Supporting Information

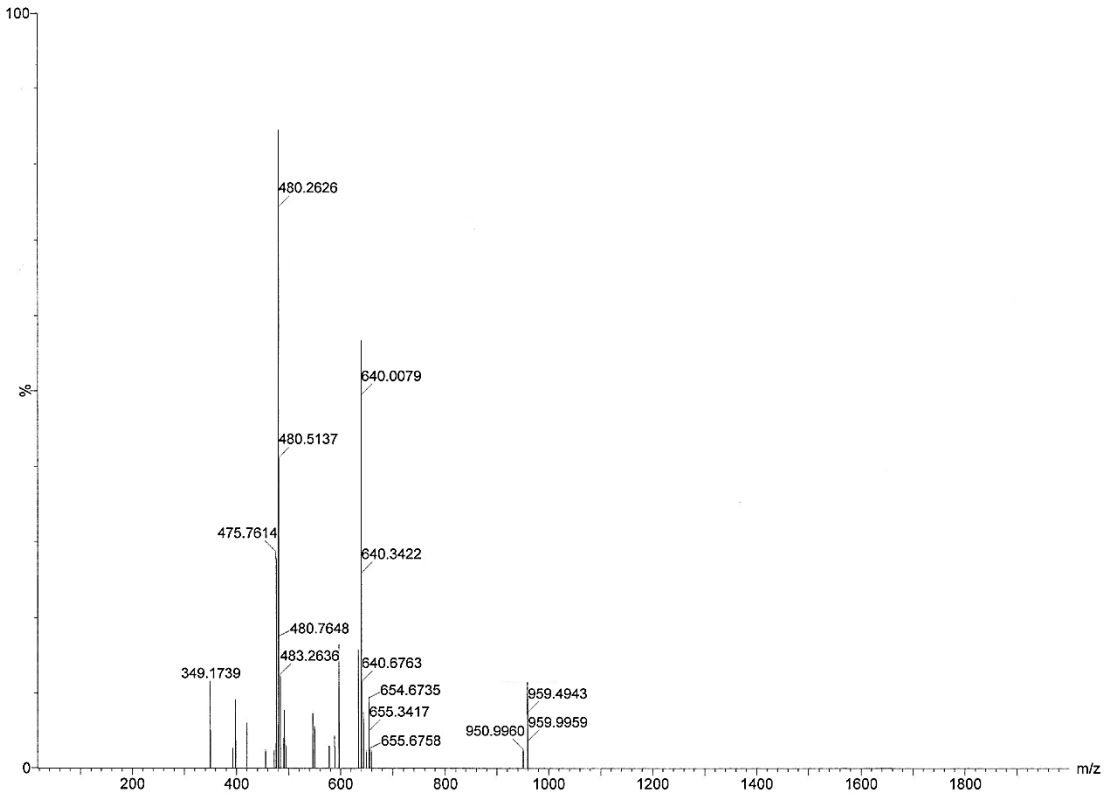
for

Development of Substrate-Selective Probes for Affinity Pulldown of Histone Demethylases

*Laura J. Marholz, Le Chang, William M. Old, and Xiang Wang**

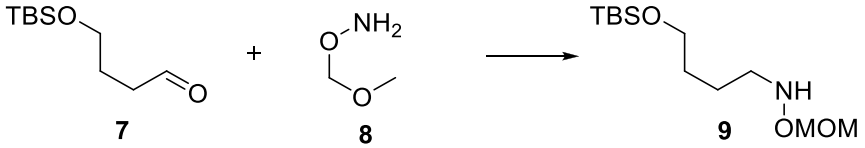
Department of Chemistry and Biochemistry, University of Colorado Boulder, Boulder, Colorado 80309, United States

*To whom correspondence should be addressed. Phone: (303) 492 6266. Fax: (303) 492 5894. Email: xiang.wang@colorado.edu



Supplementary Figure 1. High-resolution mass spectrum of peptidic probe **6**. HRMS (ESI): *m*/*z*: Calcd for $C_{89}H_{140}N_{22}O_{23}S$ $[M+4H]^{4+}$ 480.2620, Found 480.2626.

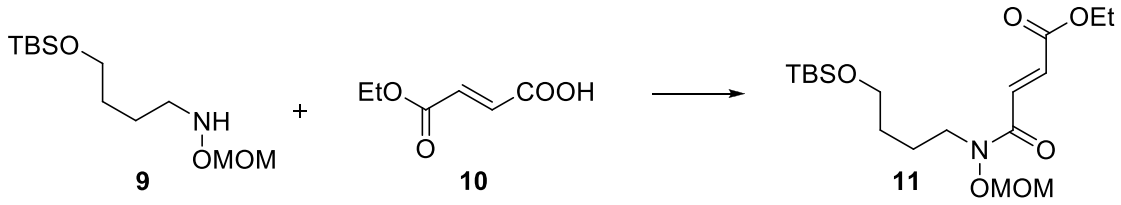
General Methods. Unless otherwise noted, reagents were obtained commercially and used without further purification. TLC analysis of reaction mixtures was performed on Dynamic adsorbents silica gel F-254 TLC plates. Flash chromatography was carried out on Zeoprep 60 ECO silica gel. ^{1}H and ^{13}C NMR spectra were recorded on Varian INOVA 300 or 500 MHz spectrometers and referenced to $CDCl_3$ or CD_3OD. Mass spectral and analytical data were obtained *via* the PE SCIEX/ABI API QSTAR Pulsar i Hybrid LC/MS/MS, Applied Biosystems operated by the Central Analytical Laboratory, University of Colorado Boulder. Infrared (IR) spectra were recorded on a Thermo Nicolet Avatar 370 FT-IR spectrometer. For the purification of peptidic probe **6**, high performance liquid chromatography was performed with a C_{18} reverse phase semi-preparative column (Beckman Ultrasphere, 235328) with solvent A (0.1% TFA in H_2O) and solvent B (0.1% TFA in CH_3CN) as eluents. The gradient used was 5-40% solvent B over 25 min with a flow rate of 5 mL/min.



***N*-(4-(*tert*-butyldimethylsilyloxy)butyl)-*O*-(methoxymethyl)hydroxylamine 9**.

Aldehyde **7** (1.629 g, 8.05 mmol, 1 eq.) was dissolved in methanol to make a 1 molar solution. The amine **8** (0.827 g, 8.05 mmol, 1.0 eq.) was also dissolved in methanol to a concentration of 1 M and slowly added dropwise to the solution of the aldehyde at 25 °C under constant stirring. The reaction was monitored by thin layer chromatography until complete conversion of the starting materials to oxime was observed (~20 min). Sodium cyanoborohydride was added (0.506 g, 8.05 mmol, 1.0 eq.) followed by addition of the acetic acid (0.461 mL, 8.05 mmol, 1.0 eq.). The reaction mixture was stirred for one hour at ambient temperature. One more equivalent of sodium cyanoborohydride and acid was added every proceeding hour for another 5 hours. The mixture was then quenched with sodium bicarbonate and the aqueous layer was extracted with ethyl acetate (3 X 20mL). The organic layer was dried over sodium sulfate, filtered, concentrated under vacuum,

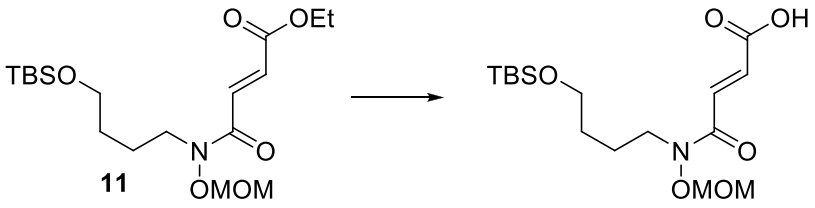
and purified by silica gel chromatography (20:1 hexanes/ethyl acetate) to give the product as a clear oil (1.085g, 4.12mmol) in 51% yield: IR (thin film) υ 3269, 2930, 2858, 1472, 1388, 1361, 1255, 1209, 1152, 1098, 1042 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$) δ 5.94 (bs, 1H), 4.76 (s, 2H), 3.65 (t, *J* = 6.0 Hz, 2H), 3.43 (s, 3H), 3.01 (t, *J* = 6.6 Hz, 2H), 1.60 (m, 4H), 0.91 (s, 9H), 0.07 (s, 6H) ppm; ^{13}C NMR (75MHz, $CDCl_3$) δ 99.3, 63.1, 55.8, 52.4, 30.5, 26.1, 23.9, 18.5, -5.2 ppm; HRMS (ESI): *m*/*z*: Calcd for $C_{12}H_{29}NO_3Si$ $[M+H]^+$ 264.1985, Found 264.1990.



(*E*)-Ethyl 4-((4-(tert-butyldimethylsilyloxy)butyl)(methoxymethoxy)amino)-4-oxobut-2-enoate 11.

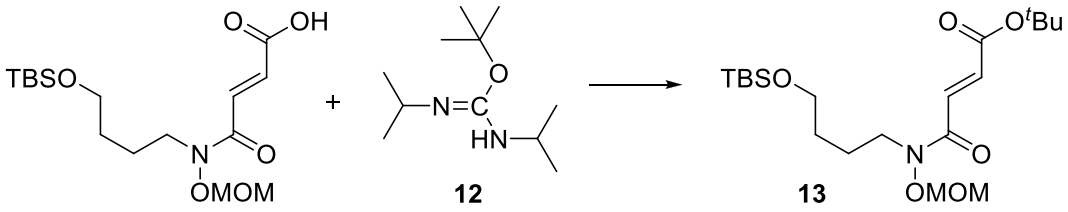
Acid **10** (0.410 g, 2.85 mmol, 1.5 eq.) was dissolved in anhydrous dichloromethane to a concentration of 0.5 M. The solution was cooled to 0 °C and the oxalyl chloride (0.482 g, 3.80 mmol, 2.0 eq.) was added dropwise followed by three drops of N,N-dimethylformamide. The reaction was allowed to warm to ambient temperature and stirred for one hour. The solvent was removed in vacuo, and the residue was redissolved in anhydrous dichloromethane to 0.5 M. A solution of the amine **9** (0.501 g, 1.898 mmol, 1.0 eq.) was prepared in anhydrous dichloromethane to 0.5 M concentration. The triethylamine (0.576 g, 5.69 mmol, 3.0 eq.) was added to the amine solution along with a catalytic amount of 4-dimethylaminopyridine. The acid chloride solution was added dropwise at 0 °C and stirred for ten minutes. The mixture was quenched with saturated sodium bicarbonate and the aqueous layer was extracted with dichloromethane (3 X 20mL) and the organic phase was dried over sodium sulfate. The solution was filtered, concentrated in vacuo, and purified by silica gel chromatography (5:1 hexanes/ethyl acetate) to give the product as a clear oil (0.623g, 1.599mmol) in 89 % yield: IR (thin film) υ 2954, 2929, 2856, 1724, 1663, 1409, 1364, 1299, 1254, 1160, 1099, 1033 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, *J* = 15.6 Hz, 1H), 6.85 (d, *J* = 15.6 Hz, 1H), 4.92

(s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 3.78 (t, J = 7.2 Hz, 2H), 3.61 (t, J = 6.2 Hz, 2H), 3.53 (s, 3H), 1.74 (m, 2H), 1.52 (m, 2H), 1.30 (t, J = 7.2 Hz, 3H), 0.87 (s, 9H), 0.03 (s, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.8, 165.2, 132.7, 132.2, 101.6, 62.7, 61.3, 58.0, 47.9, 30.1, 26.1, 23.6, 18.5, 14.3, -5.2 ppm; HRMS (ESI): *m/z*: Calcd for $C_{18}H_{35}NO_6Si$ $[M+Na]^+$ 412.2121, Found 412.2126.



(*E*)-4-((4-(*Tert*-butyldimethylsilyloxy)butyl)(methoxymethoxy) amino)-4-oxobut-2-enoic acid.

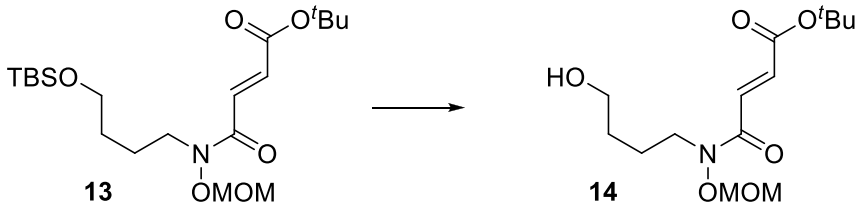
Ethyl ester **11** (1.160 g, 2.98 mmol, 1.0 eq.) was dissolved in a 9:1 mixture of tetrahydrofuran: H_2O to 0.27 M. The lithium hydroxide monohydrate (0.125 g, 2.98 mmol, 1.0 eq.) was added and the reaction mixture was stirred at room temperature for two and a half hours. The reaction was diluted with water then acidified with 2 N hydrochloric acid to a pH of 2-3. The aqueous phase was extracted with ethyl acetate (3 X 20mL) and the organic phase was dried over sodium sulfate. The salt was filtered and the solvent removed from the filtrate under vacuum to yield a clear oil that was used without further purification.



(*E*)-*Tert*-butyl 4-((4-(tert-butyldimethylsilyloxy)butyl)(methoxymethoxy)amino)-4-oxobut-2-enoate 13.

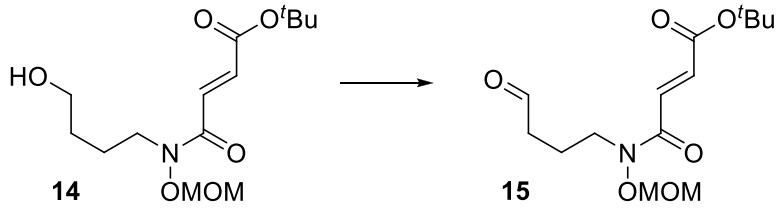
The acid (0.574 g, 1.588 mmol, 1.0 eq.) was dissolved in anhydrous 1,4-dioxane to a final concentration of 1 M. The tert-butyl *N*,*N*'-diisopropylcarbamimidate **12** (0.954 g, 4.76 mmol, 3.0 eq.) was added to the reaction and the mixture was stirred for 16 hours at

50 °C. The solid was removed by filtration and the solvent was removed in vacuo. The residue was purified by silica gel chromatography (10:1 hexanes/ethyl acetate) to yield a clear oil (0.453 g, 1.085 mmol) in 68 % yield: IR (thin film): υ 2950, 2925, 2852, 1716, 1658, 1634, 1388, 1368, 1298, 1254, 1151, 1094 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, *J* = 15.3 Hz, 1H), 6.79 (d, *J* = 15.3 Hz, 1H), 4.92 (s, 2H), 3.78 (t, *J* = 7.2 Hz, 2H), 3.62 (t, *J* = 6.3 Hz, 2H), 3.54 (s, 3H), 1.74 (m, 2H), 1.52 (m, 2H), 1.50 (s, 9H), 0.88 (s, 9H), 0.04 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6, 165.0, 134.2, 131.7, 101.6, 81.7, 62.7, 58.0, 48.0, 30.1, 28.2, 26.1, 23.6, 18.5, -5.2 ppm; HRMS (ESI): *m/z*: Calcd for $C_{20}H_{39}NO_6Si$ $[M+H]^+$ 418.2622, Found 418.2620.



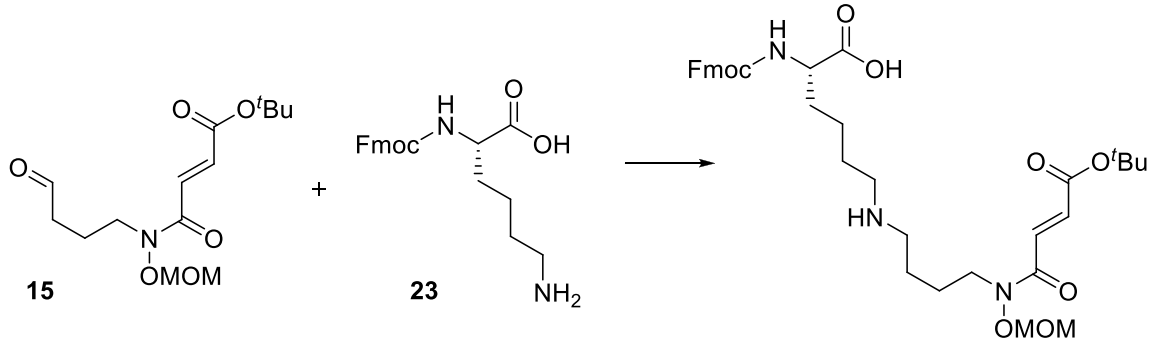
(*E*)-*Tert*-butyl 4-((4-hydroxybutyl)(methoxymethoxy)amino)-4-oxobut-2-enoate 14.

The ester **13** (0.220 g, 0.527 mmol, 1.0 eq.) was dissolved in tetrahydrofuran to a final concentration of 0.2 M. The tetrabutylammonium fluoride solution (0.207 g, 0.790 mmol, 1.5 eq) was added dropwise at 0 °C. The reaction was allowed to warm to ambient temperature and stirred 16 hours. The reaction was then stopped by the addition of water, and the aqueous layer was extracted with ethyl acetate (3 X 20mL). The organic phase was dried over sodium sulfate, and filtered. The solvent was removed in vacuo and the residue purified by silica gel chromatography (2:1 ethyl acetate/hexanes) to yield a clear oil (0.134 g, 0.442 mmol) in 96 % yield: IR (thin film): υ 3422, 2985, 2940, 2870, 1665, 1550, 1489, 1349, 1219, 1105, 913, 772 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$) δ 7.36 (d, *J* = 15.6 Hz, 1H), 6.77 (d, *J* = 15.6 Hz, 1H), 4.91 (s, 2H), 3.80 (t, *J* = 7.1 Hz, 2H), 3.65 (t, *J* = 6.3 Hz, 2H), 3.53 (s, 3H), 1.92 (bs, 1H), 1.76 (m, 2H), 1.56 (m, 2H), 1.48 (s, 9H) ppm; ^{13}C NMR (75MHz, $CDCl_3$) δ 165.7, 164.9, 134.4, 131.5, 101.6, 81.7, 62.4, 58.0, 47.9, 29.6, 28.1, 23.5 ppm; HRMS (ESI): *m/z*: Calcd for $C_{14}H_{25}NO_6$ $[M+H]^+$ 304.1758, Found 304.1755.



(*E*)-*Tert*-butyl 4-((methoxymethoxy)(4-oxobutyl)amino)-4-oxobut-2-enoate 15.

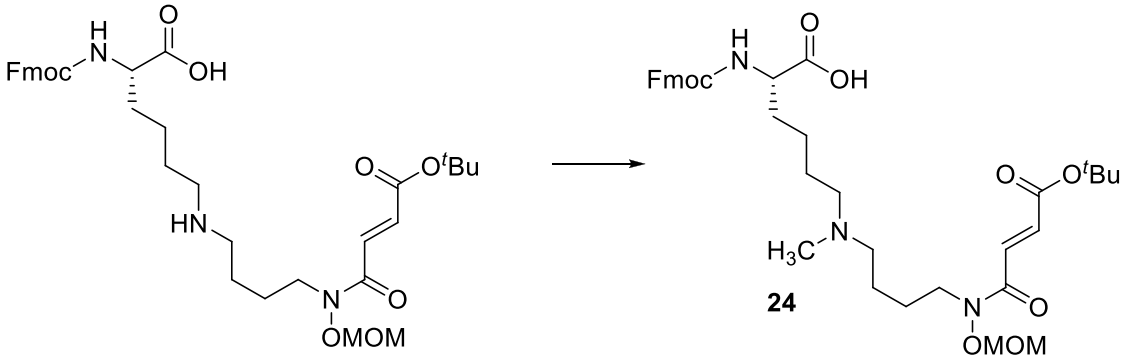
The oxalyl chloride (0.155 g, 1.220 mmol, 2.0 eq.) was dissolved in anhydrous dichloromethane to a concentration of 0.2 M. A solution of the dimethyl sulfoxide (0.191 g, 2.439 mmol, 4.0 eq.) in 10 mL anhydrous dichloromethane was added dropwise at -78 °C and the reaction was stirred for 30 min. A solution of alcohol **14** (0.185 g, 0.610 mmol, 1.0 eq.) in 5 mL anhydrous dichloromethane was added at -78 °C and the reaction was stirred for 15 min. Finally the triethylamine (0.370 g, 3.66 mmol, 6.0 eq.) was added and the reaction was allowed to warm to ambient temperature and stirred an additional 15 min. The reaction was quenched by the addition of sodium bicarbonate and water and stirred for 20 min. The aqueous layer was then extracted with dichloromethane (3 X 20mL) and the combined organic phase was dried over sodium sulfate. The salt was filtered and the solvent was removed by vacuum. The remaining byproducts were removed by filtration through a silica gel packed pipette (1:1 hexanes/ethyl acetate) to yield a clear oil that was used without further purification.



(*2S*)-6-({4-[(*2E*)-4-(*Tert*-butoxy)-*N*-(methoxymethoxy)-4-oxobut-2-enamido]butyl} amino)-2-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}hexanoic acid.

Aldehyde **15** (0.335 g, 1.112 mmol, 1.0 eq.) was dissolved in methanol to 0.03 M. The acid **23** (0.614 g, 1.668 mmol, 1.5 eq.) was added and the mixture was stirred at ambient

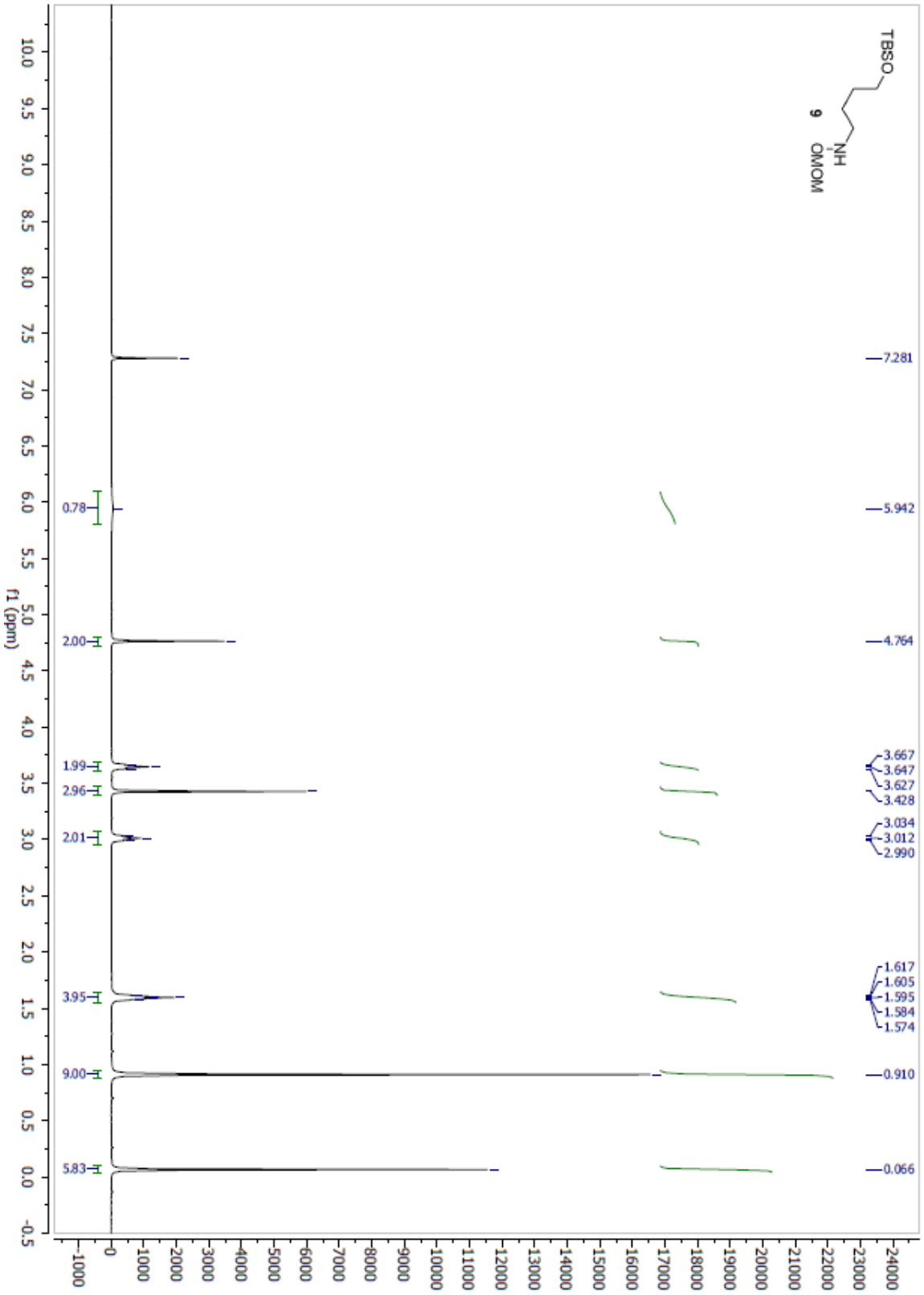
temperature for 3 hours. Then the sodium cyanoborohydride (0.140 g, 2.223 mmol, 2.0 eq.) and acetic acid (0.067 g, 1.112 mmol, 1.0 eq.) were added and the reaction was stirred at ambient temperature for 16 hours. Then the reaction was diluted with DCM and the solvent was removed under vacuum with about 5 mL residue left. The residue was purified by silica gel chromatography (100:1 to 3:1 dichloromethane/methanol) to yield a clear oil (0.428 g, 0.656 mmol) in 59 % yield. IR (thin film): υ 3318, 3042, 2937,2869,1715, 1657, 1637, 1529, 1450, 1395, 1368, 1305, 1152, 1108,1085, 978, 925, 740 cm^{-1}; [α] = 6.7 (c = 1.0 g/L, 23 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.63 (t, *J* = 8.4 Hz, 2H), 7.43–7.35 (m, 3H), 7.30 (dd, *J* = 8.0, 6.8 Hz, 2H), 6.79 (d, *J* = 15.5 Hz, 1H), 6.01 (d, *J* = 5.8 Hz, 1H), 4.90 (s, 2H), 4.39–4.28 (m, 2H), 4.21 (t, *J* = 7.2 Hz, 1H), 4.14 (d, *J* = 5.1 Hz, 1H), 3.78 (s, 2H), 3.52 (s, 3H), 3.00–2.76 (m, 4H), 1.99–1.92 (m, 1H), 1.84 (ddd, *J* = 13.7, 9.3, 4.6 Hz, 1H), 1.76–1.61 (m, 7H), 1.50 (s, 9H), 1.47 (m, 1H); ^{13}C NMR (75MHz, $CDCl_3$) δ 176.5, 165.8, 164.7, 155.6, 144.2, 144.1, 141.3, 134.5, 131.1, 127.6, 127.0, 127.0, 125.3, 125.2, 120.0, 101.6, 81.7, 66.5, 57.9, 2,56.1, 55.1, 47.3, 28.0, 24.8, 24.1, 22.1, 20.7; HRMS (ESI): *m/z*: Calcd for $C_{35}H_{48}N_3O_9$ $[M+H]^+$ 654.3385, Found 654.3385.



(*S,E*)-15-(((*9H*-Fluoren-9-yl)methoxy)carbonylamino)-5-(4-tert-butoxy-4-oxobut-2-enoyl)-10-methyl-2,4-dioxa-5,10-diazahexadecan-16-oic acid 24.

To a solution of the acid (0.727 g, 1.112 mmol, 1.0 eq.) in methanol was added CH_2O (2.0 eq., 37% in water solution). Then the mixture was stirred at ambient temperature for 30 min followed by the addition of the sodium cyanoborohydride (0.140 g, 2.223 mmol, 2.0 eq.) and acetic acid (1.0 eq.). After the reaction was completed (monitored by LC/MS), most of the solvent was removed by vacuum. Water was then added and the

reaction was diluted with ethyl acetate. The aqueous layer was extracted with ethyl acetate (3 X 20mL) and the organic layer was dried over sodium sulfate. Then removal of the solvent by vacuum yielded an oil (0.713 g, 1.068 mmol) in 96 % yield without further purification. IR (thin film): υ 3401, 3054, 2939, 1715, 1659, 1634, 1451, 1394, 1368, 1306, 1258, 1153, 1082, 978, 926, 761, 741 cm^{-1}; [α] = 7.9 (c = 1.0 g/L, 23 °C); ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (d, *J* = 7.5 Hz, 2H), 7.67 (dd, *J* = 11.1, 7.5 Hz, 2H), 7.48–7.37 (m, 3H), 7.31 (t, *J* = 7.4 Hz, 2H), 6.68 (d, *J* = 15.6 Hz, 1H), 4.98 (s, 2H), 4.36 (qd, *J* = 10.6, 7.0 Hz, 2H), 4.22 (t, *J* = 6.9 Hz, 1H), 4.15 (s, 1H), 3.83 (t, *J* = 6.4 Hz, 2H), 3.54 (s, 3H), 3.18–3.06 (m, 4H), 2.82 (s, 3H), 1.92 (m, 1H), 1.80–1.71 (m, 8H), 1.50 (s, 9H), 1.46 (m, 1H); ^{13}C NMR (75MHz, $CDCl_3$) δ 176.5, 164.6, 155.6, 144.2, 144.0, 141.2, 134.5, 131.0, 127.6, 127.0, 125.3, 125.2, 119.9, 101.6, 81.7, 66.4, 57.9, 56.0, 55.1, 48.5, 47.2, 32.4, 29.7, 28.0, 24.6, 24.0, 22.0, 20.6; HRMS (ESI): *m/z*: Calcd for $C_{36}H_{50}N_3O_9$ $[M+H]^+$ 668.3542, Found 668.3539.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1.595
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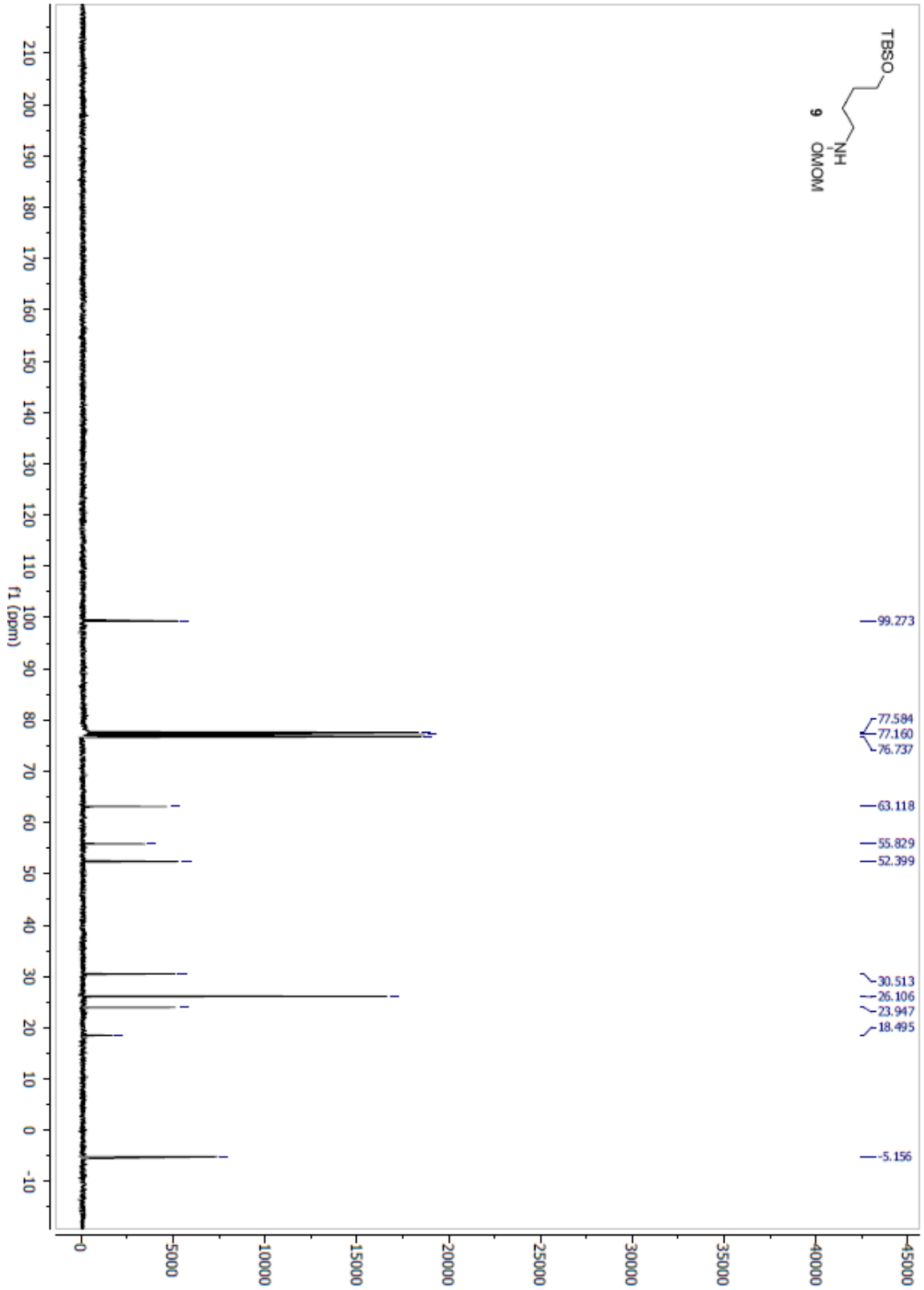
f1 (ppm)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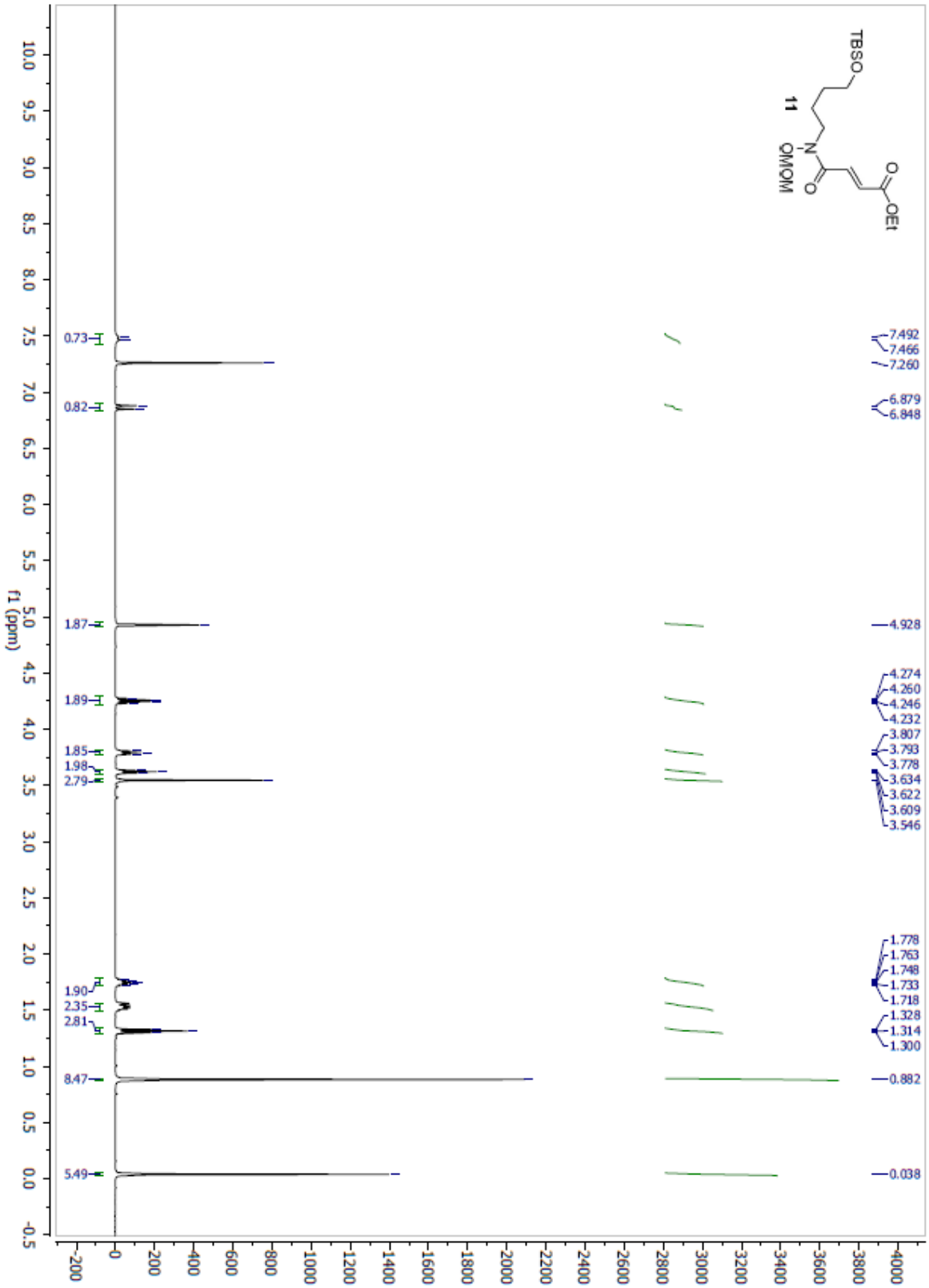
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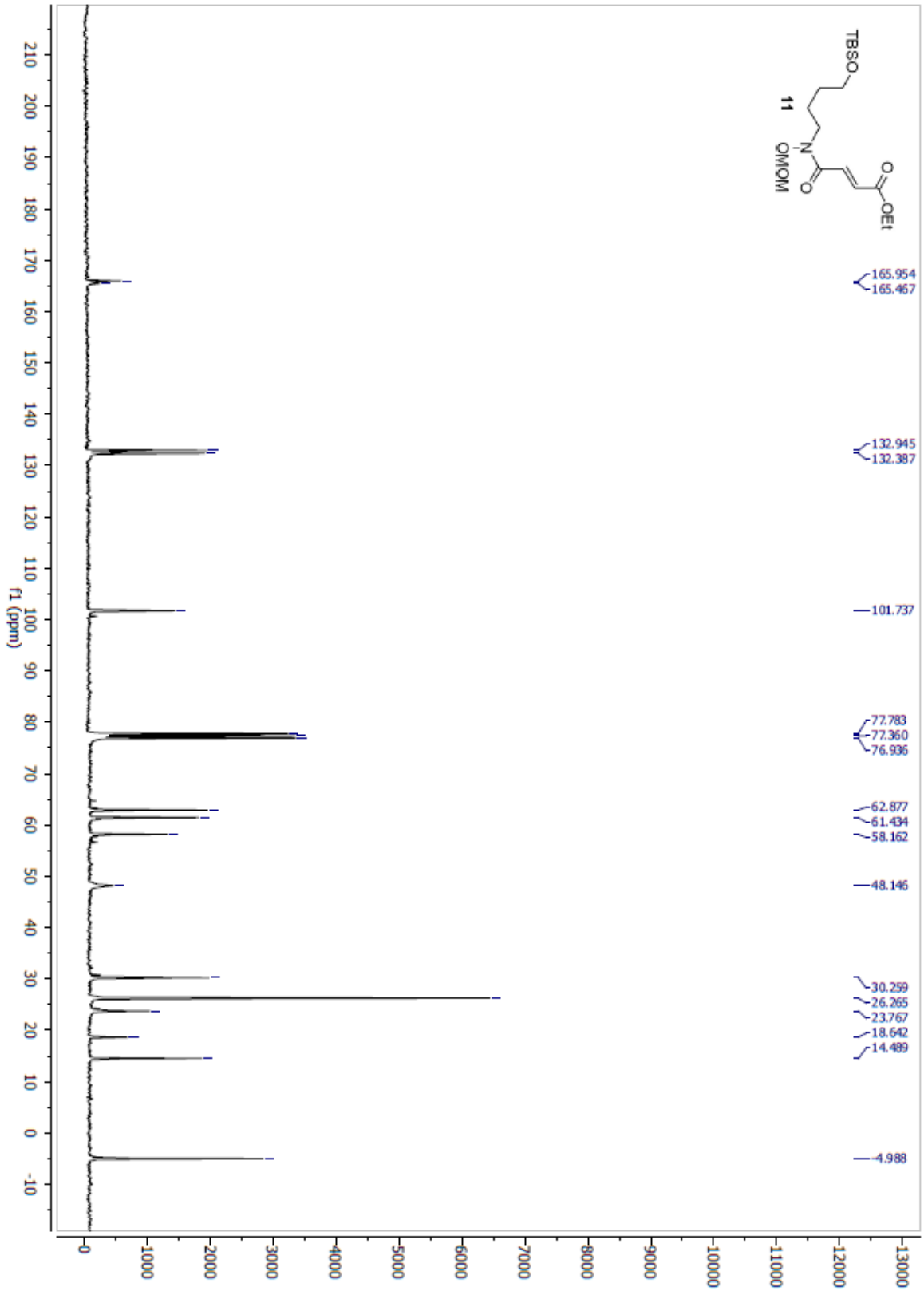
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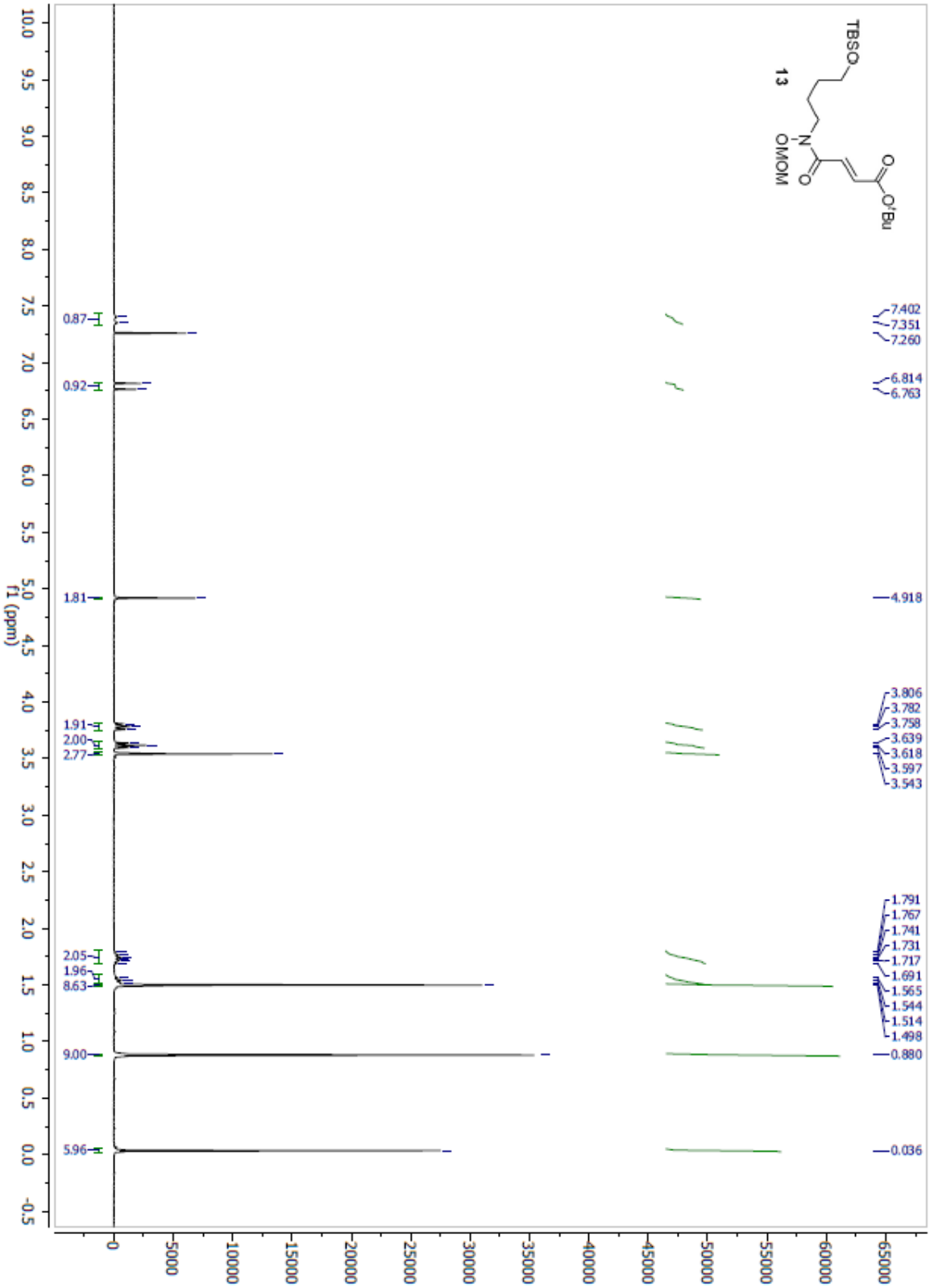
f1 (ppm)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OMOM
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O
O'Bu
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5.96
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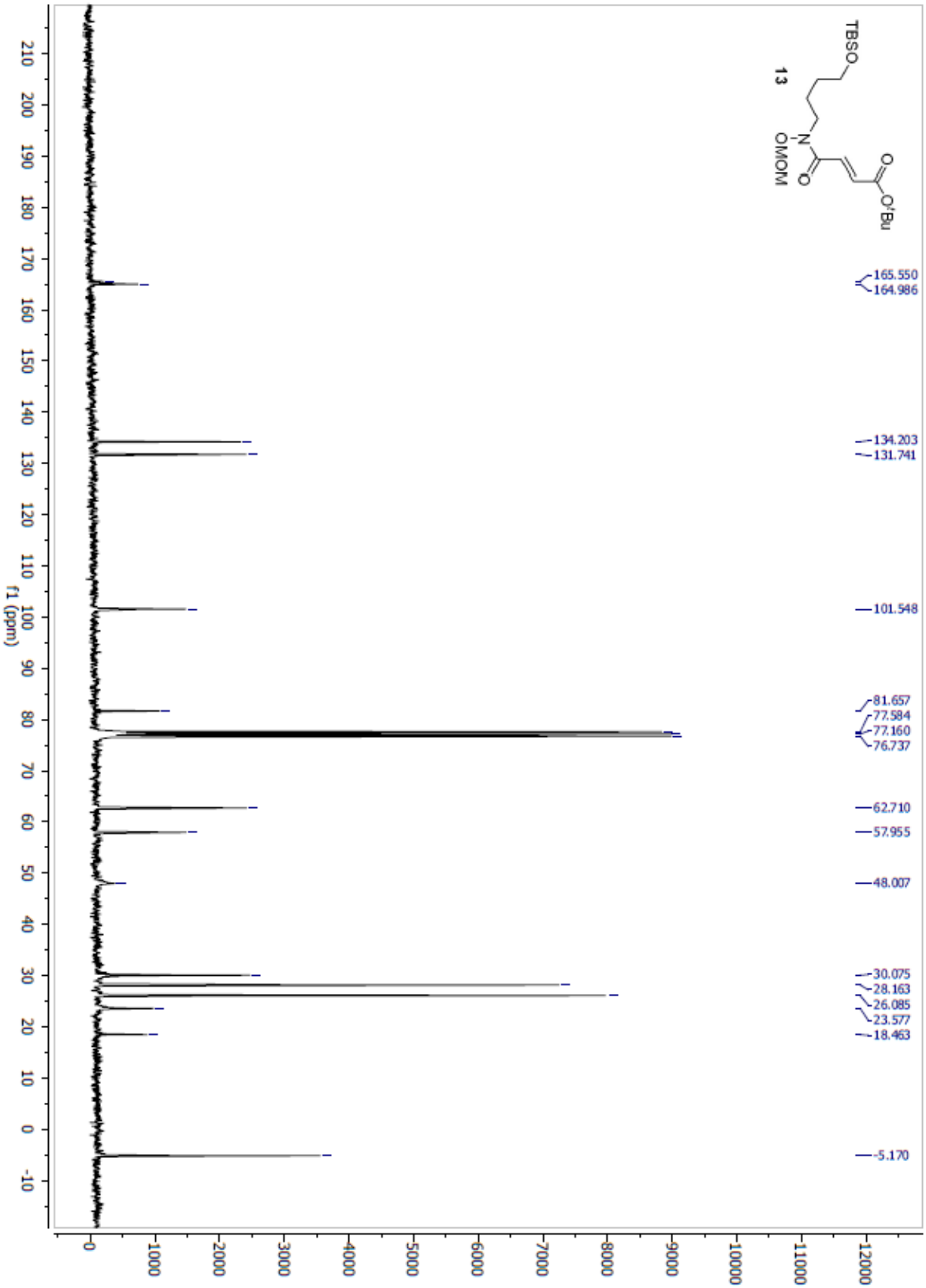
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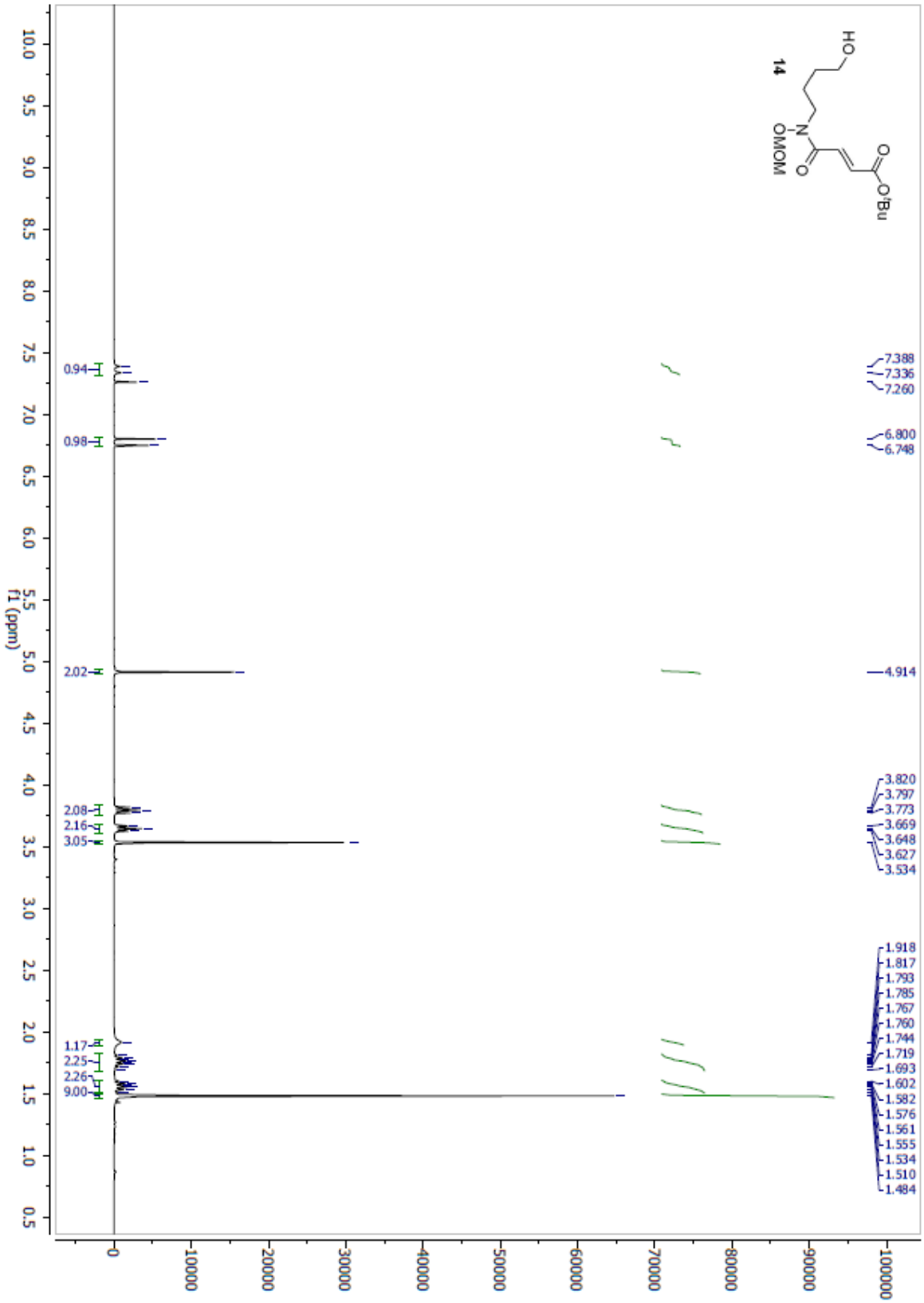
f1 (ppm)


14
HO
N
OMOM
O
O
O'Bu
f1 (ppm)
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2.08
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2.25
2.26
9.00
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7.336
7.260
6.800
6.748
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3.820
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3.773
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1.760
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1.582
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1.561
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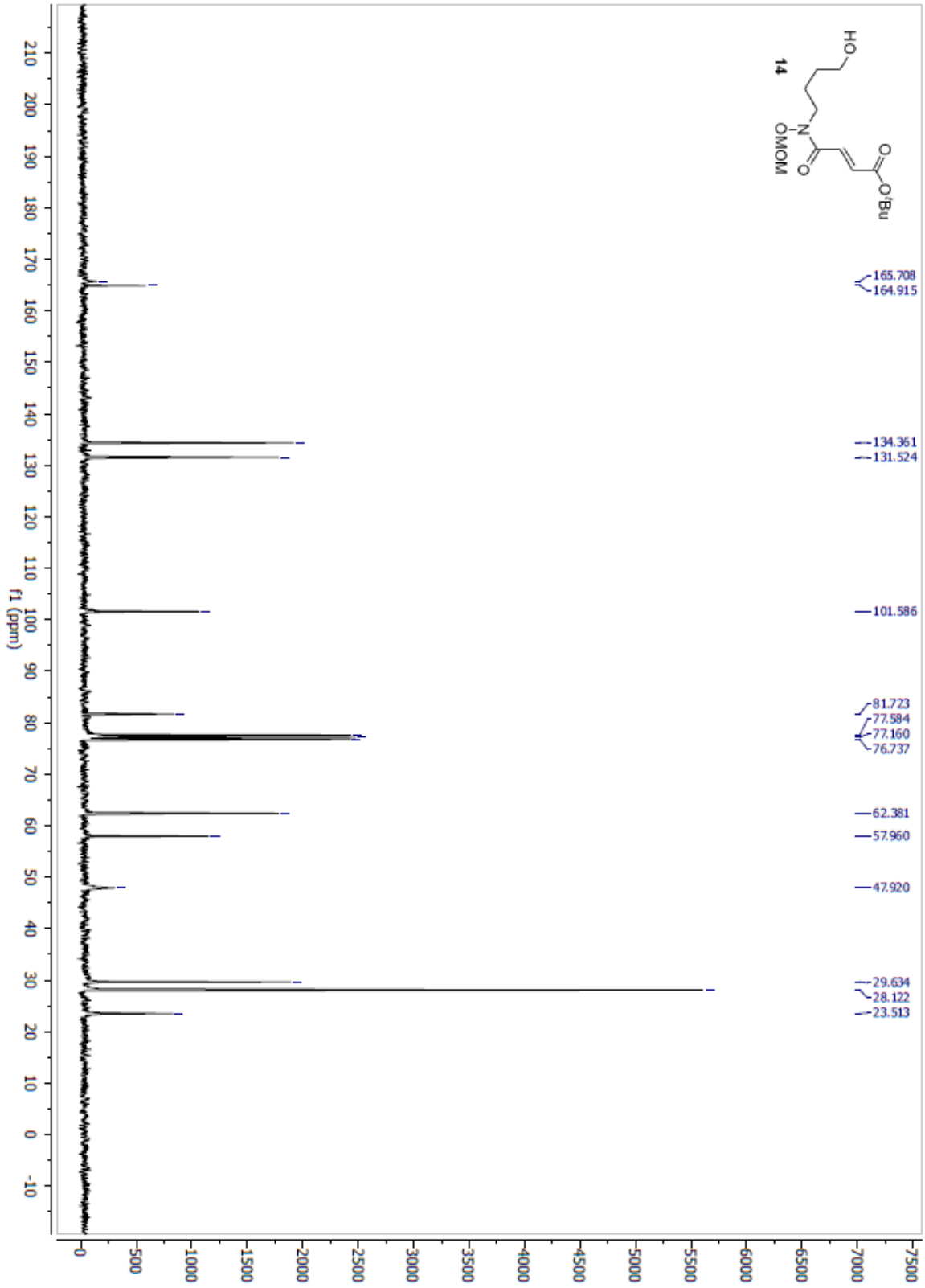
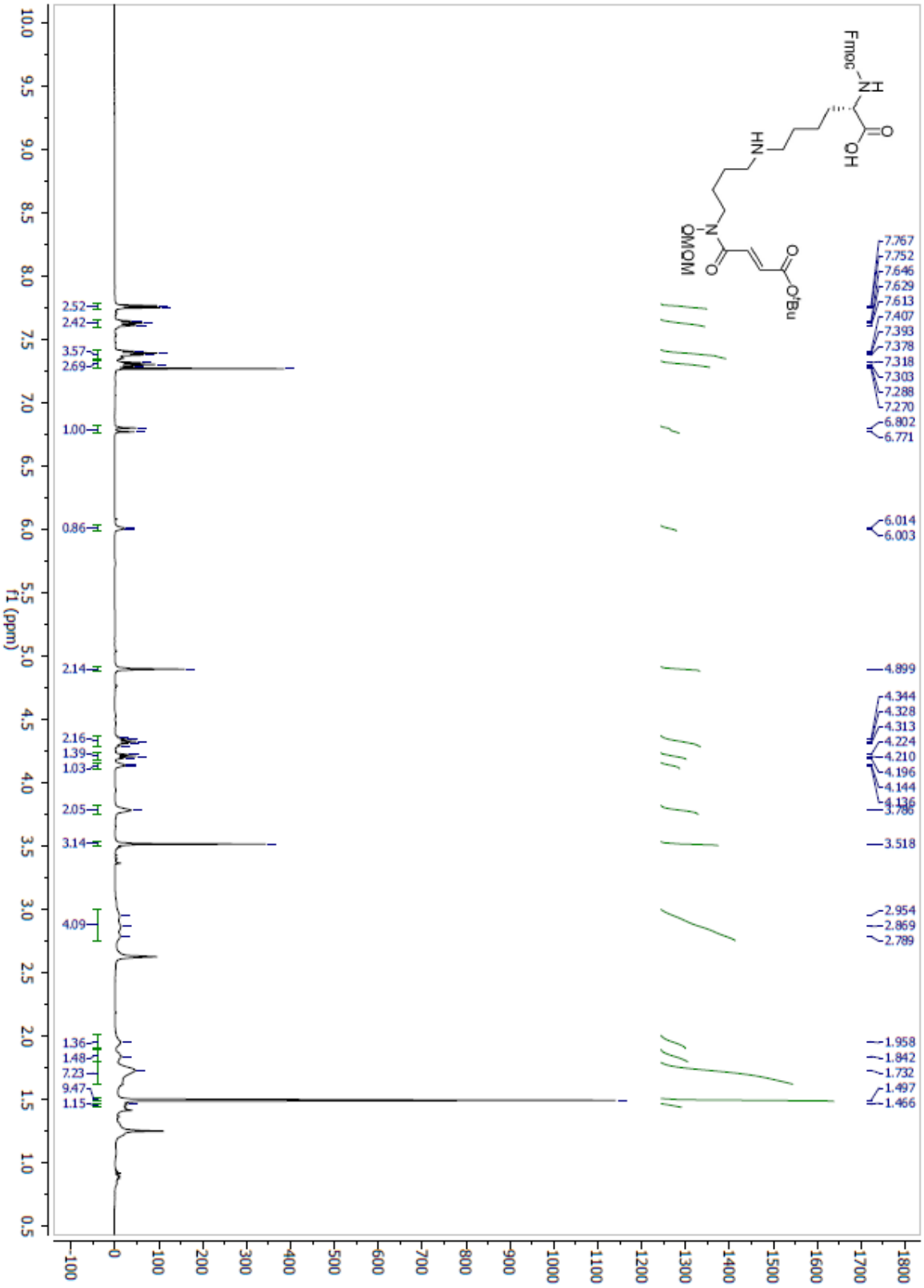
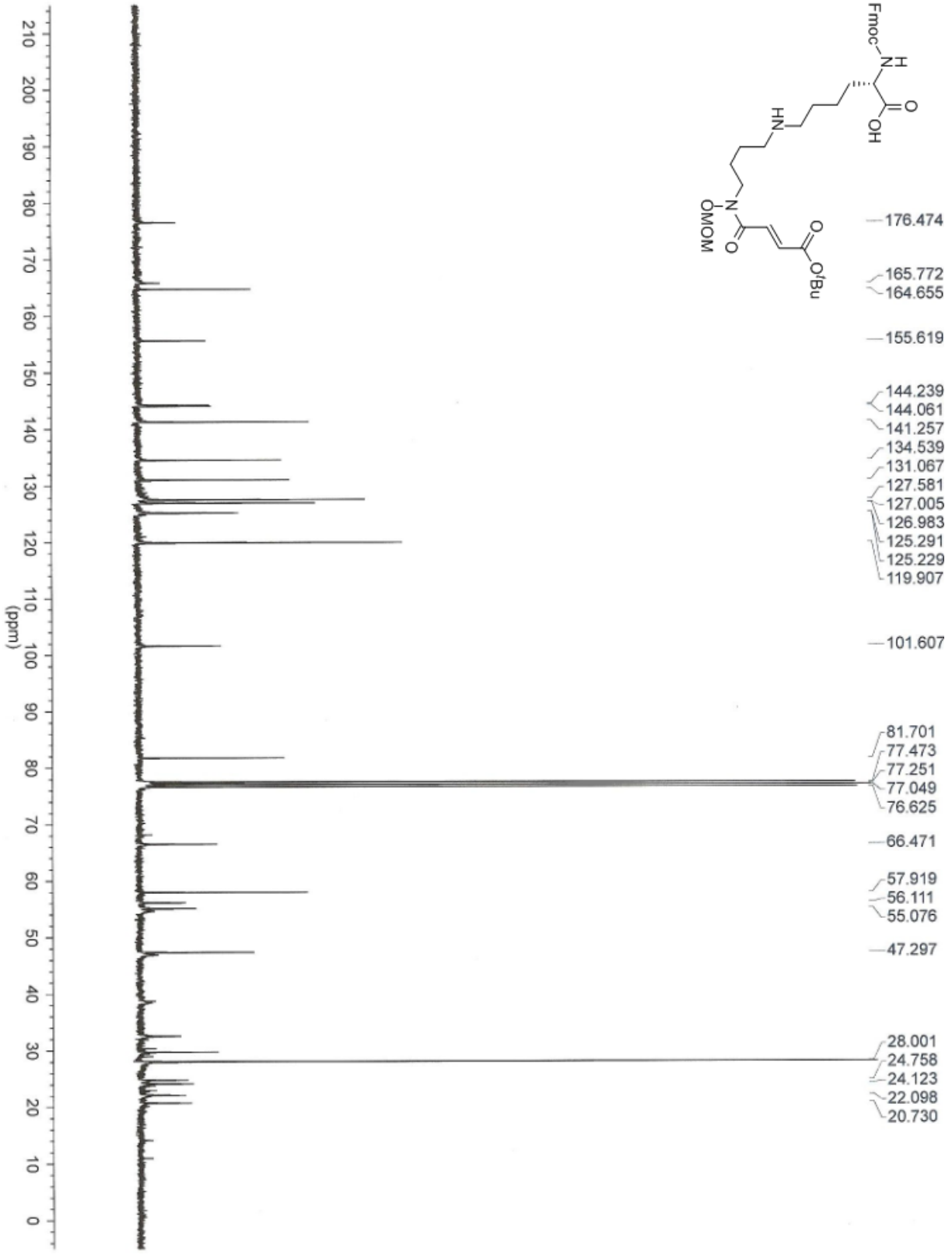
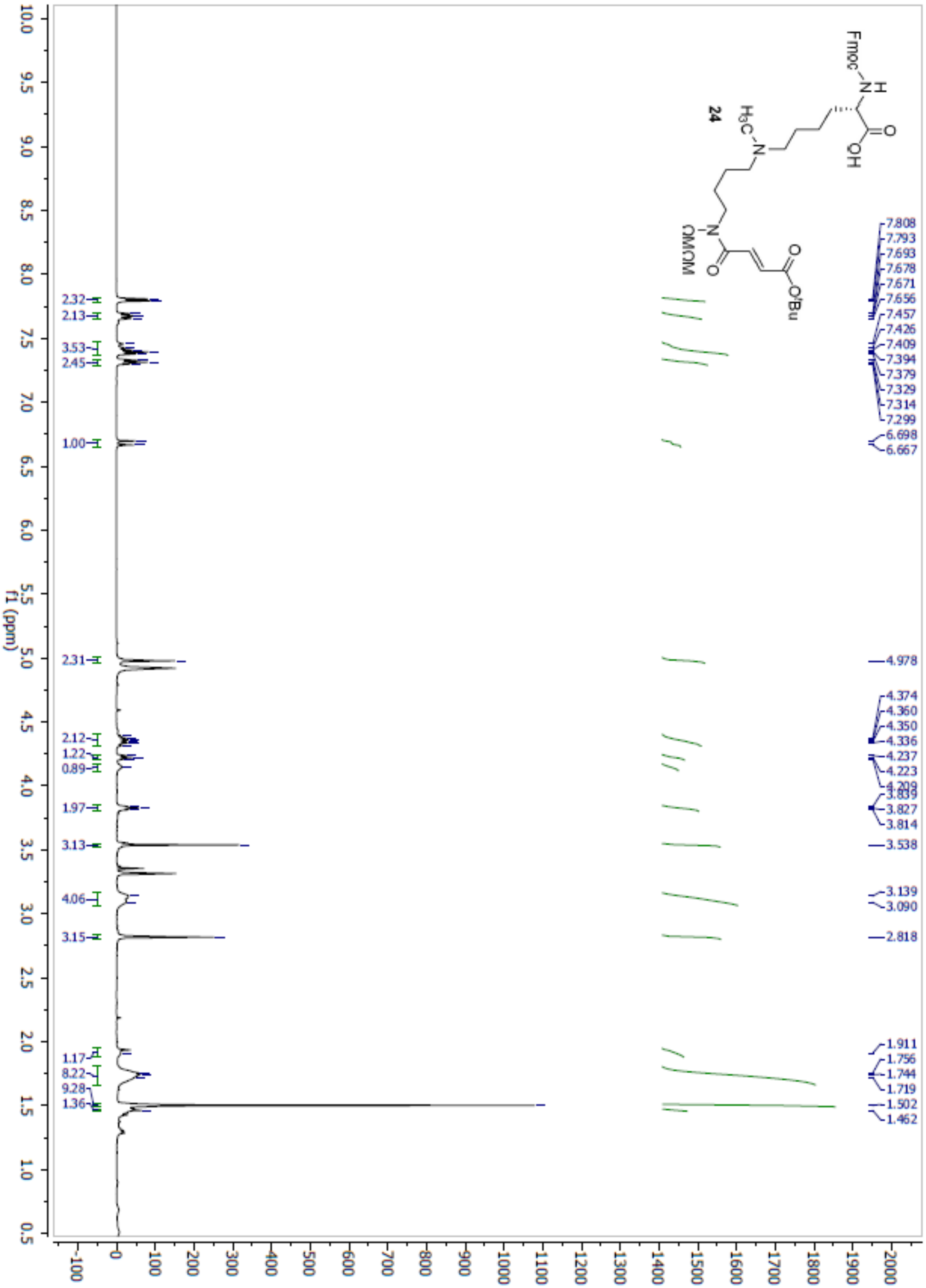
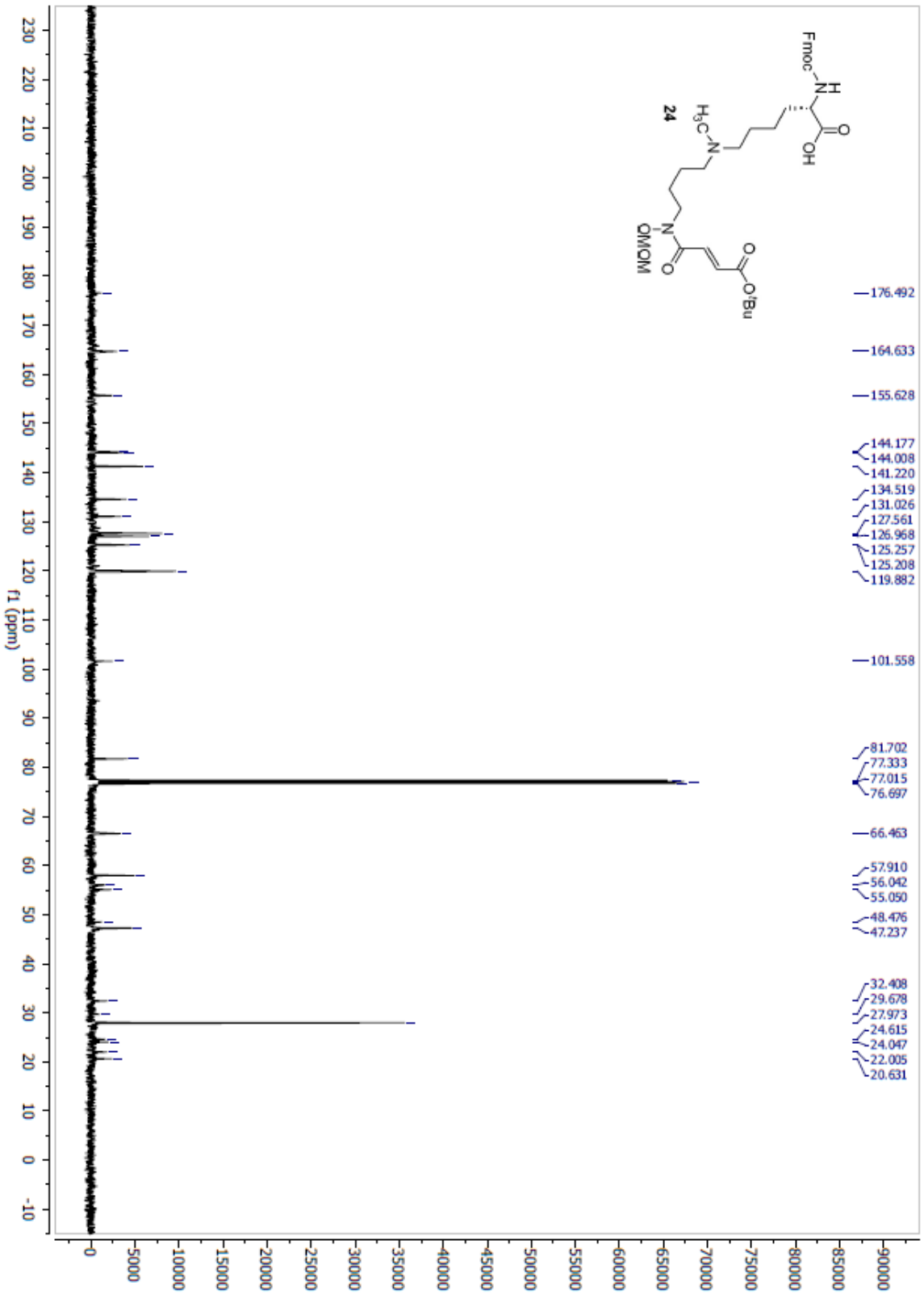
1.510
1.484


14
HO
N
OMOM
O
O
O
tBu
165.708
164.915
134.361
131.524
101.586
81.723
77.584
77.160
76.737
62.381
57.960
47.920
29.634
28.122
23.513
f1 (ppm)


Fmoc
NH
O
OH
HN
N
OMOM
O
O
O'Bu
7.767
7.752
7.646
7.629
7.613
7.407
7.393
7.378
7.318
7.303
7.288
7.270
6.802
6.771
6.014
6.003
4.899
4.344
4.328
4.313
4.224
4.210
4.196
4.144
4.126
3.786
3.518
2.954
2.869
2.789
1.958
1.842
1.732
1.497
1.466
2.52
2.42
3.57
2.69
1.00
0.86
2.14
2.16
1.39
1.03
2.05
3.14
4.09
1.36
1.48
7.23
9.47
1.15
f1 (ppm)


Fmoc
N
H
O
OH
HN
N
O
OMOM
O
O
tBu
176.474
165.772
164.655
155.619
144.239
144.061
141.257
134.539
131.067
127.581
127.005
126.983
125.291
125.229
119.907
101.607
81.701
77.473
77.251
77.049
76.625
66.471
57.919
56.111
55.076
47.297
28.001
24.758
24.123
22.098
20.730
(ppm)


Fmoc
OH
H3C
24
OMOM
O'Bu
7.808
7.793
7.693
7.678
7.671
7.656
7.457
7.426
7.409
7.394
7.379
7.329
7.314
7.299
6.698
6.667
4.978
4.374
4.360
4.350
4.336
4.237
4.223
4.209
3.839
3.827
3.814
3.538
3.139
3.090
2.818
1.911
1.756
1.744
1.719
1.502
1.462
2.32
2.13
3.53
2.45
1.00
2.31
2.12
1.22
0.89
1.97
3.13
4.06
3.15
1.17
8.22
9.28
1.36
f1 (ppm)


Fmoc
N
H
O
OH
H3C
N
24
N
OMOM
O
O
O'Bu
176.492
164.633
155.628
144.177
144.008
141.220
134.519
131.026
127.561
126.968
125.257
125.208
119.882
101.558
81.702
77.333
77.015
76.697
66.463
57.910
56.042
55.050
48.476
47.237
32.408
29.678
27.973
24.615
24.047
22.005
20.631
f1 (ppm)