SUPPORTING INFORMATION

3α,7-Dihydroxy-14(13→12)*abeo*-5β,12α(H),13β(H)-cholan-24-oic Acids Display Neuroprotective Properties in Common Forms of Parkinson's Disease

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[2] Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, United Kingdom
[3] Department of Neurology, St Olav's Hospital, Trondheim, Norway
[4] ICE Pharma, 68 Weld Street, RD2, Palmerston North 4472, New Zealand
[†] deceased
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1. Additional Table S1.

Compound	sPD		LRRK2	
	ATP	MMP	ATP	MMP
3	170	105	138	136
4	120	105	148	118
7	130	107	136	116
24	140	105	130	105
25	97	98	94	98
29	95	94	98	96
UDCA	146	120	125	125

Table S1. Primary screening data from sPD and LRRK2 mutant patient fibroblasts at one concentration of 100 nM. Data shown are % of DMSO vehicle. Activity shown by compounds **3**, **4**, **7** and **24**. Compounds **25** and **29** were not active.

2. X-Ray Diffraction Data of compound 18

The dataset was collected on an Agilent SuperNova diffractometer fitted with an EOS S2 detector at 120 K and using CuKα radiation (λ = 1.54184 Å). The ORTEP plot is with 30% probability ellipsoids. Minor disordered components and/or solvent molecules have been omitted for clarity.

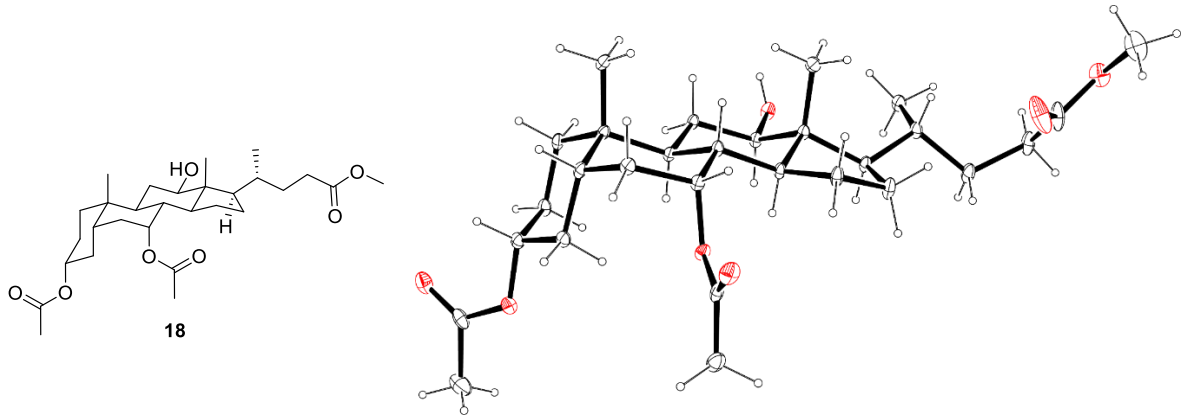
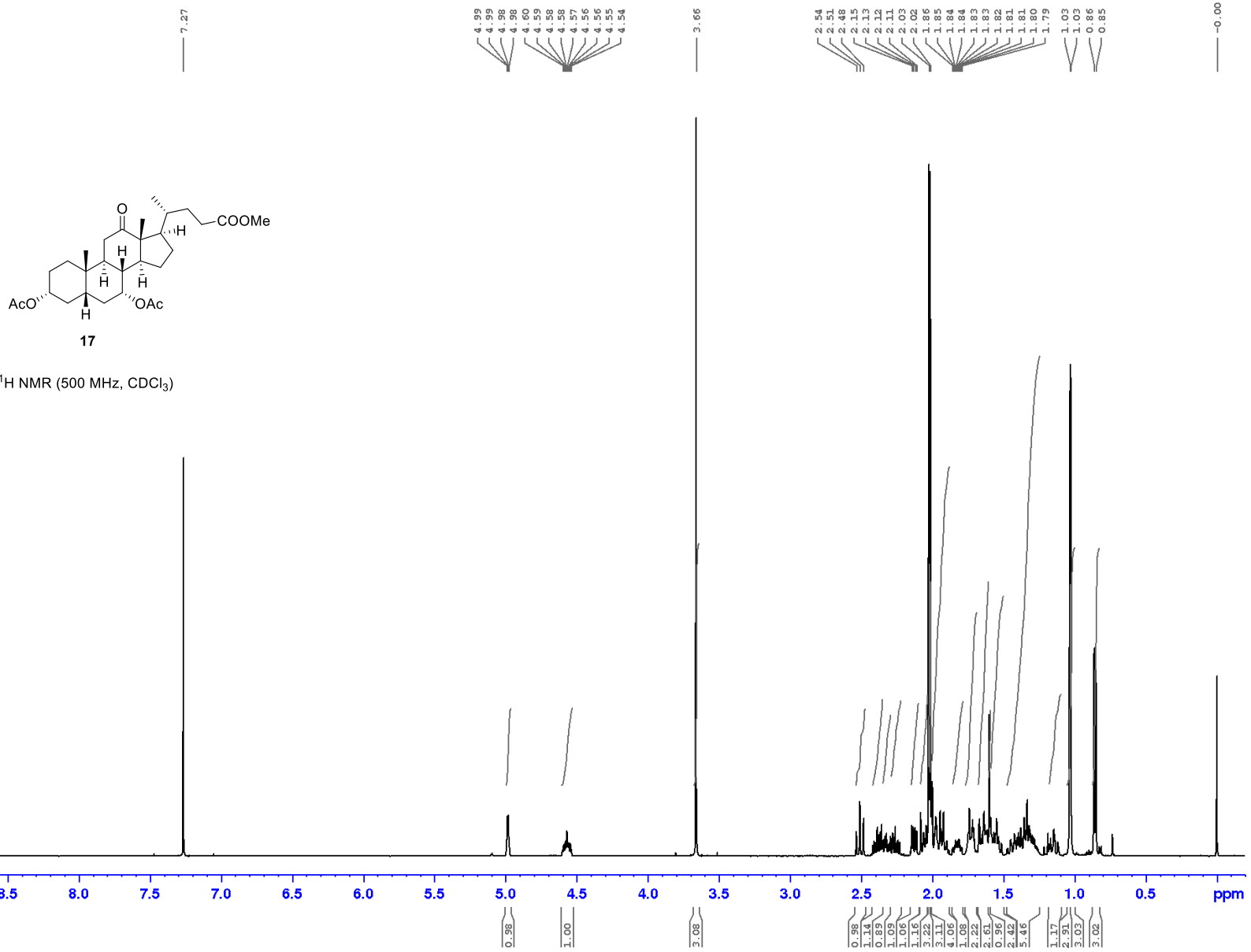


Figure S1. ORTEP diagram of **18** (CCDC 2203402) with 30% probability ellipsoids.

Table S2. Crystal data and structure refinement for compound **18**.

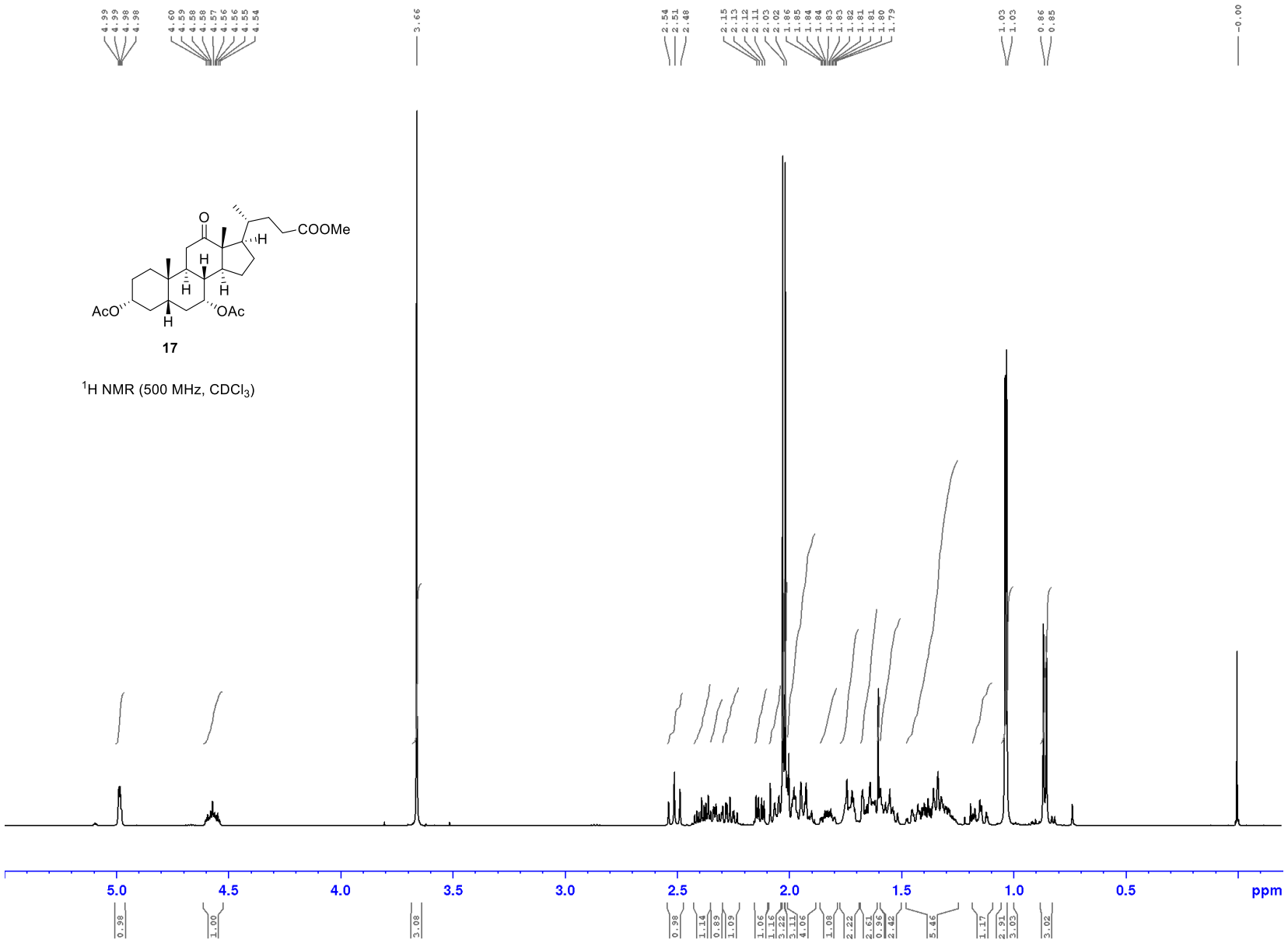
Identification code	**18**
CCDC identifier	2203402
Empirical formula	$C_{29}H_{46}O_7$
Formula weight (g/mol)	506.68
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	
a (Å)	6.08670(10)
b (Å)	16.0750(3)
c (Å)	14.0971(2)
α (°)	90
β (°)	99.0140(10)
γ (°)	90
Volume ($Å^3$)	1362.28(4)
Z	2
Calculated density (Mg/m^3)	1.235
Absorption coefficient (mm^{-1})	0.700
F (000)	552
Crystal size (mm^3)	0.680 × 0.190 × 0.077
Theta range for data collection	4.201 to 71.623°
Index ranges	$-7 \le h \le 7$ $-17 \le k \le 19$ $-17 \le l \le 17$
Reflections collected	14904
Independent reflections	4853 [R(int) = 0.0358]
Completeness to theta	99.9% to 67.684°
Data / restraints / parameters	4853 / 1 / 345
Goodness-of-fit on F^2	1.038
Final R indices [I>2sigma(I)]	R1 = 0.0349 wR2 = 0.0905
R indices (all data)	R1 = 0.0358 wR2 = 0.0919
Absolute structure parameter	0.06(10)
Largest diff. peak and hole ($e.Å^{-3}$)	0.176 and -0.184

3. NMR Spectra



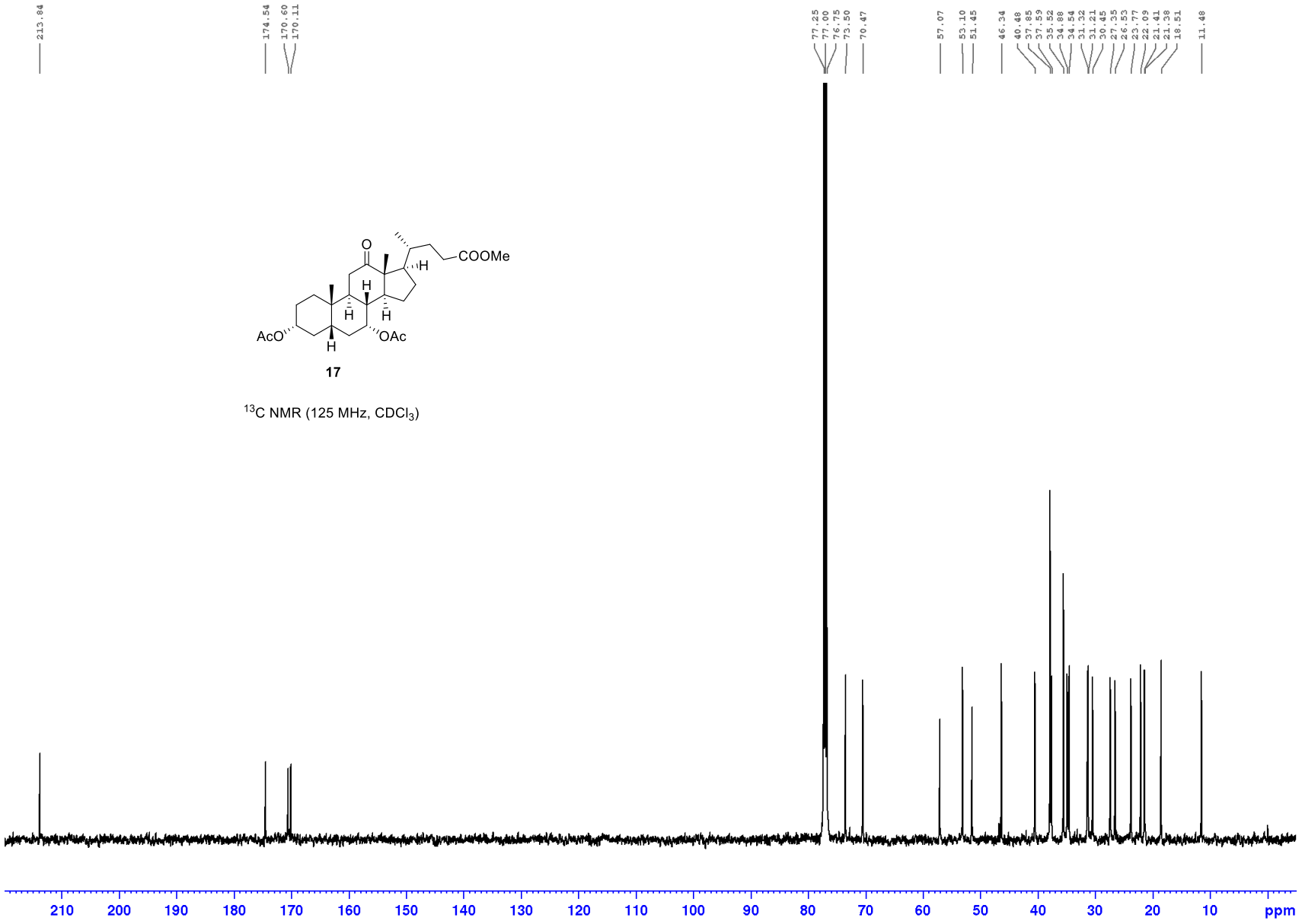
17

^{1}H NMR (500 MHz, $CDCl_3$)



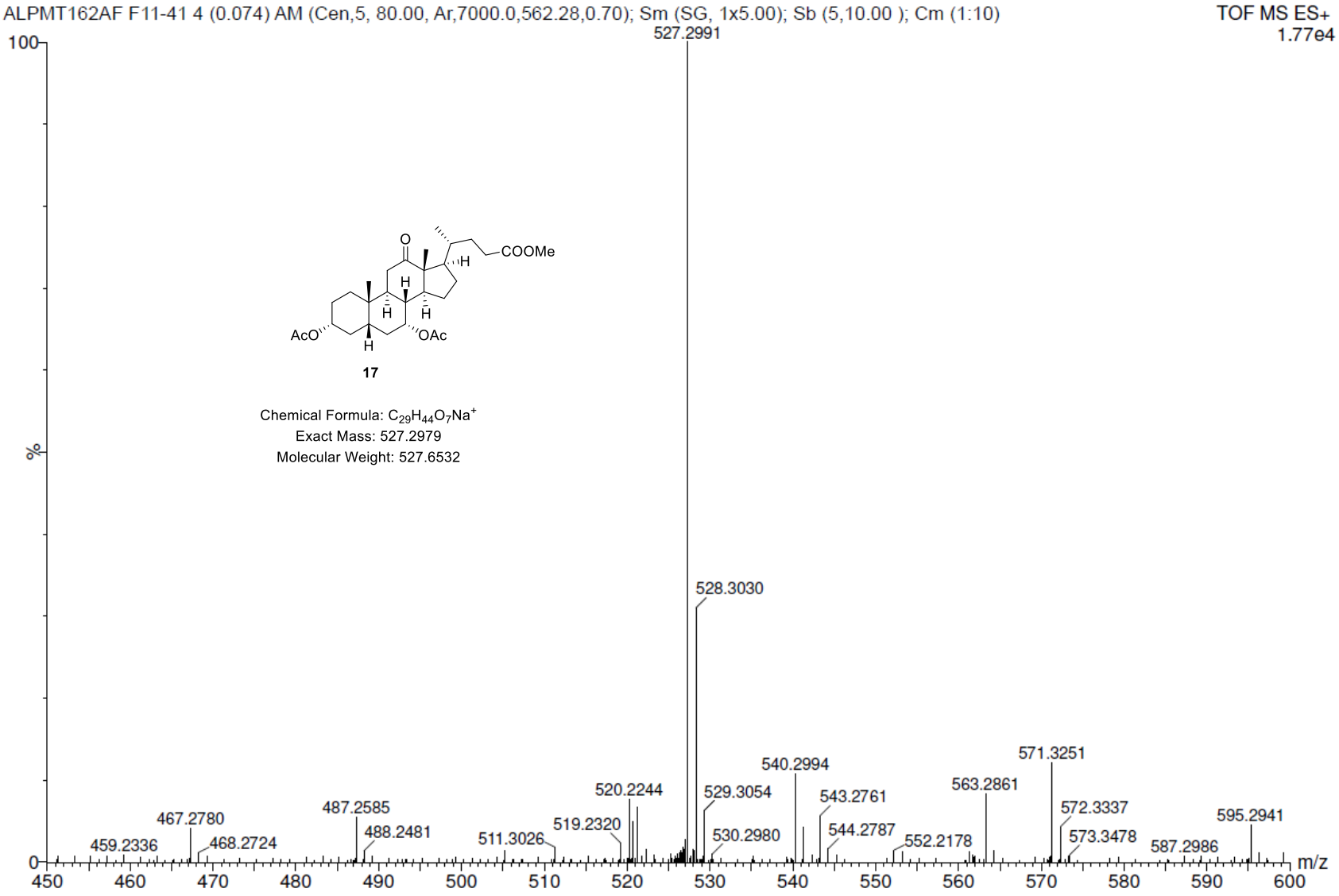
17

^{1}H NMR (500 MHz, $CDCl_3$)



17

^{13}C NMR (125 MHz, $CDCl_3$)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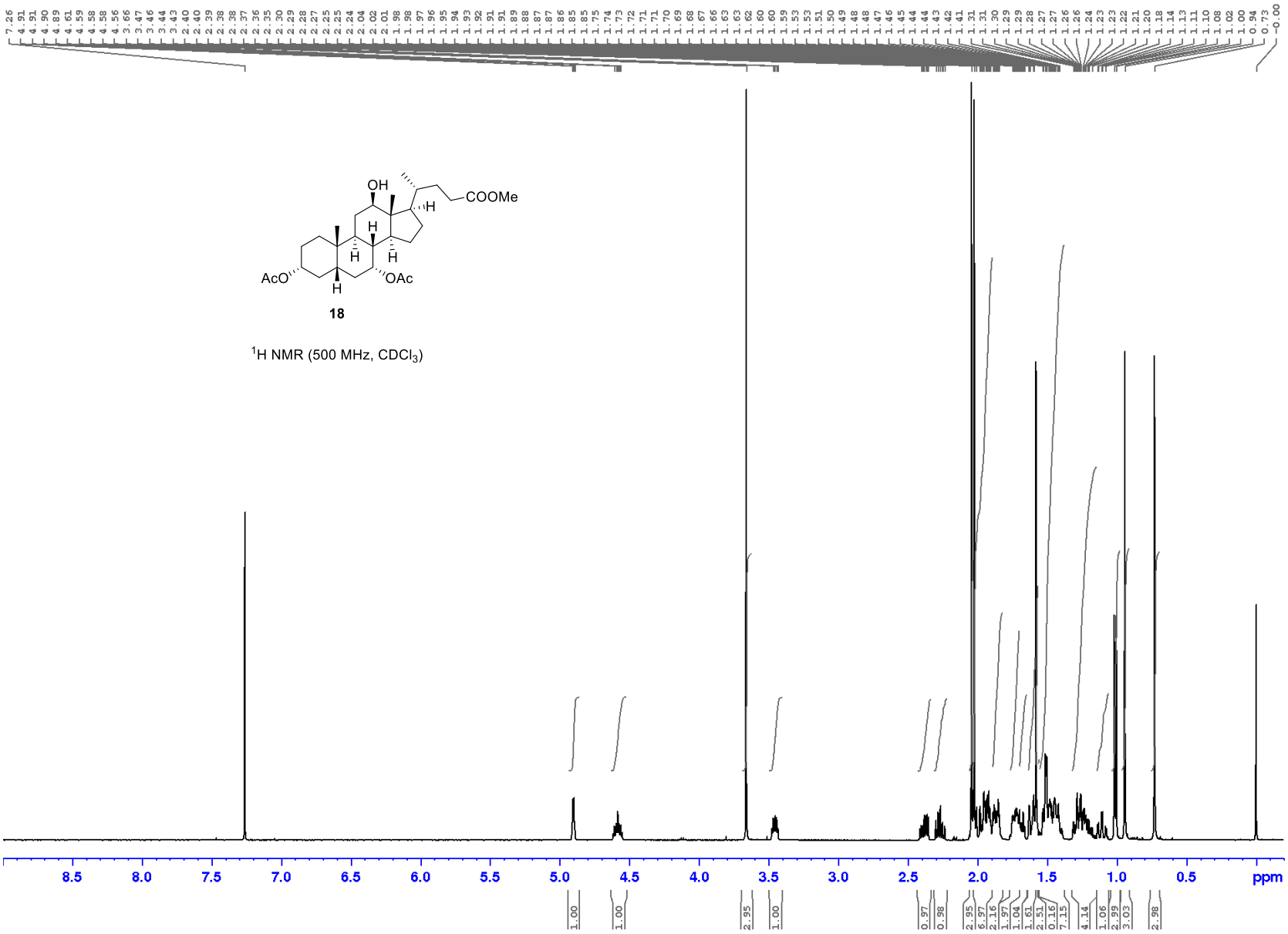
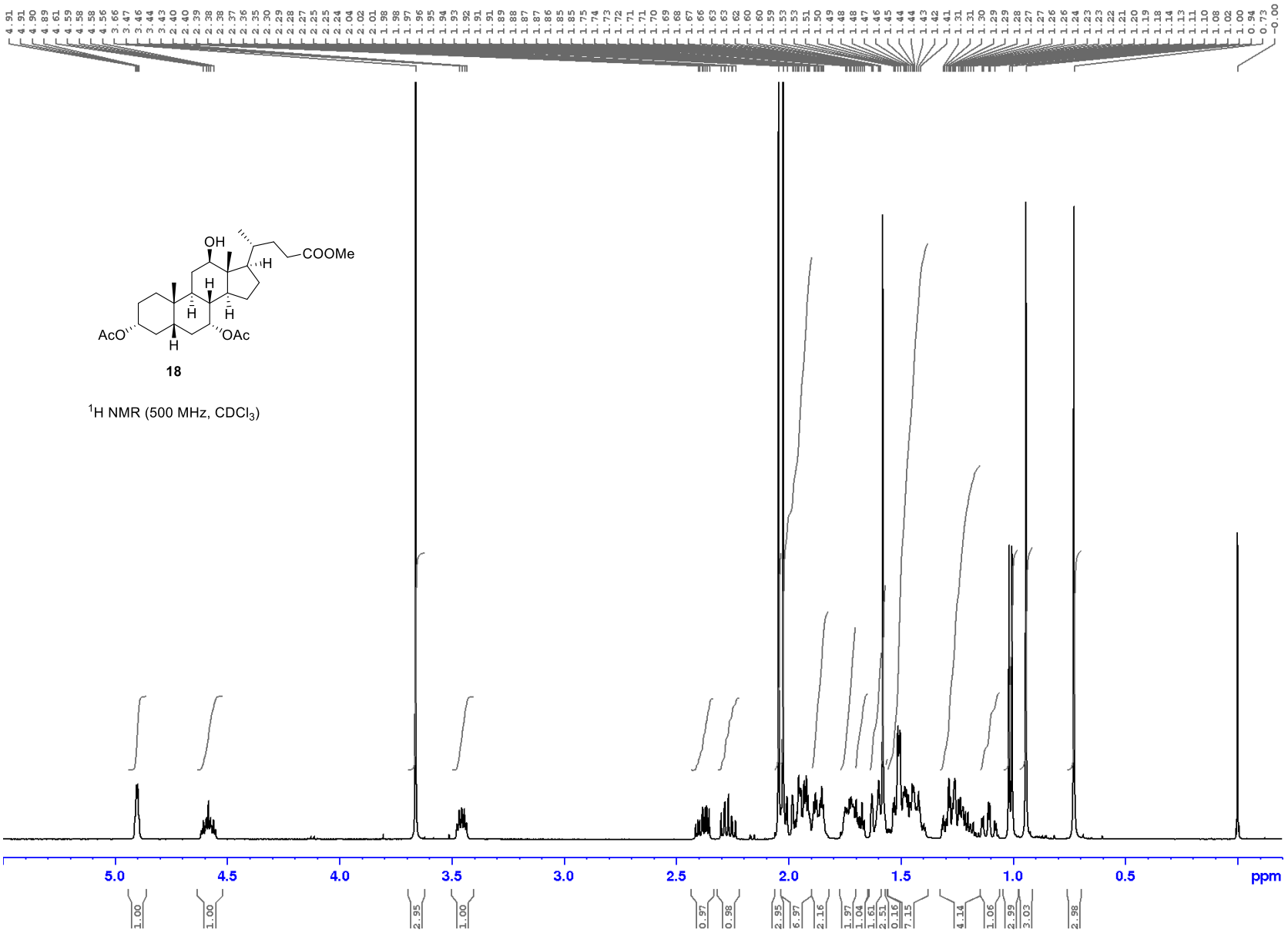
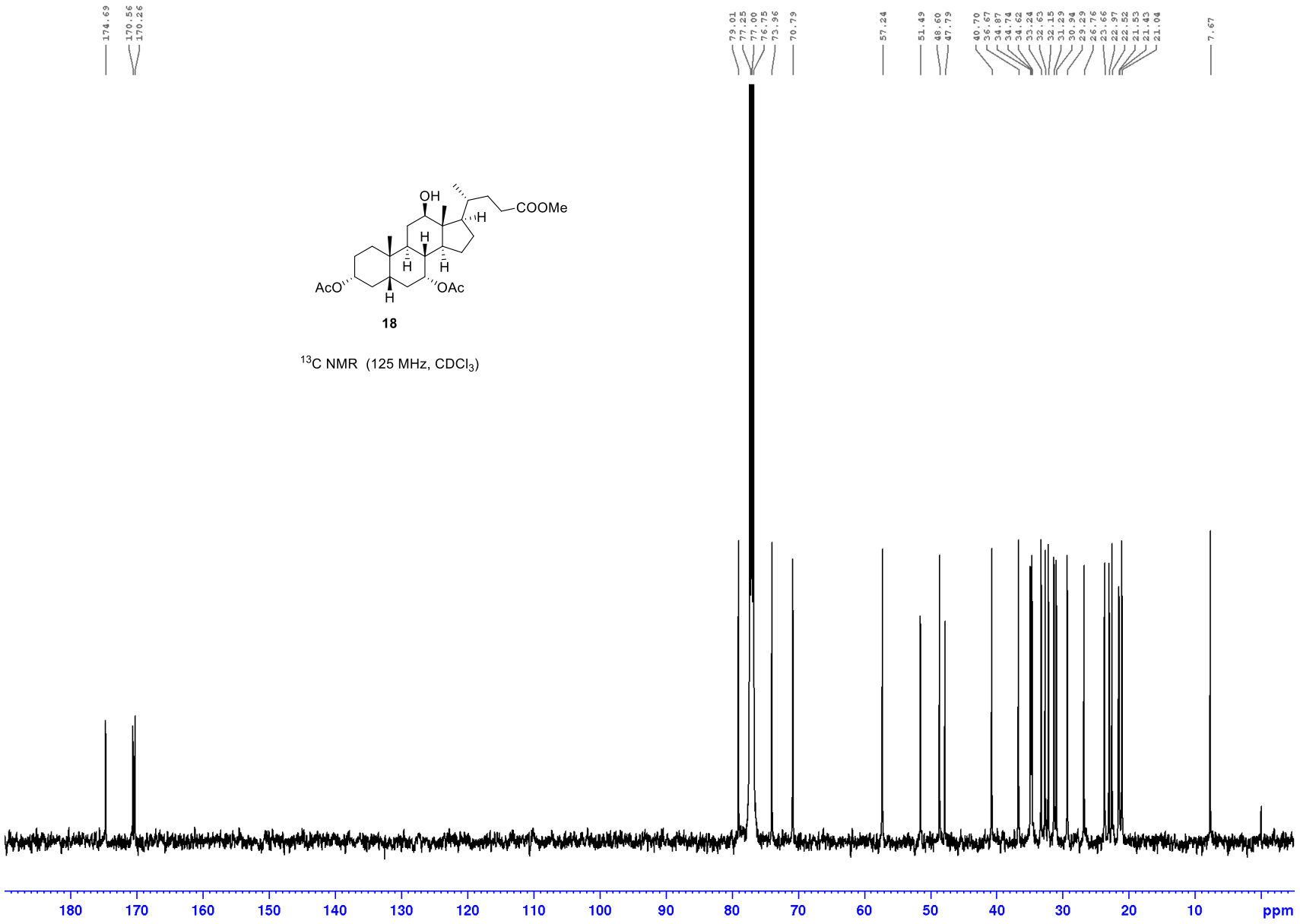
TOF MS ES+
1.77e4
100
%
0
527.2991
528.3030
540.2994
571.3251
563.2861
520.2244
529.3054
543.2761
572.3337
595.2941
487.2585
467.2780
519.2320
544.2787
573.3478
488.2481
530.2980
552.2178
587.2986
459.2336
468.2724
511.3026
450
460
470
480
490
500
510
520
530
540
550
560
570
580
590
600
m/z
O
COOMe
H
AcO
OAc
17
Chemical Formula: $C_{29}H_{44}O_7Na^+$
Exact Mass: 527.2979
Molecular Weight: 527.6532

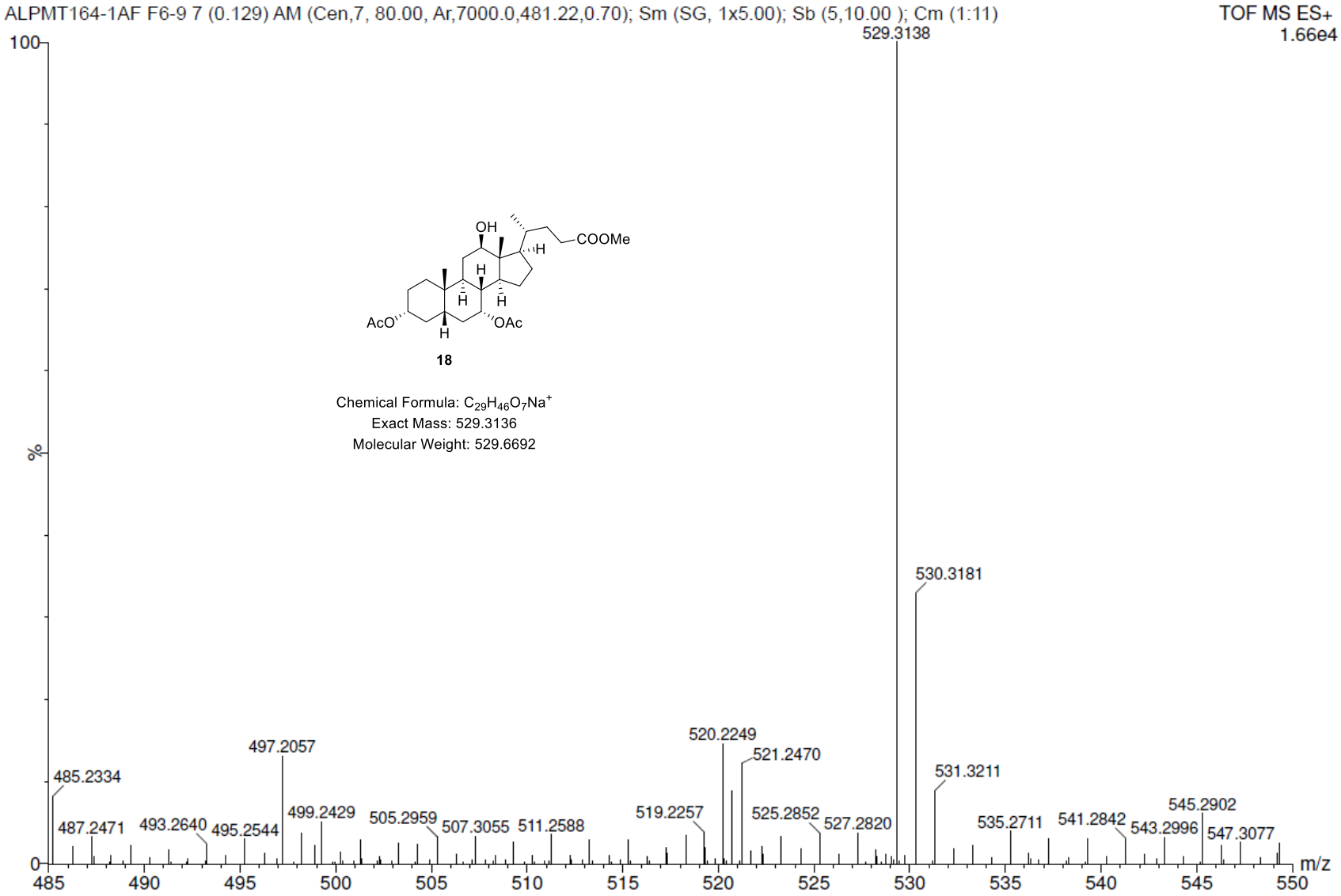

OH
COOMe
H
H
H
H
AcO
OAc
H
18
1H NMR (500 MHz, CDCl3)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm


OH
COOMe
H
H
H
H
AcO
OAc
H
18
1H NMR (500 MHz, CDCl3)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm



18

^{13}C NMR (125 MHz, $CDCl_3$)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TOF MS ES+
1.66e4
529.3138
530.3181
520.2249
521.2470
497.2057
485.2334
531.3211
545.2902
487.2471
493.2640
495.2544
499.2429
505.2959
507.3055
511.2588
519.2257
525.2852
527.2820
535.2711
541.2842
543.2996
547.3077
100
%
0
485
490
495
500
505
510
515
520
525
530
535
540
545
550
m/z
OH
COOMe
H
H
H
H
AcO
OAc
H
18
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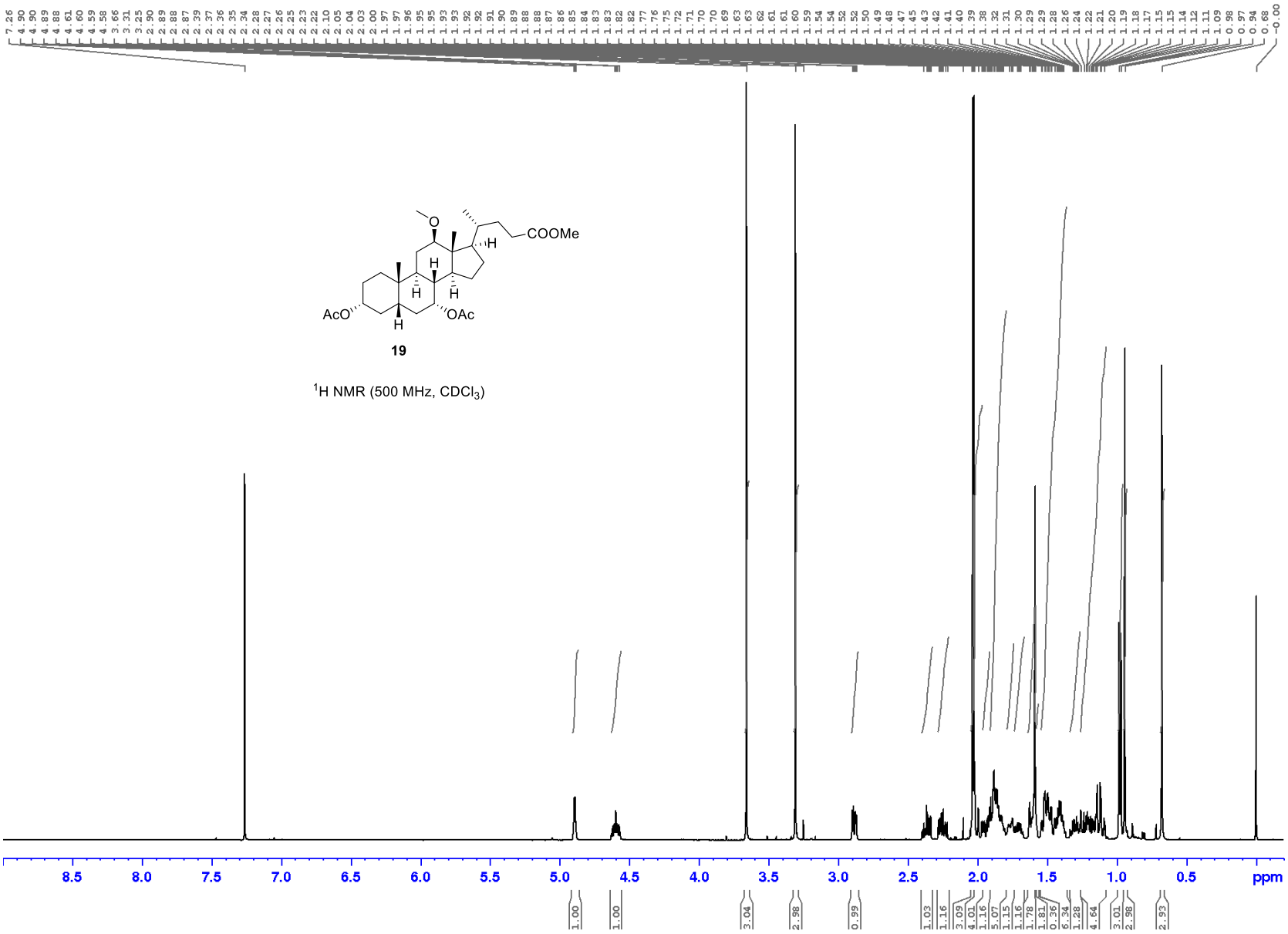
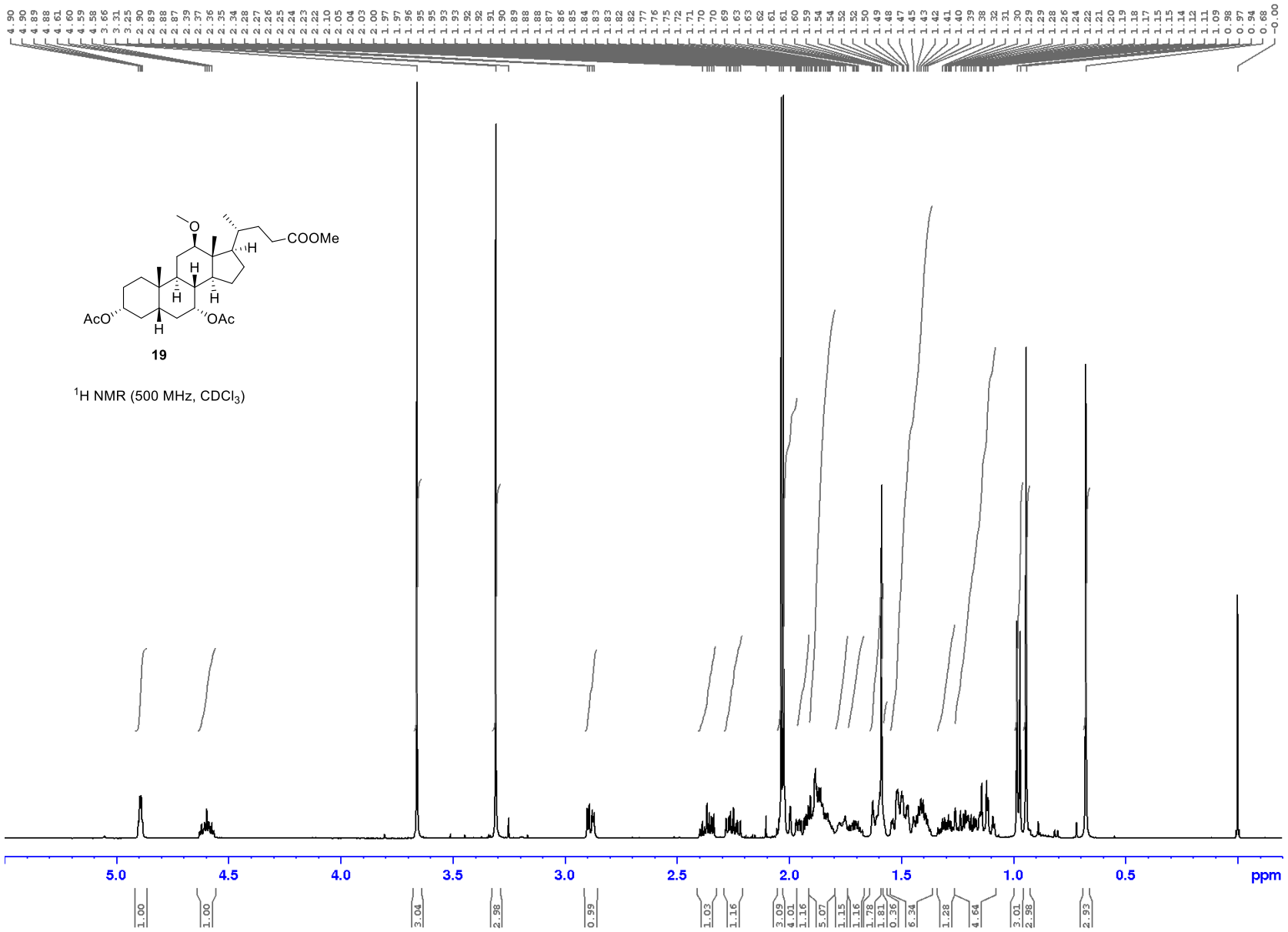
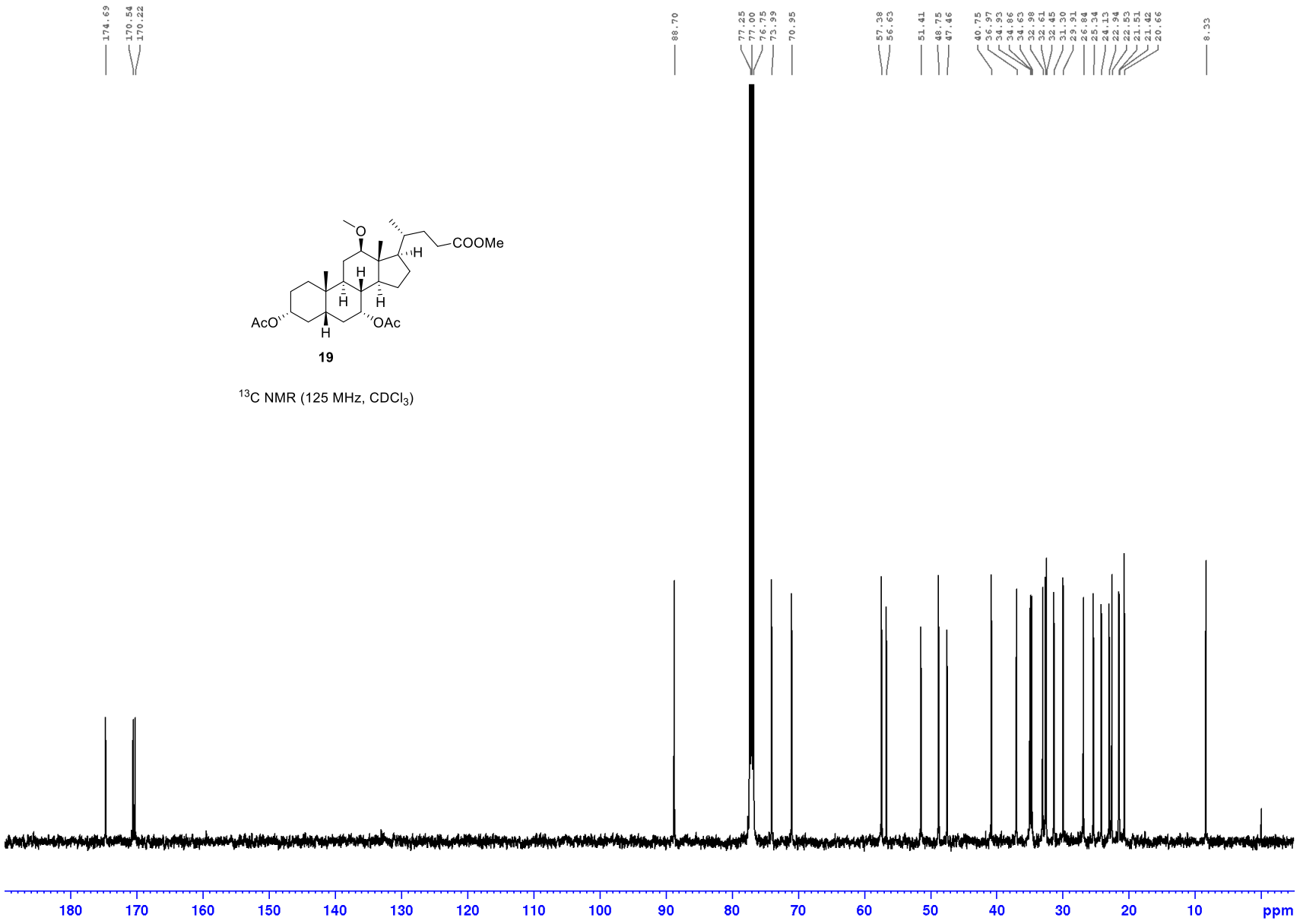
Exact Mass: 529.3136
Molecular Weight: 529.6692

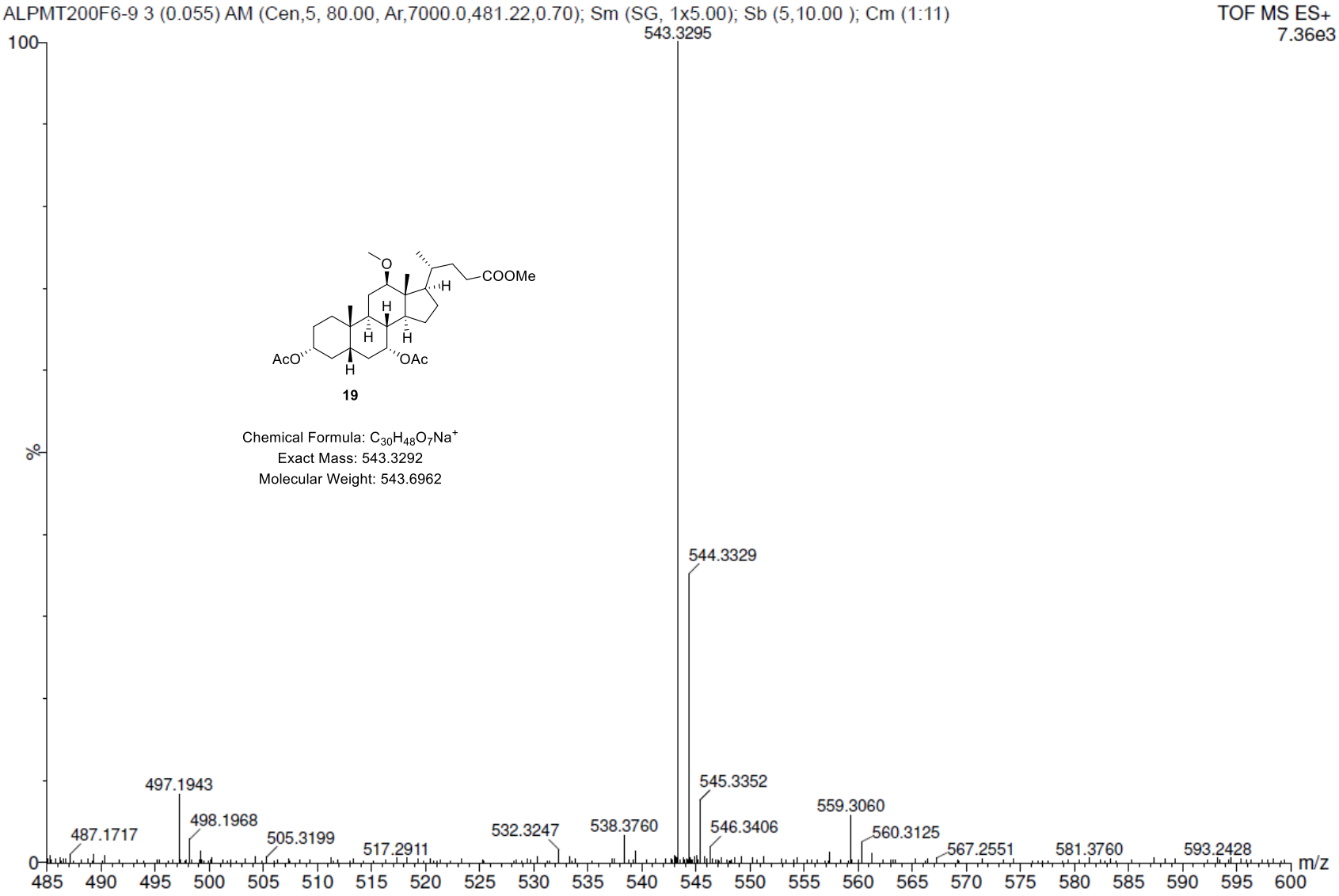

COOMe
AcO
OAc
19
^{1}H NMR (500 MHz, $CDCl_3$)
ppm


COOMe
O
H
H
H
H
AcO
OAc
H
19
^{1}H NMR (500 MHz, $CDCl_3$)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm



19

^{13}C NMR (125 MHz, $CDCl_3$)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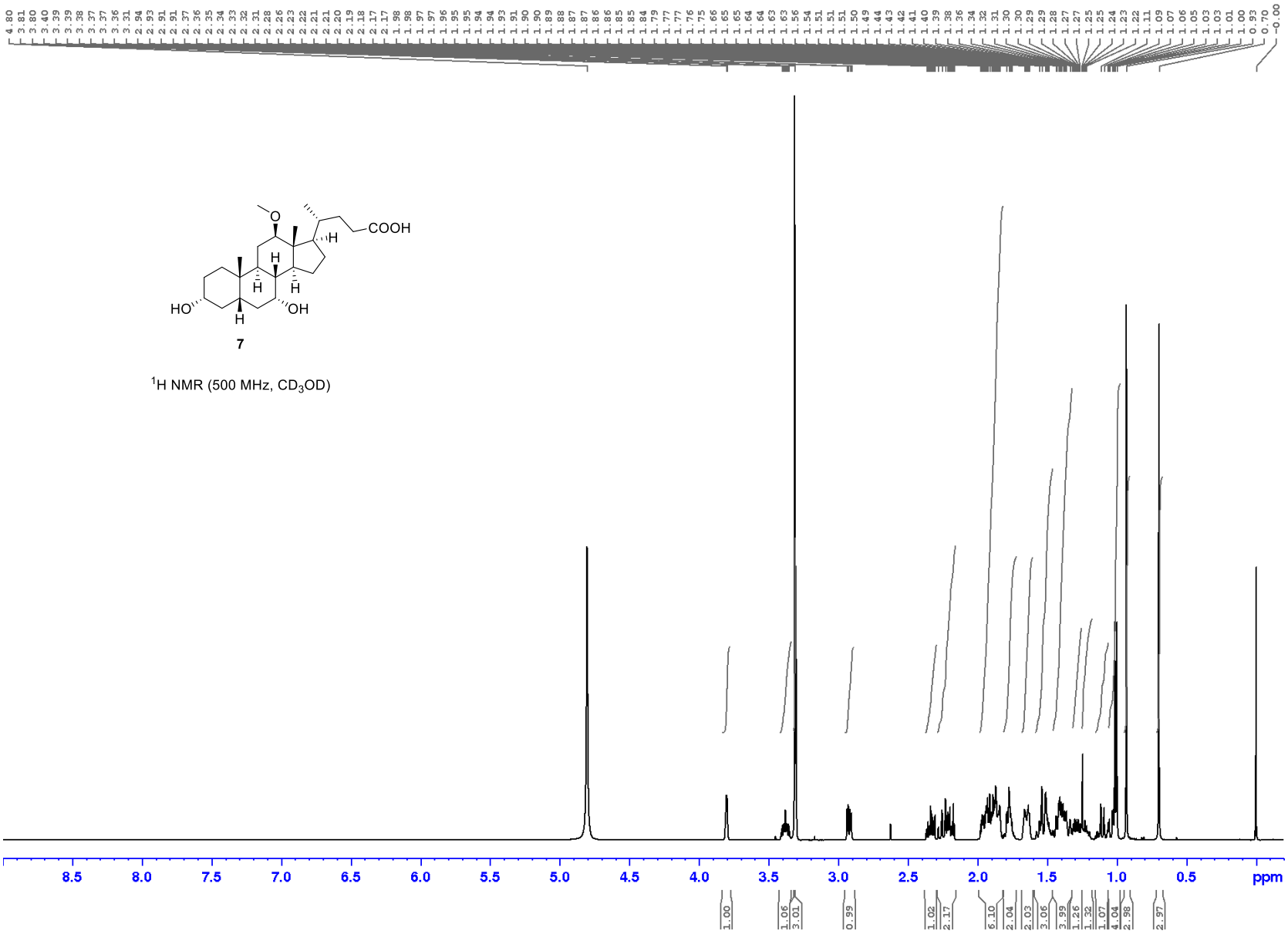
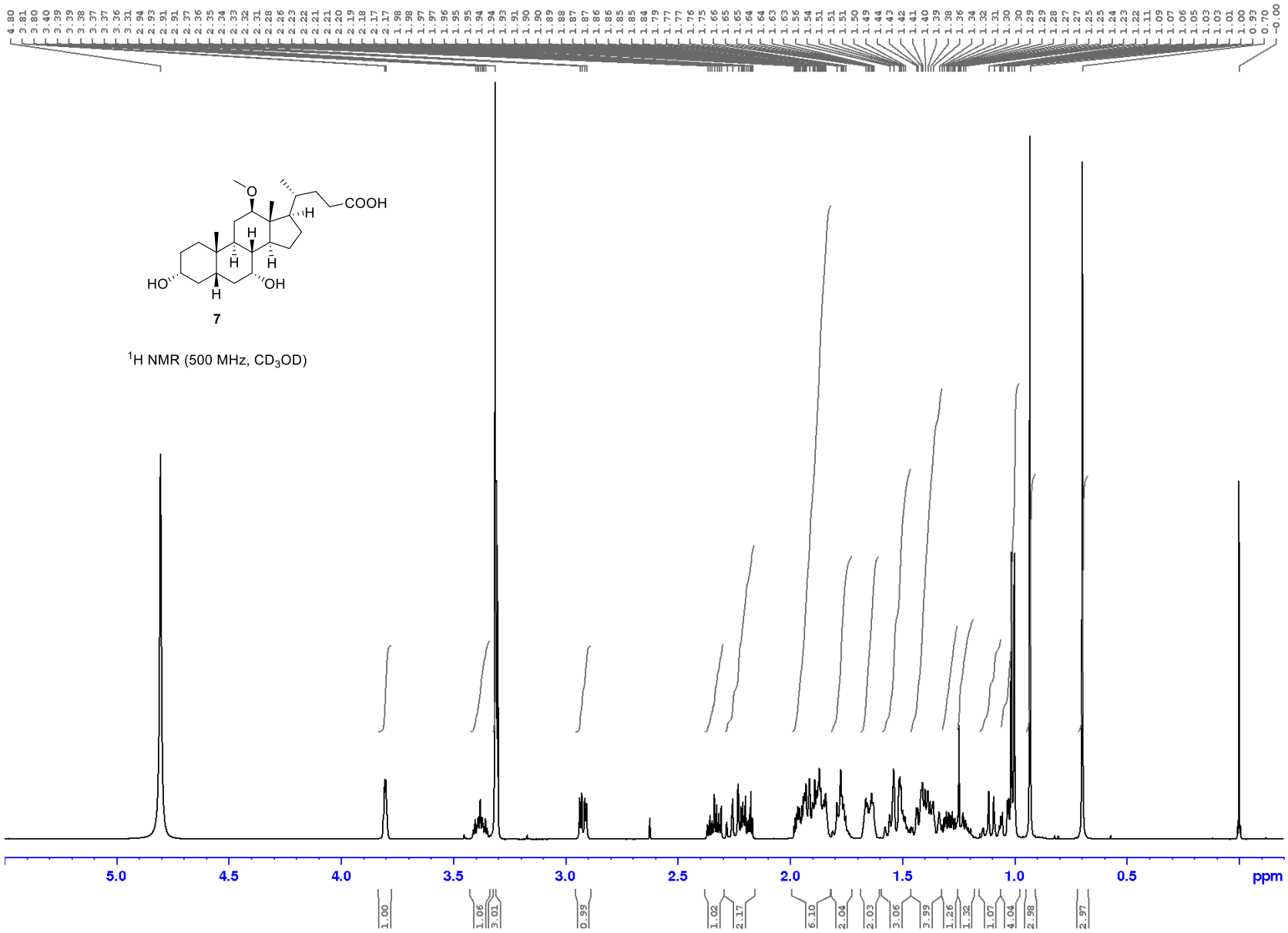
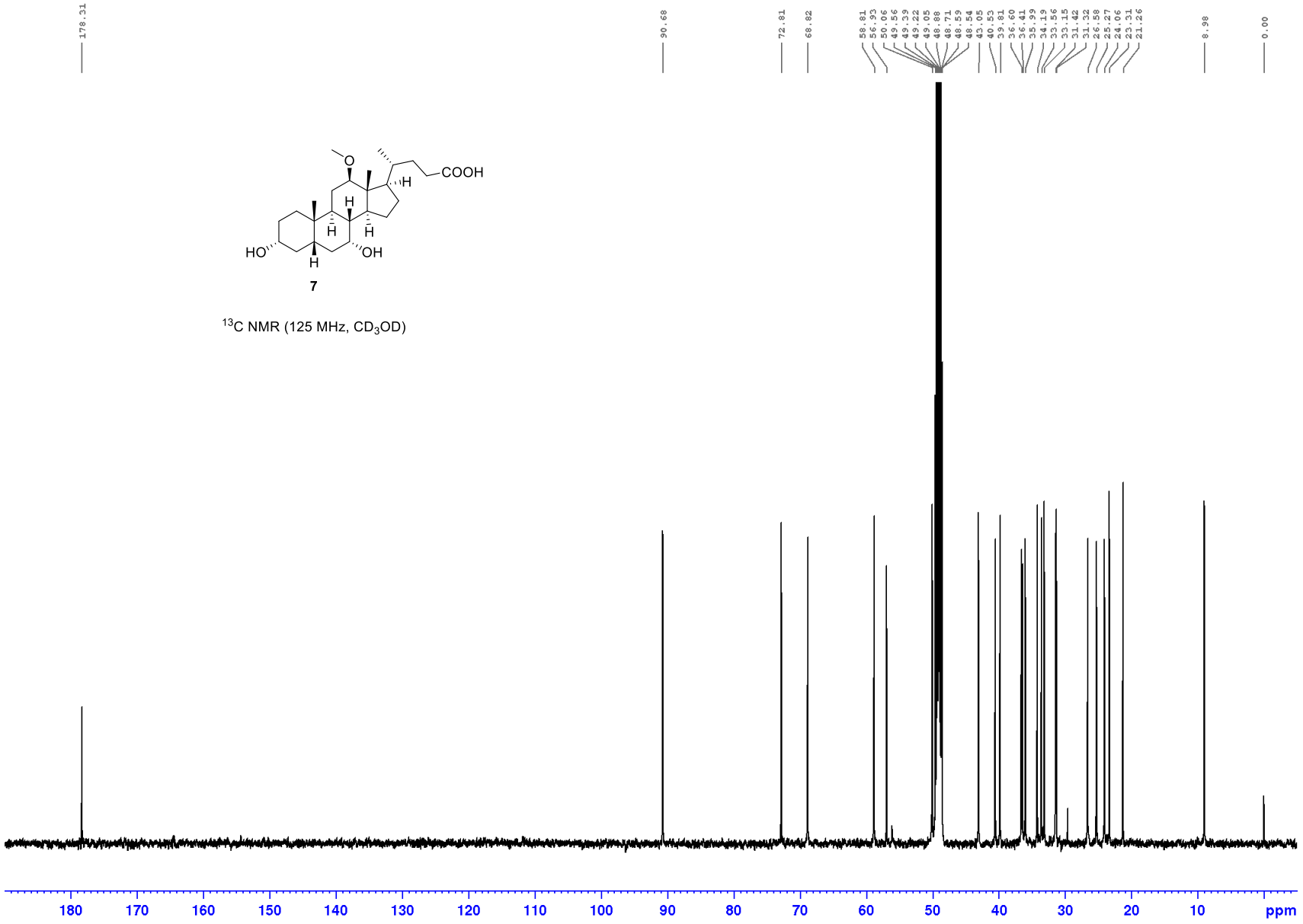
TOF MS ES+
7.36e3
543.3295
544.3329
545.3352
546.3406
559.3060
560.3125
567.2551
581.3760
593.2428
497.1943
498.1968
487.1717
505.3199
517.2911
532.3247
538.3760
100
%
0
m/z
485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600
COOMe
AcO
OAc
19
Chemical Formula: $C_{30}H_{48}O_7Na^+$
Exact Mass: 543.3292
Molecular Weight: 543.6962



7

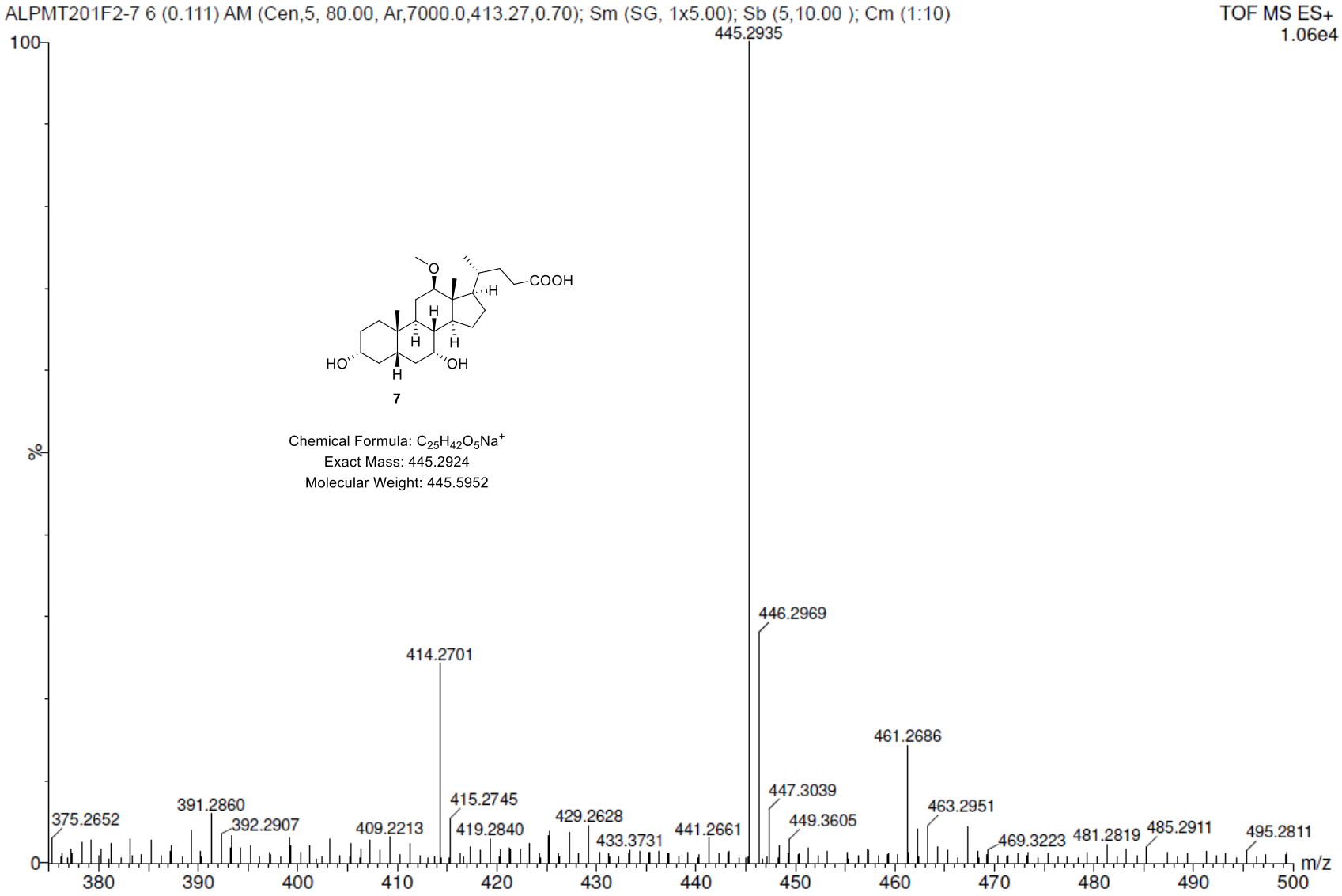
^{1}H NMR (500 MHz, CD_3OD)

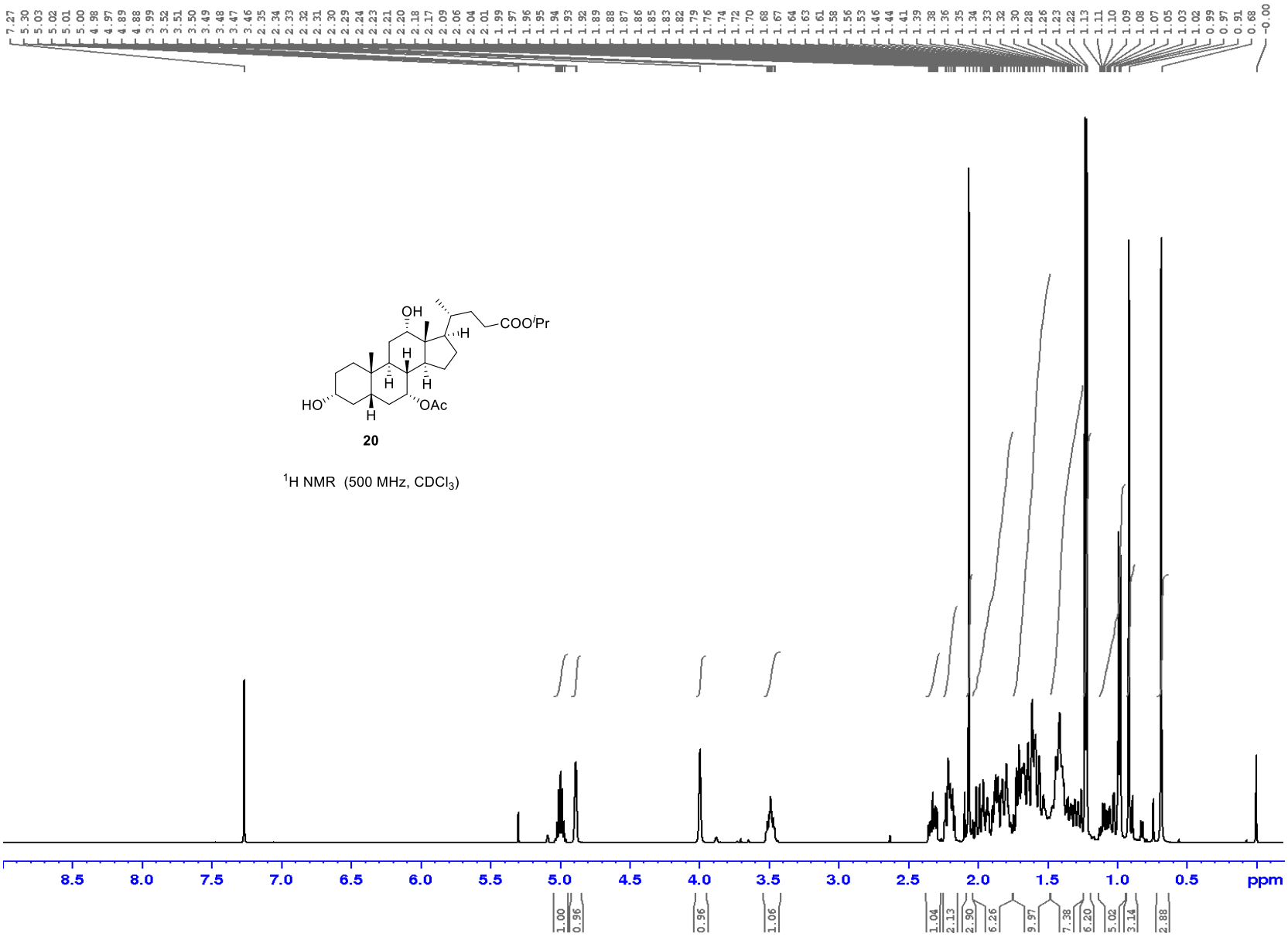

COOH
O
H
H
H
H
HO
OH
H
7
^{1}H NMR (500 MHz, CD_3OD)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm



7

^{13}C NMR (125 MHz, CD_3OD)


ALPMT201F2-7 6 (0.111) AM (Cen,5, 80.00, Ar,7000.0,413.27,0.70); Sm (SG, 1x5.00); Sb (5,10.00); Cm (1:10)
TOF MS ES+
1.06e4
100
%
0
445.2935
446.2969
414.2701
461.2686
447.3039
463.2951
415.2745
391.2860
449.3605
375.2652
392.2907
429.2628
409.2213
419.2840
433.3731
441.2661
469.3223
481.2819
485.2911
495.2811
380
390
400
410
420
430
440
450
460
470
480
490
500
m/z
O
COOH
H
HO
OH
7
Chemical Formula: $C_{25}H_{42}O_5Na^+$
Exact Mass: 445.2924
Molecular Weight: 445.5952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5.02
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3.99
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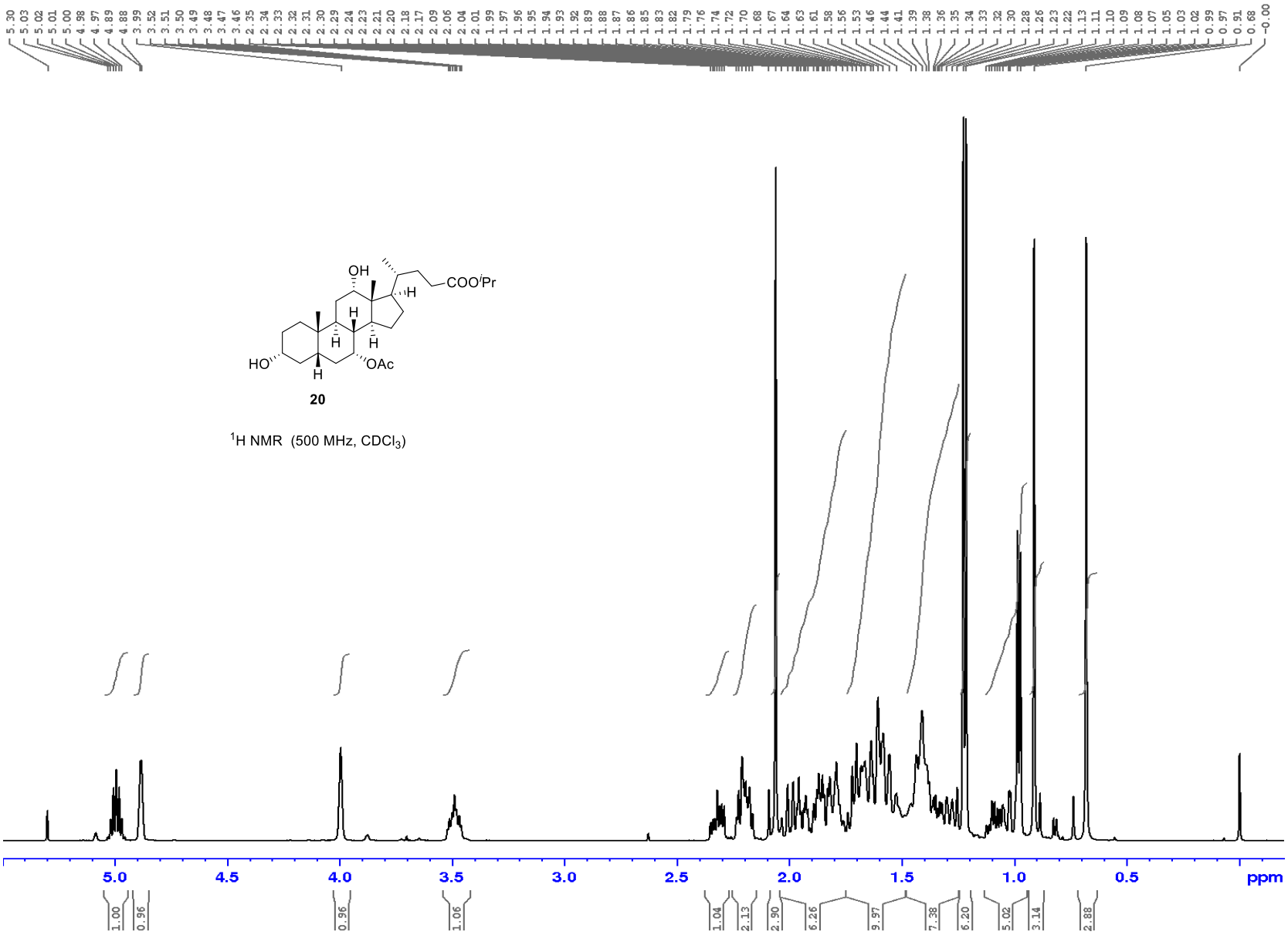
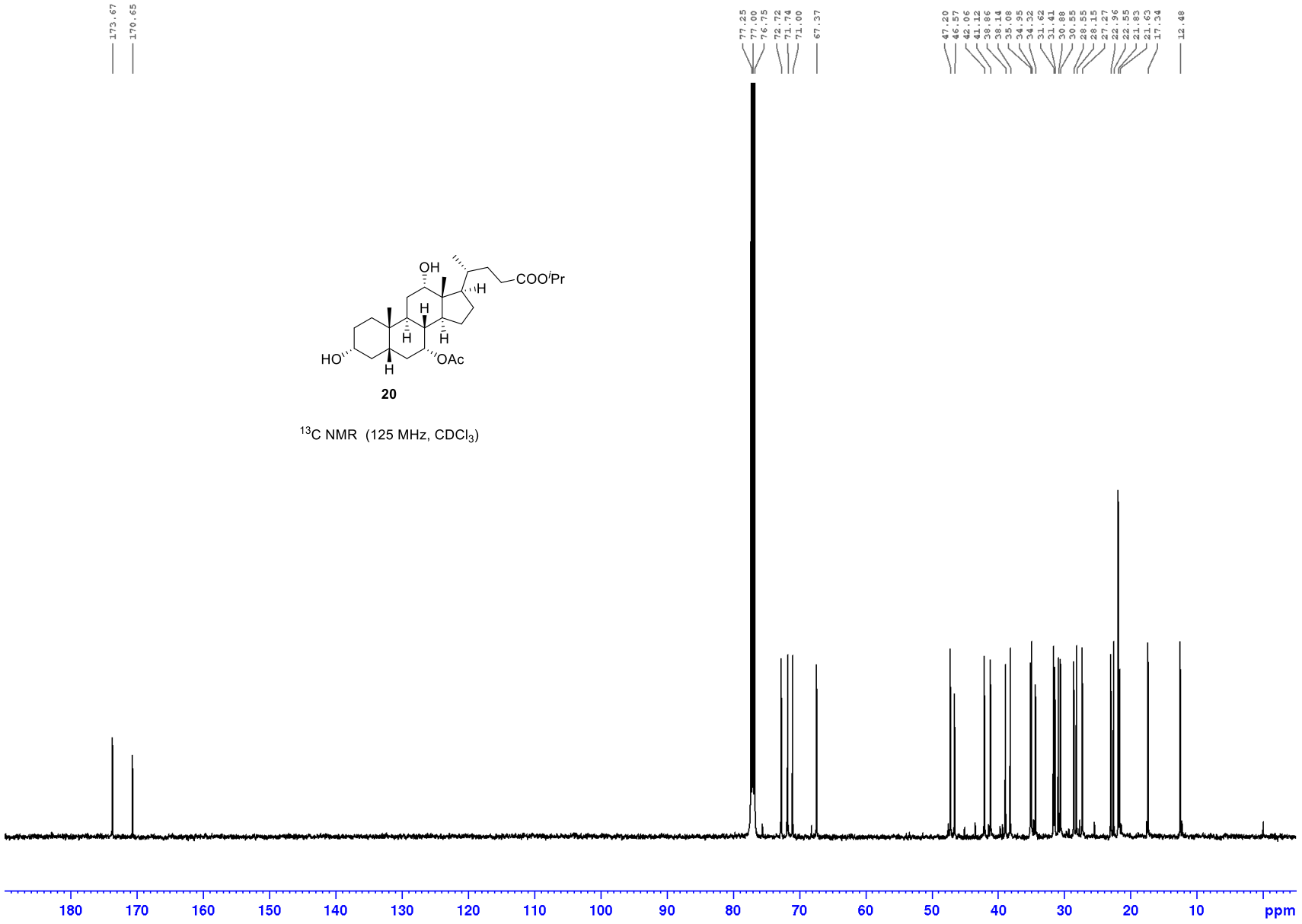
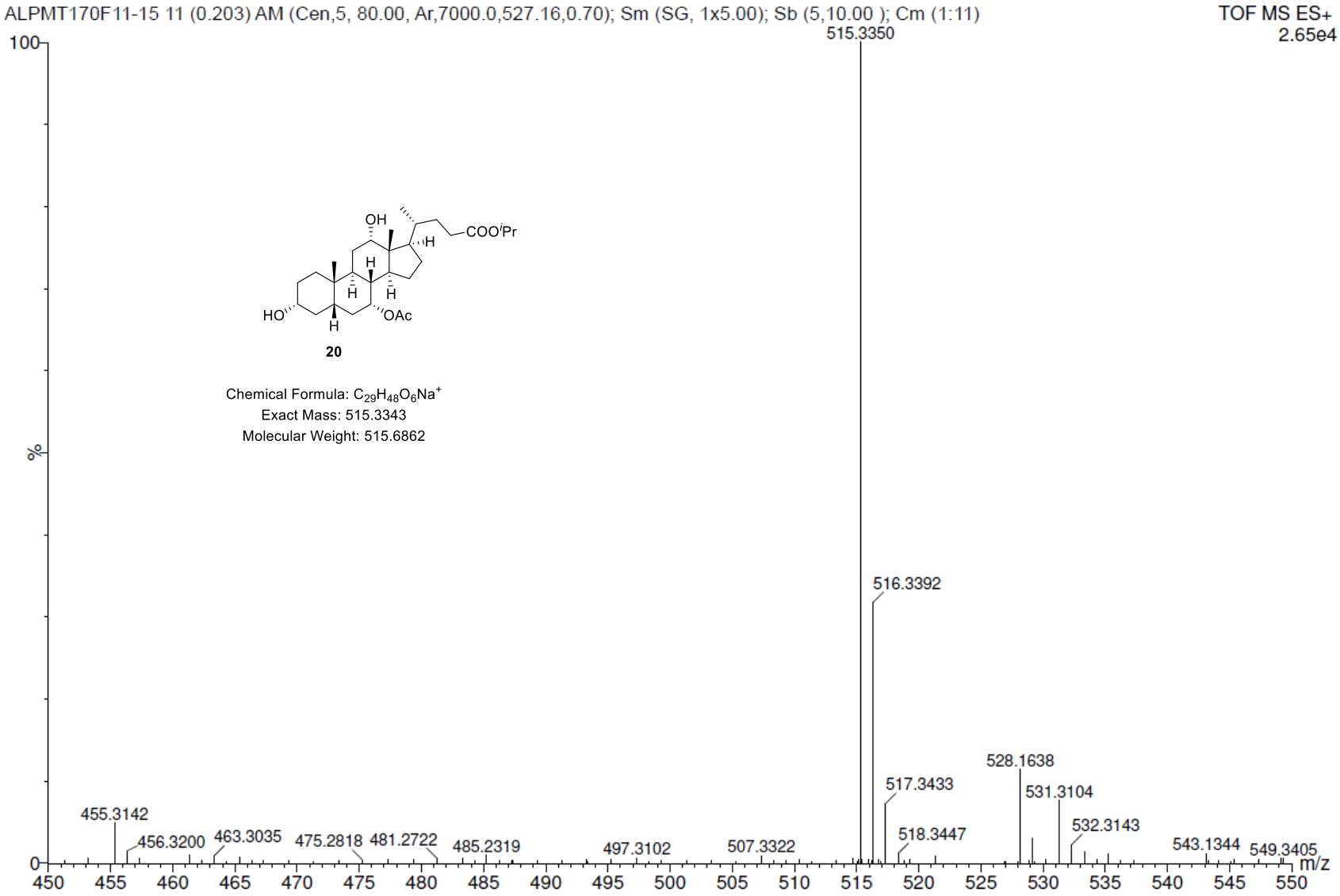
3.50
3.49
3.48
3.47
3.46
2.35
2.34
2.33
2.32
2.31
2.30
2.29
2.24
2.23
2.21
2.20
2.18
2.17
2.09
2.06
2.04
2.01
1.99
1.97
1.96
1.95
1.94
1.93
1.92
1.89
1.88
1.87
1.86
1.85
1.83
1.82
1.79
1.76
1.74
1.72
1.70
1.68
1.67
1.64
1.63
1.61
1.58
1.56
1.53
1.46
1.44
1.41
1.39
1.38
1.36
1.35
1.34
1.33
1.32
1.30
1.28
1.26
1.23
1.22
1.13
1.11
1.10
1.09
1.08
1.07
1.05
1.03
1.02
0.99
0.97
0.91
0.68
-0.00
OH
COO^{i}Pr
H
H
H
H
HO
OAc
H
20
^{1}H NMR (500 MHz, $CDCl_3$)
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
ppm
1.00
0.96
0.96
1.06
1.04
2.13
2.90
6.26
9.97
7.38
6.20
5.02
3.14
2.88


5.30 5.03 5.02 5.01 5.00 4.98 4.97 4.89 4.88 3.99 3.52 3.51 3.50 3.49 3.48 3.47 3.46 2.35 2.34 2.33 2.32 2.31 2.30 2.29 2.24 2.23 2.21 2.20 2.18 2.17 2.09 2.06 2.04 2.01 1.99 1.97 1.96 1.95 1.94 1.93 1.92 1.89 1.88 1.87 1.86 1.85 1.83 1.82 1.79 1.76 1.74 1.72 1.70 1.68 1.67 1.64 1.63 1.61 1.58 1.56 1.53 1.46 1.44 1.41 1.39 1.38 1.36 1.35 1.34 1.33 1.32 1.30 1.28 1.26 1.23 1.22 1.13 1.11 1.10 1.09 1.08 1.07 1.05 1.03 1.02 0.99 0.97 0.91 0.68 -0.00
OH
COO^{i}Pr
H
H
H
H
HO
OAc
H
20
^{1}H NMR (500 MHz, $CDCl_3$)
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00 0.96 0.96 1.06 1.04 2.13 2.90 6.26 9.97 7.38 6.20 5.02 3.14 2.88



20

^{13}C NMR (125 MHz, $CDCl_3$)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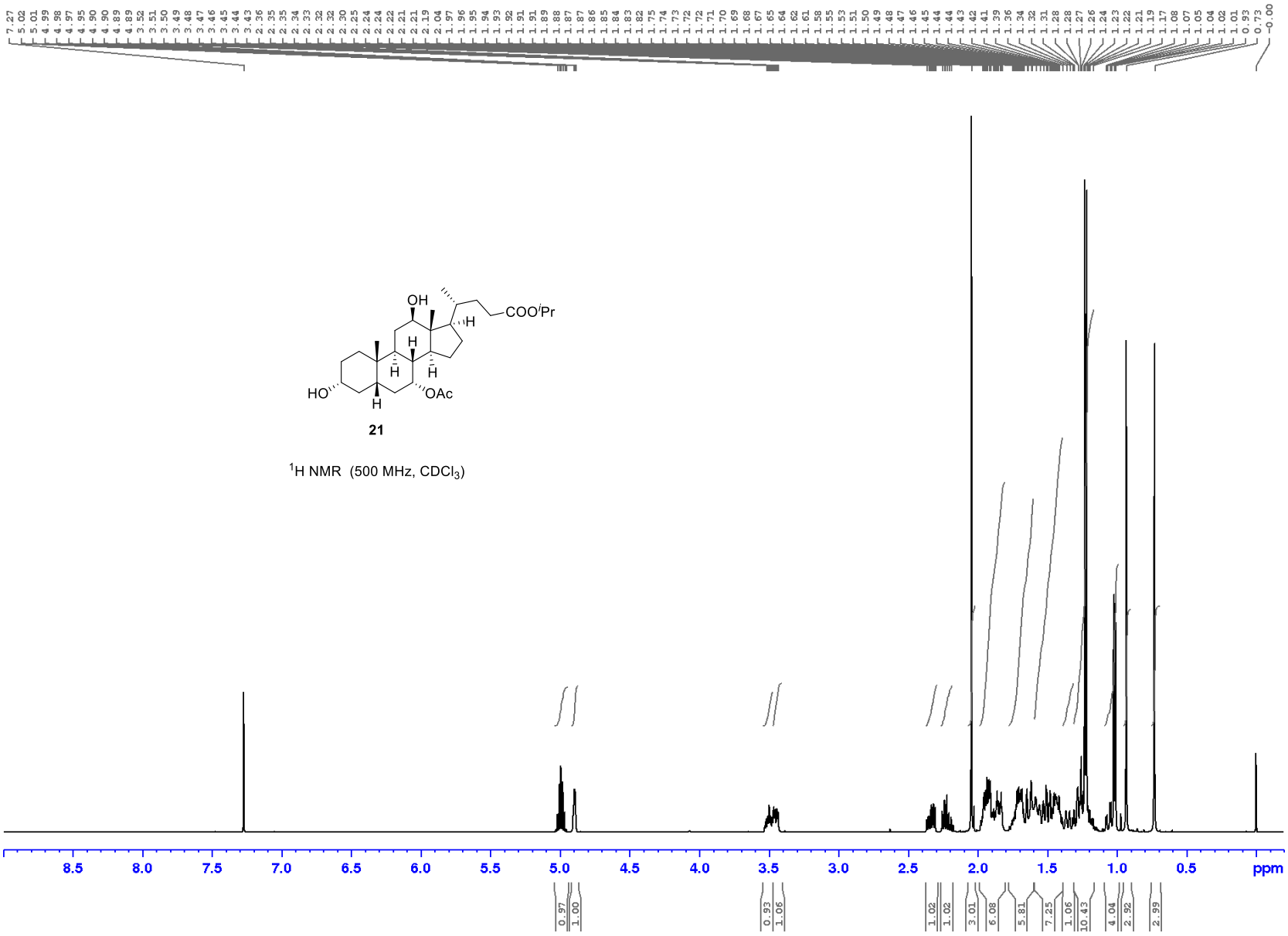
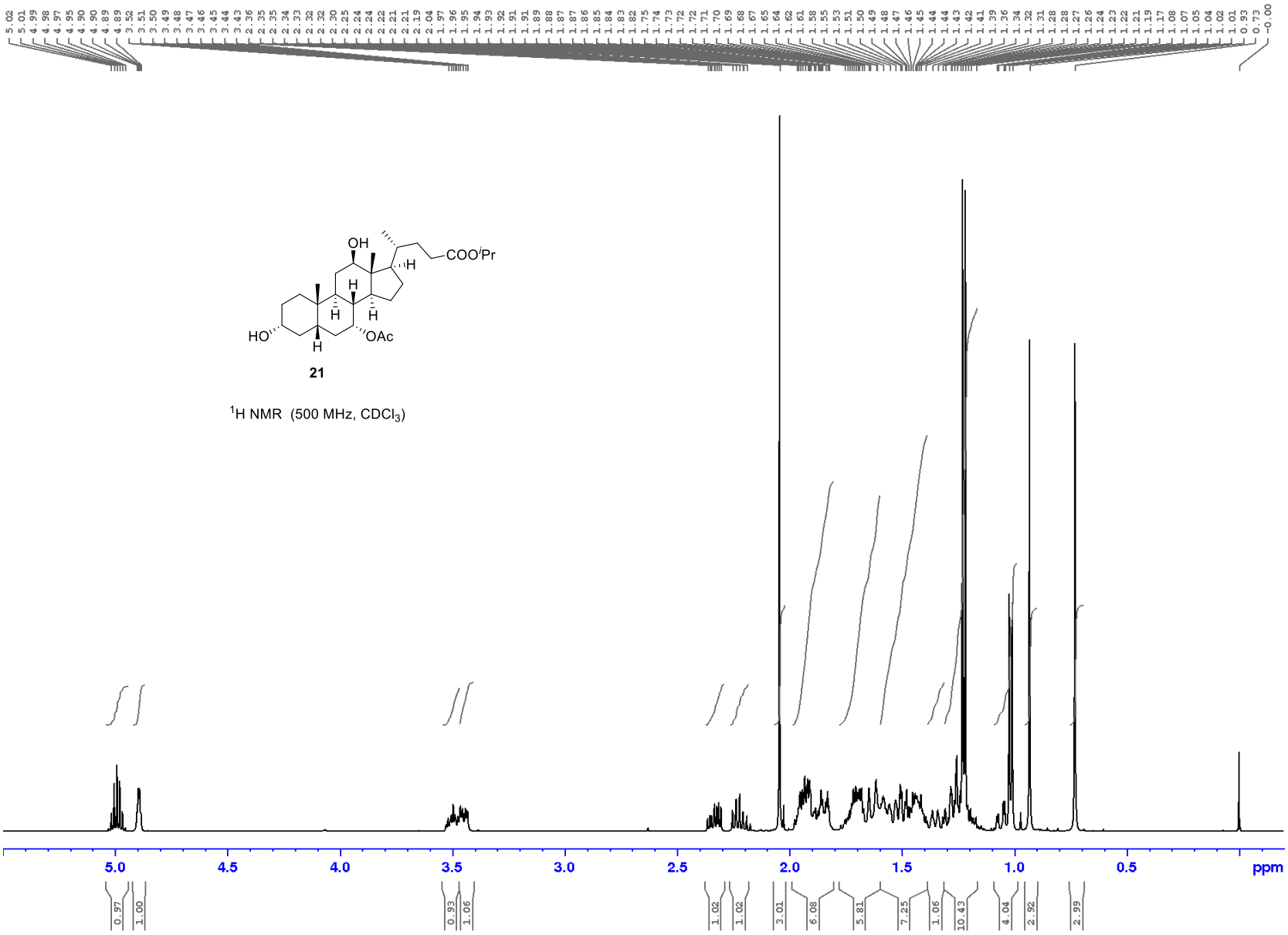
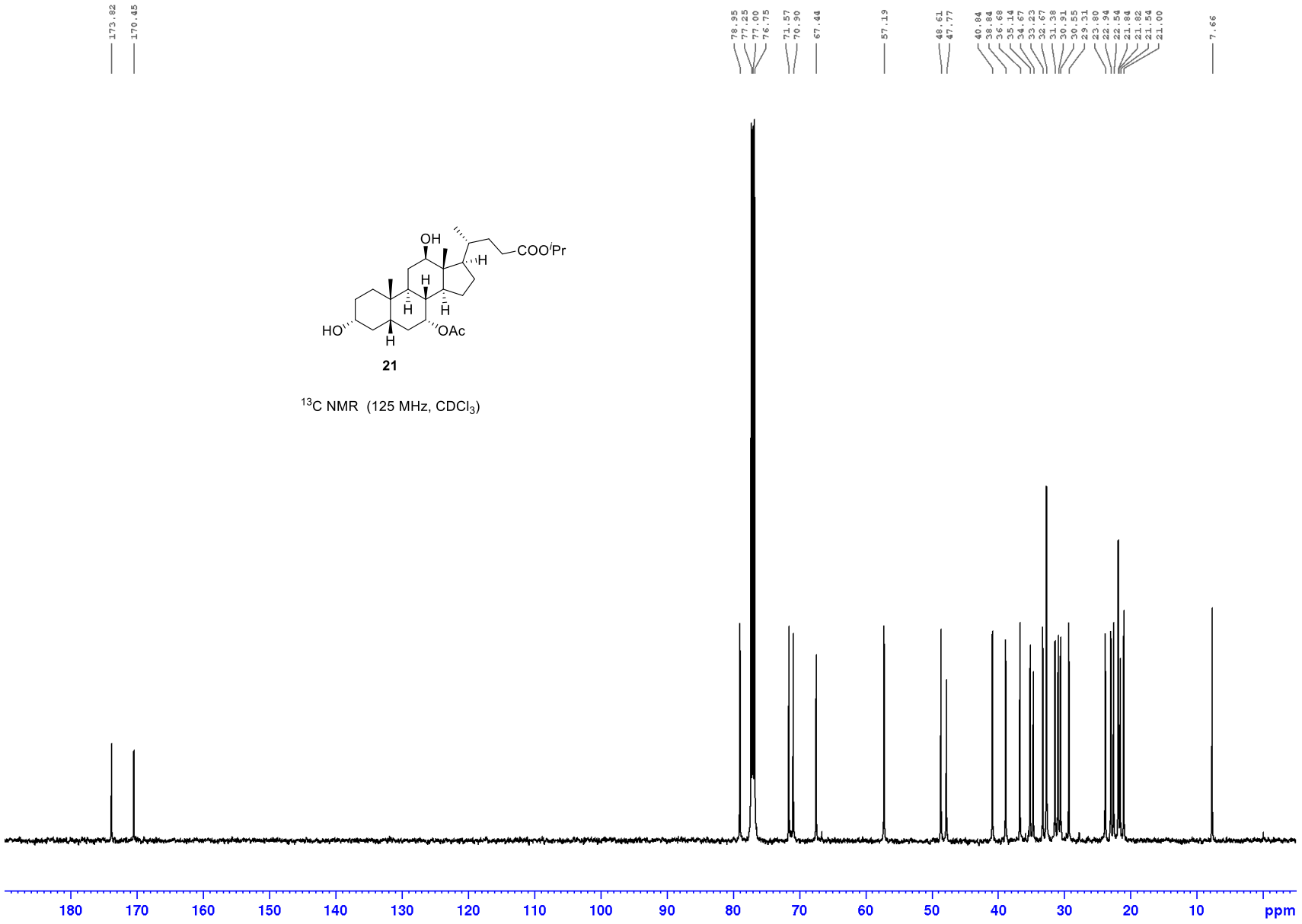
TOF MS ES+
2.65e4
100
%
0
OH
COO^{i}Pr
H
H
H
H
HO
OAc
H
20
Chemical Formula: $C_{29}H_{48}O_6Na^+$
Exact Mass: 515.3343
Molecular Weight: 515.6862
515.3350
516.3392
528.1638
517.3433
531.3104
532.3143
518.3447
455.3142
456.3200
463.3035
475.2818
481.2722
485.2319
497.3102
507.3322
543.1344
549.3405
m/z
450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550

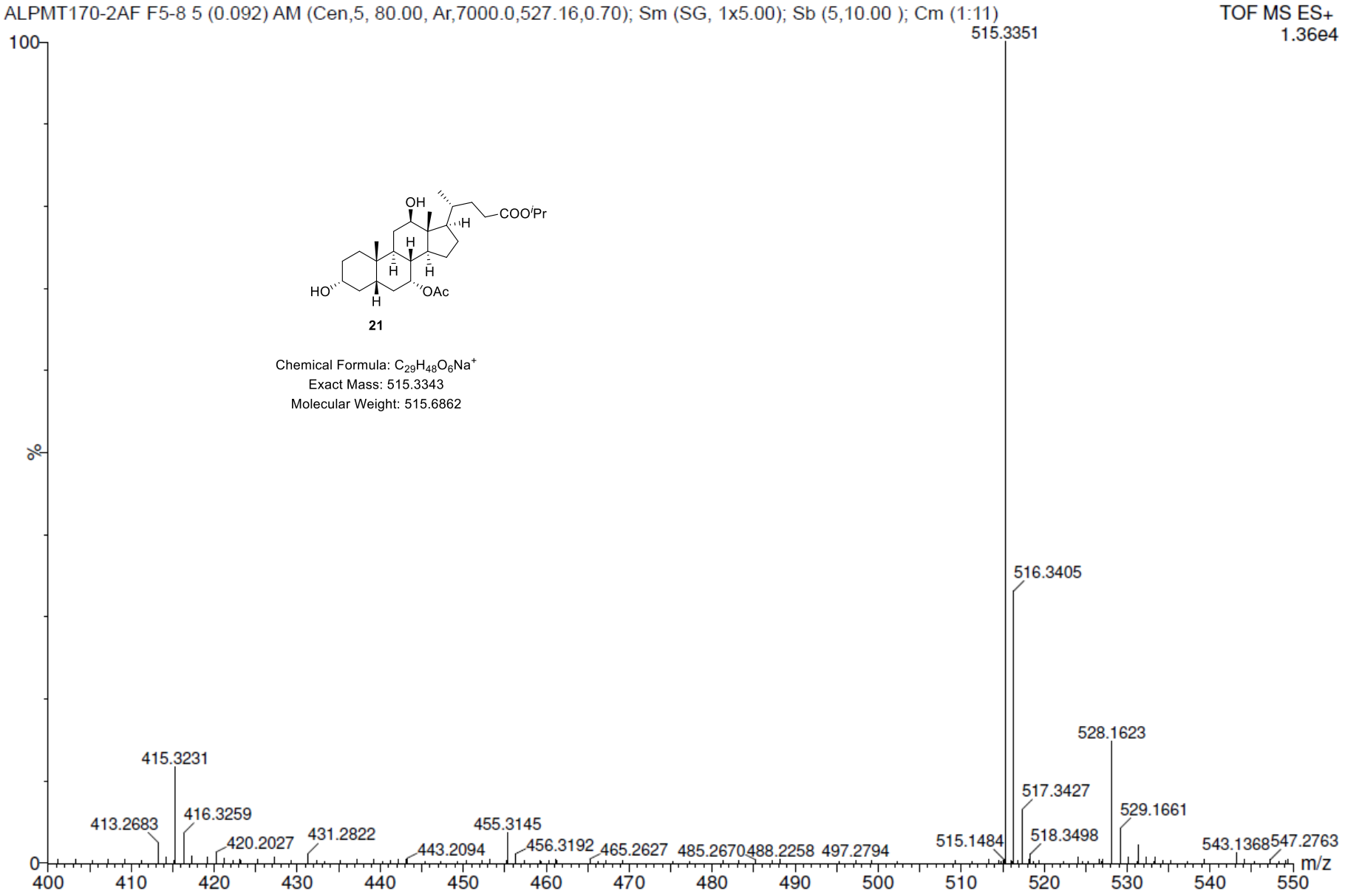

7.27 5.02 5.01 4.99 4.98 4.97 4.95 4.90 4.90 4.89 4.89 3.52 3.51 3.50 3.49 3.48 3.47 3.46 3.45 3.44 3.43 2.36 2.35 2.35 2.34 2.33 2.32 2.32 2.30 2.25 2.24 2.24 2.22 2.21 2.21 2.19 2.04 1.97 1.96 1.95 1.94 1.93 1.92 1.91 1.91 1.89 1.88 1.87 1.87 1.86 1.85 1.84 1.83 1.82 1.75 1.74 1.73 1.72 1.72 1.71 1.70 1.69 1.68 1.67 1.65 1.64 1.62 1.61 1.58 1.55 1.53 1.51 1.50 1.49 1.48 1.47 1.46 1.45 1.44 1.44 1.43 1.42 1.41 1.39 1.36 1.34 1.32 1.31 1.28 1.28 1.27 1.26 1.24 1.23 1.22 1.21 1.19 1.17 1.08 1.07 1.05 1.04 1.02 1.01 0.93 0.73 -0.00
OH
COO^{i}Pr
H
H
H
H
HO
OAc
H
21
^{1}H NMR (500 MHz, $CDCl_3$)
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 ppm
0.97 1.00 0.93 1.06 1.02 1.02 3.01 6.08 5.81 7.25 1.06 10.43 4.04 2.92 2.99

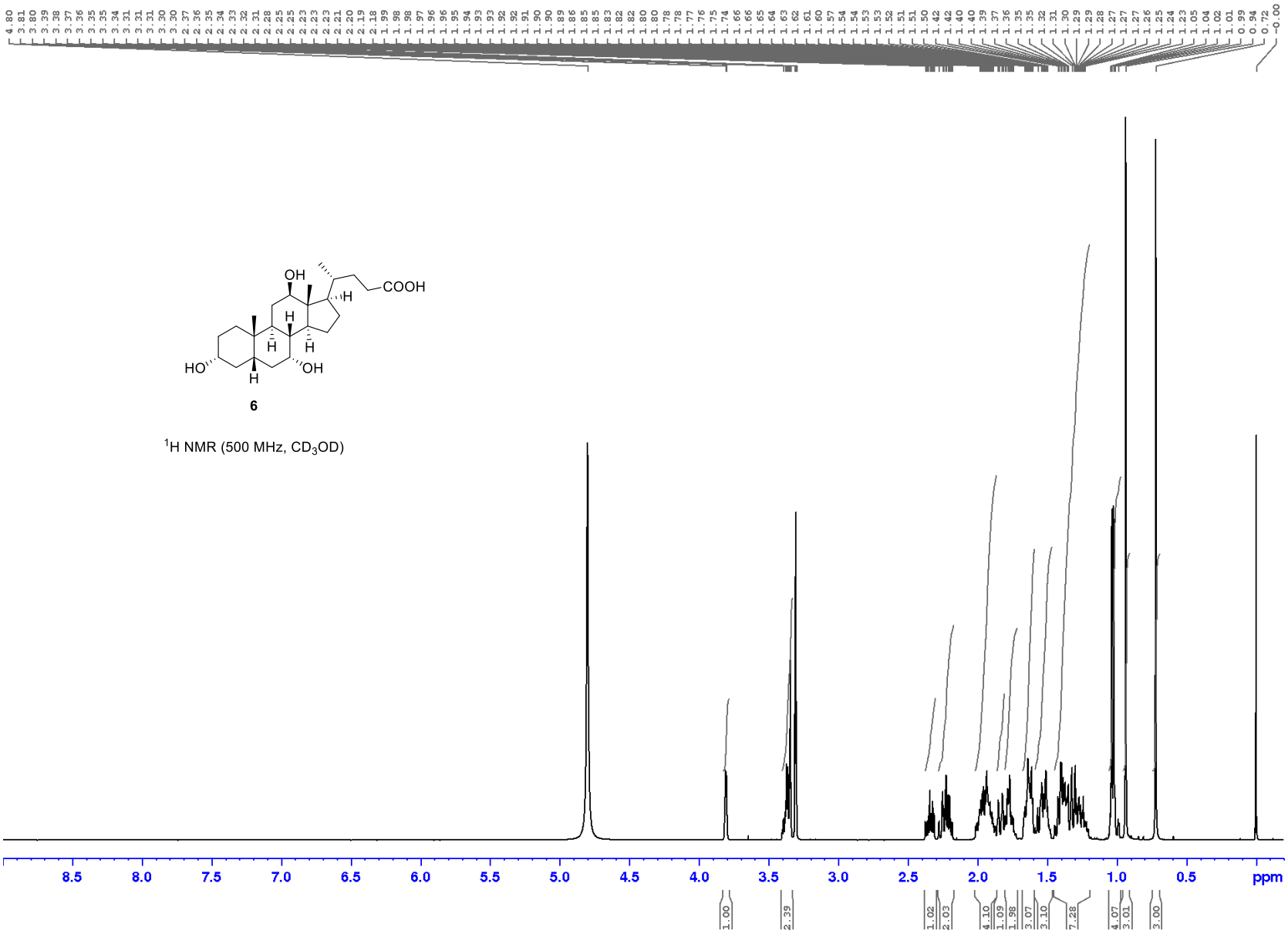

OH
COO^{i}Pr
H
H
H
H
HO
OAc
H
21
^{1}H NMR (500 MHz, $CDCl_3$)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.97
1.00
0.93
1.06
1.02
1.02
3.01
6.08
5.81
7.25
1.06
10.43
4.04
2.92
2.99

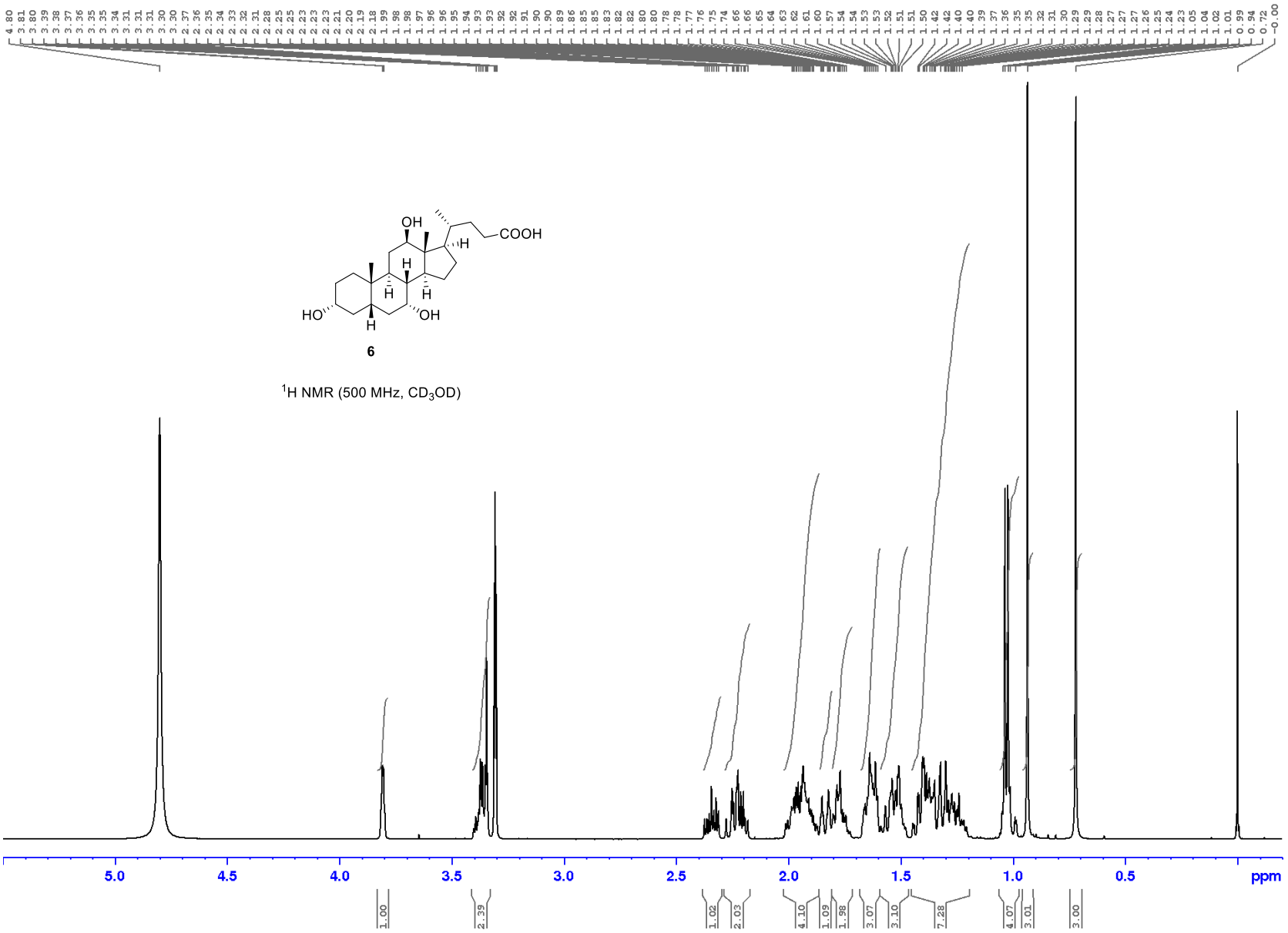


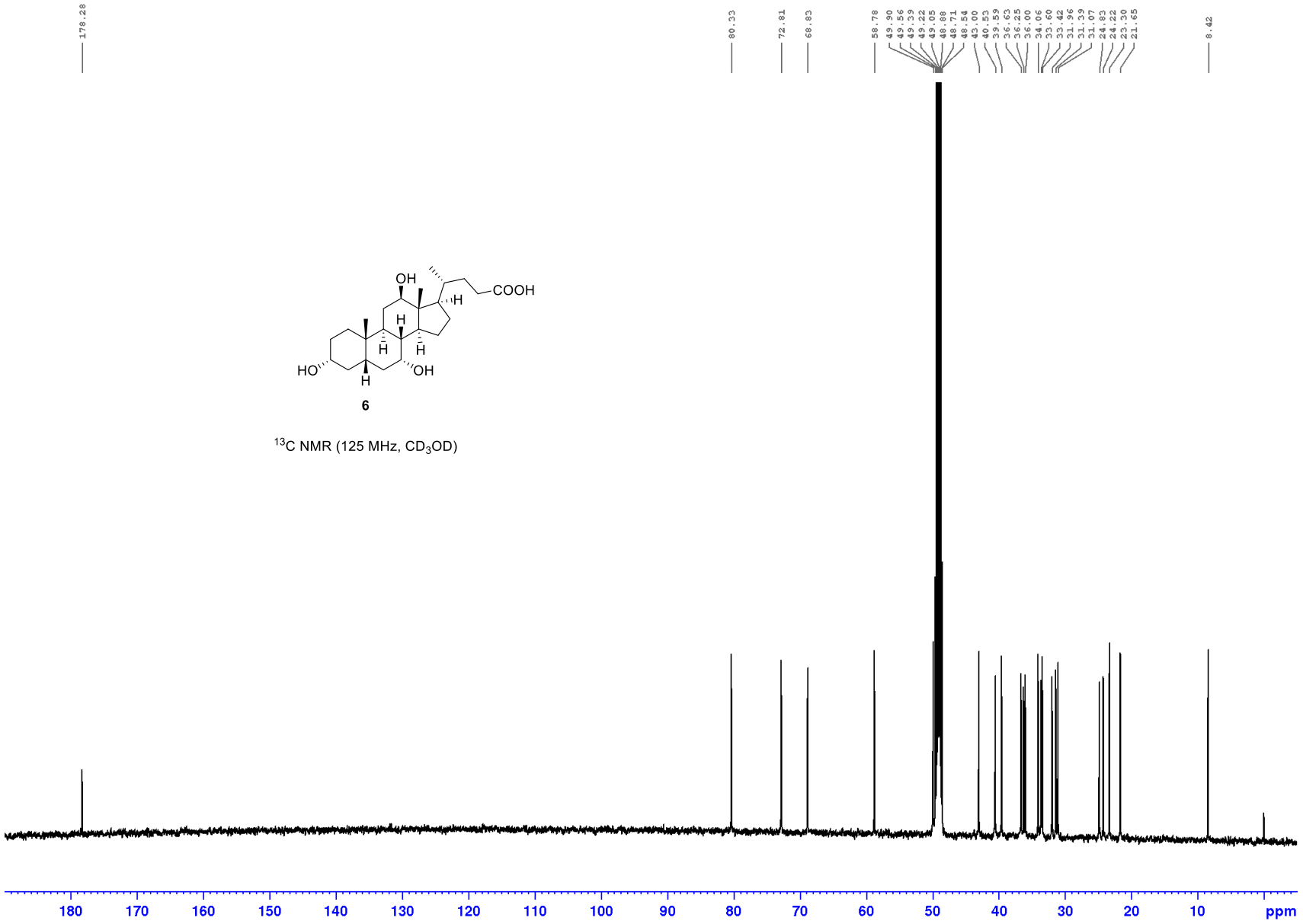
21

^{13}C NMR (125 MHz, $CDCl_3$)


ALPMT170-2AF F5-8 5 (0.092) AM (Cen,5, 80.00, Ar,7000.0,527.16,0.70); Sm (SG, 1x5.00); Sb (5,10.00); Cm (1:11)
TOF MS ES+
1.36e4
100
%
0
515.3351
516.3405
528.1623
517.3427
529.1661
518.3498
515.1484
543.1368
547.2763
415.3231
413.2683
416.3259
420.2027
431.2822
443.2094
455.3145
456.3192
465.2627
485.2670
488.2258
497.2794
m/z
400
410
420
430
440
450
460
470
480
490
500
510
520
530
540
550
OH
COO^{i}Pr
H
HO
OAc
21
Chemical Formula: $C_{29}H_{48}O_6Na^+$
Exact Mass: 515.3343
Molecular Weight: 515.6862

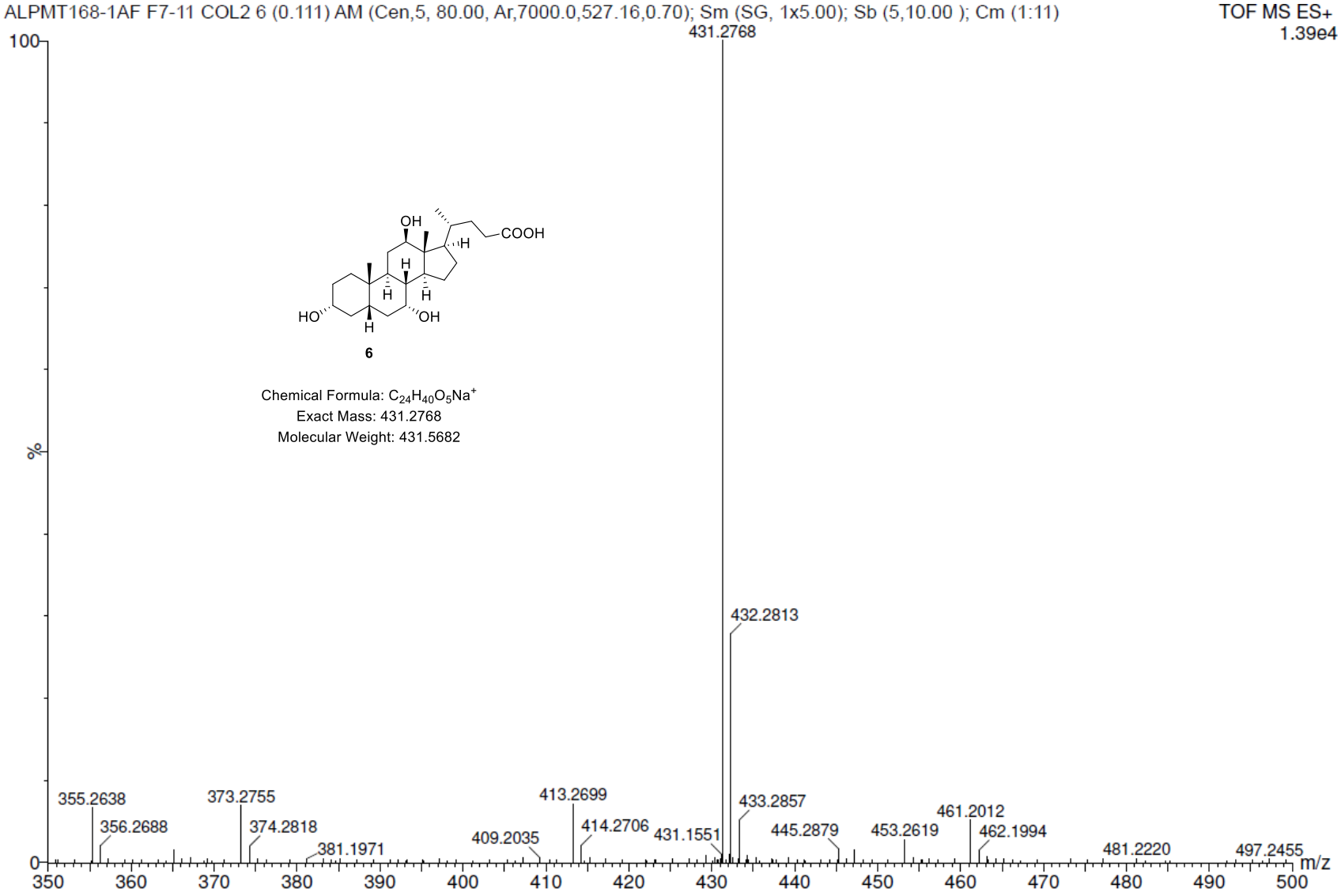

OH
COOH
H
H
H
H
HO
H
OH
6
1H NMR (500 MHz, CD3OD)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

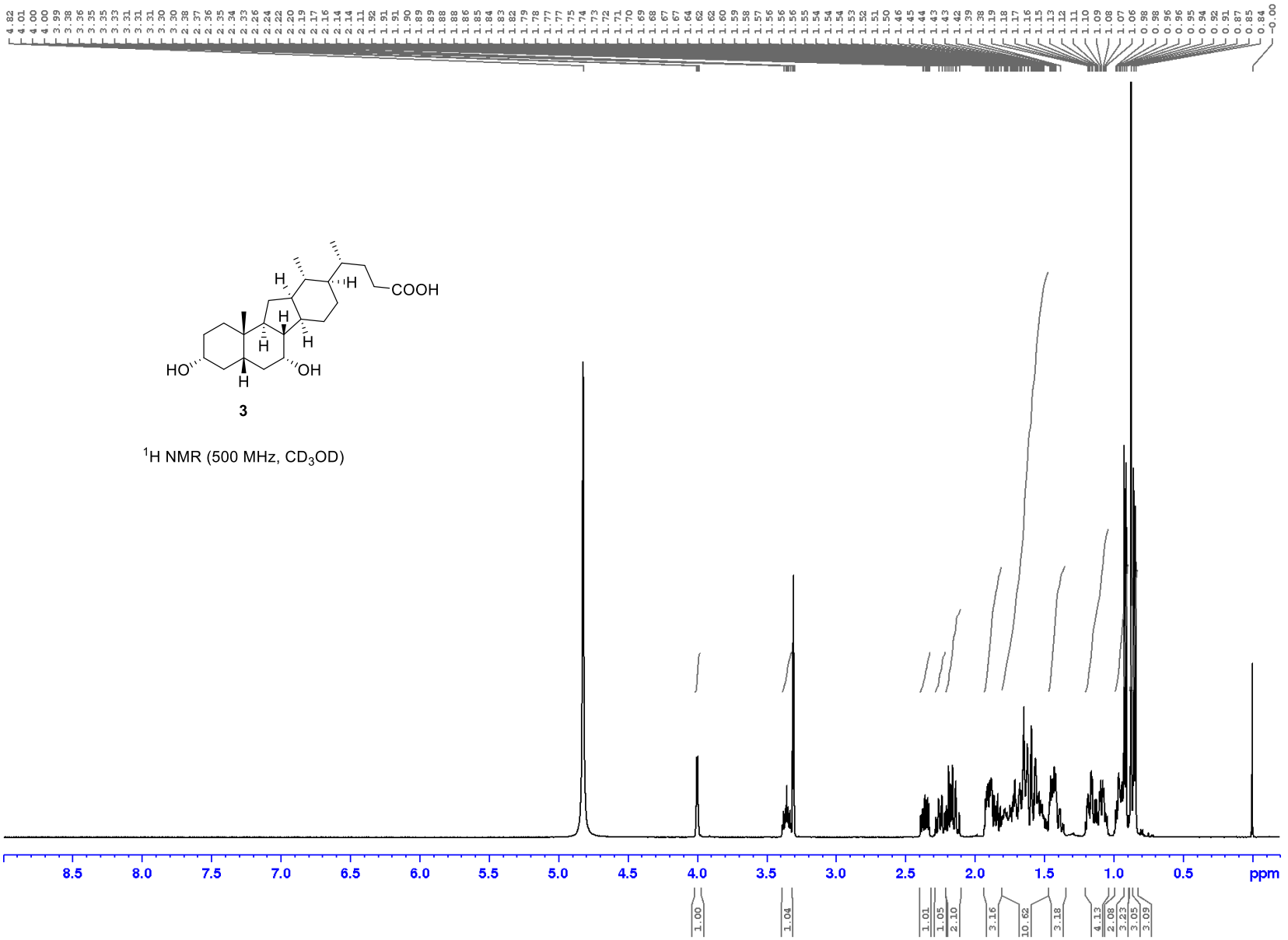

OH
COOH
H
H
H
H
HO
OH
H
6
1H NMR (500 MHz, CD3OD)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm



6

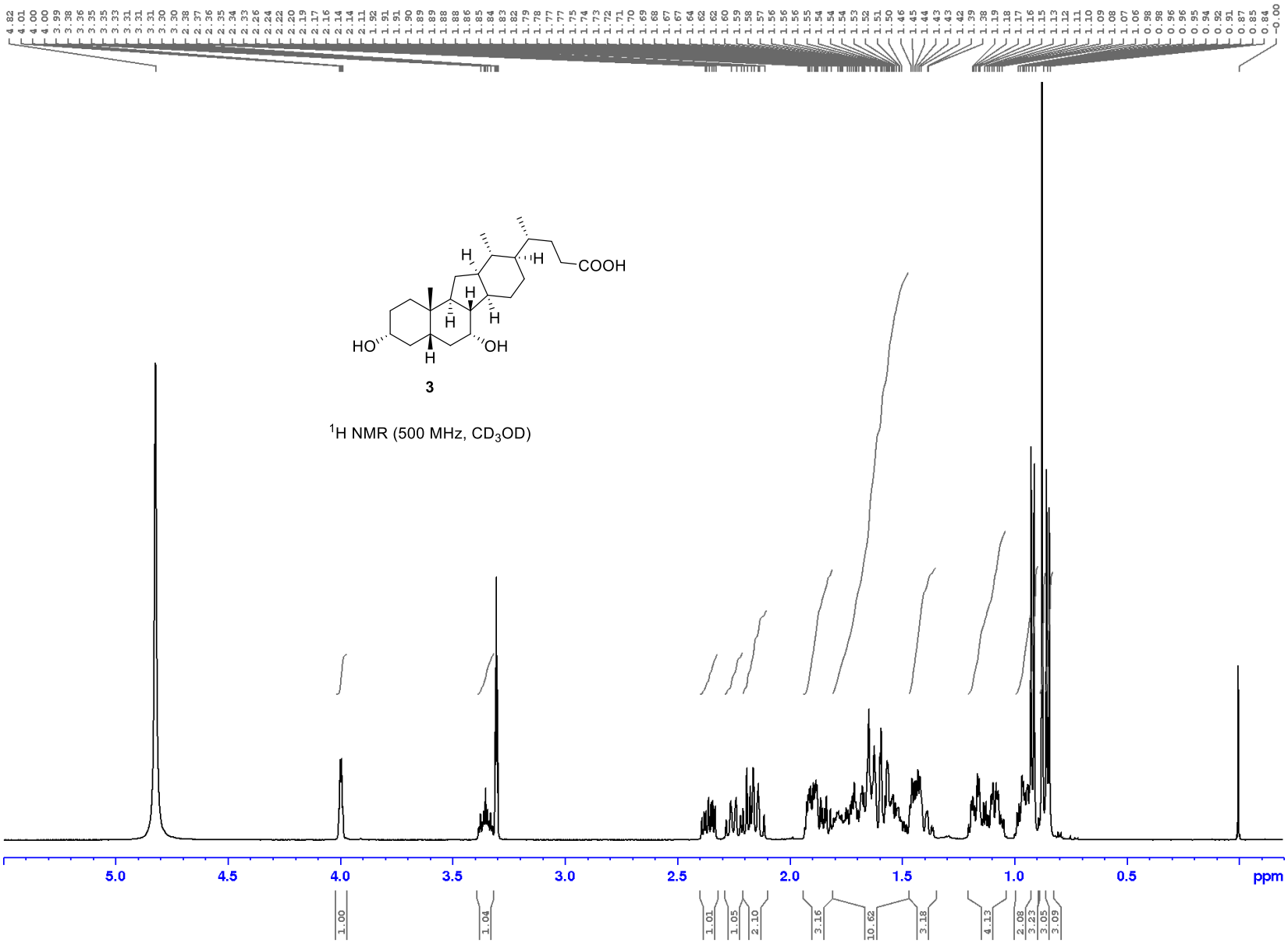
^{13}C NMR (125 MHz, CD_3OD)


ALPMT168-1AF F7-11 COL2 6 (0.111) AM (Cen,5, 80.00, Ar,7000.0,527.16,0.70); Sm (SG, 1x5.00); Sb (5,10.00); Cm (1:11)
TOF MS ES+
1.39e4
100
%
0
431.2768
432.2813
355.2638
356.2688
373.2755
374.2818
381.1971
409.2035
413.2699
414.2706
431.1551
433.2857
445.2879
453.2619
461.2012
462.1994
481.2220
497.2455
m/z
350
360
370
380
390
400
410
420
430
440
450
460
470
480
490
500
OH
COOH
H
H
H
H
HO
OH
H
6
Chemical Formula: C24H40O5Na+
Exact Mass: 431.2768
Molecular Weight: 431.5682



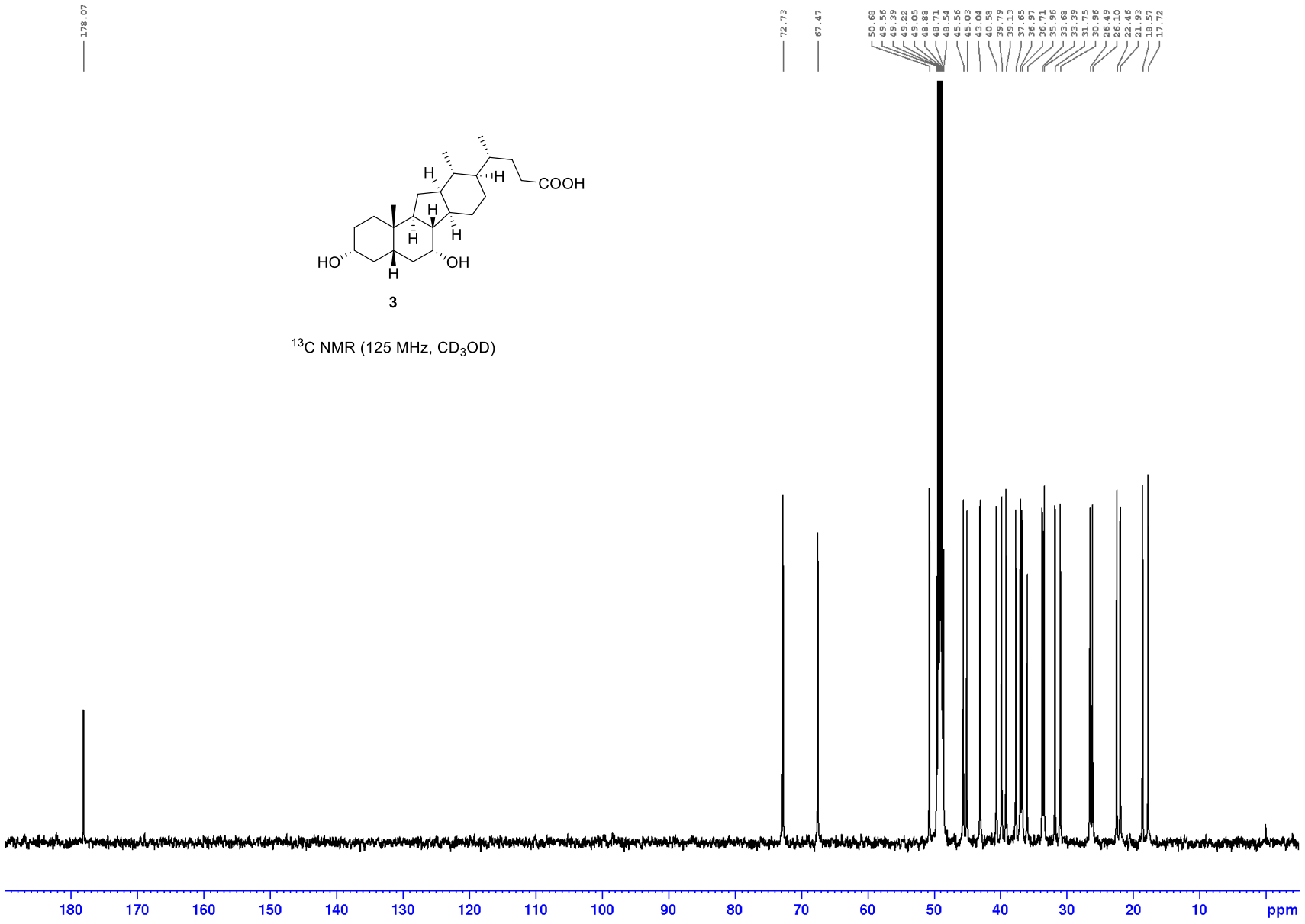
3

^{1}H NMR (500 MHz, CD_3OD)



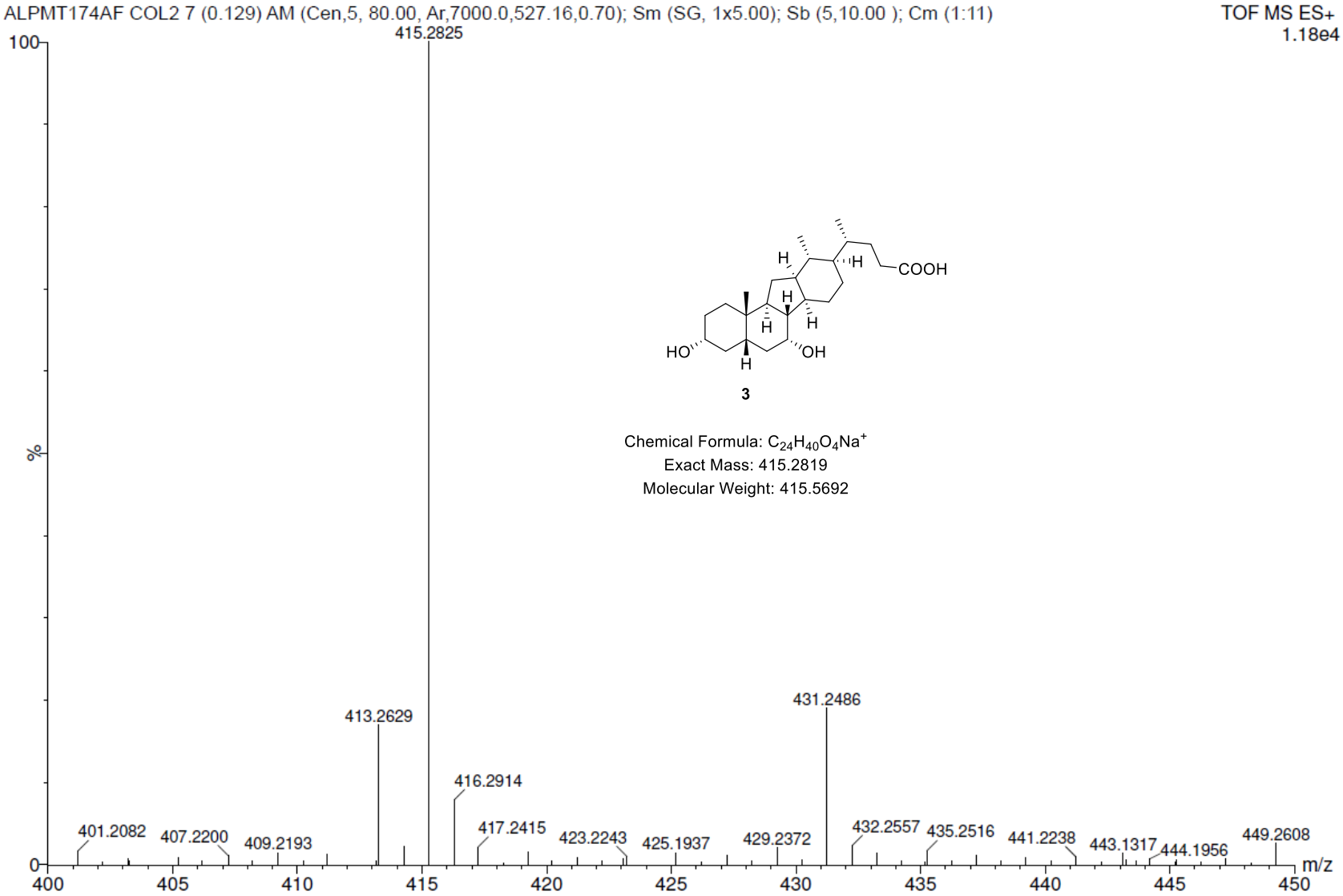
3

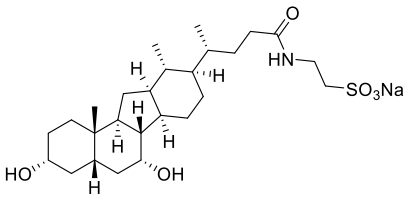
^{1}H NMR (500 MHz, CD_3OD)



3

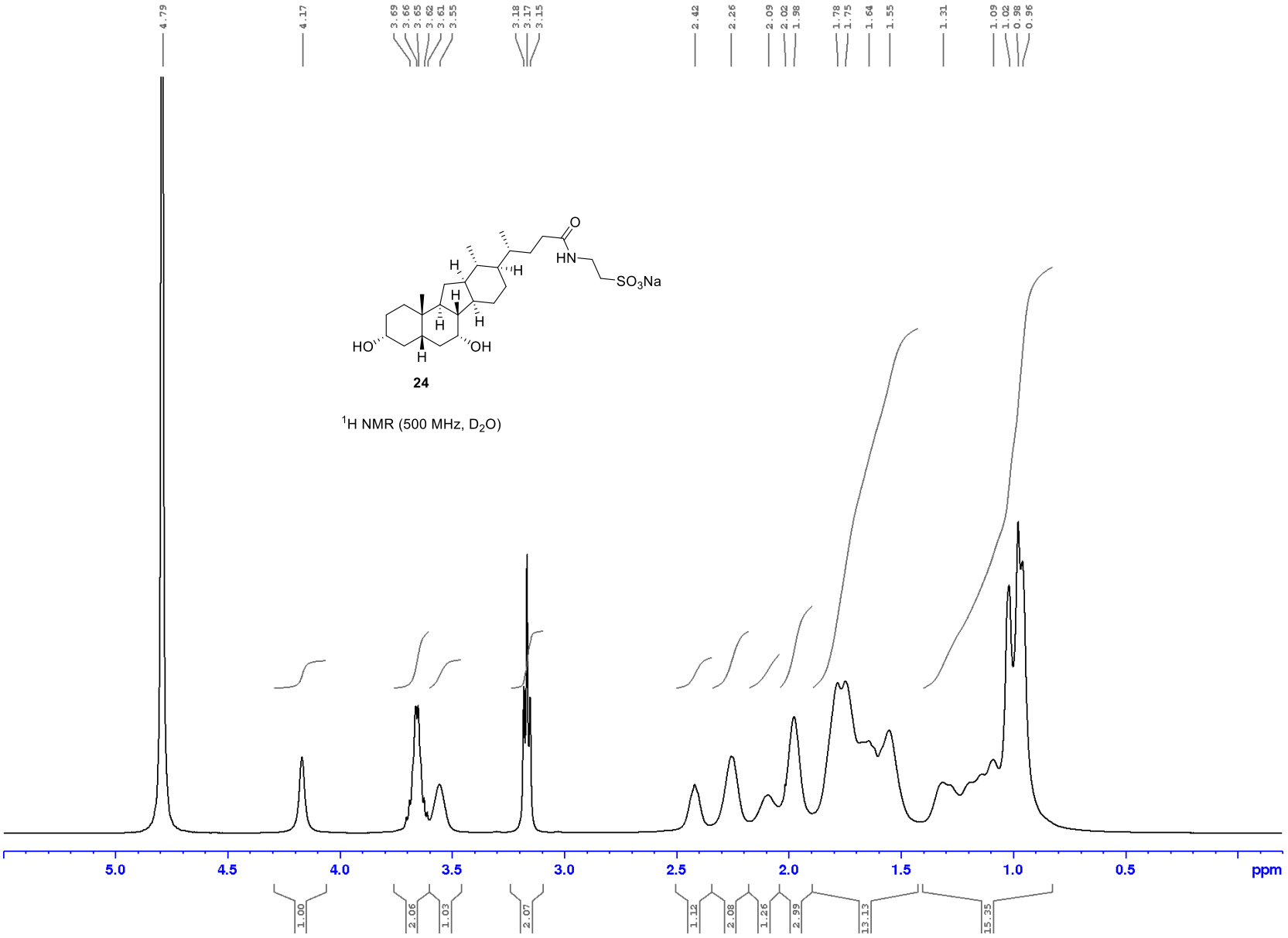
^{13}C NMR (125 MHz, CD_3OD)


ALPMT174AF COL2 7 (0.129) AM (Cen,5, 80.00, Ar,7000.0,527.16,0.70); Sm (SG, 1x5.00); Sb (5,10.00); Cm (1:11)
TOF MS ES+
1.18e4
100
%
0
415.2825
413.2629
431.2486
416.2914
401.2082
407.2200
409.2193
417.2415
423.2243
425.1937
429.2372
432.2557
435.2516
441.2238
443.1317
444.1956
449.2608
400
405
410
415
420
425
430
435
440
445
450
m/z
HO
OH
COOH
H
3
Chemical Formula: $C_{24}H_{40}O_4Na^+$
Exact Mass: 415.2819
Molecular Weight: 415.5692



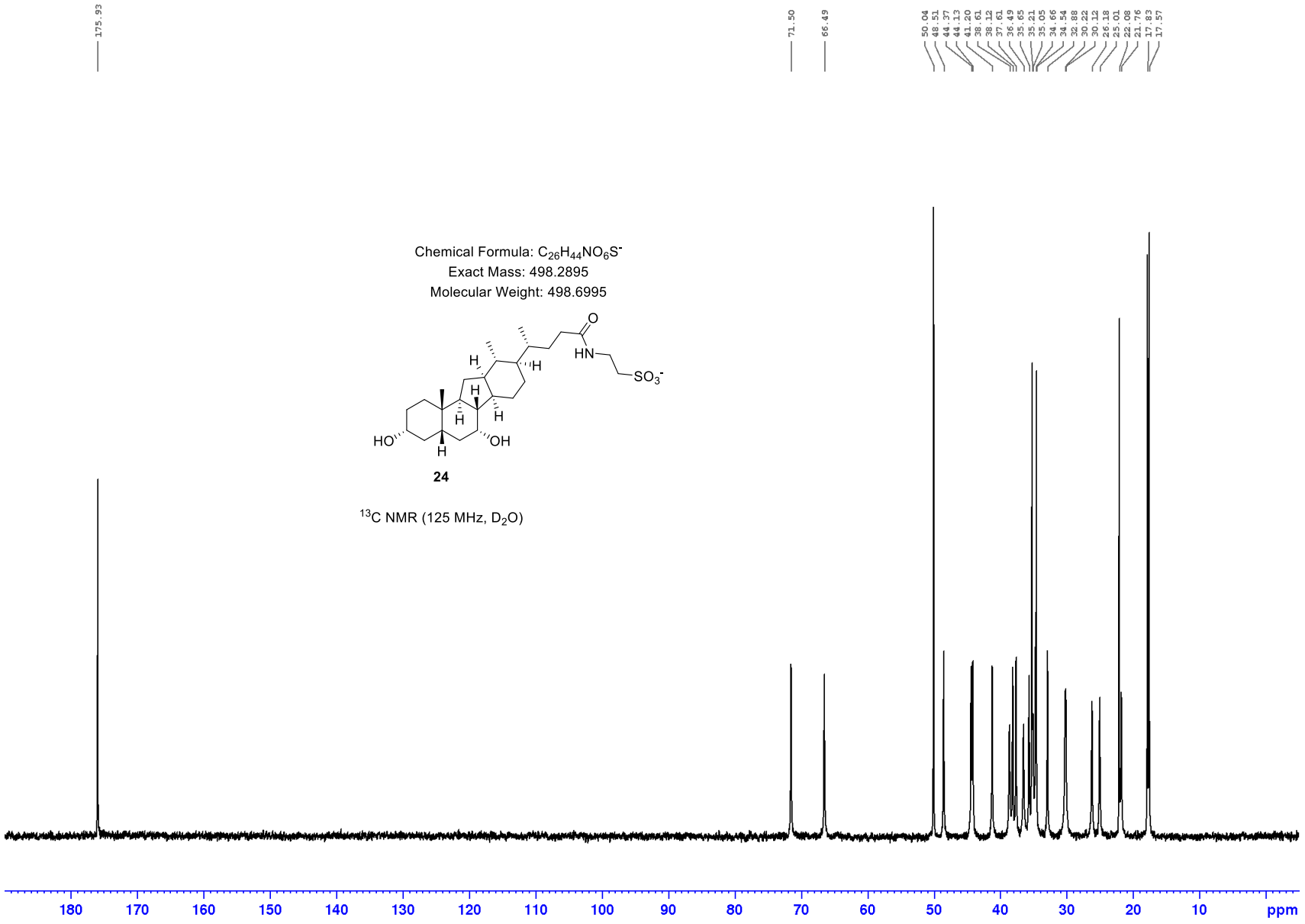
24

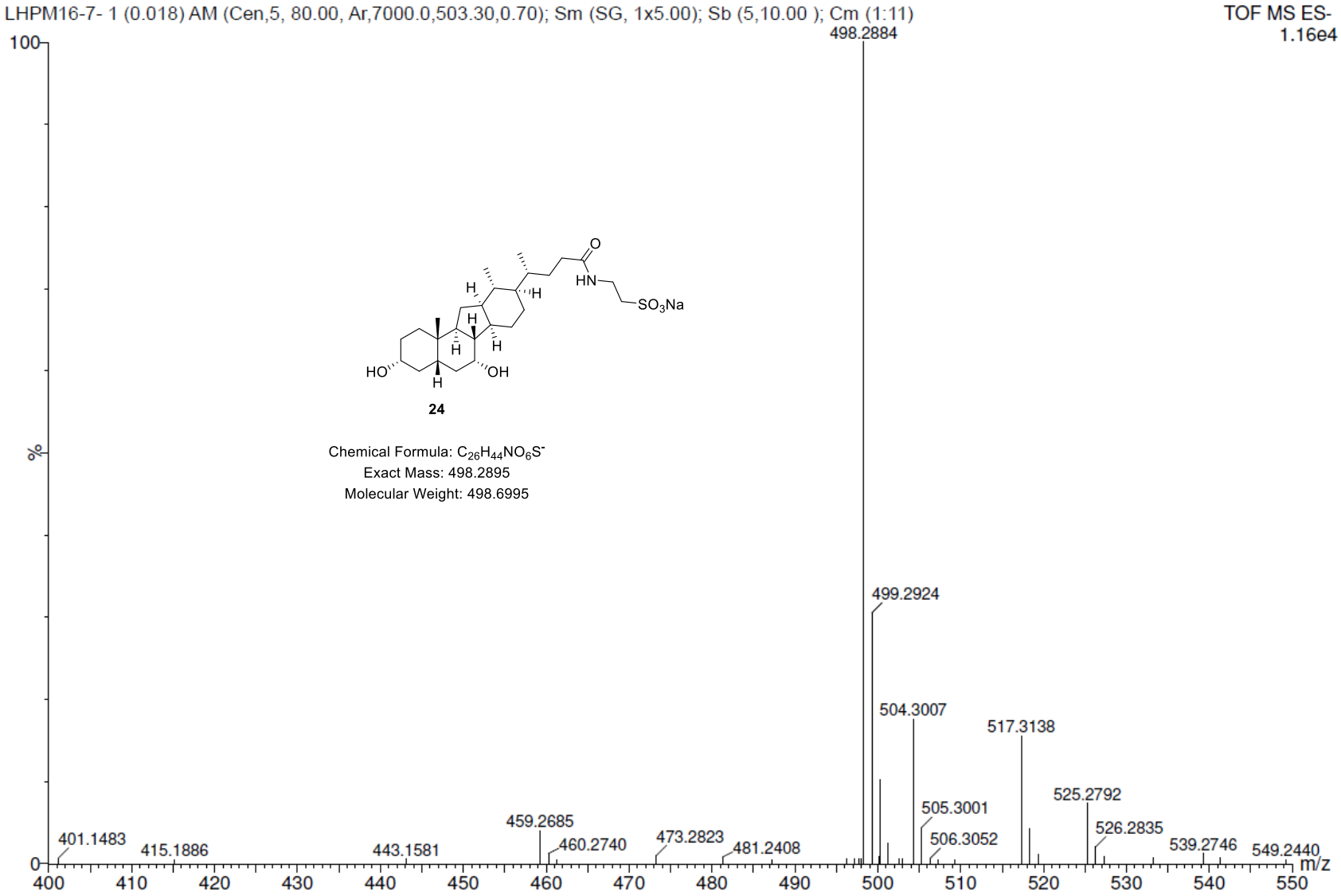
^{1}H NMR (500 MHz, D_2O)

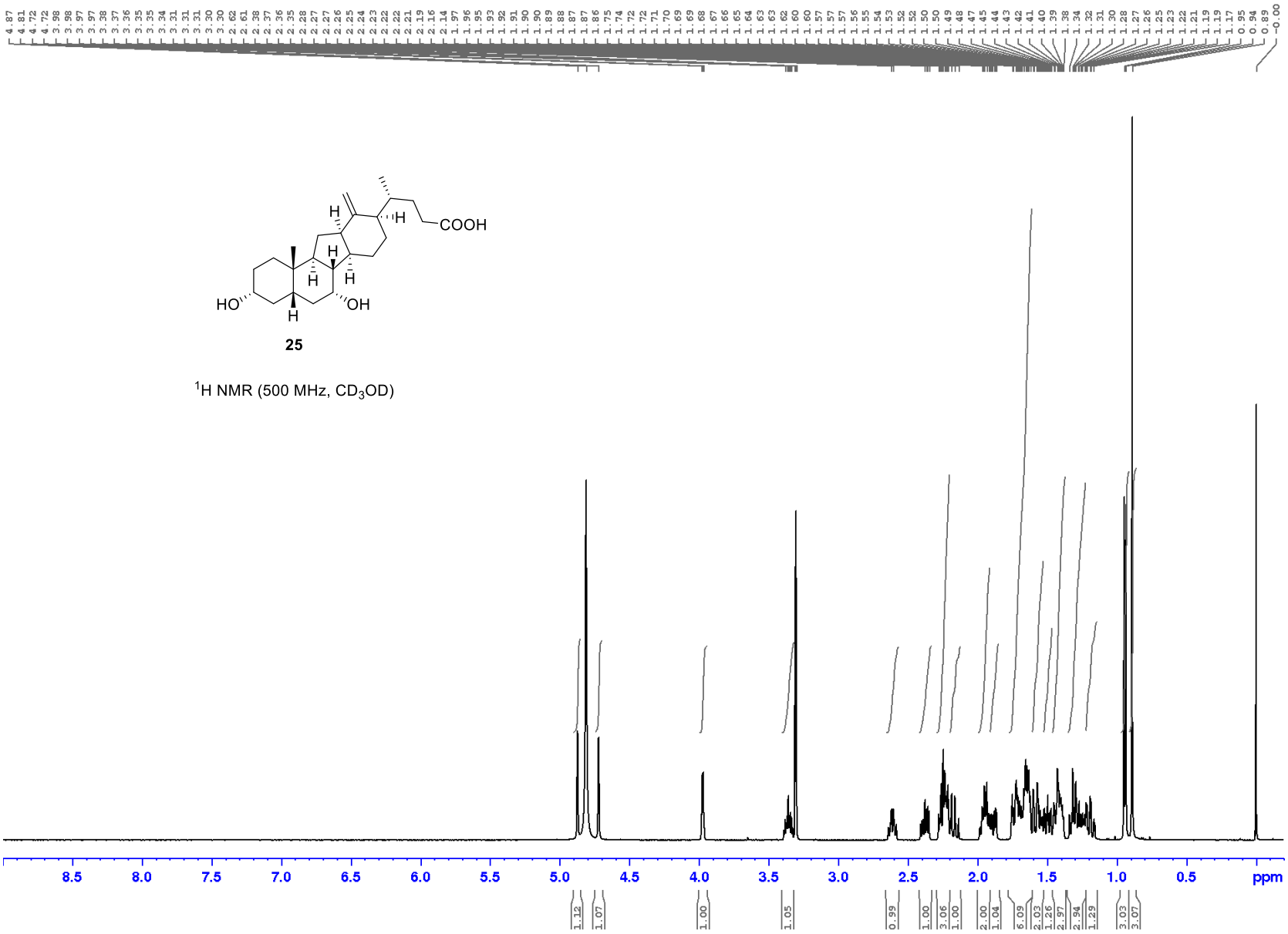


24

^{1}H NMR (500 MHz, D_2O)

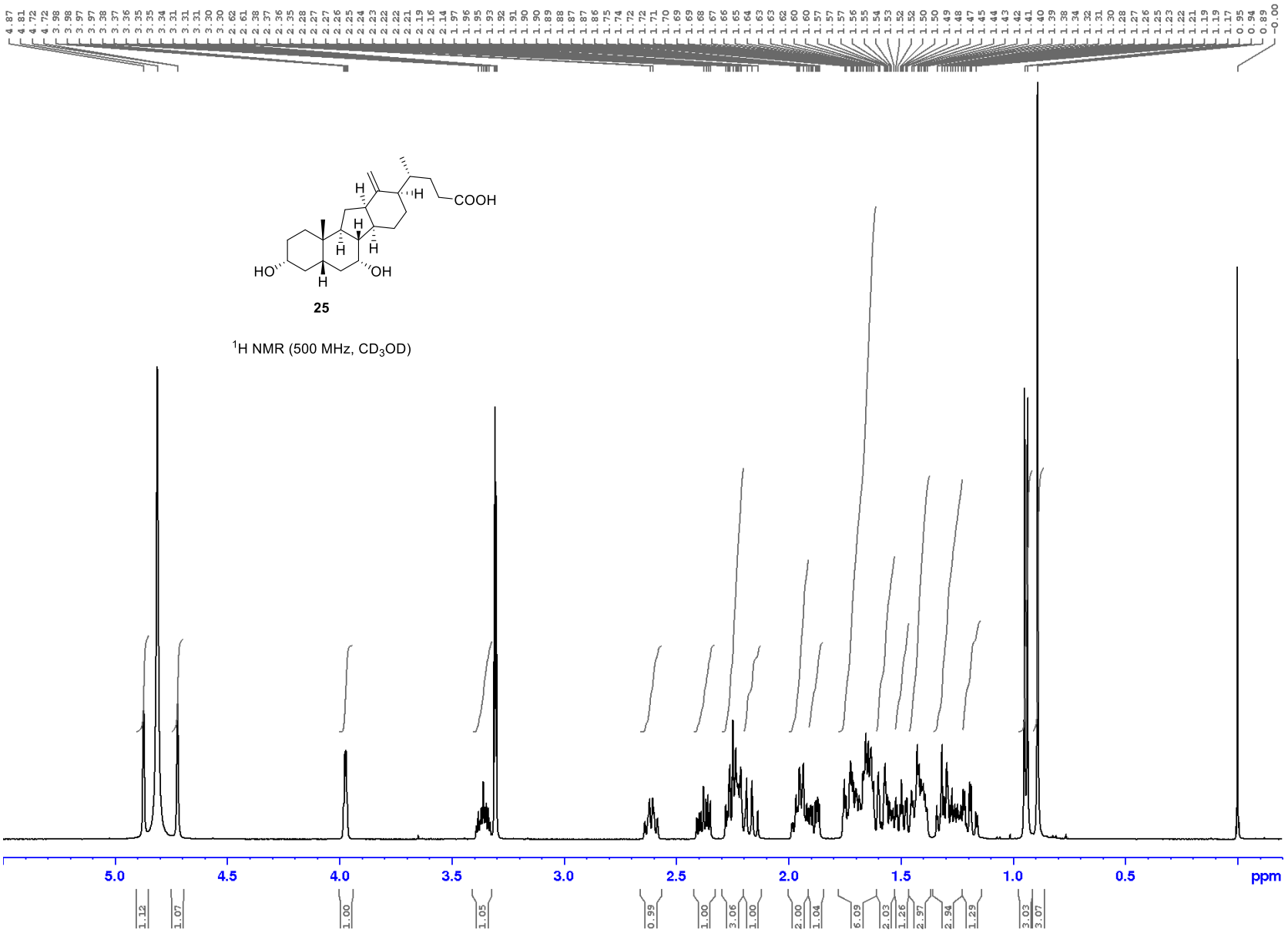

175.93
71.50
66.49
50.04
48.51
44.37
44.13
41.20
38.61
38.12
37.61
36.49
35.65
35.21
35.05
34.66
34.54
32.88
30.22
30.12
26.18
25.01
22.08
21.76
17.83
17.57
Chemical Formula: C26H44NO6S-
Exact Mass: 498.2895
Molecular Weight: 498.6995
HO
OH
HN
SO3-
24
13C NMR (125 MHz, D2O)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

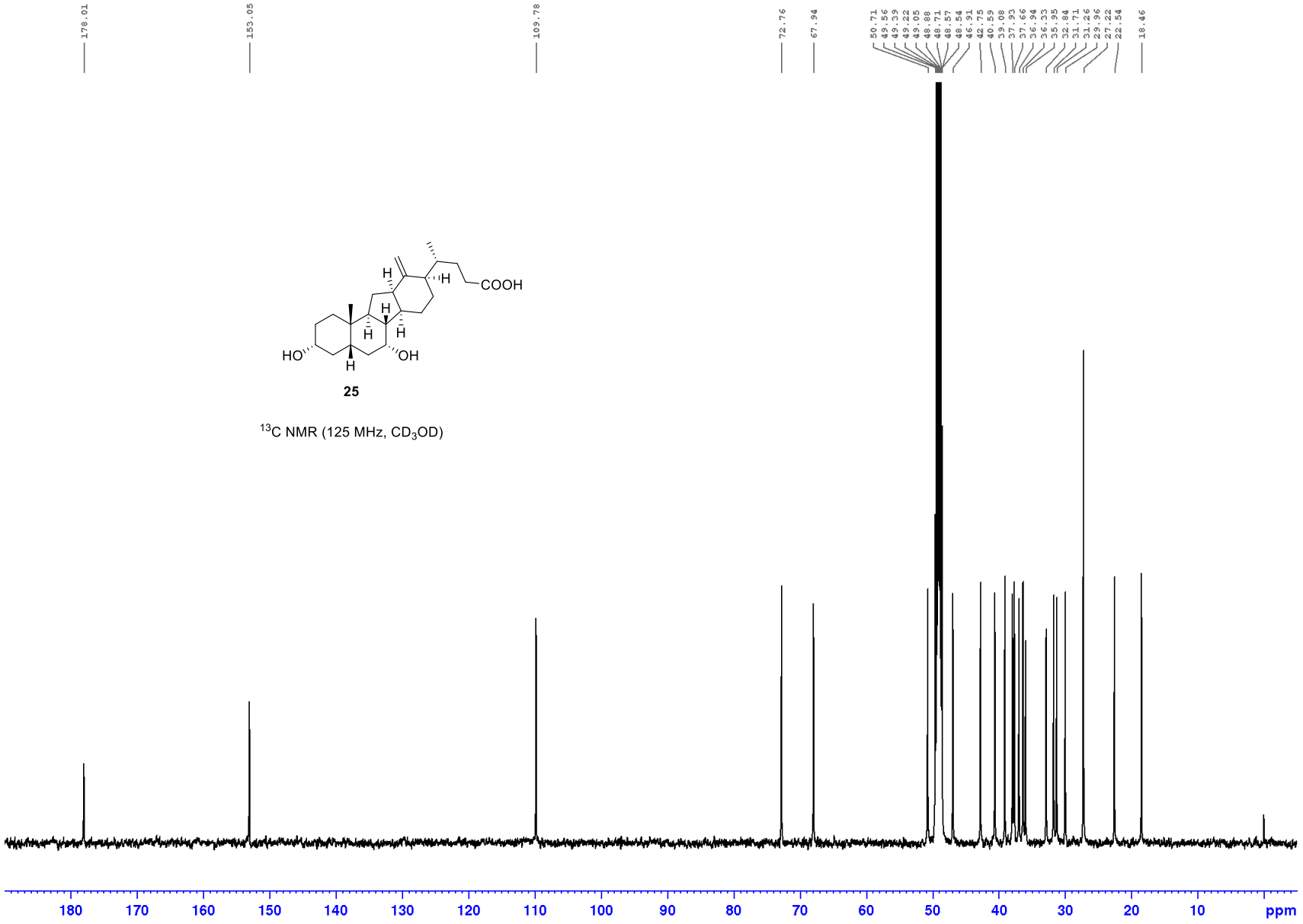

LHPM16-7- 1 (0.018) AM (Cen,5, 80.00, Ar,7000.0,503.30,0.70); Sm (SG, 1x5.00); Sb (5,10.00); Cm (1:11)
TOF MS ES-
1.16e4
100
%
0
498.2884
499.2924
504.3007
517.3138
525.2792
505.3001
526.2835
506.3052
539.2746
549.2440
459.2685
460.2740
473.2823
481.2408
401.1483
415.1886
443.1581
400
410
420
430
440
450
460
470
480
490
500
510
520
530
540
550
m/z
HO
OH
HN
O
SO3Na
24
Chemical Formula: C26H44NO6S-
Exact Mass: 498.2895
Molecular Weight: 498.6995



25

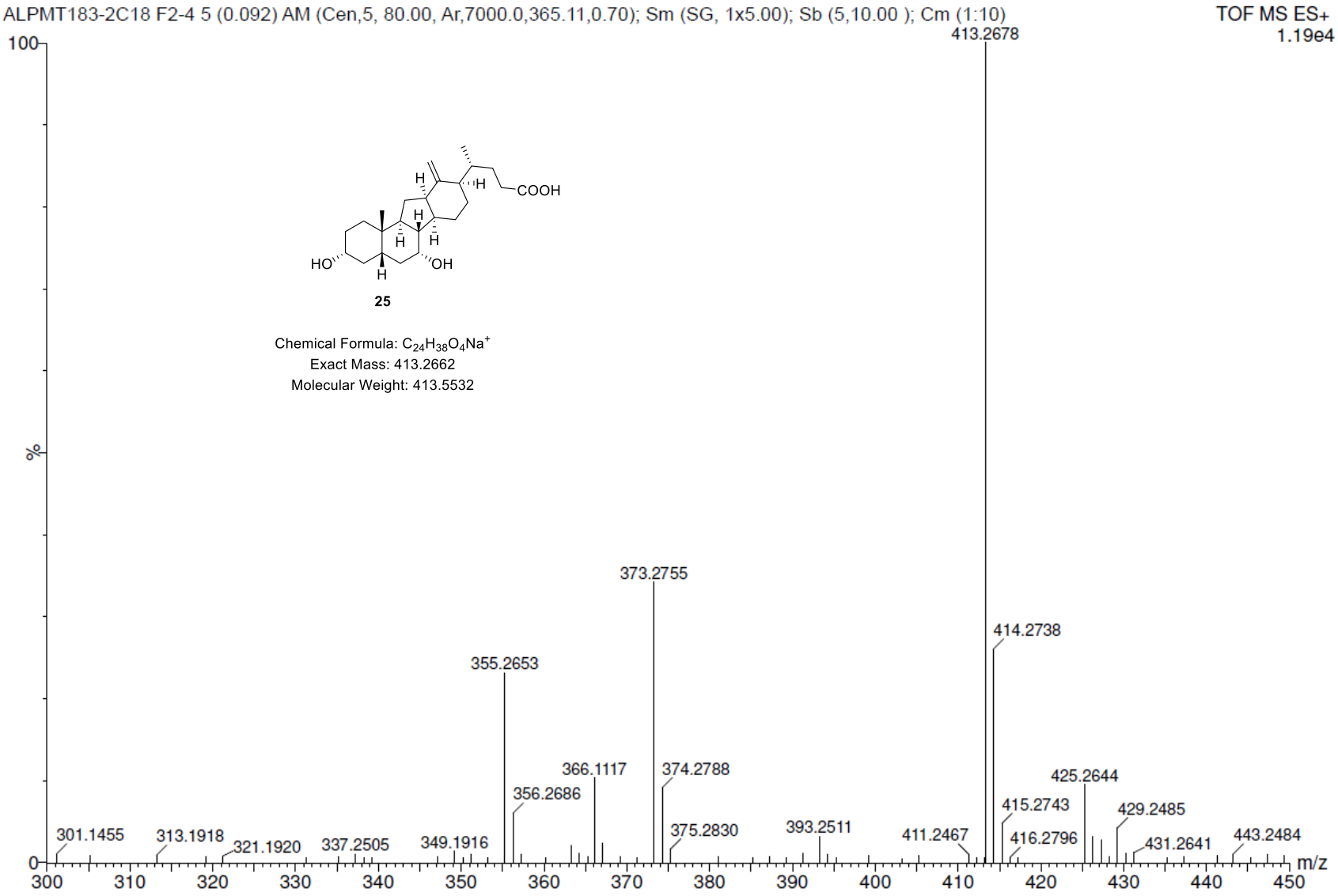
^{1}H NMR (500 MHz, CD_3OD)

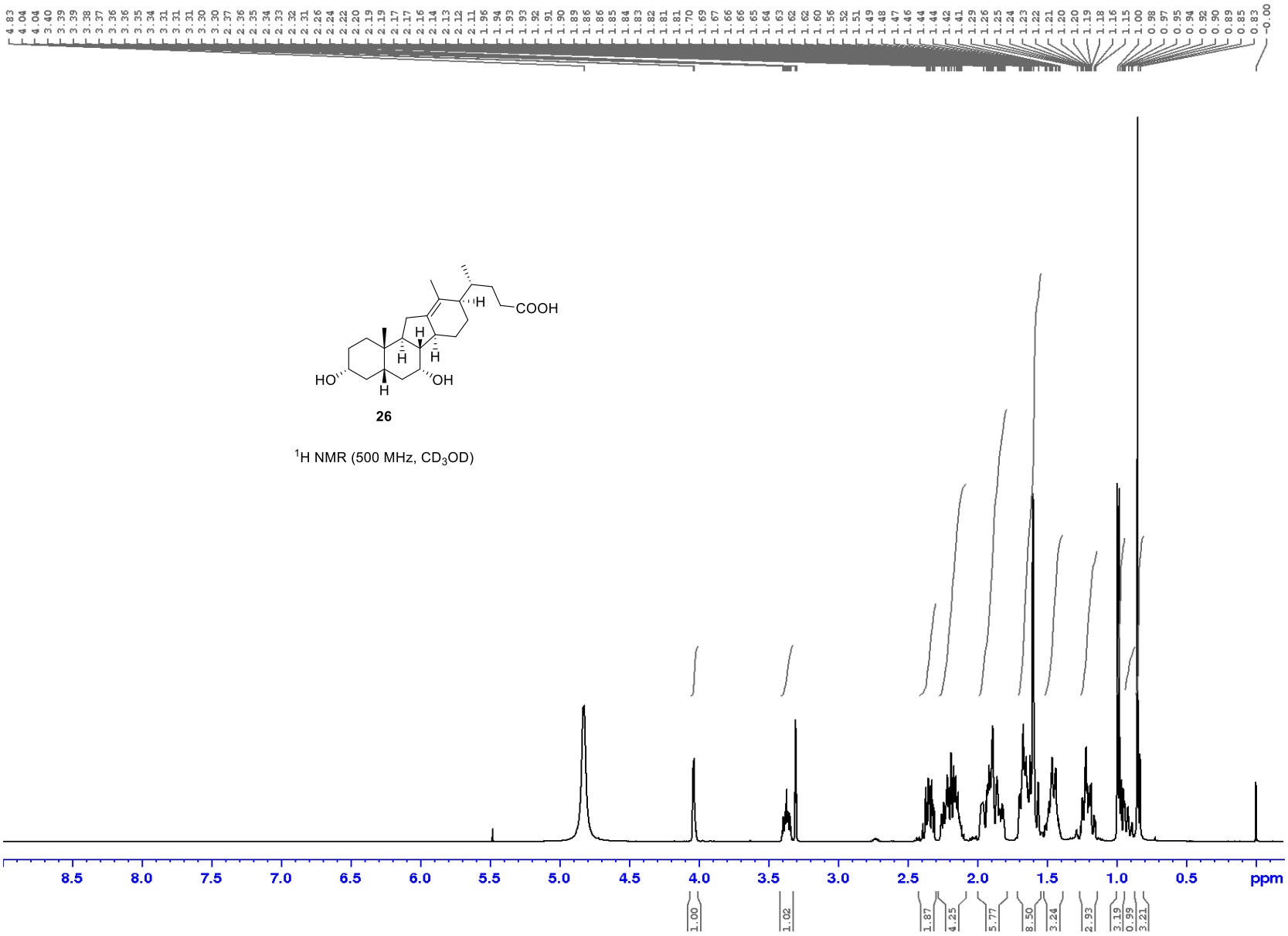

HO
OH
COOH
H
25
1H NMR (500 MHz, CD3OD)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm



25

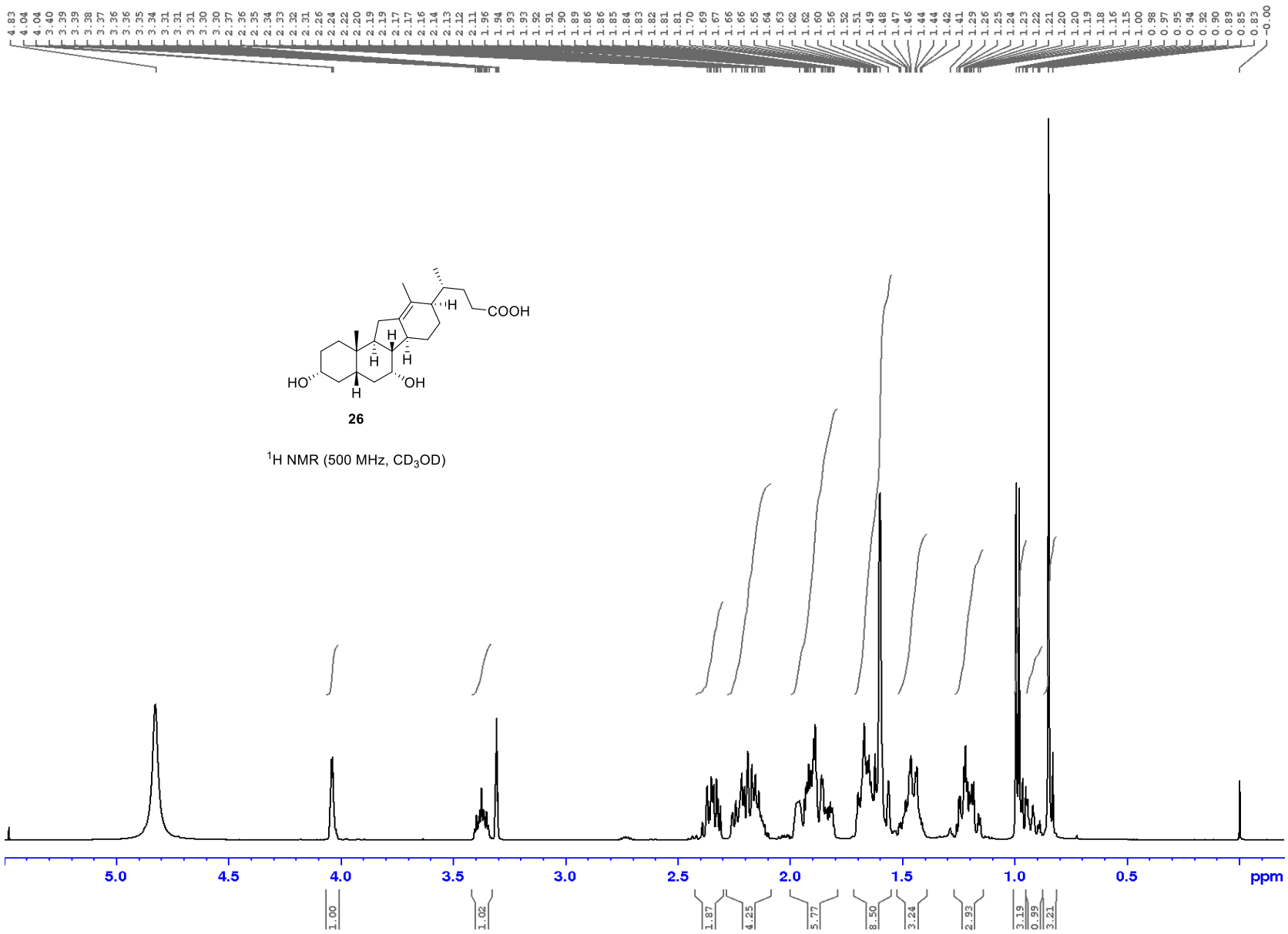
^{13}C NMR (125 MHz, CD_3OD)


ALPMT183-2C18 F2-4 5 (0.092) AM (Cen,5, 80.00, Ar,7000.0,365.11,0.70); Sm (SG, 1x5.00); Sb (5,10.00); Cm (1:10)
TOF MS ES+
1.19e4
100
%
0
413.2678
373.2755
414.2738
355.2653
366.1117
374.2788
356.2686
425.2644
415.2743
429.2485
375.2830
393.2511
411.2467
416.2796
431.2641
443.2484
301.1455
313.1918
321.1920
337.2505
349.1916
300
310
320
330
340
350
360
370
380
390
400
410
420
430
440
450
m/z
HO
OH
COOH
H
25
Chemical Formula: $C_{24}H_{38}O_4Na^+$
Exact Mass: 413.2662
Molecular Weight: 413.5532



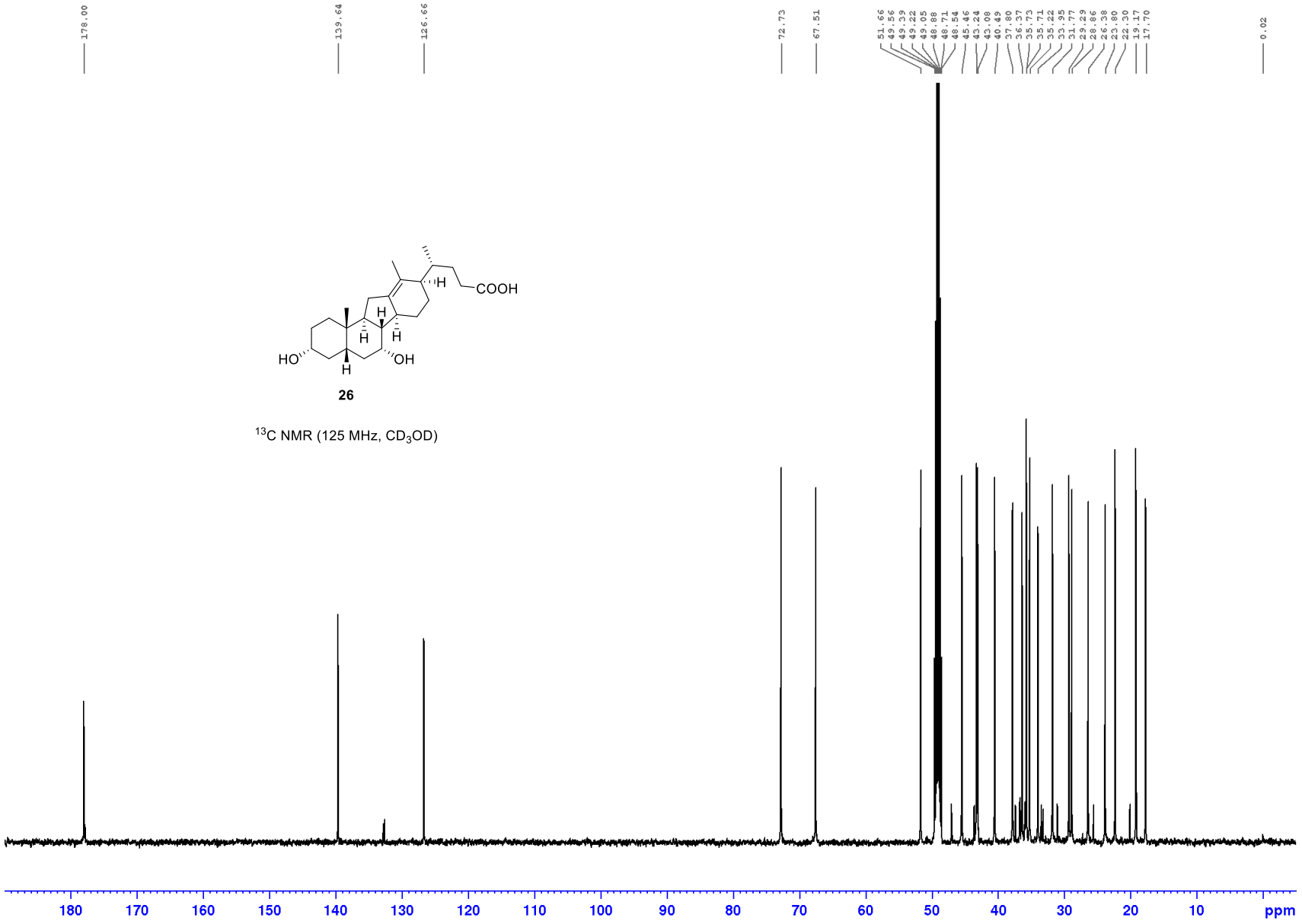
26

^{1}H NMR (500 MHz, CD_3OD)



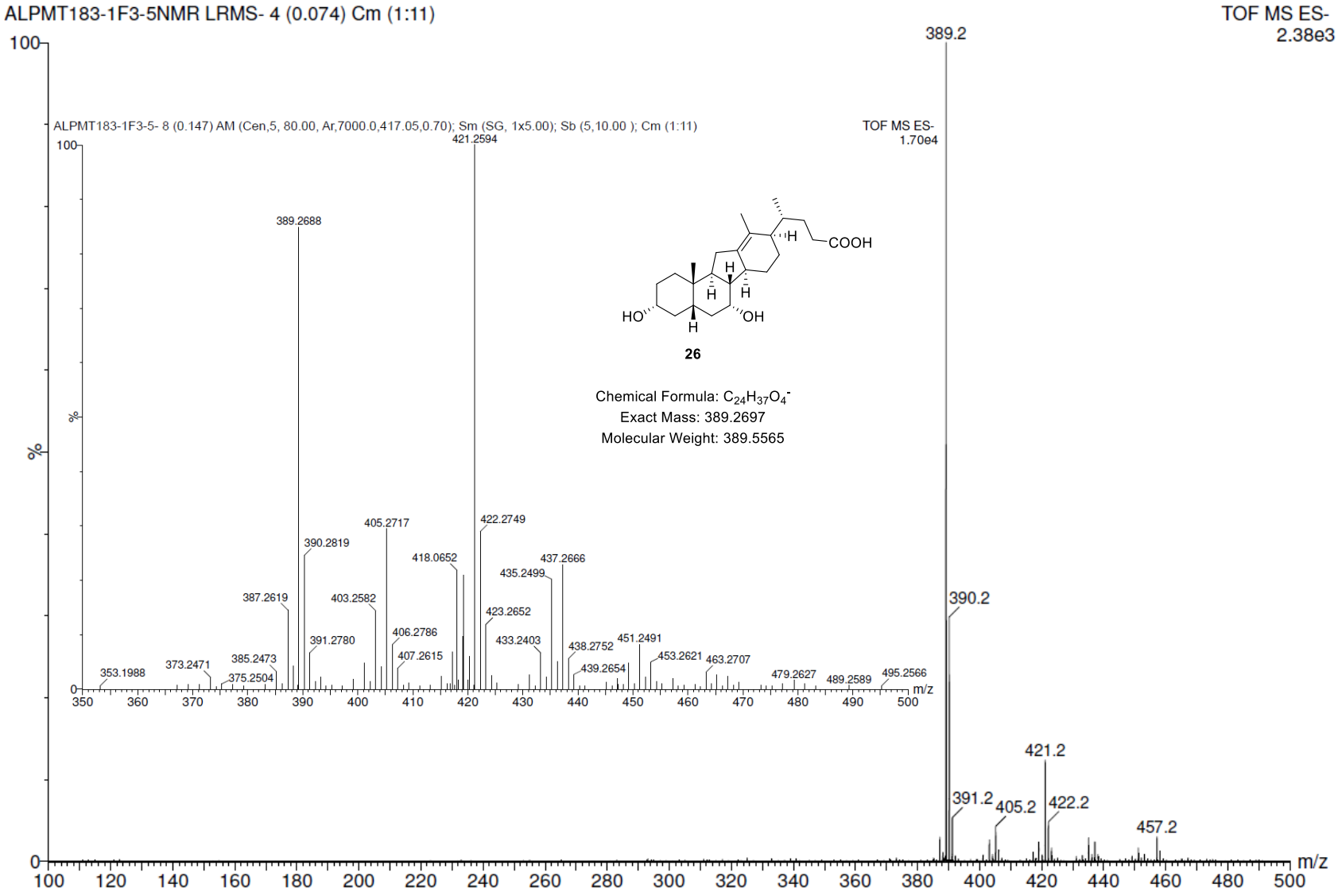
26

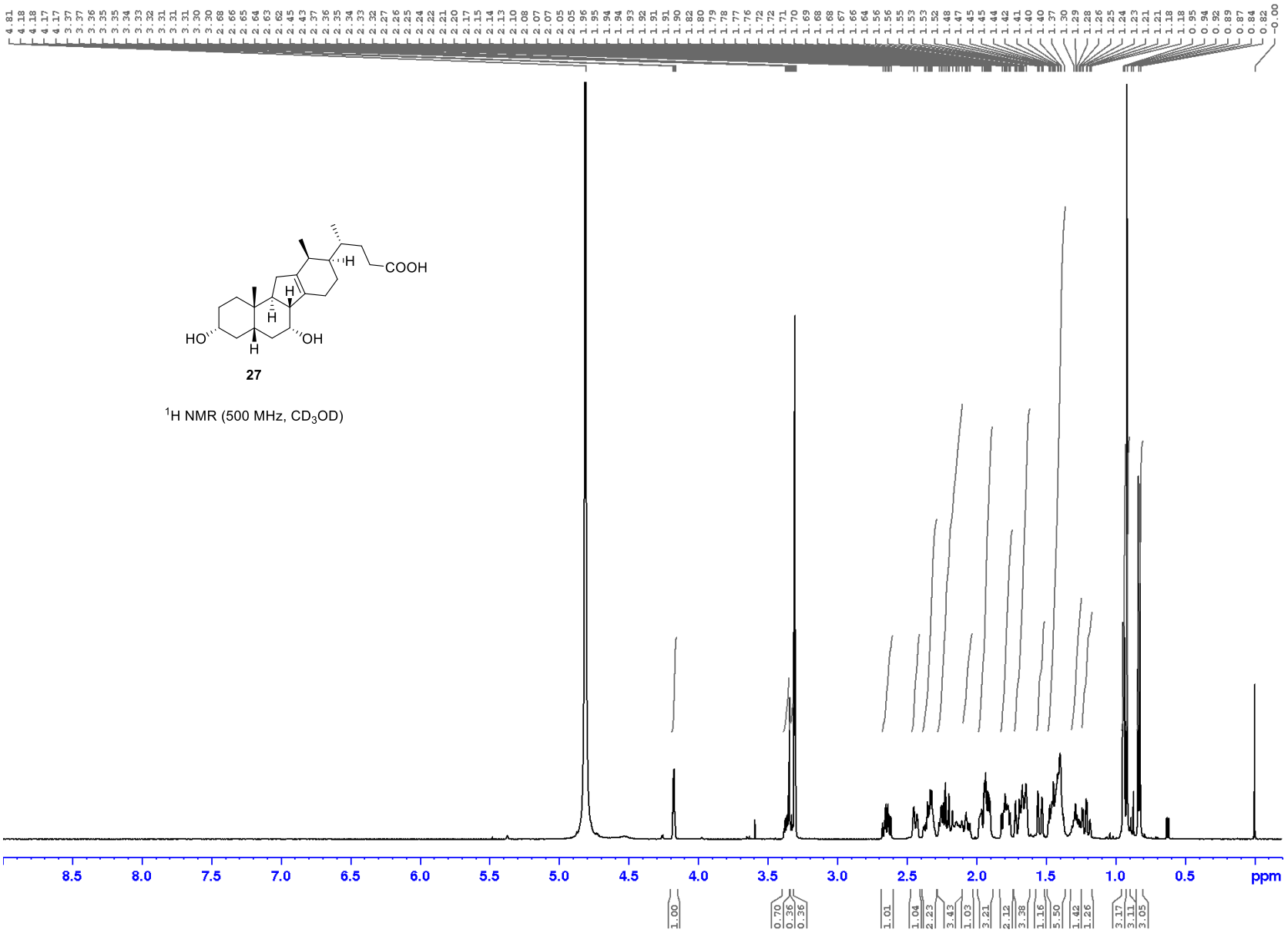
^{1}H NMR (500 MHz, CD_3OD)

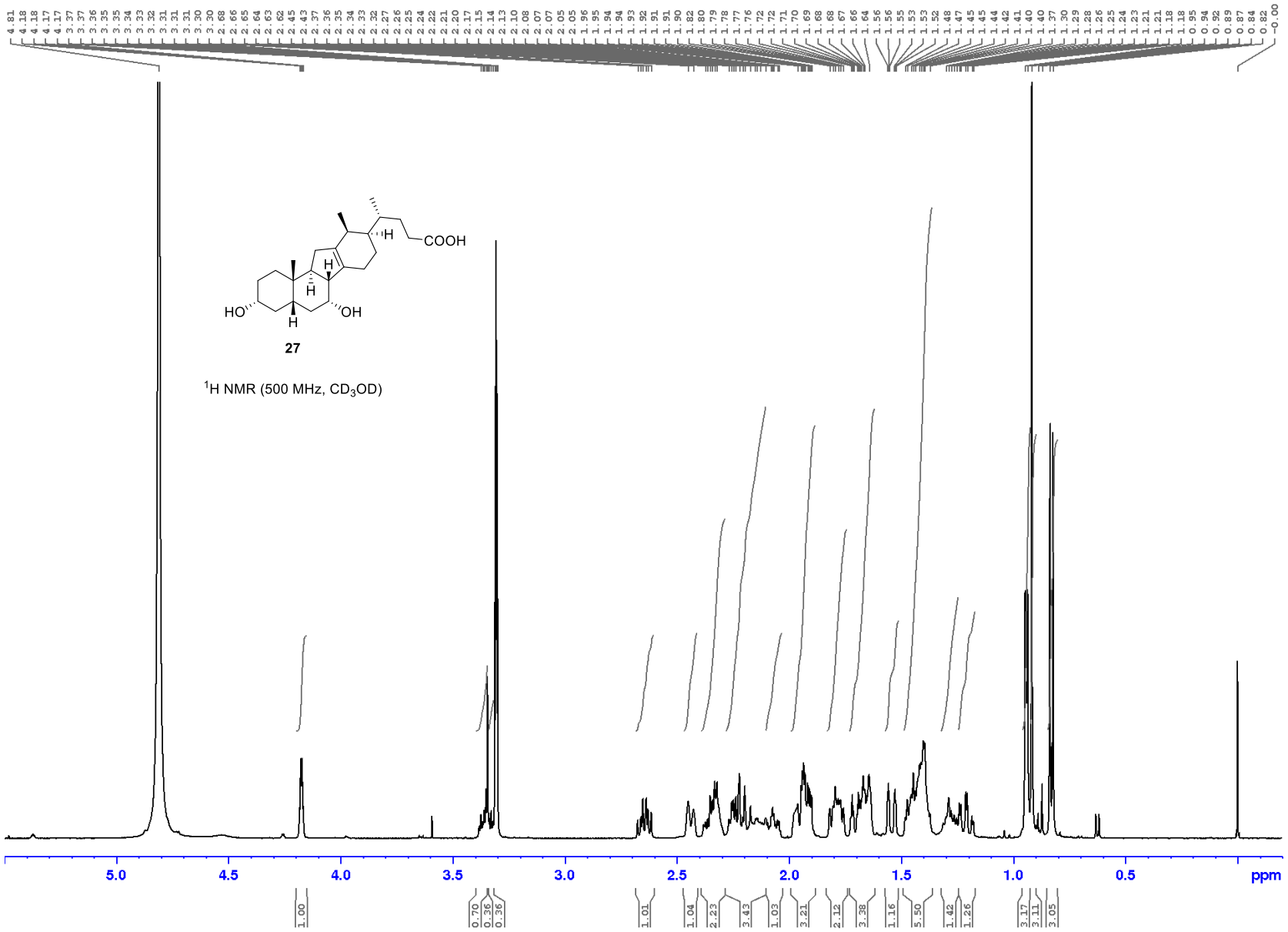


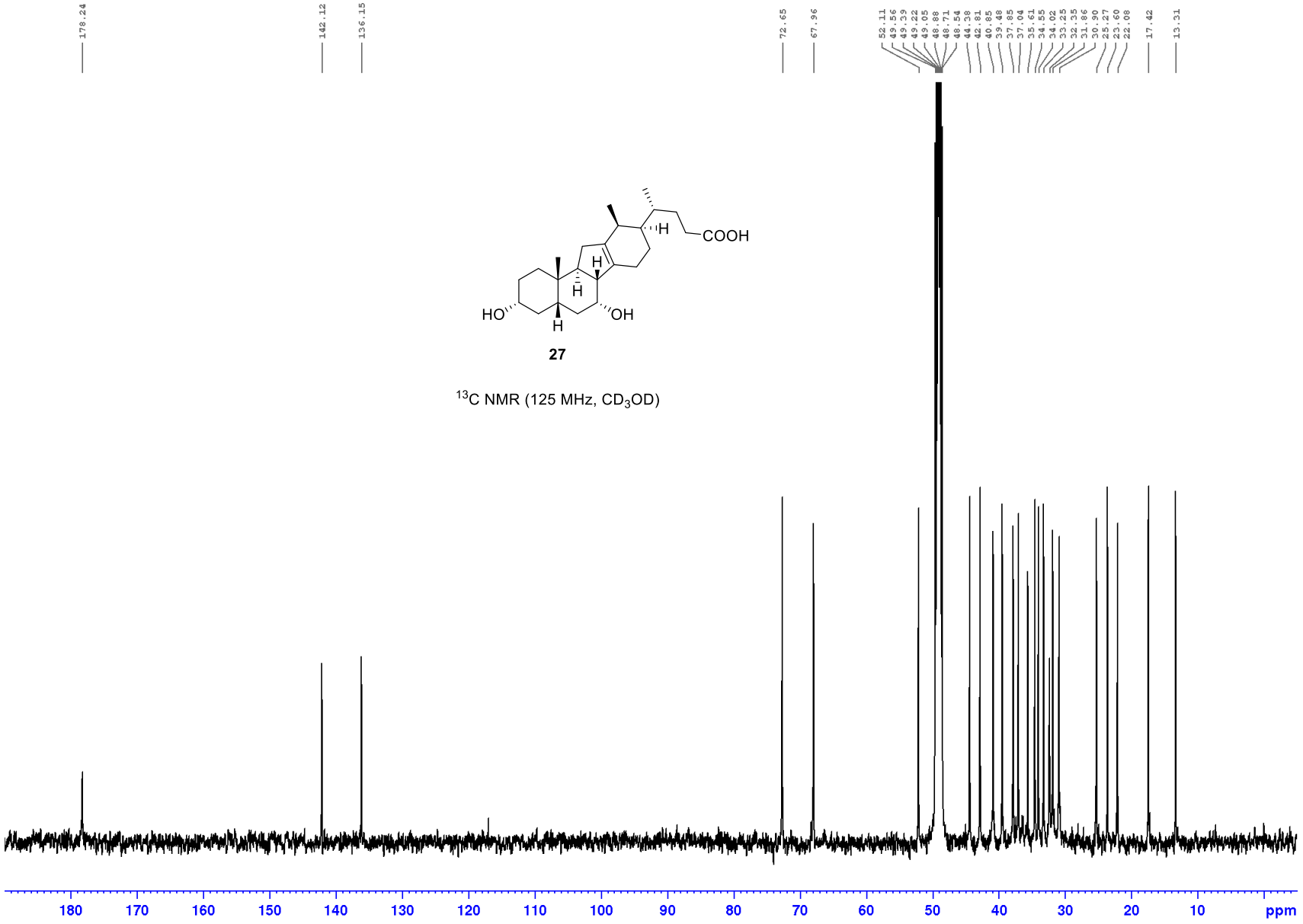
26

^{13}C NMR (125 MHz, CD_3OD)


ALPMT183-1F3-5NMR LRMS- 4 (0.074) Cm (1:11)
TOF MS ES-
2.38e3
389.2
390.2
421.2
391.2
405.2
422.2
457.2
m/z
ALPMT183-1F3-5- 8 (0.147) AM (Cen,5, 80.00, Ar,7000.0,417.05,0.70); Sm (SG, 1x5.00); Sb (5,10.00); Cm (1:11)
TOF MS ES-
1.70e4
421.2594
389.2688
405.2717
422.2749
390.2819
418.0652
437.2666
435.2499
387.2619
403.2582
423.2652
406.2786
391.2780
433.2403
438.2752
451.2491
407.2615
439.2654
453.2621
463.2707
353.1988
373.2471
385.2473
375.2504
479.2627
489.2589
495.2566
COOH
HO
OH
26
Chemical Formula: $C_{24}H_{37}O_4^-$
Exact Mass: 389.2697
Molecular Weight: 389.5565

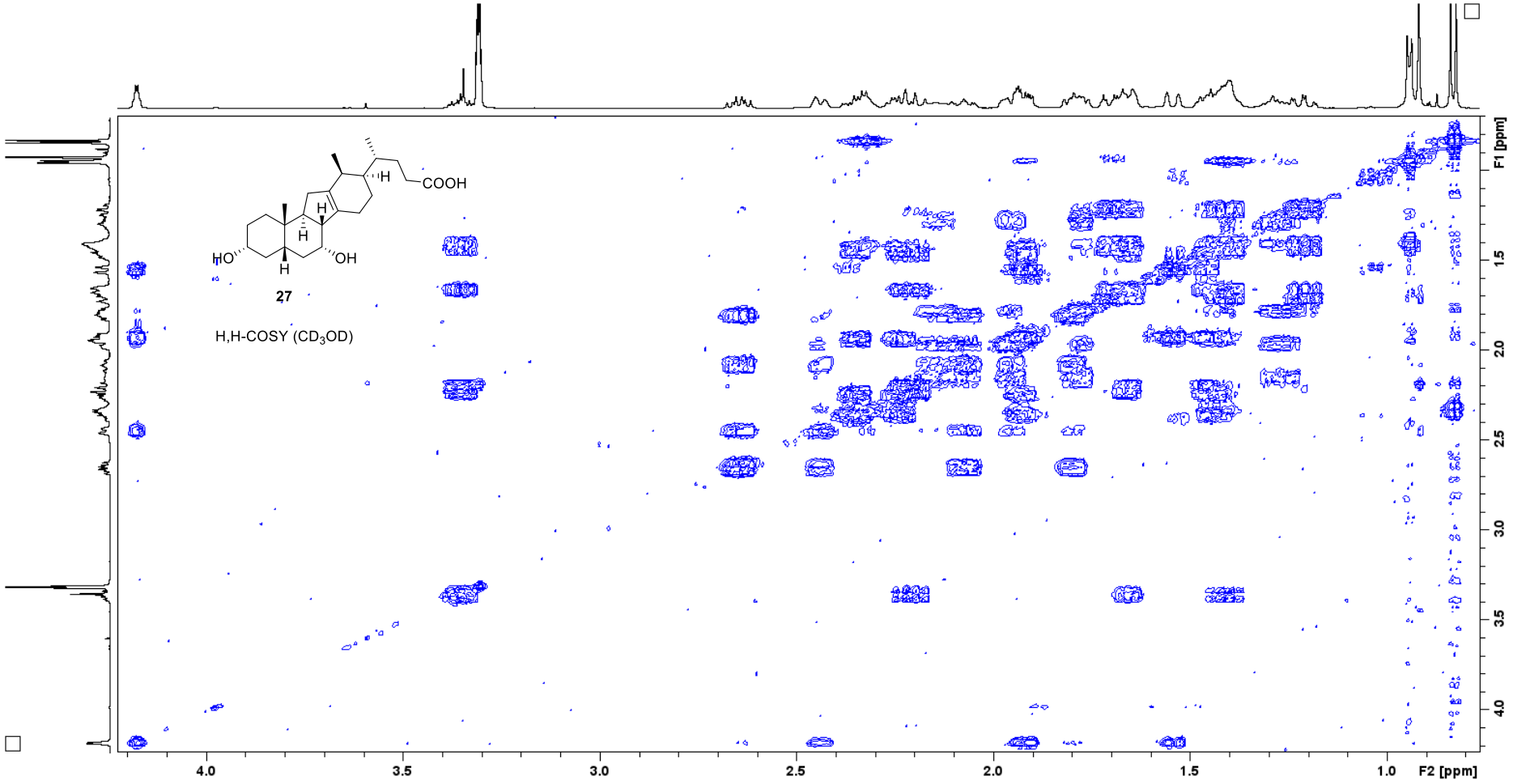

HO
OH
COOH
H
H
H
H
27
1H NMR (500 MHz, CD3OD)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

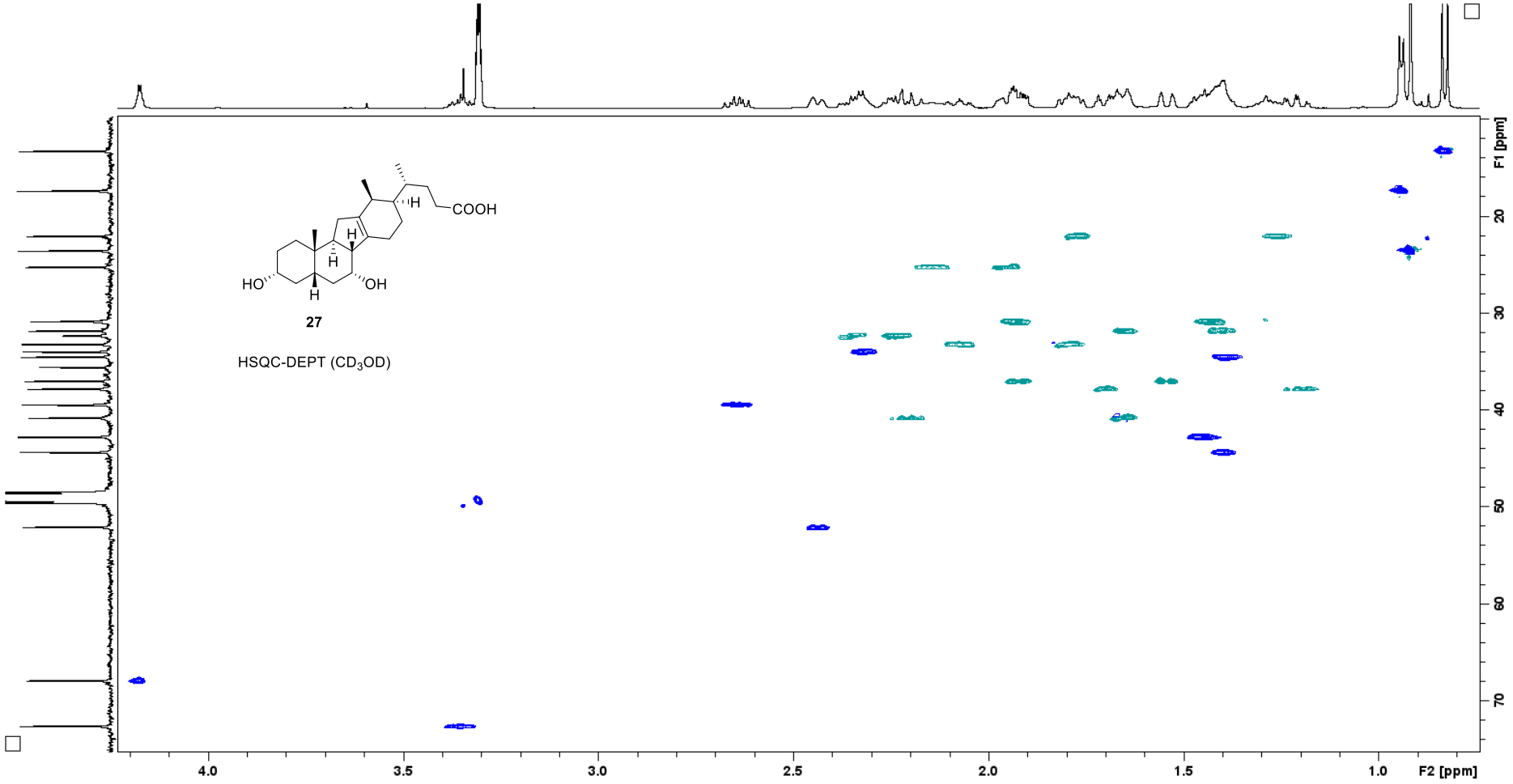

4.81 4.18 4.18 4.17 4.17 3.37 3.37 3.36 3.35 3.35 3.34 3.33 3.32 3.31 3.31 3.31 3.30 3.30 2.68 2.66 2.65 2.64 2.63 2.62 2.45 2.43 2.37 2.36 2.35 2.34 2.33 2.32 2.27 2.26 2.25 2.24 2.22 2.21 2.20 2.17 2.15 2.14 2.13 2.10 2.08 2.07 2.07 2.05 2.05 1.96 1.95 1.94 1.94 1.93 1.92 1.91 1.91 1.90 1.82 1.80 1.79 1.78 1.77 1.76 1.72 1.72 1.71 1.70 1.69 1.68 1.68 1.67 1.66 1.64 1.56 1.56 1.55 1.53 1.53 1.52 1.48 1.47 1.45 1.45 1.44 1.42 1.41 1.40 1.40 1.37 1.30 1.29 1.28 1.26 1.25 1.24 1.23 1.21 1.21 1.18 1.18 0.95 0.94 0.92 0.89 0.87 0.84 0.82 -0.00
COOH
H
H
H
HO
OH
H
27
^{1}H NMR (500 MHz, CD_3OD)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
0.70
0.36
0.36
1.01
1.04
2.23
3.43
1.03
3.21
2.12
3.38
1.16
5.50
1.42
1.26
3.17
3.11
3.05

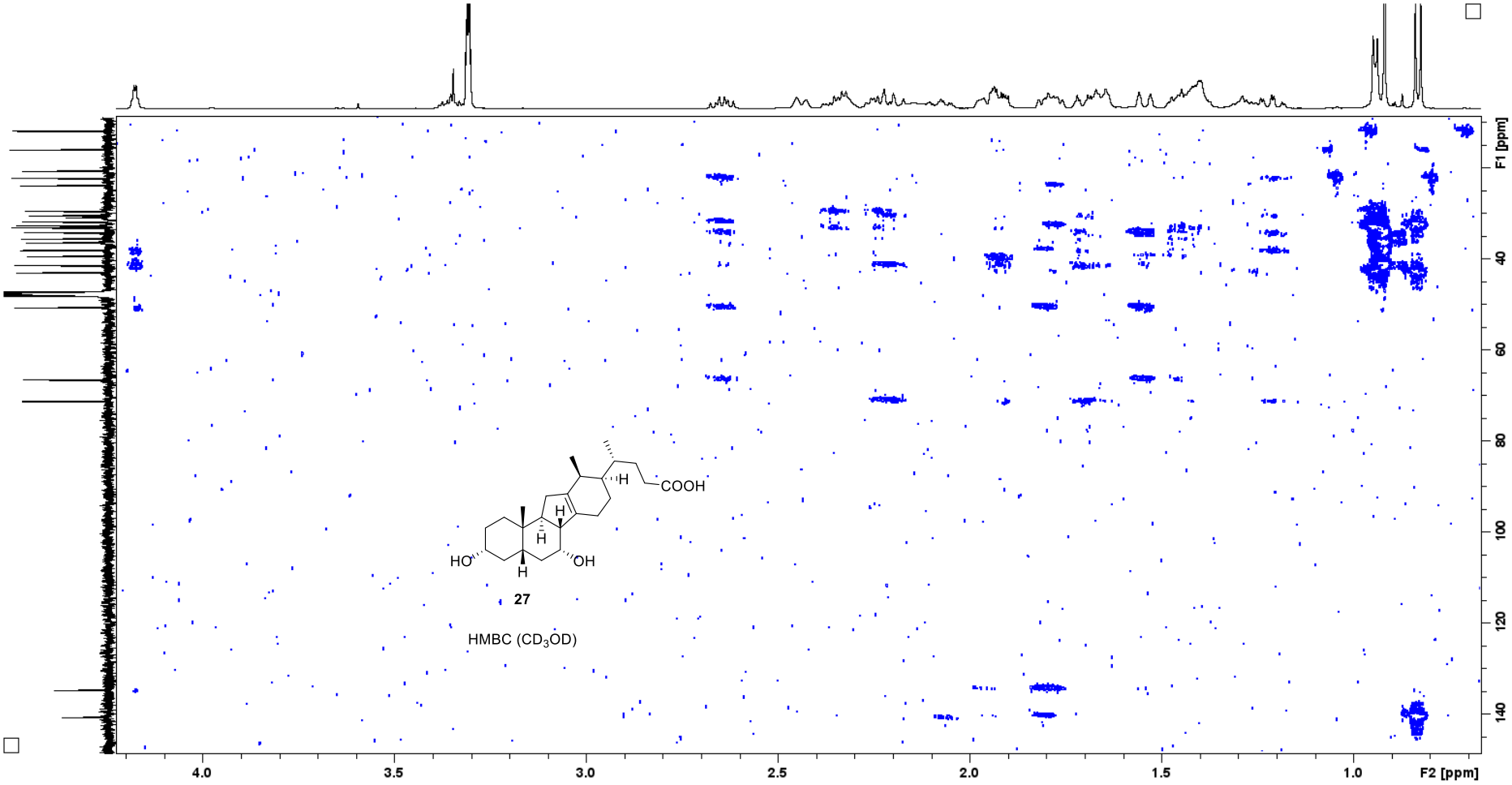


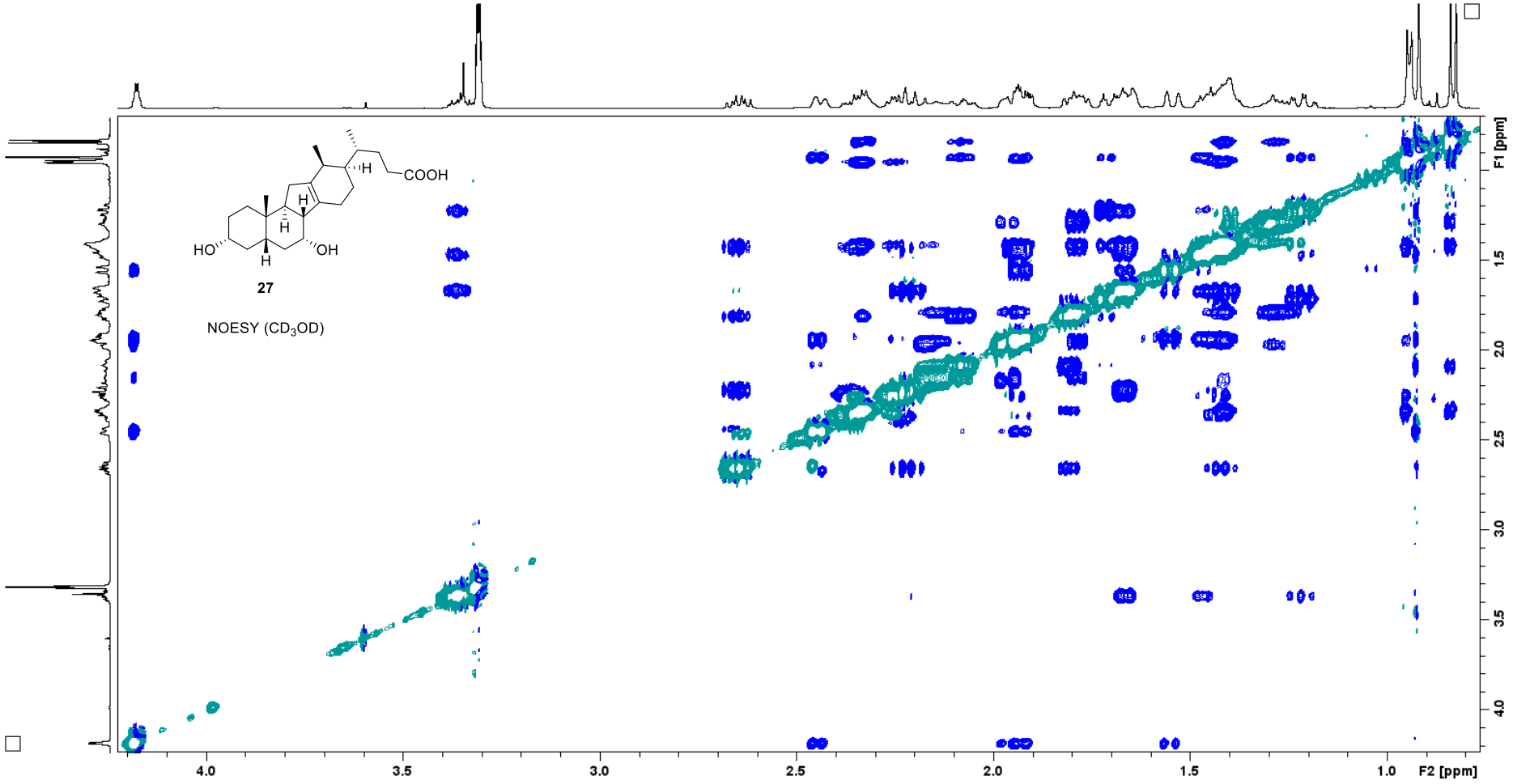
27

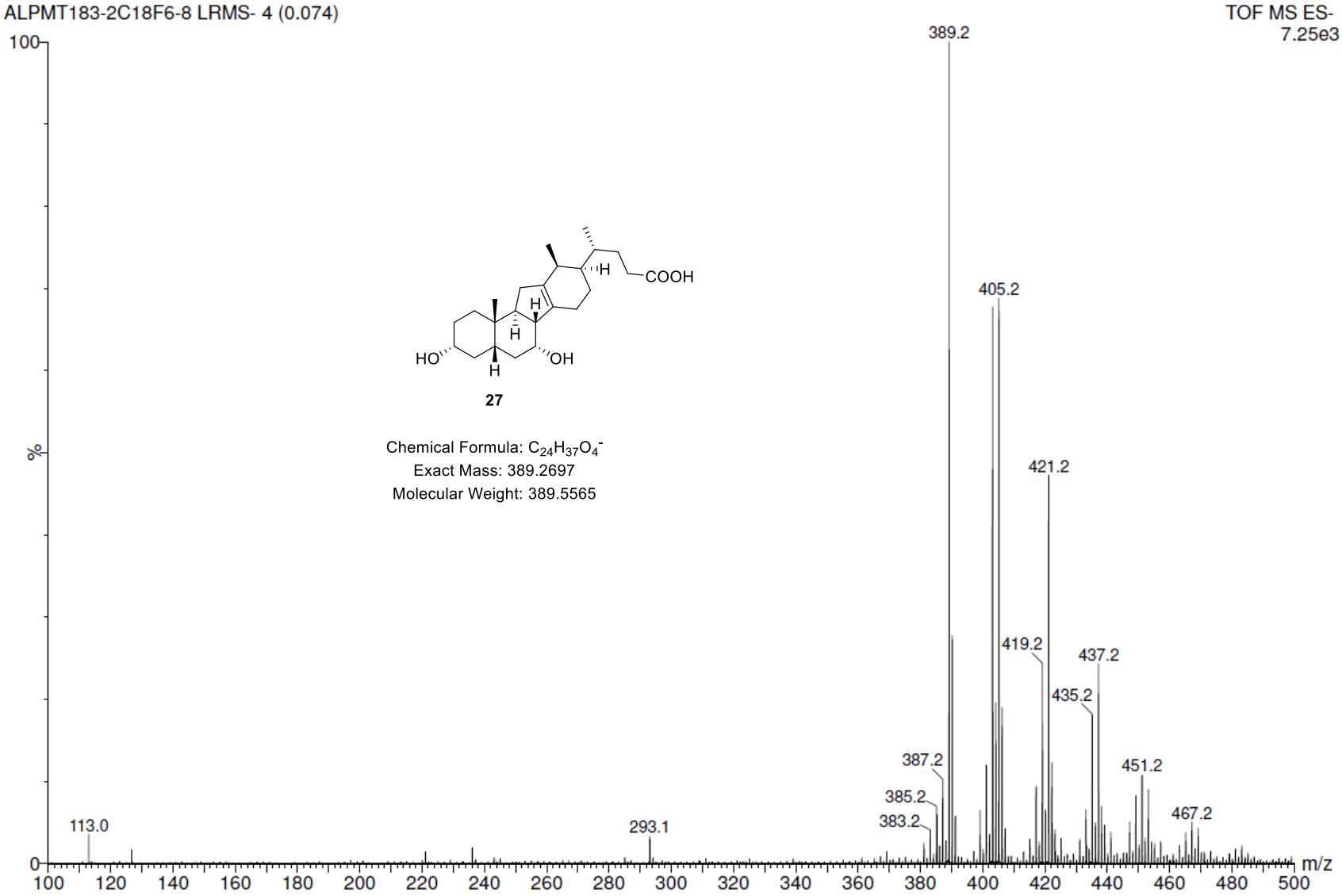
^{13}C NMR (125 MHz, CD_3OD)

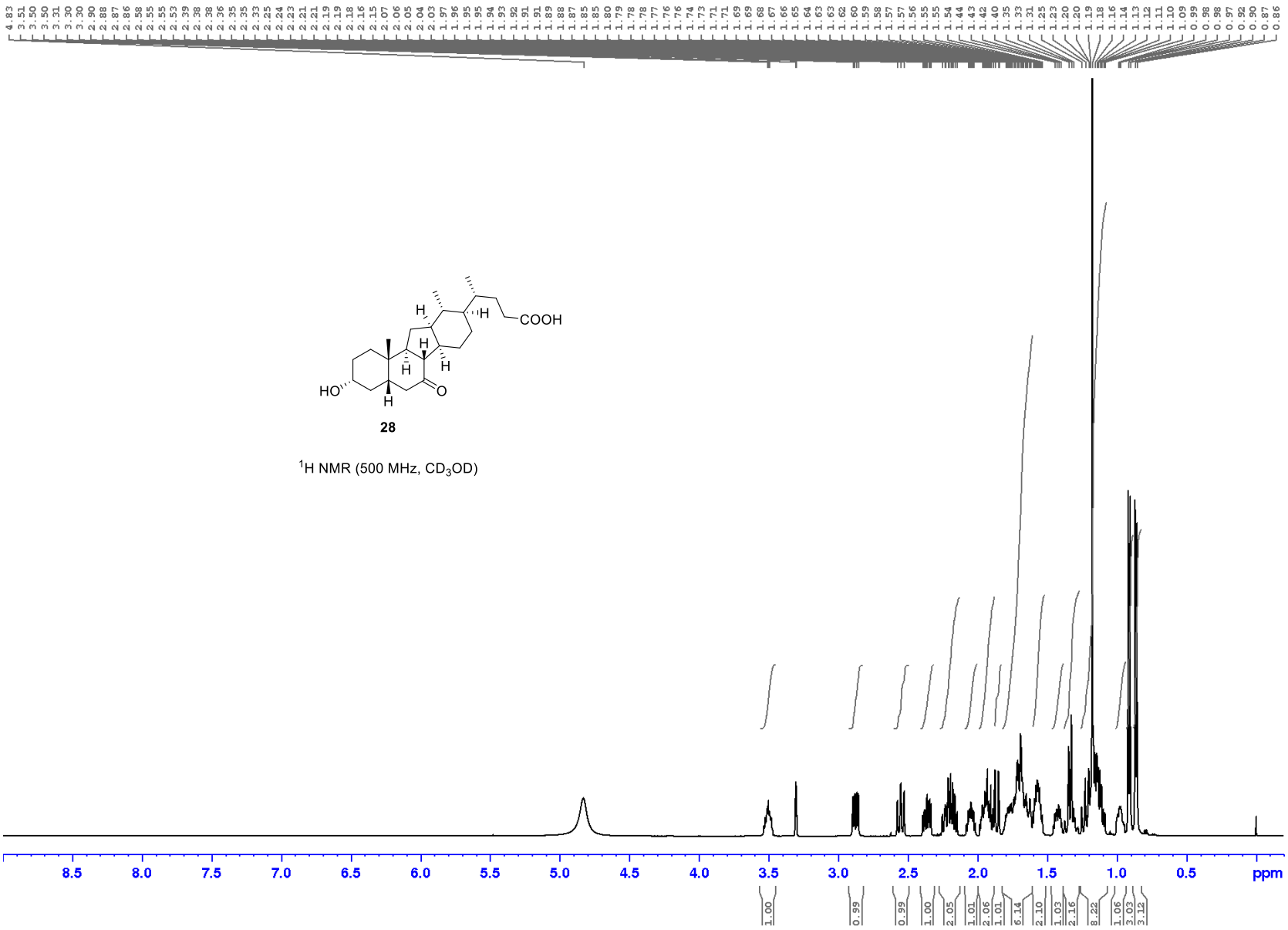

COOH
HO
OH
27
H,H-COSY (CD3OD)
F1 [ppm]
F2 [ppm]
4.0
3.5
3.0
2.5
2.0
1.5
1.0


HO
OH
COOH
H
H
H
H
27
HSQC-DEPT (CD_3OD)
F1 [ppm]
F2 [ppm]
4.0
3.5
3.0
2.5
2.0
1.5
1.0
20
30
40
50
60
70


F1 [ppm]
40
60
80
100
120
140
4.0
3.5
3.0
2.5
2.0
1.5
1.0
F2 [ppm]
COOH
H
H
H
HO
OH
H
27
HMBC (CD3OD)

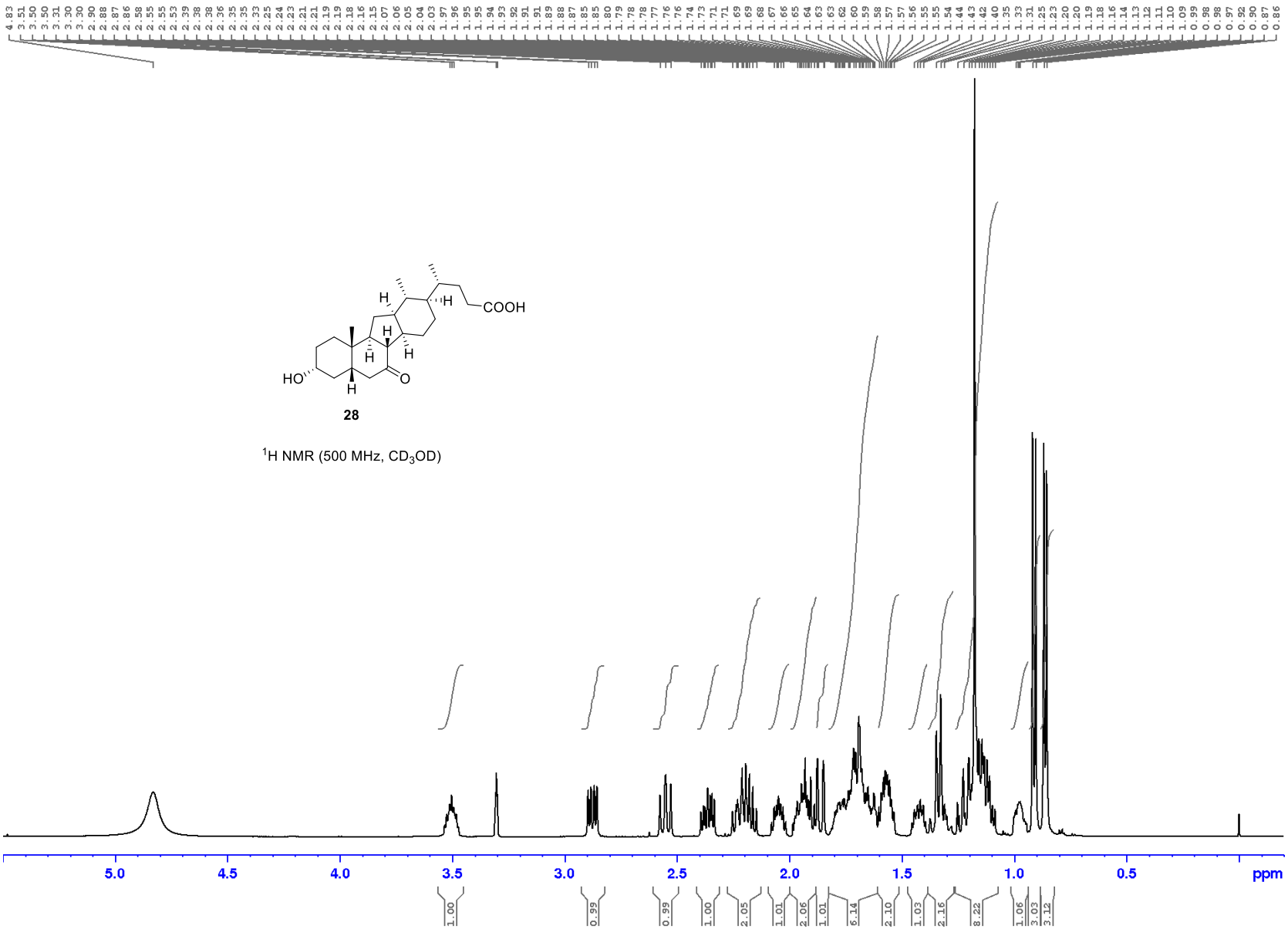

COOH
H
H
H
HO
OH
27
NOESY (CD3OD)
F1 [ppm]
1.5
2.0
2.5
3.0
3.5
4.0
4.0
3.5
3.0
2.5
2.0
1.5
1.0
F2 [ppm]


ALPMT183-2C18F6-8 LRMS- 4 (0.074)
TOF MS ES-
7.25e3
100
%
0
389.2
405.2
421.2
419.2
437.2
435.2
387.2
451.2
385.2
467.2
383.2
113.0
293.1
m/z
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
HO
OH
COOH
H
H
H
H
27
Chemical Formula: $C_{24}H_{37}O_4^-$
Exact Mass: 389.2697
Molecular Weight: 389.5565



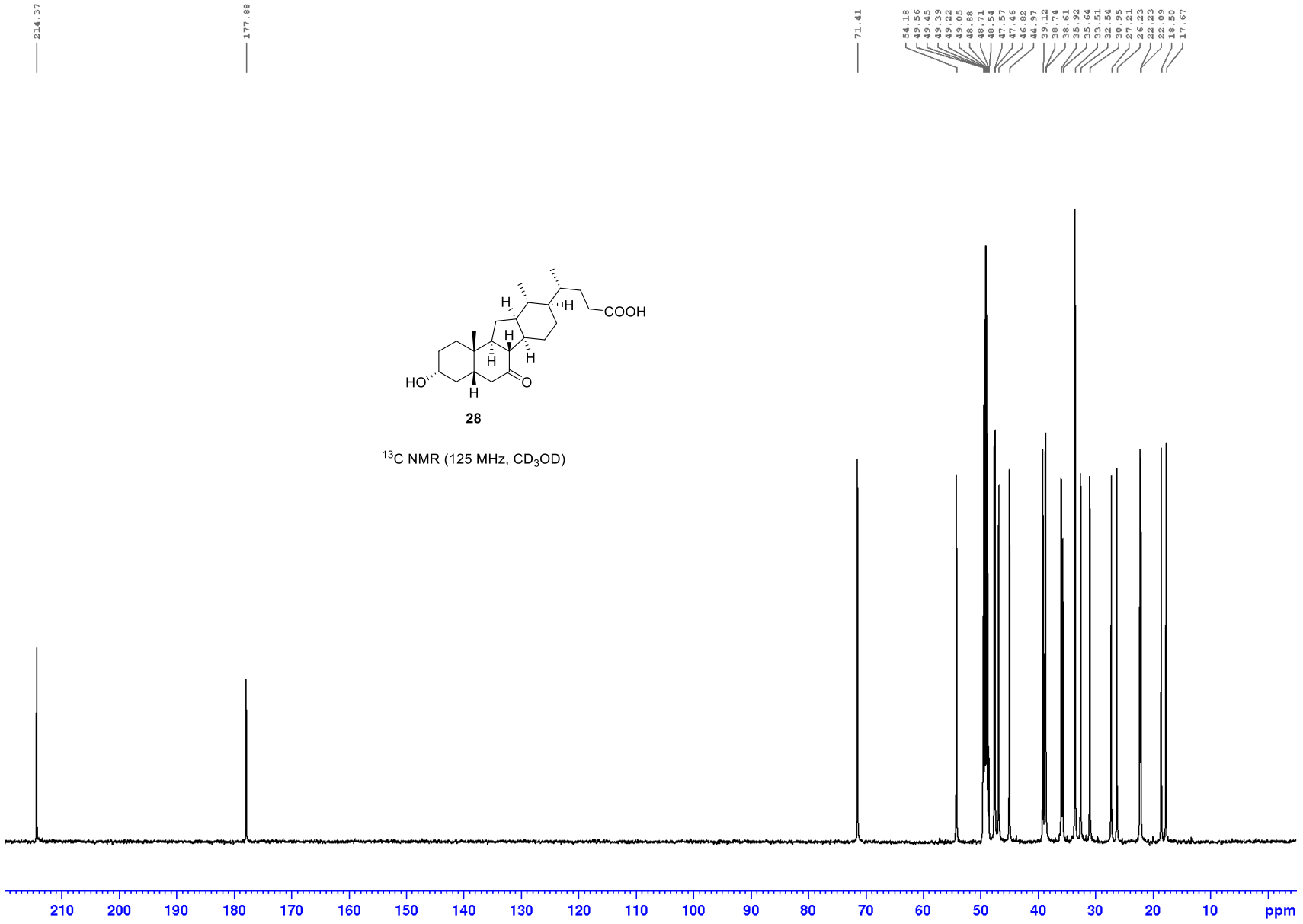
28

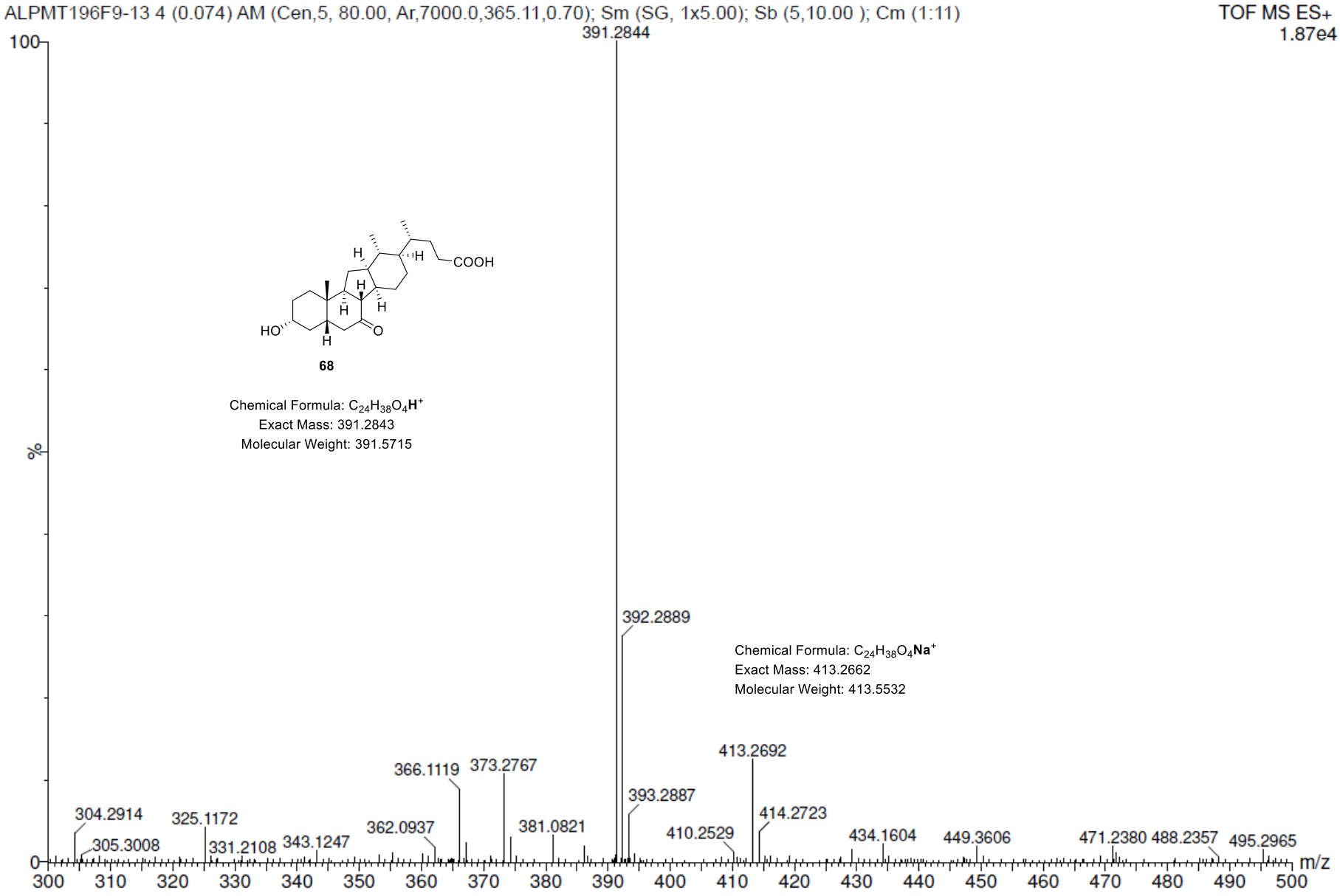
^{1}H NMR (500 MHz, CD_3OD)

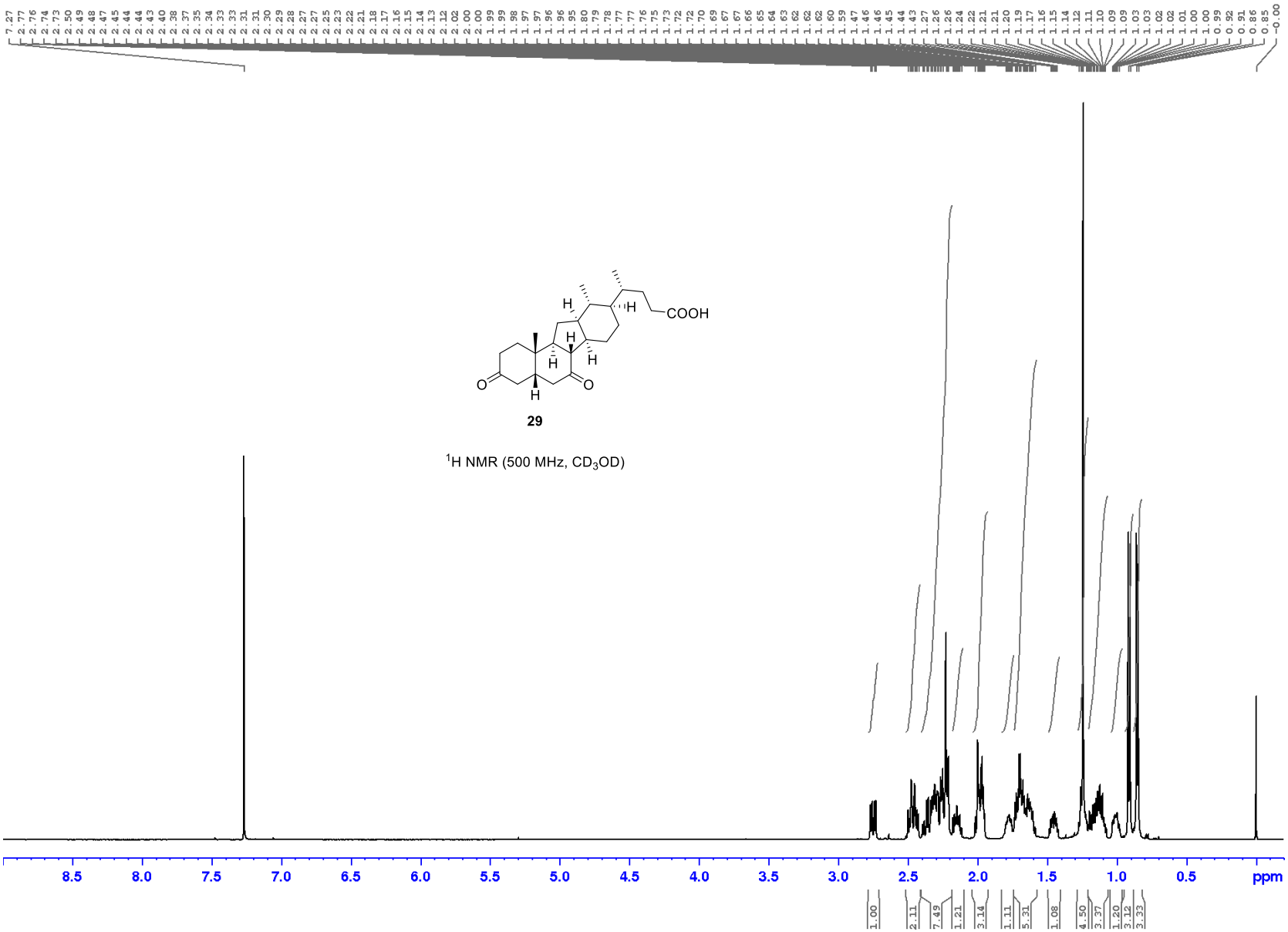


28

^{1}H NMR (500 MHz, CD_3OD)

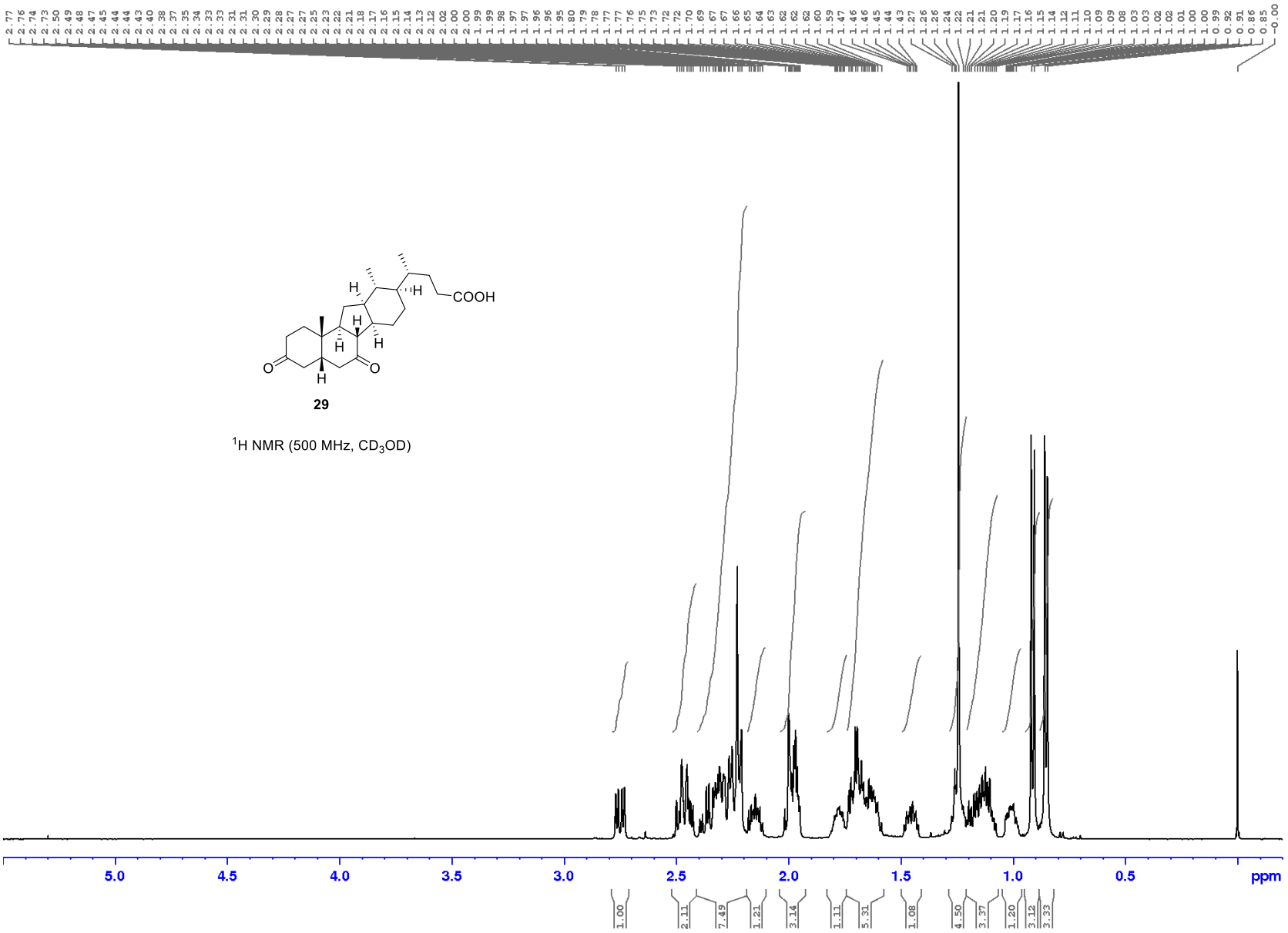

214.37
177.88
71.41
54.18
49.56
49.45
49.39
49.22
49.05
48.88
48.71
48.54
47.57
47.46
46.82
44.97
39.12
38.74
38.61
35.92
35.64
33.51
32.54
30.95
27.21
26.23
22.23
22.09
18.50
17.67
H
H
H
H
H
COOH
HO
H
O
28
13C NMR (125 MHz, CD3OD)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


ALPMT196F9-13 4 (0.074) AM (Cen,5, 80.00, Ar,7000.0,365.11,0.70); Sm (SG, 1x5.00); Sb (5,10.00); Cm (1:11)
TOF MS ES+
1.87e4
100
%
0
391.2844
392.2889
413.2692
366.1119
373.2767
393.2887
414.2723
304.2914
325.1172
305.3008
331.2108
343.1247
362.0937
381.0821
410.2529
434.1604
449.3606
471.2380
488.2357
495.2965
m/z
300
310
320
330
340
350
360
370
380
390
400
410
420
430
440
450
460
470
480
490
500
HO
O
H
COOH
68
Chemical Formula: $C_{24}H_{38}O_4\mathbf{H}^+$
Exact Mass: 391.2843
Molecular Weight: 391.5715
Chemical Formula: $C_{24}H_{38}O_4\mathbf{Na}^+$
Exact Mass: 413.2662
Molecular Weight: 413.5532



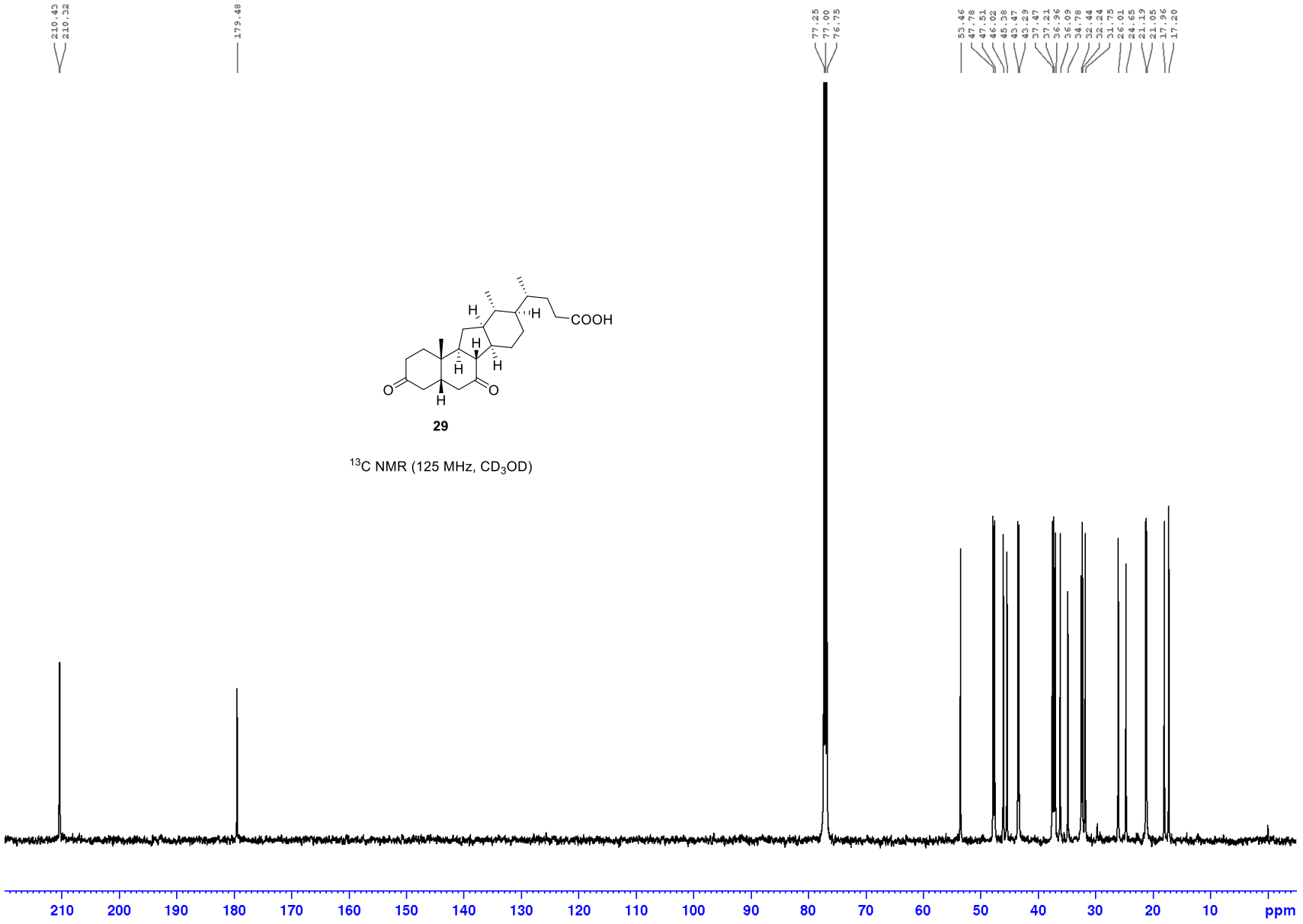
29

^{1}H NMR (500 MHz, CD_3OD)



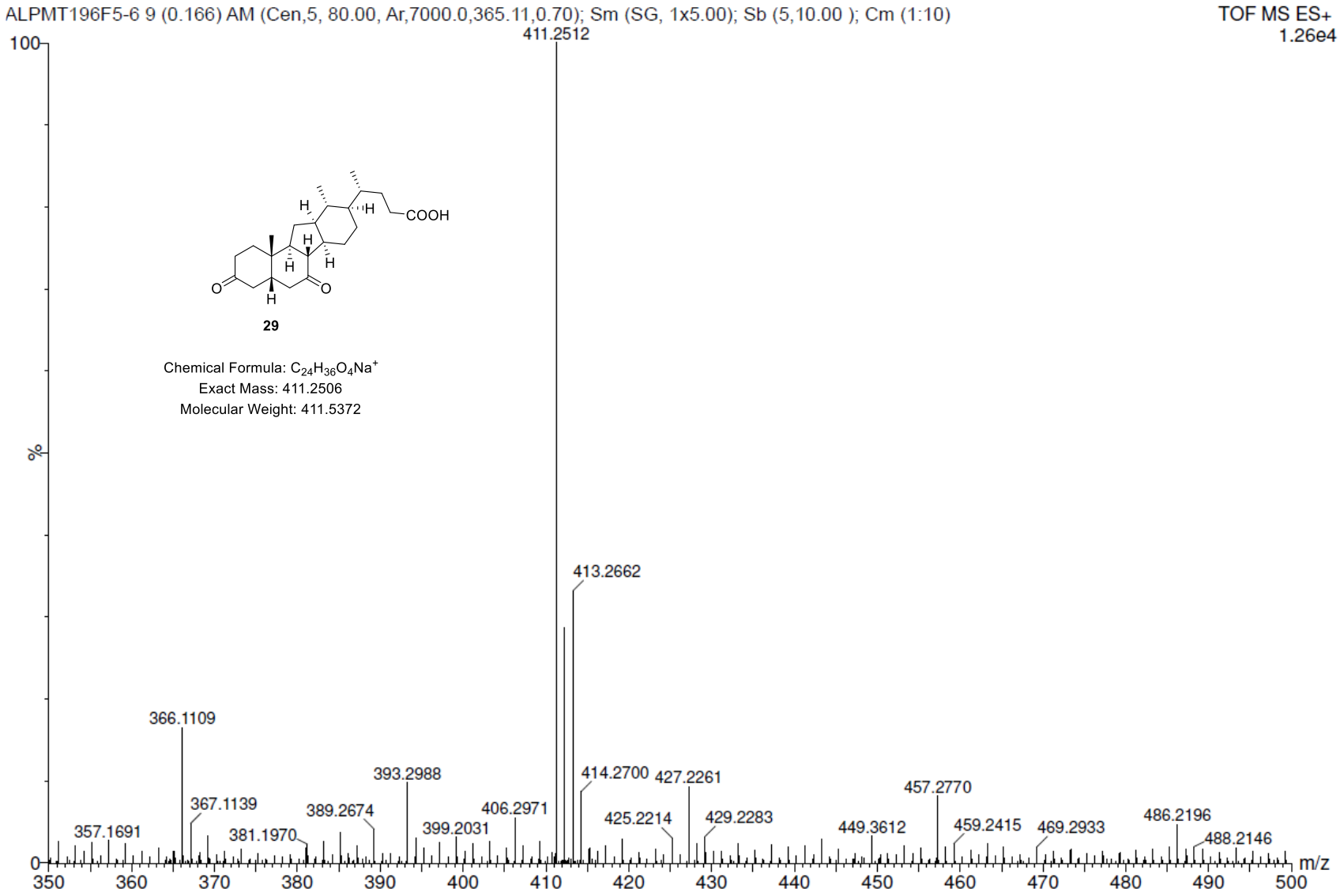
29

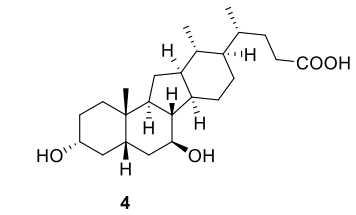
^{1}H NMR (500 MHz, CD_3OD)



29

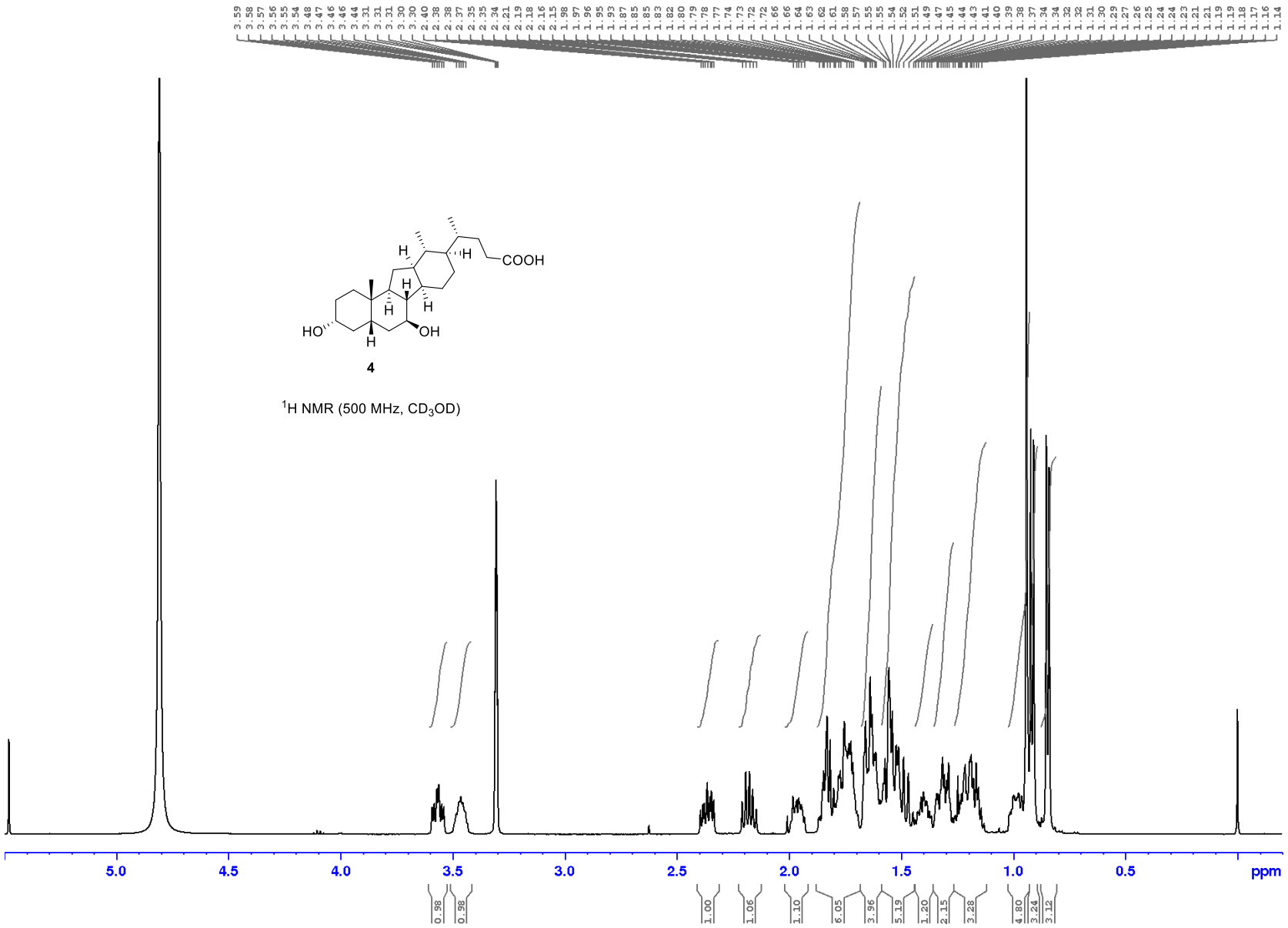
^{13}C NMR (125 MHz, CD_3OD)

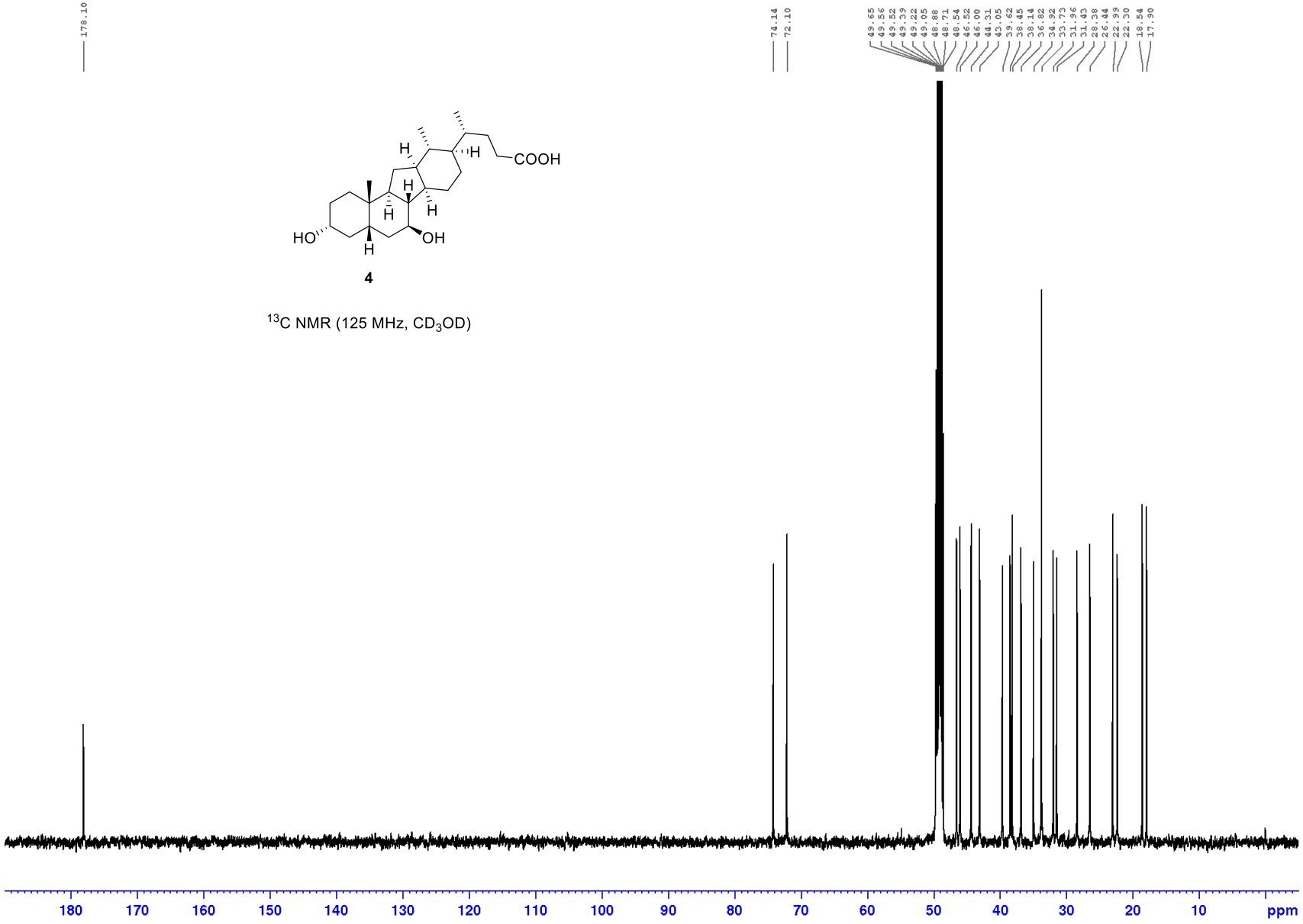

ALPMT196F5-6 9 (0.166) AM (Cen,5, 80.00, Ar,7000.0,365.11,0.70); Sm (SG, 1x5.00); Sb (5,10.00); Cm (1:10)
TOF MS ES+
1.26e4
100
%
0
411.2512
413.2662
366.1109
367.1139
357.1691
381.1970
389.2674
393.2988
399.2031
406.2971
414.2700
425.2214
427.2261
429.2283
449.3612
457.2770
459.2415
469.2933
486.2196
488.2146
350
360
370
380
390
400
410
420
430
440
450
460
470
480
490
500
m/z
COOH
O
O
H
29
Chemical Formula: $C_{24}H_{36}O_4Na^+$
Exact Mass: 411.2506
Molecular Weight: 411.5372



4

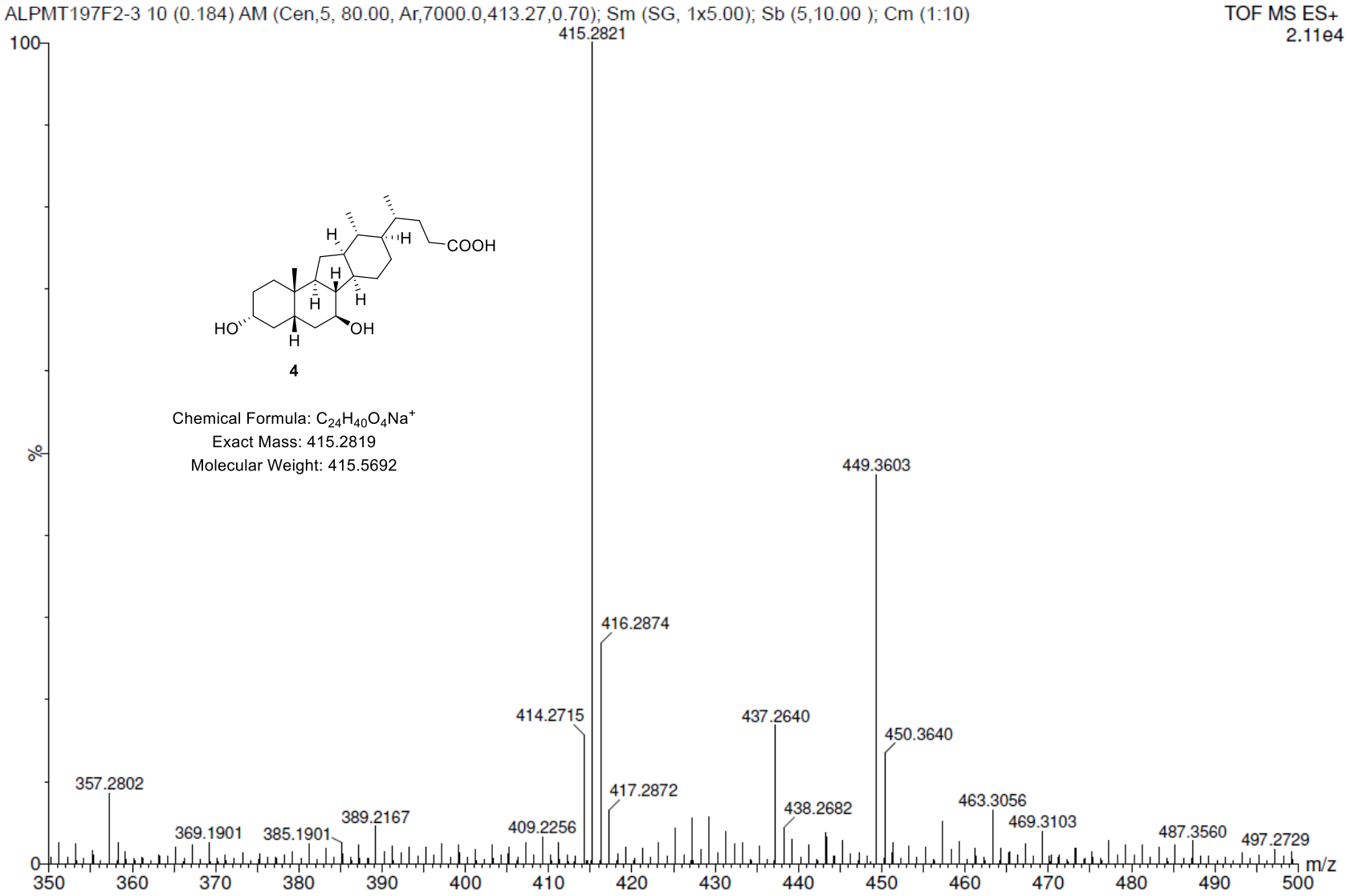
^{1}H NMR (500 MHz, CD_3OD)


3.59 3.58 3.57 3.56 3.55 3.54 3.48 3.47 3.46 3.46 3.44 3.31 3.31 3.31 3.30 3.30 2.40 2.38 2.38 2.37 2.35 2.34 2.21 2.19 2.18 2.16 2.15 1.98 1.97 1.96 1.95 1.93 1.87 1.85 1.85 1.83 1.82 1.80 1.79 1.78 1.77 1.74 1.73 1.72 1.72 1.66 1.66 1.64 1.63 1.62 1.61 1.58 1.57 1.55 1.55 1.54 1.52 1.51 1.49 1.47 1.45 1.44 1.43 1.41 1.40 1.39 1.38 1.37 1.34 1.34 1.34 1.32 1.32 1.31 1.30 1.29 1.27 1.26 1.25 1.24 1.24 1.23 1.21 1.21 1.19 1.19 1.18 1.17 1.16 1.14
COOH
H
H
H
H
H
HO
OH
4
^{1}H NMR (500 MHz, CD_3OD)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.98
0.98
1.00
1.06
1.10
6.05
3.96
5.19
1.20
2.15
3.28
4.80
3.24
3.12



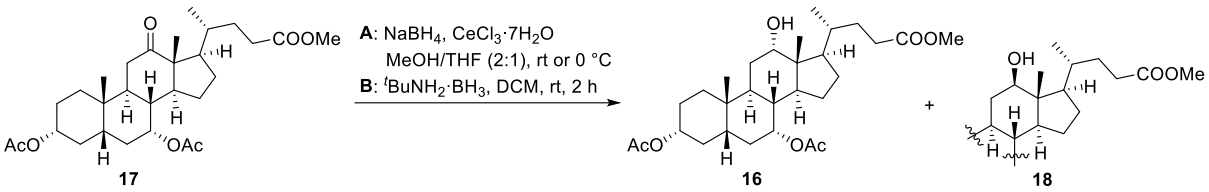
4

^{13}C NMR (125 MHz, CD_3OD)


ALPMT197F2-3 10 (0.184) AM (Cen,5, 80.00, Ar,7000.0,413.27,0.70); Sm (SG, 1x5.00); Sb (5,10.00); Cm (1:10)
TOF MS ES+
2.11e4
100
%
0
m/z
350
360
370
380
390
400
410
420
430
440
450
460
470
480
490
500
415.2821
449.3603
416.2874
414.2715
437.2640
450.3640
357.2802
417.2872
438.2682
463.3056
469.3103
389.2167
369.1901
385.1901
409.2256
487.3560
497.2729
HO
OH
COOH
H
4
Chemical Formula: $C_{24}H_{40}O_4Na^+$
Exact Mass: 415.2819
Molecular Weight: 415.5692

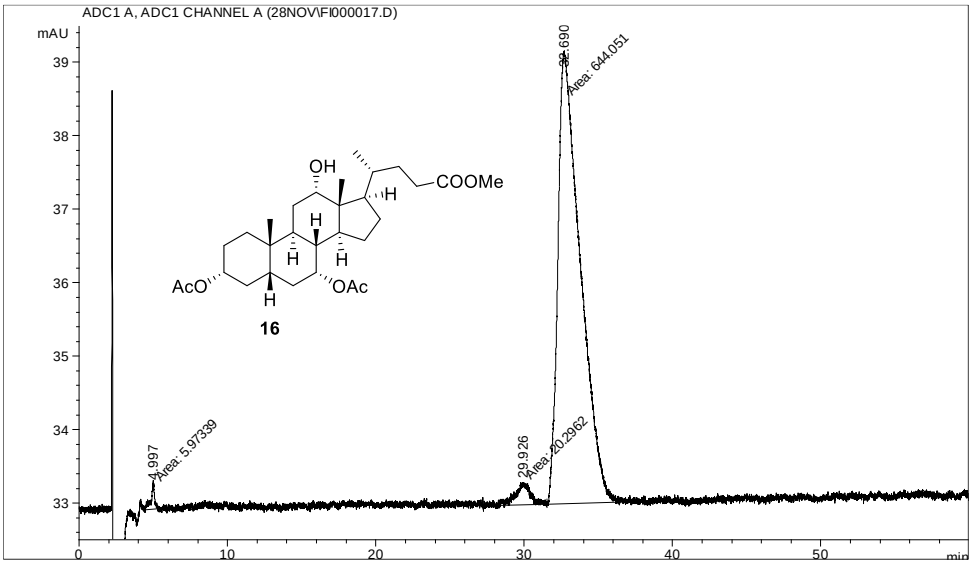
4. HPLC chromatograms

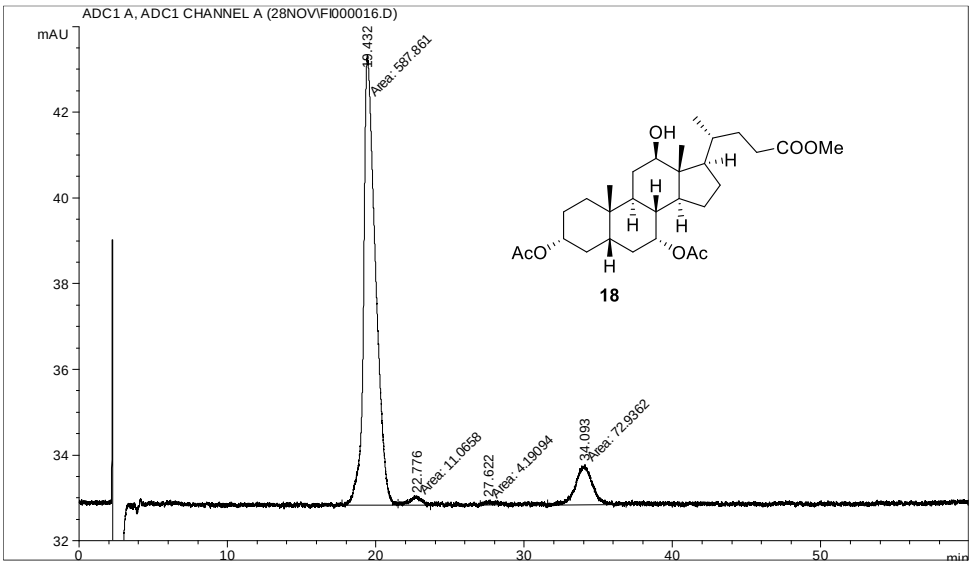
4.1. HPLC analysis of 3,7-diacetate-12-oxo methyl ester 17 reductions:

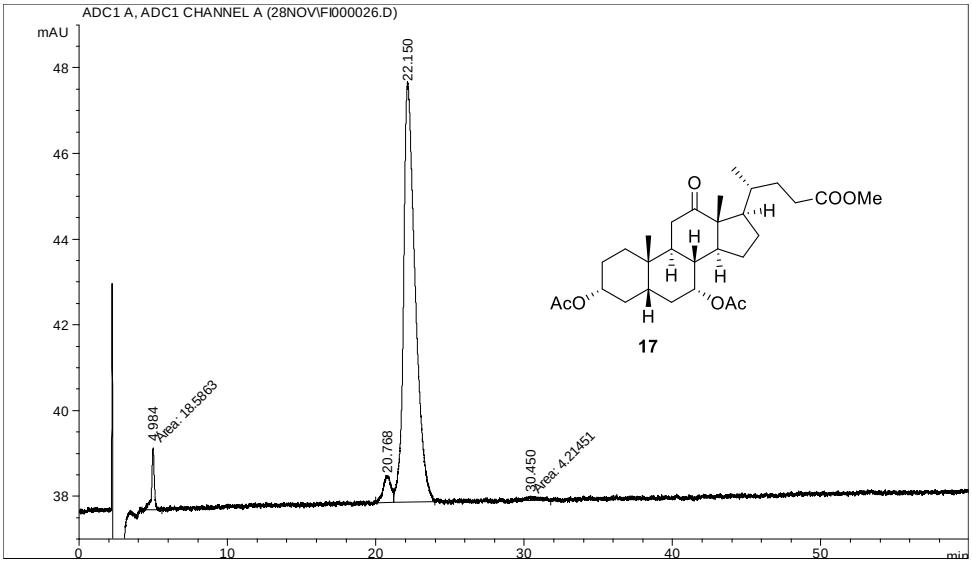


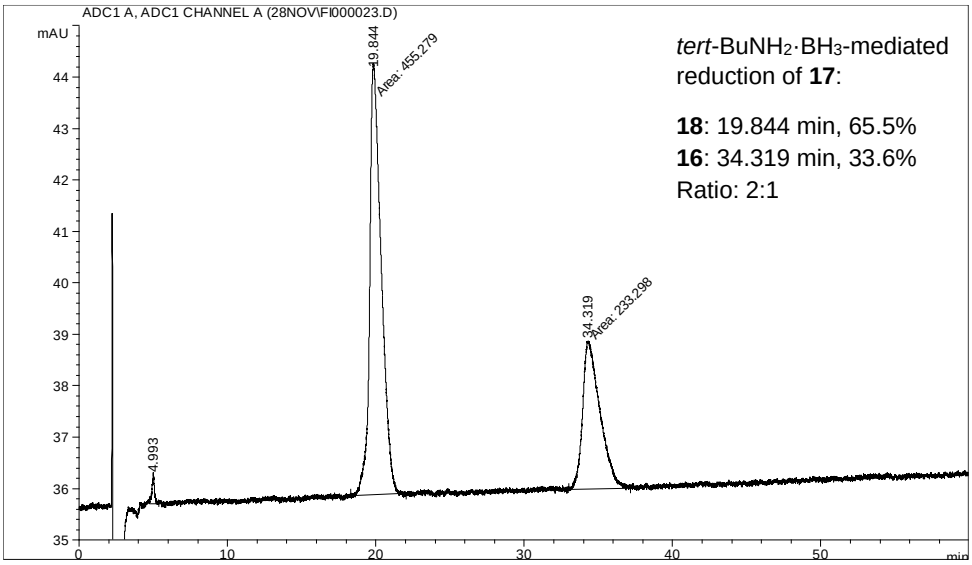
Method conditions:

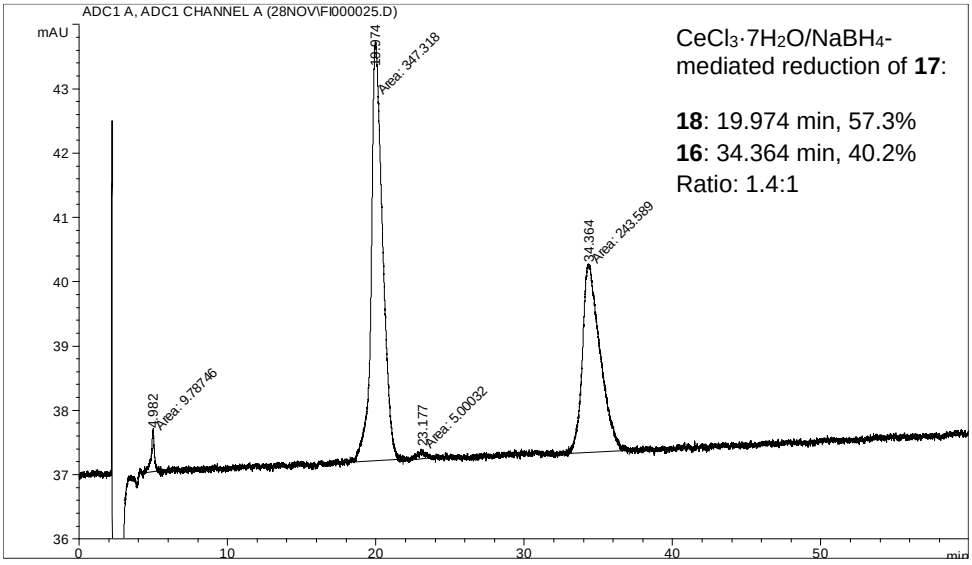
Column:	Waters Spherisorb ODS2 5 µm 250×4.6 mm		
Guard:	Phenomenex Security Guard C18 RP 4×3 mm		
Mobile Phase:	30:70:0.05 Water/Methanol/Trifluoroacetic acid		
Gradient:	Isocratic;	Flow Rate:	1 mL/min
Sample Solvent:	Methanol;	Detection:	*Refractive index*
Column Temp:	20 °C;	Injection volume:	30 µL



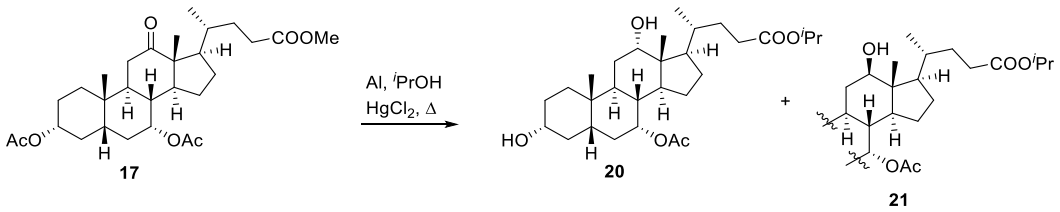



ADC1 A, ADC1 CHANNEL A (28NOV\FI000026.D)
mAU
4.984
Area: 18.5863
20.768
22.150
30.450
Area: 4.21451
min
O
COOMe
H
AcO
OAc
17


ADC1 A, ADC1 CHANNEL A (28NOV\FI000023.D)
mAU
19.844
Area: 455.279
4.993
34.319
Area: 233.298
min
tert-BuNH2·BH3-mediated reduction of 17:
18: 19.844 min, 65.5%
16: 34.319 min, 33.6%
Ratio: 2:1

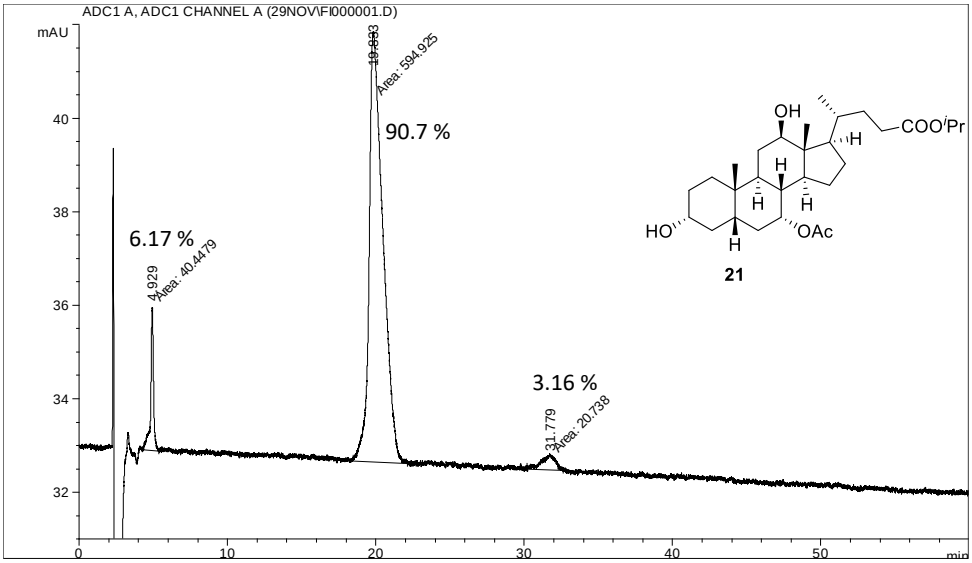

ADC1 A, ADC1 CHANNEL A (28NOV\FI000025.D)
mAU
19.974
Area: 347.318
4.982
Area: 9.78746
23.177
Area: 5.00032
34.364
Area: 243.589
min
CeCl3·7H2O/NaBH4-mediated reduction of 17:
18: 19.974 min, 57.3%
16: 34.364 min, 40.2%
Ratio: 1.4:1

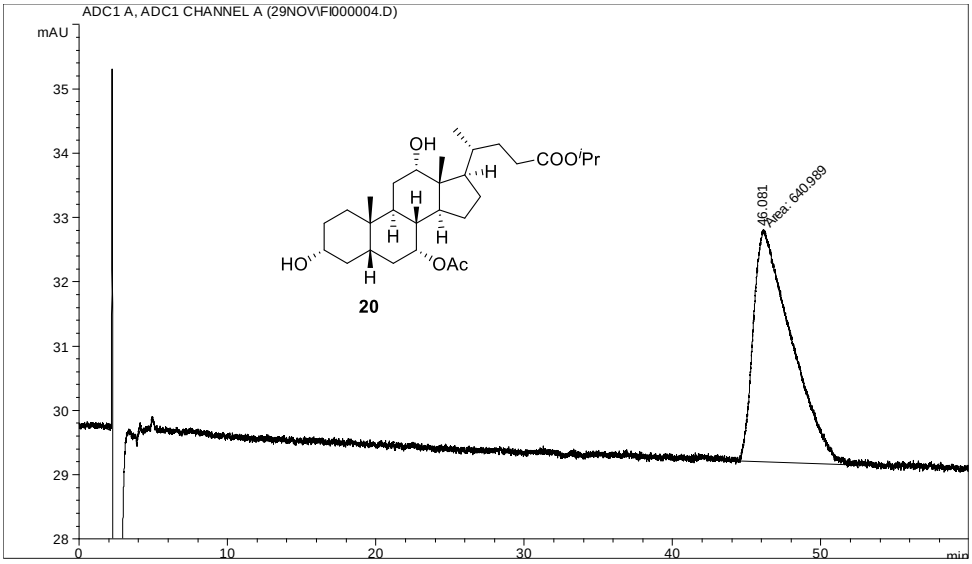
4.2. HPLC analysis of Meerwein-Ponndorf-Verley (MPV) reduction of 3,7-diacetate-12-oxo methyl ester 17 reductions:

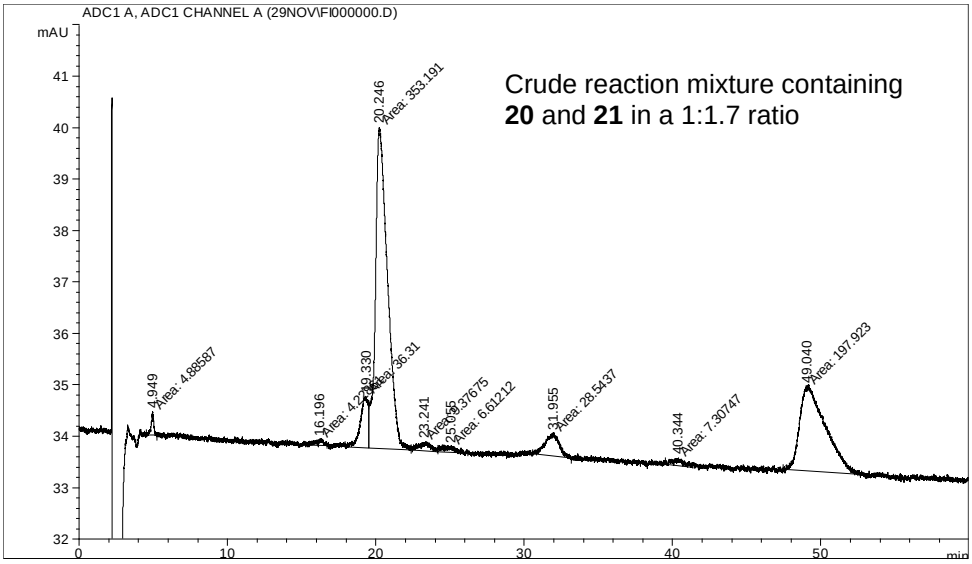


Method conditions:

Column:	Waters Spherisorb ODS2 5 µm 250×4.6 mm		
Guard:	Phenomenex Security Guard C18 RP 4×3 mm		
Mobile Phase:	30:70:0.05 Water/Methanol/Trifluoroacetic acid		
Gradient:	Isocratic;	Flow Rate:	1 mL/min
Sample Solvent:	Methanol;	Detection:	*Refractive index*
Column Temp:	20 °C;	Injection volume:	30 µL

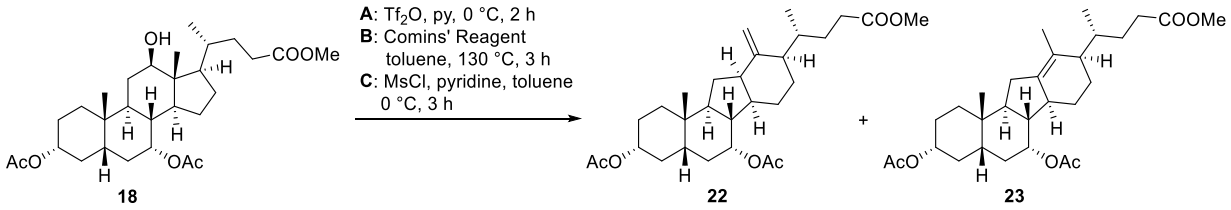






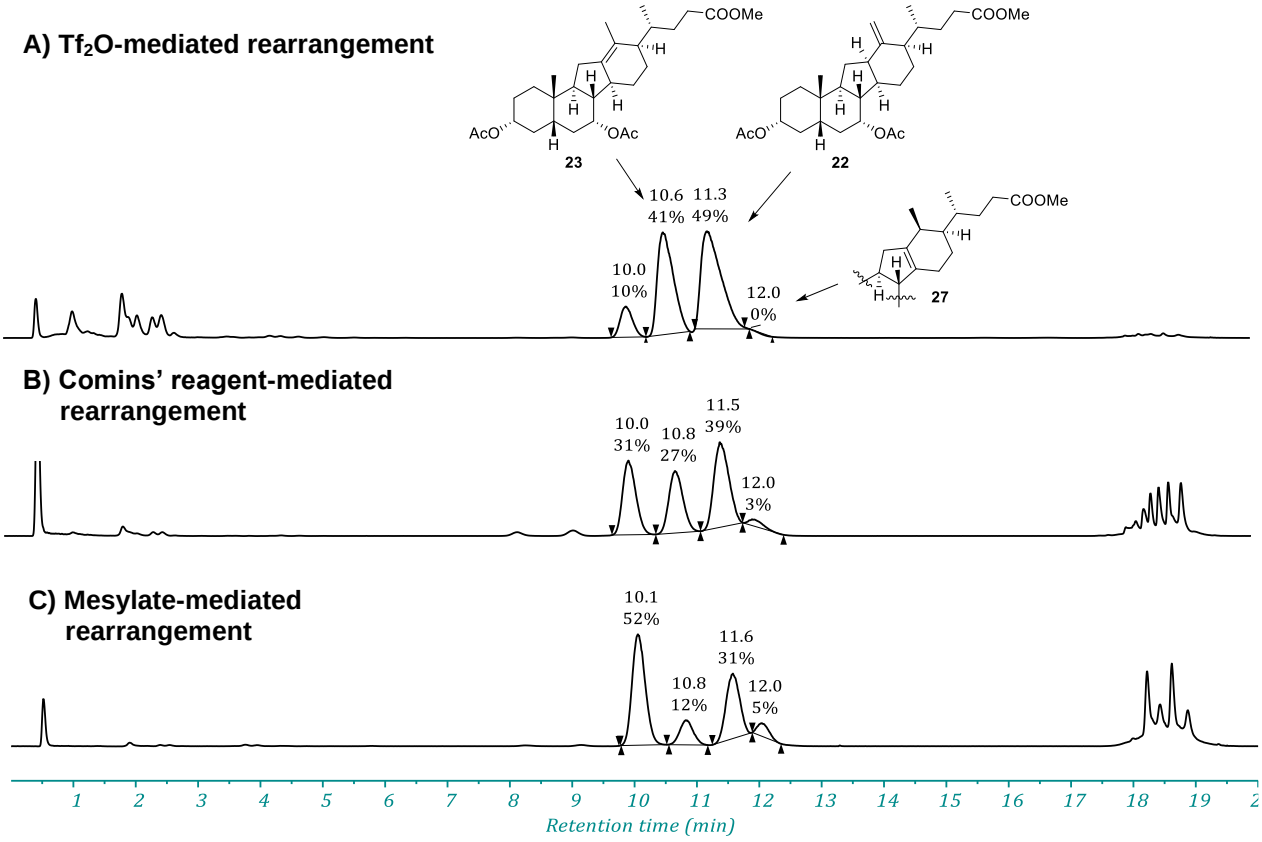
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.949	MM	0.1838	4.88587	4.42979e-1	0.7536
2	16.196	MM	0.6348	4.22861	1.11023e-1	0.6522
3	19.330	MF	0.6027	36.31005	1.00410	5.6001
4	20.246	FM	0.9425	353.19110	6.24580	54.4730
5	23.241	MF	0.9257	9.37675	1.68830e-1	1.4462
6	25.055	FM	1.0066	6.61212	1.09479e-1	1.0198
7	31.955	MM	1.0994	28.54368	4.32699e-1	4.4023
8	40.344	MM	0.9193	7.30747	1.32488e-1	1.1270
9	49.040	MM	2.0256	197.92293	1.62849	30.5258
Totals :				648.37860	10.27589	

4.3. HPLC analysis of crude rearrangement mixture 22/23:



Method conditions:

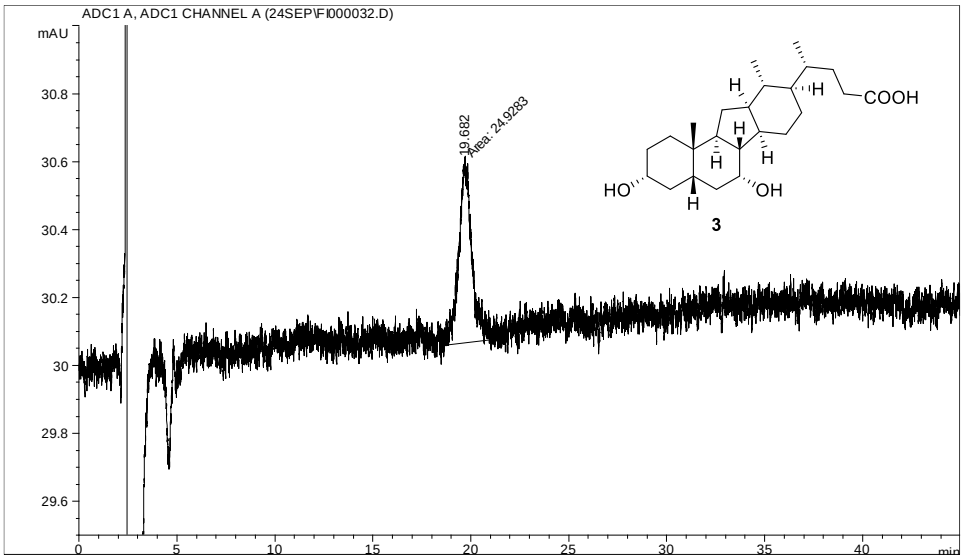
Column: Phenomenex Kinetex, 2.6 µM C-18, pore size = 100 Å, Dimensions = 50 × 30 mm
Mobile Phase A: Water (+ 0.1% Formic acid)
Mobile Phase B: Methanol
Method (% B): T0=72, T12=72, T13=100, T14=100, T16=72, T18=72
Flow Rate: 0.5 mL/min
Sample Solvent: Methanol; Detection: CAD
Column Temp: 40 °C; Injection volume: 1 µL

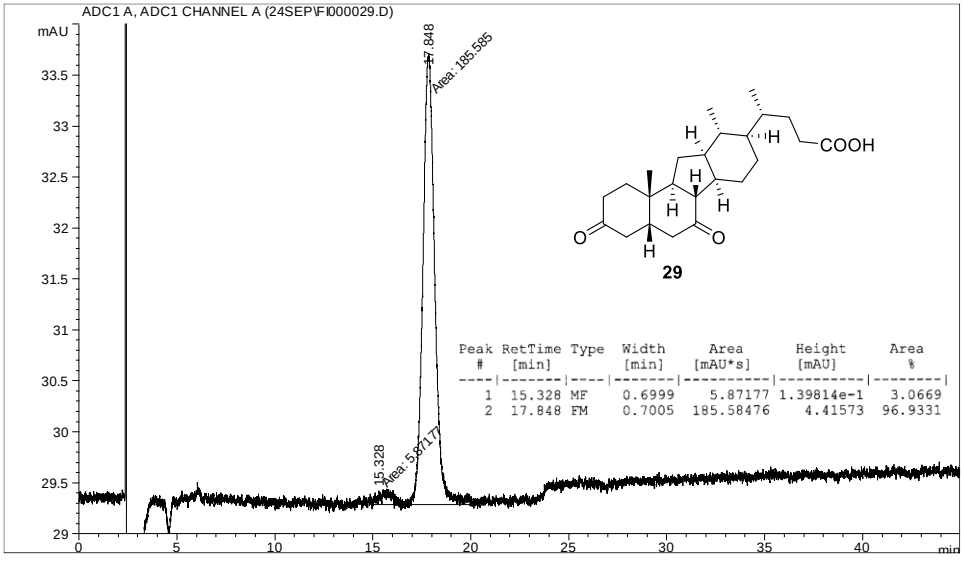


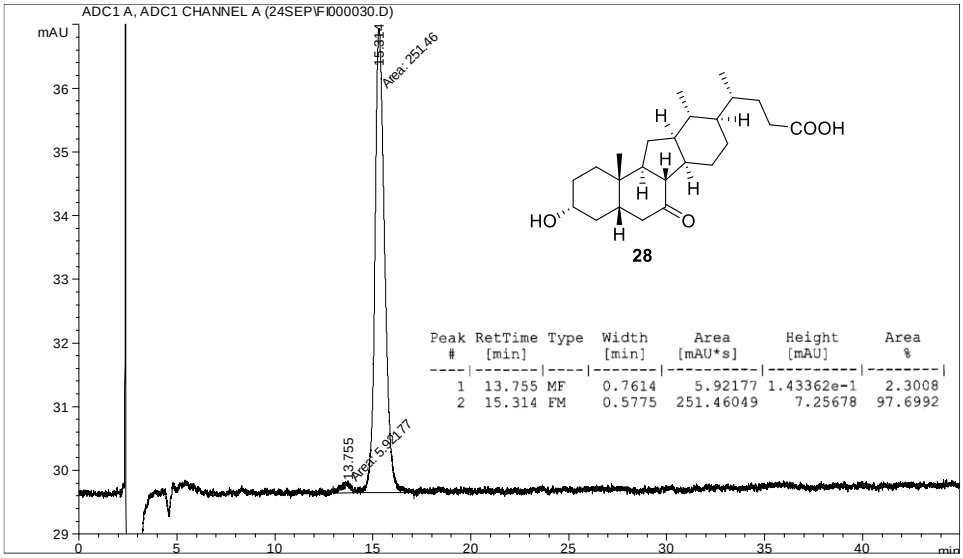
4.4. Individual HPLC traces of compounds 4, 6, 28 and 29:

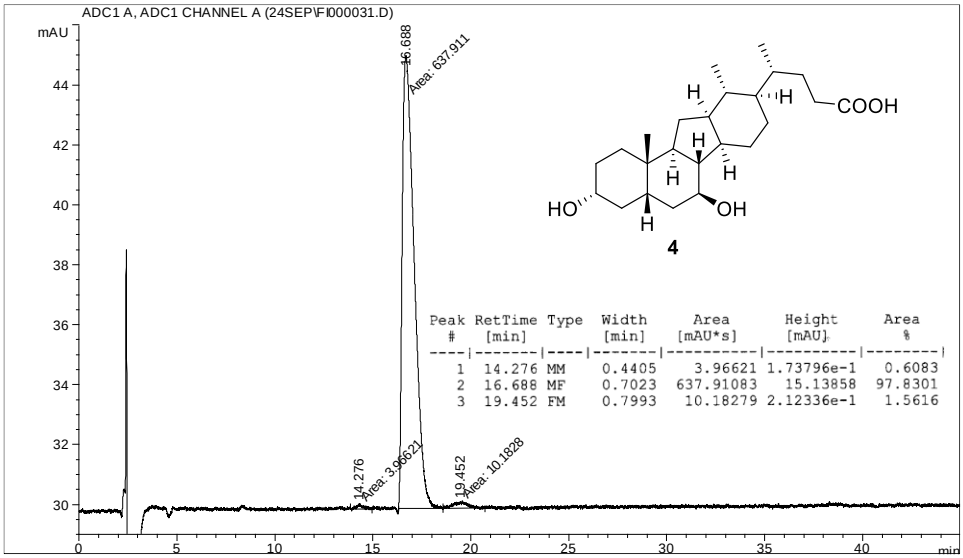
Method conditions:

Column: Phenomenex Luna C18(2) 5 µm 250×4.6 mm
Guard: Phenomenex Security Guard C18 RP 4×3 mm
Mobile Phase: 30:70:0.05 Water/Methanol/Trifluoroacetic acid
Gradient: Isocratic
Flow Rate: 1 mL/min
Sample Solvent: Methanol
Detection: *Refractive index*
Column Temp: 20 °C
Injection volume: 30 µL





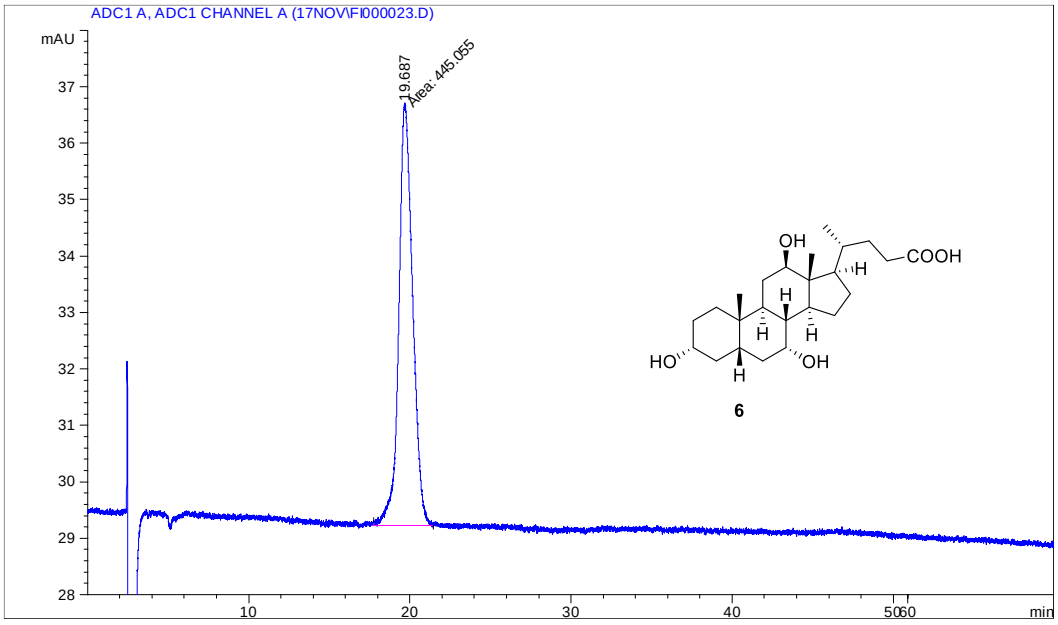

ADC1 A, ADC1 CHANNEL A (24SEP\FI000030.D)
mAU
15.314
Area: 251.46
13.755
Area: 5.92177
COOH
HO
O
28
Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area %
1 | 13.755 | MF | 0.7614 | 5.92177 | 1.43362e-1 | 2.3008
2 | 15.314 | FM | 0.5775 | 251.46049 | 7.25678 | 97.6992
min


ADC1 A, ADC1 CHANNEL A (24SEP\FI000031.D)
mAU
16.688
Area: 637.911
14.276
Area: 3.96621
19.452
Area: 10.1828
COOH
HO
OH
4
Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area %
1 | 14.276 | MM | 0.4405 | 3.96621 | 1.73796e-1 | 0.6083
2 | 16.688 | MF | 0.7023 | 637.91083 | 15.13858 | 97.8301
3 | 19.452 | FM | 0.7993 | 10.18279 | 2.12336e-1 | 1.5616
min

Individual HPLC trace of compounds 6:

Method conditions:

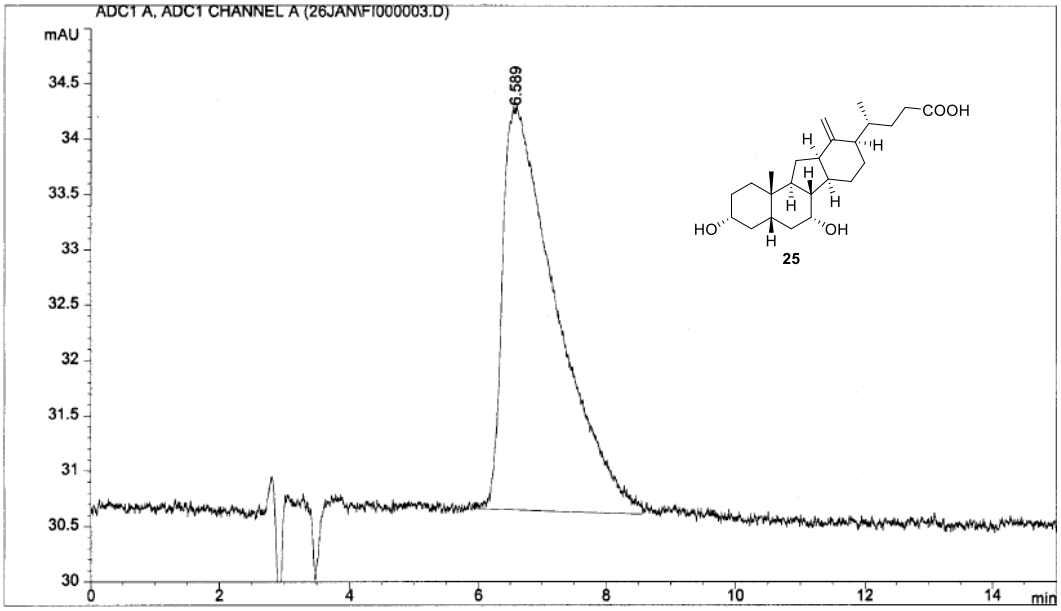
Column: Phenomenex Luna C18(2) 5 µm 250×4.6 mm
Guard: Phenomenex Security Guard C18 RP 4×3 mm
Mobile Phase: 40:60:0.05 Water/Methanol/Trifluoroacetic acid
Gradient: Isocratic
Flow Rate: 1 mL/min
Sample Solvent: Methanol
Detection: *Refractive index*
Column Temp: 20 °C
Injection volume: 20 µL

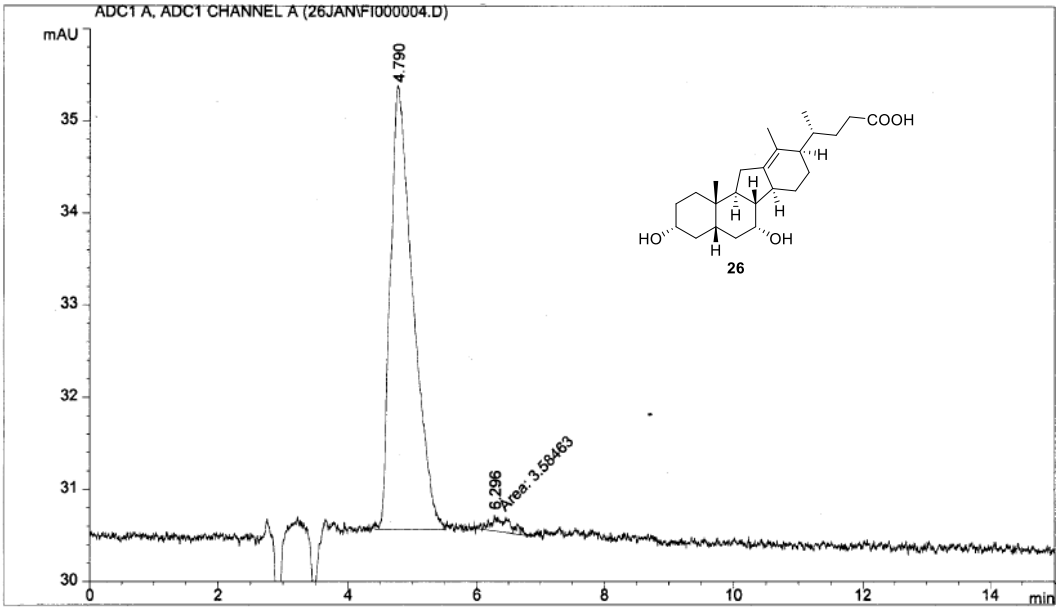


Individual HPLC traces of compounds 25 and 26:

Method conditions:

Column: Phenomenex Lux Amylose-2 5 µm 250×4.6 mm
Guard: Phenomenex Security Guard Amylose-2 4×3 mm
Mobile Phase: 40:60 Water/Acetonitrile
Gradient: Isocratic
Flow Rate: 1 mL/min
Sample Solvent: Acetonitrile
Detection: *Refractive index*
Column Temp: 20 °C
Injection volume: 10 µL

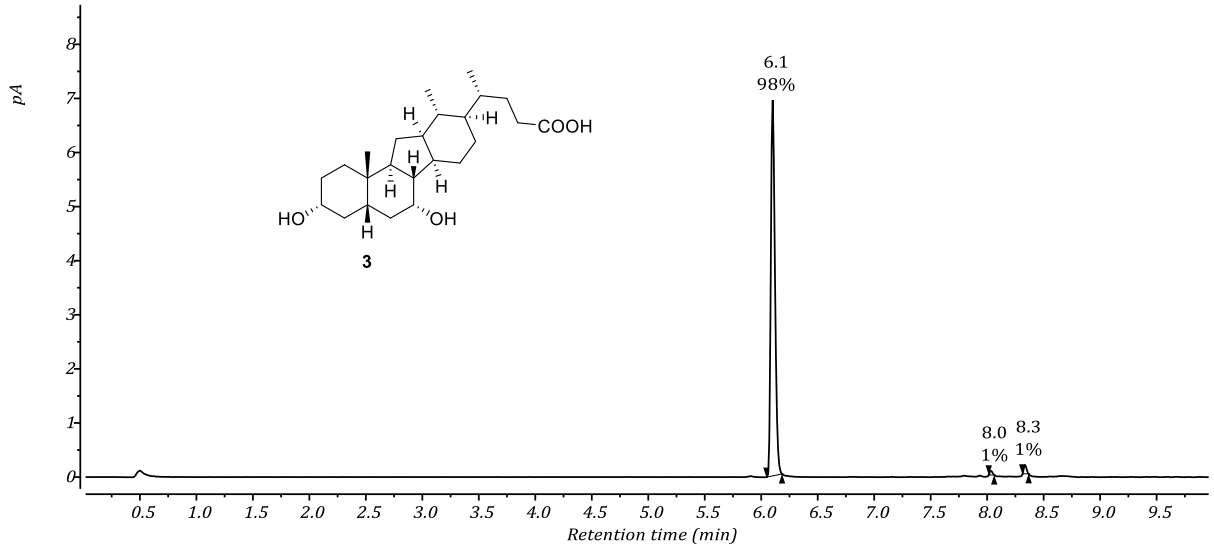




4.6. HPLC trace of 3:

Method conditions:

Column:	Phenomenex Kinetex 2.6 µM C-18, pore size = 100 Å C-18 100 × 30 mm
Column Temp:	40 °C
Detection:	CAD
Flow Rate:	0.5 mL/min
Sample Solvent:	Methanol (1 µL injection)
Mobile Phase A:	Water + 0.1% Formic acid
Mobile Phase B:	Methanol
Gradient: (% B):	T0=30, T7=100, T9=100, T10=30, T12=30



4.7. LCMS trace of compound 24:

Column: Poroshell 2.6 µM C-18, pore size = 100 Å C-18 100 × 30 mm
Column Temp: 40 °C
Detection: ELSD (temp 60 °C)
Flow Rate: 1 mL/min
Sample Solvent: Methanol (1 µL injection)
Mobile Phase A: 5 mM Ammonium formate and 0.012% Formic acid in Water
Mobile Phase B: 5 mM Ammonium formate and 0.012% Formic acid in Methanol
Gradient: (% B): T0=50, T7=100, T9=100, T9.5=50, T10=50

