

Supporting information

Effects of rigidity on the selectivity of protein kinase inhibitors

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Table S1. Kinase Inhibition Profiles of compounds **1** and **2a**^a

Kinase	1	2a	Kinase	1	2a	Kinase	1	2a	Kinase	1	2a
ABL1	98	96	EPH-A4	99	95	MAPK3	1	1	PKC-ZETA	2	4
AKT1	-1	0	EPH-A5	100	95	MAPKAPK-2	-1	5	PKN1	-2	6
AKT2	0	-10	EPH-A8	100	97	MAPKAPK-3	13	3	PKN2	3	8
AKT3	2	3	EPH-B1	97	84	MARK1	1	-2	PLK1	1	1
ALK	6	1	EPH-B2	99	97	MARK3	-1	1	PLK3	3	0
AMP-A1B1G1	2	0	EPH-B3	98	86	MARK4	-4	-1	PLK4	74	5
AMP-A2B1G1	3	1	EPH-B4	97	80	MEK1	13	-8	PRAK	-1	7
ARG	99	94	ERB-B2	58	0	MEK2	25	6	PRKACA	2	4
ARK5	14	1	ERB-B4	88	4	MELK	-3	2	PRKD1	4	3
AURORA-A	90	29	FAK	-8	3	MER	6	0	PRKD2	1	2
AURORA-B	82	27	FER	40	12	MET	8	-1	PRKD3	-1	1
AURORA-C	33	-2	FES	21	0	MKNK1	0	2	PRKG1	1	1
AXL	12	2	FGFR1	98	91	ALPHA	0	2	PRKG2	24	9
BLK	95	57	FGFR2	99	96	MRCK-BETA	-1	1	PRKX	2	-4
BMX	98	36	FGFR3	52	34	MSK1	-11	5	PTK5	95	18
BRAF	101	92	FGFR4	89	62	MSK2	3	5	PYK2	74	1
BRK	97	86	FGR	99	71	MSSK1	9	0	RET	100	98
BRSK1	-10	0	FLT-1	99	89	MST1	10	4	RIPK2	98	82
BRSK2	-3	0	FLT-3	29	7	MST2	2	2	ROCK1	5	1
BTK	71	5	FLT-4	99	97	MST3	0	3	ROCK2	18	4
CAMK1A	2	1	FMS	99	87	MST4	9	-1	RON	2	1
CAMK1D	7	10	FYN	101	85	MUSK	8	3	ROS	18	5
CAMK2A	3	6	GRK6	-9	3	NDR2	1	-1	RSK1	9	1
CAMK2B	1	5	GRK7	1	5	NDRG1	-3	6	RSK2	0	0
CAMK2D	-1	0	GSK-3-ALPHA	5	6	NEK1	-1	-5	RSK3	10	2
CAMK2G	0	2	GSK-3-BETA	19	3	NEK2	-1	0	RSK4	2	0
CAMK4	11	6	HASPIN	5	1	NEK6	0	-5	SGK1	-1	0
CDK1	6	0	HCK	107	81	NEK7	1	-2	SGK2	0	-2
CDK2	3	1	HIPK1	-3	-2	NEK9	2	-1	SGK3	-2	-3
CYCLINE	1	-1	HIPK2	0	-1	P38-ALPHA	70	15	SIK	43	7
CYCLINE	3	3	HIPK3	0	-1	P38-BETA	23	3	SLK	92	18
CYCLIND	-1	0	HIPK4	29	9	P38-DELTA	0	2	SNF1LK2	7	10
CDK5	2	3	IGF1R	1	2	P38-GAMMA	1	0	SPHK1	2	4
CDK5-P25	2	2	IKK-ALPHA	26	-3	P70S6K1	5	1	SPHK2	0	20
CYCLIND3	8	1	IKK-BETA	22	2	P70S6K2	5	3	SRC	100	80
CYCLINT1	5	-2	IKK-EPSILON	17	8	PAK1	0	-1	SRMS	55	20
CHEK1	-1	-1	INSR	12	5	PAK2	1	-2	SRPK1	1	2
CHEK2	3	-4	IRAK1	28	-3	PAK3	2	1	SRPK2	-1	-1
CK1	10	2	IRAK4	2	3	PAK4	0	2	STK16	8	2
CK1-EPSILON	27	8	IRR	2	-3	PAK5	0	-4	STK25	-5	3
CK1-GAMMA1	9	-5	ITK	23	10	PAK6	-1	3	SYK	-4	6
CK1-GAMMA2	1	2	JAK1	97	-3	ALPHA	0	0	TAK1-TAB1	62	5
CK1-GAMMA3	2	-16	JAK2	77	3	PASK	-4	1	TAOK2	17	17
CLK1	2	-1	JAK3	30	10	ALPHA	100	94	TAOK3	37	33
CLK2	5	-1	JNK1	-9	2	BETA	100	71	TBK1	6	1
CLK3	-1	6	JNK2	26	8	PDK1	4	5	TEC	40	2
CRAF	118	101	JNK3	-8	5	PERK	29	0	TIE2	96	61
CSK	93	31	KDR	109	98	GAMMA1	-3	10	TNIK	71	4
DAPK1	-2	-2	KIT	98	97	GAMMA2	-8	4	TNK1	84	19
DAPK3	11	11	LATS1	-4	3	ALPHA	36	-8	TNK2	13	3
DCAMKL2	0	-1	LATS2	0	-2	PI4-K-BETA	1	-5	TRKA	22	2
DDR2	93	96	LCK	100	85	PIM2	-1	0	TRKB	43	13
DYRK1A	2	2	LOK	97	59	PIM3	0	1	TRKC	59	14
DYRK1B	0	1	G2019S	36	3	PKACB	2	3	TSSK1	0	-1
DYRK2	11	-3	LTK	4	8	PKC-ALPHA	-7	2	TSSK2	-10	1
DYRK3	7	1	LYNA	99	95	PKC-BETA1	-3	2	TTK	33	-3
DYRK4	-1	-7	LYNB	100	94	PKC-BETA2	2	5	TXK	94	5
EGFR	55	3	MAP4K2	67	24	PKC-ETA	-5	2	TYK2	31	2
EPH-A1	97	46	MAP4K4	69	3	PKC-GAMMA	-1	2	TYRO3	67	2
EPH-A2	100	90	MAP4K5	66	33	PKC-IOTA	-3	2	YES	101	88
EPH-A3	98	76	MAPK1	0	2	PKC-THETA	0	-2	ZAP70	3	4

^aSelectivity profiling of **1** and **2a** was performed using Nanosyn Kinase Profiling Service. Value in each cell represents mean percentage inhibition (from duplicates) of the kinase by the drug (1µM). Red: inhibition >90%; yellow: inhibition 40-90%; green: inhibition <40%.

Figure S1. Percentage inhibition of Abl kinase by compounds **1** and **2a-g** at a fixed concentration of 30 nM.

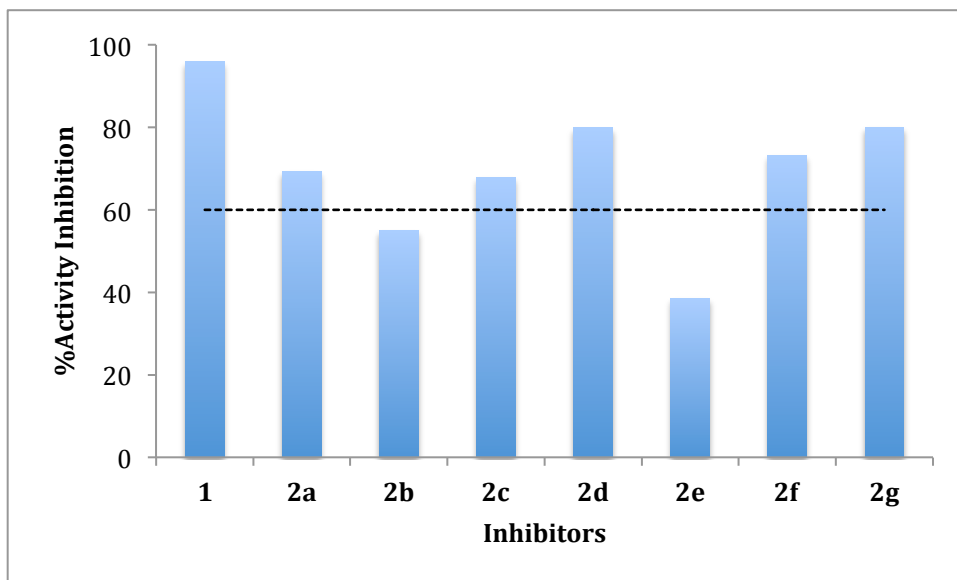


Table S2. BRAF:2a crystal structure refinement statistics

	BRAF:2a
Search model	4R5Y
Space Group	P2 ₁ 2 ₁ 2
Wavelength (Å)	0.97910
Unit Cell	
a	85.47
b	114.53
c	55.59
α	90
β	90
γ	90
Molecules/ASU	2
Data processing	
Resolution (Å)	50.00 – 2.55 (2.70 – 2.55)
Total no. of reflections	117664
Total no. of unique reflections	18363
Redundancy	6.4 (6.6)
I/σI	10.52 (0.6)
Completeness (%)	99.7 (99.9)
R _{meas}	0.15 (3.50)
CC (1/2)	(0.557)
Refinement*	
R _{work} /R _{Free}	25.3/28.3
No. Atoms	
Protein	3672
Ligand (2a)	70
Water	3
Average B-factors	
Protein	101.7
Ligand (2a)	83.8
Water	88.8
RMSD from ideal geometry	
Bond Lengths (Å)	0.002
Bond Angles (°)	0.546
Ramachandran statistics	
Residues in favoured regions (%)	97.7
Residues in allowed regions (%)	2.3
Residues in disallowed regions (%)	0
PDB ID	

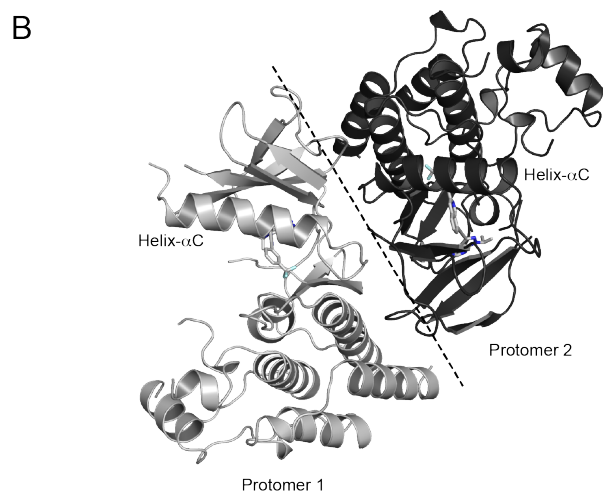
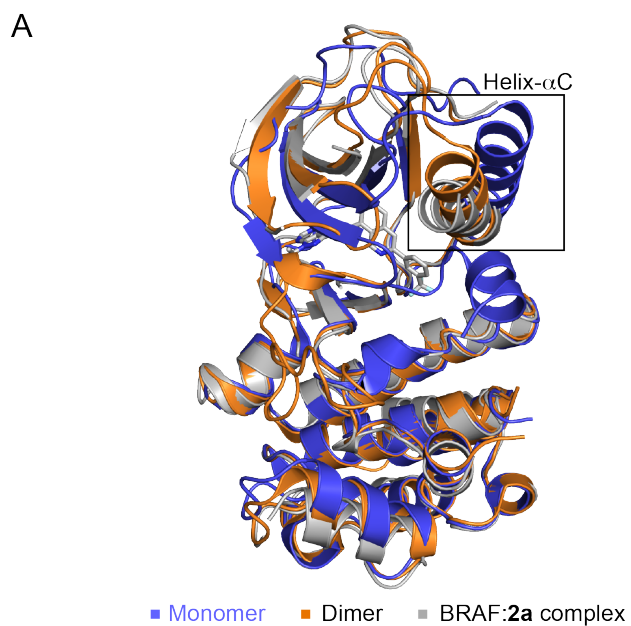


Figure S2. Structural analysis of the BRAF:2a complex. (A) Comparison of the BRAF:2a complex structure with an off-state (monomer) and an on-state (dimer) of BRAF (PDB 4WO5 and PDB 4H58, respectively). (B) The asymmetric unit of the BRAF:2a complex forms a side-to-side dimer reflective of the kinase active state.

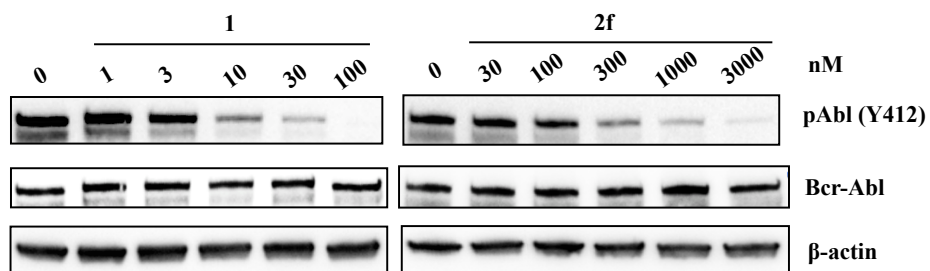


Figure S3. Effects of **1** and compound **2f** on downstream signaling of Bcr-Abl^{WT} in Ba/F3 cells. Ba/F3 cells expressing Bcr-Abl^{WT} were cultured at a density of 8×10^5 cells/mL and treated for 3 h at indicated concentrations of **1** and **2f**. The cell lysates were resolved by PAGE, transferred to nitrocellulose membrane, and sequentially probed with antibodies for Abl, pY412-Abl, and β-actin.

Table S3. Solubility of Compound 1

Std Concentration (uM)	AUC			Average	StD
	Inj 1	Inj2	Inj3		
1.5625	45901	45817	45535	45751	191.7185
6.25	181708	184151	182559	182806	1240.088
25	720683	723684	725498	723288.3	2431.763
100	2859788	2871196	2839198	2856727	16217.08
400	11676645	11536201	11562619	11591822	74637.3
1000	27266144	27041258	27130304	27145902	113251.5
Unknown (2 folds diluted)	2359566	2307116	2315327	2327336	28212.03

Unknown concentration (uM)

80.56

Solubility of 1 in 37 C (uM)

161.12

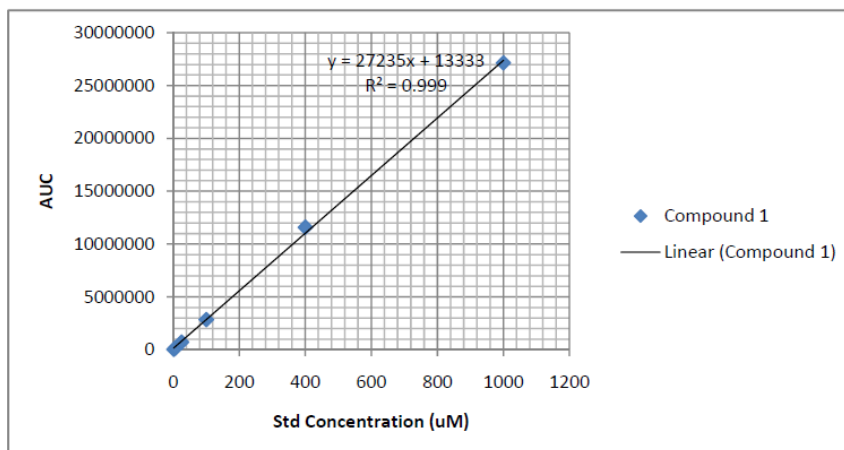


Table S4. Solubility of compound 2a

Std Concentration (uM)	AUC			Average	StD
	Inj 1	Inj2	Inj3		
0.625	20122	22373	19675	20723.33	1446.03
2.5	75798	75285	76054	75712.33	391.592
10	300519	302139	299106	300588	1517.677
40	1233407	1209382	1200864	1214551	16876.04
160	4764443	4737779	4718575	4740266	23034.89
Unknown (2 folds diluted)	889624	917639	883567	896943.3	18177.04

Unknown concentration (uM)

30.01

Solubility of 2a in 37 C (uM)

60.02

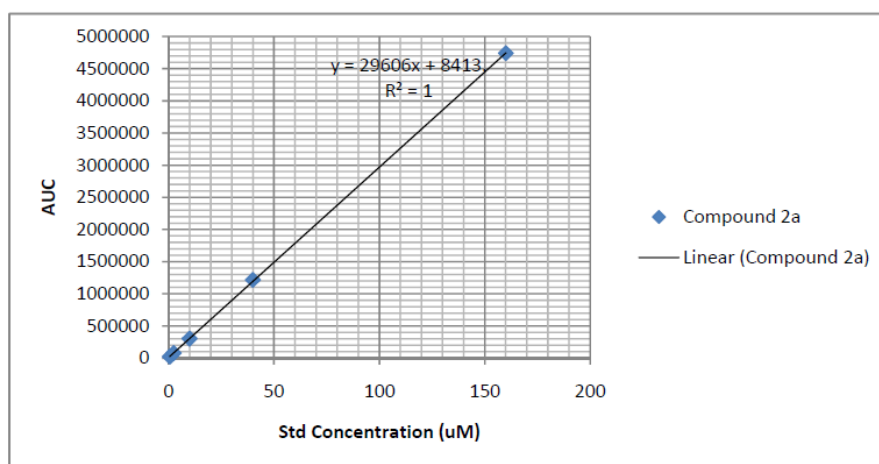


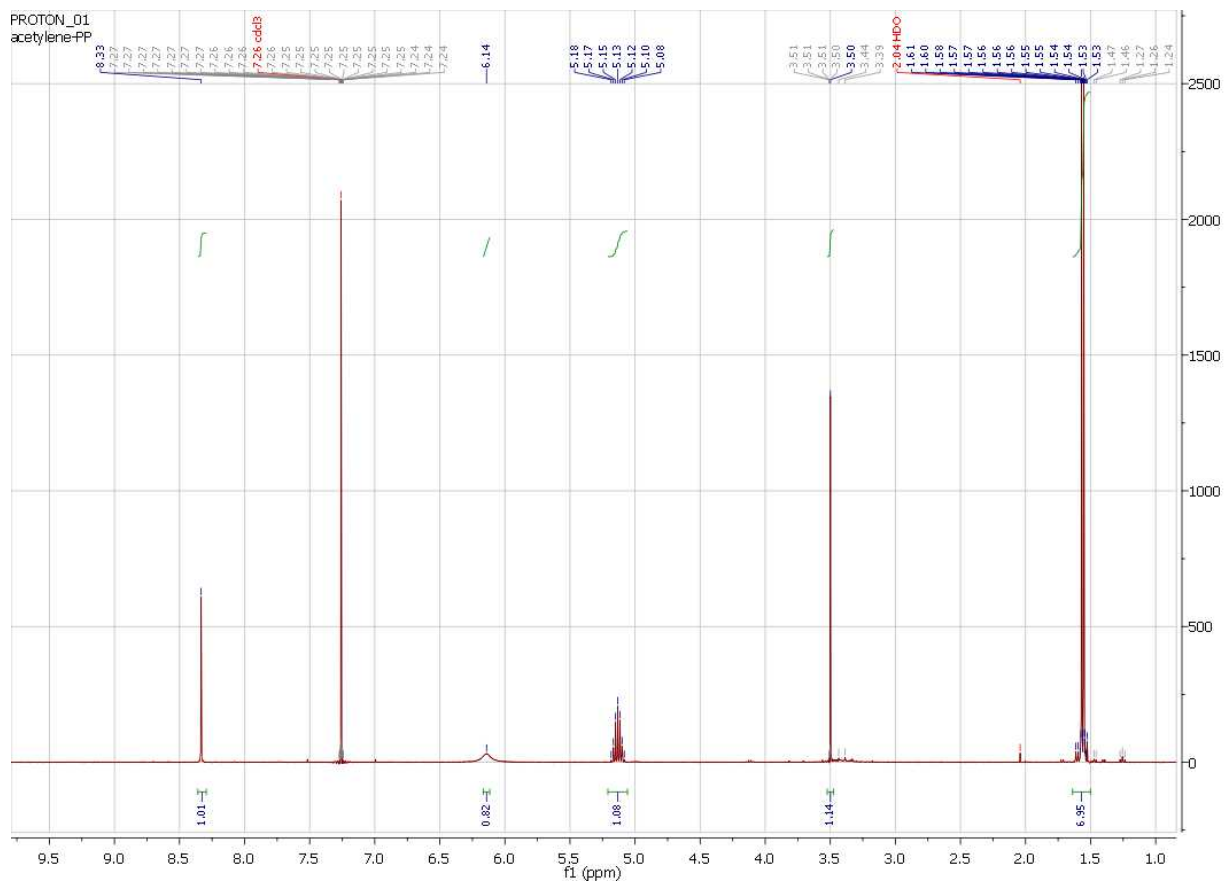
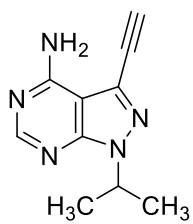
Table S5. Permeability of compound **1** and **2a**

Permeability Assay in Caco-2 Data Report									
Sample ID	M.W	cell number/well	Donor buffer pH	Pgp inhibitor	Papp (x10 ⁻⁶ cm/s) (N=2)		Efflux ratio	Permeability Classification	Pgp or BCRP substrate
					A→B	B→A			
Digoxin	780.940	60000	7.4	minus	1.2	32.4	26.1	low	yes
				plus	8.9	9.3	1.0		
Propranolol	295.800			minus	33.0	20.9	0.6	Medium	No
				plus	44.4	20.0	0.5		
1	464.157			minus	0.5	1.0	1.9	low	No
				plus	0.6	0.3	0.5		
2a	472.162			minus	0.8	2.6	3.2	low	yes
				plus	0.2	0.3	1.1		

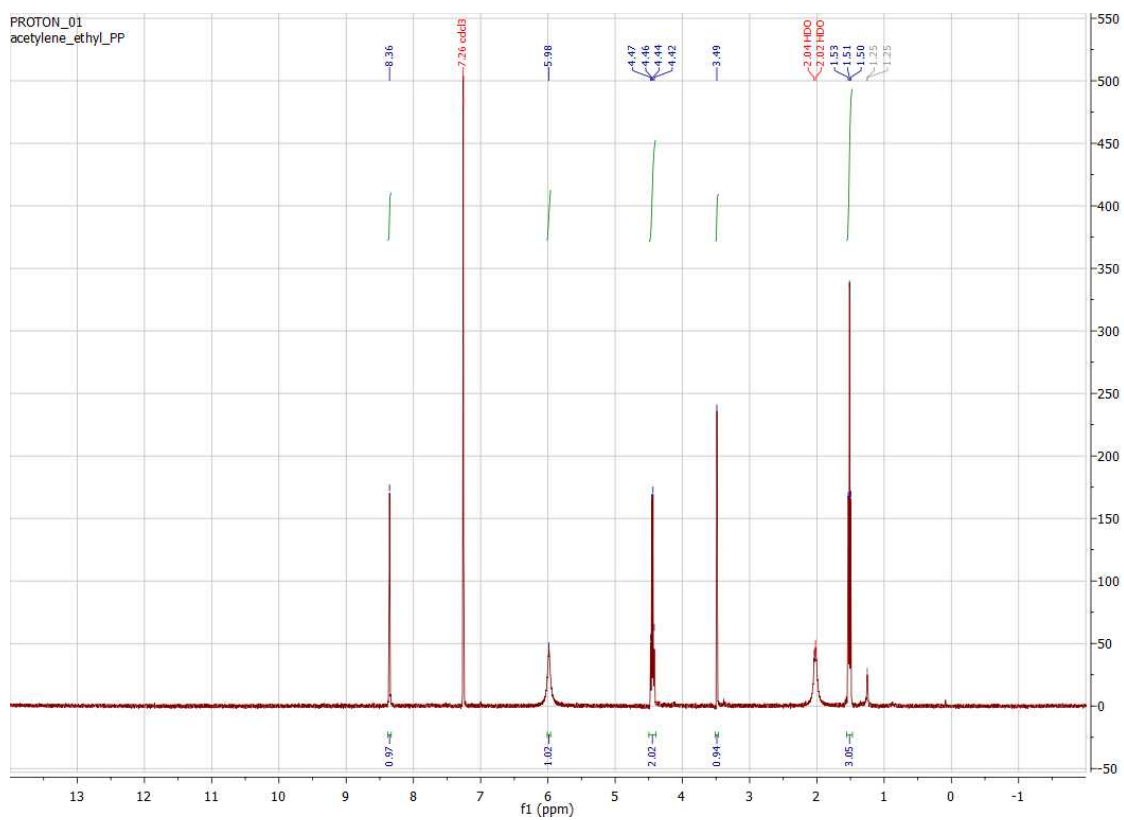
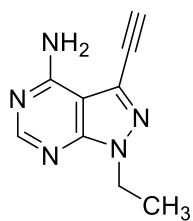
Papp A→B > 25(10⁻⁶ cm/s) high
 Papp A→B 0-25 (10⁻⁶ cm/s) med
 Papp A→B < 10 (10⁻⁶ cm/s) low

Efflux > 3.0 minus Pgp inhibitor Pgp substrate
 Efflux ≤ 1.0 plus Pgp inhibitor

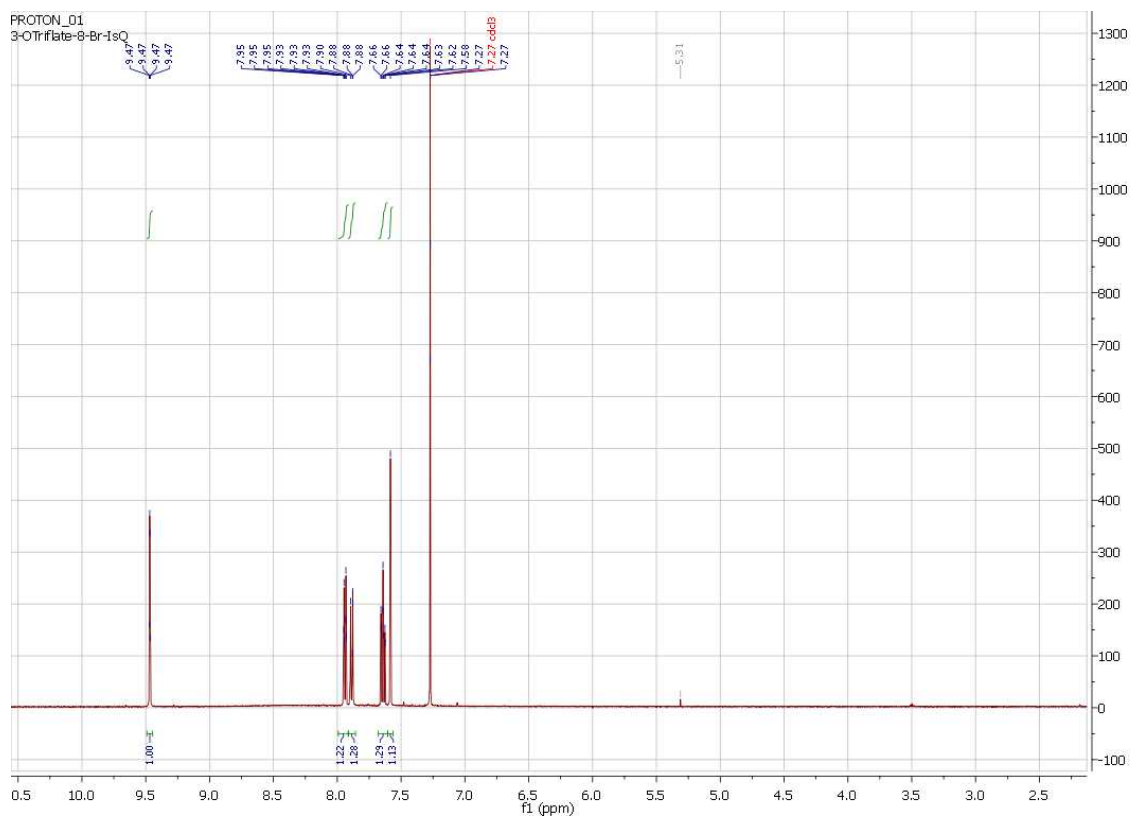
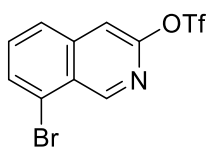
¹H NMR Compound 4a



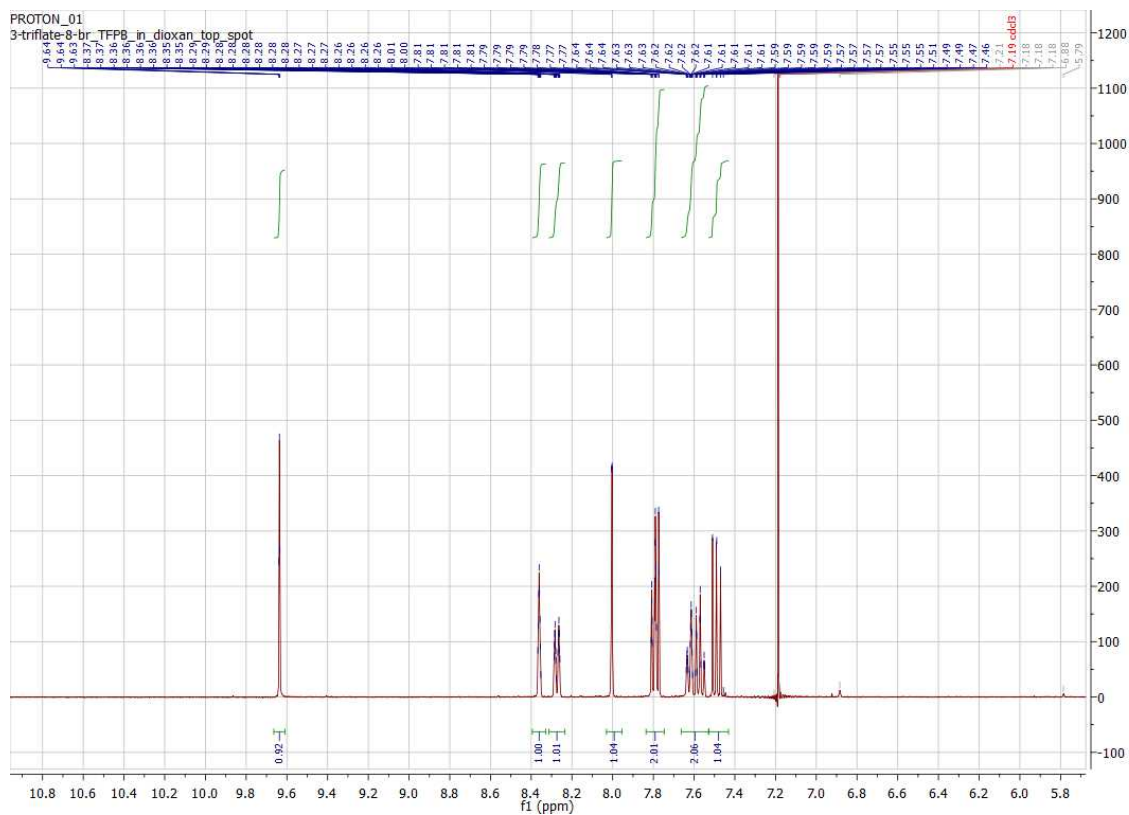
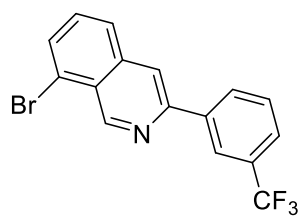
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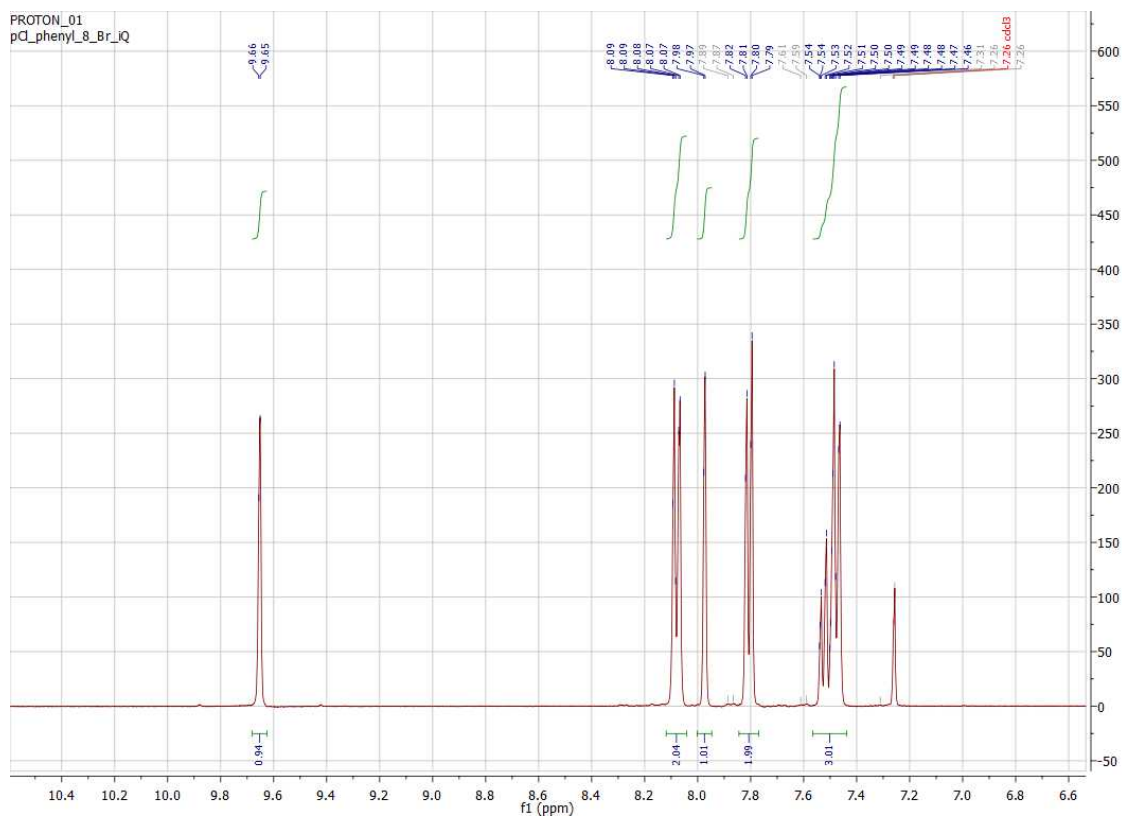
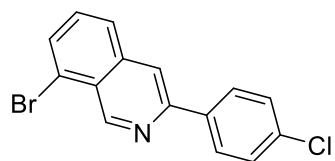
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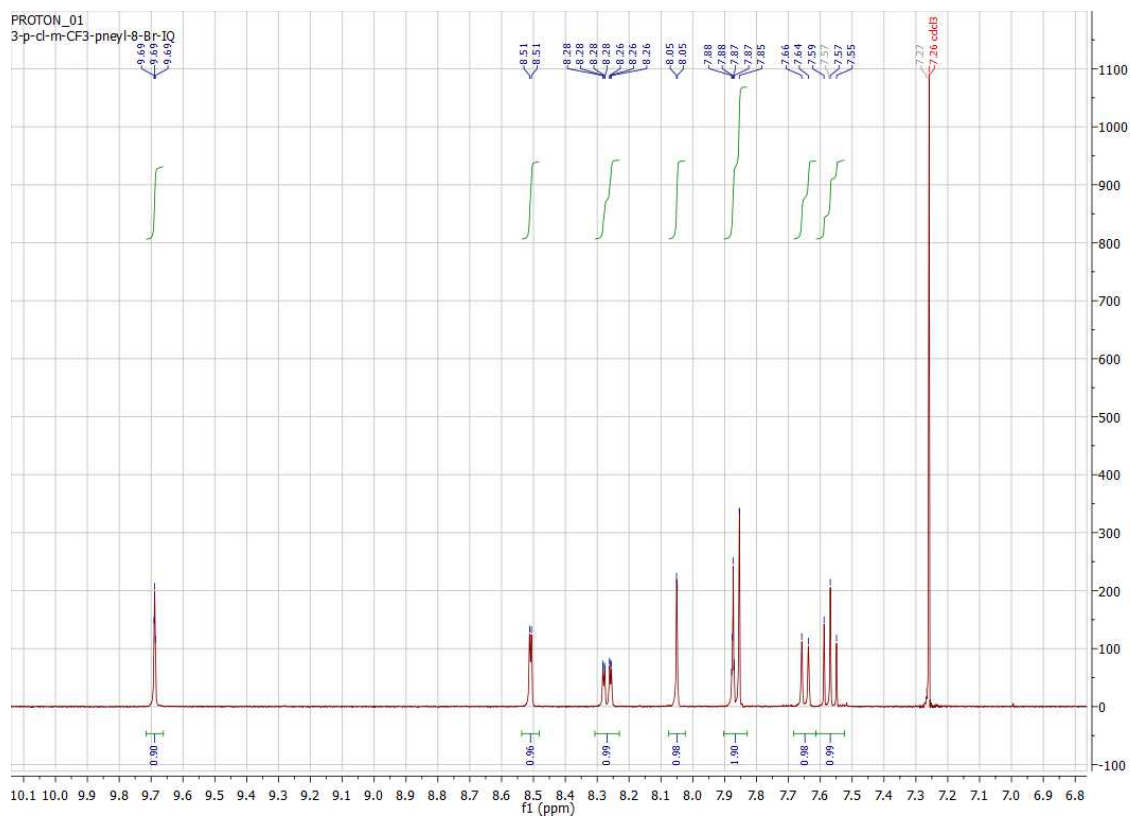
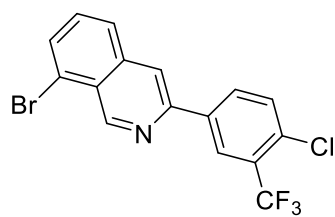
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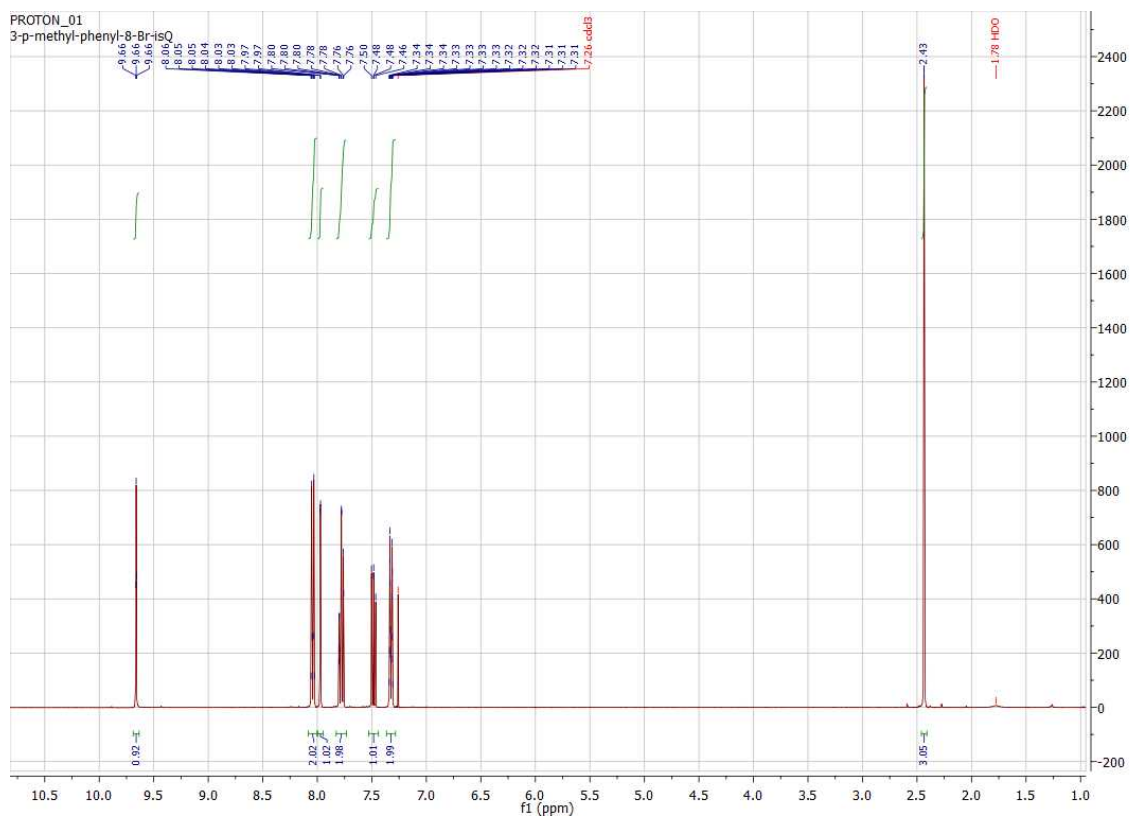
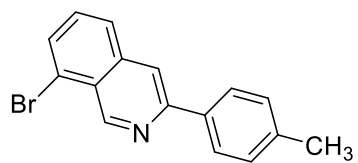
¹H NMR Compound **6b**



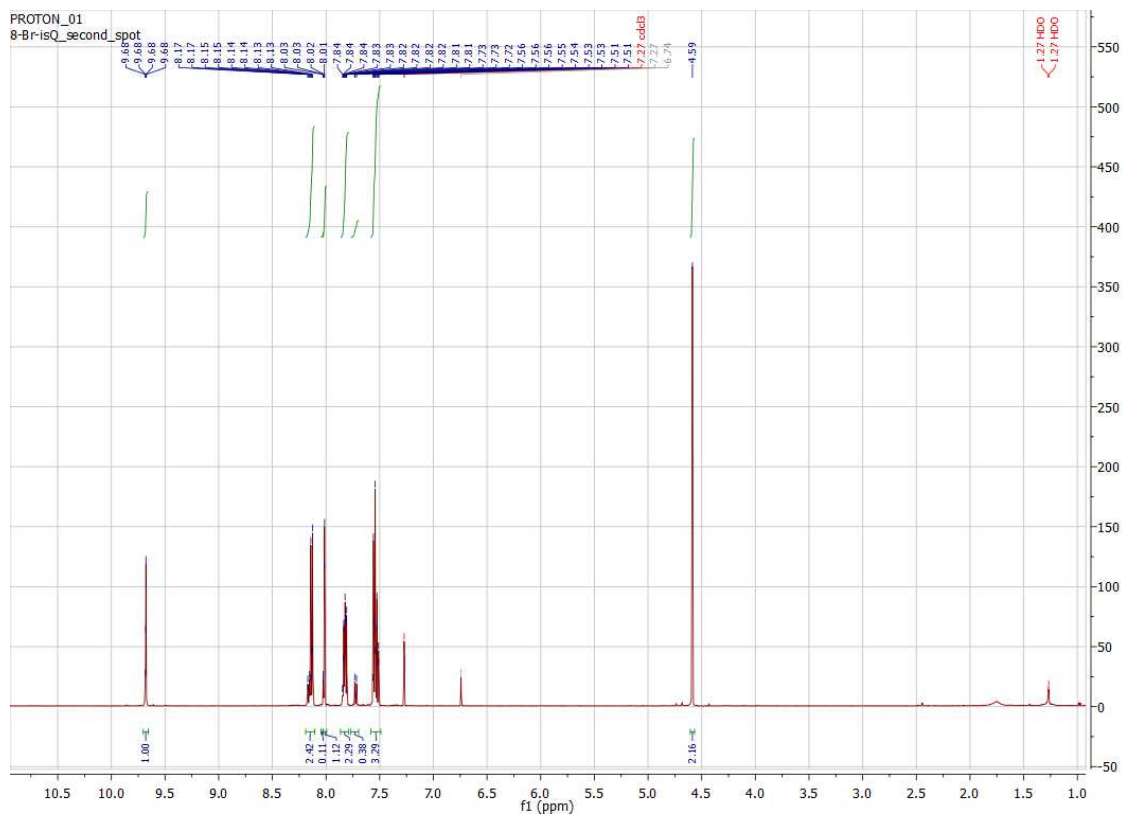
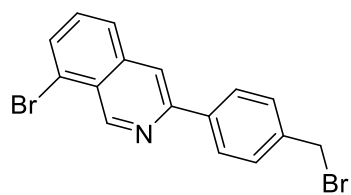
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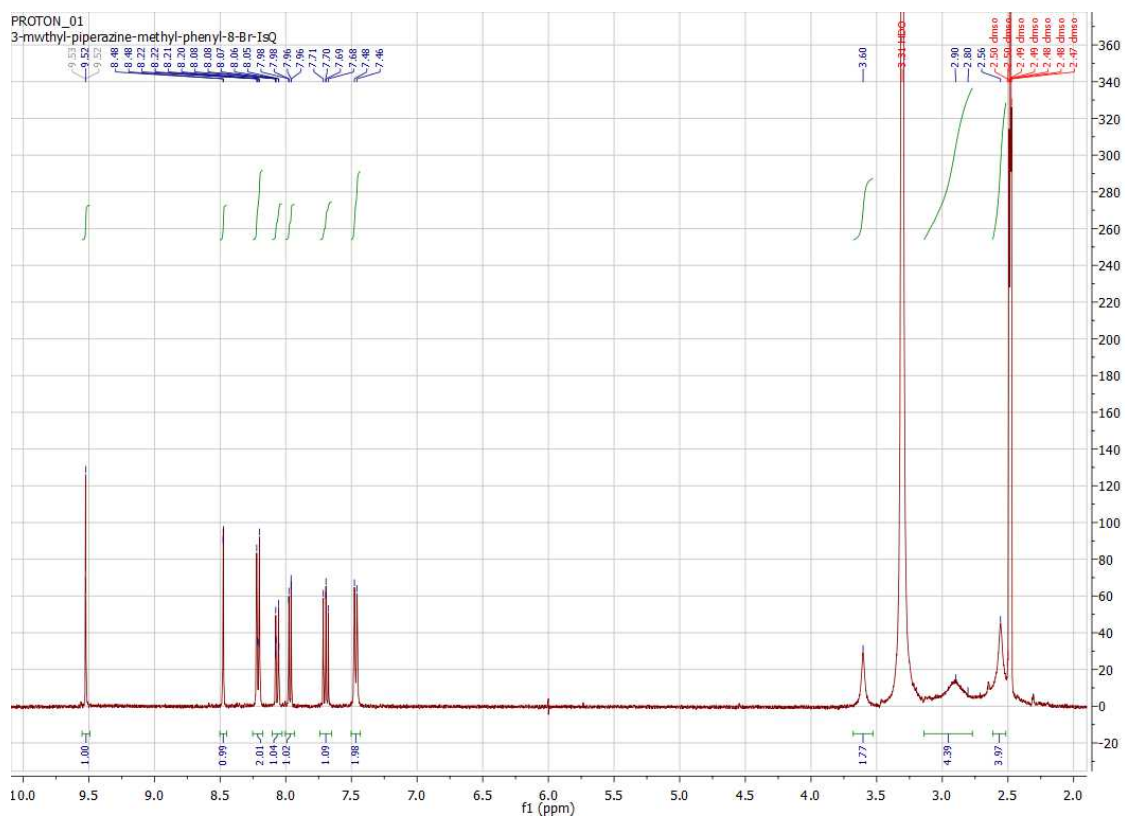
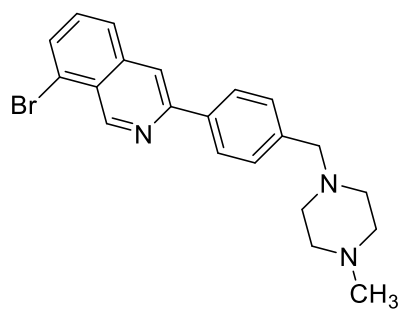
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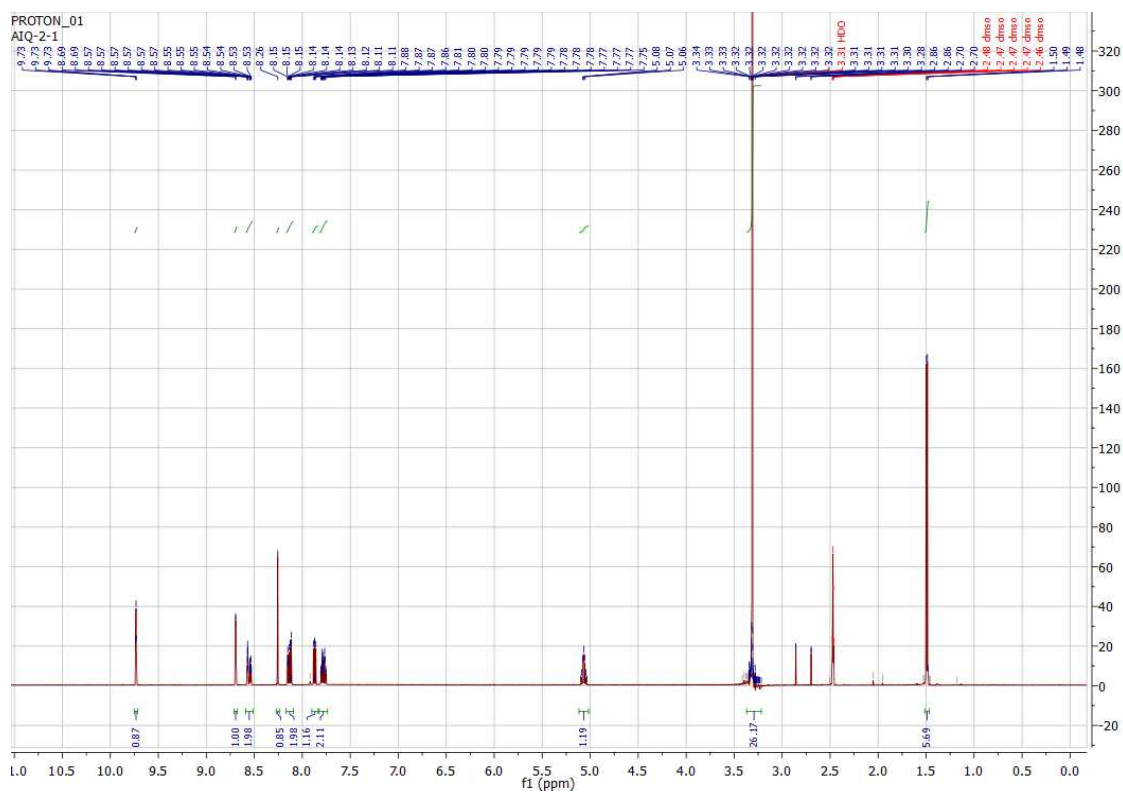
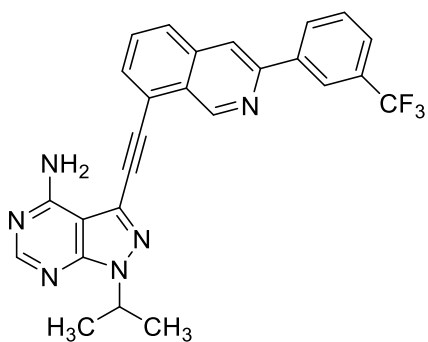
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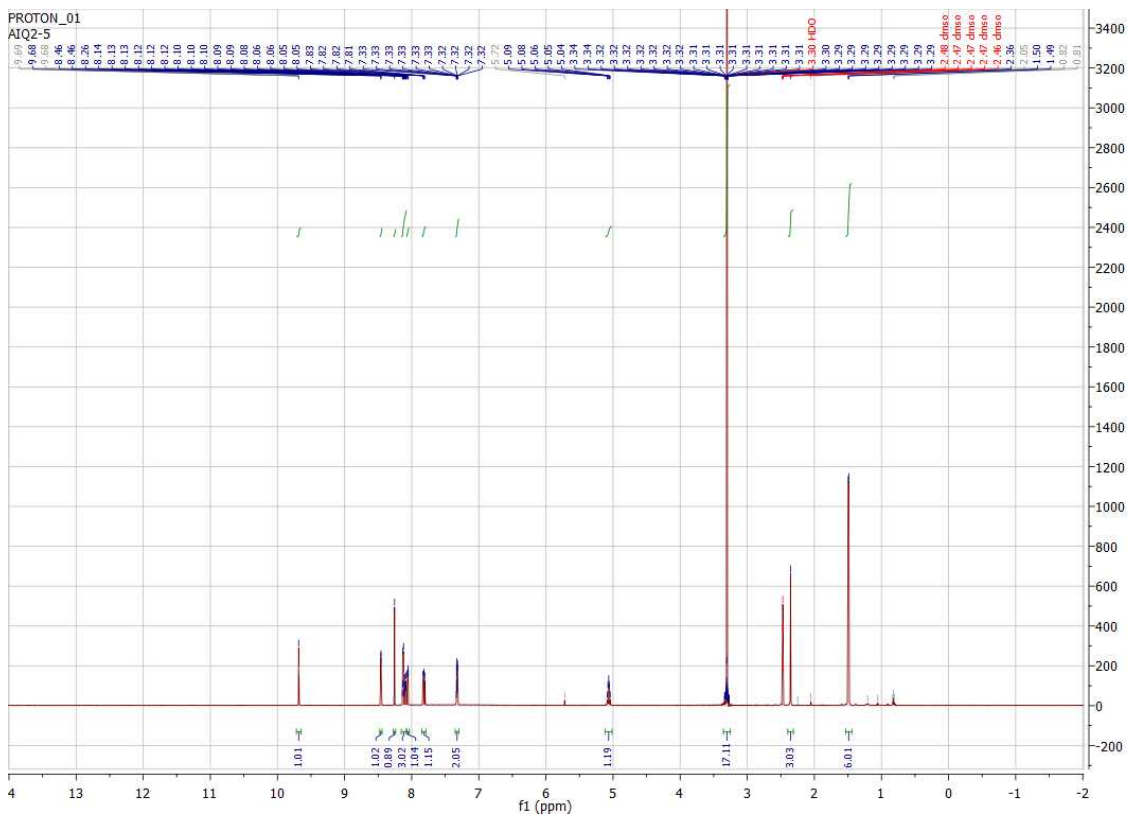
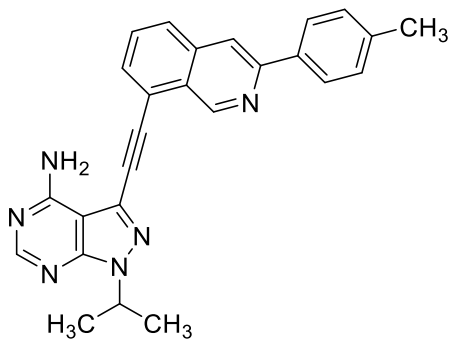
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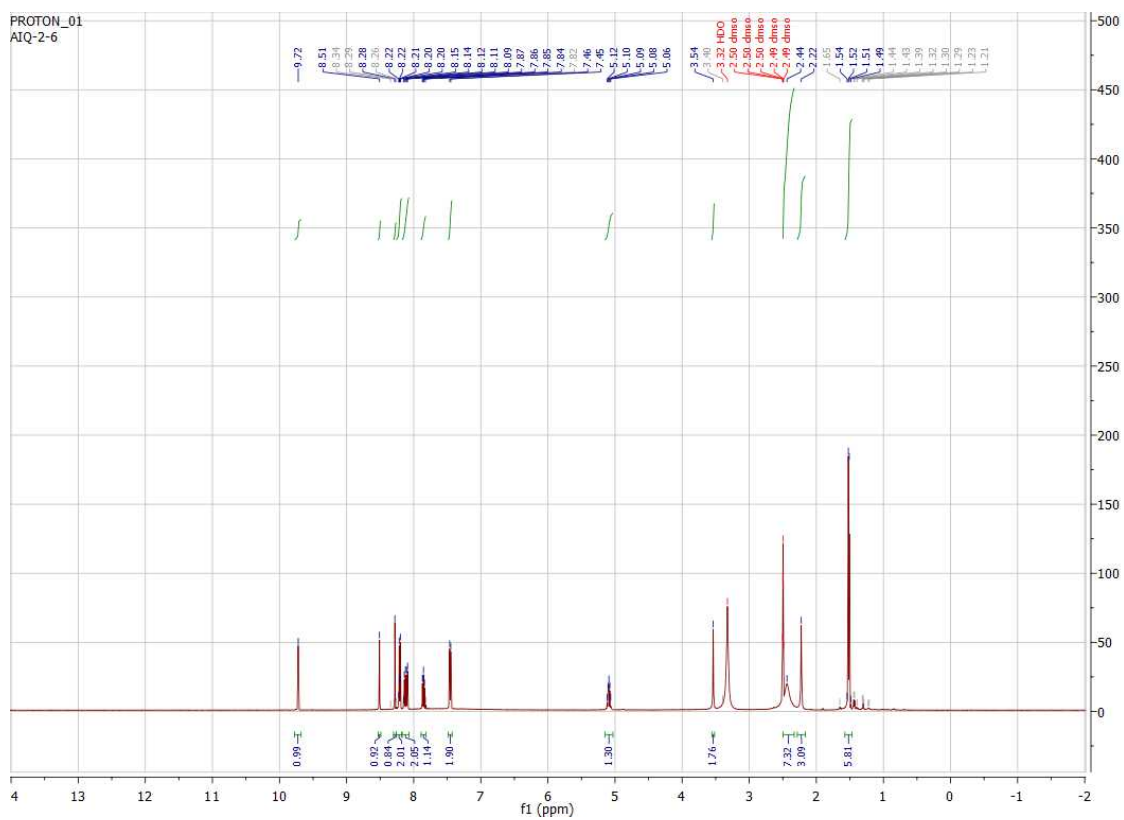
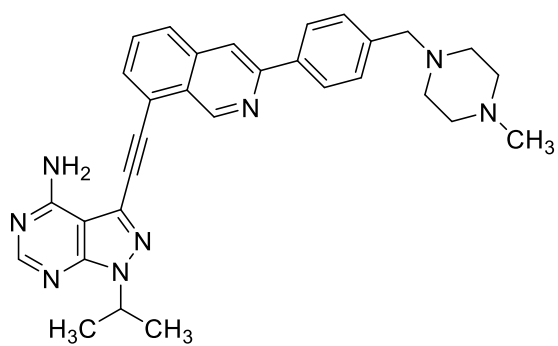
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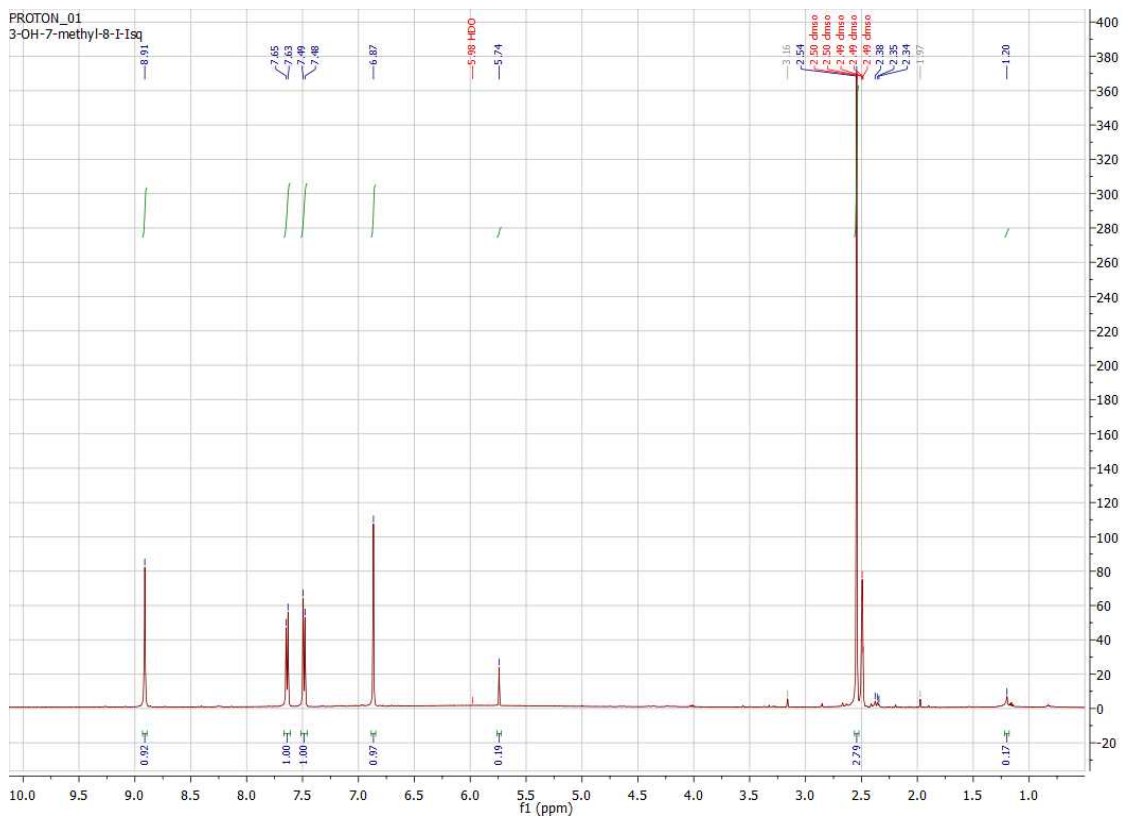
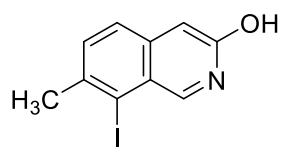
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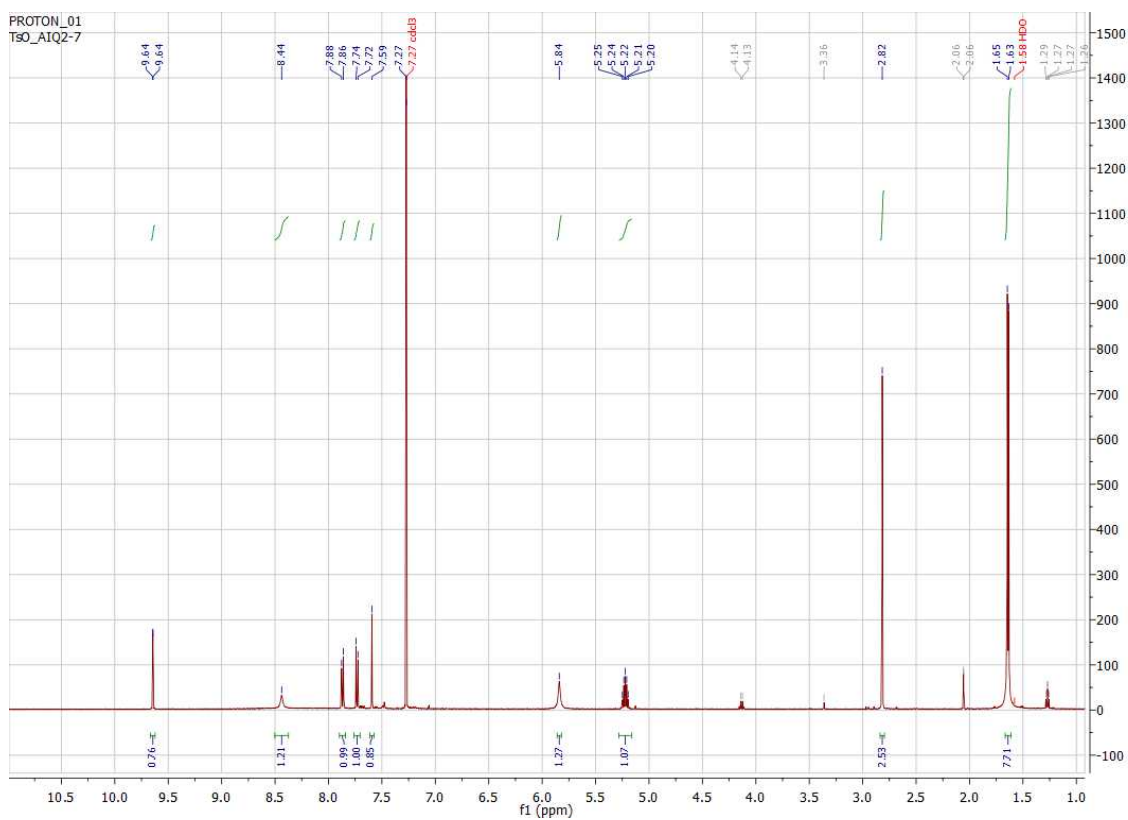
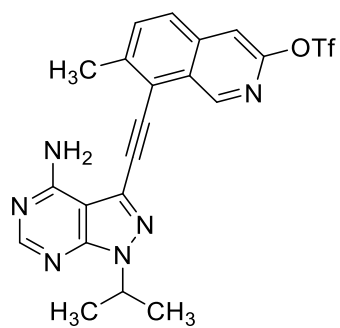
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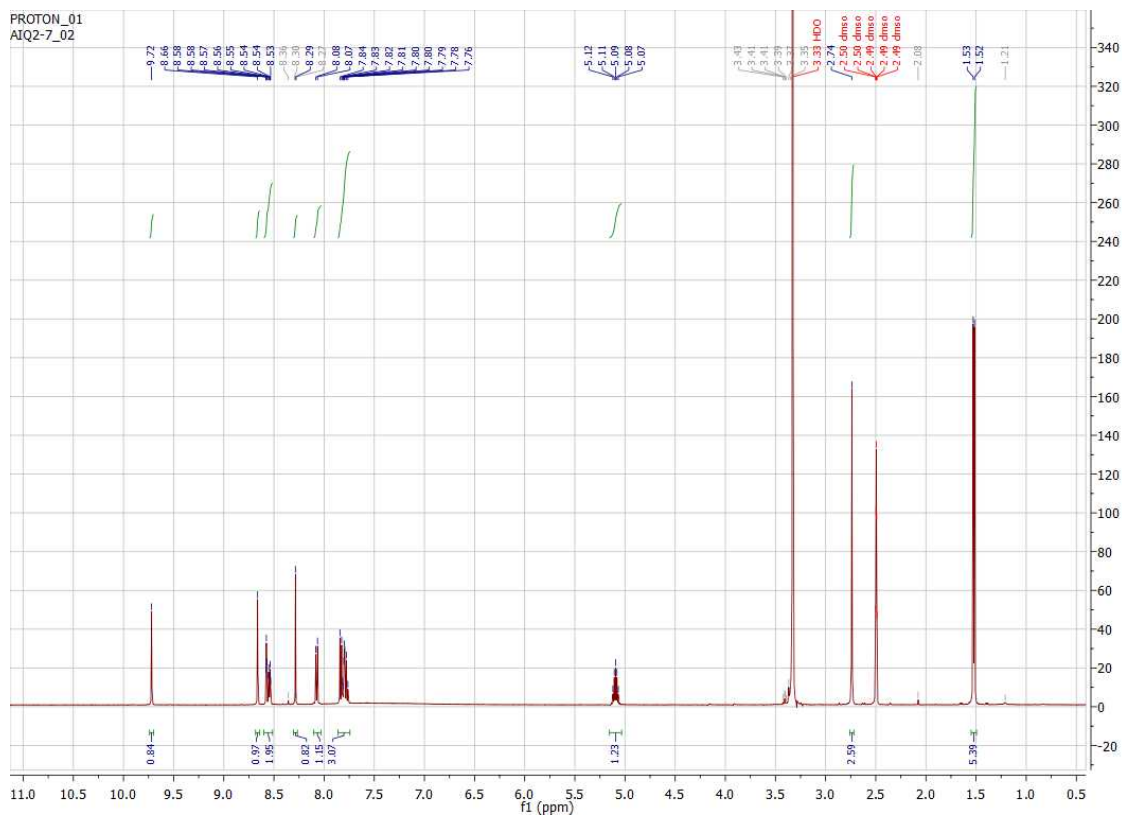
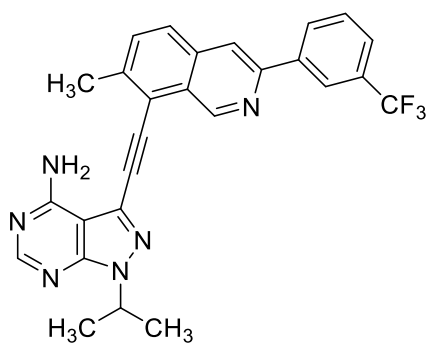
¹H NMR Compound 9



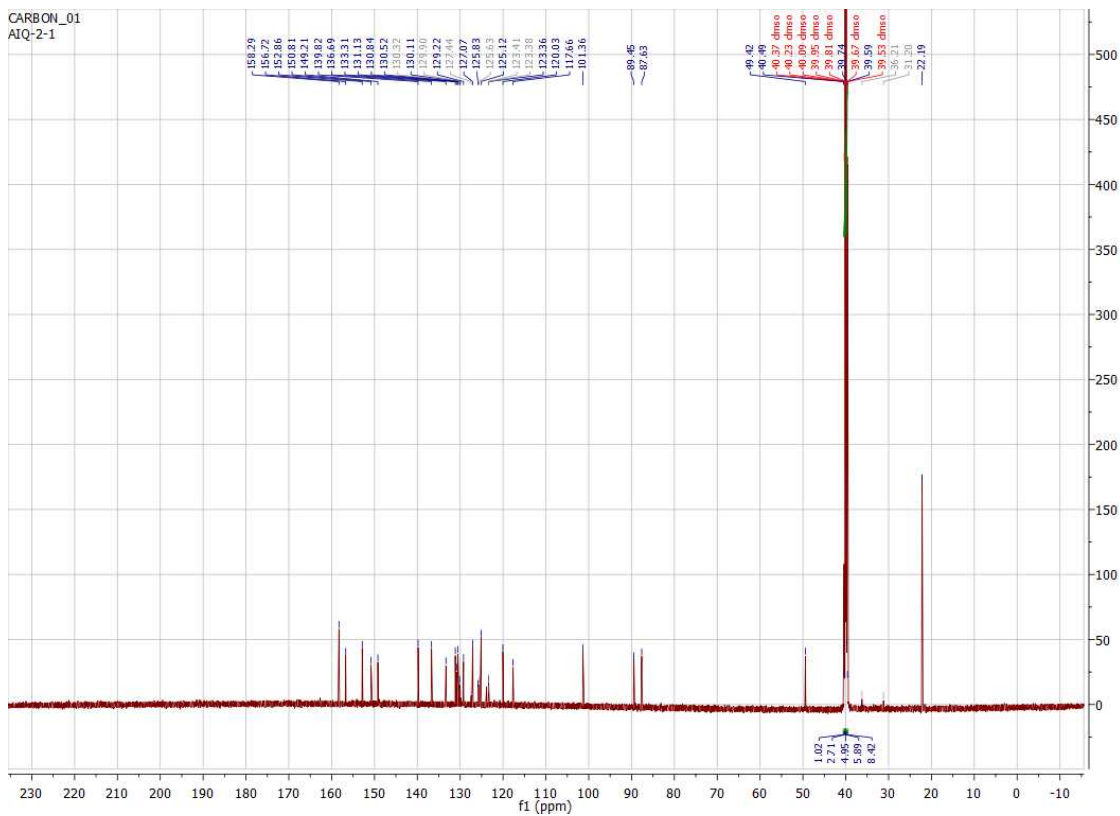
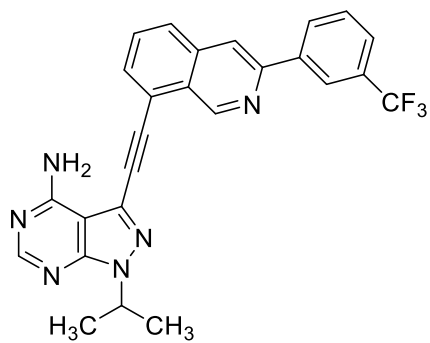
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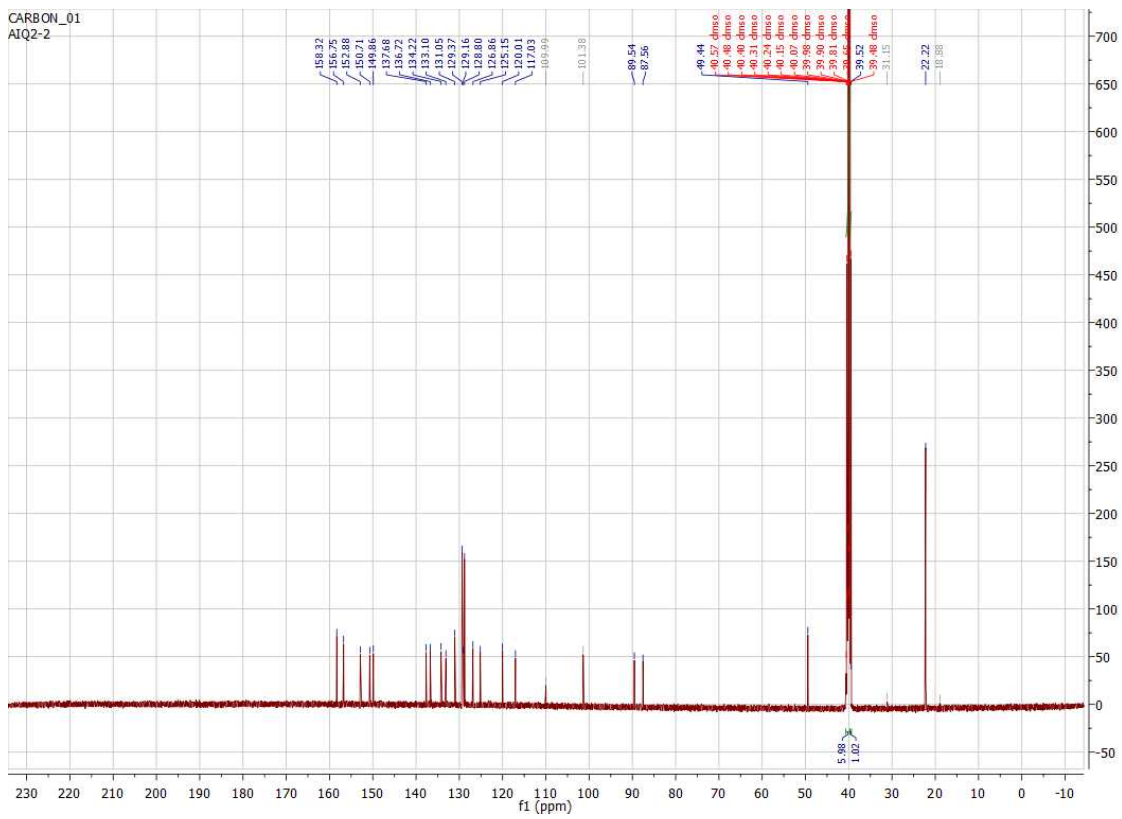
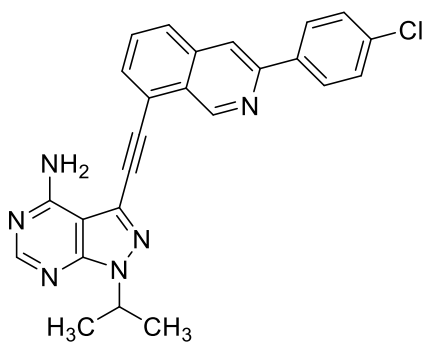
¹H NMR Compound 2g



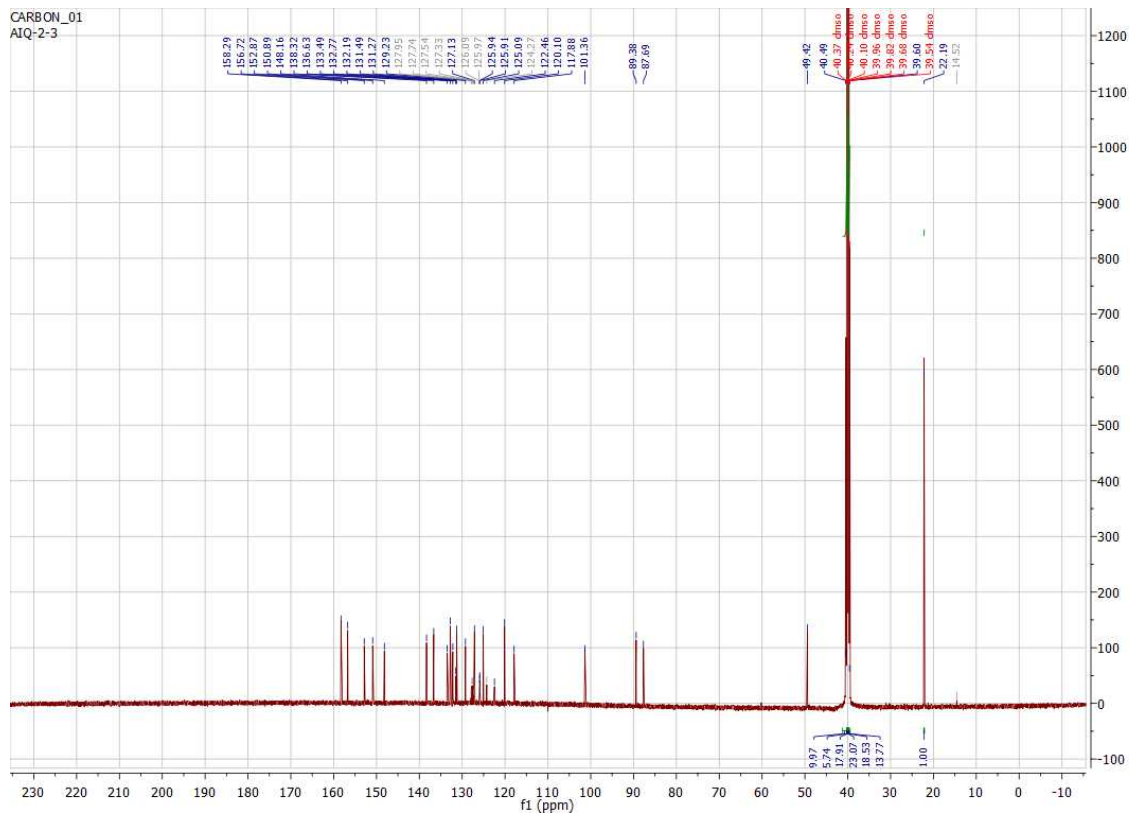
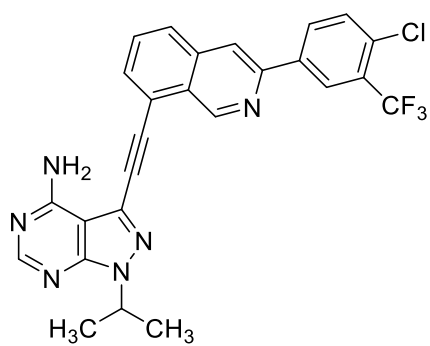
¹³CNMR Compound **2a**



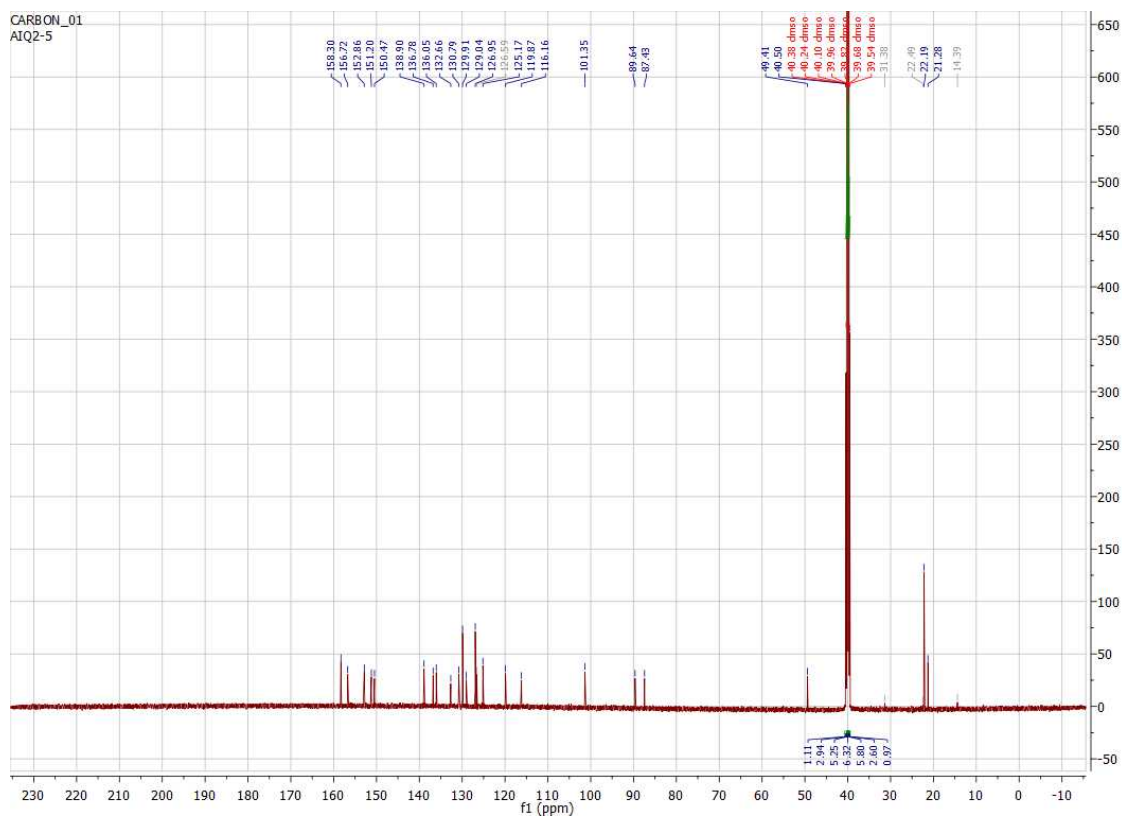
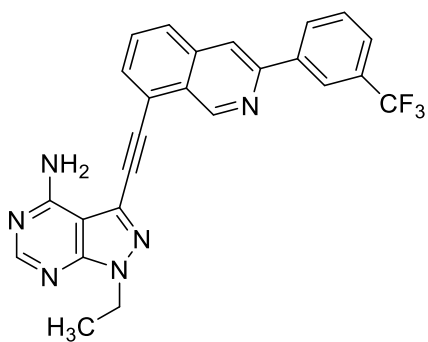
¹³CNMR Compound **2b**



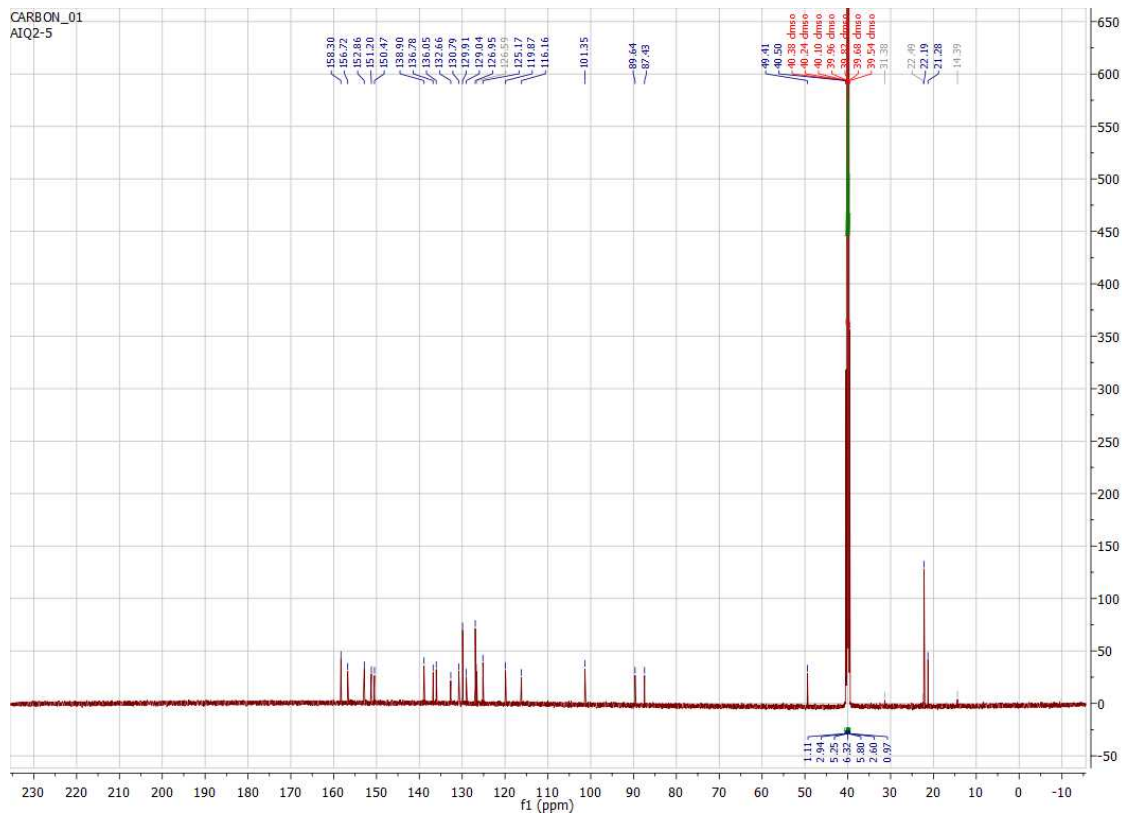
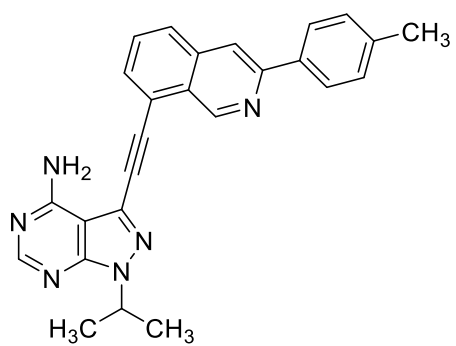
¹³CNMR Compound 2c



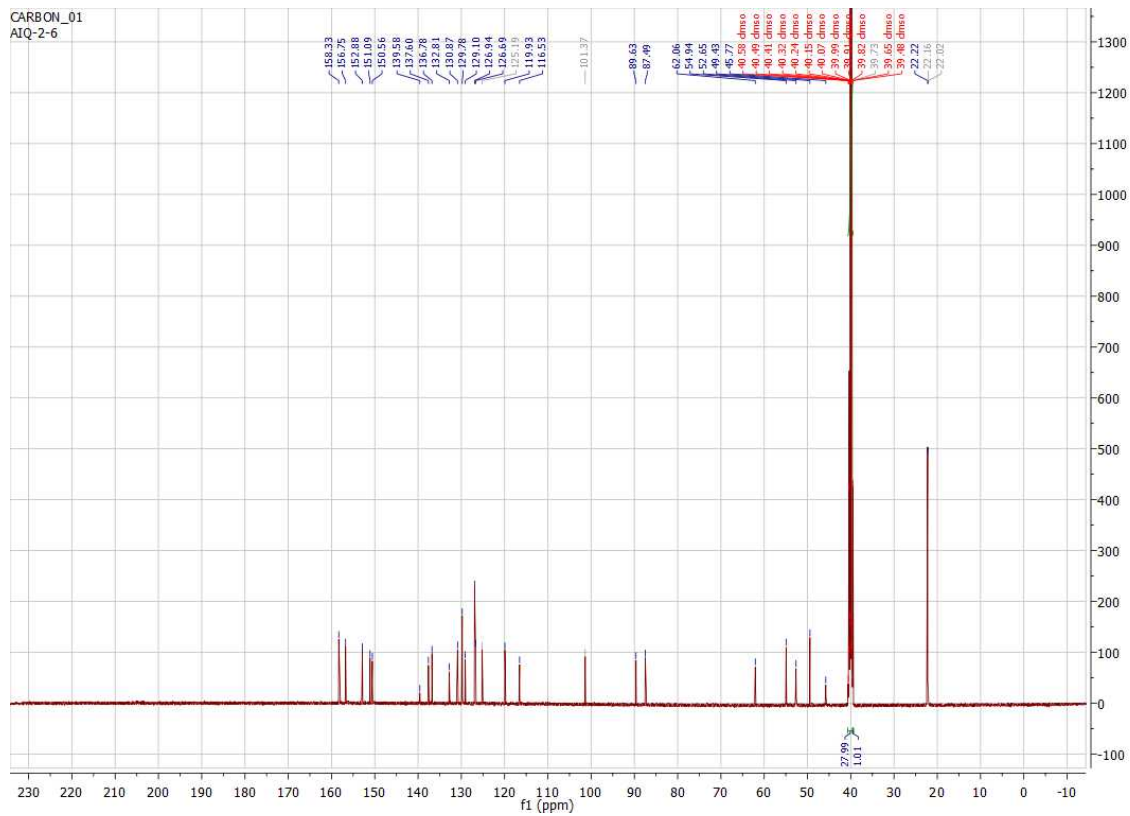
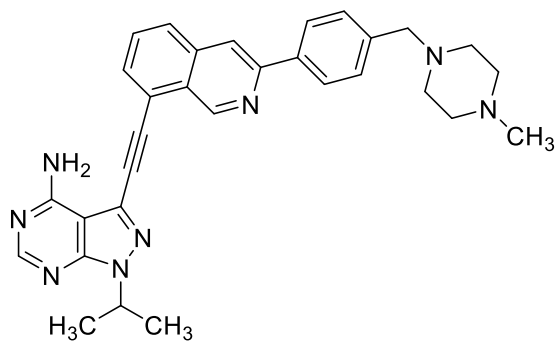
¹³CNMR Compound **2d**



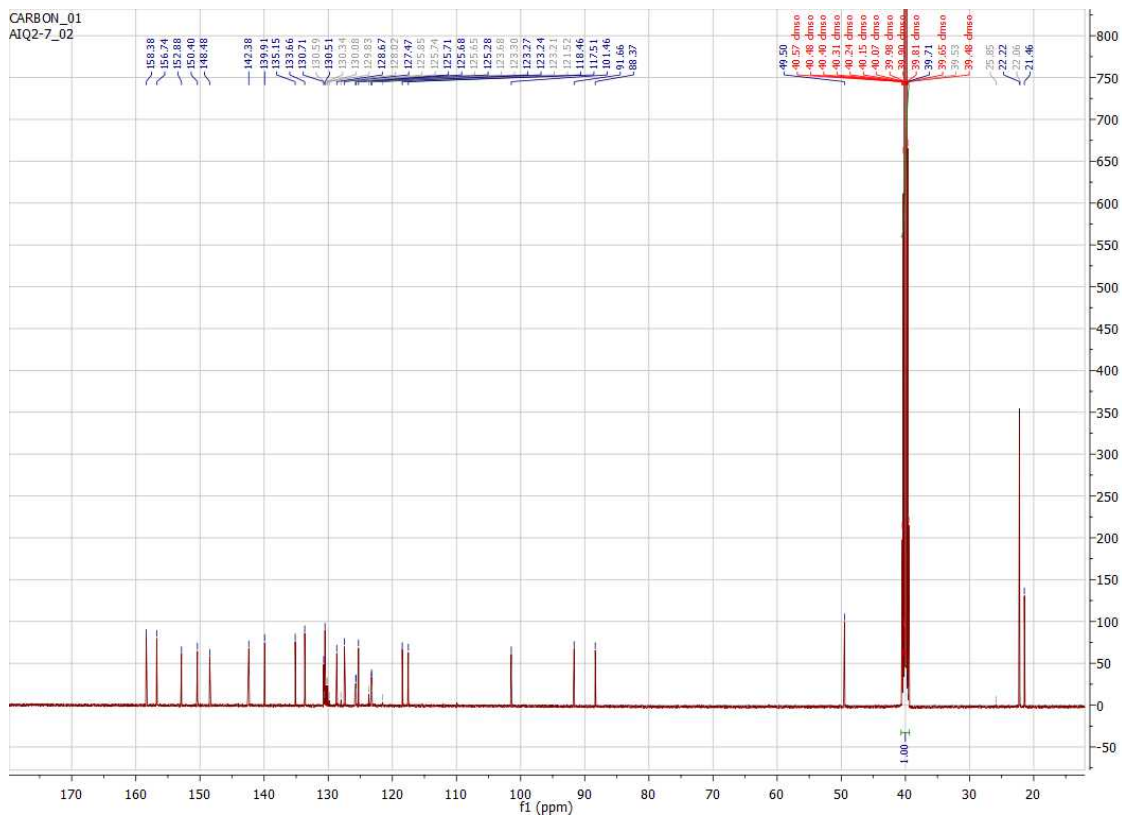
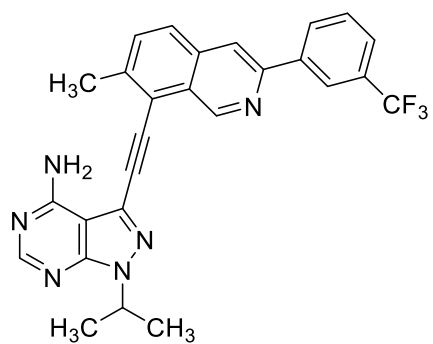
¹³CNMR Compound 2e



¹³CNMR Compound **2f**



¹³CNMR Compound **2g**

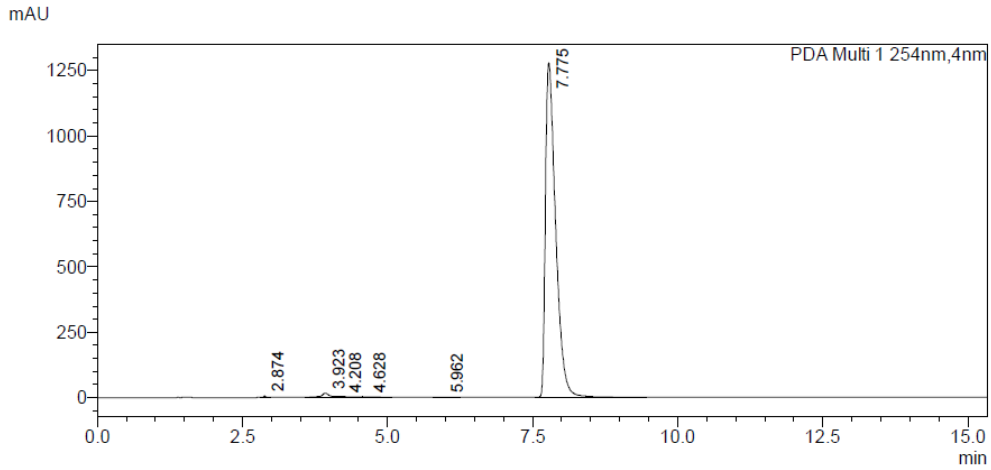


SHIMADZU
LabSolutions Analysis Report

<Sample Information>

Sample Name	: AIQ2-1 Purity 1mM in Acnitril	Sample Type	: Unknown
Sample ID	: 1		
Data Filename	: AIQ2-1 Purity 1mM in Acnitril1.lcd	Acquired by	: System Administrator
Method Filename	: AIQ2 100B-isocratic.lcm	Processed by	: System Administrator
Batch Filename	:		
Vial #	: 1-1		
Injection Volume	: 20 uL		
Date Acquired	: 2/3/2017 3:13:04 PM		
Date Processed	: 2/3/2017 3:28:27 PM		

<Chromatogram>



<Peak Table>

PDA Ch1 254nm			
Peak#	Ret. Time	Area	Area%
1	2.874	17491	0.109
2	3.923	198369	1.236
3	4.208	63588	0.396
4	4.628	42072	0.262
5	5.962	11979	0.075
6	7.775	15713460	97.922
Total		16046959	100.000

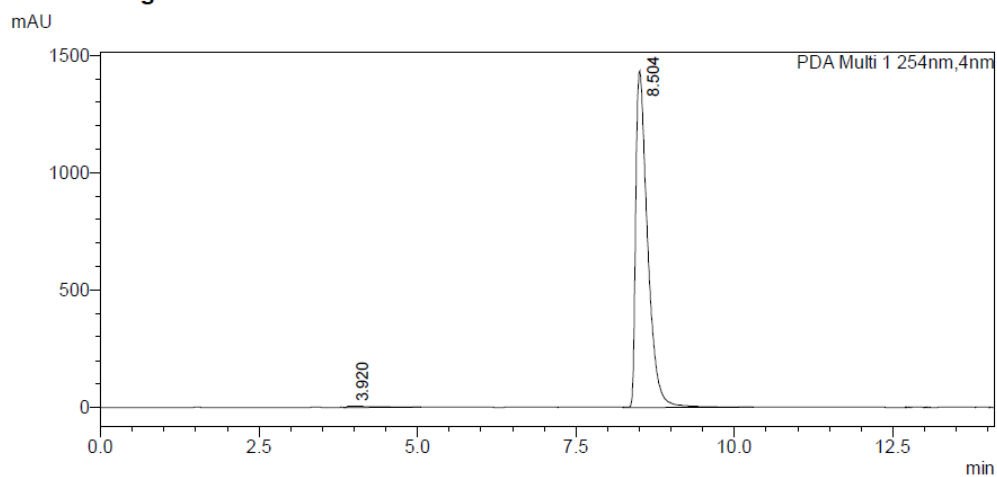


Analysis Report

<Sample Information>

Sample Name	: AIQ2-2 Purity 1mM in Acnitril	Sample Type	: Unknown
Sample ID	: 1		
Data Filename	: AIQ2-2 Purity 1mM in Acnitril1.lcd		
Method Filename	: AIQ2 100B-isocratic.lcm		
Batch Filename	:		
Vial #	: 1-1		
Injection Volume	: 20 uL	Acquired by	: System Administrator
Date Acquired	: 2/3/2017 4:01:49 PM	Processed by	: System Administrator
Date Processed	: 2/3/2017 4:15:59 PM		

<Chromatogram>



<Peak Table>

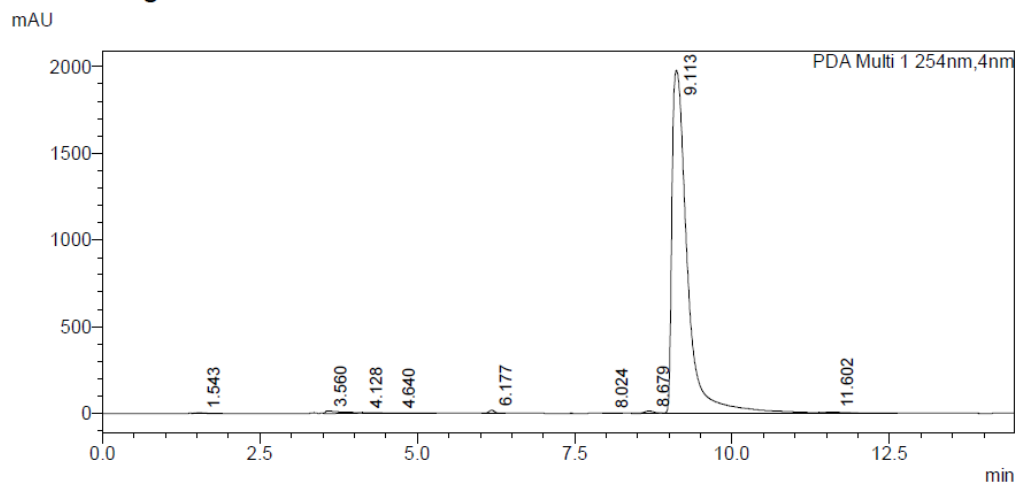
PDA Ch1 254nm			
Peak#	Ret. Time	Area	Area%
1	3.920	185690	0.986
2	8.504	18653934	99.014
Total		18839624	100.000

SHIMADZU
LabSolutions Analysis Report

<Sample Information>

Sample Name	: AIQ2-3 Purity 1mM in Acnitril	Sample Type	: Unknown
Sample ID	: 1		
Data Filename	: AIQ2-3 Purity 1mM in Acnitril.lcd	Acquired by	: System Administrator
Method Filename	: AIQ2 100B-isocratic.lcm	Processed by	: System Administrator
Batch Filename	:		
Vial #	: 1-1		
Injection Volume	: 20 uL		
Date Acquired	: 2/3/2017 4:16:53 PM		
Date Processed	: 2/10/2017 6:01:34 PM		

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	1.543	35708	0.103
2	3.560	294953	0.852
3	4.128	89454	0.258
4	4.640	40036	0.116
5	6.177	119263	0.345
6	8.024	21276	0.061
7	8.679	151410	0.437
8	9.113	33806980	97.684
9	11.602	49395	0.143
Total		34608473	100.000



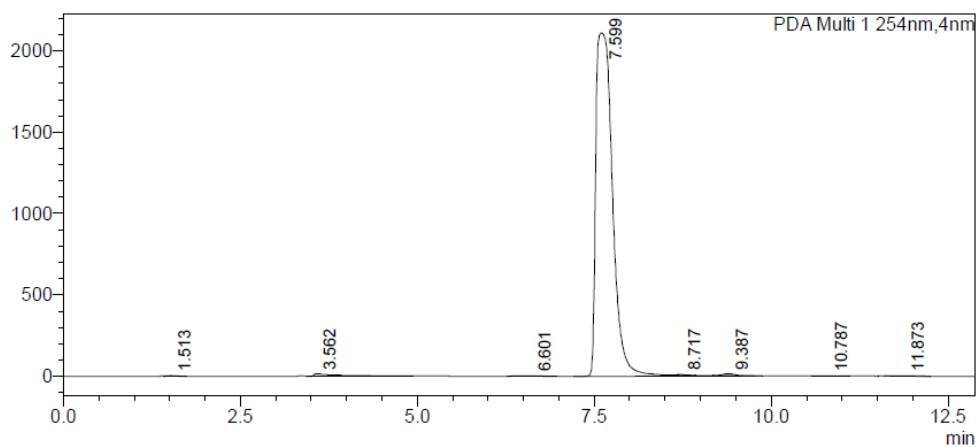
Analysis Report

<Sample Information>

Sample Name	: AIQ2-4 Purity 1mM in Acnitril	Sample Type	: Unknown
Sample ID	: 1	Acquired by	: System Administrator
Data Filename	: AIQ2-4 Purity 1mM in Acnitril1.lcd	Processed by	: System Administrator
Method Filename	: AIQ2 100B-isocratic.lcm		
Batch Filename	:		
Vial #	: 1-1		
Injection Volume	: 20 uL		
Date Acquired	: 2/3/2017 4:32:23 PM		
Date Processed	: 2/3/2017 4:45:20 PM		

<Chromatogram>

mAU



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	1.513	27182	0.077
2	3.562	432736	1.223
3	6.601	28901	0.082
4	7.599	34628101	97.862
5	8.717	40811	0.115
6	9.387	162120	0.458
7	10.787	24004	0.068
8	11.873	40914	0.116
Total		35384769	100.000

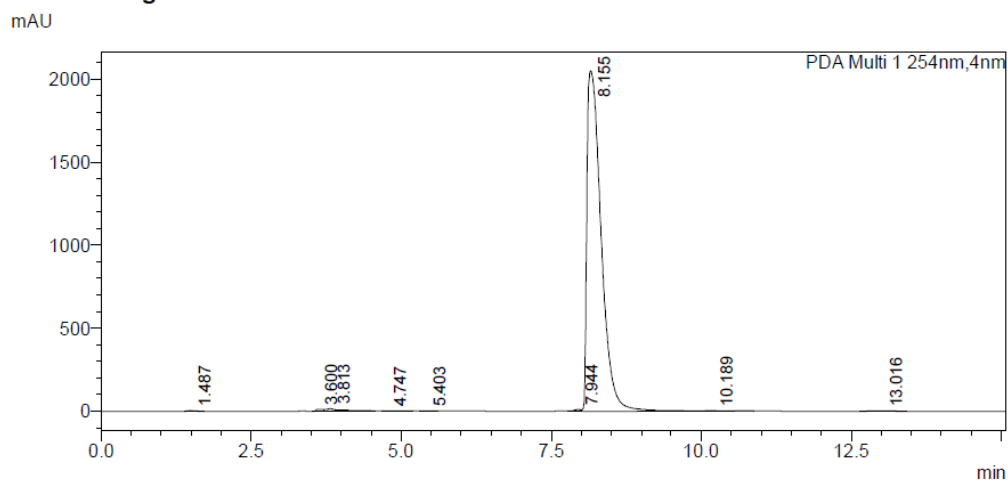


Analysis Report

<Sample Information>

Sample Name	: AIQ2-5 Purity 1mM in Acnitril	Sample Type	: Unknown
Sample ID	: 1		
Data Filename	: AIQ2-5 Purity 1mM in Acnitril1.lcd	Acquired by	: System Administrator
Method Filename	: AIQ2 100B-isocratic.lcm	Processed by	: System Administrator
Batch Filename	:		
Vial #	: 1-1		
Injection Volume	: 20 uL		
Date Acquired	: 2/3/2017 4:46:41 PM		
Date Processed	: 2/3/2017 5:01:51 PM		

<Chromatogram>



<Peak Table>

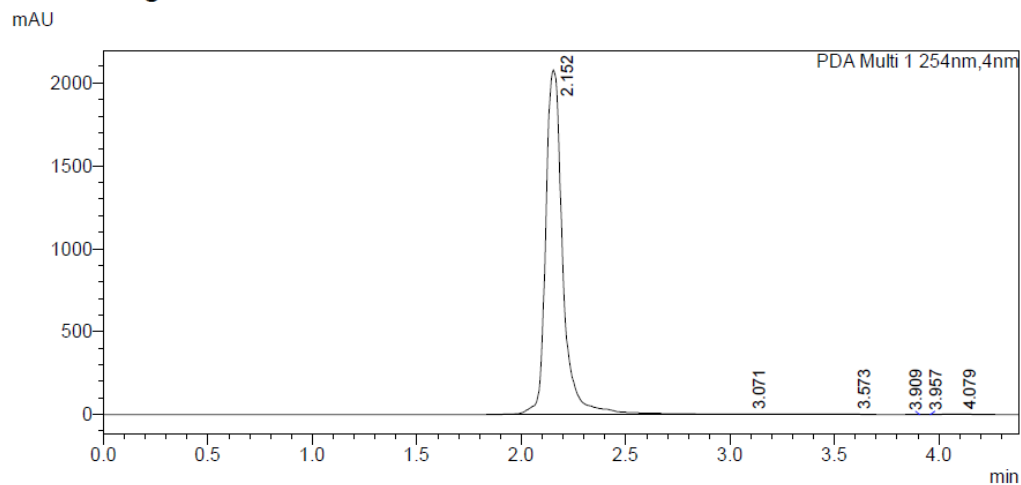
PDA Ch1 254nm			
Peak#	Ret. Time	Area	Area%
1	1.487	26811	0.078
2	3.600	83820	0.243
3	3.813	354149	1.025
4	4.747	52830	0.153
5	5.403	20353	0.059
6	7.944	81136	0.235
7	8.155	33874330	98.035
8	10.189	37379	0.108
9	13.016	22346	0.065
Total		34553153	100.000

SHIMADZU
LabSolutions Analysis Report

<Sample Information>

Sample Name : AIQ2-6 purity 1mM in Acetonitril
 Sample ID : 1
 Data Filename : AIQ2-6 purity 1mM in Acnitril5.lcd
 Method Filename : AIQ2 100B-isocratic.lcm
 Batch Filename :
 Vial # : 1-1
 Injection Volume : 20 uL
 Date Acquired : 2/6/2017 6:49:22 PM
 Date Processed : 2/6/2017 6:53:49 PM
 Sample Type : Unknown
 Acquired by : System Administrator
 Processed by : System Administrator

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	2.152	11938415	99.874
2	3.071	5822	0.049
3	3.573	6	0.000
4	3.909	498	0.004
5	3.957	523	0.004
6	4.079	8175	0.068
Total		11953438	100.000

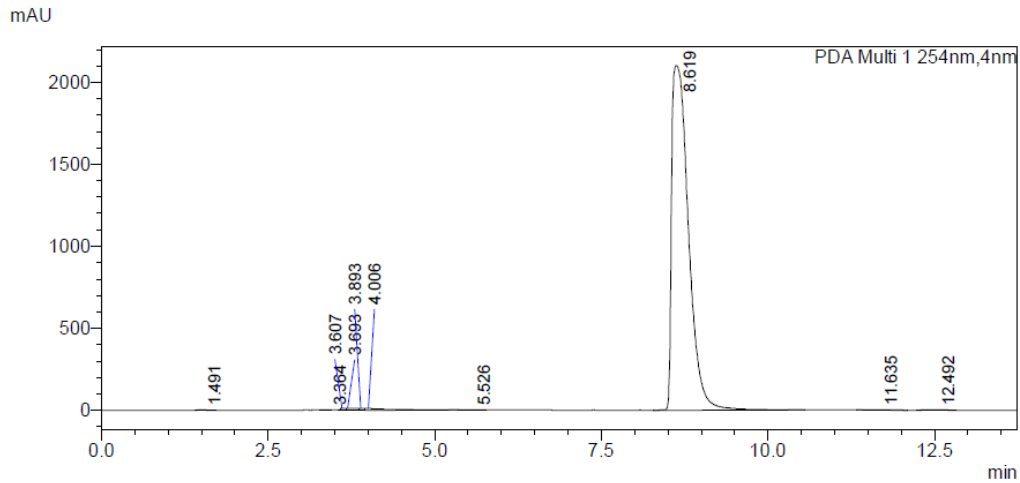


Analysis Report

<Sample Information>

Sample Name	: AIQ2-7 Purity 1mM in Acnitril	Sample Type	: Unknown
Sample ID	: 1		
Data Filename	: AIQ2-7 Purity 1mM in Acnitril1.lcd	Acquired by	: System Administrator
Method Filename	: AIQ2 100B-isocratic.lcm	Processed by	: System Administrator
Batch Filename	:		
Vial #	: 1-1		
Injection Volume	: 20 uL		
Date Acquired	: 2/3/2017 5:38:52 PM		
Date Processed	: 2/3/2017 5:52:40 PM		

<Chromatogram>



<Peak Table>

Peak#	Ret. Time	Area	Area%
1	1.491	12512	0.033
2	3.364	15832	0.042
3	3.607	62989	0.168
4	3.693	132644	0.354
5	3.893	54667	0.146
6	4.006	325347	0.867
7	5.526	16897	0.045
8	8.619	36827373	98.159
9	11.635	49536	0.132
10	12.492	20336	0.054
Total		37518133	100.000