

**Supporting Information**  
**for**

**Acyclic nucleoside phosphonates with adenine nucleobase inhibit *Trypanosoma brucei*  
adenine phosphoribosyltransferase *in vitro***

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File S1. List of oligonucleotides used in this study

File S2. Spectra of the final compounds

File S3. Raw Images. Original images supporting blot results reported in Figs. 2A, 3 and 4C.

**Supplementary file S1.** List of oligonucleotides used in this study.

APRT-I p2T7-TA Blue RNAi forward primer: 5'- ATGTCACTTGTGGAGGTTTTG-3'

APRT-I p2T7- TA Blue RNAi reverse primer: 5'- ATACCCGCCACTTCCACAAG-3'.

APRT-II p2T7-177 RNAi forward primer: 5'- TCACAGTATGATGCGATTCTGA-3'

APRT-II p2T7-177 RNAi reverse primer: 5'- GGCCACATCACAGACGGTAA-3'.

APRT-I pT7-V5 N-term forward primer: 5'-CACAGATCTTCACTTGTGGAGGTTTTG -3'

APRT-I pT7-V5 N-term reverse primer: 5'- CACTCTAGATTAATCAATTTTTTTGCCTCG -3'

APRT-II pT7-V5 N-term forward primer: 5'-CACGGATCCTCACAGTATGATGCGATTCTGA -3'

APRT-II pT7-V5 N-term reverse primer: 5'-CACTCTAGATCACAGTCGTGACCTGGTAATG -3'

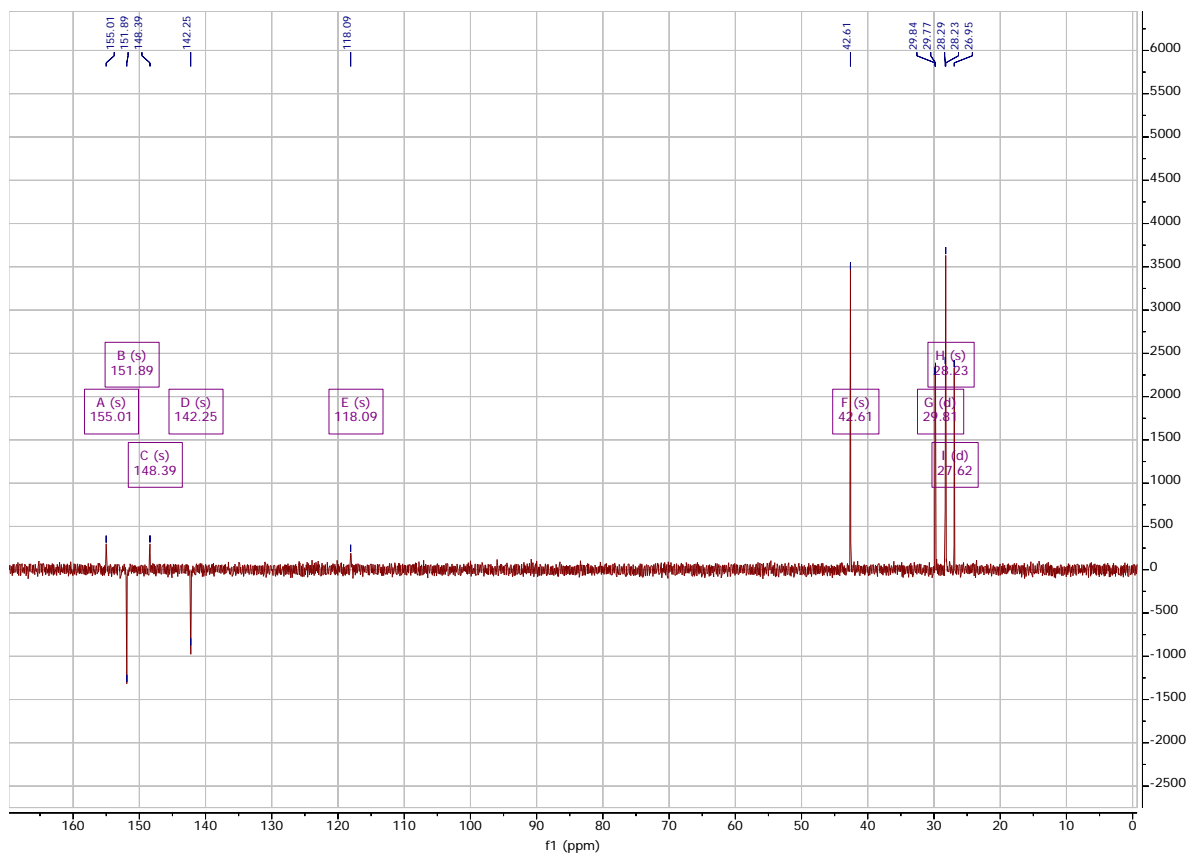
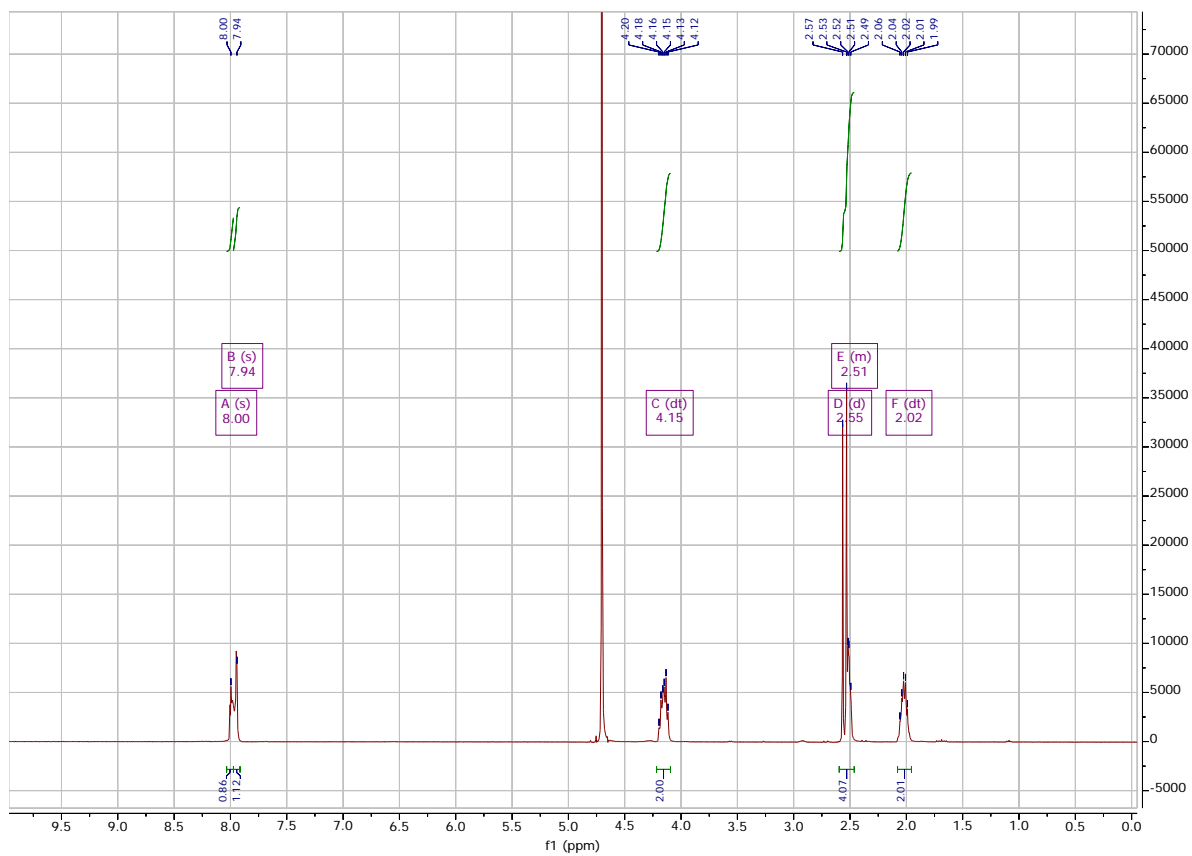
APRT-I pSKB3 overexpression forward primer: 5'- CACAGATCTTCACTTGTGGAGGTTTTG -3'

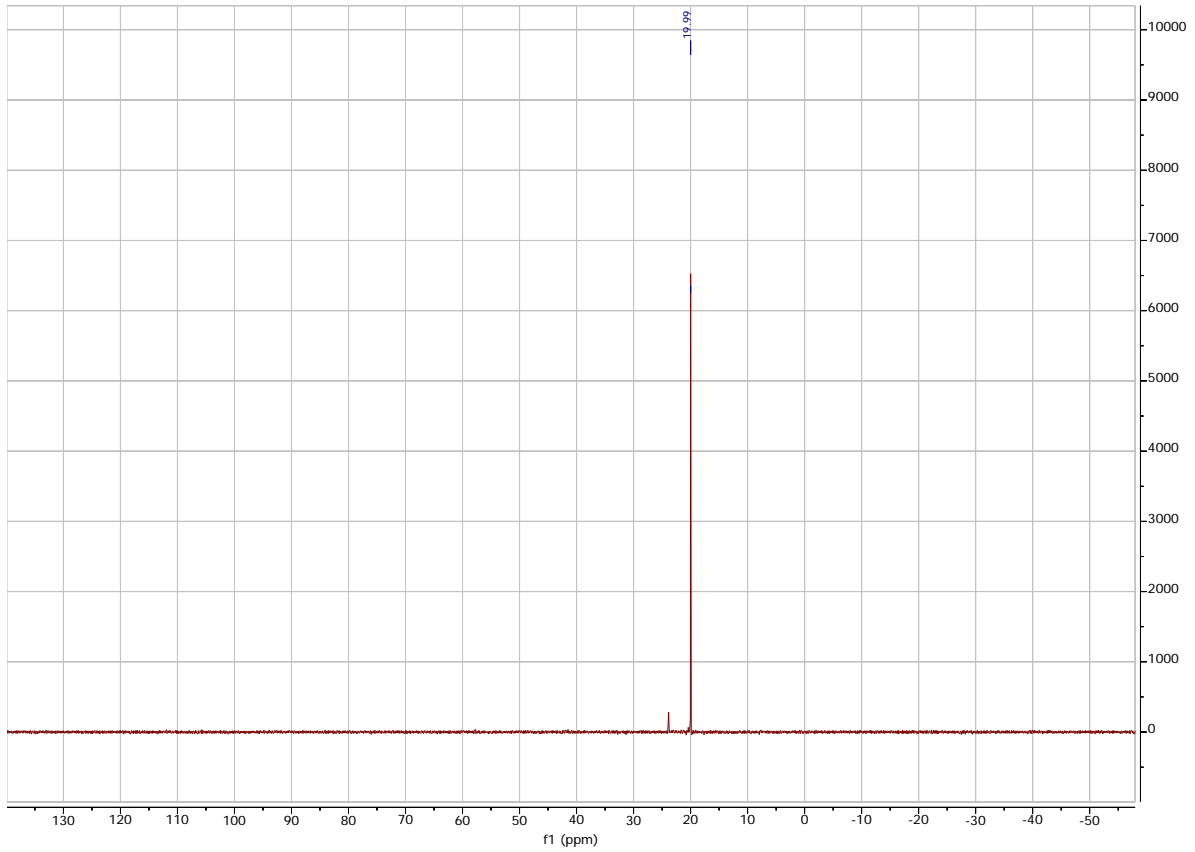
APRT-I pSKB3 overexpression reverse primer: 5'- CACCTCGAGTTAAATCAATTTTTTTGCCTCG -3'

**Supplementary file S2.** Spectra of the final compounds.

# NMR Spectra and HRMS of tested compounds

3a



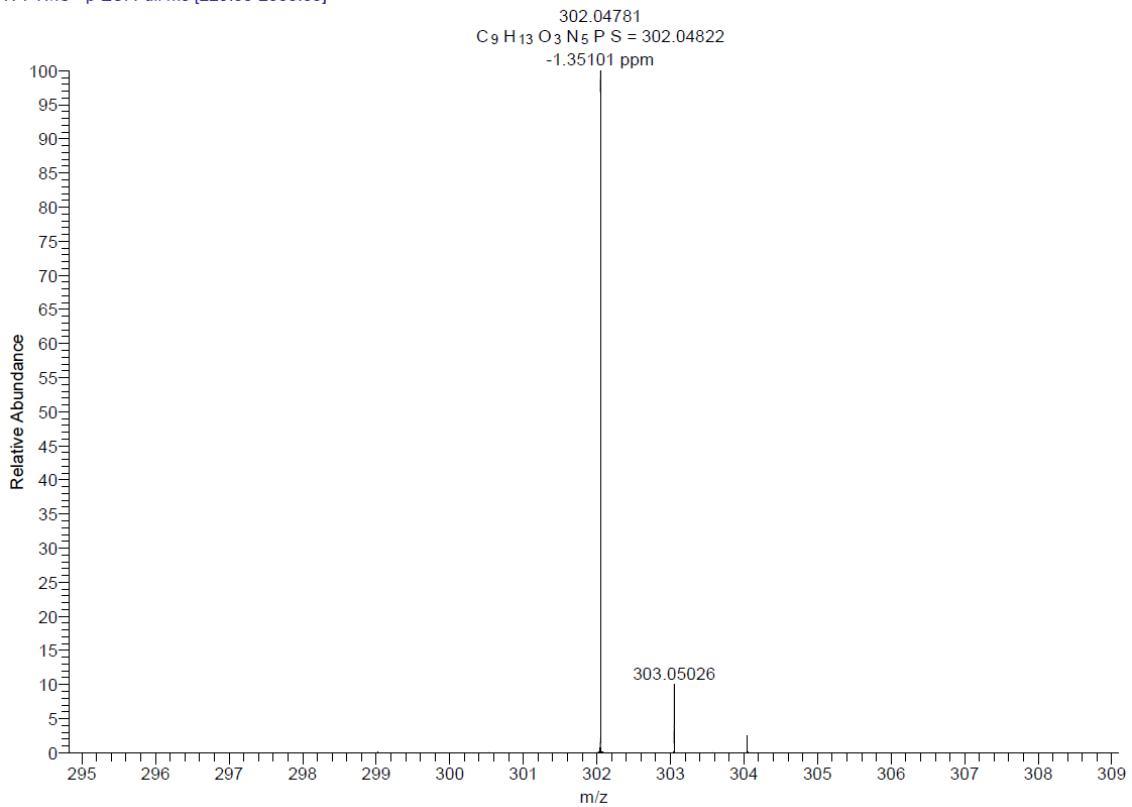


X:\SERVISHR\duben\_2019\040419servisHR\_33

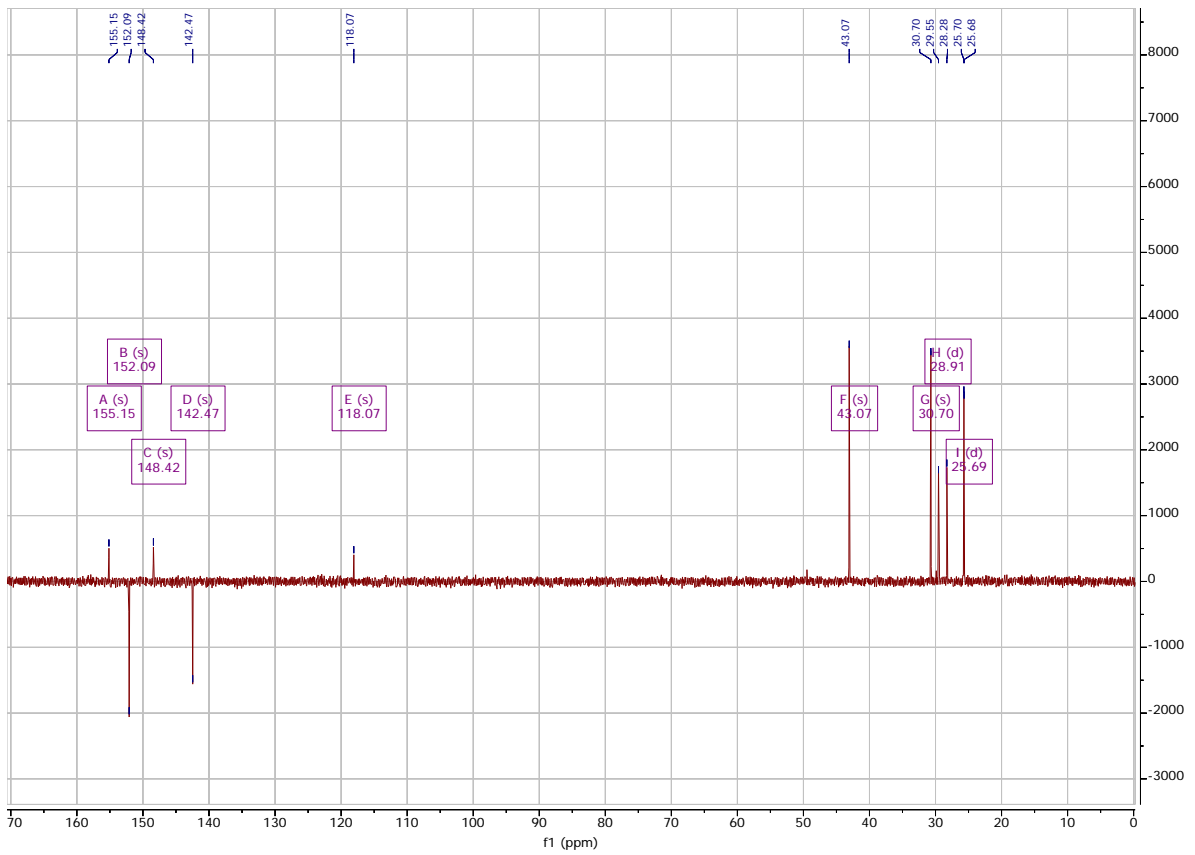
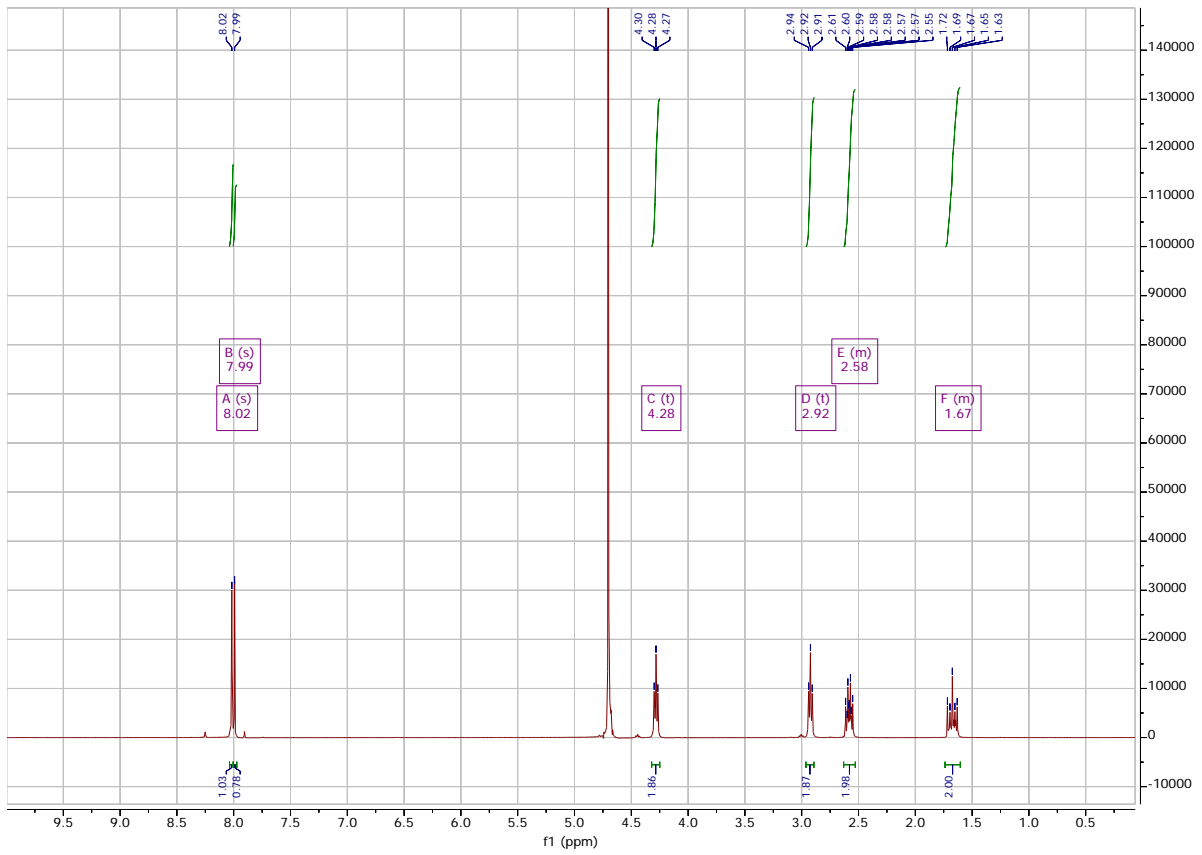
04/04/19 17:31:31

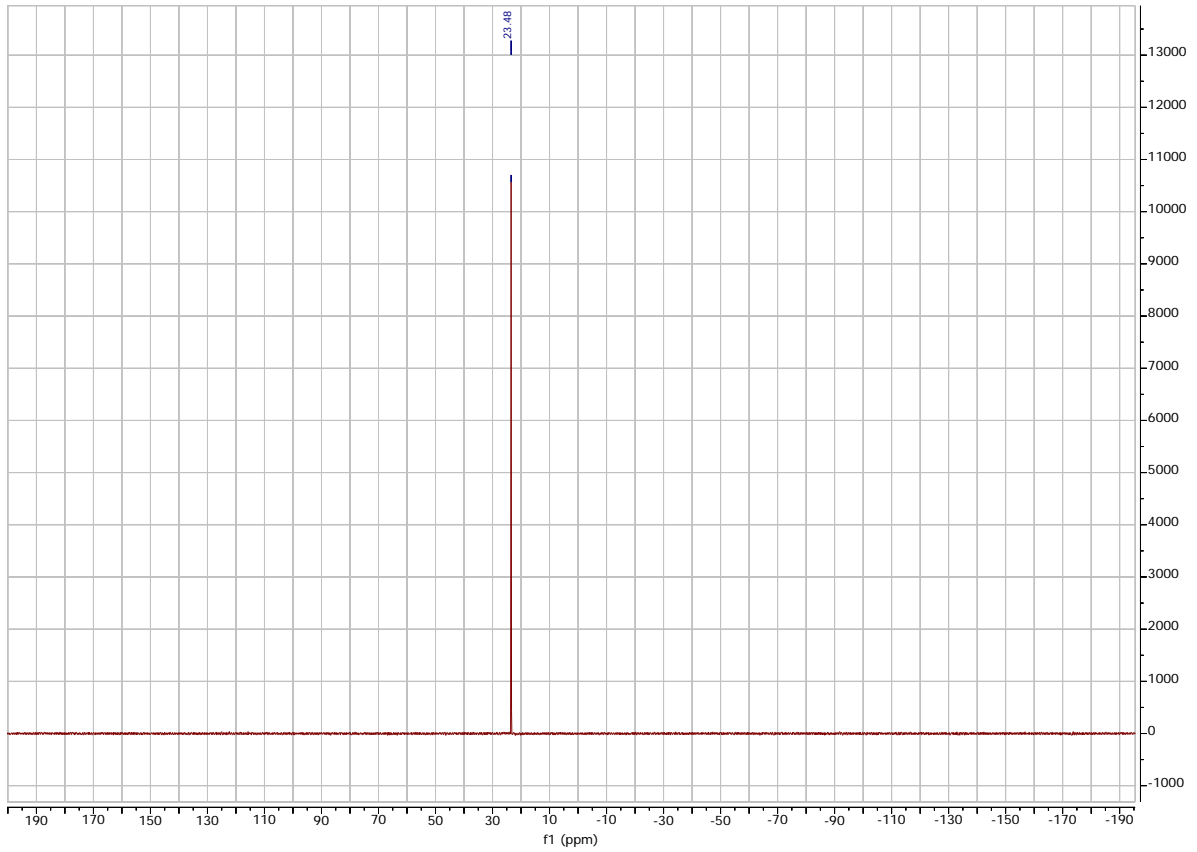
Klejch, TK371

040419servisHR\_33 #30-31 RT: 1.68-1.74 AV: 2 SB: 9 0.10-0.45, 0.27-0.33 NL: 2.76E6  
T: FTMS - p ESI Full ms [220.00-2000.00]



3b



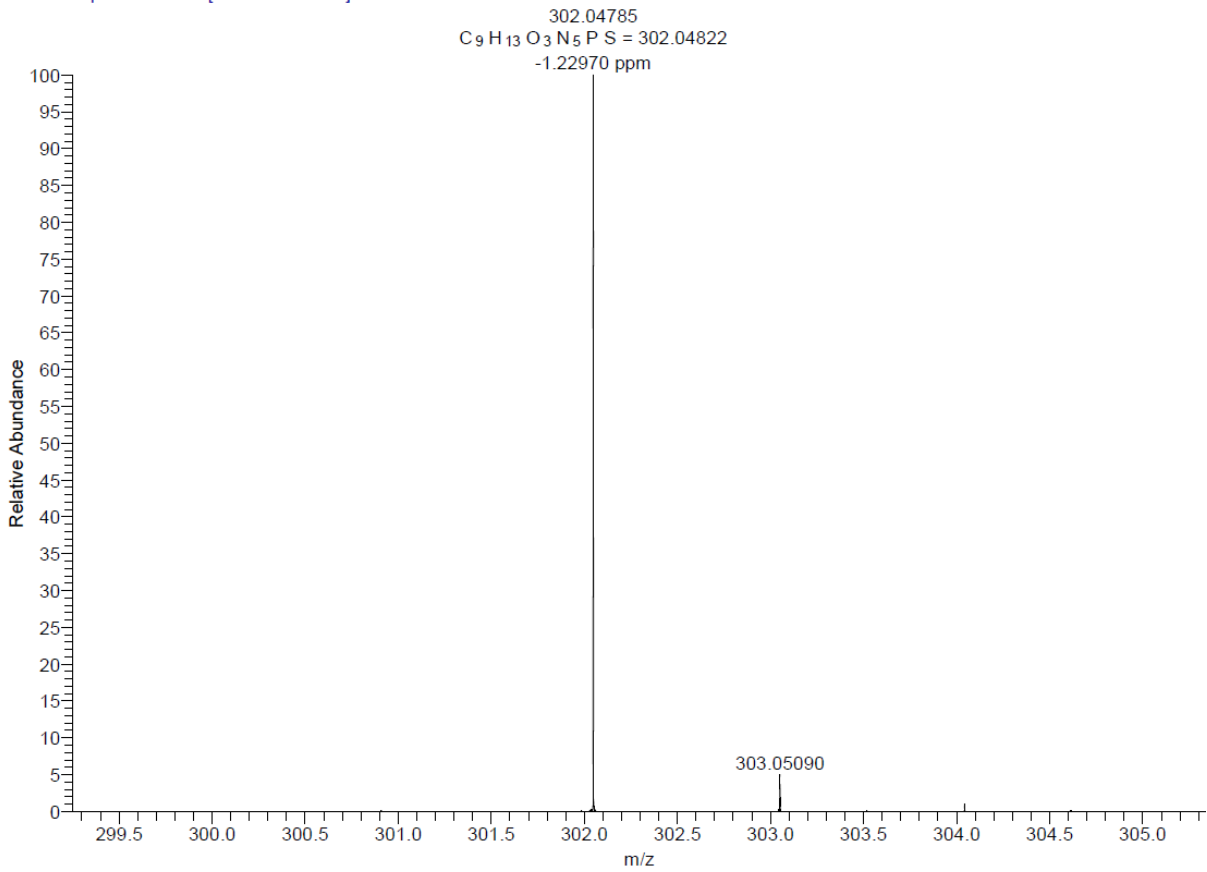


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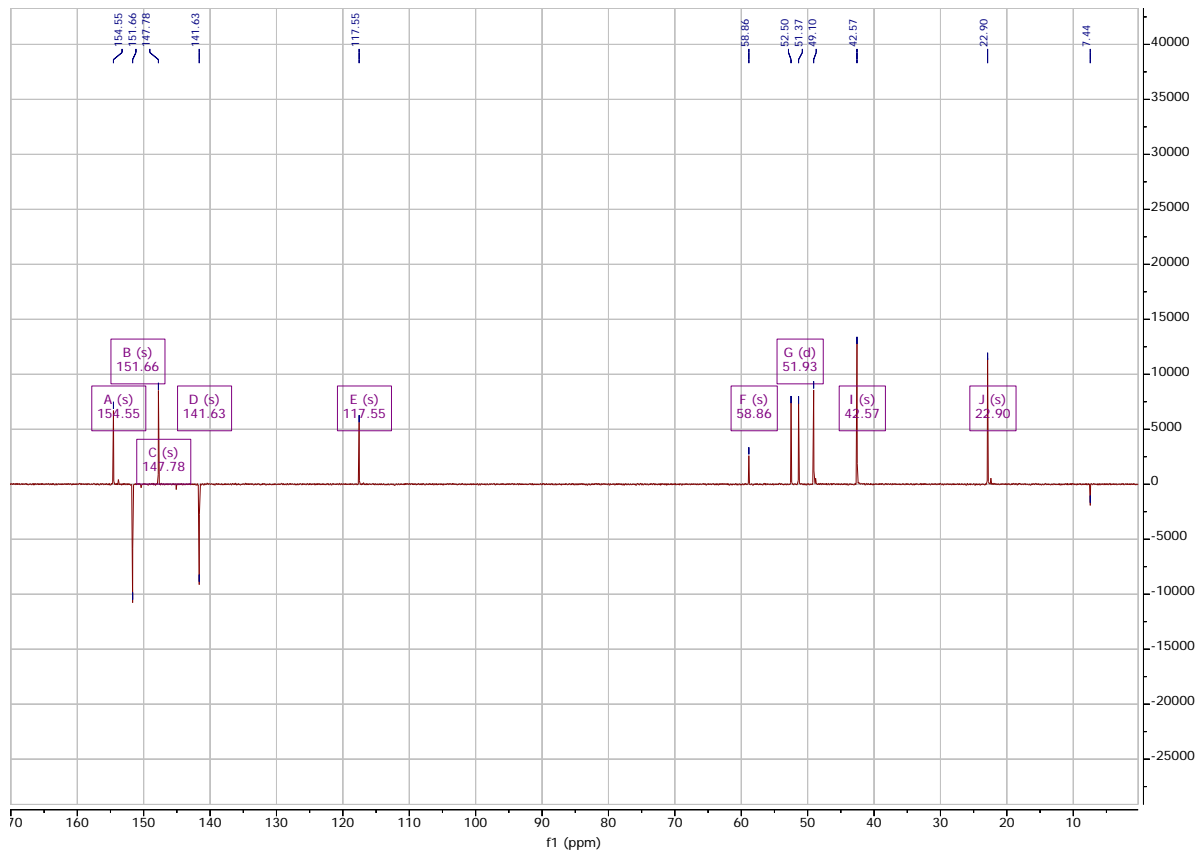
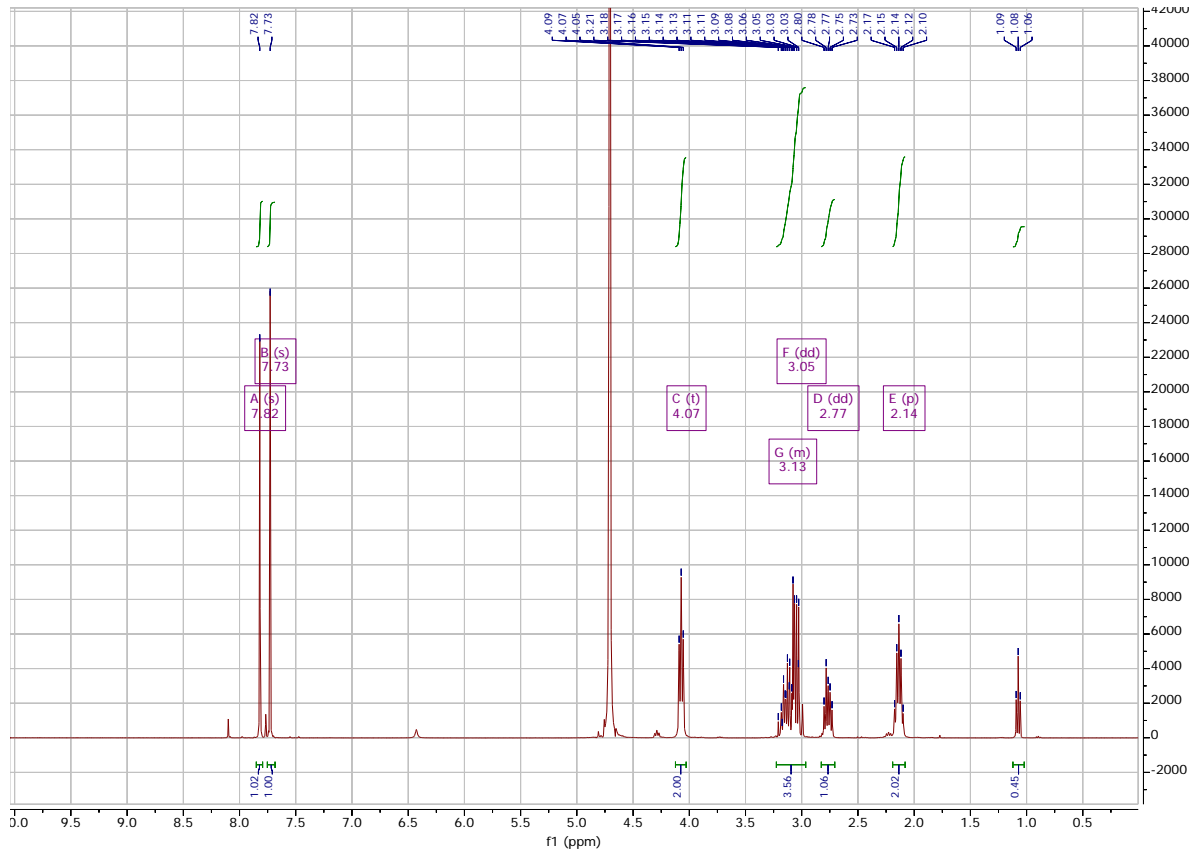
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Klejch, TK370

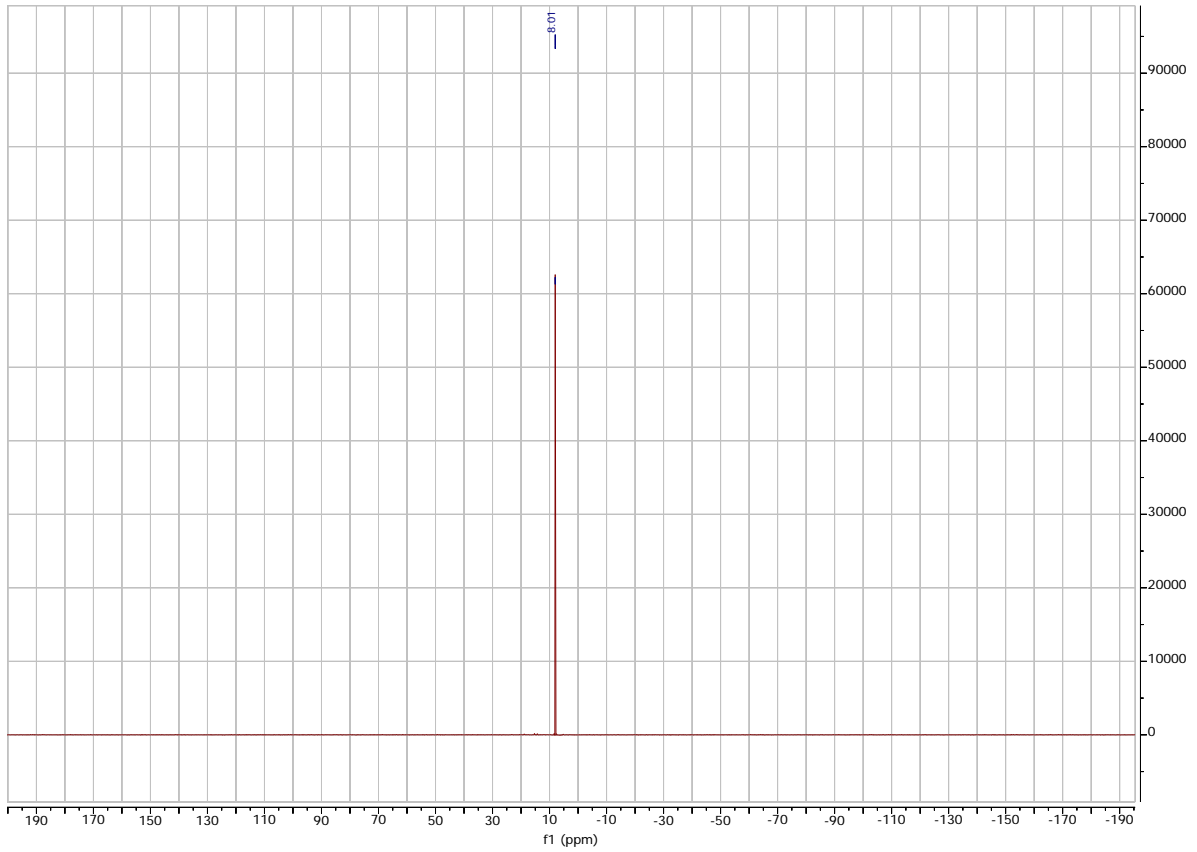
040419servisHR\_32 #28-29 RT: 1.56-1.62 AV: 2 SB: 14 0.04-0.57, 0.10-0.27 NL: 2.13E5  
T: FTMS - p ESI Full ms [220.00-2000.00]



4a





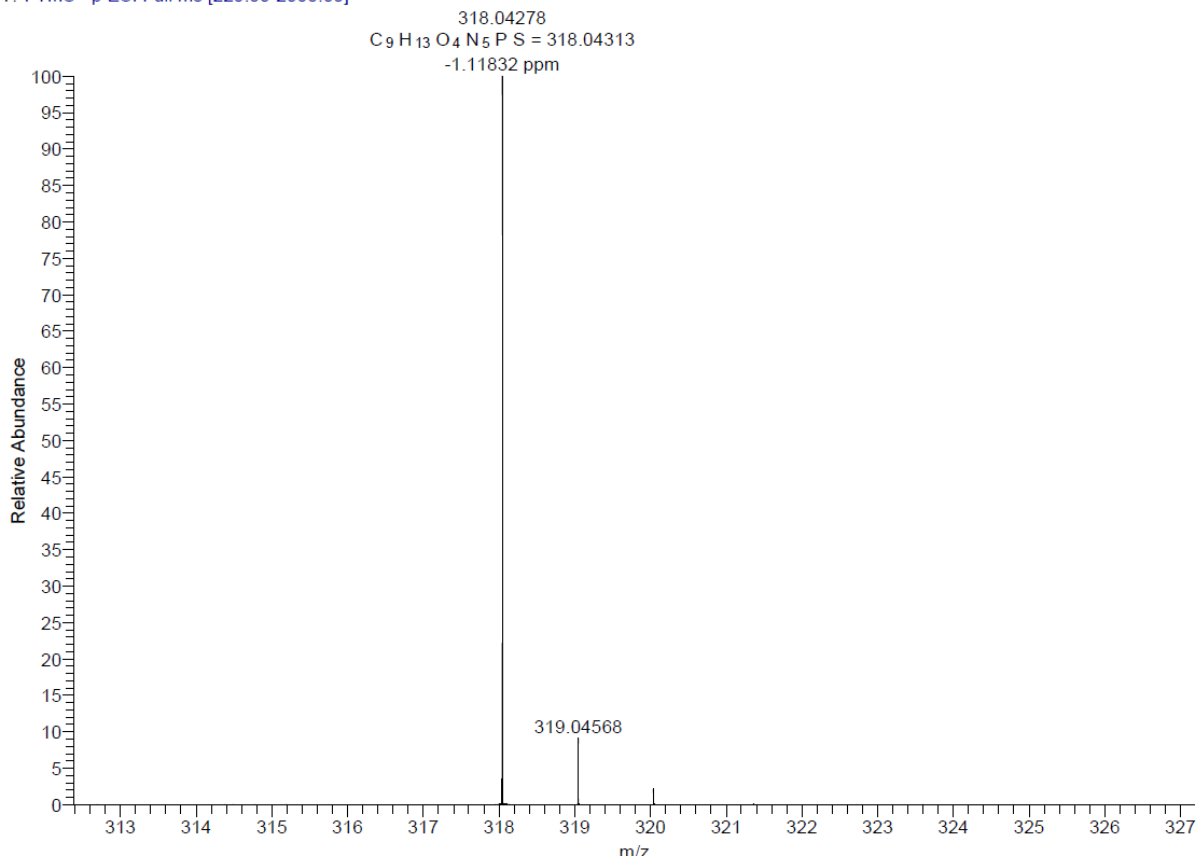


X:\SERVISHR\duben\_2019\040419servisHR\_35

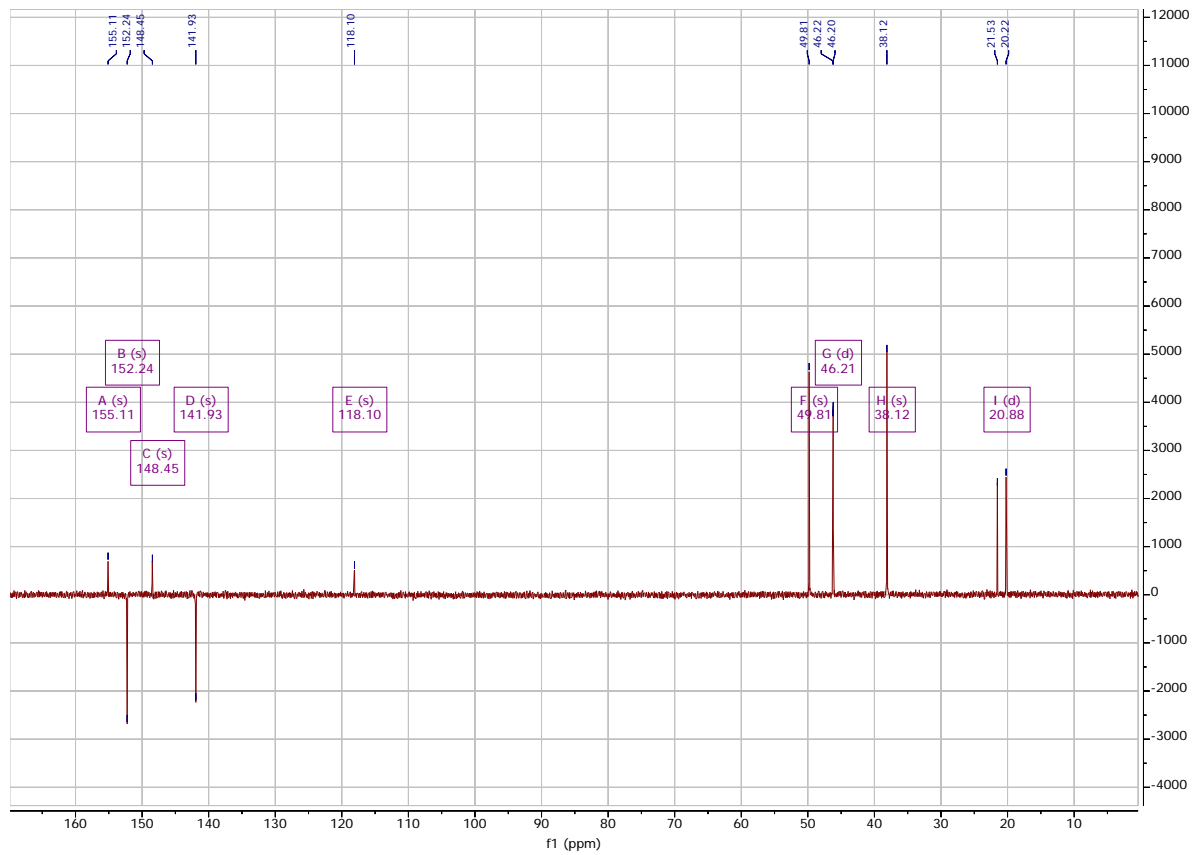
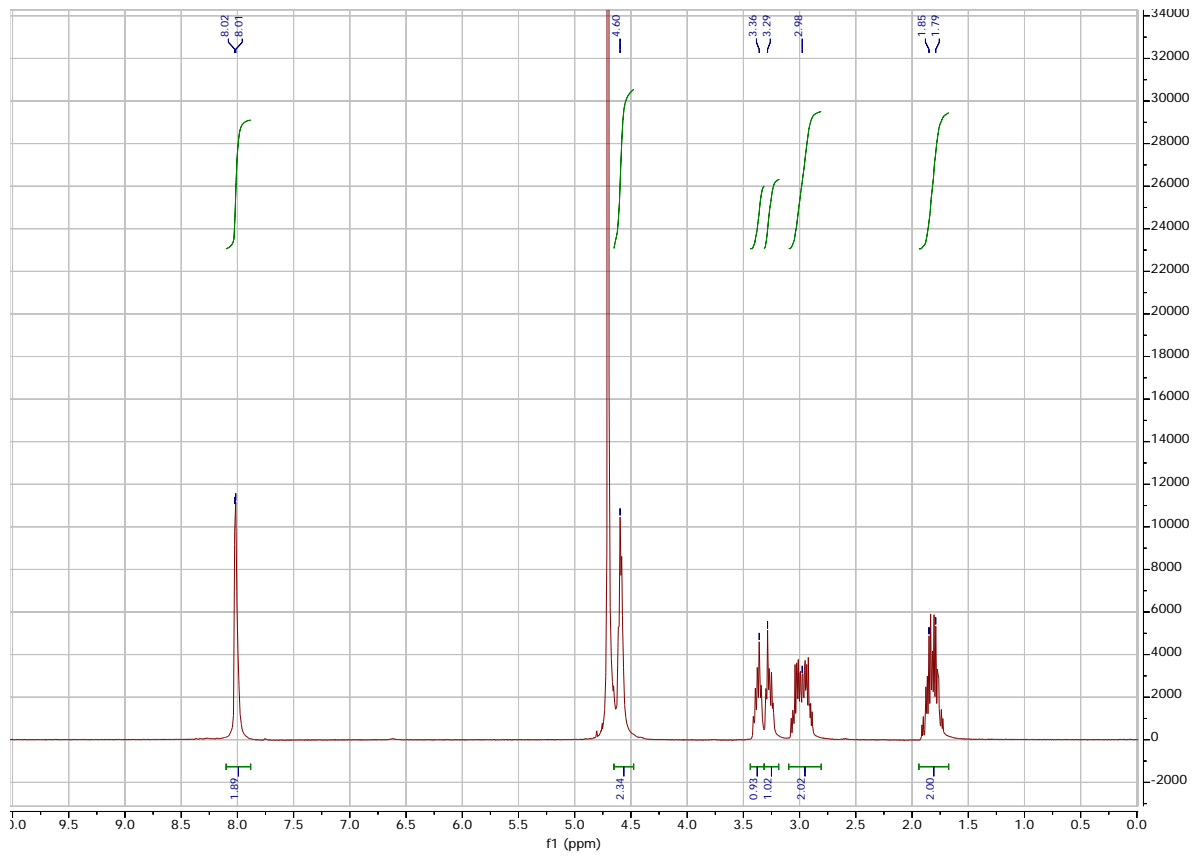
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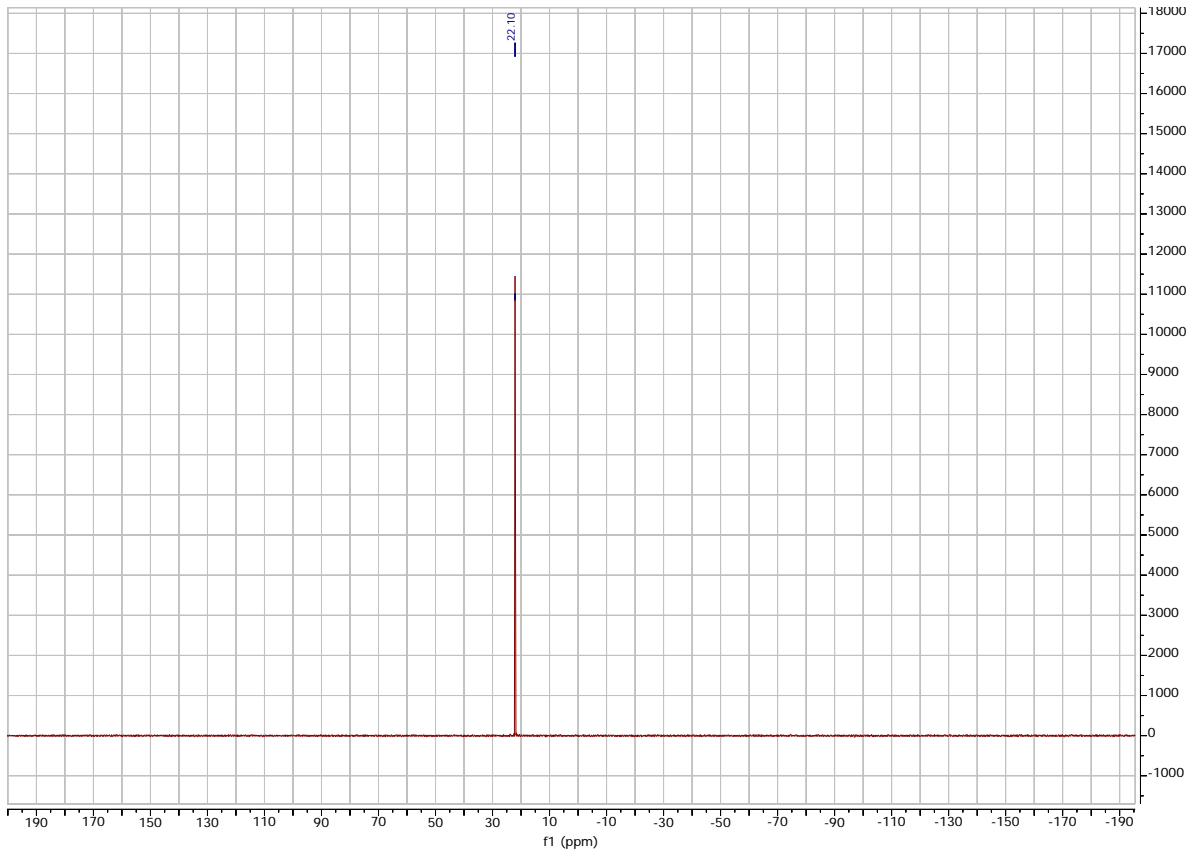
Klejš, TK373

040419servisHR\_35 #30 RT: 1.68 AV: 1 SB: 8 0.04-0.22 , 0.10-0.27 NL: 1.96E6  
T: FTMS - p ESI Full ms [220.00-2000.00]



4b



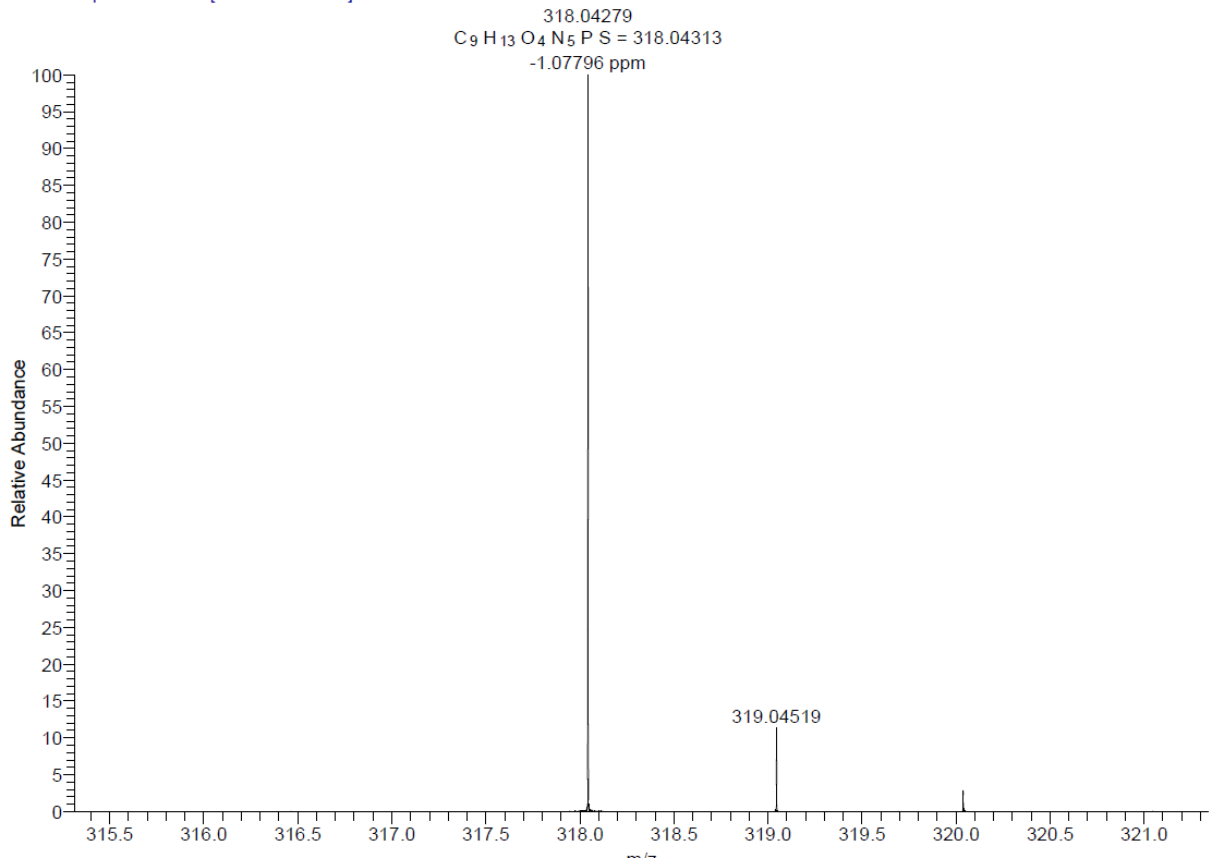


X:\SERVISHR\duben\_2019\040419servisHR\_34

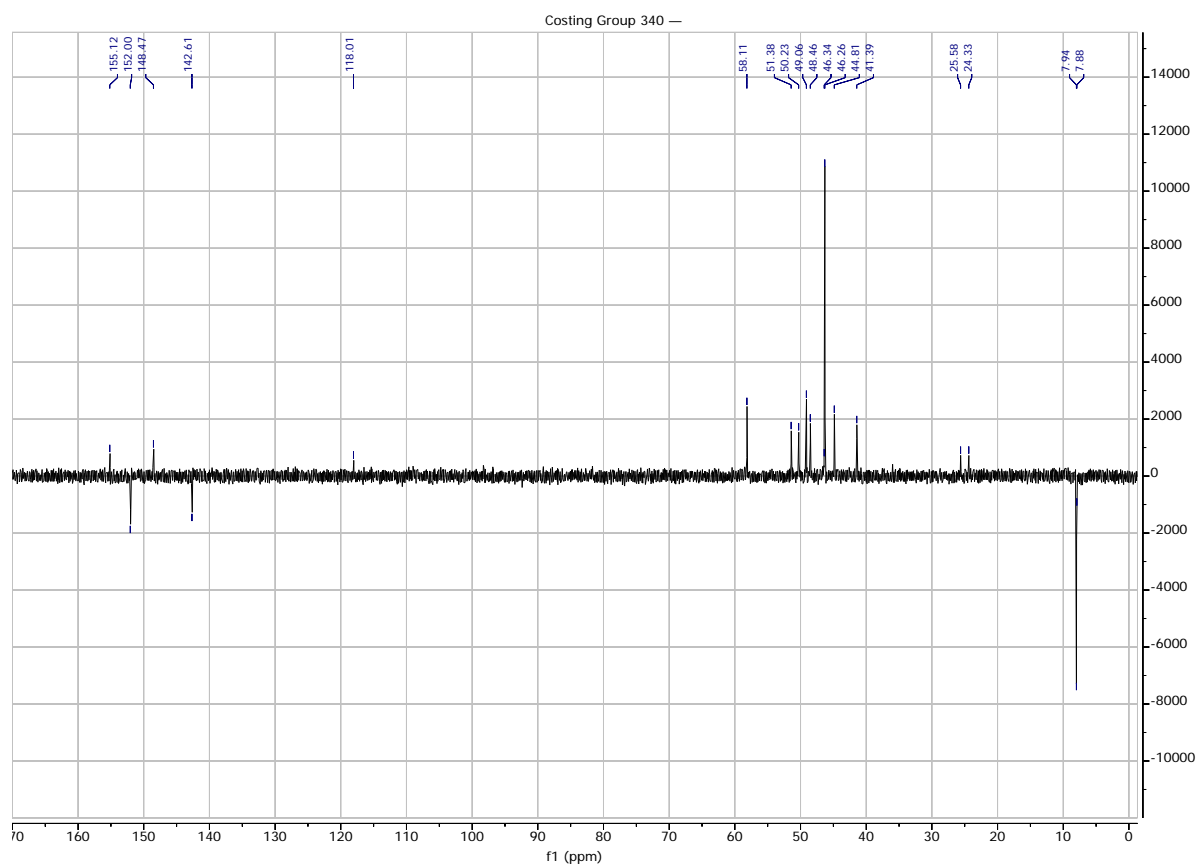
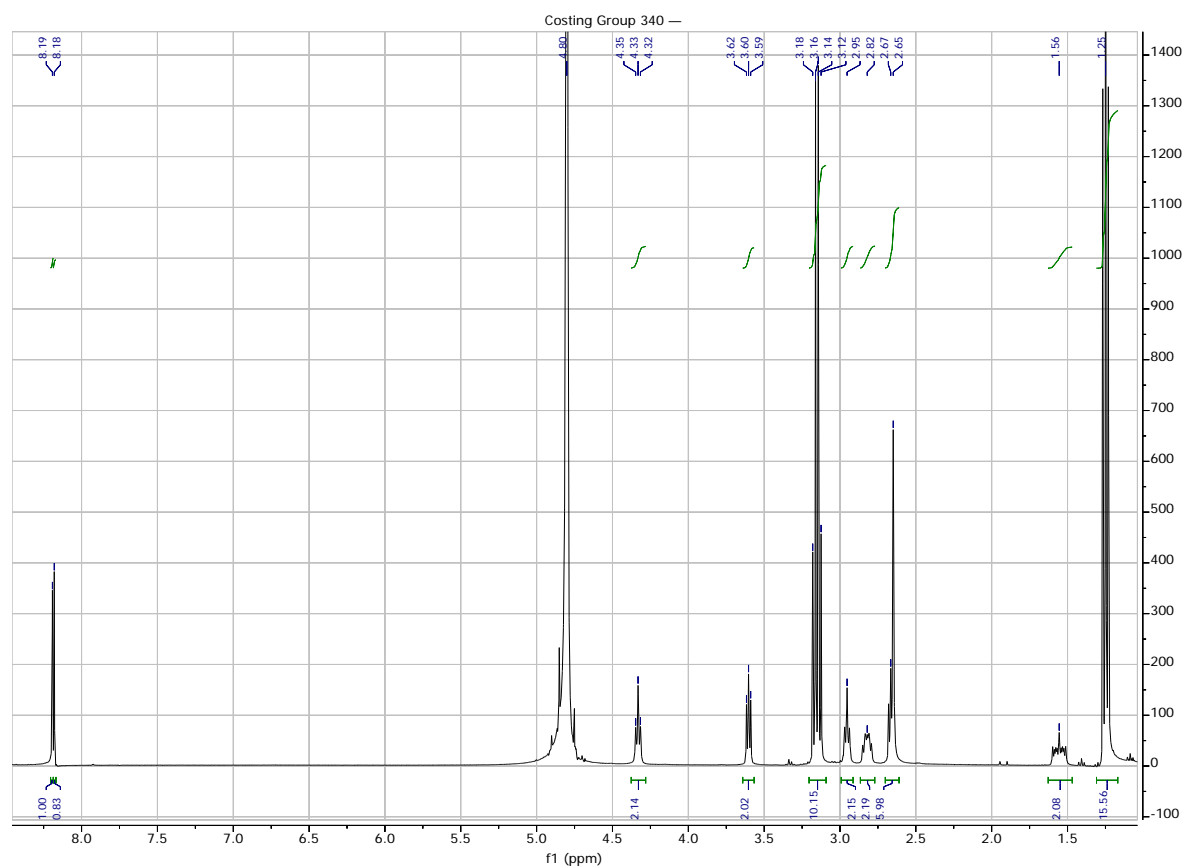
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Klejš, TK372

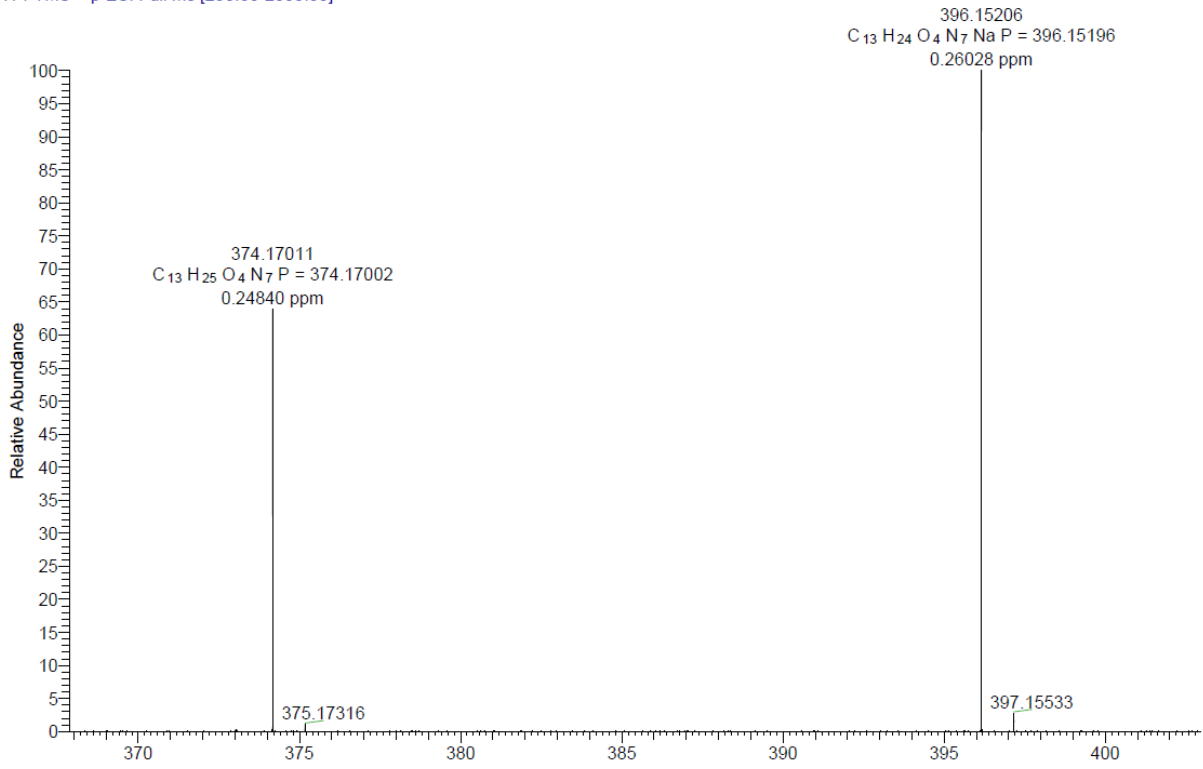
040419servisHR\_34 #29 RT: 1.62 AV: 1 SB: 17 0.04-0.22 , 0.10-0.80 NL: 2.69E6  
T: FTMS - p ESI Full ms [220.00-2000.00]



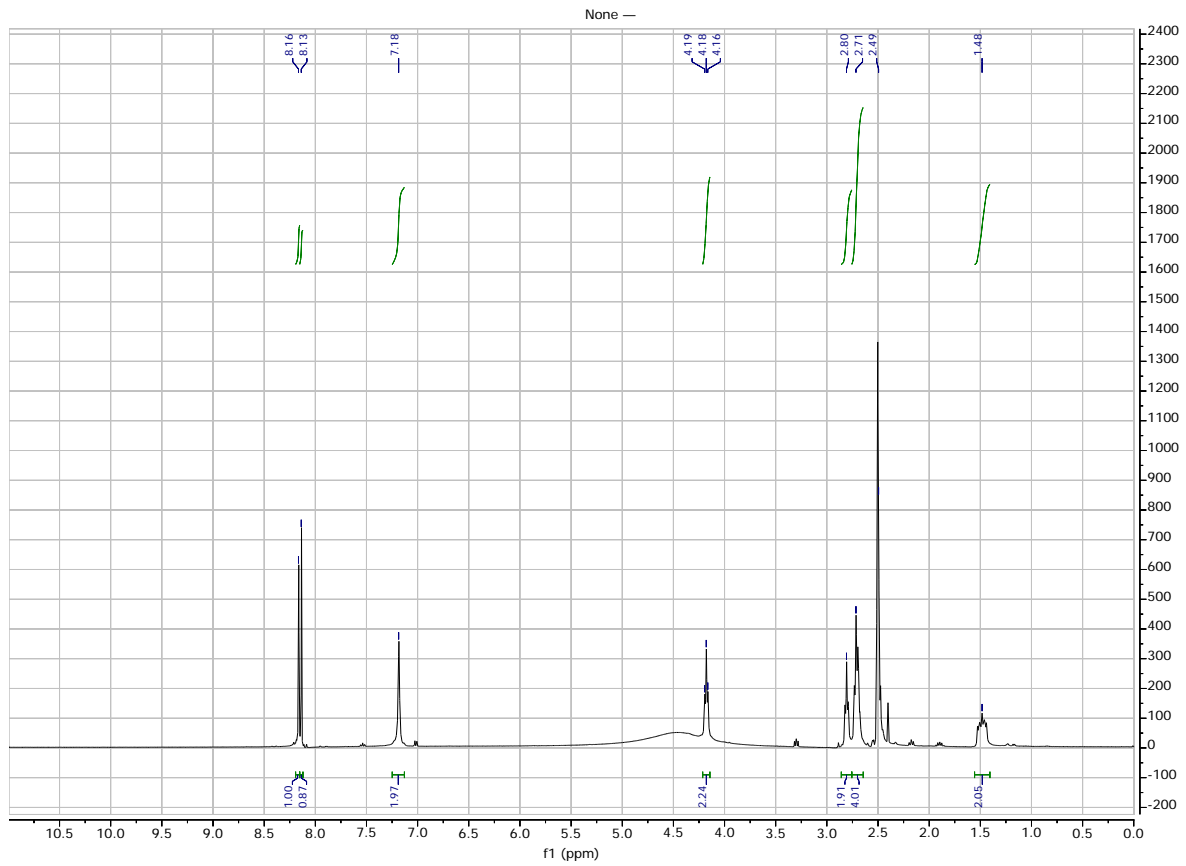
9a

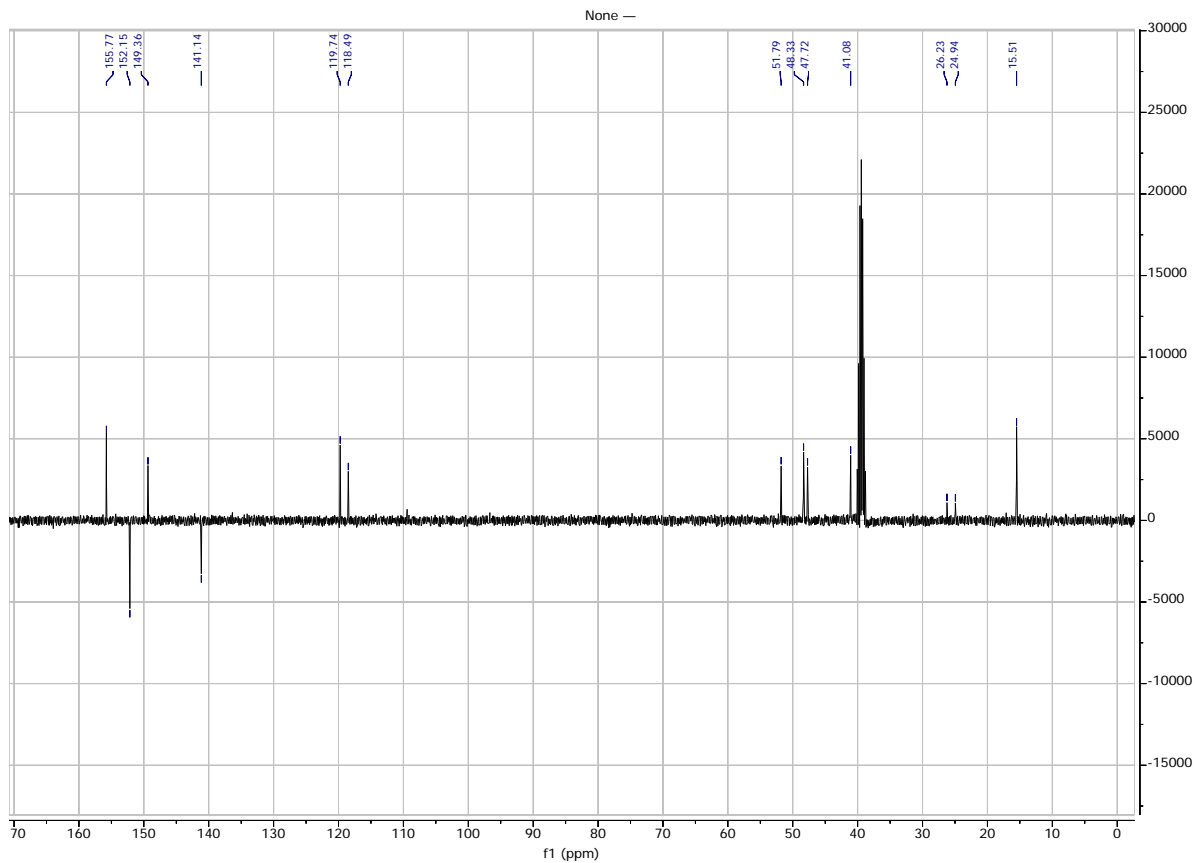


200820\_servisHR\_+23 #71-79 RT: 1.89-2.11 AV: 9 NL: 1.29E6  
T: FTMS + p ESI Full ms [200.00-2000.00]



9b



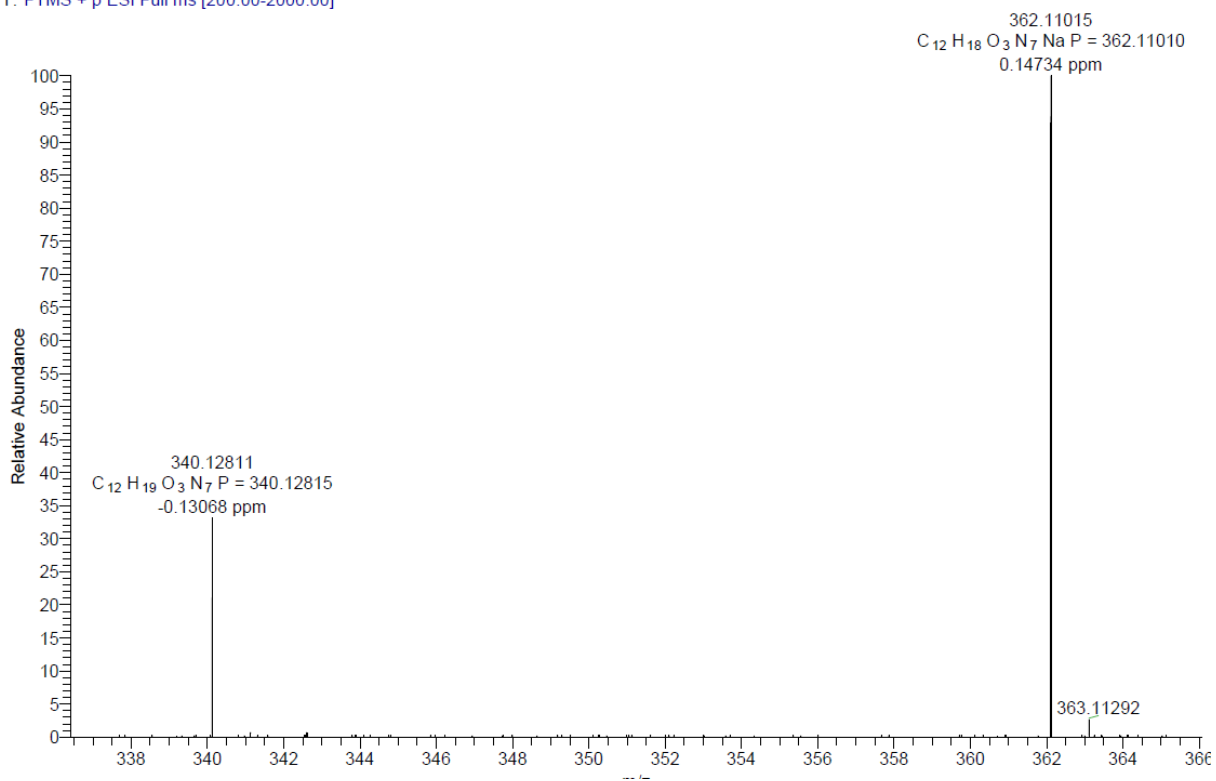


Z:\SERVISHR\...200820\_servisHR\_+22

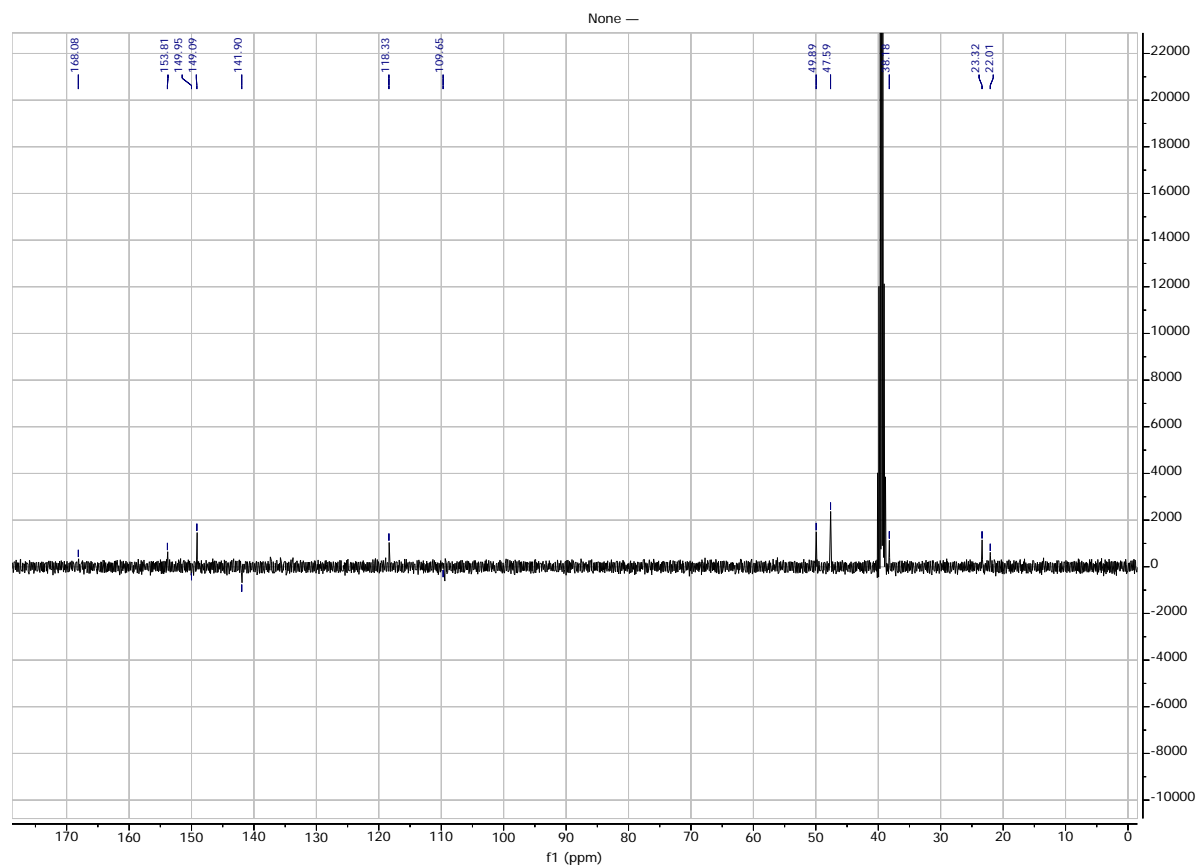
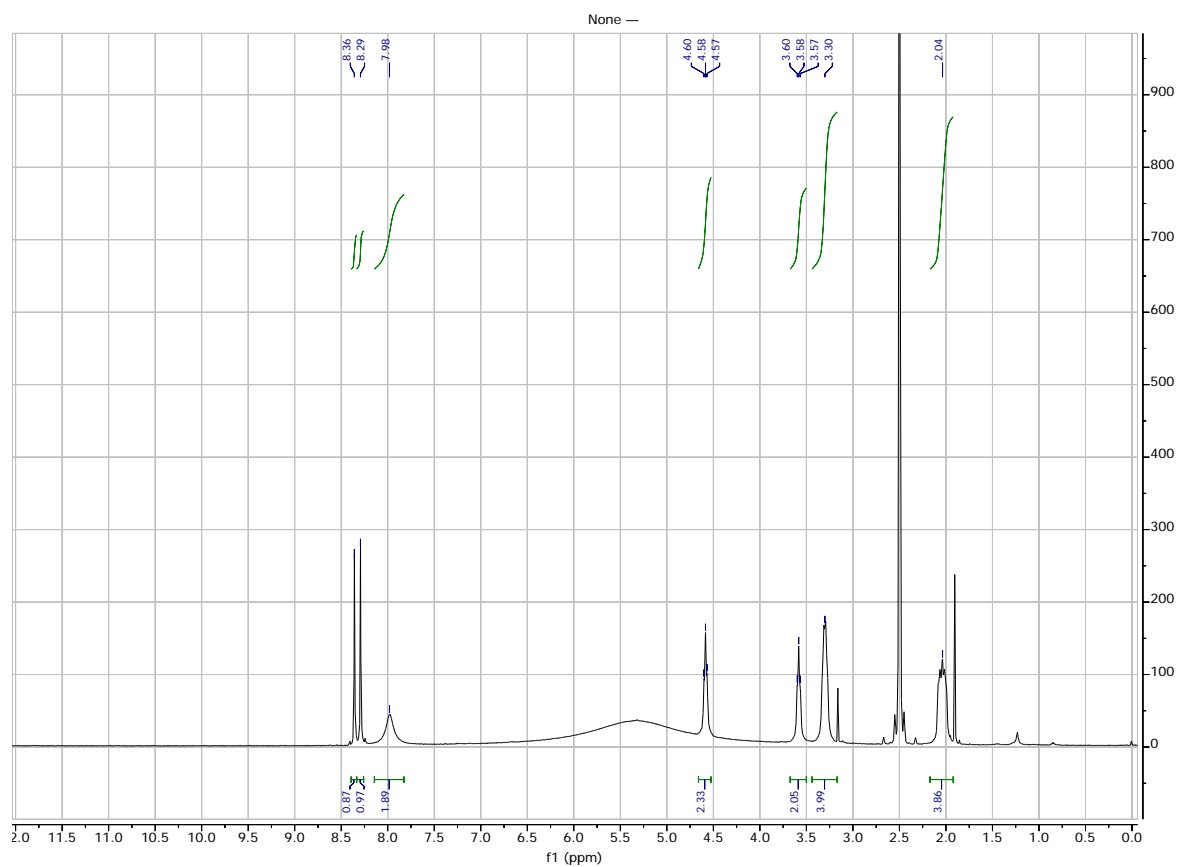
08/20/20 16:17:02

Hockova, DA-XII-5

200820\_servisHR\_+22 #75-81 RT: 2.00-2.16 AV: 7 NL: 6.21E5  
T: FTMS + p ESI Full ms [200.00-2000.00]

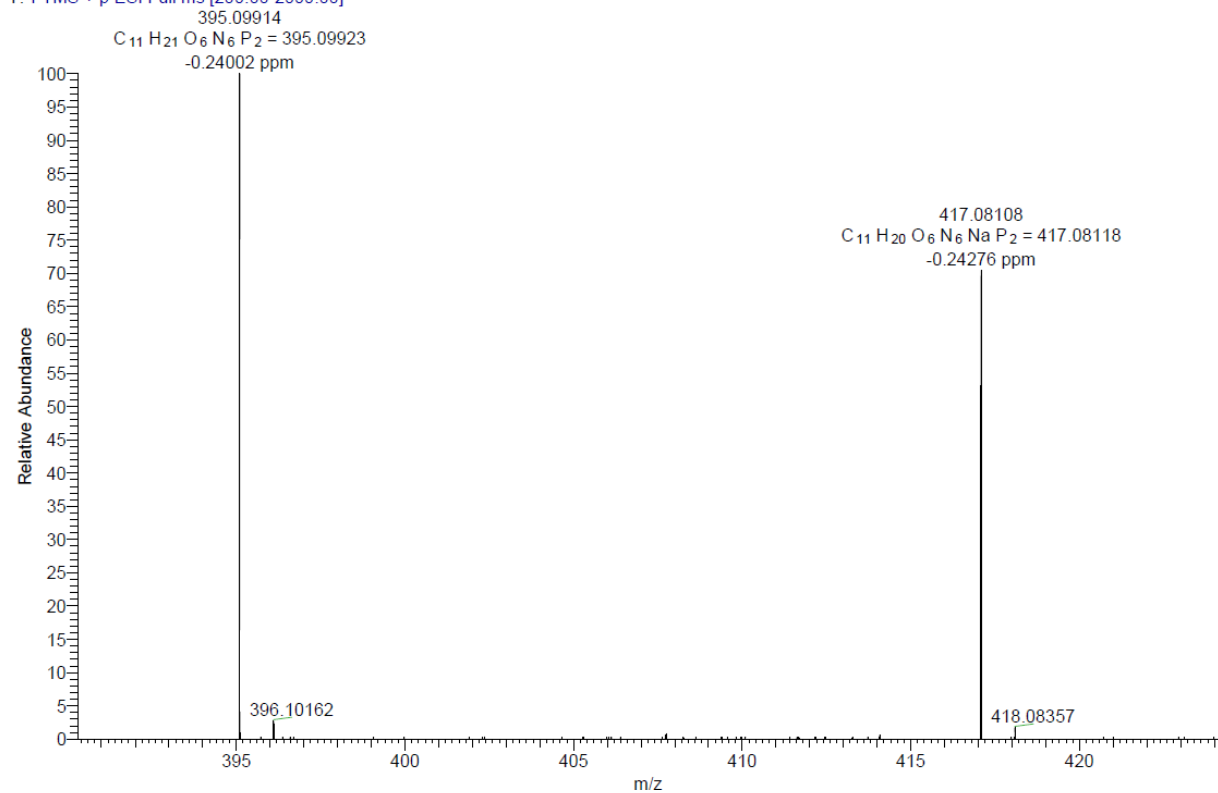


9c

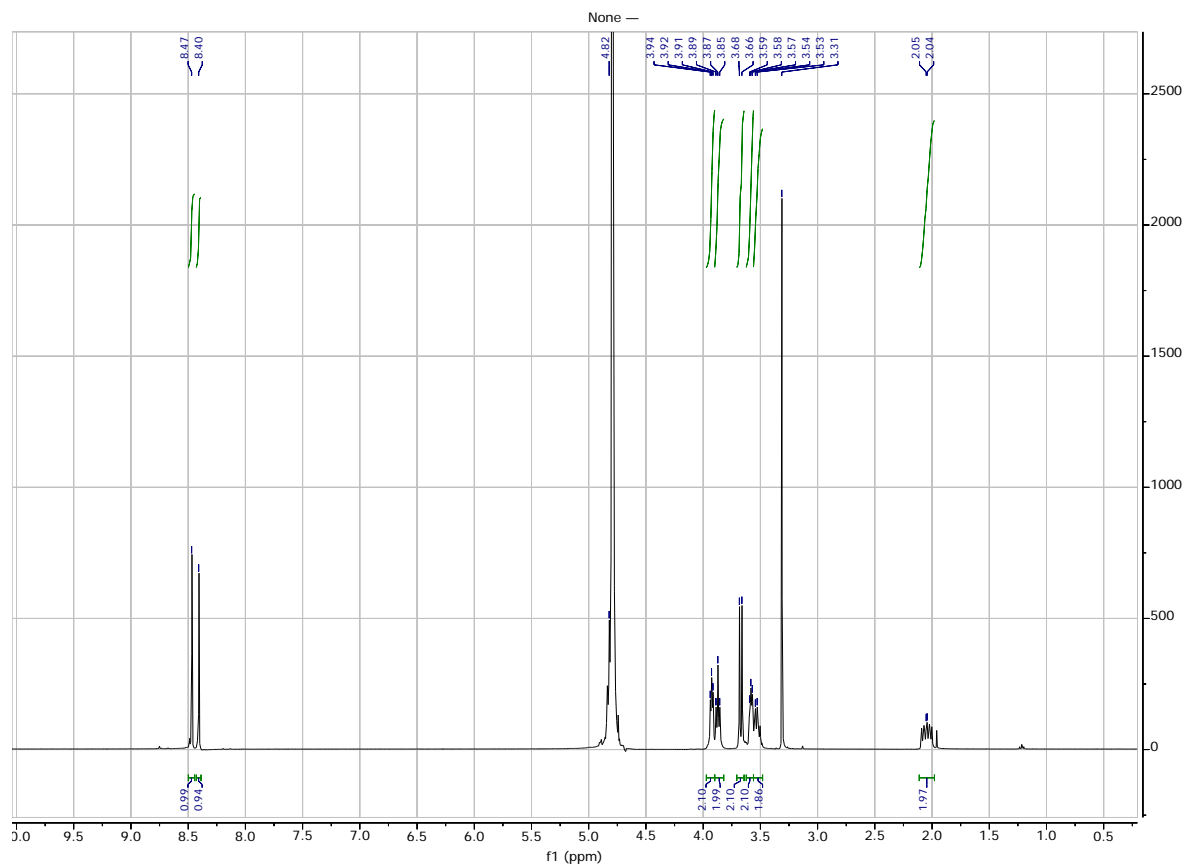


200820\_servisHR\_+21 #67-71 RT: 1.78-1.89 AV: 5 NL: 7.06E5

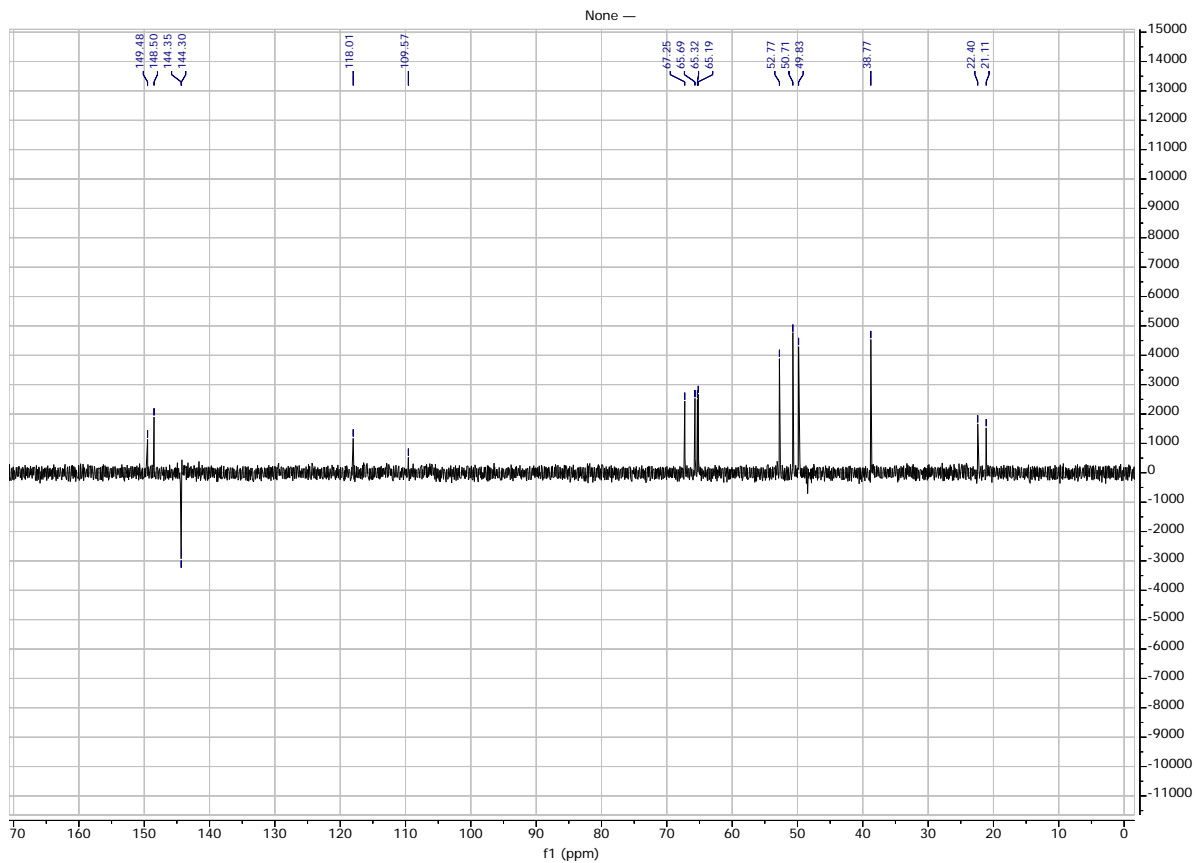
T: FTMS + p ESI Full ms [200.00-2000.00]



9d





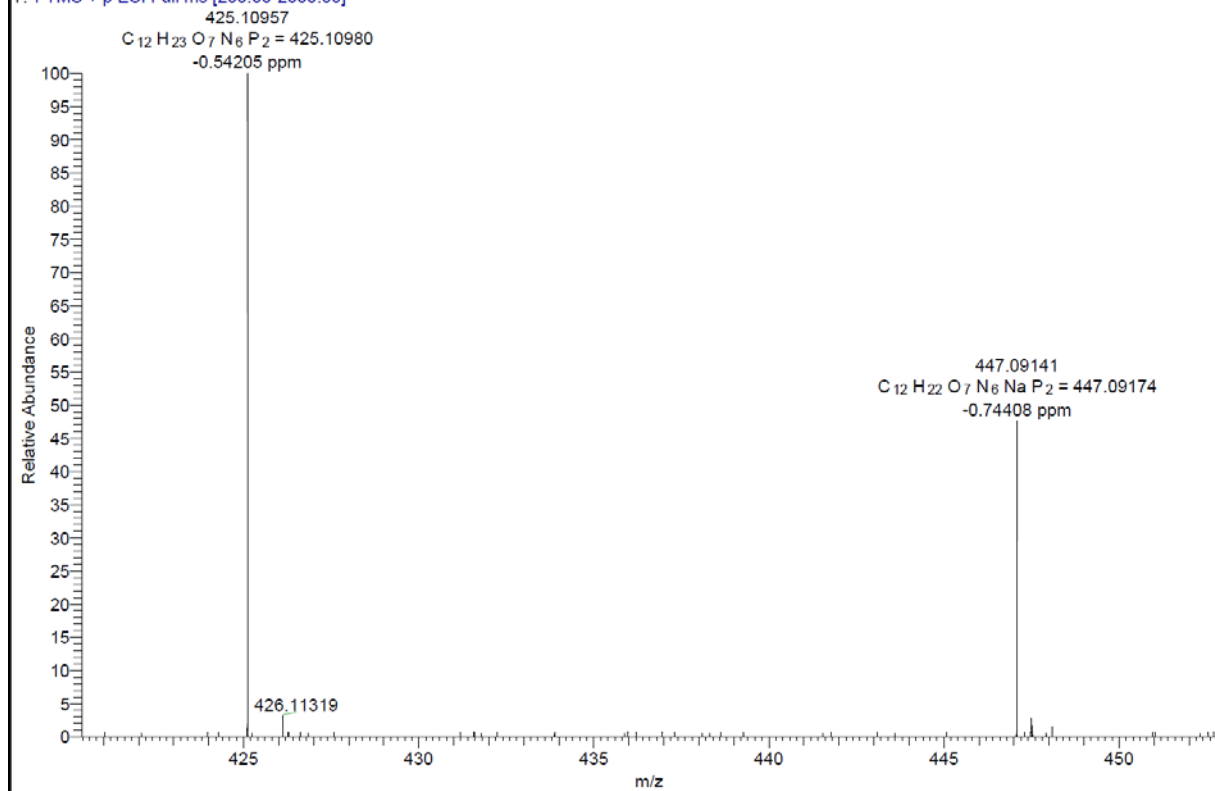


Z:\SERVISHR\...200820\_servisHR\_+20

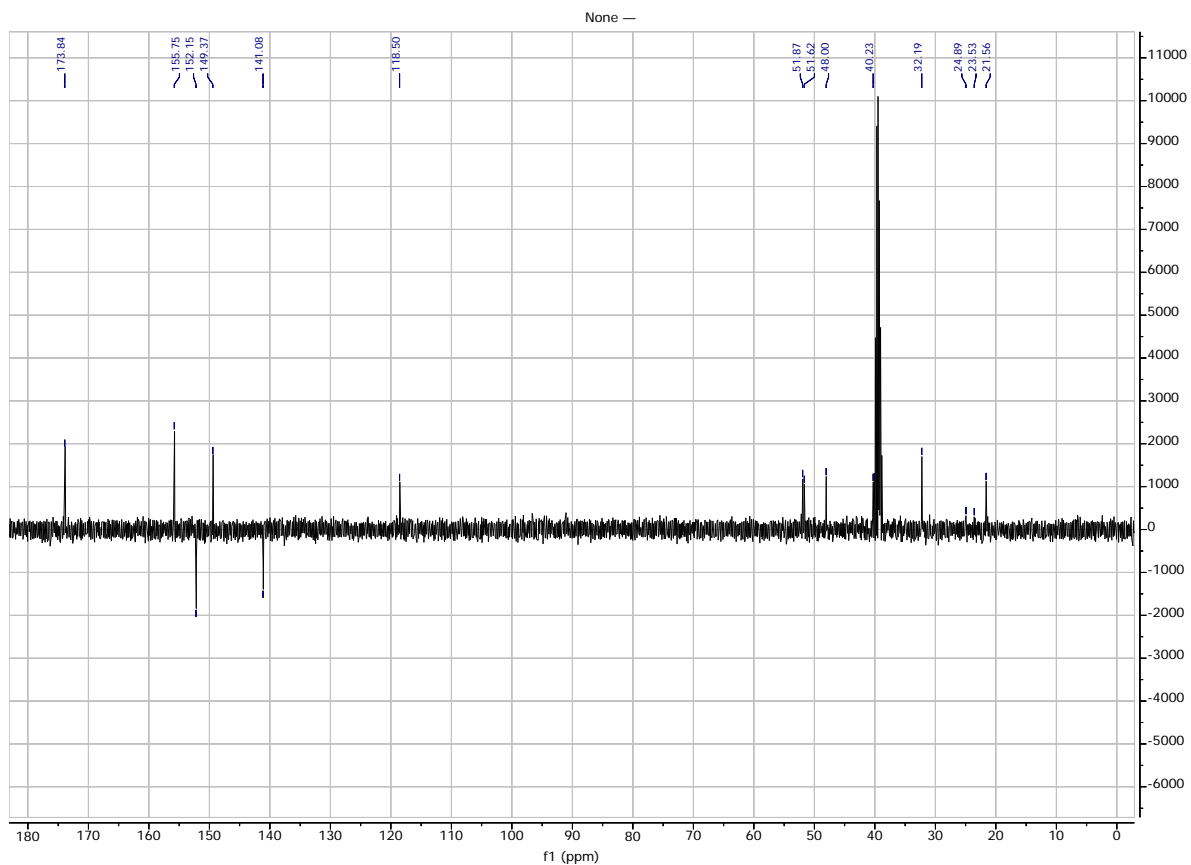
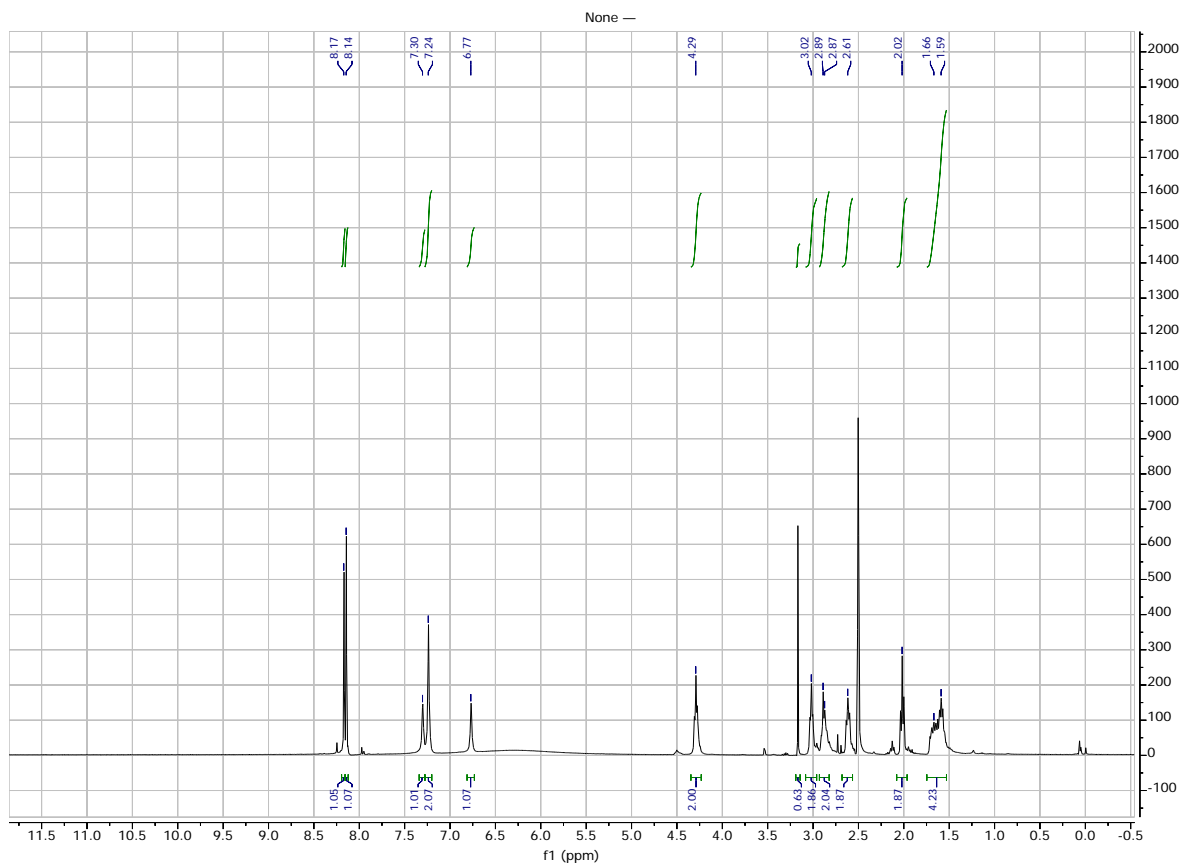
08/20/20 16:08:53

Hockova, DA-XIII-48

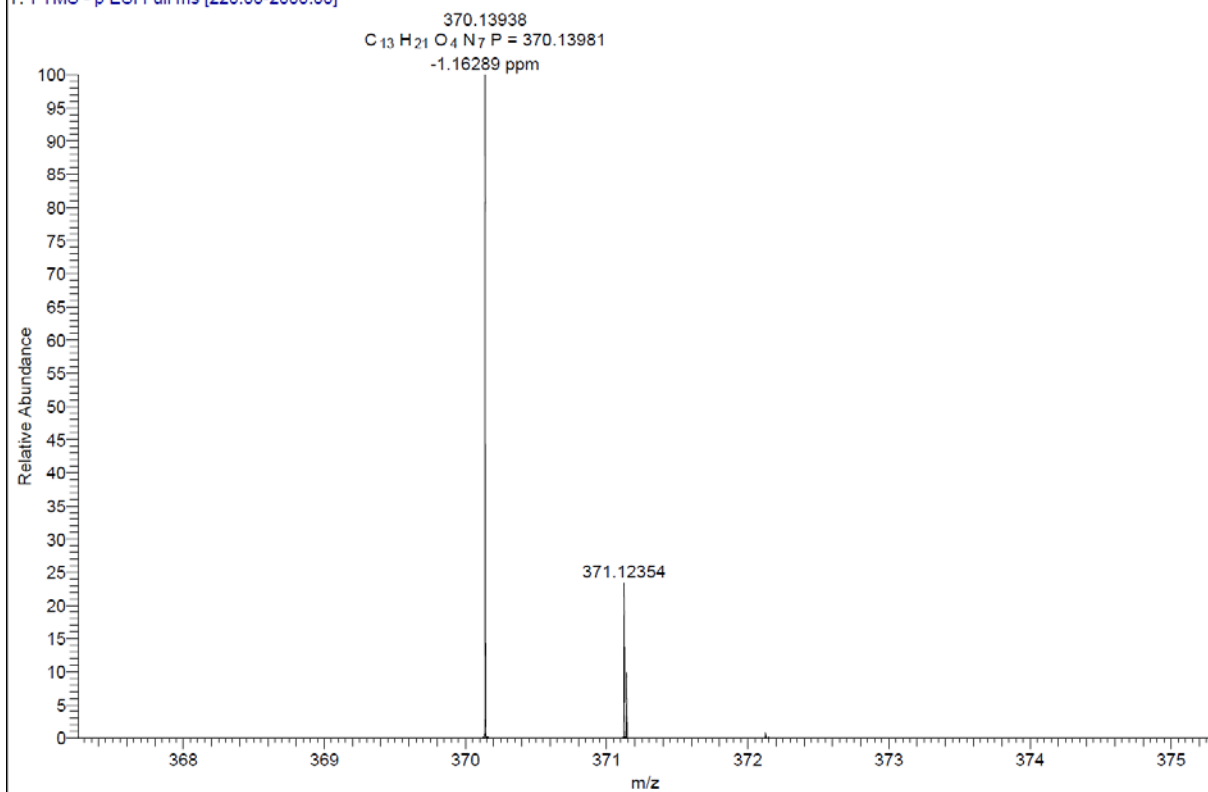
200820\_servisHR\_+20 #84-88 RT: 2.24-2.35 AV: 5 NL: 3.16E5  
T: FTMS + p ESI Full ms [200.00-2000.00]



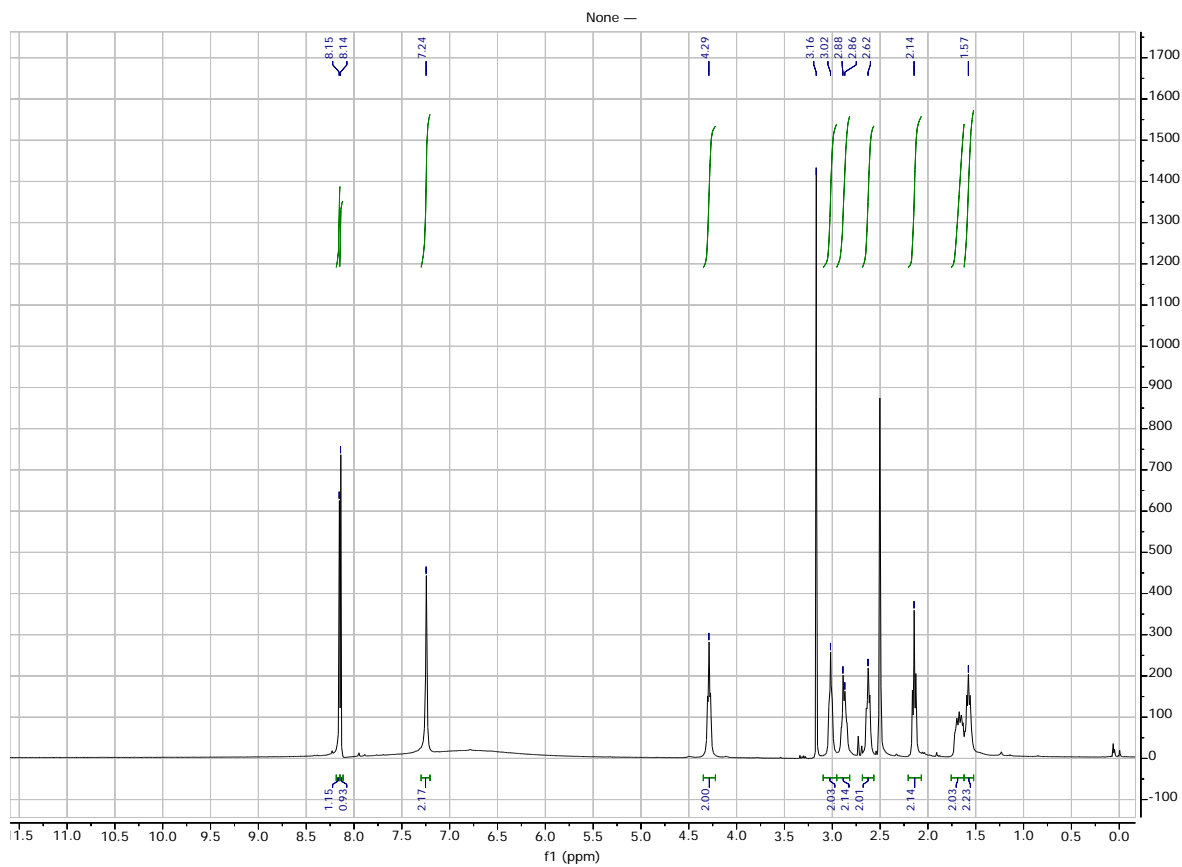
9e

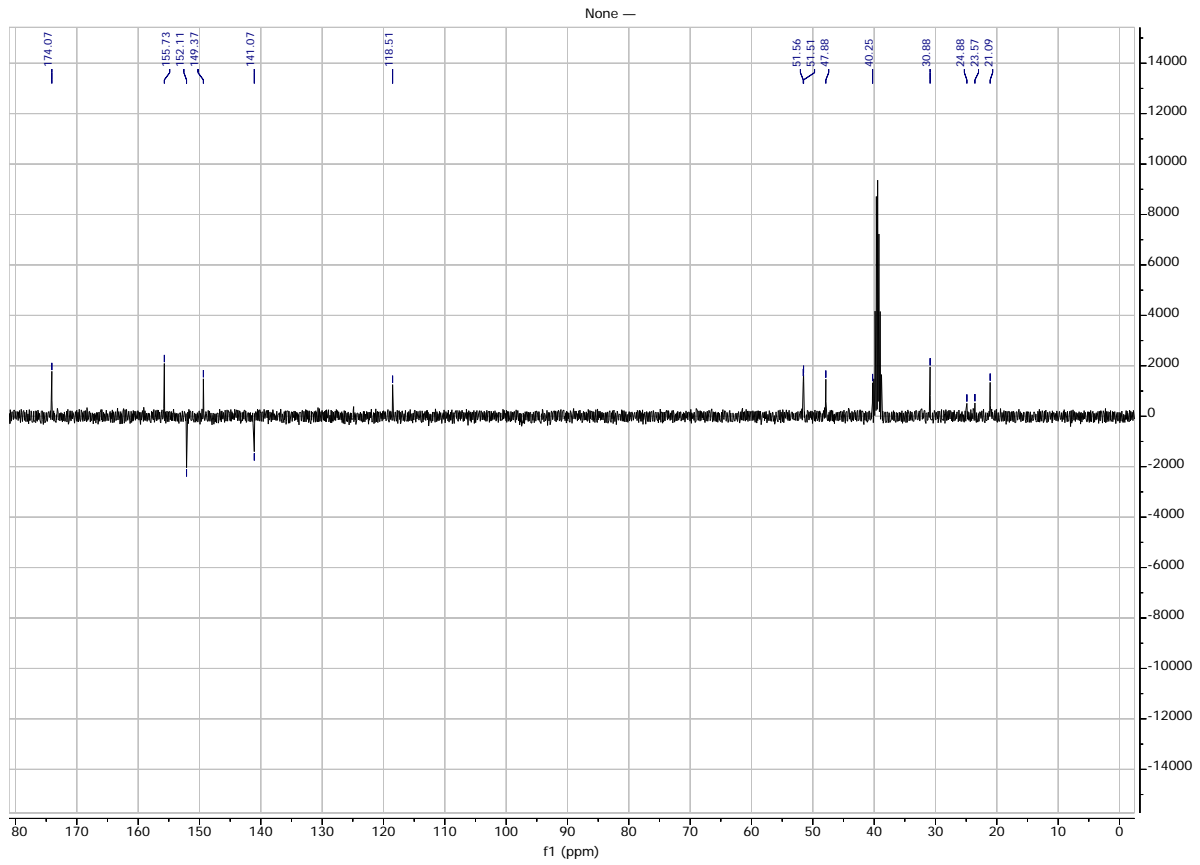


210820\_servisHR\_-3\_200821120937 #34-35 RT: 1.91-1.97 AV: 2 SB: 44 0.04-1.62, 0.10-0.98 NL: 7.30E5  
T: FTMS - p ESI Full ms [220.00-2000.00]



9f



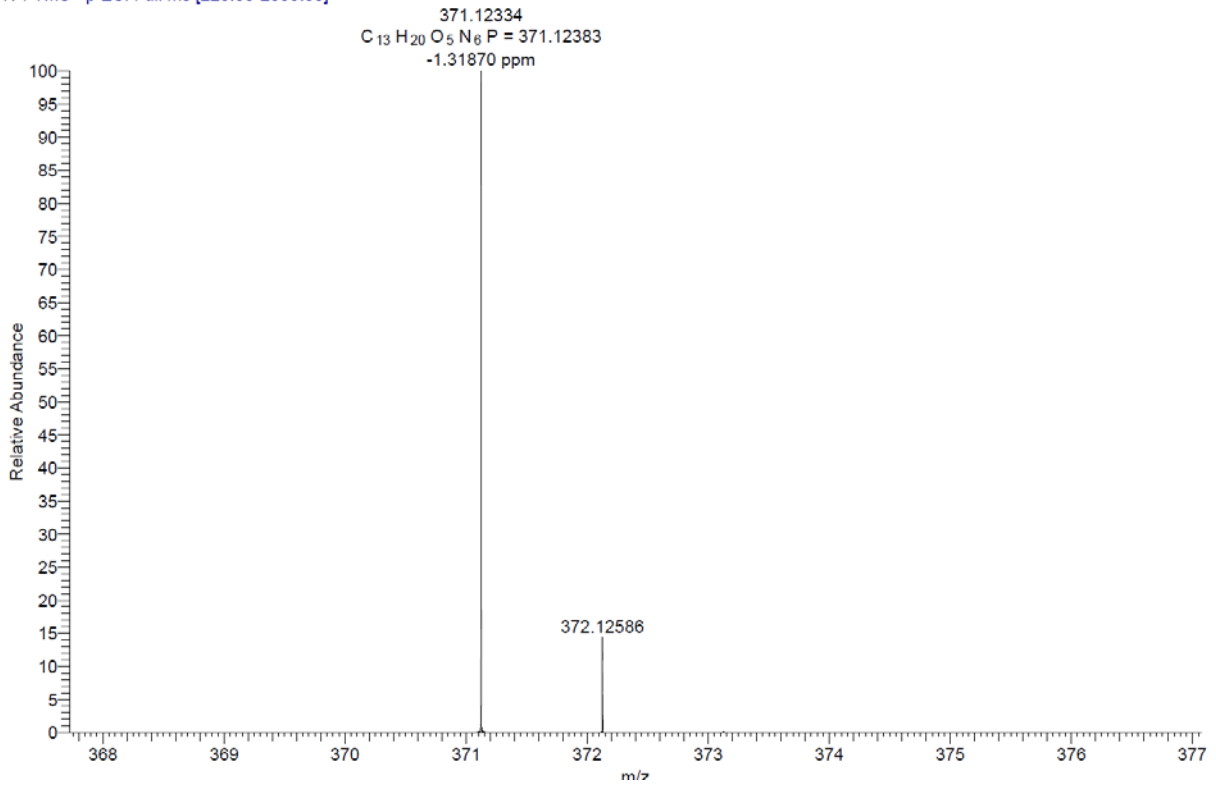


210820\_servisHR\_-2\_200821120531

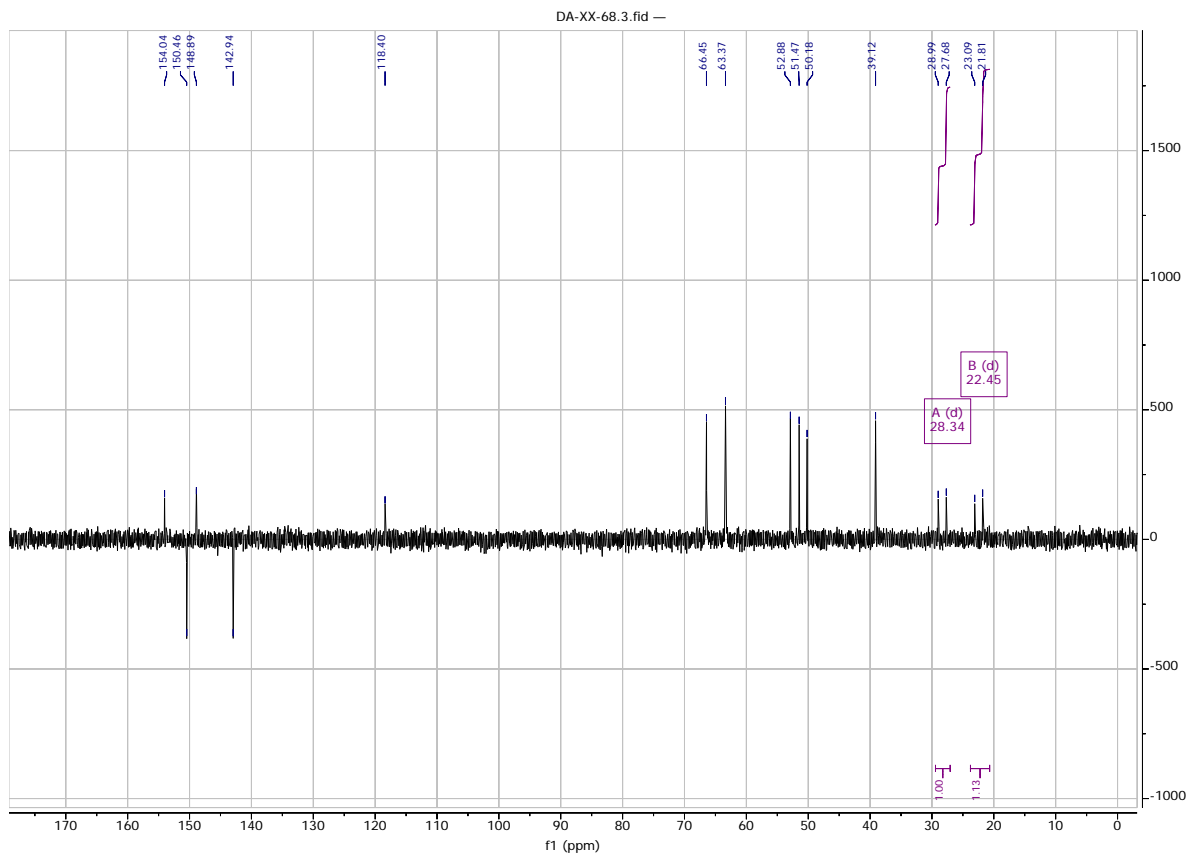
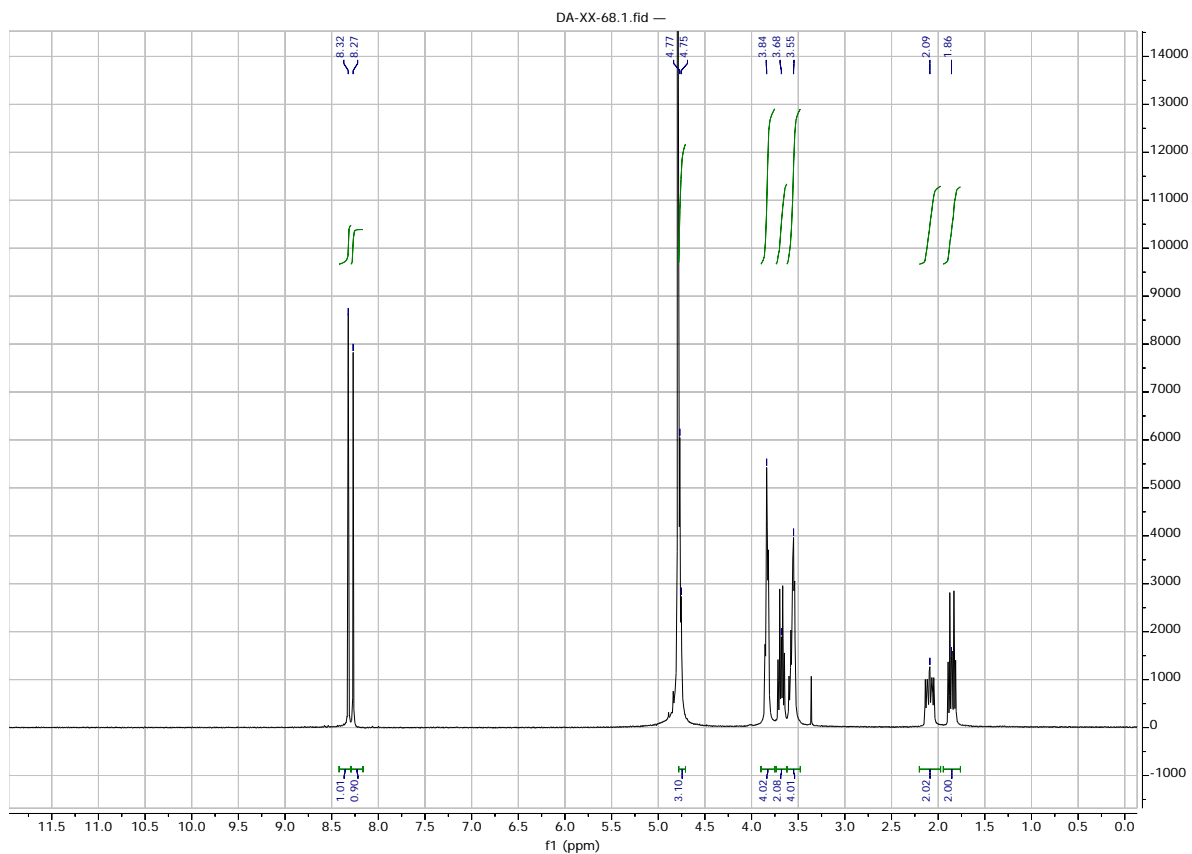
08/21/20 12:05:31

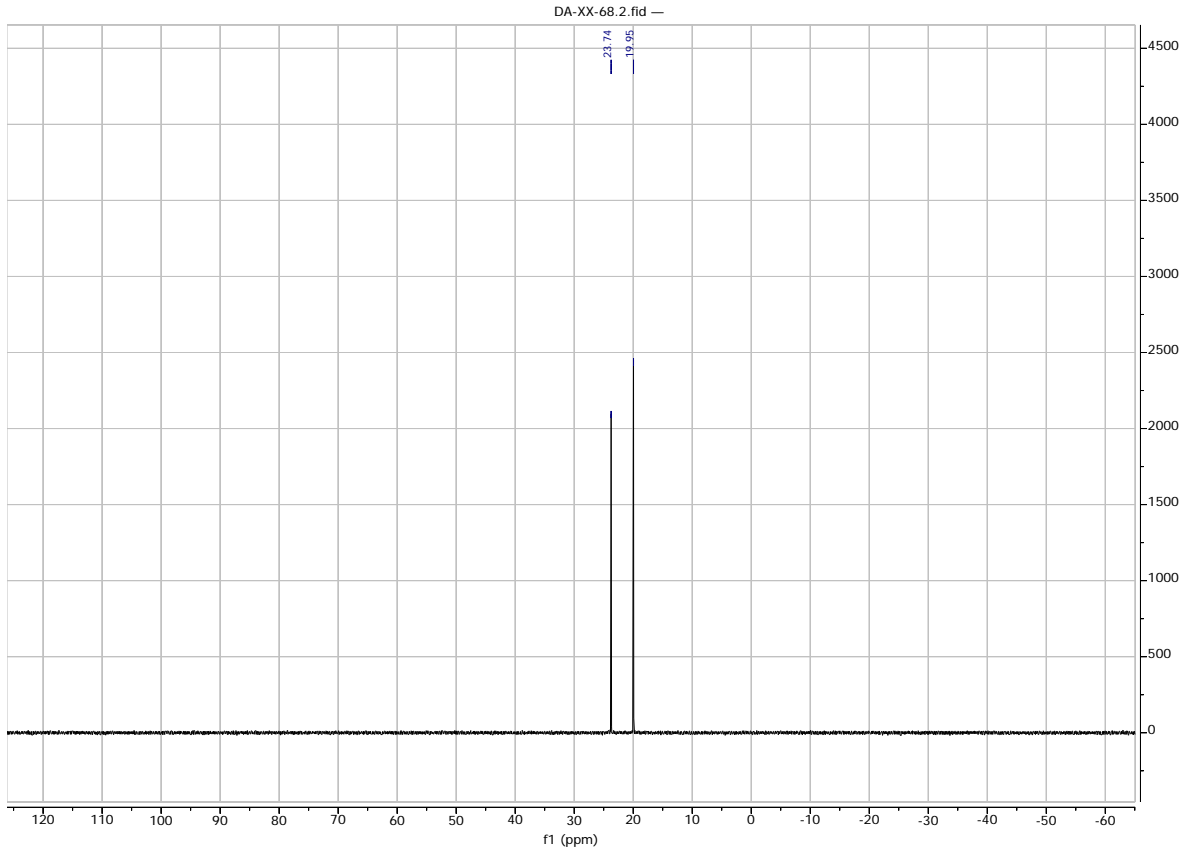
Hockova, DA-XII-54/3

210820\_servisHR\_-2\_200821120531 #32-33 RT: 1.79-1.85 AV: 2 NL: 2.06E6  
T: FTMS - p ESI Full ms [220.00-2000.00]



9g



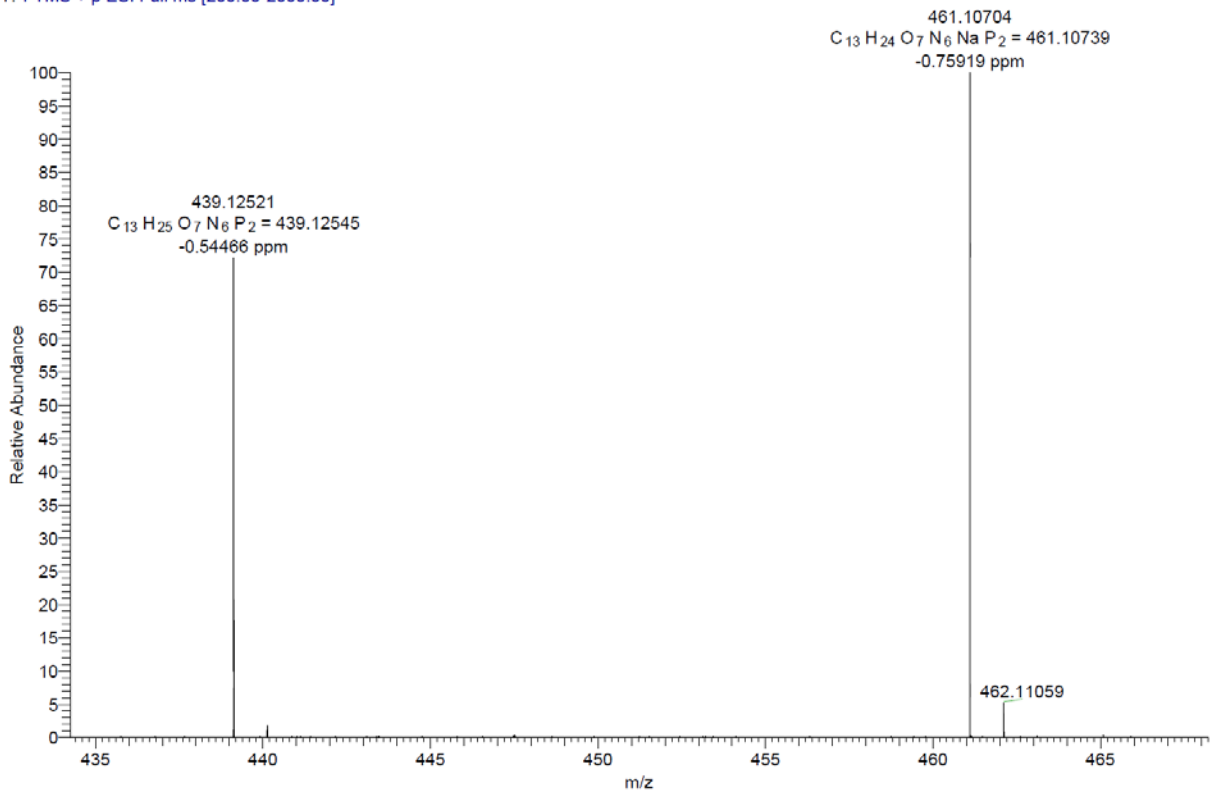


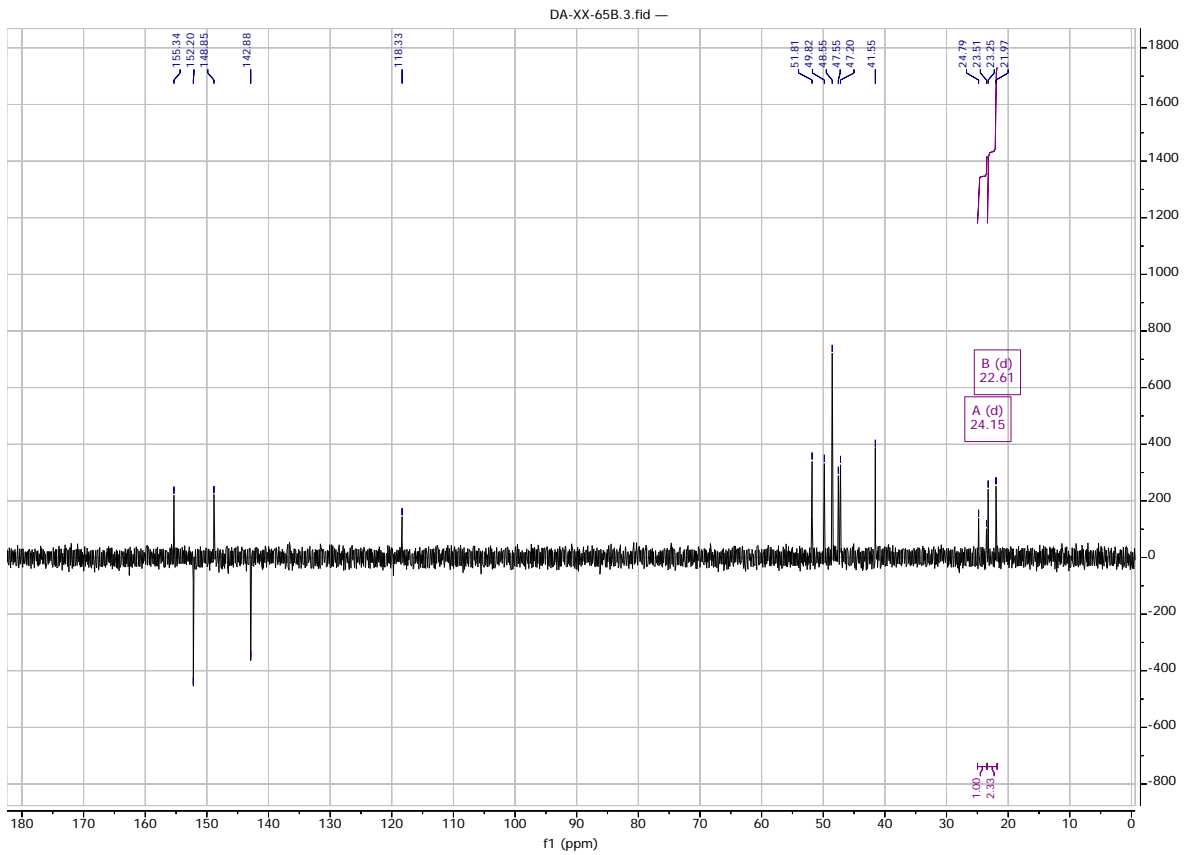
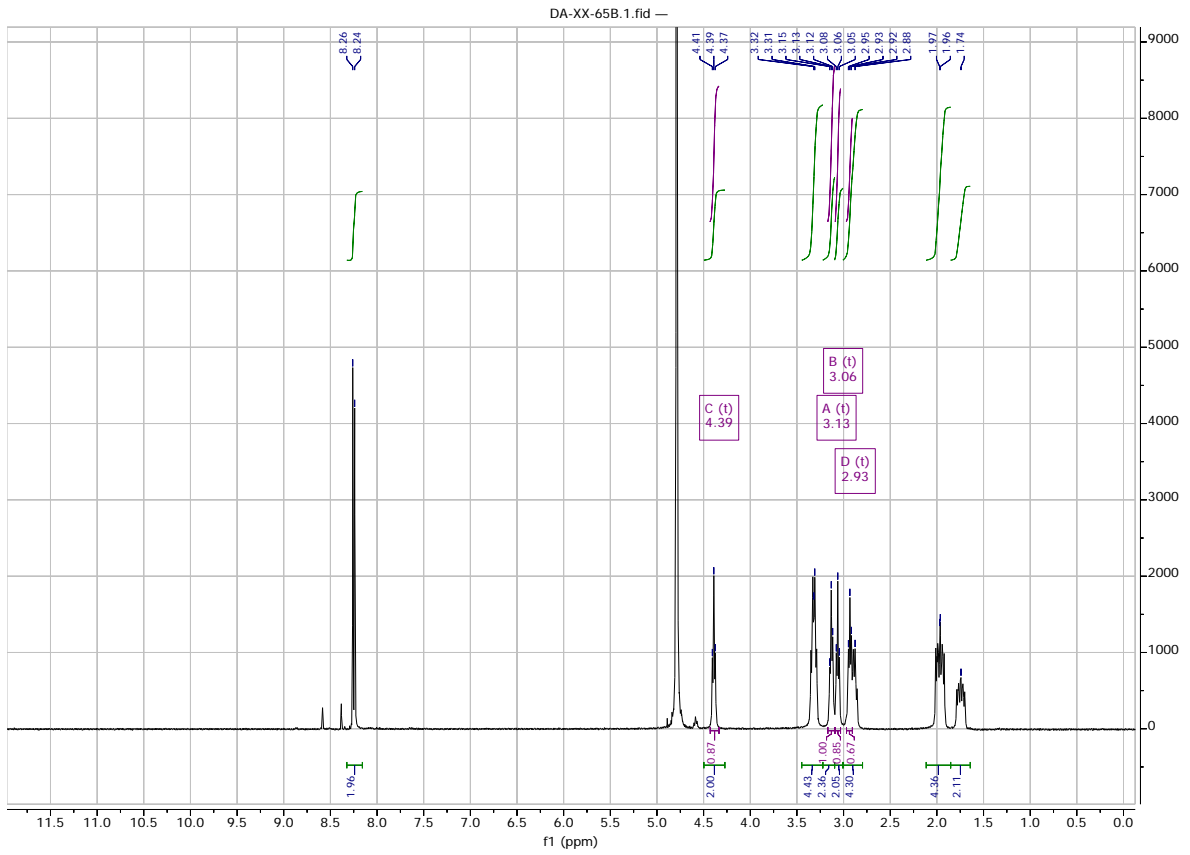
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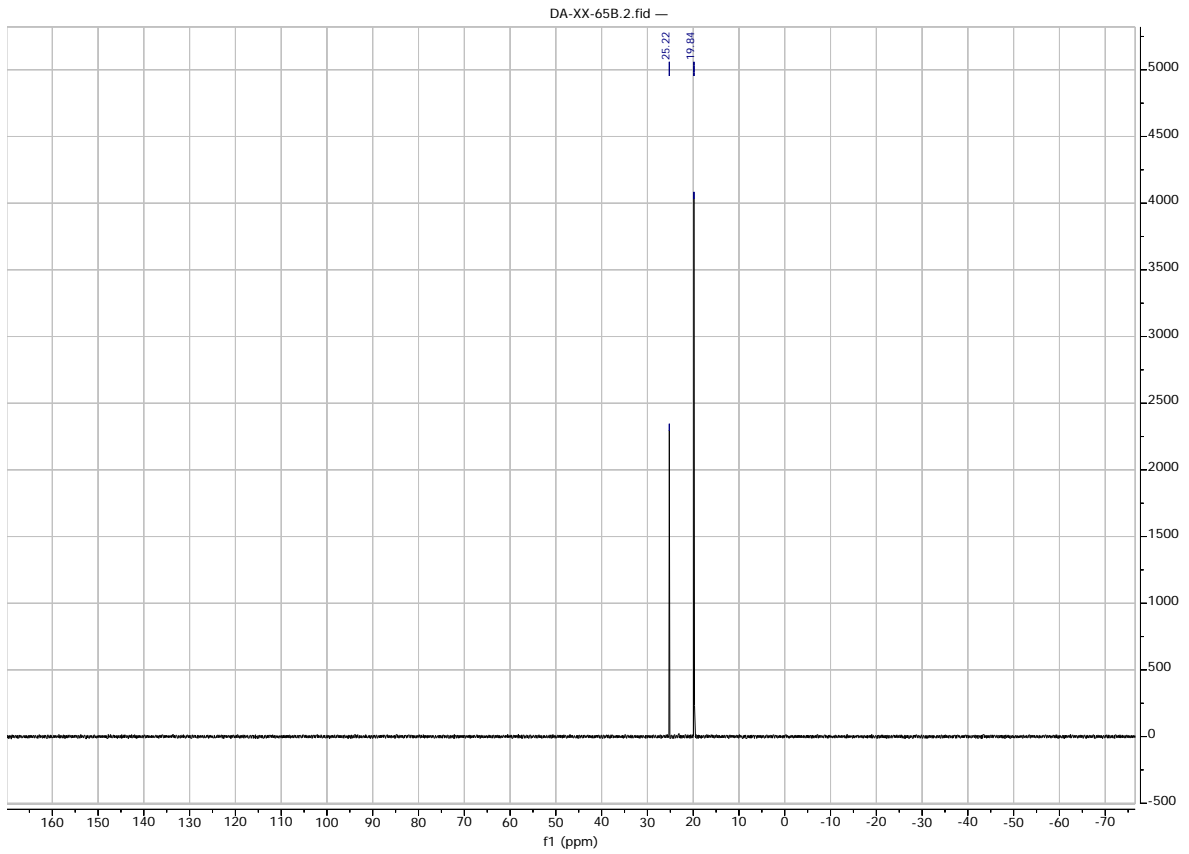
08/20/20 16:00:44

Hockova, DA-XX-68

200820\_servisHR\_+18 #70-73 RT: 1.86-1.94 AV: 4 NL: 2.62E6  
T: FTMS + p ESI Full ms [200.00-2000.00]





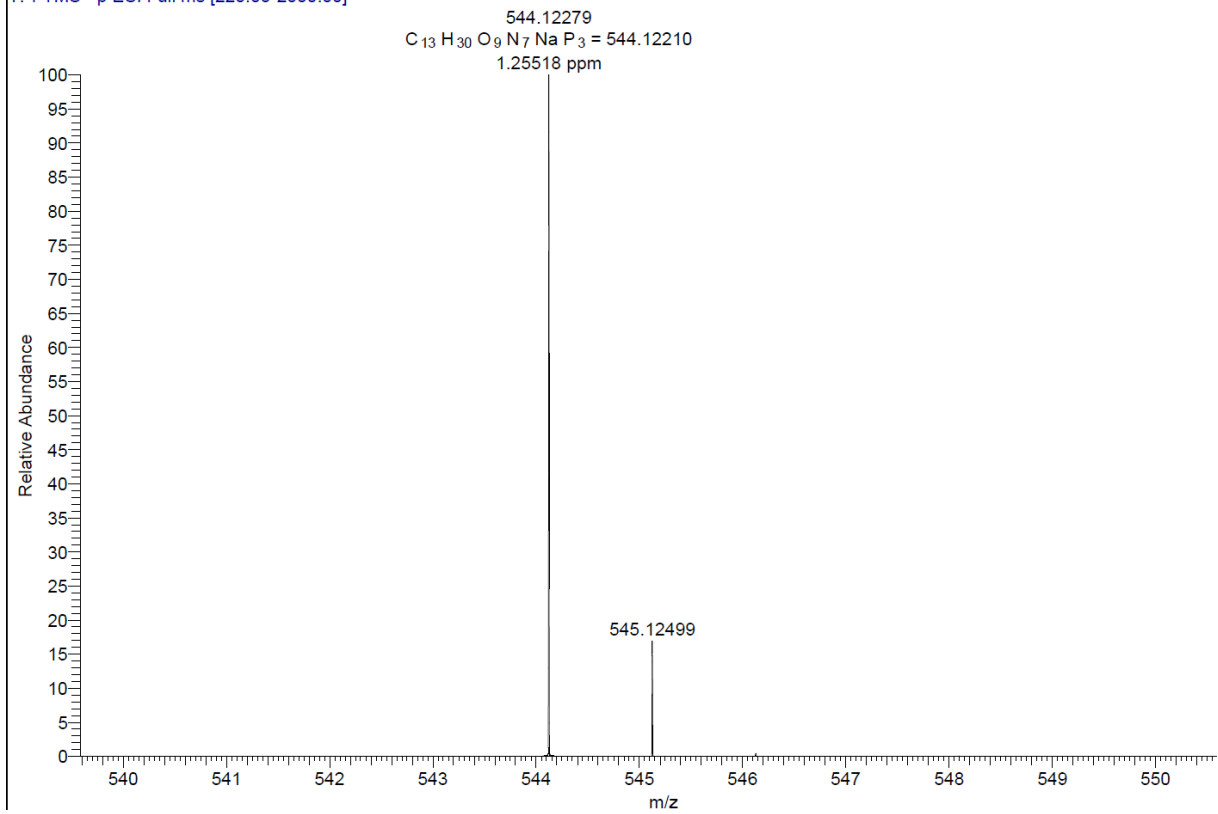


210820\_servisHR\_4\_200821121341

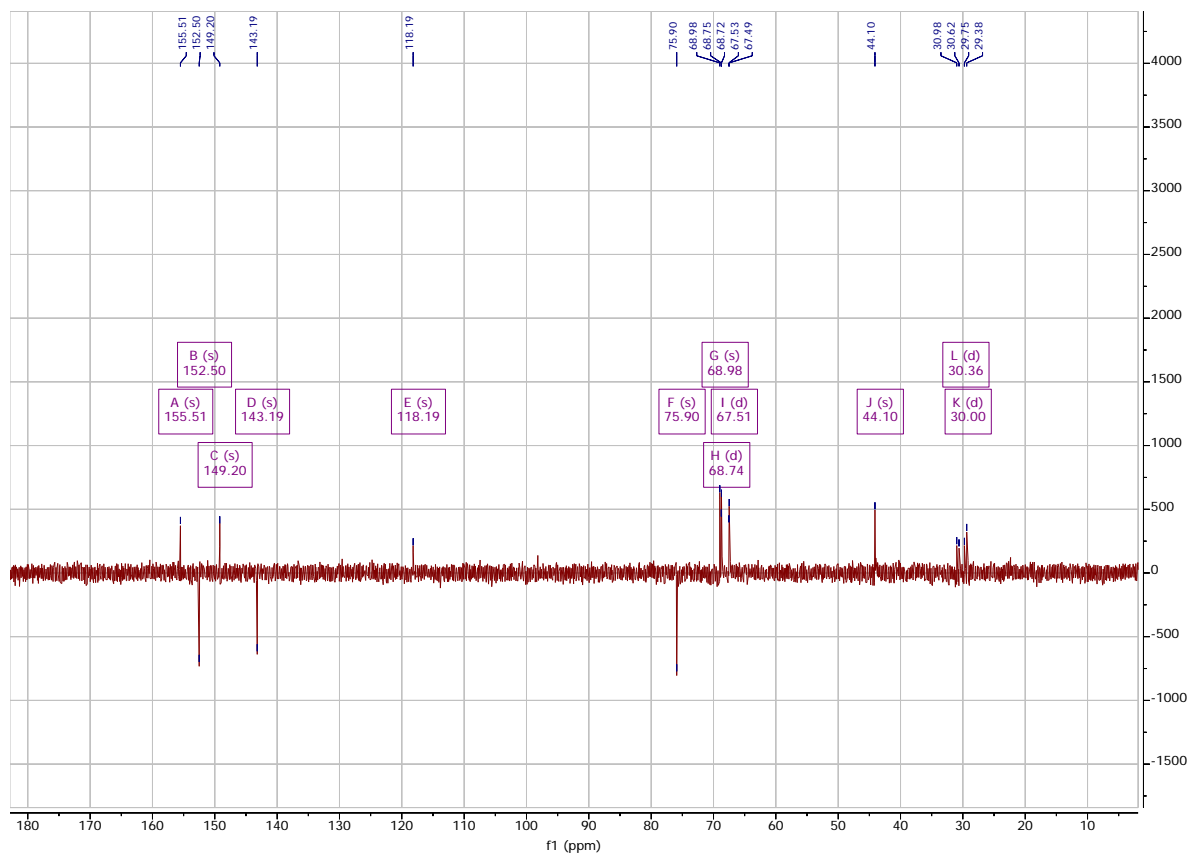
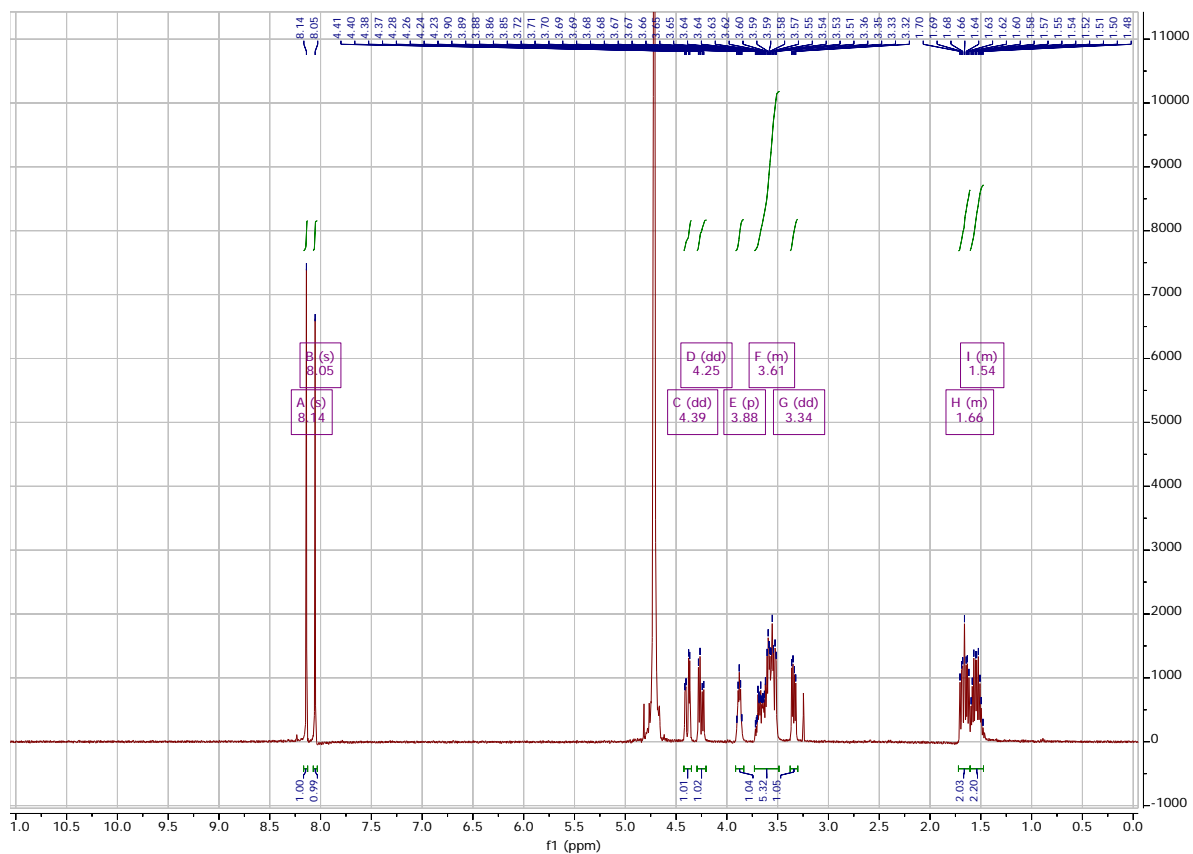
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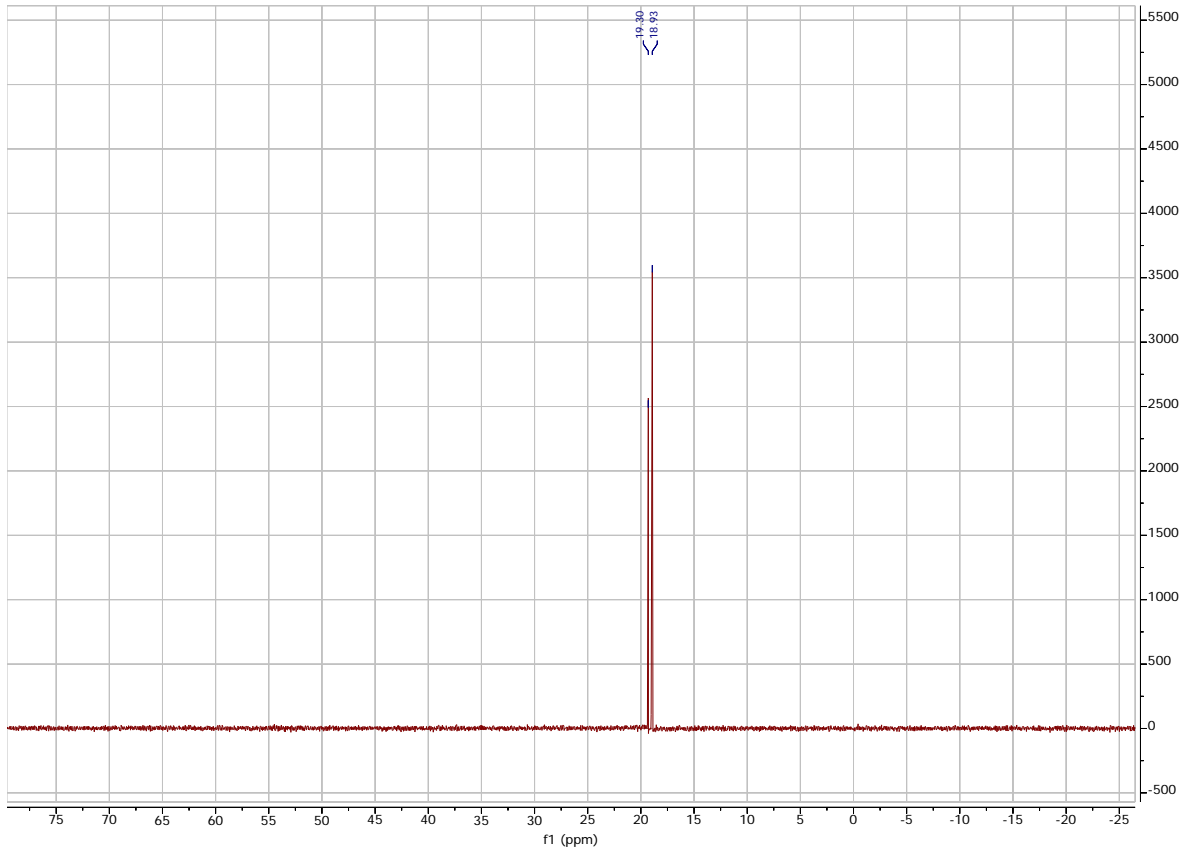
Hockova, DA-XX-65

210820\_servisHR\_4\_200821121341 #34-35 RT: 1.91-1.97 AV: 2 SB: 41 0.01-1.56, 0.04-0.74 NL: 2.00E6  
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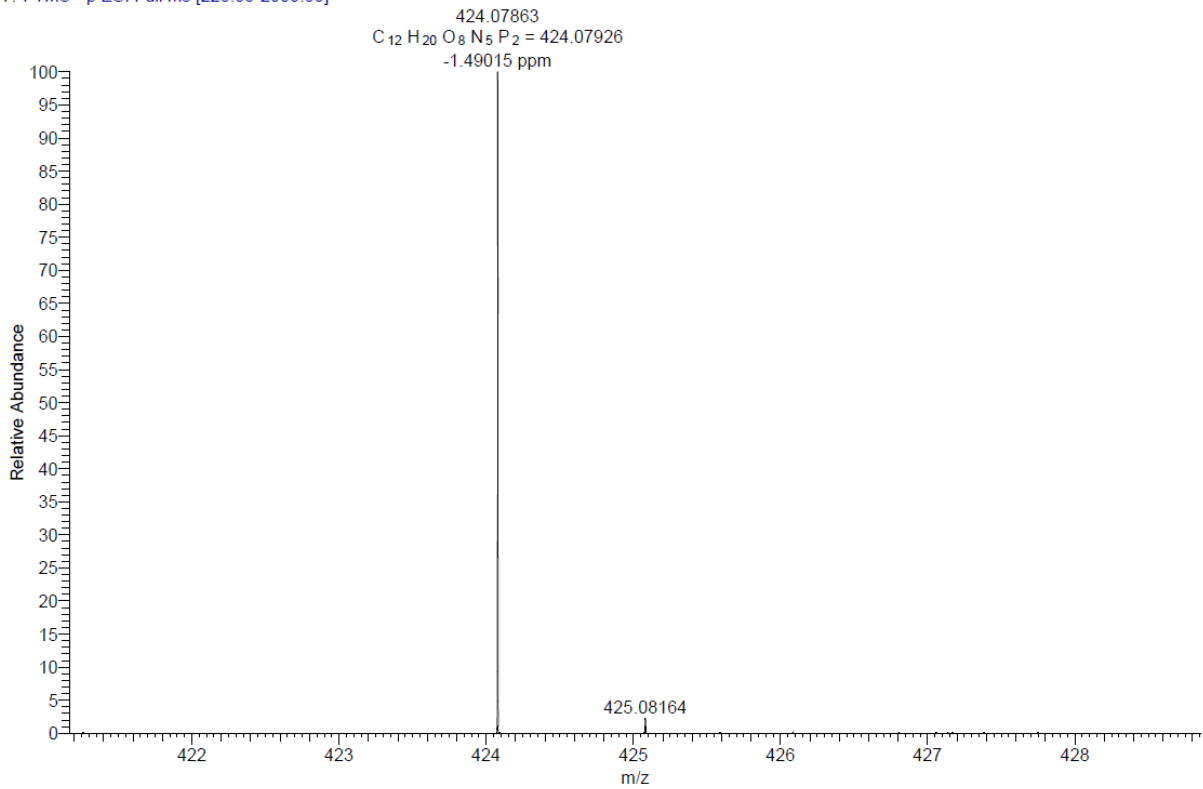


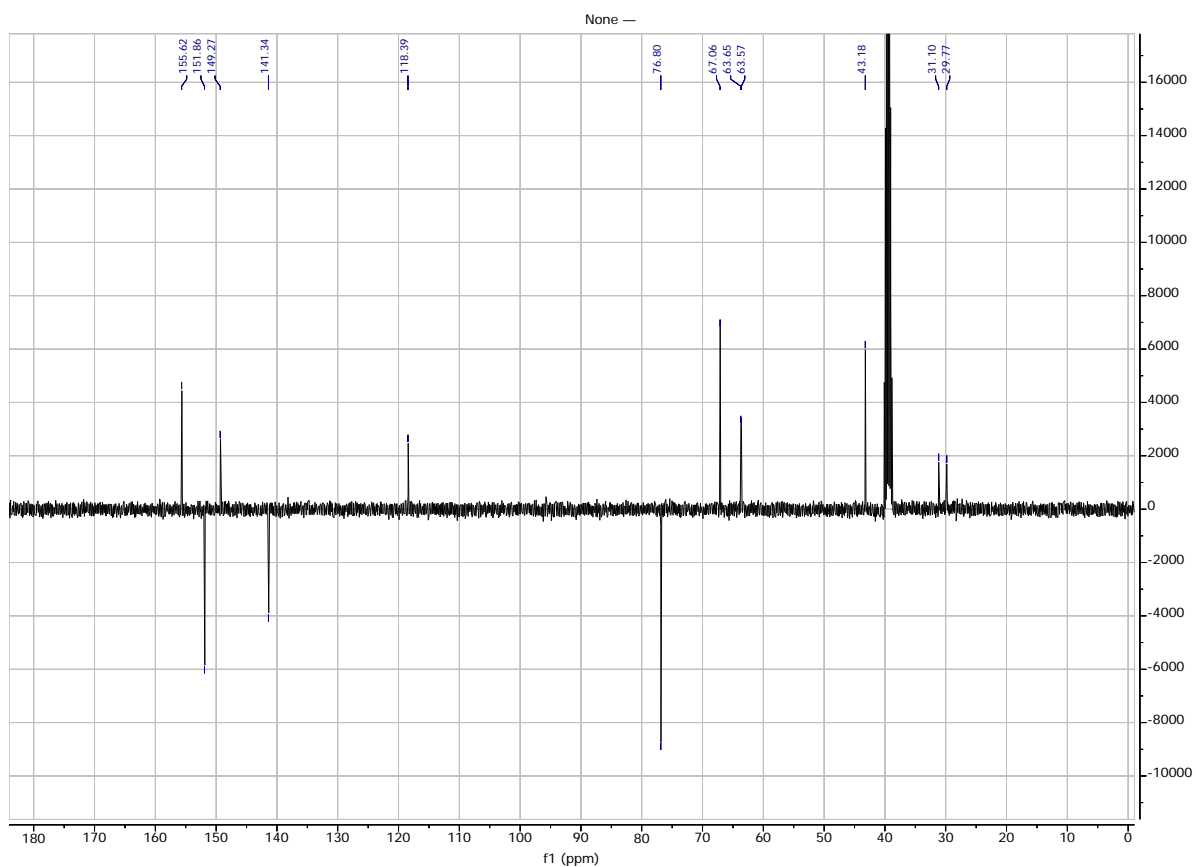
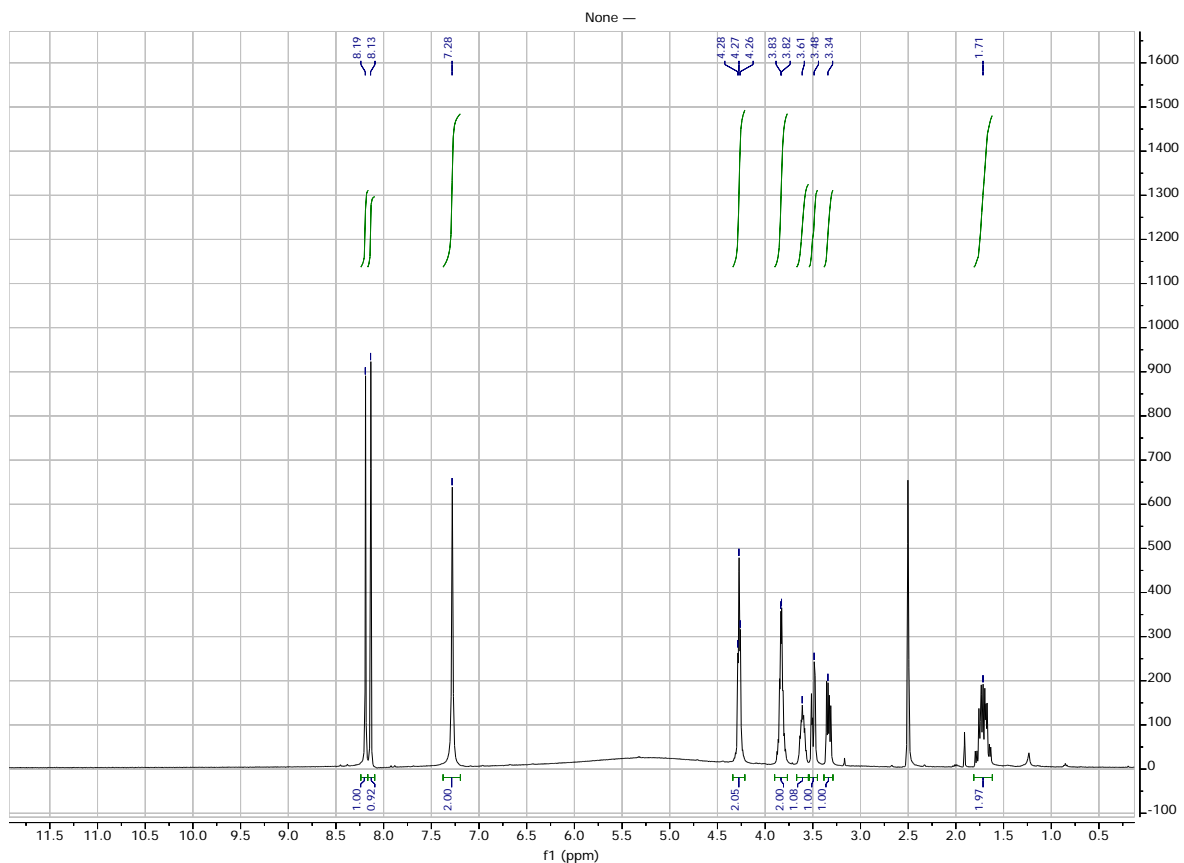
210820\_servisHR\_-5\_200821121746

08/21/20 12:17:46

Hockova, PP-P372

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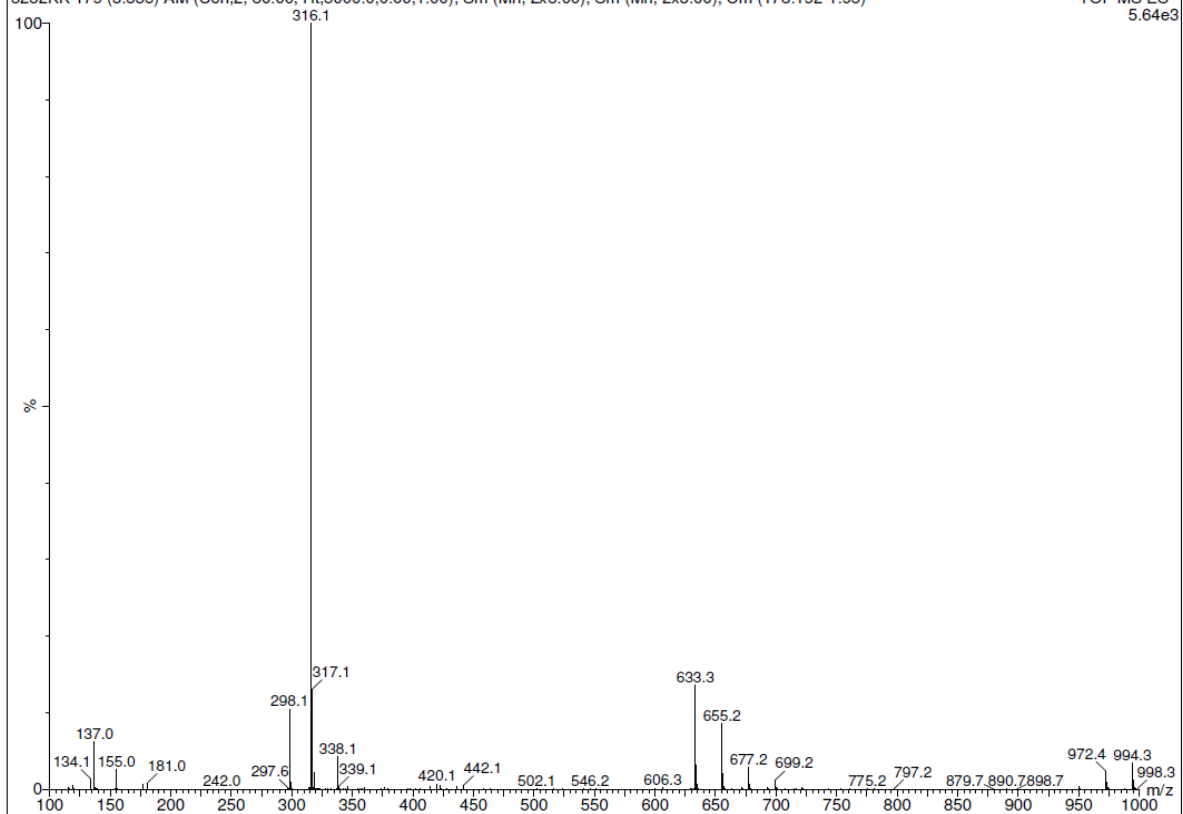




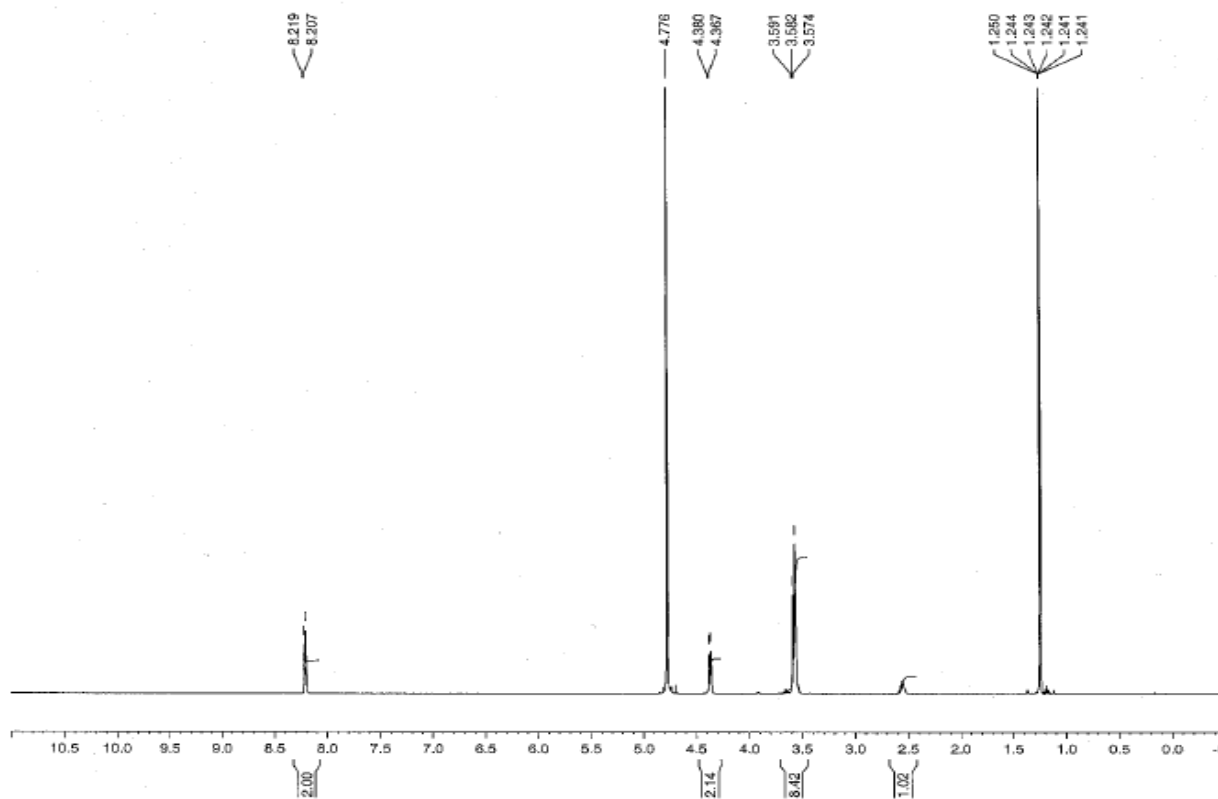
Hockova, DA-XI-3

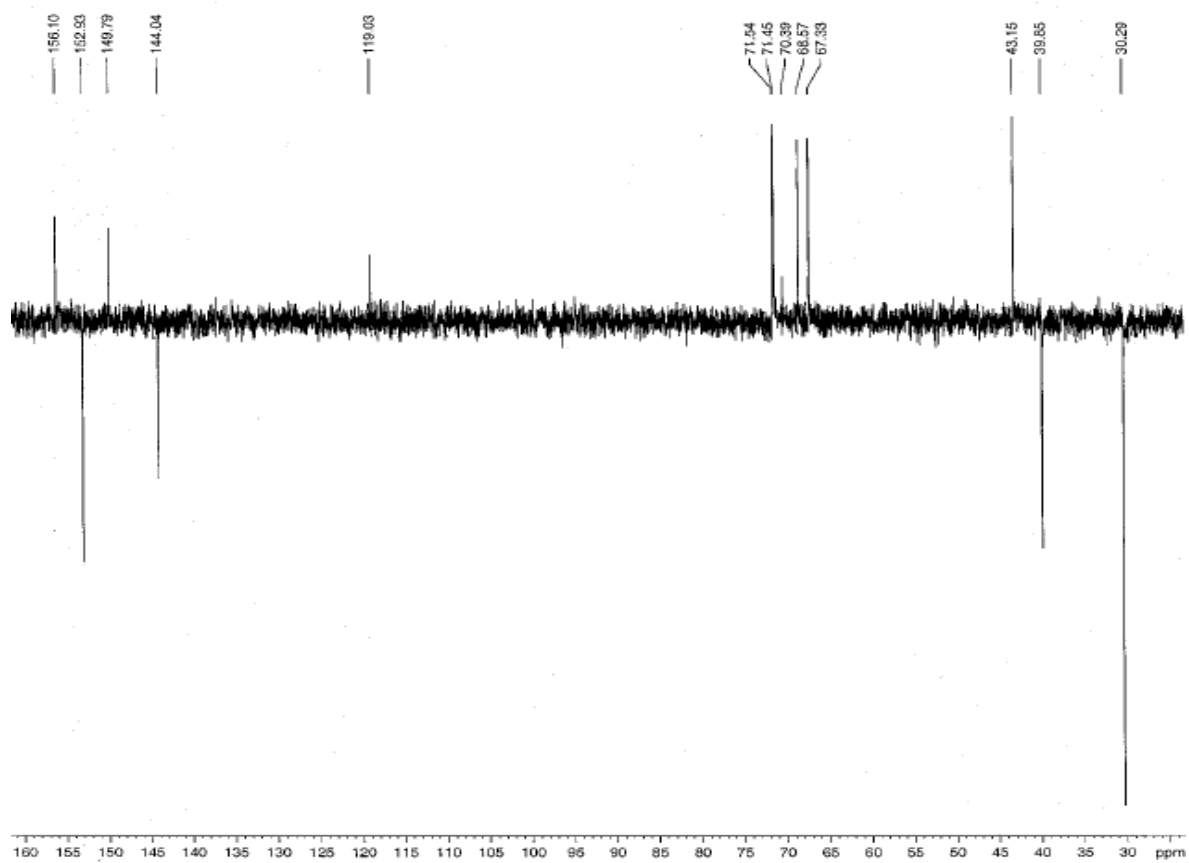
3232KK 179 (3.335) AM (Cen,2, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Sm (Mn, 2x3.00); Cm (178:192-1:95)

TOF MS ES-  
5.64e3



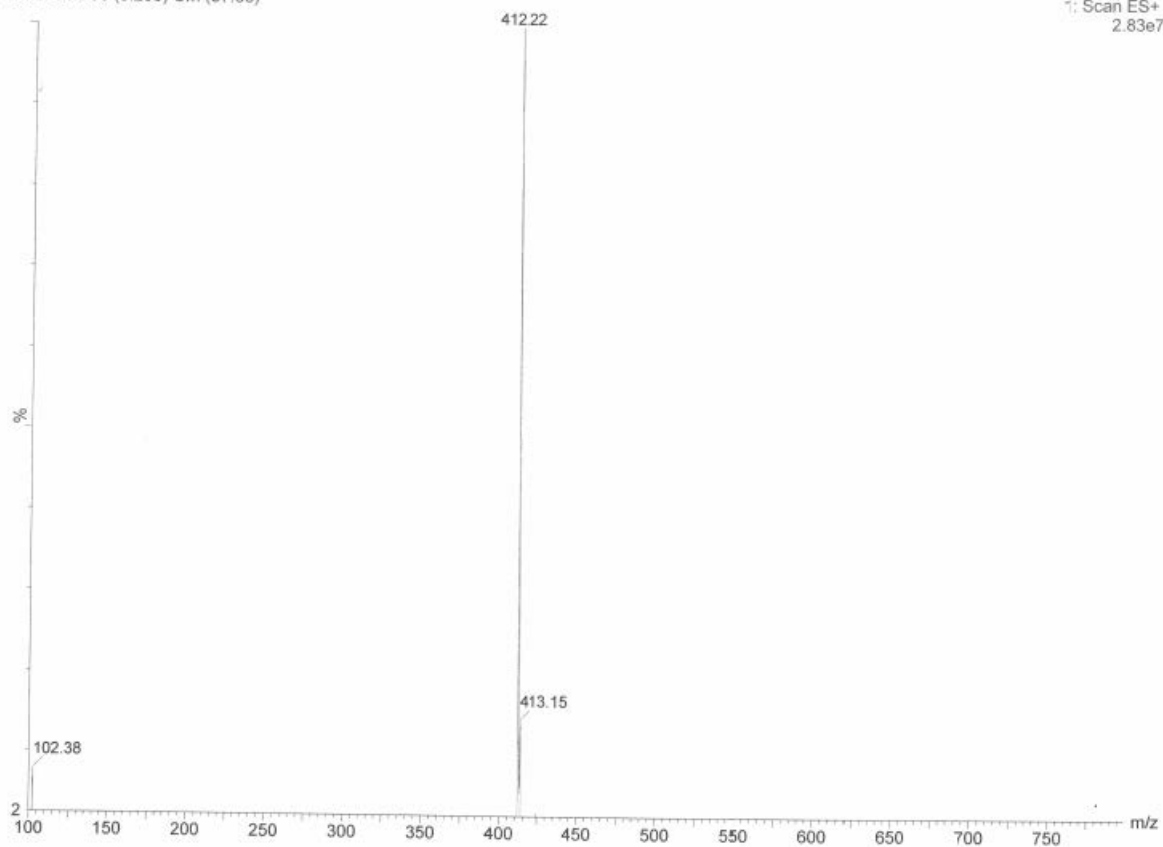
22



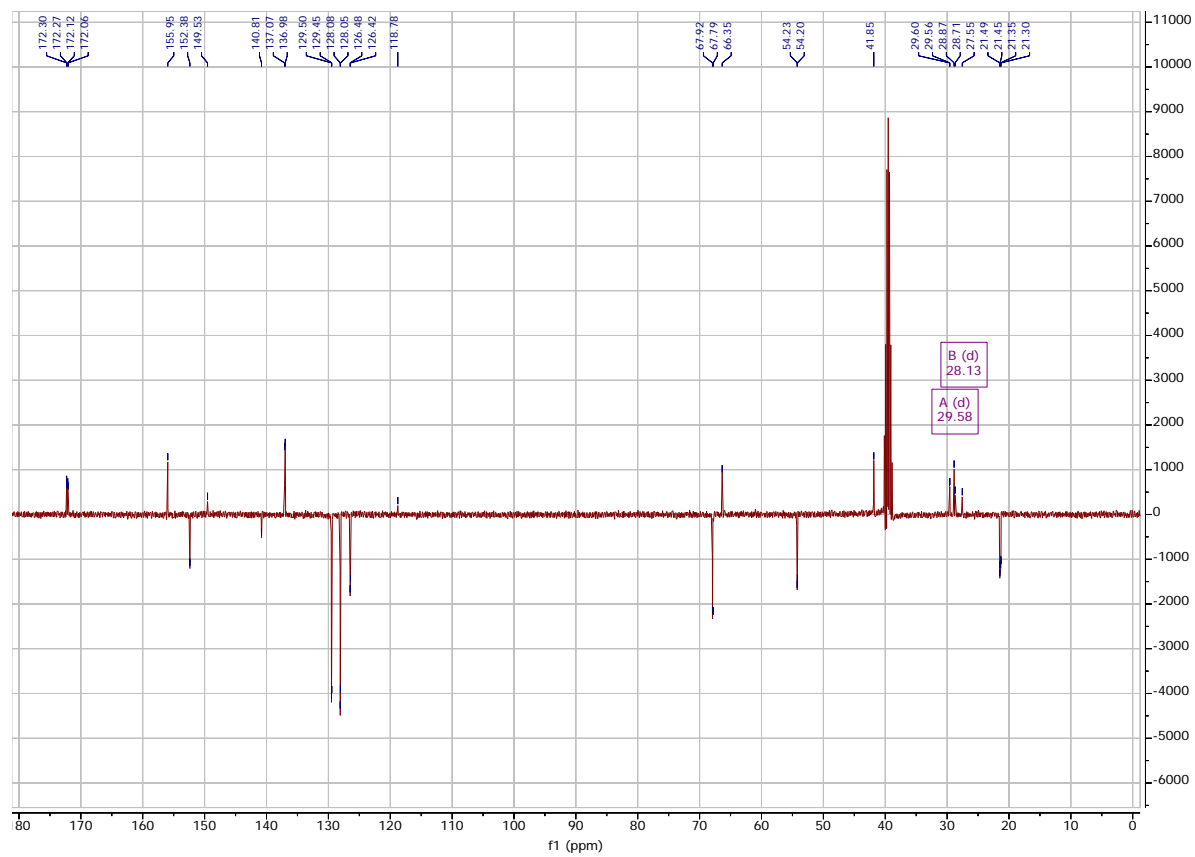
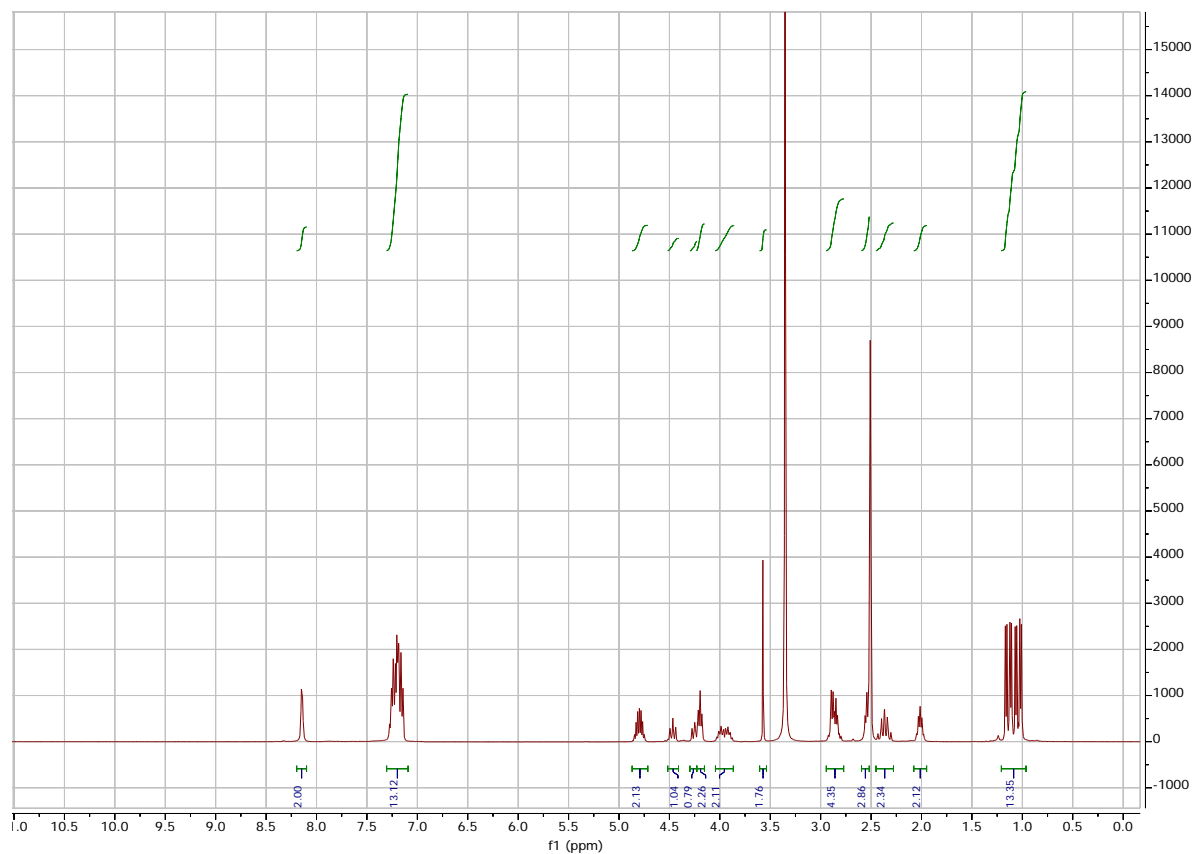


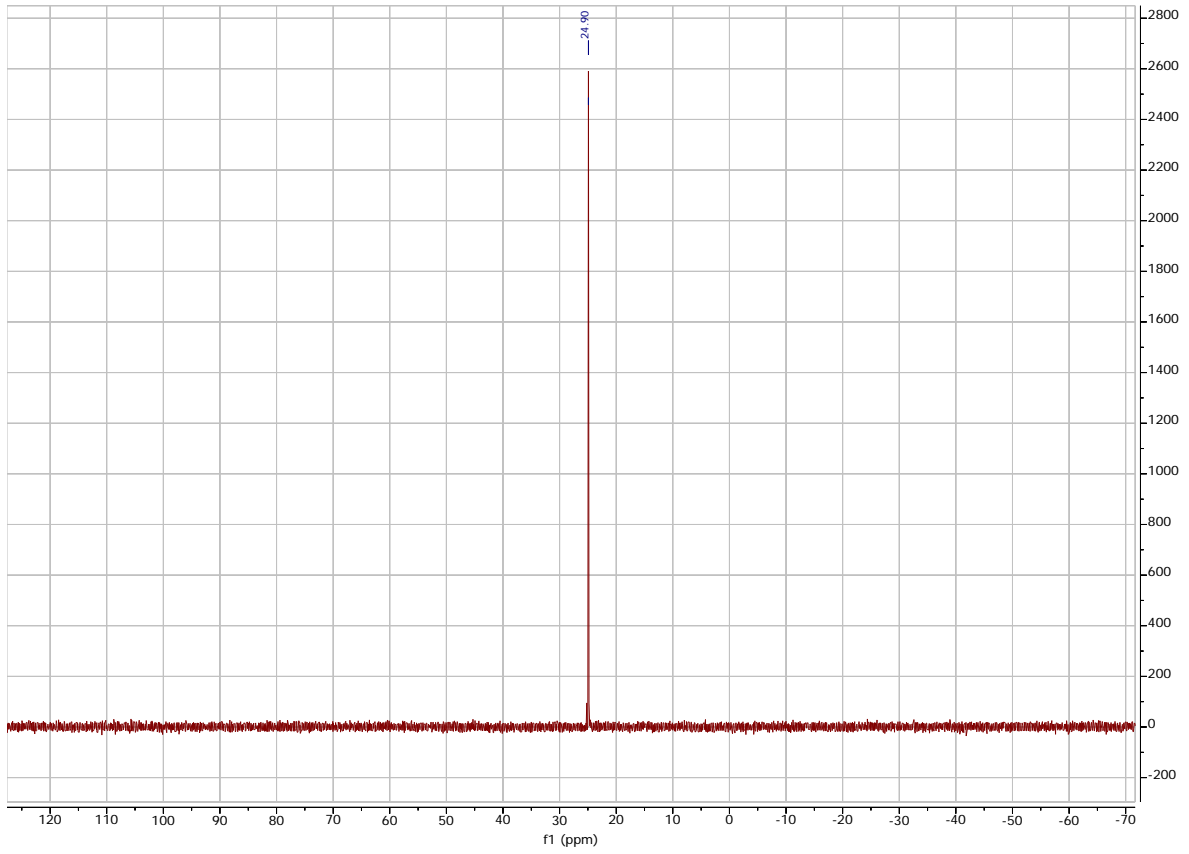
DA-SV-280 60 (0.259) Cm (57.63)

1: Scan ES+  
2.83e7



24a



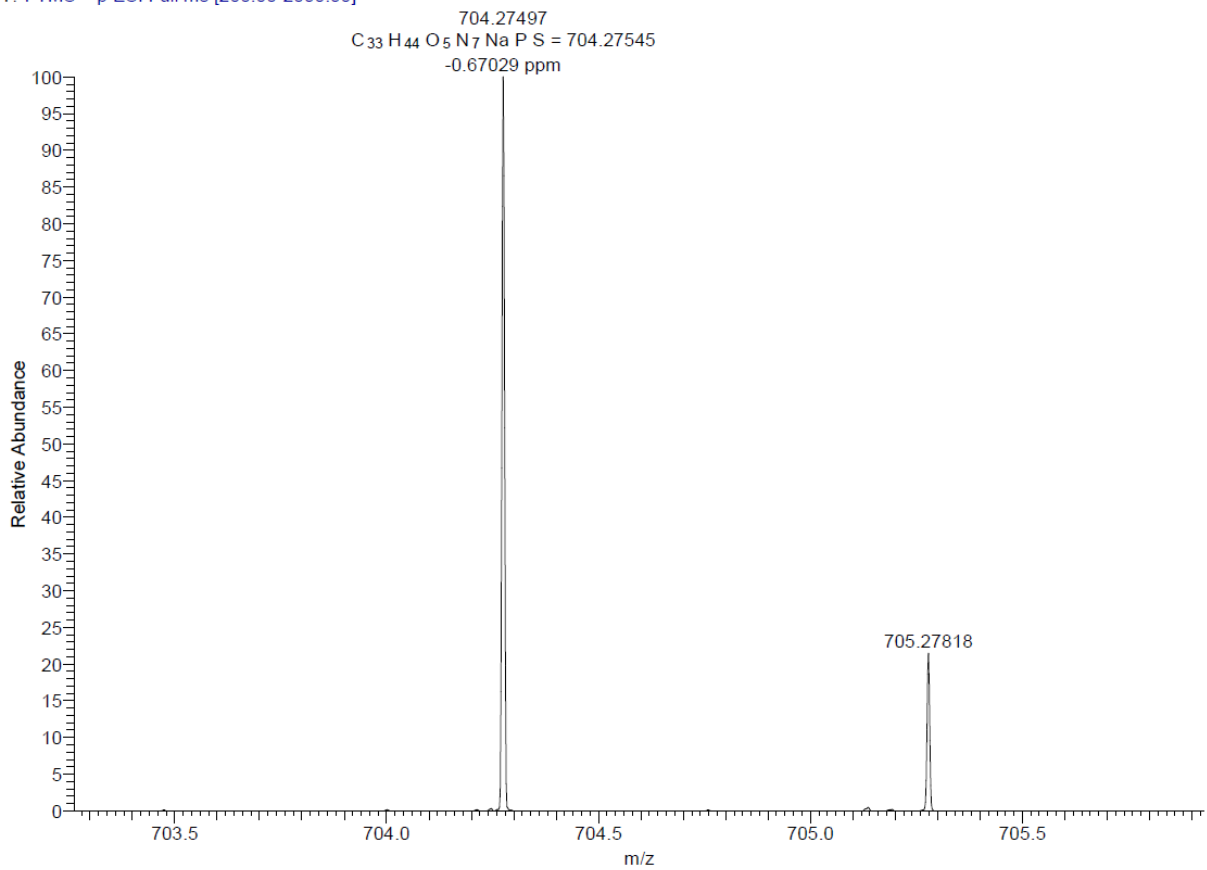


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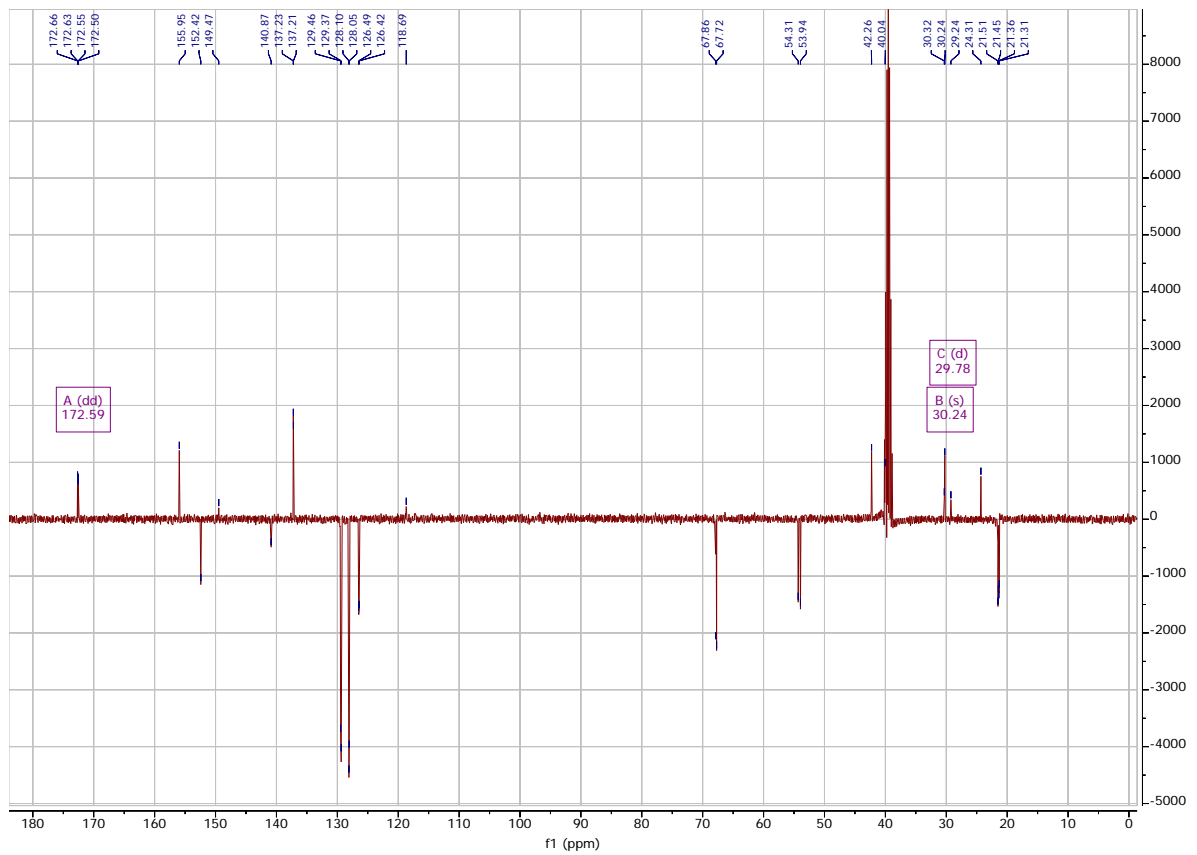
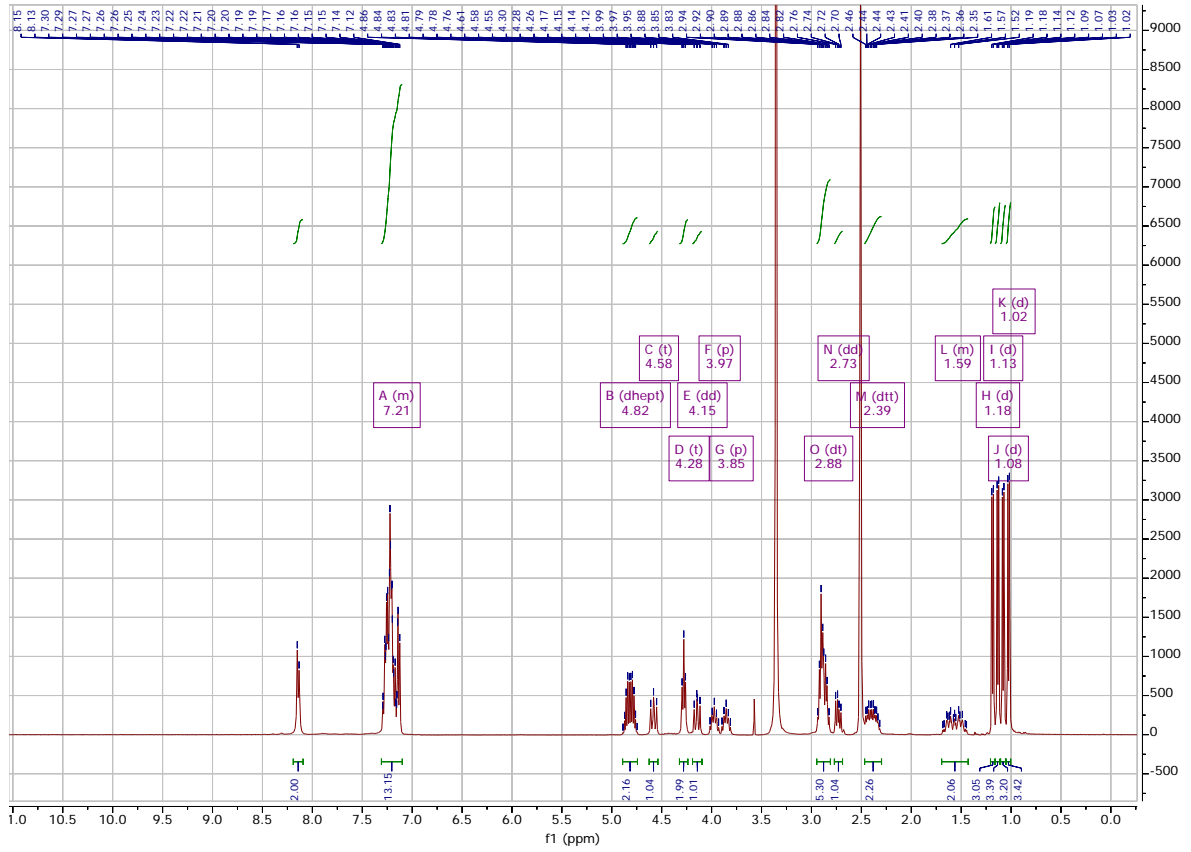
06/11/20 18:37:15

Klejci, IK403

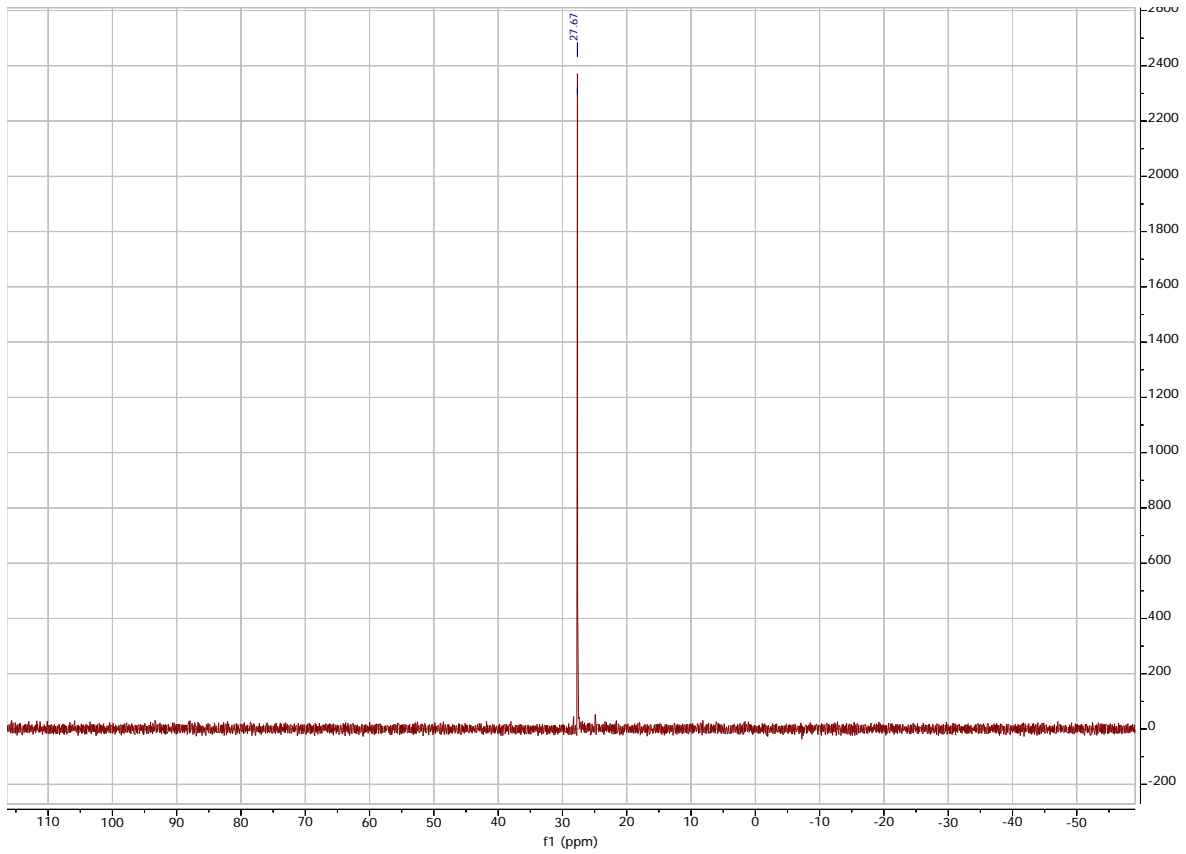
090620servis+HR\_10\_200617183715 #60-66 RT: 1.59-1.75 AV: 7 NL: 2.00E6  
T: FTMS + p ESI Full ms [200.00-2000.00]



24b





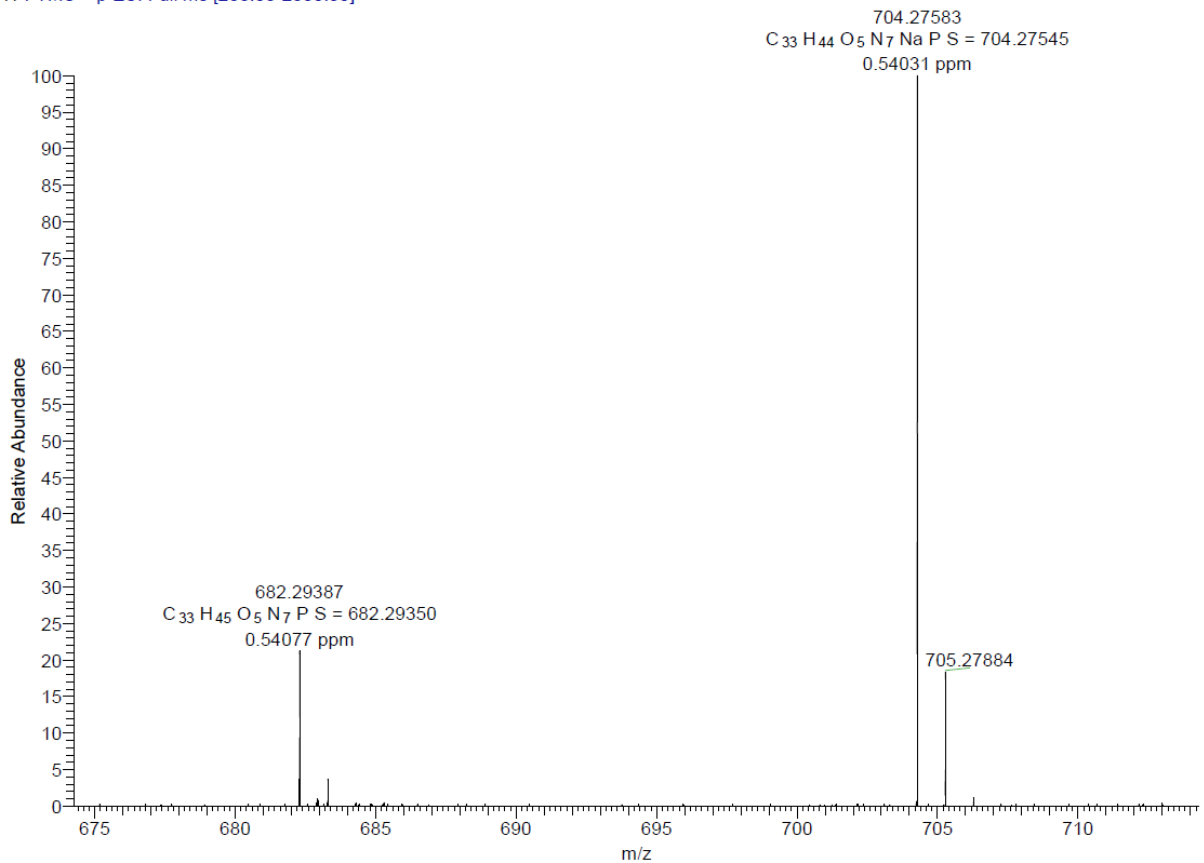


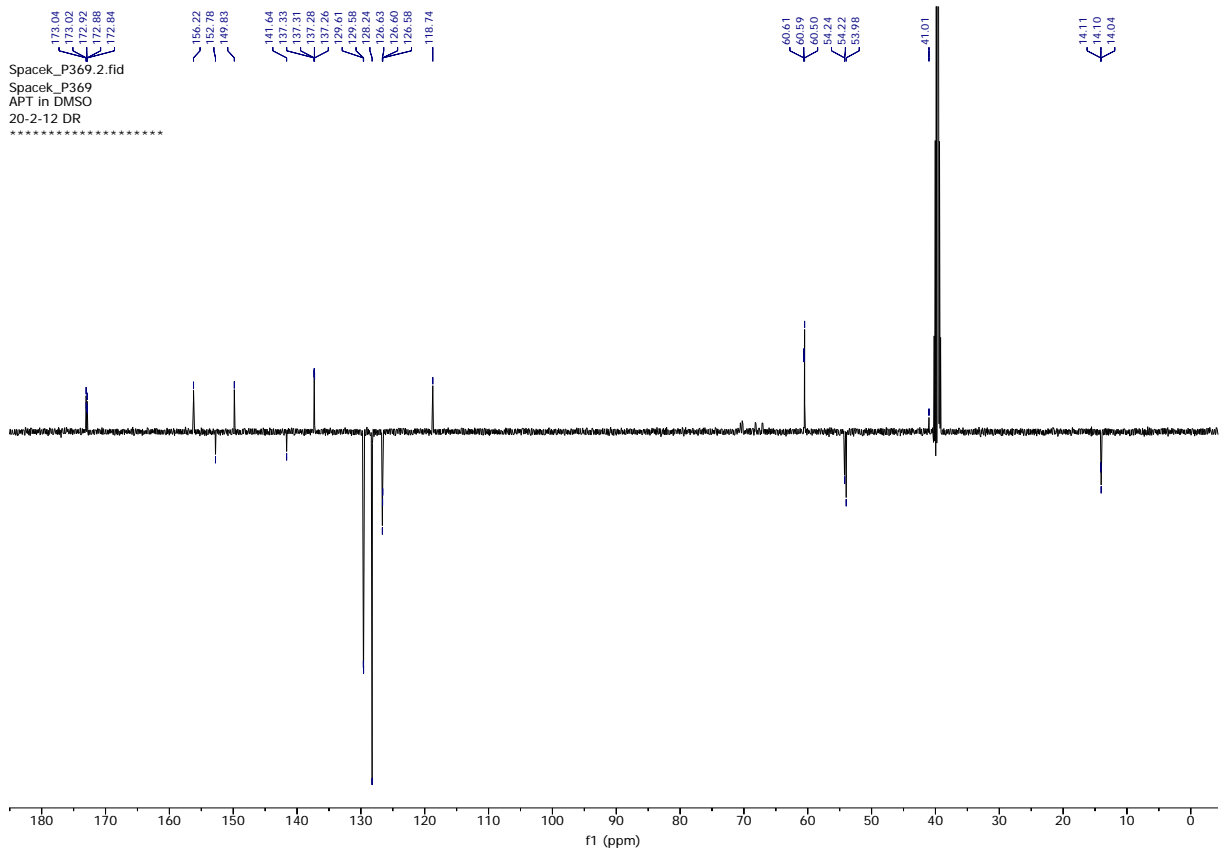
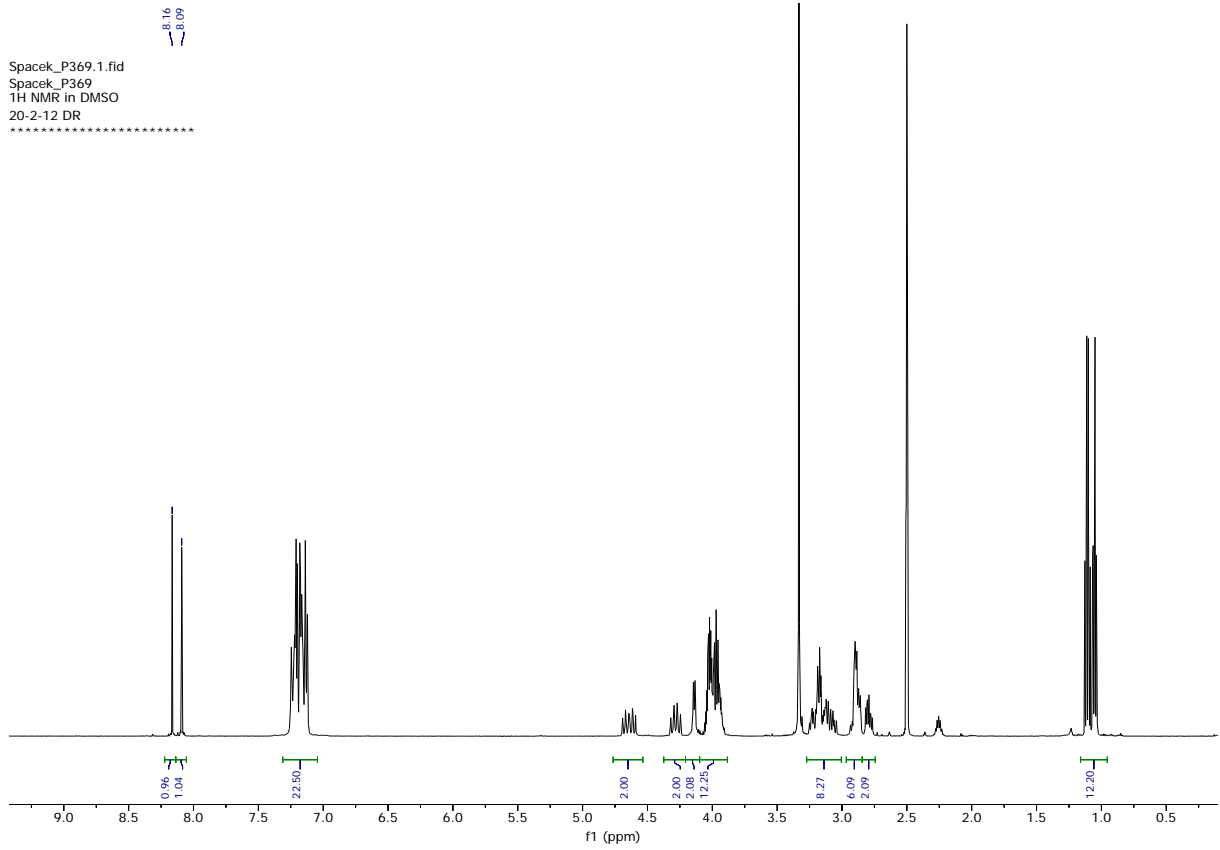
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06/09/20 10:36:45

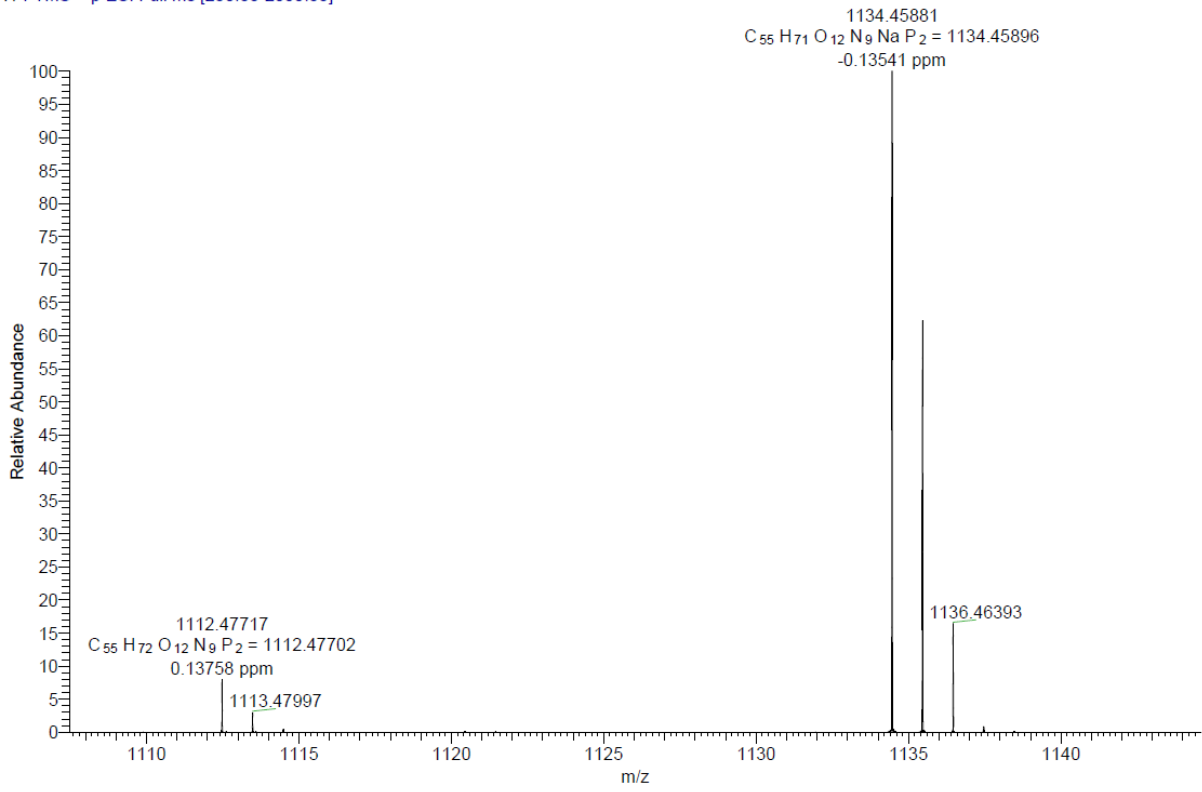
Klejš, TK402

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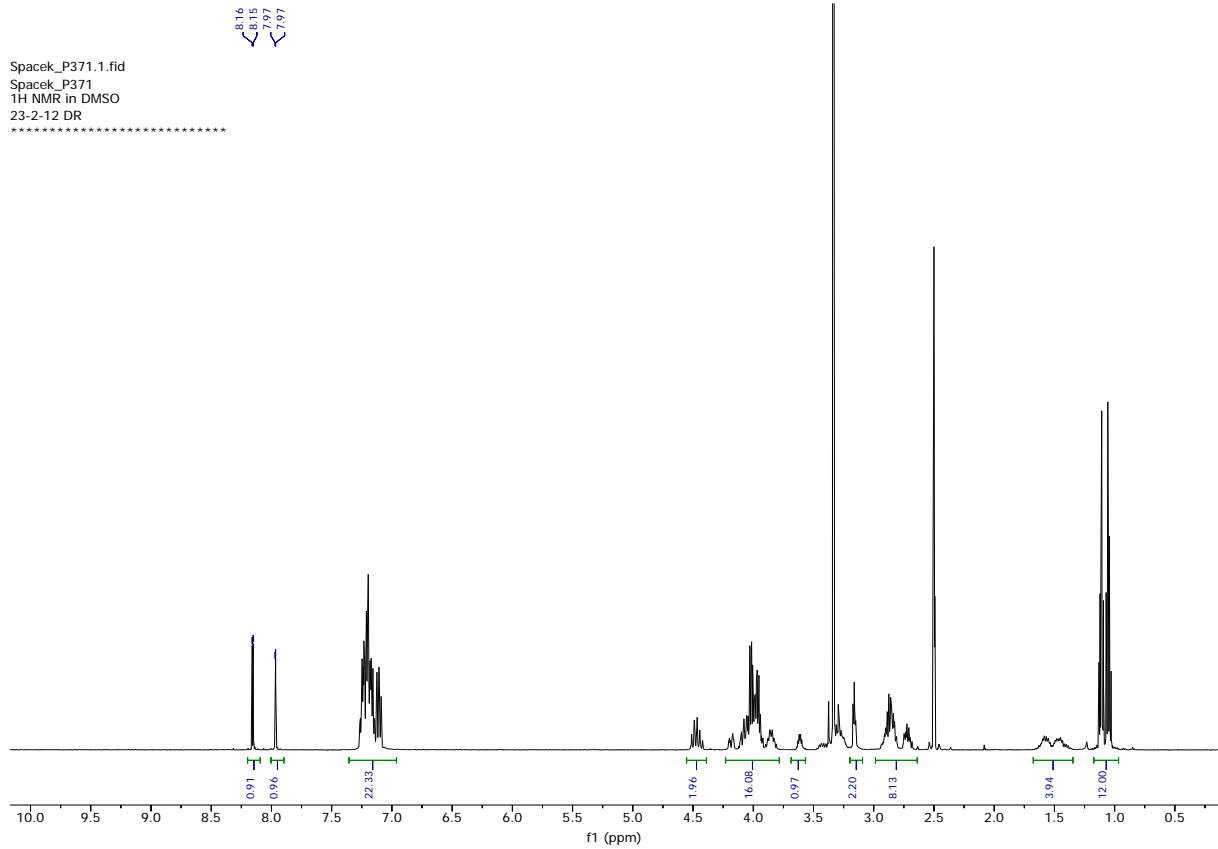




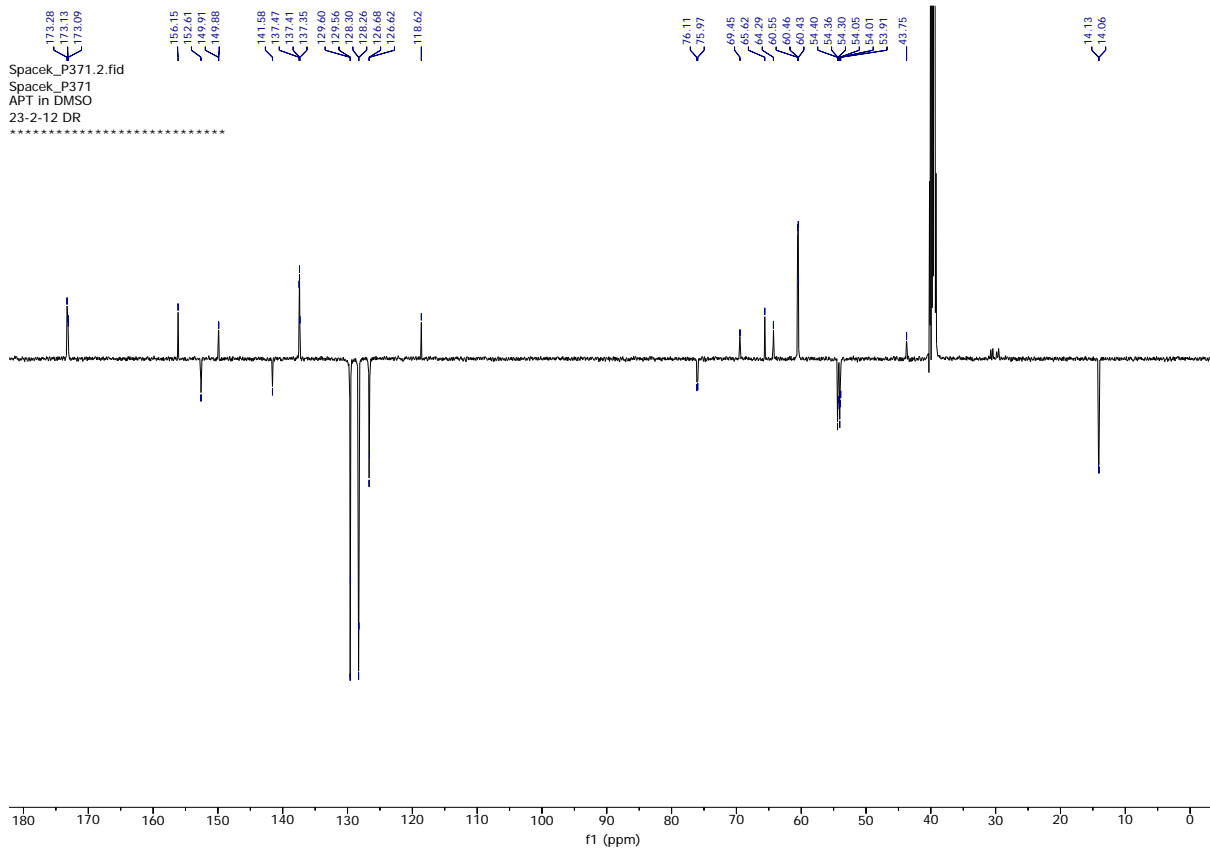
200820\_servisHR\_+12 #72-75 RT: 1.91-2.00 AV: 4 NL: 1.32E7  
T: FTMS + p ESI Full ms [200.00-2000.00]



26



Spacek\_P371.1.fid  
Spacek\_P371  
1H NMR in DMSO  
23-2-12 DR  
\*\*\*\*\*

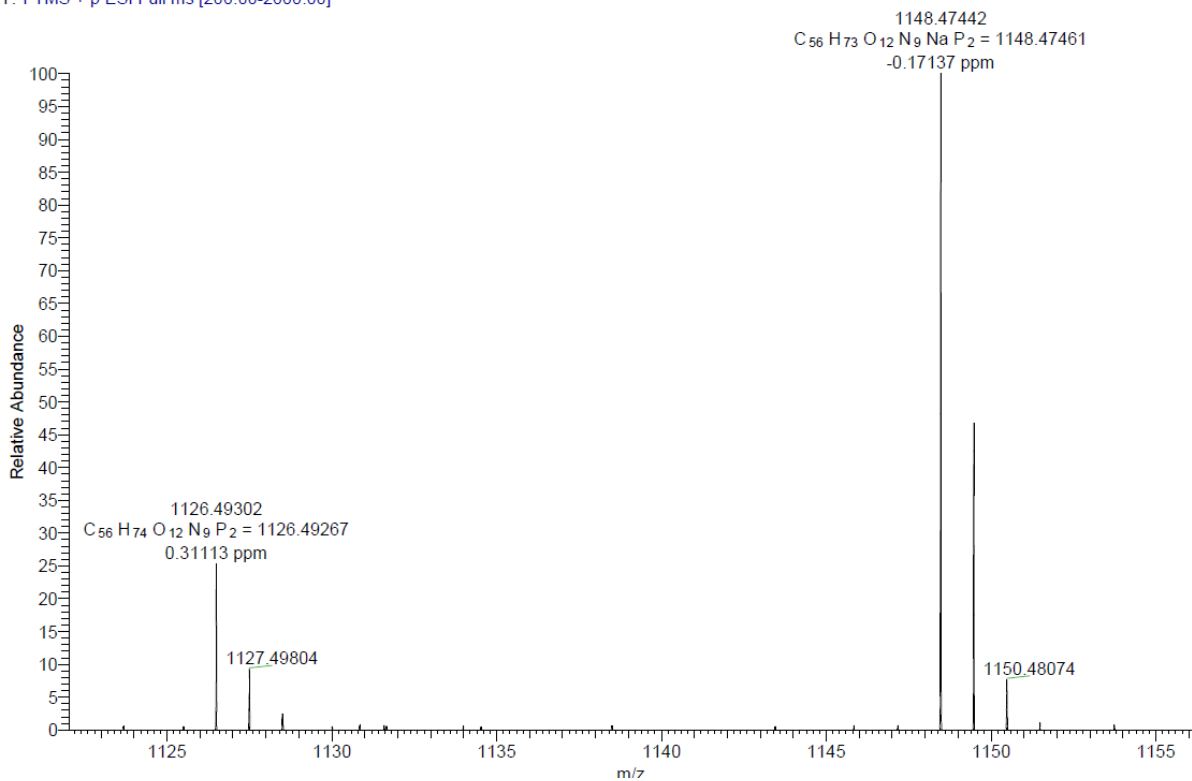


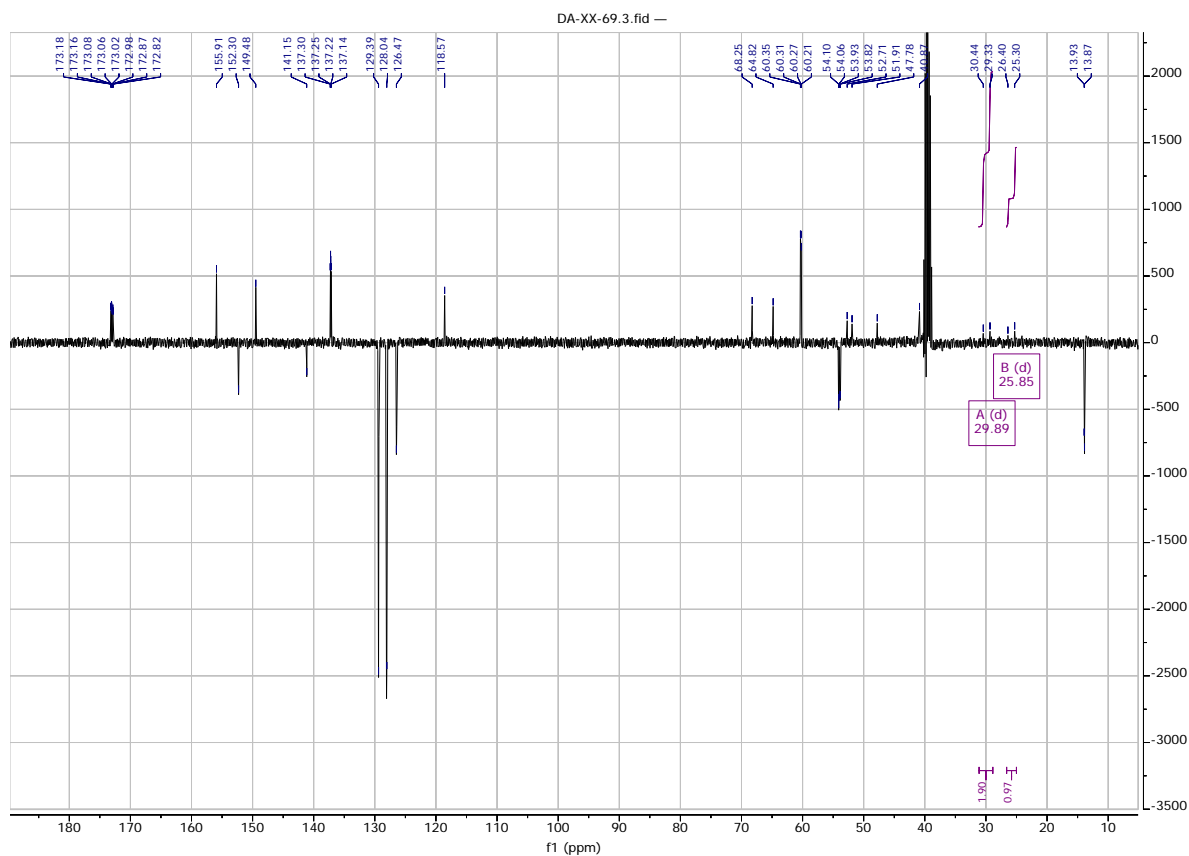
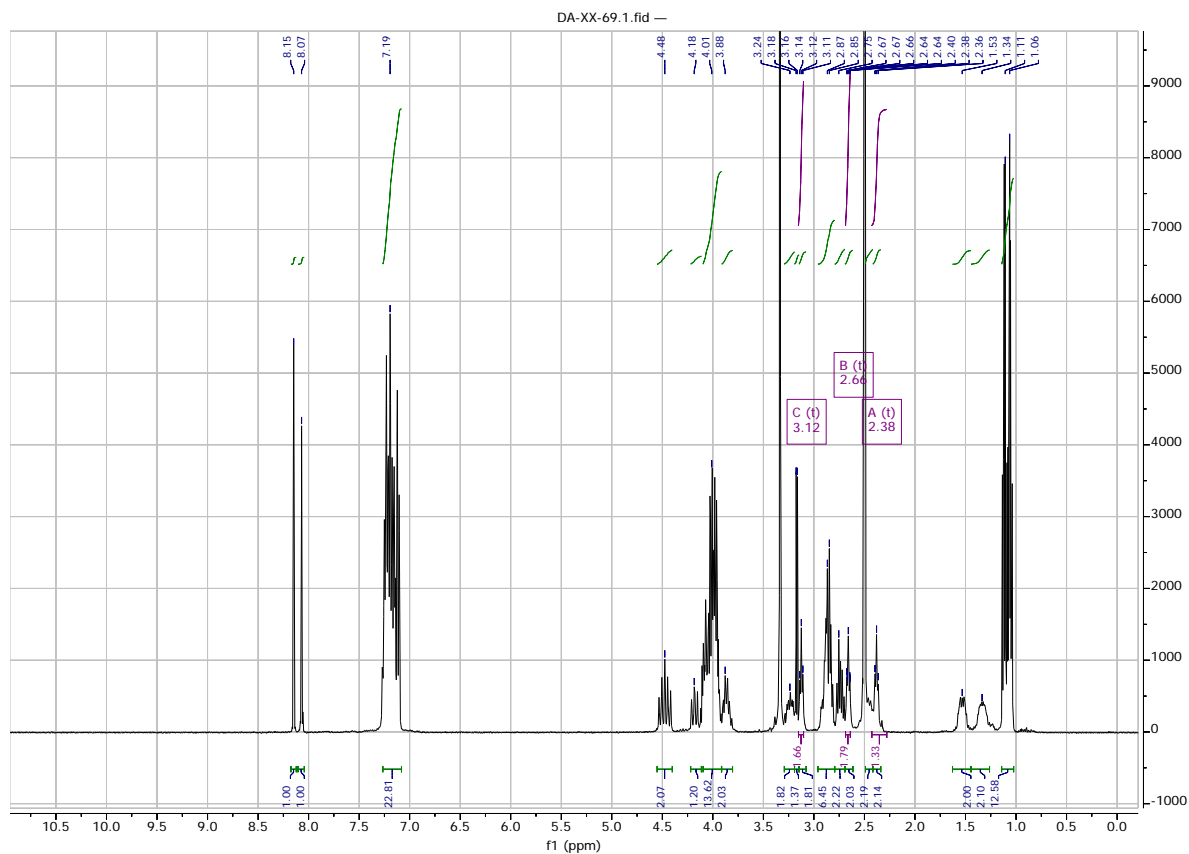
Z:\SERVISHR\...200820\_servisHR\_+13

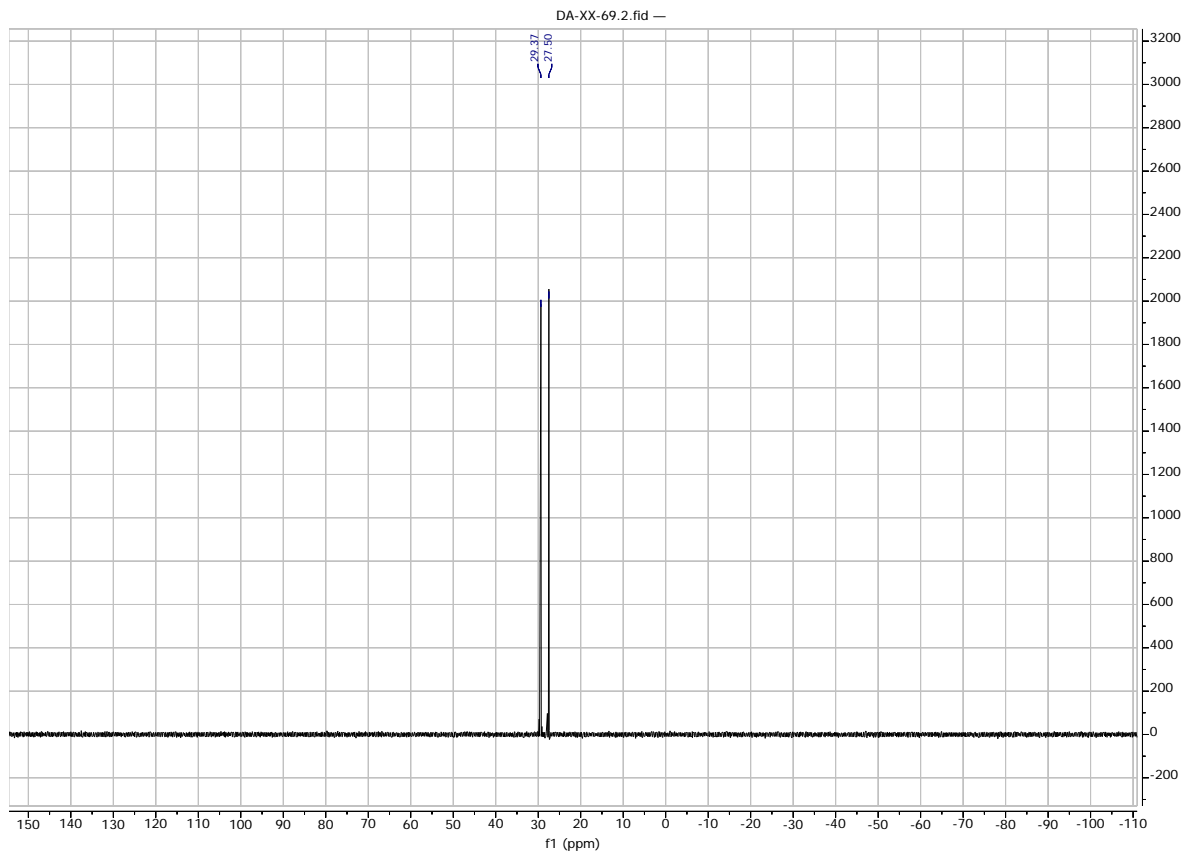
08/20/20 15:16:44

Hockova, PP-P371

200820\_servisHR\_+13 #80-83 RT: 2.13-2.21 AV: 4 NL: 4.45E5  
 T: FTMS + p ESI Full ms [200.00-2000.00]





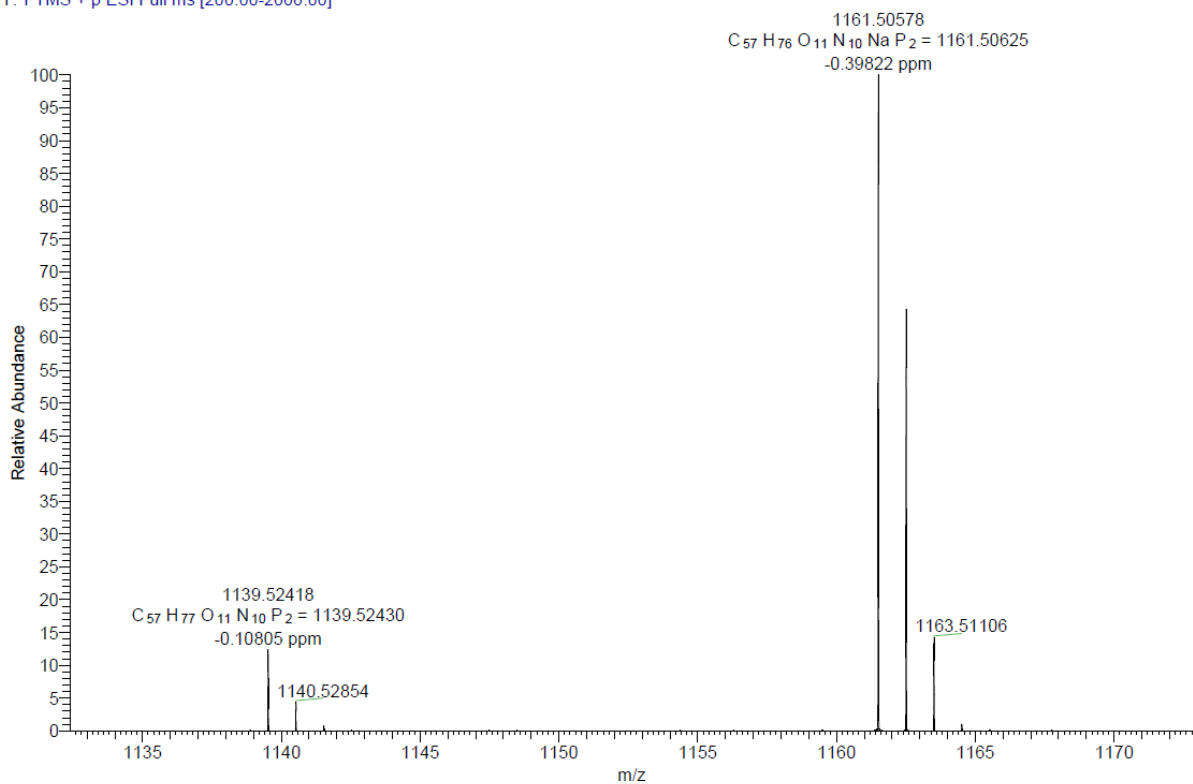


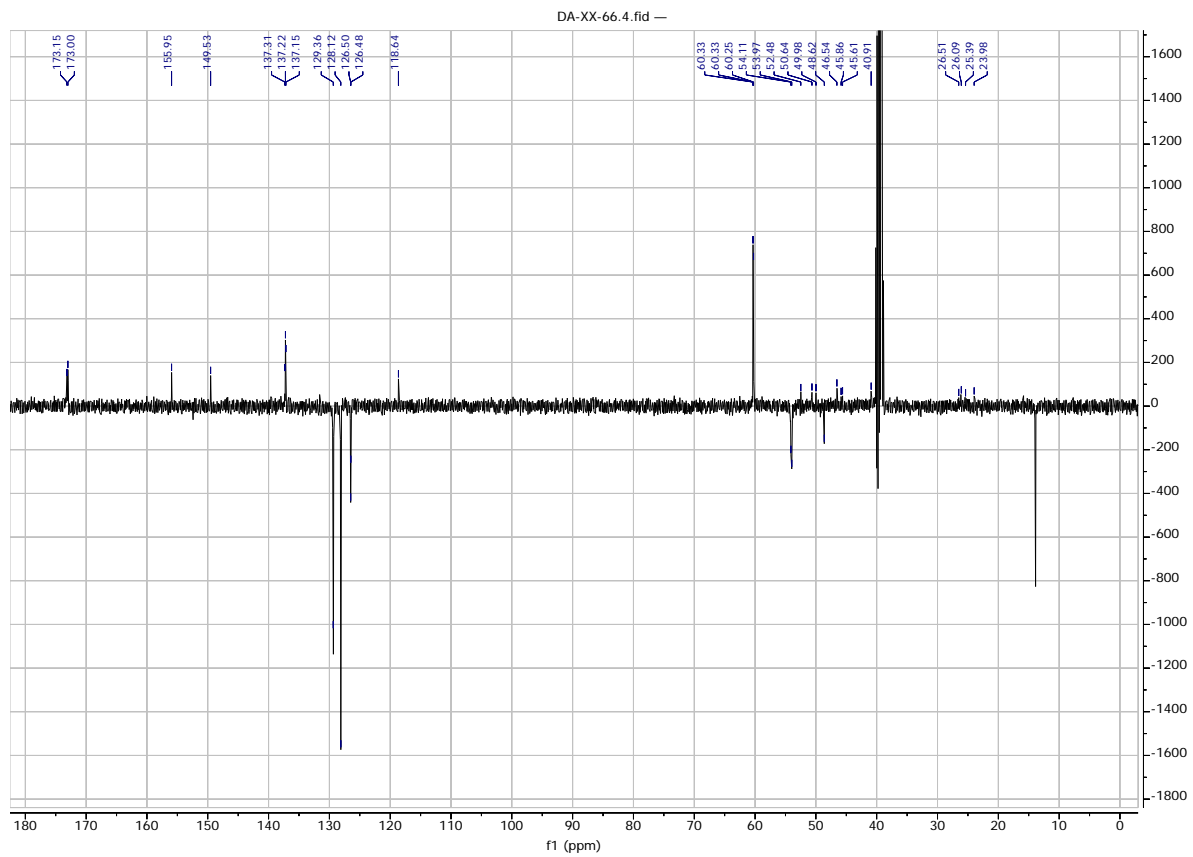
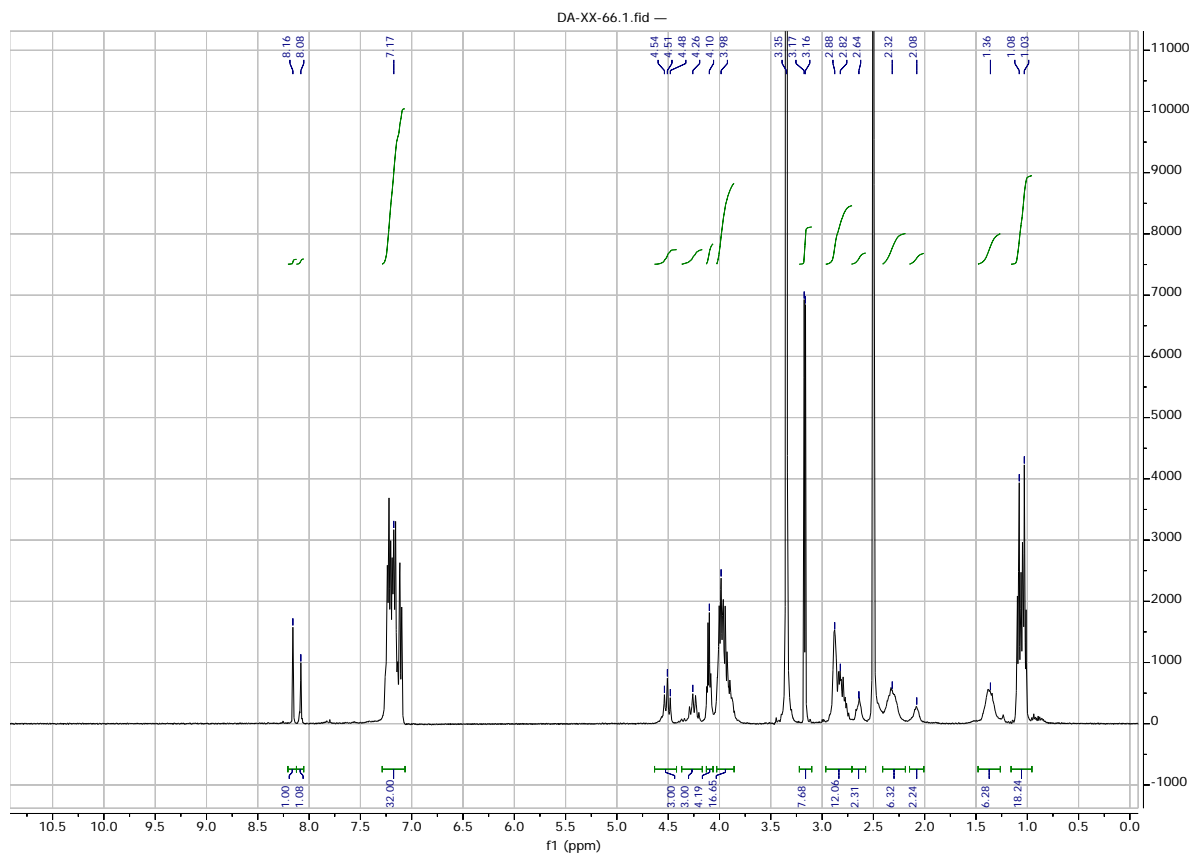
Z:\SERVISHR\...200820\_servisHR\_+16

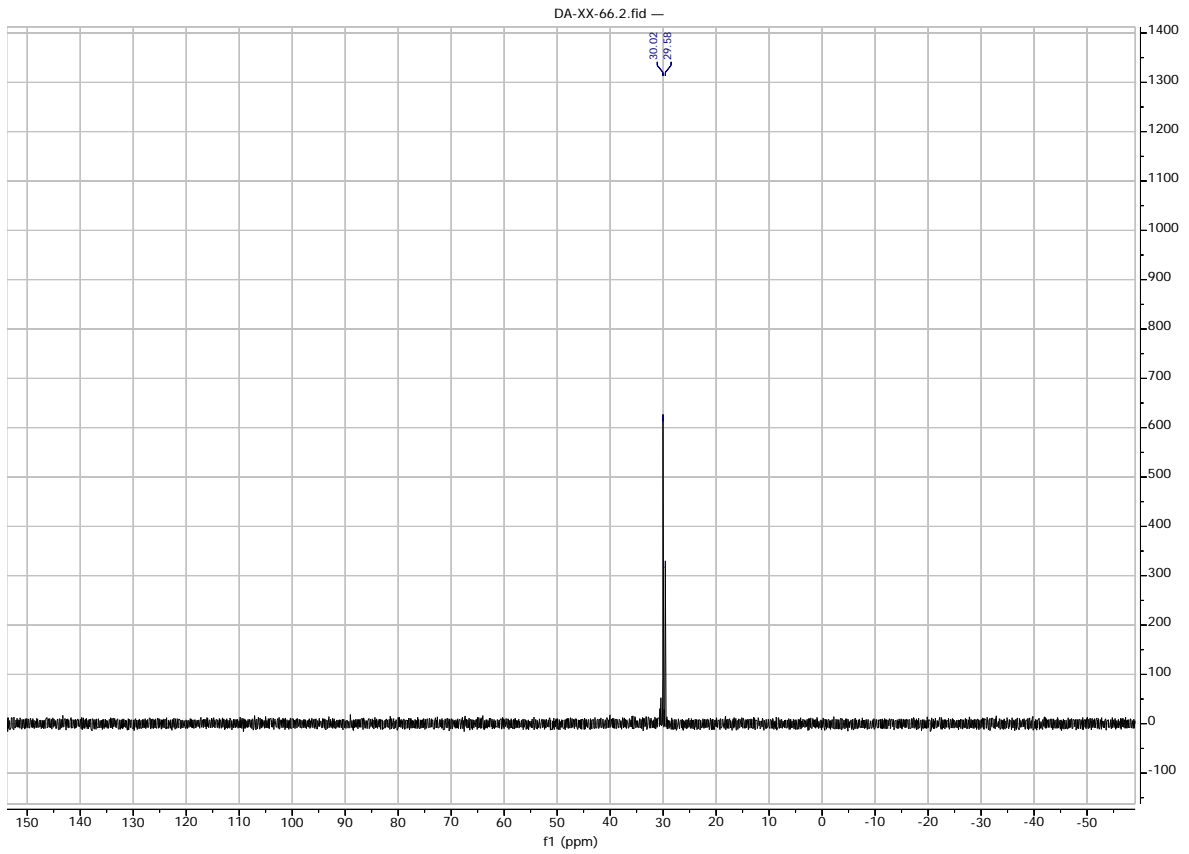
08/20/20 15:52:30

Hockova, DA-XX-69

200820\_servisHR\_+16 #73-75 RT: 1.94-2.00 AV: 3 SB: 84 0.01-1.70, 0.03-0.55 NL: 8.48E6  
T: FTMS + p ESI Full ms [200.00-2000.00]





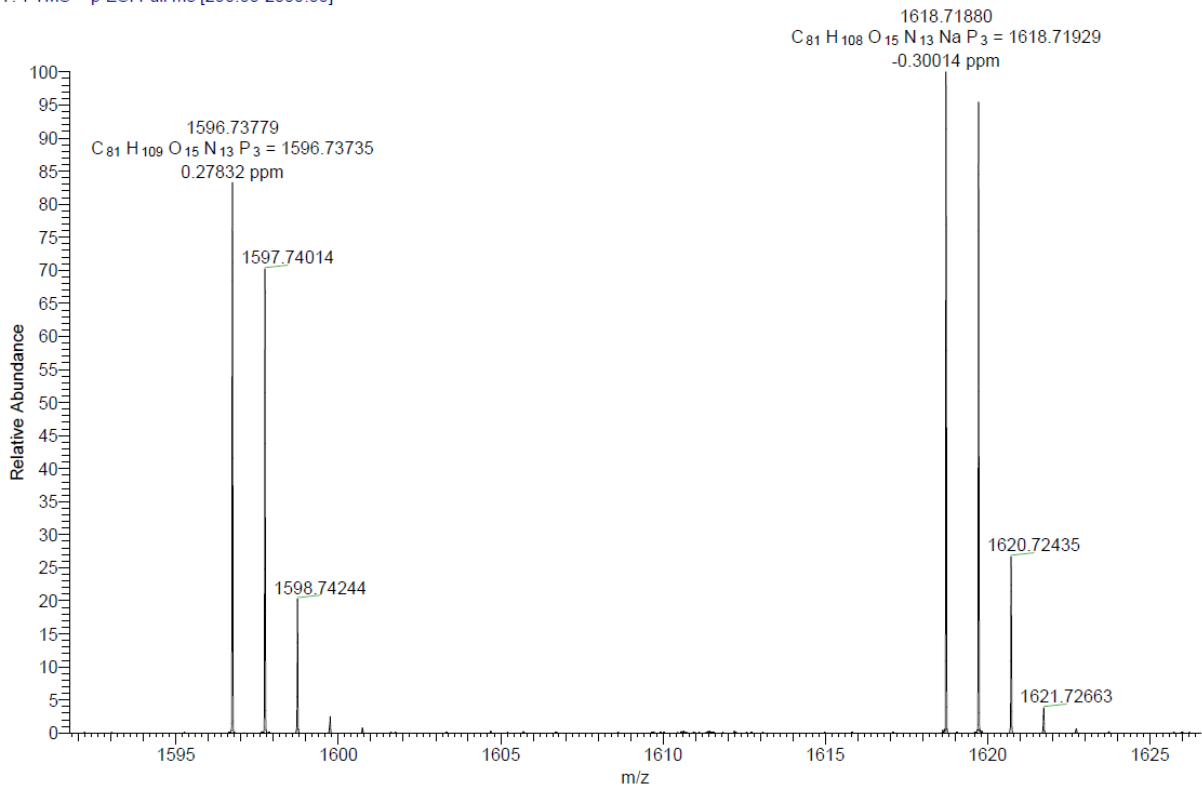


Z:\SERVISHR\...200820\_servisHR\_+15

08/20/20 15:24:55

Hockova, DA-XX-66

200820\_servisHR\_+15 #74-76 RT: 1.97-2.02 AV: 3 SB: 77 0.09-1.51, 0.09-0.72 NL: 4.25E6  
T: FTMS + p ESI Full ms [200.00-2000.00]



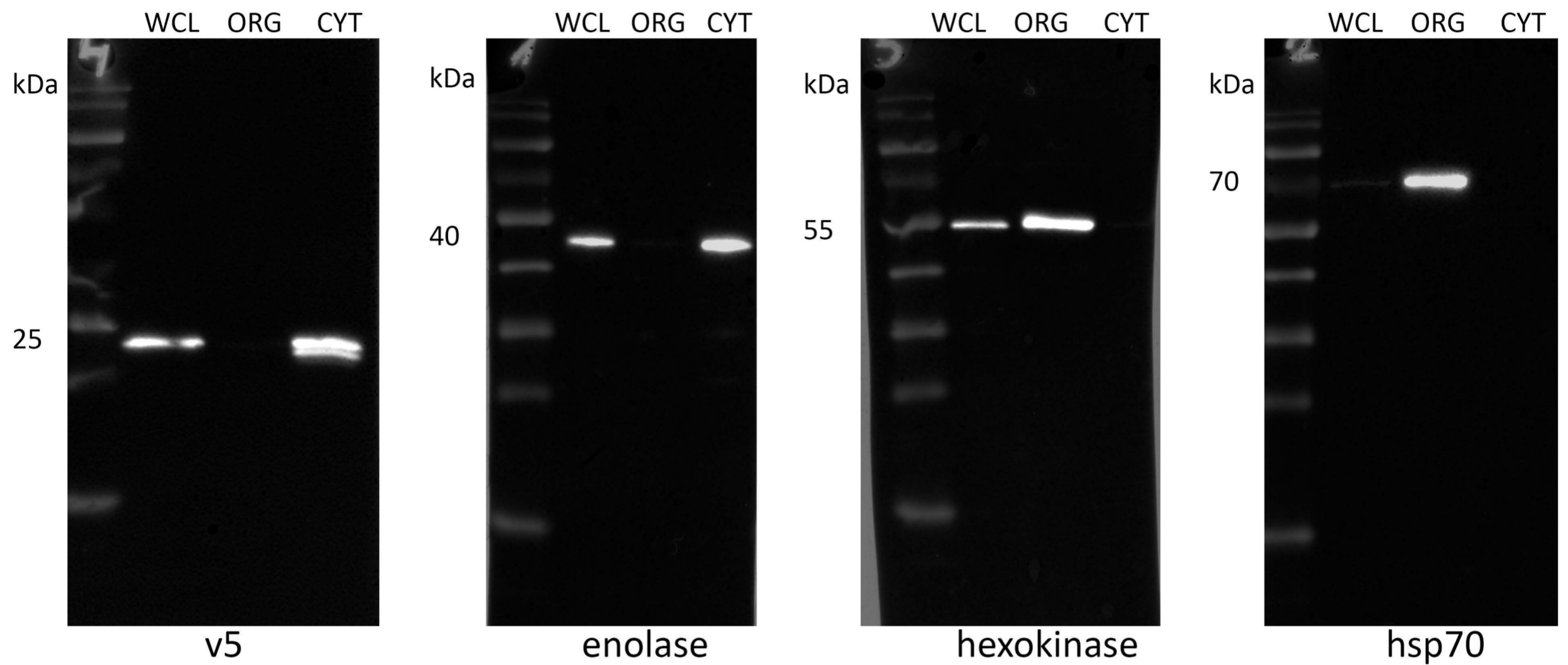


**Supplementary file S3.** Raw Images. Original images supporting blot results reported in Figs. 2A, 3 and 4C

# S1\_raw images

Fig. 2A

## v5-tagged APRT1



## v5-tagged APRT2

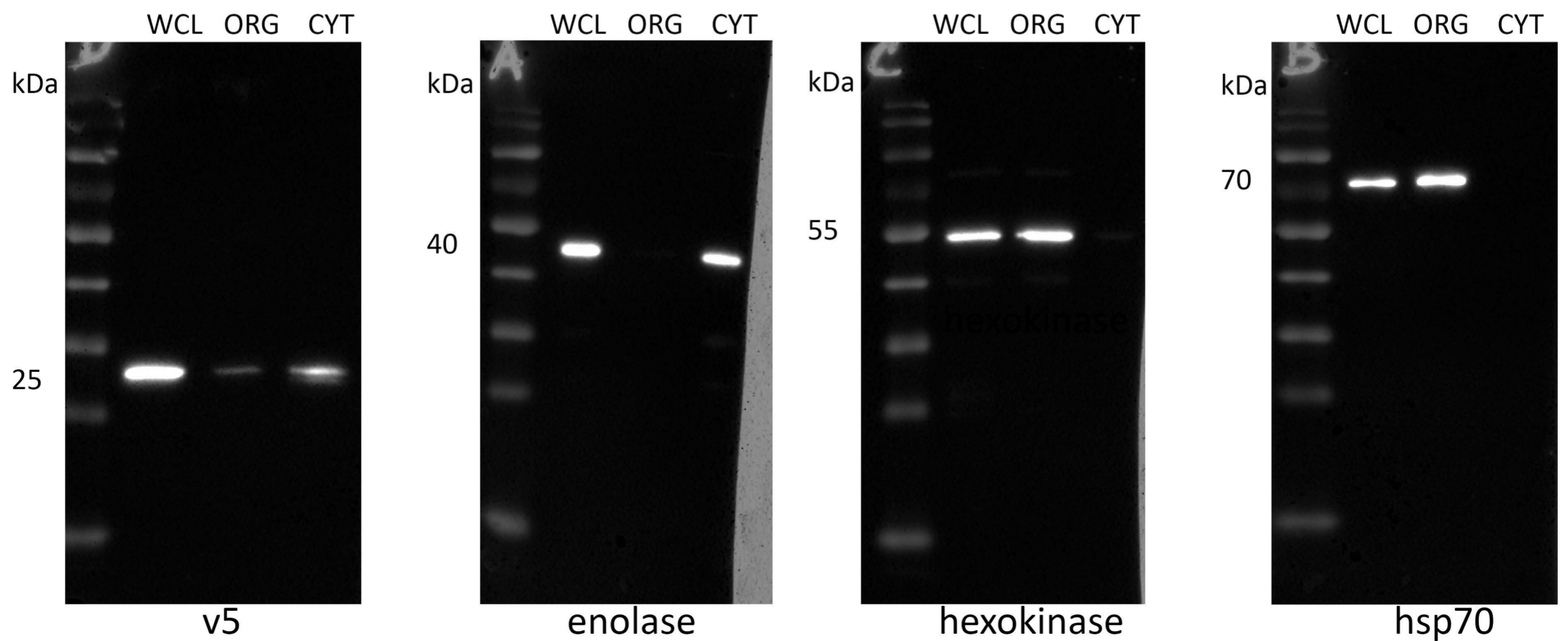
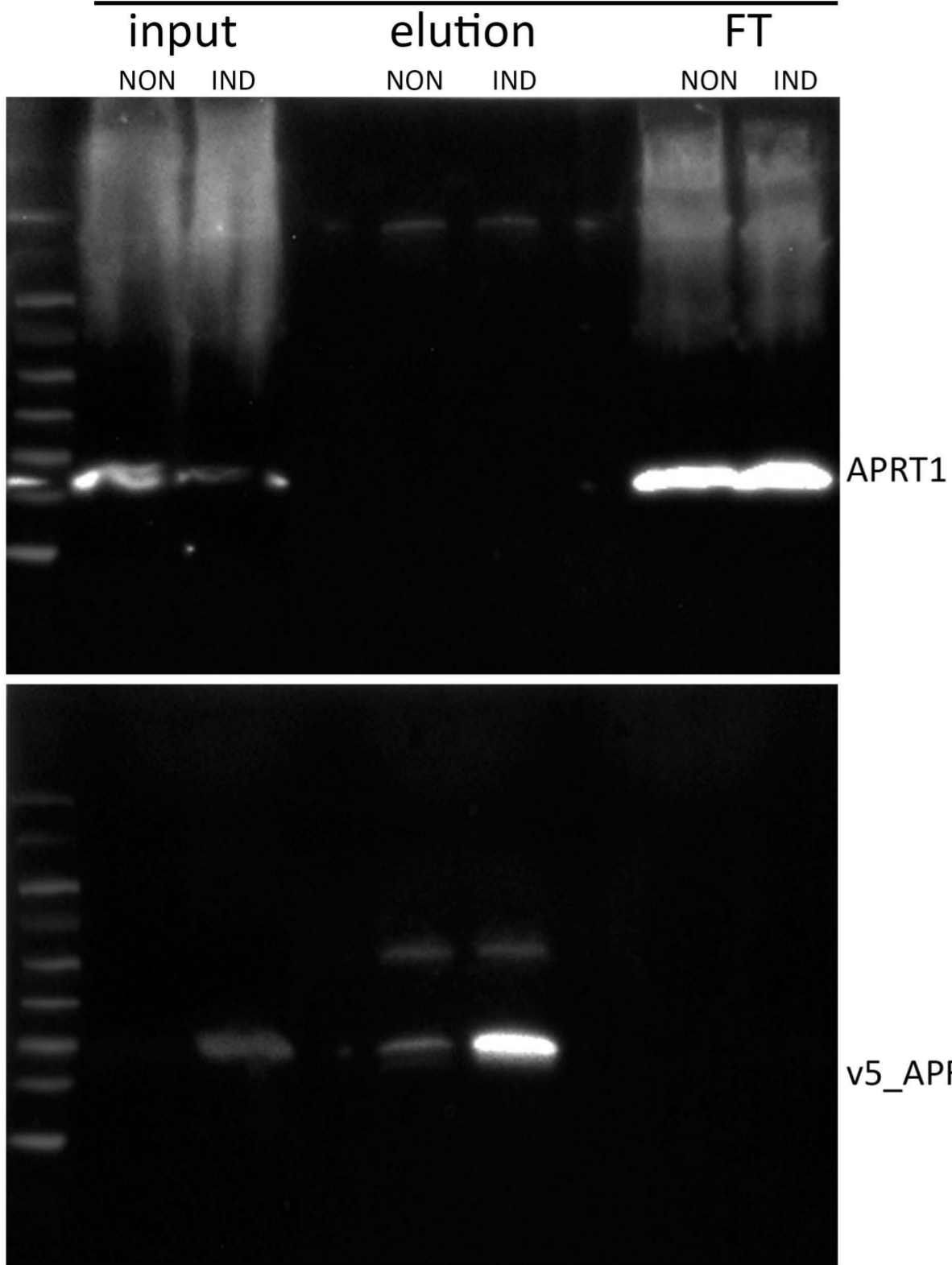


Fig. 3

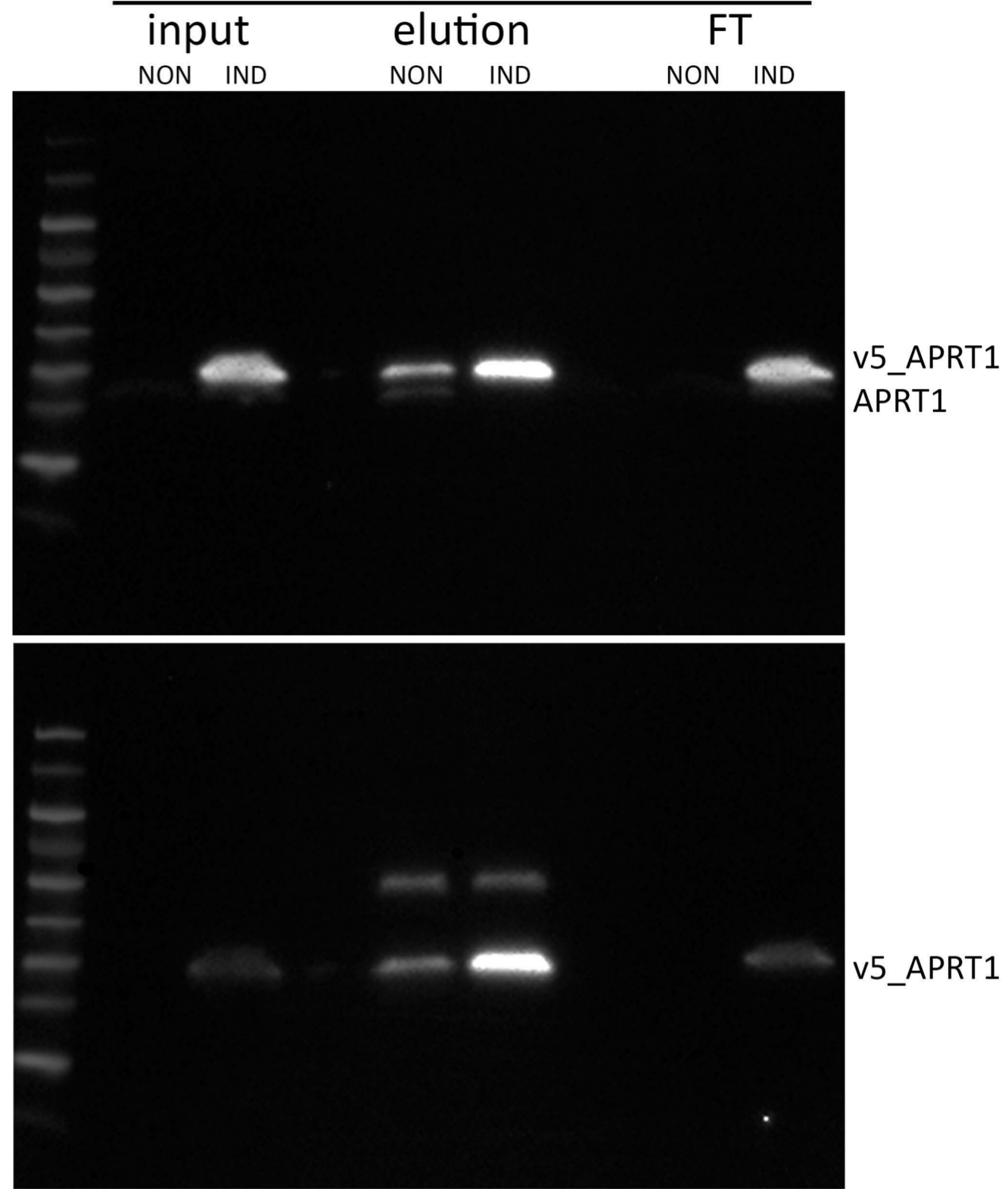
A

BSF v5-tagged APRT2



B

BSF v5-tagged APRT1



C

recombinant APRT1

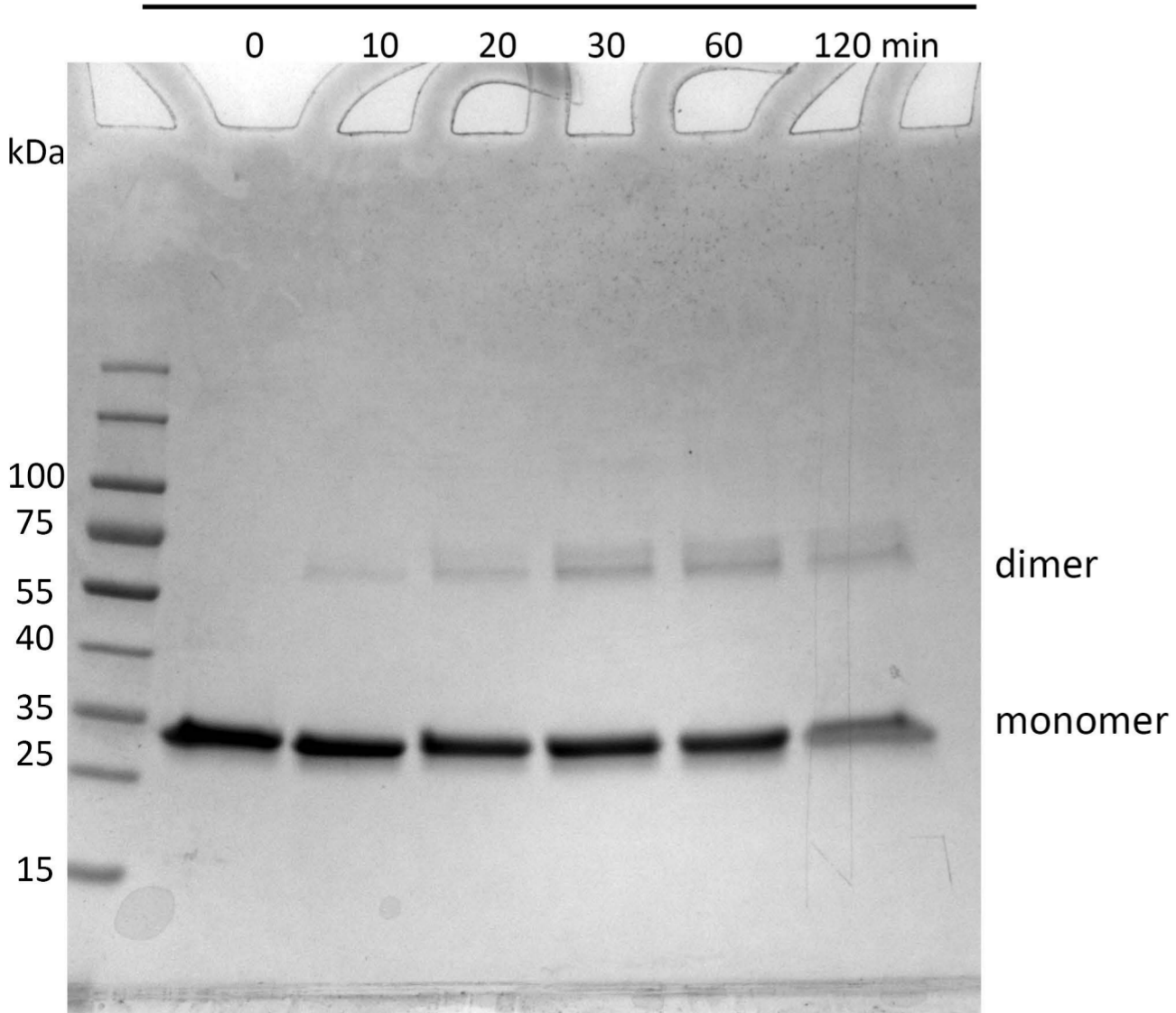


Fig. 4B

