

H-Rubies, a New Family of Red Emitting Fluorescent pH sensors for Living Cells

Guillaume Despras⁺, Alsu Zamaleeva⁺, Lucie Dradevet, Céline Tisseyre, Joao Gamelas Magalhaes, Charlotte Garner, Michel De Waard, Sebastian Amigorena, Anne Feltz, Jean-Maurice Mallet, Mayeul Collot*

Supplementary information

NMR and Mass spectra

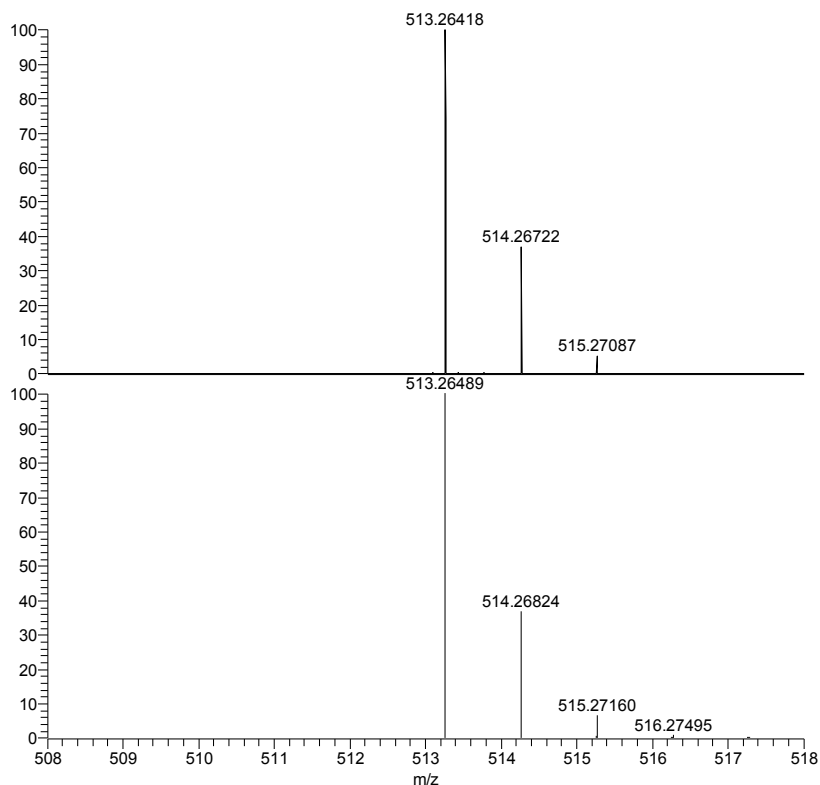
NMR and mass spectra of non phenolic X-rhodamines and intermediates
HRMS of the first set of H-Rubies
NMR spectra of functionalisable H-Rubies and their intermediates
HRMS spectra of functionalisable H-Rubies and their intermediates

NMR and mass spectra of non phenolic X-rhodamines and intermediates

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12/02/14 19:18:42

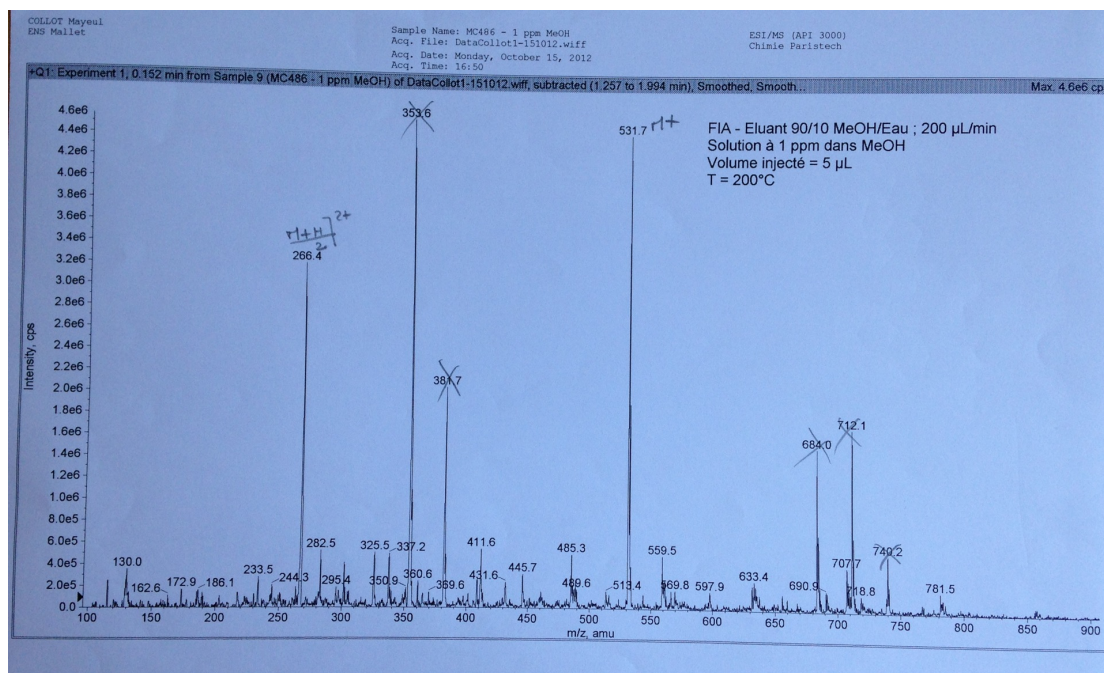
DESPRAS Guillaume MC506



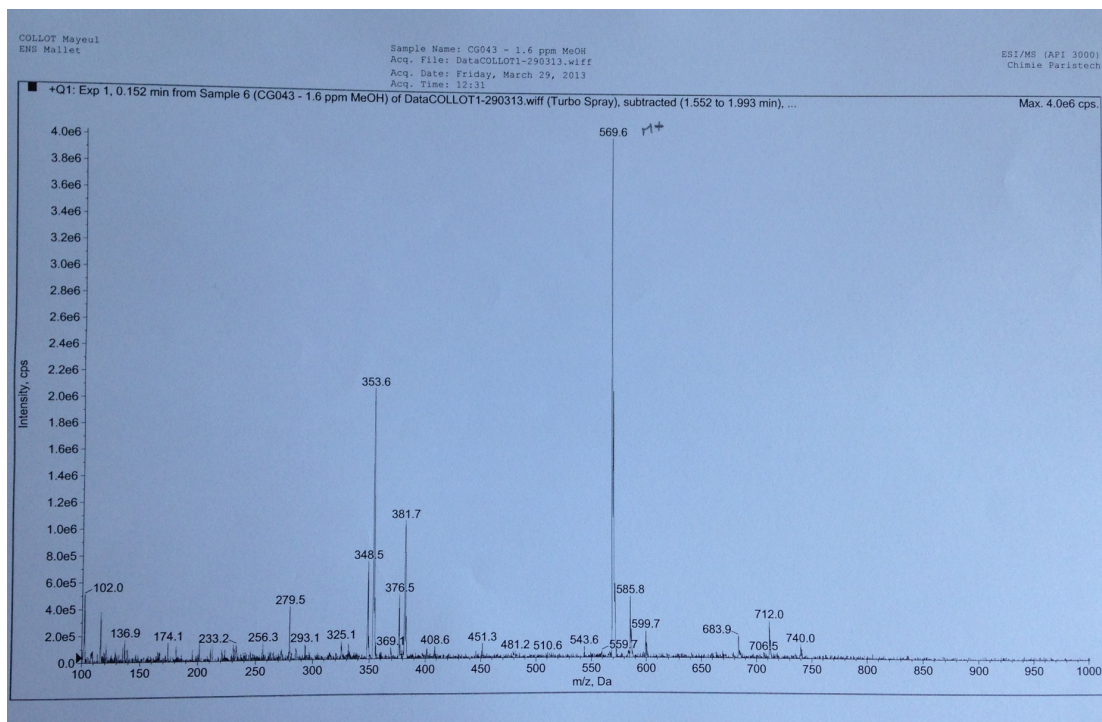
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pa Chrg 1

HRMS Spectrum of Imidazole Based X-Rhodamine



MS Spectrum of Pip-H

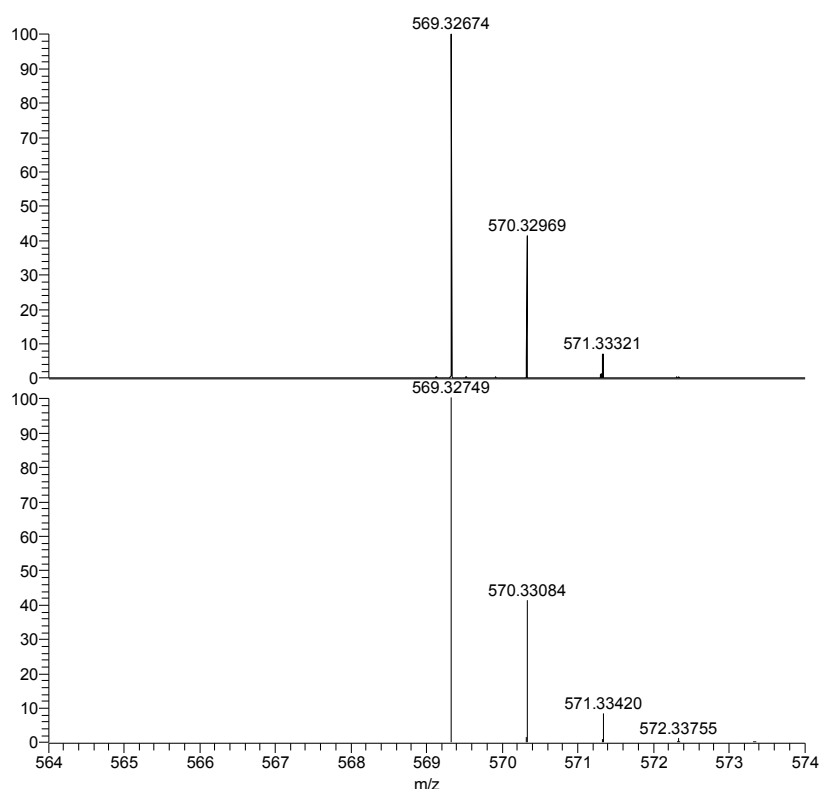


MS Spectrum of Pip-Alkyne

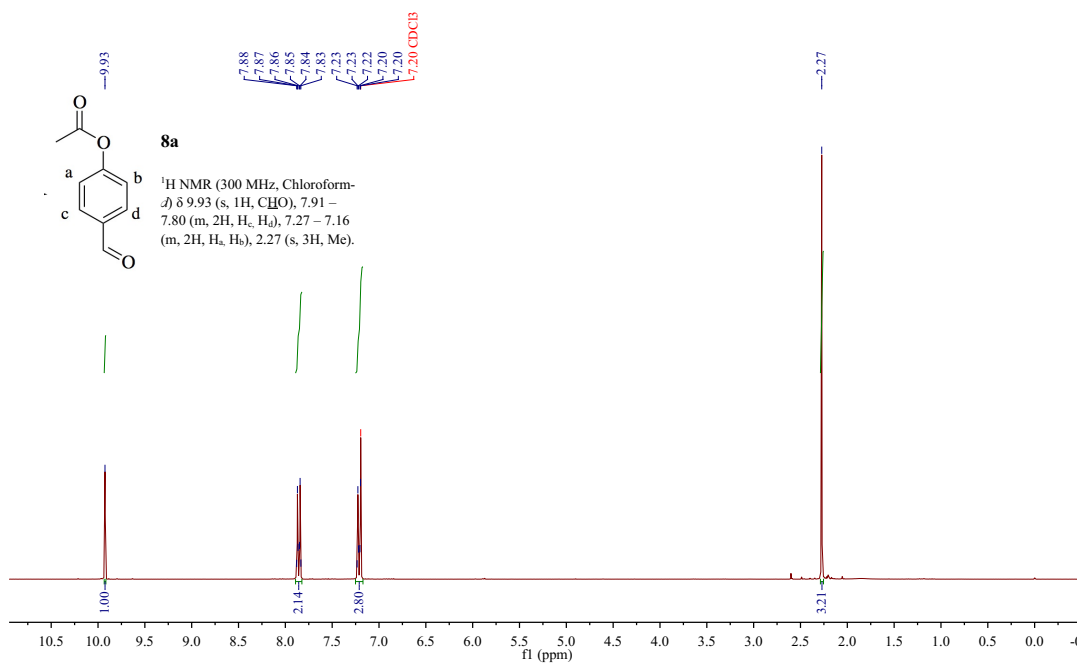
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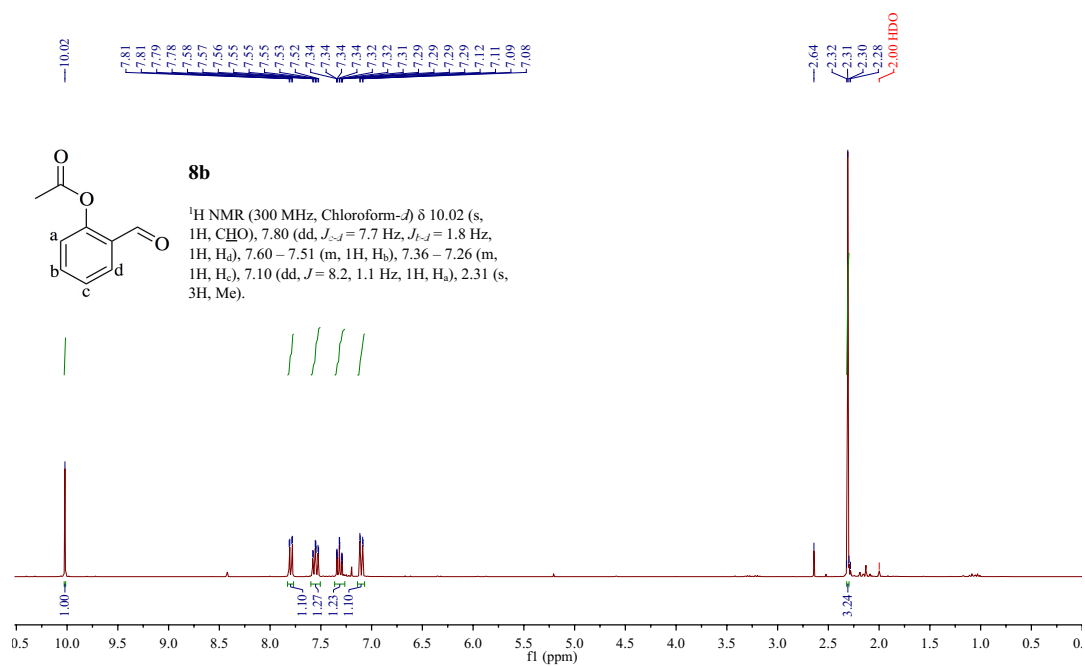
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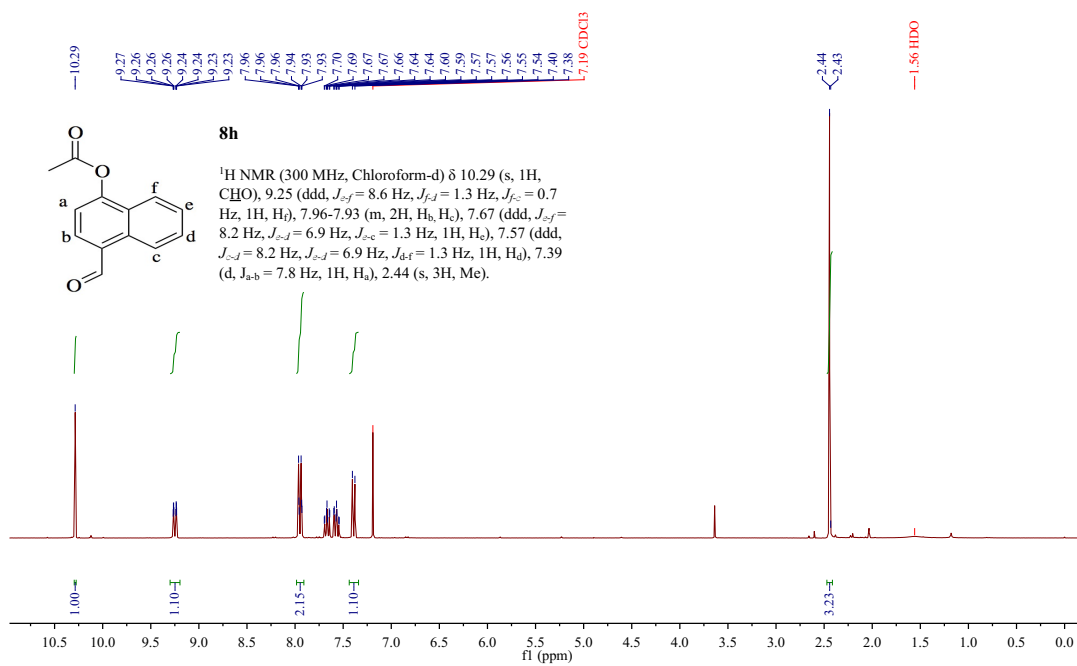
HRMS Spectrum of Pip-Alkyne



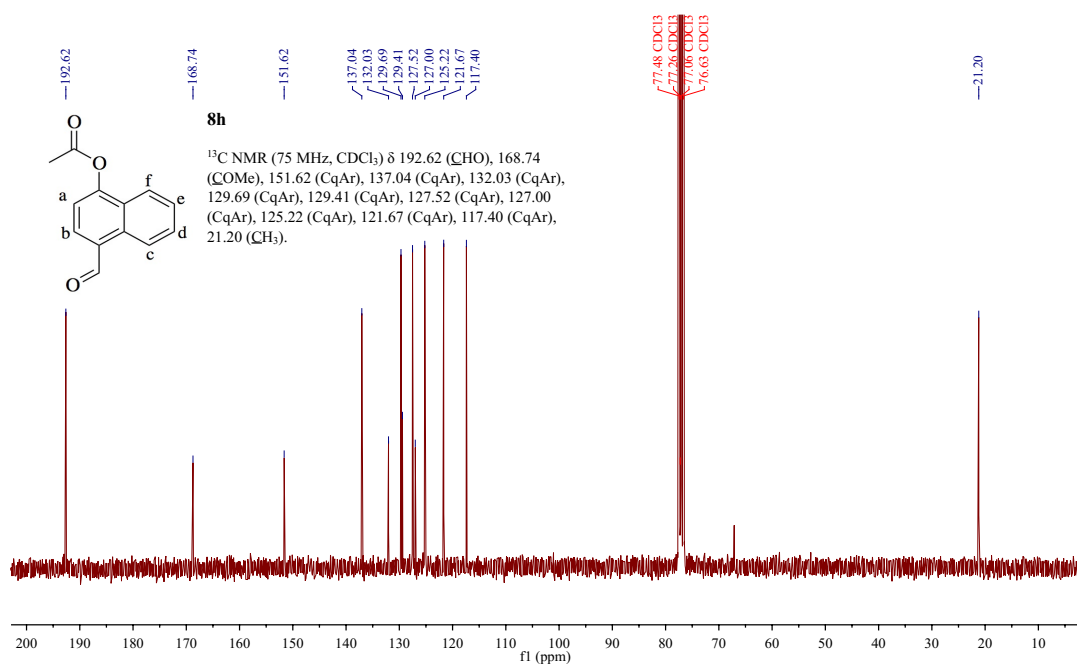
$^1\text{H NMR}$ spectrum of **4-formylphenyl acetate**



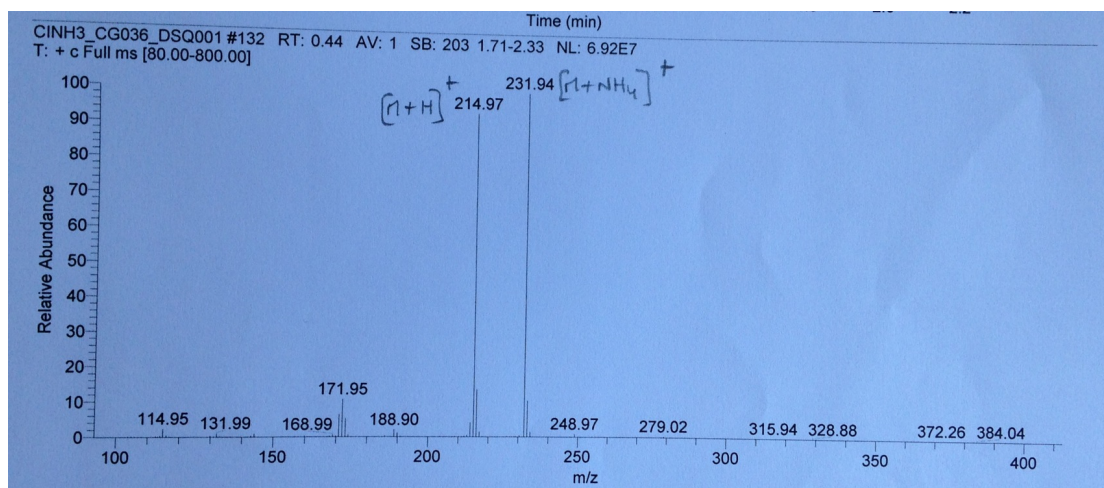
$^1\text{H NMR}$ spectrum of **2-formylphenyl acetate**



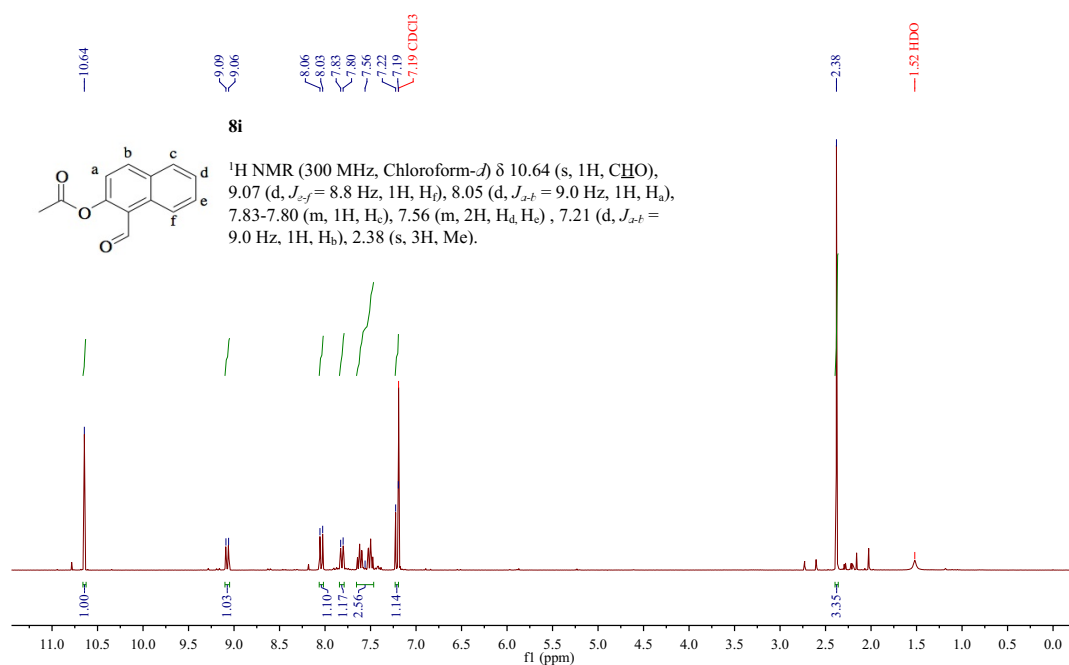
¹H NMR spectrum of 4-formylnaphthalen-1-yl acetate



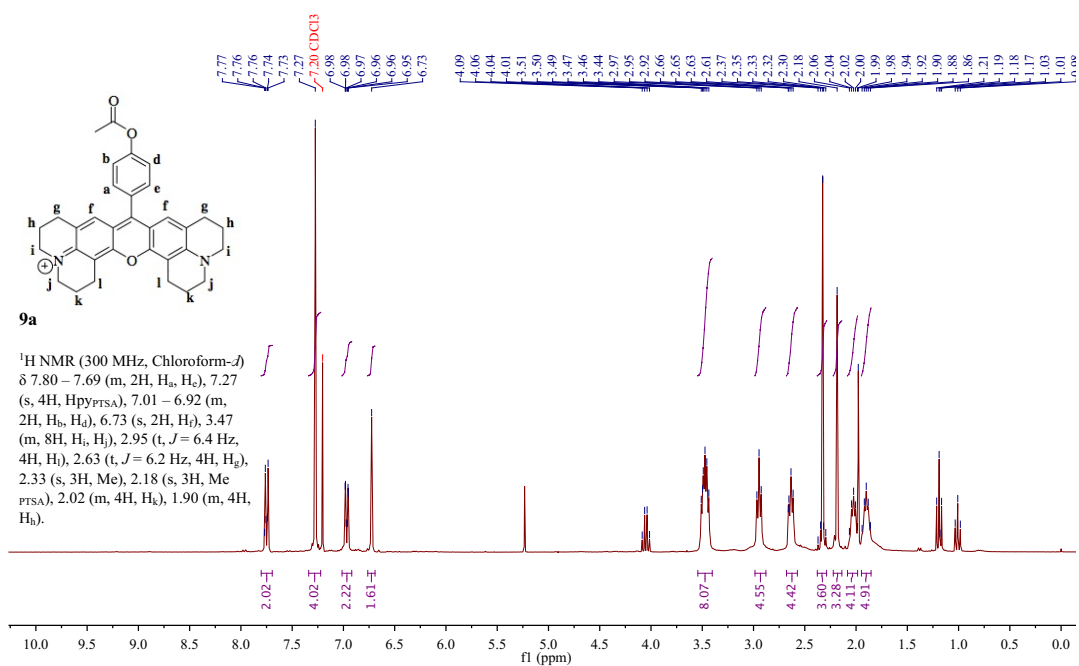
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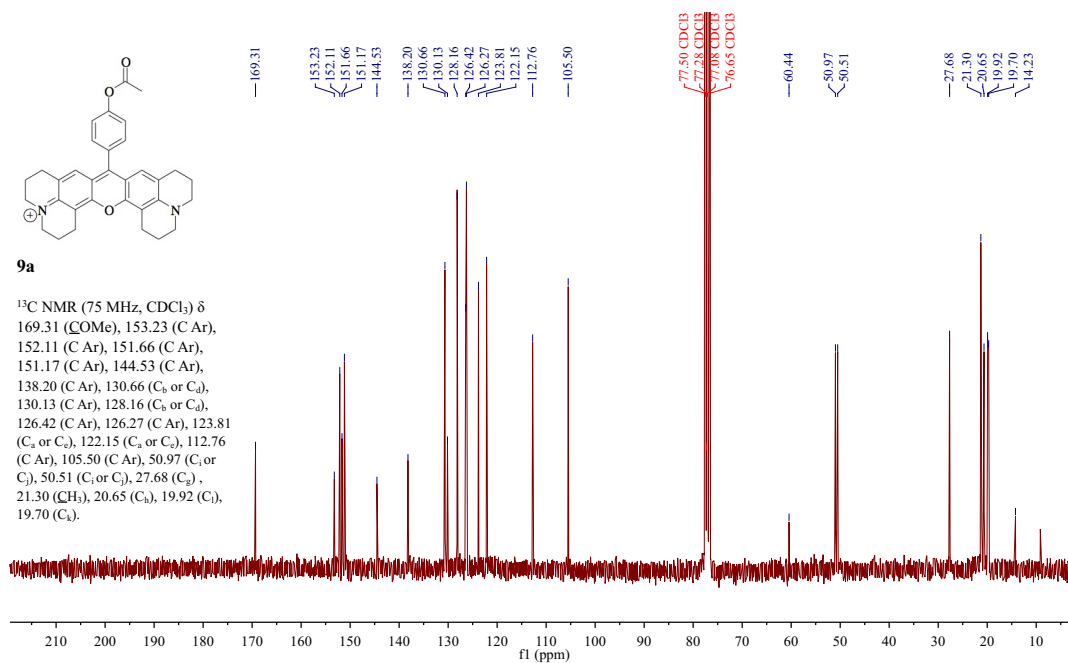
MS Spectrum of 4-formylnaphthalen-1-yl acetate



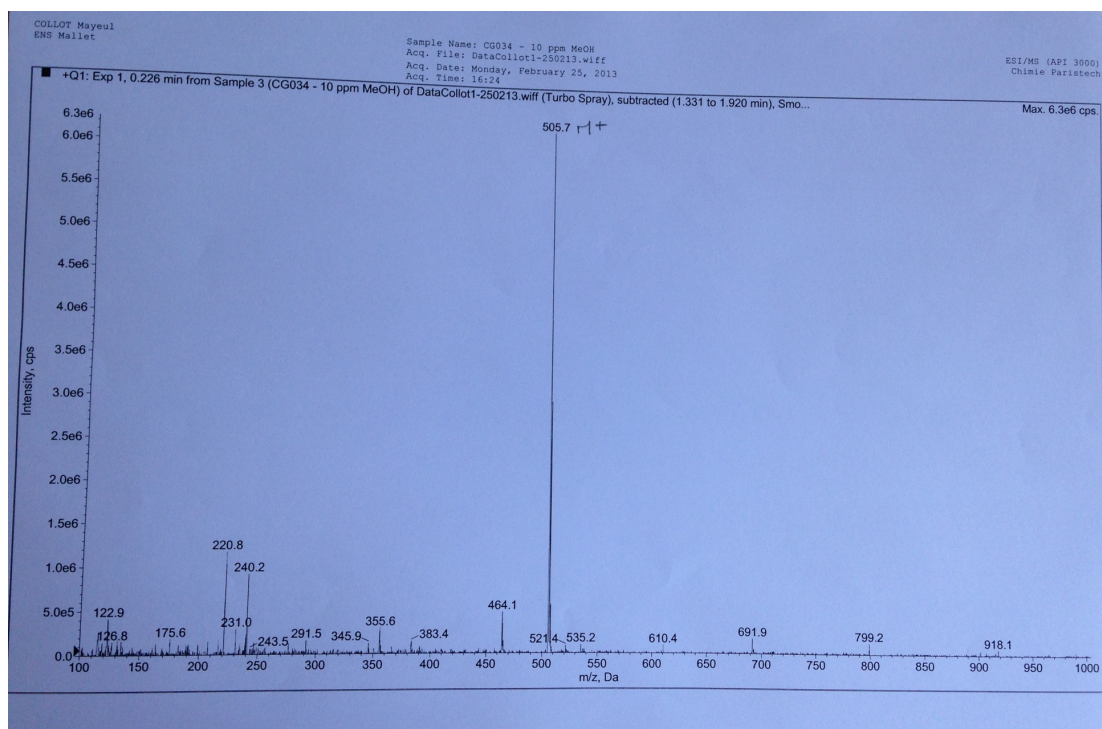
$^1\text{H NMR}$ spectrum of 1-formylnaphthalen-2-yl acetate



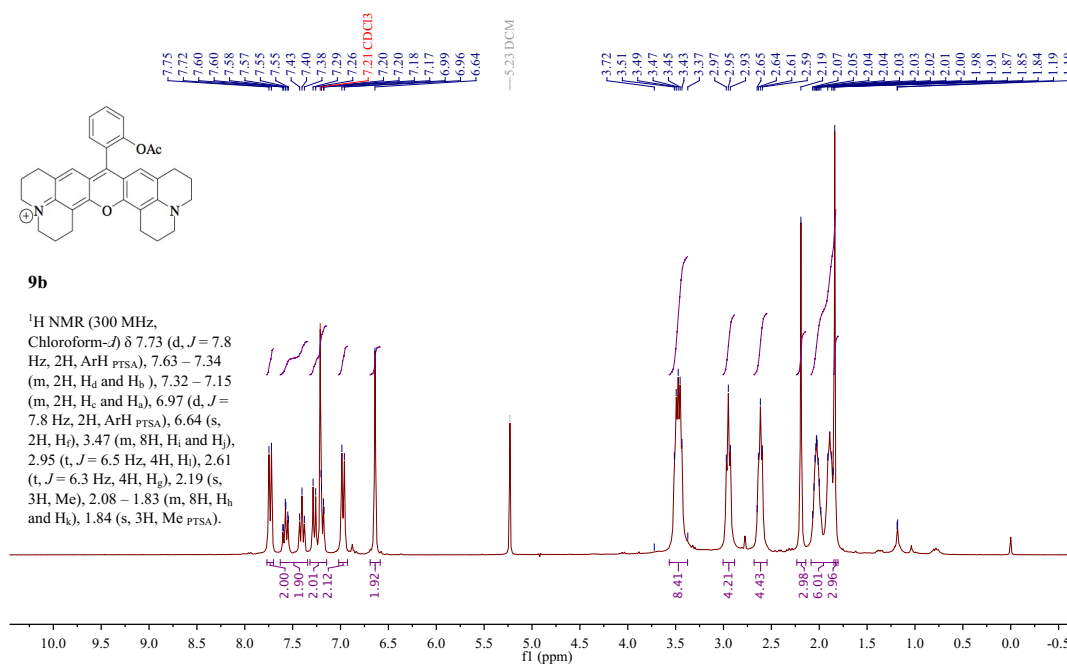
¹H NMR spectrum of Acetylated HR-*p*OH



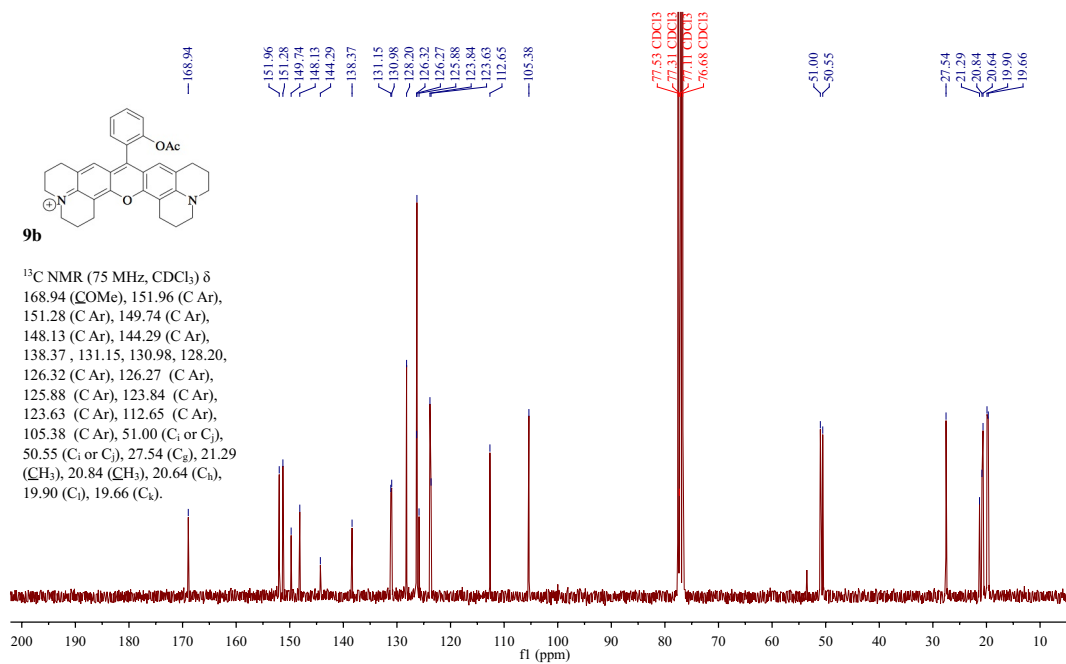
¹³C NMR spectrum of Acetylated HR-*p*OH



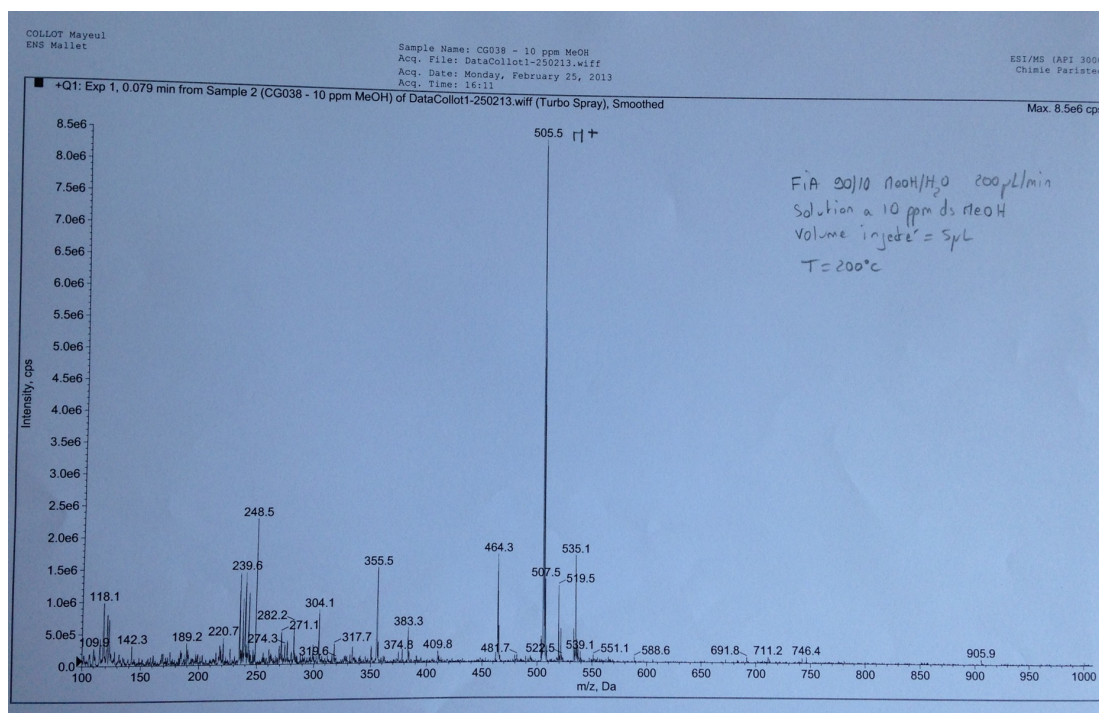
MS spectrum of Acetylated HR-*p*OH



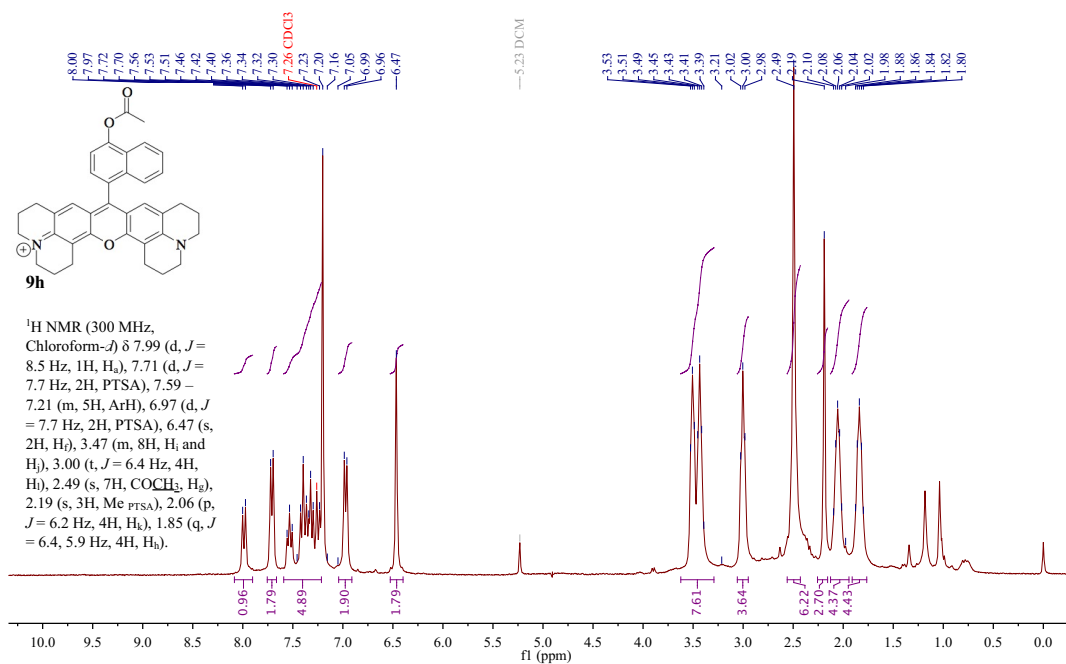
¹H NMR spectrum of Acetylated HR-*o*OH



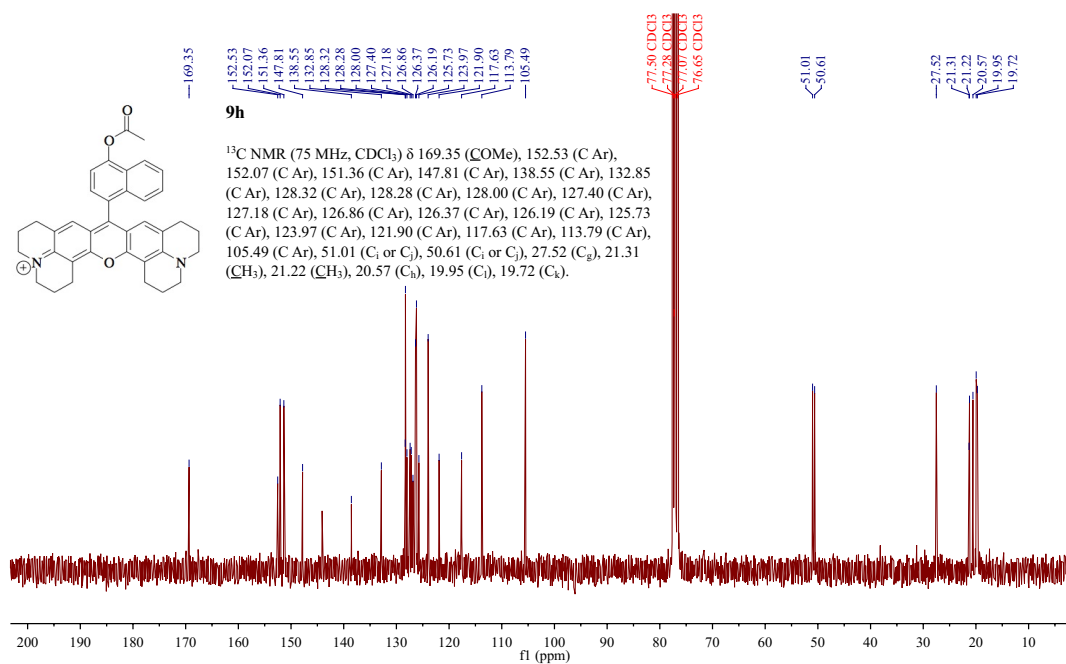
^{13}C NMR spectrum of Acetylated HR-*o*OH



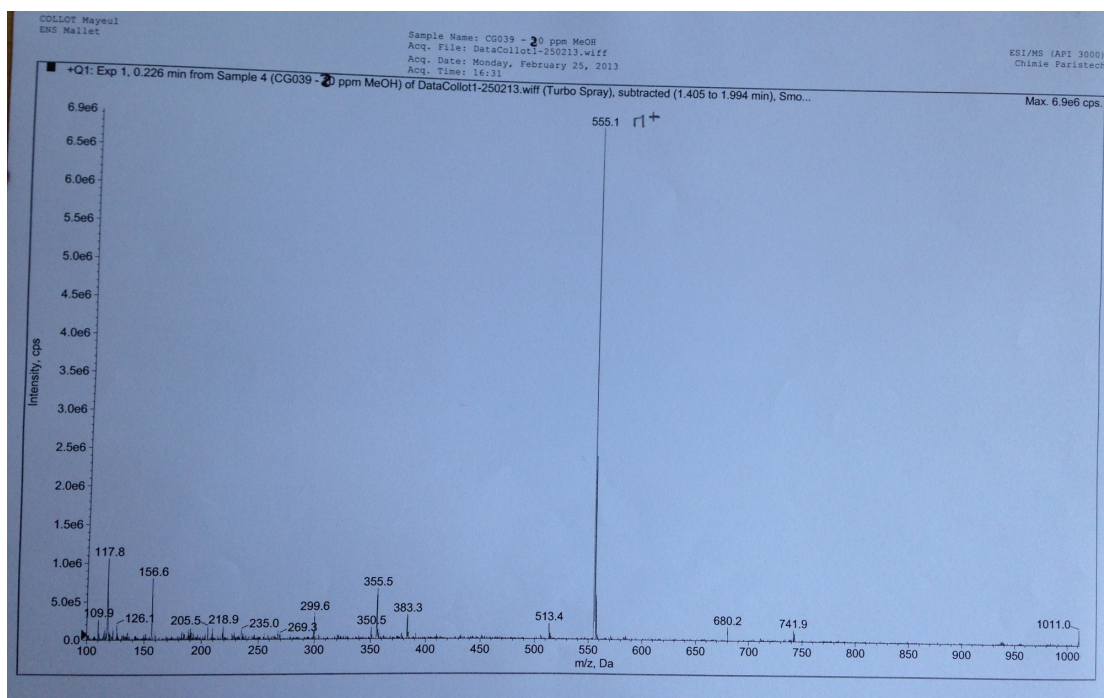
MS spectrum of Acetylated HR-*o*OH



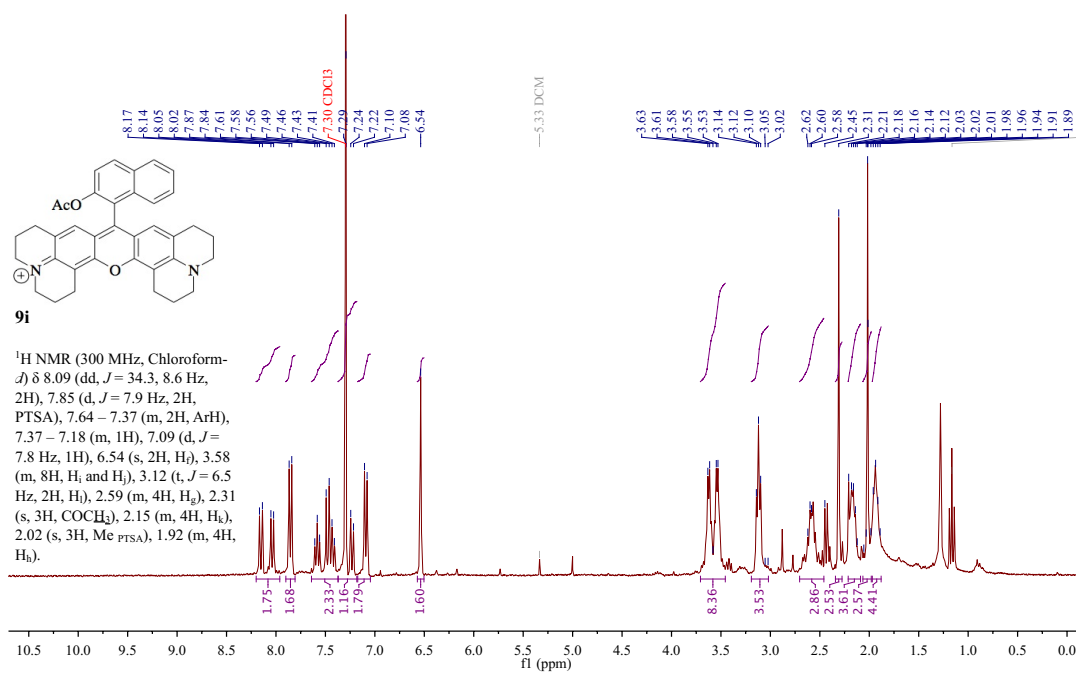
¹H NMR spectrum of Acetylated *p*-Nph



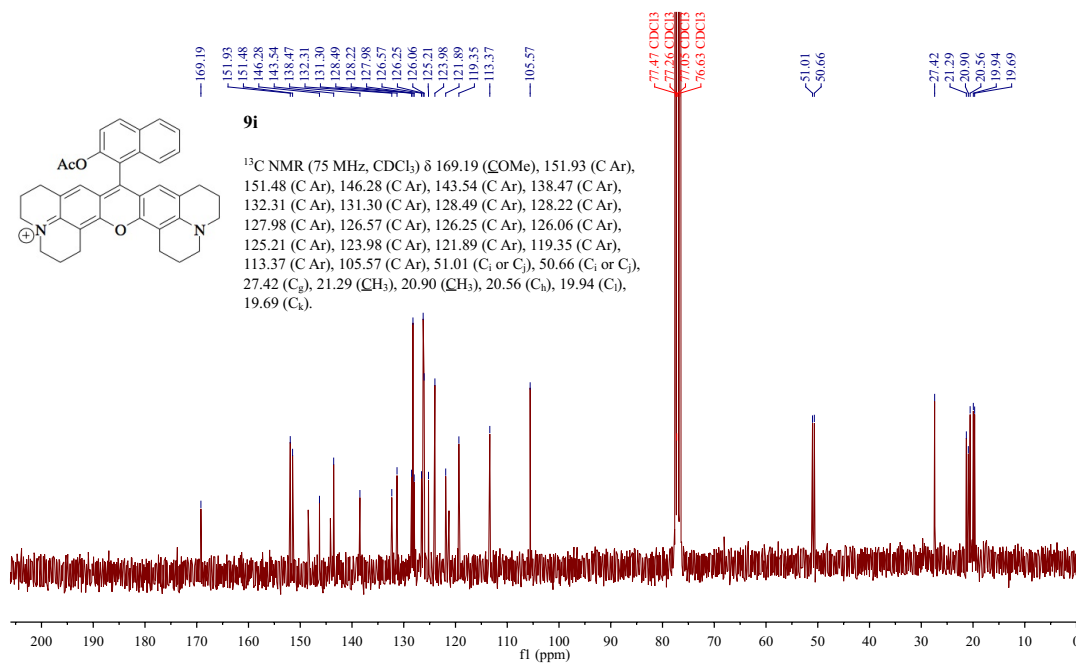
¹³C NMR spectrum of Acetylated *p*-Nph



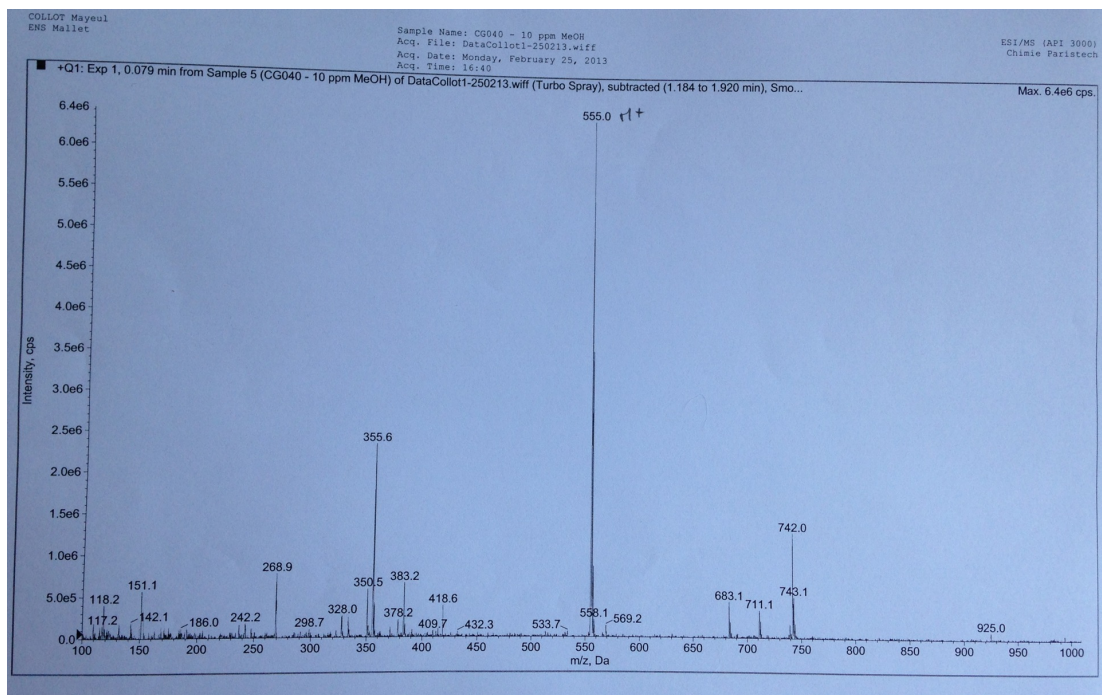
MS spectrum of Acetylated *p*-Nph



¹H NMR spectrum of Acetylated *o*-Nph



^{13}C NMR spectrum of Acetylated *o*-Nph



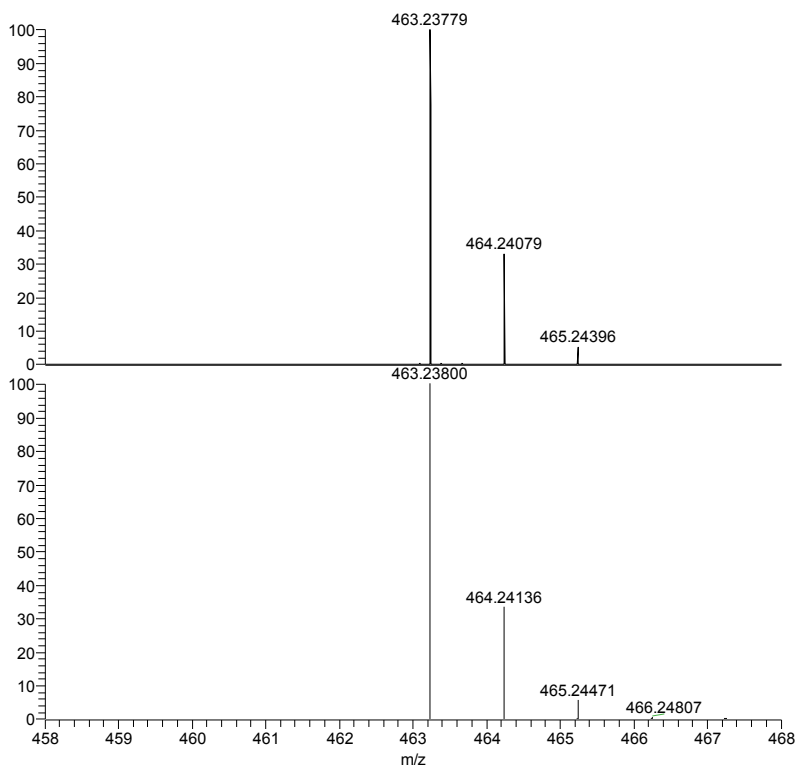
MS spectrum of Acetylated *o*-Nph

HRMS of the first set of H-Rubies

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12/02/14 18:35:56

DESPRAS Guillaume CG041



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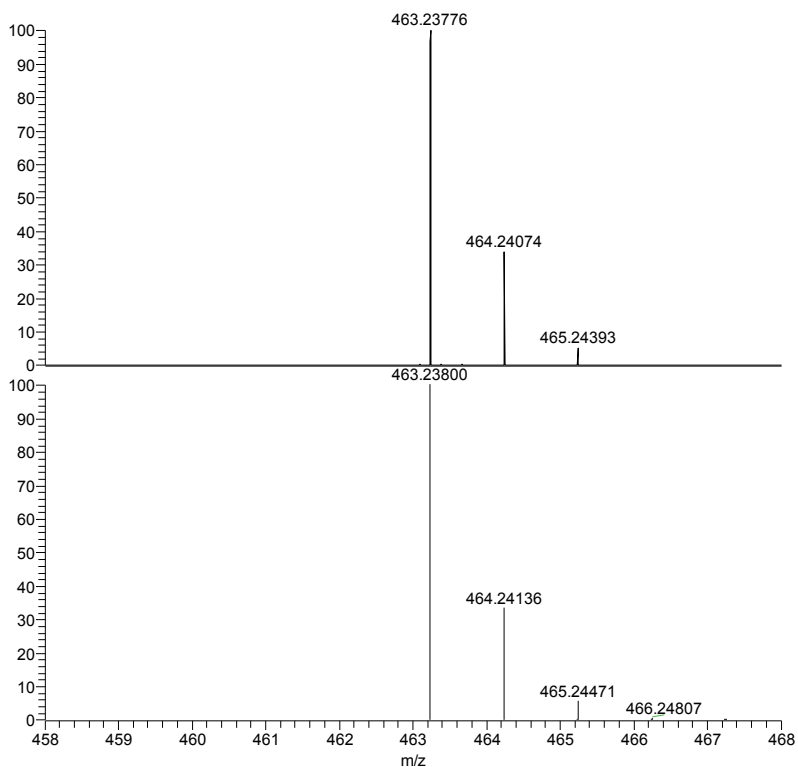
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pa Chrg 1

HRMS spectrum of HR-pOH

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12/02/14 18:25:14

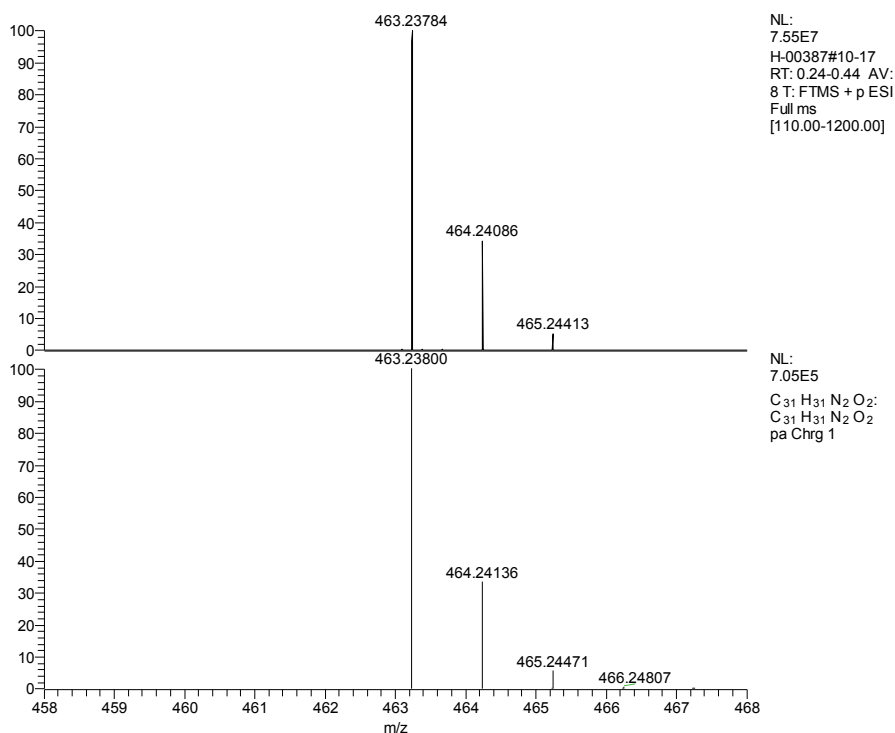
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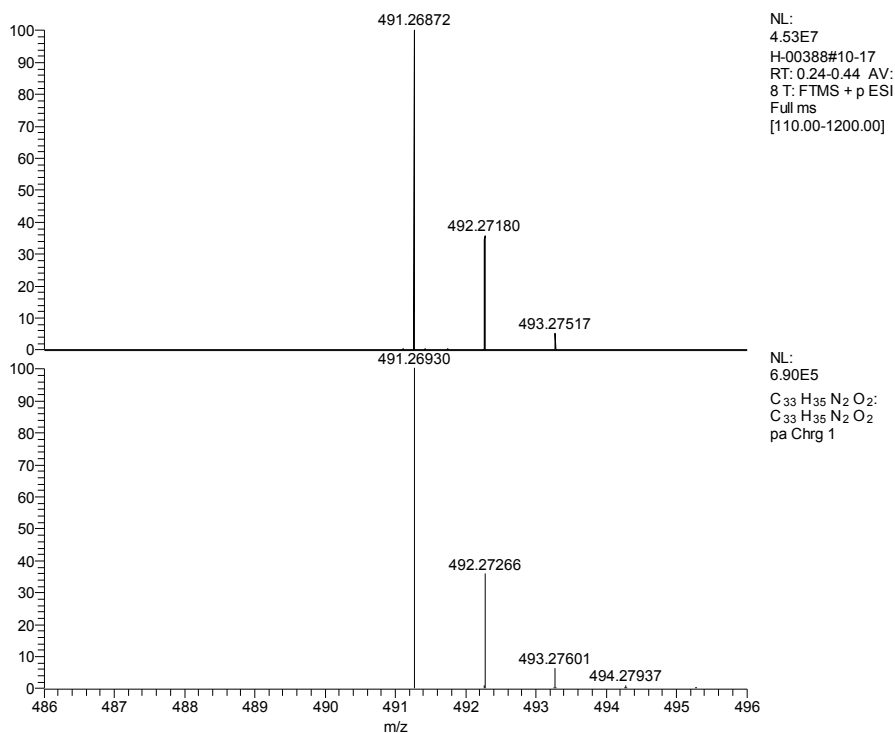
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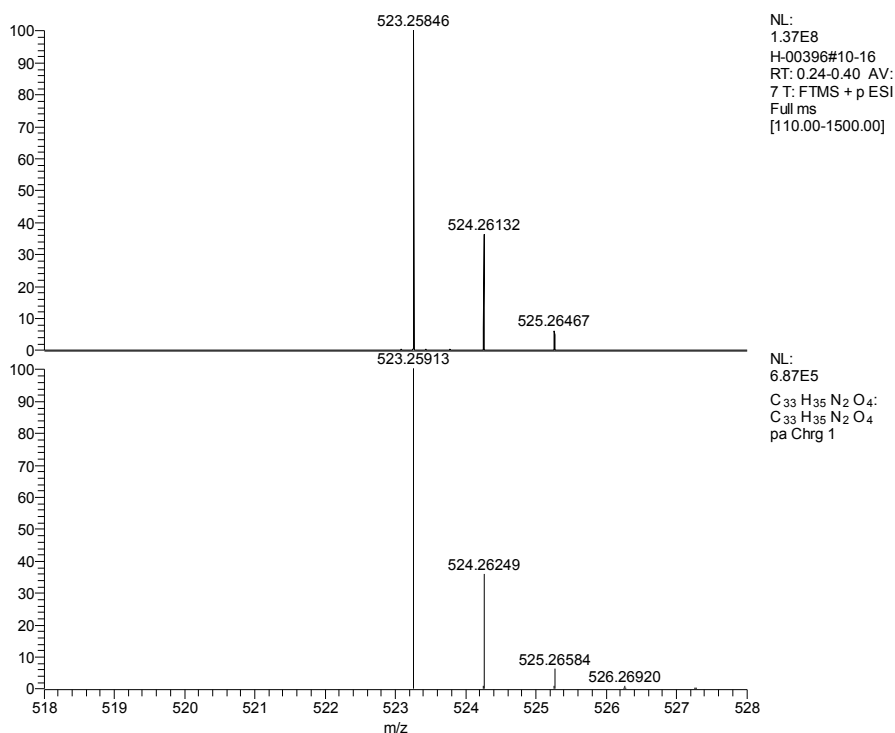
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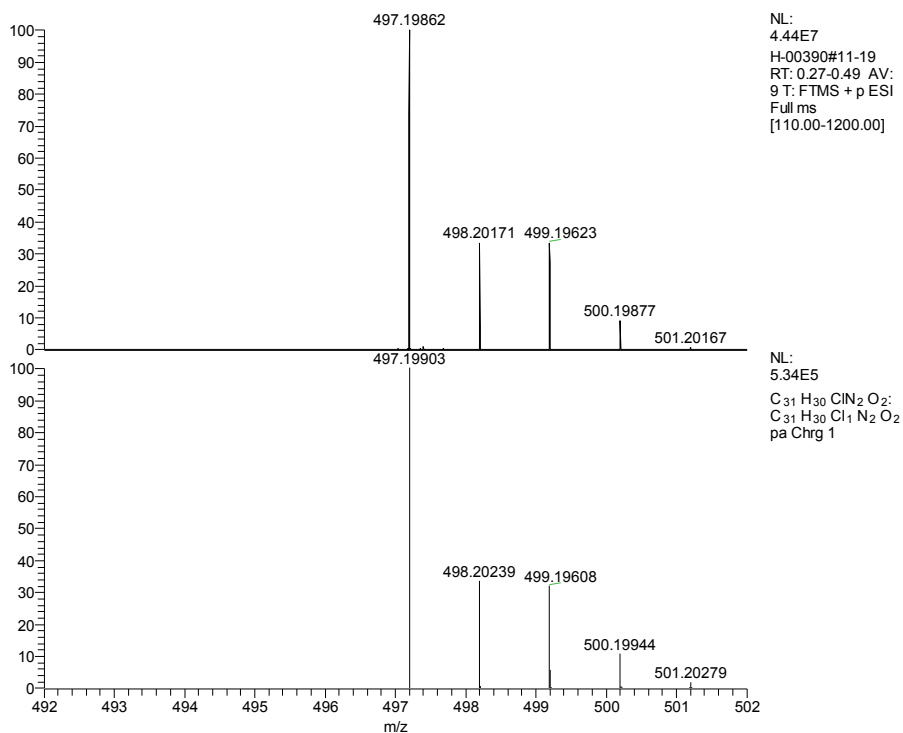
HRMS spectrum of **HR-oOH**



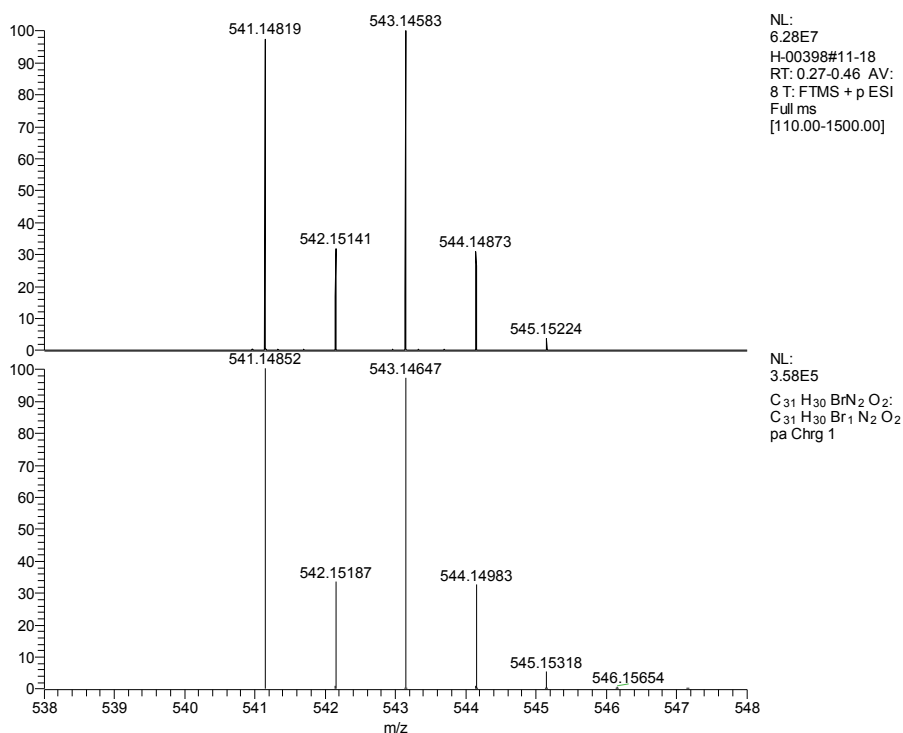
HRMS spectrum of **HR-Me**



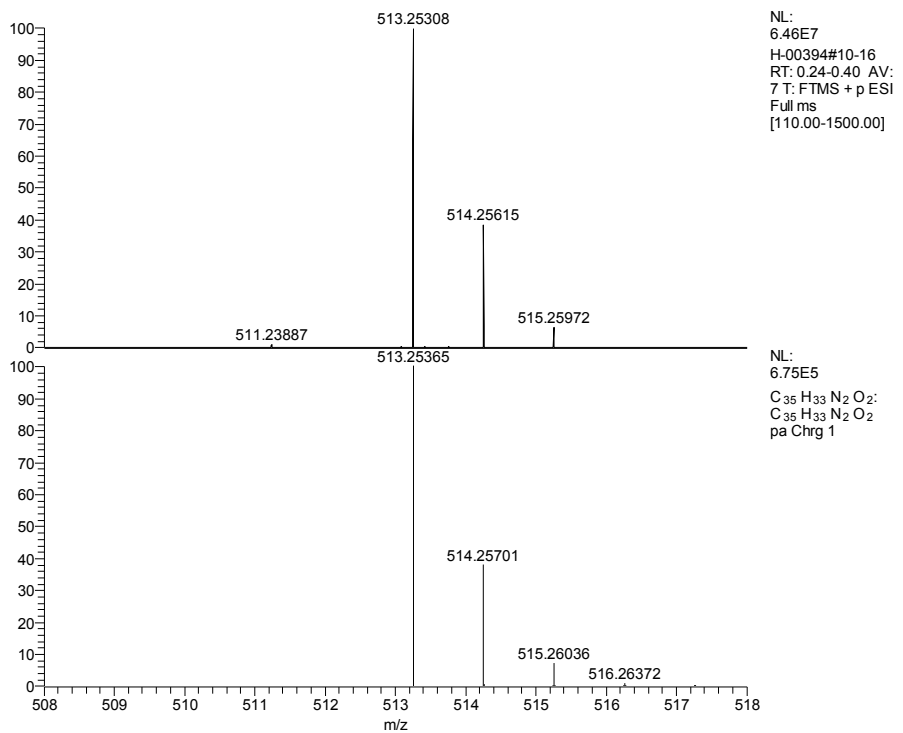
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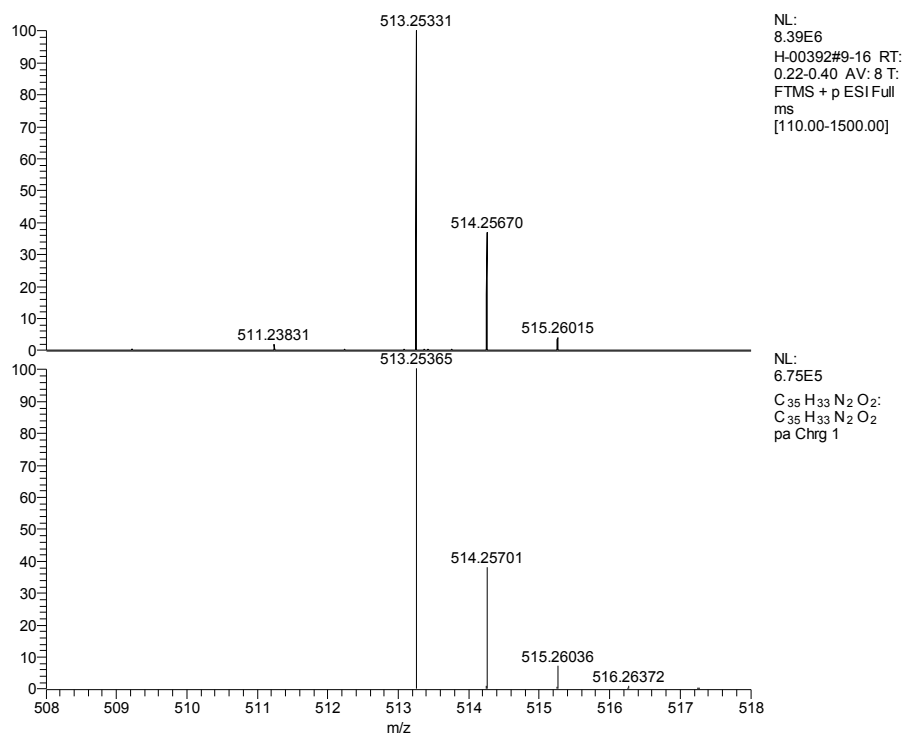
HRMS spectrum of HR-Cl



HRMS spectrum of HR-Br

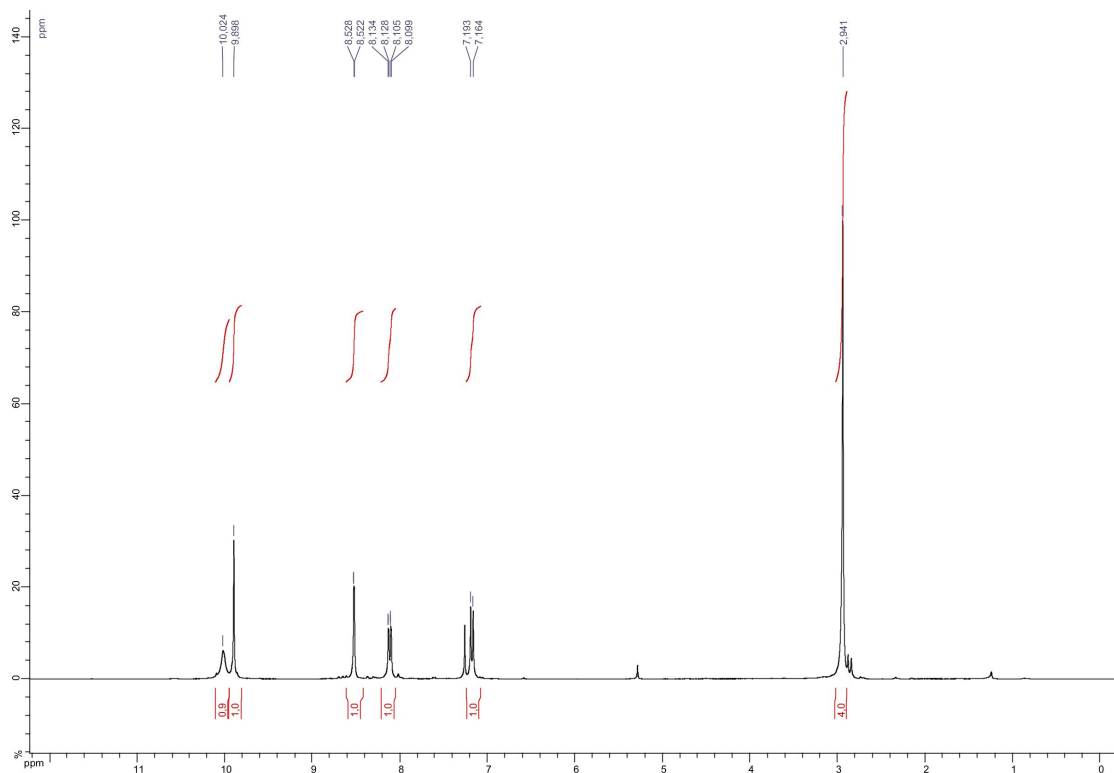


HRMS spectrum of p-Nph

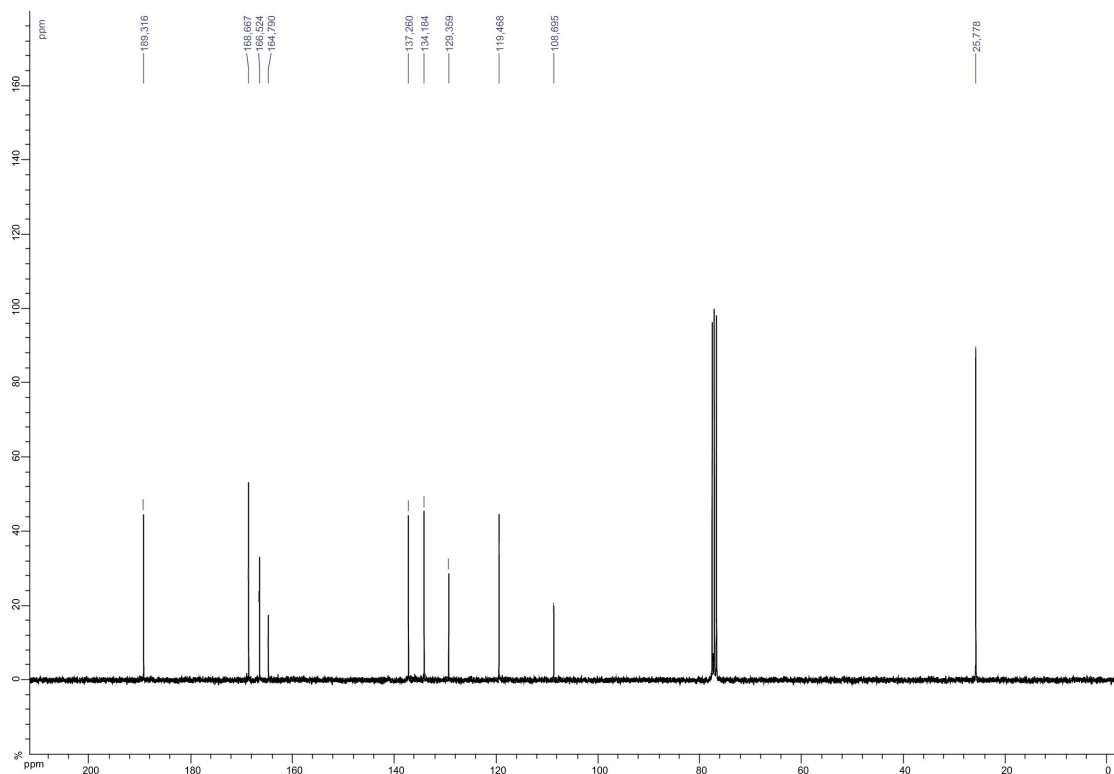


HRMS spectrum of *o*-Nph

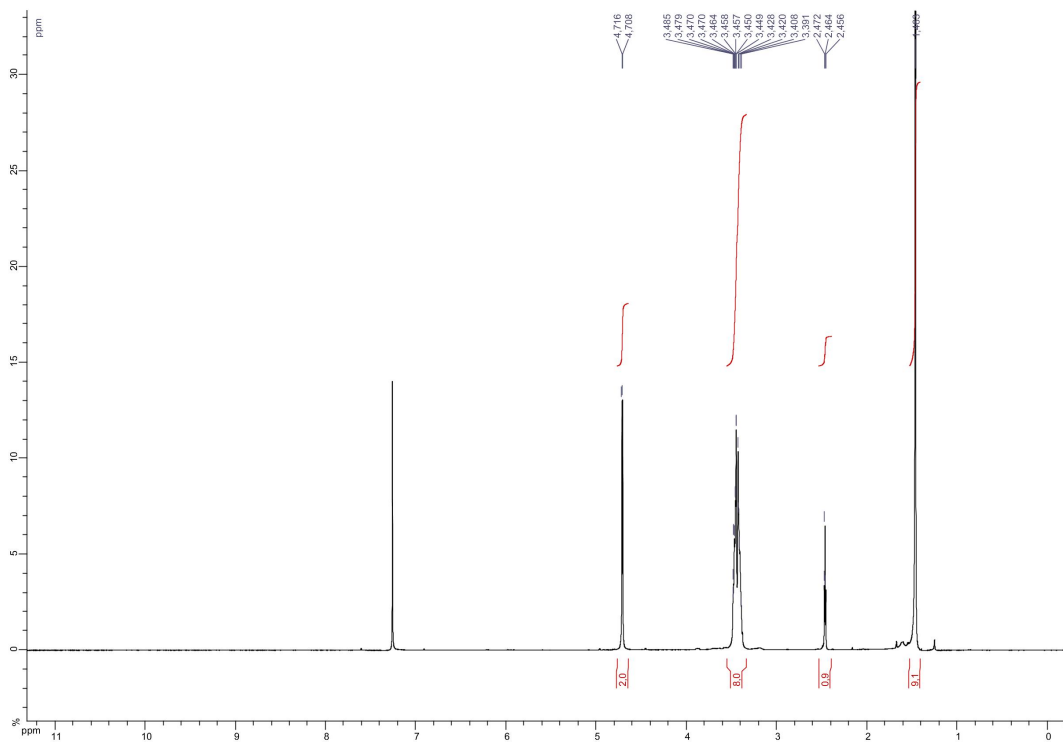
NMR spectra of functionalisable H-Rubies and their intermediates



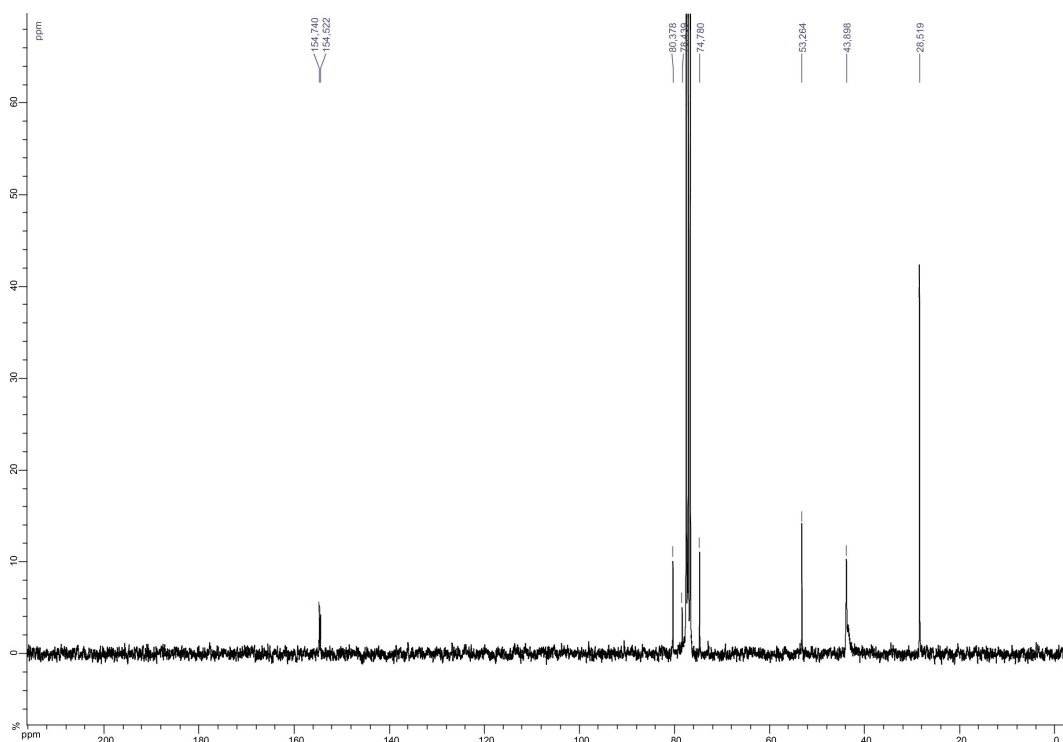
¹H NMR spectrum of **1** (CDCl₃, 300 MHz)



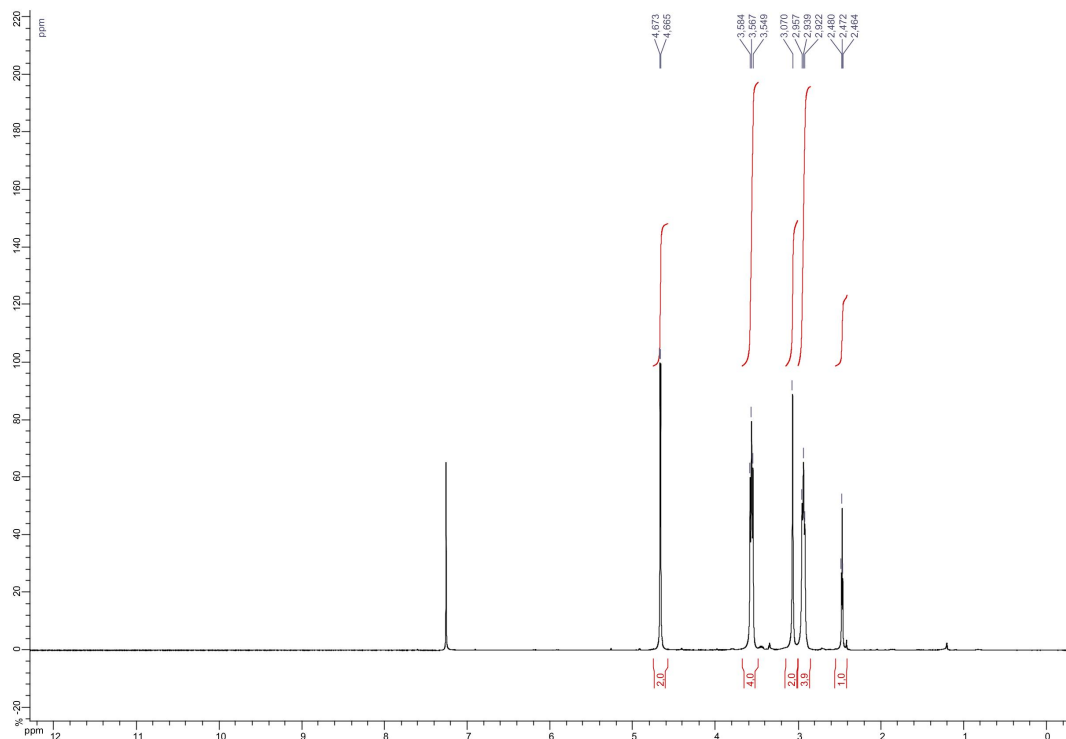
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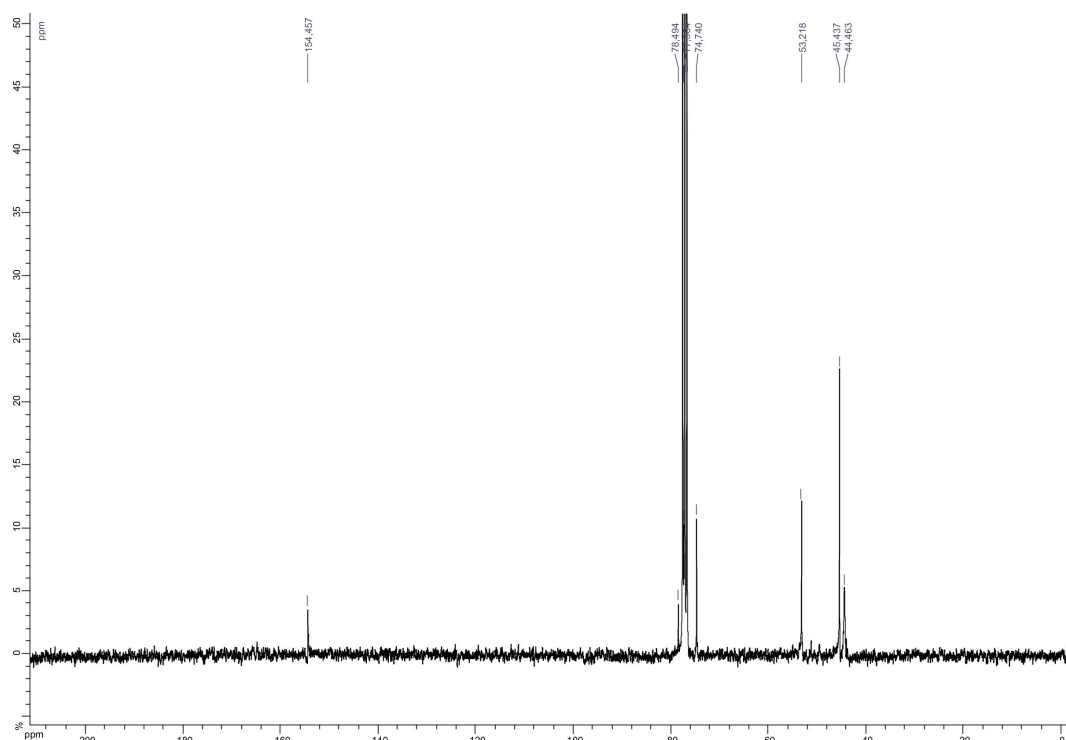
^1H NMR spectrum of 1-tert-butyl 4-prop-2-yn-1-yl piperazine-1,4-dicarboxylate (CDCl₃, 300 MHz)



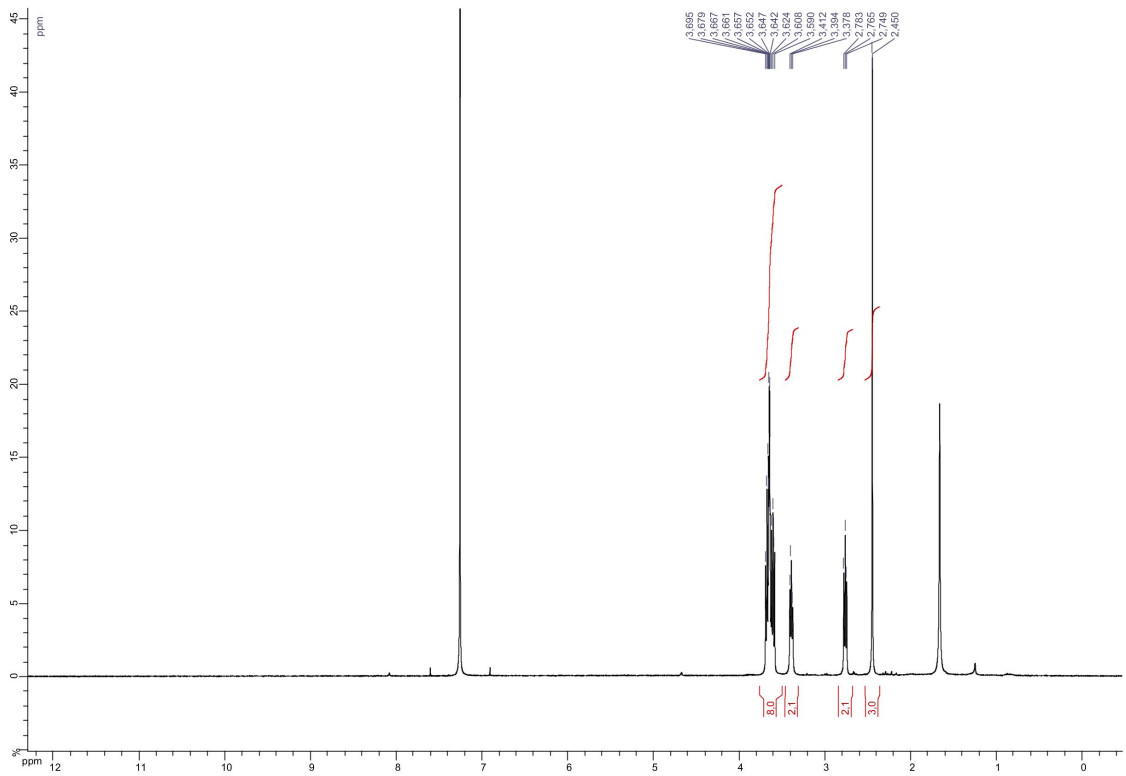
^{13}C NMR spectrum of 1-tert-butyl 4-prop-2-yn-1-yl piperazine-1,4-dicarboxylate (CDCl₃, 75 MHz)



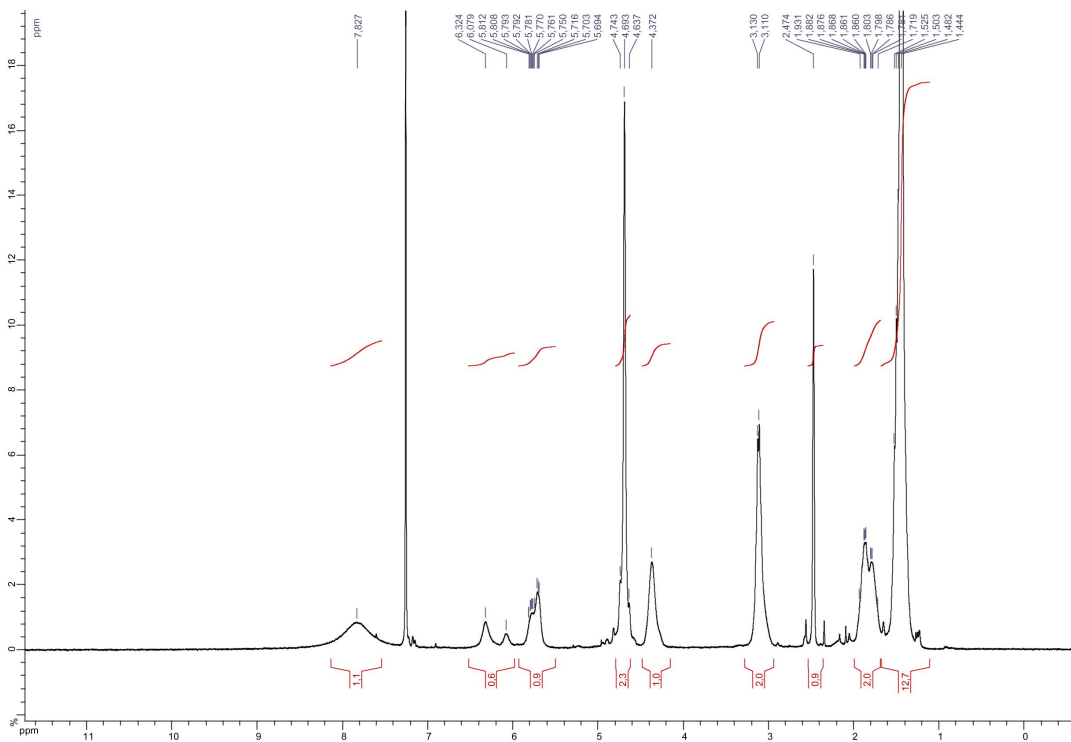
^1H NMR spectrum of **4-((prop-2-yn-1-yloxy)carbonyl)piperazin-1-ium 2,2,2-trifluoroacetate** (CDCl_3 , 300 MHz)



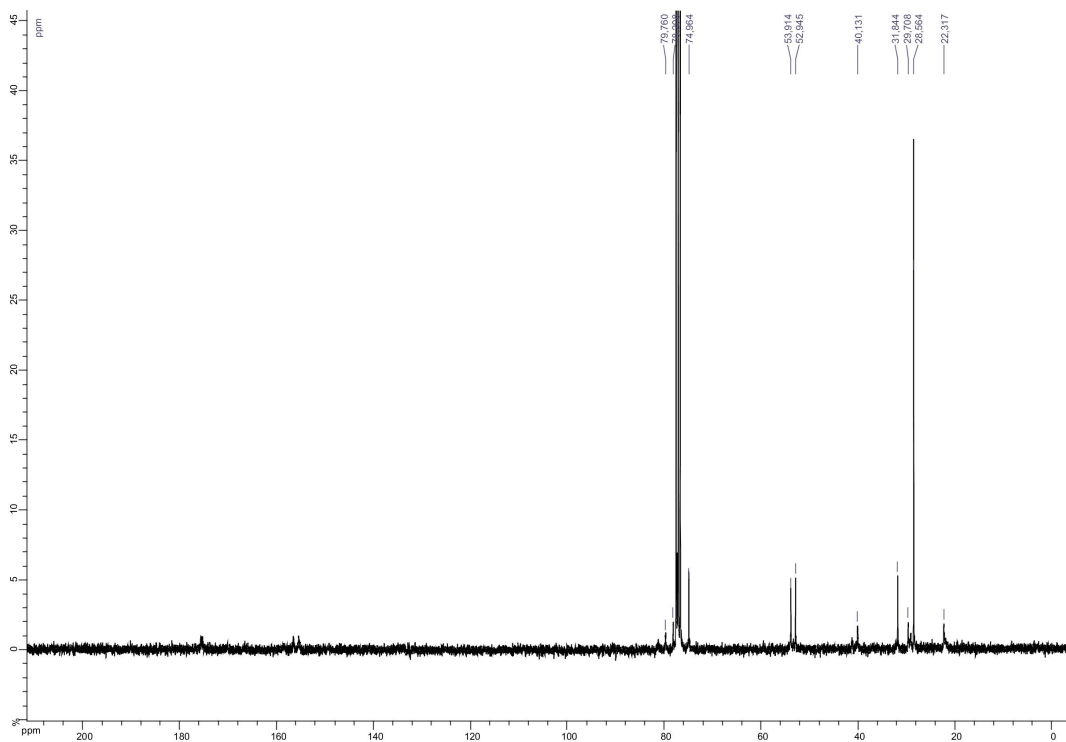
^{13}C NMR spectrum of **4-((prop-2-yn-1-yloxy)carbonyl)piperazin-1-ium 2,2,2-trifluoroacetate** (CDCl_3 , 75 MHz)



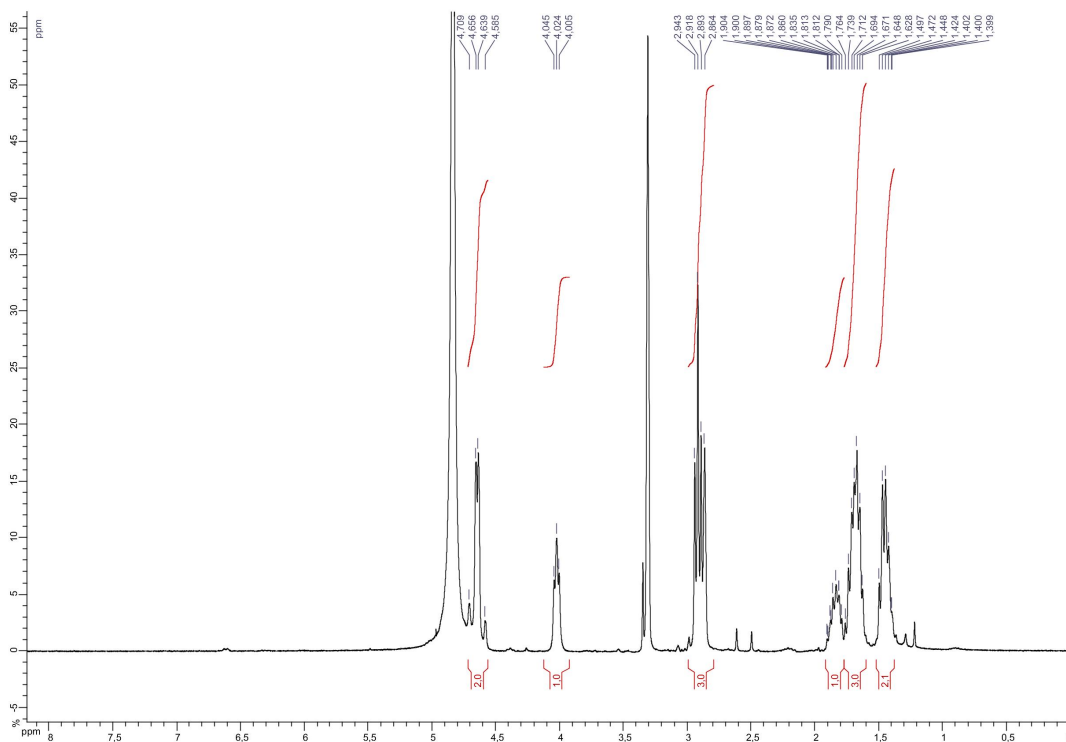
^1H NMR spectrum of **2-(2-(2-azidoethoxy)ethoxy)-N-methylethanamine** (CDCl_3 , 300 MHz)



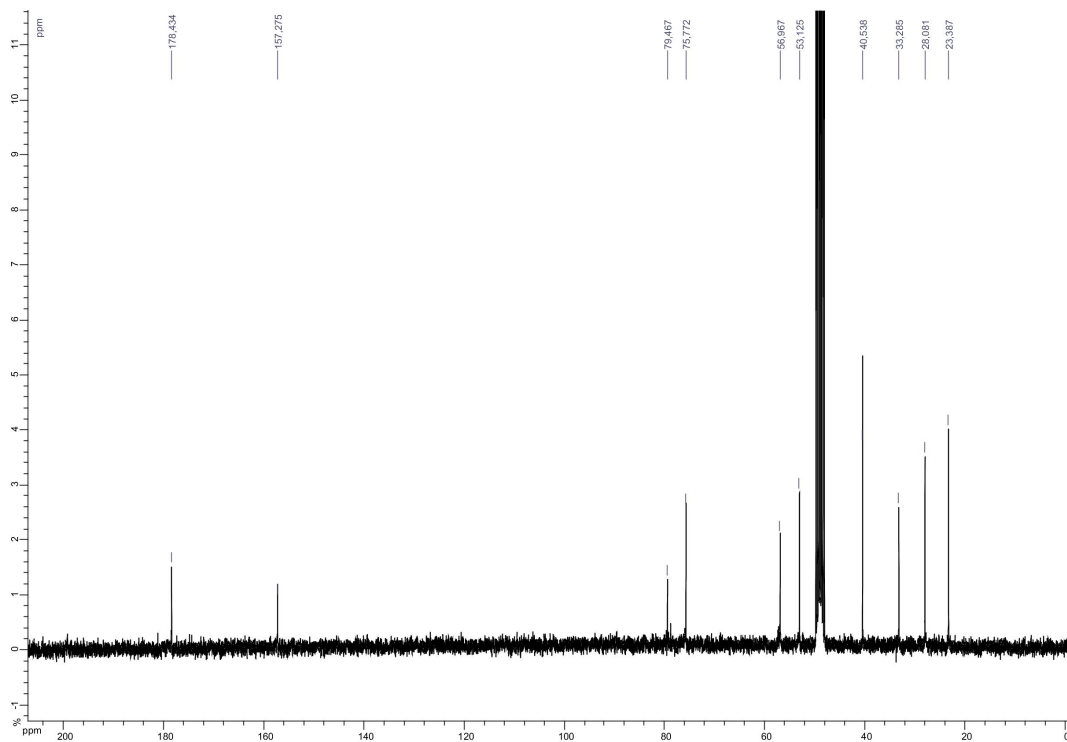
^1H NMR spectrum of **6-((tert-butoxycarbonyl)amino)-2-(((prop-2-yn-1-yloxy)carbonyl)amino)hexanoic acid** (CDCl_3 , 300 MHz)



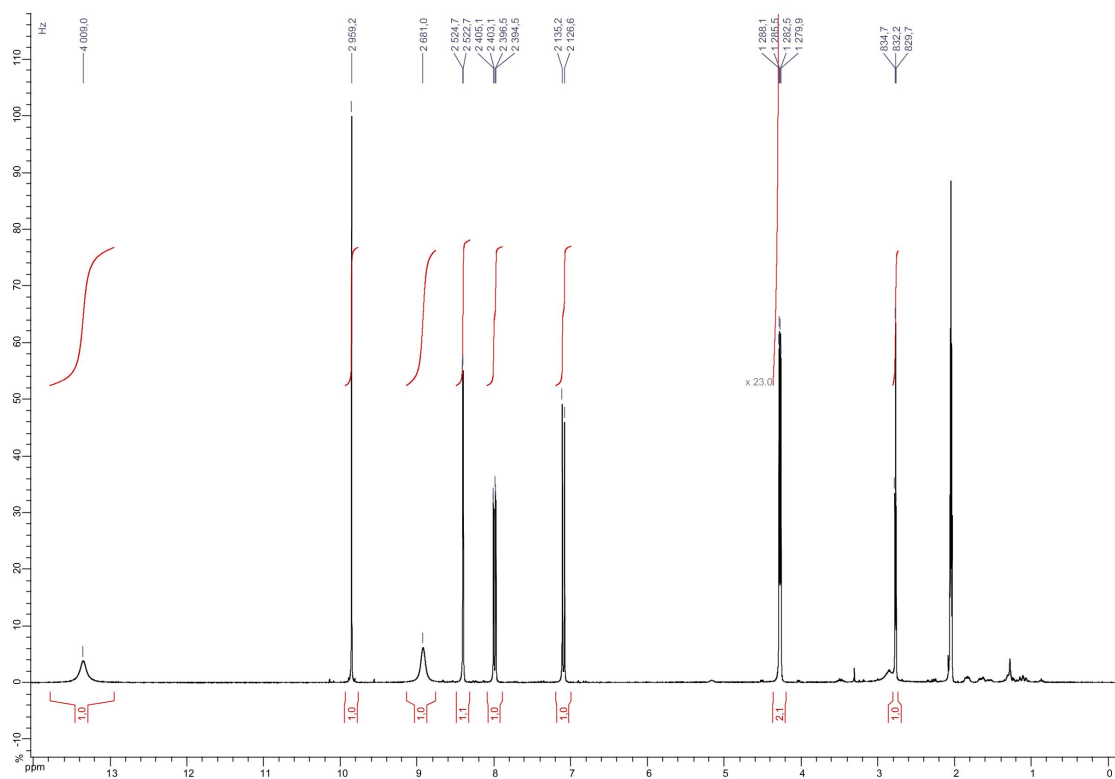
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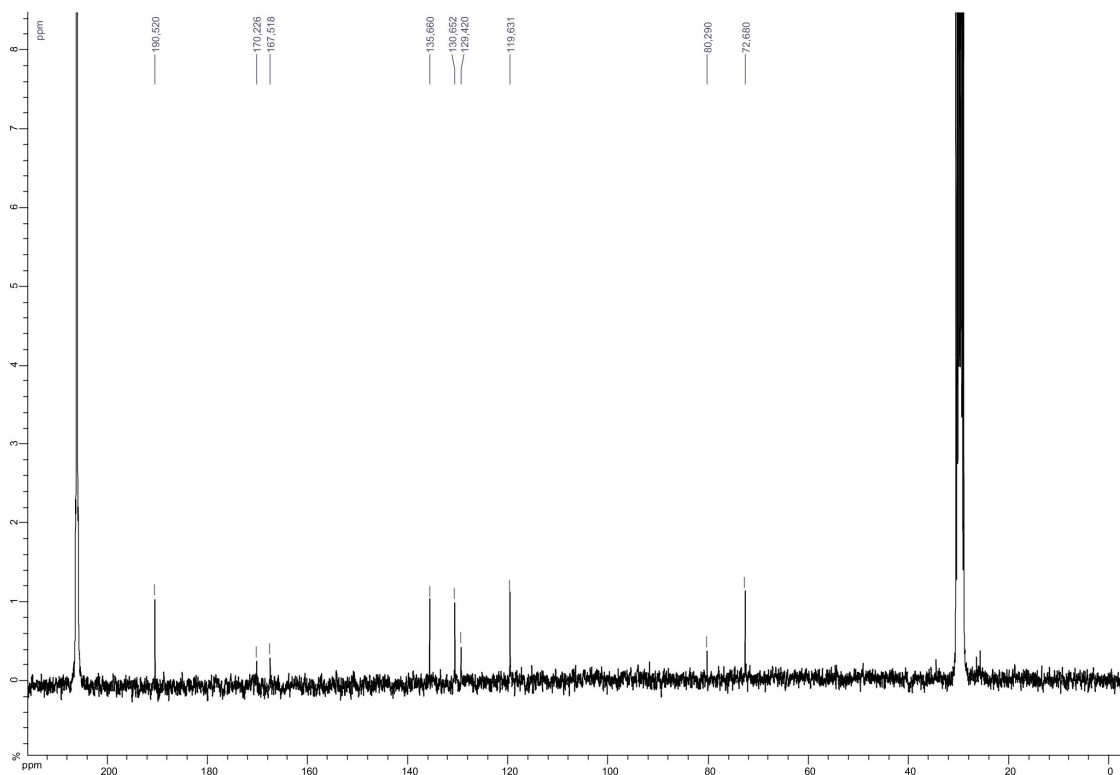
¹H NMR spectrum of **5-carboxy-5-(((prop-2-yn-1-yloxy)carbonyl)amino)pentan-1-aminium 2,2,2-trifluoroacetate** (CDCl₃, 300 MHz)



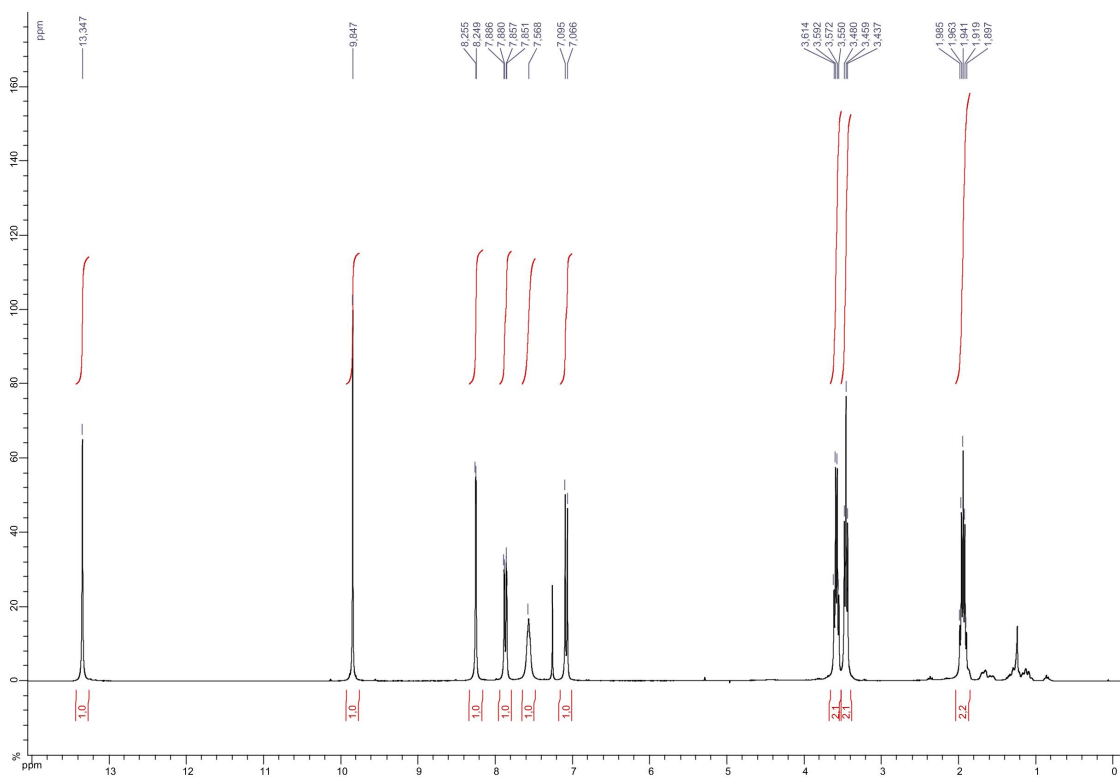
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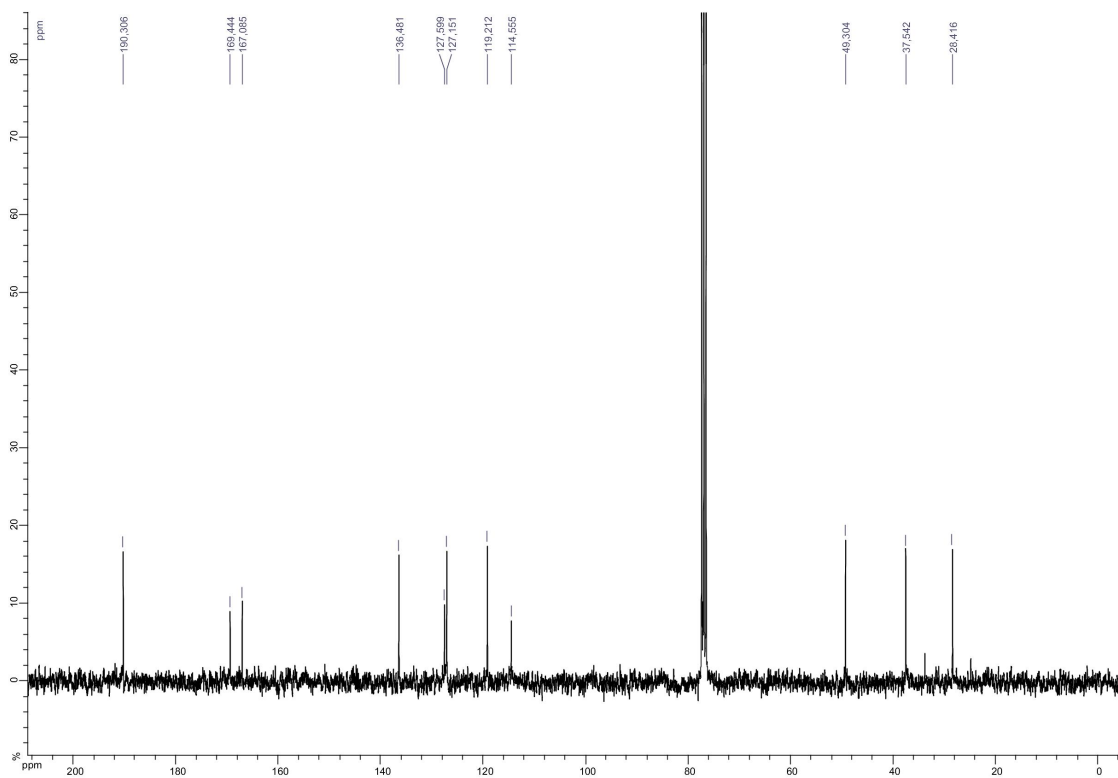
^1H NMR spectrum of **2a** ((CD₃)₃CO, 300 MHz)



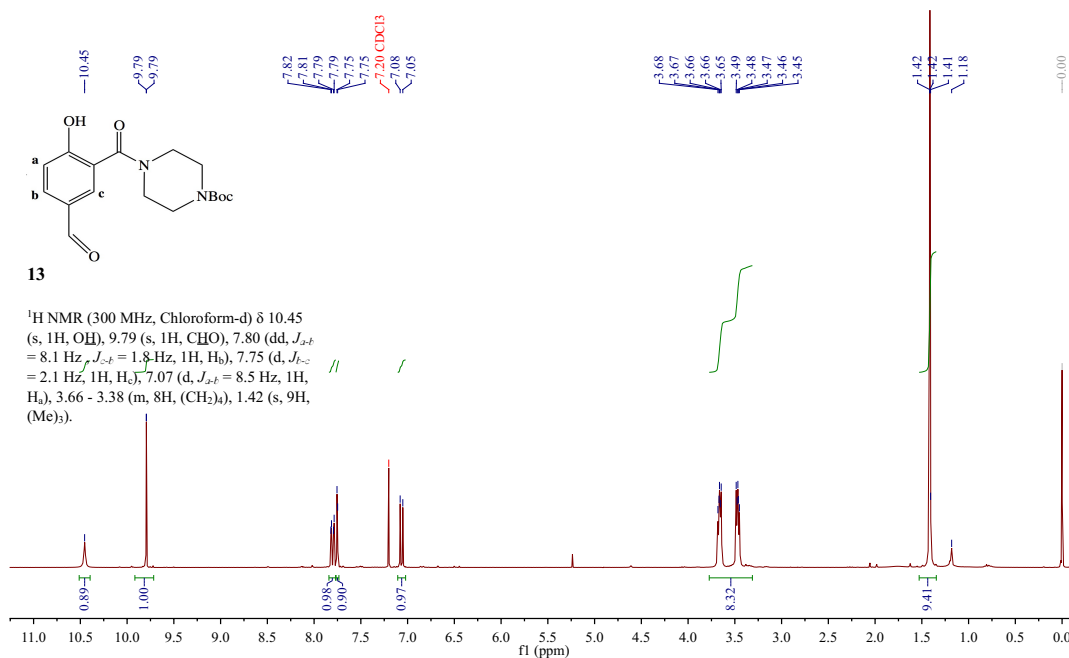
^{13}C NMR spectrum of **2a** ($(\text{CD}_3)_3\text{CO}$, 75 MHz)



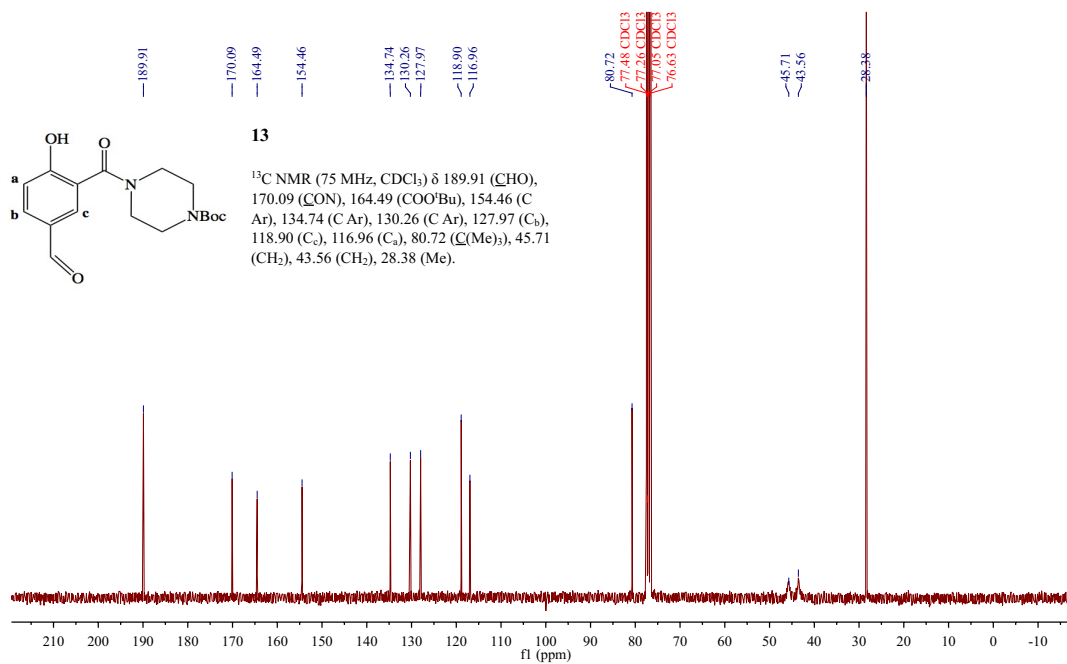
^1H NMR spectrum of **2b** (CDCl_3 , 300 MHz)



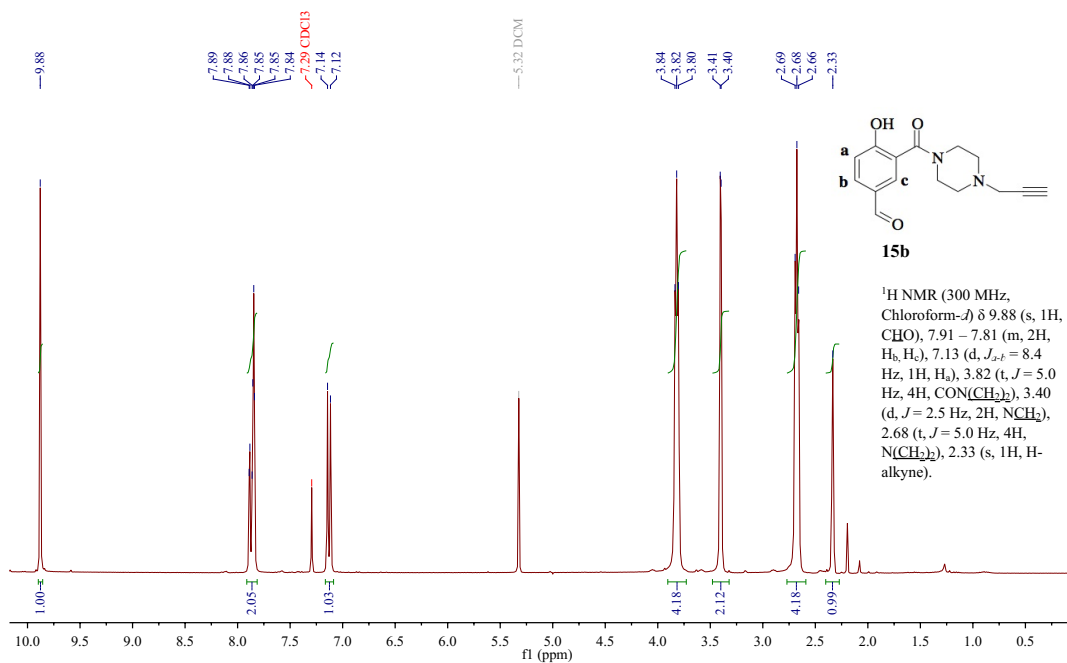
^{13}C NMR spectrum of **2b** (CDCl_3 , 75 MHz)



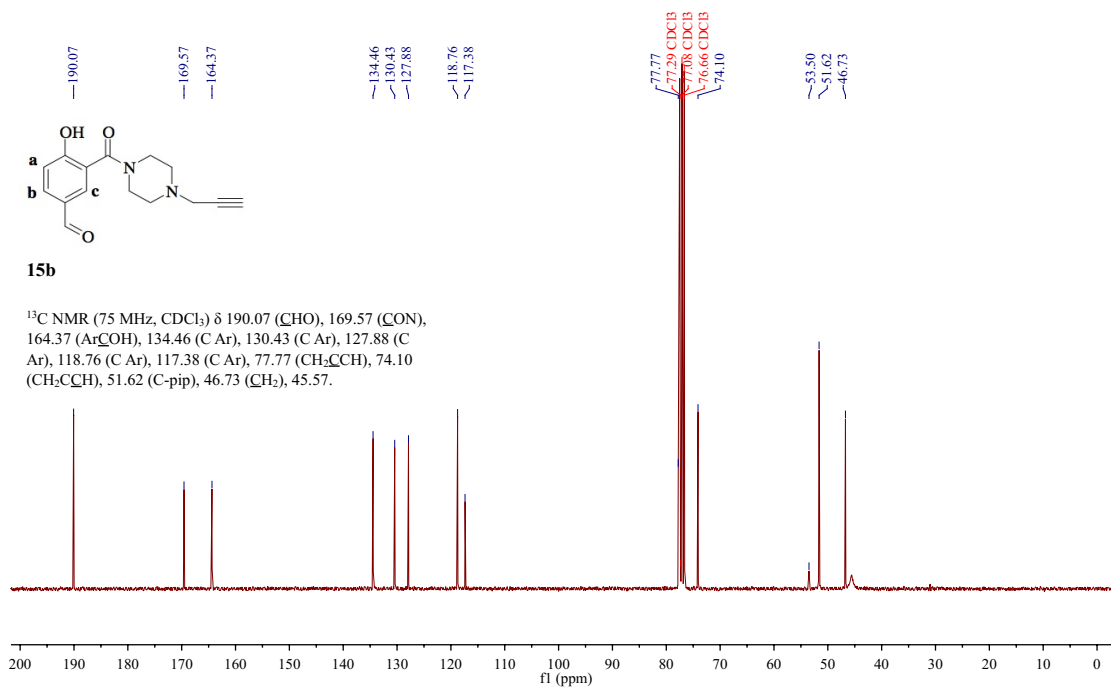
^1H NMR spectrum of **2c'** (CDCl_3 , 300 MHz)



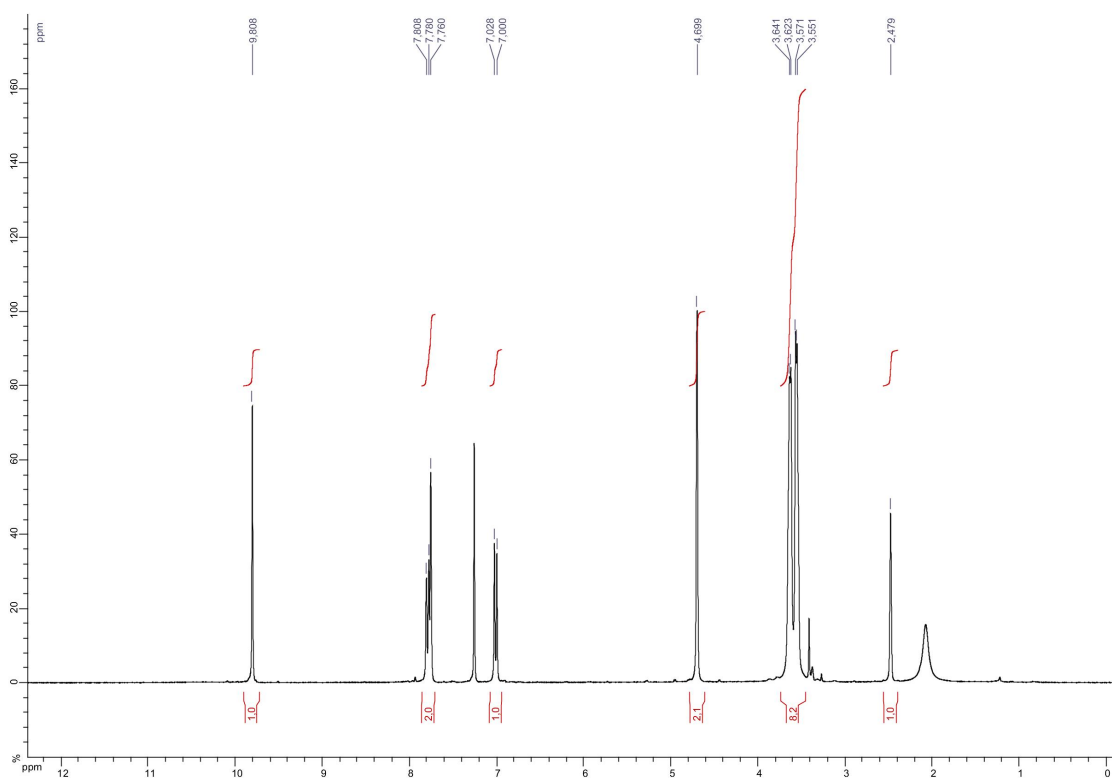
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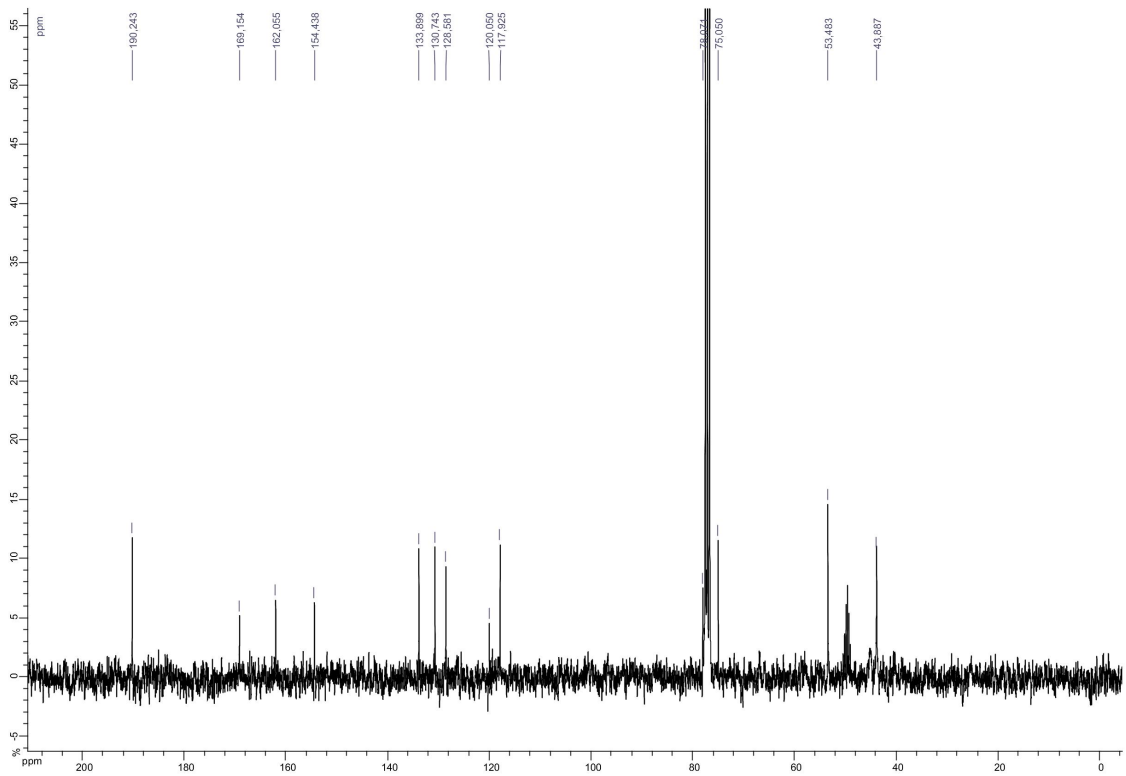
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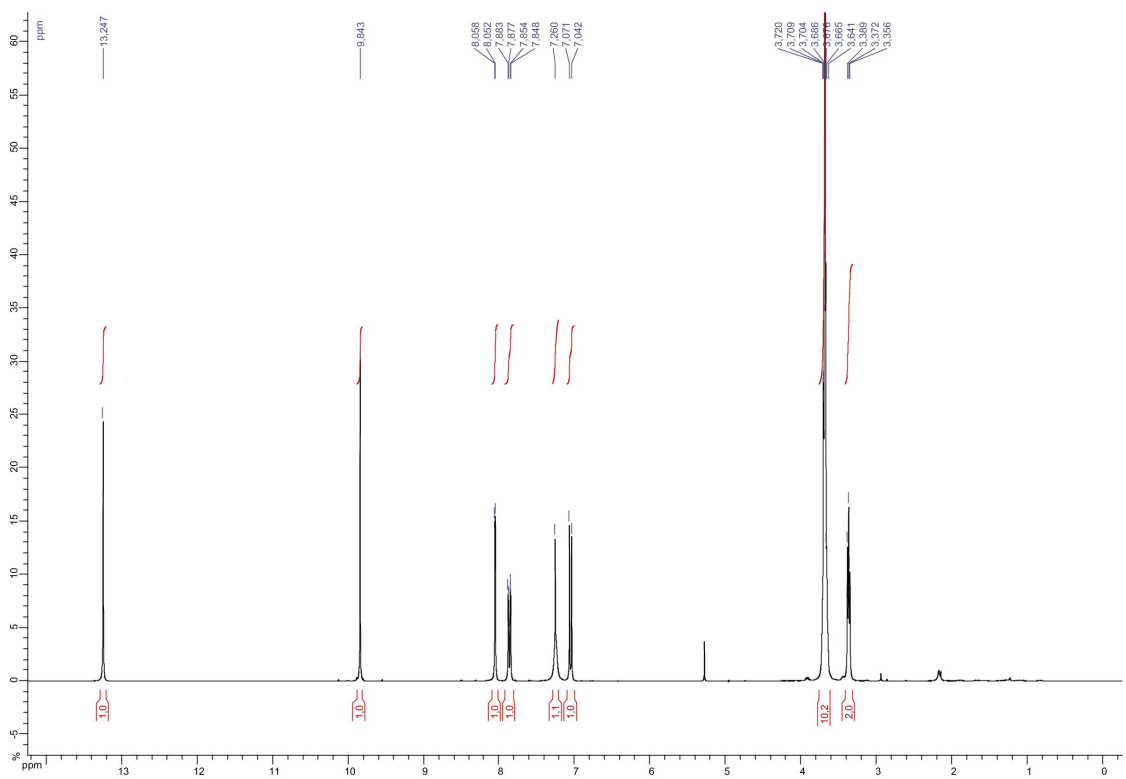
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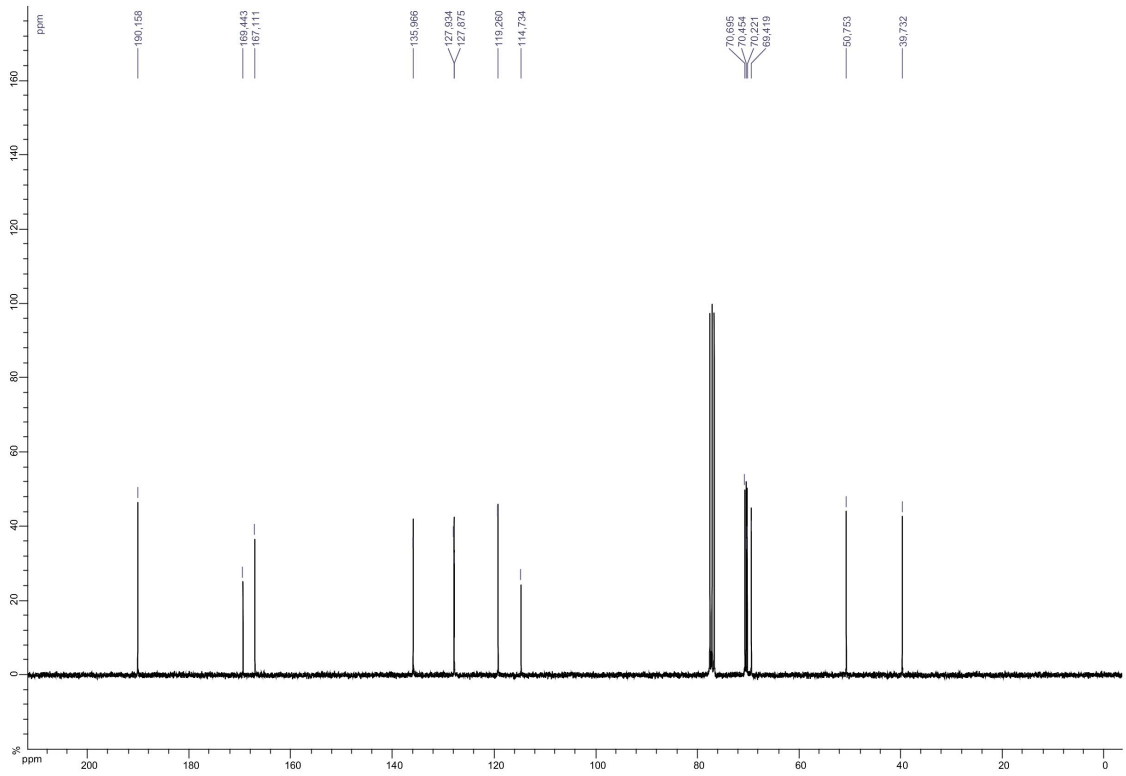
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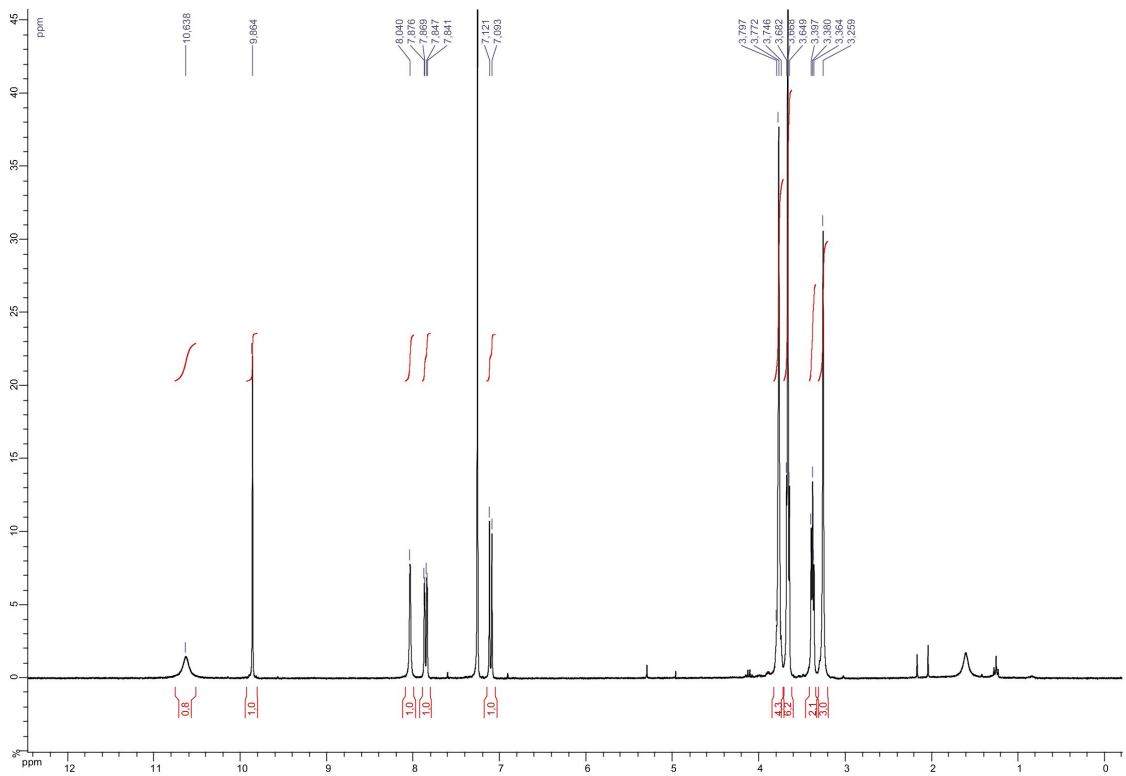
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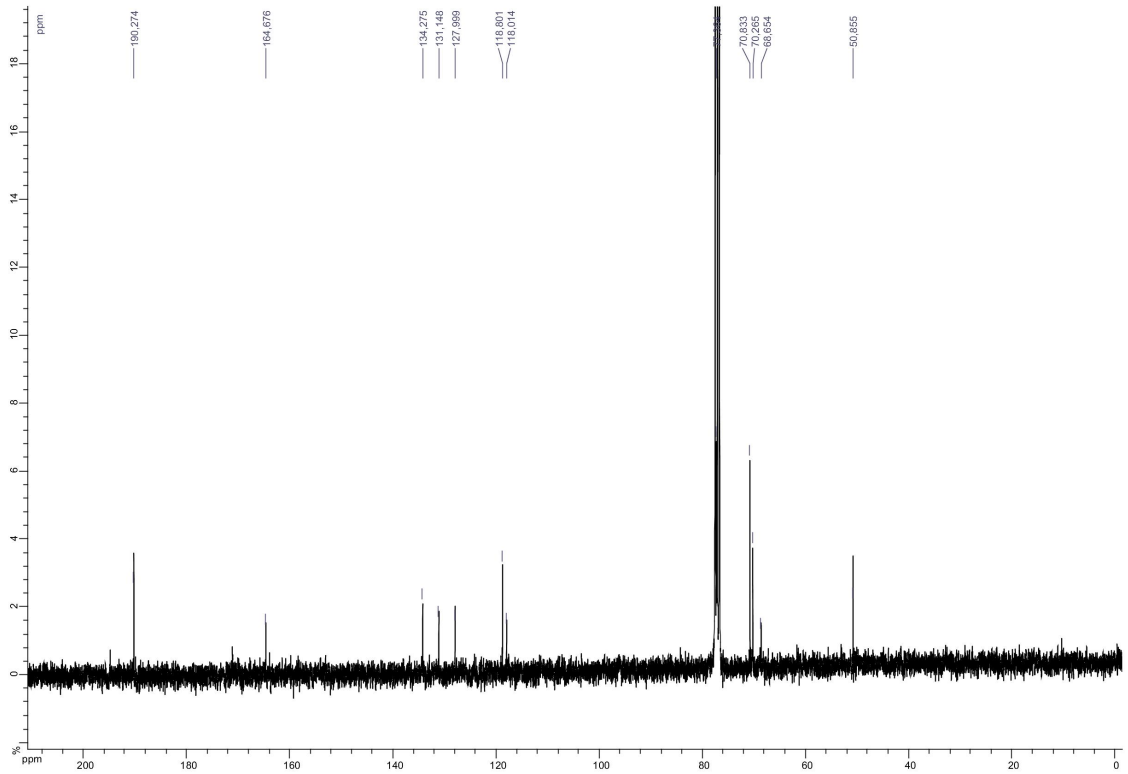
¹H NMR spectrum of **2e** (CDCl₃, 300 MHz)



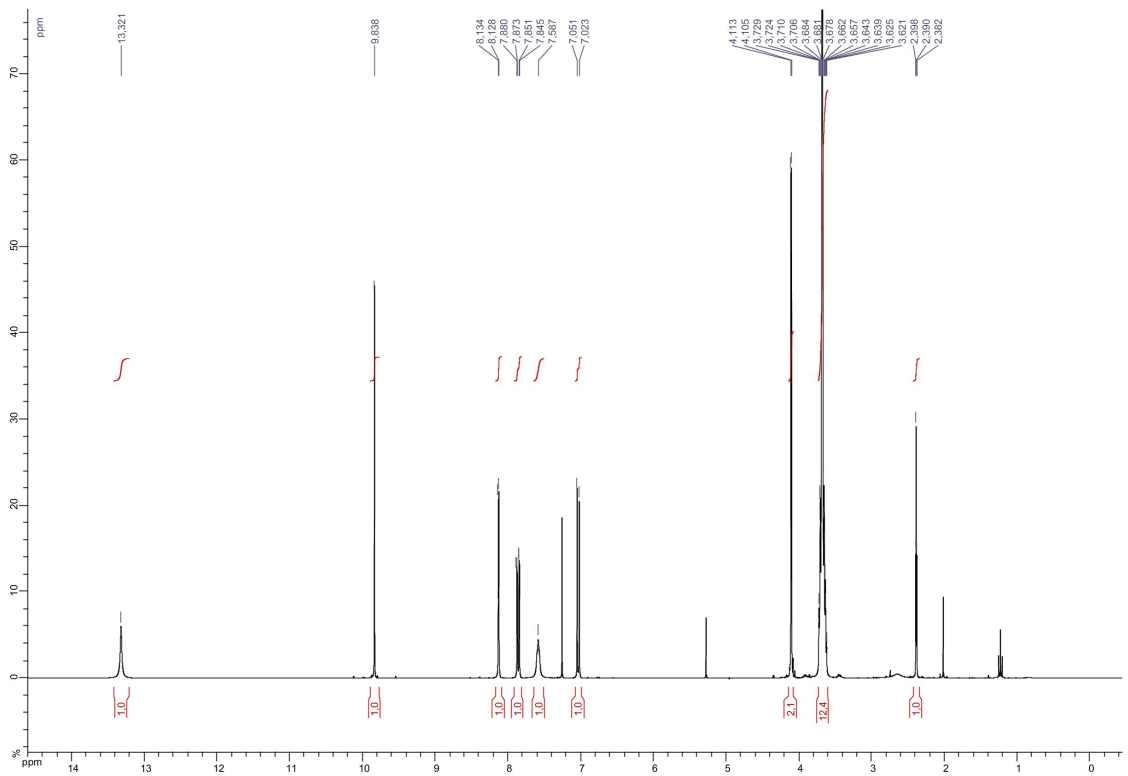
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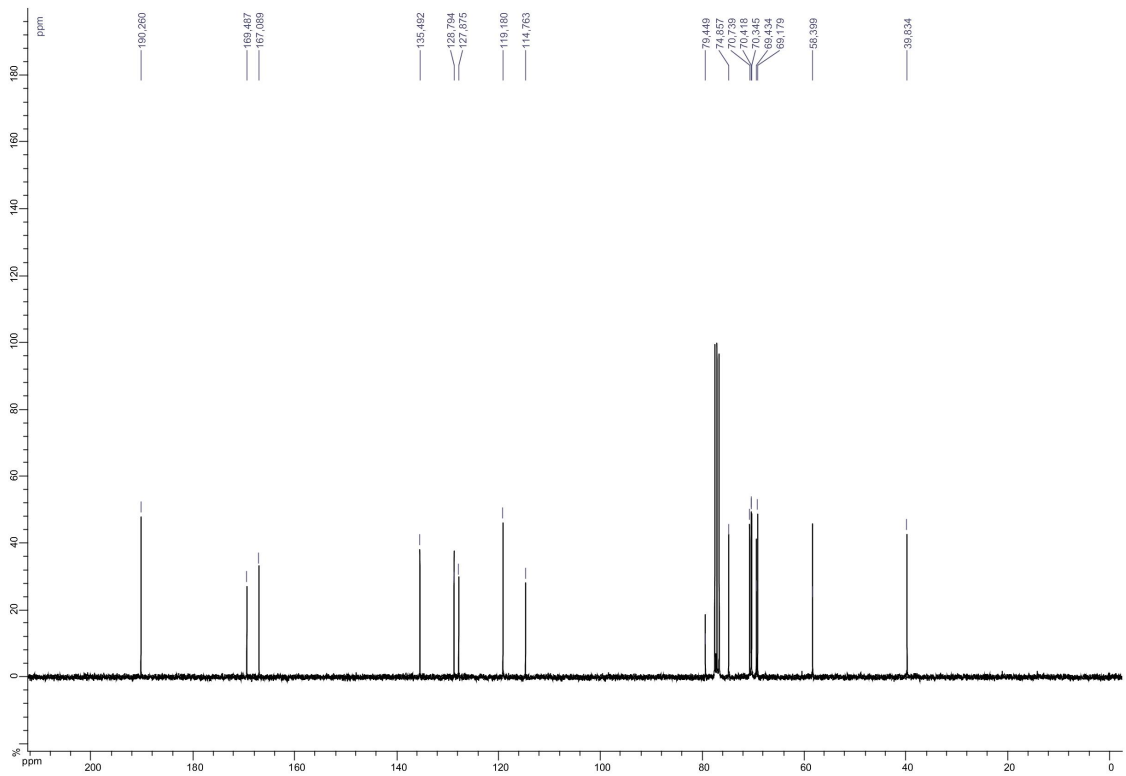
^1H NMR spectrum of **2f** (CDCl_3 , 300 MHz)



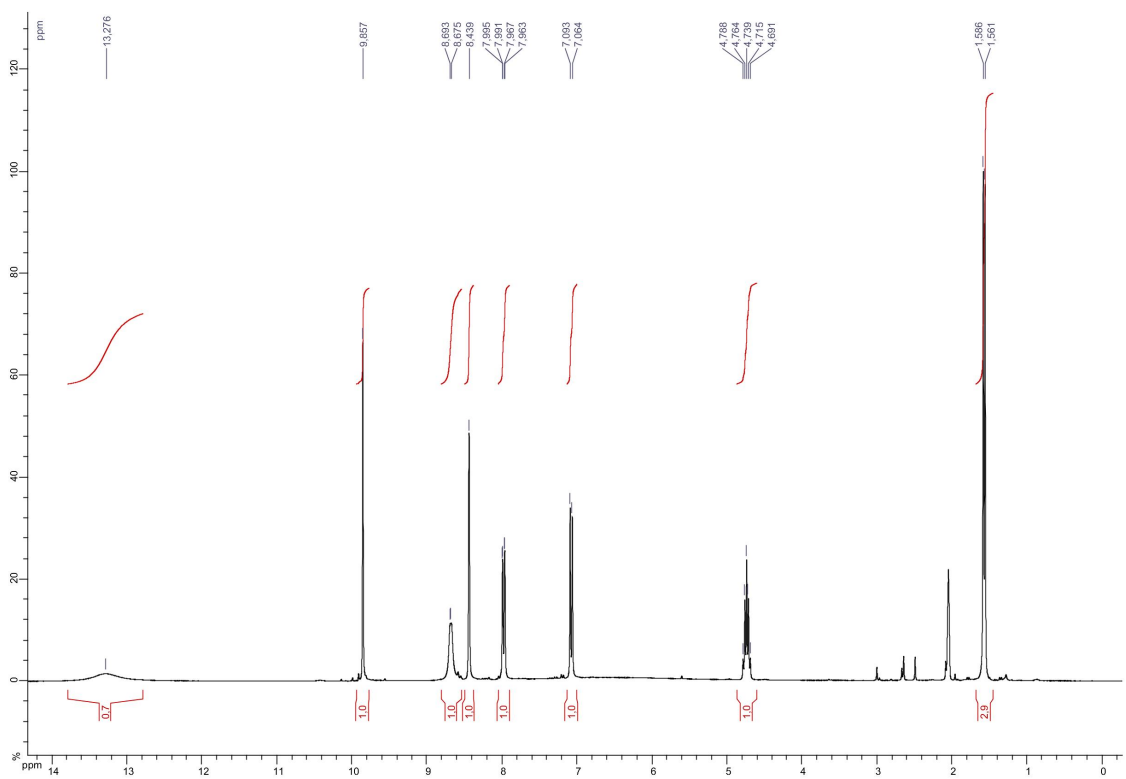
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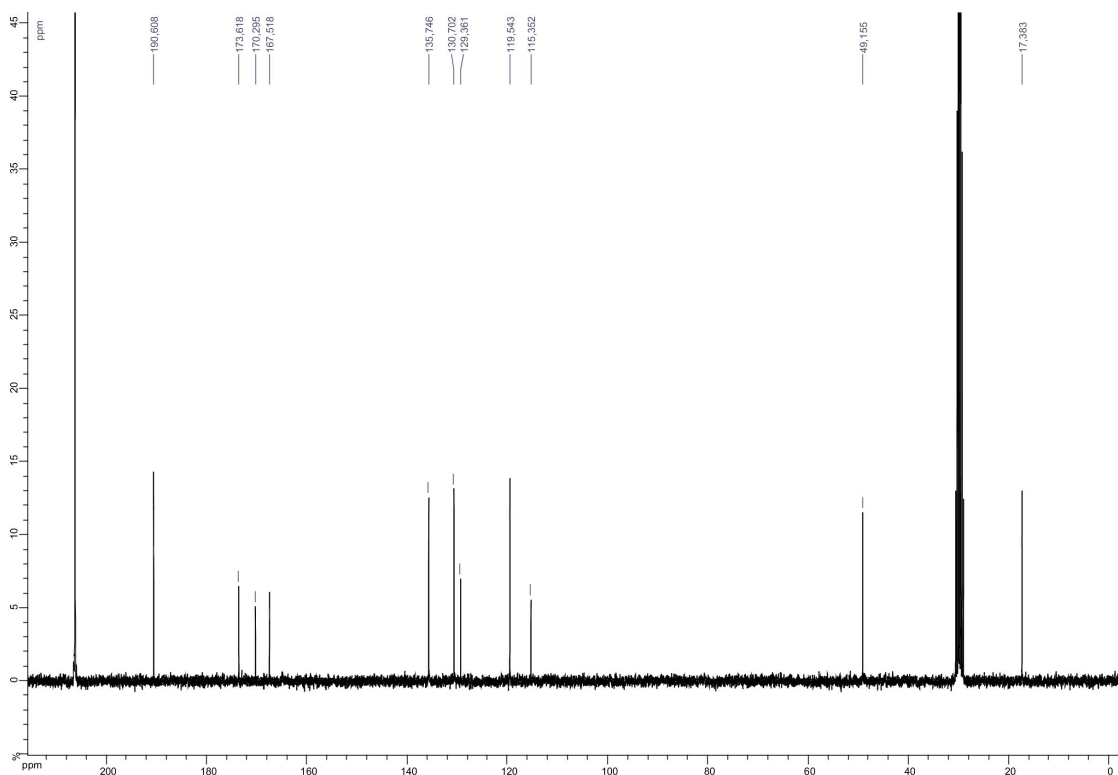
^1H NMR spectrum of **2g** (CDCl_3 , 300 MHz)



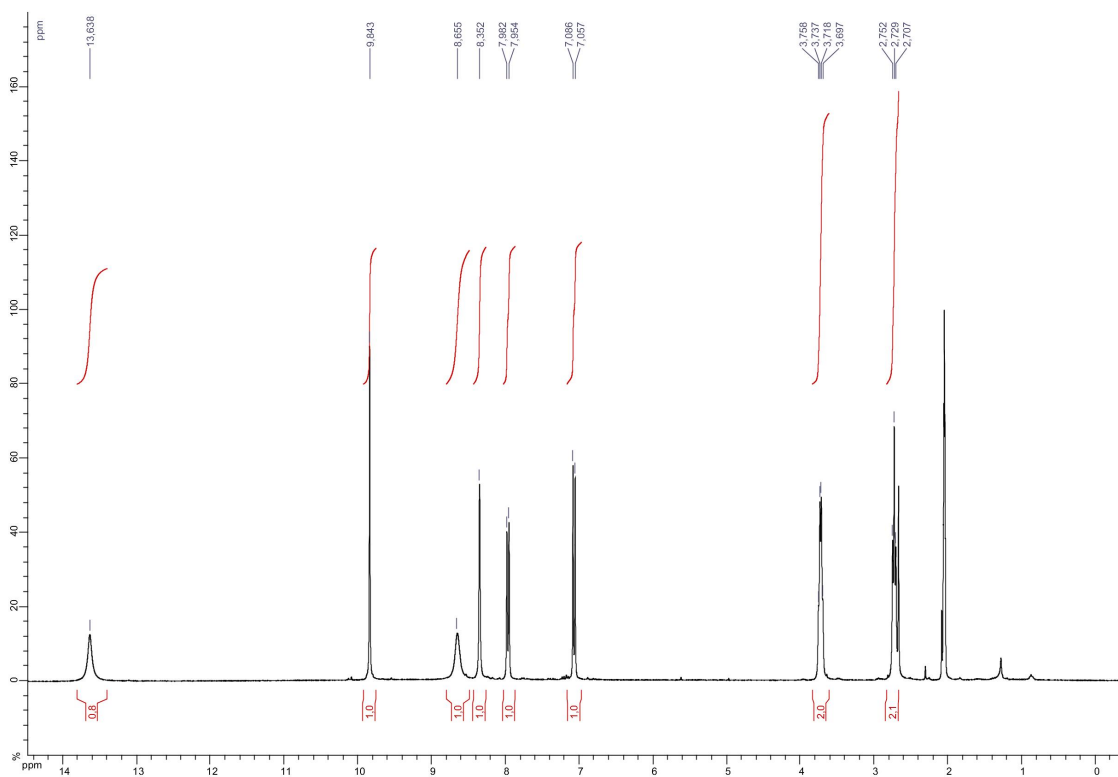
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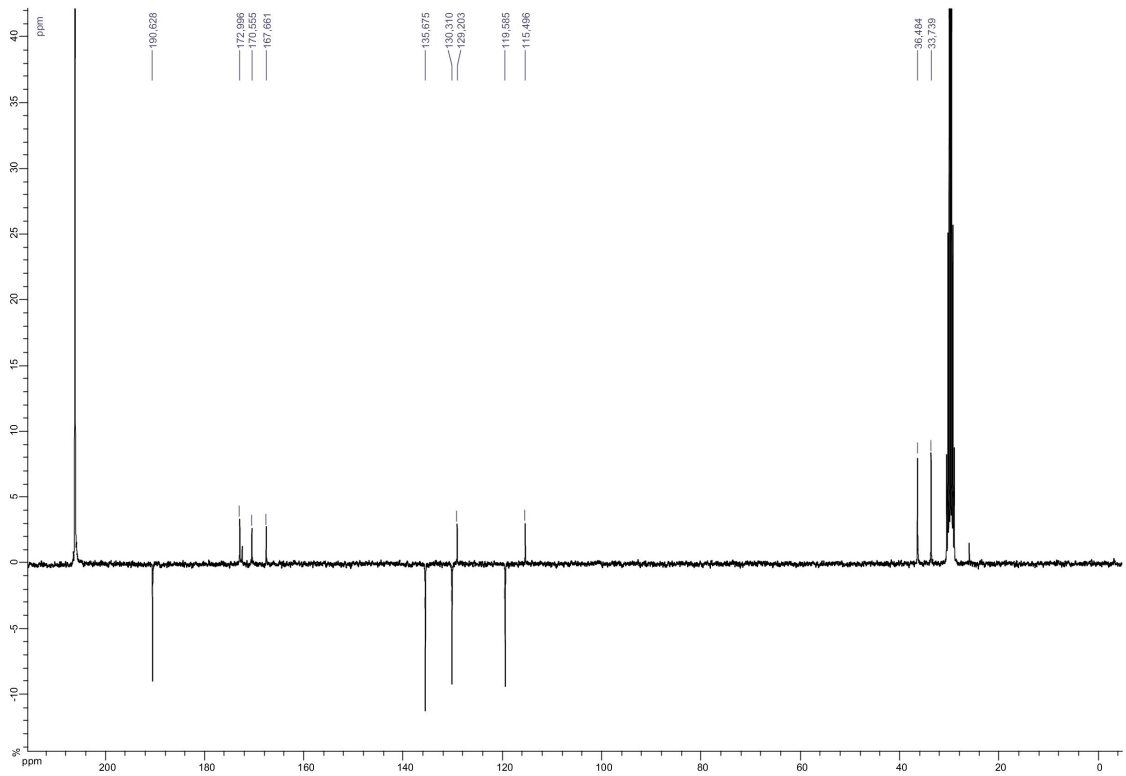
¹H NMR spectrum of **2h** (CDCl₃, 300 MHz)



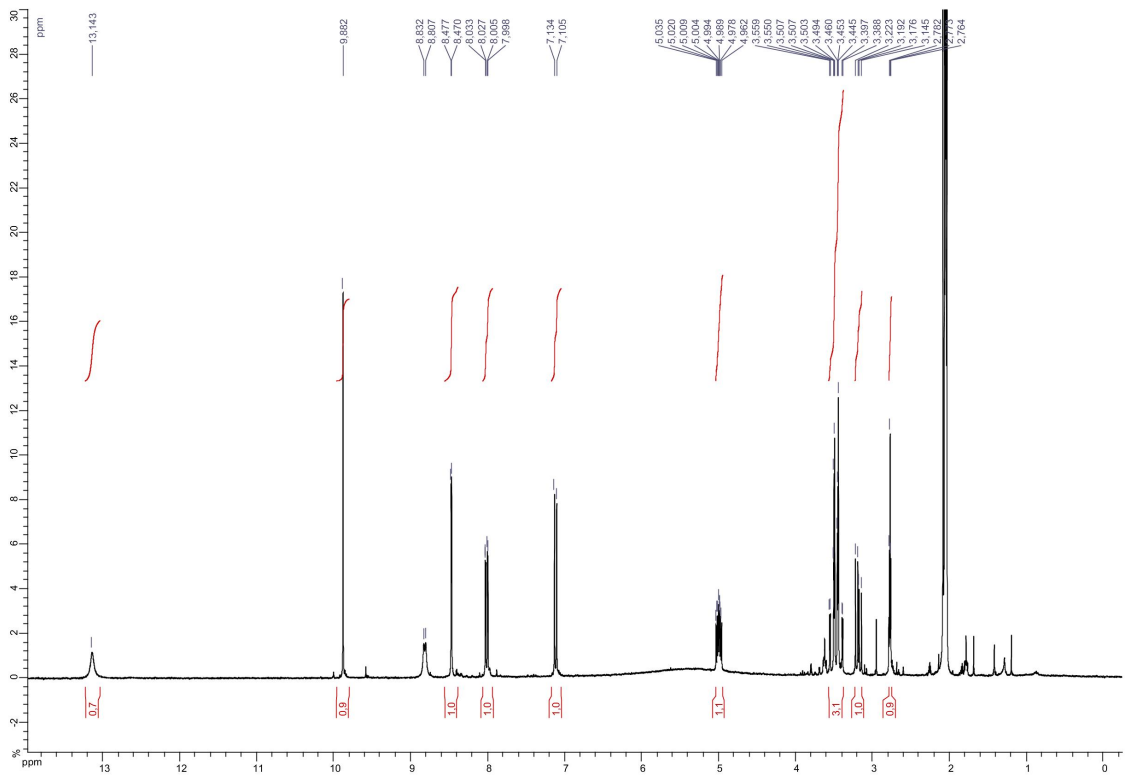
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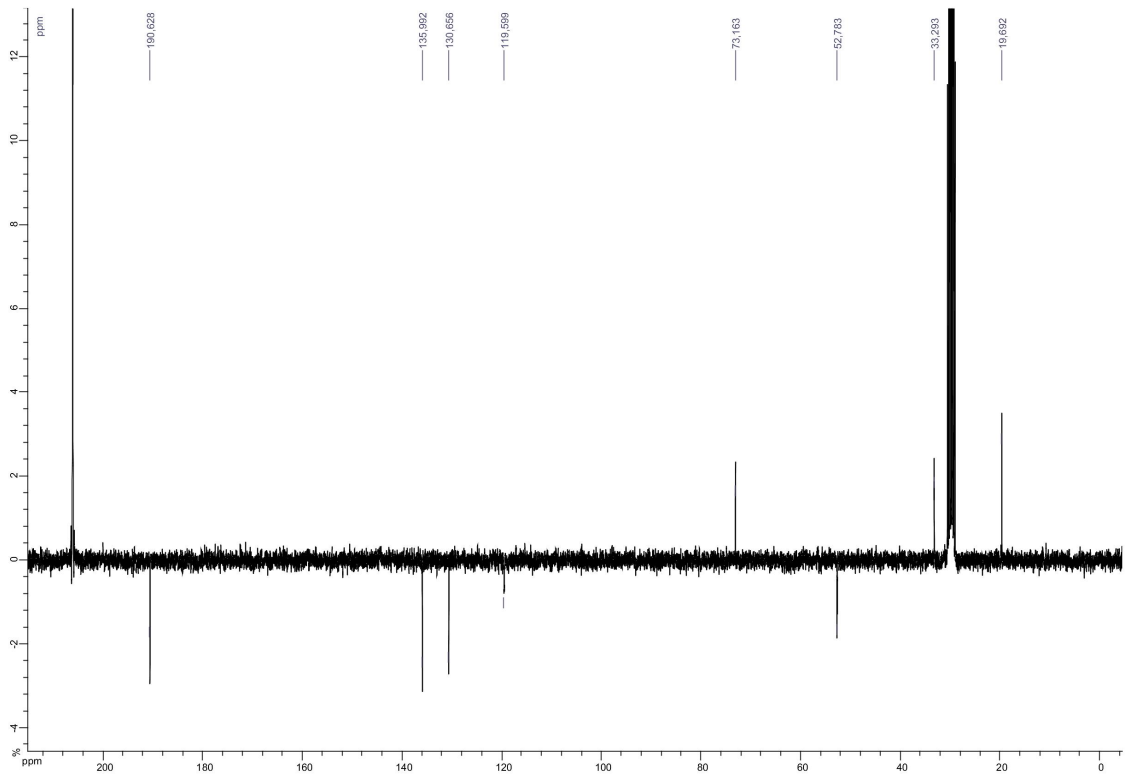
¹H NMR spectrum of **2i** (CDCl₃, 300 MHz)



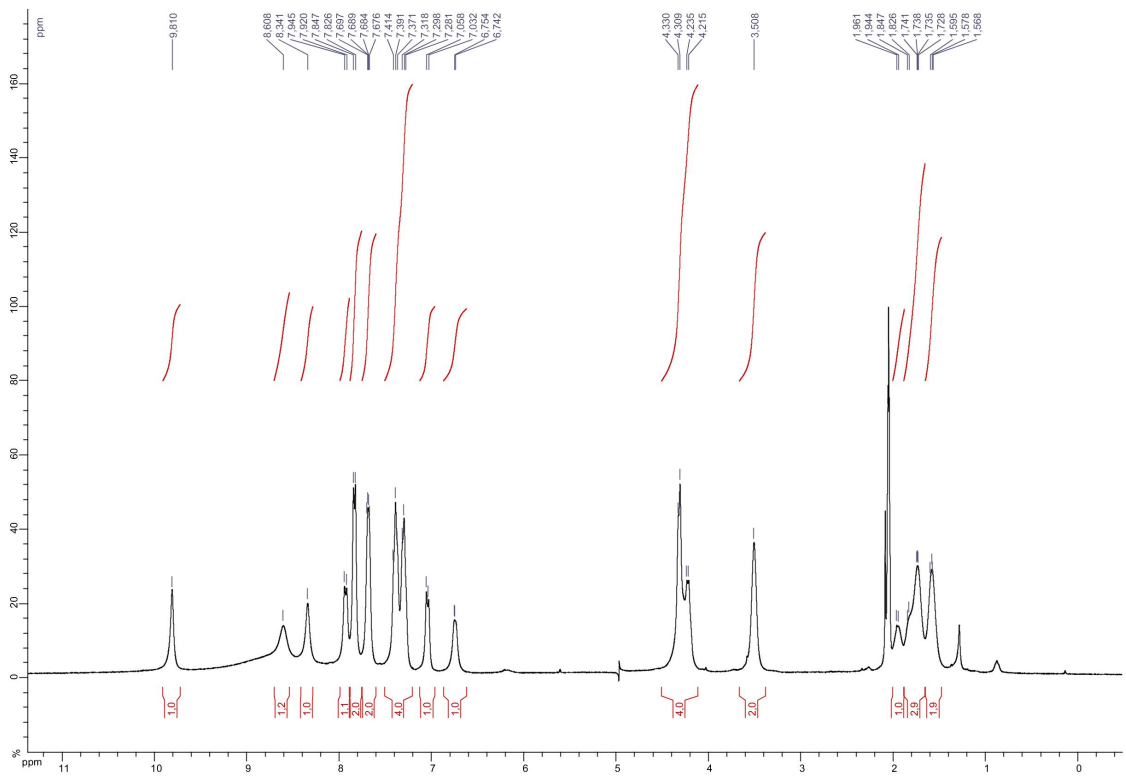
^{13}C NMR (JMOD) spectrum of **2i** (CDCl_3 , 75 MHz)



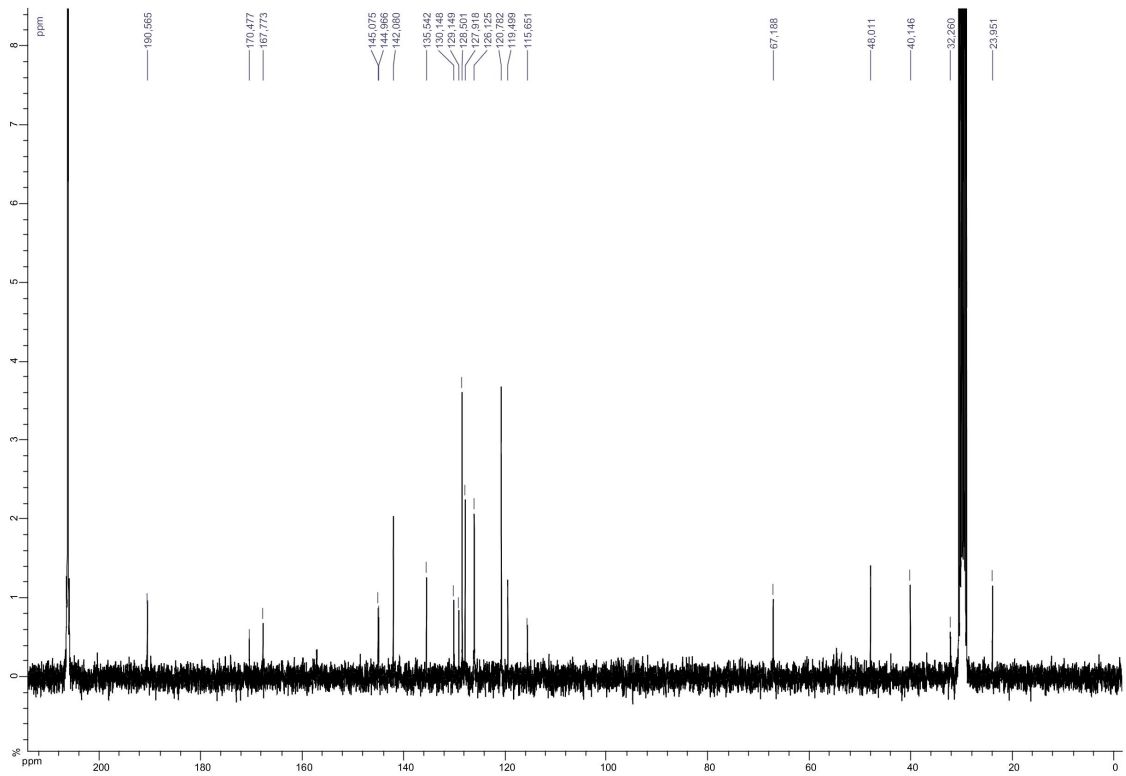
^1H NMR spectrum of **2j** (CDCl_3 , 300 MHz)



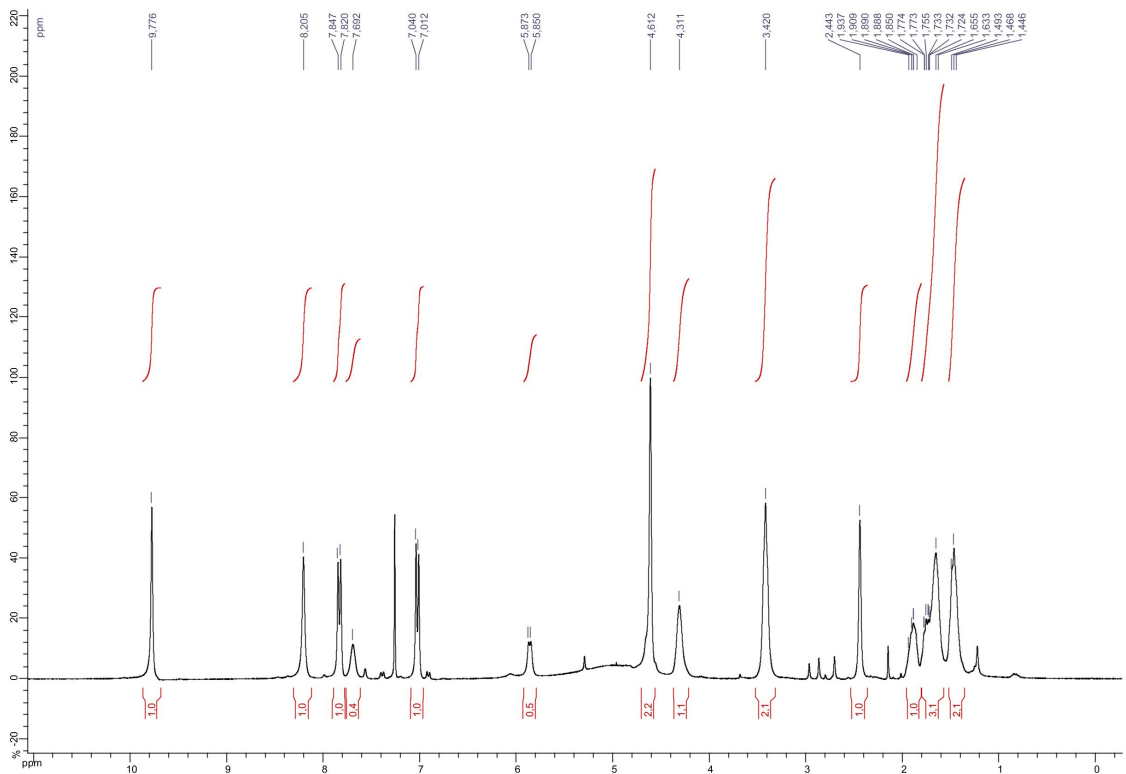
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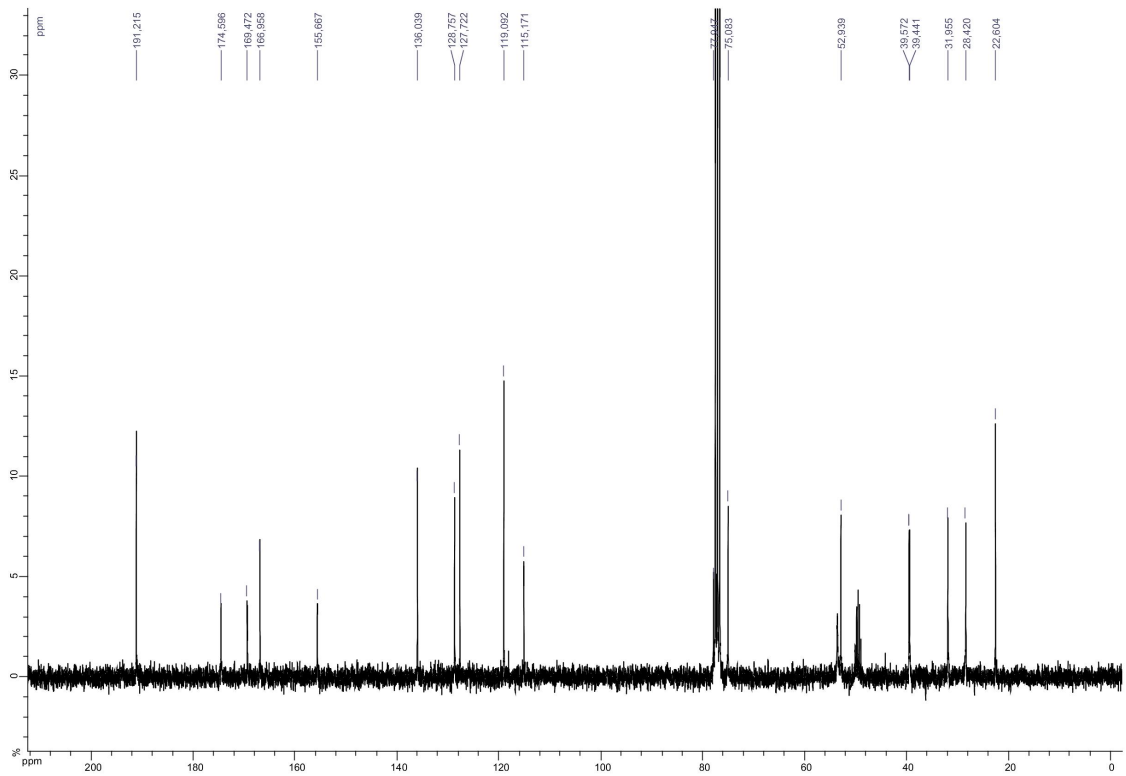
^1H NMR spectrum of **2k** ($(\text{CD}_3)_3\text{CO}$, 300 MHz)



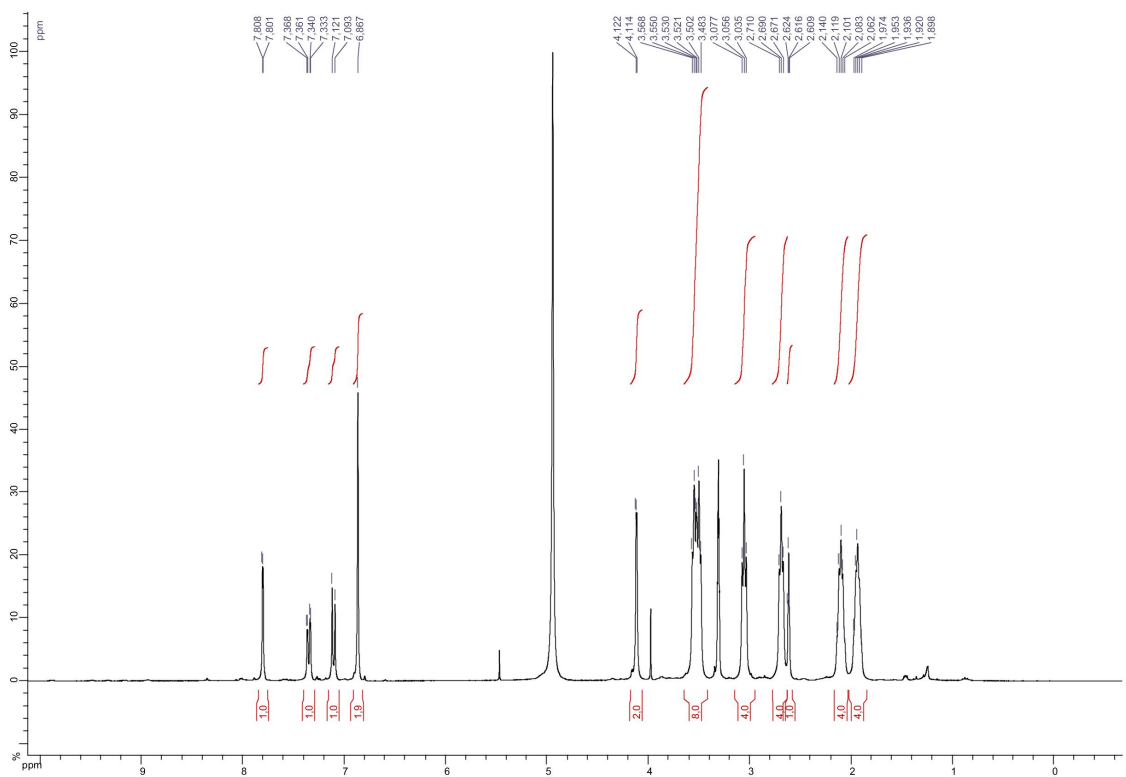
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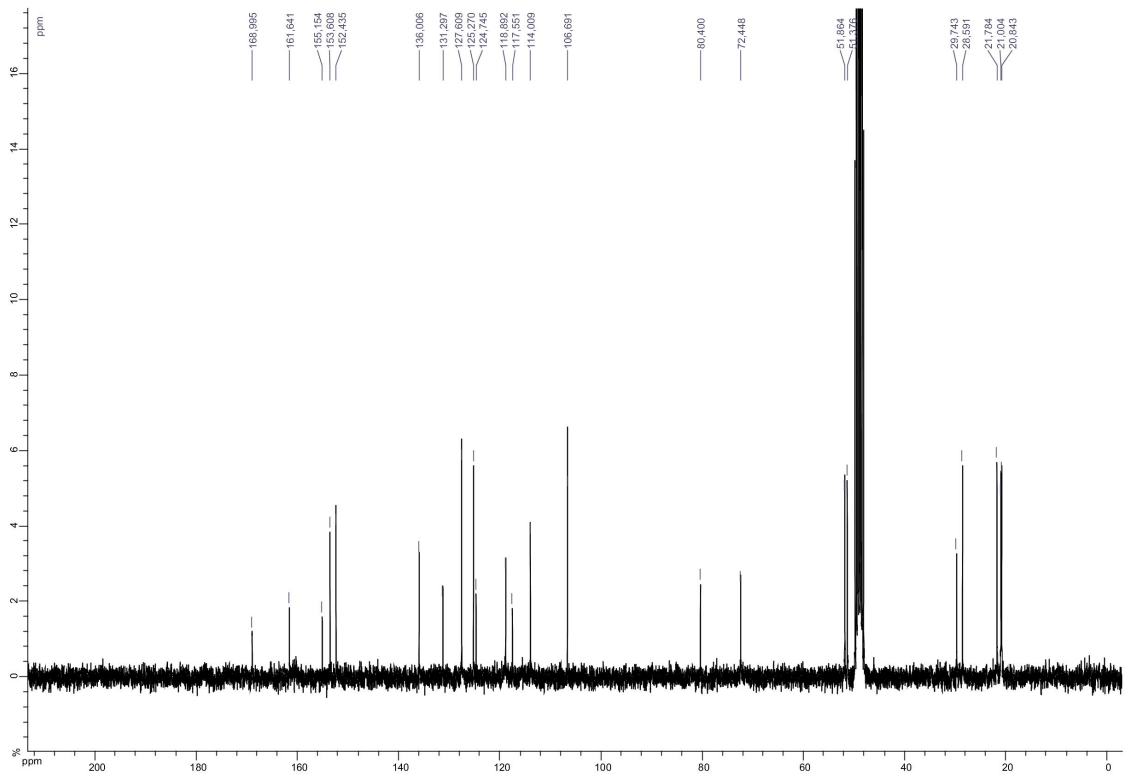
^1H NMR spectrum of **2l** (CDCl_3 , 300 MHz)



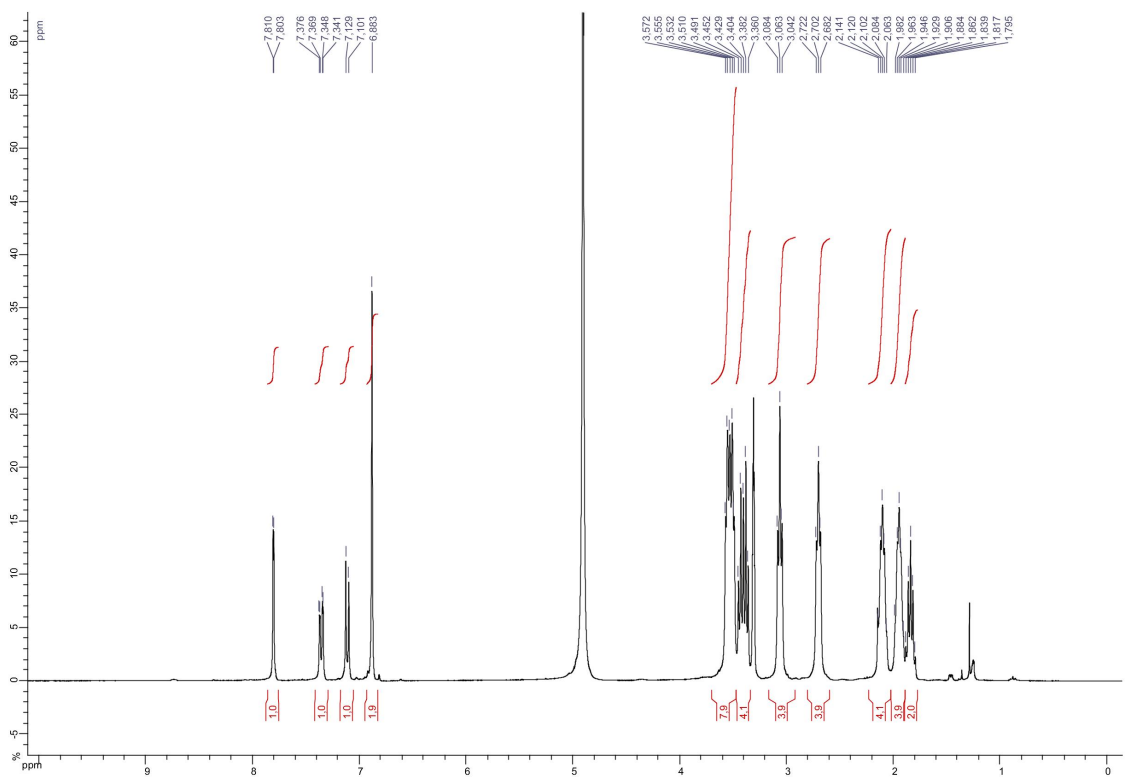
^{13}C NMR spectrum of **2l** ($\text{CDCl}_3 + \epsilon\text{CD}_3\text{OD}$, 75 MHz)



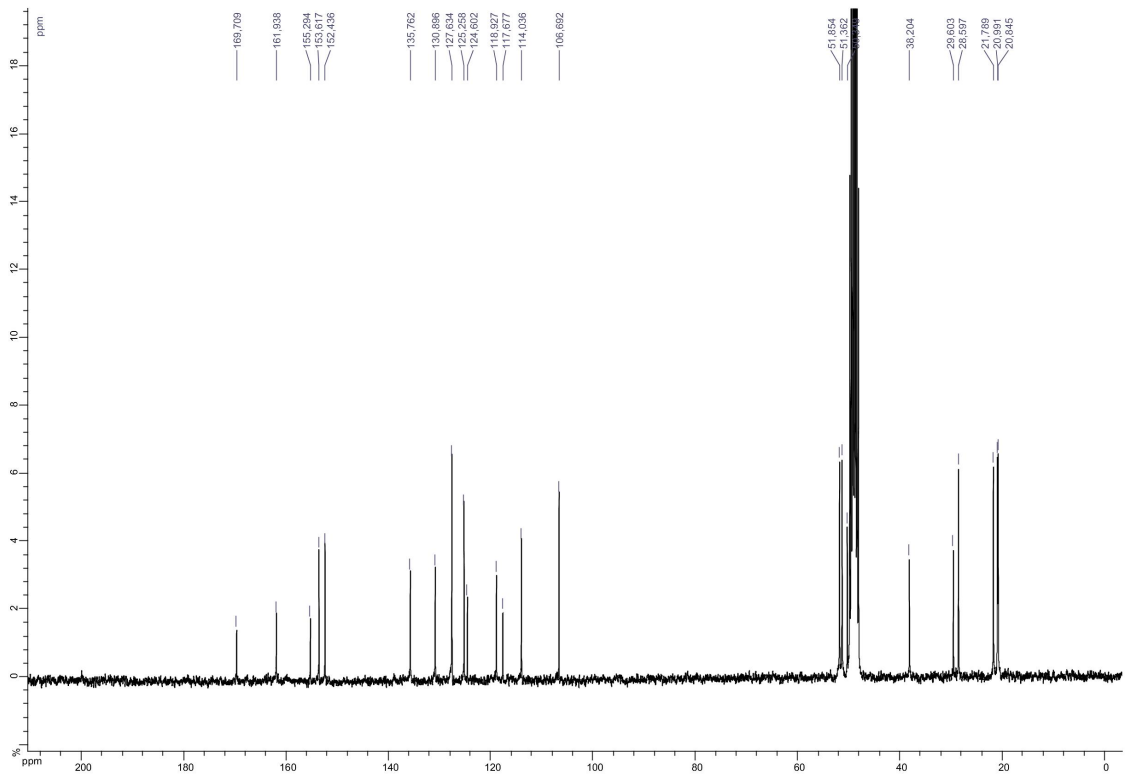
^1H NMR spectrum of **2b** (CD_3OD , 300 MHz)



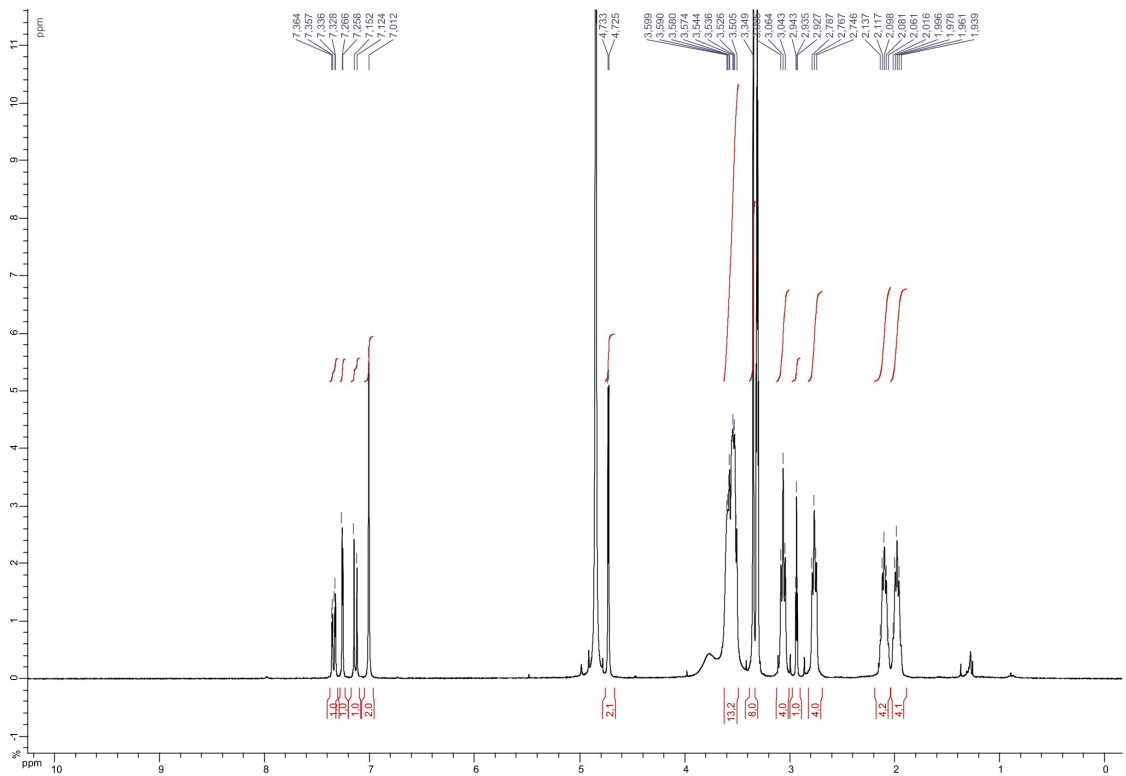
^{13}C NMR spectrum of **2b** (CD_3OD , 75 MHz)



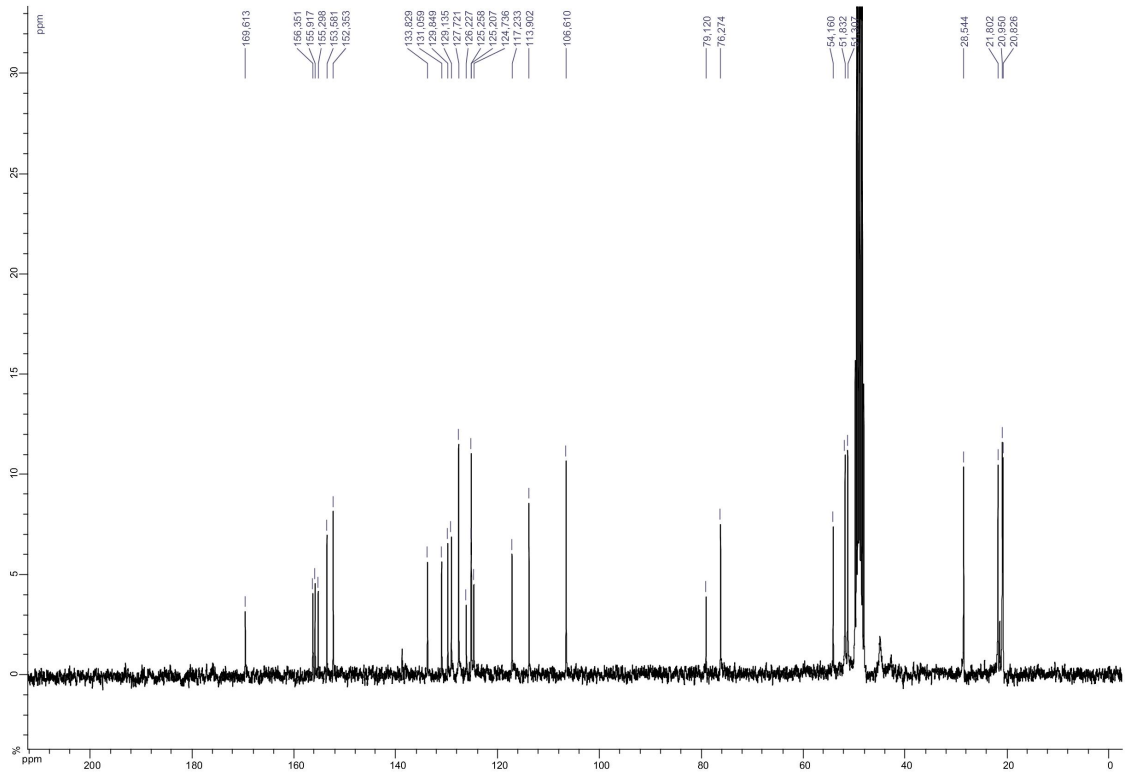
^1H NMR spectrum of **3b** (CD_3OD , 300 MHz)



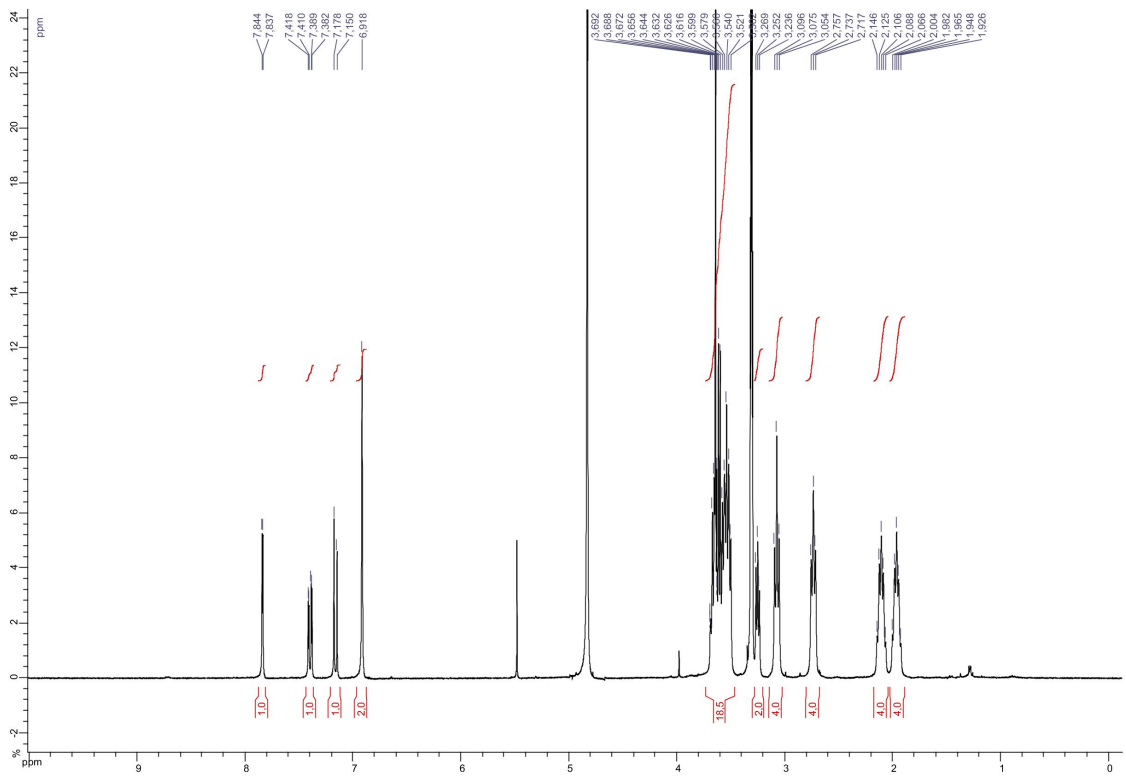
¹³C NMR spectrum of **3b** (CD₃OD, 75 MHz)



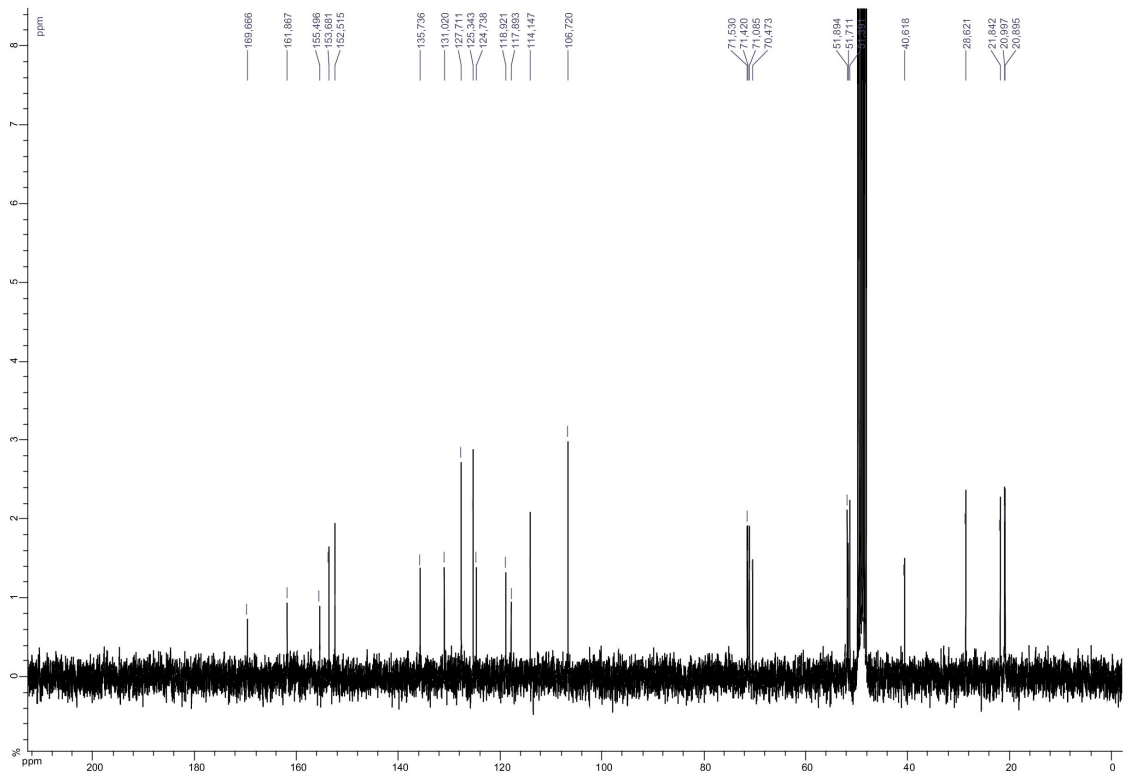
¹H NMR spectrum of **4b** (CD₃OD, 300 MHz)



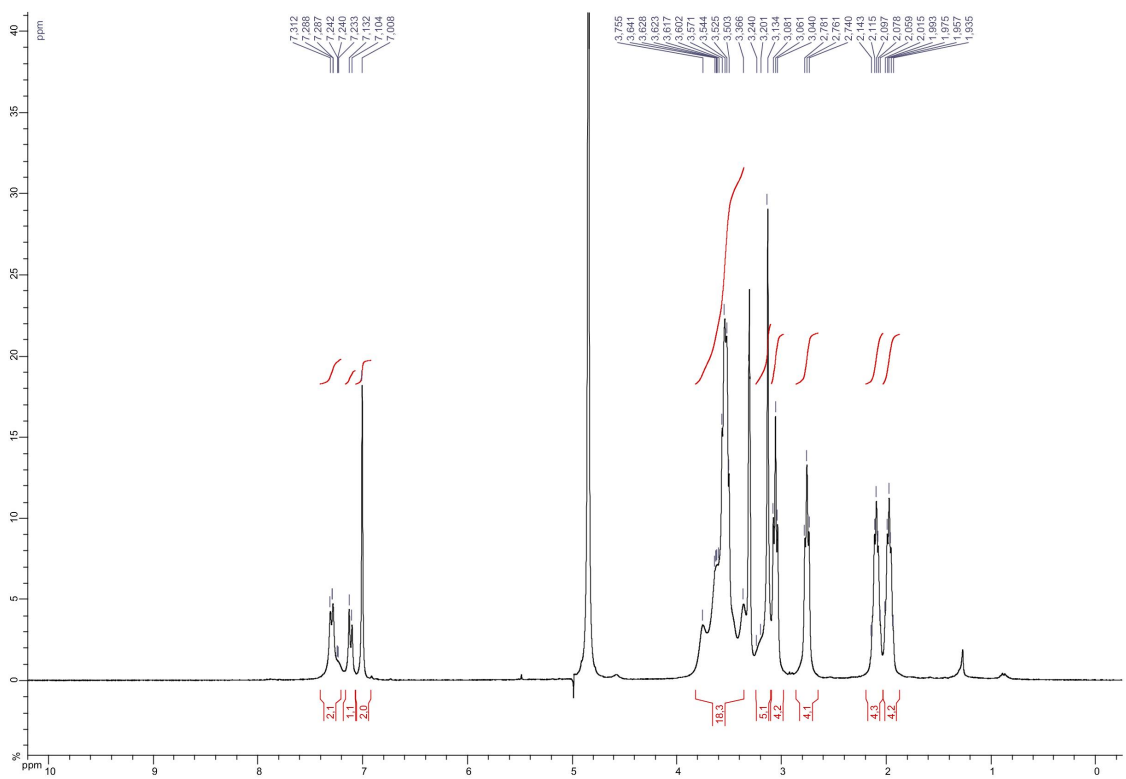
¹³C NMR spectrum of **4b** (CD₃OD, 75 MHz)



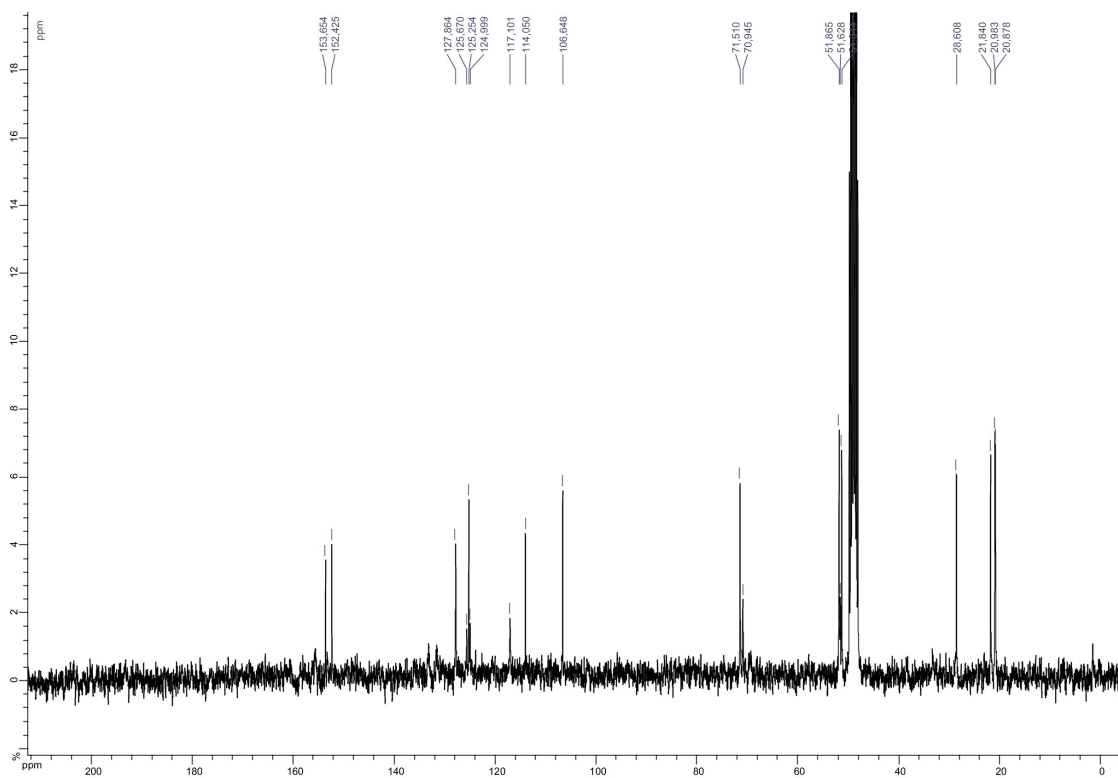
¹H NMR spectrum of **5b** (CD₃OD, 300 MHz)



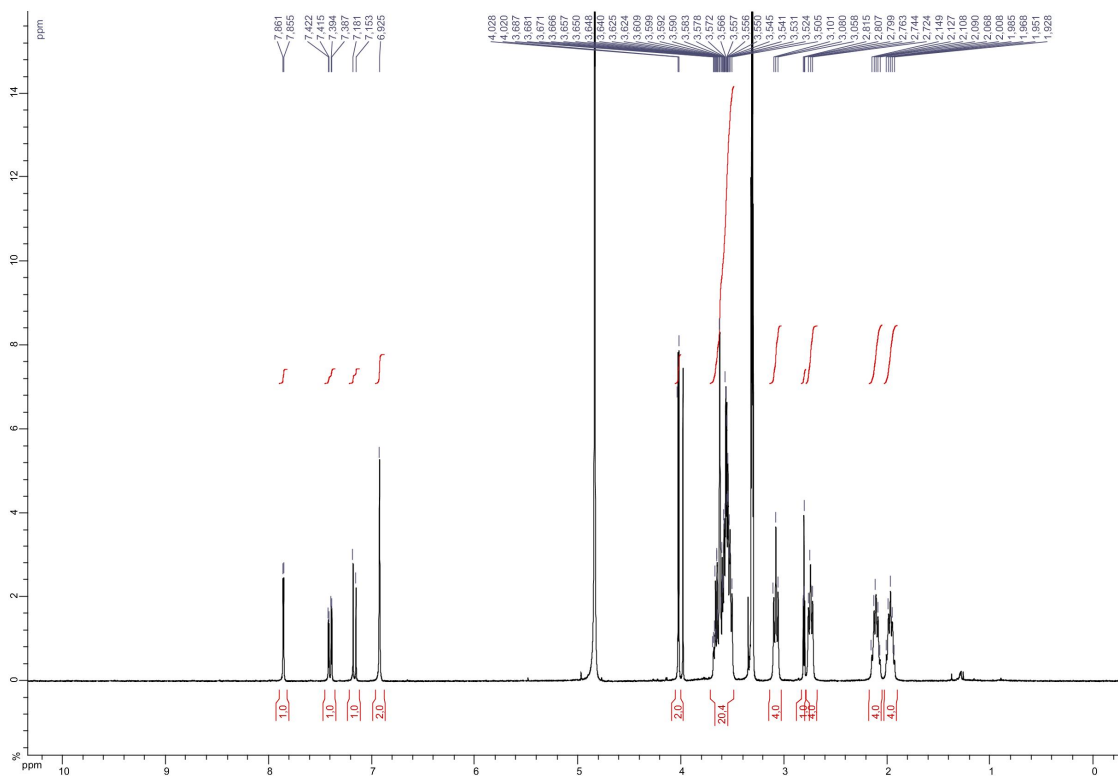
^{13}C NMR spectrum of **5b** (CD_3OD , 75 MHz)



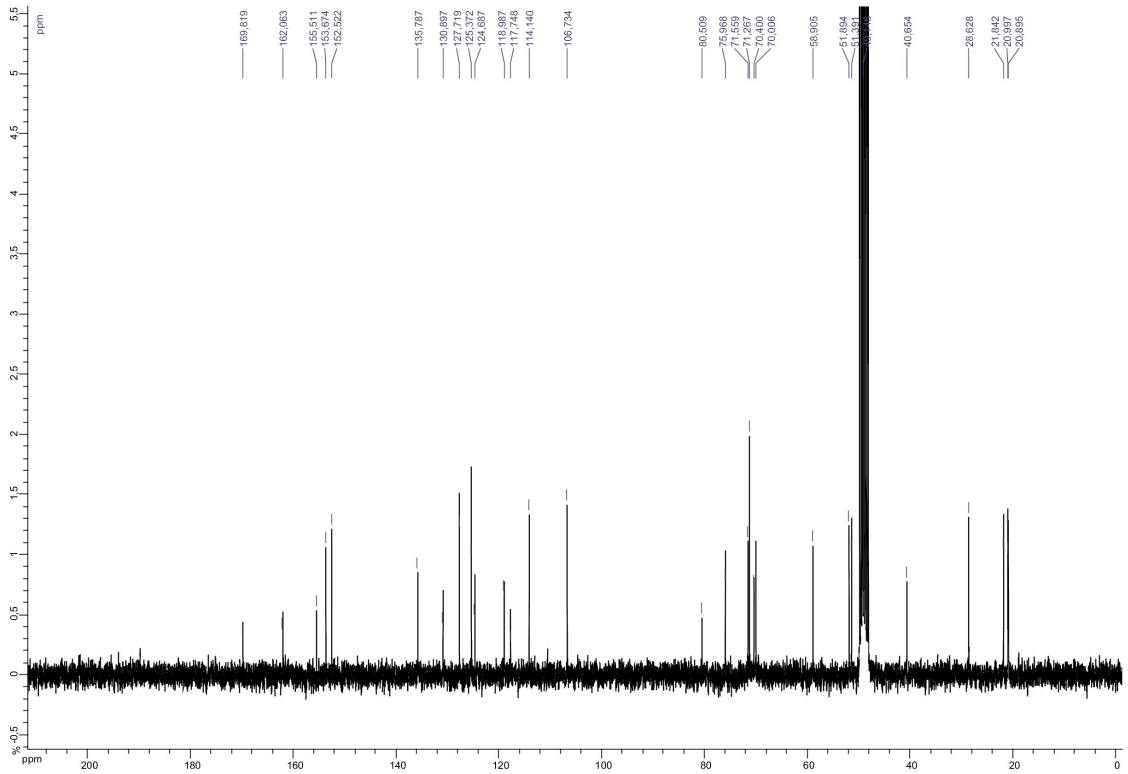
^1H NMR spectrum of **6b** (CD_3OD , 300 MHz)



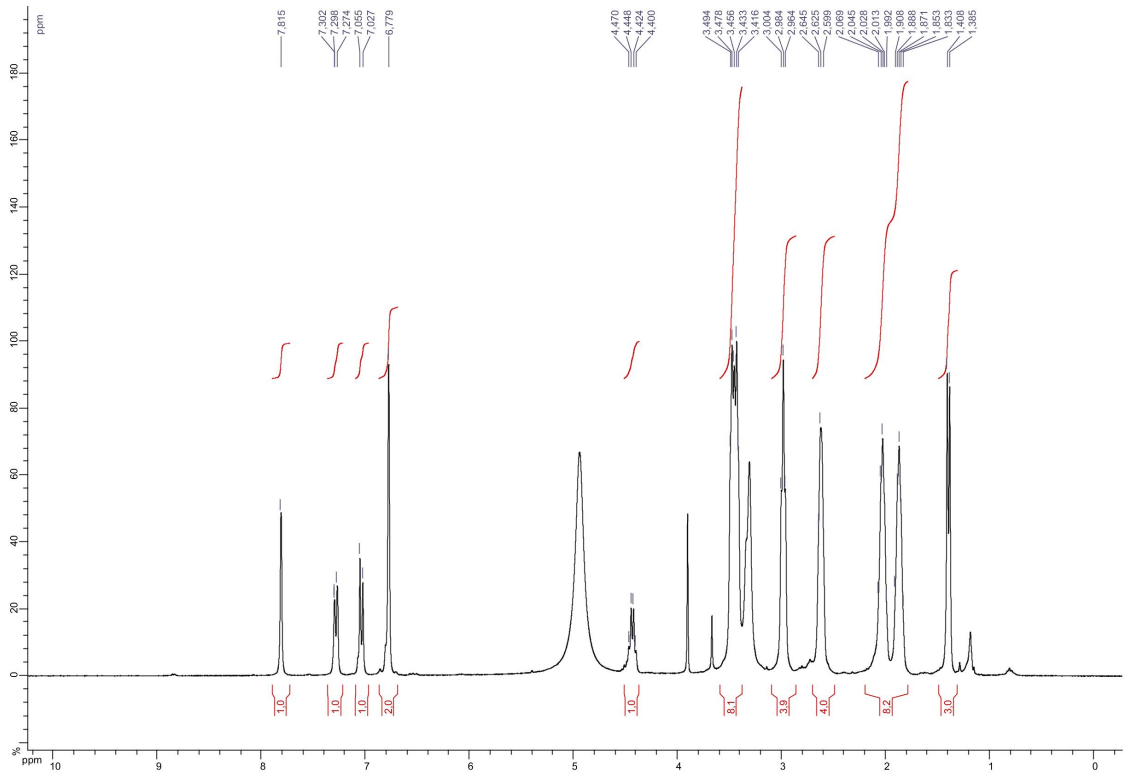
^{13}C NMR spectrum of **6b** (CD₃OD, 75 MHz)



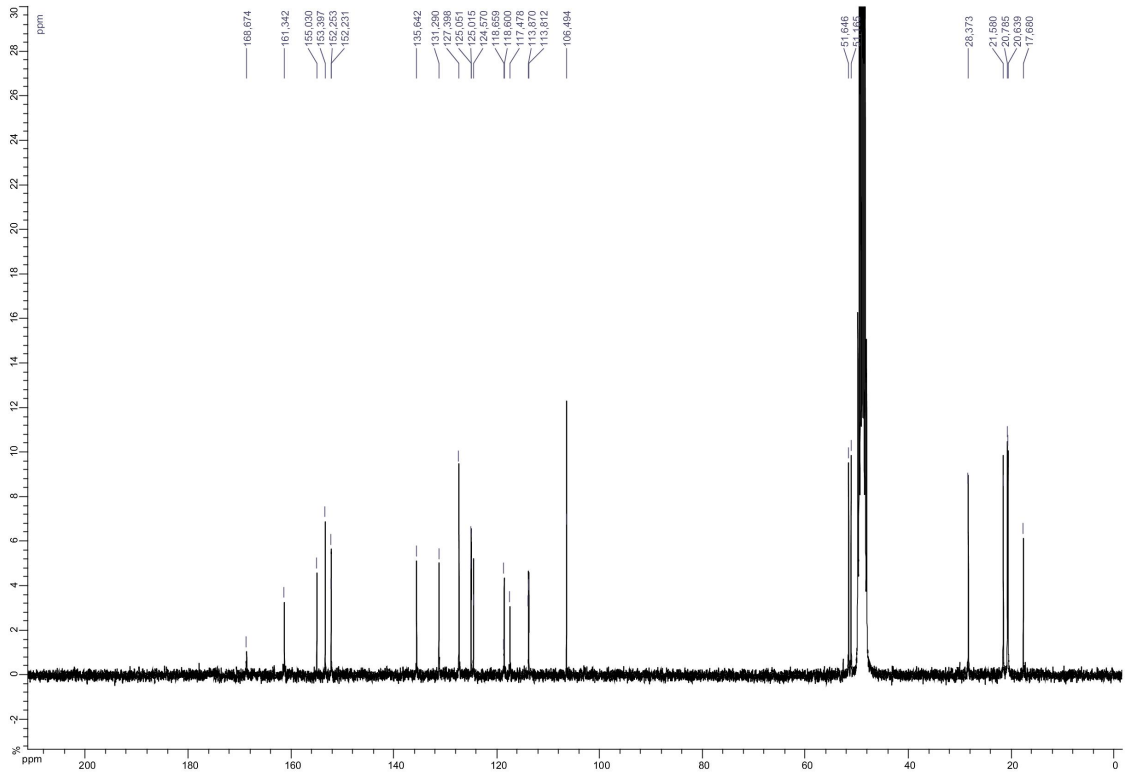
^1H NMR spectrum of **7b** (CD₃OD, 300 MHz)



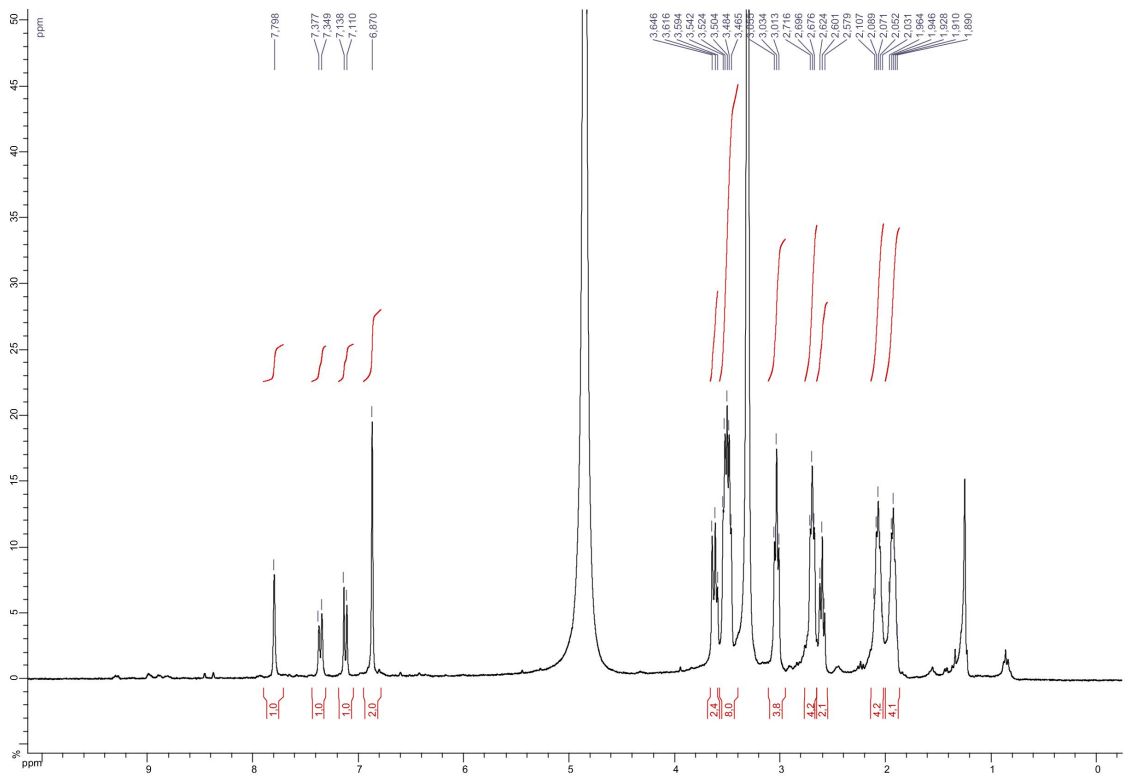
^{13}C NMR spectrum of **7b** (CD_3OD , 75 MHz)



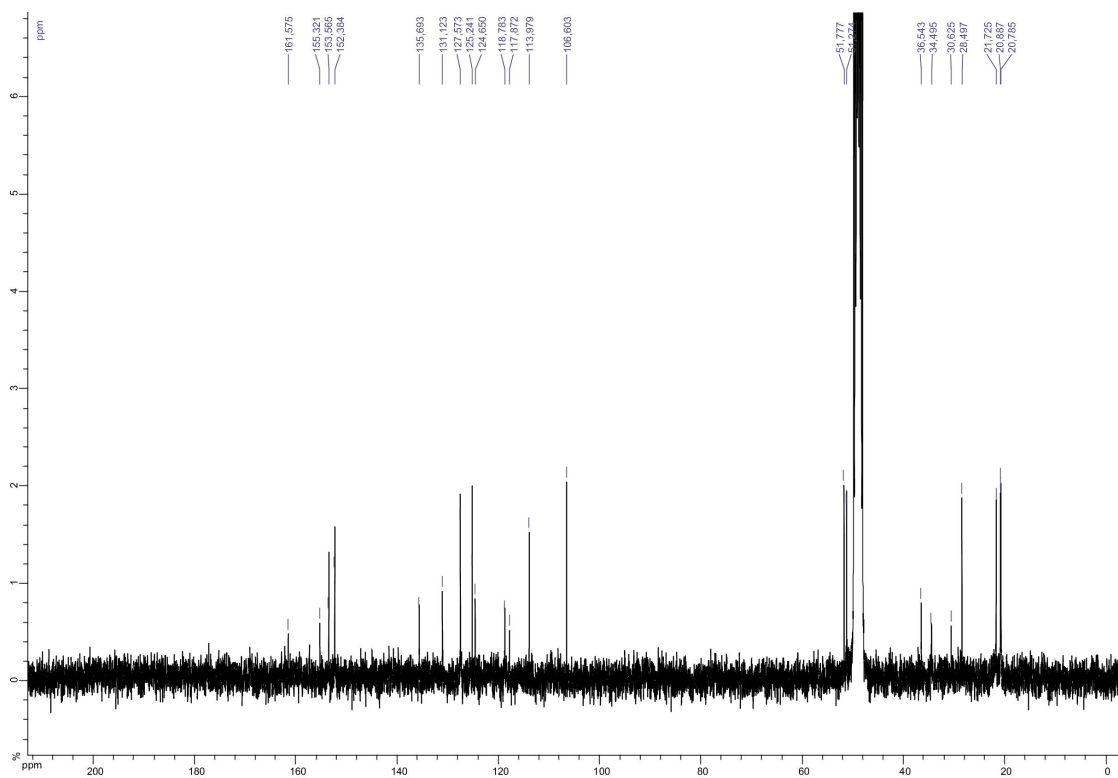
^1H NMR spectrum of **8b** (CD_3OD , 300 MHz)



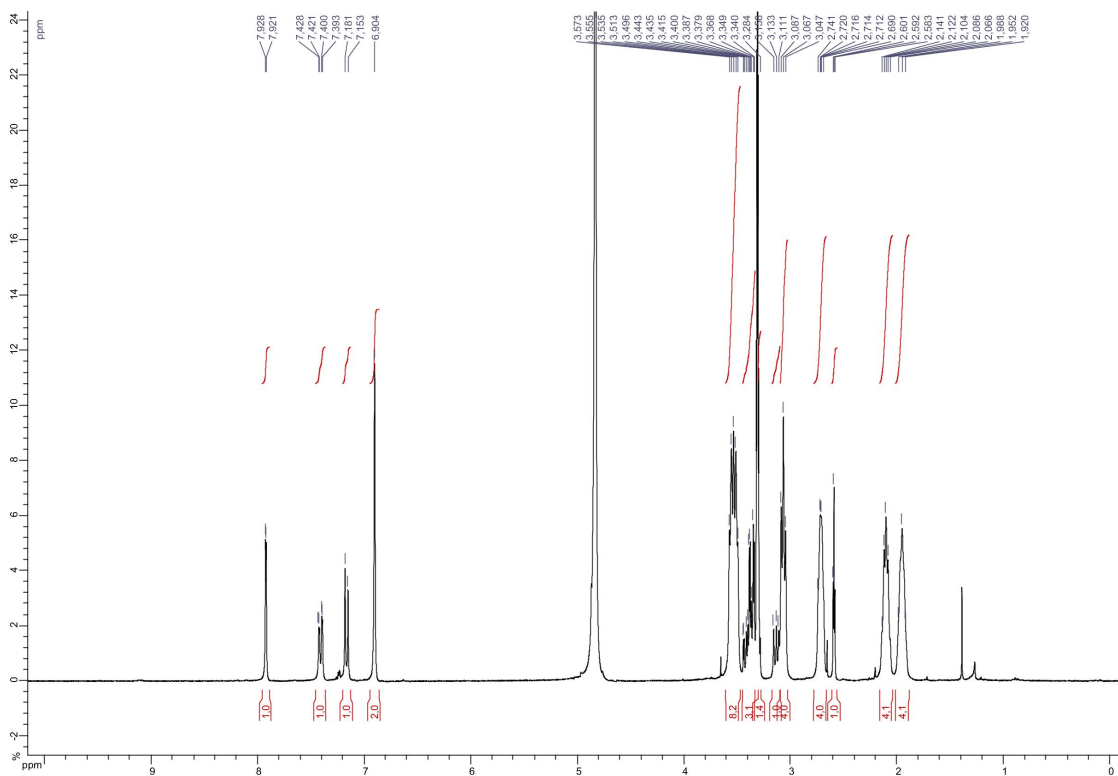
¹³C NMR spectrum of **8b** (CD₃OD, 75 MHz)



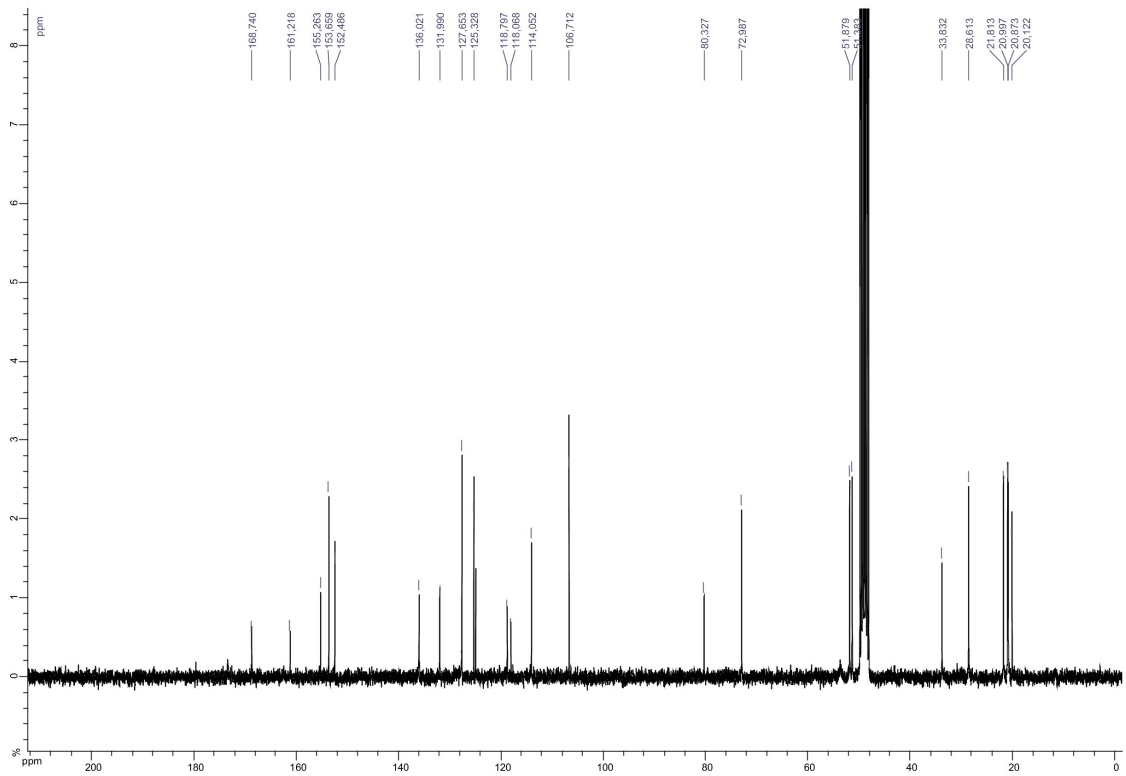
¹H NMR spectrum of **9b** (CD₃OD, 300 MHz)



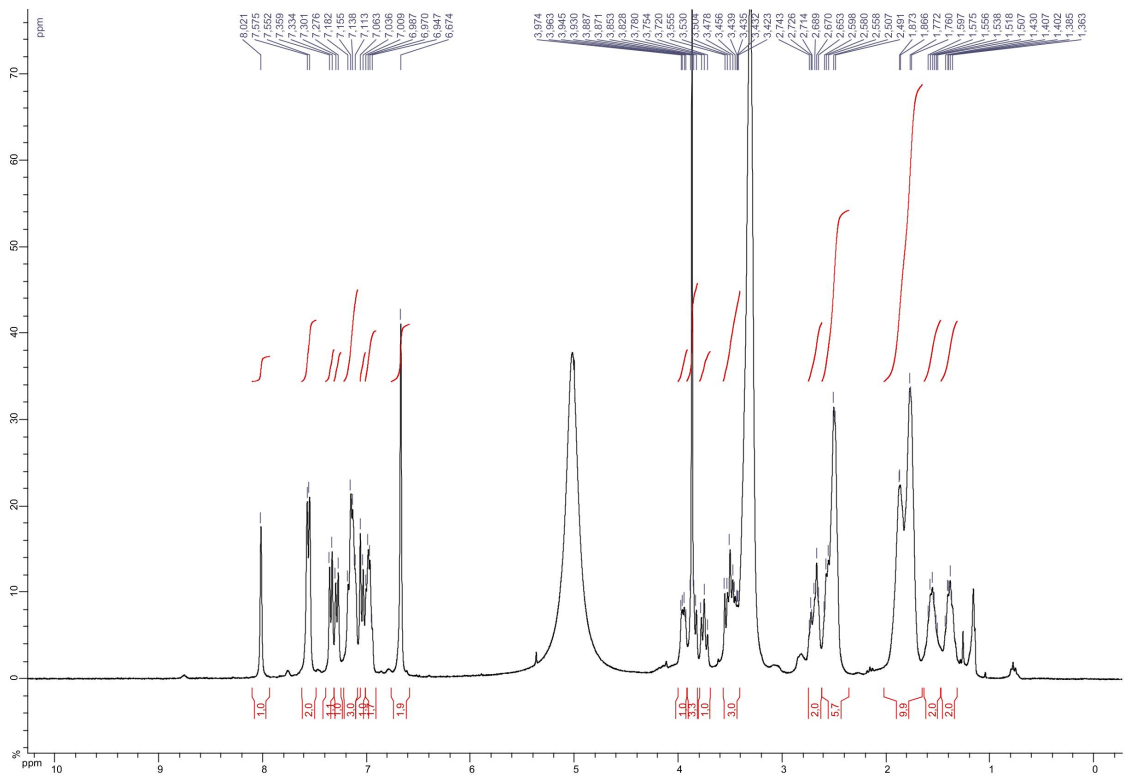
^{13}C NMR spectrum of **9b** (CD_3OD , 75 MHz)



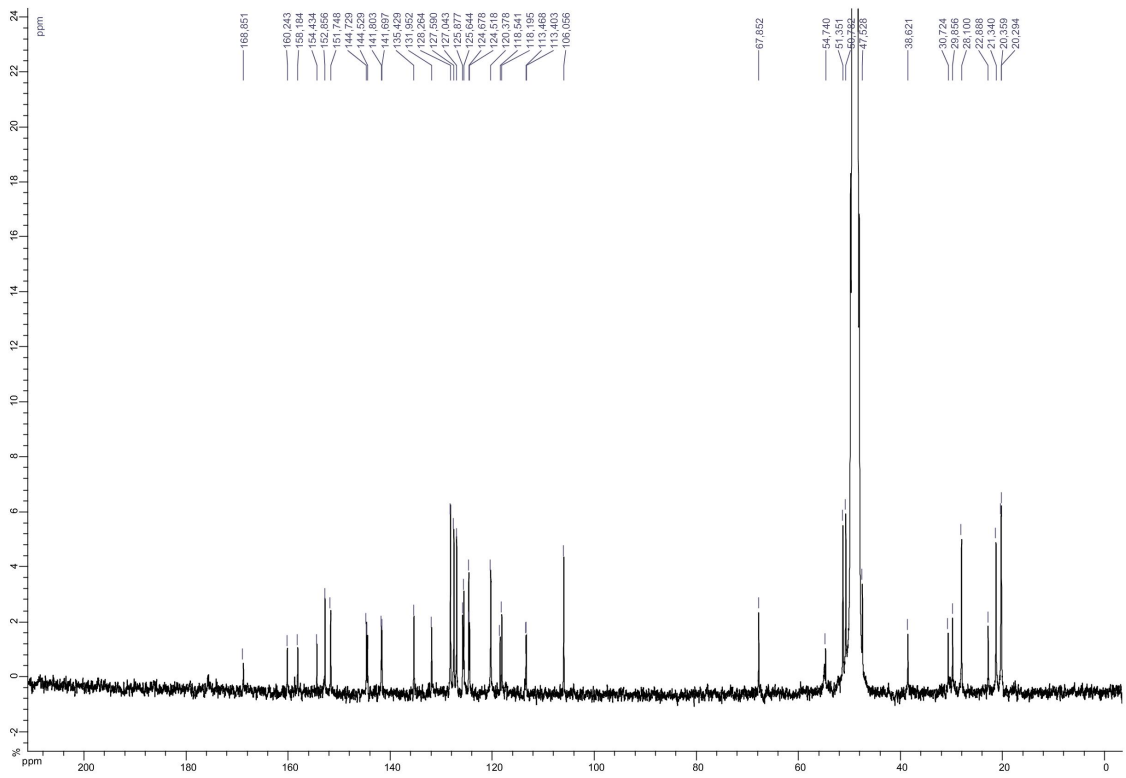
^1H NMR spectrum of **10b** (CD_3OD , 300 MHz)



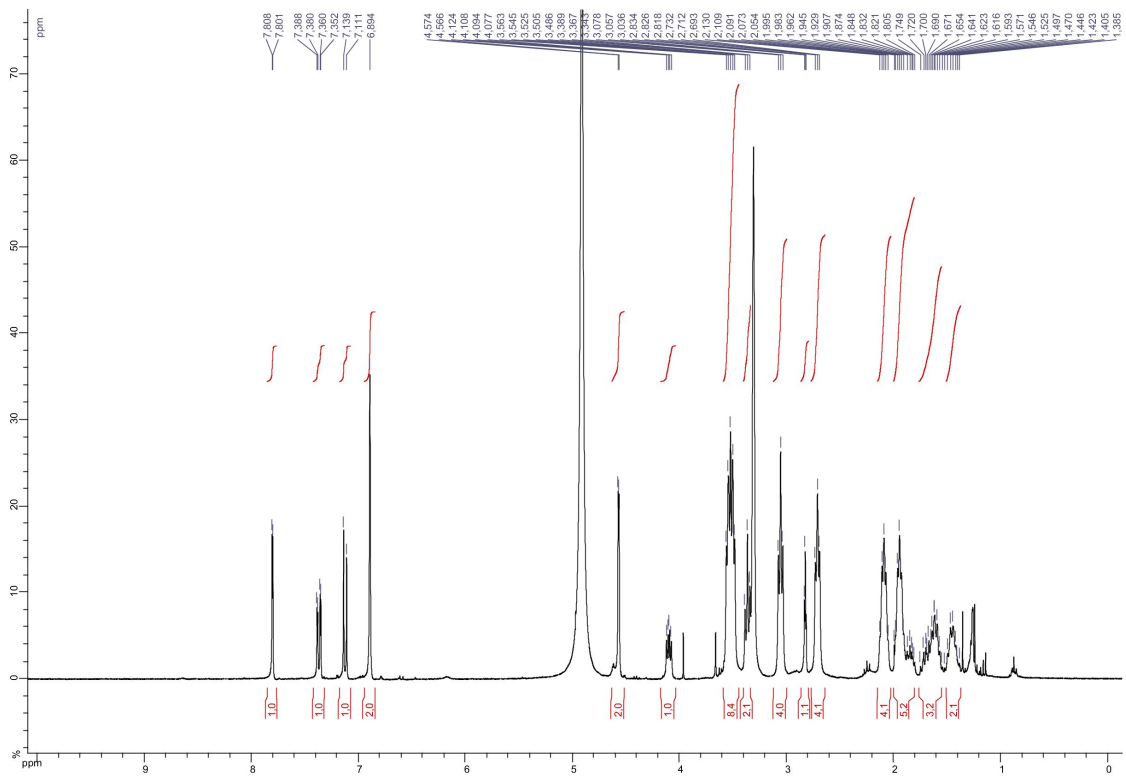
¹³C NMR spectrum of **10b** (CD₃OD, 75 MHz)



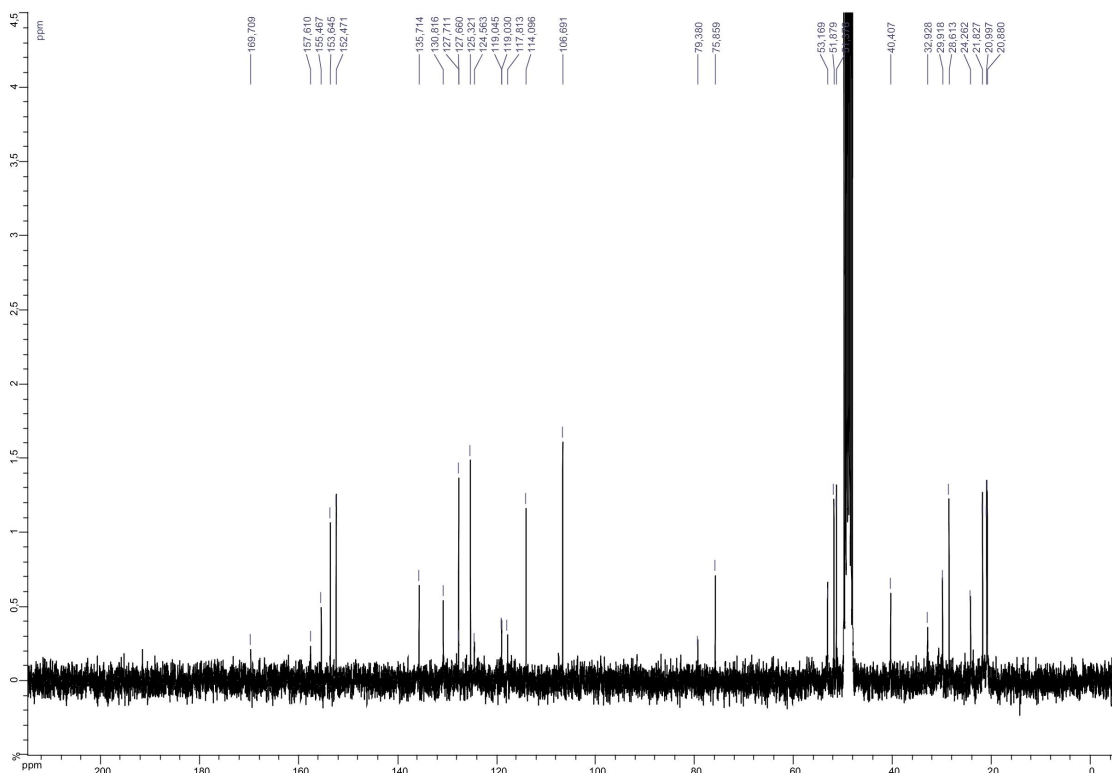
¹H NMR spectrum of **11b** (CD₃OD, 300 MHz)



¹³C NMR spectrum of **11b** (CD₃OD, 75 MHz)



¹H NMR spectrum of **12b** (CD₃OD, 300 MHz)



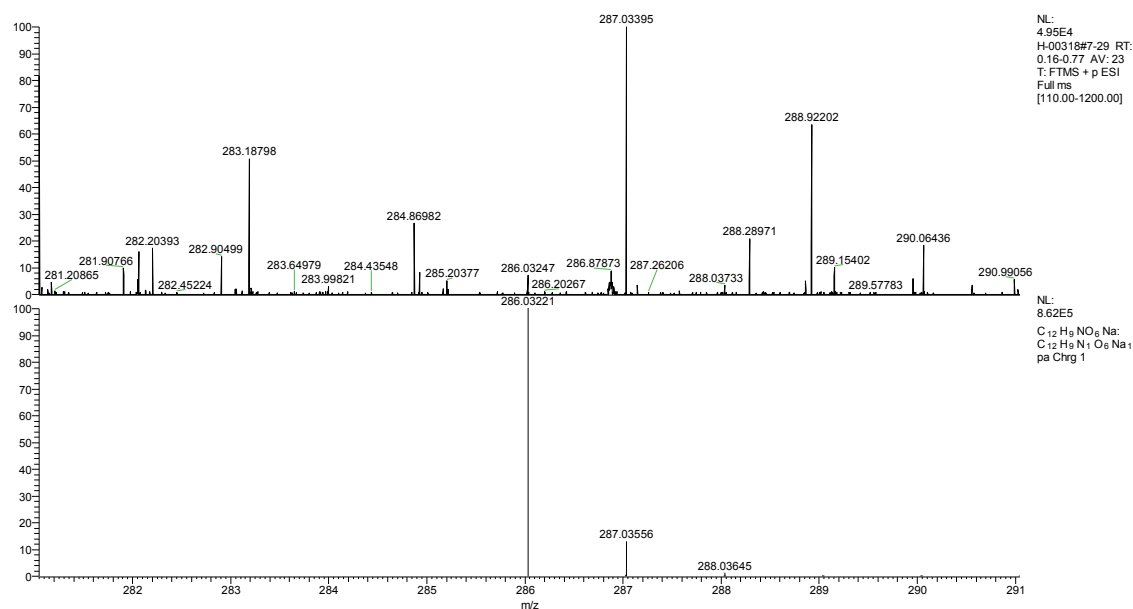
^{13}C NMR spectrum of **12b** (CD_3OD , 75 MHz)

HRMS spectra of functionalisable H-Rubies and their intermediates

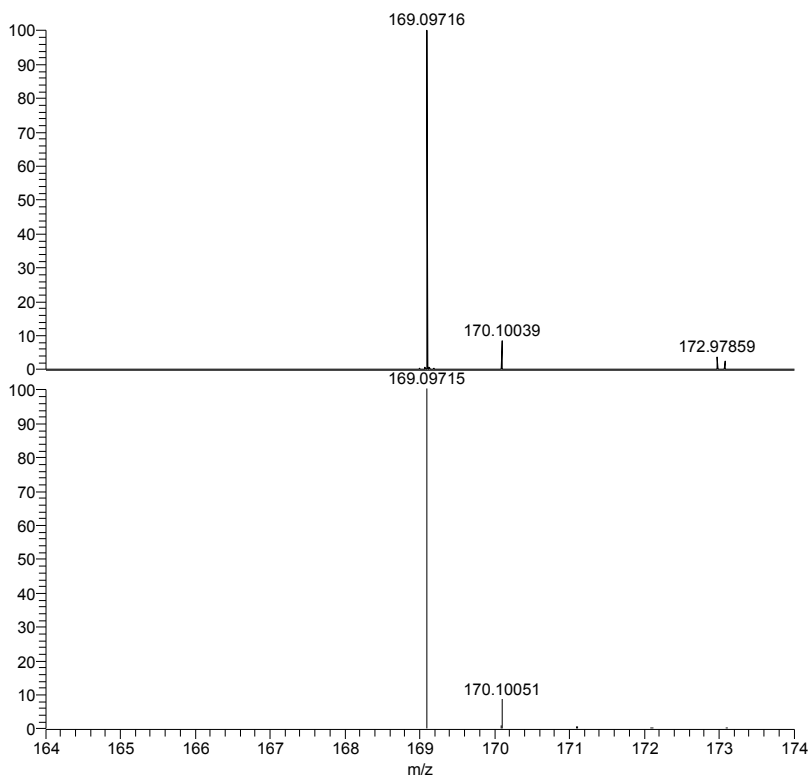
C:\Xcalibur\data\Analyses\H-00318
MeCN

11/25/2014 3:59:21 PM

DESPRAS Guillaume GD1-29



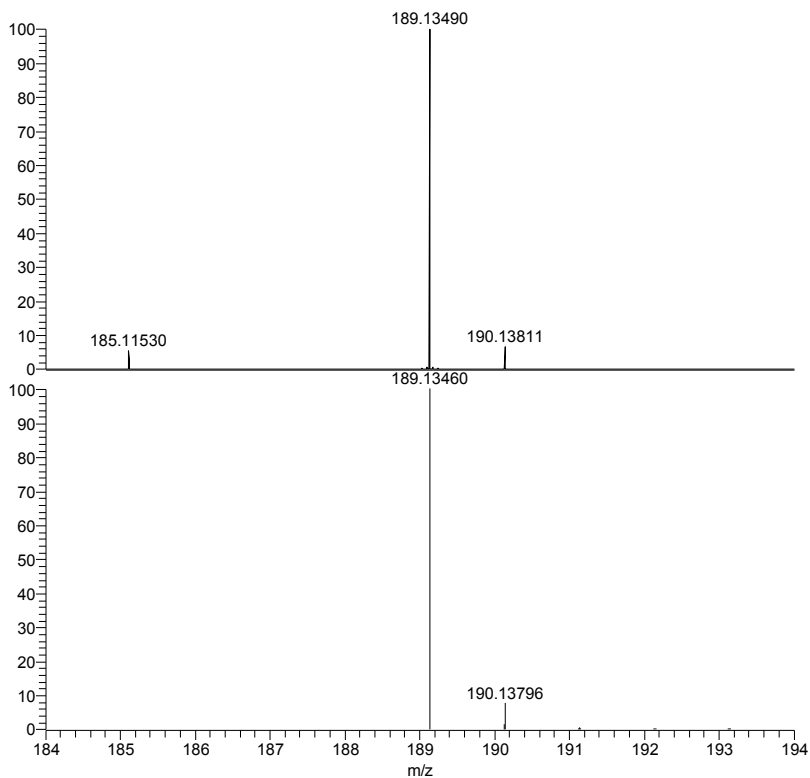
HRMS spectrum of **1**



NL:
7.31E7
H-00326#7-29 RT:
0.16-0.77 AV: 23
T: FTMS + p ESI
Full ms
[110.00-1000.00]

NL:
9.05E5
C₈ H₁₃ N₂ O₂:
C₈ H₁₃ N₂ O₂
pa Chrg 1

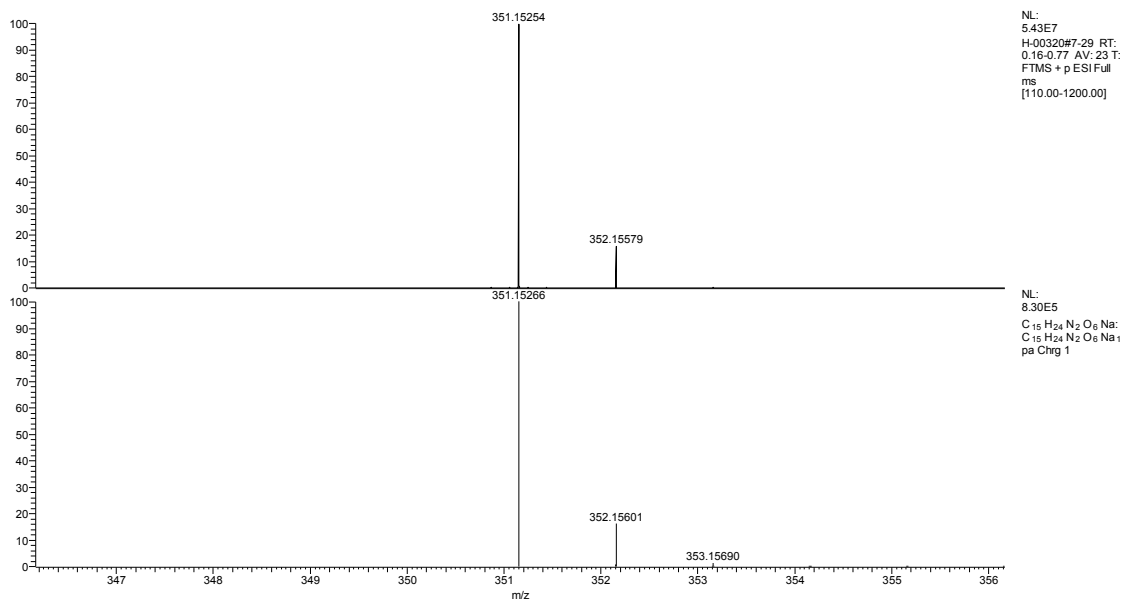
HRMS spectrum of 4-((prop-2-yn-1-yloxy)carbonyl)piperazin-1-ium



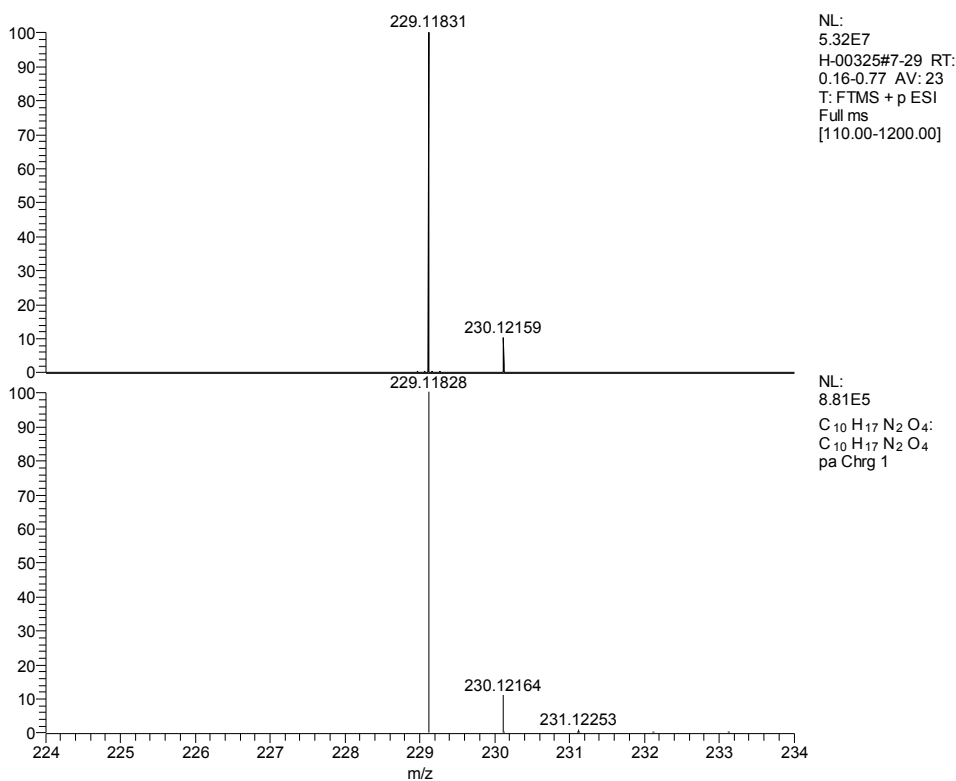
NL:
5.94E7
H-00321#21-25
RT: 0.55-0.66 AV:
5 T: FTMS + p ESI
Full ms
[110.00-1000.00]

NL:
9.08E5
C₇ H₁₆ N₄ O₂ H:
C₇ H₁₇ N₄ O₂
pa Chrg 1

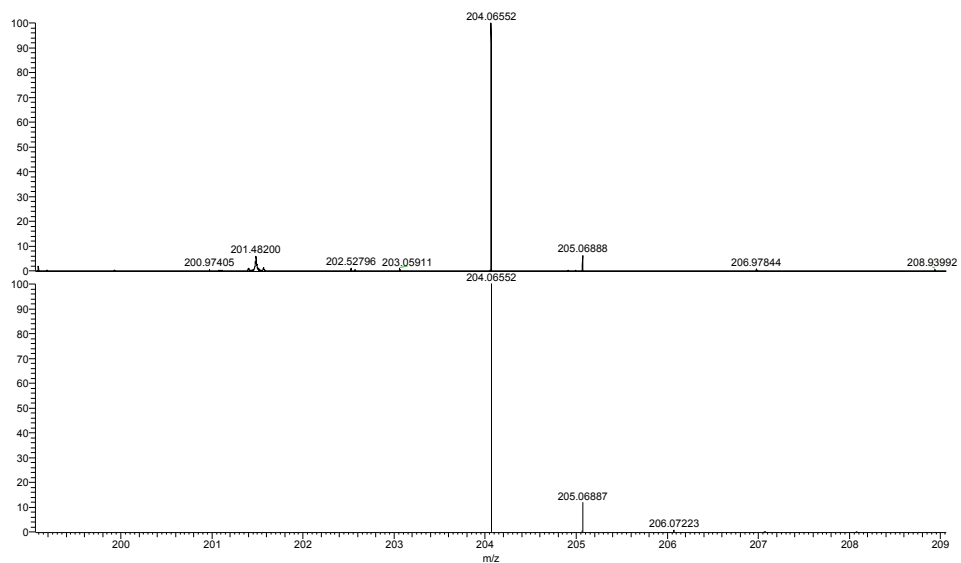
HRMS spectrum of 2-(2-(2-azidoethoxy)ethoxy)-N-methylethanamine



HRMS spectrum of 6-((tert-butoxycarbonyl)amino)-2-(((prop-2-yn-1-yloxy)carbonyl)amino)hexanoic acid



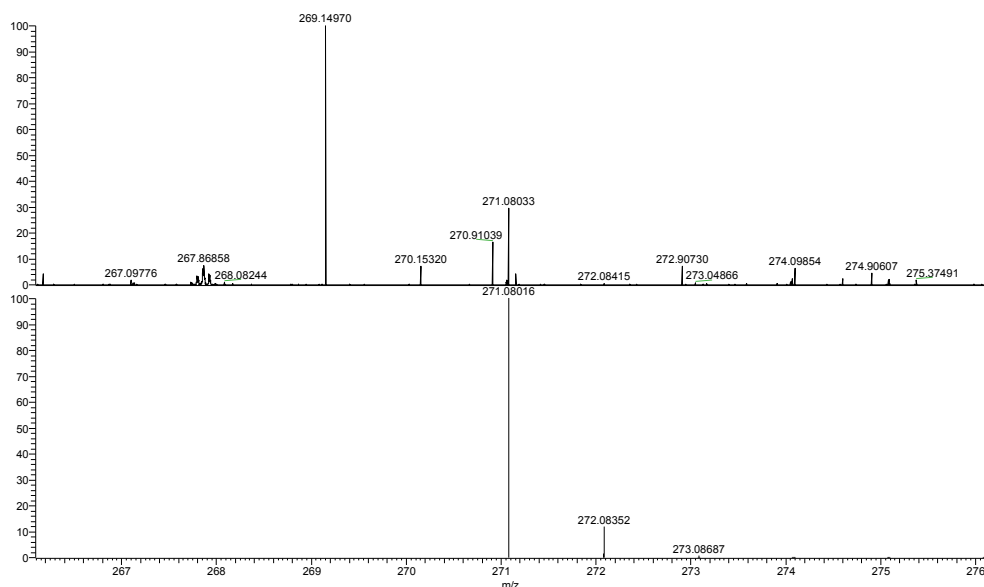
HRMS spectrum of 5-carboxy-5-(((prop-2-yn-1-yloxy)carbonyl)amino)pentan-1-aminium



NL:
1.15E6
H-00317#7-29 RT:
0.16-0.77 AV: 23
T: FTMS + p ESI
Full.ms
[110.00-1200.00]

NL:
8.78E5
C₁₁H₉NO₃H
C₁₁H₁₀N₁O₃
pa Chrg 1

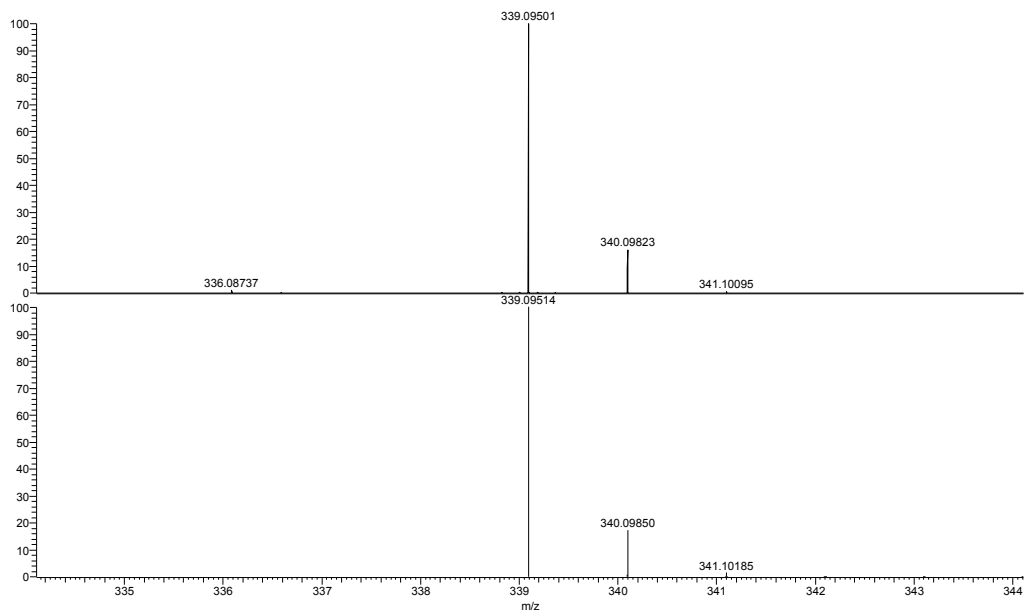
HRMS spectrum of 2a



NL:
7.60E5
H-00386#7-29 RT:
0.15-0.76 AV: 23 T:
FTMS + p ESI Full
ms
[110.00-1200.00]

NL:
8.68E5
C₁₁H₁₂N₄O₃Na
C₁₁H₁₂N₄O₃Na₁
pa Chrg 1

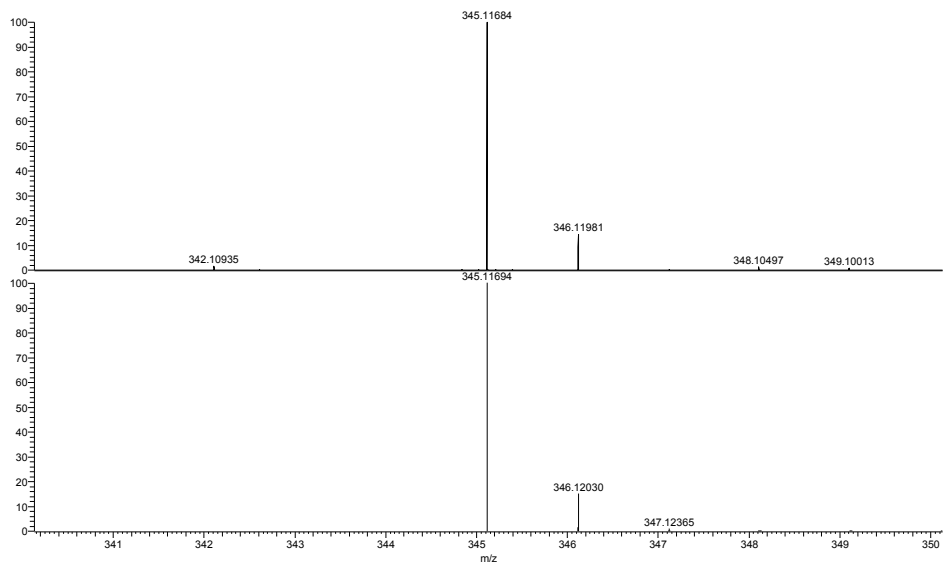
HRMS spectrum of 2b



NL:
3.98E7
H-00323#7-29 RT:
0.16-0.77 AV: 23 T:
FTMS + p ESI Full
ms
[110.00-1200.00]

NL:
8.24E5
C₁₆H₁₆N₂O₅Na:
C₁₆H₁₆N₂O₅Na:
pa Chrg 1

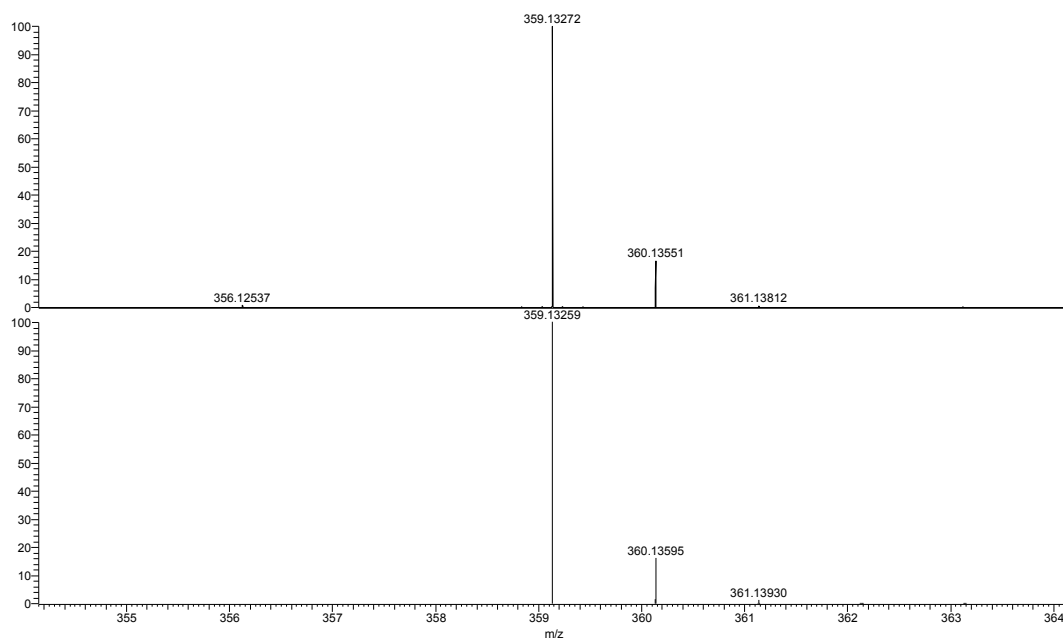
HRMS spectrum of 2d



NL:
6.22E7
H-00324#7-29 RT:
0.16-0.77 AV: 23 T:
FTMS + p ESI Full
ms
[110.00-1200.00]

NL:
8.36E5
C₁₄H₁₆N₄O₅Na:
C₁₄H₁₆N₄O₅Na:
pa Chrg 1

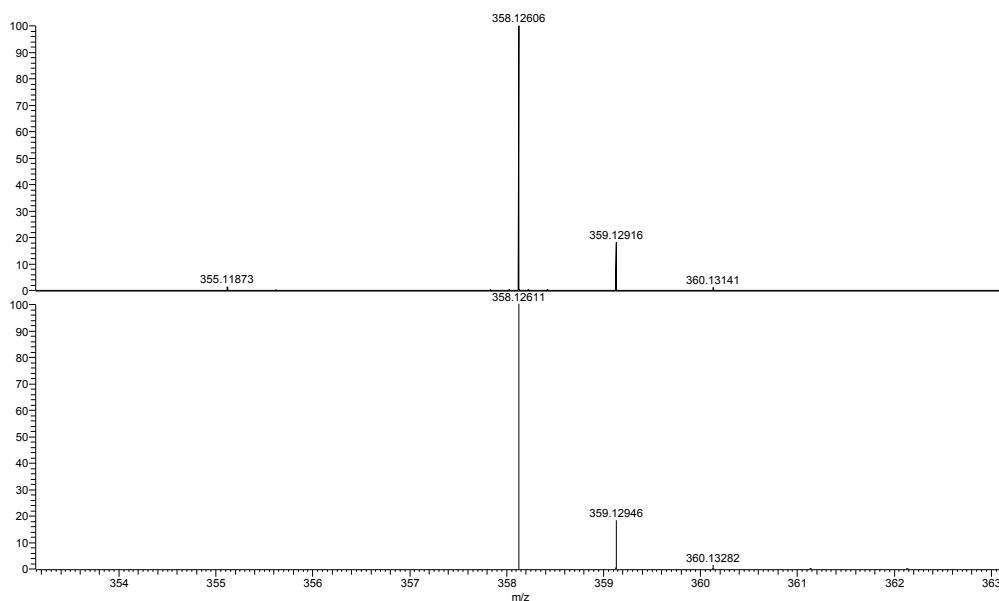
HRMS spectrum of 2e



NL:
9.83E7
H-00322#7-29 RT:
0.16-0.77 AV: 23 T:
FTMS + p ESI Full
ms
[110.00-1200.00]

NL:
8.26E5
C₁₅H₂₀N₄O₅Na:
C₁₅H₂₀N₄O₅Na:
pa Chrg 1

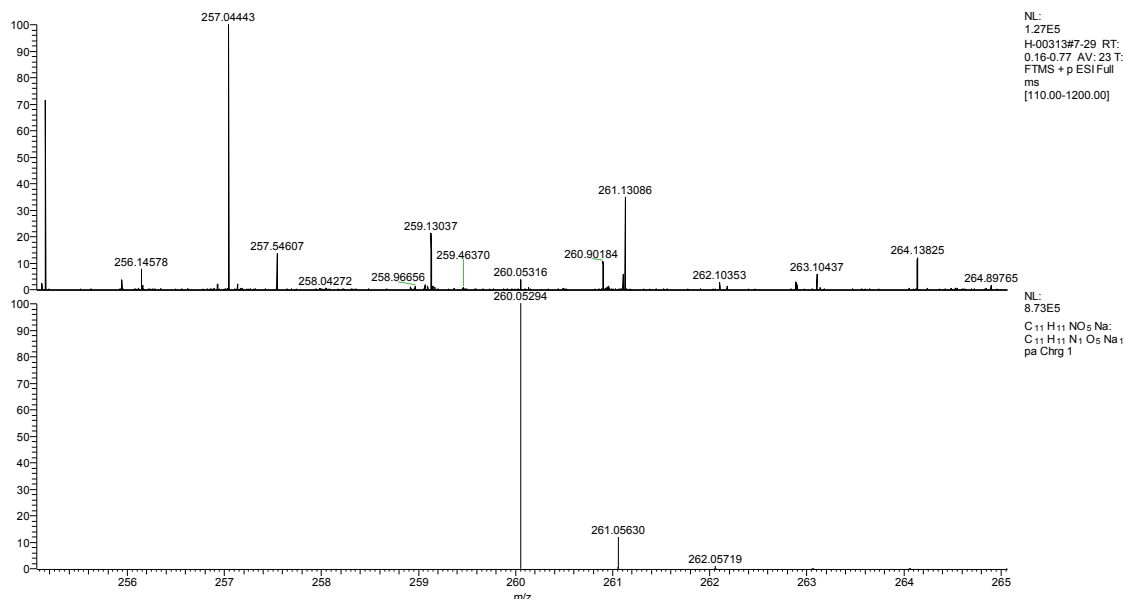
HRMS spectrum of 2f



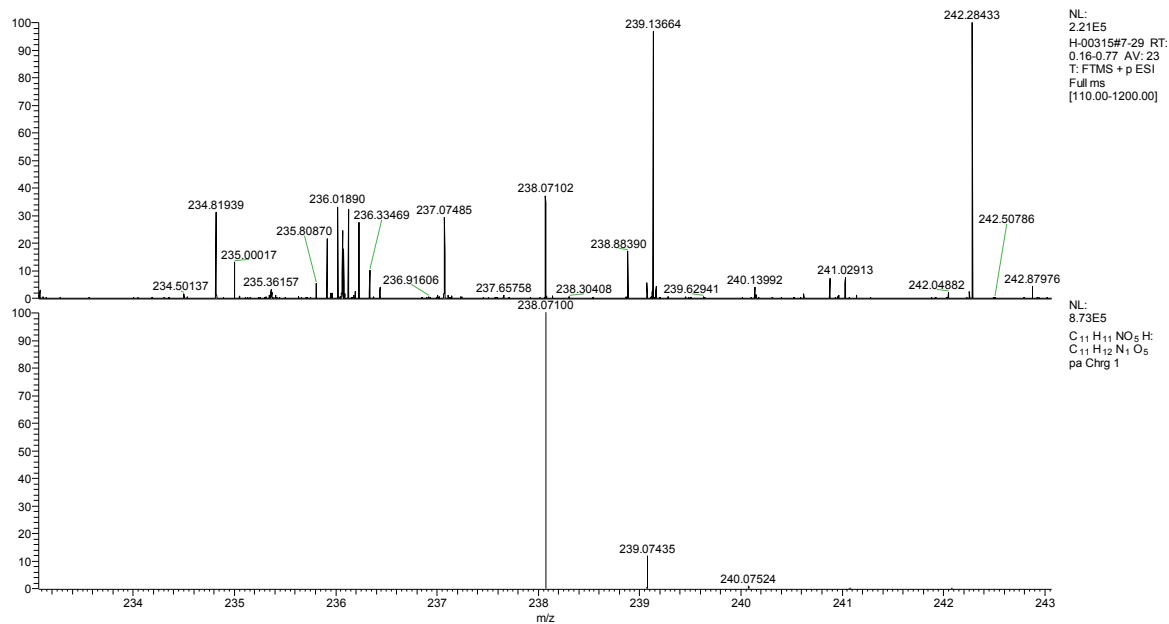
NL:
1.58E8
H-00319#7-29 RT:
0.16-0.77 AV: 23 T:
FTMS + p ESI Full
ms
[110.00-1200.00]

NL:
8.16E5
C₁₇H₂₁NO₅Na:
C₁₇H₂₁N₁O₅Na:
pa Chrg 1

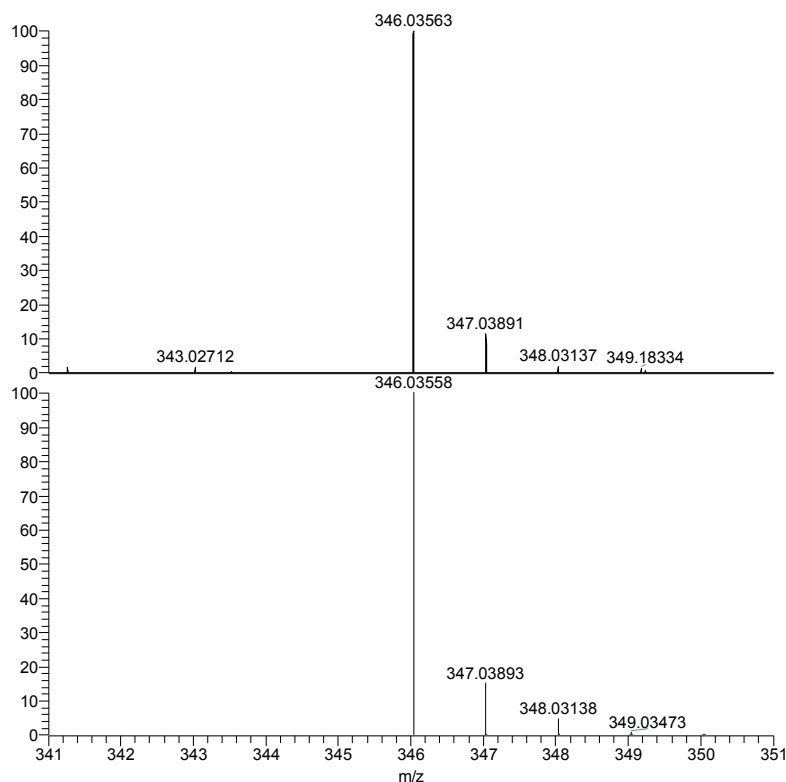
HRMS spectrum of 2g



HRMS spectrum of **2h**



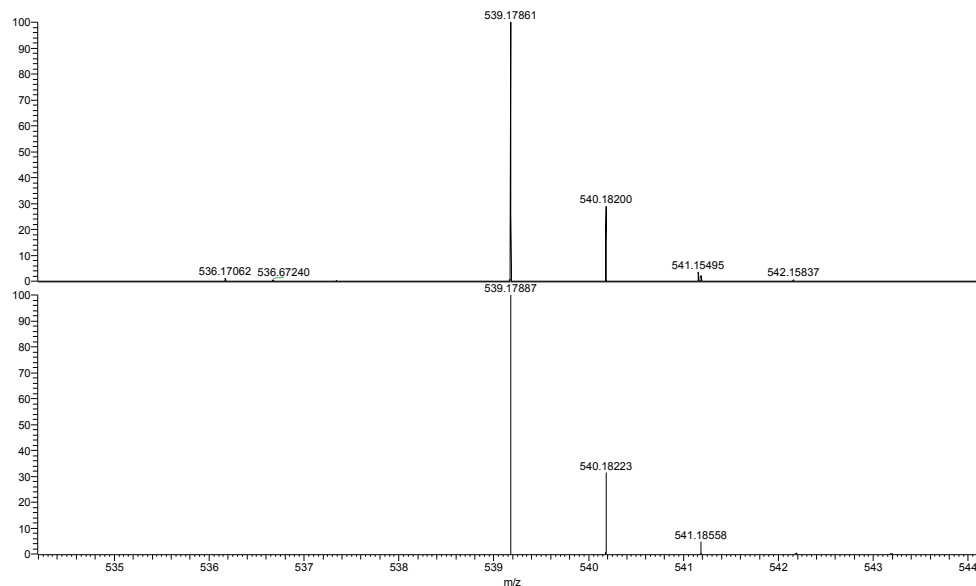
HRMS spectrum of **2i**



NL:
1.85E6
H-00314b#14-21 RT:
0.35-0.55 AV: 8 T:
FTMS + p ESI Full ms
[110.00-1200.00]

NL:
8.01E5
C₁₄H₁₃NO₆SNa:
C₁₄H₁₃N₁O₆S₁Na₁
pa Chrg 1

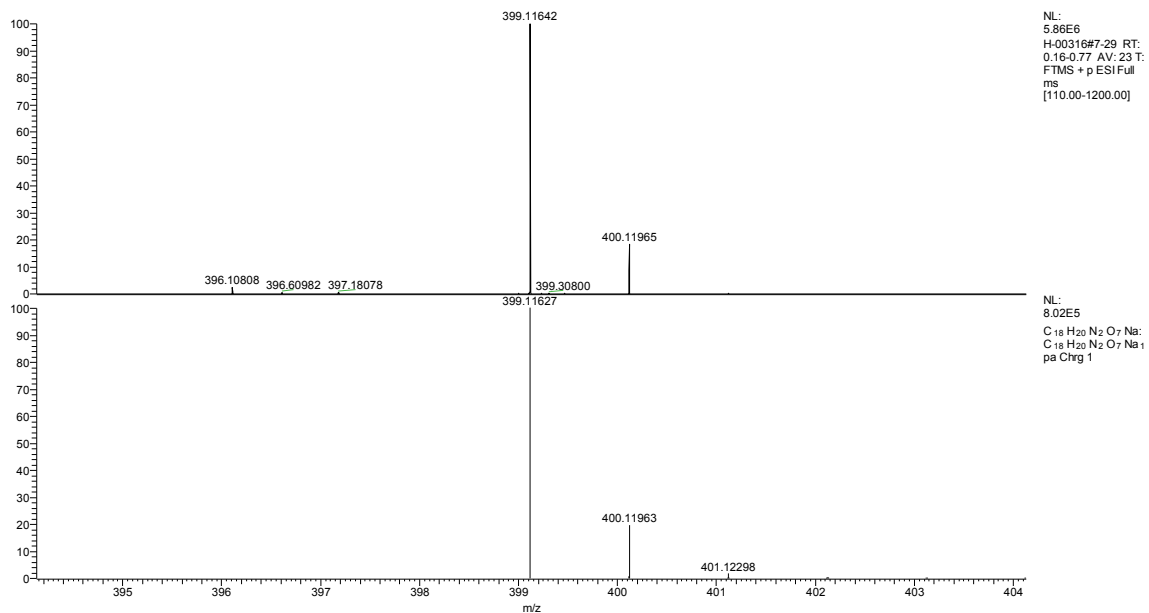
HRMS spectrum of 2j (sulfoxyde)



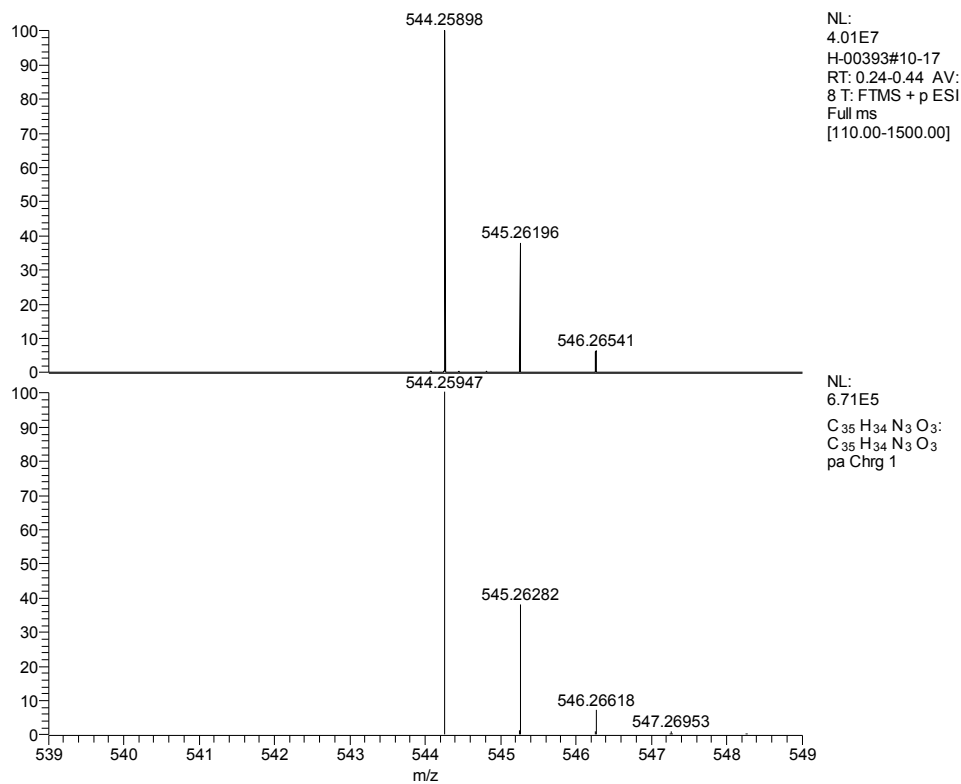
NL:
3.05E6
H-00384#7-29 RT:
0.16-0.77 AV: 23 T:
FTMS + p ESI Full
ms
[110.00-1500.00]

NL:
7.12E5
C₂₉H₂₈N₂O₇Na:
C₂₉H₂₈N₂O₇Na₁
pa Chrg 1

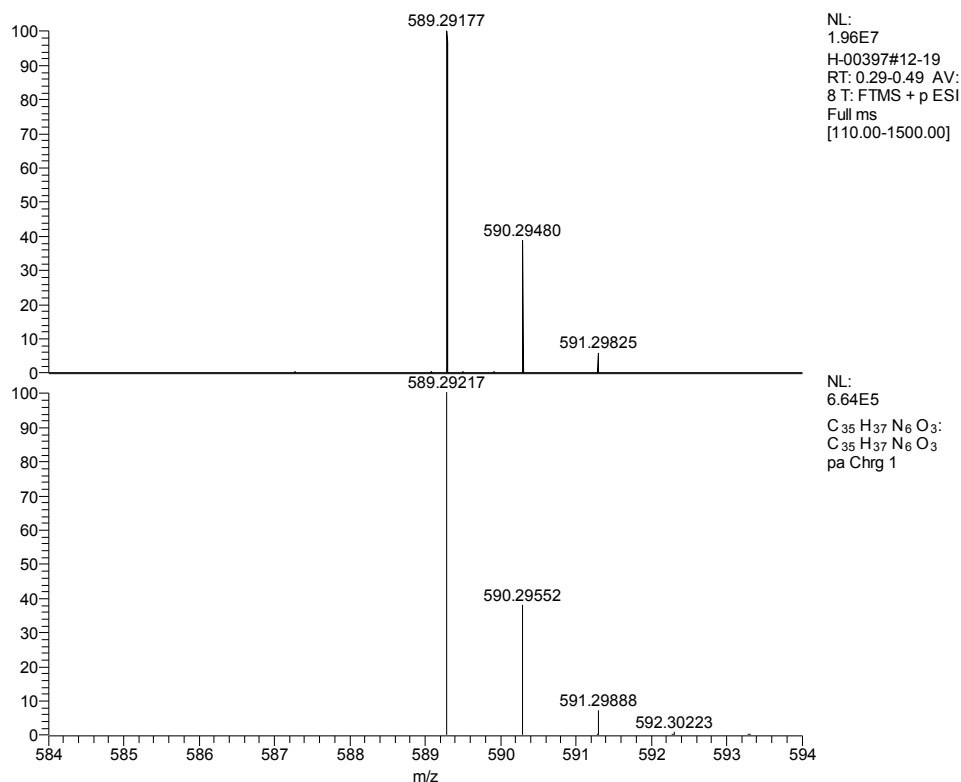
HRMS spectrum of 2k



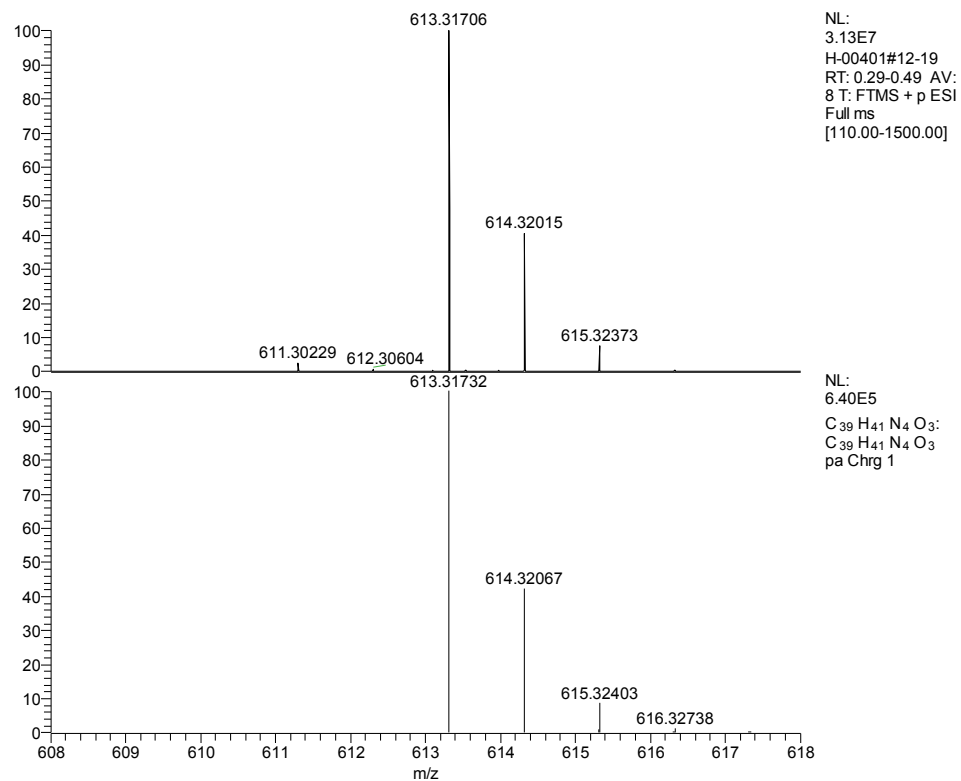
HRMS spectrum of **2I**



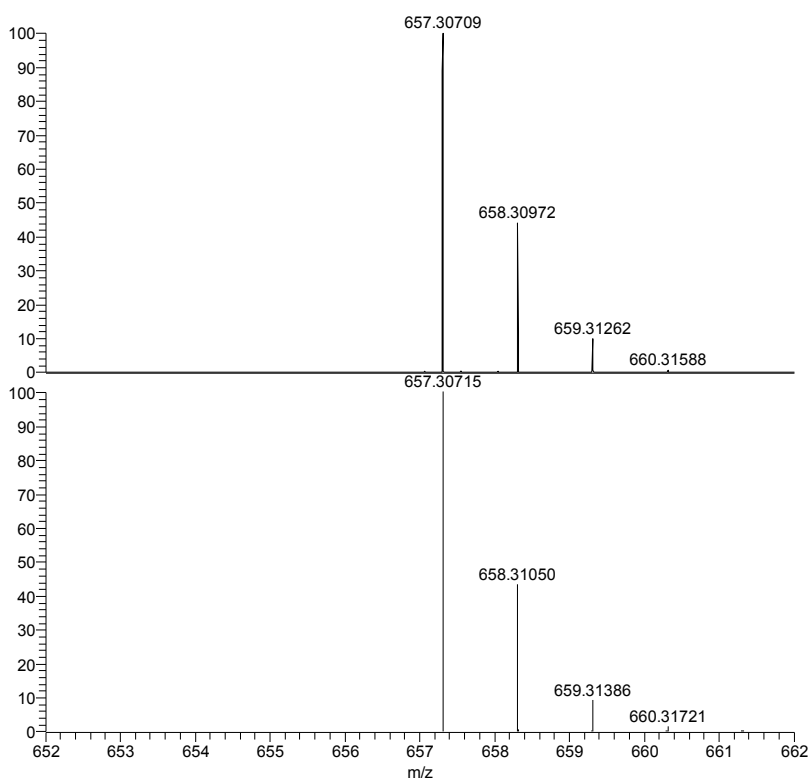
HRMS spectrum of **HR-A**



HRMS spectrum of HR-N3



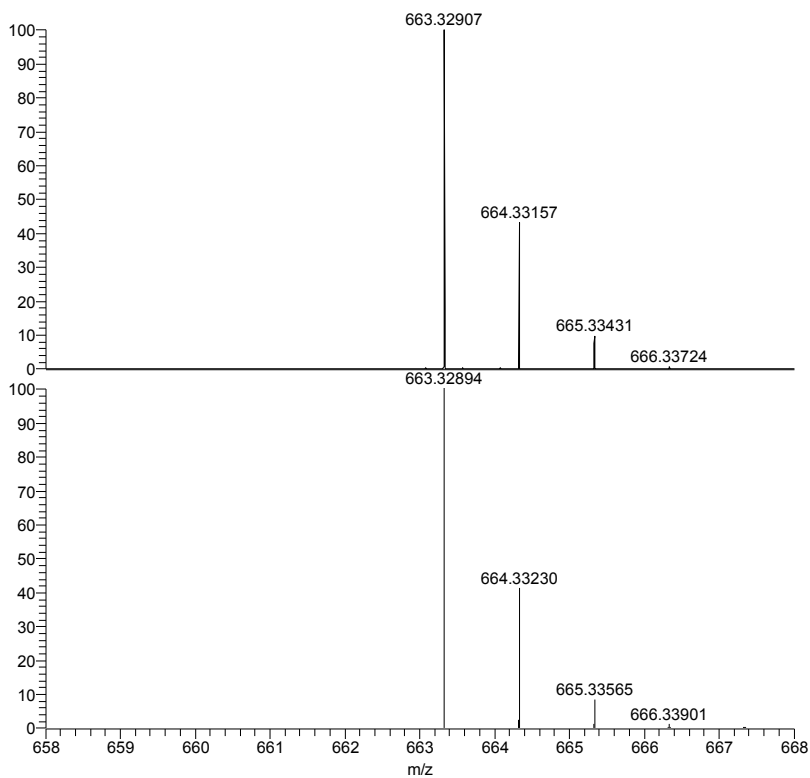
HRMS spectrum of HR-PiA



NL:
7.19E7
H-00334#7-29 RT:
0.16-0.77 AV: 23
T: FTMS + p ESI
Full ms
[110.00-1500.00]

NL:
6.30E5
C₄₀ H₄₁ N₄ O₅:
C₄₀ H₄₁ N₄ O₅
pa Chrg 1

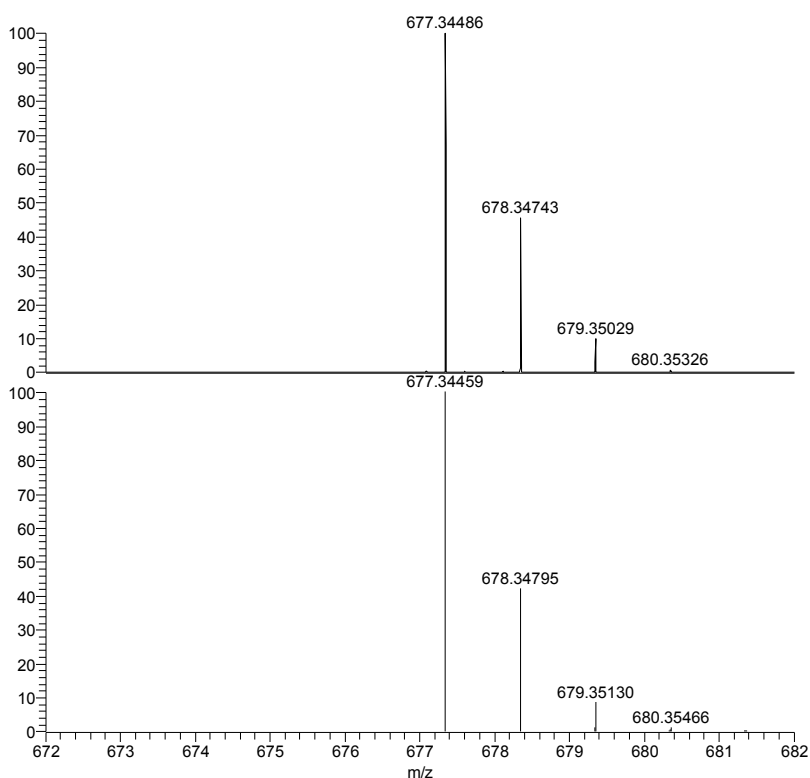
HRMS spectrum of HR-PiAC



NL:
1.37E8
H-00333#7-29 RT:
0.16-0.77 AV: 23
T: FTMS + p ESI
Full ms
[110.00-1500.00]

NL:
6.39E5
C₃₈ H₄₃ N₆ O₅:
C₃₈ H₄₃ N₆ O₅
pa Chrg 1

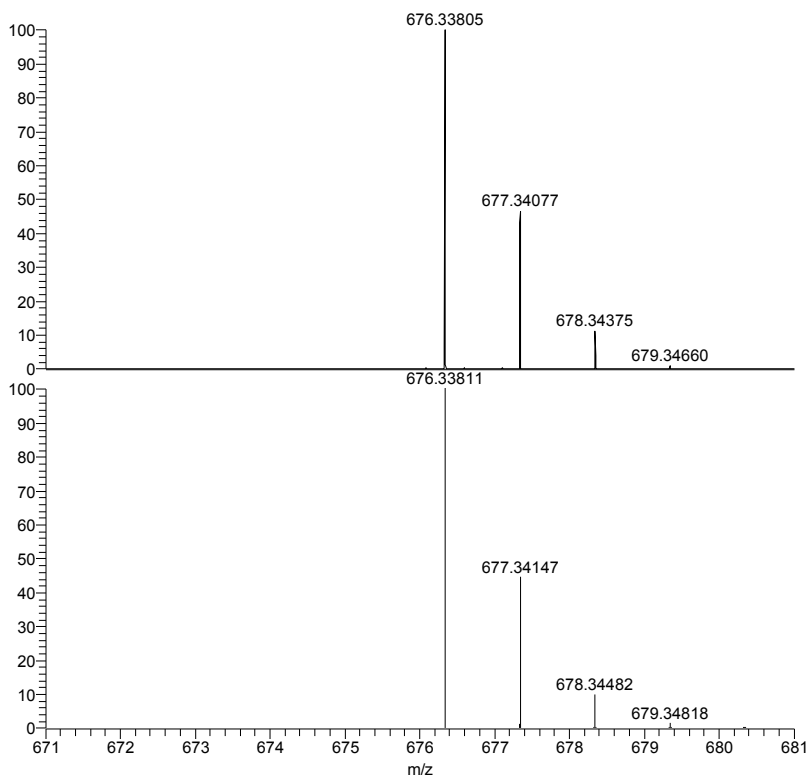
HRMS spectrum of HR-PN₃



NL:
1.13E8
H-00331#7-29 RT:
0.16-0.77 AV: 23
T: FTMS + p ESI
Full ms
[110.00-1500.00]

NL:
6.32E5
C₃₉ H₄₅ N₆ O₅:
C₃₉ H₄₅ N₆ O₅
pa Chrg 1

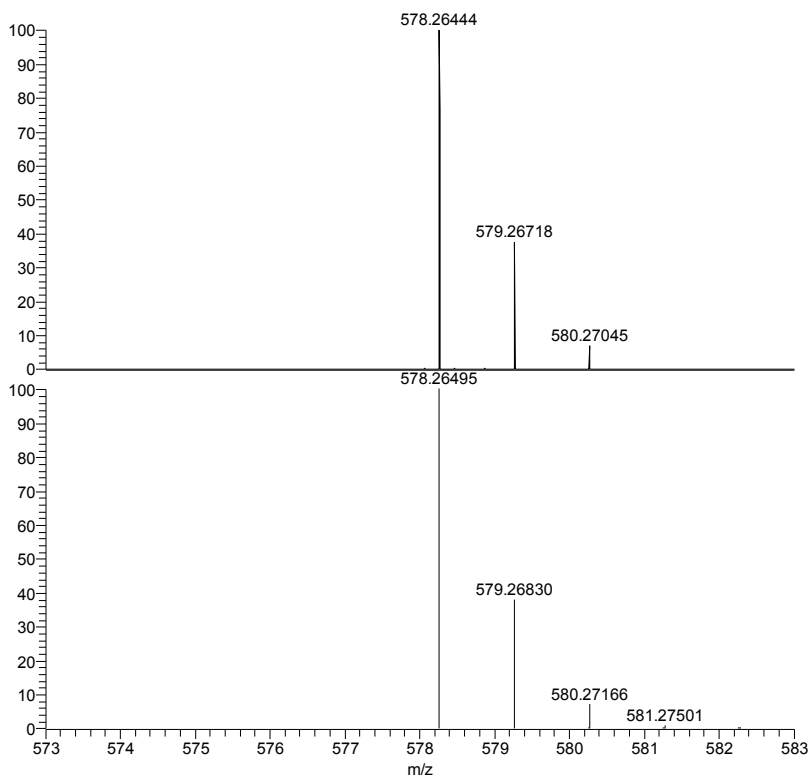
HRMS spectrum of **HR-MPN₃**



NL:
1.85E8
H-00329#7-29 RT:
0.16-0.77 AV: 23
T: FTMS + p ESI
Full ms
[110.00-1500.00]

NL:
6.24E5
C₄₁ H₄₆ N₃ O₆:
C₄₁ H₄₆ N₃ O₆
pa Chrg 1

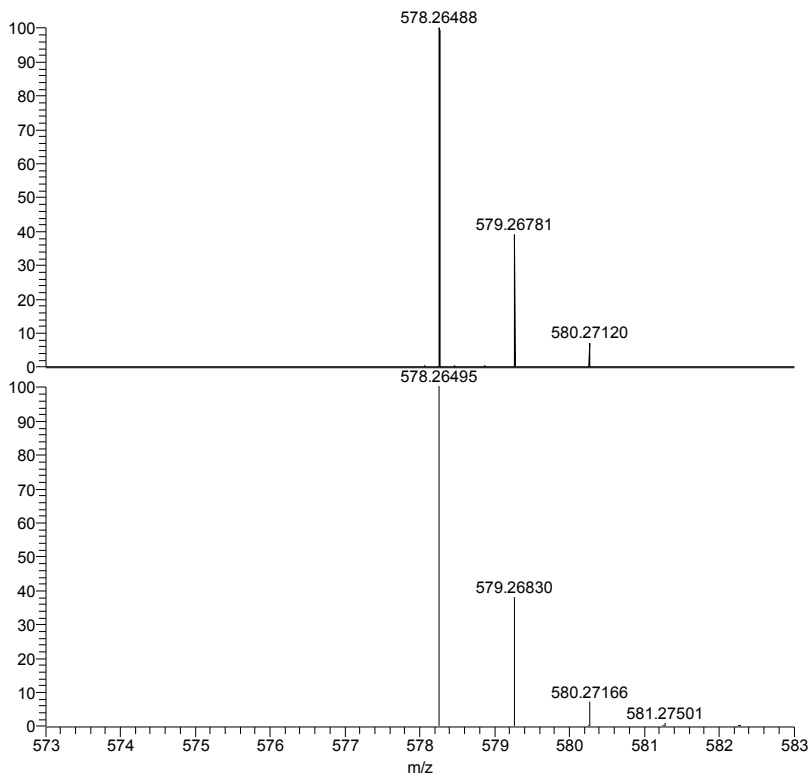
HRMS spectrum of **HR-PA**



NL:
8.54E7
H-00328#7-29 RT:
0.16-0.77 AV: 23
T: FTMS + p ESI
Full ms
[110.00-1500.00]

NL:
6.68E5
C₃₅ H₃₆ N₃ O₅:
C₃₅ H₃₆ N₃ O₅:
pa Chrg 1

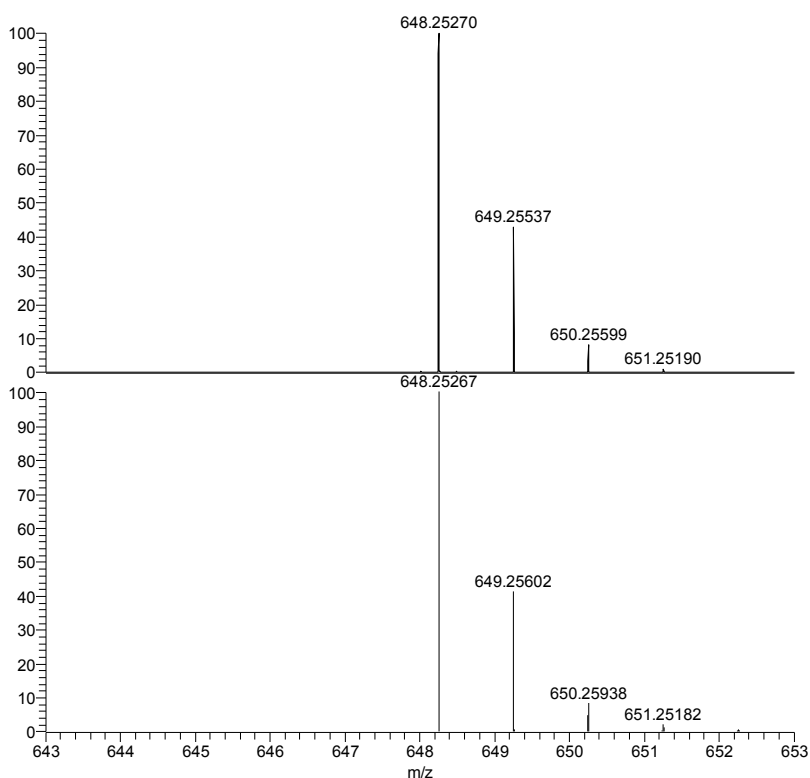
HRMS spectrum of HR-Ala



NL:
5.24E7
H-00332#7-29 RT:
0.16-0.77 AV: 23
T: FTMS + p ESI
Full ms
[110.00-1500.00]

NL:
6.68E5
C₃₅ H₃₆ N₃ O₅:
C₃₅ H₃₆ N₃ O₅:
pa Chrg 1

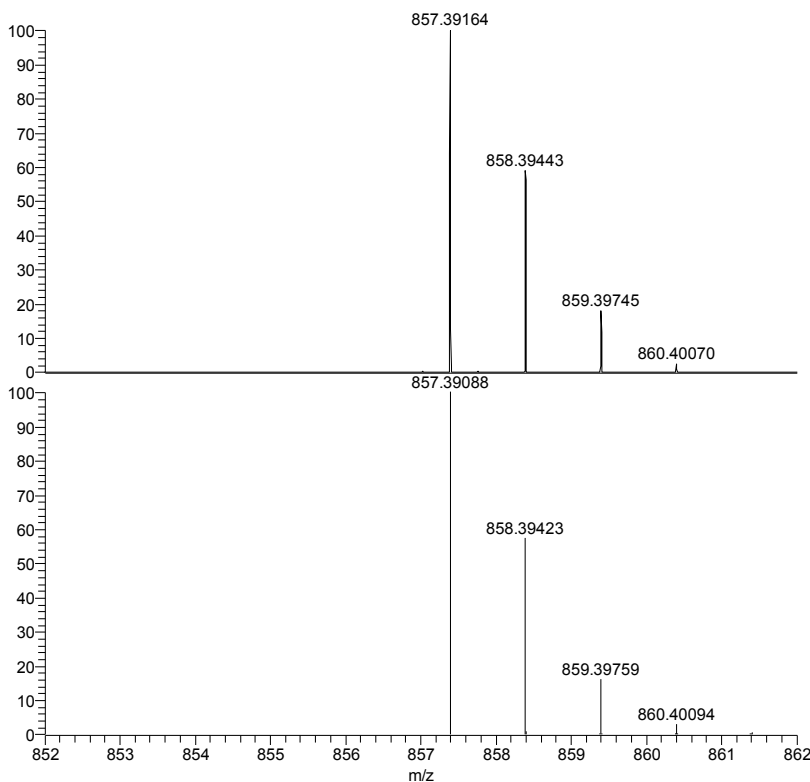
HRMS spectrum of HR-βAla



NL:
5.95E7
H-00327#7-29 RT:
0.16-0.77 AV: 23
T: FTMS + p ESI
Full ms
[110.00-1500.00]

NL:
6.14E5
C₃₈ H₃₈ N₃ O₅ S:
C₃₈ H₃₈ N₃ O₅ S₁
pa Chrg 1

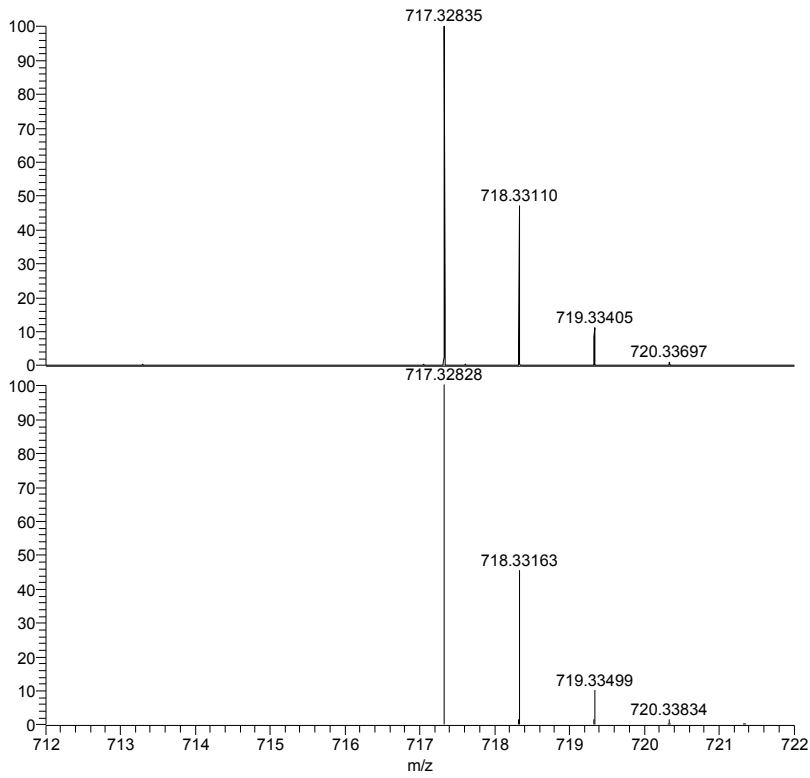
HRMS spectrum of HR-CysA



NL:
6.05E7
H-00403#11-19
RT: 0.27-0.49 AV:
9 T: FTMS + p ESI
Full ms
[110.00-1500.00]

NL:
5.44E5
C₅₃ H₅₃ N₄ O₇:
C₅₃ H₅₃ N₄ O₇
pa Chrg 1

HRMS spectrum of HR-LysF



NL:
5.87E7
H-00330#7-29 RT:
0.16-0.77 AV: 23
T: FTMS + p ESI
Full ms
[110.00-1500.00]

NL:
6.13E5
C₄₂ H₄₅ N₄ O₇:
C₄₂ H₄₅ N₄ O₇
pa Chrg 1

HRMS spectrum of HR-LysA