Supplementary Data

Synthesis, Biological, and Biophysical Studies of DAG-indololactones Designed as Selective Activators of RasGRP.

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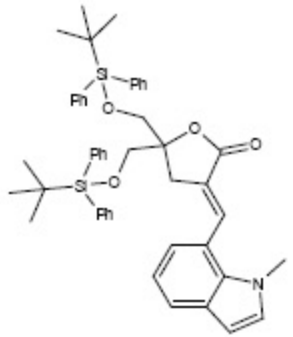
General Information

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 400 instrument at 400 and 100 MHz, respectively. Spectra are referenced to the solvent in which they were run (7.26 ppm for $CDCl_3$). High resolution MS analysis was conducted on a Thermo-Fisher LTQ-XL Orbitrap hybrid mass spectrometer operated at a resolution of 30,0000 (FWHM) using either FIA or LC/MS sample introduction, depending on which mode was the most suitable based on previous low resolution analyses. For LC/MS analyses on the Orbitrap, a narrow-bore (50 X 2.1 mm) Zorbax Rapid-Resolution reversed-phase C18 column coupled with a C18 guard column was eluted with a 5-90% gradient of CH_3CN/H_2O containing 0.1% HCOOH at 250 µl/min. The resulting accurate mass measurement of a molecular species ($[M+H]^+$, $[M+Na]^+$ or $M+NH_4]^+$) was then used to determine a unique elemental composition for each particular compound. Where appropriate, ^{1}H and ^{13}C NMR data were used to set elemental constraints for this calculation. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA and by UMYMFOR-CONICET, Argentina.

7a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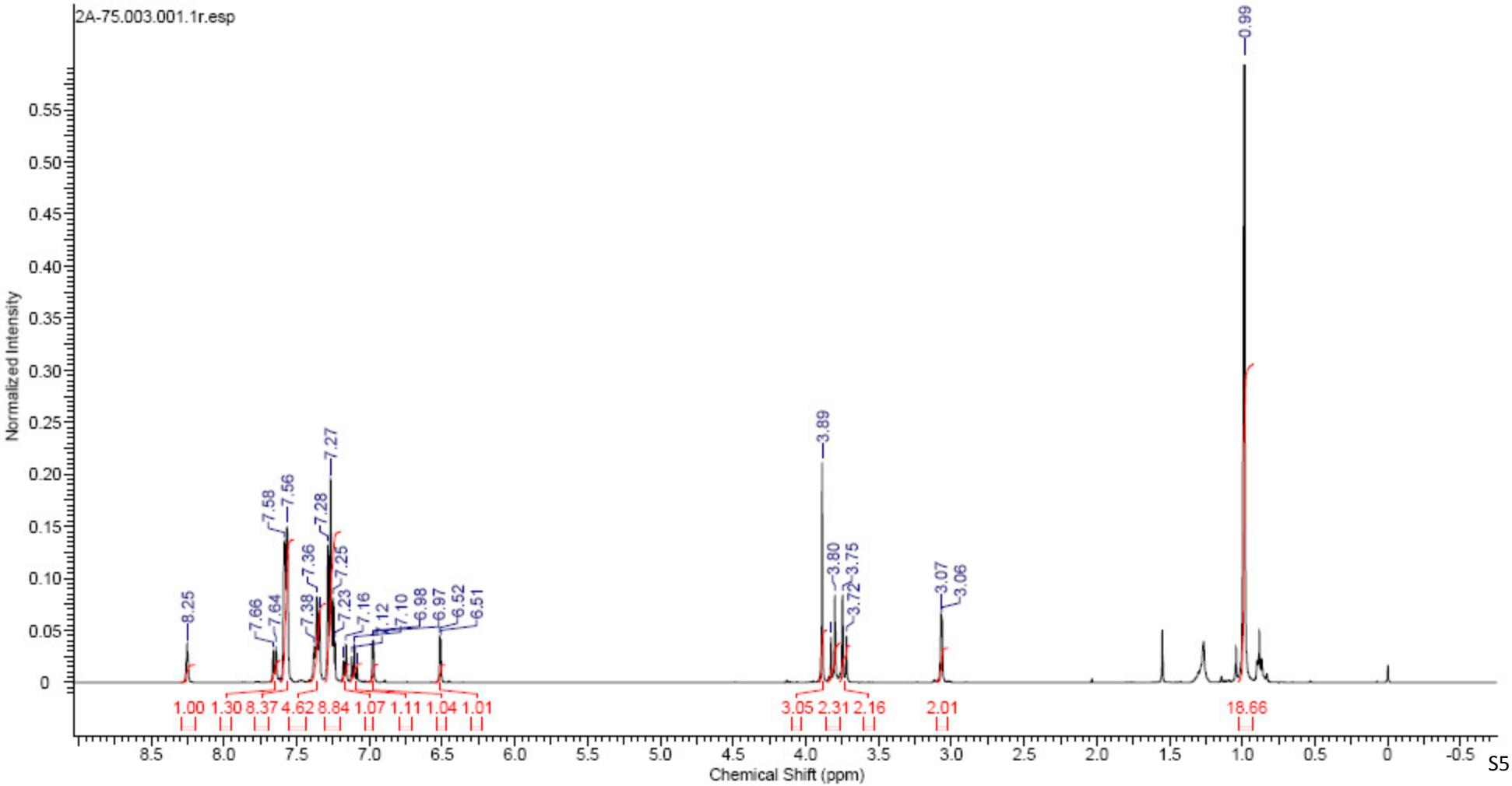
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7.36
7.28
7.27
7.25
7.23
7.16
7.12
7.10
6.98
6.97
6.52
6.51
3.89
3.80
3.75
3.72
3.07
3.06
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2.01
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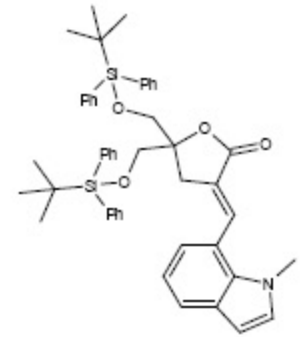
7a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Ph
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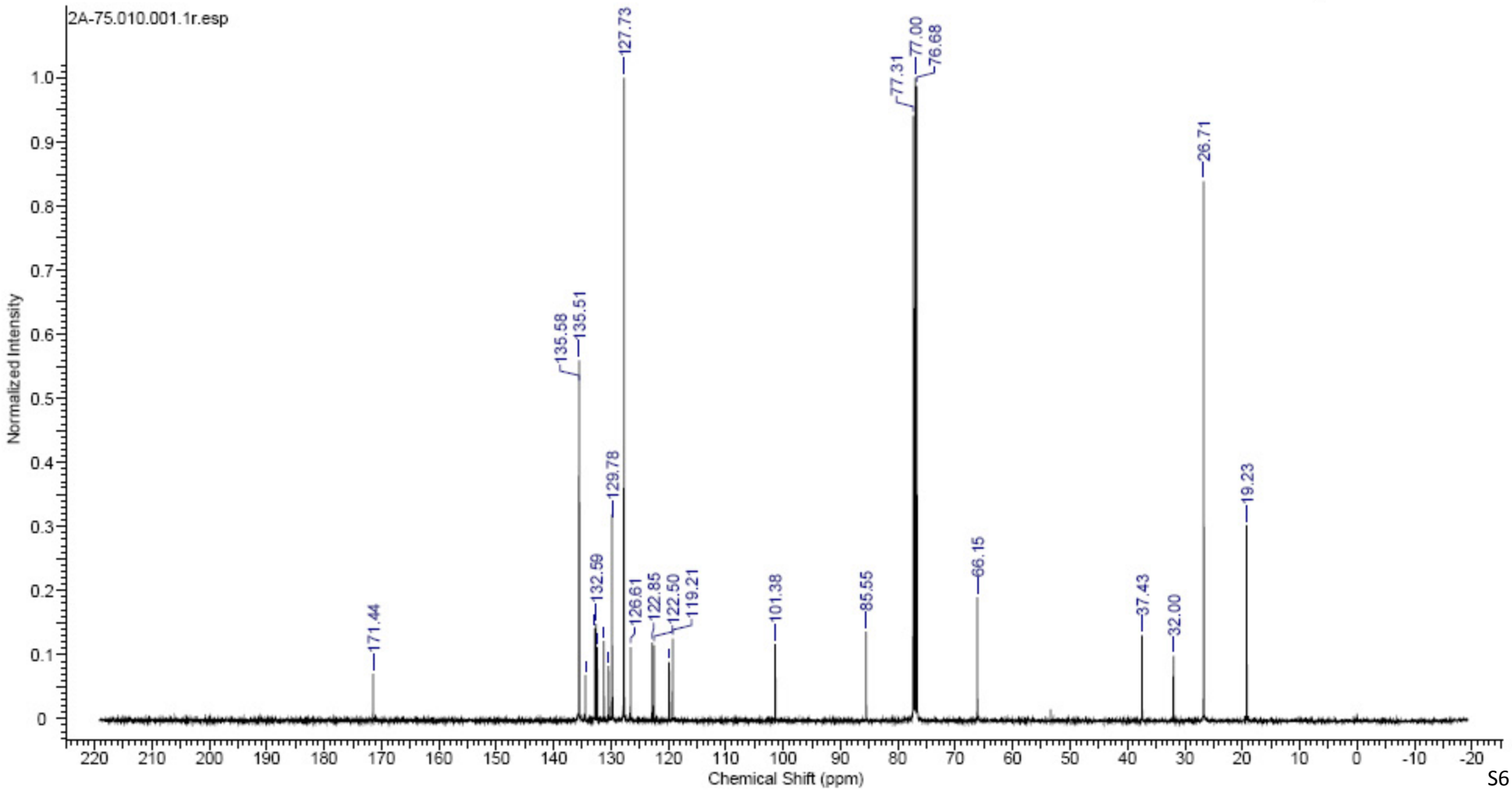
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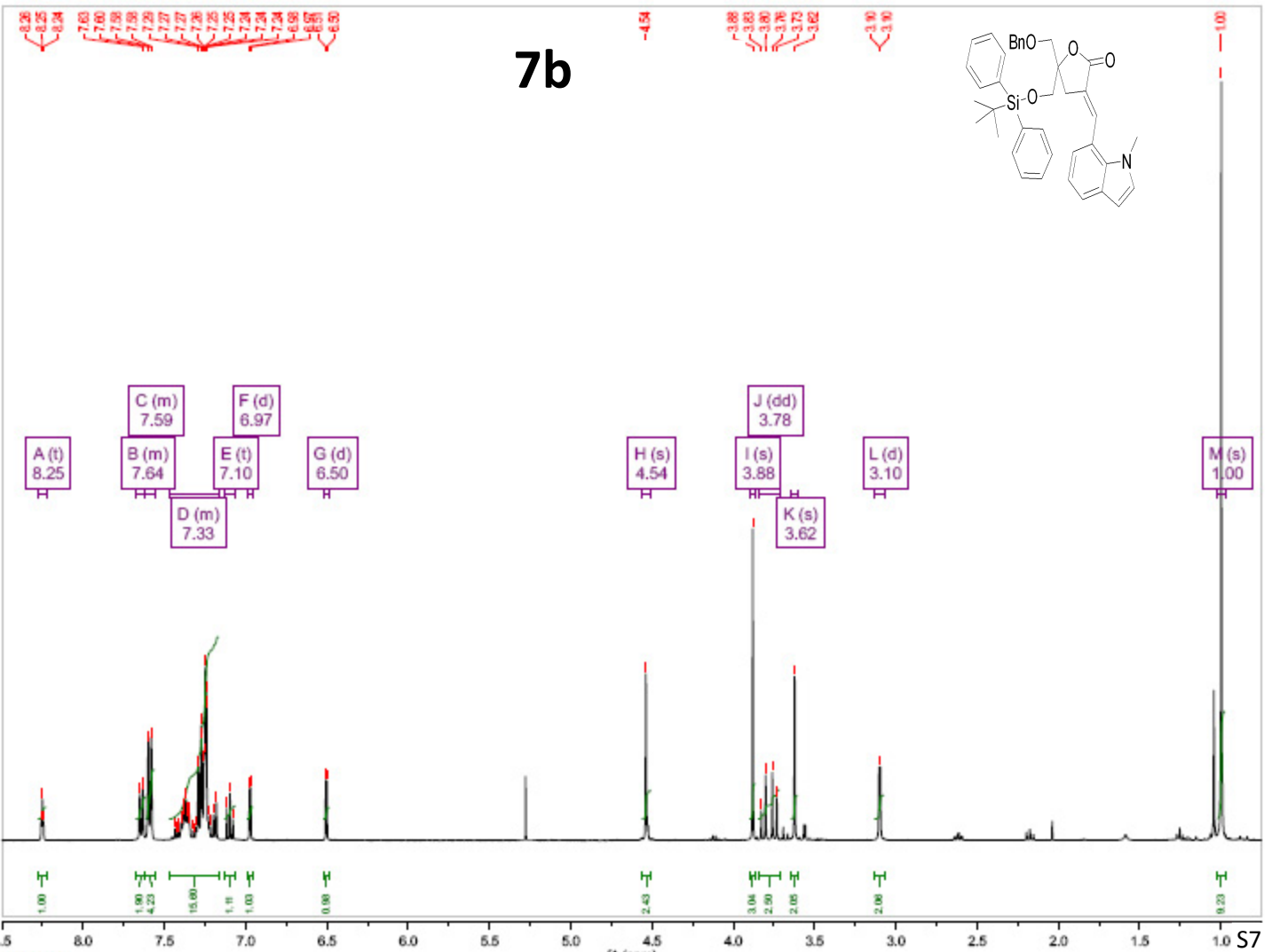
32.00
26.71
19.23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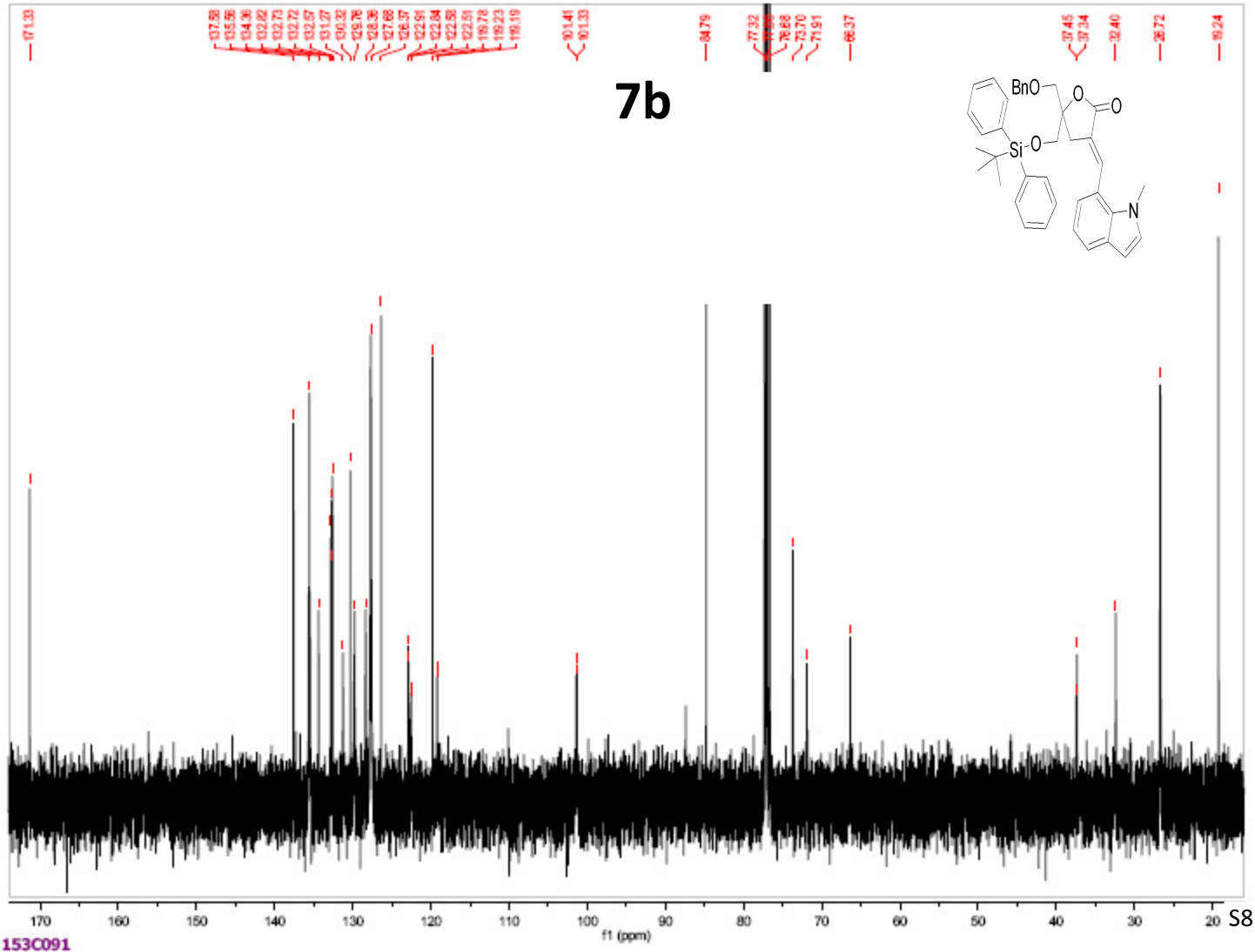
BnO
O
O
Si
O
N
A (t) 8.25
B (m) 7.64
C (m) 7.59
D (m) 7.33
E (t) 7.10
F (d) 6.97
G (d) 6.50
H (s) 4.54
I (s) 3.88
J (dd) 3.78
K (s) 3.62
L (d) 3.10
M (s) 1.00
f1 (ppm)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131.27
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128.36
127.88
126.37
122.91
122.84
122.58
122.51
119.78
119.23
119.19
101.41
101.33
84.79
77.32
76.68
73.70
71.91
66.37
37.45
37.34
32.40
26.72
19.24
BnO
O
O
Si
O
N
170
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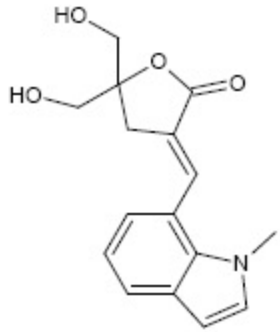
90
80
70
60
50
40
30
20
f1 (ppm)
153C091

8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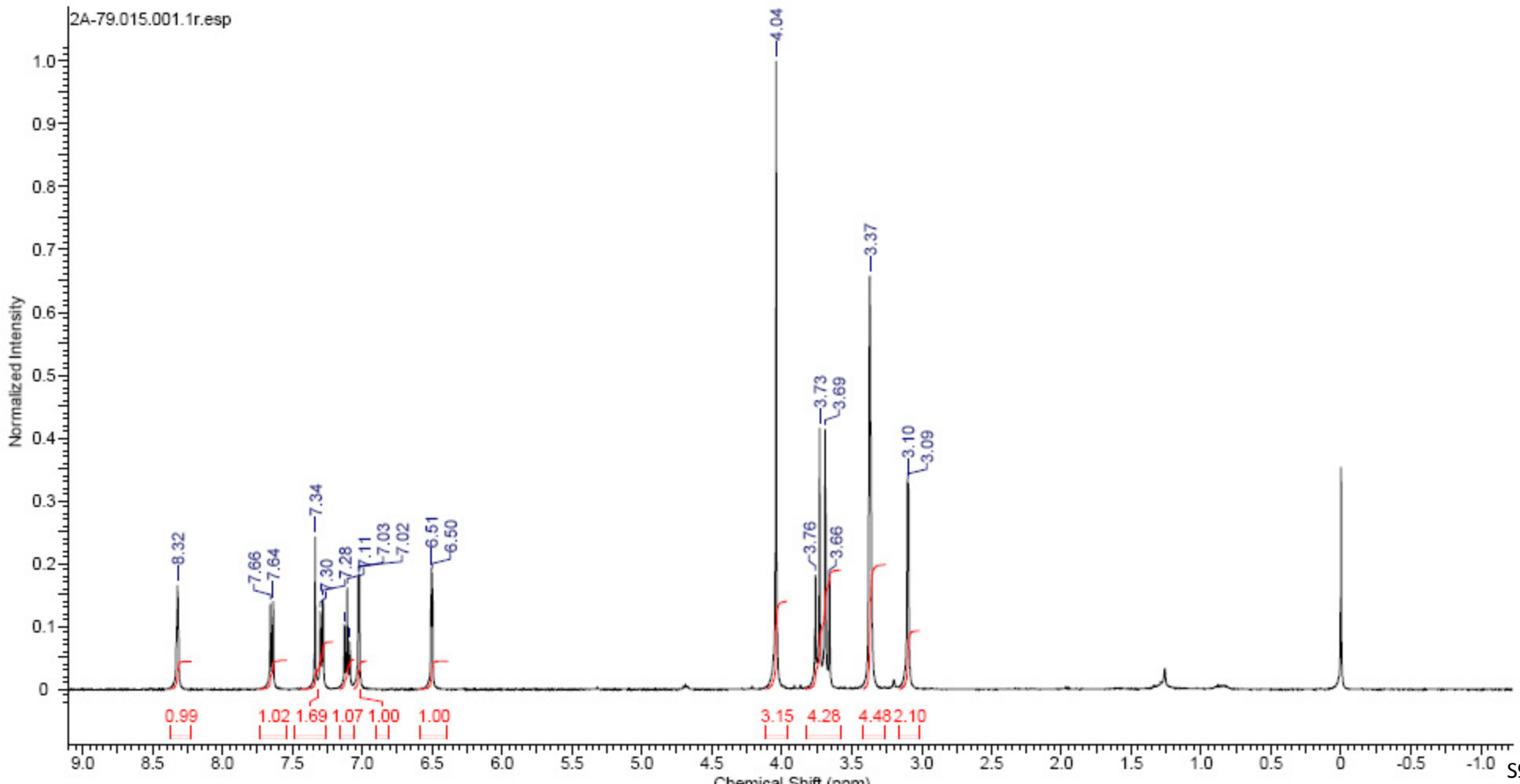
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7.02
6.51
6.50
4.04
3.76
3.73
3.69
3.66
3.37
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1.00
3.15
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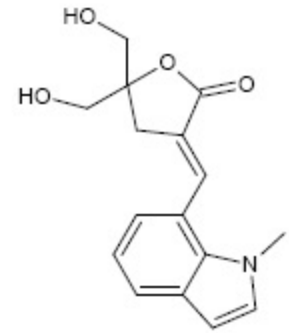
4.48
2.10

8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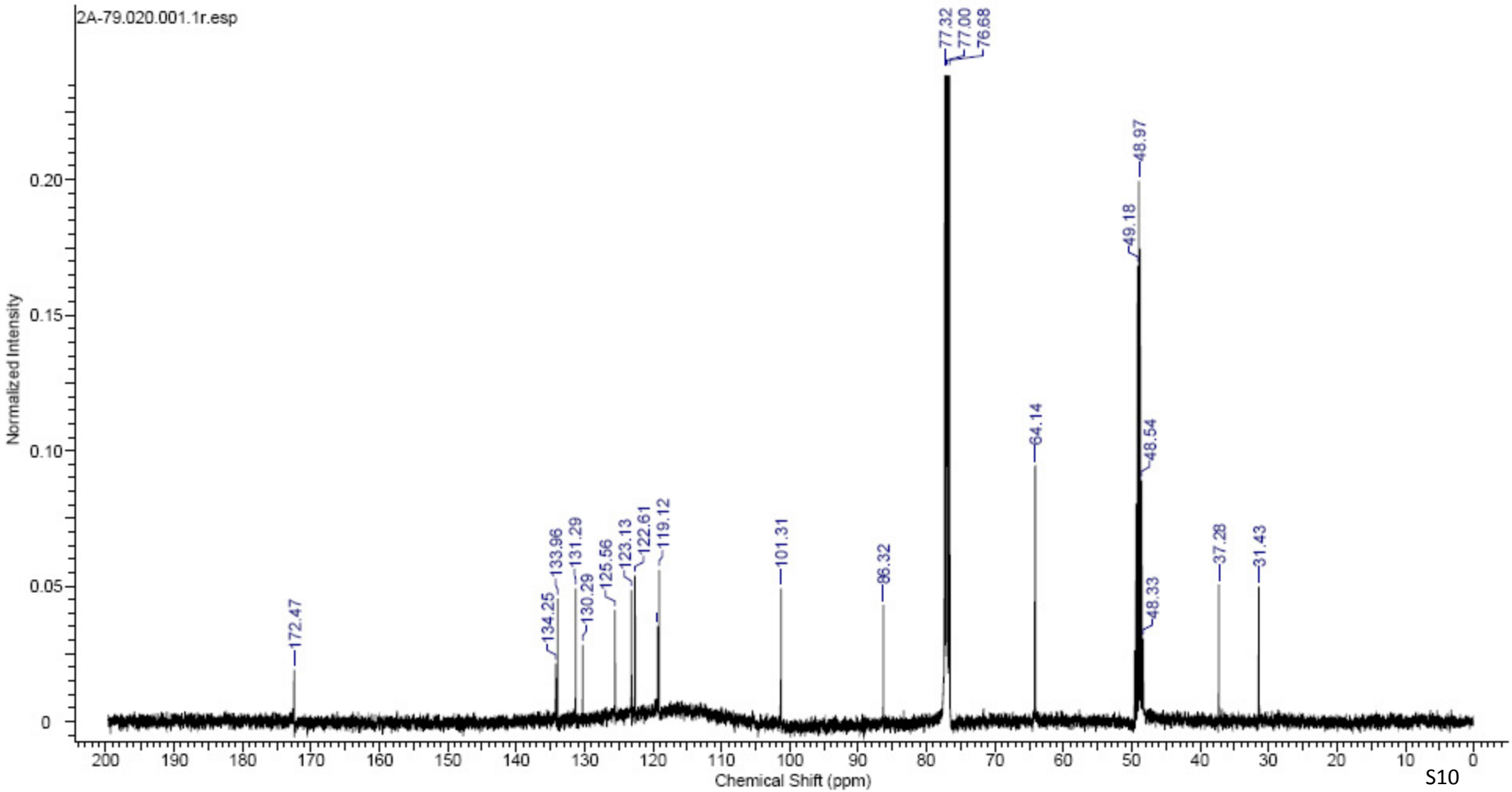
HO
O
O
N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0
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48.97
49.18
64.14
48.54
48.33
37.28
31.43
172.47
134.25
133.96
131.29
130.29
125.56
123.13
122.61
119.12
101.31
86.32
200
190
180
170
160
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110
100
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70
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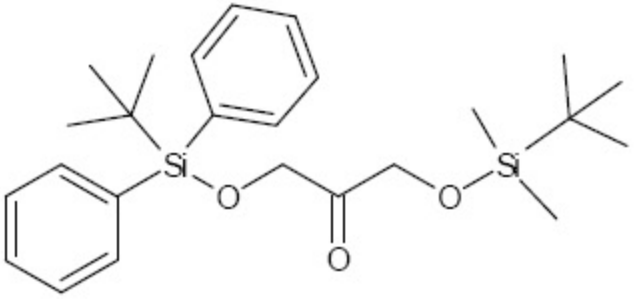
50
40
30
20
10
0
Chemical Shift (ppm)

9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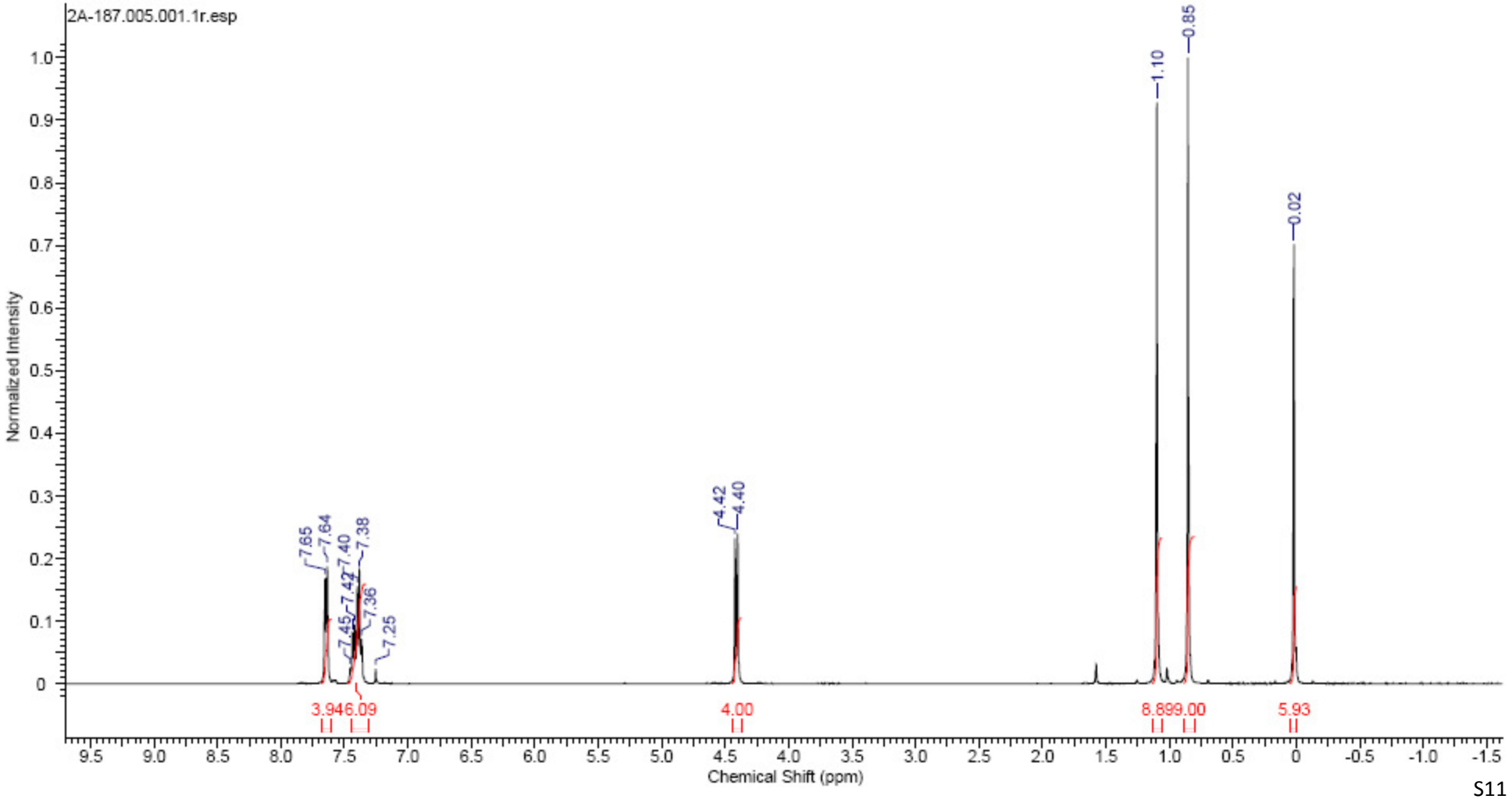
O
O
Si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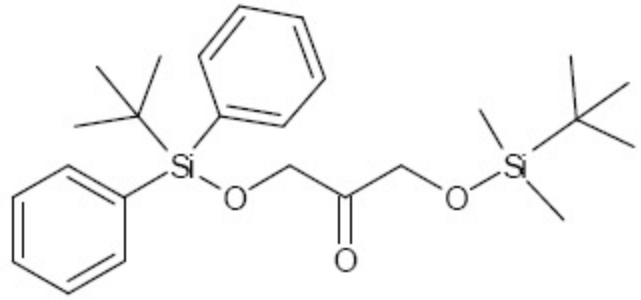
0.02
3.94
6.09
4.00
8.89
9.00
5.93

9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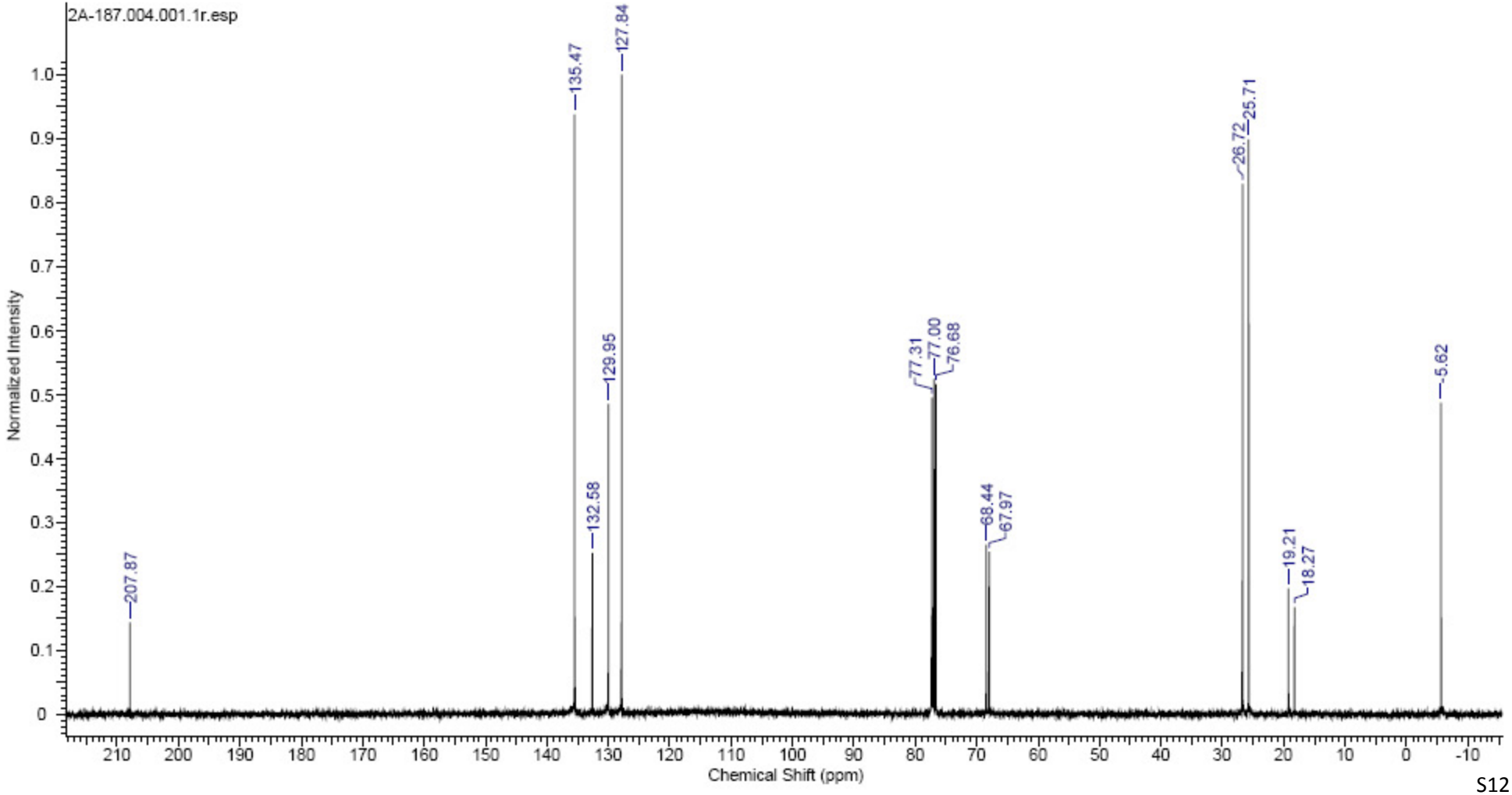
O
O
O
Si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25.71
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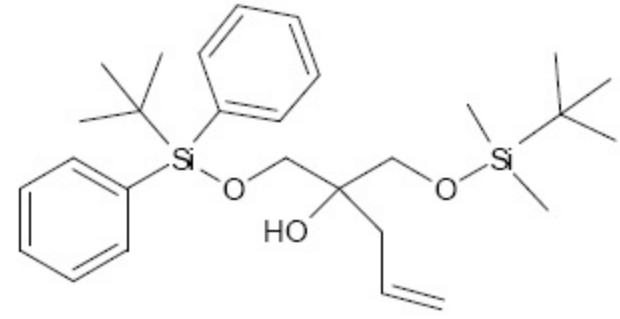
-5.62

10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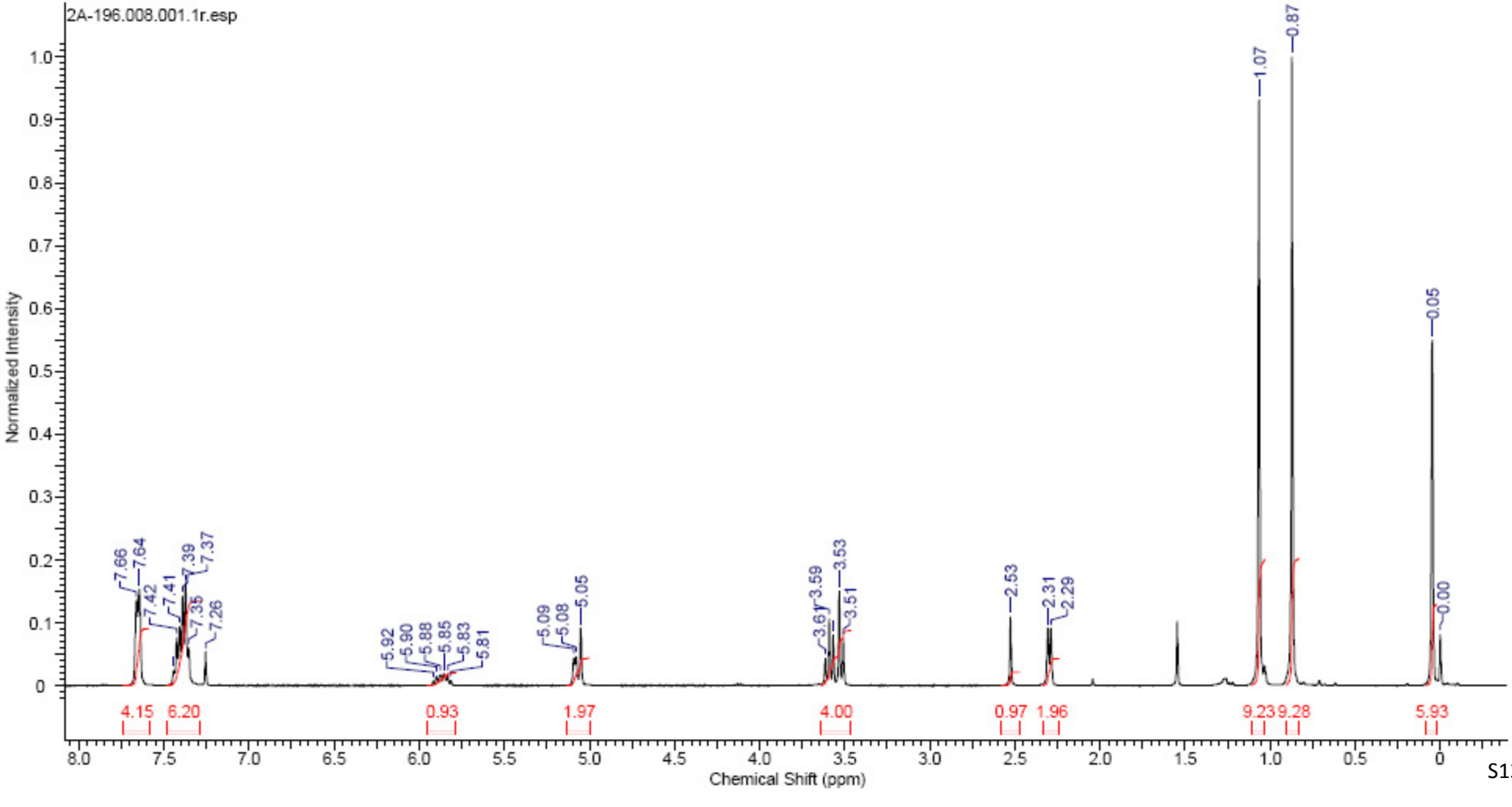
O
HO
O
Si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3.59
3.53
3.51
2.53
2.31
2.29
1.07
0.87
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9.28
5.93

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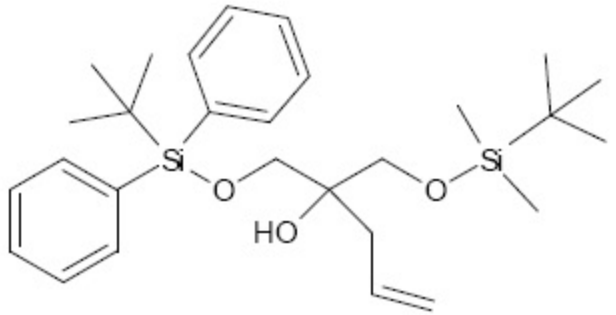
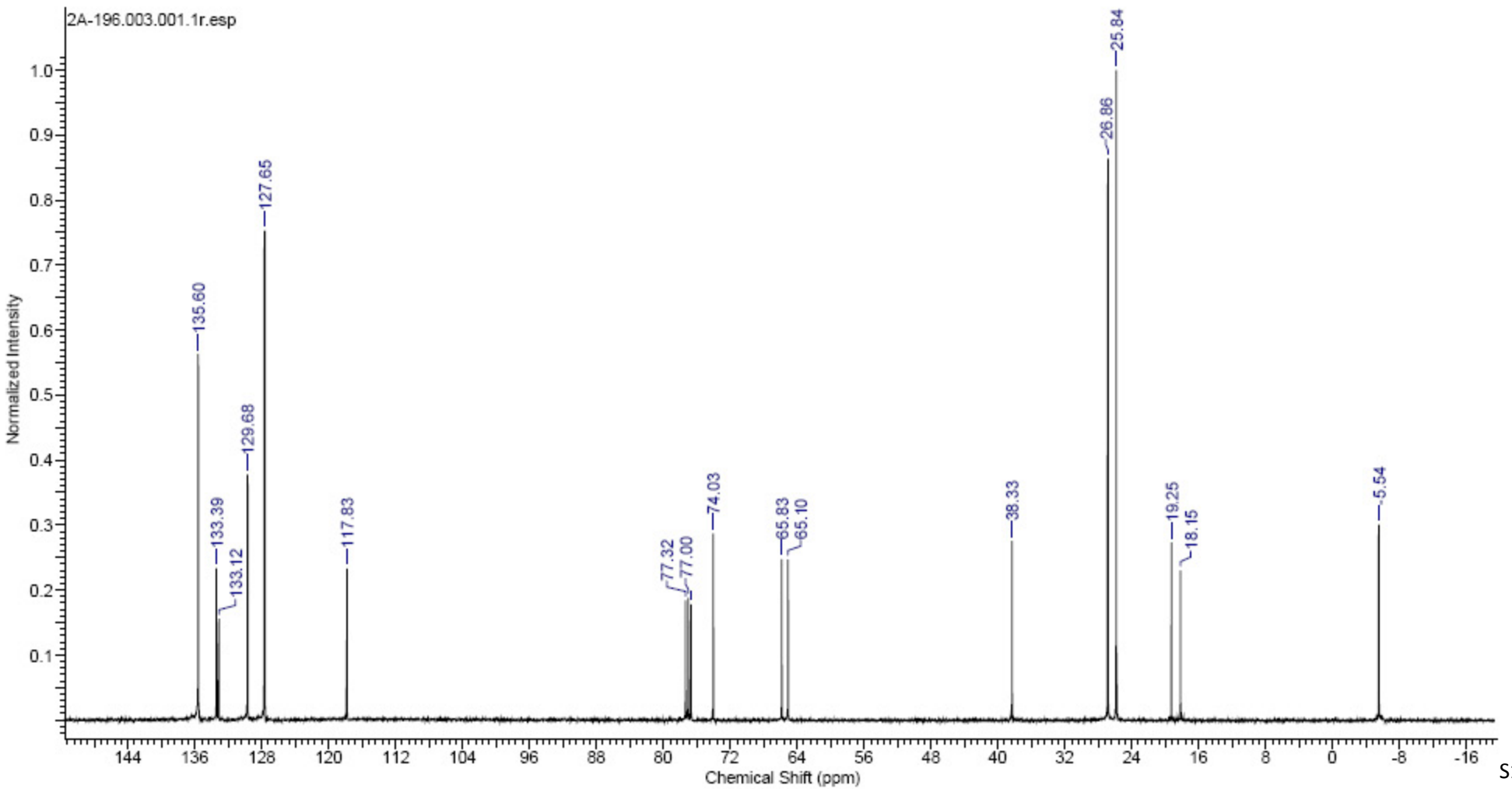

Si
O
HO
O
Si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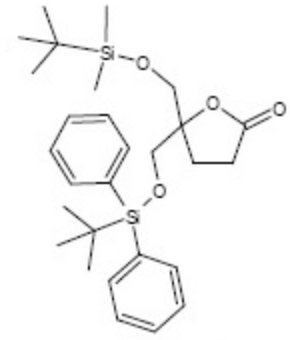
18.15
5.54

11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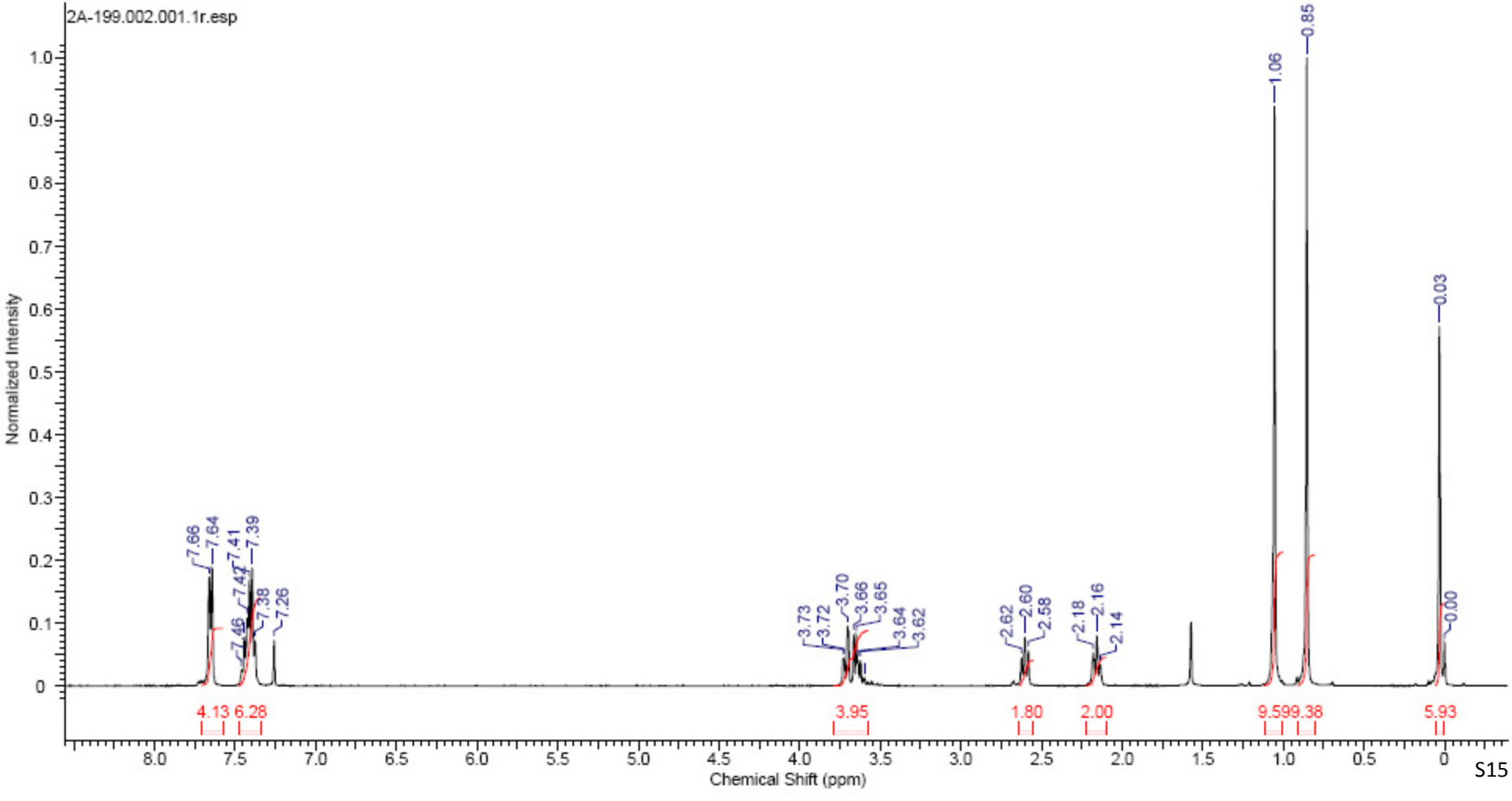
O
O
O
Si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3.66
3.65
3.64
3.62
2.62
2.60
2.58
2.18
2.16
2.14
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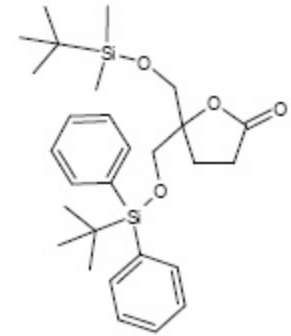
2.00
9.59
9.38
5.93

11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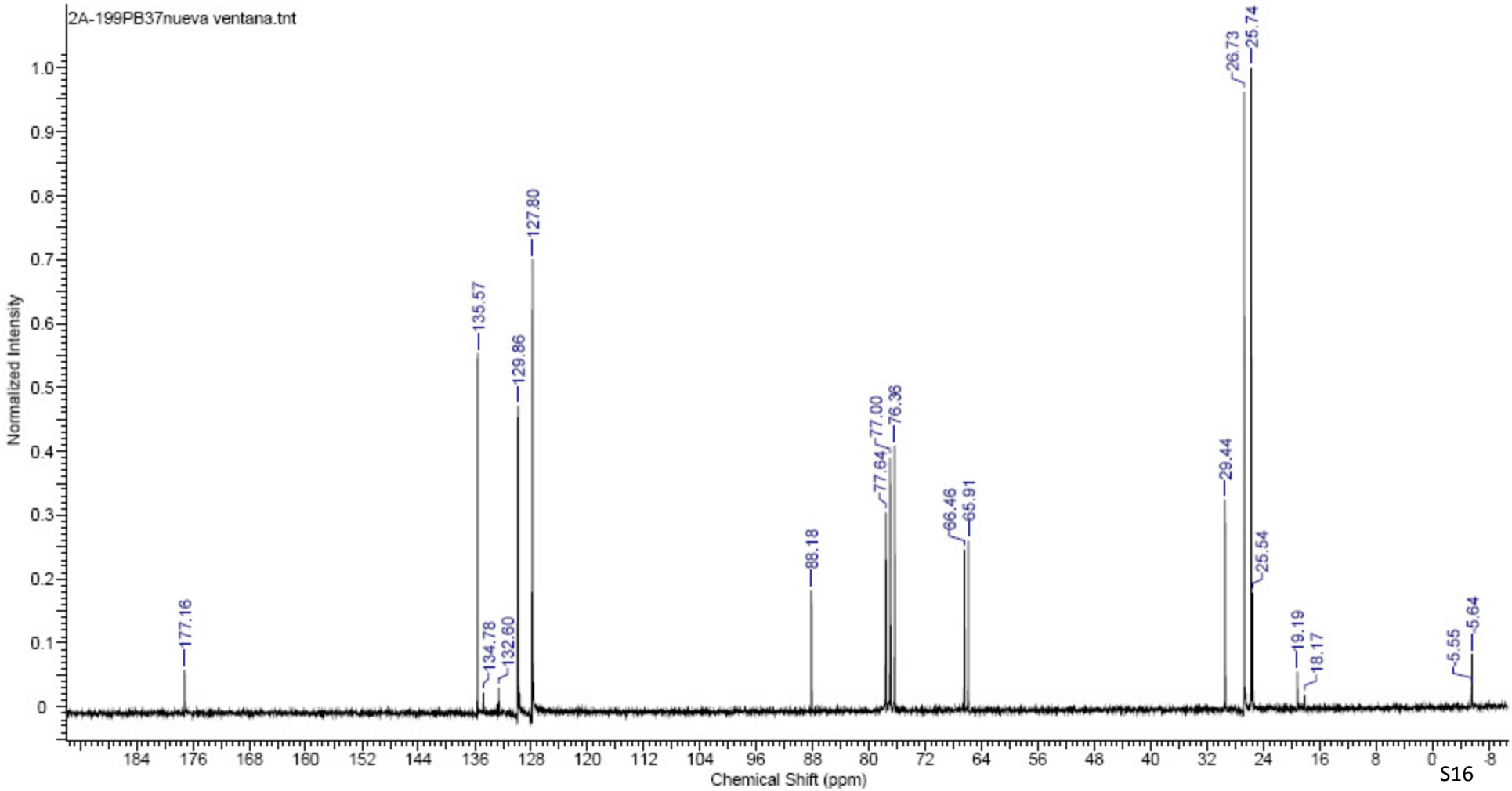
O
O
O
Si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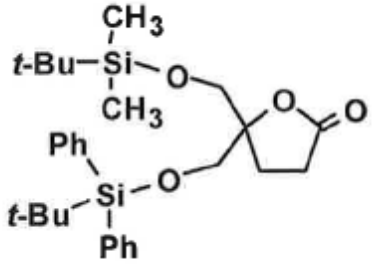
5.64

11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CH3
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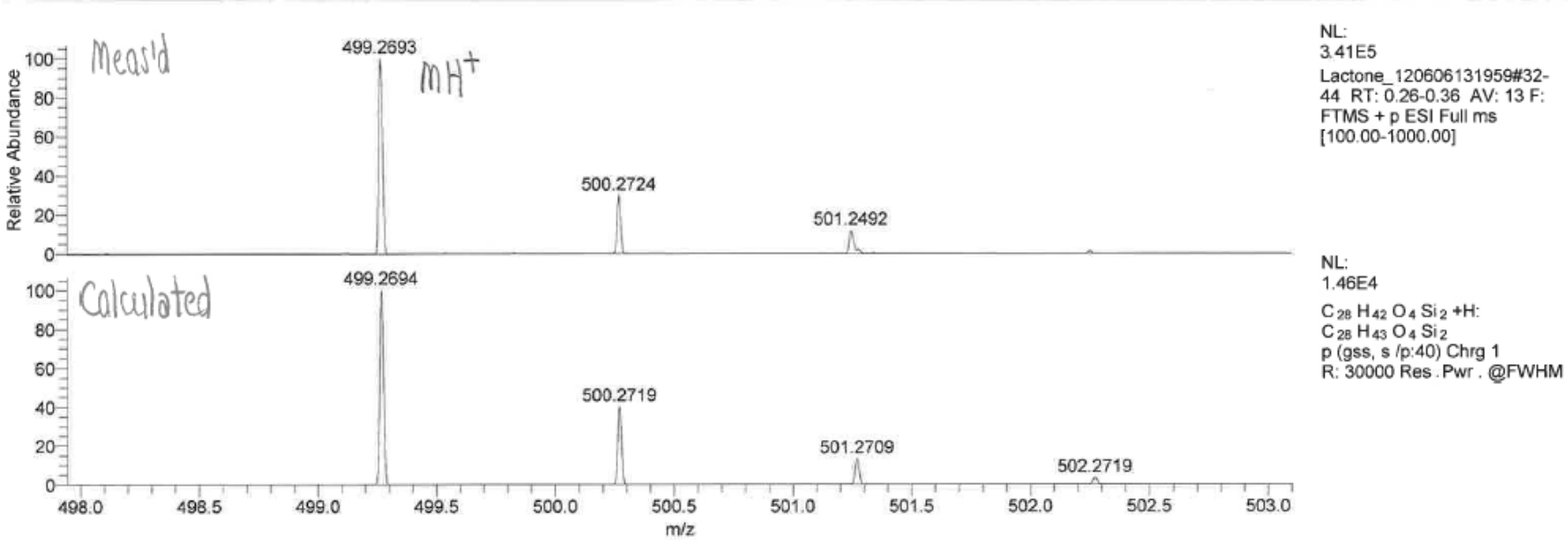
t-Bu
Ph


Meas'd
499.2693
MH+
500.2724
501.2492
Relative Abundance
100
80
60
40
20
0
NL:
3.41E5
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Calculated
499.2694
500.2719
501.2709
502.2719
NL:
1.46E4
C28 H42 O4 Si2 +H:
C28 H43 O4 Si2
p (gss, s /p:40) Chrg 1
R: 30000 Res . Pwr . @FWHM
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498.5
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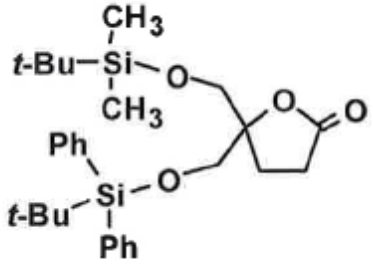
500.5
501.0
501.5
502.0
502.5
503.0
m/z

11



UMYMFOR

CONICET

FCEN - UBA

UNIDAD DE MICROANALISIS Y
METODOS FÍSICOS APLICADOS
A QUÍMICA ORGANICA

Page 818 27 Apr 2012 15:57:38

ANALIZADOR ELEMENTAL EXETER CE 440

ANALISIS NRO.: 5754
MUESTRA: 2A-199PB37
Código: U12-042306
Fórmula Empírica: $C_{28}H_{42}O_4Si_2$
Calculado: %C 67.422 %H 8.487 %N .000

RESULTADOS
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%C: 67.48
%H: 8.30
%N: --

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ANALIZADOR ELEMENTAL EXETER CE 440

ANALISIS NRO.. 5756
MUESTRA: 2A-199PB37
Código: U12-042306 (Duplicado)
Fórmula Empírica: $C_{28}H_{42}O_4Si_2$
Calculado: %C 67.422 %H 8.487 %N .000

RESULTADOS
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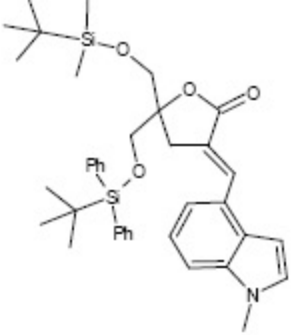
12a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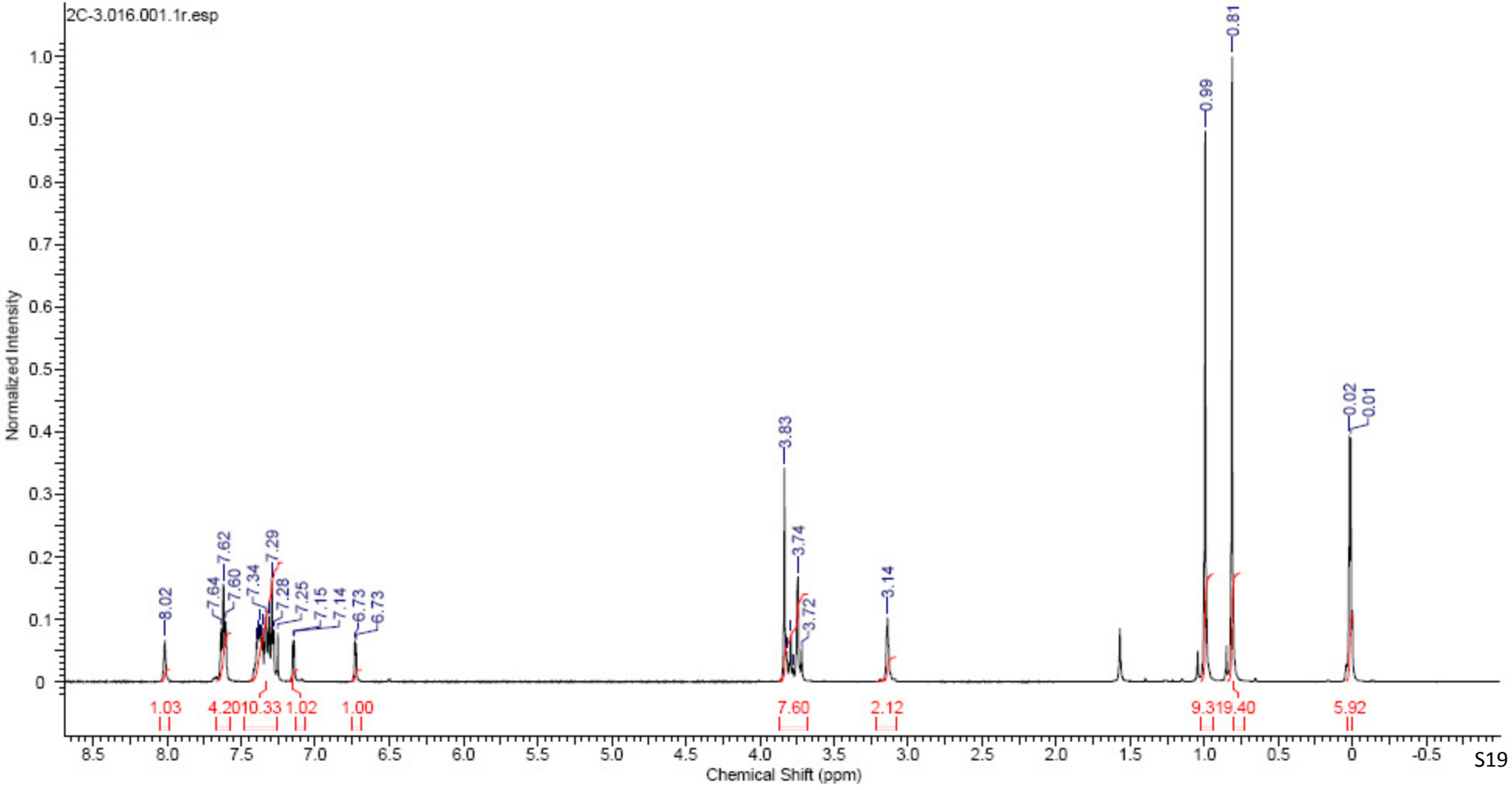
O
Ph
Si
Ph
N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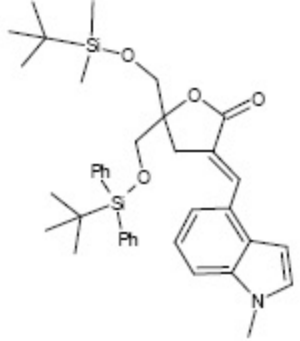
7.28
7.25
7.15
7.14
6.73
6.73
3.83
3.74
3.72
3.14
0.99
0.81
0.02
0.01
1.03
4.20
10.33
1.02
1.00
7.60
2.12
9.31
9.40
5.92

12a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O
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Ph
Si
Ph
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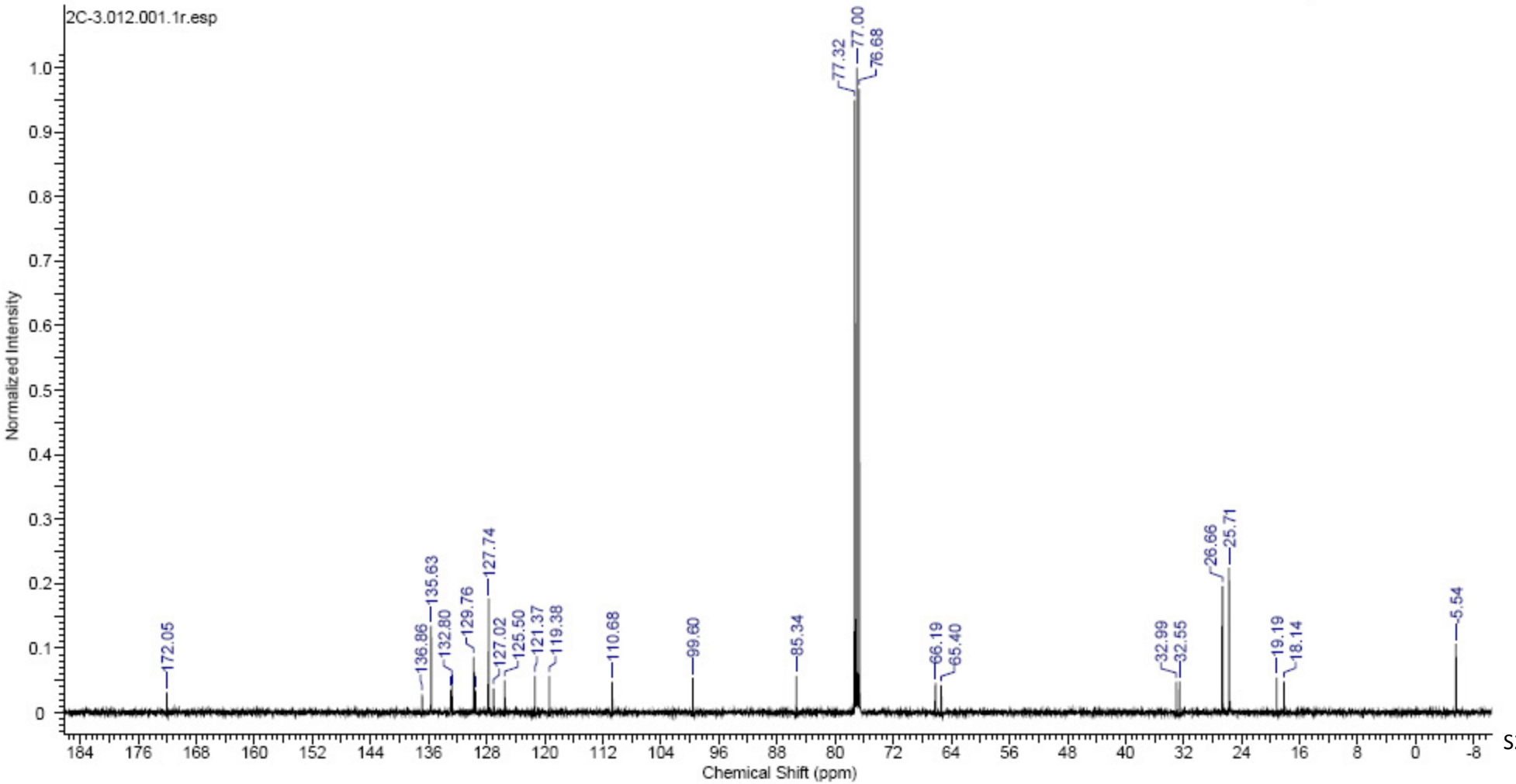
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Chemical Shift (ppm)
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135.63
132.80
129.76
127.74
127.02
125.50
121.37
119.38
110.68
99.60
85.34
77.32
77.00
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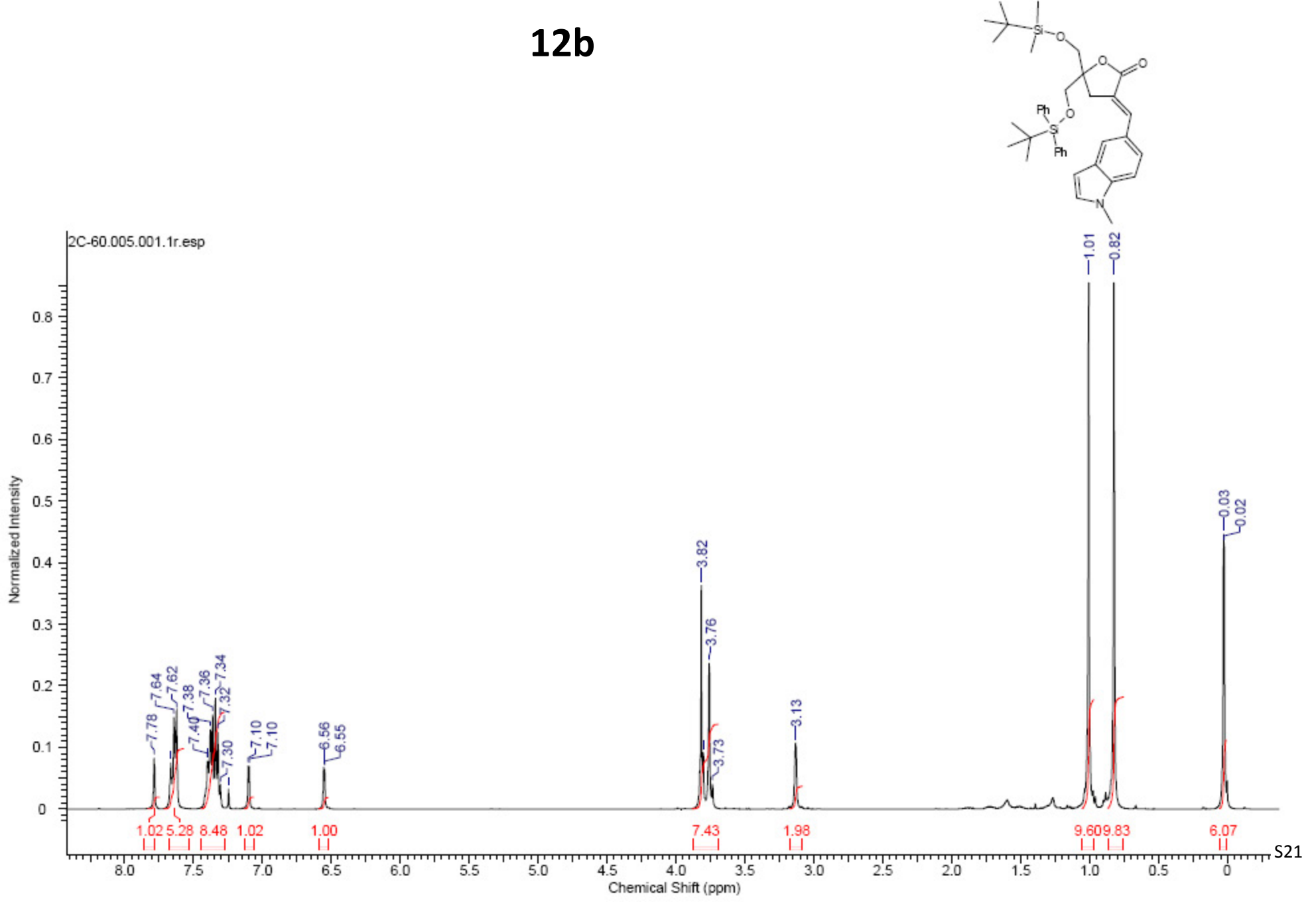
26.66
25.71
19.19
18.14
5.54

12b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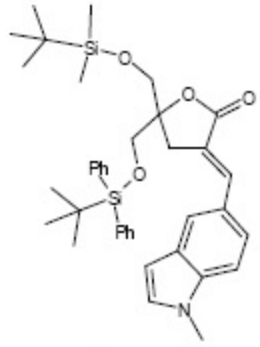
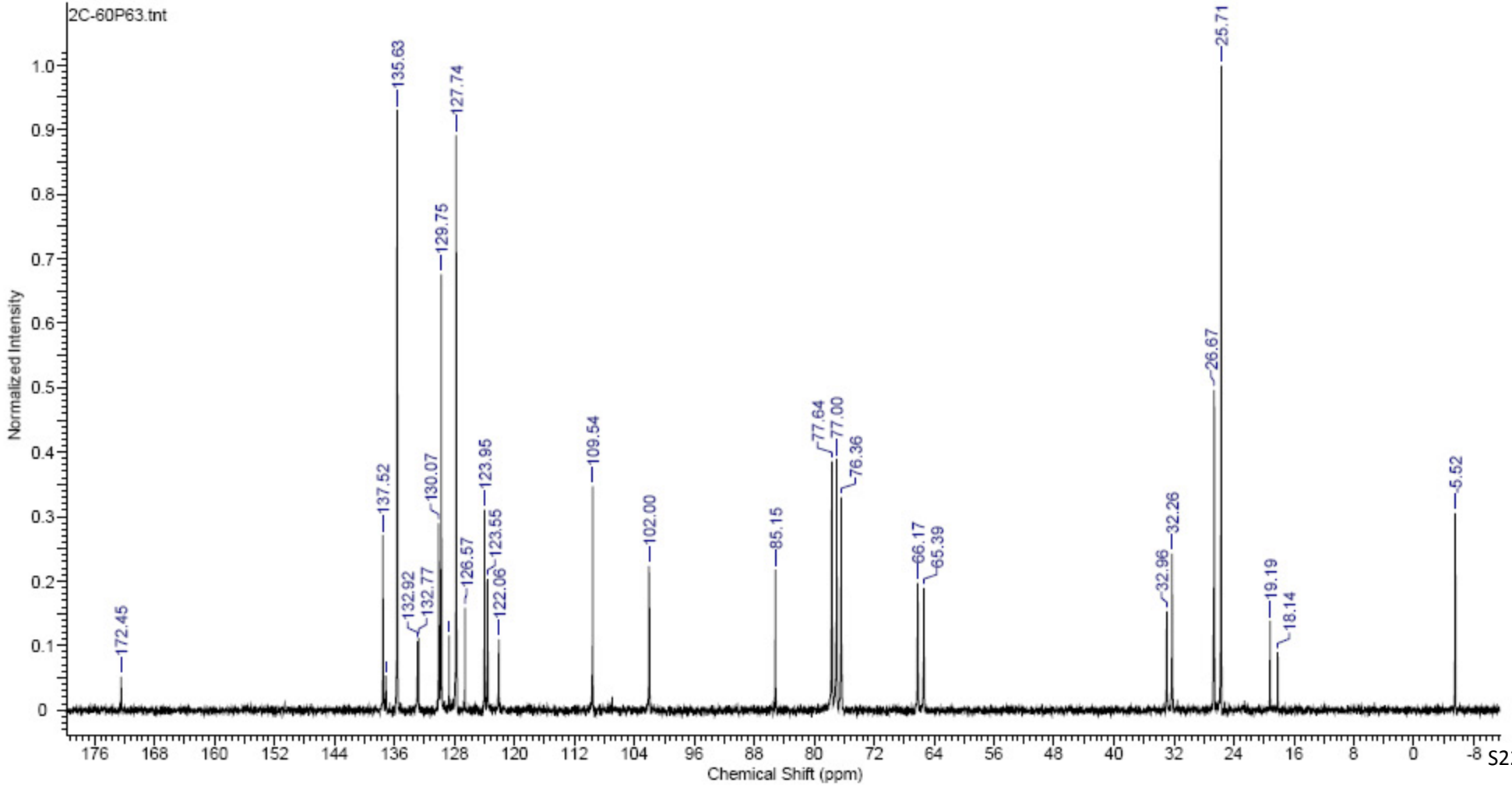
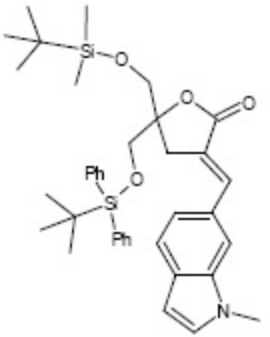
7.34
7.32
7.30
7.10
7.10
6.56
6.55
3.82
3.76
3.73
3.13
1.01
0.82
0.03
0.02
1.02
5.28
8.48
1.02
1.00
7.43
1.98
9.60
9.83
6.07

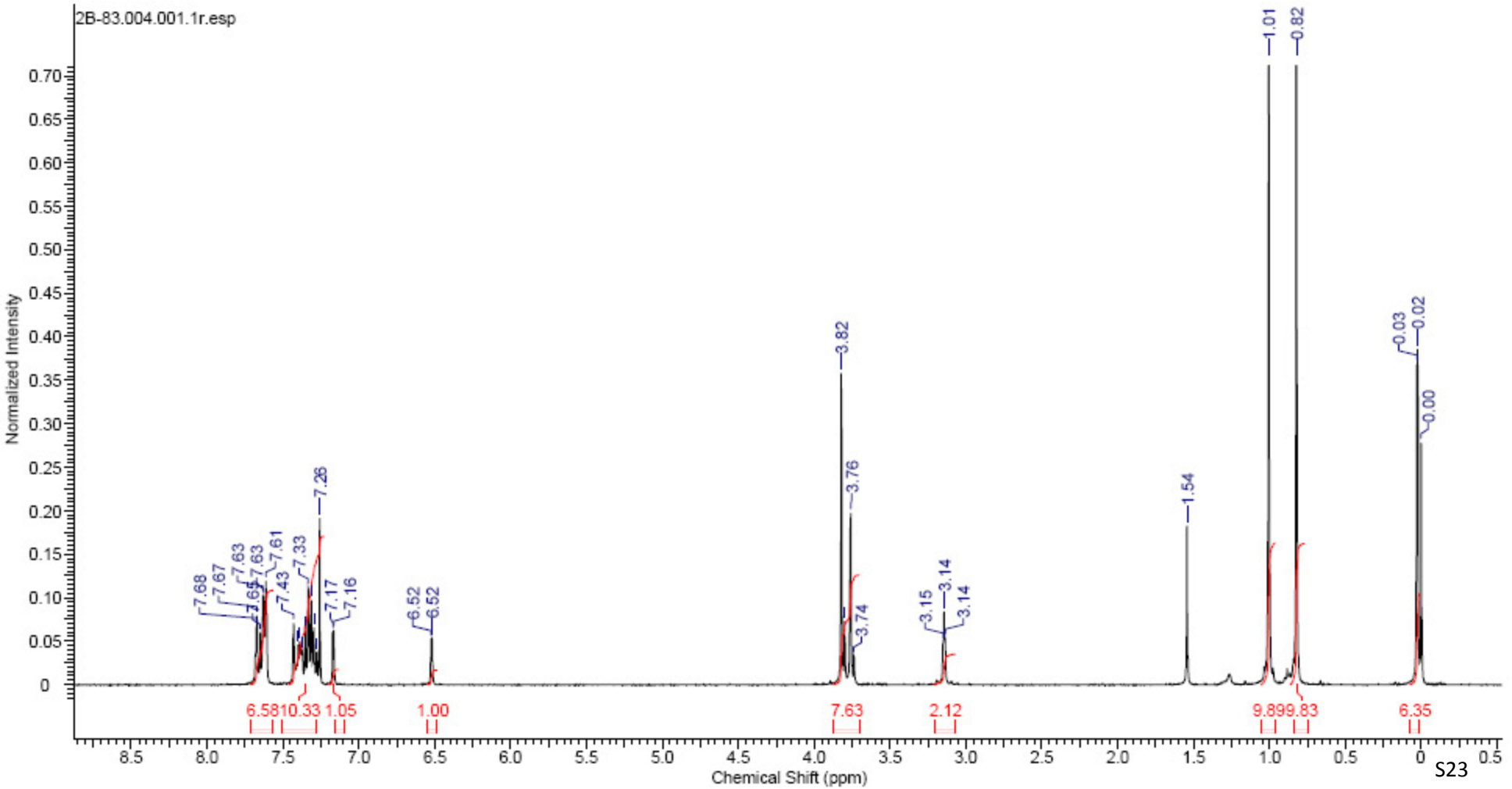
12b


Si
O
O
O
Ph
Si
Ph
N

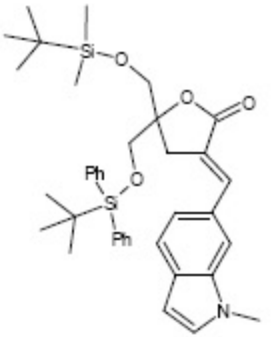

2C-60P63.tnt
Normalized Intensity
Chemical Shift (ppm)
172.45
137.52
135.63
132.92
132.77
130.07
129.75
127.74
126.57
123.95
123.55
122.06
109.54
102.00
85.15
77.64
77.00
76.36
66.17
65.39
32.96
32.26
26.67
25.71
19.19
18.14
5.52

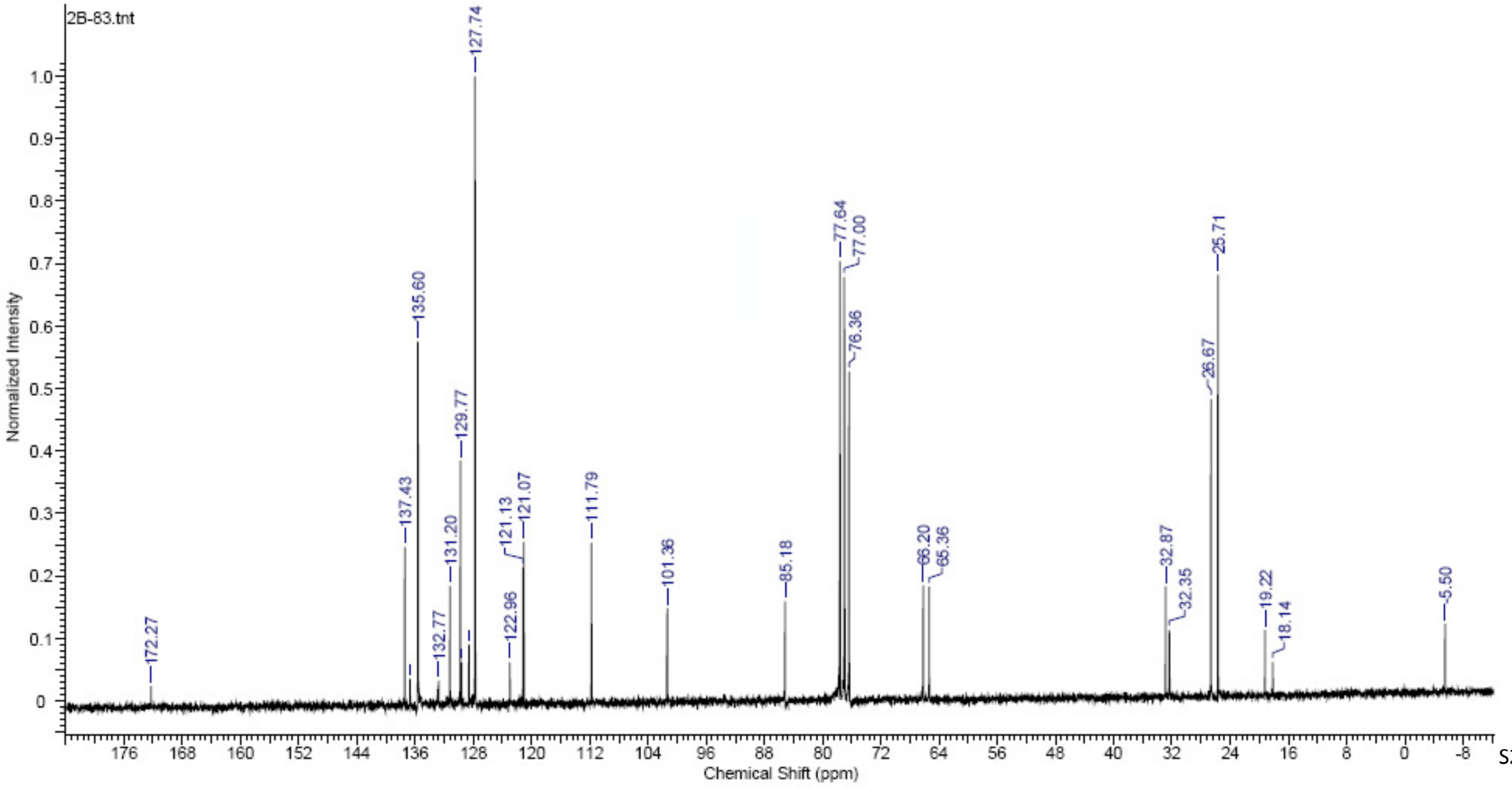
12c


Si
O
O
O
Ph
Si
Ph
N

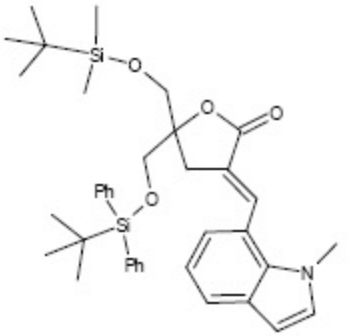

2B-83.004.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
7.68
7.67
7.63
7.63
7.61
7.43
7.33
7.26
7.17
7.16
6.52
6.52
3.82
3.76
3.74
3.15
3.14
3.14
1.54
1.01
0.82
0.03
0.02
0.00
6.58
10.33
1.05
1.00
7.63
2.12
9.89
9.83
6.35

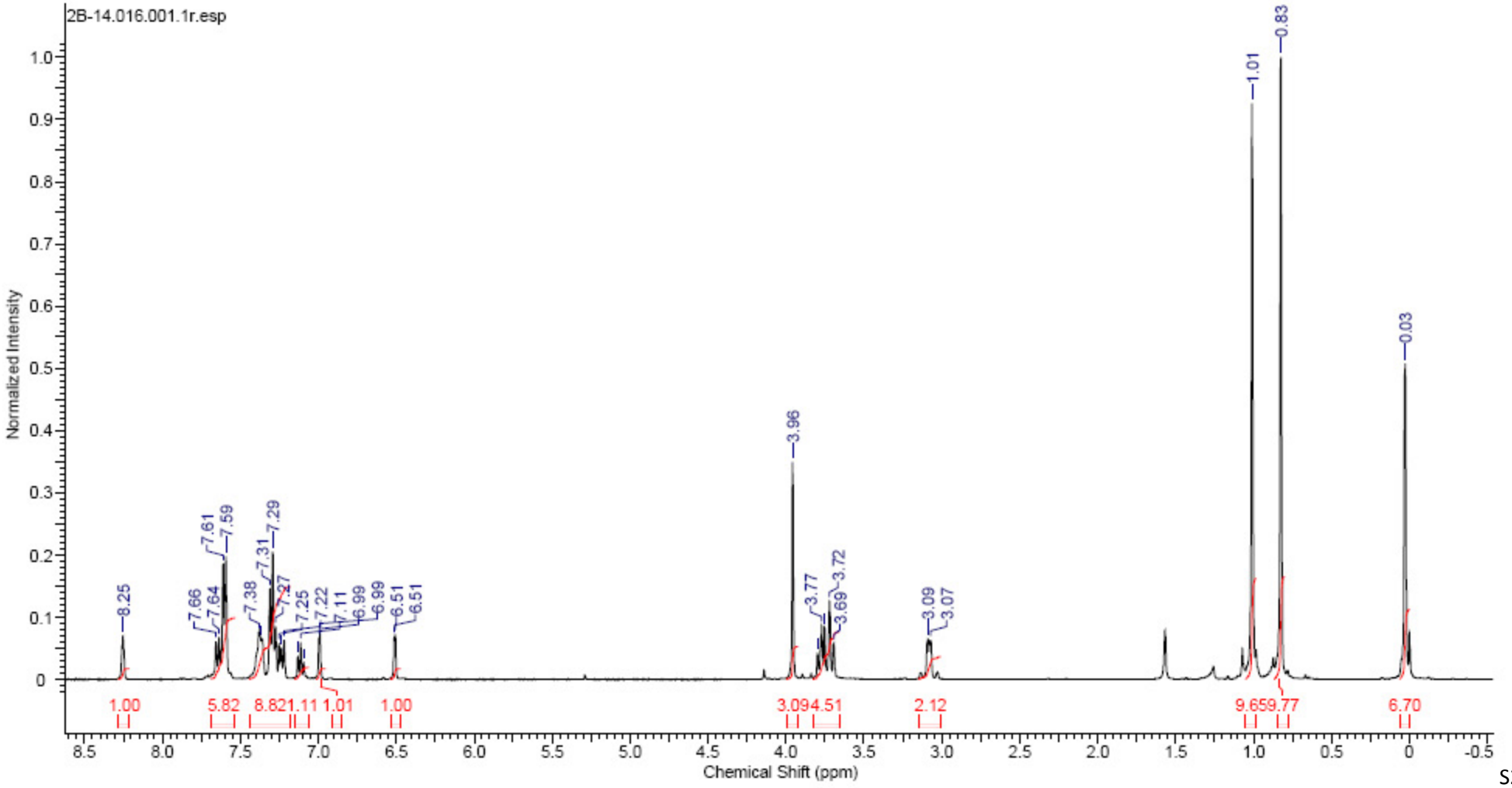
12c


Si
O
O
O
Ph
Si
Ph
N

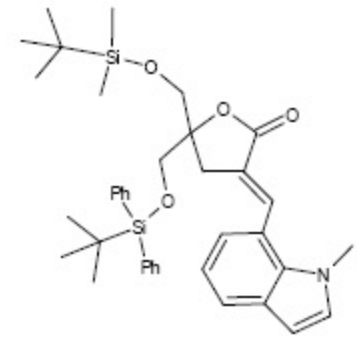

2B-83.tnt
Normalized Intensity
Chemical Shift (ppm)
172.27
137.43
135.60
132.77
131.20
129.77
127.74
122.96
121.13
121.07
111.79
101.36
85.18
77.64
77.00
76.36
66.20
65.36
32.87
32.35
26.67
25.71
19.22
18.14
-5.50

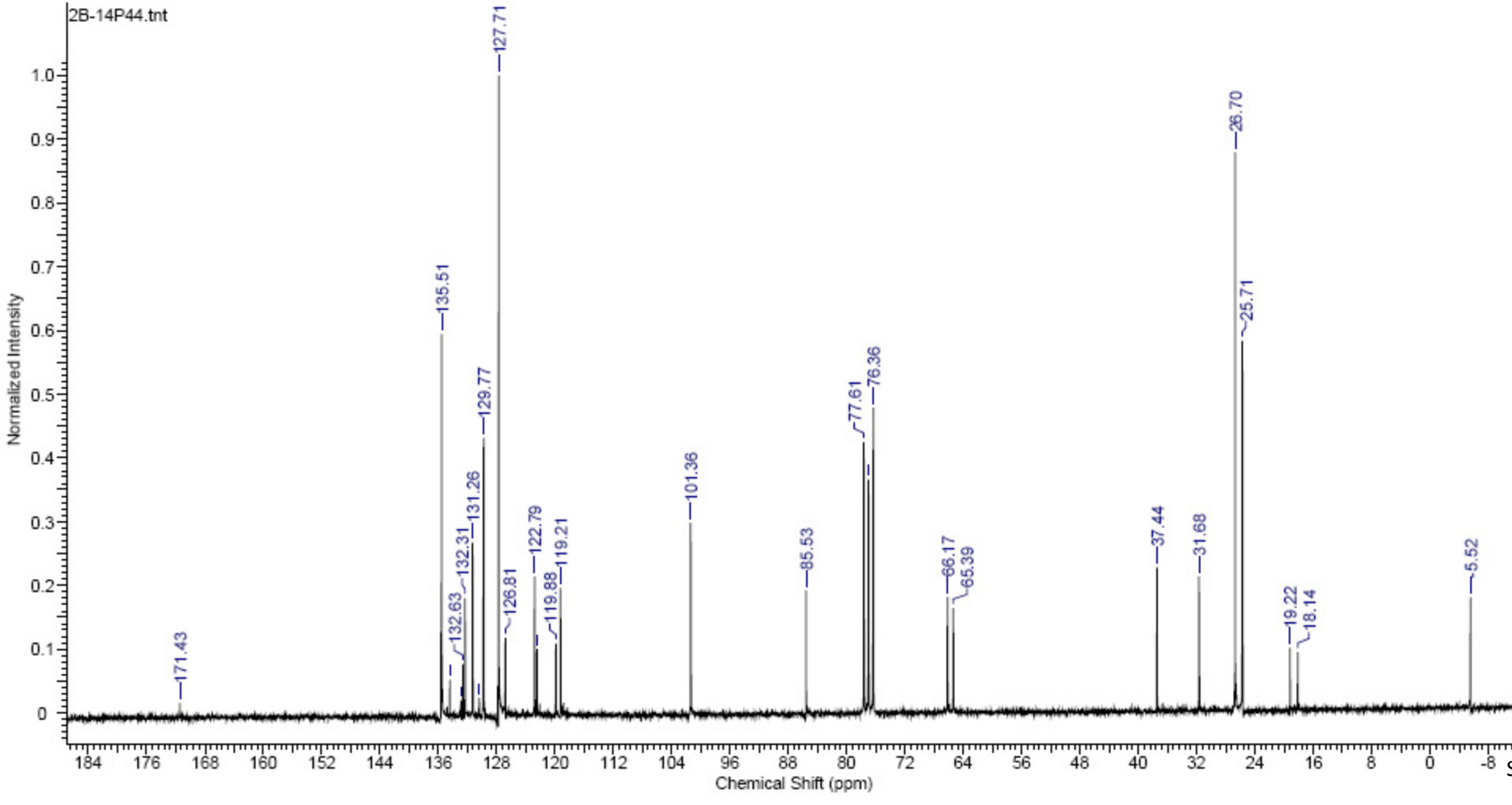
12d


Si
O
O
O
Ph
Si
O
Ph
N

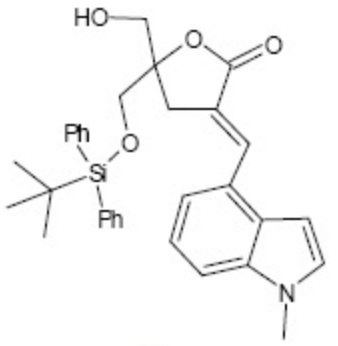

2B-14.016.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
8.25
7.66
7.64
7.61
7.59
7.38
7.31
7.29
7.27
7.25
7.22
7.11
6.99
6.99
6.51
6.51
3.96
3.77
3.72
3.69
3.09
3.07
1.01
0.83
0.03
1.00
5.82
8.82
1.11
1.01
1.00
3.09
4.51
2.12
9.65
9.77
6.70

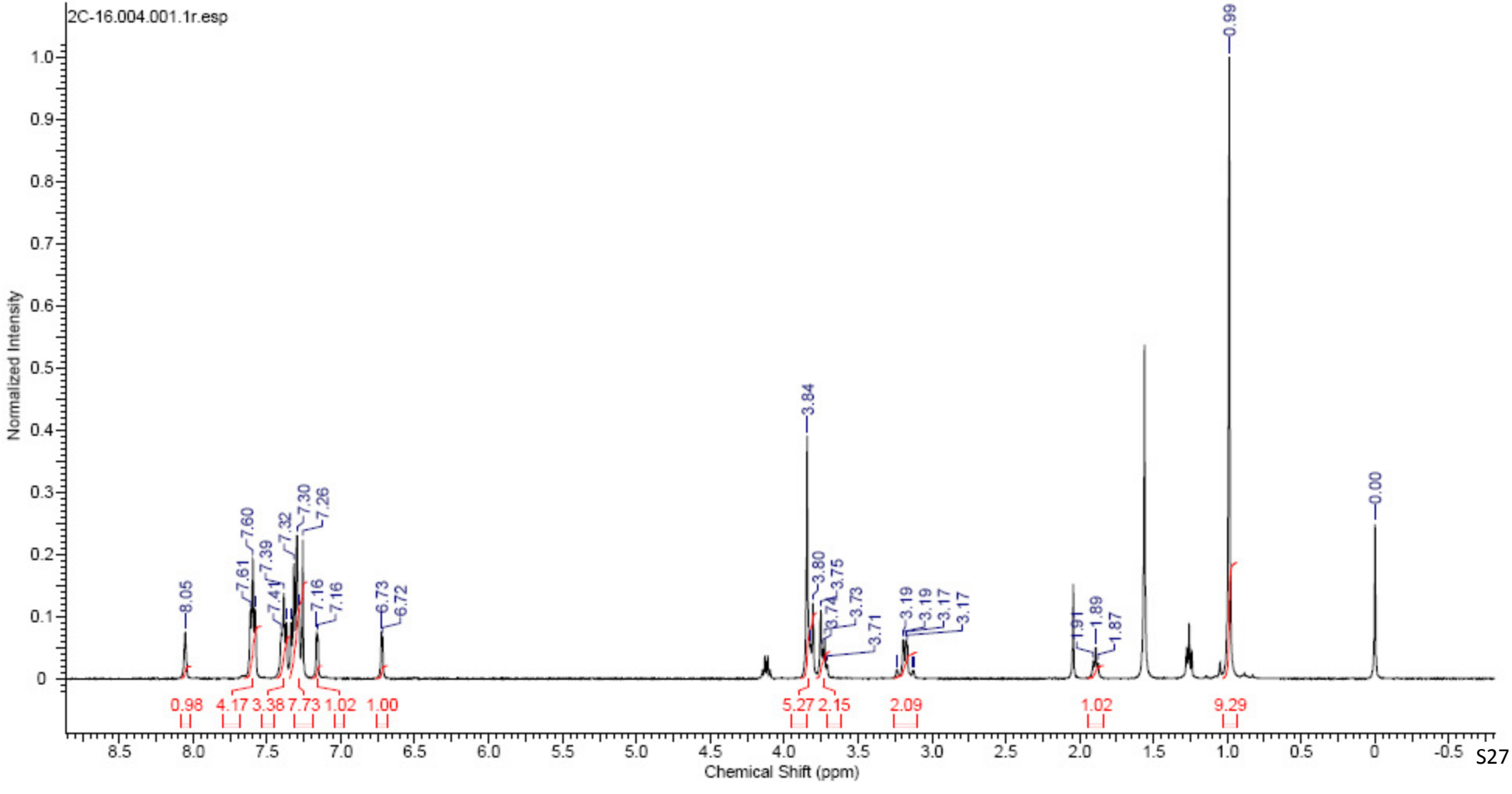
12d


Si
O
O
O
Ph
Si
Ph
N

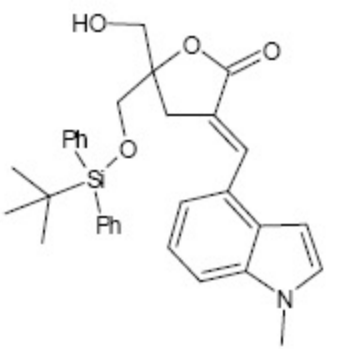

2B-14P44.tnt
Normalized Intensity
Chemical Shift (ppm)
171.43
135.51
132.63
132.31
131.26
129.77
127.71
126.81
122.79
119.88
119.21
101.36
85.53
77.61
76.36
66.17
65.39
37.44
31.68
26.70
25.71
19.22
18.14
5.52

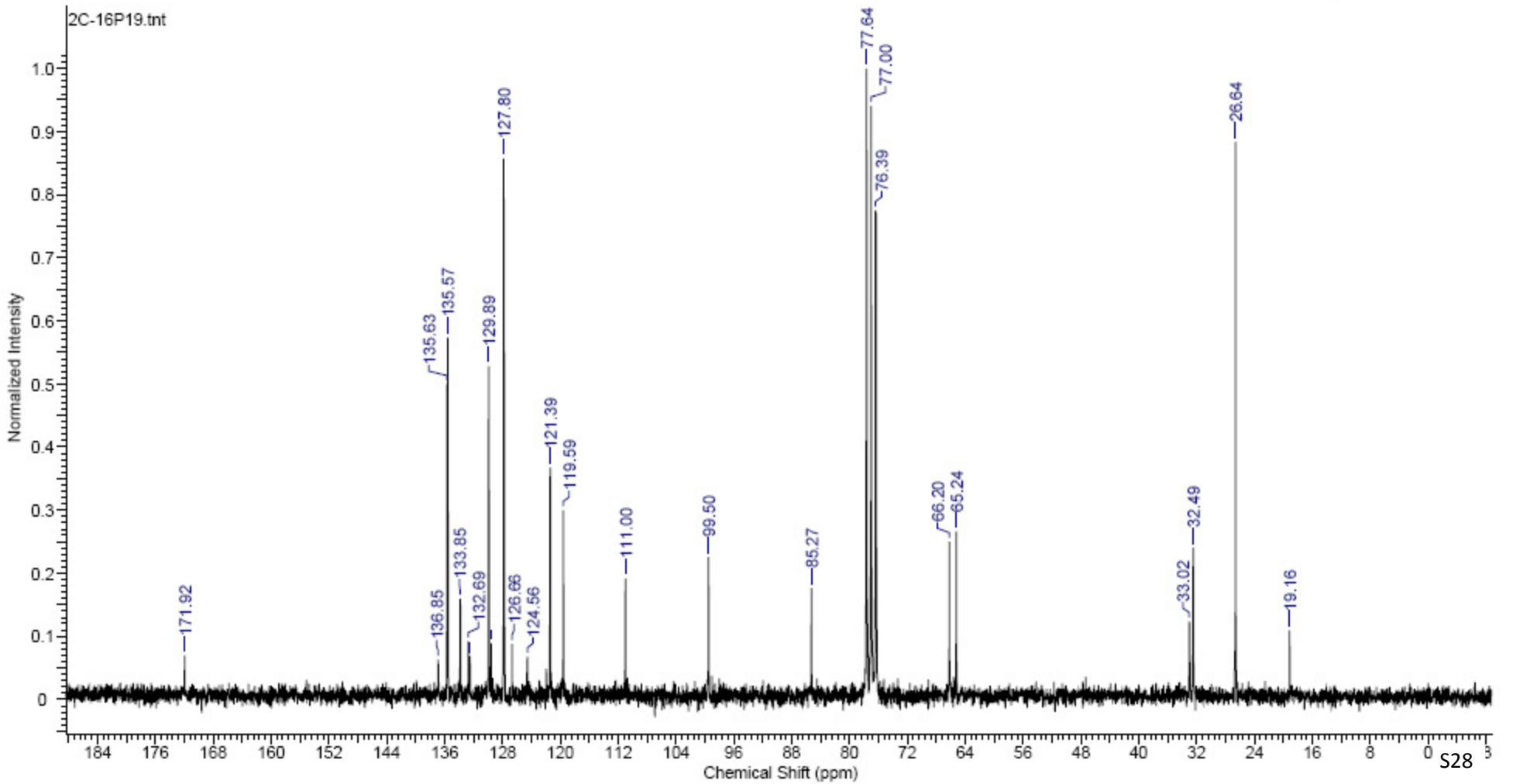
13a


HO
O
O
Ph
O
Si
Ph
N

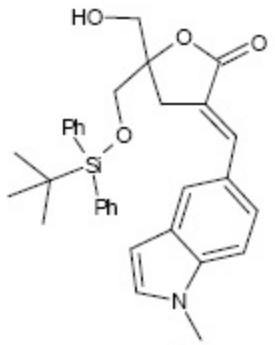

2C-16.004.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
8.05
7.61
7.60
7.41
7.39
7.32
7.30
7.26
7.16
7.16
6.73
6.72
3.84
3.80
3.75
3.74
3.73
3.71
3.19
3.19
3.17
3.17
1.91
1.89
1.87
0.99
0.00
0.98
4.17
3.38
7.73
1.02
1.00
5.27
2.15
2.09
1.02
9.29

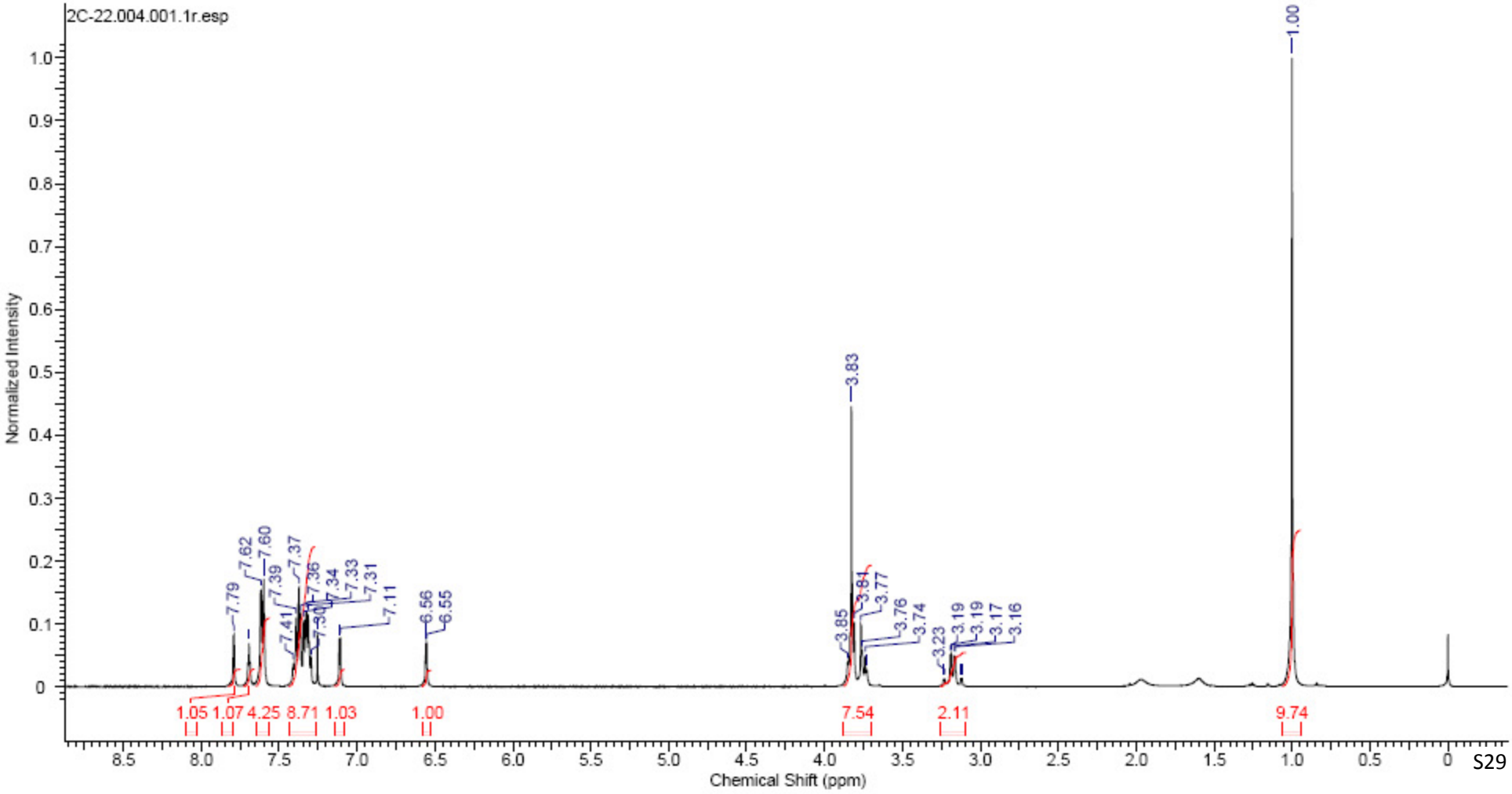
13a


HO
O
O
Ph
O
Si
Ph
N

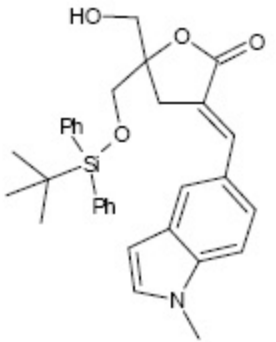

2C-16P19.tnt
Normalized Intensity
Chemical Shift (ppm)
171.92
136.85
135.63
135.57
133.85
132.69
129.89
127.80
126.66
124.56
121.39
119.59
111.00
99.50
85.27
77.64
77.00
76.39
66.20
65.24
33.02
32.49
26.64
19.16

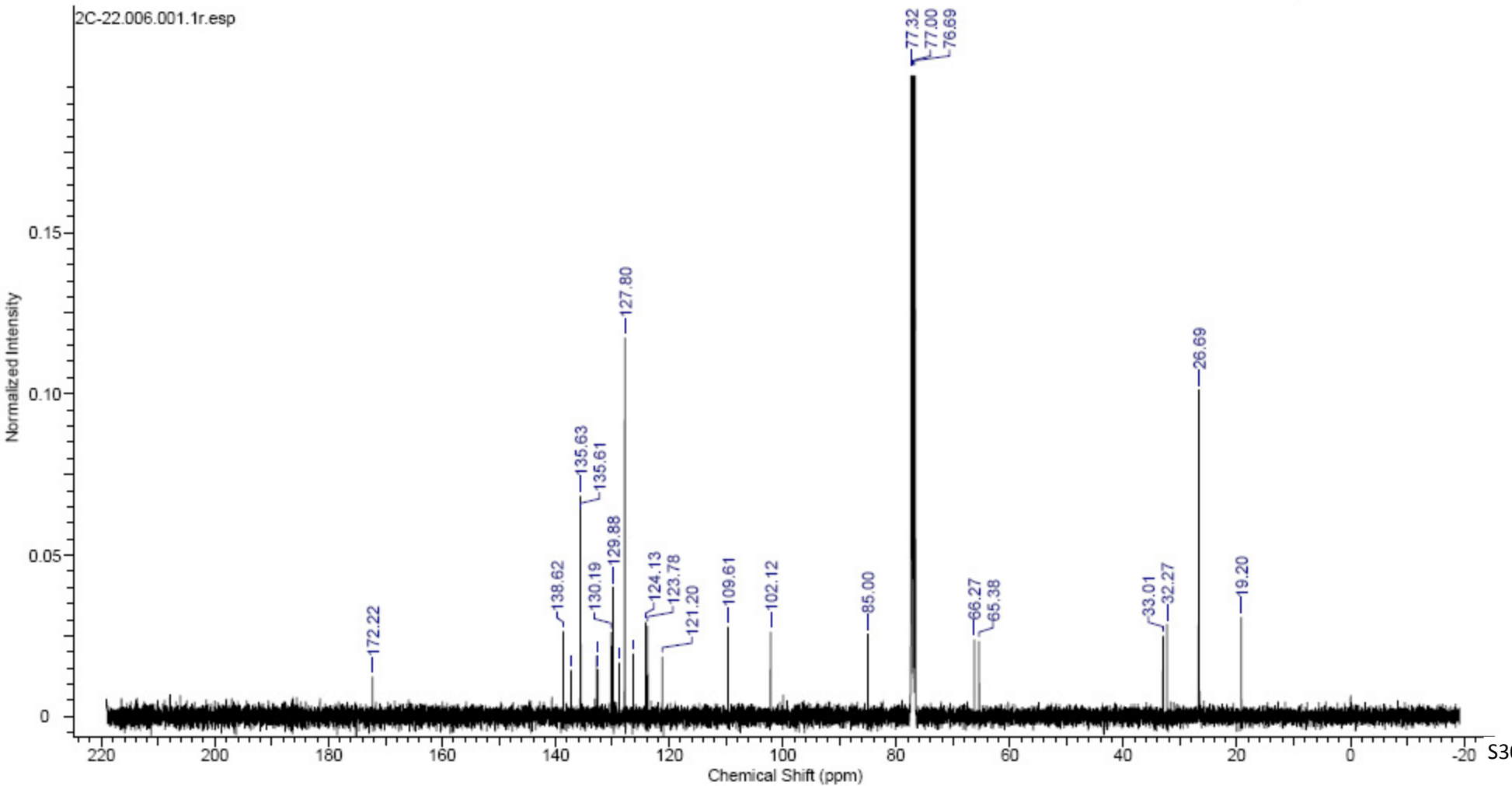
13b


HO
O
O
Ph
O
Si
Ph
N

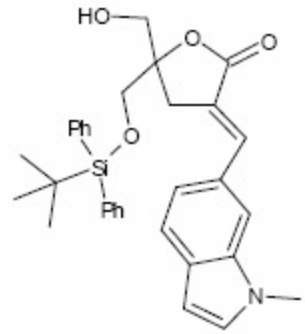

2C-22.004.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
7.79
7.62
7.60
7.41
7.39
7.37
7.36
7.34
7.33
7.31
7.11
6.56
6.55
3.85
3.83
3.81
3.77
3.76
3.74
3.23
3.19
3.19
3.17
3.16
1.00
1.05
1.07
4.25
8.71
1.03
1.00
7.54
2.11
9.74

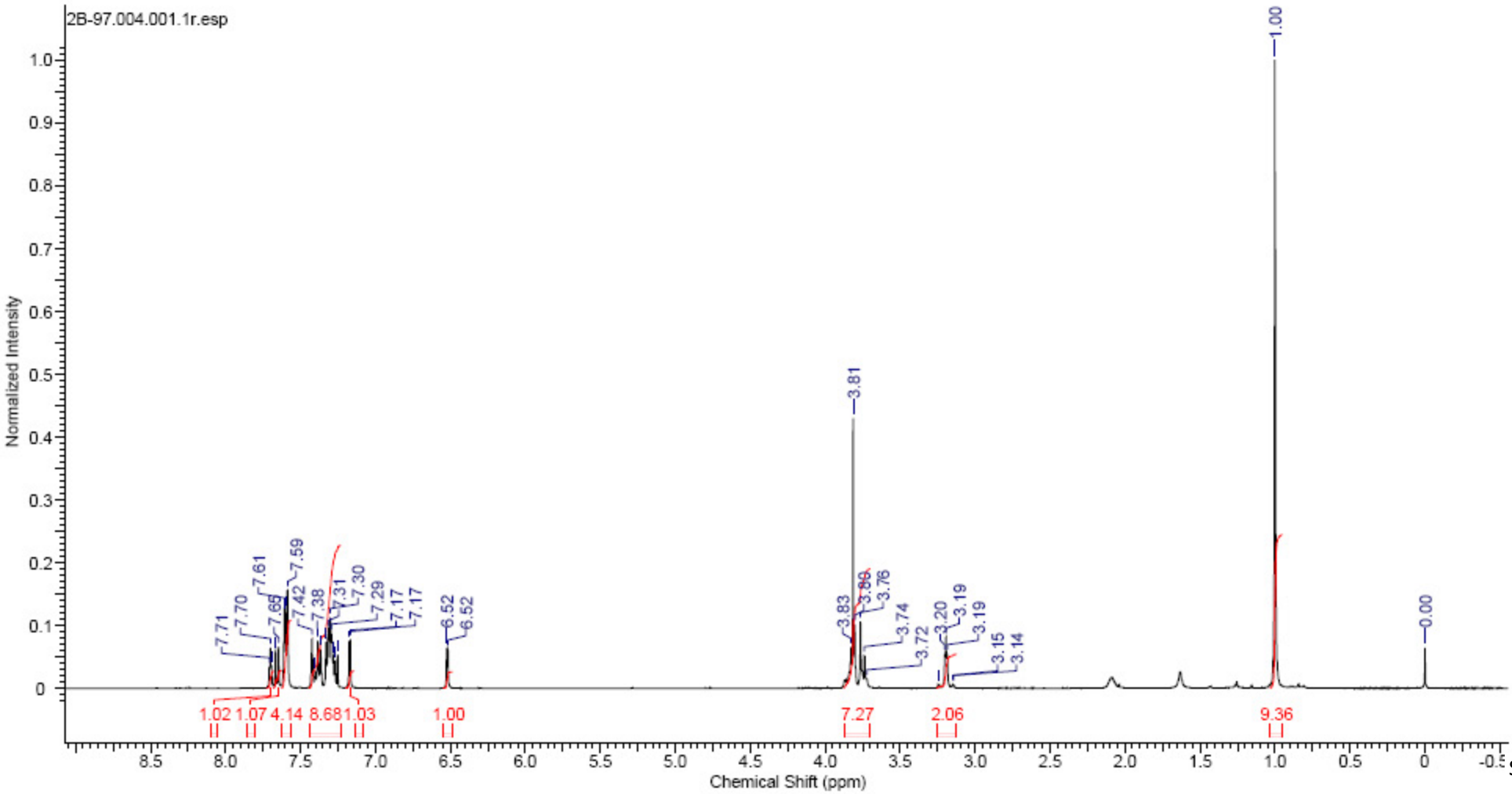
13b


HO
O
O
Ph
O
Si
Ph
N

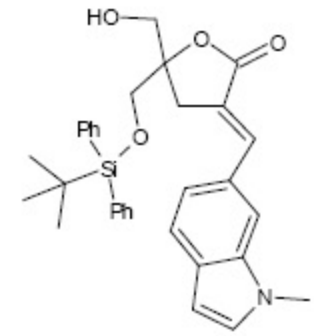

2C-22.006.001.1r.esp
Normalized Intensity
0.15
0.10
0.05
0
220
200
180
160
140
120
100
80
60
40
20
0
-20
Chemical Shift (ppm)
77.32
77.00
76.69
127.80
135.63
135.61
129.88
138.62
130.19
124.13
123.78
121.20
109.61
102.12
85.00
66.27
65.38
33.01
32.27
26.69
19.20
172.22

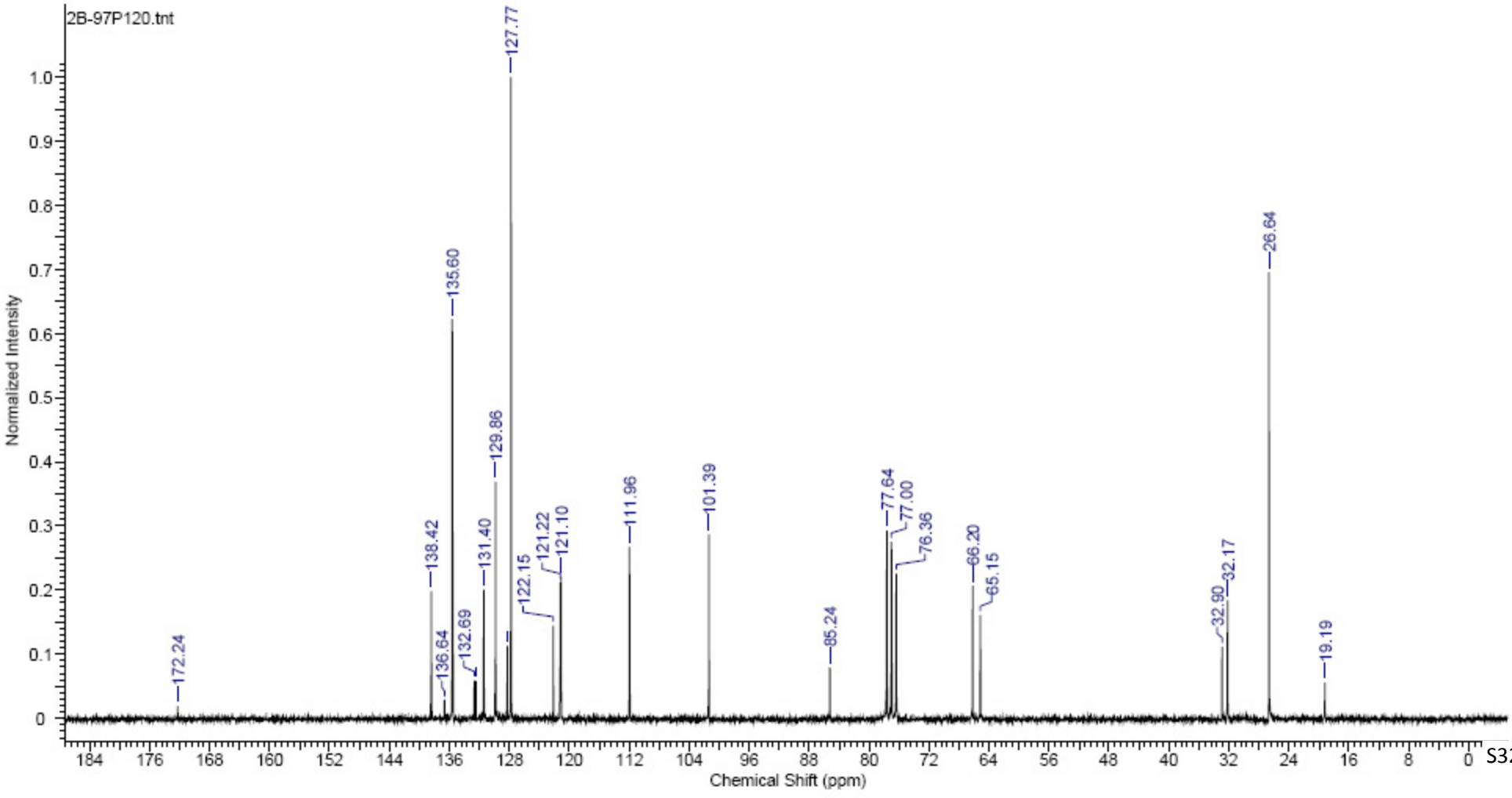
13c


HO
O
O
Ph
O
Si
Ph
N

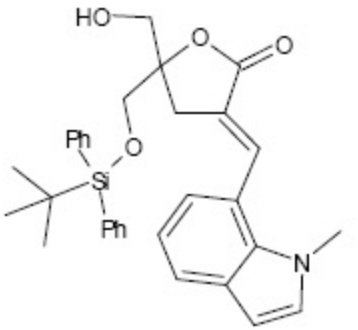

2B-97.004.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
7.71
7.70
7.69
7.61
7.59
7.42
7.38
7.31
7.30
7.29
7.17
7.17
6.52
6.52
3.83
3.81
3.76
3.74
3.72
3.20
3.19
3.19
3.15
3.14
1.00
0.00
1.02
1.07
4.14
8.68
1.03
1.00
7.27
2.06
9.36

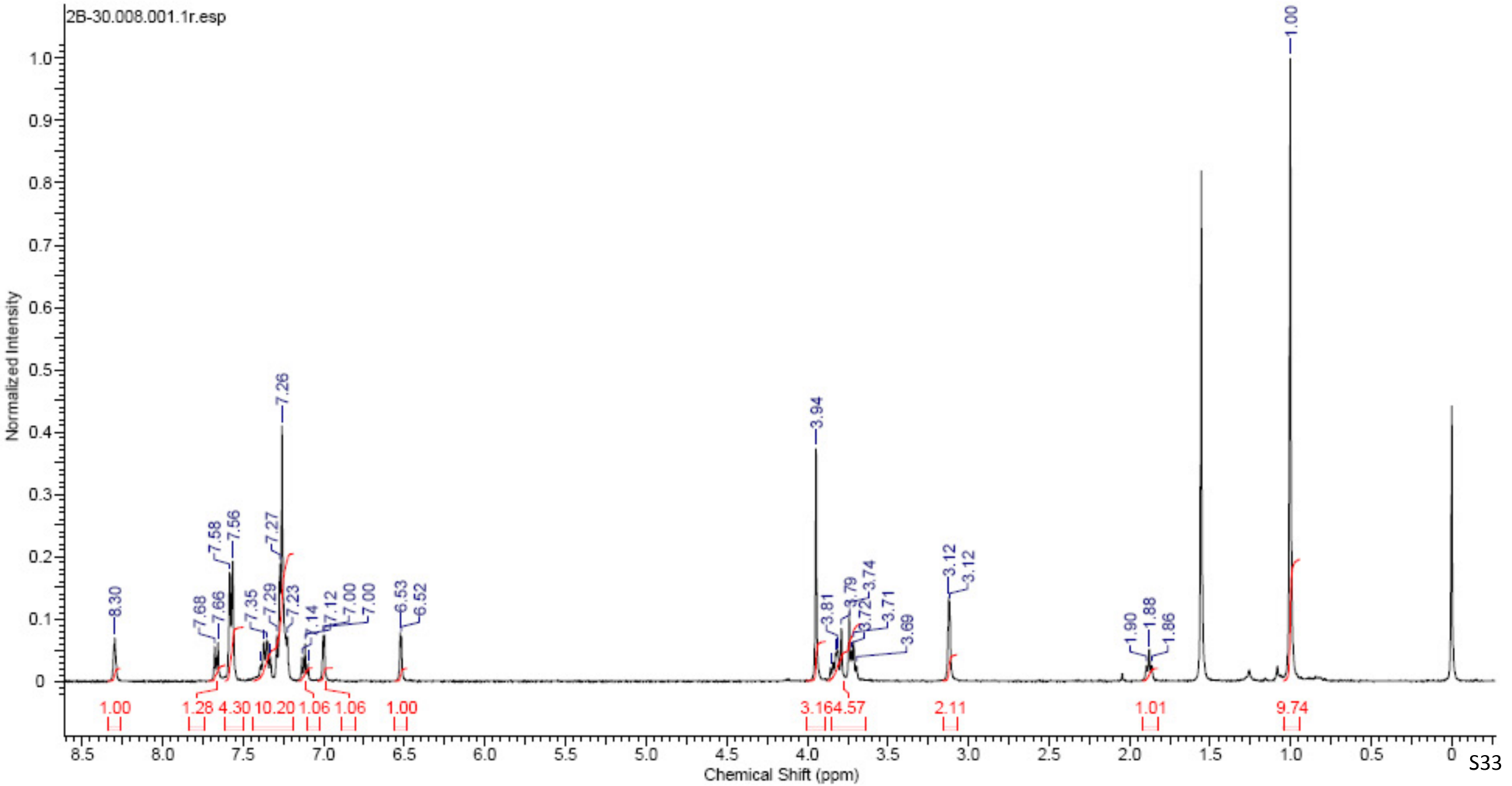
13c


HO
O
O
Ph
O
Si
Ph
N

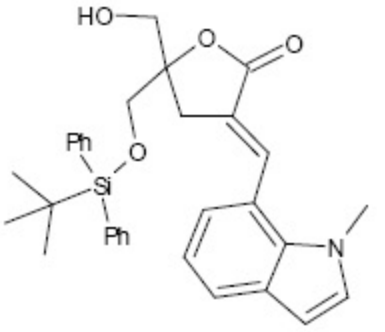

2B-97P120.tnt
Normalized Intensity
Chemical Shift (ppm)
172.24
136.64
138.42
135.60
132.69
131.40
129.86
127.77
122.15
121.22
121.10
111.96
101.39
85.24
77.64
77.00
76.36
66.20
65.15
32.90
32.17
26.64
19.19

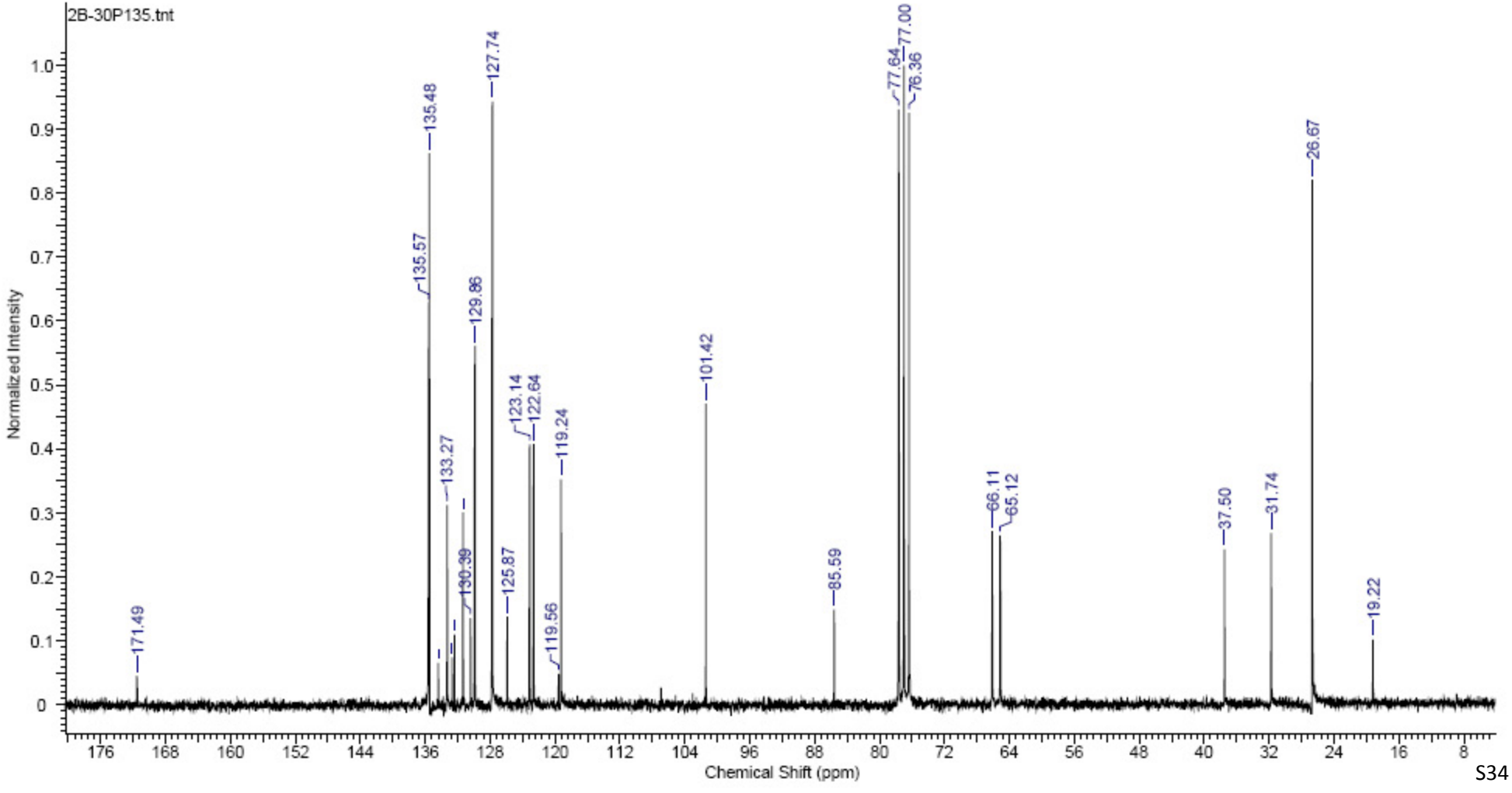
13d


HO
O
O
Ph
O
Si
Ph
N

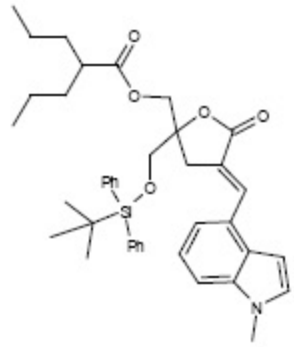

2B-30.008.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
8.30
7.68
7.66
7.58
7.56
7.35
7.29
7.27
7.26
7.23
7.14
7.12
7.00
7.00
6.53
6.52
3.94
3.81
3.79
3.74
3.72
3.71
3.69
3.12
3.12
1.90
1.88
1.86
1.00
1.00
1.28
4.30
10.20
1.06
1.06
1.00
3.16
4.57
2.11
1.01
9.74

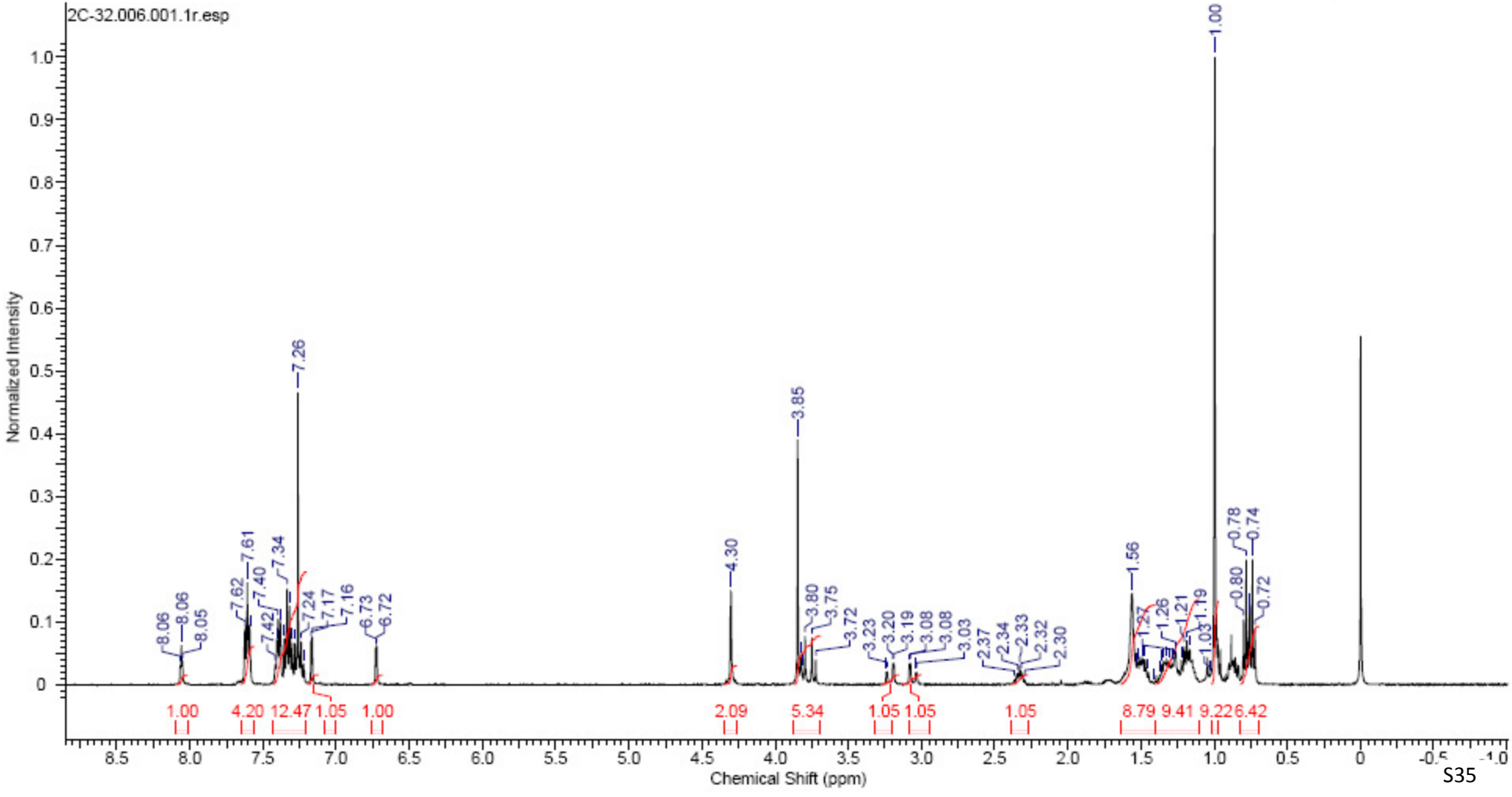
13d


HO
O
O
Ph
O
Si
Ph
N

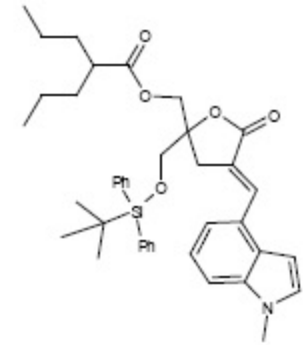

2B-30P135.tnt
Normalized Intensity
Chemical Shift (ppm)
171.49
135.57
135.48
133.27
130.39
129.86
127.74
125.87
123.14
122.64
119.56
119.24
101.42
85.59
77.64
77.00
76.36
66.11
65.12
37.50
31.74
26.67
19.22

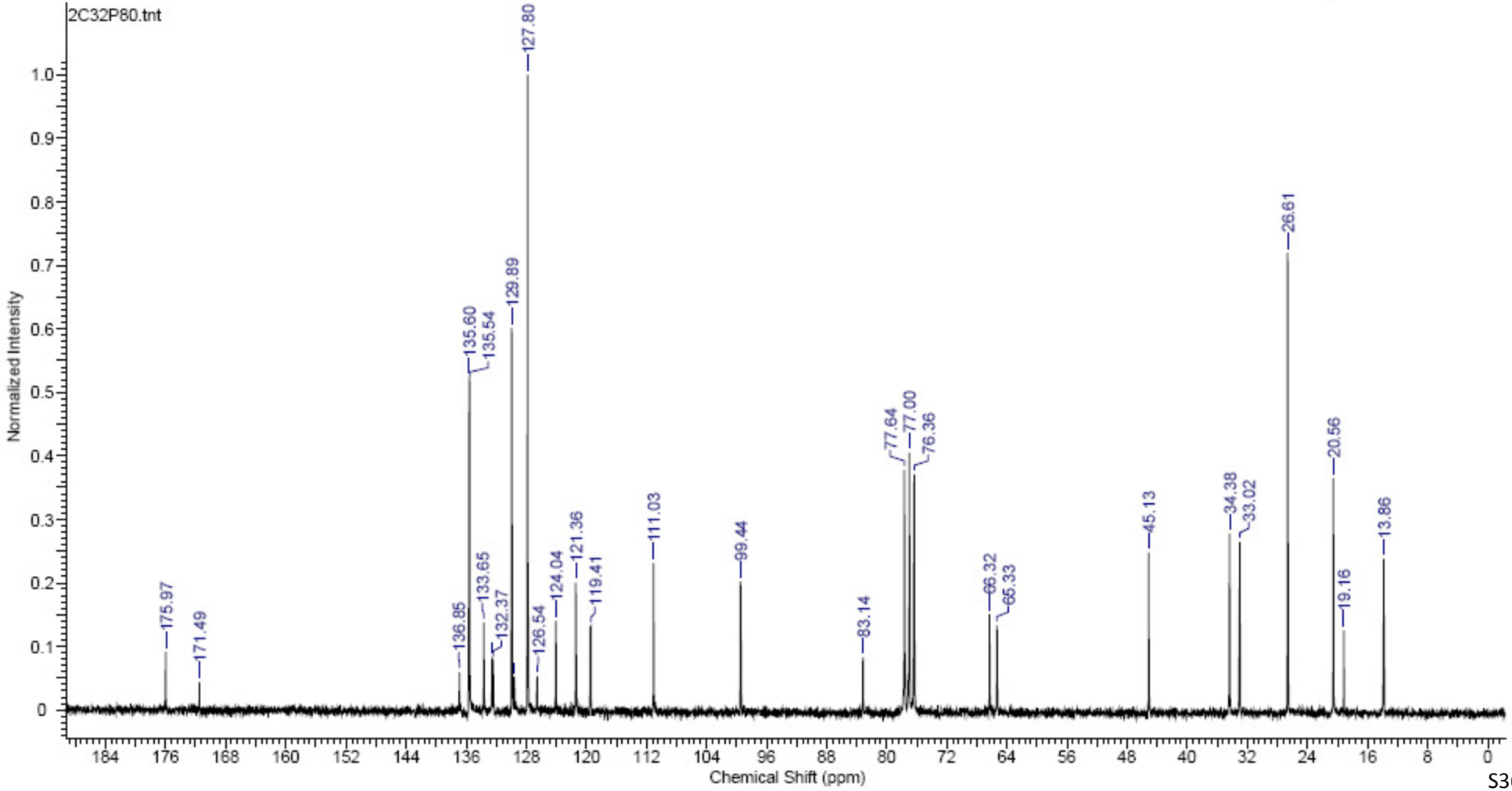
14a



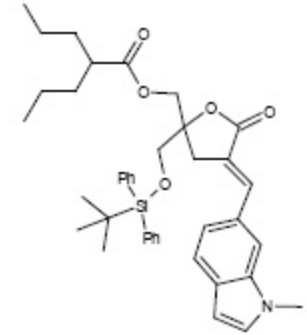

2C-32.006.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
1.00
4.20
12.47
1.05
1.00
2.09
5.34
1.05
1.05
1.05
8.79
9.41
9.22
6.42

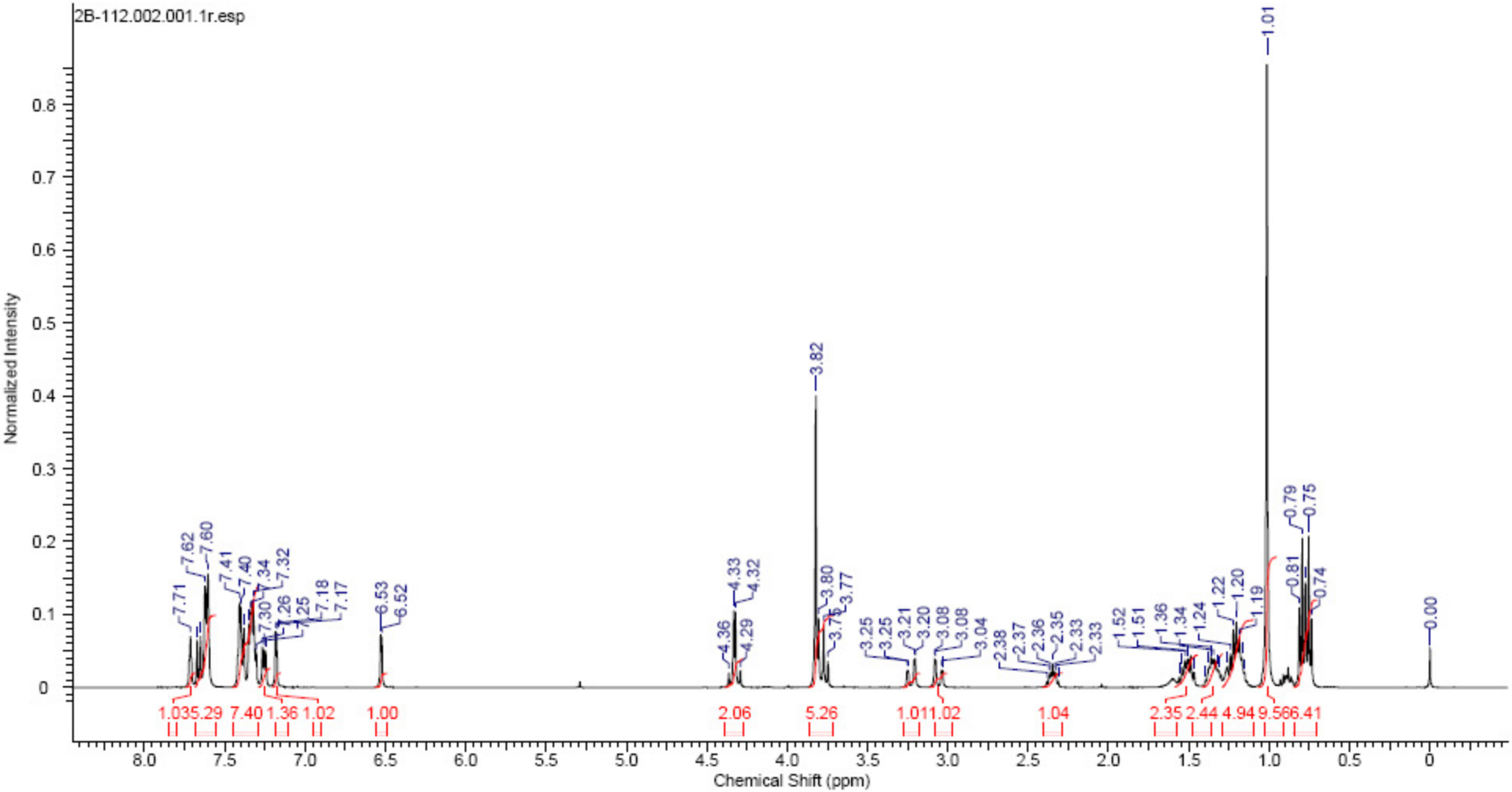
14a


O
O
O
O
O
Si
Ph
Ph
N

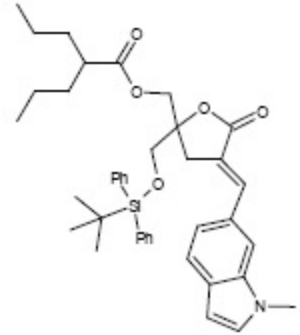

2C32P80.tnt
Normalized Intensity
Chemical Shift (ppm)
175.97
171.49
136.85
135.60
135.54
133.65
132.37
129.89
127.80
126.54
124.04
121.36
119.41
111.03
99.44
83.14
77.64
77.00
76.36
66.32
65.33
45.13
34.38
33.02
26.61
20.56
19.16
13.86

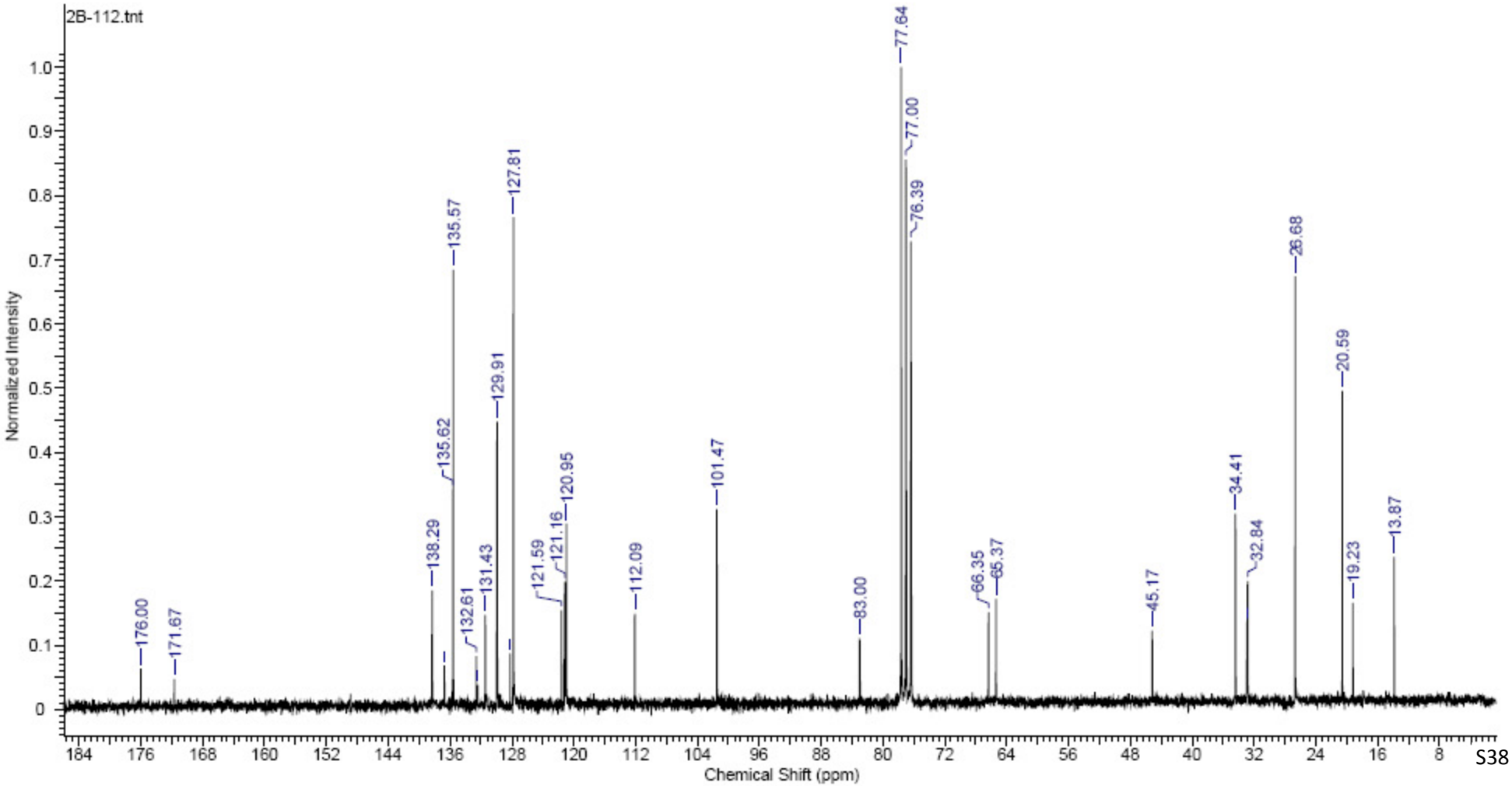
14c



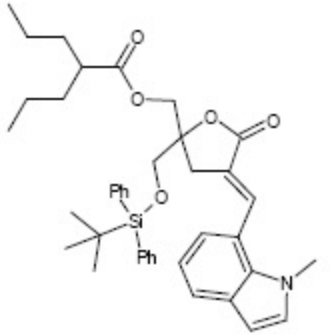

2B-112.002.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
1.01
3.82
7.71
7.62
7.60
7.41
7.40
7.34
7.32
7.30
7.26
7.25
7.18
7.17
6.53
6.52
4.36
4.33
4.32
4.29
3.80
3.77
3.25
3.25
3.21
3.20
3.08
3.08
3.04
2.38
2.37
2.36
2.35
2.33
2.33
1.52
1.51
1.36
1.34
1.24
1.22
1.20
1.19
0.81
0.79
0.75
0.74
0.00
1.03 5.29 7.40 1.36 1.02
1.00
2.06
5.26
1.01 1.02
1.04
2.35 2.44 4.94 9.56 6.41

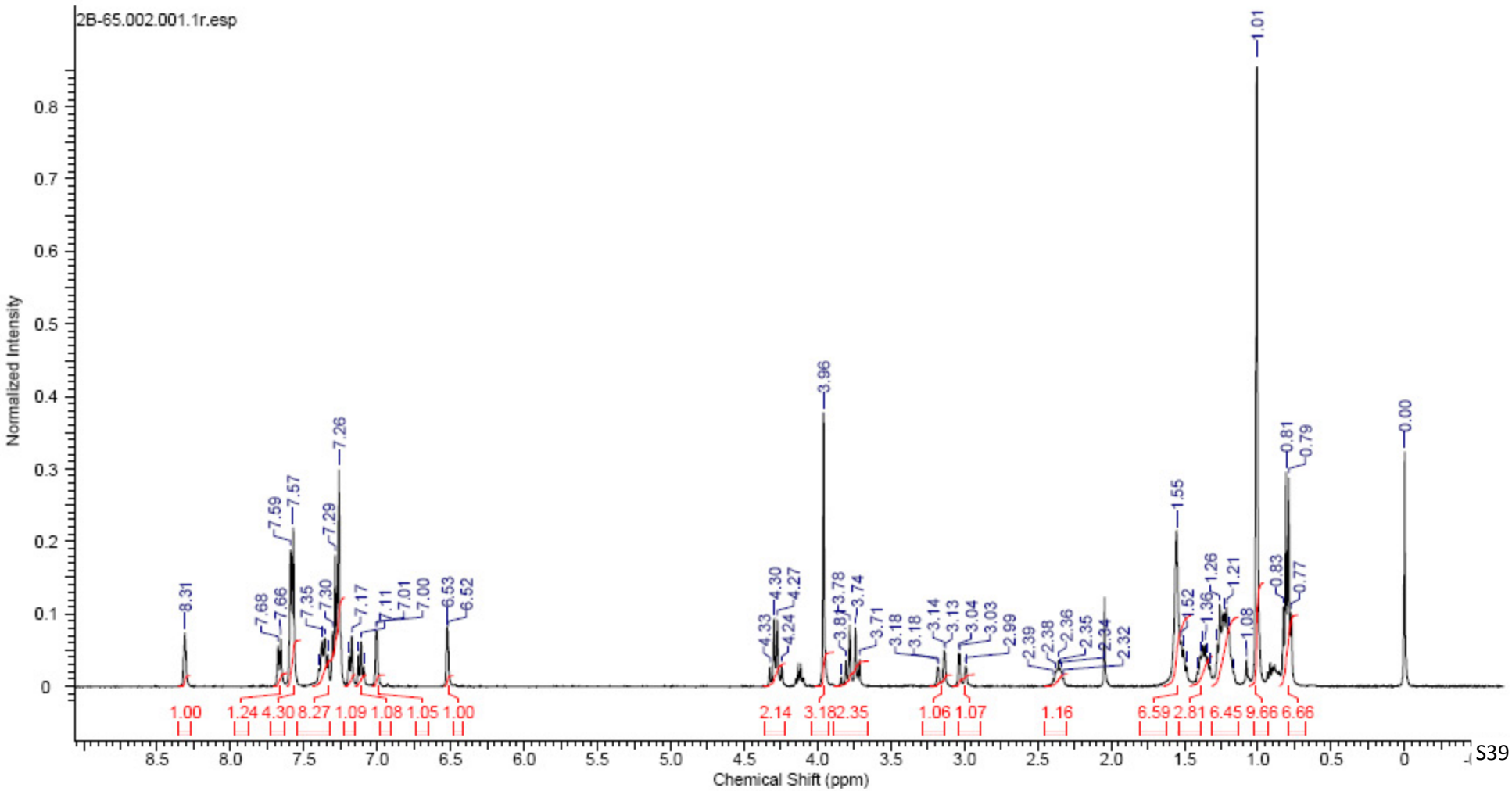
14c


O
O
O
O
Ph
Si
O
Ph
N

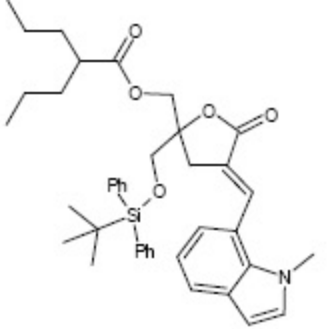

2B-112.tnt
Normalized Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
176.00
171.67
138.29
135.62
135.57
132.61
131.43
129.91
127.81
121.59
121.16
120.95
112.09
101.47
83.00
77.64
77.00
76.39
66.35
65.37
45.17
34.41
32.84
26.68
20.59
19.23
13.87
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
Chemical Shift (ppm)

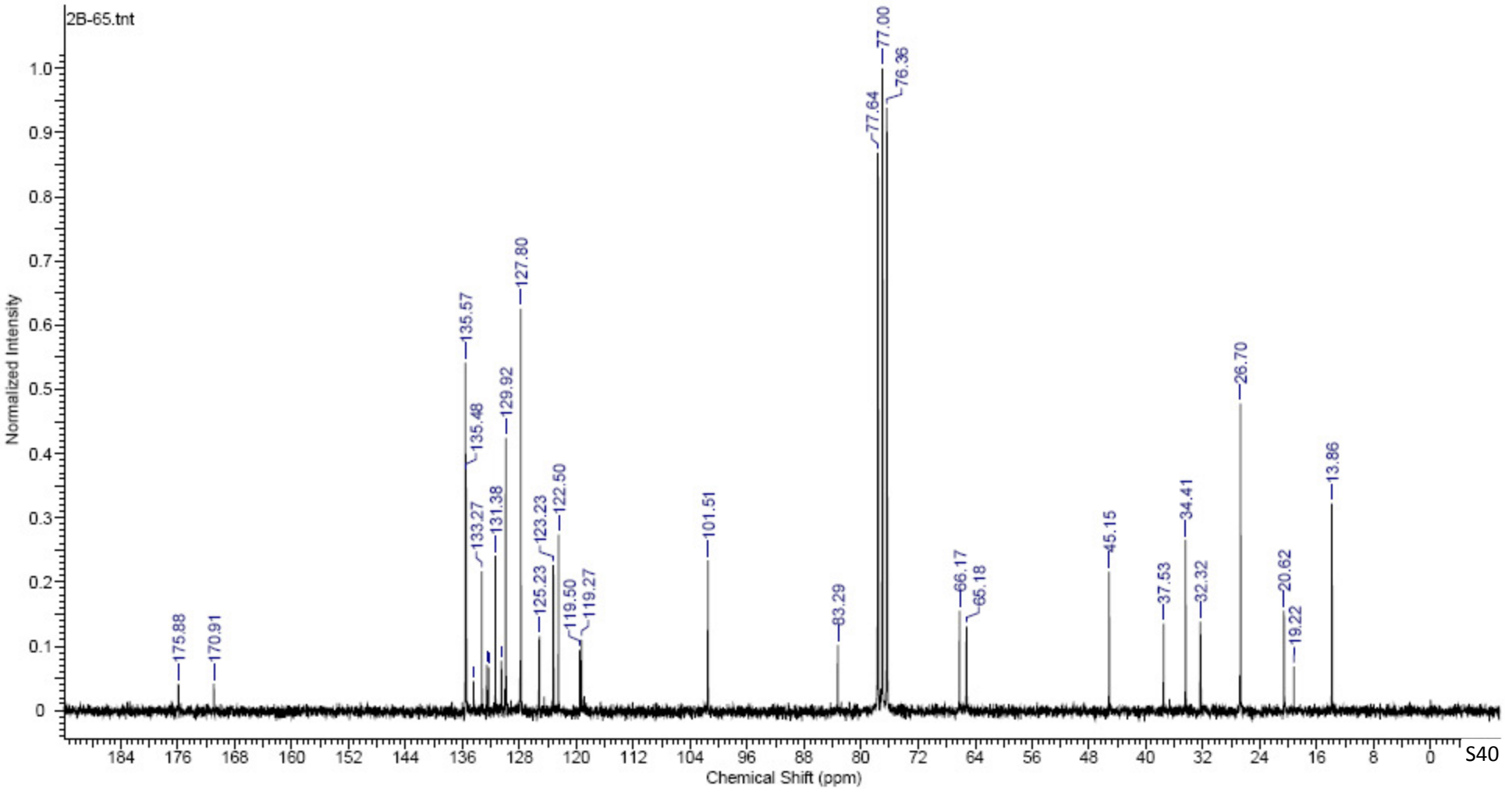
14d


O
O
O
O
Ph
Si
Ph
N

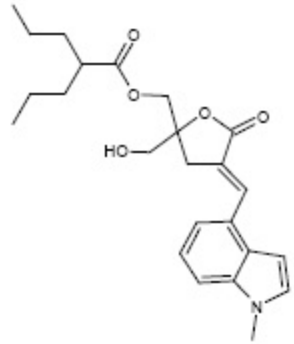

2B-65.002.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
8.31
7.68
7.66
7.59
7.57
7.35
7.30
7.29
7.26
7.17
7.11
7.01
7.00
6.53
6.52
4.33
4.30
4.27
4.24
3.96
3.81
3.78
3.74
3.71
3.18
3.18
3.14
3.13
3.04
3.03
2.99
2.39
2.38
2.36
2.35
2.34
2.32
1.55
1.52
1.36
1.26
1.21
1.08
1.01
0.83
0.81
0.79
0.77
0.00
1.00
1.24
4.30
8.27
1.09
1.08
1.05
1.00
2.14
3.18
2.35
1.06
1.07
1.16
6.59
2.81
6.45
9.66
6.66

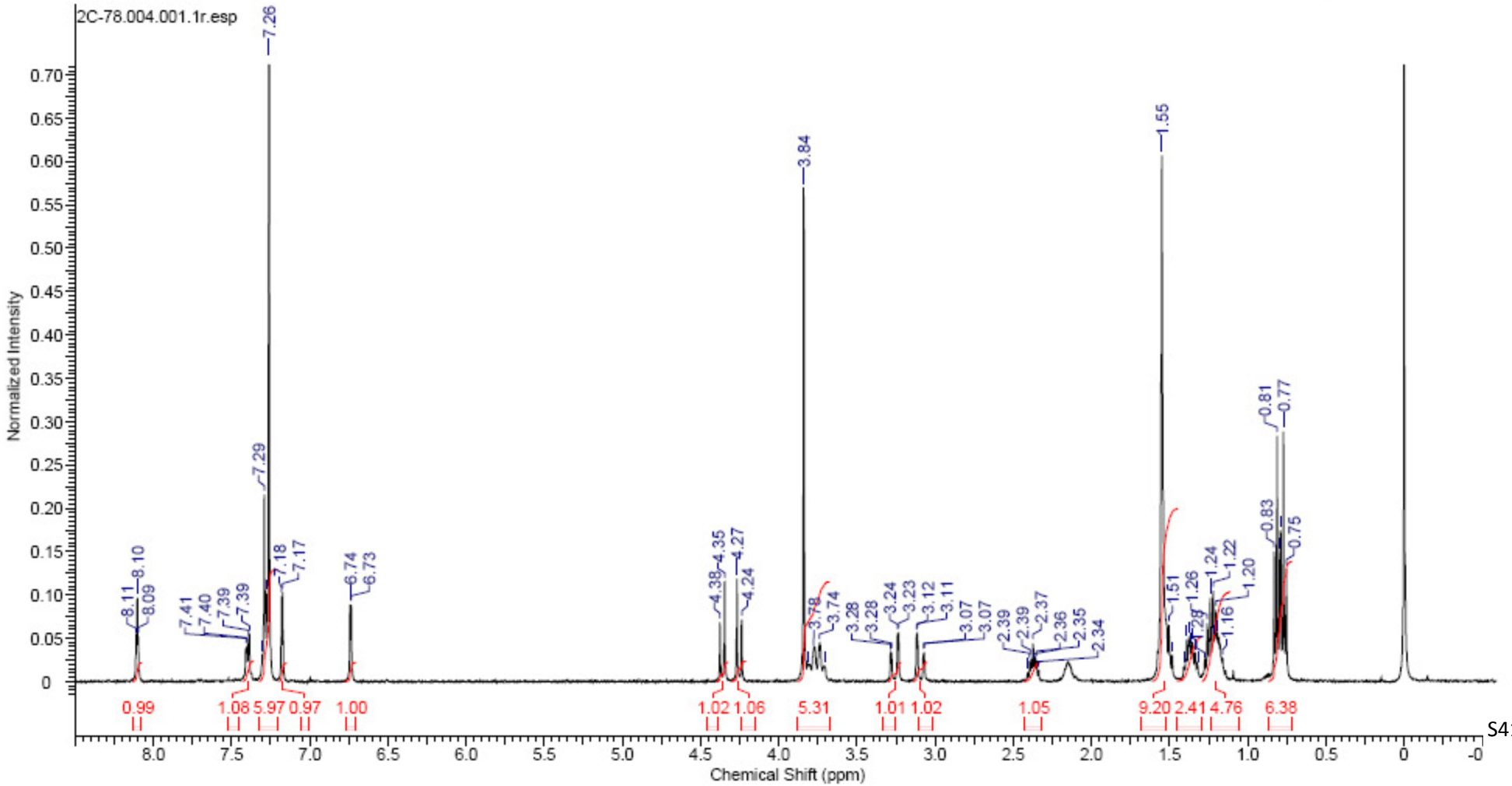
14d


O
O
O
O
O
Ph
Si
Ph
N

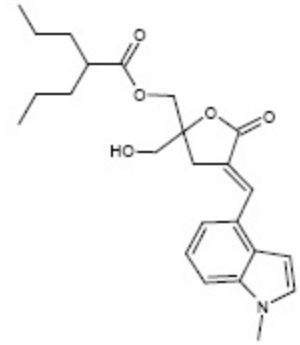

2B-65.tnt
Normalized Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
175.88
170.91
135.57
135.48
133.27
131.38
129.92
127.80
125.23
123.23
122.50
119.50
119.27
101.51
83.29
77.64
77.00
76.36
66.17
65.18
45.15
37.53
34.41
32.32
26.70
20.62
19.22
13.86
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)

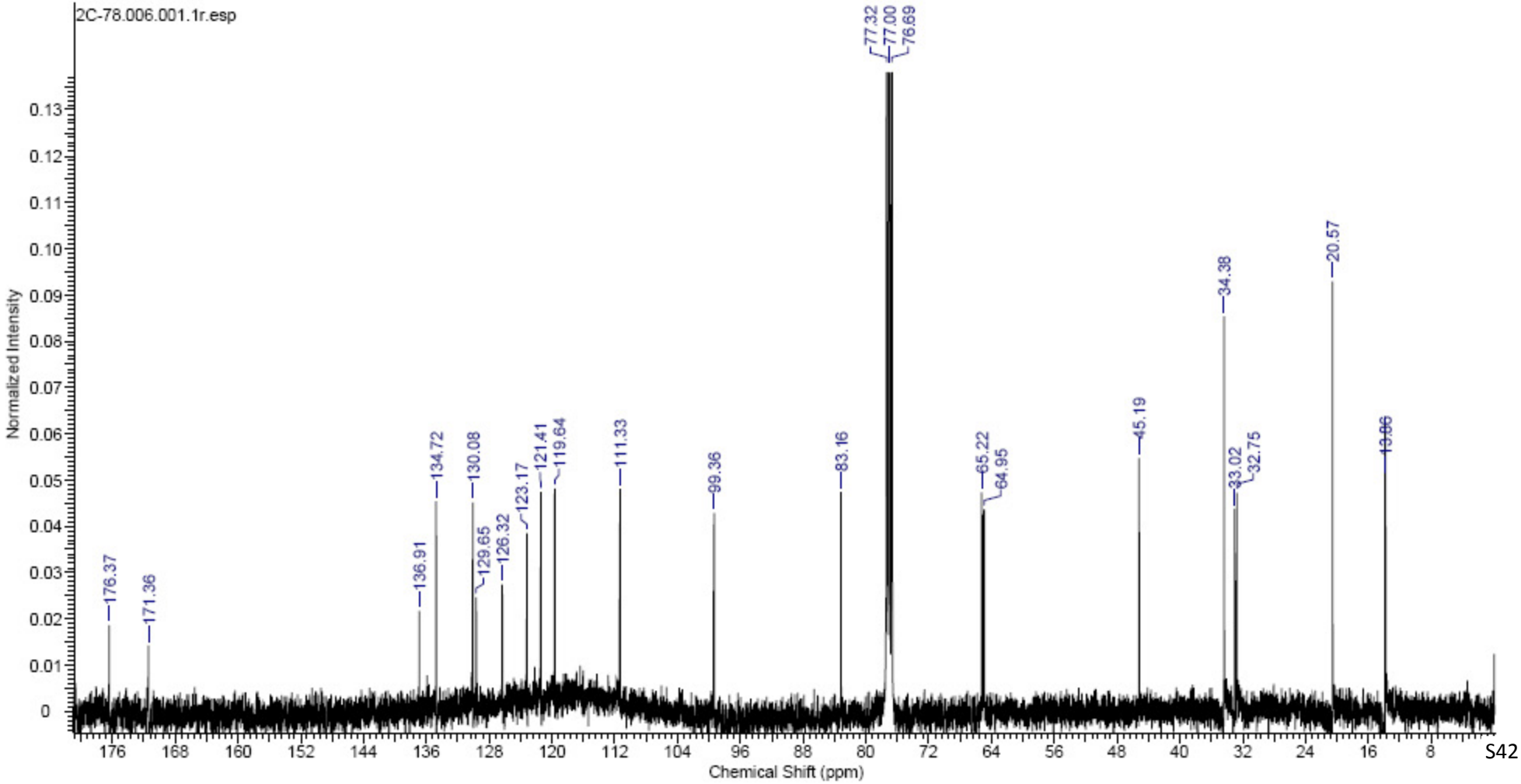
2


HO
O
O
O
O
N

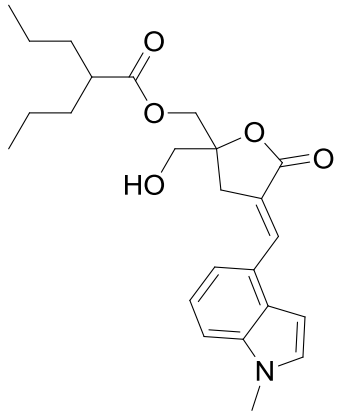

2C-78.004.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
7.26
7.29
8.11
8.10
8.09
7.41
7.40
7.39
7.39
7.18
7.17
6.74
6.73
4.38
4.35
4.27
4.24
3.84
3.78
3.74
3.28
3.28
3.24
3.23
3.12
3.11
3.07
3.07
2.39
2.39
2.37
2.36
2.35
2.34
1.55
1.51
1.26
1.24
1.22
1.20
1.16
0.81
0.77
0.83
0.75
0.99
1.08
5.97
0.97
1.00
1.02
1.06
5.31
1.01
1.02
1.05
9.20
2.41
4.76
6.38

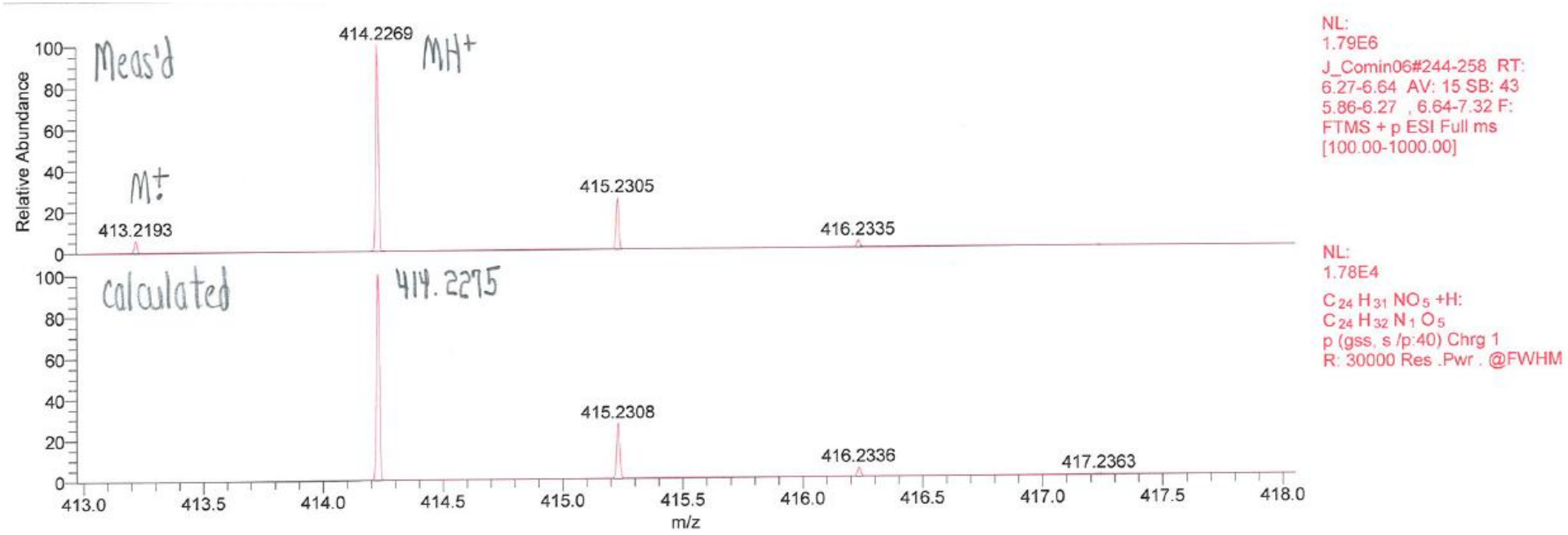
2


O
O
O
HO
O
N

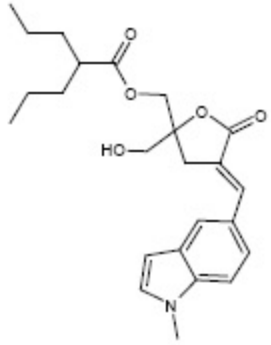

2C-78.006.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
0.11
0.12
0.13
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
176.37
171.36
136.91
134.72
130.08
129.65
126.32
123.17
121.41
119.64
111.33
99.36
83.16
77.32
77.00
76.69
65.22
64.95
45.19
34.38
33.02
32.75
20.57
13.86

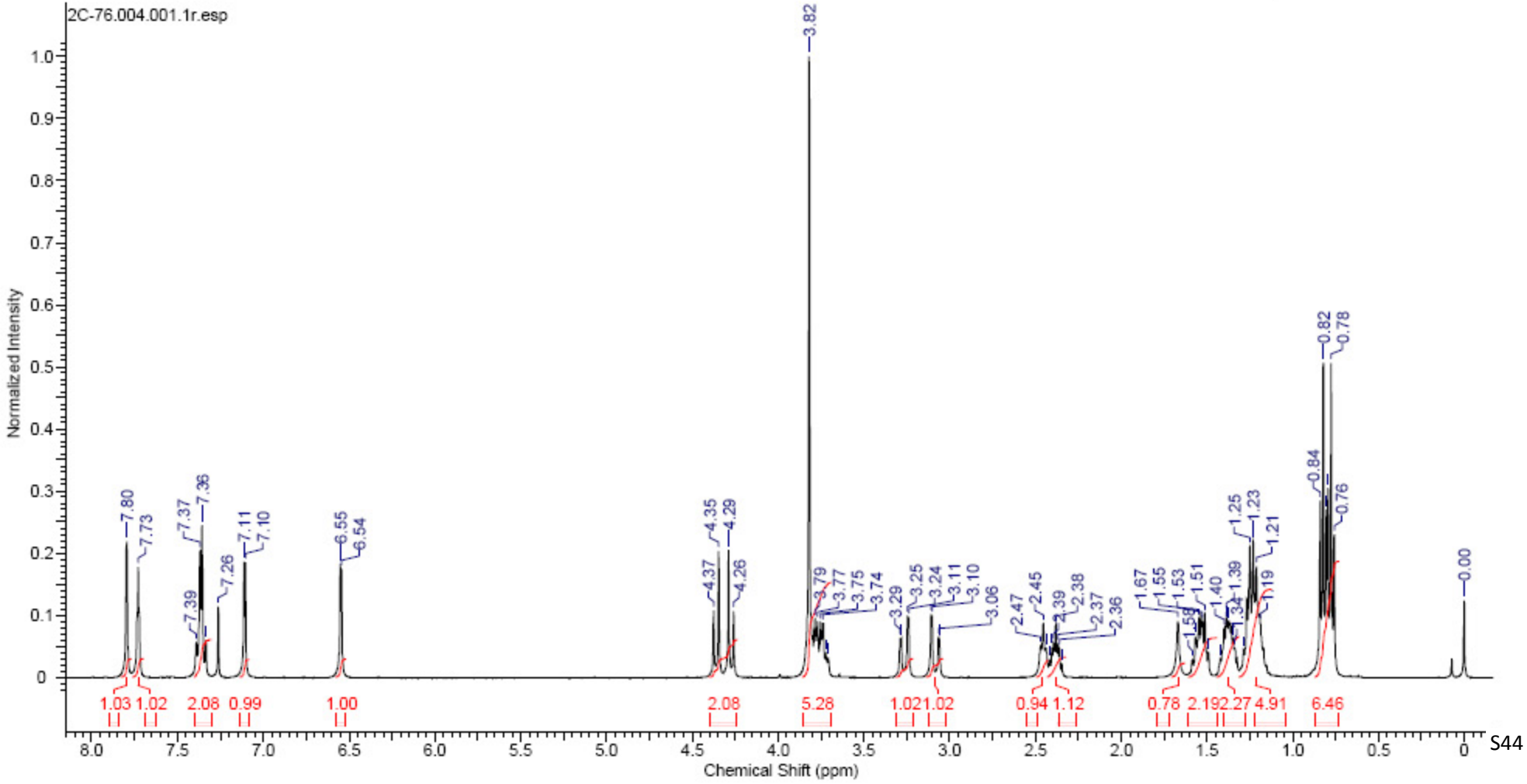
2


O
O
O
O
HO
N

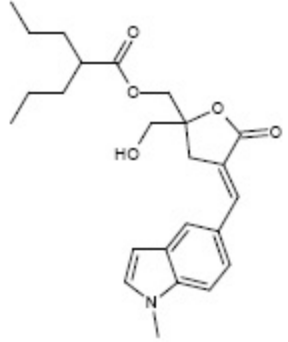

Meas'd
414.2269
MH+
M+
413.2193
415.2305
416.2335
calculated
414.2275
415.2308
416.2336
417.2363
Relative Abundance
m/z
NL:
1.79E6
J_Comin06#244-258 RT:
6.27-6.64 AV: 15 SB: 43
5.86-6.27 , 6.64-7.32 F:
FTMS + p ESI Full ms
[100.00-1000.00]
NL:
1.78E4
C24 H31 NO5 +H:
C24 H32 N1 O5
p (gss, s /p:40) Chrg 1
R: 30000 Res .Pwr . @FWHM

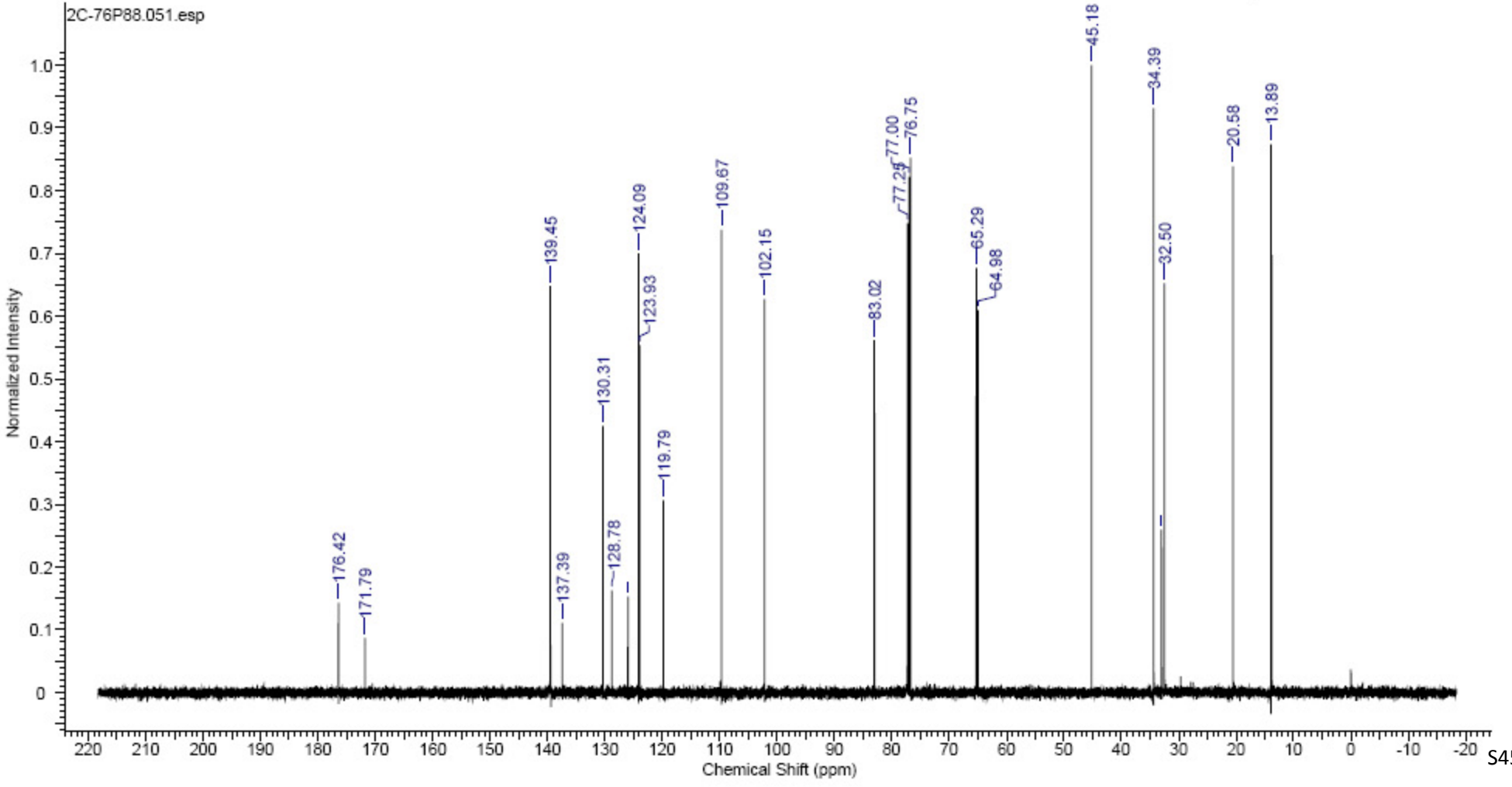
3


O
O
O
HO
O
N

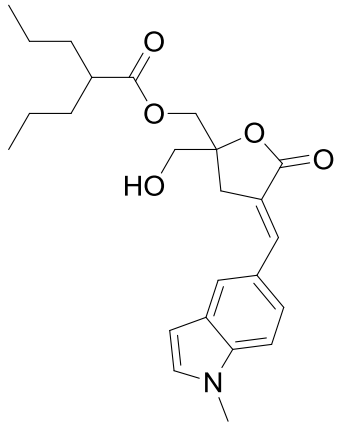

2C-76.004.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
7.80
7.73
7.39
7.37
7.36
7.26
7.11
7.10
6.55
6.54
4.37
4.35
4.29
4.26
3.82
3.79
3.77
3.75
3.74
3.29
3.25
3.24
3.11
3.10
3.06
2.47
2.45
2.39
2.38
2.37
2.36
1.67
1.55
1.53
1.51
1.40
1.39
1.34
1.25
1.23
1.21
0.84
0.82
0.78
0.76
0.00
1.03
1.02
2.08
0.99
1.00
2.08
5.28
1.02
1.02
0.94
1.12
0.78
2.19
2.27
4.91
6.46

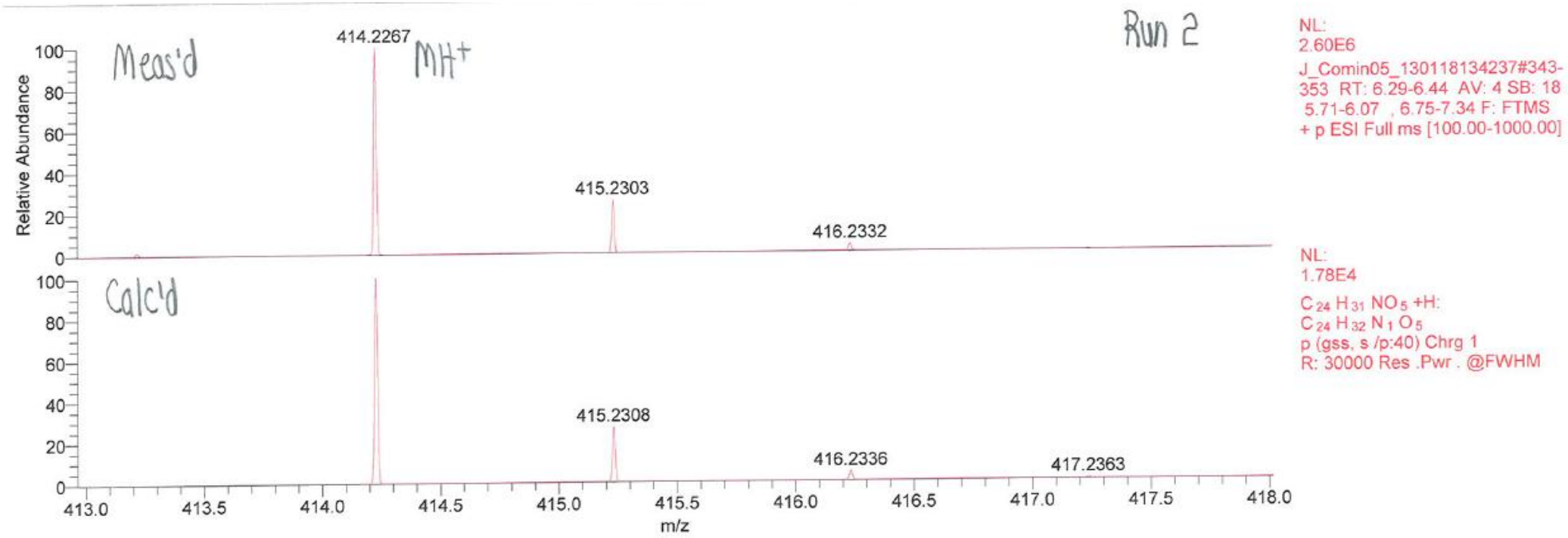
3


O
O
O
HO
O
O
N

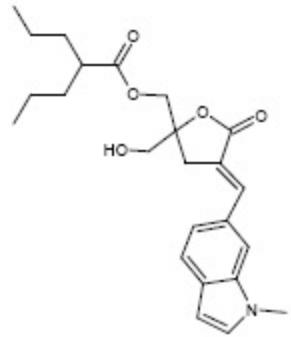

2C-76P88.051.esp
Normalized Intensity
Chemical Shift (ppm)
176.42
171.79
139.45
137.39
130.31
128.78
124.09
123.93
119.79
109.67
102.15
83.02
77.25
77.00
76.75
65.29
64.98
45.18
34.39
32.50
20.58
13.89

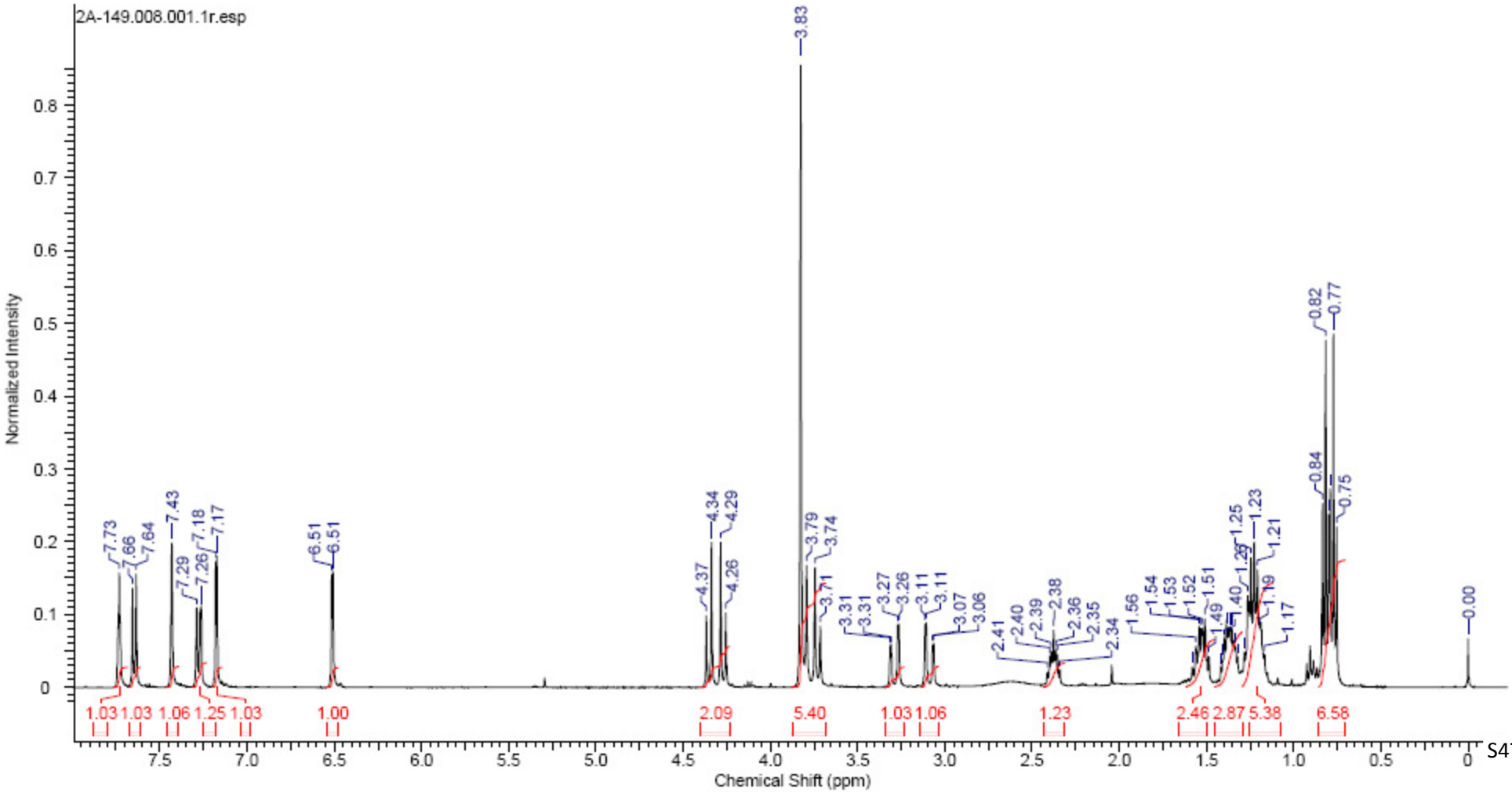
3


O
O
O
O
HO
N

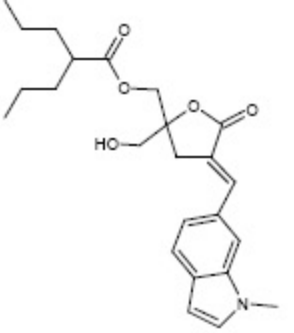

Meas'd
414.2267
MH+
Run 2
415.2303
416.2332
Calc'd
415.2308
416.2336
417.2363
Relative Abundance
m/z
NL:
2.60E6
J_Comin05_130118134237#343-353 RT: 6.29-6.44 AV: 4 SB: 18 5.71-6.07 , 6.75-7.34 F: FTMS + p ESI Full ms [100.00-1000.00]
NL:
1.78E4
C24 H31 NO5 +H:
C24 H32 N1 O5
p (gss, s /p:40) Chrg 1
R: 30000 Res .Pwr . @FWHM

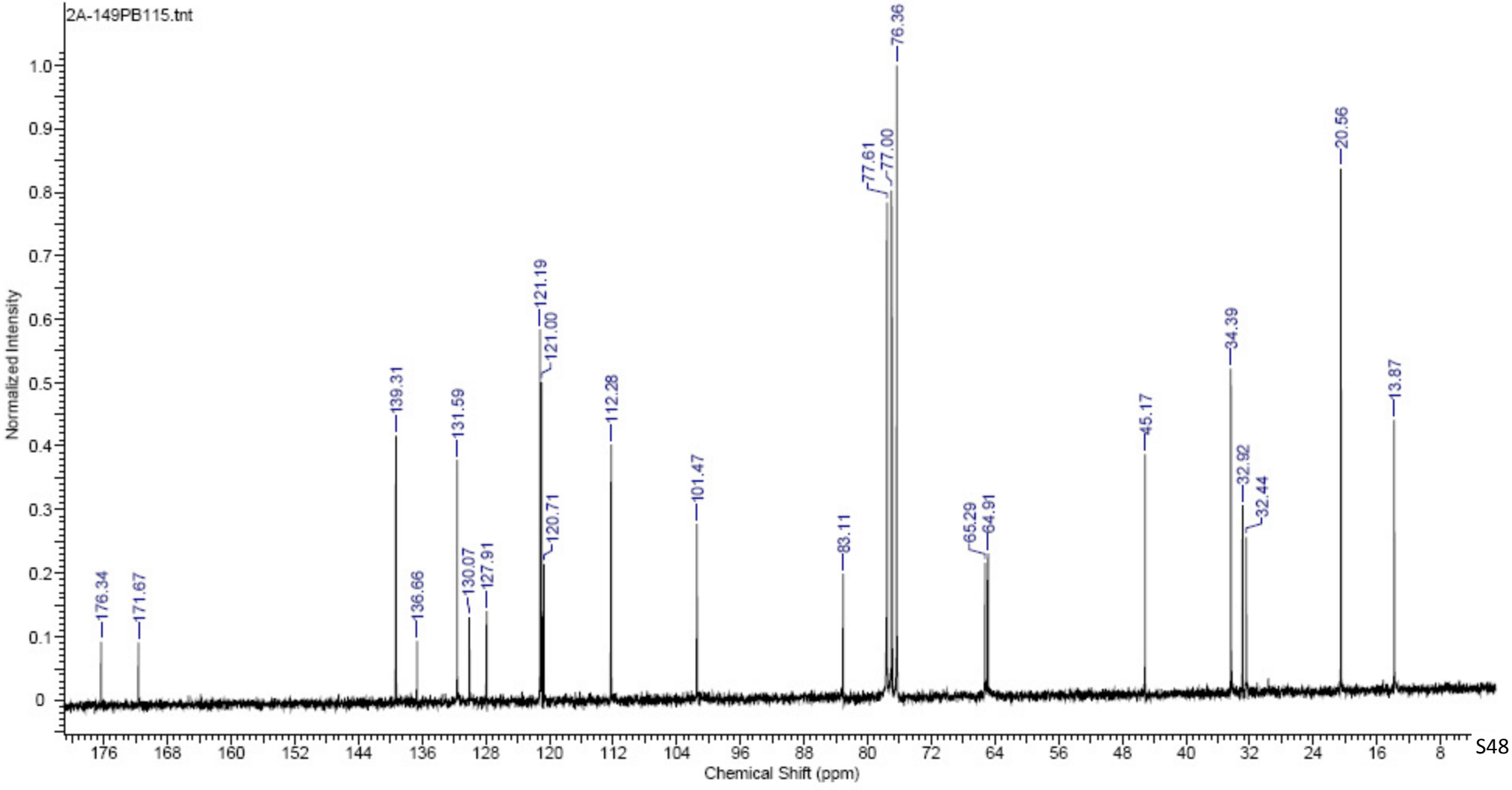
4


O
O
O
HO
O
O
N

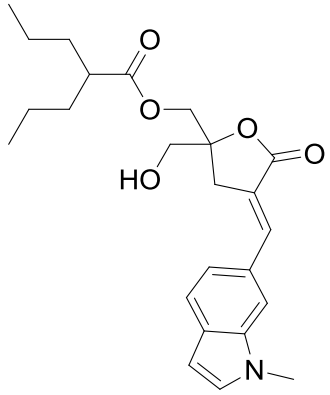

2A-149.008.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
7.73
7.66
7.64
7.43
7.29
7.26
7.18
7.17
6.51
6.51
4.37
4.34
4.29
4.26
3.83
3.79
3.74
3.71
3.31
3.31
3.27
3.26
3.11
3.11
3.07
3.06
2.41
2.40
2.39
2.38
2.36
2.35
2.34
1.56
1.54
1.53
1.52
1.51
1.49
1.40
1.25
1.23
1.21
1.19
1.17
0.82
0.77
0.84
0.75
0.00
1.03 1.03 1.06 1.25 1.03
1.00
2.09
5.40
1.03 1.06
1.23
2.46 2.87 5.38
6.58

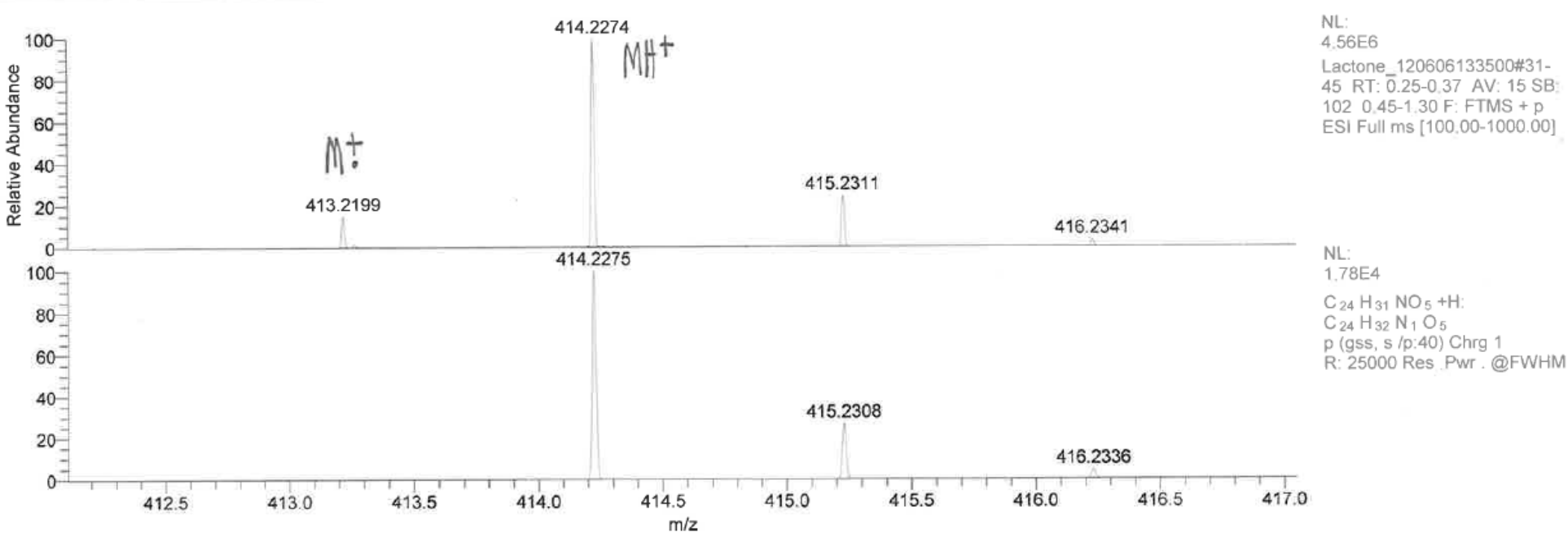
4


O
O
O
HO
N

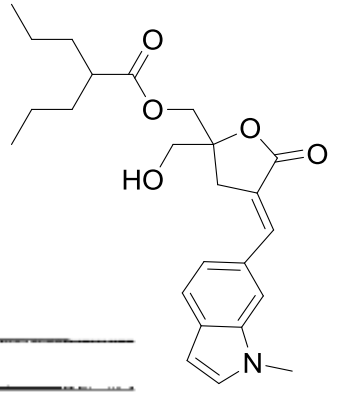

2A-149PB115.tnt
Normalized Intensity
Chemical Shift (ppm)
176.34
171.67
139.31
136.66
131.59
130.07
127.91
121.19
121.00
120.71
112.28
101.47
83.11
77.61
77.00
76.36
65.29
64.91
45.17
34.39
32.92
32.44
20.56
13.87

4


O
O
O
O
HO
N


414.2274
MH+
M+
413.2199
415.2311
416.2341
414.2275
415.2308
416.2336
Relative Abundance
m/z
NL:
4.56E6
Lactone_120606133500#31-45 RT: 0.25-0.37 AV: 15 SB: 102 0.45-1.30 F: FTMS + p ESI Full ms [100.00-1000.00]
NL:
1.78E4
C24 H31 NO5 +H:
C24 H32 N1 O5
p (gss, s /p:40) Chrg 1
R: 25000 Res .Pwr . @FWHM

4



ATLANTIC MICROLAB, INC.

Sample No. 115D141-H (2A-149)

6180 Atlantic Blvd. Suite M
Norcross, GA 30071

www.atlanticmicrolab.com

PROFESSOR/SUPERVISOR: Joe Barchi 301-846-1514

P.O. #: Pls call Lora Main @ 301-846-1514 for credit card paym

SUBMITTER

Company / School NIH/NCI

Address 376 Boyles St, Room 220

Frederick, MD 21702

NAME Dr. Dina M. Sigano DATE 5/30/12

Element	Theory	Found	
C	69.7100	69.70	
	7.5600	7.49	
N	3.3900	3.40	

Single ☑ Duplicate ☐

Elements Present: C, H, N, O

Analyze for: C, H, N

Hygroscopic ☐ Explosive ☐

M.P. _____ B.P. _____

To be dried: Yes ☐ No ☑

Temp. _____ Vac. _____ Time _____

FAX Service ☐ EMAIL Service ☑

FAX# /EMAIL SIGANOD@MAIL.NIH.GOV

Rush Service ☐ (SEE CURRENT

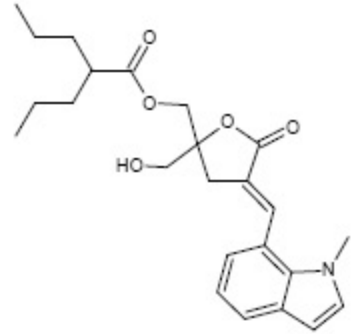
Phone Service ☐ PRICE LIST)

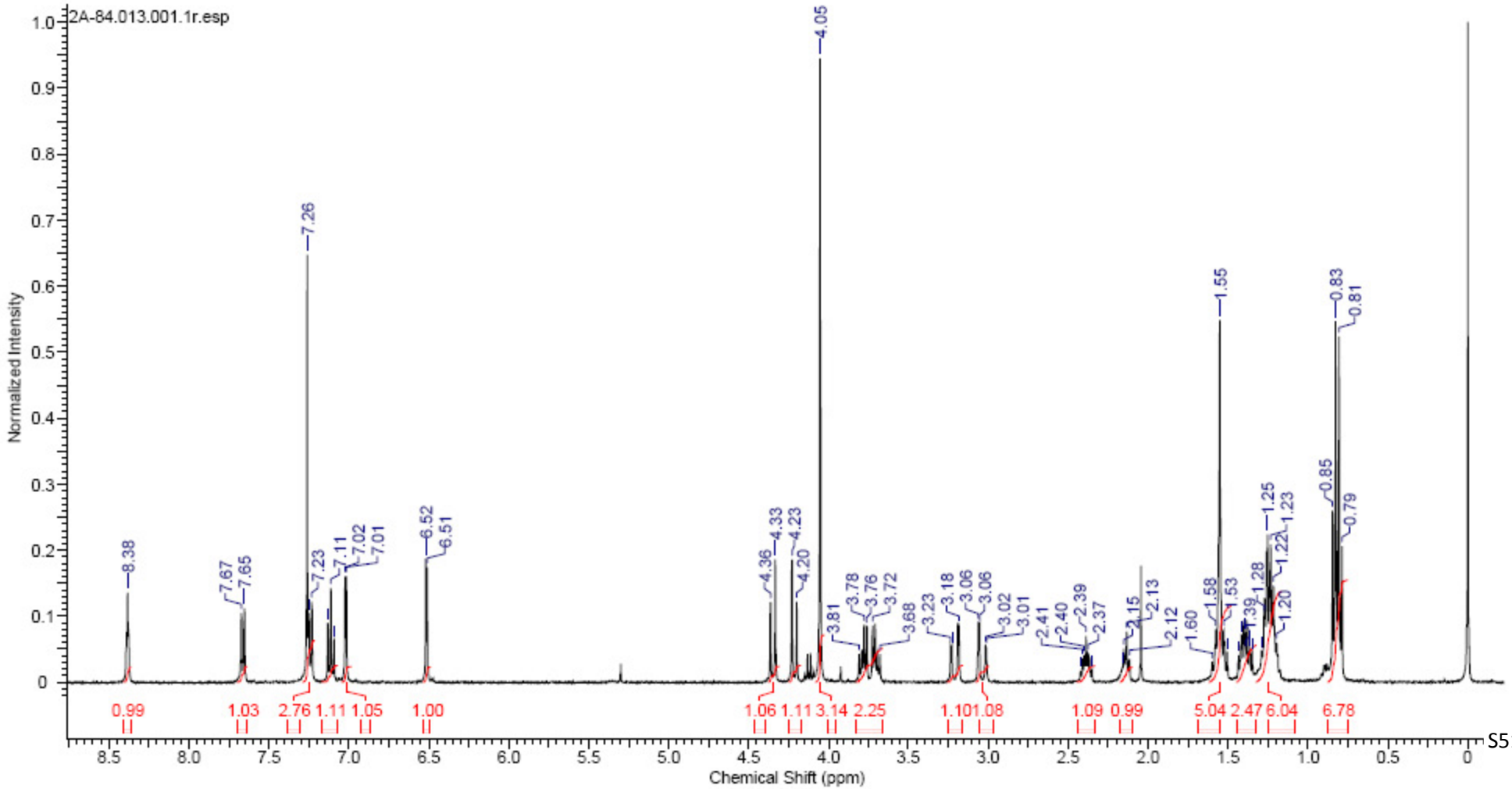
Phone No. _____

Date Received JUN 0 1 2012 Date Completed JUN 0 4 2012

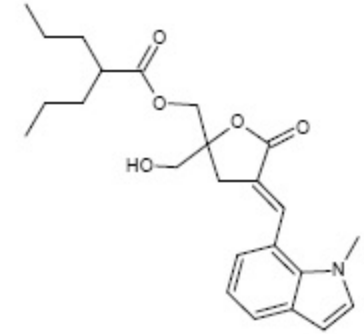
Remarks:

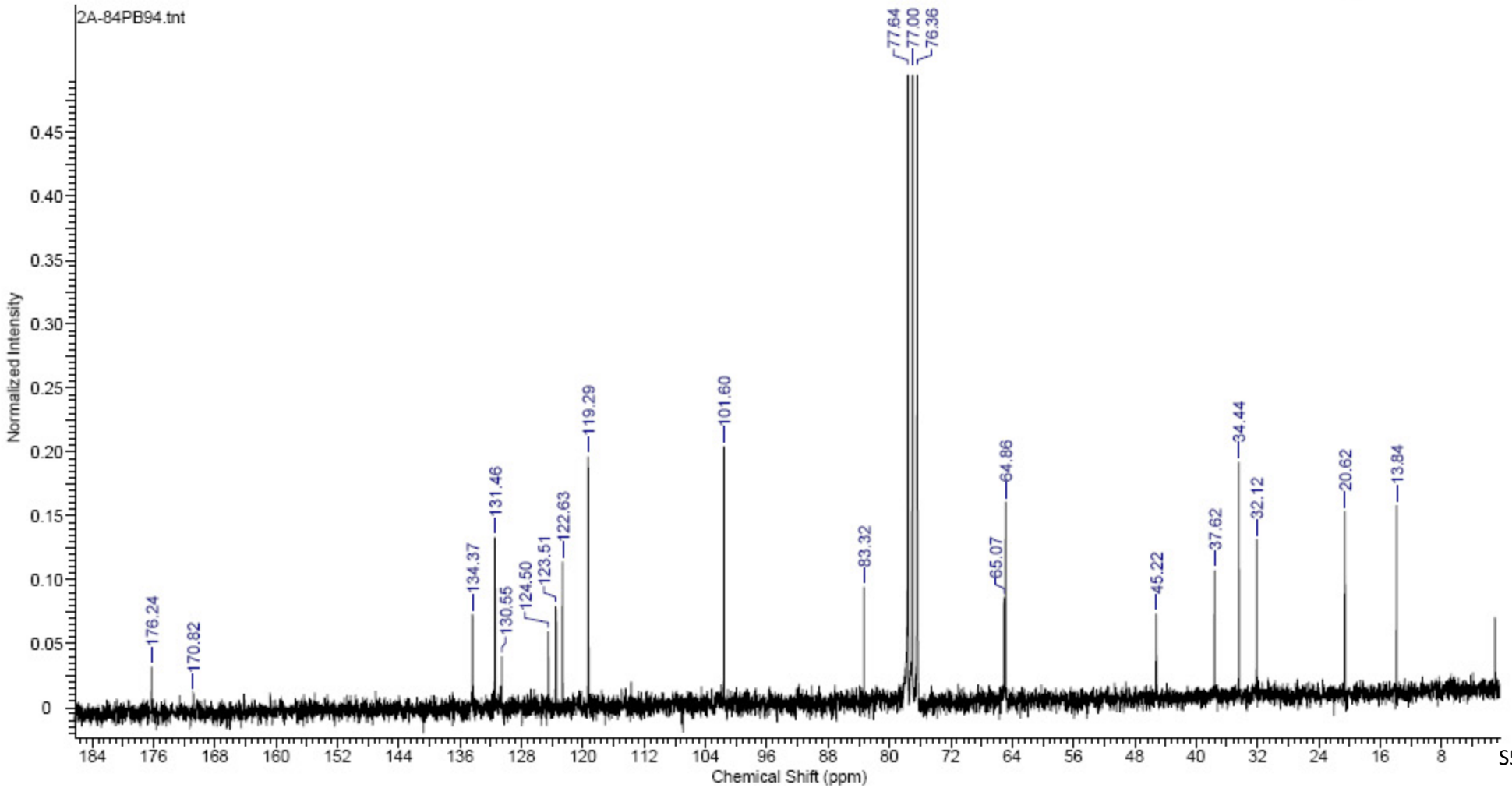
5


O
O
O
HO
O
N

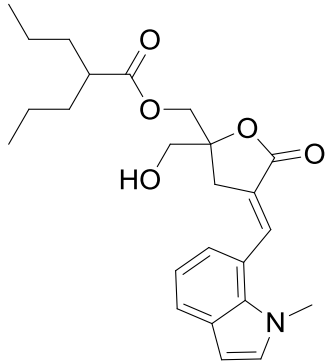

2A-84.013.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
8.38
7.67
7.65
7.26
7.23
7.11
7.02
7.01
6.52
6.51
4.36
4.33
4.23
4.20
4.05
3.81
3.78
3.76
3.72
3.68
3.23
3.18
3.06
3.06
3.02
3.01
2.41
2.40
2.39
2.37
2.15
2.13
2.12
1.60
1.58
1.55
1.53
1.39
1.28
1.25
1.23
1.22
1.20
0.85
0.83
0.81
0.79
0.99
1.03
2.76
1.11
1.05
1.00
1.06
1.11
3.14
2.25
1.10
1.08
1.09
0.99
5.04
2.47
6.04
6.78

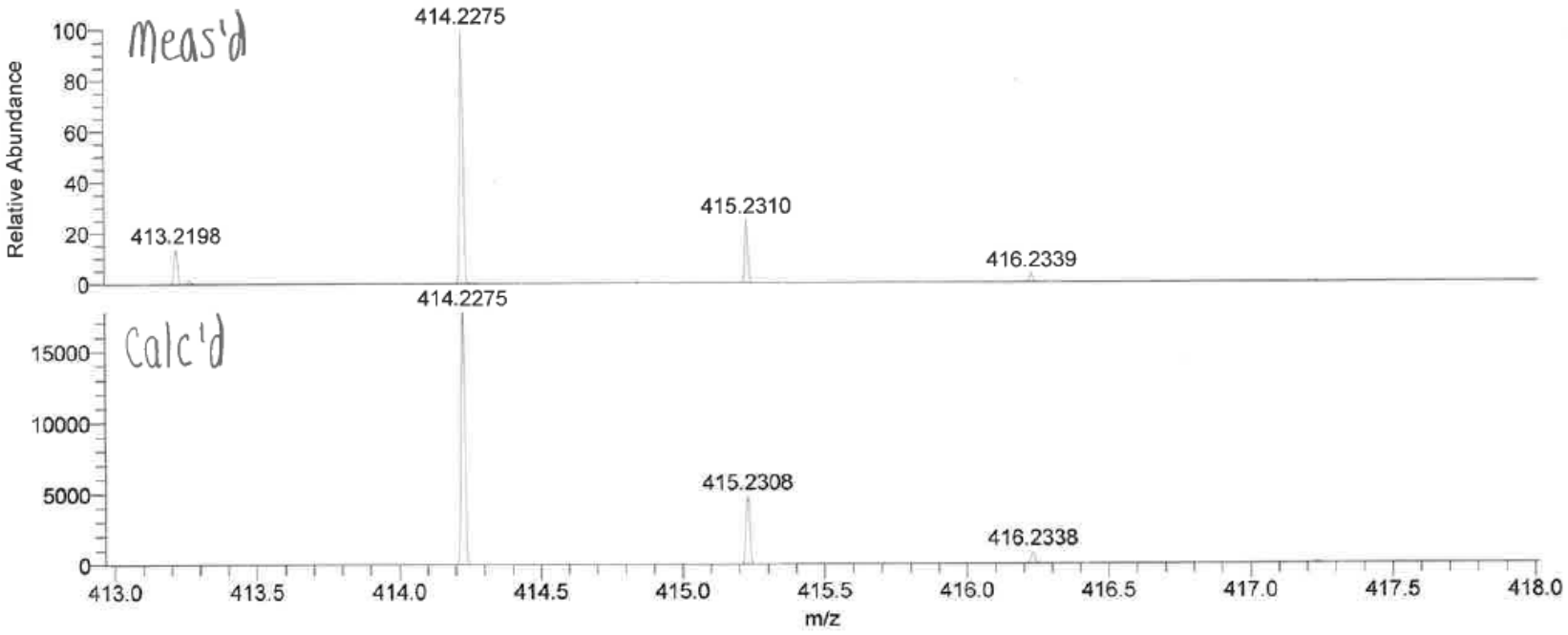
5

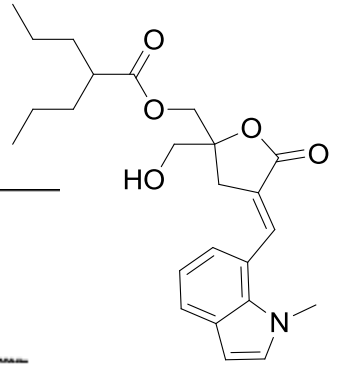

O
O
O
O
HO
N


2A-84PB94.tnt
Normalized Intensity
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
77.64
77.00
76.36
176.24
170.82
134.37
131.46
130.55
124.50
123.51
122.63
119.29
101.60
83.32
65.07
64.86
45.22
37.62
34.44
32.12
20.62
13.84
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
Chemical Shift (ppm)

5


O
O
O
O
HO
N


Meas'd
414.2275
413.2198
415.2310
416.2339
Calc'd
414.2275
415.2308
416.2338
Relative Abundance
m/z
100
80
60
40
20
0
15000
10000
5000
0
413.0
413.5
414.0
414.5
415.0
415.5
416.0
416.5
417.0
417.5
418.0
NL:
3.20E6
Lactone_120606135002#32-42
RT: 0.26-0.35 AV: 11 SB: 40
0.10-0.24 , 0.45-0.63 F: FTMS
+ p ESI Full ms
[100.00-1000.00]
NL:
1.78E4
C24 H31 NO5 +H:
C24 H32 N1 O5
p (gss, s /p.40) Chrg 1
R: 30000 Res .Pwr . @FWHM



ATLANTIC MICROLAB, INC.

Sample No. 115D141-D (2A-84)

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PROFESSOR/SUPERVISOR: Joe Barchi 301-846-1574

P.O. #: Pls call Lora Main @ 301-846-1574 for credit card paym

SUBMITTER

Company / School NIH/NCI

Address 376 Boyles St, Room 220
Frederick, MD 21702

NAME Dr. Dina M. Sigano DATE 5/30/12

Element	Theory	Found	
C	69.7100	69.44	
	7.5600	7.51	
N	3.3900	3.43	

Single ☑ Duplicate ☐

Elements Present: C, H, N, O

Analyze for: C, H, N

Hygroscopic ☐ Explosive ☐
M.P. _____ B.P. _____

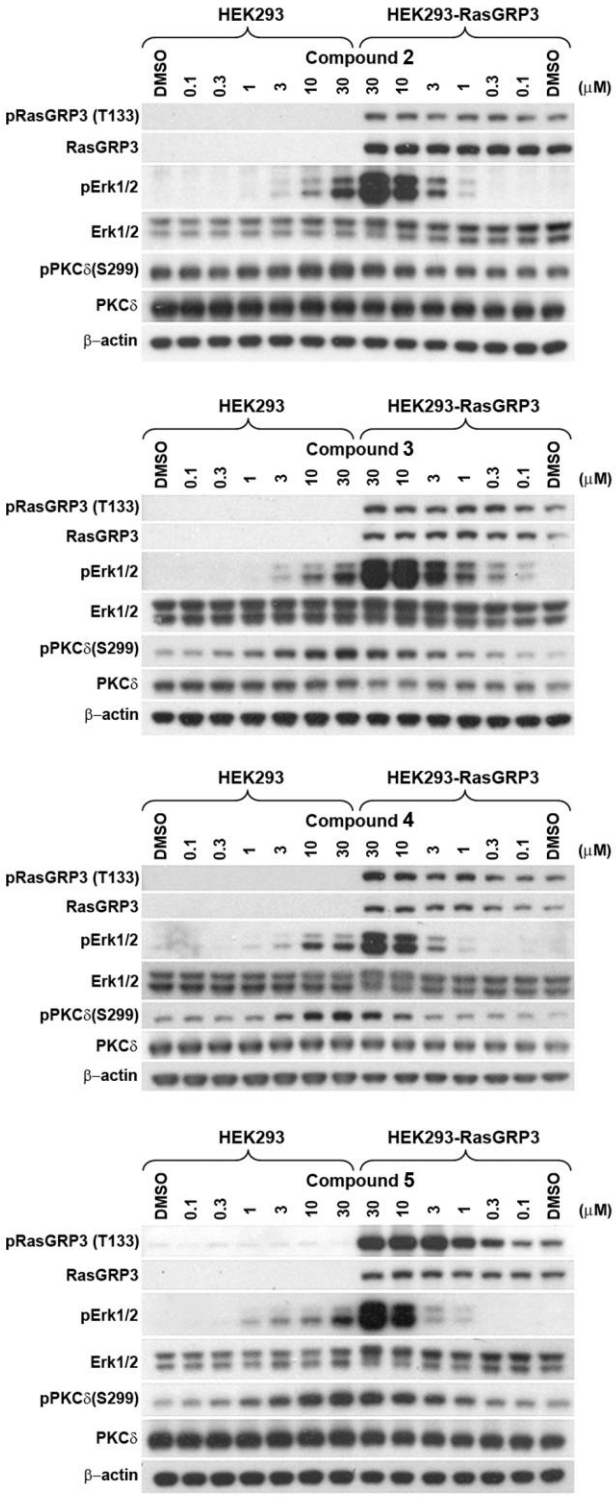
To be dried: Yes ☐ No ☑
Temp. _____ Vac. _____ Time _____

FAX Service ☐ EMAIL Service ☑
FAX# /EMAIL SIGANOD@MAIL.NIH.GOV

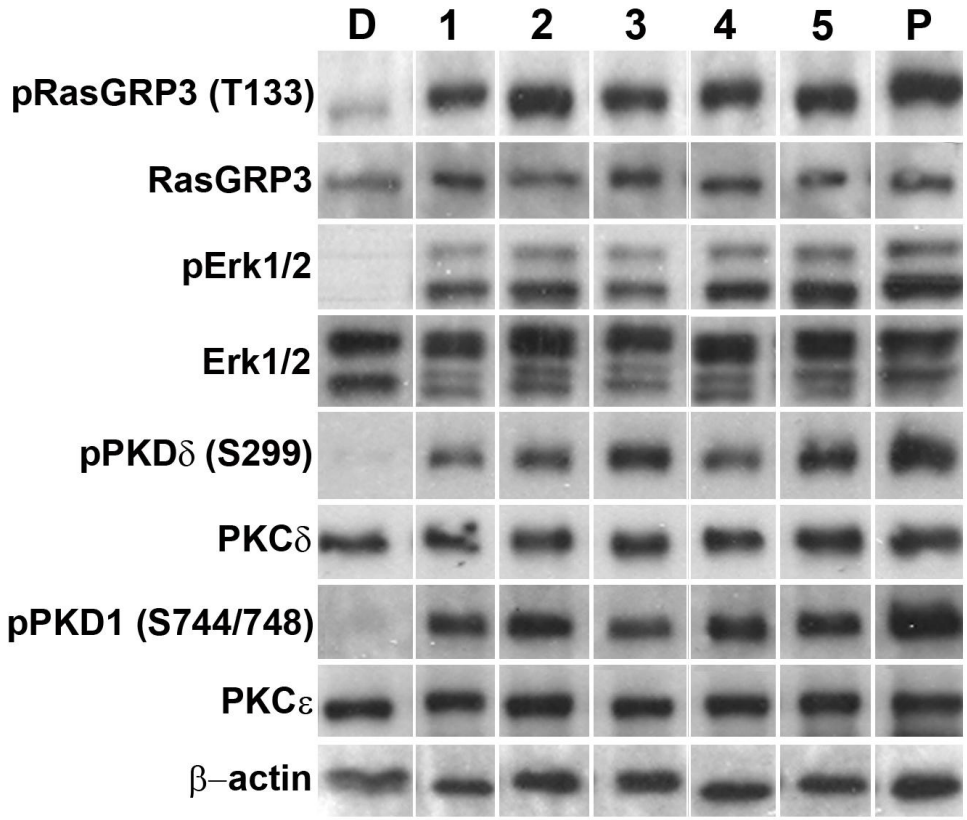
Rush Service ☐ (SEE CURRENT
Phone Service ☐ PRICE LIST)
Phone No. _____

Date Received JUN 0 1 2012 Date Completed JUN 0 4 2012

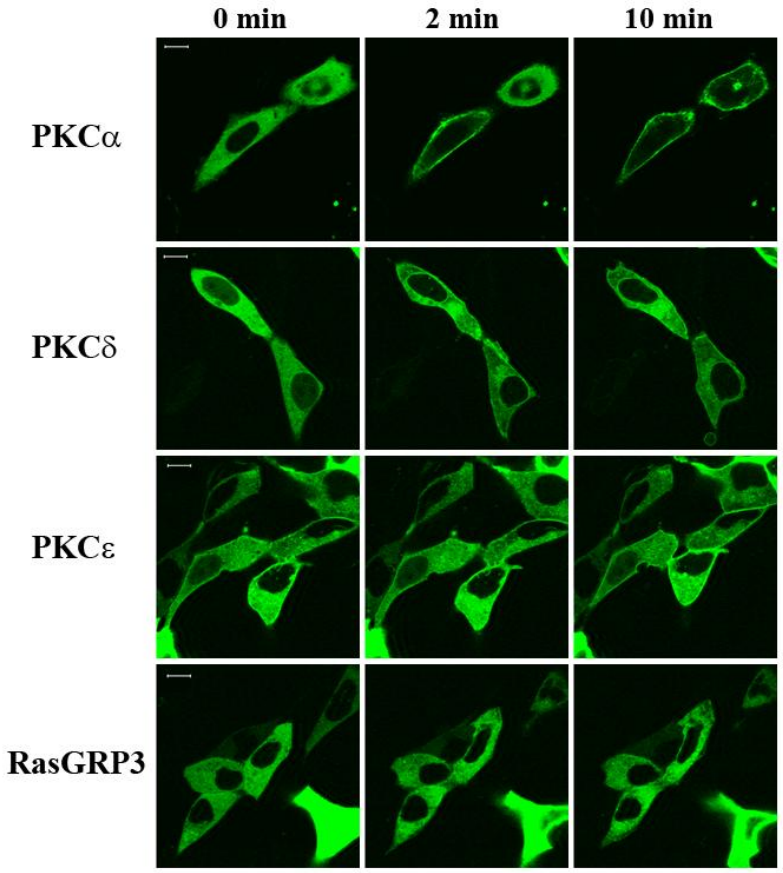
Remarks:



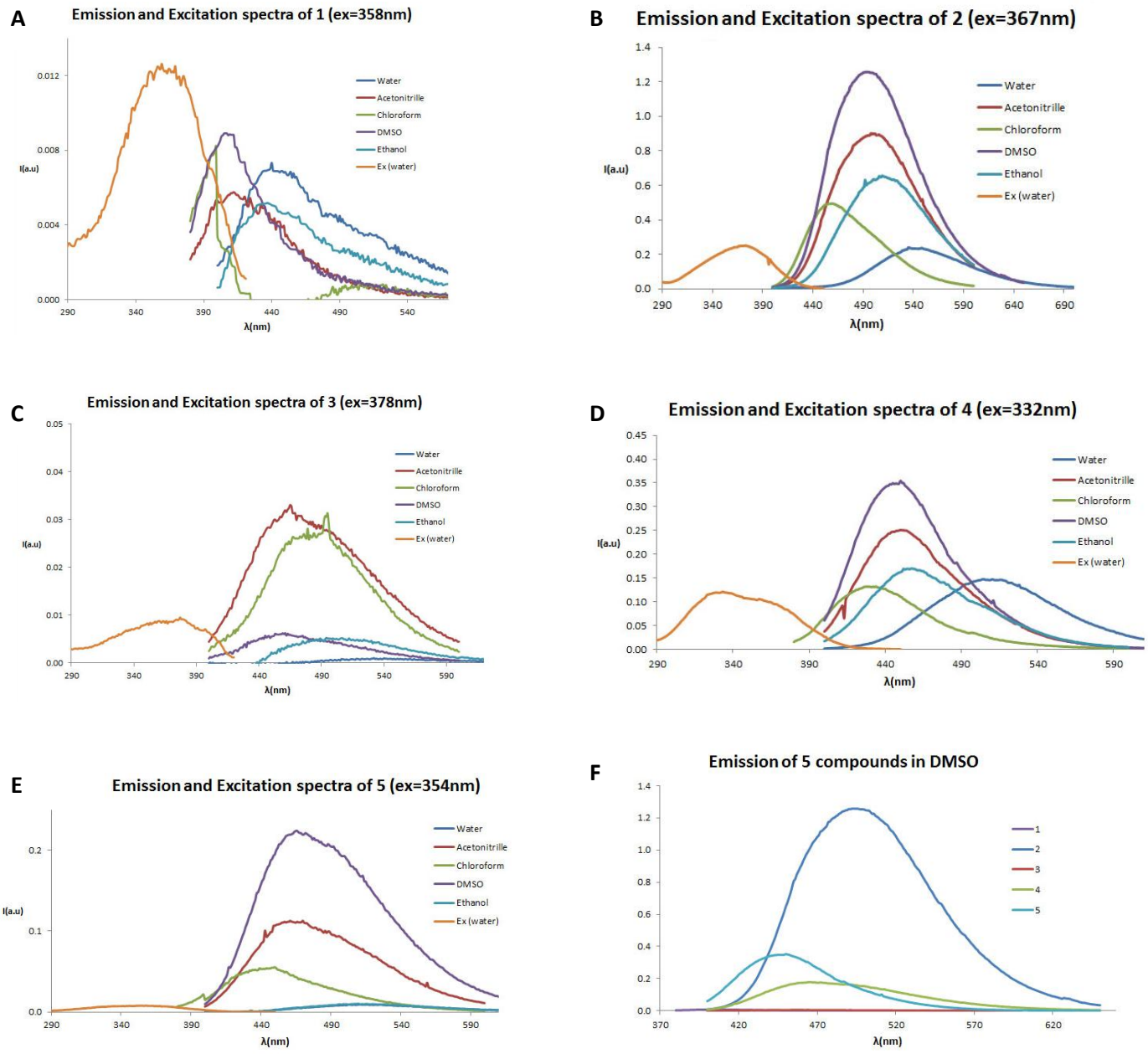
Supplementary Figure 1. Response of HEK293 and HEK293 cells overexpressing RasGRP3 to treatment with compounds **2** - **5**. Cells were treated for 30 min with the indicated concentrations of compound, after which the cells were lysed and the lysates subjected to electrophoresis and immunoblotting with the antibodies as indicated. Results are representative of the triplicate experiments performed.



Supplemental Figure 2. Biological activity in Ramos cells. Ramos cells were incubated with DMSO (D), the individual compounds **1**-**5** (10,000 nM), or PMA (P) (1,000 nM) for 30 minutes. Lysates were subjected to western blot to compare compound activities as described in Materials and Methods. The experiment shown is one of three independent experiments.



Supplementary Figure 3. Translocation of different PKC isoforms and RasGRP3 in living cells in response to PDBu. CHO-K1 cells were transiently transfected with GFP-PKCα and LNCaP cells were transiently transfected with GFP- PKCδ, YFP-PKCε, or GFP-RasGRP3. The translocation pattern was examined by confocal microscopy as a function of time after treatment with PDBu (1000 nM). Each panel represents images typical of the experiments performed (n = 3). Bars are 10 μm.



Supplementary Figure 4. Fluorescence measurements of compounds **1** to **5** in different solvents (6.05 μM). A) Compound **1**; B) Compound **2**; C) Compound **3**; D) Compound **4**; E) Compound **5**; F) Comparison of fluorescence emission of compounds **1** to **5** in DMSO.

Fluorescence measurements. Fluorescence measurements of the compound's intrinsic emission and excitation was measured by dissolving the compounds (1mg/ml in DMSO) in 1mL of the required solvent up to a final concentration of 6.05μM. Fluorescence excitation and emission spectra were acquired at 27^{o}C on a FL920 spectrofluorimeter (Edinburgh, Co., Edinburgh, UK) by using the compound's excitation and emissionwavelengths. Solutions were placed in a quartz cell with a 0.5 cm optical path-length. Light scattering from the vesicles was confirmed to account for less than 5% of the emission intensity.