Supporting Information

Targeted Degradation of Oncogenic KRAS^{G12C} by VHL Recruiting PROTACs

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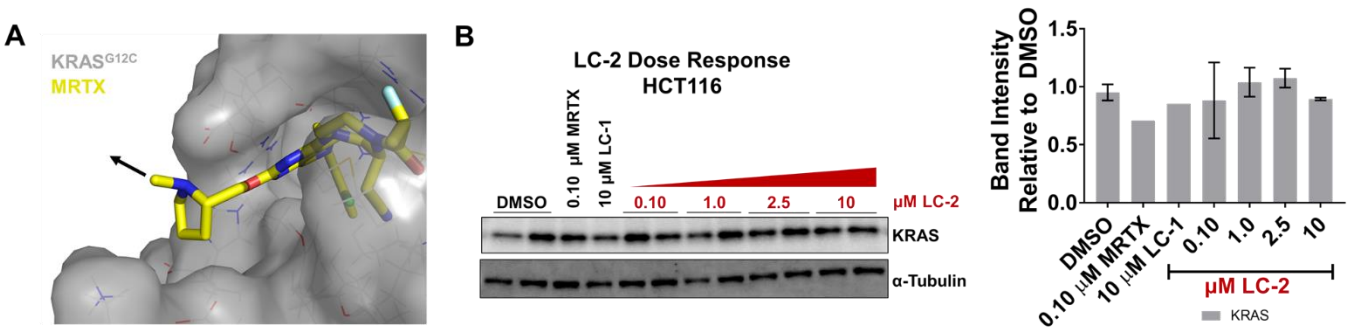
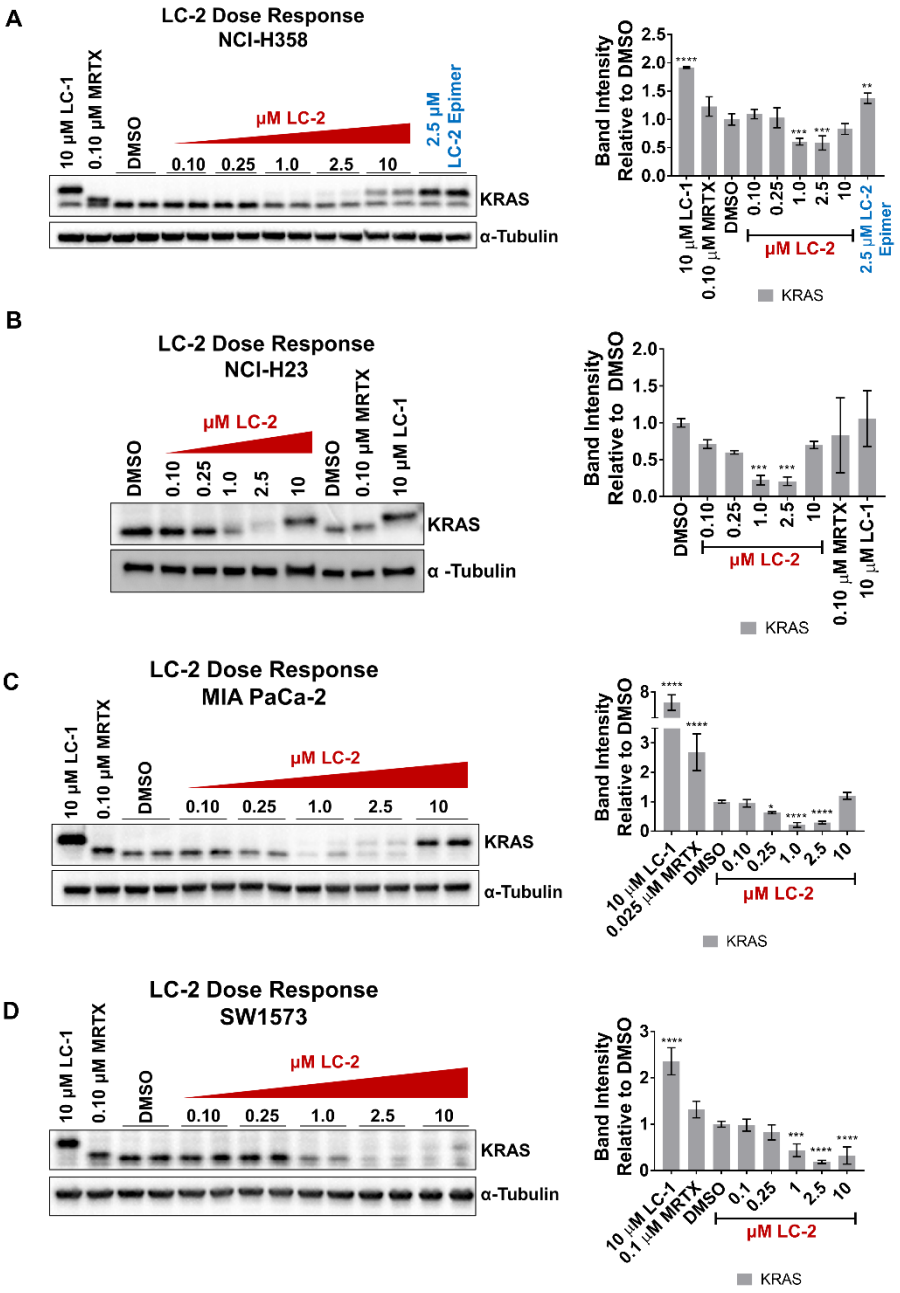
Materials and Methods

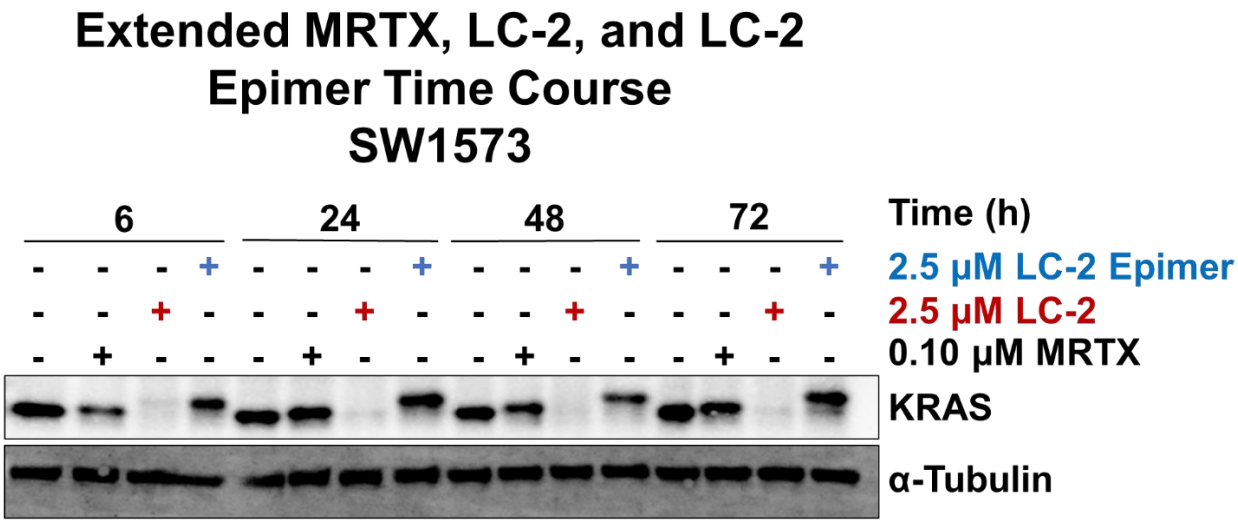
Chemical Synthesis



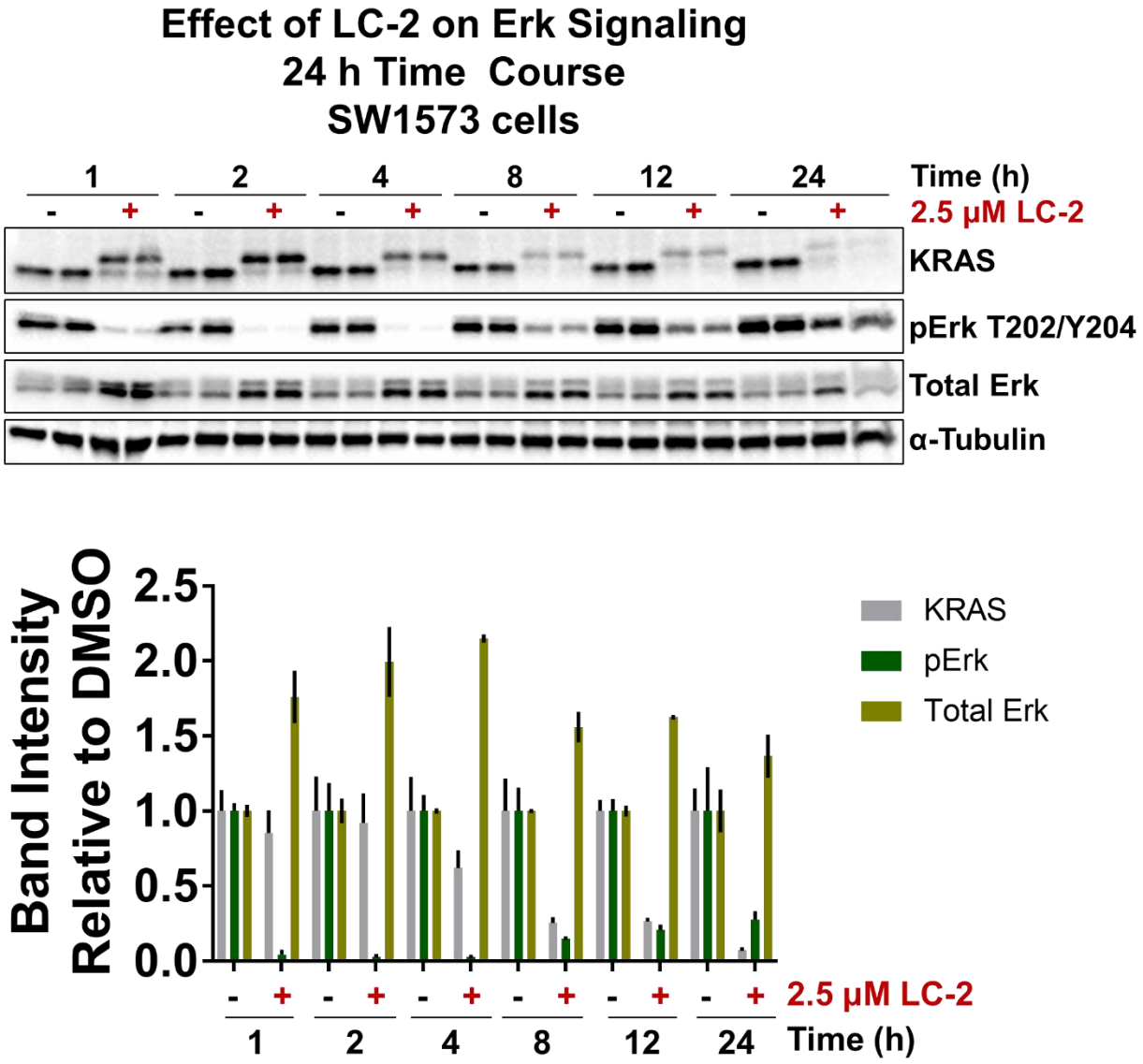
Supporting Information Figure 1: LC-2 degradation is specific for KRAS^{G12C}. A) Crystal structure of MRTX849 bound to KRAS^{G12C} (PDB: 6UT0). MRTX849 is shown in yellow, the black arrow indicates the point of linker attachment. B) LC-2 does not degrade KRAS^{G13D} in HCT116 cells. Immunoblot shows lysates from two independent wells harvested side-by-side on the same day. Quantitation on the right. Quantified data represents mean ± SD.



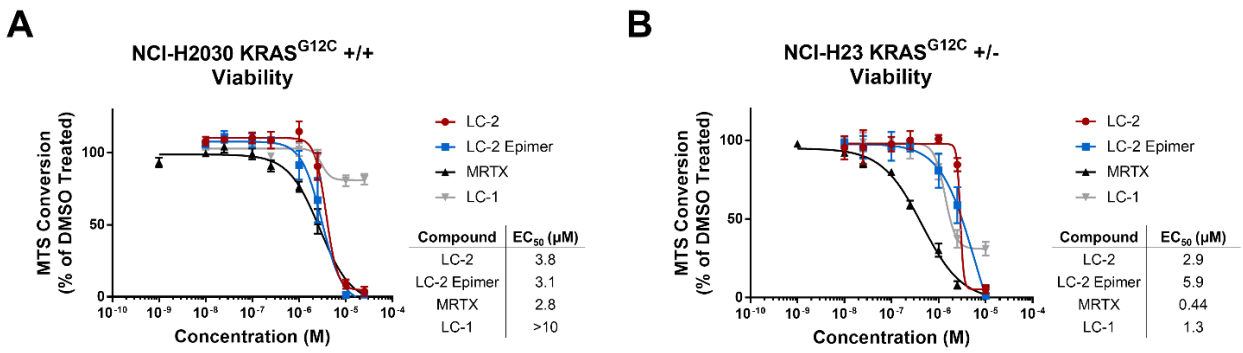
Supporting Information Figure 2: LC-2 induces KRAS^{G12C} degradation in A) heterozygous H358 cells, B) heterozygous NCI-H23 cells, C) homozygous MIA PaCa-2 cells, and D) homozygous, MRTX849 resistant, SW1573 cells. Immunoblots show lysates from two independent wells harvested side-by-side on the same day (except B). Quantitation on the right. Quantified data represents mean +/- SD for two biological replicates. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.001$



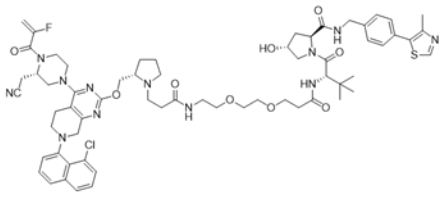
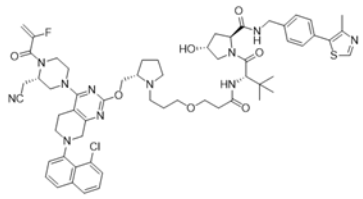
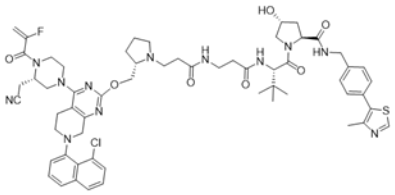
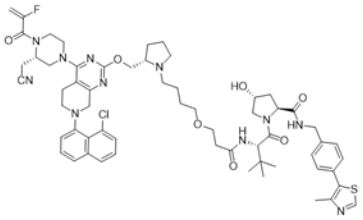
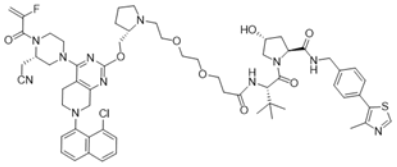
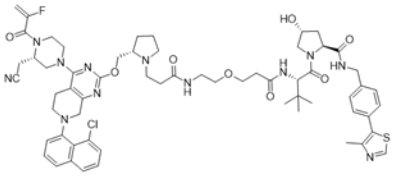
Supporting Information Figure 3: LC-2 induced $KRAS^{G12C}$ degradation is maintained over 72 h in SW1573. LC-2 induced $KRAS^{G12C}$ occurs within 6 h and is maintained for 72 h. No change is observed for LC-2 Epimer.



Supporting Information Figure 4: Changes in Erk signaling during a 24 h LC-2 treatment in SW1573 cells. Erk signaling is modulated by LC-2. pErk is decreased throughout the time course. Total Erk is increased at all time points for LC-2 treated samples. Immunoblot shows lysates from two independent wells harvested side-by-side on the same day. Quantitation on the right. Quantified data represents mean ± SD. For statistical analysis see SI table 6.



Supporting Information Figure 5: Effects of KRAS^{G12C} degradation vs inhibition on cell viability in NCI-H2030 and NCI-H23 cells**.** As expected for a covalent, non-catalytic PROTAC, MRTX is more potent than LC-2 in (A) homozygous NCI-H2030 cells and (B) heterozygous NCI-H23 cells. LC-2 Epimer and MRTX have similar potency. MTS reagent was used to measure cell viability after 72 h of compound treatment. Quantified data represents mean +/- SD.

Name	Structure	Linker Length (Atoms)	DC_{50} (μM)	D_{max} (%)
LC-1		12	N/A	~30[a]
LC-2		6	0.59 0.76	~75[a] ~90[b]
LC-3		6	6.4	~85[a]
LC-4		7	0.88	~90[b]
LC-5		8	>10	~50[b]
LC-6		9	N/A	~35[a]

SI Table 1: MRTX-based, VHL recruiting PROTAC library. Structures, linker length (from the carbon adjacent to the pyrrolidine nitrogen to the carbon adjacent to the VHL carbonyl), and activity are presented. Shorter linker lengths induce higher levels of degradation. [a]Data from NCI-H2030 cells. [b]Data from SW1573 cells.

Effects of LC-2 on KRAS Signaling
NCI-H2030

	DMSO	10 µM LC-1	0.10 µM MRTX	0.10 µM LC-2	0.25 µM LC-2	1.0 µM LC-2	2.5 µM LC-2	10 µM LC-2
KRAS	N/A	*	*	N.S.	N.S.	**	****	****
pErk	N/A	****	****	*	N.S.	****	****	****

Supporting Information Table 2: Two-way ANOVA analysis of Figure 5A. Not Significant (N.S.); * $p < 0.05$; ** $p < 0.01$; **** $p < 0.001$.

Effects of LC-2 on KRAS Signaling
NCI-H23

	DMSO	0.10 µM LC-2	0.25 µM LC-2	1.0 µM LC-2	2.5 µM LC-2	10 µM LC-2	0.10 µM MRTX	10 µM LC-1
KRAS	N/A	N.S.	*	****	*****	N.S.	N.S.	N.S.
pErk	N/A	N.S.	N.S.	N.S.	*	N.S.	*	*

Supporting Information Table 3: Two-way ANOVA analysis of Figure 5B. Not Significant (N.S.); * $p < 0.05$; **** $p < 0.001$.

Effects of LC-2 on KRAS Signaling
MIA PaCa-2

	Time (h)					
	6			24		
	DMSO	100 nM MRTX849	2.5 µM LC-2	DMSO	100 nM MRTX849	2.5 µM LC-2
KRAS	N/A	N.S.	**	N/A	N.S.	**
pErk	N/A	****	**	N/A	*	**

Supporting Information Table 4: Two-way ANOVA analysis of Figure 6A. Not Significant (N.S.); * $p < 0.05$; ** $p < 0.01$; **** $p < 0.001$.

Effects of LC-2 on KRAS Signaling
NCI-H23

	Time (h)					
	6			24		
	DMSO	100 nM MRTX849	2.5 µM LC-2	DMSO	100 nM MRTX849	2.5 µM LC-2
KRAS	N/A	N.S.	**	N/A	****	****
pErk	N/A	****	****	N/A	****	****

Supporting Information Table 5: Two-way ANOVA analysis of Figure 6B. Not Significant (N.S.); * $p < 0.05$; ** $p < 0.01$; **** $p < 0.001$.

Effect of LC-2 on KRAS Signaling
24 h Time Course
SW1573 cells

	Time (h)											
	1		2		4		8		12		24	
	DMSO	2.5 µM LC-2	DMSO	2.5 µM LC-2	DMSO	2.5 µM LC-2	DMSO	2.5 µM LC-2	DMSO	2.5 µM LC-2	DMSO	2.5 µM LC-2
KRAS	N/A	N.S.	N/A	N.S.	N/A	N.S	N/A	**	N/A	**	N/A	***
pErk	N/A	****	N/A	****	N/A	****	N/A	**	N/A	*	N/A	N.S
Total Erk	N/A	****	N/A	****	N/A	****	N/A	***	N/A	****	N/A	*

Supporting Information Table 6: Two-way ANOVA analysis of SI Figure 4. Not Significant (N.S.); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; $p < 0.001$.

Materials and Methods:

Cell Lines and Reagents:

NCI-H2030 (CRL-5914), MIA PaCa-2 (CRL-1420), NCI-H23 (CRL-5800), NCI-H358 (CRL-5807), and HCT-116 (CCL-247) cells were obtained from ATCC, expanded immediately, and frozen down. Vials were thawed and used within 20 passages. SW1573 cells were a gift from Arvinas and were handled in the same manner. NCI-H2030, NCI-H23, and NCI-H358 were cultured in RPMI (ATCC 30-2001) supplemented with 10% FBS (Biological Industries; cat. no. S1480) and 1% Penicillin-Streptomycin (ThermoFisher; cat. no. 15140122). HCT-116 cells were maintained in high glucose DMEM (ThermoFisher; cat. no. 11965084) supplemented as above. MIA PaCa-2 cells were cultured in high glucose DMEM supplemented with 10% FBS, 2.5% horse serum (ThermoFisher; cat. no. 26050-088; lot 2109875), and 1% Penicillin-Streptomycin. SW1573 cells were maintained in DMEM/F12 Nutrient Mix with GlutaMAX supplement (ThermoFisher; cat. no. 10565018) with 10% FBS and 1% Penicillin-Streptomycin added. DPBS (ThermoFisher; cat. no. 14190250) was used to wash cells and 0.25% Trypsin-EDTA (ThermoFisher; cat. no. 252000-056) was used to detach cells for passaging. MRTX849 was purchased from ChemieTek (cat. no. CT-MRTX849), epoxomicin was purchased from Astatech (cat. no. 41576), MLN4924 (pevonedistat) was purchased from Selleckchem (cat. no. S7109), and bafilomycin A1 was purchased from Millipore Sigma (cat. no. B1793). The VHL ligand was generously provided by Arvinas.

Time Course Assays:

Between 2.5 x 10^5 and 6.0 x 10^5 cells were seeded into 6-well plates (Corning; cat. no. 353046). The next day, media was removed and cells were treated with 2.5 μM LC-2 for 1, 2, 4, 8, 12, or 24 h. Cells were treated with 0.10 μM MRTX849 and 2.5 μM LC-2 for 6, 24, 48, or 72 h for longer time course experiments. For 24-h time course experiments, cells were treated at the indicated times and concurrently lysed in RIPA buffer supplemented as described previously. For longer time course experiments and time courses in which cells were treated with LC-2 or LC-2 Epimer, cells were concurrently treated with either compound, then lysed by scraping in RIPA buffer at the indicated time

points. For an individual experiment conducted on a given day, two separate wells of cells were treated identically for every time point and harvested side-by-side.

Competition, Proteasome Inhibition, and Neddylation Inhibition Experiments

Between 2.5 x 10^5 and 5.0 x 10^5 cells were seeded into 6-well plates. The next day cells were pretreated with DMSO, 500 μM or 1 mM VHL ligand, 1 μM epoxomicin, 1 μM MLN4924, or 100 nM M bafilomycin A1 for 1 h. Media was then removed and cells were treated with DMSO, 2.5 μM LC-2 plus DMSO, 2.5 μM LC-2 Epimer plus DMSO, or co-treated with 2.5 μM LC-2 and the corresponding competitor/inhibitor. NCI-H2030 cells were treated for 4 h and NCI-H23 cells were treated for 24 h, after which cells were lysed by scraping in RIPA buffer supplemented as described previously. For an individual experiment conducted on a given day, two separate wells of cells were treated identically for every condition and harvested side-by-side.

Immunoblotting:

Cell lysates were clarified at 21,000 x g for 15 mins at 4° C. Protein levels were quantified using a 50:1 mixture of bicinchoninic acid solution (Millipore Sigma; cat. no. B9643) and 4% (w/v) copper(II) sulfate solution (Millipore Sigma; cat. no. C2284) incubated at 37° C for 30 mins. Absorbance values at 560 nm were read on an EnVision 2101 Multilabel Reader (PerkinElmer). Proteins were separated using 26 well Criterion TGX precast 4-15% (cat. no. 5671085) or 8-16% (cat. no. 5671105) gradient midi gels. After separation, proteins were transferred to nitrocellulose or PVDF membranes at 76 V for 2 h at 4° C. Blots were then blocked in 5% milk in tris-buffered saline with Tween-20 (TBST; 20 mM Tris, 150 mM NaCl, 0.02 % Tween-20) for 1 h. After blocking, blots were incubated in primary antibody overnight (12-18 h) at 4° C or for 2 h at room temperature with mild agitation at the manufacturer's indicated dilution in either 5% milk or 5% BSA in TBST. Blots were then washed thrice with TBST for 5 mins at room temperature. After washing, blots were incubated with 1:5,000-1:10,000 of donkey anti-rabbit (GE Life Sciences; NA934) or sheep anti-mouse (GE Life Sciences; NA931) secondary antibody diluted in 5% milk for 1 h at room temperature with mild agitation. Blots were again washed thrice with TBST for 5 mins. Chemiluminescent signal was generated using Amersham ECL

Prime Western Blotting Detection Reagant (GE Life Sciences; cat. no. RPN2232) or SuperSignal West Femto Maximum Sensitivity Substrate (ThermoFisher; cat. no. 34095). Images were obtained using a Bio-Rad ChemiDoc Imager. Fluorescent α-tubulin images were collected with the Bio-Rad ChemiDoc Imager using an Alexa488 filter. The primary antibodies used in this work include: KRAS (LSBio; clone 2C1; cat. no. LS-C175665), p-p42/44 MAPK (phospho T202/Y204) (pErk; Cell Signaling Technologies (CST); cat. no. 4370S or 9106S), p42/44 MAPK (Erk1/2; CST; cat. no. 4695S), alpha-tubulin (CST; 2144S), and alpha-tubulin w/ AlexaFluor488 (Millipore; cat. no. 16-232).

Quantification and Statistical Analysis for Immunoblotting:

Band intensities were quantified using BioRad's Image Lab Software. Total KRAS levels were examined by quantifying levels of both conjugated $KRAS^{G12C}$ and unbound KRAS (wild type or mutant) using an analysis box that spanned the two bands. This same sized box was used to quantify unbound KRAS in DMSO treated samples to account for background, except for LC-2 Epimer in Figure 3 (see below). KRAS band intensities were first normalized to the corresponding tubulin for each sample. For an individual experiment conducted on a given day, two separate wells of cells were treated identically for every condition or time point and harvested side-by-side. Therefore, two DMSO samples were collected for each experiment. DMSO samples were averaged and KRAS, pErk, and/or Total Erk were then normalized to the average of the corresponding protein level in DMSO samples for a given experiment. To quantify LC-2 Epimer engagement in Figure 3 only the top, PROTAC bound, band was quantified and band intensity was normalized to unbound KRAS in DMSO treated samples using a similar sized analysis box.

Data were analyzed by computing one-way ANOVAs or two-way ANOVAs (for grouped data) using multiple comparisons in which the mean of DMSO was compared to all treatment means using GraphPad Prism 7. For time courses, protein levels were compared to DMSO for each time point. DC_{50} and D_{Max} were quantified by fitting data to an [inhibitor] vs. dose response non-linear regression using GraphPad Prism 7.

Cell Viability Assay

Cells were seeded into 96-well plates at a density of 2.0 x 10^3 cells/well and incubated with the indicated concentrations of LC-2, LC-2 Epimer, LC-1, or MRTX849 for 72 h., at which time MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] (Promega Corp.) and PMS [phenazine methosulfate] (Sigma) were added to a final concentration of 330 µg/ml and 25 µM, respectively. Viable cells converted the MTS to its colored formazan derivative, which was quantitated by measuring absorbance at 490 nm using a Perkin-Elmer Envision multi-label plate reader. Dose response curves were generated by fitting the data to an [inhibitor] vs. dose response non-linear regression using GraphPad Prism 7.

Safety Statement

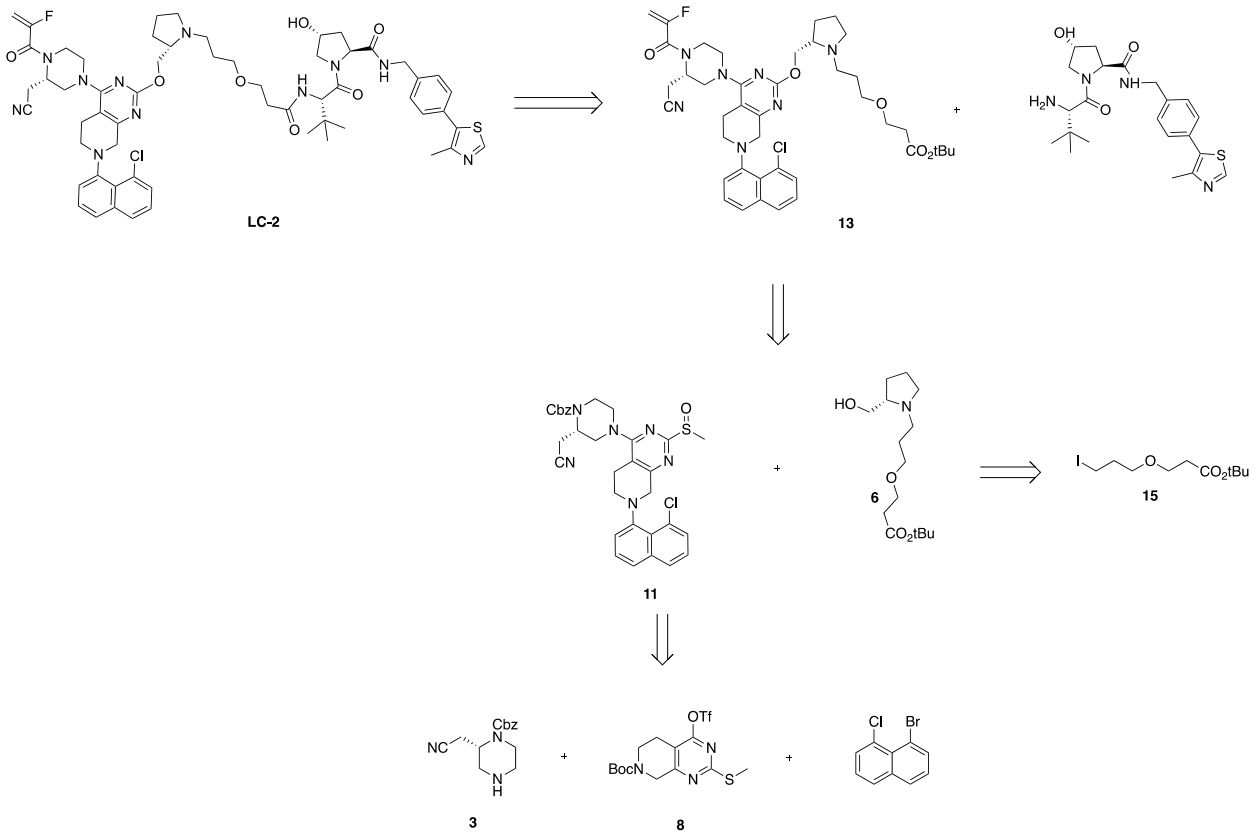
No unexpected or unusually high safety hazards were encountered during the synthesis of testing of the molecules described in the manuscript or SI.

Chemical synthesis:

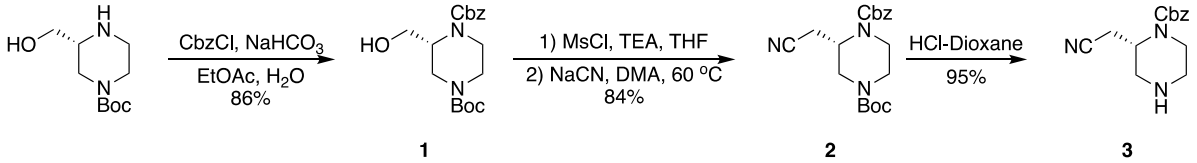
A. General considerations. Chemicals used for synthesis were purchased from commercial sources and were used without further purification. Flash chromatography was performed using Biotage flash chromatography system using pre-packed columns. ^{1}H NMR and ^{13}C NMR spectra were recorded on an Agilent DD2 600 NMR spectrometer (600 MHz for ^{1}H and 151 MHz for ^{13}C). The values of chemical shifts (δ) are reported in p.p.m. Coupling constants (*J*) are reported in Hz. High-resolution mass spectra (HRMS) were recorded on a Waters Xevo QTOF LCMS with ESI using a Waters Acquity UPLC. HPLC purifications were performed on a reverse-phase column using a Gilson HPLC system.

B. Experimental protocols.

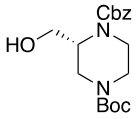
Retrosynthetic analysis of **LC-2**:



Synthesis of **3**:



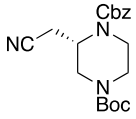
1 **2** **3**



1-benzyl 4-(*tert*-butyl) (*R*)-2-(hydroxymethyl)piperazine-1,4-dicarboxylate (**1**)[s1]

To a solution of tert-butyl (3R)-3-(hydroxymethyl)piperazine-1 -carboxylate (5.0 g, 1.0 eq) in Ethyl acetate (100 mL) was added $NaHCO_3$ (3.0 eq), H_2O (50 mL) and benzyl carbonochloridate (1.30 eq). The mixture was stirred at 25 °C. for 12 h. After completion, the organic phase was separated, washed with water (100 mLX2) dried over Na_2SO_4 and filtered. The solvent was removed under vacuum to give the title compound (7.0 g, 86% yield) as a yellow oil, which was used in the next step without further purification.

LCMS [ESI, M+1]: 351.



1-benzyl 4-(*tert*-butyl) (*S*)-2-(cyanomethyl)piperazine-1,4-dicarboxylate (**2**)[s1]

To a solution of I-benzyl 4-tert-butyl (2R)-2-(hydroxymethyl) piperazine-l,4-dicarboxylate (7.0 g, 1.0 eq) in THF (100 mL) was added TEA (3.0 eq) and methanesulfonyl chloride (1.2 eq). The mixture was stirred at 20 °C. for one h. The reaction mixture was quenched by addition H2O 50 mL at 20 °C. The reaction mixture was extracted with ethyl acetate (100 mLx2). The organic layers were washed with H_2O (100 mL), dried over Na_2SO_4, and filtered. The solvent was removed under vacuum. 1-benzyl 4-tert-butyl (2R)-2-(methylsulfonyloxymethyl) piperazine-l,4-dicarboxylate was obtained as a yellow oil. The crude product was used directly to the next step without further purification.

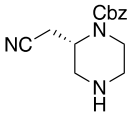
To a solution of 1-benzyl 4-tert-butyl (2R)-2-(methylsulfonyloxymethyl)piperazine-1,4-dicarboxylate in DMA (150 mL) was added NaCN (4 eq.). The mixture was stirred at 60 °C.

for 12 h. The solvent was removed under vacuum to give an oil residue. The residue was diluted with H_2O (40 mL) and extracted with ethyl acetate (50 mLx3). The combined organic layers were washed with saturated brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Hexanes/Ethyl acetate=5:1 to 3:1) to give the title compound (6.0 g, two steps yield 84 %) as a yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40-7.32 (m, 5H), 5.16 (s, 2H), 4.55 (s, 1H), 4.25-3.80 (m, 3H), 2.95-3.25 (m, 2H), 2.85 (s, 1H), 2.60 (d, *J* = 50.2 Hz, 1H), 2.74-2.40 (m, 2H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 154.9, 154.6, 135.8, 128.6, 128.4, 128.2, 116.7, 81.0, 68.0, 48.2, 45.3, 42.4, 39.2, 28.3, 19.1.

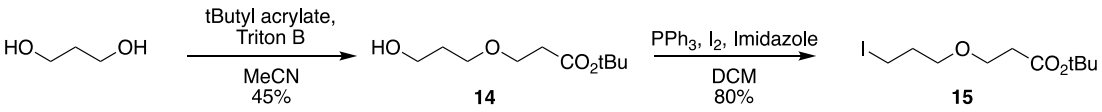
HRMS [$C_{19}H_{25}N_3O_4Na^+$] Cal: 382.1737; Obs: 382.1743.

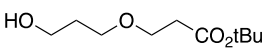


benzyl (*S*)-2-(cyanomethyl)piperazine-1-carboxylate (**3**)[s1]

To a solution of I-benzyl 4-tert-butyl (2S)-2-(cyanomethyl) piperazine-l,4-dicarboxylate (6.0 g, 1.0 eq) in dioxane (20.8 mL) was added 4.0 M HCl in dioxane (20.8 mL, 5.0 eq). The mixture was stirred at 20 °C. for 1 h. Then $NaHCO_3$ was added to the reaction mixture until a pH>7 was reached, after which the reaction was concentrated under reduced pressure to remove dioxane. The residue was diluted with H_2O (50 mL) and extracted with ethyl acetate (50 mLx3). The combined organic layers were washed with H_2O (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The product benzyl (2S)-2-(cyanomethyl) piperazine-1-carboxylate (4.1 g, 95% yield) was obtained as a yellow oil.

Synthesis of **15**:



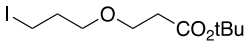


tert-butyl 3-(3-hydroxypropoxy)propanoate (**14**)

To a solution of propane 1,3-diol (20 mmol, 1.5 g, 1.0 eq.) in acetonitrile (20 mL) was added Triton B (0.3 eq.), and tert-butyl 3-(3-hydroxypropoxy)propanoate (1.0 eq.). The mixture was stirred at 20 °C overnight and concentrated under vacuum. The residue was purified by column chromatography (SiO_2, Hexanes/Ethyl acetate=5:1 to 1:1) to give the title compound as a colorless oil (1.85 g, 45% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ 3.77 – 3.72 (m, 2H), 3.67 (t, *J* = 6.2 Hz, 2H), 3.65 – 3.60 (m, 2H), 2.48 (t, *J* = 6.2 Hz, 2H), 1.83 – 1.78 (m, 2H), 1.44 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 171.1, 80.8, 70.1, 66.7, 61.8, 36.2, 31.9, 28.0.



tert-butyl 3-(3-iodopropoxy)propanoate (**15**)

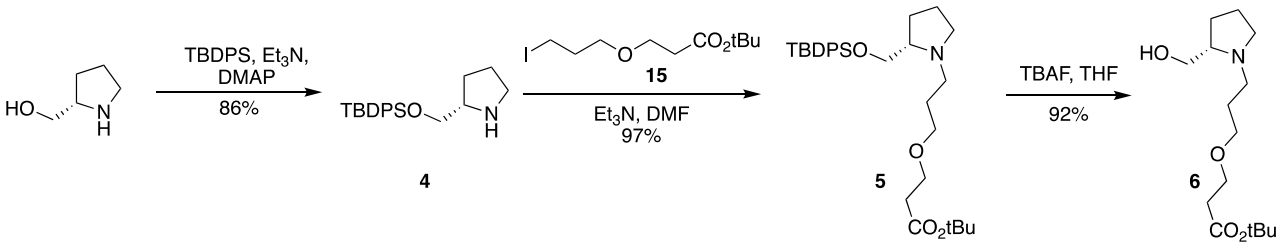
14 (1.85 g, 1.0 eq.) was dissolved in dichloromethane (30 mL) and triphenylphosphine (1.1 eq.) was added followed by imidazole (1.2 eq.). I_2 (1.1 eq.) was added portion wise and the reaction mixture was stirred overnight at 20 °C. The reaction mixture was quenched with a saturated aqueous solution of sodium thiosulfate and stirred for 20 minutes. The organic layer was separated and the aqueous layer was then extracted with dichloromethane. The organic layers were combined, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, Hexanes/Ethyl acetate=0:1 to 3:1) to yield the title compound (2.01 g, 80% yield).

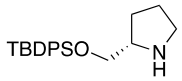
^{1}H NMR (600 MHz, $CDCl_3$) δ 3.66 (t, *J* = 6.4 Hz, 2H), 3.48 (t, *J* = 5.8 Hz, 2H), 3.25 (t, *J* = 6.8 Hz, 2H), 2.46 (t, *J* = 6.4 Hz, 2H), 2.03 (tt, *J* = 6.9, 5.8 Hz, 2H), 1.44 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8, 80.5, 70.1, 66.5, 36.3, 33.3, 28.1, 3.3.

HRMS [$C_{10}H_{19}INaO_3^+$] Cal: 337.0271; Obs: 337.2077.

Synthesis of **6**:





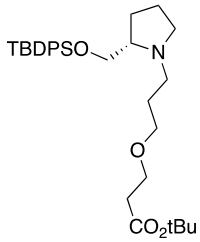
(*S*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)pyrrolidine (**4**)

To a solution of (S)-(+)-2-Pyrrolidinemethanol (1.0 g, 1.0 eq.) in anhydrous THF (20 mL) was added tert-butyl-chloro-diphenyl-silane (1.2 eq.), DMAP (0.1 eq.), and TEA (3.0 eq.). The mixture was stirred at 20 °C overnight and concentrated under vacuum. The residue was purified by column chromatography (SiO_2, DCM/Methanol=1:0 to 10:1) to give the title compound as a yellow oil (2.9 g, 86% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.68 – 7.65 (m, 4H), 7.44-7.35 (m, 6H), 3.68 (dd, *J* = 10.3, 4.8 Hz, 1H), 3.61 (dd, *J* = 10.1, 6.1 Hz, 1H), 3.32-3.26 (m, 1H), 3.04-2.98 (m, 1H), 2.93-2.87 (m, 1H), 1.84-1.70 (m, 3H), 1.55-1.47 (m, 1H), 1.06 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 135.6, 133.5, 133.4, 129.6, 127.7, 66.0, 59.9, 46.3, 27.4, 26.9, 25.16, 19.3.

HRMS [$C_{21}H_{30}NOSi^+$] Cal: 340.2091; Obs: 340.2088.



tert-butyl (*S*)-3-(3-(2-(((*tert*-butyldiphenylsilyl)oxy)methyl)pyrrolidin-1-yl)propoxy)propanoate (**5**)

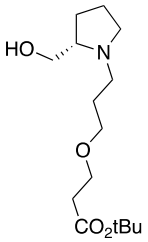
To a solution of **4** (2.0 g, 1.0 eq.) in DMF (4 mL) was added *tert*-butyl 3-(3-iodopropoxy)propanoate (1.0 eq.) and TEA (3.0 eq.). The mixture was stirred at 20 °C overnight and concentrated under vacuum. The residue was purified by column

chromatography (SiO_2, DCM/Methanol=1:0 to 10:1) to give the title compound as a yellow oil (3.0 g, 97% yield).

^1H NMR (600 MHz, $CDCl_3$) δ 7.80-7.56 (m, 4H), 7.51-7.37 (m, 6H), 4.56 – 3.79 (m, 4H), 3.66 – 3.44 (m, 5H), 3.36 (s, 1H), 3.06 (s, 1H), 2.53-2.31 (m, 3H), 2.26-1.99 (m, 5H), 1.90-1.78 (m, 1H), 1.60 (s, 2H), 1.43 (s, 9H), 1.08 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 171.1, 135.7, 135.5, 130.2, 128.1, 128.0, 80.8, 70.5, 68.2, 66.4, 62.7, 54.9, 35.9, 28.1, 26.9, 25.5, 22.07, 19.1.

HRMS [$C_{31}H_{48}NO_4Si^+$] Cal: 526.3347; Obs: 526.3353.

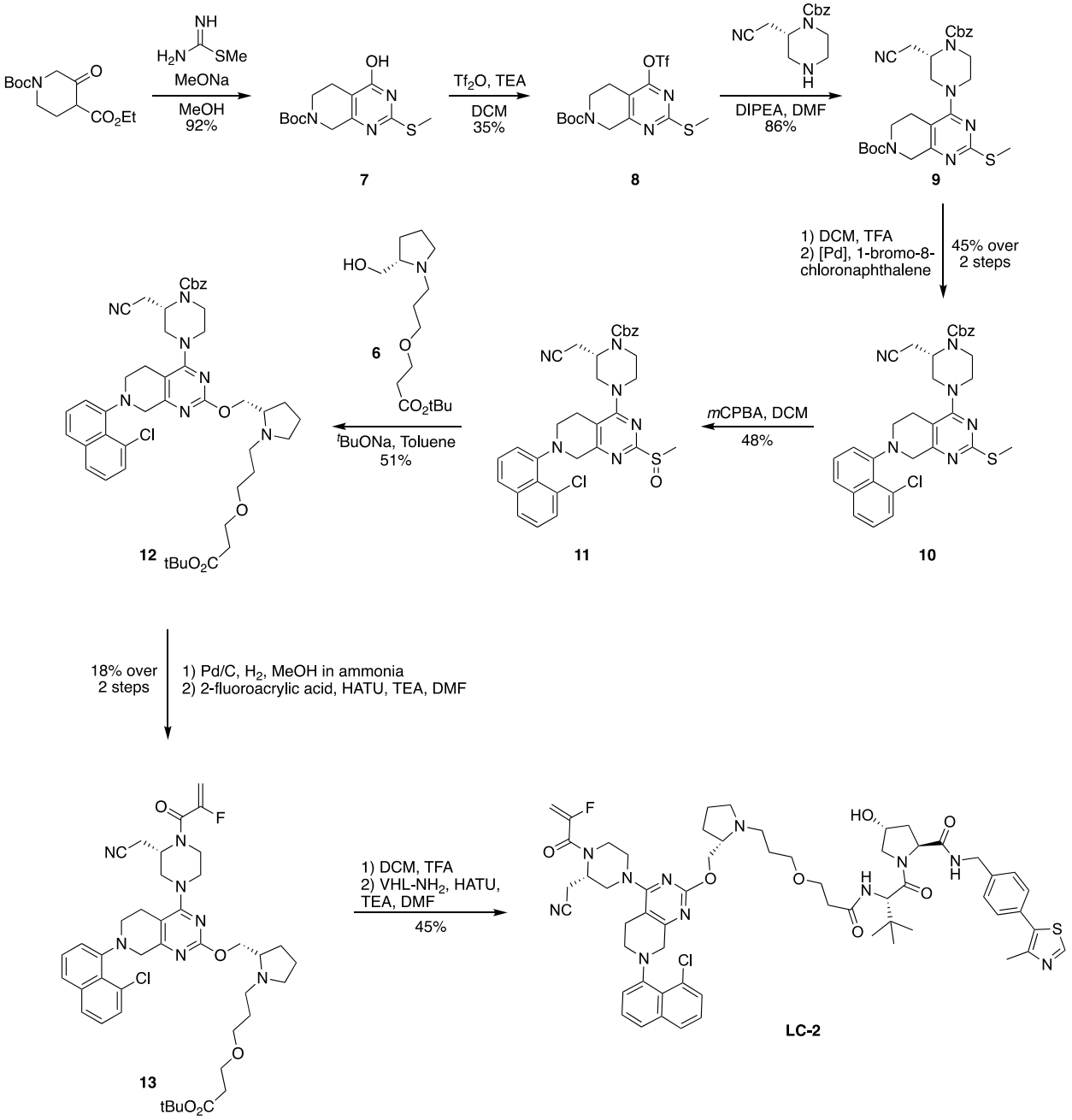


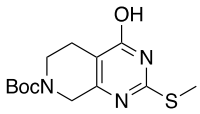
tert-butyl (*S*)-3-(3-(2-(hydroxymethyl)pyrrolidin-1-yl)propoxy)propanoate (**6**)

To a solution of **5** (3.0 g, 1.0 eq.) in anhydrous THF (10 mL) was added TBAF (1.0 M in THF, 2.0 eq.). The mixture was stirred at 20 °C overnight and concentrated under vacuum. The residue was purified by column chromatography (SiO_2, DCM/Methanol=1:0 to 5:1) to give the title compound as a yellow oil (1.5 g, 92% yield).

HRMS [$C_{15}H_{30}NO_4^+$] Cal: 288.2169; Obs: 288.2175.

Synthesis of **LC-2**[S1]:

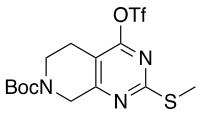




tert-butyl 4-hydroxy-2-(methylthio)-5,8-dihydropyrido[3,4-*d*]pyrimidine-7(6*H*)-carboxylate (**7**)[S1]

Step 1: To a stirred solution of 1-tert-butyl 4-ethyl 3-oxopiperidine-1,4-dicarboxylate (17.0 g, 1.0 eq) in MeOH (300 mL) at 20 °C under nitrogen was added NaOMe (5.0 eq), followed by 2-methylisothiourea (1.80 eq.) as a solid. The reaction mixture was stirred at 20 °C for 16 h. The reaction mixture was acidified with HCl (2 M) until pH~5, and then the mixture was concentrated under reduced pressure to removed MeOH. The residue was resuspended in 300 mL of ethyl acetate and 300 mL of water and stirred rapidly. The suspension was filtered and the white solid was collected. The filtrate was separated and the organics washed with water (1x300 mL) and brine (1x200 mL). The organics were isolated, dried over Na_2SO_4, filtered and concentrated to a white solid, tert-butyl 4-hydroxy-2-methylsulfanyl-6,8-dihydro-5H-pyrido[3,4-d]pyrimidine-7-carboxylate (17.2 g, 92% yield) was obtained as a white solid and used directly for next step without further purification.

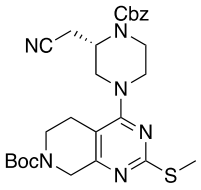
LCMS [M+1]: 298.



tert-butyl 2-(methylthio)-4-(((trifluoromethyl)sulfonyl)oxy)-5,8-dihydropyrido[3,4-*d*]pyrimidine-7(6*H*)-carboxylate (**8**)[S1]

Step 2: To a stirred suspension of **7** (10 g, 1.0 eq) in DCM (200 mL) at 0 °C was added DIEA (2.0 eq.) followed by Tf_2O (1.5 eq.) under nitrogen. Immediately a brown solution formed. After stirring at 25 °C for 16 h, the reaction was concentrated to give a brown oil. The brown oil was purified by column chromatography (SiO2, Hexanes/Ethyl acetate=1/0 to 10/1) to give the title compound (5.1 g, 35% yield) as a yellow solid.

HRMS [$C_{14}H_{19}F_3N_3O_5S_2$+] Cal: 430.0713; Obs: 430.0718.



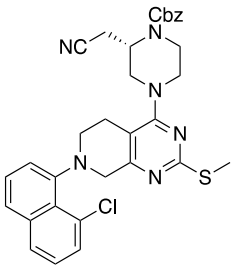
tert-butyl (*S*)-4-(4-((benzyloxy)carbonyl)-3-(cyanomethyl)piperazin-1-yl)-2-(methylthio)-5,8-dihydropyrido[3,4-*d*]pyrimidine-7(6*H*)-carboxylate (**9**)[s1]

Step 3: A mixture of **8** (1.24 g, 1.0 eq), benzyl-(2S)-2-(cyanomethyl)piperazine-1-carboxylate (1.05 eq), and DIEA (3.0 eq) in DMF (10 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 100 °C for 1 h under N_2 atmosphere. After completion, the solvent was removed under vacuum. The residue was purified by column chromatography (SiO_2, Hexanes/ Ethyl acetate=3/1 to 1:1) to give title compound (1.36 g, 86% yield) as a yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43 – 7.30 (m, 5H), 5.17 (s, 2H), 4.72 – 4.55 (m, 2H), 4.36 (d, *J* = 19.1 Hz, 1H), 4.02 – 3.71 (m, 3H), 3.40 – 3.21 (m, 4H), 2.98 (t, *J* = 12.2 Hz, 1H), 2.75 – 2.57 (m, 4H), 2.49 (s, 3H), 1.47 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 168.61, 164.66, 135.82, 128.68, 128.65, 128.45, 128.29, 128.26, 128.23, 117.00, 111.12, 68.07, 60.39, 48.50, 47.84, 28.41, 28.02, 25.88, 21.05, 19.16, 17.59, 14.19, 14.05.

HRMS [$C_{27}H_{35}N_6O_4S^+$] Cal: 539.2435; Obs: 539.2438.

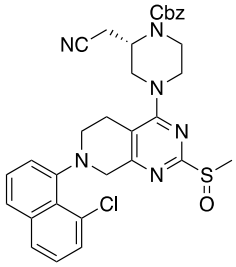


benzyl (*S*)-4-(7-(8-chloronaphthalen-1-yl)-2-(methylthio)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-4-yl)-2-(cyanomethyl)piperazine-1-carboxylate (**10**)[s1]

Step 4: A mixture of **9** (1.36 g，1.0 eq), TFA (6.8 mL) in DCM (6.8 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 20°C for 1 h under N_2 atmosphere. After completion, the reaction mixture was quenched with saturated $NaHCO_3$ solution. The mixture was extracted with ethyl acetate (3x50 mL) and the organic

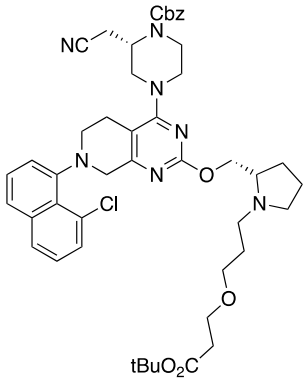
layer was dried over Na_2SO_4 and filtered. The solvent was removed under vacuum to give benzyl (*S*)-2-(cyanomethyl)-4-(2-(methylthio)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-4-yl)piperazine-1-carboxylate (1.11 g, crude) as a yellow solid which was used for the next step without further purification.

Step 5: A mixture of benzyl (2S)-2-(cyanomethyl)-4-(2-methylsulfanyl-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-l-carboxylate (1.11 g, 1.0 eq.), 1-bromo-8-chloro-naphthalene (1.8 eq.), $Pd_2(dba)_3$ (0.1 eq.), RuPhos (0.2 eq.) and Cs2CO3 (3.6 eq.) in toluene (10 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 100 °C for 12 h under N_2 atmosphere. After completion, the reaction mixture was filtered. The organic solvent was removed under vacuum to give an oil residue. The residue was purified by column chromatography (SiO_2, Hexanes/Ethyl acetate=5:1 to 3:1) to give the title compound (0.77 g, two steps yield 45%) as a dark yellow solid.
HRMS [$C_{32}H_{32}ClN_6O_2S^+$] Cal: 599.1990; Obs: 599.1996.



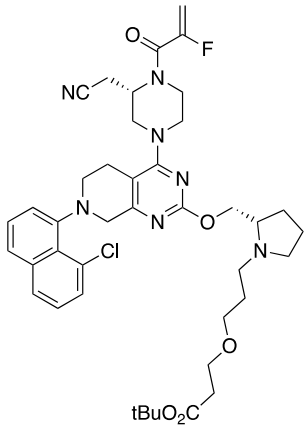
benzyl (2*S*)-4-(7-(8-chloronaphthalen-1-yl)-2-(methylsulfinyl)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-4-yl)-2-(cyanomethyl)piperazine-1-carboxylate (**11**)[s1]
Step 6: A mixture of **10** (734 mg, 1.0 eq), m-CPBA (0.6 eq.) in DCM (8 mL) was stirred at 0 °C for 30 min. After which another batch of m-CPBA (0.6 eq.) was added and the mixture was stirred for another 30 min at 0 °C. After completion, the reaction was quenched with water (10 mL). The mixture was extracted with ethyl acetate (3x10 mL). The combined organic layer was dried with Na_2SO_4 and filtered. The solvent was removed to give an oil residue. The residue was purified by column chromatography (SiO_2, Methanol/Ethyl acetate=0:1 to 1:10) to give the title compound (350 mg, 46% yield, 80% purity) as a yellow solid. The products were obtained as a mixture that was not separable by column chromatography.

LC-MS [ESI, M+1] = 616.



benzyl (*S*)-4-(2-(((*S*)-1-(3-(3-(*tert*-butoxy)-3-oxopropoxy)propyl)pyrrolidin-2-yl)methoxy)-7-(8-chloronaphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-4-yl)-2-(cyanomethyl)piperazine-1-carboxylate (**12**)

Step 7: To a solution of **11** (350 mg, 1.0 eq.) and *tert*-butyl (*S*)-3-(3-(2-(hydroxymethyl)pyrrolidin-1-yl)propoxy)propanoate (**6**) (3.0 eq.) in toluene (5 mL) was added *t*-BuONa (3.0 eq.). The mixture was stirred at 0 °C. for 0.5 h. After completion, the mixture was added to cold water (5 mL) and extracted with ethyl acetate (3x5 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated. The obtained product was purified by column chromatography (SiO_2, Ethyl acetate:Methanol=1:0 to 10:1) to give the title compound (230 mg, 44% yield, 80% purity) as a yellow solid. The products were obtained as a mixture that was not separable by column chromatography. LC-MS [ESI, M+1] = 838.

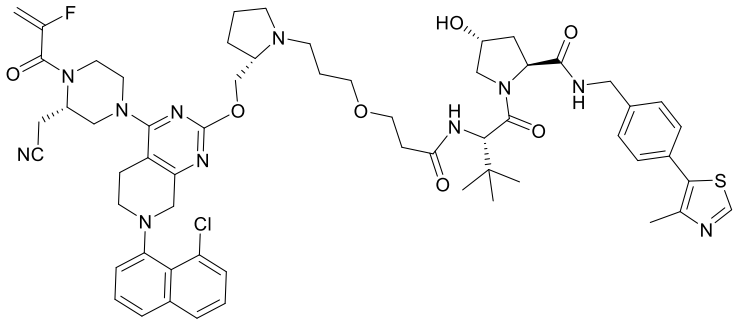


tert-butyl 3-(3-((*S*)-2-(((7-(8-chloronaphthalen-1-yl)-4-((*S*)-3-(cyanomethyl)-4-(2-fluoroacryloyl)piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-2-yl)oxy)methyl)pyrrolidin-1-yl)propoxy)propanoate (**13**)

Step 8: To a solution of **12** (230 mg, 1.0 eq.) in MeOH (3 mL) was added 7N NH_3 in MeOH (3 mL), and Pd/C (100 mg, 10% purity) under N_2. The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 (15 psi) at 20 °C for 4 h. After which another batch of Pd/C (100 mg, 10% purity) was added. The mixture was stirred under H_2 overnight. Upon completion, the catalyst was filtered off and the filtrate was concentrated under vacuum to give the title compound (100 mg, 52% yield) as a yellow solid which was used directly in the next step without further purification. LC-MS [ESI, M+1] = 704.

Step 9: To a solution of above product (141 mg, 1.0 eq.) in DMF was added sodium 2-fluoroprop-2-enoyloxy (2.0 eq.), HATU (1.5 eq.), and TEA (4.0 eq.). The mixture was stirred at room temperature for 1 h. After completion, the residue was diluted with H_2O (15 mL), extracted with EtOAc (3X15 mL), dried over Na_2SO_4, concentrated under vacuum and purified by Prep-TLC (DCM:Methanol:Ammonia = 200:10:1). The title compound (42 mg, 27% yield, 60% purity) was obtained as a colorless oil. The products were obtained as a mixture that was not separable by prep-TLC.

LC-MS [ESI, M+1] = 776.



(2*S*,4*R*)-1-((*S*)-2-(3-(3-((*S*)-2-(((7-(8-chloronaphthalen-1-yl)-4-((*S*)-3-(cyanomethyl)-4-(2-fluoroacryloyl)piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-2-yl)oxy)methyl)pyrrolidin-1-yl)propoxy)propanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**LC-2**)

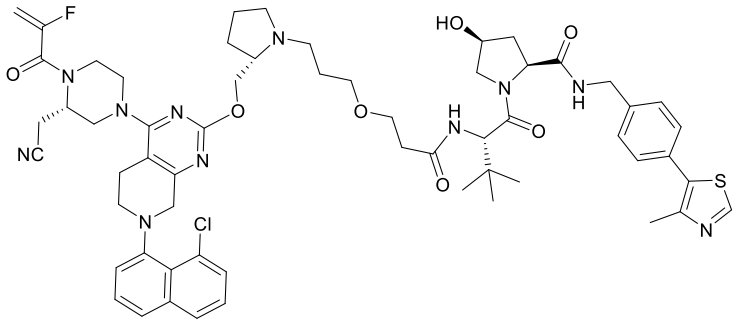
Step 10: To a solution of **13** (21 mg, 1.0 eq.) in DCM (1 mL) was added TFA (1 mL). The mixture was stirred for 0.5 h at room temperature, concentrated under vacuum and used in the next step without further purification.

To a solution of above product (1.0 eq.) in DMF (1 mL) was added (1R)-1-[(2S,4R)-4-hydroxy-2-[[4-(4-methylthiazol-5-yl)phenyl]methylcarbamoyl]pyrrolidine-1-carbonyl]-2,2-dimethyl-propyl (1.2 eq.), HATU (1.3 eq.), and TEA (5.0 eq.). The mixture was stirred for 1 h at room temperature. Upon completion, the mixture was diluted with H_2O (5 mL), extracted with EtOAc (3X5 mL), dried over Na_2SO_4, concentrated under vacuum and purified by reverse phase HPLC. The title compound (10 mg, 33% yield) was obtained as a colorless oil.

^{1}H NMR (600 MHz, Acetone-d_6) δ 8.82 (s, 1H), 7.86 (t, *J* = 7.9 Hz, 2H), 7.70 (dd, *J* = 8.0, 3.9 Hz, 1H), 7.55 (dd, *J* = 7.3, 3.6 Hz, 1H), 7.53 – 7.48 (m, 1H), 7.45 (dd, *J* = 7.8, 4.2 Hz, 2H), 7.43 – 7.39 (m, 1H), 7.39 – 7.33 (m, 3H), 7.29 – 7.24 (m, 1H), 5.33 – 5.20 (m, 2H), 4.64 (dd, *J* = 9.3, 1.6 Hz, 1H), 4.60 – 4.48 (m, 3H), 4.39 – 4.27 (m, 3H), 4.24 – 4.08 (m, 2H), 3.87 – 3.78 (m, 2H), 3.78 – 3.70 (m, 2H), 3.67 – 3.53 (m, 3H), 3.53 – 3.43 (m, 3H), 3.37 (dd, *J* = 13.8, 3.8 Hz, 1H), 3.34 – 3.24 (m, 2H), 3.25 – 3.12 (m, 4H), 3.13 – 2.96 (m, 4H), 2.81 – 2.75 (m, 1H), 2.71 – 2.65 (m, 1H), 2.48 – 2.39 (m, 4H), 2.39 – 2.32 (m, 1H), 2.26 – 2.18 (m, 1H), 2.18 – 2.11 (m, 1H), 2.11 – 2.06 (m, 1H), 1.91 – 1.82 (m, 1H), 1.79 – 1.61 (m, 6H), 0.96 (s, 9H).

^{13}C NMR (151 MHz, Acetone-d_6) δ 172.63, 171.41, 171.34, 170.96, 167.67, 166.06, 163.77, 157.79 (d, *J* = 269.1 Hz), 151.29, 149.67, 149.41, 149.18, 140.54, 138.50, 132.37, 131.35, 130.64, 130.53, 129.92, 129.43, 128.76, 127.78, 126.77, 126.71, 125.92 (d, *J* = 11.3 Hz), 119.93, 118.28, 110.17, 77.33, 70.71, 69.85, 69.83, 67.74, 63.39, 60.60, 59.97, 59.75, 57.59, 57.46, 54.95, 53.23, 51.27, 51.21, 43.20, 38.44, 38.43, 37.41, 36.50, 32.70, 26.98, 26.86, 26.41, 24.05, 23.39, 20.89, 19.43, 16.41, 14.56, 14.41.

HRMS [$C_{59}H_{72}ClFN_{11}O_7S^+$] Cal: 1132.5004; Obs: 1132.5010.



(2*S*,4*S*)-1-((*S*)-2-(3-(3-((*S*)-2-(((7-(8-chloronaphthalen-1-yl)-4-((*S*)-3-(cyanomethyl)-4-(2-fluoroacryloyl)piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-2-yl)oxy)methyl)pyrrolidin-1-yl)propoxy)propanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**LC-2- Epimer**)

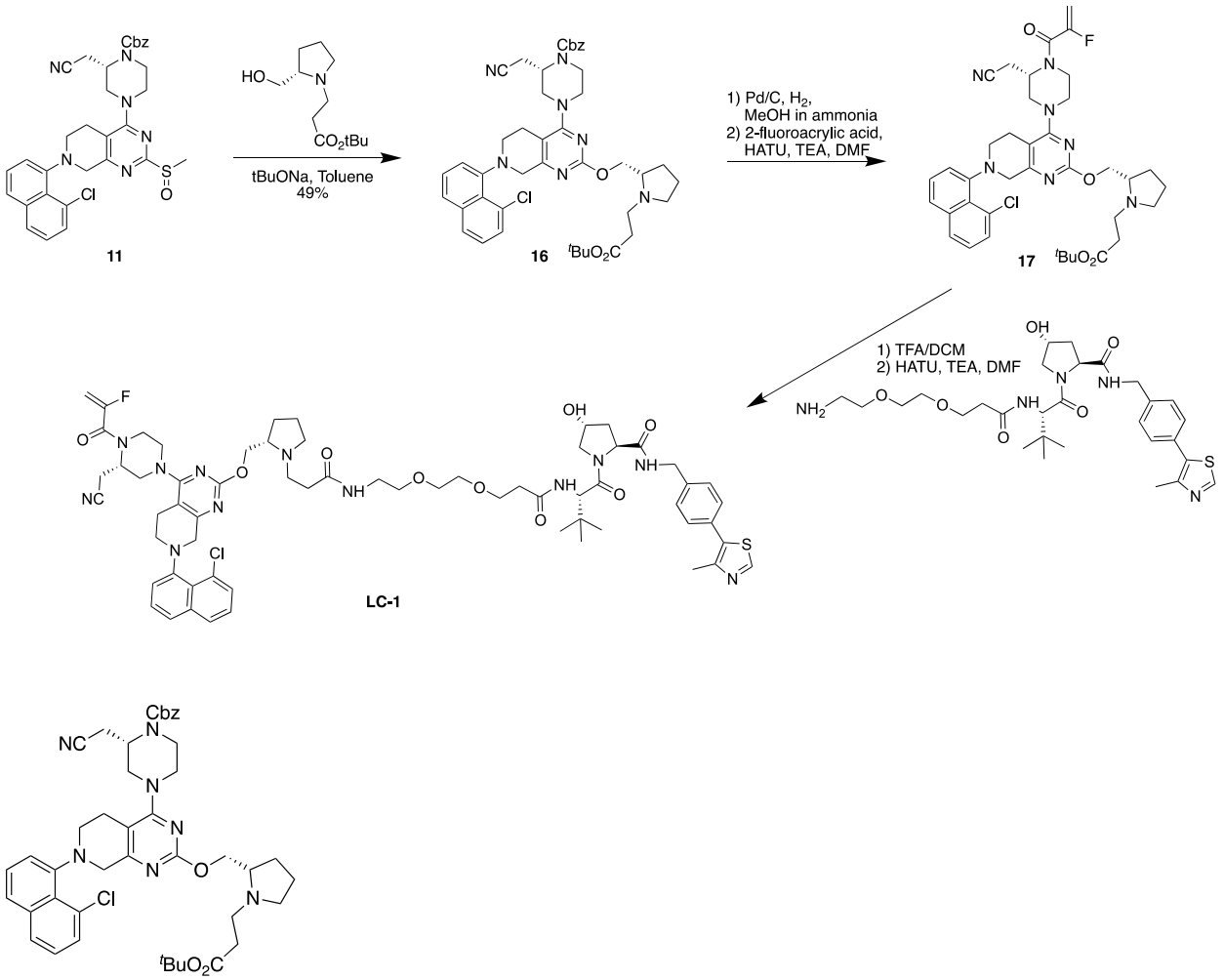
To a solution of **13** (21 mg, 1.0 eq.) in DCM (1 mL) was added TFA (1 mL). The mixture was stirred for 0.5 h at room temperature, concentrated under vacuum and used in the next step without further purification.

To a solution of above product (1.0 eq.) in DMF (1 mL) was added (1R)-1-[(2S,4S)-4-hydroxy-2-[[4-(4-methylthiazol-5-yl)phenyl]methylcarbamoyl]pyrrolidine-1-carbonyl]-2,2-dimethyl-propyl (1.2 eq.), HATU (1.3 eq.), and TEA (5.0 eq.). The mixture was stirred for 1 h at room temperature. Upon completion, the mixture was diluted with H_2O (5 mL), extracted with EtOAc (3X5 mL), dried over Na_2SO_4, concentrated under vacuum and purified by reverse phase HPLC. The title compound (4.4 mg, 15% yield) was obtained as a colorless oil.

^{1}H NMR (400 MHz, Acetone-d_6) δ 8.82 (s, 1H), 7.87 (d, J = 8.5 Hz, 1H), 7.70 (dd, J = 8.1, 2.6 Hz, 1H), 7.59 – 7.53 (m, 1H), 7.52 – 7.32 (m, 7H), 7.24 (dd, J = 9.4, 3.3 Hz, 1H), 5.37 – 5.17 (m, 2H), 5.00 – 4.83 (m, 1H), 4.67 – 4.57 (m, 2H), 4.51 (d, J = 9.6 Hz, 1H), 4.40 – 4.22 (m, 4H), 4.21 – 3.95 (m, 4H), 3.87 – 3.71 (m, 4H), 3.66 – 3.46 (m, 7H), 3.33 – 3.25 (m, 2H), 3.23 – 3.11 (m, 2H), 2.83 – 2.62 (m, 4H), 2.53 – 2.33 (m, 6H), 2.32 – 2.24 (m, 2H), 2.22 – 2.11 (m, 2H), 1.95 (d, J = 2.8 Hz, 1H), 1.91 (s, 2H), 1.78 – 1.62 (m, 4H), 0.97 (s, 9H).

HRMS [$C_{59}H_{72}ClFN_{11}O_7S^+$] Cal: 1132.5004; Obs: 1132.4999.

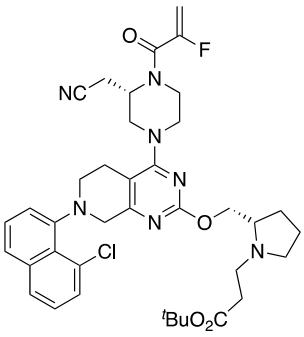
Synthesis of **LC-1**:



benzyl (S)-4-(2-(((S)-1-(3-(tert-butoxy)-3-oxopropyl)pyrrolidin-2-yl)methoxy)-7-(8-chloronaphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)-2-(cyanomethyl)piperazine-1-carboxylate (**16**)

To a solution of **11** (79 mg, 1.0 eq.) and *tert*-butyl (*S*)-3-(2-(hydroxymethyl)pyrrolidin-1-yl)propanoate (3.0 eq.) in toluene (5 mL) was added *t*-BuONa (3.0 eq.). The mixture was stirred at 0 °C. for 0.5 h. After completion, the mixture was added to cold water (5 mL) and extracted with ethyl acetate (3x5 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated. The obtained product was purified by column chromatography (SiO_2, Ethyl acetate:Methanol=1:0 to 10:1) to give the title compound (74 mg, 63% yield, 80% purity) as a yellow solid. The products were obtained as a mixture that was not separable by column chromatography.

LC-MS [ESI, M+1] = 781.

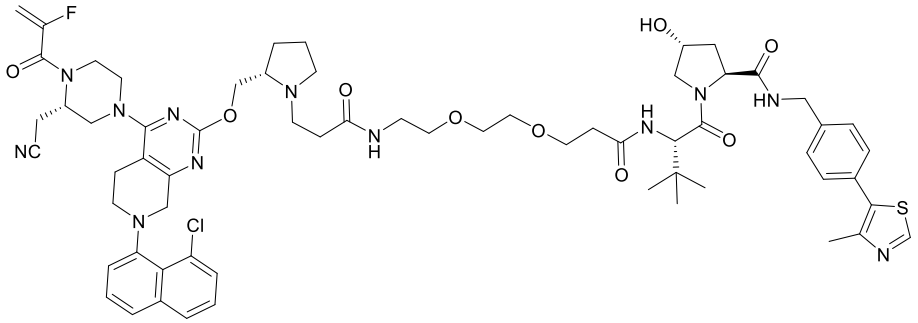


tert-butyl 3-((*S*)-2-(((7-(8-chloronaphthalen-1-yl)-4-((*S*)-3-(cyanomethyl)-4-(2-fluoroacryloyl)piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-2-yl)oxy)methyl)pyrrolidin-1-yl)propanoate (**17**)

To a solution of **12** (20 mg, 1.0 eq.) in MeOH (1 mL) was added 7N NH_3 in MeOH (1 mL), and Pd/C (20 mg, 10% purity) under N_2. The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 overnight. Upon completion, the catalyst was filtered off and the filtrate was concentrated under vacuum to give the title compound (7 mg, 44% yield) as a yellow solid which was used directly in the next step without further purification. LC-MS [ESI, M+1] = 533.

To a solution of above product (7 mg, 1.0 eq.) in DMF was added sodium 2-fluoroprop-2-enoyloxy (2.0 eq.), HATU (1.5 eq.), and TEA (4.0 eq.). The mixture was stirred at room temperature for 1 h. After completion, the residue was diluted with H_2O (5 mL), extracted with EtOAc (3X5 mL), dried over Na_2SO_4, concentrated under vacuum and purified by Prep-TLC (DCM:Methanol:Ammonia = 200:10:1). The title compound (1.8 mg, 23% yield, 60% purity) was obtained as a colorless oil. The products were obtained as a mixture that are not separable by prep-TLC.

LC-MS [ESI, M+1] = 605.



(2*S*,4*R*)-1-((*S*)-2-(*tert*-butyl)-16-((*S*)-2-(((7-(8-chloronaphthalen-1-yl)-4-((*S*)-3-(cyanomethyl)-4-(2-fluoroacryloyl)piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-2-yl)oxy)methyl)pyrrolidin-1-yl)-4,14-dioxo-7,10-dioxa-3,13-diazahexadecanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**LC-1**)

To a solution of **17** (20 mg, 1.0 eq.) in DCM (1 mL) was added TFA (1 mL). The mixture was stirred for 0.5 h at room temperature, concentrated under vacuum and used in the next step without further purification.

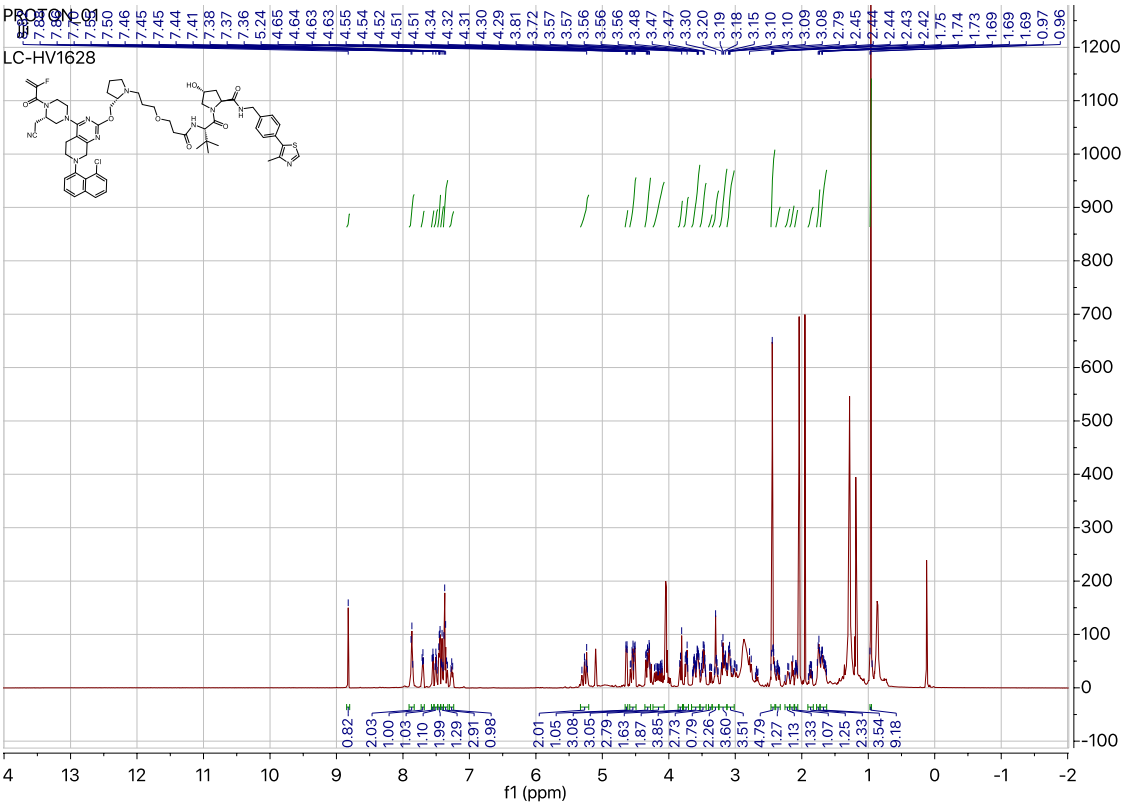
To a solution of above product (1.0 eq.) in DMF (1 mL) was added (2S,4R)-1-((S)-2-(3-(2-(2-aminoethoxy)ethoxy)propanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (1.2 eq.), HATU (1.3 eq.), and TEA (5.0 eq.). The mixture was stirred for 1 h at room temperature. Upon completion, the mixture was diluted with H_2O (5 mL), extracted with EtOAc (3X5 mL), dried over Na_2SO_4, concentrated under vacuum and purified by reverse phase HPLC. The title compound (6.2 mg, 18% yield) was obtained as a colorless oil.

^{1}H NMR (600 MHz, Methanol-d_4) δ 8.84 (s, 1H), 7.80 (d, *J* = 8.2 Hz, 1H), 7.65 (dd, *J* = 8.2, 3.7 Hz, 1H), 7.52 – 7.49 (m, 1H), 7.46 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.43 – 7.40 (m, 2H), 7.39 – 7.33 (m, 3H), 7.32 – 7.27 (m, 1H), 5.38 – 5.23 (m, 2H), 4.63 (s, 1H), 4.55 (t, *J* = 8.4 Hz, 1H), 4.48 (d, *J* = 11.6 Hz, 2H), 4.41 – 4.36 (m, 1H), 4.35 – 4.24 (m, 4H), 4.16 (d, *J* = 13.6 Hz, 1H), 4.11 – 4.02 (m, 2H), 3.87 (d, *J* = 10.9 Hz, 1H), 3.77 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.72 – 3.52 (m, 5H), 3.46 (dt, *J* = 7.4, 5.5 Hz, 2H), 3.38 – 3.27 (m, 9H), 3.25 – 3.04 (m, 6H), 2.91 (dd, *J* = 17.1, 6.9 Hz, 1H), 2.79 – 2.58 (m, 2H), 2.54 – 2.36 (m, 8H), 2.23 – 2.16 (m, 1H), 2.10 – 2.02 (m, 2H), 1.90 – 1.71 (m, 3H), 1.40 – 1.25 (m, 3H), 0.99 (d, *J* = 1.8 Hz, 9H).

^{13}C NMR (151 MHz, Methanol-d_4) δ 174.57, 174.51, 173.14, 173.07 (d, J = 220.9 Hz), 167.96, 167.80, 166.82, 163.67, 152.99, 149.85 (d, J = 21.0 Hz), 149.19, 140.42, 139.06, 133.53, 131.67, 131.08, 131.02, 130.94, 130.51, 129.73, 129.12, 127.96, 127.23, 126.96, 126.52, 126.49, 120.29, 111.58, 110.40, 71.20, 70.56, 69.91, 68.15, 64.89, 61.69, 61.01, 60.59, 60.44, 59.03, 58.23, 55.36, 52.77, 52.73, 51.29, 43.84, 40.51, 39.16, 37.78, 37.37, 36.98, 35.24, 30.93, 29.22, 27.64, 27.51, 27.13, 24.15, 21.01, 16.02, 14.61, 11.58.
HRMS [$C_{63}H_{79}ClFN_{12}O_9S^+$] Cal: 1233.5481; Obs: 1233.5490.

References:

[S1]KRAS G12C inhibitors, US 2018/0072723 A1.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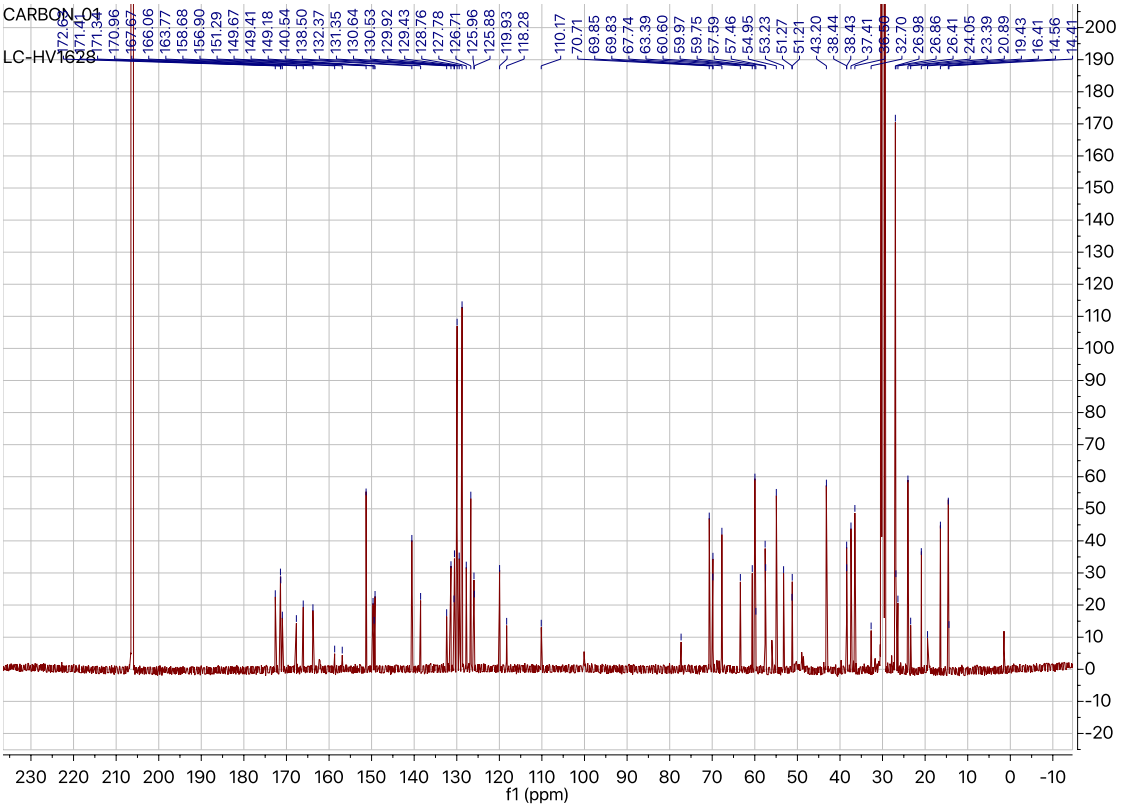
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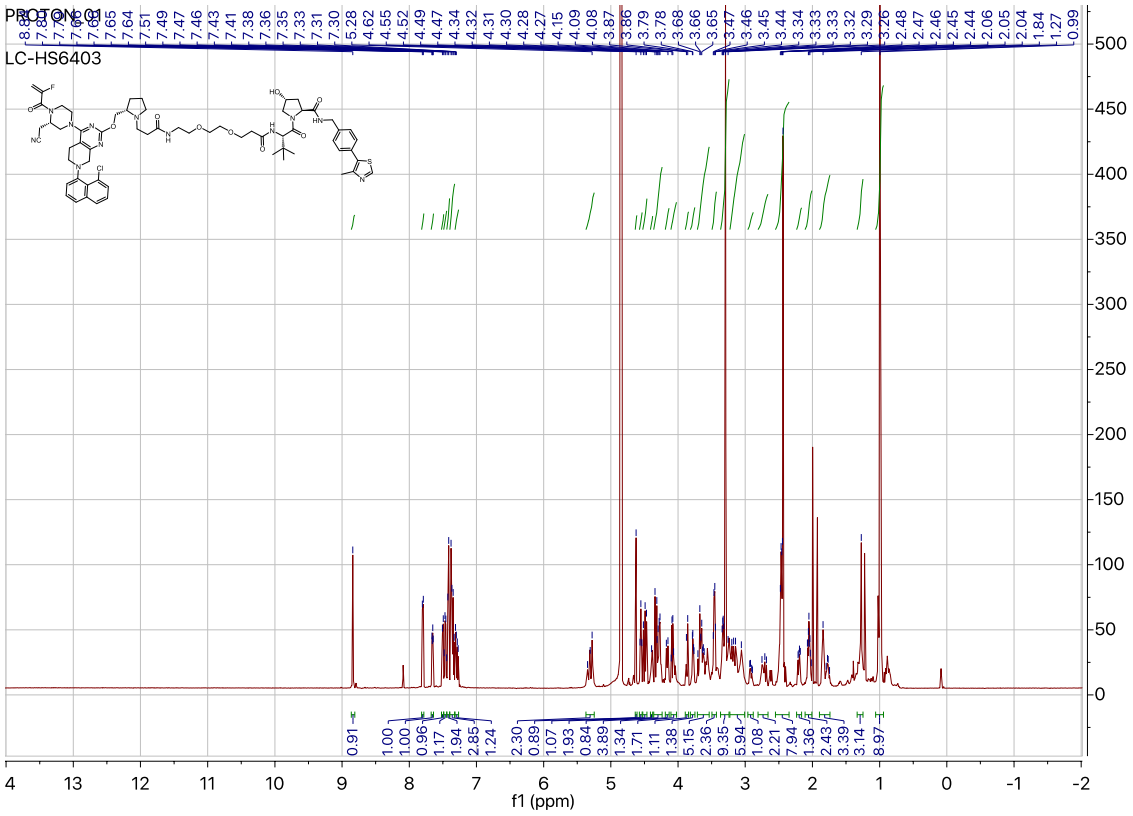
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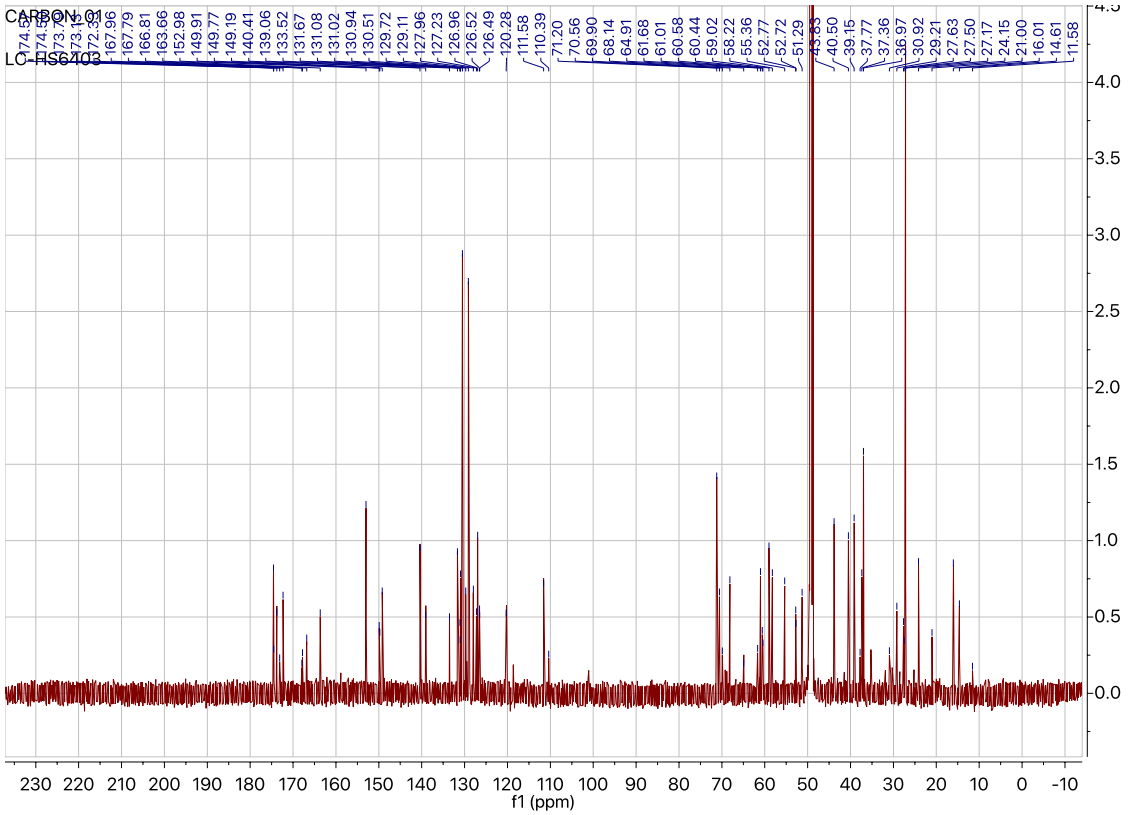
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f1 (ppm)