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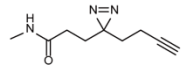

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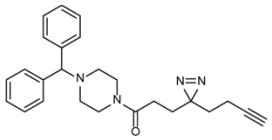

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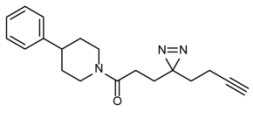

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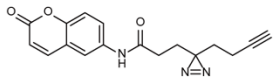

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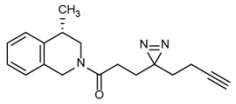

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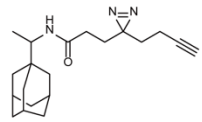

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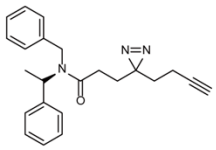

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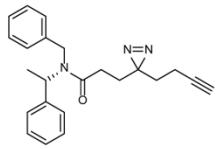

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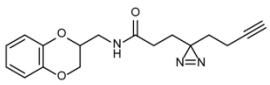

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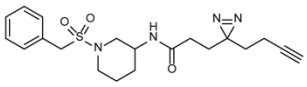

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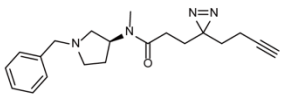

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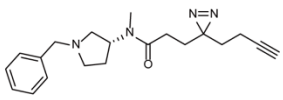

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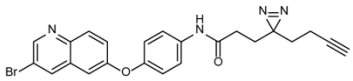

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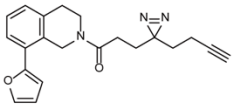

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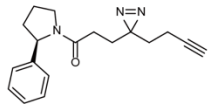

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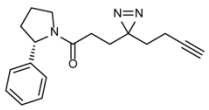

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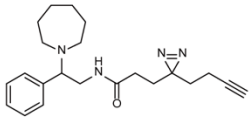

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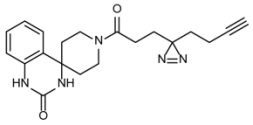

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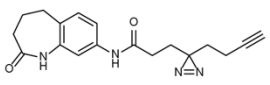

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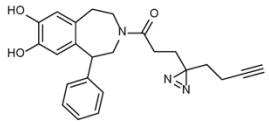

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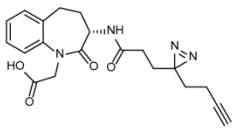

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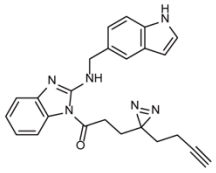

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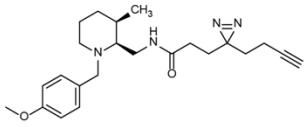

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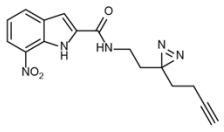

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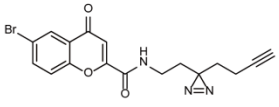

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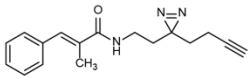

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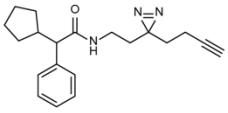

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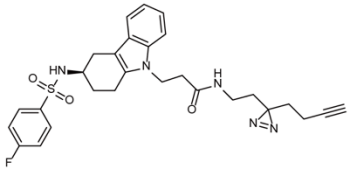

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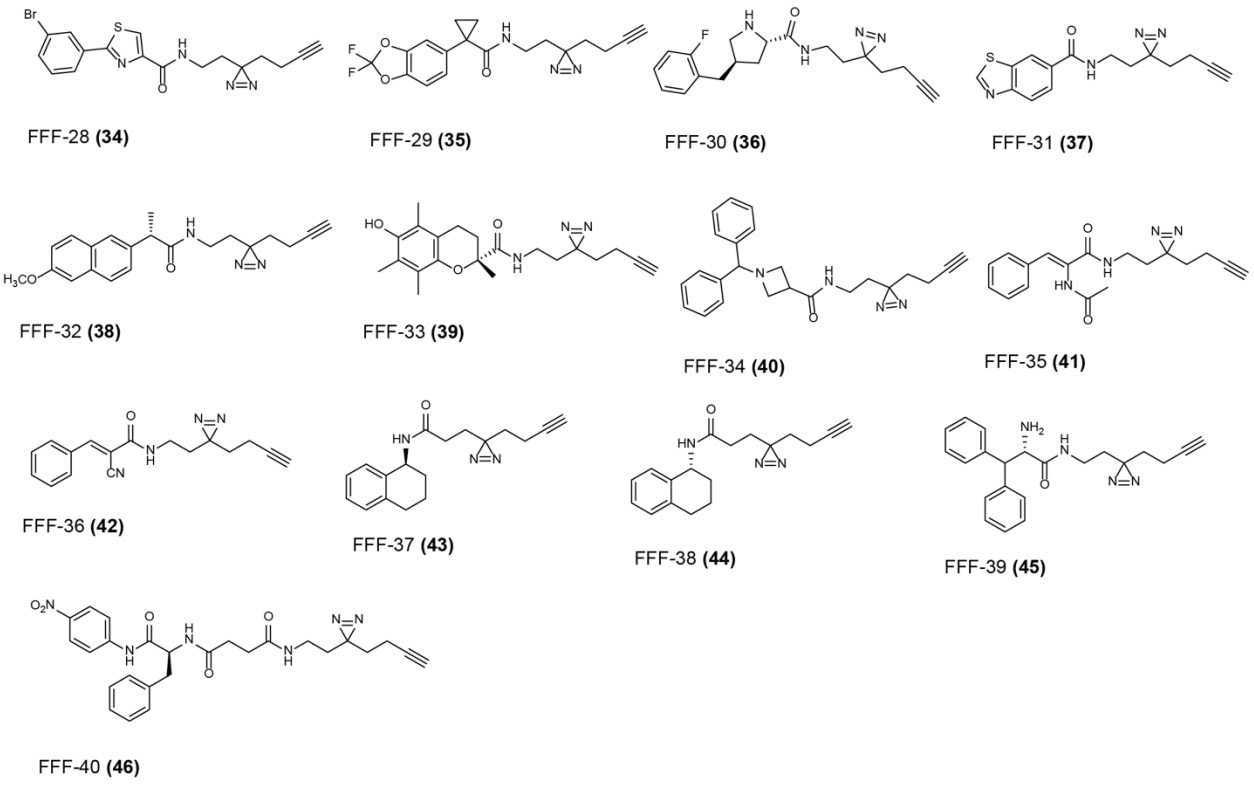
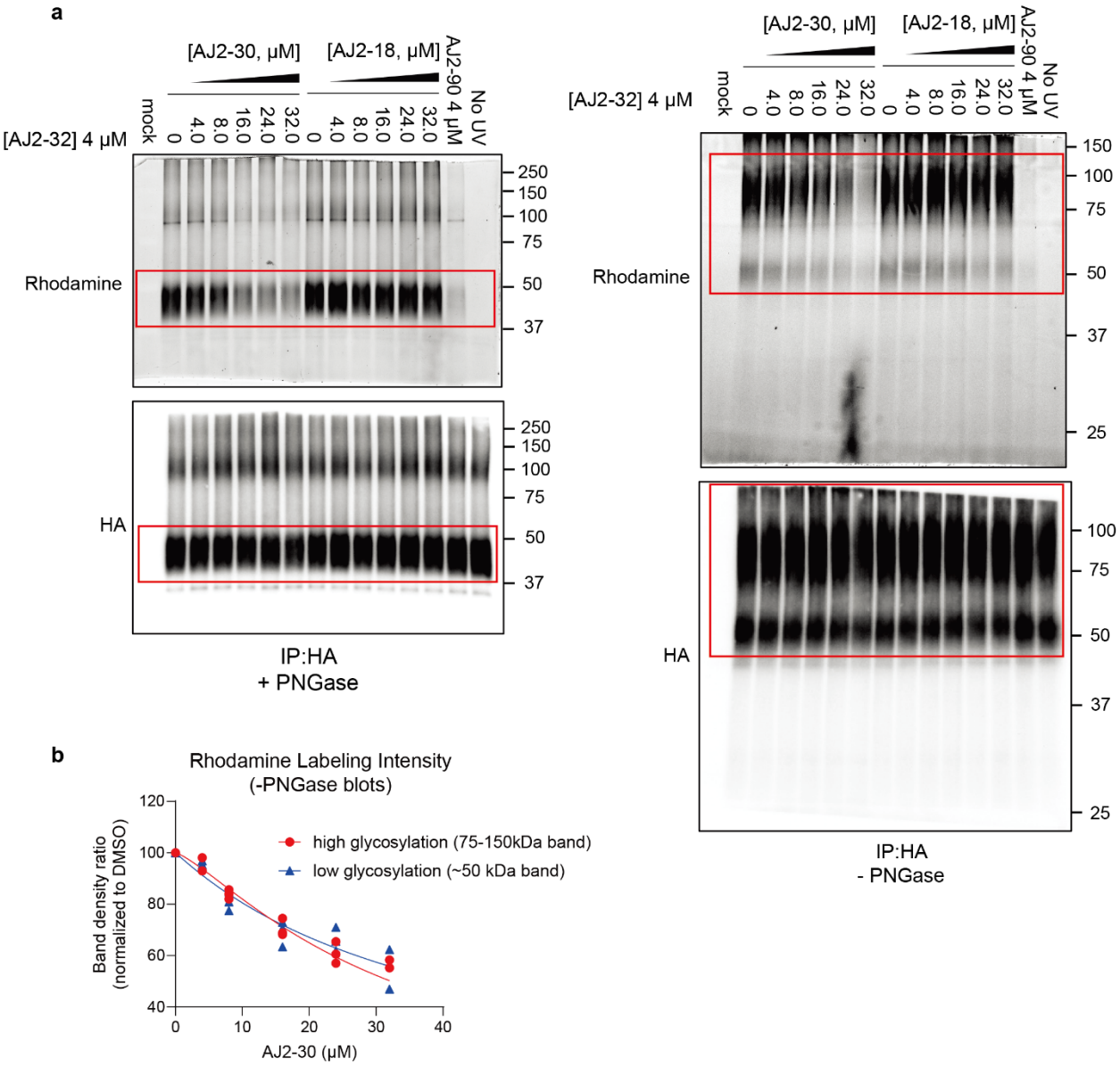


Supplementary Fig. 1: Fully functionalized fragment (FFF) probe library and cell models for SLC15A4 inhibitor characterization.

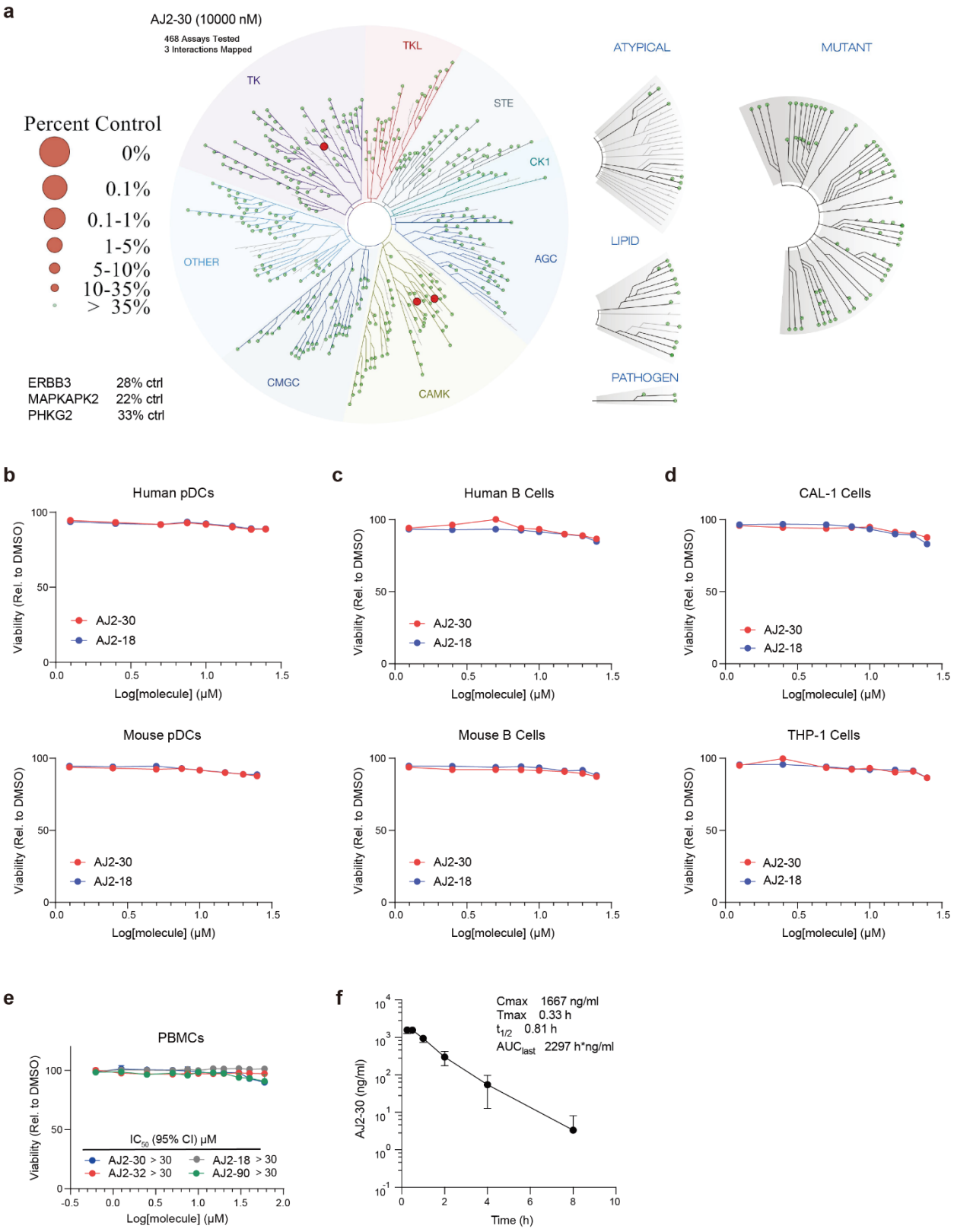
Chemical structures of FFF library. Each member of the FFF probe library possesses: 1) a 'variable' recognition element consisting of structurally diverse small-molecule fragments intended to promote interactions with distinct proteins in human cells; and (2) a structurally minimized 'constant' region bearing a photoactivatable diazirine group and alkyne handle, which together enabled UV-light-induced covalent modification and detection, enrichment, and identification of fragment-interacting protein targets.



Supplementary Fig. 2:Target engagement of HA-SLC15A4 in CAL-1 cells.

a, Immunoblot of HA-SLC15A4 engagement with or without PNGase, related to Fig. 2f.

b, high and low glycosylation SLC15A4 bands of rhodamine labeling intensity from no PNGase treatment blots were quantified. Data is plotted as mean values for replicates (*n* = 3 independent biological replicates).

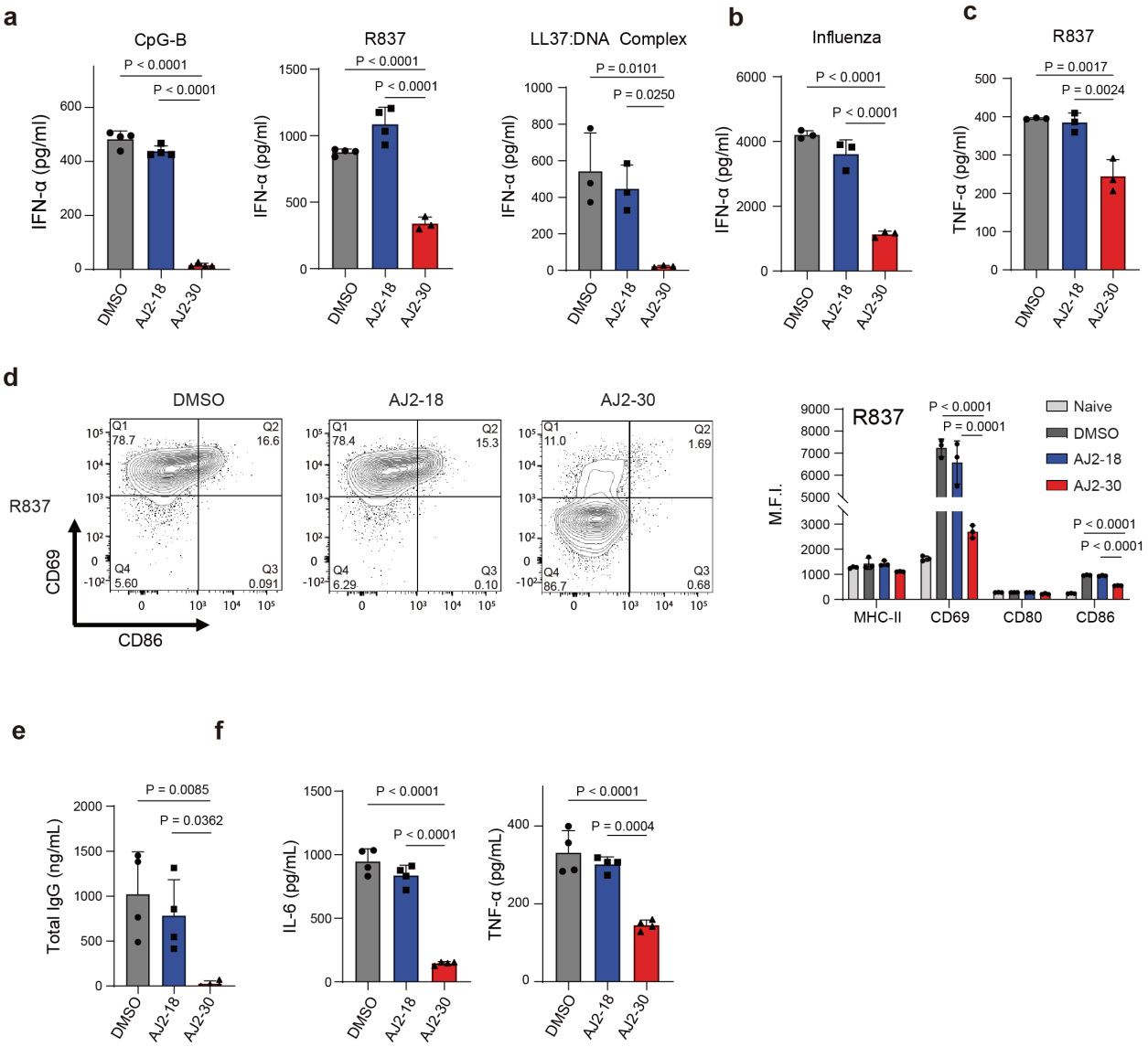

a
AJ2-30 (10000 nM)
468 Assays Tested
3 Interactions Mapped
Percent Control
0%
0.1%
0.1-1%
1-5%
5-10%
10-35%
> 35%
ERBB3 28% ctrl
MAPKAPK2 22% ctrl
PHKG2 33% ctrl
TK
TKL
STE
CK1
AGC
CAMK
CMGC
OTHER
ATYPICAL
LIPID
PATHOGEN
MUTANT
b
Human pDCs
Mouse pDCs
c
Human B Cells
Mouse B Cells
d
CAL-1 Cells
THP-1 Cells
Viability (Rel. to DMSO)
Log[molecule] (µM)
AJ2-30
AJ2-18
e
PBMCs
IC50 (95% CI) µM
AJ2-30 > 30
AJ2-32 > 30
AJ2-18 > 30
AJ2-90 > 30
f
Cmax 1667 ng/ml
Tmax 0.33 h
t1/2 0.81 h
AUClast 2297 h*ng/ml
AJ2-30 (ng/ml)
Time (h)

Supplementary Fig.3: Characterization of SLC15A4 chemical probes and control compounds.

a, AJ2-30 (10 μM) was submitted for a KinomeScan (DiscoveryX) profiling to quantify interactions with 468 human kinases. Results are displayed as a TREESPOT interaction map.

b-e, Viability profiling of chemical probes in indicated primary immune cells (b, human and mouse pDCs; c, human and mouse B cells), cell lines (d, CAL-1 and THP-1), and human PBMCs (e) 24 h after compound treatment. Viabilities were normalized to DMSO treated group. Data is plotted as the mean ± s.d. (*n* = 3 independent biological replicates).

f, Pharmacokinetics of AJ2-30 following intraperitoneal administration (30 mg/kg) to male BALB/c mice. Plasma concentration of AJ2-30 was measured at different time points by LC-MS. Data is plotted as the mean ± s.d. (*n* = 6 independent mice per group).



Supplementary Fig. 4: Pharmacological inhibition of SLC15A4 suppresses multiple innate signaling pathways in human immune cells.

a, AJ2-30 inhibits TLR7, TLR7/8, and TLR9-induced pDC IFN-α production. pDCs were isolated from human PBMCs and treated with AJ2-18 or AJ2-30 (5 μM, 24 h) along with CpG-B (1 μM), R848 (5 μg/mL), or LL37:DNA complex (10 μg/mL).

b, AJ2-30 inhibits influenza mediated IFN-α production in human pDCs. pDCs were isolated from human PBMCs and treated with AJ2-18 or AJ2-30 (5 μM, 24 h) along with influenza virus (TLR7 agonist; MOI = 1).

C, AJ2-30 inhibits TLR7/8 mediated production of TNF-α in primary human monocytes. Monocytes were isolated from PBMCs and treated with AJ2-18 or AJ2-30 (5 μM, 24 h) along with R837 (5 μg/mL).

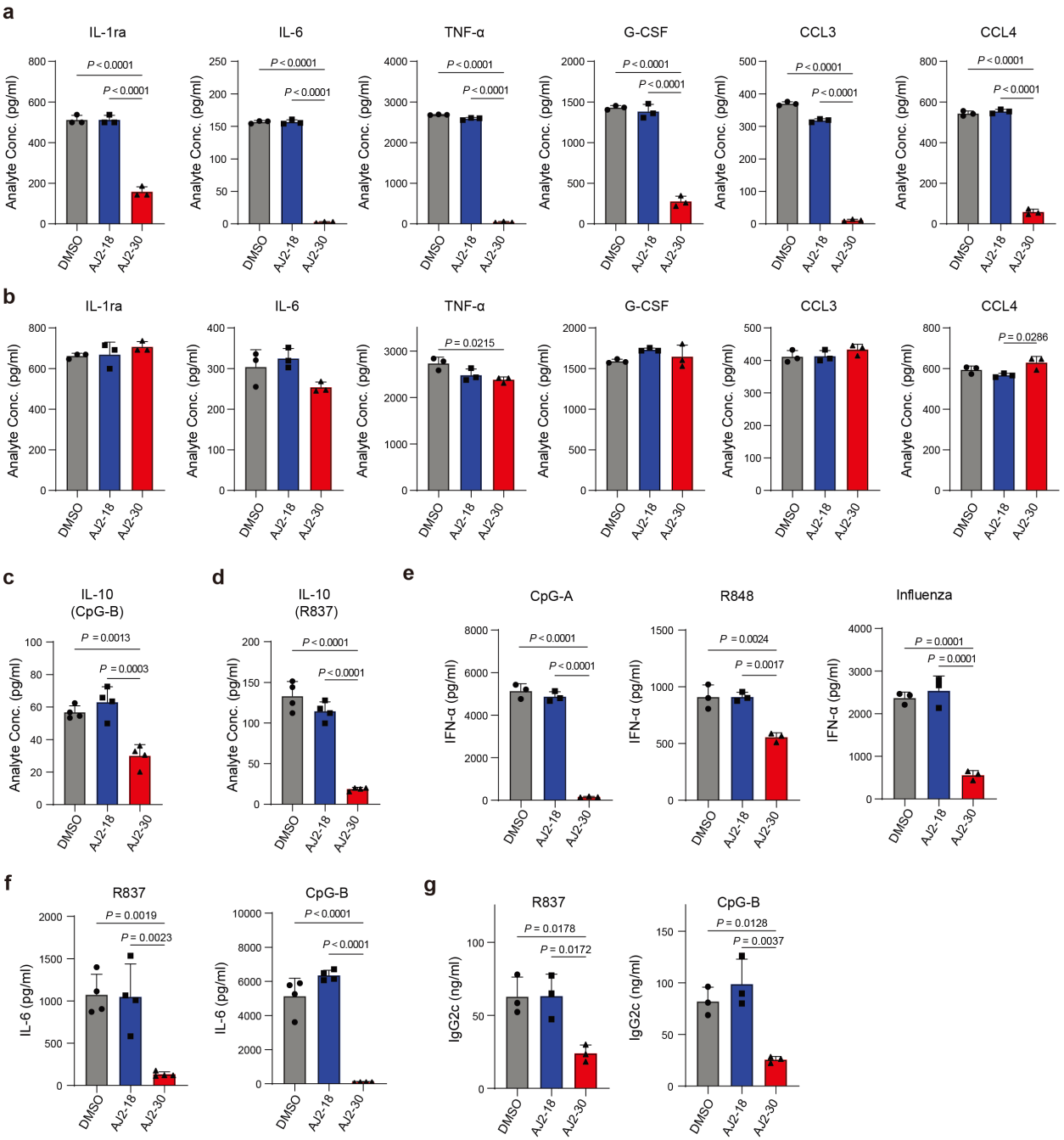
d, Primary human B cells isolated from PBMCs were treated with AJ2-18 or AJ2-30 (5 μM, 24 h) while stimulated with either R837 (10 μg/mL). B cell activation was assessed by measuring

the surface expression of CD69, CD80, CD86, and MHC-II on live cells.

e, *In vitro* IgG secretion from primary human B cells stimulated with R837 for 6 days in the presence of either AJ2-18 and AJ2-30 (5 μM). The compounds were not replenished during the treatment.

F, *In vitro* secretion of IL-6 and TNF-α from isolated primary human B cells in the presence of either AJ2-18 or AJ2-30 (5 μM) when stimulated by R837 after 24 h.

Results are presented as mean ± s.d. of at least $n = 3$ independent biological replicates from at least 3 independent experiments. Statistical analysis was performed using ANOVA analysis followed by multiple comparisons test. *P*-values are shown.



Supplementary Fig. 5: Pharmacological inhibition of SLC15A4 suppresses multiple innate signaling pathways in mouse immune cells.

a, b, Secretion of cytokines and chemokines by primary human pDCs in the presence of either AJ2-18 or AJ2-30 in response to stimulation with CpG-A (1 µM) (a) or R848 (5 µg/mL) (b) for 24 h.

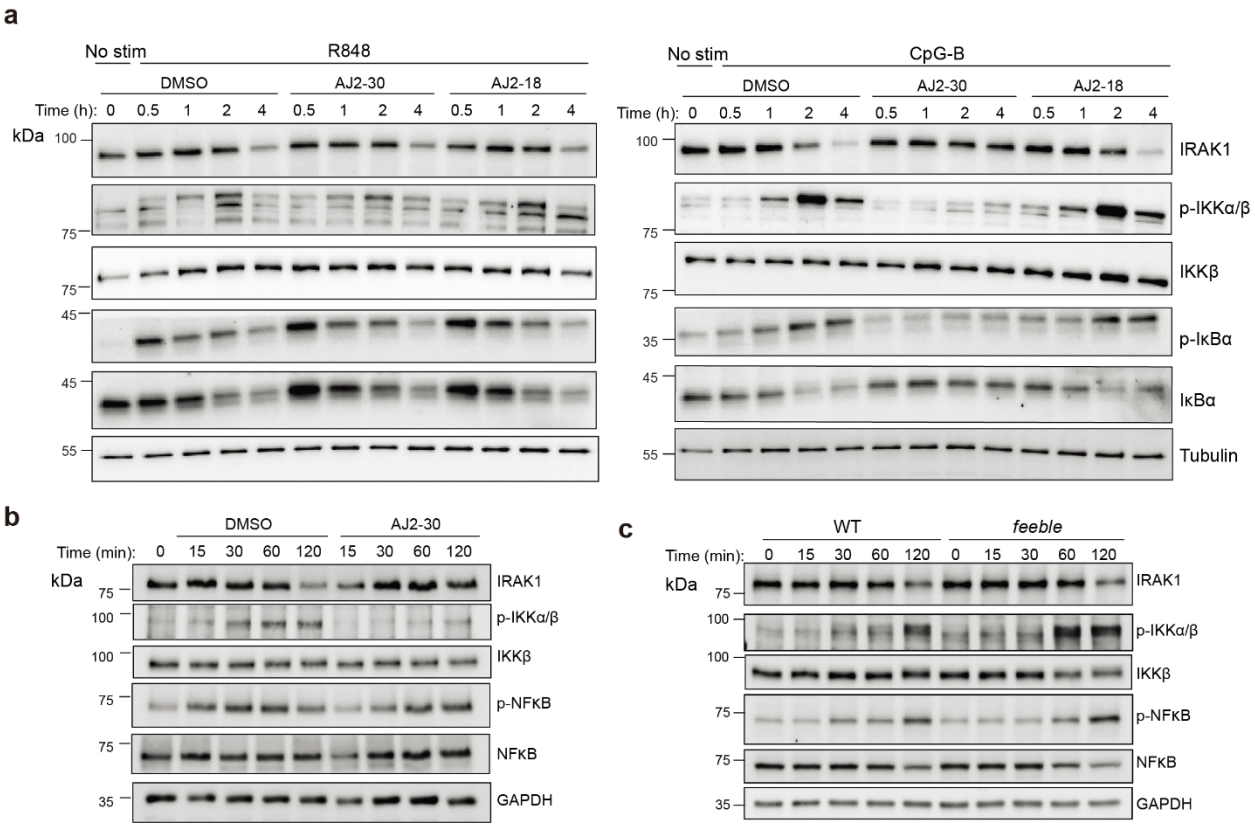
c, d, Secretion of IL-10 from isolated primary human B cells in the presence of either AJ2-18 (5 μM) or AJ2-30 (5 μM) when stimulated by either R837 (10 μg/mL) or CpG-B (1 μM). Cytokine and chemokine levels in supernatants were assessed by Luminex 24 h post-stimulation.

e, AJ2-30 inhibits TLR7/8, TLR9, and influenza-induced IFN-α production in mouse pDCs. pDCs were derived from mouse bone marrow using Flt3 ligand and treated with 5 μM AJ2-18 or AJ2-30 for 24 h along with 1 μM CpG-A, 5 μg/ml R848, or challenged with influenza (MOI=1).

f, AJ2-30 inhibits production of IL-6 induced by wild-type mouse B cells 24 h after TLR7 and TLR9 stimulation.

g, AJ2-30 inhibits production of IgG2c from mouse B cells. *In vitro* IgG2c secretion from mouse B cells was measured by ELISA following B cell stimulation with CpG-B or R837 for 6 days.

Results are presented as the mean ± s.d. of at least $n = 3$ independent biological replicates from 3 independent experiments. Statistical analysis was performed using ANOVA analysis followed by multiple comparisons test. *P*-values are shown.

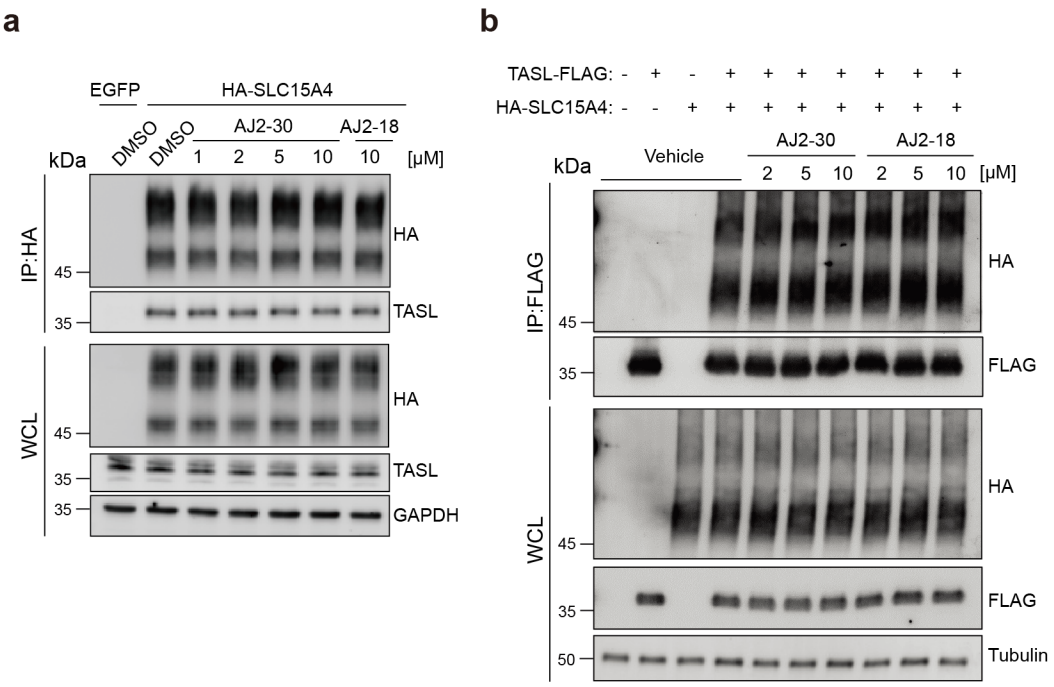


Supplementary Fig. 6: Mechanistic characterization of SLC15A4 inhibition by AJ2-30.

a, Immunoblot analysis of TLR proximal signaling in human B cells isolated from PBMCs. Cells were co-treated with compounds (5 μM) or DMSO and stimuli 5 μg/ml R848 (left) or 1 μM CpG-B (right) for indicated time points before lysis. Data are representative of three independent experiments.

b, Immunoblot analysis of TLR proximal signaling in WT mouse B cells. Cells were co-treated with AJ2-30 (5 μM) /DMSO and stimuli 1 μM CpG-B for indicated time points before lysis. Data are representative of two independent experiments.

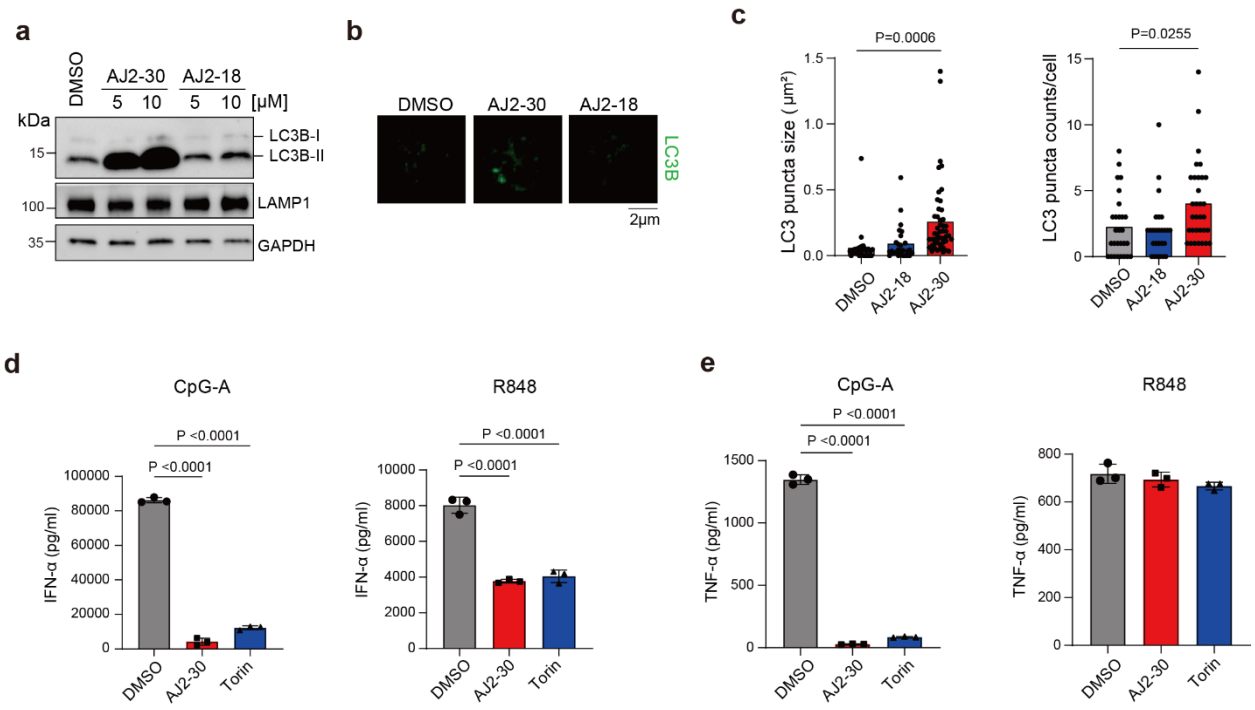
c, immunoblot analysis of TLR proximal signaling in WT and *feeble* mouse B cells. Cells were stimulated with 1 μM CpG-B for indicated time points before lysis. Data are representative of two independent experiments.



Supplementary Fig. 7: Mechanistic characterization of SLC15A4 inhibition by AJ2-30.

a, CAL-1 cells expressing EGFP, HA-SLC15A4 cells were treated with compounds or DMSO for 1h. The whole cell lysates (WCL) from each condition were immunoprecipitated (IP) with anti-HA beads. WCL and immunoprecipitates were analyzed by immunoblot with indicated antibodies. Data are representative of three independent experiments.

b, SLC15A4/TASL interaction analysis by immunoprecipitation in HEK293T cells transiently overexpressing HA-SLC15A4 and FLAG-TASL. Data are representative of two independent experiments.



Supplementary Fig. 8: Mechanistic characterization of SLC15A4 inhibition by AJ2-30.

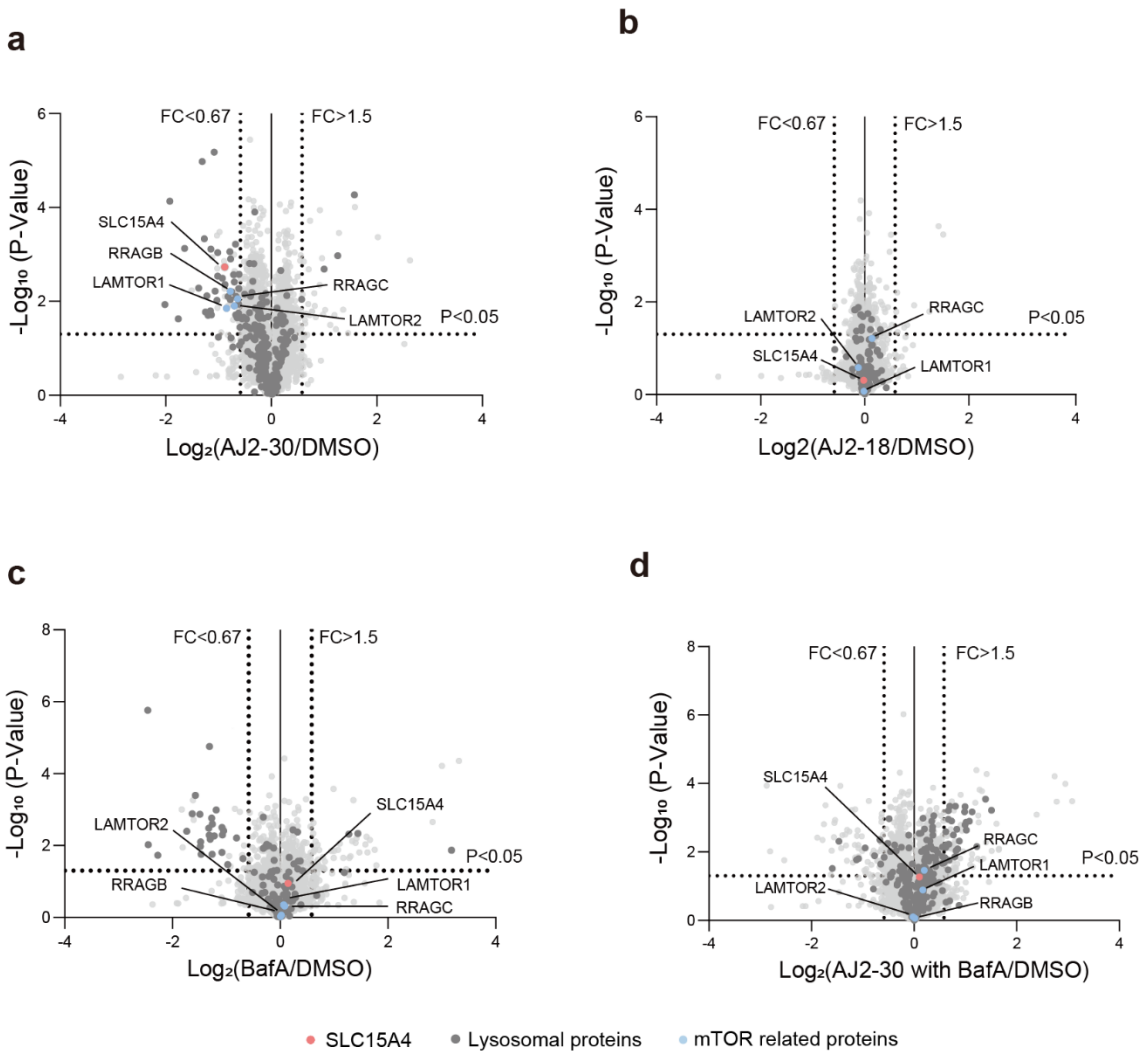
a, Immunoblot analysis of LC3B in human B cells isolated from PBMCs. Cells were treated with indicated compounds or DMSO for 16h before lysis. Images are representative of two different donors.

b, Immunostaining of LC3B in human B cells isolated from PBMCs. Cells were co-treated with compounds (5 μM) or DMSO for 16h before fixation. Scale bar: 2 μm.

c, Quantitation of LC3B puncta size and numbers per cell (n = 28, 26, 48 cells from left to right; mean ± s.d.). Images are representative of two independent experiments (two healthy donors). Statistics were performed using ANOVA analysis. *P*-values are shown.

d, AJ2-30 and torin inhibit TLR9 and TLR7/8 mediated induction of IFN-α in primary pDCs. Data is plotted as the mean ± s.d. (n = 3 independent biological replicates). Statistics were performed using ANOVA analysis. *P*-values are shown.

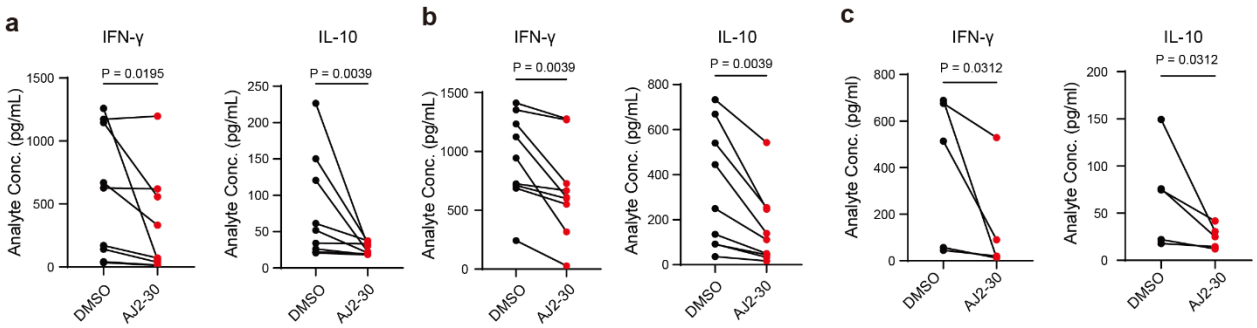
e, AJ2-30 and torin inhibit TLR9 but not TLR7/8 mediated induction of TNF-α in primary pDCs. In Fig g and h, primary human pDCs were stimulated with CpG-A (1 μM) or R848 (5 μg/mL) and co-treated with DMSO, AJ2-30 (5 μM) or torin 1 (100nM) for 24 hrs. Cytokines were measured by ELISA. Data is plotted as the mean ± s.d. (n = 3 independent biological replicates). Statistics were performed using ANOVA analysis. *P*-values are shown.



Supplementary Fig. 9: AJ2-30 down-regulates SLC15A4 in human B cells.

a, Bafilomycin A blocks AJ2-30 degradation in CAL-1 cells. CAL-1 cells stably expressing HA-SLC15A4 were pre-treated with BafA for 1h and co-incubated with AJ2-30 (10 μM) or DMSO for 4h. HA-SLC15A4 abundance was determined by immunoblot. Images are representative of two independent experiments.

b-d, Bafilomycin A blocks AJ2-30 mediated degradation in human B cells, related to Figure 5c. Scatter plots depicting the relative fold change (FC) in protein abundance following treatment of human B cells with AJ2-30 (**i**, 10 μM), AJ2-18 (**j**, 10 μM), BafA (**k**, 500nM), or AJ2-30 (10 μM) and BafA (**l**, 500nM, pretreated 1 h) for 16 h. Lysosomal proteins are depicted by dark gray circles and SLC15A4 is displayed as a red circle. mTOR-related proteins that are down-regulated by AJ2-30 and can be rescued by BafA are shown as blue circles. Data presented as mean of *n* = 2 biologically independent treatment samples. Associated data set provided in Supplementary Dataset.



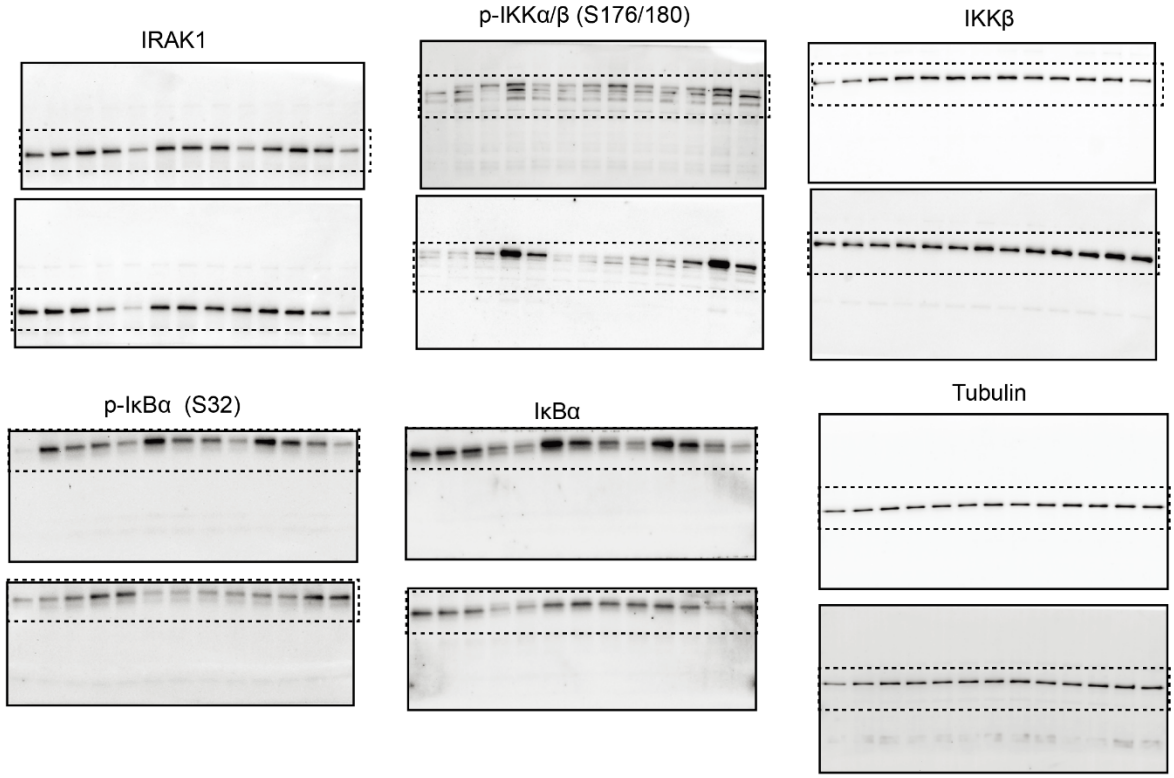
Supplementary Fig. 10: Pharmacological inhibition of SLC15A4 suppresses inflammatory cytokine production in lupus patient derived PBMCs.

a, Secretion of IFN-γ and IL-10 from PBMCs of lupus patients in the presence of either DMSO or AJ2-30 (5 μM) when stimulated by either CpG-B (1 μM) after 24 h. n = 9 independent patient samples.

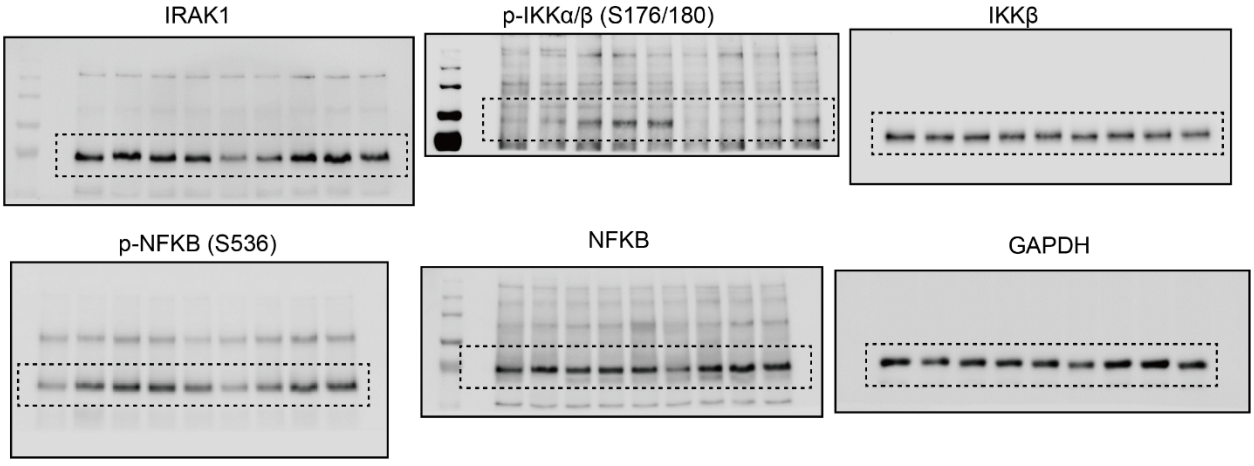
b, Secretion of IFN-γ and IL-10 from PBMCs of lupus patients in the presence of either DMSO or AJ2-30 (5 μM) when stimulated by R837 (10 μg/mL) after 24 h. n = 9 independent patient samples.

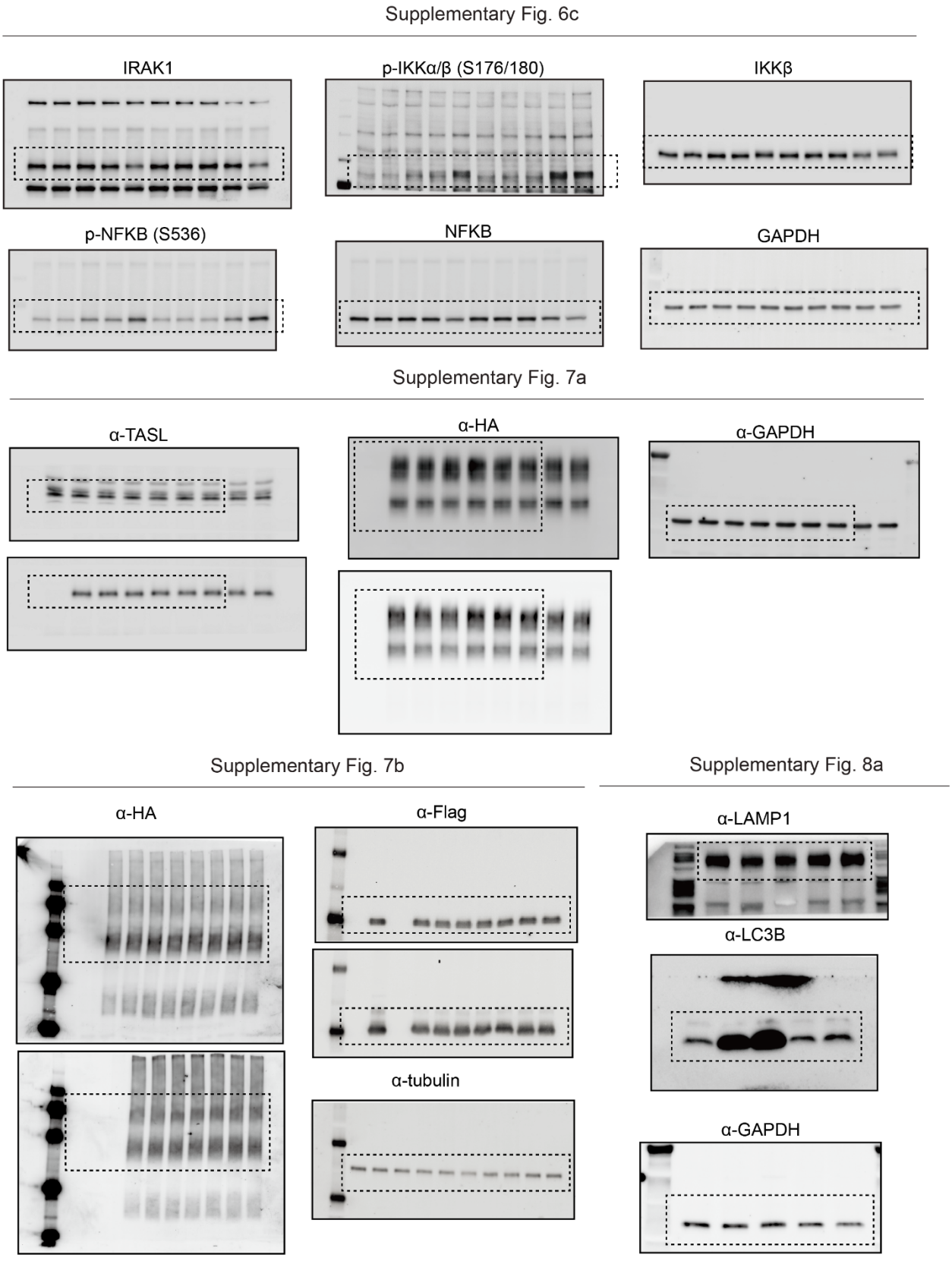
c, Secretion of IFN-γ and IL-10 of unstimulated PBMCs from lupus patients in the presence of either AJ2-30 (5 μM) or DMSO for 24 h. n = 9 independent patient samples.

Supplementary Fig. 6a

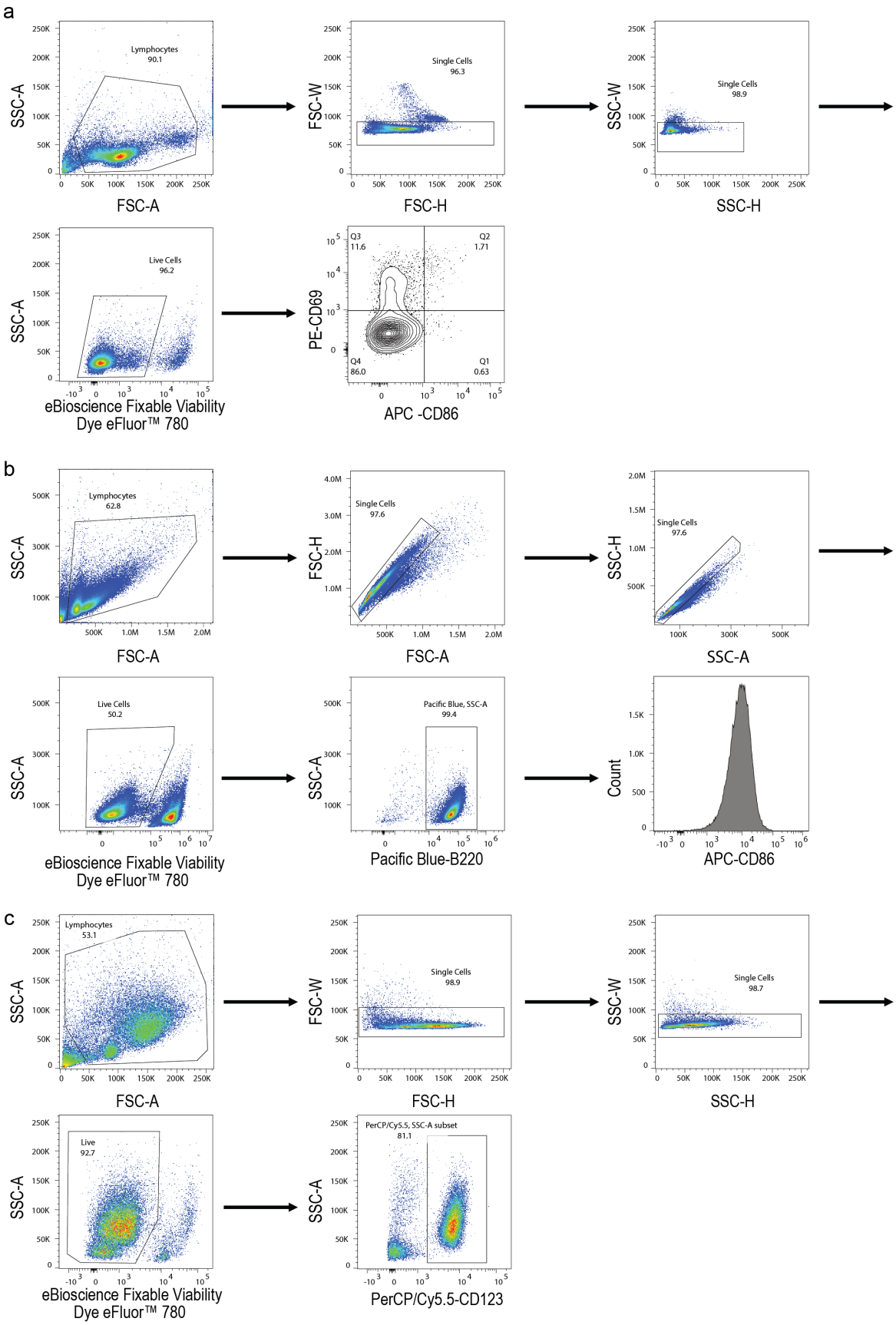


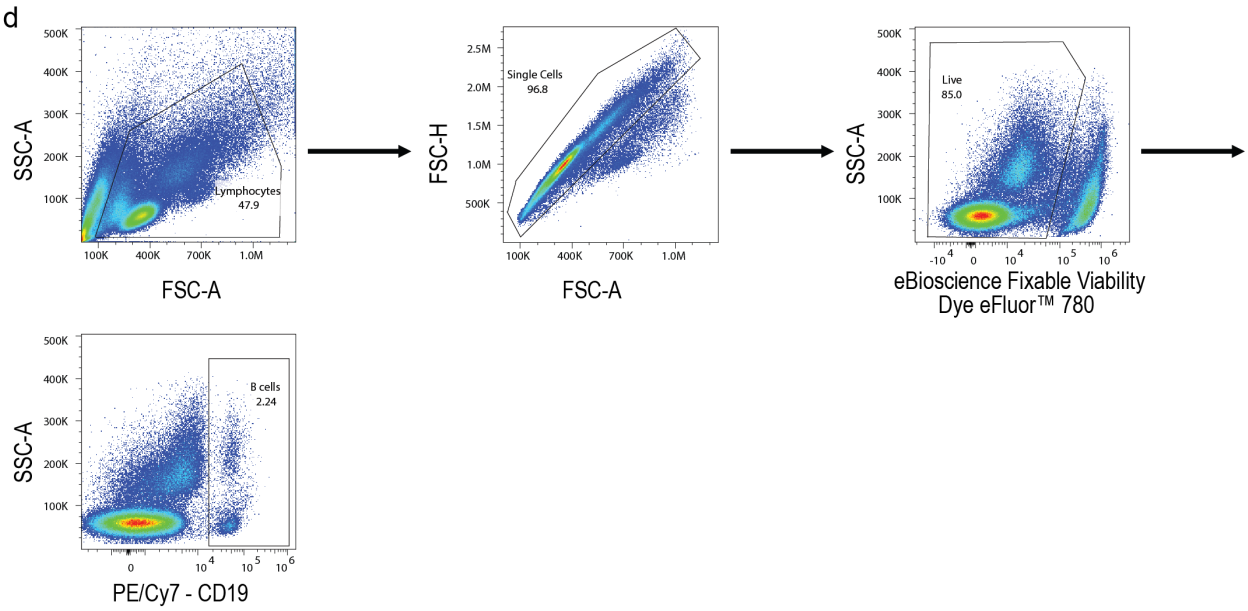
Supplementary Fig. 6b





Supplementary Fig. 11: Uncropped western blots of supplementary figures.


a
Lymphocytes
90.1
SSC-A
FSC-A
Single Cells
96.3
FSC-W
FSC-H
Single Cells
98.9
SSC-W
SSC-H
Live Cells
96.2
SSC-A
eBioscience Fixable Viability
Dye eFluor™ 780
Q3
11.6
Q2
1.71
Q4
86.0
Q1
0.63
PE-CD69
APC -CD86
b
Lymphocytes
62.8
SSC-A
FSC-A
Single Cells
97.6
FSC-H
FSC-A
Single Cells
97.6
SSC-H
SSC-A
Live Cells
50.2
SSC-A
eBioscience Fixable Viability
Dye eFluor™ 780
Pacific Blue, SSC-A
99.4
SSC-A
Pacific Blue-B220
Count
APC-CD86
c
Lymphocytes
53.1
SSC-A
FSC-A
Single Cells
98.9
FSC-W
FSC-H
Single Cells
98.7
SSC-W
SSC-H
Live
92.7
SSC-A
eBioscience Fixable Viability
Dye eFluor™ 780
PerCP/Cy5.5, SSC-A subset
81.1
SSC-A
PerCP/Cy5.5-CD123



Supplementary Fig. 12: Gating strategy for flow cytometry analysis.

a, Human B Cell gating strategy for *Fig 3d-e* and *Supplementary Fig. 4d-e*: Lymphocyte population was identified using FSC-A/SSC-A gating. From the lymphocyte population, single cells were identified using both FSC and SSC Height vs. Width gates. From the single cell population, live cells were characterized using the eBioscience Fixable Viability Dye eFluor™ 780 marker. Downstream activation markers were gated on naïve, unstimulated cells.

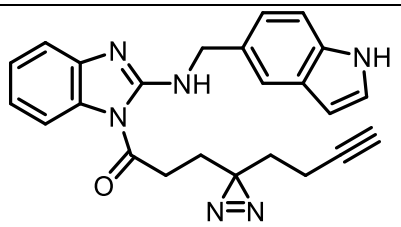
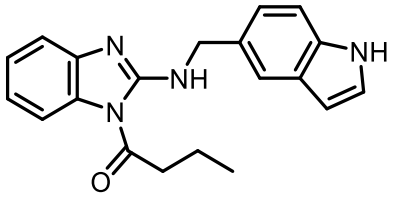
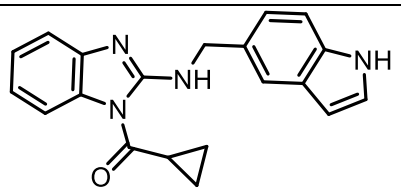
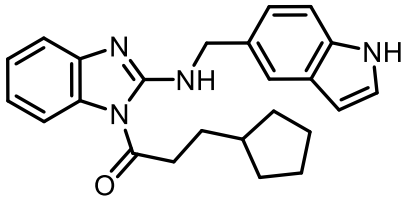
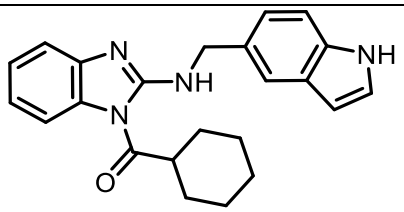
b, Mouse B Cell gating strategy for *Fig. 4d-e*, and *Extended Data Fig. 3d and 4b*: Lymphocyte population was identified using FSC-A/SSC-A gating. From the lymphocyte population, the single cell population was identified using both FSC and SSC Height vs. Area gates. Live cells were identified from the single cell population using the eBioscience Fixable Viability Dye eFluor™ 780 marker, and the pure B cell population was identified the marker B220.

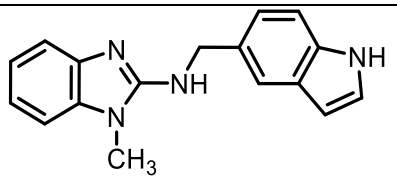
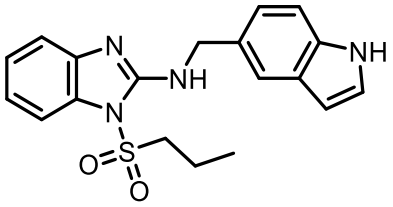
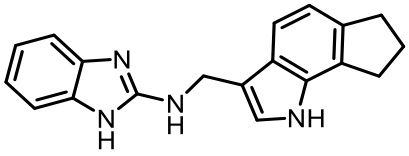
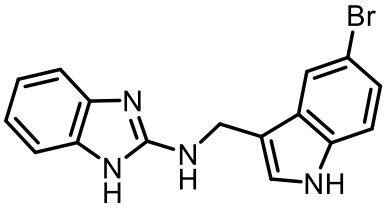
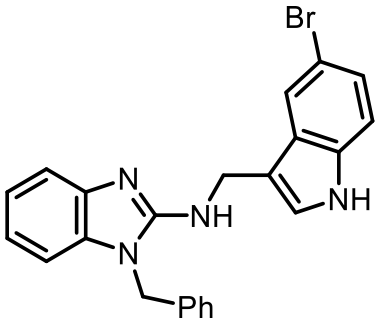
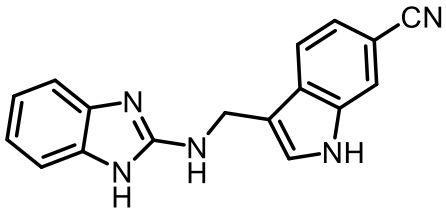
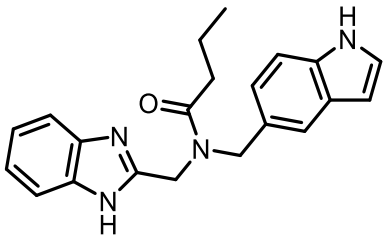
c, Human pDC gating strategy for *Extended Data Fig. 5a and 6c*: Lymphocyte population was identified using FSC-A/SSC-A gating. From the lymphocyte population, single cells were identified using both FSC and SSC Height vs. Width gates. Live cells were identified from the single cell population using the eBioscience Fixable Viability Dye eFluor™ 780 marker, and the pure pDC population was further identified using the marker CD123.

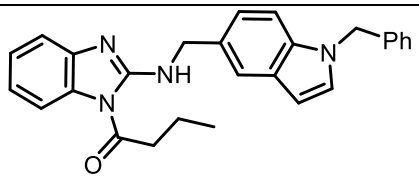
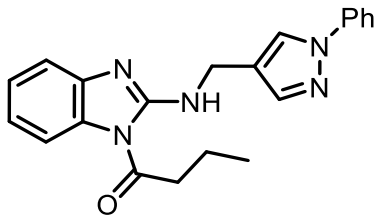
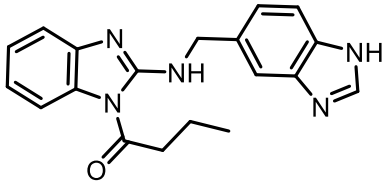
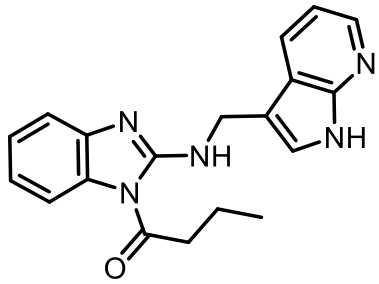
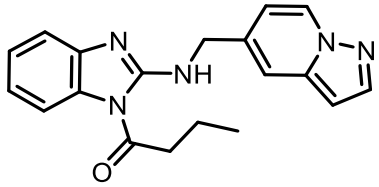
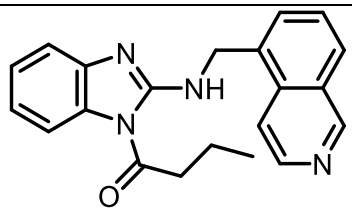
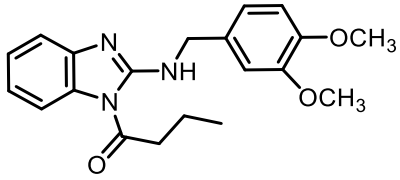
d, Representative Lupus patient gating strategy pertaining to *Extended Data Fig.* 8: Lymphocyte population was identified using FSC-A/SSC-A gating. From the lymphocyte population, single cells were identified using FSC-A versus FSC-H gating. Live cells were then identified using the eBioscience Fixable Viability Dye eFluor™ 780 marker from the single cell population. To identify the B cell population from the entire PBMC population, staining for the pan-B cell marker CD19 was employed. All subsequent FACS analysis was performed on this identified B cell population.

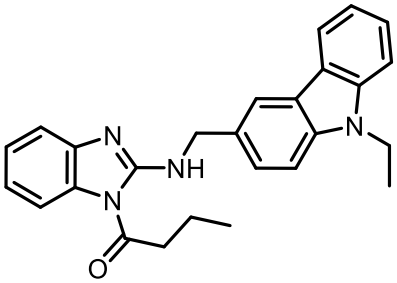
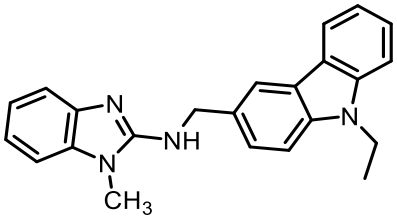
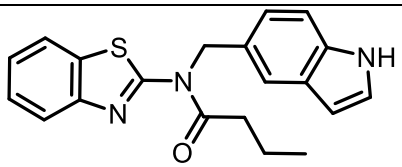
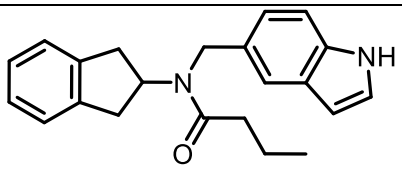
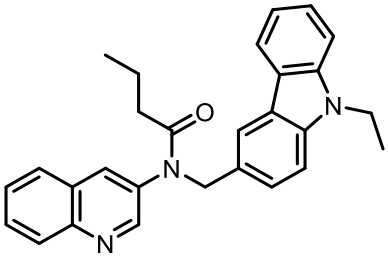
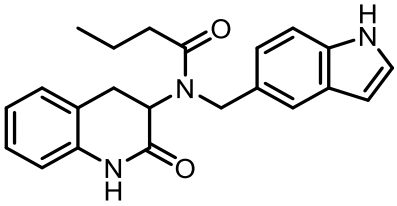
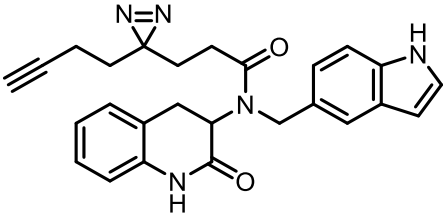
SUPPLEMENTAL TABLES

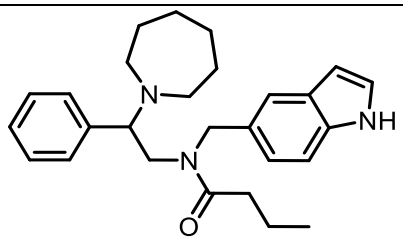
Supplementary Table 1: Inhibition of IFN-α production and SLC15A4 transport activity by analogs of FFF-15/21c. For IFN-α quantitation, human pDCs were treated with CpG-A (1 μM) and varying doses of compounds for 24 hours before analysis. For transport assay, reporter cells were treated with varying doses of compounds and MDP (500 ng ml^{-1}) for 24 hours before analysis.

Compound	Structure	IFN-I IC_{50} (μM)	MDP-NOD Reporter Assay IC_{50} (μM)
FFF-21 (**2**)		~21	~20
21c (**3**)		~32	-
AJ2-10 (**47**)		3.6	15.6
AJ2-13 (**48**)		~20	~20
AJ2-14 (**49**)		>20	>20

AJ2-29 (**50**)		3.6	7.1
AJ2-11 (**51**)		>20	>20
AJ2-38 (**52**)		0.72	-
AJ2-3A (**53**)		5.0	5.3
AJ2-66 (**54**)		>20	-
AJ2-46 (**55**)		1.5	-
AJ2-23 (**56**)		>20	-

AJ2-8 (**57**)		3.4	-
AJ2-27 (**58**)		>20	>20
AJ2-9 (**59**)		>20	-
AJ2-5 (**60**)		>20	-
AJ2-6 (**61**)		>20	>20
AJ2-4 (**62**)		>20	>20
AJ2-7 (**63**)		>20	>20

AJ2-25 (**64**)		2.6	-
AJ2-30 (**4**)		1.8	2.6
AJ2-2 (**65**)		>20	>20
AJ2-22 (**66**)		>20	>20
AJ2-74 (**67**)		>20	-
AJ2-18 (**6**)		>20	>20
AJ2-90 (**7**)		>20	>20

AJ2-26 (**68**)		>20	>20

Supplementary Table 2: SLE Patients information. Lupus patient samples were obtained through StemCell Technologies Diseased Human Peripheral Blood Mononuclear Cells Catalog.

Donor ID	Date of Draw	Age	Sex	Ethnicity	Weight (kg)	Height (cm)	Smoking Status	Blood Type	Year of Diagnosis	Current Medication
RG1817	9/17/20	47	F	Caucasian	83	157	Smoker	A-	2016	Levothyroxine
RG1814	10/29/20	55	M	Caucasian	95	178	Non-smoker	A+	2013	Topro-XL
RG2551	10/30/20	67	F	Caucasian	96	168	Non-smoker	A+	2010	Cymbalta, Hydroxychloroquine, Levothyroxine, Lisinopril, Mobic, Prilosec
RG1342	11/9/20	54	F	Caucasian	57	165	Non-smoker	O+	2013	Insulin, Loestrin
RG3041	2/5/21	48	F	Caucasian	83	168	Non-smoker	N/A	2019	Gabapentin, Lexapro, Methotrexate
RG3195	5/19/21	42	F	Caucasian	128	155	Smoker	N/A	2020	Allergy Shot, Folic acid, Hydrocodone, Levothyroxine, Linzess, Methotrexate, Tizanidine
RG1166	2/4/22	49	F	Caucasian	85	157	Non-smoker	A+	2015	Crestor, Fenobibrate, Lyrica, Metformin, Vascepa, Zyrtec, Estradile
RG3502	2/8/22	30	F	Caucasian	131	165	Non-smoker	A+	2006	Albuterol inhaler, Iron Supplement, Prenatal Vitamins, Zinc, Vitamin A, C, and D
RG2261	5/3/22	59	F	Caucasian	77	157	Non-smoker	O+	2015	None

Supplementary Table 3: reagents table

REAGENT	SOURCE	IDENTIFIER
Antibodies		
Anti-Lamtor1 (IB 1:1000)	Cell Signaling Technology	#8975
Anti-Lamtor2 (IB 1:1000)	Cell Signaling Technology	#8145
Anti-LC3B (IB 1:2000)	Cell Signaling Technology	#83506
Anti-RRAGC (IB 1:1000)	Proteintech	26989-1-AP
Anti-ATP6V1B2 (IB 1:1000)	Proteintech	15097-1-AP
Anti-ATP6V0D1 (IB 1:1000)	Proteintech	18274-1-AP
Anti-STX7 (IB 1:5000)	Proteintech	12322-1-AP
Anti-ATP6V0A3 (TCIRG1) (IB 1:2000)	Sigma-Aldrich	HPA038742
Anti-CXorf21(TASL) antibody (IB 1:2000)	ABclonal	# A20486
HA-Tag mAb (IB 1:2000)	Cell Signaling Technology	#3724
p70 S6 Kinase mAb (IB 1:1000)	Cell Signaling Technology	#34475
P-p70 S6 Kinase (T389) mAb (IB 1:500)	Cell Signaling Technology	#9234
IRF-5 mAb (IB 1:500, IF 1:200)	Cell Signaling Technology	#96527
IRF-7 mAb (IF 1:200)	Santa Cruz Biotechnology	sc-74472
IRF-7 mAb (IB:1:1000)	Abcam	Ab238137
LC3B	Abcam	ab48394
4E-BP1 mAb (IB 1:1000)	Cell Signaling Technology	#9644
P-4E-BP1 (S65) mAb (IB 1:1000)	Cell Signaling Technology	#13443
IκBα (IB 1:500)	Cell Signaling Technology	#4814
p-IκBα (S32) mAb (IB 1:500)	Cell Signaling Technology	#2859
p- IKKα/β (Ser176/180) (IB 1:500)	Cell Signaling Technology	#2697
IKKβ mAb (IB 1:1000)	Cell Signaling Technology	#8943
IRAK1 (IB 1:300)	Cell Signaling Technology	#4359
Anti-Flag mAb (IB 1:1000)	Cell Signaling Technology	#14793
Anti-Tubulin hFAB (IB 1:3000)	Bio-Rad	#12004165
Donkey anti-Mouse IgG, Alexa Fluor Plus 488 (IF 1:500)	Thermo Fisher Scientific	A32766TR
Donkey anti-Rabbit IgG, Alexa Fluor Plus 647 (IF 1:500)	Thermo Fisher Scientific	A32795TR

Rabbit anti-Mouse IgG, HRP (IB 1:10000)	Thermo Fisher Scientific	PA1-28568
Goat anti-Rabbit IgG, HRP (IB 1:10000)	Thermo Fisher Scientific	31460
NucBlue™ Fixed Cell ReadyProbes™ Reagent (DAPI)	Thermo Fisher Scientific	R37606
LysoSensor Green DND-189	Thermo Fisher Scientific	L7535
eBioscience Fixable Viability Dye eFluor™ 780	Thermo Fisher Scientific	65-0865-18
BV421 anti-human CD80 (Clone 2D10) (FC 1:200)	Biolegend	305222
AlexaFluor488 anti-human HLA-DR (Clone LN3) (FC 1:200)	Biolegend	327010
PE anti-human CD69 (Clone FN50) (FC 1:200)	Biolegend	310906
APC anti-human CD86 (Clone IT2.2) (FC 1:200)	Biolegend	305412
PE/Cy7 anti-human CD19 (Clone HIB19) (FC 1:200)	Biolegend	302216
BV421™ anti-mouse/human CD45R/B220 Antibody (Clone RA3-6B2) (FC 1:200)	Biolegend	103240
PE anti-mouse CD69 (Clone H1.2F3) (FC 1:200)	Biolegend	104508
APC anti-mouse CD86 (Clone GL-1) (FC 1:200)	Biolegend	105012
PerCP/Cy5.5 anti-mouse CD80 (Clone 16-10A1) (FC 1:200)	Biolegend	104722
PerCP/Cy5.5 anti-human CD123 (Clone 6H6) (FC 1:200)	Biolegend	306016
PE anti-human CD303 (BDCA-2) (Clone 201A) (FC 1:200)	Biolegend	354204
IRF-7 pS477/pS479 Antibody, anti-human, REAfinity™ (FC 1:100)	Miltenyi Biotec	130-104-702

Anti-hu/mo phospho-mTOR (Ser2448)-PE (FC 1:100)	Thermo Fisher Scientific	12-9718-42
phospho-S6 ribosomal Protein (S235/236) XP-PeCy7 (FC 1:100)	Cell Signaling Technology	34411S
phospho-4E-BP1 (T37/46) AlexaFluor647 (FC 1:100)	Cell Signaling Technology	5123S
LAMP2 Recombinant Rabbit Monoclonal Antibody (ARC0274)	Invitrogen	MA5-35052
hFAB™ Rhodamine Anti-GAPDH Primary Antibody (IB 1:10000)	Bio-Rad	#12004168
mTOR (7C10) Rabbit mAb (IF 1:200)	Cell Signaling Technology	2983T
Purified anti-human CD107a (LAMP-1) Antibody (IF 1:500; clone H4A3)	Biolegend	328601
TLR9 antibody (26C593.2) - BSA Free (IF 1:200)	Novus Biologicals	NBP2-24729
Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb (Alexa Fluor® 488 Conjugate) (FC 1:100)	Cell Signaling Technology	9174S
Experimental models: Cell Lines		
293T	ATCC	CRL-3216
A549	ATCC	CCL-185
CAL-1	S. Kamihira, (Nagasaki University)	PMID: 15765784
CAL-1::EGFP	In this study	CAL-1 stably overexpressing EGFP
CAL-1::HA-SLC15A4	In this study	CAL-1 stably overexpressing HA tagged SLC15A4
CAL-1::V5-APEX2-SLC15A4	In this study	CAL-1 stably overexpressing V5-APEX2 tagged SLC15A4

THP-1 Dual	InvivoGen	thpd-nfis
Critical commercial assays		
Quanti-Luc™-Gold	InvivoGen	rep-qlcg1
Quanti-Blue™	InvivoGen	rep-qbs
Trans-Blot Turbo RTA transfer Kit, LF, PVDF	BioRad	#1704275
CellTiter-Glo Luminescent Cell Viability Assay	Promega	G7570
CytoTox 96® Non-Radioactive Cytotoxicity Assay	Promega	G1780
Human IFN-Beta ELISA Kit	pbl Assay Science	41410-2
Human IFN-Alpha ELISA Kit	pbl Assay Science	41100-2
Mouse IFN-Alpha ELISA Kit	pbl Assay Science	42120-2
ELISA MAX™ Deluxe Set Human TNF-α	Biolegend	430204
ELISA MAX™ Deluxe Set Mouse IL-6	Biolegend	431304
ELISA MAX™ Deluxe Set Mouse TNF-α	Biolegend	430904
IgG (Total) Human Uncoated ELISA Kit with Plates	Thermo Fisher Scientific	88-50550-22
IgG2c Mouse Uncoated ELISA Kit with Plates	Thermo Fisher Scientific	88-50670-22
IgM Mouse Uncoated ELISA Kit with Plates	Thermo Fisher Scientific	88-50470-22
Bio-Plex Pro Mouse Cytokine 23-plex assay	Bio-Rad	M60009RDPD
Bio-Plex Pro Human Cytokine 27-plex Assay	Bio-Rad	M500KCAF0Y
RNeasy Plus Mini Kit	Qiagen	74134
TURBO DNA-free™ Kit	Thermo Fisher Scientific	AM1907
M-MLV Reverse Transcriptase (200 U/µL)	Thermo Fisher Scientific	28025013
Fast SYBR™ Green Master Mix	Thermo Fisher Scientific	4385612
Reagents, Peptides and Recombinant proteins		
TMT10plex™ Isobaric label reagent set	Thermo Fisher Scientific	90110

TMTpro™ 16plex label reagent set	Thermo Fisher Scientific	A44522
Biotin-PEG3-Azide	Click Chemistry Tools	AZ104
Pierce Streptavidin Agarose	Thermo Fisher Scientific	20353
Pierce High pH Reversed-Phase Fractionation Kit	Thermo Fisher Scientific	84868
HA peptide	GenScript	RP11735
PEI MAX	Polysciences Inc.	24765-1
Rhodamine-azide	Synthesized in lab	-
Triptolide	AdipoGen Life Sciences	501146334
GSK583, RIPK2i	MedChemExpress	HY-100339
NOD2 Signaling Inhibitor II, GSK717	EMD Millipore	5.33718.0001
R848 (Resiquimod)	InvivoGen	tlrl-848
Imiquimod (R837)	InvivoGen	tlrl-imq
VACV-70/ LyoVec™	InvivoGen	tlrl-vav70n
Pam3CSK4	InvivoGen	tlrl-pms
Standard LPS, E. coli K12	InvivoGen	tlrl-eklps
ODN 2216	InvivoGen	tlrl-2216-5
ODN 2006 (ODN 7909)	InvivoGen	tlrl-2006-5
LL-37 (Antimicrobial peptide)	InvivoGen	tlrl-l37
Muramyldipeptide (L-D isoform, active)	InvivoGen	tlrl-mdp
Tri-DAP	InvivoGen	tlrl-tdap
DNeasy Blood & Tissue Kit	Qiagen	69504
QIAprep Spin Miniprep Kit (250)	Qiagen	27106
EndoFree Plasmid Maxi Kit	Qiagen	12362
Pierce Anti-HA Magnetic Beads	Thermo Fisher Scientific	88837
Sequencing Grade Modified Trypsin	Promega	V5111
Endoproteinase LysC	New England Biolabs	P8109
DOTAP Liposomal Transfection Reagent	Roche	11202375001
CD304 (BDCA-4/Neuropilin-1) MicroBead Kit, human	Miltenyi Biotec	130-090-532
EasySep™ Human B Cell Isolation Kit	StemCell Technologies	17954
EasySep™ Mouse B Cell Isolation Kit	StemCell Technologies	19854

EasySep™ Human Monocyte Isolation Kit	StemCell Technologies	19359
EasySep™ Mouse CD11c Positive Selection Kit II	StemCell Technologies	18780
ACK lysing buffer (RBC Lysis)	Quality Biological Inc.	118-156-101
Recombinant Human IFN-gamma	R&D Systems	285-IF
Human Recombinant M-CSF	StemCell Technologies	78057.1
Flt-3L-Ig	BioXcell	BE0098
Mouse M-CSF	Fisher Scientific	416ML010
GM-CSF	Fisher Scientific	41-5ML0-05CF
eBioscience Fixable Viability Dye eFluor™ 780	Thermo Fisher Scientific	65-0865-14
Halt Protease Inhibitor	Thermo Fisher Scientific	78438
Pierce Quantitative Fluorometric Peptide Assays	Thermo Fisher Scientific	23290
ProteaseMAX™ Surfactant, Trypsin Enhancer	Promega	V2072
Pierce C18 Spin Columns	Thermo Fisher Scientific	89873
Dynabeads® M-280 Sheep anti-Rabbit IgG	Thermo Fisher Scientific	11203D
Chloroquine diphosphate salt	Sigma	C6628
Bafilomycin A1	Selleck Chemicals	S1413
EnzChek™ Phosphate Assay Kit	Thermo Fisher Scientific	E6646
Torin 1	Selleck Chemicals LLC	S2827
Recombinant DNA		
pDONR221_SLC15A4	Addgene	#131910
pLJM1-EGFP	Addgene	#19319
Cxorf21 (TASL)	GPS core UF Scripps	476835
Human SLC15A4 (Codon Optimized)	IDT	
Human SLC15A3 (Codon Optimized)	IDT	
Mouse SLC15A4 (Codon Optimized)	IDT	
Human NOD2 (Codon Optimized)	IDT	
Software and Algorithms		

Proteome Discoverer (v2.4)	Thermo Fisher Scientific	-
GraphPad	Dotmatics	-
FlowJo™ (v10)	FlowJo	-
NovoCyte (v1.2.5)	ACEA Bioscience. Inc.	-
Xcalibur (v4.1.50)	Thermo Fisher Scientific	-
Carl Zeiss AG, ZEN (v2.3)	Zeiss Microscopy	-
Imagelab (Version 6.1.0)	Bio-Rad laboratories	-
QuantStudio (v1.3)	Thermo Fisher Scientific	-
ChemDraw (21.0.0.28)	Revvity signals	-
MestReNova (14.0.0)	Mestrelab Research	-
Phoenix WinNonlin 6.3.	Certara	-
Excel (v2310)	Microsoft	-
Prism (v9.3.0).	GraphPad	-

NMR spectra

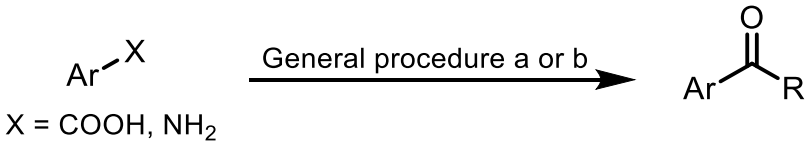
I. Synthetic Methods:

(A) Chemistry material

Chemicals and reagents were purchased from commercial vendors, including Sigma-Aldrich, Fisher Scientific, Combi-Blocks, MedChemExpress, Alfa Aesar and AstaTech, and were used as received without further purification, unless otherwise noted. Anhydrous solvents were purchased from Sigma-Aldrich in Sure/Seal™ formulations. All reactions were monitored by thin-layer chromatography (TLC, Merck silica gel 60 F-254 plates). The plates were stained either with *p*-anisaldehyde (2.5% *p*-anisaldehyde, 1% AcOH, 3.5% H_2SO_4 (conc.) in 95% EtOH), ninhydrin (0.3% ninhydrin (w/v), 97:3 EtOH-AcOH), $KMnO_4$ (1.5g of $KMnO_4$, 10g K_2CO_3, and 1.25mL 10% NaOH in 200mL water), iodine or directly visualized with UV light. Reaction purification was carried out using Flash chromatography (230 – 400 mesh silica gel), and Biotage® or preparative thin layer chromatography (pTLC, Analtech, 500-2000 µm thickness). NMR spectra were recorded on Bruker DPX-400 MHz, Bruker AV-500 MHz, Bruker AV-600 MHz spectrometers in the indicated solvent. Multiplicities are reported with the following abbreviations: s singlet; d doublet; t triplet; q quartet; p pentet; m multiplet; br broad; dd doublet of doublets; dt doublet of triplets; td triplet of doublets. Chemical shifts are reported in ppm relative to the residual solvent peak and J values are reported in Hz. Mass spectrometry data were collected on an Agilent 6120 single-quadrupole LC/MS instrument (ESI, low resolution) or an Agilent ESI-TOF instrument (ESI-TOF, HRMS).

(B) Synthetic Procedures:

1) General synthetic procedure of diazirine containing FFF (Fully Functionalized Fragments) Scheme 1:

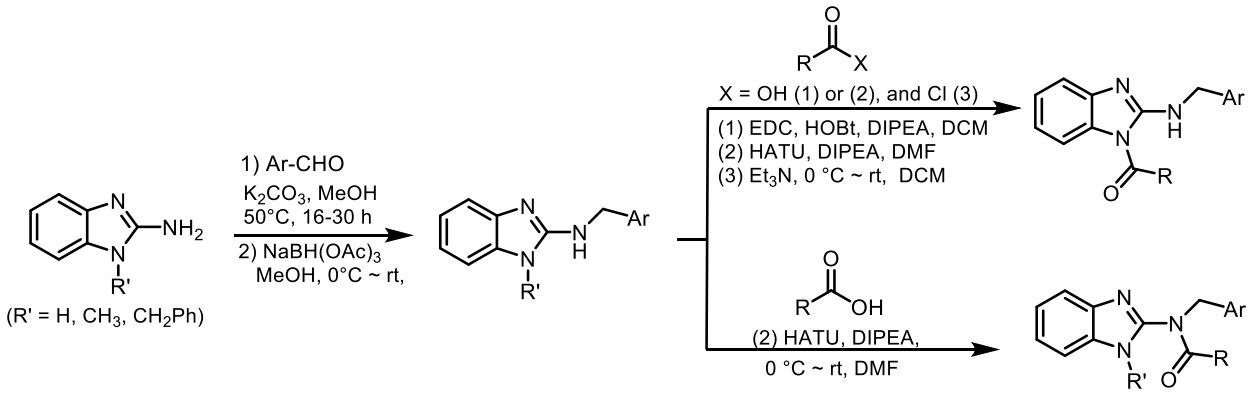


General Procedure a: To a solution of corresponding commercially available carboxylic acid (0.113 mmol) in 3 ml DCM, the corresponding diazirine amine (0.118 mmol), DIPEA (0.354 mmol), EDC-HCl (0.177 mmol), and HOBt (0.177 mmol) were added. The reaction mixtures were stirred at room temperature for 14 to 16 hr. After completion (monitored by TLC) the crude reaction mixture was diluted with DCM (20 mL) and washed first with saturated aqueous NH_4Cl (10 mL) and saturated aqueous $NaHCO_3$ (10 mL) solution, dried over anhydrous Na_2SO_4 and volatiles removed by rotary evaporation. Crude products were purified by PTLC or Biotage® SNAP Cartridge KP-Sil Snap 10g with linear gradient of ethyl acetate and hexane over 20 column volumes (CV).

General Procedure b: To a solution of corresponding commercially available amine (0.113 mmol) in 3 ml DCM, the corresponding diazirine acid (0.118 mmol), DIPEA (0.354 mmol), EDC-HCl (0.177 mmol), and HOBt (0.177 mmol) were added. The reaction mixtures were stirred at room temperature for 14 to 16 hr. After completion (monitored by TLC) the crude reaction mixture was diluted with DCM (20 mL) and washed first with saturated aqueous NH_4Cl (10 mL) and saturated aqueous $NaHCO_3$ (10 mL) solution, dried over anhydrous Na_2SO_4 and volatiles removed by rotary

evaporation. Crude products were purified by PTLC or Biotage® SNAP Cartridge, KP-Sil, 10g with linear gradient of ethyl acetate and hexane over 20 column volumes (CV).

2) General Synthetic Scheme 2:



General Procedure 1: coupling procedure for the synthesis of benzo[*d*]imidazole amine intermediate (S1)

Synthesized according to reported procedure[3], to a dried round bottom flask containing solution of commercially available 2-aminobenzimidazole, or 2-amino-1-methylbenzimidazole, or 2-aminoindane derivatives (1.0 eq.) and corresponding aldehyde (1.0 eq.) in dry methanol, potassium carbonate (3.0 eq.) was added, and the reaction mixture was heated at 50 ^{o}C for 16 to 30 h. The solvent was filtered to remove the excess potassium carbonate and sodium triacetoxyborohydride (2.0 eq.) was added at 0^{o}C to the solution and resulting mixture was stirred for 8 h at room temperature. After completion (monitored by TLC) the solvent was removed by rotary evaporation, crude mixture was diluted with water and washed with saturated aqueous $NaHCO_3$ solution extracted in ethyl acetate, the combined extract was dried over Na_2SO_4, filtered and concentrated in vacuum, purified by flash column chromatography on Biotage® isolera one instrument to give corresponding amines intermediate (S1).

General Procedure 2: Coupling of amines intermediate with acid

To a vial containing corresponding amine intermediate (1 eq.) in dichloromethane (60 mM relative to S1), commercially available butyric acid or 3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)propanoic acid (1.1 eq.), N, N-diisopropylethylamine (3.0 eq.), EDC-HCl (1.5 eq.) and HOBt (1.5 eq.) were added and stirred at room temperature for 4 h to overnight. After completion (monitored by TLC) the crude mixture was diluted with dichloromethane, saturated aqueous NH_4Cl solution was added and extracted in dichloromethane. The combined extract was washed with saturated aqueous $NaHCO_3$ solution, extracted in dichloromethane dried over anhydrous Na_2SO_4, and volatiles removed by rotary evaporation. Crude products were purified by PTLC or flash column chromatography on Biotage® isolera one instrument to give the corresponding products.

General Procedure 3: Coupling of amines intermediate with acid

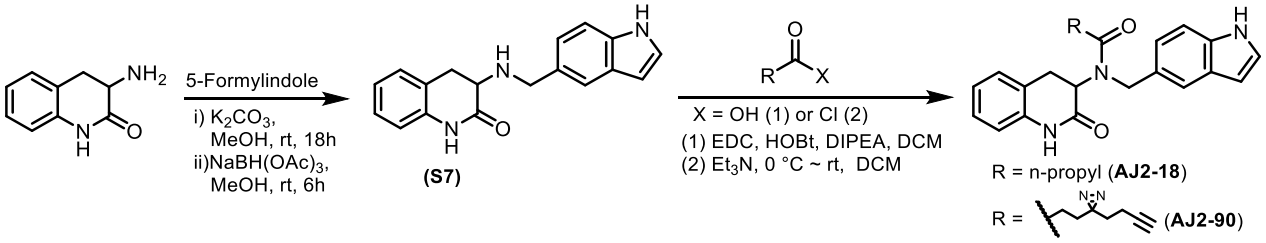
To a solution of corresponding butyric acid or 3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)propanoic acid (1.1 eq.) and amine intermediate (1.0 eq.) in dimethylformamide (60 mM relative to S1), the N, N-diisopropylethylamine (3.0 eq.) and HATU (1.1 eq.) were added at 0°C and resulting mixture was stirred at room temperature until amines were fully consumed, as indicated by TLC. The

crude mixture was diluted with cold water and extracted in ethyl acetate then combined extract were dried over anhydrous Na_2SO_4 and volatiles removed by rotary evaporation. Crude materials were purified by PTLC or flash column chromatography on Biotage® isolera one instrument to give the corresponding products.

General Procedure 4: Coupling procedure for synthesis of amide with acid chloride

To a solution of corresponding amine (1.0 eq.) and triethylamine (1.1 eq.) in dichloromethane (0.1 M), and corresponding acid chloride (1.0 eq.) solution in dichloromethane was added over 10 minutes at 0°C, and resulting mixture was allowed to stir at room temperature until starting amines was fully consumed, as indicated by TLC. The crude reaction mixture was diluted with dichloromethane, washed with saturated aqueous NH_4Cl solution followed by $NaHCO_3$ solution, the combined dichloromethane solution dried over anhydrous Na_2SO_4, and volatiles removed by rotary evaporation. The crude materials were purified by PTLC or flash column chromatography on Biotage® isolera one instrument to give the corresponding products.

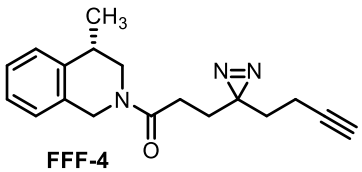
General synthetic Scheme 3:



To a solution of 3-amino-3,4-dihydroquinolin-2(1H)-one (1.0 eq.) and corresponding 5-Formylindole (1.0 eq.) in dry methanol, potassium carbonate (3.0 eq.) was added, and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was filtered on Whatman® filter paper to remove the excess potassium carbonate washed with methanol and sodium triacetoxyborohydride (2.0 eq.) was added at 0°C to the filtered solution and resulting mixture was stirred for 6 h at room temperature. After completion (monitored by TLC) the solvent was removed by rotary evaporation, crude mixture was diluted with water and washed with saturated aqueous $NaHCO_3$ solution extracted in ethyl acetate, the combined extract was dried over Na_2SO_4, filtered and concentrated in vacuum, purified by flash column chromatography on Biotage® isolera one with 5-80 % ethyl acetate gradient in hexane to obtain corresponding 3-(((1H-indol-5-yl)methyl)amino)-3,4-dihydroquinolin-2(1H)-one (**S7**) as a white solid.

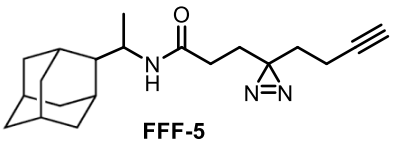
ii) Characterization data of FFF (Fully Functionalized Fragments) probes:

FFF-1, **2**, **3**, **6**, **7**, **10**, **11**, **14**, **15**, **37**, **38** and **FFF-ctrl** are synthesized according to previously reported procedure.[1, 2]



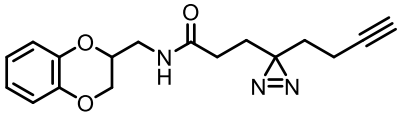
(S)-3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)-1-(4-methyl-3,4-dihydroisoquinolin-2(1H)-yl)propan-1-one (FFF-4): Synthesized according to general procedure 2, the crude residue was

purified by biotage (Hexanes/EtOAc, 6:4) to afford FFF-4 as a colorless liquid (12 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.06 (m, 7H), 4.95 (d, *J* = 17.2 Hz, 1H), 4.52 – 4.57 (m, 2H), 3.74 – 3.68 (m, 1H), 3.62 – 3.56 (m, 1H), 3.48 – 3.42 (m, 1H), 3.10 – 2.93 (m, 2H), 2.21 – 2.11 (m, 3H) 2.02 – 2.07 (m, 3H) 1.91 (dd, *J* = 7.1, 1.8 Hz, 3H), 1.69 (t, *J* = 7.5 Hz, 3H), 1.30 (d, *J* = 7.0 Hz, 3H), 1.26 (d, *J* = 7.0 Hz, 2.55H). *Note: rotameric mixtures observed*, LCMS *calcd for* $C_{18}H_{22}N_3O$, 296.18 ($M+H^+$), *found*: 296.2.



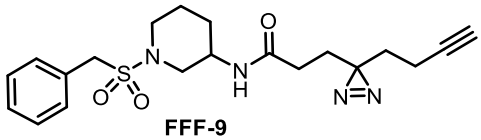
FFF-5

N-(1-((3r,5r,7r)-adamantan-1-yl)ethyl)-3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)propanamide (FFF-5): Synthesized according to general procedure 1, purified by biotage (Hexanes/EtOAc, 8:2) to afford FFF-5 as a white solid (11 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.26 – 5.24 (m, 1H), 3.74 – 3.68 (m, 1H), 2.06 – 1.96 (m, 6H), 1.97 – 1.81 (m, 4H), 1.76 – 1.58 (m, 9H), 1.58 – 1.43 (m, 7H), 1.06 – 0.98 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.76, 83.08, 69.52, 53.41, 38.73, 37.37, 36.07, 32.82, 31.03, 28.82, 28.63, 28.27, 14.89, 13.67.



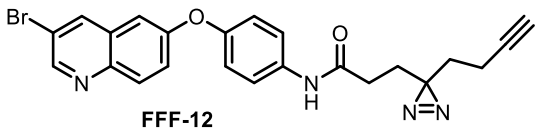
FFF-8

3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)-N-((2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl)propenamide (FFF-8): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 6:4) to afford FFF-8 as a white solid (16 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88 – 6.82 (m, 4H), 5.91 (s, 1H), 4.33 – 4.17 (m, 2H), 3.98 – 3.92 (m, 1H), 3.71 – 3.65 (m, 1H), 3.47 (t, *J* = 6.1 Hz, 1H), 2.03 – 1.94 (m, 5H), 1.85 (t, *J* = 7.4 Hz, 2H), 1.63 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.71, 143.06, 142.73, 121.68, 117.28, 117.21, 82.73, 71.91, 69.39, 65.68, 39.81, 32.33, 30.18, 28.25, 27.86, 13.26. LCMS *calcd for* $C_{17}H_{20}N_3O_3$, 314.1 ($M+H^+$), *found*: 314.1.



FFF-9

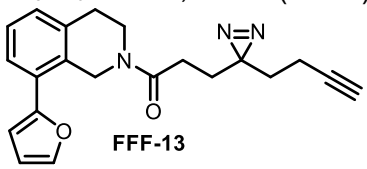
N-(1-(benzylsulfonyl)piperidin-3-yl)-3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)propanamide (FFF-9): Synthesized according to general procedure 2, the crude residue was purified by biotage (Hexanes/EtOAc, 8:2) to afford FFF-9 as a colorless liquid (14 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (q, *J* = 2.6 Hz, 5H), 5.81 (d, *J* = 7.7 Hz, 1H), 4.24 (s, 2H), 4.02 – 3.89 (m, 1H), 3.46 – 3.42 (m, 1H), 3.28 – 3.20 (m, 1H), 2.98 – 2.94 (m, 1H), 2.75 – 1.98 (m, 1H), 2.07 – 1.98 (m, 3H), 1.98 – 1.88 (m, 2H), 1.86 – 1.77 (m, 3H), 1.68 – 1.60 (m, 3H). LCMS *calcd for* $C_{20}H_{27}N_4O_3S$, 403.18 ($M+H^+$), *found*: 403.2.



FFF-12

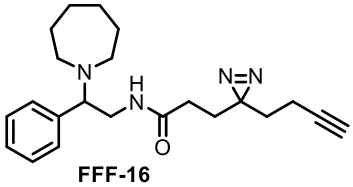
N-(4-((3-bromoquinolin-6-yl)oxy)phenyl)-3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)propanamide (FFF-12): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc,

6:4) to afford FFF-12 as an off white solid (15 mg, 74%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.79 (d, *J* = 2.3 Hz, 1H), 8.14 (d, *J* = 2.3 Hz, 1H), 8.03 (d, *J* = 9.2 Hz, 1H), 7.58 – 7.52 (m, 2H), 7.47 (d, *J* = 2.7 Hz, 1H), 7.43 (s, 1H), 7.10 – 7.01 (m, 3H), 2.14 (dd, *J* = 8.2, 6.8 Hz, 2H), 2.08 – 2.02 (m, 2H), 2.00 (t, *J* = 2.6 Hz, 1H), 2.00 – 1.94 (m, 2H), 1.70 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.46, 157.00, 152.28, 149.83, 143.05, 136.31, 134.22, 131.41, 130.11, 123.10, 121.93, 120.67, 117.97, 110.94, 82.72, 69.35, 32.48, 31.21, 28.20, 27.86, 13.33. LCMS *calcd for* $C_{23}H_{20}BrN_4O_2$, 463.1 ($M+H^+$), *found*: 463.1.



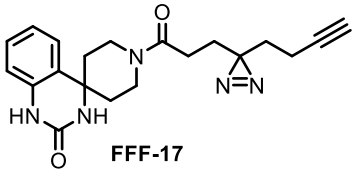
FFF-13

3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)-1-(8-(furan-2-yl)-3,4-dihydroisoquinolin-2(1H)-yl)propan-1-one (FFF-13): Synthesized according to general procedure 1, purified by biotage (Hexanes/EtOAc, 5:5) to afford FFF-13 as a colorless liquid (15 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, J = 1.9 Hz, 1H), 7.31 (s, 1.25 H), 7.27 – 7.16 (m, 7.68 H), 6.82 – 6.80 (m, 1.32 H), 6.27 – 6.24 (m, 2.27 H), 6.08 – 5.99 (m, 2.29 H), 5.98 (s, 1H), 4.60 – 4.55 (m, 1H), 3.81 – 3.76 (m 1.33 H), 3.60 – 3.52 (m, 1.35 H)), 3.06 – 2.85 (m, 5H), 2.80 – 2.75 (m, 1.12 H), 2.65 – 2.49 (m, 2.81 H), 2.36 – 2.28 (m, 1.06), 2.23 – 2.07 (m, 2.43 H), 2.09 – 2.00 (m, 3.47 H), 1.98 – 1.95 (m, 2.53 H), 1.94 – 1.86 (m, 4.31H), 1.70 – 1.65 (s, 3.74H). *Note: rotameric mixtures observed*, LCMS *calcd for* $C_{21}H_{22}N_3O_2$, 348.2 ($M+H^+$), *found*: 348.2.



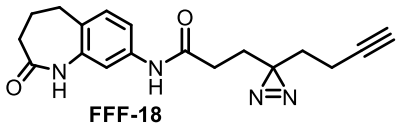
FFF-16

N-(2-(azepan-1-yl)-2-phenylethyl)-3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)propenamide (FFF-16): Synthesized according to general procedure 1, purified by biotage (Hexanes/EtOAc, 3:7) to afford FFF-16 as a colorless liquid (7 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.28 (m, 3H), 7.23 (dd, *J* = 7.9, 1.7 Hz, 2H), 6.21 (s, 1H), 3.82 – 3.76 (m, 1H), 3.55 – 3.53 (m, 2H), 2.80 – 2.66 (m, 2H), 2.59 – 2.53 (m, 2H), 2.07 – 1.91 (m, 5H), 1.90 – 1.78 (m, 2H), 1.69 – 1.53 (m, 10H). LCMS *calcd for* $C_{22}H_{31}N_4O$, 367.2 ($M+H^+$), *found*: 367.2.



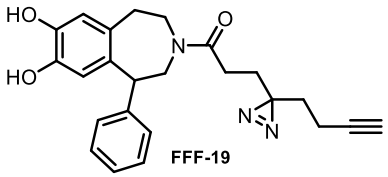
FFF-17

N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-2'-oxo-2',3'-dihydro-1'H-spiro[piperidine-4,4'-quinazoline]-1-carboxamide (FFF-17): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 8:2) to afford FFF-17 as an off white solid (12 mg, 67 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.18 – 7.09 (m, 2H), 7.05 (d, *J* = 2.1 Hz, 1H), 6.98 (dd, *J* = 7.6, 1.2 Hz, 1H), 6.78 (dd, *J* = 7.9, 1.2 Hz, 1H), 4.68 – 4.54 (m, 1H), 3.75 – 3.61 (m, 1H), 3.53 – 3.46 (m, 1H), 3.02 – 2.98 (m, 1H), 2.12 – 1.93 (m, 7H), 1.93 – 1.79 (m, 4H), 1.72 – 1.62 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.53, 154.96, 135.65, 128.61, 125.12, 123.84, 122.87, 114.80, 82.82, 69.27, 55.00, 40.52, 38.13, 36.78, 32.55, 28.03, 27.96, 26.86, 13.34. LCMS *calcd for* $C_{20}H_{25}N_5O_2$, 366.2 ($M+H^+$), *found*: 366.2.



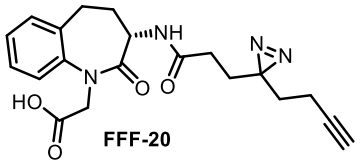
FFF-18

3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)-N-(2-oxo-2,3,4,5-tetrahydro-1H-benzo[b]azepin-8-yl)propenamide (FFF-18): Synthesized according to general procedure 1, purified by biotage (Hexanes/EtOAc, 6:4) to afford FFF-18 as a white solid (9 mg, 64%).[1]H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 8.00 (s, 1H), 7.33 – 7.23 (m, 2H), 7.13 (d, *J* = 8.1 Hz, 1H), 2.73 (t, *J* = 7.2 Hz, 2H), 2.33 (t, *J* = 7.3 Hz, 2H), 2.26 – 2.09 (m, 4H), 2.06 – 1.98 (m, 3H), 1.95 – 1.90 (m, 2H), 1.67 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.59, 169.87, 138.14, 137.26, 130.21, 130.10, 117.02, 113.51, 82.74, 69.36, 32.89, 32.40, 31.20, 29.77, 28.50, 28.17, 27.92, 13.32. LCMS *calcd for* $C_{18}H_{21}N_4O_2$, 325.2 ($M+H^+$), *found*: 325.2.



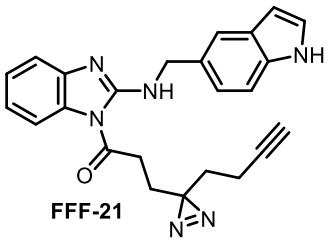
FFF-19

3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)-1-(7,8-dihydroxy-1-phenyl-1,2,4,5-tetrahydro-3H-benzo[d]azepin-3-yl)propan-1-one (FFF-19): Synthesized according to general procedure 1, purified by biotage (Hexanes/EtOAc, 6:4) to afford FFF-19 as a white solid (8 mg, 65%).[1]H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.16 (m, 5H), 7.13 – 7.03 (m, 1H), 6.99 – 6.85 (m, 2H), 6.72 (s, 1H), 6.39 (s, 1H), 4.48 – 4.43 (m, 1H), 4.37 – 4.32 (s, 1H), 4.03 – 3.88 (m, 1H), 3.70 – 3.61 (m, 1H), 3.59 – 3.49 (m, 2H), 3.38 – 3.19 (m, 2H), 2.84 – 2.67 (m, 1H), 1.96 – 1.81 (m, 7H), 1.62 – 1.52 (m, 2H), 1.52 – 1.36 (m, 4H). *Note: rotameric mixtures observed*, LCMS *calcd for* $C_{24}H_{27}N_3O_3$, 404.2 ($M+H^+$), *found*: 404.2.



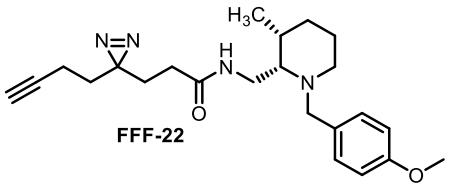
FFF-20

(S)-2-(3-(3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)propanamido)-2-oxo-2,3,4,5-tetrahydro-1H-benzo[b]azepin-1-yl)acetic acid (**FFF-20):** Synthesized according to general procedure 1, purified by biotage (Hexanes/EtOAc, 8:2) to afford FFF-20 as a brown solid (11 mg, 64 %). ^1H NMR (400 MHz, $CDCl_3$) δ 7.31 (dd, *J* = 7.8, 2.4 Hz, 1H), 7.26 – 7.19 (m, 2H), 7.14 (dd, *J* = 7.8, 1.3 Hz, 1H), 6.64 (d, *J* = 7.3 Hz, 1H), 4.68 – 4.62 (m, 1H), 4.58 – 4.51 (m, 1H), 4.52 – 4.44 (m, 1H), 3.31 – 3.19 (m, 1H), 2.73 – 2.56 (m, 2H), 2.10 (s, 1H), 2.03 – 1.89 (m, 5H), 1.82 – 1.68 (m, 2H), 1.66 – 1.53 (m, 2H). LCMS *calcd for* $C_{20}H_{23}N_4O_4$, 383.17 ($M+H^+$), *found*: 383.2.



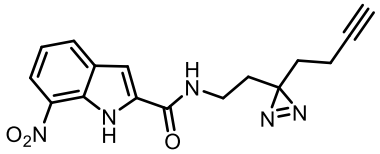
FFF-21

1-(2-(((1H-indol-5-yl)methyl)amino)-1H-benzo[d]imidazol-1-yl)-3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)propan-1-one (FFF-21): Synthesized according to scheme 1, general procedure 1 and following general procedure 2, purified on Biotage® with hexane : ethyl acetate (6:4) to afford FFF-21 as colorless liquid (12 mg, 42 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.09 – 8.04 (m, 1H), 7.67 (s, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.30 – 7.20 (m, 4H), 7.06 (t, *J* = 7.8 Hz, 1H), 6.52 (s, 1H), 4.84 (d, *J* = 5.1 Hz, 2H), 2.76 (t, *J* = 7.4 Hz, 2H), 2.10 – 1.98 (m, 5H), 1.76 – 1.72 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.13, 149.86, 140.04, 135.33, 132.21, 128.20, 127.64, 124.96, 122.19, 122.11, 120.66, 118.41, 111.64, 110.60, 102.69, 82.67, 69.19, 50.27, 32.34, 29.72, 29.48, 27.57, 13.22. HRMS (ESI-TOF) calcd for $C_{24}H_{23}N_6O$, 411.1928 ($M+H^+$), found 411.1938.



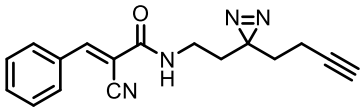
FFF-22

3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)-N-(((2S,3R)-1-(4-methoxybenzyl)-3-methylpiperidin-2-yl)methyl)propanamide (FFF-22): Synthesized according to general procedure 2. The residue obtained was purified by biotage (Hexanes/EtOAc, 8:2) to afford FFF-22 as a colorless liquid (13 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.2 Hz, 2H), 6.92 – 6.86 (m, 2H), 6.24 – 6.11 (m, 1H), 3.82 (s, 3H), 3.76 (s, 2H), 3.43 – 3.31 (m, 1H), 3.14 – 3.09 (m, 1H), 2.79 – 2.69 (m, 1H), 2.67 – 2.50 (m, 2H), 2.12 – 2.06 (m, 1H), 2.06 – 1.97 (m, 3H), 1.92 – 1.72 (m, 5H), 1.68 – 1.56 (m, 3H), 1.37 – 1.27 (m, 2H), 0.86 – 0.79 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.96, 129.86, 113.91, 82.72, 69.19, 56.71, 55.30, 55.29, 45.11, 34.31, 32.36, 30.53, 29.70, 28.57, 27.90, 27.01, 20.49, 18.17, 13.31. HRMS (ESI-TOF) *calcd for* $C_{23}H_{33}N_4O_2$, 397.5425 ($M+H^+$), *found* 397.2549.



FFF-23

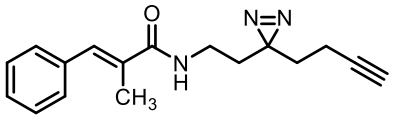
N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-7-nitro-1H-indole-2-carboxamide (FFF-23): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 6:4) to afford FFF-23 as a white solid (10 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.51 (s, 1H), 8.24 (dd, *J* = 8.1, 1.0 Hz, 1H), 8.01 (d, *J* = 7.8 Hz, 1H), 7.32 – 7.19 (m, 1H), 6.58 (t, *J* = 6.0 Hz, 1H), 3.39 (t, *J* = 6.4 Hz, 2H), 2.13 – 1.99 (m, 3H), 1.88 (t, *J* = 6.6 Hz, 3H), 1.71 (t, *J* = 7.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.41, 133.44, 133.14, 131.24, 130.22, 129.29, 121.60, 120.11, 103.31, 82.80, 69.60, 34.76, 32.55, 32.09, 26.90, 13.20. LCMS *calcd for* $C_{16}H_{15}N_5O_3$, 326.1 ($M+H^+$), *found*: 326.1.



FFF-24

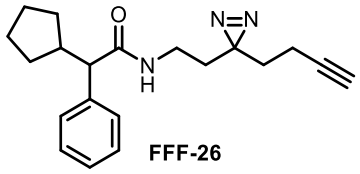
(E)-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-2-cyano-3-phenylacrylamide (FFF-24): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 6:4) to afford FFF-24 as an off white solid (14 mg, 74 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.99 –

7.86 (m, 2H), 7.59 – 7.44 (m, 3H), 6.51 (s, 1H), 3.34 – 3.30 (m, 2H), 2.10 – 1.99 (m, 3H), 1.81 (t, *J* = 6.9 Hz, 2H), 1.74 – 1.64 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.34, 153.19, 132.90, 131.75, 130.70, 129.28, 116.91, 103.76, 82.55, 69.61, 35.50, 32.48, 32.14, 26.66, 13.25. LCMS *calcd for* $C_{17}H_{17}N_4O$, 293.2 ($M+H^+$), *found*: 293.2.



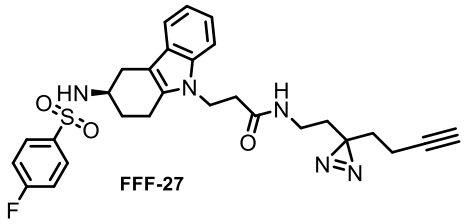
FFF-25

(E)-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-2-methyl-3-phenylacrylamide (FFF-25): Synthesized according to general procedure 2, the crude residue was purified by biotage (Hexanes/EtOAc, 7:3) to afford FFF-25 as a colorless liquid (15 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.28 (m, 6H), 6.01 (s, 1H), 3.27 – 3.24 (m, 2H), 2.14 – 2.10 (m, 3H), 2.10 – 1.98 (m, 3H), 1.80 (t, *J* = 6.6 Hz, 2H), 1.69 (t, *J* = 7.2 Hz, 2H). LCMS *calcd for* $C_{17}H_{19}N_3O$, 281.16 ($M+H^+$), *found*: 281.2.



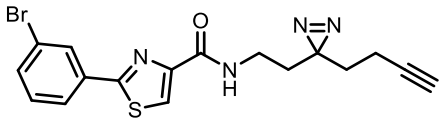
FFF-26

N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-2-cyclopentyl-2-phenylacetamide (FFF-26): Synthesized according to general procedure 2, the crude residue was purified by biotage (Hexanes/EtOAc, 9:1) to afford FFF-26 as a colorless liquid (13 mg, 68%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.34 (m, 2H), 7.34 – 7.30 (m, 2H), 7.28 – 7.23 (m, 1H), 5.65 (s, 1H), 3.13 – 3.03 (m, 2H), 3.02 – 3.00 (m, 1H), 2.63 – 2.59 (m, 1H), 2.03 – 1.95 (m, 2H), 1.93 – 1.89 (m, 2H), 1.70 – 1.58 (m, 5H), 1.56 – 1.51 (m, 2H), 1.49 – 1.42 (m, 1H), 1.28 (d, *J* = 8.5 Hz, 2H), 1.07 – 0.95 (m, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.49, 139.98, 128.59, 128.00, 127.12, 82.73, 69.35, 59.91, 43.17, 34.26, 32.43, 32.07, 31.74, 30.95, 26.77, 25.19, 24.84, 13.16. LCMS *calcd for* $C_{20}H_{26}N_3O$, 324.20 ($M+H^+$), *found*: 324.2.



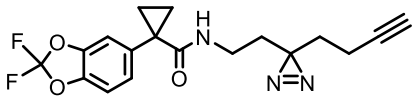
FFF-27

(R)-N-((3-(but-3-yn-1-yl)-3H-diazirin-3-yl)methyl)-3-(3-((4-fluorophenyl)sulfonamido)-1,2,3,4-tetrahydro-9H-carbazol-9-yl)propanamide (FFF-27): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 6:4) to afford FFF-27 as a colorless liquid (15 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.79 (m, 2H), 7.30 – 7.22 (m, 2H), 7.16 – 7.08 (m, 3H), 7.06 – 7.00 (m, 1H), 5.60 (t, *J* = 5.8 Hz, 1H), 5.37 (d, *J* = 8.4 Hz, 1H), 4.40 – 4.32 (m, 1H), 4.25 (t, *J* = 6.6 Hz, 1H), 3.80 (d, *J* = 8.3, Hz, 1H), 3.04 – 2.87 (m, 2H), 2.90 – 2.78 (m, 2H), 2.78 – 2.67 (m, 1H), 2.57 – 2.43 (m, 3H), 2.00 – 1.93 (m, 3H), 1.91 – 1.89 (m, 2H), 1.51 – 1.35 (m, 4H), LCMS *calcd for* $C_{27}H_{29}FN_5O_3S$, 521.2 ($M+H^+$), *found*: 521.2.



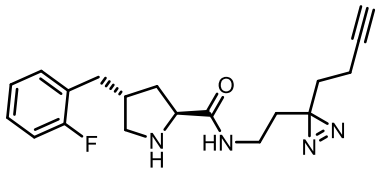
FFF-28

2-(3-bromophenyl)-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)thiazole-4-carboxamide (FFF-28): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 7:3) to afford FFF-28 as an off white solid (12 mg, 71%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (t, *J* = 1.8 Hz, 1H), 8.10 (s, 1H), 7.84 (dd, *J* = 7.8, 1.7, Hz, 1H), 7.57 (dd, *J* = 8.0, 2.0, Hz, 1H), 7.52 (d, *J* = 6.1 Hz, 1H), 7.32 (t, *J* = 7.9 Hz, 1H), 3.35 (t, *J* = 7.0 Hz, 2H), 2.04 (t, *J* = 7.4 Hz, 2H), 2.00 (t, *J* = 2.6 Hz, 1H), 1.82 (t, *J* = 7.0 Hz, 2H), 1.70 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.34, 160.96, 150.75, 134.59, 133.50, 130.61, 129.41, 125.27, 123.55, 123.23, 82.63, 69.45, 34.32, 32.85, 26.86, 13.29. LCMS *calcd for* $C_{17}H_{16}BrN_4OS$, 403.01 ($M+H^+$), *found*: 403.1.



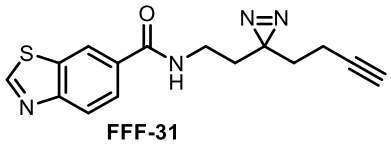
FFF-29

N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-1-carboxamide (FFF-29): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 6:4) to afford FFF-29 as a white solid (15 mg, 78%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 – 7.16 (m, 2H), 7.09 (d, *J* = 8.1 Hz, 1H), 5.39 (d, *J* = 6.2 Hz, 1H), 3.08 (t, *J* = 6.4 Hz, 2H), 2.00 – 1.93 (m, 3H), 1.64 – 1.54 (m, 6H), 1.05 (q, J = 3.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.24, 143.12, 143.51, 135.90, 133.80 (t, *J* = 256.0 Hz), 126.63, 112.50, 109.95, 82.65, 69.34, 35.26, 32.63, 32.13, 30.46, 26.84, 16.14, 16.12, 13.17. HRMS (ESI-TOF) *calcd for* $C_{18}H_{18}F_3N_3O_3$, 362.1311 ($M+H^+$), *found* 362.1312.



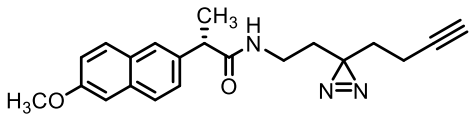
FFF-30

(2S,4R)-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-4-(2-fluorobenzyl)pyrrolidine-2-carboxamide (FFF-30): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 3:7) to afford FFF-30 as a colorless liquid (8 mg, 56%).^{1}H NMR (600 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.17 (d, *J* =7.3 Hz, 2H), 7.05 (d, *J* = 7.3 Hz, 1H), 7.02 – 6.98 (m, 1H), 3.84 – 3.86 (m, 1H), 3.05 – 3.11 (m, 2H), 2.76 – 2.79 (m, 1H), 2.65 – 2.70 (m, 2H), 2.40 – 2.33 (m, 1H), 2.10 – 2.06 (m, 1H), 2.00 – 1.97 (m, 2H), 1.93 – 1.86 (m, 2H), 1.67 – 1.62 (m, 3H), 0.93 – 0.79 (m, 3H). LCMS *calcd for* $C_{19}H_{24}FN_4O_3$, 343.2 ($M+H^+$), *found* 343.2.



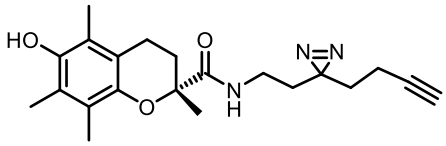
FFF-31

N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)benzo[d]thiazole-6-carboxamide (FFF-31): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 6:4) to afford FFF-31 as a white solid (16 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.42 (dd, *J* = 1.8, 0.6 Hz, 1H), 8.09 (dd, *J* = 8.5, 0.6 Hz, 1H), 7.86 (dd, *J* = 8.5, 1.8 Hz, 1H), 6.75 (t, *J* = 5.9 Hz, 1H), 3.31 (td, *J* = 6.7, 5.8 Hz, 2H), 2.06 – 1.94 (m, 3H), 1.81 (t, *J* = 6.7 Hz, 2H), 1.65 (t, *J* = 7.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.01, 156.67, 155.04, 134.12, 131.80, 124.69, 123.52, 121.69, 82.73, 69.54, 35.18, 32.42, 32.06, 26.98, 13.22. LCMS *calcd for* $C_{15}H_{15}N_4OS$, 298.09 ($M+H^+$), *found*: 298.1.



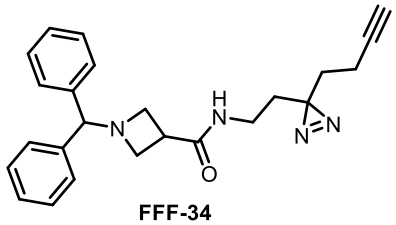
FFF-32

(S)-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-2-(6-methoxynaphthalen-2-yl)propanamide (FFF-32): Synthesized according to general procedure 1. The residue obtained was purified by biotage (Hexanes/EtOAc, 7:3) to afford FFF-32 as a white solid (14 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.65 (m, 3H), 7.39 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.21 – 7.09 (m, 2H), 5.46 (s, 1H), 3.92 (s, 3H), 3.70 (q, *J* = 7.2 Hz, 1H), 3.05 (t, *J* = 6.7 Hz, 2H), 1.93 – 1.80 (m, 3H), 1.61 (d, *J* = 7.2 Hz, 3H), 1.59 – 1.55 (m, 2H), 1.54 – 1.46 (m, 2H). LCMS *calcd for* $C_{21}H_{24}N_3O_2$, 350.2 ($M+H^+$), *found*: 350.2.



FFF-33

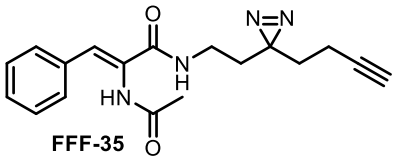
(R)-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (FFF-33): Synthesized according to general procedure 1. The residue obtained was purified by biotage (Hexanes/EtOAc, 6:4) to afford FFF-33 as a white solid (10 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.67 – 6.52 (m, 1H), 4.49 (d, *J* = 7.3 Hz, 1H), 3.11 – 3.07 (m, 2H), 2.71 – 2.49 (m, 2H), 2.42 – 2.36 (m, 1H), 2.24 (s, 3H), 2.18 (s, 3H), 2.10 (s, 3H), 1.95 (t, *J* = 2.6 Hz, 1H), 1.92 – 1.83 (m, 3H), 1.63 – 1.83 (m, 2H), 1.52 (s, 3H), 1.50 – 1.46 (m, 1H). LCMS *calcd for* $C_{21}H_{27}N_3O_3$, 370.2 ($M+H^+$), *found*: 370.2.



FFF-34

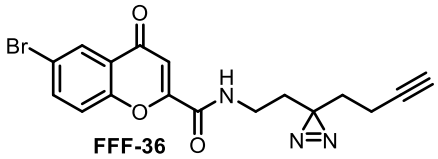
1-benzhydryl-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)azetidine-3-carboxamide (FFF-34): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 5:5) to afford FFF-34 as a colorless liquid (14 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.38 (m, 4H), 7.32 – 7.26 (m, 4H), 7.24 – 7.16 (m, 2H), 6.48 (s, 1H), 4.45 (s, 1H), 3.46 – 3.29 (m, 4H), 3.15 (t, *J* = 6.3 Hz, 2H), 3.07 (q, *J* = 6.5 Hz, 1H), 2.03 (t, *J* = 7.3 Hz, 2H), 1.98 (t, *J* = 2.6 Hz, 1H), 1.75 (t, *J* = 6.6 Hz, 2H), 1.66 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.40, 141.51, 128.55,

127.47, 127.29, 82.65, 77.63, 77.28, 69.49, 56.41, 35.83, 34.18, 32.42, 32.27, 26.91, 13.25. LCMS *calcd for* $C_{24}H_{27}N_4O$, 387.2 ($M+H^+$), *found*: 387.2.



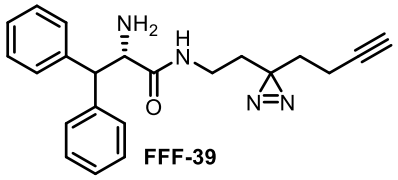
FFF-35

(Z)-2-acetamido-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-3-phenylacrylamide (FFF-35): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 8:2) to afford FFF-35 as an off white solid (12 mg, 71 %). ^{1}H NMR (600 MHz, CD_3OD_SPE) δ 7.52 (dd, *J* = 7.7, 1.7 Hz, 2H), 7.45 – 7.38 (m, 2H), 7.38 – 7.32 (m, 1H), 7.14 (s, 1H), 3.22 (t, *J* = 7.3 Hz, 2H), 2.31 (d, *J* = 2.7, 0.9 Hz, 1H), 2.12 (d, *J* = 0.9 Hz, 3H), 2.07 (tdd, *J* = 7.4, 2.7, 0.9 Hz, 2H), 1.74 – 1.65 (m, 4H). ^{13}C NMR (151 MHz, CD_3OD_SPE) δ 171.94, 166.70, 133.86, 129.17, 129.14, 129.06, 128.70, 128.37, 82.32, 69.06, 48.09, 47.94, 47.80, 47.66, 47.52, 47.38, 47.24, 34.58, 32.07, 31.84, 26.56, 21.36. LCMS *calcd for* $C_{18}H_{21}N_4O_2$, 325.2 ($M+H^+$), *found*: 325.2.



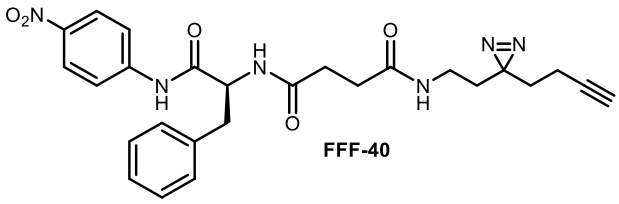
FFF-36

6-bromo-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-4-oxo-4H-chromene-2-carboxamide (FFF-36): Synthesized according to general procedure 1. The residue obtained was purified by biotage (Hexanes/EtOAc, 7:3) to afford FFF-36 as a white solid (12 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, *J* = 2.4 Hz, 1H), 7.84 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.47 (d, *J* = 8.9 Hz, 1H), 7.17 (s, 1H), 6.99 (s, 1H), 3.37 (q, *J* = 6.5 Hz, 2H), 2.12 – 1.99 (m, 3H), 1.88 (t, *J* = 6.7 Hz, 2H), 1.71 (t, *J* = 7.1 Hz, 2H). LCMS *calcd for* $C_{17}H_{14}BrN_3O_3$, 388.02 ($M+H^+$), *found*: 388.02.



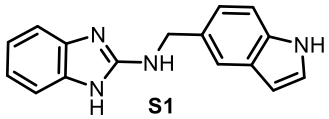
FFF-39

(S)-2-amino-N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-3,3-diphenylpropanamide (FFF-39): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 8:2) to afford FFF-39 as a white solid (8 mg, 58 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.26 (m, 8H), 7.26 – 7.19 (m, 2H), 6.70 (s, 1H), 4.59 (d, *J* = 6.5 Hz, 1H), 4.08 (d, *J* = 6.5 Hz, 1H), 3.05 – 2.88 (m, 2H), 2.01 – 1.90 (m, 3H), 1.53 (t, *J* = 7.4 Hz, 2H), 1.42 (t, *J* = 6.9 Hz, 2H). LCMS *calcd for* $C_{22}H_{25}N_4O$, 361.20 ($M+H^+$), *found*: 361.2.



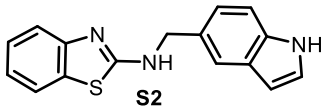
FFF-40

(S)-N1-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-N4-(1-((4-nitrophenyl)amino)-1-oxo-3-phenylpropan-2-yl)succinamide (FFF-40): Synthesized according to general procedure 2, purified by biotage (Hexanes/EtOAc, 4:6) to afford FFF-40 as a white solid (8 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.24 – 8.14 (m, 2H), 7.97 – 7.88 (m, 2H), 7.37 – 7.28 (m, 3H), 7.27 – 7.22 (m, 3H), 6.20 – 6.16 (m, 1H), 5.74 – 5.68 (m, 1H), 4.94 – 4.88 (m, 1H), 3.38 – 3.18 (m, 2H), 3.08 – 2.96 (m, 2H), 2.91 – 2.76 (m, 1H), 2.42 – 2.37 (m, 2H), 2.05 – 1.94 (m, 3H), 1.71 – 1.67 (m, 2H), 1.63 – 1.55 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.89, 172.39, 170.31, 144.10, 143.46, 136.27, 129.19, 128.90, 127.27, 124.75, 119.77, 82.66, 69.65, 54.86, 36.96, 34.60, 32.27, 32.05, 31.49, 31.11, 26.72, 13.17. LCMS *calcd for* $C_{26}H_{29}N_6O_5$, 505.2 ($M+H^+$), *found*: 505.2.



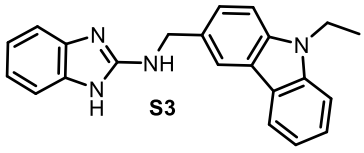
S1

N-((1H-indol-5-yl)methyl)-1H-benzo[d]imidazol-2-amine: Synthesized according to scheme 1 purified on Biotage® with 0 to 7 % methanol gradient in dichloromethane to afford S1 as an light brown solid (450 mg, 62 %). ^{1}H NMR (400 MHz, DMSO) δ 11.02 (s, 1H), 7.53 (d, *J* = 1.6 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 1H), 7.30 (t, *J* = 2.7 Hz, 1H), 7.15 – 7.09 (m, 3H), 7.05 (t, *J* = 6.1 Hz, 1H), 6.85 (dd, *J* = 5.8, 3.2 Hz, 2H), 6.37 (t, *J* = 2.6 Hz, 1H), 4.56 (d, J = 5.9 Hz, 2H). HRMS (ESI-TOF) calcd for $C_{16}H_{15}N_4$, 263.1291 ($M+H^+$), found 263.1287.



S2

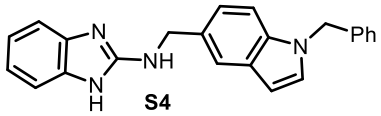
N-((1H-indol-5-yl)methyl)benzo[d]thiazol-2-amine (S2): To a solution of 2-Aminobenzothiazole (1.0 eq.) and 5-Formylindole (1.0 eq.) in dry ethanol, sodium triacetoxyborohydride (2.0 eq.) and acetic acid (2.0 eq.) was added at 0°C to the solution and resulting mixture was stirred for 14 h at room temperature. After completion (monitored by TLC) the solvent was removed by rotary evaporation, crude mixture was diluted with water and saturated aqueous $NaHCO_3$ solution, extracted in ethyl acetate, the combined extracts was dried over Na_2SO_4, filtered and concentrated in vacuum, purified by flash column chromatography on Biotage® isolera one with 5-70 % ethyl acetate gradient in hexane to obtain corresponding N-((1H-indol-5-yl)methyl)benzo[d]thiazol-2-amine (**S2**) as a light brown solid (12 mg, 22 %). ^{1}H NMR (400 MHz, CD_3OD_SPE) δ 7.58 – 7.53 (m, 2H), 7.43 (dd, *J* = 8.1, 1.2, Hz, 1H), 7.36 (dd, *J* = 8.3, 0.9 Hz, 1H), 7.27 – 7.19 (m, 2H), 7.14 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.03 (td, *J* = 7.6, 1.2 Hz, 1H), 6.41 (d, *J* = 3.1, Hz, 1H), 4.66 (s, 2H). HRMS (ESI-TOF) calcd for $C_{16}H_{14}N_3S$, 280.0903 ($M+H^+$), found 280.0901.



S3

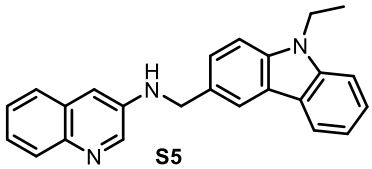
N-((9-ethyl-9H-carbazol-3-yl)methyl)-1H-benzo[d]imidazol-2-amine (S3): Synthesized according to scheme 1 purified on Biotage® with 0 to 6 % methanol gradient in dichloromethane to afford S3 as an light yellow solid (210 mg, 72 %). ^{1}H NMR (400 MHz, DMSO) δ 11.00 (s, 1H), 8.15 (dd, *J* = 1.7, 0.7 Hz, 1H), 8.10 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.62 – 7.55 (m, 2H), 7.51 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.43 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.27 (s, 1H), 7.21 – 7.11 (m, 3H), 6.92 – 6.86 (m,

2H), 4.67 (d, *J* = 5.4 Hz, 2H), 4.42 (t, *J* = 7.1 Hz, 2H), 1.28 (t, *J* = 7.1 Hz, 3H). HRMS (ESI-TOF) calcd for $C_{22}H_{21}N_4$, 341.1761 ($M+H^+$), found 341.1762.



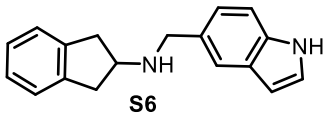
S4

N-((1-benzyl-1H-indol-5-yl)methyl)-1H-benzo[d]imidazol-2-amine (S4): Synthesized according to scheme 1 purified on Biotage® with 0 to 6 % methanol gradient in dichloromethane to afford S4 as an light yellow solid (85 mg, 62 %). ^{1}H NMR (600 MHz, DMSO) δ 7.58 (s, 1H), 7.48 – 7.46 (s, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.30 – 7.24 (m, 2H), 7.24 – 7.18 (m, 2H), 7.18 – 7.14 (m, 5H), 6.92 – 6.86 (m, 2H), 6.46 (d, *J* = 3.2 Hz, 1H), 5.40 (s, 2H), 4.59 (s, 2H). ^{13}C NMR (151 MHz, DMSO) δ 155.74, 138.80, 135.49, 131.06, 129.91, 128.97, 128.72, 127.74, 127.35, 121.76, 119.77, 119.59, 112.05, 110.47, 101.37, 49.63, 46.68, 40.40, 40.22, 40.12, 39.94, 39.84, 39.71, 39.57. HRMS (ESI-TOF) calcd for $C_{23}H_{21}N_4$, 353.1761 ($M+H^+$), found 353.1750.



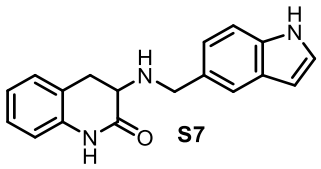
S5

N-((9-ethyl-9H-carbazol-3-yl)methyl)quinolin-3-amine (S5): Synthesized according to scheme 1 purified on Biotage® with 0 to 6 % methanol gradient in dichloromethane to afford S5 as an brown solid (90 mg, 58 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (dd, J = 2.8, 1.0 Hz, 1H), 8.20 – 8.08 (m, 2H), 8.04 – 7.98 (m, 1H), 7.62 (dd, *J* = 6.3, 3.3 Hz, 1H), 7.52 (dd, *J* = 7.0, 1.4 Hz, 2H), 7.48 – 7.42 (m, 4H), 7.27 (dd, *J* = 7.0, 1.8, Hz, 1H), 7.14 (d, *J* = 2.7 Hz, 1H), 4.59 (d, *J* = 3.6 Hz, 2H), 4.47 (s, 1H), 4.43 – 4.33 (m, 2H), 1.46 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 143.46, 143.45, 142.18, 141.63, 140.34, 139.53, 129.57, 129.03, 128.48, 128.46, 126.91, 126.07, 125.92, 125.56, 124.92, 123.26, 122.65, 120.52, 119.76, 118.97, 110.38, 108.76, 108.62, 48.53, 37.64, 13.84. HRMS (ESI-TOF) calcd for $C_{24}H_{22}N_3$, 352.1804 ($M+H^+$), found 352.1808.



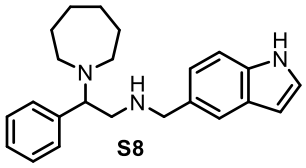
S6

N-((1H-indol-5-yl)methyl)-2,3-dihydro-1H-inden-2-amine (S6): Synthesized according to scheme 1 general procedure 1 purified on Biotage® with 0 to 6 % methanol gradient in dichloromethane to afford S6 as an brown solid (90 mg, 58 %). ^{1}H NMR (400 MHz, CDCl3) δ 8.65 (s, 1H), 7.59 – 7.51 (m, 1H), 7.20 – 7.14 (m, 3H), 7.14 – 7.08 (m, 3H), 7.04 – 6.98 (m, 1H), 6.48 – 6.42 (m, 1H), 3.92 (s, 2H), 3.74 – 3.68 (m, 1H), 3.18 – 3.14 (m, 2H), 2.84 –2.78 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.93, 135.21, 131.41, 128.10, 126.51, 124.84, 124.81, 122.73, 120.30, 111.28, 102.26, 58.85, 52.89, 40.11. HRMS (ESI-TOF) calcd for $C_{18}H_{19}N_2$, 263.1543 ($M+H^+$), found 263.1535.



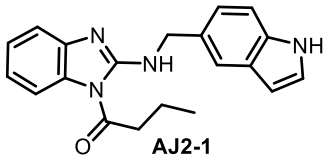
S7

3-(((1H-indol-5-yl)methyl)amino)-3,4-dihydroquinolin-2(1H)-one (S7): Synthesized according to scheme 3, purified on Biotage® with 5 to 80 % ethyl acetate gradient in hexane to afford S7 as an off white solid (90 mg, 58 %). ^{1}H NMR (400 MHz, CD_3OD_SPE) δ 7.53 (d, *J* = 1.6 Hz, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 7.20 (d, *J* = 3.1 Hz, 1H), 7.13 (td, J = 7.8, 2.8 Hz, 3H), 6.94 (dd, *J* = 7.5, 1.2 Hz, 1H), 6.87 – 6.79 (m, 1H), 6.40 (dd, *J* = 3.2, 0.9 Hz, 1H), 4.01 (d, *J* = 12.6 Hz, 1H), 3.87 (d, *J* = 12.6 Hz, 1H), 3.40 (dd, *J* = 13.4, 6.2 Hz, 1H), 3.14 (dd, *J* = 15.3, 6.3 Hz, 1H), 2.84 – 2.78 (m, 1H). ^{13}C NMR (101 MHz, CD3OD_SPE) δ 171.47, 136.80, 135.72, 129.02, 128.21, 128.00, 127.37, 124.72, 122.79, 122.53, 121.70, 119.90, 114.98, 110.98, 100.95, 54.50, 51.37, 30.95. HRMS (ESI-TOF) calcd for $C_{18}H_{19}N_3O$, 292.1445 ($M+H^+$), found 292.1446.



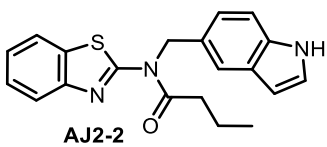
S8

N-((1H-indol-5-yl)methyl)-2-(azepan-1-yl)-2-phenylethan-1-amine (S8): Synthesized according to scheme 3, and general procedure 2 purified on Biotage® with 0 to 6 % methanol gradient in dichloromethane to afford S8 as an off colorless liquid (90 mg, 58 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.47 (d, *J* = 1.6 Hz, 1H), 7.24 – 7.14 (m, 4H), 7.09 – 7.01 (m, 4H), 6.40 (s, 1H), 4.59 (s, 1H), 3.97 – 3.94 (m, 1H), 3.90 – 3.76 (m, 2H), 3.10 – 3.04 (m, 1H), 2.82 – 2.78 (m, 1H), 2.57 – 2.51 (m, 2H), 2.42 – 2.28 (m, 2H), 2.00 – 1.96 (m, 1H), 1.53 – 1.44 (m, 2H), 1.40 (d, *J* = 2.9 Hz, 5H). LCMS calcd for $C_{23}H_{30}N_3$, 348.2 ($M+H^+$), found 348.2.



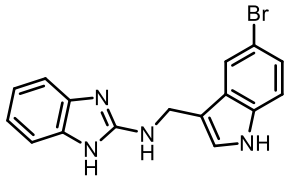
AJ2-1

1-(2-(((1H-indol-5-yl)methyl)amino)-1H-benzo[d]imidazol-1-yl)butan-1-one (AJ2-1): Synthesized according to scheme 1 general procedure 1 and following general procedure 4, purified by Biotage® with hexane : ethyl acetate, (6:4) to afford AJ2-1 as an off white solid (17 mg, 62 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 8.16 (s, 1H), 7.70 – 7.66 (m, 1H), 7.47 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.38 (dd, *J* = 8.4, 1.7 Hz, 2H), 7.26 – 7.20 (m, 4H), 7.06 (dd, *J* = 7.5, 1.3 Hz, 1H), 6.53 – 6.55 (m, 1H), 4.85 (d, *J* = 5.2 Hz, 2H), 2.99 (t, *J* = 7.2 Hz, 2H), 1.84 (p, *J* = 7.3 Hz, 2H), 1.08 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.57, 155.01, 143.89, 135.34, 130.14, 129.31, 128.08, 124.85, 124.74, 122.41, 120.21, 120.19, 117.13, 113.05, 111.33, 102.69, 47.60, 40.33, 17.27, 13.62. HRMS (ESI-TOF) calcd for $C_{20}H_{21}N_4O$, 333.1710 ($M+H^+$), found 333.1715.



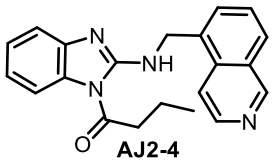
AJ2-2

N-((1H-indol-5-yl)methyl)-N-(benzo[d]thiazol-2-yl)butyramide (AJ2-2): Synthesized according to scheme 1 general procedure 1 and following general procedure 2, purified by PTLC with hexane : ethyl acetate, (7:3) to afford AJ2-2 as brown solid (8 mg, 62 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.84 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.79 (dt, *J* = 8.2, 0.9 Hz, 1H), 7.48 – 7.45 (m, 1H), 7.40 (dd, *J* = 7.2, 1.3 Hz, 1H), 7.36 – 7.27 (m, 2H), 7.24 – 7.19 (m, 1H), 7.09 (dd, *J* = 8.5, 1.8 Hz, 1H), 6.48 (d, *J* = 2.0, 1H), 5.74 (s, 2H), 2.62 (t, *J* = 7.3 Hz, 2H), 1.72 (q, *J* = 7.4 Hz, 2H), 0.92 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 173.98, 159.69, 147.59, 135.12, 132.74, 127.74, 126.99, 126.05, 125.92, 123.78, 121.47, 120.90, 119.35, 117.03, 111.77, 100.99, 50.22, 35.73, 17.35, 13.43. HRMS (ESI-TOF) calcd for $C_{20}H_{20}N_3OS$, 350.1322 ($M+H^+$), found 350.1329.



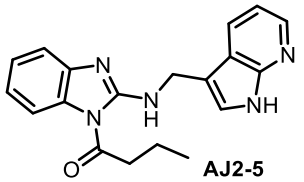
AJ2-3A

N-((5-bromo-1H-indol-3-yl)methyl)-1H-benzo[d]imidazol-2-amine (AJ2-3A): Synthesized according to scheme 1, general procedure 1 purified on Biotage® with dichloromethane : methanol (9:1) to afford AJ2-3A as light brown solid (160 mg, 64 %); ^{1}H NMR (400 MHz, CD_3OD) δ 7.74 (s, 1H), 7.28 (s, 1H), 7.23 (dd, *J* = 8.6, 0.6 Hz, 1H), 7.20 – 7.12 (m, 3H), 6.94 (dd, *J* = 5.8, 3.2 Hz, 2H), 4.64 (d, *J* = 0.8 Hz, 2H). ^{13}C NMR (151 MHz, CD_3OD_SPE) δ 156.77, 136.92, 129.81, 126.02, 125.37, 122.27, 121.36, 114.00, 113.41, 113.19, 112.77, 39.38. HRMS (ESI-TOF) calcd for $C_{16}H_{14}BrN_4$, 341.0397 ($M+H^+$), found 341.0396.



AJ2-4

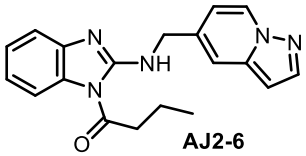
1-(2-((isoquinolin-5-ylmethyl)amino)-1H-benzo[d]imidazol-1-yl)butan-1-one (AJ2-4): Synthesized according to scheme 1, general procedure 1 and following general procedure 4, purified on Biotage® with Hexane : Ethyl acetate (6:4) to afford AJ2-4 as light brown solid (7 mg, 54 %) ^{1}H NMR (400 MHz, CD_3OD) δ 9.17 (s, 1H), 8.40 (d, *J* = 7.1 Hz, 1H), 7.96 (d, *J* = 7.2, 1.6 Hz, 2H), 7.76 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.62 – 7.54 (m, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 7.24 – 7.20 (m, 1H), 7.12 (dd, *J* = 7.7, 1.0 Hz, 1H), 7.07 – 6.99 (m, 1H), 5.09 (s, 2H), 3.00 (t, *J* = 7.1 Hz, 2H), 1.73 (q, *J* = 7.3 Hz, 2H), 0.98 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.81, 154.76, 153.16, 143.67, 134.32, 132.84, 130.23, 130.17, 128.98, 128.01, 127.77, 127.35, 126.92, 125.05, 120.66, 117.37, 116.56, 113.17, 56.00, 44.15, 40.33, 17.21, 13.59. HRMS (ESI-TOF) calcd for $C_{21}H_{21}N_4O$, 345.1710 ($M+H^+$), found 345.1720.



AJ2-5

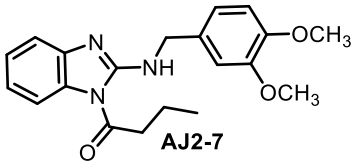
1-(2-(((1H-pyrrolo[2,3-b]pyridin-3-yl)methyl)amino)-1H-benzo[d]imidazol-1-yl)butan-1-one (AJ2-5): Synthesized according to scheme 1 general procedure 1 and following procedure 4,

purified on Biotage® with solvent Hexane : Ethyl acetate (6:4) to afford AJ2-5 as light brown solid to afford AJ2-5 as brown solid (12 mg, 62 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.42 (s, 1H), 8.33 (d, *J* = 1.5 Hz, 1H), 8.12 – 8.07 (m, 1H), 8.05 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.50 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.44 – 7.38 (m, 2H), 7.30 – 7.24 (m, 2H), 7.13 – 7.05 (m, 2H), 4.93 (d, *J* = 5.1Hz, 2H), 2.98 (t, *J* = 7.2 Hz, 2H), 1.83 (q, *J* = 7.3 Hz, 2H), 1.07 (t, *J* = 7.4 Hz, 3H). HRMS (ESI-TOF) calcd for $C_{19}H_{20}N_5O$, 334.1663 ($M+H^+$), found 334.1676.



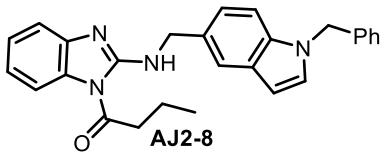
AJ2-6

1-(2-((pyrazolo[1,5-a]pyridin-5-ylmethyl)amino)-1H-benzo[d]imidazol-1-yl)butan-1-one (AJ2-6): Synthesized according to scheme 1 general procedure 1 and following general procedure 4, purified on Biotage® with hexane : ethyl acetate (6:4) to afford AJ2-6 as a brown solid (6 mg, 52 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 7.2 Hz, 1H), 8.34 (s, 1H), 7.95 (d, *J* = 2.3 Hz, 1H), 7.55 (s, 1H), 7.44 – 7.39 (m, 2H), 7.30 – 7.24 (m, 2H), 7.16 – 7.06 (m, 1H), 6.82 (dd, *J* = 7.2, 2.0 Hz, 1H), 6.49 (d, *J* = 2.3 Hz, 1H), 4.83 (d, *J* = 5.9 Hz, 2H), 3.06 (t, *J* = 7.2 Hz, 2H), 1.92 (q, *J* = 7.3 Hz, 2H), 1.14 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.89, 154.97, 143.45, 142.30, 139.95, 134.11, 130.18, 128.72, 125.02, 120.66, 117.39, 115.65, 113.14, 111.75, 96.82, 45.91, 40.36, 17.28, 13.62. HRMS (ESI-TOF) calcd for $C_{19}H_{20}N_5O$, 334.1663 ($M+H^+$), found 334.1676.



AJ2-7

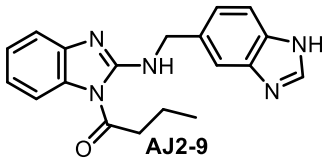
1-(2-((3,4-dimethoxybenzyl)amino)-1H-benzo[d]imidazol-1-yl)butan-1-one (AJ2-7): Synthesized according to scheme 1, general procedure 1 and following general procedure 4, purified on Biotage® with hexane : ethyl acetate (6:4) to afford AJ2-7 as a brown solid (12 mg, 72 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 – 8.13 (m, 1H), 7.45 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.41 – 7.35 (m, 1H), 7.24 (dd, *J* = 7.7, 1.0 Hz, 1H), 7.06 (dd, *J* = 8.2, 1.3 Hz, 1H), 6.98 – 6.93 (m, 2H), 6.87 – 6.81 (m, 1H), 4.70 (d, *J* = 5.4 Hz, 2H), 3.88 (s, 3H), 3.87 (s, 3H), 2.99 (t, *J* = 7.2 Hz, 2H), 1.88 – 1.84 (m, 2H), 1.09 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.68, 154.95, 149.13, 148.54, 143.67, 130.58, 130.10, 124.92, 120.36, 120.16, 117.15, 113.08, 111.31, 111.25, 55.96, 55.91, 46.79, 40.33, 17.26, 13.62. HRMS (ESI-TOF) calcd for $C_{20}H_{24}N_3O_3$, 354.1812 ($M+H^+$), found 354.1822.



AJ2-8

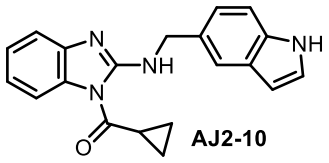
1-(2-(((1-benzyl-1H-indol-5-yl)methyl)amino)-1H-benzo[d]imidazol-1-yl)butan-1-one (AJ2-8): Synthesized according to scheme 1, general procedure 1 and following general procedure 4, purified on Biotage® with hexane : ethyl acetate (6:4) to afford AJ2-8 as an off white solid (14 mg, 74 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.71 – 7.65 (m, 1H), 7.46 (dd, *J* = 7.9, 1.2 Hz,

1H), 7.38 (d, *J* = 8.1 Hz, 1H), 7.33 – 7.27 (m, 3H), 7.25 – 7.23 (m, 2H), 7.17 – 7.14 (m, 1H), 7.13 – 7.09 (m, 1H), 7.09 – 7.02 (m, 2H), 6.56 – 6.51 (m, 1H), 5.32 (s, 2H), 4.84 (d, *J* = 5.1 Hz, 2H), 2.98 (t, *J* = 7.2 Hz, 2H), 1.87 – 1.84 (m, 2H), 1.08 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.56, 154.98, 143.89, 137.46, 135.88, 130.13, 128.83, 128.80, 127.64, 126.77, 126.72, 124.85, 122.15, 121.45, 120.51, 120.18, 119.89, 117.14, 113.03, 110.38, 110.03, 101.74, 50.22, 47.59, 40.33, 17.26, 13.62. HRMS (ESI-TOF) calcd for $C_{27}H_{27}N_4O$, 423.2180 ($M+H^+$), found 423.2176.



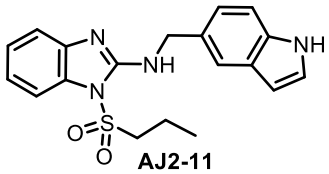
AJ2-9

1-(2-(((1H-benzo[d]imidazol-5-yl)methyl)amino)-1H-benzo[d]imidazol-1-yl)butan-1-one (AJ2-9): Synthesized according to scheme 1, general procedure 1 and following general procedure 4, purified by PTLC with dichloromethane : methanol (9.5:0.5) to afford AJ2-9 as off white solid (6 mg, 48 %) ^{1}H NMR (400 MHz, MeOD) δ 8.05 (s, 1H), 7.57 (d, *J* = 1.5 Hz, 1H), 7.50 (d, *J* = 8.3 Hz, 1H), 7.24 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.15 – 7.12 (m, 3H), 6.96 – 6.89 (m, 2H), 4.62 (s, 2H), 2.12 (t, *J* = 7.4 Hz, 2H), 1.56 – 1.47 (m, 2H), 0.84 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 178.06, 154.19, 141.60, 135.66, 133.20, 122.19, 120.83, 120.72, 114.94, 111.28, 37.01, 29.36, 18.59, 12.75. HRMS (ESI-TOF) calcd for $C_{19}H_{20}N_5O$, 334.1663 ($M+H^+$), found 334.1674.



AJ2-10

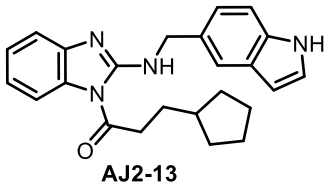
(2-(((1H-indol-5-yl)methyl)amino)-1H-benzo[d]imidazol-1-yl)(cyclopropyl)methanone (AJ2-10): Synthesized according to scheme 1, general procedure 1 and following general procedure 4, purified by PTLC with hexane : ethyl acetate (6:4) to afford AJ2-10 as off white solid (11 mg, 54 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.68 (t, *J* = 5.2 Hz, 1H), 7.58 (s, 1H), 7.51 (d, *J* = 8.1 Hz, 1H), 7.40 (d, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 1H), 7.17 – 7.13 (m, 4H), 6.98 (t, *J* = 7.8 Hz, 1H), 6.44 (d, *J* = 3.2 Hz, 1H), 4.75 (d, *J* = 4.6 Hz, 2H), 2.41 (d, *J* = 4.6 Hz, 1H), 1.32 – 1.24 (m, 2H), 1.15 – 1.11 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.96, 154.41, 143.92, 135.36, 130.69, 129.28, 129.26, 128.07, 124.78, 124.73, 124.61, 122.32, 120.14, 117.07, 112.78, 111.36, 111.31, 102.62, 102.56, 47.56, 16.72, 10.26. HRMS (ESI-TOF) calcd for $C_{20}H_{19}N_4O$, 331.1554 ($M+H^+$), found 331.1554.



AJ2-11

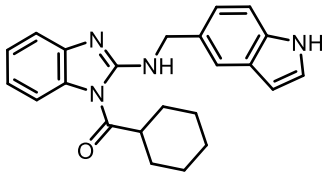
N-((1H-indol-5-yl)methyl)-1-(propylsulfonyl)-1H-benzo[d]imidazol-2-amine (AJ2-11): Synthesized according to scheme 1, general procedure 1 and following general procedure 4 with 1-Propanesulfonyl chloride, purified by PTLC (Hexane/Ethyl acetate 7:3) to afford AJ2-11 as an off white solid (5 mg, 43 %) ^{1}H NMR (400 MHz, DMSO) δ 11.05 (s, 1H), 7.56 (d, *J* = 1.6 Hz, 1H),

7.54 – 7.48 (m, 1H), 7.36 (d, *J* = 8.3 Hz, 1H), 7.34 – 7.28 (m, 2H), 7.22 – 7.14 (m, 2H), 7.09 (t, *J* = 5.9 Hz, 1H), 7.05 (td, *J* = 7.7, 1.2 Hz, 1H), 6.42 – 6.38 (m, 1H), 4.69 (d, *J* = 5.8 Hz, 2H), 3.65 – 3.56 (m, 2H), 1.60 – 1.48 (m, 2H), 0.82 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 152.60, 142.79, 135.63, 131.68, 129.76, 128.01, 126.12, 124.89, 121.49, 121.13, 119.41, 116.76, 112.22, 111.76, 101.42, 54.76, 47.20, 16.88, 12.49. HRMS (ESI-TOF) calcd for $C_{19}H_{21}N_4O_2S$, 369.1380 ($M+H^+$), found 369.1381.



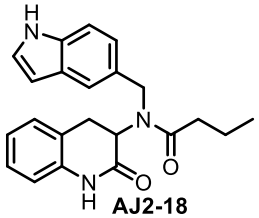
AJ2-13

1-(2-(((1H-indol-5-yl)methyl)amino)-1H-benzo[d]imidazol-1-yl)-3-cyclopentylpropan-1-one (AJ2-13): Synthesized according to scheme 1, general procedure 1 and following general procedure 4, purified by PTLC with hexane : ethyl acetate (7:3) to afford AJ2-13 as off white solid (8 mg, 47 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 8.17 – 8.14 (m, 1H), 7.69 – 7.66 (m, 1H), 7.47 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.42 – 7.35 (m, 2H), 7.25 – 7.17 (m, 4H), 7.10 – 7.04 (m, 2H), 6.54 (d, *J* = 2.0 Hz, 1H), 4.84 (d, *J* = 5.1 Hz, 2H), 3.08 – 2.97 (m, 2H), 2.37 (s, 2H), 1.85 – 1.77 (m, 4H), 1.69 – 1.63 (m, 5H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.39, 135.65, 134.62, 127.96, 127.89, 120.49, 120.05, 116.70, 109.02, 108.80, 65.90, 48.02, 37.63, 36.04, 34.97, 30.05, 28.04, 19.58, 15.51, 15.30. HRMS (ESI-TOF) calcd for $C_{24}H_{27}N_4O$, 387.2180 ($M+H^+$), found 387.2186.



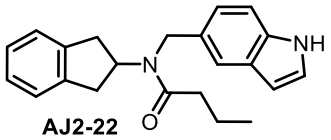
AJ2-14

(2-(((1H-indol-5-yl)methyl)amino)-1H-benzo[d]imidazol-1-yl)(cyclohexyl)methanone (AJ2-14): Synthesized according to scheme 1, general procedure 1 and following general procedure 4, purified by PTLC with hexane : ethyl acetate (6:4) to afford AJ2-14 as off white solid (6 mg, 47 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.17 – 8.14 (m, 1H), 7.58 (d, *J* = 1.6 Hz, 1H), 7.38 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.27 (d, *J* = 8.3 Hz, 1H), 7.22 – 7.13 (m, 4H), 7.04 – 6.96 (m, 2H), 6.49 – 6.42 (m, 1H), 4.74 (d, *J* = 5.1 Hz, 2H), 3.12 – 3.07 (m, 1H), 2.02 – 1.93 (m, 2H), 1.87 – 1.81 (m, 2H), 1.75 – 1.65 (m, 2H), 1.57 – 1.50 (m, 2H), 1.39 – 1.34 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.08, 155.28, 143.83, 135.37, 129.83, 129.18, 128.08, 124.87, 124.78, 122.35, 120.39, 120.17, 117.12, 112.82, 111.36, 102.62, 47.67, 44.79, 29.04, 28.73, 25.63, 25.47, 25.42. HRMS (ESI-TOF) calcd for $C_{23}H_{25}N_4O$, 373.2023 ($M+H^+$), found 373.2035.



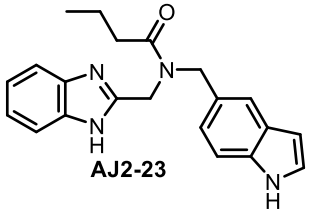
AJ2-18

N-((1H-indol-5-yl)methyl)-N-(2-oxo-1,2,3,4-tetrahydroquinolin-3-yl)butyramide (AJ2-18): Synthesized according to scheme 2 and general procedure 4, purified on Biotage® with hexane : ethyl acetate (5:5) to afford AJ2-18 as off white solid (14 mg, 62 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.31 (s, 1H), 7.48 (d, *J* = 1.6 Hz, 1H), 7.32 (d, *J* = 8.3 Hz, 1H), 7.19 – 7.13 (m, 1H), 7.02 (dd, *J* = 8.3, 1.7 Hz, 2H), 6.87 (d, *J* = 7.6 Hz, 1H), 6.82 – 6.76 (m, 1H), 6.62 (dd, *J* = 7.9, 1.1 Hz, 1H), 6.49 – 6.42 (m, 1H), 5.00 – 4.85 (m, 1H), 4.77 (d, *J* = 17.1 Hz, 1H), 4.63 (d, *J* = 17.0 Hz, 1H), 3.34 (t, *J* = 14.8 Hz, 1H), 2.69 (dd, *J* = 15.3, 6.7 Hz, 1H), 2.51 – 2.32 (m, 2H), 1.71 – 1.67 (m, 2H), 0.88 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.69, 169.14, 136.32, 135.33, 128.58, 128.32, 128.13, 127.62, 125.12, 122.95, 122.50, 120.65, 118.54, 115.14, 111.61, 102.50, 55.30, 51.88, 35.64, 30.32, 18.75, 13.90. HRMS (ESI-TOF) calcd for $C_{22}H_{23}N_3NaO_2$, 384.1682 ($M+Na^+$), found 384.1697.



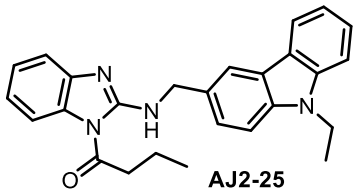
AJ2-22

N-((1H-indol-5-yl)methyl)-N-(2,3-dihydro-1H-inden-2-yl)butyramide (AJ2-22): Synthesized according to scheme 1, general procedure 1 and following and general procedure 4, purified on Biotage® with hexane : ethyl acetate (6:4) to afford AJ2-22 as brown viscous liquid (17 mg, 68 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.55 (s, 0.39 H), 7.41 (d, *J* = 1.8 Hz, 1H), 7.39 – 7.32 (m, 1.61 H), 7.26 (d, *J* = 8.4 Hz, 0.5 H), 7.22 – 7.17 (m, 1.12 H), 7.17 – 7.12 (m, 1.93 H), 7.00 (d, *J* = 8.4 Hz, 0.45 H), 6.95 – 6.93 (dd, *J* = 8.4, 1.9 Hz, 1.19 H), 6.50 (t, *J* = 2.7 Hz, 1.10 H), 6.45 (s, 0.42 H), 5.58 – 5.50 (m, 1.14 H), 4.90 (t, *J* = 8.2 Hz, 0.43 H), 4.74 (s, 0.89 H), 4.64 (s, 2.20 H), 3.17 – 2.93 (m, 6.63 H), 2.57 (t, *J* = 7.6 Hz, 0.93 H), 2.32 (t, *J* = 7.5 Hz, 2.28 H), 1.83 (q, *J* = 7.5 Hz, 1.01H), 1.74 – 1.65 (m, 2.49 H), 1.04 (t, *J* = 7.4 Hz, 1.43 H), 0.89 (t, *J* = 7.4 Hz, 3.53H). ^{13}C NMR (151 MHz, DMSO) δ 173.33, 172.89, 141.56, 141.13, 135.53, 135.29, 130.38, 129.33, 128.30, 128.07, 126.93, 126.75, 126.22, 125.83, 124.78, 124.75, 120.51, 119.71, 117.97, 117.38, 112.08, 111.54, 101.47, 101.30, 58.09, 56.00, 55.38, 48.80, 45.07, 36.88, 36.15, 35.58, 35.31, 19.11, 18.68, 14.37, 14.20. Note: rotomeric isomers observed. HRMS (ESI-TOF) calcd for $C_{22}H_{25}N_2O$, 333.1962 ($M+H^+$), found 333.1960.



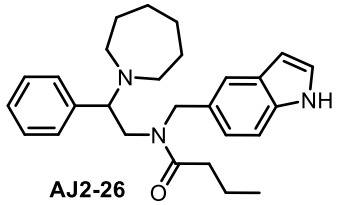
AJ2-23

N-((1H-benzo[d]imidazol-2-yl)methyl)-N-((1H-indol-5-yl)methyl)butyramide (AJ2-23): Synthesized according to scheme 1, general procedure 1 and following general procedure 4, purified by PTLC with hexane : ethyl acetate (6:4) to afford AJ2-23 as viscous liquid (13 mg, 57 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.50 (s, 1H), 9.17 (s, 1H), 7.79 – 7.69 (m, 1H), 7.47 – 7.39 (m, 2H), 7.28 – 7.23 (m, 4H), 6.89 (dd, *J* = 8.3, 1.7 Hz, 1H), 6.53 – 6.47 (m, 1H), 4.70 (s, 2H), 4.69 (s, 2H), 2.48 (t, *J* = 7.5 Hz, 2H), 1.74 – 1.73 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.42, 151.21, 135.50, 128.25, 126.69, 125.16, 120.85, 118.71, 111.72, 102.48, 52.27, 44.34, 35.10, 18.73, 13.95. HRMS (ESI-TOF) calcd for $C_{21}H_{23}N_4O$, 347.1867 ($M+H^+$), found 347.1853.



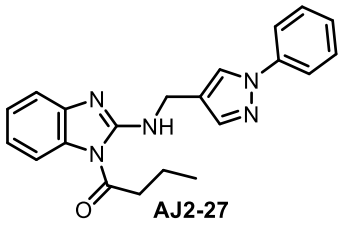
AJ2-25

1-(2-(((9-ethyl-9H-carbazol-3-yl)methyl)amino)-1H-benzo[d]imidazol-1-yl)butan-1-one (AJ2-25): Synthesized according to scheme 1, general procedure 1 and following general procedure 4, purified on Biotage® with hexane : ethyl acetate (6:4) to afford AJ2-25 as a yellow solid (16 mg, 68 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 8.14 (s, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.54 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.51 – 7.44 (m, 2H), 7.44 – 7.37 (m, 3H), 7.26 – 7.19 (m, 2H), 7.11 – 7.03 (m, 1H), 4.94 (d, *J* = 5.2 Hz, 2H), 4.38 (q, *J* = 7.2 Hz, 2H), 3.00 (t, *J* = 7.2 Hz, 2H), 1.87 – 1.84 (m, 2H), 1.49 – 1.37 (m, 3H), 1.14 – 1.04 (m, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.62, 155.00, 143.88, 140.29, 139.51, 130.16, 128.35, 125.96, 125.75, 124.88, 123.13, 122.74, 120.55, 120.24, 120.18, 118.84, 117.19, 113.06, 108.68, 108.51, 47.49, 40.35, 37.62, 17.26, 13.82, 13.62. HRMS (ESI-TOF) calcd for $C_{26}H_{27}N_4O$, 411.2180 ($M+H^+$), found 411.2180.



AJ2-26

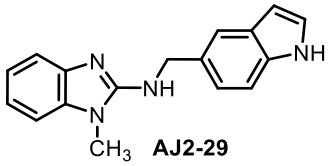
N-((1H-indol-5-yl)methyl)-N-(2-(azepan-1-yl)-2-phenylethyl)butyramide (AJ2-26): Synthesized according to scheme 1, general procedure 1 and following general procedure 4, purified PTLC with Hexane : Ethyl acetate (7:3) to afford AJ2-26 as a colorless liquid (9 mg, 56 %); ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.97 (s, 1H), 7.67 – 7.48 (m, 2H), 7.42 – 7.31 (m, 2H), 7.25 (d, *J* = 8.3 Hz, 2H), 7.16 – 7.12 (m, 2H), 6.69 (dd, *J* = 8.4, 1.7 Hz, 1H), 6.36 (d, *J* = 2.4 Hz, 1H), 4.41 – 4.29 (m, 2H), 3.62 – 3.51 (m, 2H), 3.05 (s, 2H), 2.29 – 2.09 (m, 3H), 1.79 – 1.69 (m, 3H), 1.53 – 1.48 (m, 9H), 0.80 (d, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.89, 173.54, 135.13, 128.88, 128.57, 128.27, 128.10, 125.69, 125.44, 124.86, 124.48, 122.66, 120.59, 120.31, 118.27, 111.38, 111.11, 102.56, 68.89, 66.49, 52.69, 52.56, 52.02, 48.67, 35.53, 35.00, 29.72, 26.92, 26.84, 19.00, 18.86, 14.07, 14.03. *Note: rotomeric isomers observed*, HRMS (ESI-TOF) calcd for $C_{27}H_{36}N_3O$, 418.2853 ($M+H^+$), found 418.2853.



AJ2-27

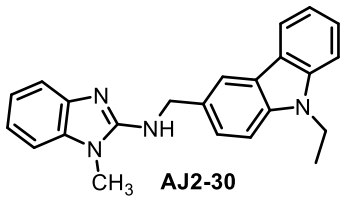
1-(2-(((1-phenyl-1H-pyrazol-4-yl)methyl)amino)-1H-benzo[d]imidazol-1-yl)butan-1-one (AJ2-27): Synthesized according to scheme 1, general procedure 1 and following general procedure 4, purified PTLC with Hexane : Ethyl acetate (6:4) to afford AJ2-27 as a colorless liquid (16 mg, 62 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 – 8.09 (m, 1H), 8.04 – 7.98 (m, 1H), 7.82 – 7.79 (m, 1H), 7.69 – 7.63 (m, 2H), 7.49 – 7.37 (m, 4H), 7.31 – 7.24 (m, 3H), 7.08 (dd, *J* = 8.5, 1.3

Hz, 1H), 4.71 (d, *J* = 5.5 Hz, 2H), 3.00 (t, *J* = 7.2 Hz, 2H), 1.89 – 1.85 (m, 2H), 1.09 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.67, 154.76, 143.61, 140.78, 140.06, 130.12, 129.42, 126.51, 126.17, 124.93, 120.46, 120.41, 119.15, 117.21, 113.11, 40.31, 37.23, 17.25, 13.62. HRMS (ESI-TOF) calcd for $C_{21}H_{22}N_5O$, 360.1819 ($M+H^+$), found 360.1816.



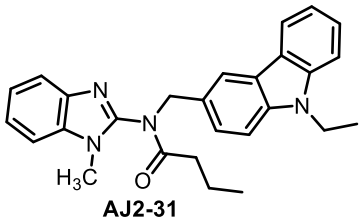
AJ2-29

N-((1H-indol-5-yl)methyl)-1-methyl-1H-benzo[d]imidazol-2-amine (AJ2-29): Synthesized according to scheme 1, purified on Biotage® with Hexane : Ethyl acetate (4:6) to afford AJ2-29 as a brown solid (165 mg, 72 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.70 (d, *J* = 1.6 Hz, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.42 – 7.36 (m, 1H), 7.31 – 7.24 (m, 2H), 7.16 – 7.12 (m, 1H), 7.10 – 7.05 (m, 2H), 6.55 (d, *J* = 1.1 Hz, 1H), 4.81 (d, *J* = 5.1 Hz, 2H), 4.24 (s, 1H), 3.46 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 155.74, 142.91, 135.78, 135.52, 130.85, 127.99, 125.93, 121.62, 120.66, 119.22, 118.73, 115.32, 111.57, 107.63, 101.37, 46.85, 28.69. HRMS (ESI-TOF) calcd for $C_{17}H_{17}N_4$, 277.1448 ($M+H^+$), found 277.1441.



AJ2-30

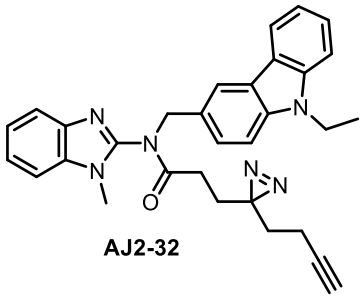
N-((9-ethyl-9H-carbazol-3-yl)methyl)-1-methyl-1H-benzo[d]imidazol-2-amine (AJ2-30): Synthesized according to scheme 1 and general procedure 1, purified on Biotage® with 0 to 6 % methanol gradient in dichloromethane to afford AJ2-30 as a white solid (248 mg, 76 %); ^{1}H NMR (400 MHz, DMSO) δ 8.17 (t, *J* = 1.1 Hz, 1H), 8.12 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.61 – 7.53 (m, 3H), 7.43 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.25 – 7.22 (m, 1H), 7.23 – 7.13 (m, 3H), 7.00 – 6.84 (m, 2H), 4.75 (d, *J* = 5.8 Hz, 2H), 4.42 (q, *J* = 7.1 Hz, 2H), 3.55 (s, 3H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.38, 142.32, 140.31, 139.55, 135.03, 128.90, 126.27, 125.89, 123.13, 122.67, 121.24, 120.52, 120.38, 119.61, 118.96, 116.53, 108.68, 108.59, 107.05, 48.26, 37.63, 28.24, 13.82. HRMS (ESI-TOF) calcd for $C_{23}H_{23}N_4$, 355.1917 ($M+H^+$), found 355.1925.



AJ2-31

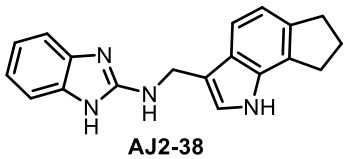
N-((9-ethyl-9H-carbazol-3-yl)methyl)-N-(1-methyl-1H-benzo[d]imidazol-2-yl)butyramide (AJ2-31): Synthesized according to scheme 1, general procedure 1 and following general procedure 3, purified on Biotage® with 0 to 5 % methanol gradient in dichloromethane to afford AJ2-31 as a white solid (64 mg, 52 %); ^{1}H NMR (400 MHz, DMSO) δ 8.15 – 7.86 (m, 2H), 7.64 (d, *J* = 7.6 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.53 – 7.49 (m, 2H), 7.45 – 7.43 (m, 1H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.28 – 7.24 (m, 2H), 7.19 – 7.12 (m, 1H), 5.07 (s, 2H), 4.40 (q, *J* = 7.1 Hz, 2H),

3.39 (s, 3H), 2.00 – 1.96 (m, 2H), 1.62 – 1.48 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H), 0.81 (d, *J* = 7.7 Hz, 3H).^{13}C NMR (151 MHz, DMSO) δ 171.61, 147.34, 139.80, 139.16, 138.30, 133.94, 126.53, 125.82, 125.15, 122.23, 121.58, 121.29, 119.82, 119.61, 118.74, 118.12, 110.28, 108.53, 108.33, 50.42, 36.34, 34.55, 28.66, 17.24, 13.05, 12.90. *Note: rotomeric isomers observed*, HRMS (ESI-TOF) calcd for $C_{27}H_{29}N_4O$, 425.2336 (M+H$^+$), found 425.2353.



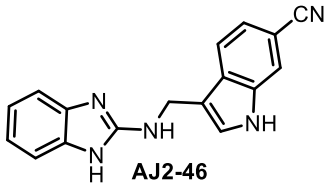
AJ2-32

3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)-N-((9-ethyl-9H-carbazol-3-yl)methyl)-N-(1-methyl-1H-benzo[d]imidazol-2-yl)propanamide (AJ2-32): Synthesized according to scheme 1 and general procedure 1 following general procedure 3, purified on Biotage® with 0 to 5 % methanol gradient in dichloromethane to afford AJ2-32 as a light brown viscous liquid (12 mg, 46 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.8 Hz, 1H), 7.97 (d, *J* = 1.6 Hz, 1H), 7.83 (dd, *J* = 6.9, 2.1 Hz, 1H), 7.50 – 7.46 (m, 1H), 7.41 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.39 – 7.26 (m, 4H), 7.25 – 7.18 (m, 2H), 5.19 (s, 2H), 4.34 (q, *J* = 7.2 Hz, 2H), 3.05 (s, 3H), 2.02 – 1.96 (m, 3H), 1.95 – 1.81 (m, 4H), 1.62 (t, *J* = 7.4 Hz, 2H), 1.42 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.28, 147.65, 140.97, 140.25, 139.62, 134.48, 127.08, 126.62, 125.93, 123.58, 123.05, 122.96, 122.63, 121.50, 120.61, 120.32, 118.99, 110.05, 108.57, 108.53, 82.69, 69.19, 52.72, 37.62, 32.42, 29.24, 28.16, 27.76, 27.67, 13.80, 13.22. Note: rotomeric isomers observed, HRMS (ESI-TOF) calcd for $C_{31}H_{31}N_6O$, 503.2554 (M+H$^+$), found 503.2556.



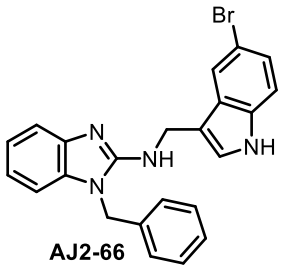
AJ2-38

N-((1,6,7,8-tetrahydrocyclopenta[g]indol-3-yl)methyl)-1H-benzo[d]imidazol-2-amine (AJ2-38): Synthesized according to scheme 1 and general procedure 1, purified on purified on Biotage® with 0 to 5 % methanol gradient in dichloromethane to afford AJ2-38 as an off white solid (64 mg, 72 %); ^{1}H NMR (400 MHz, CD_3OD) δ 7.38 (d, *J* = 8.0 Hz, 1H), 7.24 – 7.20 (m, 2H), 7.17 (s, 1H), 7.02 – 6.98 (m, 2H), 6.91 (d, *J* = 8.0 Hz, 1H), 4.70 (s, 2H), 3.01 – 2.96 (m, 4H), 2.14 – 2.07 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 154.74, 137.63, 136.51, 133.87, 125.45, 125.30, 122.36, 120.45, 116.22, 115.69, 112.07, 111.32, 47.52, 47.31, 38.70, 25.02. HRMS (ESI-TOF) calcd for $C_{19}H_{19}N_4$, 303.1604 (M+H$^+$), found 303.1602.



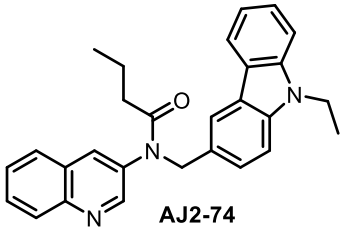
AJ2-46

3-(((1H-benzo[d]imidazol-2-yl)amino)methyl)-1H-indole-6-carbonitrile (AJ2-46): Synthesized according to scheme 1 and general procedure 1, purified on Biotage® with 10 to 70 % ethyl acetate gradient in hexane to afford AJ2-46 as an brown solid (35 mg, 58%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.83 – 7.75 (m, 2H), 7.61 – 7.57 (m, 1H), 7.30 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.25 – 7.20 (m, 2H), 7.04 – 6.98 (m, 2H), 4.77 (d, *J* = 0.8 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ 155.98, 135.58, 135.55, 130.16, 128.91, 121.68, 121.20, 120.54, 116.88, 114.67, 102.96, 37.89. HRMS (ESI-TOF) calcd for $C_{17}H_{14}N_5$, 288.1244 ($M+H^+$), found 288.1239.



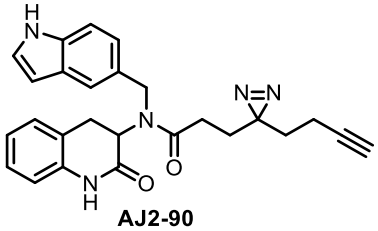
AJ2-66

1-Benzyl-N-((5-bromo-1H-indol-3-yl)methyl)-1H-benzo[d]imidazol-2-amine (AJ2-66): Synthesized according to scheme 1 and general procedure 1, purified purified on Biotage® with 5 to 70 % ethyl acetate gradient in hexane to afford AJ2-66 as a brown solid (48 mg, 63%), ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1H), 7.38 – 7.30 (m, 2H), 7.14 –7.08 (m, 3H), 7.03 – 6.97 (m, 3H), 6.98 – 6.92 (m, 4H), 6.83 (d, *J* = 2.3 Hz, 1H), 4.92 (s, 2H), 4.51 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.72, 140.70, 135.15, 134.96, 134.32, 129.16, 128.24, 128.10, 126.53, 124.88, 124.68, 121.86, 121.07, 120.48, 115.77, 113.02, 112.76, 111.71, 107.89, 45.80, 39.24. HRMS (ESI-TOF) calcd for $C_{23}H_{20}BrN_4$, 431.0866 ($M+H^+$), found 431.0850.



AJ2-74

N-((9-ethyl-9H-carbazol-3-yl)methyl)-N-(quinolin-3-yl)butyramide (AJ2-74): Synthesized according to Scheme 1 and general procedure 1 followed by general procedure 4, purified on PTLC with Hexane : Ethyl acetate (6:4) to afford AJ2-74 as a brown solid (17 mg, 68%), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 – 8.32 (m, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.92 (d, *J* = 7.7 Hz, 1H), 7.85 (d, *J* = 1.2 Hz, 1H), 7.71 – 7.57 (m, 3H), 7.46 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.41 – 7.28 (m, 2H), 7.21 – 7.16 (m, 1H), 7.10 (dd, *J* = 7.0, 1.1 Hz, 1H), 5.10 (s, 2H), 4.26 (q, *J* = 7.2 Hz, 2H), 2.06 – 1.92 (m, 2H), 1.63 – 1.59 (m, 2H), 1.34 (t, *J* = 7.2 Hz, 3H), 0.77 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.80, 150.98, 147.07, 140.23, 139.50, 135.81, 134.68, 130.14, 129.37, 127.91, 127.83, 127.46, 127.42, 126.90, 125.78, 123.07, 122.69, 121.15, 120.55, 118.85, 108.51, 108.47, 53.44, 37.61, 36.77, 18.87, 13.84, 13.82. HRMS (ESI-TOF) calcd for $C_{28}H_{28}N_3O$, 422.2227 ($M+H^+$), found 422.2217.



AJ2-90

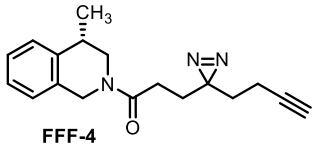
N-((1H-indol-5-yl)methyl)-3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)-N-(2-oxo-1,2,3,4-tetrahydroquinolin-3-yl)propenamide (AJ2-90): Synthesized according to general scheme 2 and general procedure 2, purified by PTLC (Hexane/Ethyl acetate; 5:5) to afford AJ2-90 as white solid (12 mg, 46%), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.87 (s, 1H), 7.48 (d, *J* = 1.7 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 2.4 Hz, 1H), 7.07 – 7.03 (m, 2H), 6.91 (d, *J* = 7.4 Hz, 1H), 6.85 – 6.81 (m, 1H), 6.64 – 6.60 (m, 1H), 6.48 – 6.49 (m, 1H), 4.88 – 4.83 (m, 1H), 4.72 – 4.59 (m, 2H), 3.42 – 3.34 (m, 1H), 2.74 – 2.70 (m, 1H), 2.27 – 2.15 (m, 2H), 1.89 – 1.77 (m, 4H), 1.56 – 1.49 (m, 2H).). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.65, 168.47, 136.18, 135.33, 128.43, 128.18, 127.71, 125.10, 123.06, 122.39, 120.73, 118.69, 114.96, 111.66, 102.69, 102.62, 82.86, 69.09, 55.58, 51.99, 32.49, 30.26, 29.72, 28.01, 27.72, 13.29. HRMS (ESI-TOF) calcd for $C_{26}H_{26}N_5NaO_2$, 462.1900 ($M+Na^+$), found 462.1908.

Supplementary references:

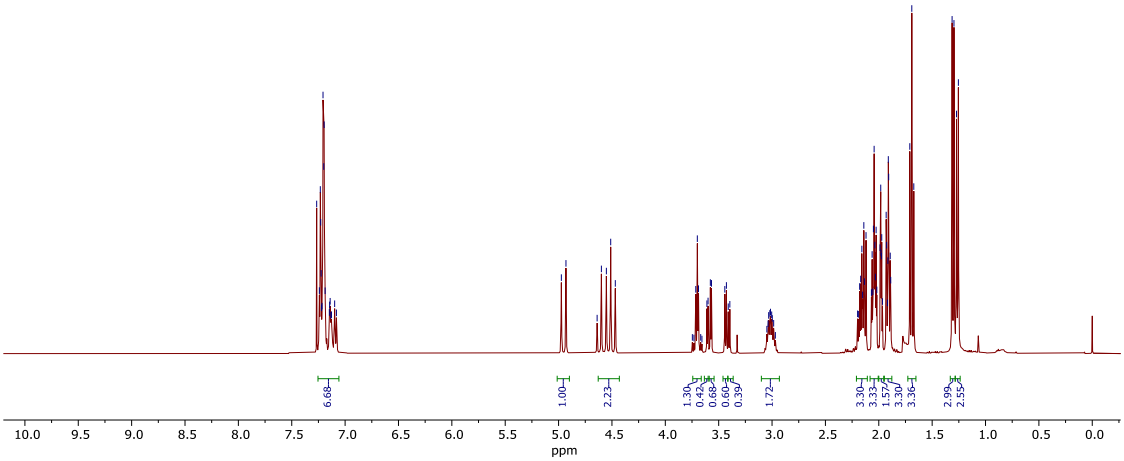
1) Parker C. G. *et al*. *Cell.* **2017**, *168*, 527 - 541.
2) Wang Y. *et al*. *Nat. Chem*., **2019**, *11*, 1113 - 1123.
3) Kuhler, T. C. *et al*. *J. Med. Chem.* **2002**, *45,* 4282 - 4299.

II. NMR spectra:

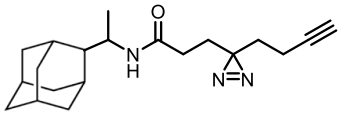
^{1}H NMR ($CDCl_3$) FFF-4



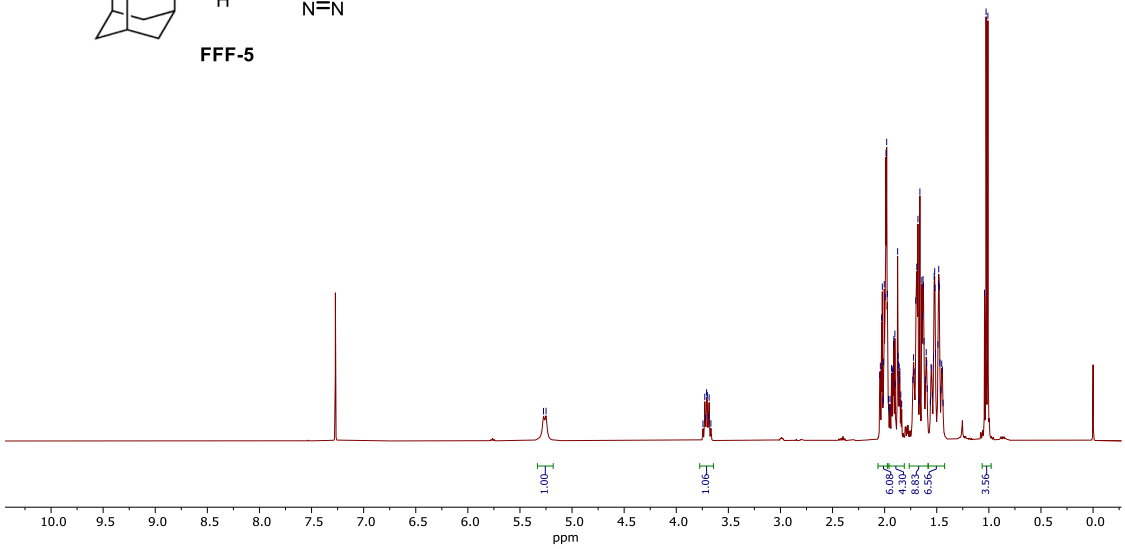
FFF-4



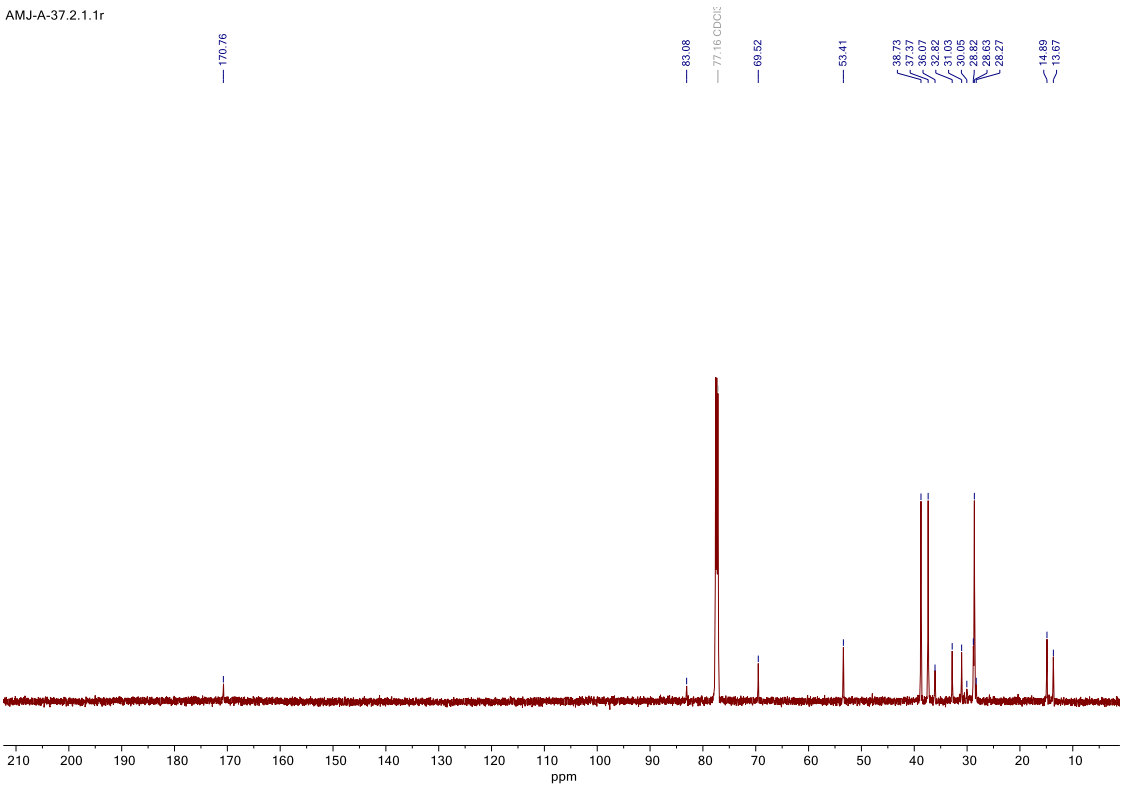
^{1}H NMR ($CDCl_3$) FFF-5



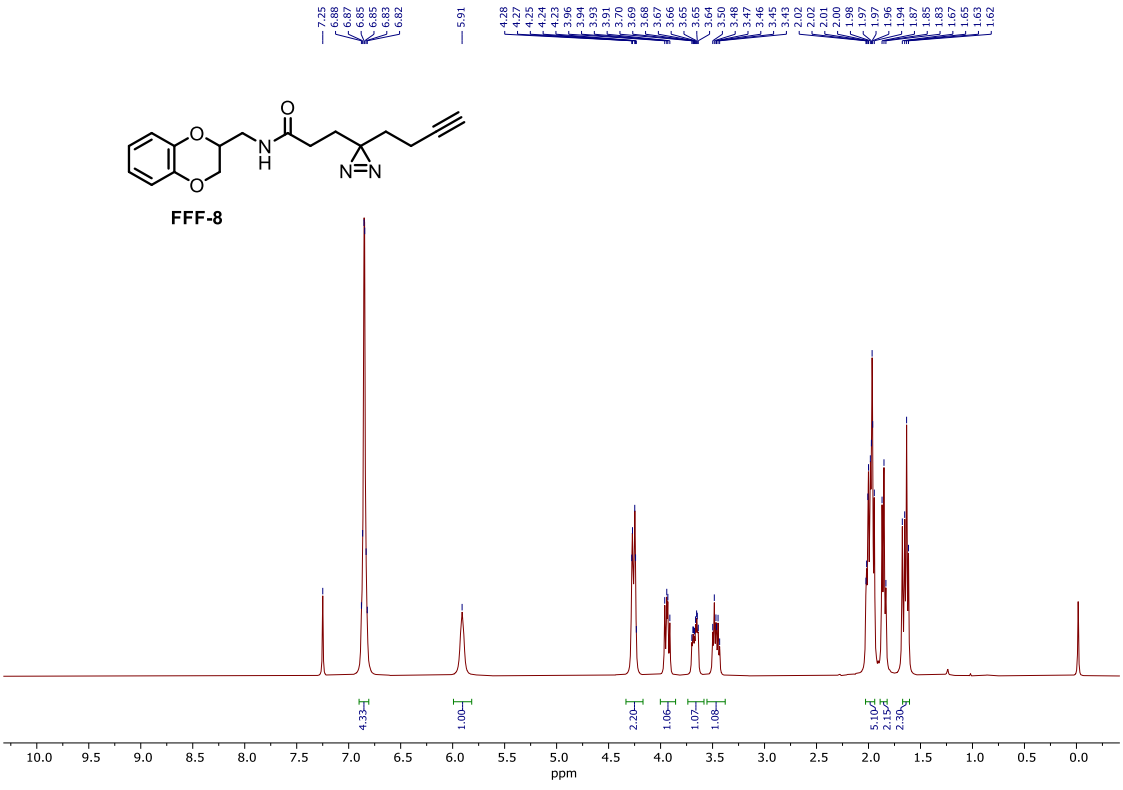
FFF-5



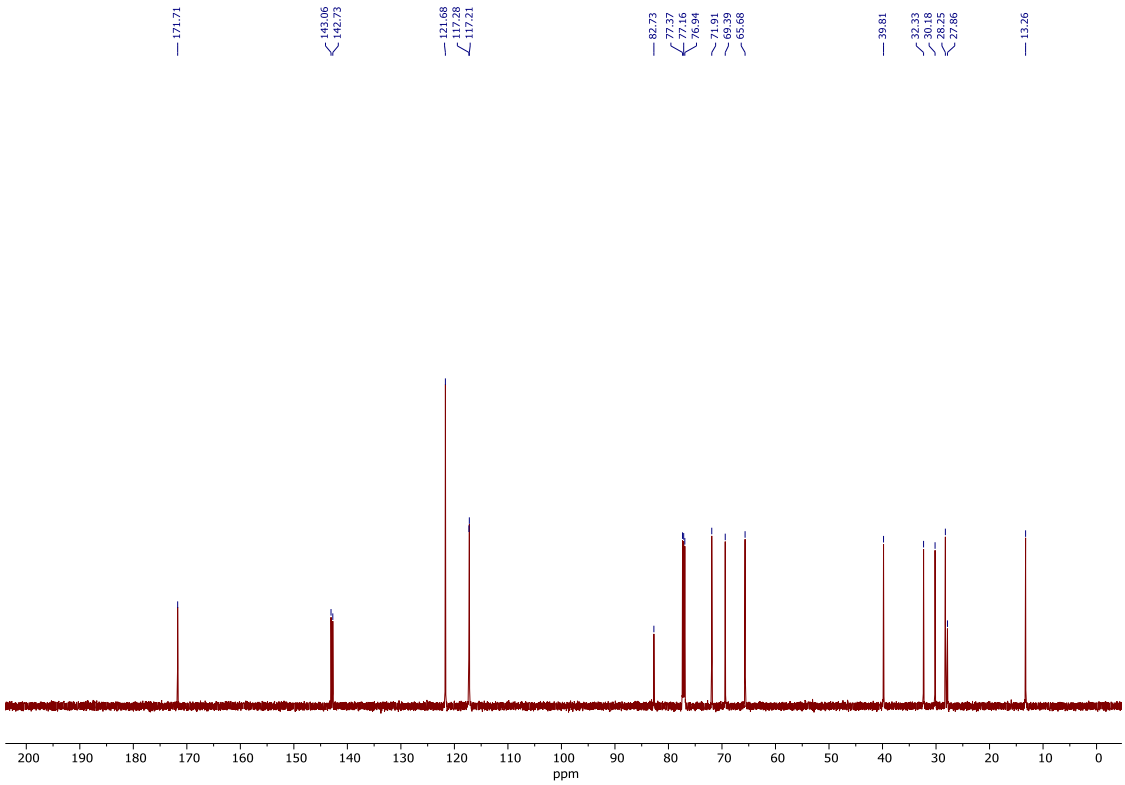
^{13}C NMR ($CDCl_3$) FFF-5



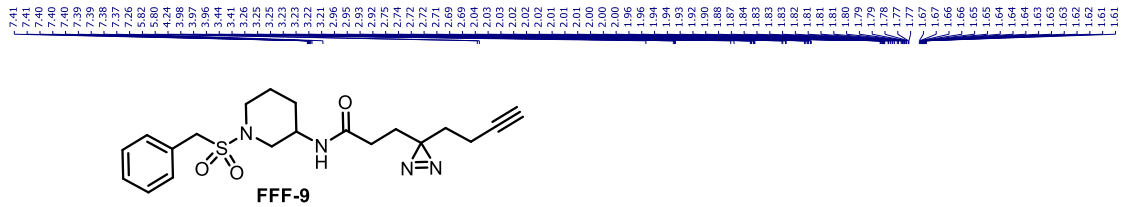
^{1}H NMR ($CDCl_3$) FFF-8



^{13}C NMR ($CDCl_3$) FFF-8



^{1}H NMR ($CDCl_3$) FFF-9



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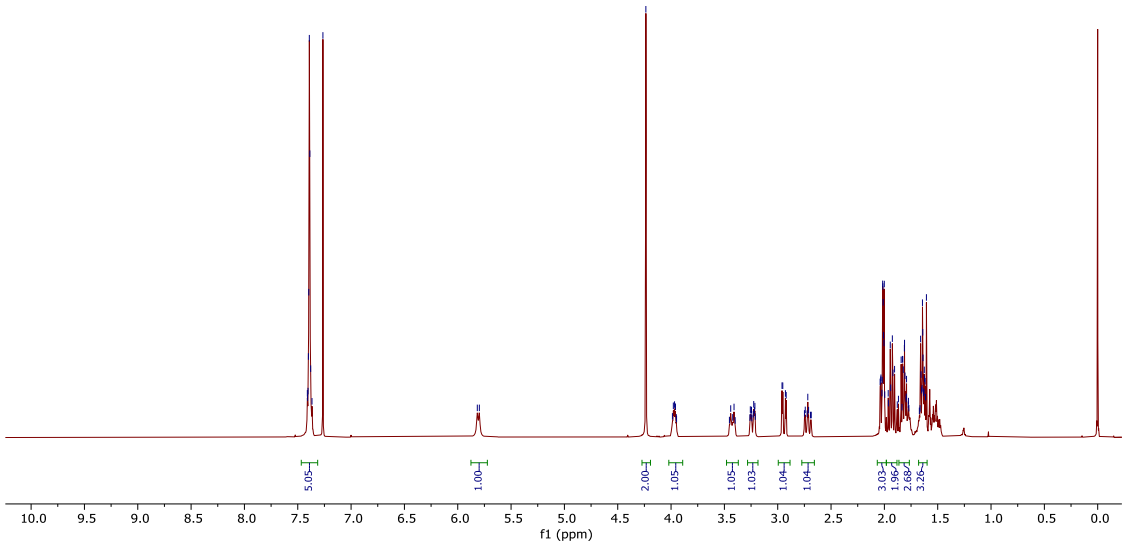
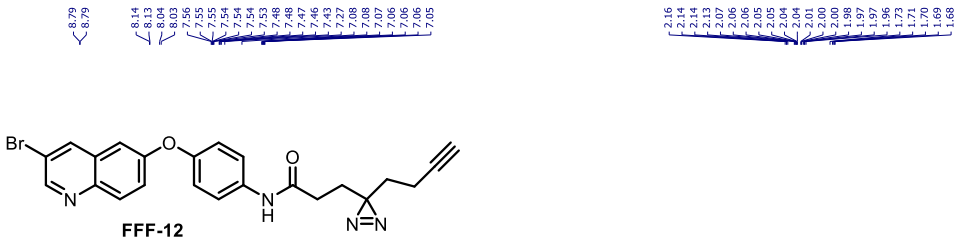
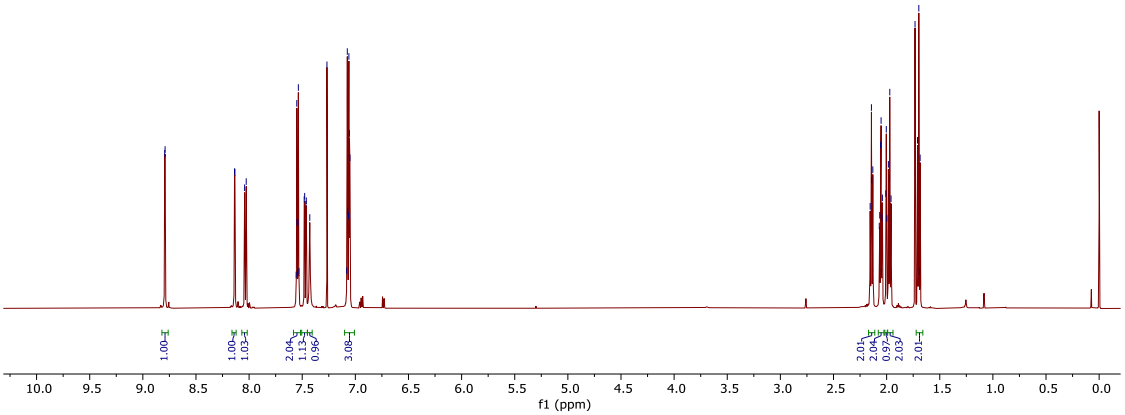


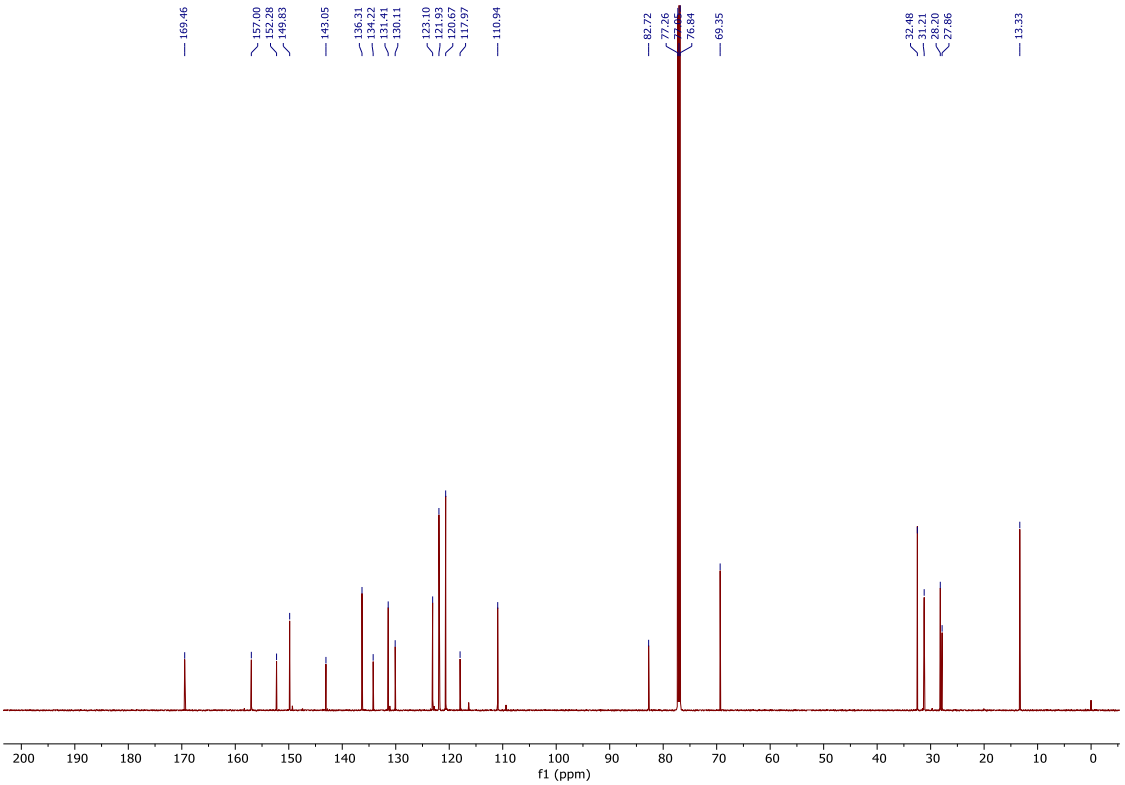
^{1}H NMR ($CDCl_3$) FFF-12



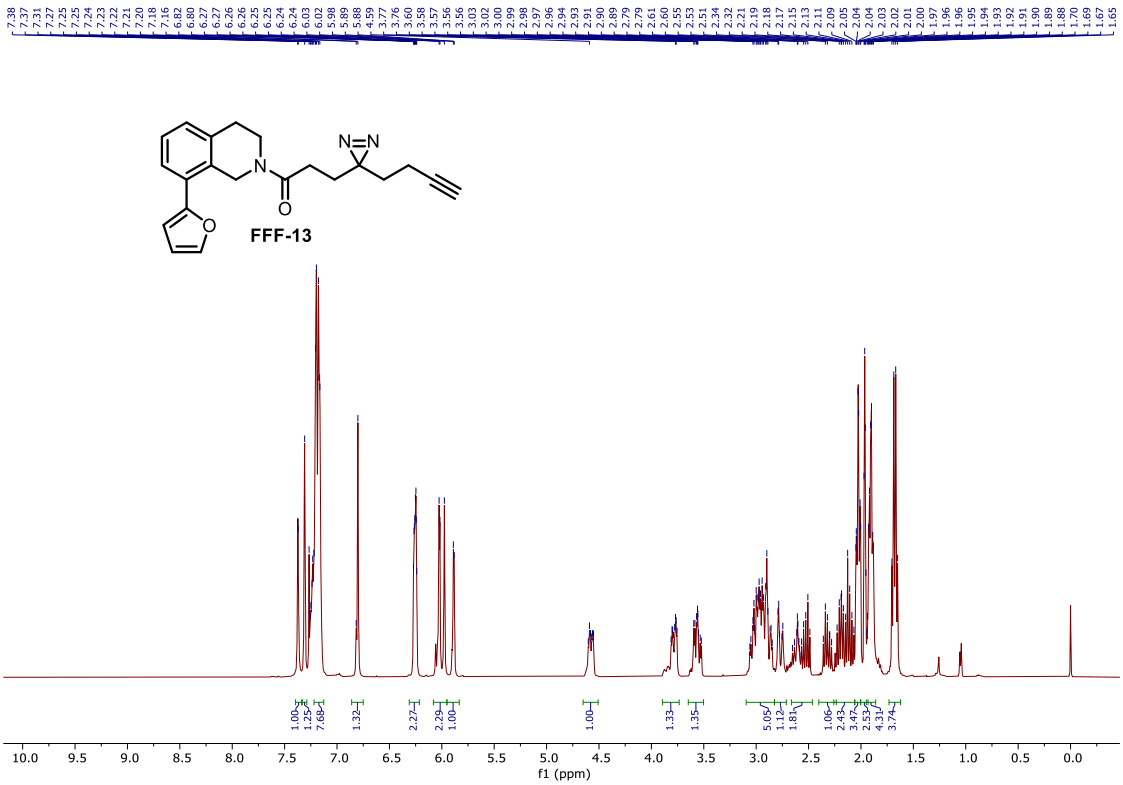
FFF-12



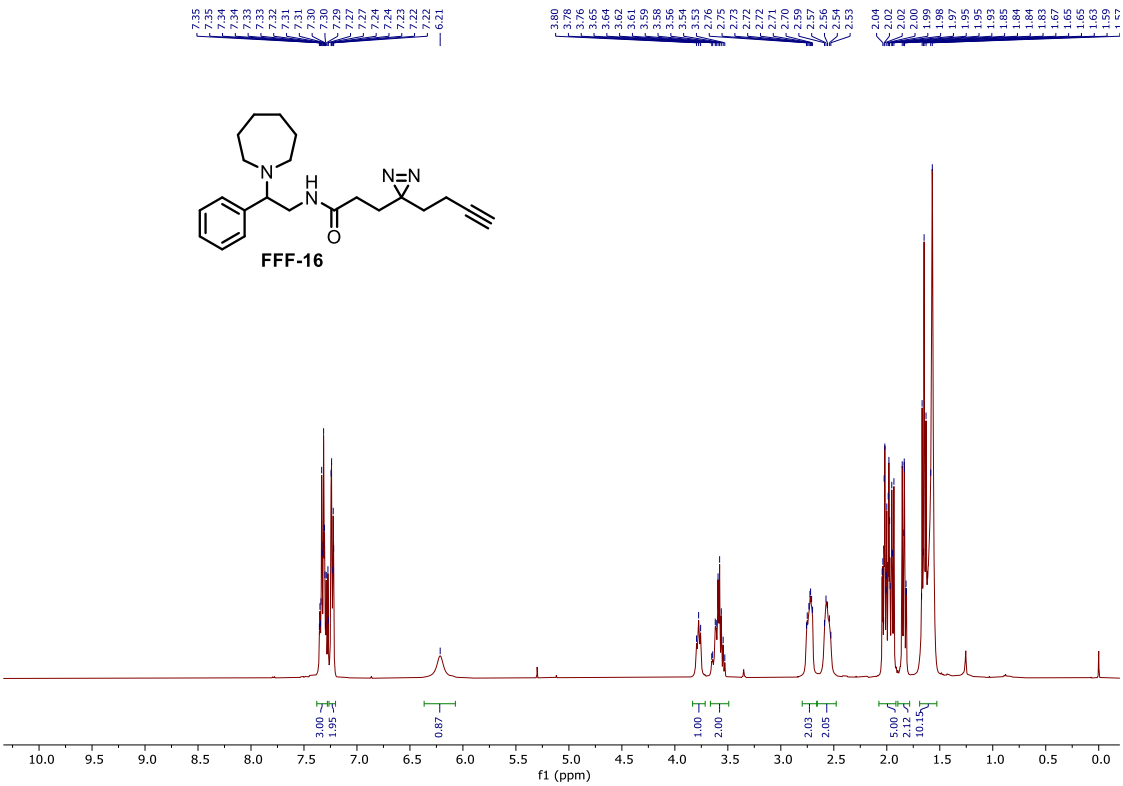
^{13}C NMR ($CDCl_3$) FFF-12



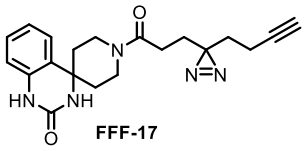
^{1}H NMR ($CDCl_3$) FFF-13



^{1}H NMR ($CDCl_3$) FFF-16



^{1}H NMR ($CDCl_3$) FFF-17



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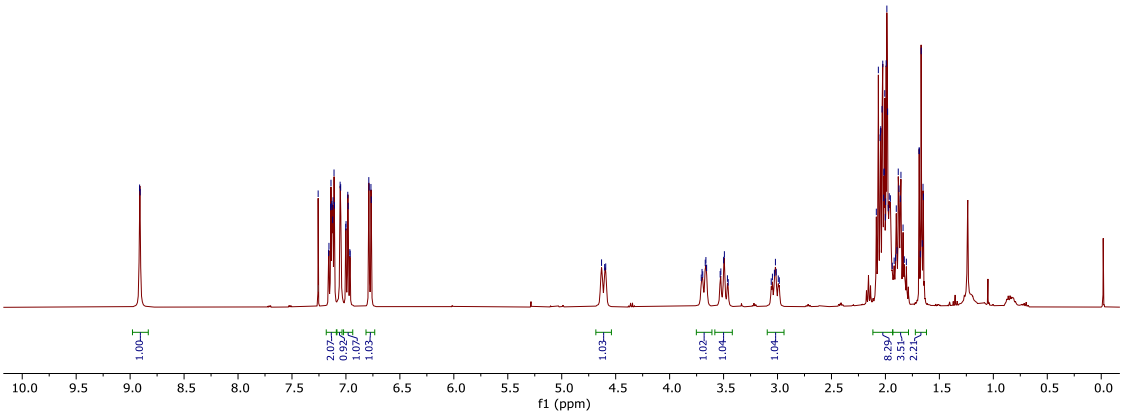
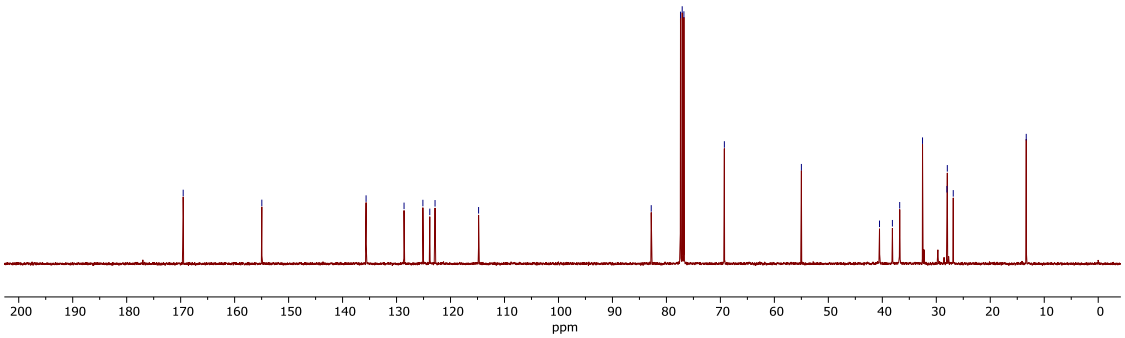
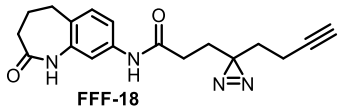


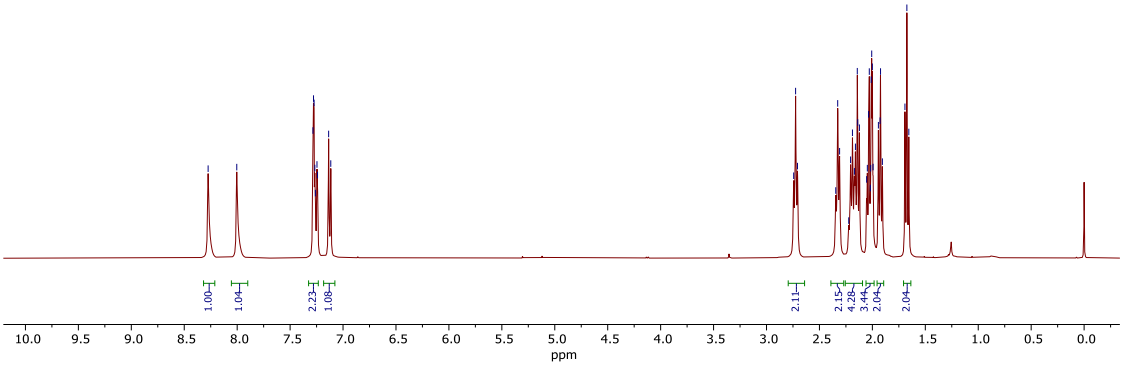
^{13}C NMR ($CDCl_3$) FFF-17



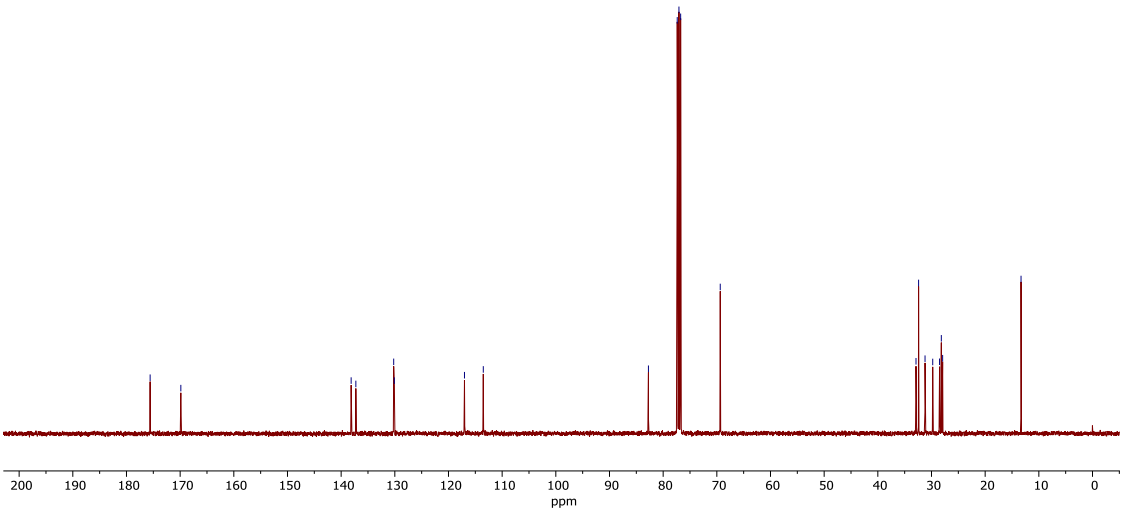
^{1}H NMR ($CDCl_3$) FFF-18



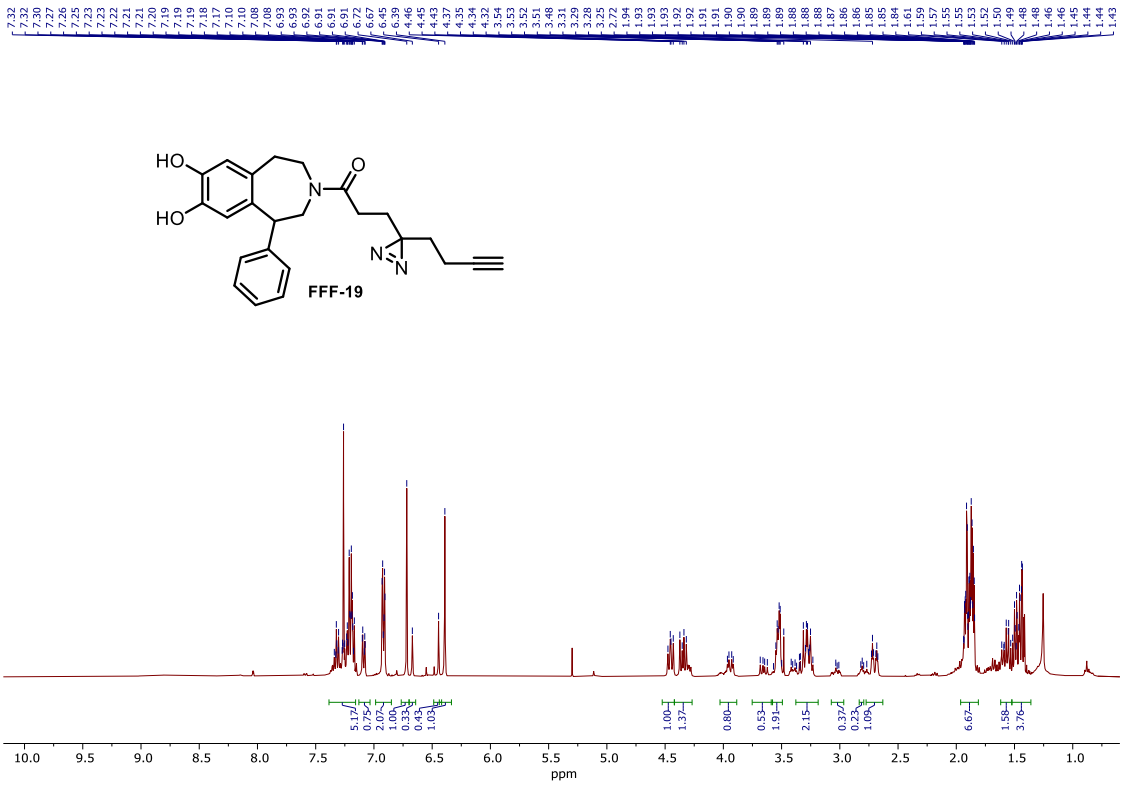
FFF-18



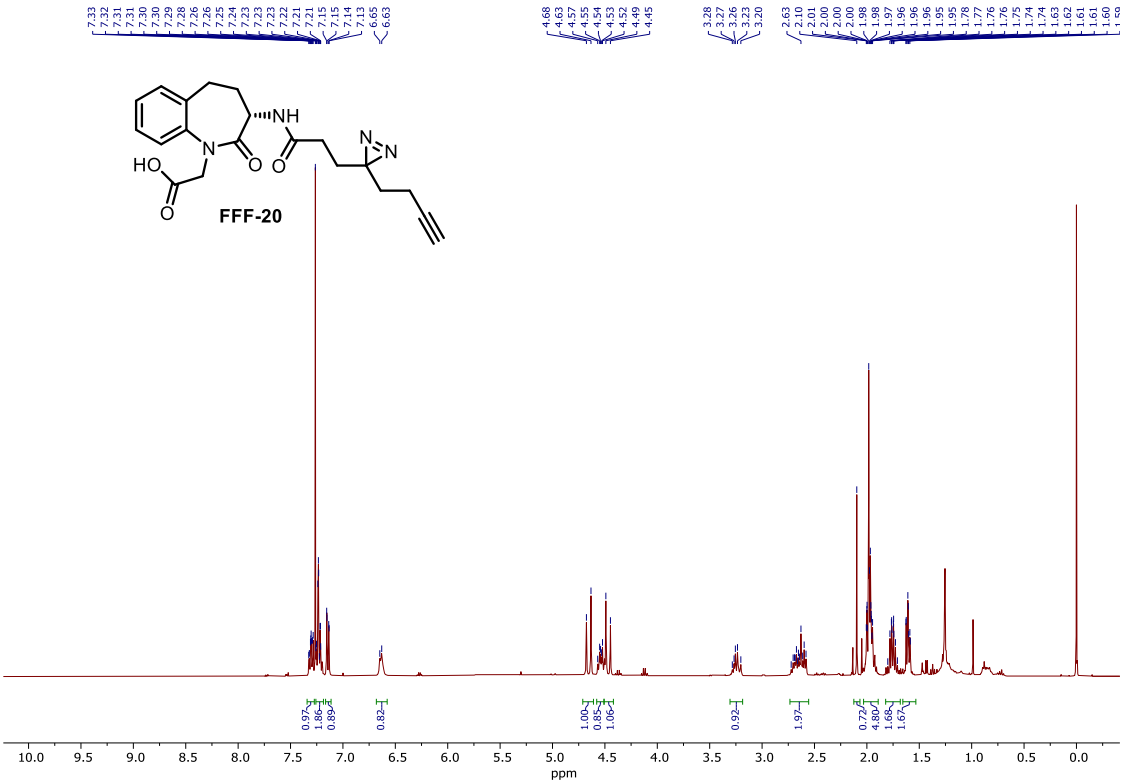
^{13}C NMR ($CDCl_3$) FFF-18



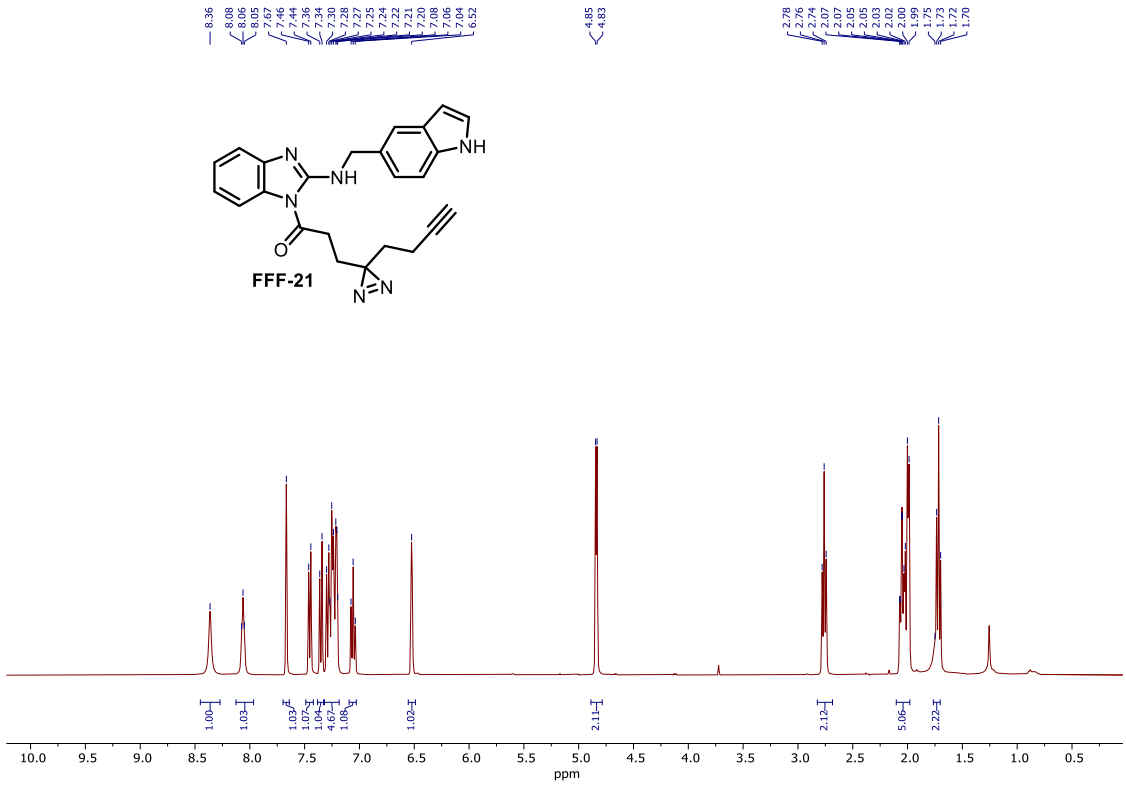
^{1}H NMR ($CDCl_3$) FFF-19



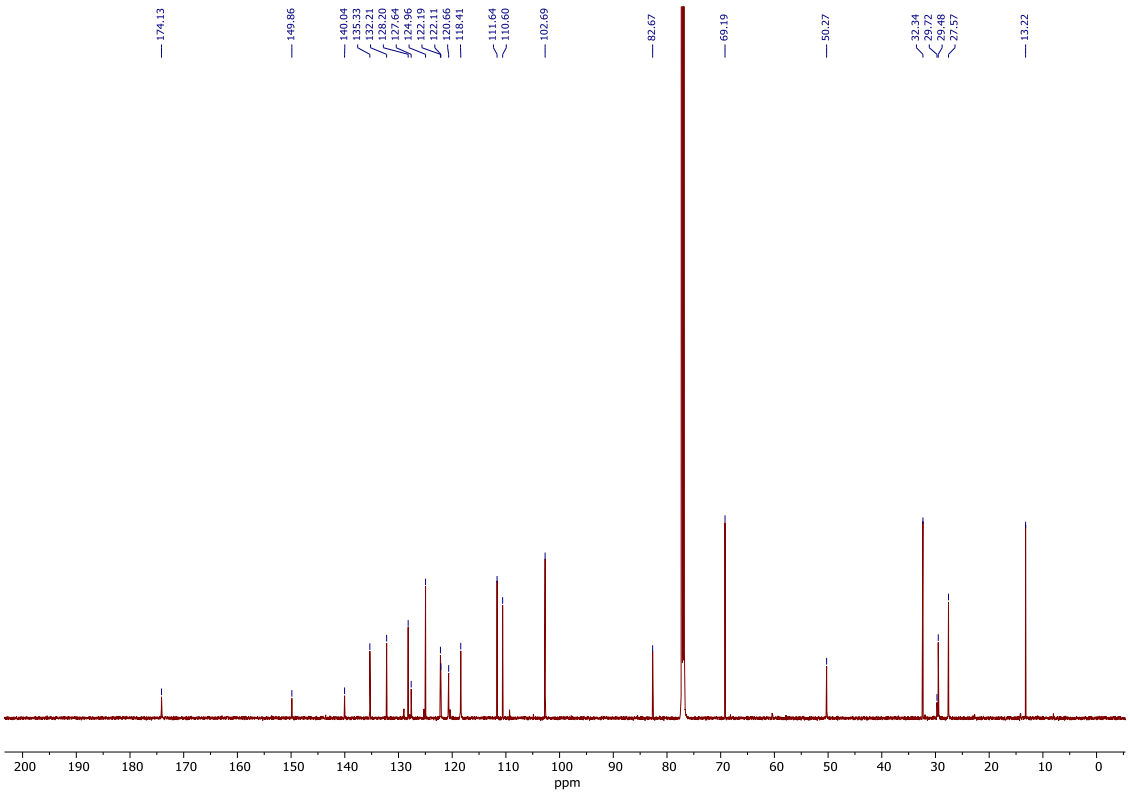
^{1}H NMR ($CDCl_3$) FFF-20



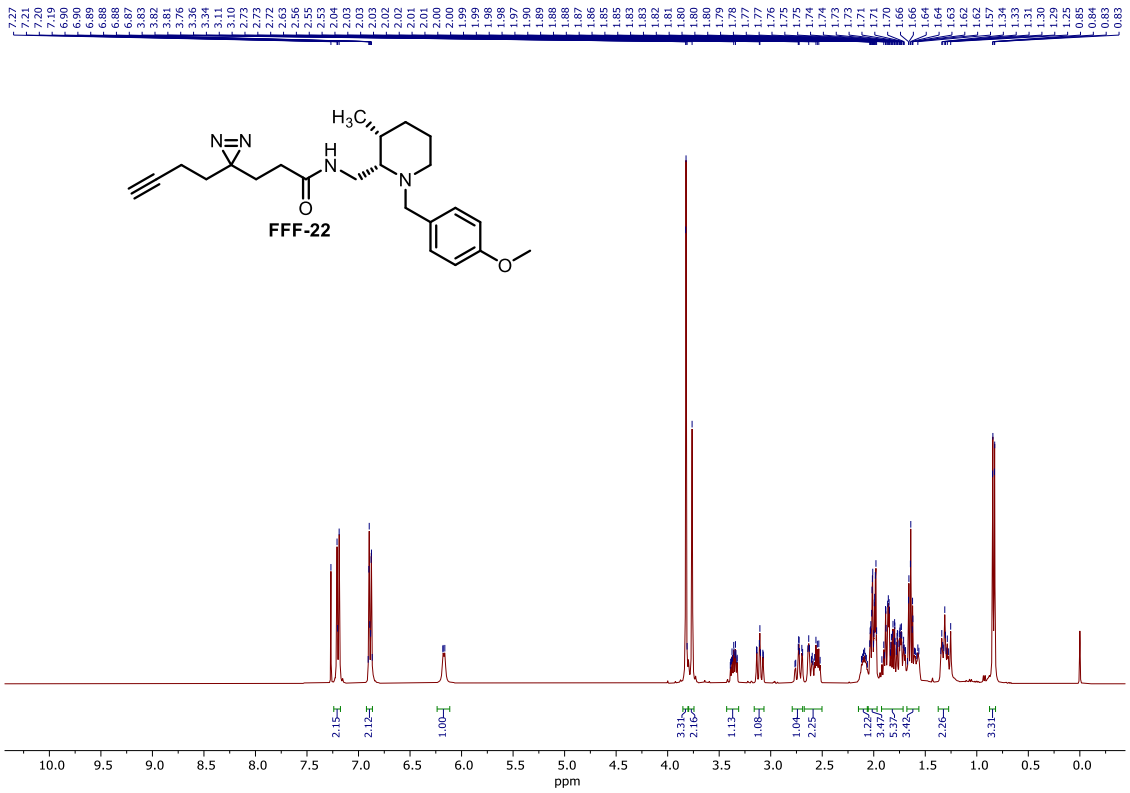
^{1}H NMR ($CDCl_3$) FFF-21



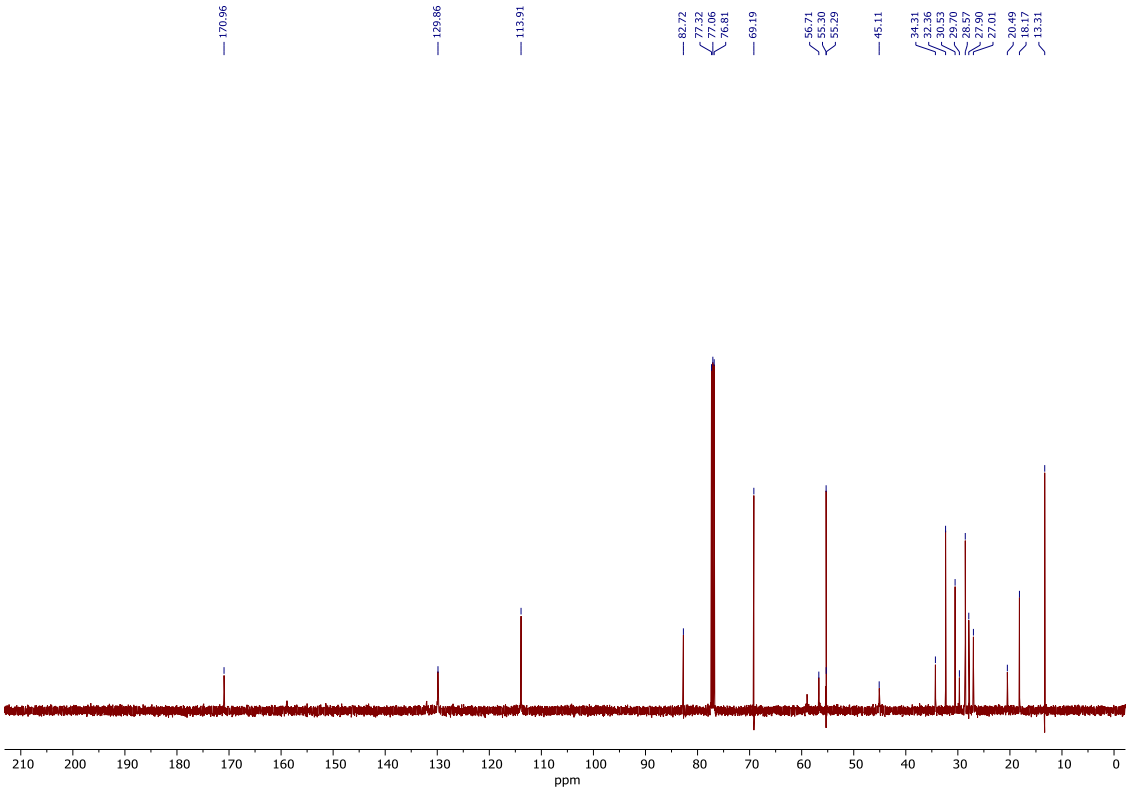
^{13}C NMR ($CDCl_3$) FFF-21



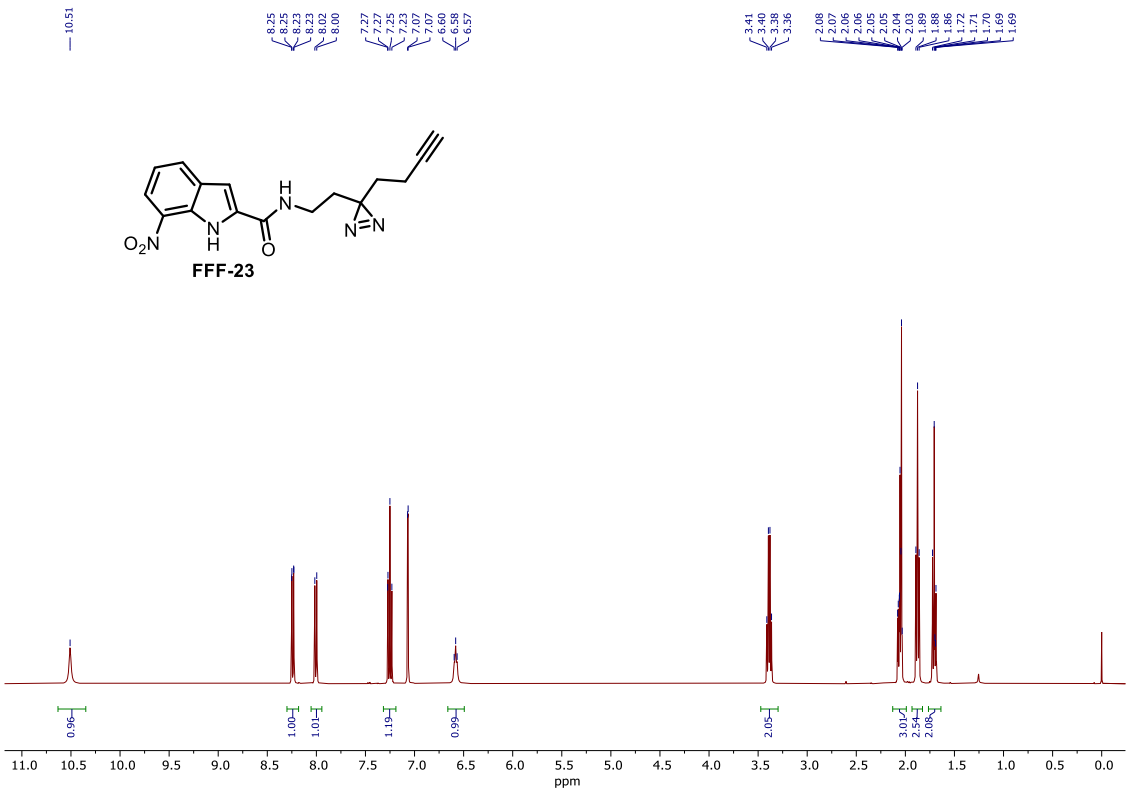
^{1}H NMR ($CDCl_3$) FFF-22



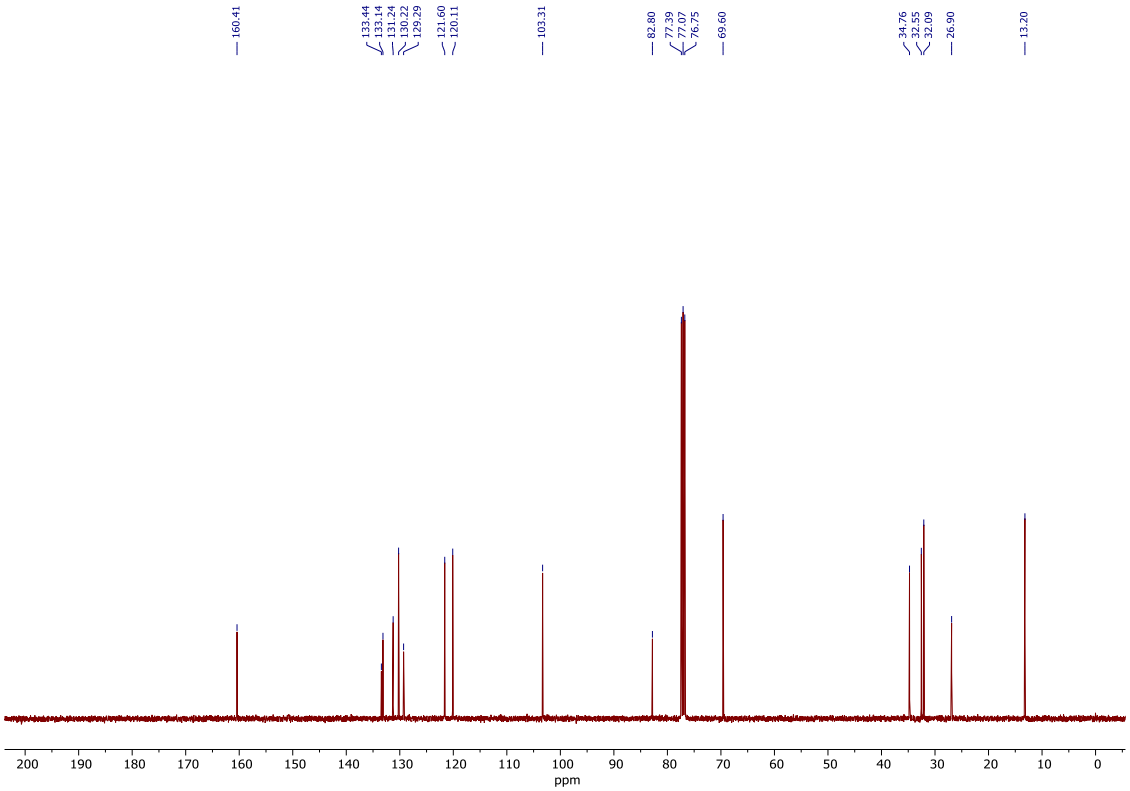
^{13}C NMR ($CDCl_3$) FFF-22



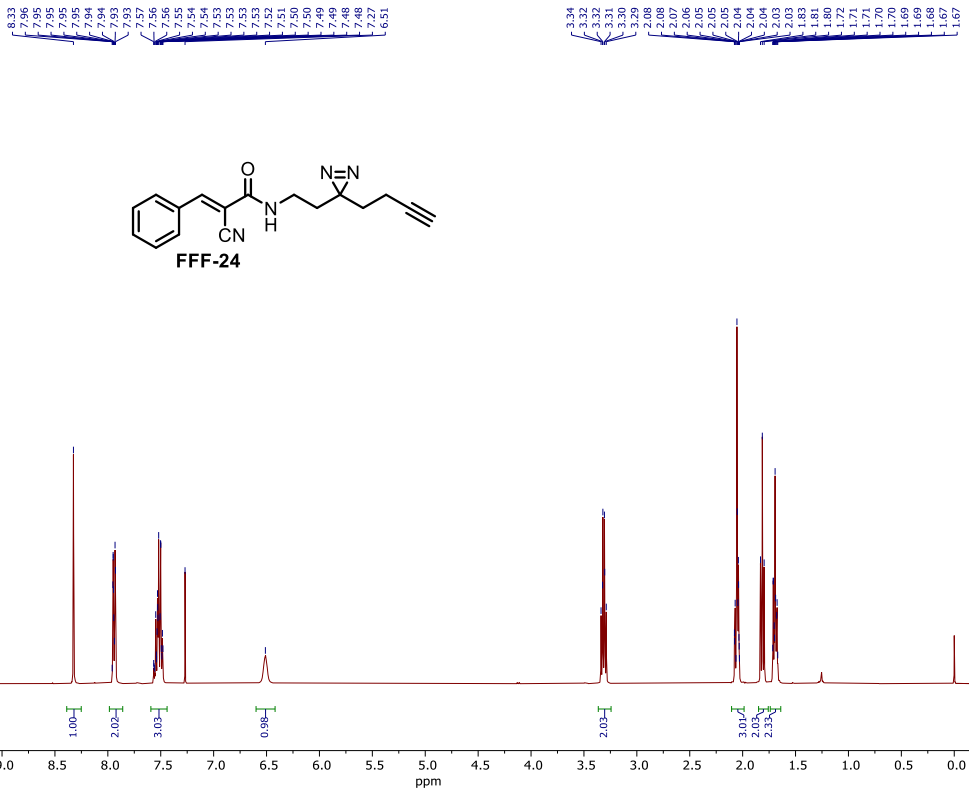
^{1}H NMR ($CDCl_3$) FFF-23



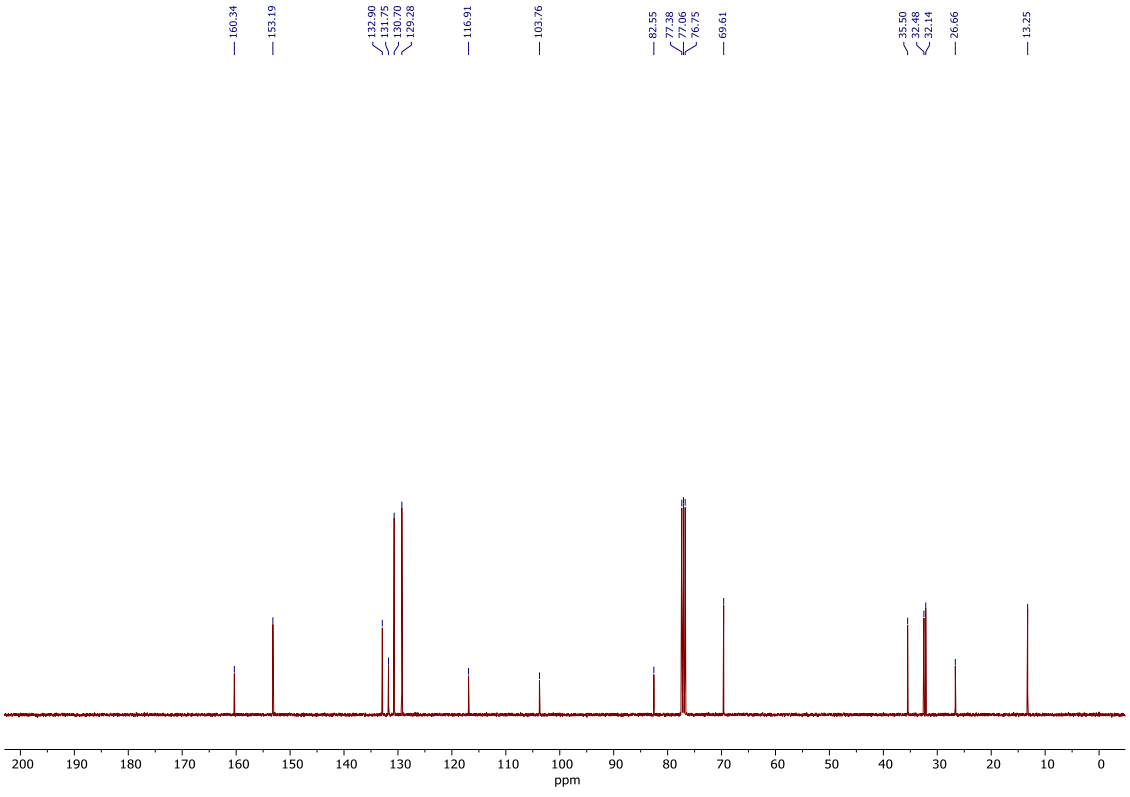
^{13}C NMR ($CDCl_3$) FFF-23



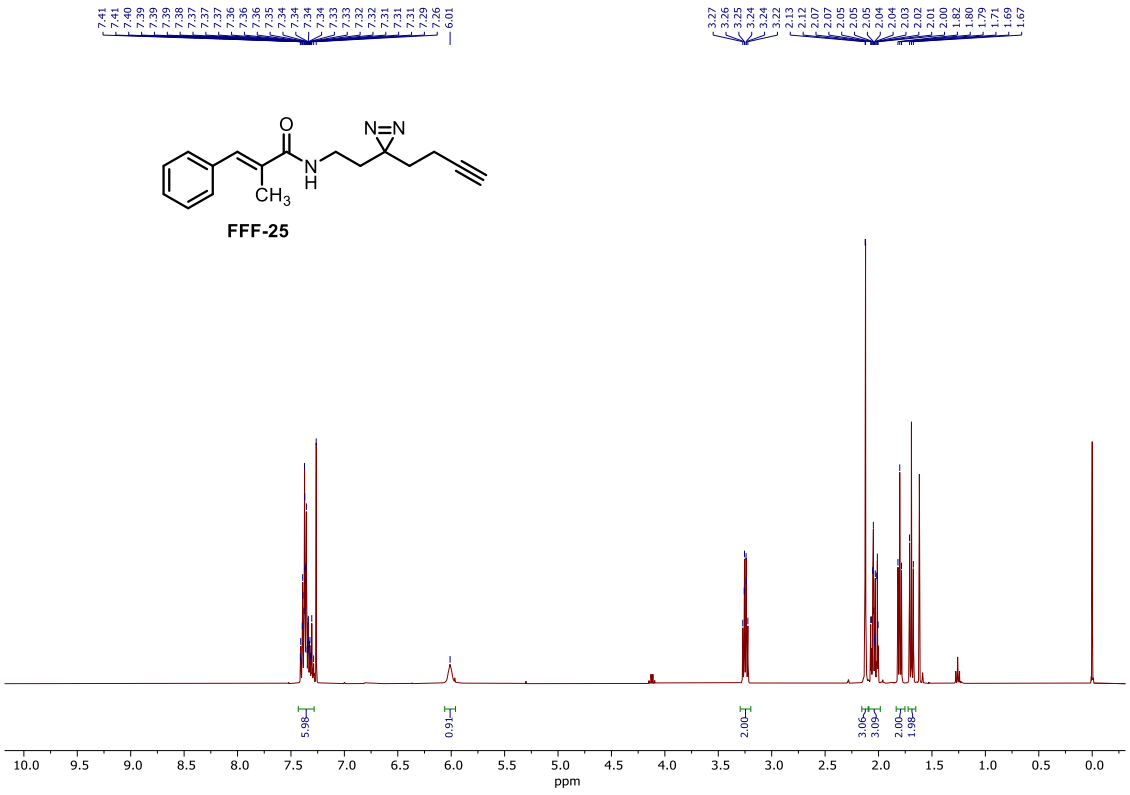
^{1}H NMR ($CDCl_3$) FFF-24



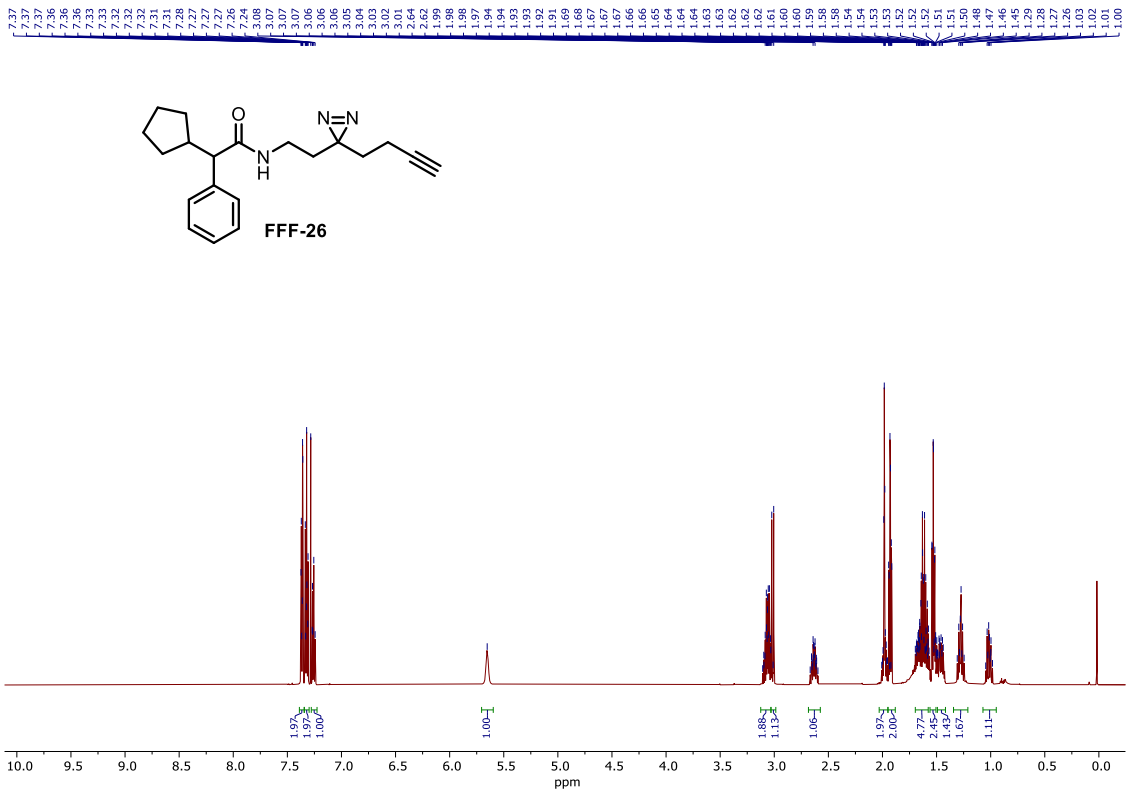
^{13}C NMR ($CDCl_3$) FFF-24



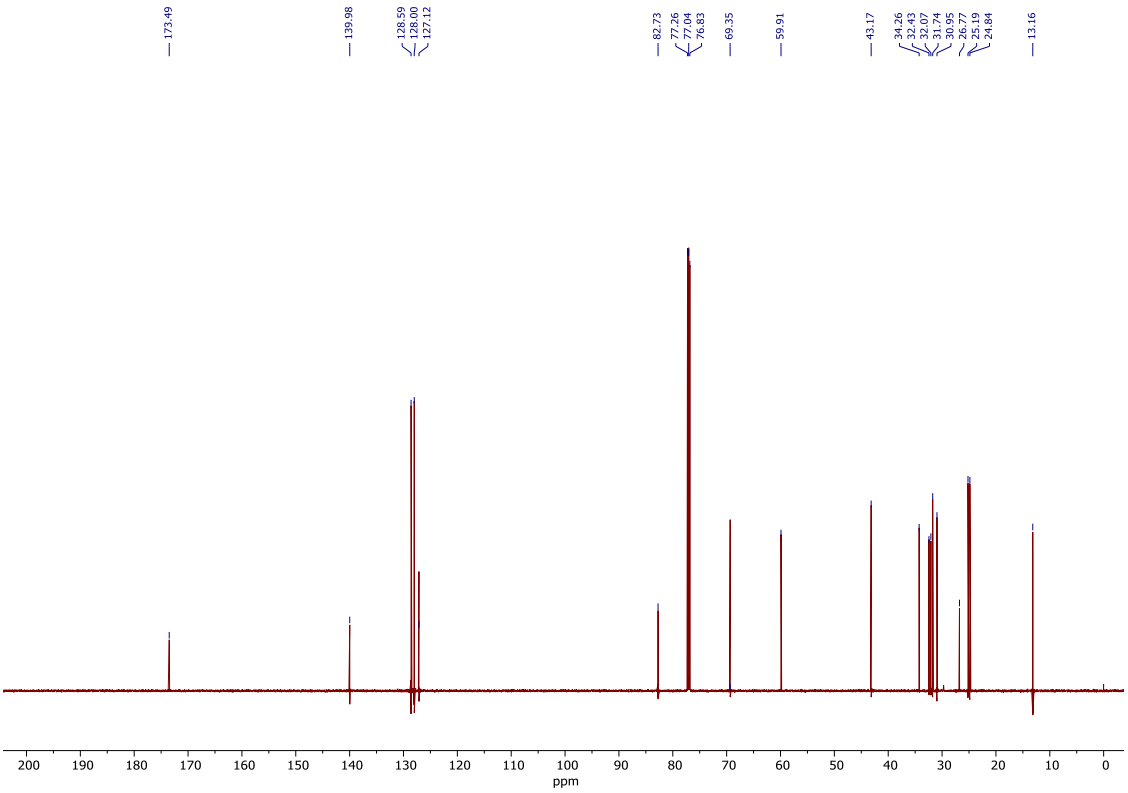
^{1}H NMR ($CDCl_3$) FFF-25



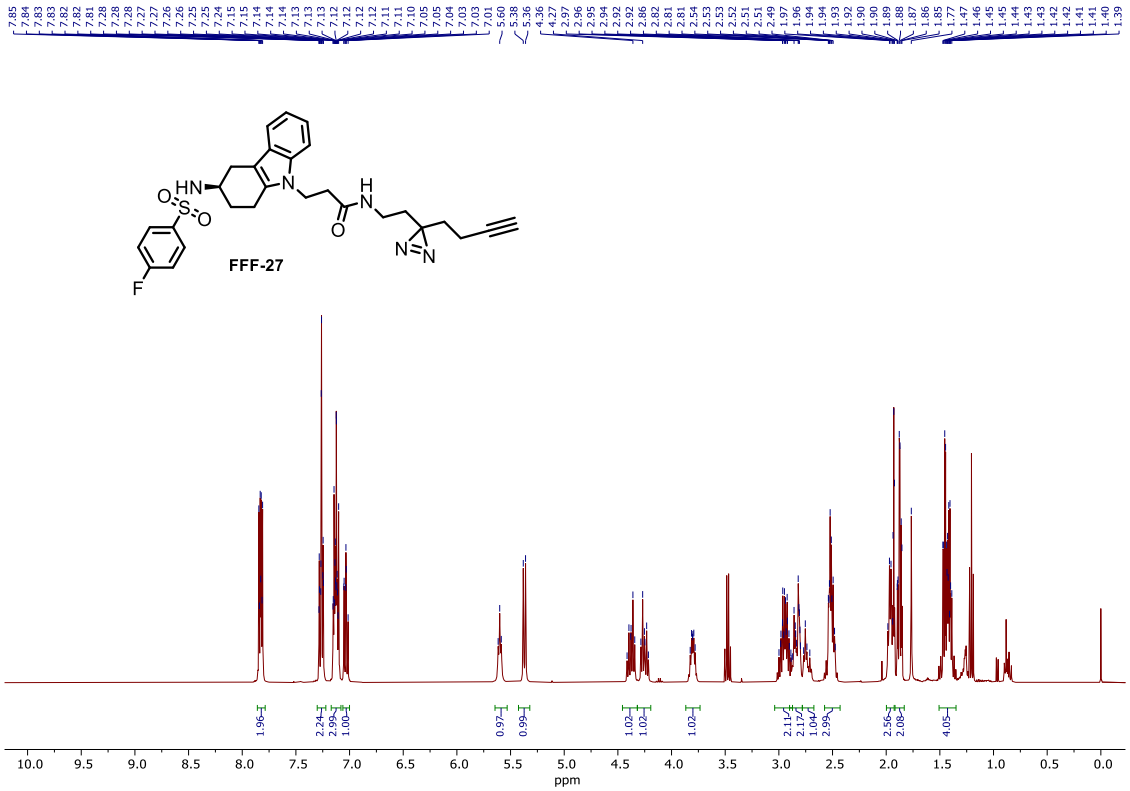
^{1}H NMR ($CDCl_3$) FFF-26



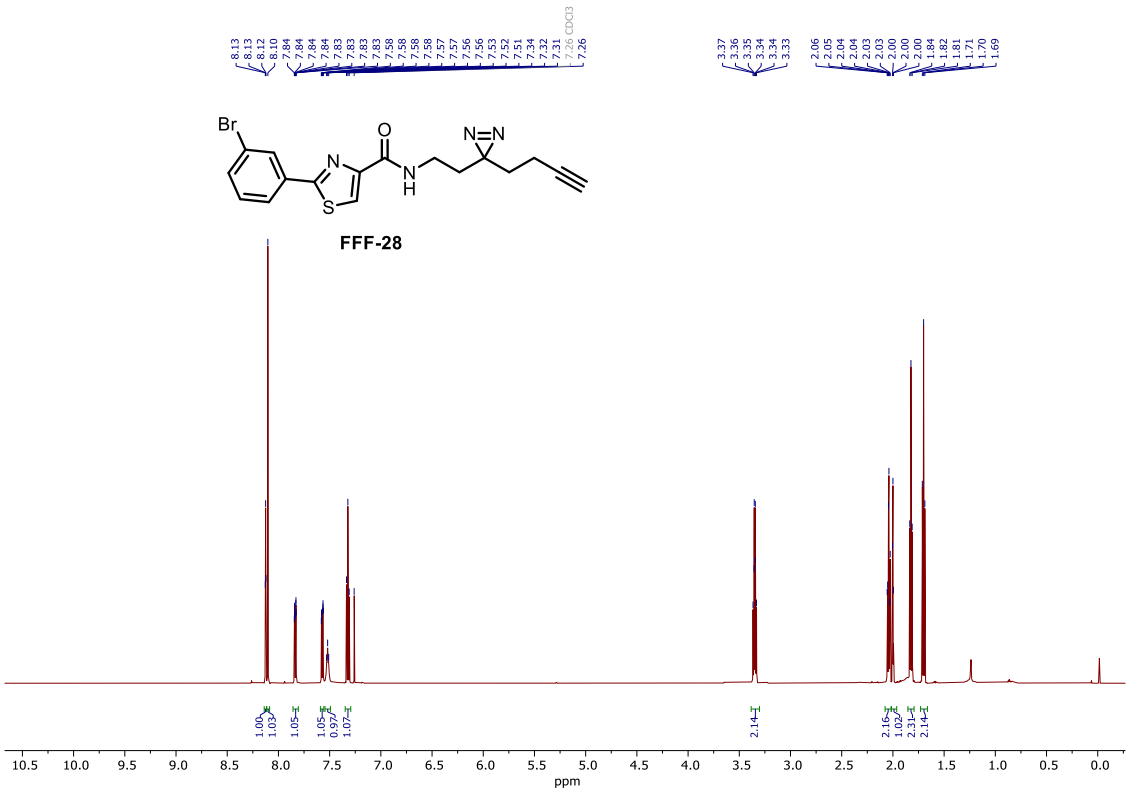
^{13}C NMR ($CDCl_3$) FFF-26



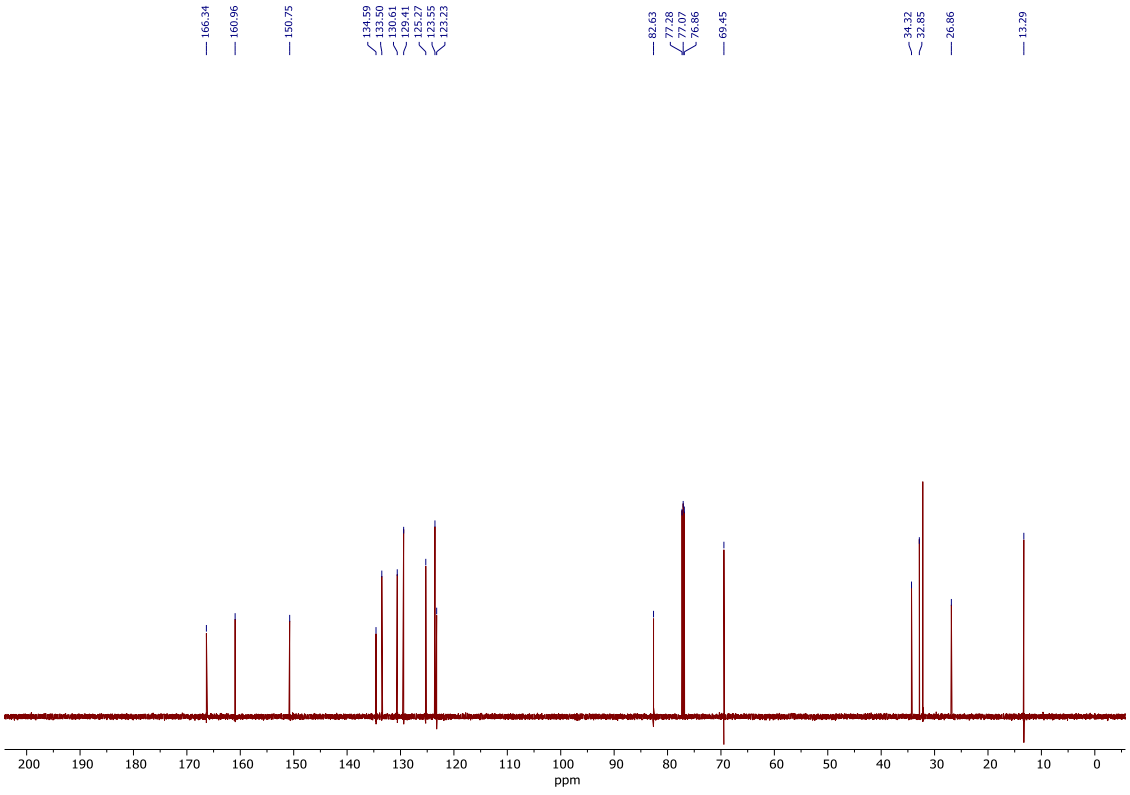
^{1}H NMR ($CDCl_3$) FFF-27



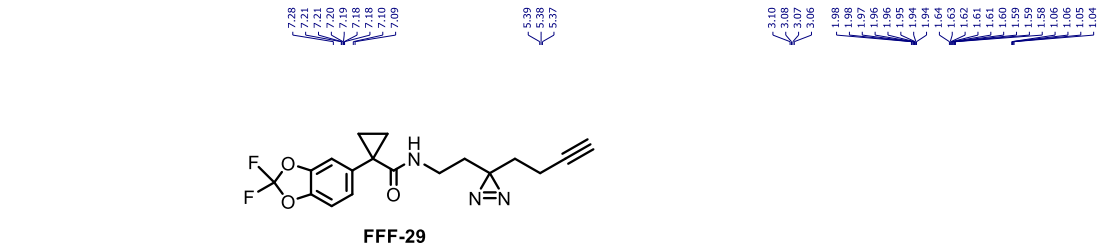
^{1}H NMR ($CDCl_3$) FFF-28



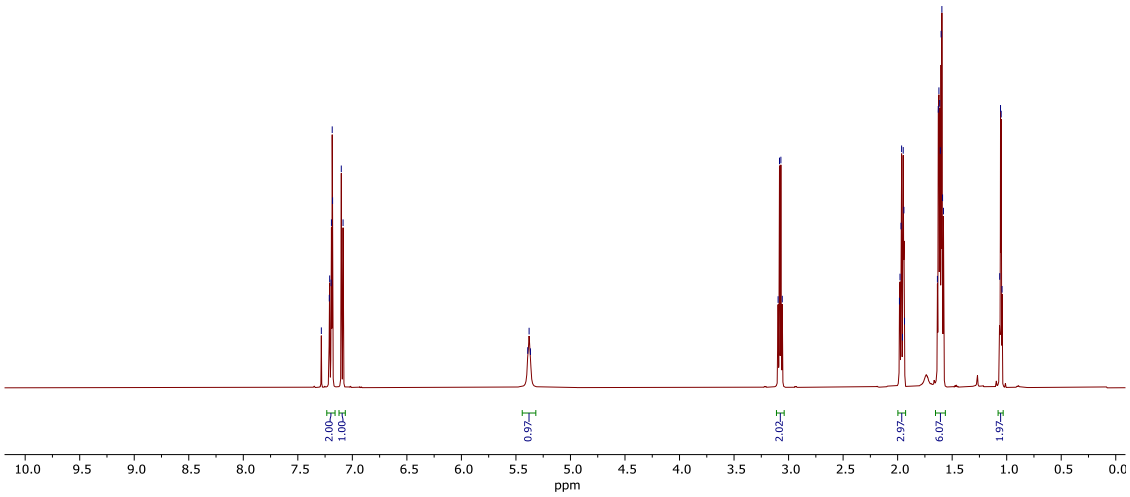
^{13}C NMR ($CDCl_3$) FFF-28



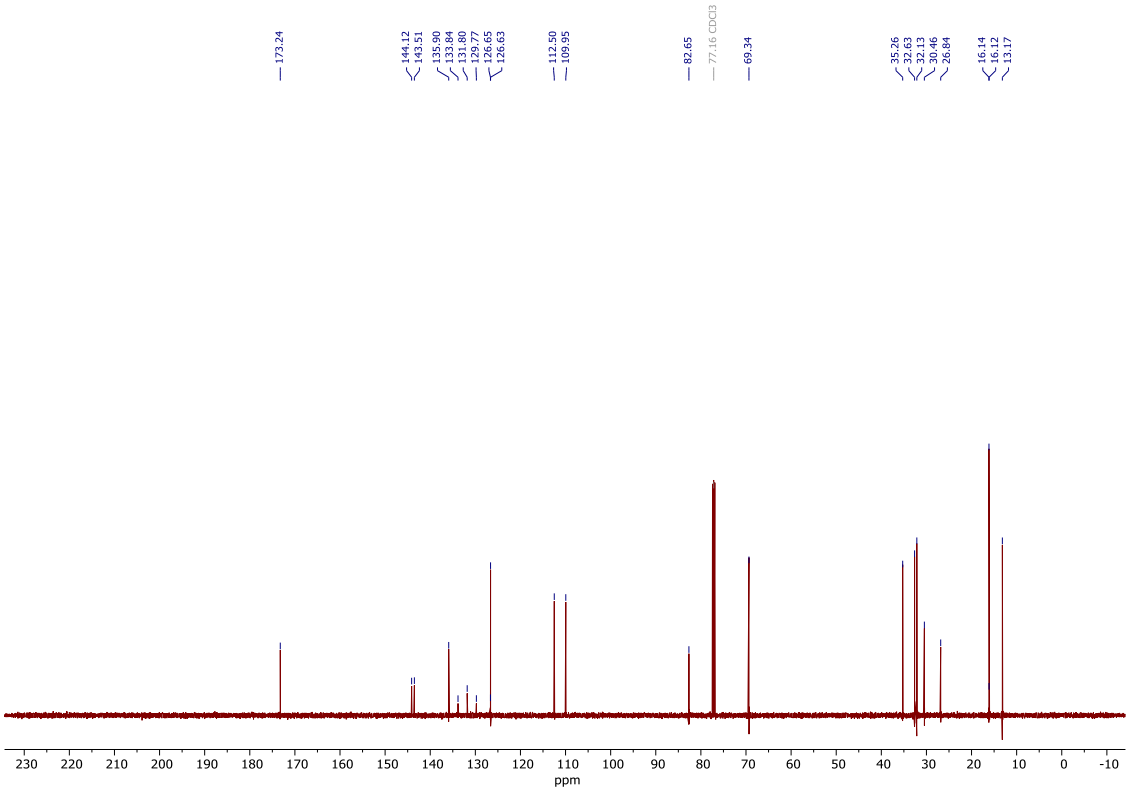
^{1}H NMR ($CDCl_3$) FFF-29



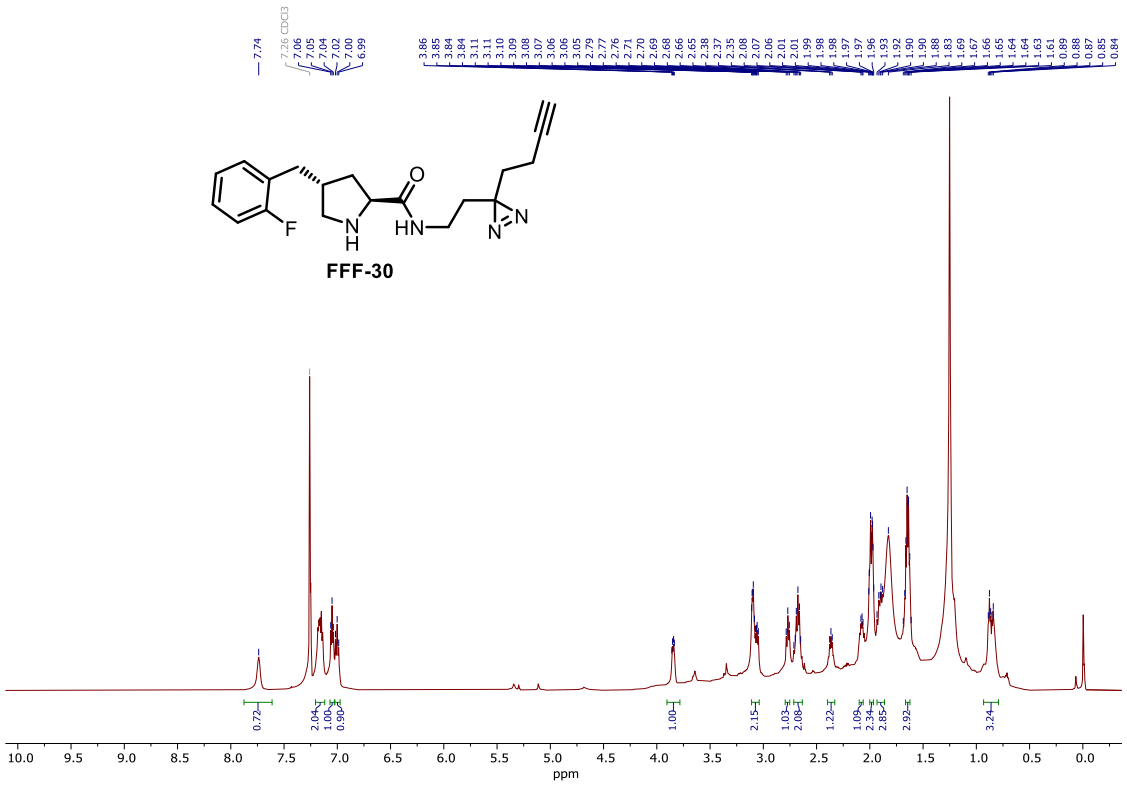
FFF-29



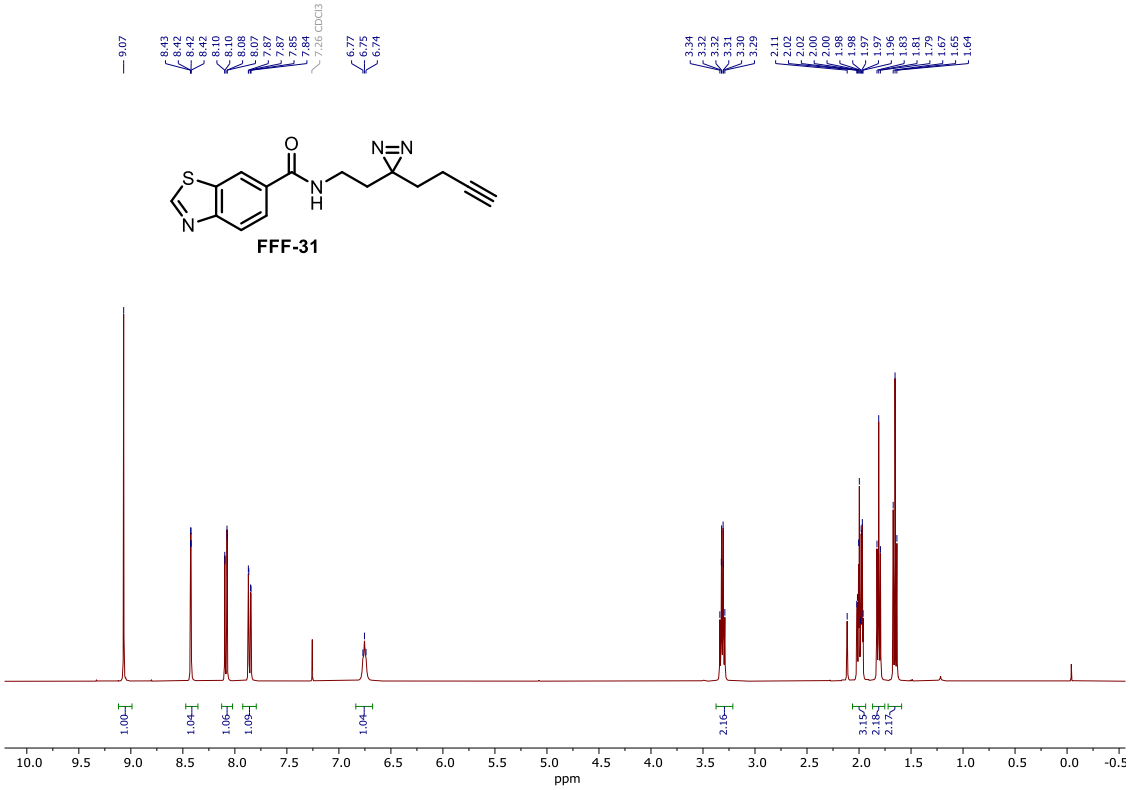
^{13}C NMR ($CDCl_3$) FFF-29



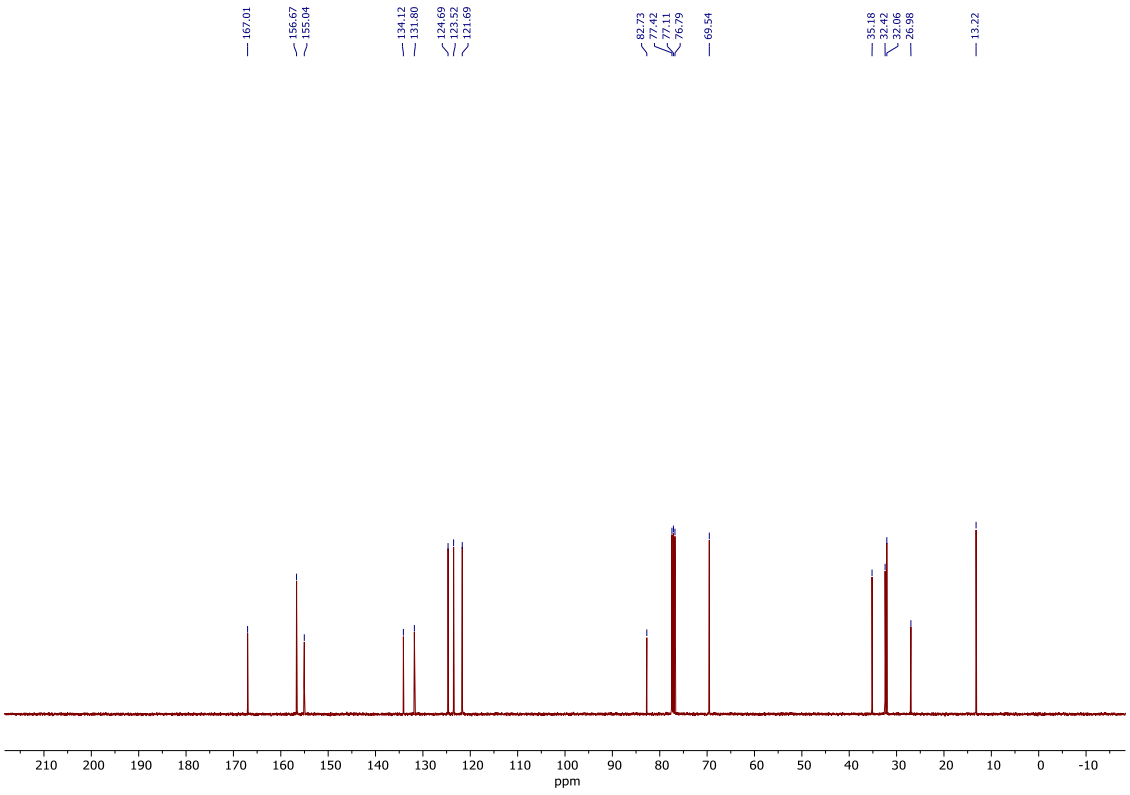
^{1}H NMR ($CDCl_3$) FFF-30



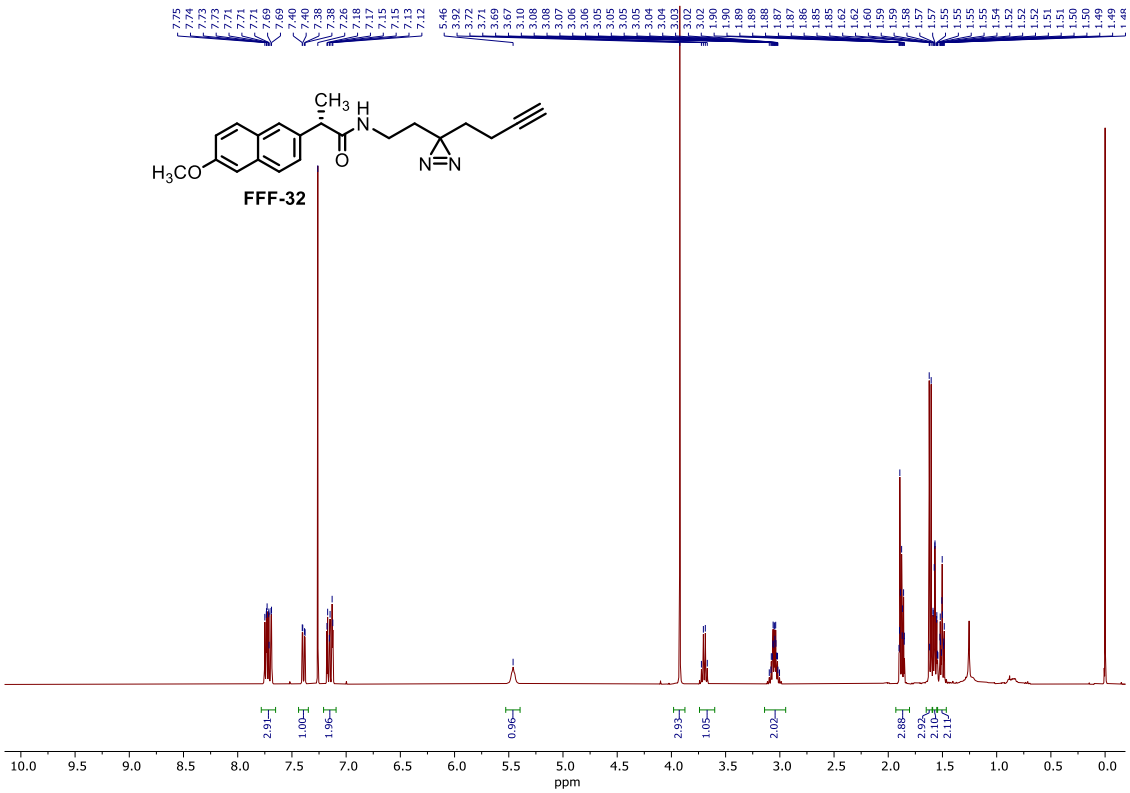
^{1}H NMR ($CDCl_3$) FFF-31



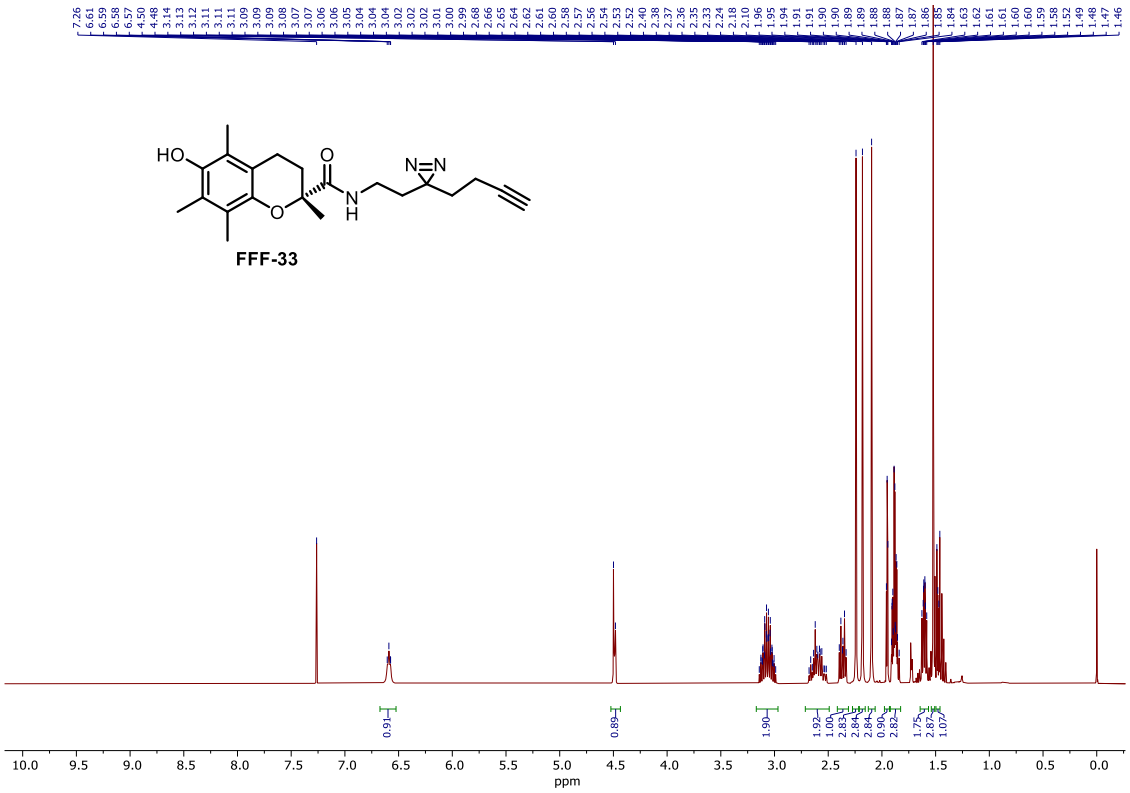
^{1}H NMR ($CDCl_3$) FFF-31



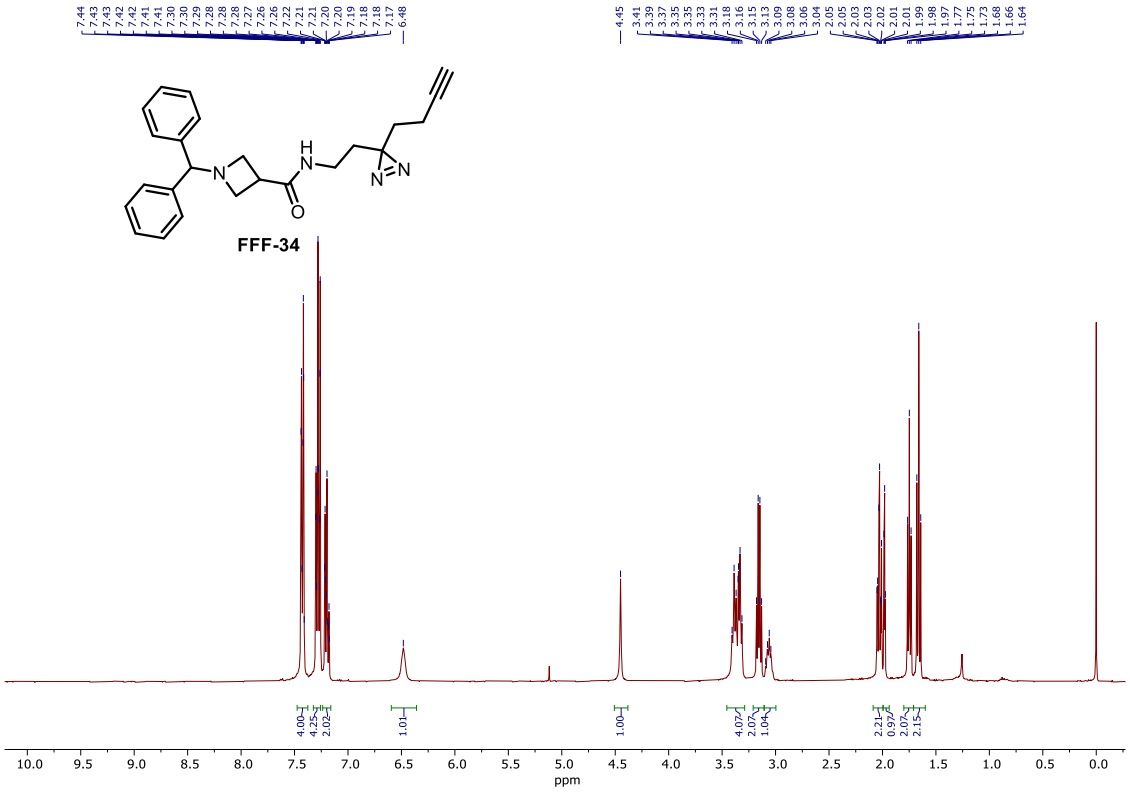
^{1}H NMR ($CDCl_3$) FFF-32



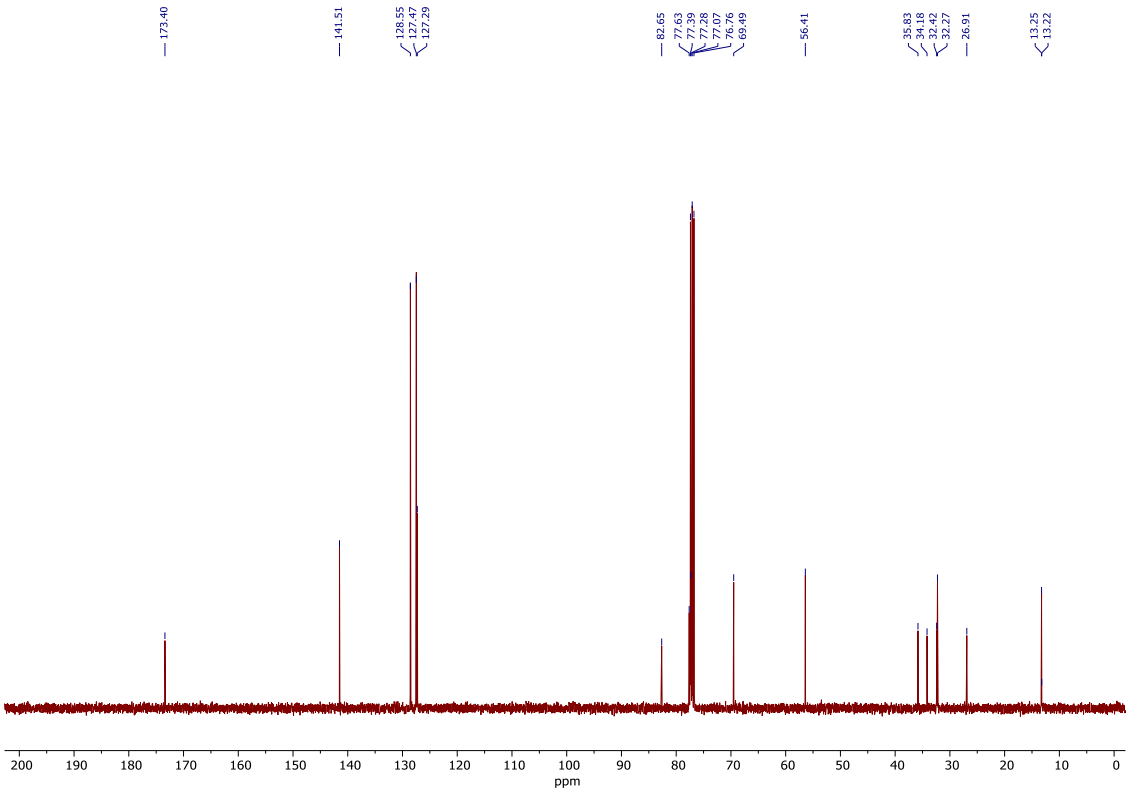
^{1}H NMR ($CDCl_3$) FFF-33



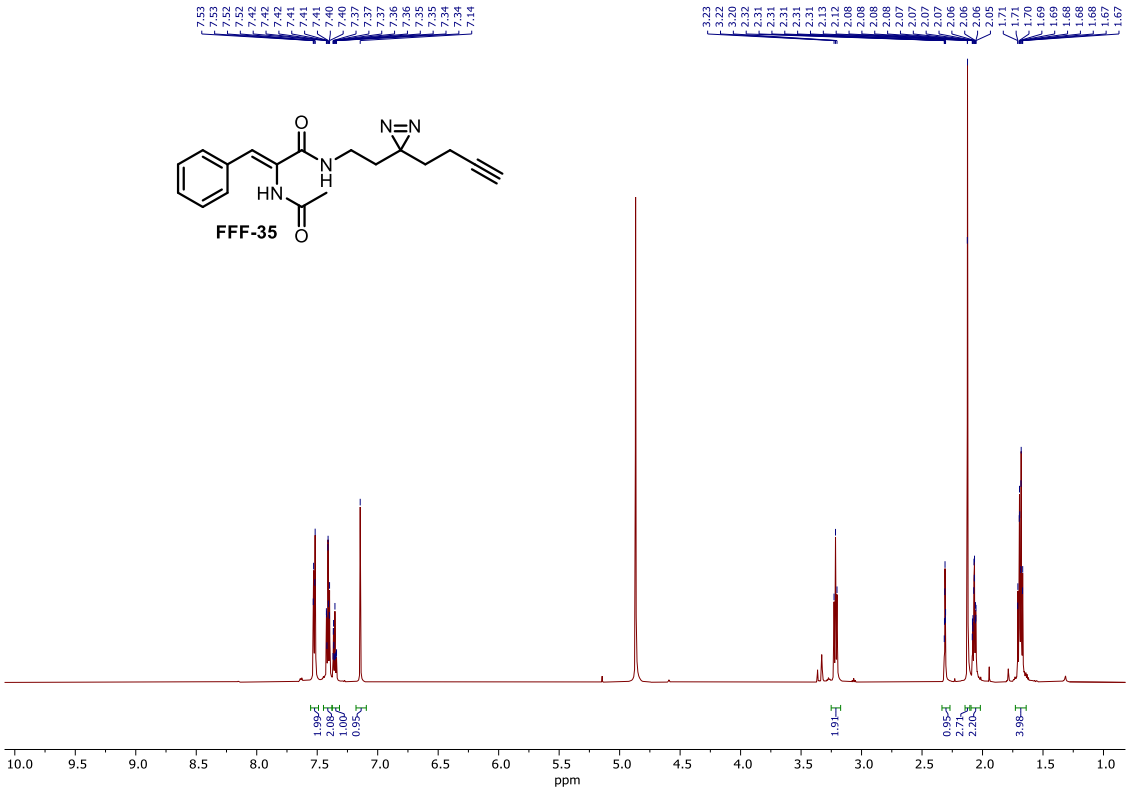
^{1}H NMR ($CDCl_3$) FFF-34



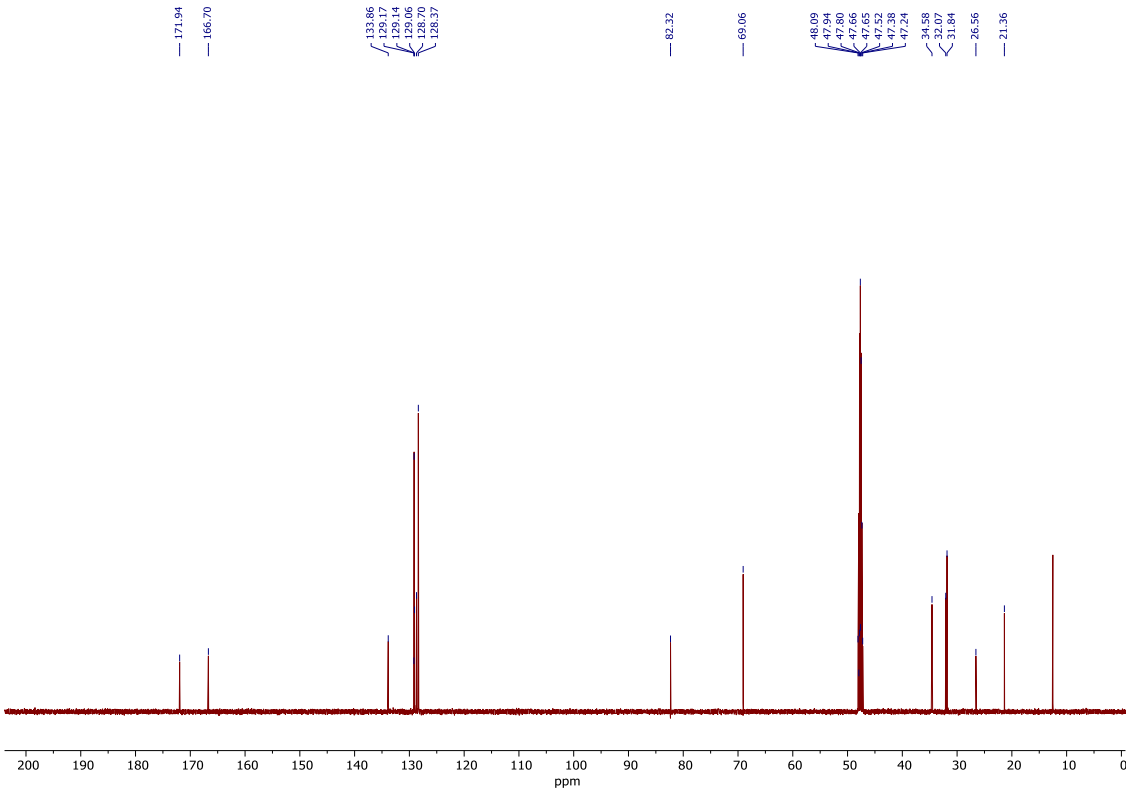
^{13}H NMR ($CDCl_3$) FFF-34



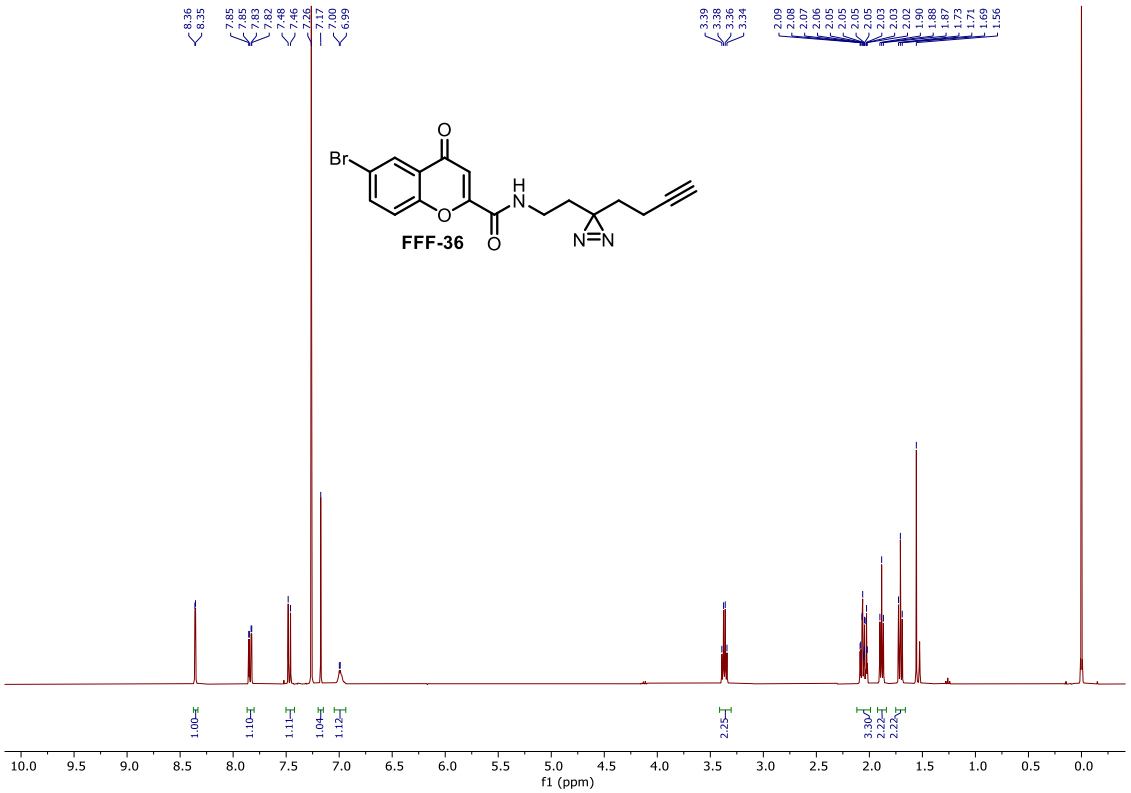
^{1}H NMR ($CDCl_3$) FFF-35



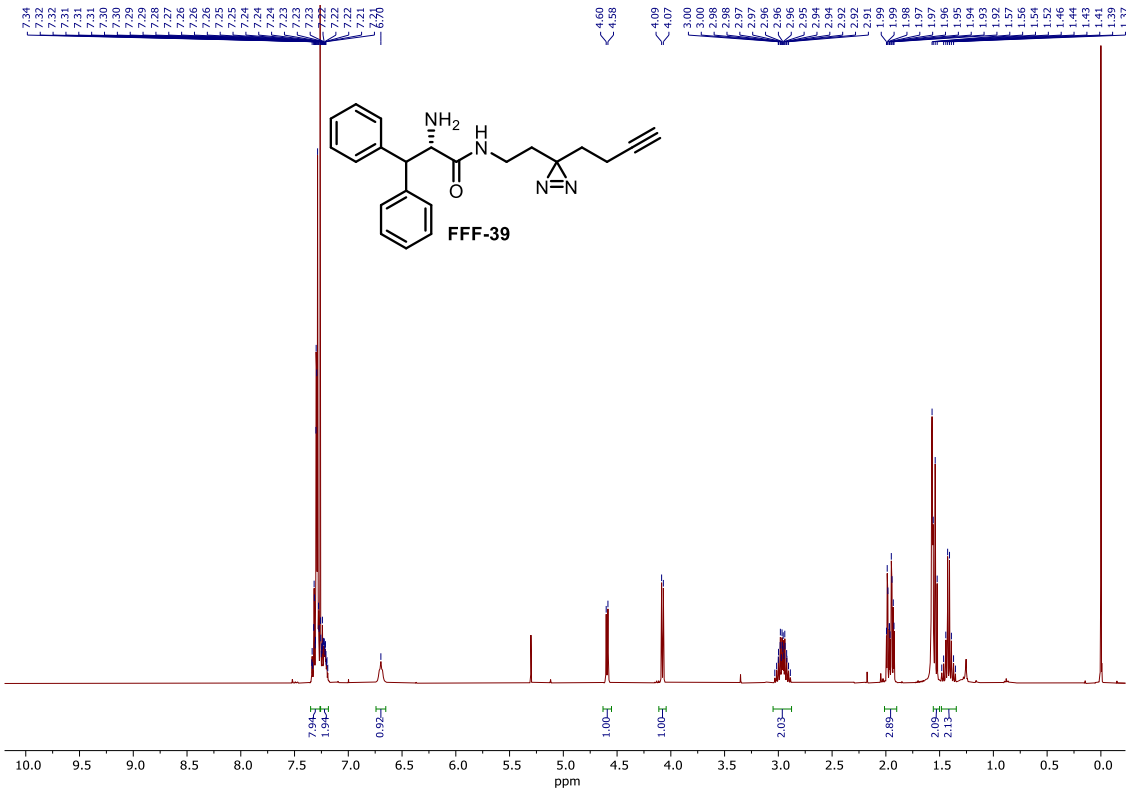
^{13}C NMR ($CDCl_3$) FFF-35



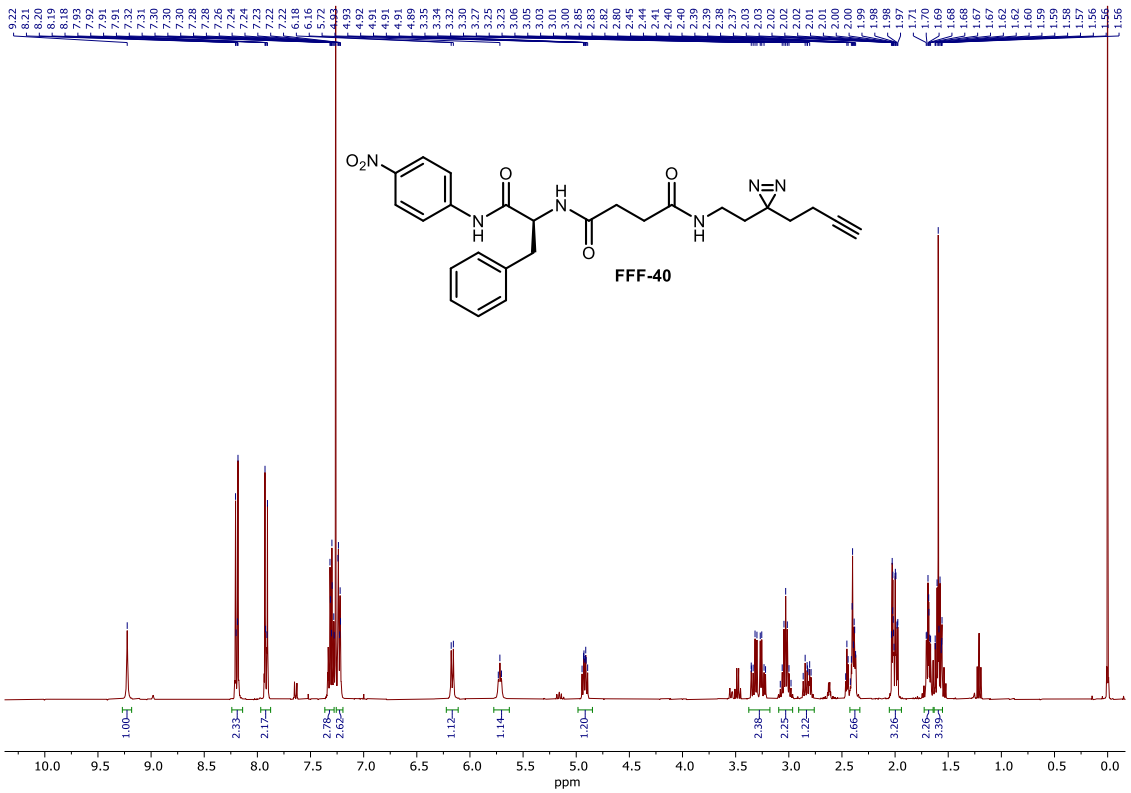
^{1}H NMR ($CDCl_3$) FFF-36



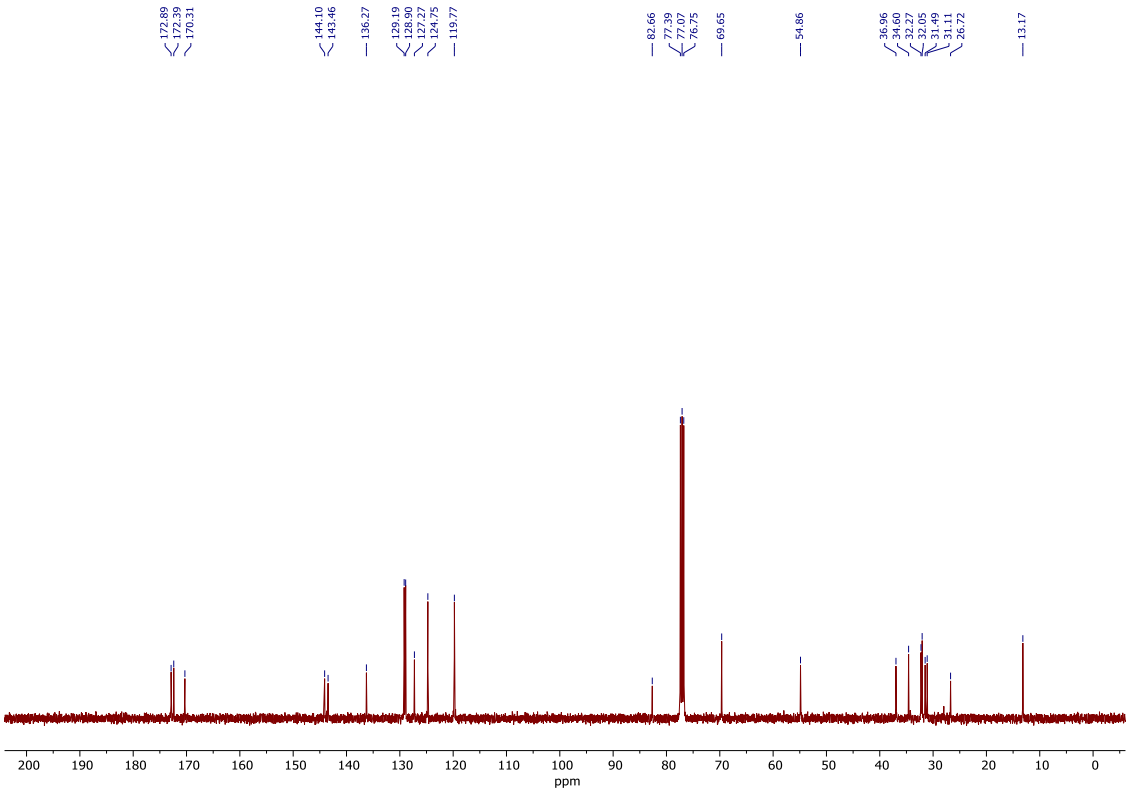
^{1}H NMR ($CDCl_3$) FFF-39



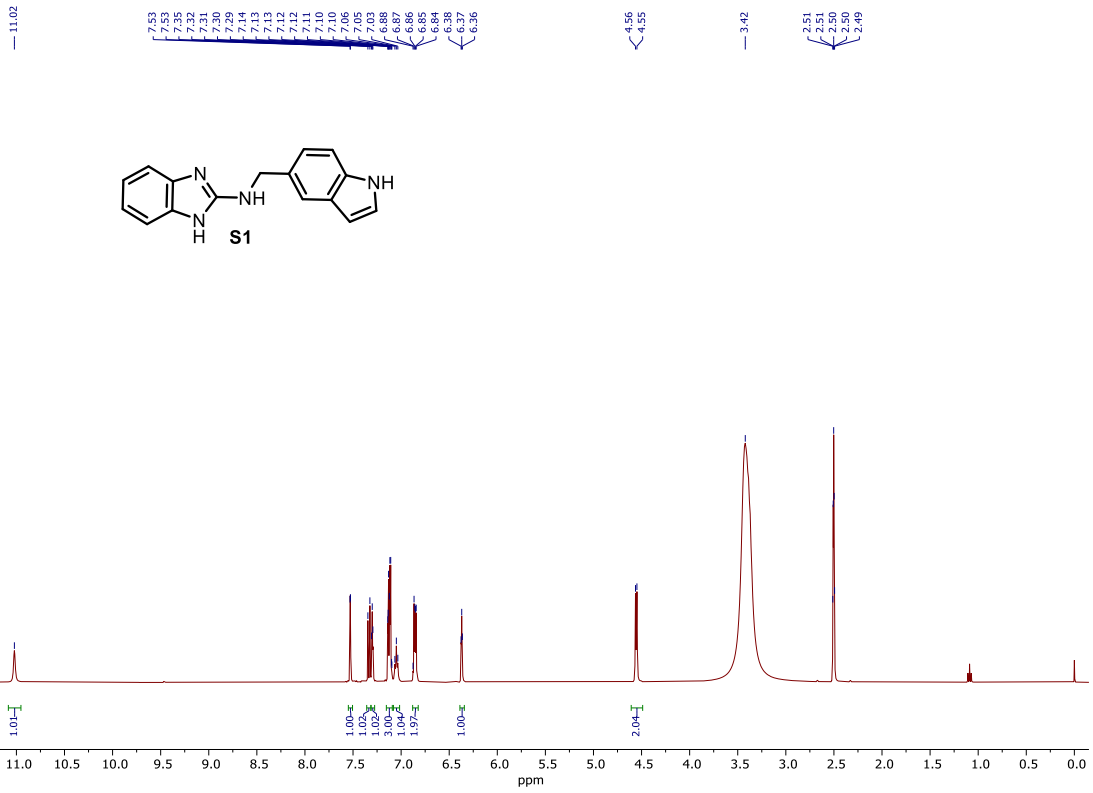
^{1}H NMR ($CDCl_3$) FFF-40



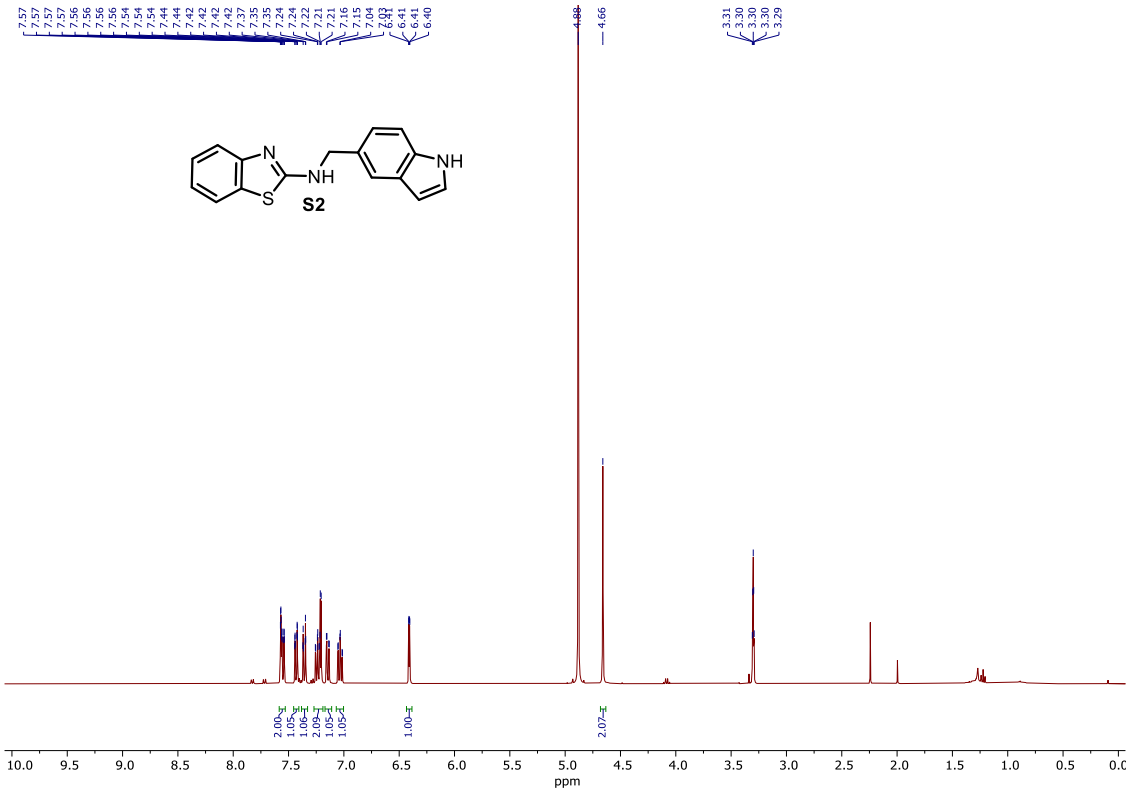
^{13}C NMR ($CDCl_3$) FFF-40



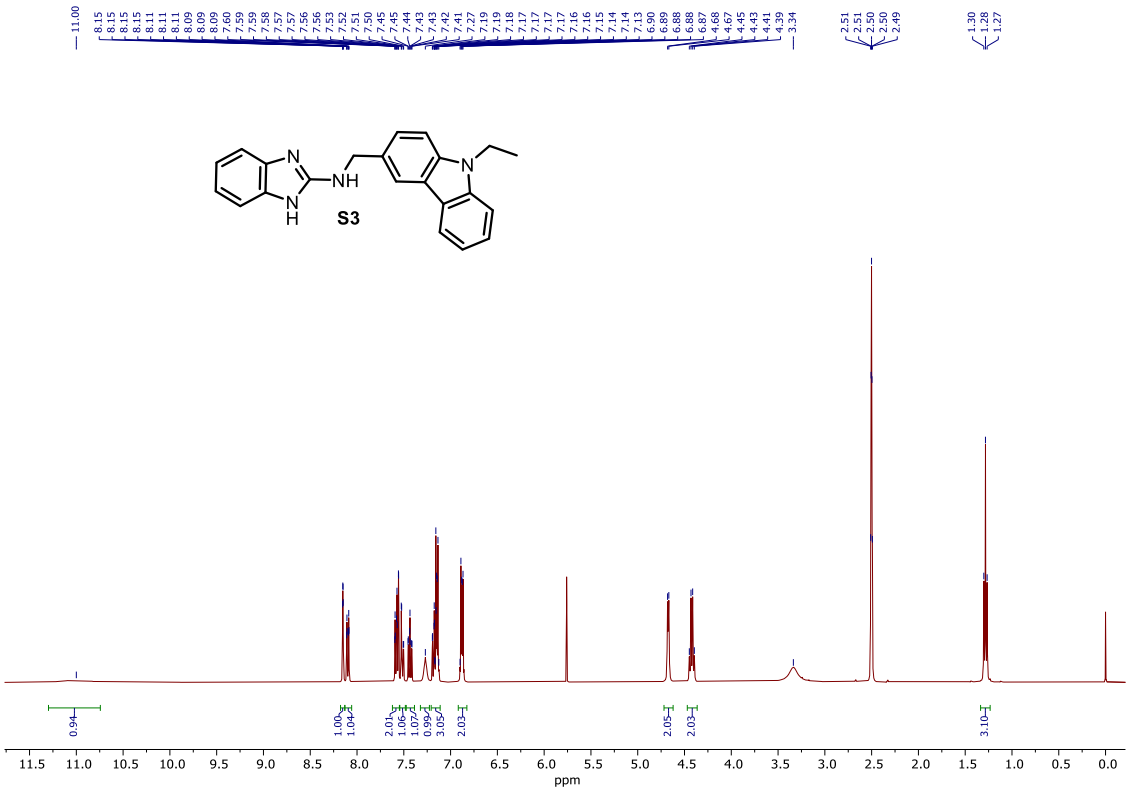
^{1}H NMR (DMSO-d_6) S1



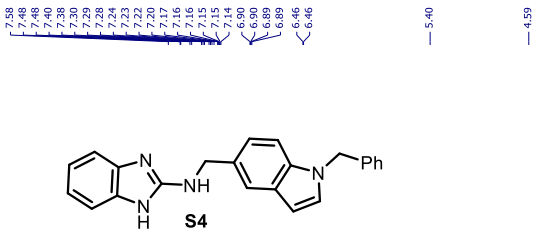
^{1}H NMR (CD_3OD) S2

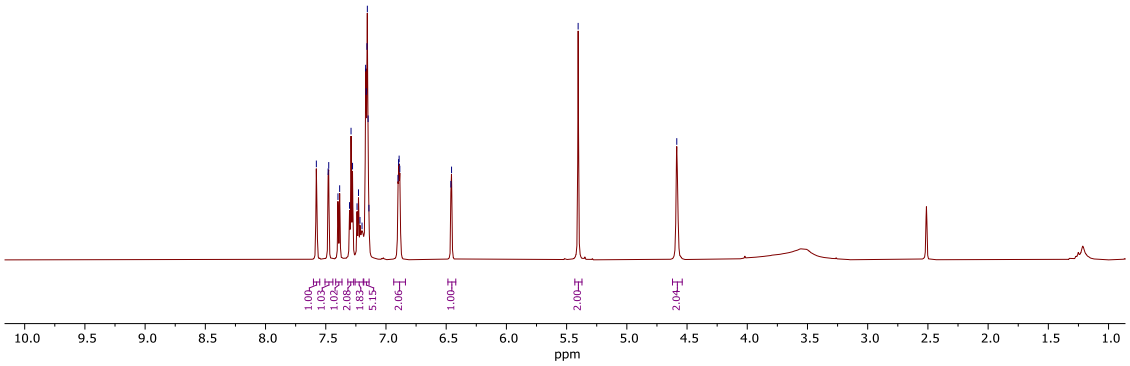


^{1}H NMR (DMSO-d$_6$) S3

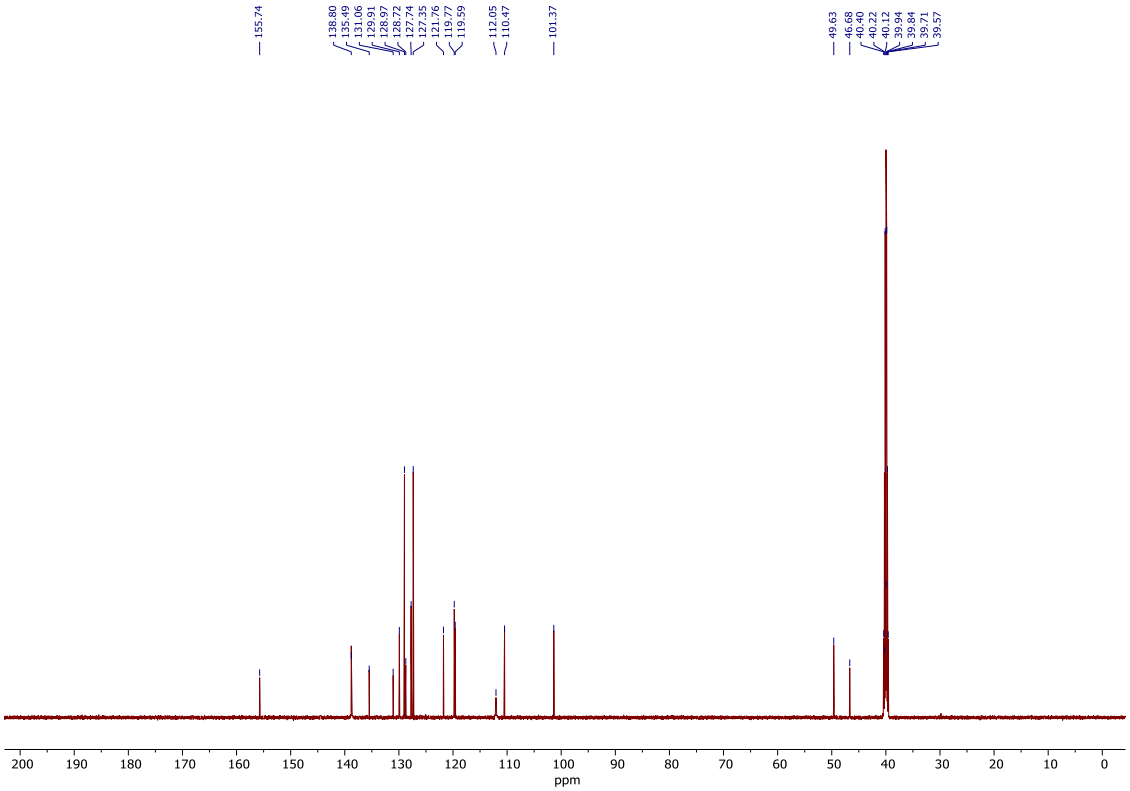


^{1}H NMR (DMSO-d$_6$) S4

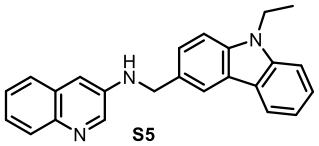




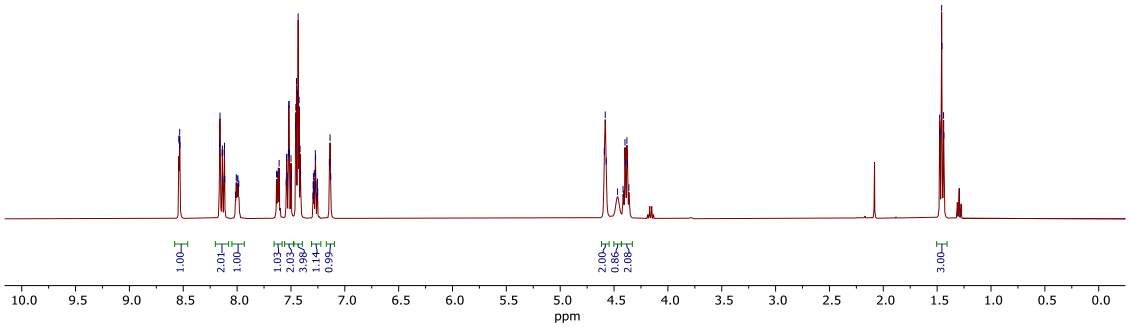
^{13}C NMR ($DMSO-d_6$) S4



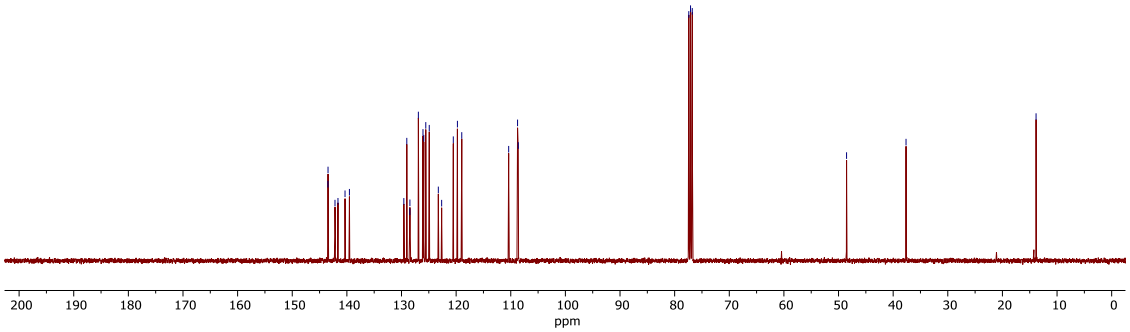
^{1}H NMR ($CDCl_3$) S5



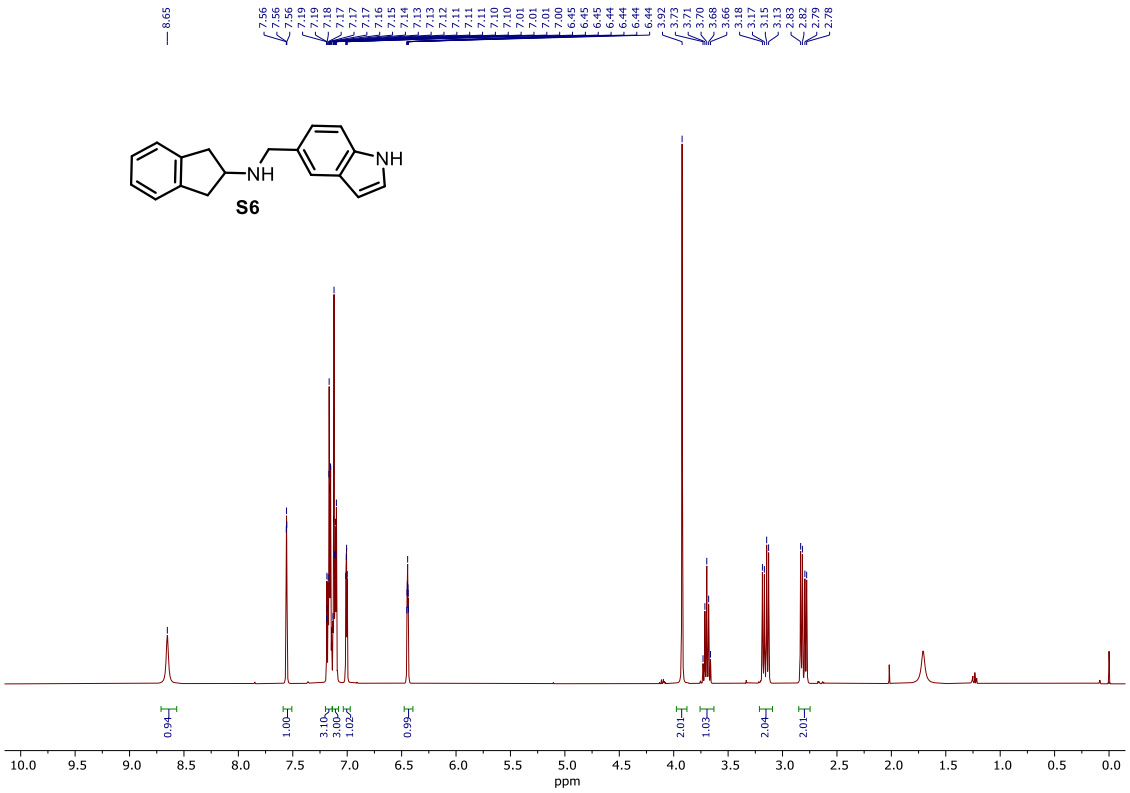
S5



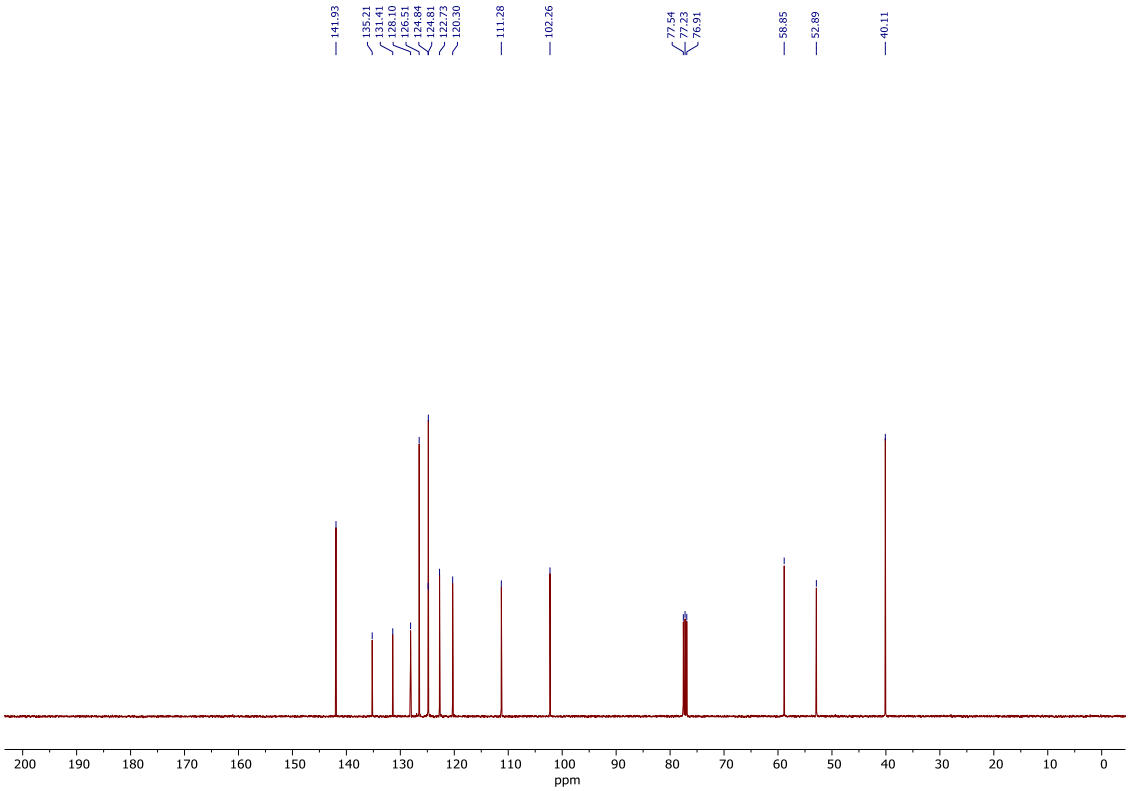
^{13}C NMR ($CDCl_3$) S5



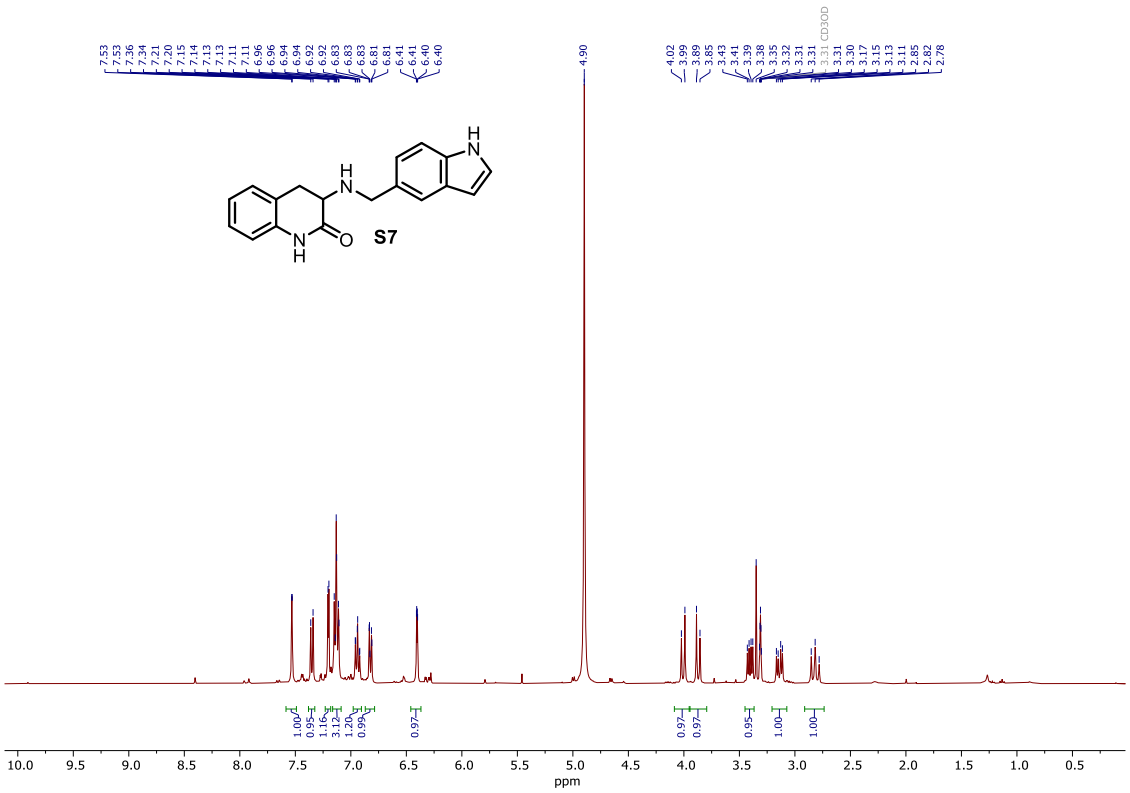
^{1}H NMR ($CDCl_3$) S6



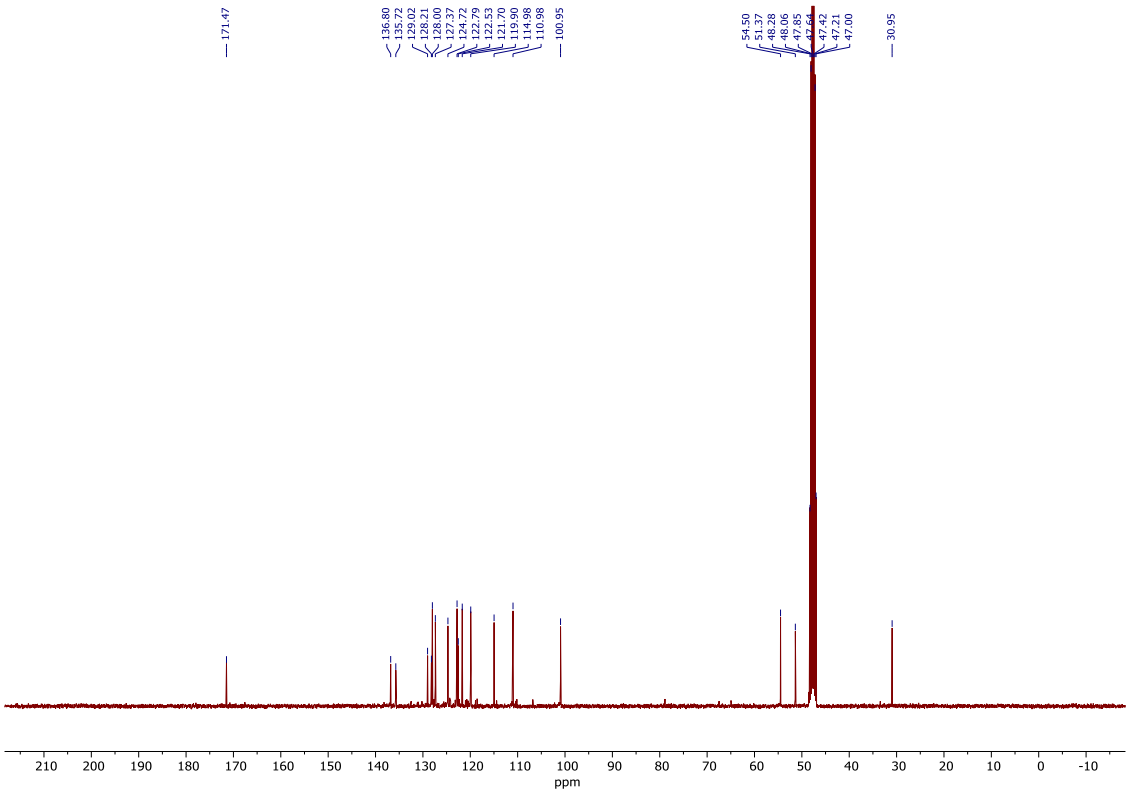
^{13}C NMR ($CDCl_3$) S6



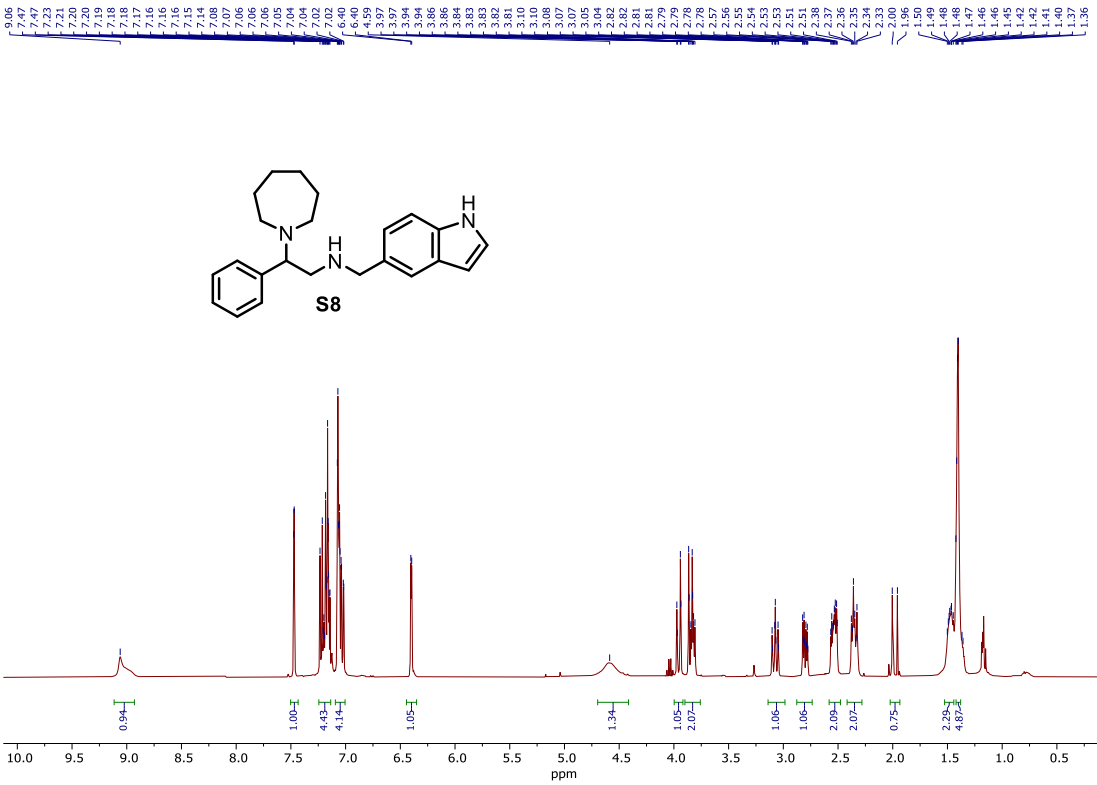
^{1}H NMR (CD_3OD) S7



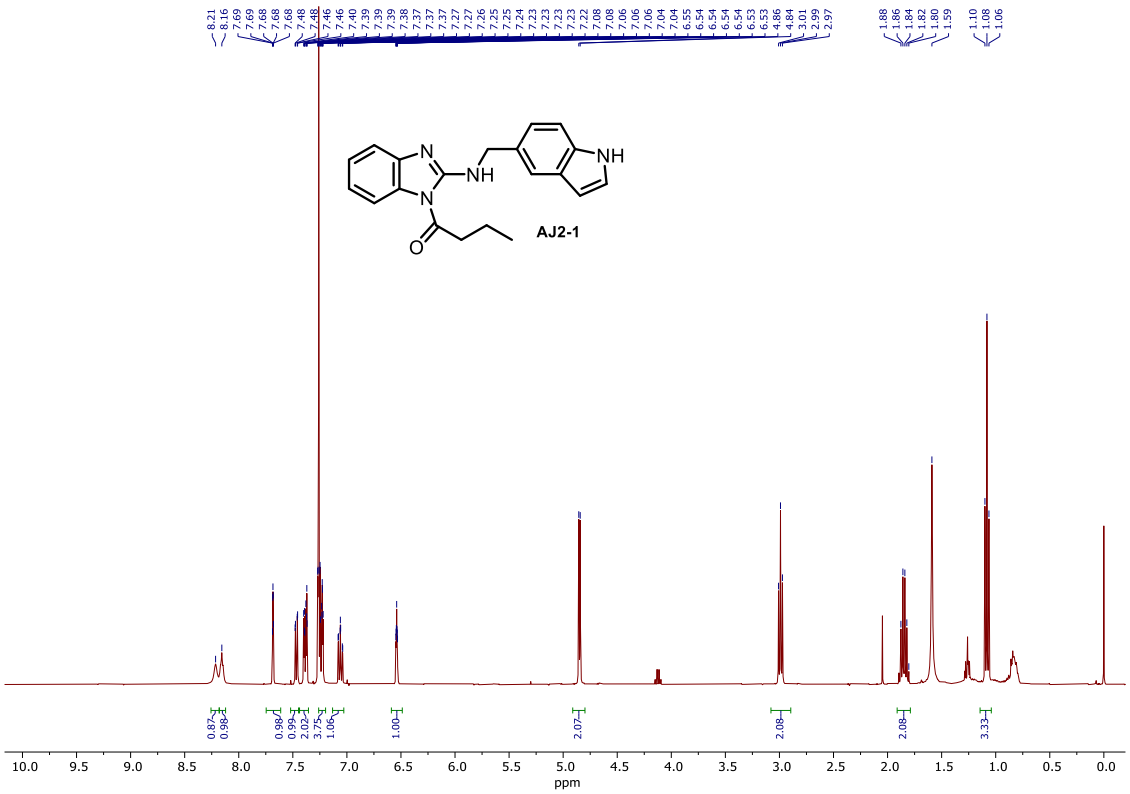
^{13}C NMR (CD_3OD) S7



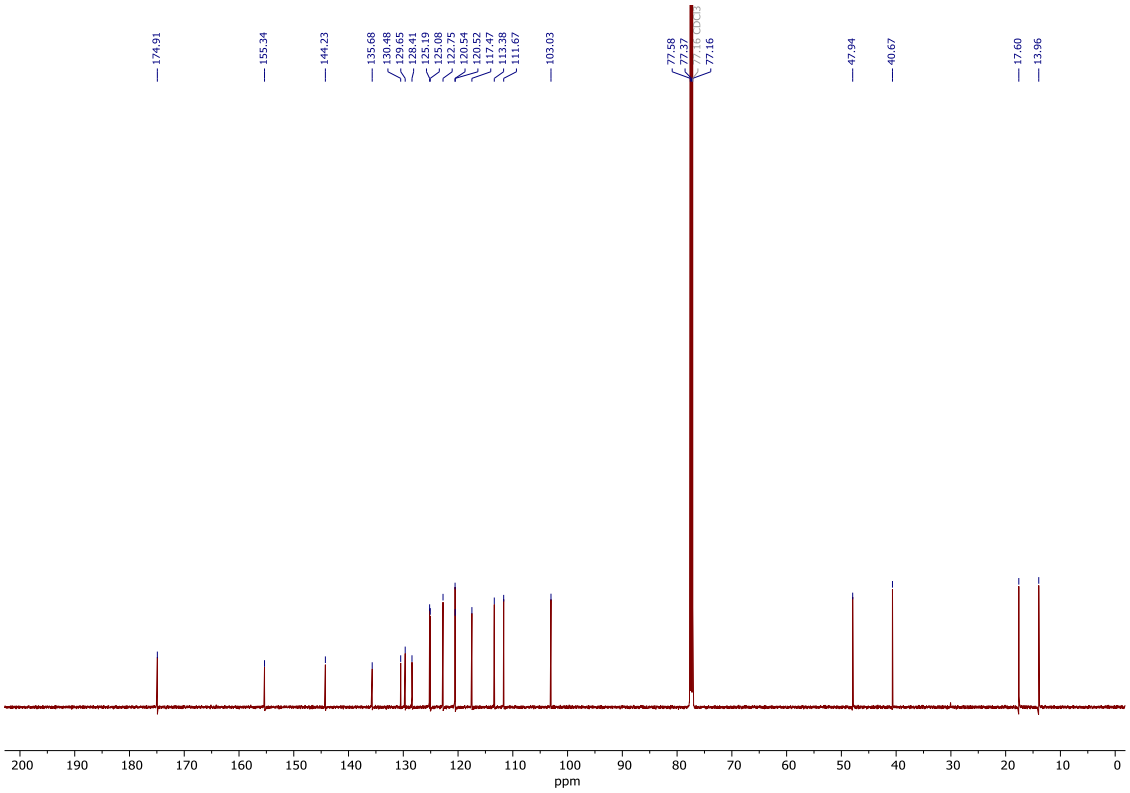
^{1}H NMR ($CDCl_3$) S8



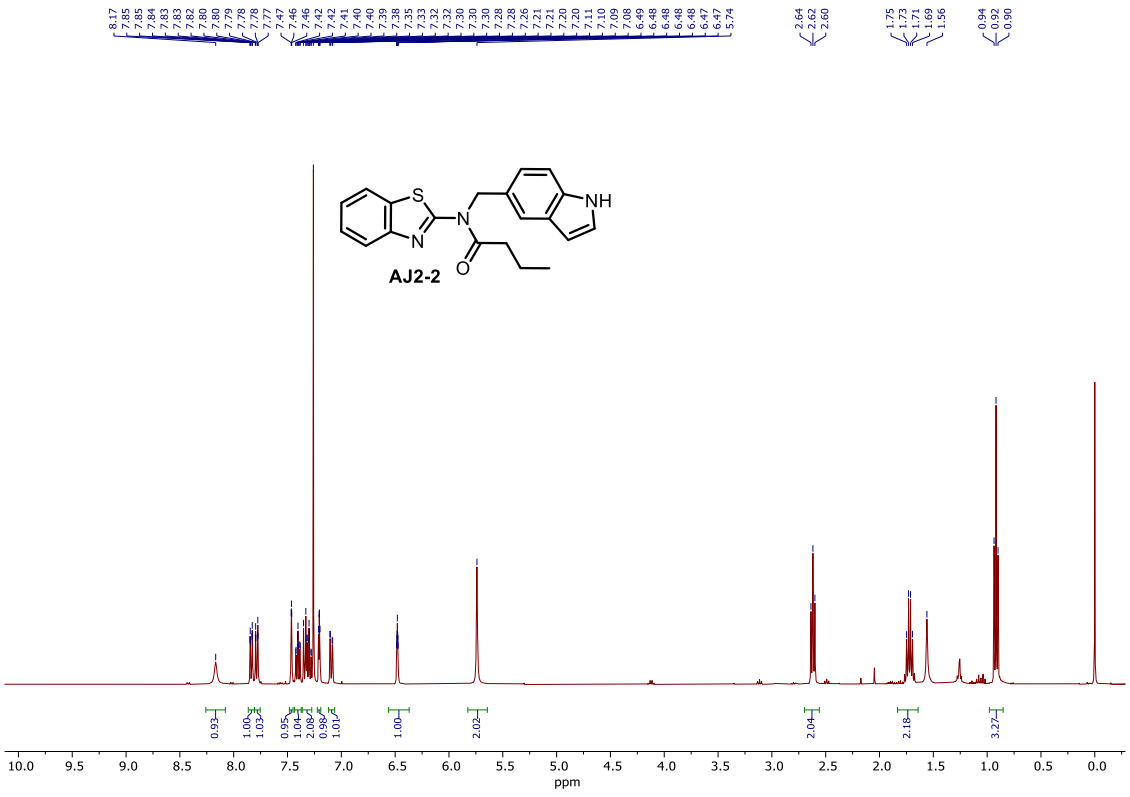
^{1}H NMR ($CDCl_3$) AJ2-1



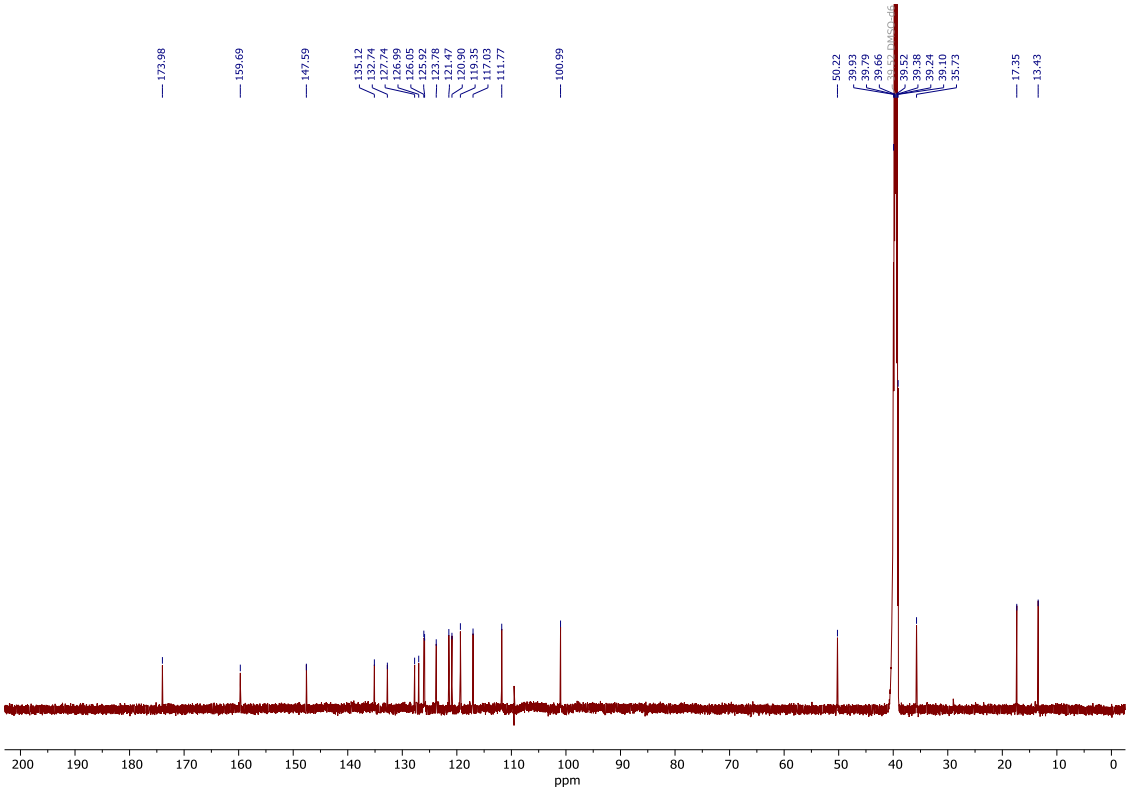
^{13}C NMR ($CDCl_3$) AJ2-1



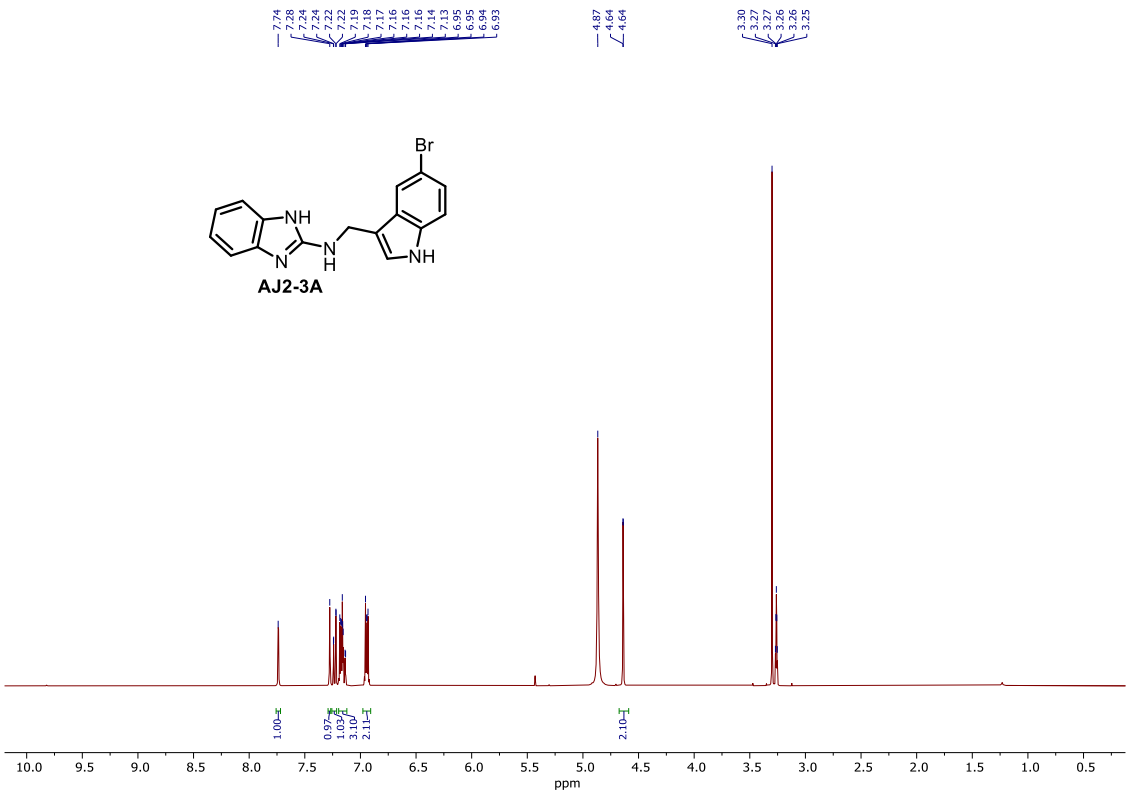
^{1}H NMR ($CDCl_3$) AJ2-2



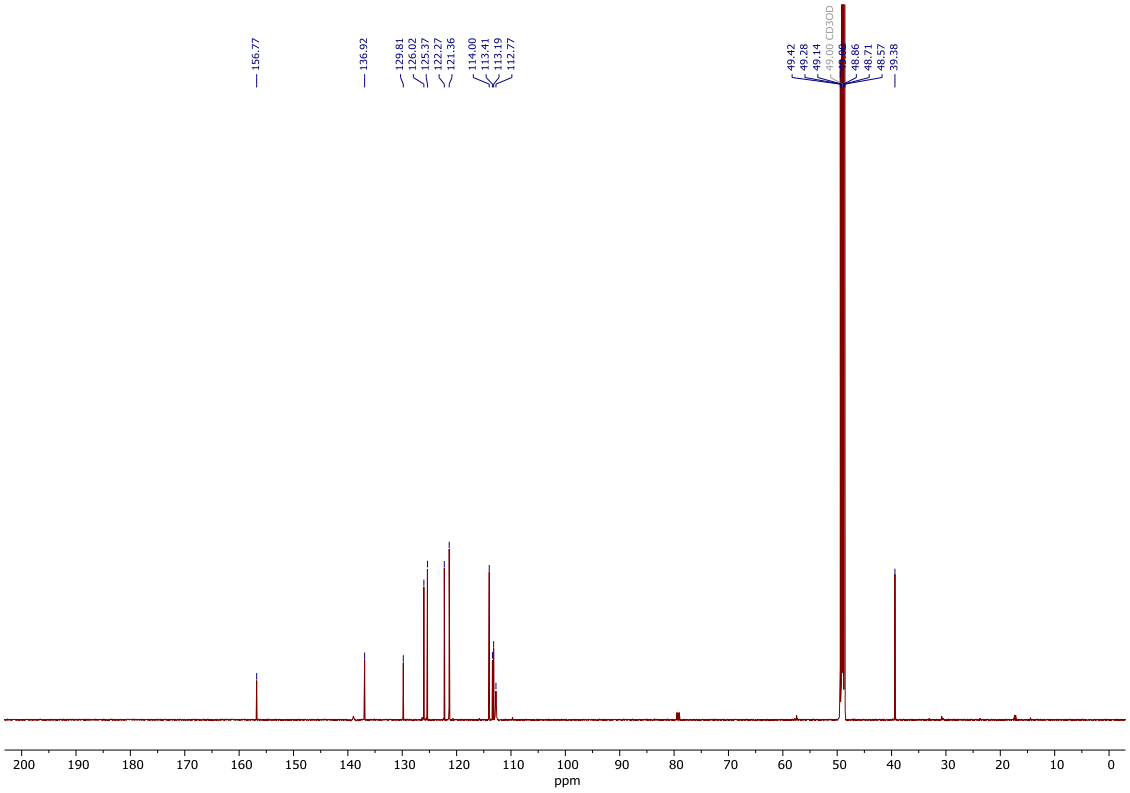
^{13}C NMR ($CDCl_3$) AJ2-2



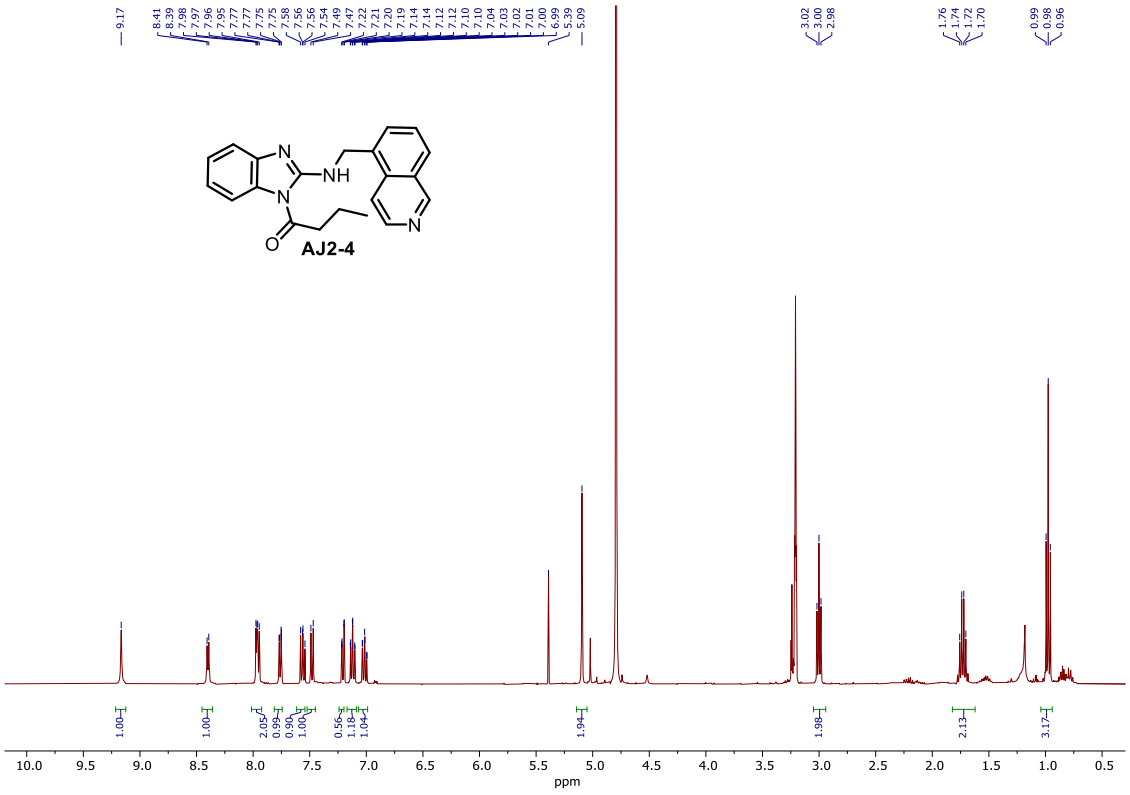
^{1}H NMR ($CDCl_3$) AJ2-3A



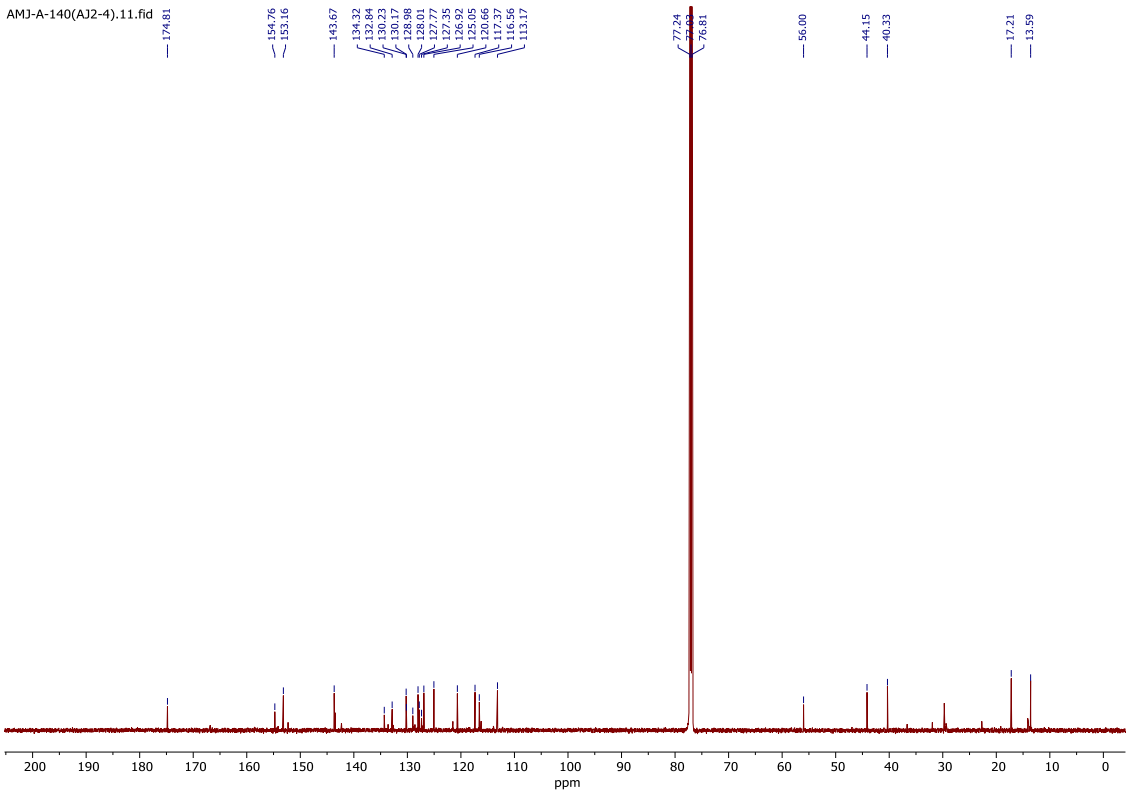
^{13}C NMR ($CDCl_3$) AJ2-3A



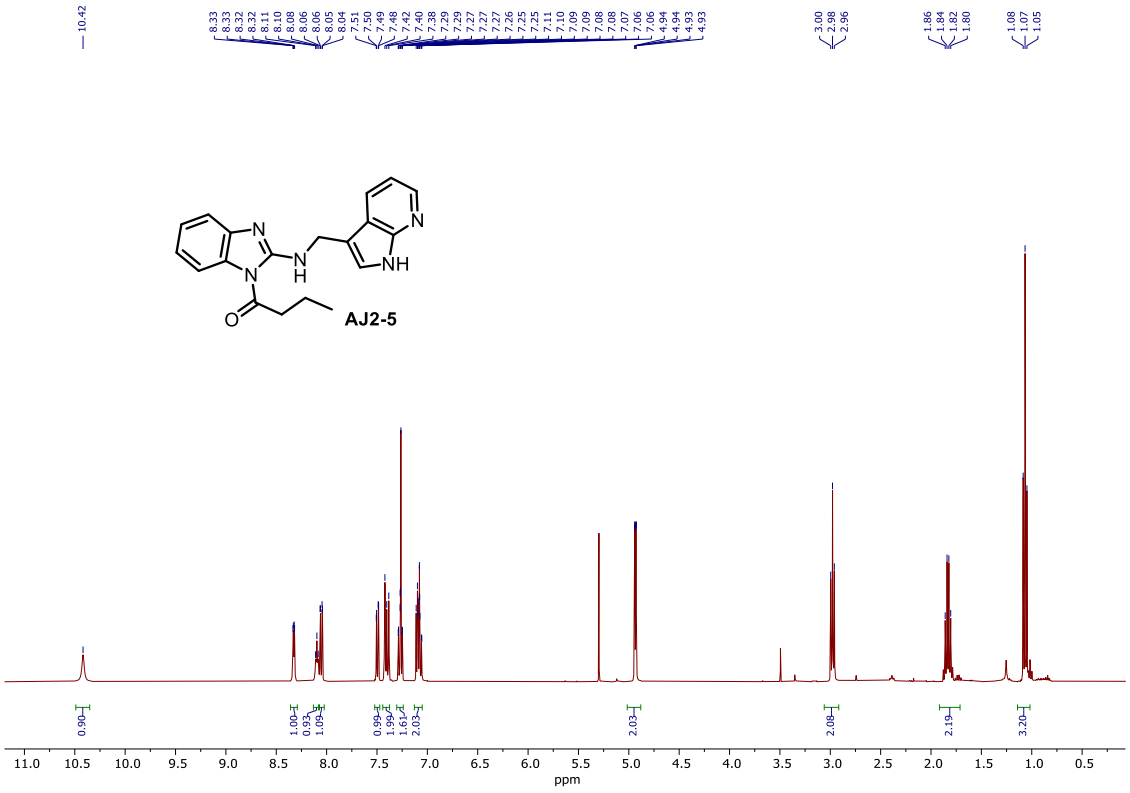
^{1}H NMR (CD_3OD) AJ2-4



^{13}C NMR ($CDCl_3$) AJ2-4

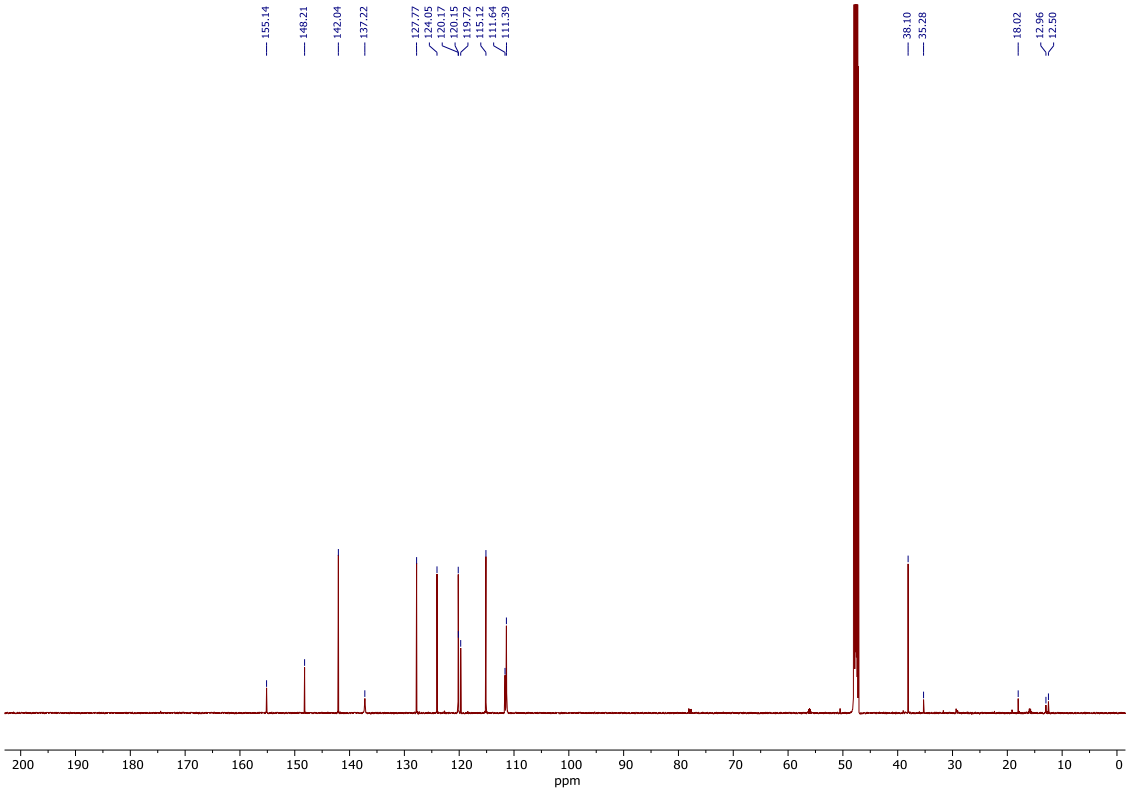


^{1}H NMR ($CDCl_3$) AJ2-5

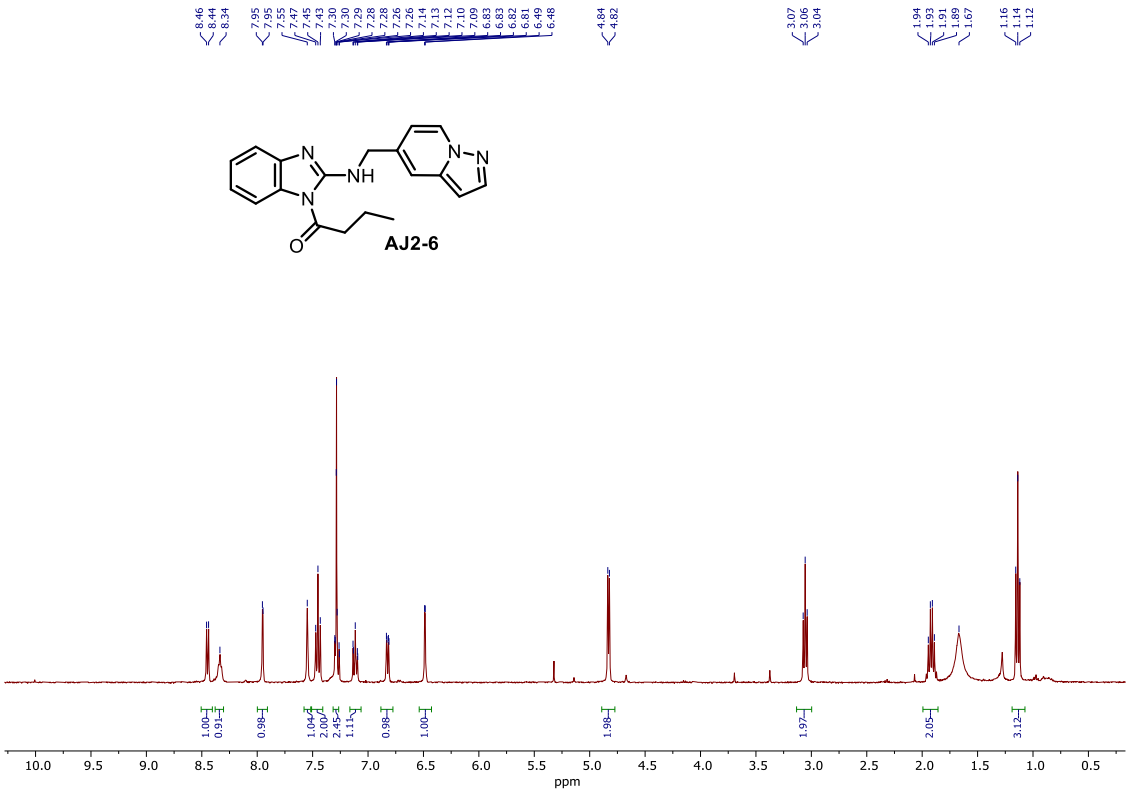


AJ2-5

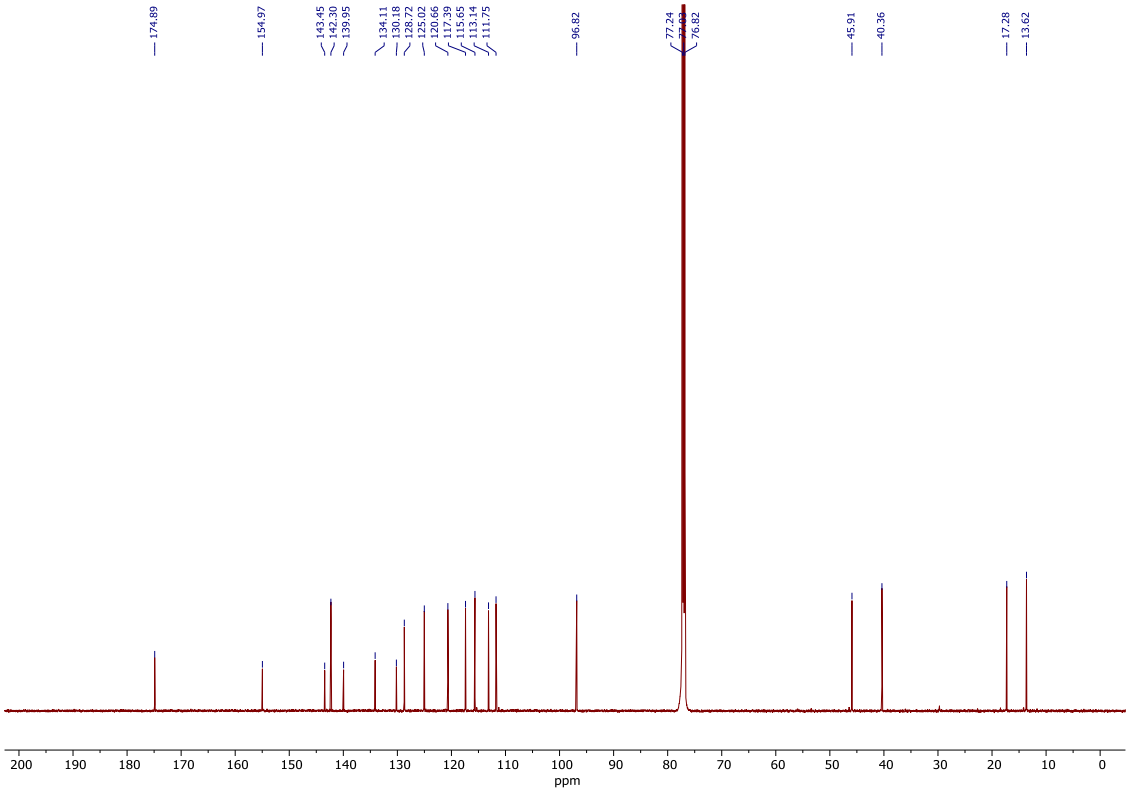
^{13}C NMR ($CDCl_3$) AJ2-5



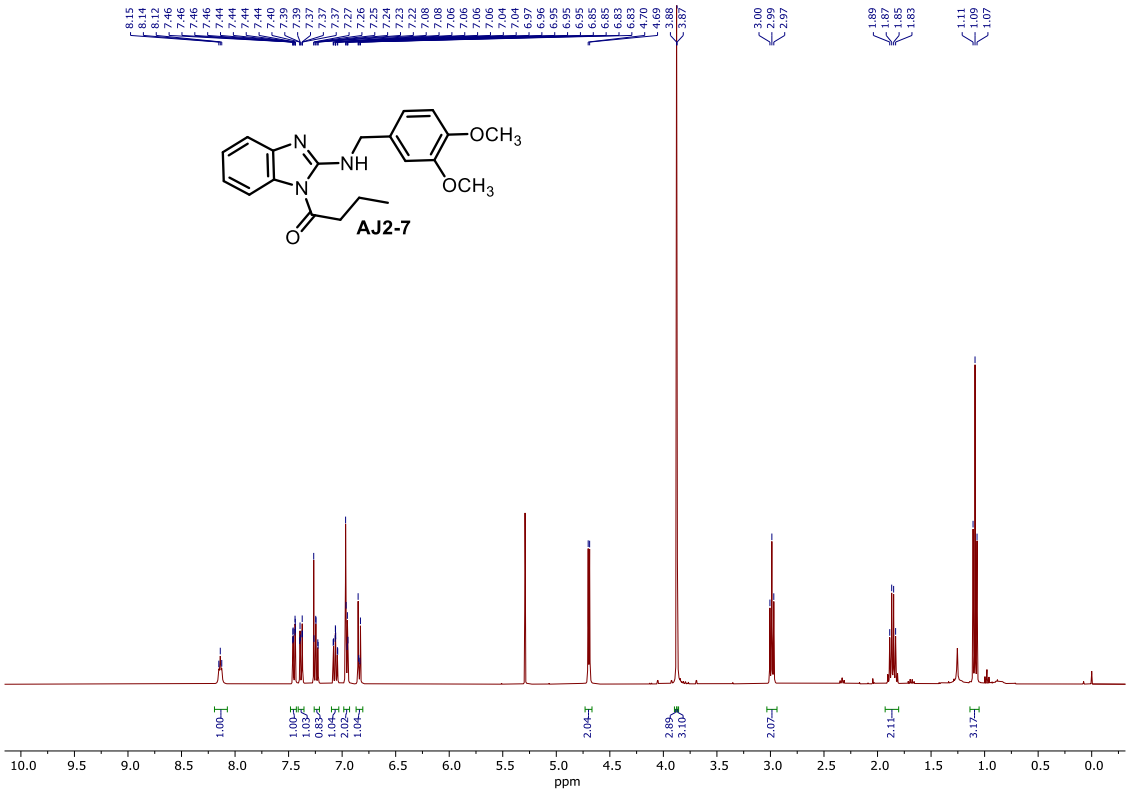
^{1}H NMR ($CDCl_3$) AJ2-6



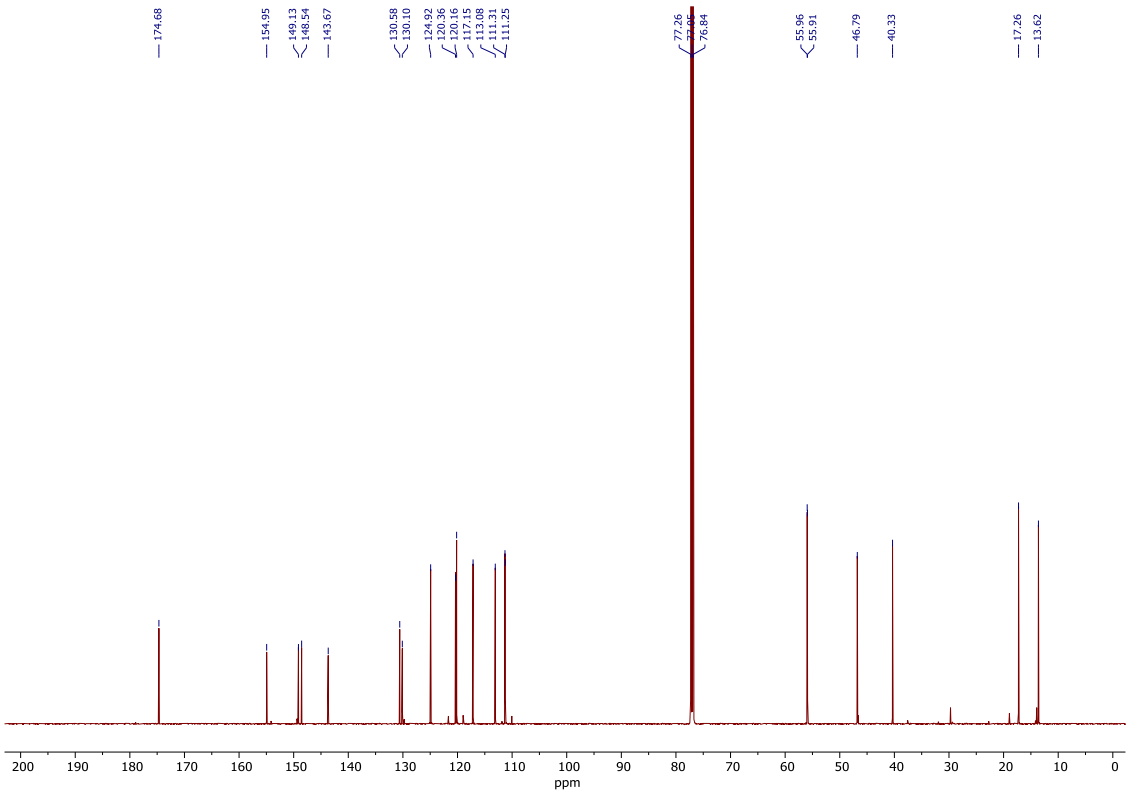
^{13}C NMR ($CDCl_3$) AJ2-6



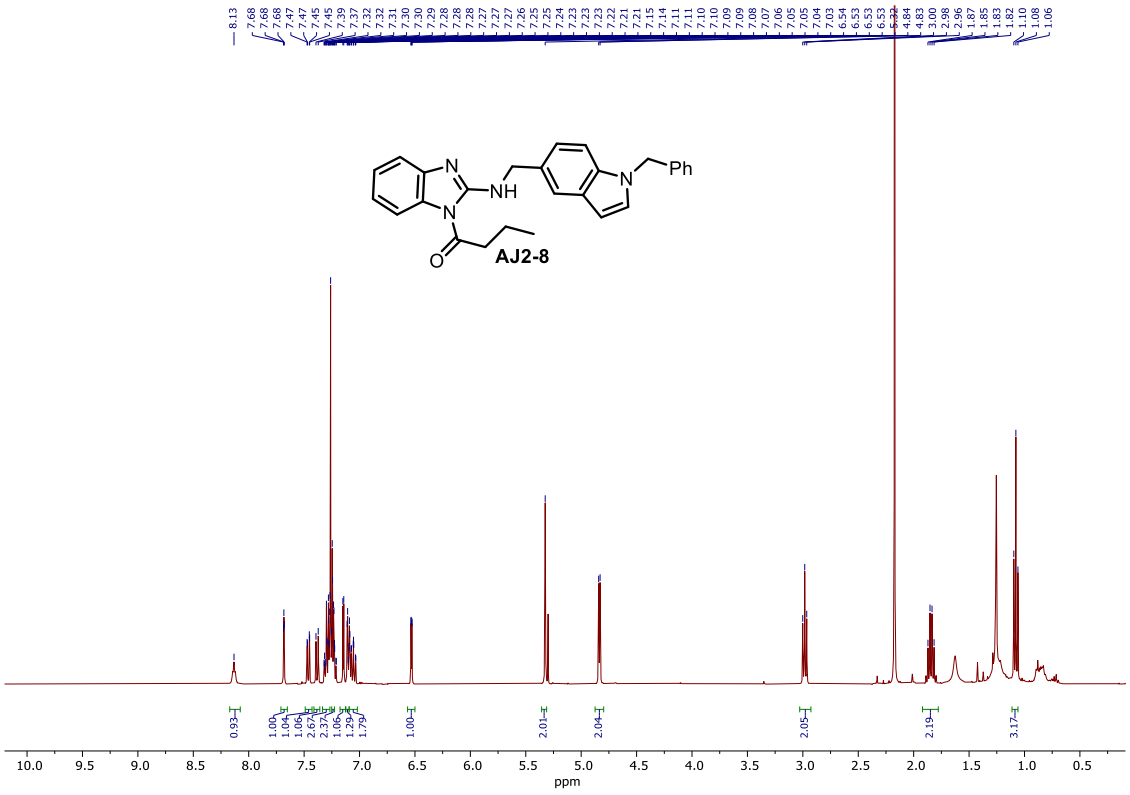
^{1}H NMR ($CDCl_3$) AJ2-7



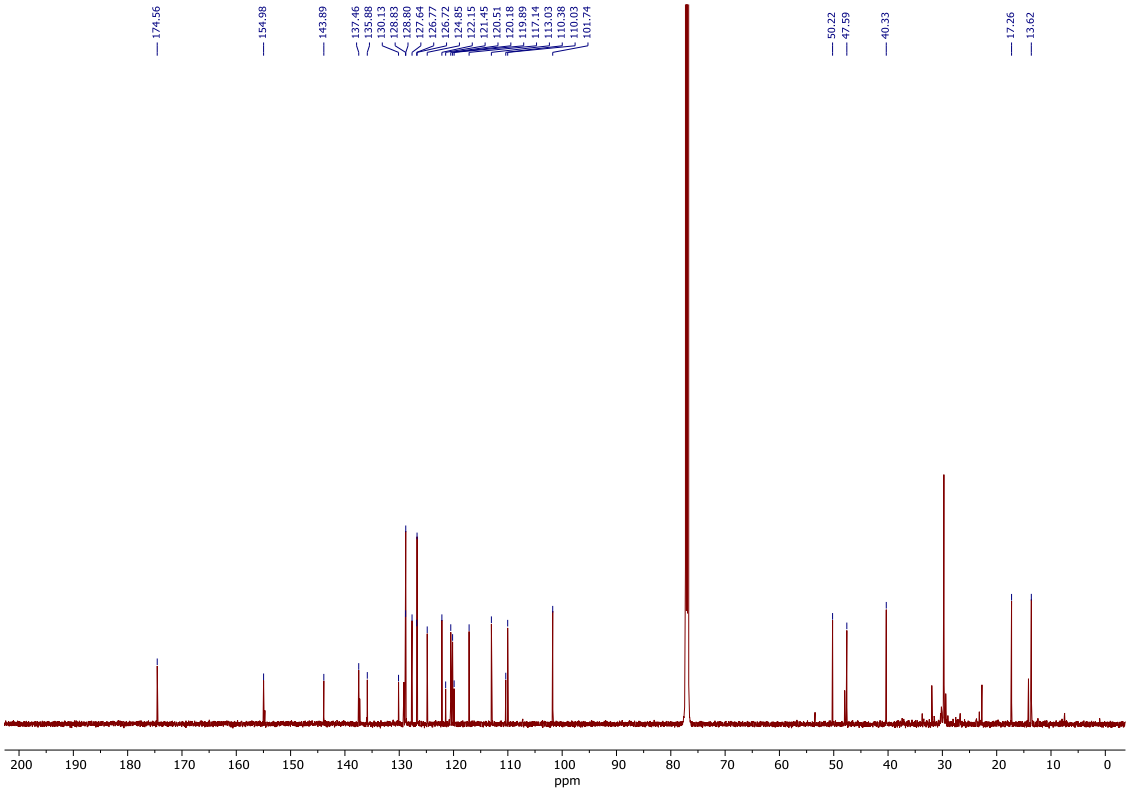
^{13}C NMR ($CDCl_3$) AJ2-7



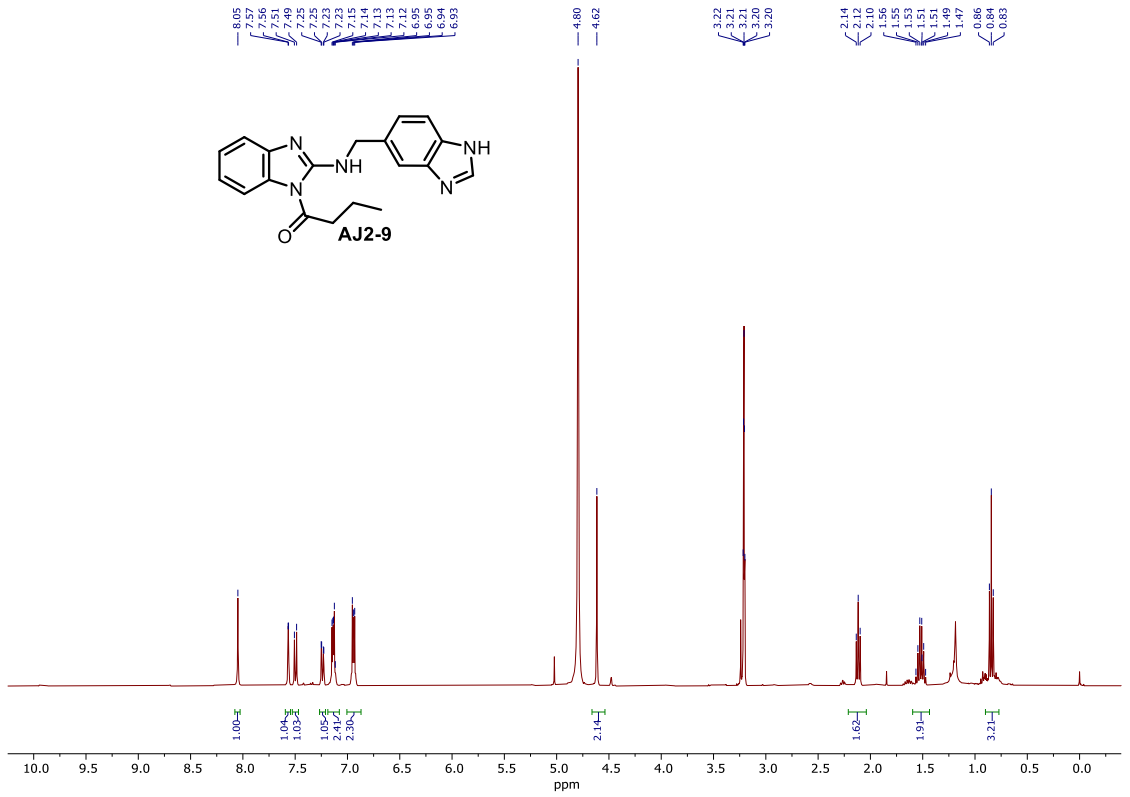
^{1}H NMR ($CDCl_3$) AJ2-8



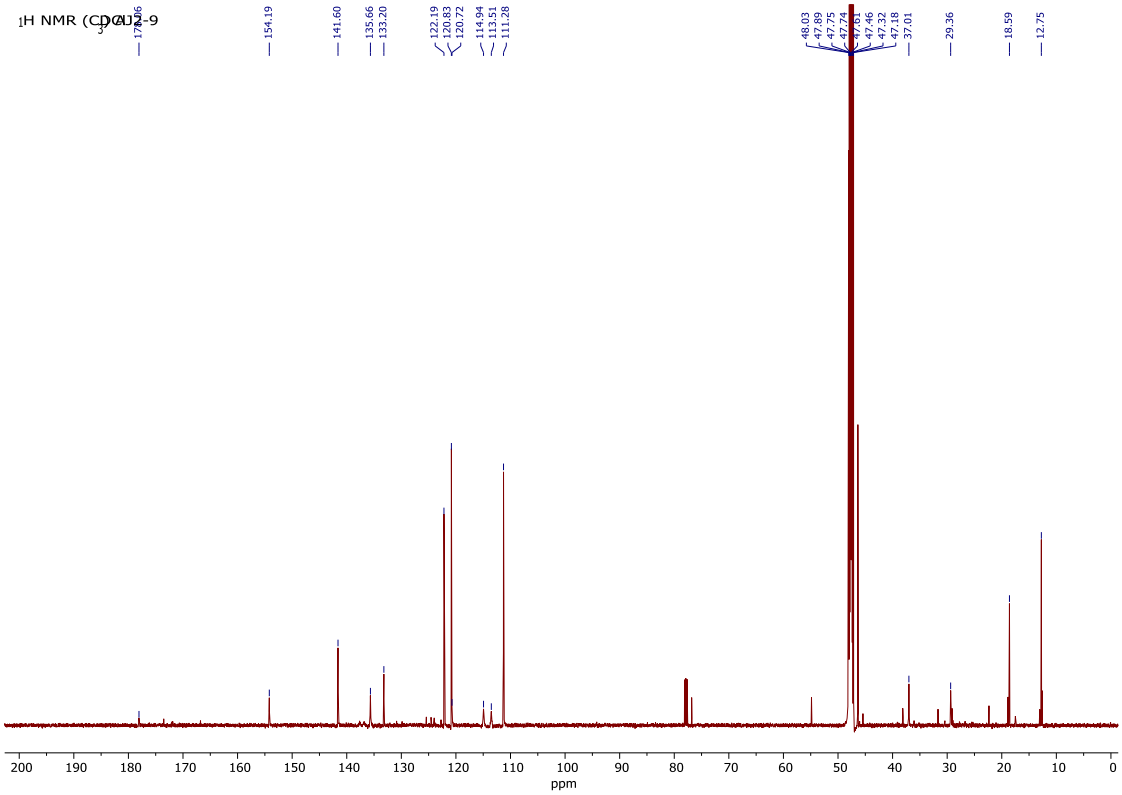
^{13}C NMR ($CDCl_3$) AJ2-8



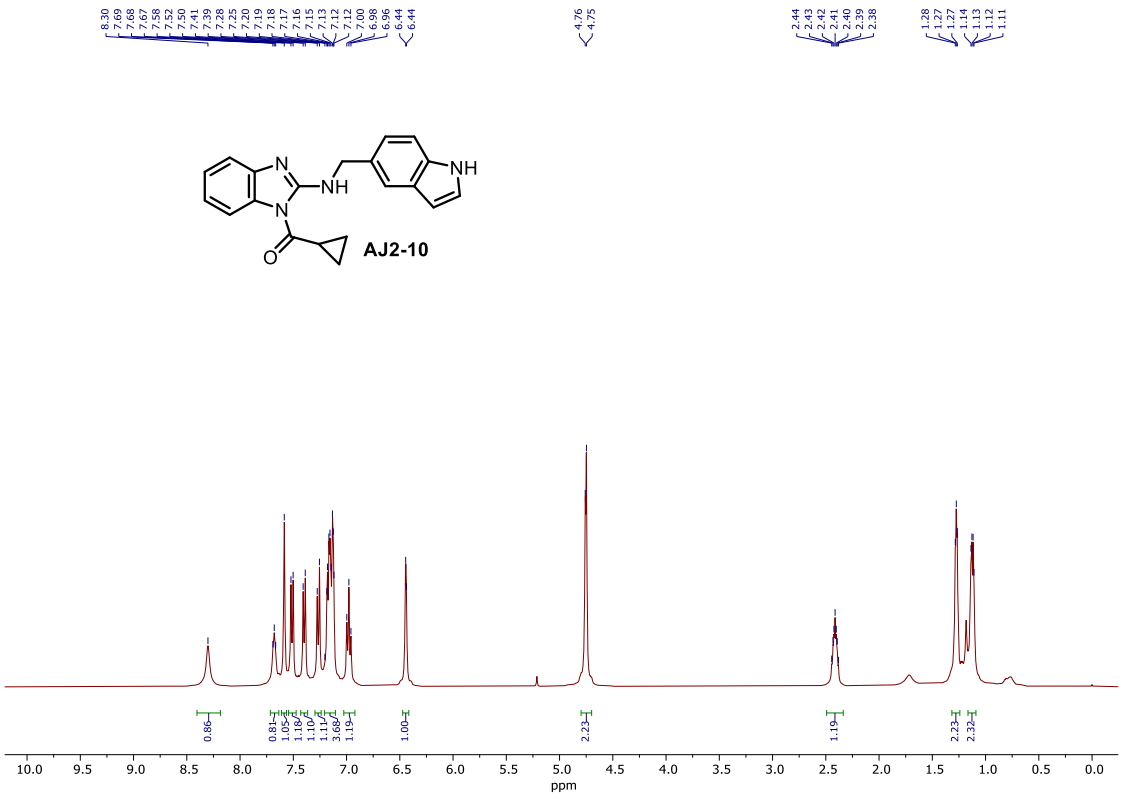
^{1}H NMR ($CDCl_3$) AJ2-9



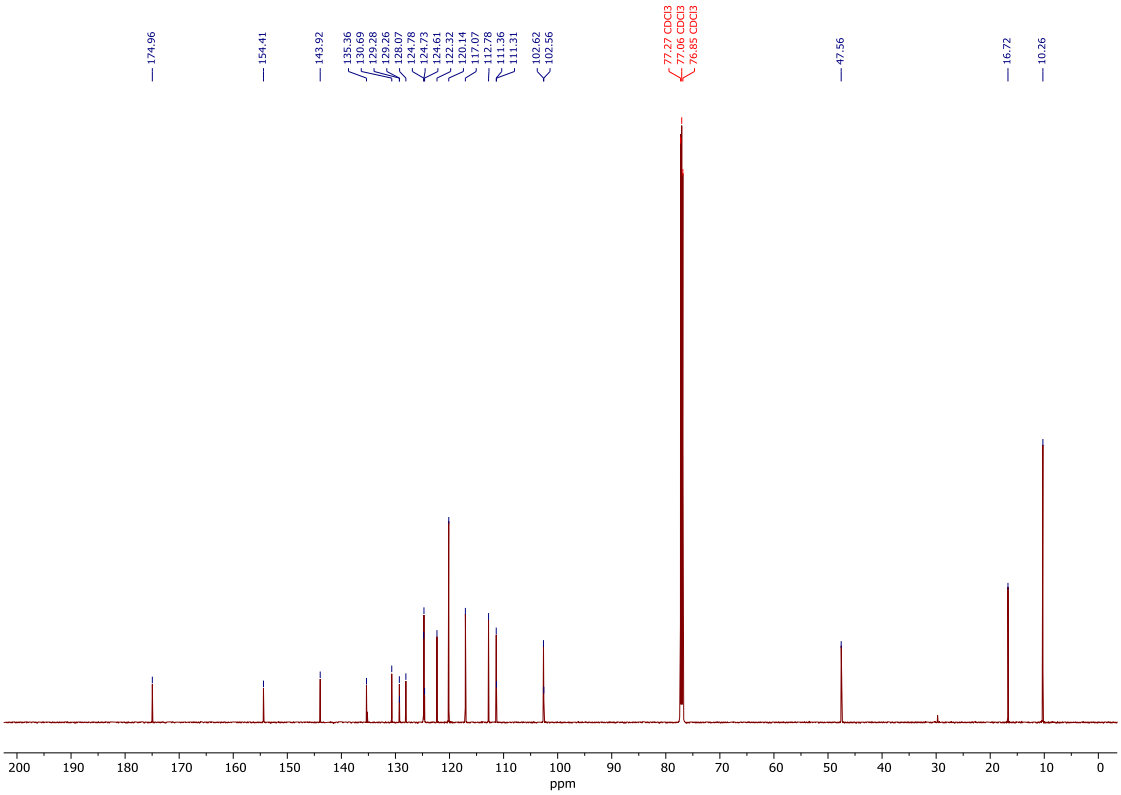
^{13}C NMR ($CDCl_3$) AJ2-9



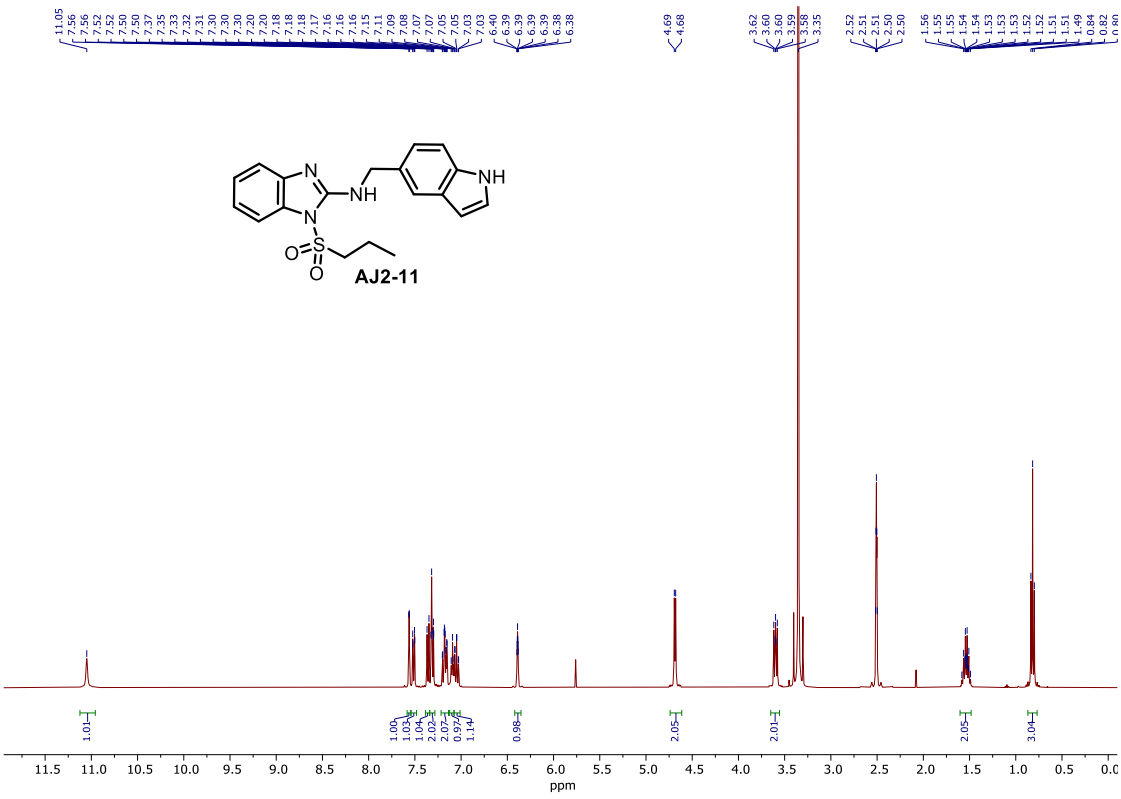
^{1}H NMR ($CDCl_3$) AJ2-10



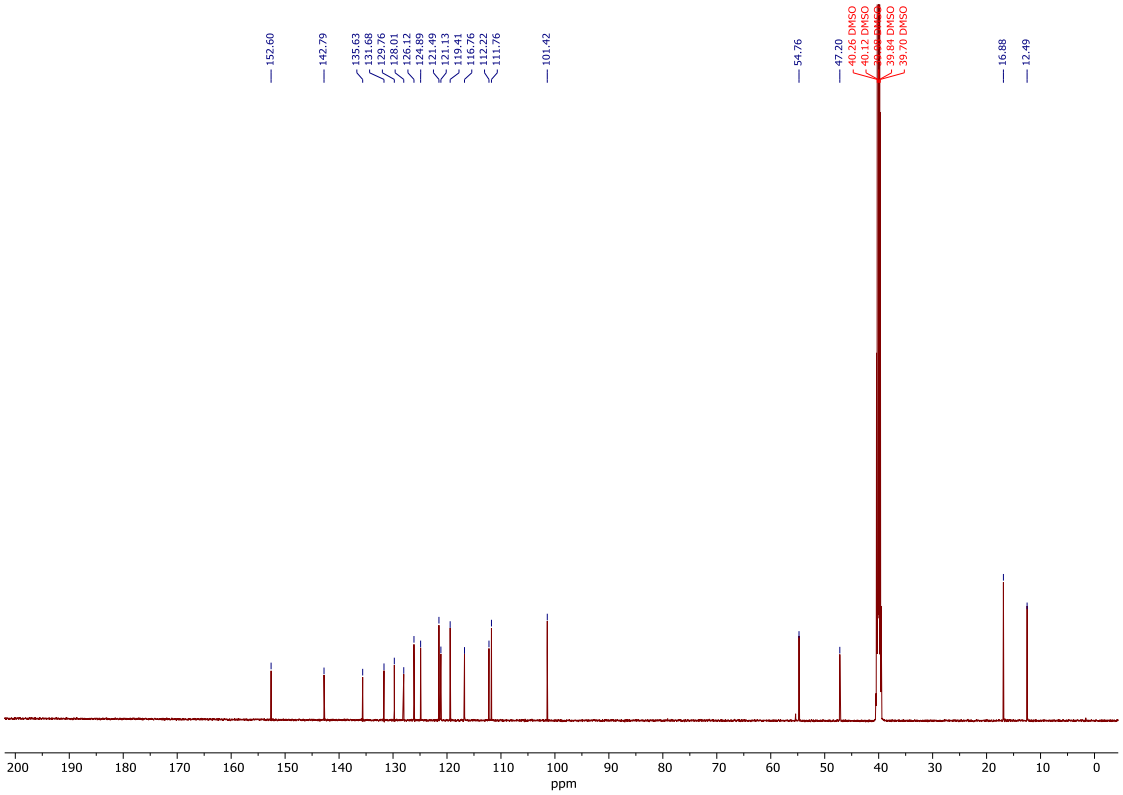
^{13}C NMR ($CDCl_3$) AJ2-10



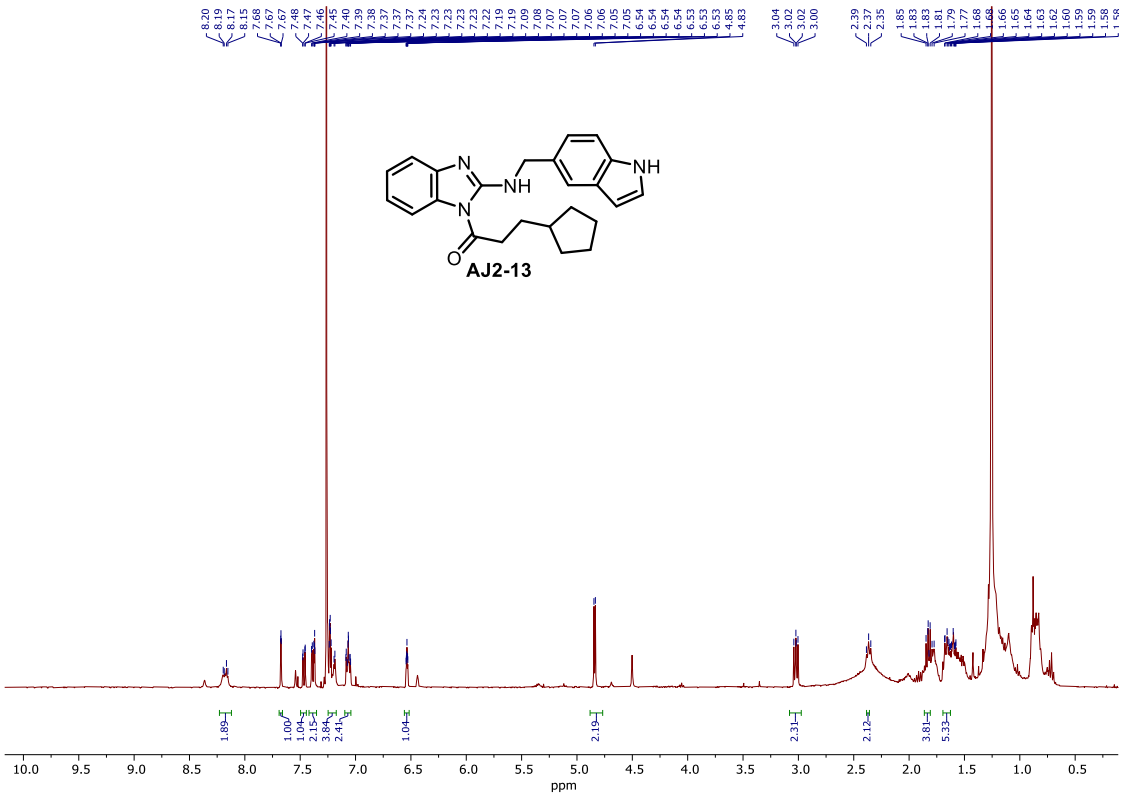
^{1}H NMR ($CDCl_3$) AJ2-11



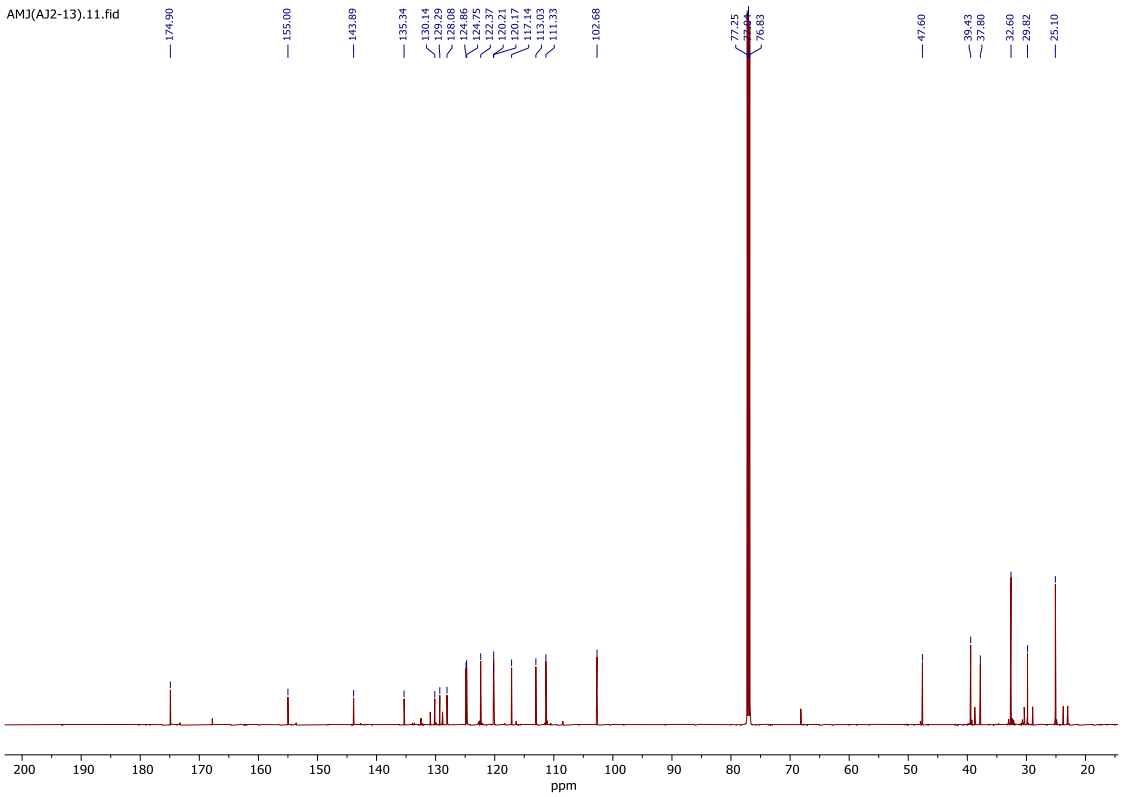
^{13}C NMR ($CDCl_3$) AJ2-11



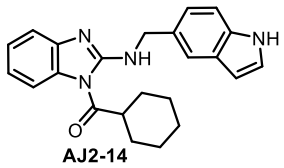
^{1}H NMR ($CDCl_3$) AJ2-13



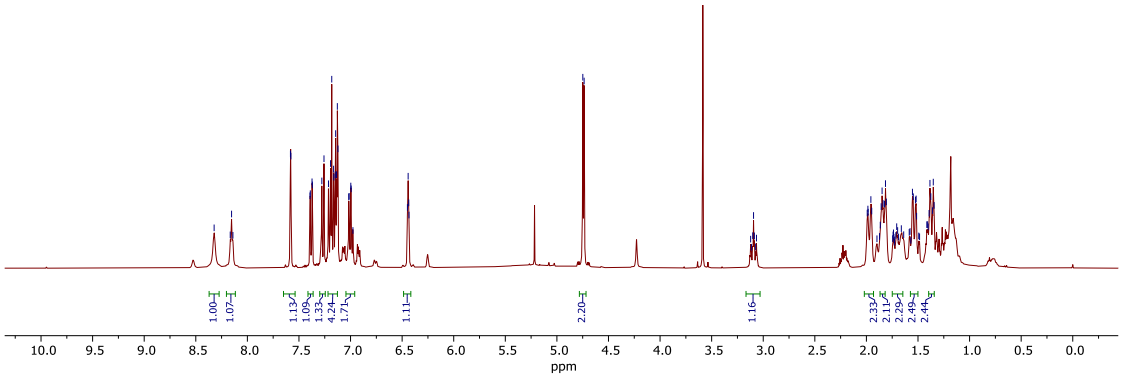
^{13}C NMR ($CDCl_3$) AJ2-13



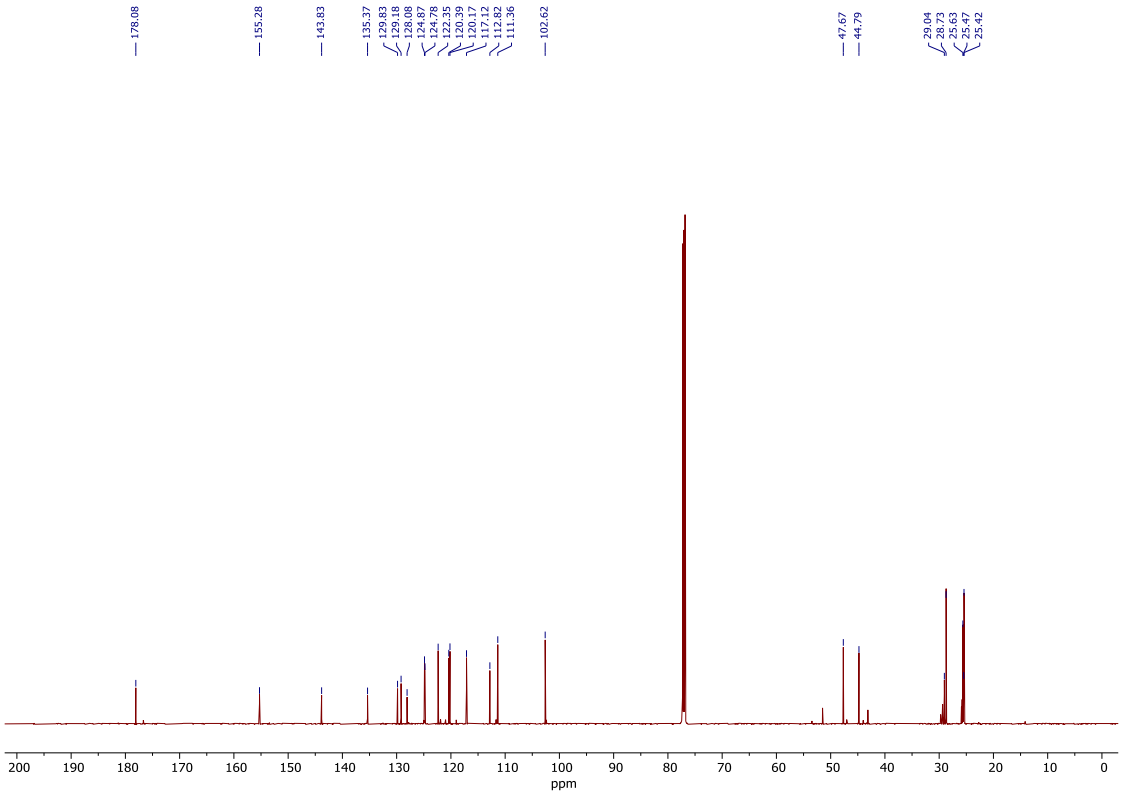
^{1}H NMR ($CDCl_3$) AJ2-14



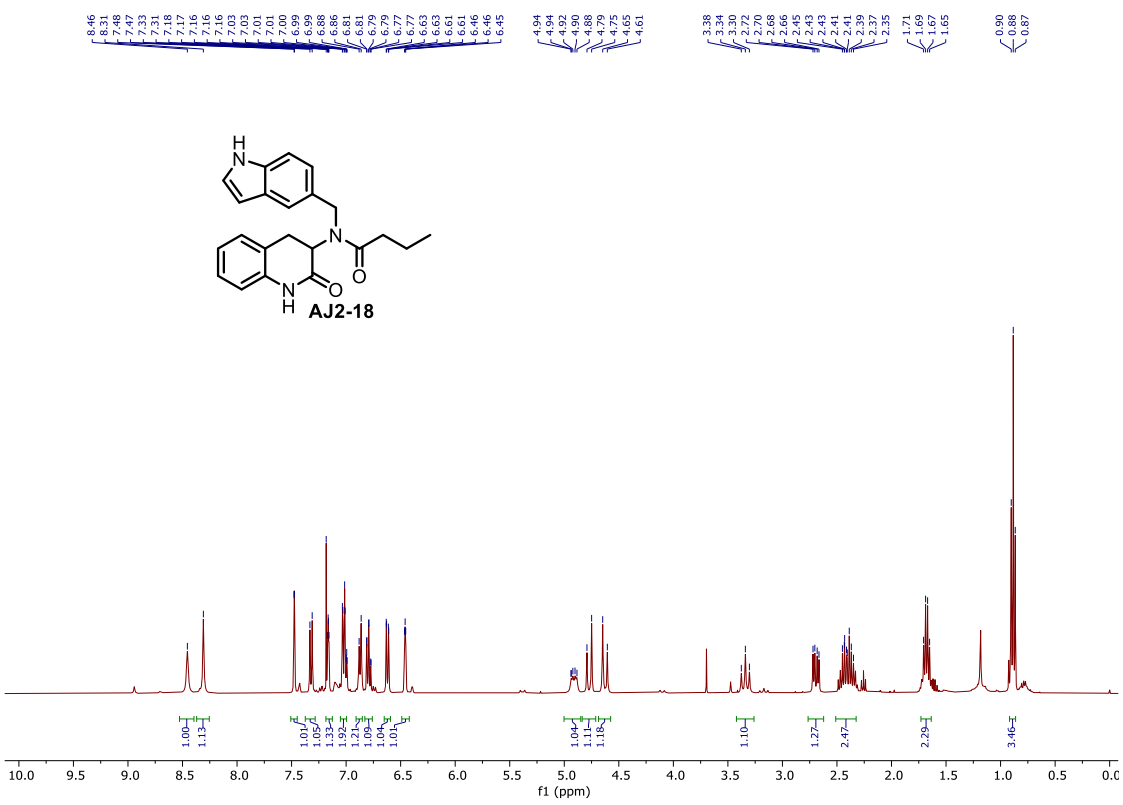
AJ2-14



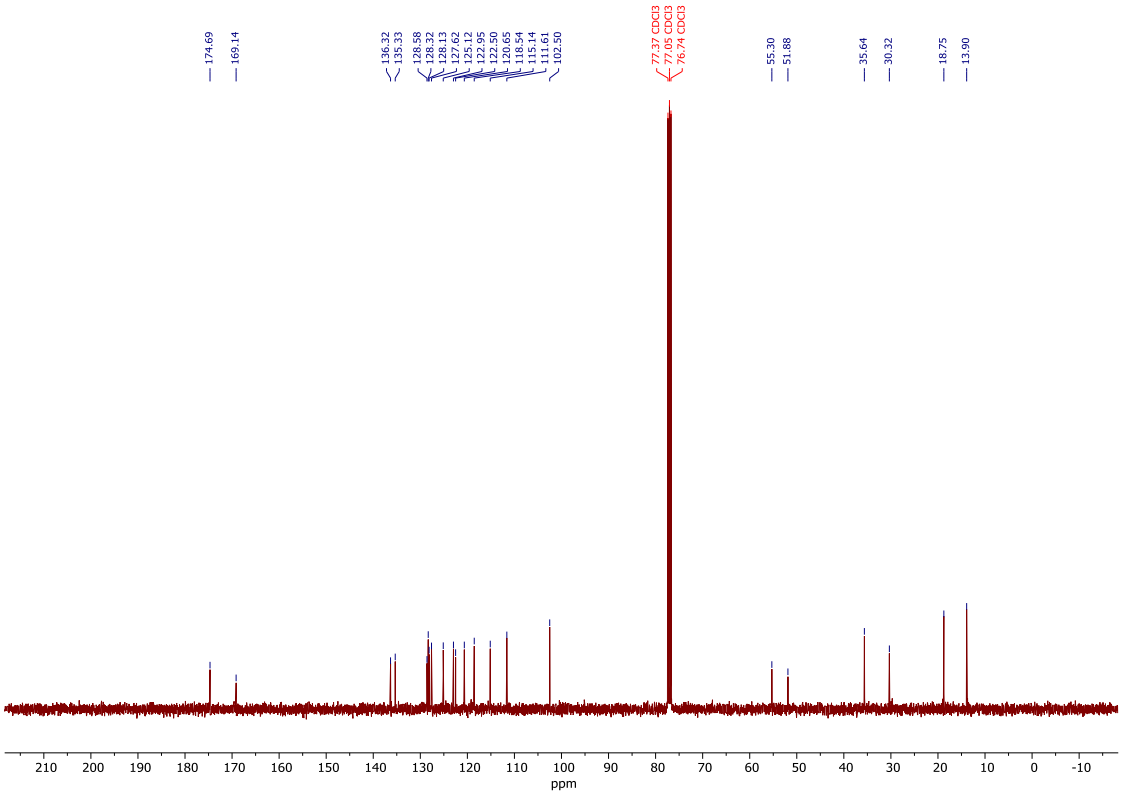
^{13}C NMR ($CDCl_3$) AJ2-14



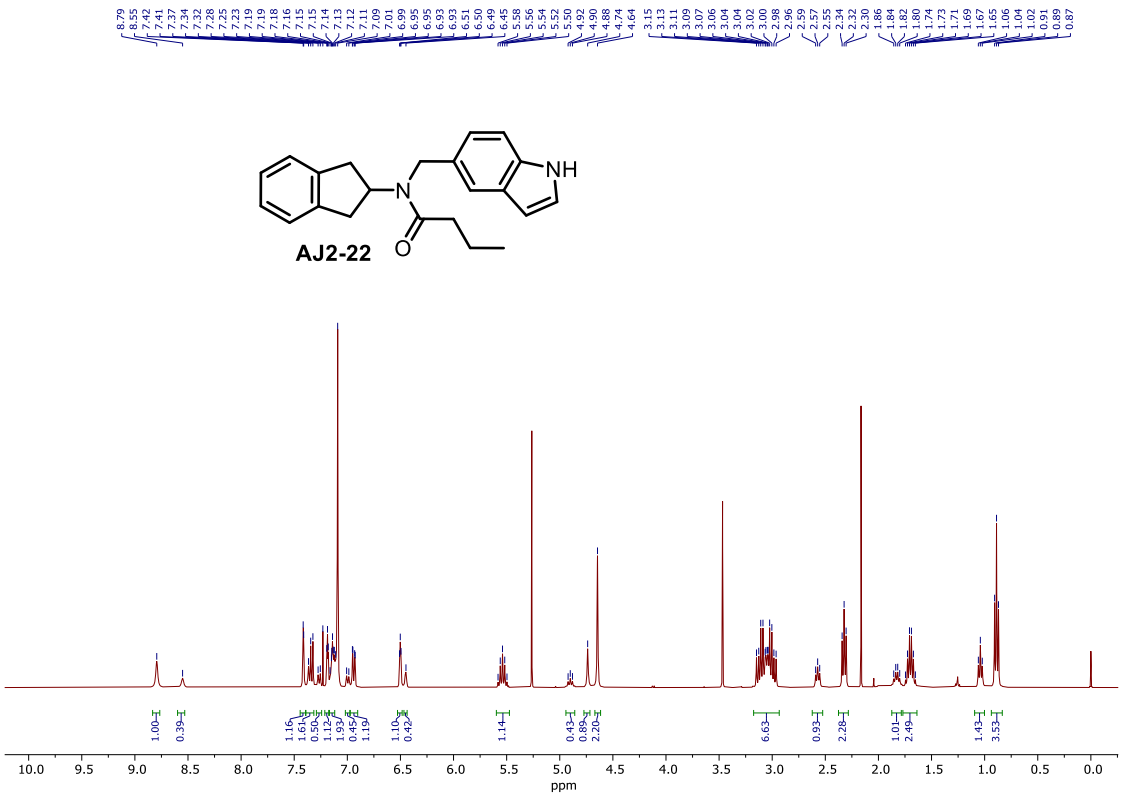
^{1}H NMR ($CDCl_3$) AJ2-18



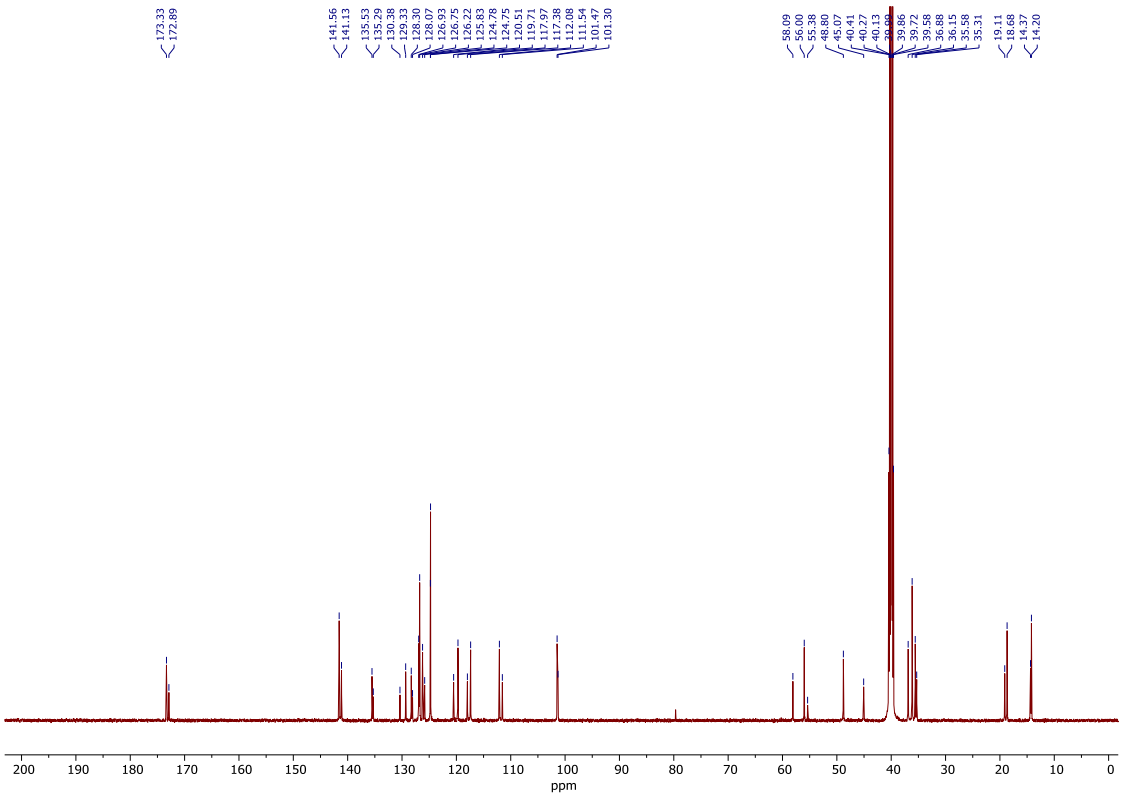
^{13}C NMR ($CDCl_3$) AJ2-18



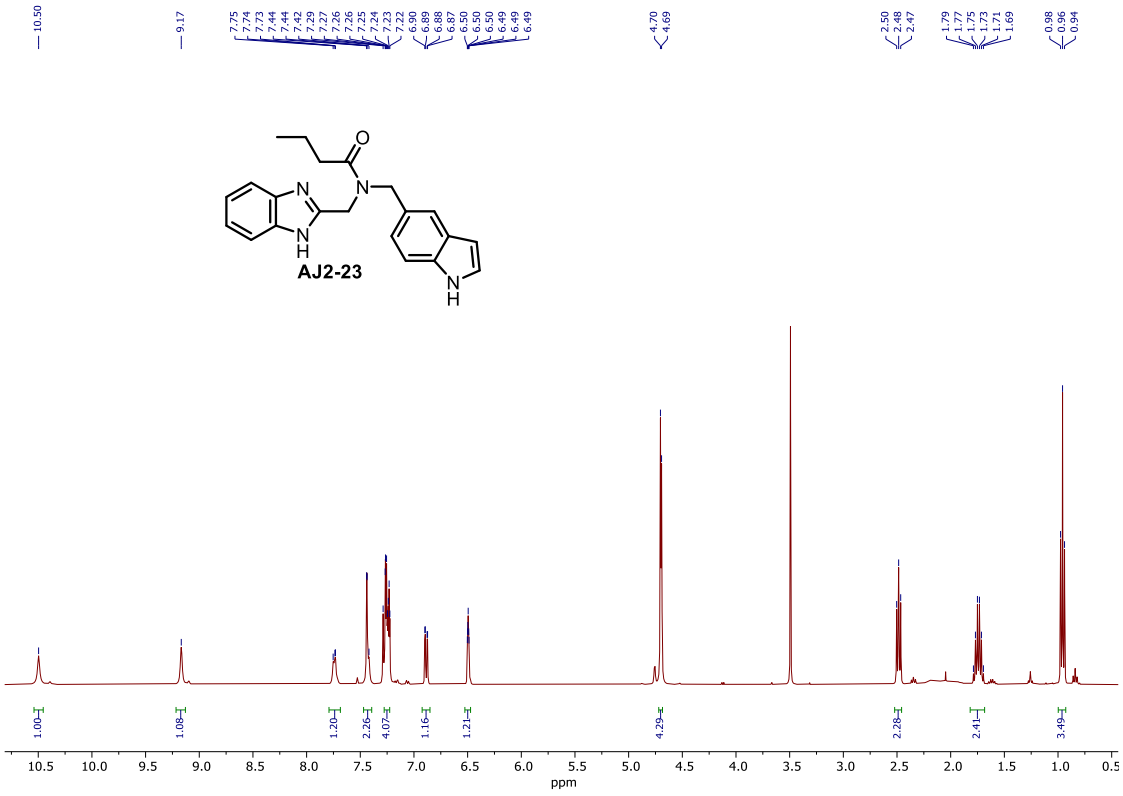
^{1}H NMR ($CDCl_3$) AJ2-22



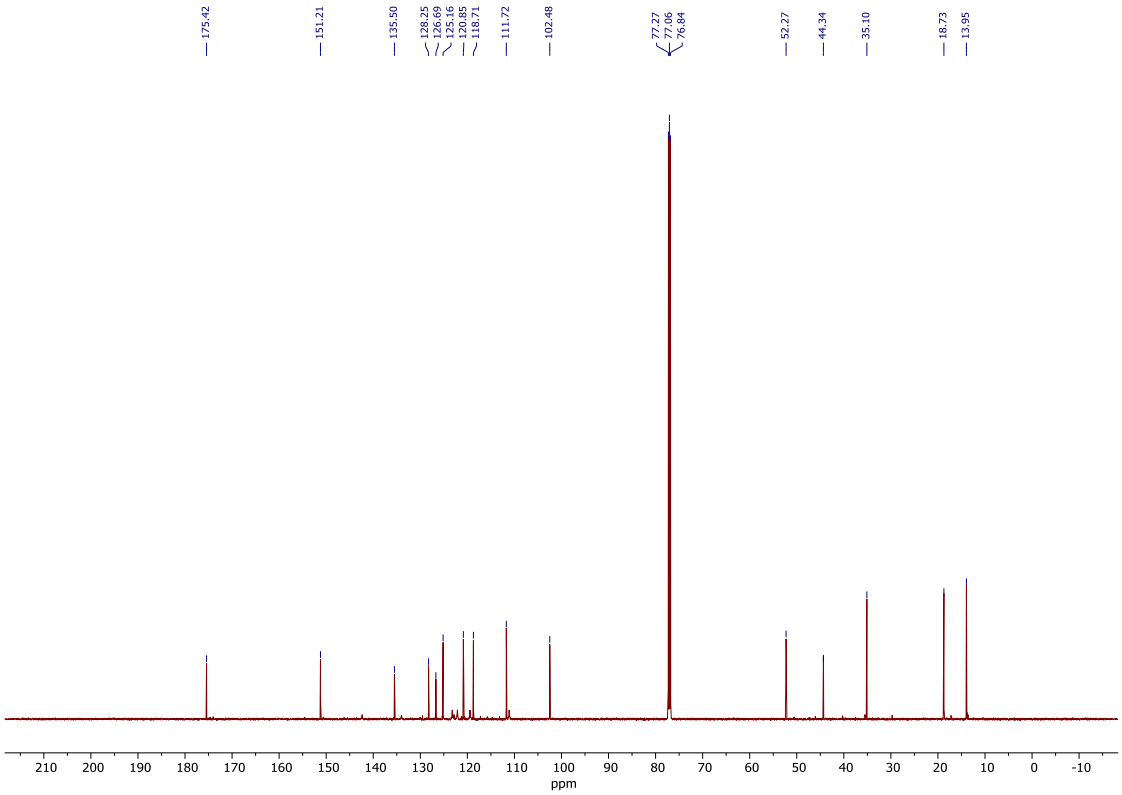
^{13}C NMR ($CDCl_3$) AJ2-22



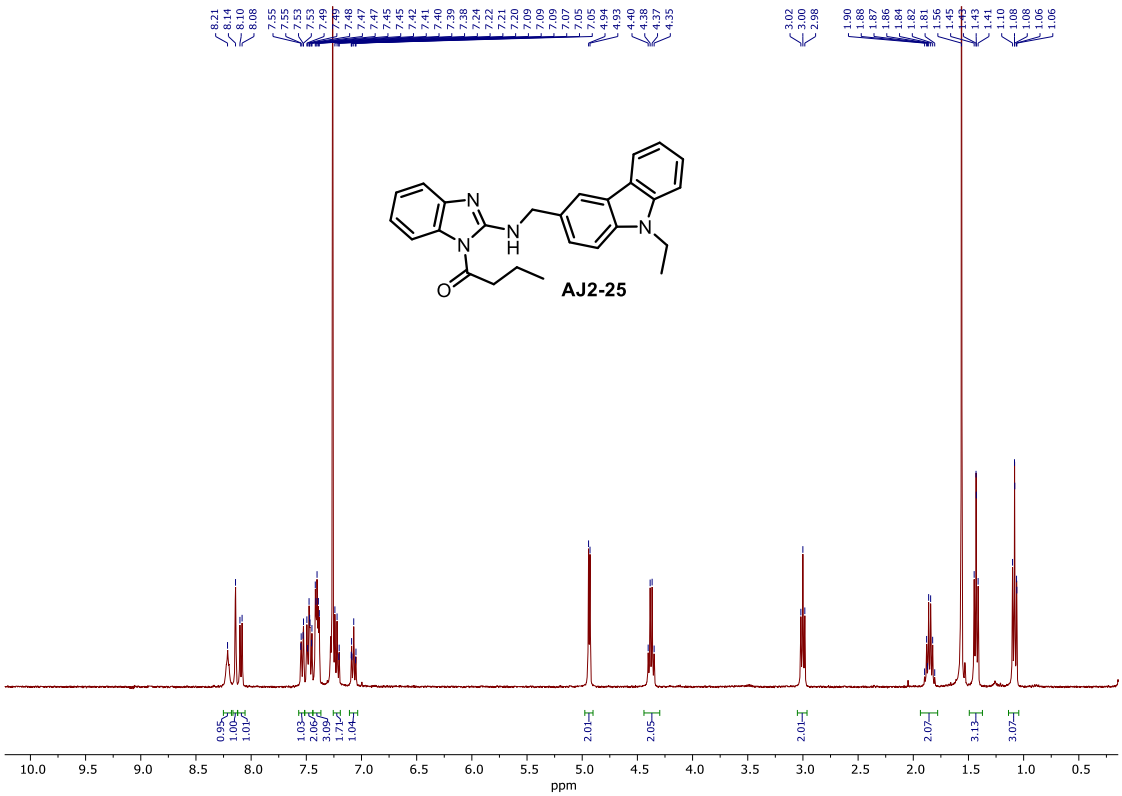
^{1}H NMR ($CDCl_3$) AJ2-23



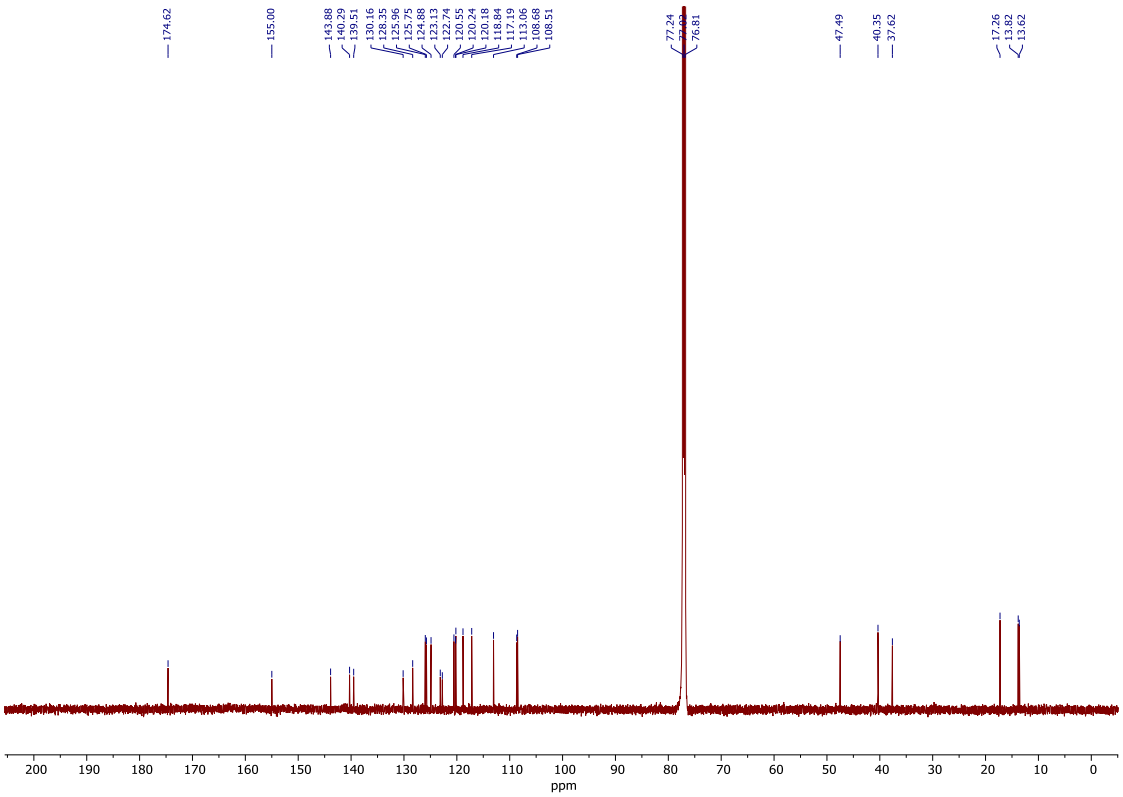
^{13}C NMR ($CDCl_3$) AJ2-23



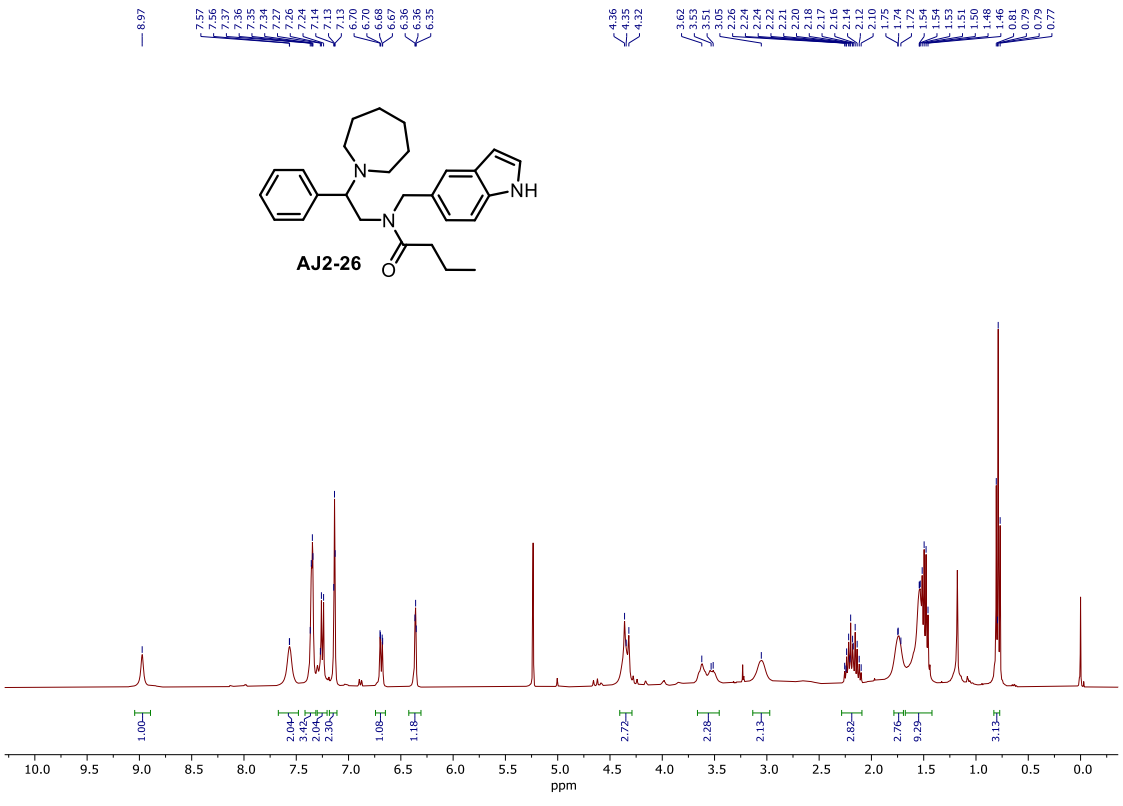
^{1}H NMR ($CDCl_3$) AJ2-25



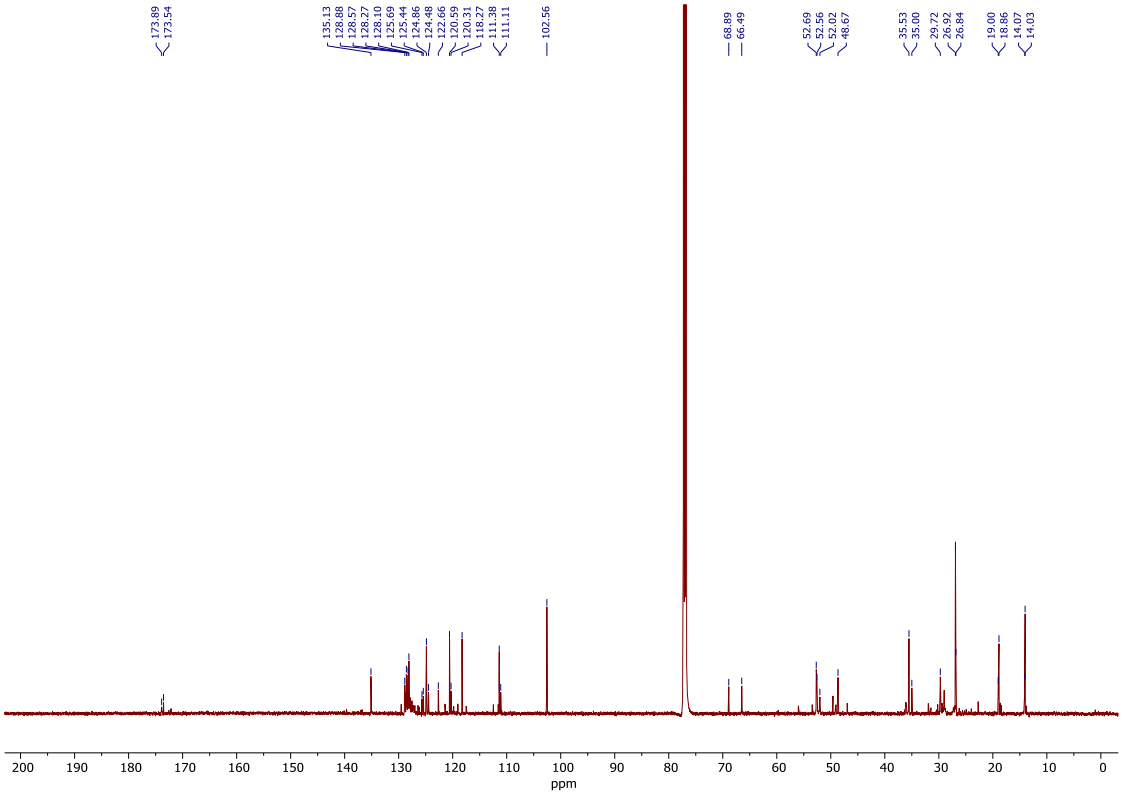
^{13}C NMR ($CDCl_3$) AJ2-25



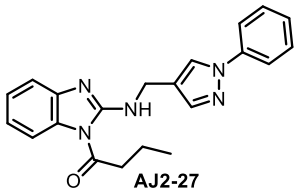
^{1}H NMR ($CDCl_3$) AJ2-26



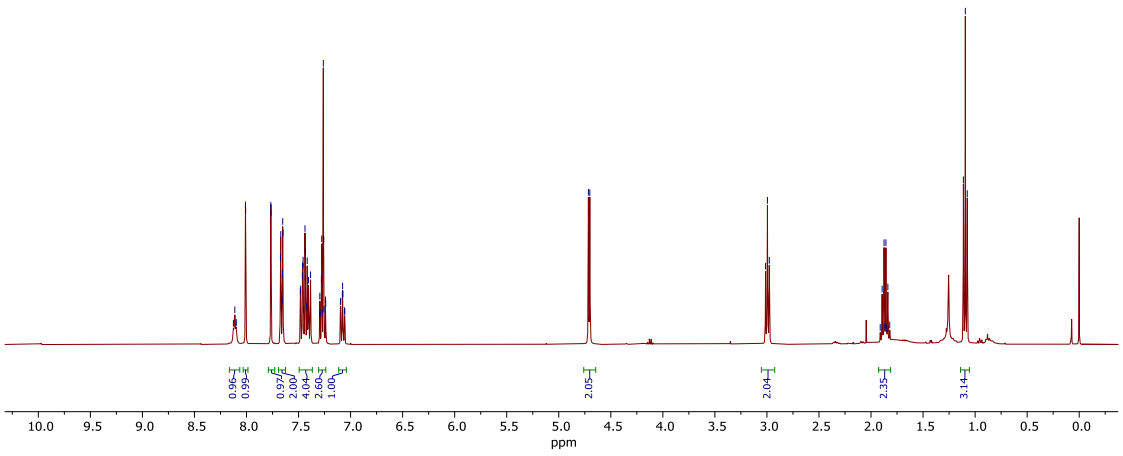
^{13}C NMR ($CDCl_3$) AJ2-26



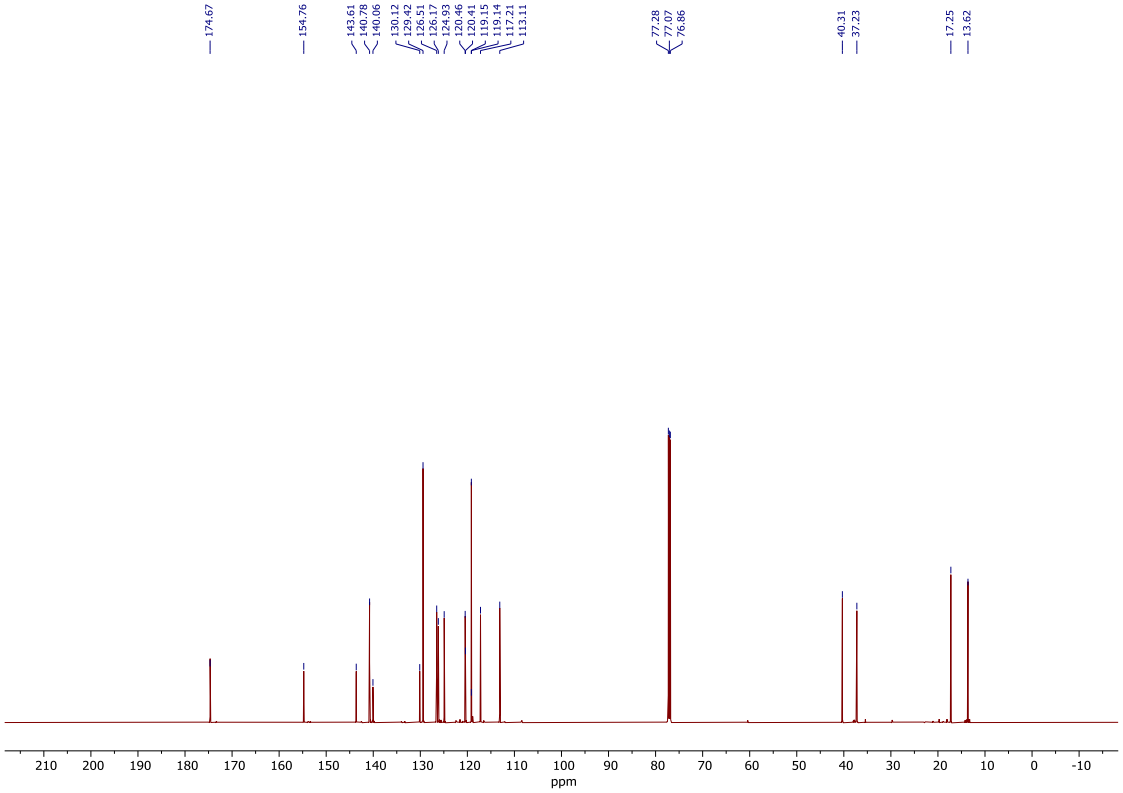
^{1}H NMR ($CDCl_3$) AJ2-27



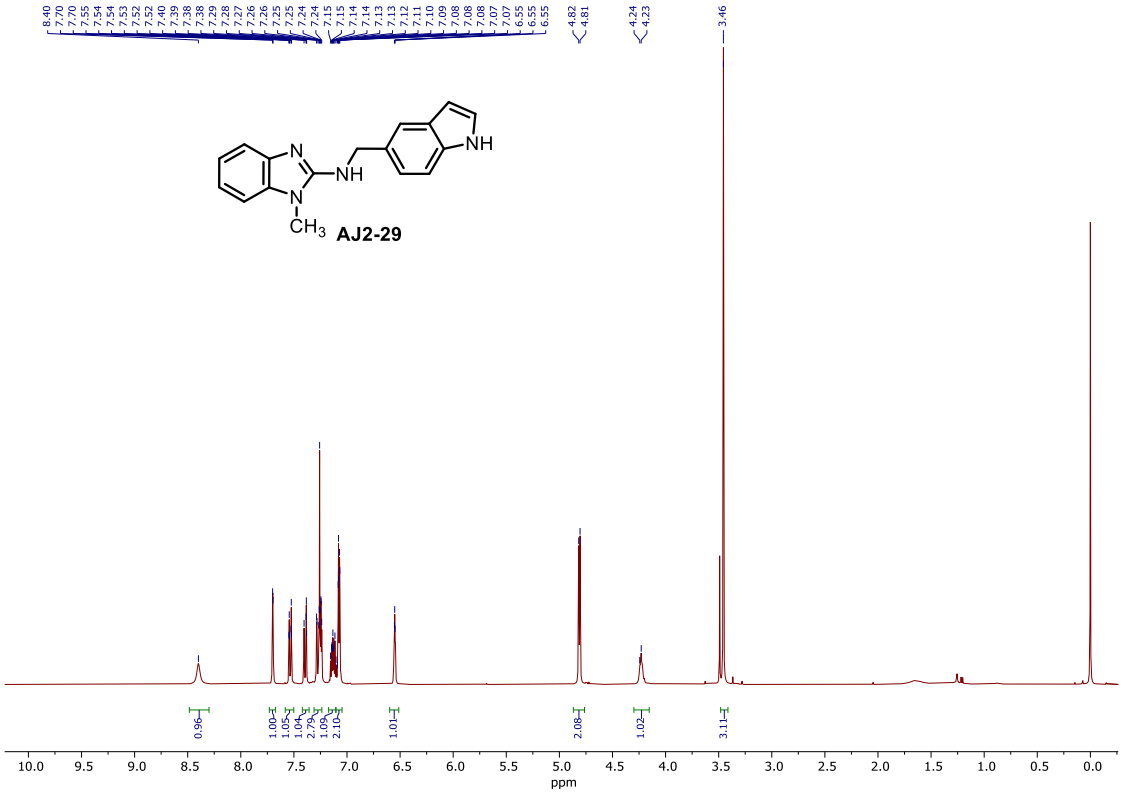
AJ2-27



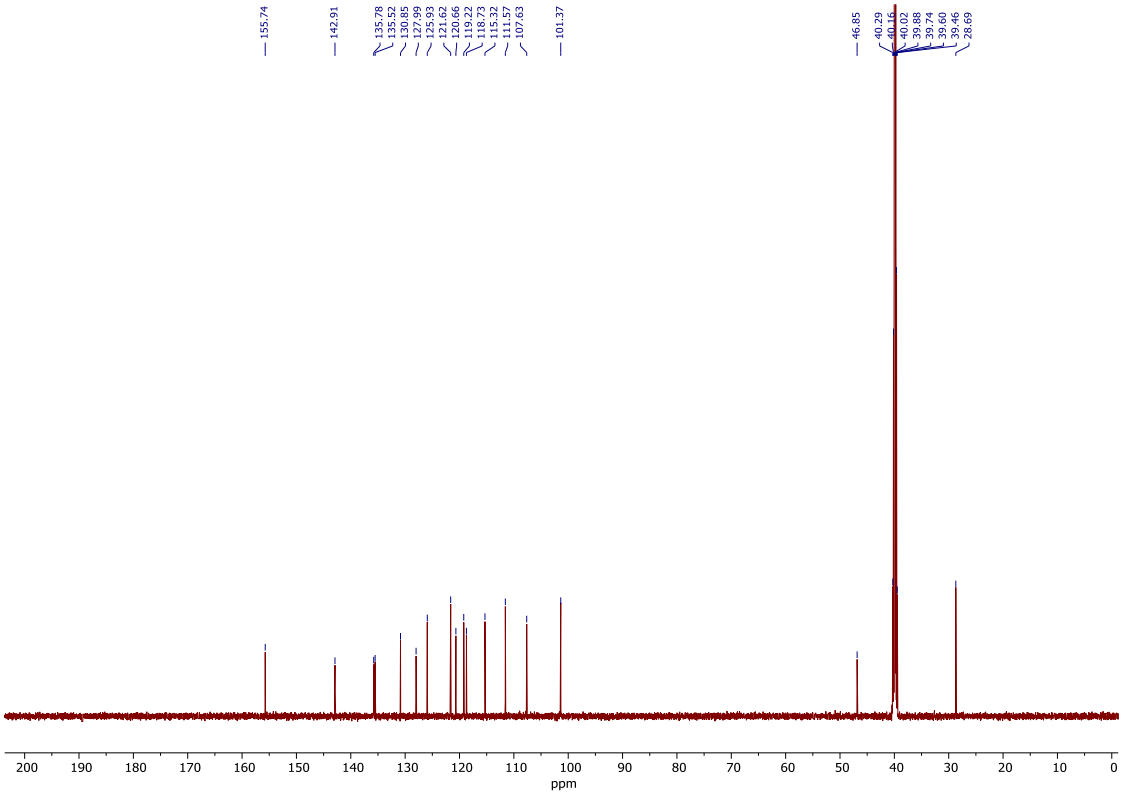
^{13}C NMR ($CDCl_3$) AJ2-27



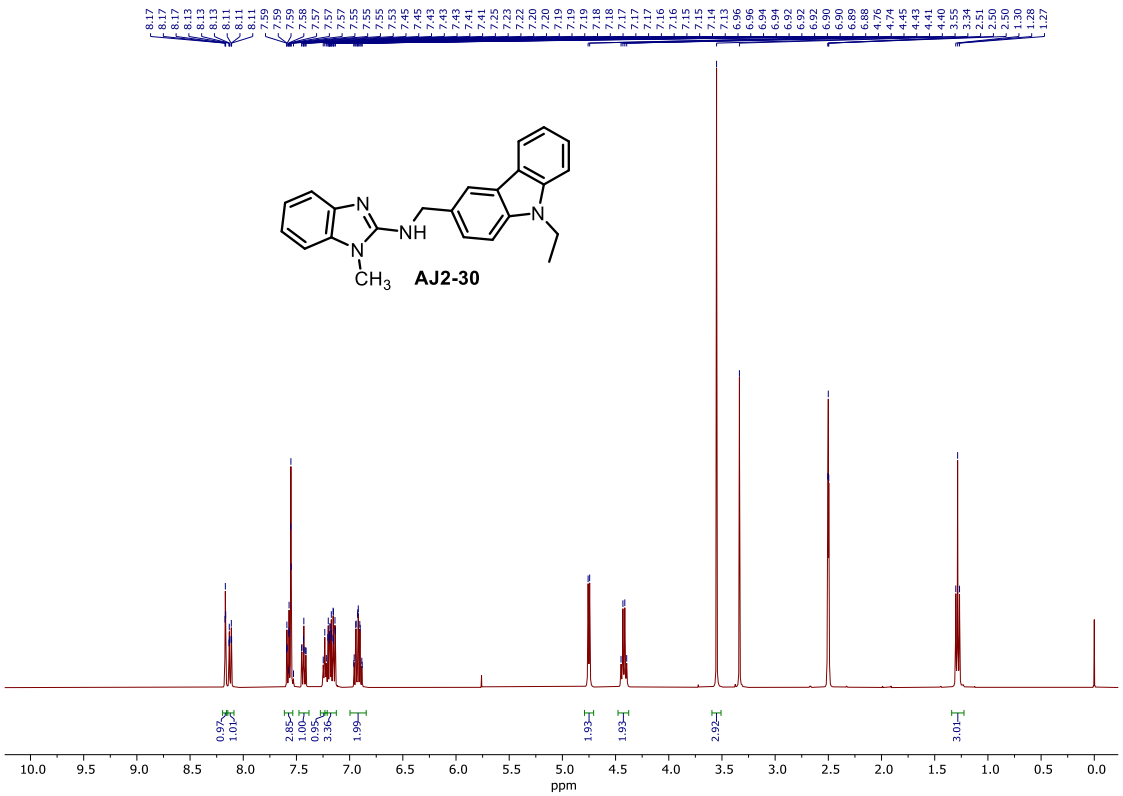
^{1}H NMR ($CDCl_3$) AJ2-29



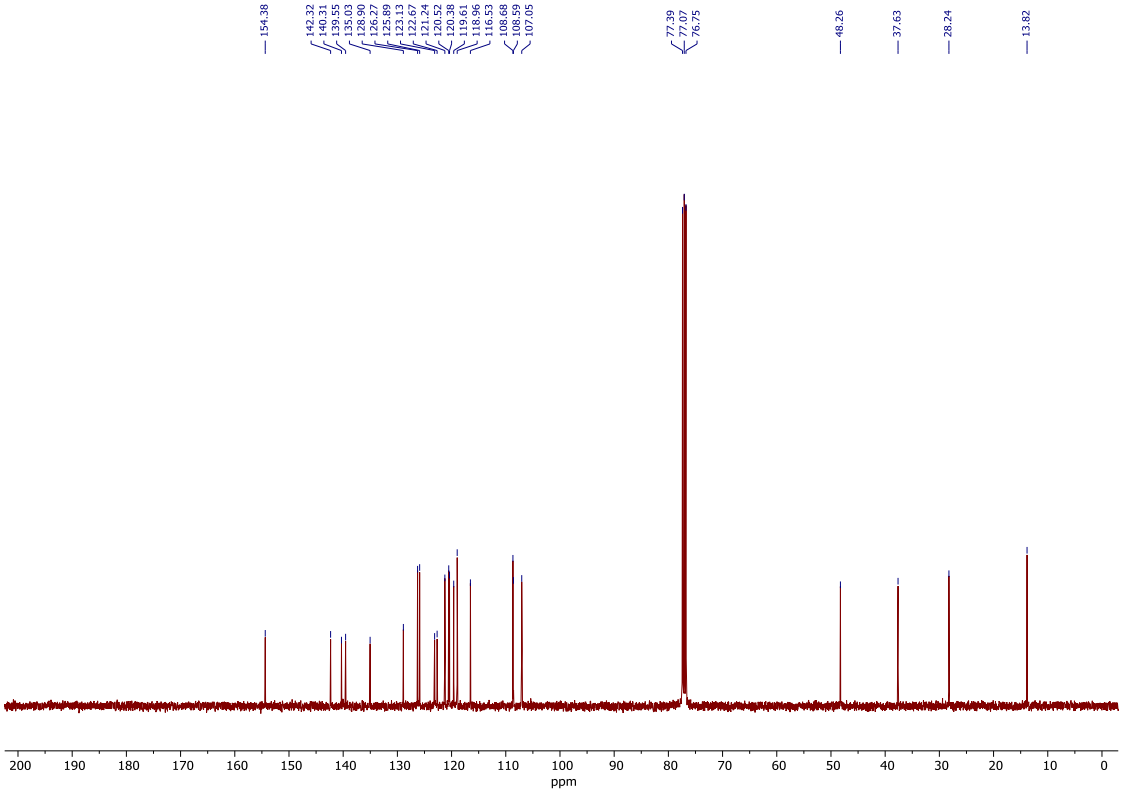
^{13}C NMR ($CDCl_3$) AJ2-29



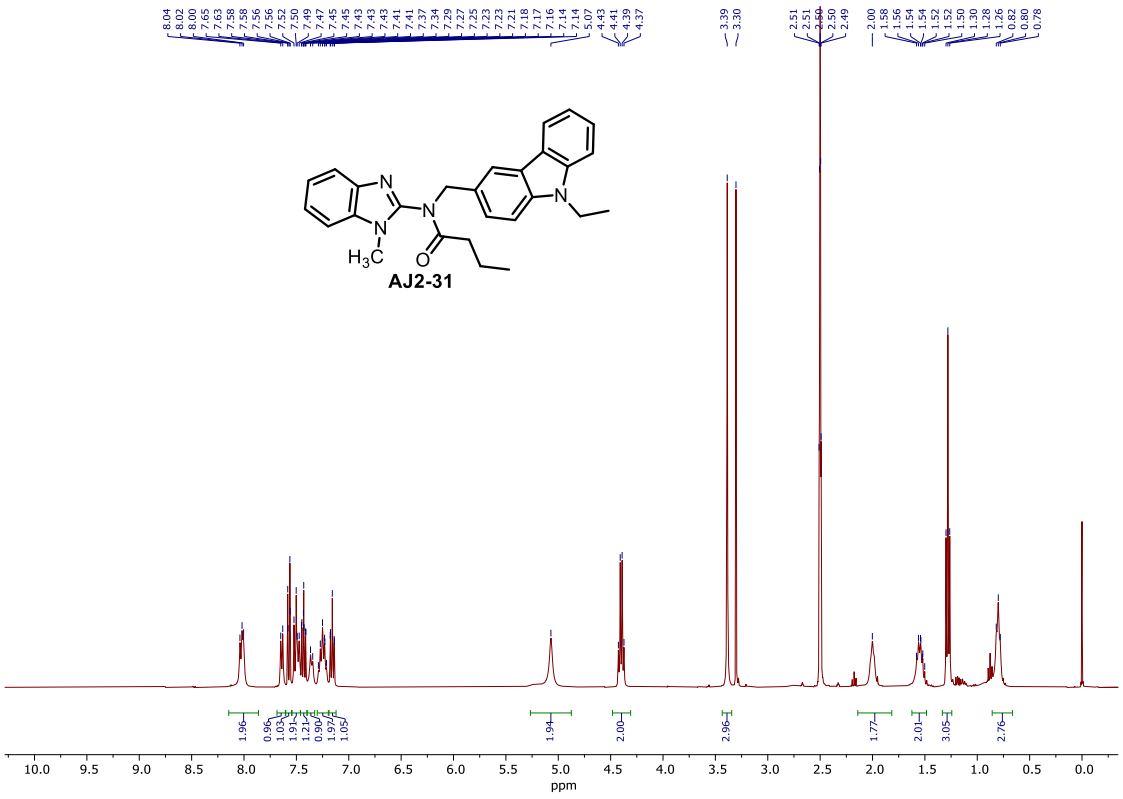
^{1}H NMR ($DMSO\text{-}d_6$) AJ2-30



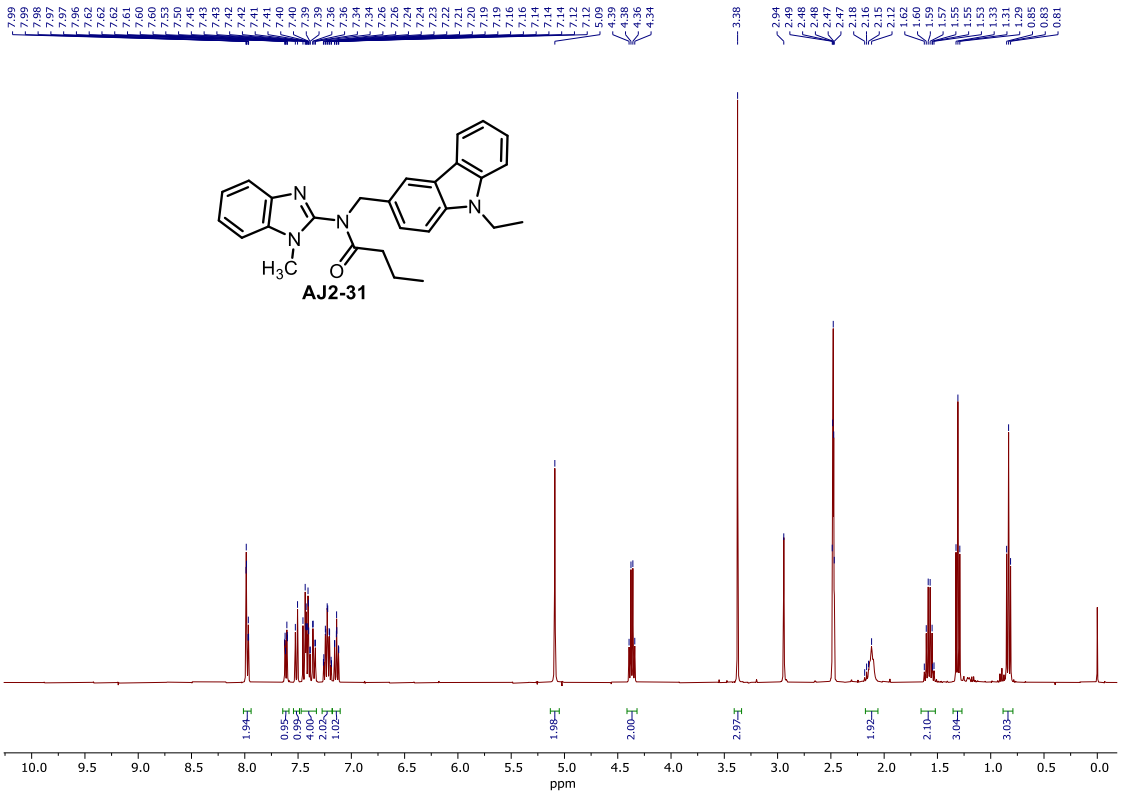
^{13}C NMR (DMSO-d_6) AJ2-30



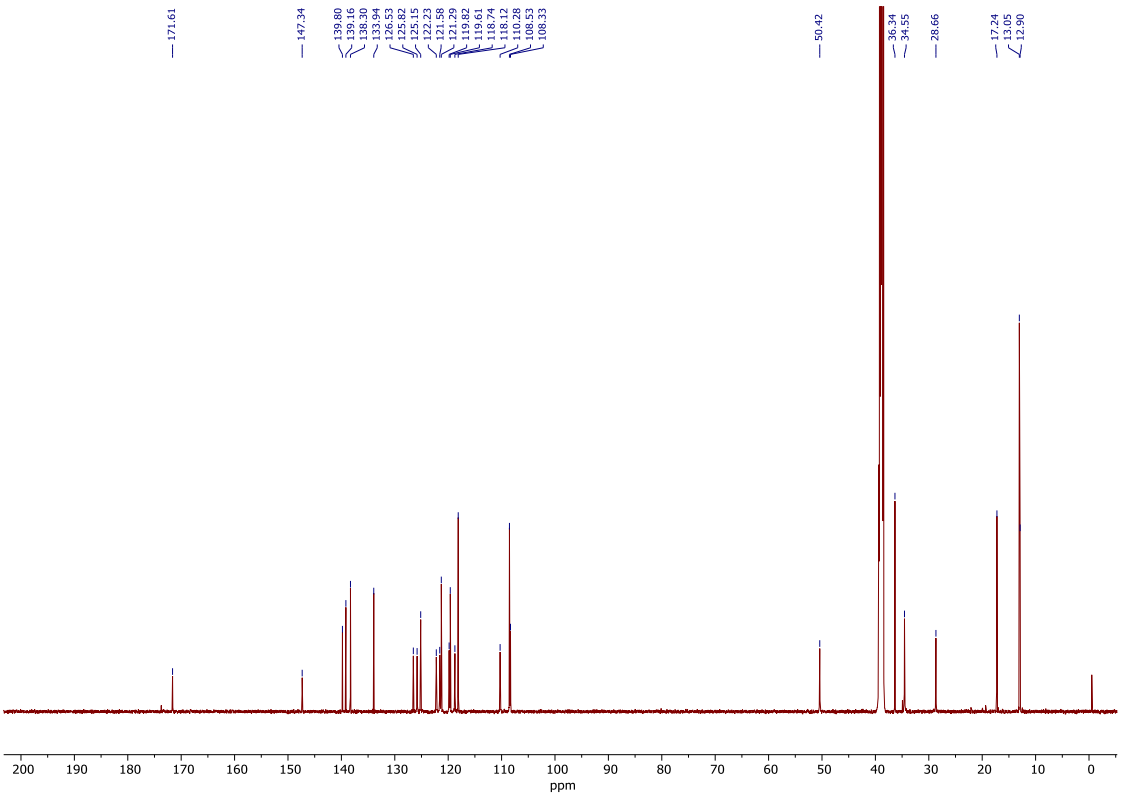
^{1}H NMR (DMSO-d_6) AJ2-31



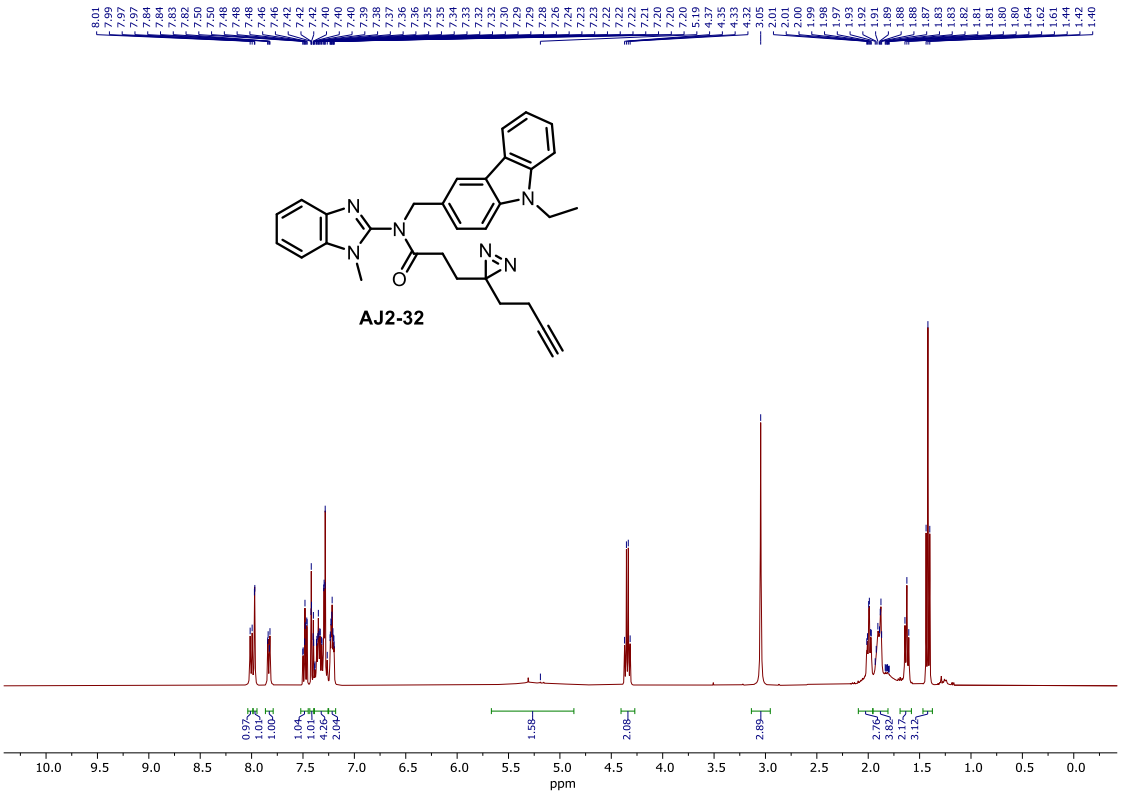
^{1}H NMR (DMSO-d_6 at 100°C) AJ2-31



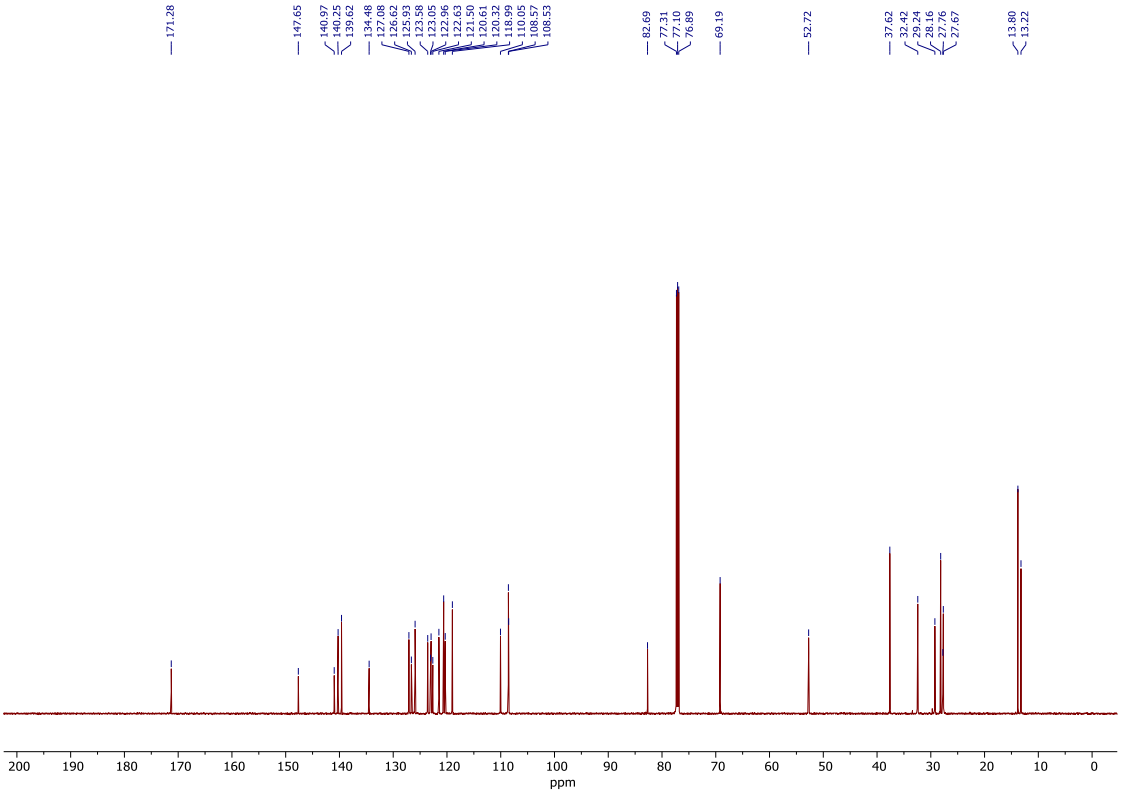
^{13}C NMR (DMSO-d_6) AJ2-31



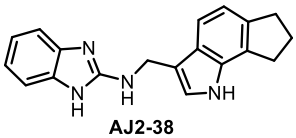
^{1}H NMR ($CDCl_3$) AJ2-32



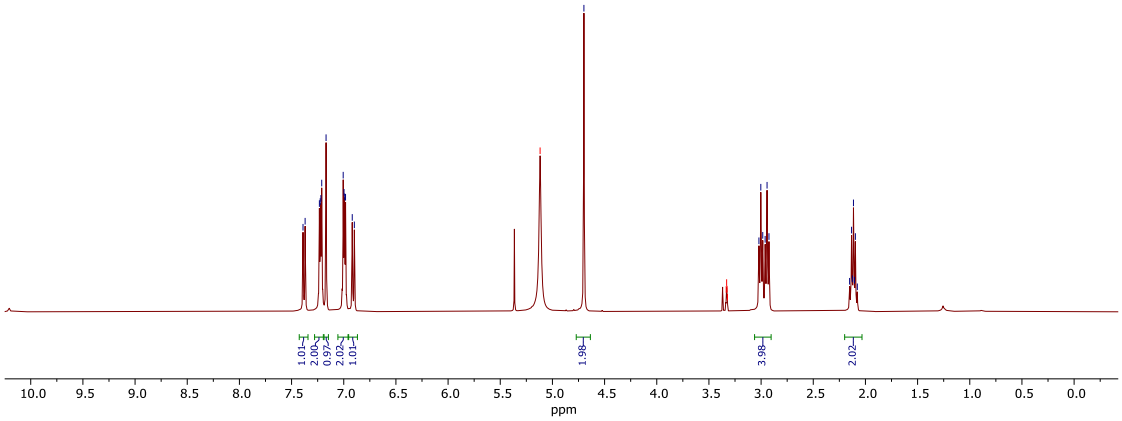
^{13}C NMR ($CDCl_3$) AJ2-32



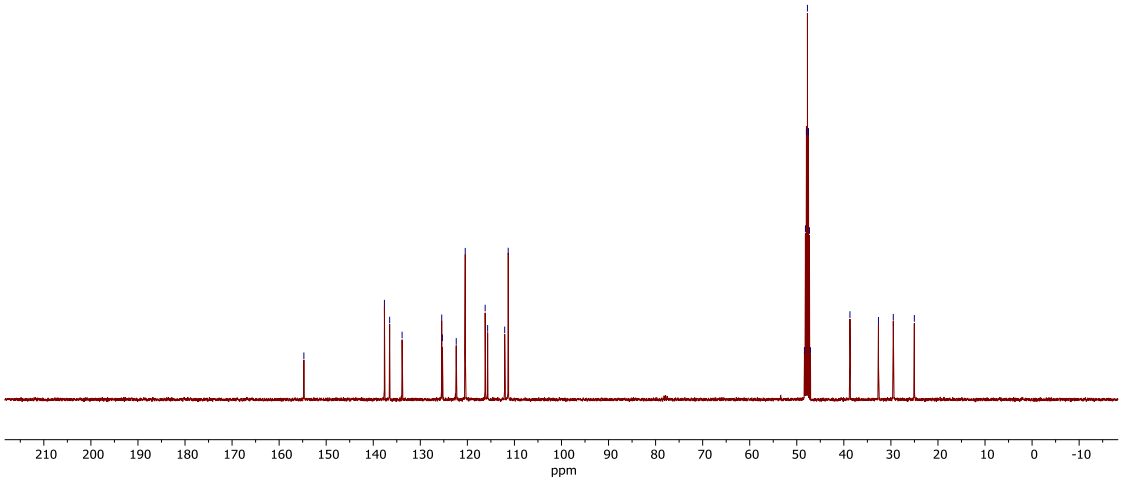
^{1}H NMR (CD_3OD) AJ2-38



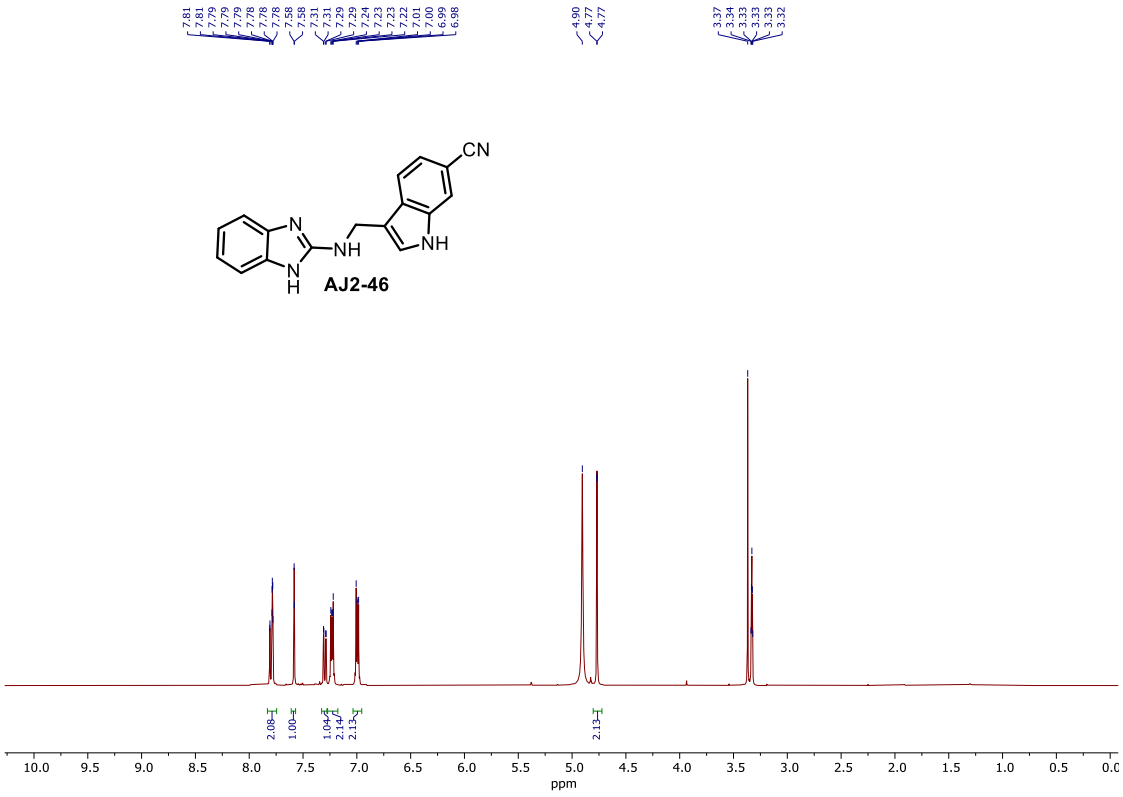
AJ2-38



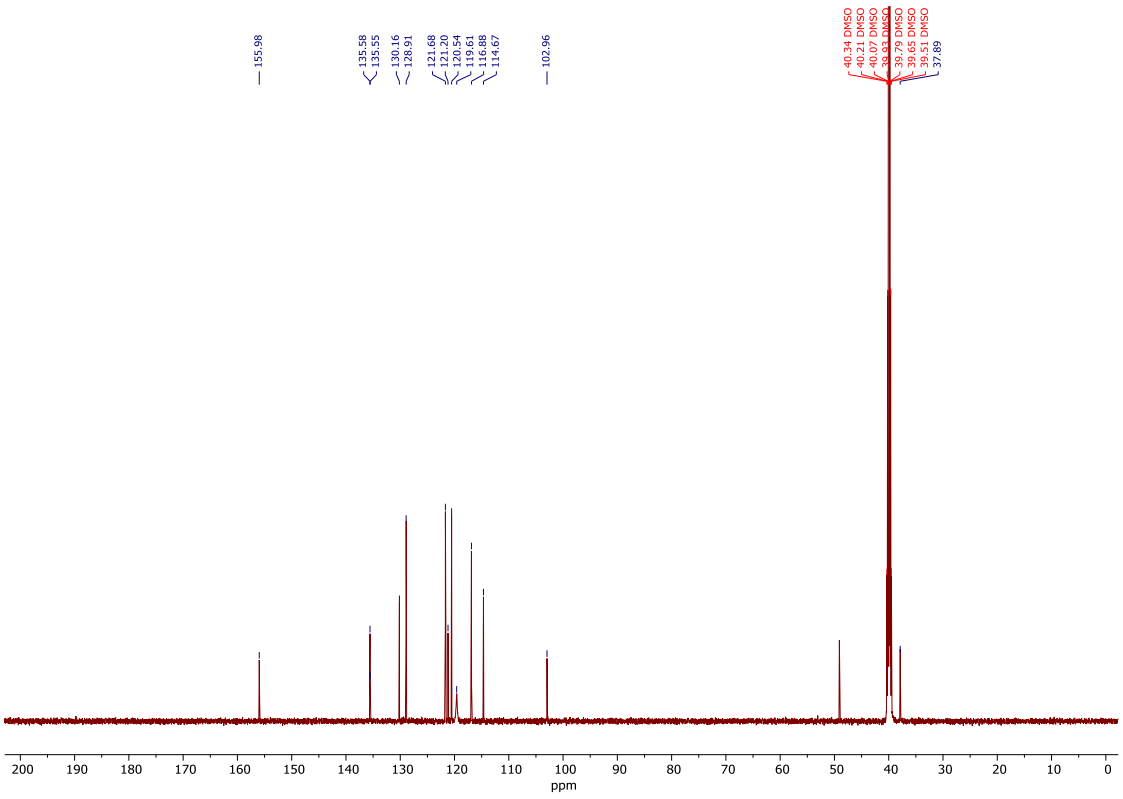
^{13}C NMR (CD_3OD) AJ2-38



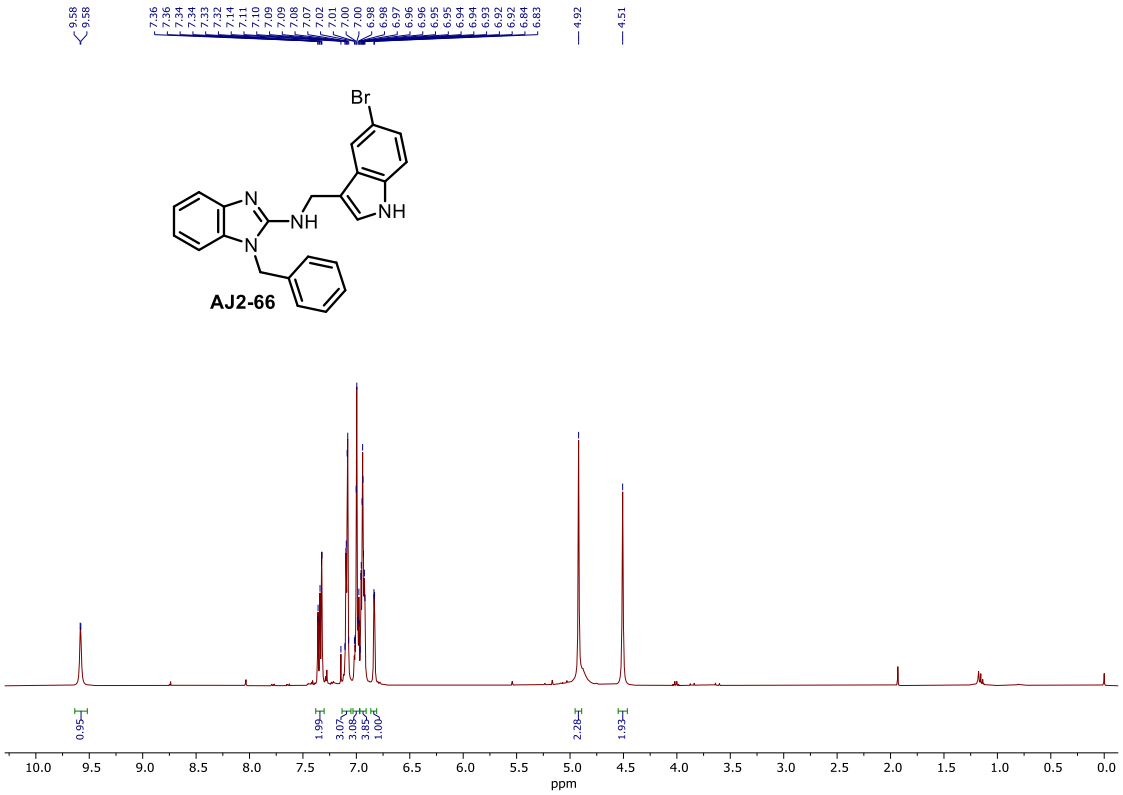
^{1}H NMR (CD_3OD) AJ2-46



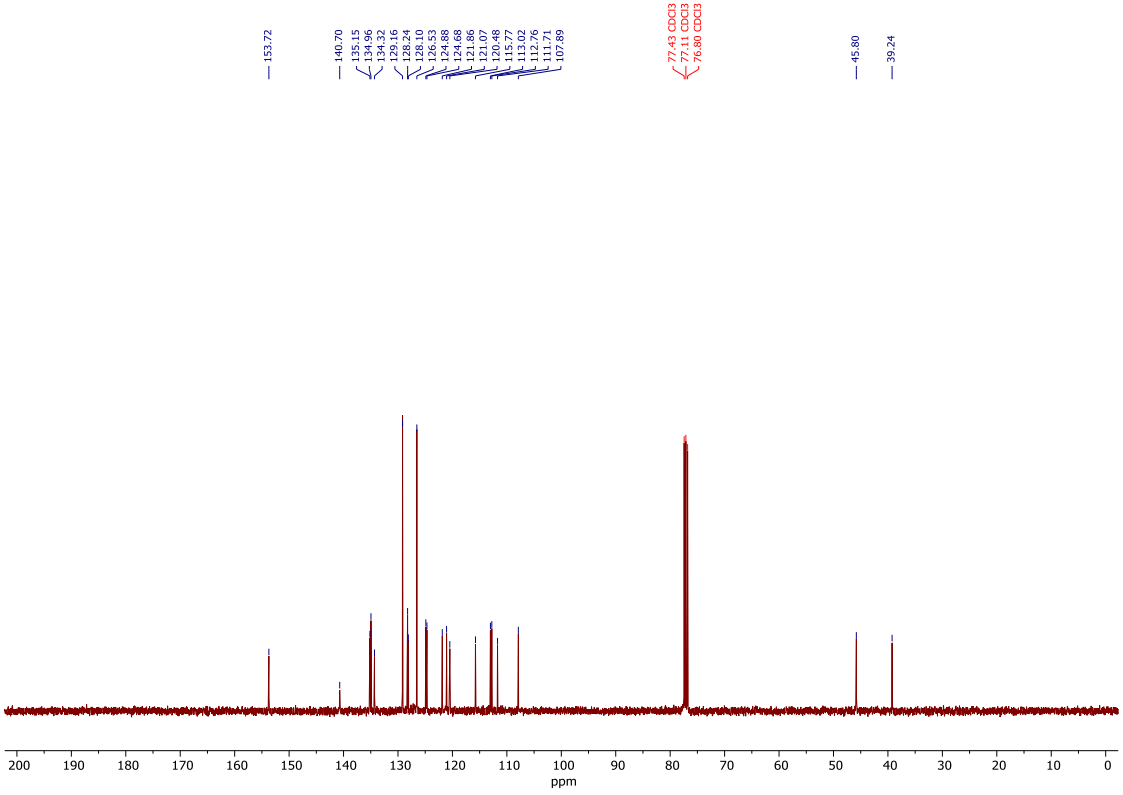
^{13}C NMR (CD_3OD) AJ2-46



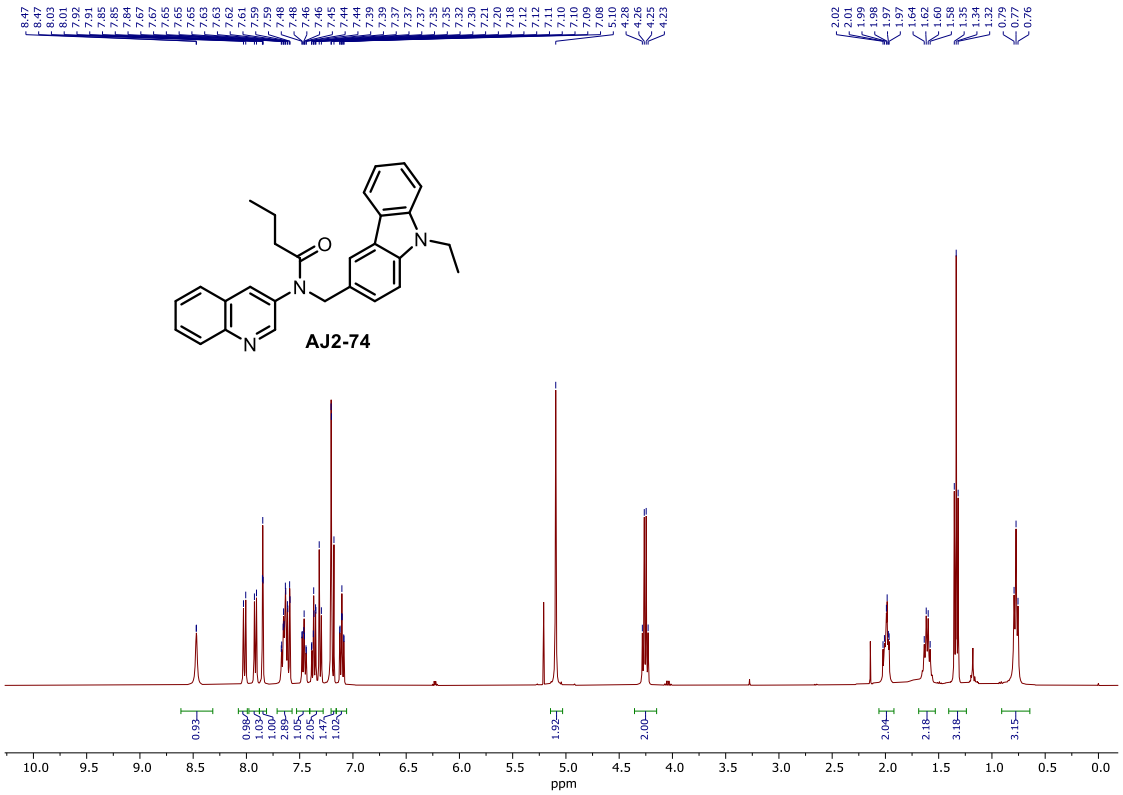
^{1}H NMR ($CDCl_3$) AJ2-66



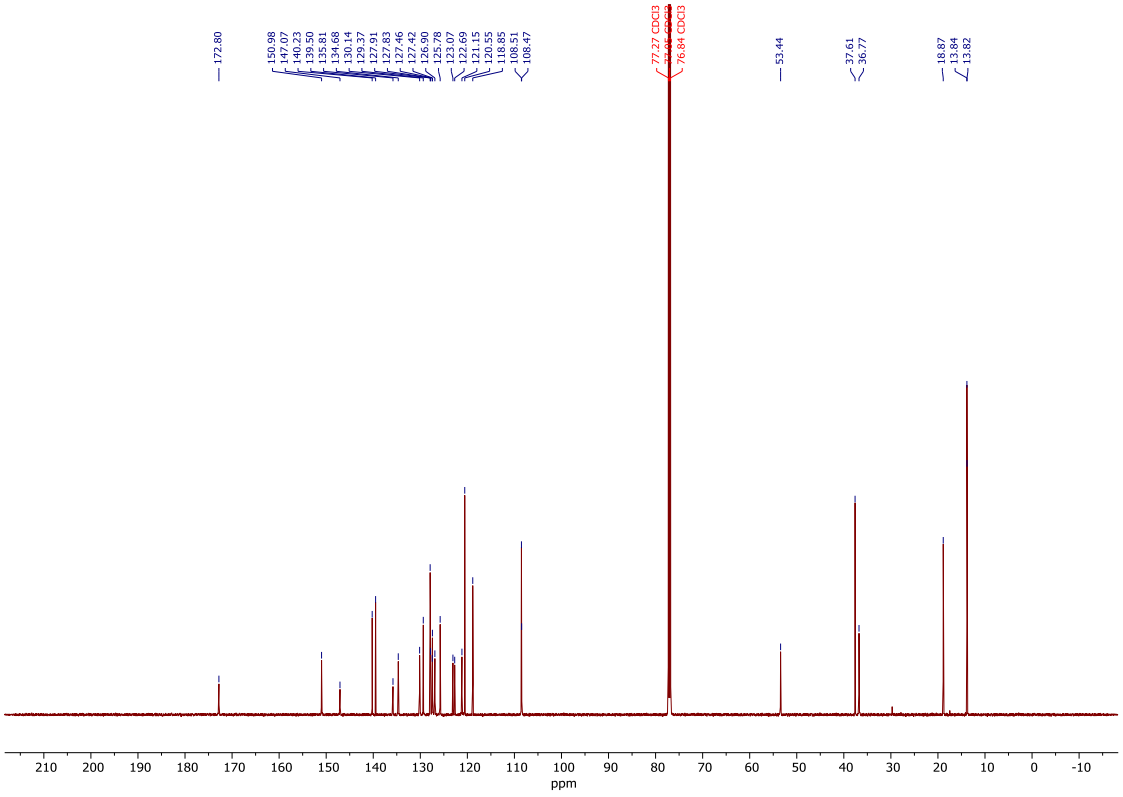
^{13}C NMR ($CDCl_3$) AJ2-66



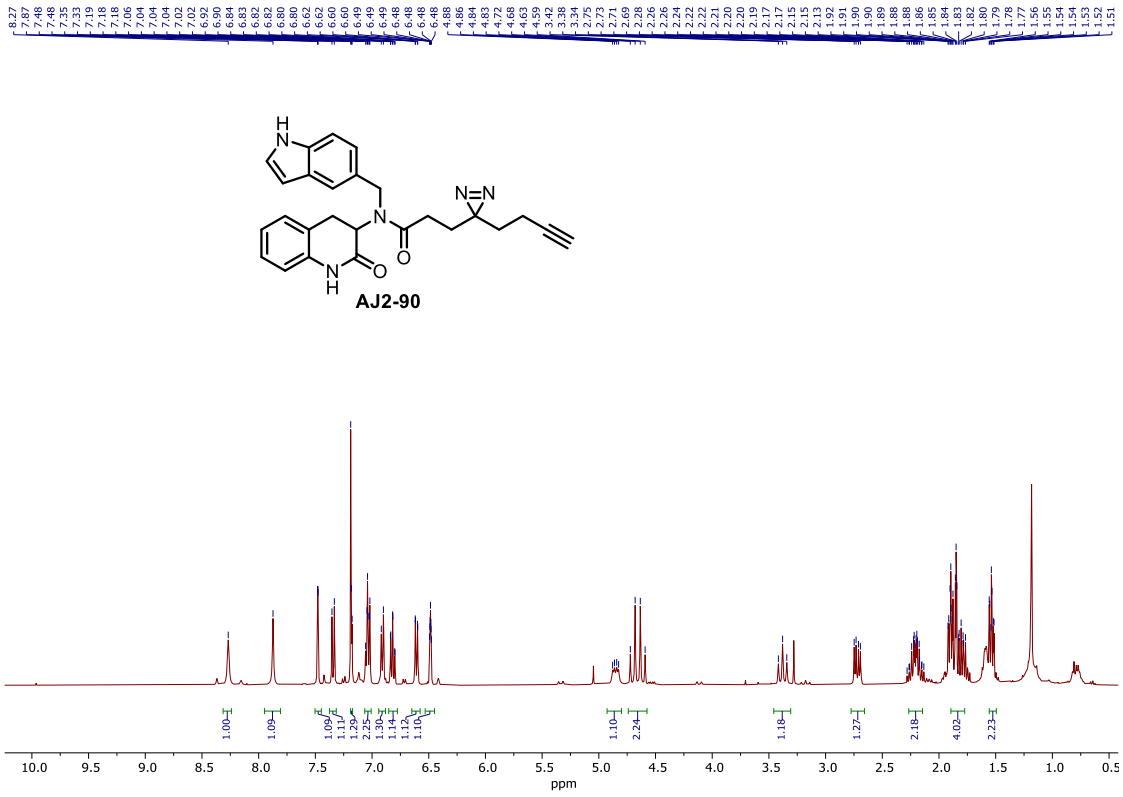
^{1}H NMR ($CDCl_3$) AJ2-74



^{13}C NMR ($CDCl_3$) AJ2-74



^{1}H NMR ($CDCl_3$) AJ2-90



^{13}C NMR ($CDCl_3$) AJ2-90