Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supporting Information

Stereoisomeric Pam_2CS Based TLR2 Agonists: Synthesis, Structural Modelling and Activity as Vaccine Adjuvants

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*Corresponding authors

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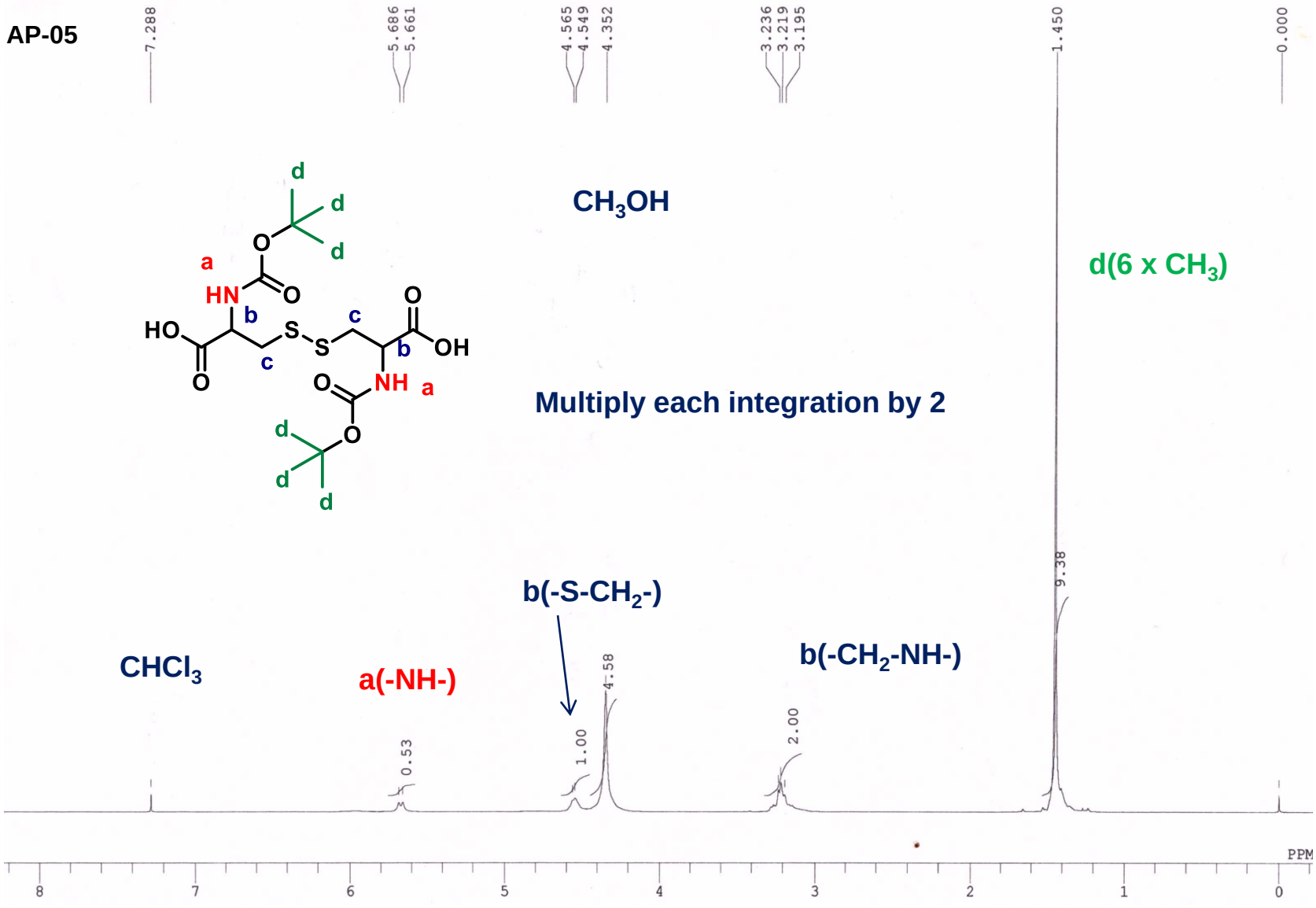
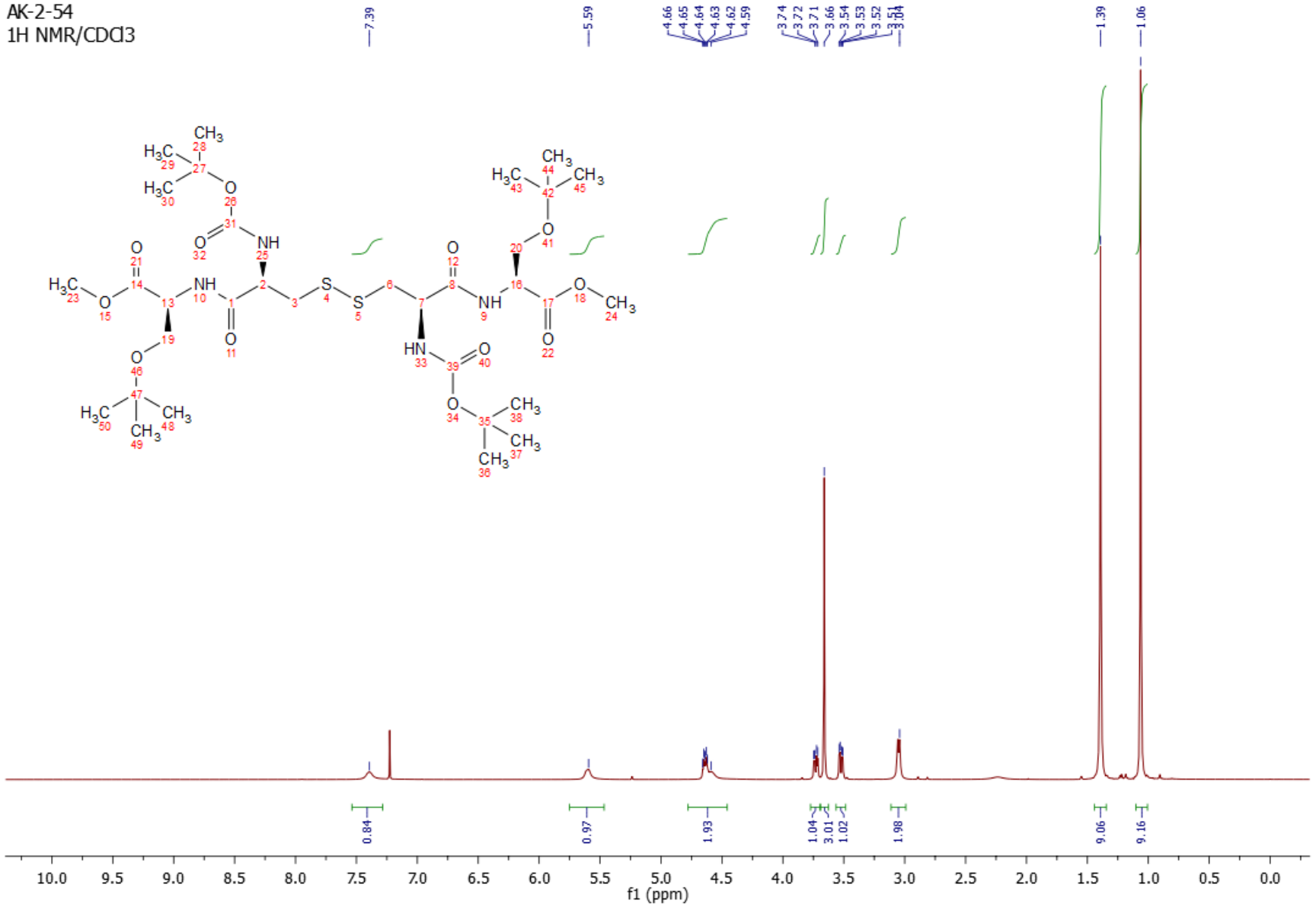
^{1}H NMR of Compound **2** (in $CDCl_3$ + CD_3OD, 300 MHz)

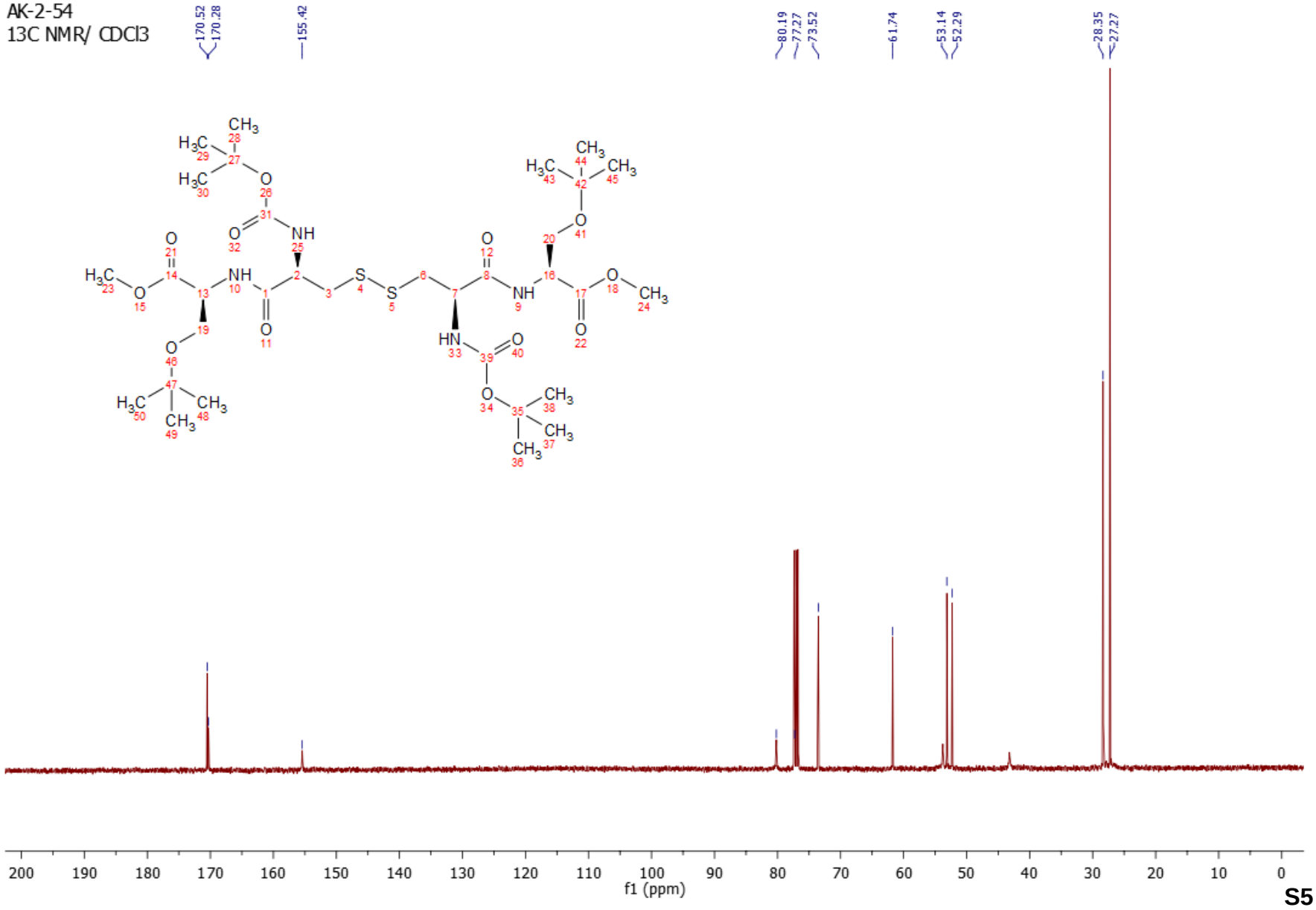
ESI



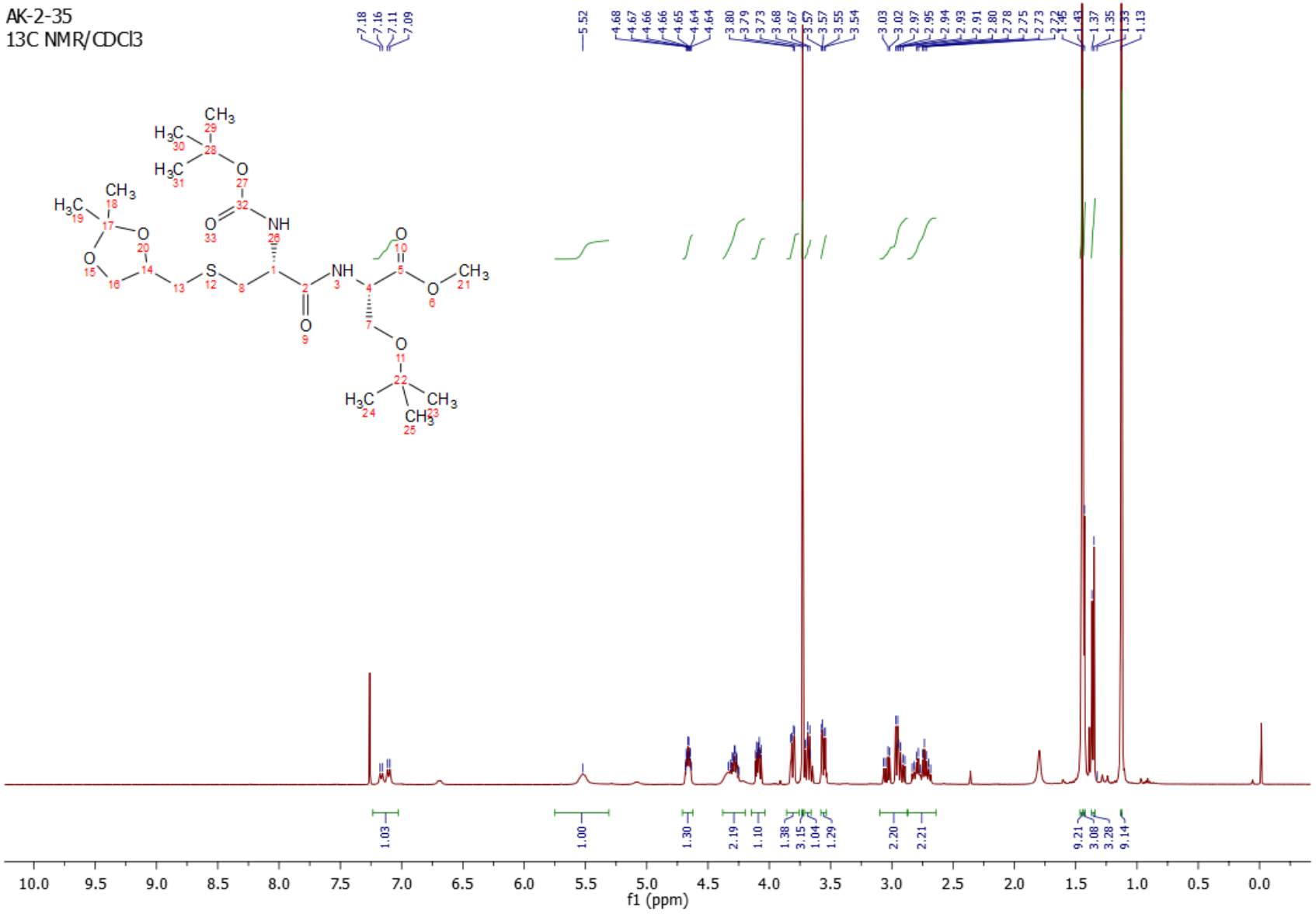
^{1}H NMR of Compound **3** (in $CDCl_3$, 400 MHz)



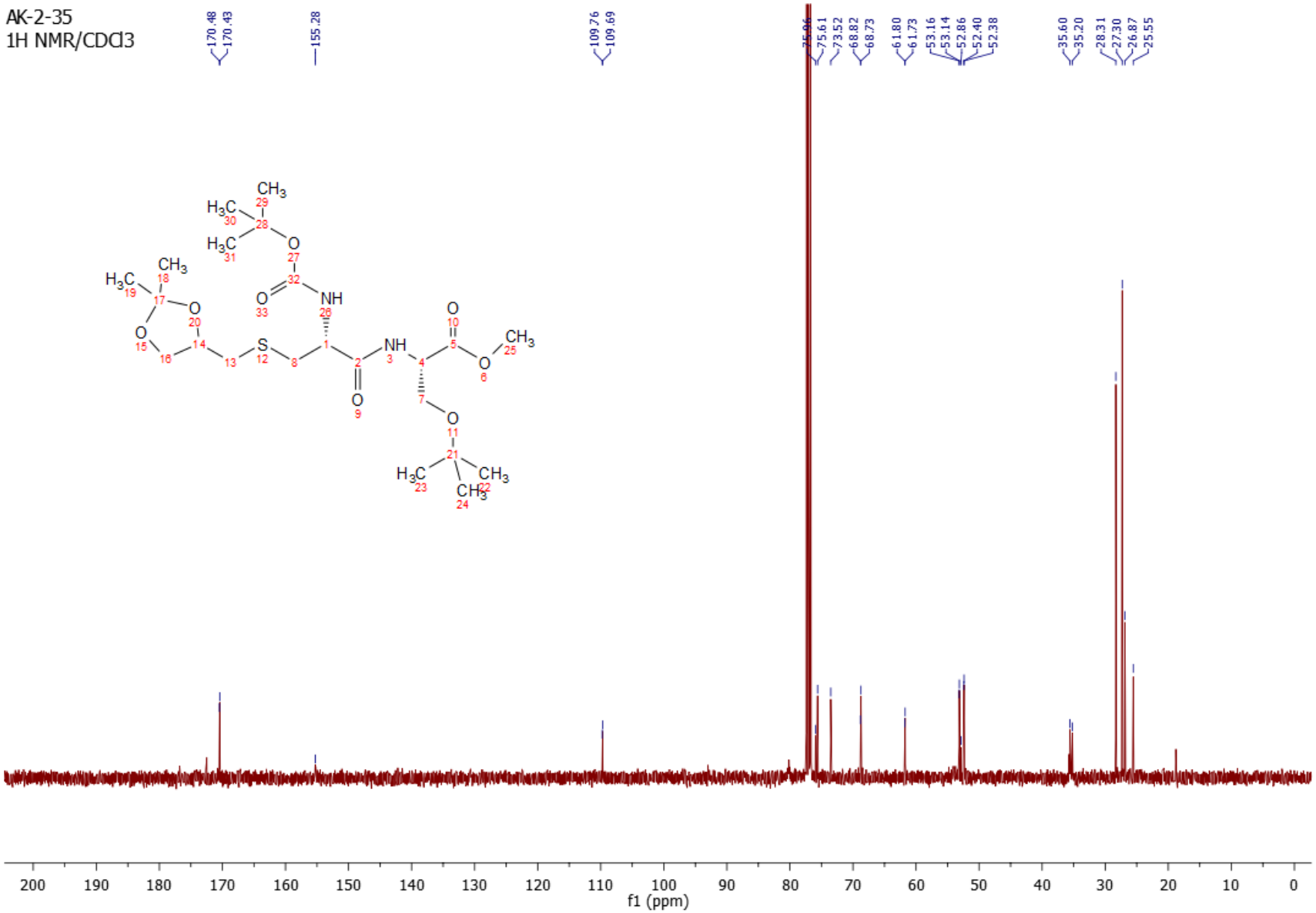
^{13}C NMR of Compound **3** (in $CDCl_3$, 100 MHz) ESI



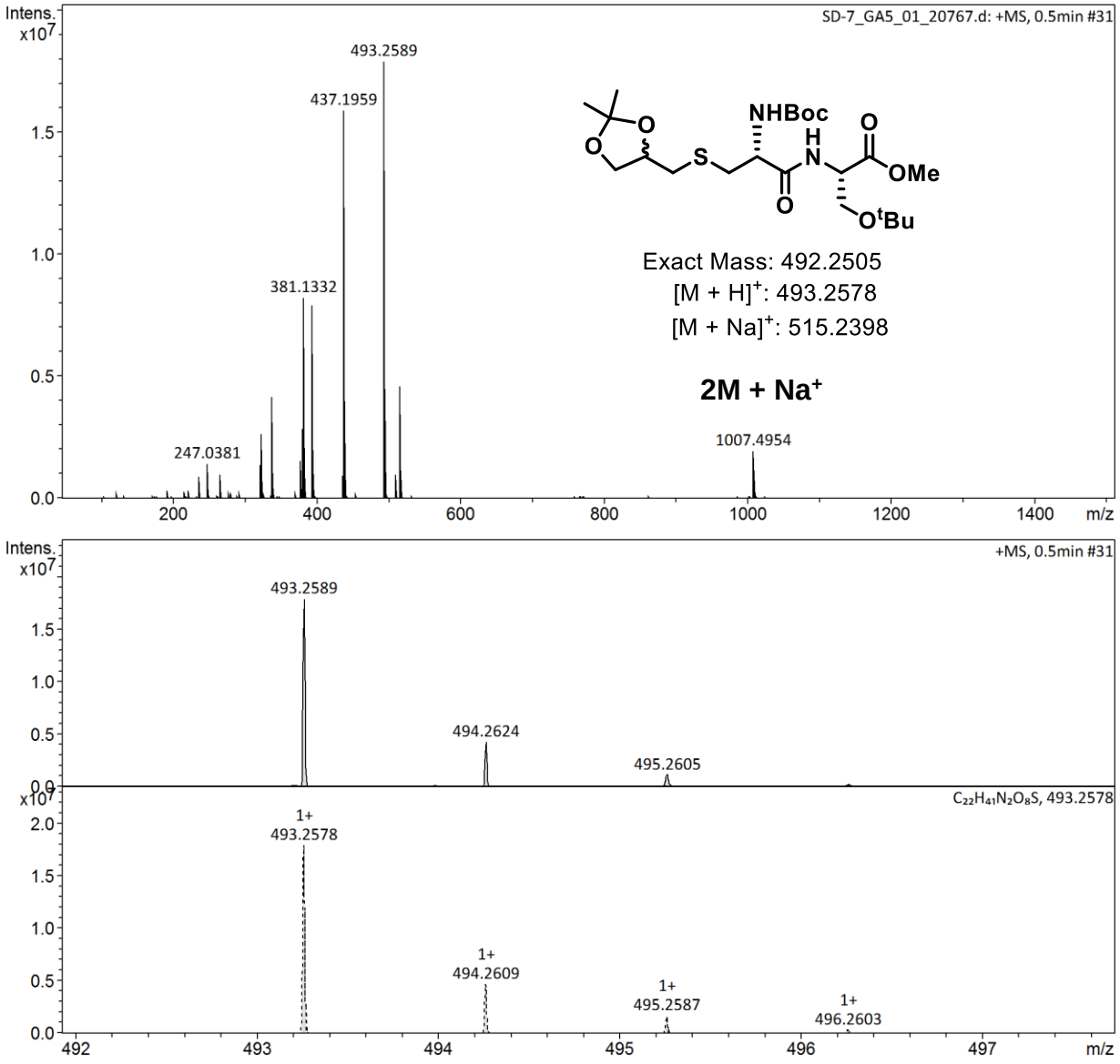
^{1}H NMR of Compound **5a** (in $CDCl_3$, 400 MHz) ESI



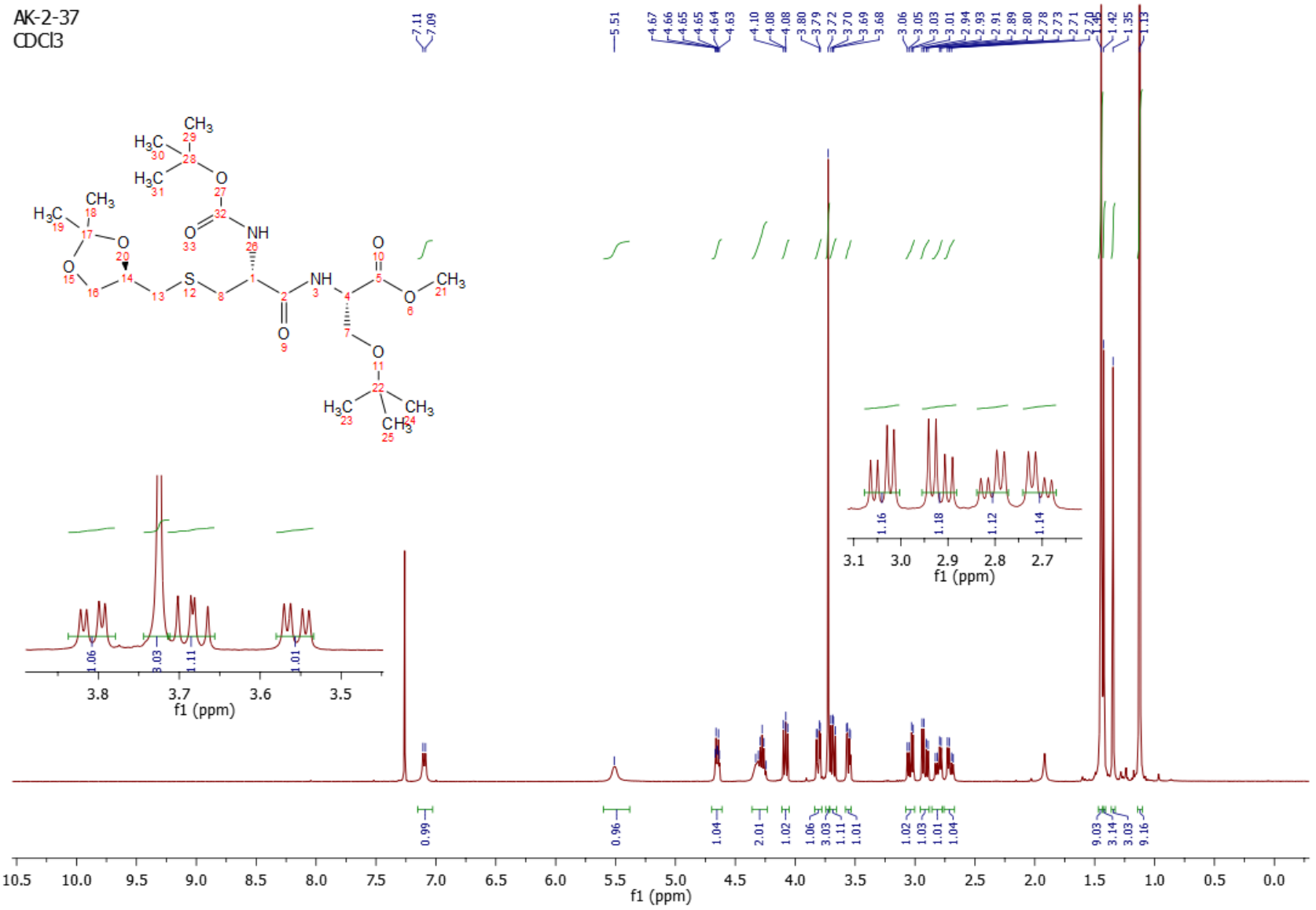
^{13}C NMR of Compound **5a** (in $CDCl_3$, 100 MHz)



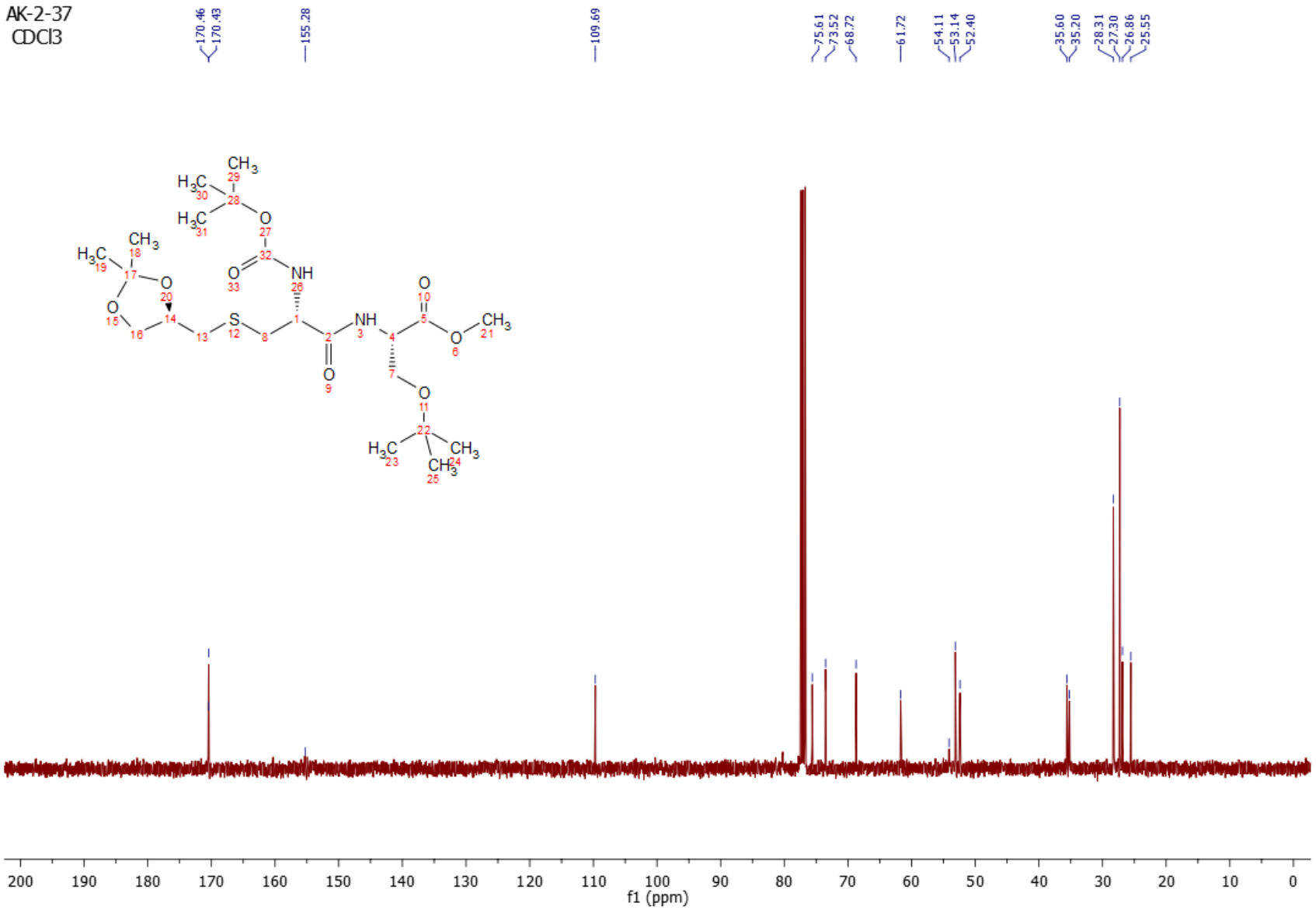
HRMS of Compound **5a** ESI



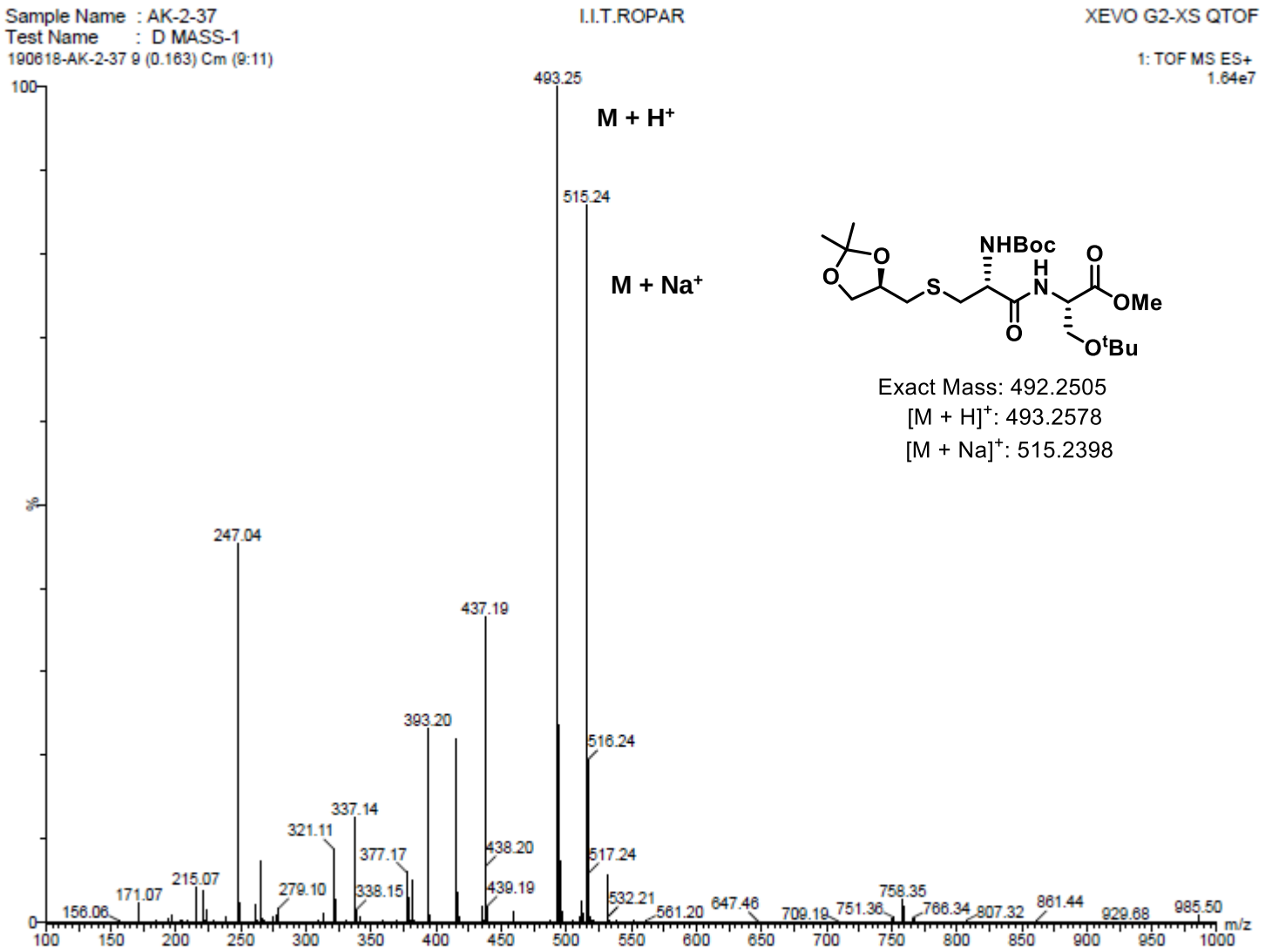
^{1}H NMR of Compound **5b** (in $CDCl_3$, 400 MHz)



^{13}C NMR of Compound **5b** (in $CDCl_3$, 100 MHz) ESI



MS of Compound **5b** ESI

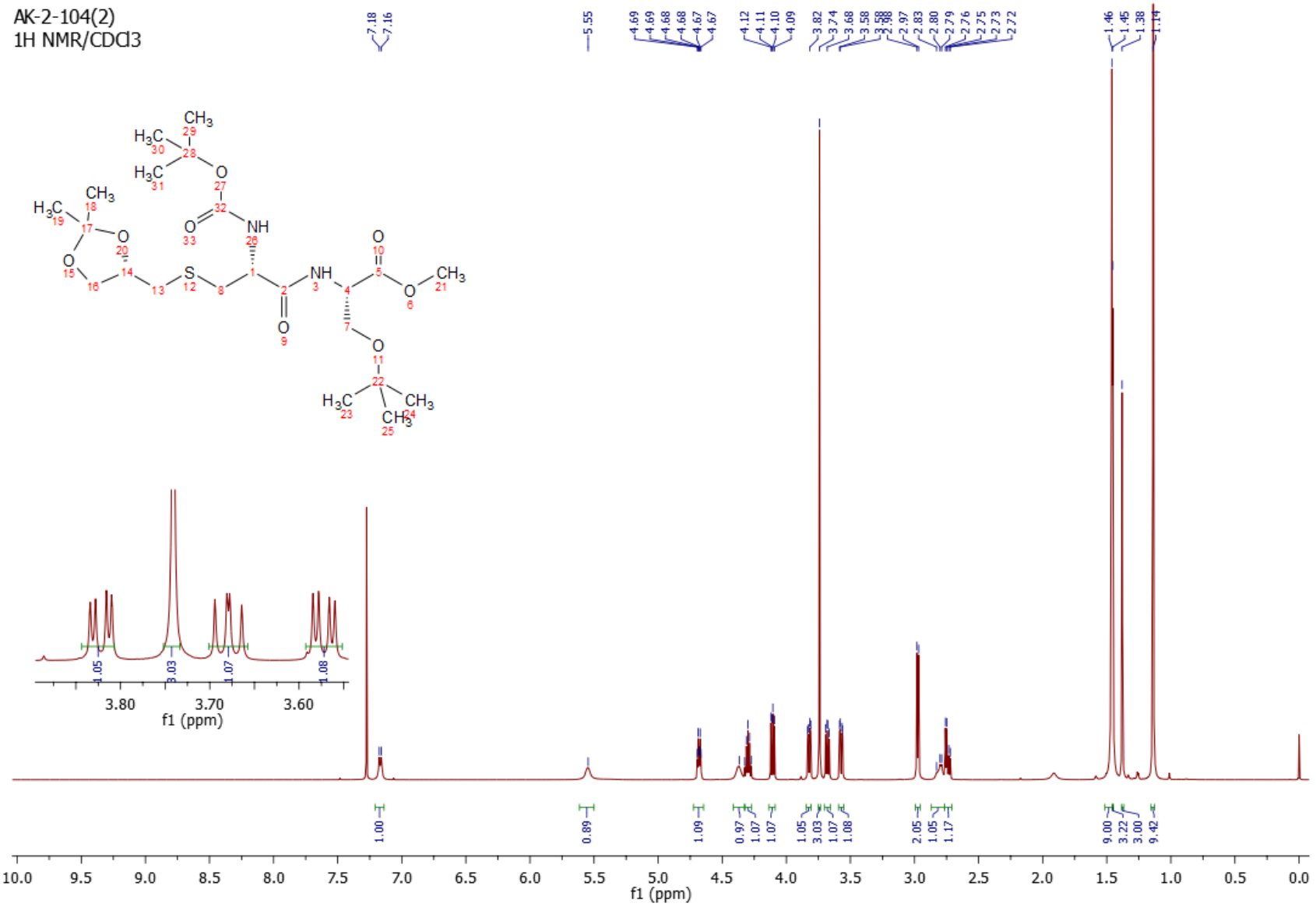


Exact Mass: 492.2505

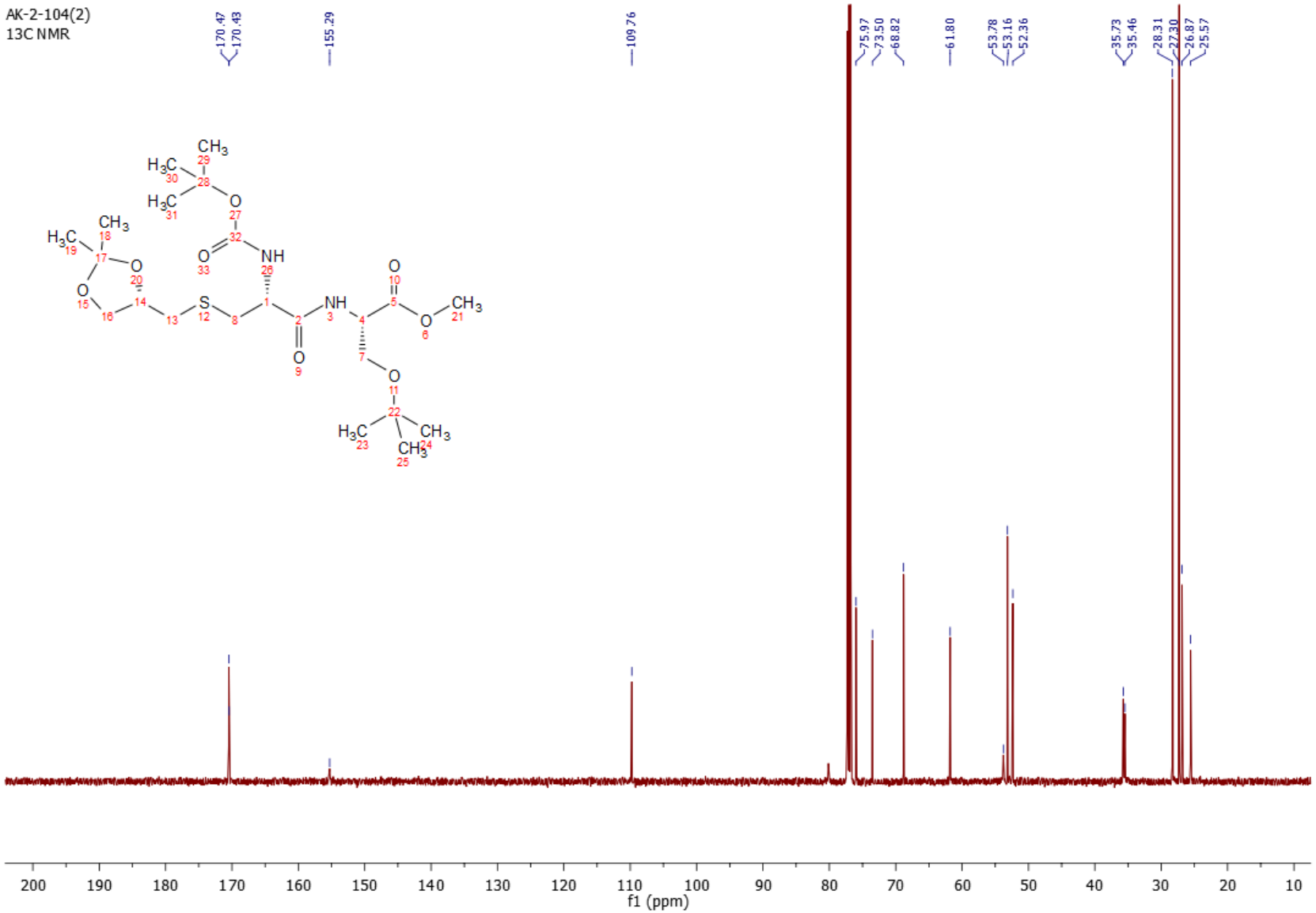
$[M + H]^+$: 493.2578

$[M + Na]^+$: 515.2398

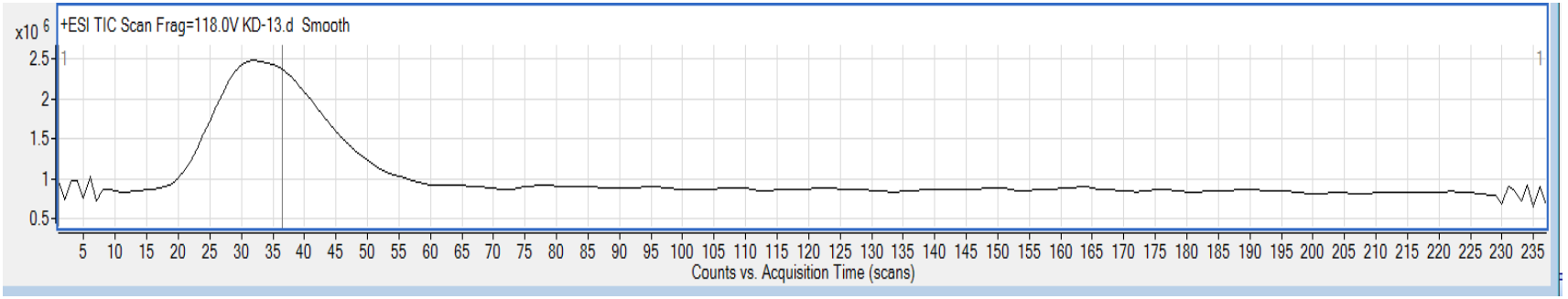
^{1}H NMR of Compound **5c** (in $CDCl_3$, 500 MHz)

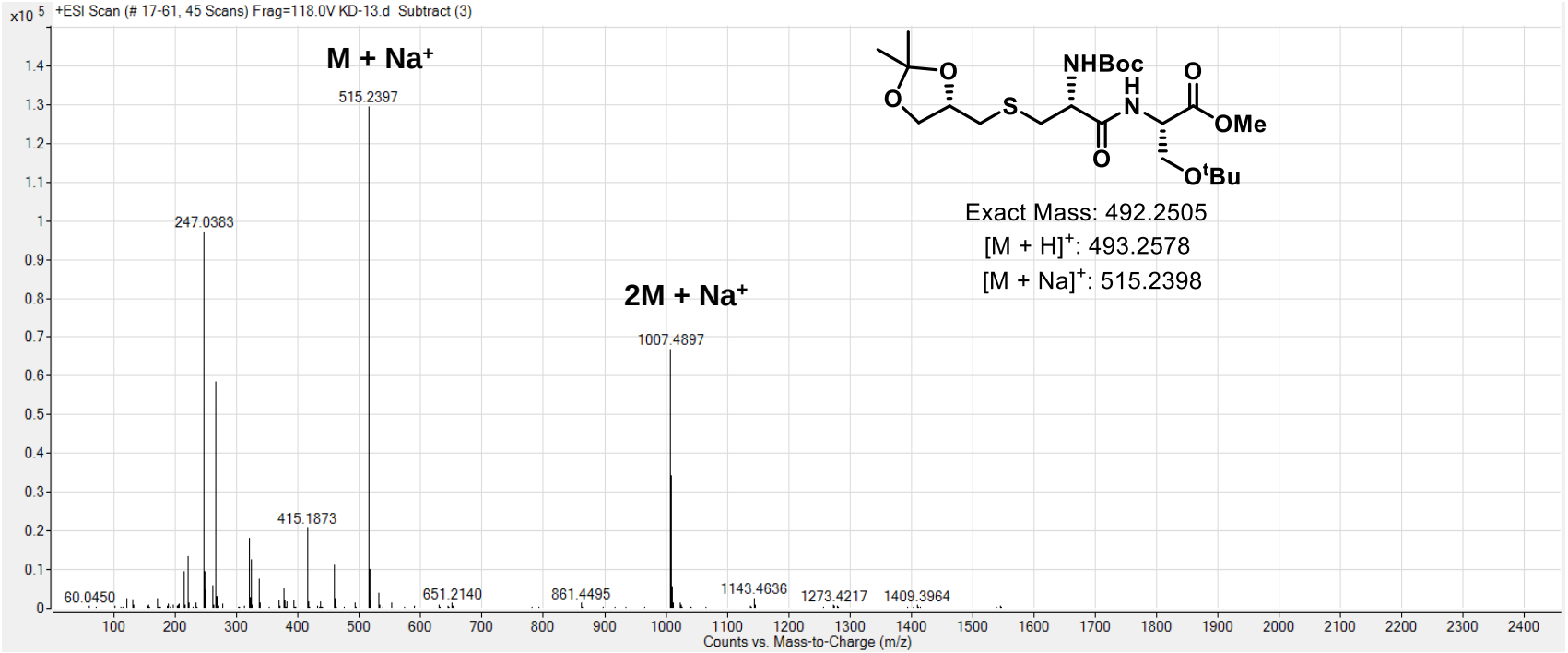


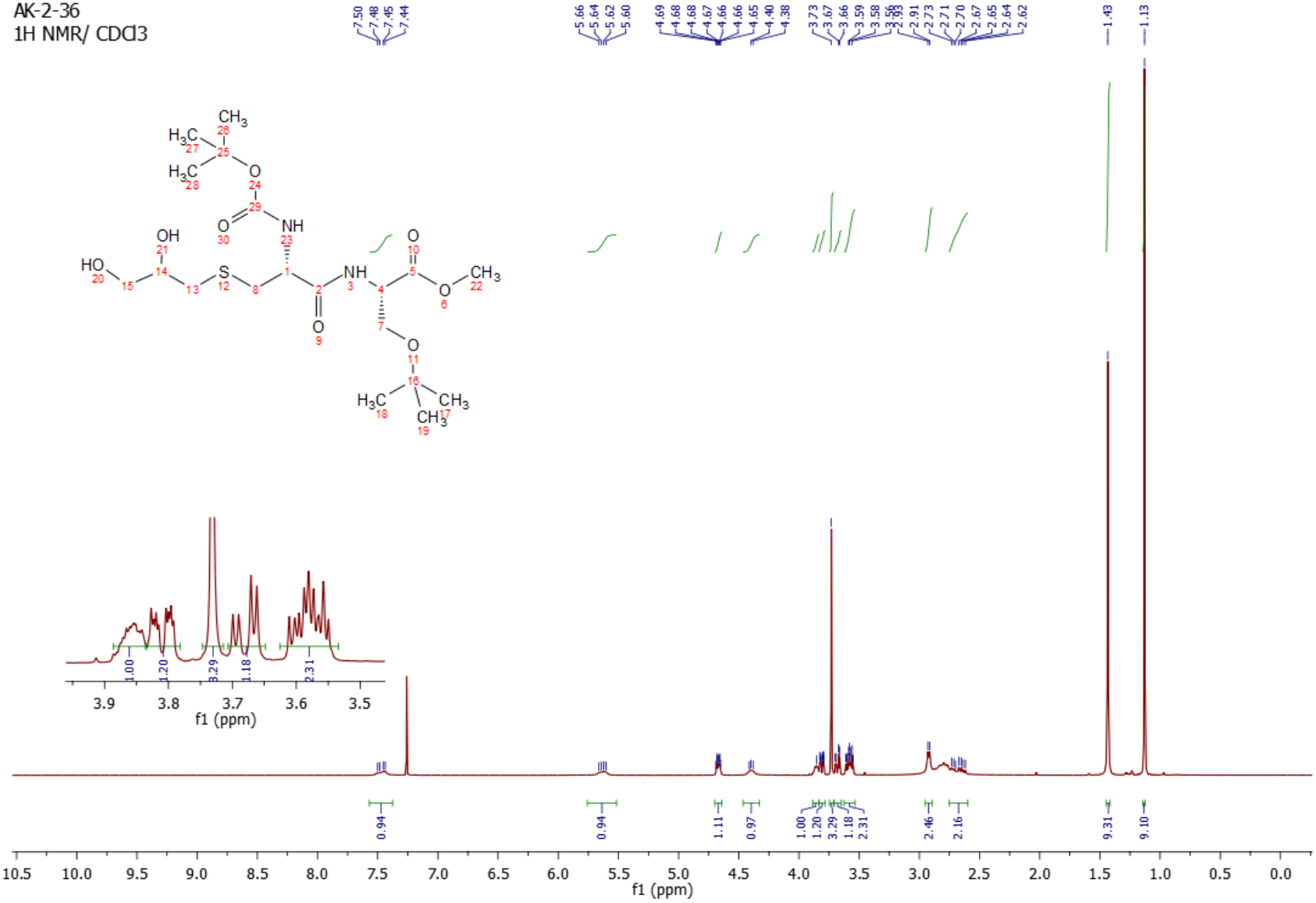
^{13}C NMR of Compound **5c** (in $CDCl_3$, 125 MHz)



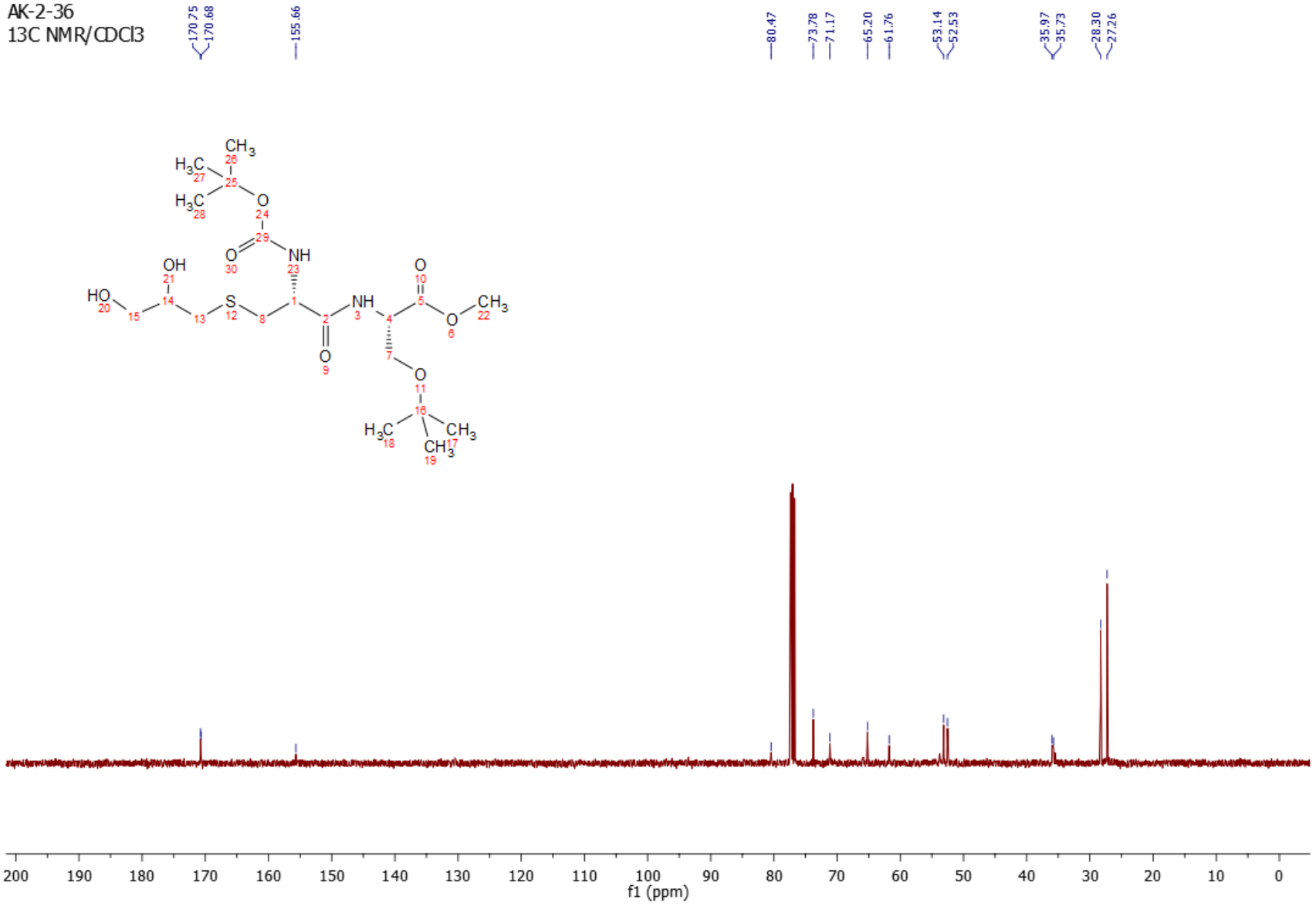
HRMS of Compound **5c** ESI



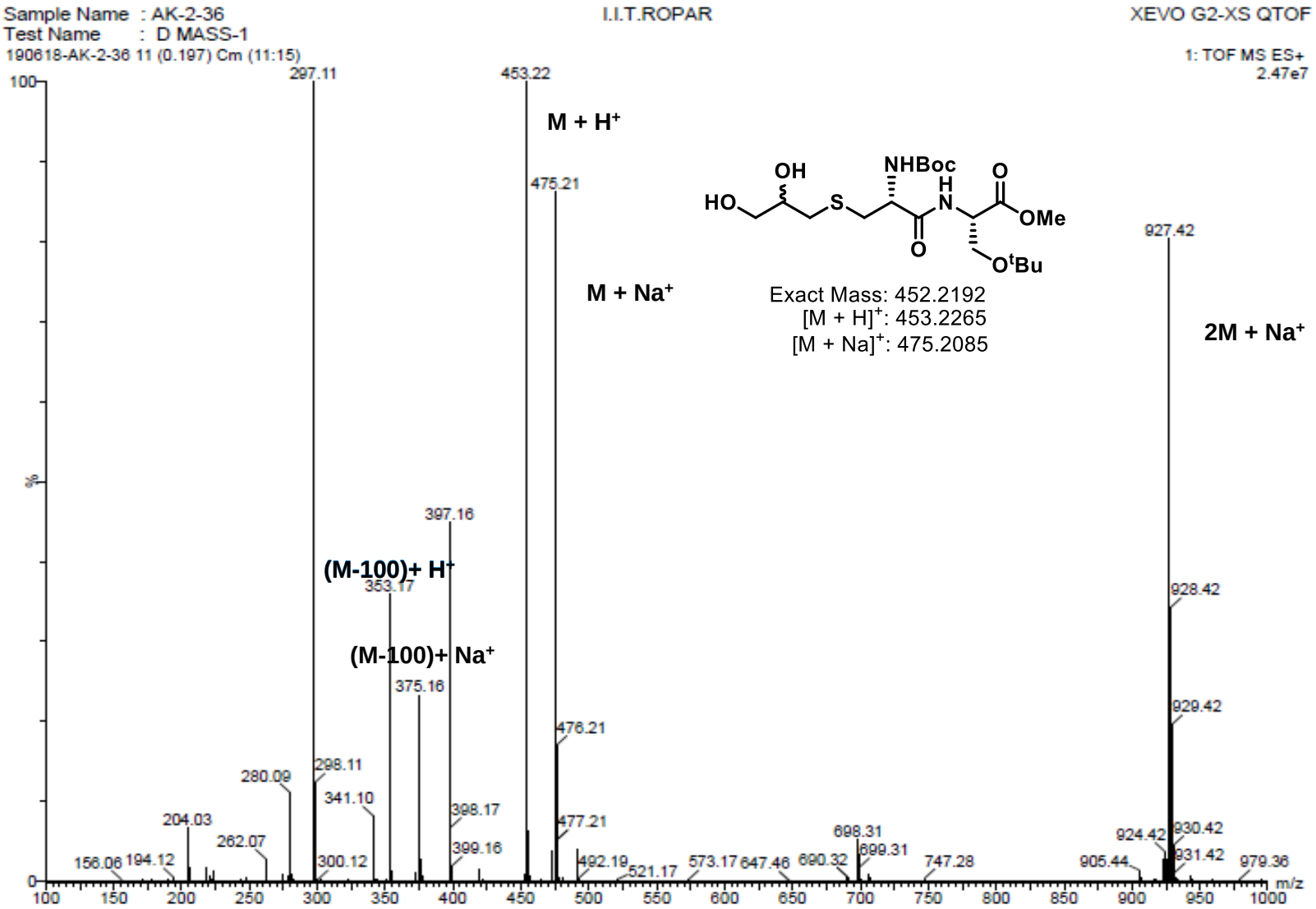



AK-2-36
1H NMR/ CDCl3
f1 (ppm)

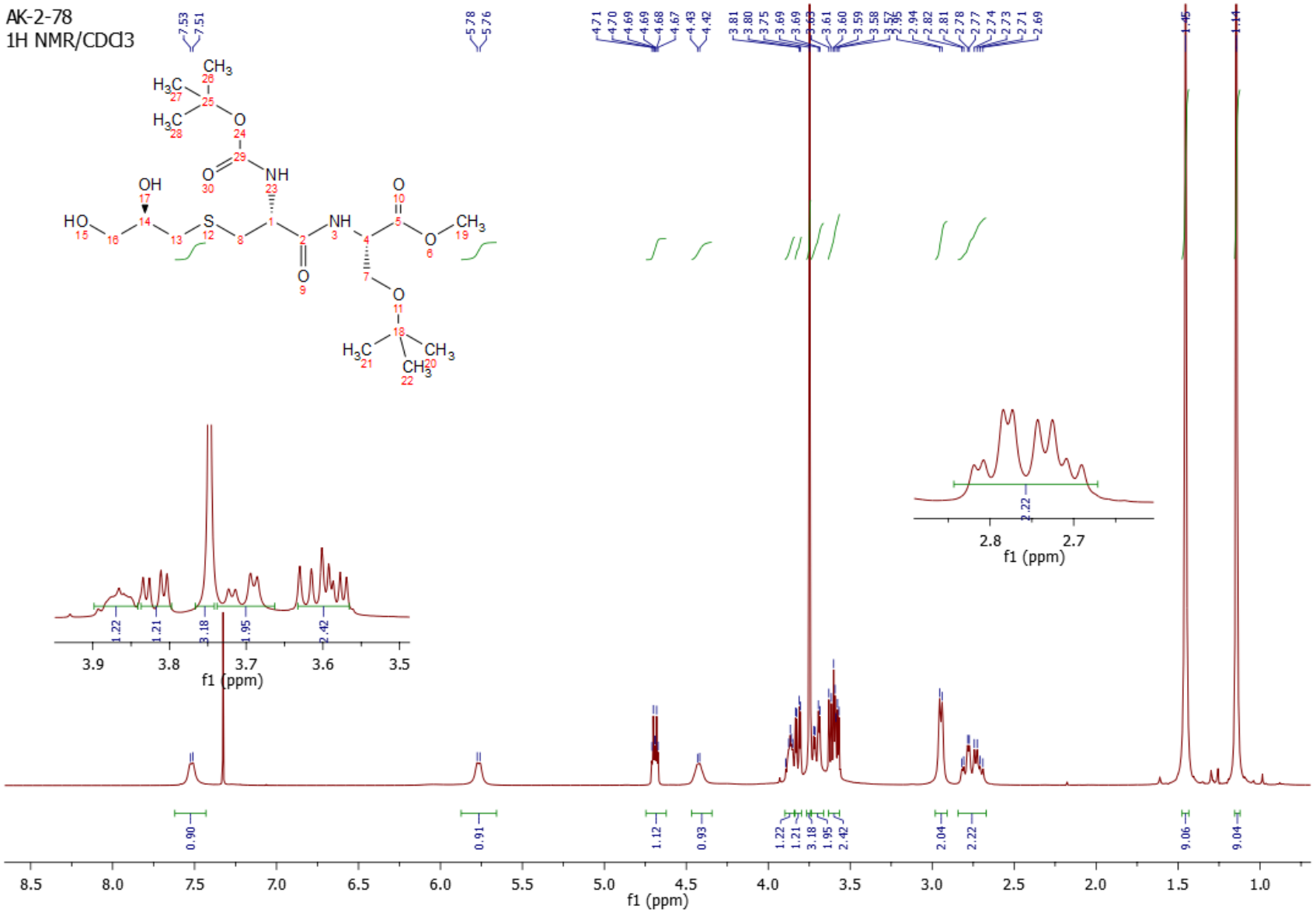
^{13}C NMR of Compound **6a** (in $CDCl_3$, 100 MHz)



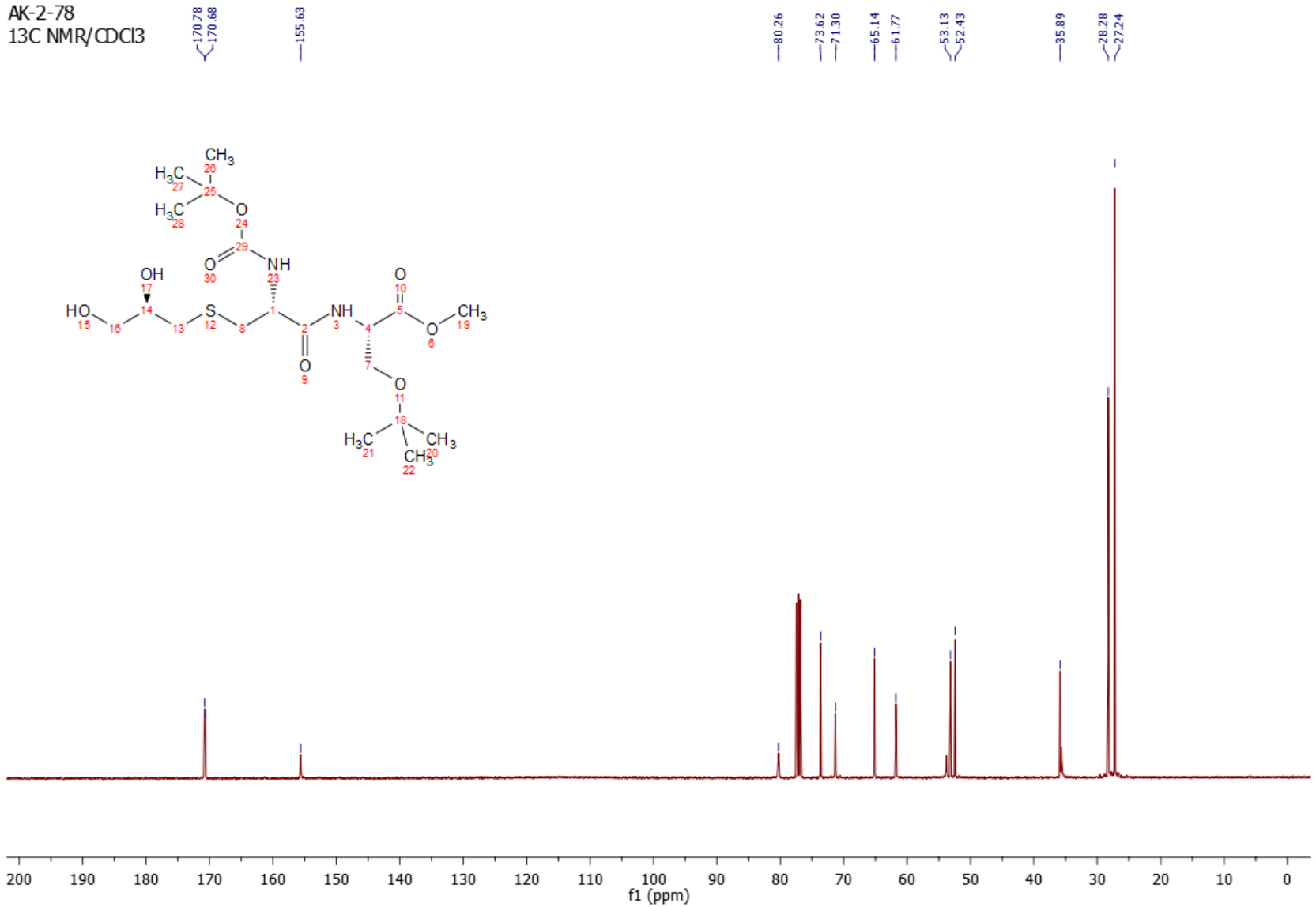
MS of Compound **6a** ESI



^{1}H NMR of Compound **6b** (in $CDCl_3$, 400 MHz) ESI



^{13}C NMR of Compound **6b** (in $CDCl_3$, 100 MHz)



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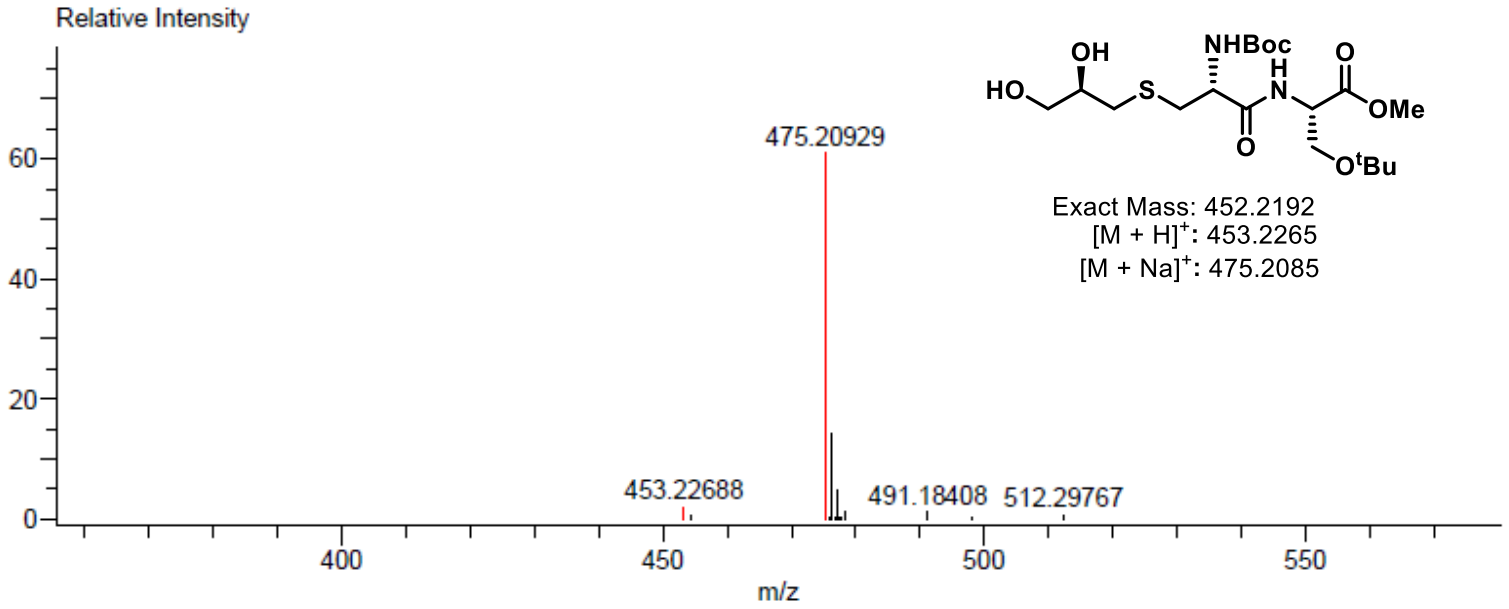
Created by:AccuTOF

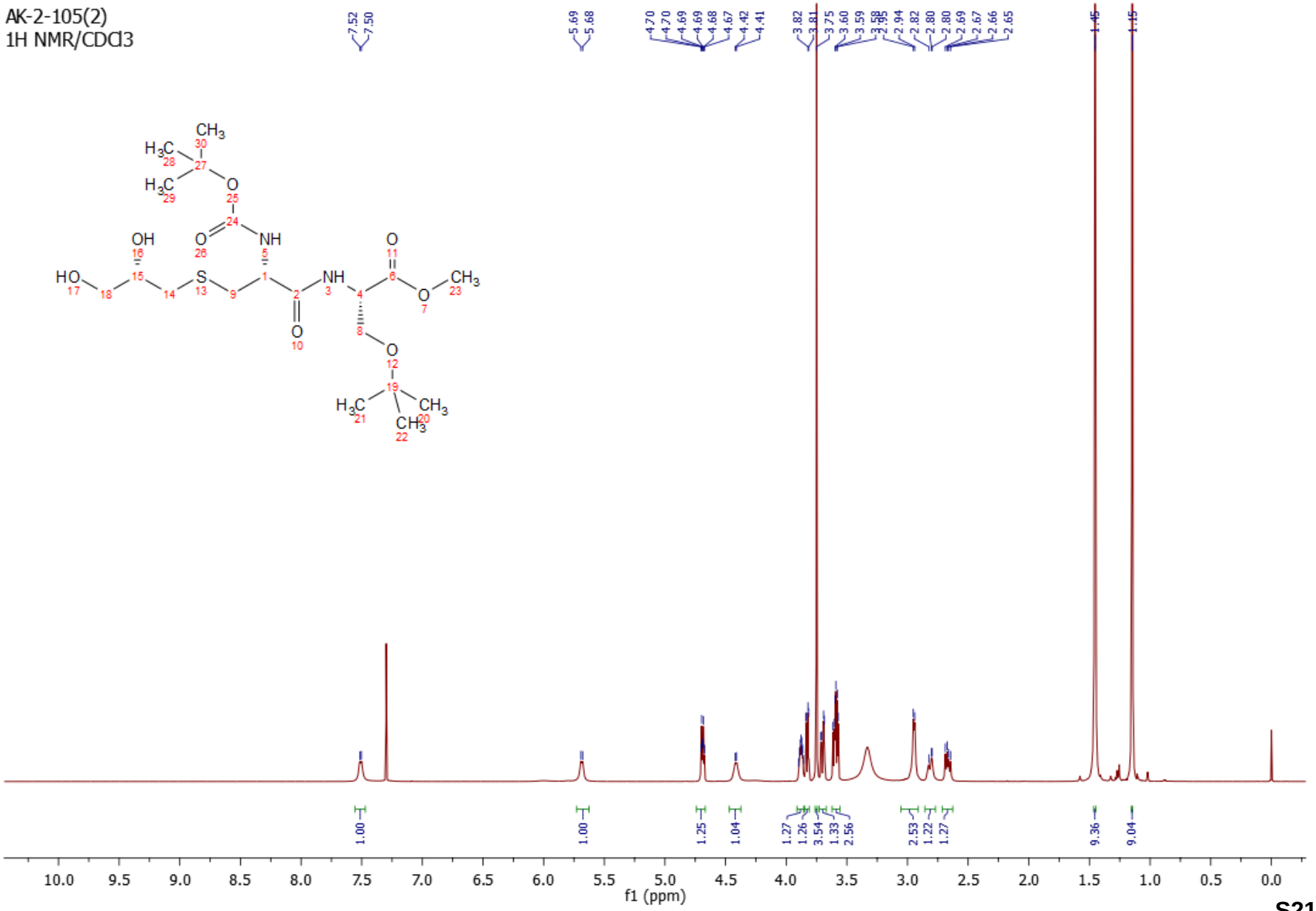
Charge number:1 Tolerance:200.00[ppm], 250.00 .. 250.... Unsaturation Number:-100.5 .. 200.0 (...
Element:^{12}C:19 .. 19, ^{1}H:36 .. 37, ^{14}N:2 .. 2, ^{23}Na:0 .. 1, ^{16}O:8 .. 8, ^{32}S:1 .. 1



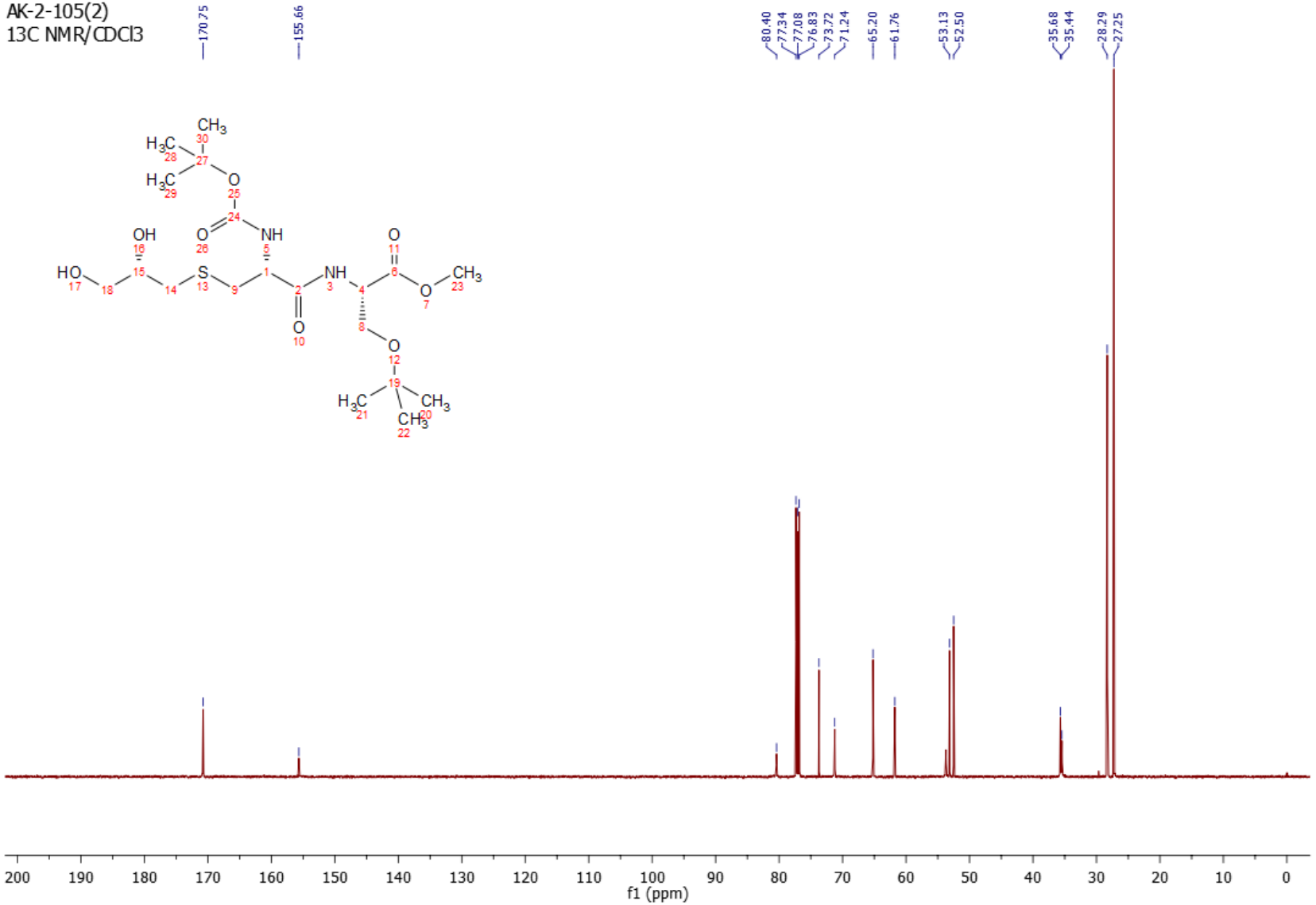
Exact Mass: 452.2192
$[M + H]^+$: 453.2265
$[M + Na]^+$: 475.2085

Mass	Intensity	Calc. Mass	Mass Difference [ppm]	Possible Formula
453.22688	4645.38	453.22706	-0.40	$^{12}C_{19}{}^{1}H_{37}{}^{14}N_2{}^{16}O_8{}^{32}S_1$
475.20929	156003.54	475.20901	0.60	$^{12}C_{19}{}^{1}H_{36}{}^{14}N_2{}^{23}Na_1{}^{16}O_8{}^{32}S_1$

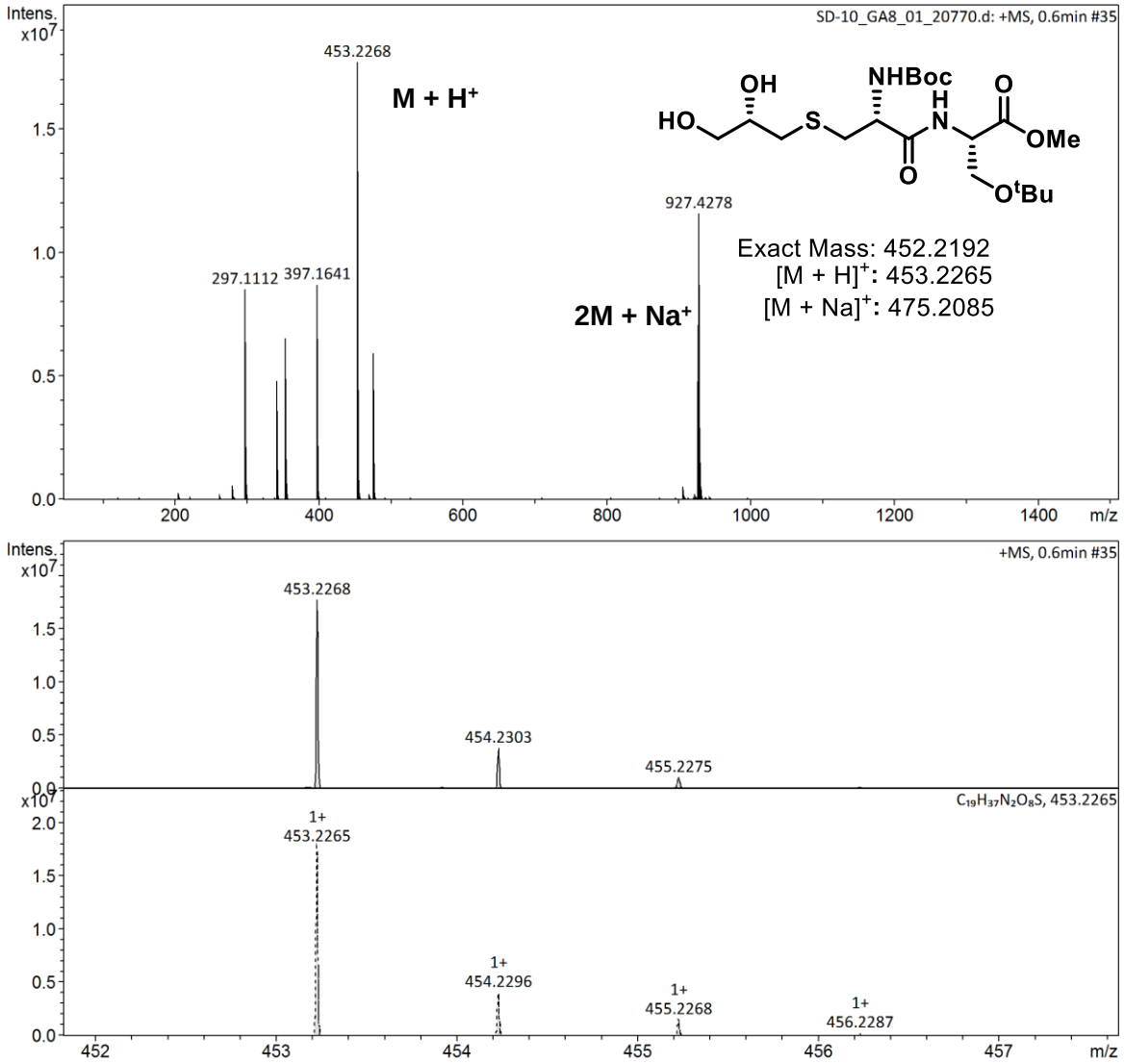
^{1}H NMR of Compound **6c** (in $CDCl_3$, 500 MHz) ESI



^{13}C NMR of Compound **6c** (in $CDCl_3$, 125 MHz)

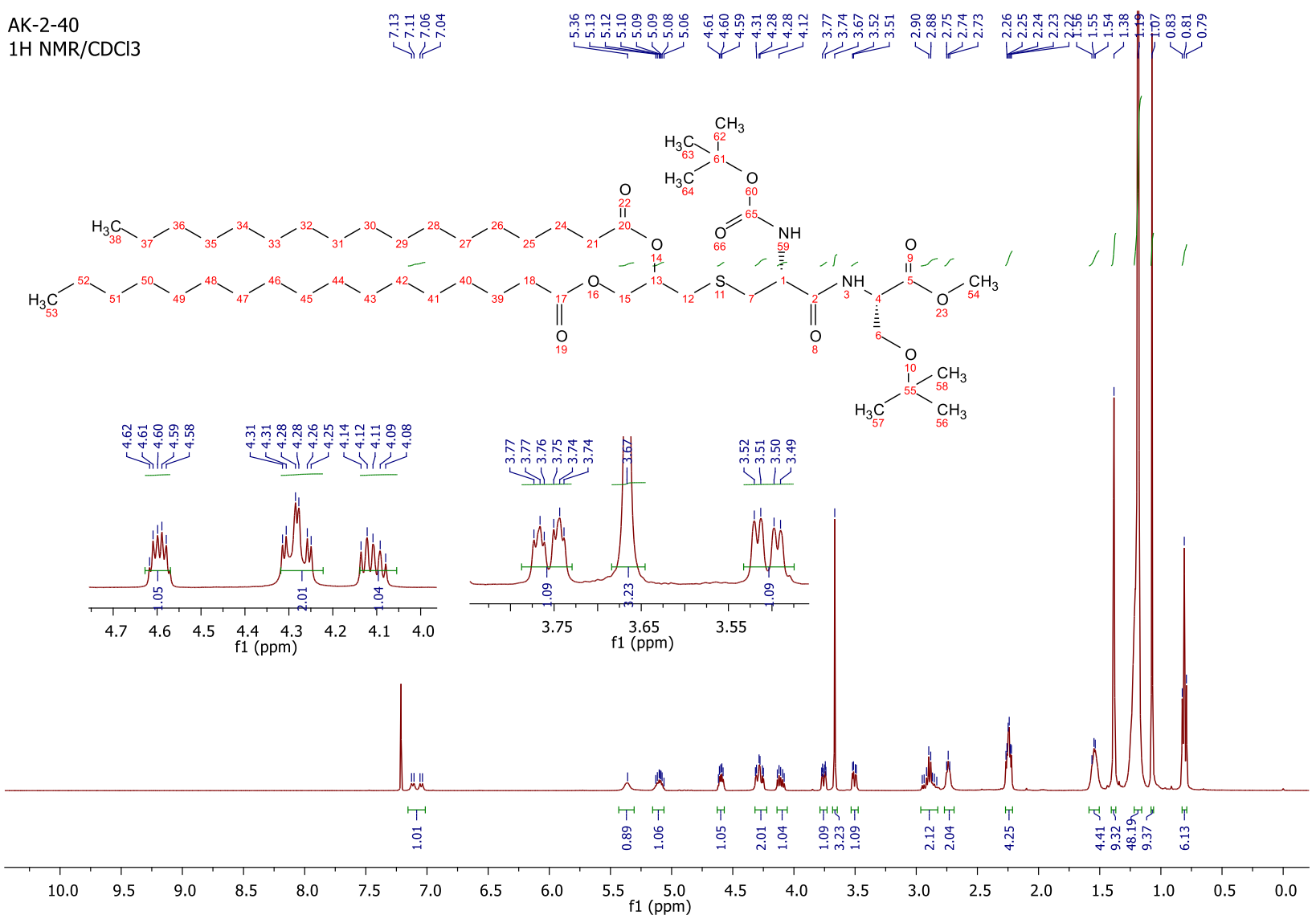


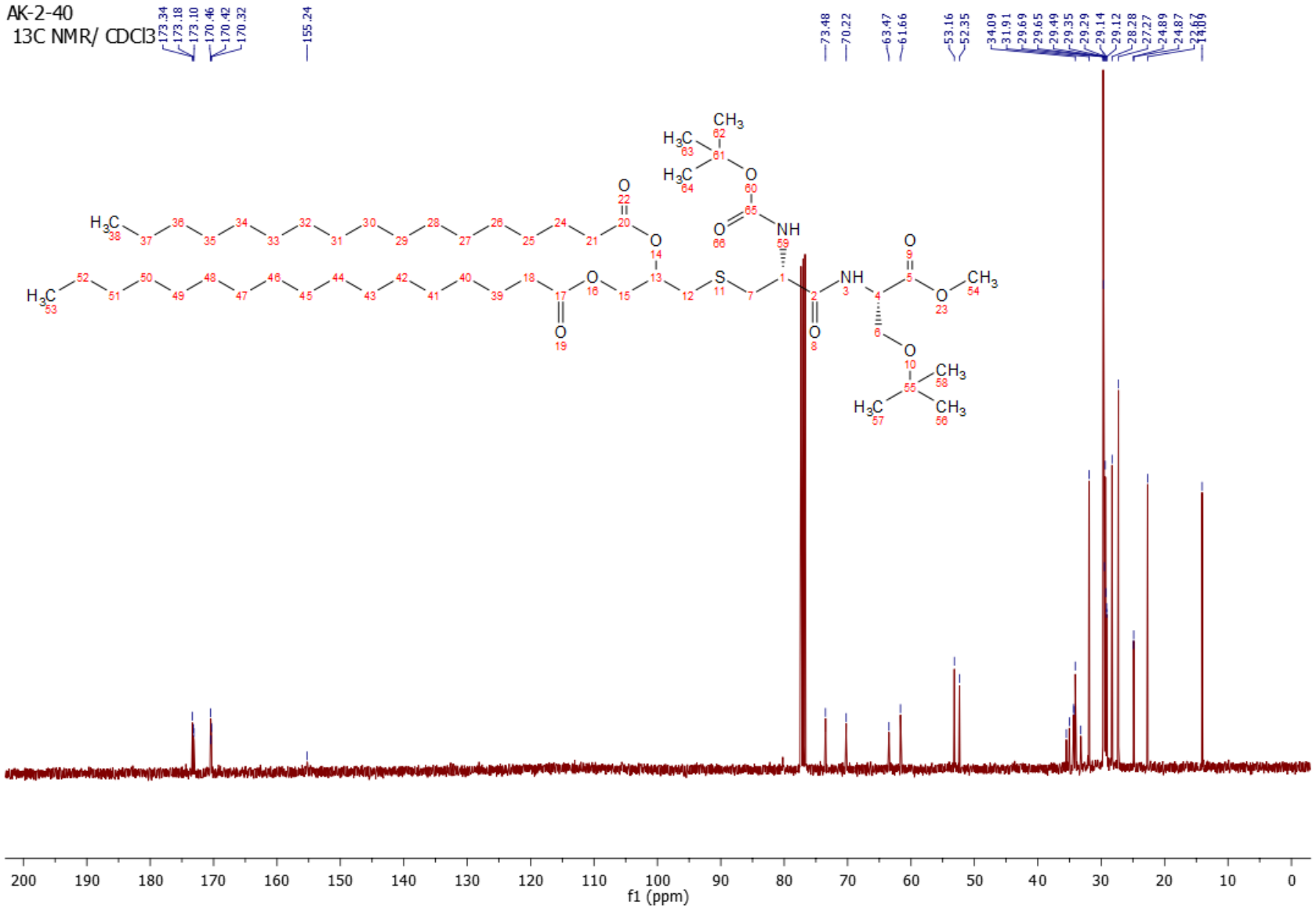
HRMS of Compound **6c** ESI



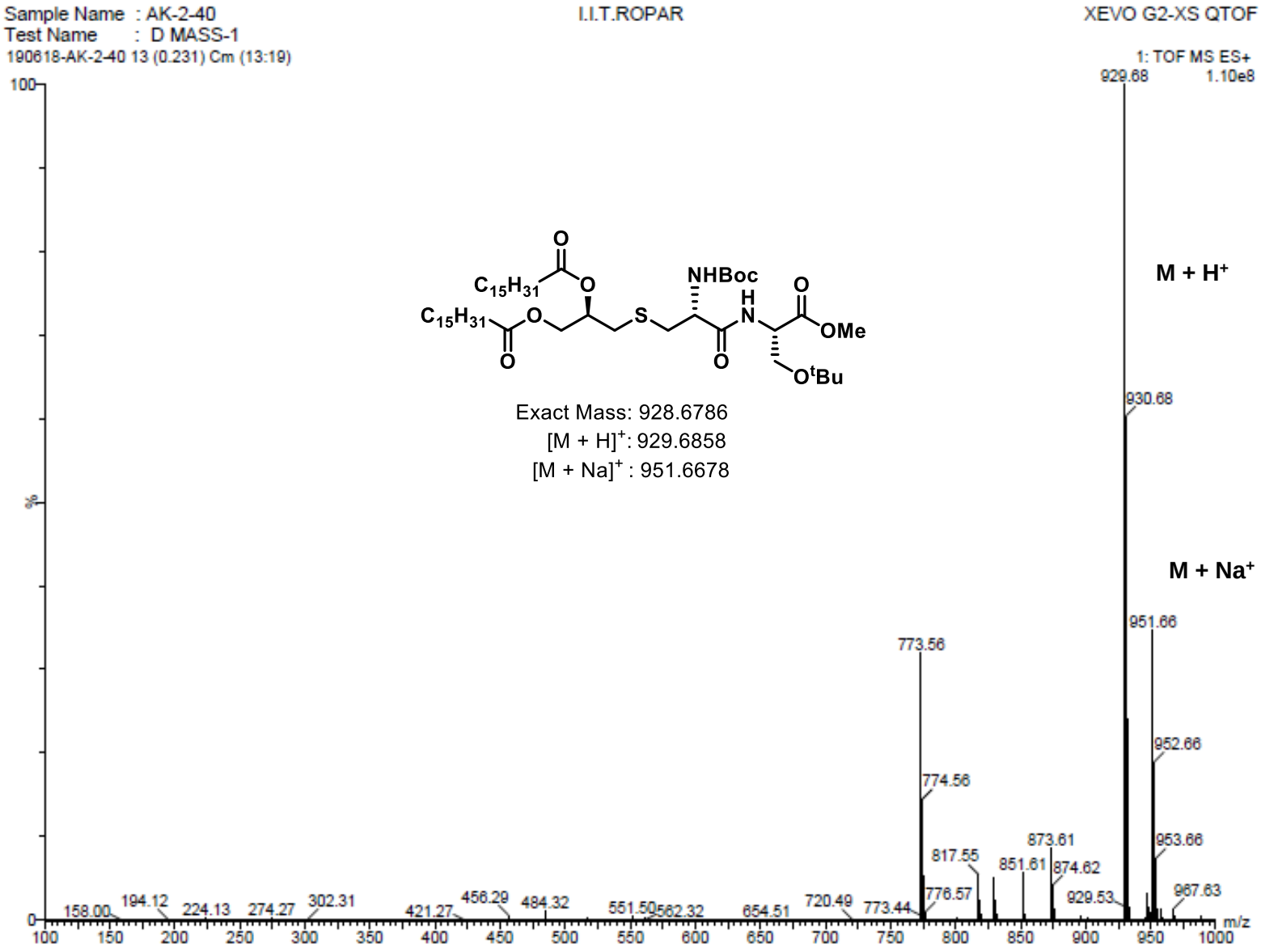
^{1}H NMR of Compound **7a** (in $CDCl_3$, 400 MHz)

ESI



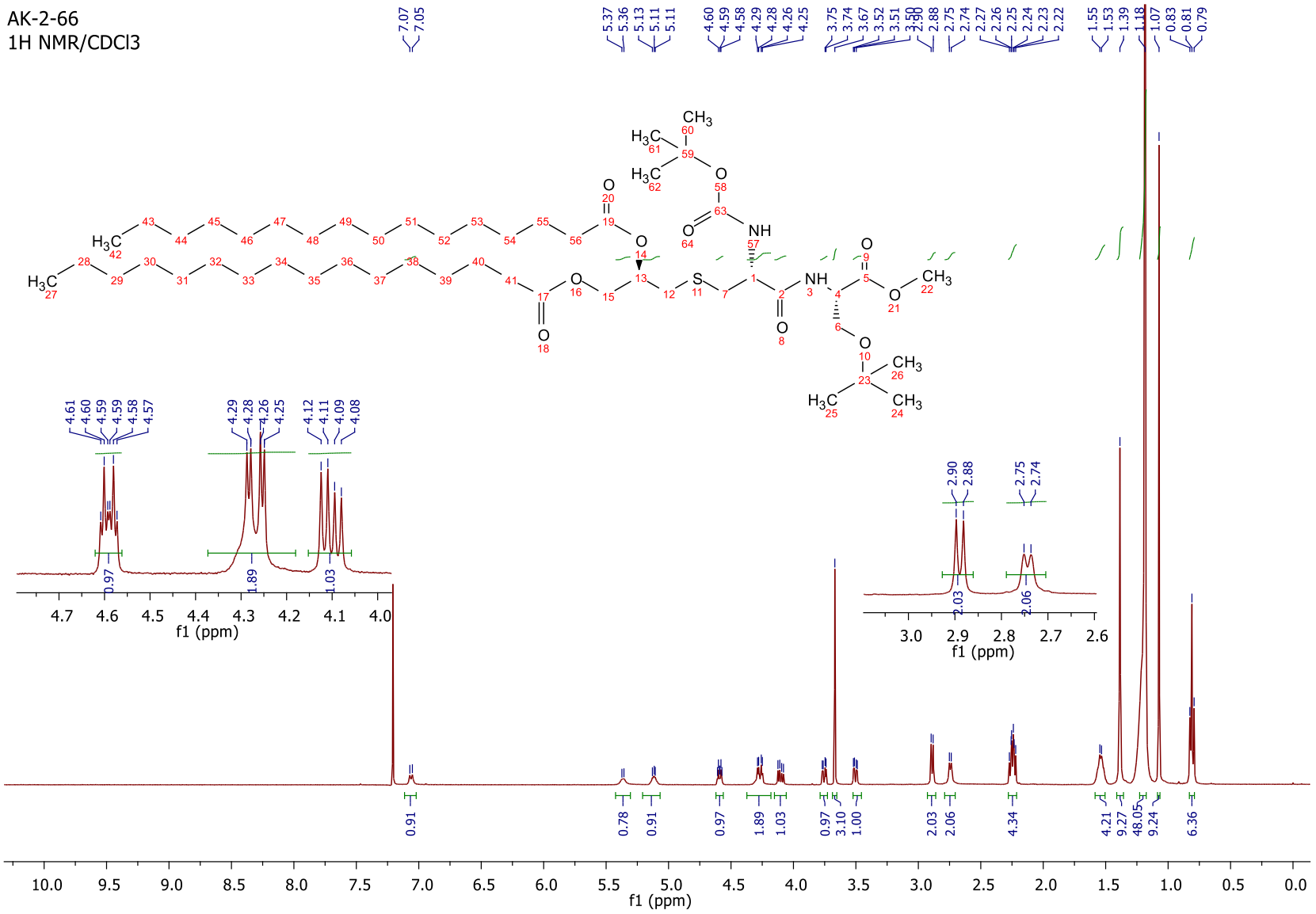

AK-2-40
13C NMR/ CDCl3
173.34
173.18
173.10
170.46
170.42
170.32
155.24
73.48
70.22
63.47
61.66
53.16
52.35
34.09
31.91
29.69
29.65
29.49
29.35
29.29
29.14
29.12
28.28
27.27
24.89
24.87
22.67
14.09
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

MS of Compound **7a** ESI

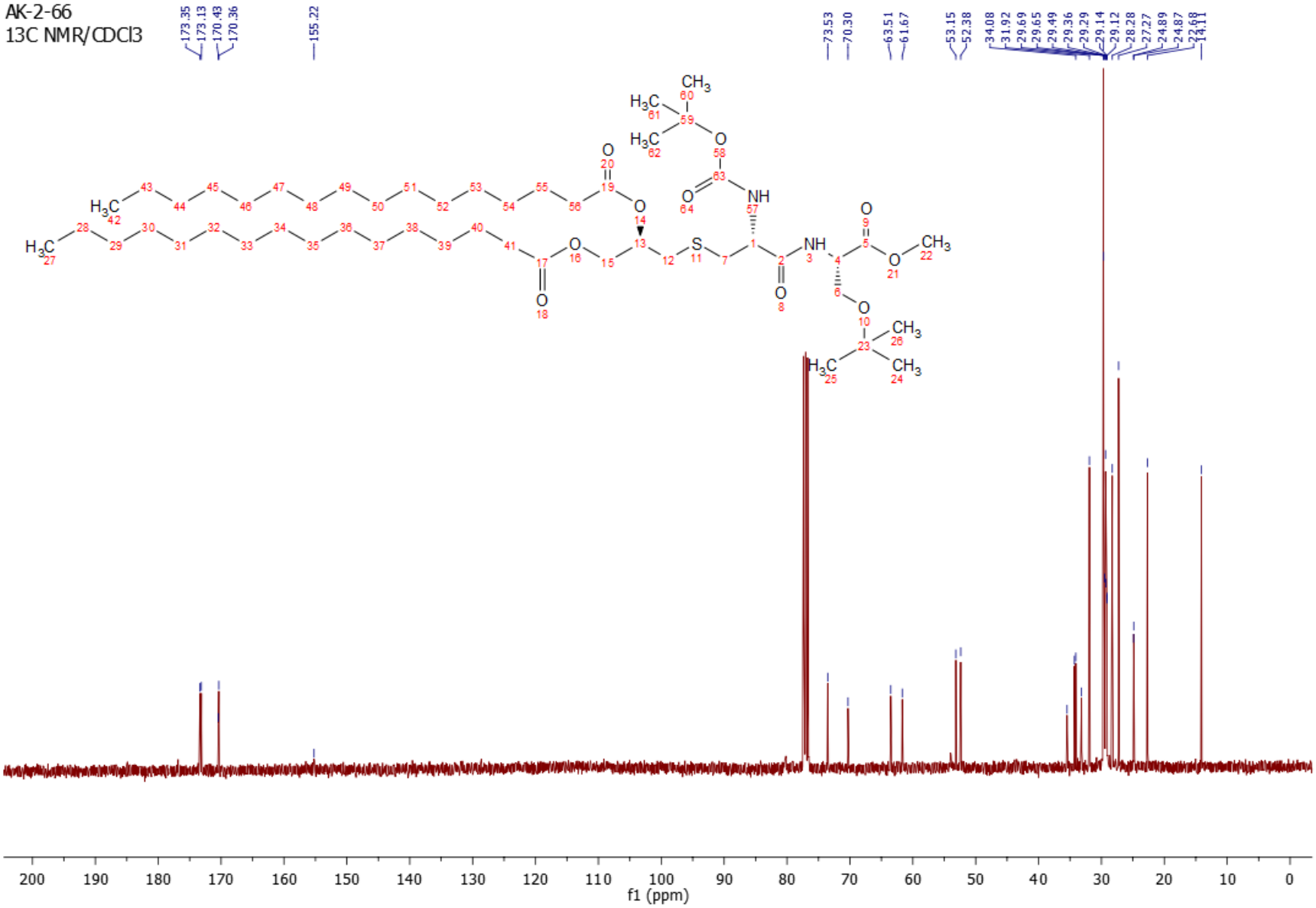


^{1}H NMR of Compound **7b** (in $CDCl_3$, 400 MHz)

ESI



^{13}C NMR of Compound **7b** (in $CDCl_3$, 100 MHz) ESI



HRMS of Compound **7b** ESI

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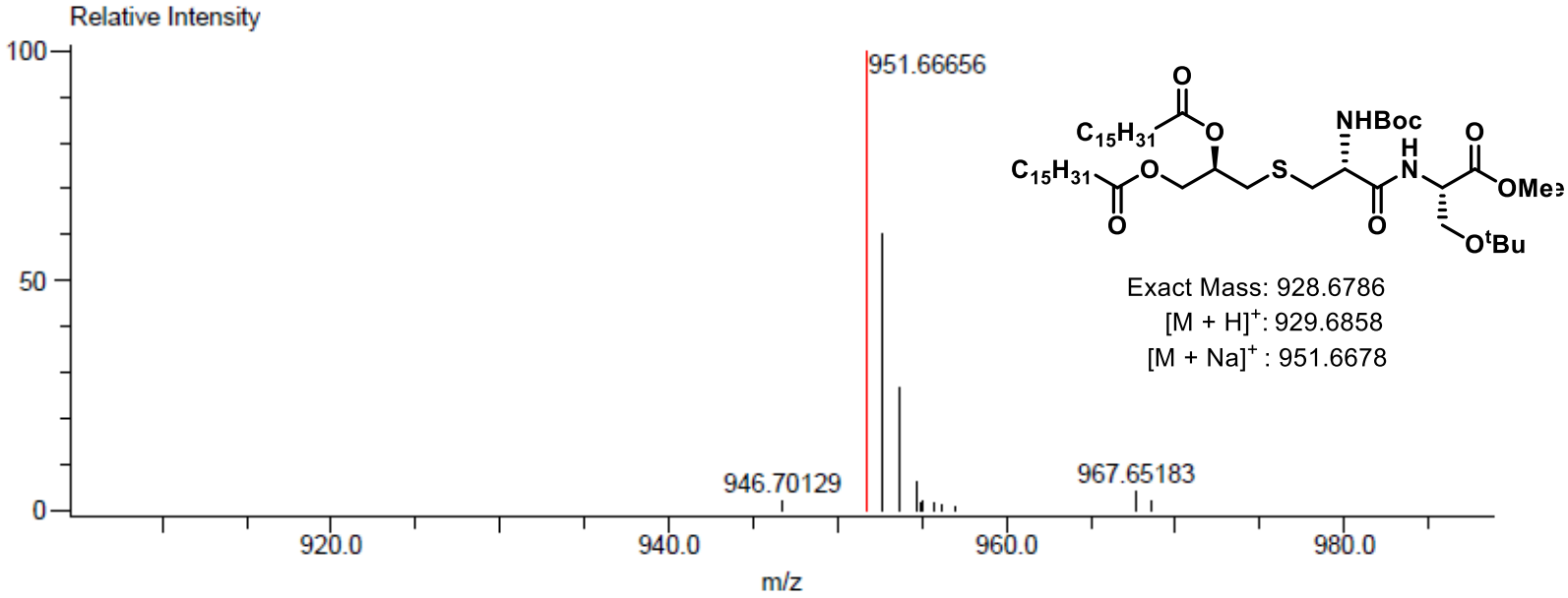
m/z Calibration File:20190422-TFANa_...
Created:4/29/2019 2:05:13 PM
Created by:AccuTOF

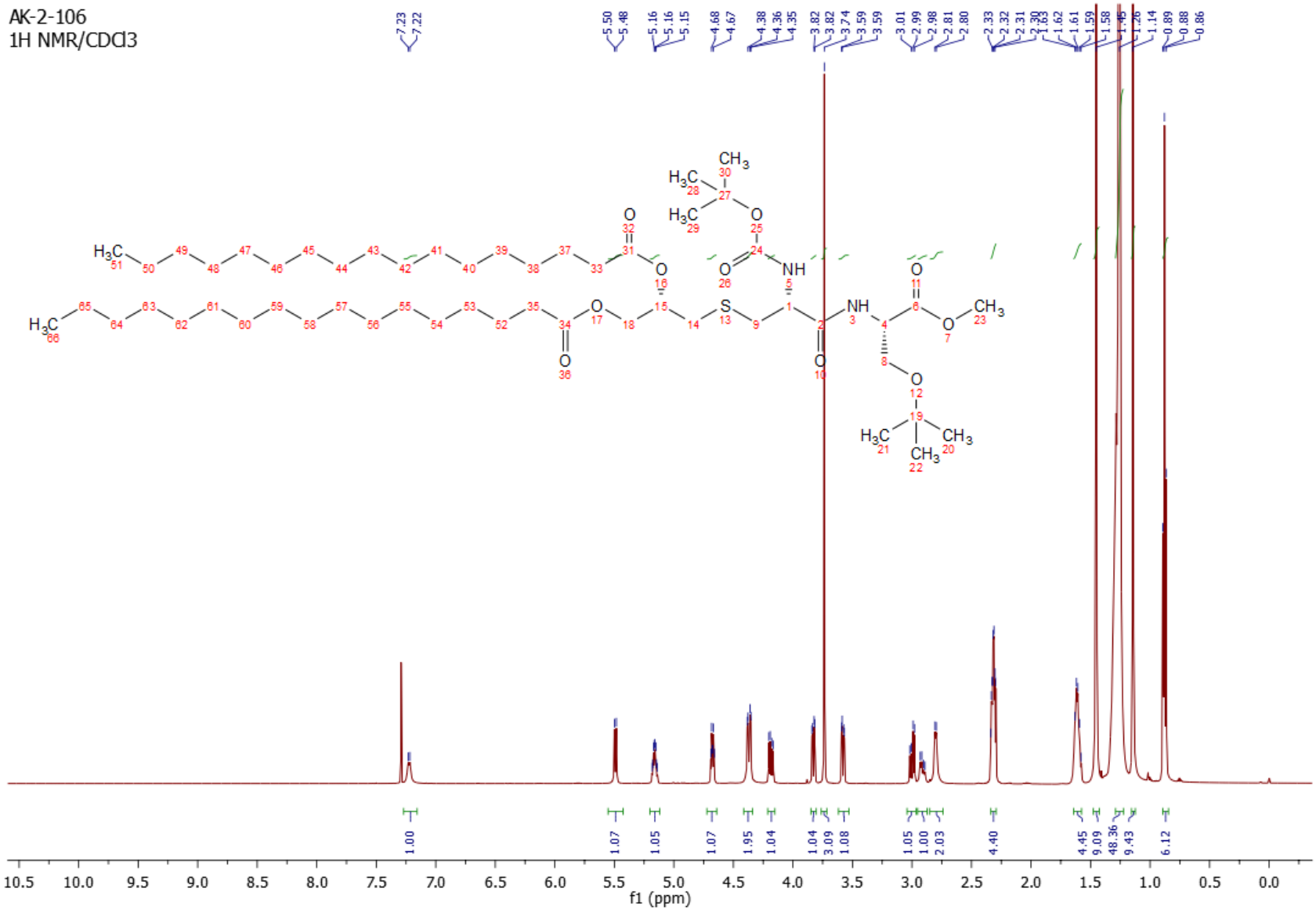
Charge number:1 Tolerance:200.00[ppm], 250.00 .. 250.... Unsaturation Number:-100.5 .. 200.0 (...
Element:^{12}C:51 .. 51, ^{1}H:96 .. 97, ^{14}N:2 .. 2, ^{23}Na:0 .. 1, ^{16}O:10 .. 10, ^{32}S:1 .. 1



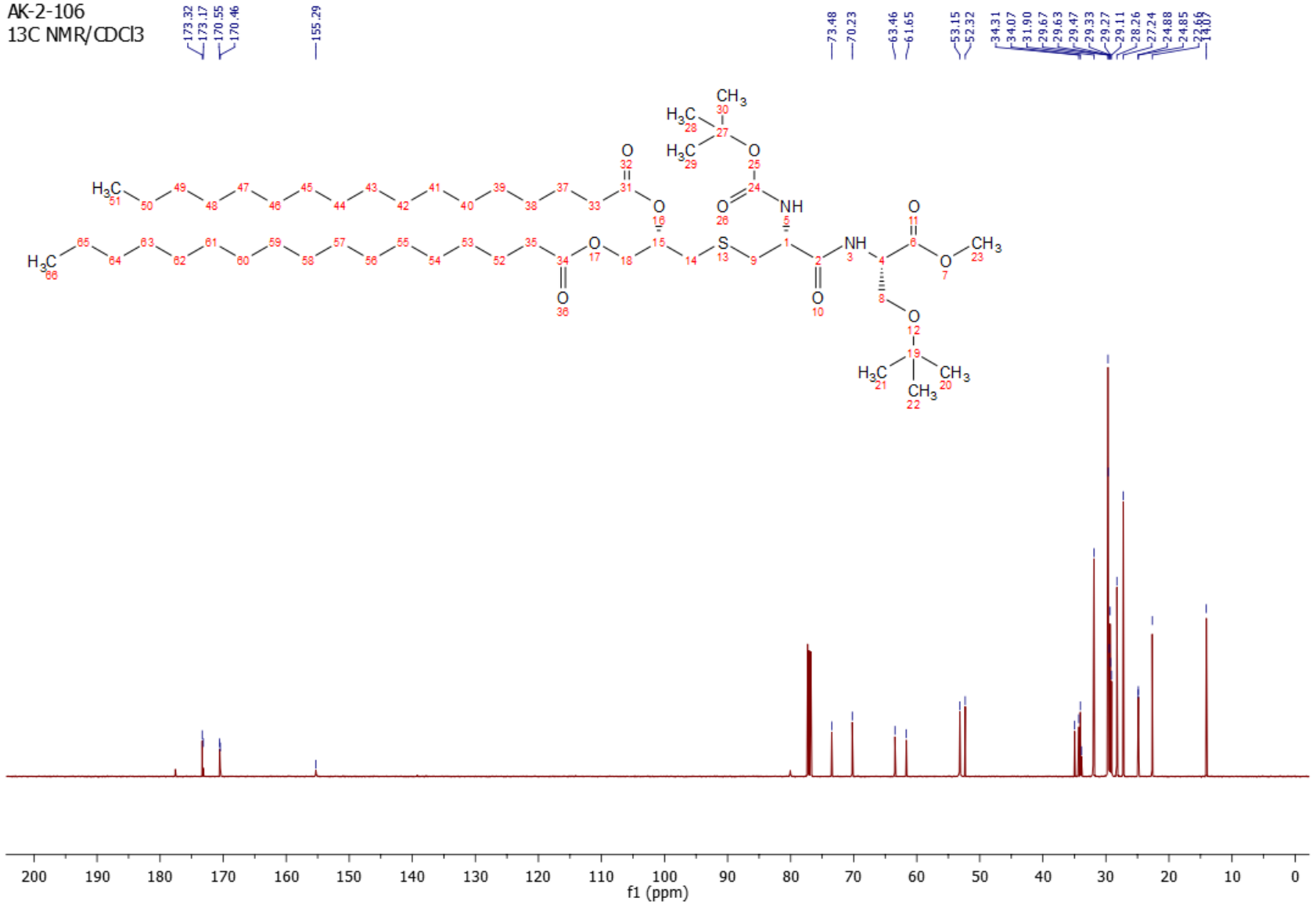
Exact Mass: 928.6786
$[M + H]^+$: 929.6858
$[M + Na]^+$: 951.6678

Mass	Intensity	Calc. Mass	Mass Difference [ppm]	Possible Formula
951.66656	72038.76	951.66833	-1.86	$^{12}C_{51}{}^{1}H_{96}{}^{14}N_{2}{}^{23}Na_{1}{}^{16}O_{10}{}^{32}S_{1}$

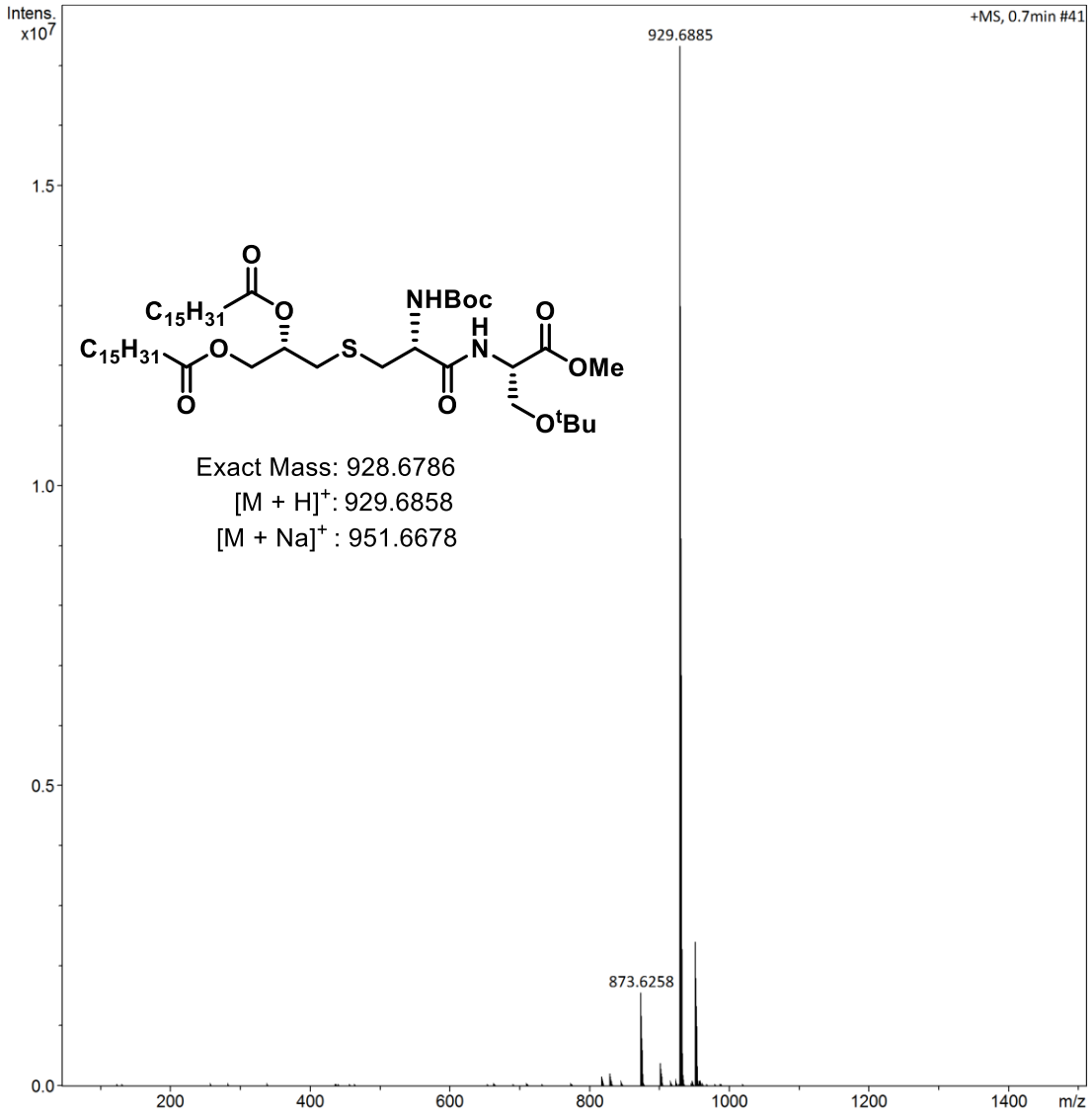
^{1}H NMR of Compound **7c** (in $CDCl_3$, 500 MHz) ESI



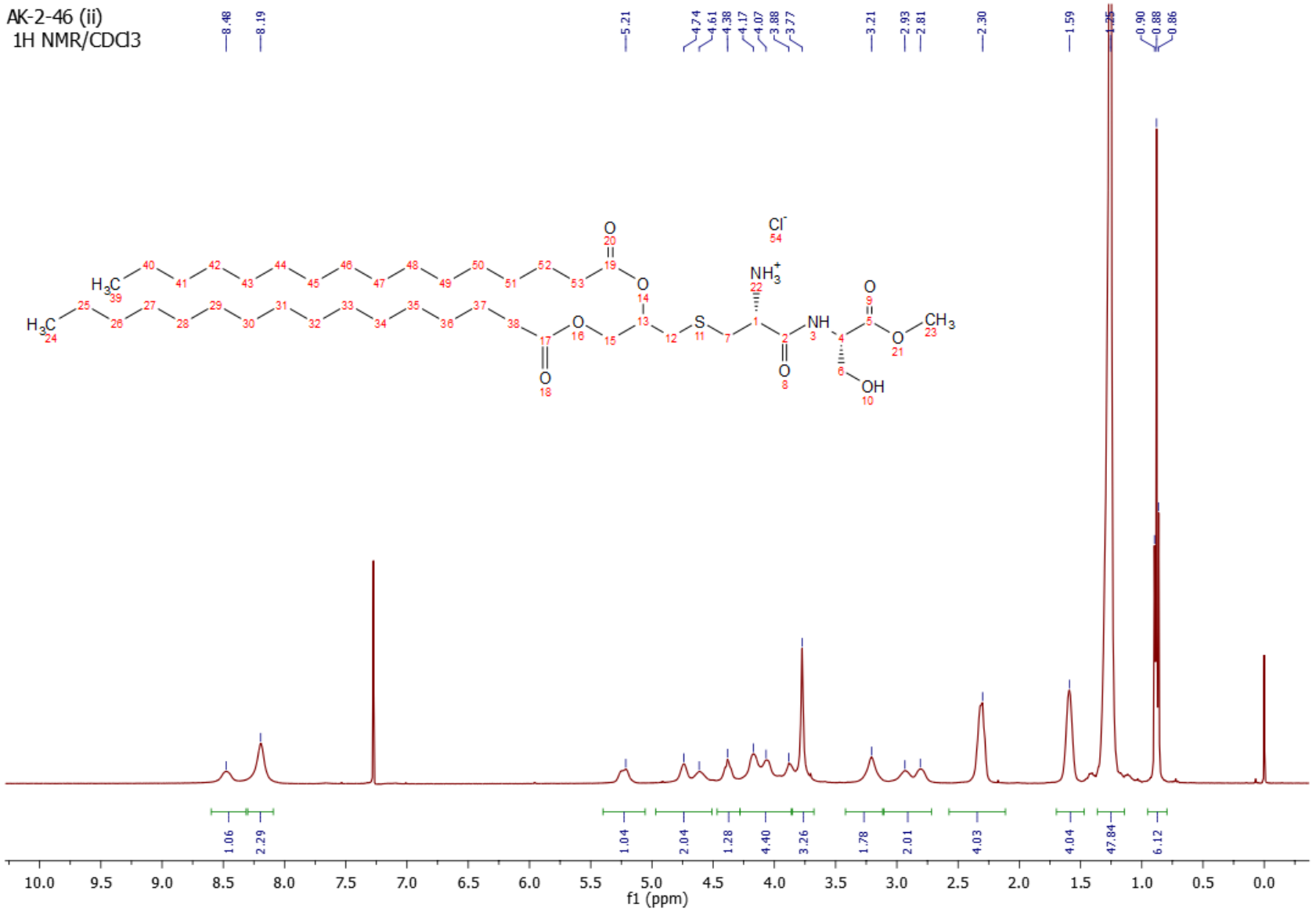
^{13}C NMR of Compound **7c** (in $CDCl_3$, 125 MHz)



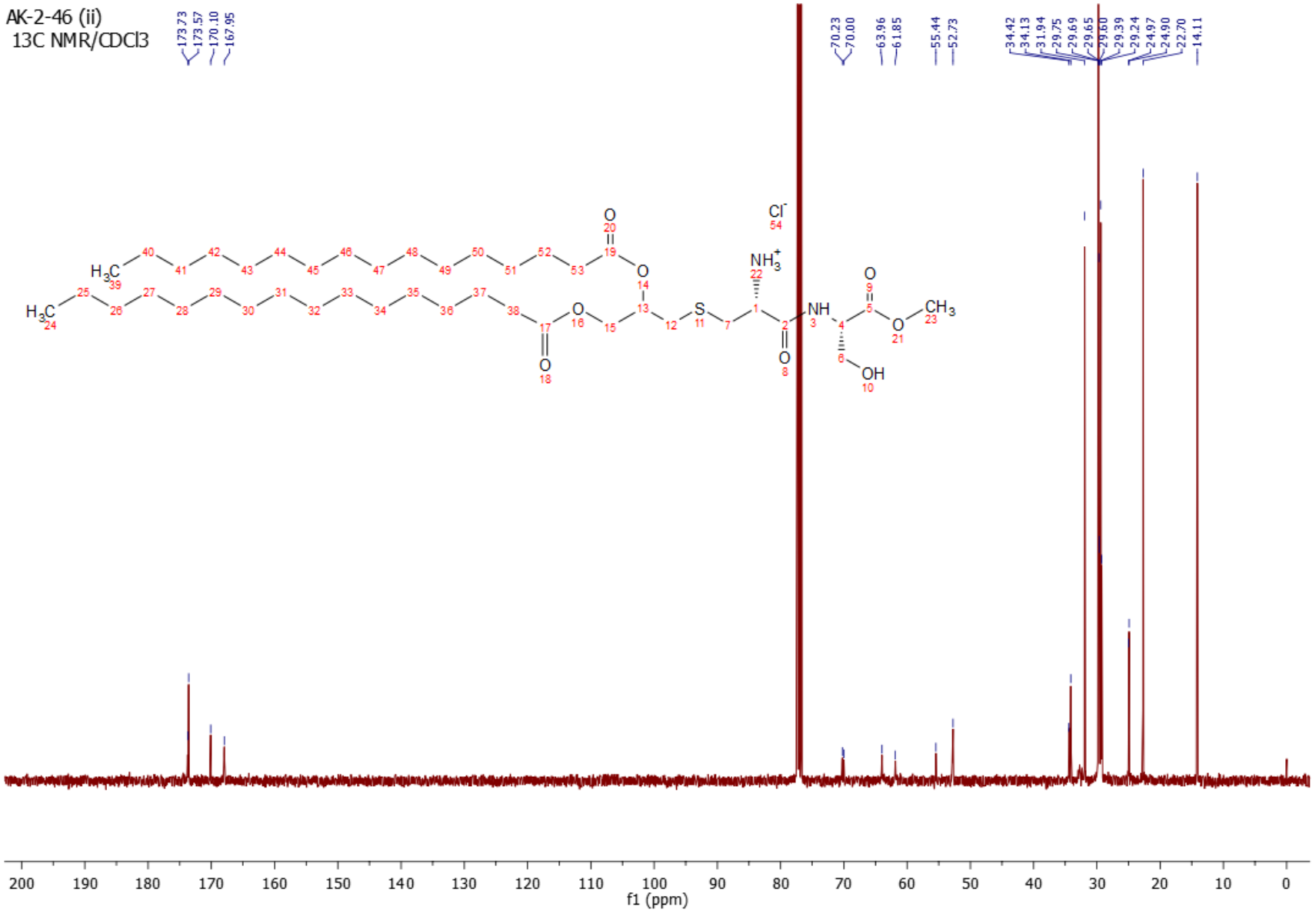
HRMS of Compound **7c** ESI



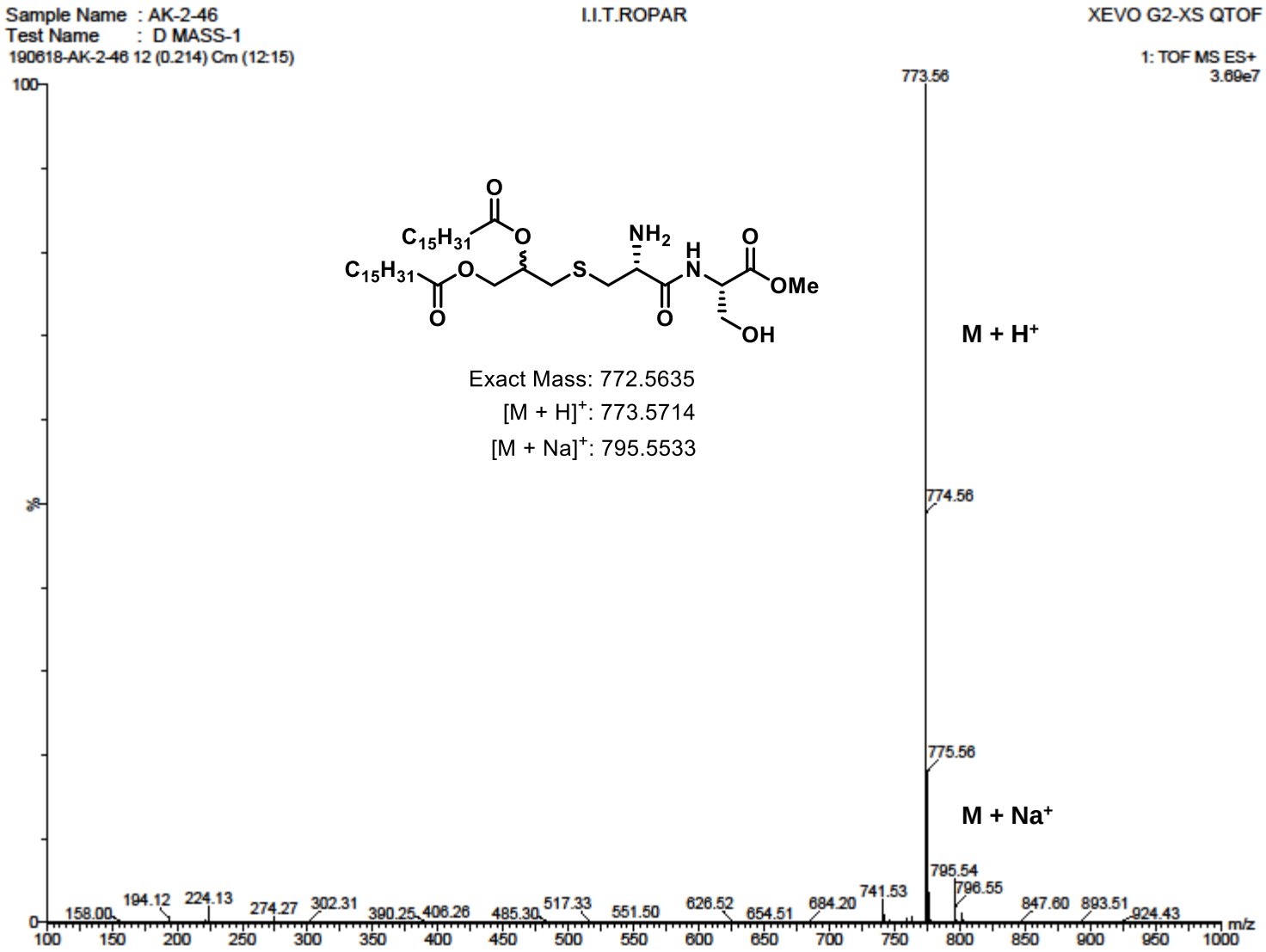
^{1}H NMR of Compound **8a** (in $CDCl_3$, 400 MHz) ESI



^{13}C NMR of Compound **8a** (in $CDCl_3$, 100 MHz) ESI



MS of Compound **8a** ESI



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Description:
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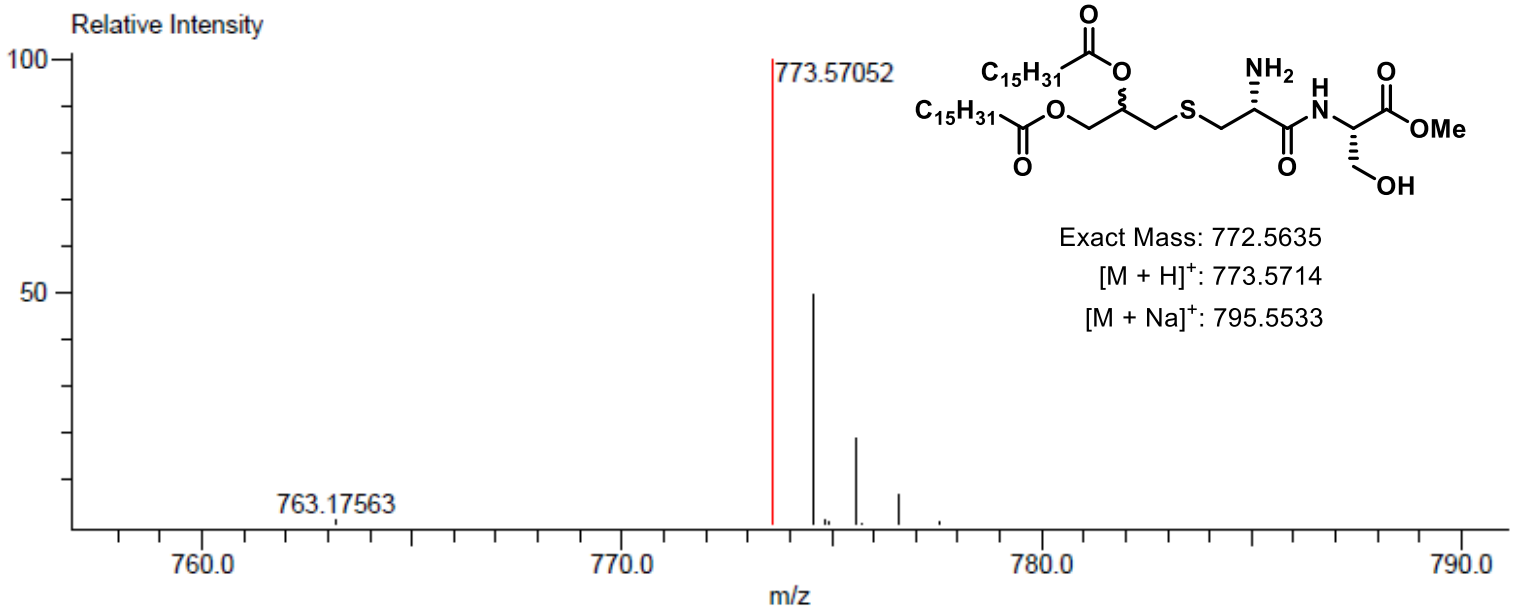
m/z Calibration File:20190422-TFANa
Created:4/29/2019 2:19:15 PM
Created by:AccuTOF

Charge number:1 Tolerance:200.00[ppm], 250.00 .. 250.... Unsaturation Number:-100.5 .. 200.0 (
Element:^{12}C:42 .. 42, ^{1}H:81 .. 85, ^{35}Cl:0 .. 1, ^{14}N:2 .. 2, ^{23}Na:0 .. 1, ^{16}O:8 .. 8, ^{32}S:1 .. 1

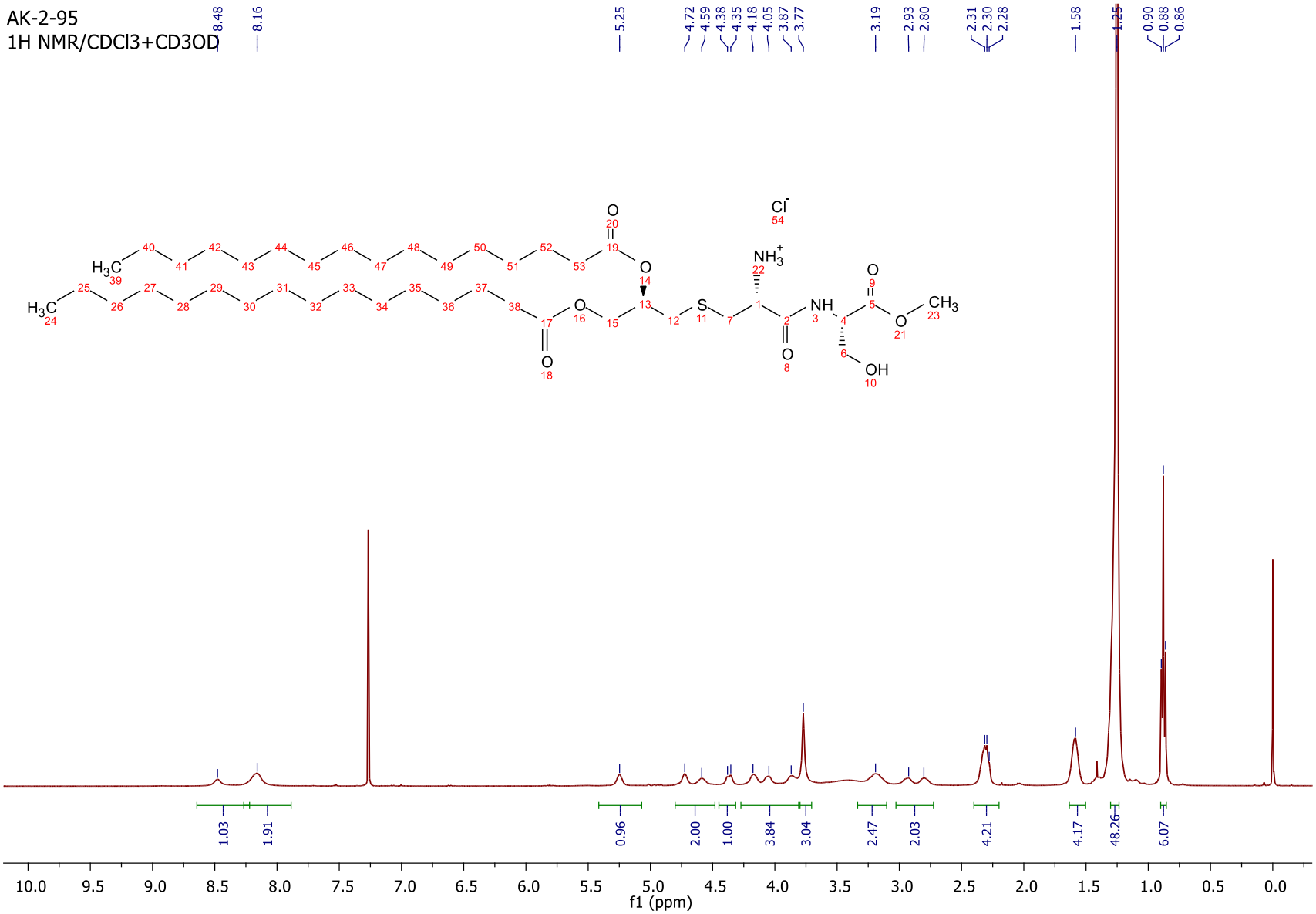


Exact Mass: 772.5635
$[M + H]^+$: 773.5714
$[M + Na]^+$: 795.5533

Mass	Intensity	Calc. Mass	Mass Difference [ppm]	Possible Formula
773.57052	33839.88	773.57136	-1.09	$^{12}C_{42}{}^{1}H_{81}{}^{14}N_2{}^{16}O_8{}^{32}S_1$

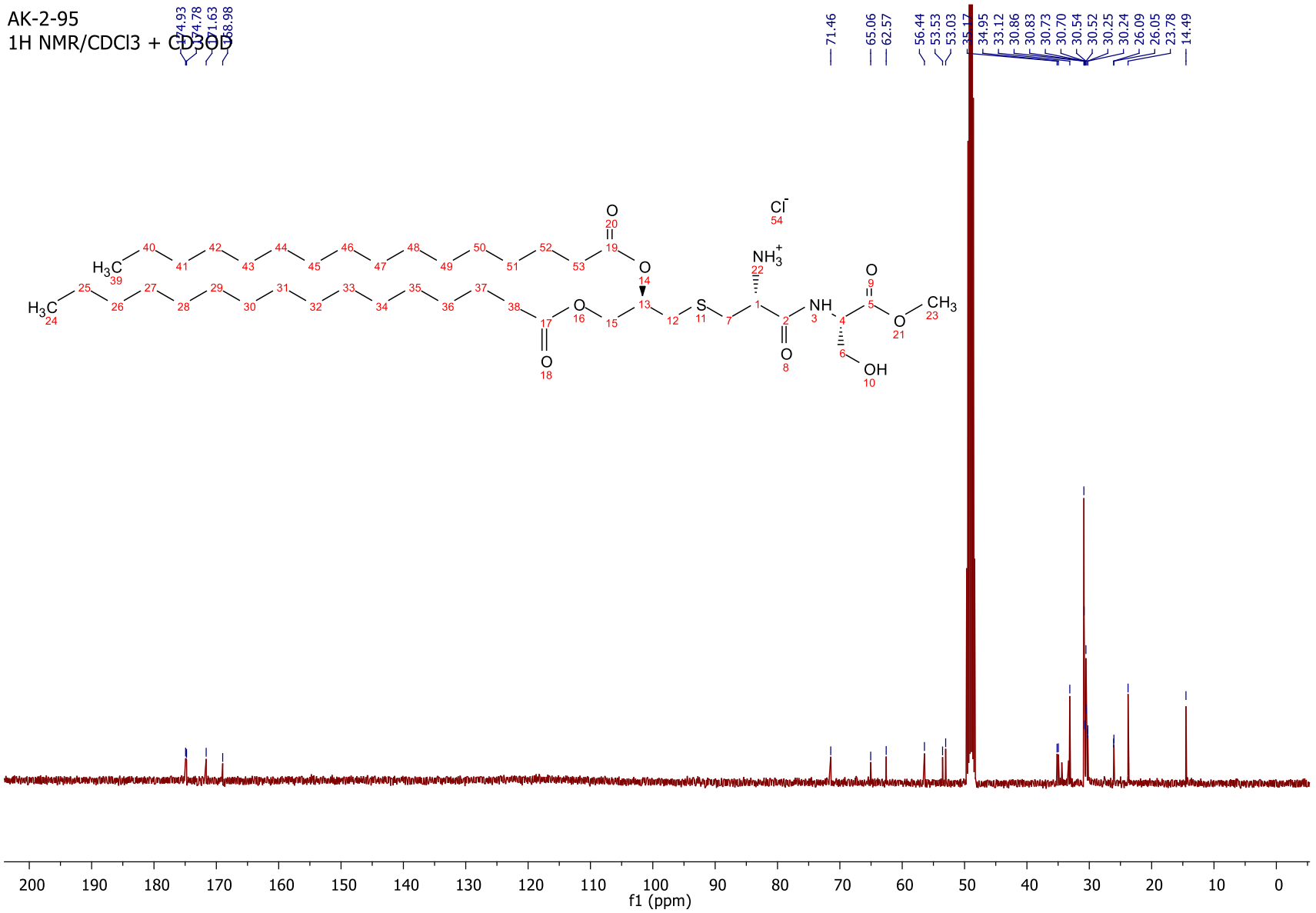
^{1}H NMR of Compound **8b** (in $CDCl_3$ + CD_3OD, 400 MHz)

ESI

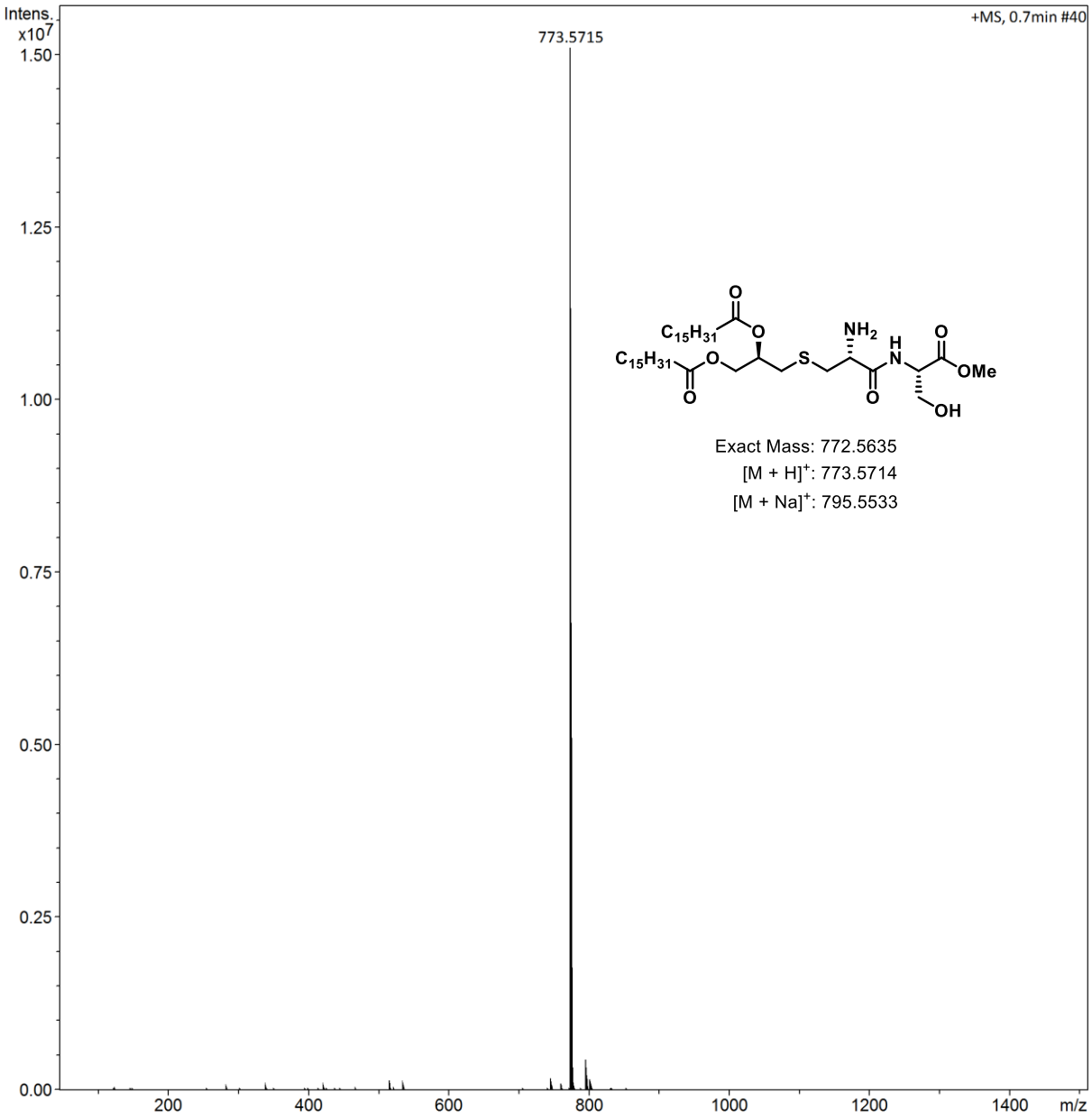


^{13}C NMR of Compound **8b** (in $CDCl_3$ + CD_3OD, 100 MHz)

ESI

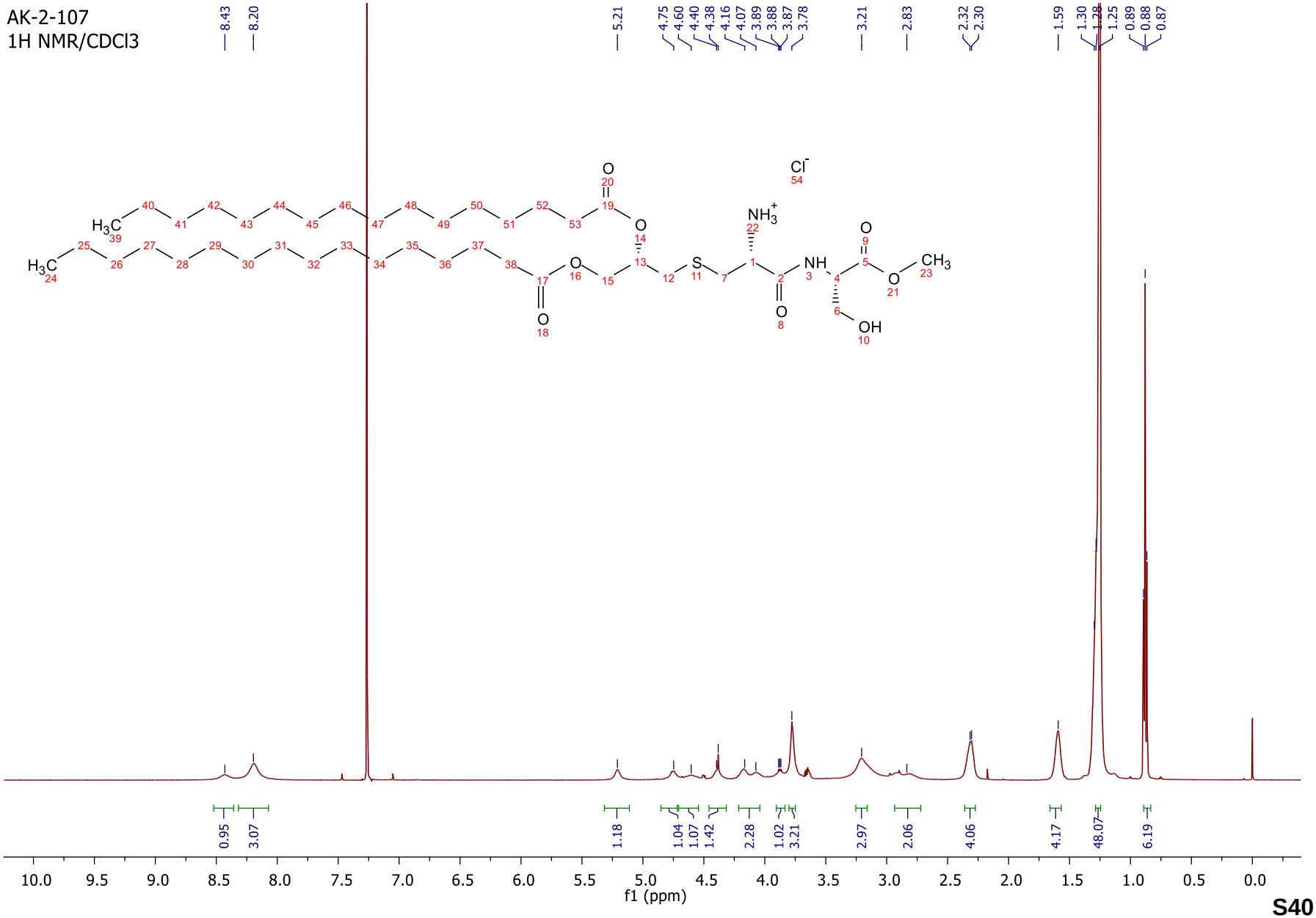


HRMS of Compound **8b** ESI

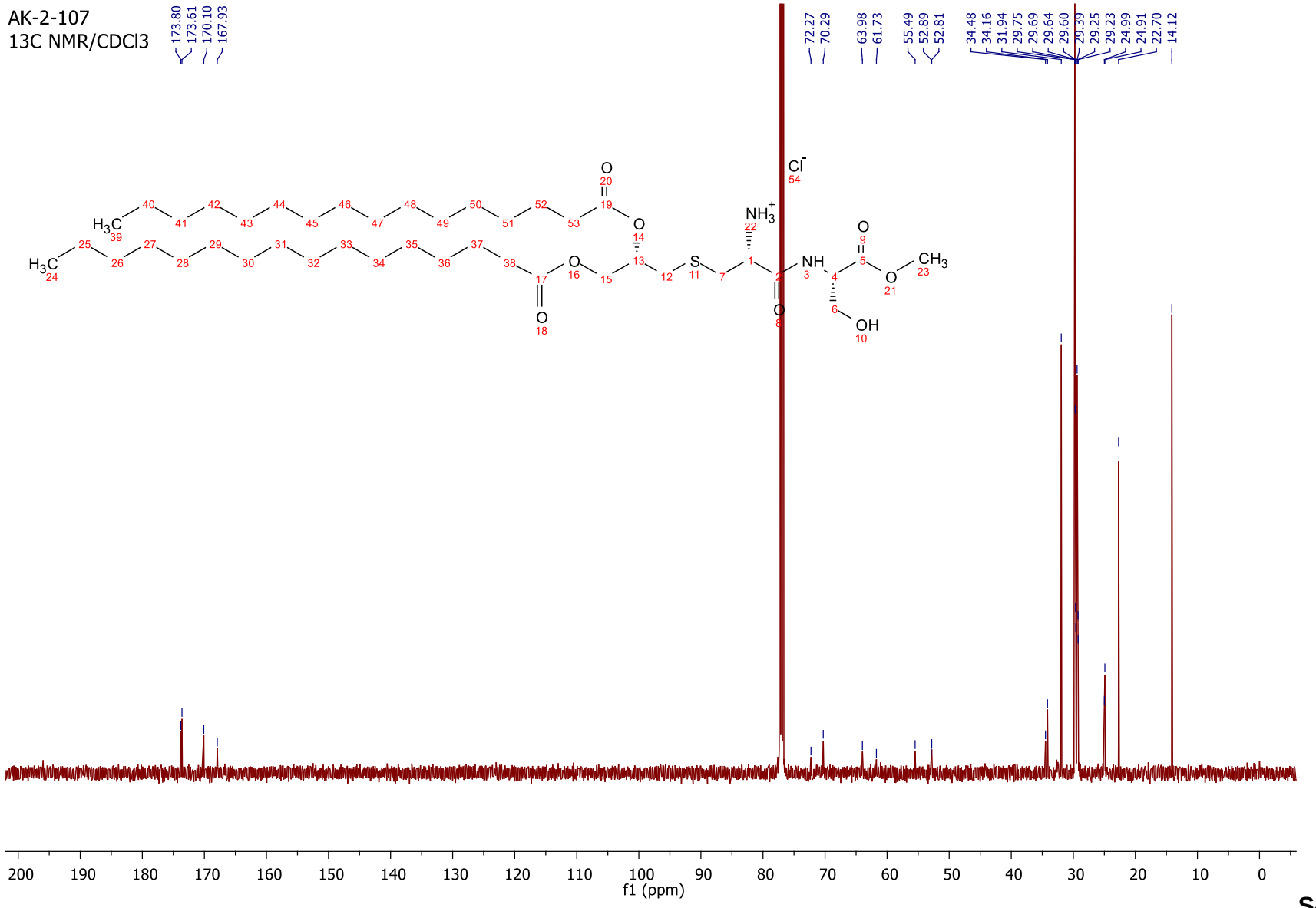


^{1}H NMR of Compound **8c** (in $CDCl_3$, 500 MHz)

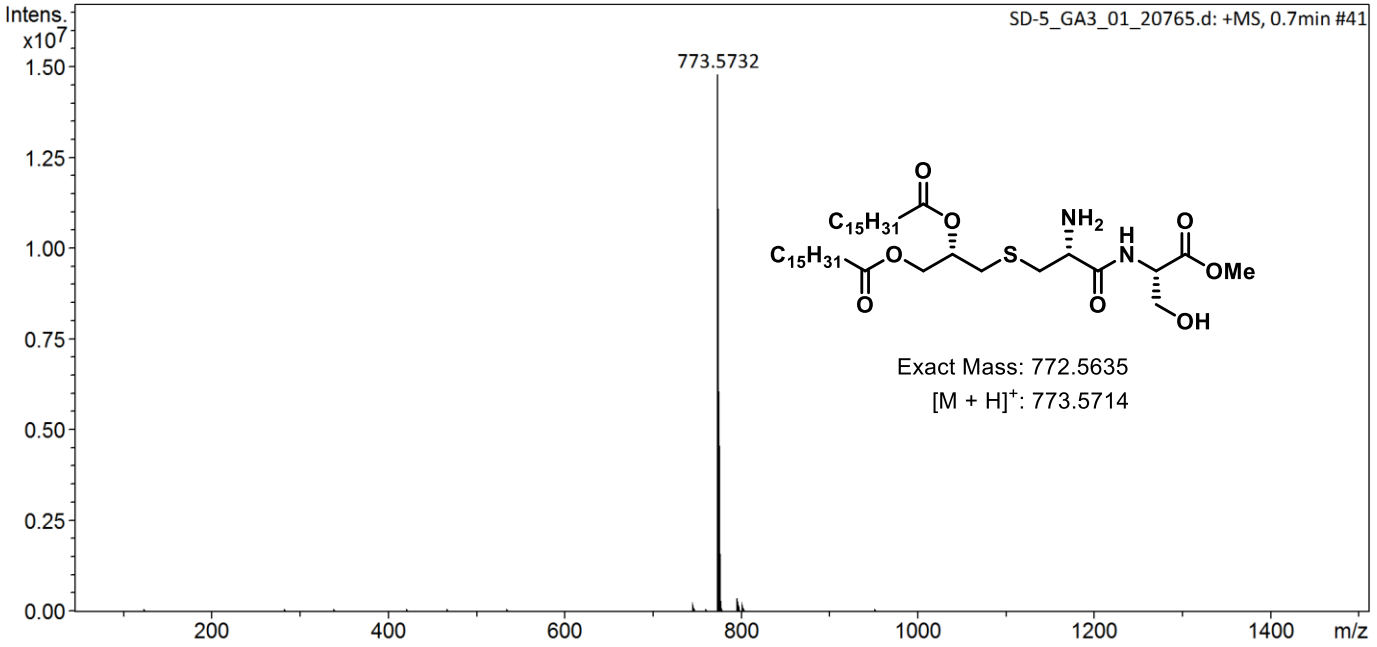
ESI

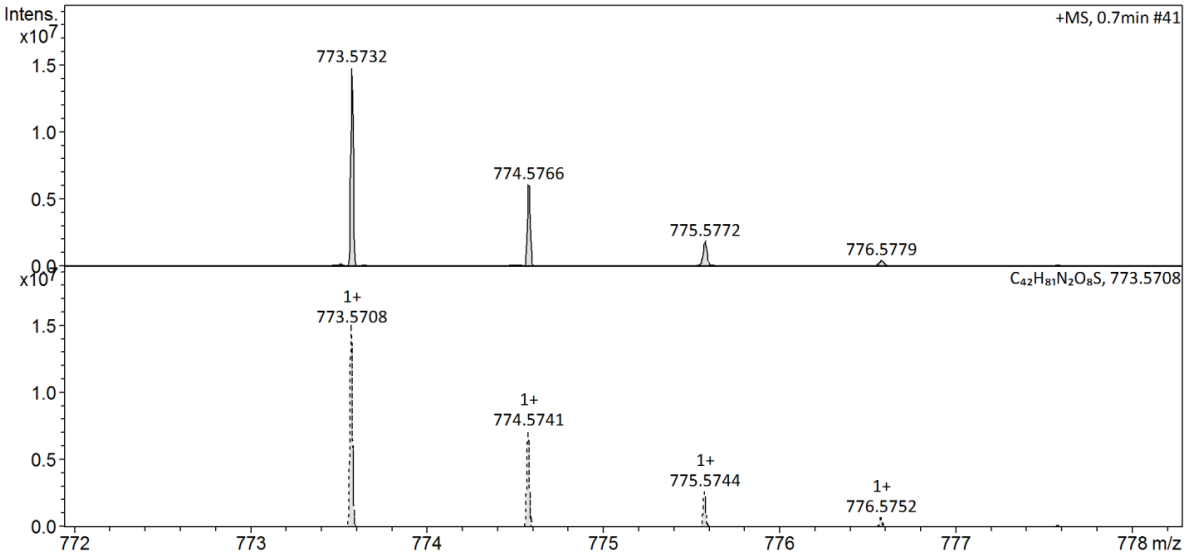


^{13}C NMR of Compound **8c** (in $CDCl_3$, 125 MHz)

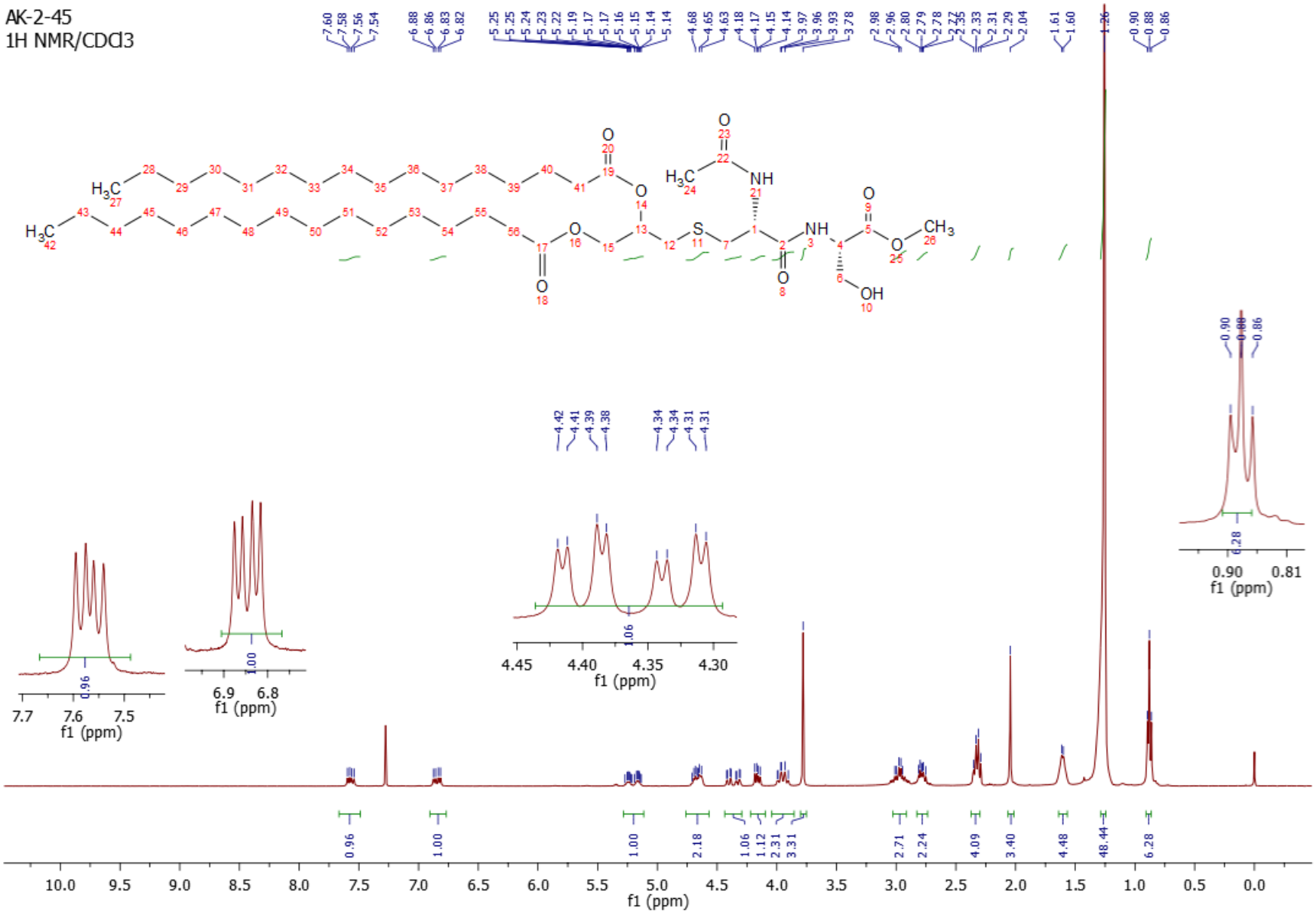


HRMS of Compound **8c** ESI

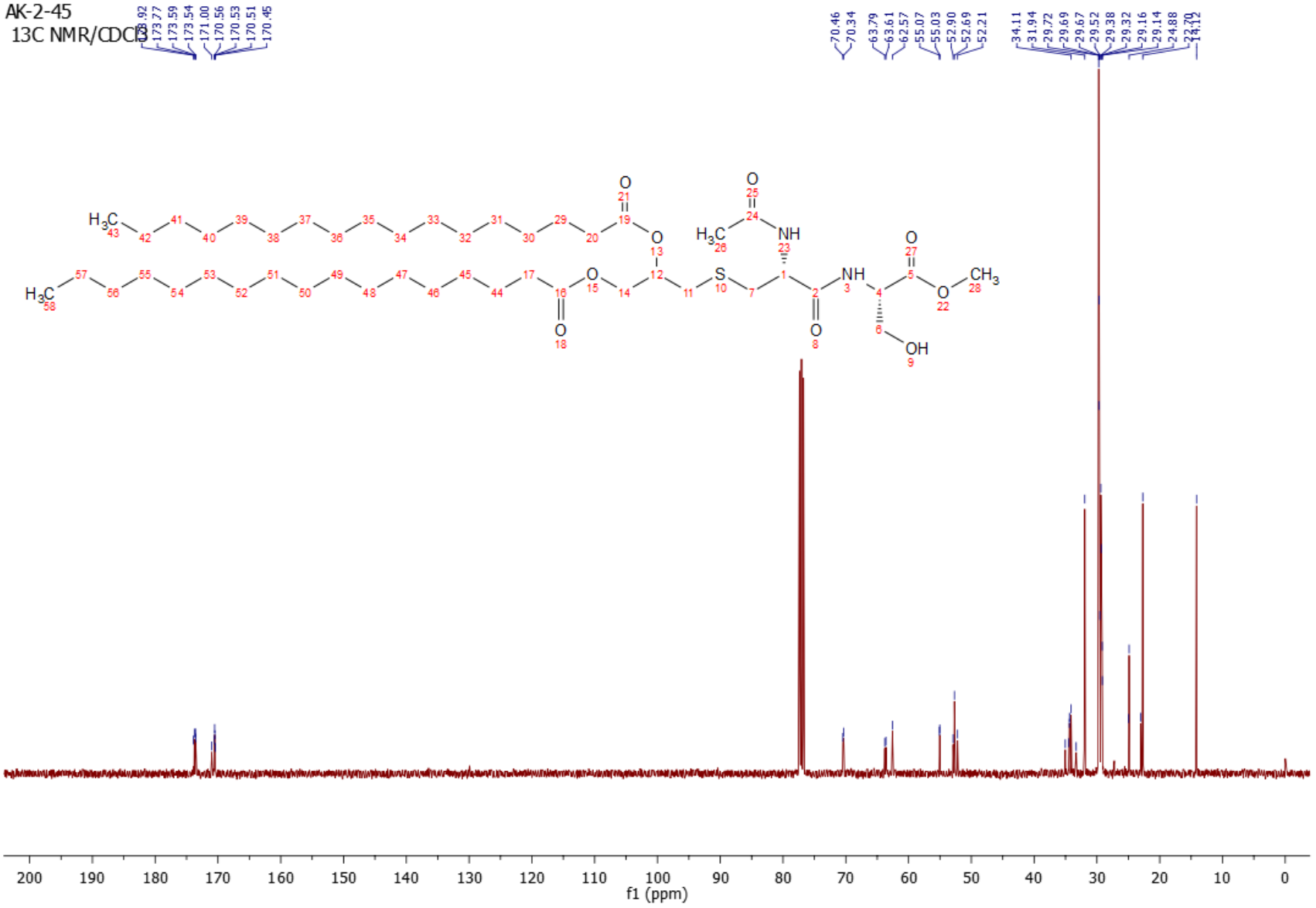


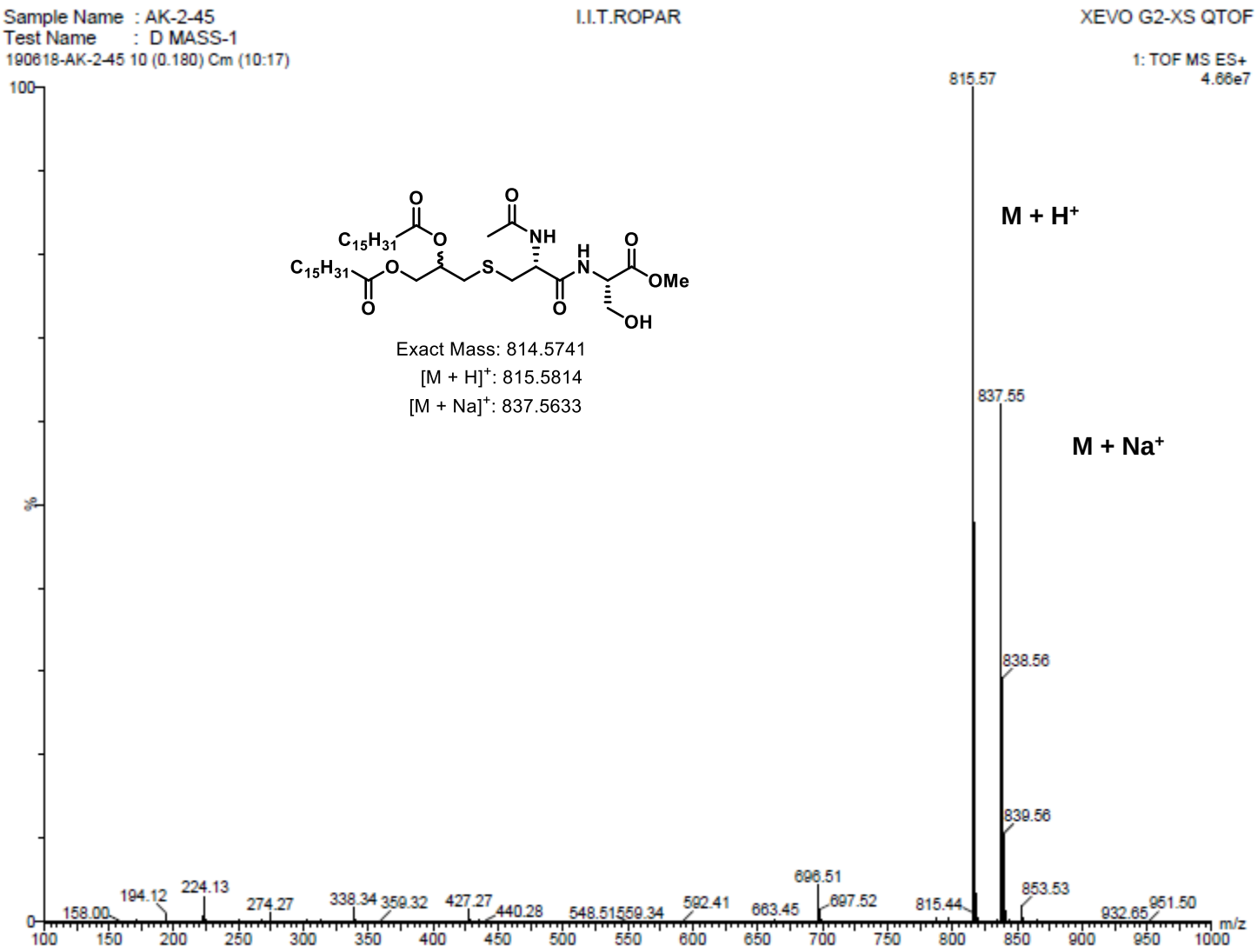


^{1}H NMR of Compound **9a** (in $CDCl_3$, 400 MHz) ESI



^{13}C NMR of Compound **9a** (in $CDCl_3$, 100 MHz) ESI

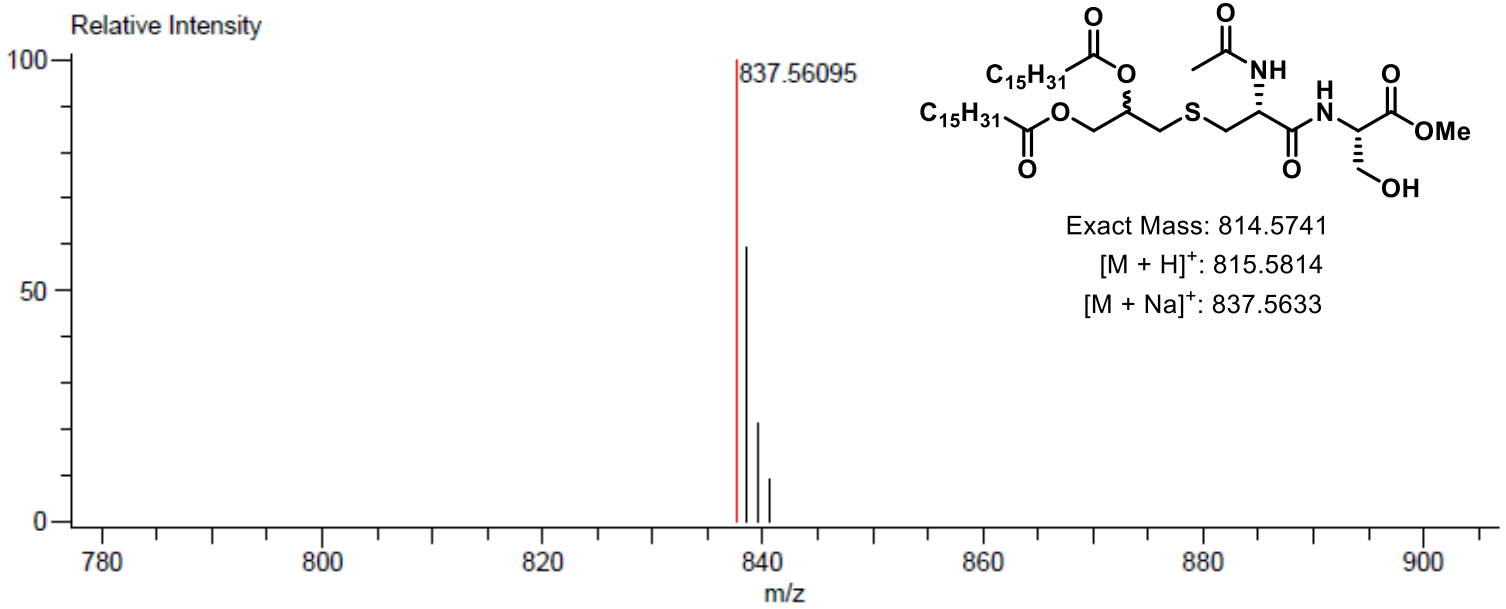



Sample Name : AK-2-45
Test Name : D MASS-1
190618-AK-2-45 10 (0.180) Cm (10:17)
I.I.T.ROPAR
XEVO G2-XS QTOF
1: TOF MS ES+
4.66e7
Exact Mass: 814.5741
[M + H]+: 815.5814
[M + Na]+: 837.5633
M + H+
M + Na+
815.57
837.55
838.56
839.56
696.51
697.52
853.53
951.50
932.65
815.44
663.45
592.41
559.34
548.51
440.28
427.27
359.32
338.34
274.27
224.13
194.12
158.00
%
m/z

Data:AK-2-45
Comment:
Description:
Ionization Mode:ESI+
History:Average(MS[1] 0.35..0.38)

Acquired:4/26/2019 4:13:52 PM
Operator:AccuTOF
m/z Calibration File:20190422-TFANa_...
Created:4/29/2019 2:14:28 PM
Created by:AccuTOF

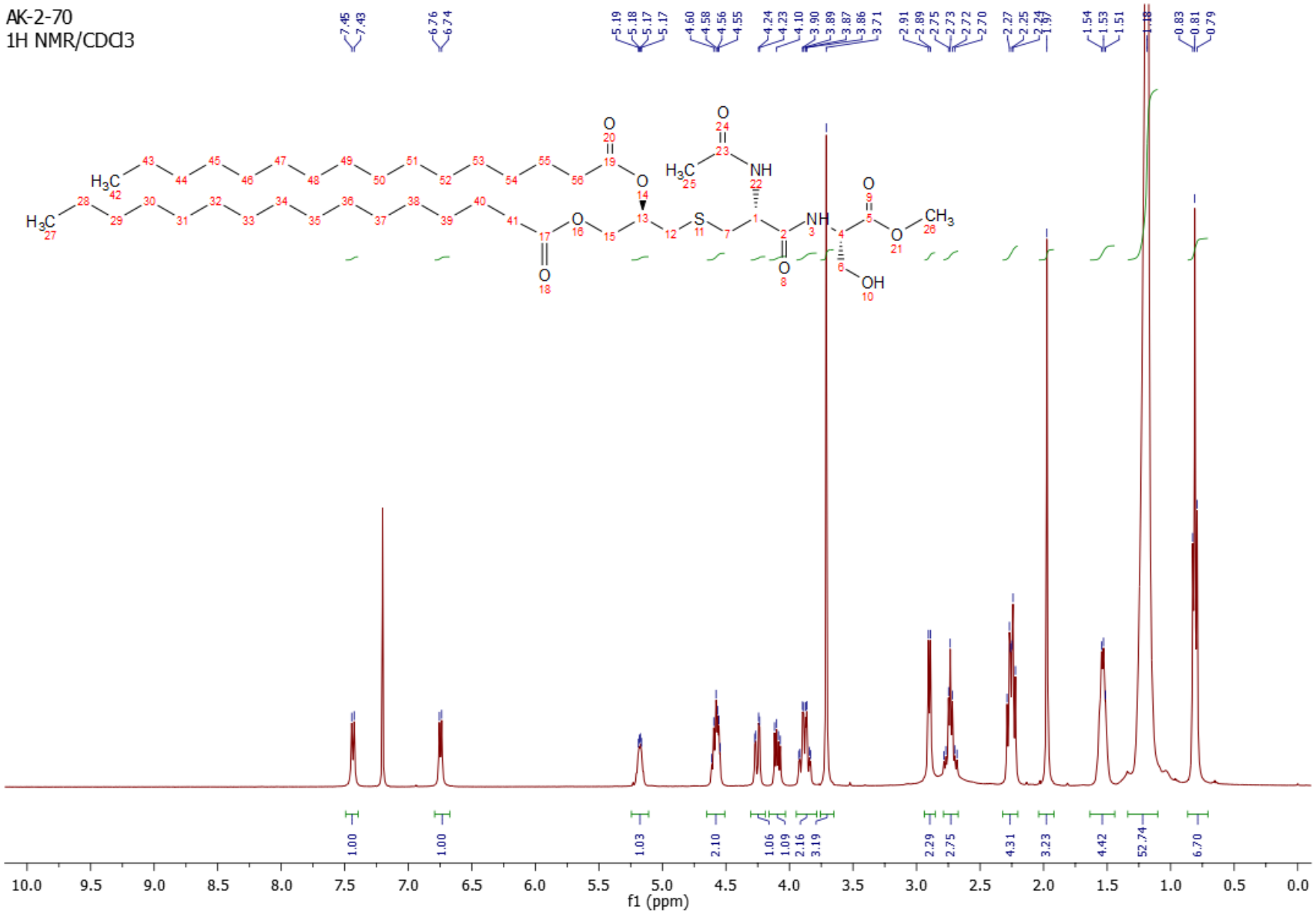
Charge number:1 Tolerance:200.00[ppm], 250.00 .. 250.... Unsaturation Number:-100.5 .. 200.0 (...
Element:^{12}C:44 .. 44, ^{1}H:0 .. 83, ^{14}N:2 .. 2, ^{23}Na:0 .. 1, ^{16}O:9 .. 9, ^{32}S:1 .. 1



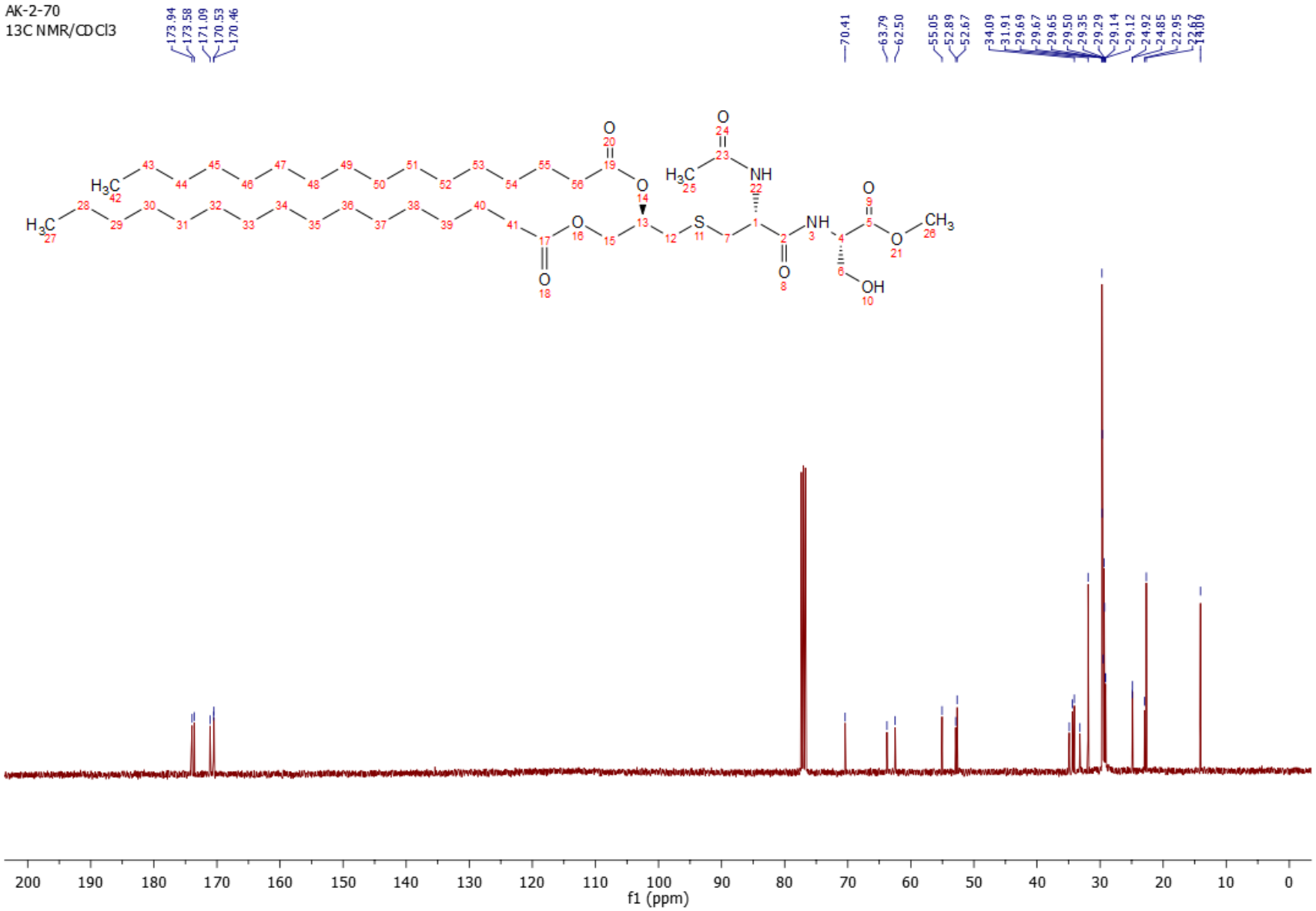
Exact Mass: 814.5741
$[M + H]^+$: 815.5814
$[M + Na]^+$: 837.5633

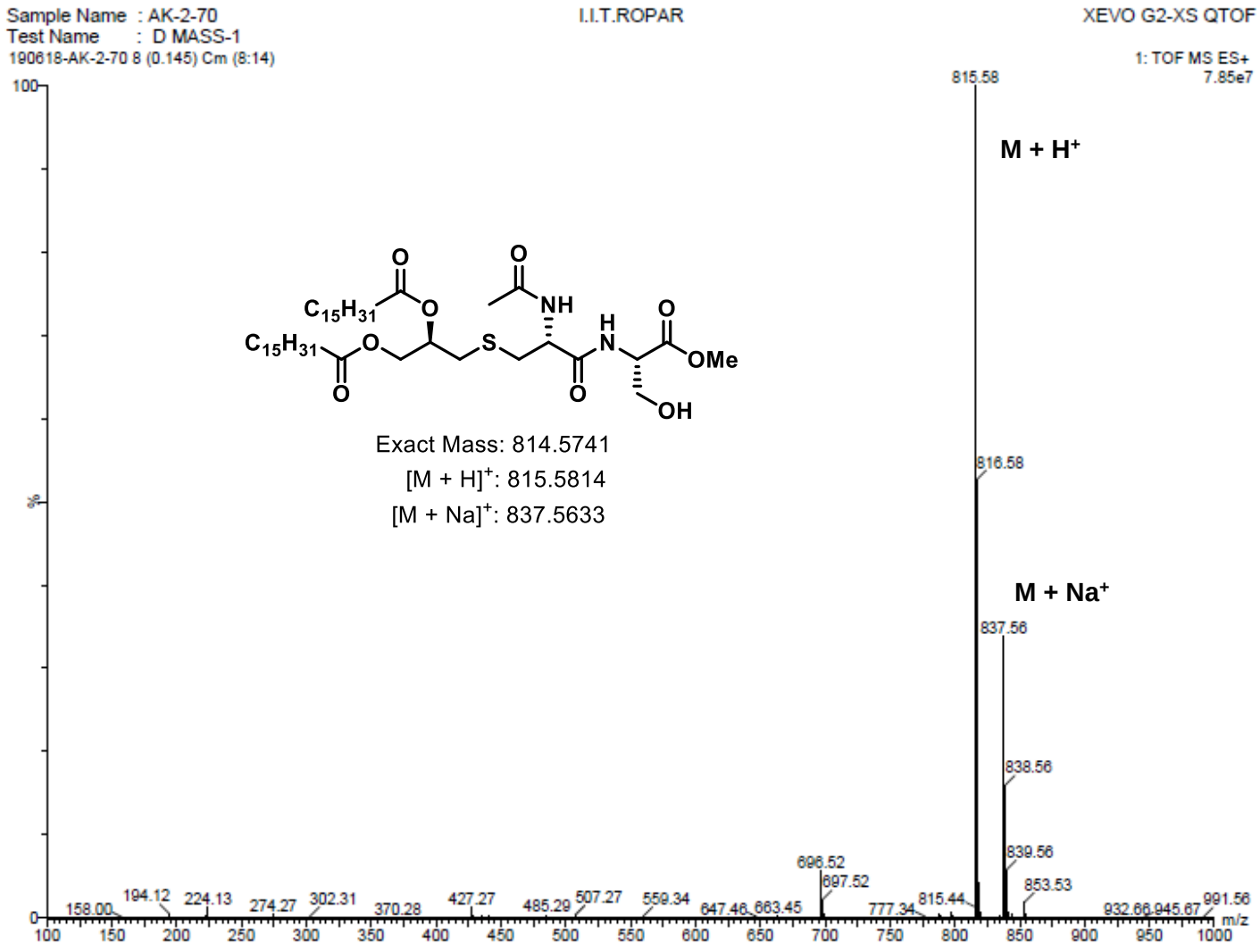
Mass	Intensity	Calc. Mass	Mass Difference [ppm]	Possible Formula
837.56095	17693.06	837.56387	-3.48	$^{12}C_{44}{}^{1}H_{82}{}^{14}N_{2}{}^{23}Na_{1}{}^{16}O_{9}{}^{32}S_{1}$

^{1}H NMR of Compound **9b** (in $CDCl_3$, 400 MHz) ESI



^{13}C NMR of Compound **9b** (in $CDCl_3$, 100 MHz)



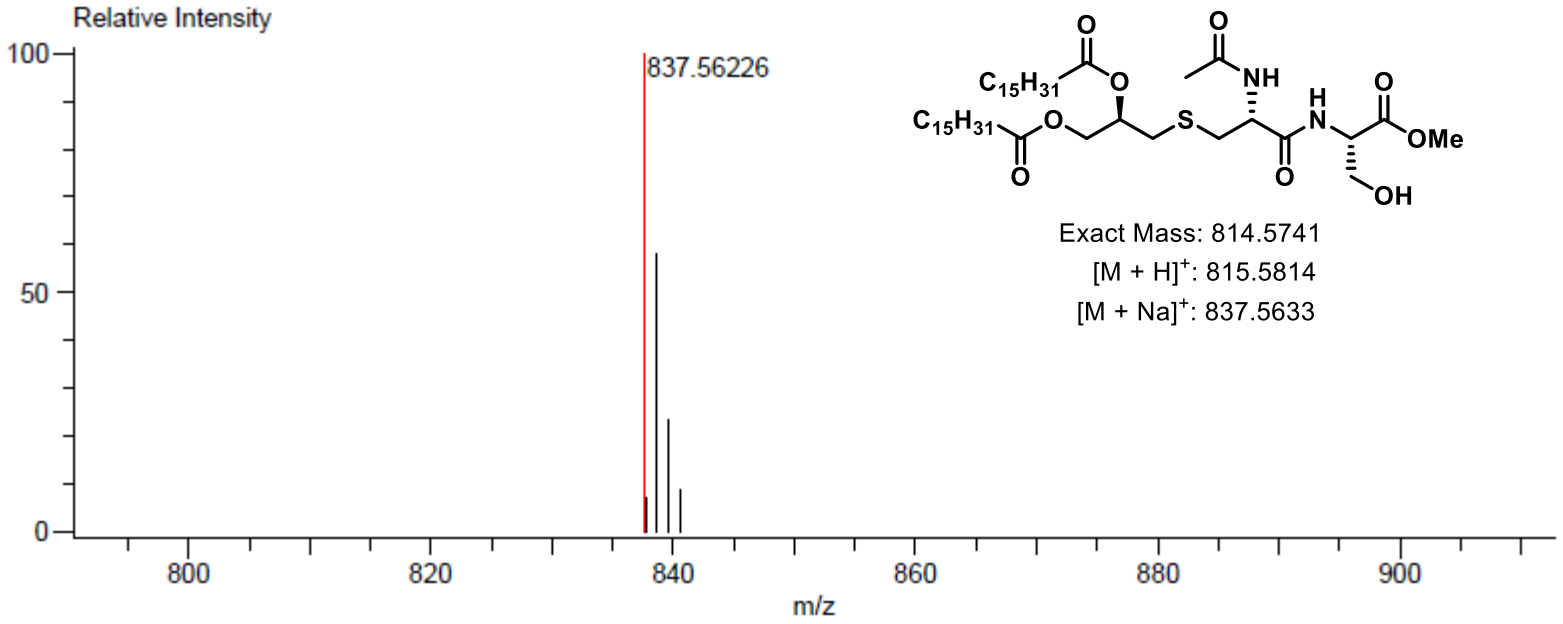

Sample Name : AK-2-70
Test Name : D MASS-1
190618-AK-2-70 8 (0.145) Cm (8:14)
I.I.T.ROPAR
XEVO G2-XS QTOF
1: TOF MS ES+
7.85e7
Exact Mass: 814.5741
[M + H]+: 815.5814
[M + Na]+: 837.5633
M + H+
M + Na+
815.58
816.58
837.56
838.56
839.56
696.52
697.52
853.53
991.56
158.00
194.12
224.13
274.27
302.31
370.28
427.27
485.29
507.27
559.34
647.46
663.45
777.34
815.44
932.66
945.67
m/z

HRMS of Compound **9b** ESI

Data:AK-2-73
Comment:
Description:
Ionization Mode:ESI+
History:Average(MS[1] 0.36..0.38)

Acquired:4/26/2019 3:37:12 PM
Operator:AccuTOF
m/z Calibration File:20190422-TFANa_...
Created:4/29/2019 2:12:09 PM
Created by:AccuTOF

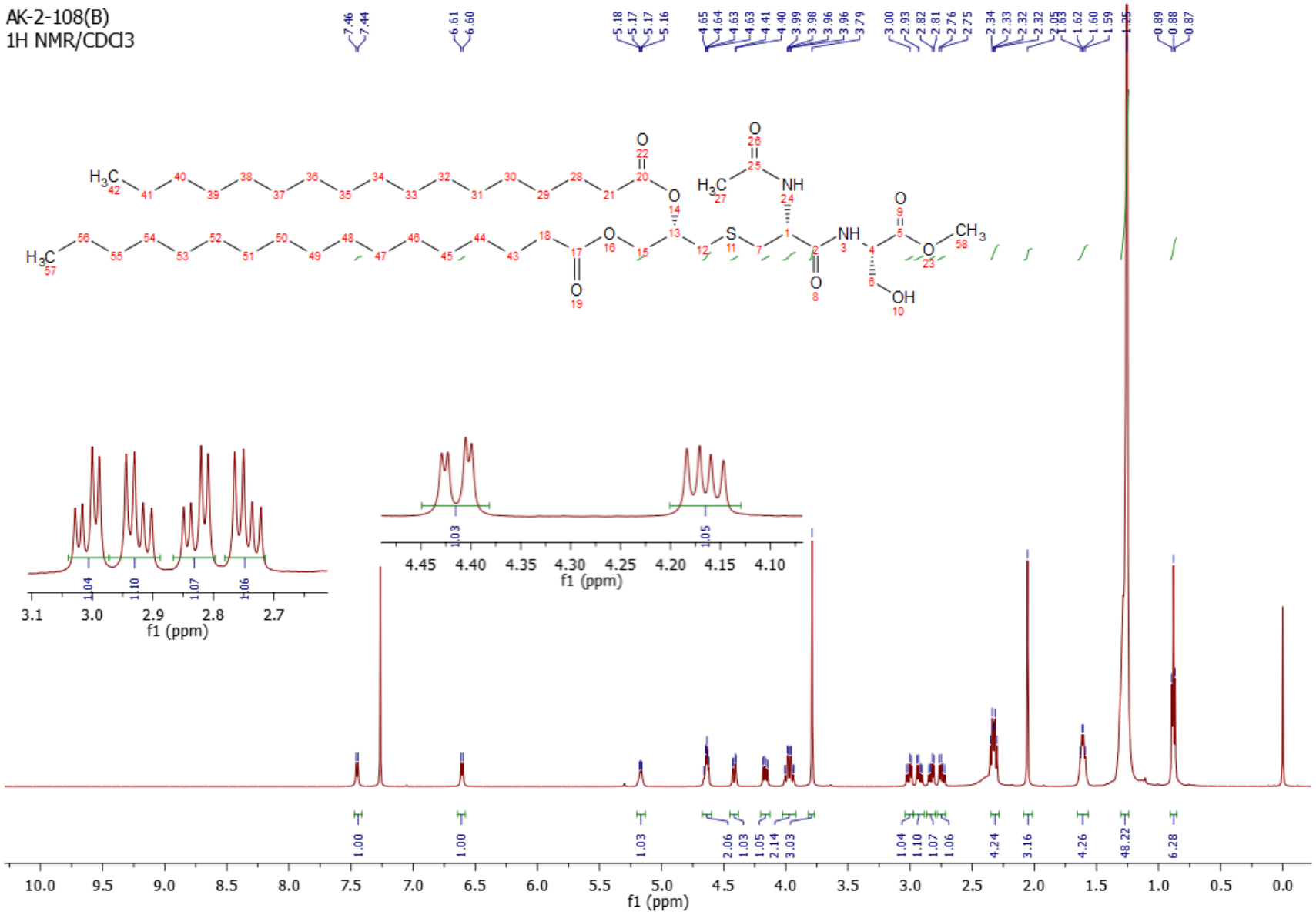
Charge number:1 Tolerance:200.00[ppm], 250.00 .. 250.... Unsaturation Number:-100.5 .. 200.0 (...
Element:^{12}C:44 .. 44, ^{1}H:0 .. 83, ^{14}N:2 .. 2, ^{23}Na:0 .. 1, ^{16}O:9 .. 9, ^{32}S:1 .. 1



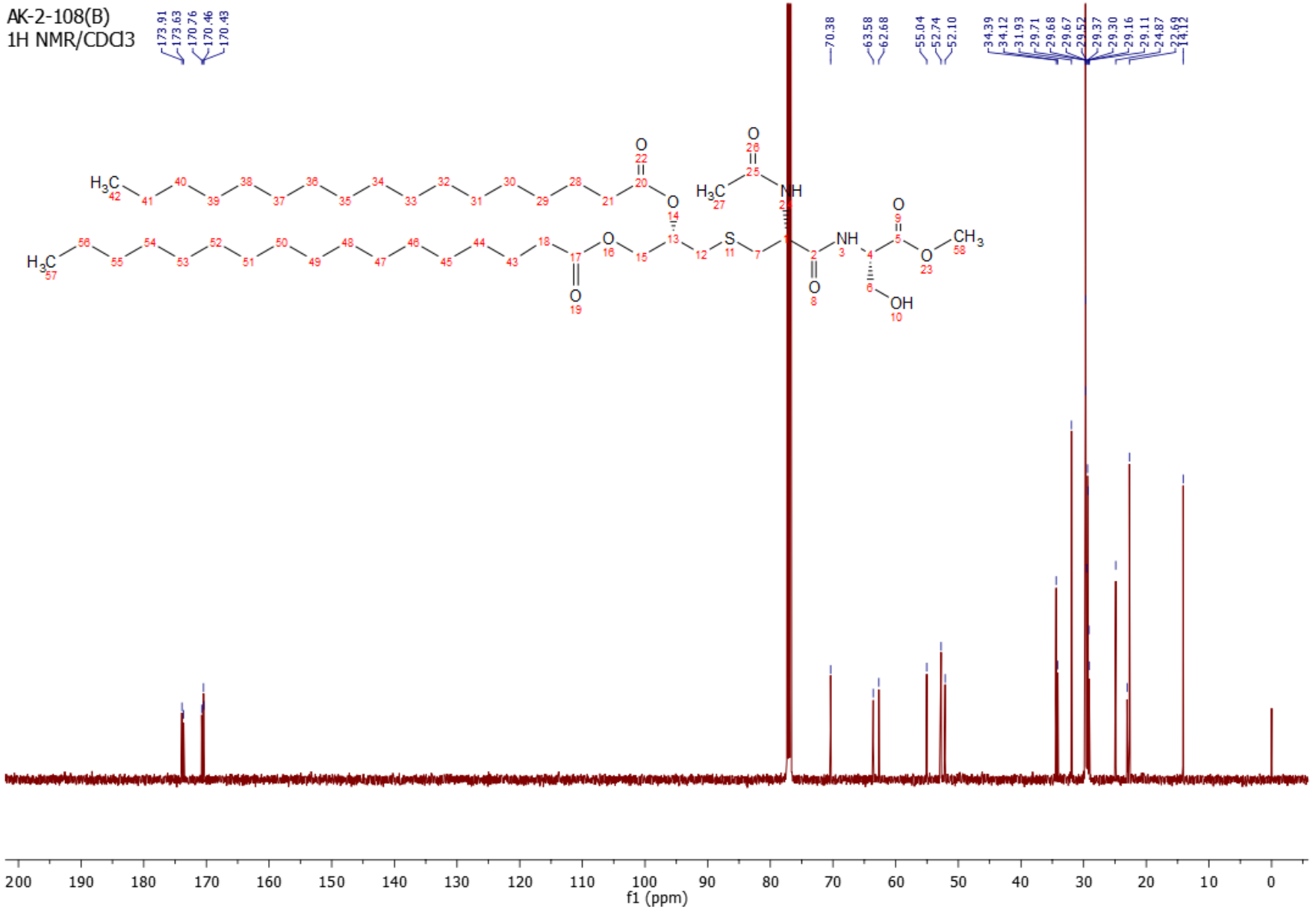
Exact Mass: 814.5741
$[M + H]^+$: 815.5814
$[M + Na]^+$: 837.5633

Mass	Intensity	Calc. Mass	Mass Difference [ppm]	Possible Formula
837.56226	21385.75	837.56387	-1.93	$^{12}C_{44}{}^{1}H_{82}{}^{14}N_{2}{}^{23}Na_{1}{}^{16}O_{9}{}^{32}S_{1}$

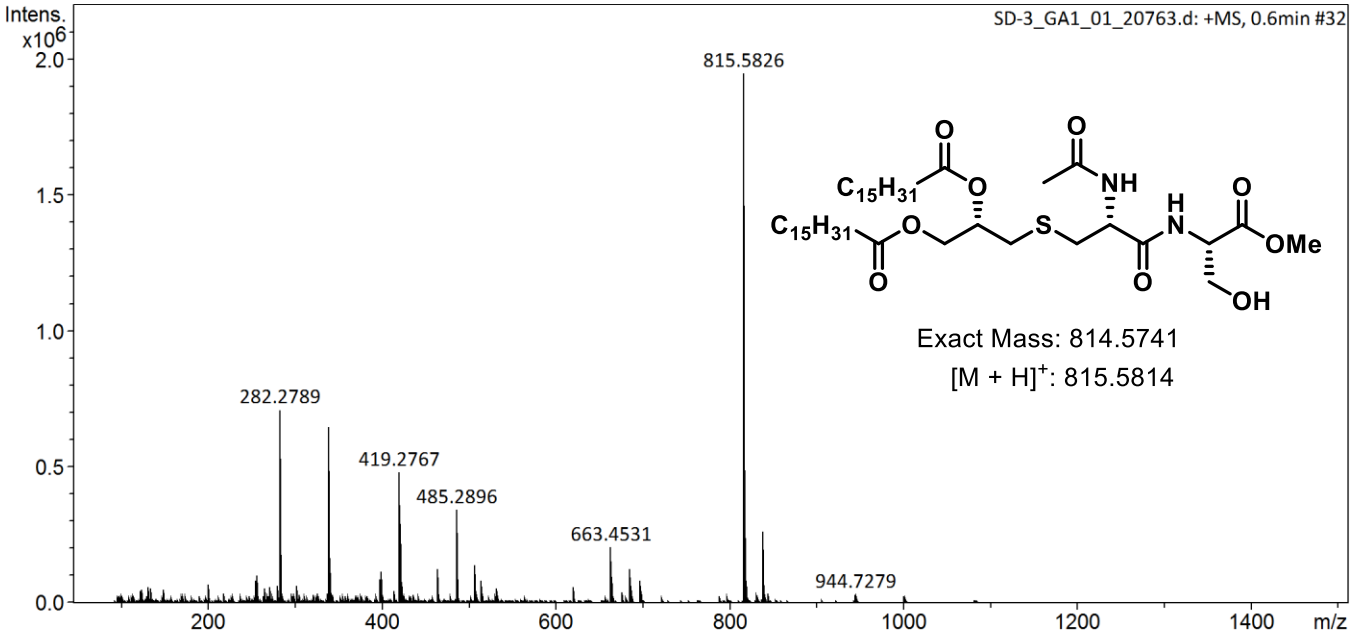
^{1}H NMR of Compound **9c** (in $CDCl_3$, 500 MHz) ESI



^{13}C NMR of Compound **9c** (in $CDCl_3$, 125MHz) ESI



HRMS of Compound **9c** ESI



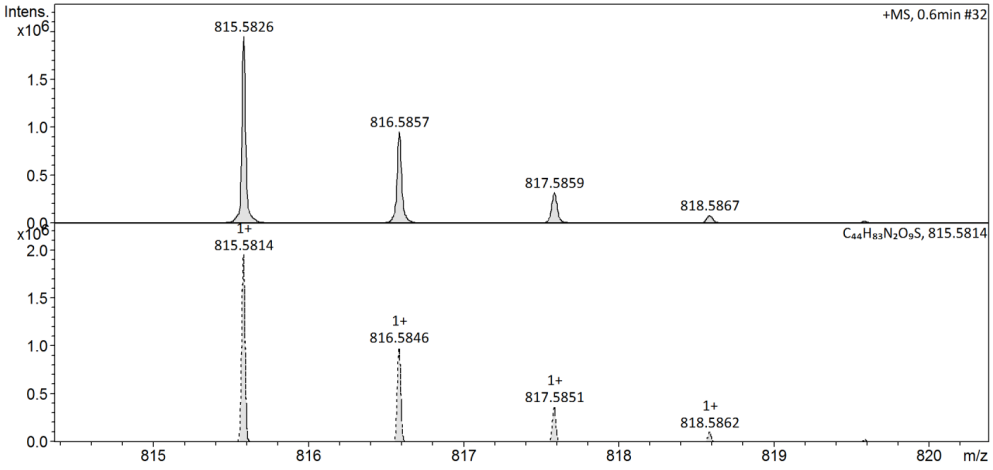


Table S1. List of interacting residues of TLR2/6 heterodimer with compounds and H-bond distances.

Compound	MMPBSA_Score (Free energy binding)	Interacting Residue	H-bond residue	H-bond distance
7b	-20.42	ChainA: Leu240, Phe258, Leu263, Phe269, Leu286, Ile288, Leu291, Ile293, Phe299, Tyr300, Asp301, Leu302, Leu305, Tyr306, Thr309, Ile315, Val317, Ser320, Lys321, Val322, Phe323, Leu324, Pro326, Leu329 ChainB: Phe317, Phe319, Ser320, Gln321	Gln321(O6-NE2)	3.16Å
7c	-25.78	ChainA: Leu263, Phe269, Leu286, Ile288, Leu291, Ile293, Phe296, Tyr297, Phe299, Tyr300, Asp301, Leu302, Leu305, Tyr306, Thr309, Ile315, Val317, Ser320, Lys321, Val322, Phe323, Leu324, Val325, Pro326, Leu329 ChainB: Phe317, Leu318, Phe319, Ser320, Gln321	Phe323(O8-N) Leu324(O8-N)	3.21Å 3.25Å
8b	-36.52	ChainA: Ile235, Leu240, Phe258, Leu263, Phe269, Leu286, Ile288, Leu291, Ile293, Phe296, Tyr297, Phe299, Leu305, Thr309, Ile315, Val317, Ser320, Lys321, Val322, Phe323, Leu324, Pro326, Leu329 ChainB: Phe317, Phe319, Ser320, Gln321	Phe296(N2-O) Tyr297(O5-O) Phe299(N2-N) Phe323(O4-N) Ser320(O5-OG) Gln321(O5-N) Gln(O5-NE2)	3.09Å 2.97Å 3.07Å 3.23Å 3.29Å 3.01Å 2.89Å
8c	-32.10	ChainA: Phe258, Leu286, Ile288, Ile293, Phe296, Tyr297, Leu298, Phe299, Asp301 Leu302, Leu305, Tyr306, Thr308, Ile315, Val317, Lys321, Val322, Phe323, Leu324, Val325, Pro326, Leu329, Leu341 ChainB: Val316, Gln321	Phe296(O8-O) Phe299(O8-O) Phe323(O4-N) Leu324(O4-N)	3.28Å 3.05Å 3.00Å 3.25Å

9b	-34.22	ChainA: Leu240, Phe258, Phe269, Leu286, Ile288, Leu291, Ile293, Tyr297, Phe299, Asp301, Leu302, Leu305, Tyr306, Leu308, Thr309, Ile315, Val317, Ser320, Val322, Phe323, Leu324, Val325, Pro326, Leu329, Leu341 ChainB: Phe317, Gln321	Phe317(O5-O) Gln321(O4-NE2)	3.09Å 2.96Å
9c	-37.91	ChainA: Ile235, Leu240, Leu263, Phe269, Leu286, Ile288, Leu291, Tyr297, Leu302, Leu305, Thr309, Ile315, Val317, Lys321, Val322, Phe323, Leu324, Val325, Pro326, Leu329 ChainB: Val316, Phe317, Ser320, Thr322, Gln321	Tyr297(O8-O) Phe323(O7-N) Leu324(O7-N) Ser320(O8-OG) Gln321(O8-N) Thr322(O8-N) Thr322(O8-OG1)	3.02Å 3.10Å 3.03Å 3.12Å 2.99Å 3.34Å 3.14Å

Figure S1. Ramachandran Plot of human TLR2/6.

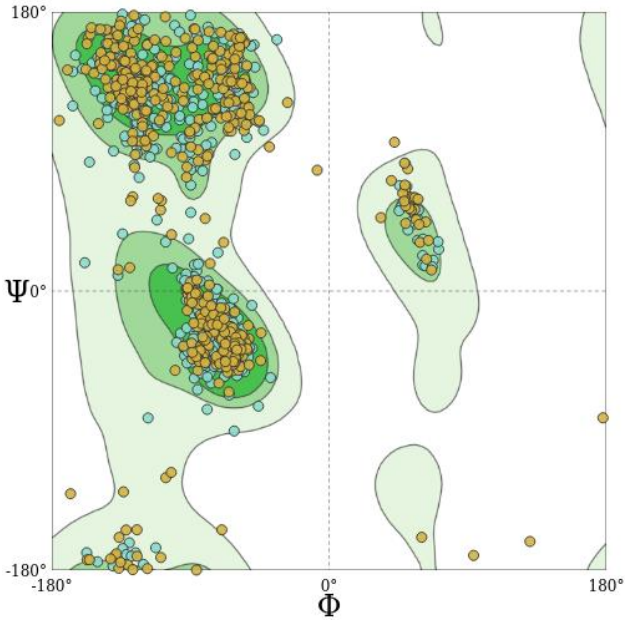


Figure S2. Surface representation of hTLR2/6 with compound 7b

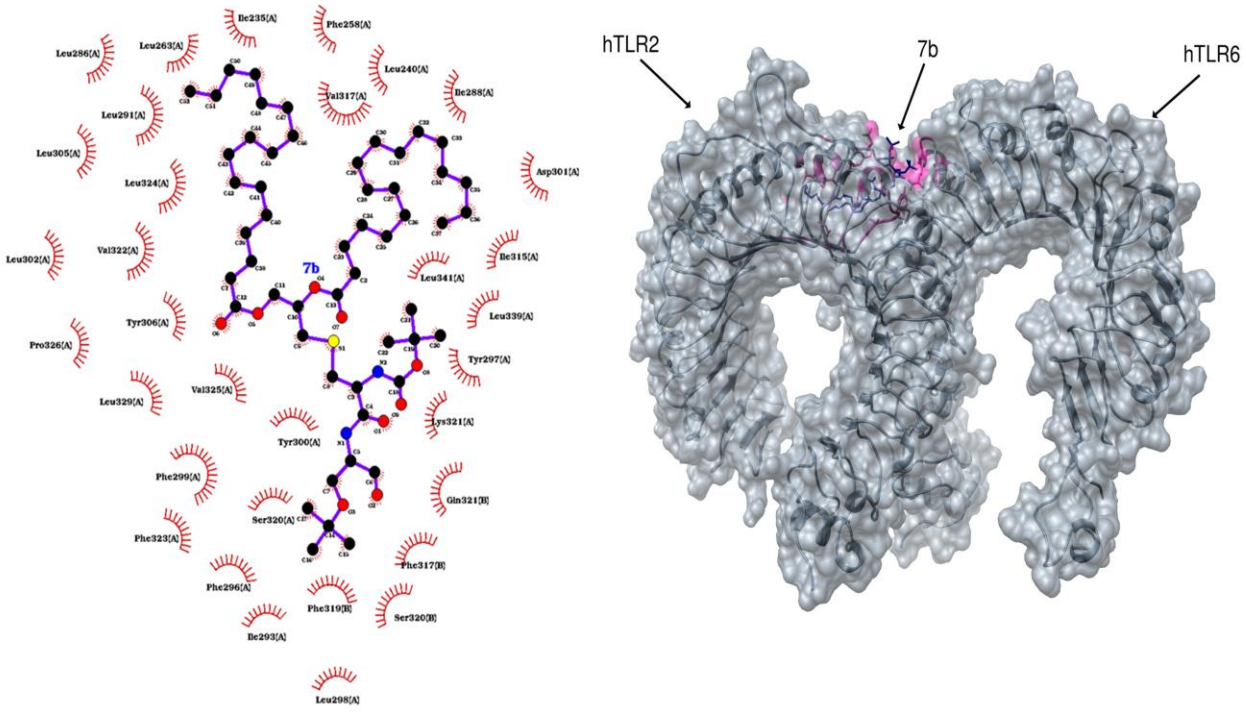


Figure S2a. Interacting amino acid residues of hTLR2/6 with compounds 7b

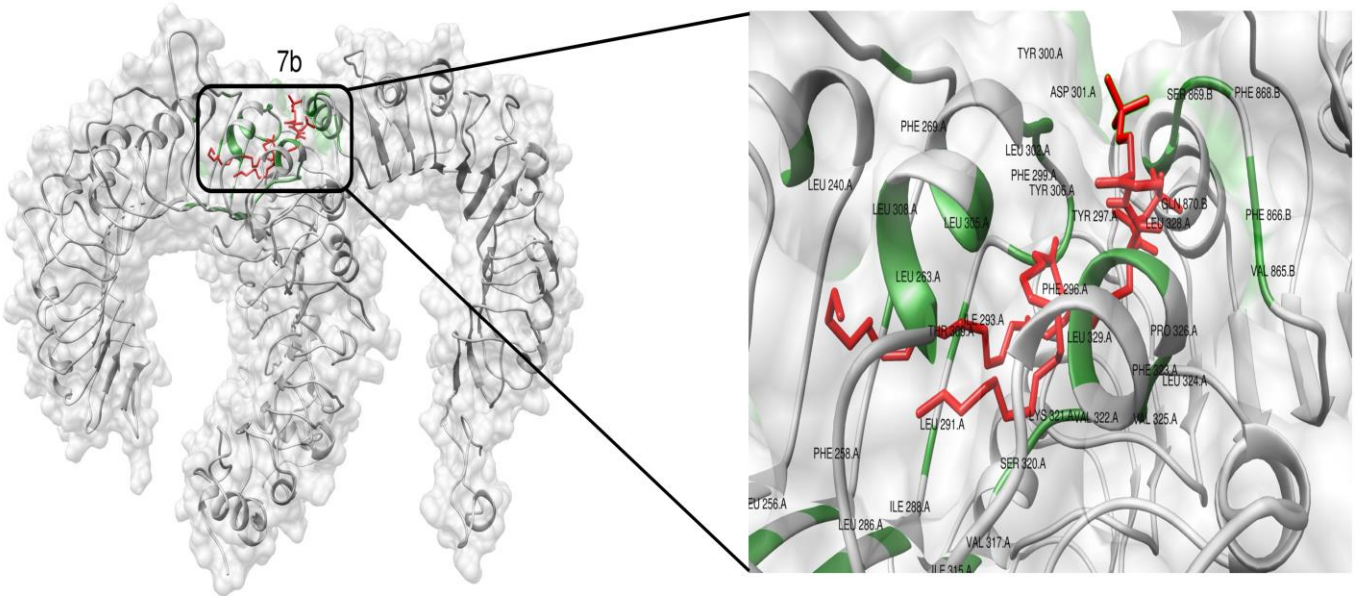


Figure S3. Surface representation of hTLR2/6 with compound 7c

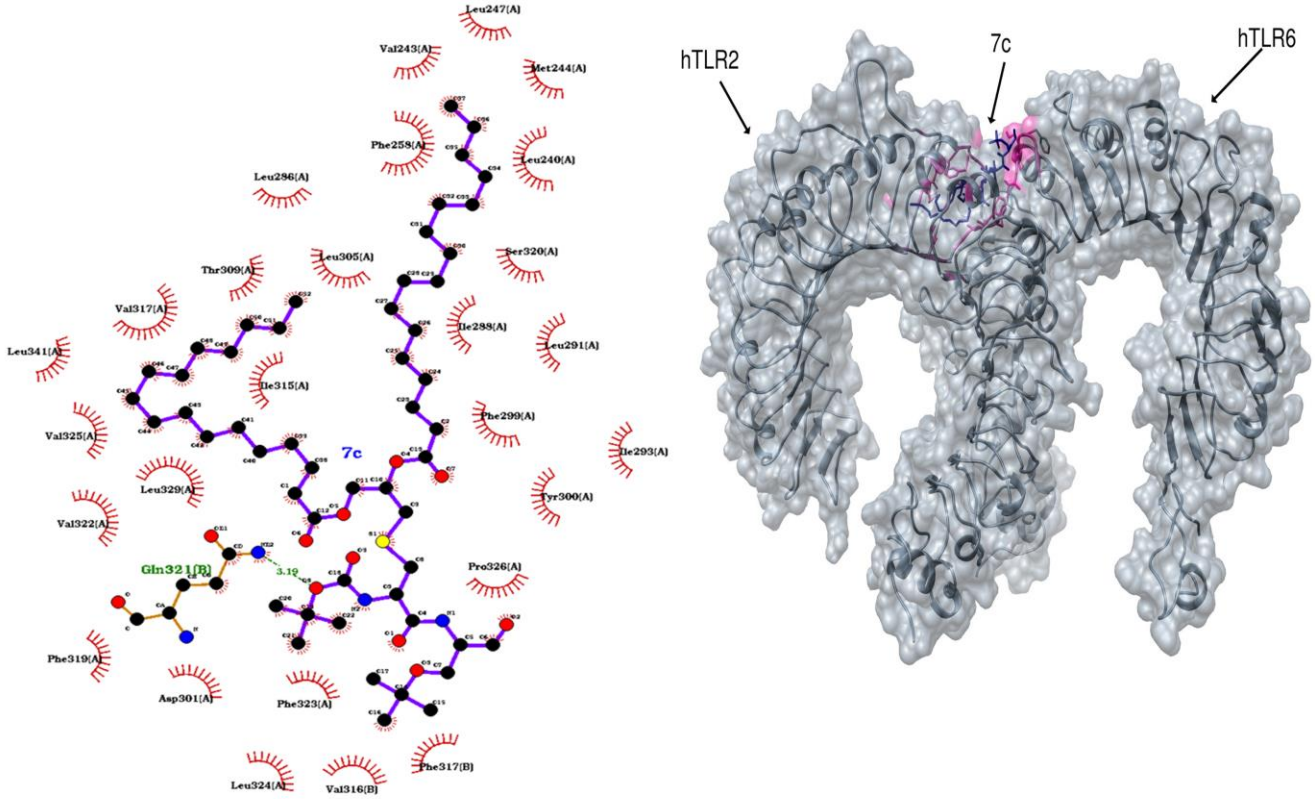


Figure S3a. Interacting amino acid residues of hTLR2/6 with compounds 7c

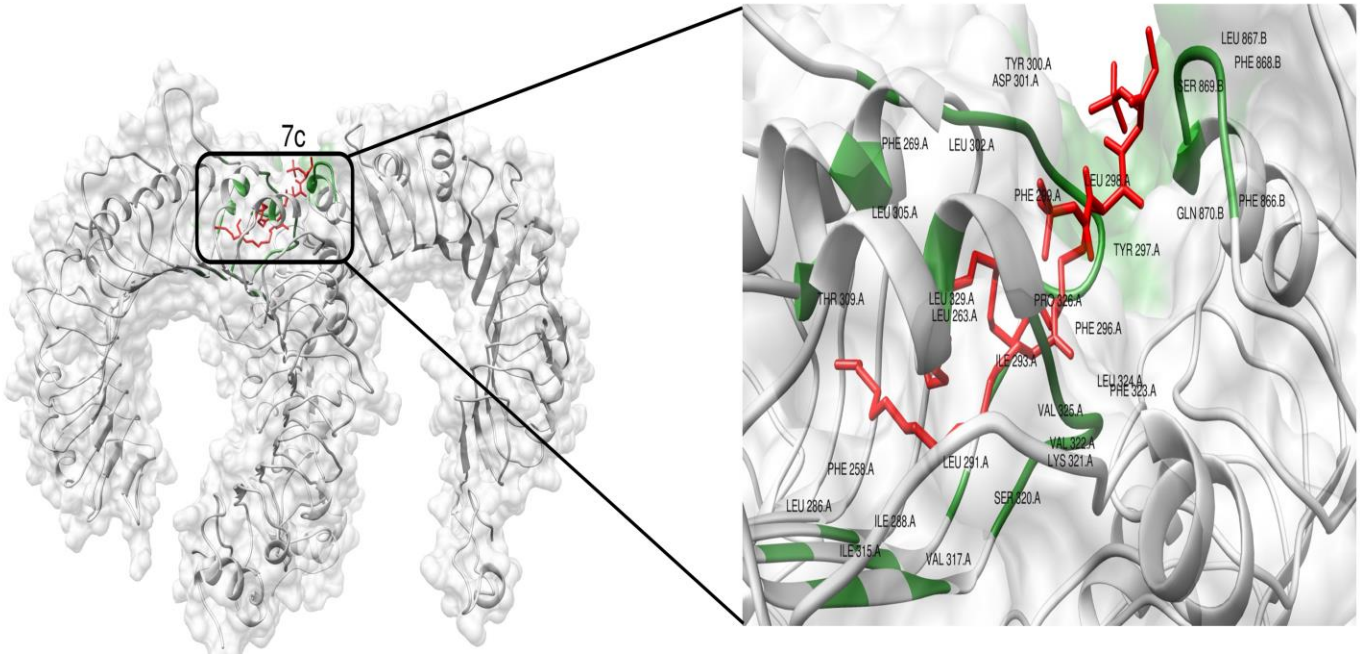


Figure S4. Surface representation of hTLR2/6 with compound 8b

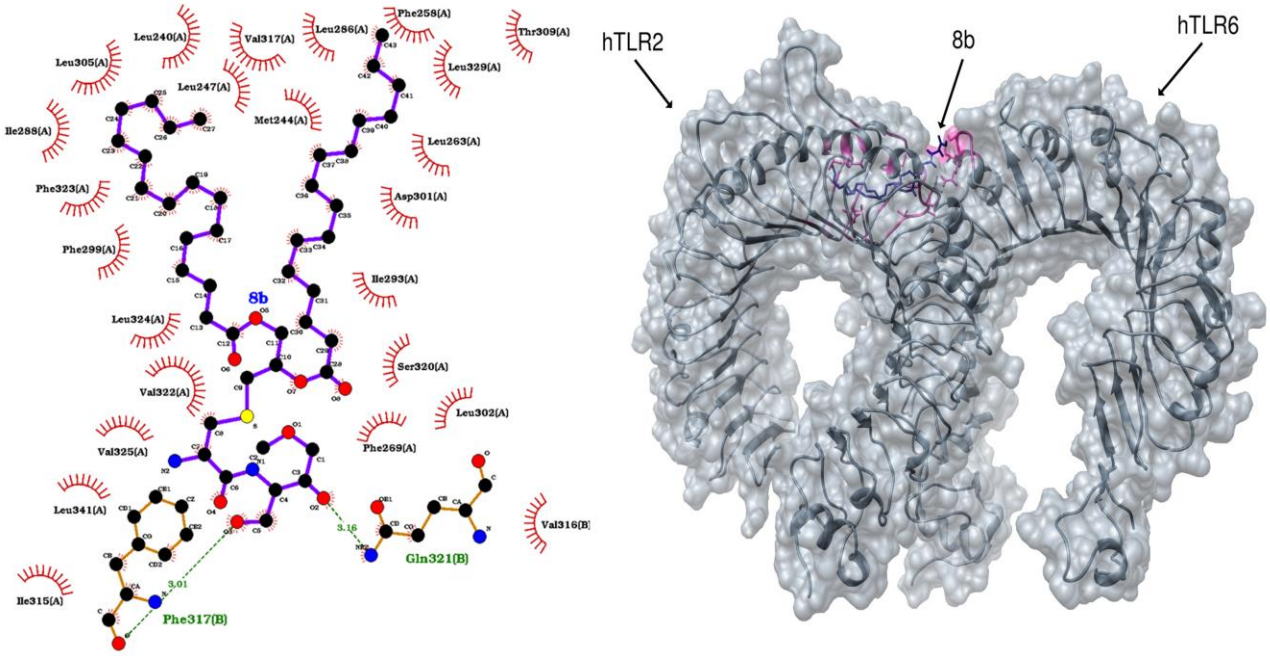


Figure S4a. Interacting amino acid residues of hTLR2/6 with compounds 8b

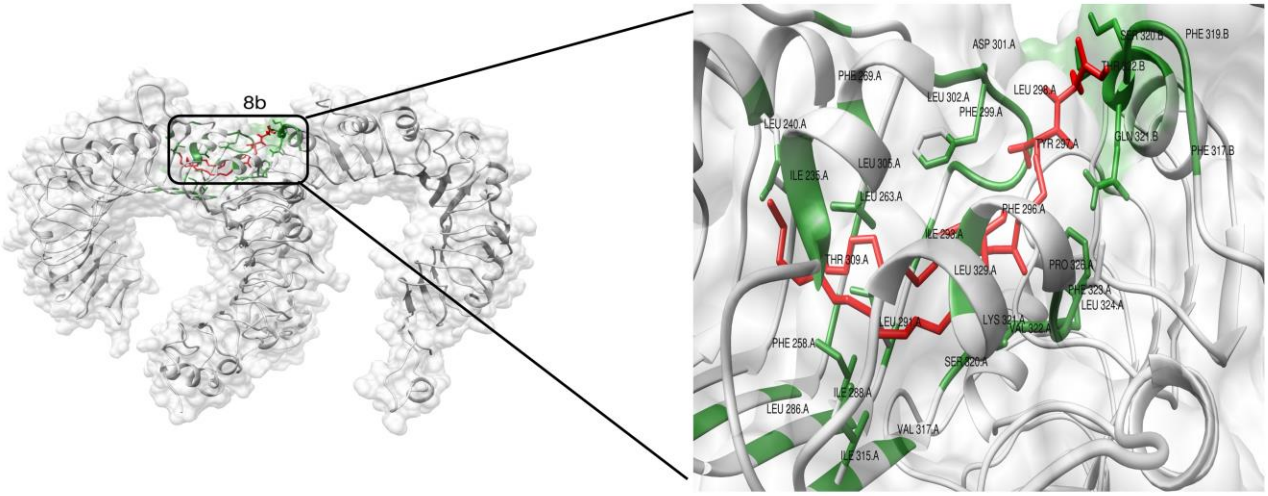


Figure S5. Surface representation of hTLR2/6 with compound 8c

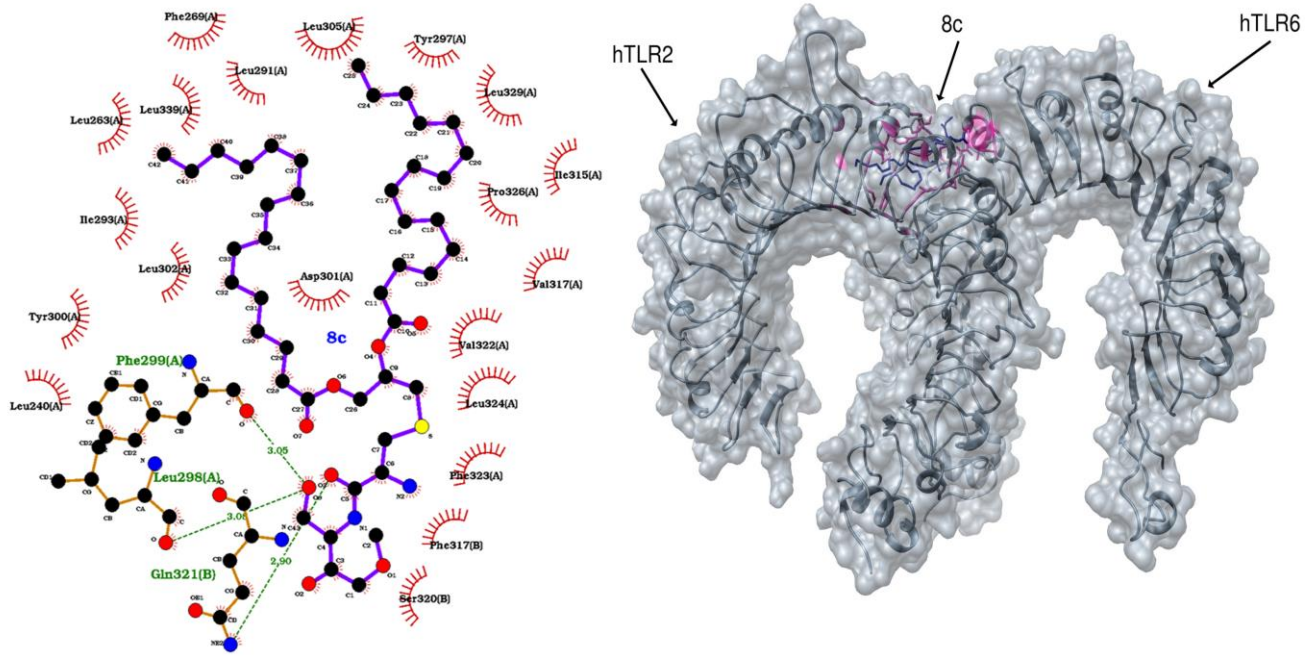


Figure S5a. Interacting amino acid residues of hTLR2/6 with compounds 8c

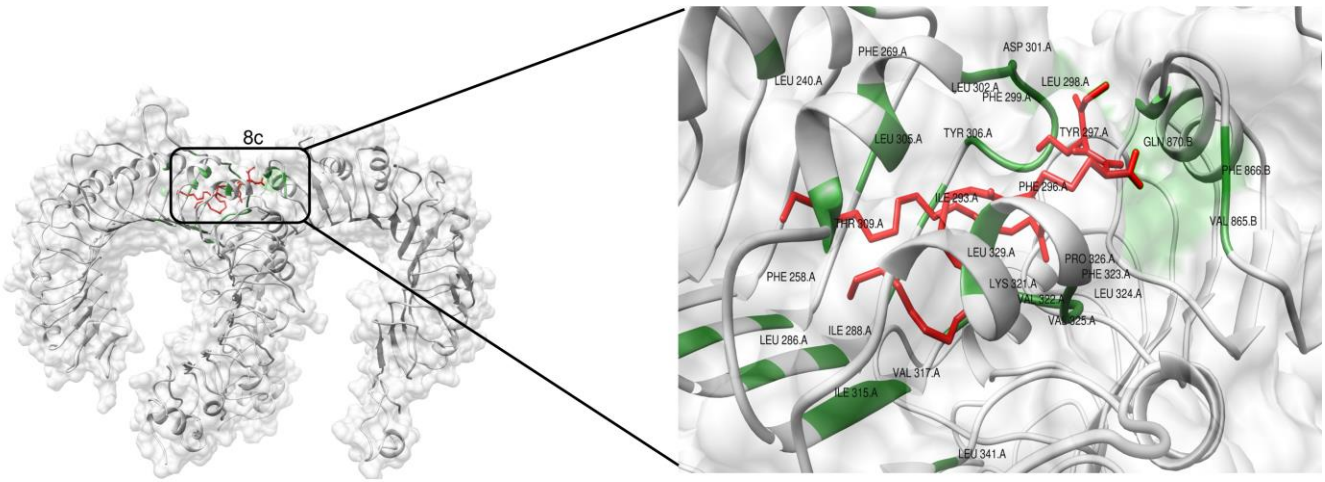


Figure S6. Surface representation of hTLR2/6 with compound 9b

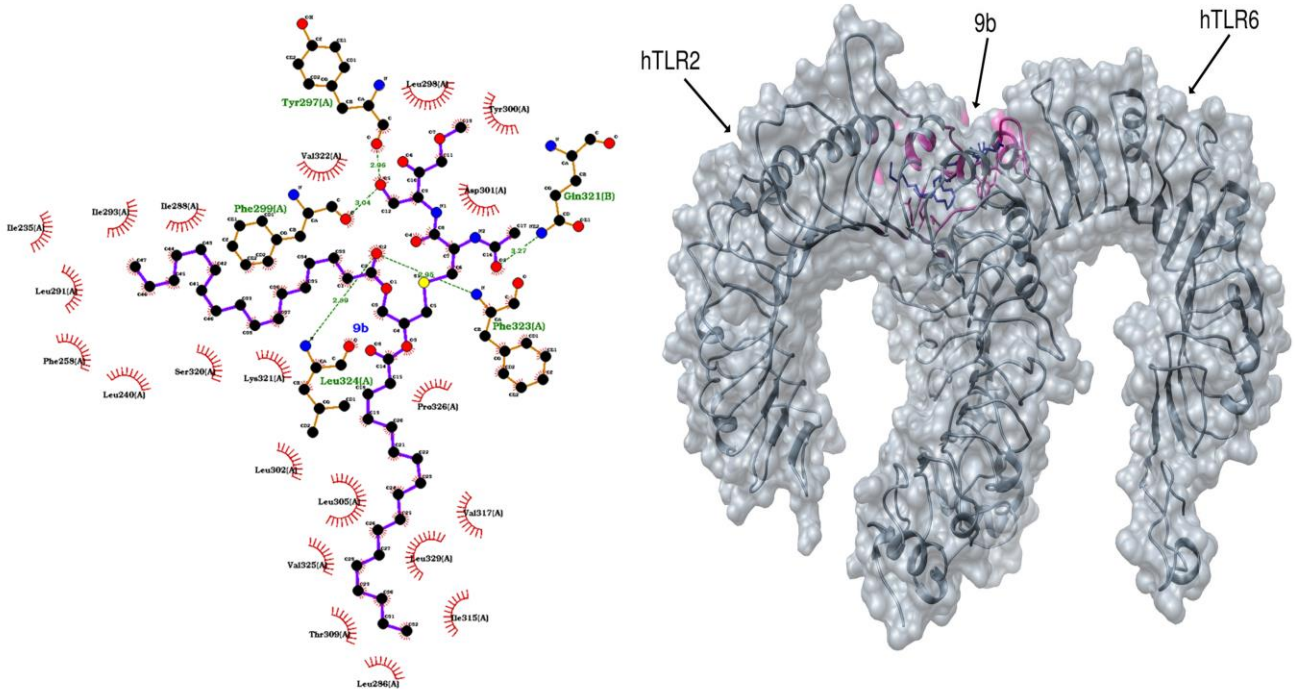


Figure S6a. Interacting amino acid residues of hTLR2/6 with compounds 9b

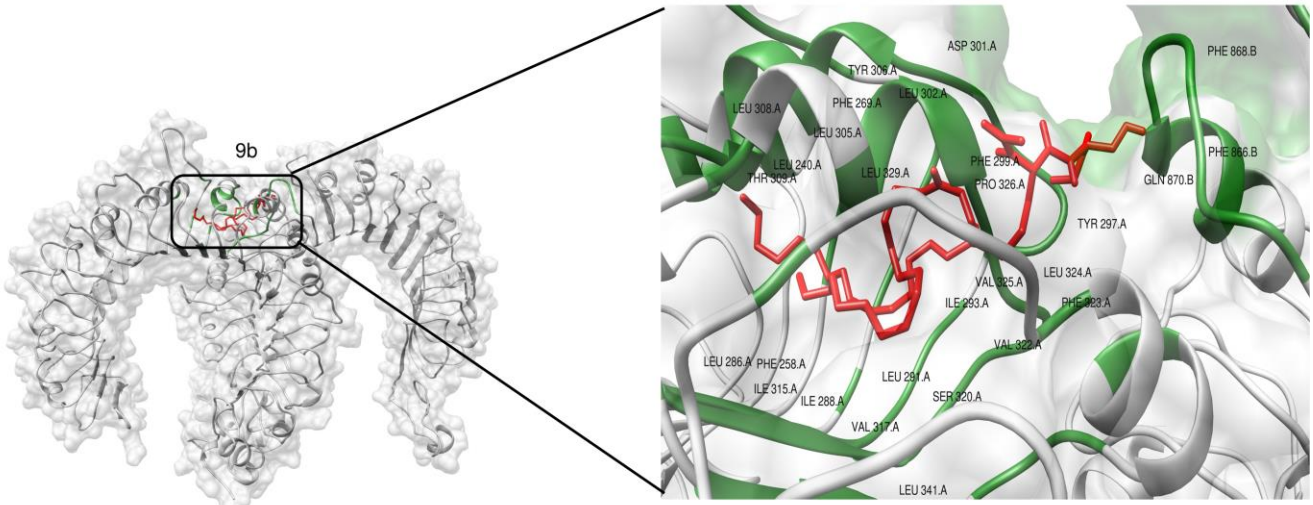


Figure S7. Surface representation of hTLR2/6 with compound 9c

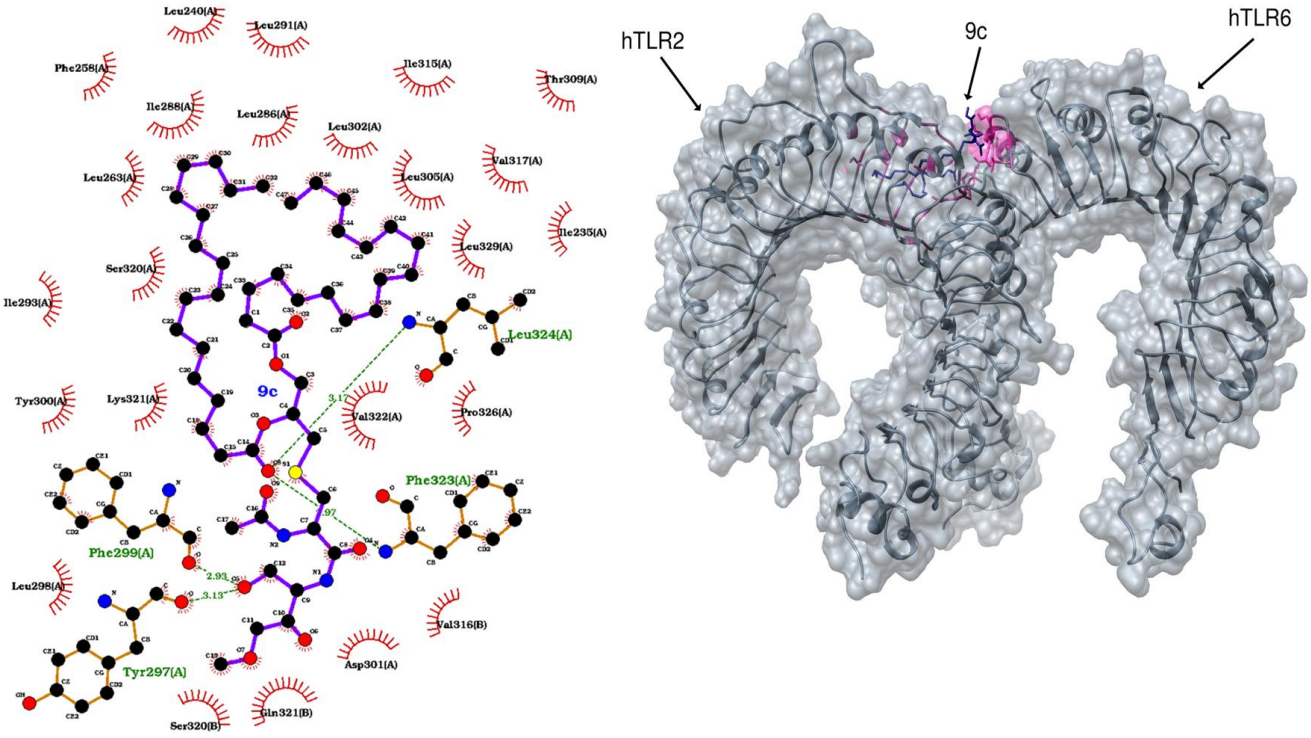


Figure S7a. Interacting amino acid residues of hTLR2/6 with compounds 9c

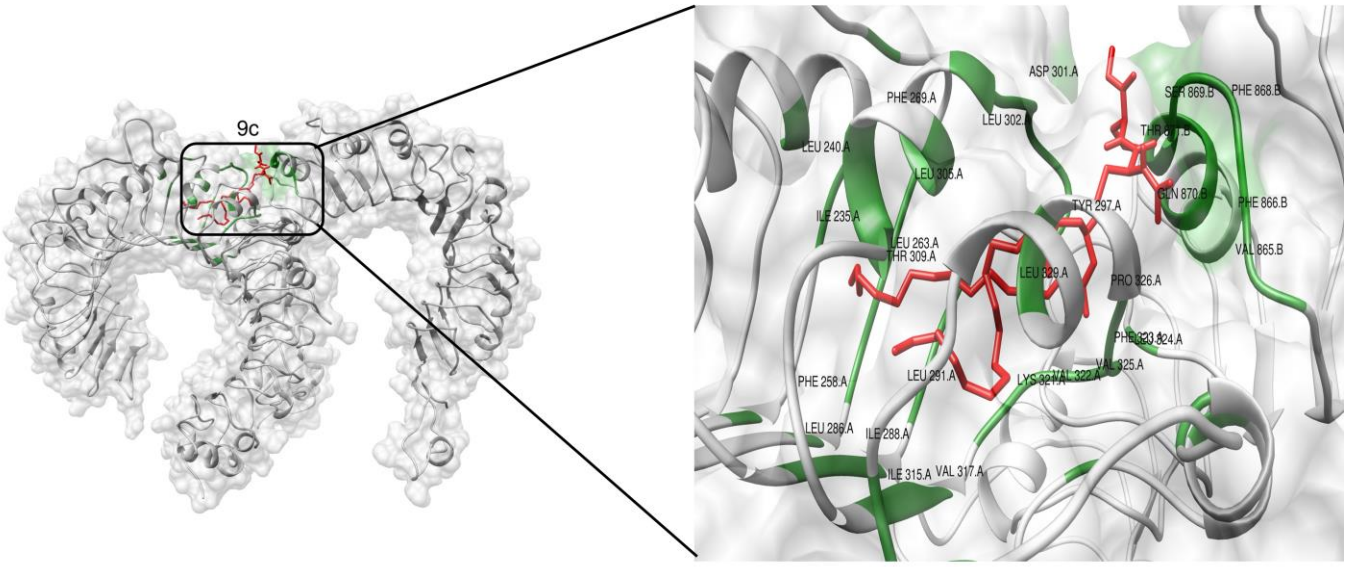


Figure S8. Modelled structure of human TLR2/6 showing the position of the ligand binding pocket

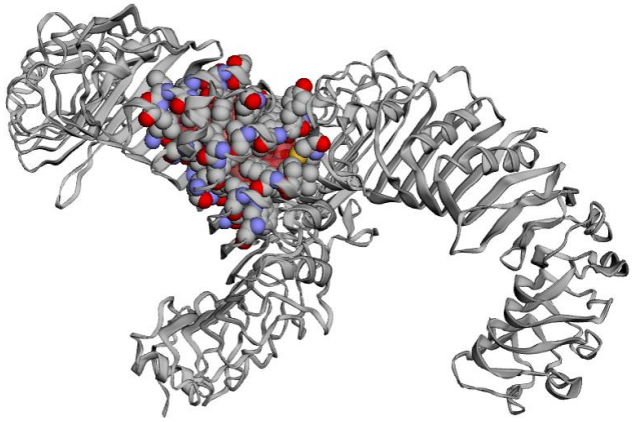
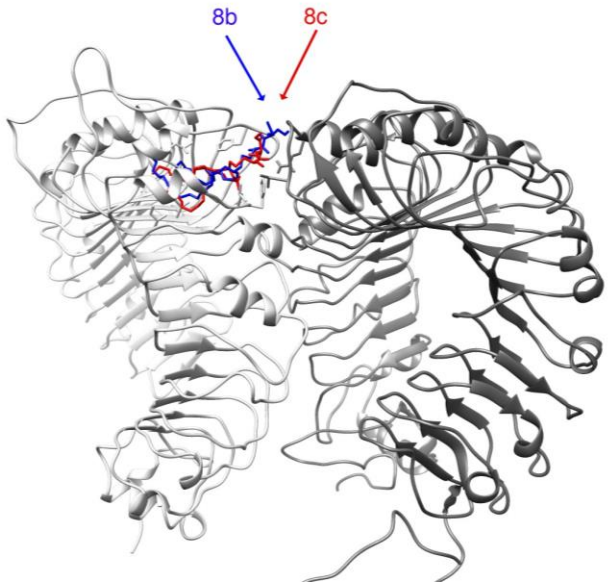


Figure S9. Superimposition of the structures of compounds 8b and 8c in hTLR2/6



Modeller Scripts for Docking Studies:

Script1:

```
from modeller import *

log.verbose()
env = environ()
#-- Prepare the input files

#-- Read in the sequence database
sdb = sequence_db(env)
sdb.read(seq_database_file='pdb_95.pir', seq_database_format='PIR',
        chains_list='ALL', minmax_db_seq_len=(30, 4000), clean_sequences=True)

#-- Write the sequence database in binary form
sdb.write(seq_database_file='pdb_95.bin', seq_database_format='BINARY',
         chains_list='ALL')

#-- Now, read in the binary database
sdb.read(seq_database_file='pdb_95.bin', seq_database_format='BINARY',
        chains_list='ALL')

#-- Read in the target sequence/alignment
aln = alignment(env)
aln.append(file='tlr2-6.ali', alignment_format='PIR', align_codes='ALL')

#-- Convert the input sequence/alignment into
#   profile format
prf = aln.to_profile()

#-- Scan sequence database to pick up homologous sequences
prf.build(sdb, matrix_offset=-450, rr_file='${LIB}/blosum62.sim.mat',
         gap_penalties_1d=(-500, -50), n_prof_iterations=1,
         check_profile=False, max_aln_evalue=0.01)

#-- Write out the profile in text format
prf.write(file='build_profile.prf', profile_format='TEXT')

#-- Convert the profile back to alignment format
aln = prf.to_alignment()

#-- Write out the alignment file
aln.write(file='build_profile.ali', alignment_format='PIR')
```

Script2:

```
from modeller import *

env = environ()
aln = alignment(env)
for (pdb, chain) in (('2z7x', 'A'), ('3a79', 'A')):
   m = model(env, file=pdb, model_segment=('FIRST:'+chain, 'LAST:'+chain))
   aln.append_model(m, atom_files=pdb, align_codes=pdb+chain)
aln.malign()
aln.malign3d()
aln.compare_structures()
aln.id_table(matrix_file='family.mat')
env.dendrogram(matrix_file='family.mat', cluster_cut=-1.0)
```

Script3:

```
from modeller import *

env = environ()
aln = alignment(env)
mdl = model(env, file='3a79', model_segment=('FIRST:A','LAST:B'))
aln.append_model(mdl, align_codes='3a79', atom_files='3a79.pdb')
aln.append(file='tlr2-6.ali', align_codes='tlr2-6')
aln.align2d()
aln.write(file='tlr-1.ali', alignment_format='PIR')
aln.write(file='tlr-1.pap', alignment_format='PAP')
```

Script4:

```
from modeller import *
from modeller.automodel import *
#from modeller import soap_protein_od

env = environ()
a = automodel(env, alnfile='tlr2-6.ali',
            knowns='3a79', sequence='tlr2-6',
            assess_methods=(assess.DOPE,
                        #soap_protein_od.Scorer(),
                        assess.GA341))
a.starting_model = 1
a.ending_model = 10
a.make()
```

Autodock Vina Scripts:

Conf.txt:

```
receptor = tlr2_6model.pdbqt

center_x =  138.161
center_y =  109.587
center_z = 33.389

size_x = 40
size_y = 80
size_z = 60

num_modes = 10
```

Vina_screen_local.sh:

```
#! /bin/bash

for f in *.pdbqt; do
   b=`basename $f .pdbqt`
   echo Processing ligand $b
   mkdir -p $b
   vina --config conf.txt --ligand $f --out ${b}/out.pdbqt --log ${b}/log.txt
done
```

Table S2 to correlate all the available data, including computation, *in vitro* and *in vivo* data

Compound No.	**Docking Score (MMPBSA)**	**TLR2/6 activity**	**Cytokine Levels**			**MHC-II$^+$ CD19$^+$ cells (B cells) †**		**MHC-II$^+$ CD11c$^+$ cells (DCs) †**		**MHC-II$^+$ CD11b$^+$ cells (Monocytes)†**		**Protection against influenza challenge‡**
	Free energy binding	EC_{50} (μM)	IL-6	IL-10	TNF-α	CD40	CD86	CD40	CD86	CD40	CD86	
Pam_2CSK_4		0.0003	+	++	+	+++	+++	+++	+++	+++	+++	-
Pam_3CSK_4		0.0193	++	+++	++	++	++	++	++	++	++	-
7a		5.63	-	-	-	vvv	+++	vvv	+++	vvv	+++	NT
7b	-20.42	5.86	-	-	-	vvv	+++	vvv	++	vvv	+++	NT
7c	-25.78	4.27	-	-	-	+vv	+++	+vv	+++	+vv	vvv	NT
8a		0.0010	+	+	+	vvv	+++	vvv	+++	vvv	++v	++
8b	-36.52	0.004	+	+	+	+++	+++	+++	+++	+++	++v	+++
8c	-32.10	0.1337	-	-	-	+++	+++	+++	+++	+++	v+v	-
9a		0.0050	+	++	+	+++	v++	+++	v++	+++	vv+	+
9b	-34.22	0.0008	++	+++	++	vvv	+++	+vv	+++	++v	+vv	+++
9c	-37.91	0.2518	+	+	+	v++	+++	v++	+++	+++	vv+	-

†CD expressions seen for three doses; 10.0, 1.0, 0.1 μg/mL; + for upregulation; v for down regulation;
‡Cumulative weight loss; +++ stands for no weight loss; - stands for weight loss equivalent to control group (saline);
NT is not tested