Return of the lysergamides. Part III: Analytical characterization of *N*^{6}-ethyl-6-norlysergic acid diethylamide (ETH-LAD) and 1-propionyl ETH-LAD (1P-ETH-LAD)

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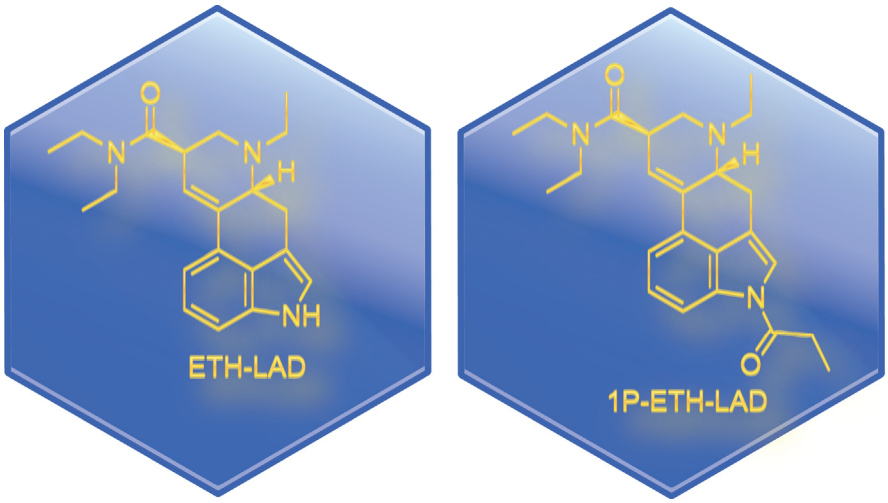

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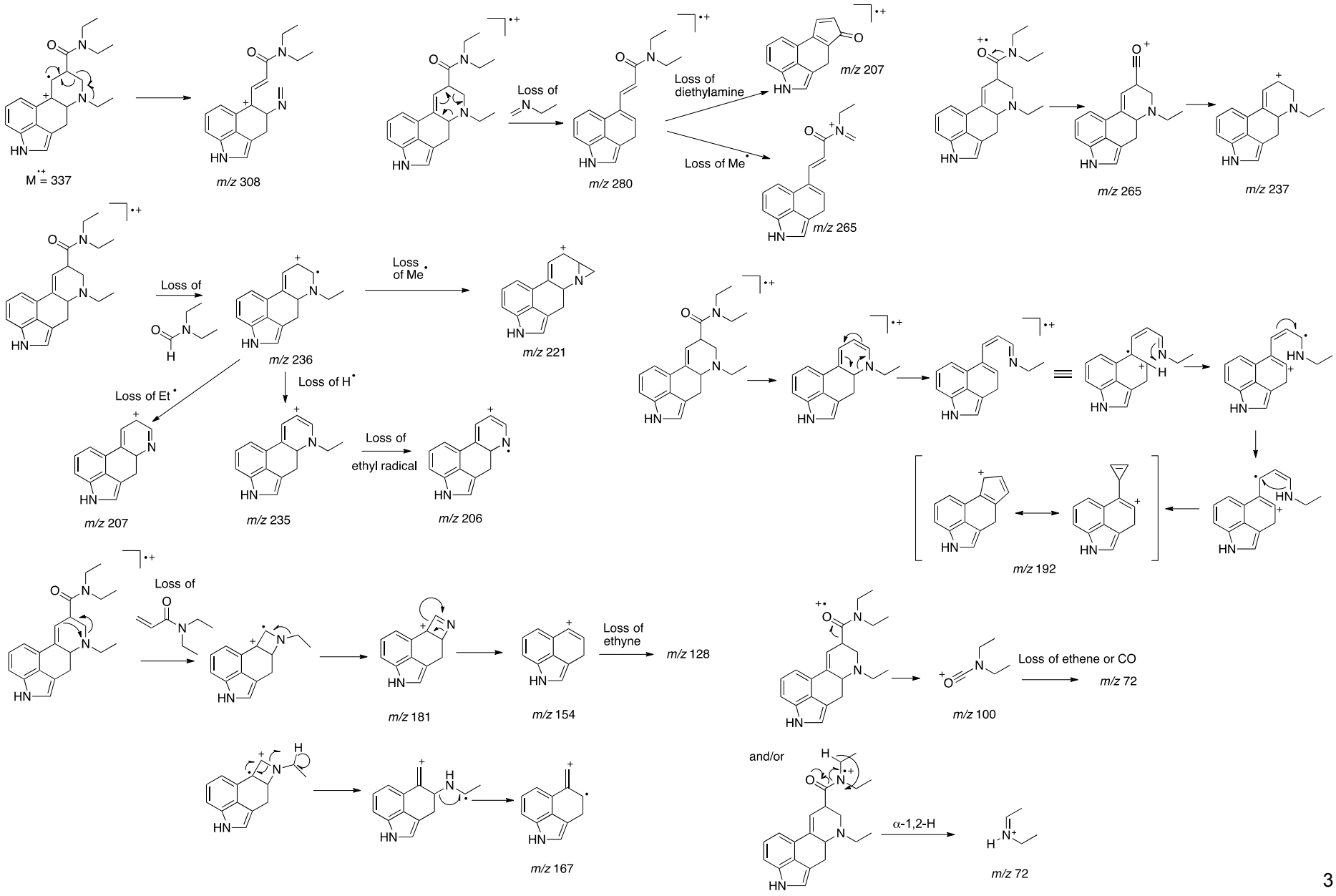
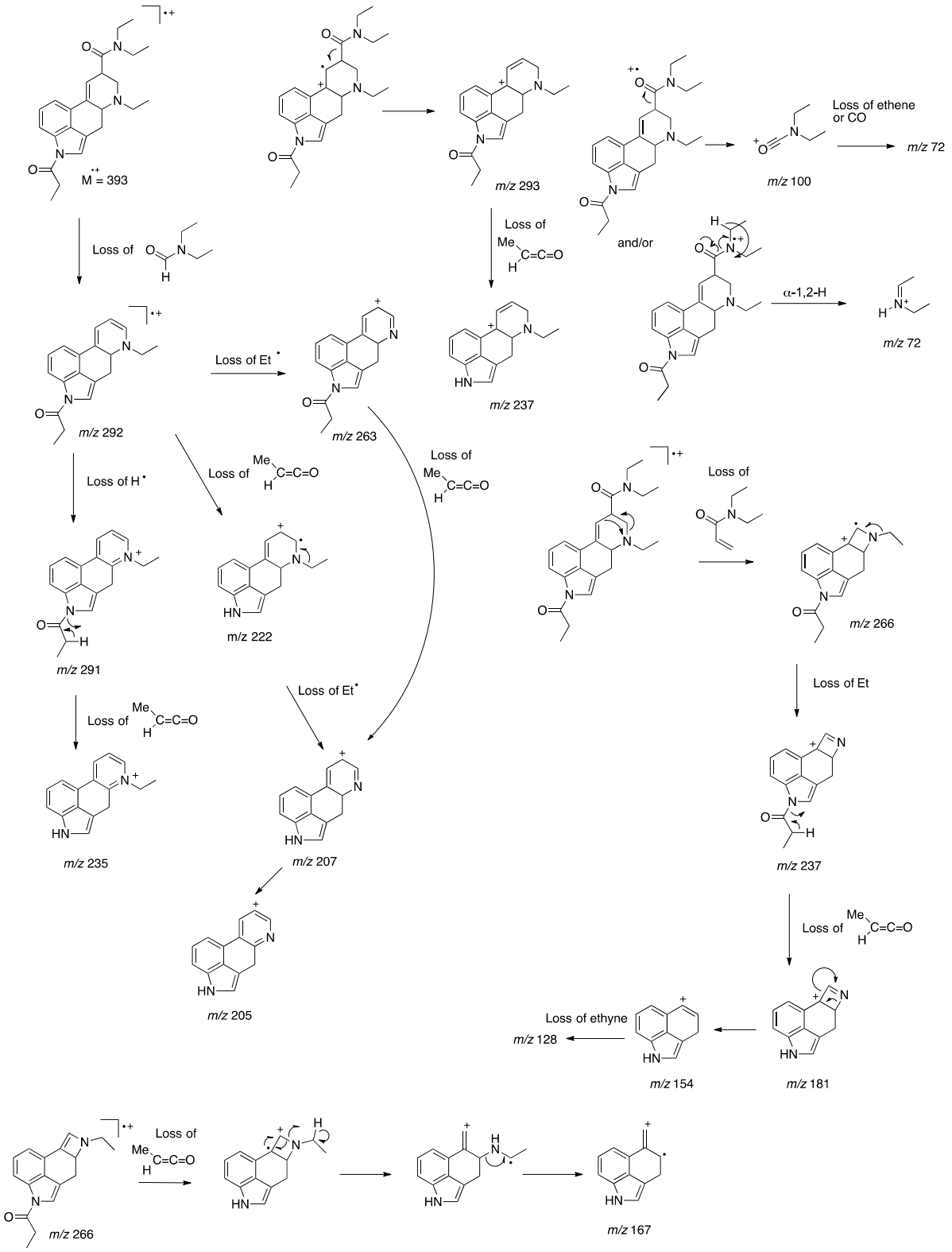
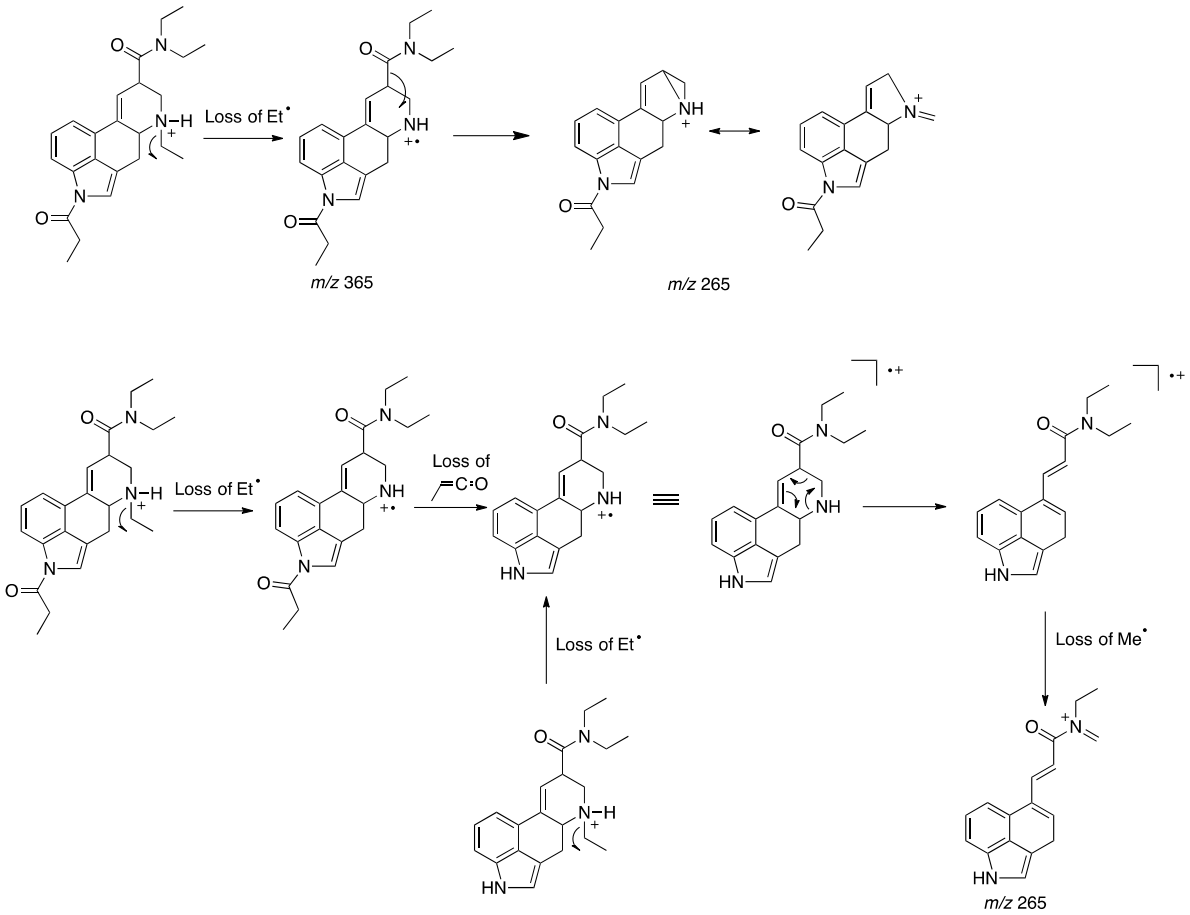
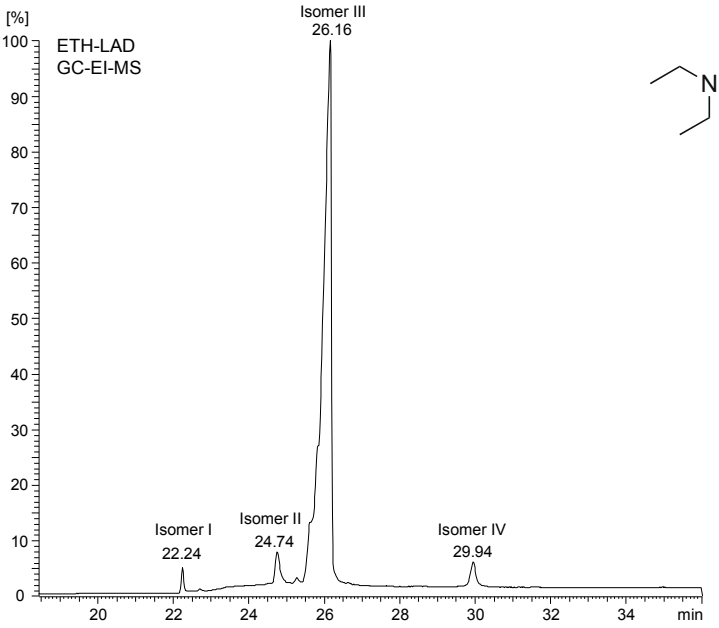
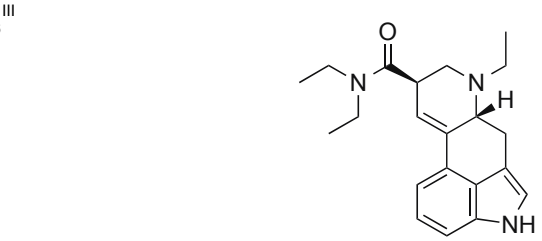
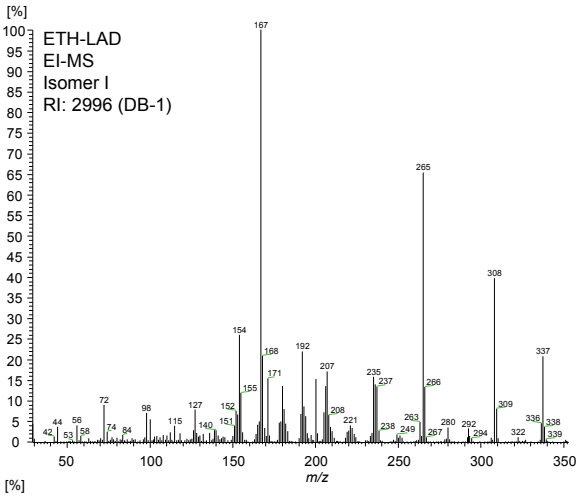

M•+ = 337
m/z 308
Loss of
Loss of diethylamine
m/z 207
Loss of Me•
m/z 280
m/z 265
m/z 265
m/z 237
Loss of
Loss of Me•
m/z 236
m/z 221
Loss of Et•
Loss of H•
m/z 207
m/z 235
Loss of
ethyl radical
m/z 206
m/z 192
Loss of
m/z 181
m/z 154
Loss of
ethyne
m/z 128
Loss of ethene or CO
m/z 72
m/z 100
m/z 167
and/or
α-1,2-H
m/z 72

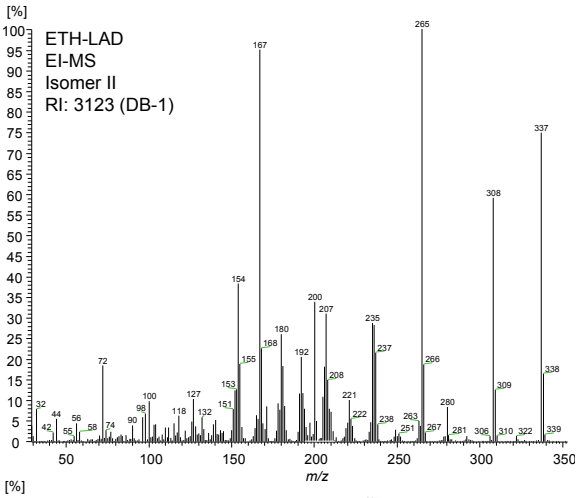

M = 393
m/z 293
Loss of ethene or CO
m/z 72
m/z 100
Loss of
and/or
α-1,2-H
m/z 72
m/z 292
Loss of Et
m/z 263
m/z 237
Loss of H
Loss of
Loss of
m/z 291
m/z 222
Loss of
m/z 266
Loss of Et
Loss of
m/z 235
Loss of Et
m/z 207
m/z 237
Loss of
m/z 205
Loss of ethyne
m/z 128
m/z 154
m/z 181
Loss of
m/z 266
m/z 167

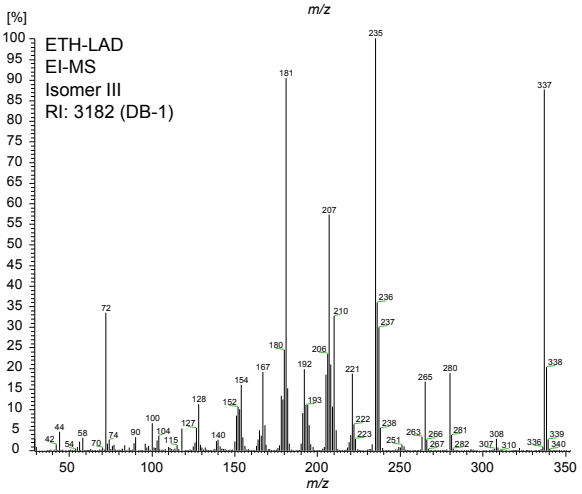

Loss of Et•
m/z 365
m/z 265
Loss of Et•
Loss of
C=C=O
Loss of Et•
Loss of Me•
m/z 265
Loss of
Me
C=C=O
H
m/z 192

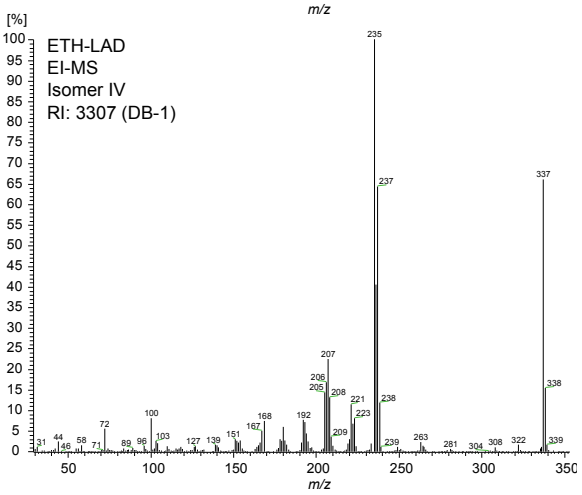

[%]
ETH-LAD
GC-EI-MS
Isomer III
26.16
Isomer I
22.24
Isomer II
24.74
Isomer IV
29.94
min

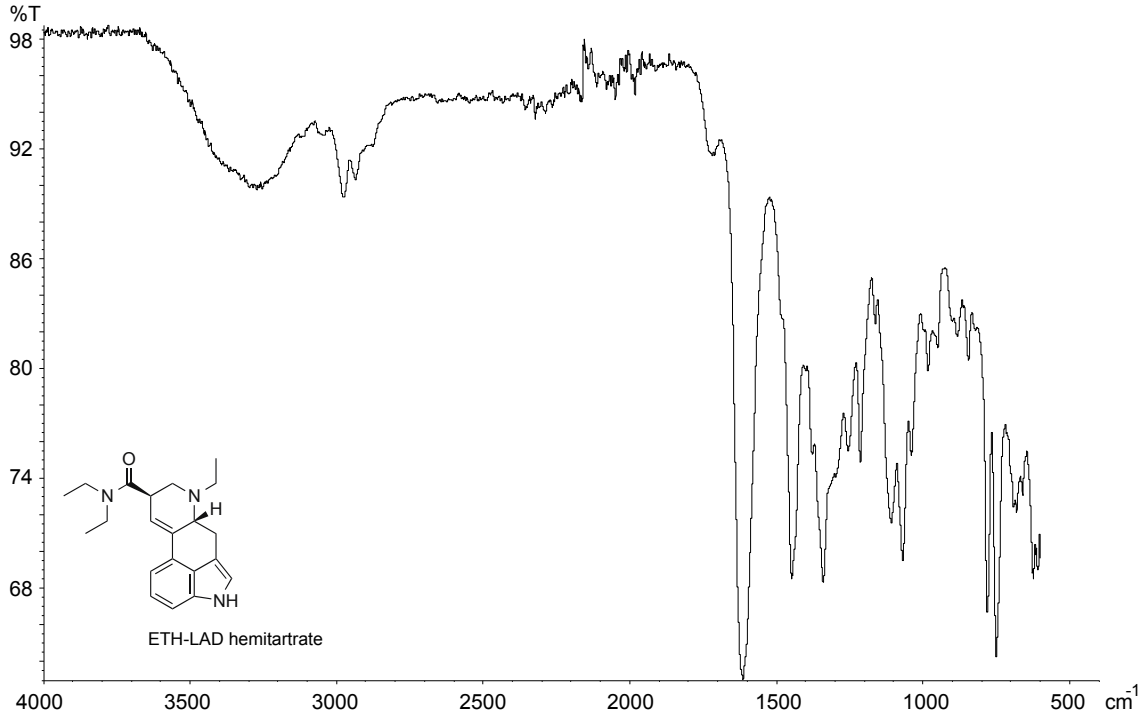

O
N
N
H
NH

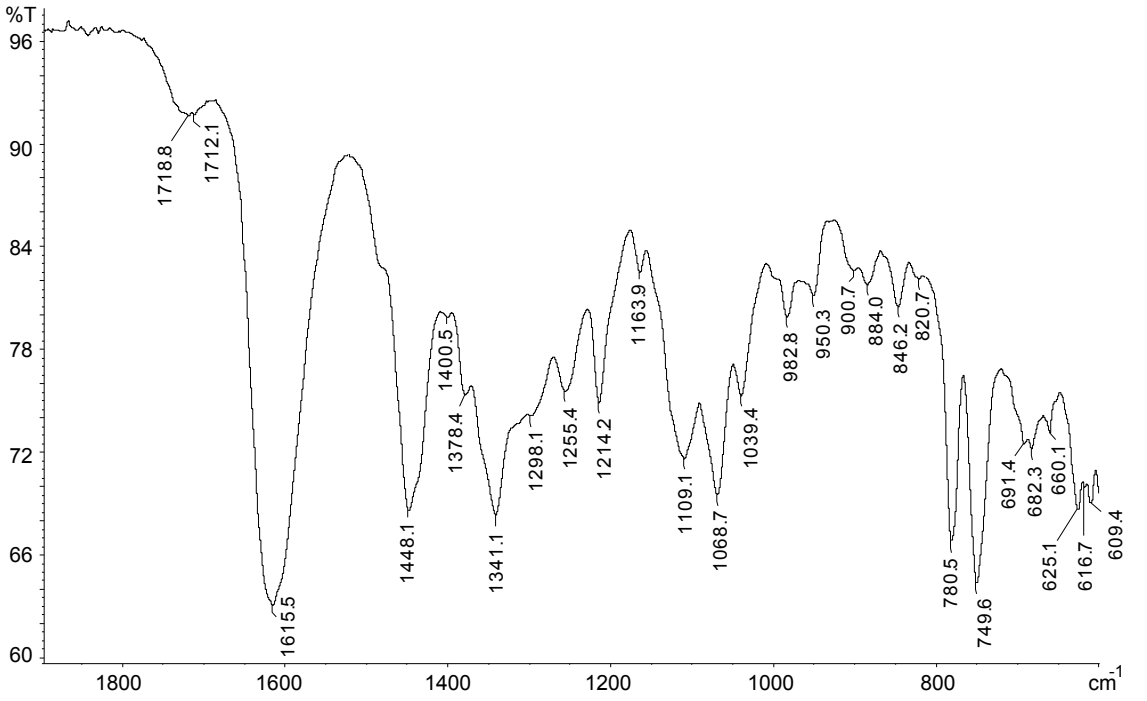

[%]
ETH-LAD
EI-MS
Isomer I
RI: 2996 (DB-1)
m/z

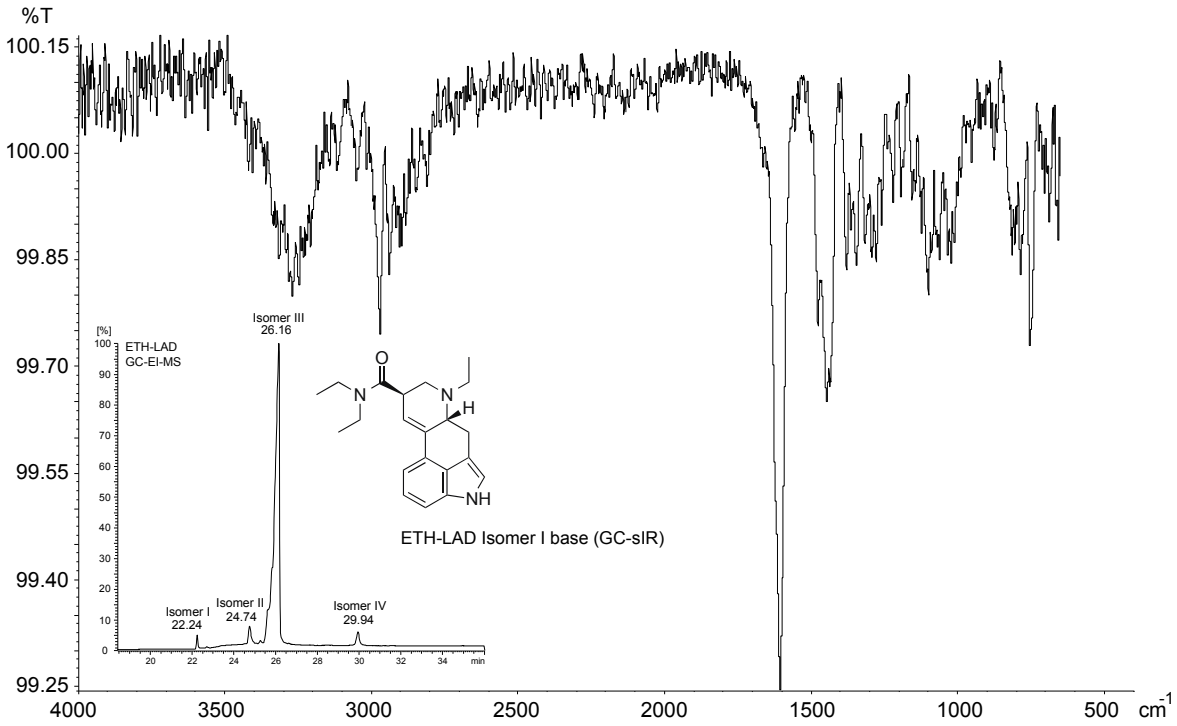

[%]
ETH-LAD
EI-MS
Isomer II
RI: 3123 (DB-1)
m/z

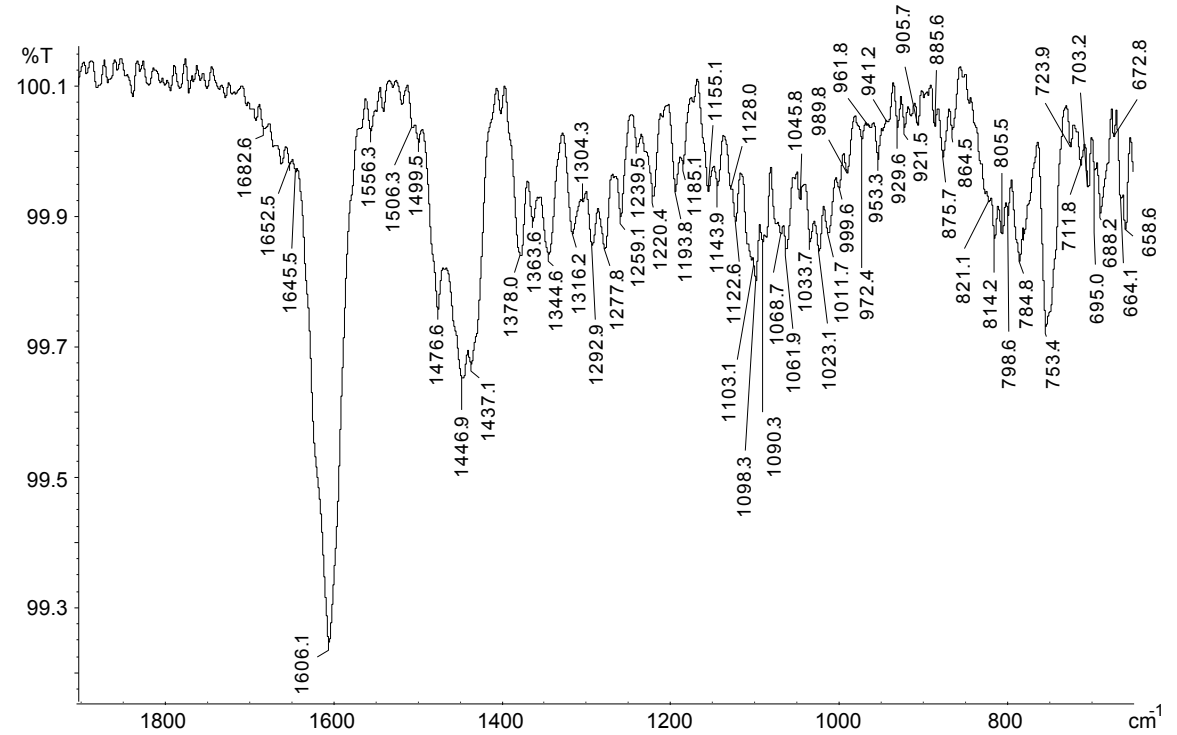

[%]
ETH-LAD
EI-MS
Isomer III
RI: 3182 (DB-1)
m/z

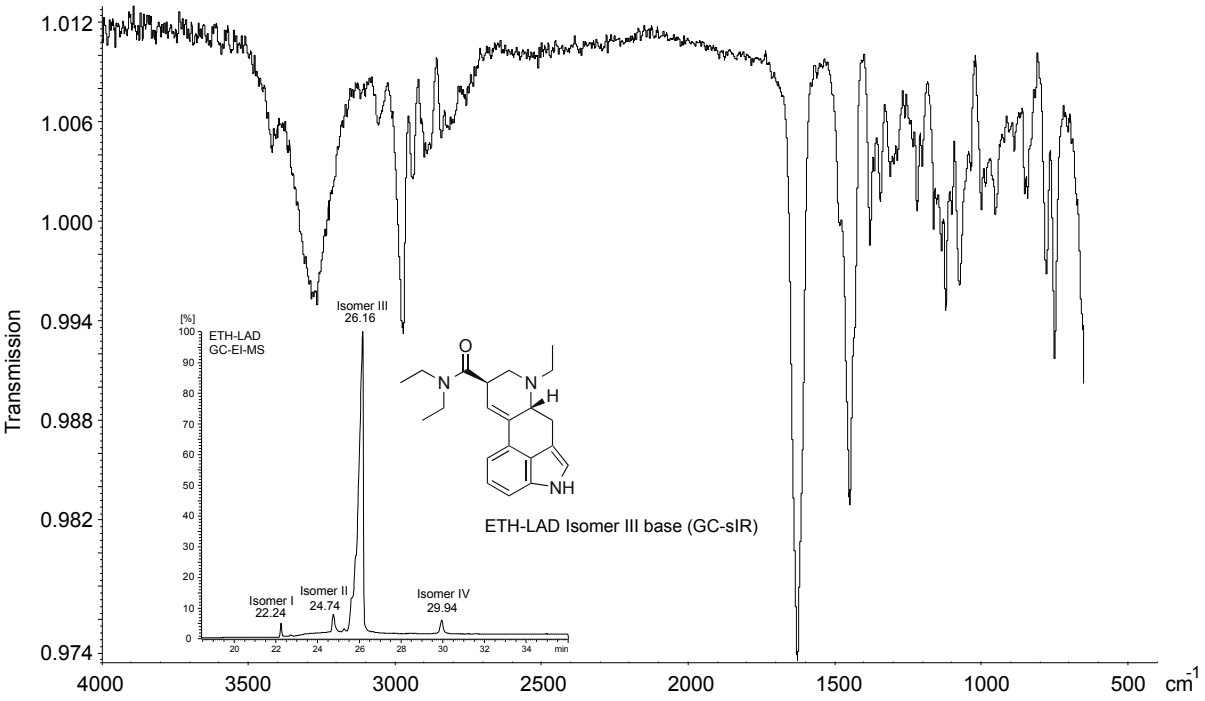

[%]
ETH-LAD
EI-MS
Isomer IV
RI: 3307 (DB-1)
m/z

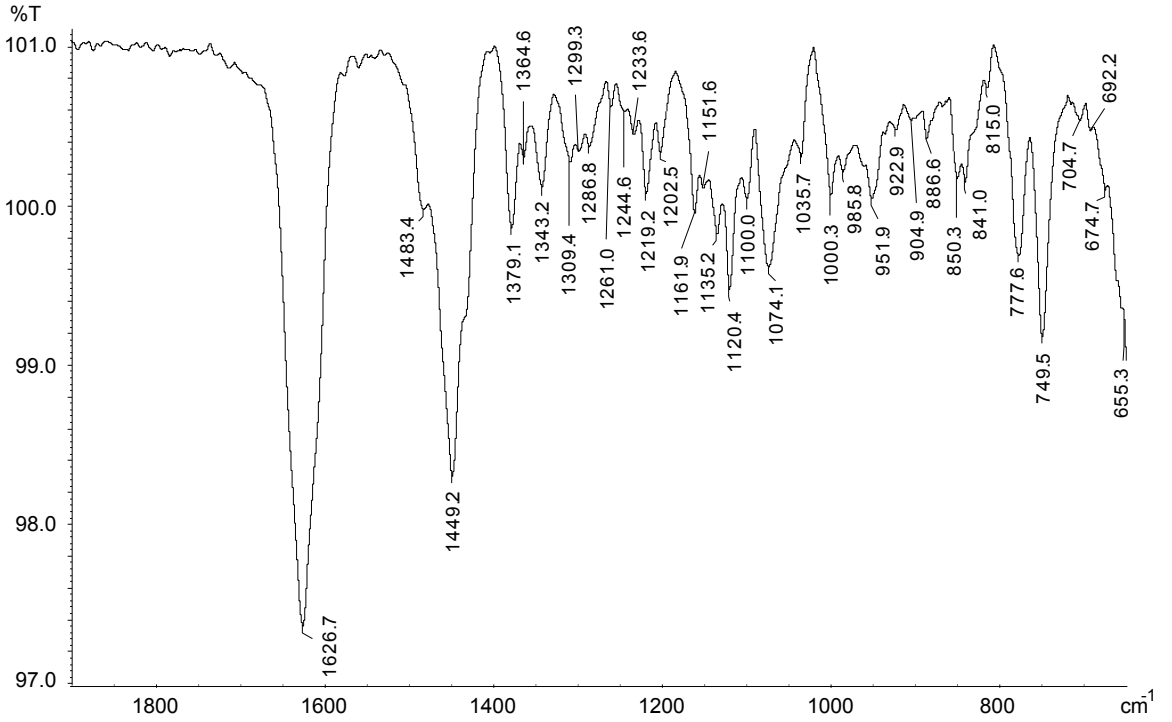

%T
98
92
86
80
74
68
4000
3500
3000
2500
2000
1500
1000
500
cm-1
O
N
N
H
NH
ETH-LAD hemitartrate

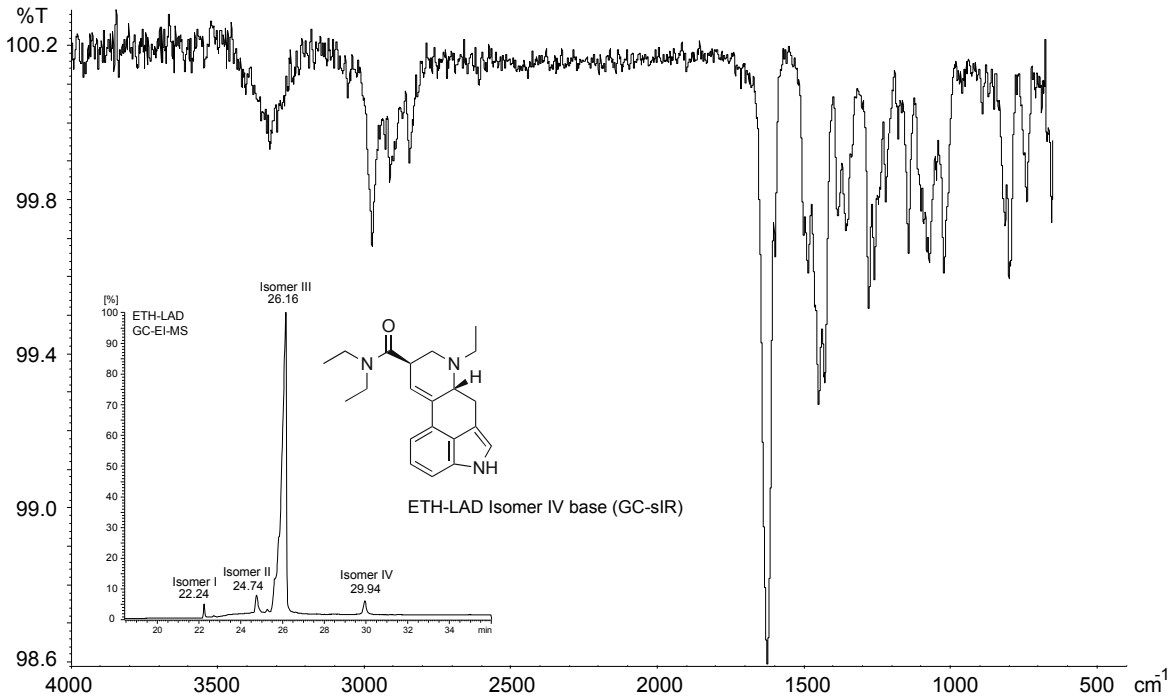

%T
96
90
84
78
72
66
60
1800
1600
1400
1200
1000
800
cm-1
1718.8
1712.1
1615.5
1448.1
1400.5
1378.4
1341.1
1298.1
1255.4
1214.2
1163.9
1109.1
1068.7
1039.4
982.8
950.3
900.7
884.0
846.2
820.7
780.5
749.6
691.4
682.3
660.1
625.1
616.7
609.4

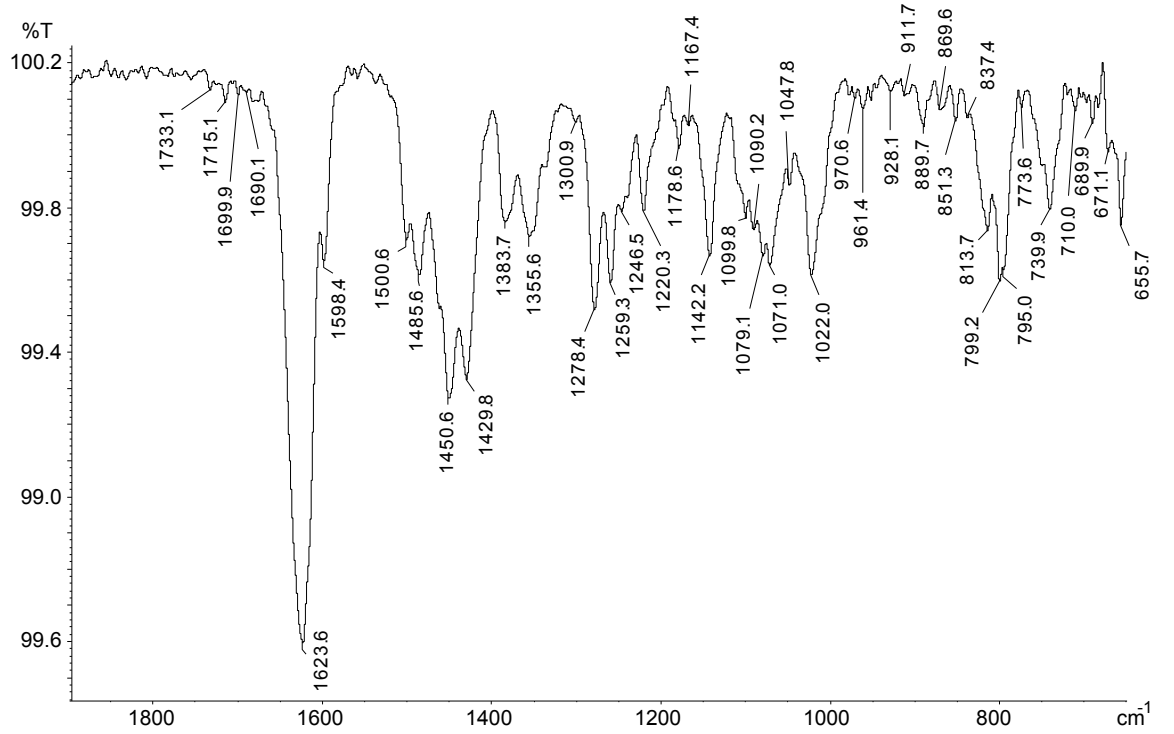

%T
100.15
100.00
99.85
99.70
99.55
99.40
99.25
4000
3500
3000
2500
2000
1500
1000
500
cm-1
[%]
ETH-LAD
GC-EI-MS
Isomer III
26.16
Isomer I
22.24
Isomer II
24.74
Isomer IV
29.94
min
ETH-LAD Isomer I base (GC-sIR)

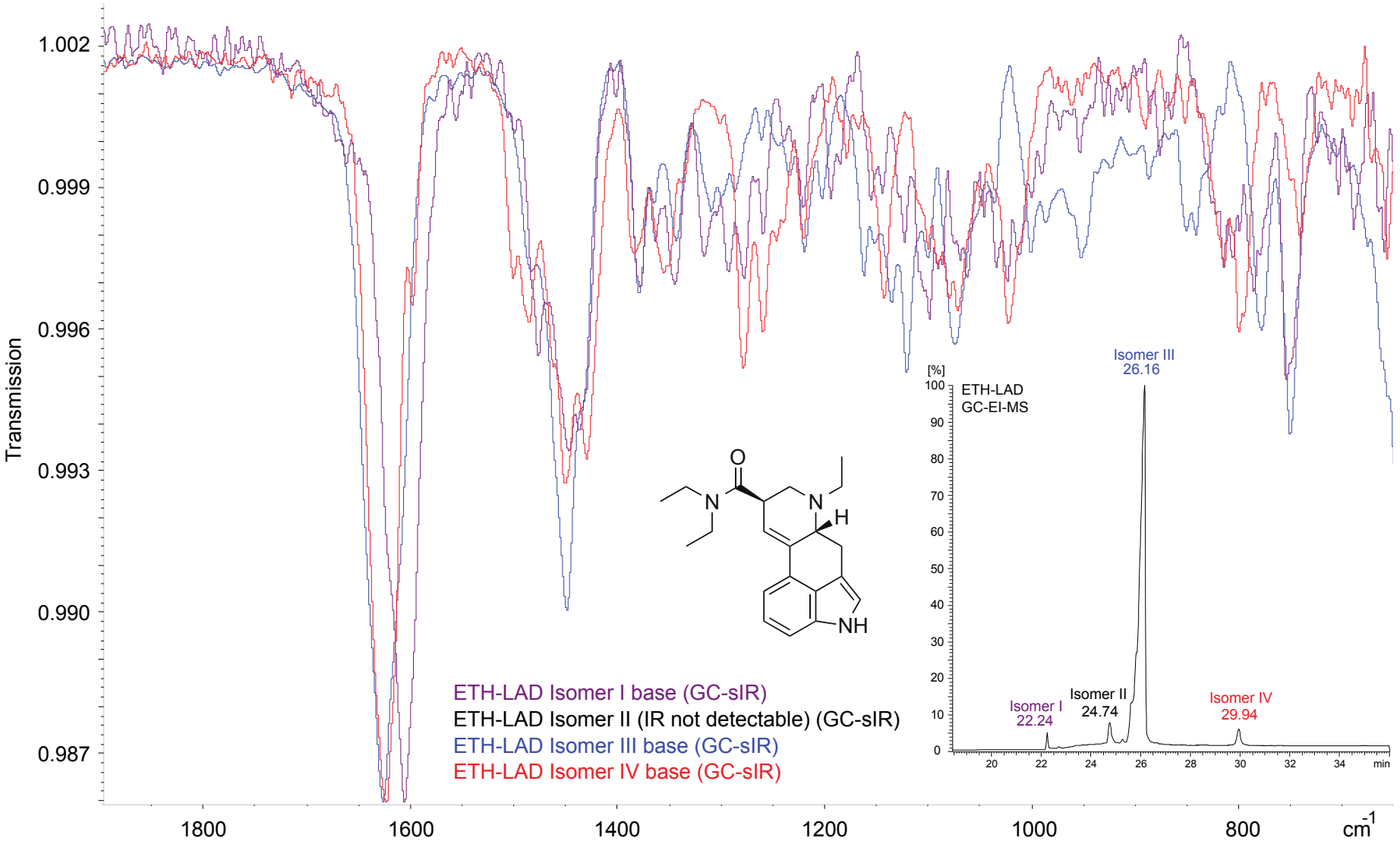

%T
100.1
99.9
99.7
99.5
99.3
1800
1600
1400
1200
1000
800
cm-1
1682.6
1652.5
1645.5
1606.1
1556.3
1506.3
1499.5
1476.6
1446.9
1437.1
1378.0
1363.6
1344.6
1316.2
1304.3
1292.9
1277.8
1259.1
1239.5
1220.4
1193.8
1185.1
1155.1
1143.9
1128.0
1122.6
1103.1
1098.3
1090.3
1068.7
1061.9
1045.8
1033.7
1023.1
1011.7
999.6
989.8
972.4
961.8
953.3
941.2
929.6
921.5
905.7
885.6
875.7
864.5
821.1
814.2
805.5
798.6
784.8
753.4
723.9
711.8
703.2
695.0
688.2
672.8
664.1
658.6

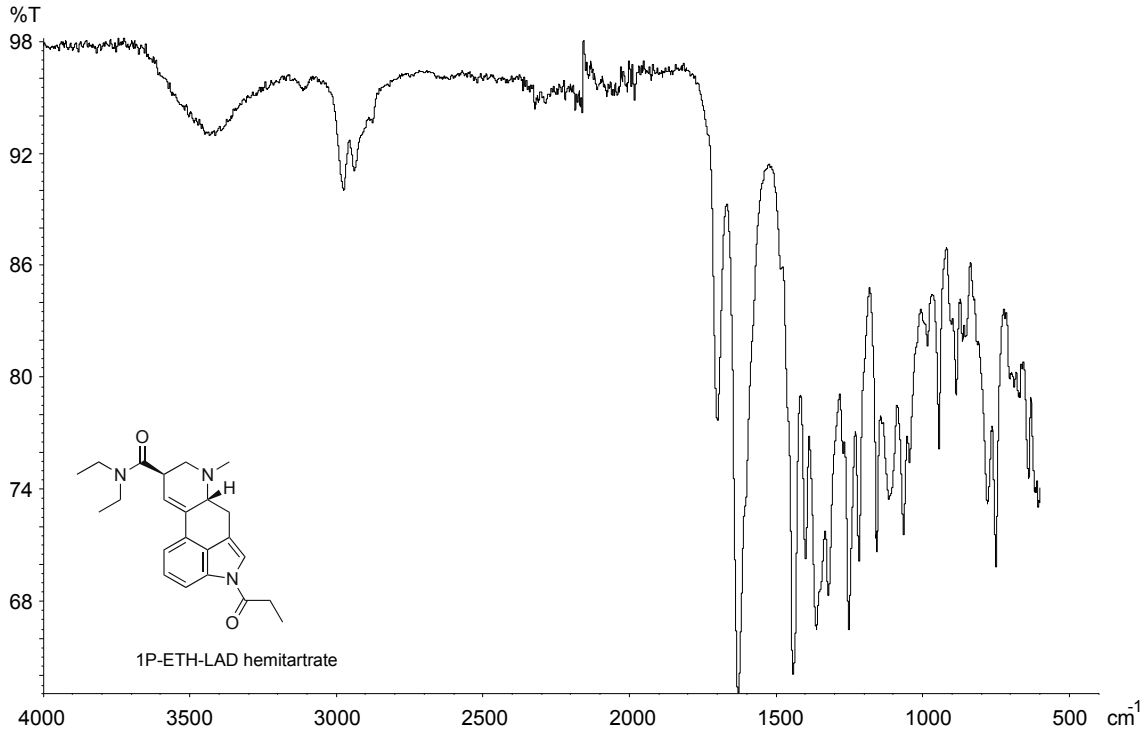

Transmission
1.012
1.006
1.000
0.994
0.988
0.982
0.974
4000
3500
3000
2500
2000
1500
1000
500
cm-1
[%]
ETH-LAD
GC-EI-MS
Isomer I
22.24
Isomer II
24.74
Isomer III
26.16
Isomer IV
29.94
min
O
N
N
H
NH
ETH-LAD Isomer III base (GC-sIR)

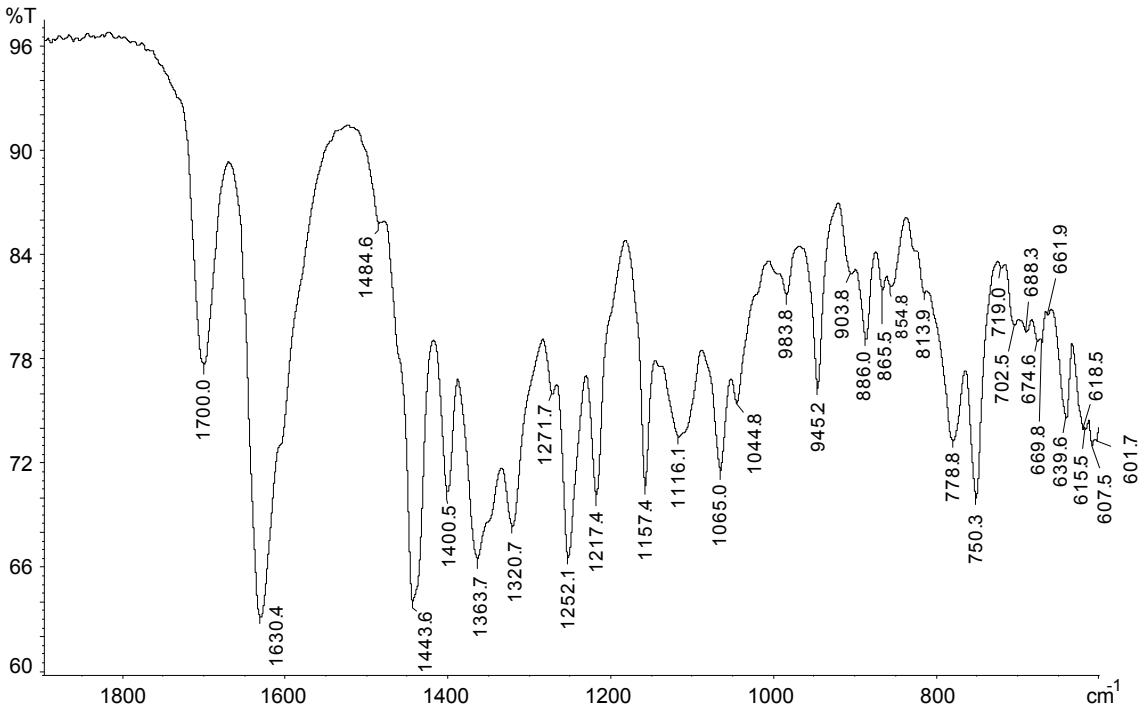

%T
101.0
100.0
99.0
98.0
97.0
1800
1600
1400
1200
1000
800
cm-1
1626.7
1483.4
1449.2
1379.1
1364.6
1343.2
1309.4
1299.3
1286.8
1261.0
1244.6
1233.6
1219.2
1202.5
1161.9
1151.6
1135.2
1120.4
1100.0
1074.1
1035.7
1000.3
985.8
951.9
922.9
904.9
886.6
850.3
841.0
815.0
777.6
749.5
704.7
692.2
674.7
655.3

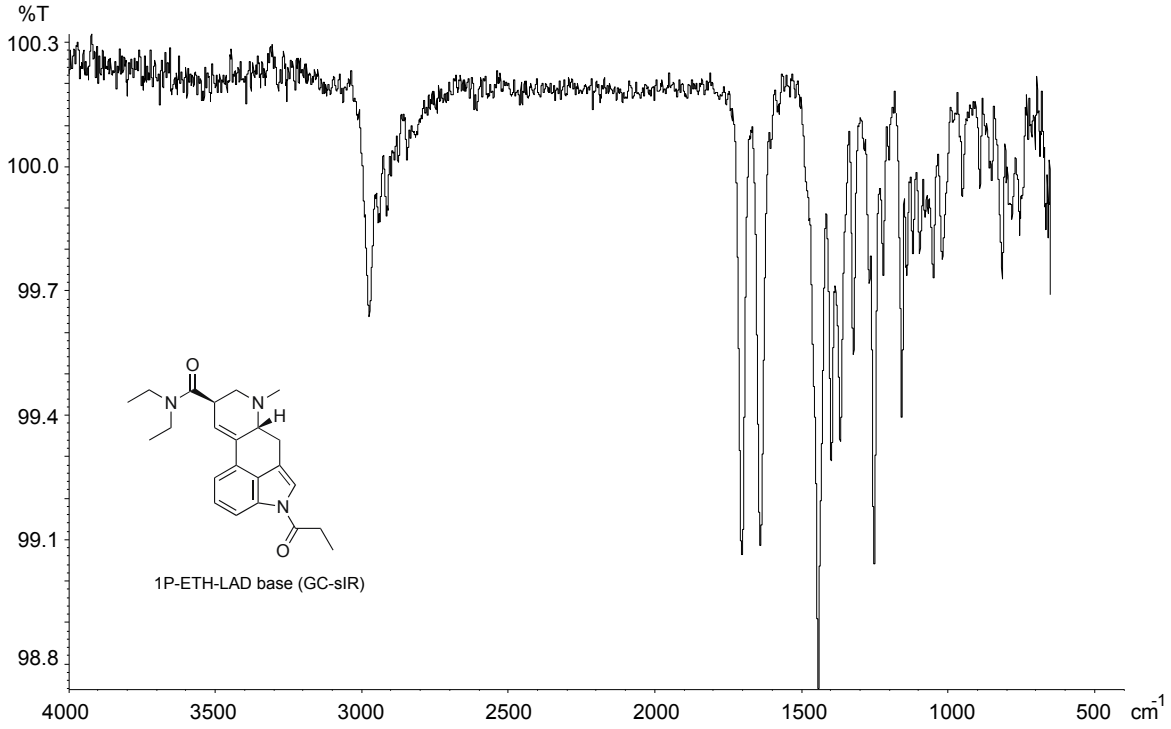

%T
100.2
99.8
99.4
99.0
98.6
4000
3500
3000
2500
2000
1500
1000
500
cm-1
[%]
ETH-LAD
GC-EI-MS
Isomer I
22.24
Isomer II
24.74
Isomer III
26.16
Isomer IV
29.94
min
ETH-LAD Isomer IV base (GC-sIR)

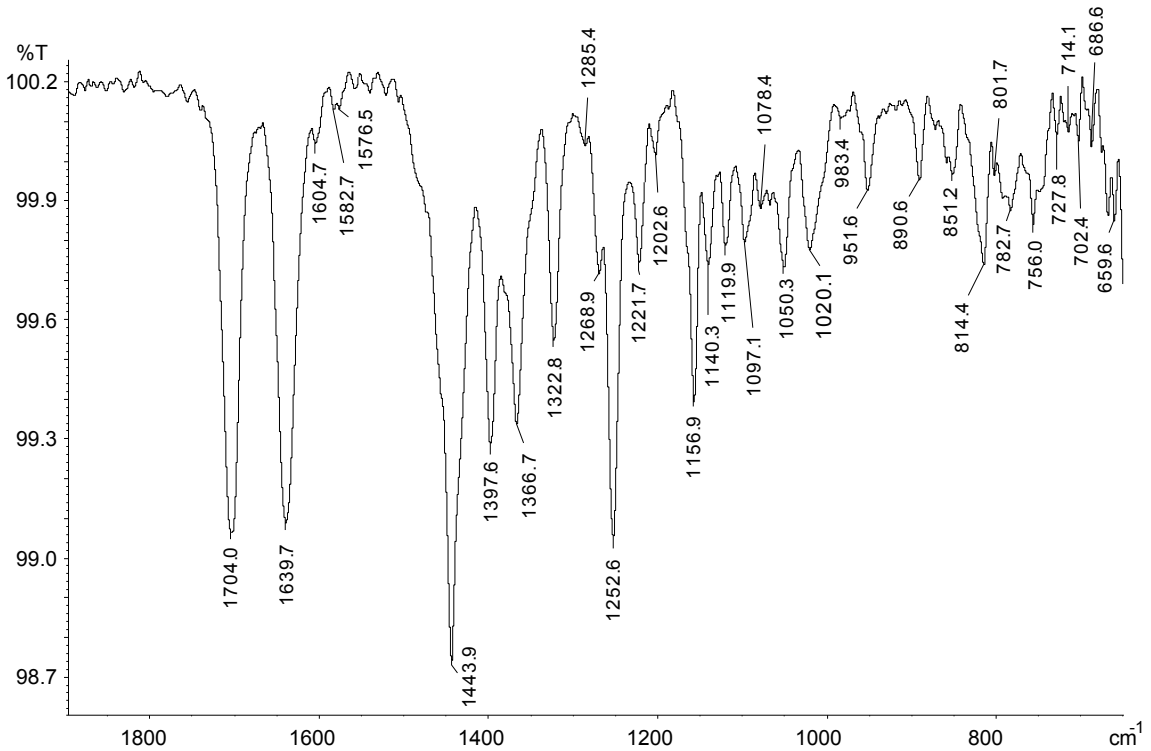

%T
100.2
99.8
99.4
99.0
99.6
1800
1600
1400
1200
1000
800
cm-1
1733.1
1715.1
1699.9
1690.1
1623.6
1598.4
1500.6
1485.6
1450.6
1429.8
1383.7
1355.6
1300.9
1278.4
1259.3
1246.5
1220.3
1178.6
1167.4
1142.2
1099.8
1090.2
1079.1
1071.0
1047.8
1022.0
970.6
961.4
928.1
911.7
889.7
869.6
851.3
837.4
813.7
799.2
795.0
773.6
739.9
710.0
689.9
671.1
655.7


Transmission
1.002
0.999
0.996
0.993
0.990
0.987
1800
1600
1400
1200
1000
800
cm-1
ETH-LAD Isomer I base (GC-sIR)
ETH-LAD Isomer II (IR not detectable) (GC-sIR)
ETH-LAD Isomer III base (GC-sIR)
ETH-LAD Isomer IV base (GC-sIR)
O
N
N
H
NH
[%]
ETH-LAD
GC-EI-MS
Isomer III
26.16
Isomer I
22.24
Isomer II
24.74
Isomer IV
29.94
min


%T
98
92
86
80
74
68
1P-ETH-LAD hemitartrate
4000
3500
3000
2500
2000
1500
1000
500
cm-1

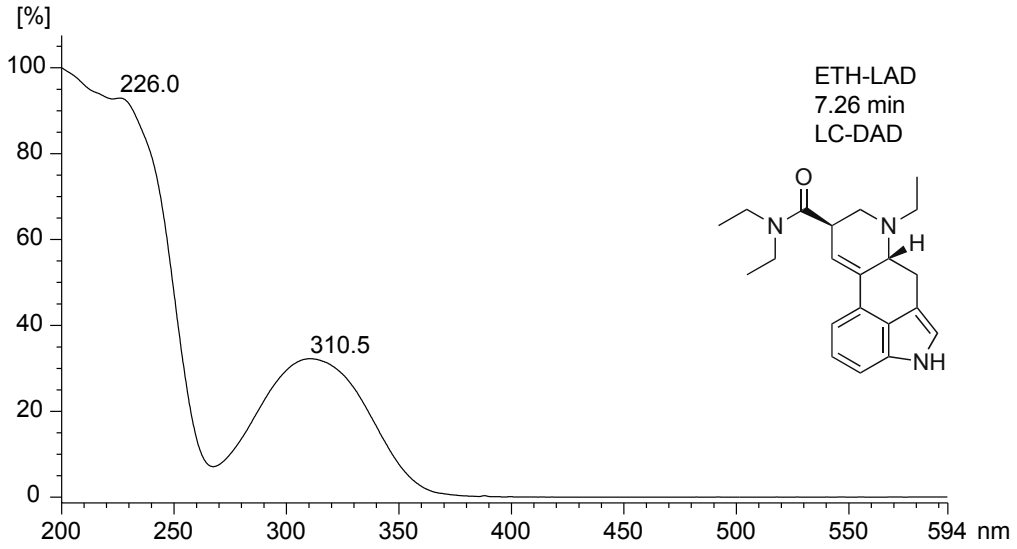

%T
96
90
84
78
72
66
60
1700.0
1630.4
1484.6
1443.6
1400.5
1363.7
1320.7
1271.7
1252.1
1217.4
1157.4
1116.1
1065.0
1044.8
983.8
945.2
903.8
886.0
865.5
854.8
813.9
778.8
750.3
719.0
702.5
688.3
674.6
669.8
661.9
639.6
618.5
615.5
607.5
601.7
1800
1600
1400
1200
1000
800
cm-1

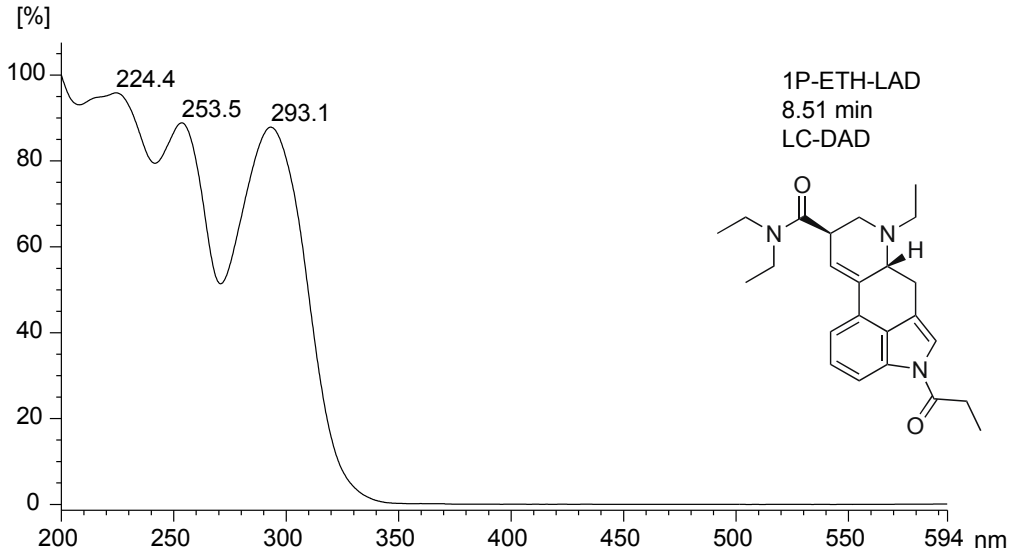

%T
100.3
100.0
99.7
99.4
99.1
98.8
4000
3500
3000
2500
2000
1500
1000
500
cm-1
1P-ETH-LAD base (GC-sIR)


%T
100.2
99.9
99.6
99.3
99.0
98.7
1800
1600
1400
1200
1000
800
cm-1
1704.0
1639.7
1604.7
1582.7
1576.5
1443.9
1397.6
1366.7
1322.8
1285.4
1268.9
1252.6
1221.7
1202.6
1156.9
1140.3
1119.9
1097.1
1078.4
1050.3
1020.1
983.4
951.6
890.6
851.2
814.4
801.7
782.7
756.0
727.8
714.1
702.4
686.6
659.6

Liquid chromatography diode array detection

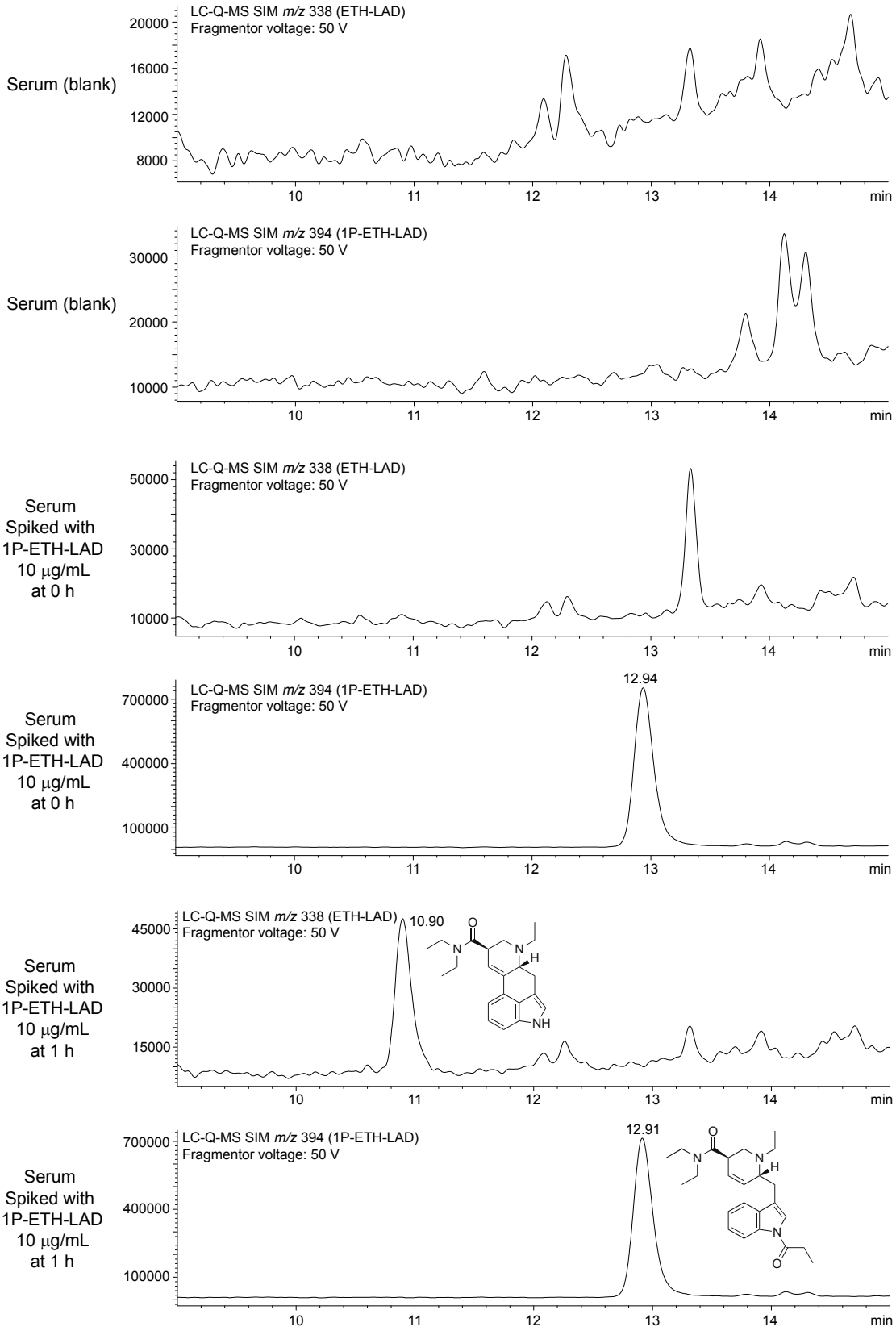
A Dionex 3000 Ultimate liquid chromatography system coupled to a UV diode array detector (Thermo Fisher, St. Albans, UK) was used for analysis using a Phenomenex Synergi Fusion column (150 mm × 2 mm, 4 µm) that was protected by a 4 mm × 3 mm Phenomenex Synergi Fusion guard column (Phenomenex, Cheshire, UK). The Mobile phases were 70% acetonitrile with 25 mM of triethylammonium phosphate buffer (TEAP) and aqueous TEAP (25 mM) buffer. The gradient elution commenced with 4% acetonitrile and ramped to 70% acetonitrile in 15 min and held for 3 min, resulting in a total acquisition time of 18 min at a flow rate of 0.6 mL/min. The diode array detection window was set at 200 nm–595 nm (collection rate 2 Hz).



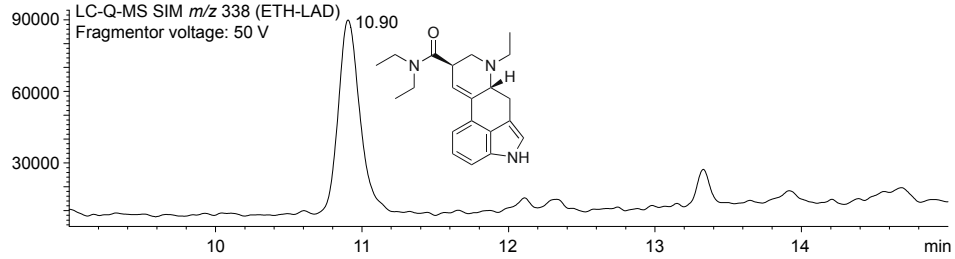


1P-ETH-LAD incubation (10 µg/mL) in human serum at 37°C.

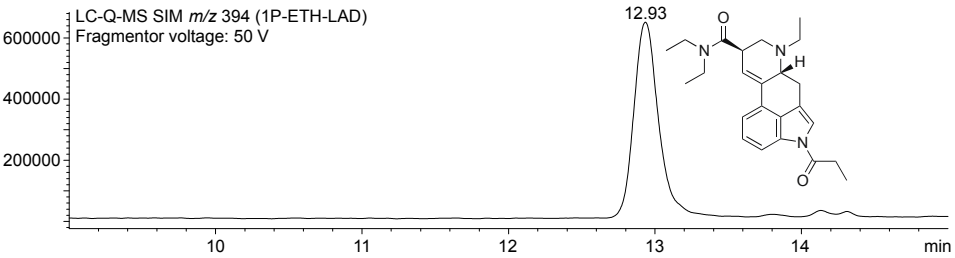
Fifty µL and 950 µL (acetonitrile/water, 1/1 + 0.1 % formic acid); centrifuged at 18,000 rpm for 3 min, then passed through a Nylon spin filter (0.2 µm). Samples were further diluted for LC-MS: 50 µL of the above and 950 µL (acetonitrile /water, 1/1 + 0.1 % formic acid).



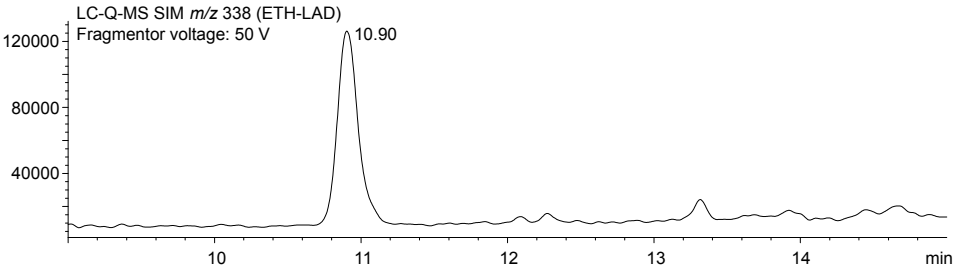
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 2 h



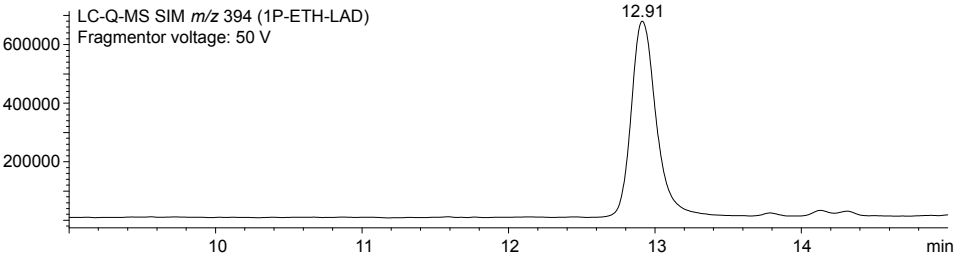
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 2 h



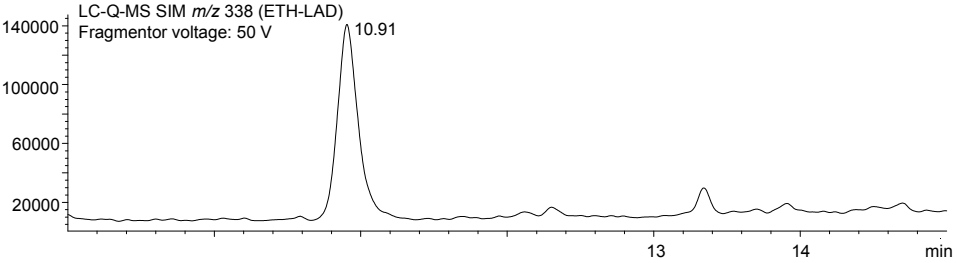
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 3 h



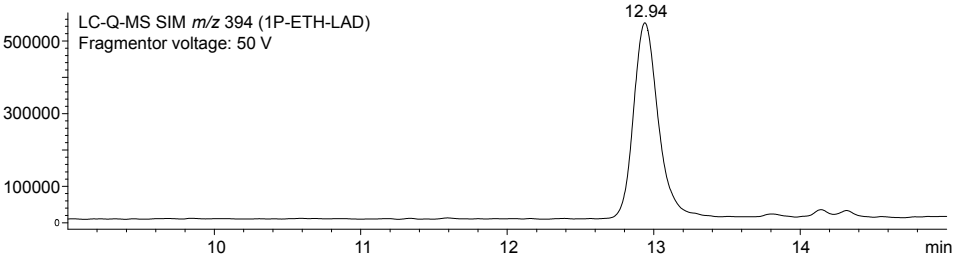
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 3 h

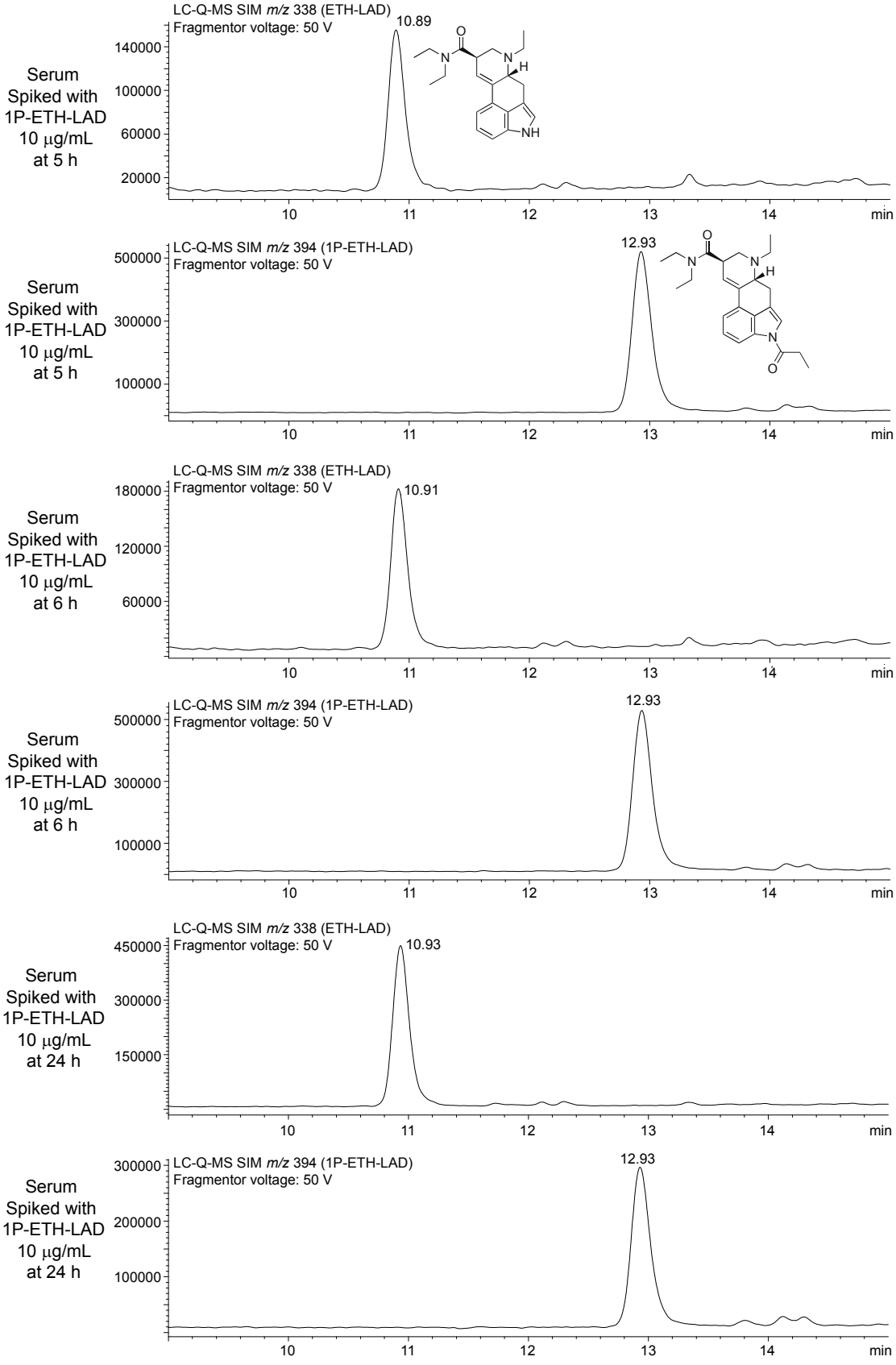


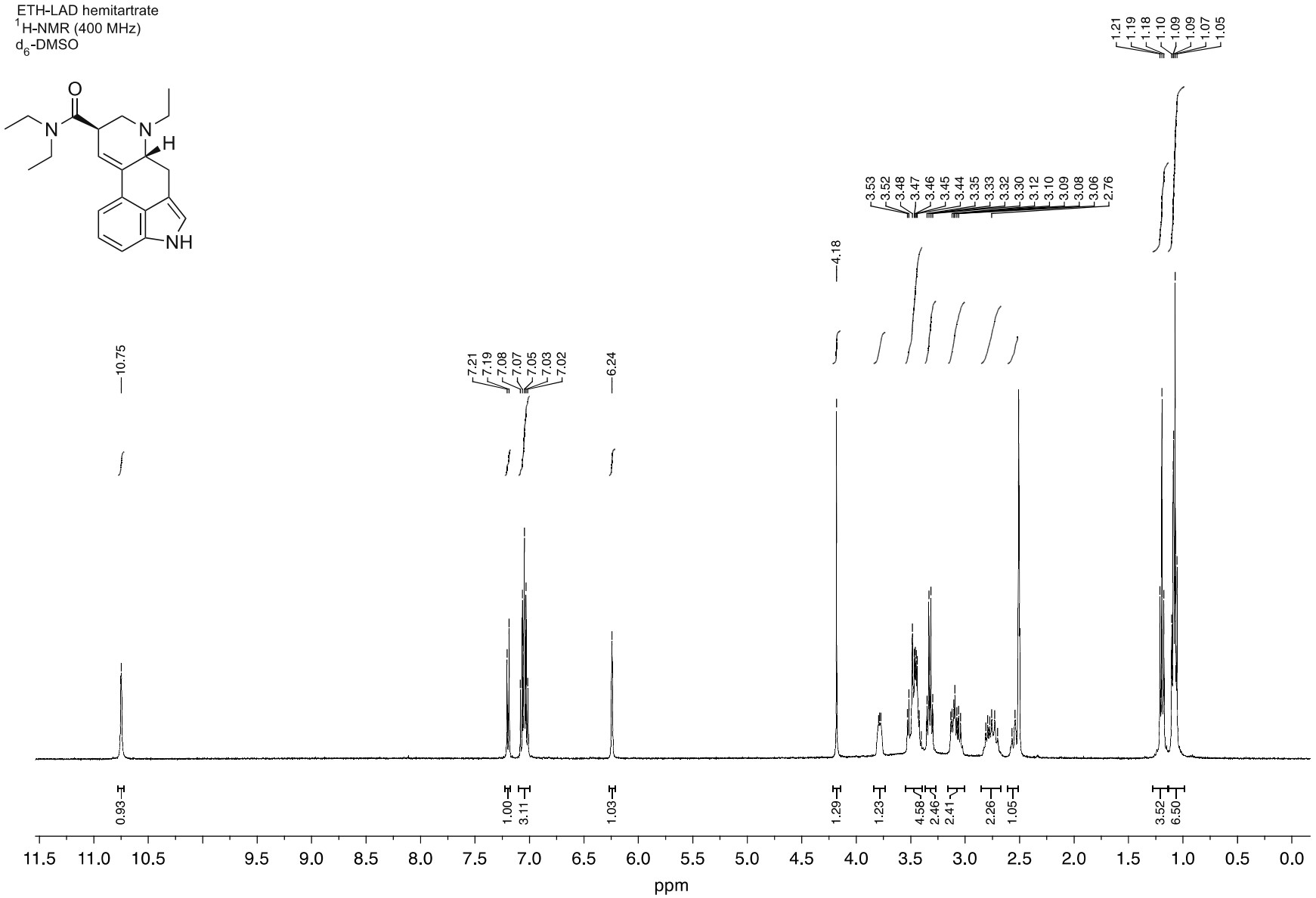
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 4 h



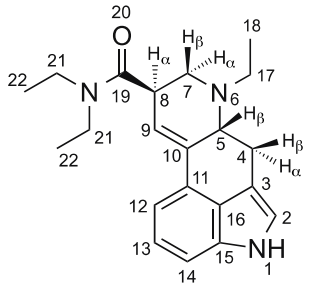
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 4 h

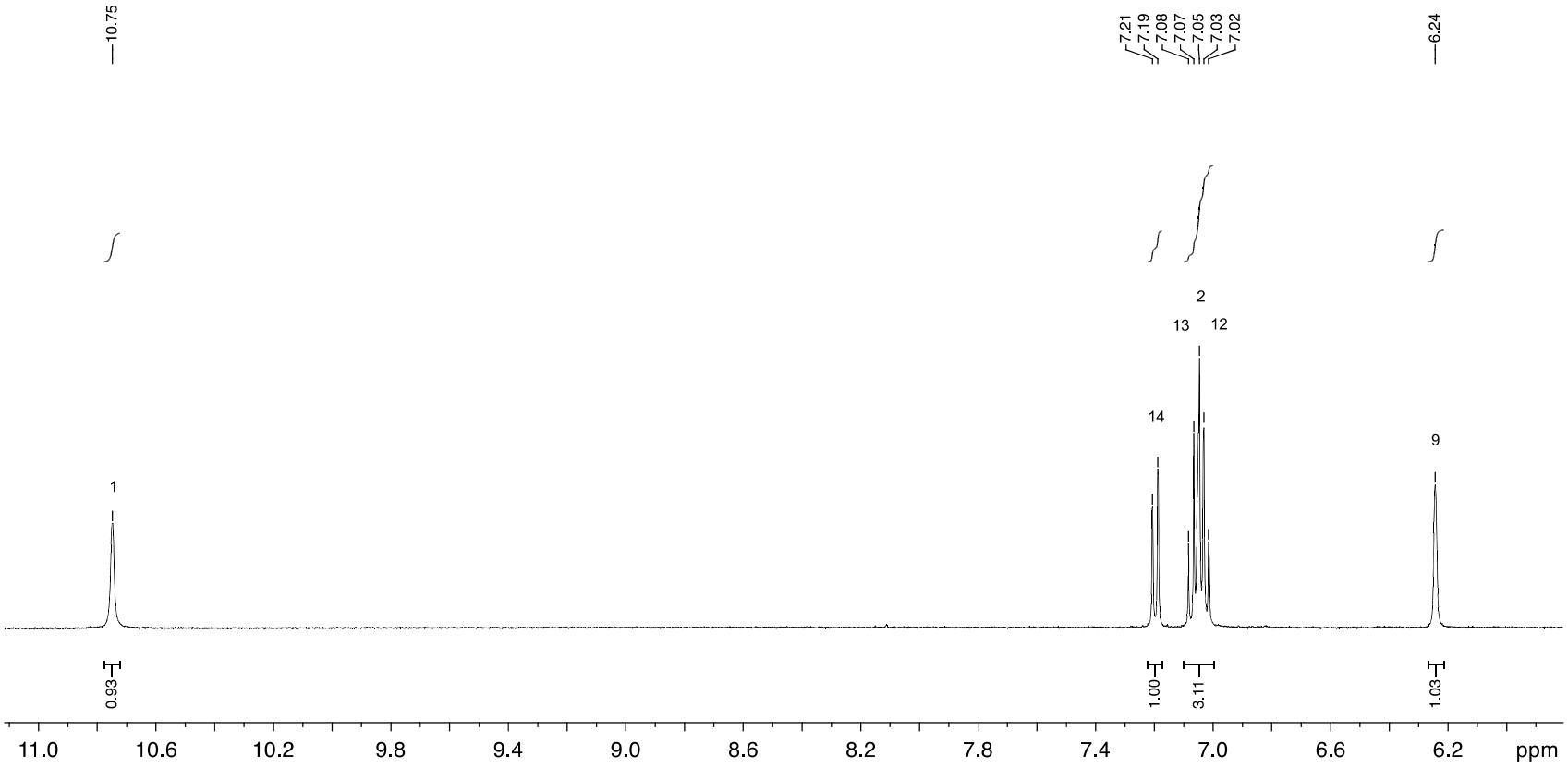


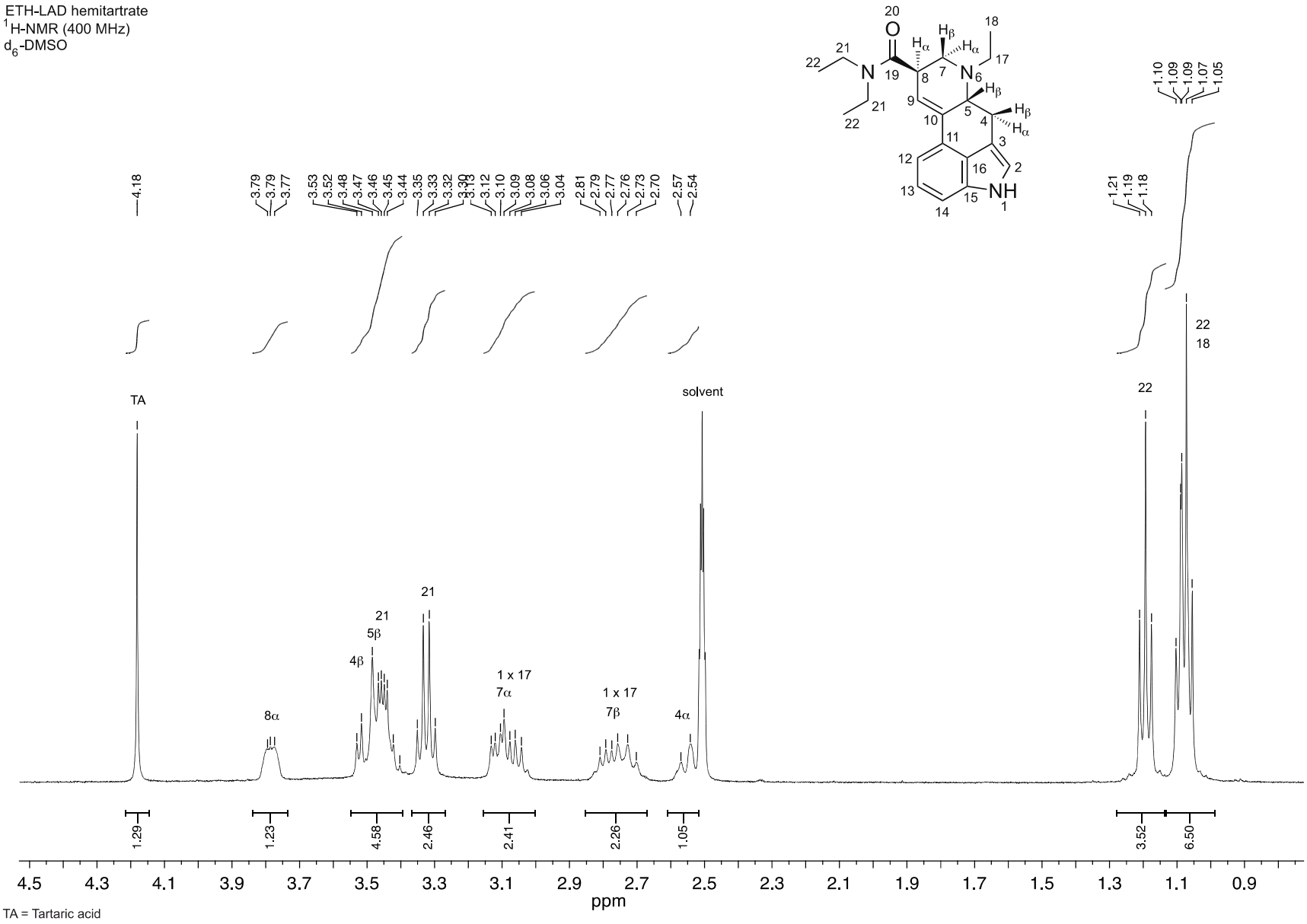

Serum Spiked with 1P-ETH-LAD 10 μg/mL at 5 h
LC-Q-MS SIM m/z 338 (ETH-LAD)
Fragmentor voltage: 50 V
10.89
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 5 h
LC-Q-MS SIM m/z 394 (1P-ETH-LAD)
Fragmentor voltage: 50 V
12.93
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 6 h
LC-Q-MS SIM m/z 338 (ETH-LAD)
Fragmentor voltage: 50 V
10.91
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 6 h
LC-Q-MS SIM m/z 394 (1P-ETH-LAD)
Fragmentor voltage: 50 V
12.93
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 24 h
LC-Q-MS SIM m/z 338 (ETH-LAD)
Fragmentor voltage: 50 V
10.93
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 24 h
LC-Q-MS SIM m/z 394 (1P-ETH-LAD)
Fragmentor voltage: 50 V
12.93
min

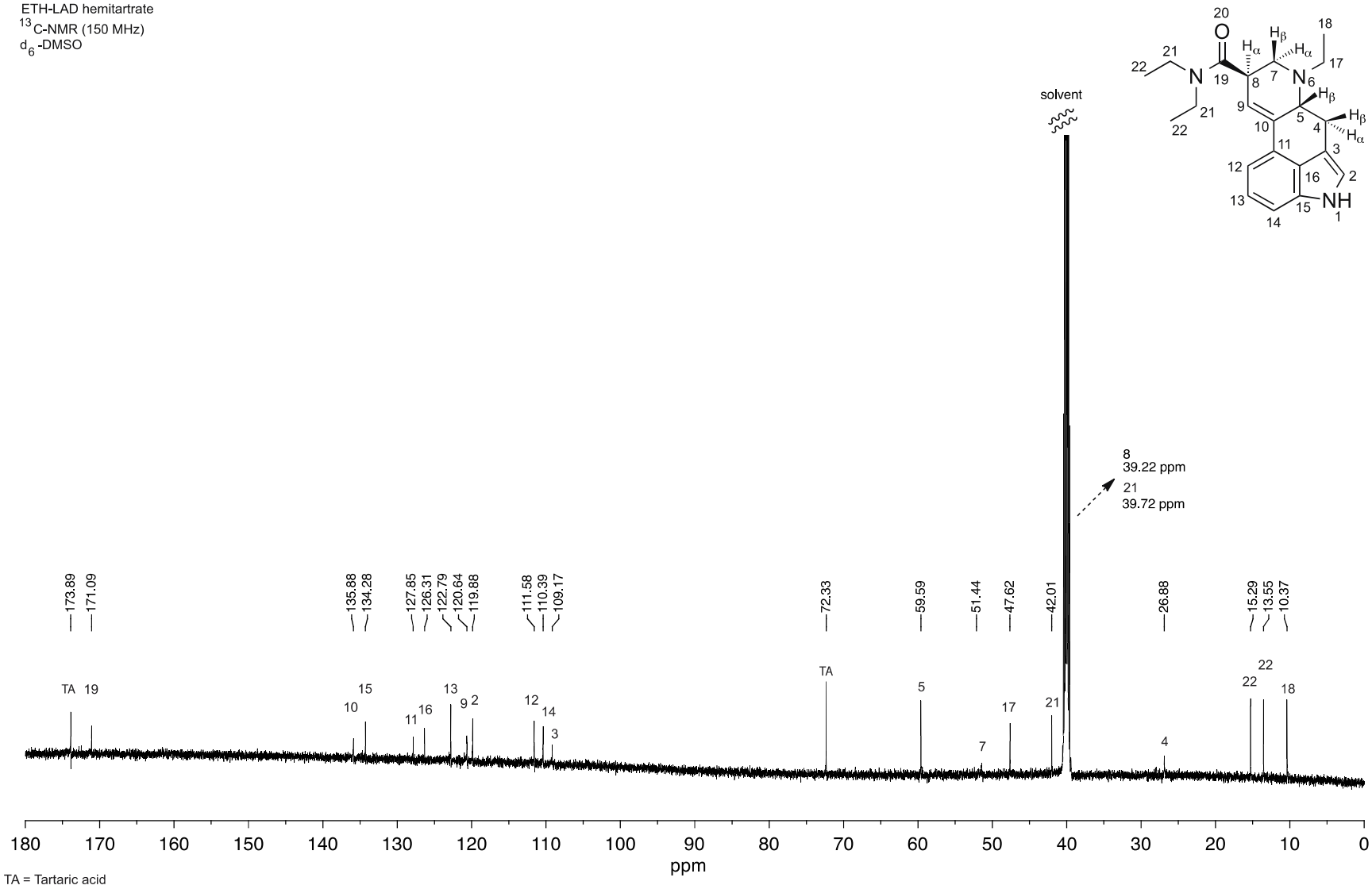

ETH-LAD hemitartrate
1H-NMR (400 MHz)
d6-DMSO
O
N
N
H
NH
10.75
7.21
7.19
7.08
7.07
7.05
7.03
7.02
6.24
4.18
3.53
3.52
3.48
3.47
3.46
3.45
3.44
3.35
3.33
3.32
3.30
3.12
3.10
3.09
3.08
3.06
2.76
1.21
1.19
1.18
1.10
1.09
1.09
1.07
1.05
0.93
1.00
3.11
1.03
1.29
1.23
4.58
2.46
2.41
2.26
1.05
3.52
6.50
11.5
11.0
10.5
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

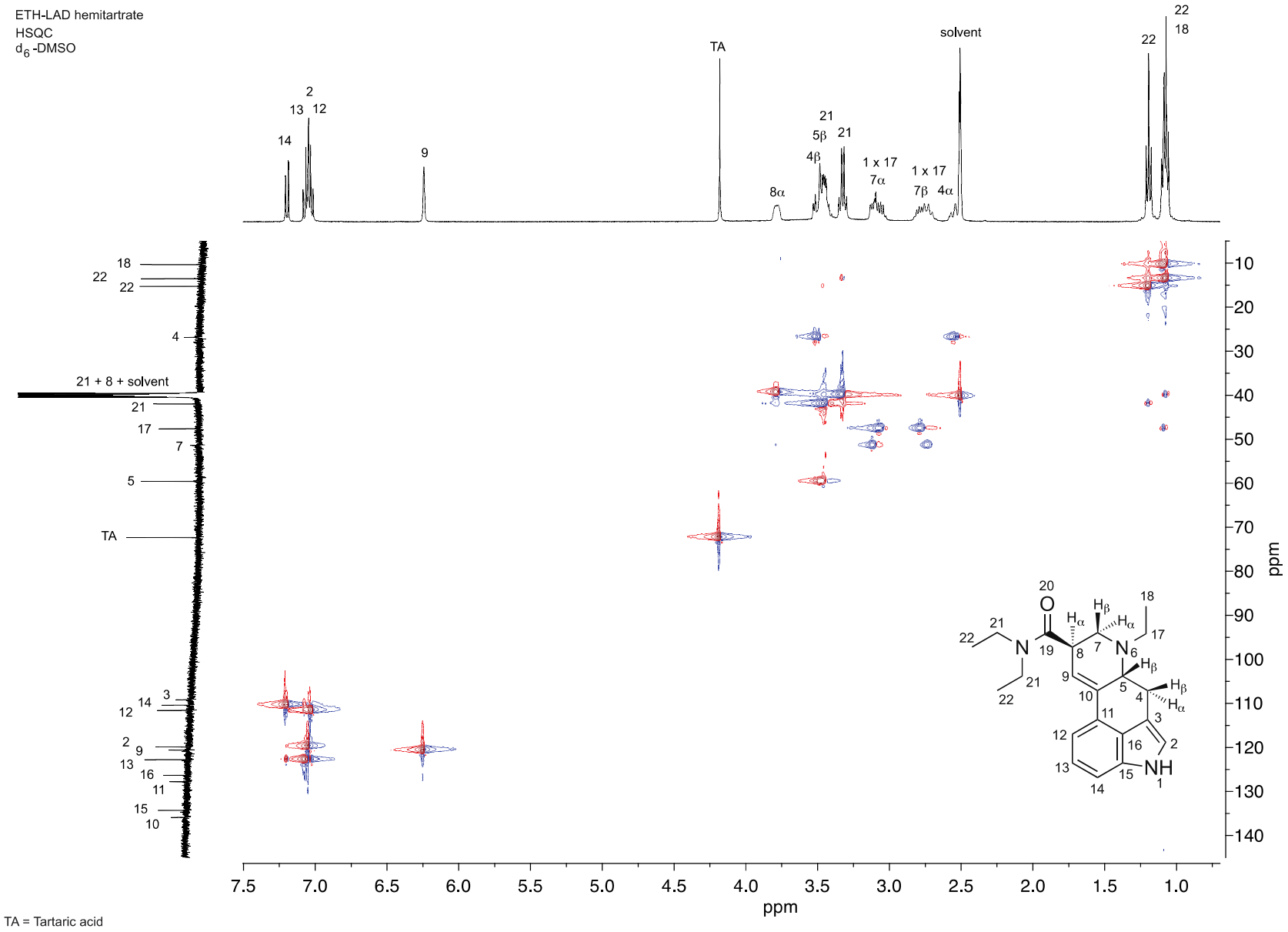
ETH-LAD hemitartrate
^{1}H-NMR (400 MHz)
d_6-DMSO



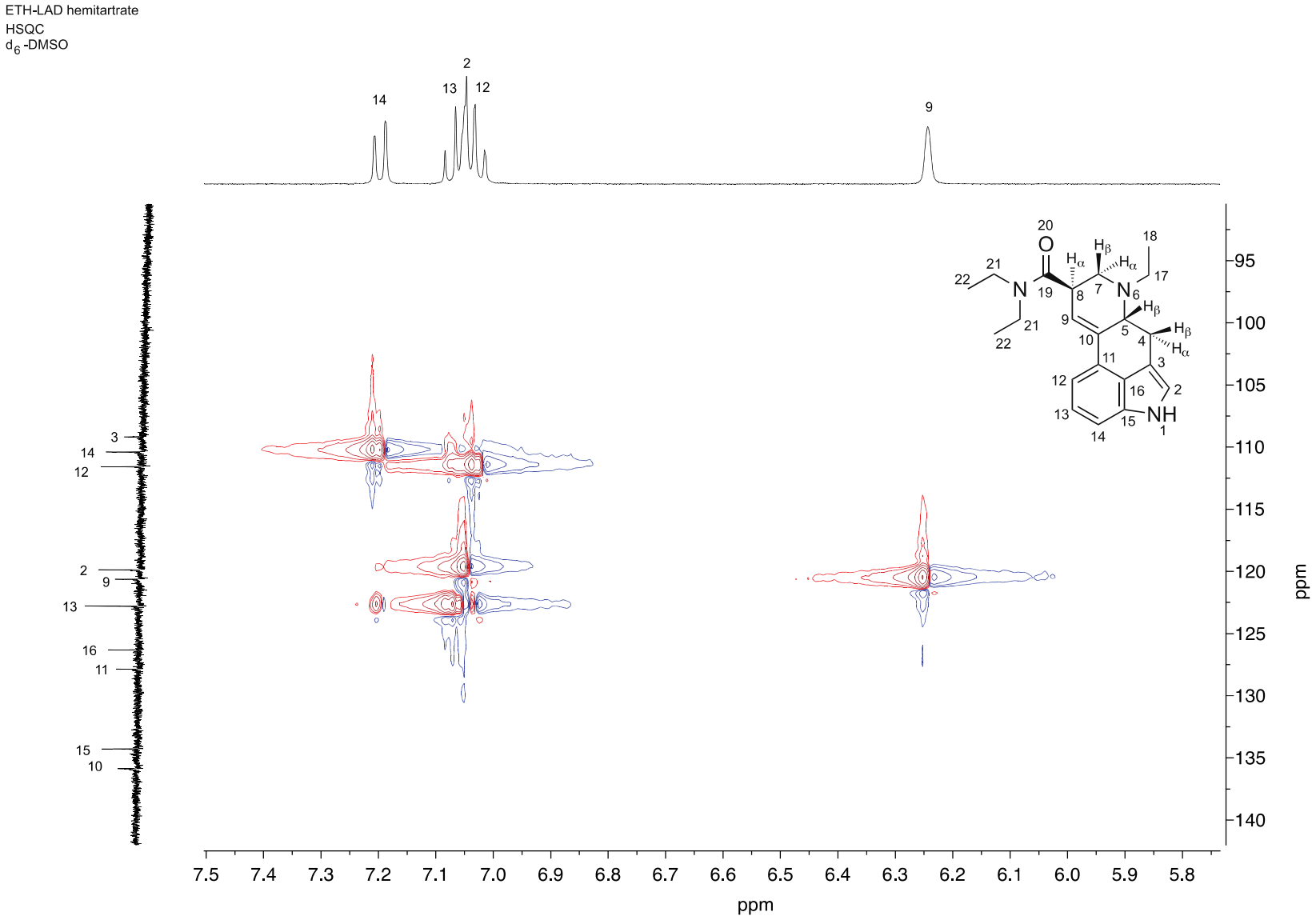


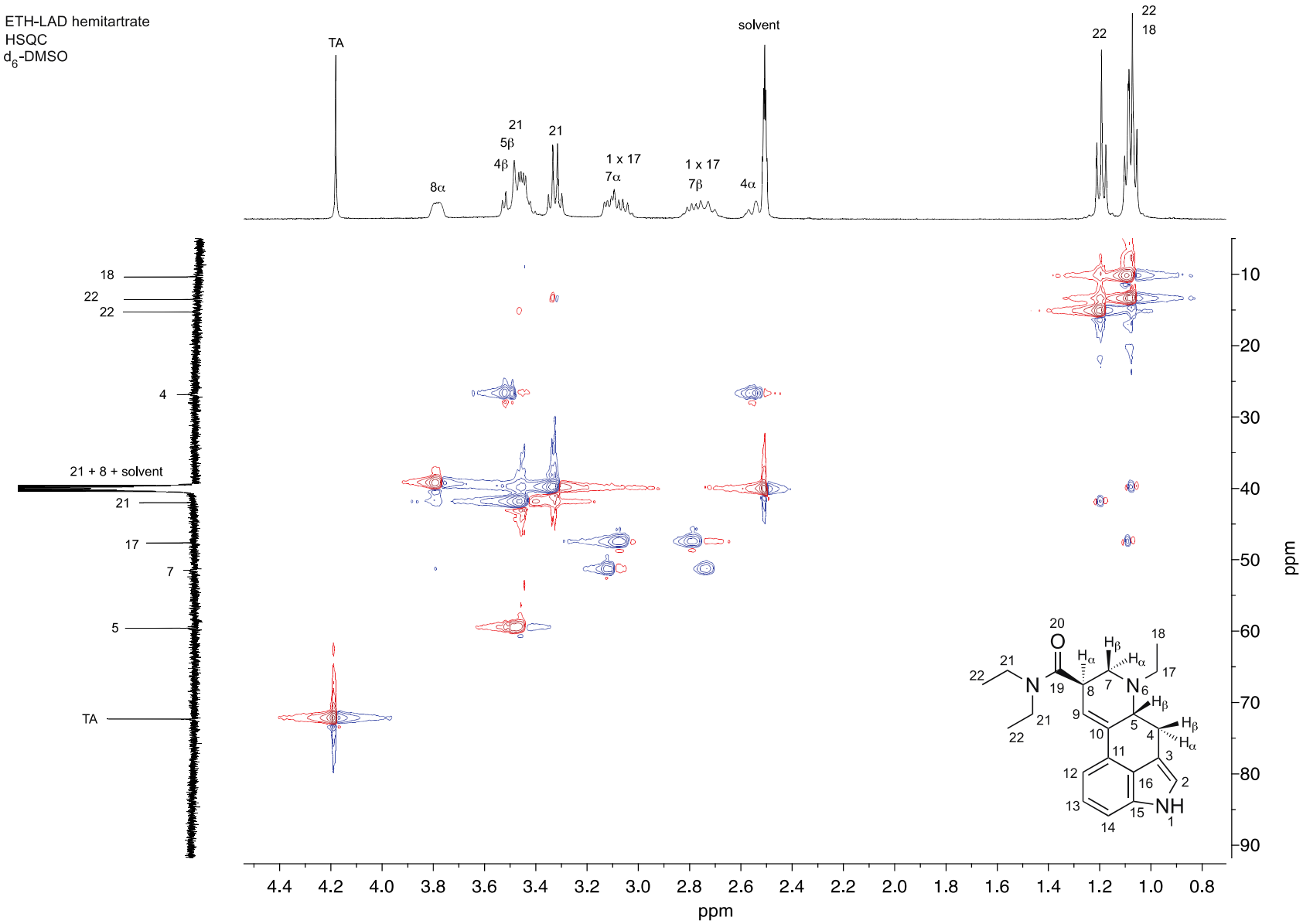

ETH-LAD hemitartrate
1H-NMR (400 MHz)
d6-DMSO
TA
solvent
8α
4β
5β
21
21
1 x 17
7α
1 x 17
7β
4α
22
22
18
ppm
TA = Tartaric acid


ETH-LAD hemitartrate
13C-NMR (150 MHz)
d6-DMSO
solvent
8
39.22 ppm
21
39.72 ppm
173.89
171.09
135.88
134.28
127.85
126.31
122.79
120.64
119.88
111.58
110.39
109.17
72.33
59.59
51.44
47.62
42.01
26.88
15.29
13.55
10.37
TA
19
15
10
16
11
13
9
2
12
14
3
5
7
17
21
4
22
22
18
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm
TA = Tartaric acid

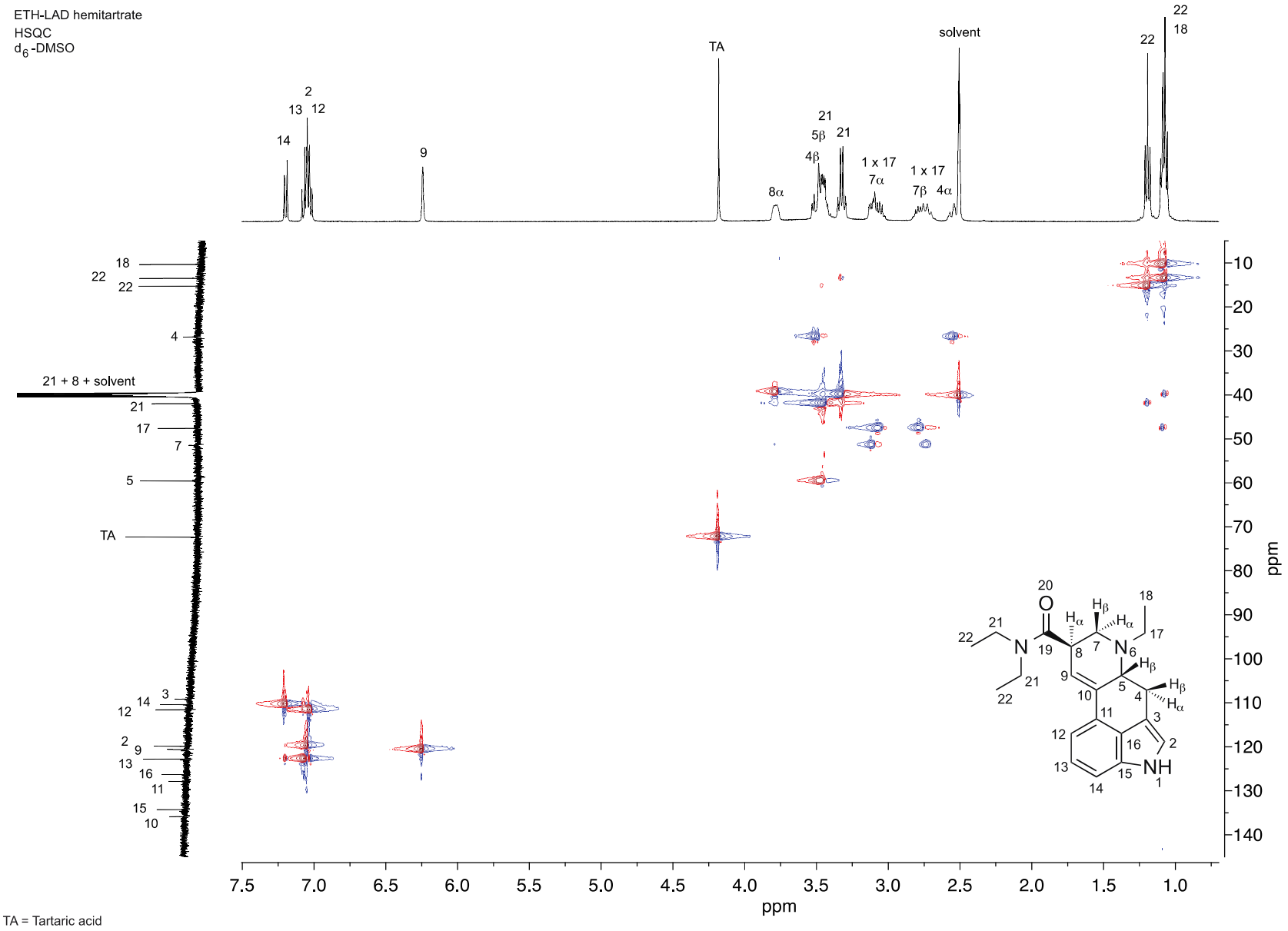


TA = Tartaric acid

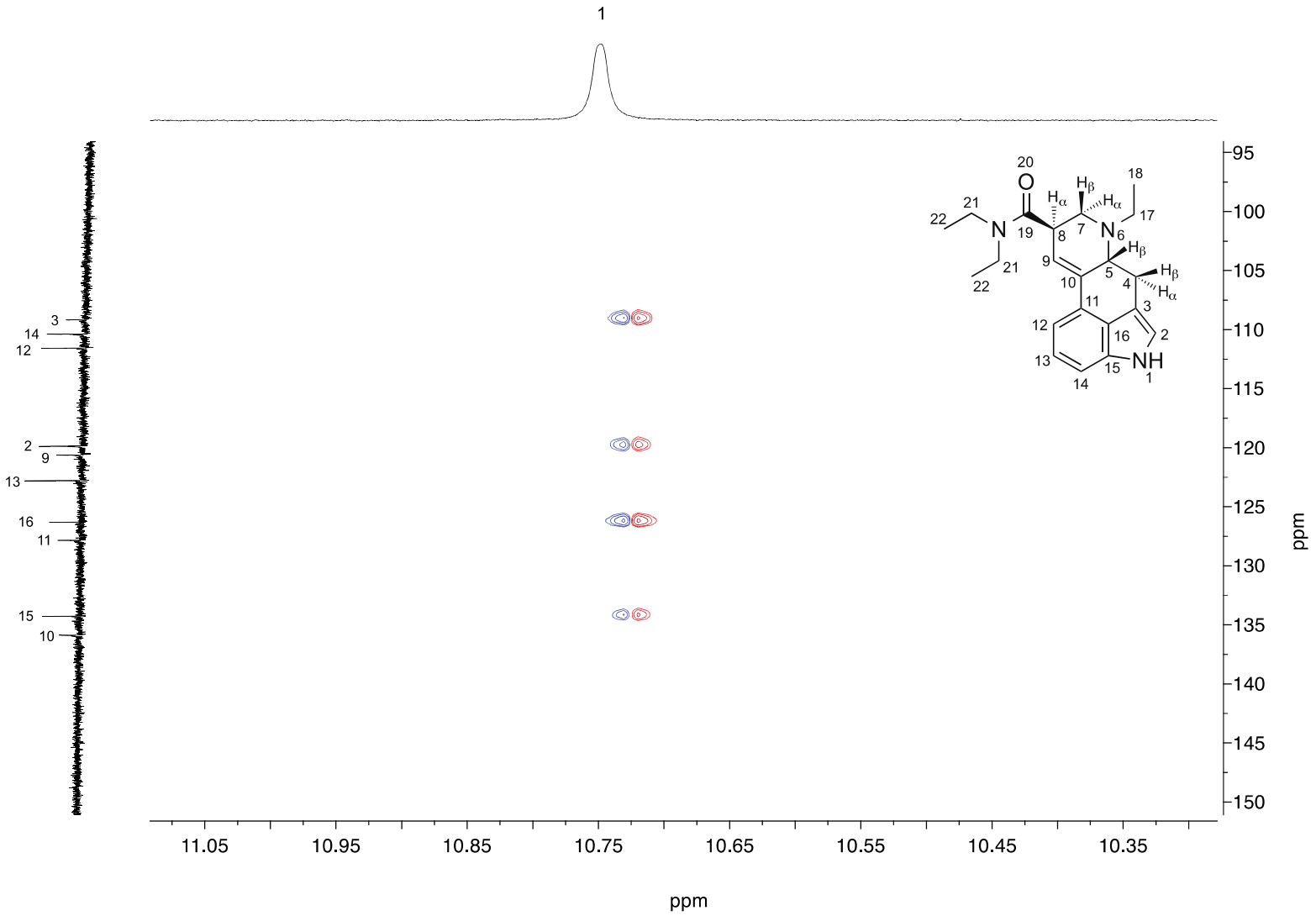

ETH-LAD hemitartrate
HSQC
d6 -DMSO
14
2
13
12
9
3
14
12
2
9
13
16
11
15
10
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6.0
5.9
5.8
ppm
95
100
105
110
115
120
125
130
135
140
ppm
O
N
NH
Hβ
Hα

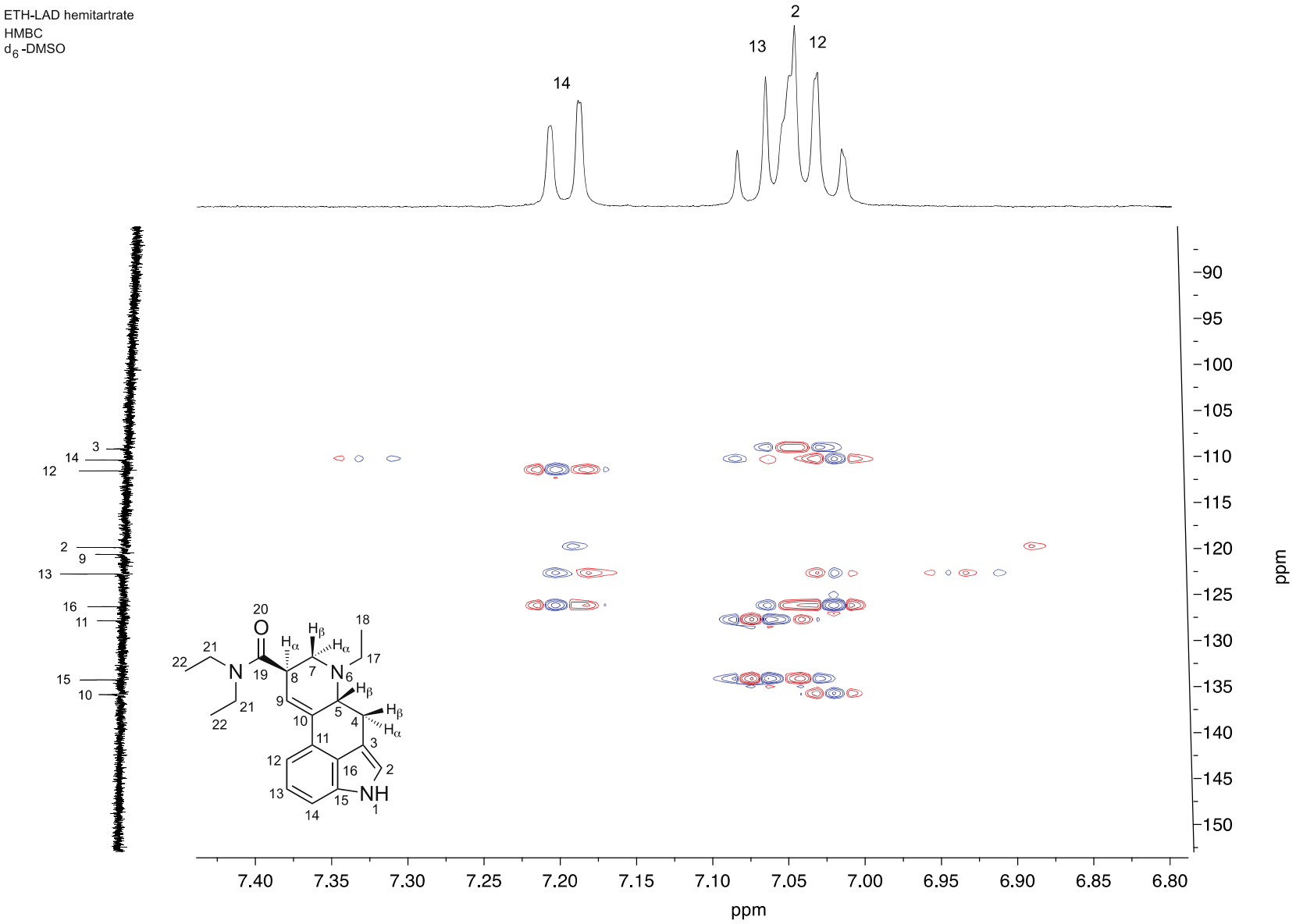


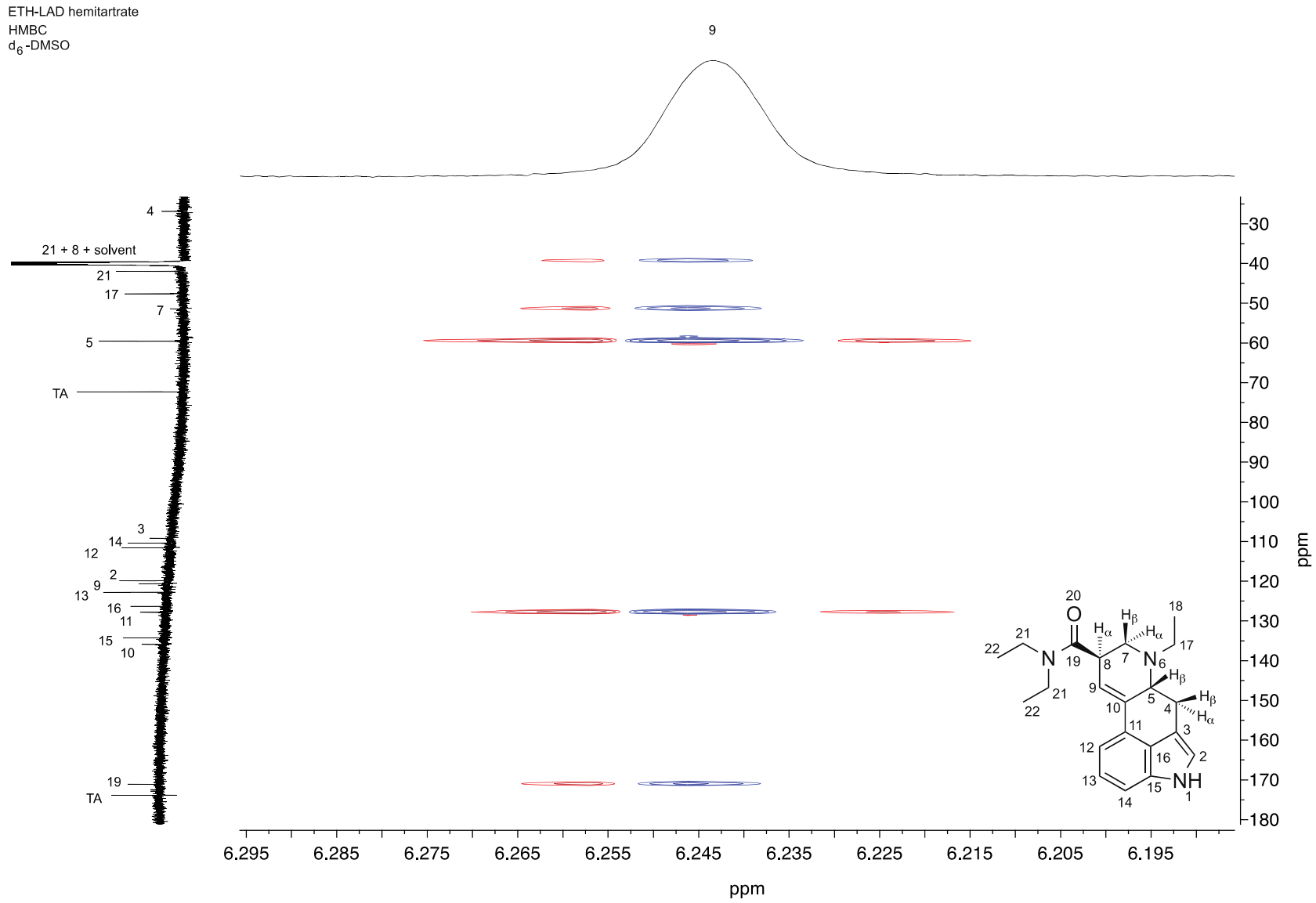
TA = Tartaric acid


ETH-LAD hemitartrate
HSQC
d_6-DMSO
TA
solvent
14
13
2
12
9
8α
5β
4β
21
21
1 x 17
7α
1 x 17
7β
4α
22
18
22
18
22
22
4
21 + 8 + solvent
21
17
7
5
TA
12
14
3
2
9
13
16
11
15
10
ppm
ppm
TA = Tartaric acid

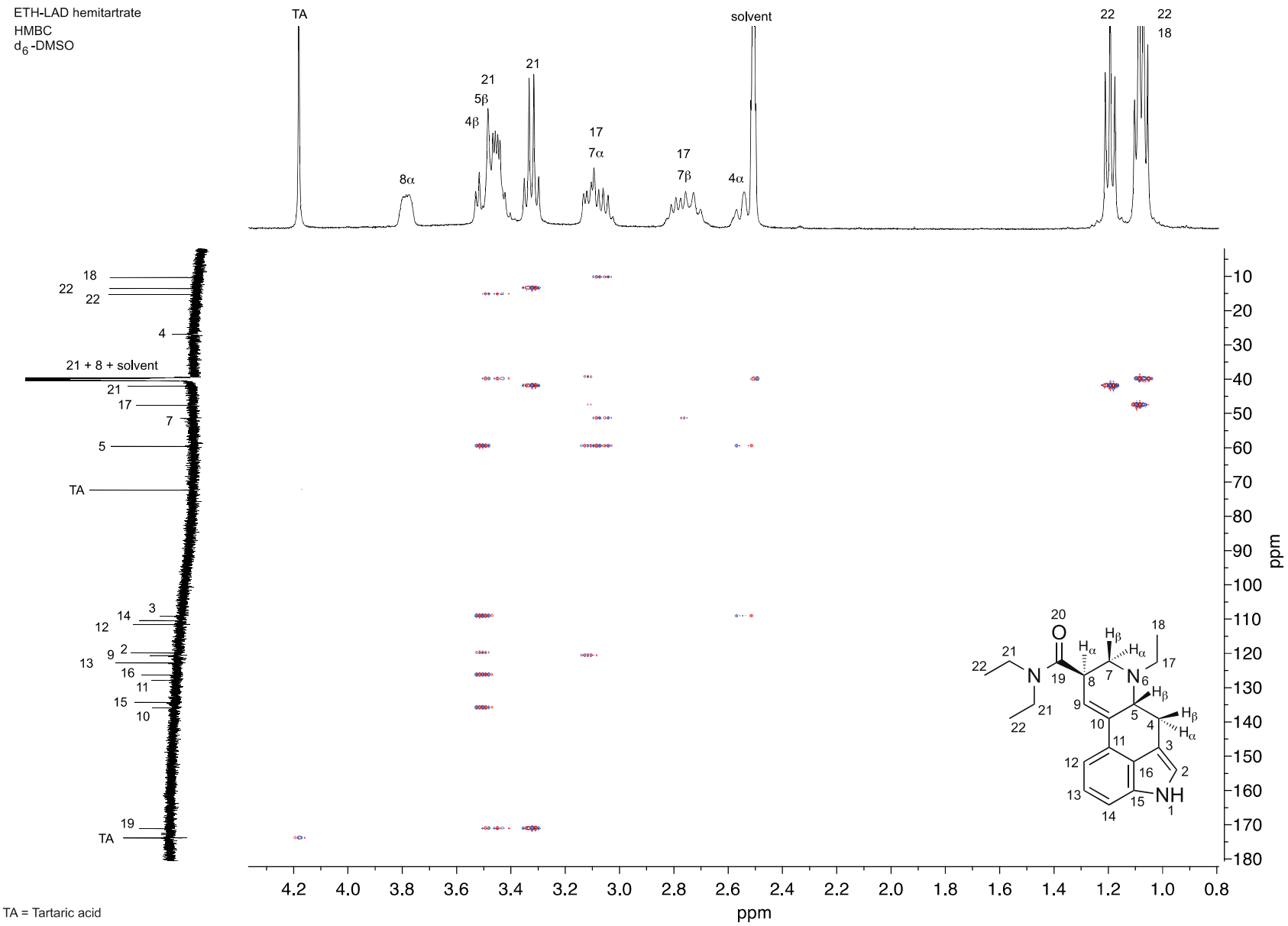
ETH-LAD hemitartrate
HMBC
d_6-DMSO



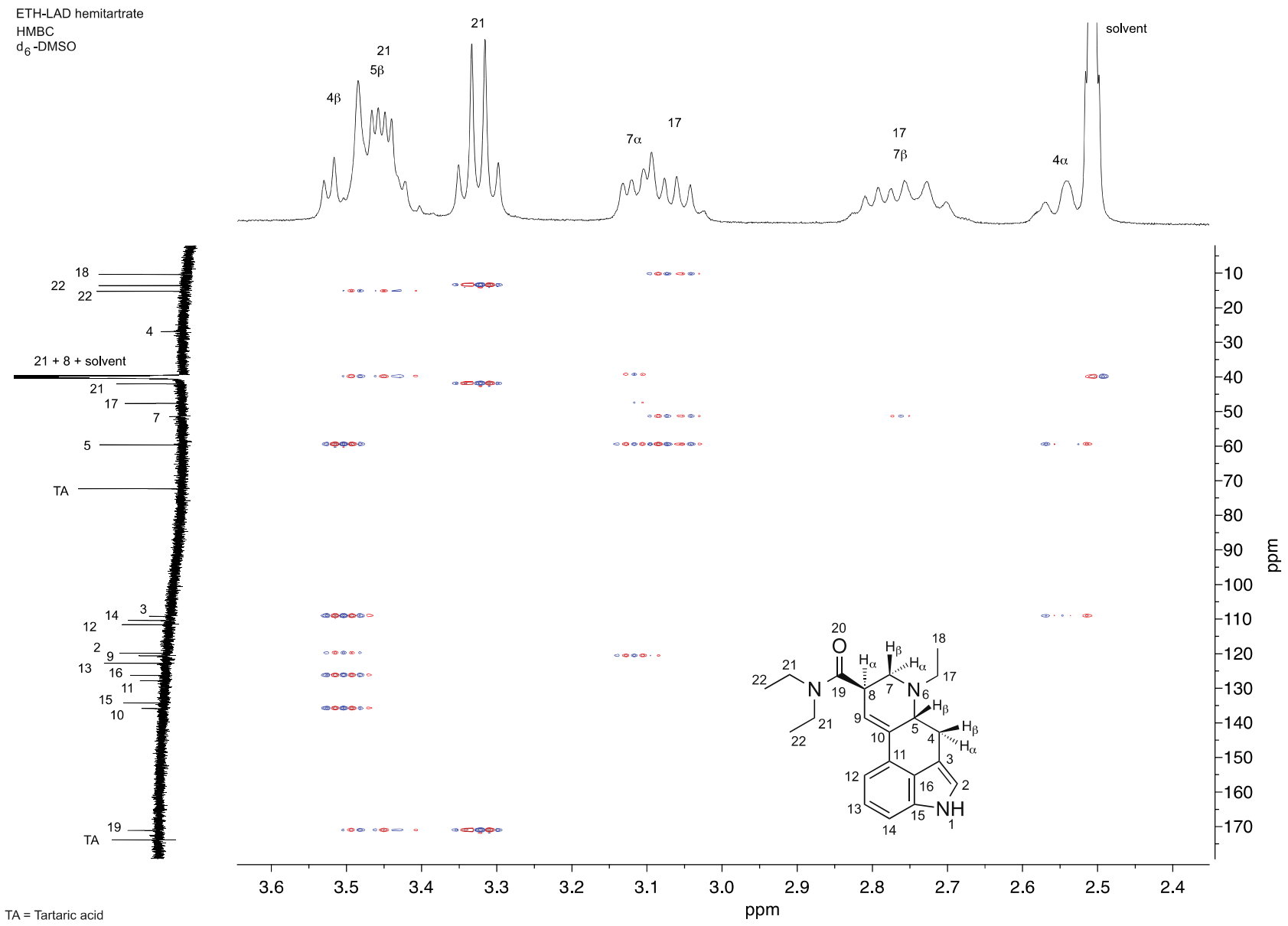

ETH-LAD hemitartrate
HMBC
d6-DMSO
14
13
2
12
ppm
7.40
7.35
7.30
7.25
7.20
7.15
7.10
7.05
7.00
6.95
6.90
6.85
6.80
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
-150

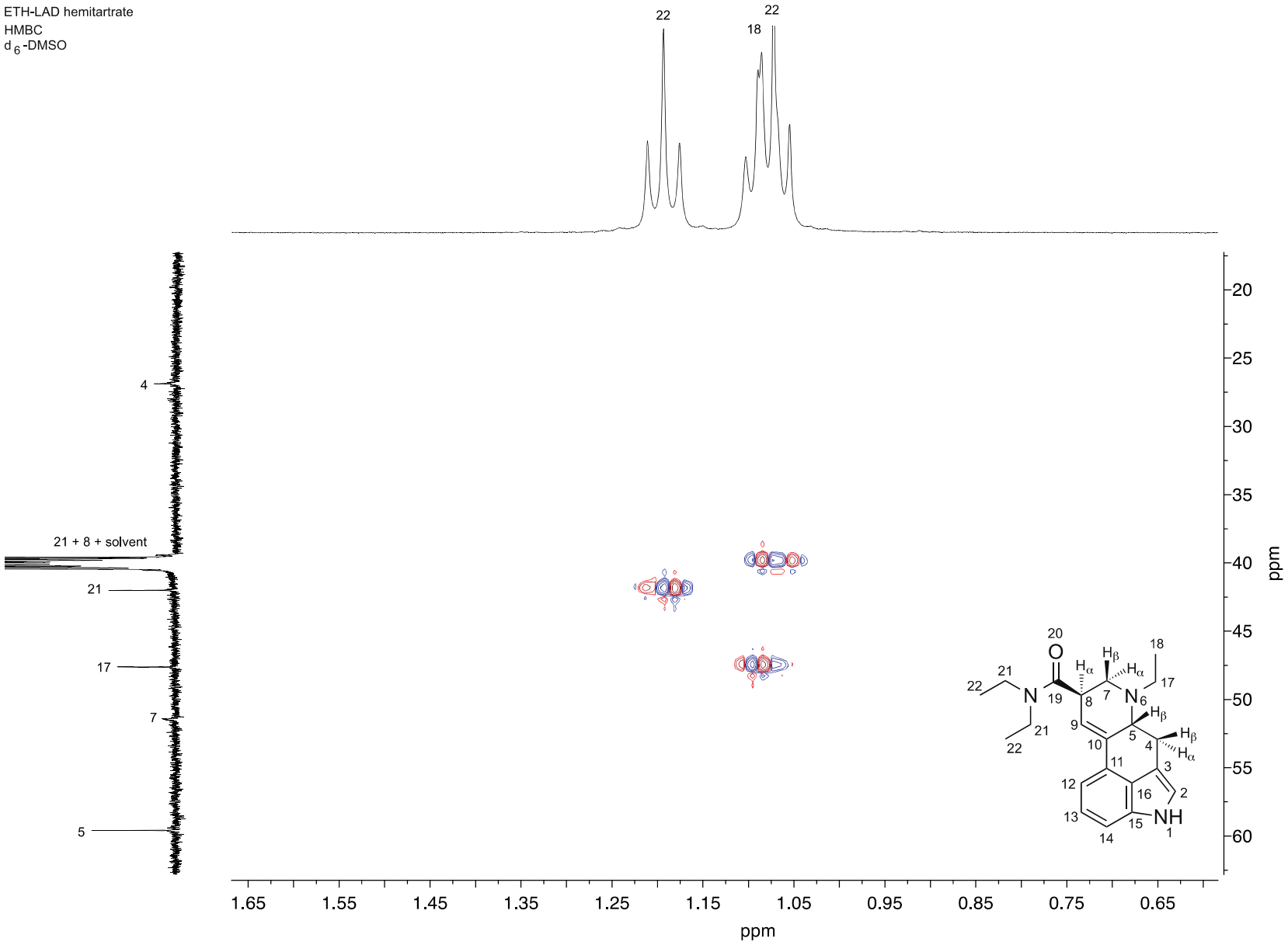


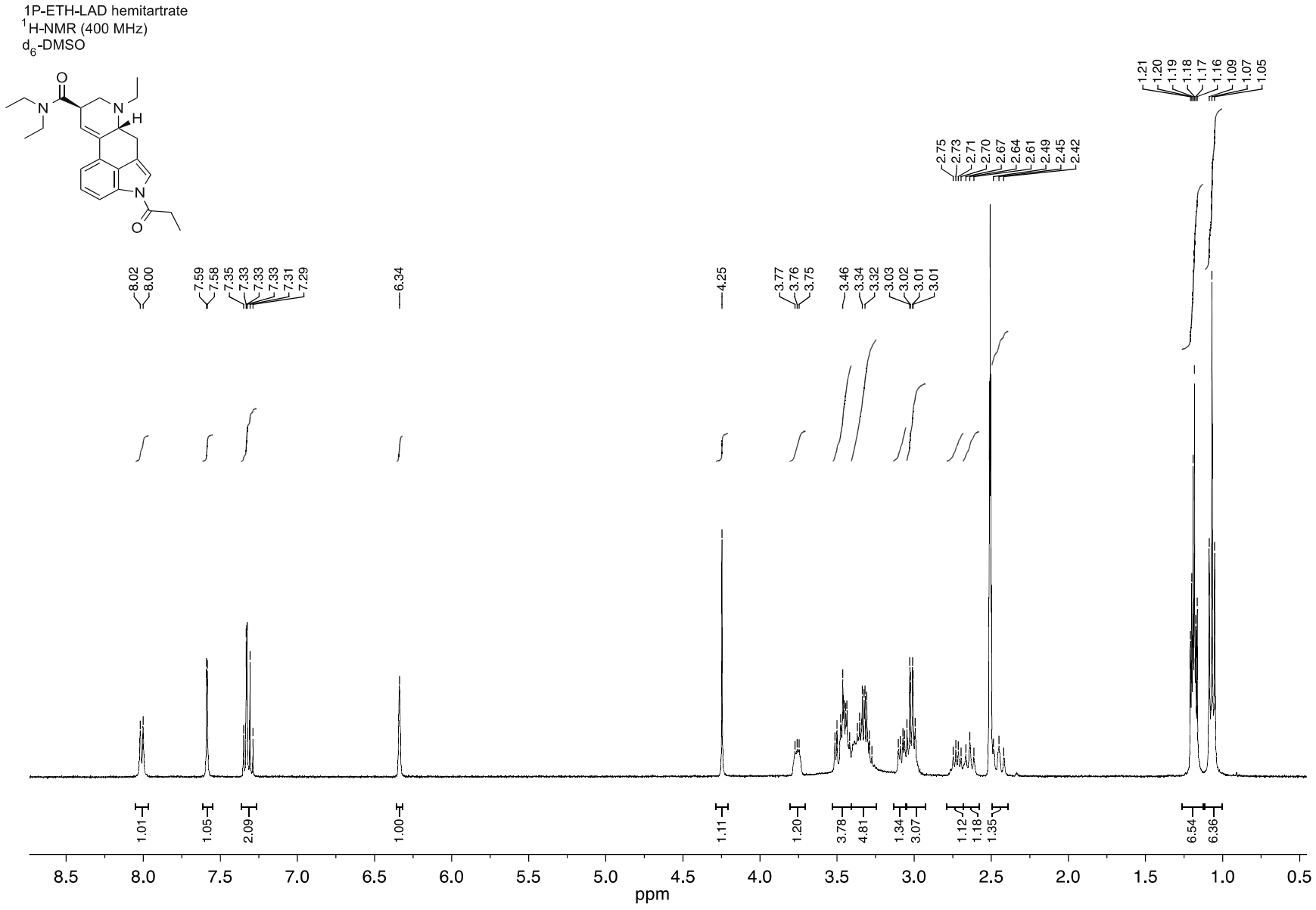
TA = Tartaric acid



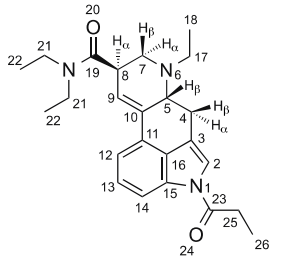
TA = Tartaric acid

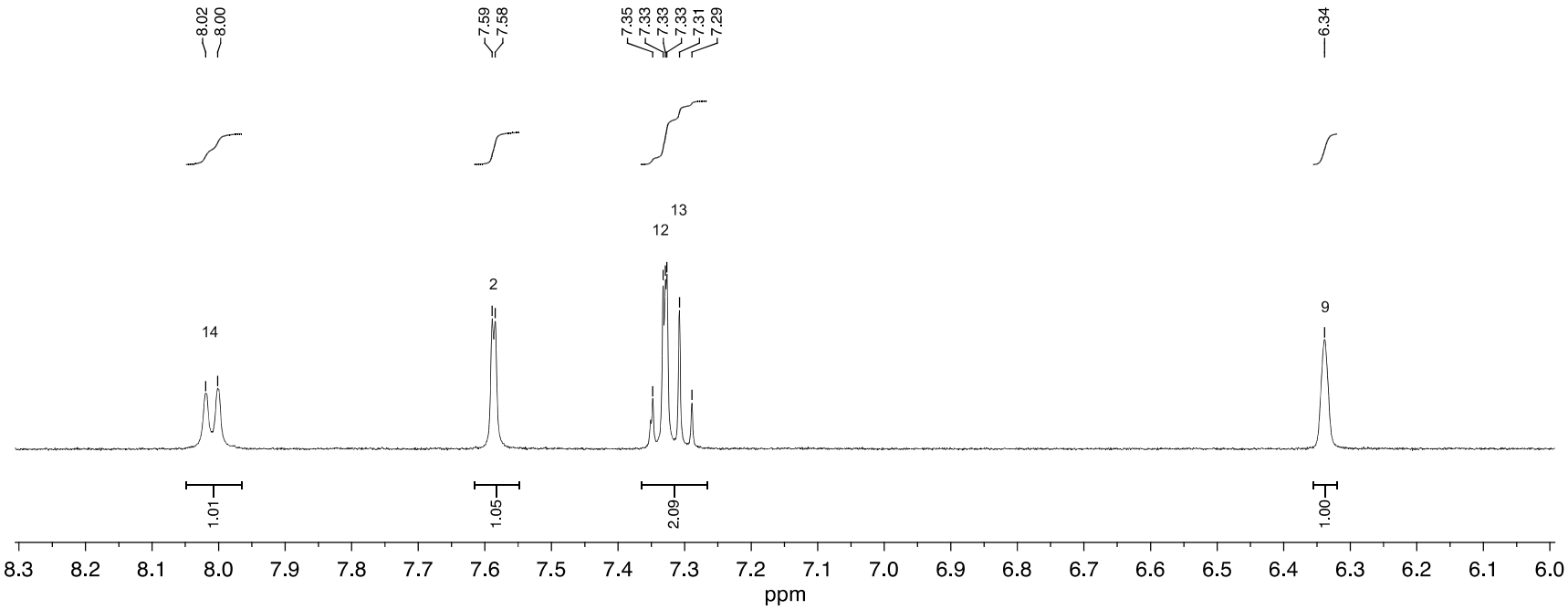

ETH-LAD hemitartrate
HMBC
d6-DMSO
4β
21
5β
21
7α
17
17
7β
4α
solvent
18
22
22
4
21 + 8 + solvent
21
17
7
5
TA
14
3
12
2
9
13
16
11
15
10
19
TA
ppm
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
ppm
TA = Tartaric acid

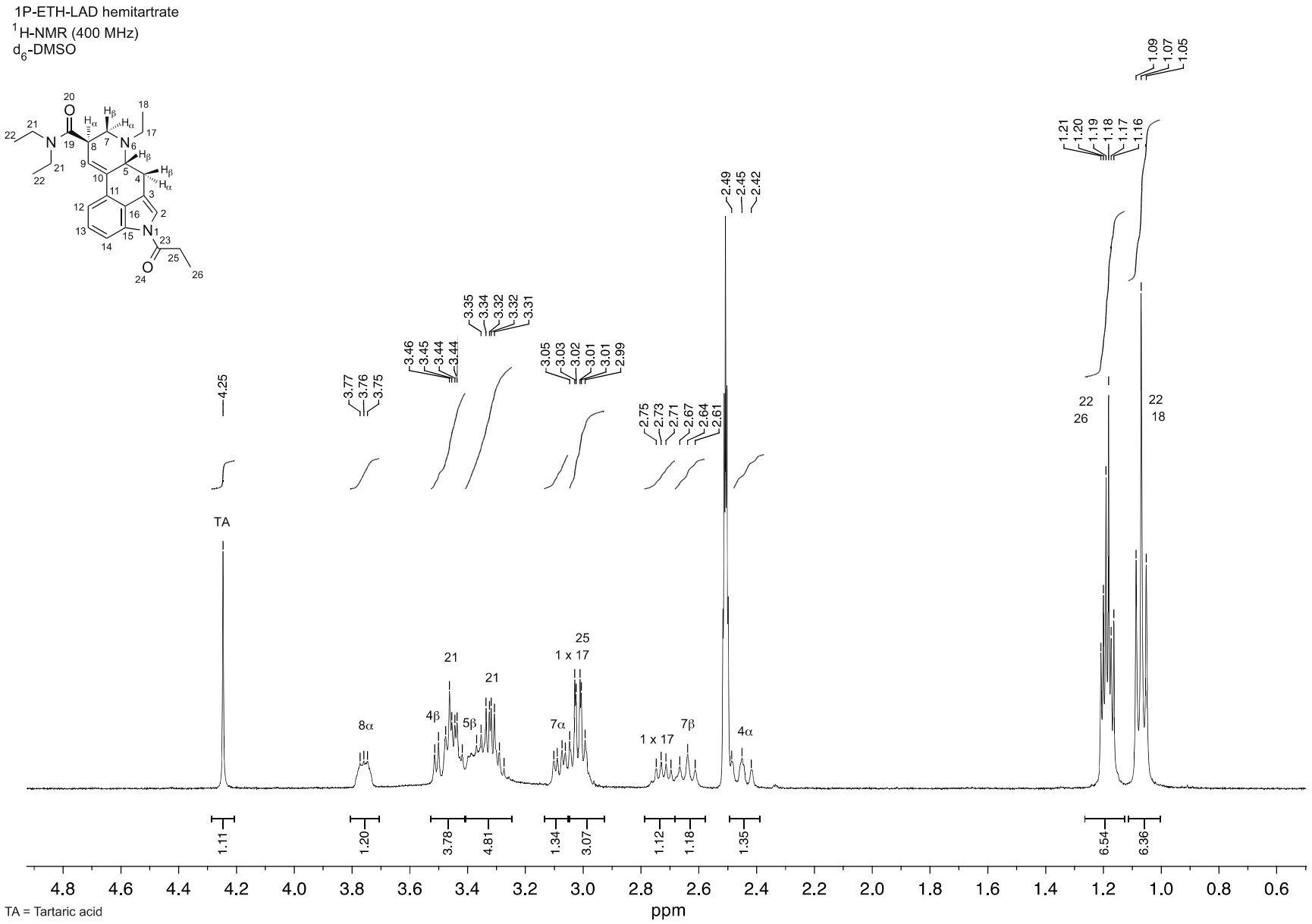

ETH-LAD hemitartrate
HMBC
d 6 -DMSO
22
18
22
4
21 + 8 + solvent
21
17
7
5
20
25
30
35
40
45
50
55
60
ppm
1.65
1.55
1.45
1.35
1.25
1.15
1.05
0.95
0.85
0.75
0.65
ppm

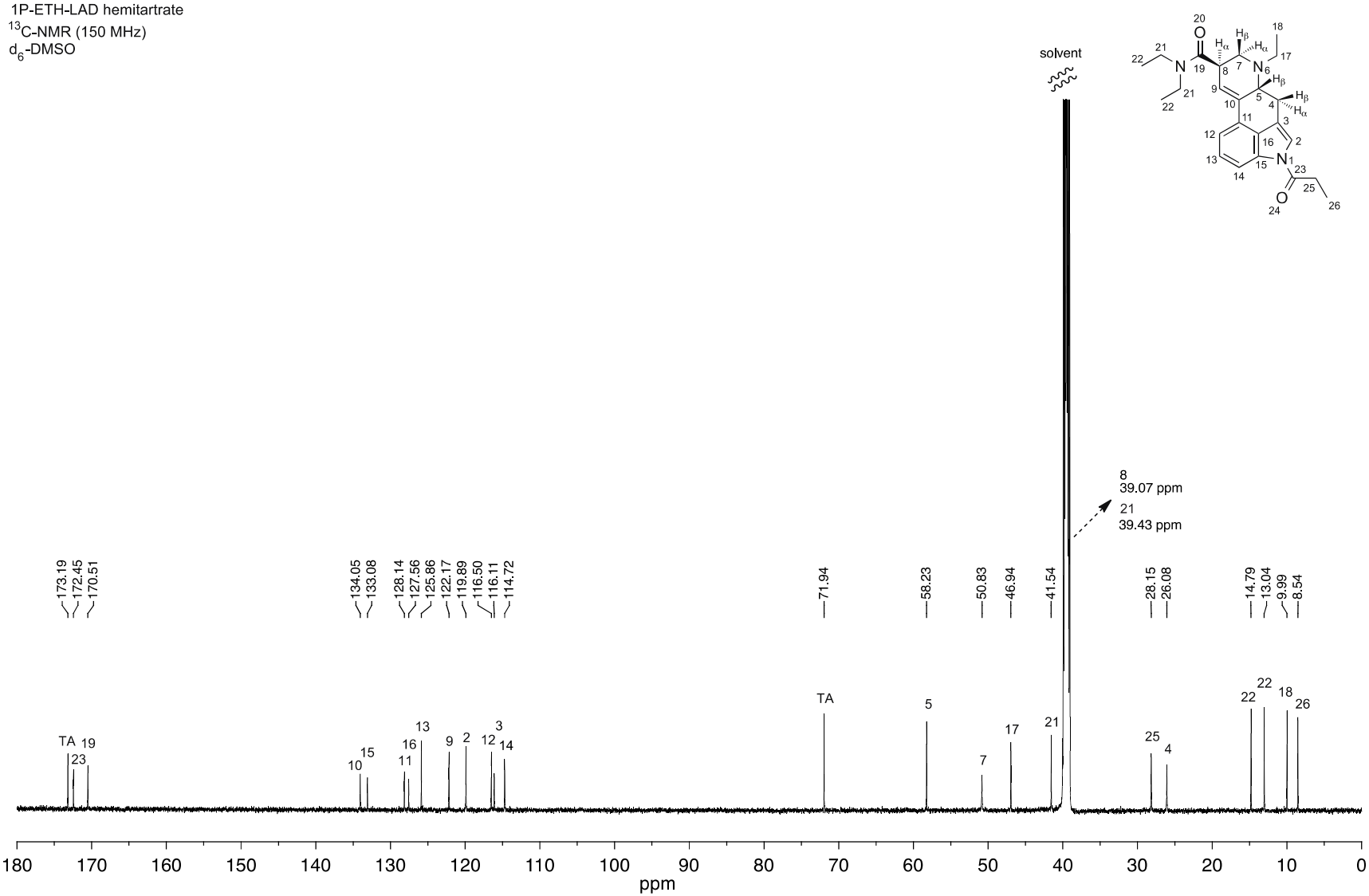

1P-ETH-LAD hemitartrate
1H-NMR (400 MHz)
d6-DMSO
8.02
8.00
7.59
7.58
7.35
7.33
7.33
7.33
7.31
7.29
6.34
4.25
3.77
3.76
3.75
3.46
3.34
3.32
3.03
3.02
3.01
3.01
2.75
2.73
2.71
2.70
2.67
2.64
2.61
2.49
2.45
2.42
1.21
1.20
1.19
1.18
1.17
1.16
1.09
1.07
1.05
1.01
1.05
2.09
1.00
1.11
1.20
3.78
4.81
1.34
3.07
1.12
1.18
1.35
6.54
6.36
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

1P-ETH-LAD hemitartrate
^{1}H-NMR (400 MHz)
d_6-DMSO

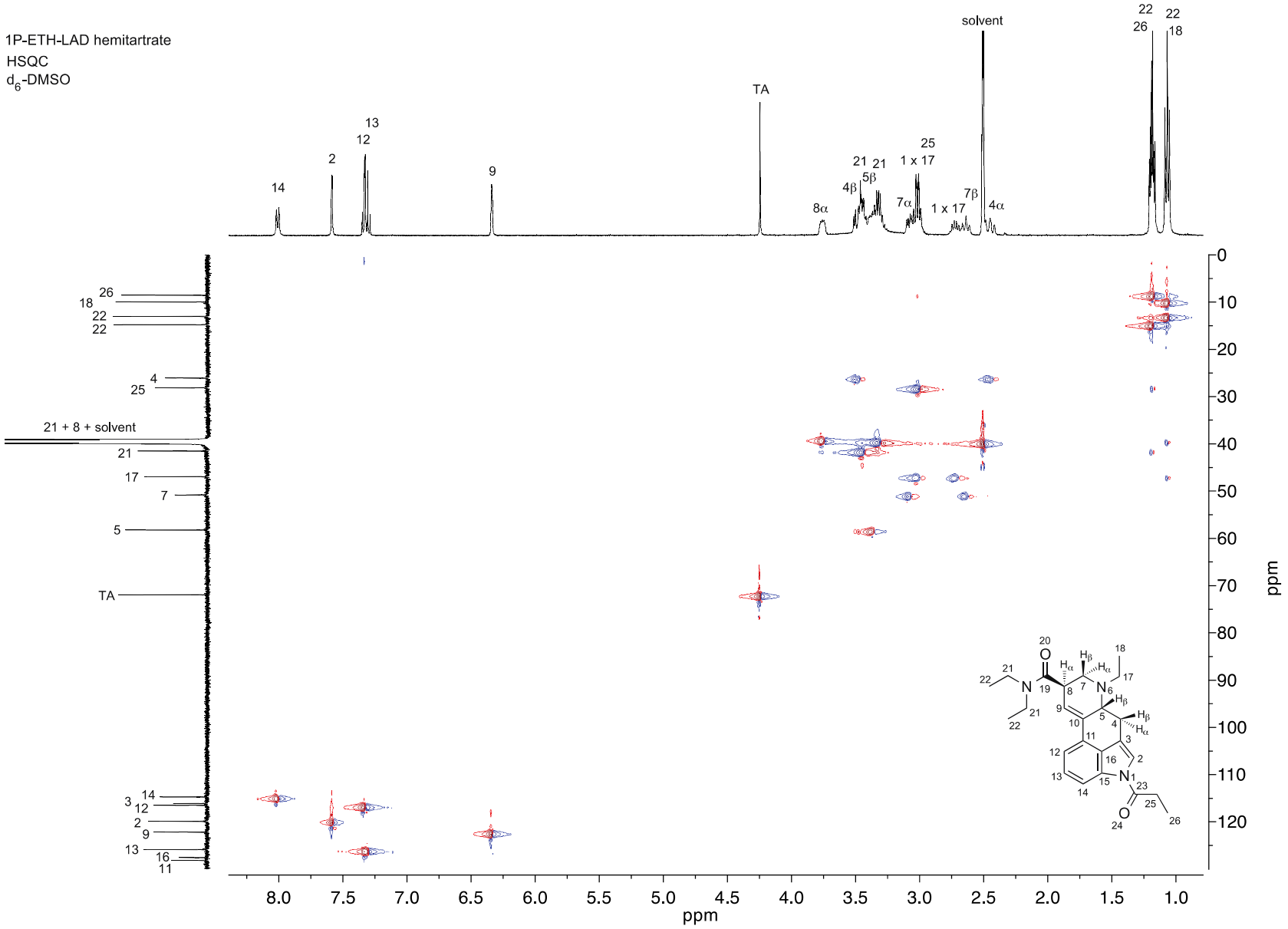


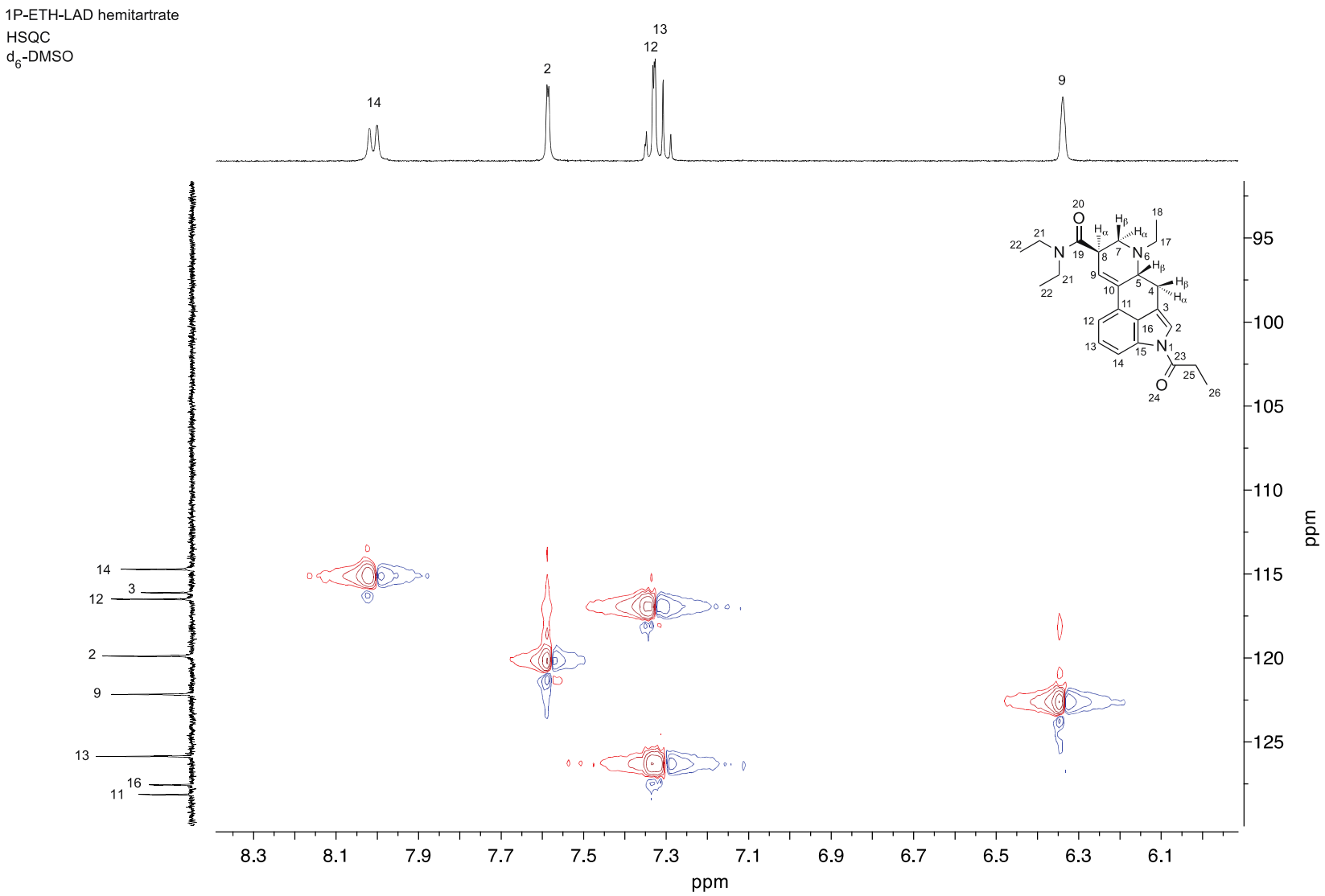


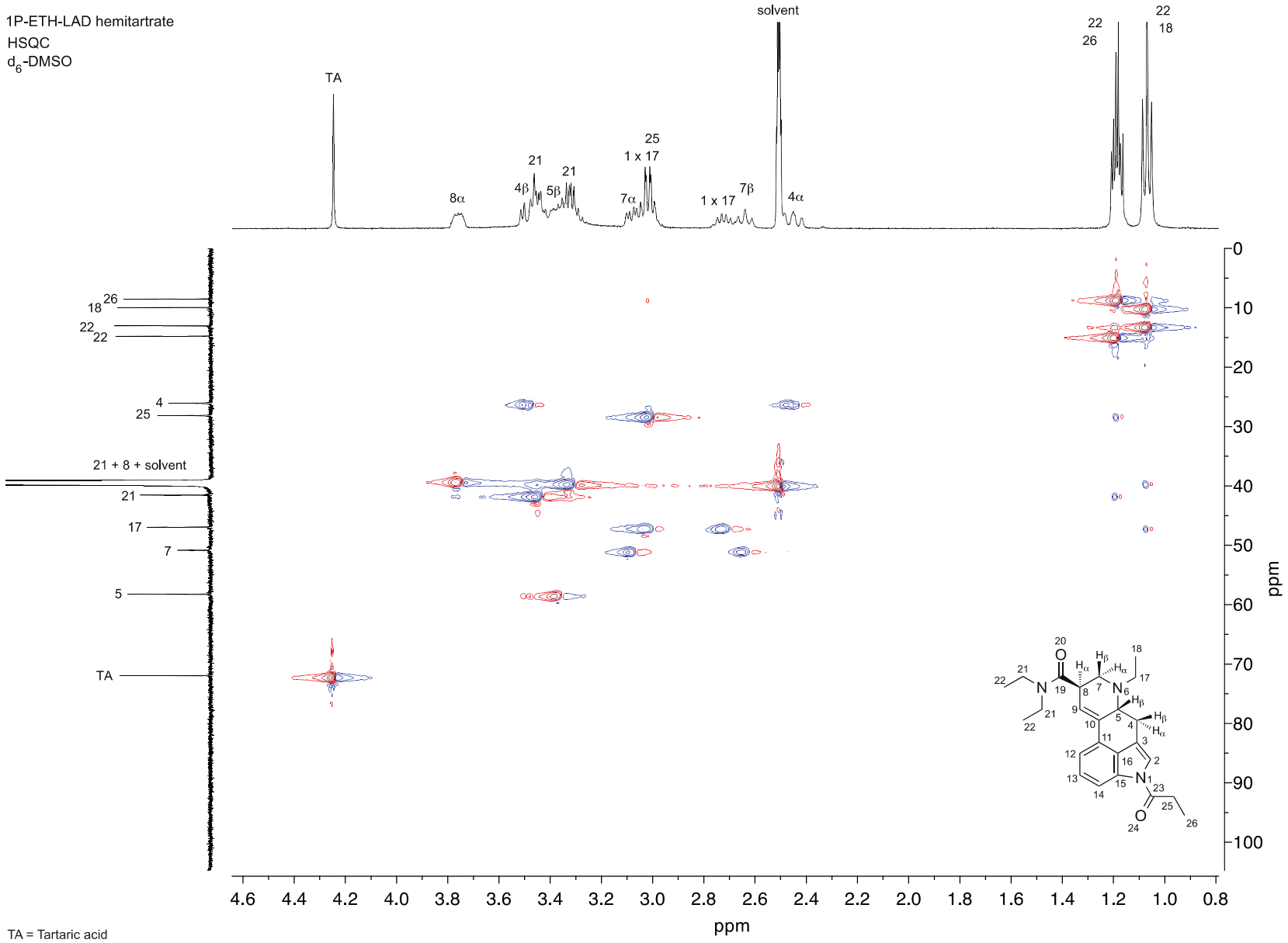

1P-ETH-LAD hemitartrate
1H-NMR (400 MHz)
d6-DMSO
TA
4.25
3.77
3.76
3.75
3.46
3.45
3.44
3.44
3.35
3.34
3.32
3.32
3.31
3.05
3.03
3.02
3.01
3.01
2.99
2.75
2.73
2.71
2.67
2.64
2.61
2.49
2.45
2.42
1.21
1.20
1.19
1.18
1.17
1.16
1.09
1.07
1.05
8α
4β
5β
21
21
21
7α
25
1 x 17
1 x 17
7β
4α
22
26
22
18
1.11
1.20
3.78
4.81
1.34
3.07
1.12
1.18
1.35
6.54
6.36
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
ppm
TA = Tartaric acid

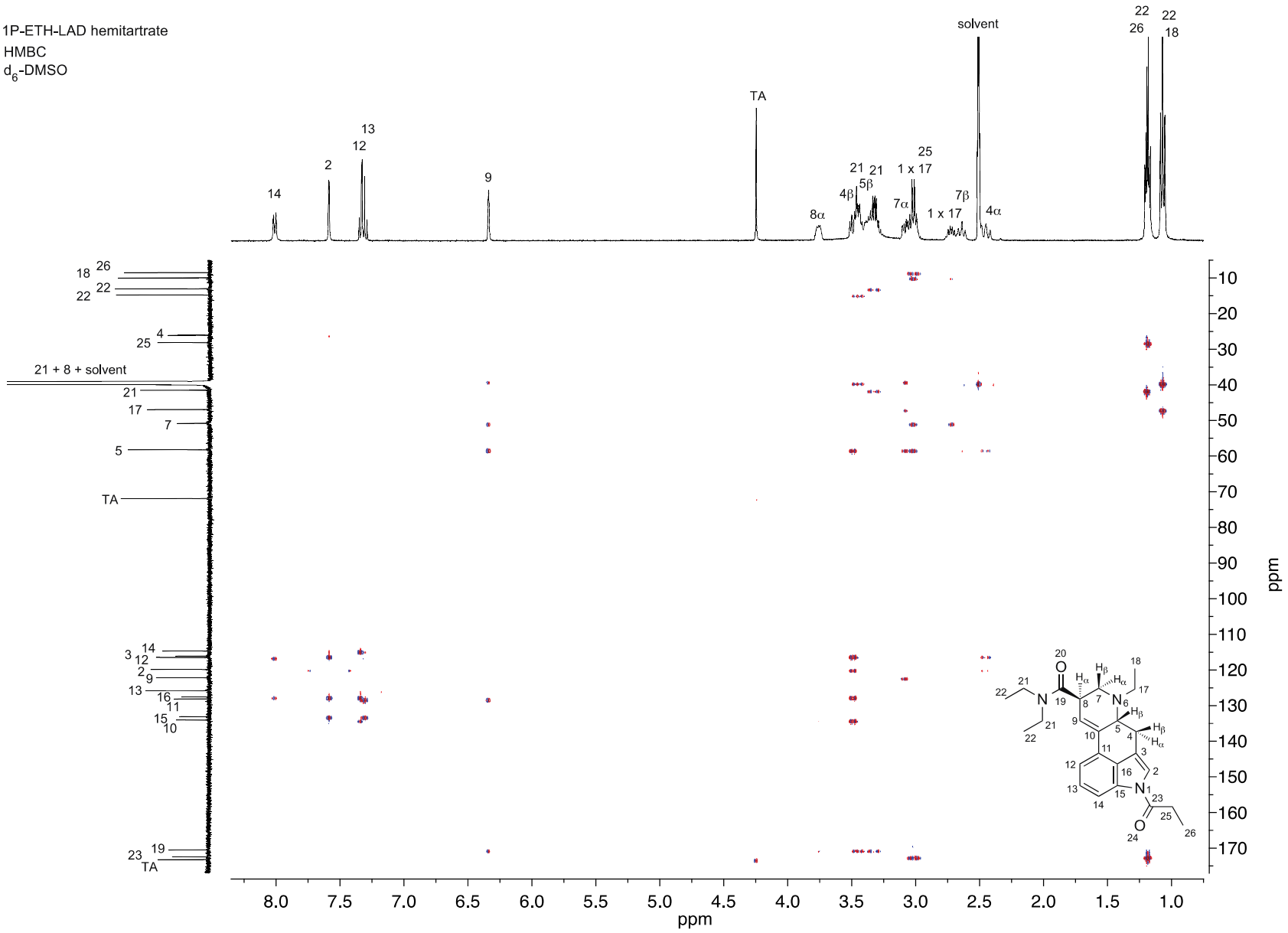


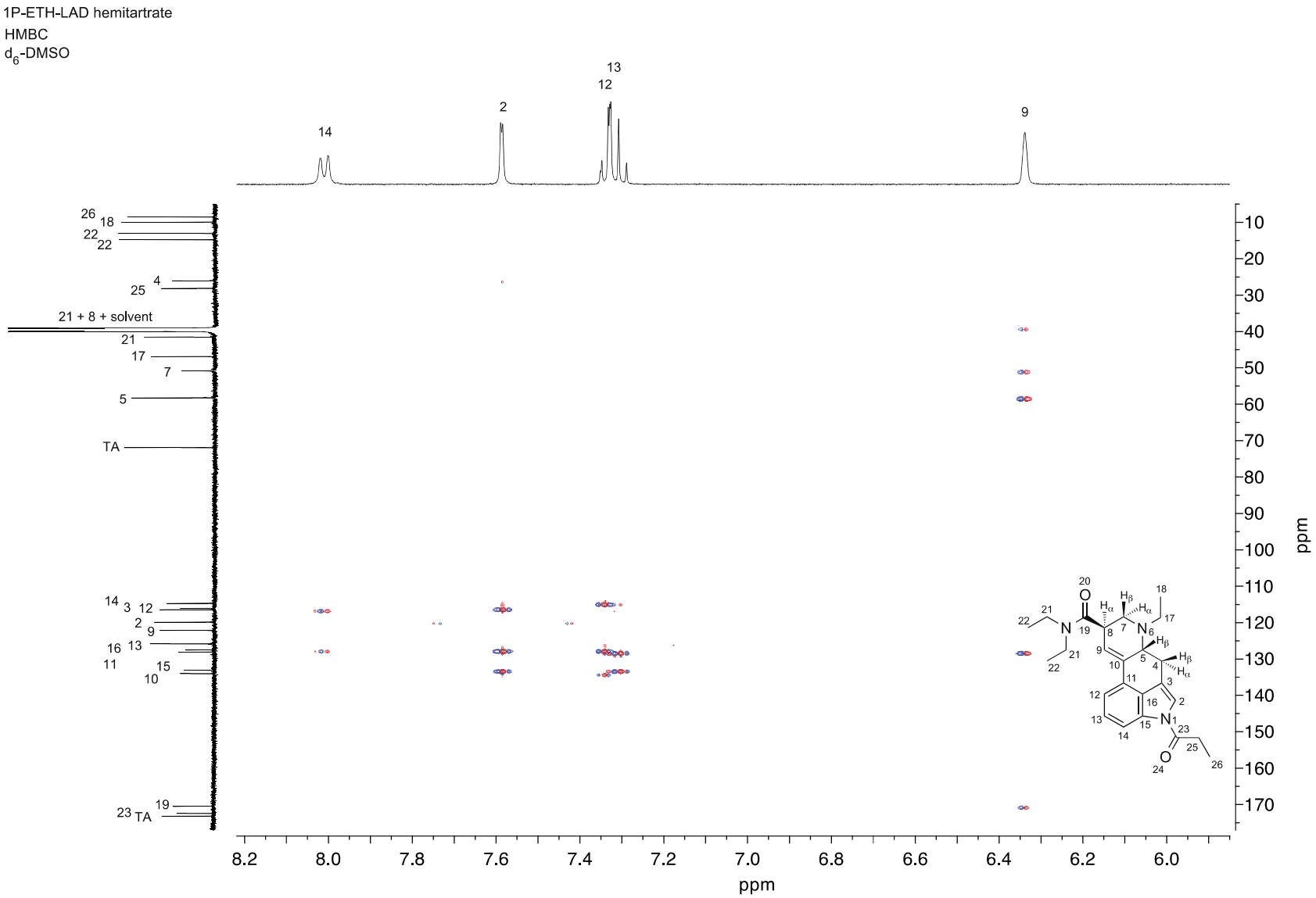
TA = Tartaric acid


1P-ETH-LAD hemitartrate
HSQC
d6-DMSO
14
2
13
12
9
TA
8α
4β
21
5β
21
7α
25
1 x 17
1 x 17
7β
solvent
4α
22
26
22
18
26
18
22
22
4
25
21 + 8 + solvent
21
17
7
5
TA
3
14
12
2
9
13
16
11
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
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
ppm

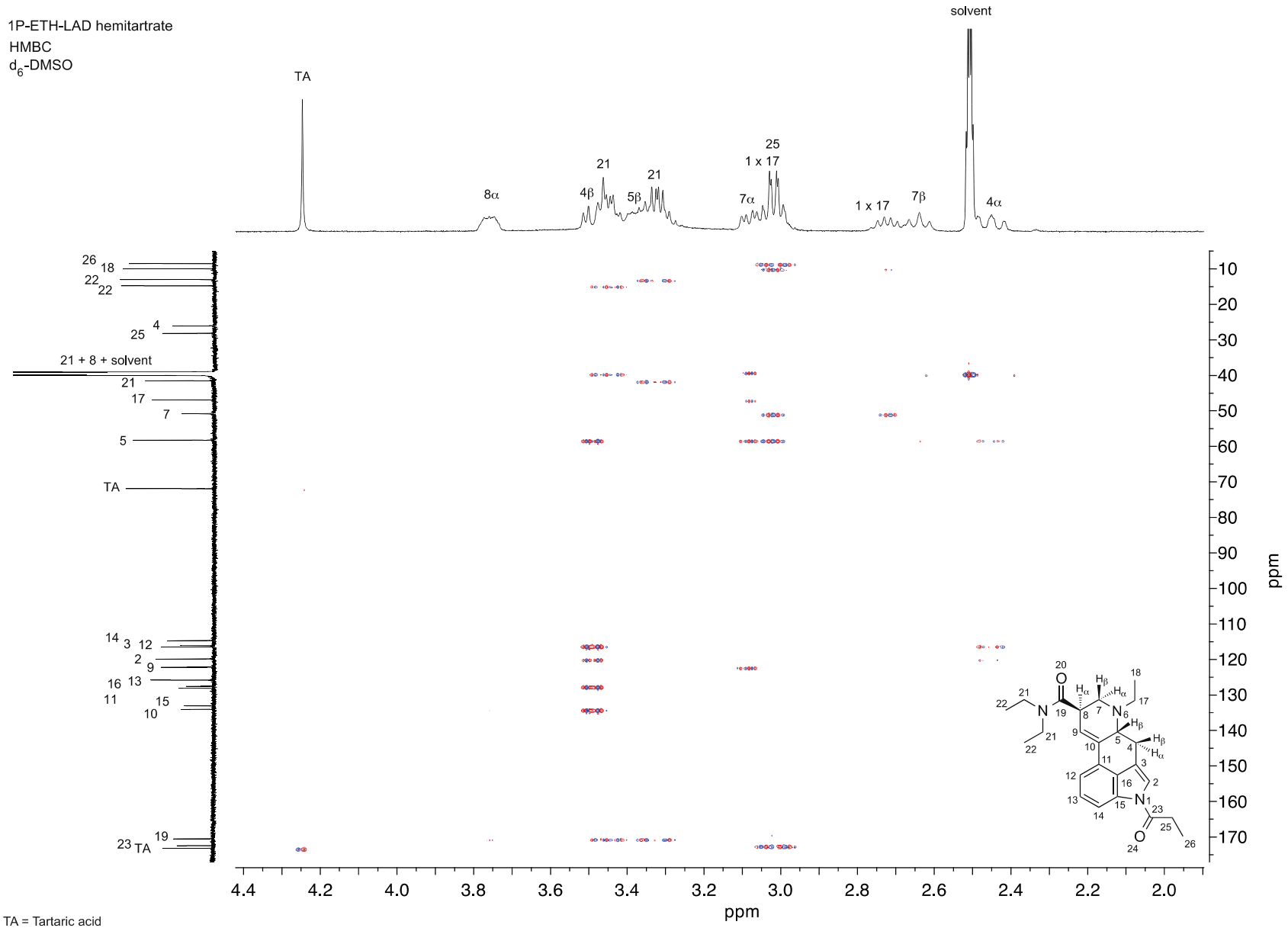

1P-ETH-LAD hemitartrate
HSQC
d6-DMSO
14
2
13
12
9
14
3
12
2
9
13
16
11
8.3
8.1
7.9
7.7
7.5
7.3
7.1
6.9
6.7
6.5
6.3
6.1
ppm
95
100
105
110
115
120
125
ppm


1P-ETH-LAD hemitartrate
HSQC
d6-DMSO
TA
8α
21
4β
5β
21
7α
25
1 x 17
1 x 17
7β
solvent
4α
22
26
22
18
18
26
22
22
4
25
21 + 8 + solvent
21
17
7
5
TA
ppm
ppm
TA = Tartaric acid

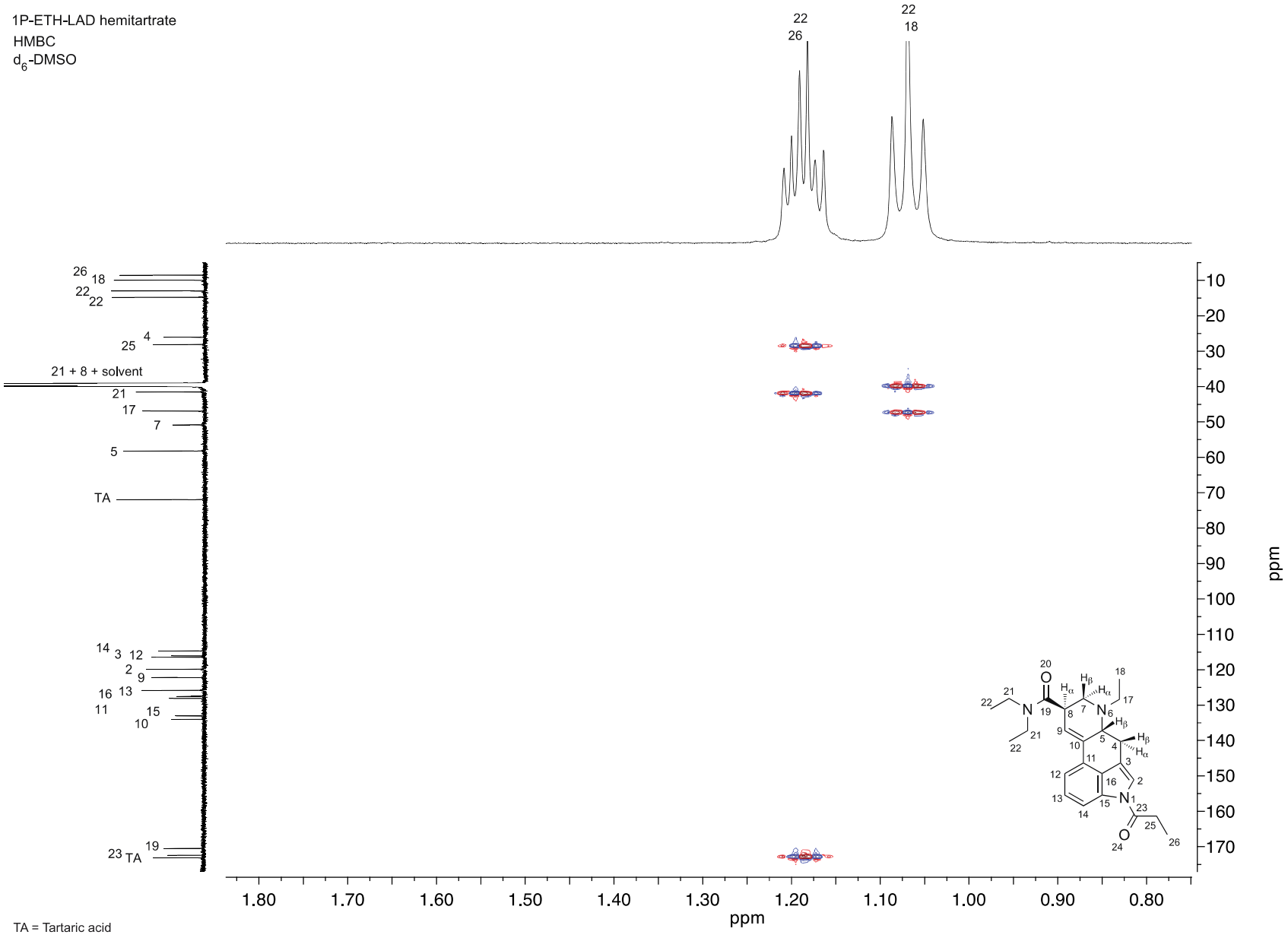

1P-ETH-LAD hemitartrate
HMBC
d6-DMSO
TA
solvent
21 + 8 + solvent
ppm



TA = Tartaric acid



TA = Tartaric acid


1P-ETH-LAD hemitartrate
HMBC
d6-DMSO
22
26
22
18
26
18
22
22
25
4
21 + 8 + solvent
21
17
7
5
TA
14
3
12
2
9
16
13
11
15
10
23
TA
19
ppm
1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.90
0.80
ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
TA = Tartaric acid