Supporting Information

Design and synthesis of soluble and cell-permeable PI3Kδ inhibitors for long-acting inhaled administration

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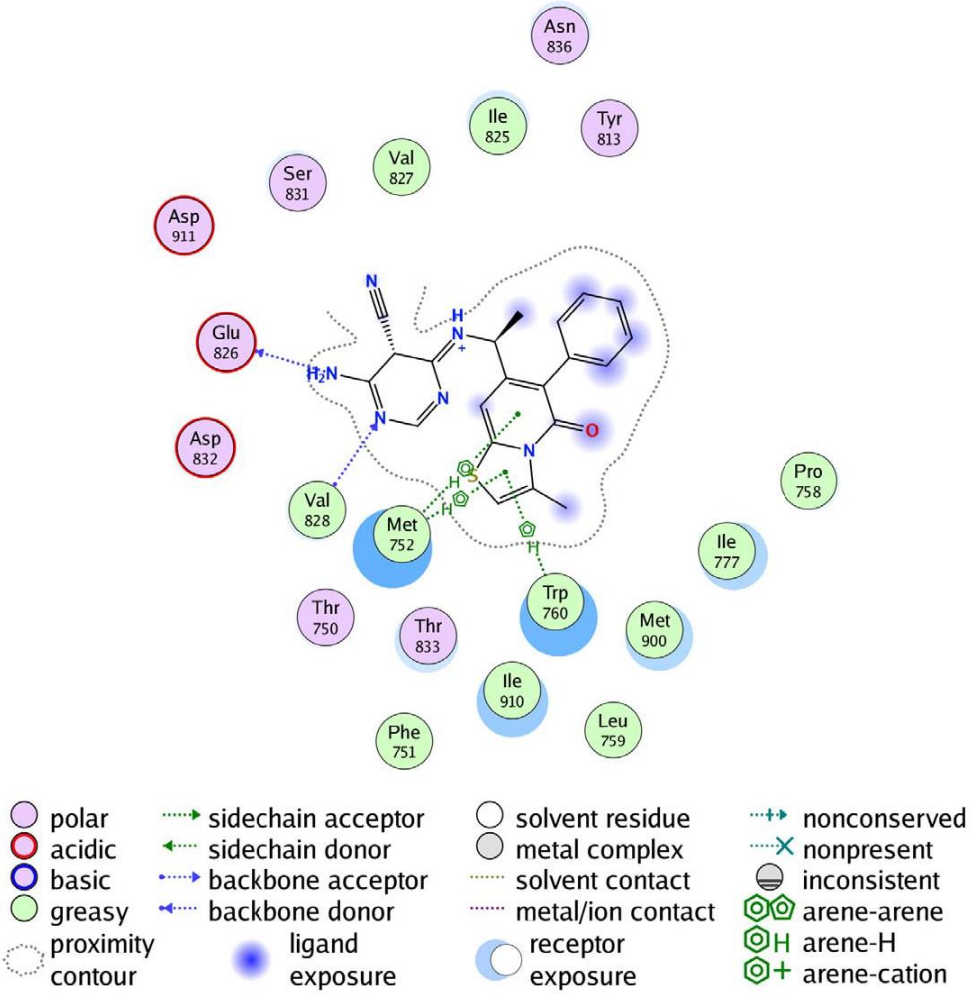
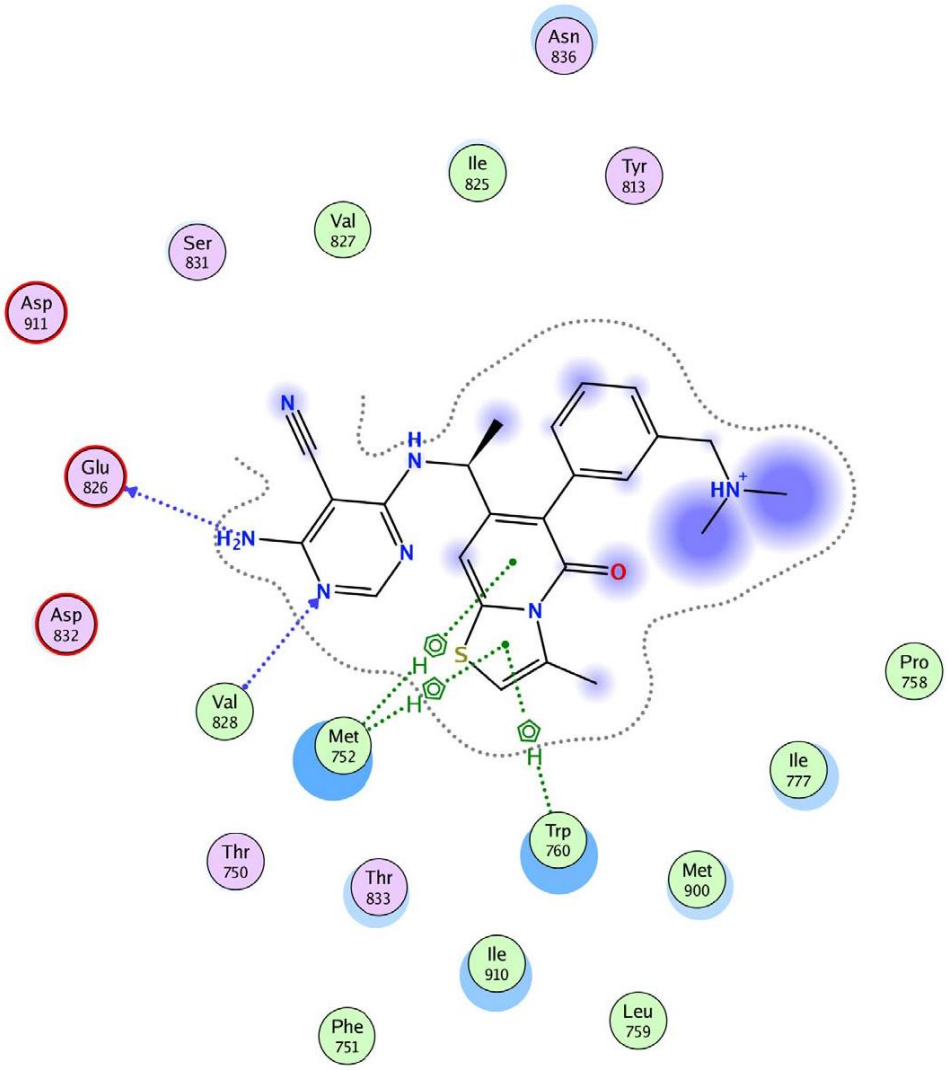
Ligand diagram for 13



Ligand diagram for 15c

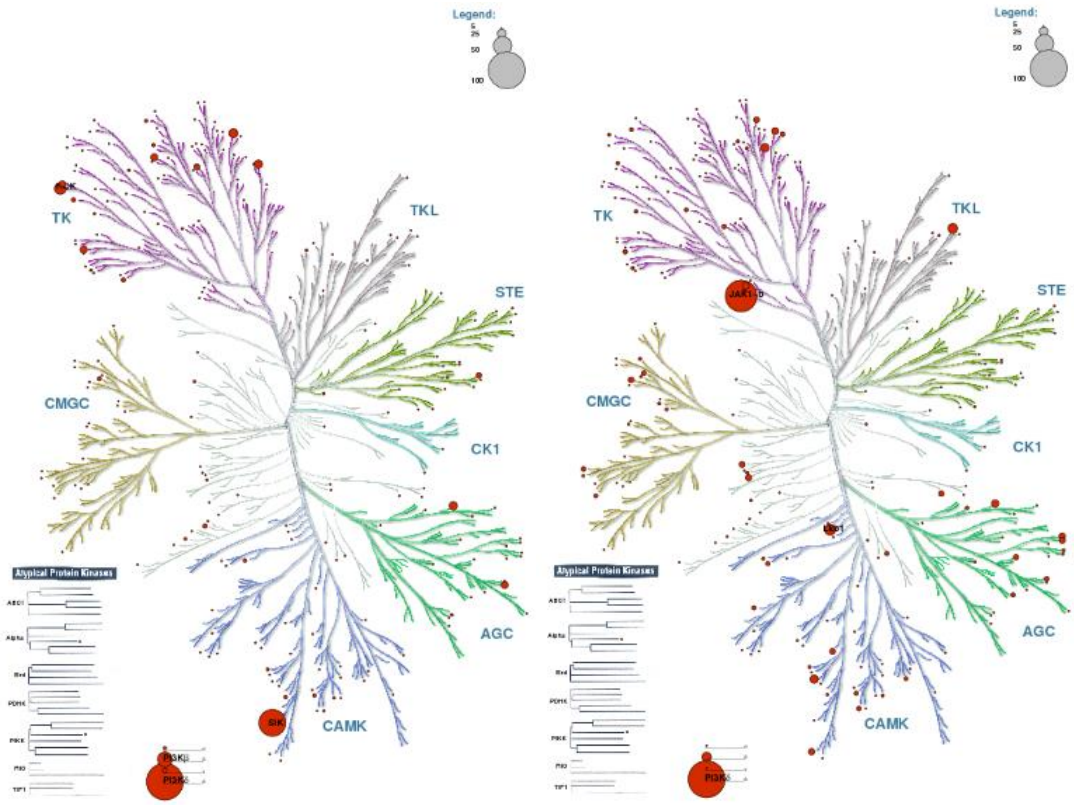


Kinase selectivity for 20b & **20f**

Both of these compounds were screened against a panel of 345 kinases; **20b** was active against one other kinase at >50% at 1 μM (SIK1, 73%, IC_{50} >1 μM on retest) whilst **20f** hit 1 enzyme (JAK1) with >50% inhibition at 1 μM (86%, IC_{50} > 30 μM on retest) (figure **S1**).

Figure S1. Kinase selectivity of **20b** and **20f**

20b **20f**



Kinome map produced using KinomeRender v1.1
Kinome illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)

Analytical HPLC conditions

System 1
XBridge C18 3.5 μm; 50 mm × 4.6 mm; Mobile phase A: 5mM NH_4HCO_3, B:Acetonitrile; gradient 10 – 95% B over 1.9 min then 95% B for 0.7 min.

System 2
Shim-pack XR-ODS 2.2 μm; 50 mm × 3.0 mm; Mobile phase A: Water/0.05% TFA, B:Acetonitrile/0.05% TFA 5 – 100% B over 1.2 min then 100% B for 1.0 min

System 3
Shim-pack XR-ODS 2.2 μm; 50 mm × 3.0 mm; Mobile phase A: Water/0.05% TFA, B:Acetonitrile/0.05% TFA 5 – 100% B over 4.2 min then 100% B for 1.0 min

System 4
Shim-pack XR-ODS 2.2 μm; 50 mm × 3.0 mm; Mobile phase A: Water/0.05% TFA, B:Acetonitrile/0.05% TFA 5 – 100% B over 2.2 min then 100% B for 1.0 min

System 5
ACQUITY UPLC CSH C18 1.7μm; 50 mm × 2.1 mm;Mobile phaseA:water/0.1% formic acid, B:Acetonitrile/0.05% formic acid 5 – 100% B over 1.1 min then 100% B for 0.5 min.

System 6
ACQUITY UPLC BEH C18 1.7μm; 50 mm × 2.1 mm;Mobile phaseA:water/0.1% formic acid, B:Acetonitrile/0.05% formic acid 5 – 95% B over 2.0 min then 95% B for 0.6 min.

System 7

Phenomenex kinetex XB-C18 1.7μm; 50 mm × 2.1 mm, Mobile phase A: Water/0.05% TFA, B:Acetonitrile/0.05% TFA 0.7 mL/min 5 – 95% B over 2.0 min then 95% B for 1.1 min.

System 8

Waters HSS C18 1.8μm; 50 mm × 2.1 mm, Mobile phase A: Water/0.05% TFA, B:Acetonitrile/0.05% TFA 0.7 mL/min 5 – 60% B over 4.0 min then 60 - 95% B over 0.5 min then 95% B for 0.7 min.

System 9
Shim-pack XR-ODS 2.2 μm; 50 mm × 3.0 mm; Mobile phase A: Water/0.05% TFA, B:Acetonitrile/0.05% TFA 5 – 60% B over 5 min then 60% B for 1.3 min

System 10
Shim-pack XR-ODS 2.2 μm; 50 mm × 3.0 mm; Mobile phase A: Water/0.05% TFA, B:Acetonitrile/0.05% TFA 5 – 95% B over 2.2 min then 95% B for 1.0 min

System 11
Geminin NX C18 3 μm; 50 mm × 3.0 mm; Mobile phase A: 6.5mM NH_4HCO_3, B:Acetonitrile; gradient 10 – 70% B over 3.5 min then 70% B for 1.3 min.

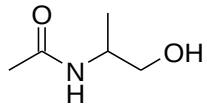
HPLCMS results

Compound	HPLC System	Retention time (min)	Ion and mass observed	HPLC purity
3	System 1	0.85	232.9 $[M-H]^-$	
4	System 2	1.37	230/232 (Cl pattern) $[M+H]^+$	
5	System 2	1.51	308/310/312 (BrCl pattern) $[M+H]^+$	
6	System 2	1.25	324/326/328 (BrCl pattern) $[M+H]^+$	
7	System 3	2.92	308 $[M+H]^+$	
8	System 2	1.68	315 $[M+H]^+$	
9	System 3	1.26	287 $[M+H]^+$	
10	System 4	1.96	421 $[M+H]^+$	
11	System 6	1.04	419 $[M+H]^+$	
12	System 7	0.70	285 $[M+H]^+$	
13	System 7	1.16	403 $[M+H]^+$	98.7
14	System 5	1.04	405 $[M+H]^+$	
15a	System 4	0.97	432 $[M+H]^+$	99.0
15b	System 4	1.26	446 $[M+H]^+$	99.7
15c	System 6	1.00	460 $[M+H]^+$	98.3
15d	System 4	1.30	490 $[M+H]^+$	98.0

15e	System 4	1.39	502 $[M+H]^+$	99.2
15f	System 4	1.29	472 $[M+H]^+$	98.0
15g	System 4	1.67	608 $[M+H]^+$	99.0
16	System 4	1.17	431 $[M+H]^+$	98.0
17	System 4	1.99	490 $[M+H]^+$	99.1
18	System 9	2.41	474 $[M+H]^+$	99.4
19	System 4	1.50	596 $[M+H]^+$	98.0
20a	System 10	1.19	545 $[M+H]^+$	97.6
20b	System 10	1.19	543 $[M+H]^+$	98.0
20c	System 12	2.25	599 $[M+H]^+$	99.6
20d	System 4	1.32	625 $[M+H]^+$	99.0
20e	System 13	1.89	614 $[M+H]^+$	96.1
20f	System 11	2.02	585 $[M+H]^+$	96.0
20g	System 4	1.26	634 $[M+H]^+$	99.5
20h	System 11	1.62	573 $[M+H]^+$	99.0
20i	System 11	2.01	627/629 (Cl pattern) $[M+H]^+$	99.0
20j	System 4	1.25	601 $[M+H]^+$	98.0
20k	System 11	2.05	621 $[M+H]^+$	99.0
20l	System 11	2.74	615 $[M+H]^+$	99.0

Synthesis procedures for compounds 1 & 2

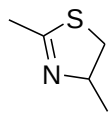
N-(1-hydroxypropan-2-yl)acetamide (1)



Three 20 L 4-necked round-bottom flasks were each charged with 2-aminopropan-1-ol (600 g, 8.0 mol) acetonitrile (12 L) and sodium carbonate (2544 g, 23.8 mol). The suspensions were cooled to 0 °C and acetyl chloride (624 g, 8.0 mol) was added dropwise to each. The resulting suspensions were stirred for 2 h at room temperature. The reaction mixtures were filtered, the filtrates were combined, dried (sodium sulfate), concentrated and chromatographed (silica gel, dichloromethane/methanol (30:1)) to give the title compound (2.31 kg, 82%).

LCMS (System 2) T_r 0.37 min; (ES, m/z): 178 $[M+H]^+$

2,4-dimethyl-4,5-dihydro-1,3-thiazole (2)

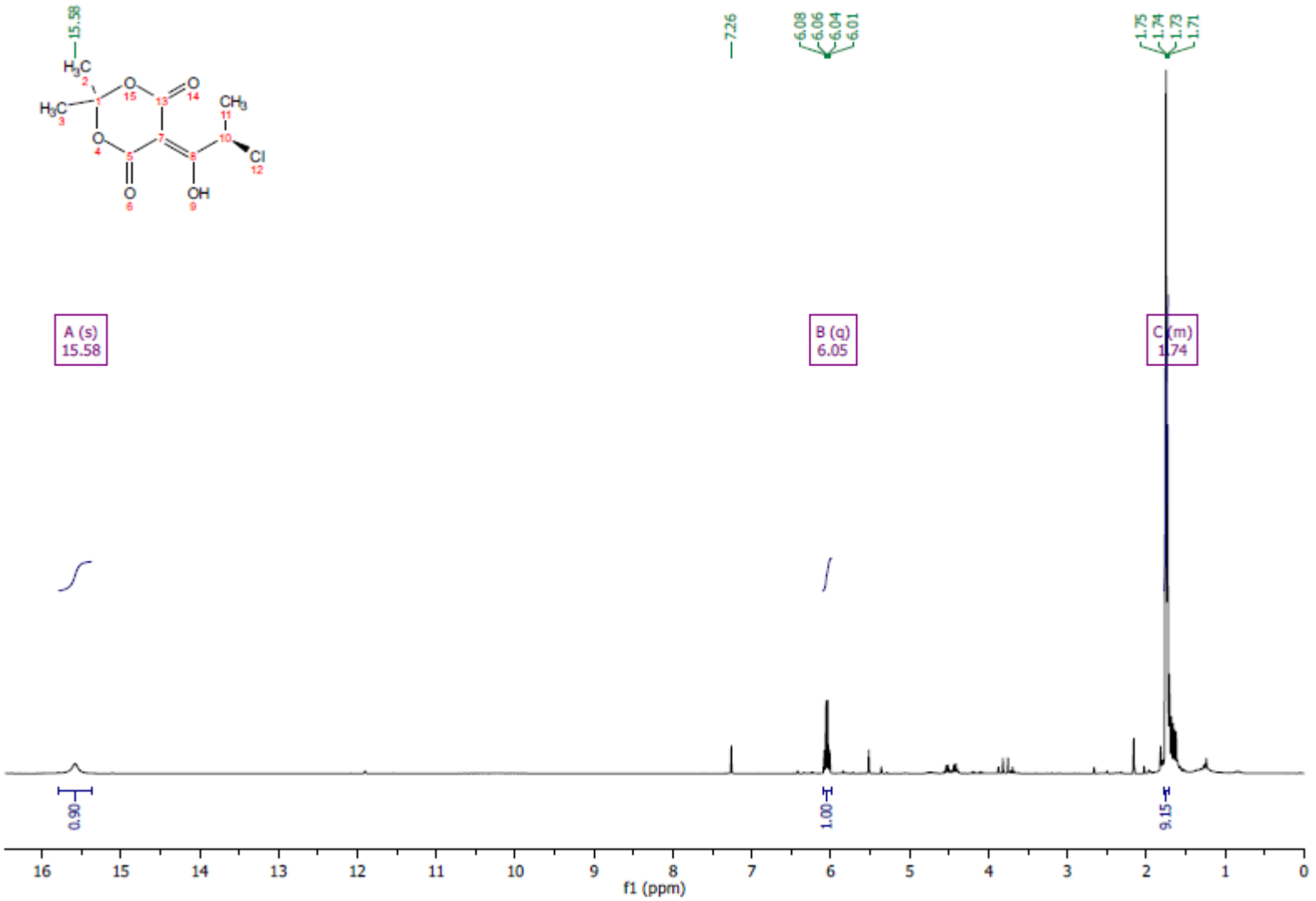


Two 20 L 4-necked round-bottom flasks were charged with N-(1-hydroxypropan-2-yl)acetamide (333 g, 2.84 mol), tetrahydrofuran (10 L) and Lawesson's reagent (1.38 kg, 3.41 mol). The solutions were heated to 65 °C for 3 h. The reaction mixtures were combined and the solvent was removed by distillation at atmospheric pressure. Saturated aqueous sodium bicarbonate solution was added until a pH of 8-9 was obtained. The resulting mixture was extracted with 3 × 15 L of dichloromethane and the organic layers were combined, dried ($MgSO_4$) and concentrated to give the title compound (300 g 46%) as a yellow oil.

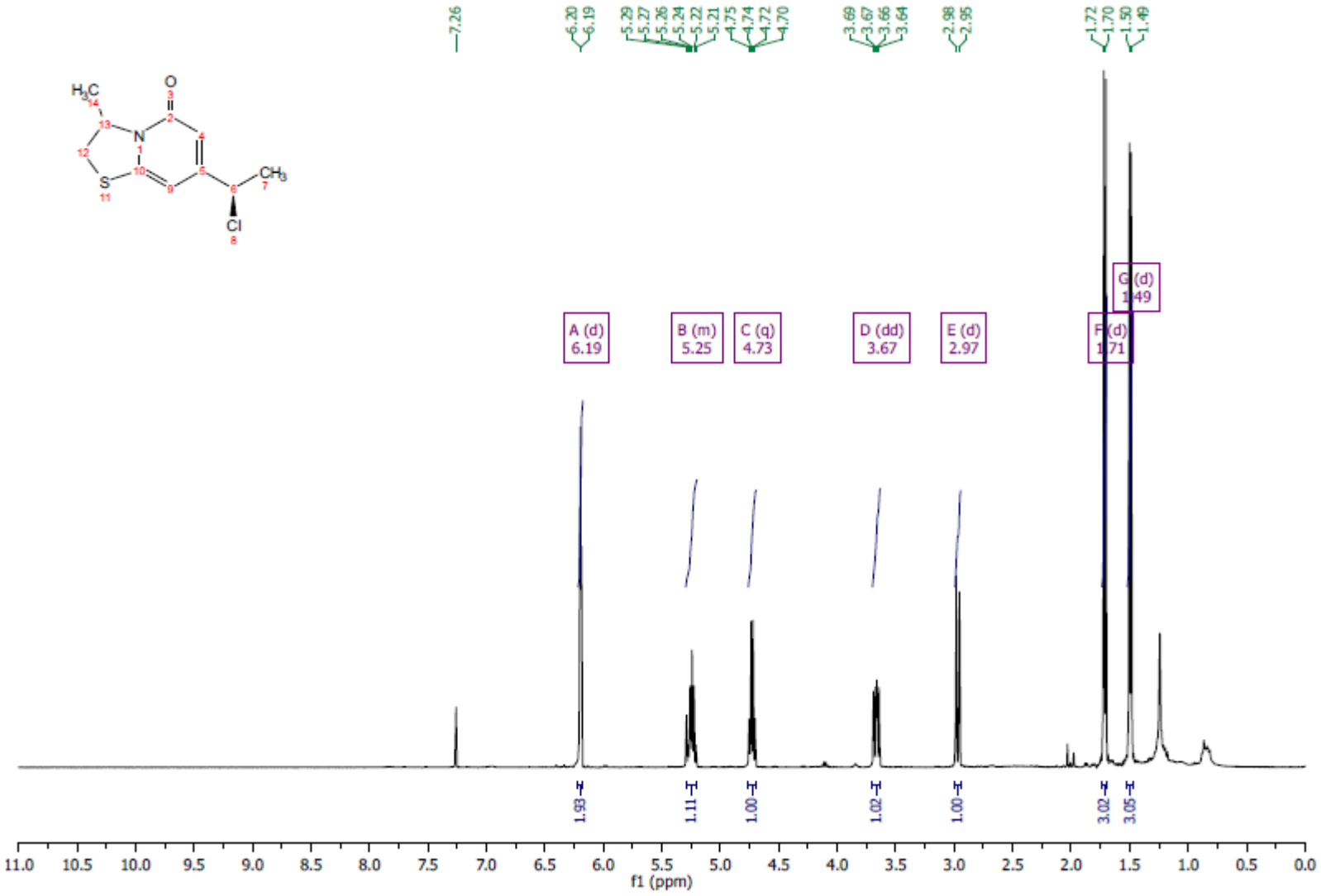
LCMS (System 2) T_r 0.40 min; (ES, m/z): 116 $[M+H]^+$

^{1}H NMR (400 MHz, CDCl3) δ 4.50 (m, 1H), 3.43 (dd, J = 8.3, 10.8 Hz, 1H), 2.93 (dd, J = 7.8, 10.8 Hz, 1H), 2.20 (d, J = 1.6 Hz, 3H), 1.35 (d, J = 6.7 Hz, 3H)

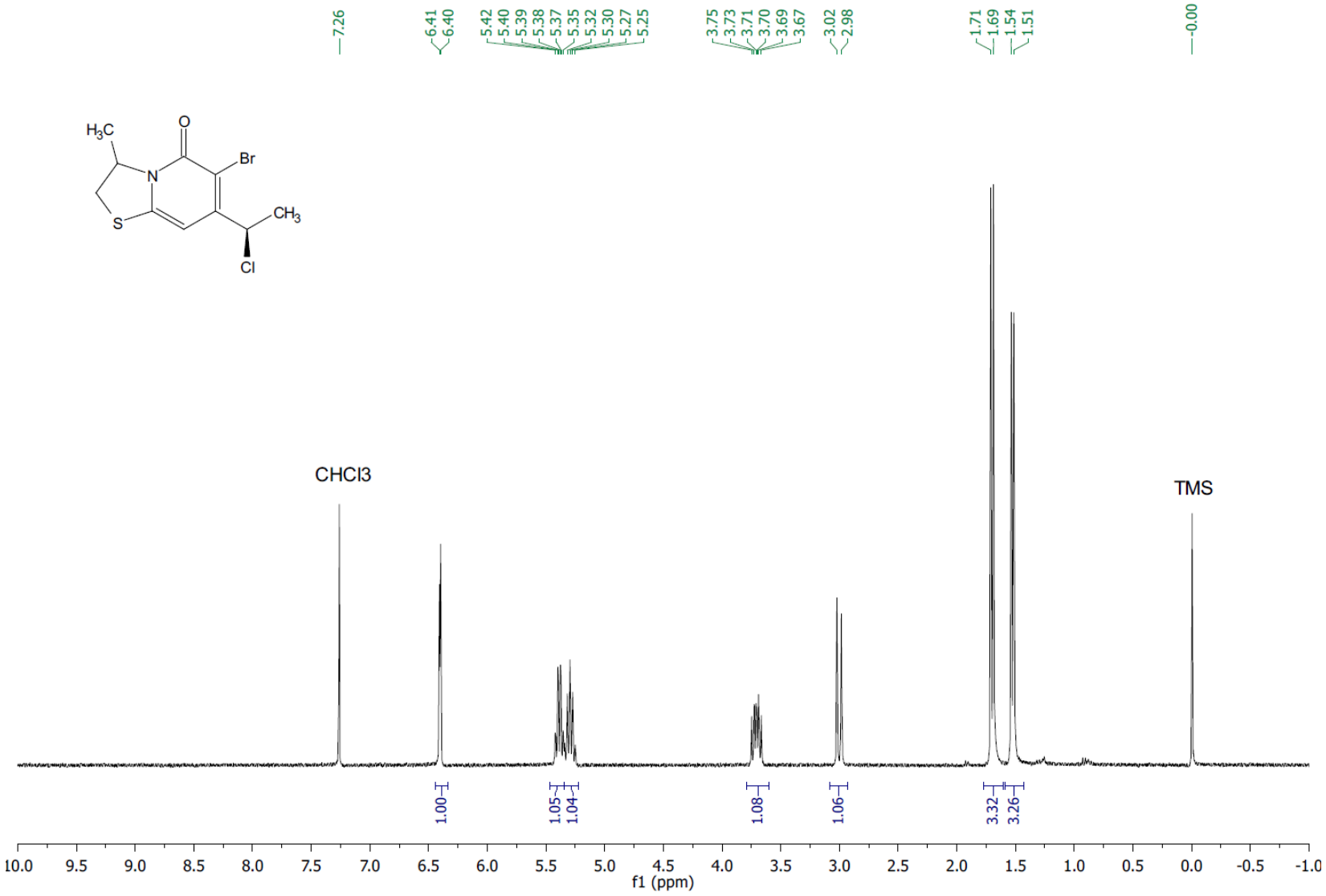
Compound 3


15.58
7.26
6.08
6.06
6.04
6.01
1.75
1.74
1.73
1.71
A (s)
15.58
B (q)
6.05
C (m)
1.74
0.90
1.00
9.15
f1 (ppm)

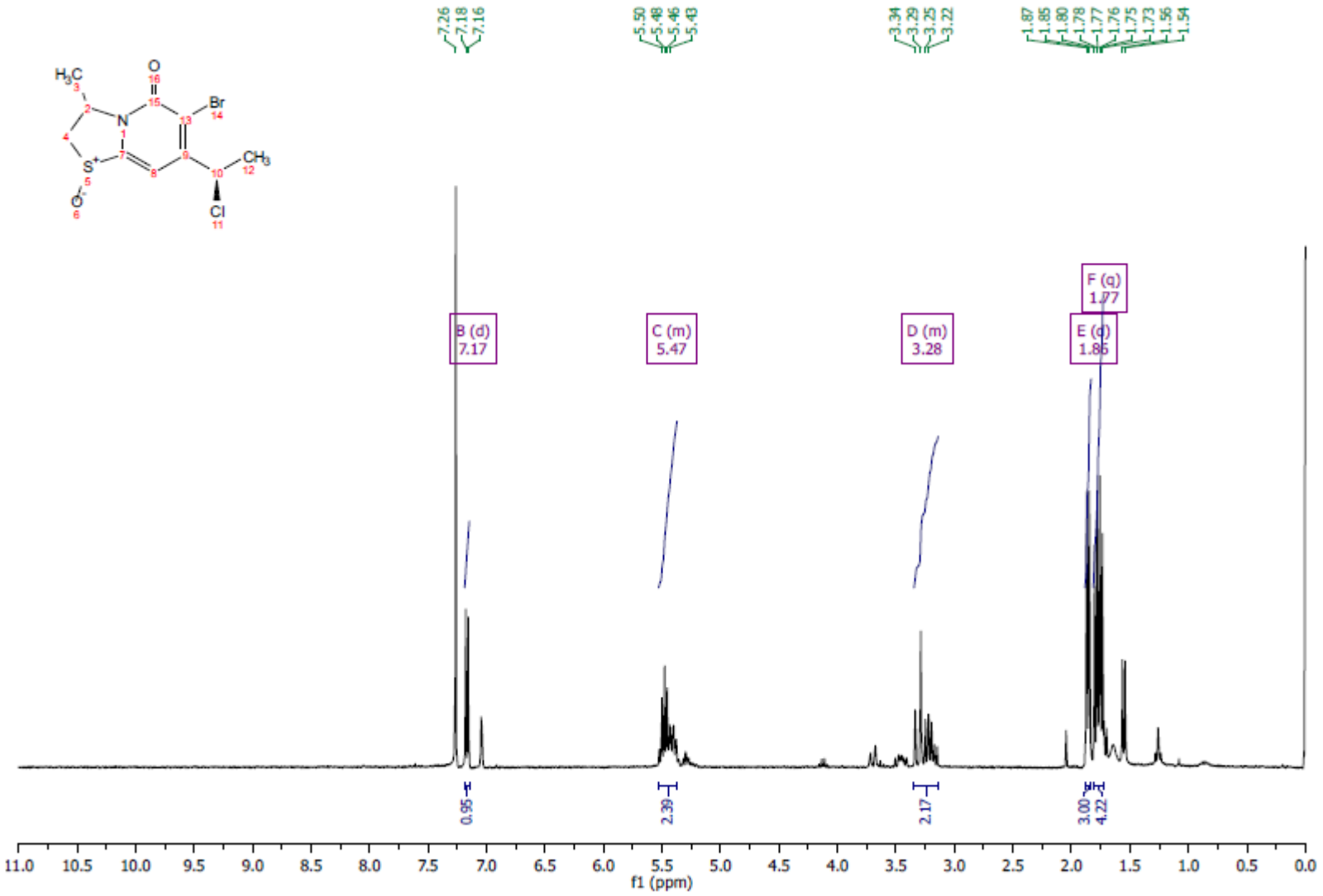
Compound 4


7.26
6.20
6.19
5.29
5.27
5.26
5.24
5.22
5.21
4.75
4.74
4.72
4.70
3.69
3.67
3.66
3.64
2.98
2.95
1.72
1.70
1.50
1.49
A (d) 6.19
B (m) 5.25
C (q) 4.73
D (dd) 3.67
E (d) 2.97
G (d) 1.49
1.93
1.11
1.00
1.02
1.00
3.02
3.05
f1 (ppm)

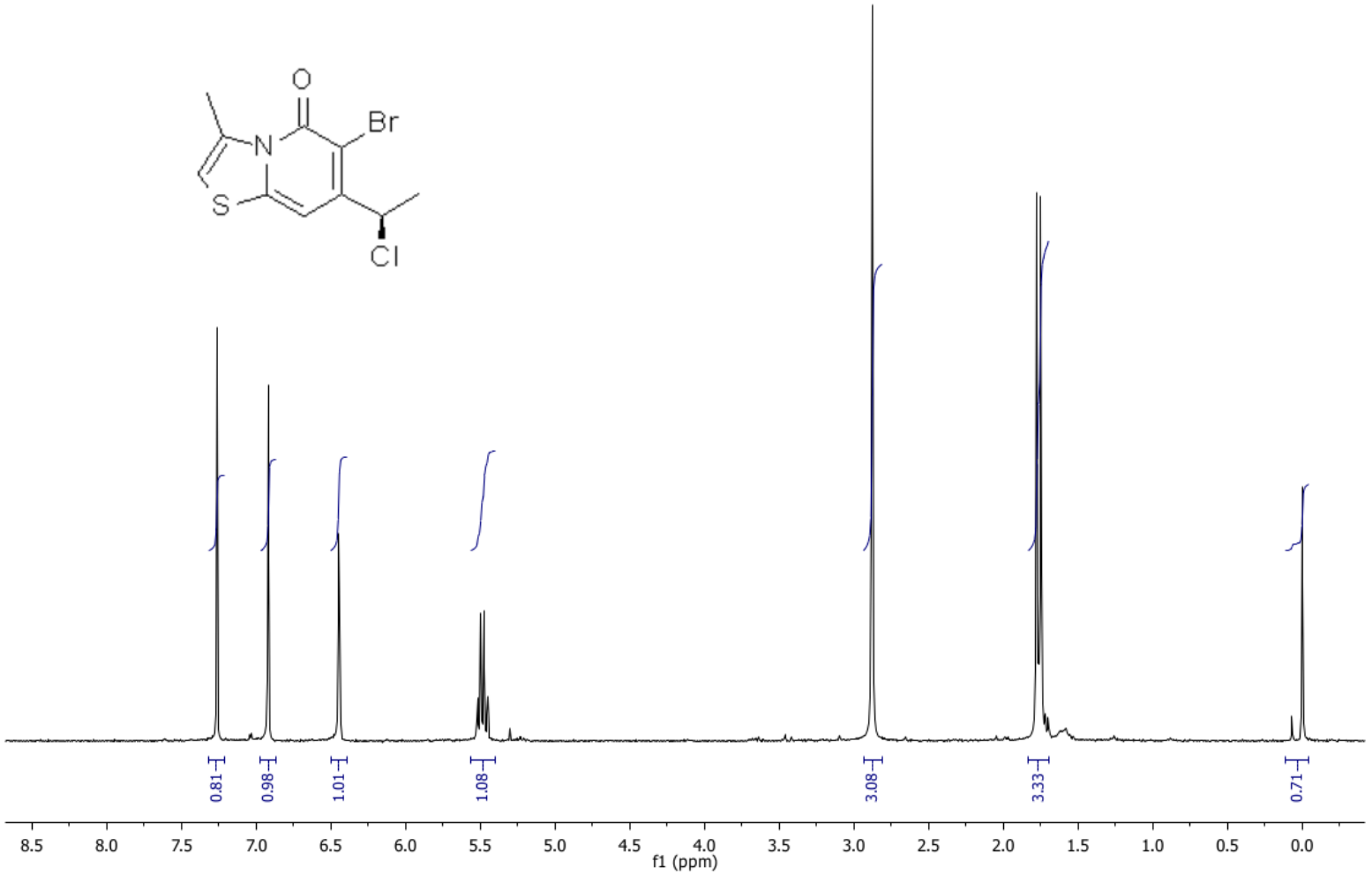
Compound 5


7.26
6.41
6.40
5.42
5.40
5.39
5.38
5.37
5.35
5.32
5.30
5.27
5.25
3.75
3.73
3.71
3.70
3.69
3.67
3.02
2.98
1.71
1.69
1.54
1.51
-0.00
H3C
O
Br
N
S
CH3
Cl
CHCl3
TMS
1.00
1.05
1.04
1.08
1.06
3.32
3.26
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
-0.5
-1.0
f1 (ppm)

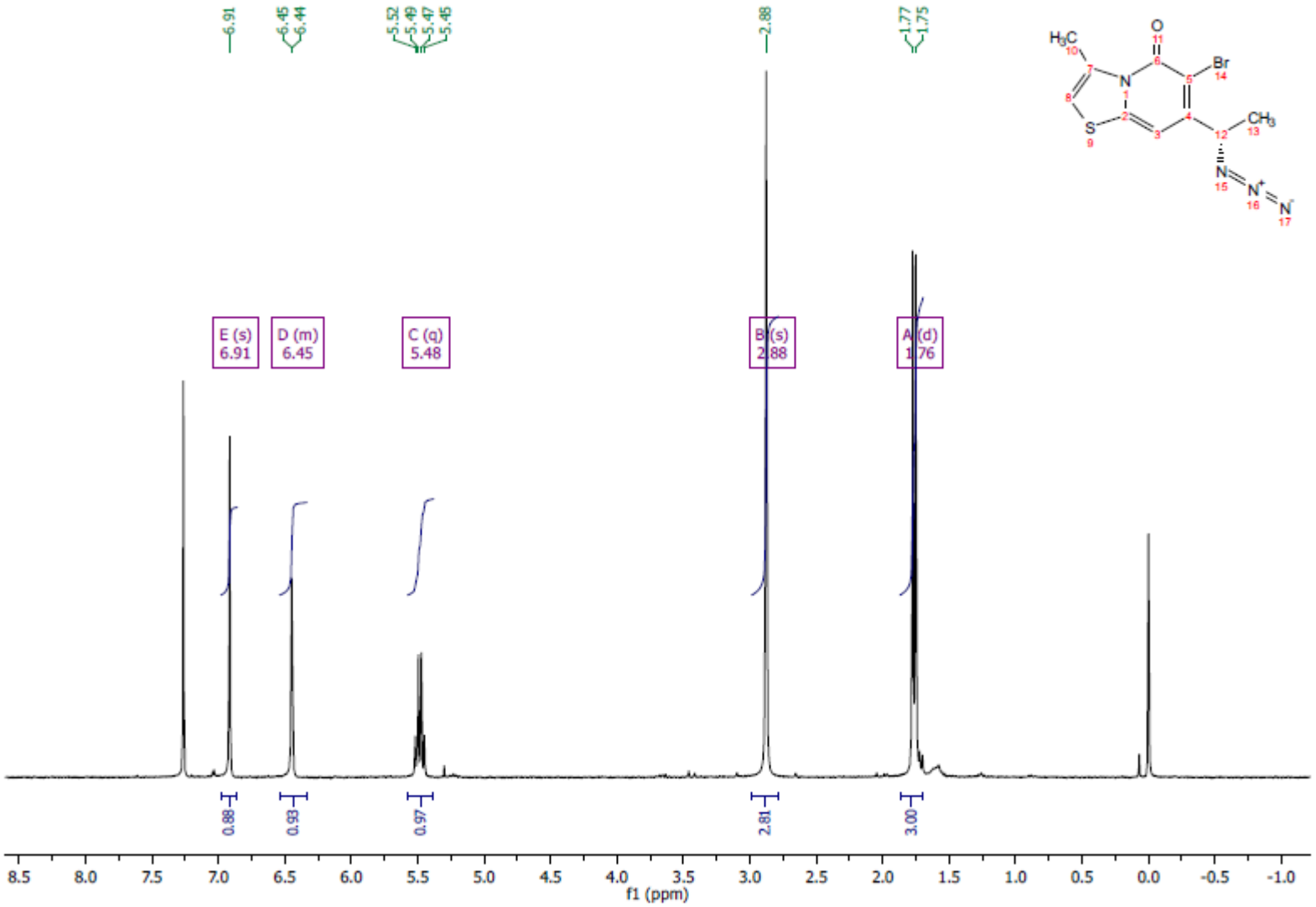
Compound 6


7.26
7.18
7.16
5.50
5.48
5.46
5.43
3.34
3.29
3.25
3.22
1.87
1.85
1.80
1.78
1.77
1.76
1.75
1.73
1.56
1.54
B (d)
7.17
C (m)
5.47
D (m)
3.28
E (q)
1.85
F (q)
1.77
0.95
2.39
2.17
3.00
4.22
11.0
10.5
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
f1 (ppm)

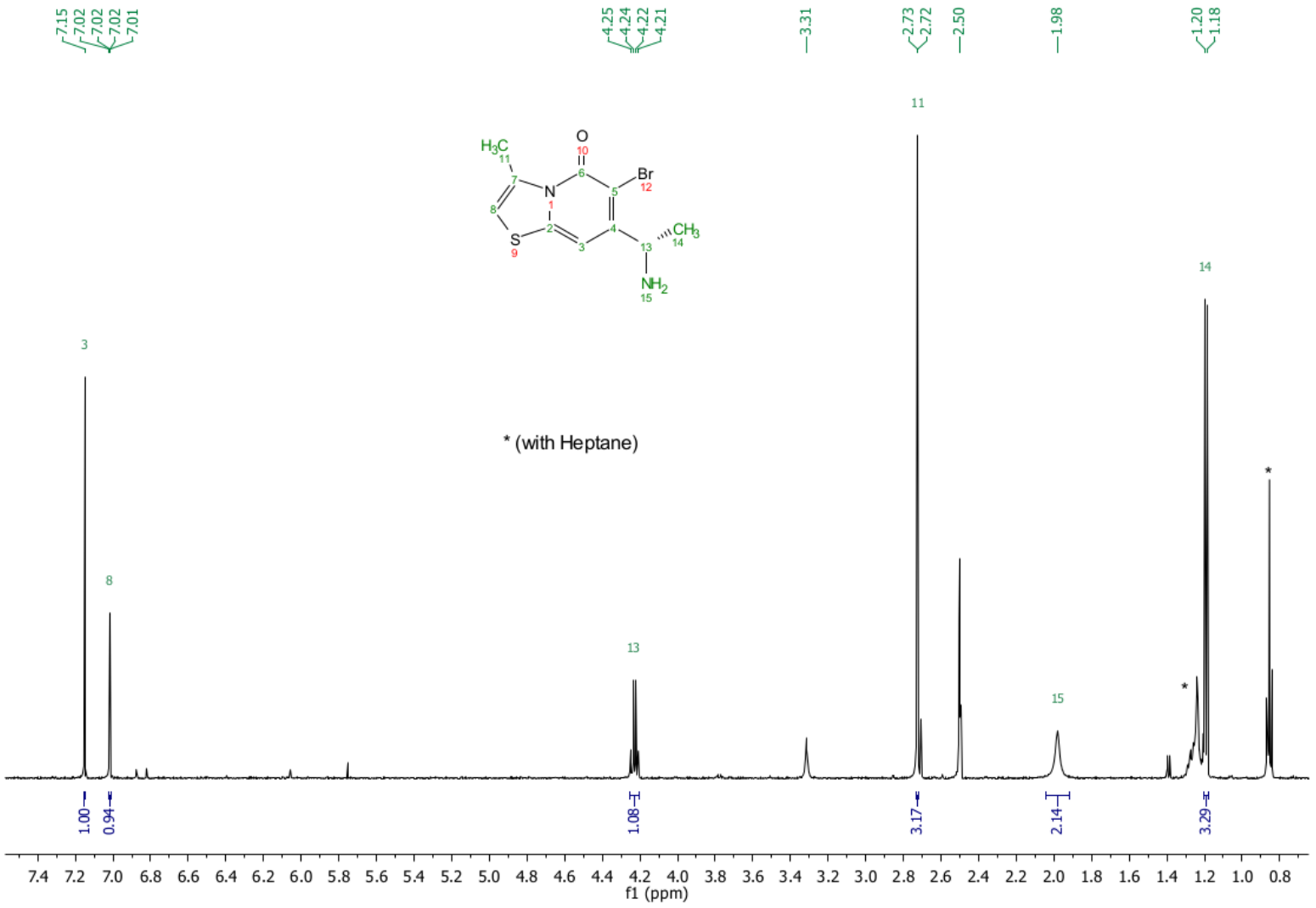
Compound 7


O
Br
N
S
Cl
0.81
0.98
1.01
1.08
3.08
3.33
0.71
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
f1 (ppm)

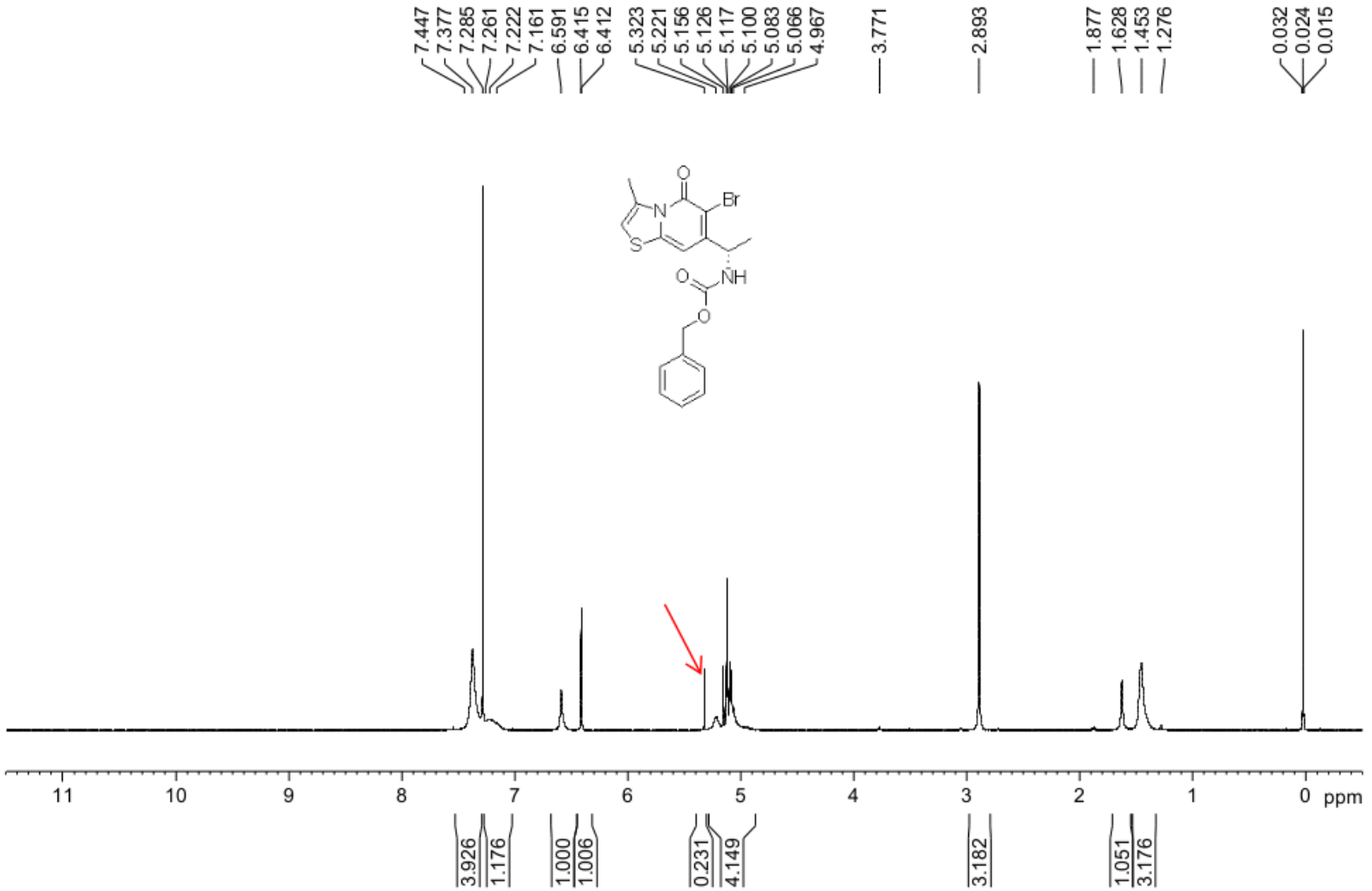
Compound 8


6.91
6.45
6.44
5.52
5.49
5.47
5.45
2.88
1.77
1.75
E (s)
6.91
D (m)
6.45
C (q)
5.48
B (s)
2.88
A (d)
1.76
0.88
0.93
0.97
2.81
3.00
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
-0.5
-1.0
f1 (ppm)

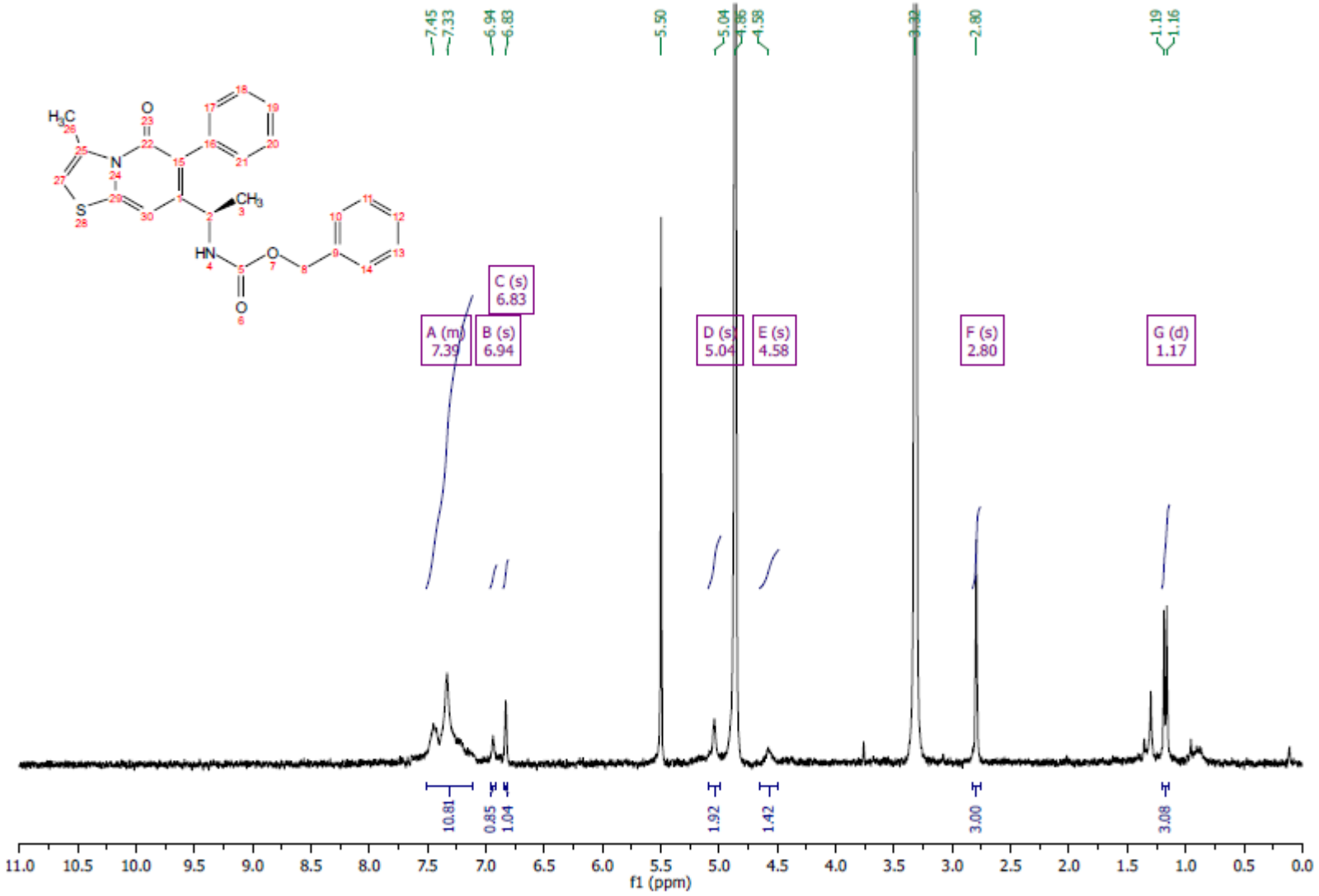
Compound 9


7.15
7.02
7.02
7.02
7.01
4.25
4.24
4.22
4.21
3.31
2.73
2.72
2.50
1.98
1.20
1.18
* (with Heptane)
1.00
0.94
1.08
3.17
2.14
3.29
f1 (ppm)

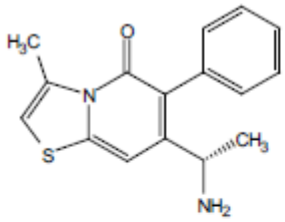
Compound 10

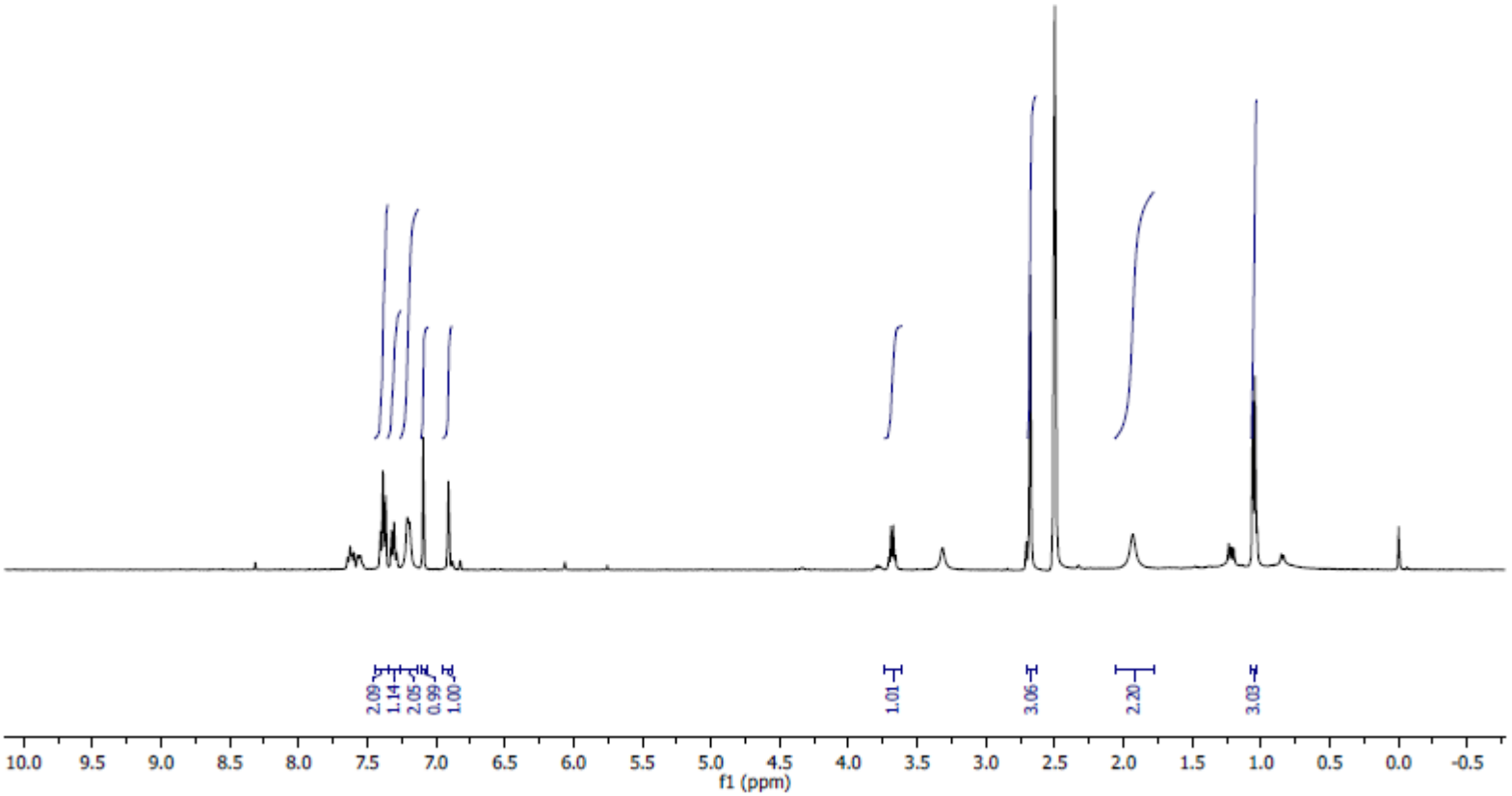

7.447
7.377
7.285
7.261
7.222
7.161
6.591
6.415
6.412
5.323
5.221
5.156
5.126
5.117
5.100
5.083
5.066
4.967
3.771
2.893
1.877
1.628
1.453
1.276
0.032
0.024
0.015
Br
N
S
O
NH
O
O
11
10
9
8
7
6
5
4
3
2
1
0 ppm
3.926
1.176
1.000
1.006
0.231
4.149
3.182
1.051
3.176

Compound 11

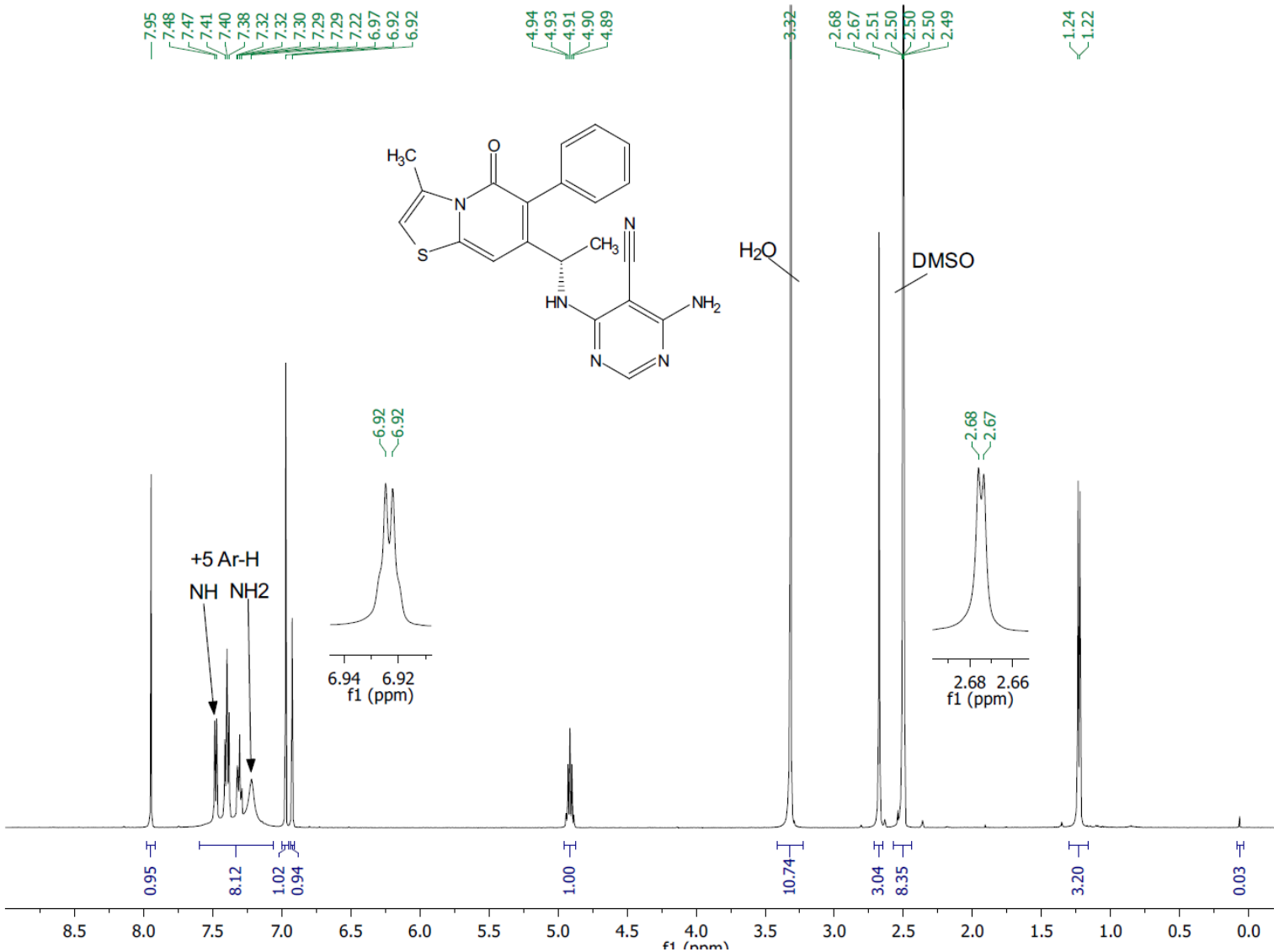

7.45
7.33
6.94
6.83
5.50
5.04
4.86
4.58
3.33
2.80
1.19
1.16
C (s)
6.83
A (m)
7.39
B (s)
6.94
D (s)
5.04
E (s)
4.58
F (s)
2.80
G (d)
1.17
10.81
0.85
1.04
1.92
1.42
3.00
3.08
f1 (ppm)

Compound 12

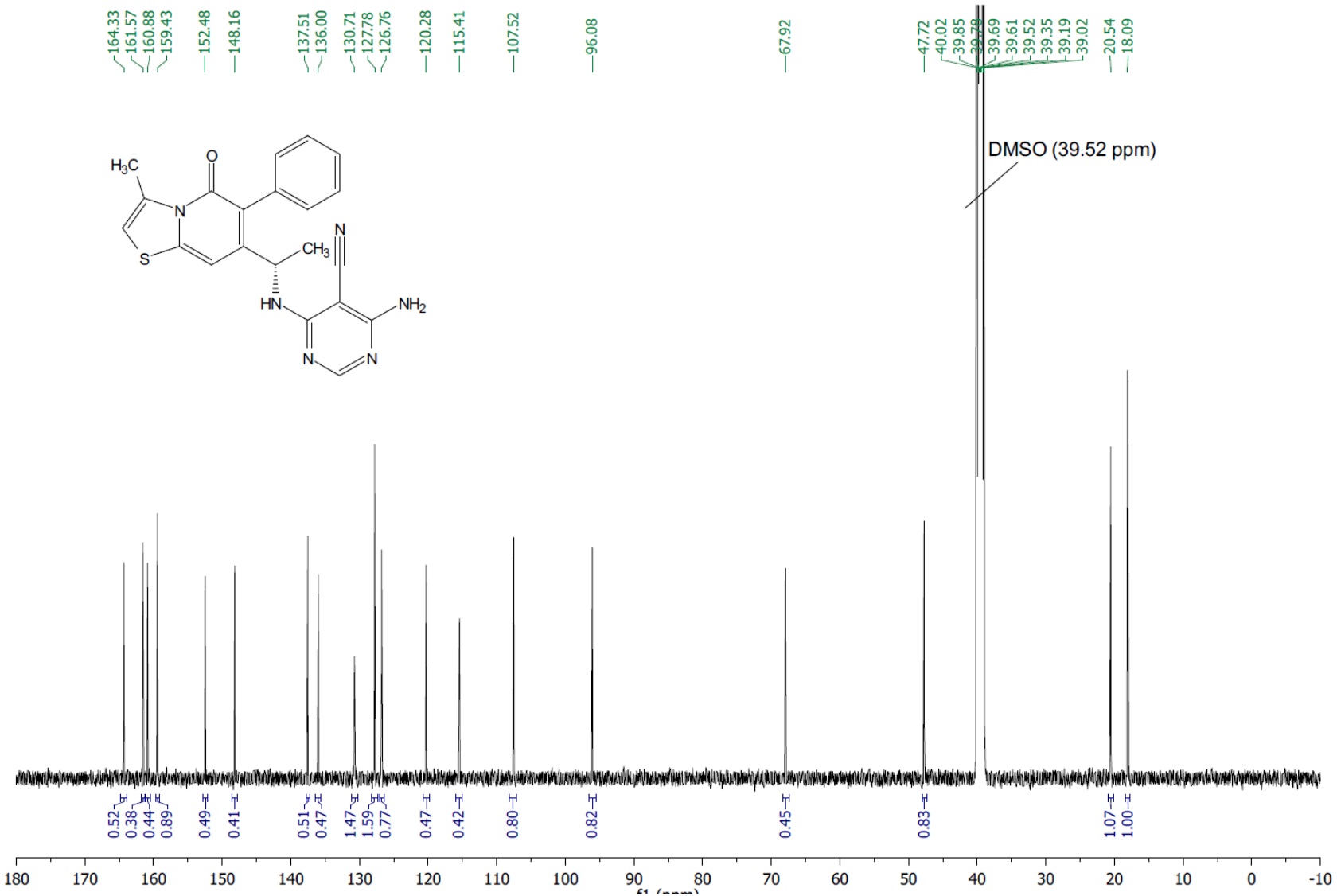

H3C
O
N
S
CH3
NH2


2.09
1.14
2.05
0.99
1.00
1.01
3.06
2.20
3.03
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
-0.5
f1 (ppm)

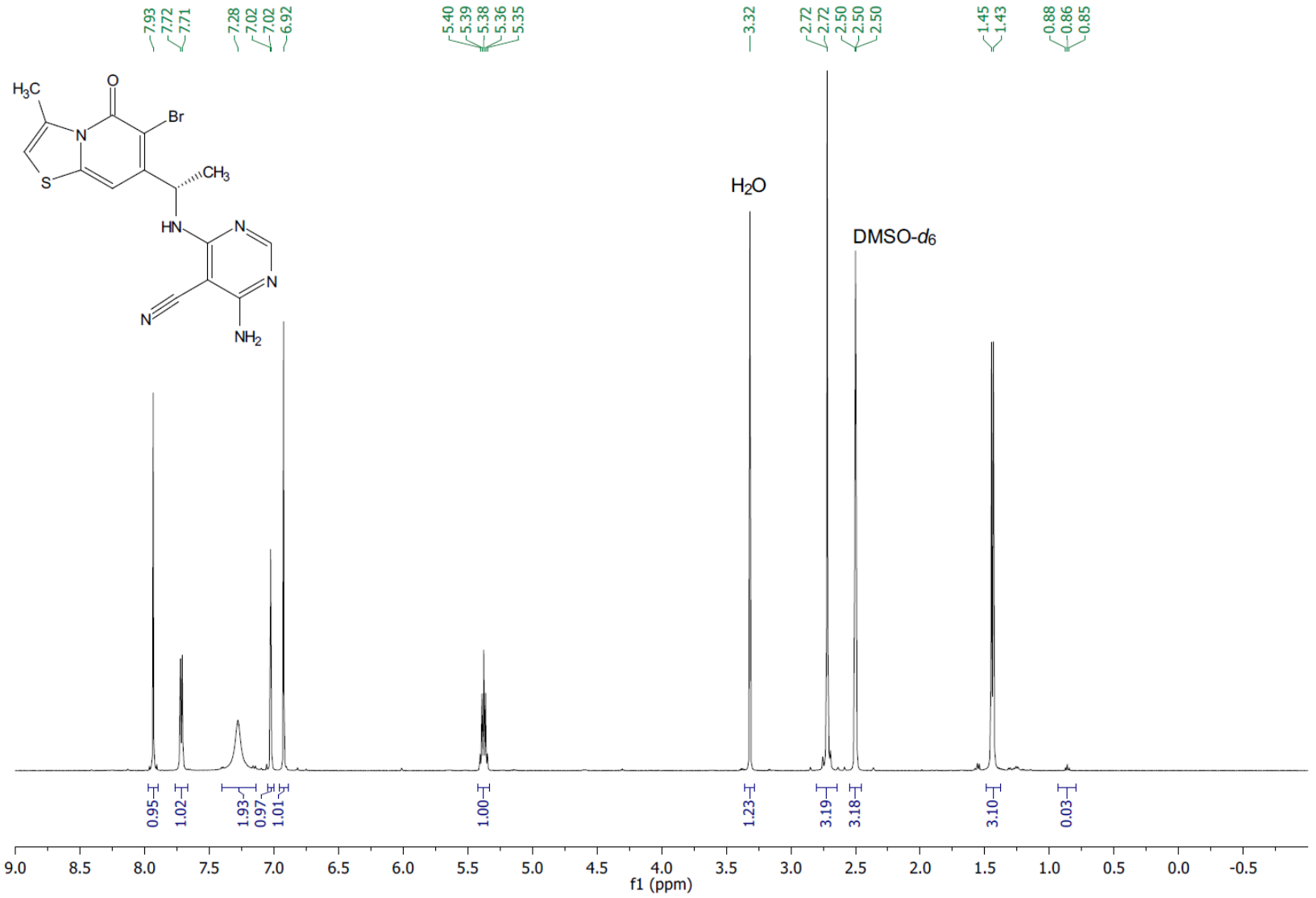
Compound 13


7.95
7.48
7.47
7.41
7.40
7.38
7.32
7.32
7.30
7.29
7.29
7.22
6.97
6.92
6.92
4.94
4.93
4.91
4.90
4.89
3.32
2.68
2.67
2.51
2.50
2.50
2.50
2.49
1.24
1.22
H2O
DMSO
+5 Ar-H
NH NH2
6.92
6.92
6.94 6.92
f1 (ppm)
2.68
2.67
2.68 2.66
f1 (ppm)
0.95
8.12
1.02
0.94
1.00
10.74
3.04
8.35
3.20
0.03
f1 (ppm)

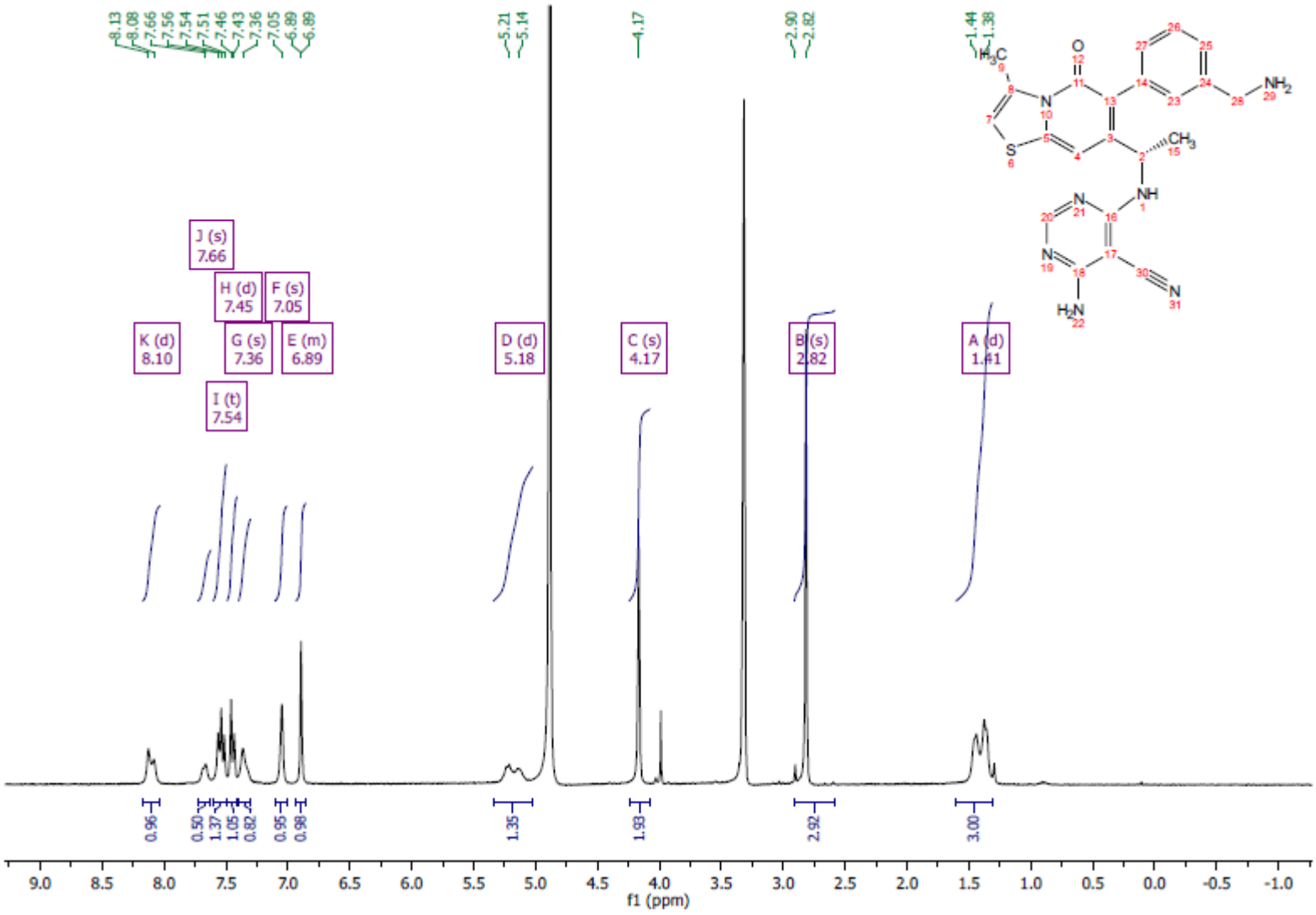
Compound 13 ^{13}C



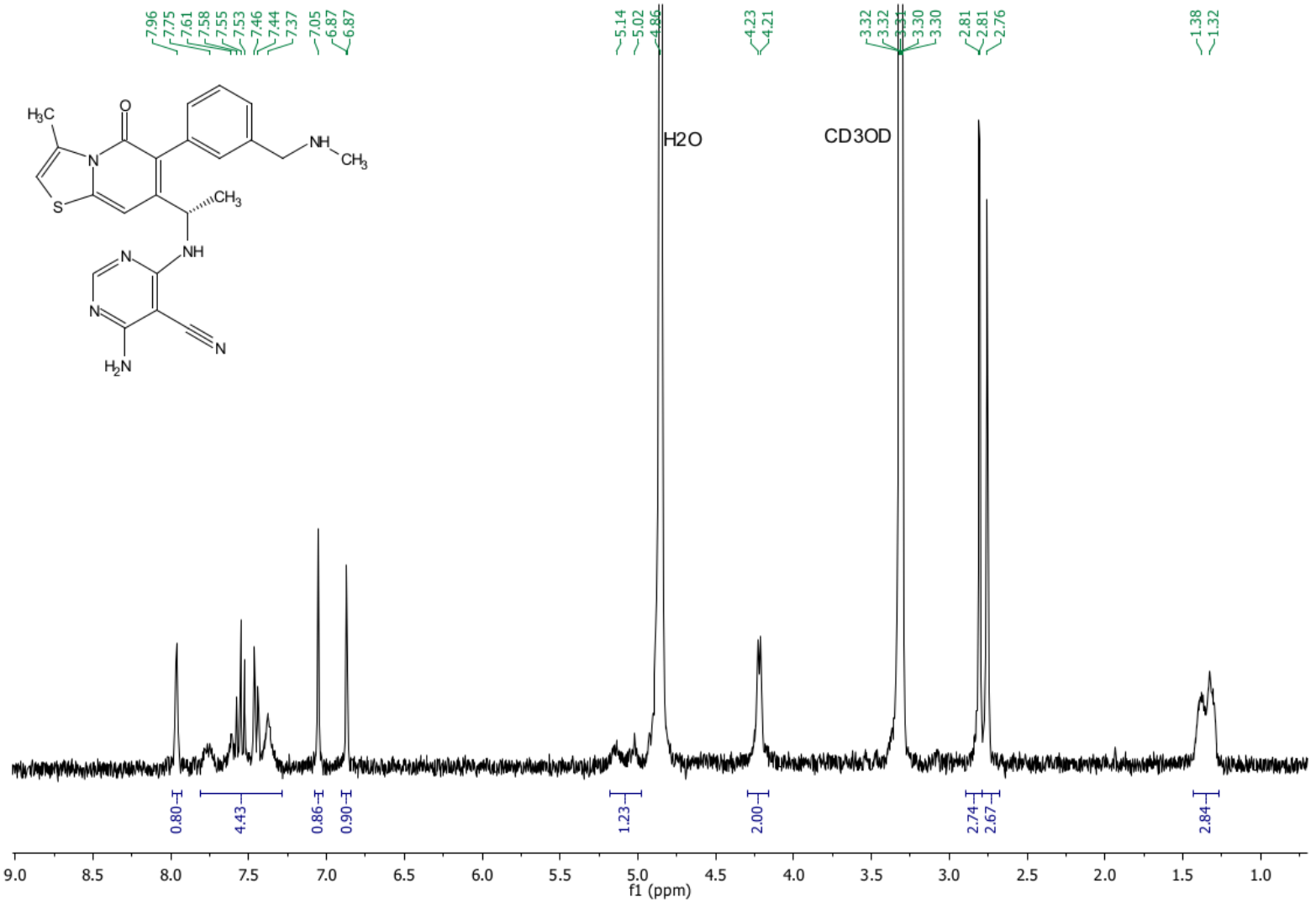
Compound 14


7.93
7.72
7.71
7.28
7.02
7.02
6.92
5.40
5.39
5.38
5.36
5.35
3.32
2.72
2.72
2.50
2.50
2.50
1.45
1.43
0.88
0.86
0.85
H2O
DMSO-d6
0.95
1.02
1.93
0.97
1.01
1.00
1.23
3.19
3.18
3.10
0.03
f1 (ppm)

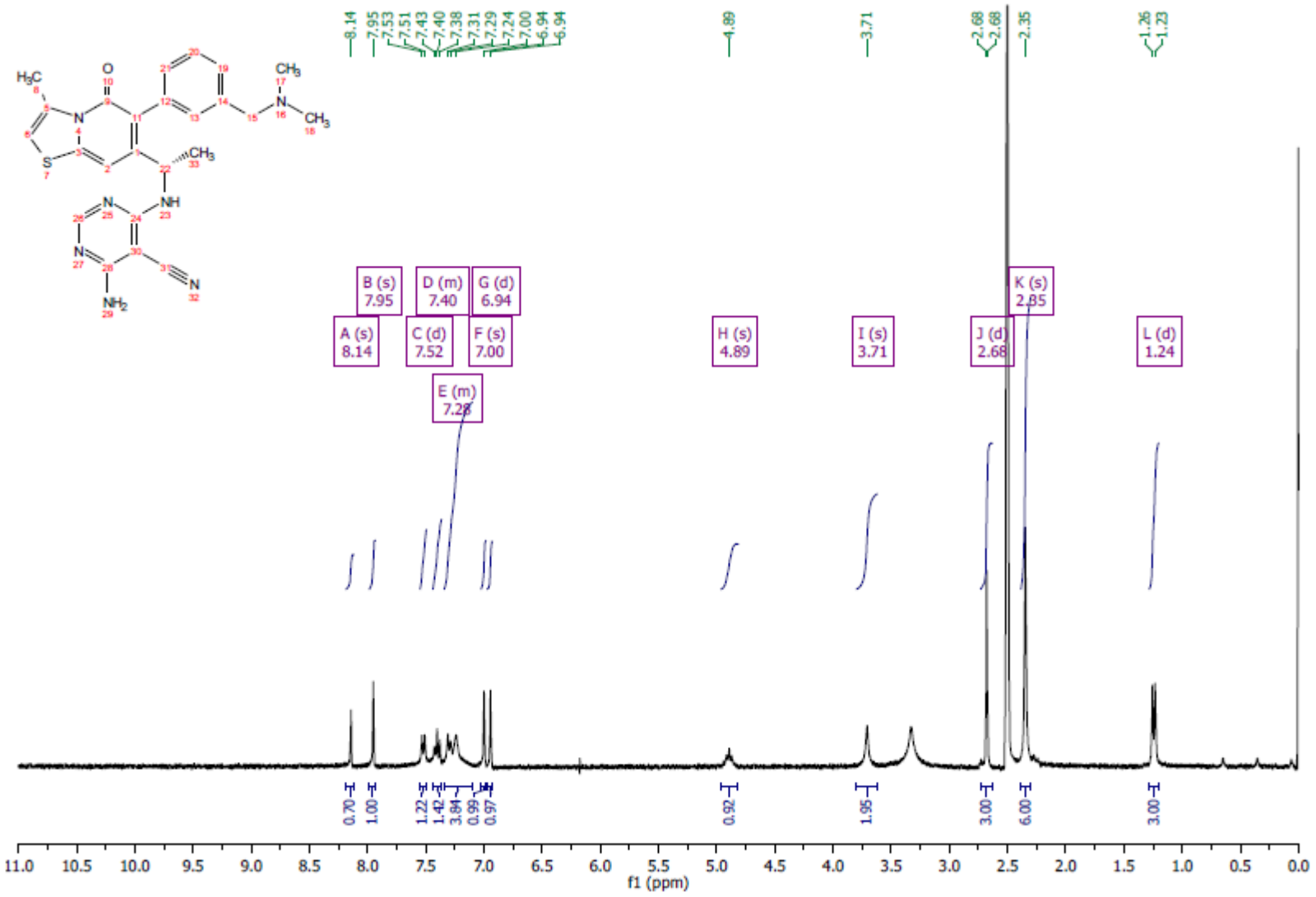
Compound 15a



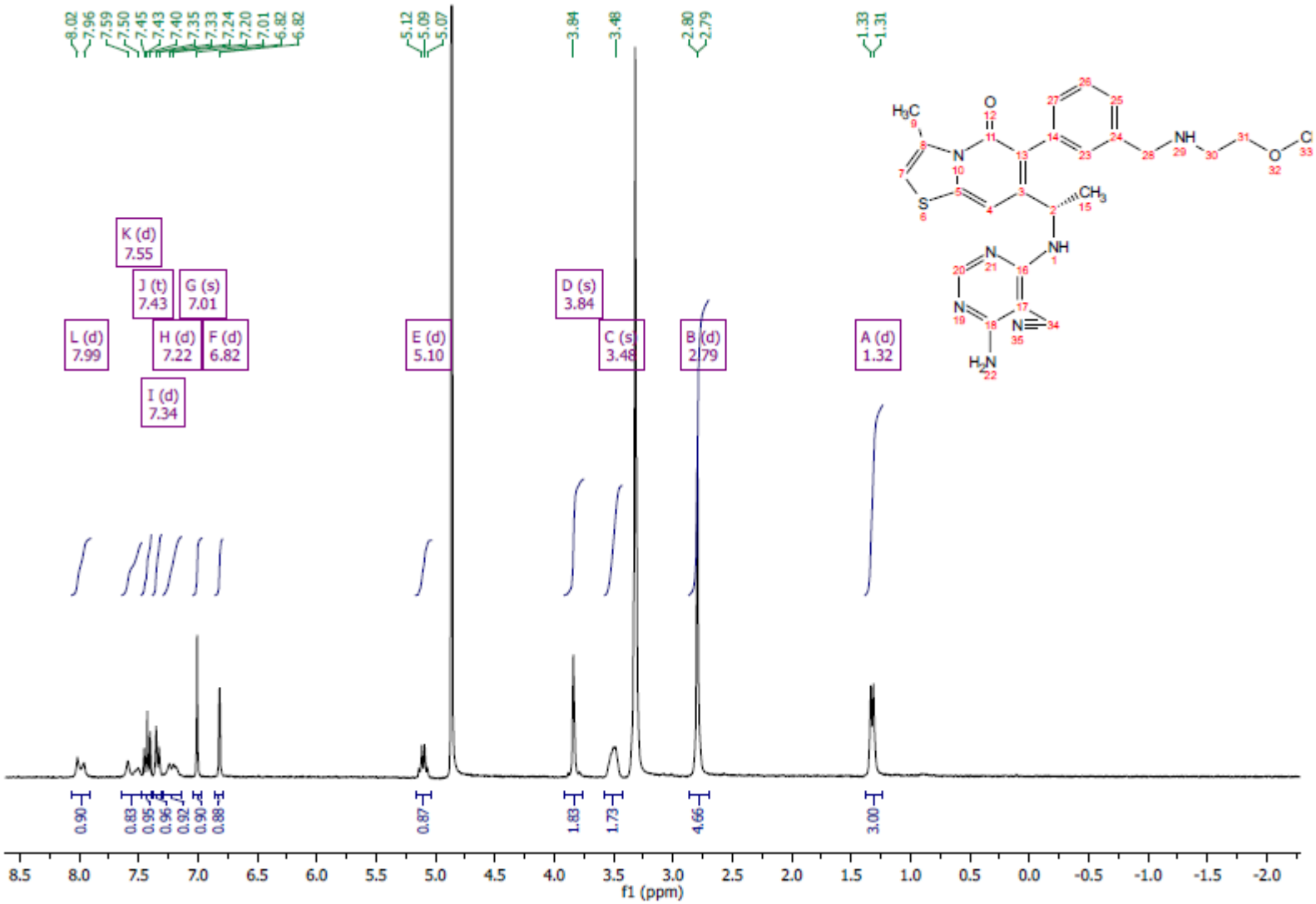
Compound 15b



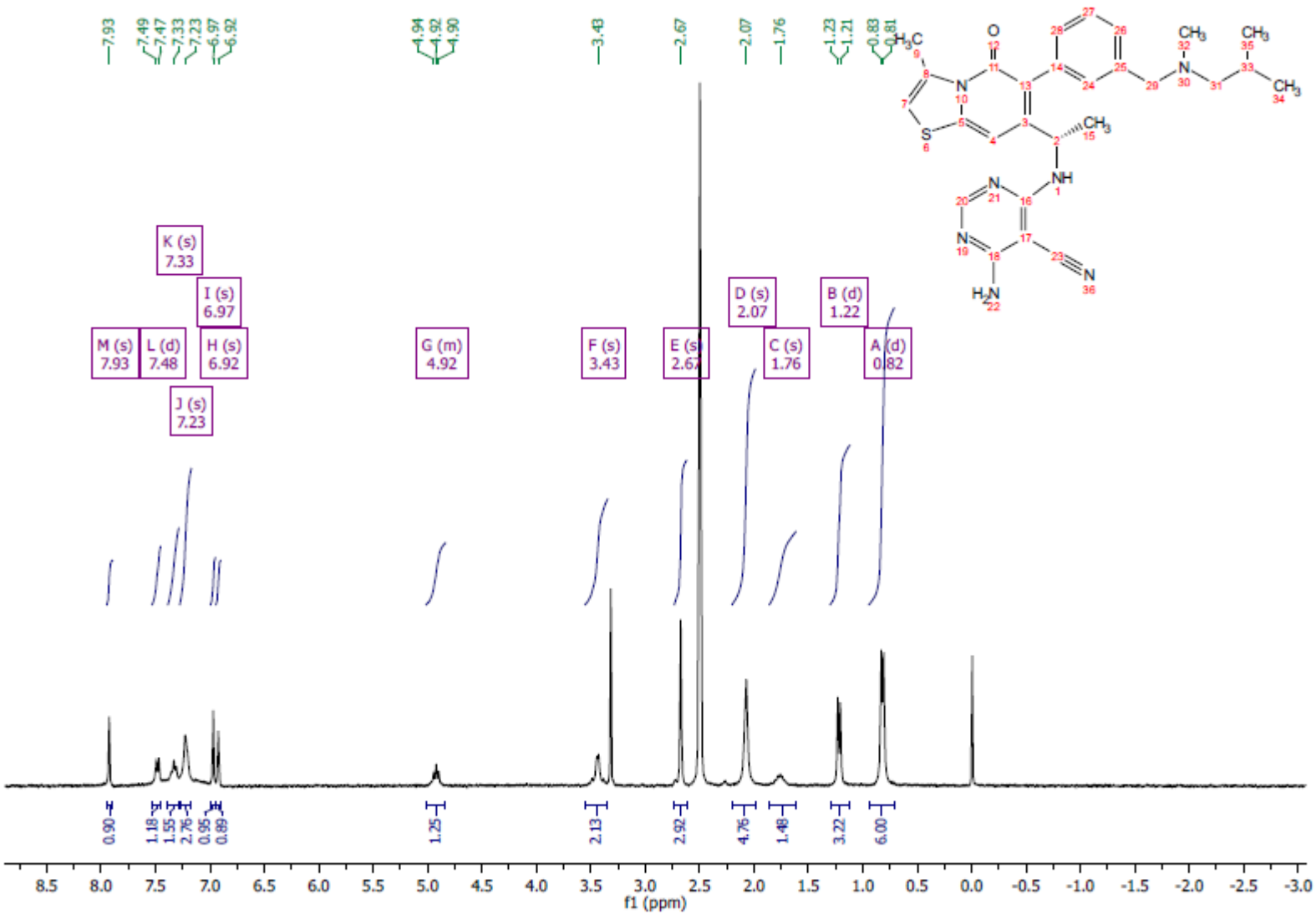
Compound 15 c



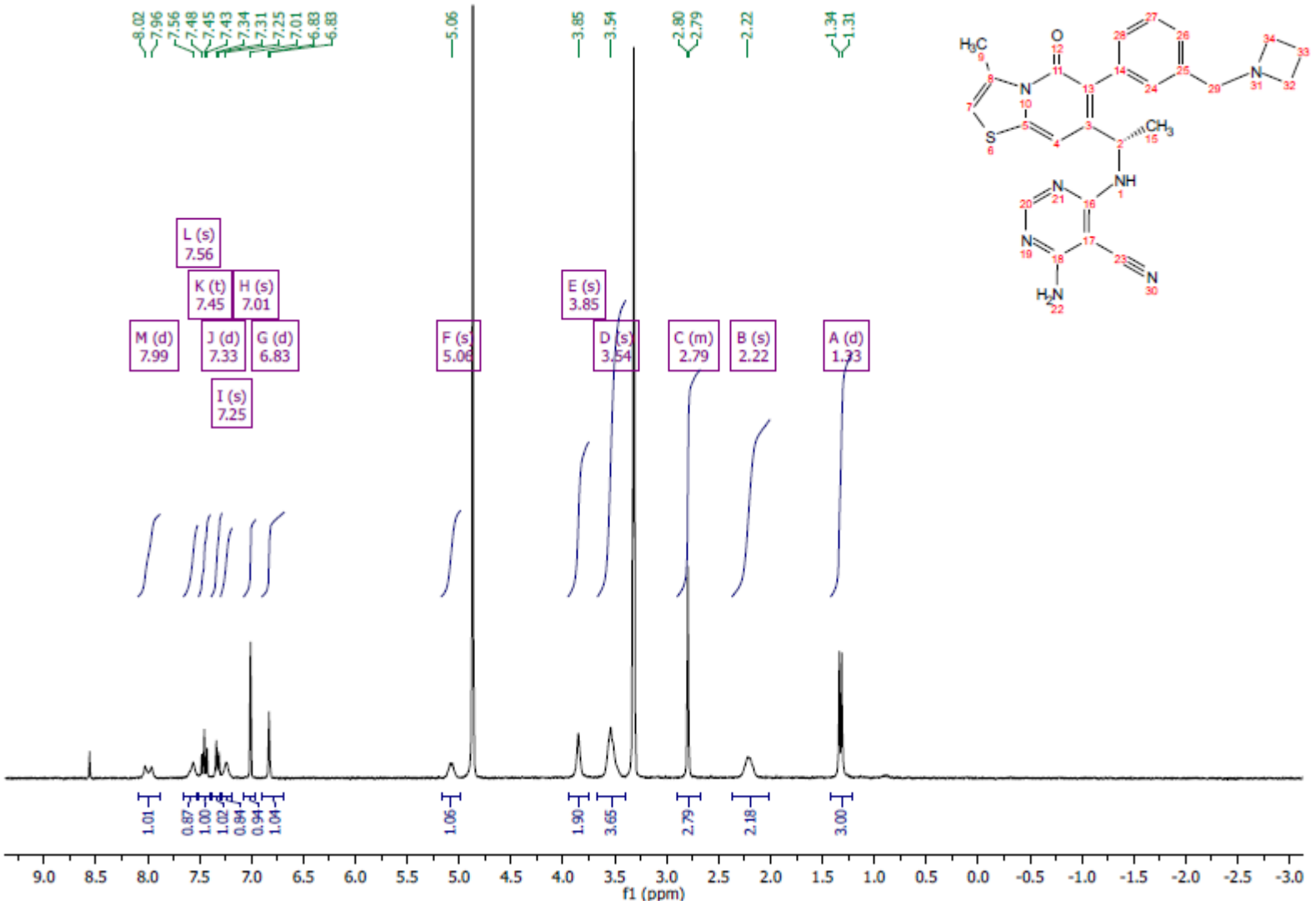
Compound 15d



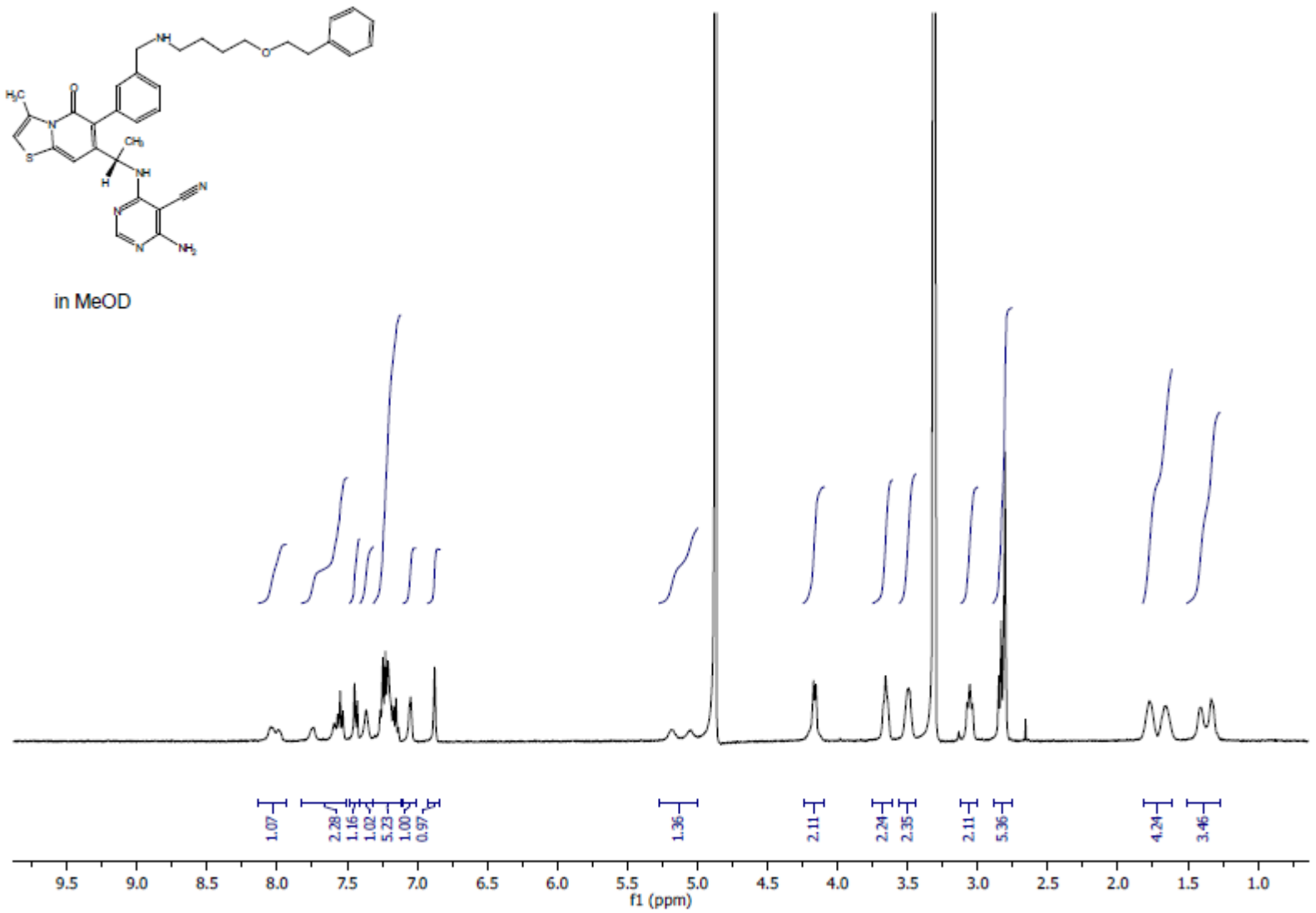
Compound 15e



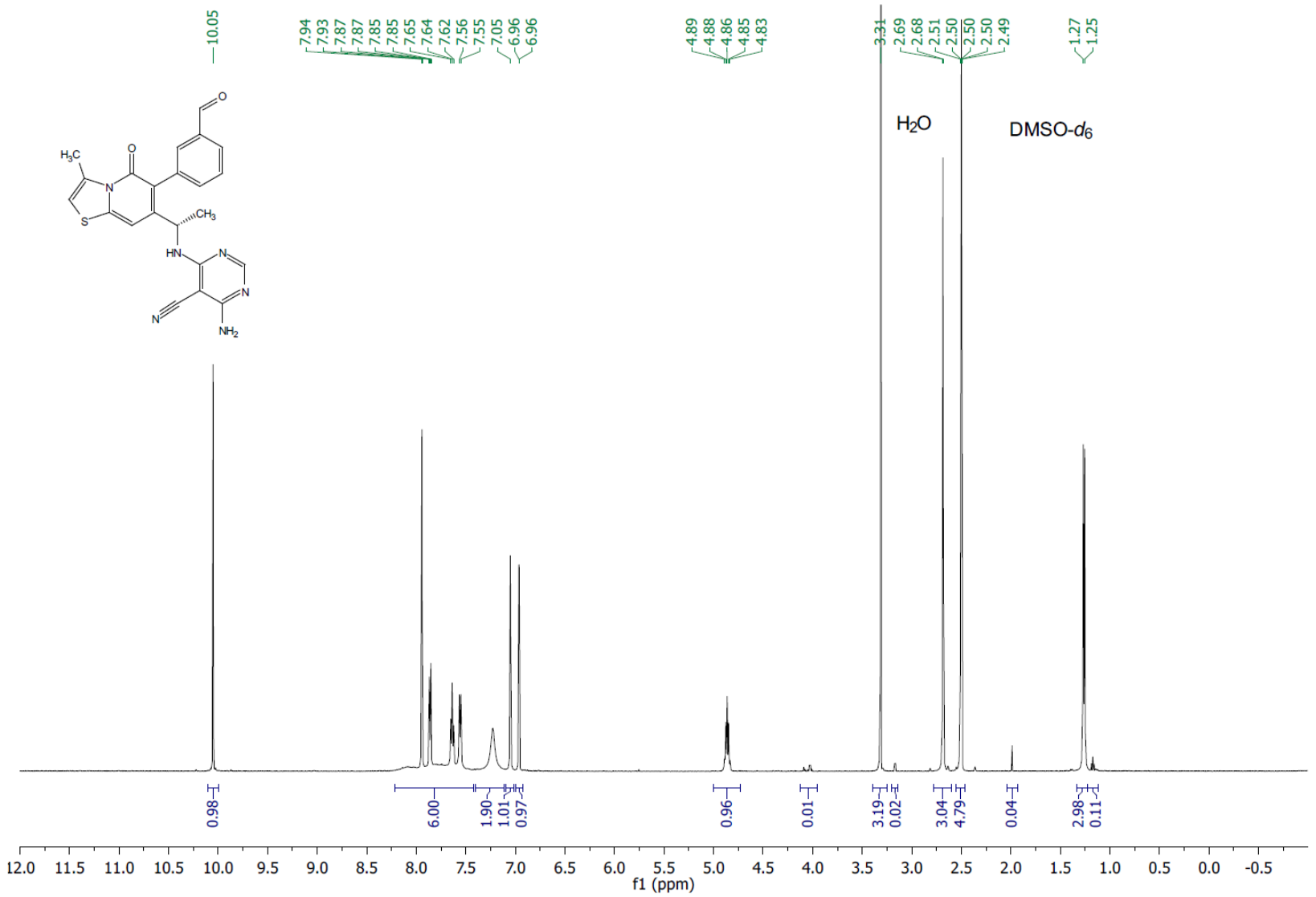
Compound 15f



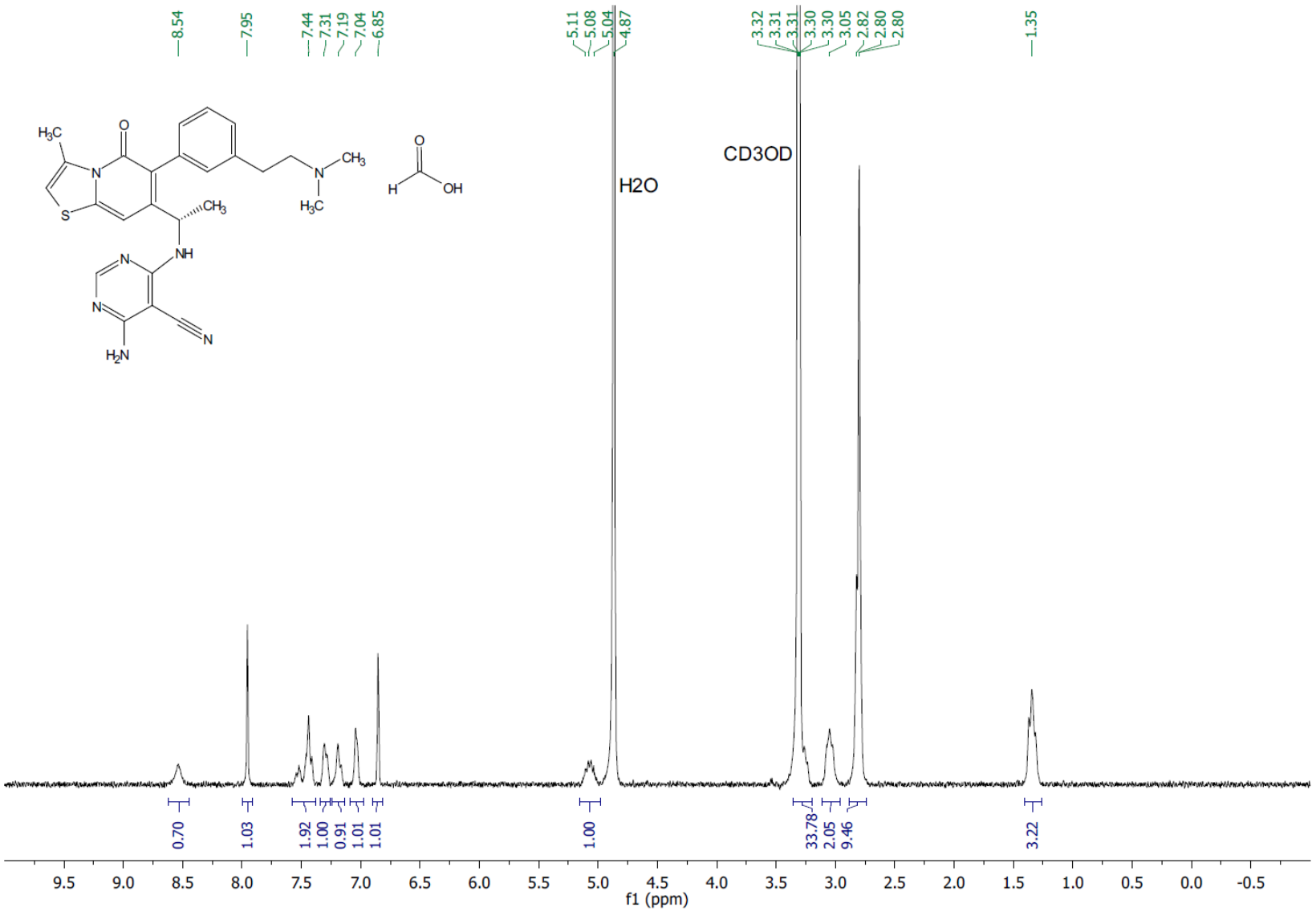
Compound 15g



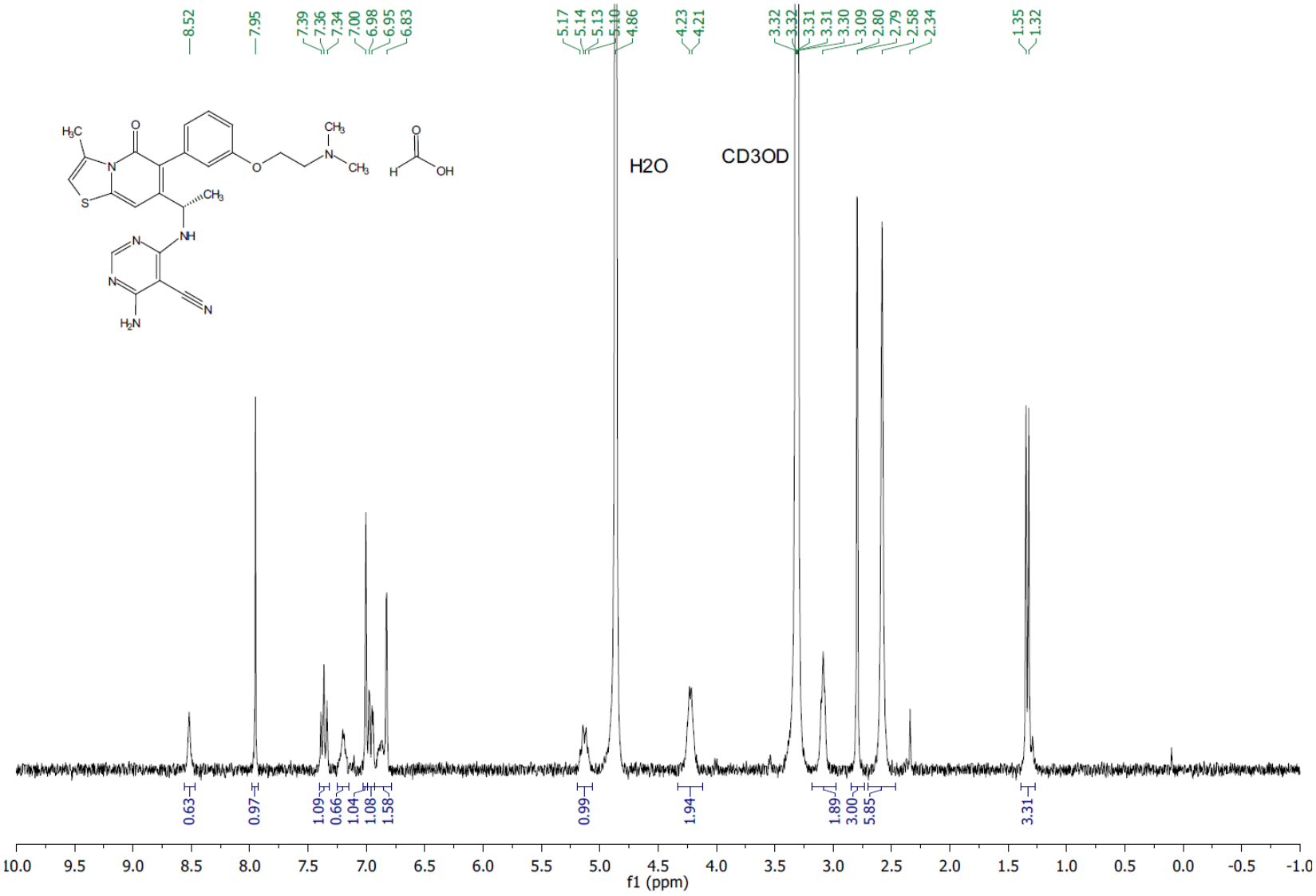
Compound 16


10.05
7.94
7.93
7.87
7.87
7.85
7.85
7.65
7.64
7.62
7.56
7.55
7.05
6.96
6.96
4.89
4.88
4.86
4.85
4.83
3.31
2.69
2.68
2.51
2.50
2.50
2.50
2.49
1.27
1.25
H2O
DMSO-d6
0.98
6.00
1.90
1.01
0.97
0.96
0.01
3.19
0.02
3.04
4.79
0.04
2.98
0.11
f1 (ppm)

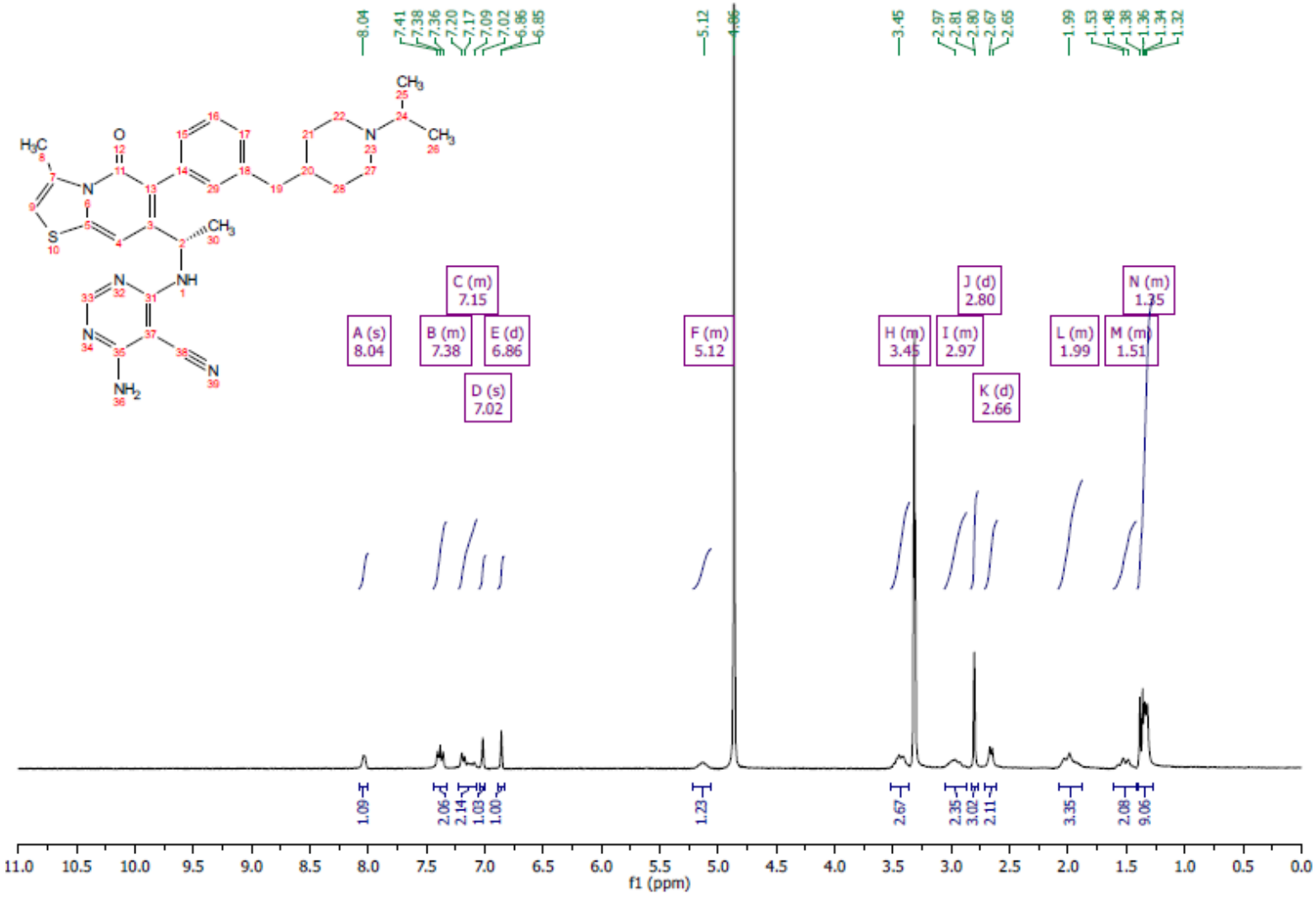
Compound 17


8.54
7.95
7.44
7.31
7.19
7.04
6.85
5.11
5.08
5.04
4.87
3.32
3.31
3.31
3.30
3.30
3.05
2.82
2.80
2.80
1.35
H2O
CD3OD
0.70
1.03
1.92
1.00
0.91
1.01
1.01
1.00
33.78
2.05
9.46
3.22
f1 (ppm)

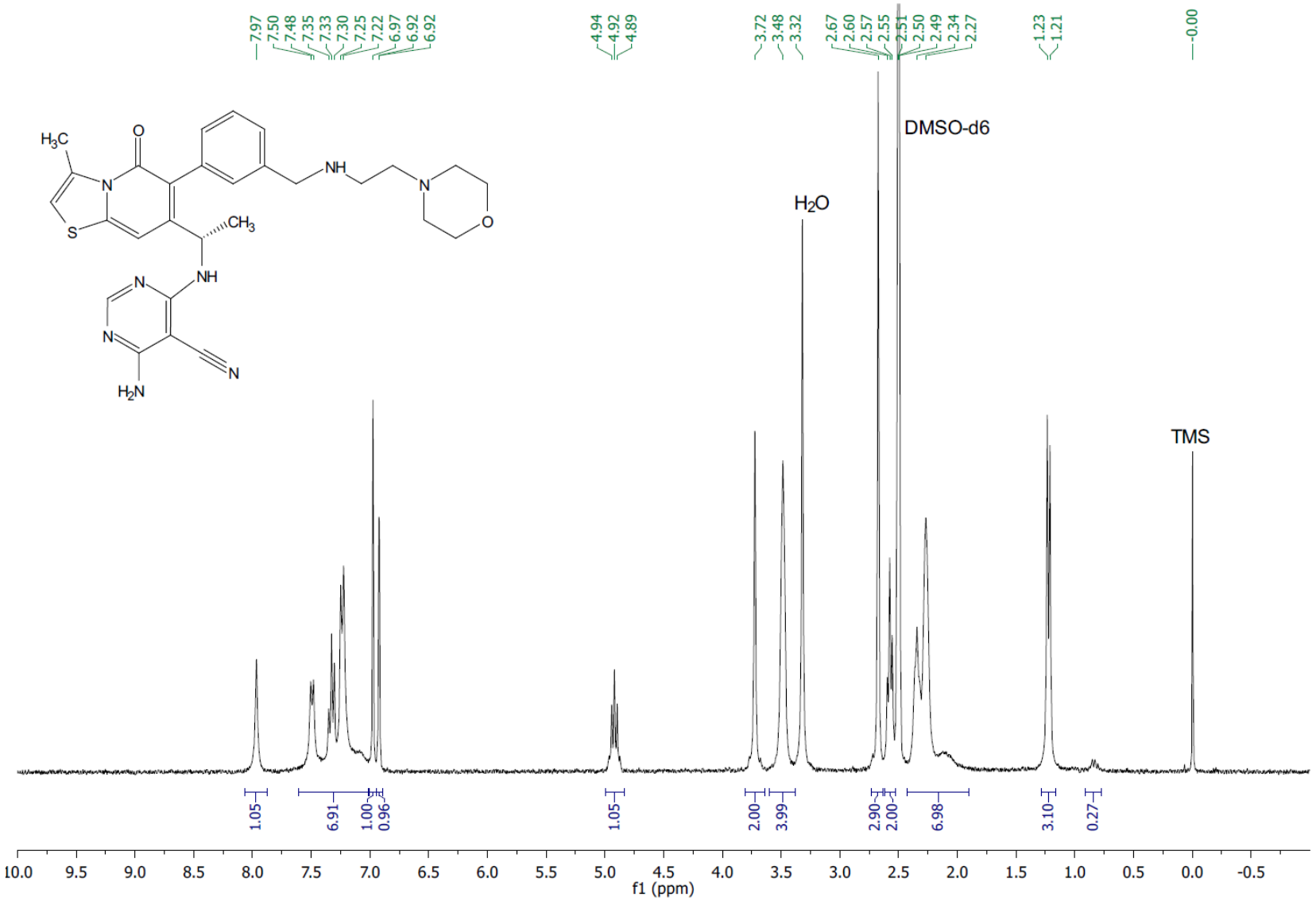
Compound 18


8.52
7.95
7.39
7.36
7.34
7.00
6.98
6.95
6.83
5.17
5.14
5.13
5.10
4.86
4.23
4.21
3.32
3.32
3.31
3.31
3.30
3.09
2.80
2.79
2.58
2.34
1.35
1.32
H2O
CD3OD
0.63
0.97
1.09
0.66
1.04
1.08
1.58
0.99
1.94
1.89
3.00
5.85
3.31
f1 (ppm)

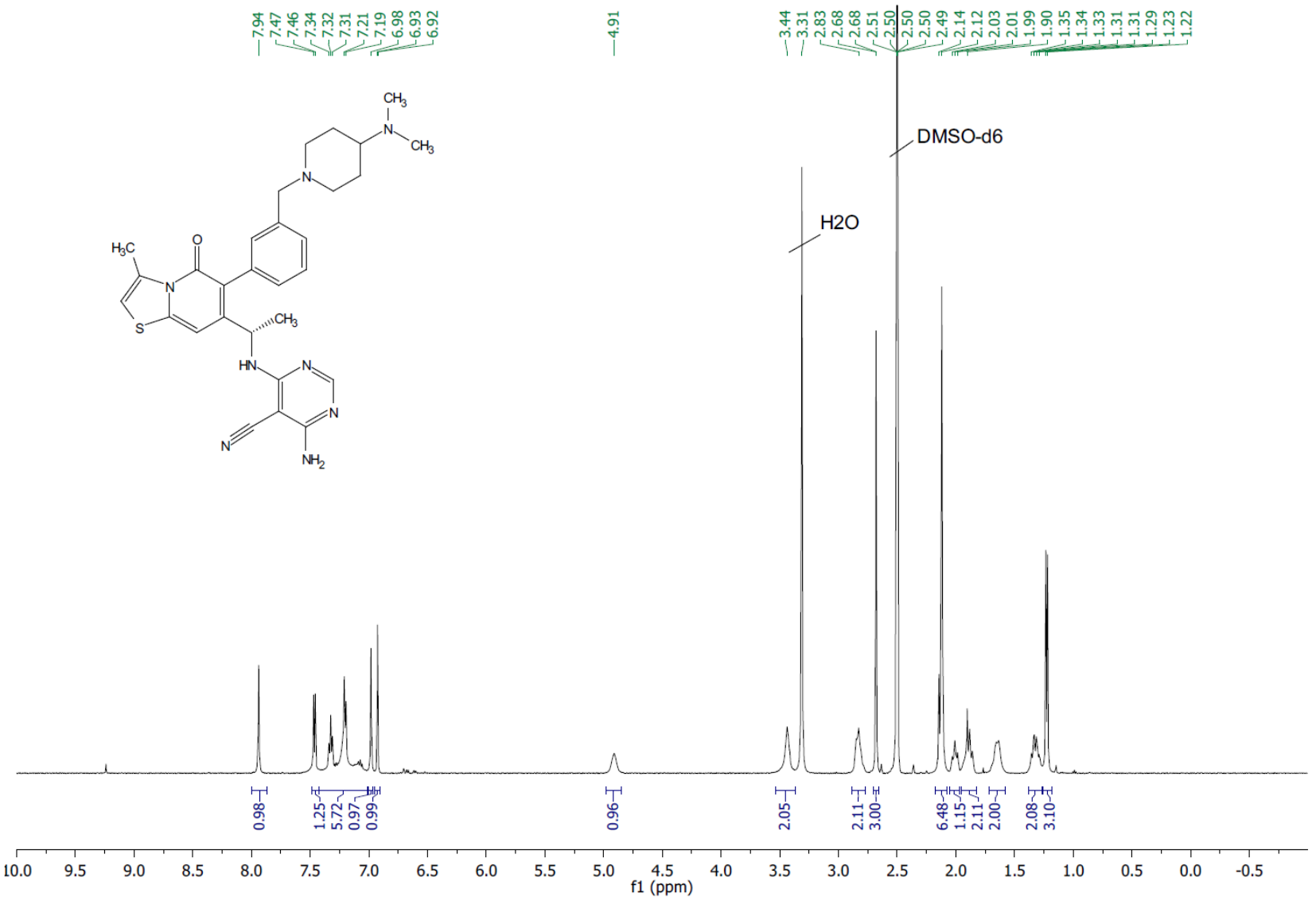
Compound 19


8.04
7.41
7.38
7.36
7.20
7.17
7.09
7.02
6.86
6.85
5.12
4.86
3.45
2.97
2.81
2.80
2.67
2.65
1.99
1.53
1.48
1.38
1.36
1.34
1.32
A (s) 8.04
B (m) 7.38
C (m) 7.15
D (s) 7.02
E (d) 6.86
F (m) 5.12
H (m) 3.45
I (m) 2.97
J (d) 2.80
K (d) 2.66
L (m) 1.99
M (m) 1.51
N (m) 1.35
1.09
2.06
2.14
1.03
1.00
1.23
2.67
2.35
3.02
2.11
3.35
2.08
9.06
f1 (ppm)

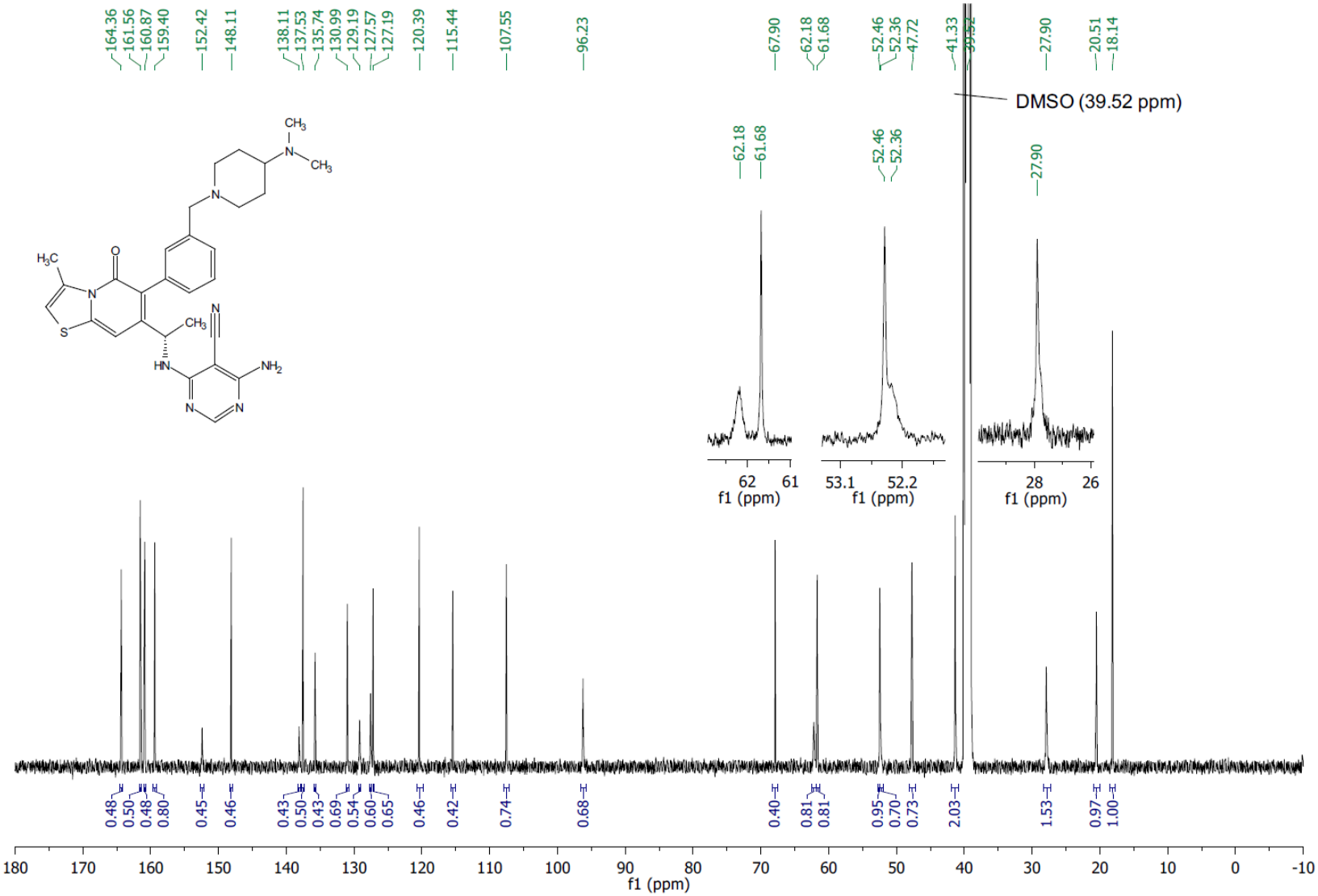
Compound 20a



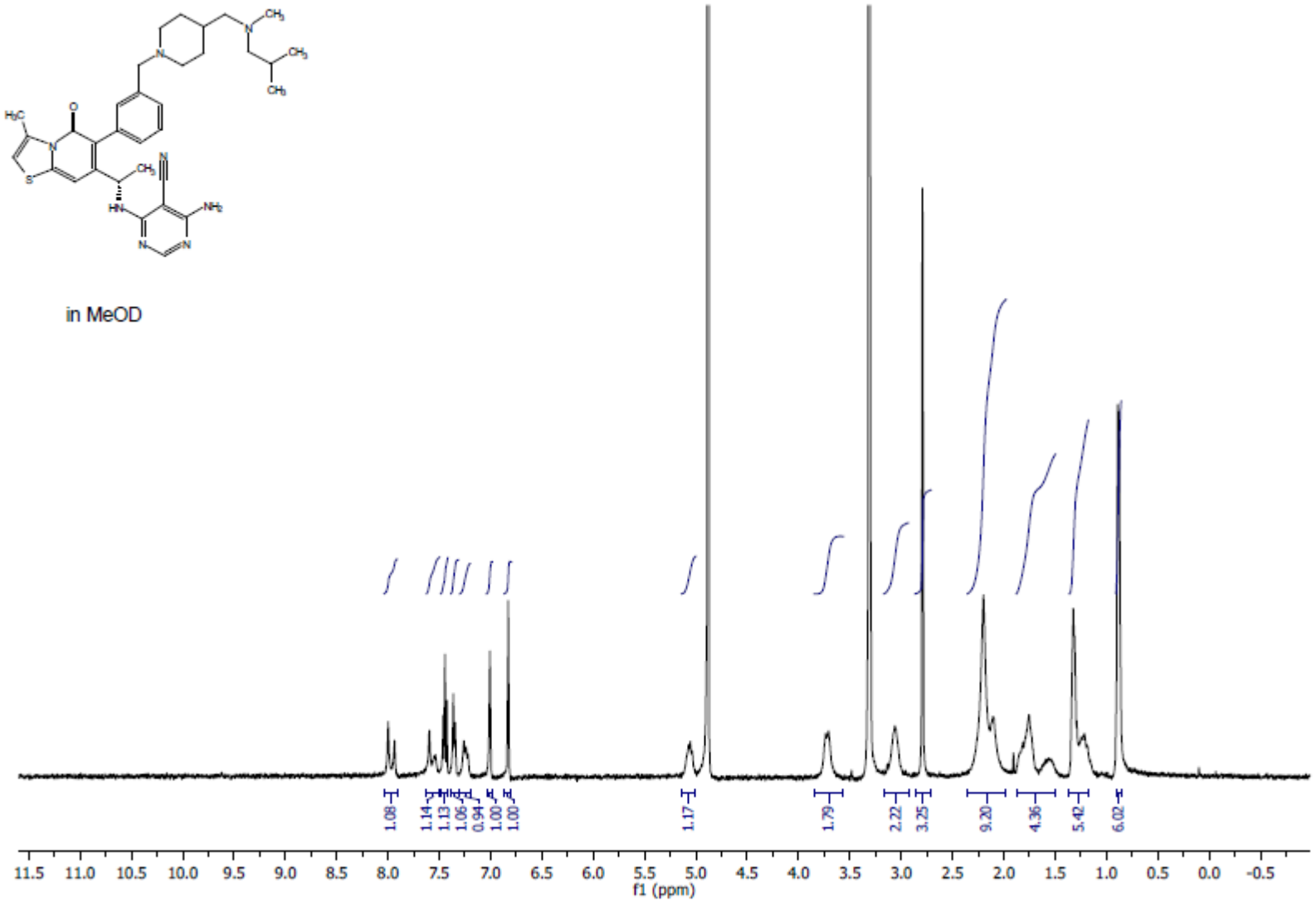
Compound 20b



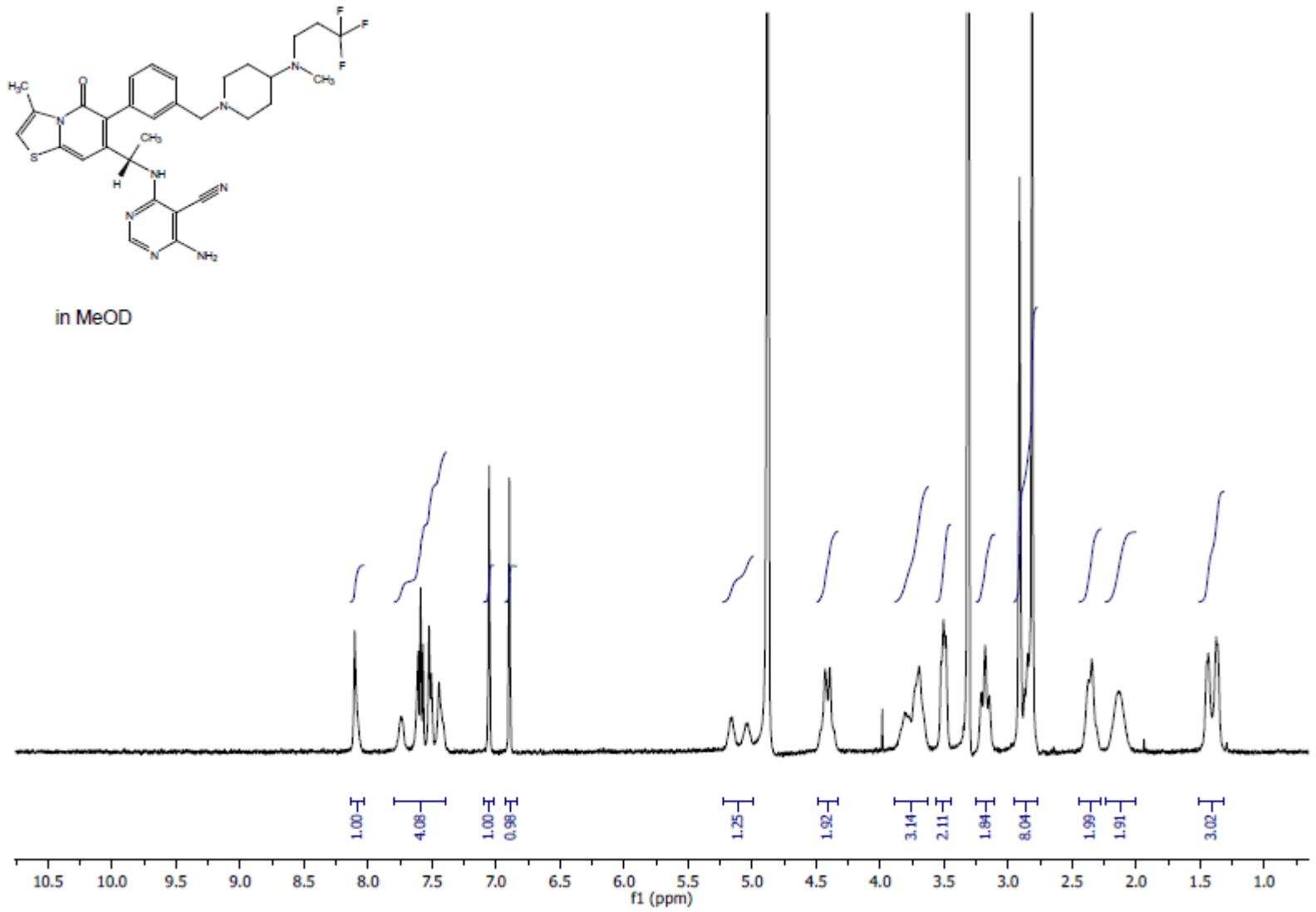
Compound 20b ^{13}C



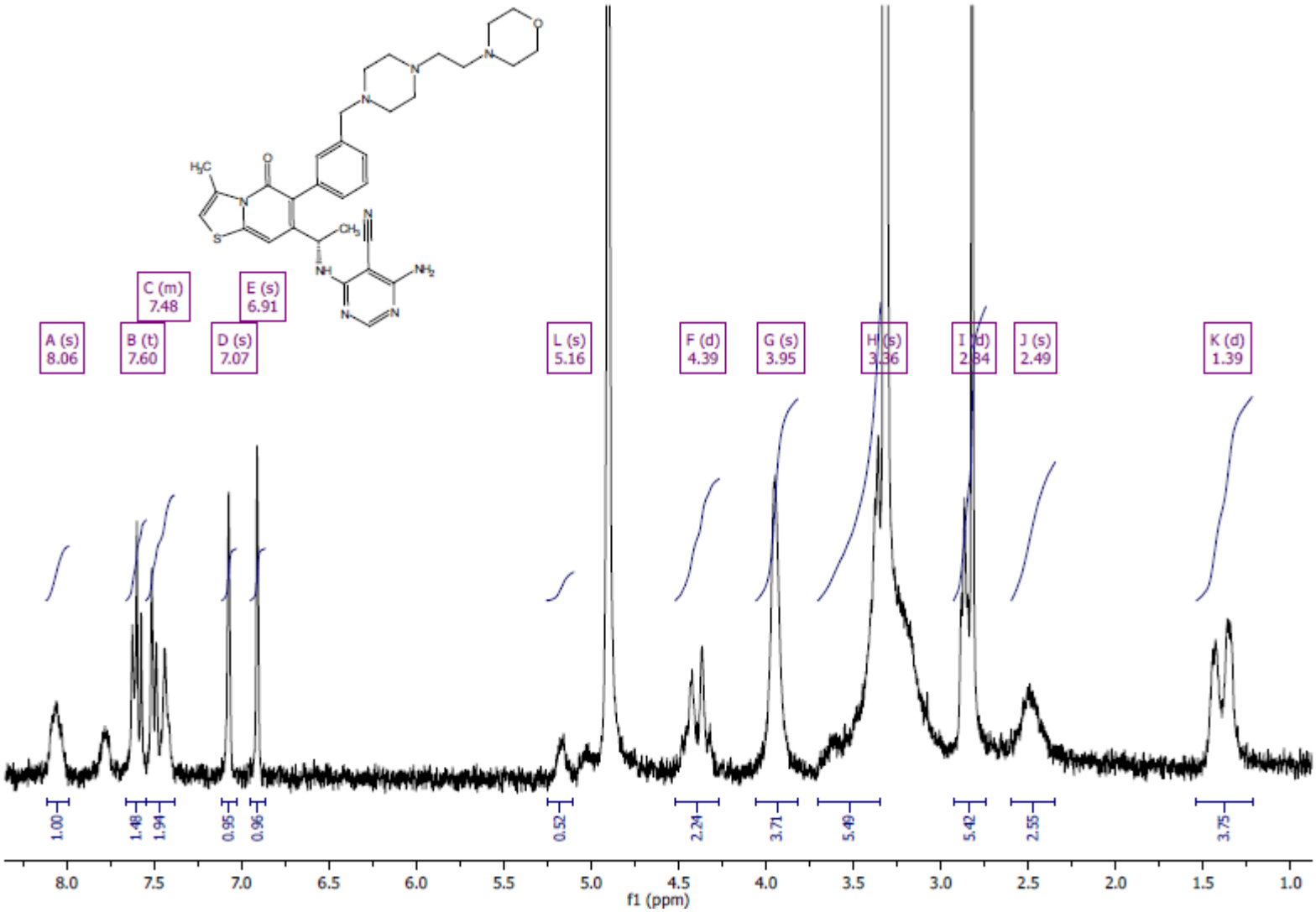
Compound 20c



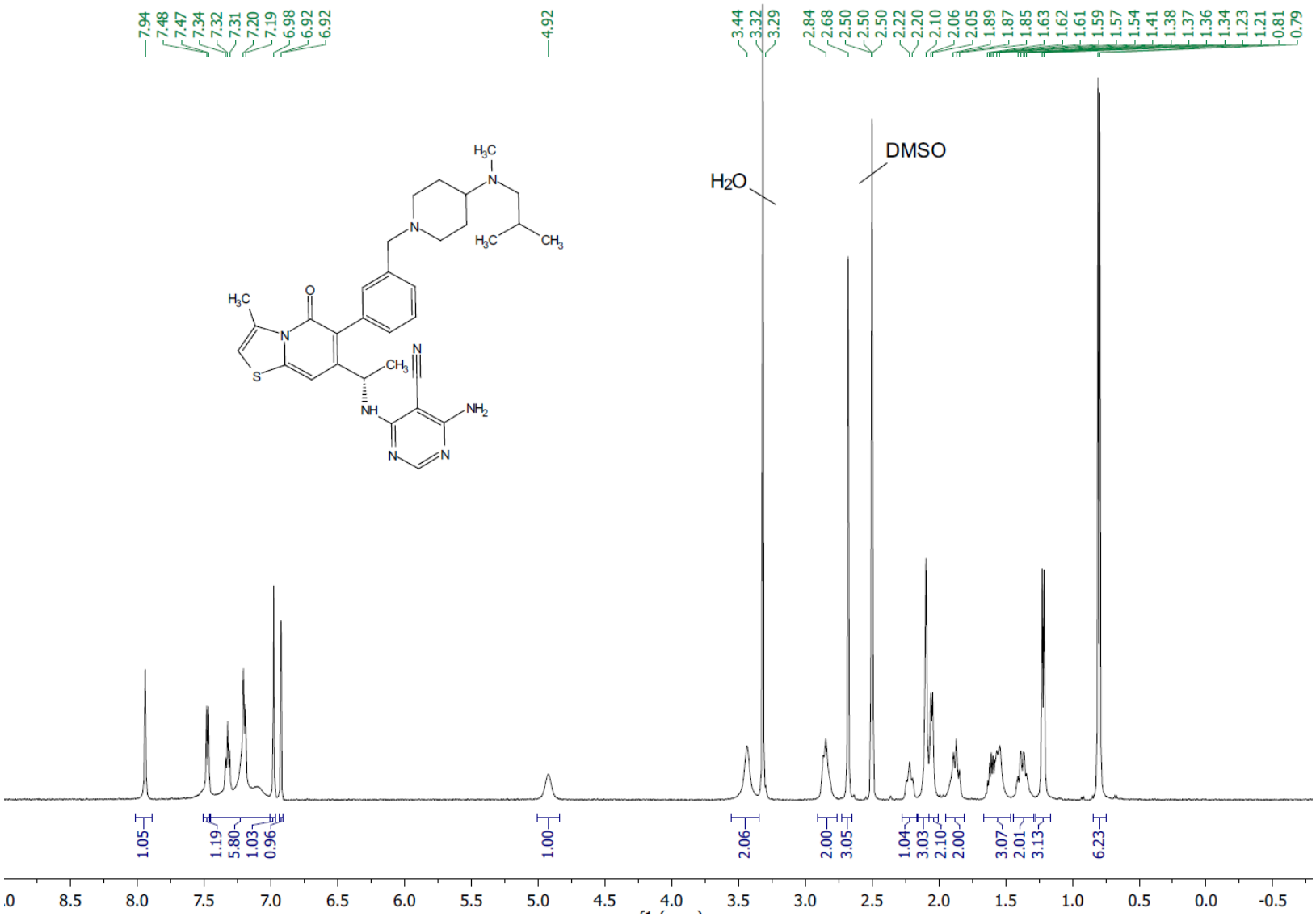
Compound 20d



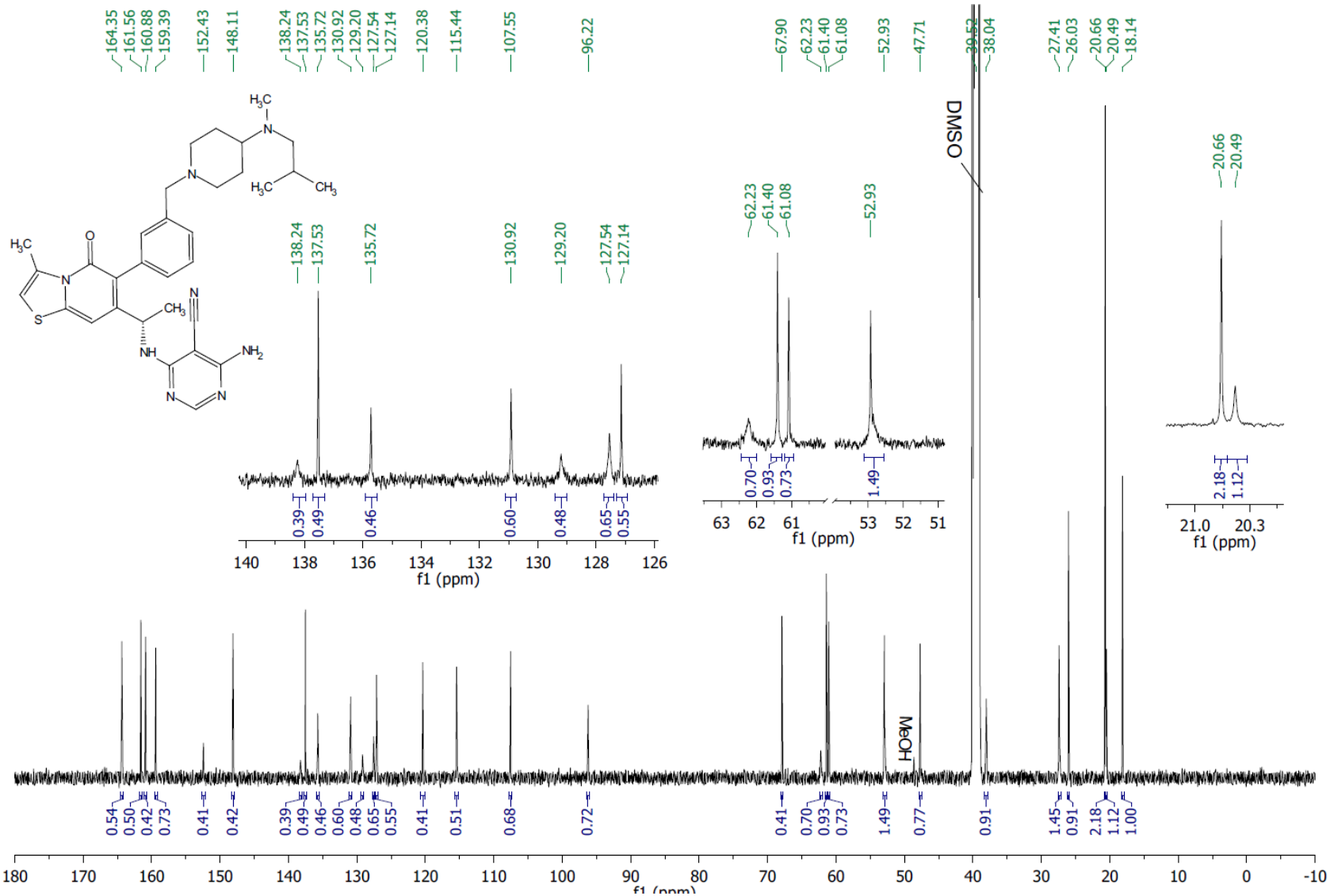
Compound 20e



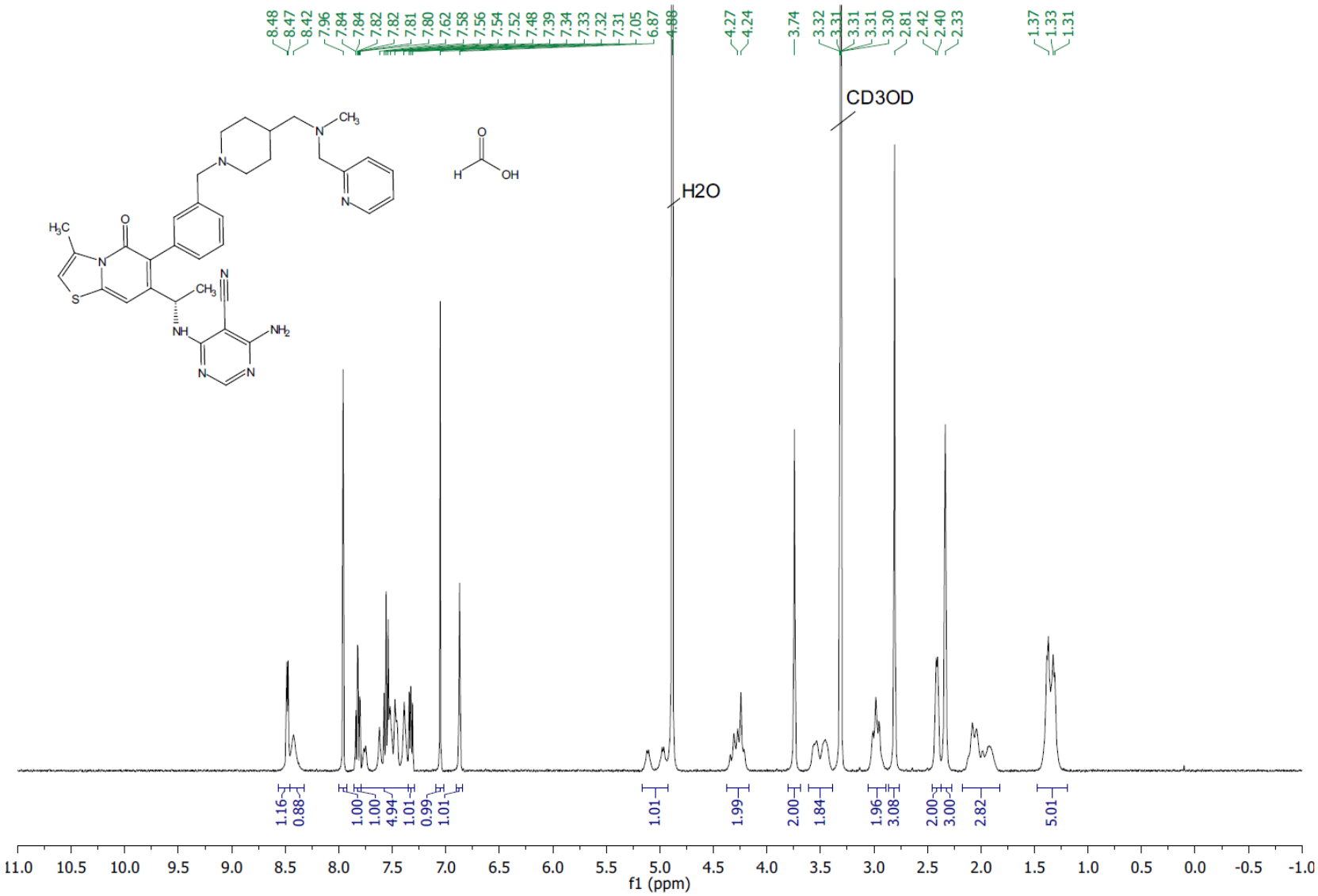
Compound 20f



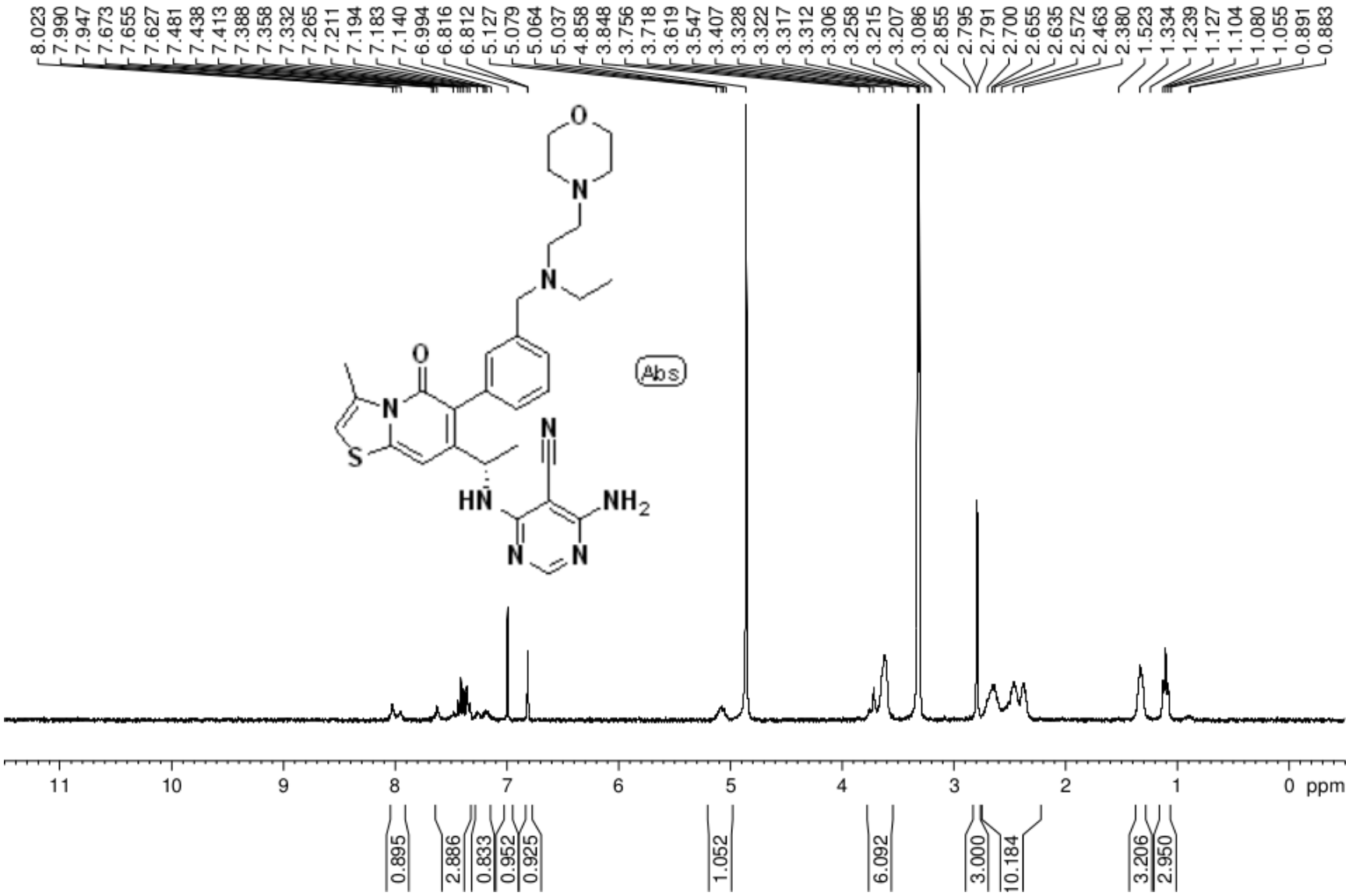
Compound 20f ^{13}C



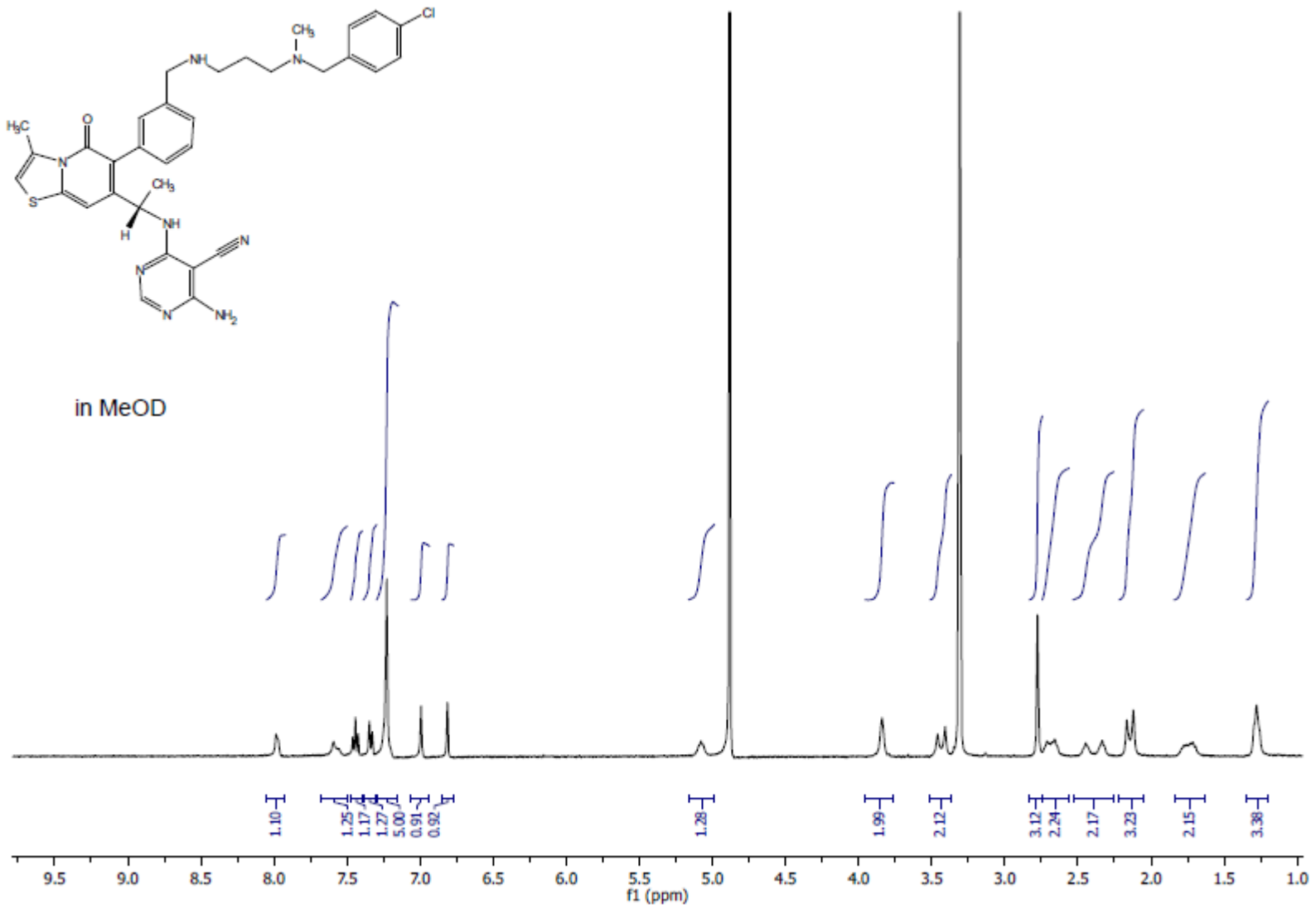
Compound 20g



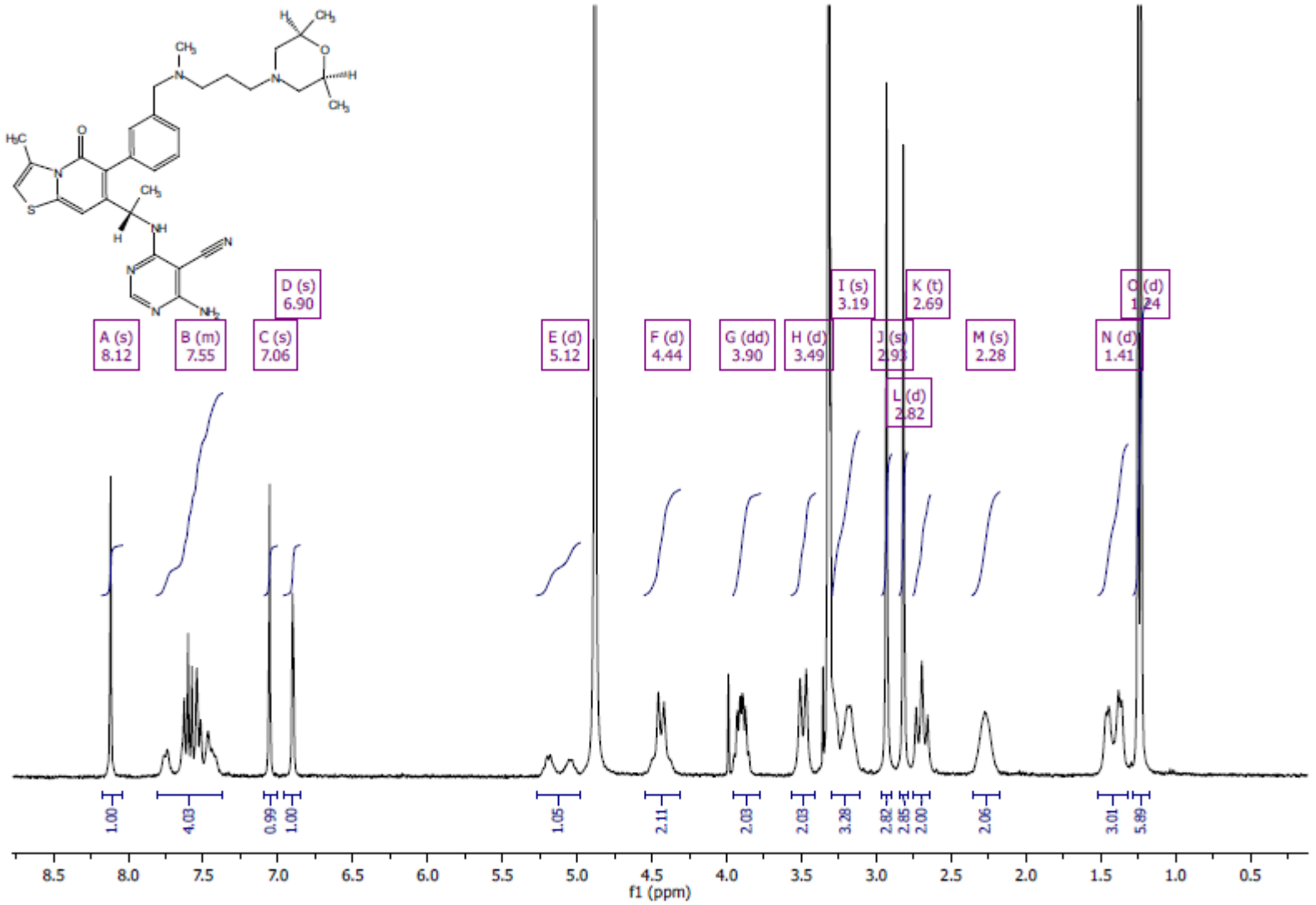
Compound 20h



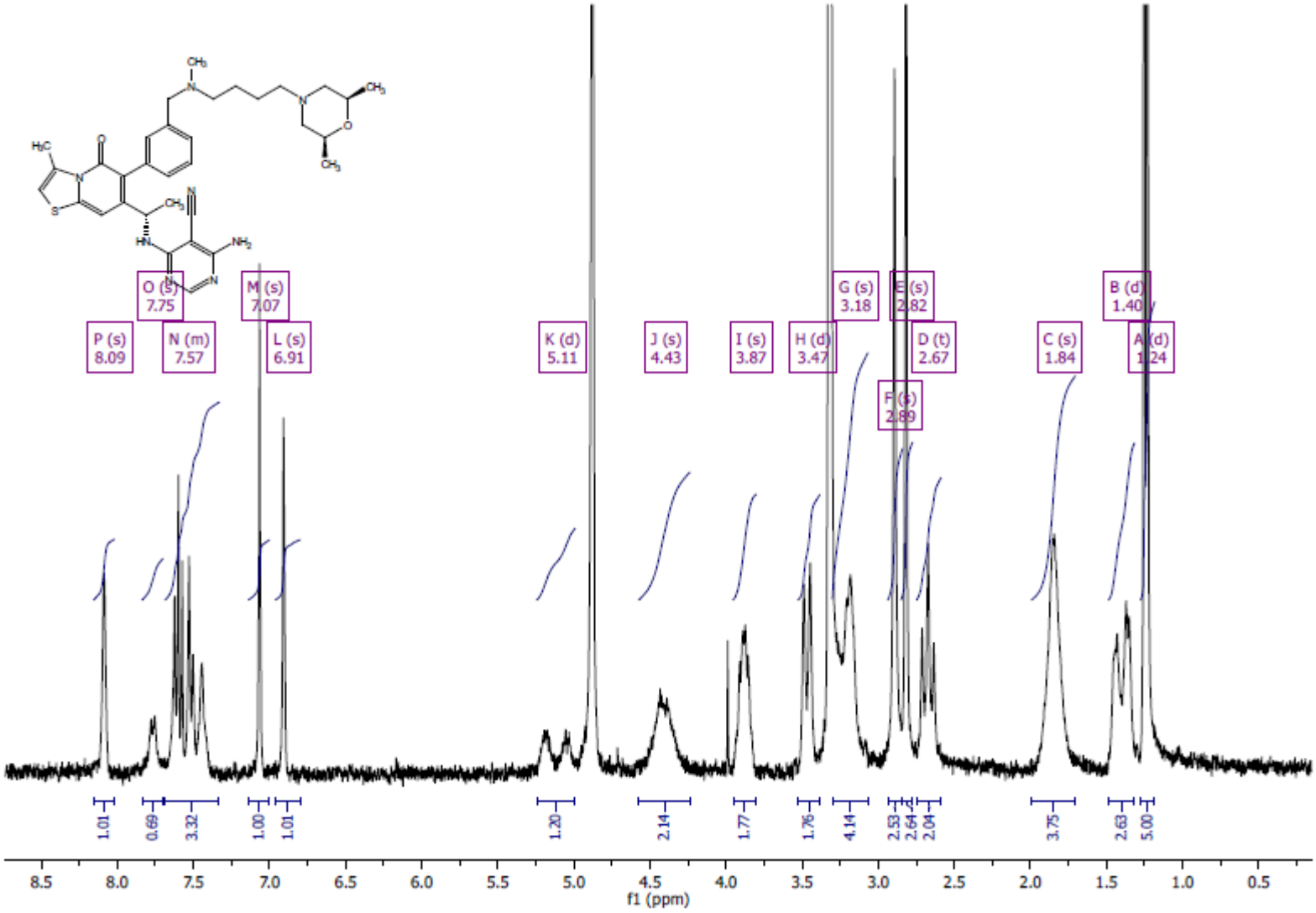
Compound 20i



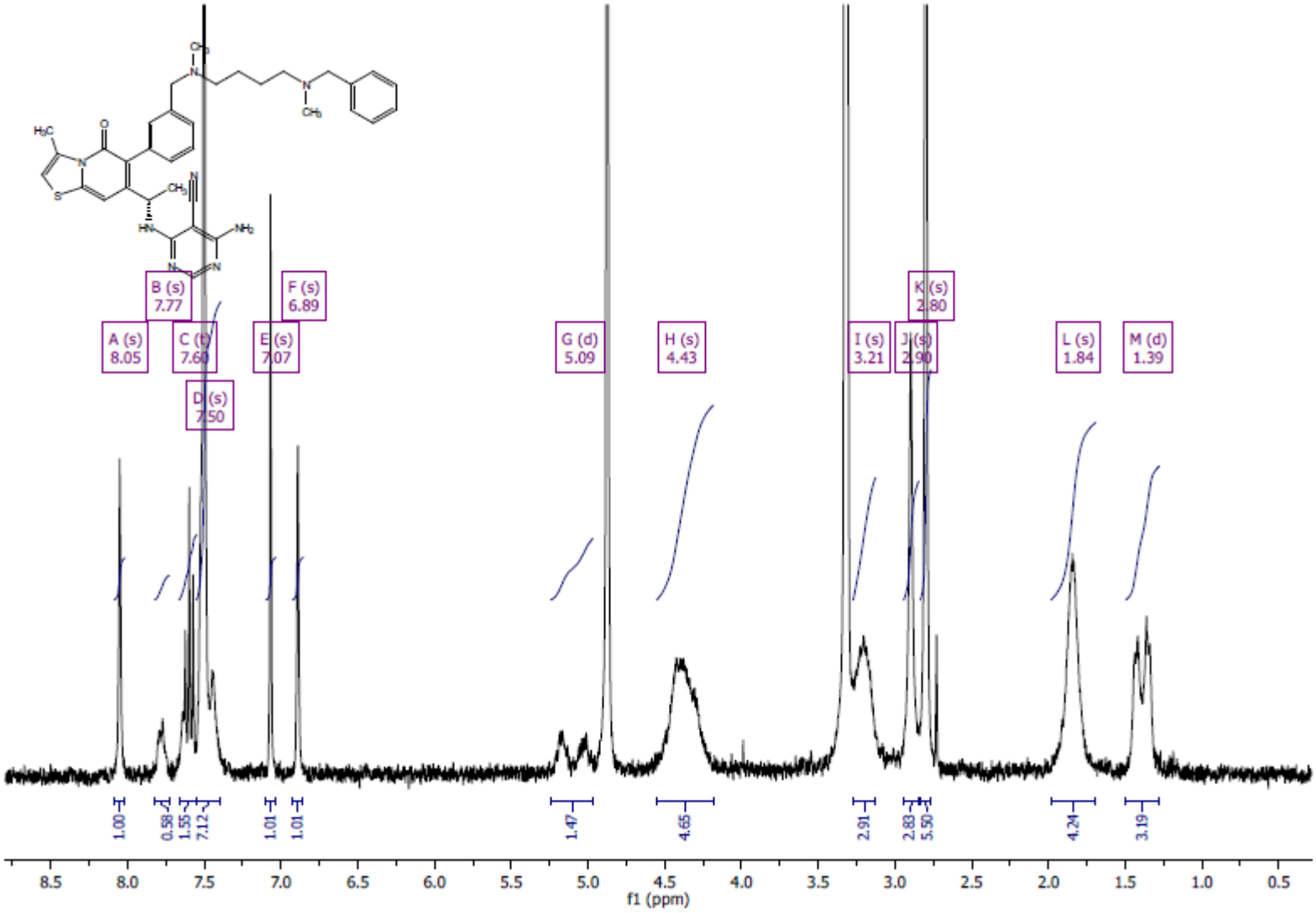
Compound 20j



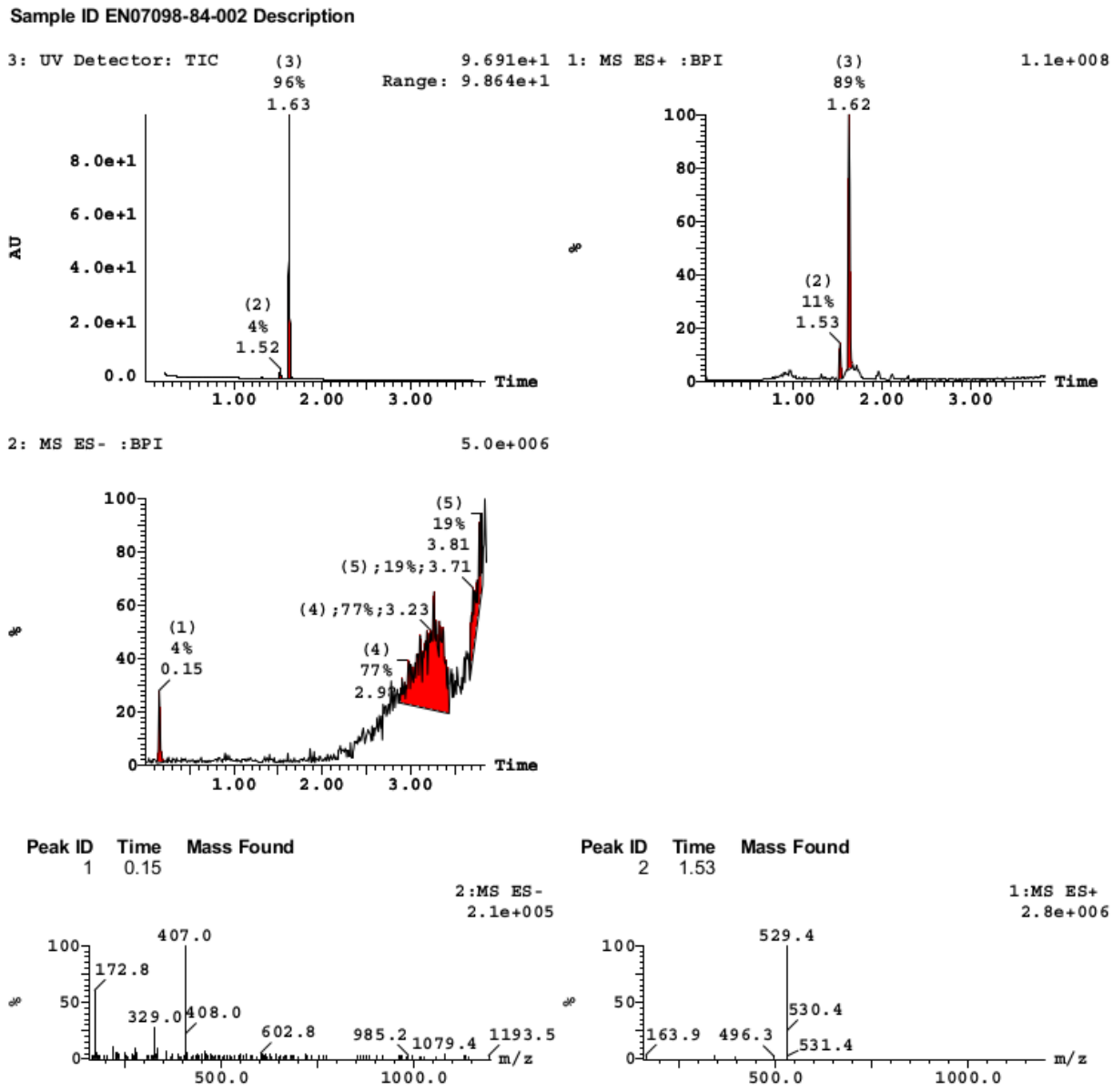
Compound 20k



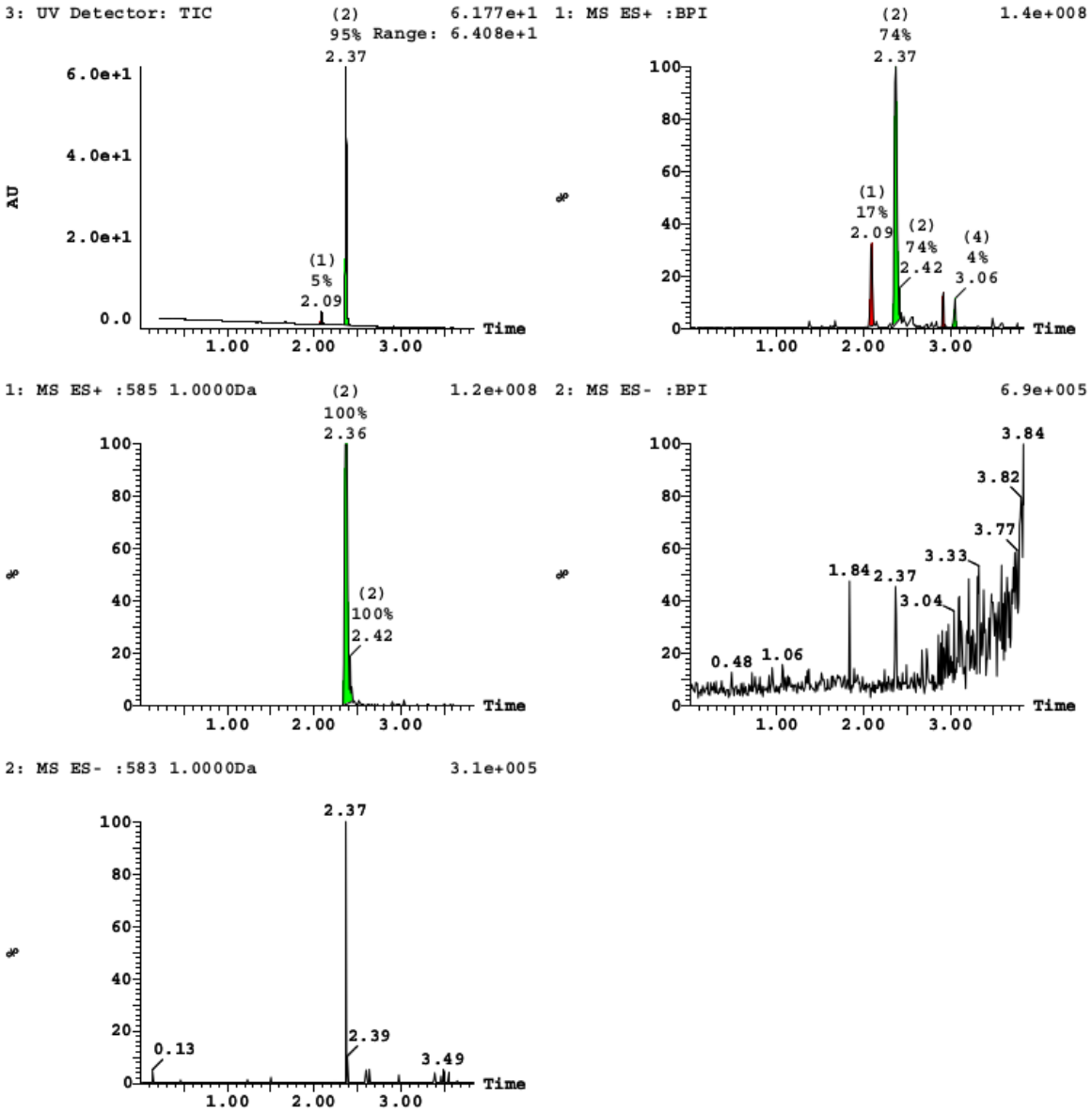
Compound 20l

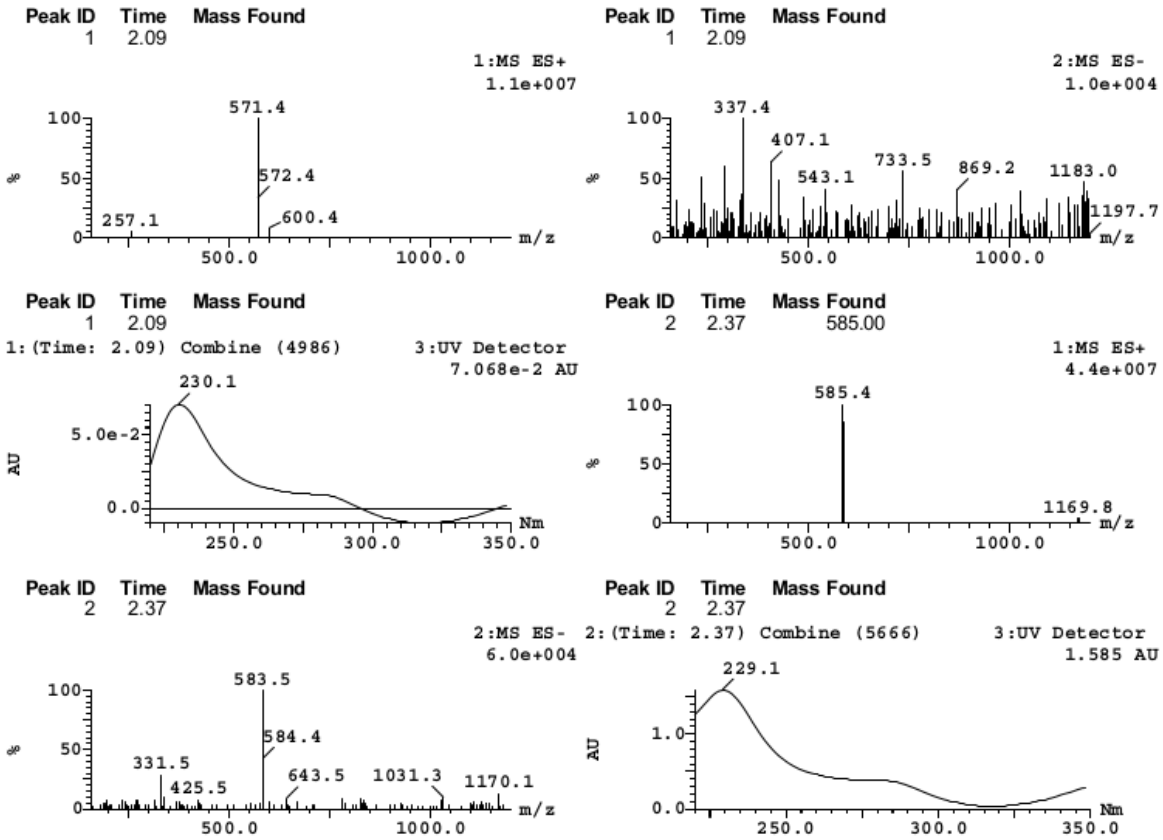


HPLC trace compound 20b



HPLC trace compound 20f




Peak ID Time Mass Found
1 2.09
1:MS ES+
1.1e+007
571.4
572.4
257.1
600.4
m/z
Peak ID Time Mass Found
1 2.09
2:MS ES-
1.0e+004
337.4
407.1
733.5
543.1
869.2
1183.0
1197.7
m/z
Peak ID Time Mass Found
1 2.09
1:(Time: 2.09) Combine (4986)
3:UV Detector
7.068e-2 AU
230.1
5.0e-2
0.0
AU
Nm
Peak ID Time Mass Found
2 2.37 585.00
1:MS ES+
4.4e+007
585.4
1169.8
m/z
Peak ID Time Mass Found
2 2.37
2:MS ES-
6.0e+004
583.5
584.4
331.5
425.5
643.5
1031.3
1170.1
m/z
Peak ID Time Mass Found
2 2.37
2:(Time: 2.37) Combine (5666)
3:UV Detector
1.585 AU
229.1
1.0
0.0
AU
Nm

Determination of the mPI3Kδ crystal structures

The expression and purification of mouse PI3Kδ followed the same procedure as previously described.[1] Compound 13 and 15C were both solubilised to 50 mM in DMSO. In each case, 0.4 µL of compound stock was mixed with 3 µL of 5% w/v n-dodecyl-β-D-maltoside (DDM) from Hampton Research® prior to the addition of 25 µL mPI3Kδ (at 5 mg/mL in 20 mM Tris pH 7.2, 50 mM AmSO4, 1% ethylene glycol, 1% betaine, 0.02% CHAPS and 5 mM DTT). The mixture was left to incubate for 1 hour and then centrifuged at 13.000 rpm for 30 minutes. Hanging drops were set up with a one to one ratio of protein solution and well solution around condition G6 of the Morpheus screen (Molecular Dimensions®): 50% ethylene glycol/PEG 8000, 0.1 M carboxylic acids mix, 0.1 M Buffer system 2 pH 7.5. Streak seeding improved the size and quality of the crystals. For data collection crystals were flash frozen in liquid nitrogen without the addition of any cryo-protection. All data was collected at the European Synchrotron Radiation Facility, France; compound beam line ID23-1 Complex.structures of mPI3Kδ were determined by Molecular replacement using the program "Molrep[2]" using internal structures as search models. Manual fitting and inspection of structures were carried out in the program Coot[3] and refinement carried out using the program Buster[4].

Compound #	13	15c
Space group	*C2*	*C2*
Unit cell dimensions		
a, b, c (Å)	140.98, 64.55, 116.01	141.65, 64.84 116.60
β () $\alpha=\gamma=90°$	103.59	103.63
Beam line	ID23	ID23
Wavelength (Å)	0.970	0.970
Resolution (Å)	50.00-1.90	42.97-1.94
High Resolution Shell (Å)	(1.97-1.90)	(2.01-1.94)
Total / Unique reflections	268 967 (77199)	234743 (73956)
Multiplicity	3.5 (3.4)	3.2 (3.0)
Completeness (%)	96.7 (90.8)	97.0 (95.6)
$\langle I/\sigma I \rangle$	13.6 (1.7)	15.9 (2.5)
R_{merge} (%)[a]	0.058 (0.0.697)	0.038 (0.358)
Refinement		
R_{work}[b] / R_{free}[c] (%)	22.2 / 26.4	22.5 / 25.9
PDB Code	5NCY	5NCZ

[a] $R_{merge} = \Sigma|I-\langle I\rangle|/\Sigma I$, where I is the integrated intensity of a given reflection and $\langle I\rangle$ is the average intensity of multiple observations of symmetry-related reflections. [b] $R_{work} = \Sigma|F_o-F_c|/\Sigma F_o)$, where F_o and F_c are observed and calculated structure factors. [c] R_{free} was calculated from a 5% subset of reflections that were excluded from the refinement. Brackets indicate highest resolution shell.

References

1. Berndt, A., et al. Nat. Chem. Biol. 2010, 6, 117-124

2. Vagin, A., Teplyakov A. J. Appl. Cryst. 1997, 30, 1022-1025.

3. Emsley P., Lohkamp B., Scott W. G. & Cowtan K. 2010 Acta. Cryst D. 66,486-501.

4. Bricogne G., Blanc E., Brandl M., Flensburg C., Keller P., Paciorek W.,Roversi P, Sharff A., Smart O.S., Vonrhein C., Womack T.O. (2016). BUSTER version 2.11.6. Cambridge, United Kingdom: Global Phasing Ltd.