

Supporting Information

Discovery of Highly Isoform Selective Orally Bioavailable Phosphoinositide-3-Kinase (PI3K)- γ Inhibitors.

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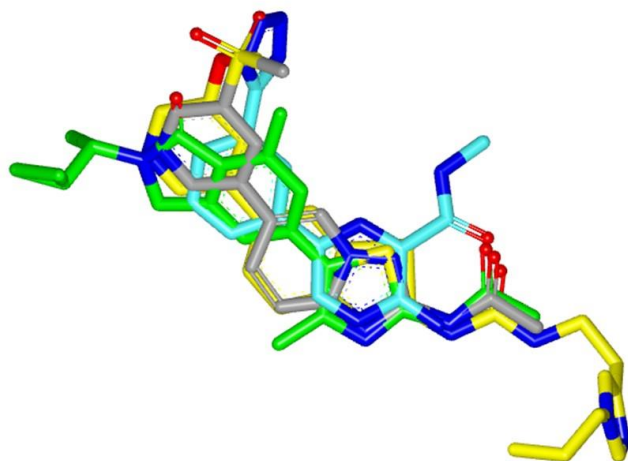


Figure S1. X-ray crystal structure of compound **9** in mouse PI3K δ (ligand in green, protein not shown, PDB code 6FTN) overlaid with PI3K γ structures (only ligands shown) of reported selective PI3K γ inhibitors.¹⁻³ PDB codes: 4ANU (cyan)¹, 4PS3 (yellow)², 4AOF (grey)³.

Table S1. Kinase selectivity profile of compound **9** at 1 μ M.

Kinase Name	Inhibition compound 9 (%)
ABL1	30
ABL2 (Arg)	6
ACVR1B (ALK4)	8
AKT1 (PKB alpha)	6
AKT2 (PKB beta)	12
ALK	2
AMPK A2/B1/G1	5
NUAK1 (ARK5)	14
AURKB (Aurora B)	7
AURKC (Aurora C)	8
AXL	3
BLK	4
BMX	5
BRAF	-4
BTB	5
CAMK1 (CaMK1)	6
CAMK2B (CaMKII beta)	5
CDK1/cyclin B	2
CDK2/cyclin A	11
CDK7/cyclin H/MNAT1	11
CDK8/cyclin C	2
CDK9/cyclin T1	-3
CHEK1 (CHK1)	12
KIT	10
CLK1	15
CLK2	31
CLK4	90
RAF1 (cRAF) Y340D Y341D	19
CSF1R (FMS)	7
CSNK1G1 (CK1 gamma 1)	-1
CSNK2A2 (CK2 alpha 2)	9
DDR2	13
DMPK	1

DNA-PK	14
DYRK2	45
EEF2K	10
EGFR (ErbB1) T790M L858R	2
EGFR (ErbB1)	0
EPHA5	-1
EPHB1	4
EPHB4	1
ERBB2 (HER2)	-2
ERBB4 (HER4)	-1
FER	4
FES (FPS)	-1
FGFR1 V561M	5
FGFR1	-3
FGFR2	0
FGFR3	0
FGFR4	0
FLT1 (VEGFR1)	-9
FLT3	6
FRAP1 (mTOR)	8
FYN	12
GSG2 (Haspin)	-4
GSK3A (GSK3 alpha)	1
GSK3B (GSK3 beta)	-10
IGF1R	12
IKBKB (IKK beta)	-6
INSR	1
INSRR (IRR)	2
IRAK1	25
IRAK4	9
JAK1	1
JAK2	-7
JAK3	-1
MAPK8 (JNK1)	-3
KDR (VEGFR2)	6
LCK	18
LYN A	26
MAP2K1 (MEK1)	13
MAP3K7/MAP3K7IP1 (TAK1-TAB1)	1
MAP3K9 (MLK1)	-4
MAPK1 (ERK2)	6
RPS6KA1 (RSK1)	16
MAPKAPK2	-1
MAPKAPK5 (PRAK)	-6
MARK1 (MARK)	6
MARK2	9
MERTK (cMER)	3
MINK1	24
MKNK2 (MNK2)	31
RPS6KA5 (MSK1)	9
MST1R (RON)	2
MET (cMet)	-4
MYLK (MLCK)	3
NEK2	2
MAPK14 (p38 alpha) Direct	7
RPS6KB1 (p70S6K)	18
PAK1	-9
PAK2 (PAK65)	-5
PAK4	7
PAK7 (KIAA1264)	-5
PDGFRB (PDGFR beta)	12
PDK1	16
PIK3CB/PIK3R1 (p110 beta/p85 alpha)	6
PIK3CD/PIK3R1 (p110 delta/p85 alpha)	60
PIK3CG (p110 gamma)	100
PI4KB (PI4K beta)	46
PIK3C2A (PI3K-C2 alpha)	14
PIK3C3 (hVPS34)	9

PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	16
PIM2	6
PRKACA (PKA)	7
PRKCA (PKC alpha)	6
PRKCE (PKC epsilon)	-7
PRKCQ (PKC theta)	0
PRKG1	3
PLK1	14
PTK2 (FAK)	8
PTK6 (Brk)	1
RET V804M	20
RET V804L	13
RET	7
ROCK1	5
ROCK2	1
ROS1	6
SGK (SGK1)	6
SRC	26
SRPK1	5
STK17A (DRAK1)	98
STK4 (MST1)	7
SYK	26
TBK1	9
TGFBR1 (ALK5)	-1
TNIK	22
NTRK1 (TRKA)	16
NTRK2 (TRKB)	1
TYRO3 (RSE)	2
ULK2	1
YES1	5
ZAP70	-3

Table S2. Kinase selectivity profile of compound **15** at 1 μ M.

Kinase name	Inhibition compound 15 (%)
AAK1	3
ABL1	-1
ABL2 (Arg)	4
ACVR1 (ALK2)	-5
ACVR1B (ALK4)	7
ACVR2A	5
ACVR2B	3
ACVRL1 (ALK1)	-10
ADCK3	6
ADRBK1 (GRK2)	8
ADRBK2 (GRK3)	-10
AKT1 (PKB alpha)	-4
AKT2 (PKB beta)	2
AKT3 (PKB gamma)	-1
ALK	-1
AMPK (A1/B1/G2)	-2
AMPK (A1/B1/G3)	-2
AMPK (A1/B2/G1)	4
AMPK (A1/B2/G3)	9
AMPK (A2/B1/G2)	8
AMPK (A2/B1/G3)	4
AMPK (A2/B2/G1)	-1
AMPK (A2/B2/G2)	0
AMPK (A2/B2/G3)	6
AMPK A1/B1/G1	5
AMPK A2/B1/G1	14
ANKK1	11
AURKA (Aurora A)	-2
AURKB (Aurora B)	9
AURKC (Aurora C)	1

AXL	-7
BLK	8
BMPRI1A (ALK3)	2
BMPRI2	4
BMX	14
BRAF	31
BRAF	0
BRSK1 (SAD1)	2
BRSK2	-4
BTK	6
CAMK1 (CaMK1)	-10
CAMK1D (CaMKI delta)	1
CAMK1G (CaMKI gamma)	3
CAMK2A (CaMKII alpha)	-13
CAMK2B (CaMKII beta)	-1
CAMK2D (CaMKII delta)	-4
CAMK2G (CaMKII gamma)	0
CAMK4 (CaMKIV)	-1
CAMKK1 (CaMKK)	-3
CAMKK2 (CaMKK beta)	0
CASK	2
CDC42 BPA (MRCKA)	-6
CDC42 BPB (MRCKB)	-7
CDC42 BPG (MRCKG)	-3
CDC7/DBF4	-5
CDK1/cyclin B	1
CDK11 (Inactive)	-1
CDK14 (PFTK1)/cyclin Y	1
CDK16 (PCTK1)/cyclin Y	-10
CDK17/cyclin Y	8
CDK18/cyclin Y	1
CDK2/cyclin A	-2
CDK2/cyclin A1	7
CDK2/cyclin E1	-1
CDK2/cyclin O	10
CDK3/cyclin E1	2
CDK5 (Inactive)	33
CDK5/p25	3
CDK5/p35	-2
CDK7/cyclin H/MNAT1	-4
CDK8/cyclin C	-9
CDK9 (Inactive)	-3
CDK9/cyclin K	-3
CDK9/cyclin T1	0
CDKL5	14
CHEK1 (CHK1)	12
CHEK2 (CHK2)	2
CHUK (IKK alpha)	-1
CLK1	2
CLK2	13
CLK3	-8
CLK4	32
CSF1R (FMS)	24
CSK	7
CSNK1A1 (CK1 alpha 1)	6
CSNK1A1L	4
CSNK1D (CK1 delta)	0
CSNK1E (CK1 epsilon)	3
CSNK1G1 (CK1 gamma 1)	3
CSNK1G2 (CK1 gamma 2)	7
CSNK1G3 (CK1 gamma 3)	2
CSNK2A1 (CK2 alpha 1)	9
CSNK2A2 (CK2 alpha 2)	8
DAPK1	2
DAPK2	-1
DAPK3 (ZIPK)	-3
DCAMKL1 (DCLK1)	2
DCAMKL2 (DCK2)	2

DDR1	-1
DDR2	-2
DMPK	-2
DNA-PK	3
DYRK1A	11
DYRK1B	6
DYRK2	15
DYRK3	4
DYRK4	0
EEF2K	6
EGFR (ErbB1)	-6
EIF2AK2 (PKR)	-2
EPHA1	11
EPHA2	5
EPHA3	6
EPHA4	9
EPHA5	-1
EPHA6	1
EPHA7	-8
EPHA8	6
EPHB1	2
EPHB2	3
EPHB3	-1
EPHB4	0
ERBB2 (HER2)	-13
ERBB4 (HER4)	-5
ERN1	4
ERN2	2
FER	13
FES (FPS)	6
FGFR1	-7
FGFR2	6
FGFR3	-4
FGFR4	0
FGR	0
FLT1 (VEGFR1)	5
FLT3	17
FLT4 (VEGFR3)	8
FRAP1 (mTOR)	24
FRK (PTK5)	4
FYN	13
FYN A	3
GAK	2
GRK1	3
GRK4	4
GRK5	0
GRK6	4
GRK7	0
GSG2 (Haspin)	2
GSK3A (GSK3 alpha)	3
GSK3B (GSK3 beta)	2
HCK	9
HIPK1 (Myak)	-3
HIPK2	9
HIPK3 (YAK1)	3
HIPK4	11
HUNK	-1
ICK	-2
IGF1R	13
IKBKB (IKK beta)	7
IKBKE (IKK epsilon)	2
INSR	-3
INSRR (IRR)	14
IRAK1	2
IRAK3	5
IRAK4	7
ITK	-3
JAK1	-2

JAK2	-7
JAK2 JH1 JH2	1
JAK3	3
KDR (VEGFR2)	2
KIT	1
KSR2	1
LATS1	1
LATS2	0
LCK	10
LIMK1	-3
LIMK2	4
LRRK2	6
LRRK2 FL	0
LTK (TYK1)	2
LYN A	-5
LYN B	2
MAP2K1 (MEK1)	33
MAP2K1 (MEK1)	4
MAP2K2 (MEK2)	2
MAP2K2 (MEK2)	27
MAP2K3 (MEK3)	-9
MAP2K4 (MEK4)	-6
MAP2K5 (MEK5)	3
MAP2K6 (MKK6)	4
MAP2K6 (MKK6)	0
MAP3K10 (MLK2)	1
MAP3K11 (MLK3)	1
MAP3K14 (NIK)	-5
MAP3K19 (YSK4)	1
MAP3K2 (MEKK2)	-3
MAP3K3 (MEKK3)	0
MAP3K5 (ASK1)	0
MAP3K7/MAP3K7IP1 (TAK1-TAB1)	-4
MAP3K8 (COT)	17
MAP3K9 (MLK1)	-3
MAP4K1 (HPK1)	0
MAP4K2 (GCK)	-3
MAP4K3 (GLK)	6
MAP4K4 (HGK)	13
MAP4K5 (KHS1)	24
MAPK1 (ERK2)	11
MAPK10 (JNK3)	2
MAPK10 (JNK3)	2
MAPK11 (p38 beta)	4
MAPK12 (p38 gamma)	19
MAPK13 (p38 delta)	7
MAPK14 (p38 alpha)	-6
MAPK14 (p38 alpha) Direct	8
MAPK15 (ERK7)	2
MAPK3 (ERK1)	6
MAPK7 (ERK5)	10
MAPK8 (JNK1)	-6
MAPK8 (JNK1)	8
MAPK9 (JNK2)	1
MAPK9 (JNK2)	25
MAPKAPK2	5
MAPKAPK3	6
MAPKAPK5 (PRAK)	8
MARK1 (MARK)	16
MARK2	8
MARK3	4
MARK4	3
MASTL	-5
MATK (HYL)	1
MELK	10
MERTK (cMER)	-2
MET (cMet)	-5
MINK1	26

MKNK1 (MNK1)	13
MKNK2 (MNK2)	-18
MLCK (MLCK2)	2
MLK4	1
MST1R (RON)	11
MST4	7
MUSK	12
MYLK (MLCK)	0
MYLK2 (skMLCK)	-4
MYLK4	8
MYO3A (MYO3 alpha)	2
MYO3B (MYO3 beta)	5
NEK1	6
NEK2	6
NEK4	5
NEK6	-3
NEK7	7
NEK8	-2
NEK9	6
NIM1K	2
NLK	-1
NTRK1 (TRKA)	12
NTRK2 (TRKB)	-17
NTRK3 (TRKC)	-1
NUAK1 (ARK5)	6
NUAK2	-11
OXSRI	-13
PAK1	5
PAK2 (PAK65)	26
PAK3	8
PAK4	17
PAK6	3
PAK7 (KIAA1264)	2
PASK	2
PDGFRA (PDGFR alpha)	8
PDGFRB (PDGFR beta)	5
PDK1	21
PDK1 Direct	13
PEAK1	2
PHKG1	-5
PHKG2	7
PI4K2A (PI4K2 alpha)	1
PI4K2B (PI4K2 beta)	0
PI4KA (PI4K alpha)	7
PI4KB (PI4K beta)	17
PIK3C2A (PI3K-C2 alpha)	4
PIK3C2B (PI3K-C2 beta)	84
PIK3C2G (PI3K-C2 gamma)	71
PIK3C3 (hVPS34)	17
PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	29
PIK3CA/PIK3R3 (p110 alpha/p55 gamma)	34
PIK3CB/PIK3R1 (p110 beta/p85 alpha)	9
PIK3CB/PIK3R2 (p110 beta/p85 beta)	13
PIK3CD/PIK3R1 (p110 delta/p85 alpha)	66
PIK3CG (p110 gamma)	95
PIM1	-6
PIM2	9
PIM3	11
PKMYT1	-12
PKN1 (PRK1)	3
PKN2 (PRK2)	-5
PLK1	7
PLK2	-3
PLK3	16
PLK4	8
PRKACA (PKA)	4
PRKACB (PRKAC beta)	7
PRKACG (PRKAC gamma)	-3

PRKCA (PKC alpha)	-16
PRKCB1 (PKC beta I)	7
PRKCB2 (PKC beta II)	-13
PRKCD (PKC delta)	-4
PRKCE (PKC epsilon)	-18
PRKCG (PKC gamma)	6
PRKCH (PKC eta)	-20
PRKCI (PKC iota)	4
PRKCN (PKD3)	9
PRKCQ (PKC theta)	23
PRKCZ (PKC zeta)	3
PRKD1 (PKC mu)	5
PRKD2 (PKD2)	4
PRKG1	2
PRKG2 (PKG2)	12
PRKX	3
PTK2 (FAK)	16
PTK2B (FAK2)	2
PTK6 (Brk)	7
RET	-4
RIPK2	1
RIPK3	1
ROCK1	10
ROCK2	-4
ROS1	5
RPS6KA1 (RSK1)	6
RPS6KA2 (RSK3)	5
RPS6KA3 (RSK2)	-10
RPS6KA4 (MSK2)	4
RPS6KA5 (MSK1)	2
RPS6KA6 (RSK4)	-7
RPS6KB1 (p70S6K)	1
RPS6KB2 (p70S6Kb)	12
SBK1	4
SGK (SGK1)	-2
SGK2	-2
SGKL (SGK3)	5
SIK1	-2
SIK3	-19
SLK	-2
SNF1LK2	11
SPHK1	5
SPHK2	15
SRC	4
SRMS (Srm)	-3
SRPK1	7
SRPK2	-1
STK16 (PKL12)	9
STK17A (DRAK1)	14
STK17B (DRAK2)	13
STK22B (TSSK2)	9
STK22D (TSSK1)	11
STK23 (MSSK1)	5
STK24 (MST3)	-1
STK25 (YSK1)	12
STK3 (MST2)	-2
STK32B (YANK2)	-5
STK32C (YANK3)	3
STK33	3
STK38 (NDR)	-4
STK38L (NDR2)	1
STK39 (STLK3)	-15
STK4 (MST1)	0
SYK	-2
TAOK1	0
TAOK2 (TAO1)	-5
TAOK3 (JIK)	3
TBK1	5

TEC	4
TEK (Tie2)	7
TESK1	-8
TESK2	-19
TGFBR1 (ALK5)	4
TGFBR2	-5
TLK1	-2
TLK2	-3
TNIK	0
TNK1	5
TNK2 (ACK)	-2
TTK	-8
TXK	3
TYK2	7
TYRO3 (RSE)	-2
ULK1	-6
ULK2	3
ULK3	0
VRK2	7
WEE1	-2
WNK2	-4
WNK3	-2
YES1	16
ZAK	0
ZAP70	0

Table S3. Selectivity profiling of compound **15** towards class II and class III PI3Ks (Thermo Fisher).

kinase	ATP conc (μ M)	pIC ₅₀ (μ M)	n
PIK3C2 α FRET-Adapta CR	25 (Km app)	<5	2
PIK3C2 β FRET-Adapta CR	100 (Km app not available)	7.5	3
PIK3C2 γ FRET-Adapta CR	150 (Km app)	5.5	2
PIK3C3 (hVPS34) *Mn* Adapta CR	10 (Km app)	5.1	3
PIK3C γ (p110 gamma) Adapta CR	25 (Km app)	>9.2	2

Table S4. Selectivity profiling of compound **15** (CEREP).

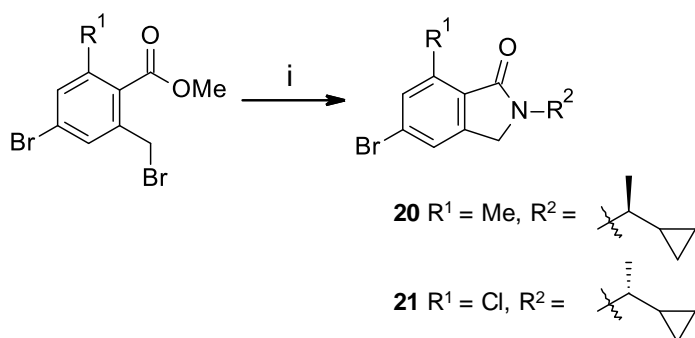
Target_Name	15 K _i or IC ₅₀ (μ M)
P2Y1 Receptor	0.55
5-Hydroxytryptamine Receptor 2B	1.1
PDE10A1	1.6
Adenosine Transporter	3.4
Fibroblast Growth Factor Receptor 1	6.3
Adenosine Receptor A2a	6.7
Phosphodiesterase 4D	10.7
Phosphodiesterase 2A	8.4
Adenosine Receptor A1	12.2
Neurokinin Receptor 2	11.9
GABAA A1B2G2 Receptor	18.1
Pyruvate dehydrogenase kinase, isoenzyme 1	29
Phosphoinositide 3 Kinase Alpha (PI3K Alpha)	29.6

Translocator Protein (18kDa)		29.8
Matrix Metalloproteinase 2	>30	
Cathepsin S	>30	
Somatostatin Receptor 4	>30	
Adrenergic Receptor Alpha 1A		30.1
Phosphodiesterase 6		31
Glycine receptor?		56.6
GABAA Receptor?		68.9
Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)		63.2
Calcium Channel, Voltage dependent, L-type (Diltizem)		71.9
TXA2 synthase		89.9
v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog		98.9
Aurora Kinase A	>100	
Opioid Receptor Kappa 1	>100	
Muscarinic Acetylcholine Receptor 1	>100	
Adrenergic Receptor Alpha 2C	>100	
Adrenergic Receptor Beta 2	>100	
Endothelin Converting Enzyme 1	>100	
Monoamine Oxidase B	>100	
Adrenergic Receptor Alpha 2A	>100	
Neurokinin Receptor 1	>100	
Peroxisome proliferative activated receptor, gamma	>100	
Opioid Receptor Mu 1	>100	
Sigma1	>100	
Muscarinic Acetylcholine Receptor 2	>100	
Cannabinoid Receptor 1	>100	
Delta 2 opioid receptor	>100	
5-Hydroxytryptamine Receptor 1A	>100	
Norepinephrine Transporter	>100	
Nicotinic Acetylcholine Receptor Alpha 7	>100	
Rho Associated, Coiled Coil Containing Protein Kinase 1	>100	
ALK4	>100	
Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	>100	
Retinoic Acid Receptor Alpha	>100	
Nicotinic Acetylcholine Receptor Alpha 1	>100	
Ghrelin Receptor	>100	
NMDA receptor (Agonism)	>100	
Nicotinic Acetylcholine Receptor Alpha 4	>100	
5-Hydroxytryptamine Receptor 4	>100	
5-Hydroxytryptamine Receptor 3A	>100	
Dopamine Receptor D3	>100	
Muscarinic Acetylcholine Receptor 5	>100	
Melatonin Receptor 2	>100	
Dopamine Receptor D1	>100	
GABA Receptor?	>100	
Dopamine Receptor D2	>100	
5-Hydroxytryptamine Receptor 2C	>100	
5-Hydroxytryptamine Receptor 1B	>100	
Kinase Insert Domain Receptor	>100	
Angiotensin II Receptor AT1	>100	
Rho Associated, Coiled Coil Containing Protein Kinase 2	>100	
eNOS	>100	
Na ⁺ /K ⁺ transporting ATPase	>100	

Calcium Channel, Voltage dependent, L-type (Verapamil)	>100
Glucocorticoid Receptor	>100
Neurotrophic Receptor Tyrosine Kinase Type 1	>100
Acetylcholinesterase	>100
Histamine Receptor H1	>100
Phosphodiesterase 3?	>100
Mitogen-Activated Protein Kinase Kinase Kinase 7	>100
GABAB Receptor	>100
5-Hydroxytryptamine Receptor 1D	>100
Serotonin Transporter	>100
NMDA receptor (Phencyclidine)	>100
EGFR kinase	>100
Histamine Receptor H2	>100
Insulin Receptor	>100
cKIT	>100
5-Hydroxytryptamine Receptor 7	>100
Bradykinin Receptor 2	>100
Glycogen Synthase Kinase-3 beta	>100
Adrenergic Receptor Alpha 1B	>100
Arginase, Type 1	>100
Dopamine Transporter	>100
Endothelin Receptor A	>100
Adrenergic Receptor Beta 1	>100

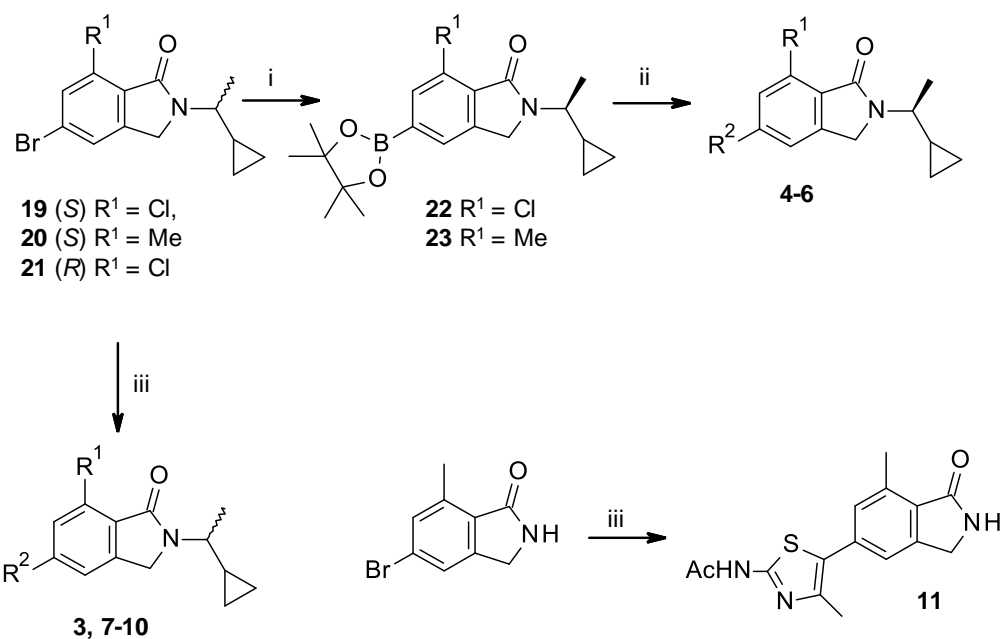
Schemes depicting synthetic routes

Scheme S1. Preparation of compounds **20**, **21**.^a



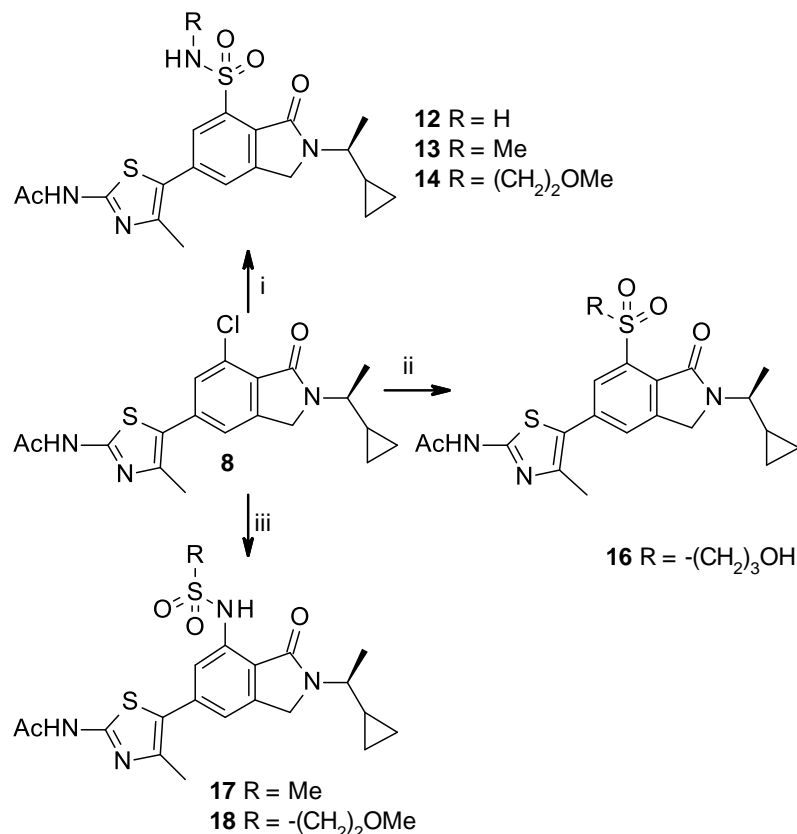
^a Reagents and conditions: (i) For **20**: B(OH)₃, K₂CO₃, MeCN, rt. For **21**: K₂CO₃, DMF, 80°C.

Scheme S2. Preparation of compounds **3** – **11**.^a



^a Reagents and conditions: (i) $\text{Pd}(\text{PPh}_3)_4$, B_2Pin_2 , KOAc, DMSO, 100°C . (ii) Suzuki coupling see experimental section below for further details. (iii) For **3**: Heteroarylboronate, $\text{PdCl}_2(\text{dtbpf})$, K_2CO_3 , water:dioxane 1:1, 70°C . For **7-11**: acetylated aminothiazole, $\text{Pd}(\text{OAc})_2$, $[(t\text{-Bu})_3\text{PH}]\text{BF}_4$, Cs_2CO_3 , DMF, $90\text{-}110^\circ\text{C}$.

Scheme S3. Preparation of sulfonamides **12-14**, sulfone **16** and reversed sulfonamides **17, 18**.



^a Reagents and conditions: (i) a) Phenylmethanethiol, sodium tert-pentoxide, DMF, 110°C. b) SO₂Cl₂, H₂O, AcOH, MeCN, 0°C. c) amine. DCM, rt. (ii) a) 3-mercaptopropan-1-ol, sodium tert-pentoxide, DMF, 110°C. b) *m*CPBA, DCM, 0°C. (iii) Buchwald-Hartwig coupling see experimental details below for further details.

Experimental Section

General Procedures. All chemicals purchased from commercial suppliers were used as received. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen atmosphere using dry solvents. All compounds were purified to ≥95% purity as judged by HPLC with UV and MS analysis. Proton (¹H) and carbon (¹³C) NMR spectra were recorded at ambient temperature. Solutions were typically prepared in deuterated dimethylsulfoxide (DMSO-d₆), deuteromethanol (CD₃OD), or deuteriochloroform (CDCl₃), with chemical shifts referenced to solvent as an internal standard. ¹H NMR data are reported indicating the chemical shift (δ), the multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; etc.), the coupling constant (J) in Hz, and the integration (e.g., 1H). MS experiments were performed on

a Waters Acquity, Waters 2777, or Waters 2700 UV–HPLC system and a Waters Xevo G2 TOF, Waters LCT Premiere, Waters LCT, or Waters QTOFmicro mass spectrometer. Compounds were named using IUPAC Name ACD (version 10.06).

5-(2-Aminopyrimidin-5-yl)-7-chloro-2-[(1S)-1-cyclopropylethyl]-2,3-dihydro-1H-isoindol-1-one (3).

A flask containing K₂CO₃ (277 mg, 2.00 mmol), **19** (210 mg, 0.67 mmol) and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-amine (148 mg, 0.67 mmol) in water (2 mL) and dioxane (2 mL) was degassed. PdCl₂(dtbpf) (21 mg, 0.03 mmol) was added and the resulting mixture was heated to 70°C. After 1h the reaction mixture was diluted with EtOAc and washed sequentially with saturated NaHCO₃ and brine. The organic phase was concentrated and the residue was chromatographed (silica gel; 70 to 100% of EtOAc in heptane followed by an isocratic gradient EtOAc with 10% EtOH) to afford the title compound (100 mg, 46%) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ 0.39 – 0.52 (m, 3H), 0.61 – 0.7 (m, 1H), 0.96 – 1.09 (m, 1H), 1.37 (d, *J* = 6.8 Hz, 3H), 3.71 – 3.85 (m, 1H), 4.45 (d, *J* = 17.3 Hz, 1H), 4.56 (d, *J* = 17.3 Hz, 1H), 5.24 (s, 2H), 7.45 (s, 1H), 7.51 (s, 1H), 8.56 (s, 2H). HRMS: *m/z* calcd 329.1169, found 329.1187.

5-(3-Aminopyrazin-2-yl)-2-[(1S)-1-cyclopropylethyl]-7-methyl-2,3-dihydro-1H-isoindol-1-one (4).

A flask containing K₂CO₃ (71 mg, 0.51 mmol), **23** (58 mg, 0.17 mmol) and 3-chloropyrazin-2-amine (22 mg, 0.17 mmol) in water (1 mL) and dioxane (1 mL) was degassed. PdCl₂(dtbpf) (5 mg, 0.09 mmol) was added and the resulting mixture was heated to 70°C for 1.5 h. The reaction mixture was diluted with EtOAc and washed sequentially with saturated NaHCO₃ and brine. The organic phase was concentrated and the residue was purified by preparative HPLC to give the title compound (16 mg, 31%). ¹H NMR (600 MHz, DMSO-d₆) δ 0.17 – 0.26 (m, 1H), 0.33 – 0.45 (m, 2H), 0.54 – 0.63 (m, 1H), 1.06 – 1.18 (m, 1H), 1.28 (d, *J* = 6.8 Hz, 3H), 2.66 (s, 3H),

3.55 – 3.63 (m, 1H), 4.50 (d, $J = 17.8$ Hz, 1H), 4.54 (d, $J = 17.8$ Hz, 1H), 6.24 (s, 2H), 7.48 (s, 1H), 7.66 (s, 1H), 7.88 (d, $J = 2.6$ Hz, 1H), 7.96 (d, $J = 2.6$ Hz, 1H). HRMS: m/z calcd 309.1715, found 309.1729.

2-[(1S)-1-Cyclopropylethyl]-7-methyl-5-(5H-pyrrolo[2,3-b]pyrazin-2-yl)-2,3-dihydro-1H-isoindol-1-one (5).

A microwave vial was charged with 2-bromo-5H-pyrrolo[2,3-b]pyrazine (232 mg, 1.17 mmol), **23** (400 mg, 1.17 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (82 mg, 0.12 mmol), 1M aqueous Na_2CO_3 (3.52 mL) and acetonitrile (7 mL). The vial was sealed, evacuated and purged three times with nitrogen and heated to 140°C for 30 minutes in a microwave reactor. Cooled to rt, EtOAc and water were added, organic phase separated and the aqueous phase was extracted with DCM. The combined organic layers were filtered through a phase separator and concentrated. The residue was chromatographed (silica gel; 20 to 100% of EtOAc in heptane) and the obtained material was further purified by preparative HPLC to give the title compound (37 mg, 10%). ^1H NMR (600 MHz, DMSO- d_6) δ 0.21 – 0.26 (m, 1H), 0.35 – 0.43 (m, 2H), 0.53 – 0.64 (m, 1H), 1.10 – 1.17 (m 1H), 1.29 (d, $J = 6.8$ Hz, 3H), 2.70 (s, 3H), 3.54 – 3.67 (m, 1H), 4.56 (s, 2H), 6.70 (d, $J = 3.4$ Hz, 1H), 7.93 (d, $J = 3.4$ Hz, 1H), 8.00 (s, 1H), 8.13 (s, 1H), 8.91 (s, 1H), 12.14 (s, 1H). HRMS: m/z calcd 333.1715, found 333.1697.

3-Amino-6-{2-[(1S)-1-cyclopropylethyl]-7-methyl-1-oxo-2,3-dihydro-1H-isoindol-5-yl}-N-methylpyrazine-2-carboxamide (6).

$\text{PdCl}_2(\text{dppf})$ (30 mg, 0.04 mmol) was added to a microwave vial containing **23** (140 mg, 0.41 mmol), Cs_2CO_3 (535 mg, 1.64 mmol), and 3-amino-6-bromo-*N*-methylpyrazine-2-carboxamide (95 mg, 0.41 mmol) in DMF (4 mL) and water (0.05 mL). The vial was sealed and heated to 100°C for 30 min in a microwave reactor. The crude reaction mixture was filtered through Celite and washed with DCM. The filtrate was concentrated and chromatographed (silica gel; 50 to 100% of EtOAc in heptane) and the obtained material was further purified by

preparative HPLC to give the title compound (27 mg, 18%). NMR (600 MHz, DMSO-d₆) δ 0.21-0.26 (m, 1H), 0.35 – 0.43 (m, 2H), 0.55-0.60 (m, 1H), 1.10 – 1.17 (m, 1H), 1.28 (d, J = 6.8 Hz, 3H), 2.68 (s, 3H), 2.87 (d, J = 4.8 Hz, 3H), 3.54 – 3.62 (m, 1H), 4.52 (s, 2H), 8.04 (s, 1H), 8.19 (s, 1H), 8.81 – 8.87 (m, 1H), 8.90 (s, 1H). HRMS: m/z calcd 366.1930, found 366.1929.

***N*-(5-{7-chloro-2-[(1*S*)-1-cyclopropylethyl]-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl}-1,3-thiazol-2-yl)acetamide (7).**

A mixture of *N*-(thiazol-2-yl)acetamide (0.75 g, 5.24 mmol), **19** (1.50 g, 4.77 mmol), Cs₂CO₃ (3.11 g, 9.54 mmol), tri-*tert*-butylphosphonium tetrafluoroborate (0.28 g, 0.95 mmol) and Pd(OAc)₂ (0.107 g, 0.48 mmol) in DMF (20 mL) was heated to 110°C. After 18h the reaction mixture was filtered through Celite, washed with DCM and the filtrate was collected. Water was added and the pH was adjusted to 5 by adding 3.8 M HCl. The Organic phase separated and aqueous phase extracted with DCM. The combined organic phases were filtered through a phase separator and concentrated. The residue was chromatographed (silica gel; 50 to 100% of EtOAc in heptane) to give the title compound (900 mg, 50%). ¹H NMR (600 MHz, DMSO-d₆) δ 0.18 – 0.29 (m, 1H), 0.35 – 0.43 (m, 2H), 0.51 – 0.61 (m, 1H), 1.08-1.15 (m, 1H), 1.28 (d, J = 6.8 Hz, 3H), 2.18 (s, 3H), 3.47 – 3.62 (m, 1H), 4.54 (s, 2H), 7.72 – 7.8 (m, 2H), 8.10 (s, 1H), 12.31 (s, 1H). HRMS: m/z calcd 376.0886, found 376.0879.

***N*-(5-{2-[(1*S*)-1-cyclopropylethyl]-7-methyl-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (9).**

A mixture of **20** (201 mg, 0.68 mmol), *N*-(4-methylthiazol-2-yl)acetamide (148 mg, 0.94 mmol) and Cs₂CO₃ (605 mg, 1.86 mmol) in DMF (4 mL) was degassed. Pd(OAc)₂ (9 mg, 0.04 mmol) and tri-*tert*-butylphosphonium tetrafluoroborate (27 mg, 0.09 mmol) were added and then heated to 90°C and allowed to stir overnight. The reaction mixture was cooled to rt and was partitioned between EtOAc and water. The organic layer was washed sequentially with water

and brine. Na₂SO₄ and Silicycle Thiol scavenger (271 mg, 2.63 mmol) were added and then filtered and concentrated. The residue was recrystallized from ethanol. The obtained residue was further purified by preparative HPLC to give the title compound (111 mg, 44%) as a white solid. ¹H-NMR (500 MHz, DMSO-d₆) δ 0.18 – 0.25 (m, 1H), 0.33 – 0.44 (m, 2H), 0.53 – 0.6 (m, 1H), 1.08 – 1.14 (m, 1H), 1.27 (d, *J* = 6.8 Hz, 3H), 2.15 (s, 3H), 2.38 (s, 3H), 2.63 (s, 3H), 3.52 – 3.6 (m, 1H), 4.51 (s, 2H), 7.29 (s, 1H), 7.47 (s, 1H), 12.17 (s, 1H). HRMS: *m/z* calcd 370.1589, found 370.1600.

***N*-(5-{7-chloro-2-[(1*R*)-1-cyclopropylethyl]-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (10).**

Cs₂CO₃ (22.8 g, 69.9 mmol) was added to a flask containing **21** (11.0 g, 35.0 mmol), *N*-(4-methylthiazol-2-yl)acetamide (6.6 g, 42.0 mmol), tri-*tert*-butylphosphonium tetrafluoroborate (2.0 g, 7.0 mmol) and Pd(OAc)₂ (0.8 g, 3.5 mmol) in DMF (100 mL). The resulting mixture was heated to 100°C. After 2 h the reaction mixture was cooled to rt, filtered through Celite and concentrated. The residue was chromatographed (silica gel; 0 to 25% of MeOH in DCM) to afford the title compound (10.0 g, 73%) as a white solid. ¹H-NMR (500 MHz, DMSO-d₆) δ 0.20 – 0.27 (m, 1H), 0.35 – 0.43 (m, 2H), 0.52 – 0.61 (m, 1H), 1.08 – 1.14 (m, 1H), 1.28 (d, *J* = 6.8 Hz, 3H), 2.16 (s, 3H), 2.40 (s, 3H), 3.52 – 3.63 (m, 1H), 4.56 (s, 2H), 7.50 (s, 1H), 7.64 (s, 1H), 12.23 (s, 1H). HRMS: *m/z* calcd 390.1043, found 390.1053.

***N*-[4-methyl-5-(7-methyl-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl)-1,3-thiazol-2-yl]acetamide (11).**

tri-*tert*-butylphosphonium tetrafluoroborate (37 mg, 0.11 mmol) was added to a flask containing 5-bromo-7-chloro-2,3-dihydro-1*H*-isoindol-1-one (120 mg, 0.53 mmol), *N*-(4-methylthiazol-2-yl)acetamide (108 mg, 0.69 mmol), Cs₂CO₃ (346 mg, 1.06 mmol) and Pd(OAc)₂ (12 mg, 0.05 mmol) in DMF (6 mL). The resulting mixture was stirred at 100°C for 2 h and then cooled to rt, filtered through Celite and concentrated. The residue was purified by preparative HPLC to

afford the title compound (20 mg, 13%) as a white solid. ¹H-NMR (400 MHz, DMSO-d₆) δ 2.15 (s, 3H), 2.38 (s, 3H), 2.63 (s, 3H), 4.33 (s, 2H), 7.28 (s, 1H), 7.43 (s, 1H), 8.45 (s, 1H), 12.16 (s, 1H). HRMS: *m/z* calcd 302.0963, found 302.0938.

***N*-(5-{7-(Benzylsulfanyl)-2-[(1*S*)-1-cyclopropylethyl]-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide.**

In a flask was added **8** (20.0 g, 51.3 mmol), phenylmethanethiol (12.7 g, 102.6 mmol), and sodium 2-methylbutan-2-olate (11.3 g, 102.6 mmol) in DMF (500 mL). The reaction mixture was stirred for 2 h at 110°C and was then filtered through Celite and concentrated under reduced pressure. The residue was chromatographed (silica gel; 0 to 25% of EtOAc in DCM) to afford the title compound (18 g, 74%) as a yellow solid. ¹H NMR (500 MHz, DMSO-d₆) δ 0.18 – 0.25 (m, 1H), 0.33 – 0.43 (m, 2H), 0.52 – 0.64 (m, 1H), 1.01 – 1.14 (m, 1H), 1.26 (d, *J* = 6.8 Hz, 3H), 2.15 (s, 3H), 2.24 (s, 3H), 3.43 – 3.59 (m, 1H), 4.35 (s, 2H), 4.52 (s, 2H), 7.24 – 7.28 (m, 2H), 7.32 – 7.37 (m, 3H), 7.48 (d, *J* = 7.3 Hz, 2H), 12.16 (s, 1H). *m/z* (ESI) 478 [M+H]⁺.

6-[2-(Acetylamino)-4-methyl-1,3-thiazol-5-yl]-2-[(1*S*)-1-cyclopropylethyl]-3-oxo-2,3-dihydro-1*H*-isoindole-4-sulfonyl chloride.

Sulfonyl chloride (8.5 g, 62.8 mmol) was added portionwise to *N*-(5-{7-(benzylsulfanyl)-2-[(1*S*)-1-cyclopropylethyl]-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (10.0 g, 20.9 mmol) in acetic acid (60 mL), acetonitrile (400 mL), and water (4 mL) at 0°C. The resulting mixture was stirred at 5°C for 1 h. The solvent was removed under reduced pressure and the reaction mixture was diluted with DCM (500 mL) and washed sequentially with saturated NaHCO₃ (100 mL) and saturated brine (200 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was suspended in diethyl ether and the solid was collected by filtration and dried under vacuum to afford the title compound (8.0 g, 84%) which was used for further synthesis without further purification. ¹H NMR (500 MHz, DMSO-d₆) δ 0.24 – 0.32 (m, 1H), 0.37 – 0.48 (m, 2H), 0.52 – 0.64 (m, 1H), 1.11 – 1.20

(m, 1H), 1.31 (d, $J = 6.8$ Hz, 3H), 2.17 (s, 3H), 2.41 (s, 3H), 3.56 – 3.66 (m, 1H), 4.70 (s, 2H), 7.76 (d, $J = 1.4$ Hz, 1H), 7.90 (d, $J = 1.4$ Hz, 1H), 12.26 (s, 1H). m/z (ESI) 454 $[M + H]^+$.

***N*-(5-{2-[(1*S*)-1-Cyclopropylethyl]-1-oxo-7-sulfamoyl-2,3-dihydro-1*H*-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (12).**

A mixture of 6-[2-(acetylamino)-4-methyl-1,3-thiazol-5-yl]-2-[(1*S*)-1-cyclopropylethyl]-3-oxo-2,3-dihydro-1*H*-isoindole-4-sulfonyl chloride (2.0 g, 4.41 mmol) and 30% Ammonium hydroxide solution (5.15 g, 44.06 mmol) in DCM (40 mL) was allowed to stir at rt for 2 h and was then concentrated. The crude product was purified by preparative HPLC to afford the title compound (0.89 g, 47%) as a yellow solid. $^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ 0.21 – 0.31 (m, 1H), 0.37 – 0.49 (m, 2H), 0.60 – 0.63 (m, 1H), 1.14 – 1.23 (m, 1H), 1.33 (d, $J = 6.9$ Hz, 3H), 2.17 (s, 3H), 2.43 (s, 3H), 3.53 – 3.67 (m, 1H), 4.75 (s, 2H), 7.73 (s, 2H), 7.87 (s, 1H), 7.98 (s, 1H), 12.29 (s, 1H). HRMS: m/z calcd 435.1161, found 435.1150.

***N*-(5-{2-[(1*S*)-1-Cyclopropylethyl]-7-(methylsulfamoyl)-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (13).**

Methylamine in THF (2M, 22 mL, 44 mmol) was added dropwise to 6-[2-(acetylamino)-4-methyl-1,3-thiazol-5-yl]-2-[(1*S*)-1-cyclopropylethyl]-3-oxo-2,3-dihydro-1*H*-isoindole-4-sulfonyl chloride (2.0 g, 4.41 mmol), in DCM (40 mL) at 25°C over a period of 30 min under nitrogen. The resulting mixture was stirred at 25°C for 12 h. The reaction mixture was concentrated and the crude product was chromatographed (silica gel; 30 to 50% of EtOAc in petroleum ether) to afford the title compound (1.8 g, 91%) as a yellow solid. $^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ 0.22 – 0.34 (m, 1H), 0.38 – 0.49 (m, 2H), 0.57-0.66 (m, 1H), 1.11 – 1.22 (m, 1H), 1.33 (d, $J = 6.8$ Hz, 3H), 2.18 (s, 3H), 2.45 (s, 3H), 2.52 (d, $J = 5.2$ Hz, 3H), 3.60 – 3.71 (m, 1H), 4.75 (s, 2H), 7.59 (q, $J = 5.2$ Hz, 1H), 7.88 (d, $J = 1.4$ Hz, 1H), 8.02 (d, $J = 1.4$ Hz, 1H), 12.31 (1H, s). HRMS: m/z calcd 449.1317, found 449.1323.

***N*-(5-{2-[(1*S*)-1-cyclopropylethyl]-7-[(2-methoxyethyl)sulfamoyl]-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (14).**

A mixture of (S)-6-(2-acetamido-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisindoline-4-sulfonyl chloride (120 mg, 0.26 mmol), pyridine (0.043 mL, 0.53 mmol), and 2-methoxyethanamine (40 mg, 0.53 mmol) in DCM (2 mL) was stirred at 25°C for 1h. The reaction mixture was concentrated and the crude product was purified by preparative HPLC to afford the title compound (30 mg, 23%) as a white solid. ¹H-NMR (300 MHz, DMSO-*d*₆) δ 0.21 – 0.32 (m, 1H), 0.35 – 0.48 (m, 2H), 0.53 – 0.65 (m, 1H), 1.14 – 1.20 (m, 1H), 1.34 (d, *J* = 6.8 Hz, 3H), 2.17 (s, 3H), 2.44 (s, 3H), 2.89 (s, 3H), 3.06 – 3.21 (m, 2H), 3.21 – 3.31 (m, 2H), 3.63 – 3.66 (m, 1H), 4.74 (s, 2H), 7.84 – 7.89 (m, 2H), 7.99 (s, 1H), 12.27 (s, 1H). HRMS: *m/z* calcd 493.1579, found 493.1567.

***N*-(5-{2-[(1*S*)-1-cyclopropylethyl]-7-[(3-hydroxypropyl)sulfanyl]-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide.**

A mixture of **8** (400 mg, 1.03 mmol), 3-mercaptopropan-1-ol (189 mg, 2.05 mmol) and sodium-*t*-amylate (226 mg, 2.05 mmol) in DMF (8 mL) in a sealed microwave vial was heated to 110°C for 90 minutes in a microwave reactor. The reaction mixture was cooled to rt, diluted with DCM and washed sequentially with water and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated. The crude product was chromatographed (silica gel; 0 to 100% of EtOAc in petroleum ether) to afford the title compound (350 mg, 77%) as a white solid. ¹H-NMR (300 MHz, MeOD) δ 0.25 – 0.54 (m, 3H), 0.60 – 0.71 (m, 1H), 1.06 – 1.19 (m, 1H), 1.36 (d, *J* = 6.8 Hz, 3H), 1.88 – 2.01 (m, 2H), 2.22 (s, 3H), 2.41 (s, 3H), 3.11 (t, *J* = 7.3 Hz, 2H), 3.53 – 3.66 (m, 1H), 3.71 (t, *J* = 6.1 Hz, 2H), 4.53 (d, *J* = 18.2 Hz, 1H), 4.61 (d, *J* = 18.2 Hz, 1H), 7.31 (s, 1H), 7.32 (s, 1H). *m/z* (ESI) 446 [M + H]⁺.

***N*-(5-{2-[(1*S*)-1-cyclopropylethyl]-7-[(3-hydroxypropyl)sulfonyl]-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (16).**

m-CPBA (70 mg, 0.40 mmol) was added portionwise to *N*-(5-{2-[(1*S*)-1-cyclopropylethyl]-7-[(3-hydroxypropyl)sulfanyl]-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (60 mg, 0.13 mmol) dissolved in DCM (5 mL) at 0°C over a period of 5 minutes. The resulting mixture was stirred at rt for 2 h and then concentrated. The crude product was purified by preparative HPLC to afford the title compound (30 mg, 47%) as a white solid. ¹H NMR (300 MHz, MeOD) δ 0.29 – 0.58 (m, 3H), 0.64 – 0.75 (m, 1H), 1.11 – 1.24 (m, 1H), 1.40 (d, *J* = 6.8 Hz, 3H), 1.85 – 1.99 (m, 2H), 2.24 (s, 3H), 2.47 (s, 3H), 3.62 (t, *J* = 6.2 Hz, 2H), 3.66 – 3.76 (m, 1H), 3.85 – 3.99 (m, 2H), 4.70 (d, *J* = 18.7 Hz, 1H), 4.78 (d, *J* = 18.7 Hz, 1H), 8.01 (s, 1H), 8.15 (s, 1H). HRMS: *m/z* calcd 478.1470, found 478.1466.

***N*-(5-{2-[(1*S*)-1-Cyclopropylethyl]-7-[(methylsulfonyl)amino]-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (17).**

A mixture of **8** (2.00 g, 5.13 mmol), methanesulfonamide (1.46 g, 15.39 mmol), sodium-*t*-butoxide (1.48 mL, 15.39 mmol), Pd(OAc)₂ (115 mg, 0.51 mmol) and *t*BuXPhos (479 mg, 1.13 mmol) in DMF (60 mL) was heated to 140°C for 7 h. After being cooled to rt the mixture was filtered through Celite and the solvent was removed under reduced pressure. The crude product was chromatographed (silica gel; 0 to 25% of EtOAc in DCM) and the obtained material was further purified by preparative HPLC to afford the title compound **17** (1.20 g, 52%) as a yellow solid. ¹H-NMR (600 MHz, DMSO-*d*₆) δ 0.21 – 0.27 (m, 1H), 0.36 – 0.45 (m, 2H), 0.55 – 0.61 (m, 1H), 1.10 – 1.18 (m, 1H), 1.30 (d, *J* = 6.8 Hz, 3H), 2.15 (s, 3H), 2.40 (s, 3H), 3.25 (s, 3H), 3.48 – 3.56 (m, 1H), 4.61 (s, 2H), 7.37 (s, 1H), 7.44 (s, 1H), 9.56 (s, 1H), 12.20 (s, 1H). HRMS: *m/z* calcd 449.1317, found 449.1312.

***N*-[5-(2-[(1*S*)-1-Cyclopropylethyl]-7-[(2-methoxyethyl)sulfonyl]amino)-1-oxo-2,3-dihydro-1*H*-isoindol-5-yl]-4-methyl-1,3-thiazol-2-yl]acetamide (18).**

8 (92 mg, 0.24 mmol), 2-methoxyethanesulfonamide (53 mg, 0.38 mmol), K₂CO₃ (98 mg, 0.71 mmol), allylpalladium(II) chloride dimer (2 mg, 5.90 μmol) and *t*BuXPhos (50 mg, 0.12 mmol) in Methyl THF (1 mL) in a sealed vial was heated to 80°C over night. The reaction mixture was filtered through a silica plug, concentrated and then dissolved in DCM and washed with water. The organic phase was filtered through a phase separator and concentrated. The crude was further purified by preparative HPLC to afford the title compound **18** (50 mg, 55%). ¹H-NMR (600 MHz, DMSO-d₆) δ 0.24 (m, 1H), 0.40 (m, 2H), 0.5 – 0.67 (m, 1H), 1.14 (m, 1H), 1.30 (d, 3H), 2.15 (s, 3H), 2.39 (s, 3H), 3.12 (s, 3H), 3.46 – 3.57 (m, 1H), 3.59 (t, 2H), 3.67 (t, 2H), 4.61 (s, 2H), 7.36 (s, 1H), 7.51 (s, 1H), 9.59 (s, 1H), 12.20 (s, 1H). HRMS: *m/z* calcd 493.1579, found 493.1585.

5-Bromo-2-[(1S)-1-cyclopropylethyl]-7-methyl-2,3-dihydro-1H-isoindol-1-one (20).

K₂CO₃ (37.3 g, 270 mmol), boric acid (5.6 g, 90 mmol) and (*S*)-1-Cyclopropylethanamine (10.0 g, 117 mmol) were added to a flask containing Methyl 4-bromo-2-(bromomethyl)-6-methylbenzoate (29.0 g, 90 mmol) in acetonitrile (450 mL). The reaction mixture was allowed to stir at rt for 20 h and was then filtered through Celite, washed with acetonitrile. The filtrate was concentrated and the resulting residue was recrystallised from heptane:MTBE 10:3 to give the title compound (10.8 g, 41%). ¹H-NMR (500 MHz, CDCl₃) δ 0.30 – 0.48 (m, 3H), 0.60-0.65 (m, 1H), 0.93 – 1.04 (m, 1H), 1.32 (d, *J* = 6.8 Hz, 3H), 2.69 (s, 3H), 3.68-3.75 (m, 1H), 4.33 (d, *J* = 17.2 Hz, 1H), 4.44 (d, *J* = 17.2 Hz, 1H), 7.35 (s, 1H), 7.41 (s, 1H). *m/z* (ESI) 296 [M + H]⁺.

5-Bromo-7-chloro-2-[(1R)-1-cyclopropylethyl]-2,3-dihydro-1H-isoindol-1-one (21).

K₂CO₃ (0.88 g, 6.4 mmol) was added to a flask containing methyl 4-bromo-2-(bromomethyl)-6-chlorobenzoate (2.20 g, 6.4 mmol) and (*R*)-1-Cyclopropylethanamine (0.55 g, 6.4 mmol) in DMF (10 mL) and the resulting mixture was heated to 80°C and stirred for 2 h. The reaction mixture was diluted with EtOAc and washed sequentially with water, brine and the organic

layer was dried over Na₂SO₄, filtered and concentrated. The residue was chromatographed (silica gel; 2 to 30% of EtOAc in heptane) to afford the title compound (1,50 g, 74%) as a yellow solid. ¹H NMR (500 MHz, DMSO-d₆) δ 0.15 – 0.26 (m, 1H), 0.32 – 0.45 (m, 2H), 0.51 – 0.61 (m, 1H), 1.06 – 1.14 (m, 1H), 1.26 (d, *J* = 6.8 Hz, 3H), 3.48 – 3.58 (m, 1H), 4.52 (s, 2H), 7.76 (d, *J* = 1.3 Hz, 1H), 7.84 (d, *J* = 1.3 Hz, 1H). *m/z* (ESI) 316 [M + H]⁺.

7-Chloro-2-[(1S)-1-cyclopropylethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3-dihydro-1H-isoindol-1-one (22).

A mixture of bis(pinacolato)diboron (3.6 g, 14.3 mmol), **19** (3.0 g, 9.5 mmol) and KOAc (4.2 g, 42.9 mmol) in DMSO (190 mL) was stirred for 15 minutes. Pd(PPh₃)₄ (0.55 g, 0.5 mmol) was added and the solution was heated to 100°C. After 2.5 h the solution was cooled to rt, diluted with EtOAc and washed sequentially with water and brine. The combined organic phases were dried over MgSO₄, filtered and concentrated. The residue was chromatographed (silica gel; 0 to 25% of EtOAc in heptane) to afford the title compound (1.3 g, 38%) as a yellow solid. ¹H NMR (500 MHz, CDCl₃) δ 0.37 – 0.47 (m, 3H), 0.59 – 0.68 (m, 1H), 0.97 – 1.06 (m, 1H), 1.35 (d, *J* = 6.8 Hz, 3H), 1.37 (s, 12H), 3.68 – 3.82 (m, 1H), 4.36 (d, *J* = 17.2 Hz, 1H), 4.47 (d, *J* = 17.2 Hz, 1H), 7.76 (s, 1H), 7.82 (s, 1H). *m/z* (ESI) 362/364 [M + H]⁺.

2-[(1S)-1-Cyclopropylethyl]-7-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3-dihydro-1H-isoindol-1-one (23).

Pd(PPh₃)₄ (220 mg, 0.19 mmol) was added to a flask containing **20** (800 mg, 2.72 mmol) KOAc (1121 mg, 11.42 mmol) and Bis(Pinacolato)Diboron (1036 mg, 4.08 mmol) in DMSO (16 mL). The reaction mixture was heated to 100°C. After 14 h the reaction mixture was cooled to rt, diluted with EtOAc and washed with brine. Organic phase filtered through a phase separator and concentrated. The residue was chromatographed (silica gel; 5 to 40% of EtOAc in heptane) to afford the title compound (700 mg, 75%) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ 0.32 – 0.48 (m, 3H), 0.59 – 0.68 (m, 1H), 0.98 – 1.08 (m, 1H), 1.34 (d, *J* = 6.8 Hz,

3H), 1.37 (s, 12H), 2.74 (s, 3H), 3.71 – 3.79 (m, 1H), 4.35 (d, $J = 16.9$ Hz, 1H), 4.46 (d, $J = 16.9$ Hz, 1H), 7.64 (s, 1H), 7.71 (s, 1H). m/z (ESI) 342 $[M + H]^+$.

Biological assays.

PI3K biochemical assays:

Enzymatic activity assay for rec human PI3K α , β , γ , and δ : The activity of recombinant human PI3K γ ((aa144-1102)-6His) and α , β , δ (6-His(p110-p85a)) was determined by measuring the ADP level after phosphorylation of DiC₈-PIP₂ using a commercially available ADPGlo™ kit from Promega. The assay was carried out in white low volume 384 well plates in a final volume of 14 μ L at rt. The assay contained the following: 50 mM Tris buffer pH 7.4, 2.1 mM DTT, 5 mM MgCl₂, 0.05% CHAPS, 20 μ M ATP, 80 μ M DiC₈-PIP₂ and 1.2 nM PI3K α , β , γ or 0.6 nM PI3K δ . Potential inhibitors were dissolved in DMSO and then diluted in the assay to give a final concentration of not exceeding 1% (v/v) DMSO. A 10-point half-log dilution series of the inhibitors (highest concentration typically 1 μ M for γ and 33 μ M for α , β , and δ) was tested and the pIC₅₀ determined using a 4-parameter logistic equation in a non-linear curve fitting routine. Routinely, inhibitors were pre-incubated with 3 μ L of enzyme for 10 min prior to the addition of 2 μ L substrate mixture for a further 60 min enzyme reaction. The phosphorylation was stopped with the addition of 3 μ L ADP-Glo™ reagent (stop solution) followed by a 40 min incubation. Prior to detection 6 μ L of ADP-Glo™ Kinase Detection Reagent was added and the plates were read in a micro plate reader using a Luminescence filter. All additions were followed by a short centrifugation step.

A 10-point half-log dilution series of the inhibitors (highest concentration typically 1 μ M for γ and 33 μ M for α , β , and δ) was tested and raw data output was analyzed in Screener software (Genedata AG) and pIC₅₀ values calculated using a four parameter logistic fit. Variability of the enzymatic activity assays for a reference compound (pIC₅₀ (mean \pm SD)): PI3K γ 7.40 ± 0.12 ($n = 100$), PI3K α 7.65 ± 0.10 ($n = 144$), PI3K β 6.87 ± 0.11 ($n = 136$), PI3K δ 7.34 ± 0.09 ($n = 161$).

PI3K γ cell assay:

Inhibition of p-Akt in in RAW264 cells: PI3K γ inhibitors were identified by measuring the phosphorylation of AKT (at Ser473) using HTRF® technology (Phospho-AKT (Ser473) kit, Cisbio) in RAW264 cells – mouse monocytic cell line (ATCC). Cryo preserved cells were thawed rapidly in DMEM + Glutamax medium (10% FBS) at 37°C. Cells were plated in white low volume 384 well plates at a density of 2.5E6 cells/mL in assay buffer (DMEM + 1% FBS). Potential inhibitors were made up in DMSO and then diluted in the assay to give a final concentration of not exceeding 1% (v/v) DMSO. Inhibitors were incubated with 8 μ L of cells for 15 minutes at 37°C prior stimulated with 2 μ L C5a (2 nM) in assay buffer. After an incubation of 3 min at 37°C the cells were lysed for 30 min at rt on a plate shaker at 500 rpm. A 5 μ L mix of d2/cryptate were added to the plates and the FRET signal were read in a micro plate reader after 3 – 4 h at 620 nm & 665 nm. The signal at 665 nm was divided by the signal at 612 nm and multiplied by 10,000 to generate a signal ratio value for each well. A 10-point half-log dilution series of the inhibitors (highest concentration typically 1 μ M) was tested and

raw data output was analyzed in Screener software (Genedata AG) and pIC₅₀ values calculated using a four parameter logistic fit.

PI3K α , β , γ , and δ enzyme and PI3K γ cell inhibitory potencies with standard deviations (SD) for compounds **3-18**.

Cmpd	PI3K γ pIC50 (SD)	PI3K α pIC50 (SD)	PI3K β pIC50 (SD)	PI3K δ pIC50 (SD)	PI3K γ Cell pIC50 (SD)
3	8.03 (0.21)	<4.5	<4.5	NV	6.22 (0.12)
4	7.57 (0.07)	<4.5	<4.5	5.02 ^a	5.12 ^a
5	8.22 (0.08)	4.62 (0.03)	<4.5	5.20 (0.04)	6.4 ^a
6	8.12 (0.17)	5.22 (0.15)	<4.5	5.48 (0.13)	6.44 (0.13)
7	8.94 (0.22)	5 (0.09)	<4.5	5.81 ^a	7.53 (0.02)
8	9.14 (0.09)	5.62 (0.09)	4.96 (0.04)	6.51 (0.05)	8.24 (0.17)
9	9.18 (0.16)	5.54 (0.11)	4.72 (0.05)	6.67 (0.20)	8.16 (0.14)
10	8.85 (0.05)	5.58 (0.05)	4.82 (0.06)	6.31 ^a	7.4 (0.03)
11	7.19 (0.05)	7.14 (0.14)	5.82 (0.04)	6.59 (0.09)	<6
12	9.24 (0.23)	5.16 (0.07)	<4.5	6.61 (0.06)	7.67 (0.18)
13	9.12 (0.08)	5.39 (0.09)	4.64 (0.06)	6.8 (0.09)	8.33 (0.26)
14	8.93 (0.05)	4.87 (0.13)	<4.5	6.37 (0.17)	7.71 ^a
15	9.06 (0.07)	5.11 (0.06)	<4.5	6.45 (0.17)	8.08 (0.27)
16	8.91 (0.08)	5.14 (0.02)	<4.5	6.72 (0.16)	6.57 ^a
17	9.23 (0.10)	5.23 (0.09)	4.68 (0.05)	6.59 (0.10)	8.59 (0.10)
18	8.98 (0.11)	5.32 (0.08)	4.63 (0.04)	6.64 (0.10)	8.22 ^a

SD = standard deviation, ^a n=1.

PI3K α cell assay: inhibition of p-Akt in BT474 cells

PI3K β cell assay: inhibition of p-Akt in MDA-MB-468 cells

PI3K δ cell assay: inhibition of p-Akt in Jeko B cells

See reference 4 for experimental details of PI3K α and β assays and reference 5 for PI3K δ assay.

Rat LPS in vivo model

The present study was approved by the local ethical committee in Gothenburg (188-2012) and the approved site number is 31-5373/11.

We tested compound **15** in the rat lipopolysaccharide (LPS) model. Female Wistar (substrain Hannover GALAS) were obtained from Taconic Europe, Denmark. The rats were challenged with 0.125 mL LPS (E. coli (026:B6, powder, Sigma-Aldrich, Sweden Lot: 091M4054), 5 μ g/mL diluted in sterile NaCl, 0.9% solution (B. Braun medical AB, Sweden)) using intratracheal route of administration. 2h prior to challenge, the rats were orally treated with 0.1, 0.5, and 2.5 mg/kg of compound **15** and sacrificed 4h later. Broncho-alveolar lavage (BAL) was performed by manual perfusion of the whole lung and the lung neutrophil infiltration for compound treated LPS challenged animals (n = 8 per group) was compared to LPS vehicle controls (n = 10) using total and differential cell count with SYSMEX XT-1800i Vet (SYSMEX, Kobe Japan).

Determination of the mPI3K δ crystal structure.

The expression and purification of mouse PI3K δ followed the same procedure as previously described.⁶ Compound **9** was solubilised to 50 mM in DMSO and 0.4 μ L of compound stock was mixed with 3 μ L of 5% w/v n-dodecyl- β -D-maltoside (DDM) from Hampton Research® prior to the addition of 25 μ L mPI3K δ (at 5 mg/mL in 20 mM Tris pH 7.2, 50 mM ammonium sulfate, 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 13000 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 ID29 complex. Structure of mPI3K δ was determined by Molecular replacement using the program “Molrep”⁷ using internal structures as search models. Manual fitting and inspection of structures were carried out in the program Coot⁸ and refinement carried out using the program Refmac.⁹ The X-ray structure has been deposited with deposition code 6FTN.

Analytical HPLC/MS

Analytical HPLC/MS was conducted using UV detection, and conditions described below:

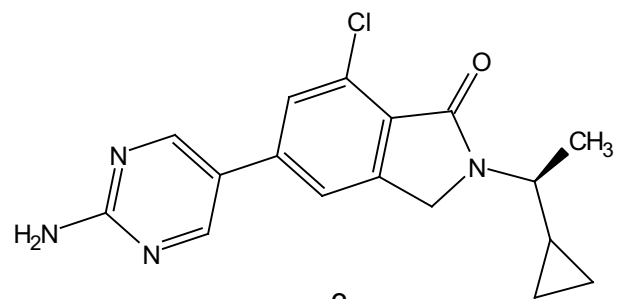
- (A) BEH C18 column (2.1mm \times 50 mm, 1.7 μ m,) using a gradient of 5% v/v MeCN in H₂O (ammonium carbonate buffer, pH 10) to 90% v/v MeCN in H₂O over 2.5 minutes. Detection at 210 nm. Ionisation mode was ESI⁺.
- (B) CSH C18 column (2.1 \times 50 mm, 1.7 μ m), using a gradient of 5% v/v MeCN in H₂O (ammonium formate buffer, pH 3) to 90% v/v MeCN in H₂O over 2.5 minutes. Detection at 230 nm. Ionisation mode was ESI⁺.

All tested compounds were determined to be \geq 95% pure based on the peak area percentage.

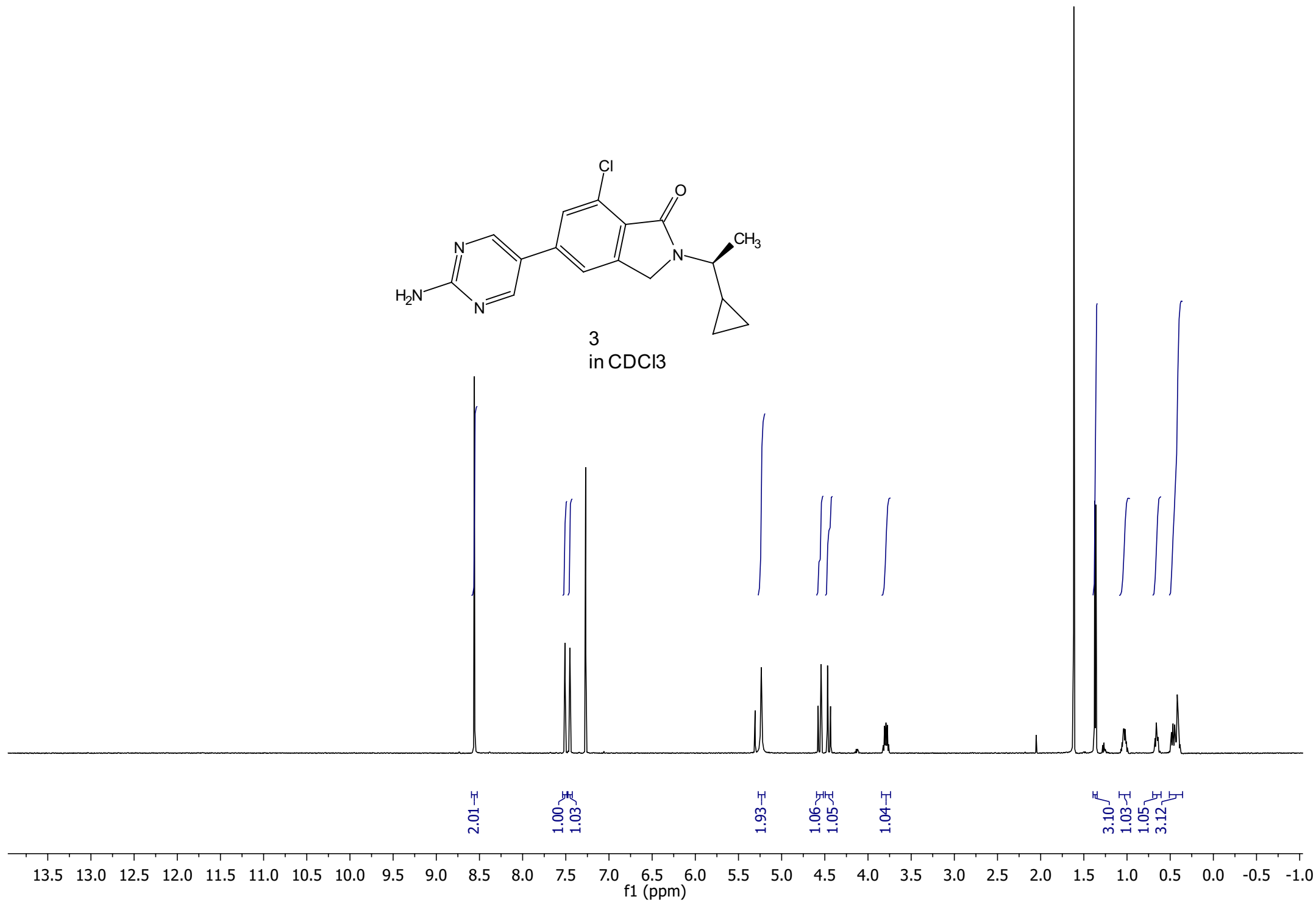
Compound	HPLC conditions	HPLC purity (%)	mass observed, [M + H] ⁺
3	A	98	329
4	A	99	309
5	A	98	333
6	A	98	366
7	A	99	376
8	A	95	390
9	A	99	370
10	A	99	390
11	A	99	302
12	A	98	435
13	A	99	449
14	A	97	493
15	A	98	434
16	A	95	478
17	A	99	449
18	B	96	493

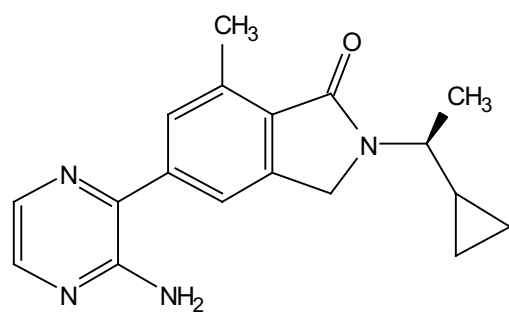
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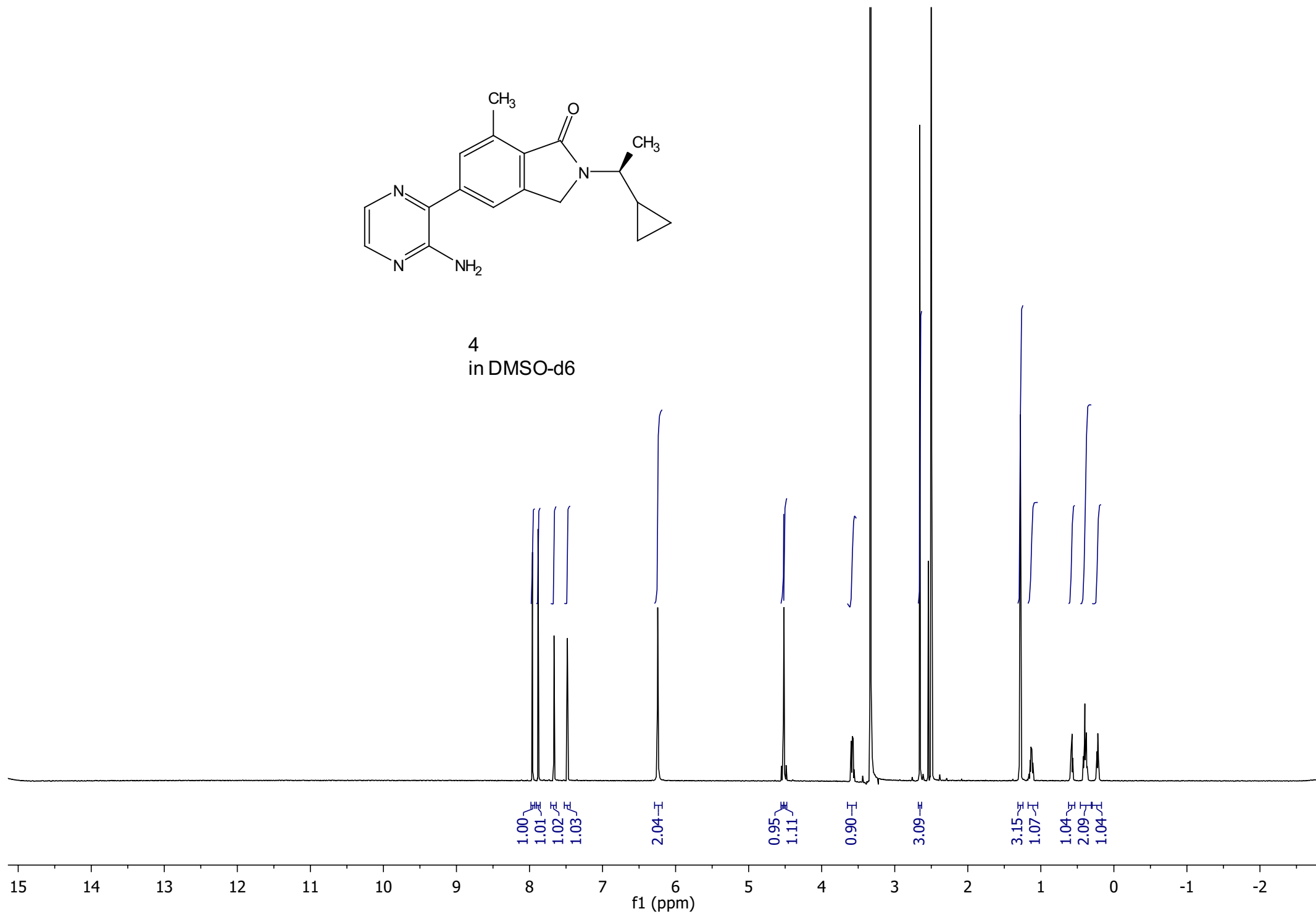


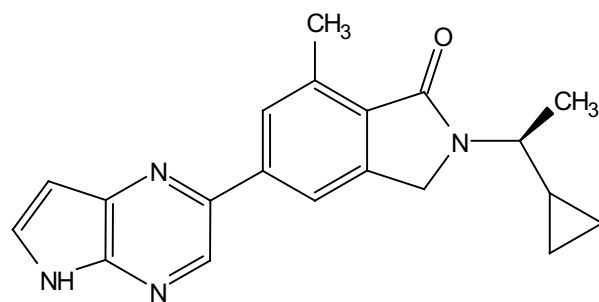
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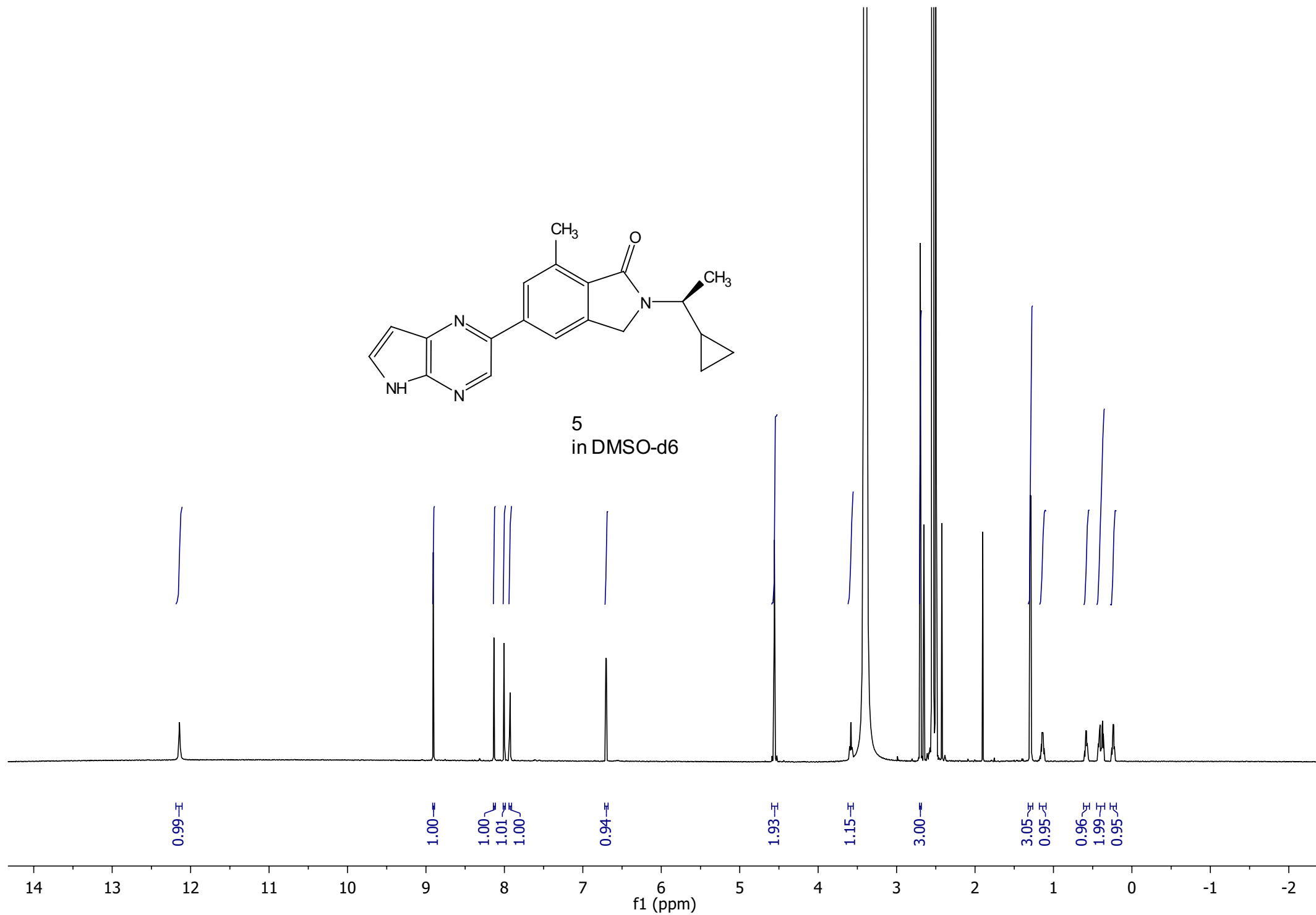


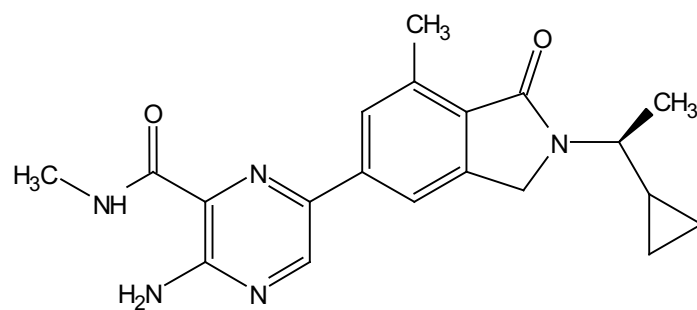
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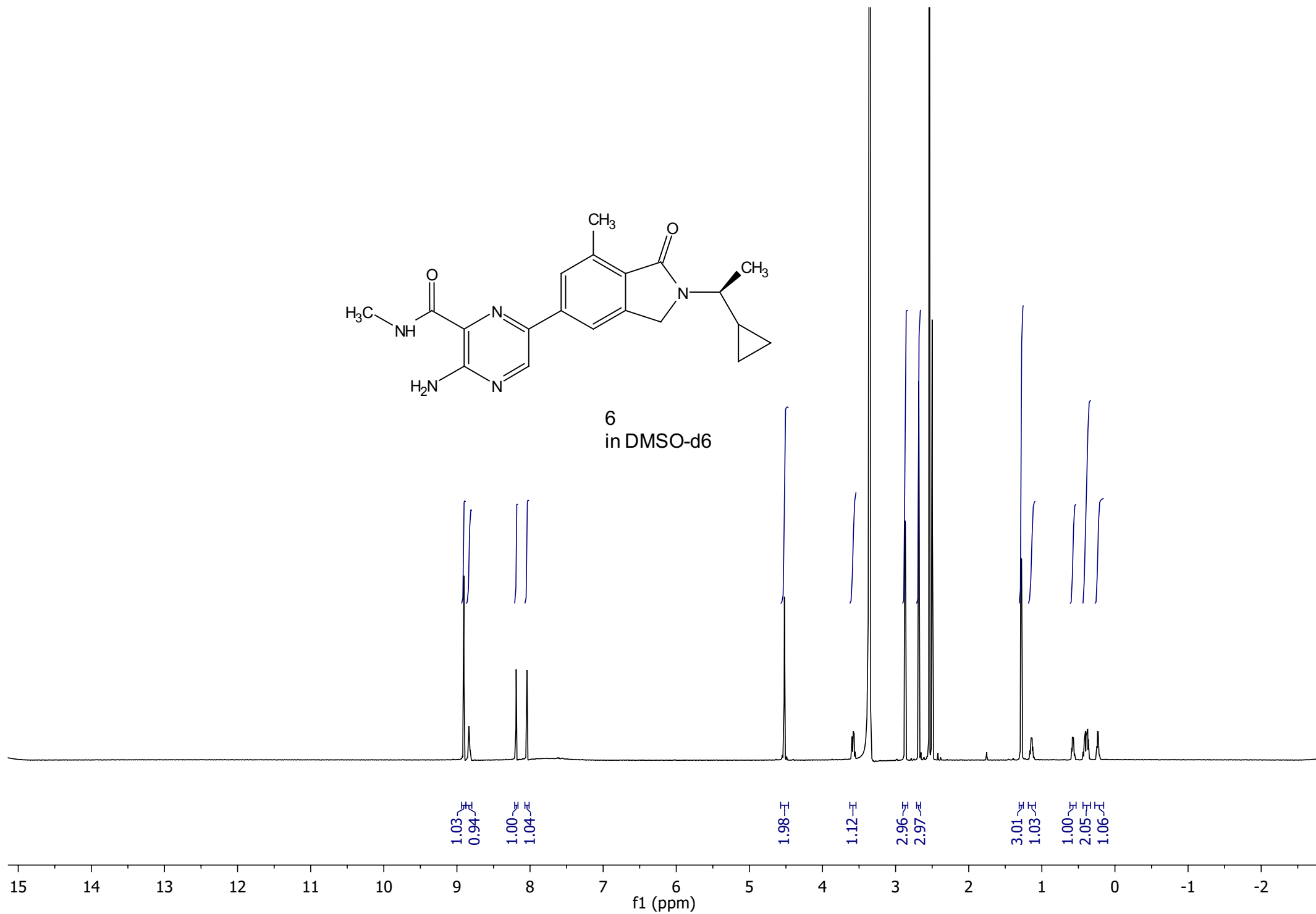


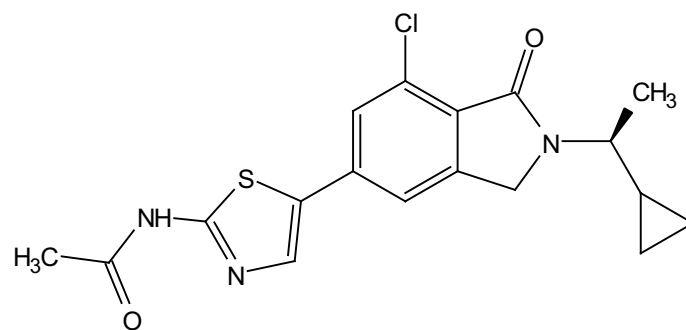
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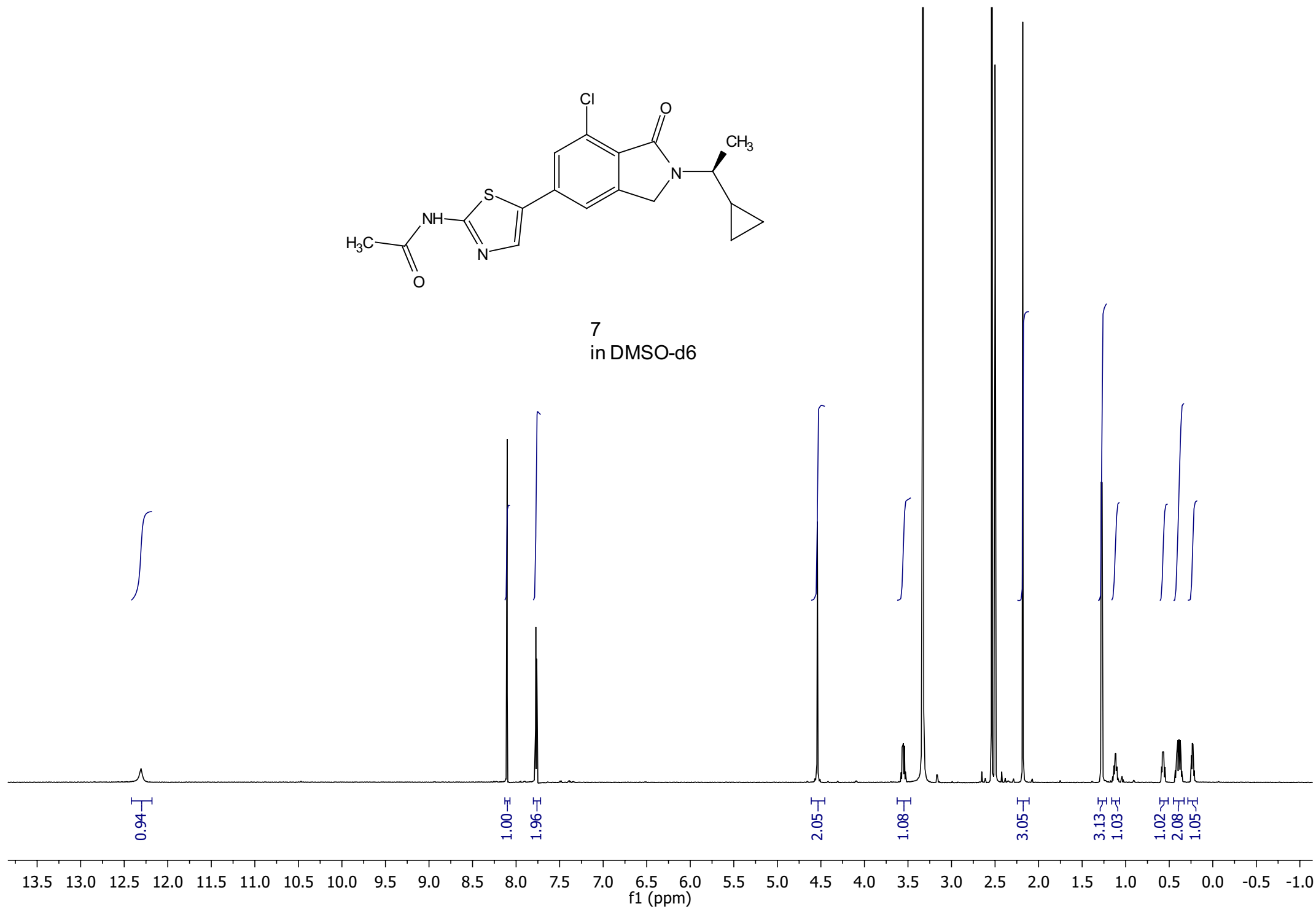


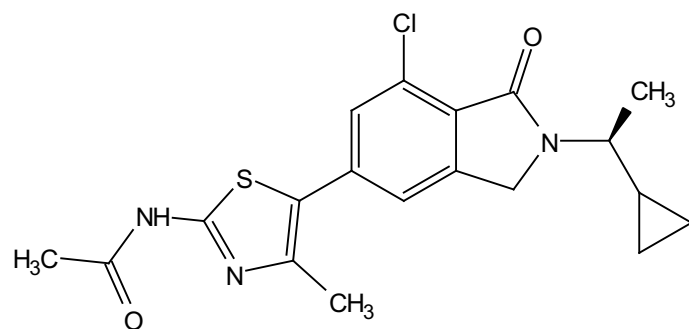
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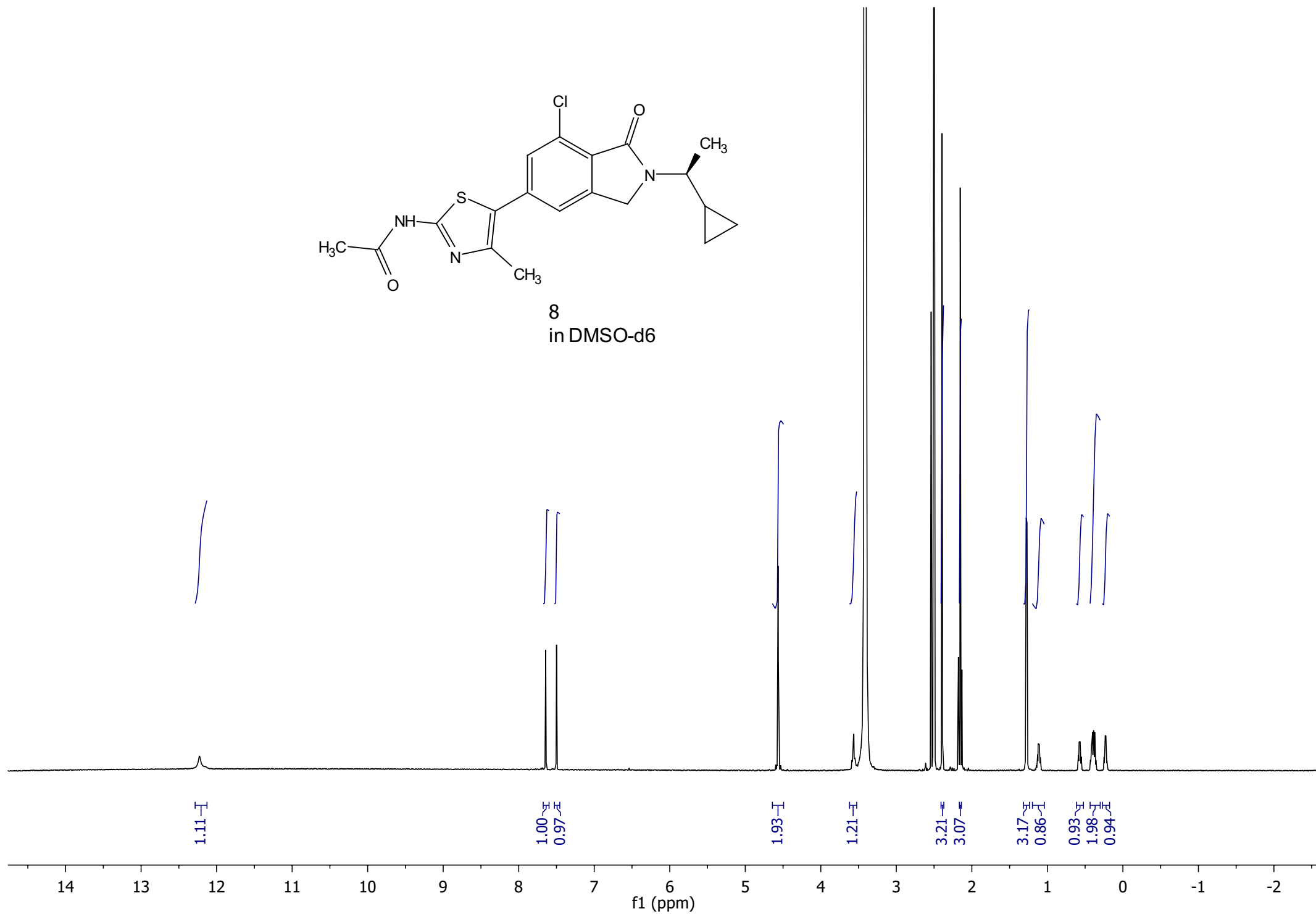


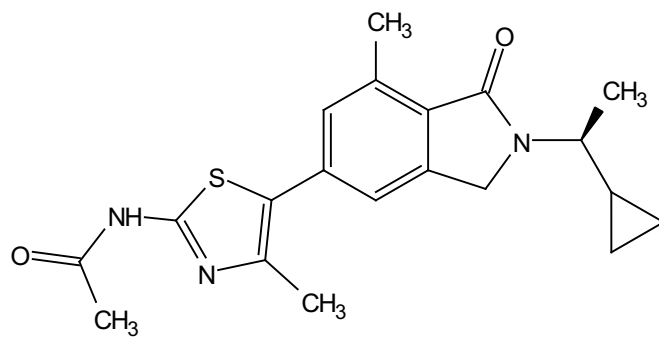
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in DMSO-d₆



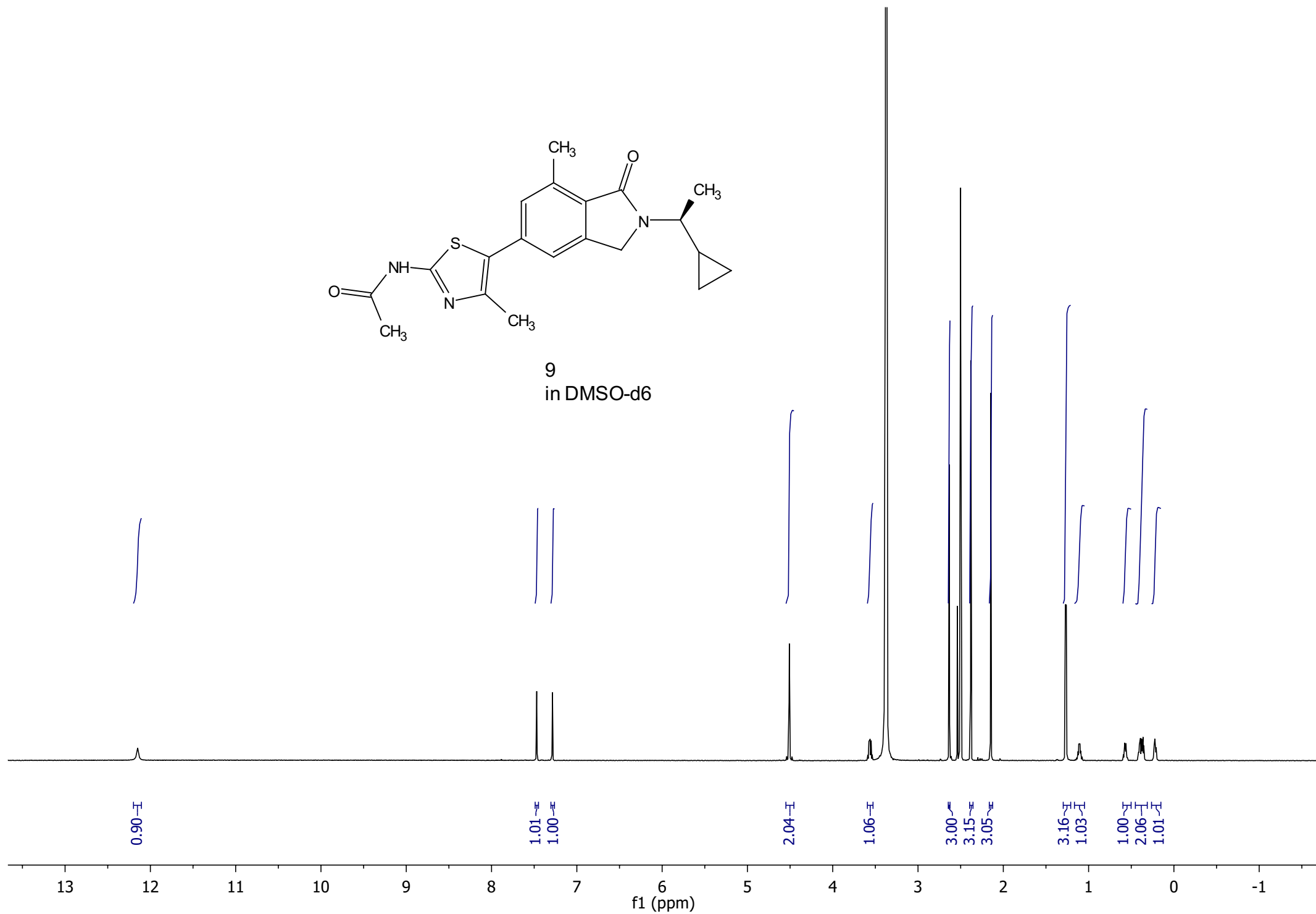


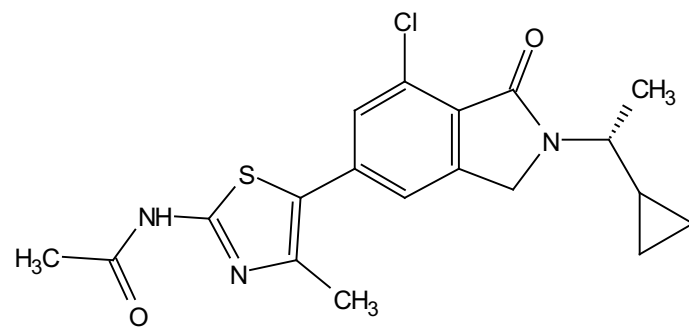
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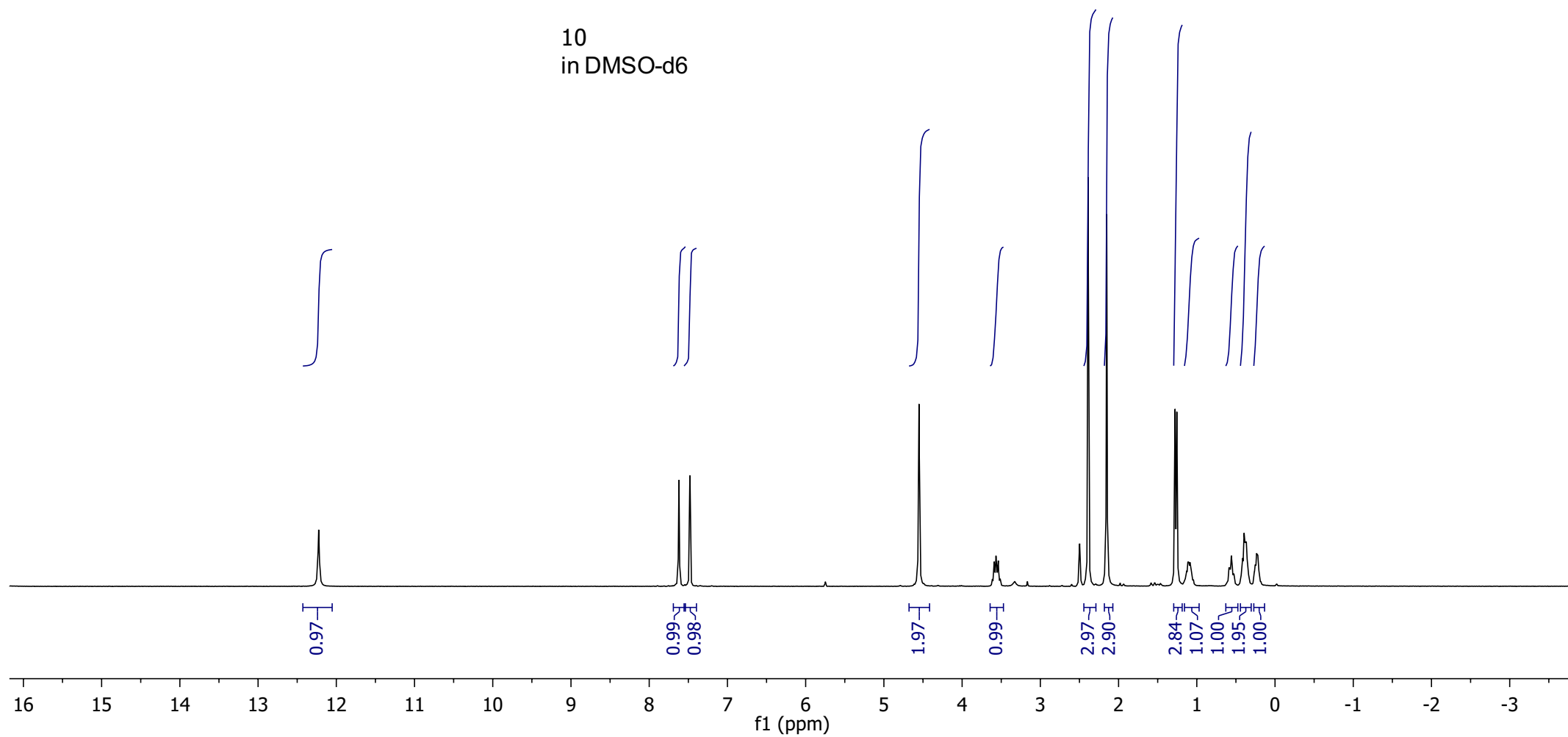


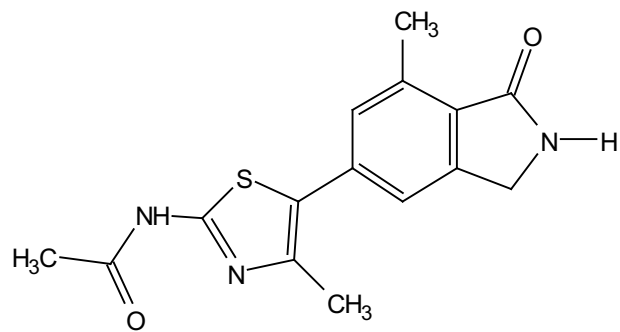
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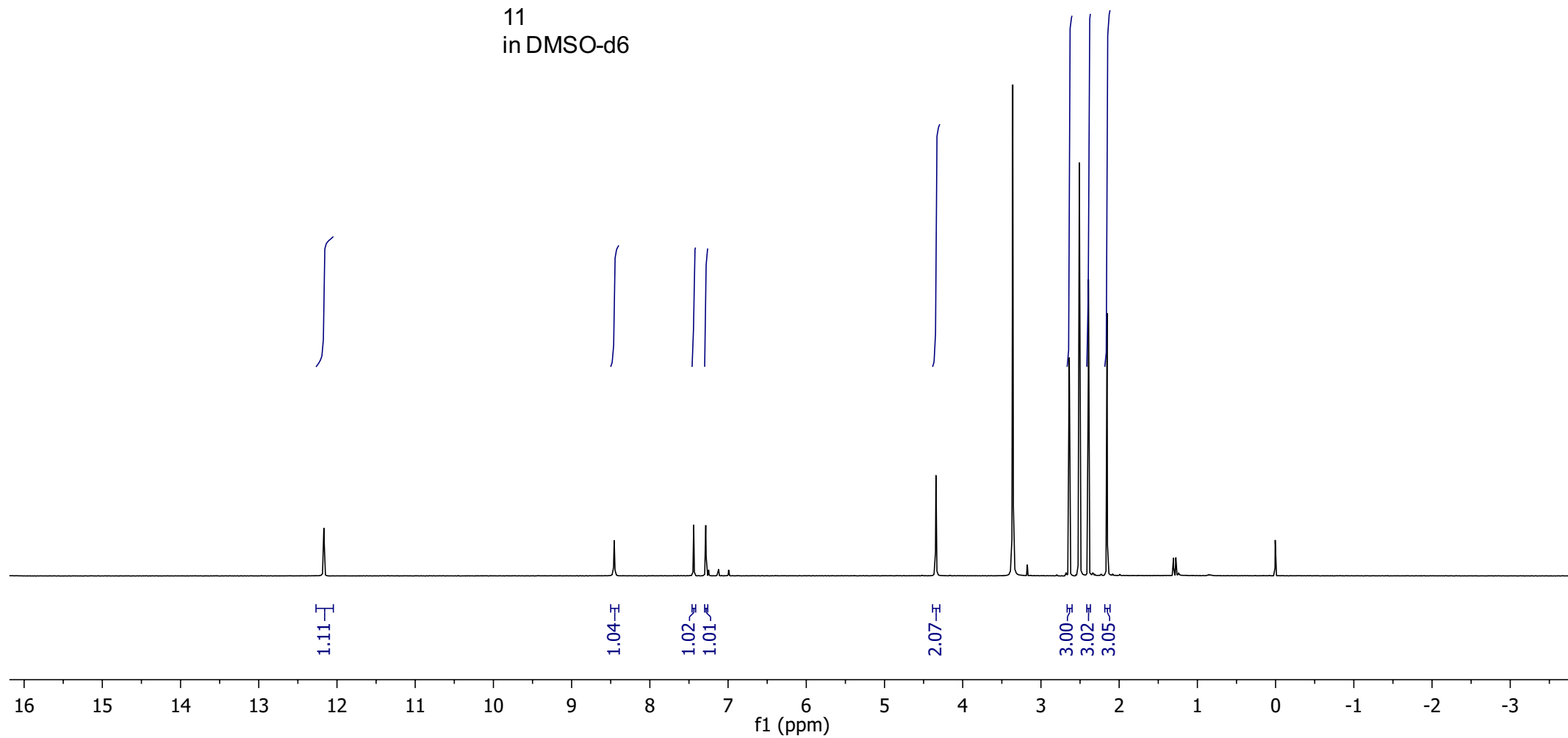


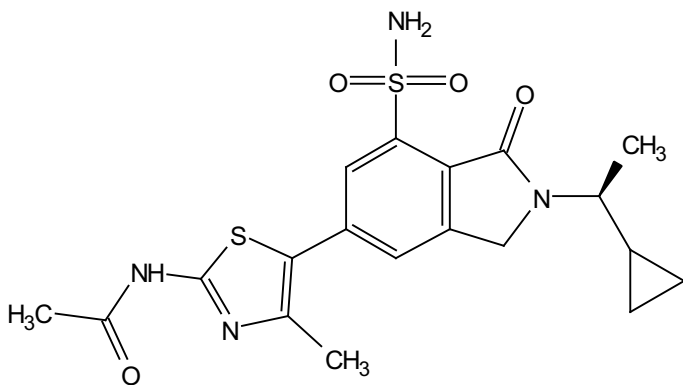
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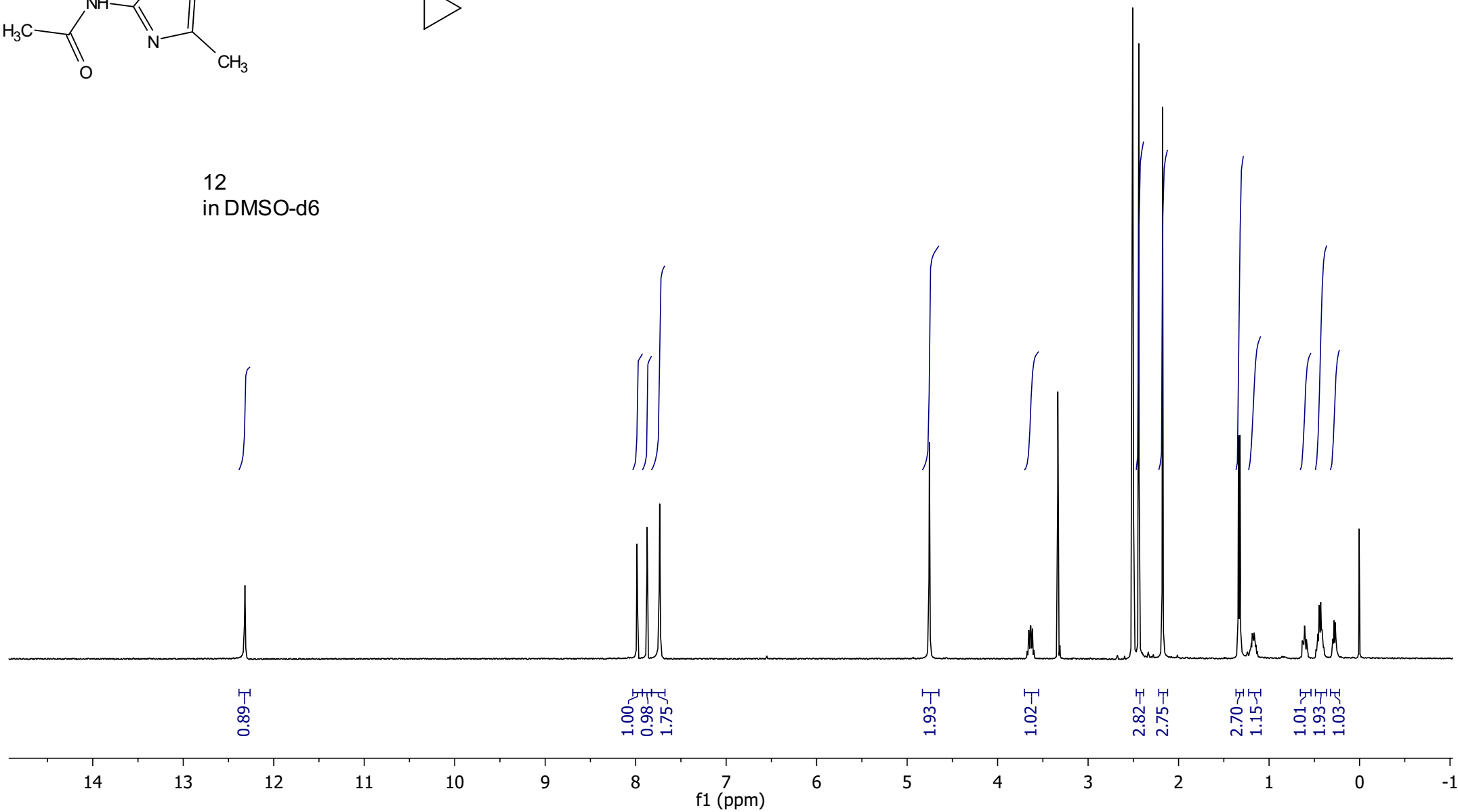


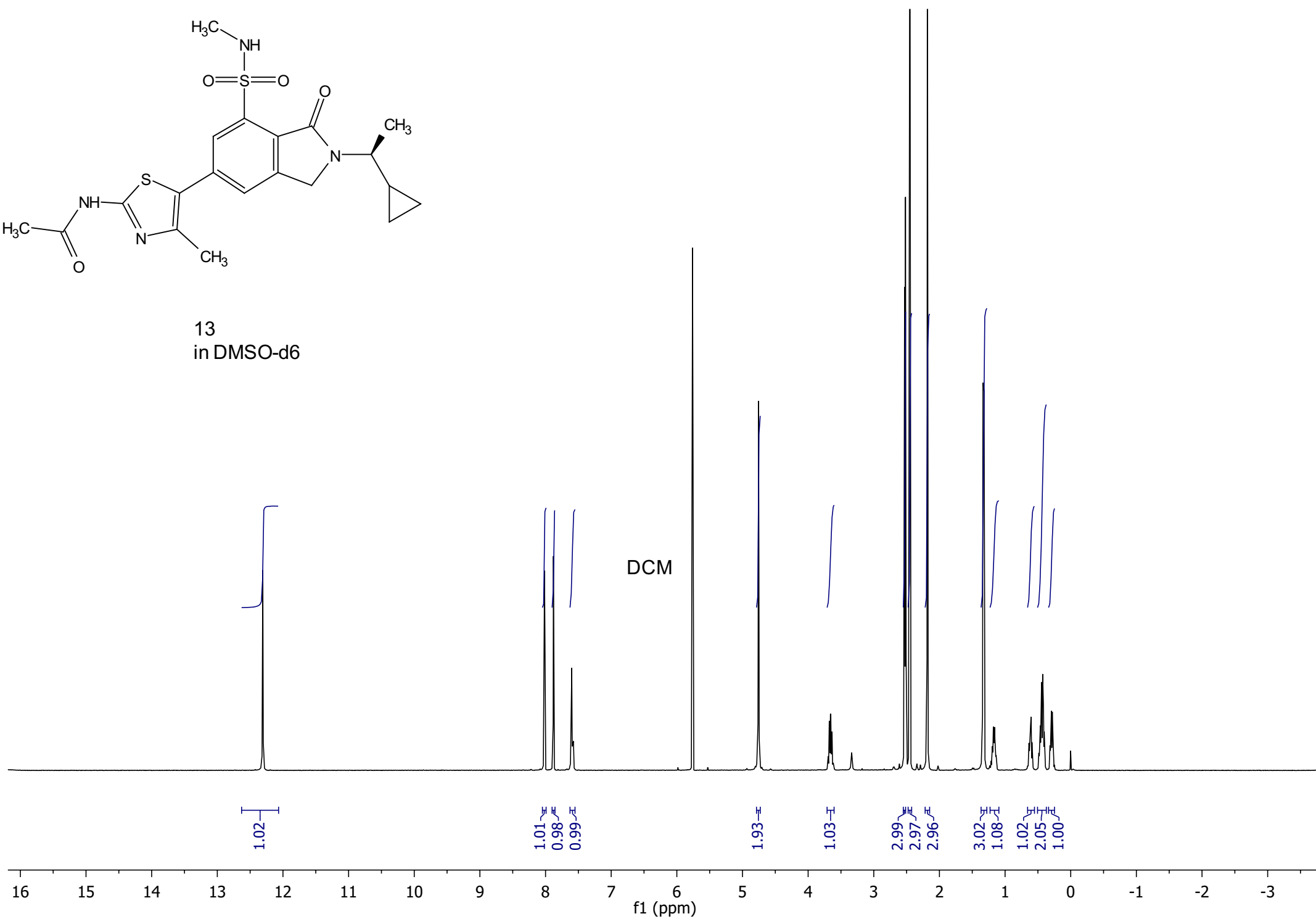
11
in DMSO-d6

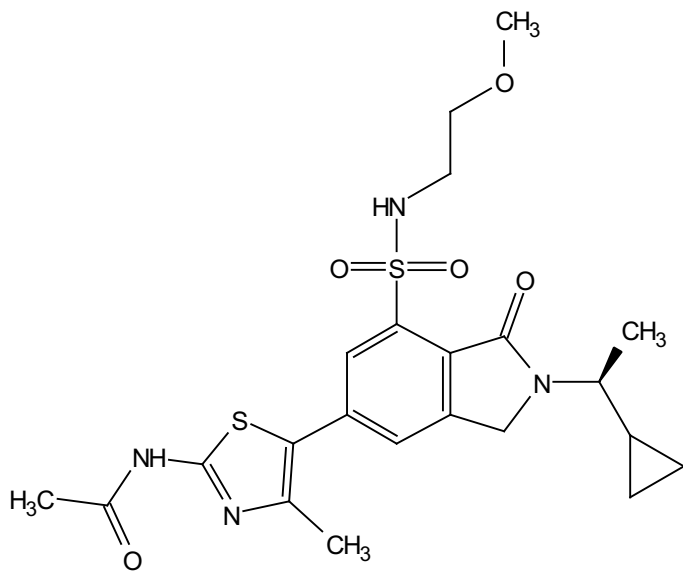




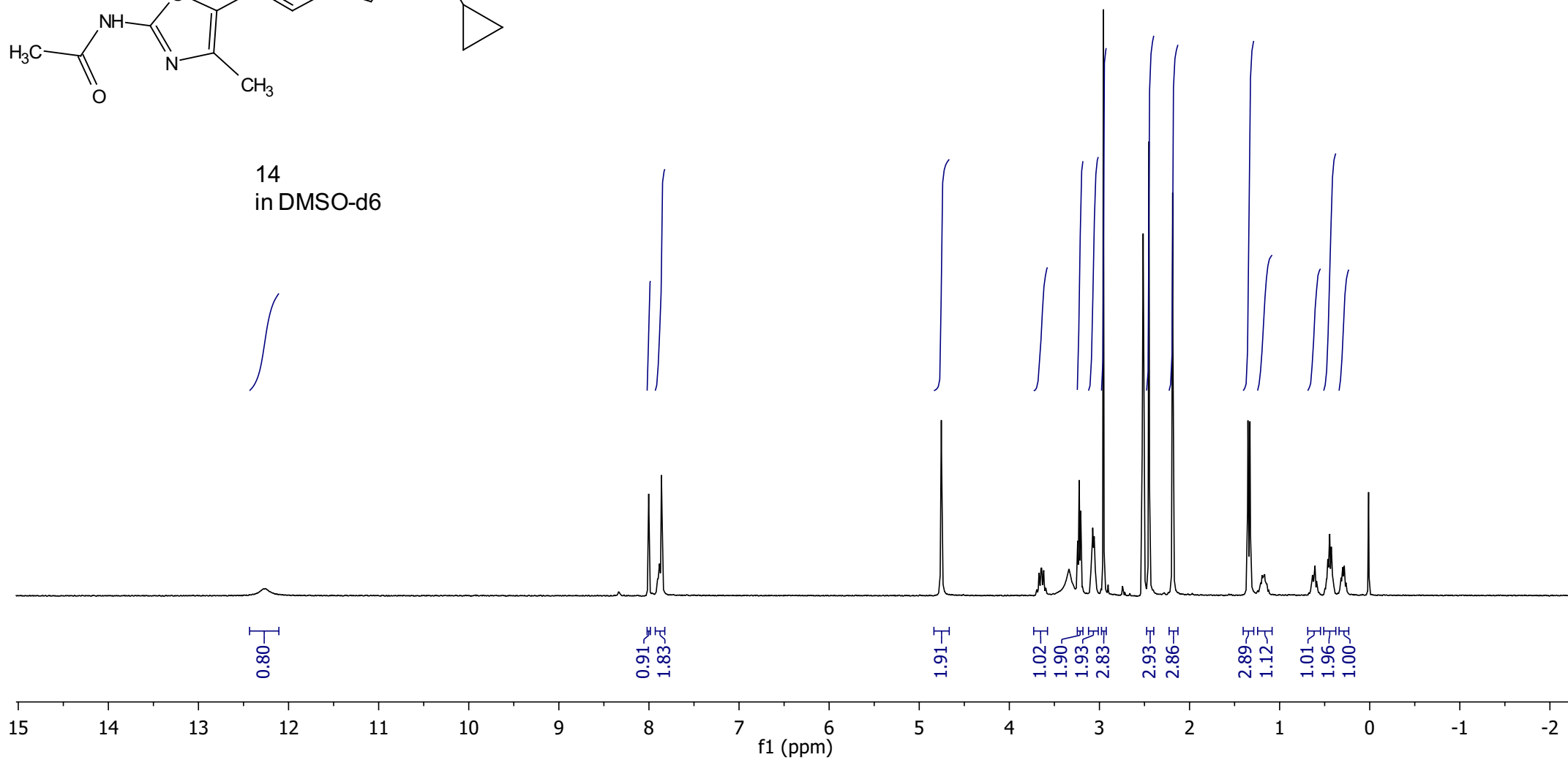
12
in DMSO-d6

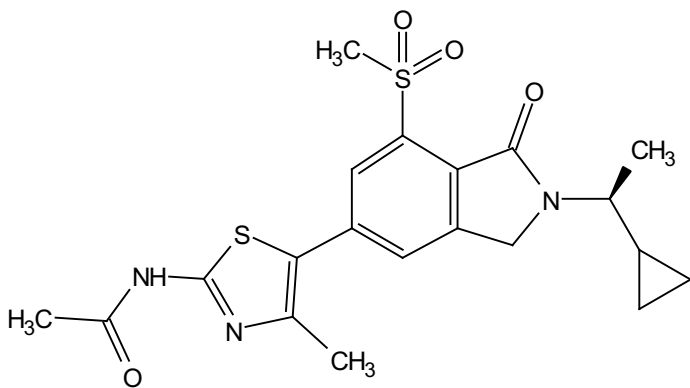




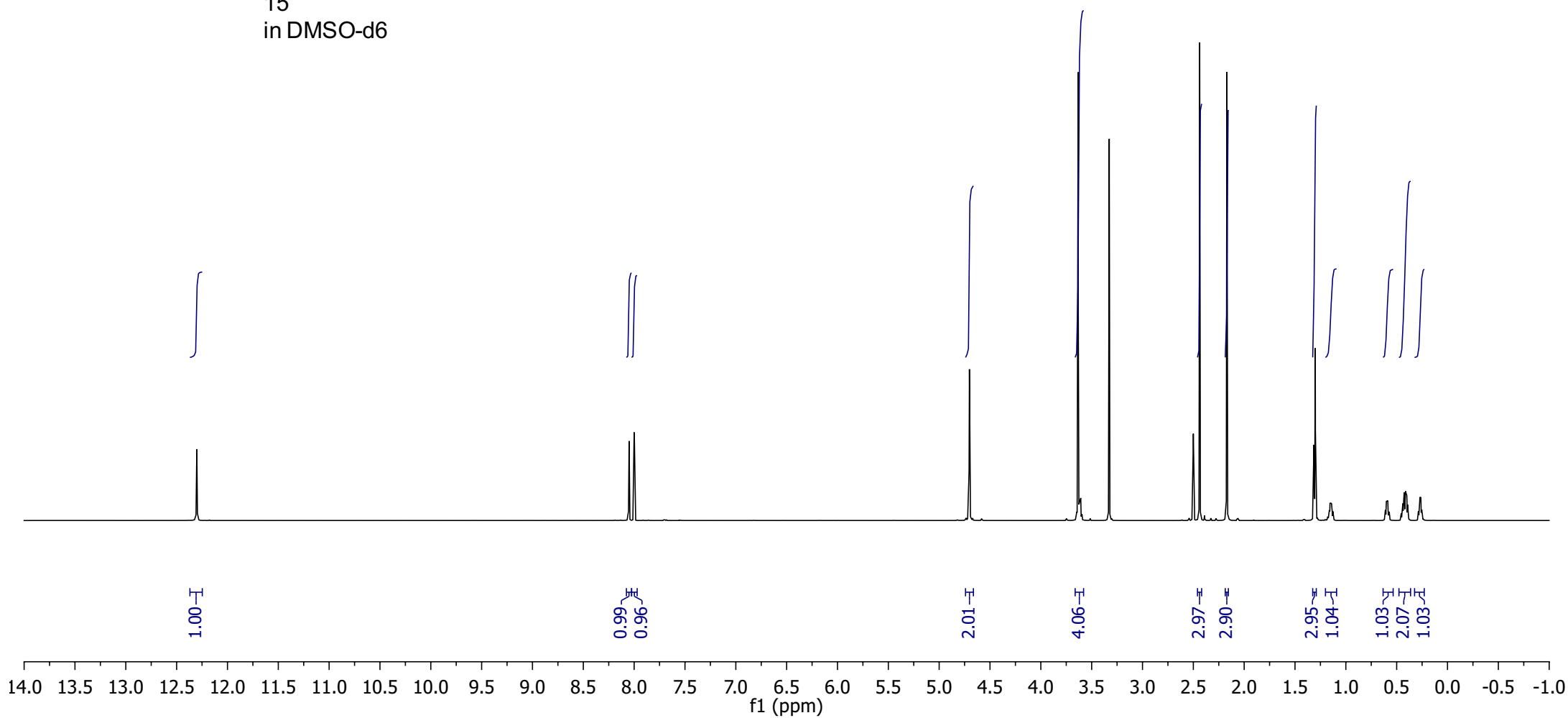


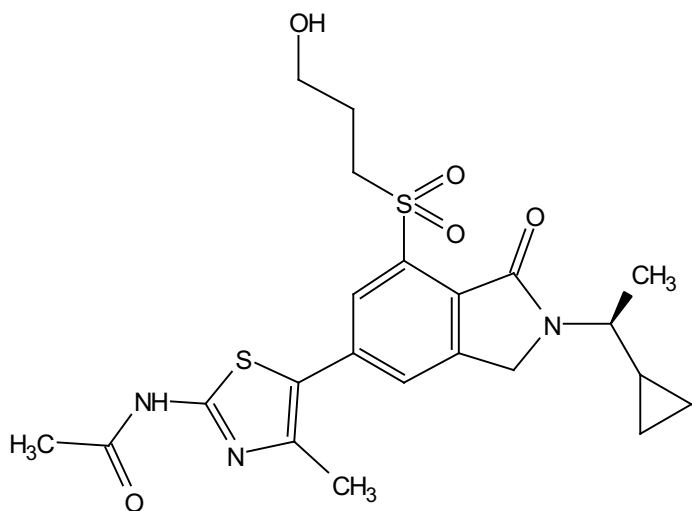
14
in DMSO-d₆



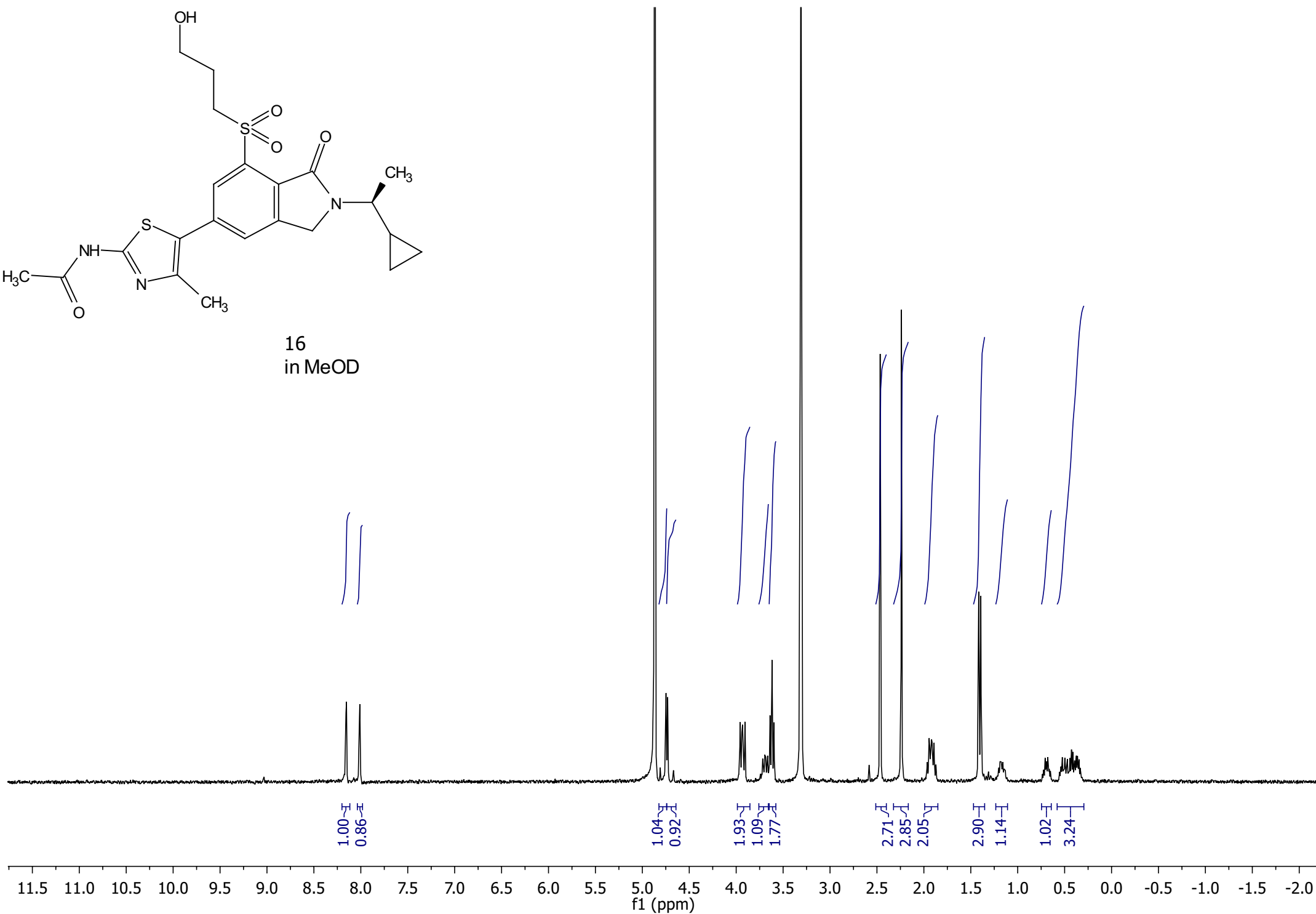


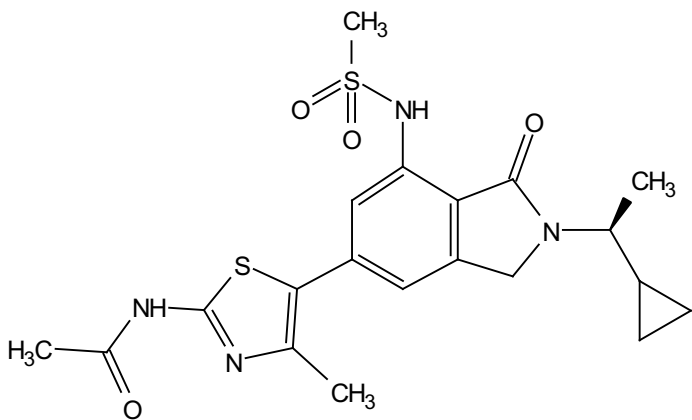
15
in DMSO-d6



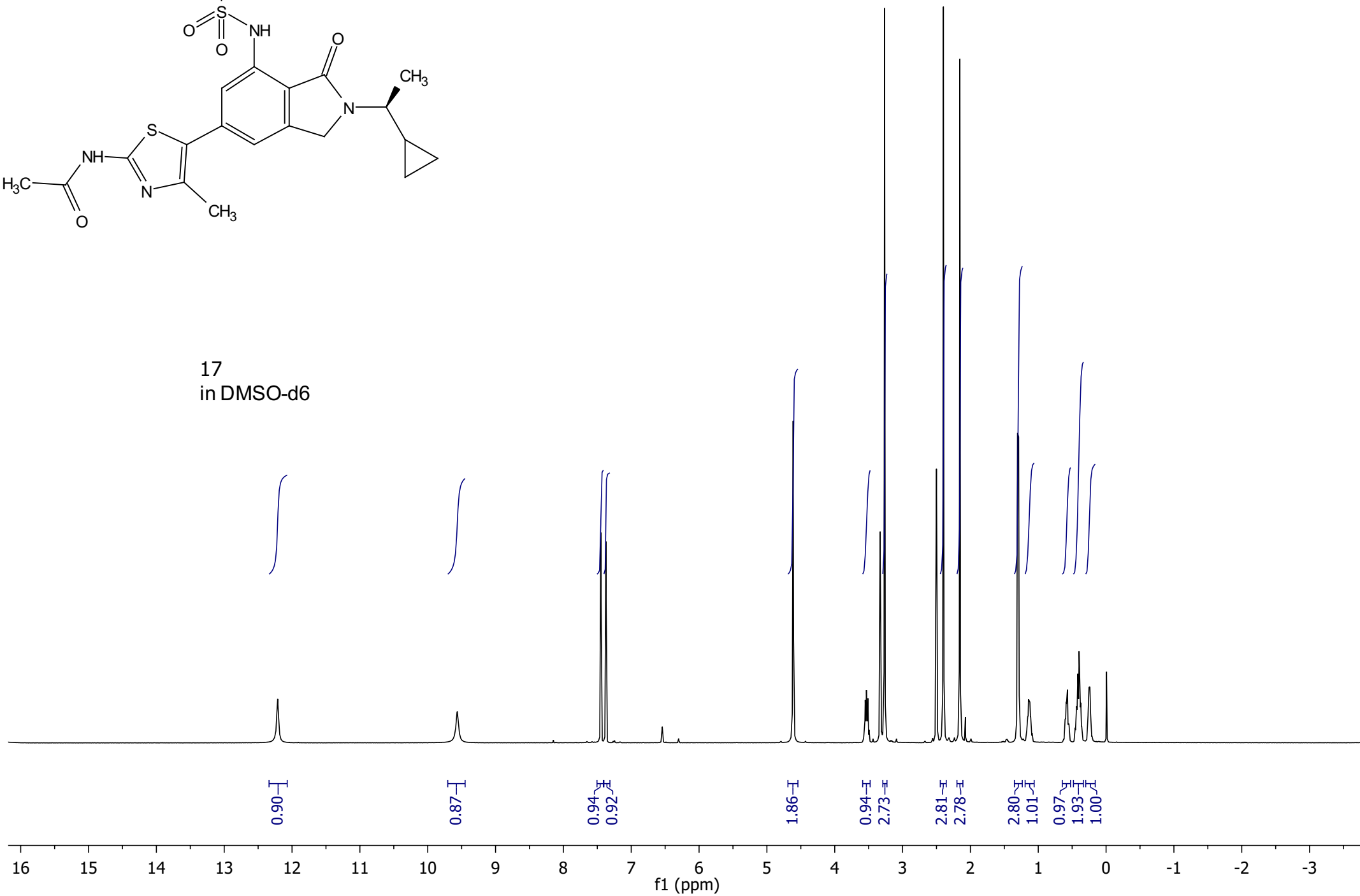


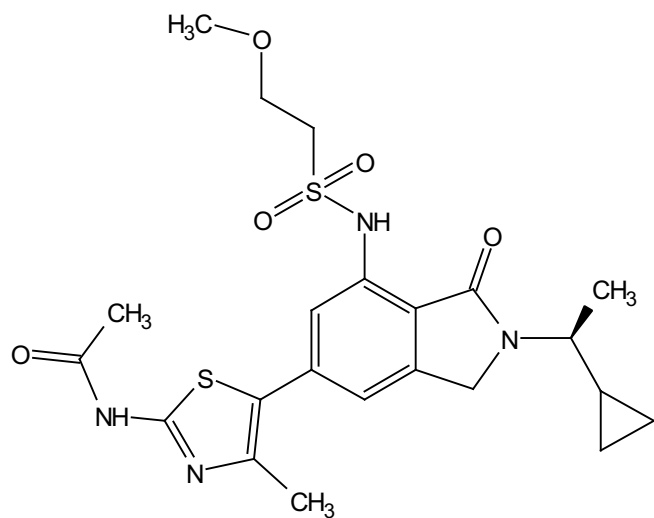
16
in MeOD





17
in DMSO-d6





18
in DMSO-d₆

