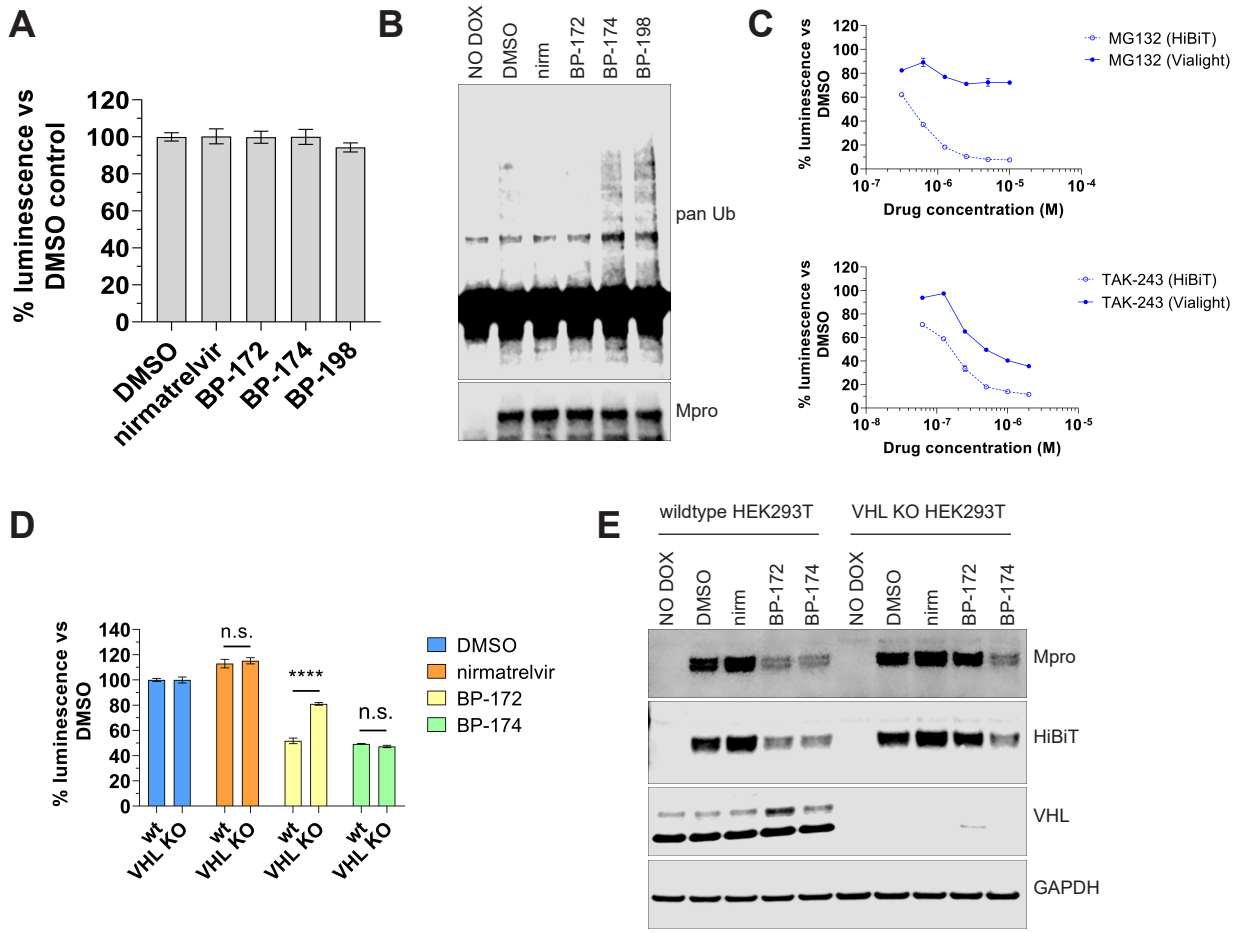
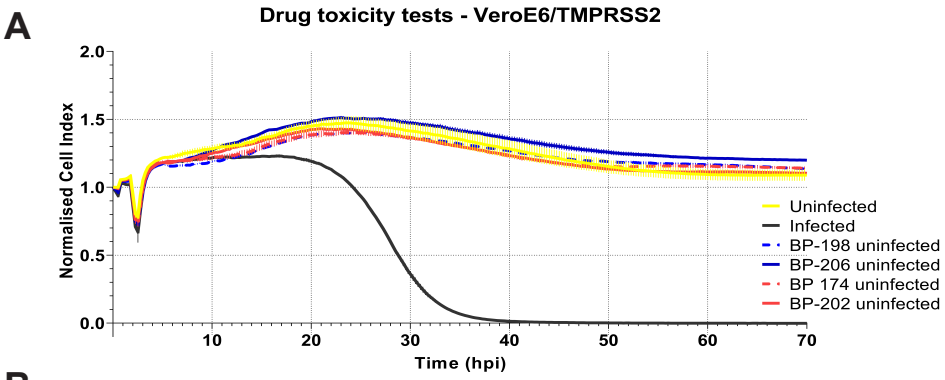
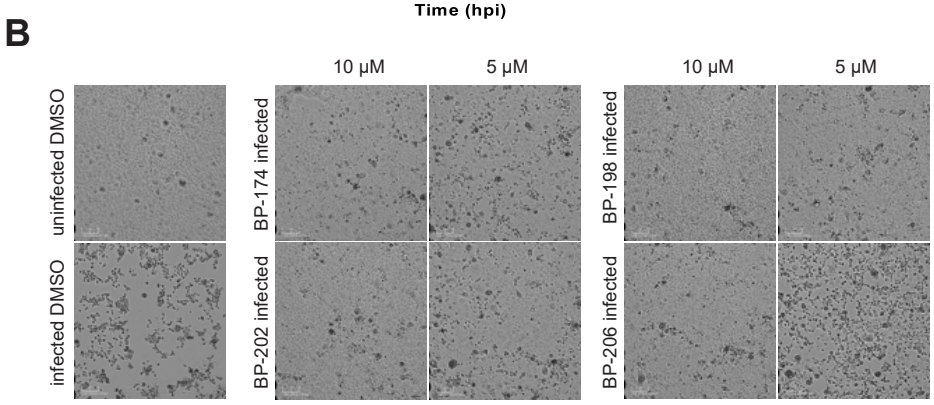
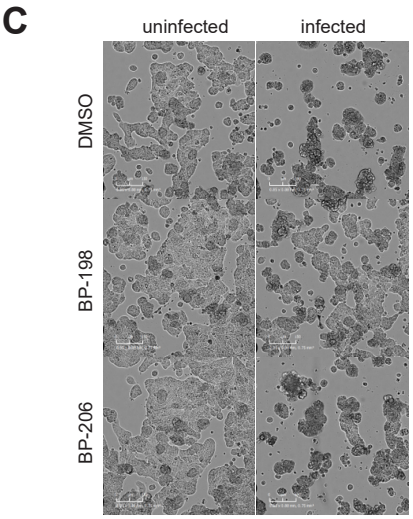


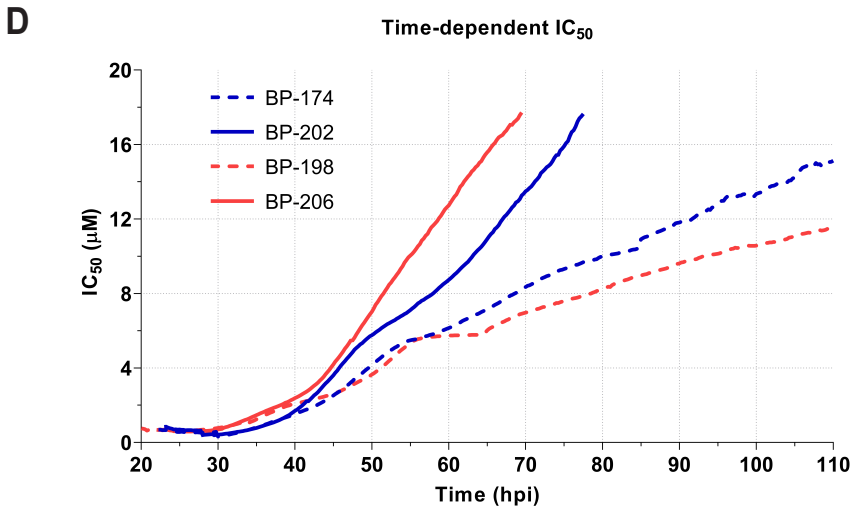
Supplementary Figure 1

(A) Vialight cell viability results demonstrating no toxicity of the TPD candidates at 20 µM. Data is presented as the % luminescence vs DMSO controls ± S.E.M. **(B)** Western blot analysis demonstrating the ubiquitination of Mpro following 4 h of drug treatment in the presence of 5 µM of proteasomal inhibitor MG132 followed by HiBiT immunoprecipitation and blotting for pan Ub and Mpro. **(C)** Combined HiBiT detection and Vialight cell viability analysis of HEK293T LVX Mpro-HiBiT cells treated with titrated MG132 or TAK-243 for 24 h. Data is presented as % luminescence vs DMSO controls ± S.E.M. **(D)** HiBiT detection results and **(E)** orthogonal western blot verification results for singular TPD testing at 20 µM for 24 h in the HEK293T LVX Mpro-HiBiT reporter expressed on a wildtype or VHL KO background. Data for HiBiT detection are presented as % luminescence vs DMSO controls ± S.E.M. **** indicates P<0.0001 significant difference to matched wildtype control by one-way ANOVA. n.s. = not significant.

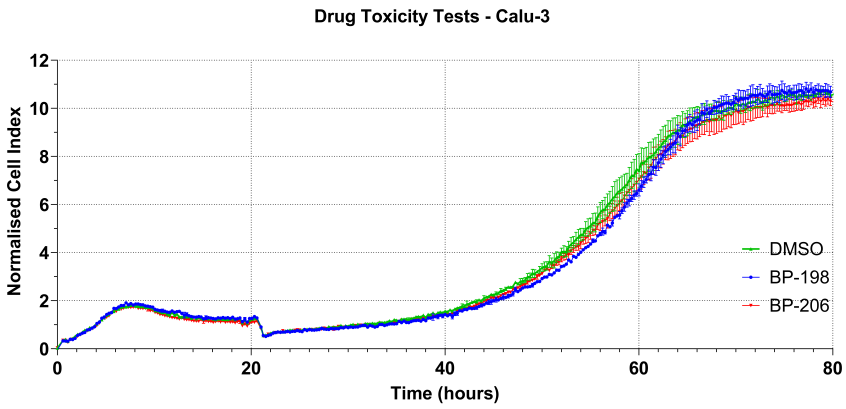

A
Drug toxicity tests - VeroE6/TMPRSS2
Normalised Cell Index
Time (hpi)
Uninfected
Infected
BP-198 uninfected
BP-206 uninfected
BP 174 uninfected
BP-202 uninfected


B
10 µM
5 µM
10 µM
5 µM
uninfected DMSO
infected DMSO
BP-174 infected
BP-202 infected
BP-198 infected
BP-206 infected


C
uninfected
infected
DMSO
BP-198
BP-206


D
Time-dependent IC50
IC50 (µM)
Time (hpi)
BP-174
BP-202
BP-198
BP-206

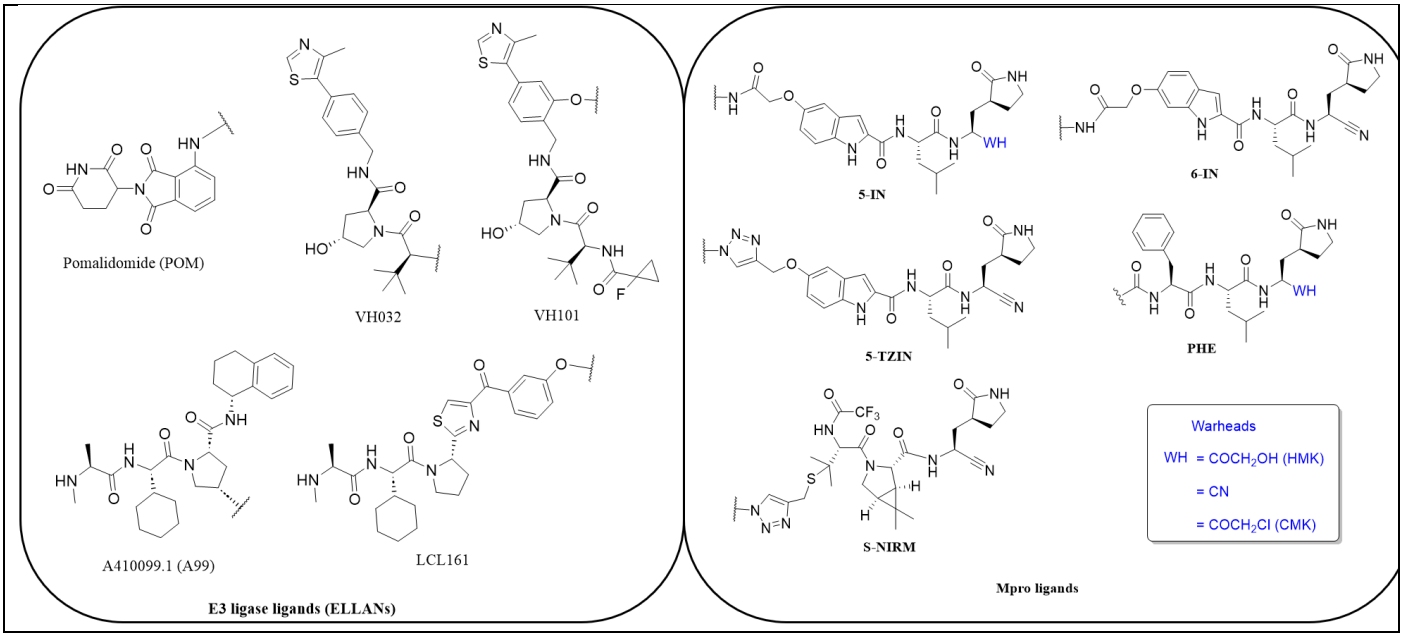
E

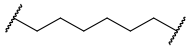
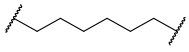
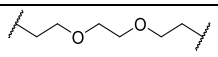
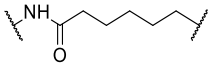
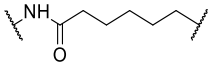
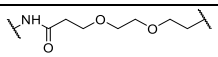
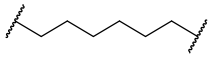
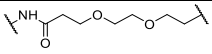
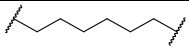
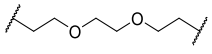
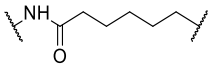
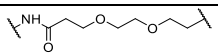
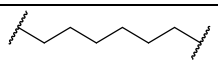
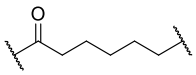
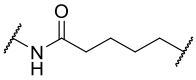
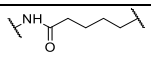


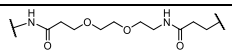
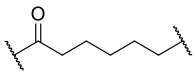
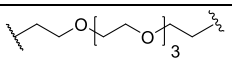
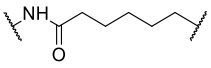
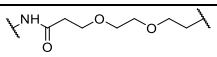
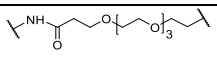
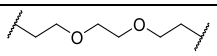
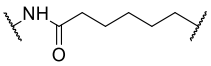
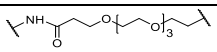
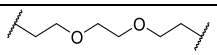
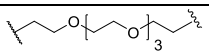
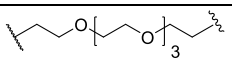
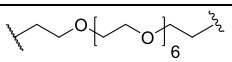
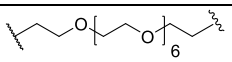
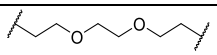
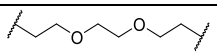
Supplementary Figure 2

(A) xCELLigence real time cellular response profiles of VeroE6/TMPRSS2 cells treated with 10 µM of each drug to confirm a lack of toxicity of the candidates in this cell model. Data are presented as normalised cell index (measuring cellular impedance) over time ± S.E.M. **(B)** Microscopic images of VeroE6/TMPRSS2 cells treated with DMSO, BP-198 or BP-206 control at multiple concentrations at 51 hpi. Cytopathic effect of the virus can be observed in DMSO infected vs non-infected cells. Scale bar = 100 µm. **(C)** Microscopic images of Calu-3 cells treated with DMSO, BP-198 or BP-206 control at 10 µM at 37 hpi. Cytopathic effect of the virus can be observed in DMSO infected vs non-infected cells. Scale bar = 100 µm. **(D)** IC_{50} for each drug calculated at 15 min intervals over the course of infection from experiments in Figure 3A and 3B. Data is presented as IC_{50} of each drug in µM ± S.E.M over time. **(E)** xCELLigence real time cellular response profiles of Calu-3 cells treated with DMSO or 30 µM of each drug in the absence of SARS-CoV-2 infection confirming a lack of toxicity of the candidates in this cell model. Data are presented as normalised cell index (measuring cellular impedance) over time ± S.E.M.

Supplementary Table 1: Summary of biochemical and degrader screening assay data for test compounds.



				Enzyme[a]	% Mpro[b]
BP-#	**ELLAN**	**Linker**	**Mpro ligand**	**IC_{50}/nM**	**% vs DMSO**
121	-	-	Nirmatrelvir	7.3	124
95	-	-	PF-008353231	4.3	89
100	POM		5IN-HMK	165	96.7
82	POM		5IN-CN	3336	98.1
108	POM		5IN-CN	1910	91.1
22	VH032		5IN-CMK	45[aa]	90
96	VH032		5IN-CN	1590	80
110	VH032		5IN-CN	2570	80
138	VH101		5IN-CN	5750	86
114	A99		5IN-CN	1490	89
148	POM		6IN-CN	1890	78.8
157	POM		6IN-CN	2550	87.5
158	VH032	direct	6IN-CN	1770	88.5
147	VH032		6IN-CN	948	57.8
153	VH032		6IN-CN	1760	77.9
151	VH101		6IN-CN	6820	84.7
75	POM		5TZIN-CN	1030	93.3
84	VH032		5TZIN-CN	1080	73.3
28	VH032		Phe-CMK	15	89

122	VH032		Phe-CN	570	90
155	POM		S-NIRM	8.7	98.2
181	POM		S-NIRM	7.1	85.7
142	VH032		S-NIRM	8.6	88.9
177	VH032		S-NIRM	10.1	97.8
178	VH032		S-NIRM	7.4	88.6
172	VH101		S-NIRM	7.6	61.5
164	A99		S-NIRM	7.0	72.9
179	A99		S-NIRM	5.6	78
174	LCL161		S-NIRM	6.7	36.8
196	VH101		S-NIRM	9.9	96[bb]
197	VH101		S-NIRM	7.3	95[bb]
198	LCL161		S-NIRM	7.3	28
199	LCL161		S-NIRM	8.1	80[bb]
203	VH101		S-NIRM	9.3	100
204	LCL161		S-NIRM	8.3	98.2[bb]
200	VH101[cis-Hyp]		S-NIRM	8.0	92.3
202	LCL161[N-Me]		S-NIRM	8.2	67.6
206	LCL161[N-Me]		S-NIRM	8.8	65

[a] Enzyme inhibition assays were performed as described in the Methods section. All results are n ≥ 2, (SEM pIC50 < 0.1); [aa] n=1

[b] Mpro degradation assays were performed as described in the Methods section. %Mpro refers to % HiBiT luminescence readout relative to DMSO control @ 20 µM ([bb] @ 10 µM). Lower percentage indicates greater degradation and target loss.

Supplementary Methods

1 Compound Synthesis and Analysis General Methods

Commercial solvents and reagents were used as received unless otherwise indicated. Anhydrous tetrahydrofuran (THF), dichloromethane (DCM), toluene, and *N*,*N*-Dimethylformamide (DMF) were purchased from Sigma Aldrich. All air- and/or moisture-sensitive liquid reagents were transferred via a syringe. Solvents were concentrated under reduced pressure by rotary evaporation or using a stream of N_2. Thin layer chromatography (TLC) was performed using Aluminium TLC plate, silica gel coated with fluorescent indicator F_{254} (Merck KGA). TLC plates were visualised under ultraviolet light or by potassium permanganate ($KMnO_4$) stain. Flash column chromatography were carried out following the procedure described by Still et al.,[1] using Silica gel 60 Å, 0.04-0.063 mm (400-230 mesh, Carl Roth). Preparative reversed-phase high-performance liquid chromatography (RP-HPLC) was performed on a Phenomenex Luna C18 column (5 µm, 100 Å, 150 x 21.2 mm) using a water 600 semi-prep HPLC incorporating a water 486 UV detector, with 0.1% trifluoroacetic acid (TFA) in H2O (mobile phase A) and 0.1% TFA in acetonitrile (MeCN) (mobile phase B) as eluents, purified fractions were combined and concentrated by lyophilisation.

^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and ^{19}F NMR spectra were recorded using Bruker Avance III Nanobay 400 MHz NMR spectrometer coupled to the BACS 60 automatic sample changer at room temperature. The spectrometer was equipped with a 5 mm PABBO BB-1H/D Z- GRD probe. All chemical shifts were reported in parts per million (ppm, δ scale). According to Fulmer et al.,[2] ^{1}H NMR spectra were referenced relative to residual protium in the deuterated solvent ($CHCl_3$, δ 7.26 ppm; Methanol-d_3, 3.31 ppm; DMSO-d_5, δ 2.50 ppm; Acetone-d_5, δ 2.05 ppm; Acetonitrile-d_2, δ 1.94 ppm). ^{13}C NMR spectra were referenced to carbon resonances of deuterated solvent ($CDCl_3$, δ 77.16 ppm; CD_3OD, δ 49.00 ppm; DMSO-d_6, δ 39.52 ppm; Acetone-d_6, δ 29.84 ppm; CD_3CN, δ 118.26 ppm). ^{1}H NMR spectra were reported in the following order: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dddd = doublet of doublet of doublet of doublets, ddt = doublet of doublet of triplets, dt = doublet of triplets, dq = doublet of quartets, td = triplet of doublets, tdd = triplet of doublet of doublets, tt = triplet of triplets, app = apparent, m = multiplet), coupling constant in Hz (if available), and integration. ^{13}C NMR spectra were reported in the following order: chemical shift, distortionless enhancement by polarization transfer quaternary (DEPTQ, if available), multiplicity (if available), and coupling constant in Hz (if available). ^{19}F NMR spectra were reported in the following order: chemical shift, multiplicity (if available), and coupling constant in Hz (if available). All literature compounds have references after the chemical names, the NMR spectra are in agreement with the reported data unless otherwise stated.

Liquid chromatography-mass spectrometry (LCMS) were performed with an Agilent 1260 Infinity II LCMS SQ. Pump: 1260 Infinity II G7111B Quat pump. Autosampler: 1260 Infinity II G7129A 1260 Vialsampler. Detector: 1260 Infinity II G7117C DAD HS. Column: Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm. Column temperature: 35°C. Solvent A: 0.1% formic acid in H_2O; solvent B: 0.1% formic acid in MeCN. Gradient: 5-100% B over 5 mins. Ion source: Quadrupole. Ion mode: MM-ES-APCI. Drying gas temperature: 350°C. Capillary voltage (V): 4000 (positive and negative).

High resolution mass spectrometry (HRMS) were performed with an Agilent 6224 TOF LC/MS Mass Spectrometer coupled to an Agilent 1290 Infinity (Agilent, Palo Alto, CA). All data were acquired, and reference mass was corrected via a dual-spray electrospray ionisation (ESI) source.

Purities were determined by Method A (Agilent 1260 Infinity Analytical HPLC coupled with a Zorbax Eclipse Plus C18 column (95 Å, 4.6 x 100 mm, 3.5 µm)) or Method B (Shimadzu LCMS-2020 system coupled with a

Phenomenex Luna C8 column (100 Å, 2.0 x 100 mm, 3 µm)). The purities of all final compounds are >95% unless otherwise indicated.

Reaction general procedures

General procedure A: *O*-alkylation

A reaction vial was charged with phenol, cesium carbonate (Cs_2CO_3), and anhydrous DMF. The mixture was stirred under an N_2 atmosphere at room temperature for 10 mins before the alkyl halide/pseudohalide was added. The reaction mixture was stirred at room temperature for the indicated period. The resulting mixture was quenched with H_2O and concentrated. The mixture was partitioned between ethyl acetate (EtOAc) and H_2O, the separate aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over magnesium sulfate ($MgSO_4$), filtered, and concentrated under reduced pressure to give a crude residue, which was purified by flash column chromatography.

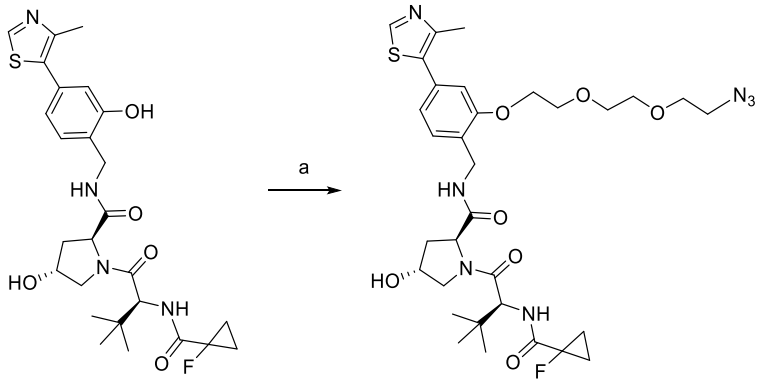
General procedure B: *tert*-butyloxycarbonyl (Boc) deprotection

A reaction vial was charged with Boc-amine, 4 M hydrochloride (HCl) in 1,4-dioxane, and DCM (if indicated). The reaction mixture was stirred at room temperature for 2 h. The resulting mixture was concentrated and dried under reduced pressure to provide the amine hydrochloride salt product, which was used for next step without further purification.

General procedure C: copper-catalysed azide–alkyne cycloaddition (CuAAC) reaction

A reaction vial was charged with alkyne, azide, sodium ascorbate, copper sulfate pentahydrate ($CuSO_4.5H_2O$), and a 2:1 mixture of *tert*-butanol (t-BuOH) and H_2O. The reaction vial was capped and sealed. The reaction mixture was stirred at 40-45°C for indicated period. The resulting mixture was cooled to room temperature and concentrated. The residue was partitioned between EtOAc and H_2O, the separate aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a crude residue, which was purified by flash column chromatography (or preparative TLC and preparative RP-HPLC if indicated).

1.1 Synthesis of Azido-Linked VH101 Derivative



S1 **S2**

Scheme S1 *Reagents and conditions*: (a) Cs_2CO_3, alkyl bromide, DMF, rt, 95%.

(2*S*,4*R*)-*N*-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (**S2**)

Following general procedure A, (2*S*,4*R*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, **S1**[3] (50.0 mg, 0.94 mmol), Cs_2CO_3 (55.1 mg, 0.17 mmol), 1-azido-2-(2-(2-bromoethoxy)ethoxy)ethane (42.5 mg, 0.18 mmol), and DMF (0.3 mL) gave a light-yellow clear mixture. After 48 h, workup and flash column chromatography (EtOAc/petroleum ether/MeOH (methanol), gradient, 80:20:0 to 90:0:10) provided **S2** as a colourless clear solid (61.5 mg, 95%).

TLC: R_f = 0.26 (EtOAc/MeOH, 90:10)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.33 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 6.1 Hz, 1H), 7.02 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.97 (dd, *J* = 7.7, 1.6 Hz, 1H), 6.91 (d, *J* = 1.6 Hz, 1H), 4.68 (t, *J* = 7.9 Hz, 1H), 4.52 (app br d, *J* = 8.0 Hz, 2H), 4.48 (dd, *J* = 6.0, 2.7 Hz, 2H), 4.27 – 4.15 (m, 2H), 4.02 – 3.97 (m, 1H), 3.96 – 3.88 (m, 2H), 3.80 – 3.75 (m, 2H), 3.72 – 3.62 (m, 5H), 3.37 (t, *J* = 5.0 Hz, 2H), 2.53 (s, 3H), 2.44 (ddd, *J* = 12.8, 7.7, 4.7 Hz, 1H), 2.14 – 2.06 (m, 1H), 1.36 – 1.25 (m, 4H, assumed; partially obscured by solvent peak), 0.96 (s, 9H).

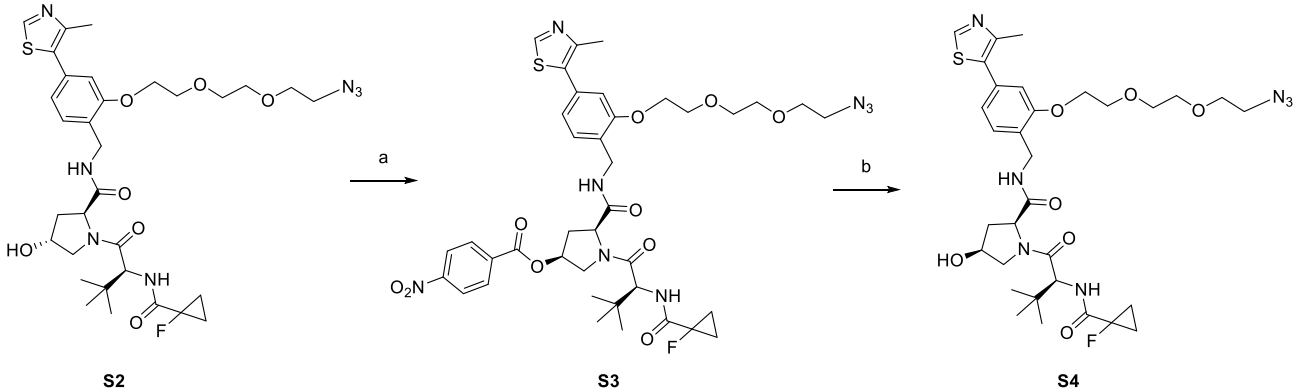
^{19}F NMR (377 MHz, $CDCl_3$) δ -197.51.

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.6, 170.3, 157.0, 150.5, 132.2, 130.0, 122.2, 113.0, 71.0, 70.9, 70.5, 70.2, 69.9, 68.2, 58.6, 57.7, 56.7, 50.9, 39.2, 36.4, 35.4, 26.5, 16.1, 13.9, 13.8. Four carbons not observed.

HPLC t_R (Method A): 8.8 min.

HRMS: Calc *m/z* for $([C_{32}H_{44}FN_7O_7S] + H)^+$ 690.3080, found 690.3086.

1.2 Synthesis of Azido-Linked cis-Hyp VH101 Derivative by Mitsunobu Inversion



Scheme S2 *Reagents and conditions*: (a) Di-*tert*-butyl azodicarboxylate (DTBAD), diphenyl-2-pyridylphosphine (Ph_2PyP), 4-nitrobenzoic acid, THF, 0°C to rt. (b) Lithium hydroxide monohydrate ($LiOH.H_2O$), THF, MeOH, H_2O, rt, 60% over two steps.

(2*S*,4*S*)-*N*-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (cis-VH101 **S4**)

A reaction vial was charged with DTBAD (58.4 mg, 0.25 mmol), Ph_2PyP (66.8 mg, 0.25 mmol), and anhydrous THF (1.0 mL). The yellow clear mixture was stirred under an N_2 atmosphere at 0°C for 5 mins to form the light-yellow cloudy betaine, which was transferred to a separated reaction vial containing **S2** (50 mg, 0.072 mmol) and 4-nitrobenzoic acid (20.6 mg, 0.12 mmol). The yellow clear mixture was stirred at 0℃ to room temperature for 18 h. The resulting light-yellow clear mixture was concentrated using a stream of N_2, diluted in DCM, and purified by flash column chromatography (EtOAc/petroleum ether/MeOH, gradient, 80:20:0 to 95:0:5). **S3** was obtained as a colourless clear gum and used for the next step without further purification.

A reaction vial was charged with **S3**, $LiOH.H_2O$ (5.5 mg, 0.13 mmol), and MeOH/THF/H_2O (0.3 mL, 1:1:1). The slightly yellow clear mixture was stirred at 0℃ to room temperature for 18 h. The resulting light yellow clear mixture was placed at 0℃ and acidified by HCl (0.27 mL, 0.27 mmol, 1 M in H_2O). The mixture was concentrated using a stream of N_2, partitioned between EtOAc (20 mL) and H_2O (10 mL). The separate aqueous layer was extracted with EtOAc (15 mL x 3). The combined organic layer was washed with brine (5 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a slightly yellow clear residue, which was purified by flash column chromatography (EtOAc/petroleum ether/MeOH, gradient, 80:20:0 to 90:0:10 followed by DCM/MeOH, gradient, 100:0 to 95:0). **S4** was obtained as a colourless clear solid (29.9 mg, 60% over two steps).

TLC: R_f = 0.17 (DCM/MeOH, 95:5)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.58 (t, *J* = 6.2 Hz, 1H), 7.33 (d, *J* = 7.7 Hz, 1H), 6.97 (dd, *J* = 7.7, 1.6 Hz, 1H), 6.95 – 6.90 (m, 2H), 5.81 (d, *J* = 10.3 Hz, 1H), 4.68 (d, *J* = 8.6 Hz, 1H), 4.60 – 4.51 (m, 2H), 4.47 – 4.38 (m, 2H), 4.29 – 4.17 (m, 2H), 3.99 – 3.89 (m, 2H), 3.88 – 3.80 (m, 2H), 3.80 – 3.75 (m, 2H), 3.72 – 3.64 (m, 4H), 3.37 (t, *J* = 5.0 Hz, 2H), 2.52 (s, 3H), 2.26 (d, *J* = 14.1 Hz, 1H), 2.17 (ddd, *J* = 14.0, 9.0, 4.8 Hz, 1H), 1.40 – 1.23 (m, 4H, assumed; partially obscured by solvent peak), 0.91 (s, 9H).

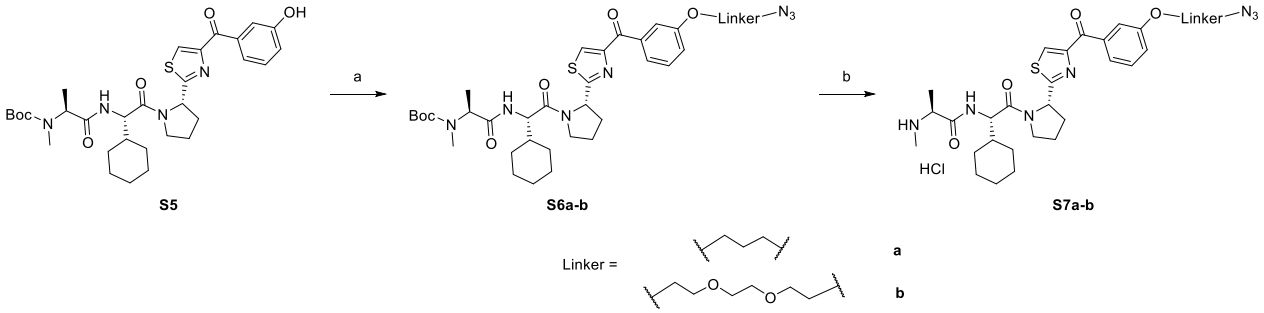
^{19}F NMR (377 MHz, $CDCl_3$) δ -197.56.

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 171.3, 169.9, 169.7, 156.9, 150.5, 148.6, 132.7, 131.8, 130.0, 126.2, 122.2, 113.0, 82.9, 81.7, 79.6, 78.8, 71.2, 71.0, 70.9, 70.2, 69.9, 68.1, 60.0, 58.6, 57.0, 50.8, 42.4, 41.0, 39.4, 35.5, 26.3, 25.6, 24.0, 21.0, 20.3, 17.6, 17.4, 16.2, 14.8, 13.8, 13.7, 13.6.

HPLC t_R (Method A): 9.2 min.

HRMS: Calc *m/z* for $([C_{32}H_{44}FN_7O_7S] + H)^+$ 690.3080, found 690.3089.

1.3 Synthesis of Azido-Linked LCL-161 Derivatives



Scheme S3 *Reagents and conditions*: (a) Cs_2CO_3, alkyl tosylate/bromide, DMF, rt, 59-77%. (b) HCl, 1,4-dioxane, DCM, rt, quant.

tert-Butyl ((*S*)-1-(((*S*)-2-((*S*)-2-(4-(3-(3-azidopropoxy)benzoyl)thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**S6a**)

Following general procedure A, *tert*-butyl ((*S*)-1-(((*S*)-1-cyclohexyl-2-((*S*)-2-(4-(3-hydroxybenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate, **S5**[4] (50.0 mg, 0.084 mmol), Cs_2CO_3 (54.4 mg, 0.17 mmol), 3-azidopropyl 4-methylbenzenesulfonate (46.9 mg, 0.18 mmol), and DMF (0.4 mL) gave a brown-orange opaque mixture. After 18 h, workup and flash column chromatography (EtOAc/petroleum ether, 50:50 to 100:0) provided **S6a** as a light yellow clear solid (43.9 mg, 77%).

TLC: R_f = 0.28 (EtOAc/petroleum ether, 70:30).

^{1}H NMR (400 MHz, MeOD) reporting the major isomer δ 8.33 (s, 1H), 7.78 – 7.63 (m, 2H), 7.44 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.23 (ddd, *J* = 8.3, 2.6, 1.0 Hz, 1H), 5.49 (dd, *J* = 7.8, 3.1 Hz, 1H), 4.63 – 4.45 (m, 2H), 4.15 (t, *J* = 6.0 Hz, 2H), 4.03 – 3.92 (m, 1H), 3.95 – 3.85 (m, 1H), 3.54 (t, *J* = 6.6 Hz, 2H), 2.86 (s, 3H), 2.44 – 2.11 (m, 4H), 2.08 (p, *J* = 6.3 Hz, 2H), 1.76 – 1.54 (m, 6H), 1.46 (s, 9H), 1.36 – 1.32 (m, 3H), 1.17 – 0.99 (m, 5H).

LCMS (APCI): Calc *m/z* for $([C_{34}H_{47}N_7O_6S] + H)^+$ 682.3, found 682.3.

HRMS: Calc *m/z* for $([C_{34}H_{47}N_7O_6S] + Na)^+$ 704.3201, found 704.3214.

(*S*)-*N*-((*S*)-2-((*S*)-2-(4-(3-(3-Azidopropoxy)benzoyl)thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-2-(methylamino)propanamide hydrochloride (**S7a**)

Following general procedure B, **S6a** (17.0 mg, 0.025 mmol), HCl (0.1 mL, 0.40 mmol, 4 M in 1,4-dioxane), and DCM (0.1 mL) gave a slightly yellow clear turned opaque mixture. Concentration and drying under reduced pressure provided **S7a** as an off-white foamy solid, which was used for the next step without further purification.

tert-Butyl ((*S*)-1-(((*S*)-2-((*S*)-2-(4-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)benzoyl)thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**S6b**)

Following general procedure A, **S5** (52.3 mg, 0.087 mmol), Cs_2CO_3 (68.3 mg, 0.21 mmol), 1-azido-2-(2-(2-bromoethoxy)ethoxy)ethane (54.0 mg, 0.23 mmol), and DMF (0.4 mL) gave a brown-orange opaque mixture. After 18 h, workup and flash column chromatography (EtOAc/petroleum ether, 50:50 to 100:0) provided **S6b** as a pale yellow clear residue (39.1 mg, 59%).

TLC: R_f = 0.30 (EtOAc, 100).

^{1}H NMR (400 MHz, MeOD) reporting the major isomer δ 8.32 (s, 1H), 7.78 – 7.68 (m, 2H), 7.44 (t, *J* = 7.9 Hz, 1H), 7.24 (ddd, *J* = 8.3, 2.7, 1.0 Hz, 1H), 5.49 (dd, *J* = 7.8, 3.2 Hz, 1H), 4.64 – 4.46 (m, 2H), 4.25 – 4.18 (m, 2H), 4.03 – 3.86 (m, 4H), 3.77 – 3.70 (m, 2H), 3.71 – 3.64 (m, 4H), 3.35 (t, *J* = 4.9 Hz, 2H), 2.86 (s, 3H), 2.43 – 2.09 (m, 4H), 1.78 – 1.55 (m, 6H), 1.47 (s, 9H), 1.34 (d, *J* = 7.2 Hz, 3H), 1.21 – 1.00 (m, 5H).

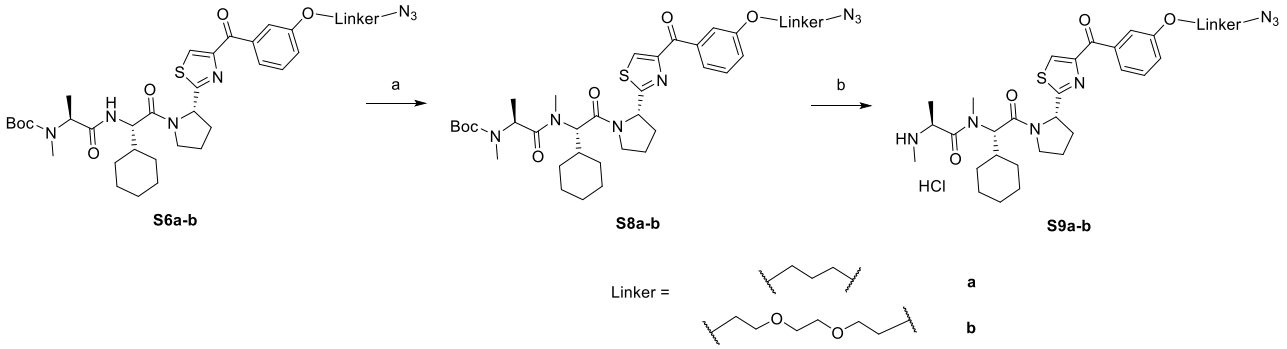
LCMS (APCI): Calc *m/z* for ($[C_{37}H_{53}N_7O_8S]$ + H)$^+$ 756.4, found 756.3.

HRMS: Calc *m/z* for ($[C_{37}H_{53}N_7O_8S]$ + Na)$^+$ 778.3569, found 778.3576.

(*S*)-*N*-((*S*)-2-((*S*)-2-(4-(3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)benzoyl)thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-2-(methylamino)propanamide hydrochloride (**S7b**)

Following general procedure B, **S6** (17.7 mg, 0.023 mmol), HCl (0.1 mL, 0.40 mmol, 4 M in 1,4-dioxane), and DCM (0.1 mL) gave a colourless clear turned opaque mixture. Concentration and drying under reduced pressure provided **S7b** as a pale yellow solid, which was used for the next step without further purification.

1.4 **Synthesis of Azido-Linked *N*-Methyl LCL-161 Derivatives**



Scheme S4 *Reagents and conditions*: (a) Sodium hydride (NaH), iodomethane (MeI), DMF, rt, 72-79%. (b) HCl, 1,4-dioxane, DCM, rt, quant.

tert-Butyl ((*S*)-1-(((*S*)-2-((*S*)-2-(4-(3-(3-azidopropoxy)benzoyl)thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)(methyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**S8a**)

A reaction vial was charged with **S6a** (31.1mg, 0.046 mmol), NaH (4.6 mg, 0.11 mmol, 60 % dispersion in mineral oil), and anhydrous DMF (0.15 mL). The yellow turned brown-red clear mixture was stirred under an N_2 atmosphere at 0°C for 5 mins before MeI (19.4 mg, 0.14 mmol) was added. The red-brown clear reaction mixture was stirred at room temperature for 1 h. Another NaH (3.1 mg, 0.078 mmol, 60 % dispersion in mineral oil) was again added, stirring was continued at room temperature for another 3 h. The resulting yellow cloudy mixture was placed at ice bath, quenched with saturated NH_4Cl solution (0.5 mL), and concentrated using a stream of N_2. The mixture was partitioned between EtOAc (20 mL) and H_2O (10 mL), the separate aqueous layer was extracted with EtOAc (15 mL x 3). The combined organic layer was washed with brine (5 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a light-brown crude residue, which was purified by flash column chromatography (EtOAc/petroleum ether, gradient, 20:80 to 80:20). **S8a** was obtained as a light-yellow clear gum (22.7 mg, 72%). The product was co-eluted with impurities and used for the next step without further purification.

TLC: R_f = 0.43 (EtOAc/petroleum ether, 70:30).

^{1}H NMR (400 MHz, MeOD) reporting characteristic signals δ 8.32 (s, 1H), 7.77 – 7.61 (m, 2H), 7.44 (t, *J* = 7.9 Hz, 1H), 7.25 – 7.21 (m, 1H), 5.47 – 5.41 (m, 1H, assumed; partially obscured by impurities), 5.17 – 5.07 (m, 2H, assumed; partially obscured by impurities), 4.15 (t, *J* = 6.0 Hz, 2H), 4.01 – 3.75 (m, 2H), 3.58 – 3.52 (m, 2H, assumed; partially obscured by impurities), 3.05 (s, 3H), 2.82 (s, 3H), 2.11 – 1.92 (m, 6H, assumed; partially obscured by impurities), 1.67 – 1.50 (m, 6H), 1.48 – 1.44 (m, 9H), 1.30 – 1.28 (m, 3H, assumed; partially obscured by impurities), 1.20 – 1.12 (m, 5H, assumed; partially obscured by impurities).

LCMS (APCI): Calc *m/z* for $([C_{35}H_{49}N_7O_6S] + H)^+$ 696.4, found 696.3.

HRMS: Calc *m/z* for $([C_{35}H_{49}N_7O_6S] + Na)^+$ 718.3357, found 718.3357.

(*S*)-*N*-((*S*)-2-((*S*)-2-(4-(3-(3-Azidopropoxy)benzoyl)thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-N-methyl-2-(methylamino)propenamide hydrochloride (*N*-Me-LCL161 **S9a**)

Following general procedure B, **S8a** (16.0 mg, 0.023 mmol), HCl (0.1 mL, 0.40 mmol, 4 M in 1,4-dioxane), and DCM (0.1 mL) gave a light-yellow clear turned opaque mixture. Concentration and drying under reduced pressure provided **S9a** as a light yellow clear solid, which was used for the next step without further purification.

tert-Butyl ((*S*)-1-(((*S*)-2-((*S*)-2-(4-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)benzoyl)thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)(methyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**S8b**)

A reaction vial was charged with **S6b** (35.4mg, 0.047 mmol), NaH (4.7 mg, 0.12 mmol, 60 % dispersion in mineral oil), and anhydrous DMF (0.16 mL). The yellow turned brown clear mixture was stirred under an N_2 atmosphere at room temperature for 5 mins before the MeI (19.9 mg, 0.14 mmol) was added. The brown reaction mixture was stirred at room temperature for 1 h. The resulting yellow cloudy mixture was placed at ice bath, quenched with saturated NH_4Cl solution (0.5 mL), and concentrated using a stream of N_2. The mixture was partitioned between EtOAc (20 mL) and H_2O (10 mL), the separate aqueous layer was extracted with EtOAc (15 mL x 3). The combined organic layer was washed with brine (5 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a dark-brown crude residue, which was purified by flash column chromatography (EtOAc/petroleum ether, gradient, 1:2 to 100:0). **S8b** was obtained as a yellow clear gum (28.5 mg, 79%). The product was co-eluted with impurities and used for the next step without further purification.

TLC: R_f = 0.32 (EtOAc/petroleum ether, 80:20).

^{1}H NMR (400 MHz, MeOD) reporting the major isomer δ 8.31 (s, 1H), 7.79 – 7.63 (m, 2H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.24 (ddd, *J* = 8.3, 2.7, 1.0 Hz, 1H), 5.44 (dd, *J* = 8.1, 3.1 Hz, 1H), 5.15 (d, *J* = 11.0 Hz, 1H), 5.10 – 5.01 (m, 1H), 4.24 – 4.19 (m, 2H), 3.93 – 3.80 (m, 4H), 3.75 – 3.71 (m, 2H), 3.69 – 3.65 (m, 4H), 3.35 (t, *J* = 4.9 Hz, 2H), 3.05 (s, 3H), 2.82 (s, 2H), 2.44 – 2.34 (m, 1H), 2.33 – 2.24 (m, 1H), 2.23 – 2.14 (m, 1H), 2.13 – 2.04 (m, 1H, assumed; partially obscured by impurities), 1.78 – 1.53 (m, 6H, assumed; partially obscured by impurities), 1.47 (s, 6H), 1.45 (s, 3H), 1.30 – 1.28 (m, 3H), 1.22 – 0.93 (m, 5H, assumed; partially obscured by impurities).

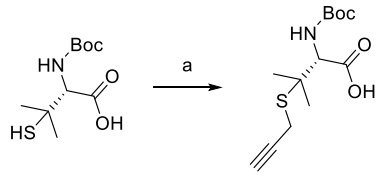
LCMS (APCI): Calc *m/z* for $([C_{38}H_{55}N_7O_8S] + H)^+$ 770.4, found 770.3.

HRMS: Calc *m/z* for $([C_{38}H_{55}N_7O_8S] + 5Na)^{5+}$ 176.8659, found 176.8657.

(*S*)-*N*-((*S*)-2-((*S*)-2-(4-(3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)benzoyl)thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-*N*-methyl-2-(methylamino)propenamide hydrochloride (*N*-Me-LCL161 **S9b**)

Following general procedure B, **S8b** (19.4 mg, 0.025 mmol), HCl (0.1 mL, 0.40 mmol, 4 M in 1,4-dioxane), and DCM (0.1 mL) gave a light-yellow clear turned opaque mixture. Concentration and drying under reduced pressure provided **S9b** as a light yellow clear solid, which was used for the next step without further purification.

1.5 Synthesis of Nirmatrelvir Derivative (S15)



S10 **S11**

Scheme S5 *Reagents and conditions*: (a) Propargyl bromide, sodium hydroxide (NaOH), H_2O, ethanol (EtOH), rt, 96%.

(*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-methyl-3-(prop-2-yn-1-ylthio)butanoic acid (**S11**)

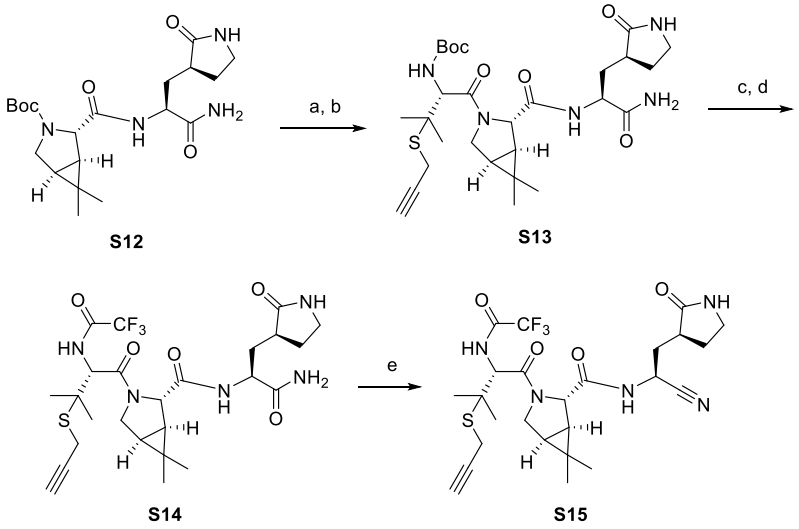
A reaction vial was charged with (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-mercapto-3-methylbutanoic acid, **S10** (176 mg, 0.71 mmol), NaOH (0.51 mL, 1.41 mmol, 10% aqueous solution), and EtOH (2.5 mL). The mixture was stirred at room temperature for 5 mins before propargyl bromide (0.080 mL, 0.71 mmol, 80% in toluene) was added. The light-yellow clear mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated using a stream of N_2. The resulting mixture was partitioned between EtOAc (40 mL) and 0.1 M HCl (7.0 mL), aqueous layer was extracted with EtOAc (30 mL x 3). The combined organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give **S11** as a slightly yellow clear gum (195 mg, 96%).

TLC: R_f = 0.39 (EtOAc/petroleum ether/AcOH, 69:30:1)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (d, *J* = 7.6 Hz, 1H), 4.39 (d, *J* = 8.6 Hz, 1H), 3.34 (d, *J* = 2.7 Hz, 2H), 2.24 (t, *J* = 2.7 Hz, 1H), 1.47 (s, 6H), 1.45 (s, 9H).

LCMS (APCI): Calc *m/z* for $([C_{13}H_{21}NO_4S] - H)^-$ 286.1, found 286.0.

HRMS: Calc *m/z* for $(2[C_{13}H_{21}NO_4S] + K)^+$ 613.2014, found 613.2023.



S12 **S13** **S14** **S15**

Scheme S6 *Reagents and conditions*: (a) HCl, 1,4-dioxane, DCM, rt. (b) **S11**, hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU), *N*-methylmorpholine (NMM), DMF, 0°C to rt, 76% over two steps. (c) TFA, DCM, rt. (d) (Trifluoroacetyl)benzotriazole (TFABt), triethylamine (Et_3N), THF, rt, 99% over two steps. (e) Burgess reagent, DCM, rt, 74%.

tert-Butyl ((*R*)-1-((1*R*,2*S*,5*S*)-2-(((*S*)-1-amino-1-oxo-3-((*S*)-2-oxopyrrolidin-3-yl)propan-2-yl)carbamoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-3-yl)-3-methyl-1-oxo-3-(prop-2-yn-1-ylthio)butan-2-yl)carbamate (**S13**)

Following general procedure B, *tert*-butyl (1*R*,2*S*,5*S*)-2-(((*S*)-1-amino-1-oxo-3-((*S*)-2-oxopyrrolidin-3-yl)propan-2-yl)carbamoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-3-carboxylate, **S12**[5] (260 mg, 0.64 mmol) in DCM (0.5 mL), and HCl (2.39 mL, 0.96 mmol, 4 M in 1,4-dioxane) gave a light-yellow clear turned white cloudy mixture. After concentration and drying under reduced pressure provided (1*R*,2*S*,5*S*)-*N*-((*S*)-1-amino-1-oxo-3-((*S*)-2-oxopyrrolidin-3-yl)propan-2-yl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide hydrochloride as an off-white solid and used in the next step without further purification.

A reaction vial was charged with **S11** (192 mg, 0.67 mmol), HATU (266 mg, 0.70 mmol), NMM (193 mg, 0.21 mmol), and anhydrous DMF (1 mL) and was stirred under an N_2 atmosphere at room temperature for 5 mins. The preactivated carboxylic acid cocktail was then transferred to (1*R*,2*S*,5*S*)-*N*-((*S*)-1-amino-1-oxo-3-((*S*)-2-oxopyrrolidin-3-yl)propan-2-yl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide hydrochloride and rinsed with anhydrous DMF (1 mL x 2). The resulting yellow-opaque mixture was stirred at room temperature for 18 h. The reaction mixture was poured into pre-cooled saturated $NaHCO_3$ solution (3 mL) and concentrated using a stream of N_2. The resulting mixture was partitioned between EtOAc (60 mL) and H_2O (10 mL), aqueous layer was extracted with EtOAc (30 mL x 3). The combined organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a crude residue, which was purified by flash column chromatography (EtOAc/MeOH/H_2O, gradient, 100:0:0 to 85:10:5). **S13** was obtained as an off-white solid (279 mg, 76% over two steps).

TLC: R_f = 0.30 (EtOAc/MeOH/H_2O, 85:10:5)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 7.2 Hz, 1H), 7.09 (s, 1H), 5.71 (s, 1H), 5.40 (s, 1H), 5.27 (d, *J* = 10.4 Hz, 1H), 4.62 (d, *J* = 10.2 Hz, 1H), 4.46 (ddd, *J* = 10.4, 7.1, 5.4 Hz, 1H), 4.29 (s, 1H), 4.18 (dd, *J* = 10.4, 5.5 Hz, 1H), 4.01 (d, *J* = 10.4 Hz, 1H), 3.44 (t, *J* = 2.4 Hz, 2H), 3.40 – 3.37 (m, 1H), 3.37 – 3.35 (m, 1H), 2.45 – 2.41 (m, 1H), 2.41 – 2.37 (m, 1H), 2.30 (t, *J* = 2.6 Hz, 1H), 2.12 – 2.07 (m, 2H), 1.96 – 1.87 (m, 1H, assumed; partially obscured by solvent peak), 1.49 – 1.44 (m, 8H), 1.41 (s, 9H), 1.04 (s, 3H), 0.88 (s, 3H).

HRMS: Calc *m/z* for $([C_{28}H_{43}N_5O_6S] + H)^+$ 578. 3007, found 578.3011.

(1*R*,2*S*,5*S*)-*N*-((*S*)-1-Amino-1-oxo-3-((*S*)-2-oxopyrrolidin-3-yl)propan-2-yl)-6,6-dimethyl-3-((*R*)-3-methyl-3-(prop-2-yn-1-ylthio)-2-(2,2,2-trifluoroacetamido)butanoyl)-3-azabicyclo[3.1.0]hexane-2-carboxamide (**S14**)

A reaction vial was charged with **S13** (147 mg, 0.25 mmol), DCM (0.6 mL), and TFA (768 mg, 7.61 mmol). The resulting light-yellow clear mixture was stirred at room temperature for 2.5 h. After concentration and drying under reduced pressure provided the residue was treated with TFABt (246 mg, 1.14 mmol, prepared from 1*H*-benzo[*d*][1,2,3]triazole and trifluoroacetic anhydride (TFAA)[6]), Et_3N (77.0 mg, 0.76 mmol), and THF (1.5 mL). The resulting yellow clear mixture was stirred under an N_2 atmosphere at room temperature for 18 h. The reaction mixture was concentrated using a stream of N_2 and then purified by flash column chromatography (EtOAc/MeOH, gradient, 100:0 to 90:10). **S14** was obtained as an off-white solid (144 mg, 99% over two steps).

TLC: R_f = 0.27 (EtOAc/MeOH/H_2O, 85:10:5)

^{1}H NMR (400 MHz, DMSO) δ 9.58 (d, *J* = 8.3 Hz, 1H), 8.29 (d, *J* = 8.8 Hz, 1H), 7.55 (s, 1H), 7.32 (s, 1H), 7.03 (s, 1H), 4.78 (d, *J* = 8.2 Hz, 1H), 4.34 – 4.27 (m, 1H), 4.26 (s, 1H), 3.96 (dd, *J* = 10.3, 5.4 Hz, 1H), 3.69 (d, *J* = 10.4

Hz, 1H), 3.40 (d, *J* = 2.6 Hz, 2H), 3.18 – 3.07 (m, 2H), 3.04 (q, *J* = 8.6 Hz, 1H), 2.44 – 2.31 (m, 1H), 2.19 – 2.09 (m, 1H), 1.97 – 1.89 (m, 1H), 1.71 – 1.61 (m, 1H), 1.55 – 1.45 (m, 5H), 1.40 (d, *J* = 7.7 Hz, 1H), 1.36 (s, 3H), 1.02 (s, 3H), 0.84 (s, 3H).

^{19}F NMR (377 MHz, DMSO) δ -73.01.

HRMS: Calc *m/z* for ($[C_{25}H_{34}F_3N_5O_5S]$ + H)$^+$ 574.2306, found 574.2308.

(1*R*,2*S*,5*S*)-*N*-((*S*)-1-Cyano-2-((*S*)-2-oxopyrrolidin-3-yl)ethyl)-6,6-dimethyl-3-((*R*)-3-methyl-3-(prop-2-yn-1-ylthio)-2-(2,2,2-trifluoroacetamido)butanoyl)-3-azabicyclo[3.1.0]hexane-2-carboxamide (**S15**)

A reaction vial was charged with **S14** (141 mg, 0.25 mmol), Burgess reagent (199 mg, 0.84 mmol), and anhydrous DCM (1.2 mL). The light-yellow clear mixture was stirred under an N_2 atmosphere for 2 h before quenching with MeOH (0.5 mL). The resulting mixture was concentrated under reduced pressure and purified by flash column chromatography (EtOAc/petroleum ether, gradient, 80:20 to 100:0). **S15** was obtained as a white foamy solid (101 mg, 74%).

TLC: R_f = 0.28 (EtOAc, 100)

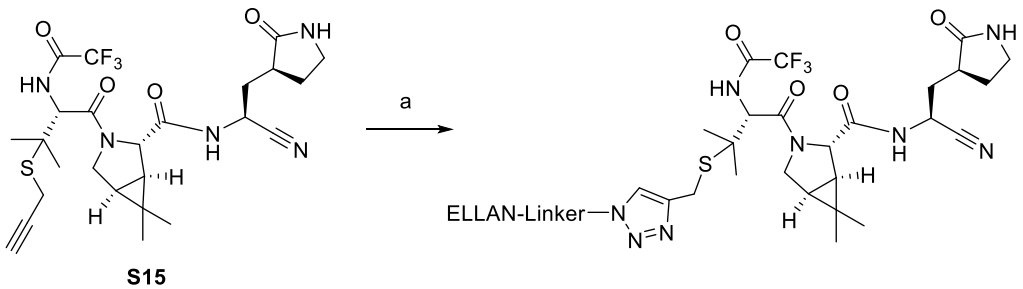
^{1}H NMR (400 MHz, DMSO) δ 9.60 (d, *J* = 8.3 Hz, 1H), 9.01 (d, *J* = 8.5 Hz, 1H), 7.67 (s, 1H), 4.97 (ddd, *J* = 10.6, 8.4, 5.3 Hz, 1H), 4.77 (d, *J* = 8.4 Hz, 1H), 4.14 (s, 1H), 3.99 (dd, *J* = 10.4, 5.5 Hz, 1H), 3.71 (d, *J* = 10.4 Hz, 1H), 3.40 (d, *J* = 2.7 Hz, 2H), 3.17 – 3.11 (m, 2H), 3.09 – 3.01 (m, 1H), 2.44 – 2.34 (m, 1H), 2.18 – 2.05 (m, 2H), 1.78 – 1.67 (m, 2H), 1.59 (dd, *J* = 7.6, 5.4 Hz, 1H), 1.50 (s, 3H), 1.37 – 1.32 (m, 4H), 1.03 (s, 3H), 0.85 (s, 3H).

^{19}F NMR (377 MHz, DMSO) δ -73.00.

^{13}C NMR (100 MHz, DMSO) δ 177.4, 170.4, 166.1, 120.2, 119.5, 80.9, 73.5, 60.2, 56.1, 48.8, 47.6, 37.9, 36.8, 34.0, 30.2, 27.2, 26.8, 25.6, 25.6, 23.8, 18.8, 16.0, 12.2. Two carbons not observed.

HRMS: Calc *m/z* for ($[C_{25}H_{32}F_3N_5O_4S]$ + H)$^+$ 556.2200, found 556.2209.

1.6 Synthesis of Target Compounds (BP-172, 198, and 174)



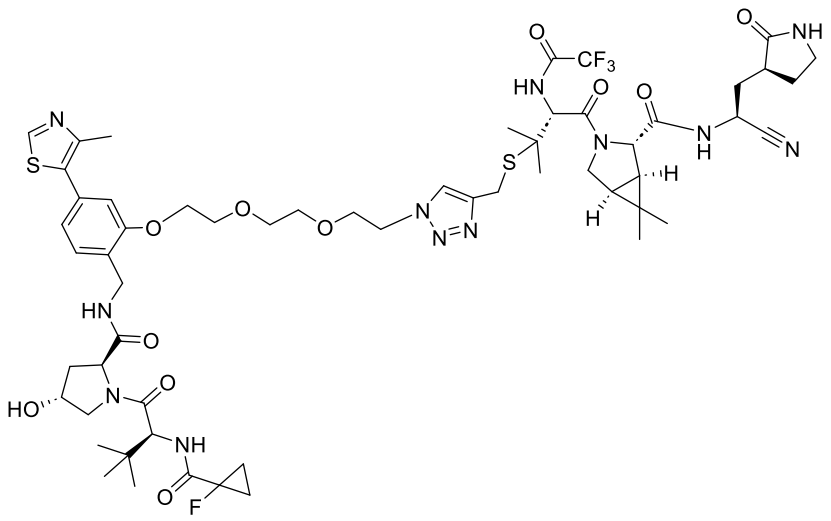
S15

BP-172 (from **S2**) **BP-200** (from **S4**)
BP-198 (from **S7a**) **BP-206** (from **S9a**)
BP-174 (from **S7b**) **BP-202** (from **S9b**)

Scheme S7 (a) ELLAN-Linker-N_3, $CuSO_4.5H_2O$, sodium ascorbate, t-BuOH, H_2O, 40-45°C.

(1*R*,2*S*,5*S*)-*N*-((*S*)-1-Cyano-2-((*S*)-2-oxopyrrolidin-3-yl)ethyl)-3-((*R*)-3-(((1-(2-(2-(2-(2-(((2*S*,4*R*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-

5-(4-methylthiazol-5-yl)phenoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)-3-methyl-2-(2,2,2-trifluoroacetamido)butanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide (**BP-172**)



Following general procedure C, **S15** (13.7 mg, 0.025 mmol), **S2** (17.2 mg, 0.025 mmol), sodium ascorbate (2.9 mg, 0.015 mmol), $CuSO_4.5H_2O$ (1.9 mg, 0.0074 mmol), and a degassed mixture of t-BuOH and H_2O (1 mL) gave a slightly yellow clear mixture. After 18 h, workup and flash column chromatography ($CHCl_3$/MeOH, gradient, 100:0 to 94:6) provided **BP-172** as a white solid (12.7 mg, 41%).

TLC: R_f = 0.27 ($CHCl_3$/MeOH, 90:10)

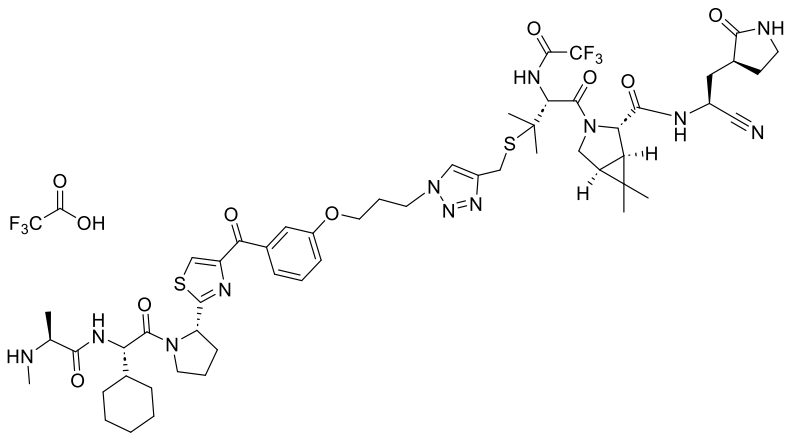
^{1}H NMR (400 MHz, DMSO) δ 9.58 (d, *J* = 8.3 Hz, 1H), 9.05 – 8.94 (m, 2H), 8.46 (t, *J* = 5.8 Hz, 1H), 7.89 (s, 1H), 7.64 (s, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.27 (br d, *J* = 9.2 Hz, 1H), 7.03 (d, *J* = 1.2 Hz, 1H), 6.97 (d, *J* = 7.4 Hz, 1H), 5.15 (d, *J* = 3.6 Hz, 1H), 5.04 – 4.91 (m, 1H), 4.72 (d, *J* = 8.1 Hz, 1H), 4.59 (d, *J* = 9.4 Hz, 1H), 4.51 (t, *J* = 8.2 Hz, 1H), 4.46 (t, *J* = 5.2 Hz, 2H), 4.36 – 4.31 (m, 1H), 4.29 (d, *J* = 6.1 Hz, 1H), 4.23 (d, *J* = 5.6 Hz, 1H), 4.19 – 4.15 (m, 2H), 4.15 (s, 1H), 3.97 (dd, *J* = 10.3, 5.5 Hz, 1H), 3.84 (s, 2H), 3.80 (t, *J* = 5.2 Hz, 2H), 3.76 (t, *J* = 4.6 Hz, 2H), 3.70 – 3.63 (m, 2H), 3.62 – 3.58 (m, 3H), 3.57 – 3.53 (m, 2H), 3.12 (t, *J* = 8.9 Hz, 1H), 3.03 (q, *J* = 8.7 Hz, 1H), 2.46 (s, 3H), 2.41 – 2.33 (m, 1H), 2.18 – 2.03 (m, 3H), 1.96 – 1.88 (m, 1H), 1.77 – 1.64 (m, 2H), 1.57 (app t, *J* = 6.5 Hz, 1H), 1.47 (s, 3H), 1.41 – 1.31 (m, 6H), 1.25 – 1.20 (m, 2H), 1.03 (s, 3H), 0.95 (s, 9H), 0.84 (s, 3H).

^{19}F NMR (377 MHz, DMSO) δ -73.01, -196.20.

HPLC t_R (Method B): 16.1 min.

HRMS: Calc *m/z* for $([C_{57}H_{76}F_4N_{12}O_{11}S_2] + H)^+$ 1245.5238, found 1245.5207.

(1*R*,2*S*,5*S*)-*N*-((*S*)-1-Cyano-2-((*S*)-2-oxopyrrolidin-3-yl)ethyl)-3-((*R*)-3-(((1-(3-(3-(2-((S)-1-((S)-2-cyclohexyl-2-((*S*)-2-(methylamino)propanamido)acetyl)pyrrolidin-2-yl)thiazole-4-carbonyl)phenoxy)propyl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)-3-methyl-2-(2,2,2-trifluoroacetamido)butanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide trifluoroacetic acid (**BP-198**)



Following general procedure C, **S15** (14.1 mg, 0.025 mmol), **S7a** (0.025 mmol), sodium ascorbate (9.9 mg, 0.050 mmol), $CuSO_4.5H_2O$ (9.3 mg, 0.037 mmol), and a degassed mixture of t-BuOH and H_2O (0.6 mL) gave a light-yellow-brown opaque mixture. After 18 h, workup (neutralised using $NaHCO_3$ solution), flash column chromatography ($CHCl_3$/7M NH_3 in MeOH, gradient, 100:0 to 96:4) and preparative RP-HPLC provided **BP-198** as a white solid (4.8 mg, 15% over two steps).

TLC: R_f = 0.22 ($CHCl_3$/7M NH_3 in MeOH, 95:5)

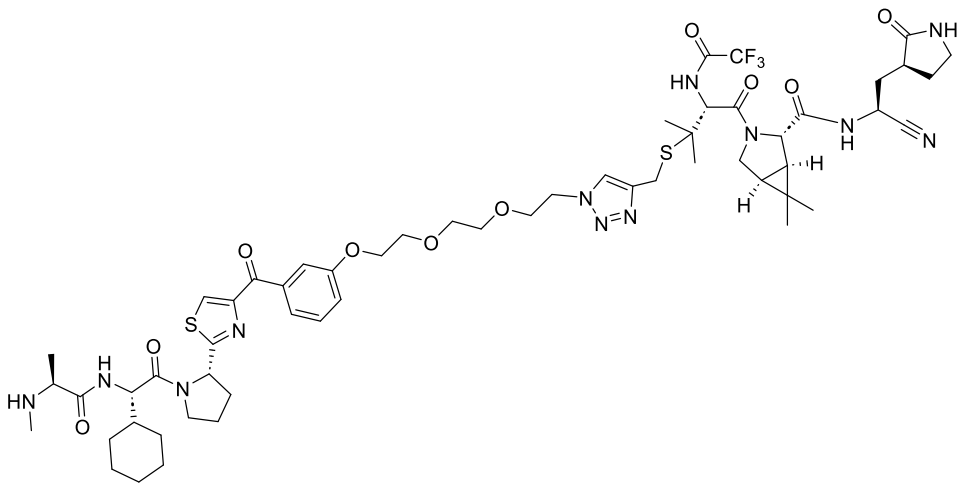
^{1}H NMR (400 MHz, DMSO) δ 9.61 (d, *J* = 8.3 Hz, 1H), 9.01 (d, *J* = 8.6 Hz, 1H), 8.78 (br s, 2H), 8.72 (d, *J* = 8.0 Hz, 1H), 8.49 (s, 1H), 7.99 (s, 1H), 7.73 – 7.64 (m, 2H), 7.62 (t, *J* = 2.1 Hz, 1H), 7.46 (t, *J* = 7.9 Hz, 1H), 7.23 (dd, *J* = 8.1, 2.7 Hz, 1H), 5.39 (dd, *J* = 7.3, 3.6 Hz, 1H), 4.96 (ddd, *J* = 10.4, 8.3, 5.4 Hz, 1H), 4.69 (d, *J* = 8.5 Hz, 1H), 4.55 – 4.43 (m, 3H), 4.14 (s, 1H), 4.05 (t, *J* = 6.0 Hz, 2H), 3.96 (dd, *J* = 10.6, 4.9 Hz, 1H), 3.91 – 3.71 (m, 5H), 3.66 (d, *J* = 10.6 Hz, 1H), 3.15 – 3.09 (m, 1H), 3.06 – 2.99 (m, 1H), 2.51 – 2.50 (m, 3H, assumed; partially obscured by solvent peak), 2.40 – 2.34 (m, 1H), 2.32 – 2.26 (m, 2H), 2.25 – 2.17 (m, 2H), 2.14 – 2.02 (m, 4H), 1.83 – 1.51 (m, 9H), 1.48 (s, 3H), 1.42 – 1.19 (m, 7H), 1.16 – 0.91 (m, 8H), 0.83 (s, 3H).

^{19}F NMR (377 MHz, DMSO) δ -72.99, -74.04.

HPLC t_R (Method B): 15.4 min.

HRMS: Calc *m/z* for $([C_{54}H_{71}F_3N_{12}O_8S_2] + H)^+$ 1137.4984, found 1137.5003.

(1*R*,2*S*,5*S*)-*N*-((*S*)-1-Cyano-2-((*S*)-2-oxopyrrolidin-3-yl)ethyl)-3-((*R*)-3-(((1-(2-(2-(2-(3-(2-((*S*)-1-((*S*)-2-cyclohexyl-2-((*S*)-2-(methylamino)propanamido)acetyl)pyrrolidin-2-yl)thiazole-4-carbonyl)phenoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)-3-methyl-2-(2,2,2-trifluoroacetamido)butanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide (**BP-174**)



Following general procedure C, **S15** (13.0 mg, 0.023 mmol), **S7b** (0.023 mmol), sodium ascorbate (9.3 mg, 0.047 mmol), $CuSO_4.5H_2O$ (8.8 mg, 0.035 mmol), and a degassed mixture of t-BuOH and H_2O (1 mL) gave a light-yellow-brown clear mixture. After 18 h, workup (neutralised using $NaHCO_3$ solution) and flash column chromatography ($CHCl_3$/7M NH_3 in MeOH, gradient, 100:0 to 96:4) provided **BP-174** as an off-white foamy solid (7.7 mg, 27% over two steps).

TLC: R_f = 0.23 ($CHCl_3$/7M NH_3 in MeOH, 95:5)

^{1}H NMR (400 MHz, DMSO) δ 9.60 (s, 1H), 9.00 (d, *J* = 8.4 Hz, 1H), 8.48 (s, 1H), 7.97 – 7.85 (m, 2H), 7.75 – 7.60 (m, 3H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.24 (dd, *J* = 8.3, 2.3 Hz, 1H), 5.38 (dd, *J* = 7.9, 3.0 Hz, 1H), 5.01 – 4.93 (m, 1H), 4.72 (s, 1H), 4.54 – 4.42 (m, 3H), 4.19 – 4.10 (m, 3H), 3.97 (dd, *J* = 10.7, 5.6 Hz, 1H), 3.88 – 3.75 (m, 6H), 3.74 (t, *J* = 4.6 Hz, 2H), 3.68 (d, *J* = 10.6 Hz, 1H), 3.60 – 3.52 (m, 4H), 3.14 – 3.08 (m, 1H, assumed; partially obscured by solvent peak), 3.06 – 3.01 (m, 1H, assumed; partially obscured by solvent peak), 2.96 (q, *J* = 6.8 Hz, 1H, assumed; partially obscured by solvent peak), 2.40 – 2.36 (m, 1H), 2.30 – 2.22 (m, 2H), 2.17 (s, 3H), 2.14 – 1.98 (m, 4H), 1.80 – 1.51 (m, 9H), 1.47 (s, 3H), 1.37 – 1.30 (m, 4H), 1.18 – 1.05 (m, 6H), 1.02 (s, 3H), 1.00 – 0.94 (m, 2H), 0.83 (s, 3H).

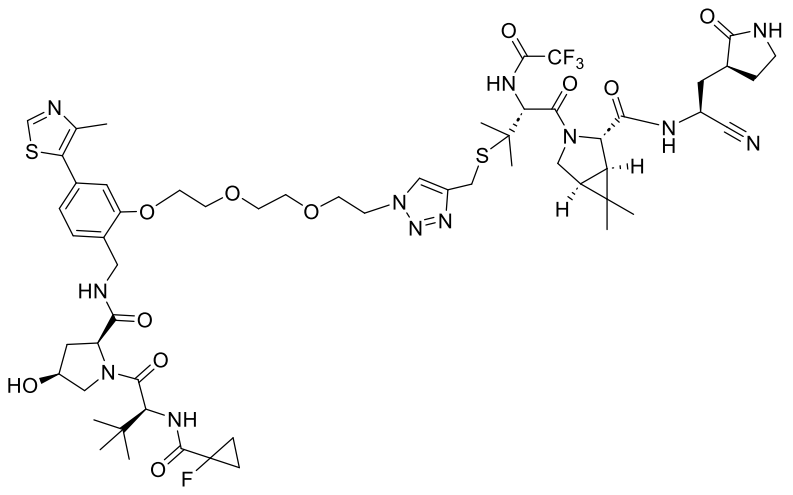
^{19}F NMR (377 MHz, DMSO) δ -73.00.

HPLC t_R (Method B): 15.6 min.

HRMS: Calc *m/z* for $([C_{57}H_{77}F_3N_{12}O_{10}S_2] + H)^+$ 1211.5352, found 1211.5342.

1.7 Synthesis of Non-PROTAC Control Compounds (BP-200, 206, and 202)

(1*R*,2*S*,5*S*)-*N*-((*S*)-1-Cyano-2-((*S*)-2-oxopyrrolidin-3-yl)ethyl)-3-((*R*)-3-(((1-(2-(2-(2-(2-(((2*S*,4*S*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)-3-methyl-2-(2,2,2-trifluoroacetamido)butanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide (**BP-200,** cis-VH101 linked)



Following general procedure C, **S15** (13.5 mg, 0.024 mmol), **S4** (16.0 mg, 0.023 mmol), sodium ascorbate (2.8 mg, 0.014 mmol), $CuSO_4.5H_2O$ (1.7 mg, 0.0070 mmol), and a degassed mixture of t-BuOH and H_2O (0.6 mL) gave a slightly yellow clear mixture. After 18 h, workup and preparative TLC (EtOAc/MeOH/H_2O, 85:10:5) provided **BP-200** as a white solid (13.9 mg, 48%).

TLC: R_f = 0.28 (EtOAc/MeOH/H_2O, 85:10:5)

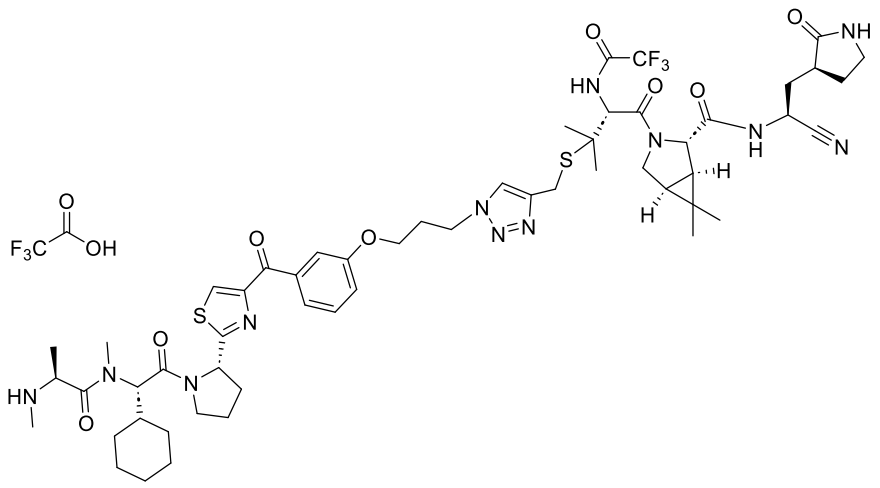
^{1}H NMR (400 MHz, DMSO) δ 9.61 (d, *J* = 8.2 Hz, 1H), 9.01 (d, *J* = 8.5 Hz, 1H), 8.98 (s, 1H), 8.55 (t, *J* = 6.0 Hz, 1H), 7.89 (s, 1H), 7.65 (s, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.30 (dd, *J* = 8.9, 2.7 Hz, 1H), 7.04 (d, *J* = 1.7 Hz, 1H), 6.95 (dd, *J* = 7.8, 1.6 Hz, 1H), 5.44 (d, *J* = 7.3 Hz, 1H), 5.01 – 4.92 (m, 1H), 4.72 (d, *J* = 8.0 Hz, 1H), 4.55 (d, *J* = 8.9 Hz, 1H), 4.49 – 4.41 (m, 3H), 4.33 (dd, *J* = 16.5, 6.2 Hz, 1H), 4.27 – 4.19 (m, 2H), 4.17 (t, *J* = 4.7 Hz, 2H), 4.14 (s, 1H), 3.97 (dd, *J* = 10.4, 5.4 Hz, 1H), 3.90 – 3.82 (m, 3H), 3.80 (t, *J* = 5.2 Hz, 2H), 3.76 (t, *J* = 4.7 Hz, 2H), 3.68 (d, *J* = 10.5 Hz, 1H), 3.62 – 3.57 (m, 2H), 3.57 – 3.52 (m, 2H), 3.46 (dd, *J* = 10.1, 5.3 Hz, 1H), 3.12 (t, *J* = 9.0 Hz, 1H), 3.02 (q, *J* = 9.2, 8.7 Hz, 1H), 2.46 (s, 3H), 2.40 – 2.32 (m, 2H), 2.18 – 2.02 (m, 2H), 1.79 – 1.65 (m, 3H), 1.59 – 1.55 (m, 1H), 1.47 (s, 3H), 1.40 – 1.28 (m, 6H), 1.25 – 1.20 (m, 2H), 1.02 (s, 3H), 0.97 (s, 9H), 0.84 (s, 3H).

^{19}F NMR (377 MHz, DMSO) δ -72.98, -196.17.

HPLC t_R (Method B): 16.1 min.

HRMS: Calc *m/z* for ($[C_{57}H_{76}F_4N_{12}O_{11}S_2]$ + H)$^+$ 1245.5207, found 1245.5265.

(1*R*,2*S*,5*S*)-*N*-((*S*)-1-Cyano-2-((*S*)-2-oxopyrrolidin-3-yl)ethyl)-3-((*R*)-3-(((1-(3-(3-(2-((*S*)-1-((*S*)-2-cyclohexyl-2-((*S*)-*N*-methyl-2-(methylamino)propanamido)acetyl)pyrrolidin-2-yl)thiazole-4-carbonyl)phenoxy)propyl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)-3-methyl-2-(2,2,2-trifluoroacetamido)butanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide trifluoroacetic acid (**BP-206,** *N*-Me-LCL161 linked)



Following general procedure C, **S15** (12.8 mg, 0.023 mmol), **S9a** (0.023 mmol), sodium ascorbate (9.1 mg, 0.046 mmol), $CuSO_4.5H_2O$ (7.5 mg, 0.030 mmol), and a degassed mixture of t-BuOH and H_2O (0.5 mL) gave a light-yellow opaque mixture. After 18 h, workup (neutralised using $NaHCO_3$ solution), flash column chromatography ($CHCl_3$/7M NH_3 in MeOH, gradient, 100:0 to 96:4), and preparative RP-HPLC provided **BP-206** as a white solid (3.9 mg, 13% over two steps, NMR indicated 10% residual ammonium trifluoroacetate).

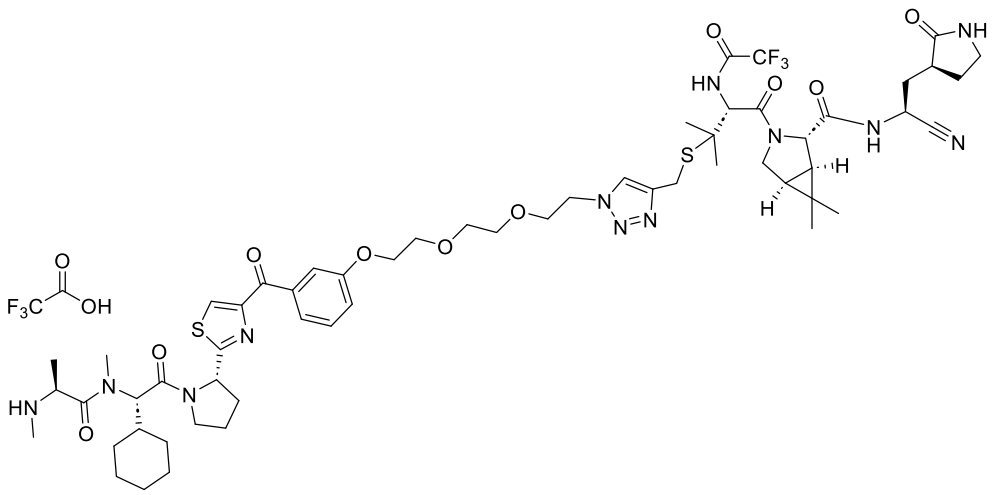
TLC: R_f = 0.18 ($CHCl_3$/7M NH_3 in MeOH, 95:5)

^{1}H NMR (400 MHz, DMSO) δ 9.61 (d, *J* = 8.4 Hz, 1H), 9.01 (d, *J* = 8.4 Hz, 1H), 8.93 (br s, 1H), 8.74 (br s, 1H), 8.48 (s, 1H), 7.99 (s, 1H), 7.72 – 7.63 (m, 2H), 7.61 (app s, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 5.39 – 5.34 (m, 1H), 5.05 (d, *J* = 10.8 Hz, 1H), 4.99 – 4.94 (m, 1H), 4.69 (d, *J* = 8.2 Hz, 1H), 4.55 – 4.49 (m, 2H), 4.41 – 4.36 (m, 1H), 4.14 (s, 1H), 4.06 (t, *J* = 6.0 Hz, 2H), 3.99 – 3.94 (m, 1H), 3.85 (s, 2H), 3.82 – 3.75 (m, 2H), 3.66 (d, *J* = 9.9 Hz, 1H), 3.15 – 3.12 (m, 1H), 3.06 – 3.01 (m, 1H), 2.94 (s, 3H), 2.50 (s, 3H, assumed; partially obscured by solvent peak), 2.31 – 2.22 (m, 3H), 2.20 – 2.13 (m, 2H), 2.11 – 2.04 (m, 2H), 2.00 – 1.92 (m, 2H), 1.79 – 1.51 (m, 9H), 1.48 (s, 3H), 1.38 (d, *J* = 6.8 Hz, 3H), 1.35 – 1.26 (m, 4H), 1.20 – 1.04 (m, 5H), 1.02 (s, 3H), 0.83 (s, 3H).

^{19}F NMR (377 MHz, DMSO) δ -72.99, -73.47.

HPLC t_R (Method B): 15.6 min.

HRMS: Calc *m/z* for ($[C_{55}H_{73}F_3N_{12}O_8S_2]$ + H)$^+$ 1151.5141, found 1151.5140.(1*R*,2*S*,5*S*)-*N*-((*S*)-1-Cyano-2-((*S*)-2-oxopyrrolidin-3-yl)ethyl)-3-((*R*)-3-(((1-(2-(2-(2-(3-(2-((*S*)-1-((*S*)-2-cyclohexyl-2-((*S*)-*N*-methyl-2-(methylamino)propanamido)acetyl)pyrrolidin-2-yl)thiazole-4-carbonyl)phenoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)-3-methyl-2-(2,2,2-trifluoroacetamido)butanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide trifluoroacetic acid (**BP-202,** *N*-Me-LCL161 linked)



Following general procedure C, **S15** (13.0 mg, 0.023 mmol), **S9b** (0.023 mmol), sodium ascorbate (10.0 mg, 0.050 mmol), $CuSO_4.5H_2O$ (9.4 mg, 0.038 mmol), and a degassed mixture of t-BuOH and H_2O (0.6 mL) gave a light-yellow-brown clear mixture. After 18 h, workup (neutralised using $NaHCO_3$ solution), preparative-TLC ($CHCl_3$/7M NH_3 in MeOH, 95:5) and preparative RP-HPLC provided **BP-202** as a white solid (6.2 mg, 18% over two step).

TLC: R_f = 0.17 ($CHCl_3$/7M NH_3 in MeOH, 95:5)

^{1}H NMR (400 MHz, DMSO) δ 9.62 (d, *J* = 8.5 Hz, 1H), 9.01 (d, *J* = 8.5 Hz, 1H), 8.94 (br s, 1H), 8.75 (br s, 1H), 8.47 (s, 1H), 7.90 (s, 1H), 7.73 – 7.64 (m, 2H), 7.64 – 7.59 (m, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.27 – 7.22 (m, 1H), 5.36 (dd, *J* = 8.1, 2.9 Hz, 1H), 5.05 (d, *J* = 10.9 Hz, 1H), 4.99 – 4.92 (m, 1H), 4.72 (d, *J* = 8.5 Hz, 1H), 4.47 (t, *J* = 5.3 Hz, 2H), 4.39 (q, *J* = 6.3 Hz, 1H), 4.18 – 4.10 (m, 3H), 3.96 (dd, *J* = 10.4, 5.5 Hz, 1H), 3.84 (s, 2H), 3.83 – 3.70 (m, 5H), 3.67 (d, *J* = 10.6 Hz, 1H), 3.55 (m, 2H), 3.50 – 3.44 (m, 1H, assumed; partially obscured by solvent peak), 3.37 – 3.37 (m, 2H, assumed; partially obscured by solvent peak), 3.15 – 3.09 (m, 1H), 3.06 – 2.99 (m, 1H), 2.93 (s, 3H), 2.51 – 2.50 (m, 3H, assumed; partially obscured by solvent peak), 2.40 – 2.34 (m, 1H), 2.30 – 2.19 (m, 2H), 2.17 – 1.92 (m, 5H), 1.79 – 1.49 (m, 8H), 1.47 (s, 3H), 1.38 (d, *J* = 6.9 Hz, 3H), 1.35 – 1.25 (m, 4H), 1.24 – 1.10 (m, 3H), 1.02 (s, 3H), 0.96 – 0.88 (m, 2H), 0.83 (s, 3H).

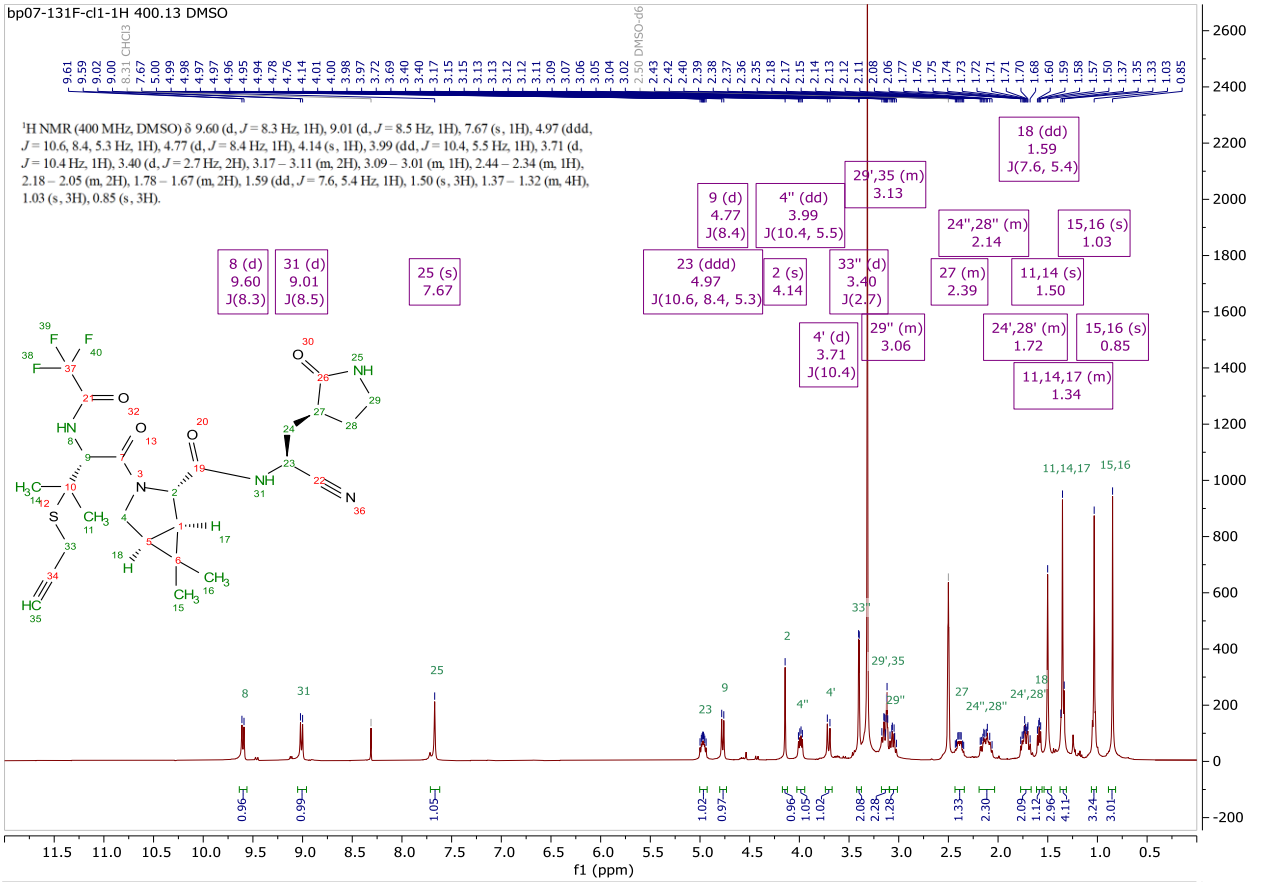
^{19}F NMR (377 MHz, DMSO) δ -72.98, -73.99.

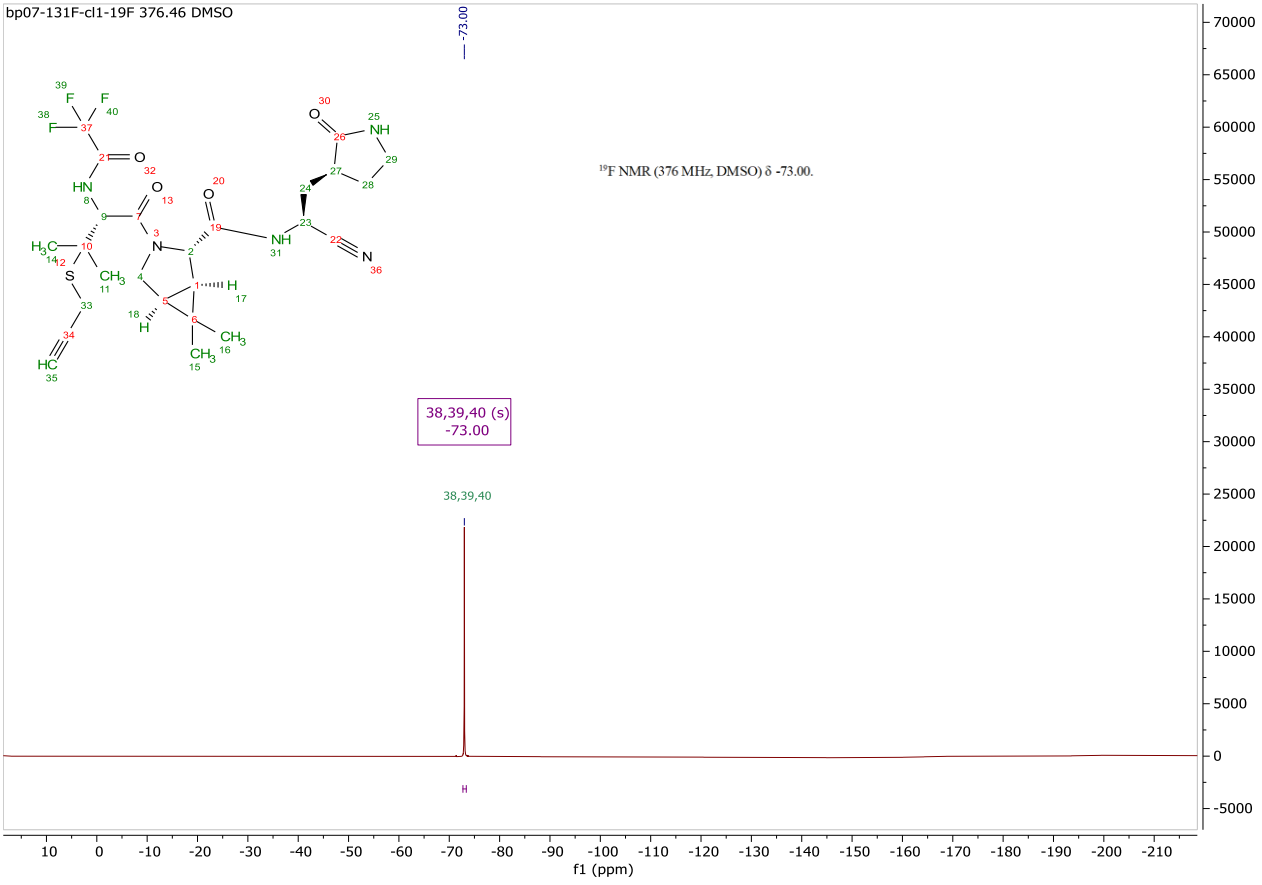
HPLC t_R (Method B): 15.5 min.

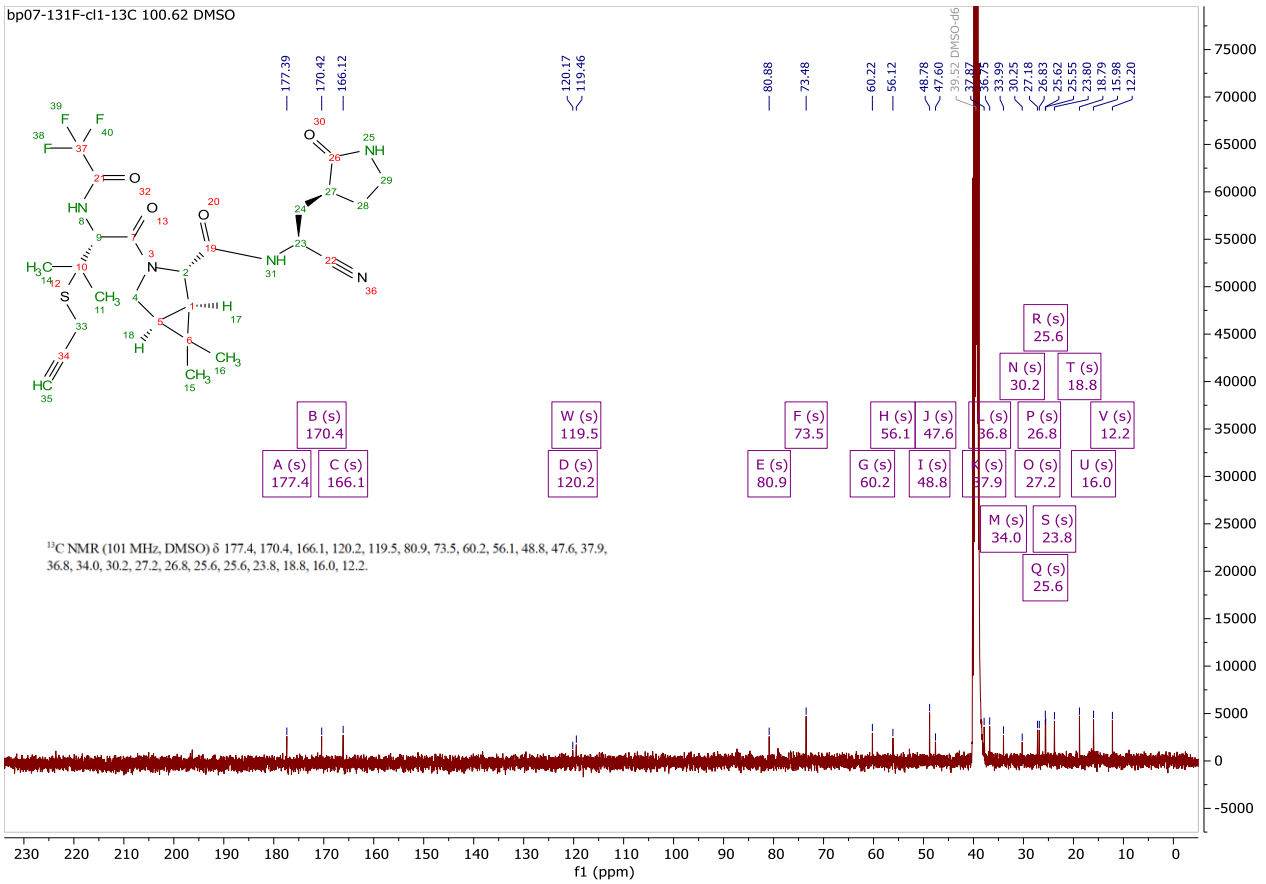
HRMS: Calc *m/z* for $([C_{58}H_{79}F_3N_{12}O_{10}S_2] + H)^+$ 1225.5508, found 1225.5515.

2 Selected NMR, HPLC, and HRMS spectra

2.1 S15







^{13}C NMR (101 MHz, DMSO) δ 177.4, 170.4, 166.1, 120.2, 119.5, 80.9, 73.5, 60.2, 56.1, 48.8, 47.6, 37.9, 36.8, 34.0, 30.2, 27.2, 26.8, 25.6, 25.6, 23.8, 18.8, 16.0, 12.2.

Compound Report

Agilent Trusted Answers

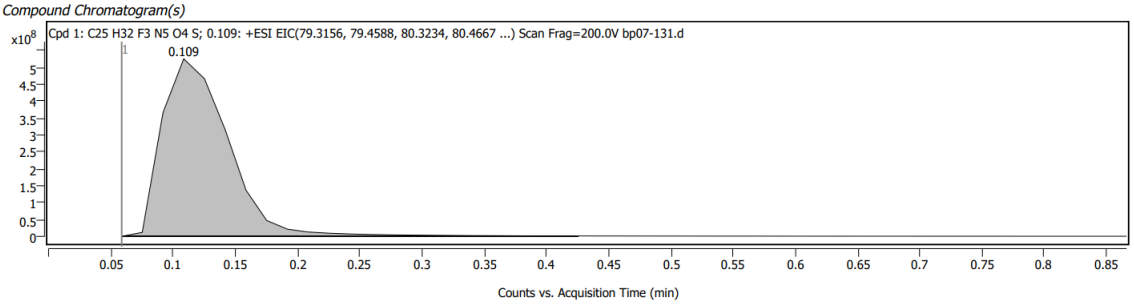
Sample Information

Name	bp07-131	**Data File Path**	D:\DATA\2022\May 23\bp07-131.d
Sample ID		**Acq. Time (Local)**	23/05/2022 11:30:14 AM (UTC+10:00)
Instrument	Instrument 1	**Method Path (Acq)**	D:\MassHunter\Methods\Monash_Direct.m
MS Type	TOF	**Version (Acq SW)**	6200 series TOF/6500 series Q-TOF 10.1 (48.0)
Inj. Vol. (ul)	0.2	**IRM Status**	Success
Position	P2-D5	**Method Path (DA)**	D:\DATA\2022\May 23\bp07-131.d\AcqData\MethodDA\Monash_Accuracy.m
Plate Pos.		**Target Source Path**	C25H32F3N5O4S
Operator	Dr Jason Dang	**Result Summary**	1 qualified (1 targets)

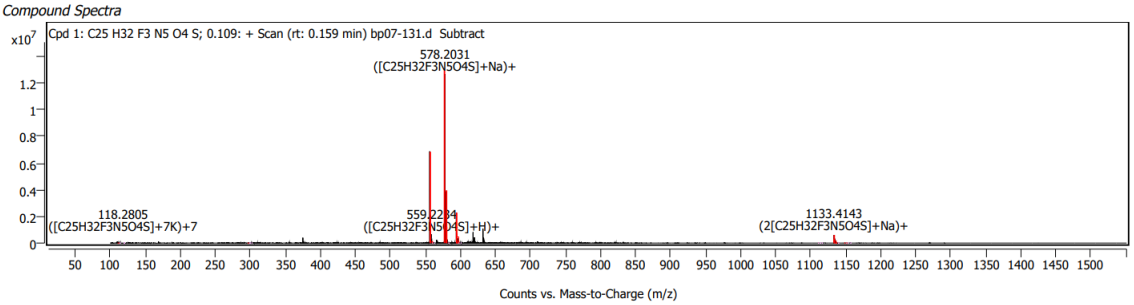
Compound Summary

Formula	RT	Mass	Area	Algorithm	Score	Diff (Tgt, ppm)
C25 H32 F3 N5 O4 S	0.109	555.2139	1930097431	FBF	99.85	2.18

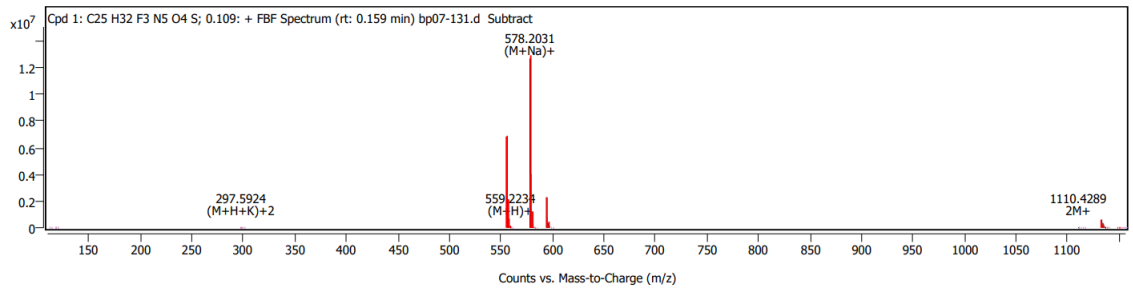
Compound Chromatogram(s)



Compound Spectra



Compound Spectra



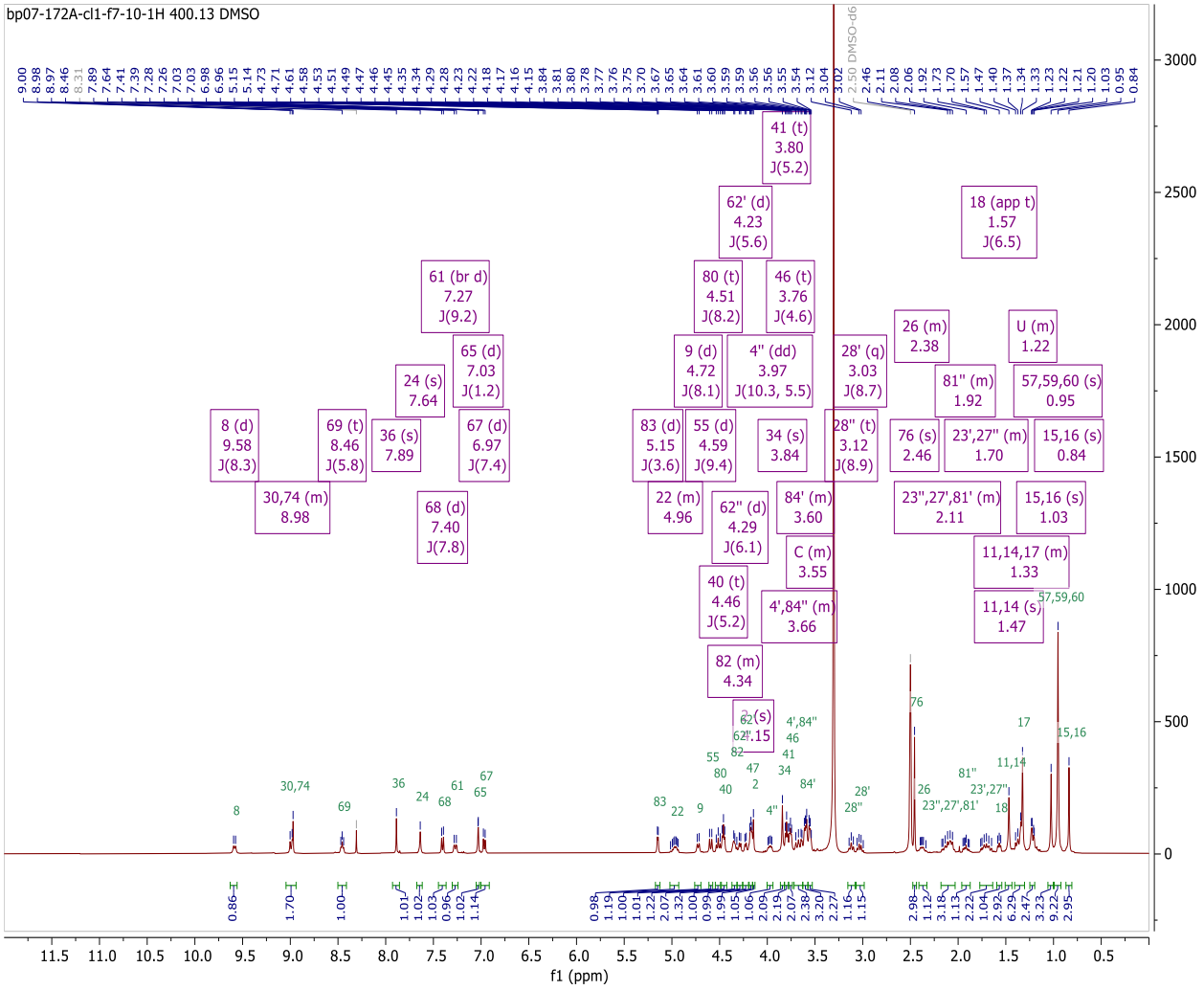
Spectrum Peaks (Max. 3)

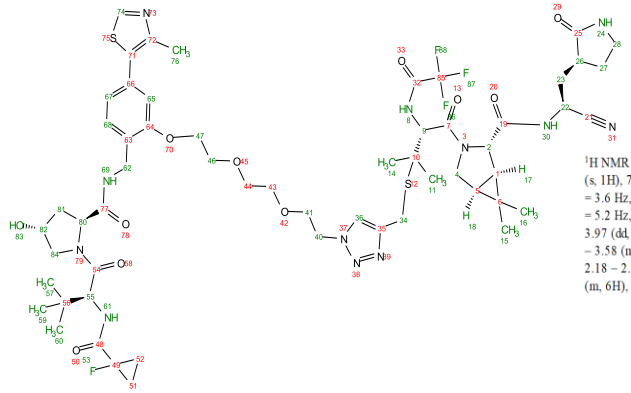
m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
556.2209	556.2200	1.73	6910750	100.00	100.00	(M+H)+	1
578.2031	578.2019	2.07	12711490	100.00	100.00	(M+Na)+	1
579.2058	579.2048	1.72	4044811	31.82	30.18	(M+Na)+	1

Spectrum Peaks (Max. 3)

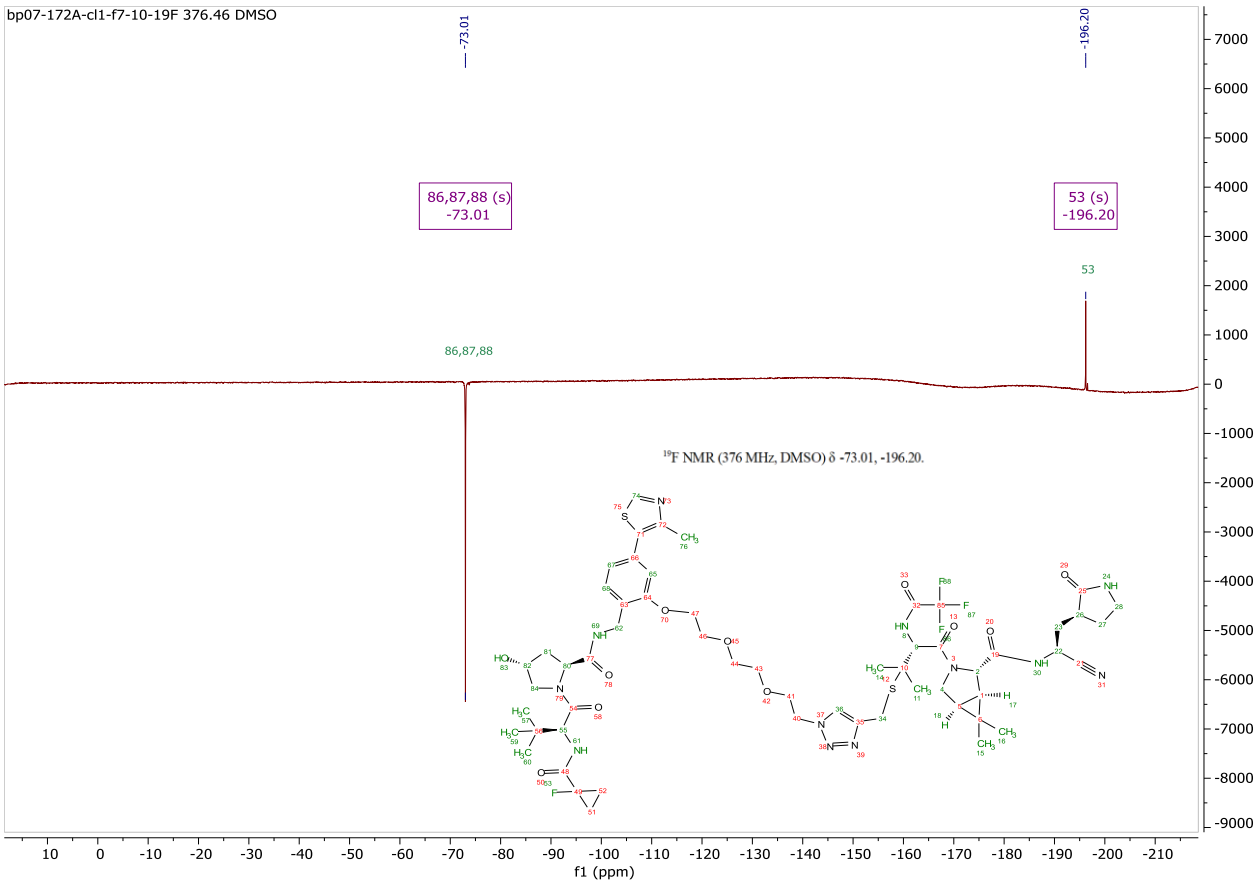
m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
556.2209	556.2200	1.73	6910750	100.00	100.00	(M+H)+	1
578.2031	578.2019	2.07	12711490	100.00	100.00	(M+Na)+	1
579.2058	579.2048	1.72	4044811	31.82	30.18	(M+Na)+	1

2.2 BP-172

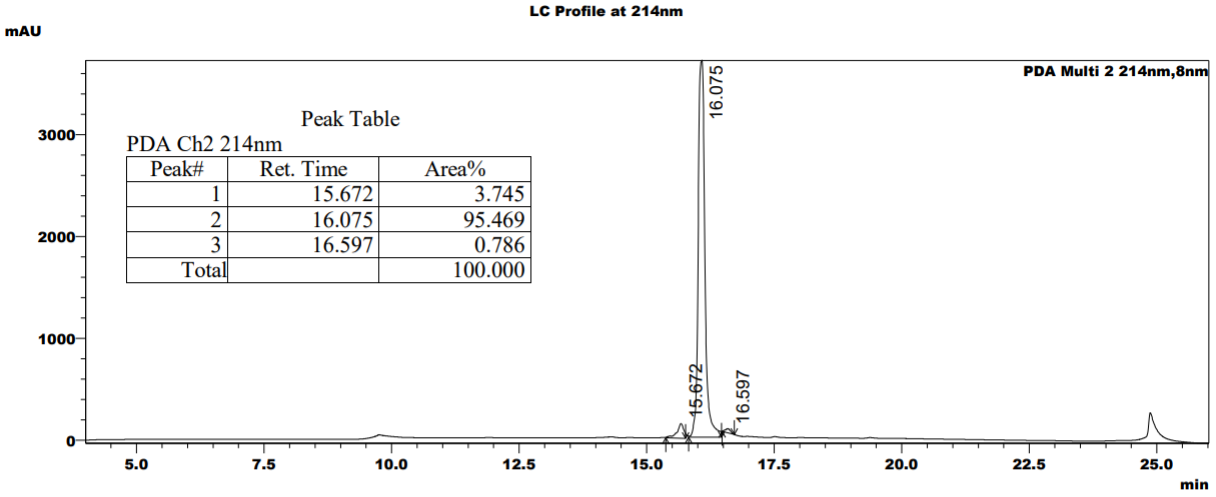




^{1}H NMR (400 MHz, DMSO) δ 9.58 (d, J = 8.3 Hz, 1H), 9.05 – 8.94 (m, 2H), 8.46 (t, J = 5.8 Hz, 1H), 7.89 (s, 1H), 7.64 (s, 1H), 7.40 (d, J = 7.8 Hz, 1H), 7.27 (br d, J = 9.2 Hz, 1H), 7.03 (d, J = 1.2 Hz, 1H), 6.97 (d, J = 7.4 Hz, 1H), 5.15 (d, J = 3.6 Hz, 1H), 5.04 – 4.91 (m, 1H), 4.72 (d, J = 8.1 Hz, 1H), 4.59 (d, J = 9.4 Hz, 1H), 4.51 (t, J = 8.2 Hz, 1H), 4.46 (t, J = 5.2 Hz, 2H), 4.36 – 4.31 (m, 1H), 4.29 (d, J = 6.1 Hz, 1H), 4.23 (d, J = 5.6 Hz, 1H), 4.19 – 4.15 (m, 2H), 4.15 (s, 1H), 3.97 (dd, J = 10.3, 5.5 Hz, 1H), 3.84 (s, 2H), 3.80 (t, J = 5.2 Hz, 2H), 3.76 (t, J = 4.6 Hz, 2H), 3.70 – 3.63 (m, 2H), 3.62 – 3.58 (m, 3H), 3.57 – 3.53 (m, 2H), 3.12 (t, J = 8.9 Hz, 1H), 3.03 (q, J = 8.7 Hz, 1H), 2.46 (s, 3H), 2.41 – 2.33 (m, 1H), 2.18 – 2.03 (m, 3H), 1.96 – 1.88 (m, 1H), 1.77 – 1.64 (m, 2H), 1.57 (app t, J = 6.5 Hz, 1H), 1.47 (s, 3H), 1.41 – 1.31 (m, 6H), 1.25 – 1.20 (m, 2H), 1.03 (s, 3H), 0.95 (s, 9H), 0.84 (s, 3H).

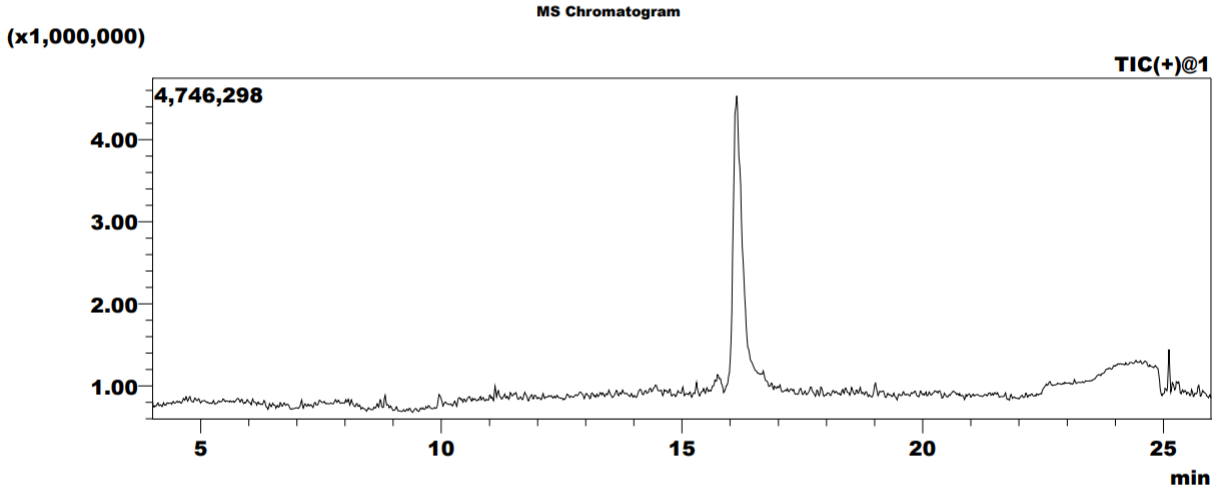

bp07-172A-cl1-f7-10-19F 376.46 DMSO
-73.01
-196.20
86,87,88 (s)
-73.01
53 (s)
-196.20
53
86,87,88
^{19}F NMR (376 MHz, DMSO) δ -73.01, -196.20.
f1 (ppm)

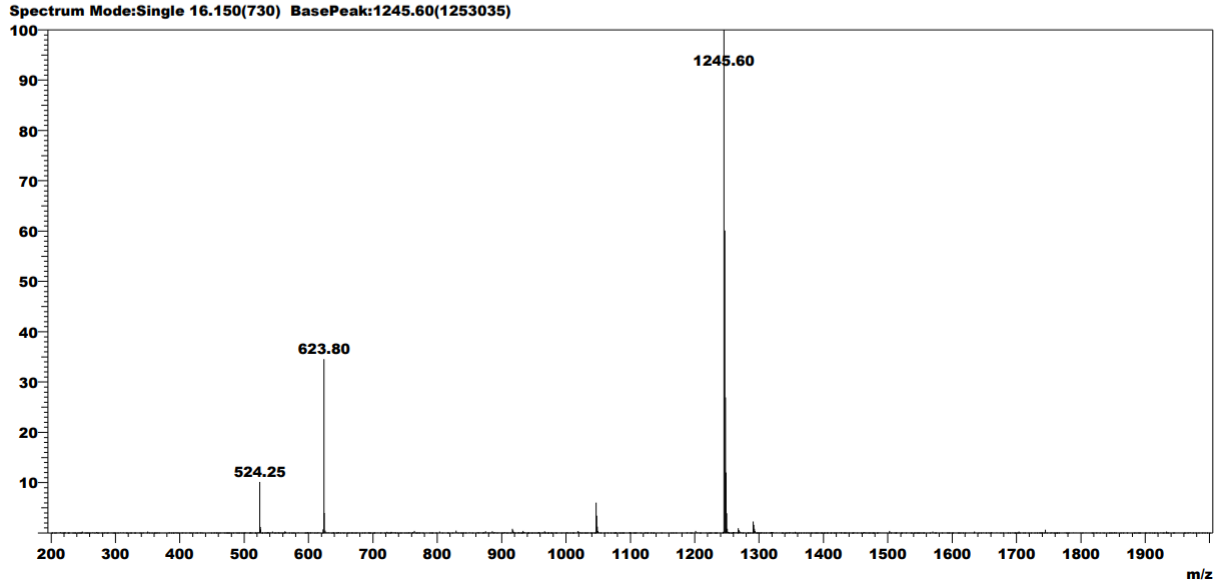
Sample Name : bp07-172A-cl1-f7-10
Month-Day Acquired : 8/07/2022
Time Acquired : 8:28:52 AM
Method File : LCMS 0-100B 15 min Pos.lcm



Peak Table
PDA Ch2 214nm

Peak#	Ret. Time	Area%
1	15.672	3.745
2	16.075	95.469
3	16.597	0.786
Total		100.000





Compound Report

Agilent | Trusted Answers

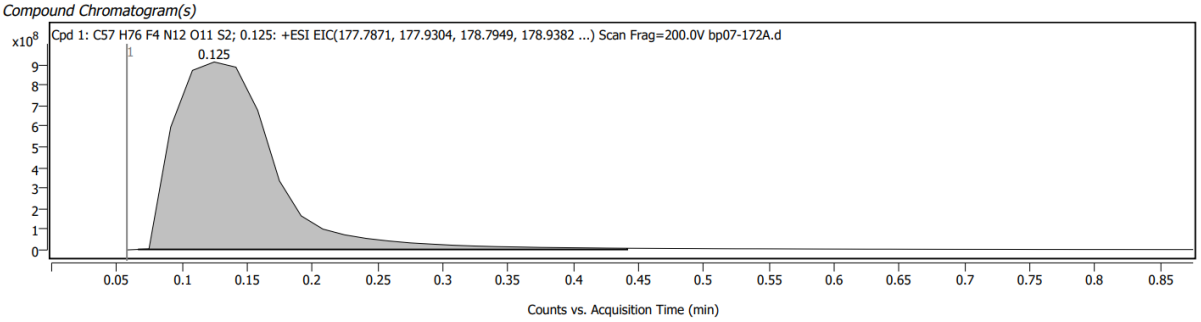
Sample Information

Name	bp07-172A	**Data File Path**	D:\DATA\2022\July 18\bp07-172A.d
Sample ID		**Acq. Time (Local)**	18/07/2022 8:37:50 PM (UTC+10:00)
Instrument	Instrument 1	**Method Path (Acq)**	D:\MassHunter\Methods\Monash_Direct.m
MS Type	TOF	**Version (Acq SW)**	6200 series TOF/6500 series Q-TOF 10.1 (48.0)
Inj. Vol. (ul)	0.1	**IRM Status**	Some ions missed
Position	P1-A1	**Method Path (DA)**	D:\DATA\2022\July 18\bp07-172A.d\AcqData\MethodDA\Monash_Accuracy.m
Plate Pos.		**Target Source Path**	C57H76F4N12O11S2
Operator	Dr Jason Dang	**Result Summary**	1 qualified (1 targets)

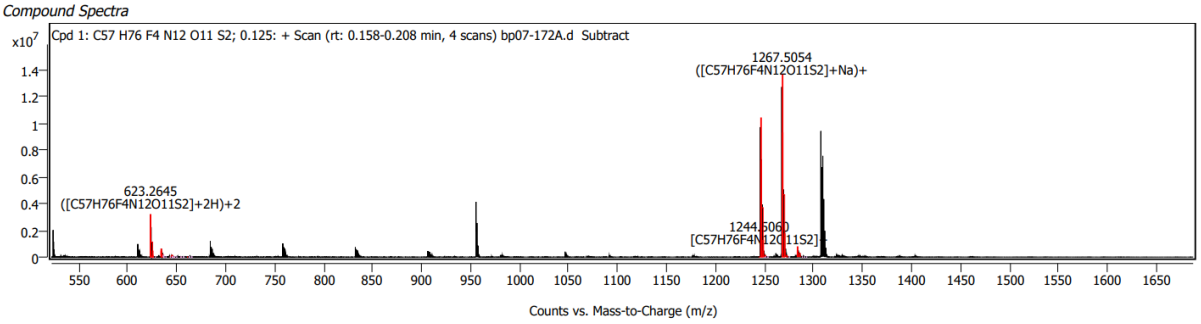
Compound Summary

Formula	RT	Mass	Area	Algorithm	Score	Diff (Tgt, ppm)
C57 H76 F4 N12 O11 S2	0.125	1244.5152	4846794679	FBF	99.48	1.46

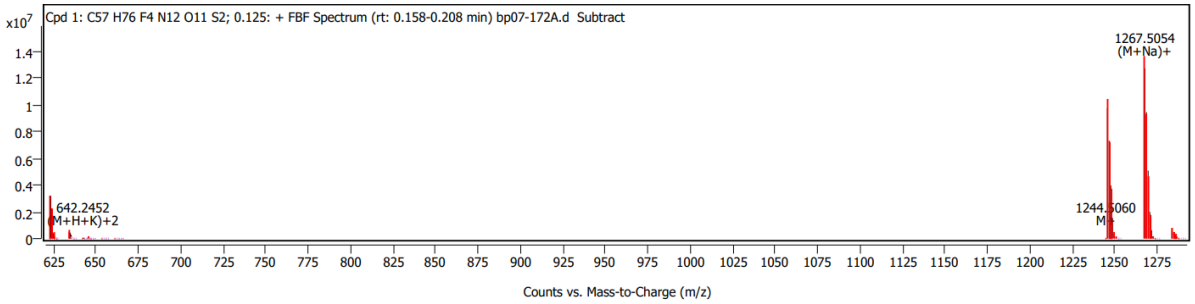
Compound Chromatogram(s)



Compound Spectra



Compound Spectra



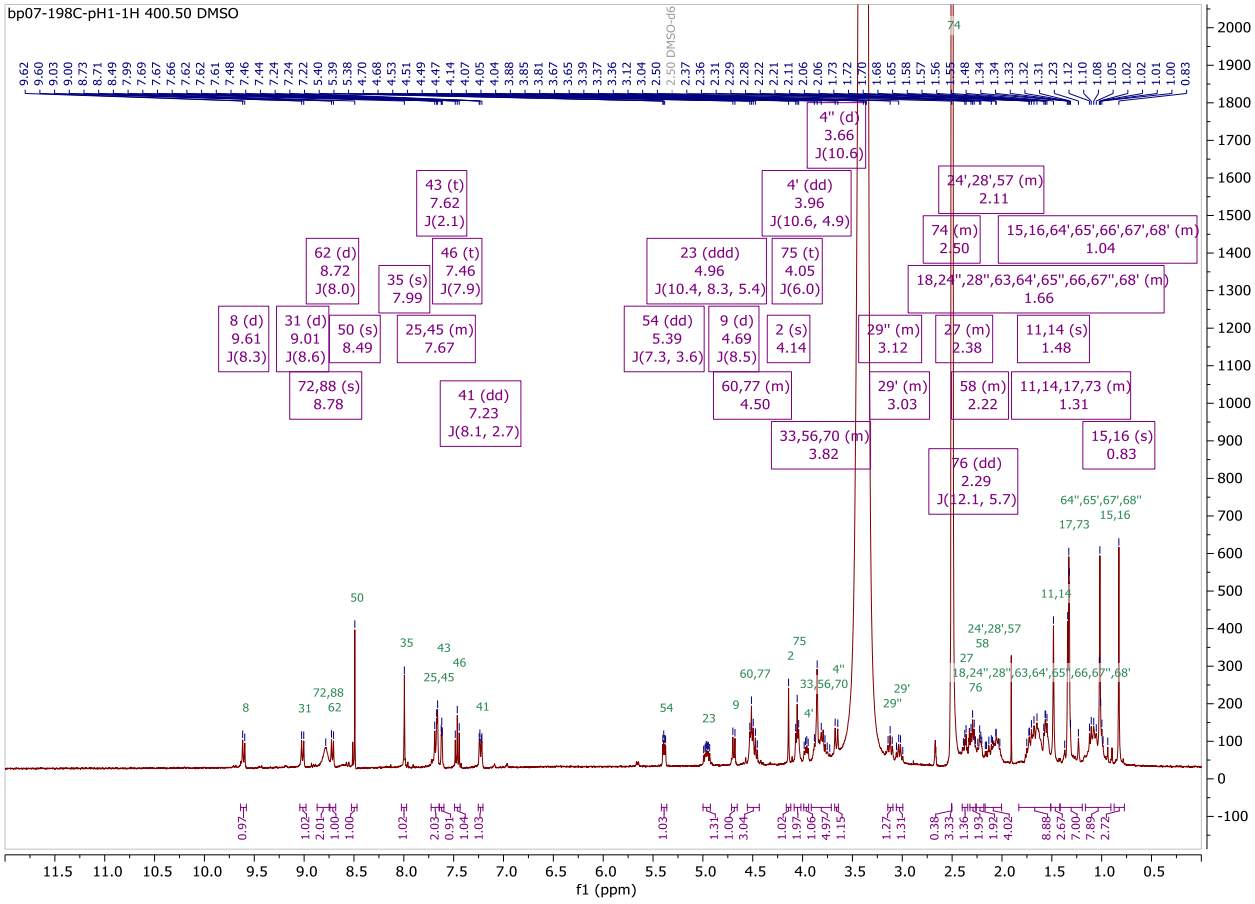
Spectrum Peaks (Max. 3)

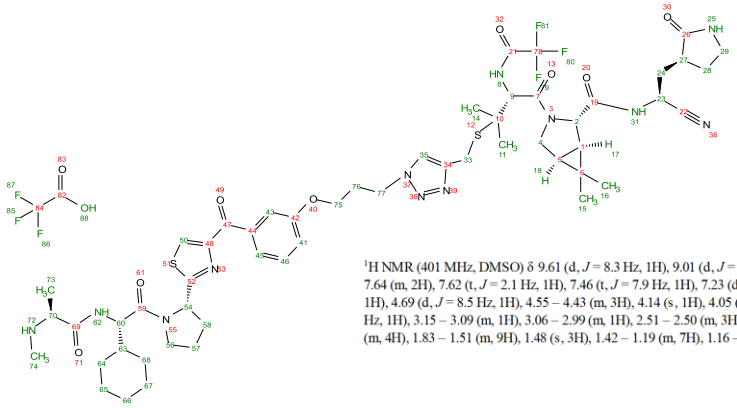
m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
1245.5238	1245.5207	2.53	9744758	100.00	100.00	(M+H)+	1
1267.5054	1267.5026	2.16	12732351	100.00	100.00	(M+Na)+	1
1268.5067	1268.5055	0.94	9457869	74.28	68.91	(M+Na)+	1

Spectrum Peaks (Max. 3)

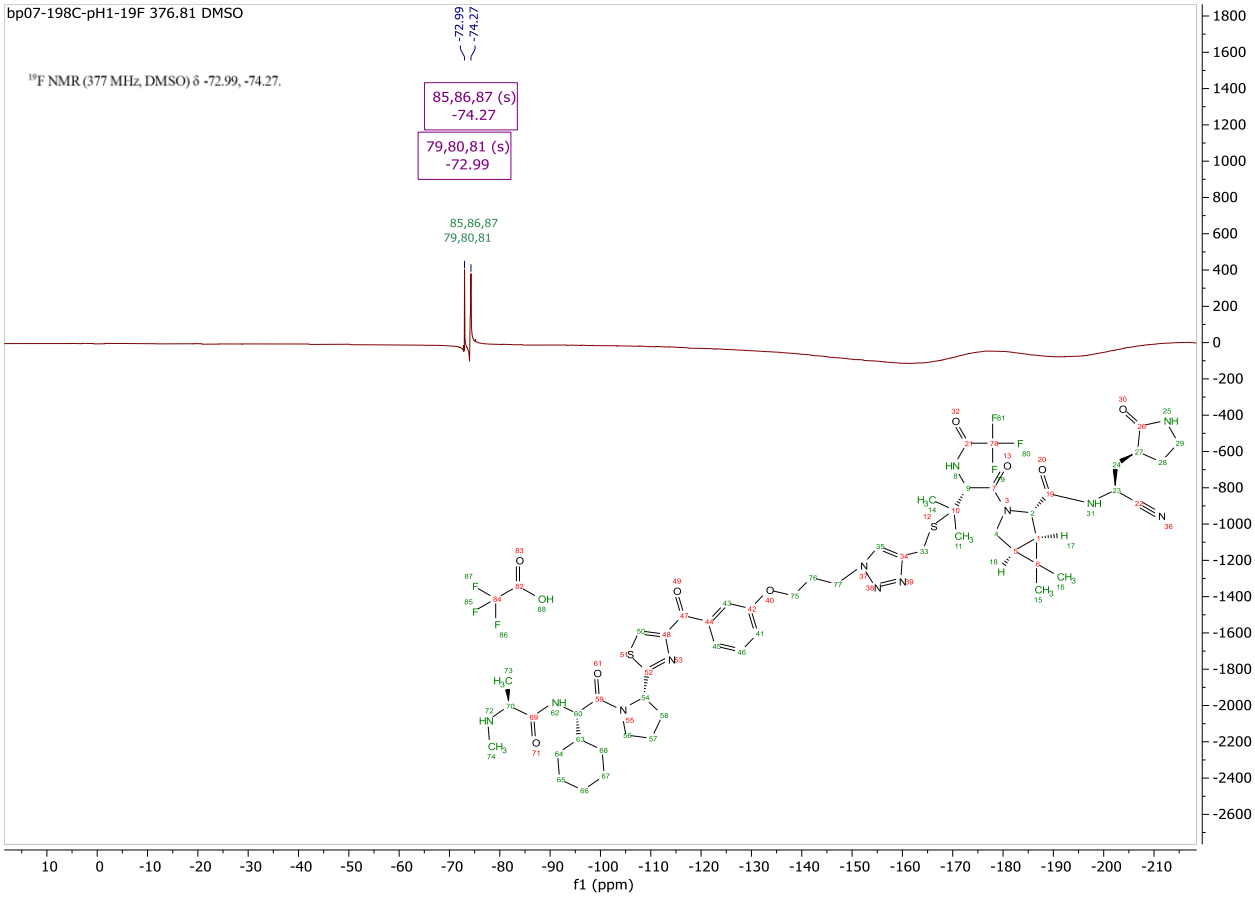
m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
1245.5238	1245.5207	2.53	9744758	100.00	100.00	(M+H)+	1
1267.5054	1267.5026	2.16	12732351	100.00	100.00	(M+Na)+	1
1268.5067	1268.5055	0.94	9457869	74.28	68.91	(M+Na)+	1

2.3 BP-198





^{1}H NMR (401 MHz, DMSO) δ 9.61 (d, J = 8.3 Hz, 1H), 9.01 (d, J = 8.6 Hz, 1H), 8.78 (s, 2H), 8.72 (d, J = 8.0 Hz, 1H), 8.49 (s, 1H), 7.99 (s, 1H), 7.73 – 7.64 (m, 2H), 7.62 (t, J = 2.1 Hz, 1H), 7.46 (t, J = 7.9 Hz, 1H), 7.23 (dd, J = 8.1, 2.7 Hz, 1H), 5.39 (dd, J = 7.3, 3.6 Hz, 1H), 4.96 (ddd, J = 10.4, 8.3, 5.4 Hz, 1H), 4.69 (d, J = 8.5 Hz, 1H), 4.55 – 4.43 (m, 3H), 4.14 (s, 1H), 4.05 (t, J = 6.0 Hz, 2H), 3.96 (dd, J = 10.6, 4.9 Hz, 1H), 3.91 – 3.71 (m, 5H), 3.66 (d, J = 10.6 Hz, 1H), 3.15 – 3.09 (m, 1H), 3.06 – 2.99 (m, 1H), 2.51 – 2.50 (m, 3H, assumed), 2.40 – 2.34 (m, 1H), 2.32 – 2.26 (m, 2H), 2.25 – 2.17 (m, 2H), 2.14 – 2.02 (m, 4H), 1.83 – 1.51 (m, 9H), 1.48 (s, 3H), 1.42 – 1.19 (m, 7H), 1.16 – 0.91 (m, 8H), 0.83 (s, 3H).

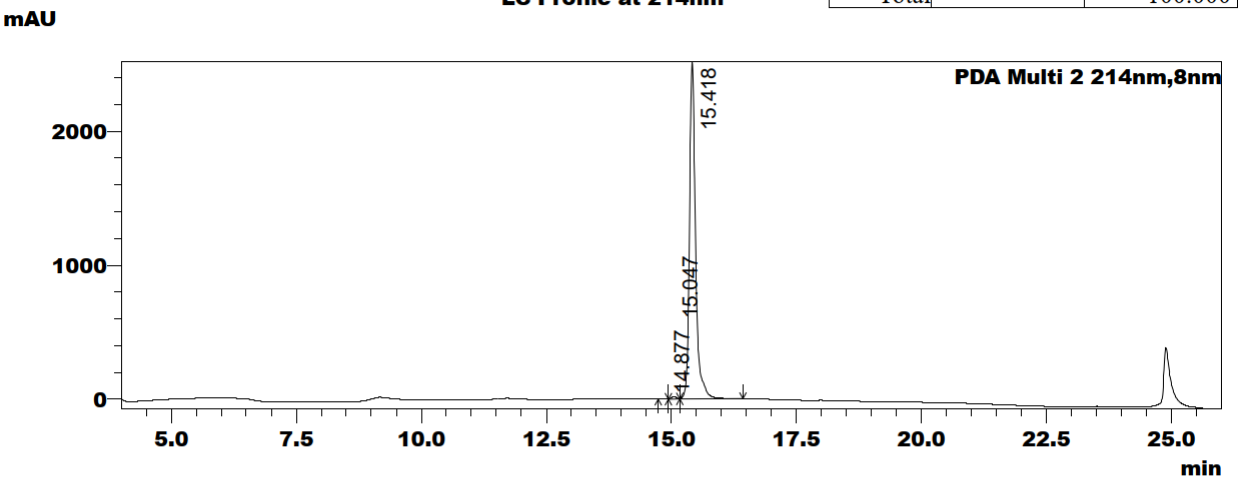

bp07-198C-pH1-19F 376.81 DMSO
-72.99
-74.27
19F NMR (377 MHz, DMSO) δ -72.99, -74.27.
85,86,87 (s)
-74.27
79,80,81 (s)
-72.99
85,86,87
79,80,81
f1 (ppm)

Sample Name : bp07-198C-p1-ccc
Month-Day Acquired : 4/19/2023
Time Acquired : 5:24:52 PM
Method File : LCMS 0-100B 15 min Pos.lcm

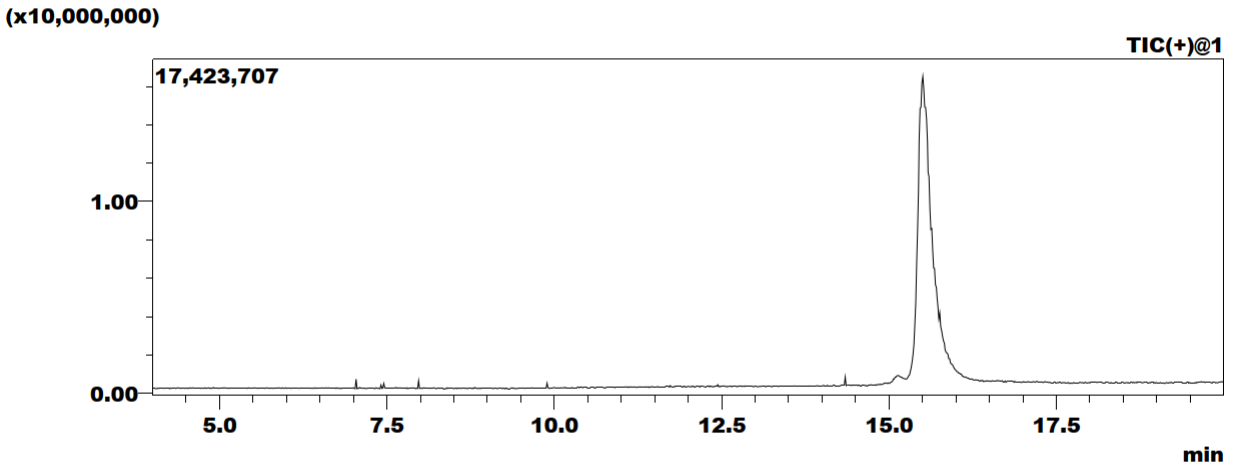
Peak Table
PDA Ch2 214nm

Peak#	Ret. Time	Area%
1	14.877	0.068
2	15.047	0.681
3	15.418	99.251
Total		100.000

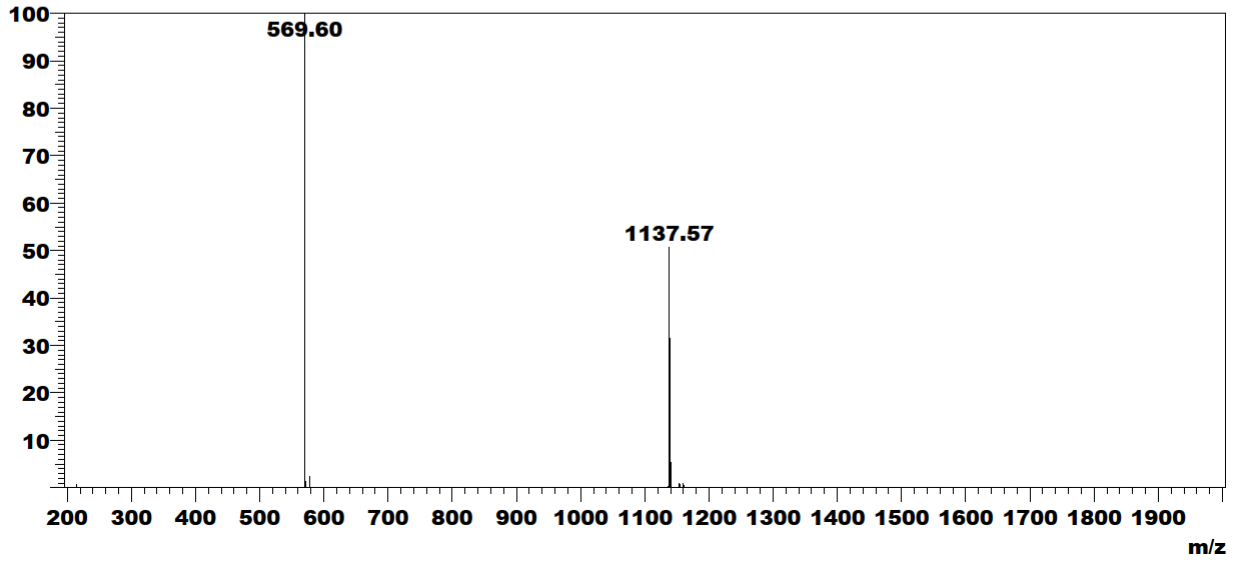
LC Profile at 214nm



MS Chromatogram



Spectrum Mode:Single 15.507(864) BasePeak:569.60(6988195)



Compound Report

Agilent Trusted Answers

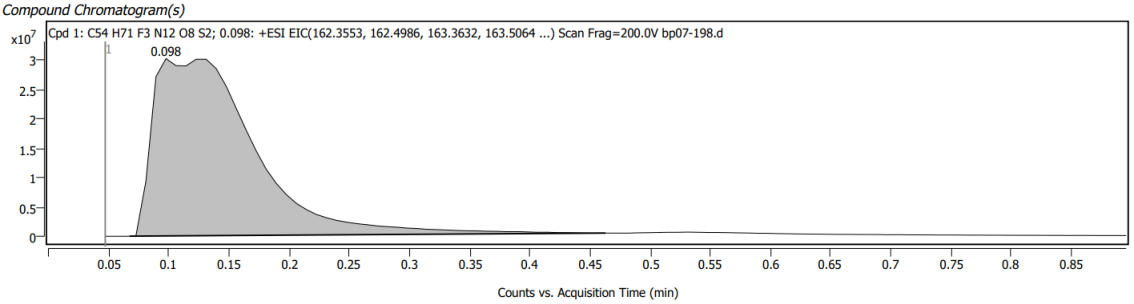
Sample Information

Name	bp07-198	**Data File Path**	D:\DATA\2023\April26\bp07-198.d
Sample ID		**Acq. Time (Local)**	26/04/2023 10:45:11 PM (UTC+10:00)
Instrument	Instrument 1	**Method Path (Acq)**	D:\MassHunter\Methods\Monash_Direct.m
MS Type	TOF	**Version (Acq SW)**	6200 series TOF/6500 series Q-TOF 10.1 (48.0)
Inj. Vol. (ul)	0.2	**IRM Status**	Some ions missed
Position	P1-D4	**Method Path (DA)**	D:\DATA\2023\April26\bp07-198.d\AcqData\MethodDA\Monash_Accuracy.m
Plate Pos.		**Target Source Path**	C54H71F3N12O8S2
Operator	Dr Jason Dang	**Result Summary**	1 qualified (1 targets)

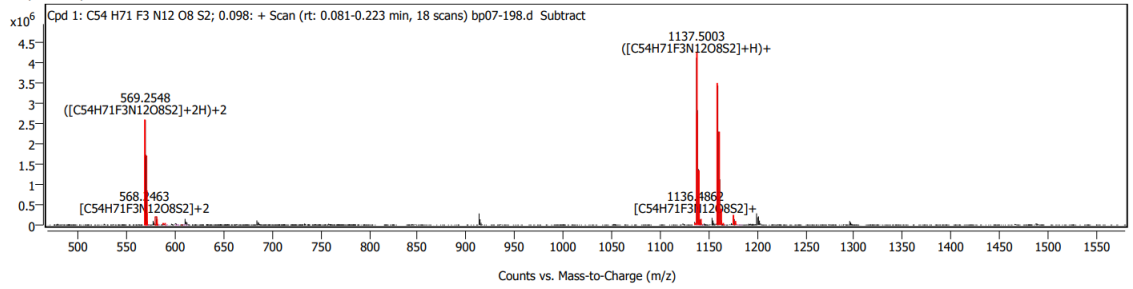
Compound Summary

Formula	RT	Mass	Area	Algorithm	Score	Diff (Tgt, ppm)
C54 H71 F3 N12 O8 S2	0.098	1136.4937	177980078	FBF	97.69	2.29

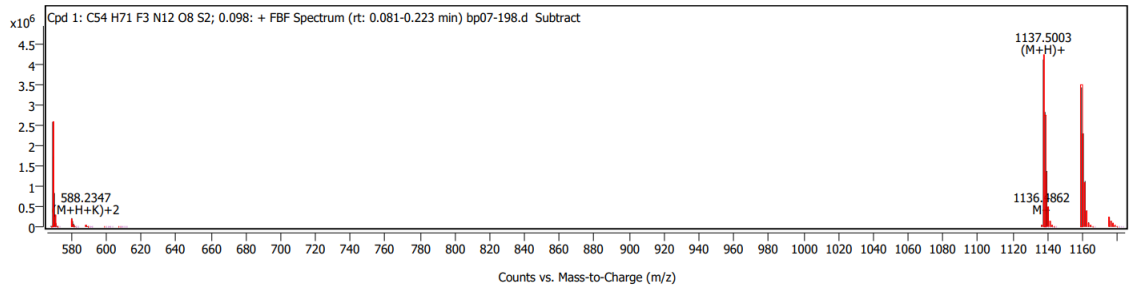
Compound Chromatogram(s)



Compound Spectra



Compound Spectra



Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
1137.5003	1137.4984	1.64	4124054	100.00	100.00	(M+H)+	1
1138.5032	1138.5013	1.65	2831444	68.66	65.50	(M+H)+	1
1159.4821	1159.4804	1.54	3439468	100.00	100.00	(M+Na)+	1

Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
1137.5003	1137.4984	1.64	4124054	100.00	100.00	(M+H)+	1
1138.5032	1138.5013	1.65	2831444	68.66	65.50	(M+H)+	1
1159.4821	1159.4804	1.54	3439468	100.00	100.00	(M+Na)+	1

3 References

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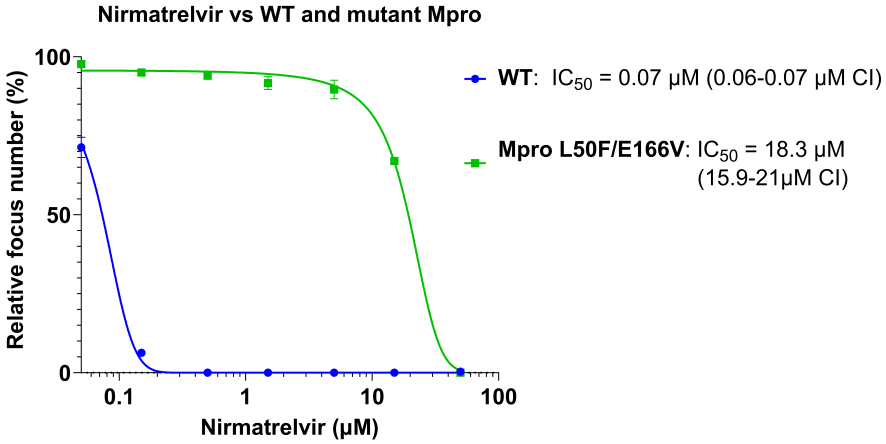

Supplementary Figure 3

Focus reduction assays in VeroE6/TMPRSS2 cells infected with SARS-CoV-2 Delta variant harbouring either wildtype Mpro or Mpro possessing the nirmatrelvir-resistant mutations E166V + L50F and treated with increasing doses of nirmatrelvir. Data is presented as the number of foci per dose of drug as a % of the DMSO control ± S.E.M. The IC_{50} dose response for each drug was calculated ± 95% CI.