Interaction of human drug-metabolizing CYP3A4 with rationally designed ritonavir analogues: Impact of steric constraints imposed on the heme-ligating group and the end-pyridine attachment

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SUPPLEMENTARY MATERIALS

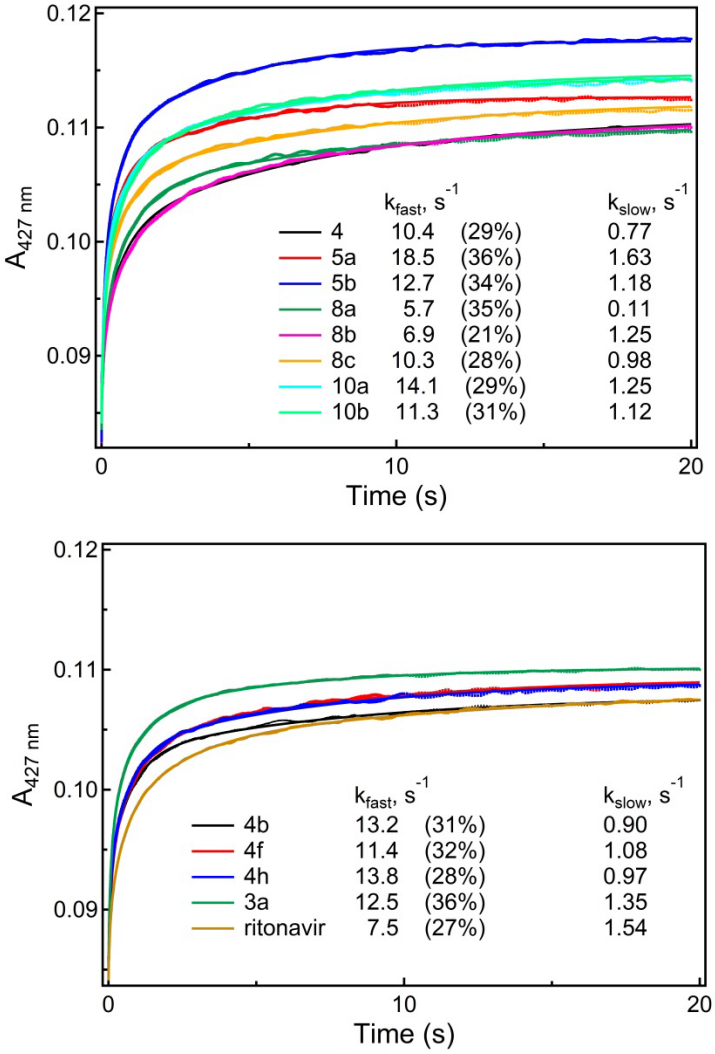


Figure S1. Kinetics of ligand binding to CYP3A4. Kinetic traces were recorded by mixing 2 μM CYP3A4 with 20 μM solutions of series VI analogues (upper panel) or previously reported compounds and ritonavir (lower panel). Within the studied time interval, all reactions were biphasic. Fittings are shown as solid lines. The derived rate constants for the fast and slow phases and the percentage of the fast phase are indicated.

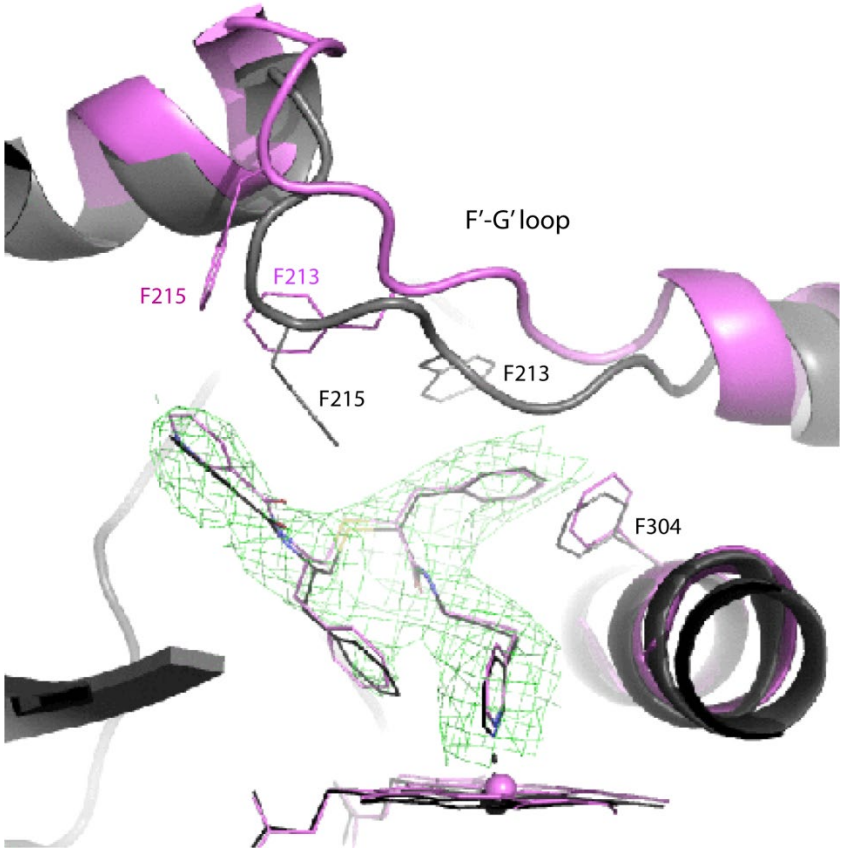


Figure S2. Superposition of **10a**–bound CYP3A4 crystallized in the I222 (magenta) and C2 space group (gray; molecule A). The ligand binding mode is only slightly affected by differences in crystal packing. Green mesh is polder omit electron density map contoured at 3 σ level.

Table S1. Data collection and refinement statistics

Ligand	**5a**	**5b**	**8a**	**8b**
CYP3A4	WT	WT	WT	WT
PDB ID	7UF9	7UFA	7UFB	7UFC
Data statistics				
Space group	I222	I222	I222	I222
Unit cell	*a, b, c* = 77 x 101 x 128 Å; α, β, γ = 90°	*a, b, c* = 77 x 102 x 126 Å; α, β, γ = 90°	*a, b, c* = 77 x 102 x 128 Å; α, β, γ = 90°	*a, b, c* = 78 x 103 x 128 Å; α, β, γ = 90°
Resolution range (Å)	79.53-2.45 (2.58-2.45)[a]	50.86-2.50 (2.64-2.50)	79.62-2.25 (2.37-2.25)	80.35-2.35 (2.48-2.35)
Total reflections	82,809 (12,979)	124,746 (17,945)	186,616 (26,677)	82,093 (12,824)
Unique reflections	18,319 (2,694)	17,305 (2,503)	24,033 (3,473)	20,611 (3,070)
Redundancy	4.5 (4.6)	7.2 (7.2)	7.8 (7.7)	4.0 (4.2)
Completeness	98.1 (99.5)	99.8 (100.0)	99.5 (99.6)	95.3 (98.0)
Average $I/\sigma I$	12.5 (1.1)	9.8 (1.0)	13.8 (1.3)	9.2 (1.1)
R_{merge}	0.047 (1.630)	0.089 (2.524)	0.059 (1.604)	0.066 (2.695)
R_{pim}	0.024 (0.842)	0.036 (1.006)	0.022 (0.610)	0.036 (1.362)
CC ½	0.999 (0.416)	0.998 (0.403)	0.999 (0.395)	0.994 (0.480)
Refinement statistics				
R/R_{free}[b]	22.5/25.5	24.7/28.4	21.0/25.8	20.8/25.4
Number of atoms:				
Protein	3645	3735	3350	3727
Solvent	0	6	20	7
R.m.s. deviations				
Bond lengths, Å	0.004	0.003	0.003	0.003
Bond angles, °	0.634	0.558	0.517	0.535
Wilson B-factor, Å^2	85	79	70	77
Average *B*-factor, Å^2:				
Protein	119	119	101	107
Ligand	137	119	102	107
Ramachandran plot[d] (residues; %)				
Preferred	413 (94.3%)	421 (94.6%)	445 (96.7%)	434 (96.2%)
Allowed	23 (5.2%)	23 (5.2%)	15 (3.3%)	27 (3.8%)
Outliers	2 (0.5%)	1 (0.2%)	none	none

[a]Values in brackets are for the highest resolution shell.
[b]R_{free} was calculated from a subset of 5% of the data that were excluded during refinement.
[c]Values for two molecules in the asymmetric unit.
[d]Analyzed with PROCHECK.

Table S2. Data collection and refinement statistics

Ligand	**8c**	**10a**	**10b**
CYP3A4	WT	K421A/K424A	K421A/K424A
PDB ID	7UFD	7UFE	7UFF
Data statistics			
Space group	I222	C2	C2
Unit cell	*a, b, c* = 78 x103 x 130 Å; α, β, γ = 90	*a, b, c* = 154 x 97 x 94 Å; α, β, γ = 90 x 124 x 90°	*a, b, c* = 157 x 98 x 95 Å; α, β, γ = 90 x 125 x 90°
Resolution range (Å)	80.52-2.90 (3.06-2.90) [a]	77.63-2.40 (2.53-2.40)	77.75-2.70 (2.85-2.70)
Total reflections	57,848 (8,841)	148,105 (22,323)	183,071 (22,905)
Unique reflections	11,818 (1,714)	43,861 (6,382)	30,683 (4,113)
Redundancy	4.9 (5.2)	3.4 (3.5)	6.0 (5.5)
Completeness	99.8 (99.9)	98.7 (98.9)	95.1 (88.6)
Average $I/\sigma I$	6.3 (2.5)	8.3 (1.1)	8.5 (1.0)
R_{merge}	0.174 (1.203)	0.063 (1.257)	0.115 (1.774)
R_{pim}	0.089 (0.570)	0.039 (0.777)	0.050 (0.818)
CC ½	0.972 (0.354)	0.998 (0.506)	0.997 (0.405)
Refinement statistics			
R/R_{free} [b]	20.1/27.8	22.1/25.3	24.4/28.1
Number of atoms:			
Protein	3727	3706/3575 [c]	3685/3575 [c]
Solvent	0	53	13
R.m.s. deviations0.003	0.002	0.002	0.002
Bond angles, °	0.476	0.503	0.511
Wilson B-factor, Å^2	94	71	83
Average *B*-factor, Å^2:			
Protein	107	90/111 [c]	92/123 [c]
Ligand	103	80/90 [c]	83/115 [c]
Ramachandran plot [d] (residues; %)			
Preferred	429 (95.1%)	820 (92.3%)	822 (93.3%)
Allowed	22 (4.9%)	67 (7.6%)	58 (6.6%)
Outliers	none	1 (0.1%)	1 (0.1%)

[a] Values in brackets are for the highest resolution shell.
[b] R_{free} was calculated from a subset of 5% of the data that were excluded during refinement.
[c] Values for two molecules in the asymmetric unit.
[d] Analyzed with PROCHECK.

Table S3. Local correlation coefficients (CC) and CC_{peak} between three polder maps (F_{obs} = $|F_{model}|$)

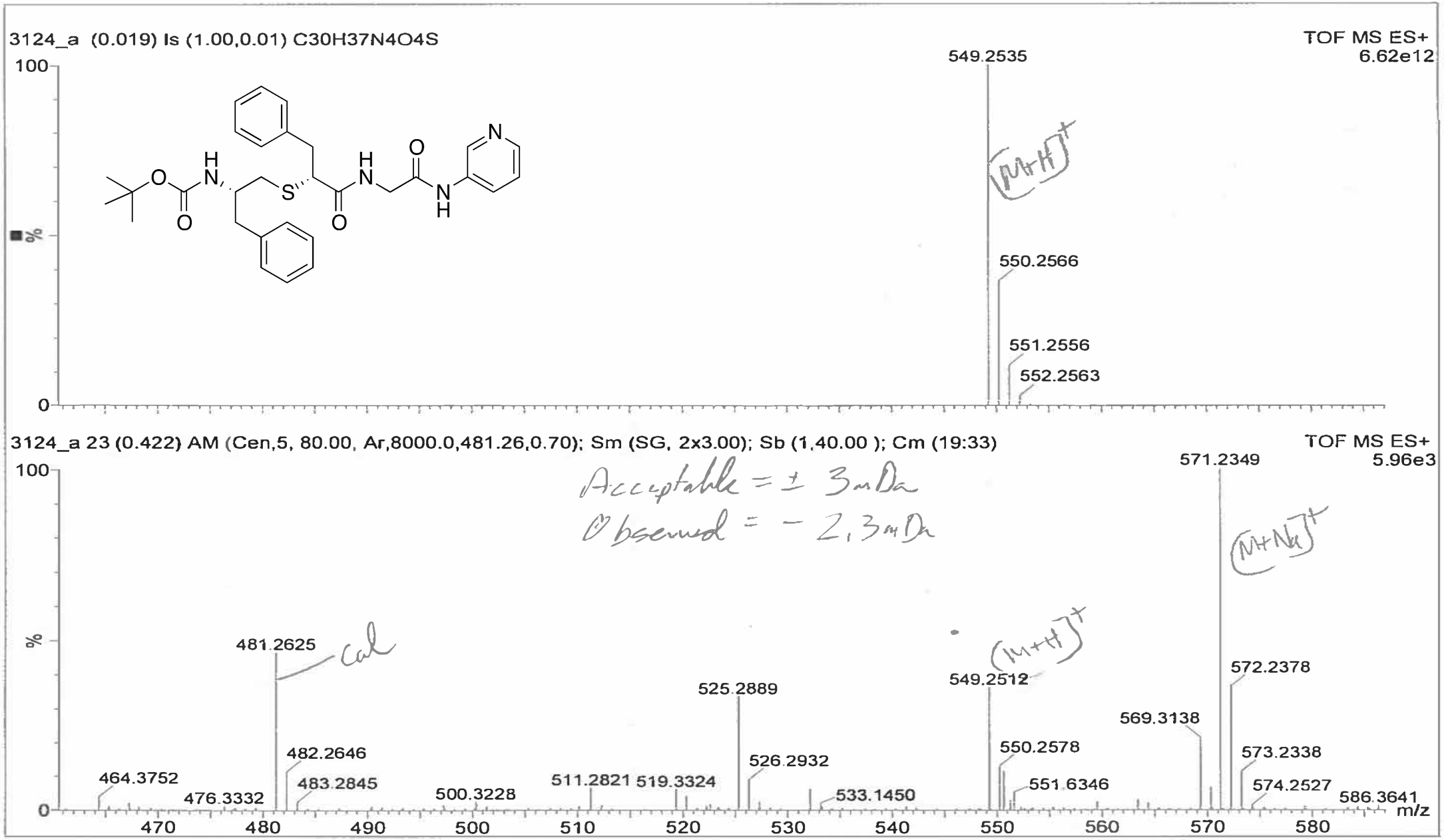
Map 1 (m1), calculated F_{obs} assuming that the omitted ligand atoms are present. Map 2 (m2), calculated F_{obs} assuming that the omitted ligand atoms are not present. Map 3 (m3), polder map calculated using the experimental data.

		m1-m2		m1-m3		m2-m3	
Ligand/PDB ID		CC	CC_{peak}	CC	CC_{peak}	CC	CC_{peak}
5a	7UF9	0.6992	0.6672	0.9230	0.8715	0.6336	0.5914
5b	7UFA	0.4962	0.4570	0.8256	0.7698	0.4460	0.4101
8a	7UFB	0.7025	0.7522	0.9062	0.8803	0.7123	0.7491
8b	7UFC	0.5861	0.5493	0.8972	0.8544	0.5939	0.5425
8c	7UFD	0.6629	0.6702	0.9049	0.8783	0.5949	0.6046
10a	7UFE	0.5353	0.5100	0.9200	0.8496	0.5071	0.4826
10b	7UFF	0.5257	0.5584	0.8700	0.8496	0.4717	0.4749

Correlation coefficients for polder maps were calculated with PHENIX.

For all ligands, pairwise comparisons of correlation coefficients indicate that m3 correlates best with m1. This means that the polder maps m3 (displayed in Figures 3, 5 and 7 of the main text) show the omitted ligand atoms.*

*Liebschner D., Afonine P.V.; Moriarty N.W.; Poon B.K.; Sobolev O.V.; Terwilliger T.C.; Adams P.D. Polder maps: improving OMIT maps by excluding bulk solvent. *Struct. Biol.* **2016**, D73, 148-157.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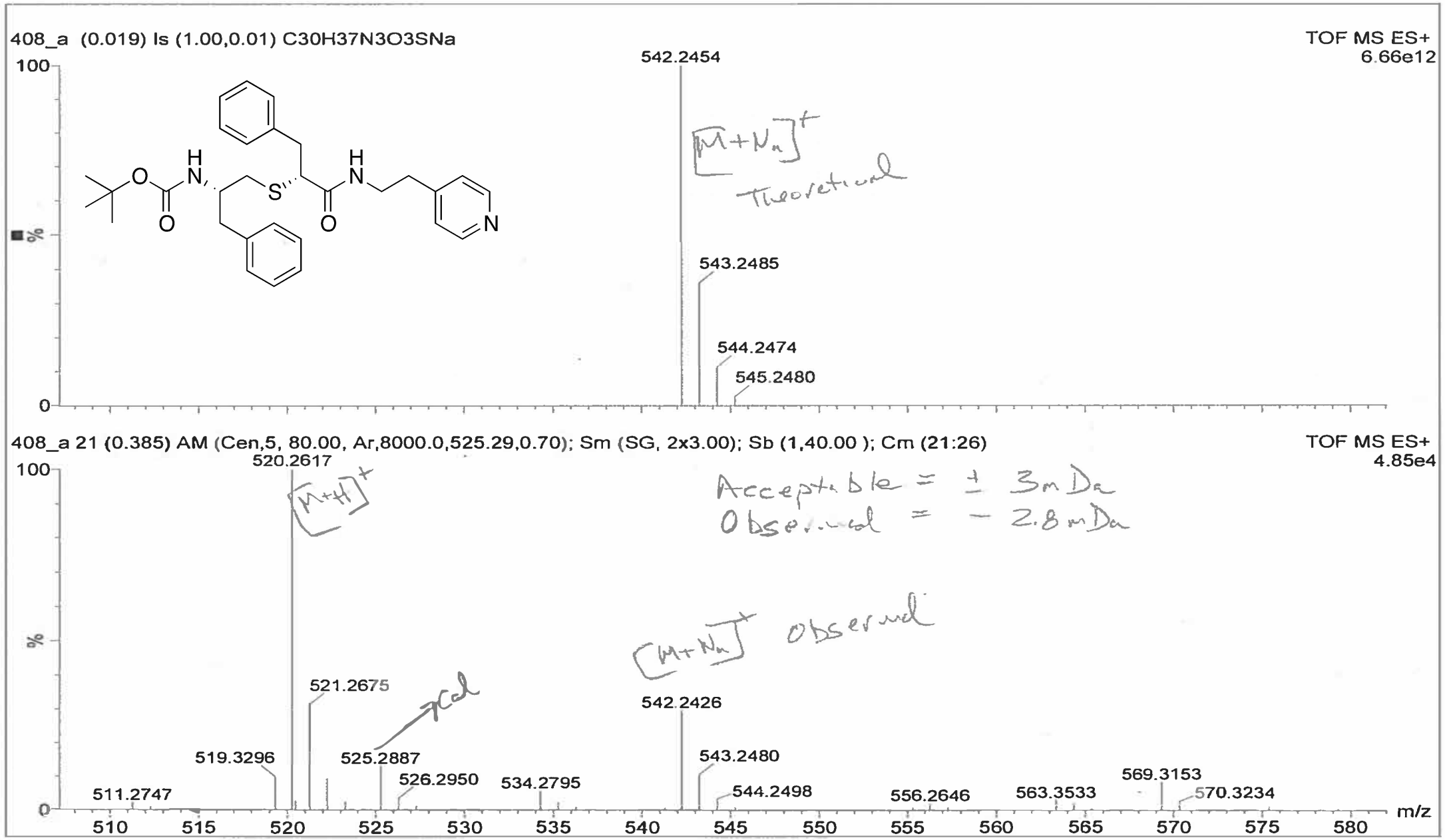
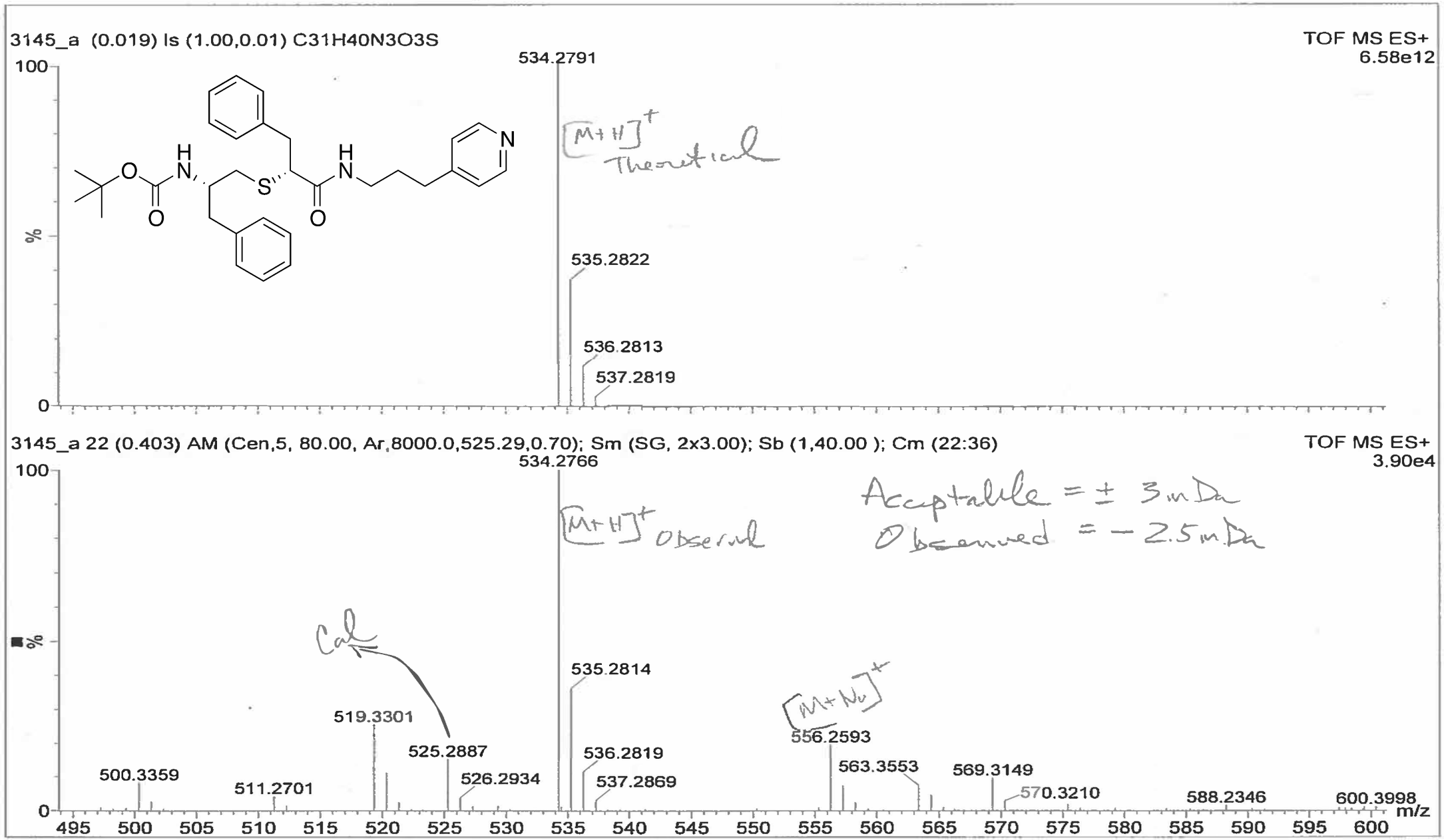
TOF MS ES+
6.62e12
549.2535
550.2566
551.2556
552.2563
(M+H)+
3124_a 23 (0.422) AM (Cen,5, 80.00, Ar,8000.0,481.26,0.70); Sm (SG, 2x3.00); Sb (1,40.00); Cm (19:33)
TOF MS ES+
5.96e3
Acceptable = ± 3mDa
Observed = − 2,3mDa
571.2349
(M+Na)+
481.2625
cal
(M+H)+
549.2512
572.2378
525.2889
569.3138
550.2578
482.2646
526.2932
573.2338
464.3752
483.2845
551.6346
574.2527
476.3332
500.3228
511.2821
519.3324
533.1450
586.3641
m/z


408_a (0.019) Is (1.00,0.01) C30H37N3O3SNa
TOF MS ES+
6.66e12
100
%
0
542.2454
543.2485
544.2474
545.2480
[M+Na]+
Theoretical
408_a 21 (0.385) AM (Cen,5, 80.00, Ar,8000.0,525.29,0.70); Sm (SG, 2x3.00); Sb (1,40.00); Cm (21:26)
TOF MS ES+
4.85e4
520.2617
[M+H]+
Acceptable = ± 3mDa
Observed = − 2.8 mDa
[M+Na]+ Observed
521.2675
Cal
542.2426
519.3296
525.2887
543.2480
526.2950
534.2795
544.2498
511.2747
556.2646
563.3533
569.3153
570.3234
m/z
510
515
520
525
530
535
540
545
550
555
560
565
570
575
580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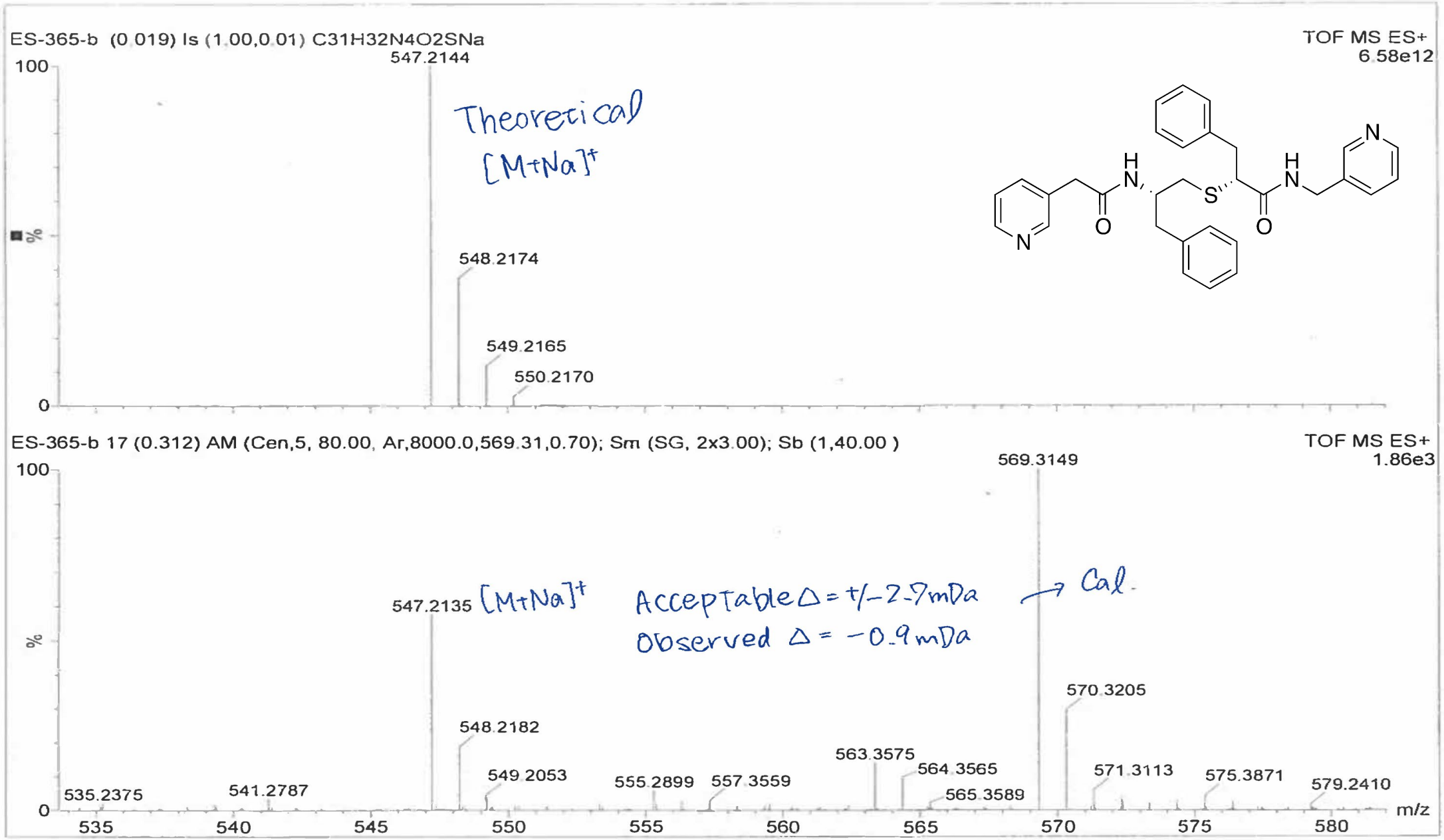
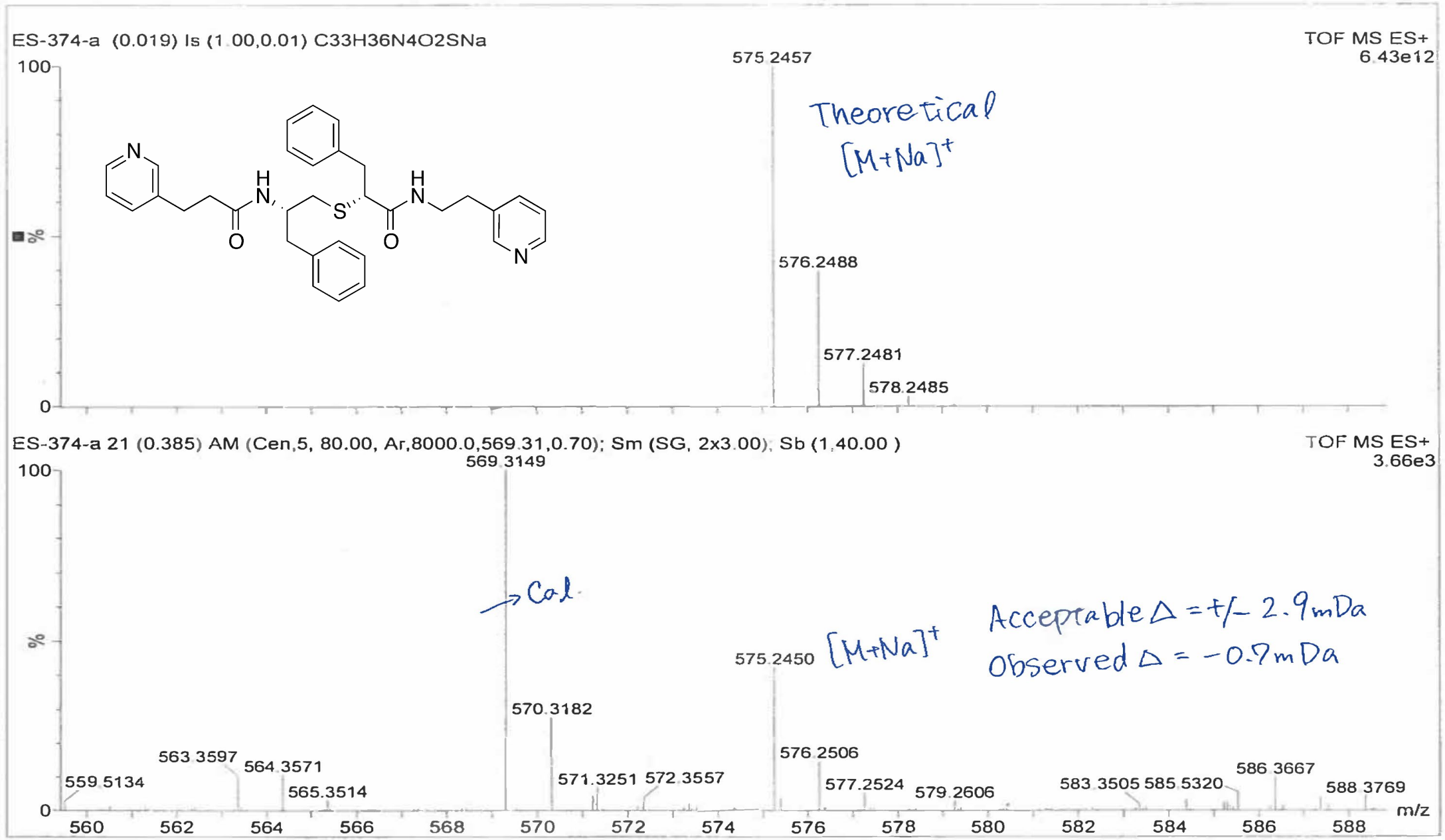
TOF MS ES+
6.58e12
534.2791
[M+H]+ Theoretical
535.2822
536.2813
537.2819
3145_a 22 (0.403) AM (Cen,5, 80.00, Ar,8000.0,525.29,0.70); Sm (SG, 2x3.00); Sb (1,40.00); Cm (22:36)
TOF MS ES+
3.90e4
534.2766
[M+H]+ Observed
Acceptable = ± 3 mDa
Observed = −2.5 mDa
Cal
535.2814
519.3301
525.2887
[M+Na]+
556.2593
536.2819
563.3553
569.3149
500.3359
511.2701
526.2934
537.2869
570.3210
588.2346
600.3998
m/z


ES-365-b (0.019) Is (1.00,0.01) C31H32N4O2SNa
TOF MS ES+
6.58e12
100
%
0
547.2144
548.2174
549.2165
550.2170
Theoretical
[M+Na]+
ES-365-b 17 (0.312) AM (Cen,5, 80.00, Ar,8000.0,569.31,0.70); Sm (SG, 2x3.00); Sb (1,40.00)
TOF MS ES+
1.86e3
569.3149
547.2135 [M+Na]+
Acceptable Δ = +/-2.9mDa
Observed Δ = -0.9mDa
→ Cal.
570.3205
548.2182
563.3575
564.3565
571.3113
575.3871
579.2410
549.2053
555.2899
557.3559
565.3589
535.2375
541.2787
m/z
535
540
545
550
555
560
565
570
575
580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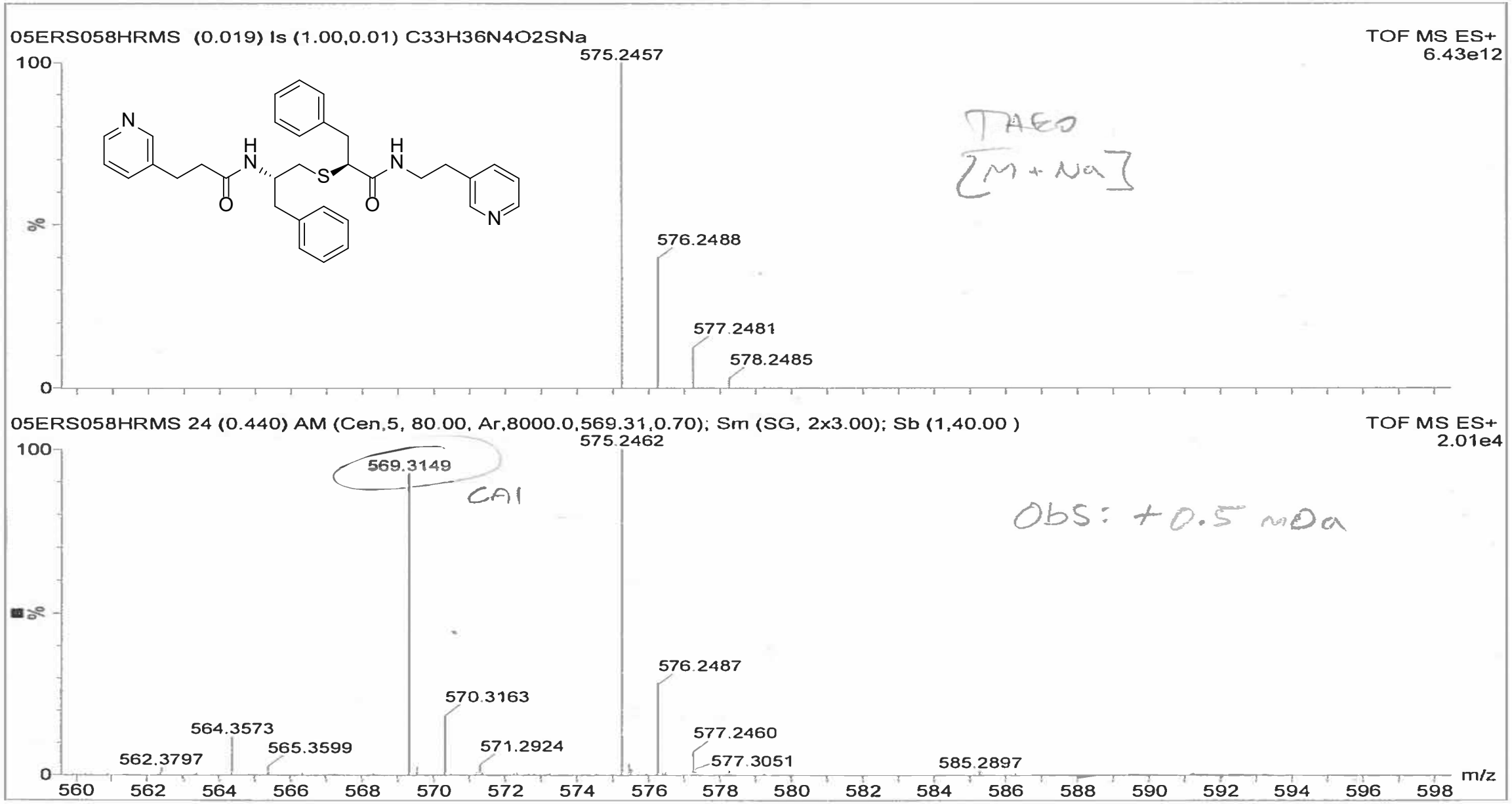
6.43e12
100
%
0
575.2457
576.2488
577.2481
578.2485
Theoretical
[M+Na]+
ES-374-a 21 (0.385) AM (Cen,5, 80.00, Ar,8000.0,569.31,0.70); Sm (SG, 2x3.00); Sb (1.40.00)
TOF MS ES+
3.66e3
569.3149
Cal.
575.2450 [M+Na]+
Acceptable Δ = +/- 2.9mDa
Observed Δ = -0.7mDa
570.3182
563.3597
564.3571
559.5134
565.3514
571.3251
572.3557
576.2506
577.2524
579.2606
583.3505
585.5320
586.3667
588.3769
m/z
560
562
564
566
568
570
572
574
576
578
580
582
584
586
588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TOF MS ES+
6.43e12
100
%
0
575.2457
576.2488
577.2481
578.2485
TAED
[M + Na]
05ERS058HRMS 24 (0.440) AM (Cen,5, 80.00, Ar,8000.0,569.31,0.70); Sm (SG, 2x3.00); Sb (1,40.00)
TOF MS ES+
2.01e4
575.2462
569.3149
CAI
Obs: +0.5 mDa
576.2487
570.3163
564.3573
565.3599
562.3797
571.2924
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577.3051
585.2897
m/z
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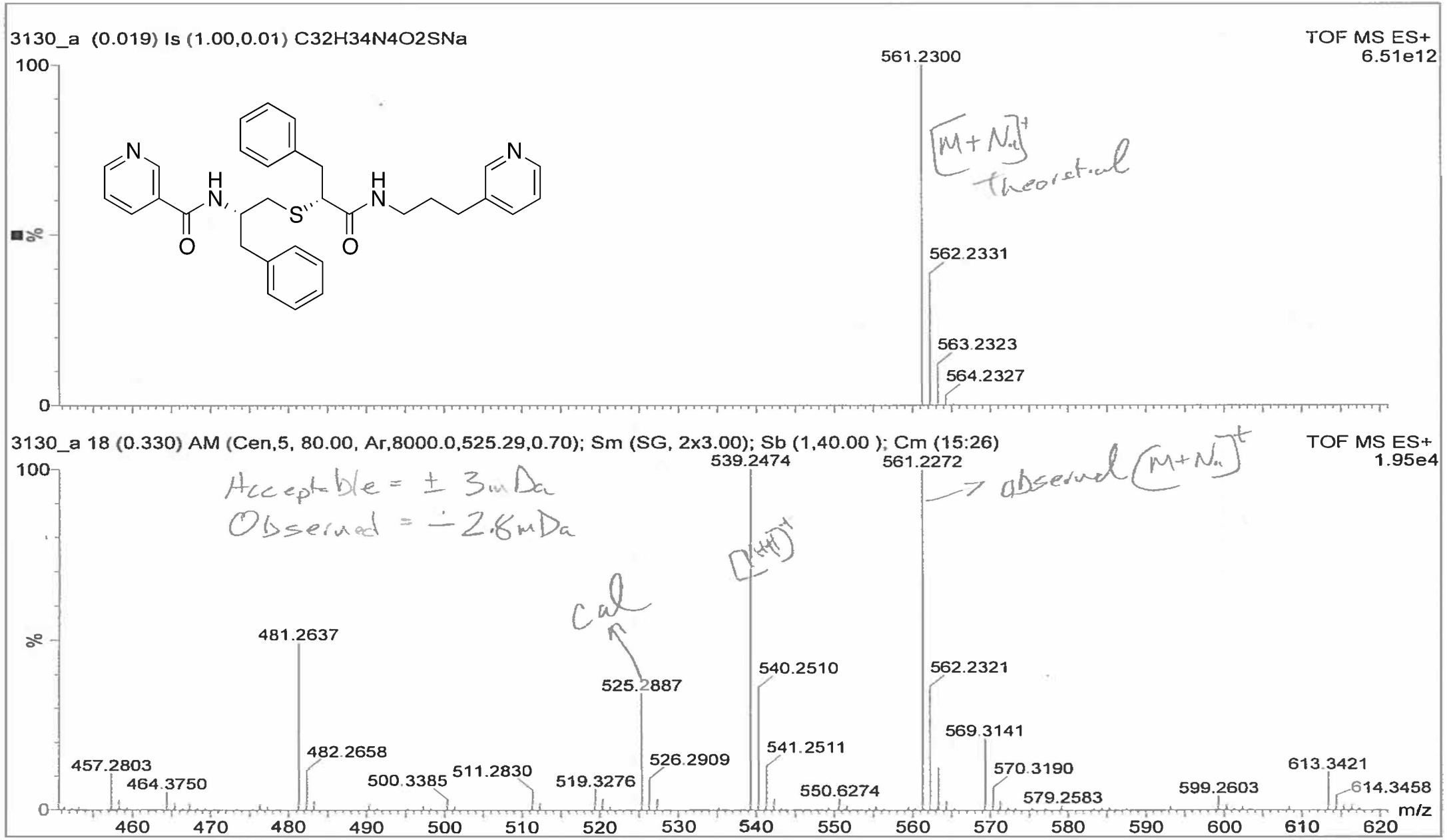
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6.51e12
100
%
0
561.2300
562.2331
563.2323
564.2327
[M+Na]+
Theoretical
3130_a 18 (0.330) AM (Cen,5, 80.00, Ar,8000.0,525.29,0.70); Sm (SG, 2x3.00); Sb (1,40.00); Cm (15:26)
TOF MS ES+
1.95e4
Acceptable = ± 3mDa
Observed = -2.6mDa
539.2474
561.2272
observed [M+Na]+
[M+H]+
cal
481.2637
525.2887
540.2510
562.2321
569.3141
457.2803
464.3750
482.2658
500.3385
511.2830
519.3276
526.2909
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579.2583
599.2603
613.3421
614.3458
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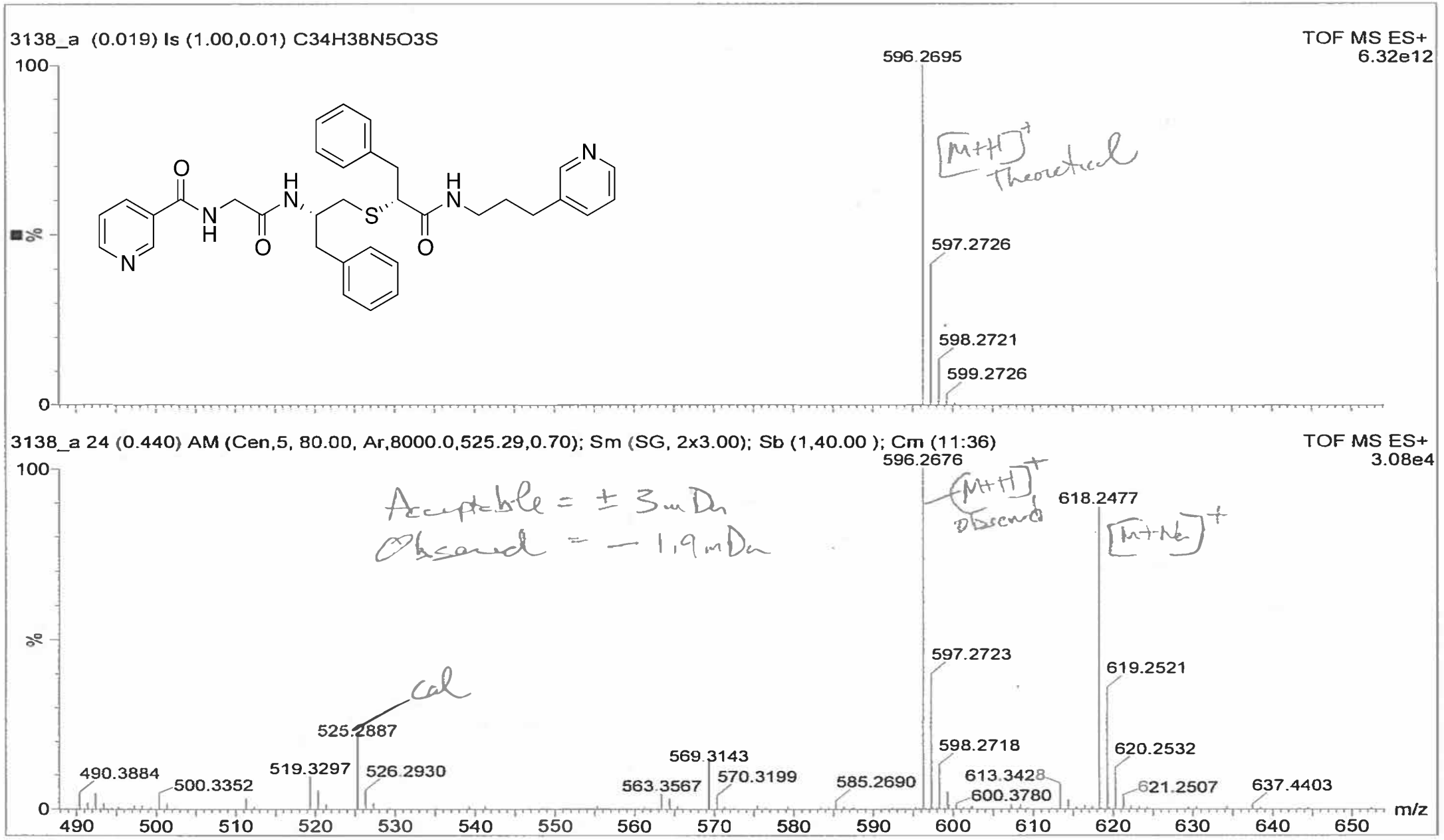
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TOF MS ES+
6.32e12
596.2695
597.2726
598.2721
599.2726
[M+H]+ Theoretical
3138_a 24 (0.440) AM (Cen,5, 80.00, Ar,8000.0,525.29,0.70); Sm (SG, 2x3.00); Sb (1,40.00); Cm (11:36)
TOF MS ES+
3.08e4
596.2676
618.2477
[M+H]+ Observed
[M+Na]+
Acceptable = ± 5mDa
Observed = −1.9mDa
597.2723
619.2521
598.2718
620.2532
613.3428
600.3780
621.2507
637.4403
525.2887
cal
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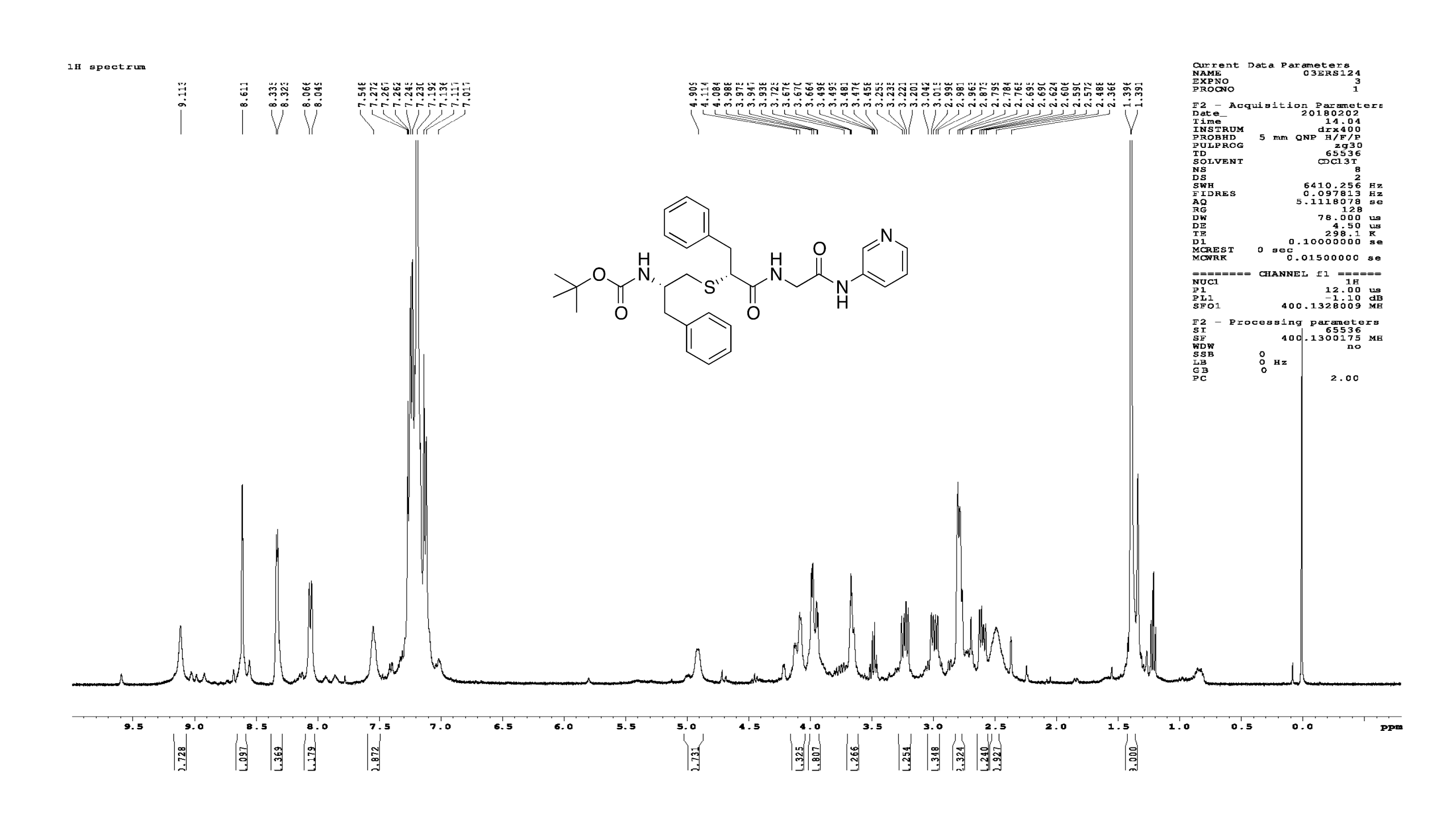
585.2690
%
m/z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TD 65536
SOLVENT CDCl3T
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DS 2
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FIDRES 0.097813 Hz
AQ 5.1118078 se
RG 128
DW 78.000 us
DE 4.50 us
TE 298.1 K
D1 0.10000000 se
MCREST 0 sec
MCWRK 0.01500000 se
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NUC1 1H
P1 12.00 us
PL1 -1.10 dB
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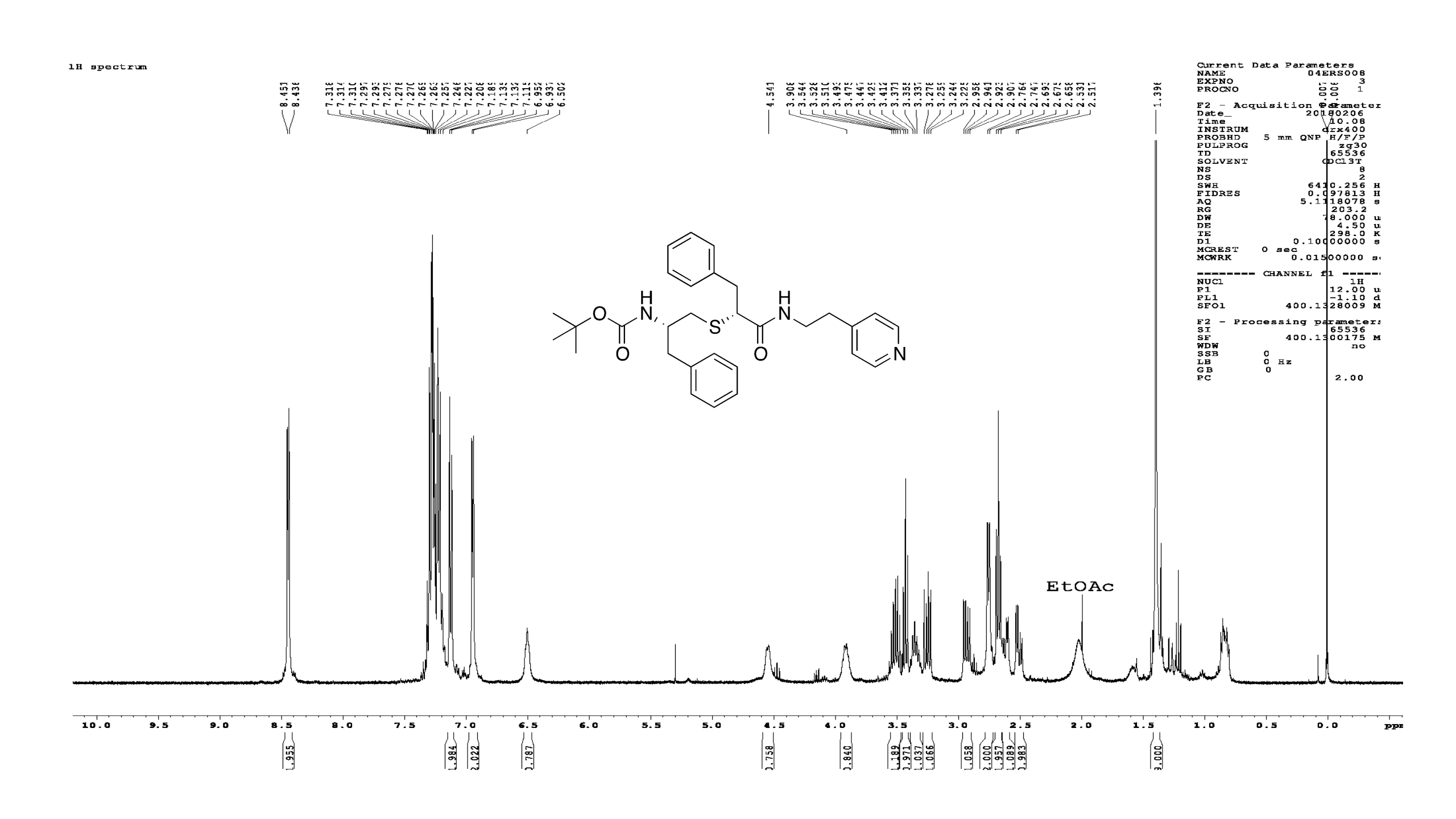
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DW 78.000
DE 4.50
TE 298.0 K
D1 0.10000000 s
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P1 12.00
PL1 -1.10 d
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SF 400.1300175 M
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SSB 0
LB 0 Hz
GB 0
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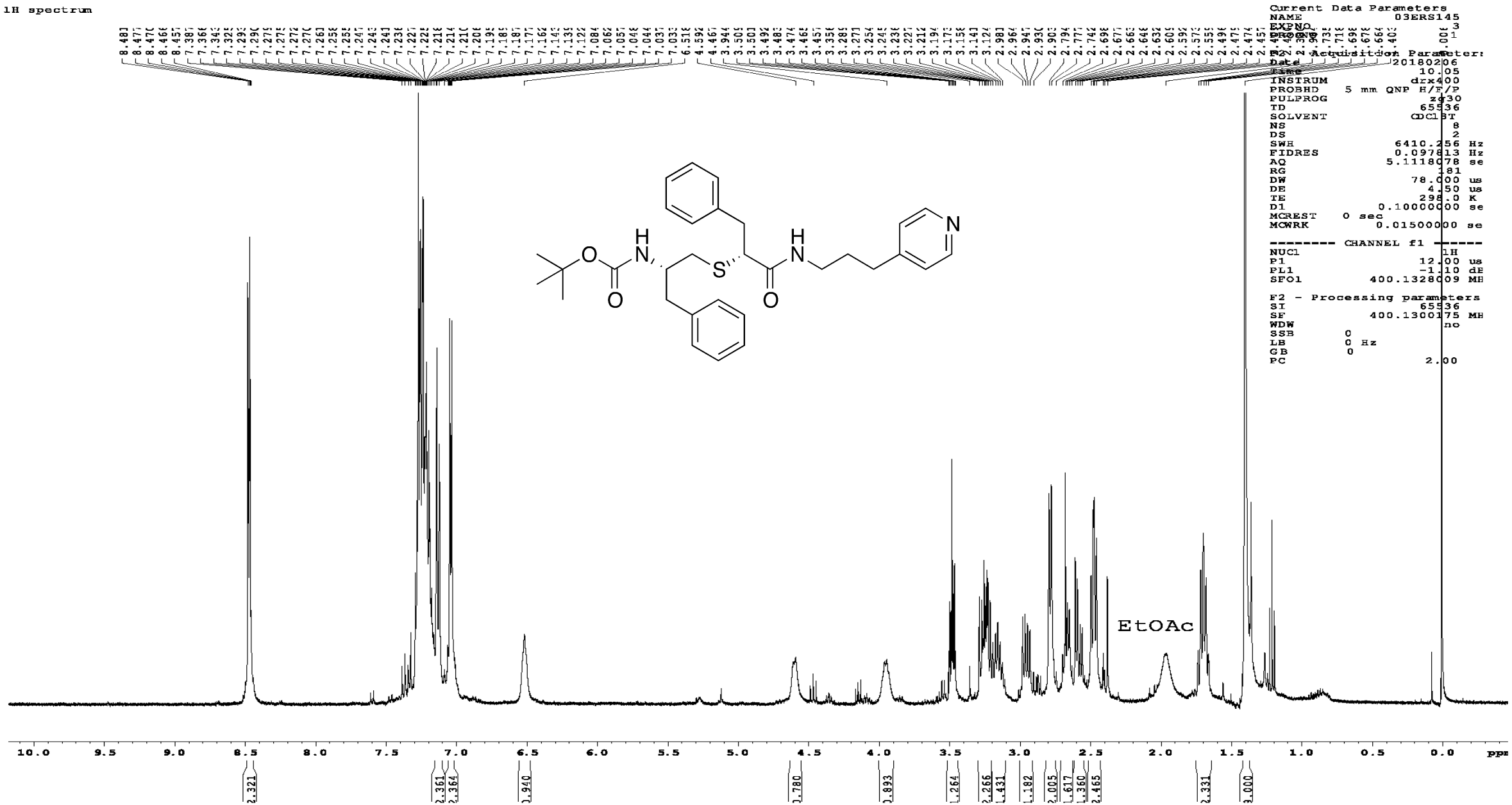
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm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TD 65536
SOLVENT CDCl3T
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DS 2
SWH 6410.256 Hz
FIDRES 0.097813 Hz
AQ 5.1118078 se
RG 181
DW 78.000 us
DE 4.50 us
TE 298.0 K
D1 0.10000000 se
MCREST 0 sec
MCWRK 0.01500000 se
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P1 12.00 us
PL1 -1.10 dB
SFO1 400.1328009 MH
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SSB 0
LB 0 Hz
GB 0
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EtOAc
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 0.0 ppm
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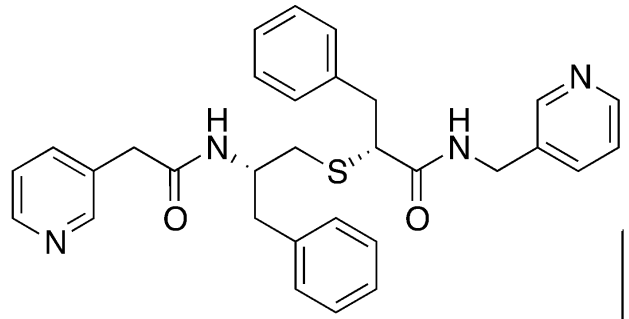
1H spectrum

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PROCNO                       1

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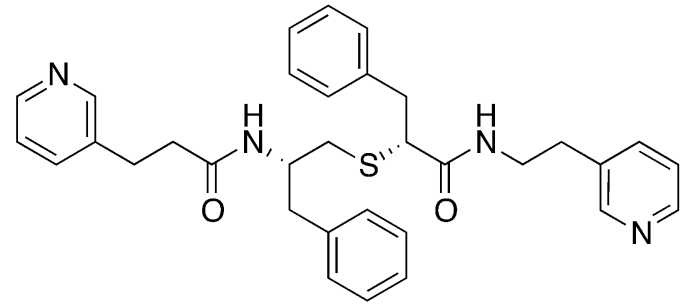
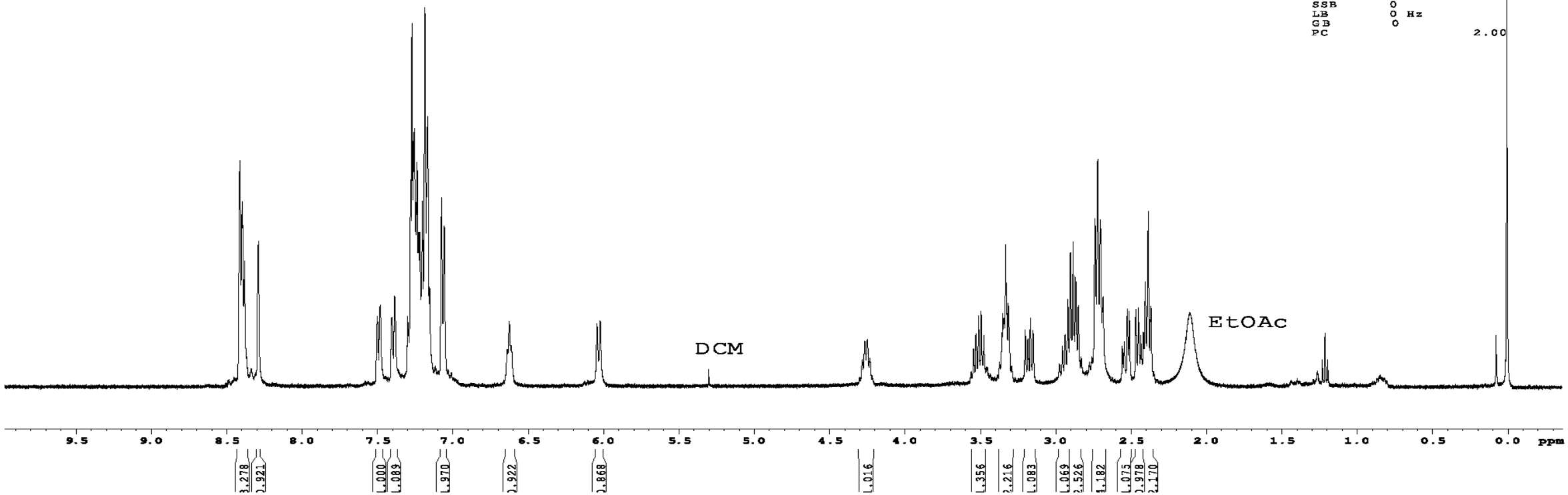
PULPROG                 zg30
TD                     65536
SOLVENT                CDCl3T
NS                           8
DS                           2
SWH                 6410.256 Hz
FIDRES              0.097813 Hz
AQ                  5.1118078 sec
RG                      228.1
DW                     78.000 usec
DE                      4.50 usec
TE                     298.1 K
D1                0.10000000 sec
MCREST    0 sec
MCWRK             0.01500000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                      12.00 usec
PL1                     -1.10 dB
SFO1           400.1328009 MHz

F2 - Processing parameters
SI                         65536
SF             400.1300175 MHz
WDW                           no
SSB                0
LB                 0 Hz
GB                 0
PC                          2.00
```

1H spectrum



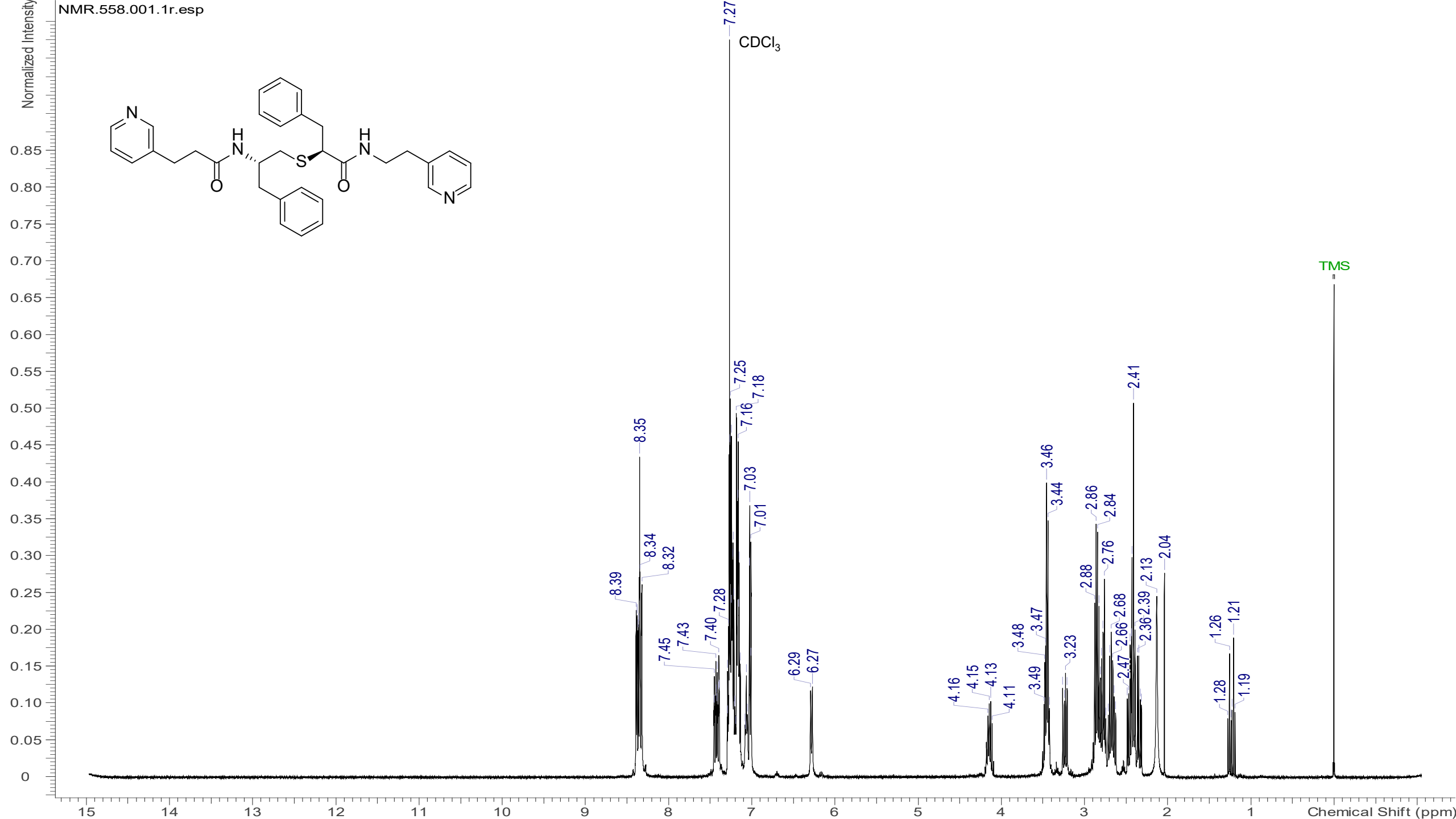


Current Data Parameters
NAME 03ERS074
EXPNO 3
PROCNO 1

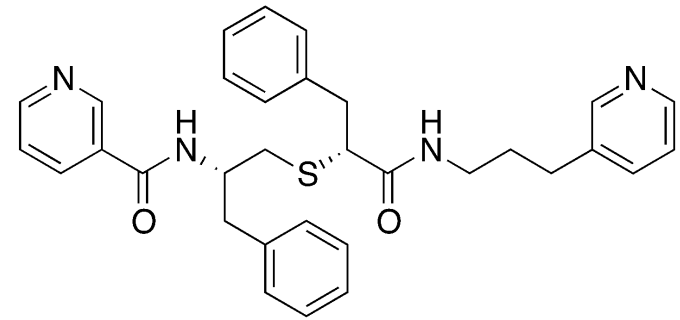
F2 - Acquisition Parameters
Date_ 20180130
Time 12.44
INSTRUM drx400
PROBHD 5 mm QNP H/F/P
PULPROG zg30
TD 65536
SOLVENT CDCl3T
NS 8
DS 2
SWH 6410.256 Hz
FIDRES 0.097813 Hz
AQ 5.1118078 sec
RG 287.4
DW 78.000 use
DE 4.50 use
TE 298.1 K
D1 0.10000000 sec
MCREST 0 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 12.00 use
PL1 -1.10 dB
SFO1 400.1328009 MHz

F2 - Processing parameters
SI 65536
SF 400.1300175 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 2.00


NMR.558.001.1r.esp
Normalized Intensity
CDCl3
TMS
Chemical Shift (ppm)
8.39
8.35
8.34
8.32
7.45
7.43
7.40
7.28
7.27
7.25
7.18
7.16
7.03
7.01
6.29
6.27
4.16
4.15
4.13
4.11
3.48
3.47
3.49
3.46
3.44
3.23
2.88
2.86
2.84
2.76
2.68
2.66
2.47
2.41
2.39
2.36
2.13
2.04
1.28
1.26
1.21
1.19

1H spectrum



Current Data Parameters

NAME	03ERS130
EXPNO	3
PROCNO	1

F2 - Acquisition Parameters

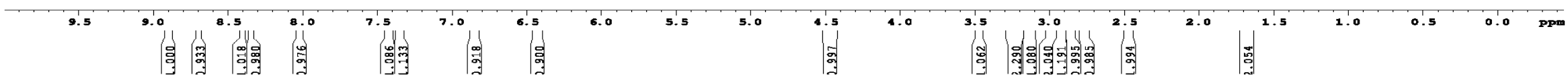
Date	20180202
Time	14.08
INSTRUM	drx400
PROBHD	5 mm QNP H/F/P
PULPROG	zg30
TD	65536
SOLVENT	CDCl3T
NS	8
DS	2
SWH	6410.256 Hz
FIDRES	0.097813 Hz
AQ	5.1118078 sec
RG	228.1
DW	78.000 use
DE	4.50 use
TE	298.0 K
D1	0.10000000 sec
MCREST	0 sec
MCWRR	0.01500000 sec

======== CHANNEL f1 ========

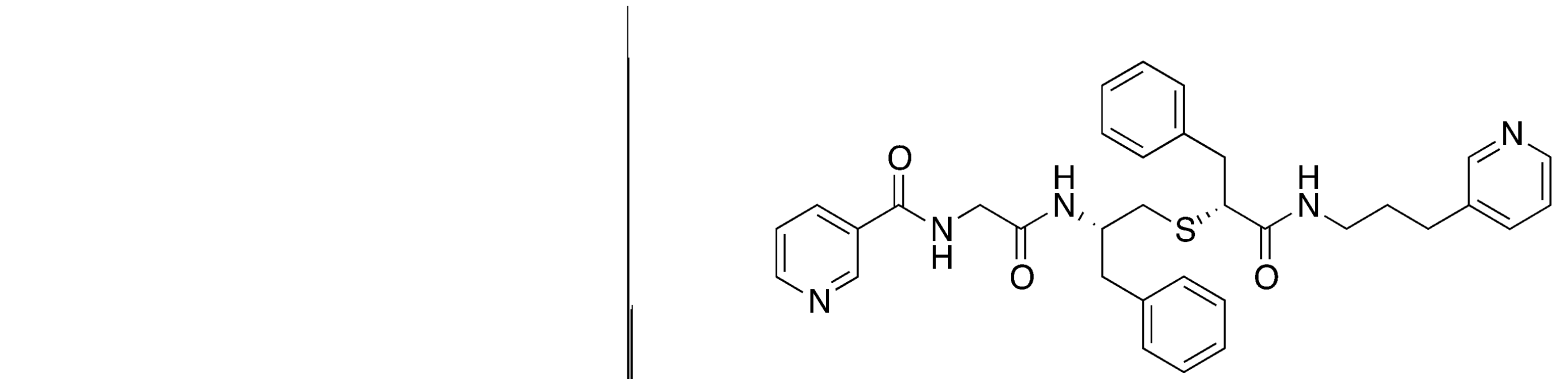
NUC1	1H
P1	12.00 use
PL1	-1.10 dB
SFO1	400.1328009 MHz

F2 - Processing parameters

SI	65536
SF	400.1300175 MHz
WDW	no
SSB	0
LB	0 Hz
GB	0
PC	2.00



1H spectrum



Current Data Parameters
NAME 03ERS138
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
Date 20180205
Time 11.49
INSTRUM drx400
PROBHD 5 mm QNP H/F/P
PULPROG zg30
TD 65536
SOLVENT CDCl3T
NS 8
DS 2
SWH 6410.256 Hz
FIDRES 0.097813 Hz
AQ 5.1118078 se
RG 143.7
DW 78.000 us
DE 4.50 us
TE 297.9 K
D1 0.10000000 se
MCREST 0 sec
MCWRK 0.01500000 se

======== CHANNEL f1 ======
NUC1 1H
P1 12.00 us
PL1 -1.10 dB
SFO1 400.1328009 MH

F2 - Processing parameters
SI 65536
SF 400.1300175 MH
WDW no
SSB 0
LB 0 Hz
GB 0
PC 2.00