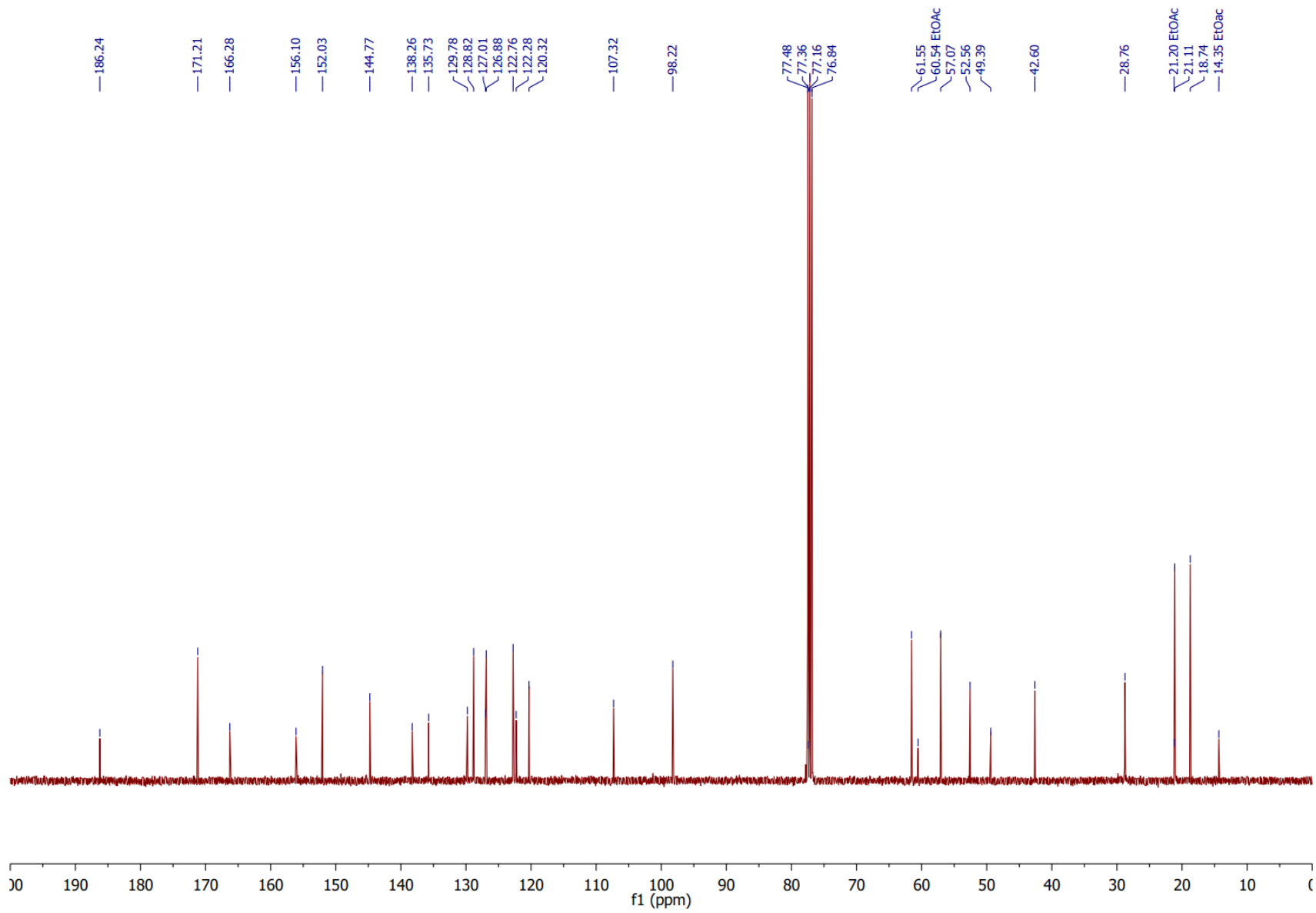


10-Ac,Et: HRMS-ESI mass spectra (positive mode)

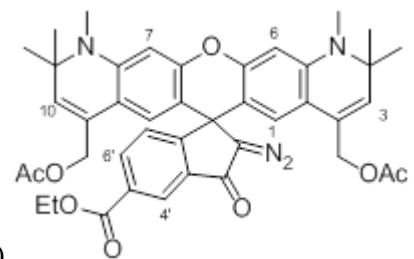
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1600 m/z			Set Divert Valve	Source



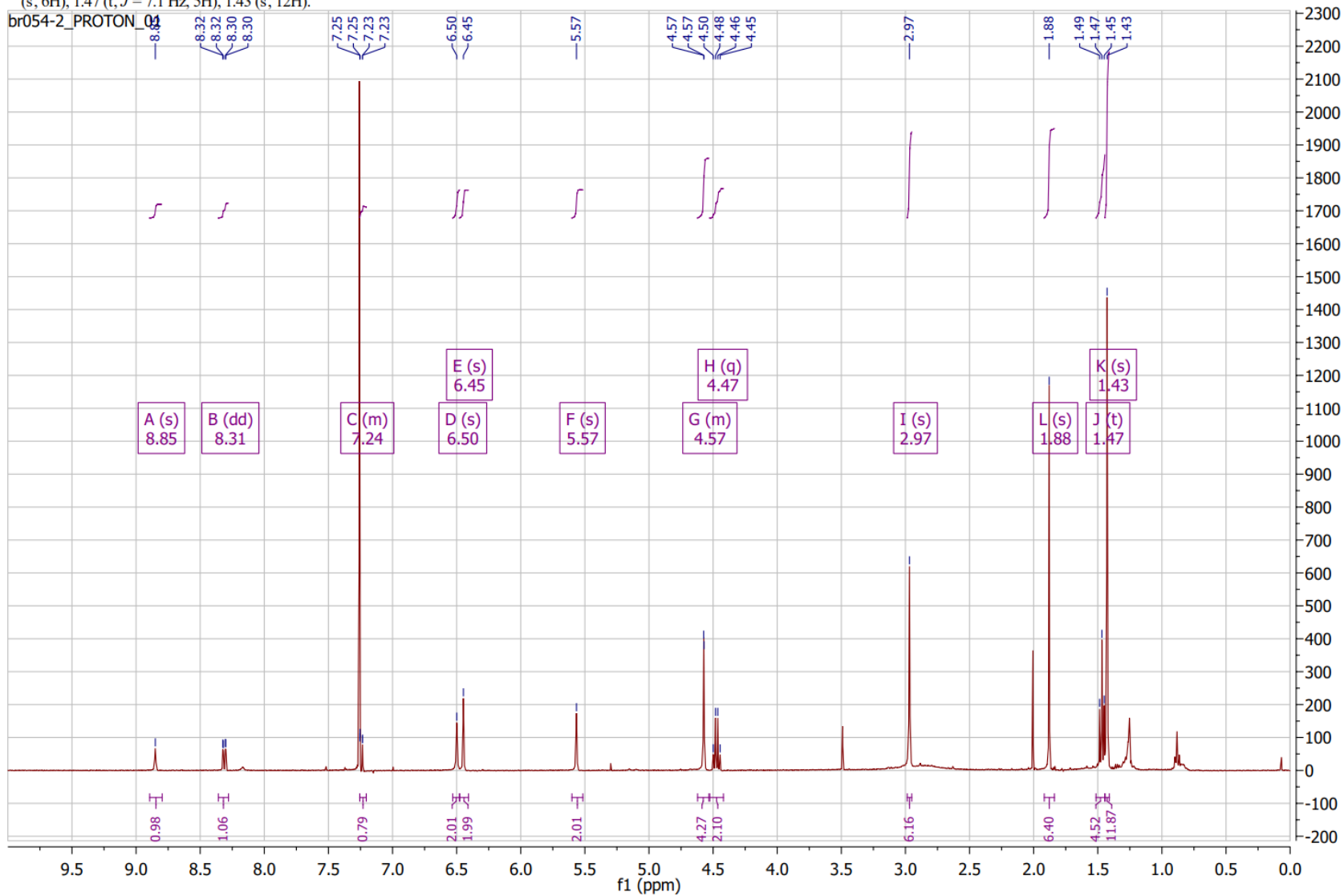


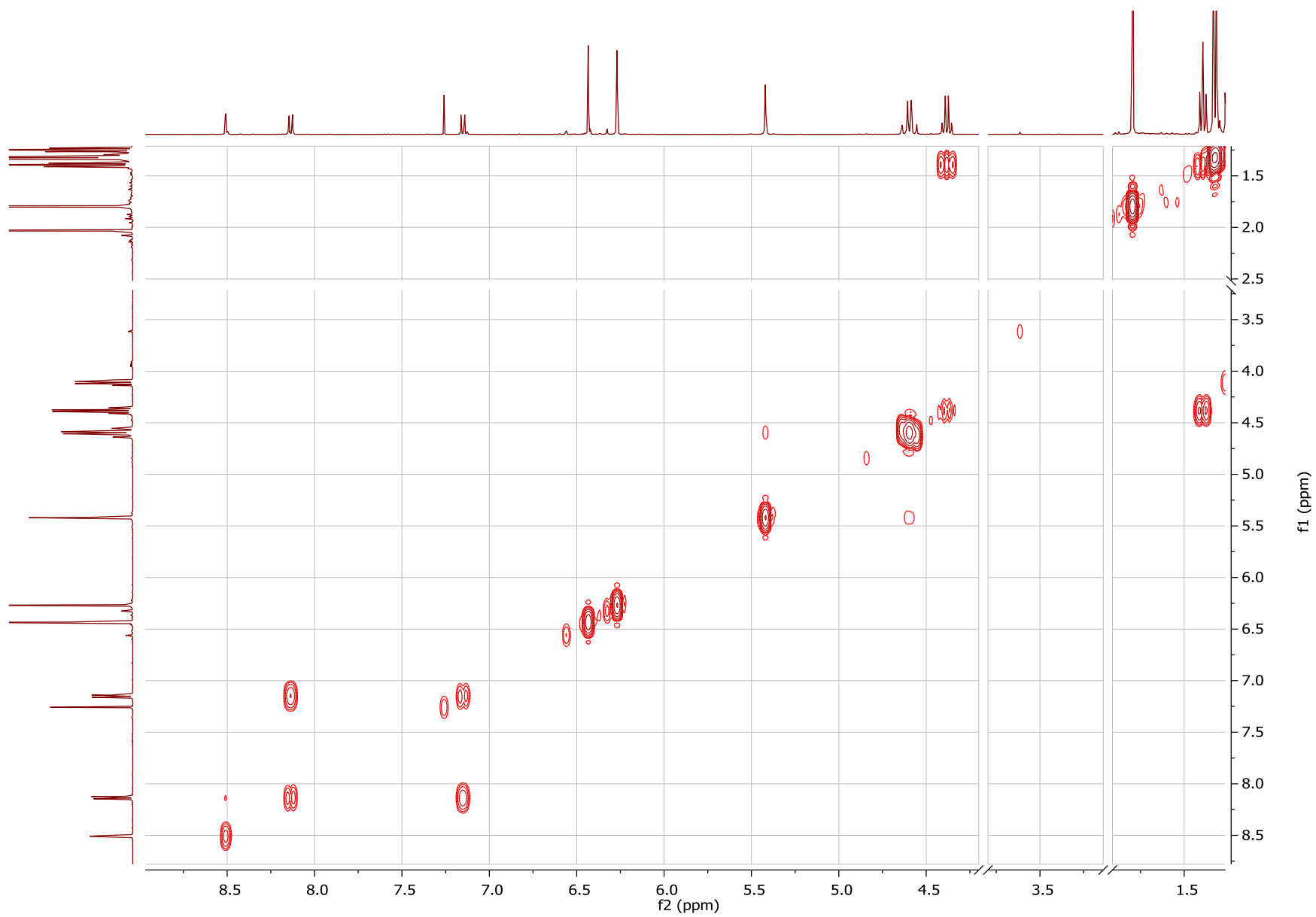
Compound 12-Ac,Et

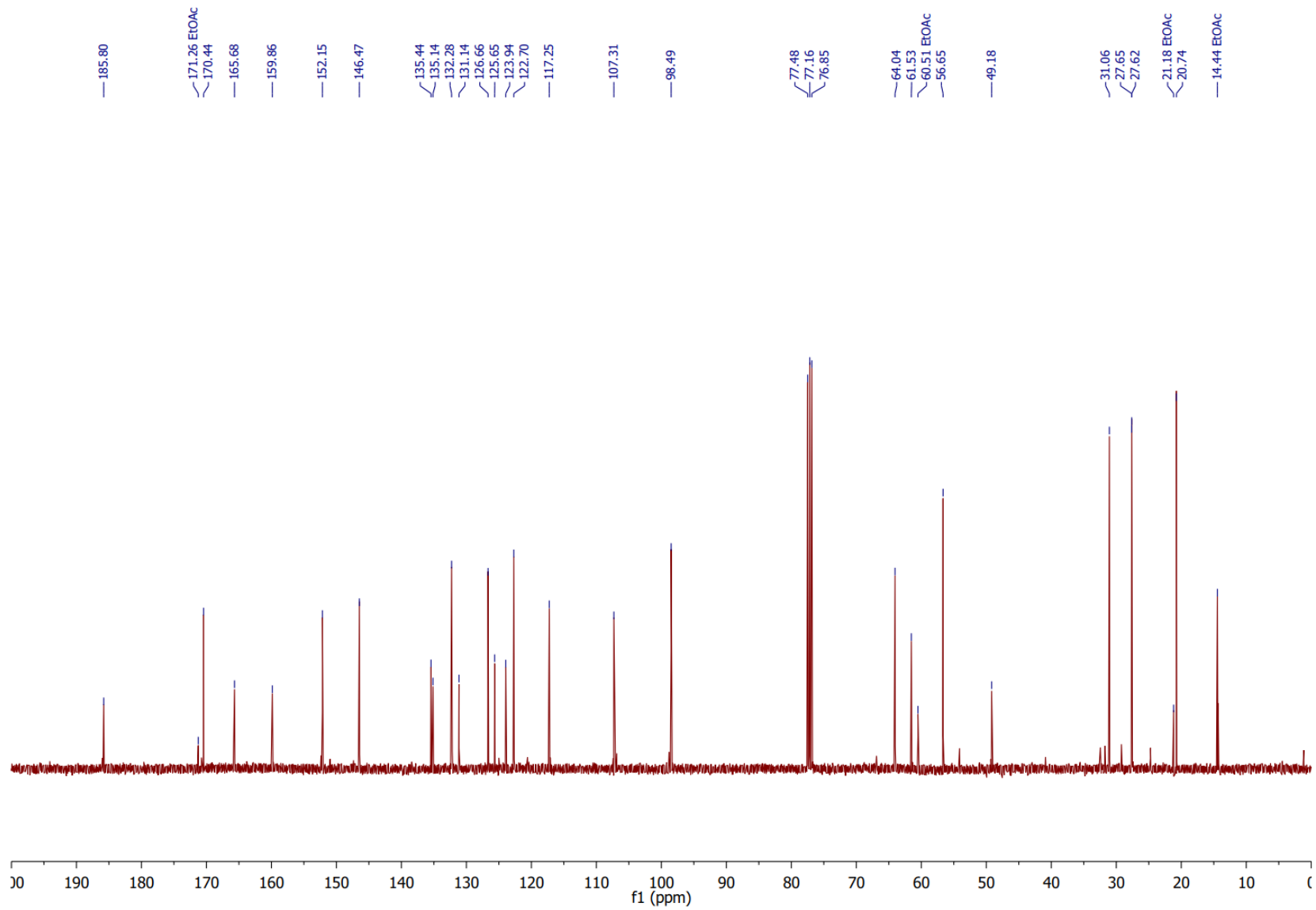


^1H , ^1H - ^1H COSY and ^{13}C NMR spectra recorded in CDCl_3 (400 and 101 MHz)

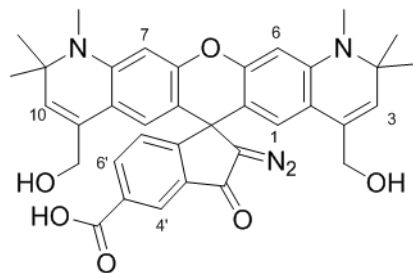
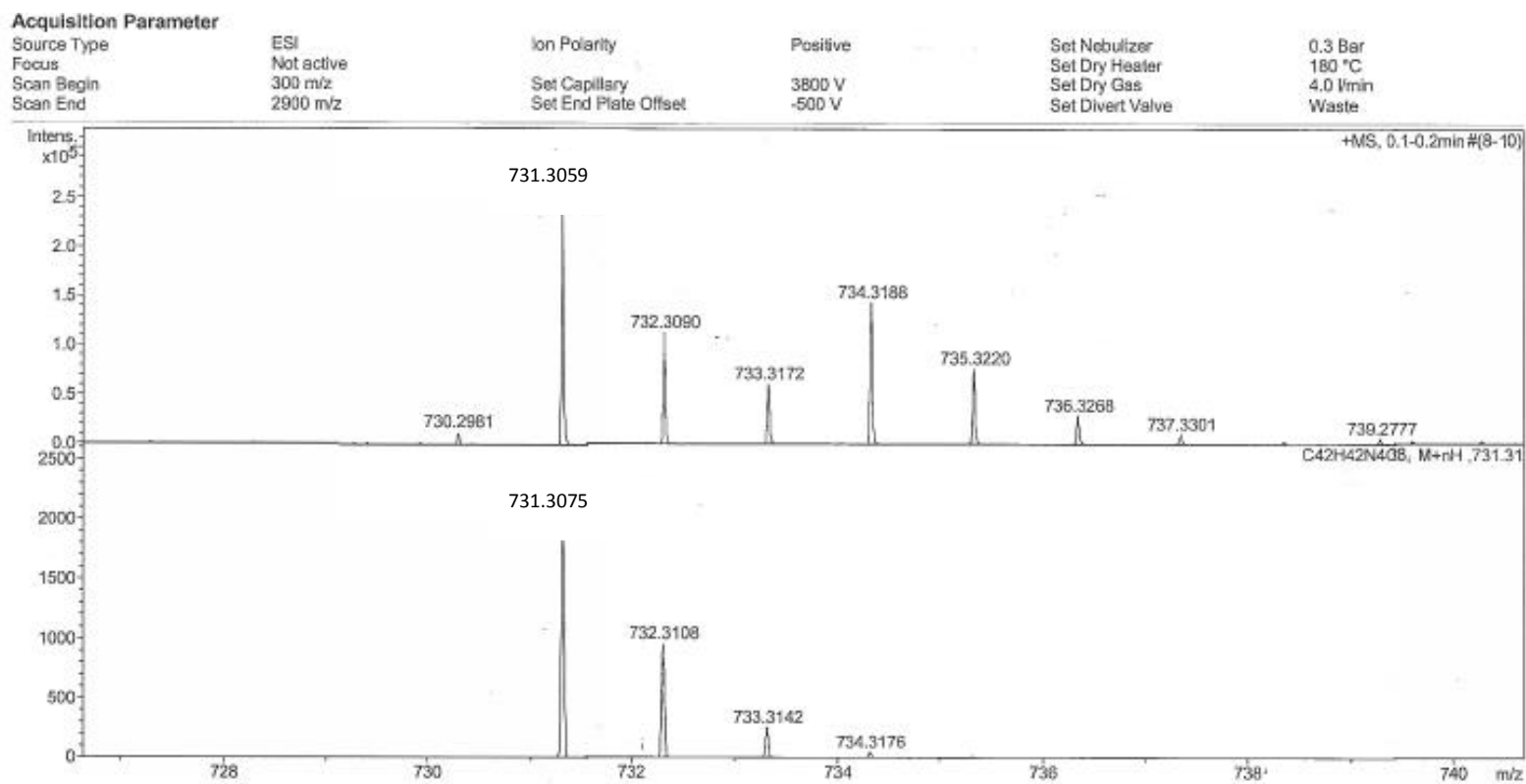
¹H NMR (400 MHz, Chloroform-*d*) δ 8.85 (s, 1H), 8.31 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.26 – 7.20 (m, 1H), 6.50 (s, 2H), 6.45 (s, 2H), 5.57 (s, 2H), 4.62 – 4.53 (m, 4H), 4.47 (q, *J* = 7.1 Hz, 2H), 2.97 (s, 6H), 1.88 (s, 6H), 1.47 (t, *J* = 7.1 Hz, 5H), 1.43 (s, 12H).







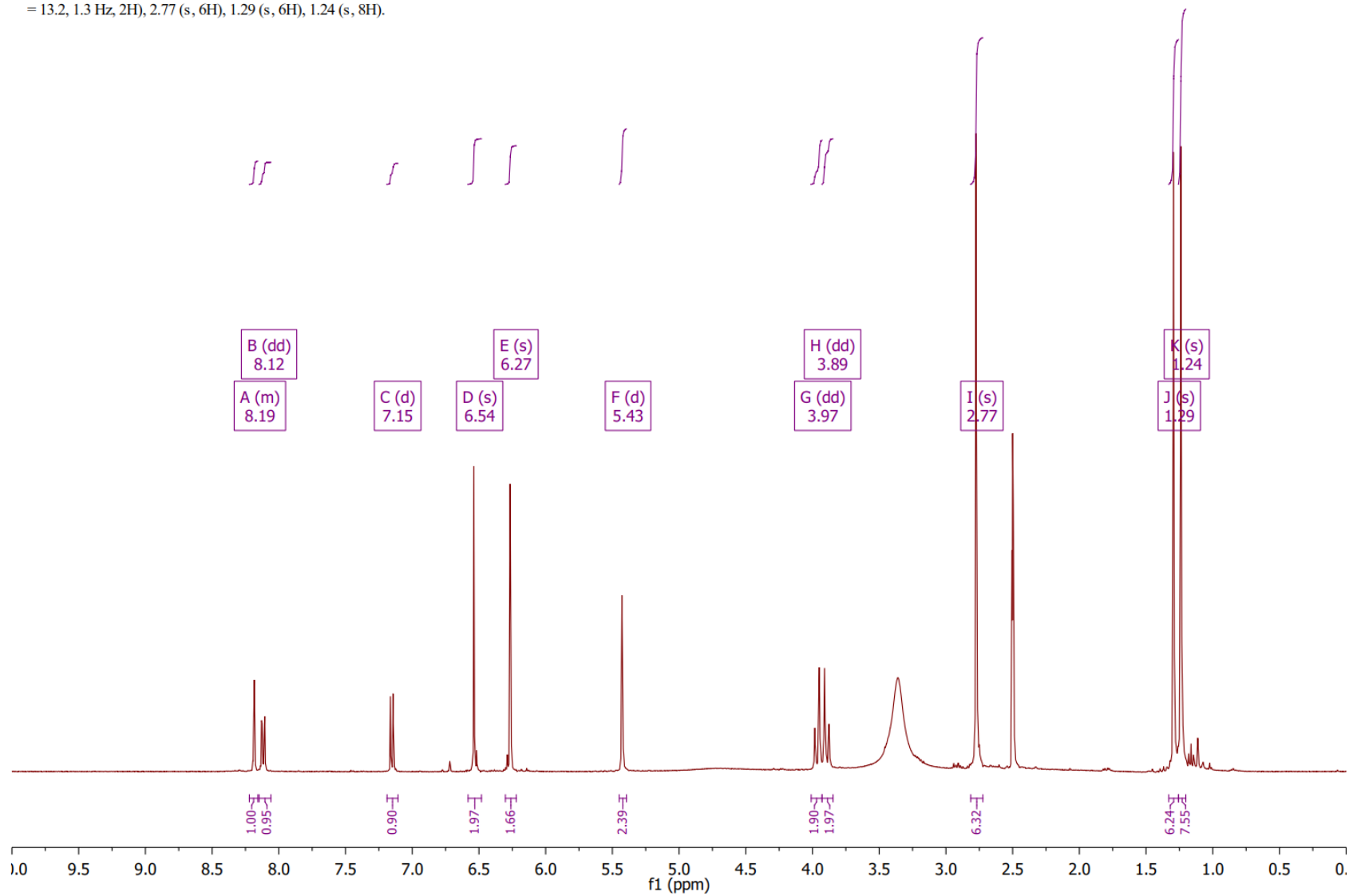
HRMS-ESI mass spectra (positive mode)

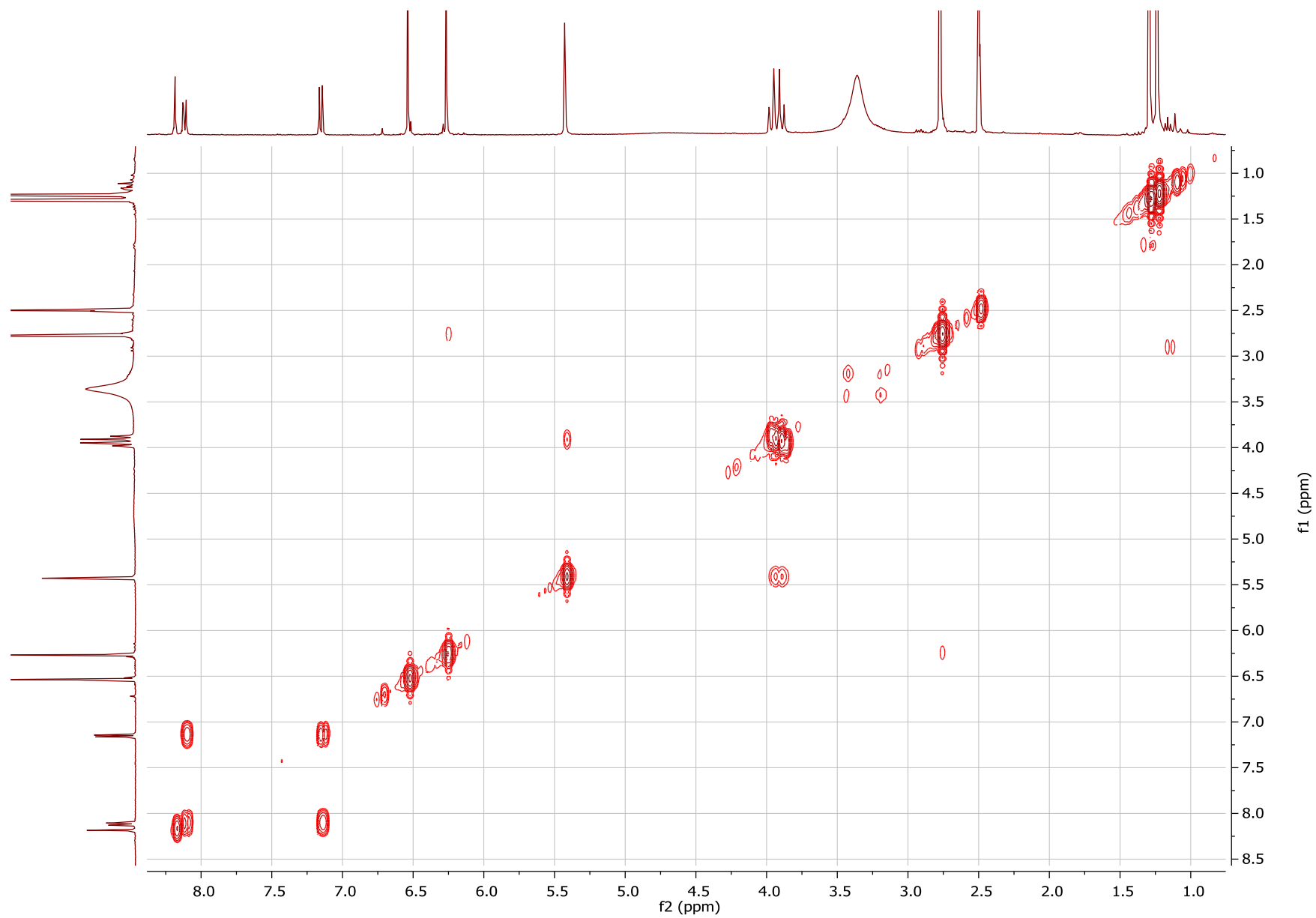


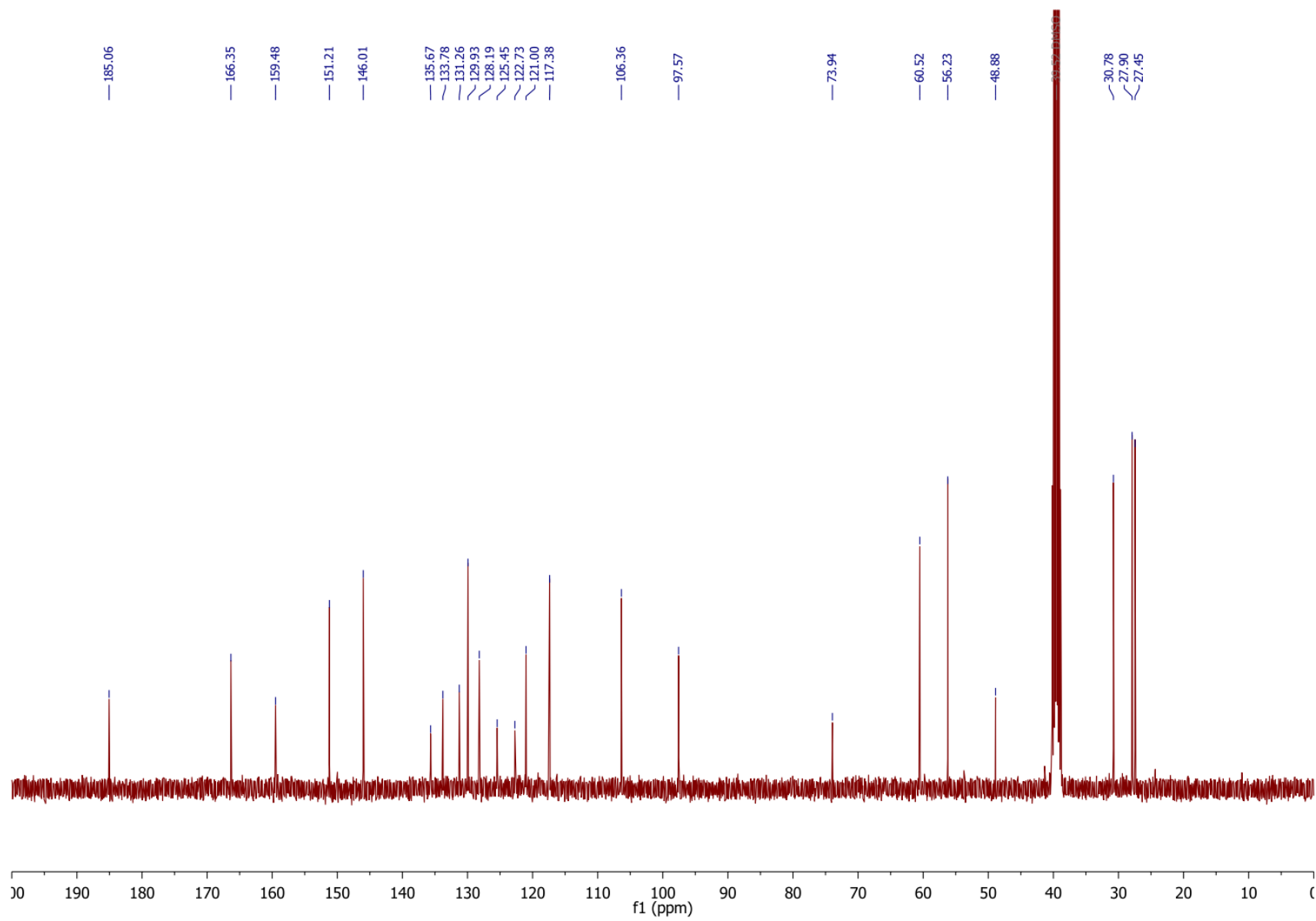
Compound 12-H,H

^1H , ^1H - ^1H COSY and ^{13}C NMR spectra recorded in DMSO- d_6 (400 and 101 MHz)

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.22 – 8.16 (m, 1H), 8.12 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 6.54 (s, 2H), 6.27 (s, 2H), 5.43 (d, *J* = 1.6 Hz, 2H), 3.97 (dd, *J* = 13.2, 1.3 Hz, 2H), 3.89 (dd, *J* = 13.2, 1.3 Hz, 2H), 2.77 (s, 6H), 1.29 (s, 6H), 1.24 (s, 8H).



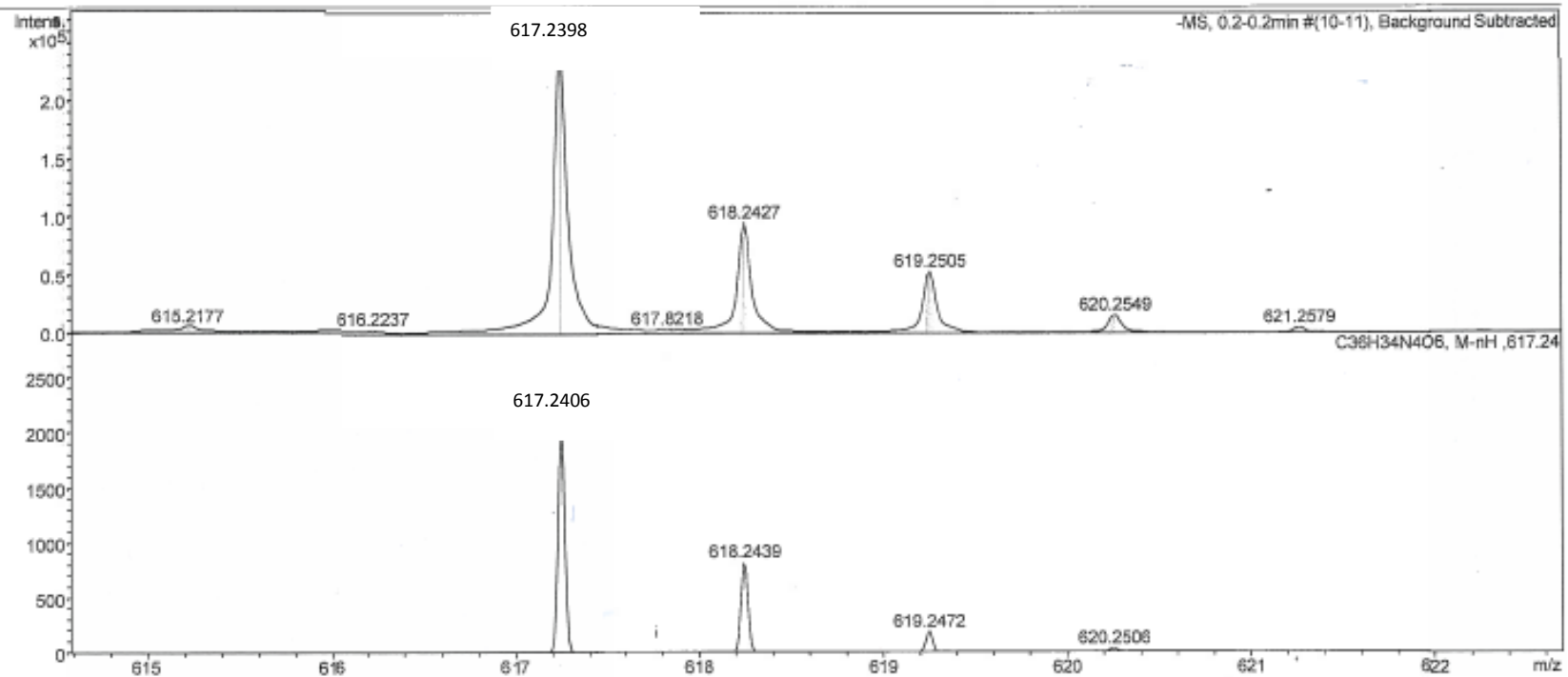


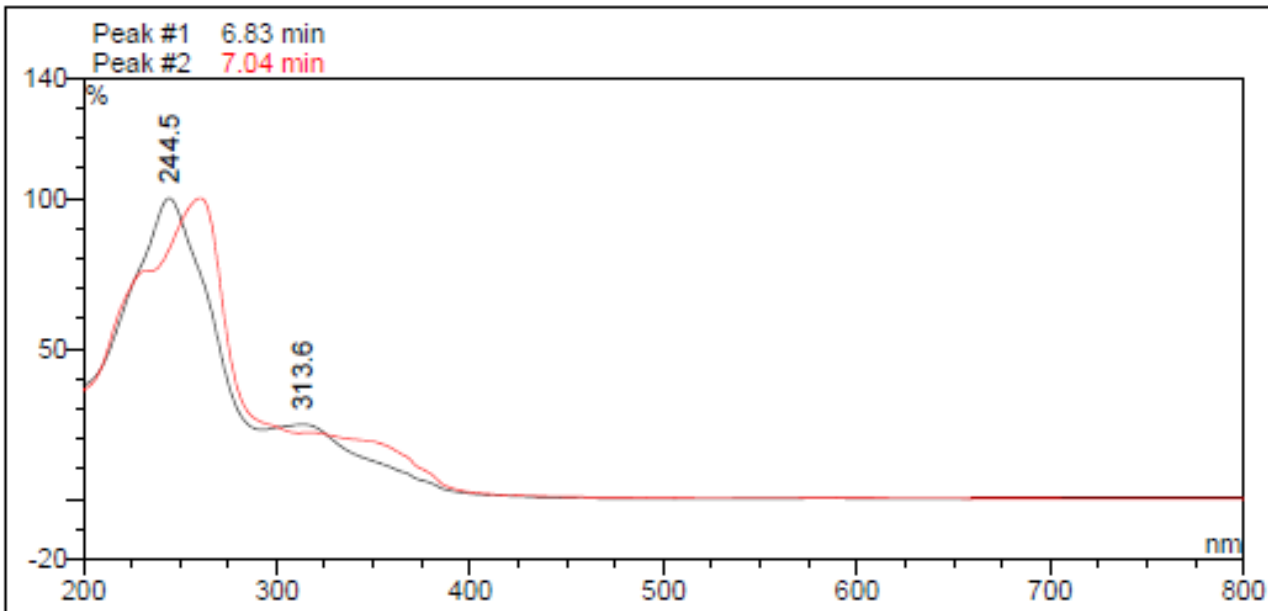
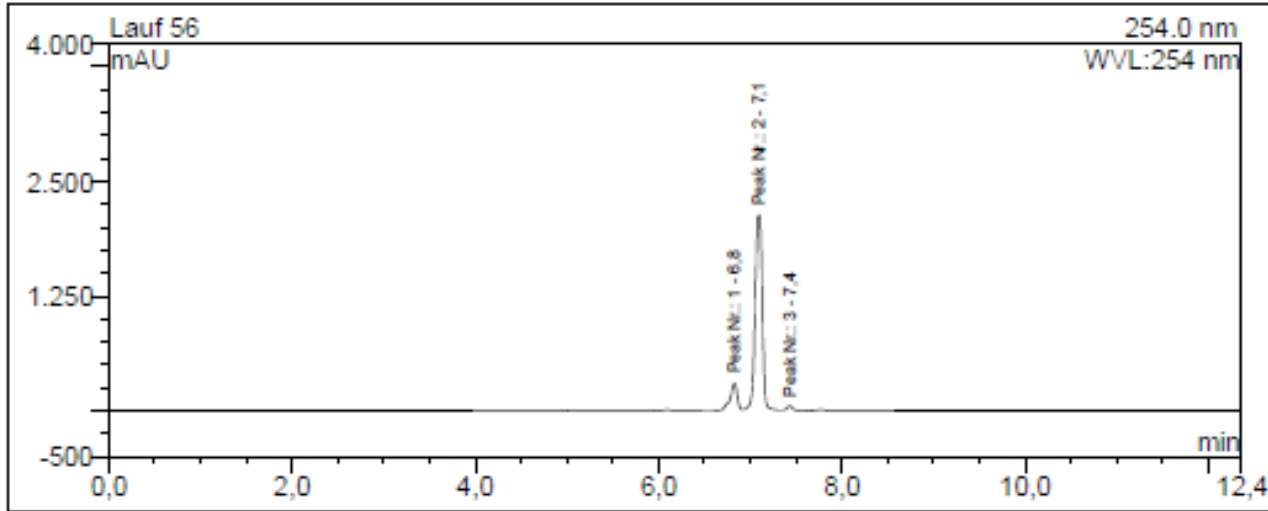


12-H,H: HRMS-ESI mass spectra (negative mode)

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	1.6 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	3800 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source





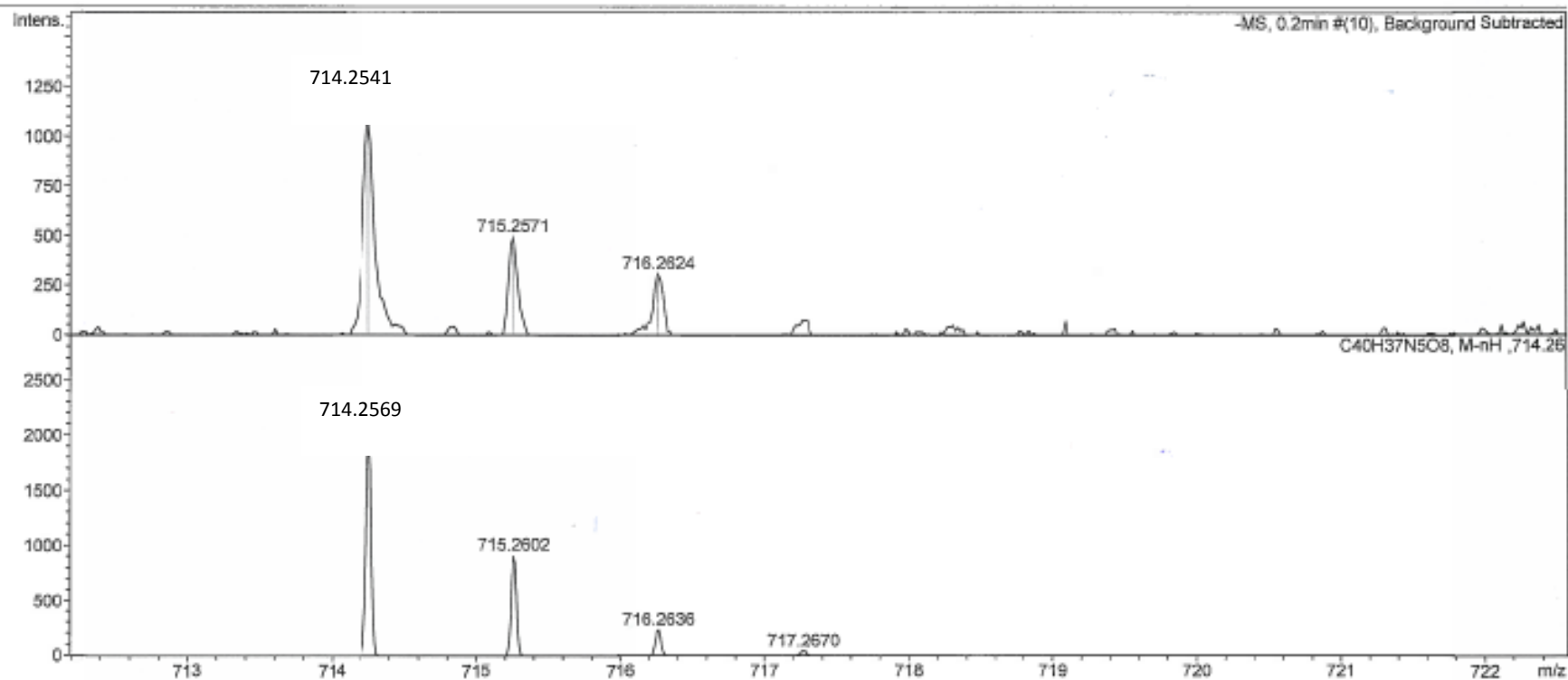
12-H,H: HPLC trace (system E)

Ester 12-H,Su

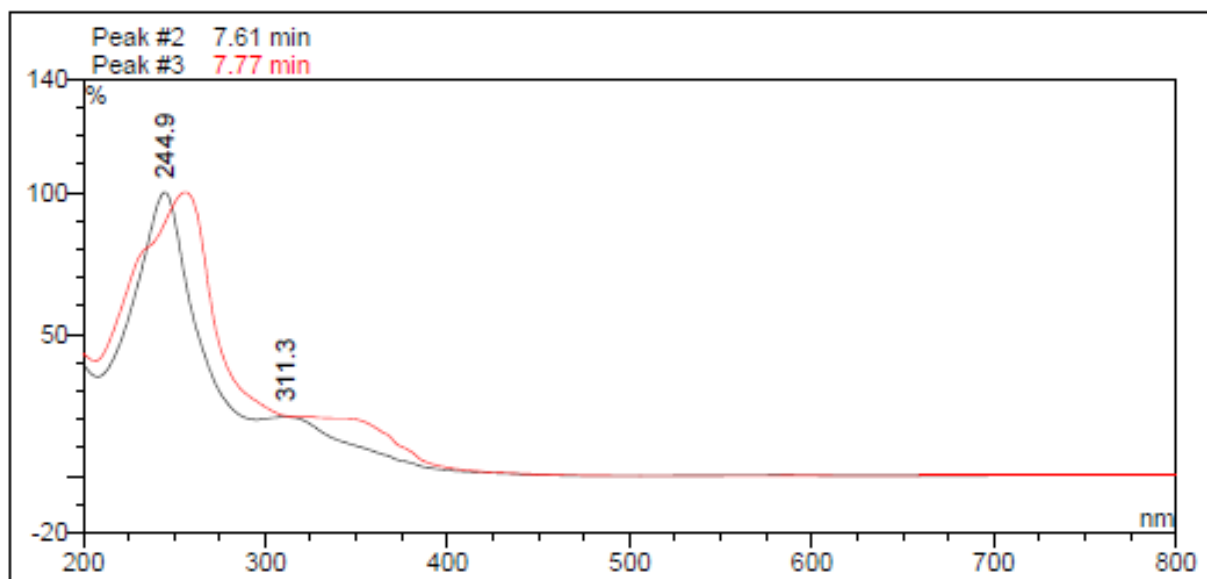
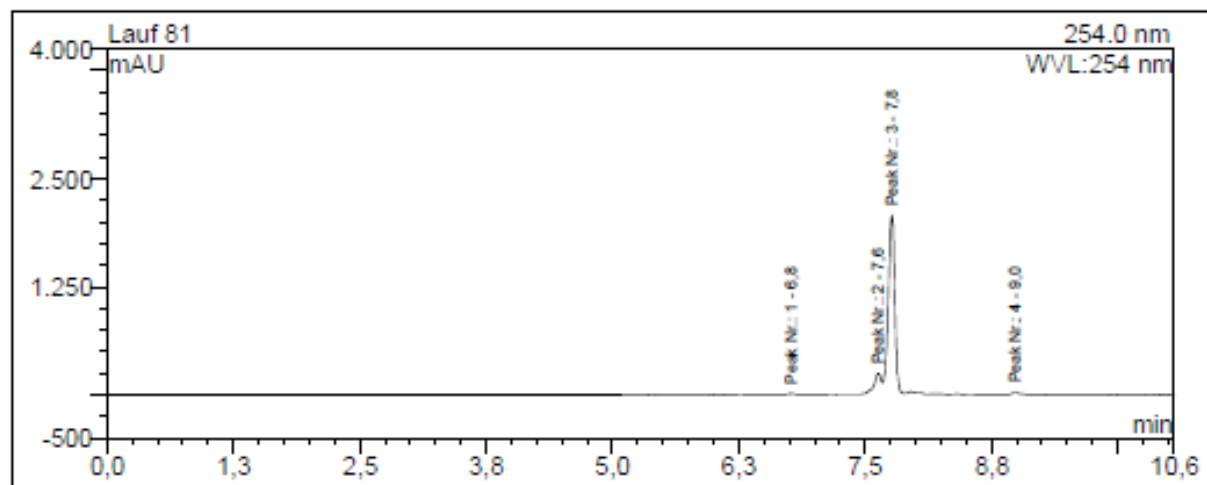
HRMS-ESI mass spectra (negative mode)

Acquisition Parameter

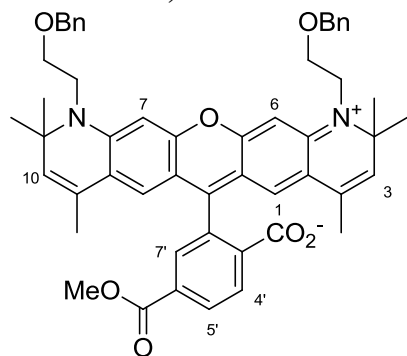
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	1.6 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	3800 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



12-H,Su: HPLC trace (system E)

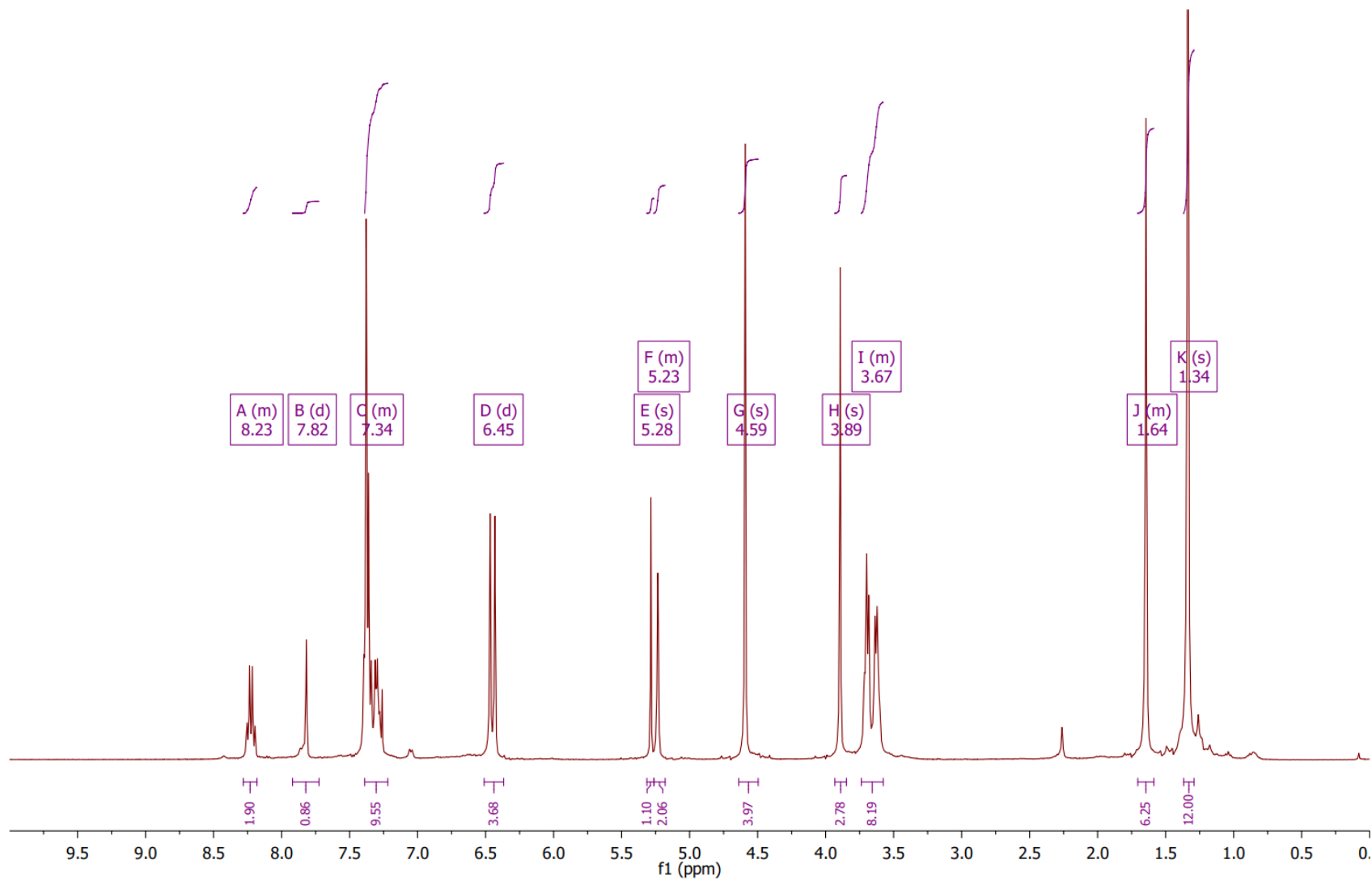


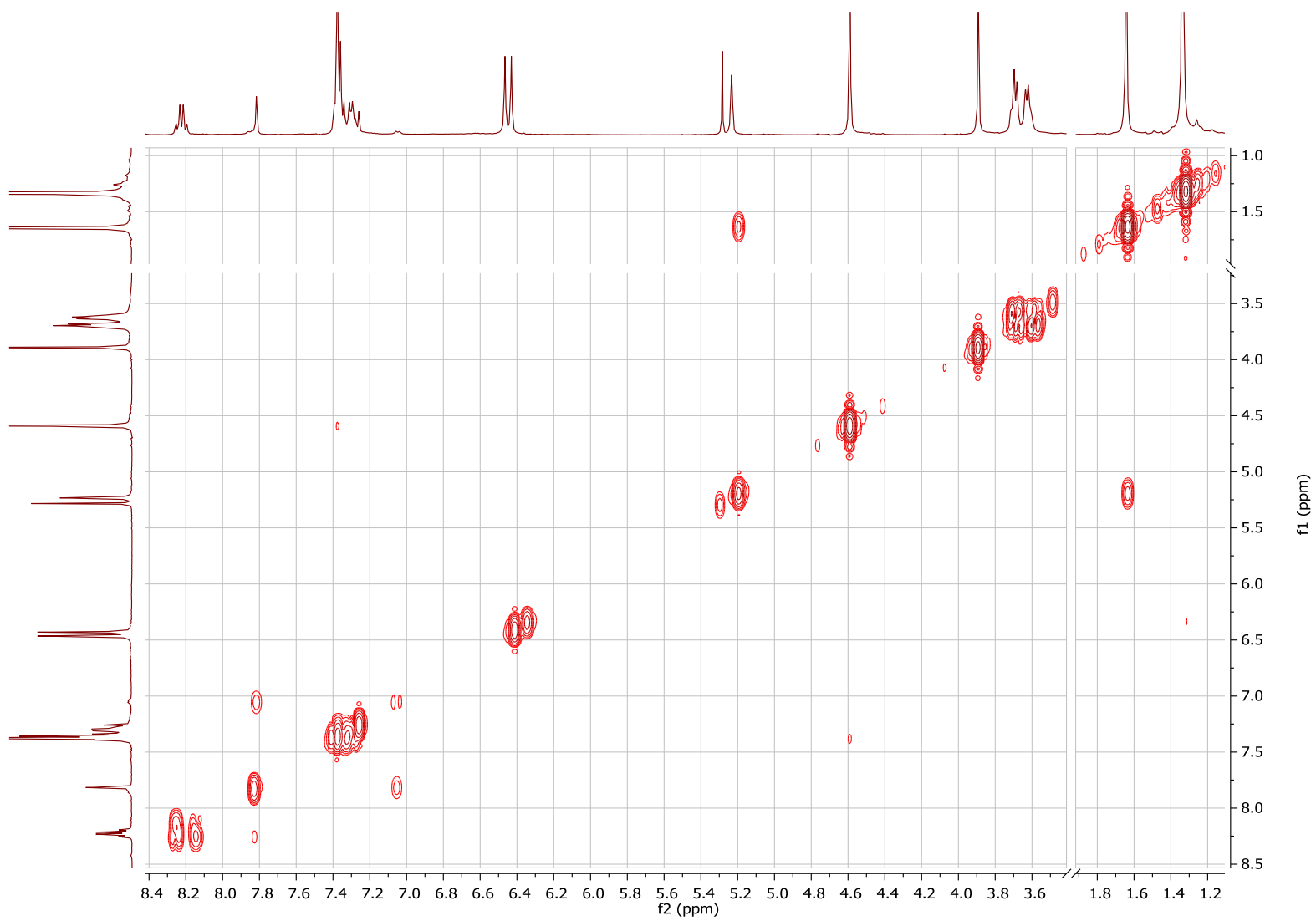
Ester 11-Bn,Me

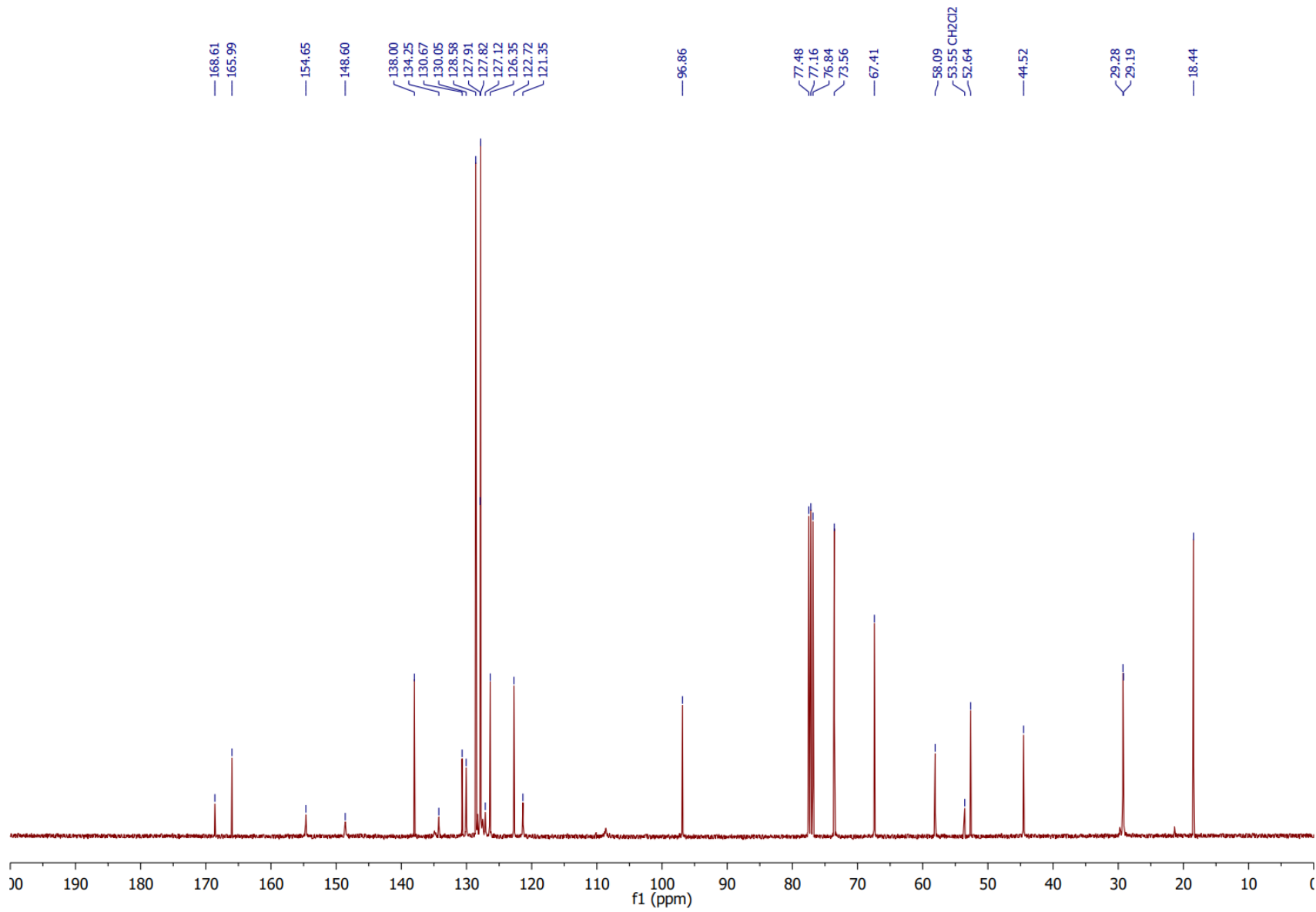


¹H, ¹H-¹H COSY and ¹³C NMR spectra recorded in CDCl₃ (400 and 101 MHz)

¹H NMR (400 MHz, Chloroform-*d*) δ 8.28 – 8.18 (m, 2H), 7.82 (d, *J* = 1.3 Hz, 1H), 7.39 – 7.22 (m, 10H), 6.45 (d, *J* = 13.9 Hz, 4H), 5.28 (s, CH₂Cl₂), 5.26 – 5.18 (m, 2H), 4.59 (s, 4H), 3.89 (s, 3H), 3.74 – 3.58 (m, 8H), 1.70 – 1.59 (m, 6H), 1.34 (s, 12H).



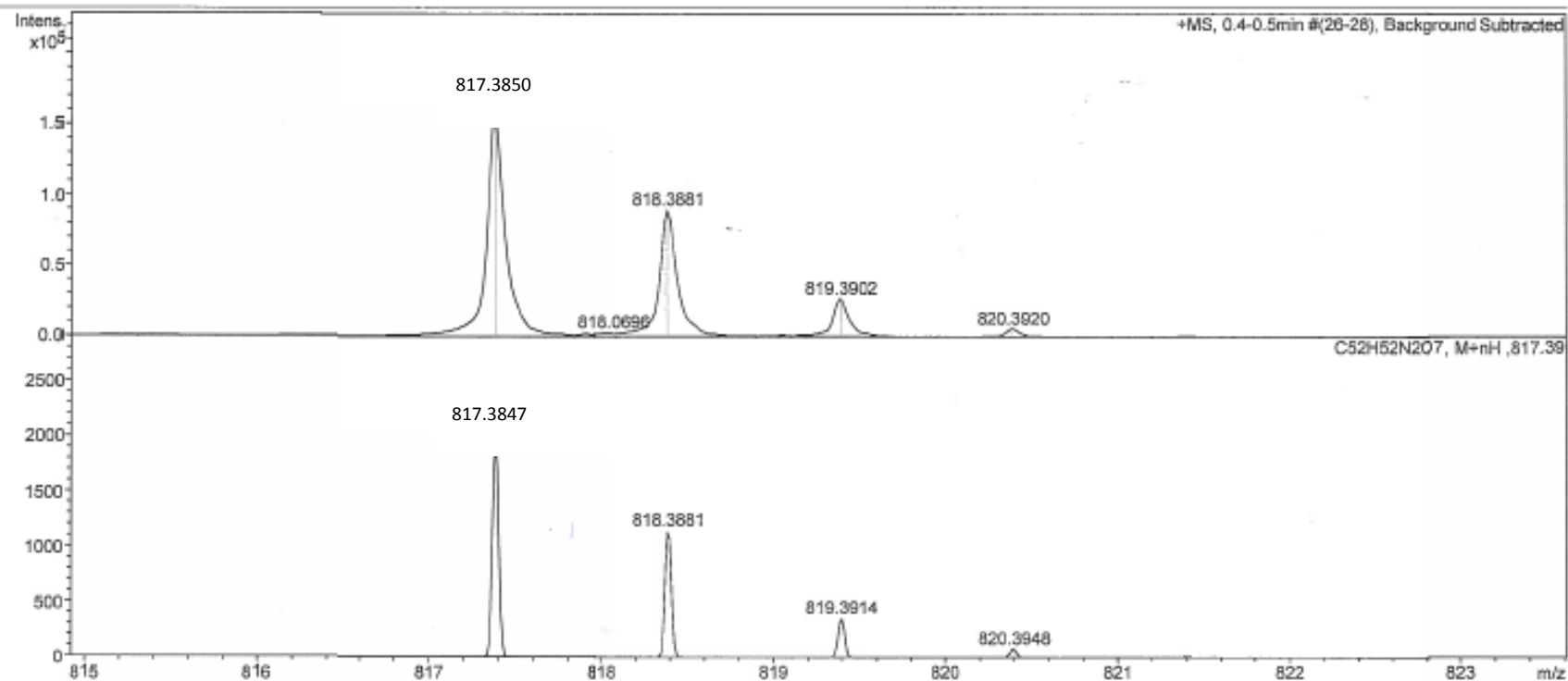




11-Bn,Me: HRMS-ESI mass spectra (positive mode)

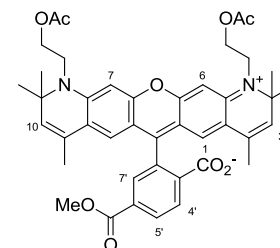
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

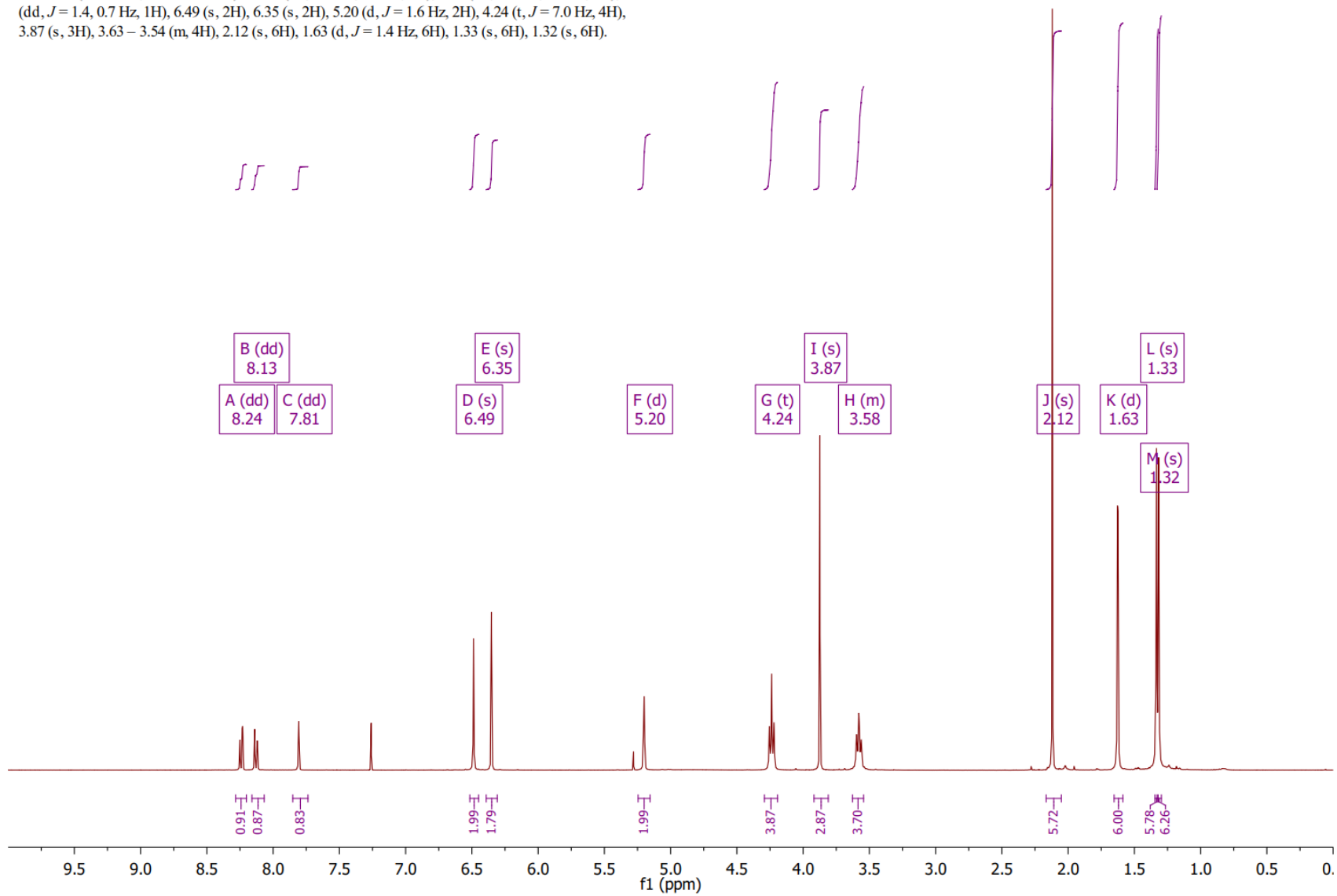


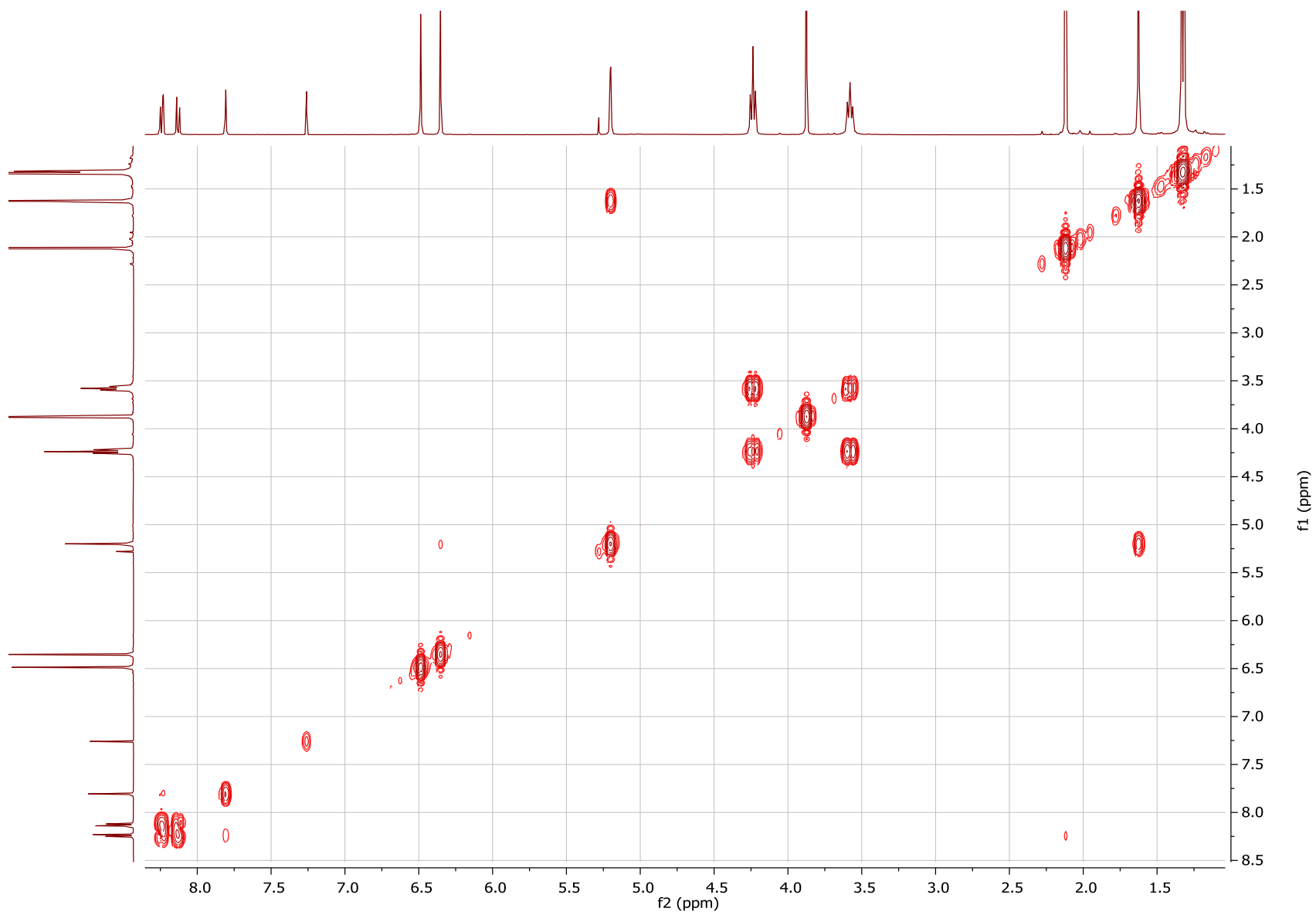
Ester 11-Ac,Me

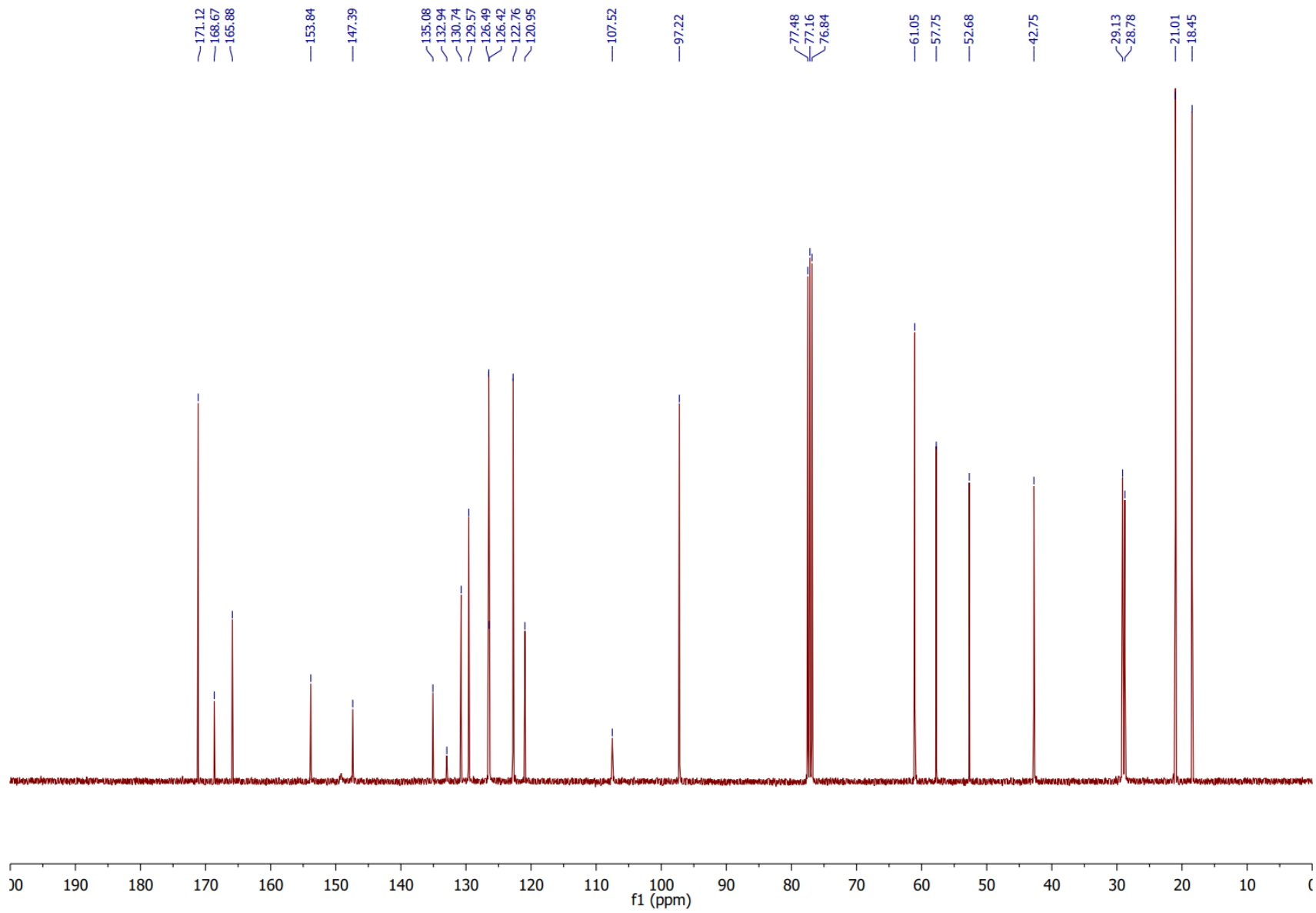
^1H , ^1H - ^1H COSY and ^{13}C NMR spectra recorded in CDCl_3 (400 and 101 MHz)



¹H NMR (400 MHz, Chloroform-*d*) δ 8.24 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.13 (dd, *J* = 8.0, 0.7 Hz, 1H), 7.81 (dd, *J* = 1.4, 0.7 Hz, 1H), 6.49 (s, 2H), 6.35 (s, 2H), 5.20 (d, *J* = 1.6 Hz, 2H), 4.24 (t, *J* = 7.0 Hz, 4H), 3.87 (s, 3H), 3.63 – 3.54 (m, 4H), 2.12 (s, 6H), 1.63 (d, *J* = 1.4 Hz, 6H), 1.33 (s, 6H), 1.32 (s, 6H).

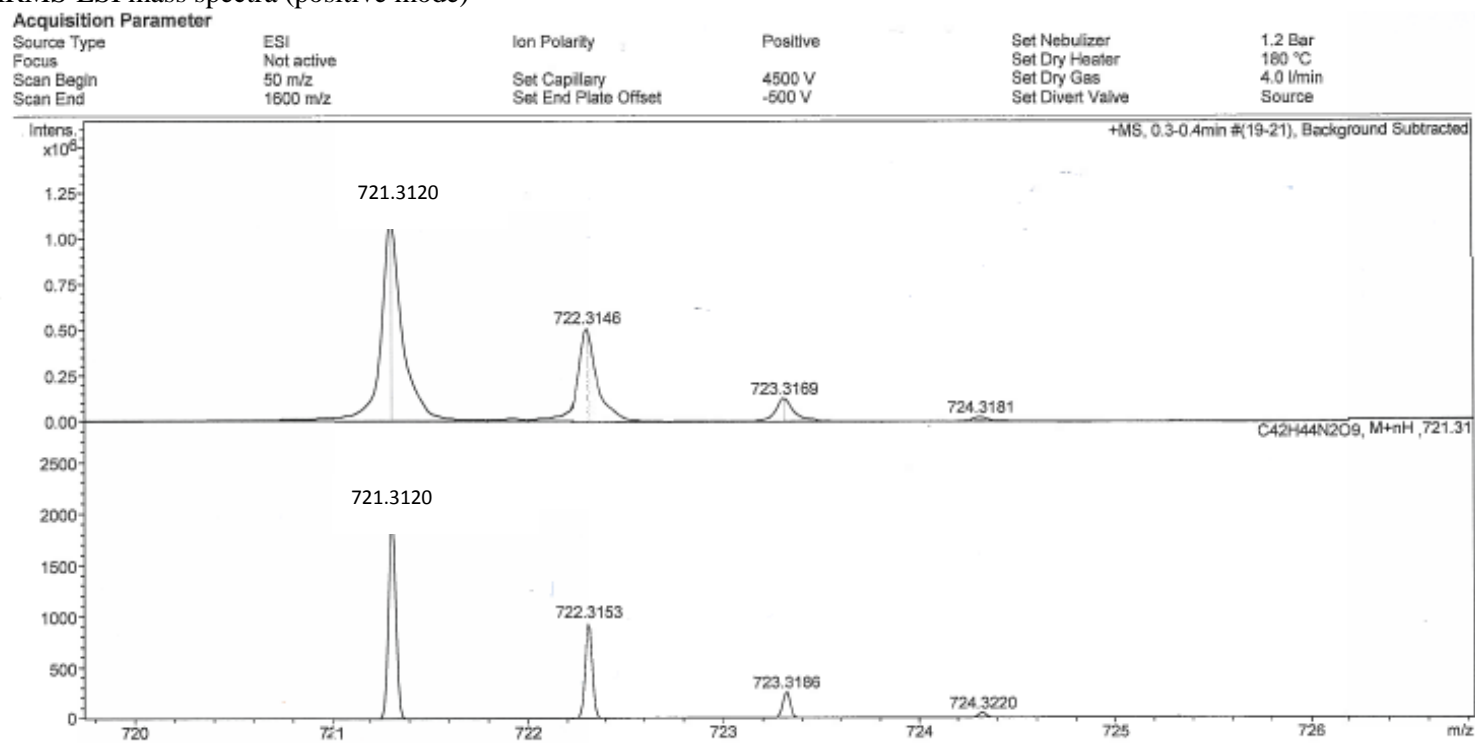




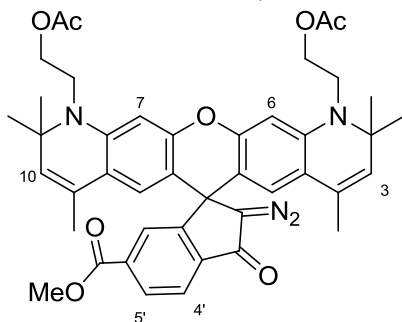


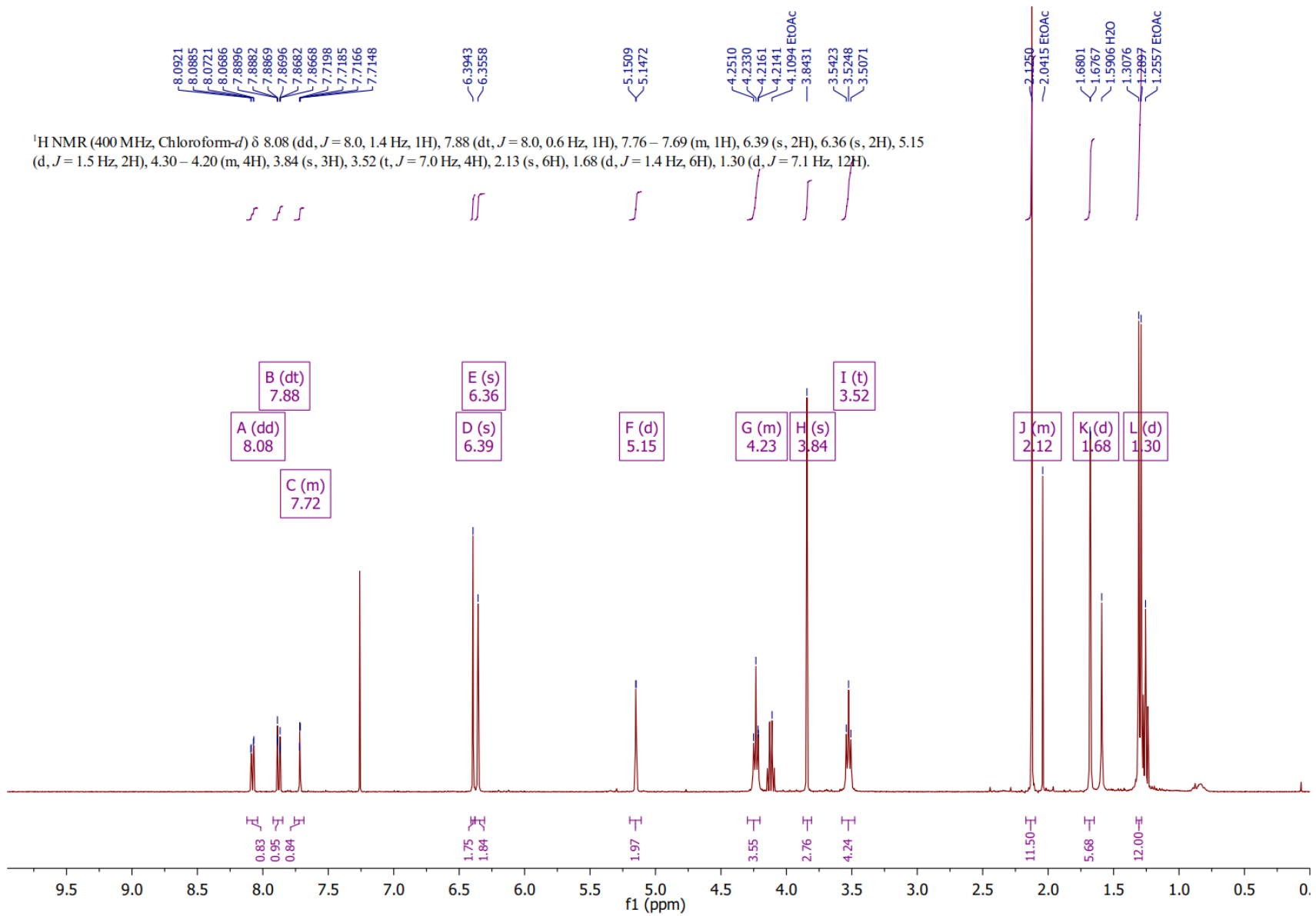
11-Ac,Me

HRMS-ESI mass spectra (positive mode)



Diazoketone 13-Ac,Me. ¹H-NMR, ¹H-¹H COSY and ¹³C NMR spectra recorded in CDCl₃ (400 and 101 MHz)







13-Ac,Me: HRMS-ESI mass spectra (positive mode)

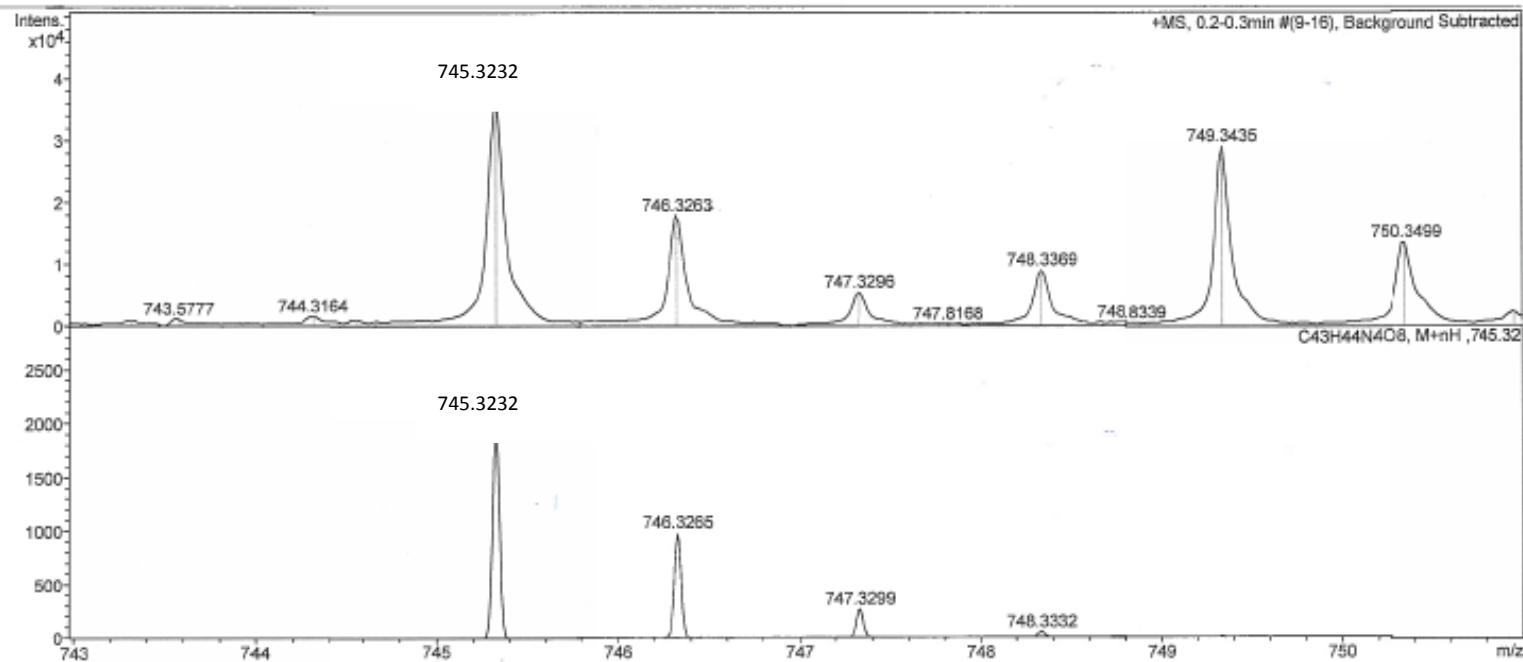
Analysis Info

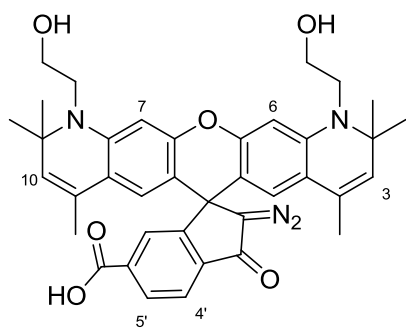
Analysis Name Z:\Data\2016\1606\sam030616\BR071_low_1_01_88717.d
 Method hystar_pl.m
 Sample Name BR071_low
 Comment

Acquisition Date 03.06.2016 09:13:58
 Operator BDAL@DE
 Instrument / Ser# micrOTOF 10237

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.2 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	1800 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

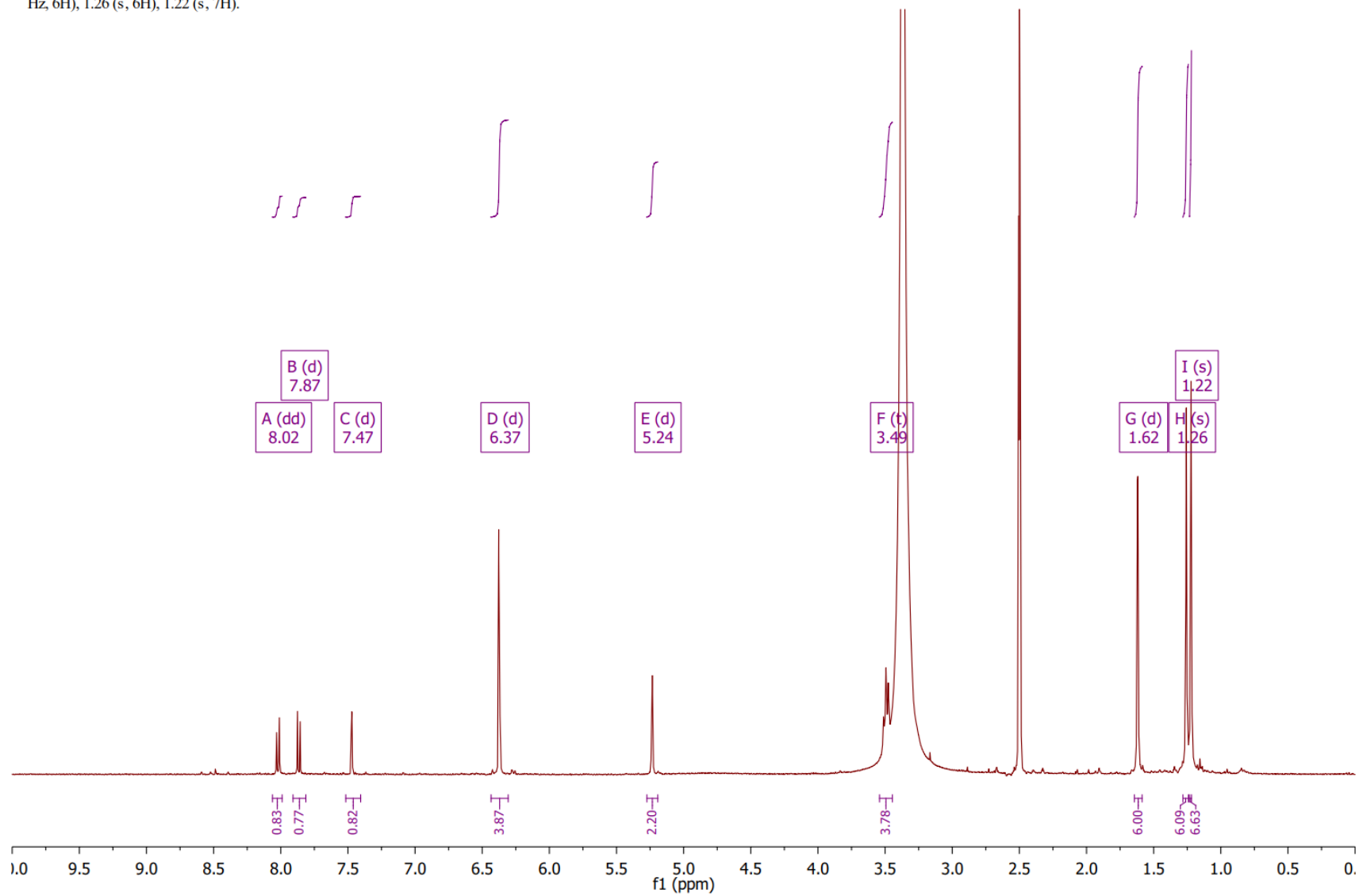


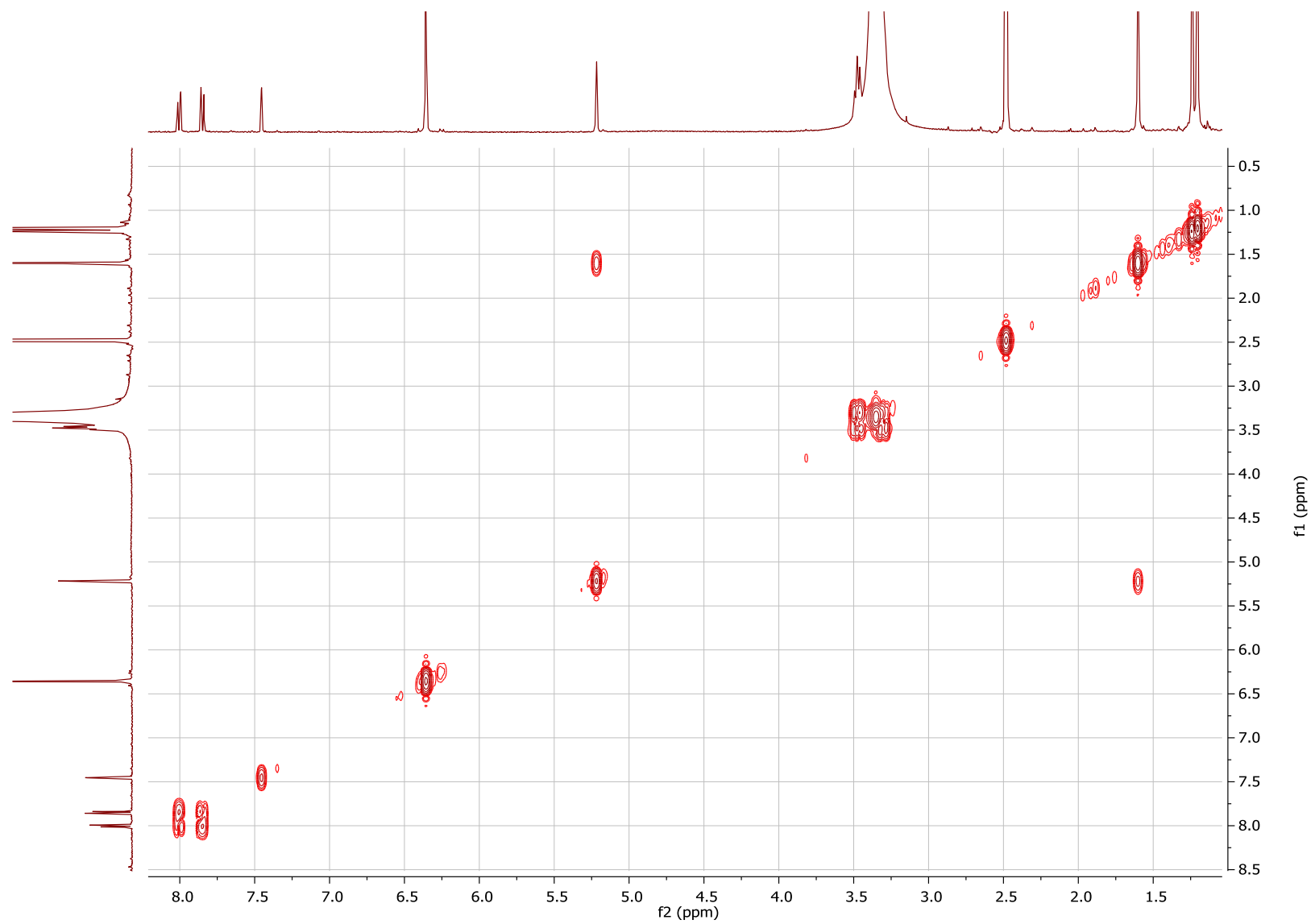


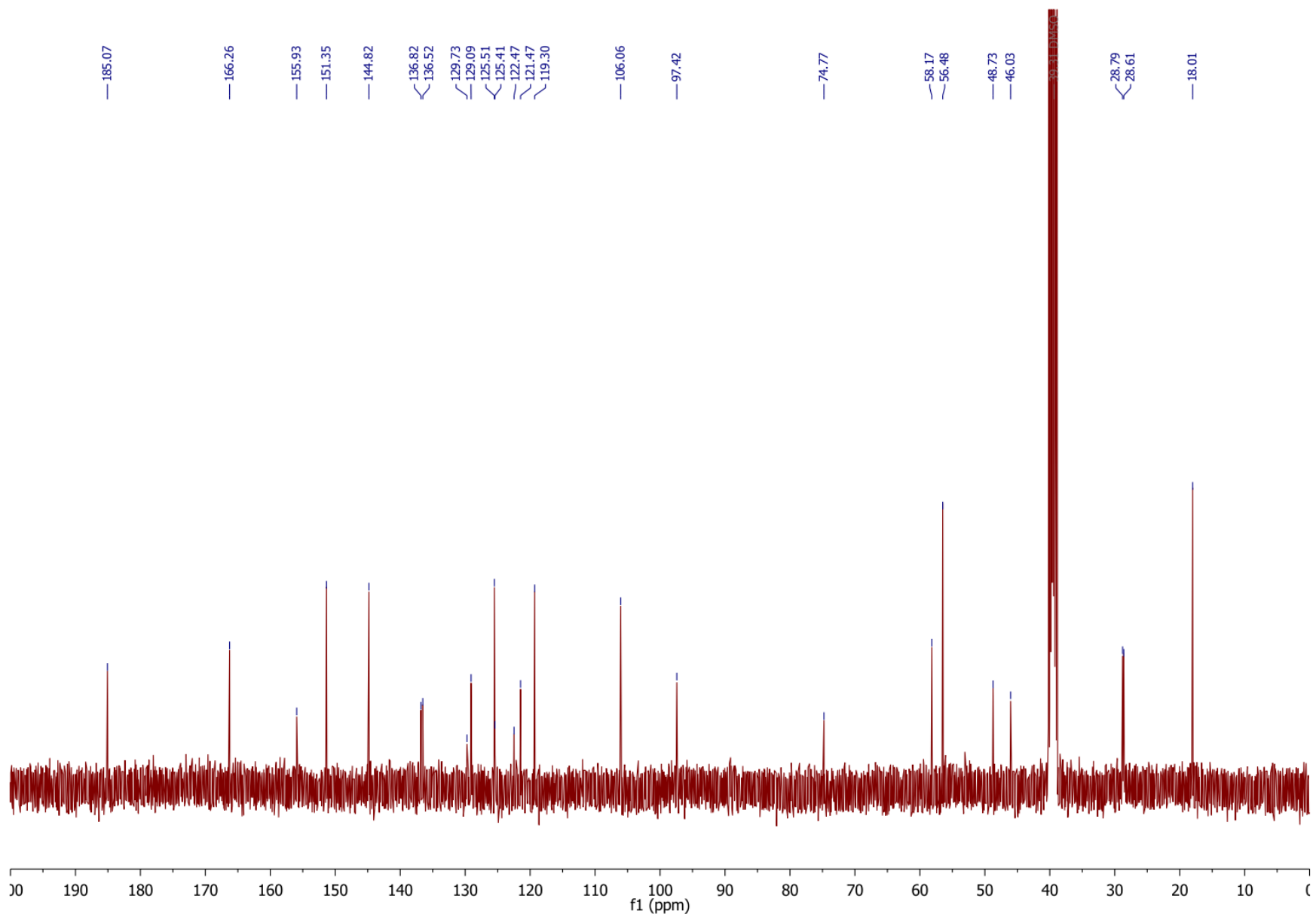
13-H,H

¹H, ¹H-¹H COSY and ¹³C NMR spectra recorded in DMSO-d₆ (400 and 101 MHz)

$^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 8.02 (dd, $J = 8.0, 1.4$ Hz, 1H), 7.87 (d, $J = 8.0$ Hz, 1H), 7.47 (d, $J = 1.3$ Hz, 1H), 6.37 (d, $J = 2.6$ Hz, 4H), 5.24 (d, $J = 1.6$ Hz, 2H), 3.49 (t, $J = 7.2$ Hz, 4H), 1.62 (d, $J = 1.4$ Hz, 6H), 1.26 (s, 6H), 1.22 (s, 7H).



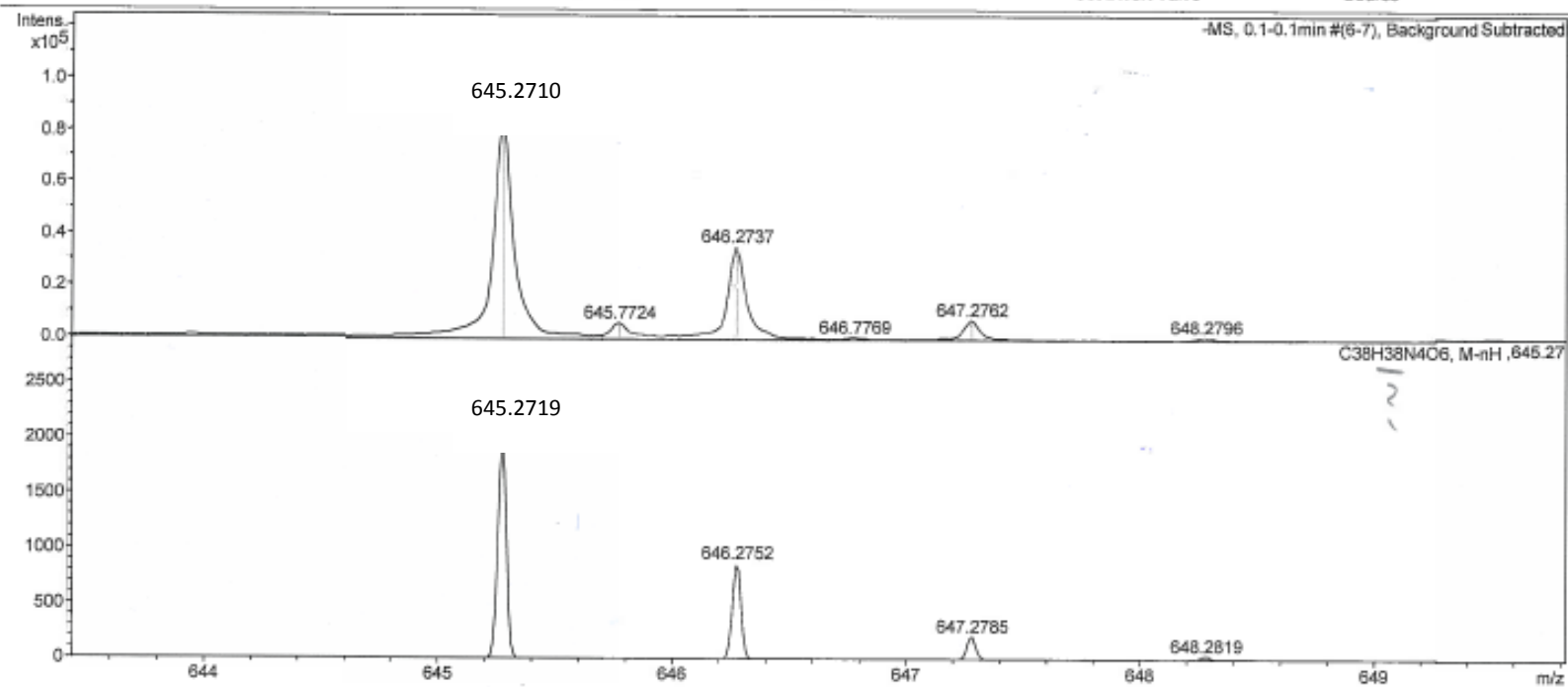




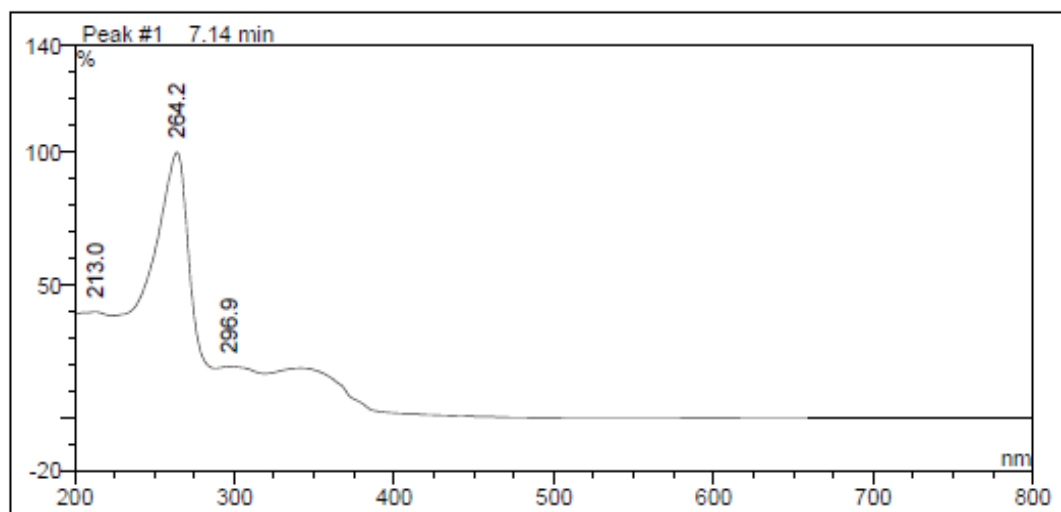
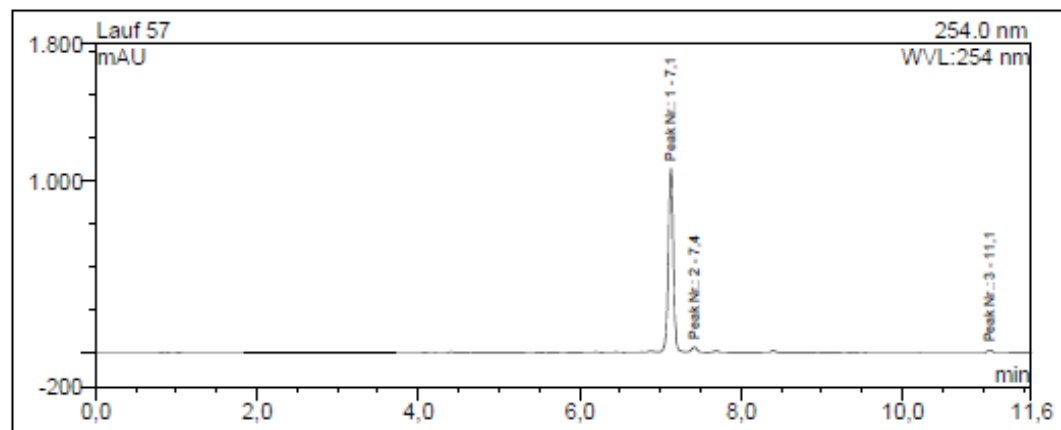
13-H,H: HRMS-ESI mass spectra (negative mode)

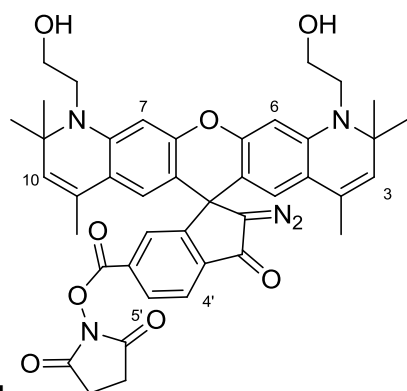
Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	1.6 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	3800 V	Set Dry Gas	8.0 l/min
Scan End	1800 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



13-H,H, HPLC (system E)





Compound 13-H,Su

HRMS-ESI mass spectra (positive mode)

