Supporting Information for

Unraveling cholesterol catabolism in *Mycobacterium tuberculosis*: the ChsE4-ChsE5 $\alpha_2\beta_2$ acyl-CoA dehydrogenase initiates β-oxidation of 3-oxo-cholest-4-en-26-oyl CoA

Meng Yang,[1] Rui Lu,[1] Kip E. Guja,[2] Matthew F. Wipperman,[1] Johnna R. St. Clair,[3] Amber C. Bonds,[2] Miguel Garcia-Diaz,[2] and Nicole S. Sampson[1,*]

[1]Department of Chemistry, Stony Brook University, Stony Brook, NY 11794-3400

[2]Department of Pharmacological Sciences, Stony Brook University, Stony Brook, NY 11794-8651

[3]Biochemistry and Structural Biology Graduate Program, Stony Brook University, Stony Brook, NY 11794-5215

Table of Contents

Table S1. Gene constructs used in this study.

construct	vector	gene	gene name	*His_6 tag*	*selection*	*source or ref*
p26N/27	*pET28b*	*Rv3504/Rv3505*	*ChsE4/ChsE5*	*N-terminal*	*Kan*	[1]
p26N	*pET28b*	*Rv3504*	*ChsE4*	*N-terminal*	*Kan*	*This work*
p27	*pET20b*	*Rv3505*	*ChsE5*	*no tag*	*Amp*	*This work*
p34N	*pET28b*	*Rv3573c*	*ChsE3*	*N-terminal*	*Kan*	*This work*
$p26N_{E247A}$/27	*pET28b*	*Rv3504/Rv3505*	*ChsE4/ChsE5*	*N-terminal*	*Kan*	*This work*

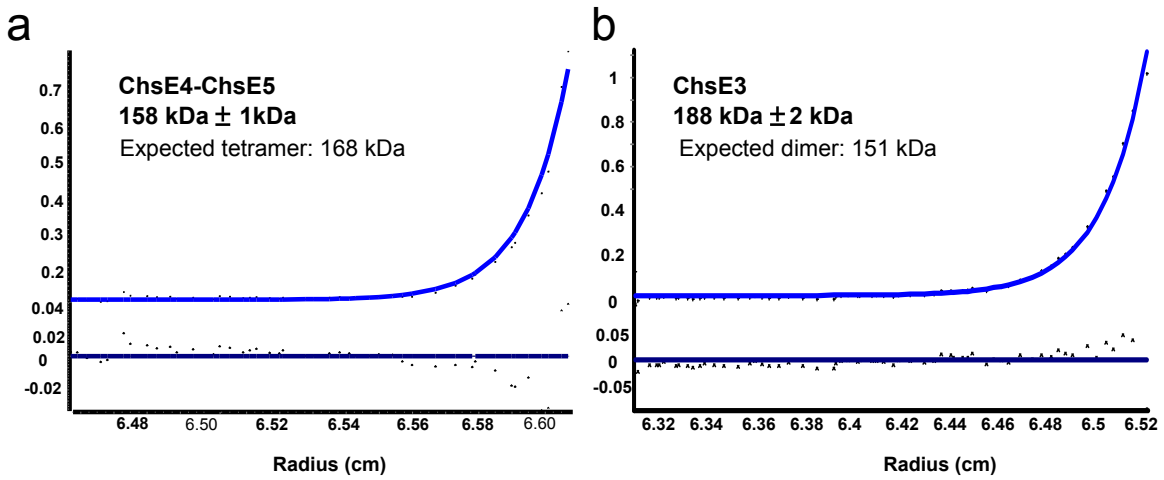


Figure S1. Analytical ultracentrifugation (AUC) sedimentation equilibrium analysis. (a) ChsE4-ChsE5 (5.18 μM, 2.28 μM, and 1.08 μM) and (b) ChsE3 (6.09 μM, 3.05 μM, and 1.52 μM) were centrifuged at speeds of 20k, 25k, and 30k rpm at 20° C in an analytical ultracentrifuge. The solid line (*light blue*) shows the fit of the data to the ideal species model and the residuals of the fit are graphed below the fit (*dark blue*). The best global fit for each protein is shown. Experimentally determined molecular weights are shown for each sample.

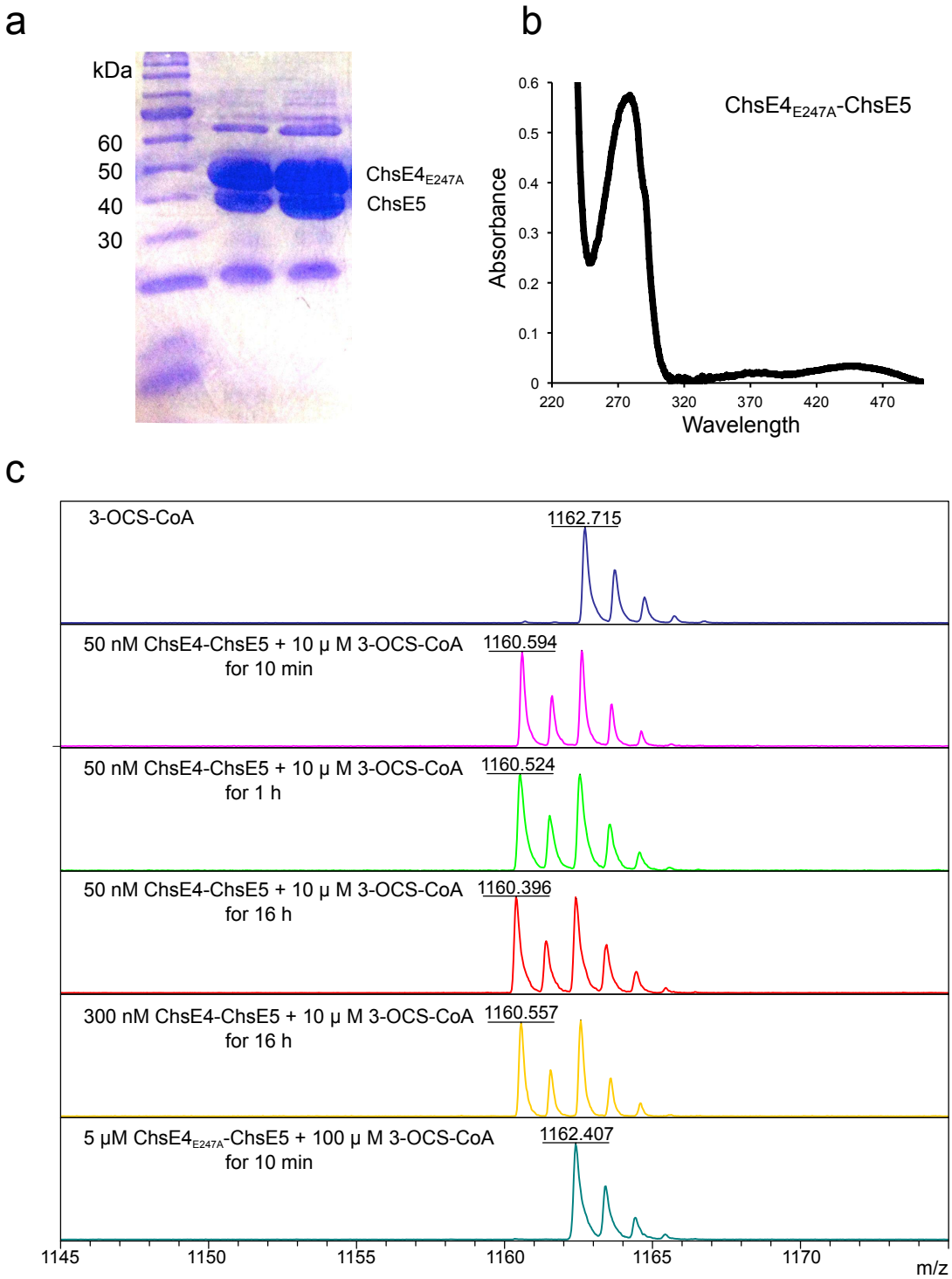


Figure S2. Purification of the ChsE4$_{E247A}$-ChsE5 mutant and its catalytic activity with 3-OCS-CoA; analysis of product formation catalyzed by ChsE4-ChsE5 with 3-OCS-CoA as the substrate. (a) SDS-PAGE gel analysis of the purified ChsE4$_{E247A}$-ChsE5 (b) The UV-visible spectrum of the purified ChsE4$_{E247A}$-ChsE5 showing the characteristic FAD absorbance. (c) Analysis of the product formation of ChsE4-ChsE5 with 3-OCS-CoA diastereomers as a function of time and increased enzyme concentration. The spectra revealed that the percent substrate conversion remained fixed at a 1:1 ratio of product to substrate.

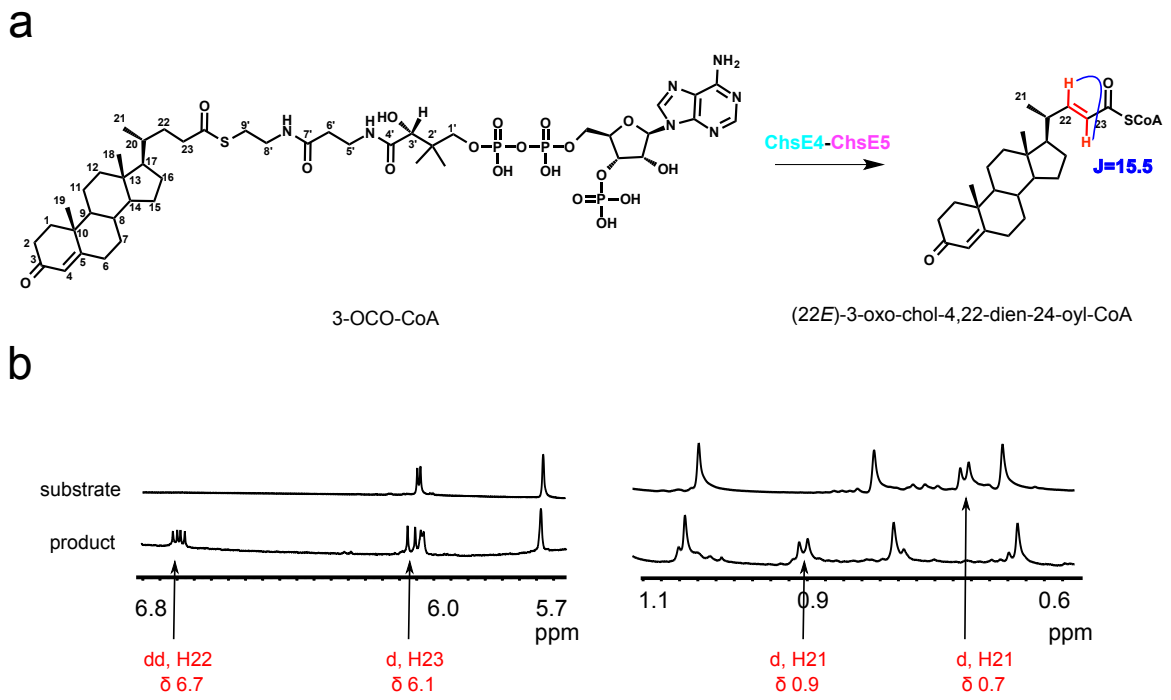


Figure S3. The dehydrogenation of 3-OCO-CoA. (a) ChsE4-ChsE5 catalyzes the formation of (22*E*)-3-oxo-chol-4,22-dien-24-oyl-CoA, (b) as determined by the observed chemical shift and coupling constant. The C22 and C23 olefinic protons appear at 6.7 and 6.1 ppm as doublets in the product spectrum (panel b, left), and the double bond induces a downfield shift of the C20 proton and C21 methyl proton resonances (panel b, right).

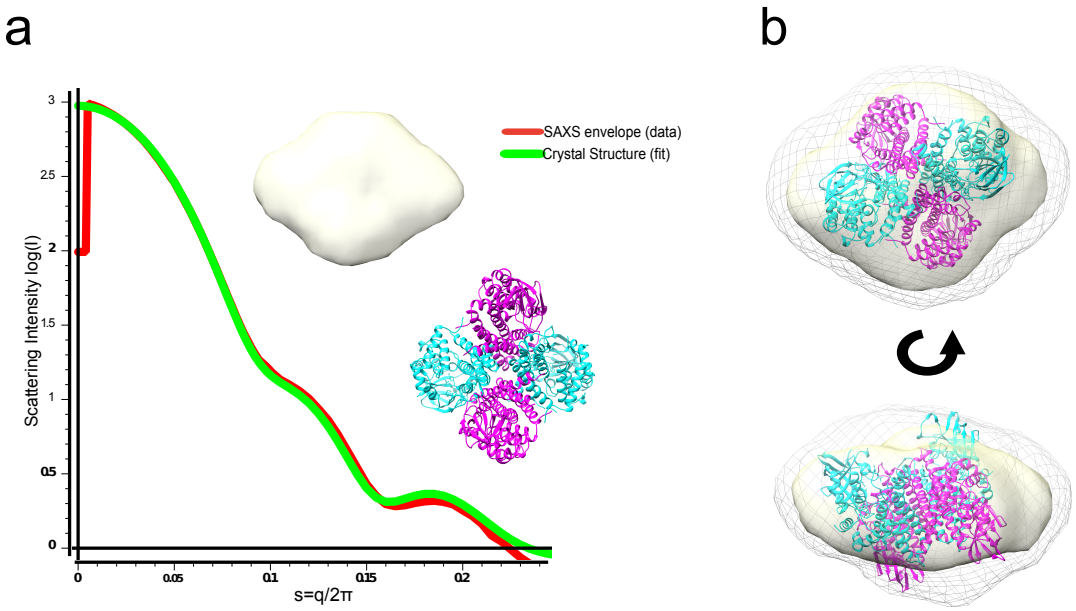


Figure S4. Small Angle X-ray Scattering (SAXS) generates a tetrameric envelope for solution state ChsE4-ChsE5. Small-angle X-ray scattering was used to determine the envelope of the solution state ChsE4-ChsE5 protein complex. (a) The theoretical scattering profile of the ChsE4-ChsE5 crystal structure, defined as scattering intensity (I), versus scattering vector (s) was calculated with CRYSOL and fit to the experimentally determined scattering profile for ChsE4-ChsE5 in solution, and the Chi-value (the square root of Chi squared) is 2.8. (b) The molecular envelope of solution state ChsE4-ChsE5 was determined from the experimental scattering profile using the ASTAS package. Then the high-resolution crystal structure was subsequently docked into the molecular envelope in Chimera, and the correlation coefficient is 0.90.

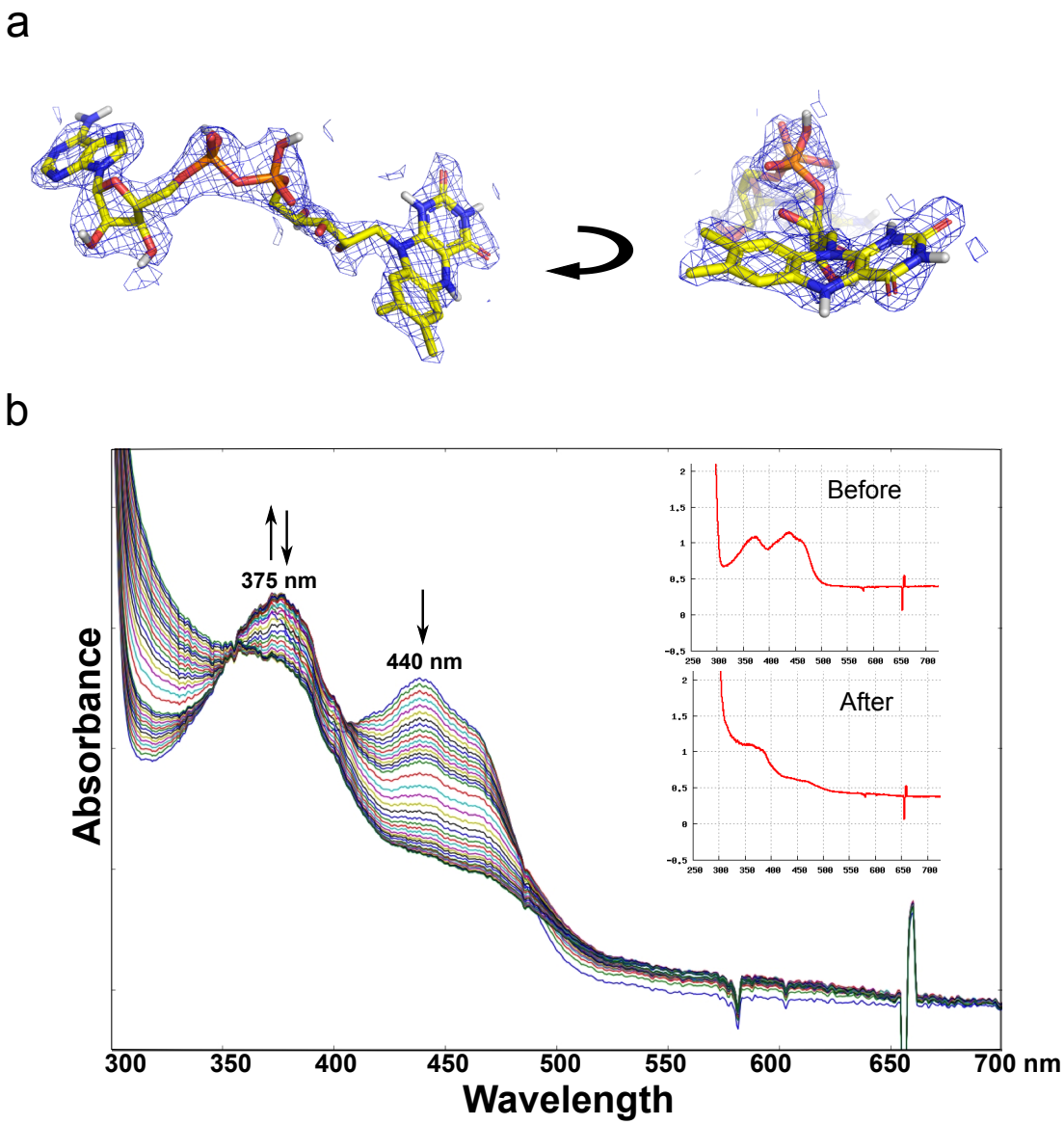


Figure S5. The FAD cofactor is reduced upon X-ray irradiation. To investigate the potential reduction of FAD cofactor during our X-ray diffraction data collection, we collected a full dataset of X-ray diffraction with simultaneous optical spectroscopy as a function of X-ray exposure on beamline X26-C of the NSLS. The optical absorption spectra were collected during the readout of each X-ray diffraction image and an overlay of spectra during the diffraction data collection was constructed. (a) A simulated annealing *Fo-Fc* omit map for the bound cofactor was calculated to reduce the effects of model bias. The electron density was contoured at 2.5 σ and displayed as blue mesh. The polar hydrogens are shown to clearly represent the oxidation state, but they are not present in the deposited coordinates. (b) Correlated absorption spectroscopy data for the ChsE4-ChsE5 crystal were collected as a function of X-ray exposure. The spectra were overlayed and the changes at 440 nm and 375 nm are shown. Inset. two spectra, one at the beginning and one at the end of X-ray exposure, were selected to highlight the change in absorbance.

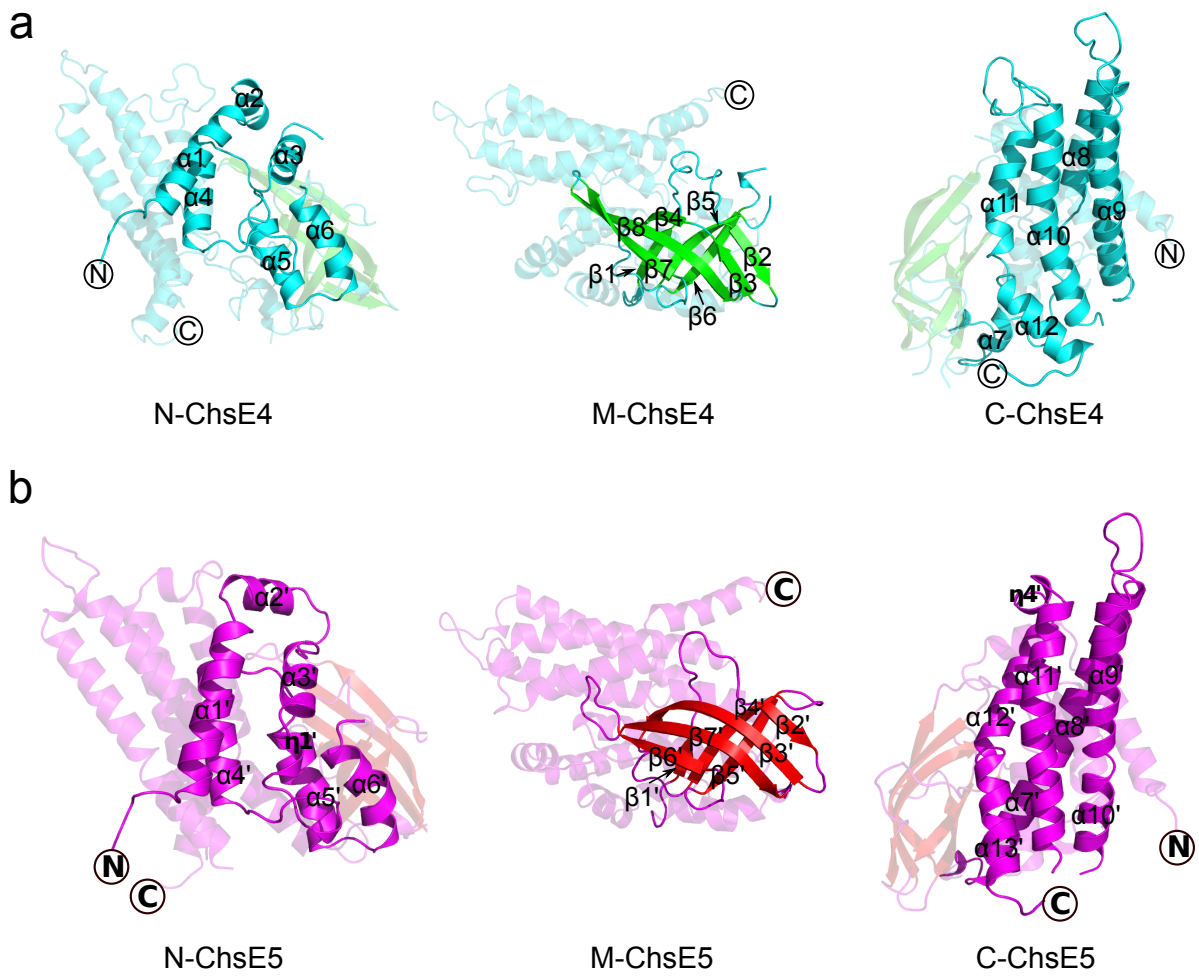
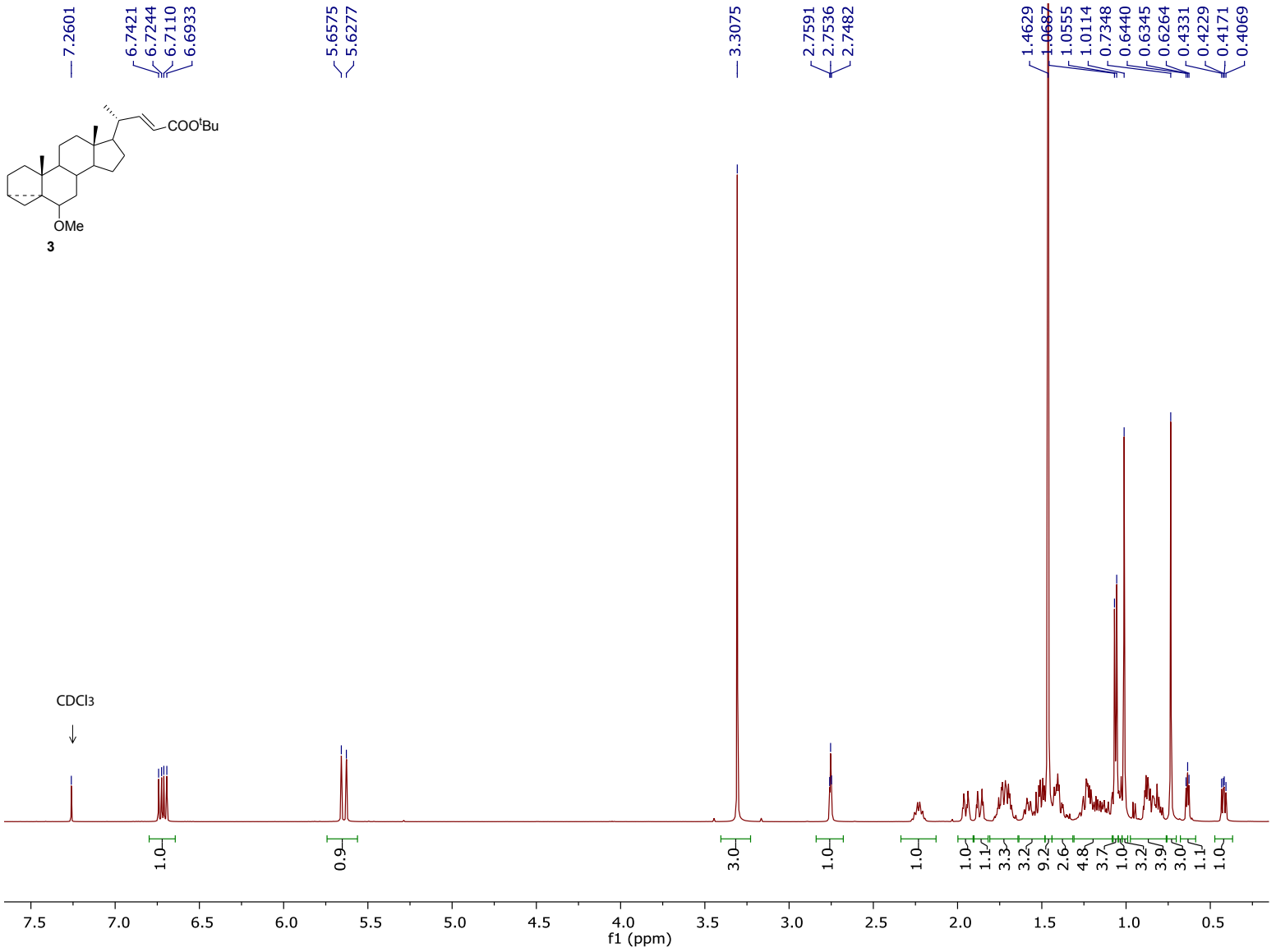
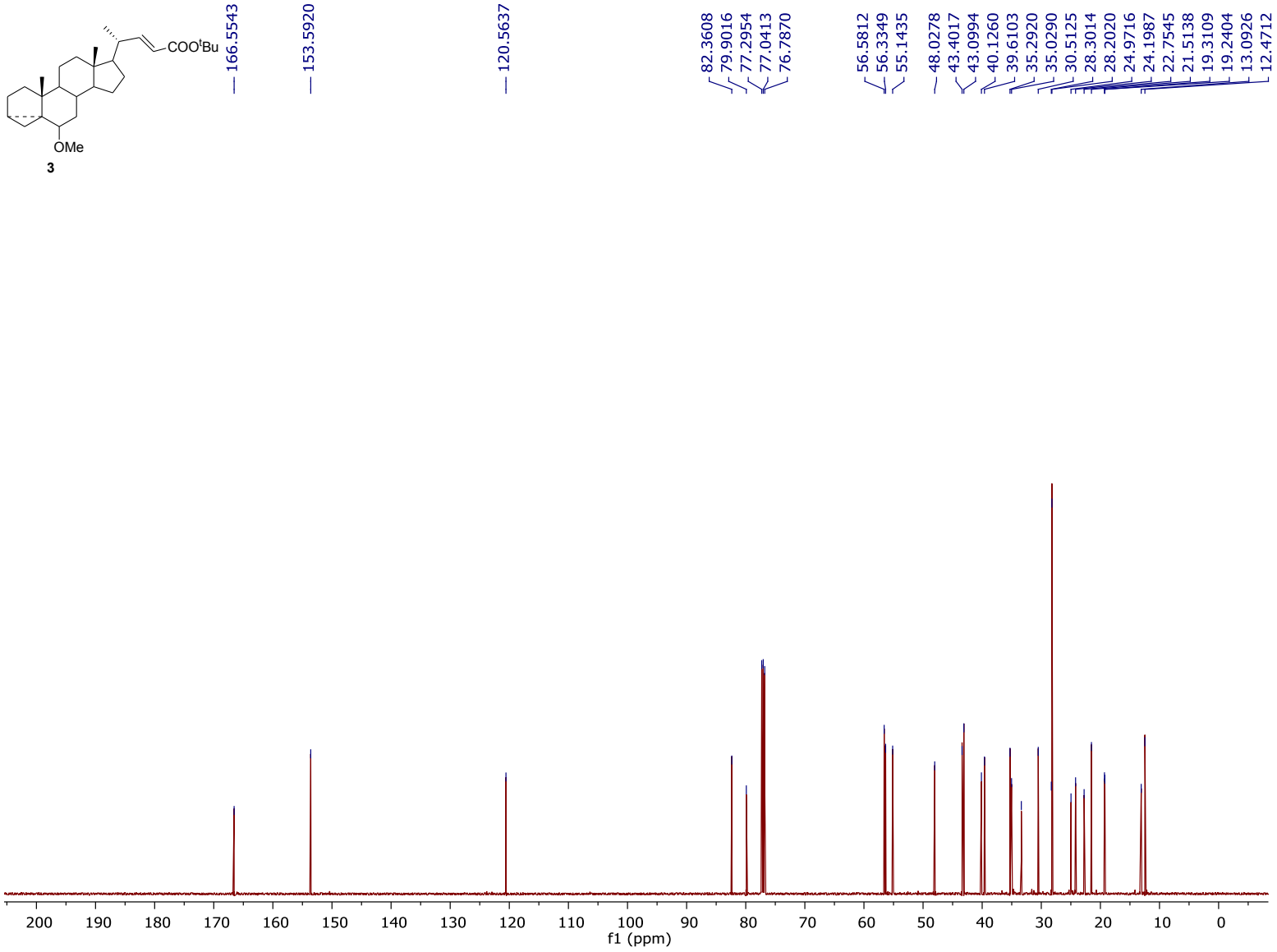
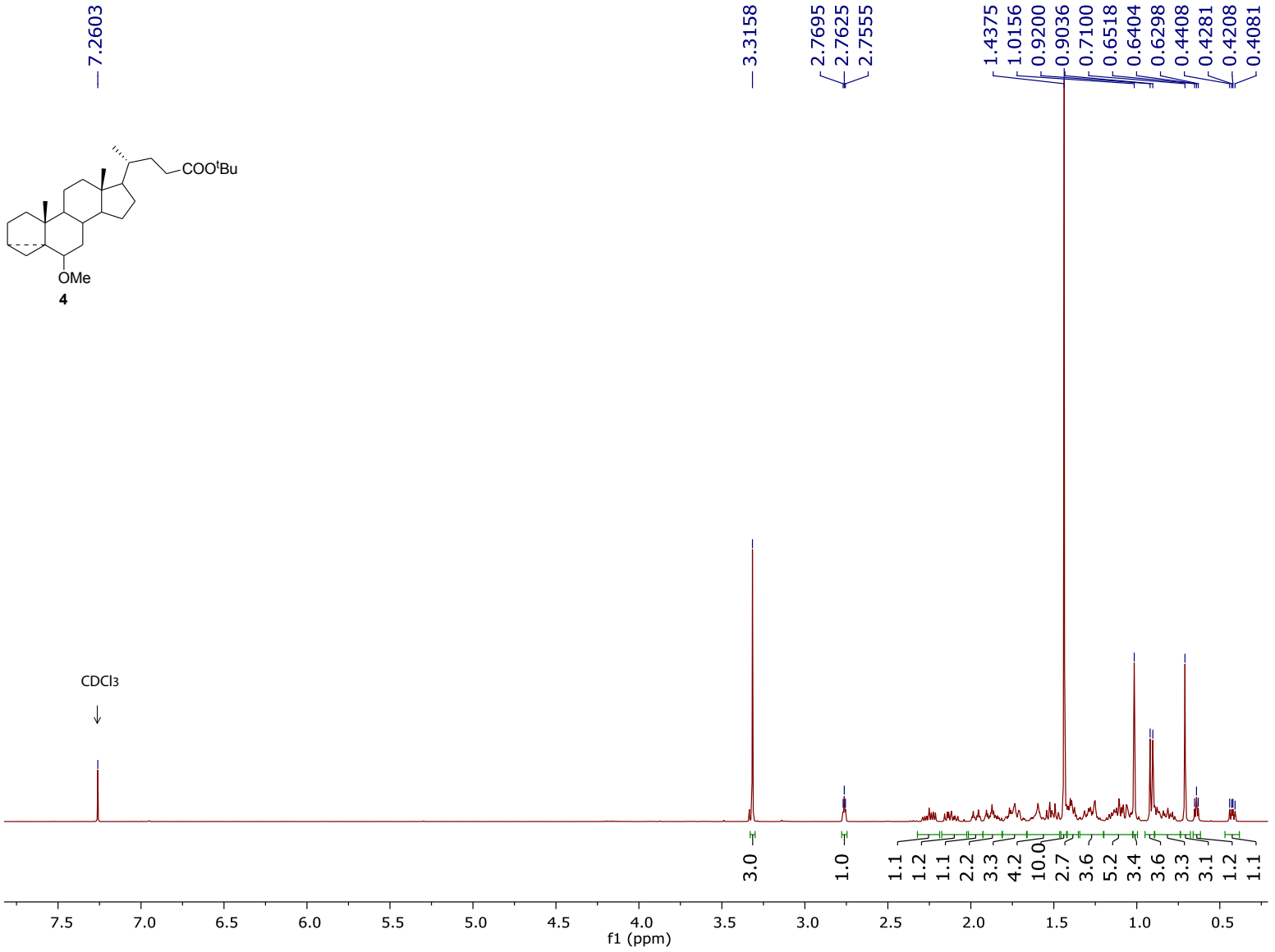
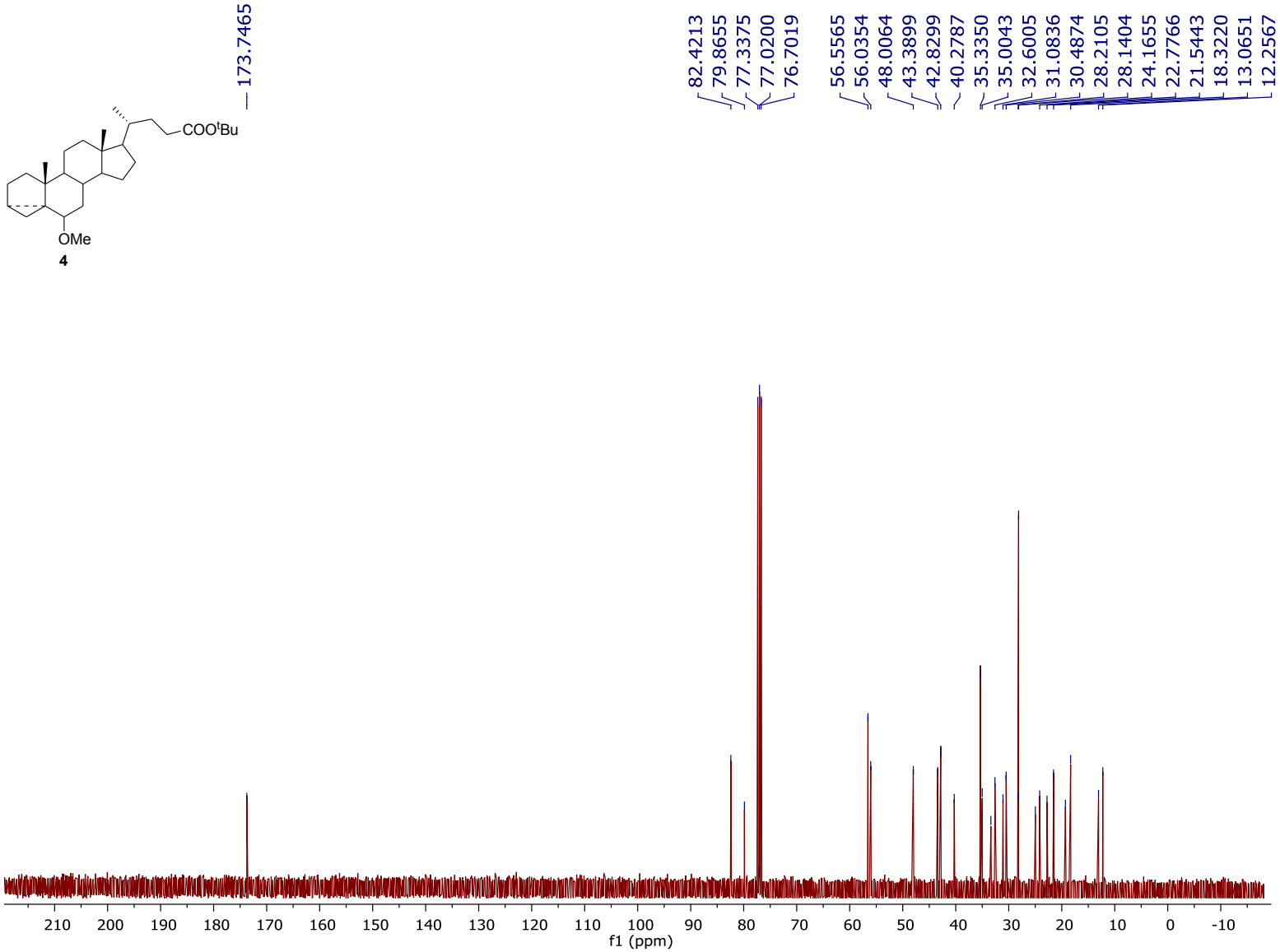


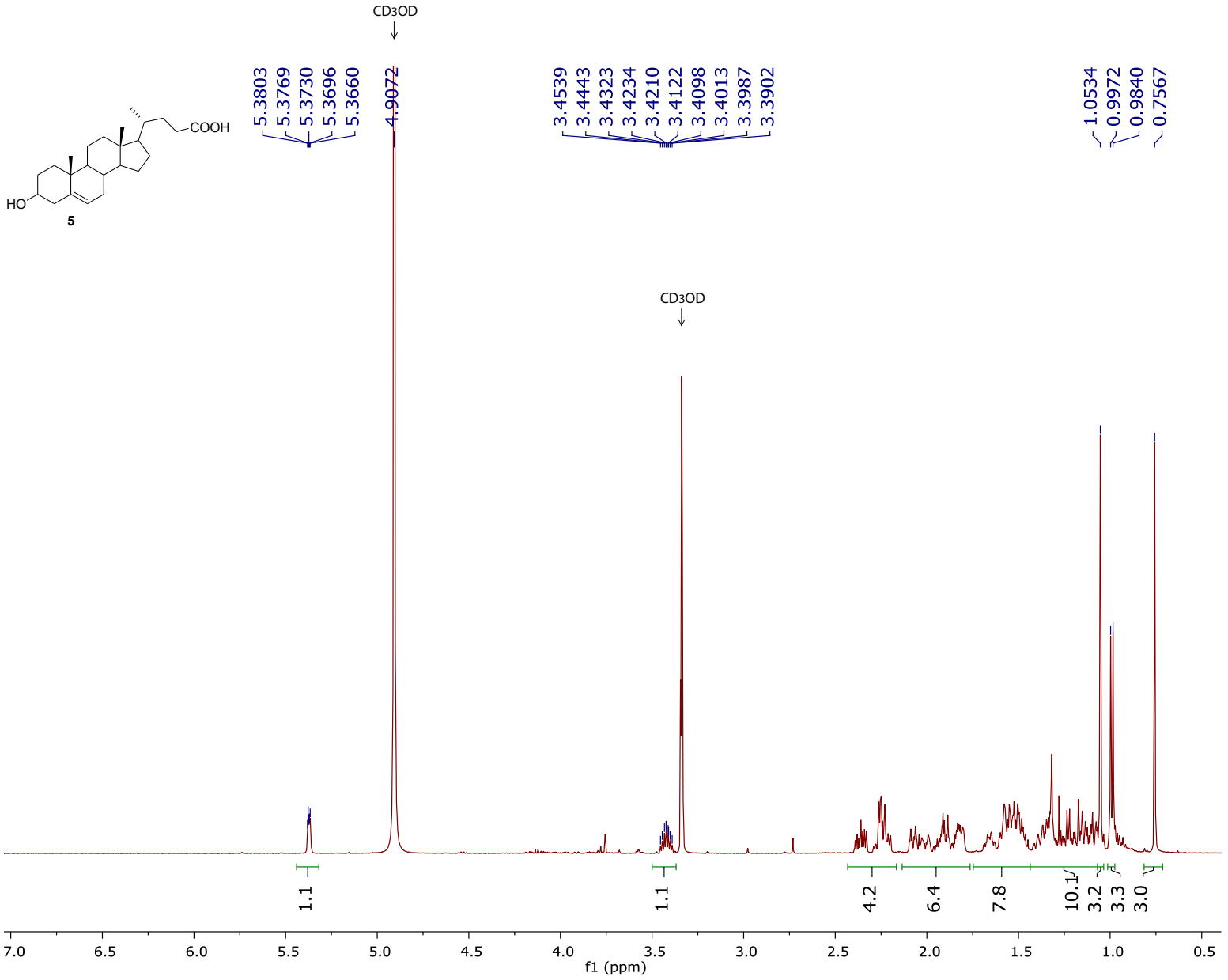
Figure S6. The three acyl-CoA dehydrogenase (ACAD) domains of ChsE4 and ChsE5. (a) ChsE4 and (b) ChsE5 both possess three ACAD domains: N-terminal domain (N-ChsE4; N-ChsE5), Middle domain (M-ChsE4; M-ChsE5), and C-terminal domain (C-ChsE5; C-ChsE5).

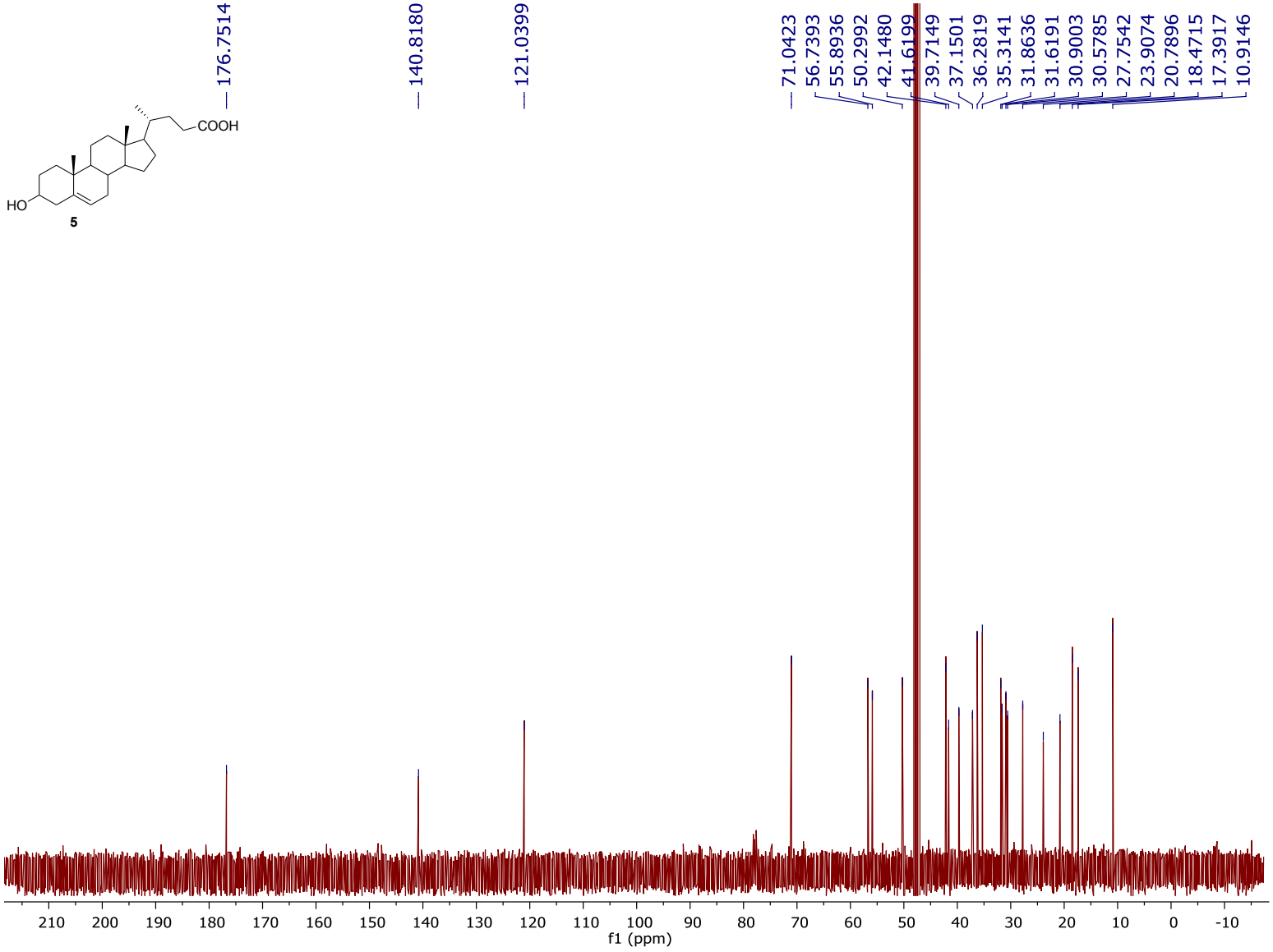

7.2601
6.7421
6.7244
6.7110
6.6933
5.6575
5.6277
3.3075
2.7591
2.7536
2.7482
1.4629
1.0687
1.0555
1.0114
0.7348
0.6440
0.6345
0.6264
0.4331
0.4229
0.4171
0.4069
COO^{t}Bu
OMe
3
CDCl3
1.0
0.9
3.0
1.0
1.0
1.0
1.1
3.3
3.2
9.2
2.6
4.8
3.7
1.0
3.2
3.9
3.0
1.1
1.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

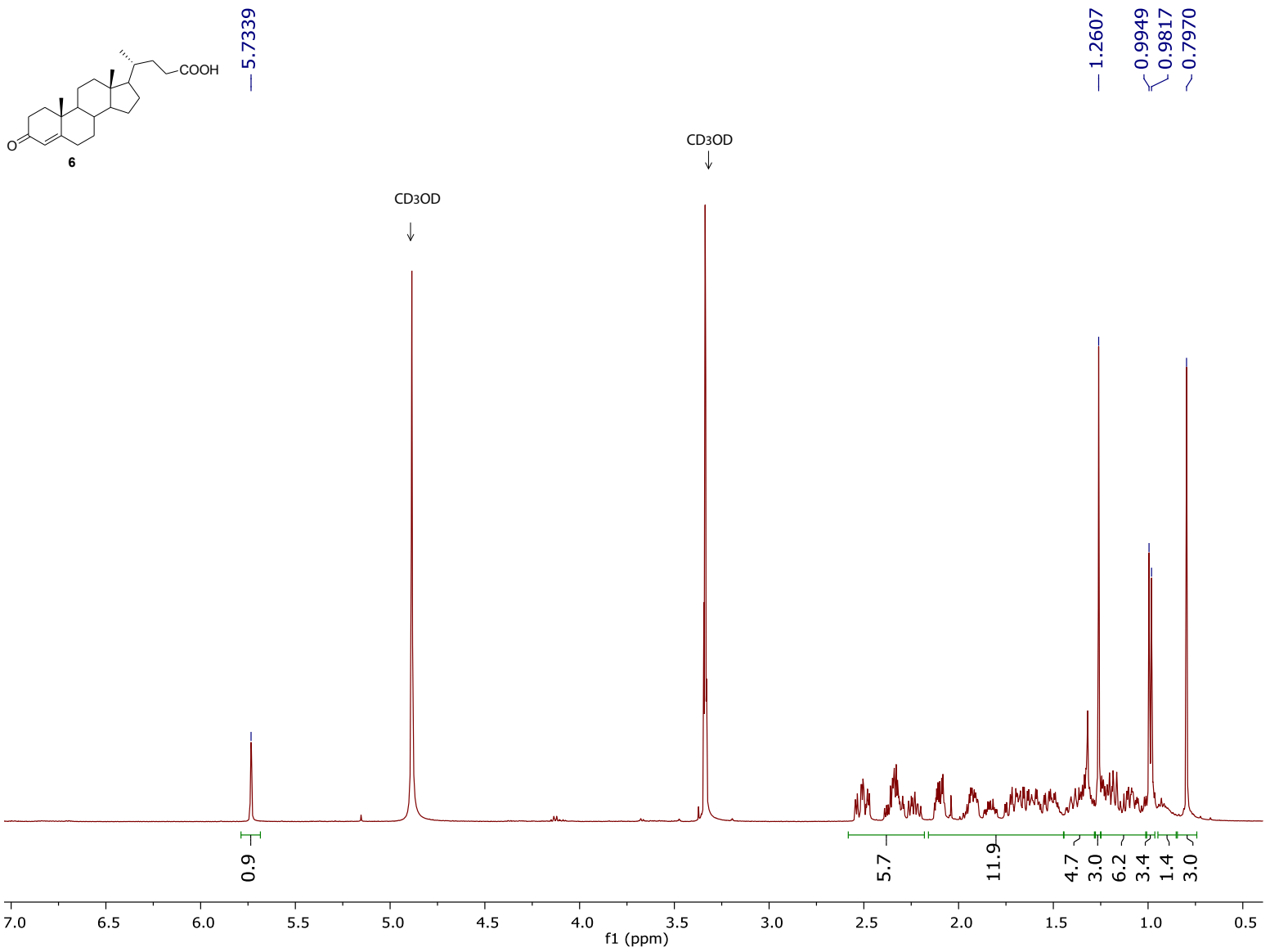

COO^{t}Bu
OMe
3
166.5543
153.5920
120.5637
82.3608
79.9016
77.2954
77.0413
76.7870
56.5812
56.3349
55.1435
48.0278
43.4017
43.0994
40.1260
39.6103
35.2920
35.0290
30.5125
28.3014
28.2020
24.9716
24.1987
22.7545
21.5138
19.3109
19.2404
13.0926
12.4712
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

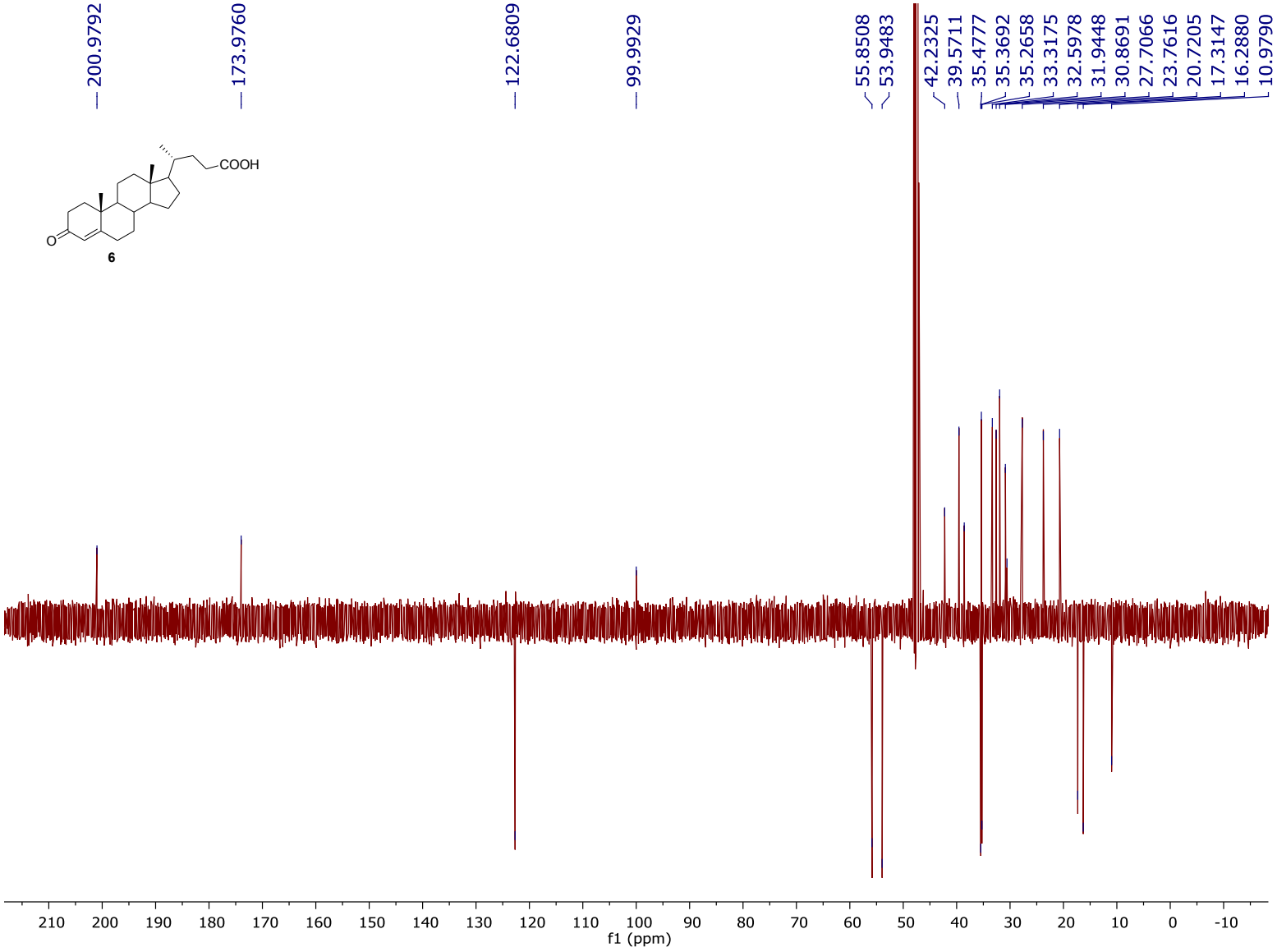

7.2603
3.3158
2.7695
2.7625
2.7555
1.4375
1.0156
0.9200
0.9036
0.7100
0.6518
0.6404
0.6298
0.4408
0.4281
0.4208
0.4081
COO^{t}Bu
OMe
4
CDCl3
3.0
1.0
1.1
1.2
1.1
2.2
3.3
4.2
10.0
2.7
3.6
5.2
3.4
3.6
3.3
3.1
1.2
1.1
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

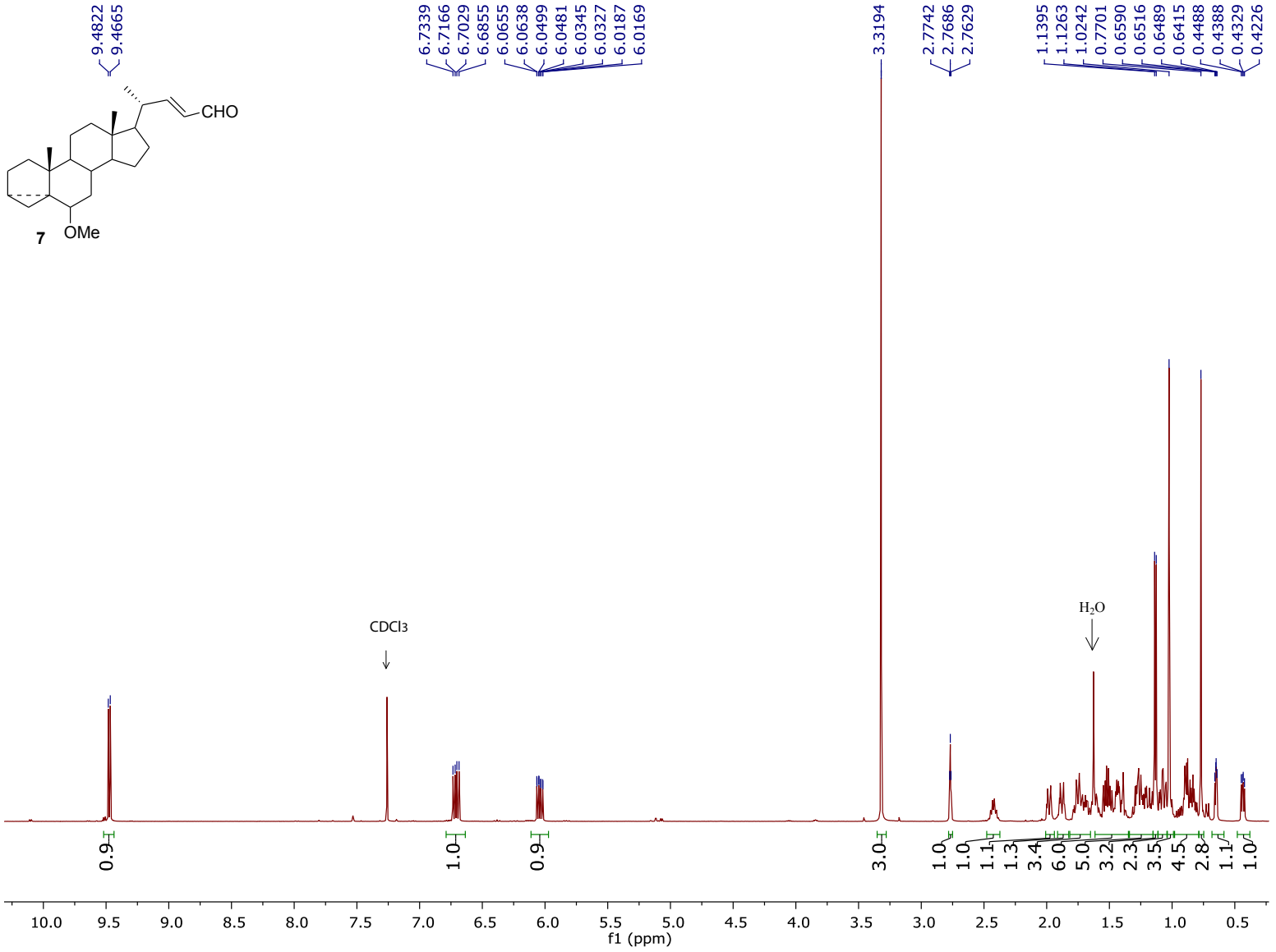

173.7465
82.4213
79.8655
77.3375
77.0200
76.7019
56.5565
56.0354
48.0064
43.3899
42.8299
40.2787
35.3350
35.0043
32.6005
31.0836
30.4874
28.2105
28.1404
24.1655
22.7766
21.5443
18.3220
13.0651
12.2567
COO^{t}Bu
OMe
4
f1 (ppm)

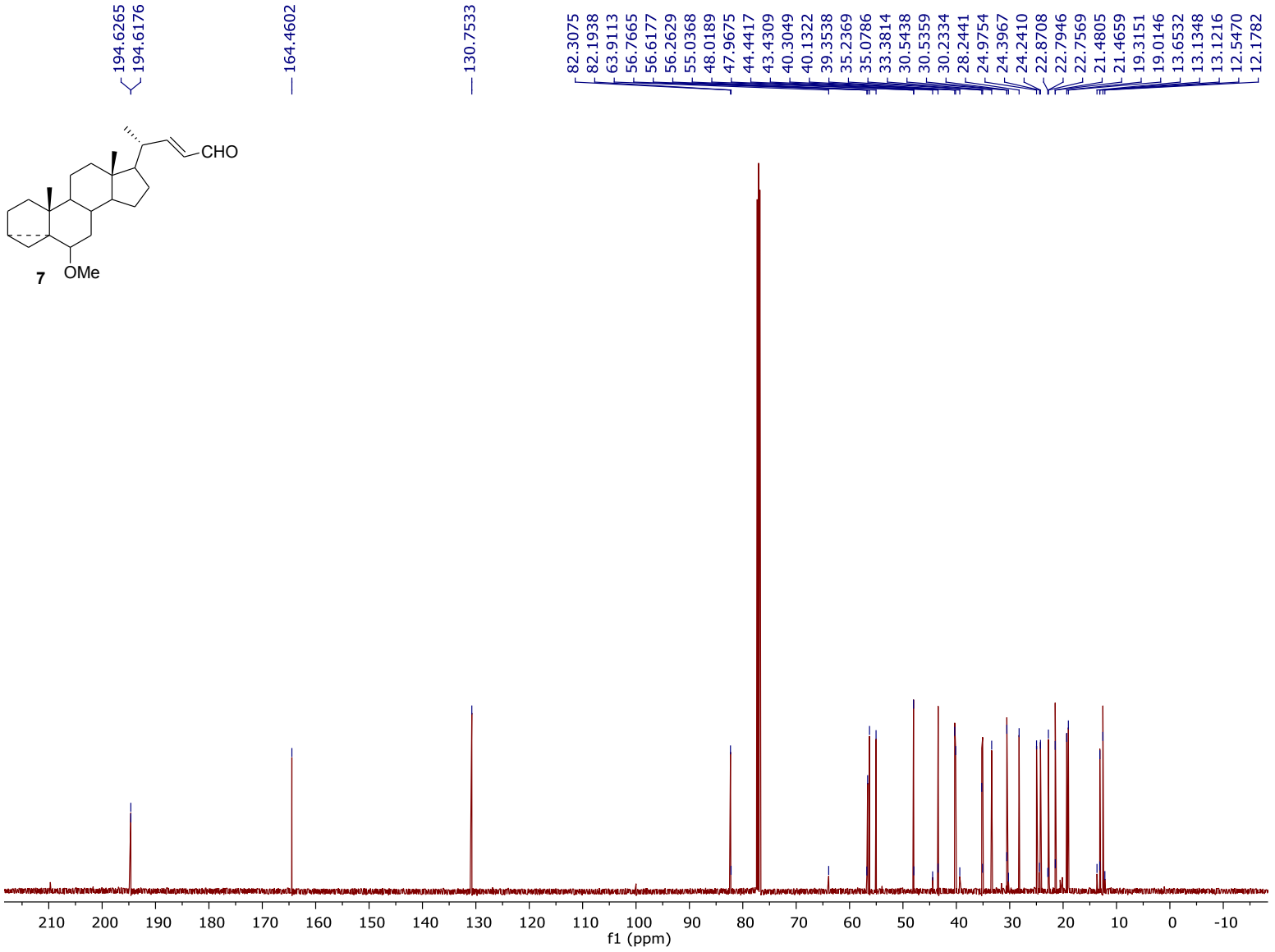

CD3OD
5.3803
5.3769
5.3730
5.3696
5.3660
4.9072
3.4539
3.4443
3.4323
3.4234
3.4210
3.4122
3.4098
3.4013
3.3987
3.3902
1.0534
0.9972
0.9840
0.7567
COOH
HO
5
CD3OD
1.1
1.1
4.2
6.4
7.8
10.1
3.2
3.3
3.0
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

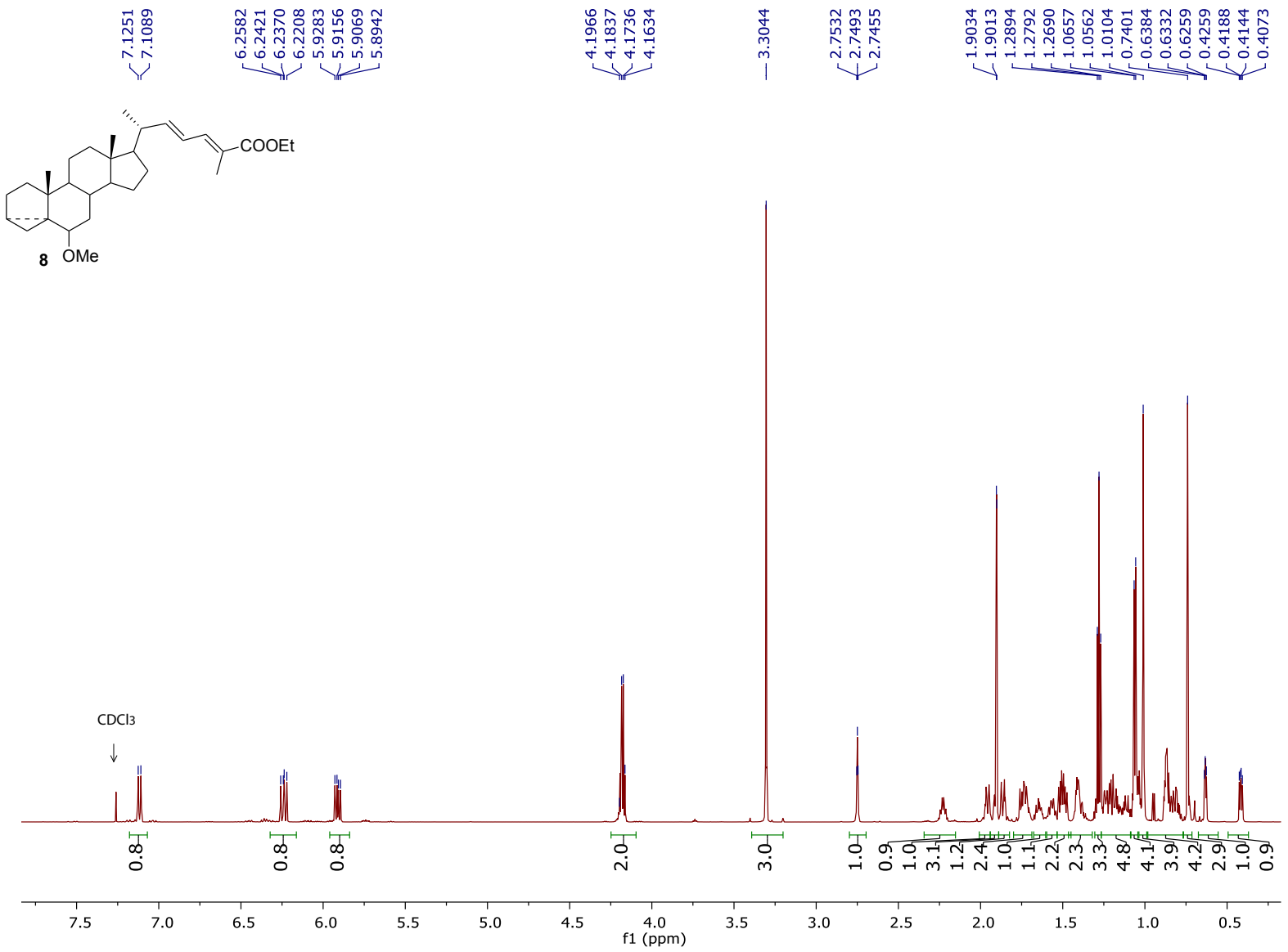

176.7514
140.8180
121.0399
71.0423
56.7393
55.8936
50.2992
42.1480
41.6199
39.7149
37.1501
36.2819
35.3141
31.8636
31.6191
30.9003
30.5785
27.7542
23.9074
20.7896
18.4715
17.3917
10.9146
COOH
HO
5
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

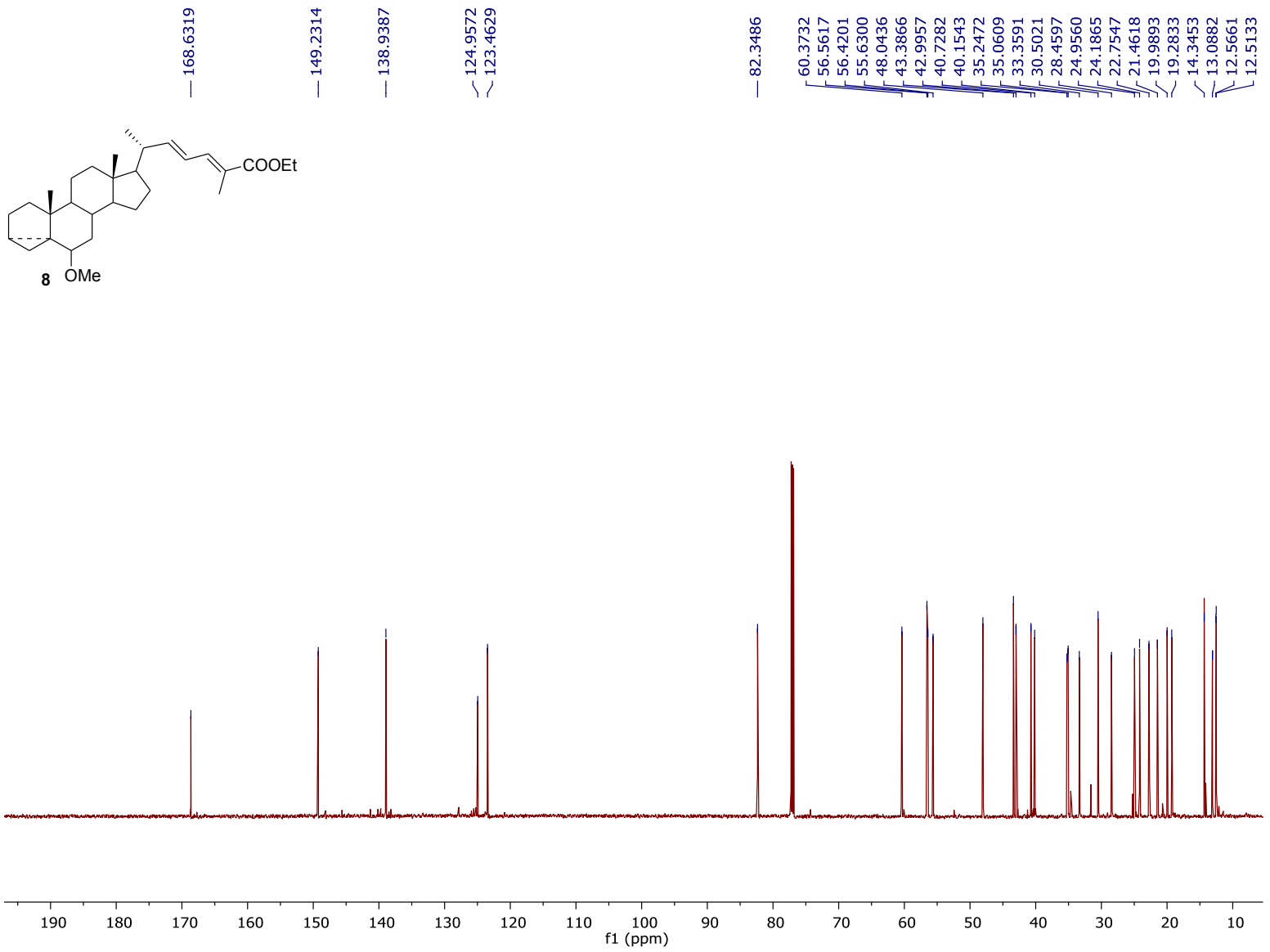

COOH
O
6
5.7339
1.2607
0.9949
0.9817
0.7970
CD3OD
CD3OD
0.9
5.7
11.9
4.7
3.0
6.2
3.4
1.4
3.0
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

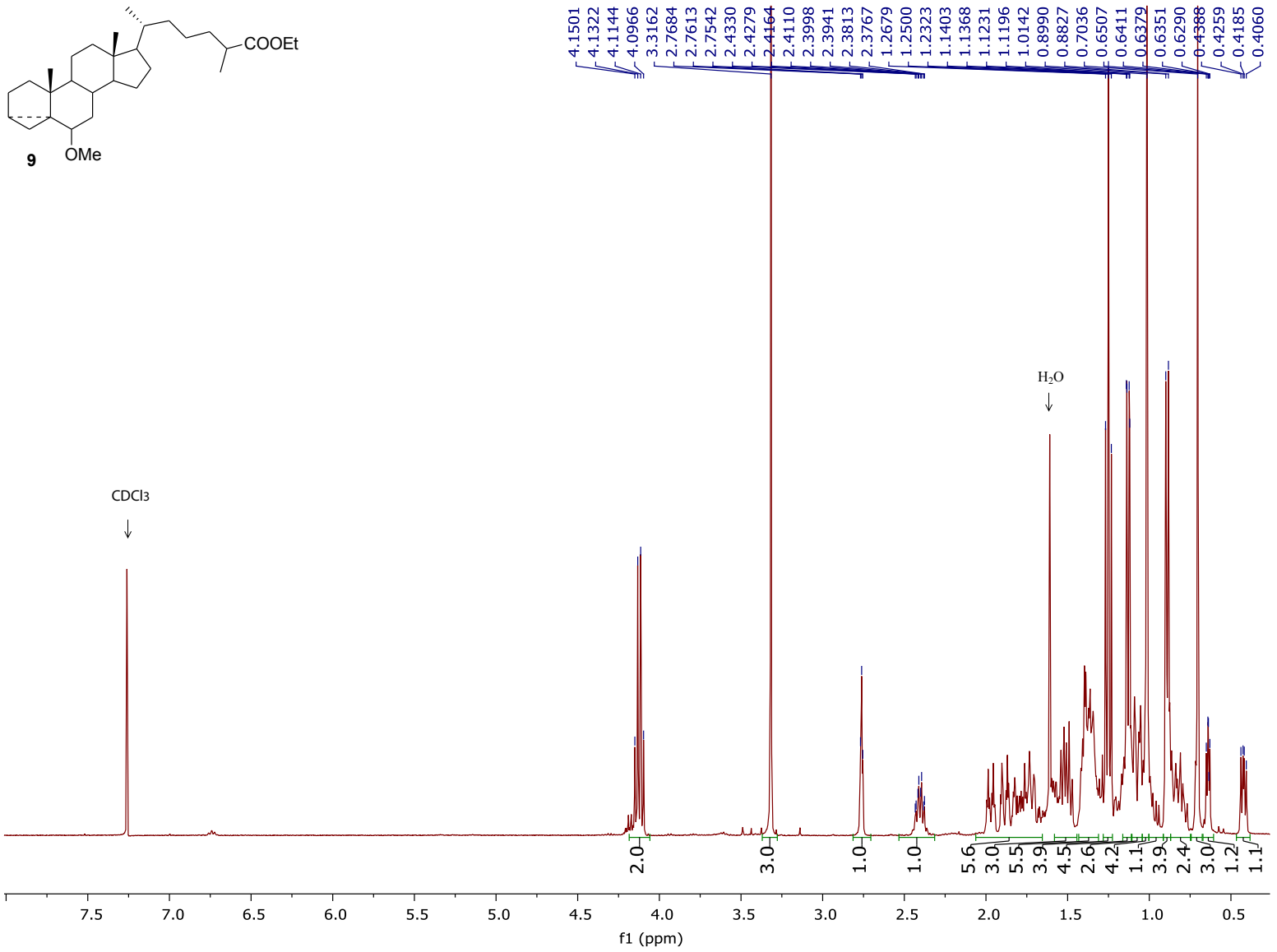

200.9792
173.9760
122.6809
99.9929
55.8508
53.9483
42.2325
39.5711
35.4777
35.3692
35.2658
33.3175
32.5978
31.9448
30.8691
27.7066
23.7616
20.7205
17.3147
16.2880
10.9790
COOH
O
6
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

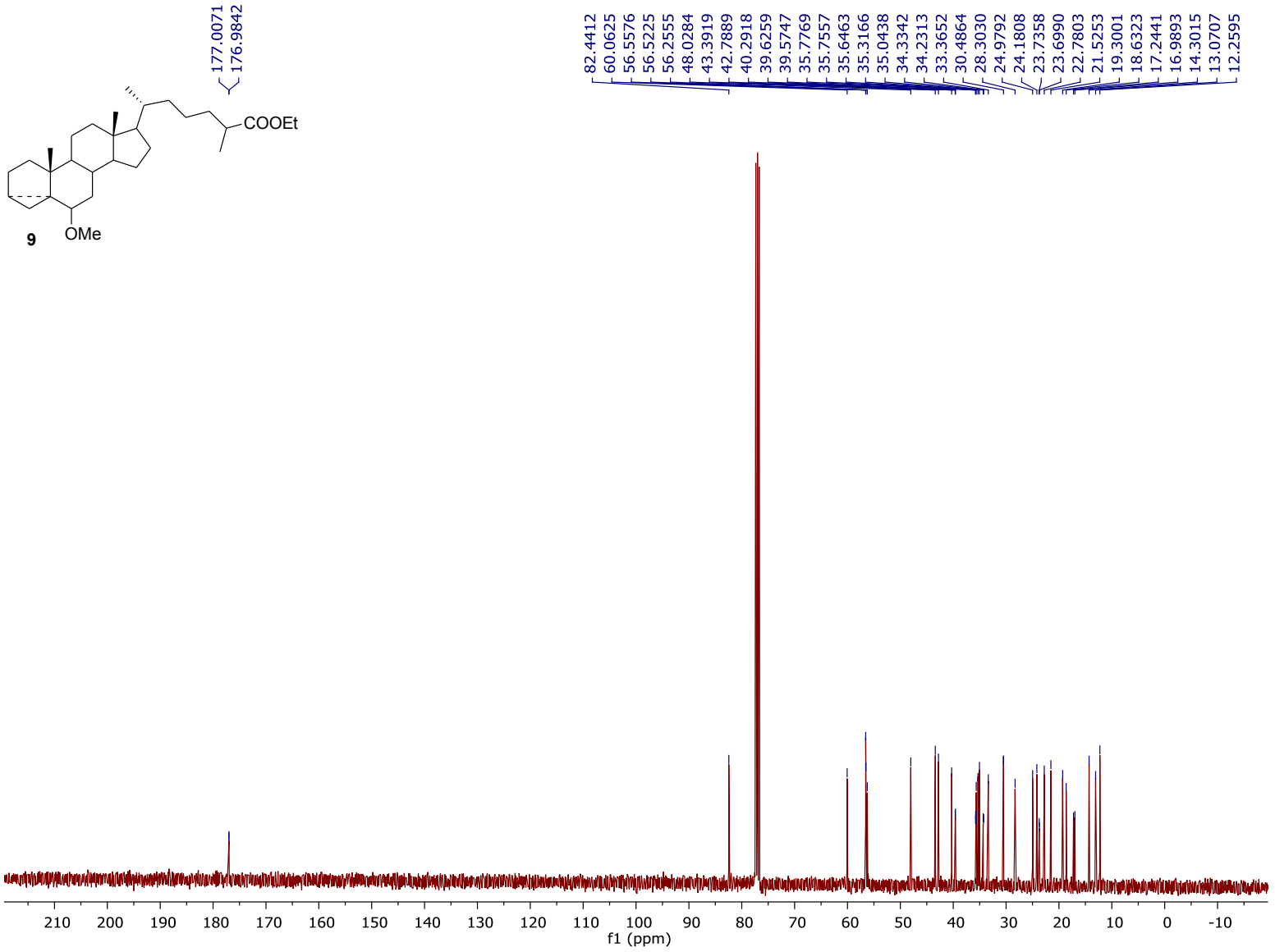

9.4822
9.4665
6.7339
6.7166
6.7029
6.6855
6.0655
6.0638
6.0499
6.0481
6.0345
6.0327
6.0187
6.0169
3.3194
2.7742
2.7686
2.7629
1.1395
1.1263
1.0242
0.7701
0.6590
0.6516
0.6489
0.6415
0.4488
0.4388
0.4329
0.4226
CHO
OMe
7
CDCl3
H2O
0.9
1.0
0.9
3.0
1.0
1.0
1.1
1.3
3.4
6.0
5.0
3.2
2.3
3.5
4.5
2.8
1.1
1.0
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

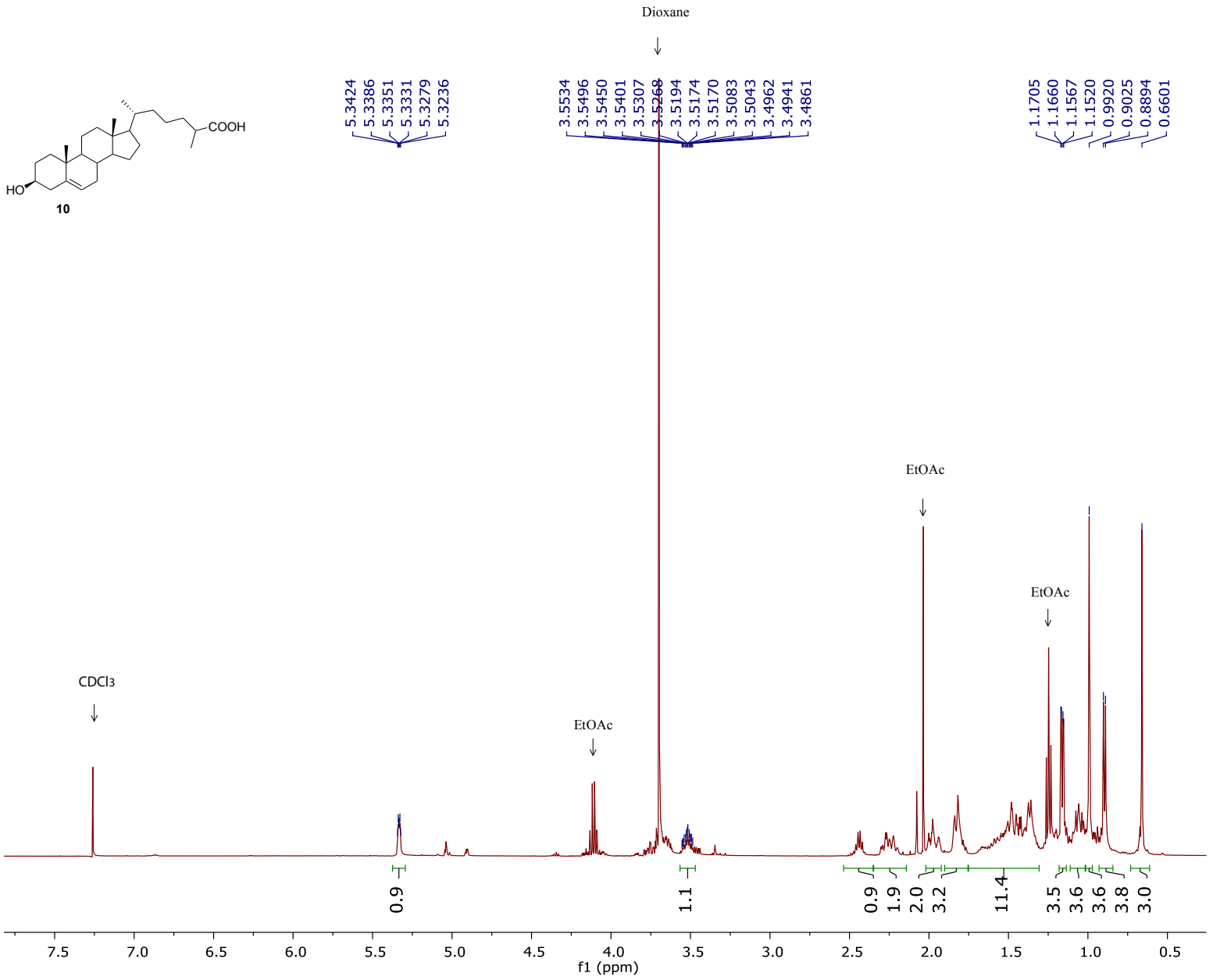

194.6265
194.6176
164.4602
130.7533
82.3075
82.1938
63.9113
56.7665
56.6177
56.2629
55.0368
48.0189
47.9675
44.4417
43.4309
40.3049
40.1322
39.3538
35.2369
35.0786
33.3814
30.5438
30.5359
30.2334
28.2441
24.9754
24.3967
24.2410
22.8708
22.7946
22.7569
21.4805
21.4659
19.3151
19.0146
13.6532
13.1348
13.1216
12.5470
12.1782
CHO
OMe
7
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

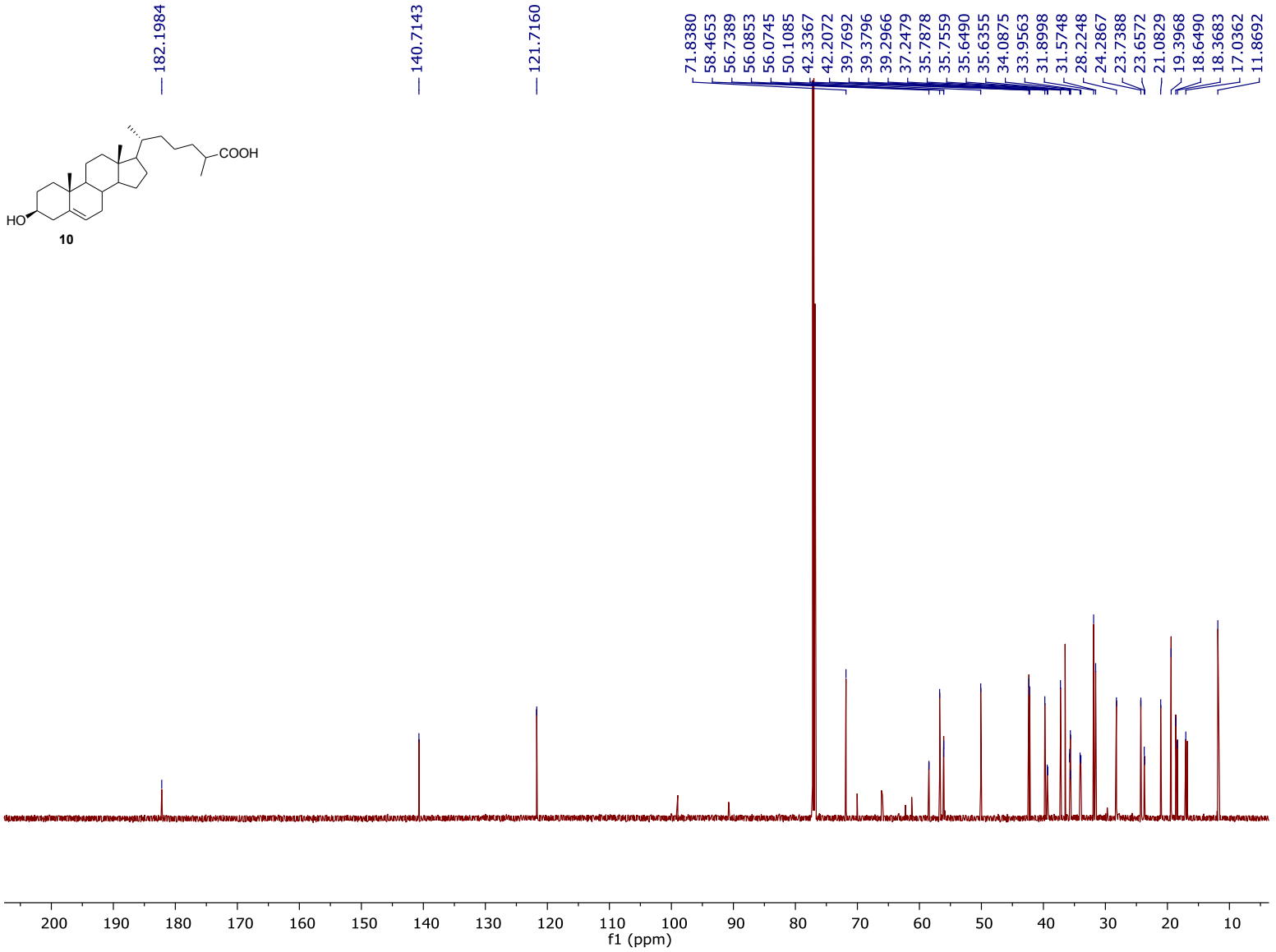

7.1251
7.1089
6.2582
6.2421
6.2370
6.2208
5.9283
5.9156
5.9069
5.8942
4.1966
4.1837
4.1736
4.1634
3.3044
2.7532
2.7493
2.7455
1.9034
1.9013
1.2894
1.2792
1.2690
1.0657
1.0562
1.0104
0.7401
0.6384
0.6332
0.6259
0.4259
0.4188
0.4144
0.4073
COOEt
OMe
8
CDCl3
0.8
0.8
0.8
2.0
3.0
1.0
f1 (ppm)

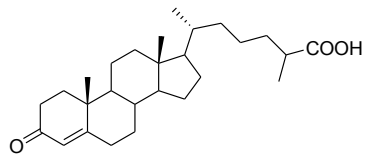

168.6319
149.2314
138.9387
124.9572
123.4629
82.3486
60.3732
56.5617
56.4201
55.6300
48.0436
43.3866
42.9957
40.7282
40.1543
35.2472
35.0609
33.3591
30.5021
28.4597
24.9560
24.1865
22.7547
21.4618
19.9893
19.2833
14.3453
13.0882
12.5661
12.5133
COOEt
OMe
8
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


COOEt
OMe
9
4.1501
4.1322
4.1144
4.0966
3.3162
2.7684
2.7613
2.7542
2.4330
2.4279
2.4164
2.4110
2.3998
2.3941
2.3813
2.3767
1.2679
1.2500
1.2323
1.1403
1.1368
1.1231
1.1196
1.0142
0.8990
0.8827
0.7036
0.6507
0.6411
0.6379
0.6351
0.6290
0.4388
0.4259
0.4185
0.4060
CDCl3
H2O
2.0
3.0
1.0
1.0
5.6
3.0
5.5
3.9
4.5
2.6
4.2
1.1
3.9
2.4
3.0
1.2
1.1
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


177.0071
176.9842
COOEt
OMe
9
82.4412
60.0625
56.5576
56.5225
56.2555
48.0284
43.3919
42.7889
40.2918
39.6259
39.5747
35.7769
35.7557
35.6463
35.3166
35.0438
34.3342
34.2313
33.3652
30.4864
28.3030
24.9792
24.1808
23.7358
23.6990
22.7803
21.5253
19.3001
18.6323
17.2441
16.9893
14.3015
13.0707
12.2595
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

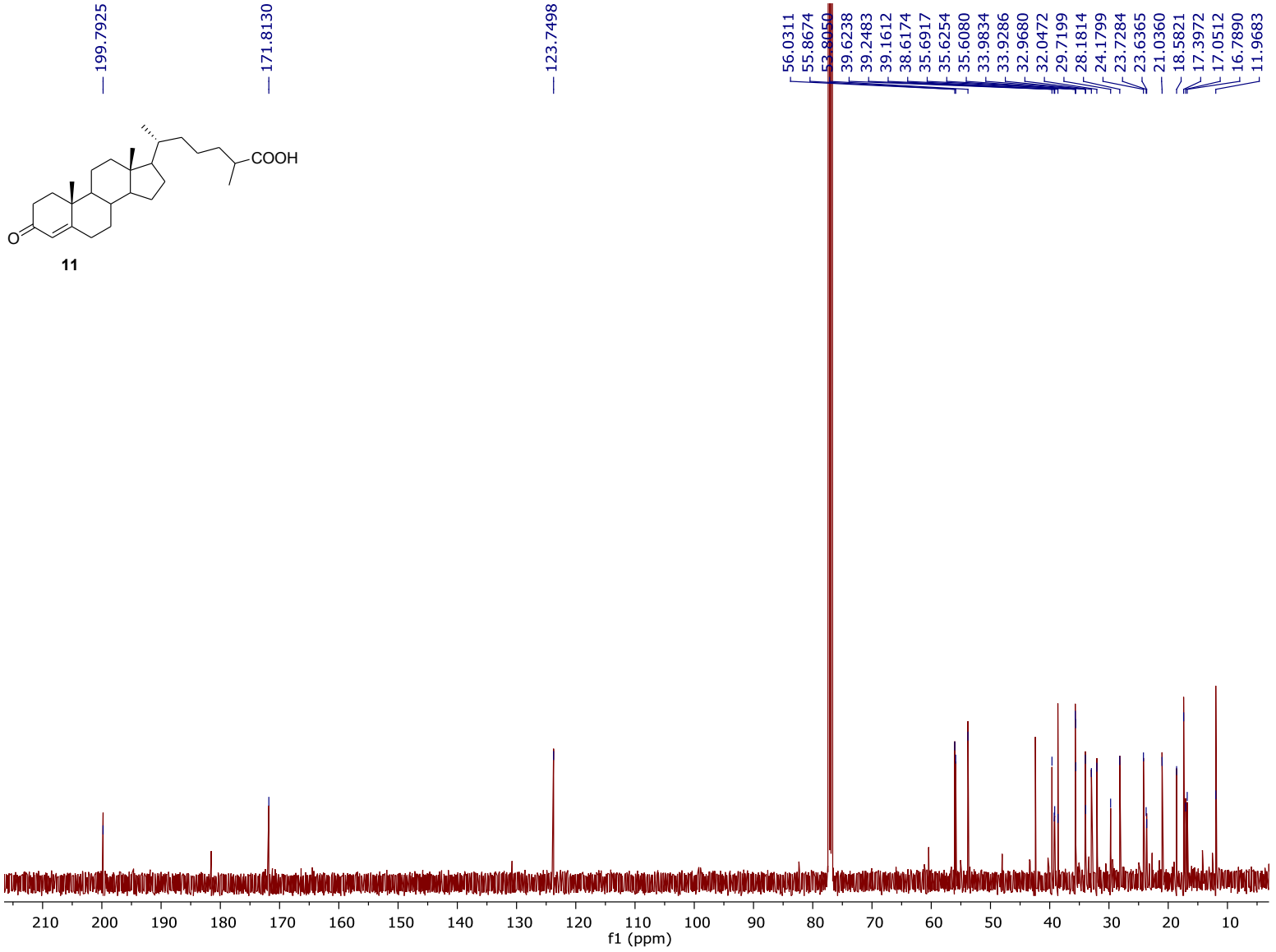

Dioxane
5.3424
5.3386
5.3351
5.3331
5.3279
5.3236
3.5534
3.5496
3.5450
3.5401
3.5307
3.5268
3.5194
3.5174
3.5170
3.5083
3.5043
3.4962
3.4941
3.4861
1.1705
1.1660
1.1567
1.1520
0.9920
0.9025
0.8894
0.6601
COOH
HO
10
EtOAc
EtOAc
CDCl3
EtOAc
0.9
1.1
0.9
1.9
2.0
3.2
11.4
3.5
3.6
3.6
3.8
3.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


182.1984
140.7143
121.7160
71.8380
58.4653
56.7389
56.0853
56.0745
50.1085
42.3367
42.2072
39.7692
39.3796
39.2966
37.2479
35.7878
35.7559
35.6490
35.6355
34.0875
33.9563
31.8998
31.5748
28.2248
24.2867
23.7388
23.6572
21.0829
19.3968
18.6490
18.3683
17.0362
11.8692
COOH
HO
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


COOH
O

11

5.7257
1.1887
1.1840
1.1759
0.9103
0.8972
0.7014
CDCl3
EtOAc
Dioxane
EtOAc
EtOAc
0.9
5.4
1.9
3.2
4.9
9.9
5.9
7.7
4.0
3.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


199.7925
171.8130
123.7498
56.0311
55.8674
53.8050
39.6238
39.2483
39.1612
38.6174
35.6917
35.6254
35.6080
33.9834
33.9286
32.9680
32.0472
29.7199
28.1814
24.1799
23.7284
23.6365
21.0360
18.5821
17.3972
17.0512
16.7890
11.9683
COOH
O
11
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)