Synthesis and Evaluation of Bioorthogonal Pantetheine Analogs for In Vivo Protein Modification

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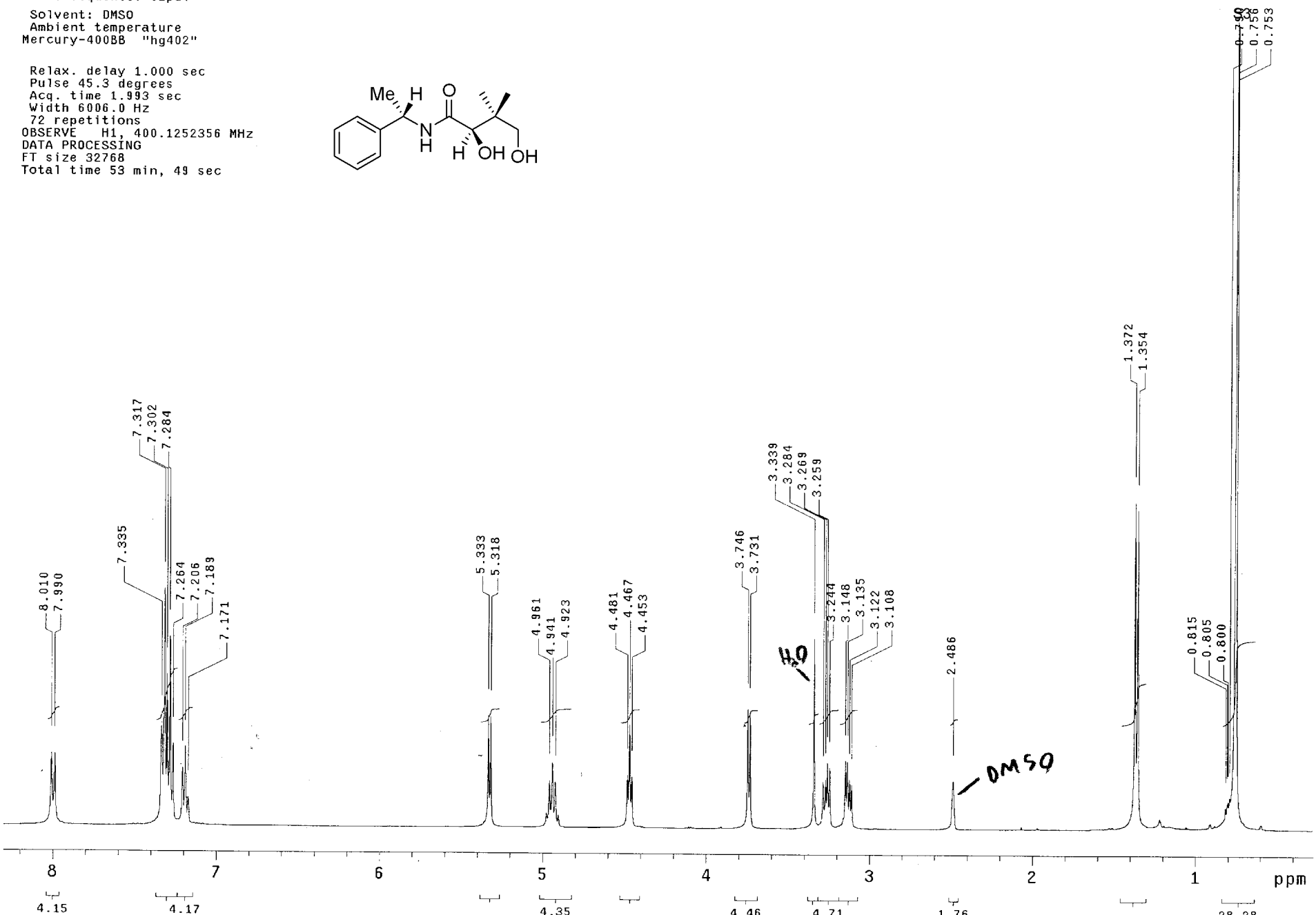
jlm3-78-chiralaminemicrowaveinETOH-DMSOHNMR-04-19-06

Pulse Sequence: s2pul

Solvent: DMSO
Ambient temperature
Mercury-400BB "hg402"

Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
72 repetitions
OBSERVE H1, 400.1252356 MHz
DATA PROCESSING
FT size 32768
Total time 53 min, 49 sec



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Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "hg402"

Relax. delay 1.000 sec
Pulse 45.3 degrees
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Width 6006.0 Hz
88 repetitions
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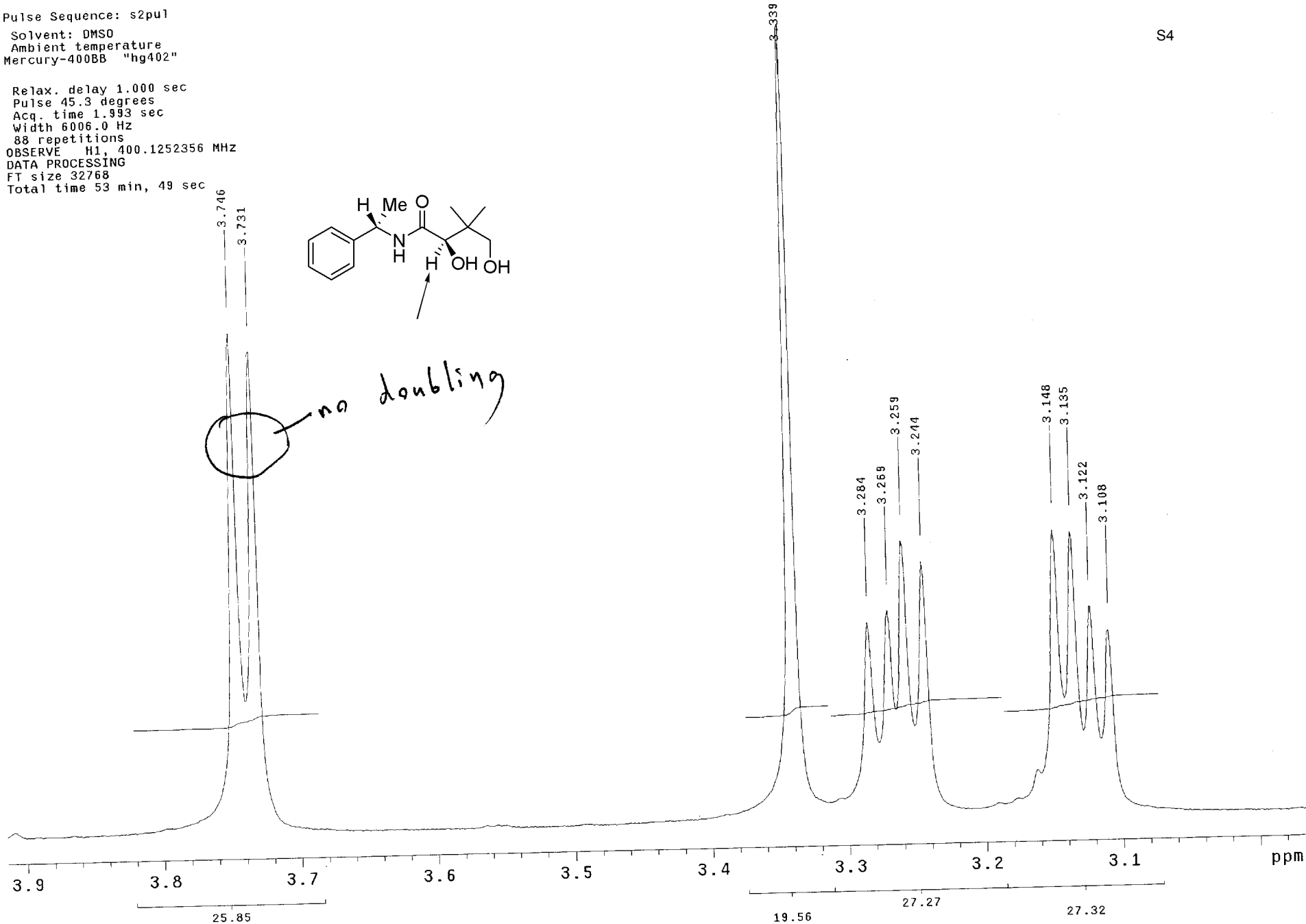

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Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "hg402"

Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
80 repetitions
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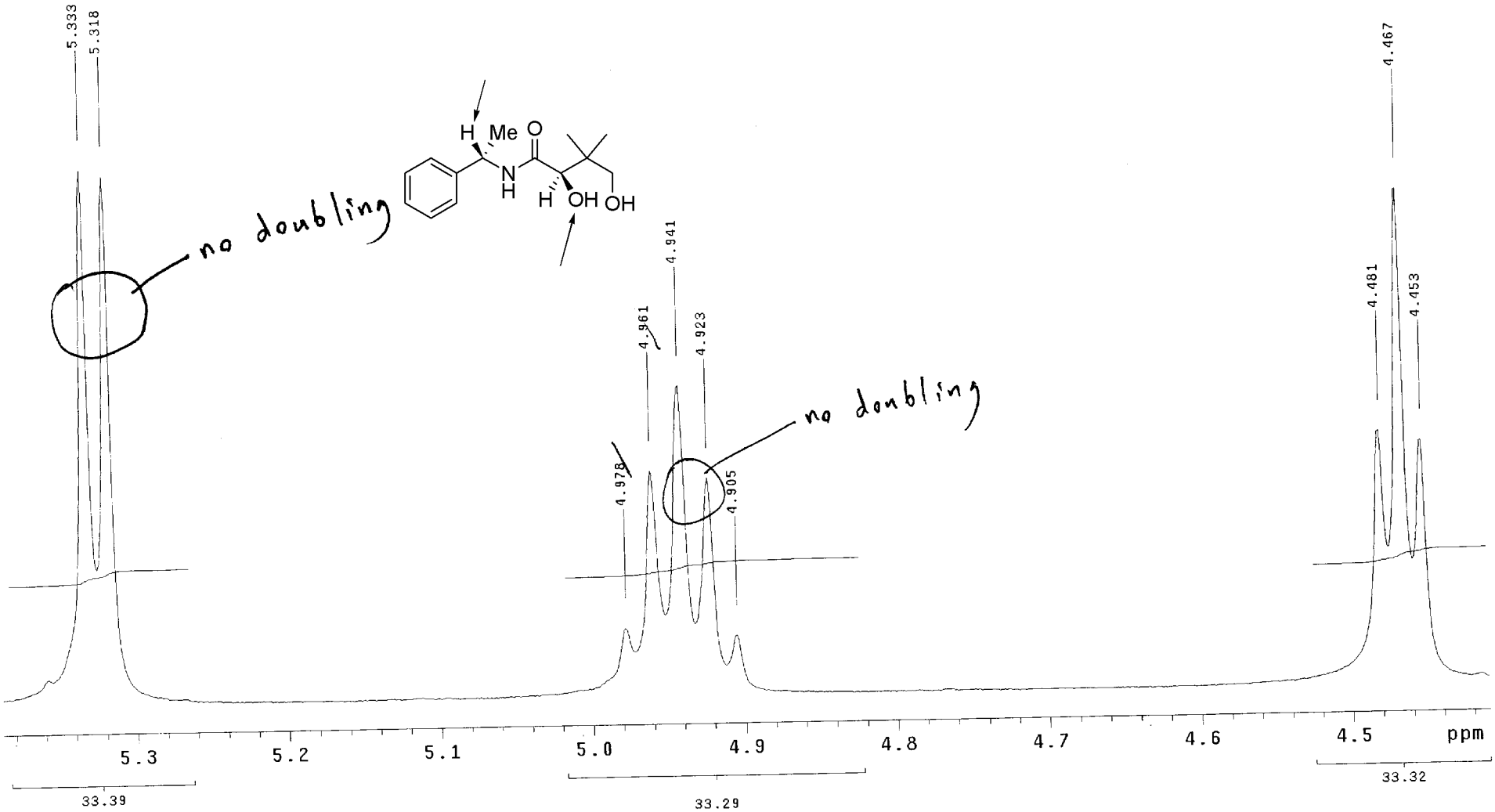

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Solvent: cd3od
Ambient temperature
Mercury-400BB "hg402"

Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
48 repetitions
OBSERVE H1, 400.1249114 MHz
DATA PROCESSING
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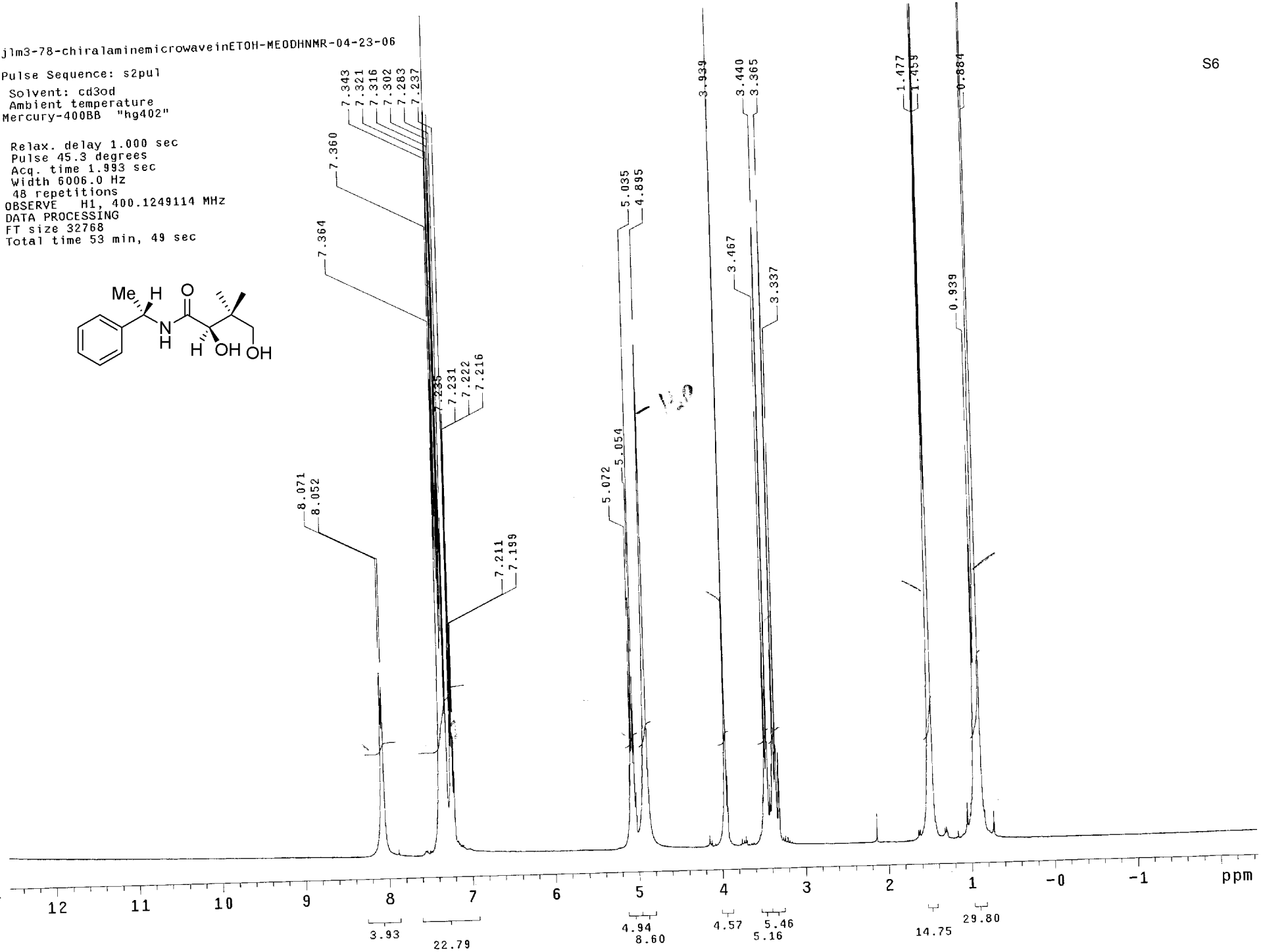
Total time 53 min, 49 sec



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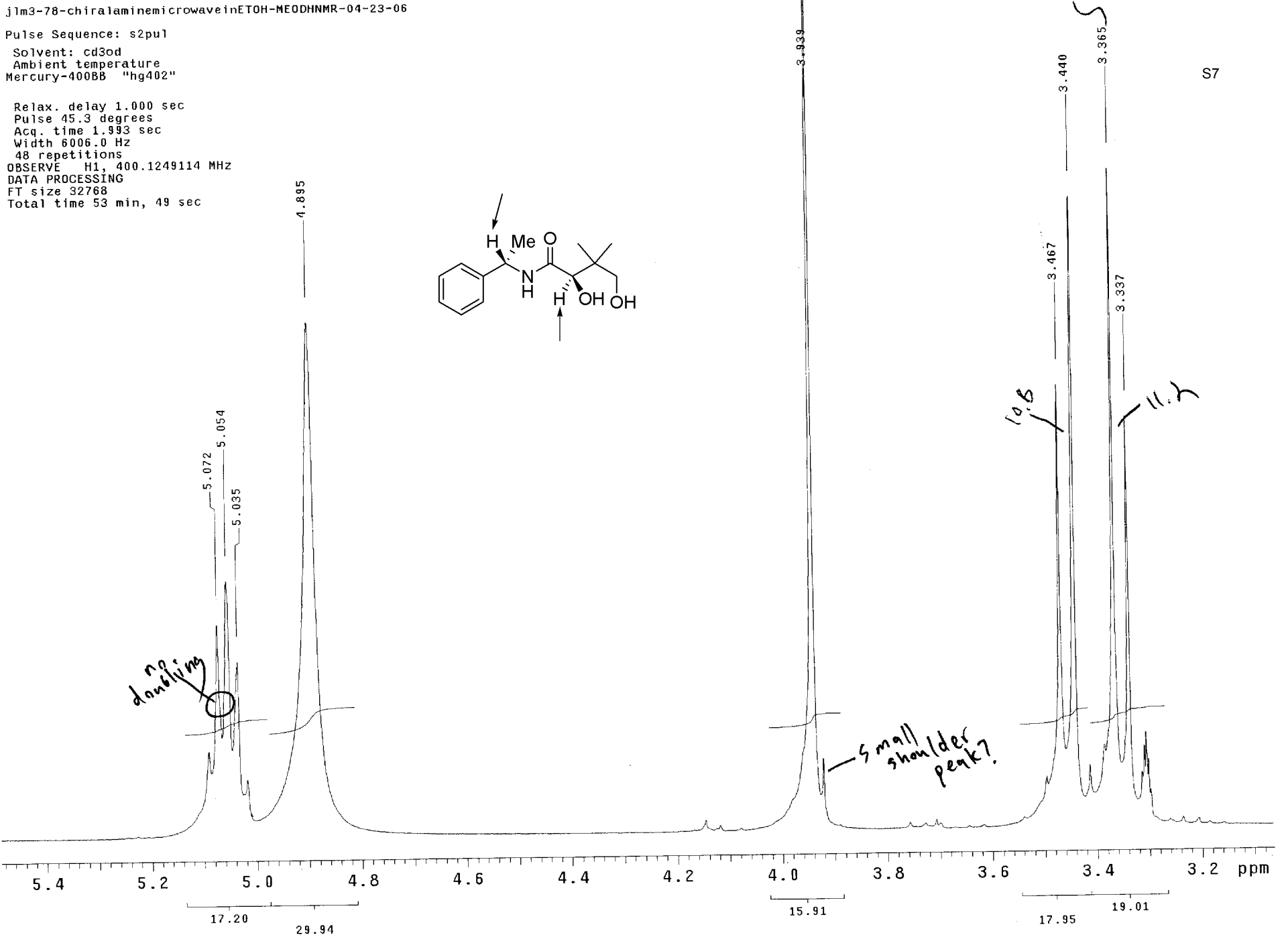
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
Mercury-400BB "hg402"

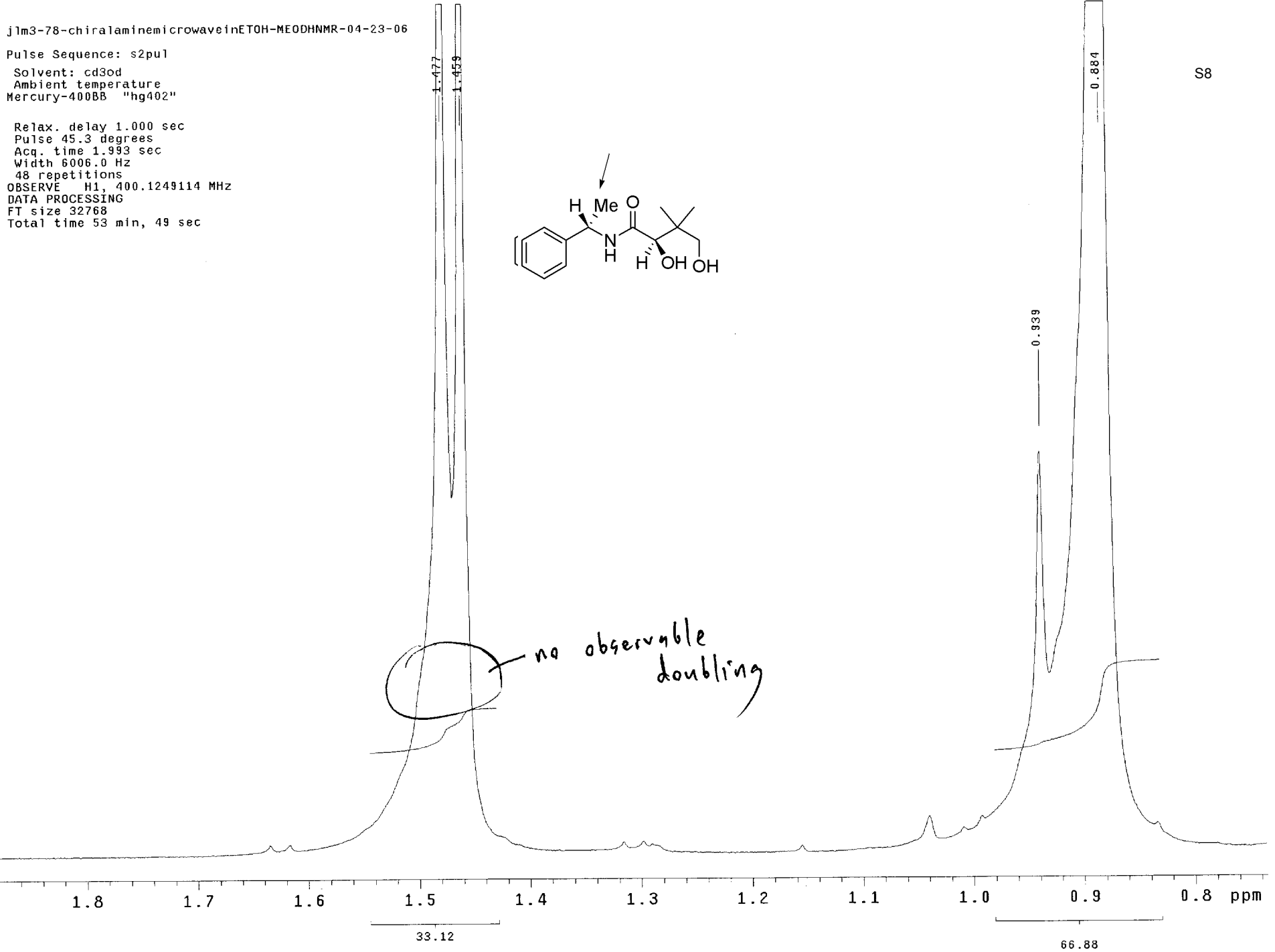
Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
48 repetitions
OBSERVE H1, 400.1249114 MHz
DATA PROCESSING
FT size 32768
Total time 53 min, 49 sec

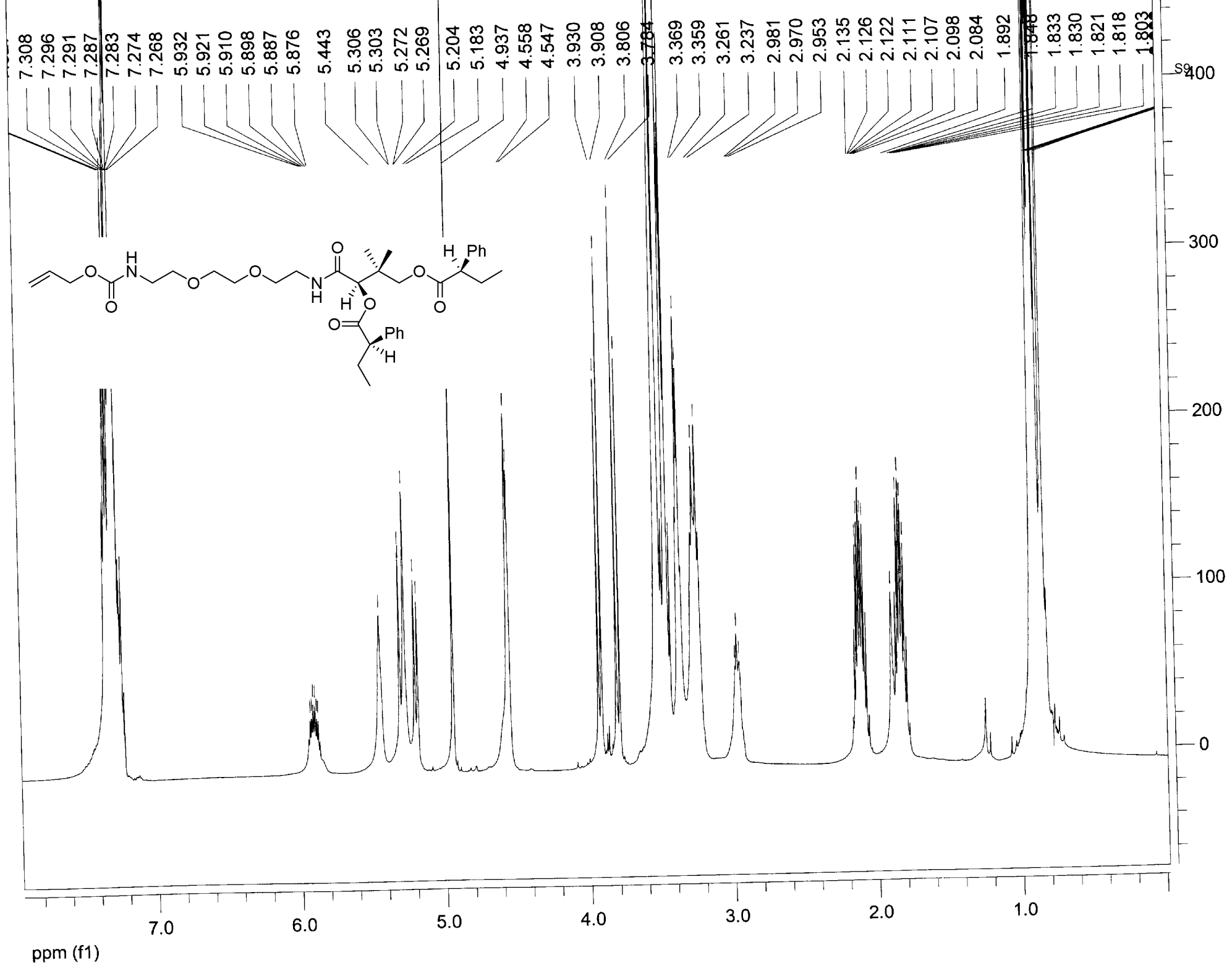


jlm3-78-chiralaminemicrowaveinETOH-MEODHNMR-04-23-06

Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
Mercury-400BB "hg402"

Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
48 repetitions
OBSERVE H1, 400.1249114 MHz
DATA PROCESSING
FT size 32768
Total time 53 min, 49 sec




7.308
7.296
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7.283
7.274
7.268
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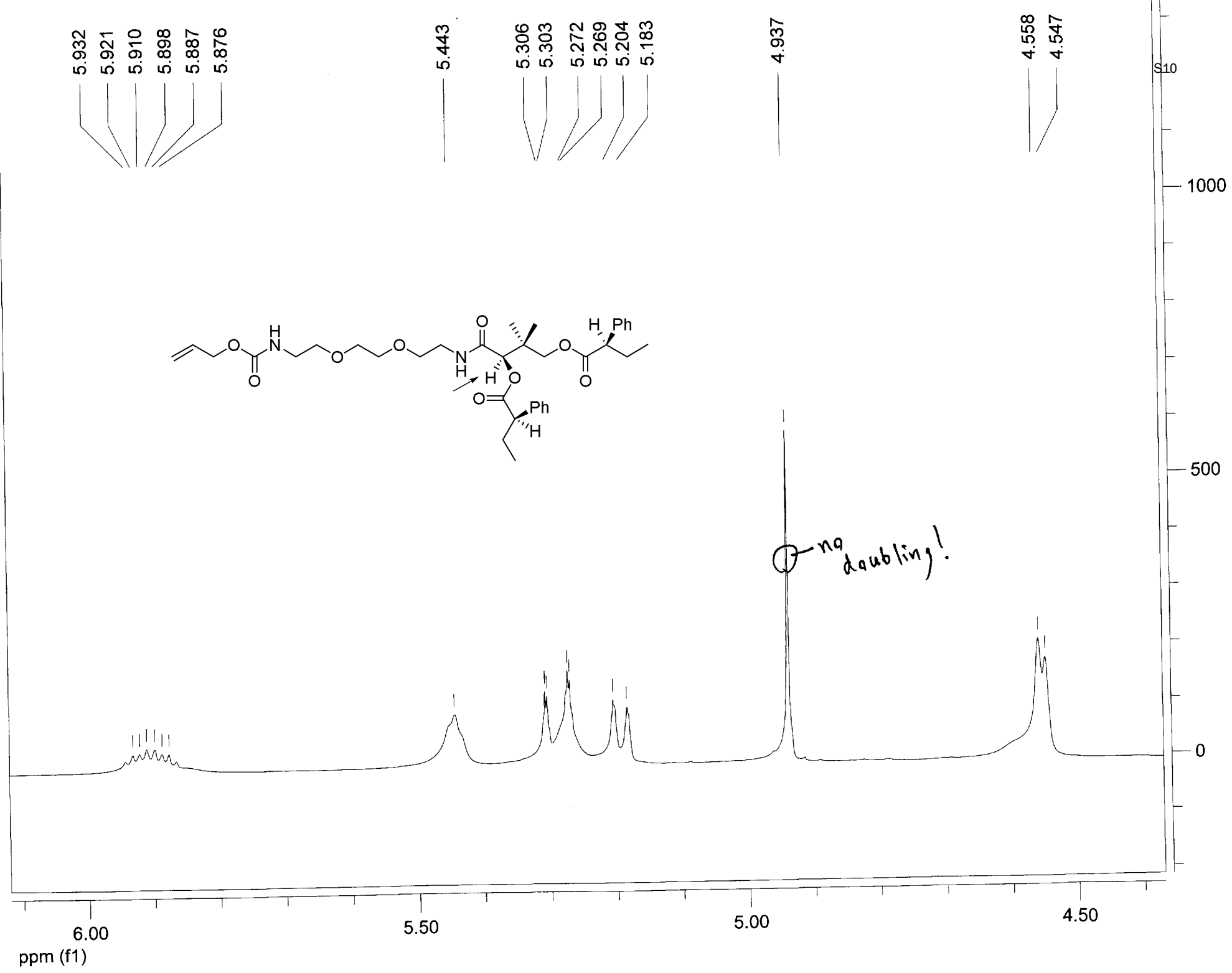
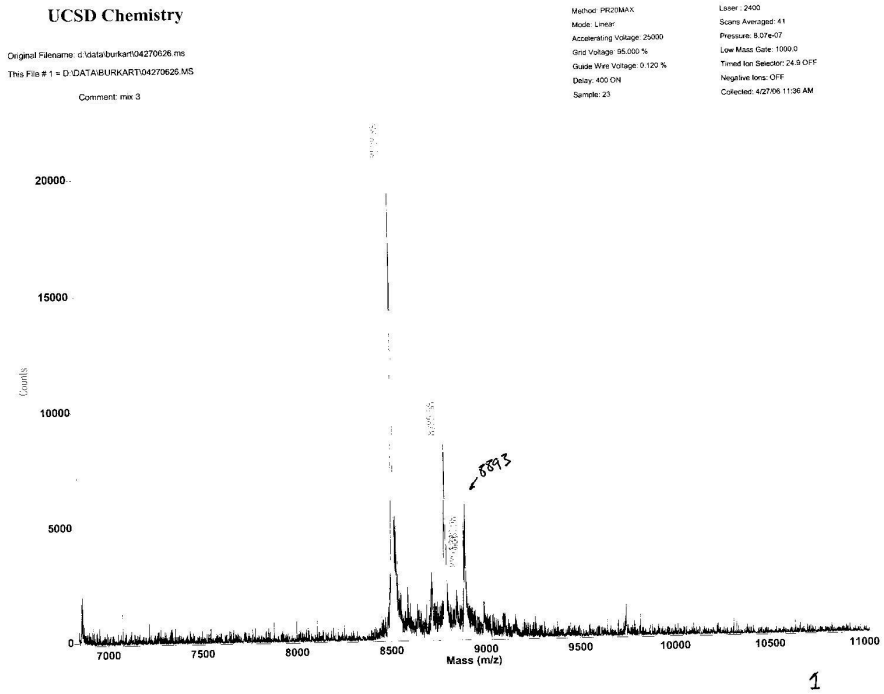
5.921
5.910
5.898
5.887
5.876
5.443
5.306
5.303
5.272
5.269
5.204
5.183
4.937
4.558
4.547
3.930
3.908
3.806
3.784
3.369
3.359
3.261
3.237
2.981
2.970
2.953
2.135
2.126
2.122
2.111
2.107
2.098
2.084
1.892
1.848
1.833
1.830
1.821
1.818
1.803
S9
400
300
200
100
0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)


5.932
5.921
5.910
5.898
5.887
5.876
5.443
5.306
5.303
5.272
5.269
5.204
5.183
4.937
4.558
4.547
1000
500
0
no doubling!
6.00
5.50
5.00
4.50
ppm (f1)

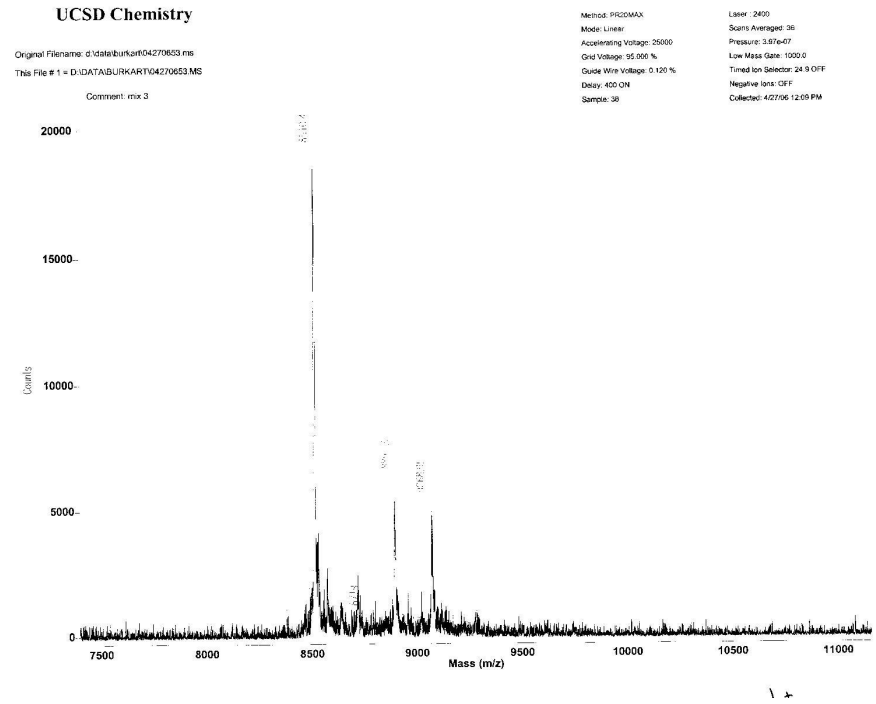
Mass Spectrometry Data

Protein mass spectrometry was performed using MALDI on an Applied Biosystems Voyager DE STR 4140. Samples were prepared in the same manner in both the in vivo and in vitro assays. For MALDI analysis doubly transformed BL21 *E. coli* with Fren (frenolicin synthase carrier protein from *Streptomyces roseofulvus*) and Sfp was used. Samples were mixed 1:5 with the ionization buffer (saturated sinapinc acid in 1:1:1 acetonitrile, TFA, water). A standard deviation of +/- 5 Da was observed over the course of 20+ runs with regards to the apo-forms of ACP and Fren. However within each run the mass shift between the *apo*-form and subsequent *crypto*-forms was consistent as described in text.

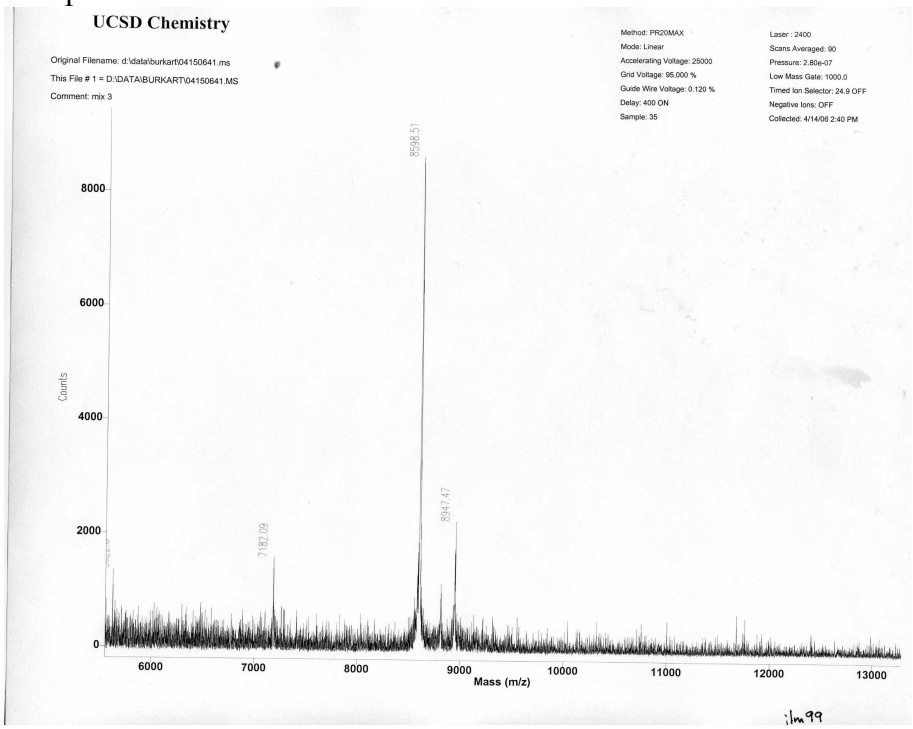
Compound **1** In vitro



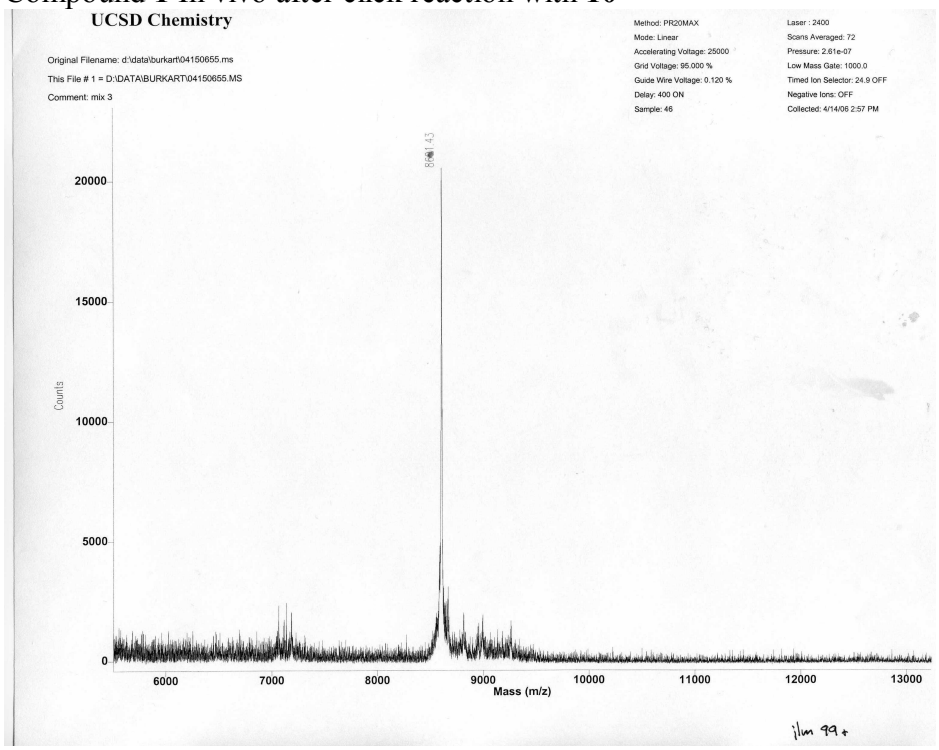
Compound **1** In vitro after click reaction with **10**



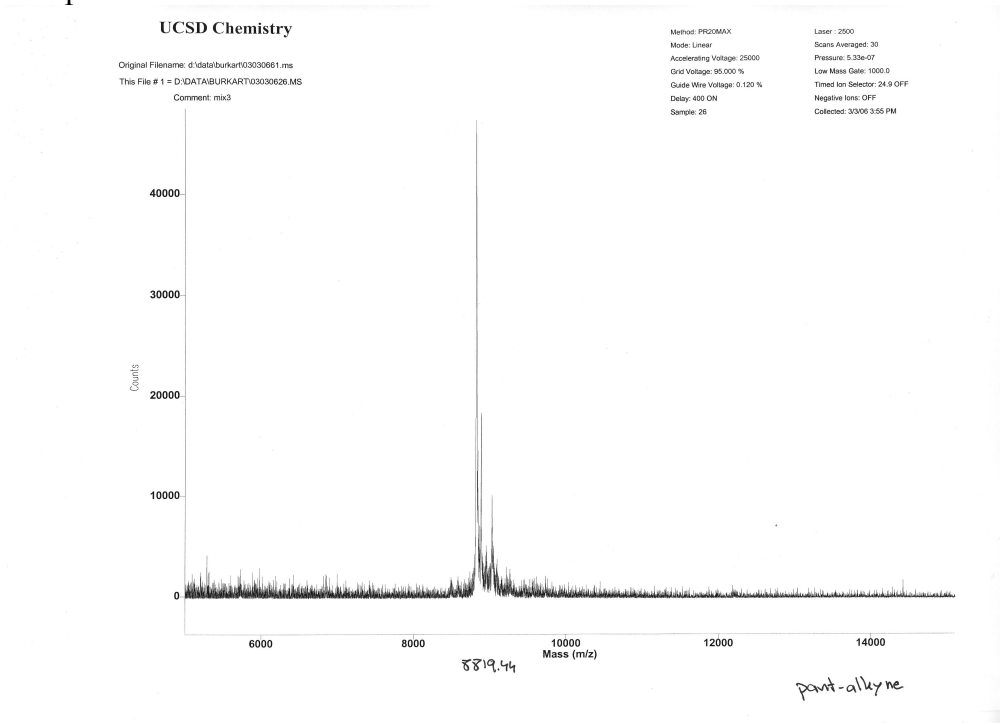
Compound **1** In vivo



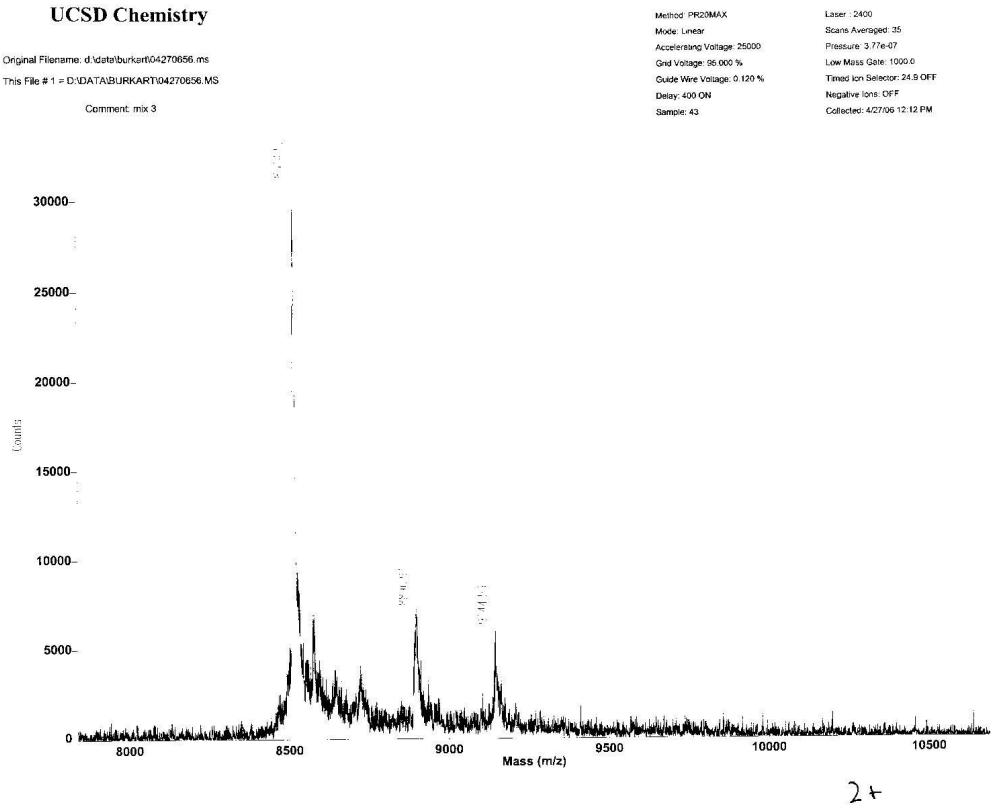
Compound **1** In vivo after click reaction with **10**



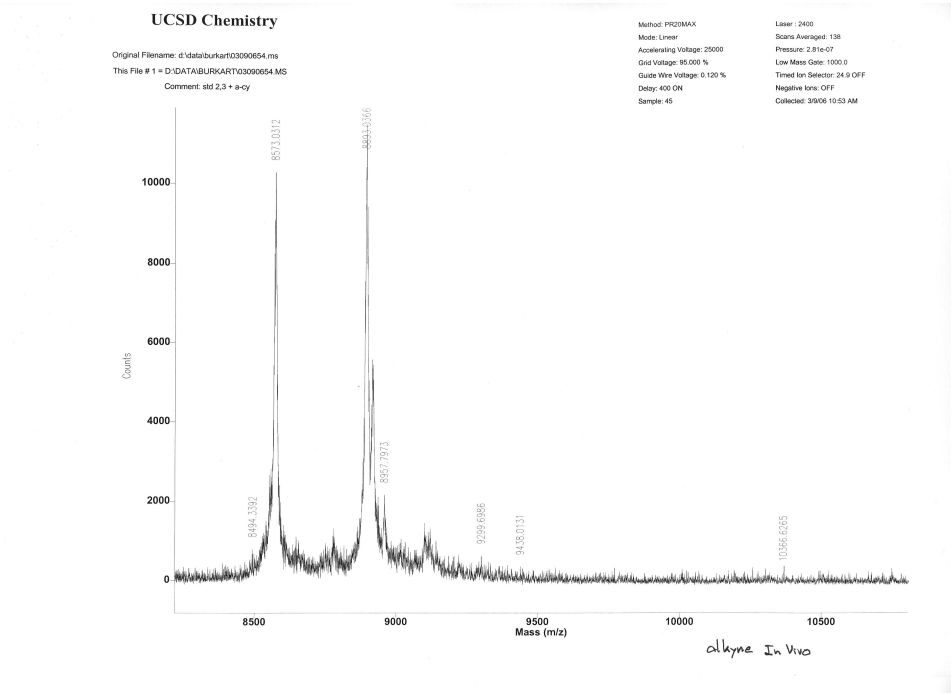
Compound **2** In vitro



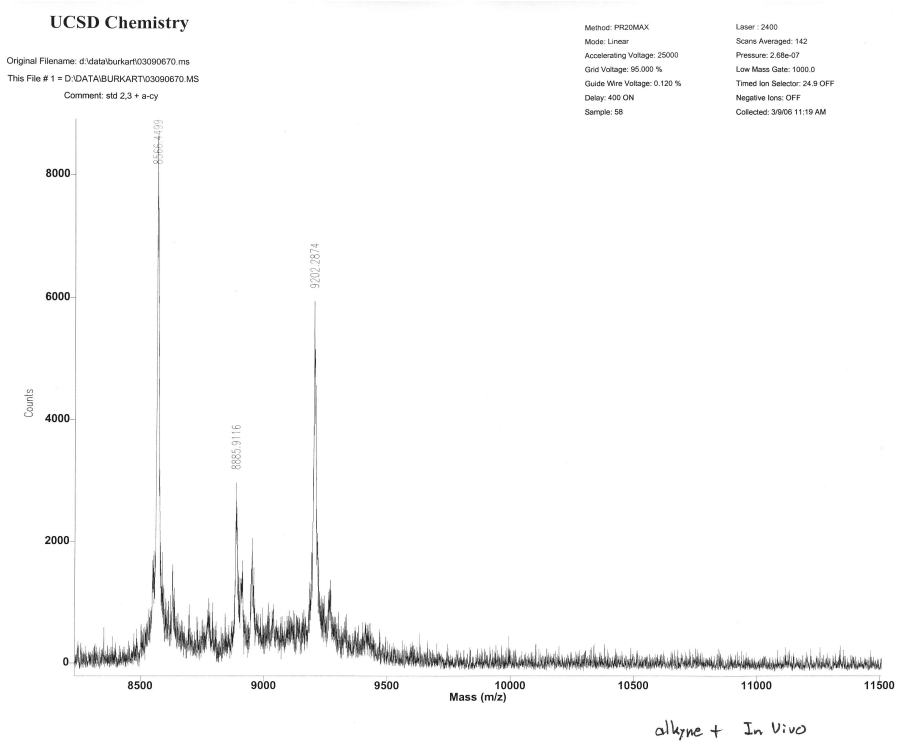
Compound **2** In vitro after click reaction with **11**



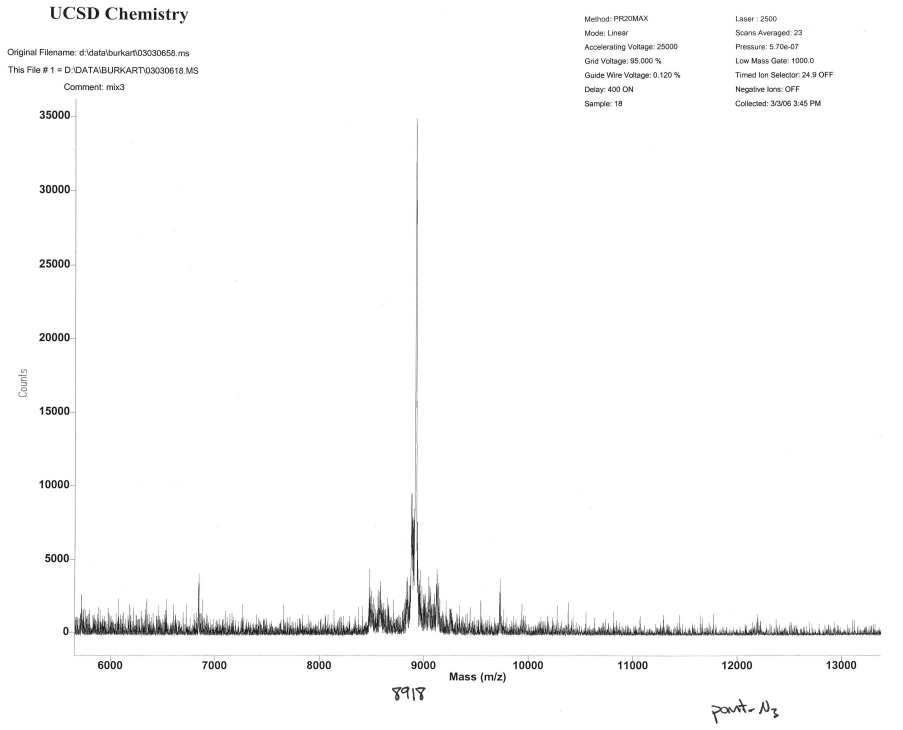
Compound **2** In vivo



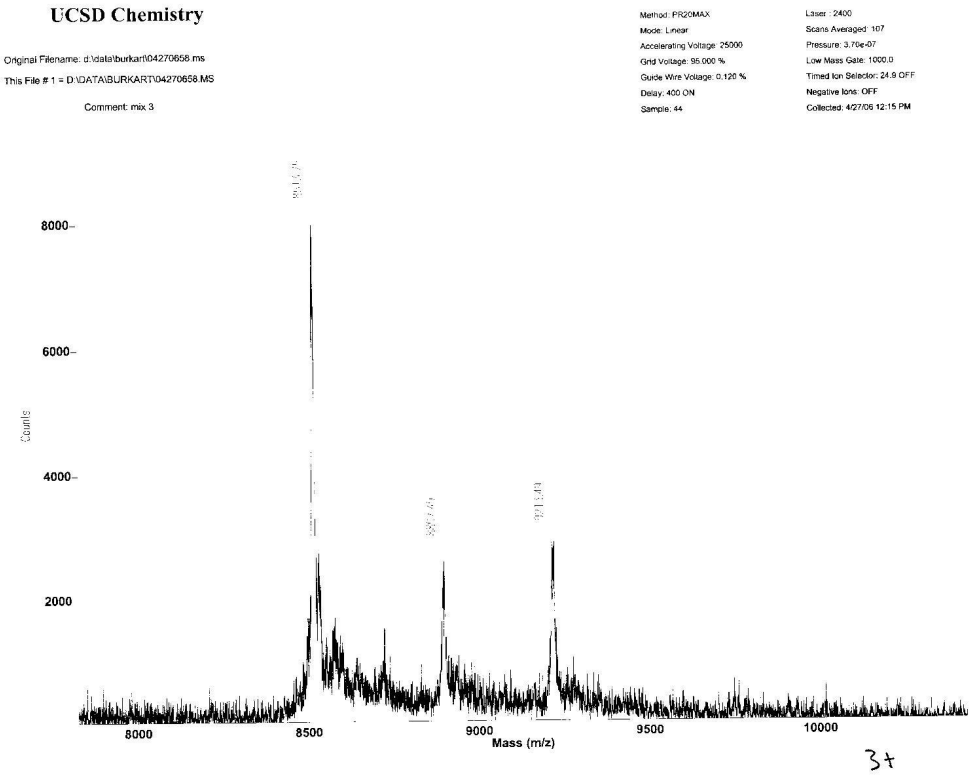
Compound **2** In vivo after click reaction with **11**



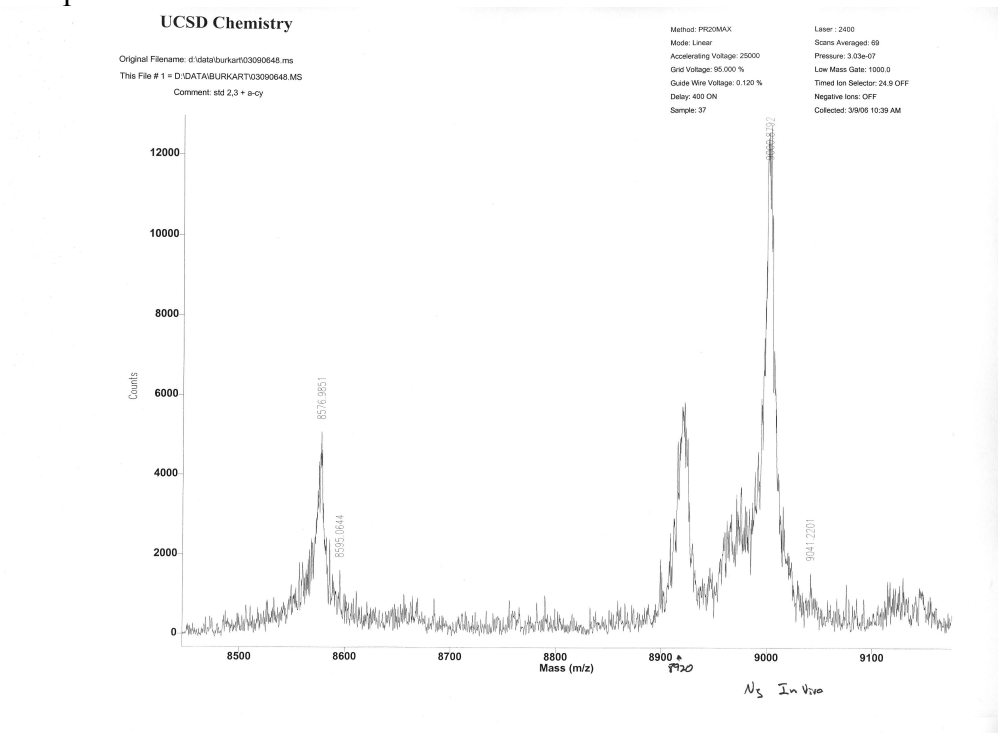
Compound **3** In vitro



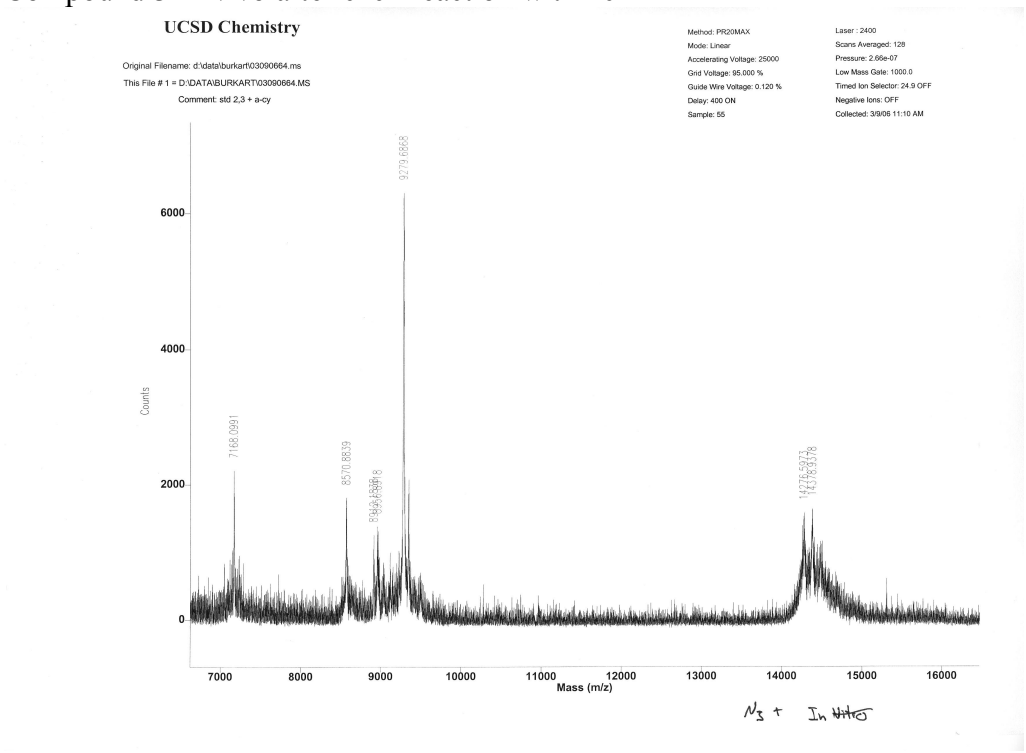
Compound **3** In vitro after click reaction with **10**



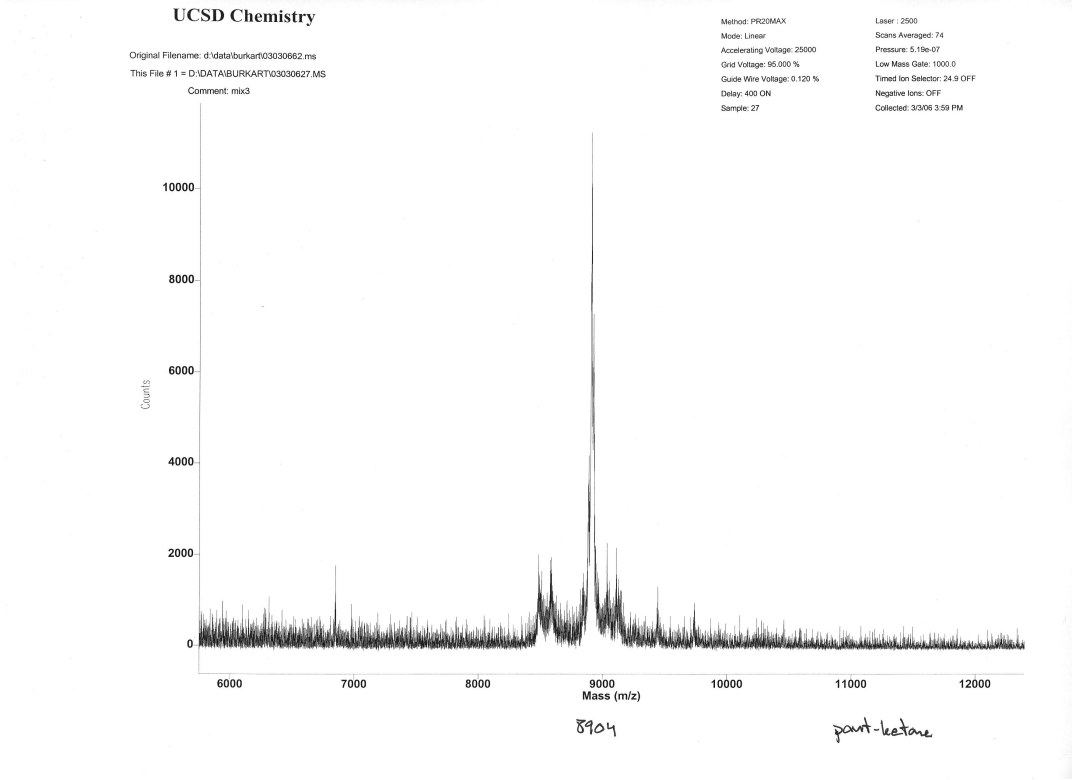
Compound **3** In vivo



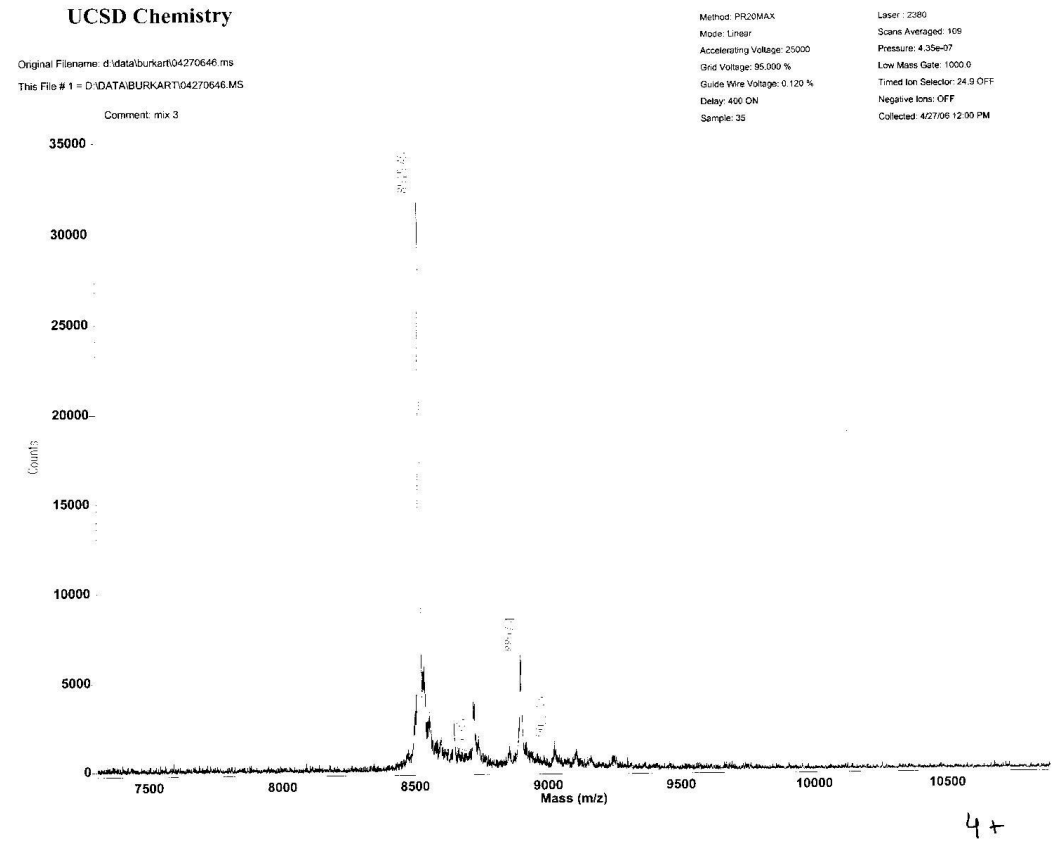
Compound **3** In vivo after click reaction with **10**



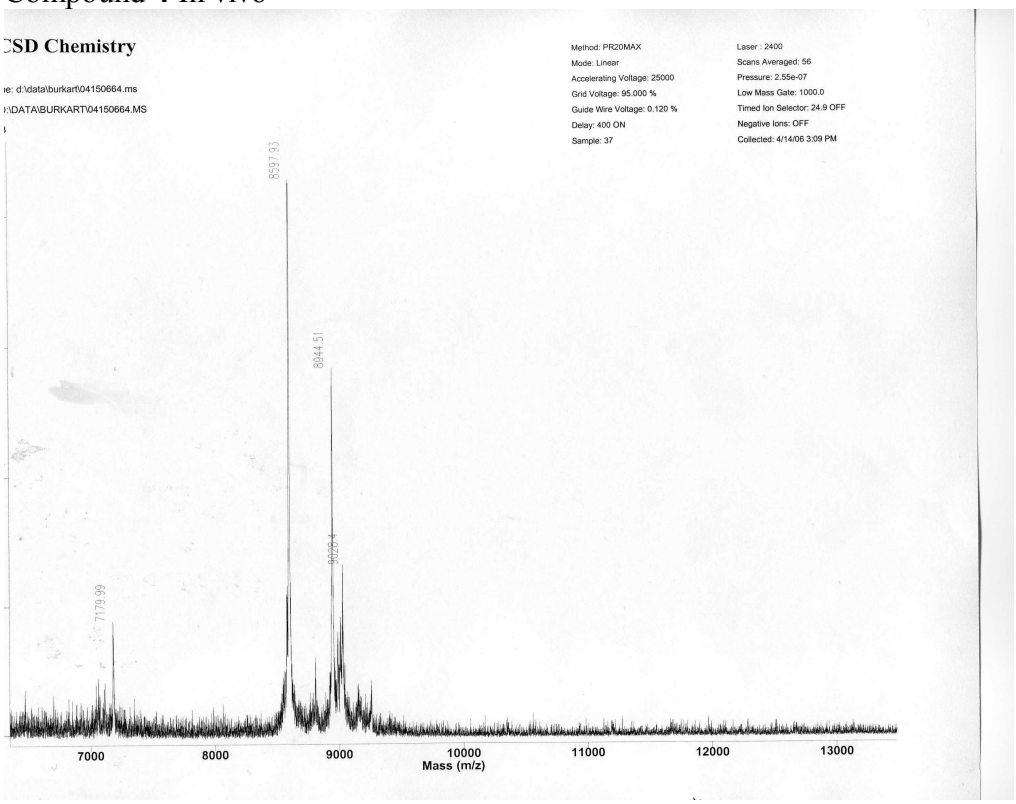
Compound **4** In vitro



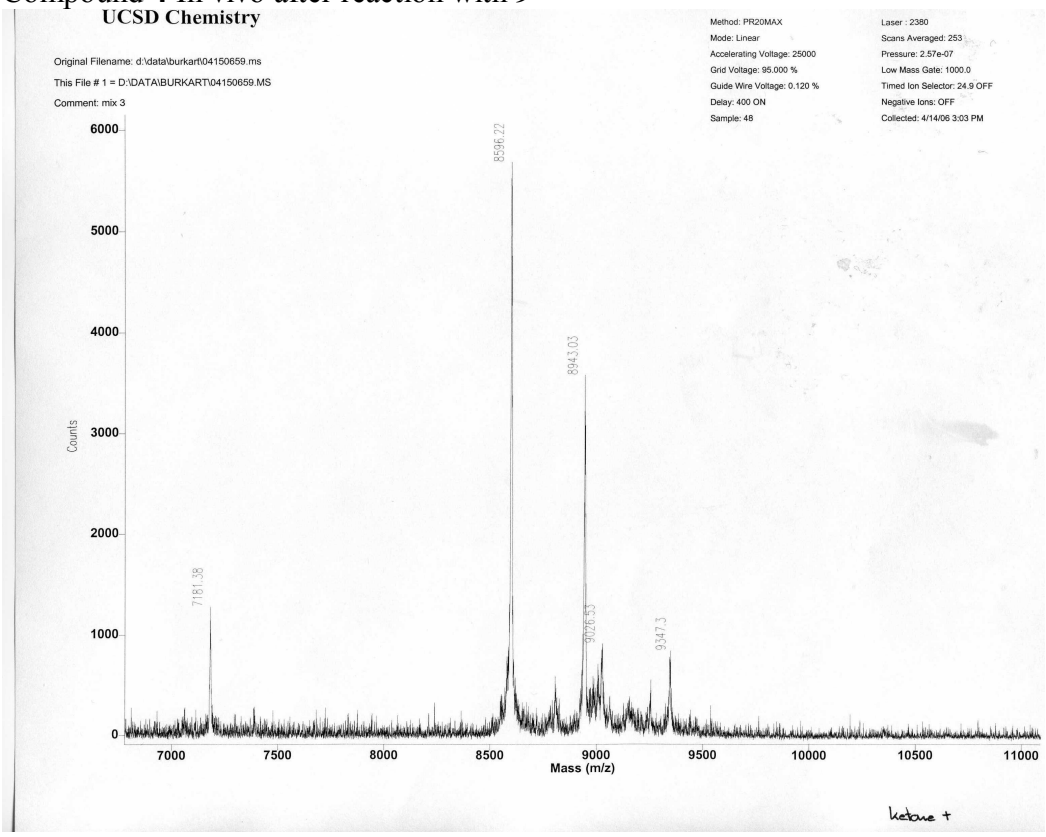
Compound **4** In vitro after reaction with **9**



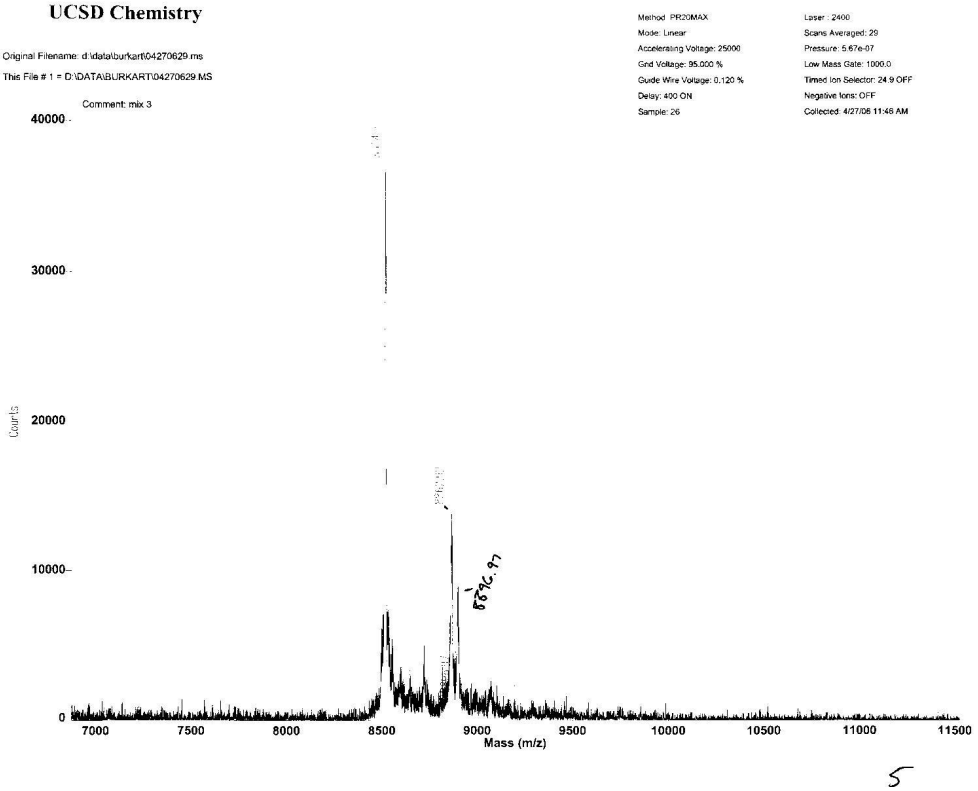
Compound **4** In vivo



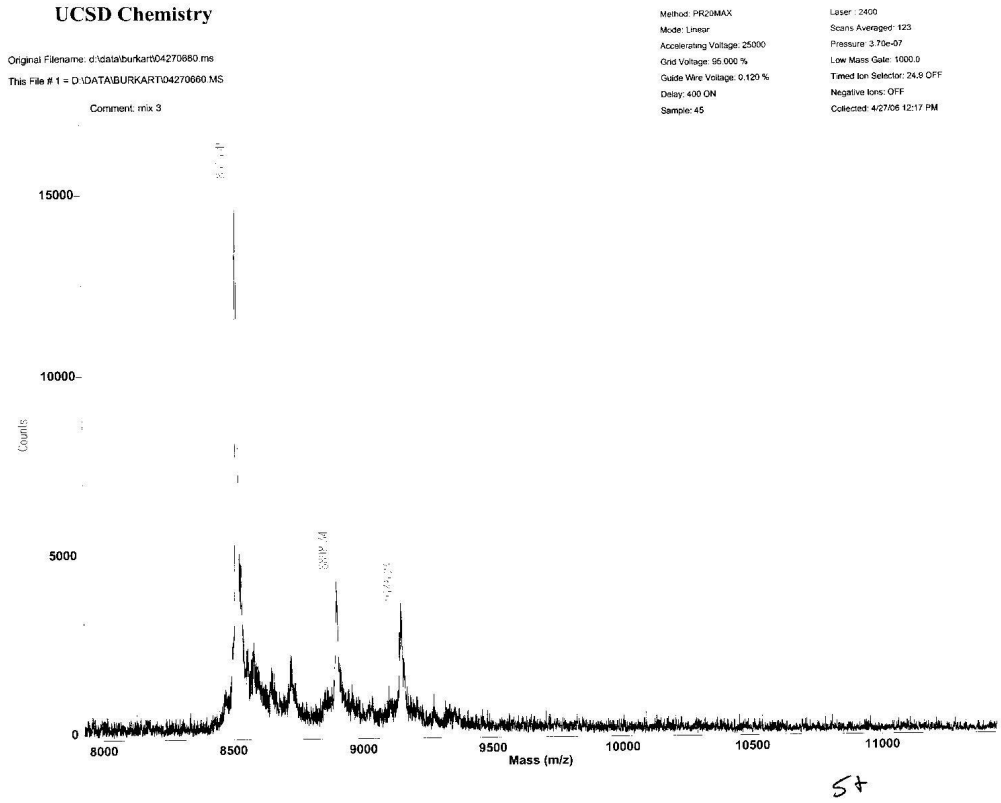
Compound **4** In vivo after reaction with **9**



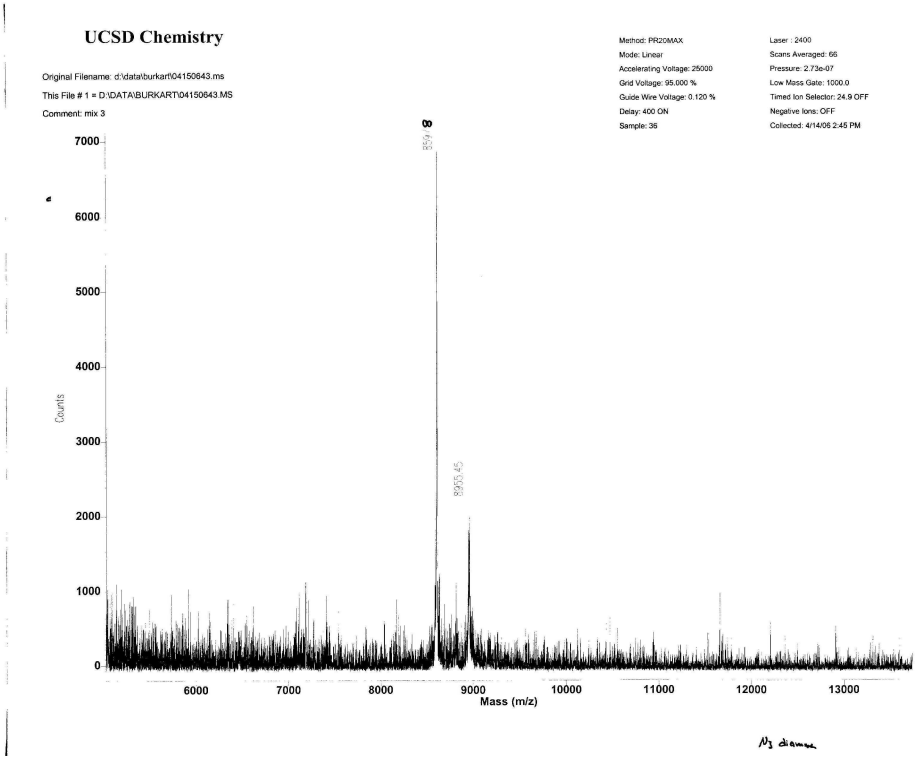
Compound **5** In vitro



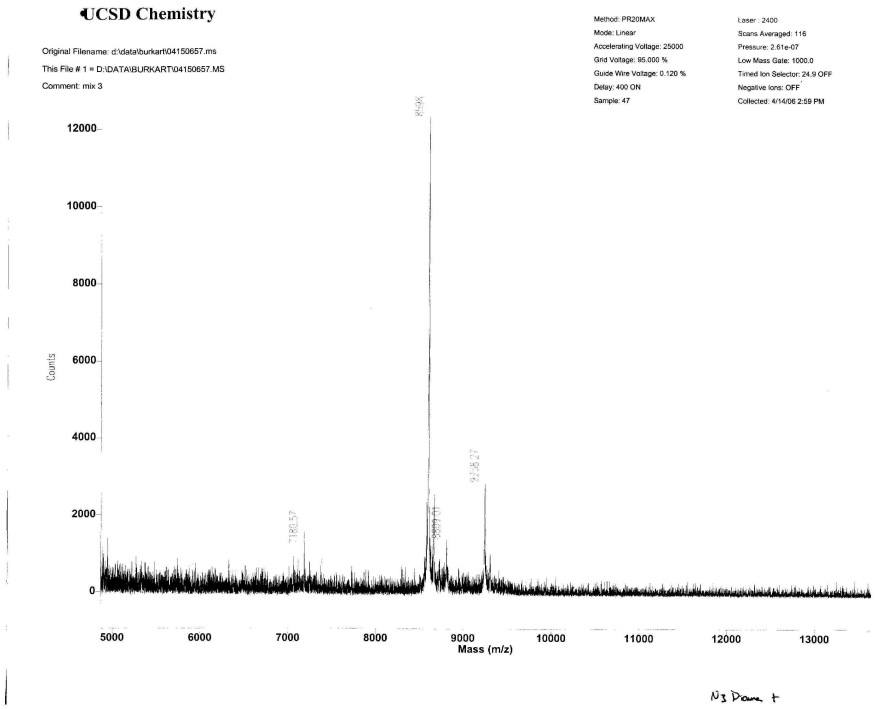
Compound **5** In vitro after click reaction with **10**



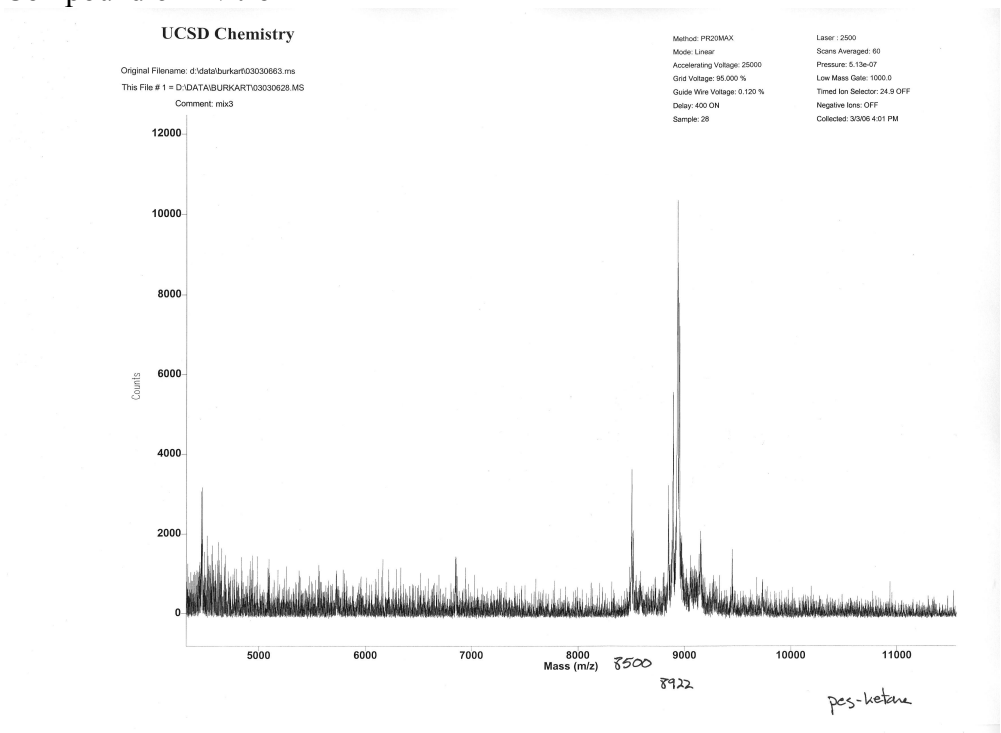
Compound **5** In vivo



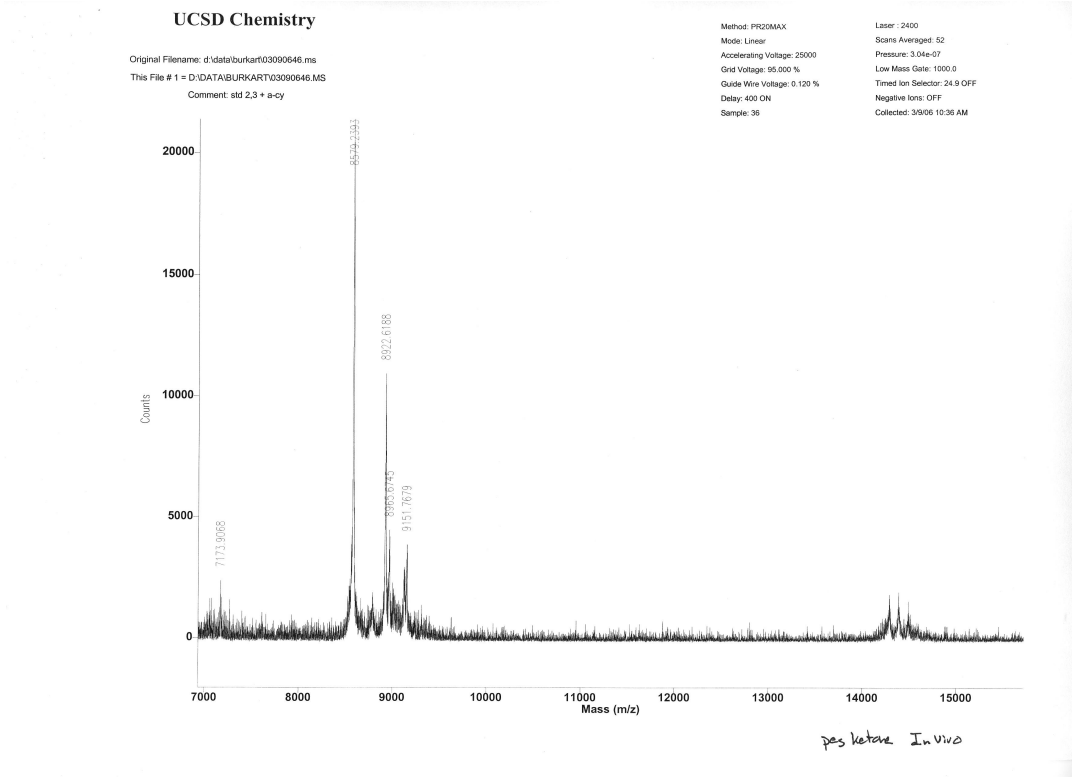
Compound **5** In vivo after click reaction with **10**



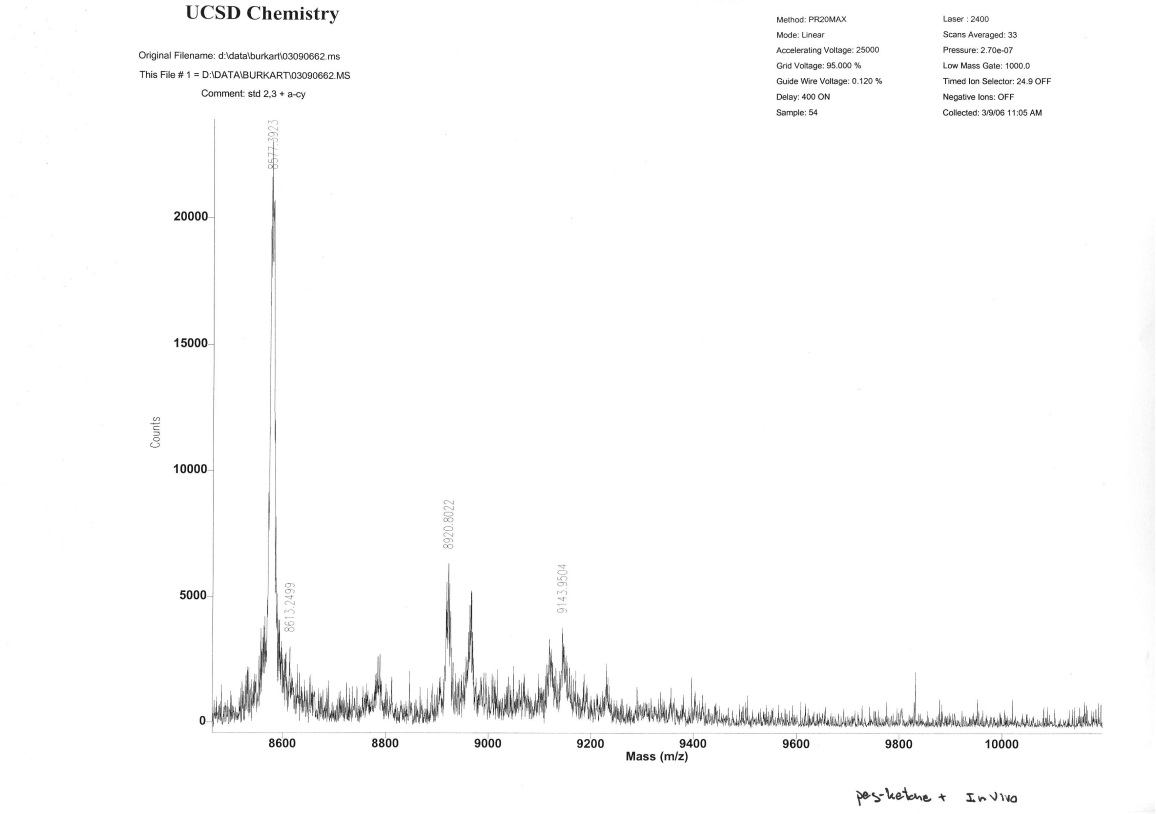
Compound **6** In vitro



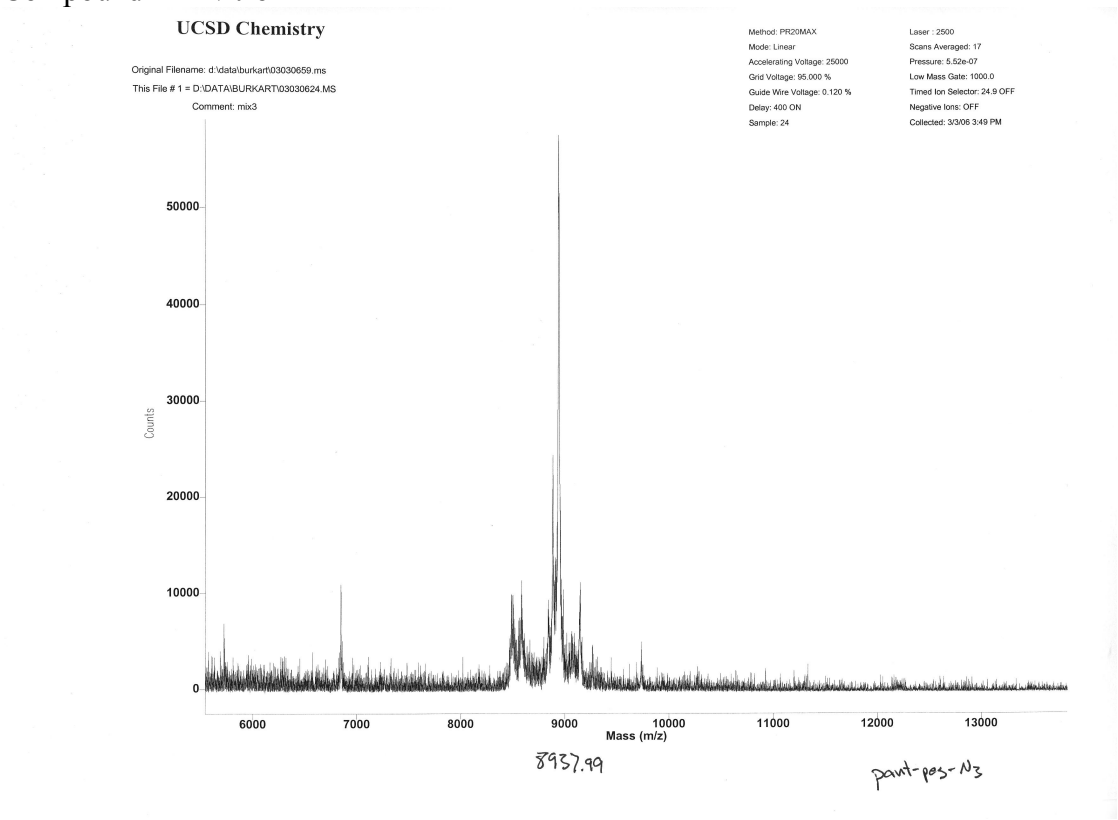
Compound **6** In vivo



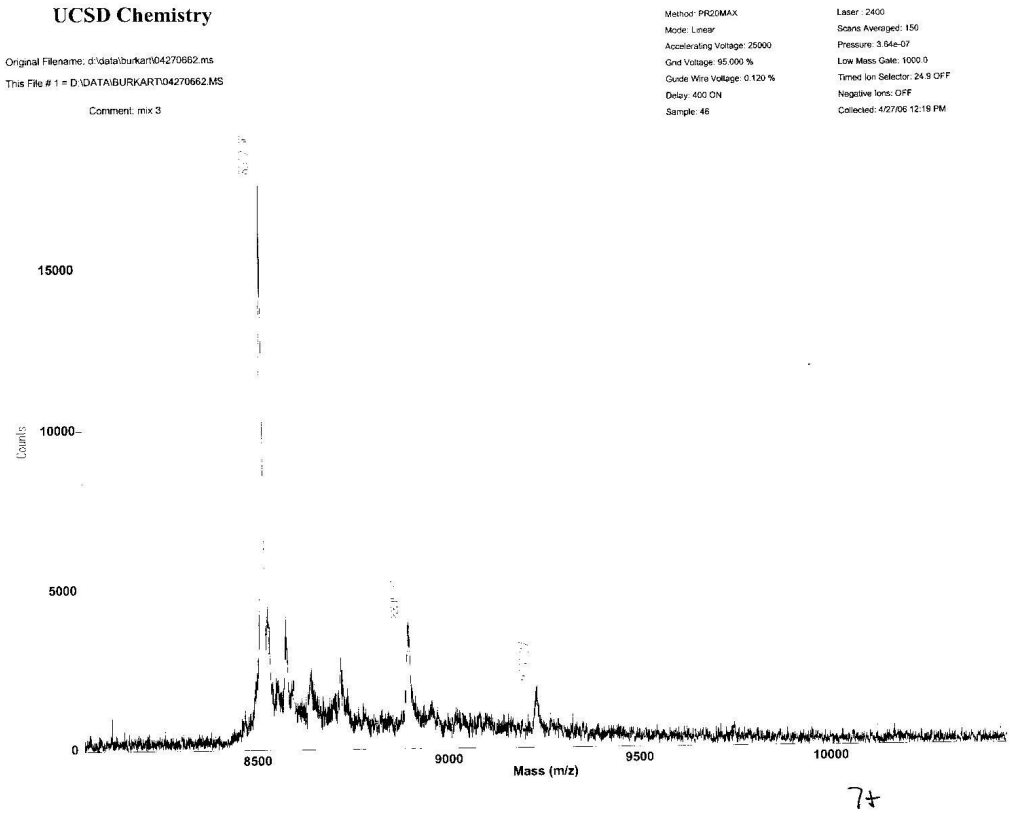
Compound **6** In vivo after reaction with **9**



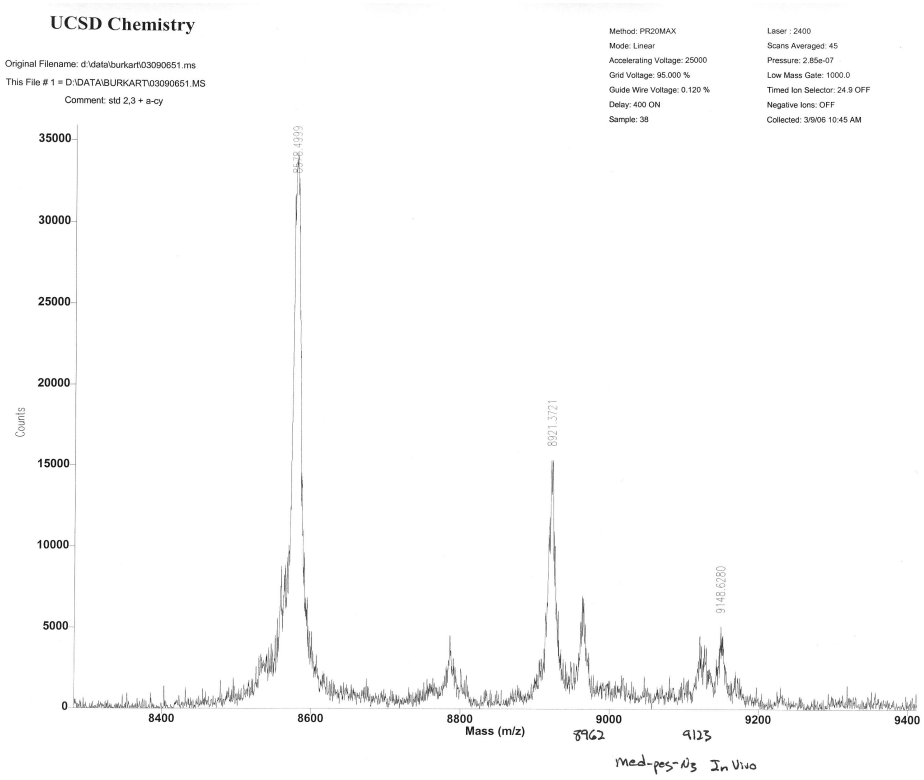
Compound **7** In vitro



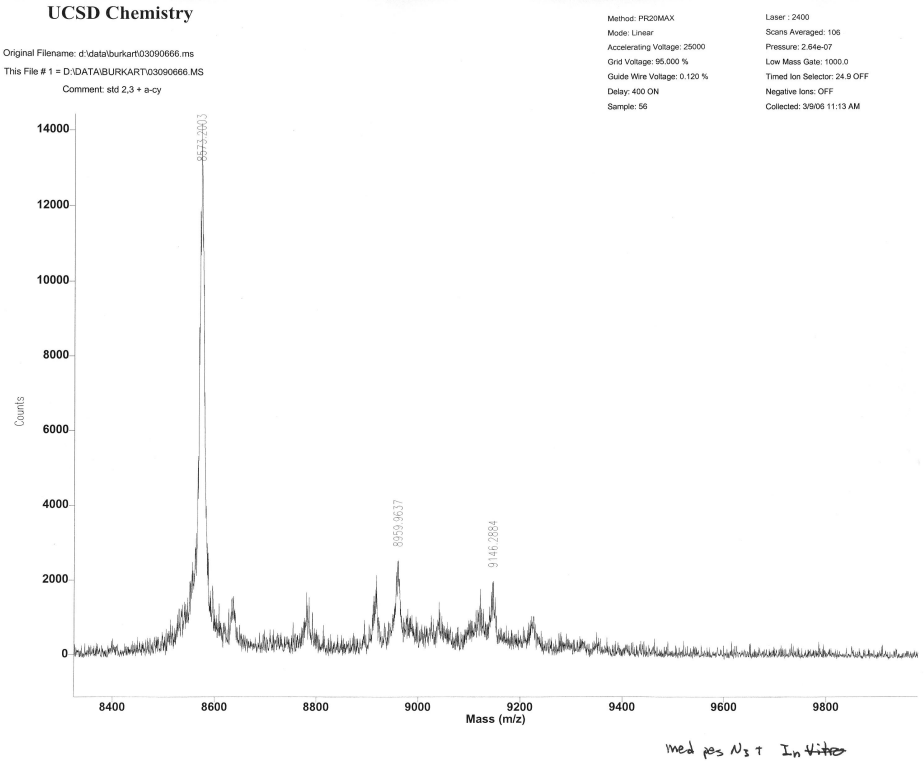
Compound **7** In vitro after click reaction with **10**



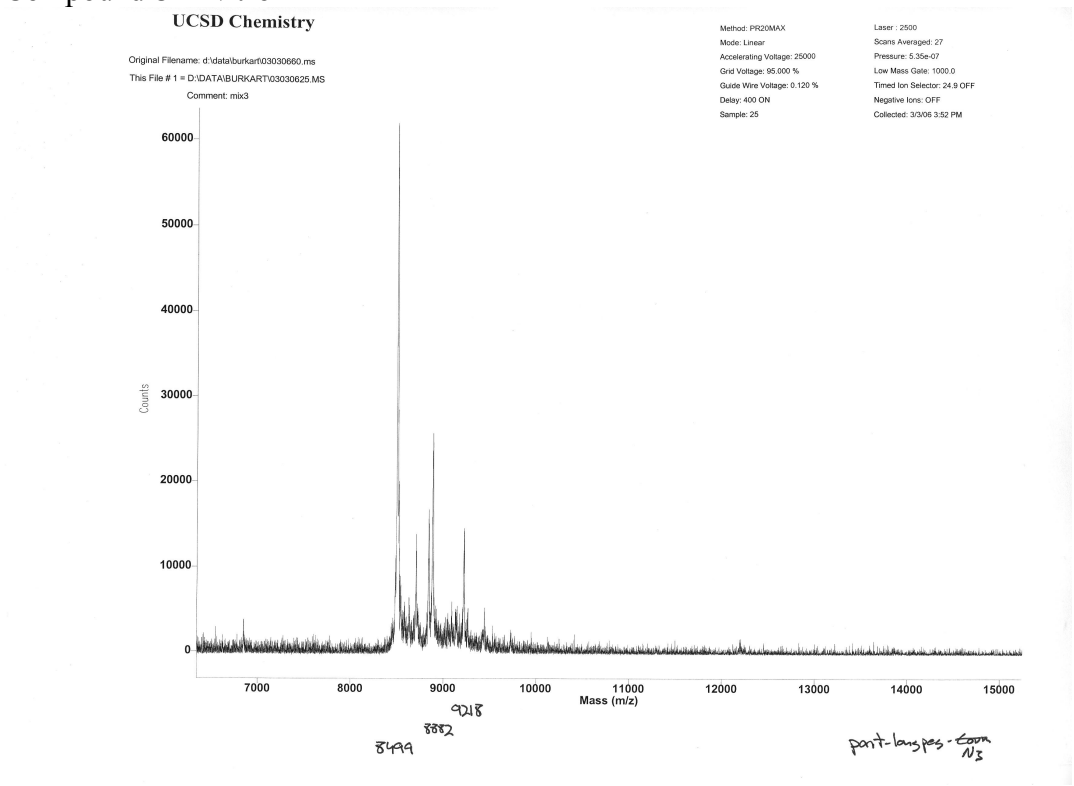
Compound **7** In vivo



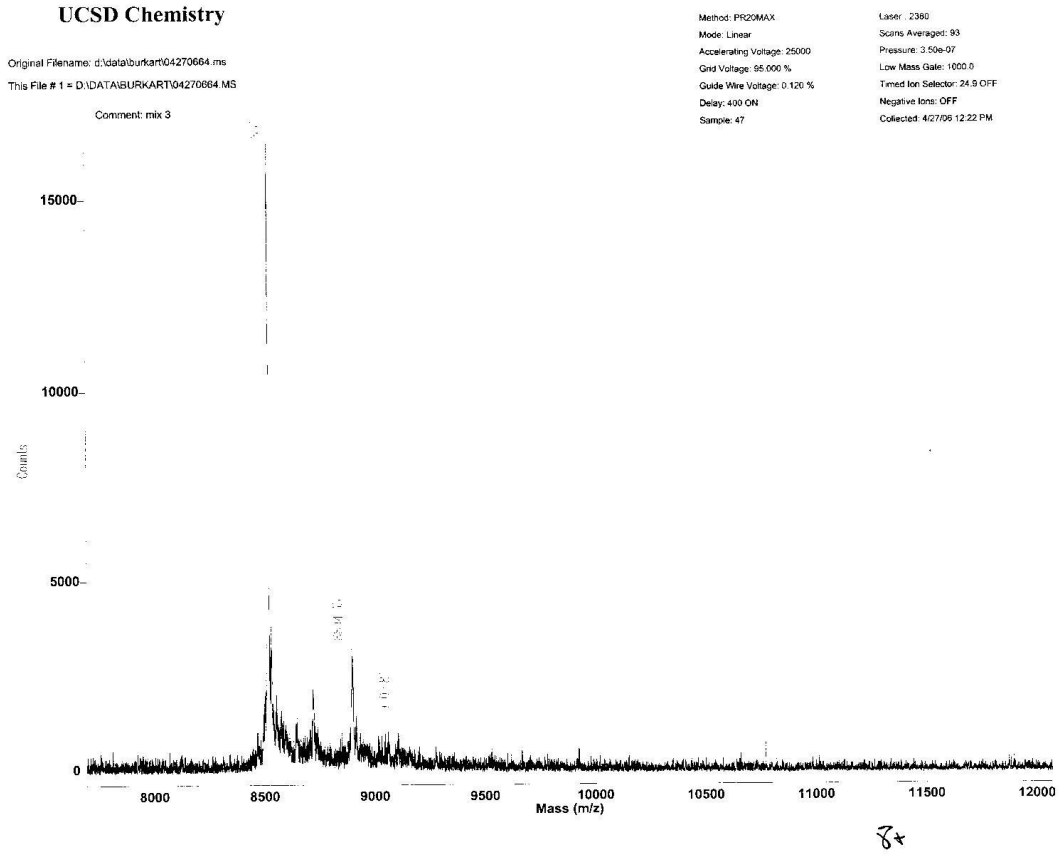
Compound **7** In vivo after click reaction with **10**



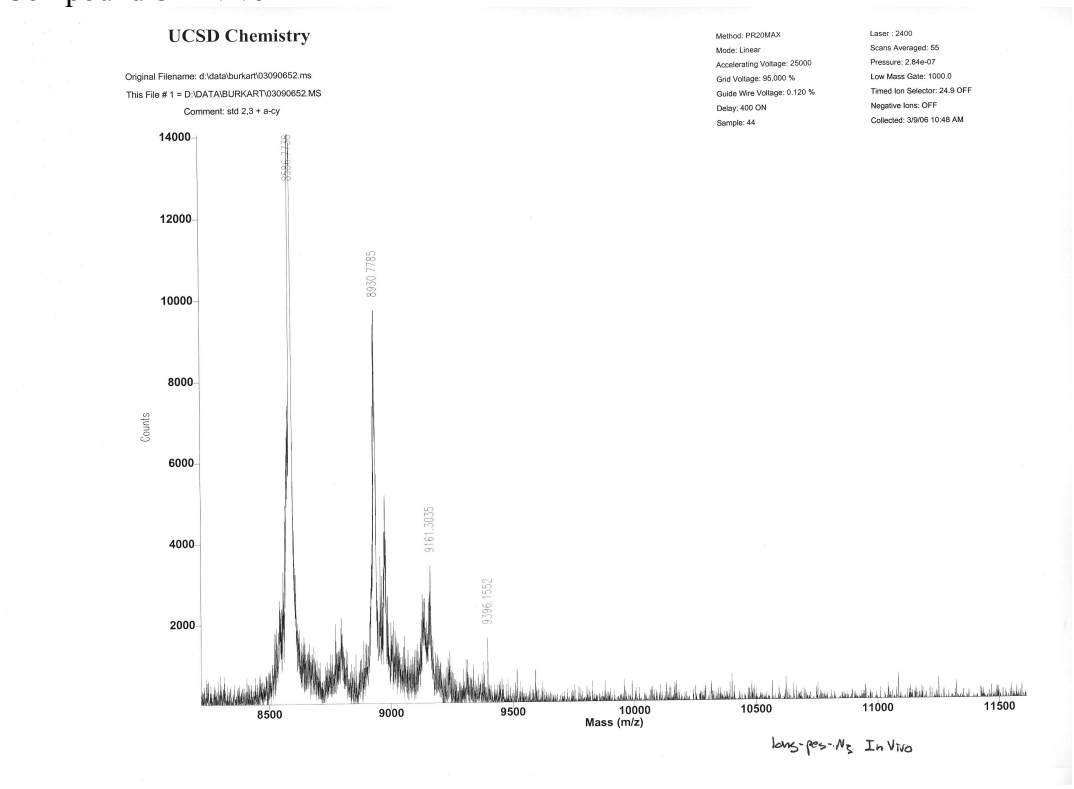
Compound **8** In vitro



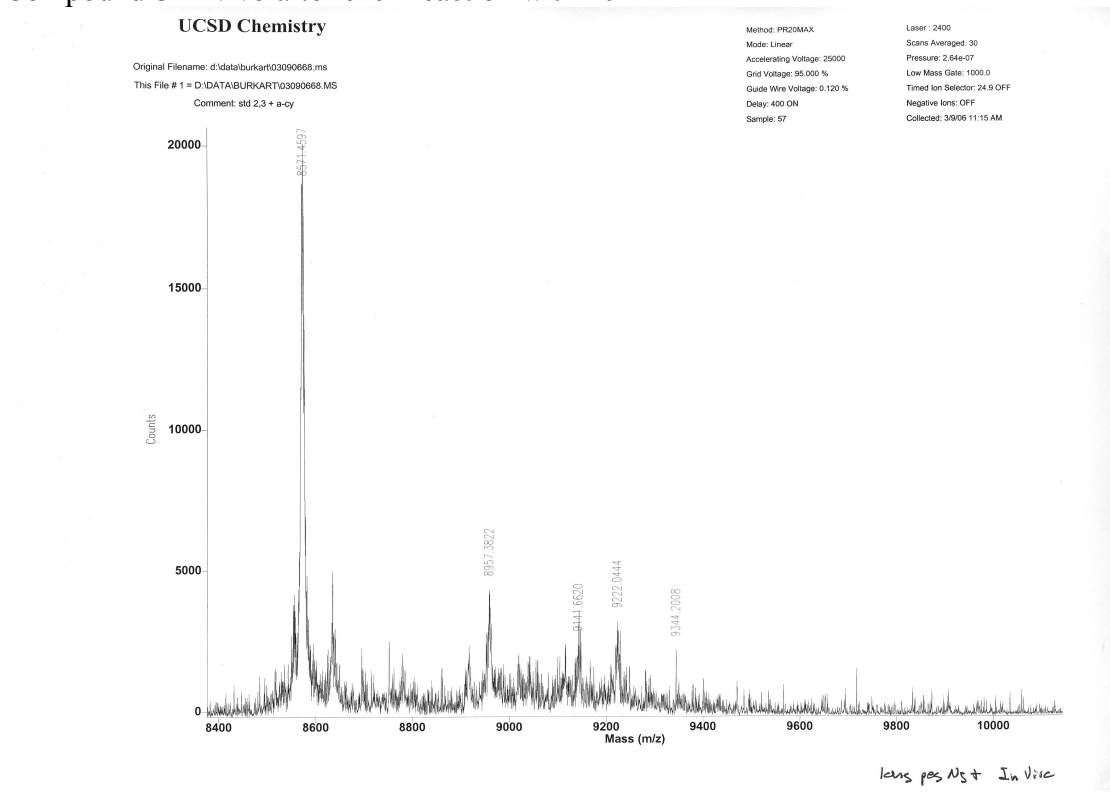
Compound **8** In vitro after click reaction with **10**



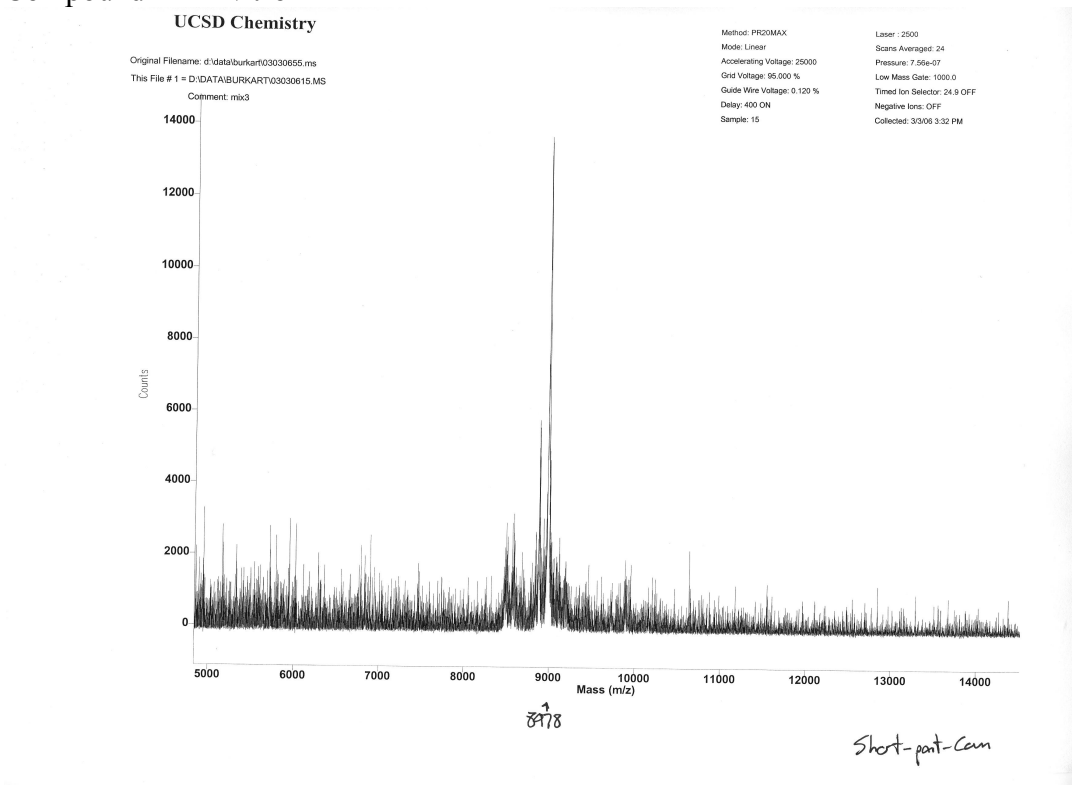
Compound **8** In vivo



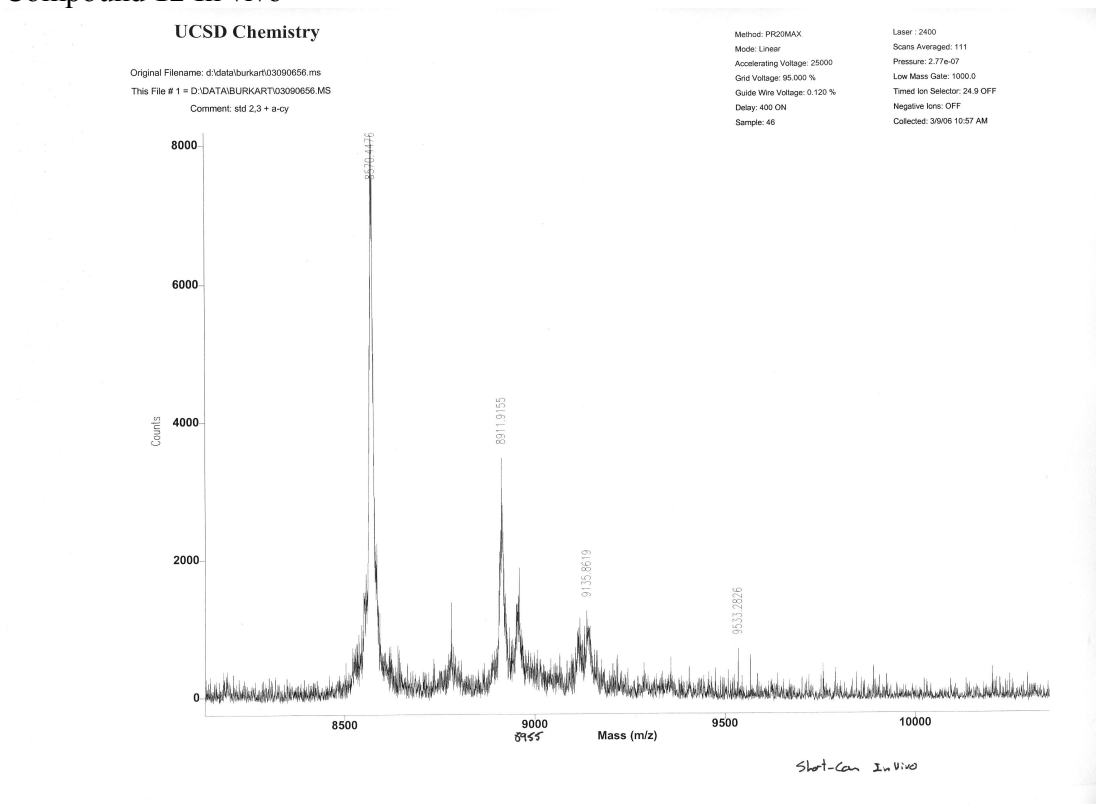
Compound **8** In vivo after click reaction with **10**



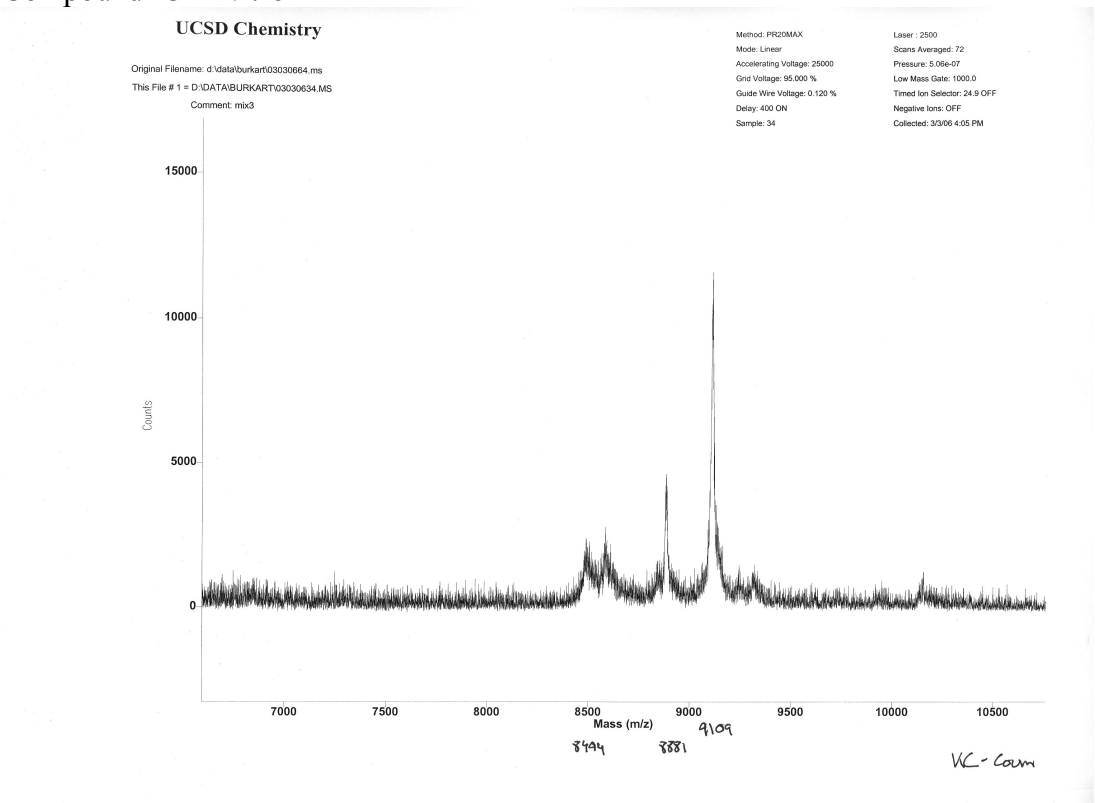
Compound **12** In vitro



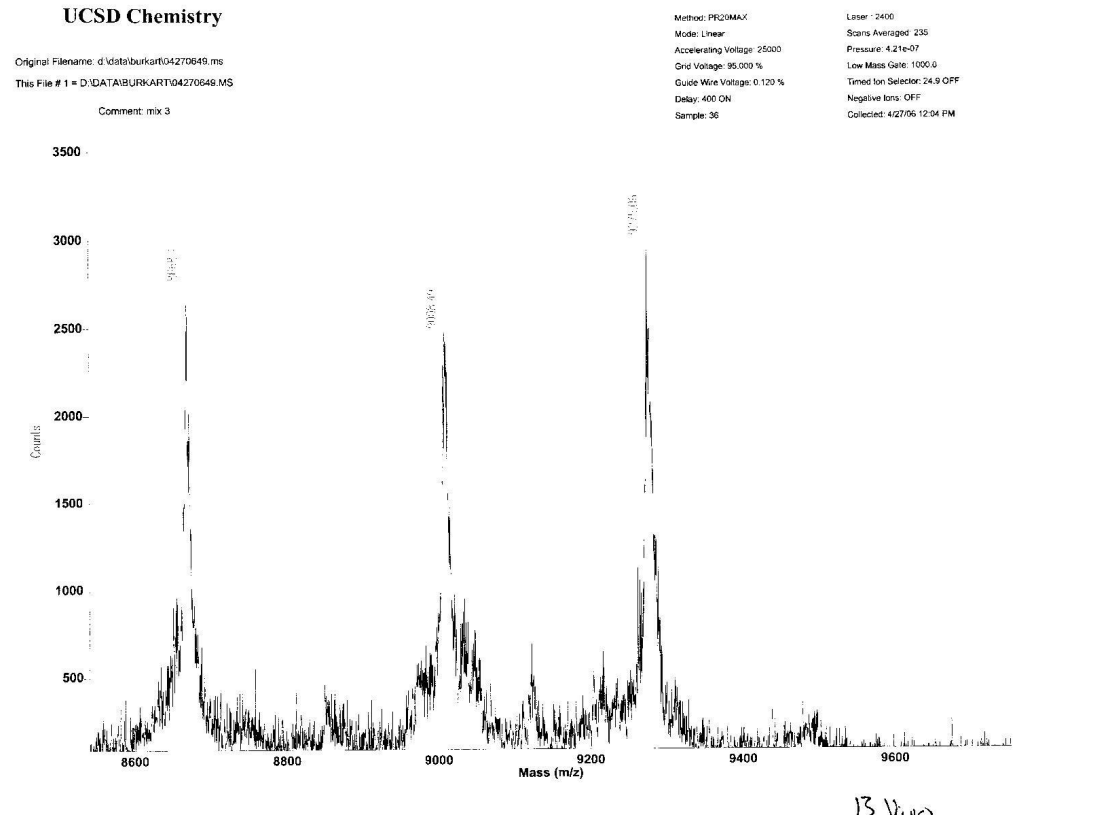
Compound **12** In vivo



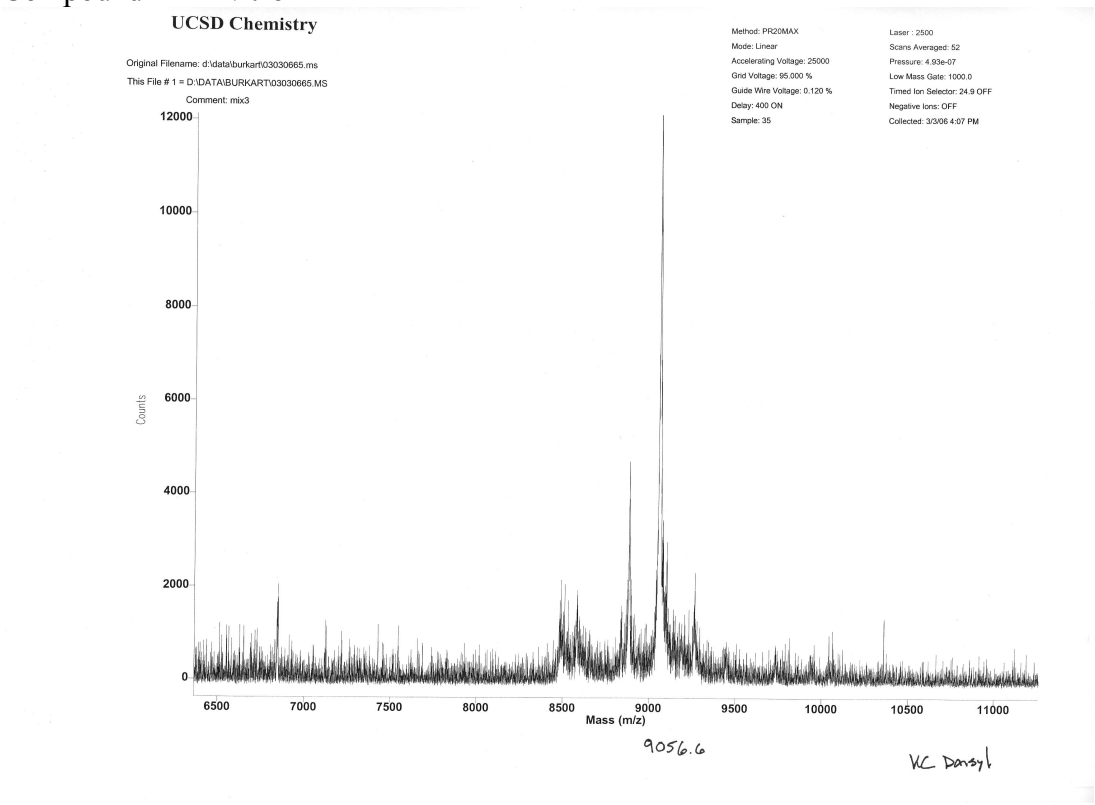
Compound **13** In vitro



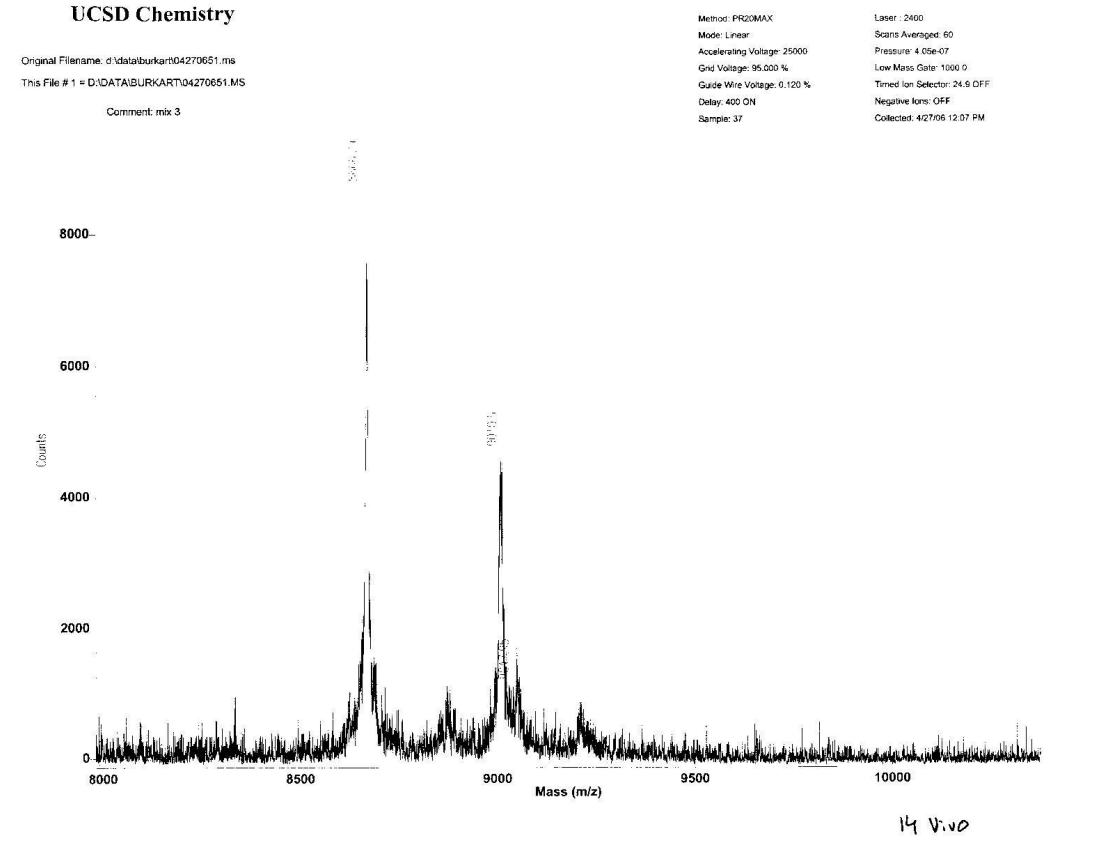
Compound **13** In vivo



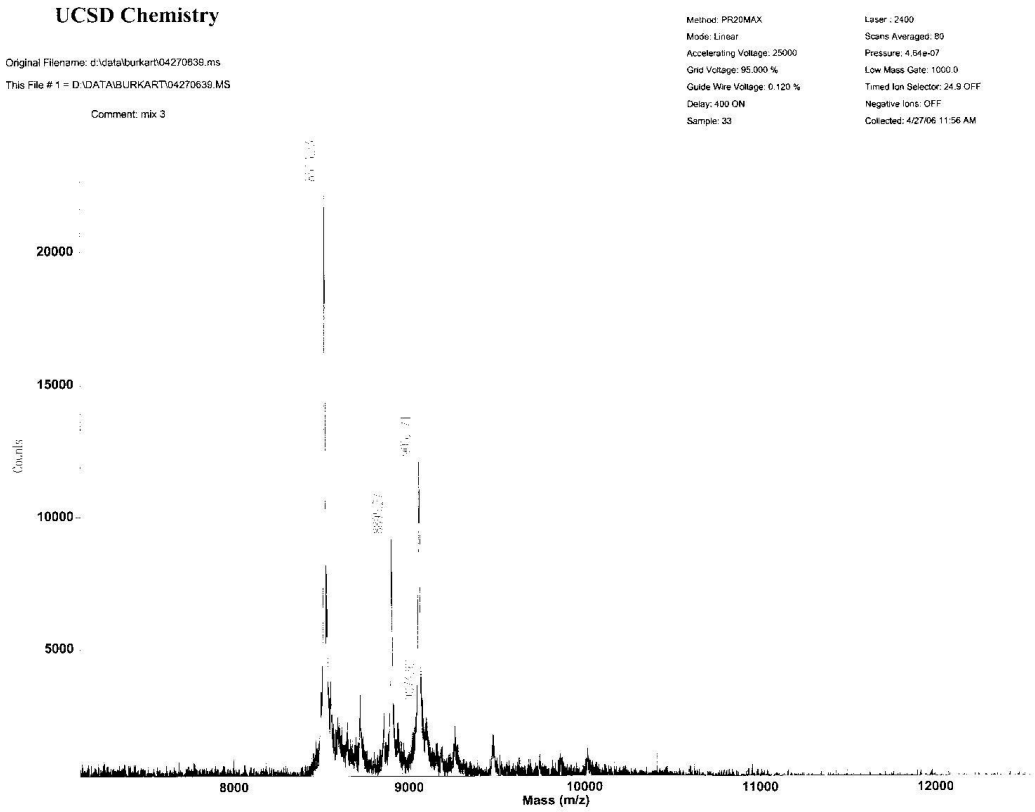
Compound **14** In vitro



Compound **14** In vivo

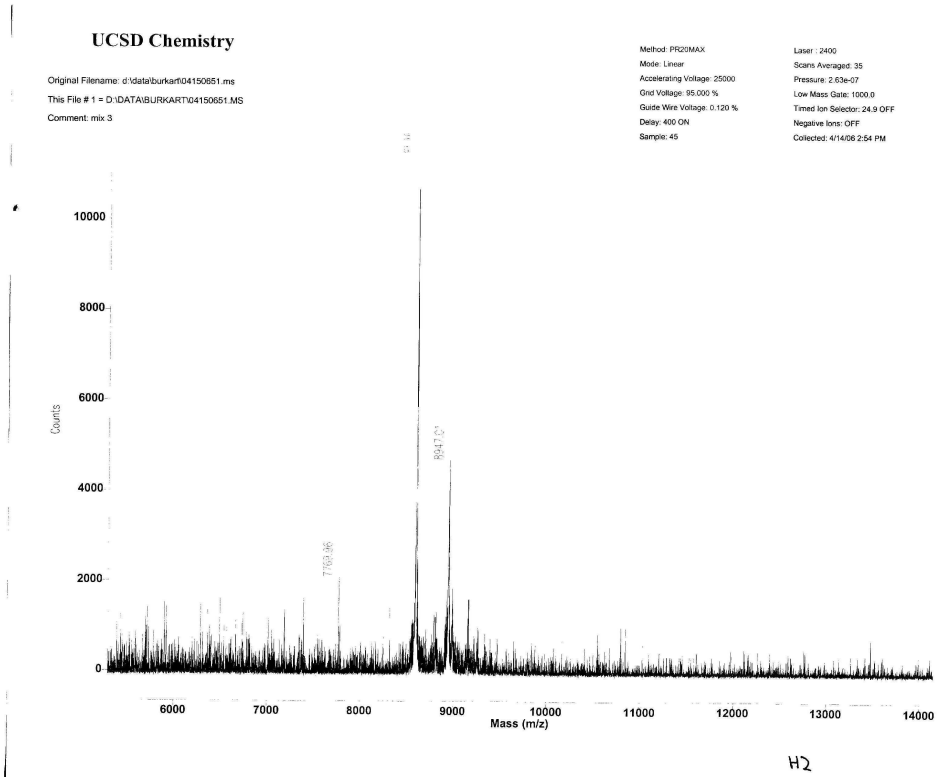


Compound **15** In vitro



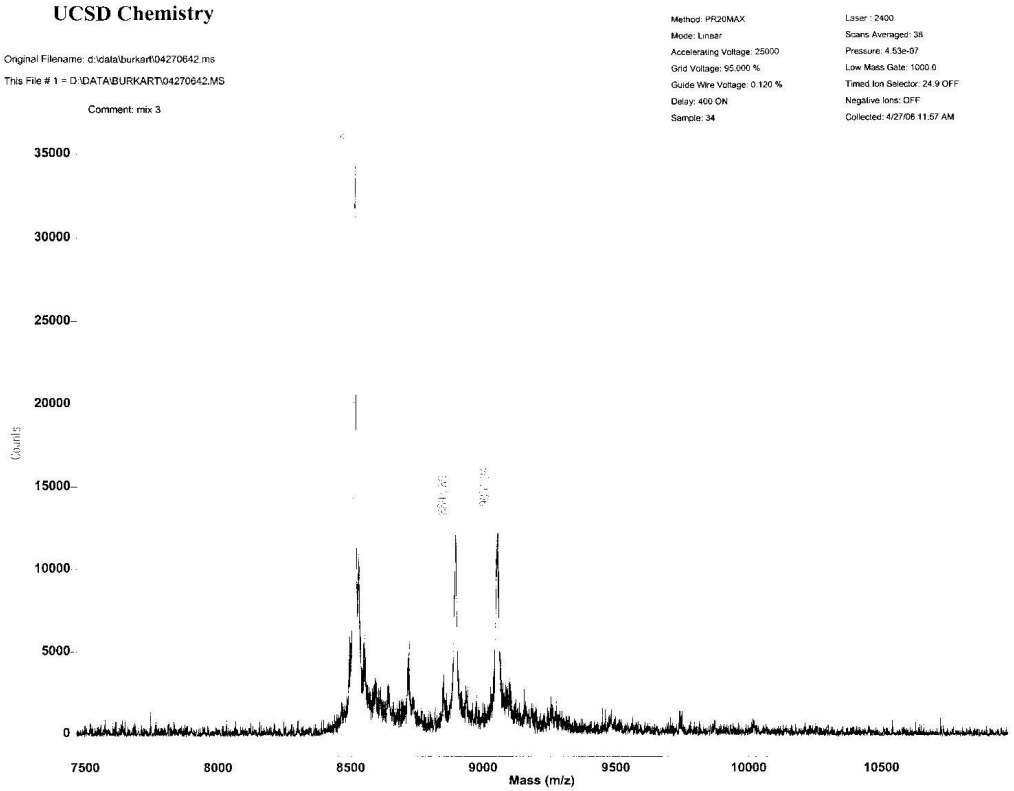
15

Compound **15** In vivo



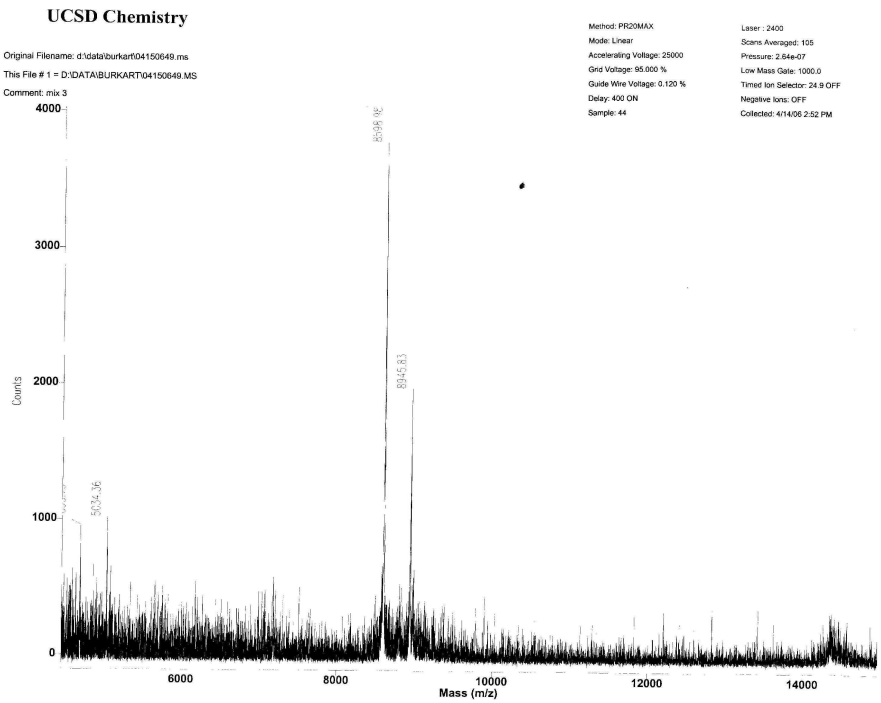
H2

Compound **16** In vitro



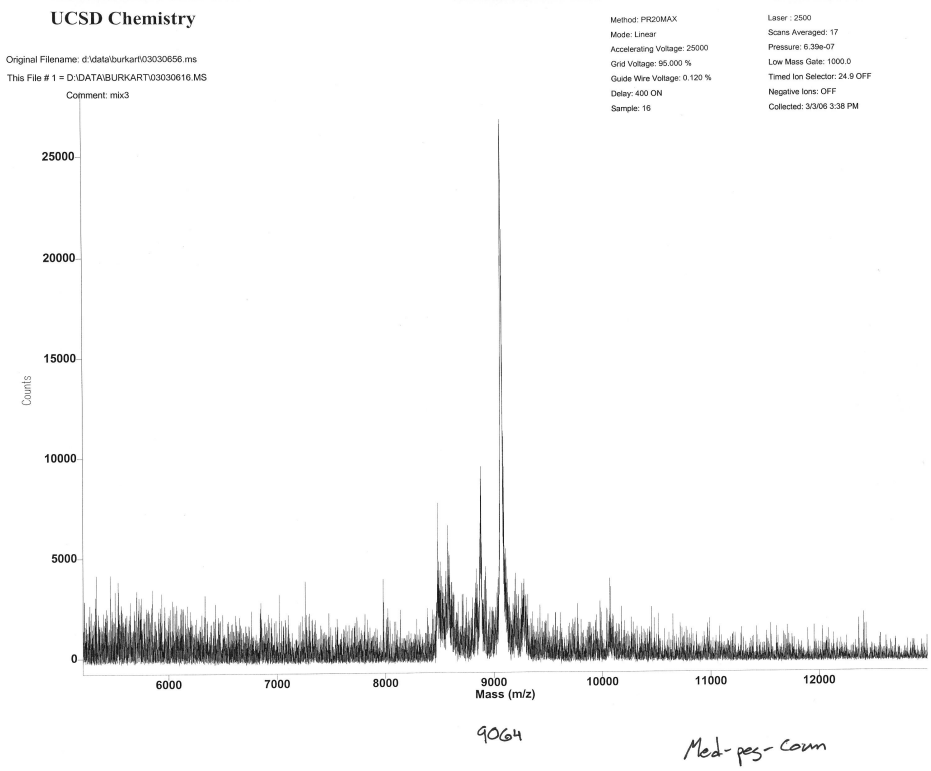
16

Compound **16** In vivo

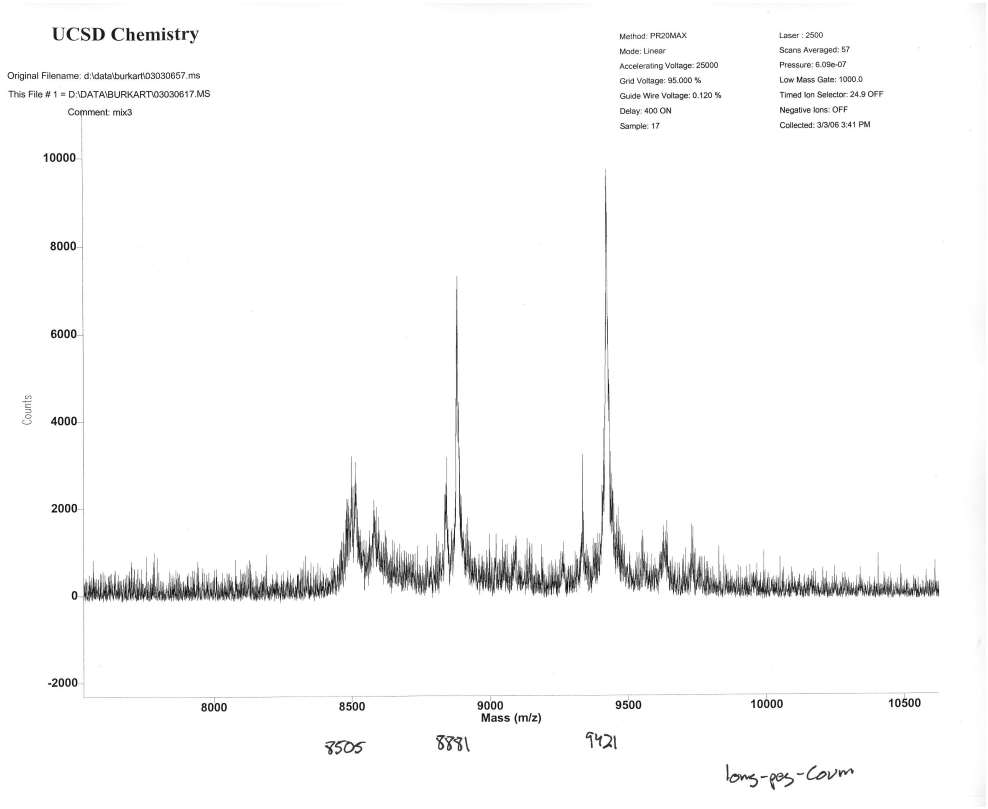


H1

Compound **17** In vitro



Compound **18** In vitro

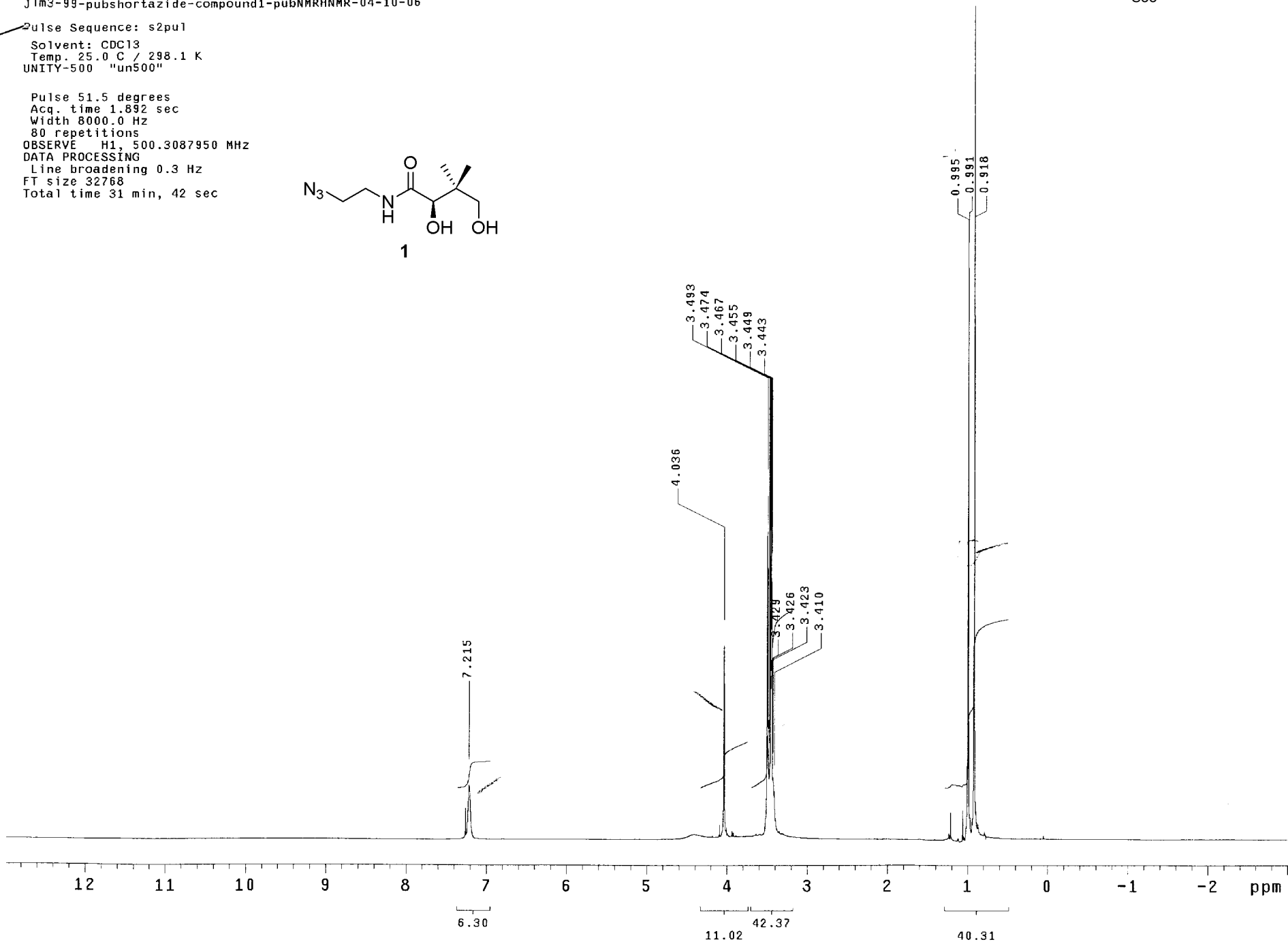


jlm3-99-pubshortazide-compound1-pubNMRHNMR-04-10-06

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

Pulse 51.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
80 repetitions
OBSERVE H1, 500.3087950 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec

1

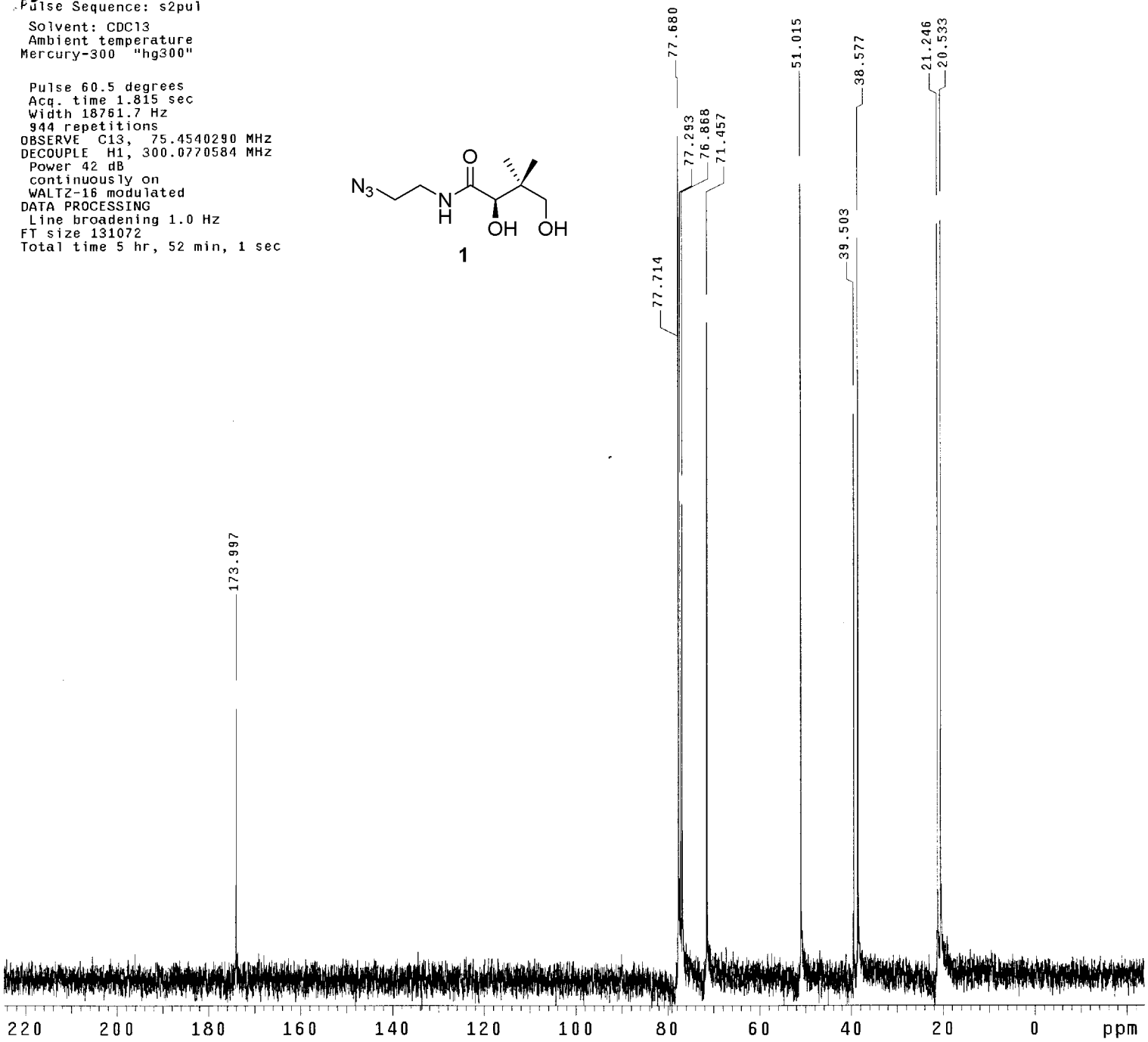


jlm3-99-monoazidopantoicacid-pubqualityCNMR-04-10-06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
944 repetitions
OBSERVE C13, 75.4540290 MHz
DECOUPLE H1, 300.0770584 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 5 hr, 52 min, 1 sec

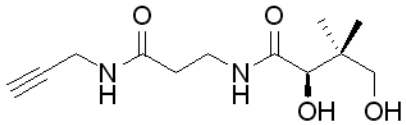
1



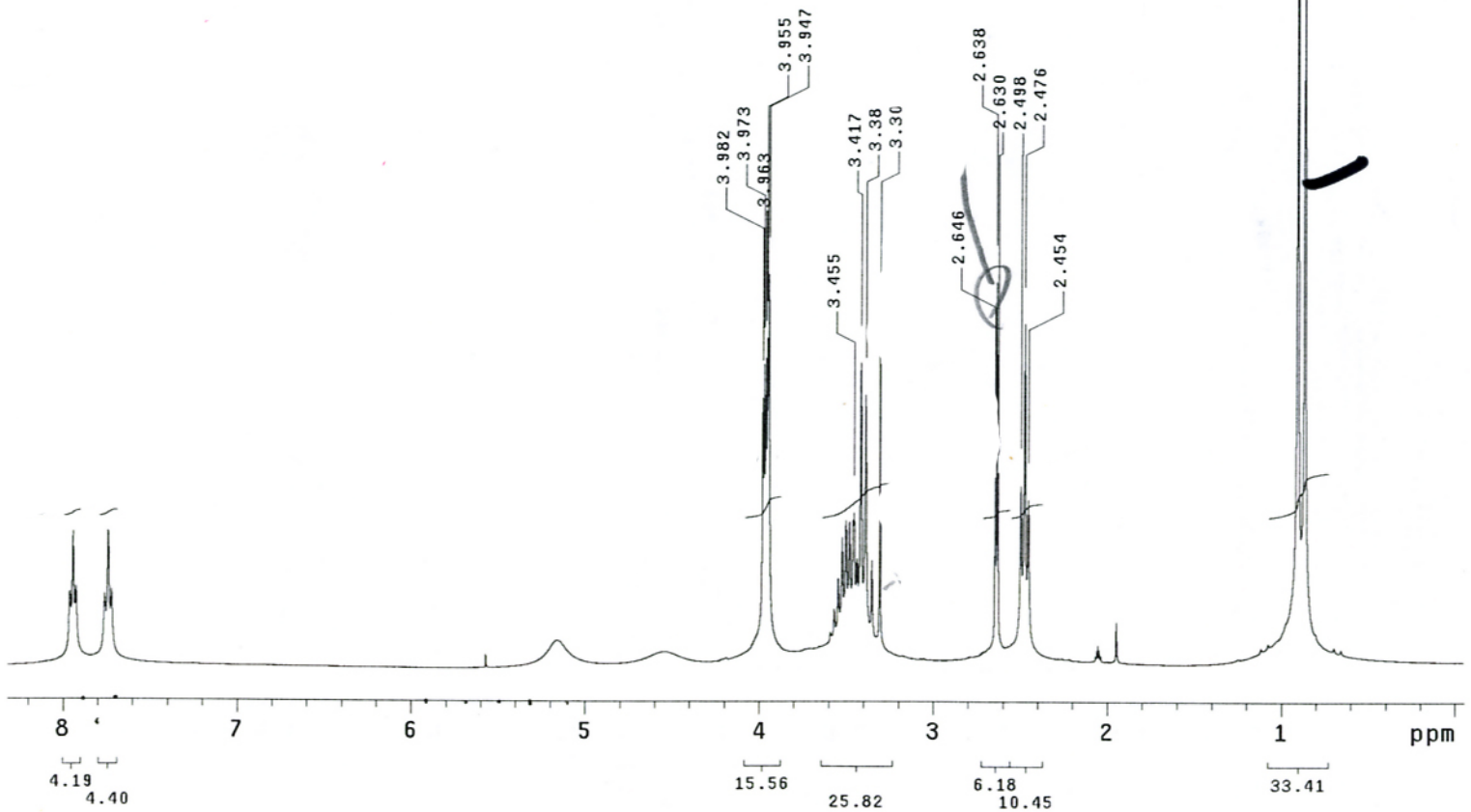
mdb3-5-propargylaminepantothenicacid-lookingforconjacetone-02-09-06

Pulse Sequence: s2pul
Solvent: Acetone
Ambient temperature
Mercury-300 "hg300"

Relax. delay 1.000 sec
Pulse 12.9 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
40 repetitions
OBSERVE H1, 300.0770676 MHz
DATA PROCESSING
FT size 32768
Total time 53 min, 11 sec



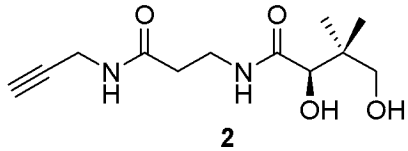
2



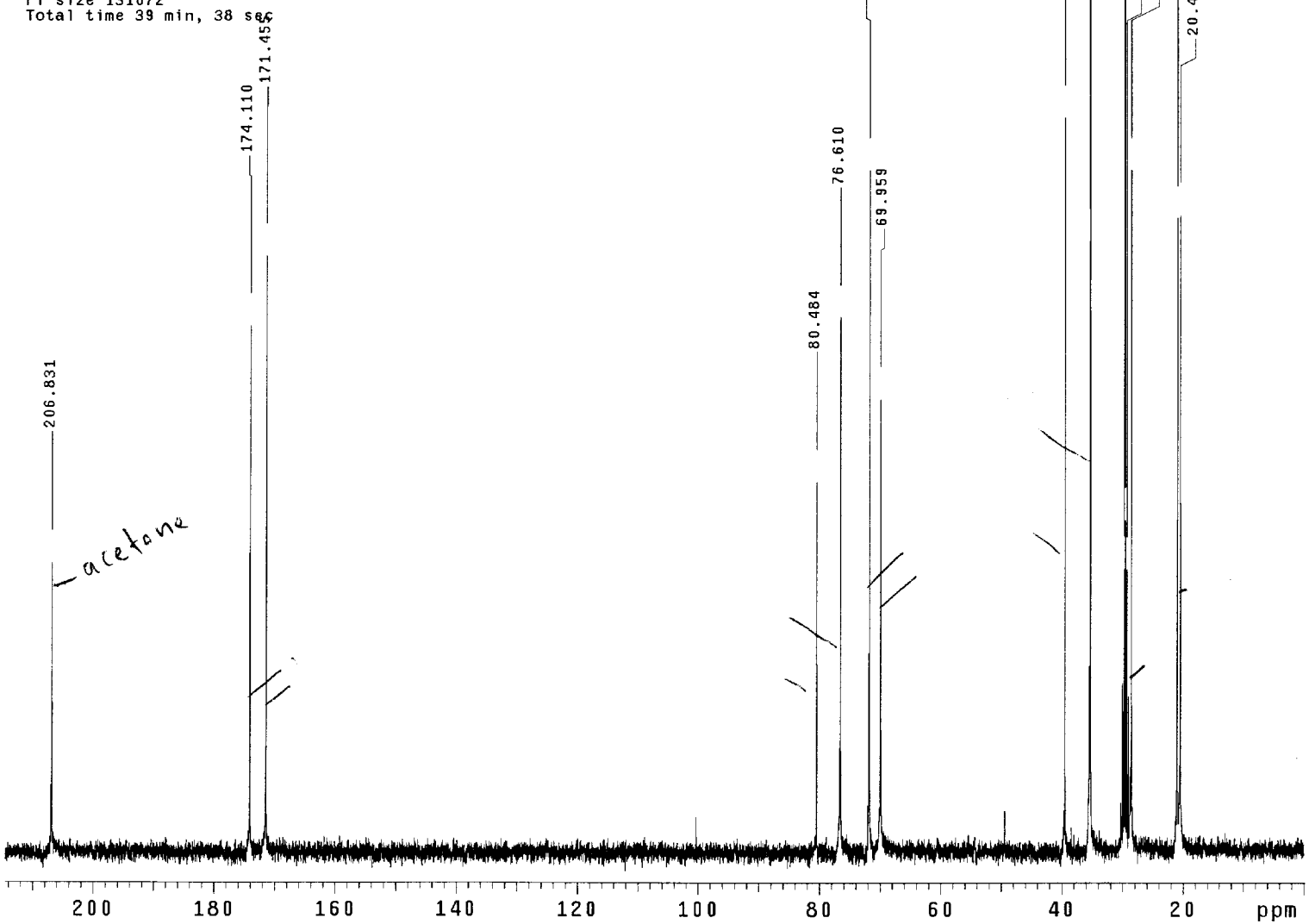
mdb3-5-pantotheinicacid-propargylamineconjugate-deprotected-acetoneCNMR-02-09-06

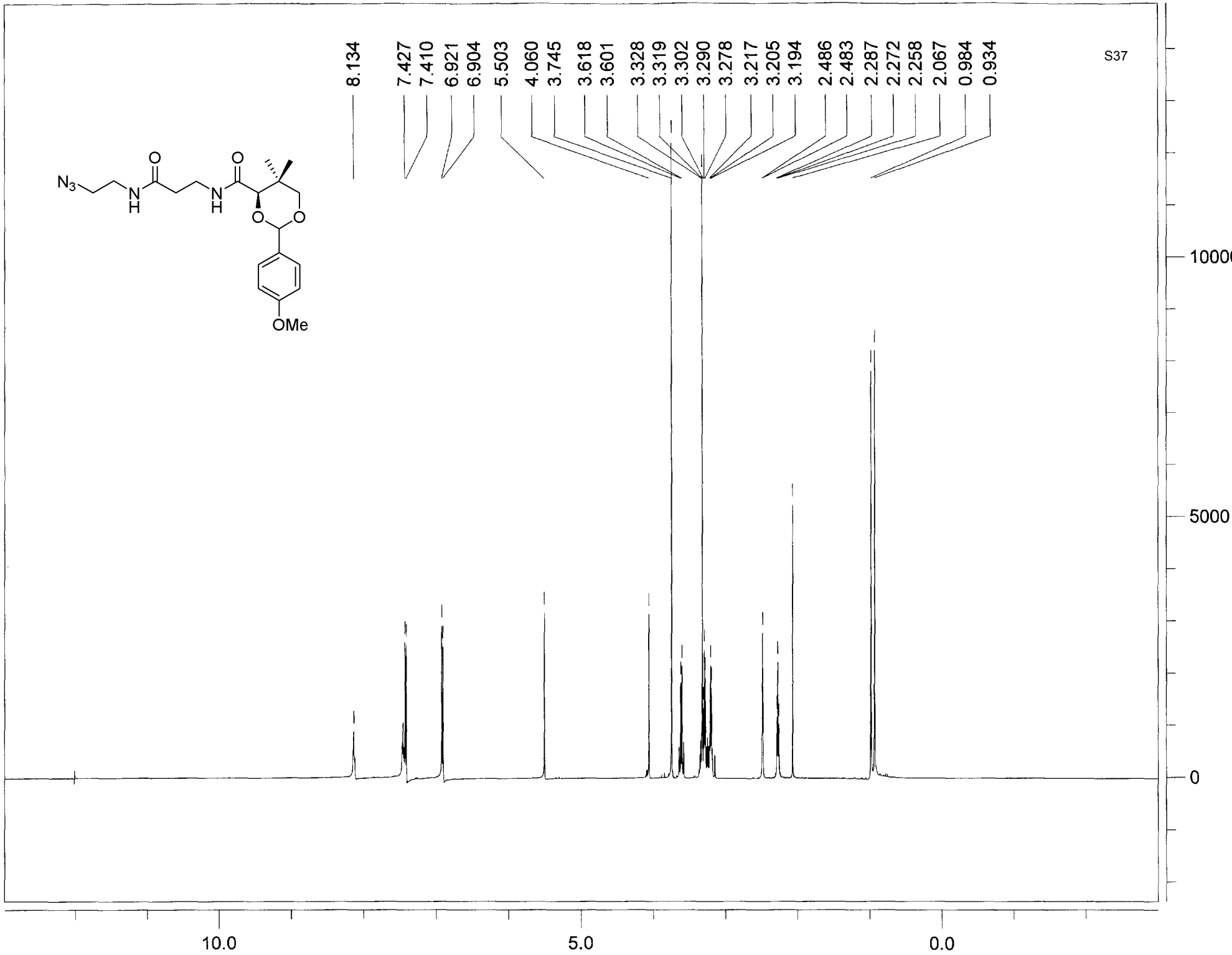
Pulse Sequence: s2pul
Solvent: Acetone
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
120 repetitions
OBSERVE C13, 75.4544206 MHz
DECOUPLE H1, 300.0786158 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 39 min, 38 sec



2

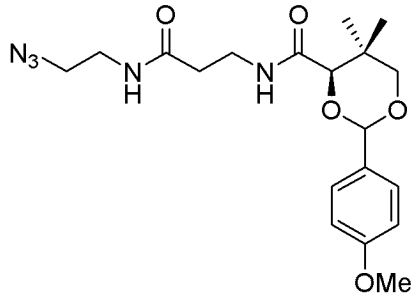


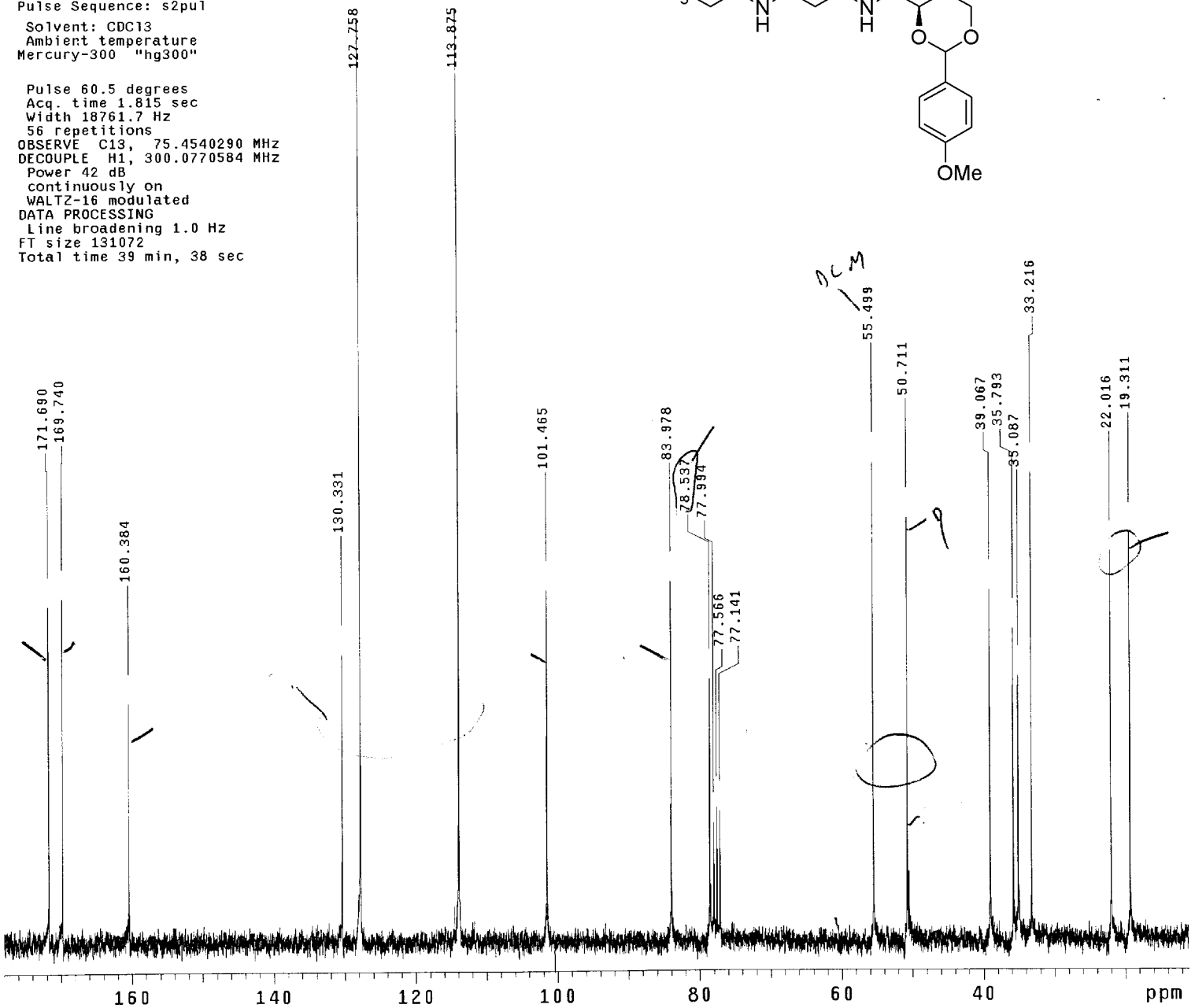

8.134
7.427
7.410
6.921
6.904
5.503
4.060
3.745
3.618
3.601
3.328
3.319
3.302
3.290
3.278
3.217
3.205
3.194
2.486
2.483
2.287
2.272
2.258
2.067
0.984
0.934
S37
N3
N
H
O
N
H
O
O
O
OMe
10000
5000
0
10.0
5.0
0.0

jlm3-61-monoazidopantothenicacid-PMPprotected-CNMRforpub-03-25-06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
56 repetitions
OBSERVE C13, 75.4540290 MHz
DECOUPLE H1, 300.0770584 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 39 min, 38 sec



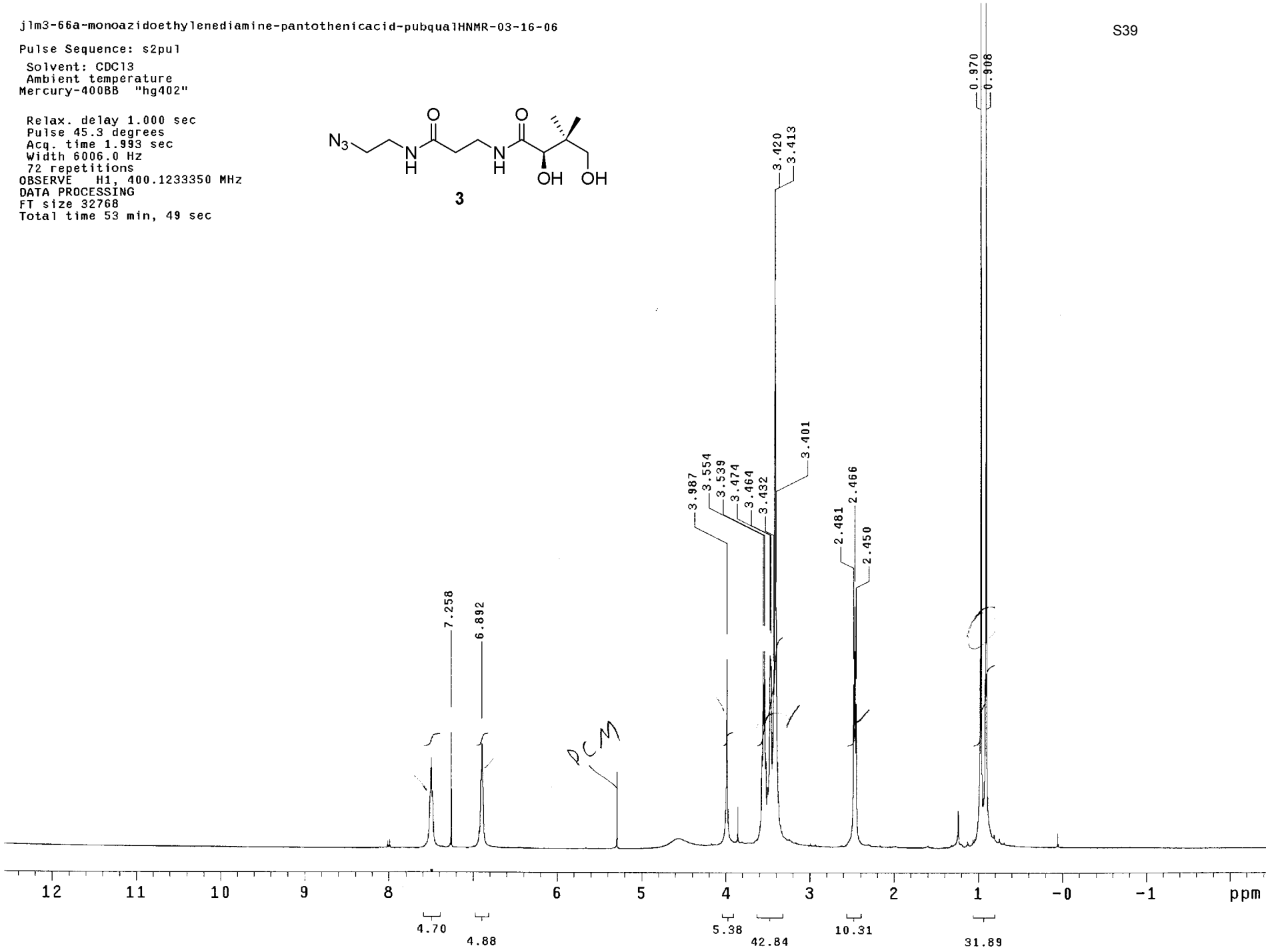


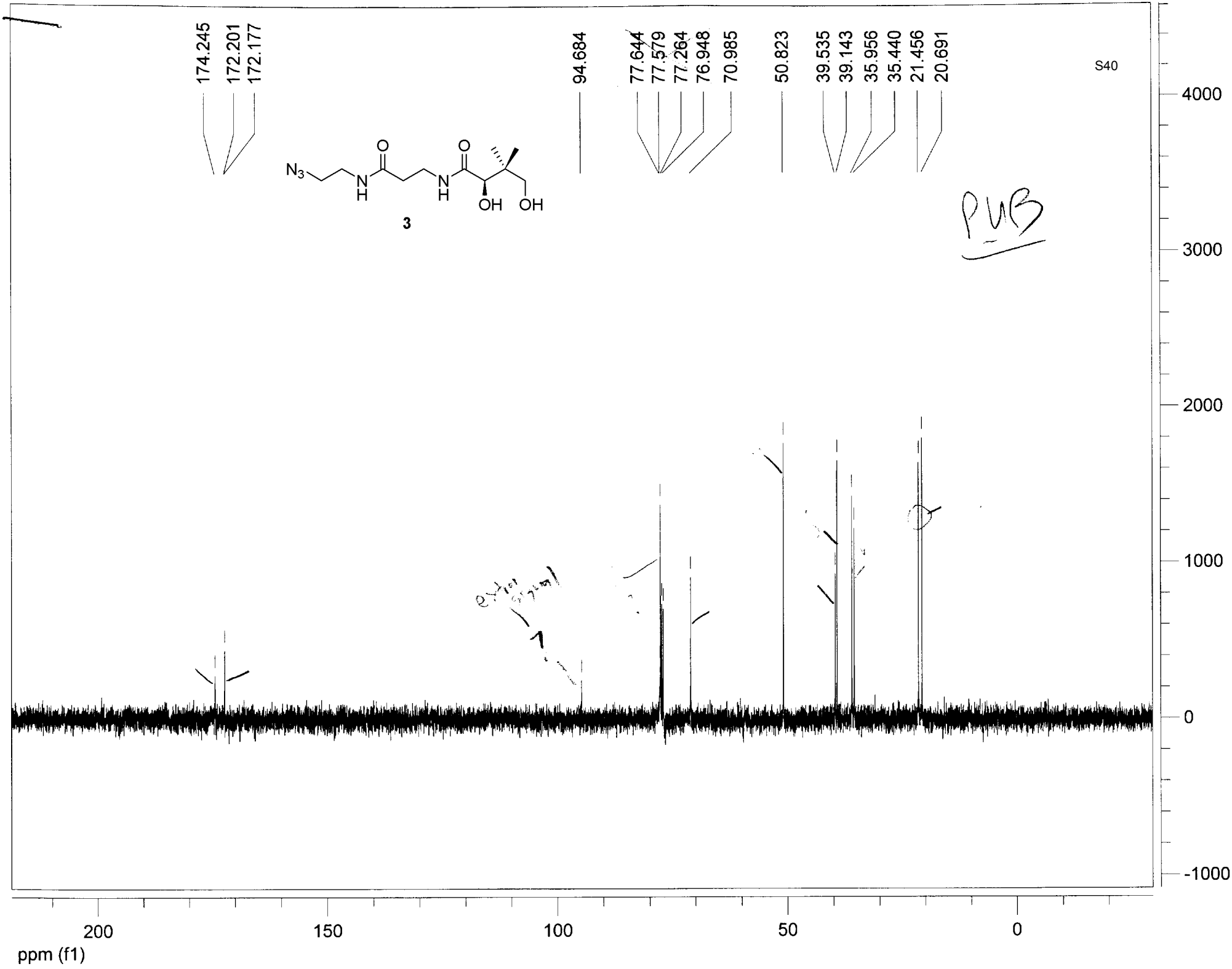
jlm3-66a-monoazidoethylenediamine-pantothenicacid-pubqualHNMR-03-16-06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "hg402"

Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
72 repetitions
OBSERVE H1, 400.1233350 MHz
DATA PROCESSING
FT size 32768
Total time 53 min, 49 sec

3

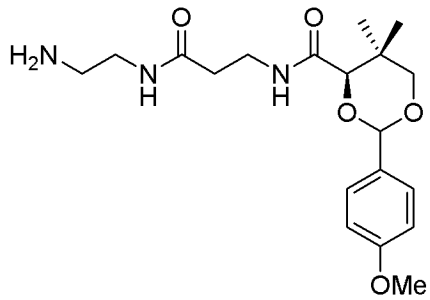


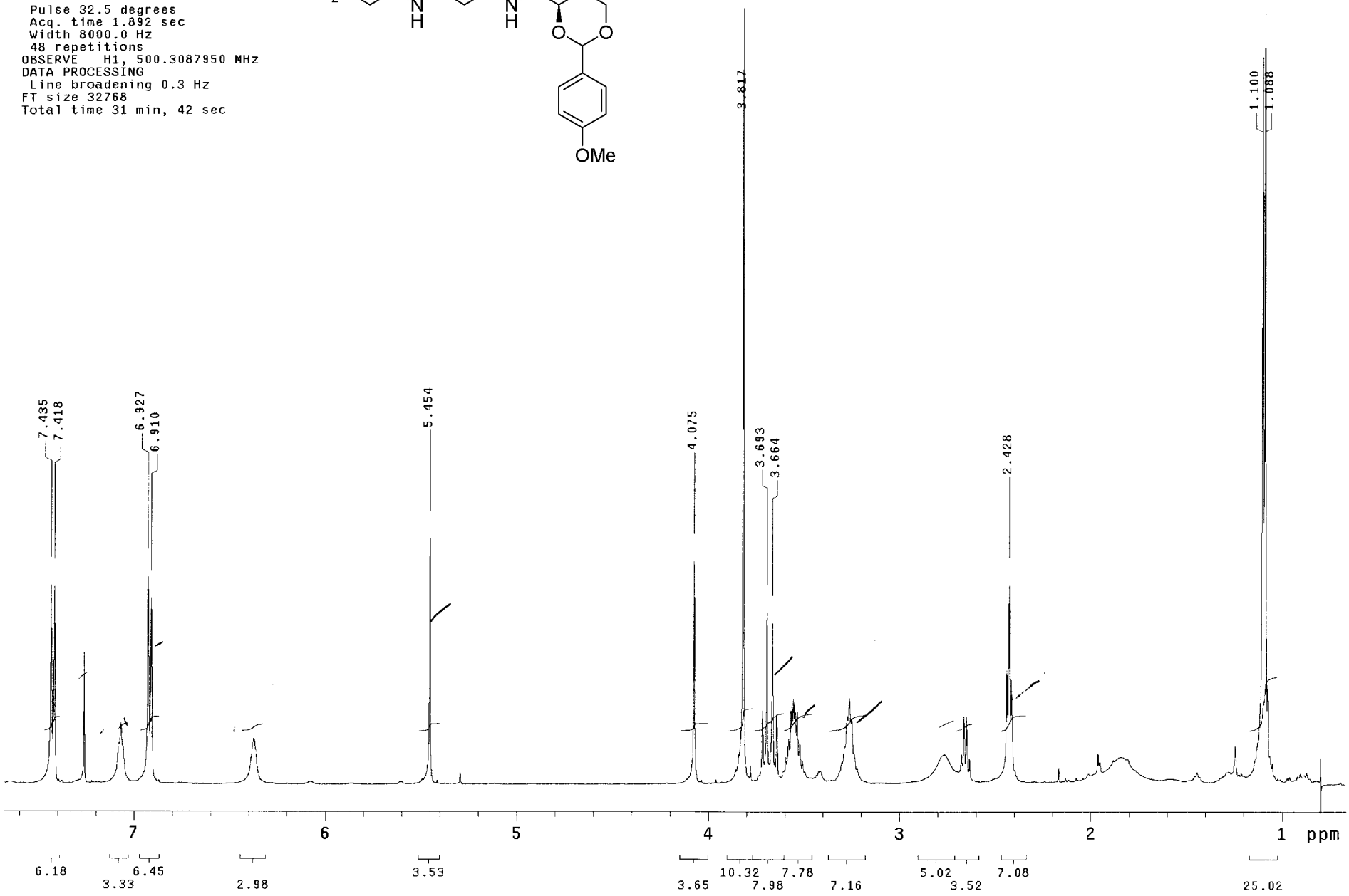

174.245
172.201
172.177
94.684
77.644
77.579
77.264
76.948
70.985
50.823
39.535
39.143
35.956
35.440
21.456
20.691
N3
N
H
O
N
H
O
OH
OH
3
PUB
4000
3000
2000
1000
0
-1000
200
150
100
50
0
ppm (f1)

jlm-19-pantotheinicacid-ethylenediamineconj-freeNH2-12-12-05

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

Pulse 32.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
48 repetitions
OBSERVE H1, 500.3087950 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec

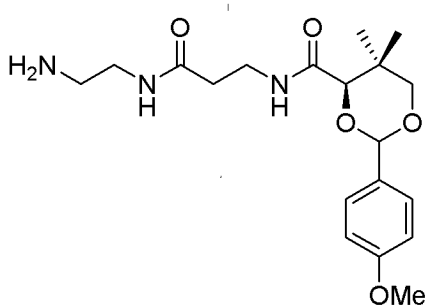


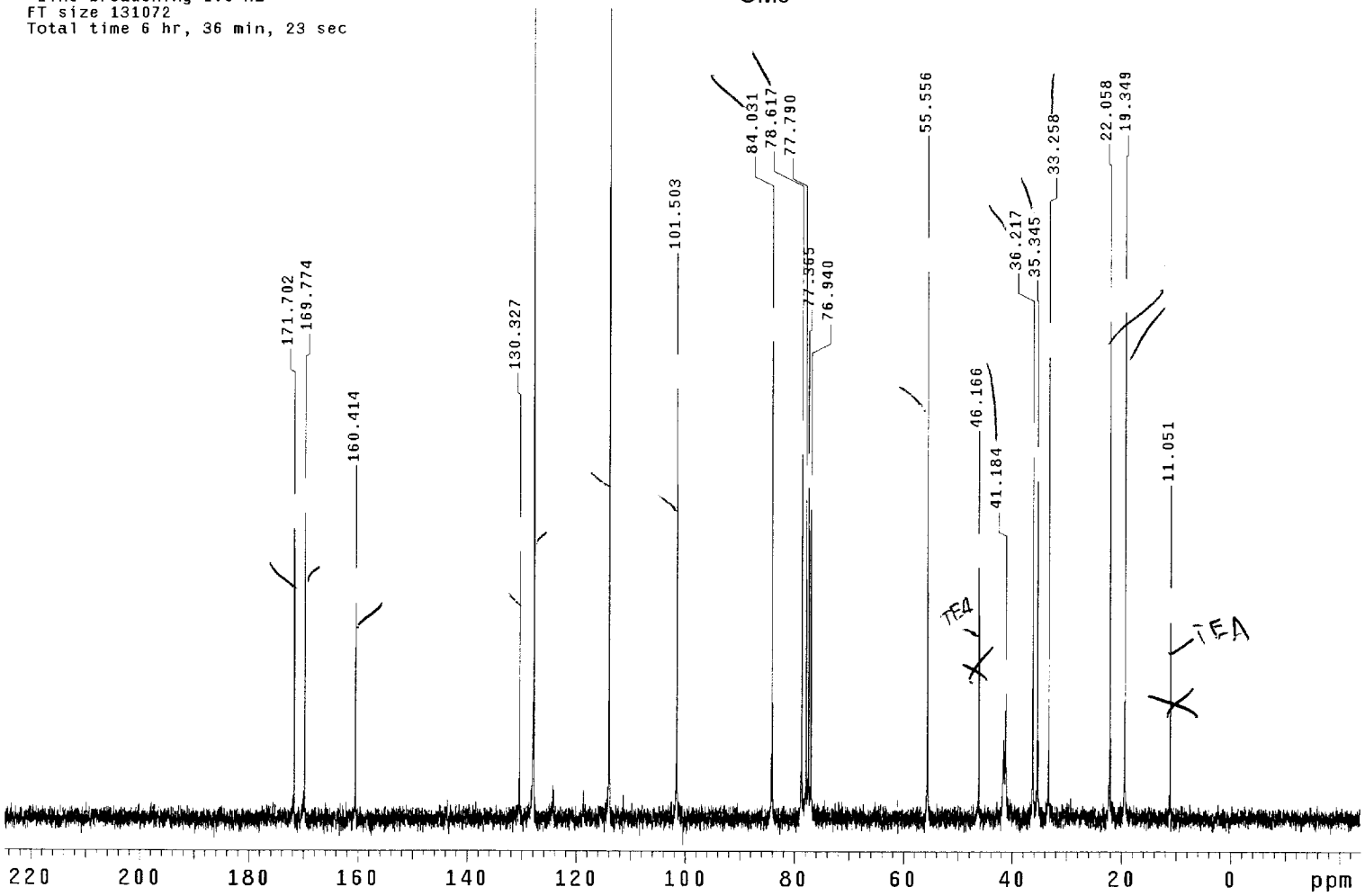


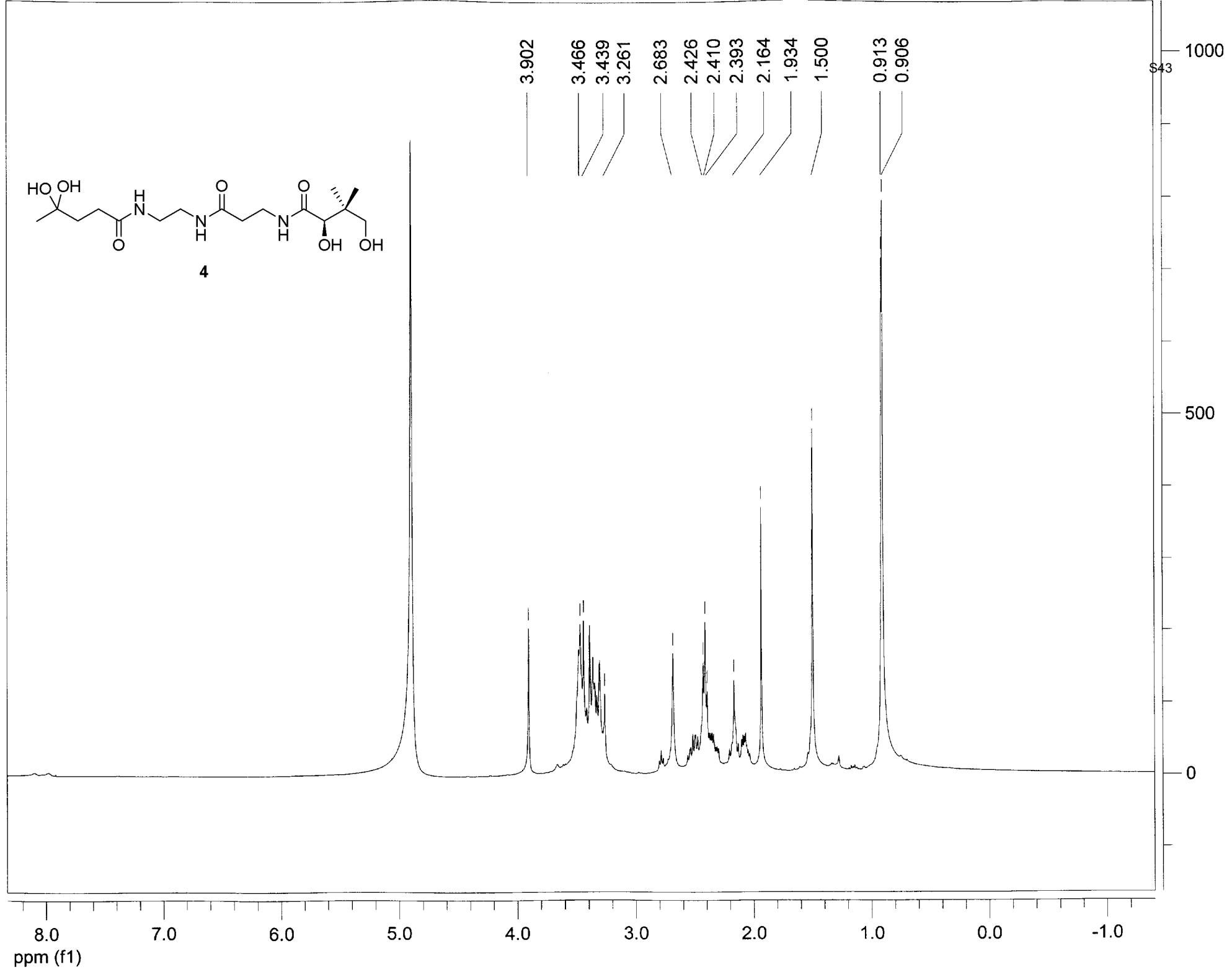
jlm3-66b-monoamino-pantothenicacid-CNMRforpub-03-25-06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
784 repetitions
OBSERVE C13, 75.4540290 MHz
DECOUPLE H1, 300.0770584 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 6 hr, 36 min, 23 sec



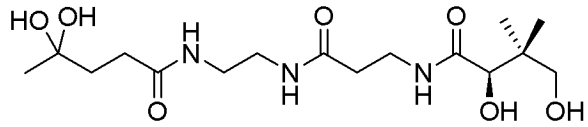



3.902
3.466
3.439
3.261
2.683
2.426
2.410
2.393
2.164
1.934
1.500
0.913
0.906
HO OH
H
N
O
O
N
H
O
N
H
O
OH
OH
4
1000
500
0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
ppm (f1)

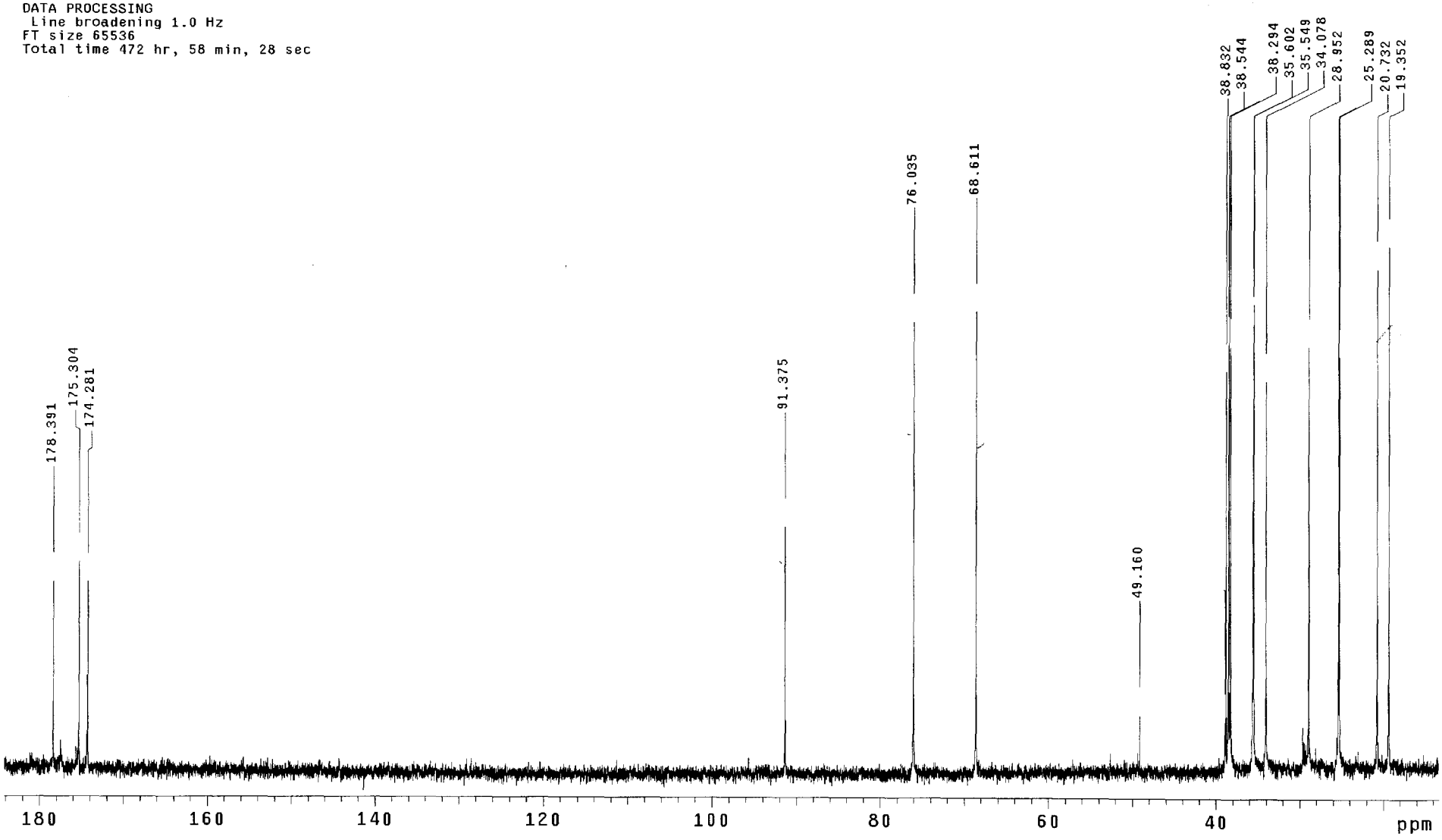
13C OBSERVE

Pulse Sequence: s2pul
Solvent: D2O
Temp. 29.0 C / 302.1 K
Mercury-400BB "hg402"

Pulse 53.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
9880 repetitions
OBSERVE C13, 100.6113272 MHz
DECOUPLE H1, 400.1263885 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 472 hr, 58 min, 28 sec



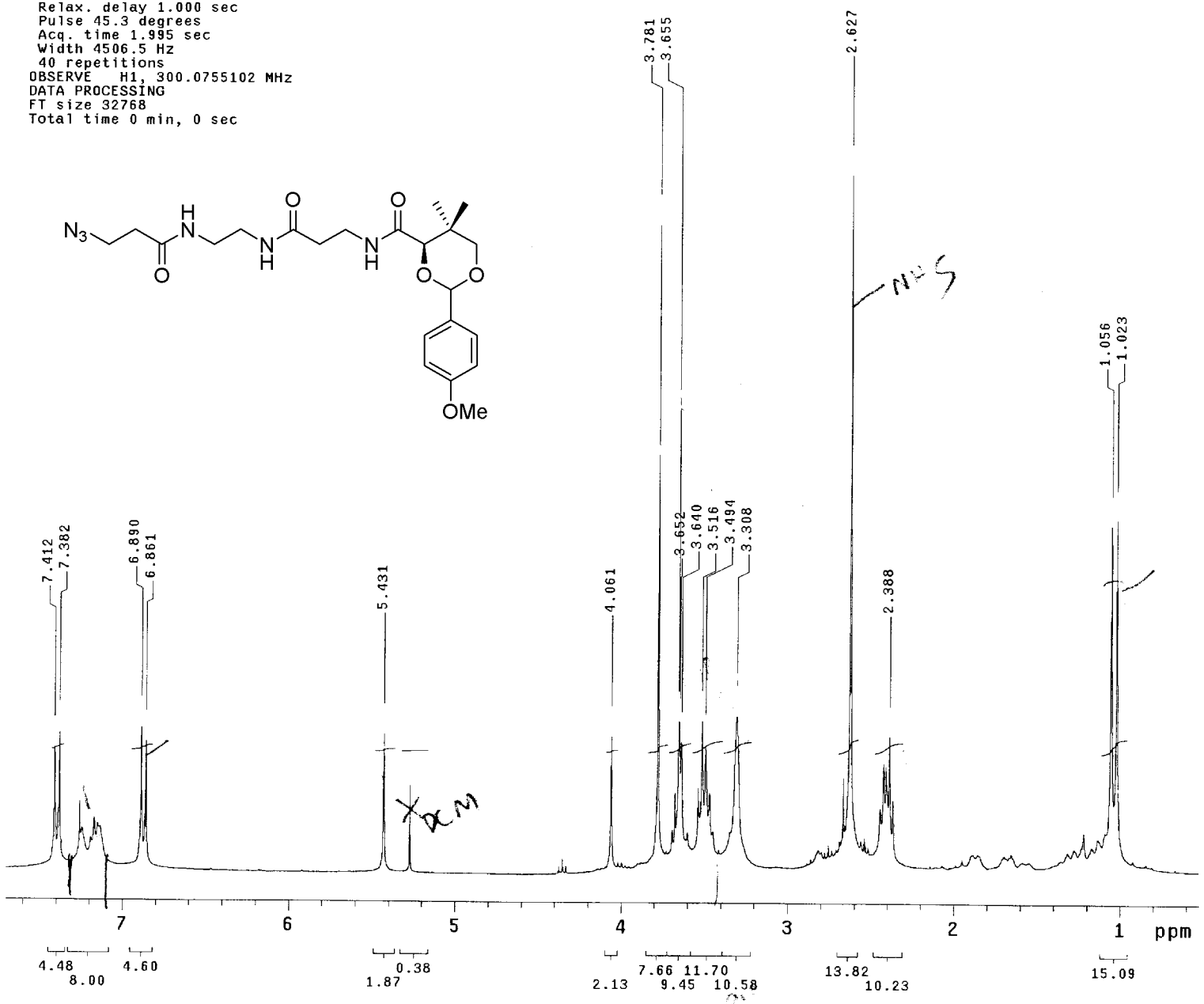
4



jlm3-59-traditionallinker-NHSazidelinkage-PMP-02-13-06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

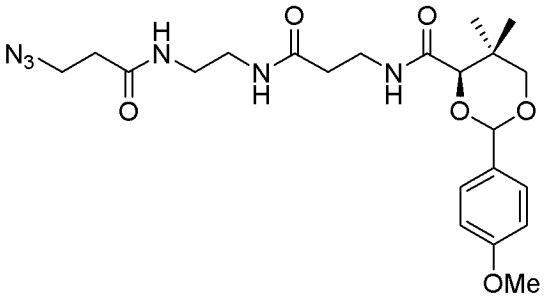
Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
40 repetitions
OBSERVE H1, 300.0755102 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec

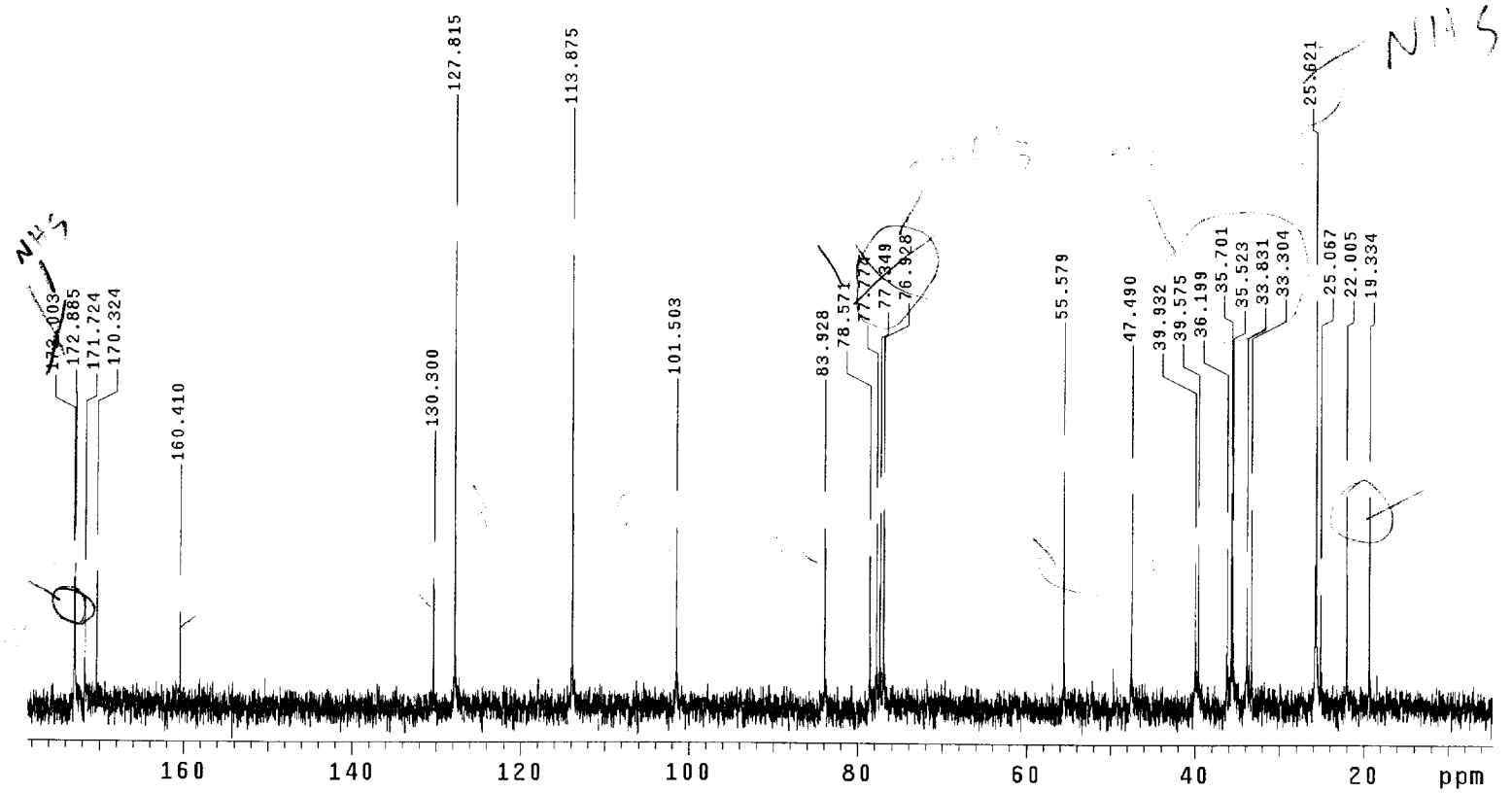


13C OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
144 repetitions
OBSERVE C13, 75.4540290 MHz
DECOUPLE H1, 300.0770584 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 39 min, 38 sec



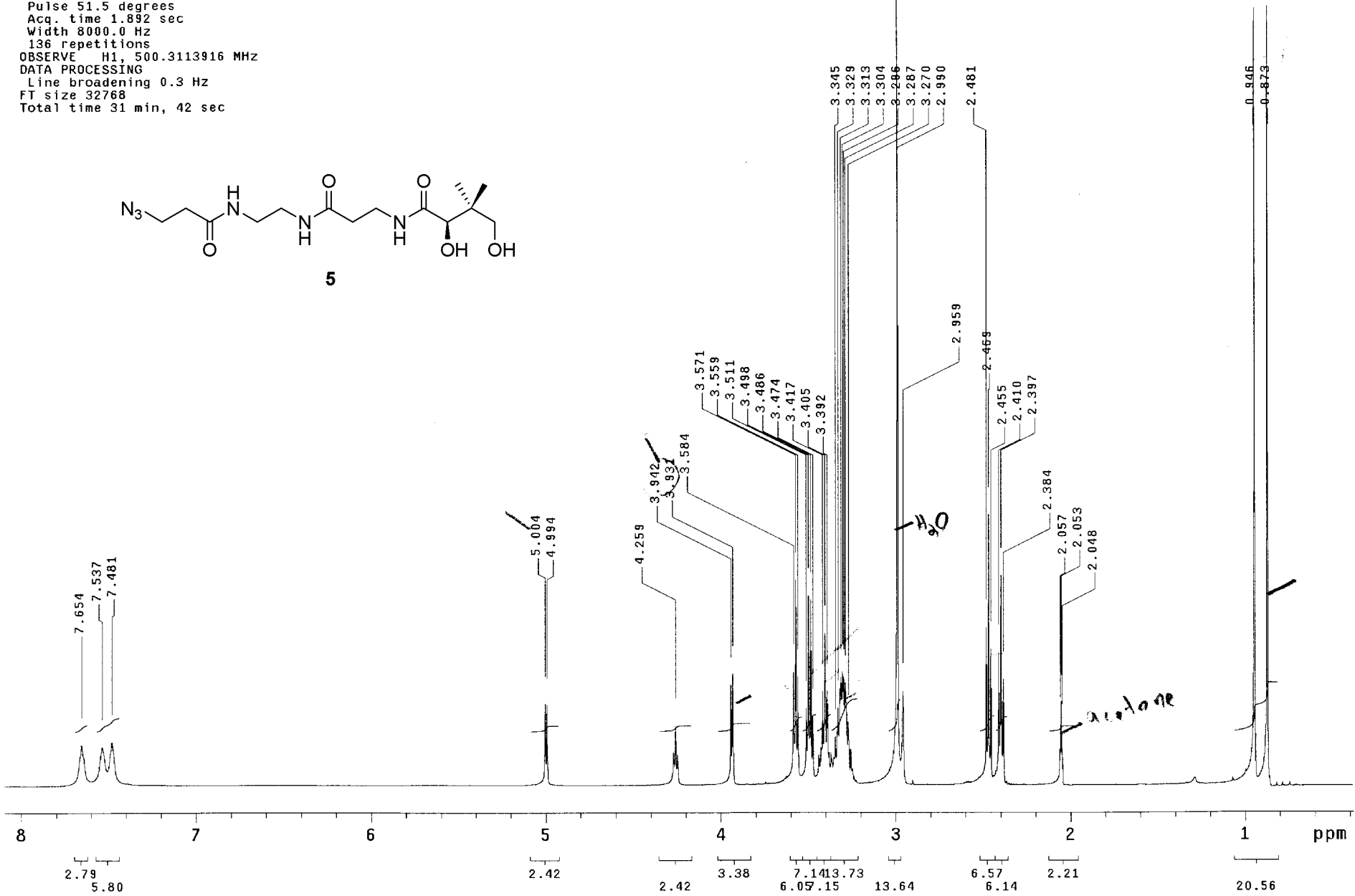


jlm3-60-traditionallinker-NHSazide-PMPdp-02-14-06

Pulse Sequence: s2pul
Solvent: Acetone
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

Pulse 51.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
136 repetitions
OBSERVE H1, 500.3113916 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec

5

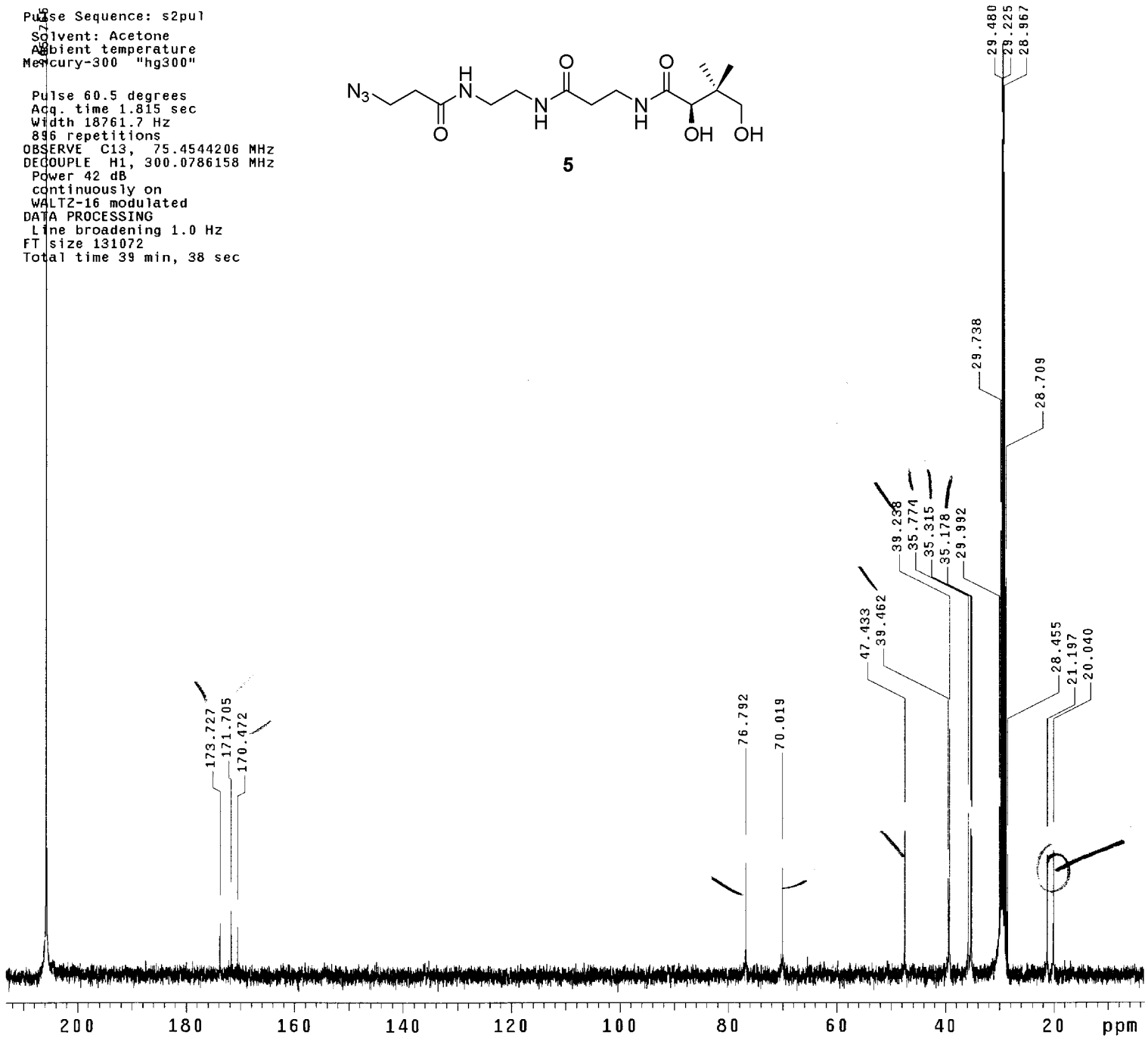


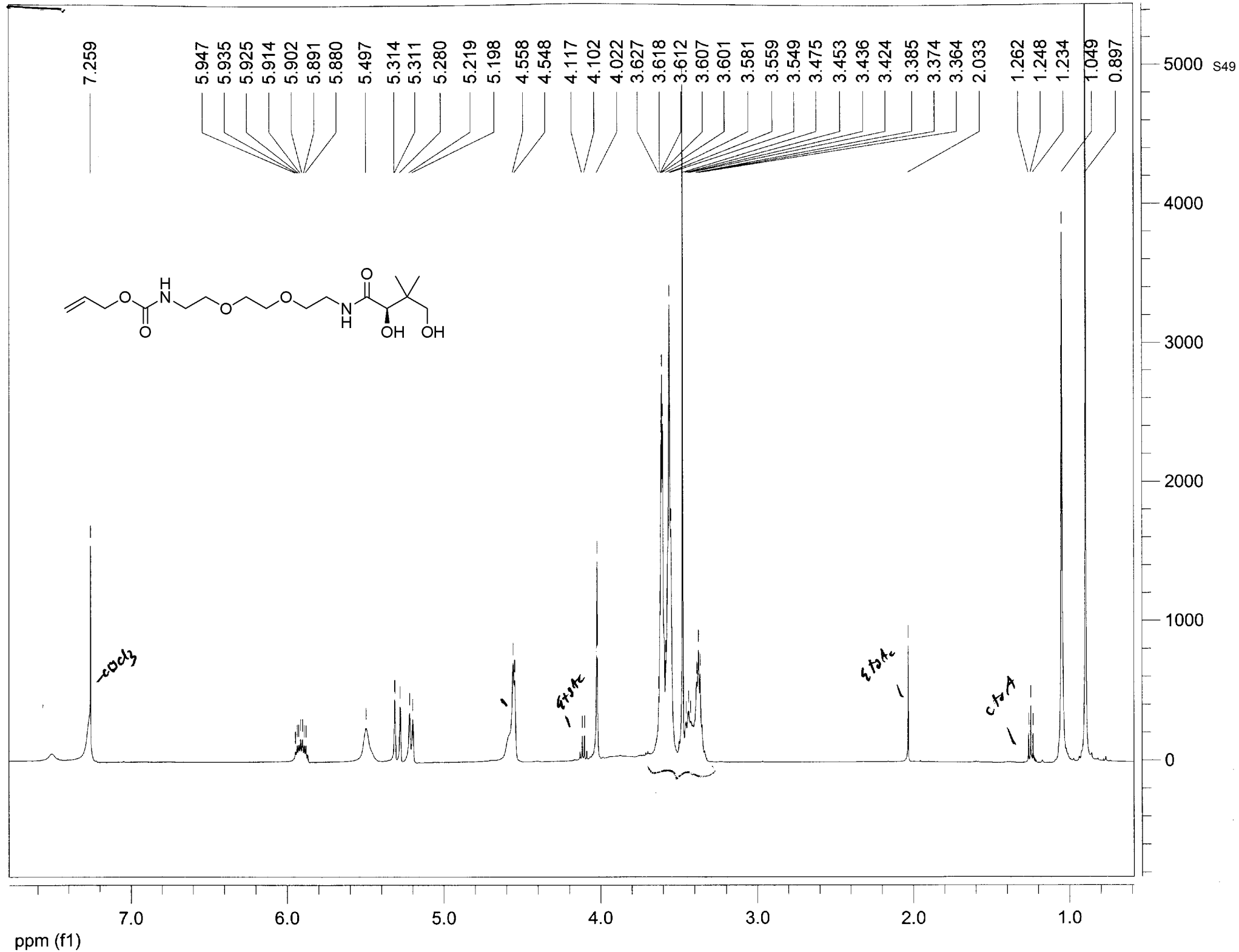
13C OBSERVE

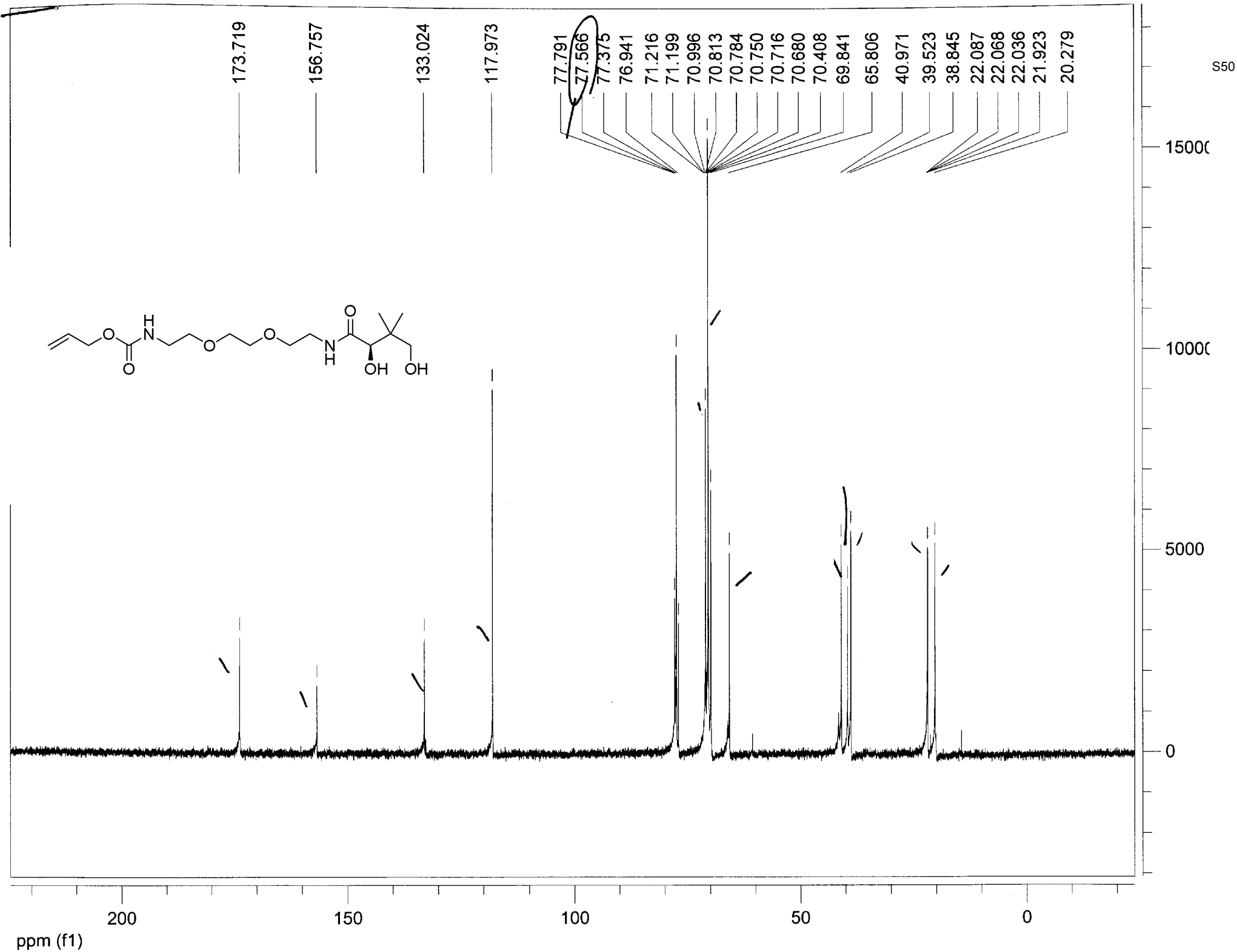
Pulse Sequence: s2pul
Solvent: Acetone
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
896 repetitions
OBSERVE C13, 75.4544206 MHz
DECOUPLE H1, 300.0786158 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 39 min, 38 sec

5



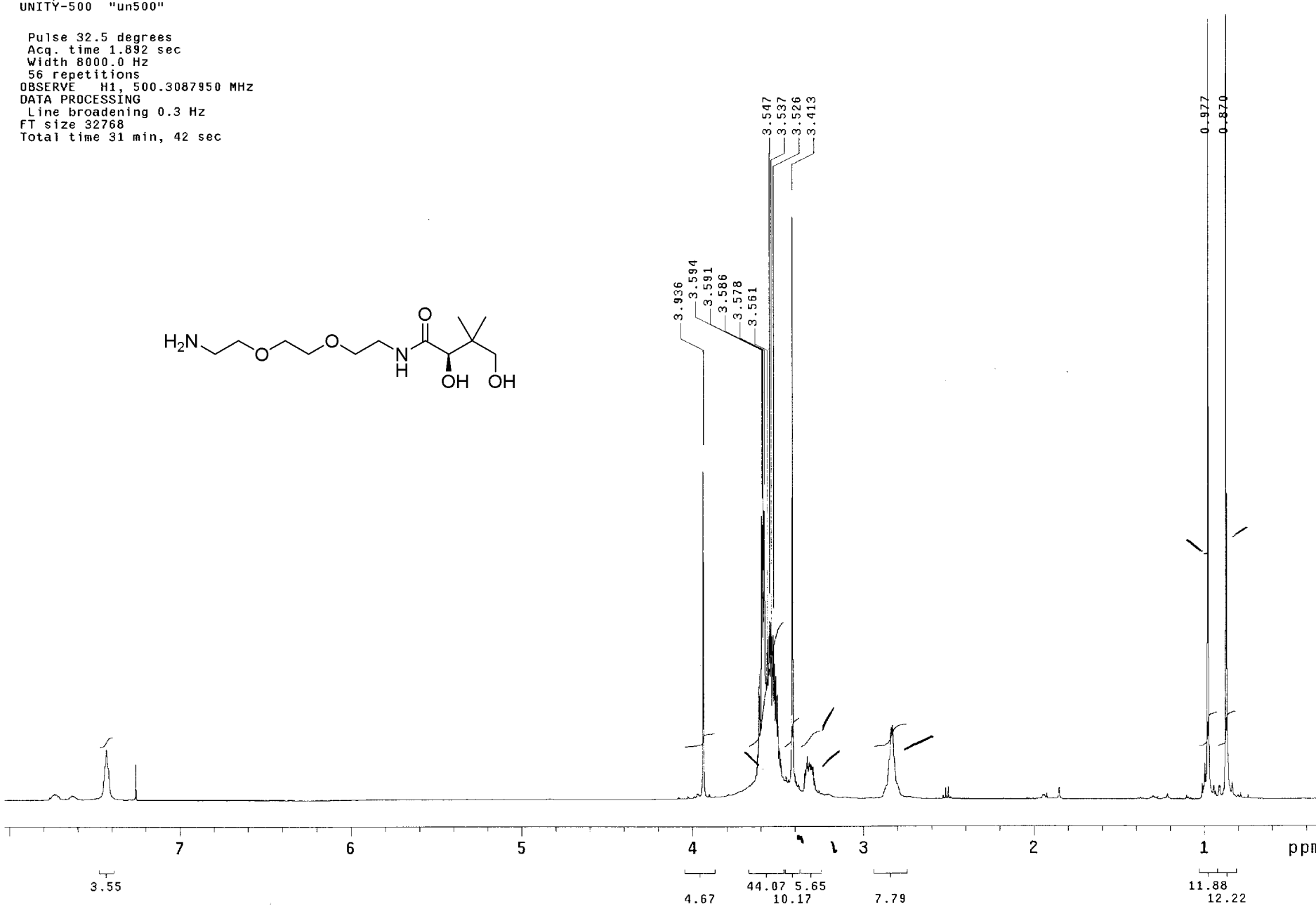

7.259
5.947
5.935
5.925
5.914
5.902
5.891
5.880
5.497
5.314
5.311
5.280
5.219
5.198
4.558
4.548
4.117
4.102
4.022
3.627
3.618
3.612
3.607
3.601
3.581
3.559
3.549
3.475
3.453
3.436
3.424
3.385
3.374
3.364
2.033
1.262
1.248
1.234
1.049
0.897
5000
4000
3000
2000
1000
0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)
H
N
O
O
O
O
O
N
H
O
OH
OH

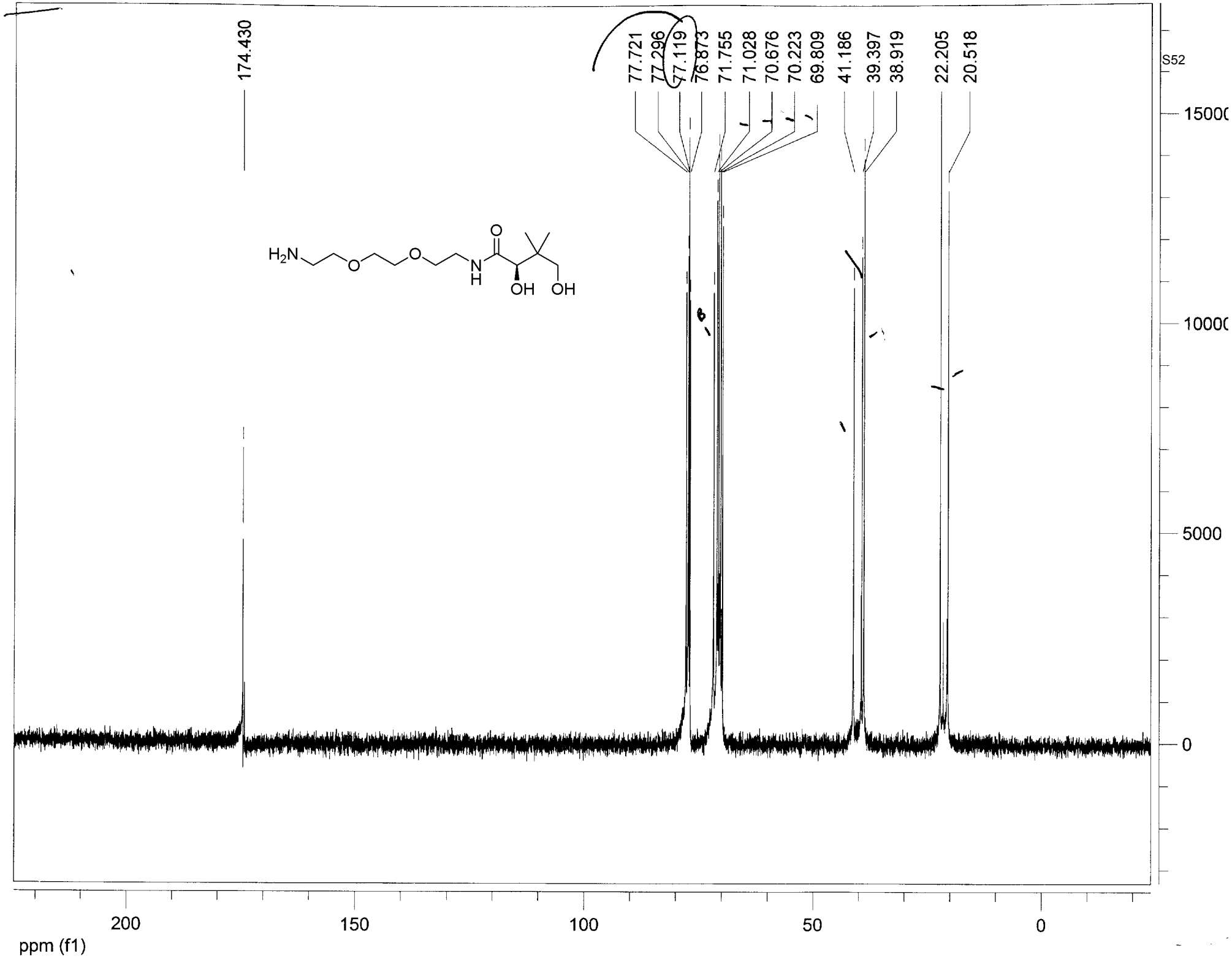

173.719
156.757
133.024
117.973
77.791
77.566
77.375
76.941
71.216
71.199
70.996
70.813
70.784
70.750
70.716
70.680
70.408
69.841
65.806
40.971
39.523
38.845
22.087
22.068
22.036
21.923
20.279
15000
10000
5000
0
200
150
100
50
0
ppm (f1)

jlm3-6-iv-dealloc-2-ethyhexanoicacidworkup-12-01-05

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

Pulse 32.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
56 repetitions
OBSERVE H1, 500.3087950 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec



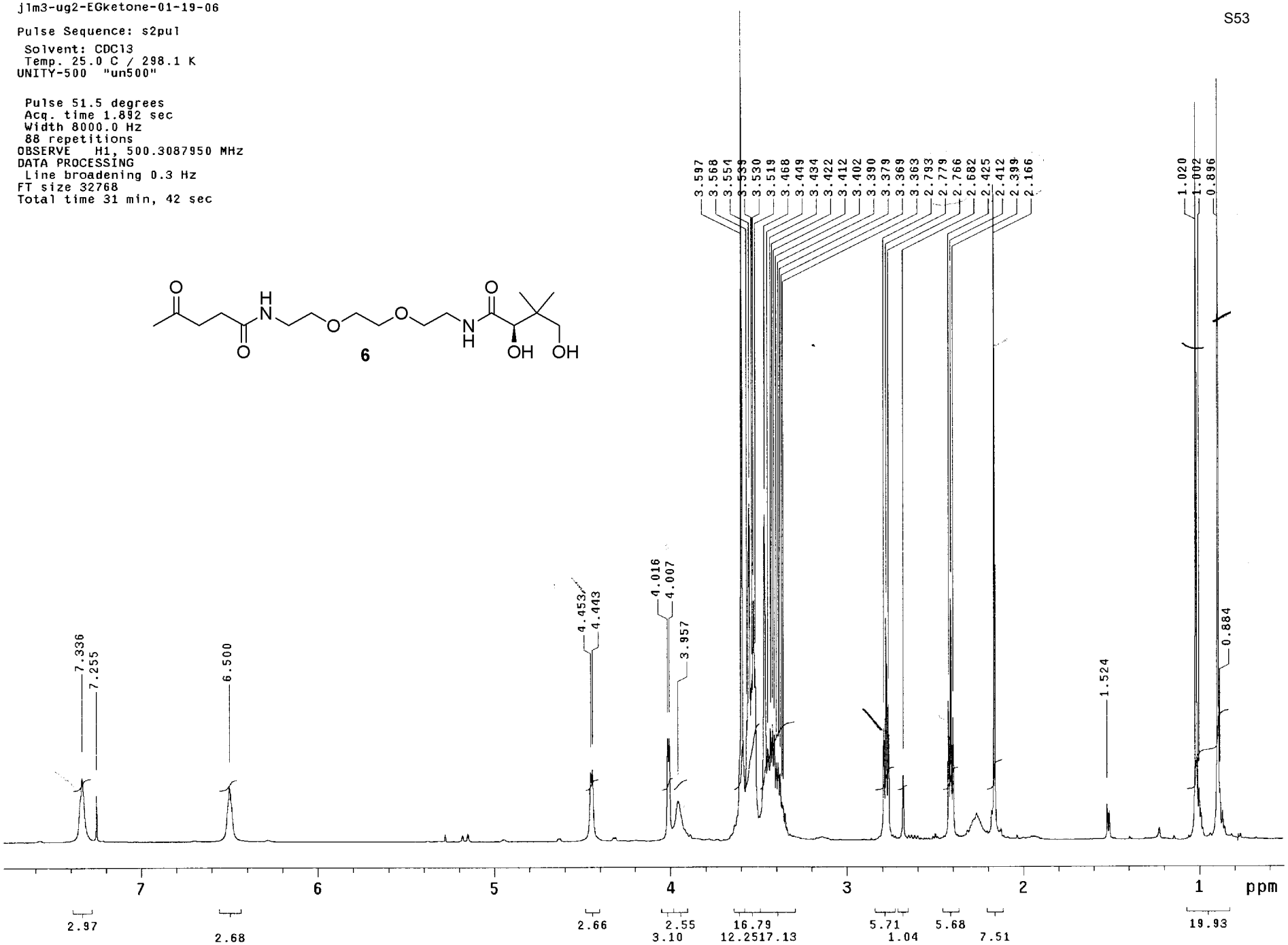

174.430
77.721
77.296
77.119
76.873
71.755
71.028
70.676
70.223
69.809
41.186
39.397
38.919
22.205
20.518
S52
15000
10000
5000
0
200
150
100
50
0
ppm (f1)
H2N
O
O
N
H
O
OH
OH

jlm3-ug2-EGketone-01-19-06

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

Pulse 51.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
88 repetitions
OBSERVE H1, 500.3087950 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec

6

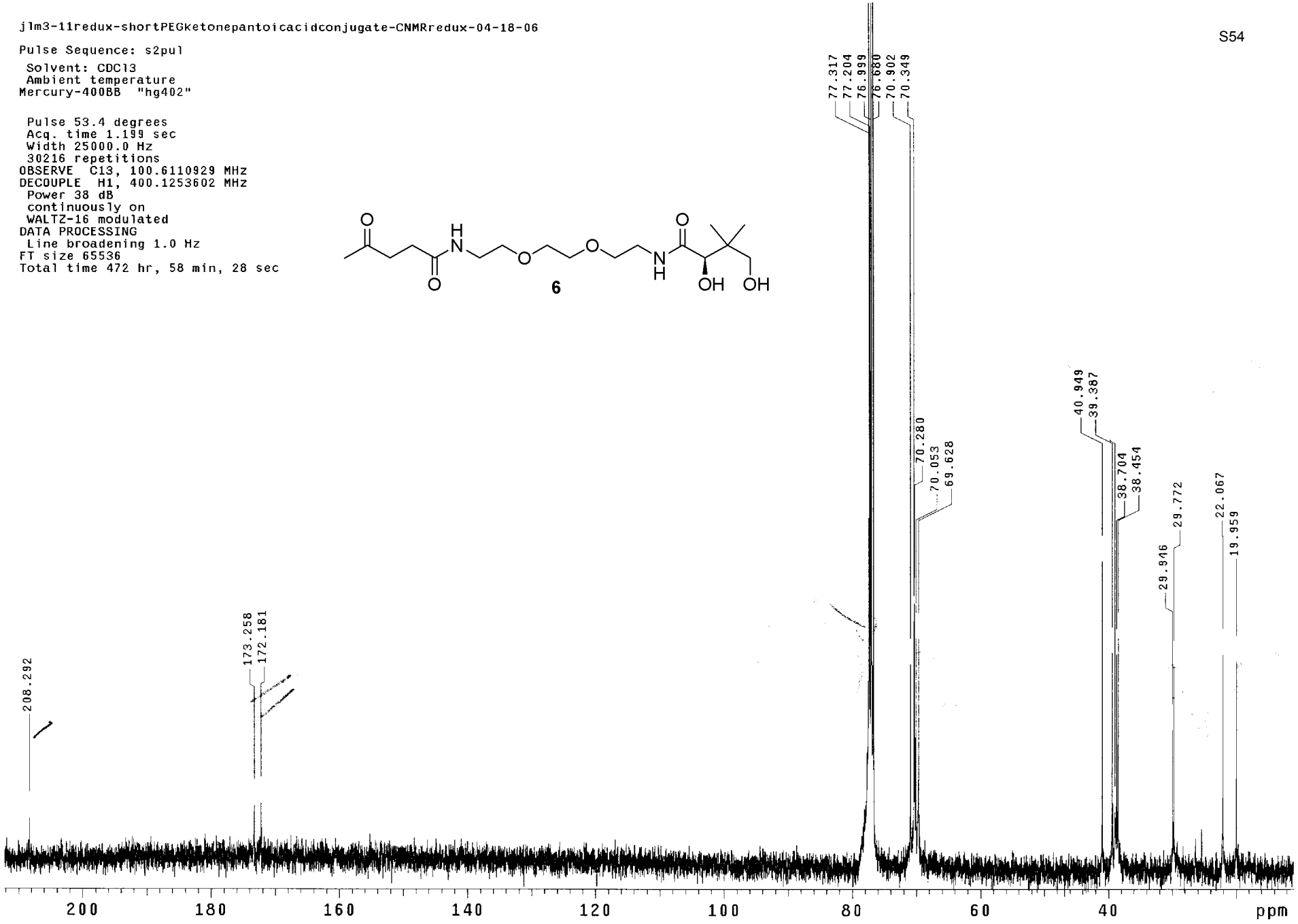


jlm3-11redux-shortPEGketonepantoicacidconjugate-CNMRredux-04-18-06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "hg402"

Pulse 53.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
30216 repetitions
OBSERVE C13, 100.6110929 MHz
DECOUPLE H1, 400.1253602 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 472 hr, 58 min, 28 sec

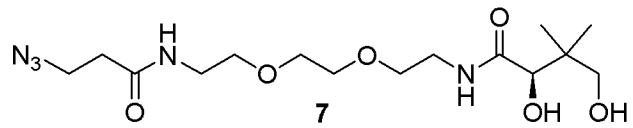
6

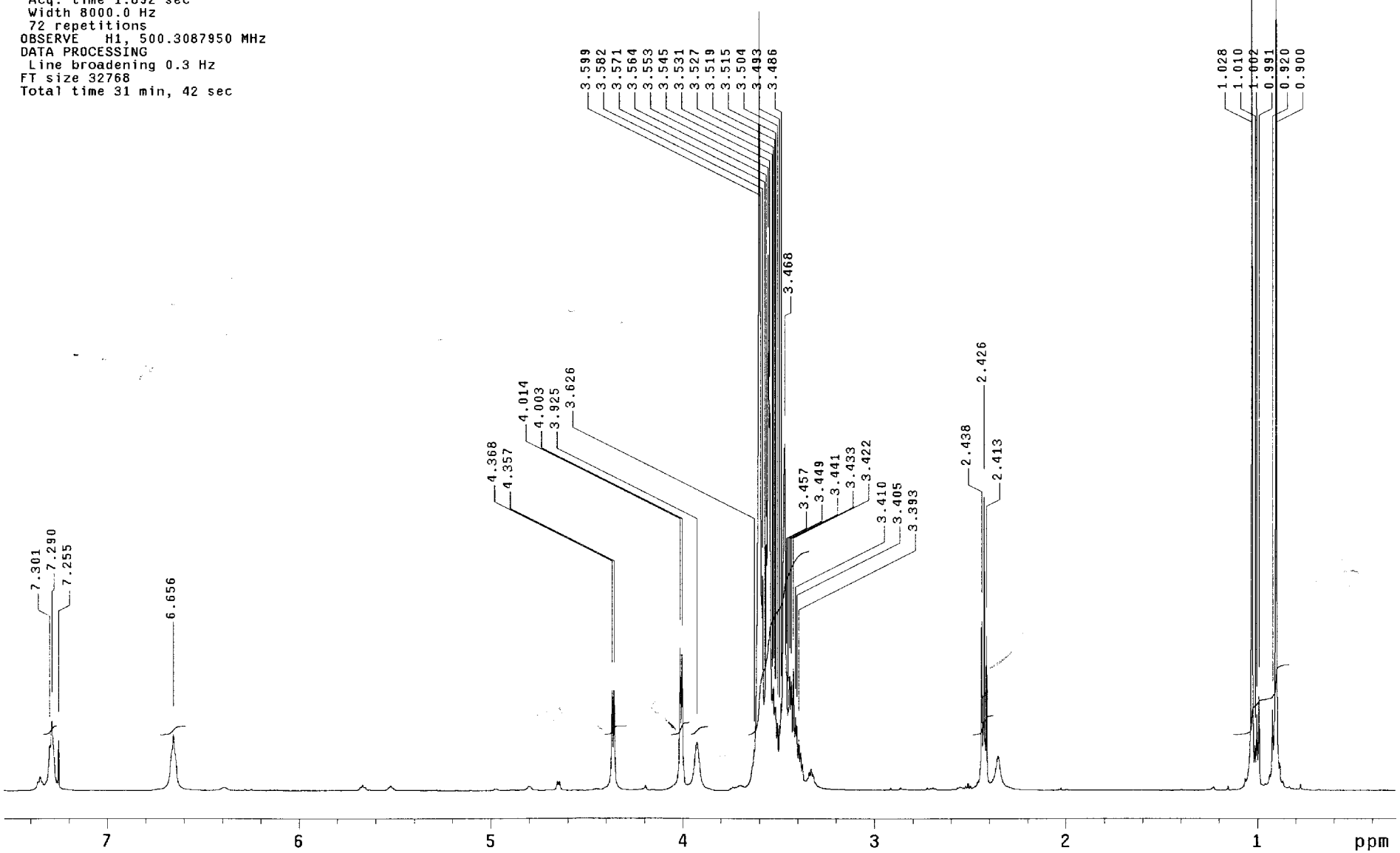


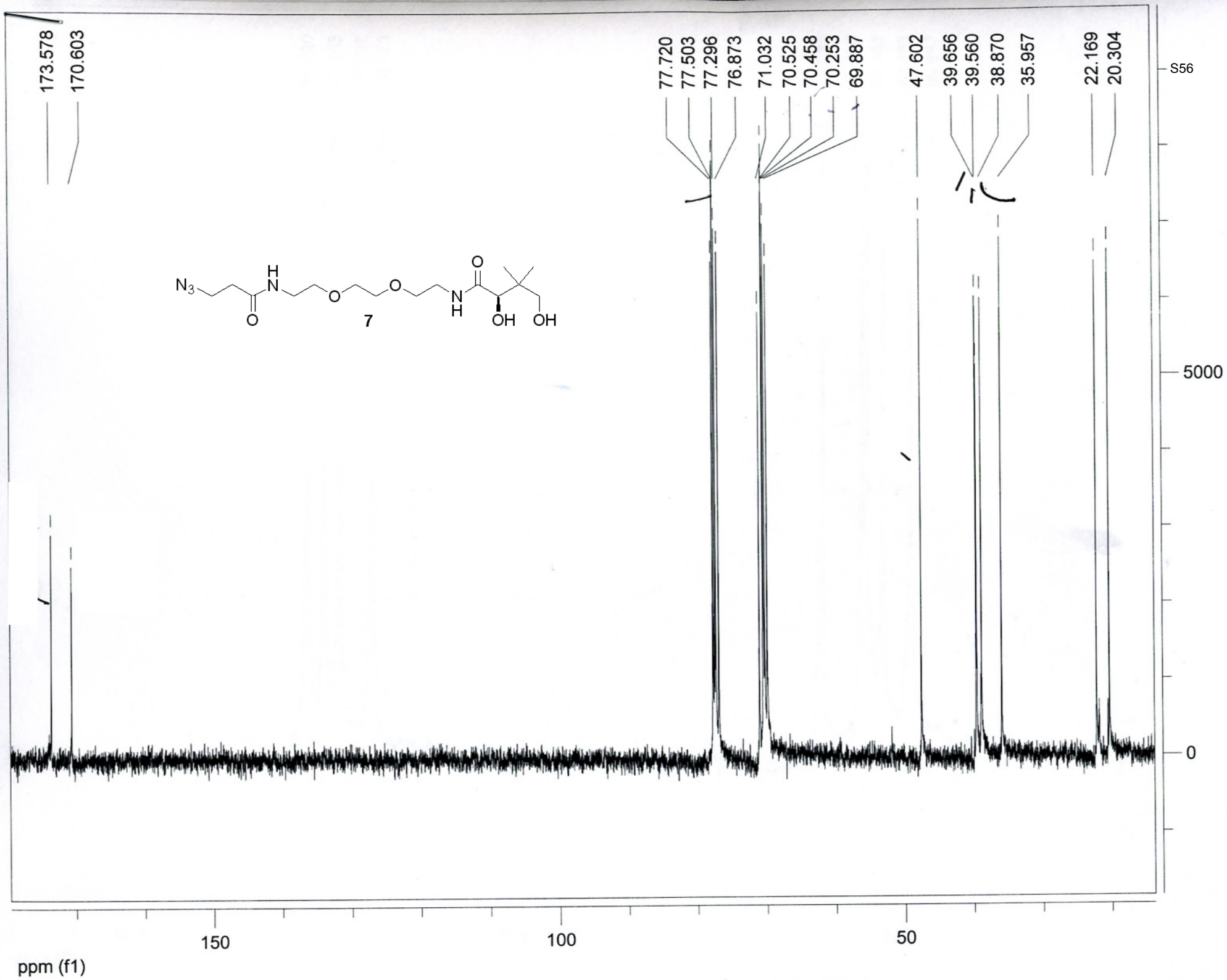
jlm3-61-azido-EG-pantoicacid-purefromminflash-pubqualityHNMR-02-18-06

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

Pulse 51.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
72 repetitions
OBSERVE H1, 500.3087950 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec



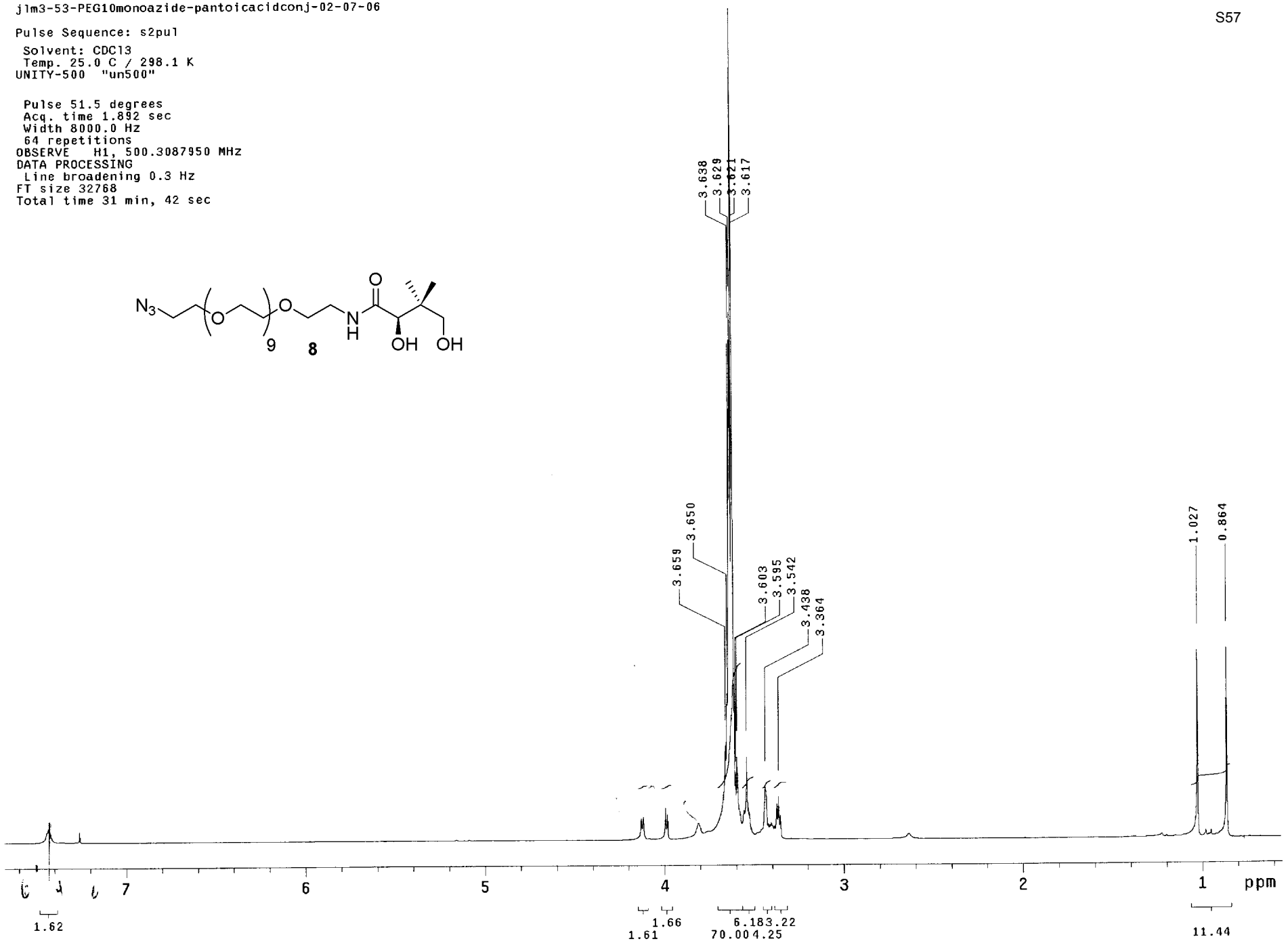


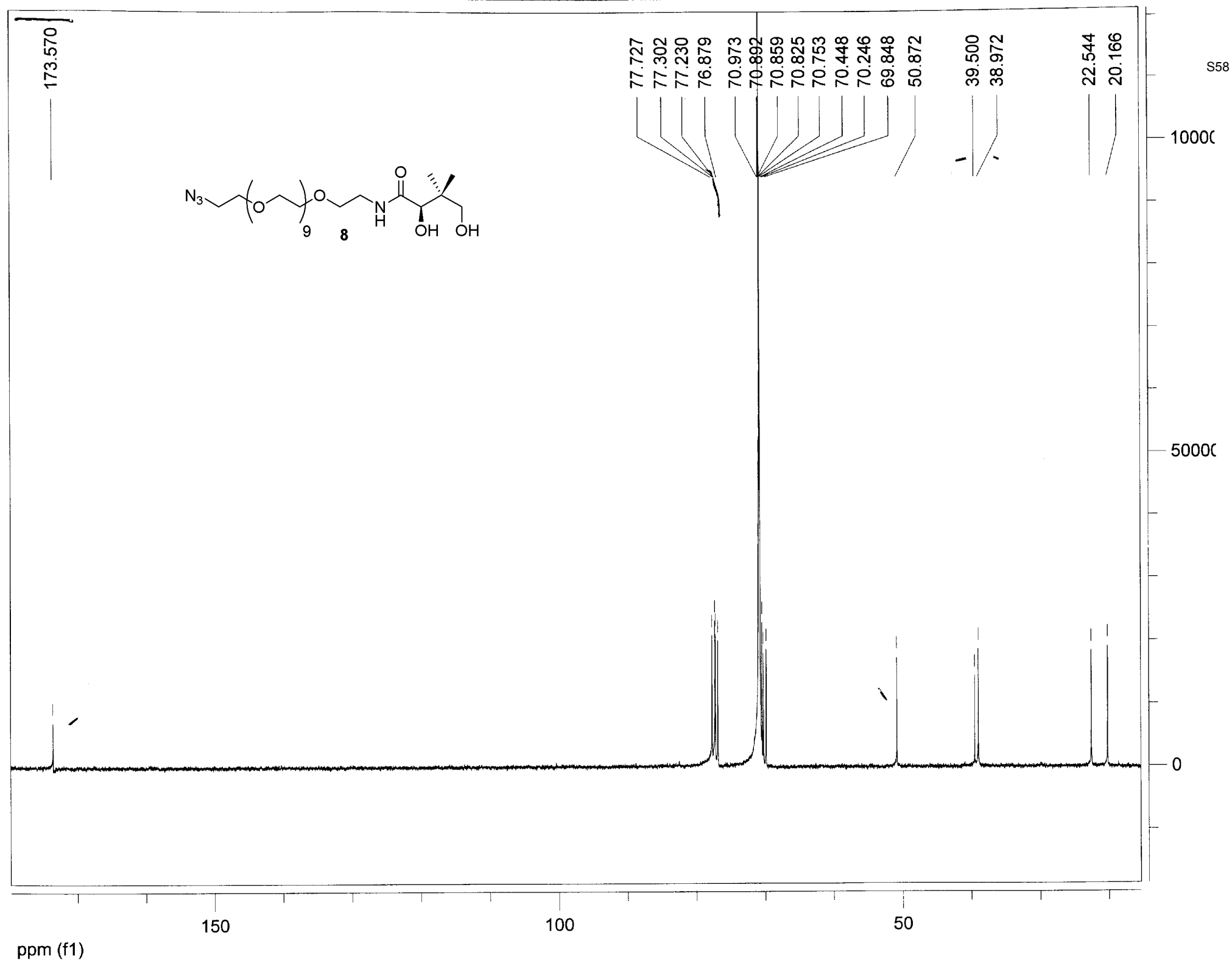

173.578
170.603
77.720
77.503
77.296
76.873
71.032
70.525
70.458
70.253
69.887
47.602
39.656
39.560
38.870
35.957
22.169
20.304
S56
5000
0
150
100
50
ppm (f1)
7

jlm3-53-PEG10monoazide-pantoicacidconj-02-07-06

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

Pulse 51.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
64 repetitions
OBSERVE H1, 500.3087950 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec



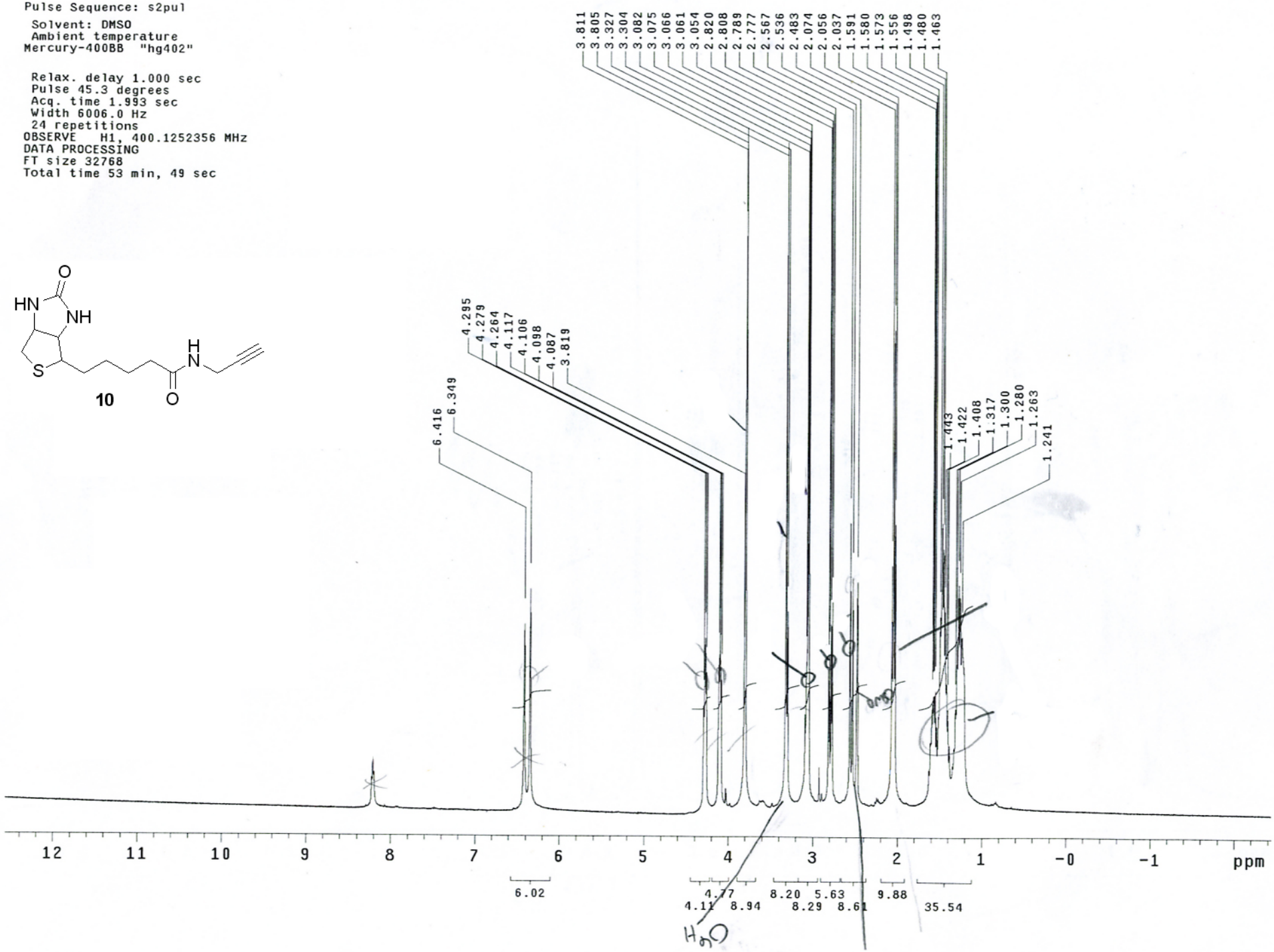

173.570
77.727
77.302
77.230
76.879
70.973
70.892
70.859
70.825
70.753
70.448
70.246
69.848
50.872
39.500
38.972
22.544
20.166
N3
O
9
8
N
H
O
OH
OH
10000
50000
0
150
100
50
ppm (f1)

jlm-biotin-propargylamineconj-pubNMRHNMR-02-07-06

Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "hg402"

Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
24 repetitions
OBSERVE H1, 400.1252356 MHz
DATA PROCESSING
FT size 32768
Total time 53 min, 49 sec

10

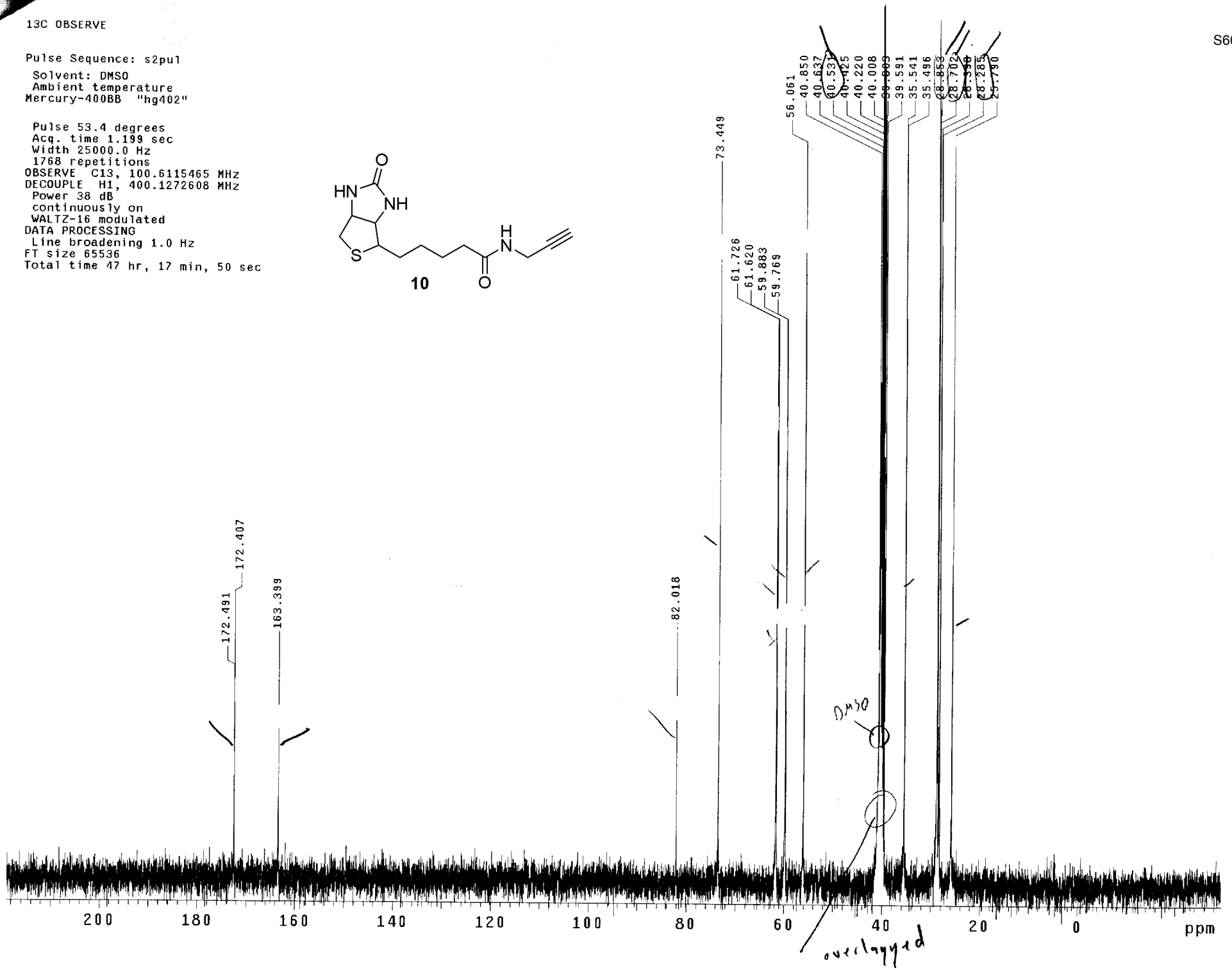


13C OBSERVE

Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "hg402"

Pulse 53.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
1768 repetitions
OBSERVE C13, 100.6115465 MHz
DECOUPLE H1, 400.1272608 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 47 hr, 17 min, 50 sec

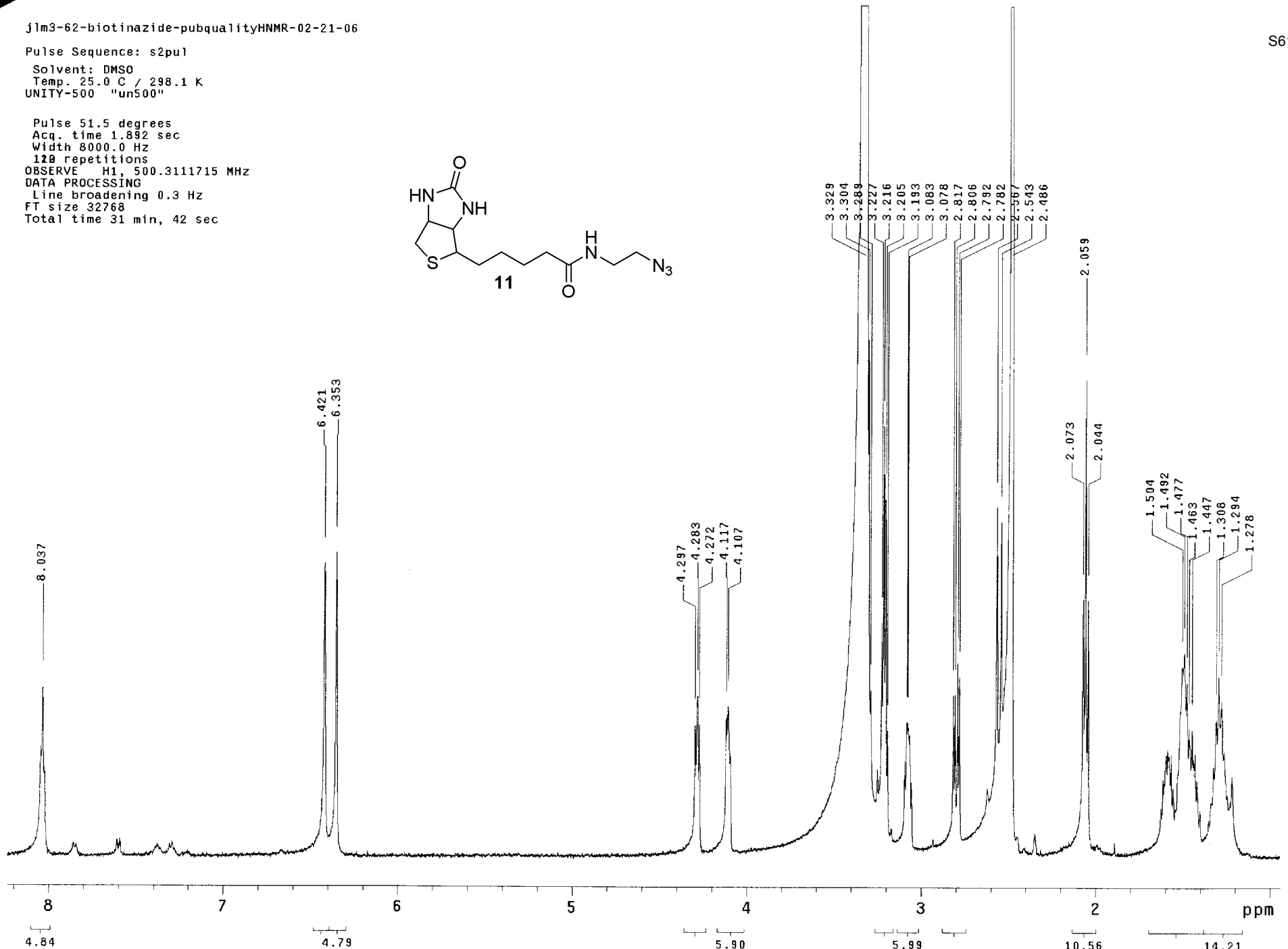
10



jlm3-62-biotinazide-pubqualityHNMR-02-21-06

Pulse Sequence: s2pul
Solvent: DMSO
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

Pulse 51.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
120 repetitions
OBSERVE H1, 500.3111715 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec

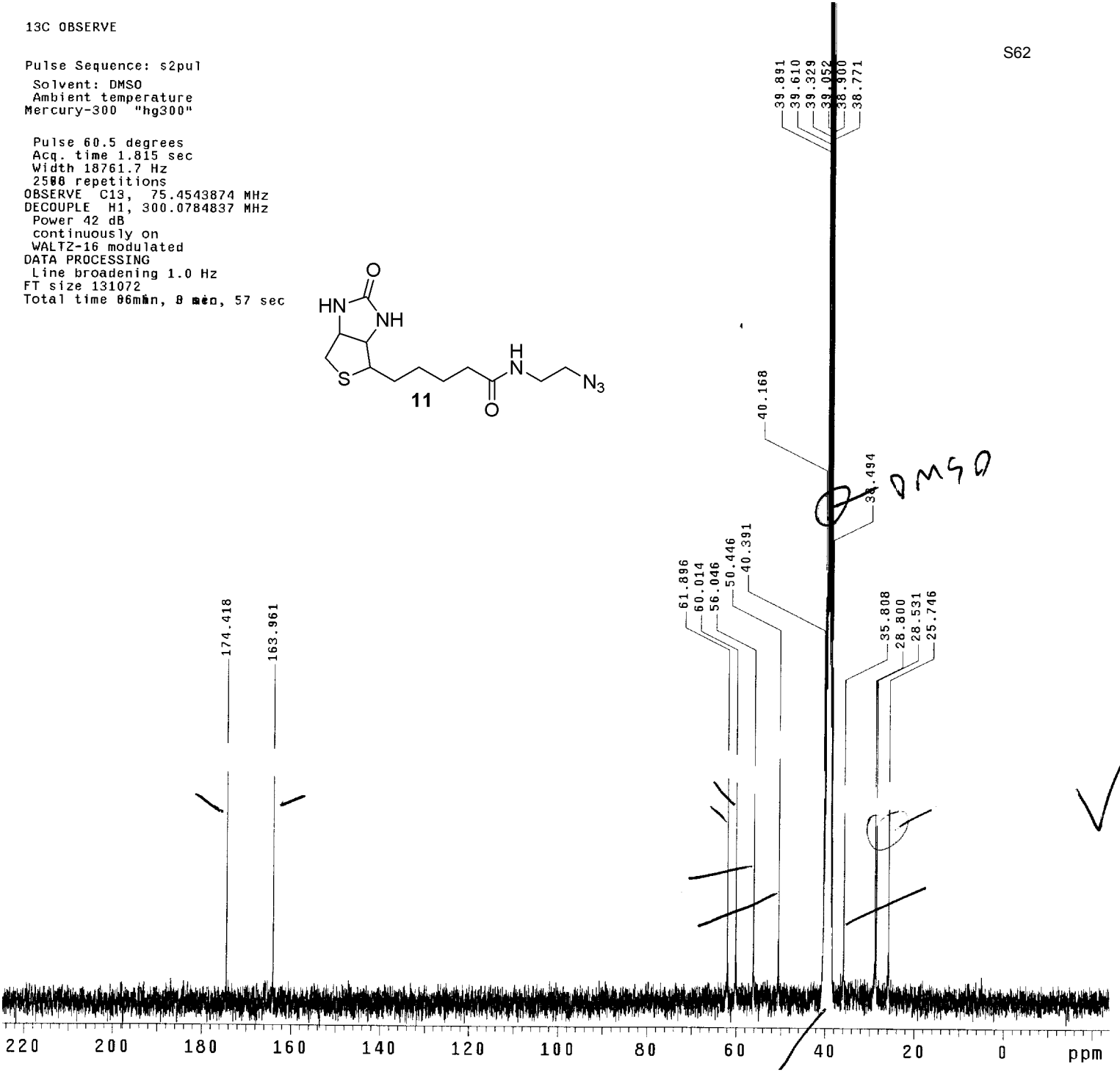


13C OBSERVE

Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
2500 repetitions
OBSERVE C13, 75.4543874 MHz
DECOUPLE H1, 300.0784837 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 06min, 0 sec, 57 sec

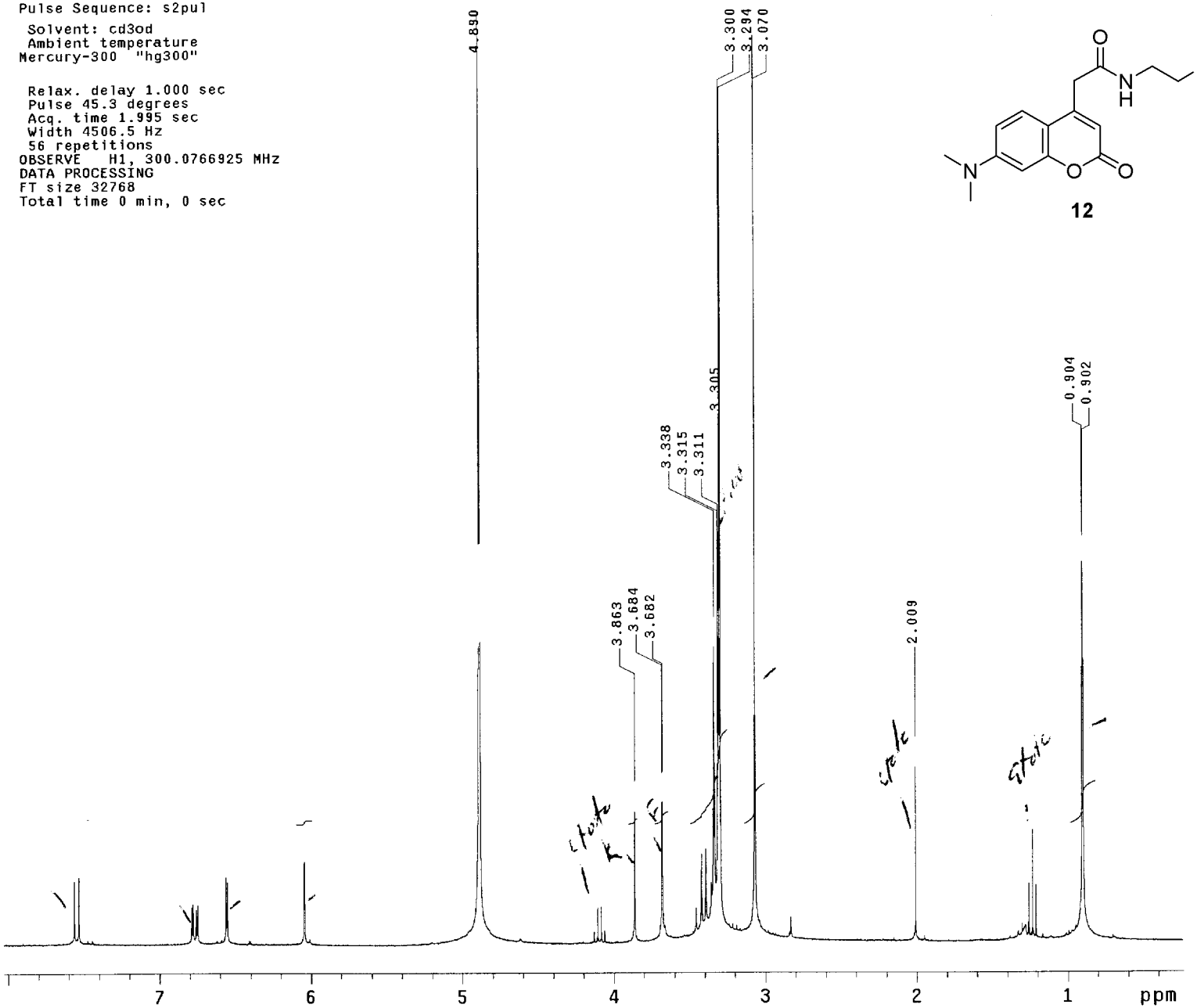
11

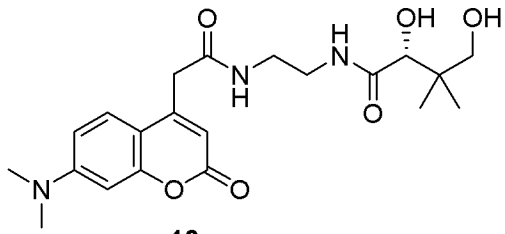


jlm3-43-DMACA-ethylenediamine-pantoicacidlinker-02-08-06

Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
Mercury-300 "hg300"

Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
56 repetitions
OBSERVE H1, 300.0766925 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec





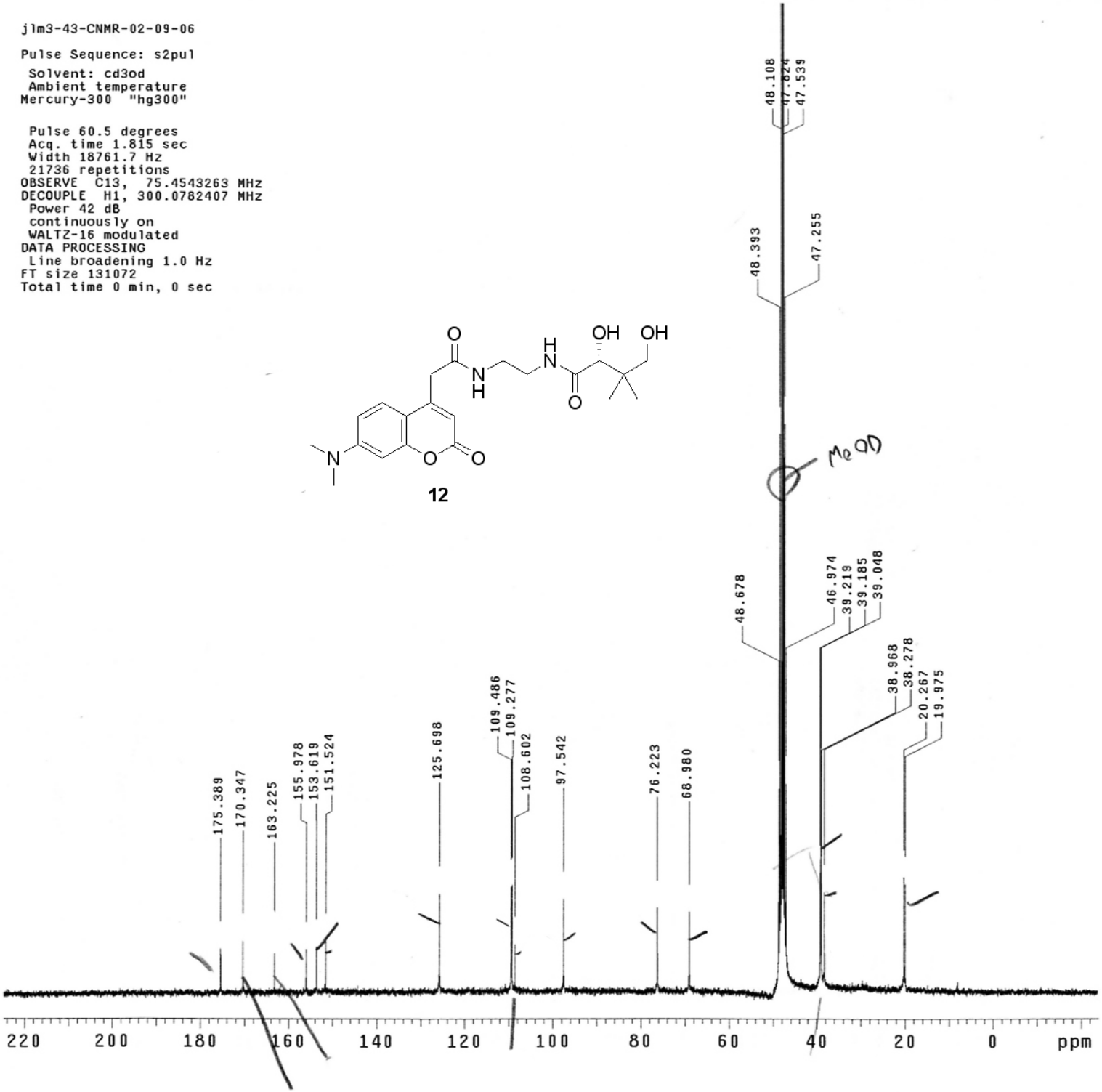
12

jlm3-43-CNMR-02-09-06

Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
21736 repetitions
OBSERVE C13, 75.4543263 MHz
DECOUPLE H1, 300.0782407 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 0 min, 0 sec

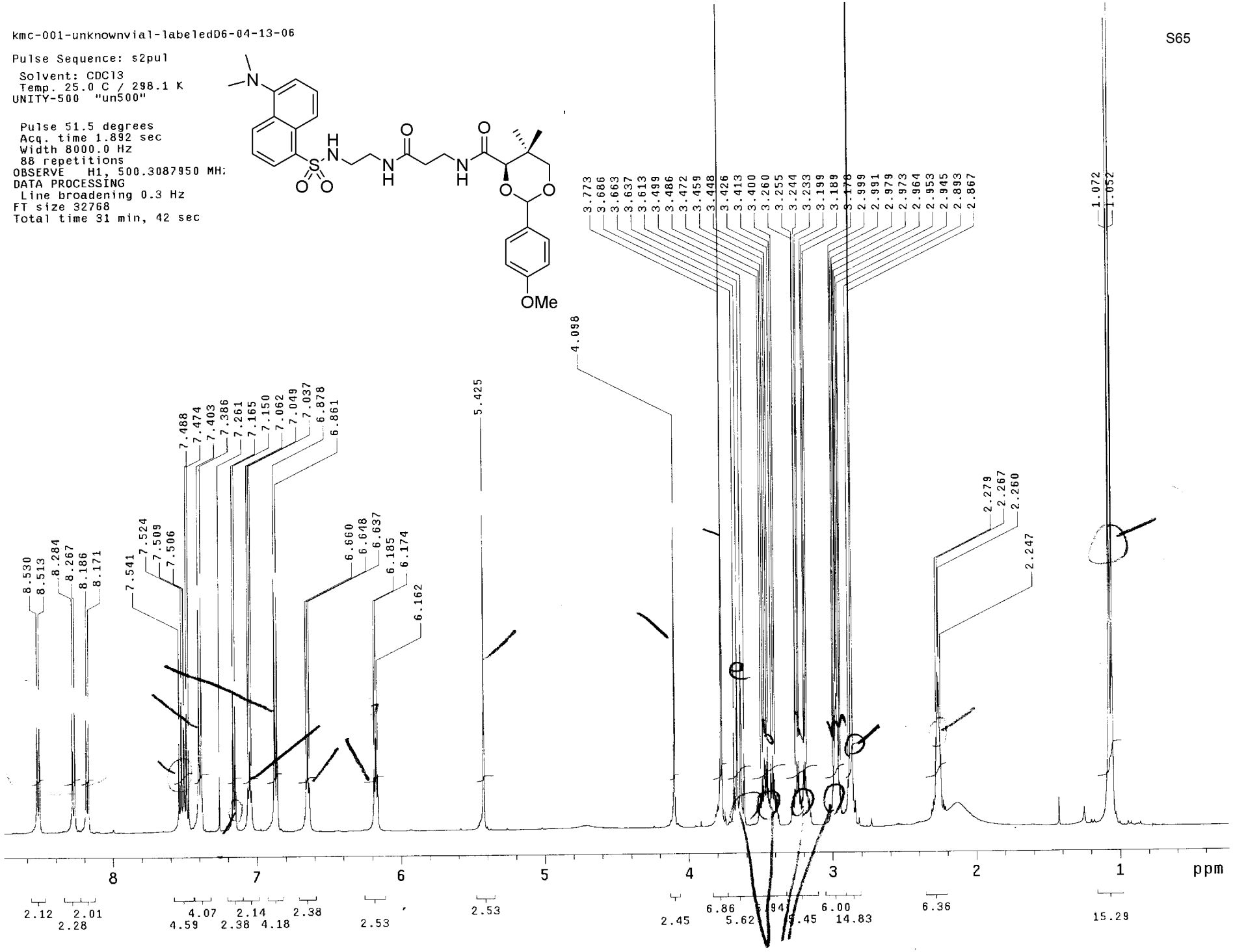
12



kmc-001-unknownvial-labeledD6-04-13-06

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

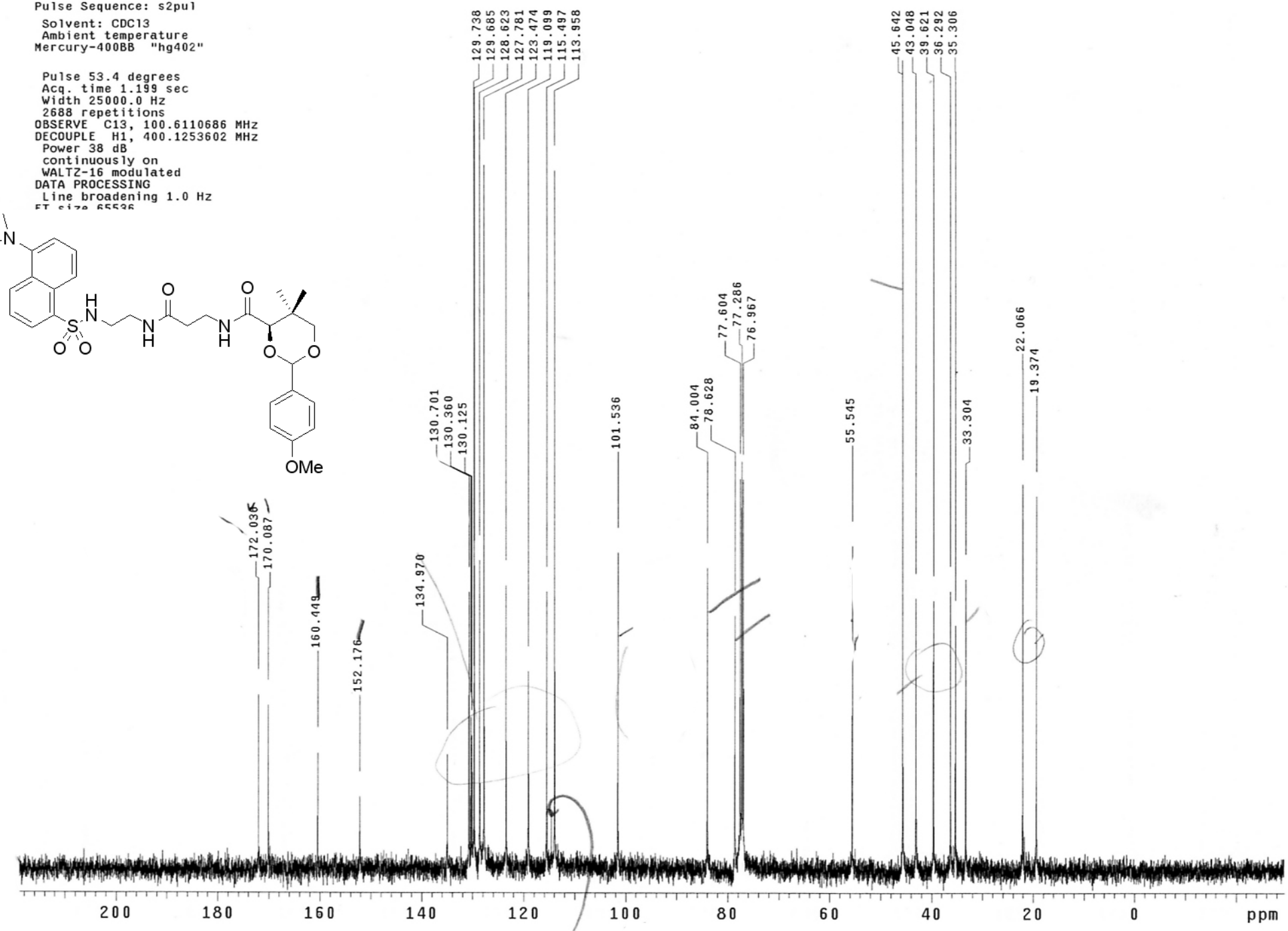
Pulse 51.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
88 repetitions
OBSERVE H1, 500.3087950 MH:
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec



kmc-002-PMPprotecteddansylfinalproduct-04-16-06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "hg402"

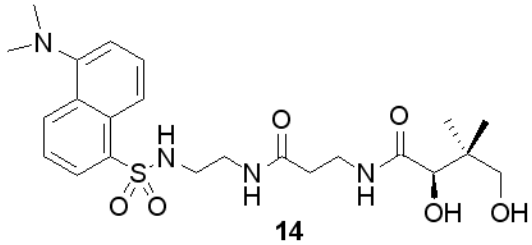
Pulse 53.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
2688 repetitions
OBSERVE C13, 100.6110686 MHz
DECOUPLE H1, 400.1253602 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536

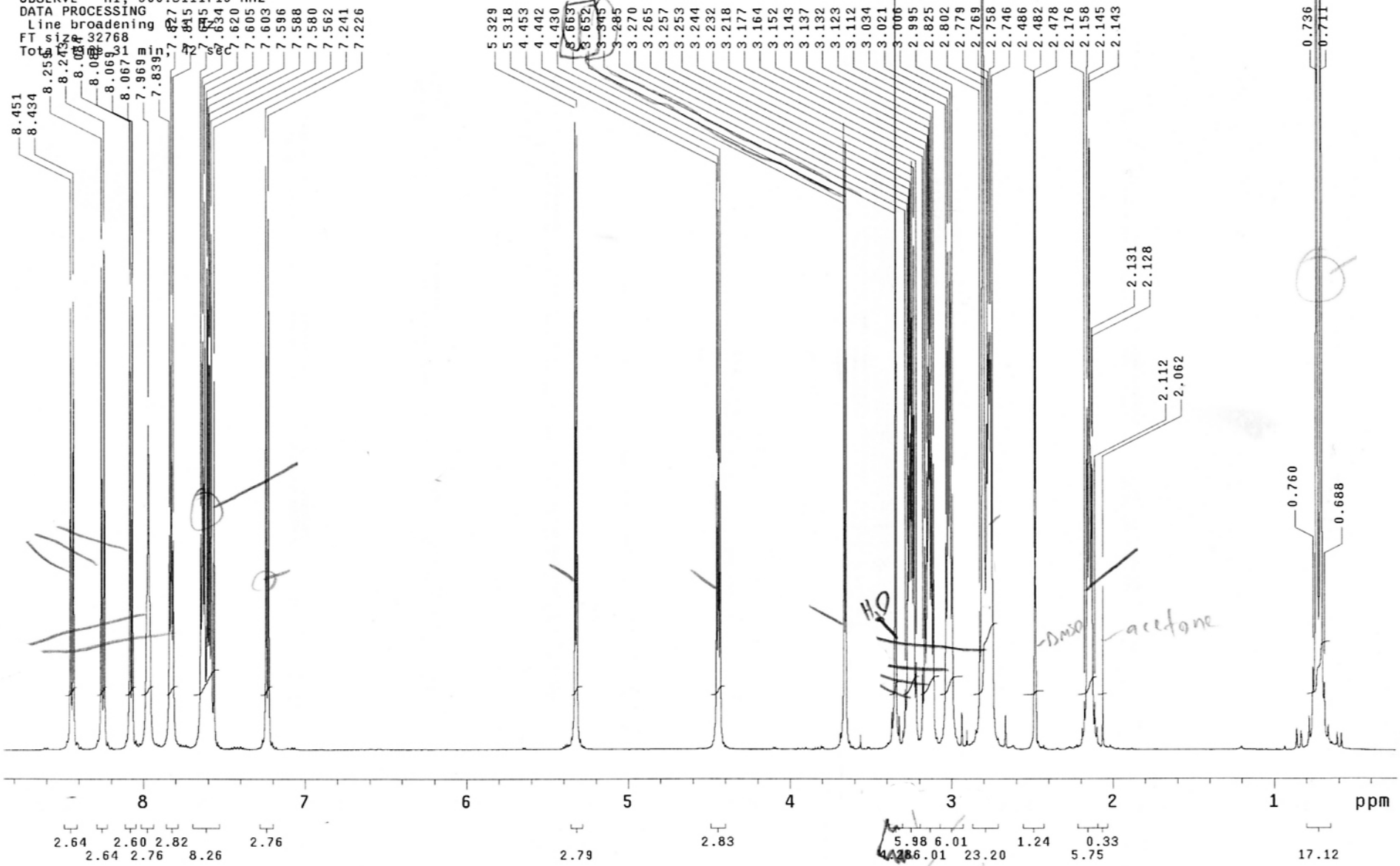


STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Solvent: DMSO
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

Pulse 51.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
96 repetitions
OBSERVE H1, 500.3111715 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec

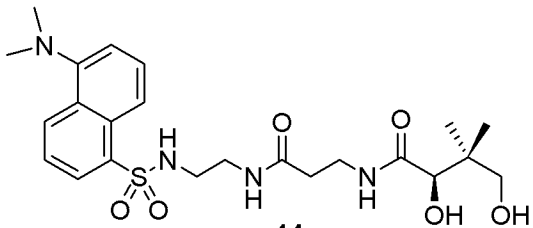




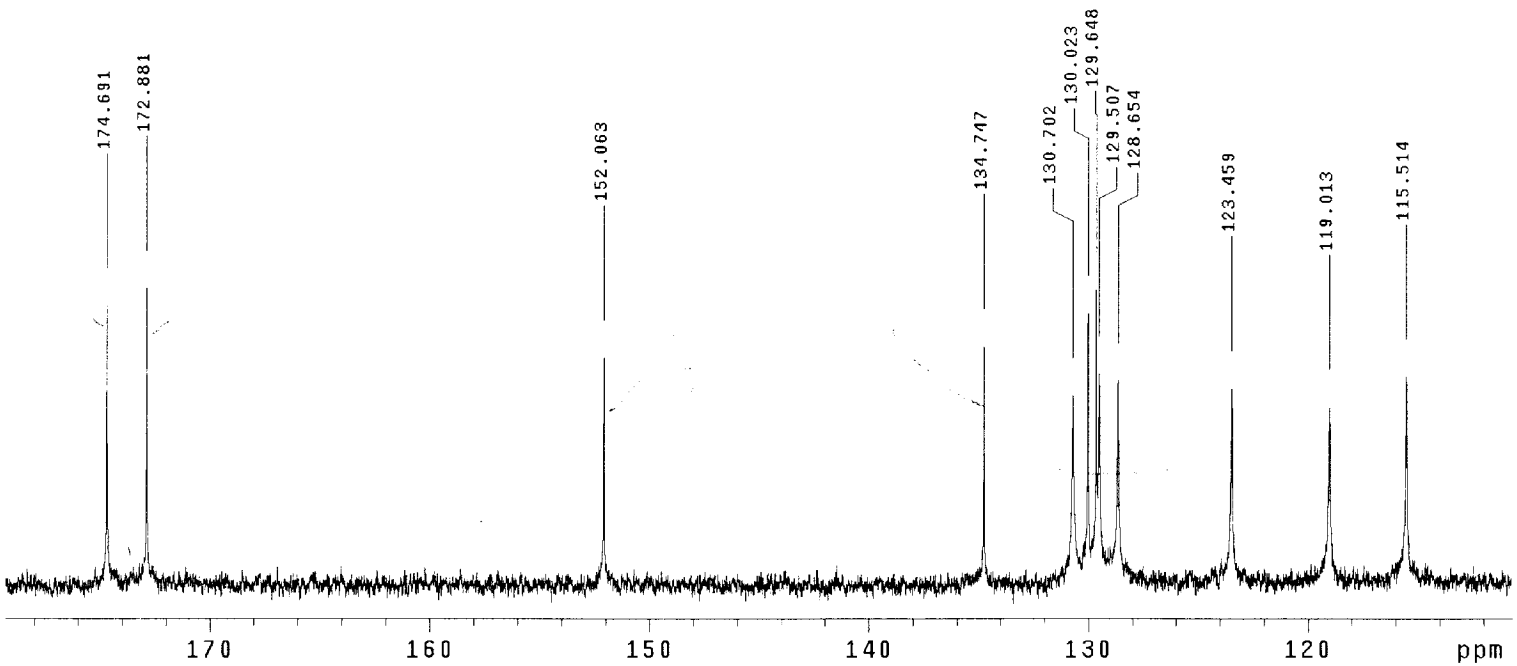
13C OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
976 repetitions
OBSERVE C13, 75.4540290 MHz
DECOUPLE H1, 300.0770584 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 6 hr, 36 min, 23 sec



14

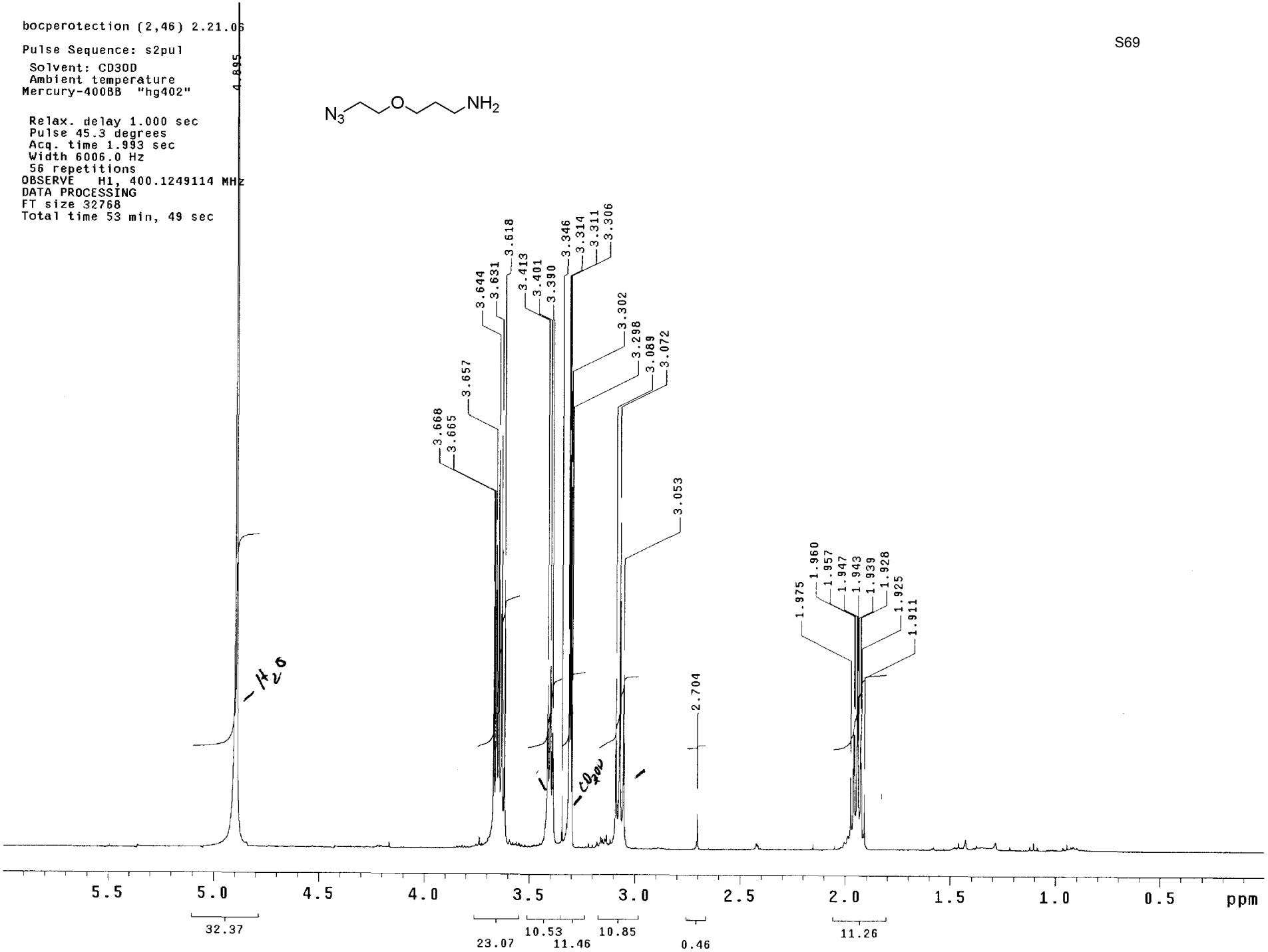


bocperotection (2,46) 2.21.06

Pulse Sequence: s2pul

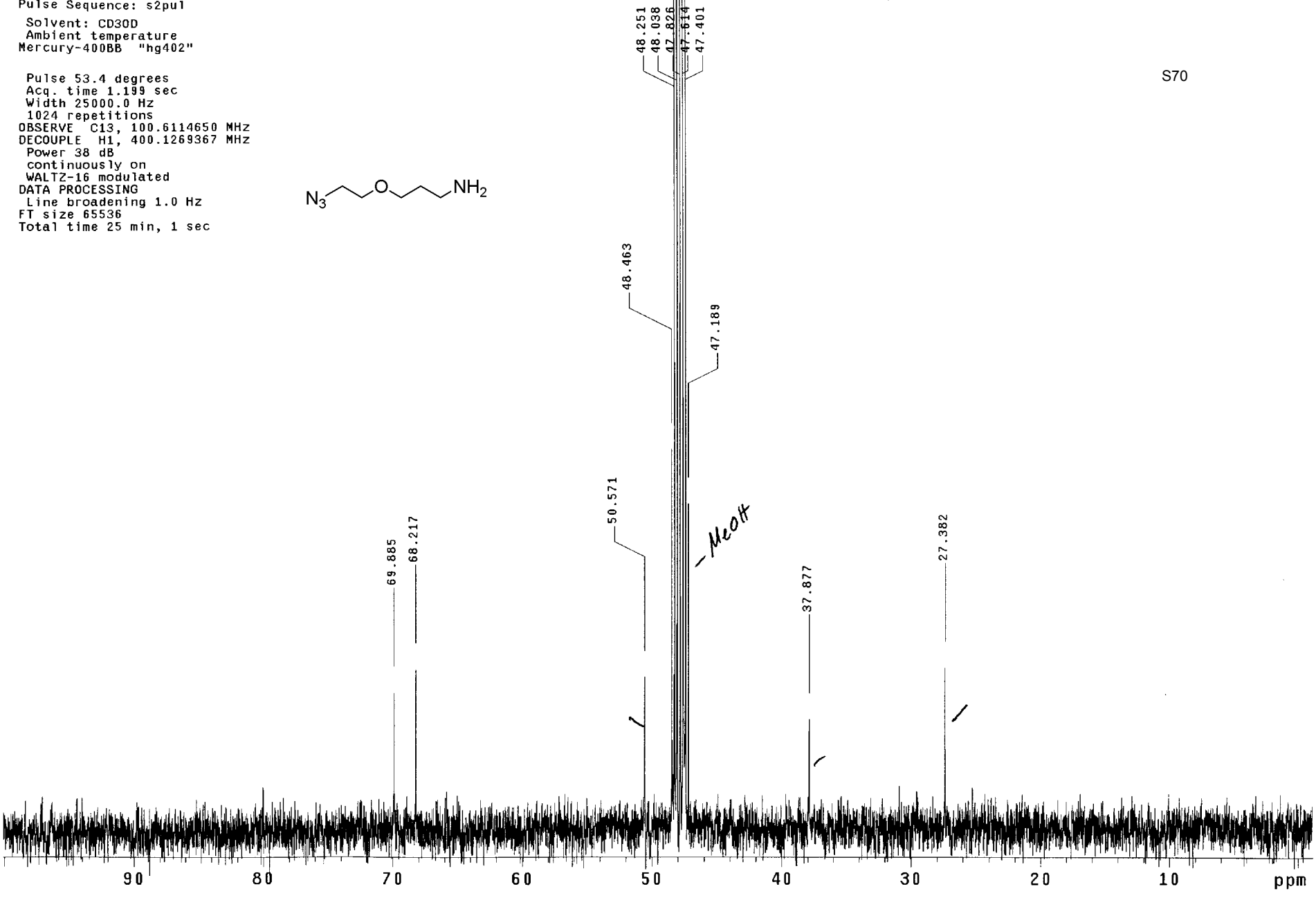
Solvent: CD3OD
Ambient temperature
Mercury-400BB "hg402"

Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
56 repetitions
OBSERVE H1, 400.1249114 MHz
DATA PROCESSING
FT size 32768
Total time 53 min, 49 sec



Pulse Sequence: s2pul
Solvent: CD3OD
Ambient temperature
Mercury-400BB "hg402"

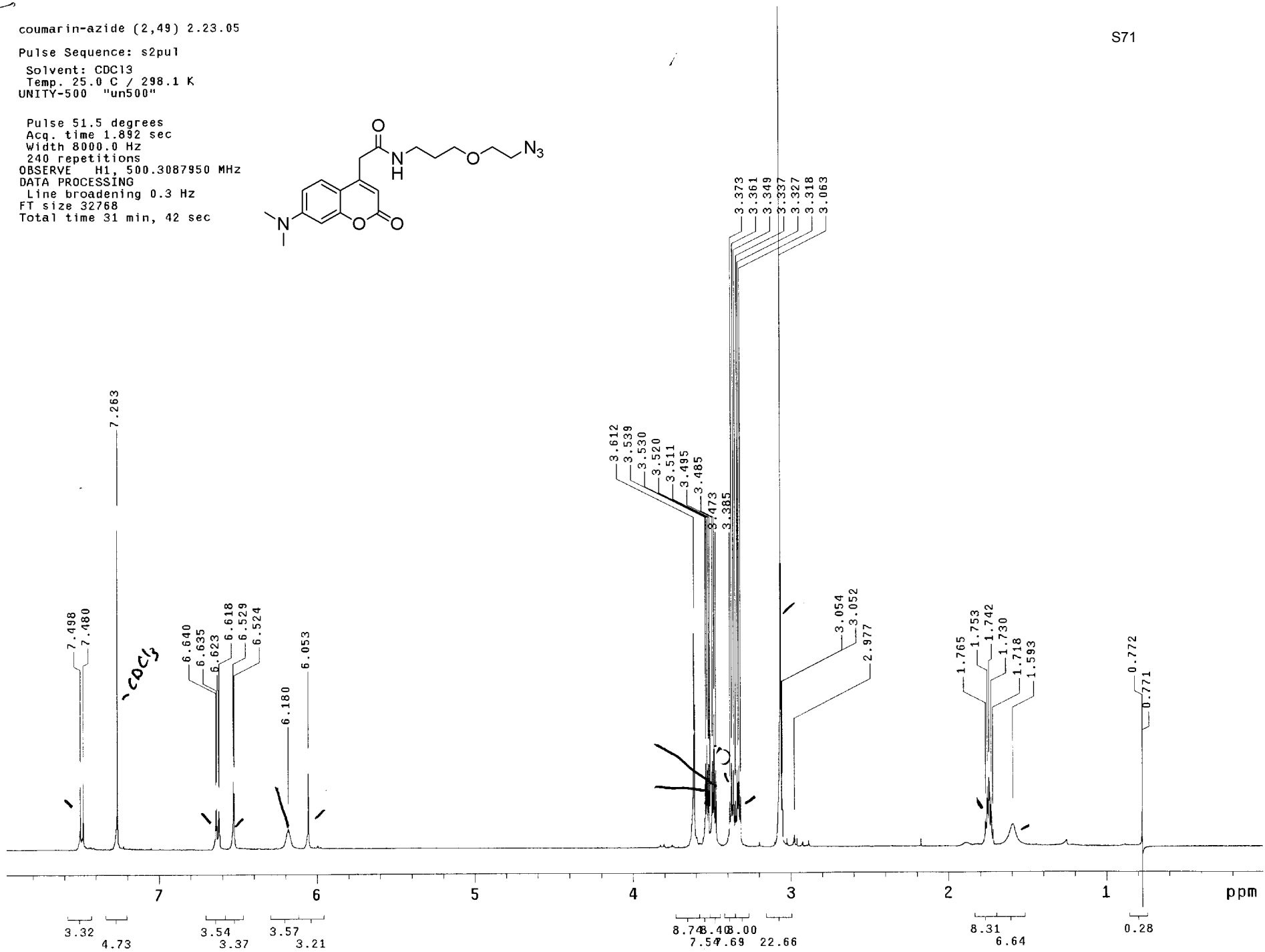
Pulse 53.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
1024 repetitions
OBSERVE C13, 100.6114650 MHz
DECOUPLE H1, 400.1269367 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 25 min, 1 sec



coumarin-azide (2,49) 2.23.05

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

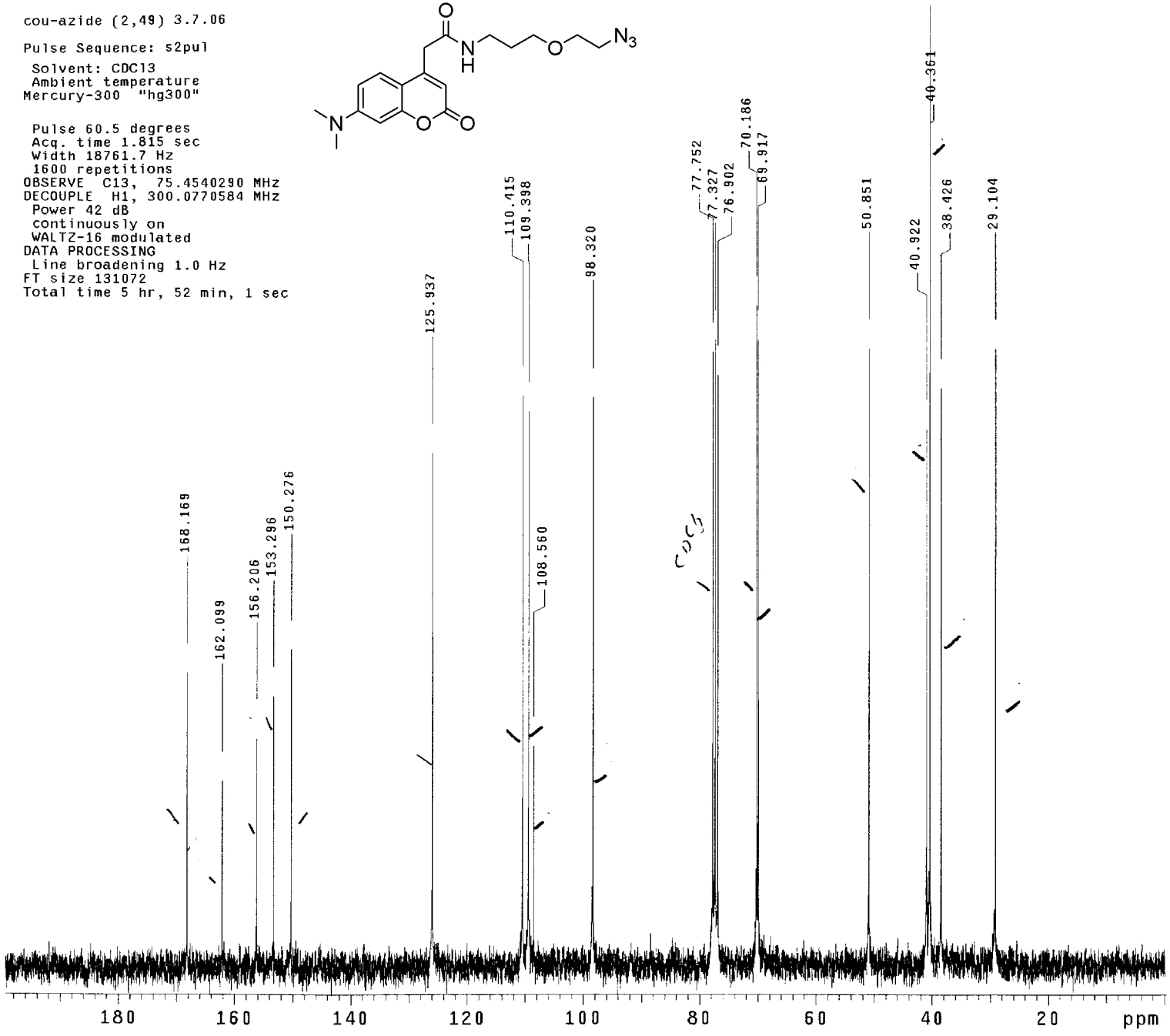
Pulse 51.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
240 repetitions
OBSERVE H1, 500.3087950 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec



cou-azide (2,49) 3.7.06

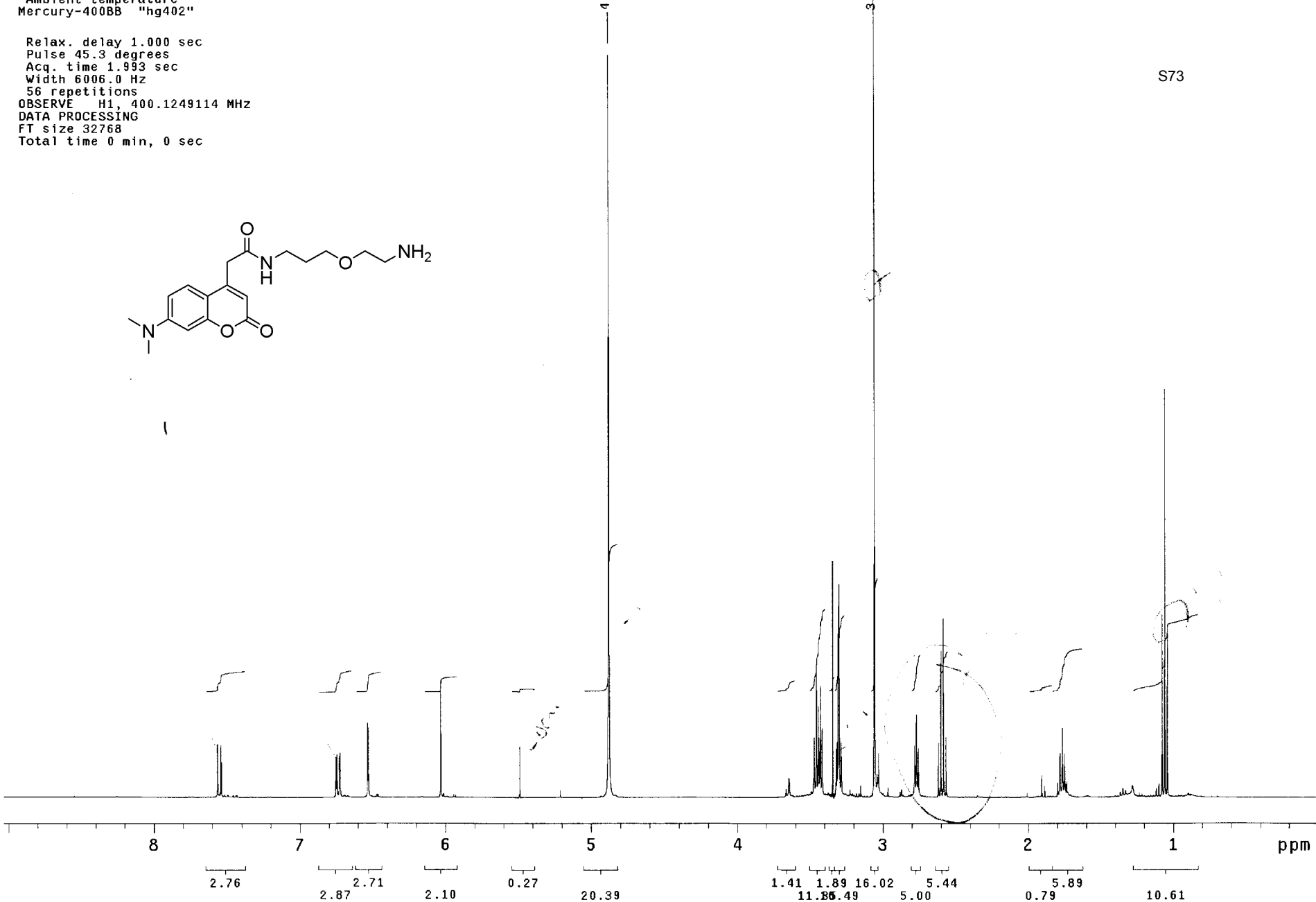
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
1600 repetitions
OBSERVE C13, 75.4540290 MHz
DECOUPLE H1, 300.0770584 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 5 hr, 52 min, 1 sec



Ambient temperature
Mercury-400BB "hg402"

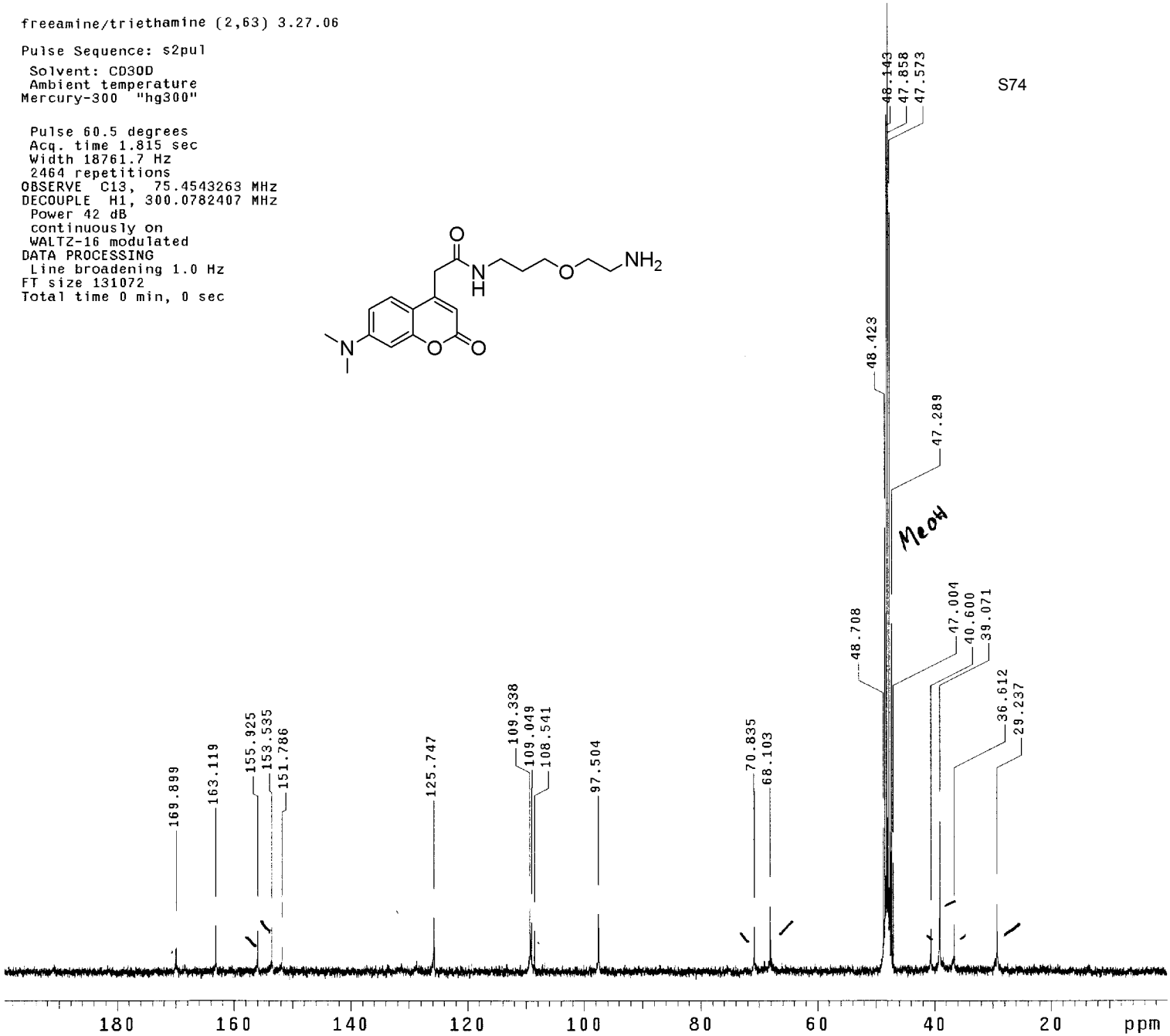
Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
56 repetitions
OBSERVE H1, 400.1249114 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec



freeamine/triethamine (2,63) 3.27.06

Pulse Sequence: s2pul
Solvent: CD3OD
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
2464 repetitions
OBSERVE C13, 75.4543263 MHz
DECOUPLE H1, 300.0782407 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 0 min, 0 sec

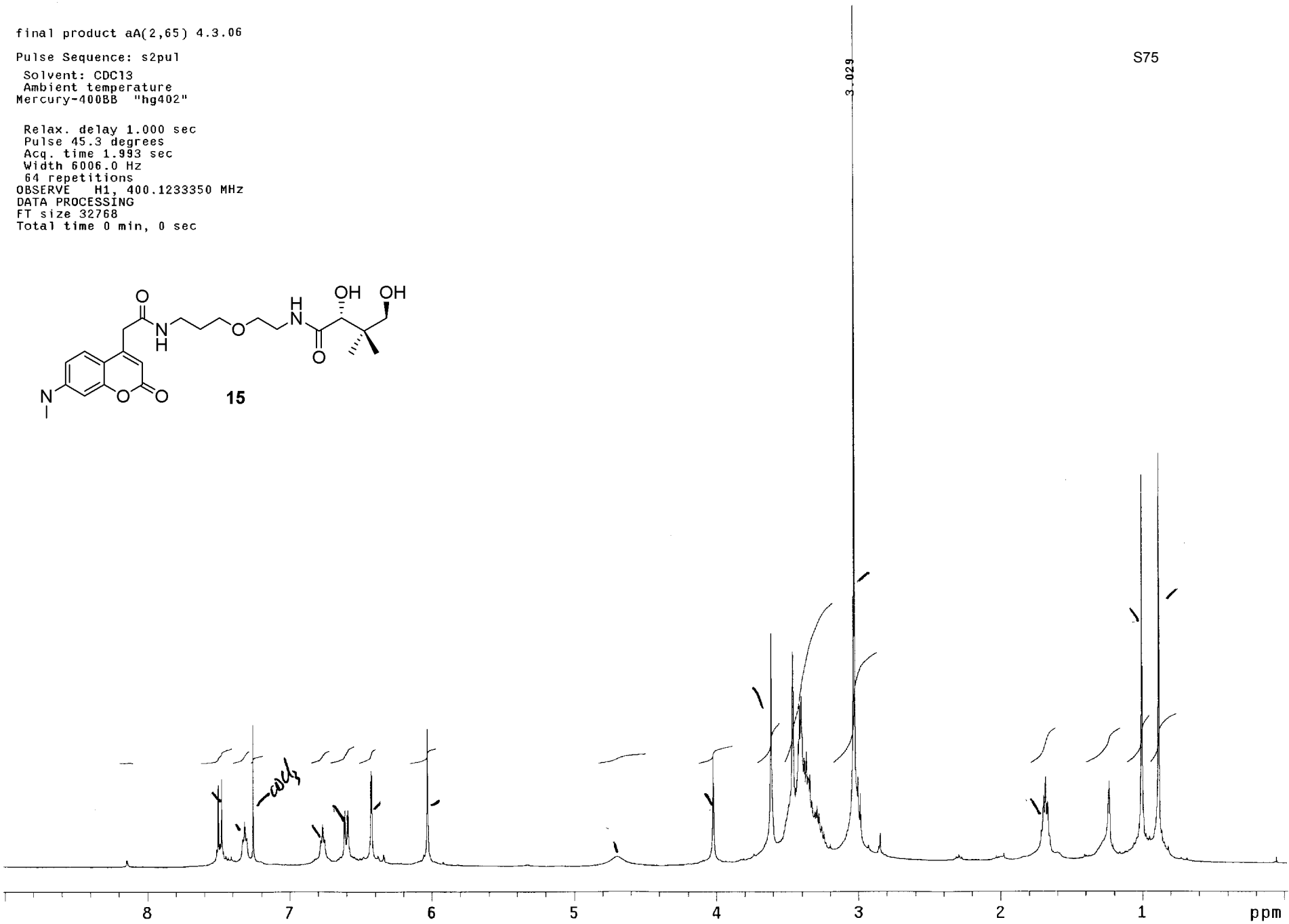


final product aA(2,65) 4.3.06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "hg402"

Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
64 repetitions
OBSERVE H1, 400.1233350 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec

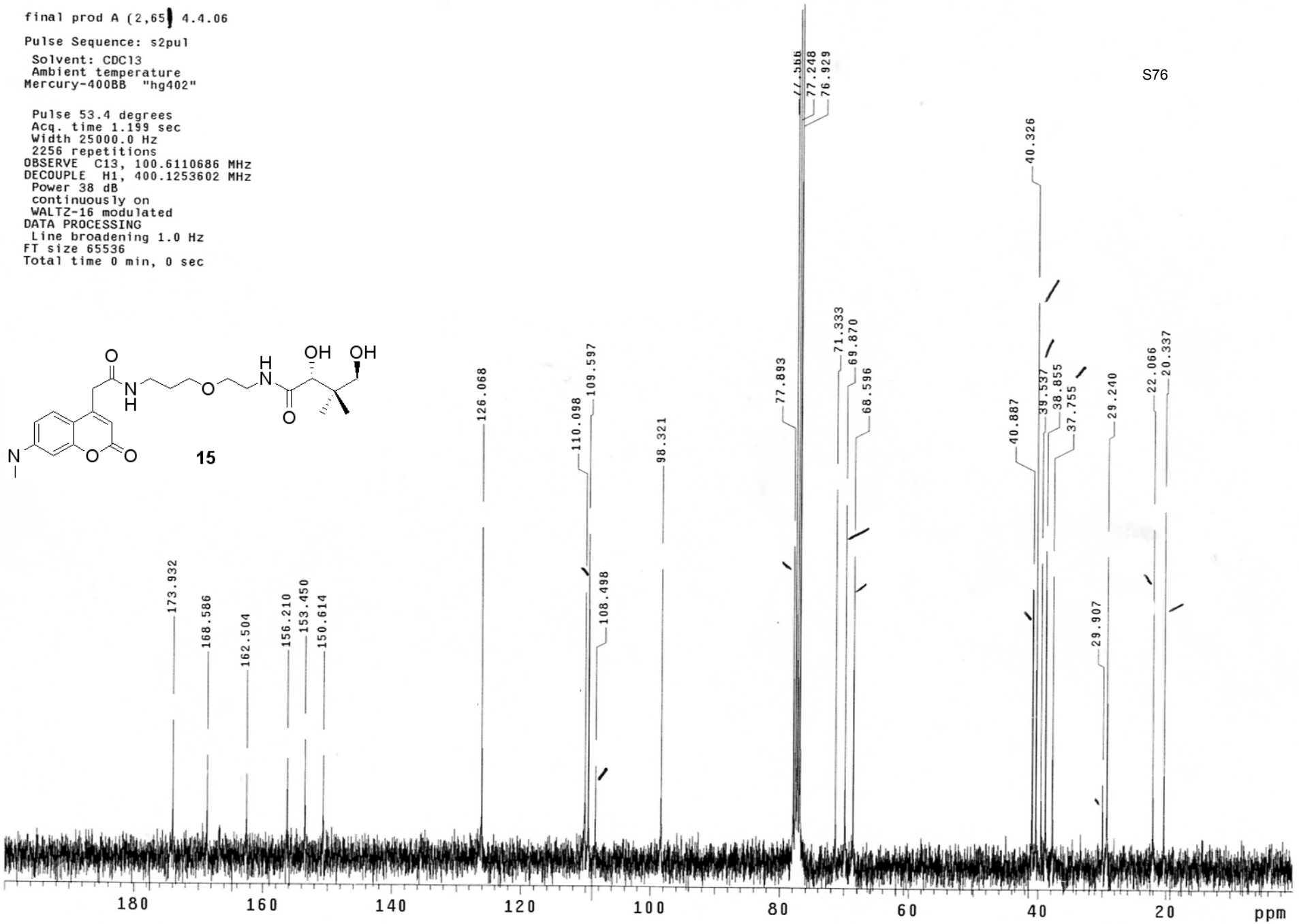
15

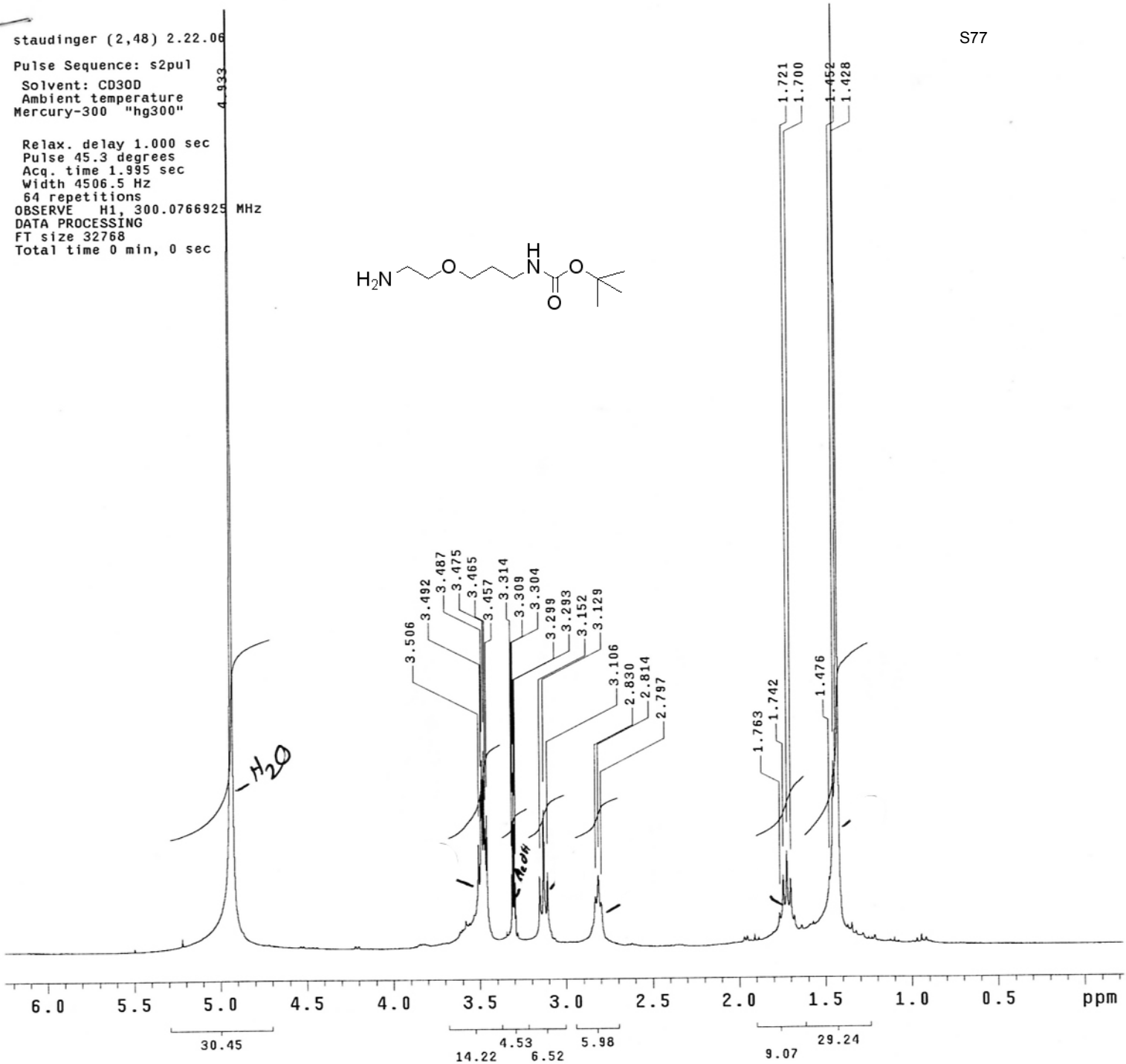


final prod A (2,65) 4.4.06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "hg402"

Pulse 53.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
2256 repetitions
OBSERVE C13, 100.6110686 MHz
DECOUPLE H1, 400.1253602 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 0 min, 0 sec

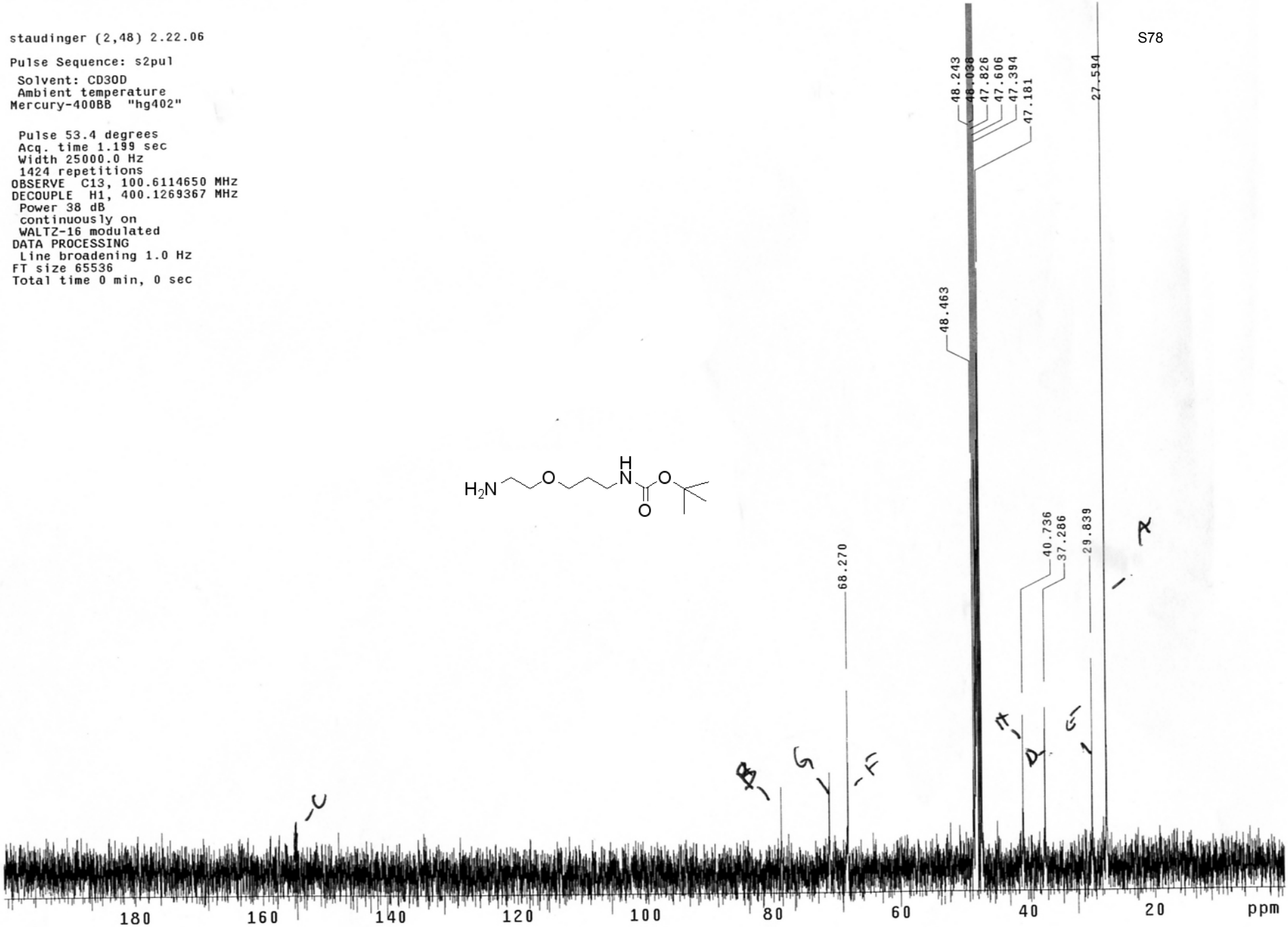



staudinger (2,48) 2.22.06
Pulse Sequence: s2pul
Solvent: CD3OD
Ambient temperature
Mercury-300 "hg300"
Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
64 repetitions
OBSERVE H1, 300.0766925 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec
4.933
H2N
O
H
N
O
O
H2O
MeOH
3.506
3.492
3.487
3.475
3.465
3.457
3.314
3.309
3.304
3.299
3.293
3.152
3.129
3.106
2.830
2.814
2.797
1.763
1.742
1.721
1.700
1.476
1.452
1.428
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
30.45
14.22
4.53
6.52
5.98
9.07
29.24

staudinger (2,48) 2.22.06

Pulse Sequence: s2pul
Solvent: CD3OD
Ambient temperature
Mercury-400BB "hg402"

Pulse 53.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
1424 repetitions
OBSERVE C13, 100.6114650 MHz
DECOUPLE H1, 400.1269367 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 0 min, 0 sec

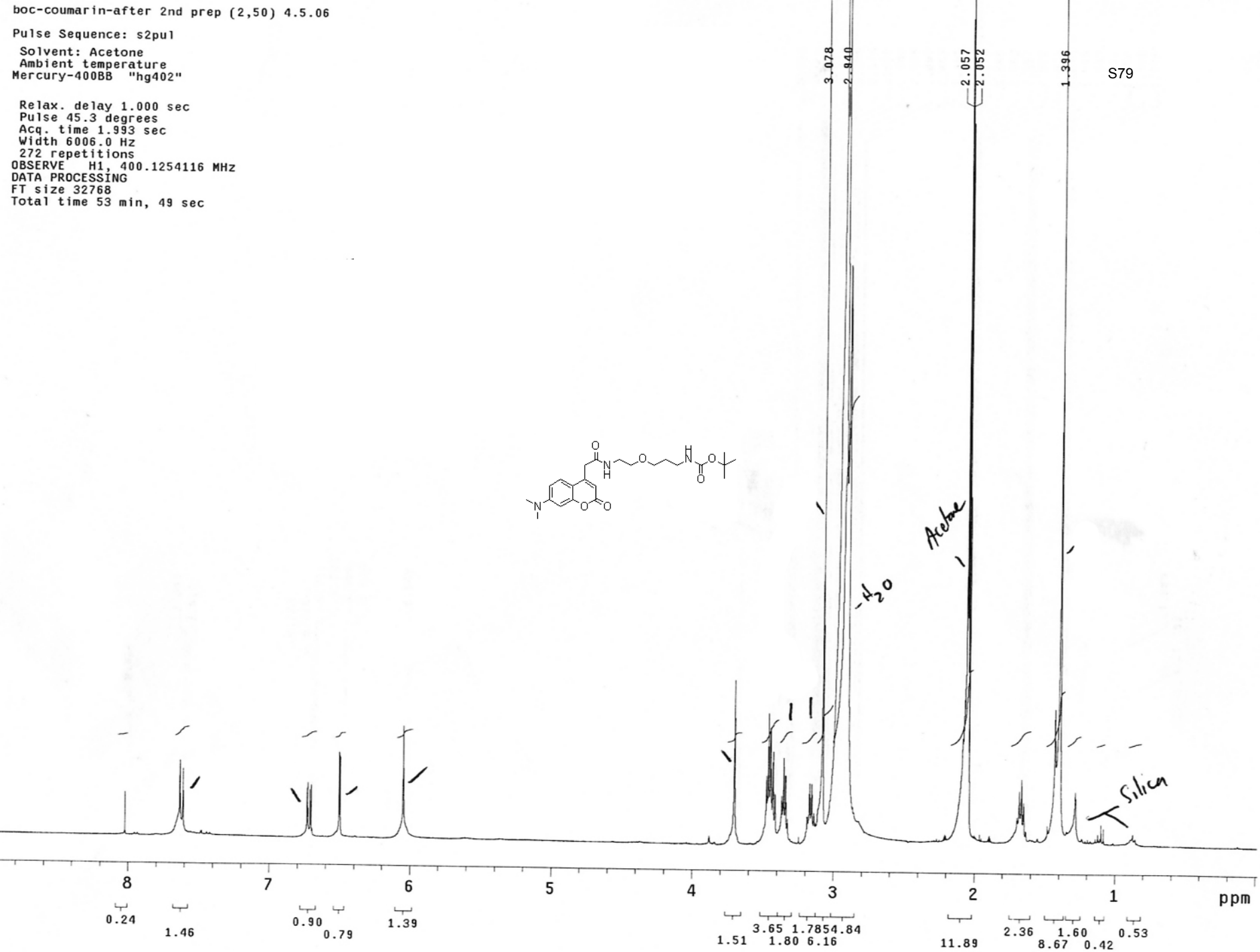


boc-coumarin-after 2nd prep (2,50) 4.5.06

Pulse Sequence: s2pul

Solvent: Acetone
Ambient temperature
Mercury-400BB "hg402"

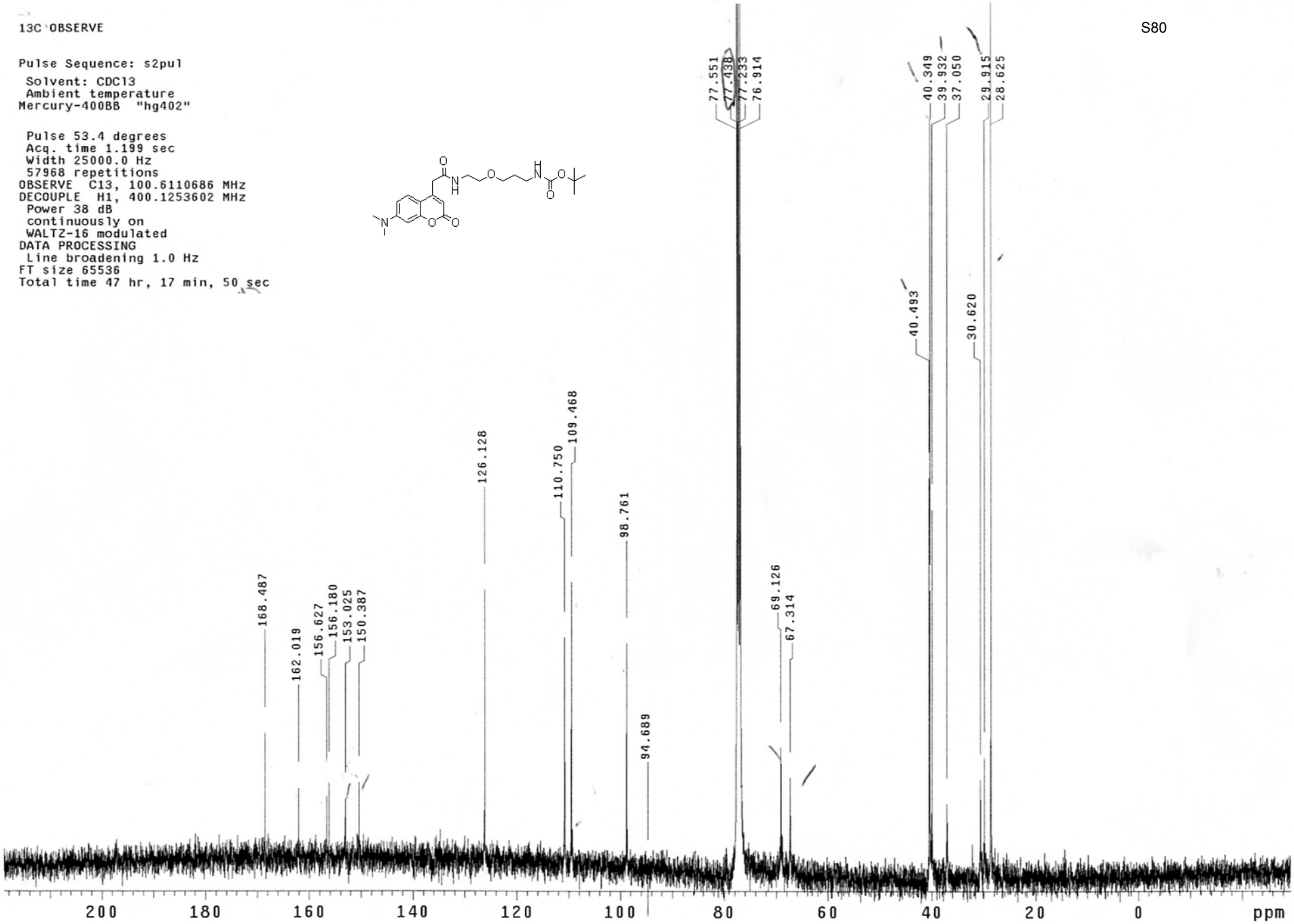
Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
272 repetitions
OBSERVE H1, 400.1254116 MHz
DATA PROCESSING
FT size 32768
Total time 53 min, 49 sec



13C OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "hg402"

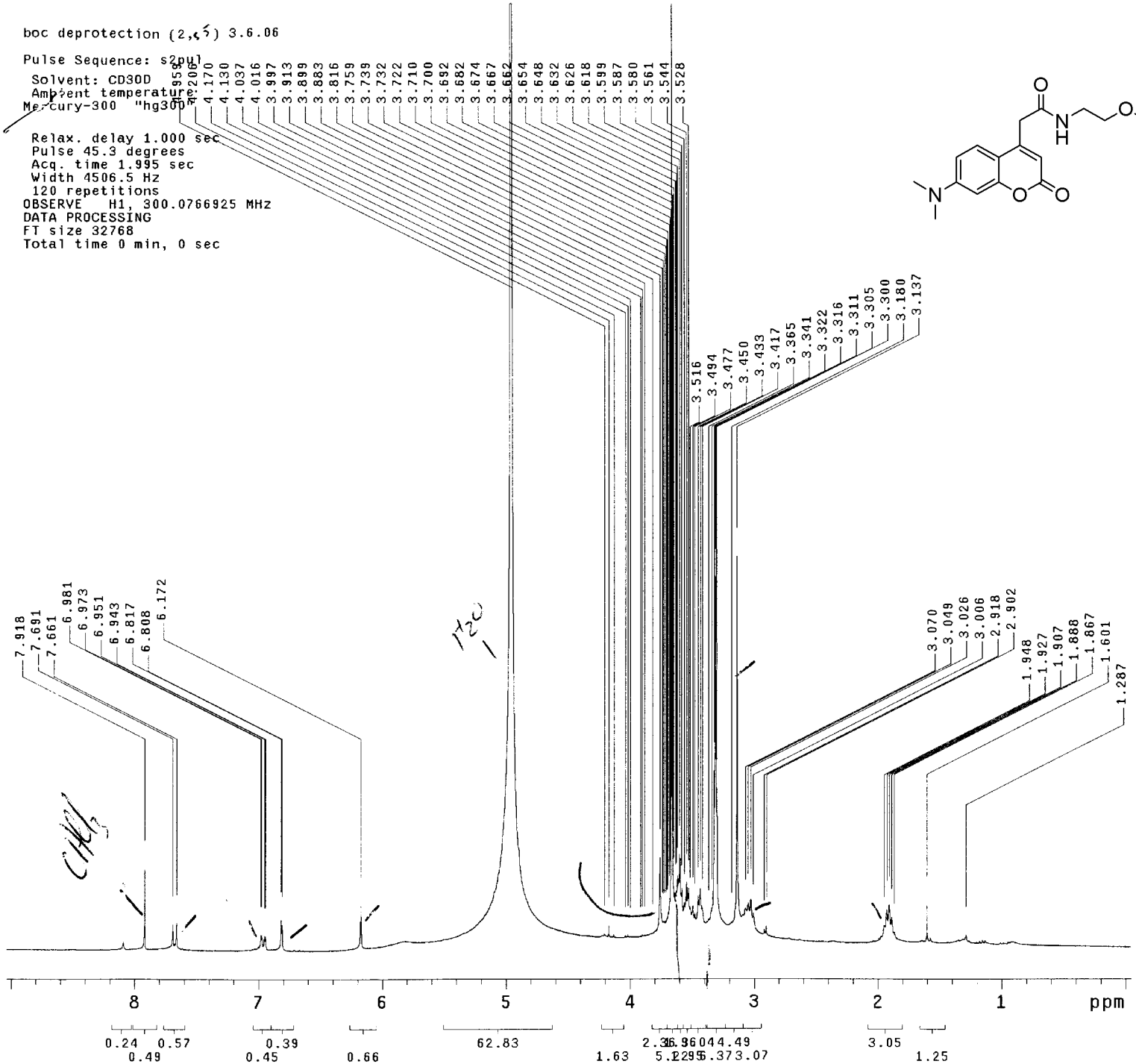
Pulse 53.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
57968 repetitions
OBSERVE C13, 100.6110686 MHz
DECOUPLE H1, 400.1253602 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 47 hr, 17 min, 50 sec

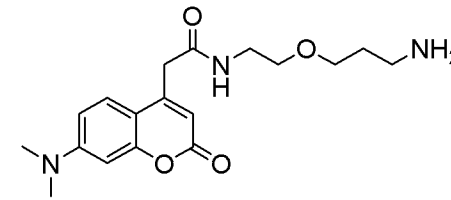


boc deprotection (2,5) 3.6.06

Pulse Sequence: s2pul
Solvent: CD3OD
Ambient temperature
Mercury-300 "hg300"

Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
120 repetitions
OBSERVE H1, 300.0766925 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec

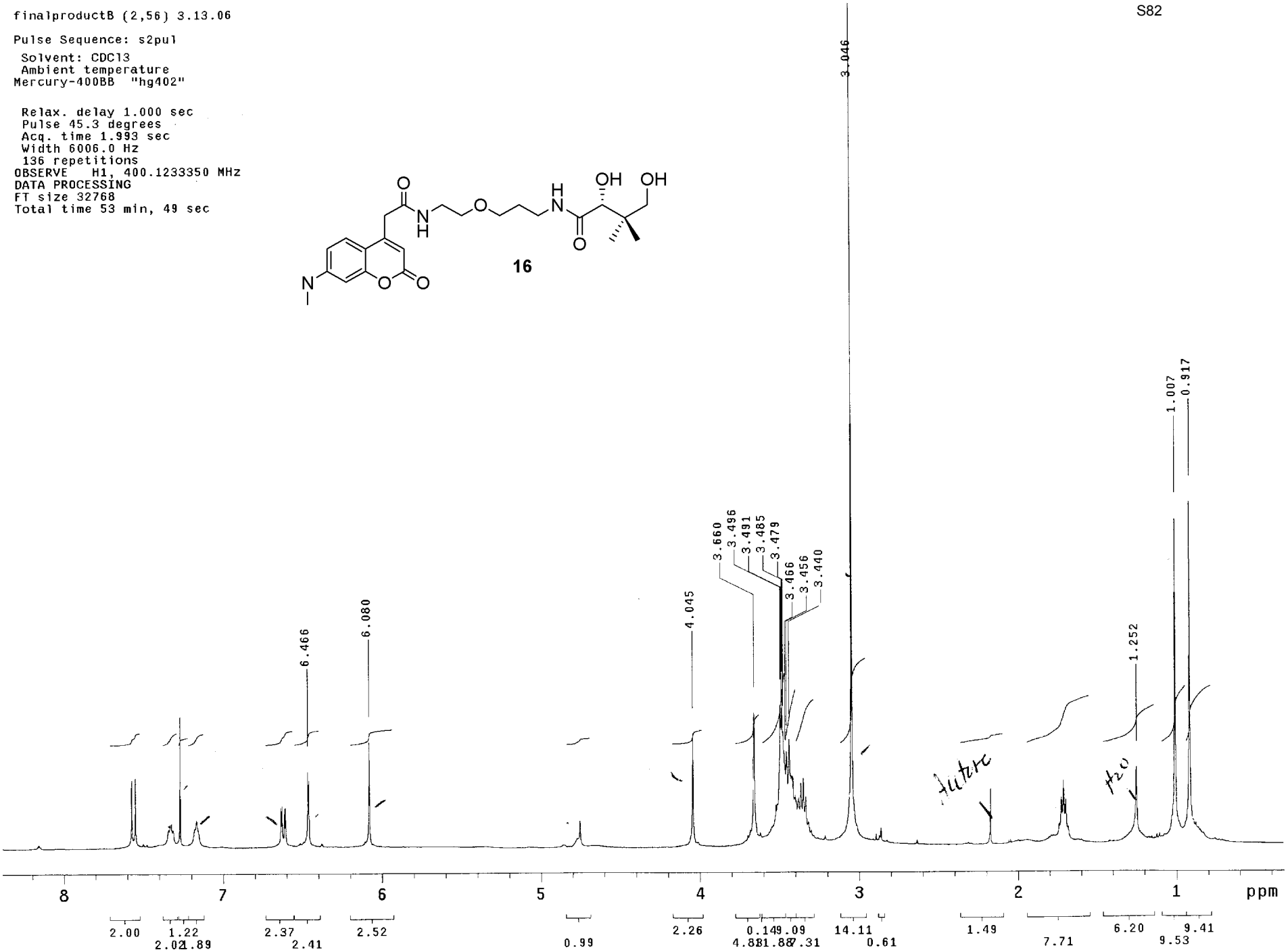




finalproductB (2,56) 3.13.06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "hg402"

Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
136 repetitions
OBSERVE H1, 400.1233350 MHz
DATA PROCESSING
FT size 32768
Total time 53 min, 49 sec

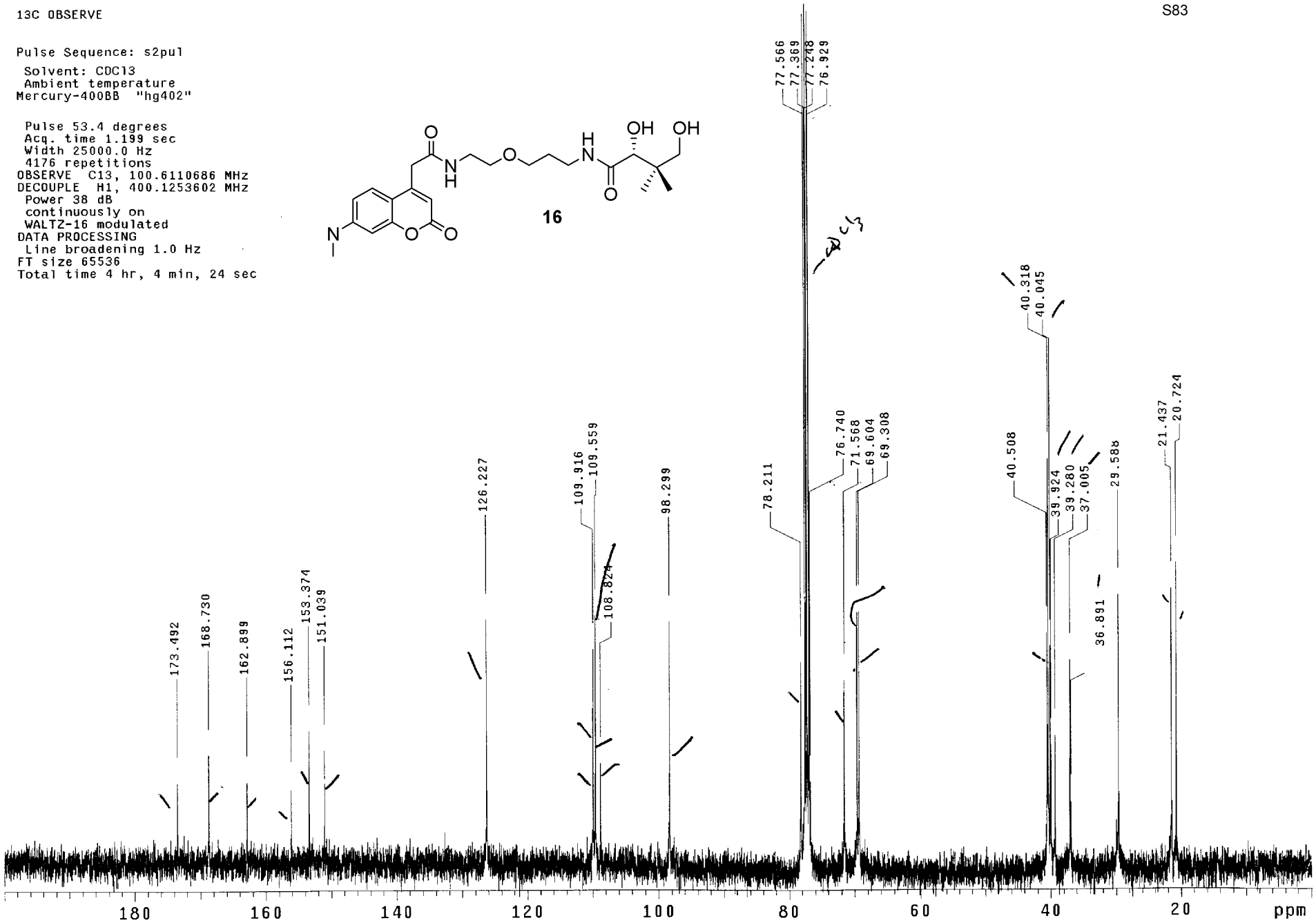


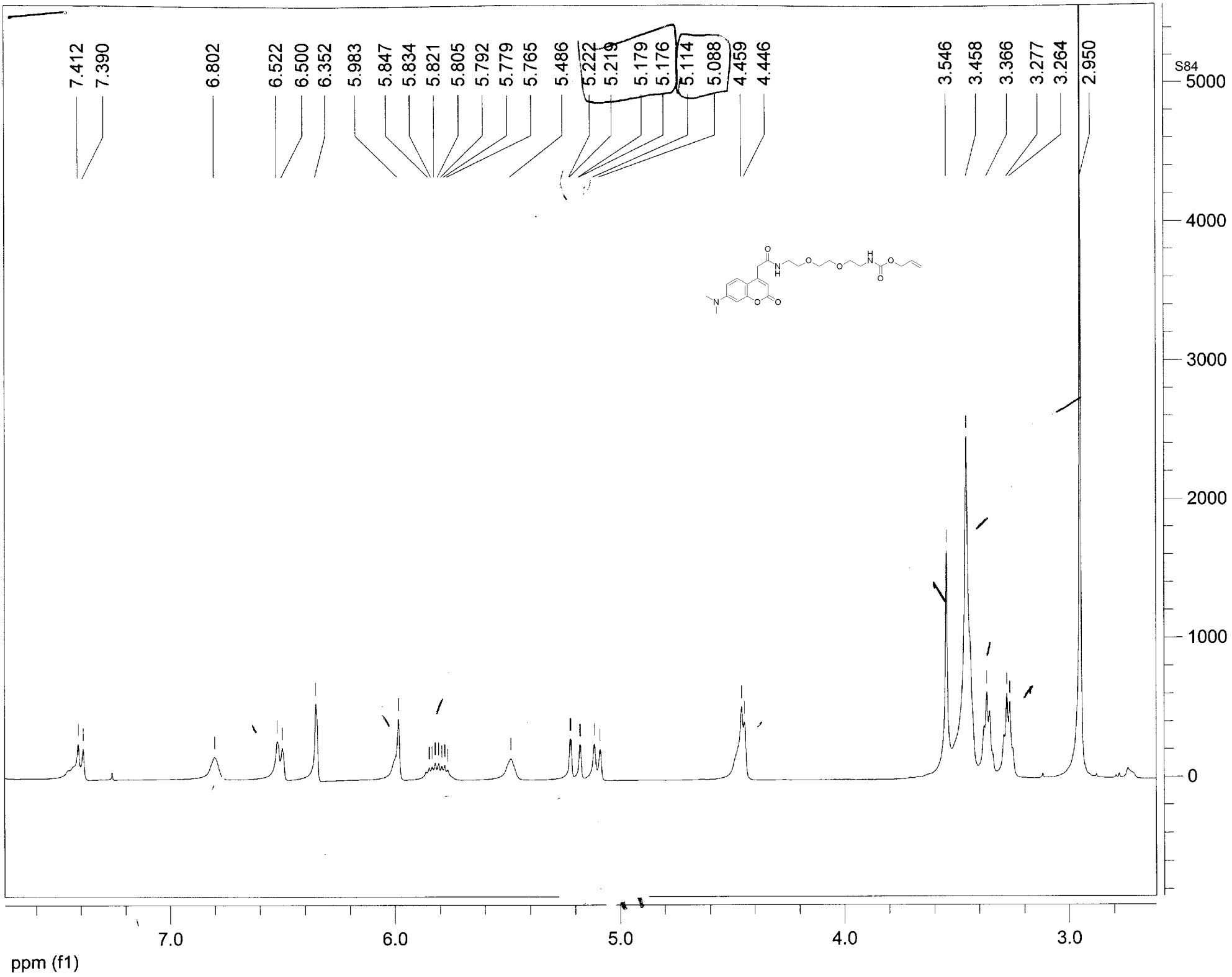
13C OBSERVE

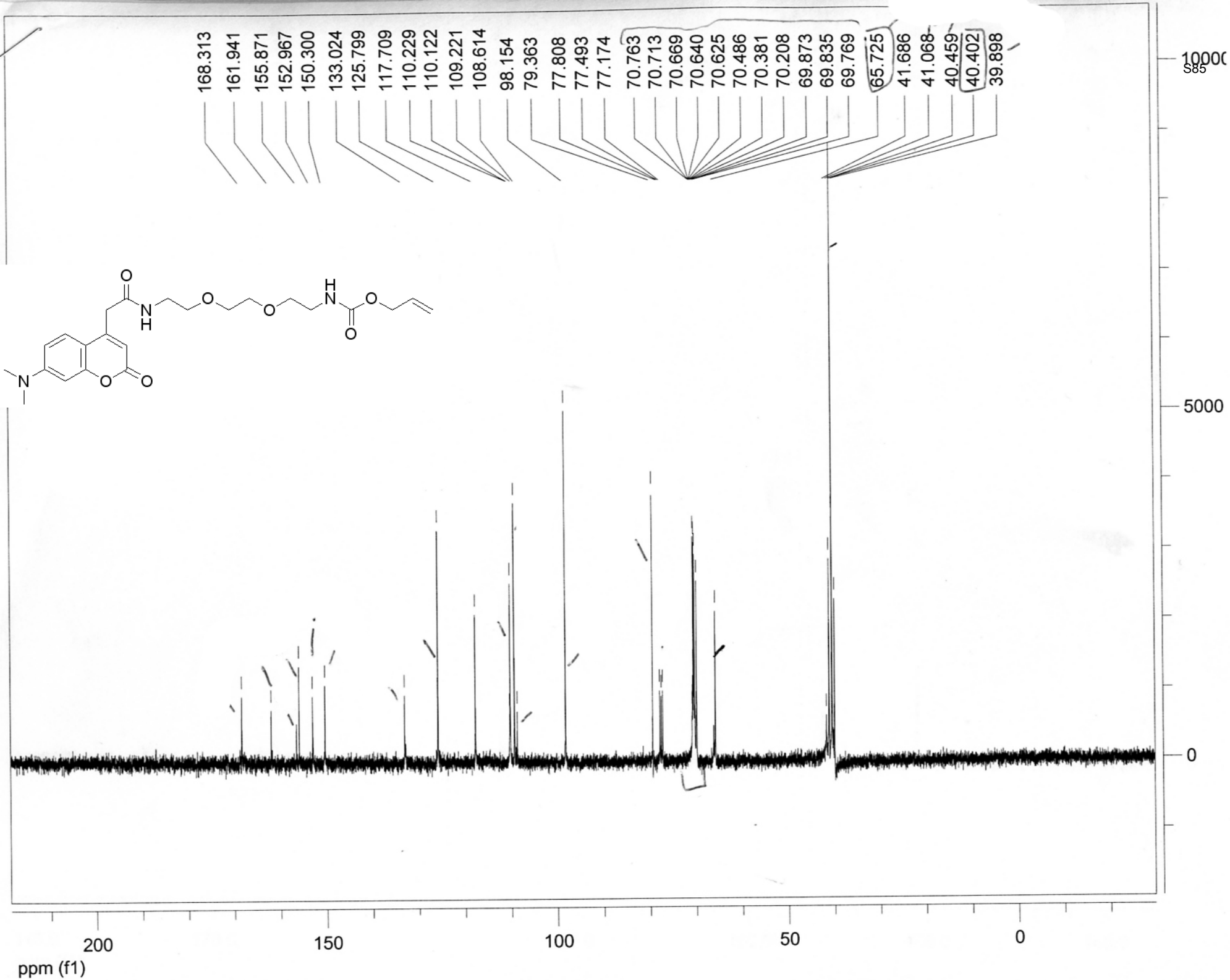
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "hg402"

Pulse 53.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
4176 repetitions
OBSERVE C13, 100.6110686 MHz
DECOUPLE H1, 400.1253602 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 4 hr, 4 min, 24 sec

16



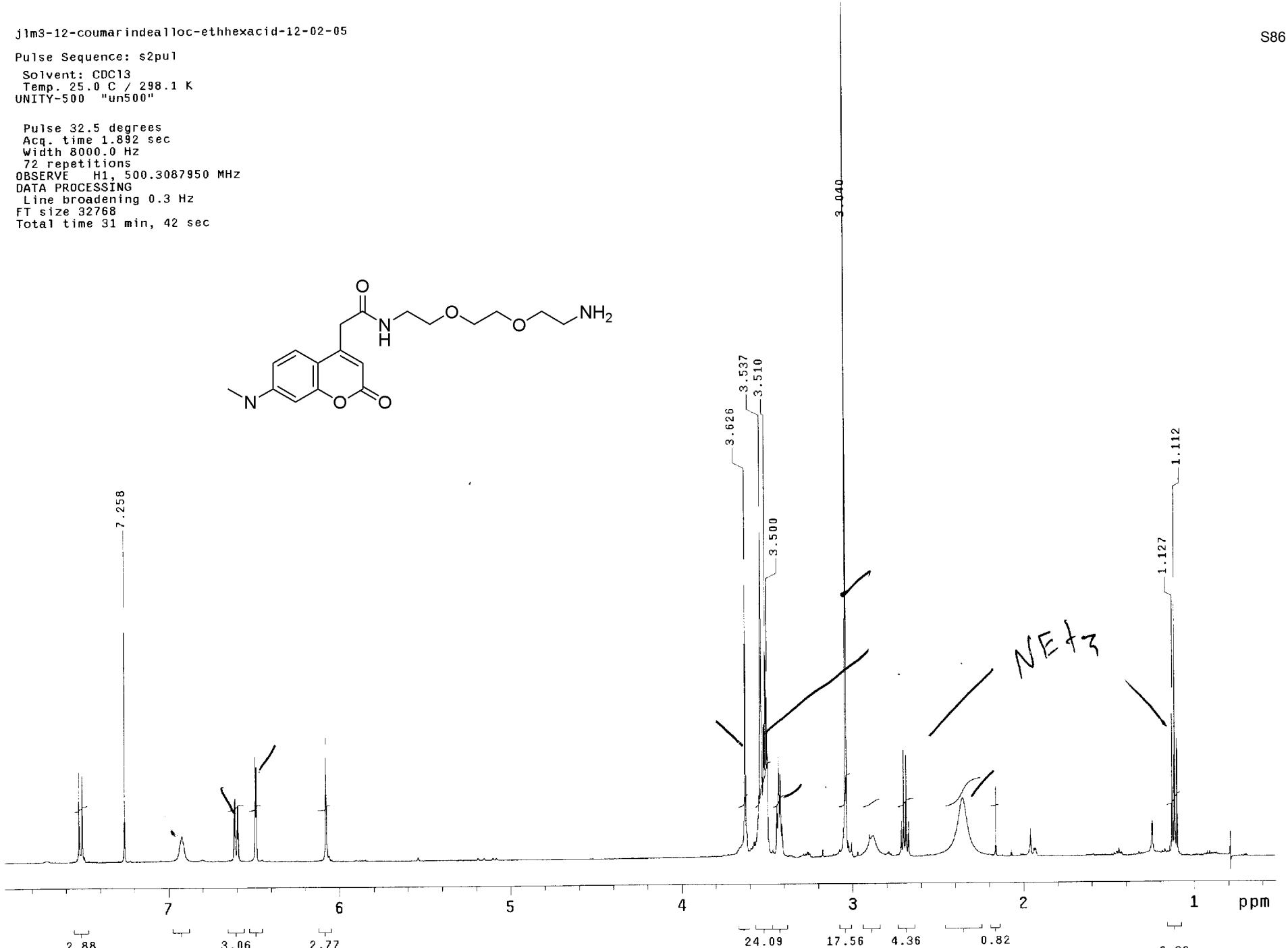

7.412
7.390
6.802
6.522
6.500
6.352
5.983
5.847
5.834
5.821
5.805
5.792
5.779
5.765
5.486
5.222
5.219
5.179
5.176
5.114
5.088
4.459
4.446
3.546
3.458
3.366
3.277
3.264
2.950
S84
5000
4000
3000
2000
1000
0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

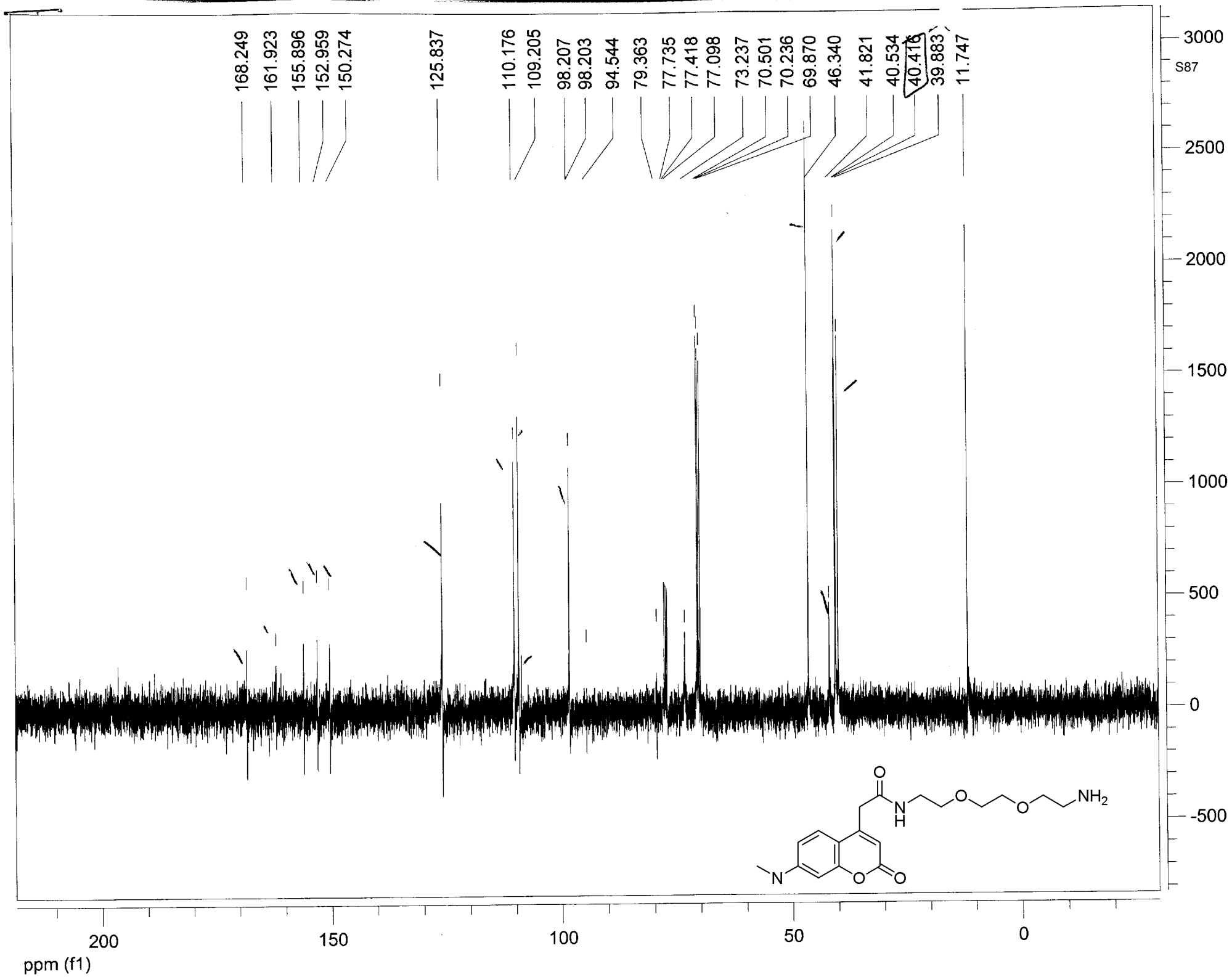

168.313
161.941
155.871
152.967
150.300
133.024
125.799
117.709
110.229
110.122
109.221
108.614
98.154
79.363
77.808
77.493
77.174
70.763
70.713
70.669
70.640
70.625
70.486
70.381
70.208
69.873
69.835
69.769
65.725
41.686
41.068
40.459
40.402
39.898
10000
5000
0
200
150
100
50
0
ppm (f1)

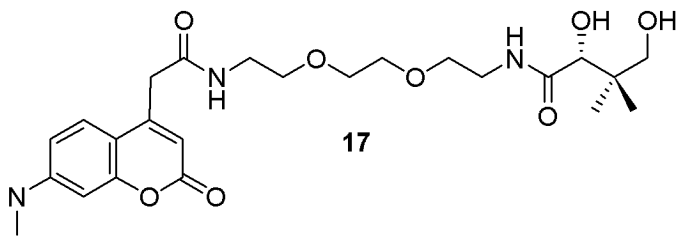
jlm3-12-coumarindealloc-ethhexacid-12-02-05

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

Pulse 32.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
72 repetitions
OBSERVE H1, 500.3087950 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec




168.249
161.923
155.896
152.959
150.274
125.837
110.176
109.205
98.207
98.203
94.544
79.363
77.735
77.418
77.098
73.237
70.501
70.236
69.870
46.340
41.821
40.534
40.416
39.883
11.747
3000
2500
2000
1500
1000
500
0
-500
200
150
100
50
0
ppm (f1)
O
N
H
O
O
NH2
N
O
O



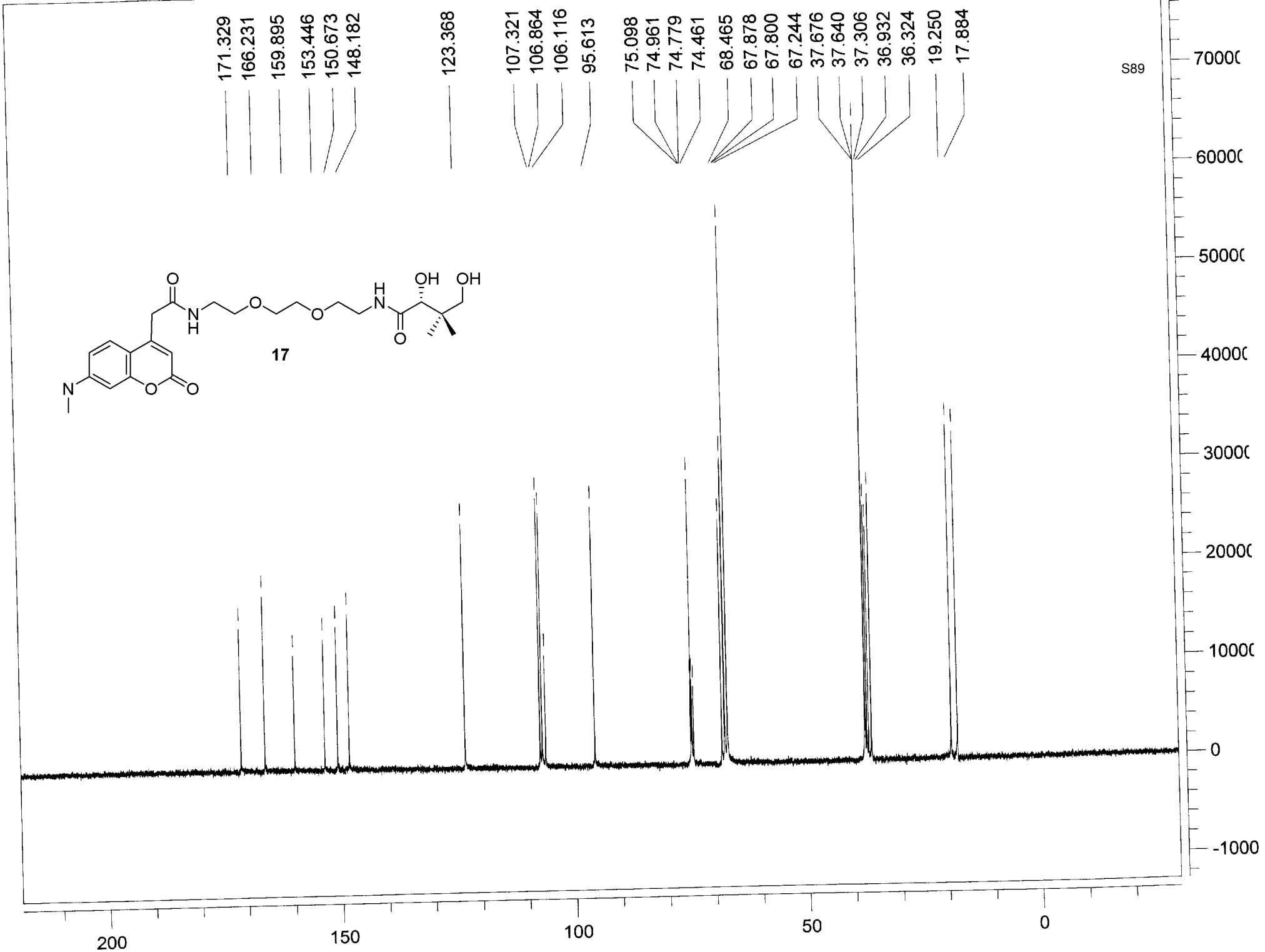
7.537 7.519 7.259 6.641 6.636 6.623 6.618 6.508 6.502 6.064 4.036 3.628 3.541 3.533 3.528 3.524 3.521 3.513 3.509 3.503 3.498 3.491 3.480 3.455 3.398 3.056 1.042 0.907

1.00 1.16 0.86 0.85 1.00 2.57 3.45 6.97 3.90 3.96 3.80

10000 5000 0

10.0 5.0 0.0

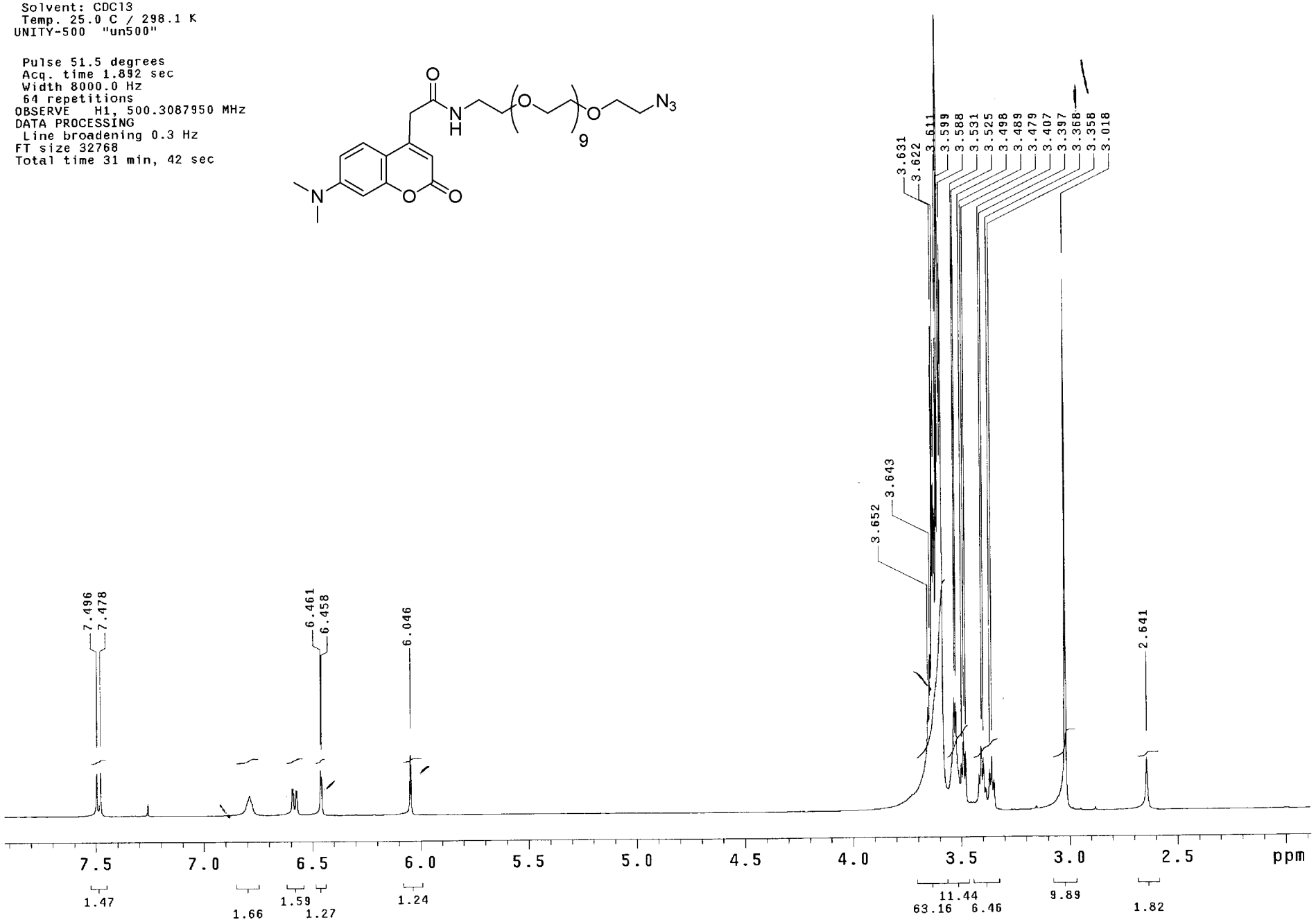
ppm (t1)


171.329
166.231
159.895
153.446
150.673
148.182
123.368
107.321
106.864
106.116
95.613
75.098
74.961
74.779
74.461
68.465
67.878
67.800
67.244
37.676
37.640
37.306
36.932
36.324
19.250
17.884
17
S89
200
150
100
50
0
70000
60000
50000
40000
30000
20000
10000
0
-1000

jlm3-51-PEG10coumarin-cutfromrow8and9-02-06-06

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

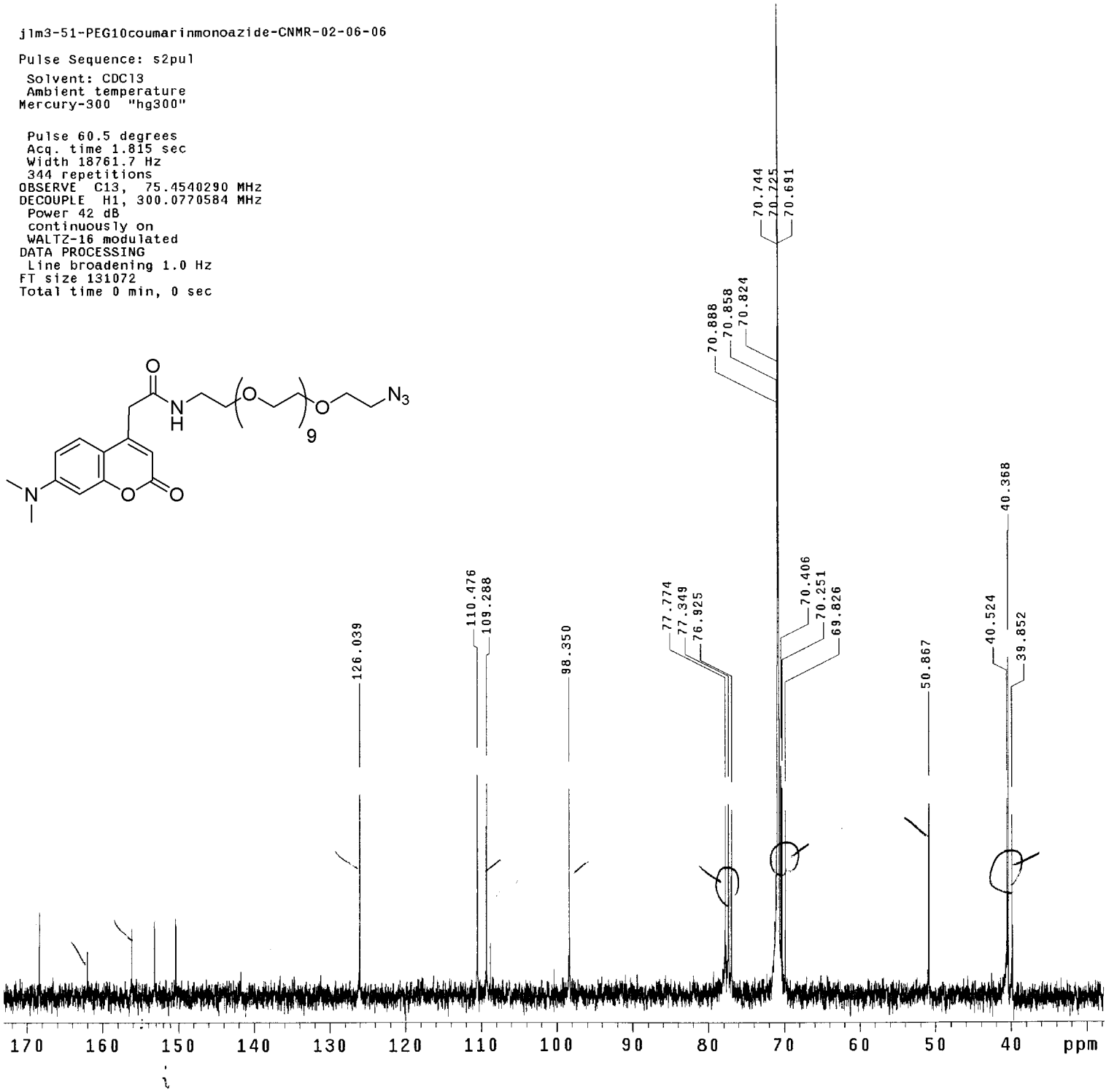
Pulse 51.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
64 repetitions
OBSERVE H1, 500.3087950 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec



jlm3-51-PEG10coumarinmonoazide-CNMR-02-06-06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

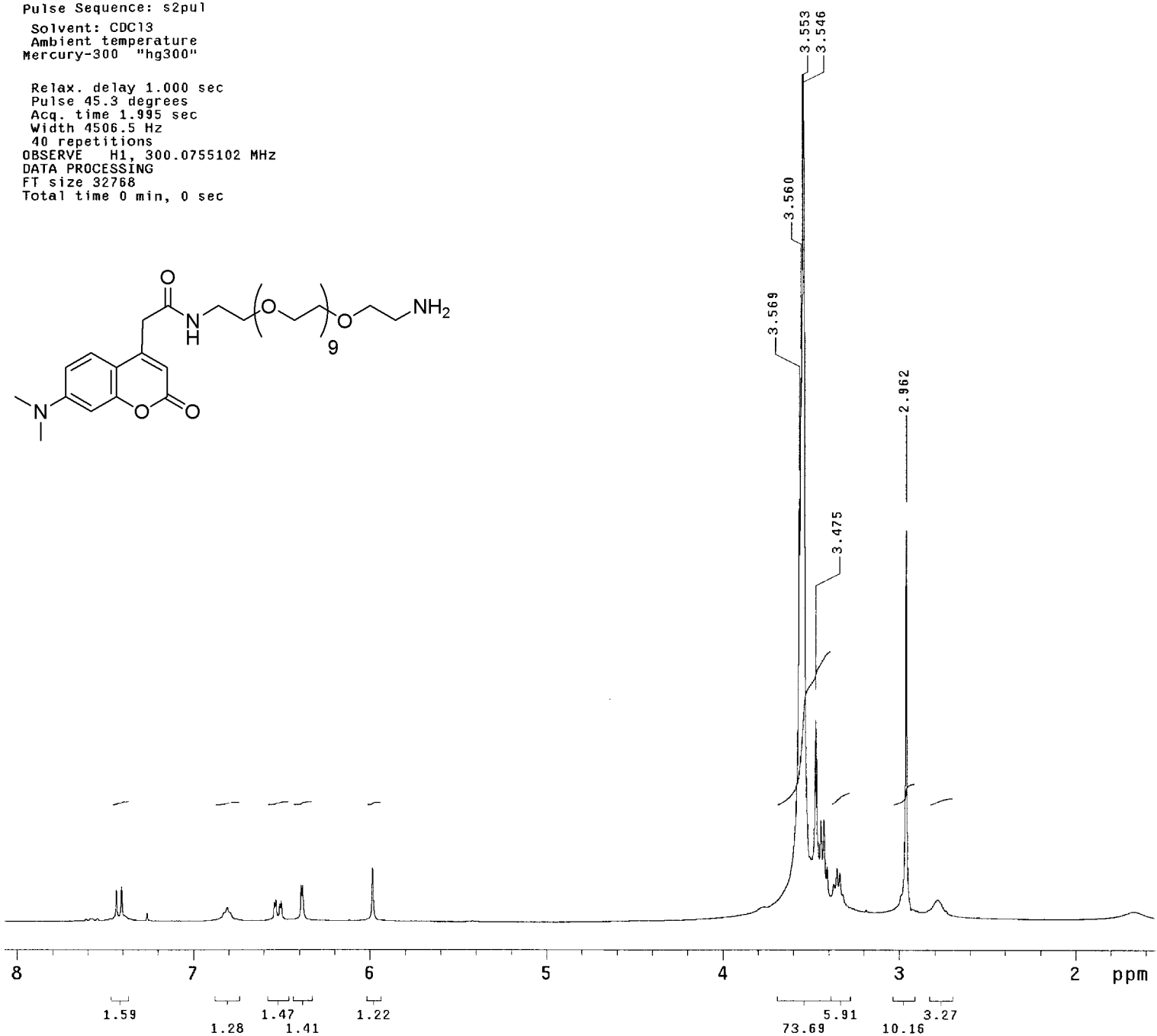
Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
344 repetitions
OBSERVE C13, 75.4540290 MHz
DECOUPLE H1, 300.0770584 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 0 min, 0 sec



jlm3-54-PEG10coumarin-staudingertoamine-lyophilate-02-07-06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

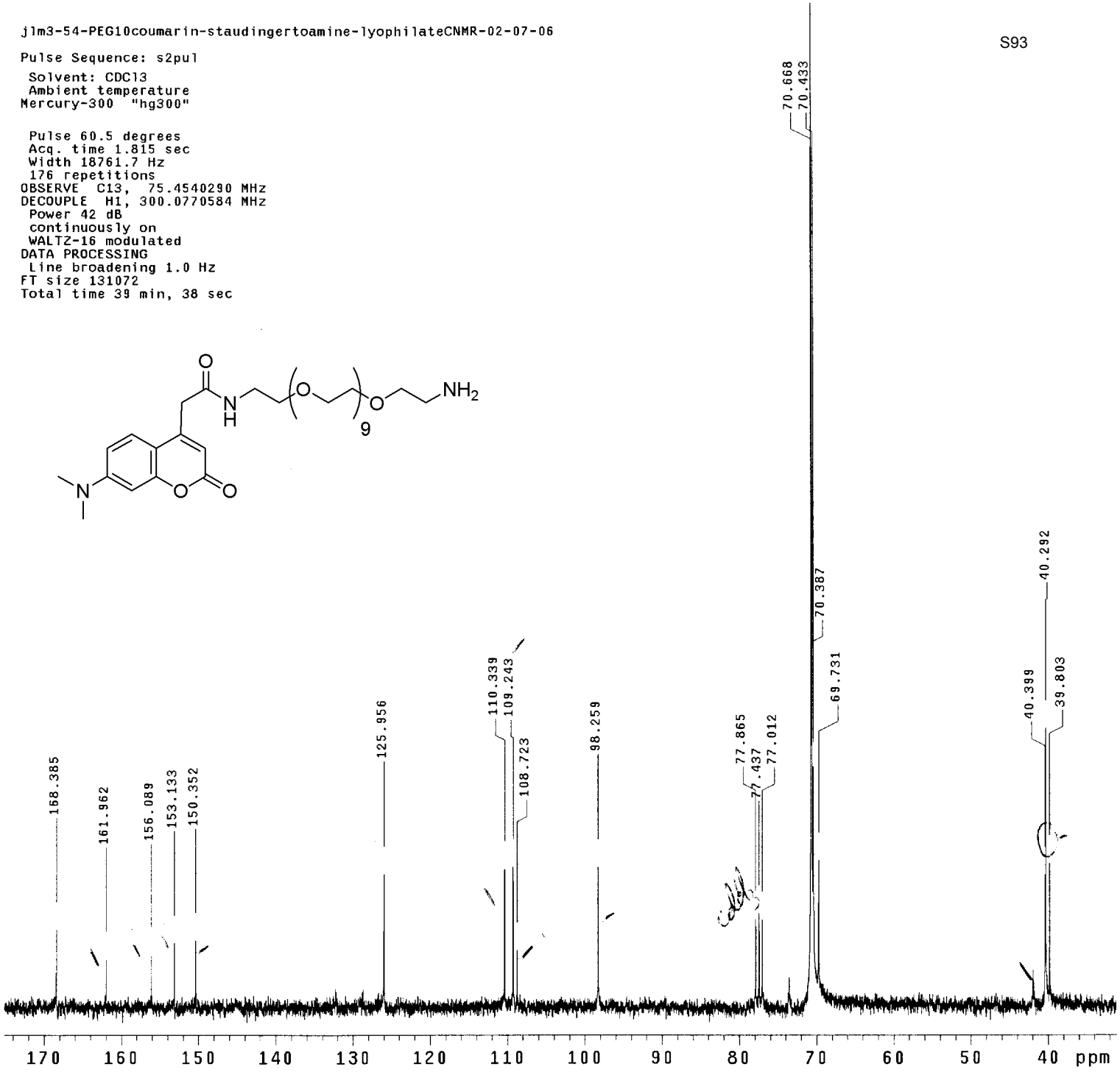
Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
40 repetitions
OBSERVE H1, 300.0755102 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec



jlm3-54-PEG10coumarin-staudingertoamine-lyophilateCNMR-02-07-06

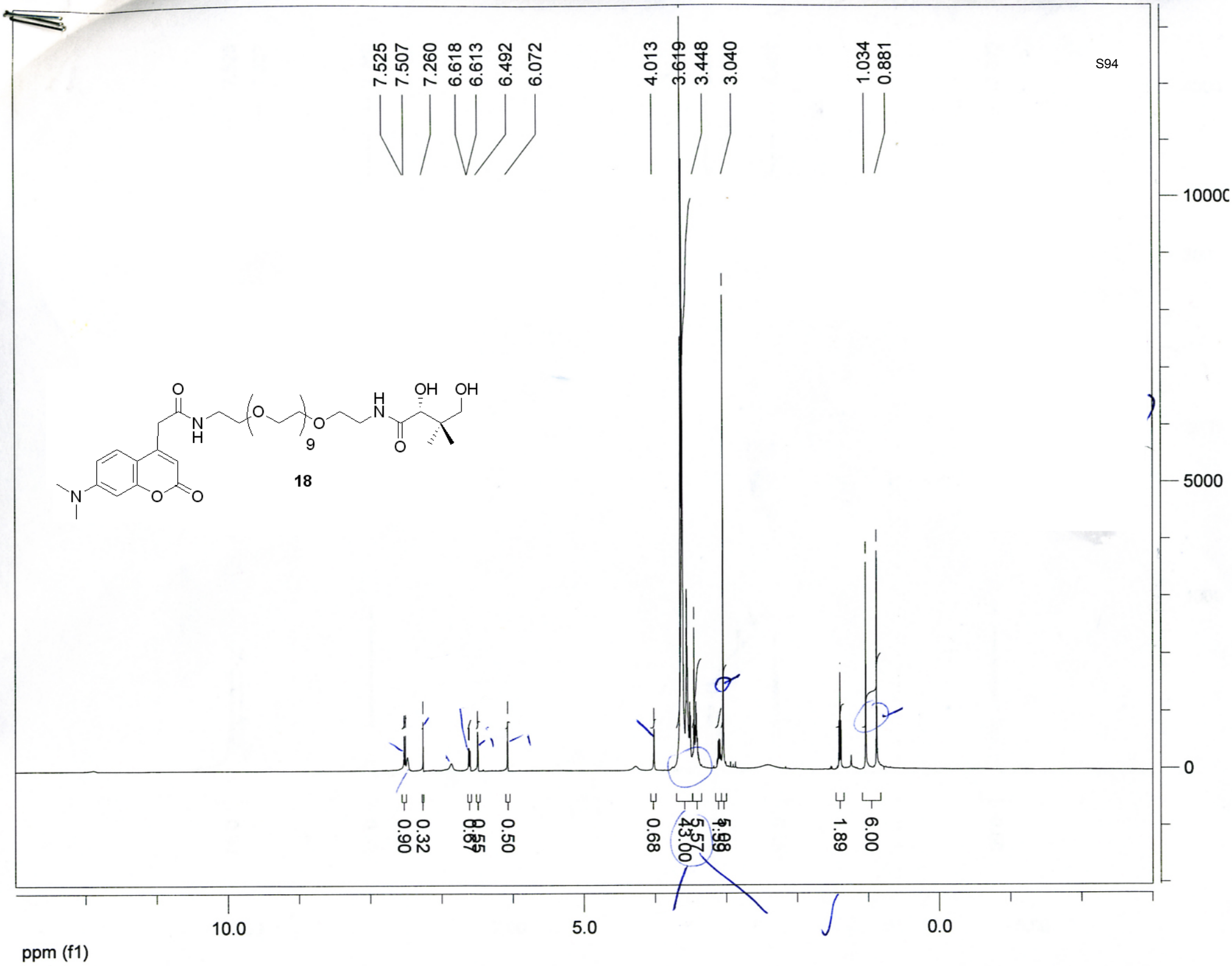
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

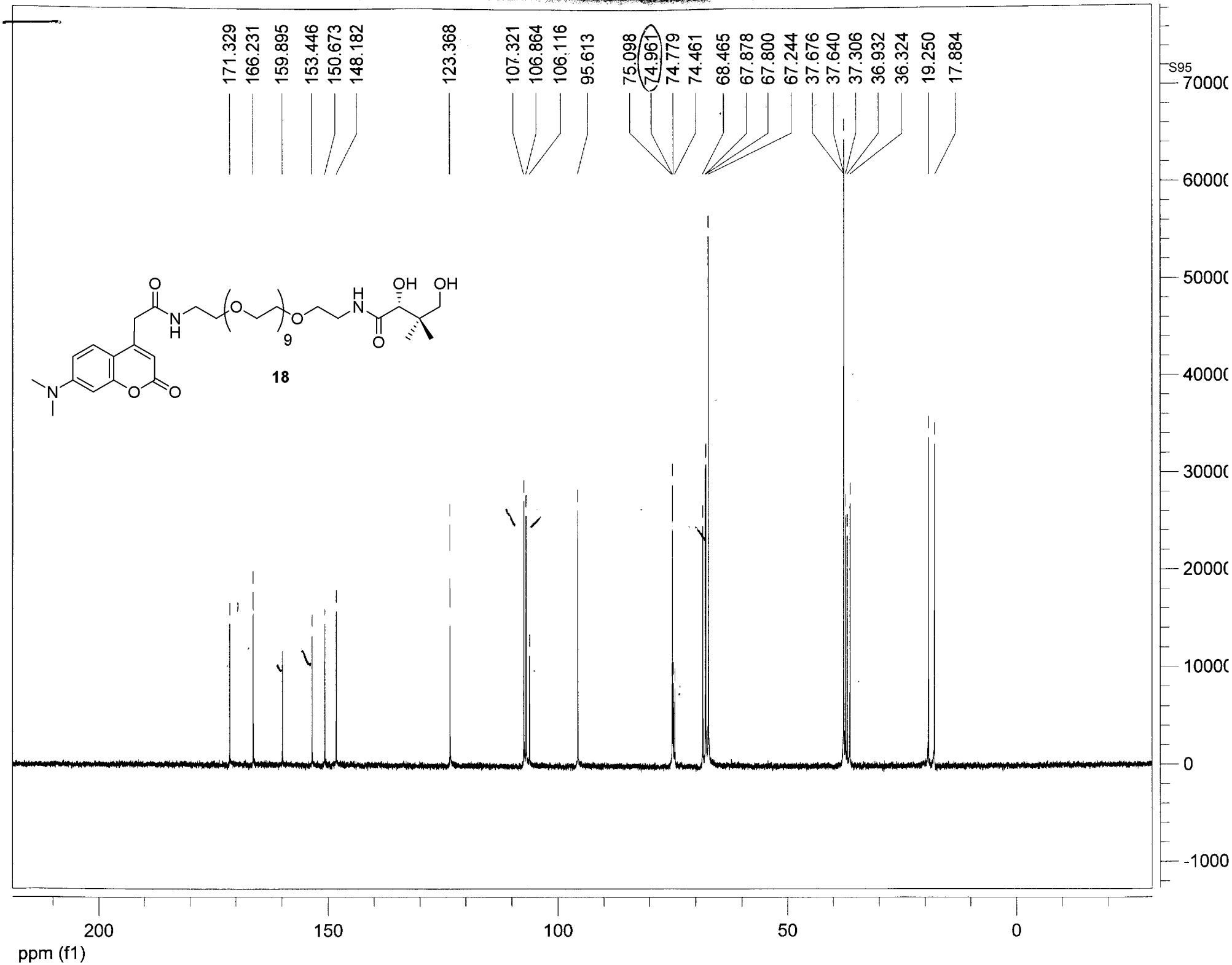
Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
176 repetitions
OBSERVE C13, 75.4540290 MHz
DECOUPLE H1, 300.0770584 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 39 min, 38 sec



O
N
H
O
9
H
N
OH
OH
O
N
O
O

18

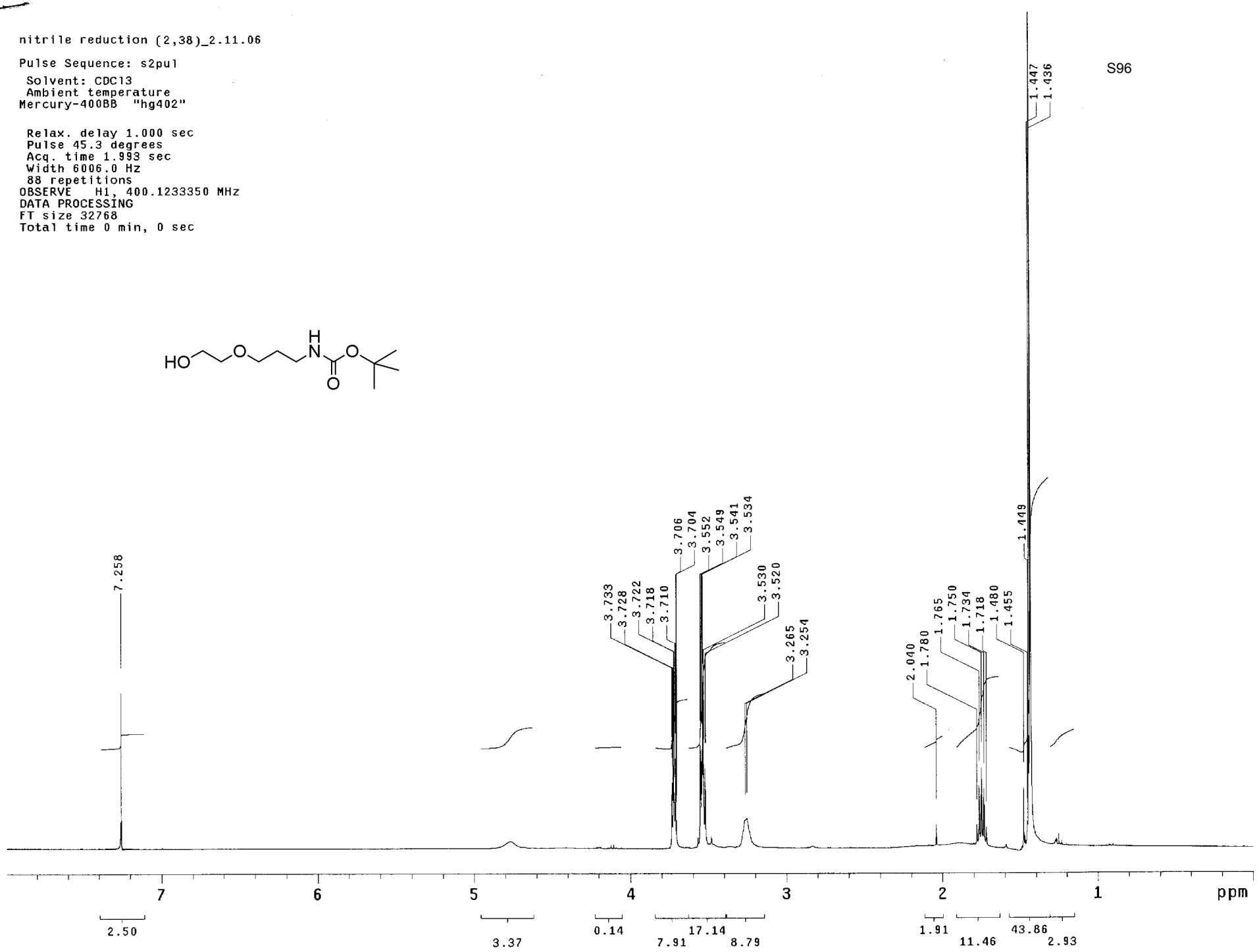

7.525
7.507
7.260
6.618
6.613
6.492
6.072
4.013
3.619
3.448
3.040
1.034
0.881
10000
5000
0
0.90
0.32
0.87
0.55
0.50
0.89
43.00
5.57
4.98
1.89
6.00
10.0
5.0
0.0
ppm (f1)


171.329
166.231
159.895
153.446
150.673
148.182
123.368
107.321
106.864
106.116
95.613
75.098
74.961
74.779
74.461
68.465
67.878
67.800
67.244
37.676
37.640
37.306
36.932
36.324
19.250
17.884
18
ppm (f1)

nitrile reduction (2,38)_2.11.06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "hg402"

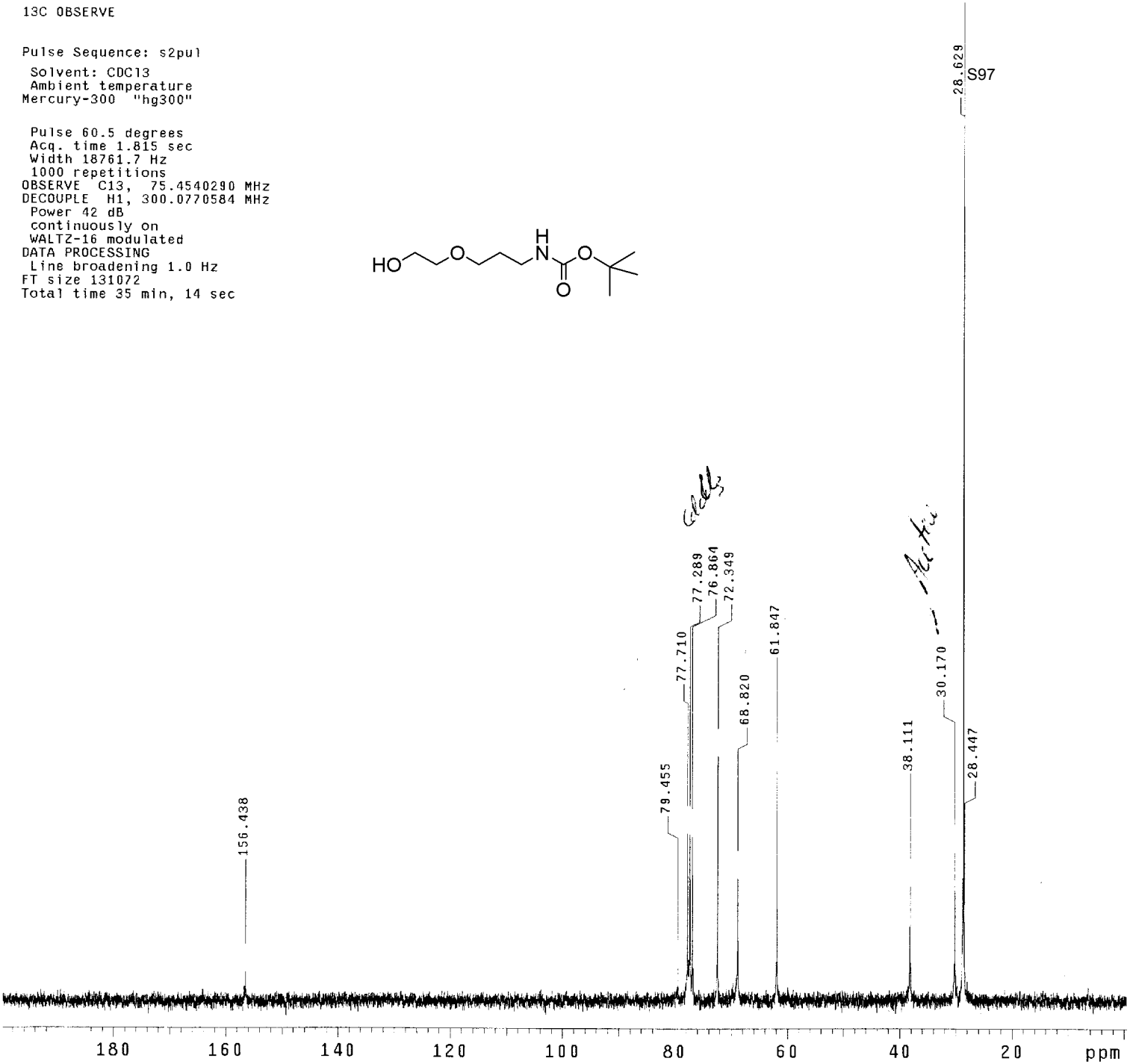
Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.993 sec
Width 6006.0 Hz
88 repetitions
OBSERVE H1, 400.1233350 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec



13C OBSERVE

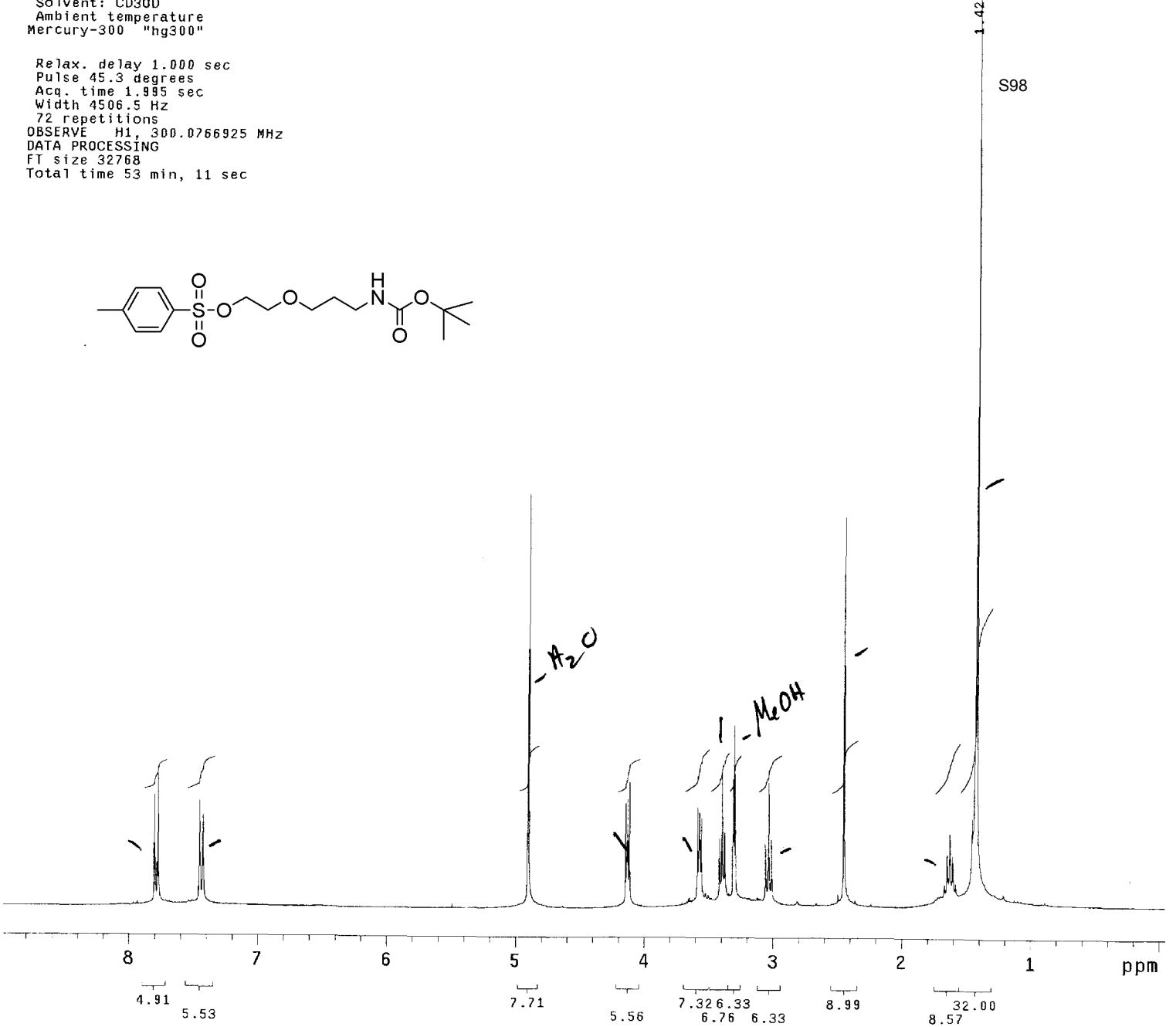
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
1000 repetitions
OBSERVE C13, 75.4540290 MHz
DECOUPLE H1, 300.0770584 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 35 min, 14 sec



Solvent: CD3OD
Ambient temperature
Mercury-300 "hg300"

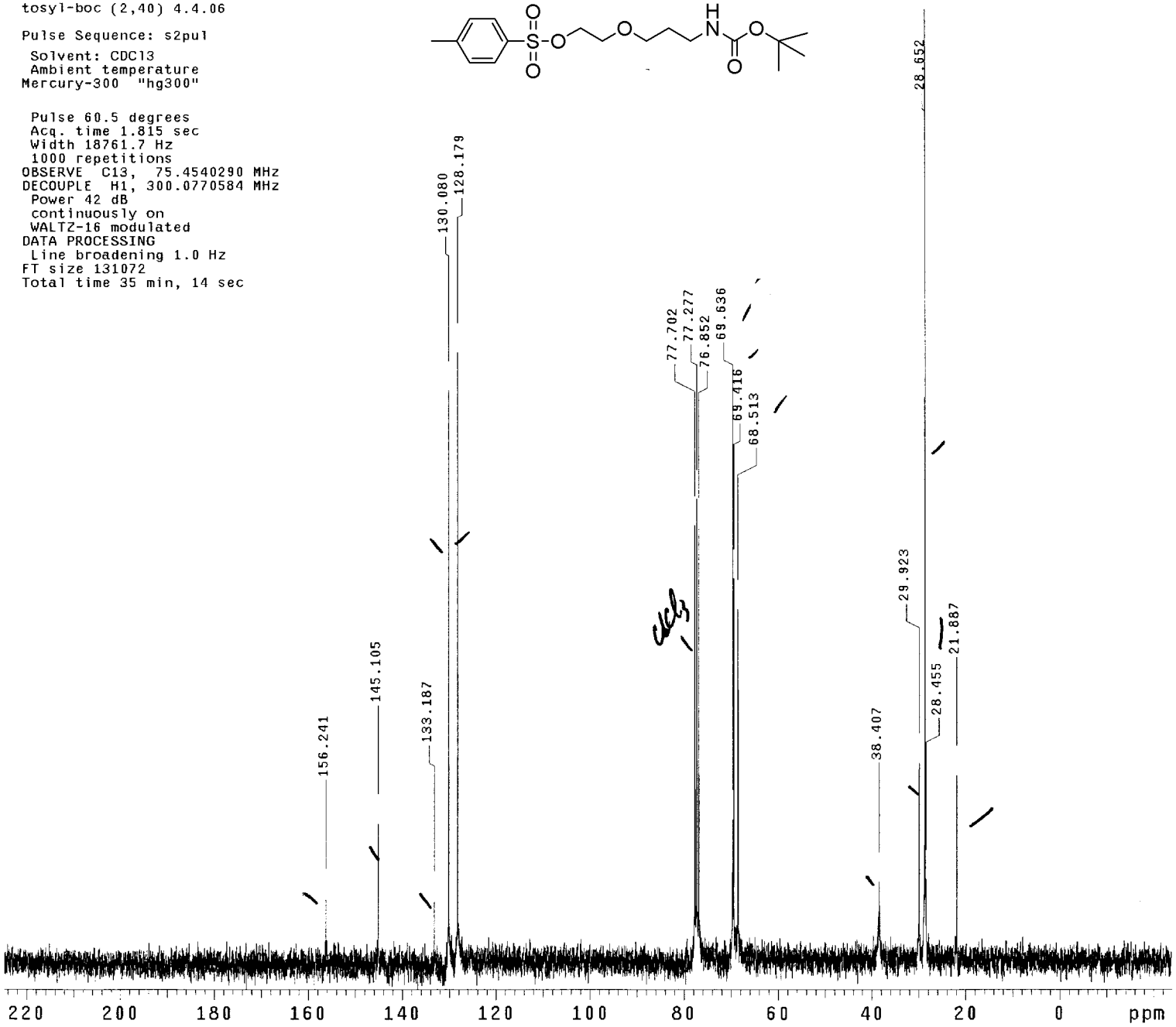
Relax. delay 1.000 sec
Pulse 45.3 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
72 repetitions
OBSERVE H1, 300.0766925 MHz
DATA PROCESSING
FT size 32768
Total time 53 min, 11 sec



tosyl-boc (2,40) 4.4.06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
1000 repetitions
OBSERVE C13, 75.4540290 MHz
DECOUPLE H1, 300.0770584 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 35 min, 14 sec

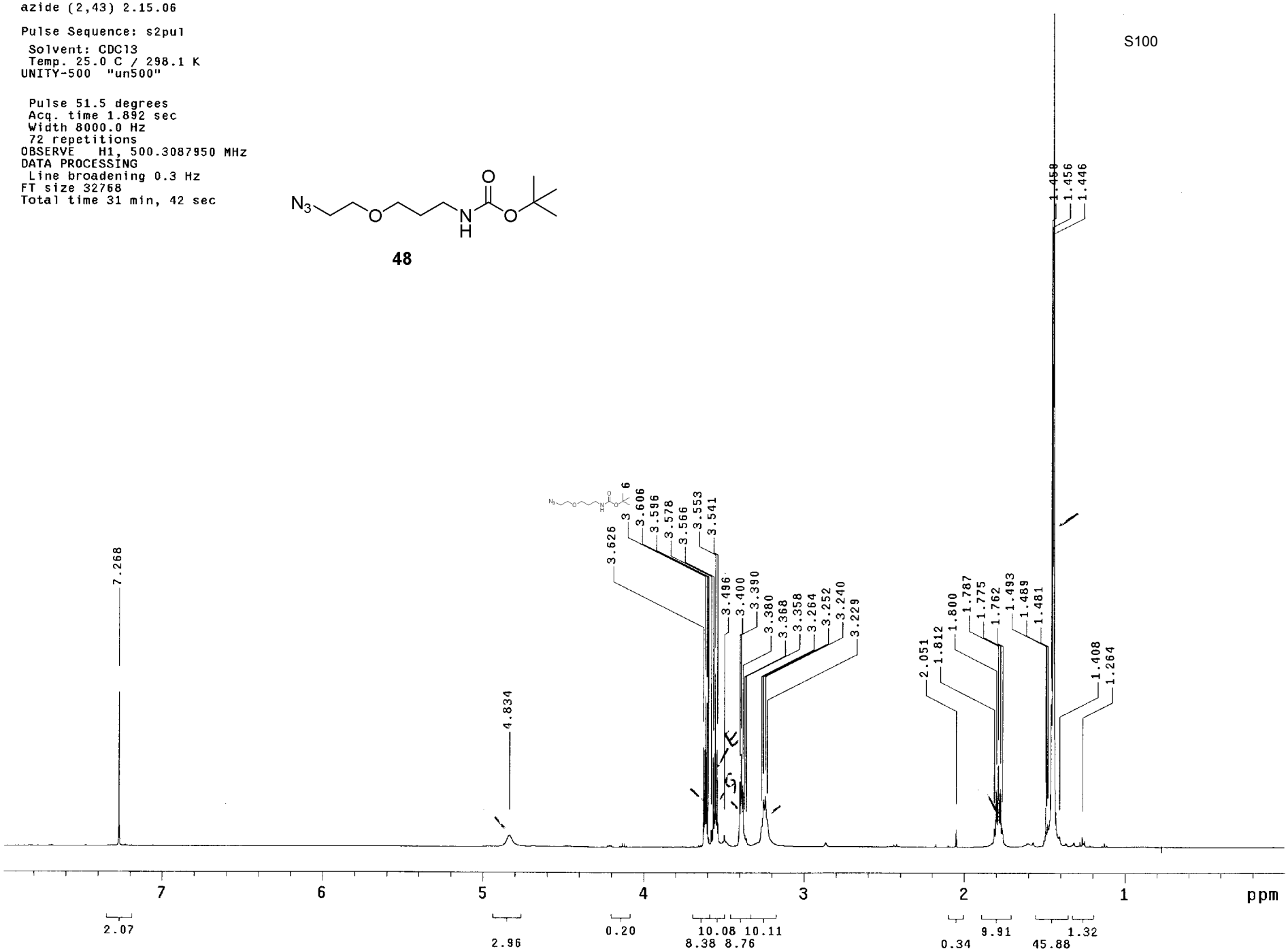


azide (2,43) 2.15.06

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
UNITY-500 "un500"

Pulse 51.5 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
72 repetitions
OBSERVE H1, 500.3087950 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 31 min, 42 sec

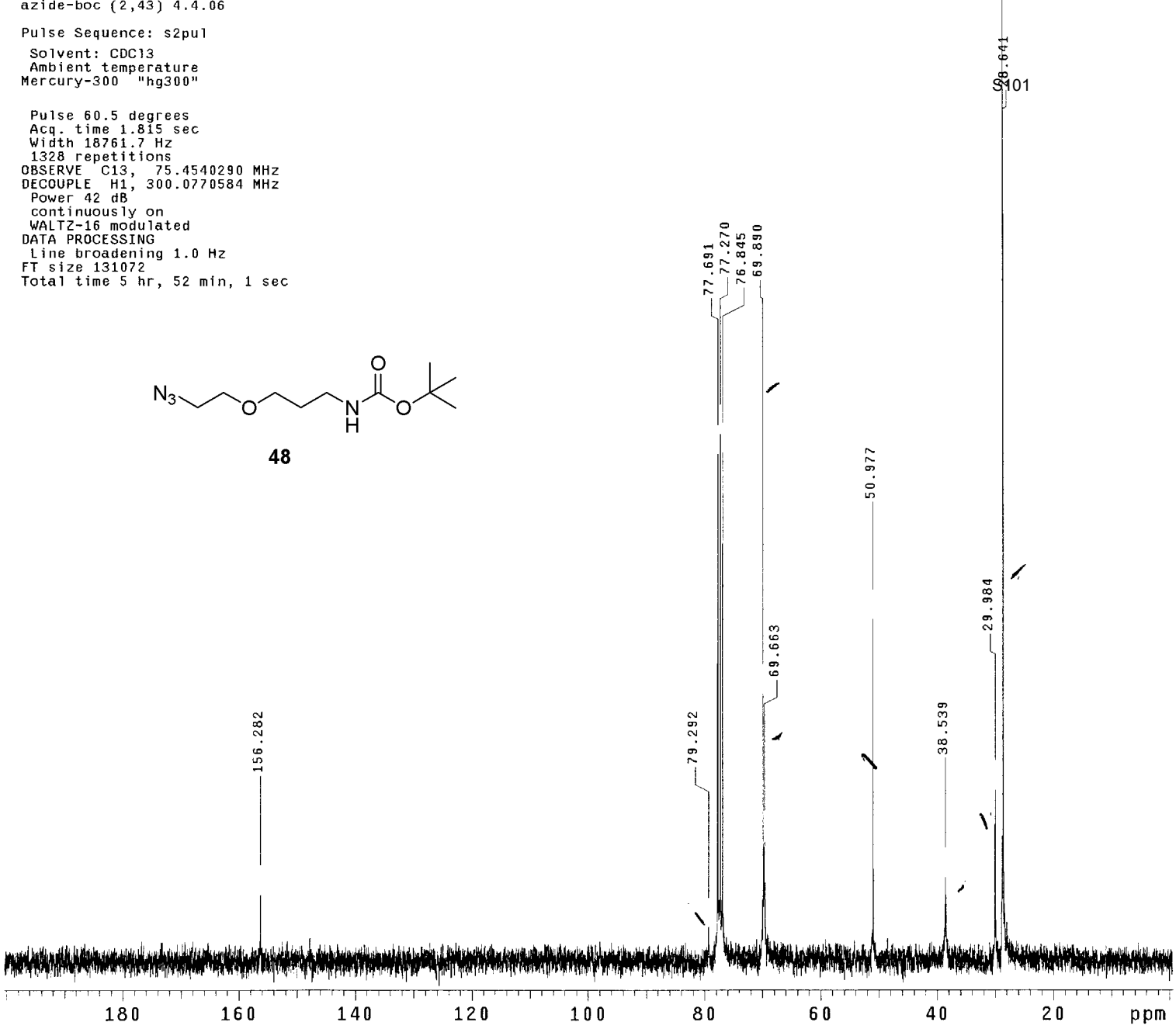
48



S100

azide-boc (2,43) 4.4.06

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300 "hg300"

Pulse 60.5 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
1328 repetitions
OBSERVE C13, 75.4540290 MHz
DECOUPLE H1, 300.0770584 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 5 hr, 52 min, 1 sec

48