Supporting Information

Modular Synthesis of Biologically Active Phosphatidic Acid Probes Using Click Chemistry

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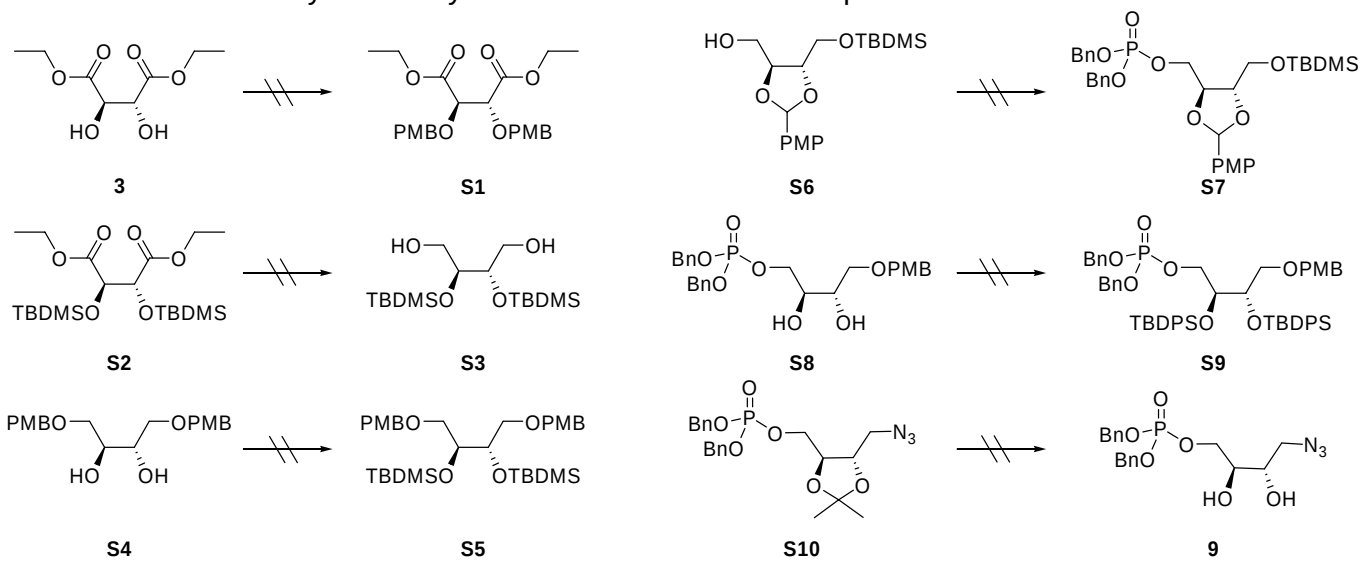
I. Protecting Group Strategies Explored for the Interior Diol of Scaffold 2

A key aspect of this synthesis was the determination of the optimal protecting group for the interior diol of **8** that could be removed without affecting the phosphotriester and azide groups. Several approaches were explored to determine that which is most effective. These routes are outlined in Scheme S1, in which the steps that were unsuccessful are indicated. First, the installation of two *p*-methoxybenzyl protecting groups onto the diol of **3** to **S1** was ineffective. While two silyl protecting groups could be introduced following literature procedures[1,2] to **S2**, these were removed during ester reduction in attempting to access **S3**. However, the introduction of two silyl groups onto the diol of **S4** to access **S5** was unsuccessful. While *p*-methoxybenzylidine-protected derivative **S6** was synthesized, all oxidation conditions attempted for the production of phosphotriester **S7** led to removal of this group. Bis-silylation of **S8** to **S9** was once again unsuccessful. Finally, deprotection of the acetonide group of **S10** under acidic conditions yielded only ~10% of diol **9** due to decomposition.

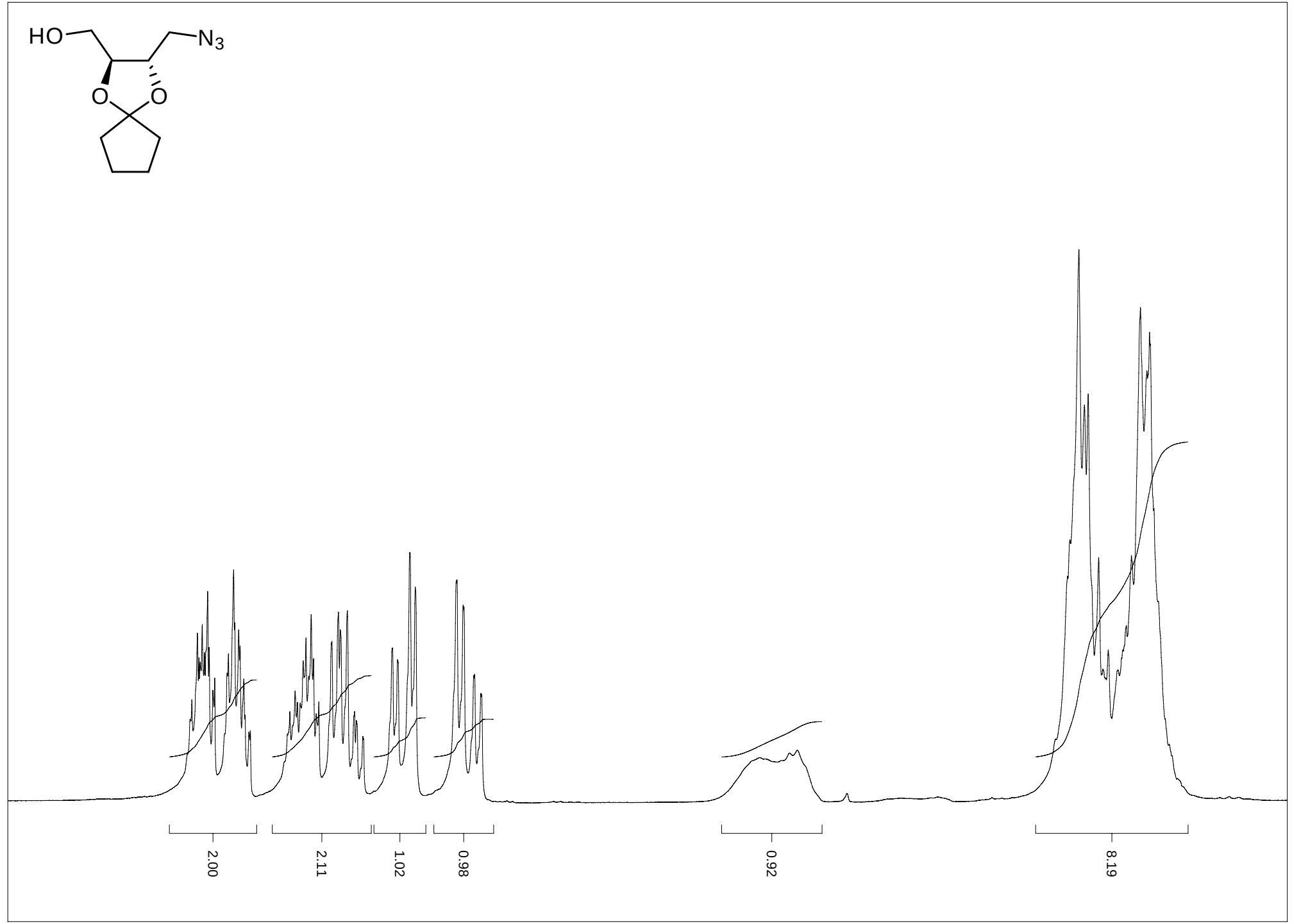


Scheme S1. Protecting group strategies explored in the synthesis of modular PA core 2.

References Cited

1 J. E. Baldwin; R. M. Adlington; A. T. Russell; M. L. Smith. *J. Chem. Soc., Chem. Commun.* 1994, 85-86.

2 T. Hiyama; T. Minami; K. Takahashi. *Bull. Chem. Soc. Japan* 1995, **68**, 364-372.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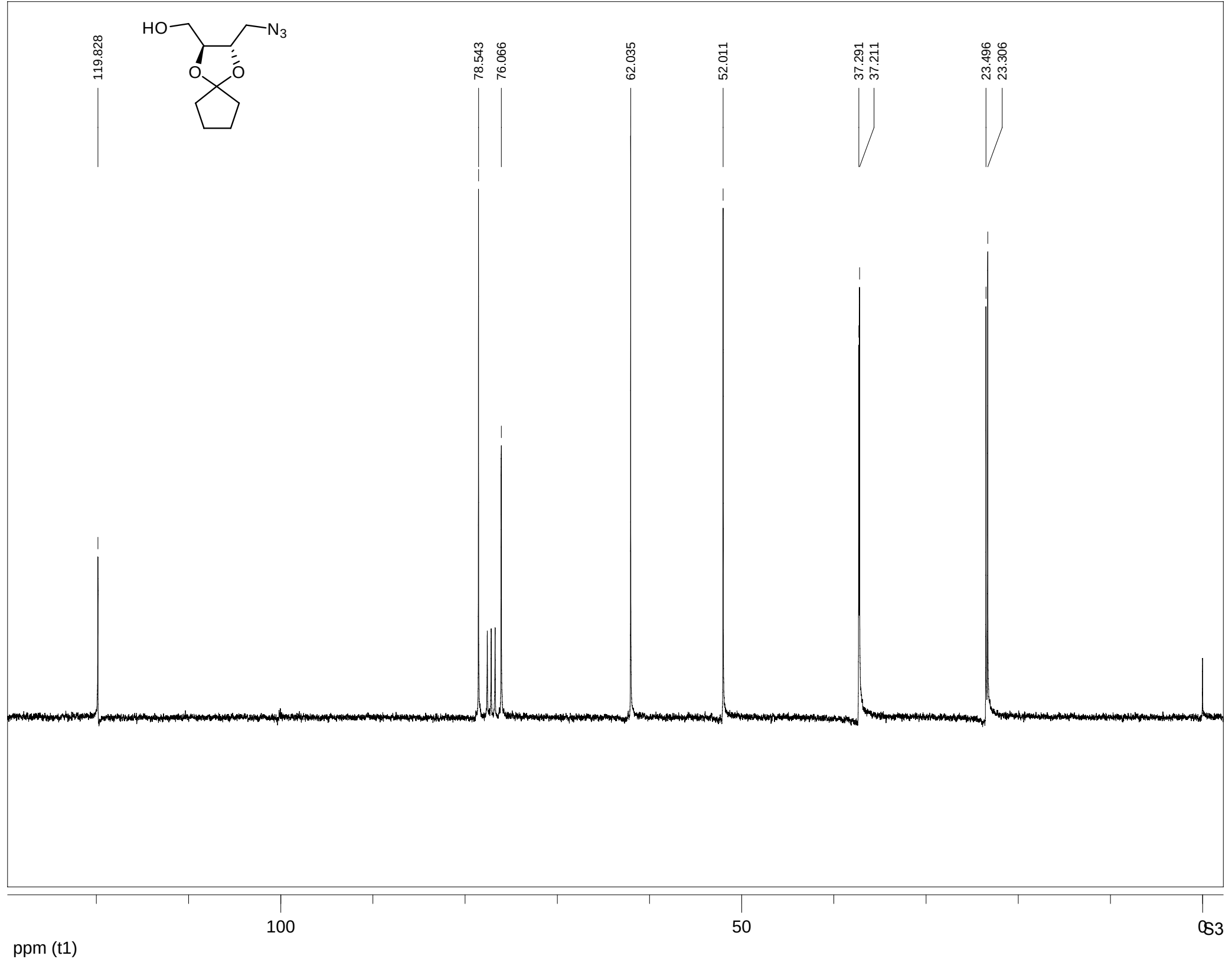
N3
O
O
2.00
2.11
1.02
0.98
0.92
8.19
4.00
3.50
3.00
2.50
2.00
1.50
ppm (t1)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N3
O
O
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76.066
62.035
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37.291
37.211
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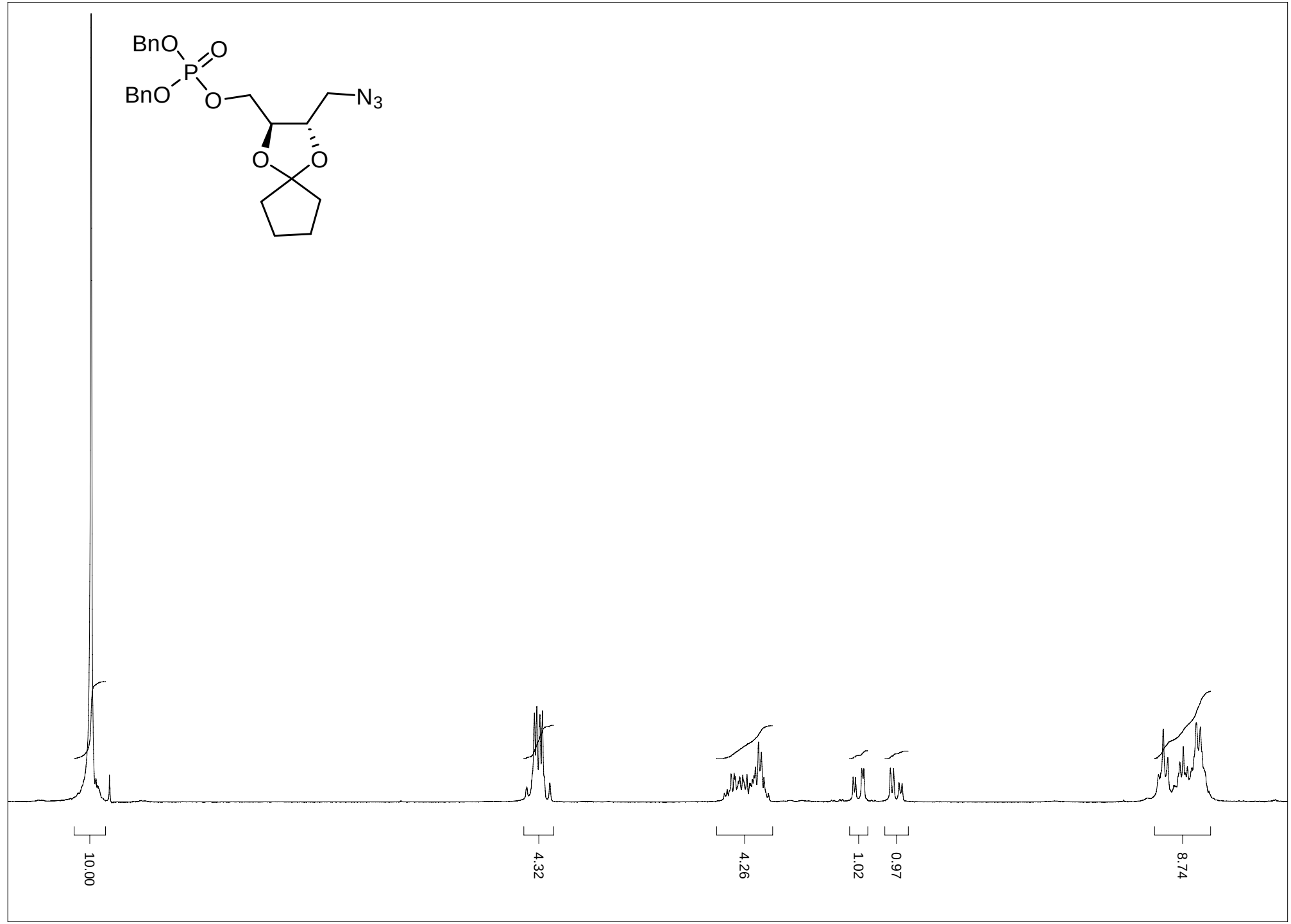
50
0
ppm (t1)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O
P
BnO
O
N3
O
O
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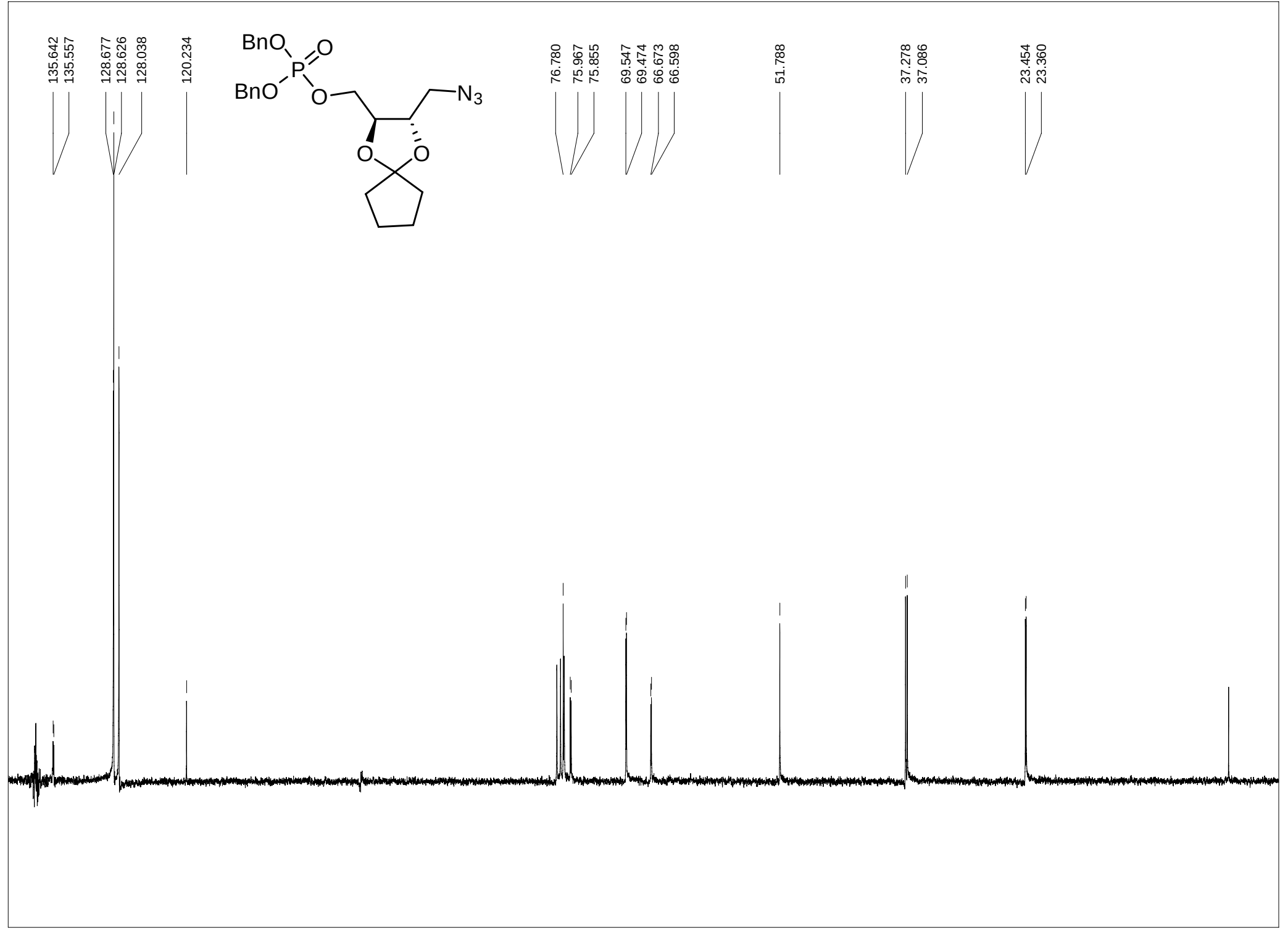
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)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128.677
128.626
128.038
120.234
BnO
BnO
P
O
O
O
O
N_3
76.780
75.967
75.855
69.547
69.474
66.673
66.598
51.788
37.278
37.086
23.454
23.360
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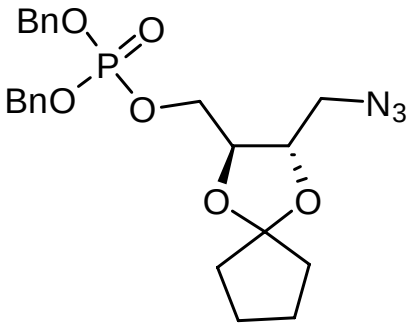
50
0
ppm (t1)



0.143

5.0

0.0

-5.0

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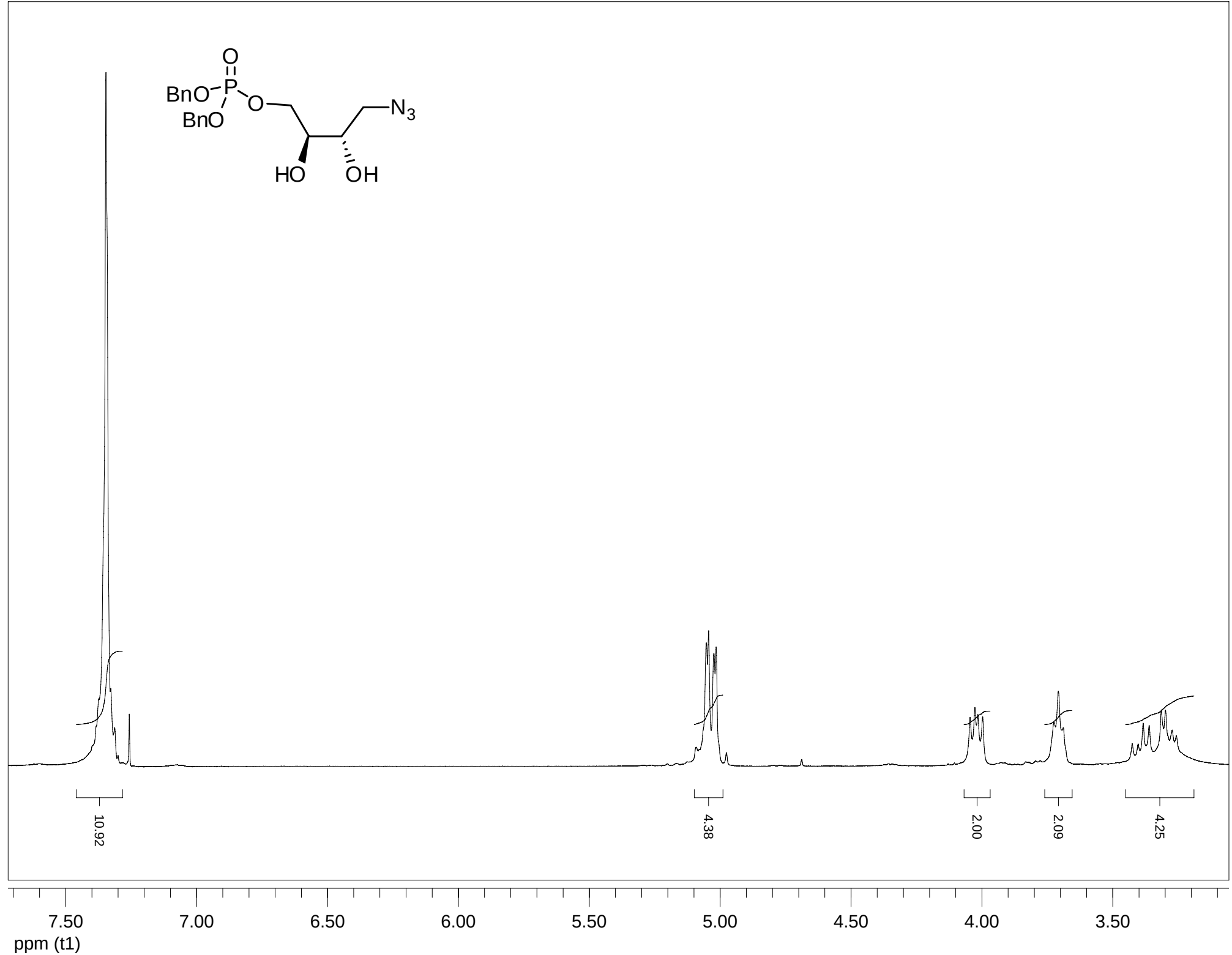
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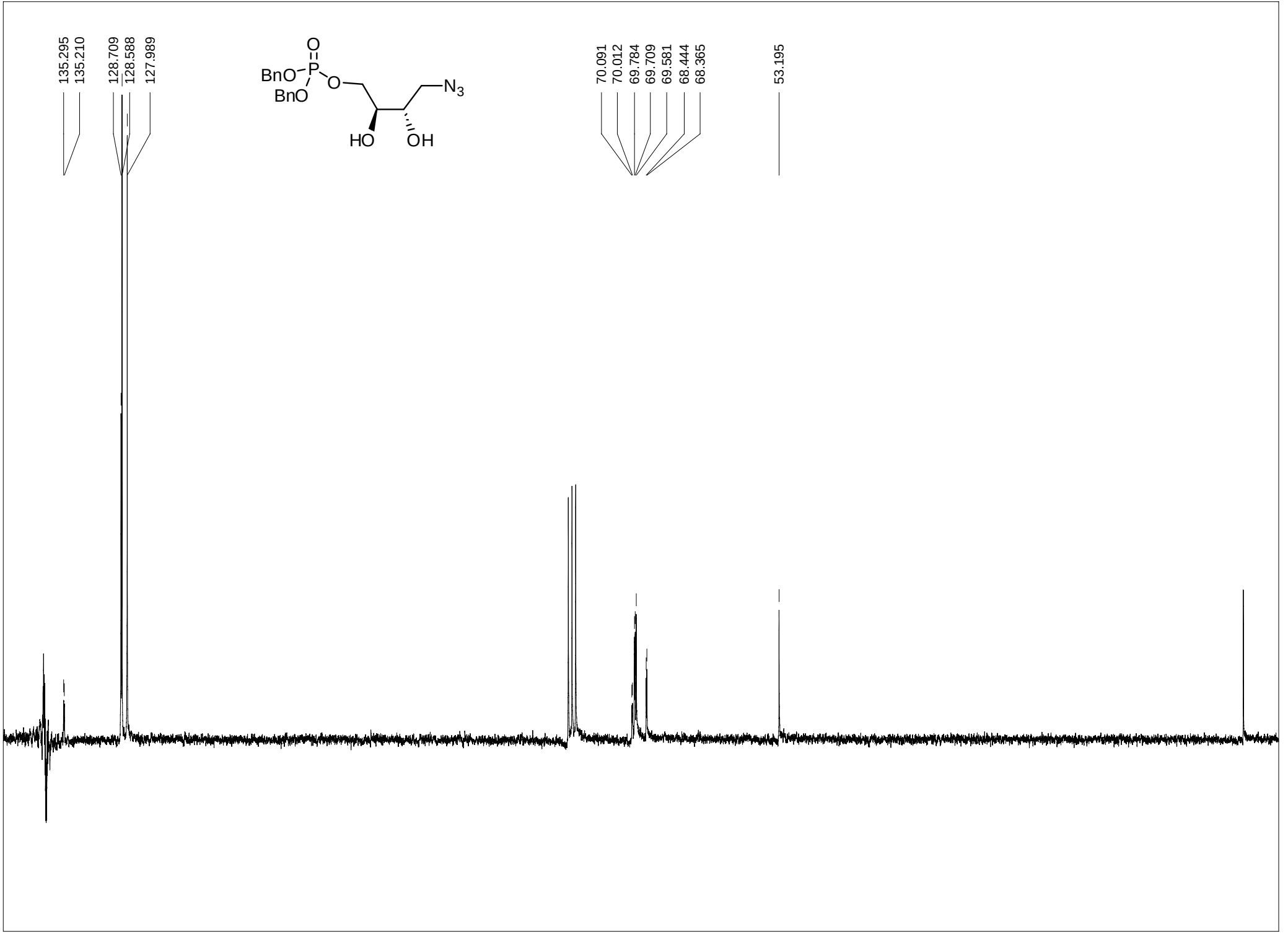
P
O
BnO
N3
HO
OH
10.92
4.38
2.00
2.09
4.25
7.50
7.00
6.50
6.00
5.50
5.00
4.50
4.00
3.50
ppm (t1)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127.989
BnO
BnO
O
P
O
O
N3
HO
OH
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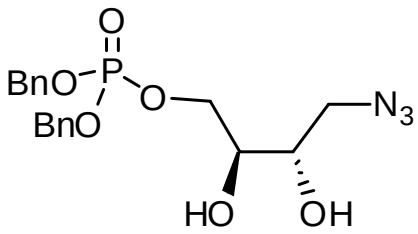
50
0
ppm (t1)



0.923

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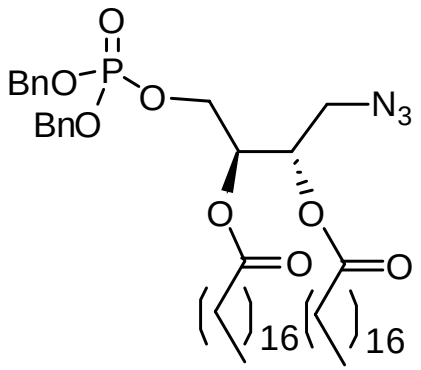
ppm (t1)


O
BnO
P
O
BnO
N3
O
O
O
O
16
16

10.00
2.04
4.07
1.74
1.68
3.93
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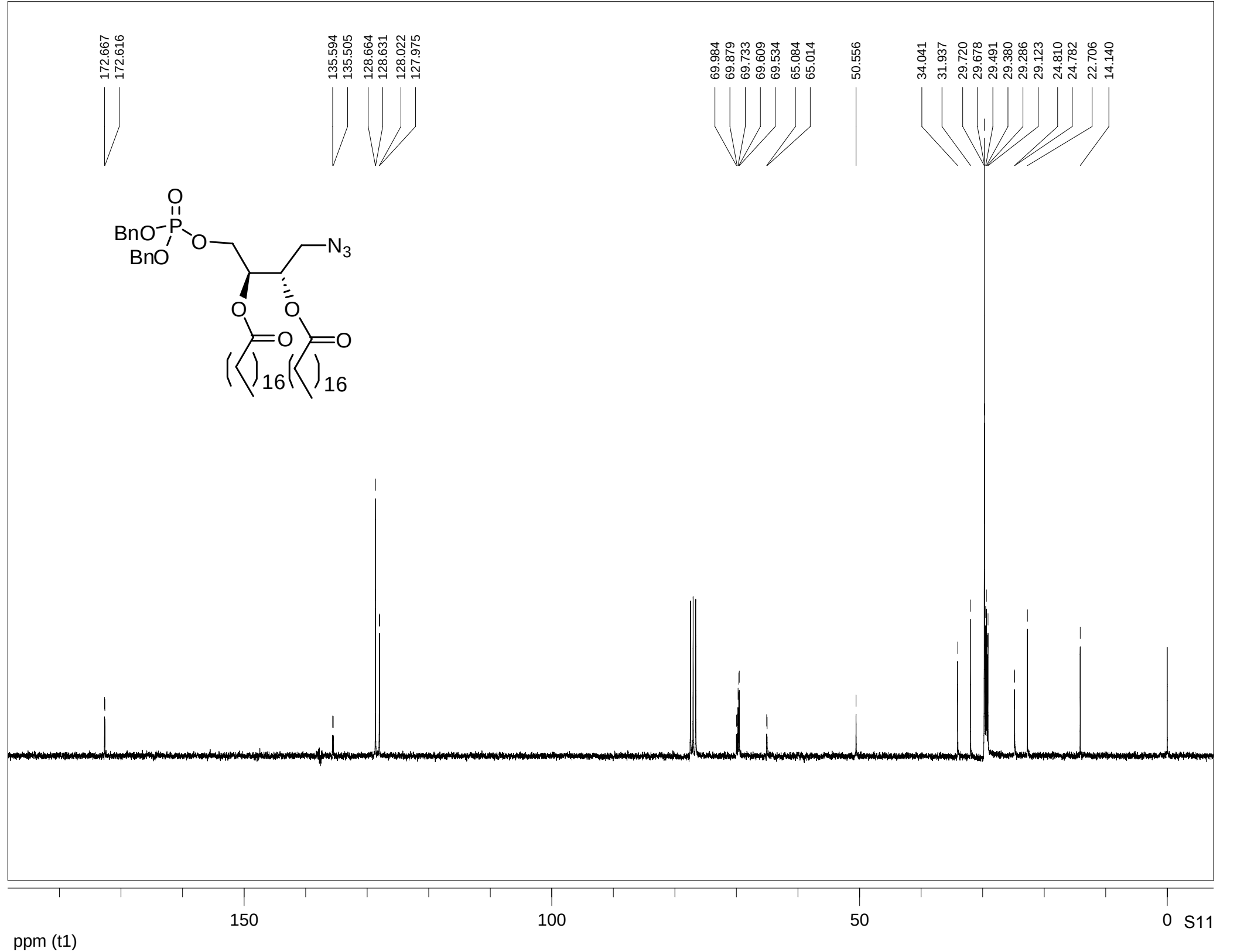
3.0
2.0
1.0
ppm (t1)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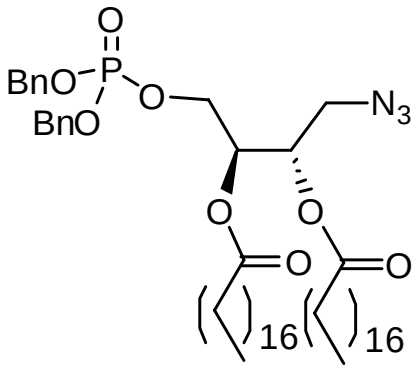
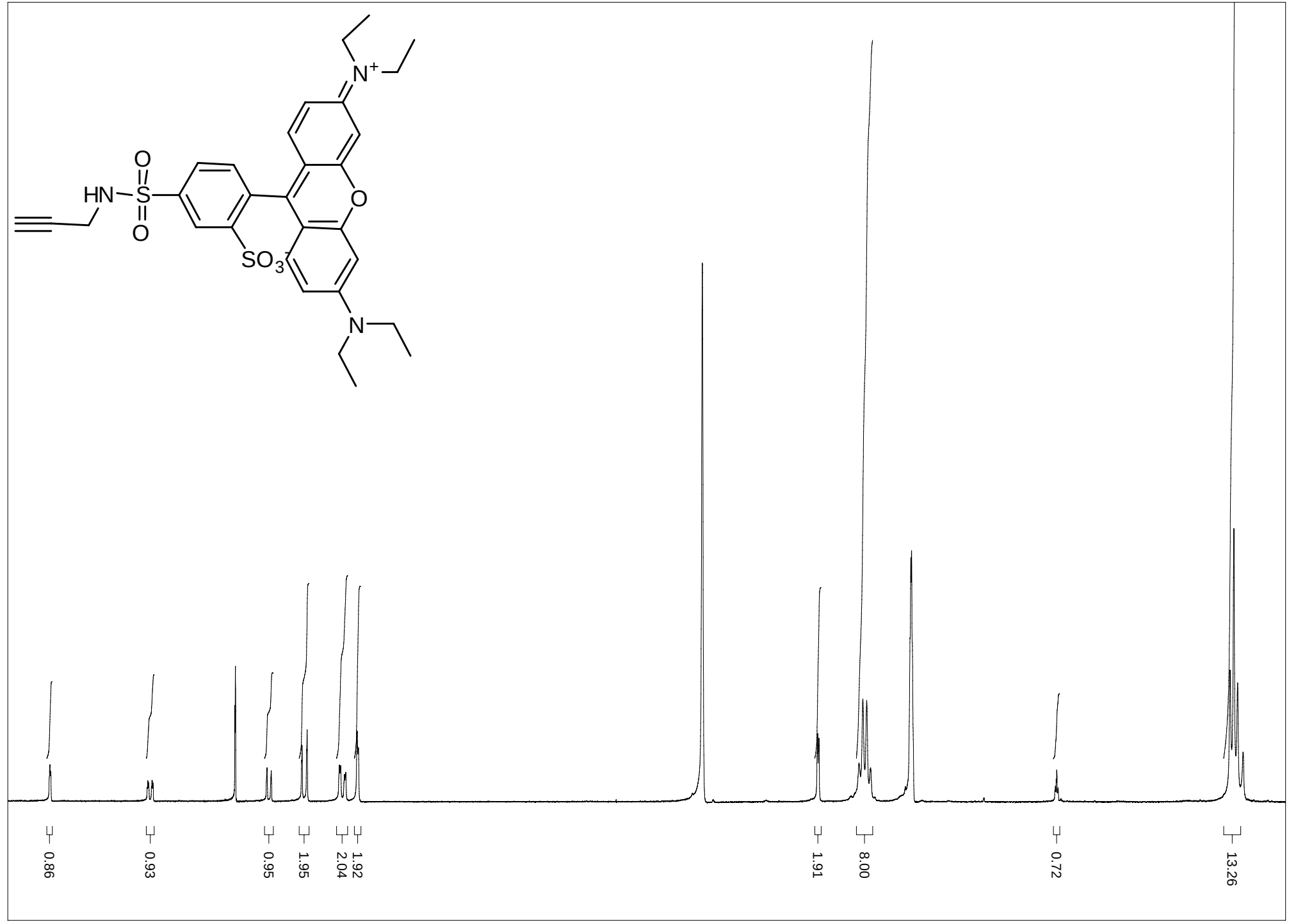
128.631
128.022
127.975
69.984
69.879
69.733
69.609
69.534
65.084
65.014
50.556
34.041
31.937
29.720
29.678
29.491
29.380
29.286
29.123
24.810
24.782
22.706
14.140
BnO
BnO
O
P
O
N3
O
O
O
O
16
16
150
100
50
0
ppm (t1)



0.041

5.0 0.0 -5.0 -10.0

ppm (t1)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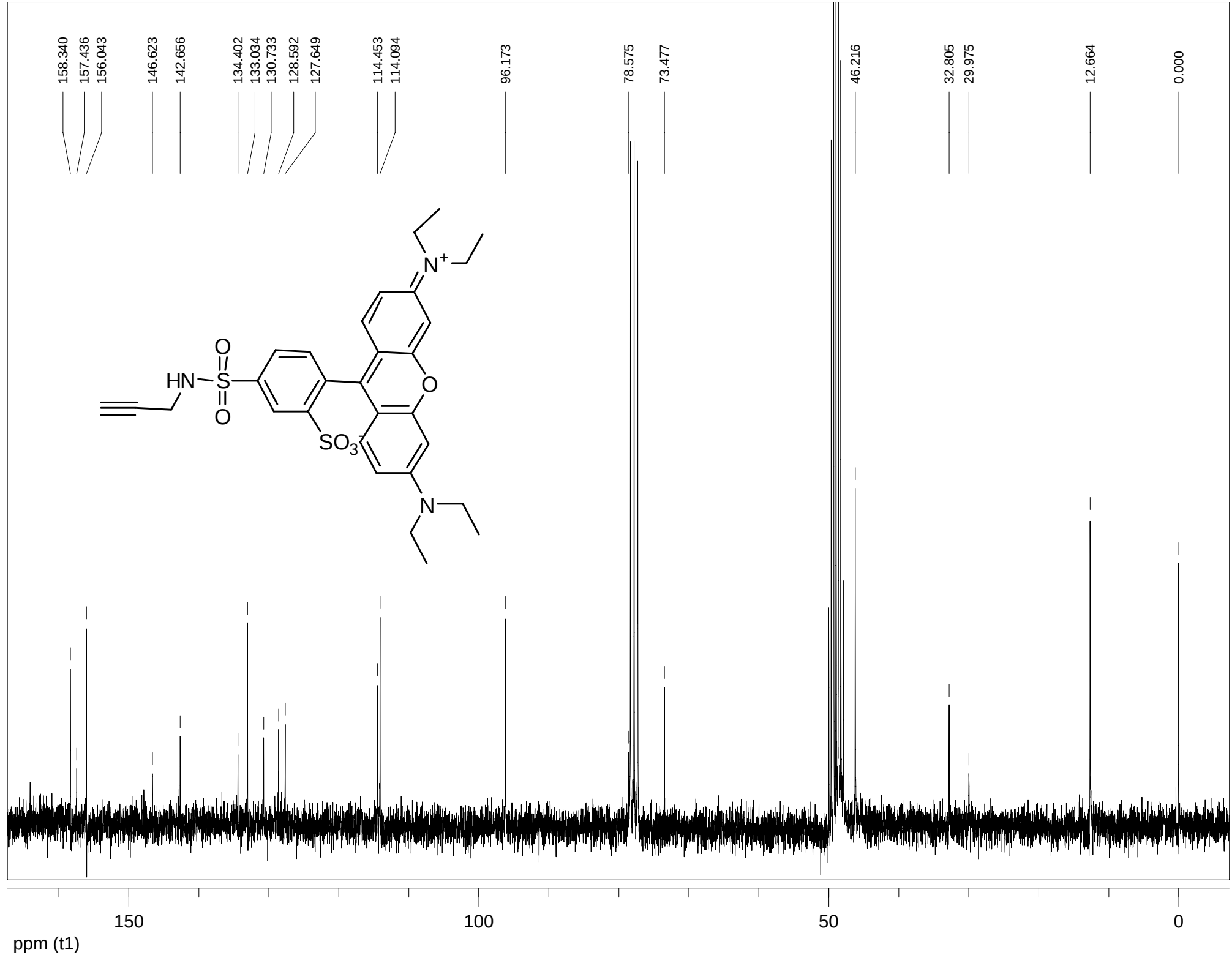
4.0
3.0
2.0
ppm (t1)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142.656
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130.733
128.592
127.649
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114.094
96.173
78.575
73.477
46.216
32.805
29.975
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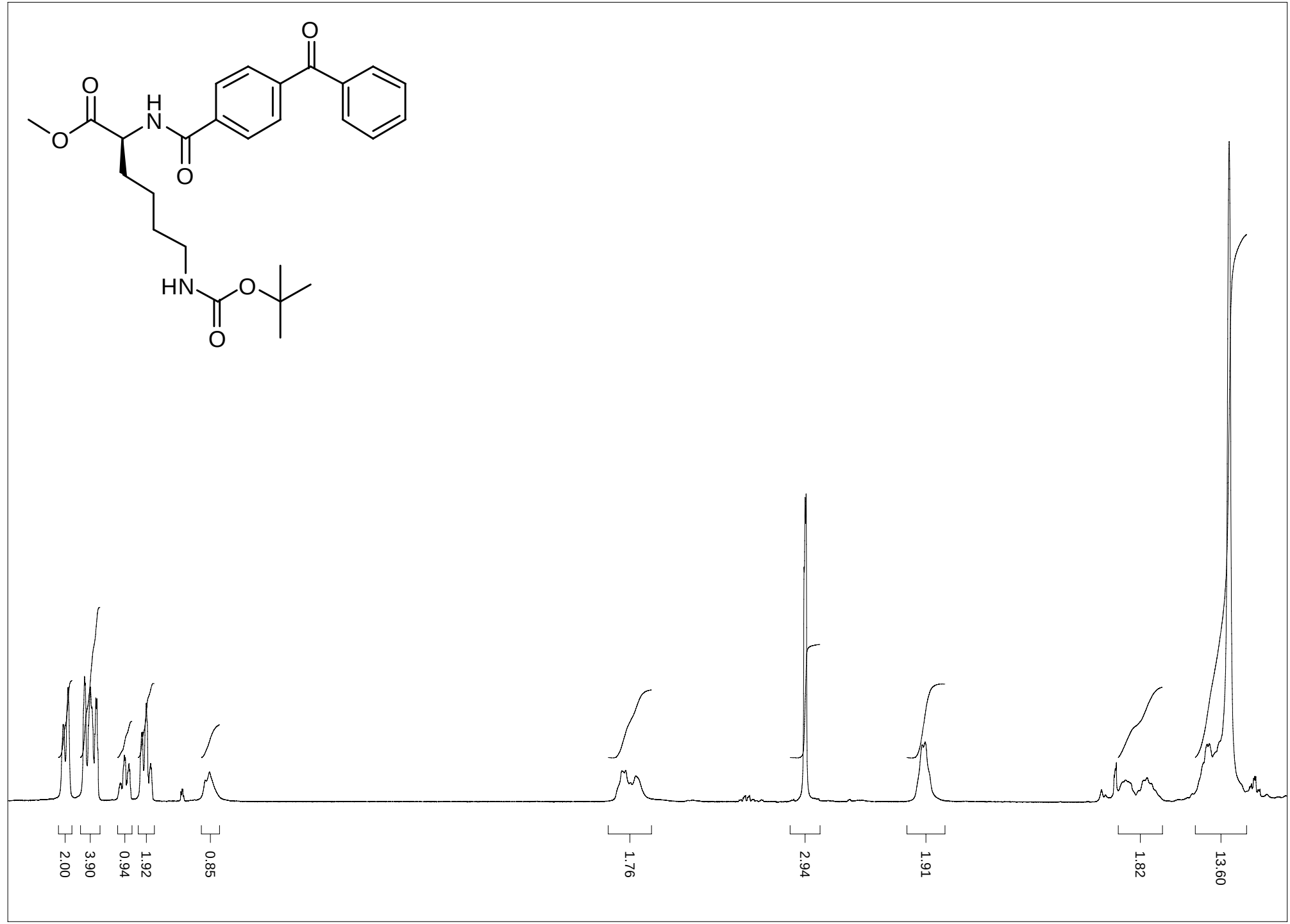
HN
S
O
O
O
SO3
N+
N
150
100
50
0
ppm (t1)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O
HN
O
O
HN
O
O
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0.85
1.76
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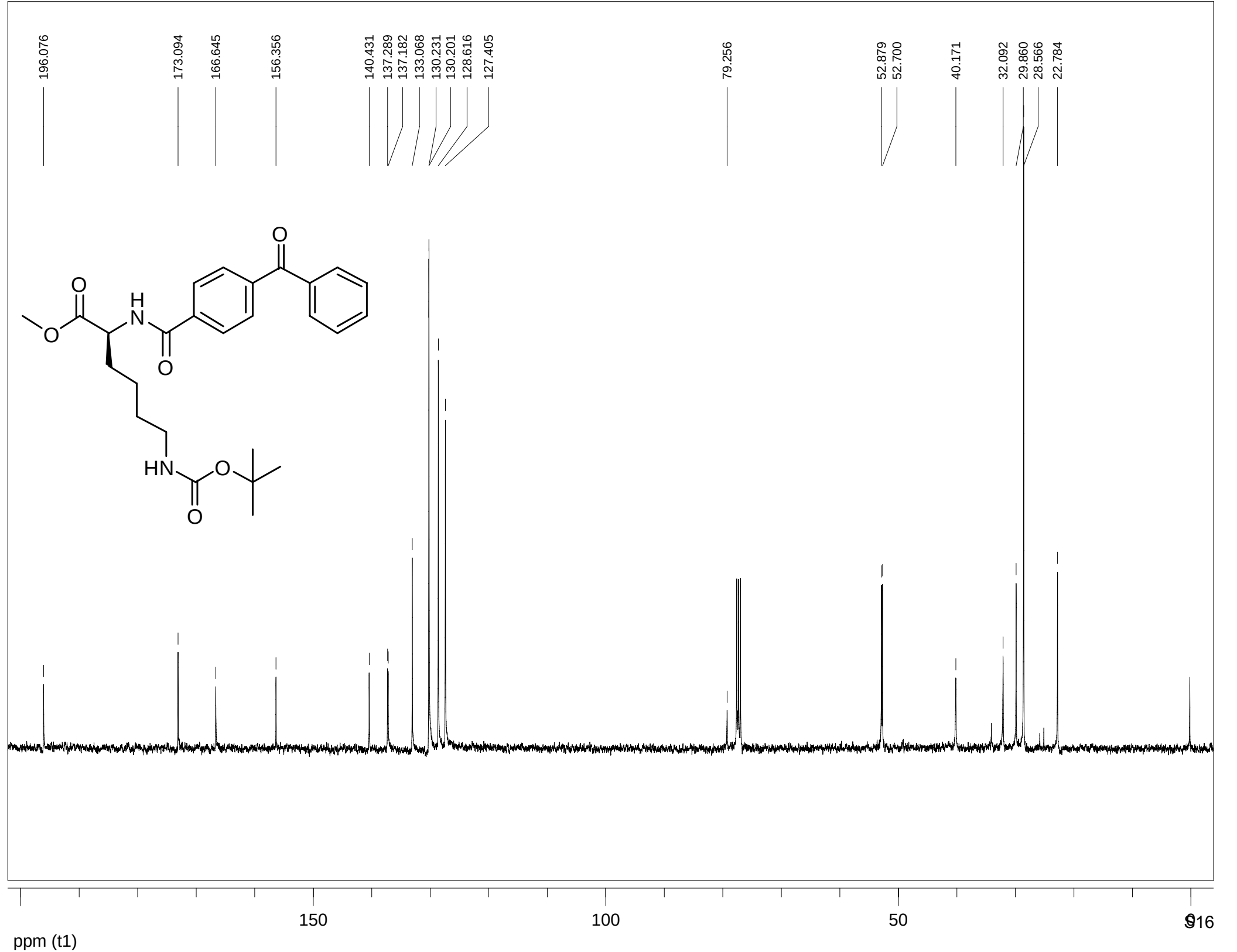
5.0
4.0
3.0
2.0
ppm (t1)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52.879
52.700
40.171
32.092
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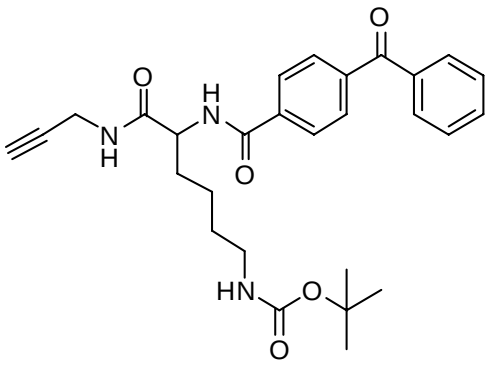
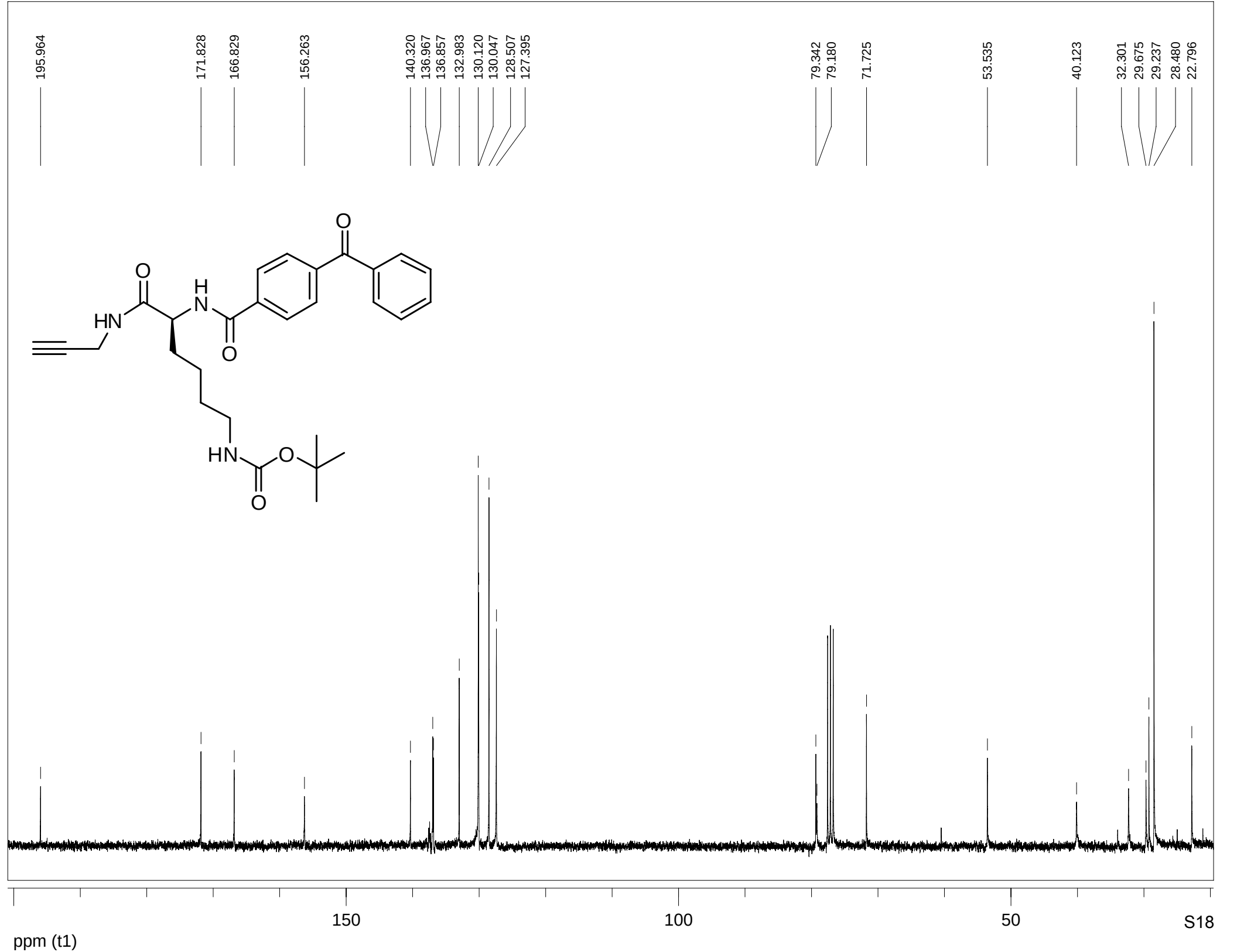
100
50
0
ppm (t1)



Proton NMR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156.263
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136.857
132.983
130.120
130.047
128.507
127.395
79.342
79.180
71.725
53.535
40.123
32.301
29.675
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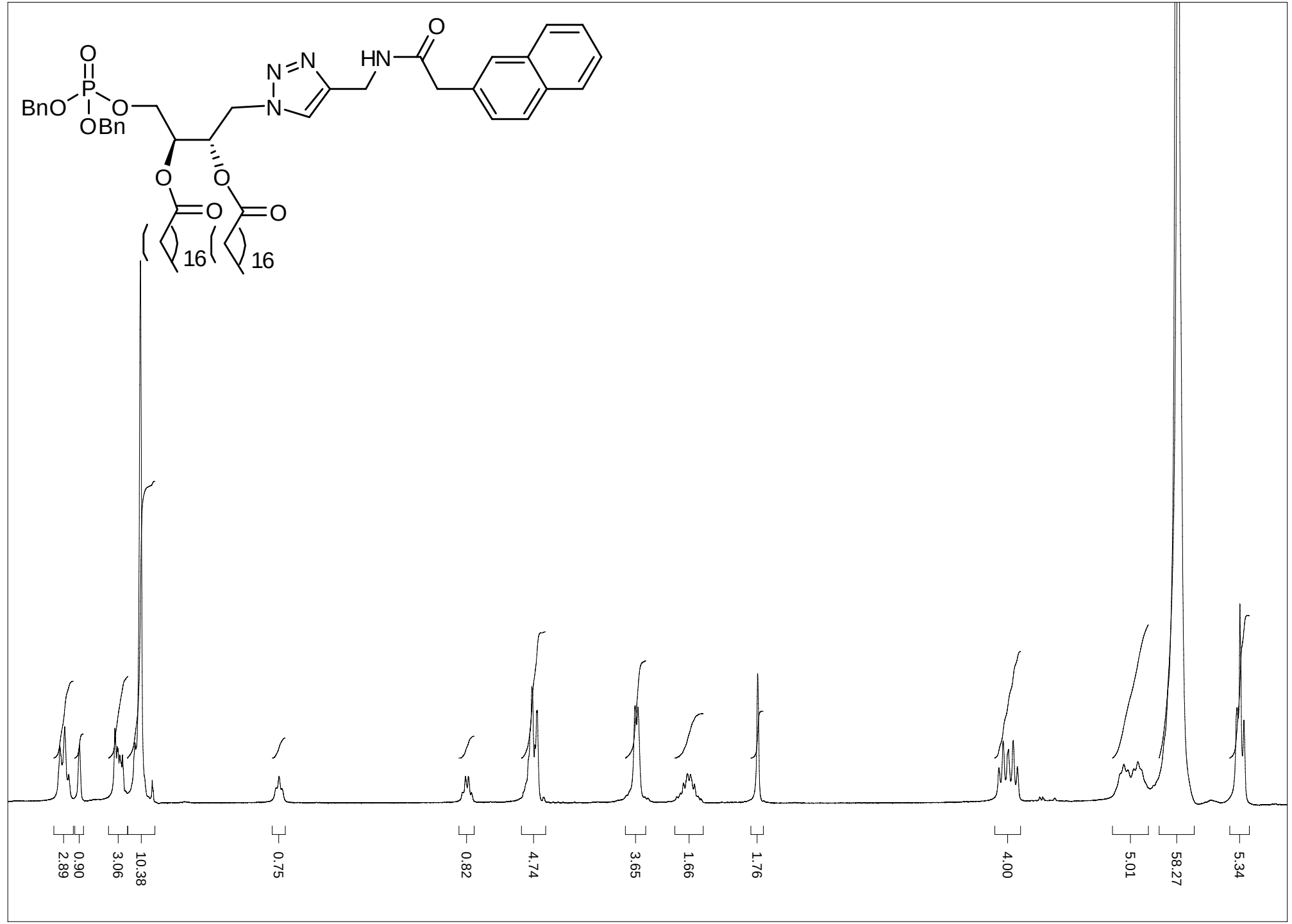
H
N
O
HN
O
O
150
100
50
ppm (t1)


BnO
O
P
O
OBn
N
N
N
HN
O
O
O
O
O
O
16
16
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0.90
3.06
10.38
0.75
0.82
4.74
3.65
1.66
1.76
4.00
5.01
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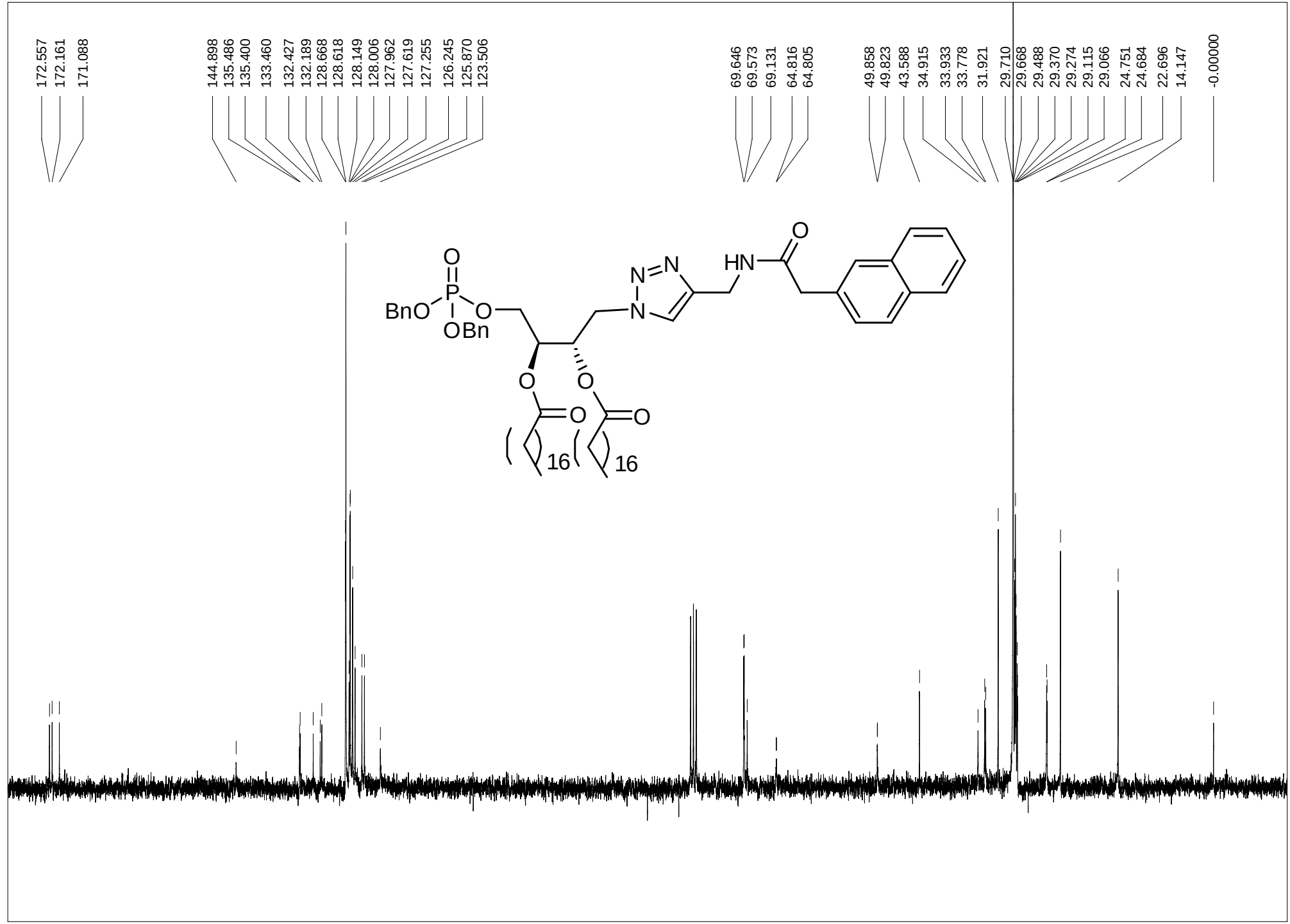
5.0
4.0
3.0
2.0
1.0
ppm (t1)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171.088
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135.486
135.400
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132.427
132.189
128.668
128.618
128.149
128.006
127.962
127.619
127.255
126.245
125.870
123.506
69.646
69.573
69.131
64.816
64.805
49.858
49.823
43.588
34.915
33.933
33.778
31.921
29.710
29.668
29.488
29.370
29.274
29.115
29.066
24.751
24.684
22.696
14.147
-0.00000
BnO
O
P
O
OBn
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N
N
HN
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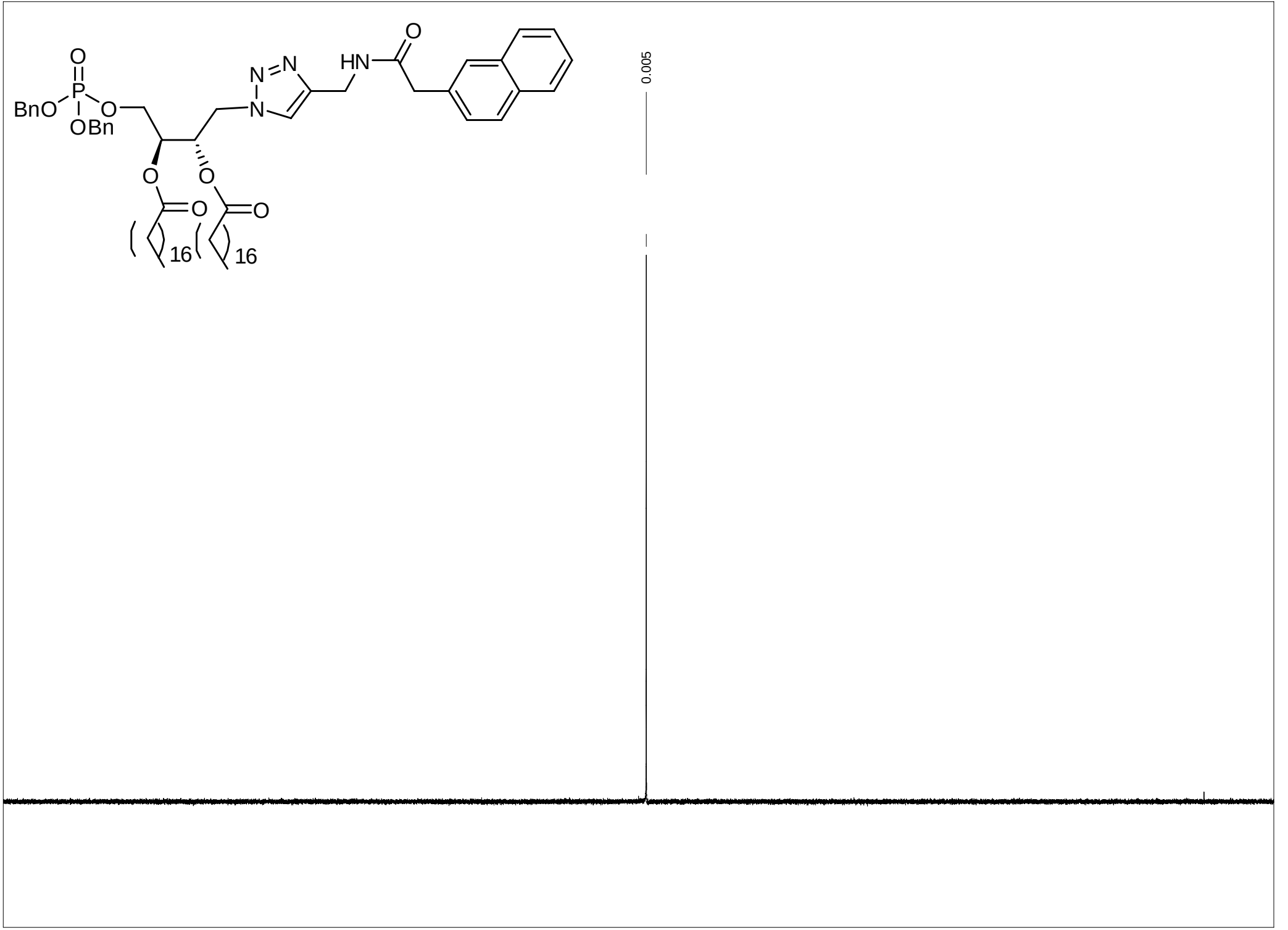
O
O
O
O
16
16
150
100
50
0
ppm (t1)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0
-100
-200
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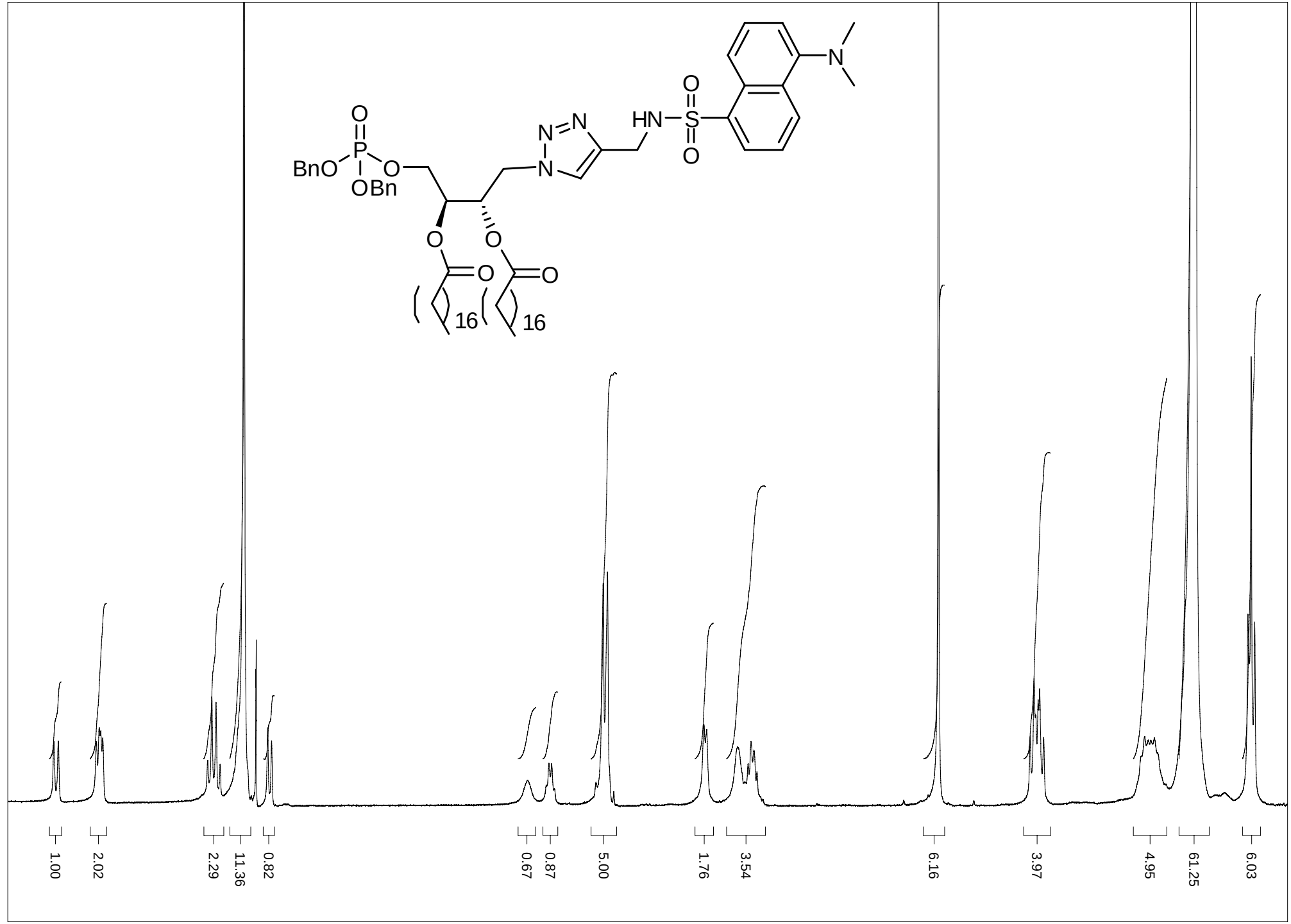
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16
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2.02
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0.67
0.87
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1.76
3.54
6.16
3.97
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5.0
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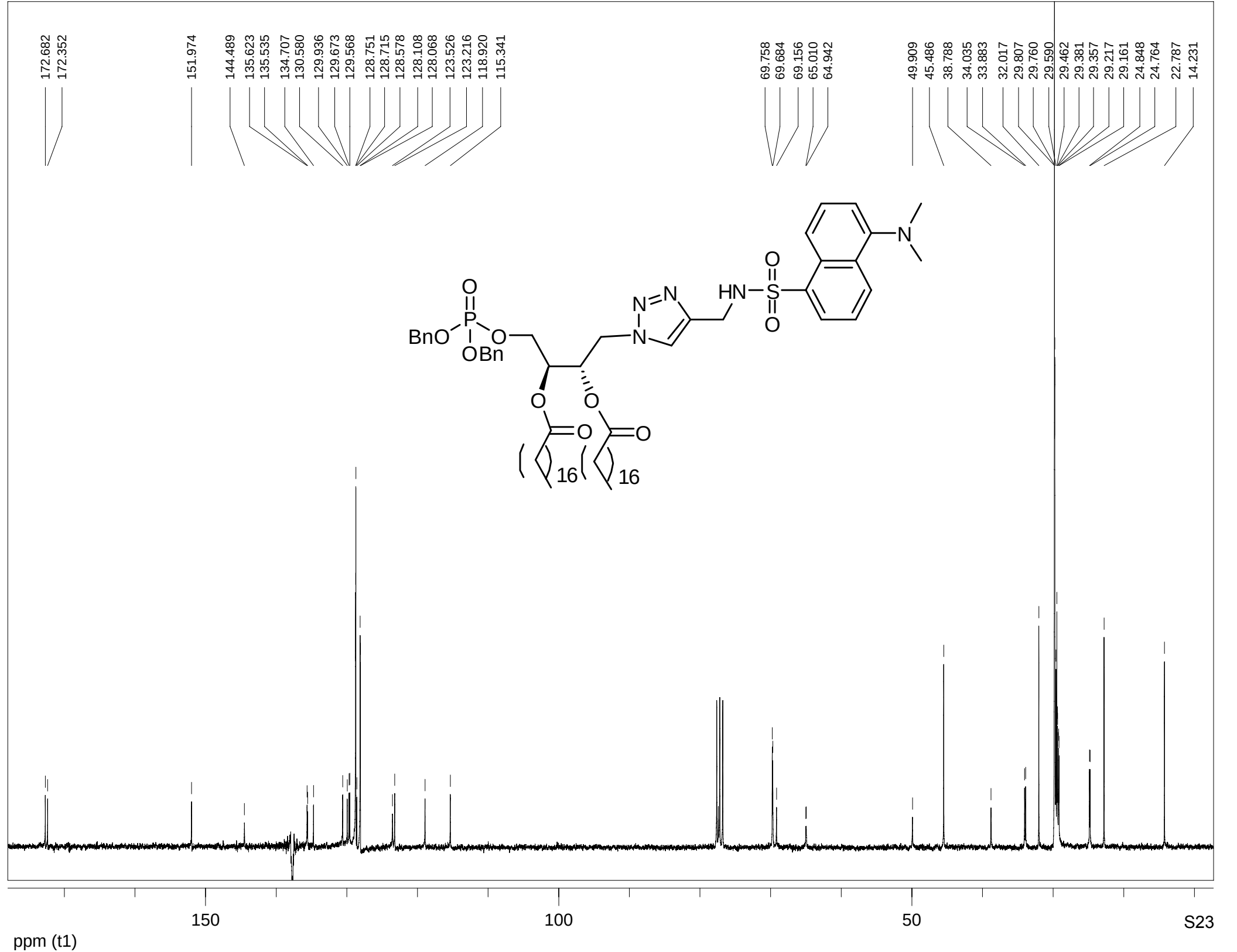
2.0
1.0
ppm (t1)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128.715
128.578
128.108
128.068
123.526
123.216
118.920
115.341
69.758
69.684
69.156
65.010
64.942
49.909
45.486
38.788
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33.883
32.017
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29.760
29.590
29.462
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29.161
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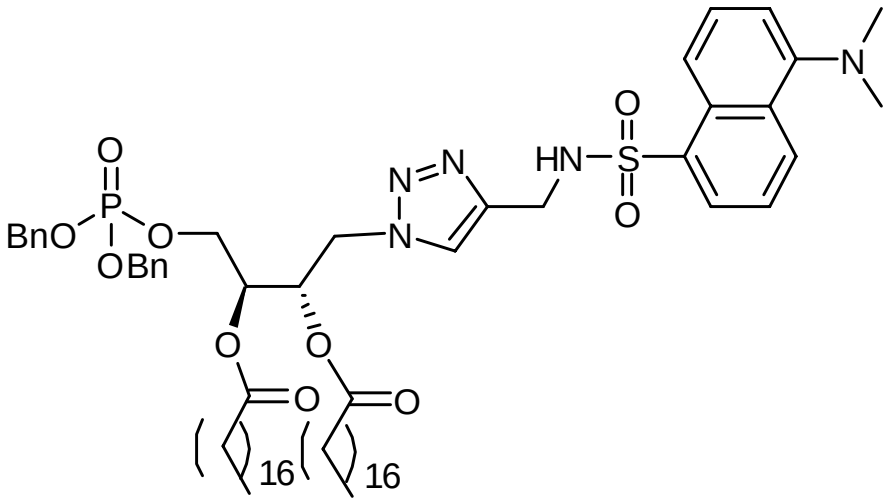
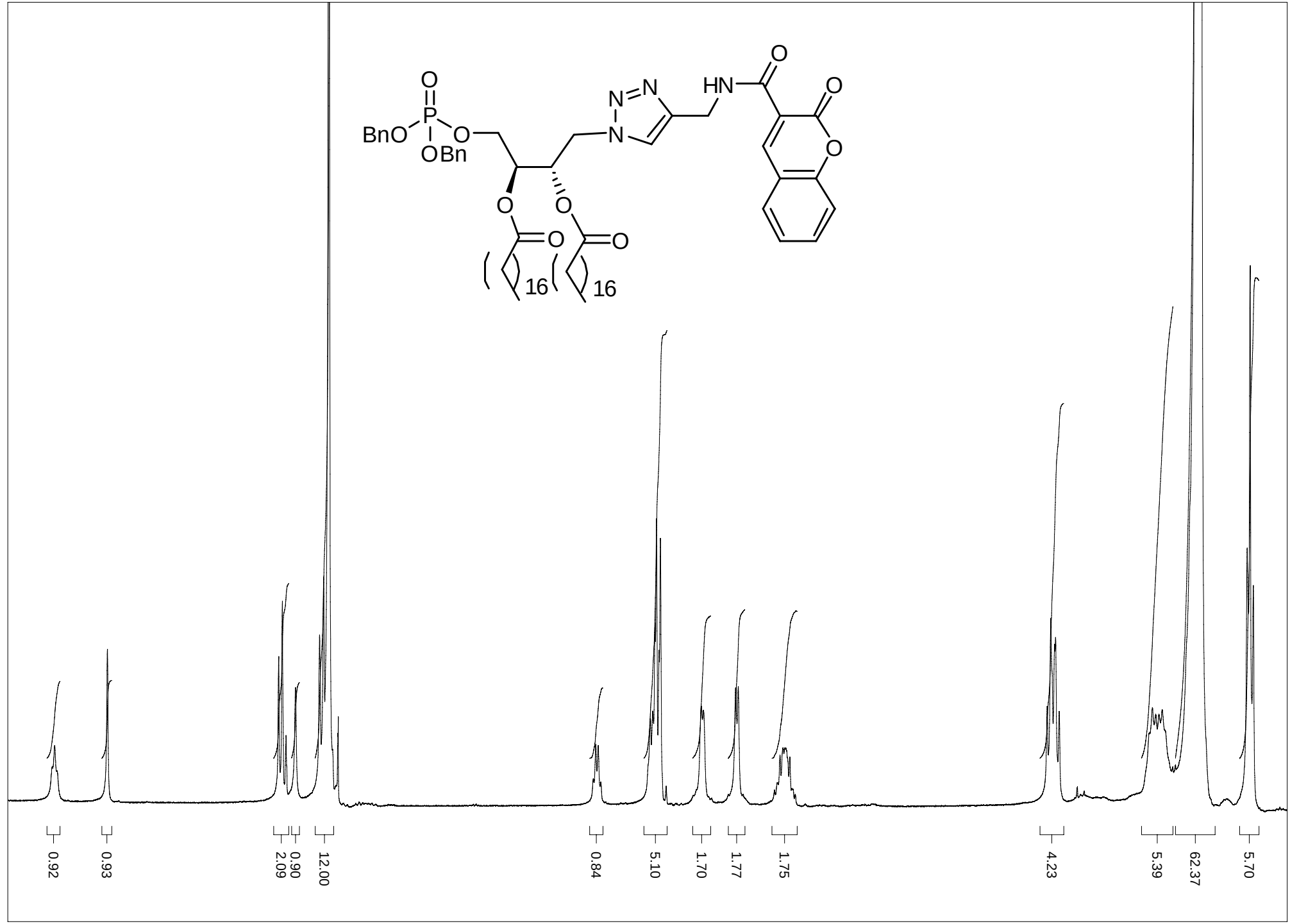
HN
16
16
150
100
50
ppm (t1)
S23



-0.053

5.0 0.0 -5.0

ppm (t1)


BnO
O
P
O
OBn
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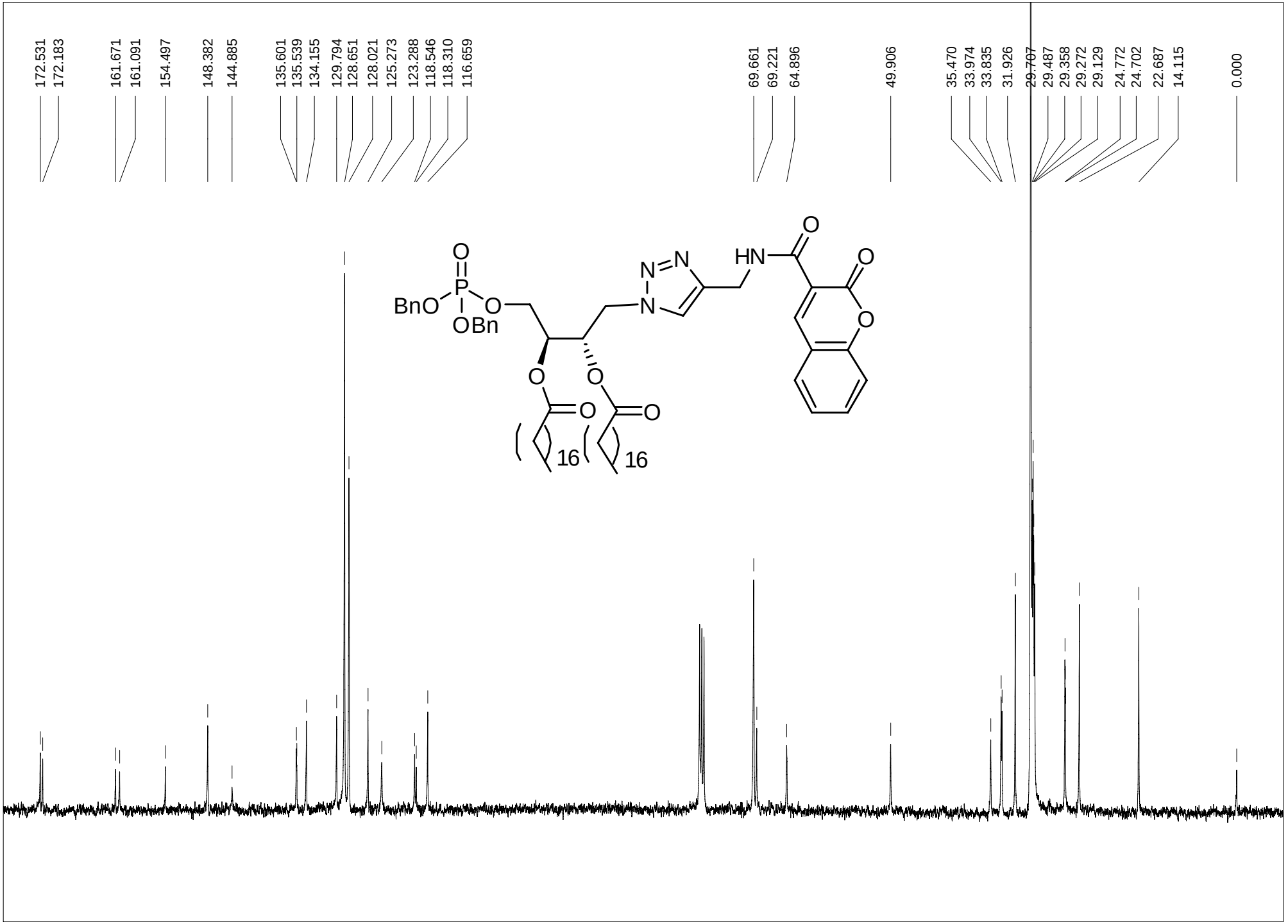
N
HN
O
O
O
O
O
O
16
16
0.92
0.93
2.09
0.90
12.00
0.84
5.10
1.70
1.77
1.75
4.23
5.39
62.37
5.70
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)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128.021
125.273
123.288
118.546
118.310
116.659
69.661
69.221
64.896
49.906
35.470
33.974
33.835
31.926
29.707
29.487
29.358
29.272
29.129
24.772
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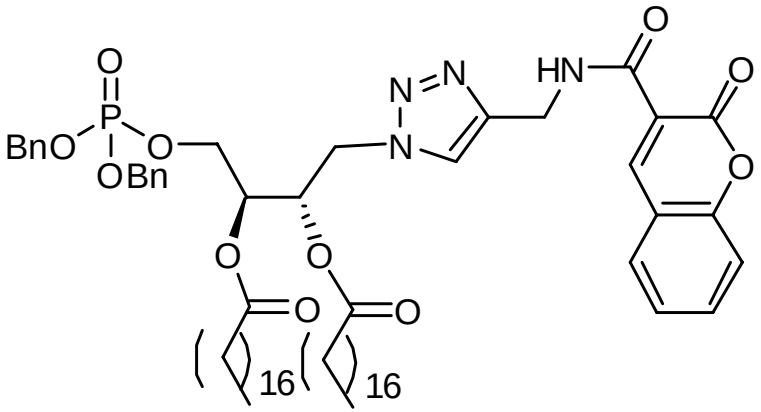
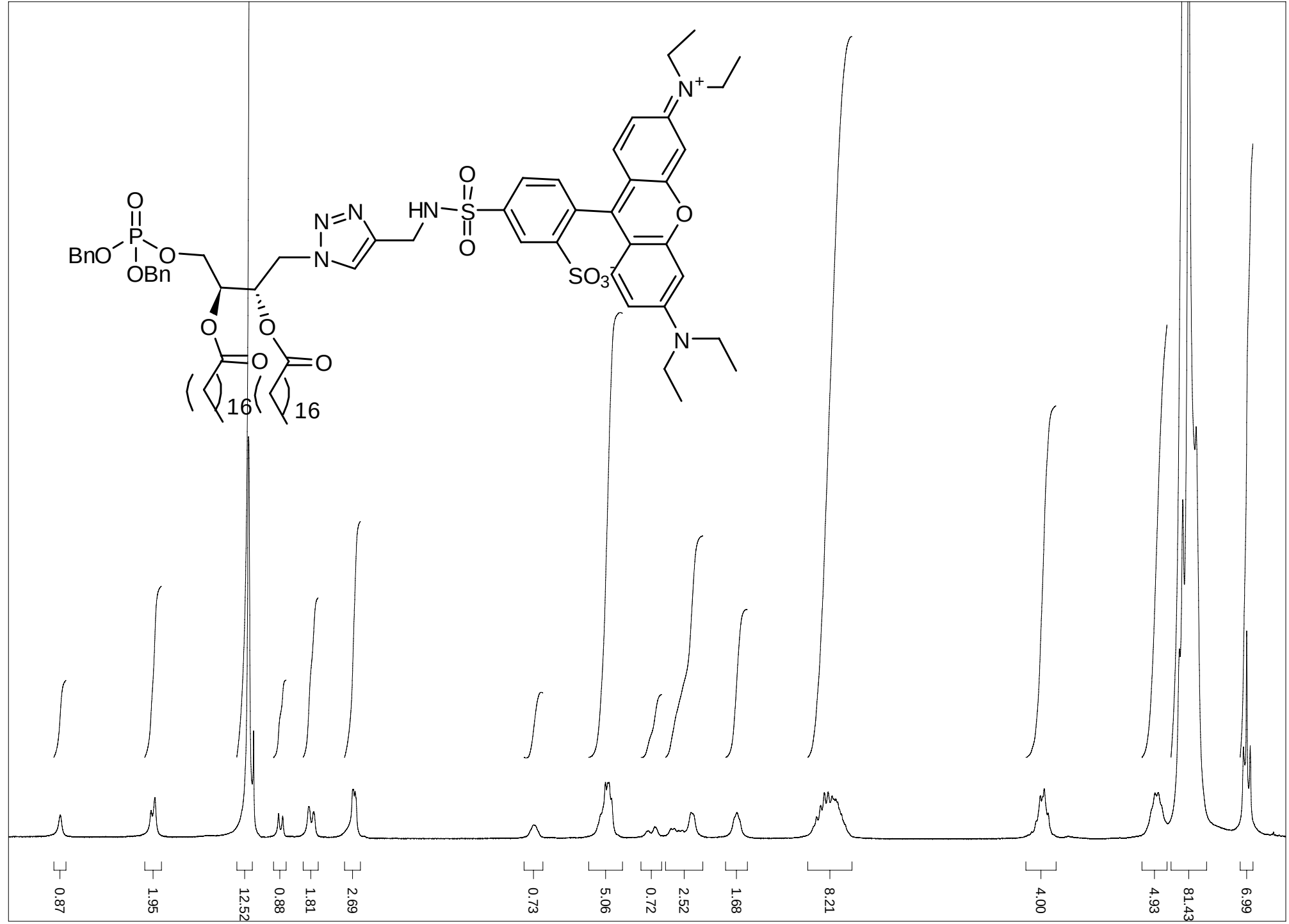
BnO
OBn
HN
16
16
150
100
50
0
ppm (t1)


O
P
BnO
O
OBn
N
N
N
HN
O
O
O
O
O
O
O
16
16

0.031
50
0
-50
ppm (t1)


0.87
1.95
12.52
0.88
1.81
2.69
0.73
5.06
0.72
2.52
1.68
8.21
4.00
4.93
81.43
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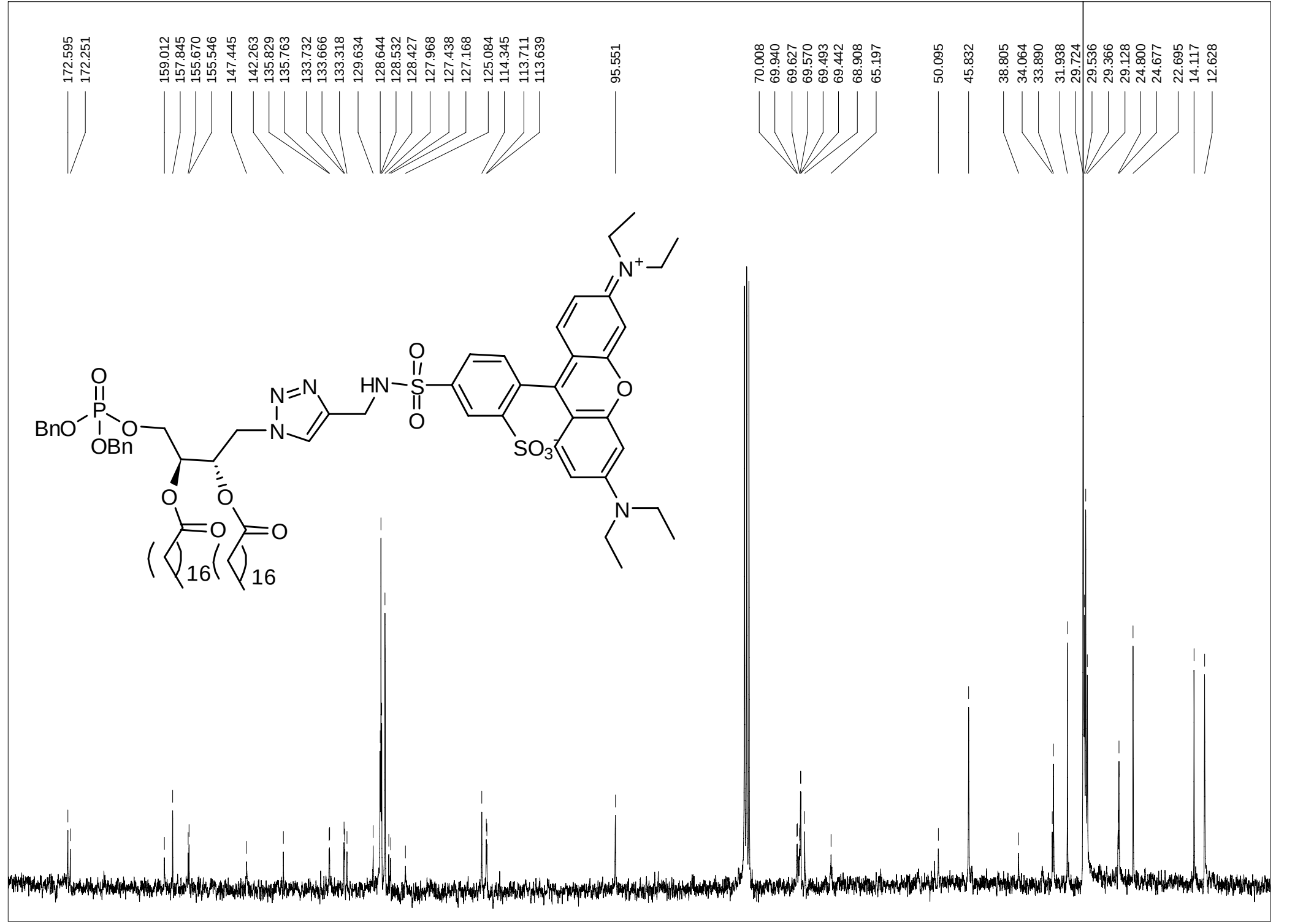
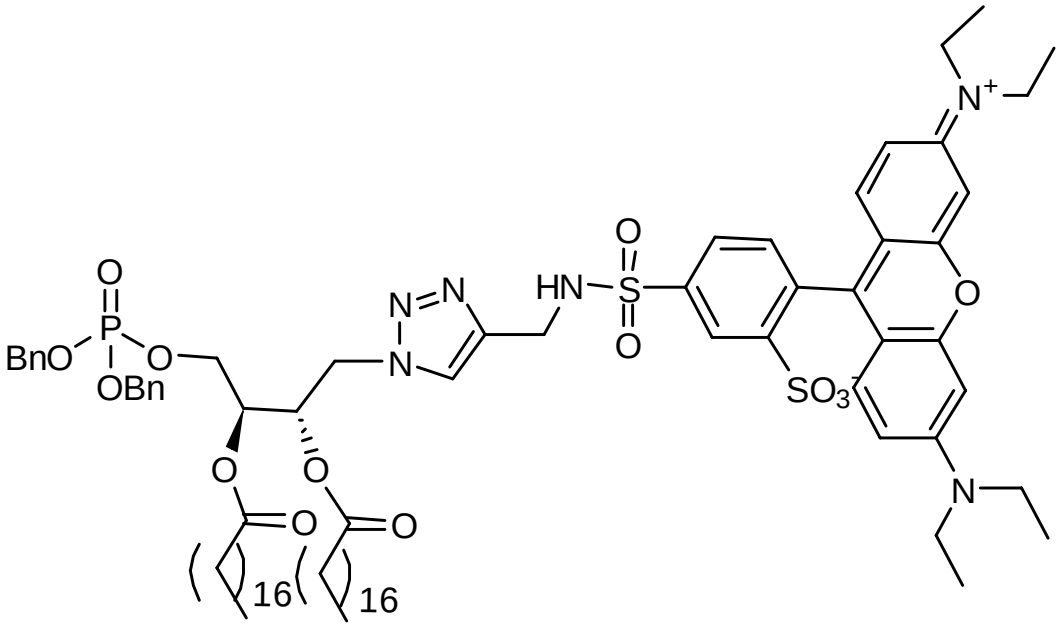
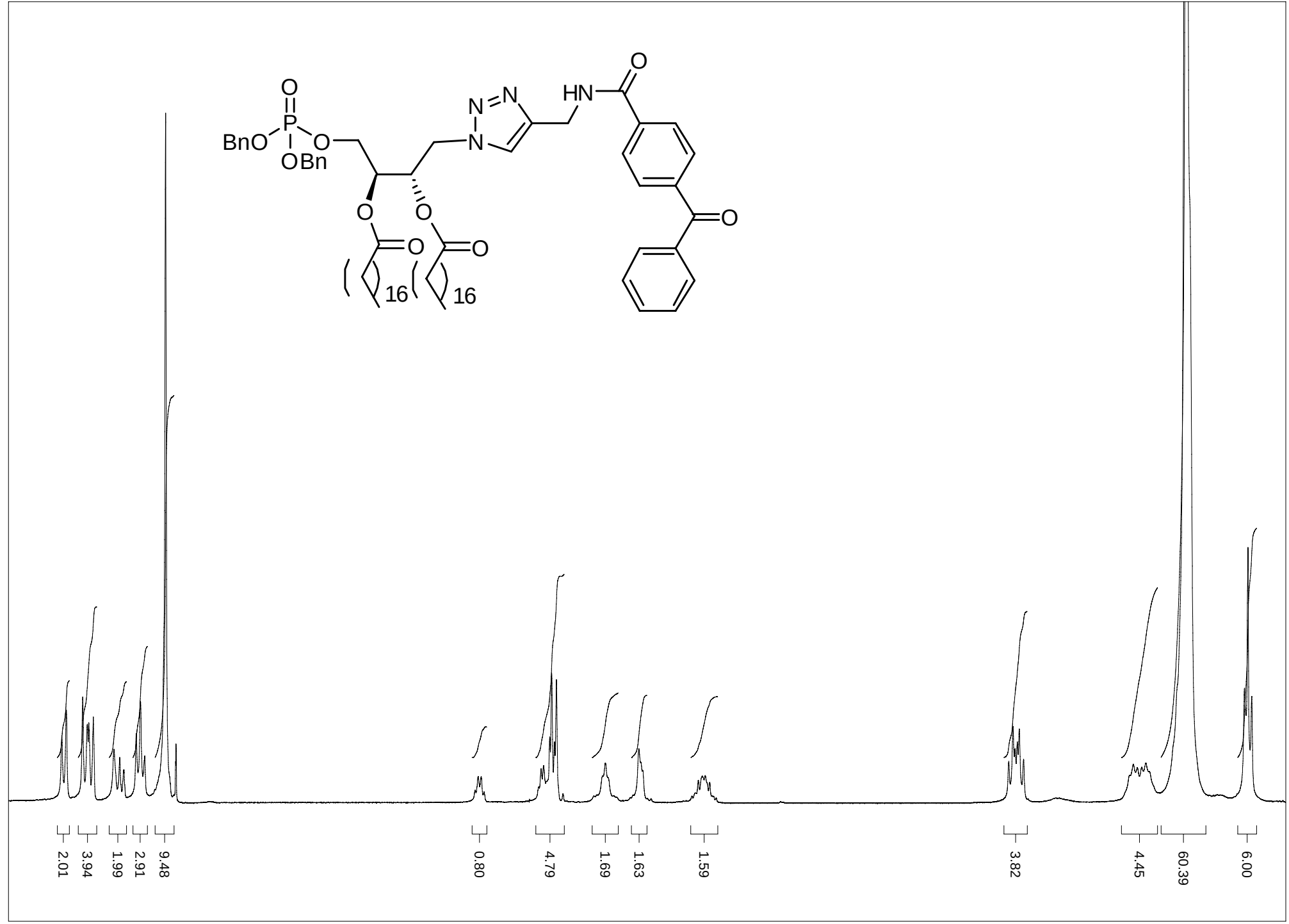
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

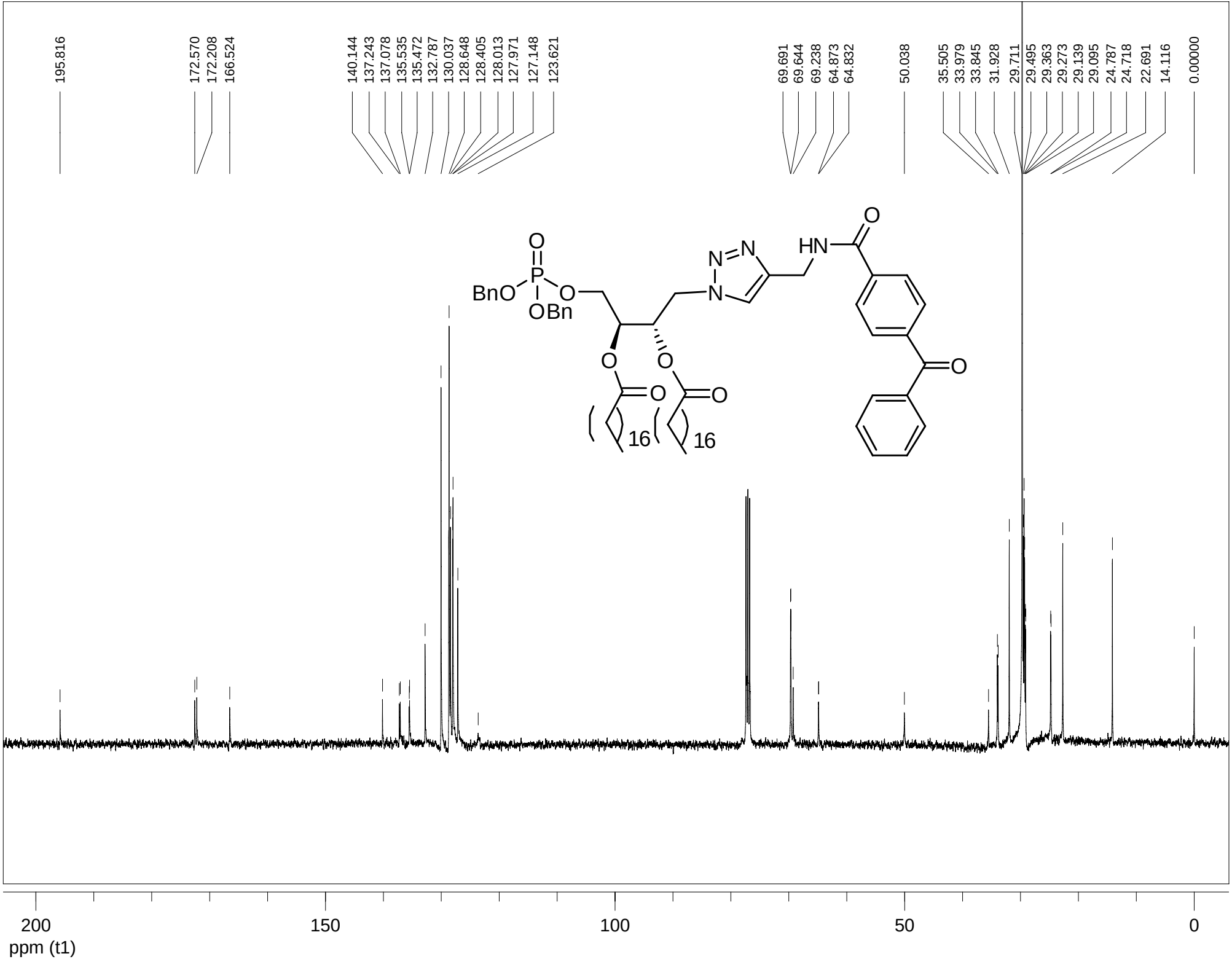

172.595
172.251
159.012
157.845
155.670
155.546
147.445
142.263
135.829
135.763
133.732
133.666
133.318
129.634
128.644
128.532
128.427
127.968
127.438
127.168
125.084
114.345
113.711
113.639
95.551
70.008
69.940
69.627
69.570
69.493
69.442
68.908
65.197
50.095
45.832
38.805
34.064
33.890
31.938
29.724
29.536
29.366
29.128
24.800
24.677
22.695
14.117
12.628
BnO
OBn
HN
SO3
16
16
150
100
50
ppm (t1)

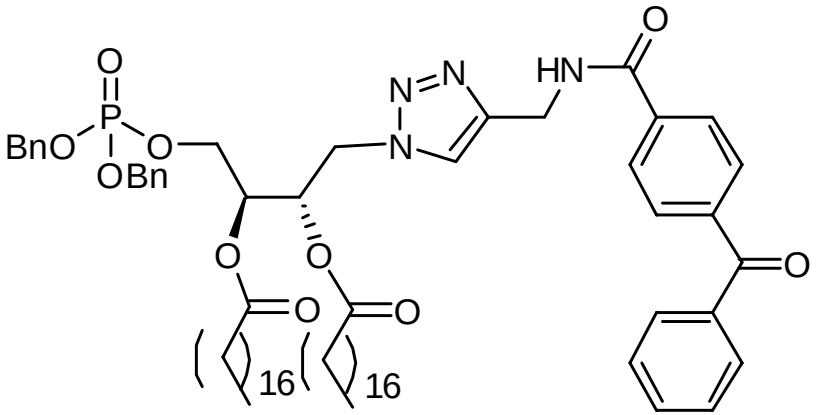


10.0 5.0 0.0 -5.0

ppm (t1)


BnO
OBn
HN
16
16
2.01
3.94
1.99
2.91
9.48
0.80
4.79
1.69
1.63
1.59
3.82
4.45
60.39
6.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


195.816
172.570
172.208
166.524
140.144
137.243
137.078
135.535
135.472
132.787
130.037
128.648
128.405
128.013
127.971
127.148
123.621
69.691
69.644
69.238
64.873
64.832
50.038
35.505
33.979
33.845
31.928
29.711
29.495
29.363
29.273
29.139
29.095
24.787
24.718
22.691
14.116
0.00000
BnO
OBn
HN
16
16
200
150
100
50
0
ppm (t1)

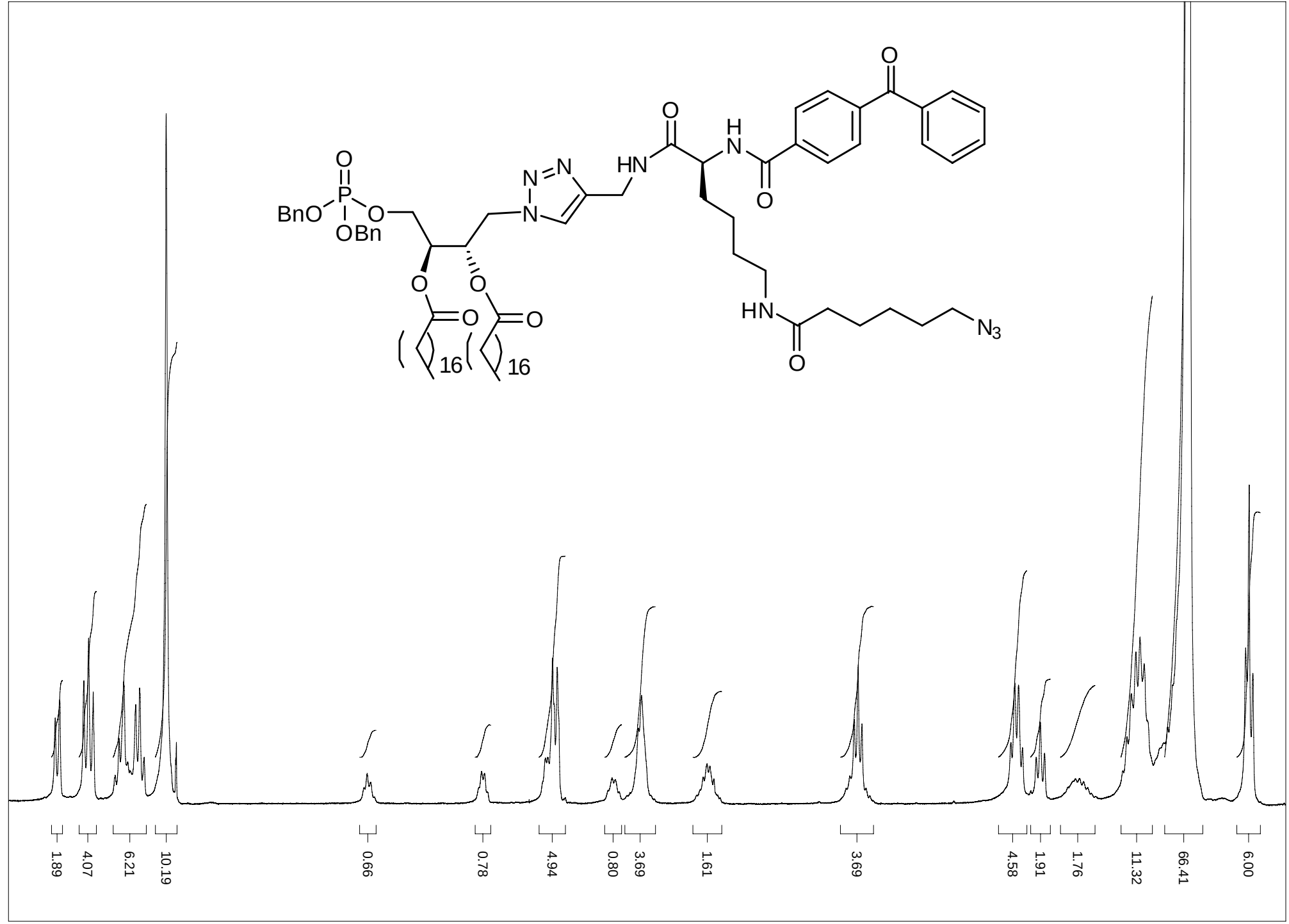


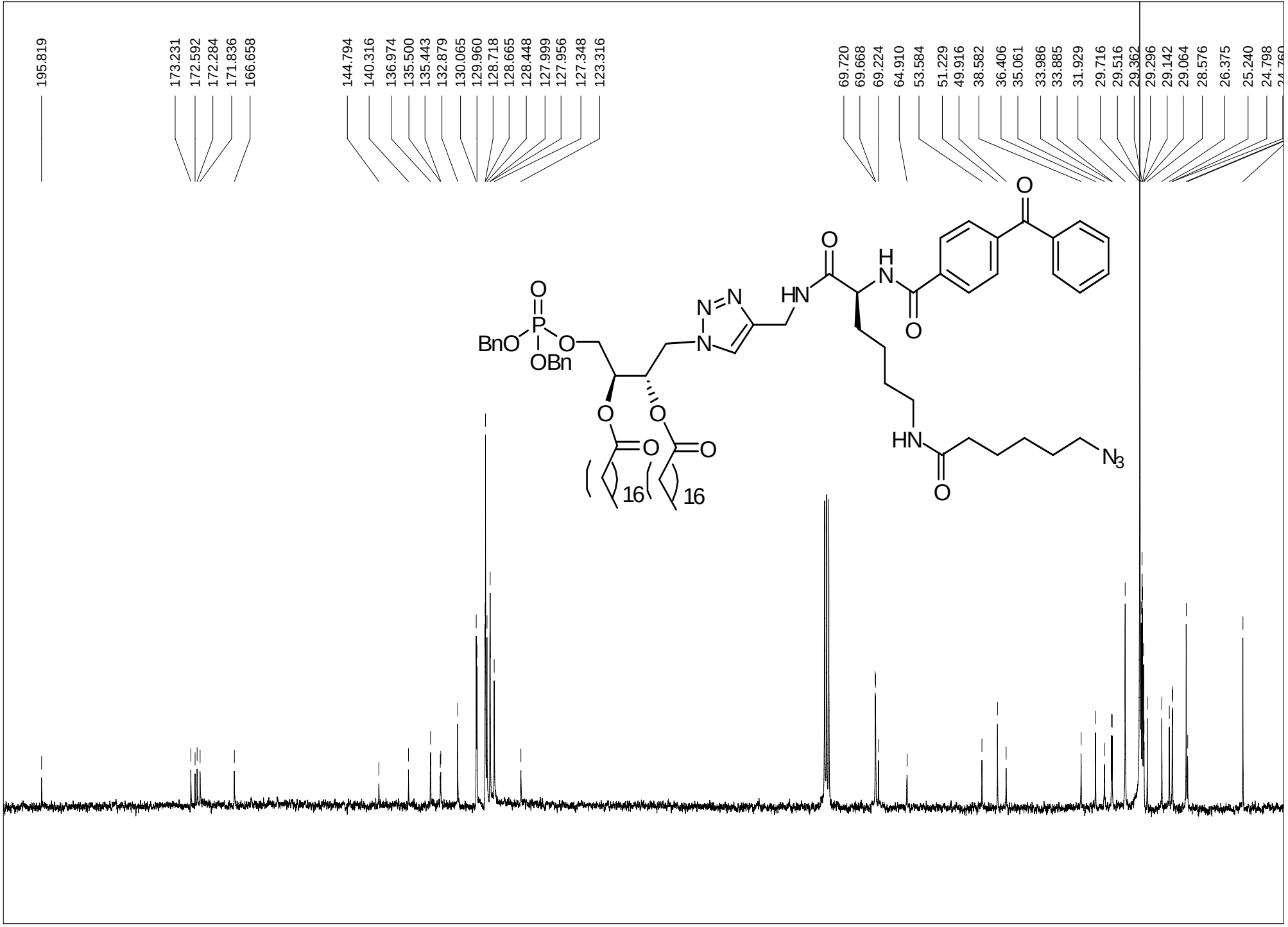
-0.056

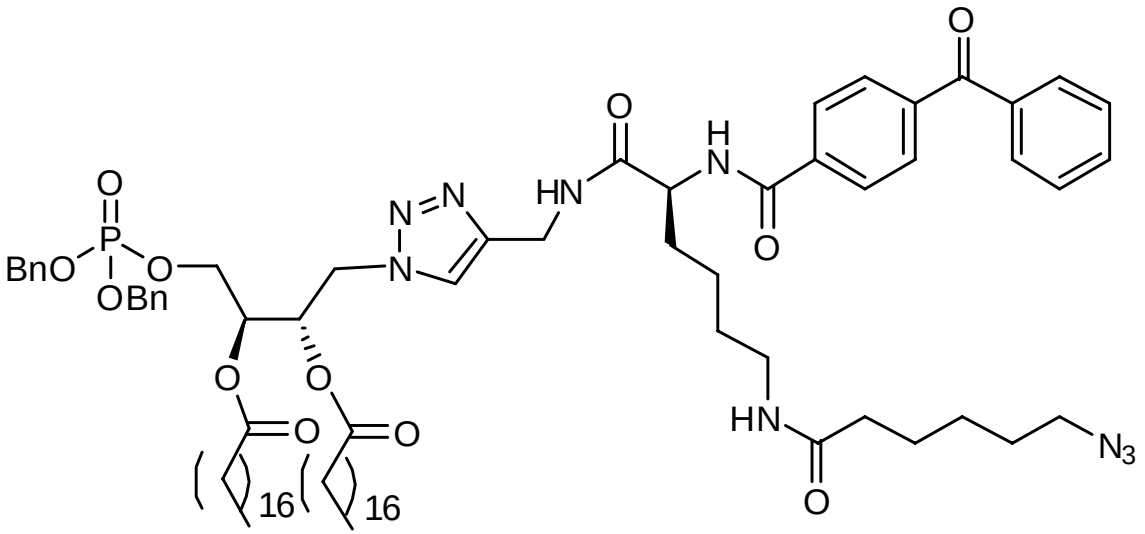
5.0

0.0

ppm (t1)


O
BnO
P
O
OBn
O
O
16
O
O
16
N
N
N
HN
O
H
N
O
O
HN
O
N3
1.89
4.07
6.21
10.19
0.66
0.78
4.94
0.80
3.69
1.61
3.69
4.58
1.91
1.76
11.32
66.41
6.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

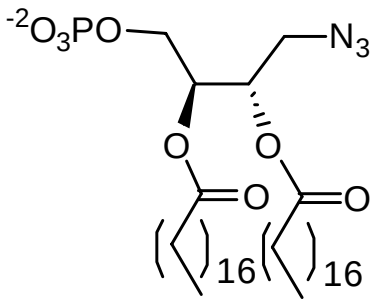

195.819
173.231
172.592
172.284
171.836
166.658
144.794
140.316
136.974
135.500
135.443
132.879
130.065
129.960
128.718
128.665
128.448
127.999
127.956
127.348
123.316
69.720
69.668
69.224
64.910
53.584
51.229
49.916
38.582
36.406
35.061
33.986
33.885
31.929
29.716
29.516
29.362
29.296
29.142
29.064
28.576
26.375
25.240
24.798
BnO
OBn
HN
HN
N3
16
16
150
100
50
ppm (t1)



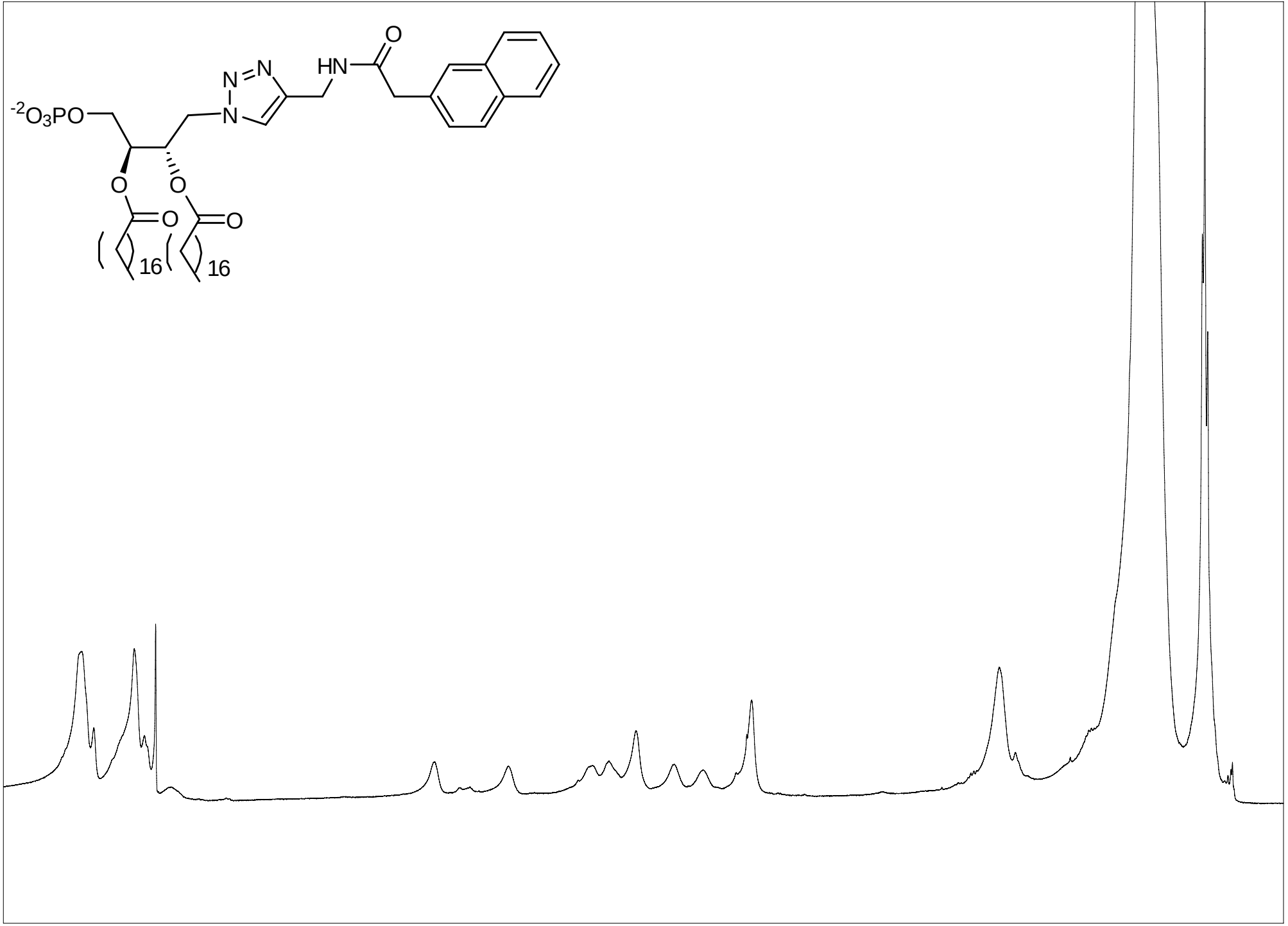
-0.251

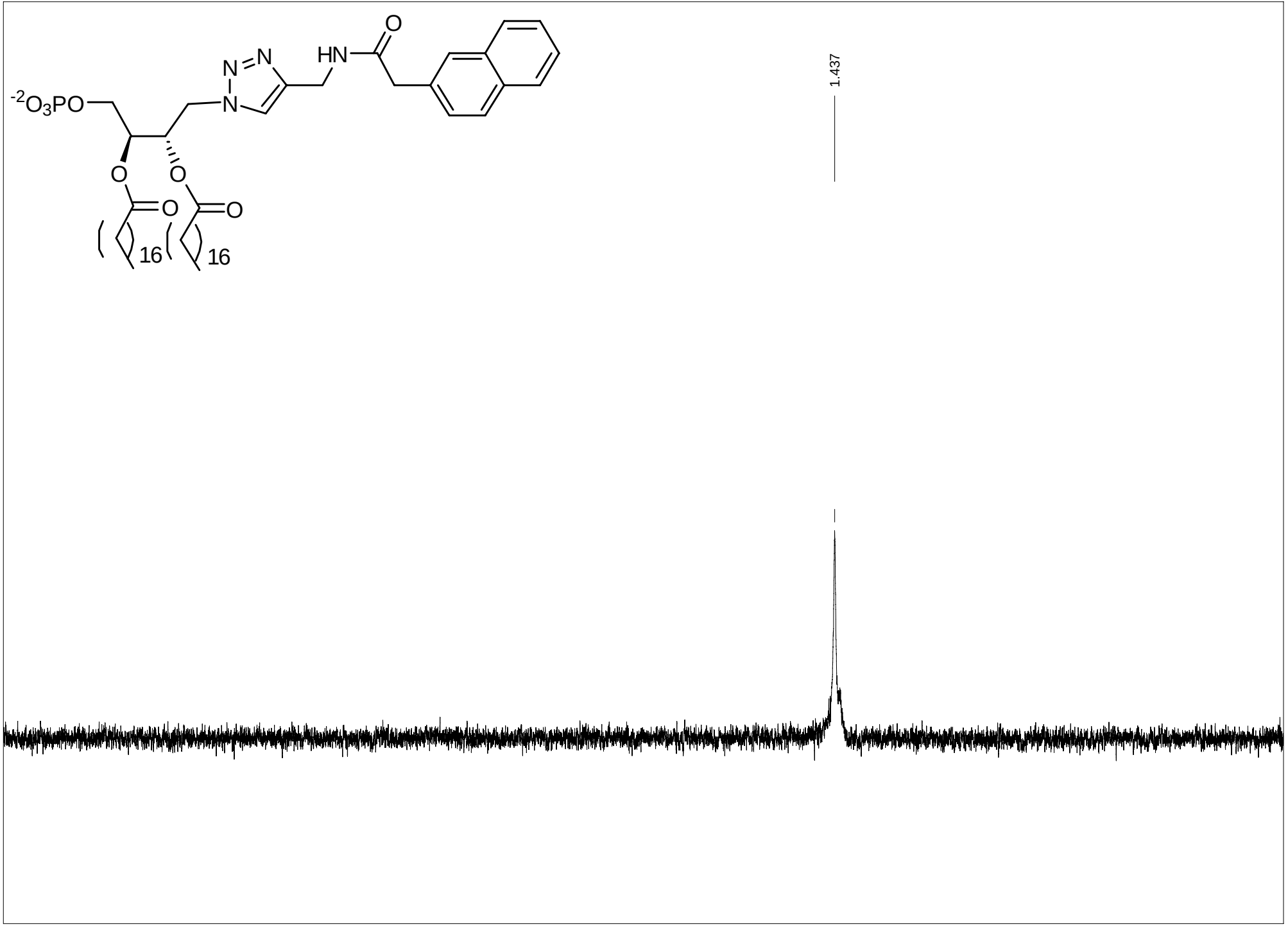
10.0 5.0 0.0 -5.0

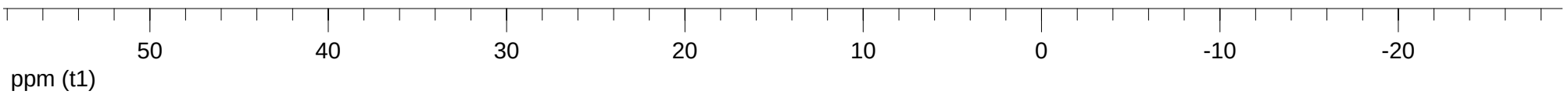
ppm (t1)

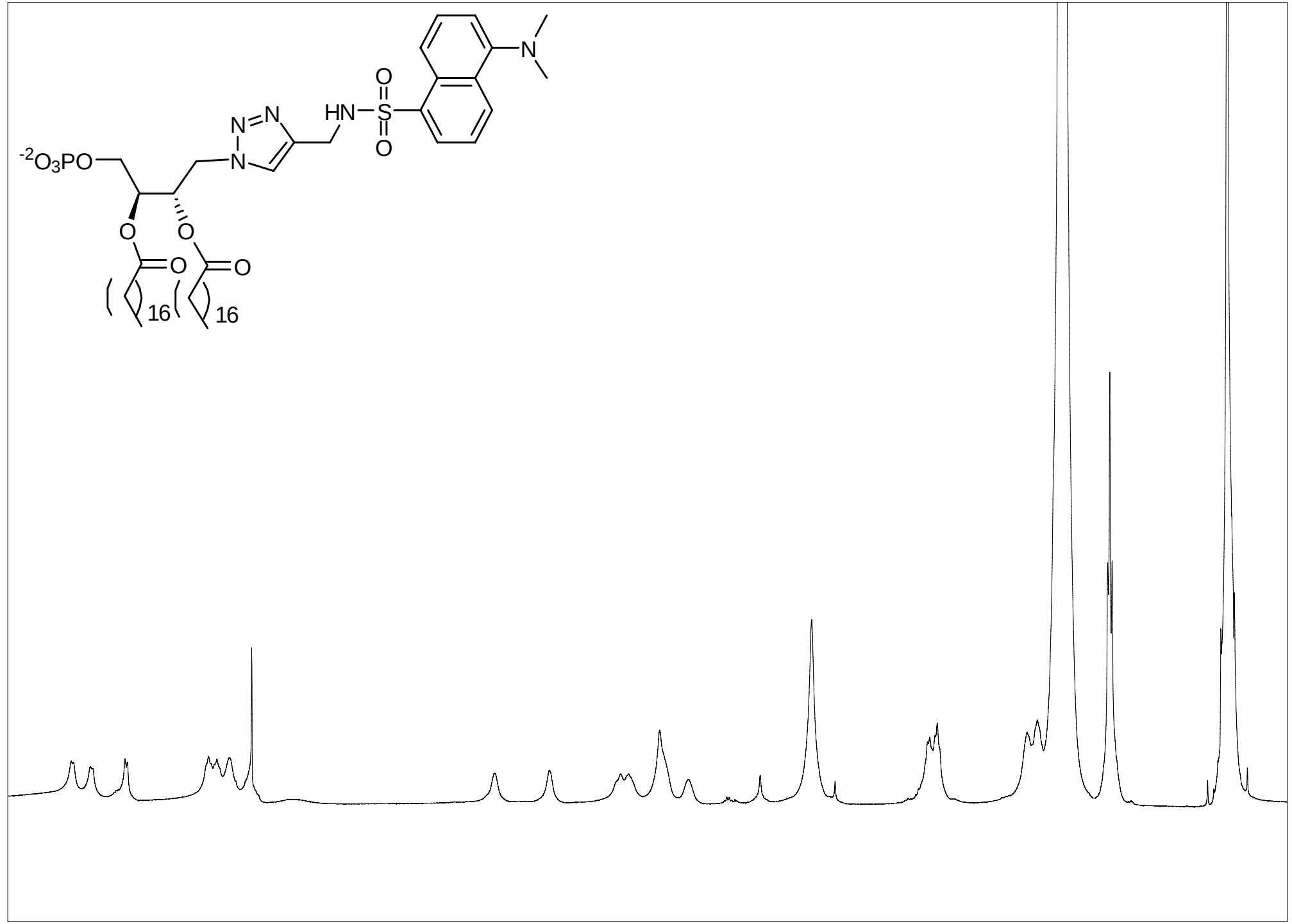

-2O3PO
N3
O
O
O
O
16
16

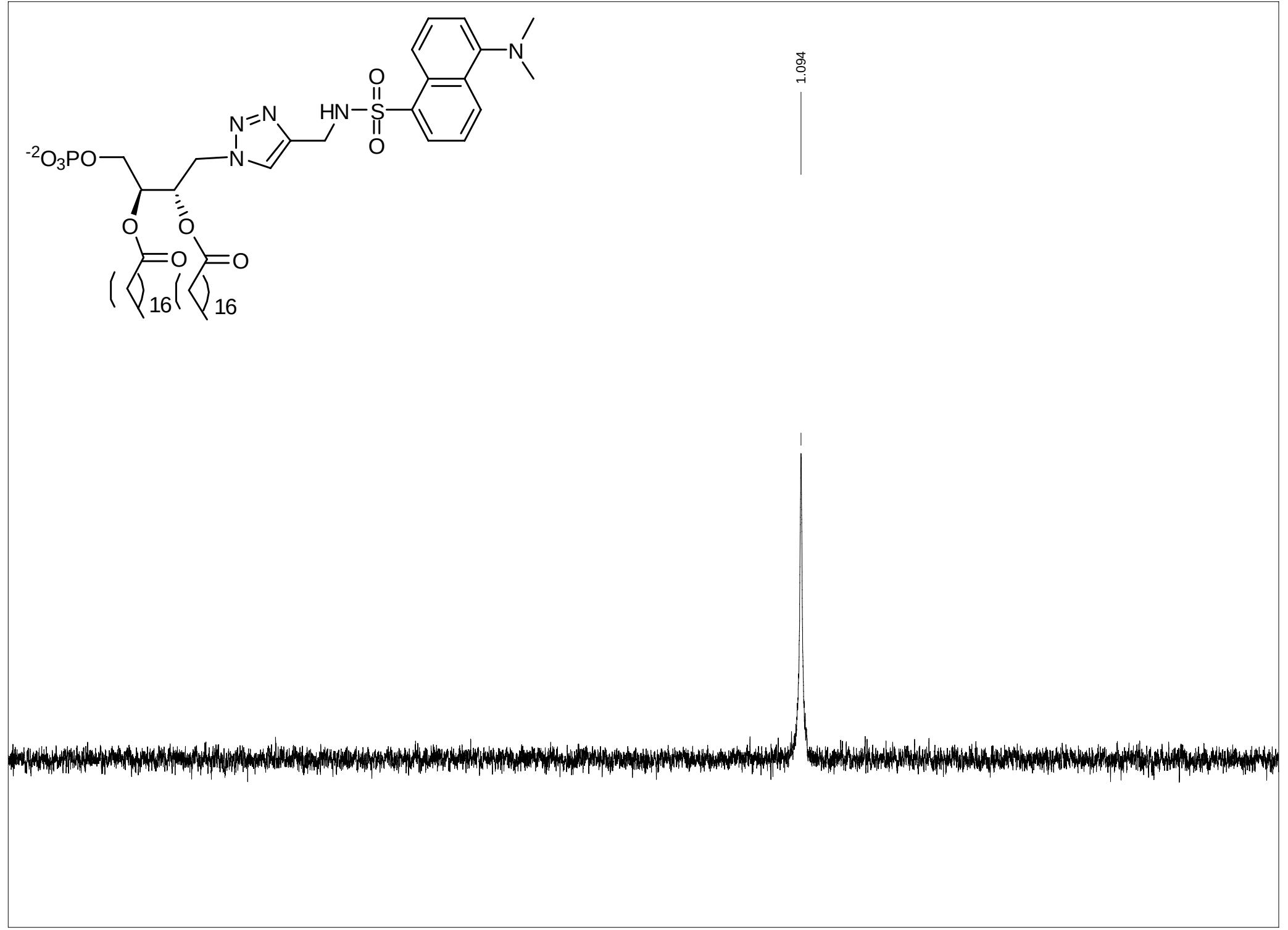
2.00
1.56
1.92
3.74
4.07
57.50
6.12
5.0
4.0
3.0
2.0
1.0
ppm (t1)

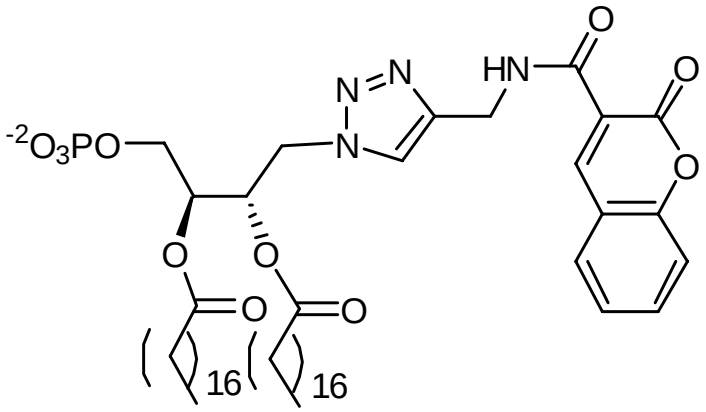

-2O3PO
N
N
N
HN
O
O
O
O
O
16
16
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


-2O3PO
N
N
N
HN
O
O
O
O
O
16
16
1.437

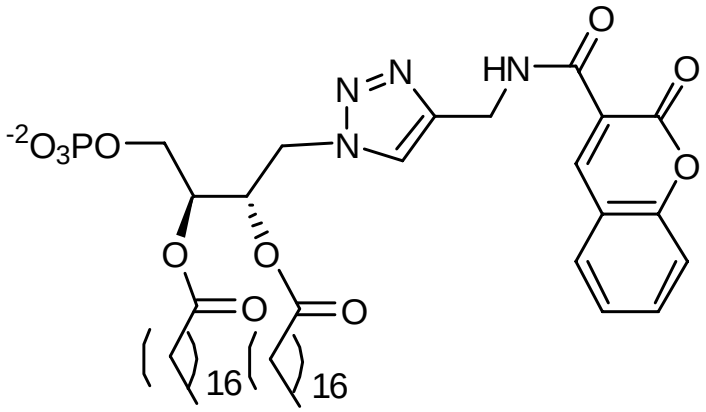

50
40
30
20
10
0
-10
-20
ppm (t1)


-2O3PO
N
N
N
HN
S
O
O
O
O
O
O
O
16
16
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


1.094
40
30
20
10
0
-10
-20
ppm (t1)


-2O3PO
N
N
N
HN
O
O
O
O
O
O
O
16
16

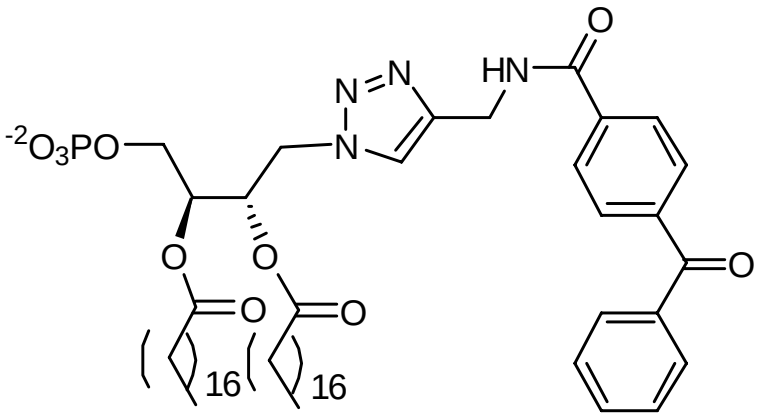
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)



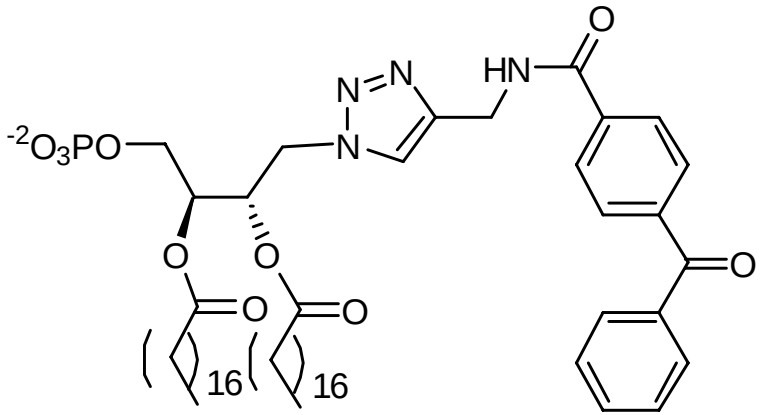
1.394

40 30 20 10 0 -10

ppm (t1)


-2O3PO
N
N
N
HN
O
O
O
O
O
O
16
16

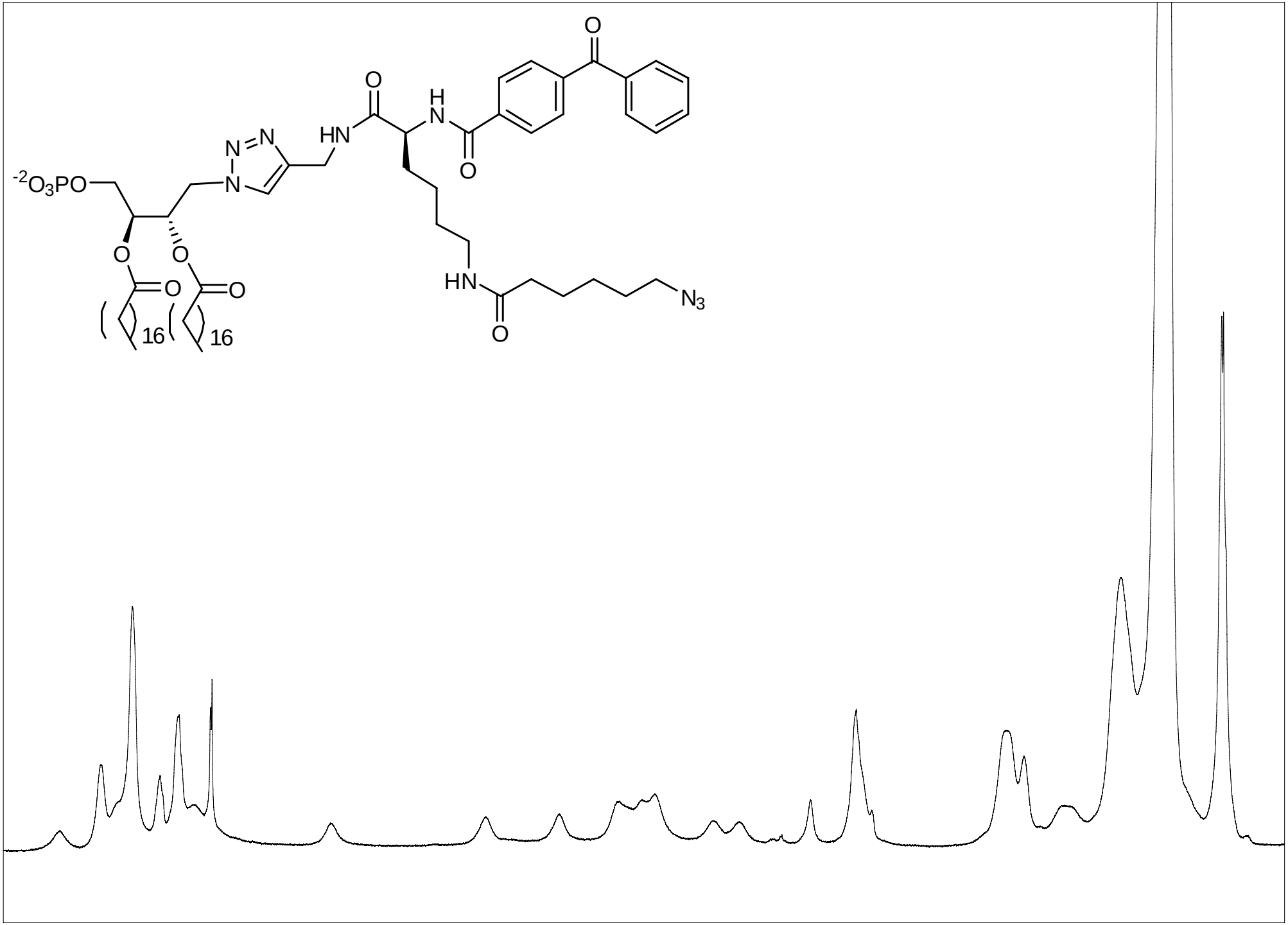
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

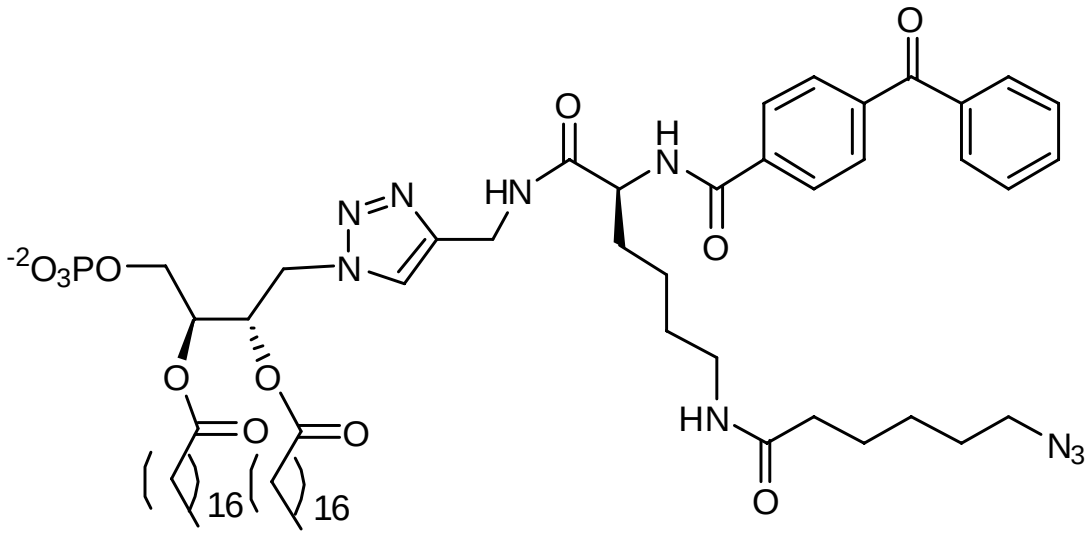


1.250

15.0 10.0 5.0 0.0

ppm (t1)


O
H
N
O
HN
O
N
N
N
-2O3PO
O
O
O
O
16
16
HN
O
N3
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)



1.124

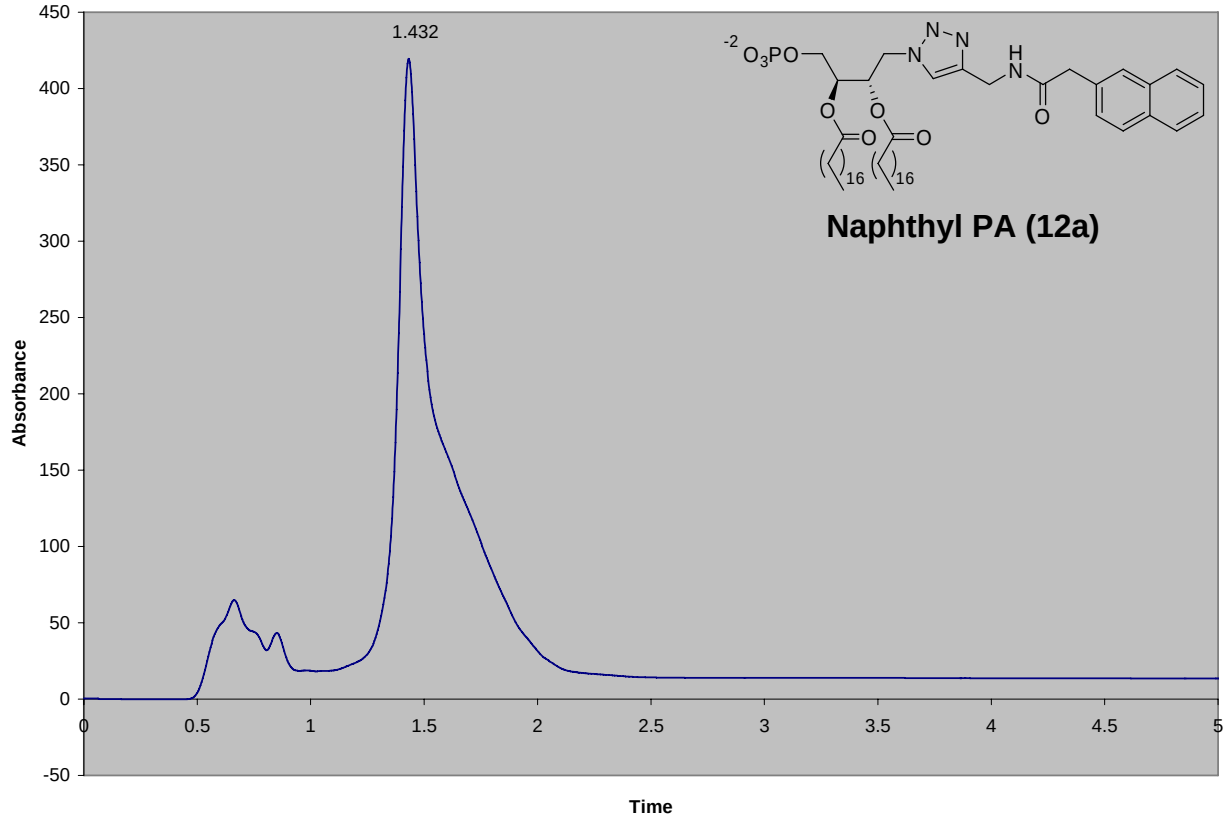
10.0

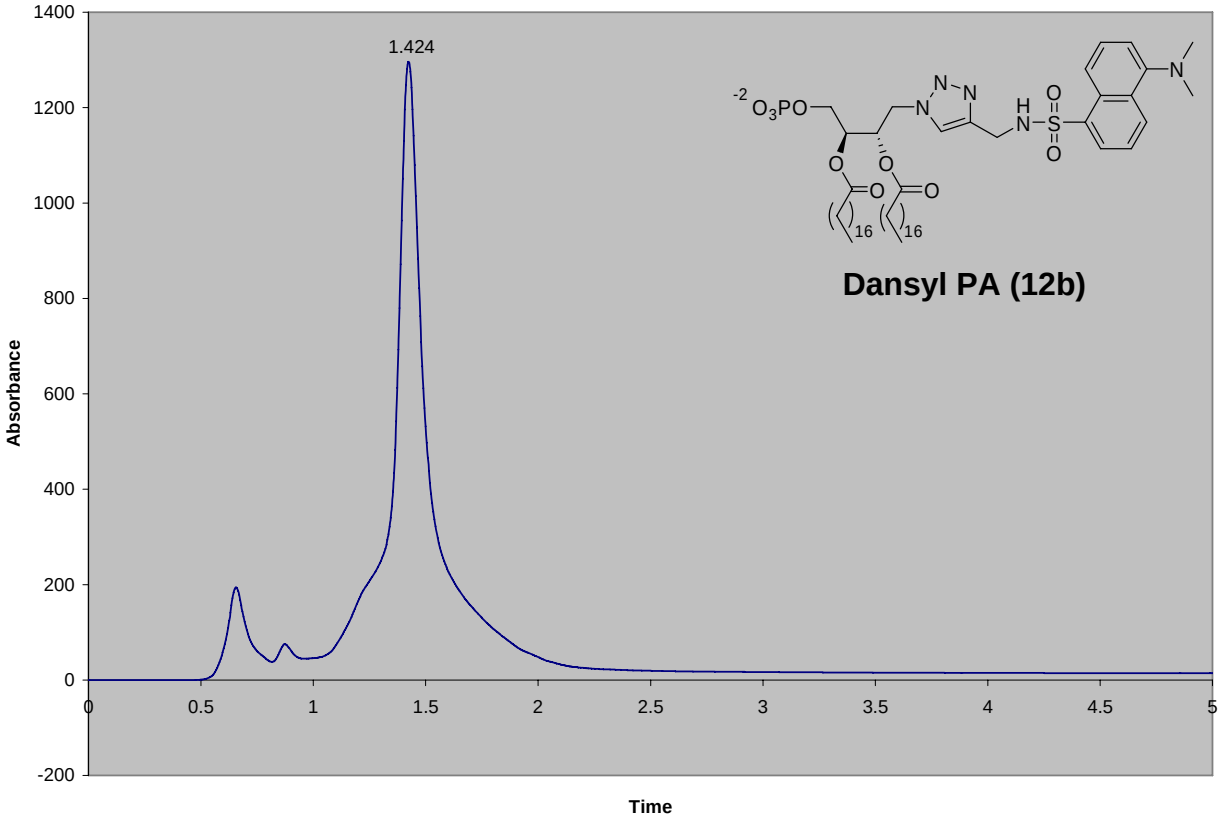
5.0

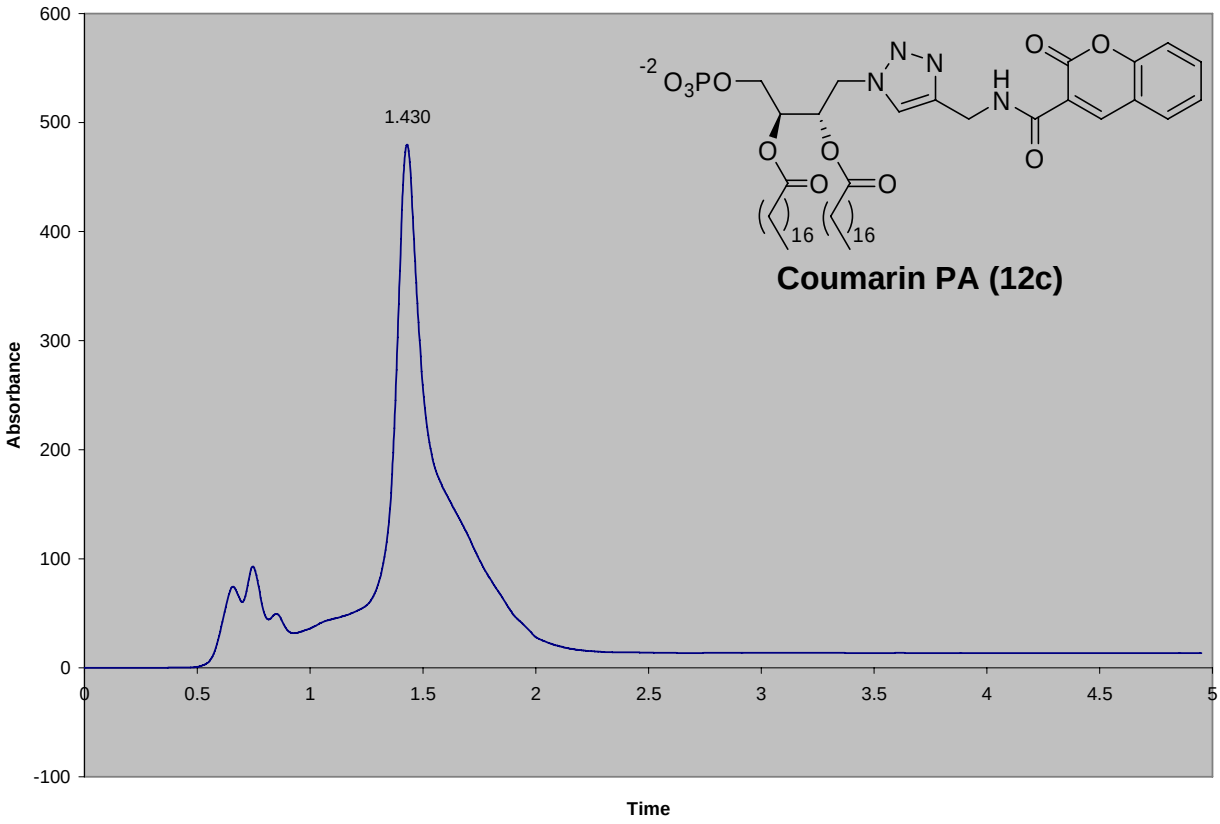
0.0

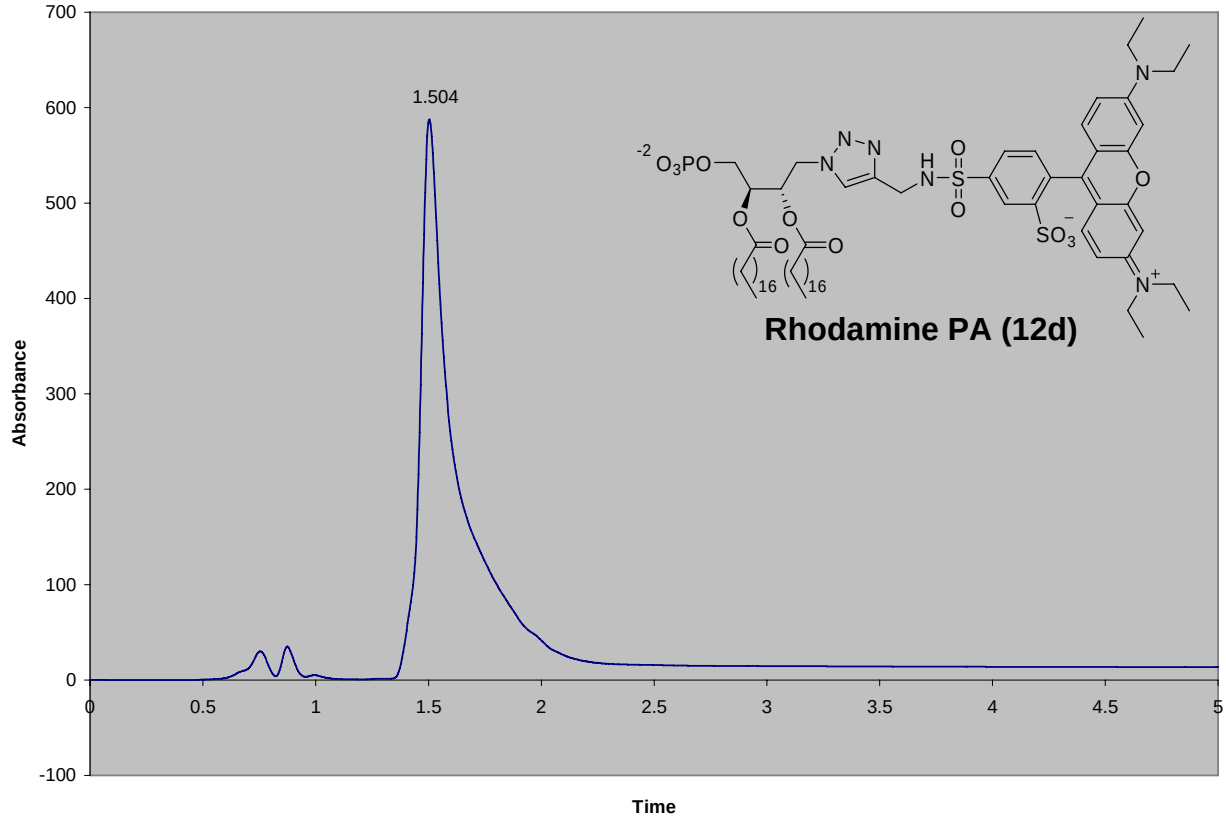
ppm (t1)

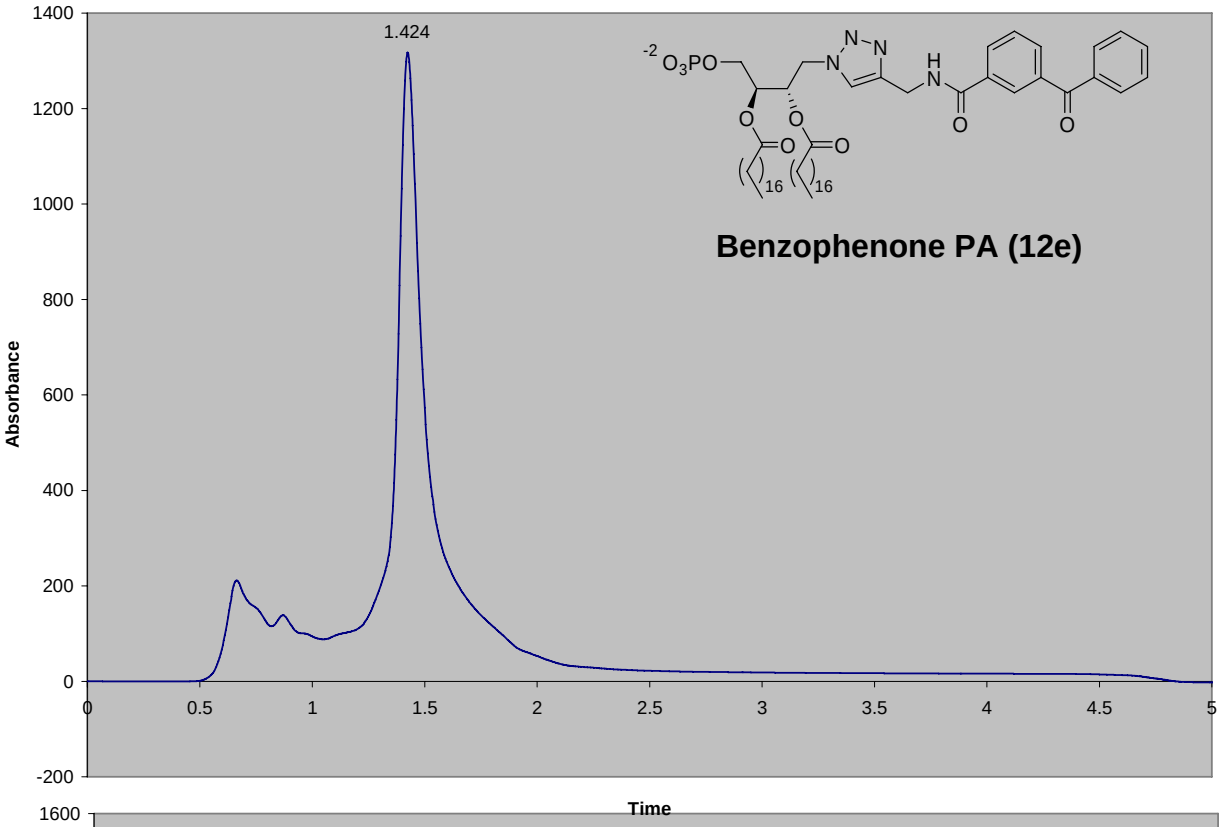
III. HPLC Traces of Derivatized PA Probes

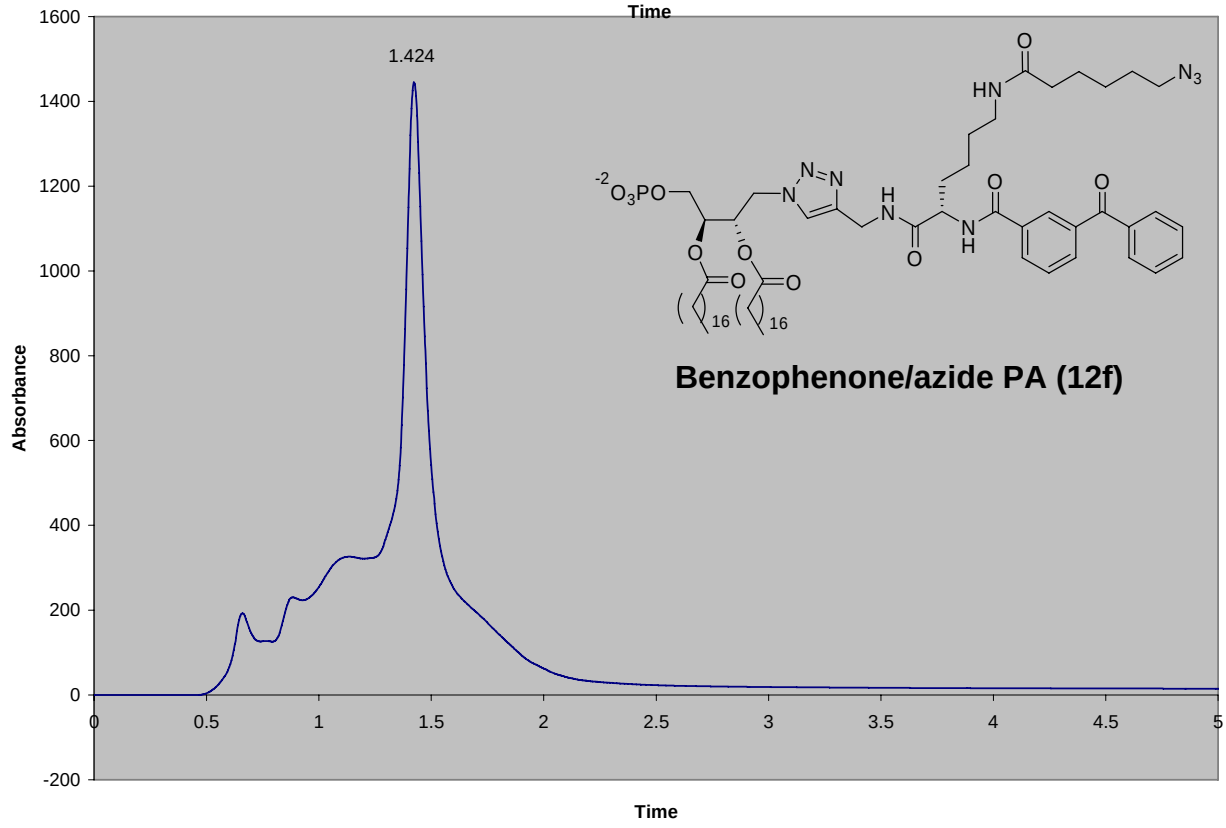





1.430
Absorbance
Time
Coumarin PA (12c)


1.504
Absorbance
Time
Rhodamine PA (12d)


1.424
Absorbance
Time
Benzophenone PA (12e)


1.424
Absorbance
Time
Benzophenone/azide PA (12f)

Raw SPR spectrograms

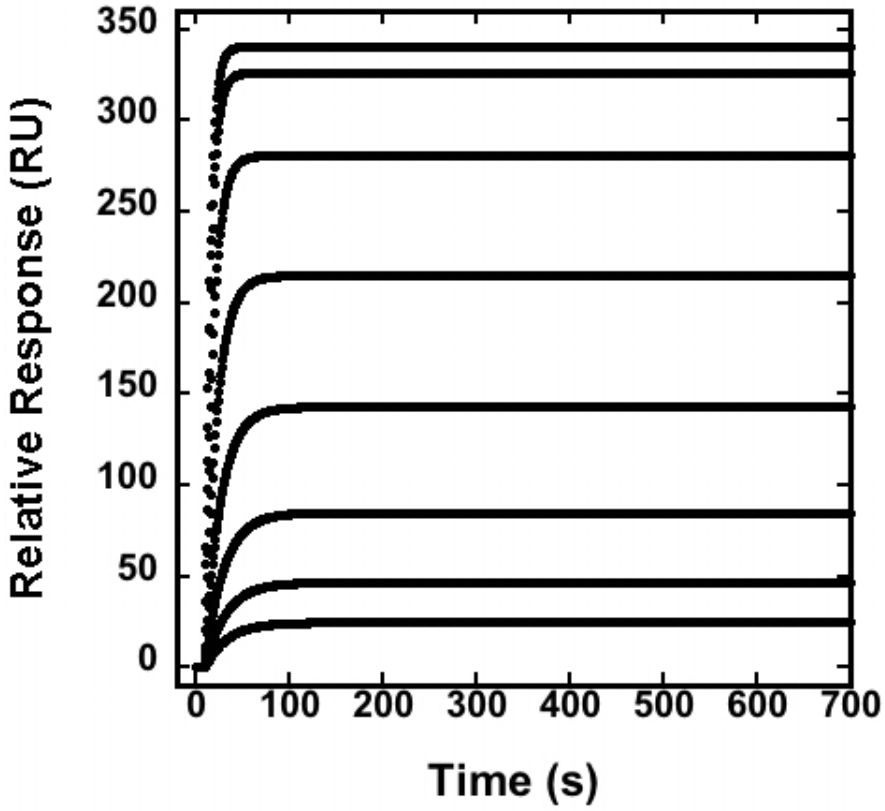


Figure S51. Equilibrium SPR analyses of PKCα-C2. The PKCα-C2 was injected at 10 μl/min at varying concentrations (50, 100, 200, 400, 800, 1500, 3000, and 4000 nM from bottom to top) over the POPC:POPE:Dansyl-PA (40:40:20) surface using a POPC:POPE (50:50) surface as a control. Subtraction of the POPC:POPE (50:50) response for each protein concentration was performed to yield the binding sensorgrams shown. R_{eq} values were then measured to determine K_d by a nonlinear least-squares analysis of the binding isotherm (See Figure 3).