Supplemental Information

PROTAC Linkerology Leads to an Optimized Bivalent Chemical Degrader of Polycomb Repressive Complex 2 (PRC2) Components

Frances M. Potjewyd[1], Caroline A. Foley[1], Han-Wee Ong[1], Justin M. Rectenwald[1], Ronan P. Hanley[1], Jacqueline L. Norris-Drouin[1], Stephanie H. Cholensky[1], Christine A. Mills[2], Kenneth H. Pearce[1], Laura E. Herring[2], Dmitri Kireev[1], Stephen V. Frye[1], Lindsey I. James[1,3 *].

1. Center for Integrative Chemical Biology and Drug Discovery, Division of Chemical Biology and Medicinal Chemistry, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.
2. UNC Proteomics Core Facility, Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.
3. Lineberger Comprehensive Cancer Center, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.

[*]Correspondence: ingerman@email.unc.edu

Chemistry

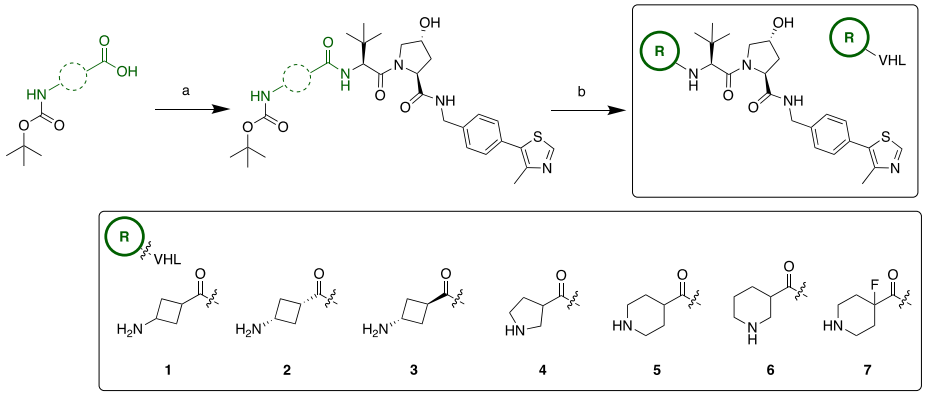
1.1. General Chemistry Procedures

All reagents and solvents were obtained from commercial suppliers and were used without further purification unless otherwise stated. Reactions were carried out using conventional glassware and room temperature was generally 22 °C. Reactions were carried out at elevated temperatures using a temperature regulated hot plater-stirrer. Thin layer chromatography (TLC) was carried out using Merck silica plates coated with fluorescent indicator UV254. TLC plates were analysed under 254 nm UV light. Analytical LCMS data for all compounds was acquired using an Agilent 6110 Series system with the UV detector set to 254 nm. Samples were injected (<10 µL) onto an Agilent Eclipse Plus 4.6 × 50 mm, 1.8 µm, C18 column at room temperature. Mobile phases A (H_2O + 0.1% acetic acid) and B (MeCN + 0.1% acetic acid) were used with a linear gradient from 10% to 100% B in 5.0 min, followed by a flush at 100% B for another 2 minutes with a flow rate of 1.0 mL/min. Mass spectra (MS) data were acquired in positive ion mode using an Agilent 6110 single quadrupole mass spectrometer with an electrospray ionization (ESI) source. Normal phase column chromatography was performed with a Teledyne Isco CombiFlash®R_f 200 using RediSep®R_f SILICA columns with the UV detector set to 254 nm and 280 nm. Reverse phase column chromatography was performed with a Teledyne Isco CombiFlash®R_f 200 using C18 RediSep®R_f Gold columns with the UV detector set to 220 nm and 254 nm. Preparative HPLC was performed using an Agilent Prep 1200 series with the UV detector set to 220 nm and 254 nm. Samples were injected onto either a Phenomenex Luna 250 × 30 mm (5 µm) C18 column or a Phenomenex Luna 75 x 30 mm (5 µm) C18 column at room temperature. Analytical LCMS (at 254 nm) was used to establish the purity of targeted compounds. All compounds that were evaluated in biochemical and biophysical assays had >95% purity as determined by LCMS.

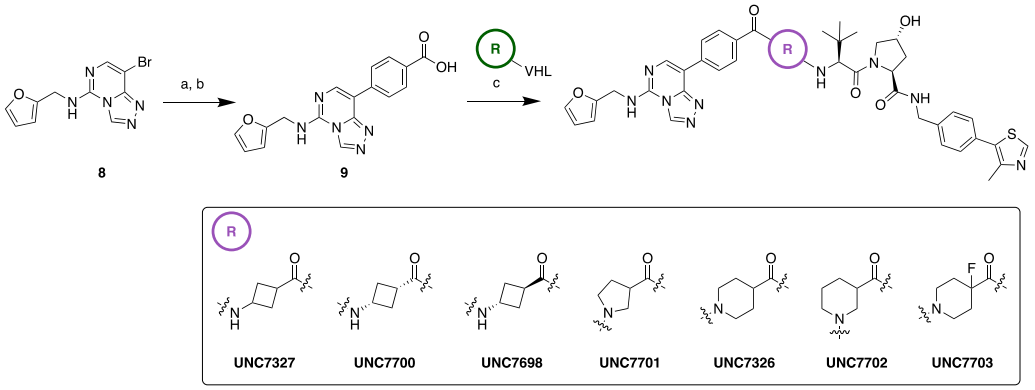
1.2. Analysis of products

^1H and ^{13}C NMR spectra were obtained on a Bruker AV 400 at 400 MHz, 101 MHz respectively. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J* values) are reported in hertz (Hz) with $CDCl_3$ referenced at 7.26 (^1H) and 77.1 ppm (^{13}C), DMSO-d_6 referenced at 2.50 (^1H) and 39.5 ppm (^{13}C), acetone-d_6 referenced at 2.05 (^1H) and 29.8 ppm (^{13}C), and CD_3OD-d_4 referenced at 3.31 (^1H) and 49.0 ppm (^{13}C). The multiplicities are included as: singlet (s), doublet (d), doublet of doublets/triplets/quartets (dd/dt/dq), triplet (t), triplet of doublets/triplets (td/tt), quartet (q), quartet of doublets (qd), pentent (p), and multiplet (m).

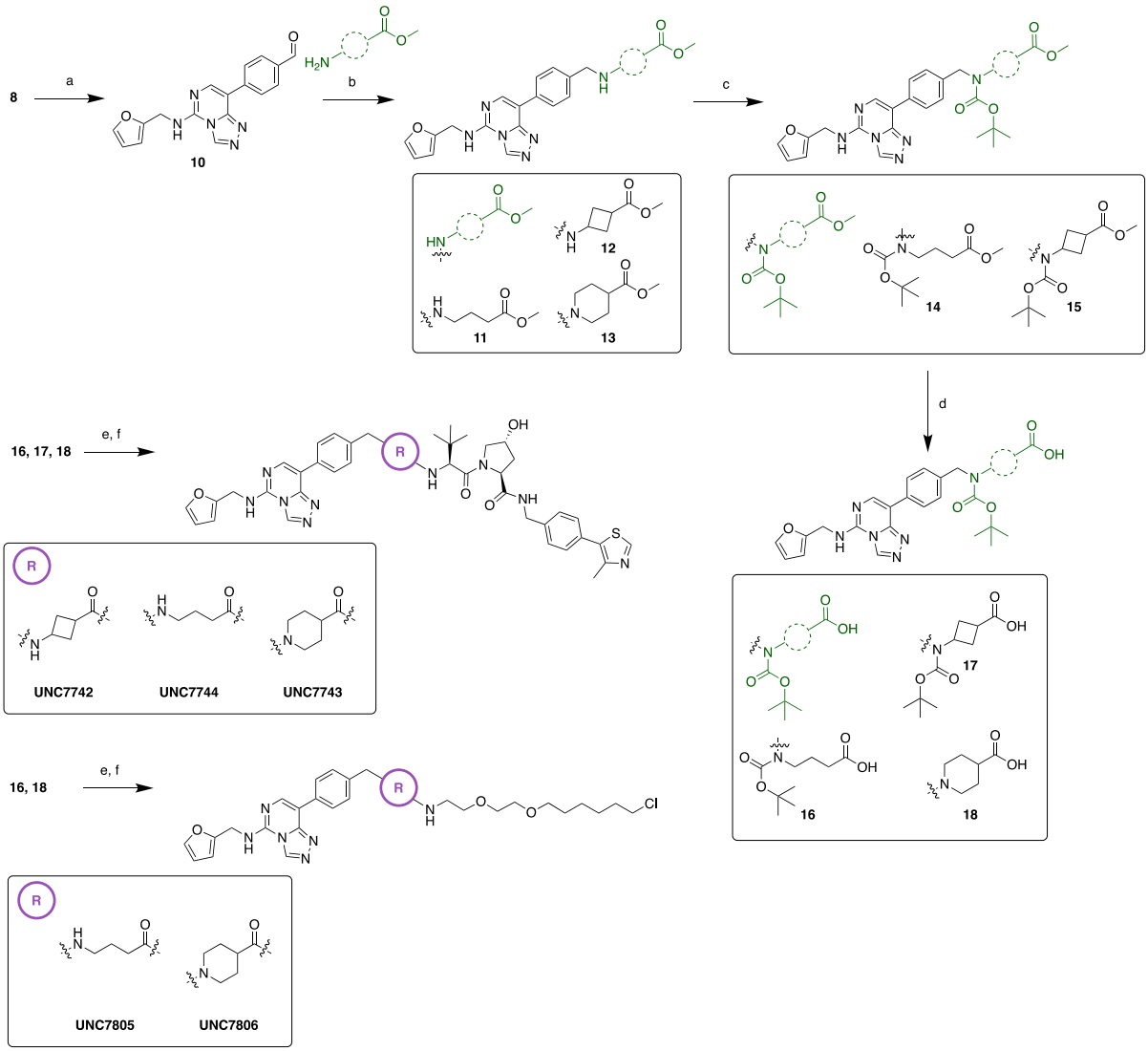
1.3. Synthetic Schemes



Scheme S1. Synthesis of R-VHL building blocks **1** – **7**. a) The corresponding *N*-Boc-R-CO2H precursor (1.0 equiv), TBTU (1.3 equiv), VHL-amine ligand (1.0 equiv), DIPEA (3.2 equiv), DMF, 16 hr; b) 20% TFA in CH_2Cl_2.

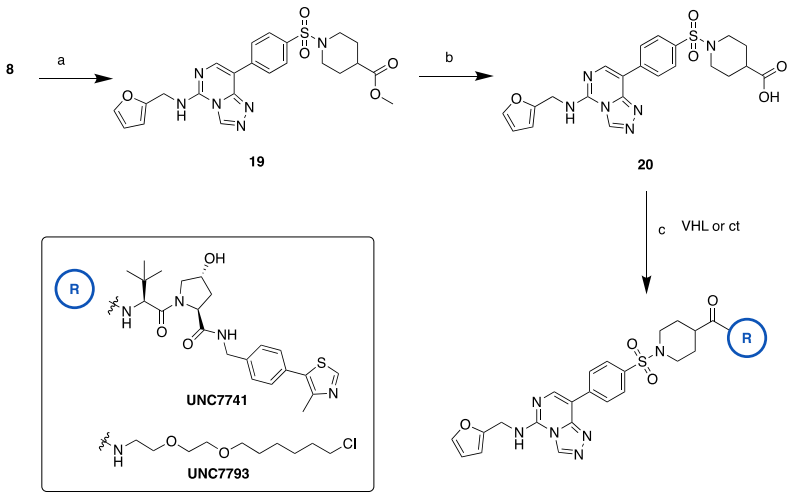


Scheme S2. Synthesis of an amide series of EED-targeted degraders. a) Triazolo[4,3-*c*]pyrimidin-5-amine (**8**) (1.0 equiv), $Pd(dppf)Cl_2.CH_2Cl_2$ (0.2 equiv), methyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (1.5 equiv), and sodium bicarbonate (2.5 equiv), N_2, THF (4 mL), H_2O (2 mL), 110 °C, 18 hr; b) LiOH (3.0 equiv), THF, H_2O, 50 °C, 16 hr; c) **9** (1.0 equiv), **1** – **7** (1.0 equiv), TBTU (1.3 equiv), DIPEA (3.0 equiv), DMF.

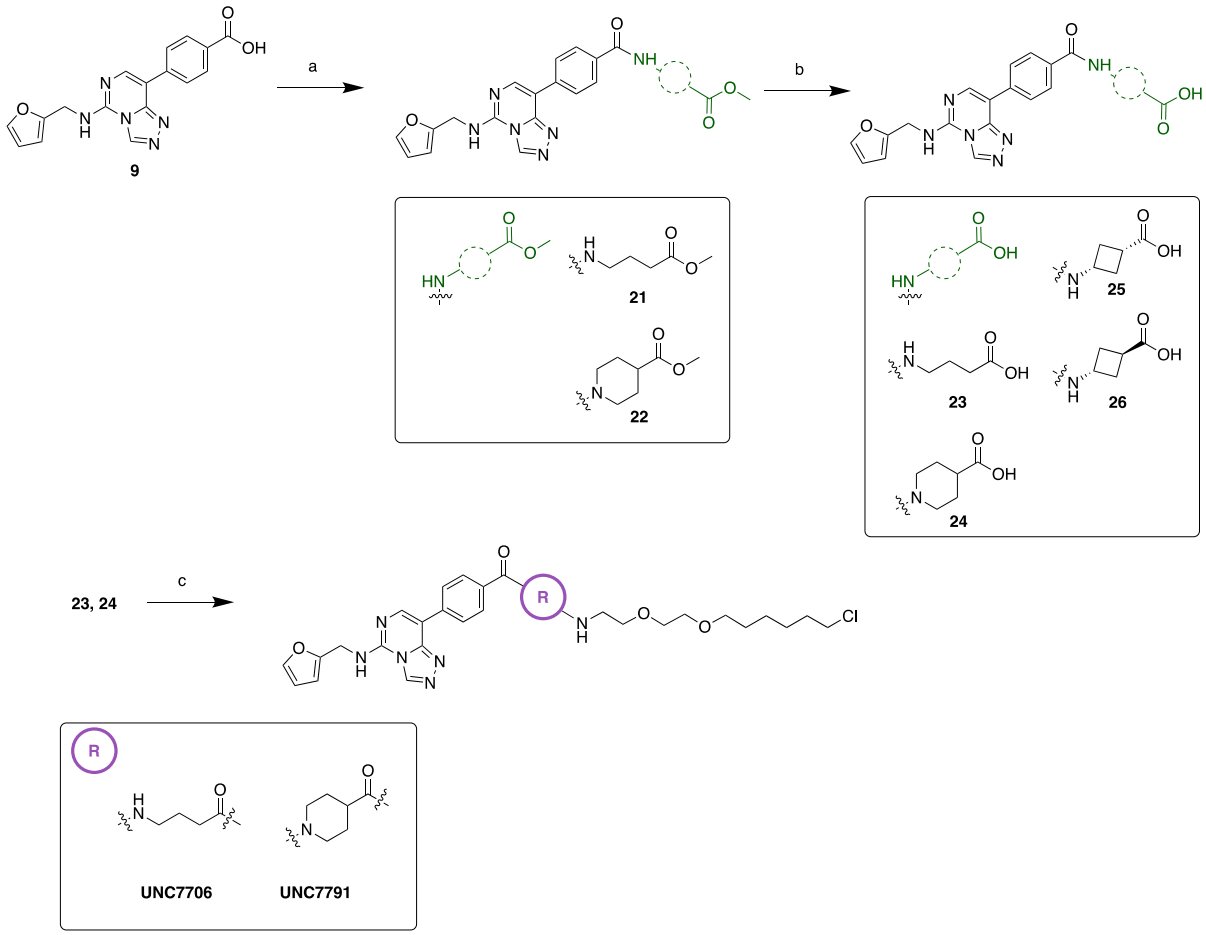


Scheme S3. Synthesis of a primary and secondary amine series of EED-targeted degraders and chloro-tagged compounds. a) To a microwave vial was added 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-

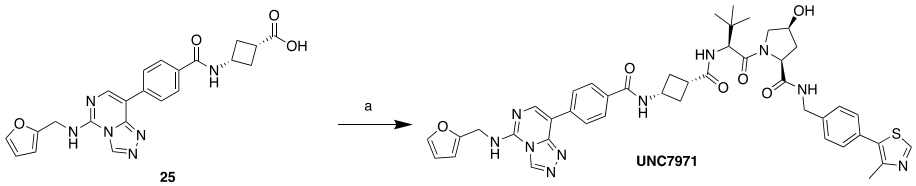
yl)benzaldehyde (1.5 equiv), **8** (1.0 equiv), $Pd(dppf)Cl_2.CH_2Cl_2$ (0.2 equiv), sodium bicarbonate (2.5 equiv), THF and H_2O, 90 °C, 16 hr; b) Amine (1.1 equiv), **10** (1.0 equiv) , sodium sulfate (1.1 equiv) , DIPEA (2.1 equiv), CH_2Cl_2, 40 °C for 1hr. Followed by MeOH, sodium tetrahydroborate (1.2 equiv), rt, 16 hr; c) **11** – **12** (1.0 equiv), CH_2Cl_2, Boc anhydride (1.1 equiv), NEt_3 (2.5 equiv), rt, 16 hr; d) **13** – **15** (1.0 equiv), $LiOH.H_2O$ (3.0 equiv), H_2O, THF, rt, 16 hr; e) **16, 18** (1.0 equiv), DIPEA (3.0 equiv), TBTU (1.3 equiv), DMF, 16 hr; f) 20% TFA in CH_2Cl_2.



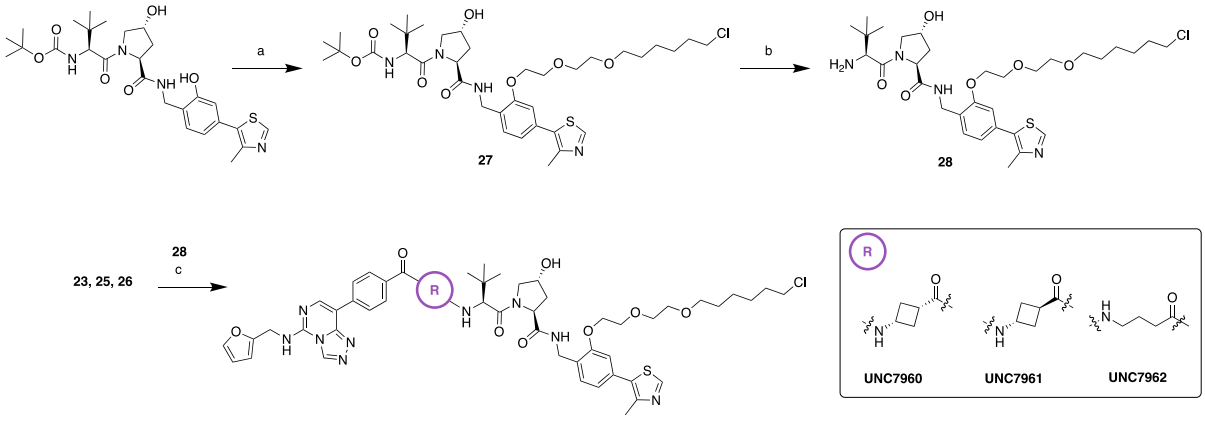
Scheme S4. Synthesis of a sulfonamide series of EED-targeted degraders and ct compounds. a) 1-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperidine-4-carboxylate (1.5 equiv), **8** (1.0 equiv), $Pd(dppf)Cl_2.CH_2Cl_2$ (0.2 equiv), sodium bicarbonate (2.5 equiv), THF, H_2O, 90 °C, 16 hr; b) **19** (1.0 equiv), $LiOH.H_2O$ (3.0 equiv), THF, H_2O, rt, 16 hr; c) **20** (1.0 equiv), DIPEA (3.0 equiv), TBTU (1.3 equiv), DMF, amine (1.1 equiv), rt, 16 hr.



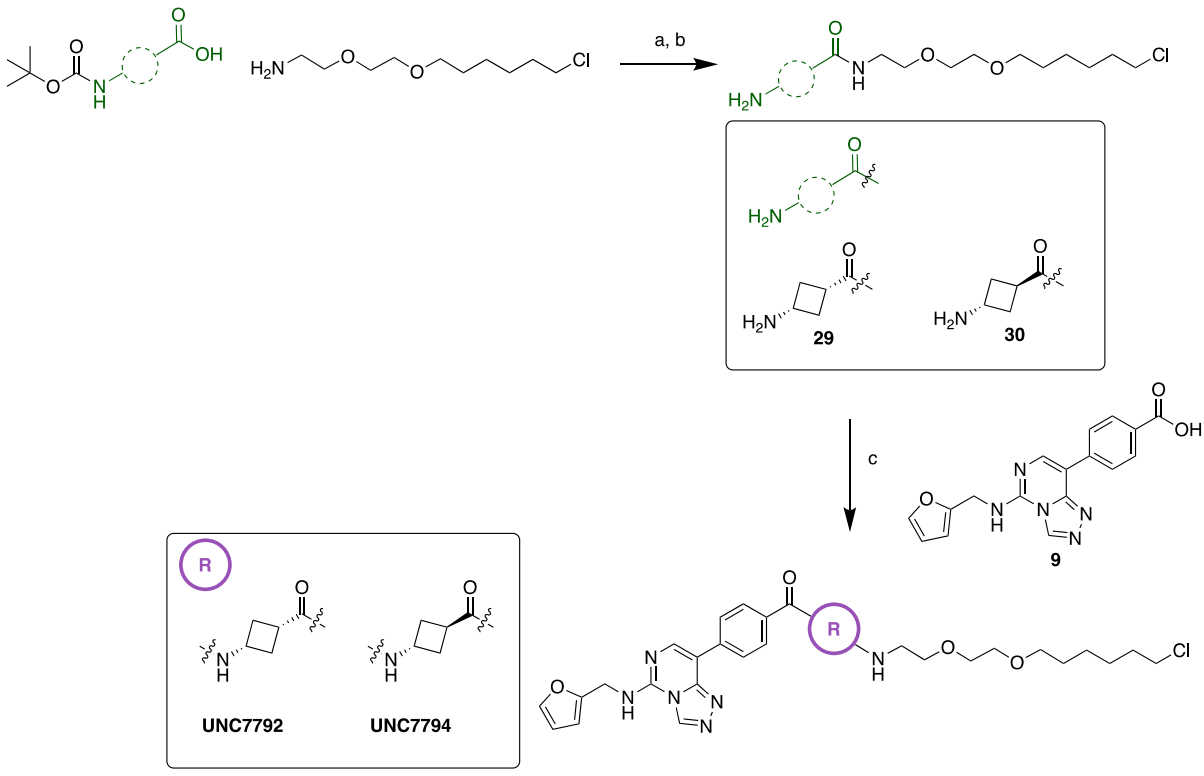
Scheme S5. Synthesis of amide linked EED-R-ct compounds. a) **9** (1.0 equiv), DIPEA (3.0 equiv), TBTU (1.3 equiv), DMF, amine (1.1 equiv), rt, 16 hr; b) **21** – **22** (1.0 equiv), LiOH.H_2O (3.0 equiv), THF, H_2O, rt, 16 hr; c) **23** – **24** (1.0 equiv), DIPEA (3.0 equiv), TBTU (1.3 equiv), DMF, amine (1.1 equiv), rt, 16 hr. **25** – **26** synthesized via a) and b) over two steps.



Scheme S6. Synthesis of a negative control degrader UNC7971. a) **25** (1.1 equiv), DIPEA (3.2 equiv), TBTU (1.3 equiv), DMF, amine (1.0 equiv), rt, 16 hr.



Scheme S7. Synthesis of PROTAC-O-ct compounds for the CAPA assay. a) *tert*-butyl ((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (1.0 equiv), Cs_2CO_3 (1.5 equiv), followed by 2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl 4-methylbenzenesulfonate (1.3 equiv), DMF, rt, 16 hr; b) 20% TFA in CH_2Cl_2; c) **23**, **25**, **26** (1.1 equiv), TBTU (1.30 equiv), DIPEA (3.2 equiv), **28** (1.0 equiv), DMF, rt, 16 hr.



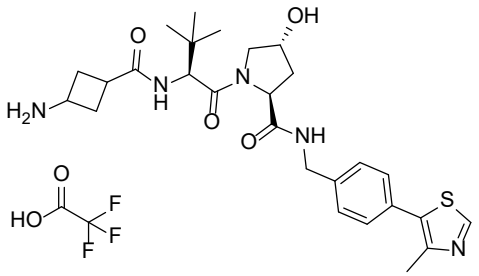
Scheme S8. Synthesis of *cis*- and *trans*- EED-R-ct cyclobutane isomers for the CAPA assay. a) Corresponding carboxylic acid (1.0 equiv), TBTU (1.3 equiv), DIPEA (3.2 equiv), 2-(2-((6-chlorohexyl)oxy)ethoxy)ethan-1-amine 2,2,2-trifluoroacetate (1.0 equiv), DMF, rt, 16 hr; b) 20% TFA in CH_2Cl_2; c) **9** (1.0 equiv), TBTU (1.3 equiv), DIPEA (3.2 equiv), **29** – **30** (1.0 equiv), DMF, rt, 16 hr.

Abbreviations

Trifluoroacetic acid (TFA), *N*,*N*-Diisopropylethylamine (DIPEA), Dimethyl formamide (DMF), Tetrahydrofuran (THF), 2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), trifluoroacetic acid (TFA), chloroalkane tag (ct).

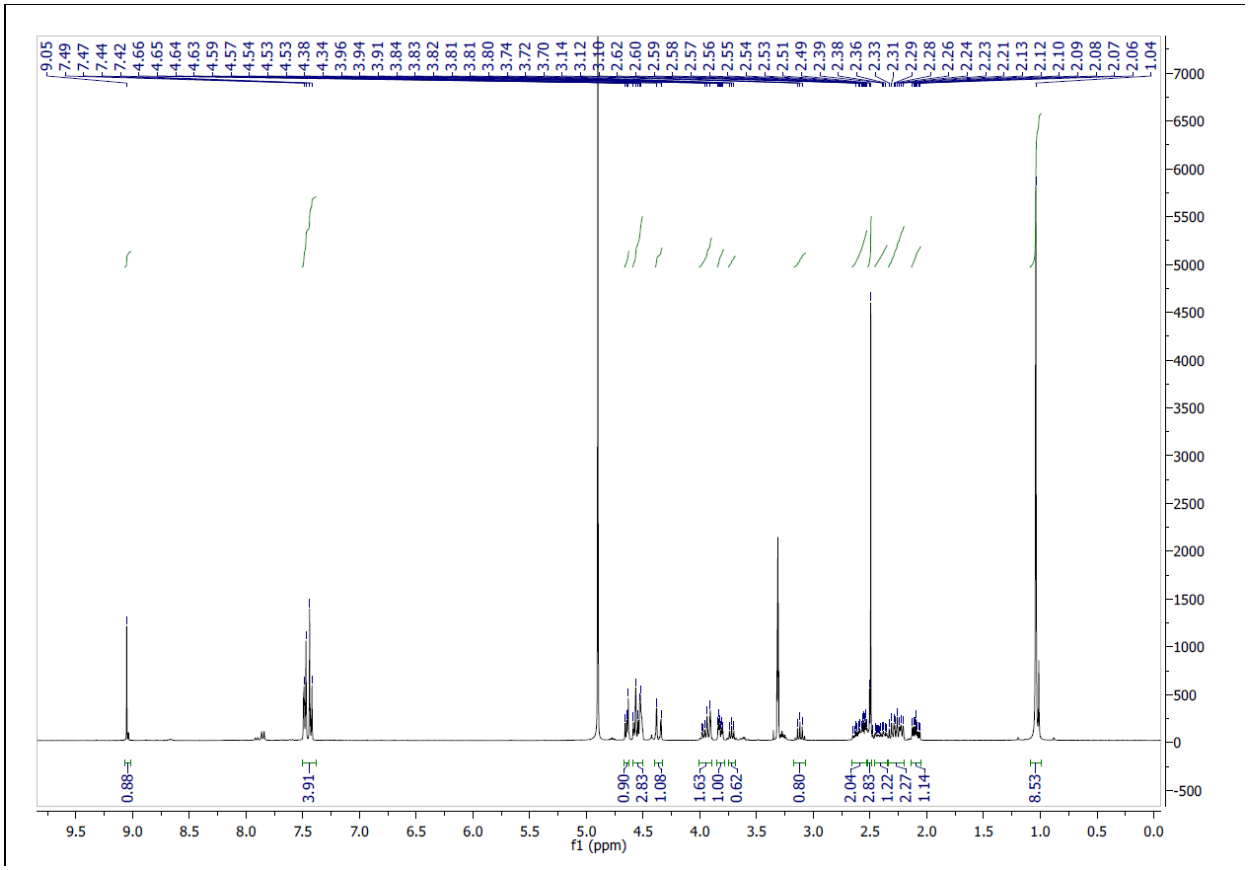
1.4. Chemistry Experimental and Spectra

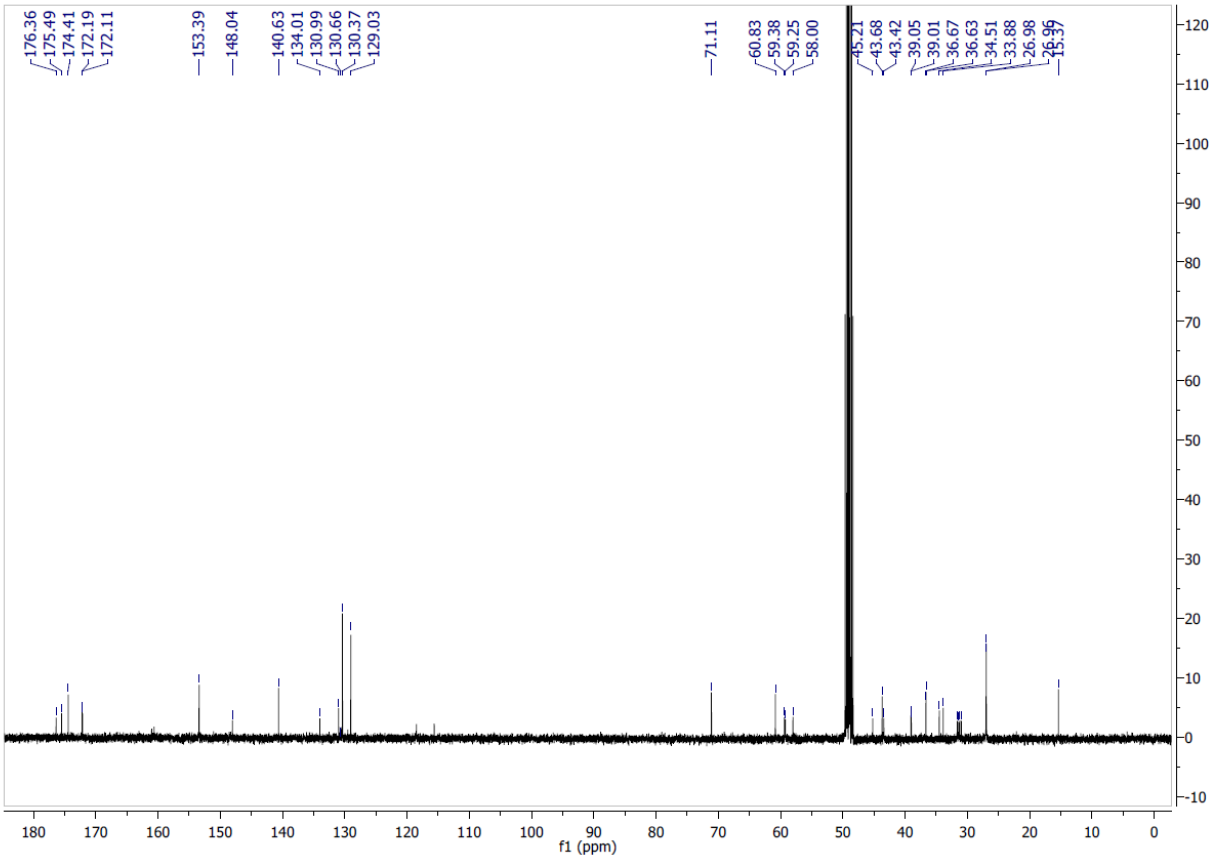
(2*S*,4*R*)-1-((*S*)-2-(3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (1)

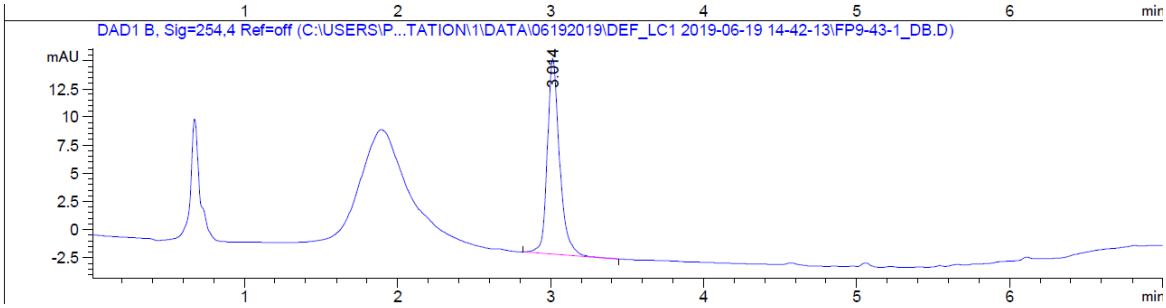


To a flask was added 3-((*tert*-butoxycarbonyl)amino)cyclobutane-1-carboxylic acid (20 mg, 92 µmol, 1.0 equiv), DIPEA (51 µL, 0.29 mmol, 3.2 equiv), and TBTU (38 mg, 0.12 mmol, 1.3 equiv) in DMF (1.0 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (50 mg, 92 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by column chromatography (silica, 0-10% MeOH in CH_2Cl_2). The reaction was telescoped into the *N*-Boc deprotection with 20% TFA in CH_2Cl_2 (2 mL), concentrated *in vacuo* and purified by reverse phase column chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a clear oil (2*S*,4*R*)-1-((*S*)-2-(3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**1**) (34 mg, 58 %) over two steps.

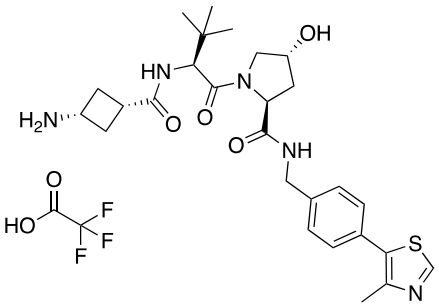
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.05 (s, 1H), 7.50 – 7.41 (m, 4H), 4.67- 4.62 (m, 1H), 4.60 – 4.51 (m, 3H), 4.36 (d, *J* = 15.6 Hz, 1H), 4.00 – 3.90 (m, 2H), 3.82 (dt, *J* = 10.9, 3.4 Hz, 1H), 3.75 – 3.68 (m, 1H), 3.17 – 3.07 (m, 1H), 2.66 – 2.53 (m, 2H), 2.49 (s, 3H), 2.46 – 2.35 (m, 1H), 2.34 – 2.20 (m, 2H), 2.13 – 2.05 (m, 1H), 1.04 (s, 9H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 176.36, 175.49, 174.41, 172.19, 172.11, 153.39, 148.04, 140.63, 134.01, 130.99, 130.37, 129.03, 71.11, 60.83, 59.38, 59.25, 58.00, 45.21, 43.68, 43.42, 39.05, 39.01, 36.67, 36.63, 34.51, 33.88, 31.62, 31.39, 31.22, 30.96, 26.98, 26.96, 15.37. Extra carbon peaks observed due to diasteromeric mixture. LCMS: expected mass for $[M+H]^+$ ($C_{27}H_{38}N_5O_4S$) requires 528.26 m/z, found 528.20 m/z.


9.05
7.49
7.47
7.44
7.42
4.66
4.65
4.64
4.63
4.59
4.57
4.54
4.53
4.53
4.38
4.34
3.96
3.94
3.91
3.84
3.83
3.82
3.81
3.81
3.80
3.74
3.72
3.70
3.14
3.12
3.10
2.62
2.60
2.59
2.58
2.57
2.56
2.55
2.54
2.53
2.51
2.49
2.39
2.38
2.36
2.33
2.31
2.29
2.28
2.26
2.24
2.23
2.21
2.13
2.12
2.10
2.09
2.08
2.07
2.06
1.04
0.88
3.91
0.90
2.83
1.08
1.63
1.00
0.62
0.80
2.04
2.83
1.22
2.27
1.14
8.53
f1 (ppm)


176.36
175.49
174.41
172.19
172.11
153.39
148.04
140.63
134.01
130.99
130.66
130.37
129.03
71.11
60.83
59.38
59.25
58.00
45.21
43.68
43.42
39.05
39.01
36.67
36.63
34.51
33.88
26.98
26.96
15.37
f1 (ppm)

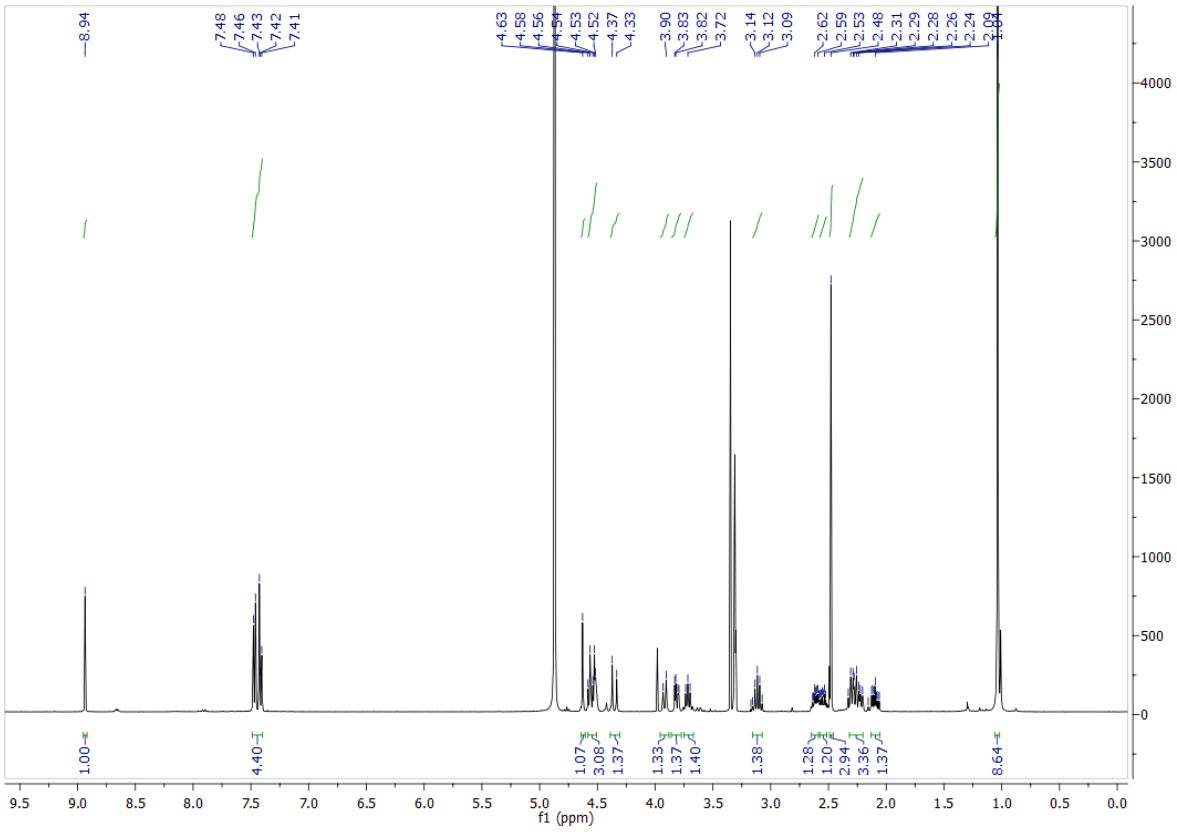


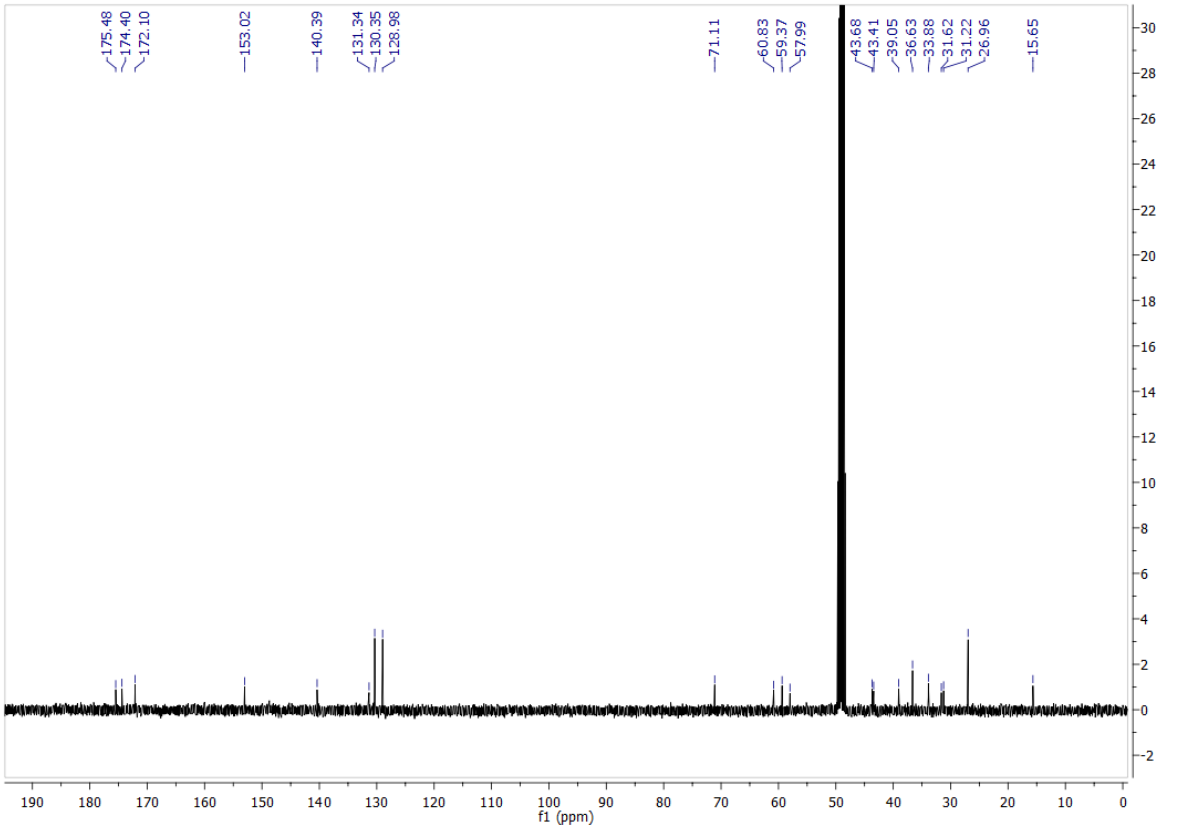
(2*S*,4*R*)-1-((*S*)-2-((1*S*,3*R*)-3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (2)

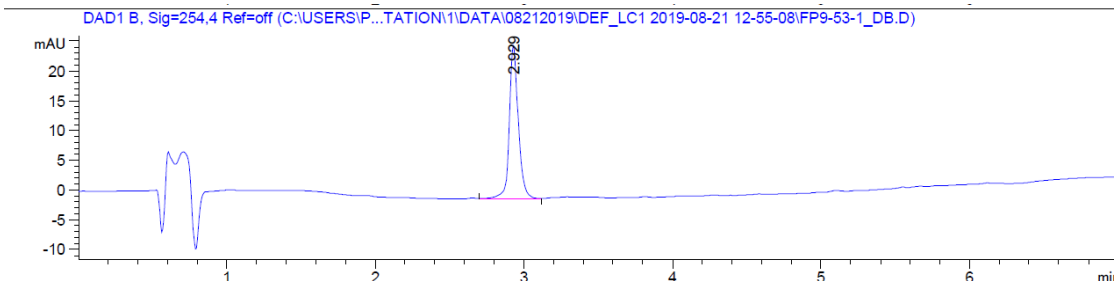


To a flask was added (1*S*,3*S*)-3-((*tert*-butoxycarbonyl)amino)cyclobutane-1-carboxylic acid (9.9 mg, 46 µmol, 1.0 equiv), DIPEA (26 µL, 0.15 mmol, 3.2 equiv), and TBTU (19 mg, 60 µmol, 1.3 equiv) in DMF (1.0 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (25 mg, 46 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by column chromatography (silica, 0-10% MeOH in CH_2Cl_2). The reaction was telescoped into the *N*-Boc deprotection with 20% TFA in CH_2Cl_2 (2 mL), concentrated *in vacuo* and purified by reverse phase column chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a clear oil (2*S*,4*R*)-1-((*S*)-2-((1*S*,3*R*)-3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**2**) (13 mg, 43 %) over two steps.

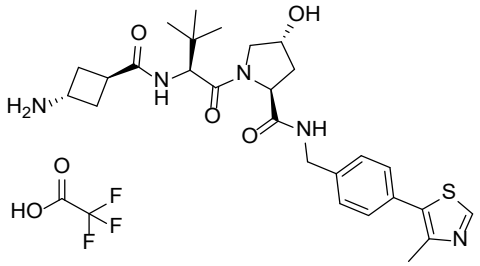
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 8.94 (s, 1H), 7.49 – 7.40 (m, 4H), 4.63 (s, 1H), 4.59 – 4.50 (m, 3H), 4.35 (d, *J* = 15.53 Hz, 1H), 3.92 (d, *J* = 11.04 Hz, 1H), 3.81 (dd, *J* = 3.85, 10.95 Hz, 1H), 3.76 – 3.66 (m, 1H), 3.16 – 3.07 (m, 1H), 2.65 – 2.58 (m, 1H), 2.57 – 2.52 (m, 1H), 2.48 (s, 3H), 2.33 – 2.20 (m, 3H), 2.09 (ddd, *J* = 4.42, 9.21, 13.33 Hz, 1H), 1.04 (s, 9H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 175.49, 174.41, 172.11, 153.03, 140.40, 131.35, 130.36, 128.99, 71.12, 60.84, 59.37, 58.00, 43.69, 43.41, 39.05, 36.64, 33.88, 31.62, 31.22, 26.96, 15.65. LCMS: expected mass for $[M+H]^+$ ($C_{27}H_{38}N_5O_4S$) requires 528.26 m/z, found 528.20 m/z.


8.94
7.48
7.46
7.43
7.42
7.41
4.63
4.58
4.56
4.54
4.53
4.52
4.37
4.33
3.90
3.83
3.82
3.72
3.14
3.12
3.09
2.62
2.59
2.53
2.48
2.31
2.29
2.28
2.26
2.24
2.09
1.84
1.00
4.40
1.07
3.08
1.37
1.33
1.37
1.40
1.38
1.28
1.20
2.94
3.36
1.37
8.64
f1 (ppm)


175.48
174.40
172.10
153.02
140.39
131.34
130.35
128.98
71.11
60.83
59.37
57.99
43.68
43.41
39.05
36.63
33.88
31.62
31.22
26.96
15.65
f1 (ppm)

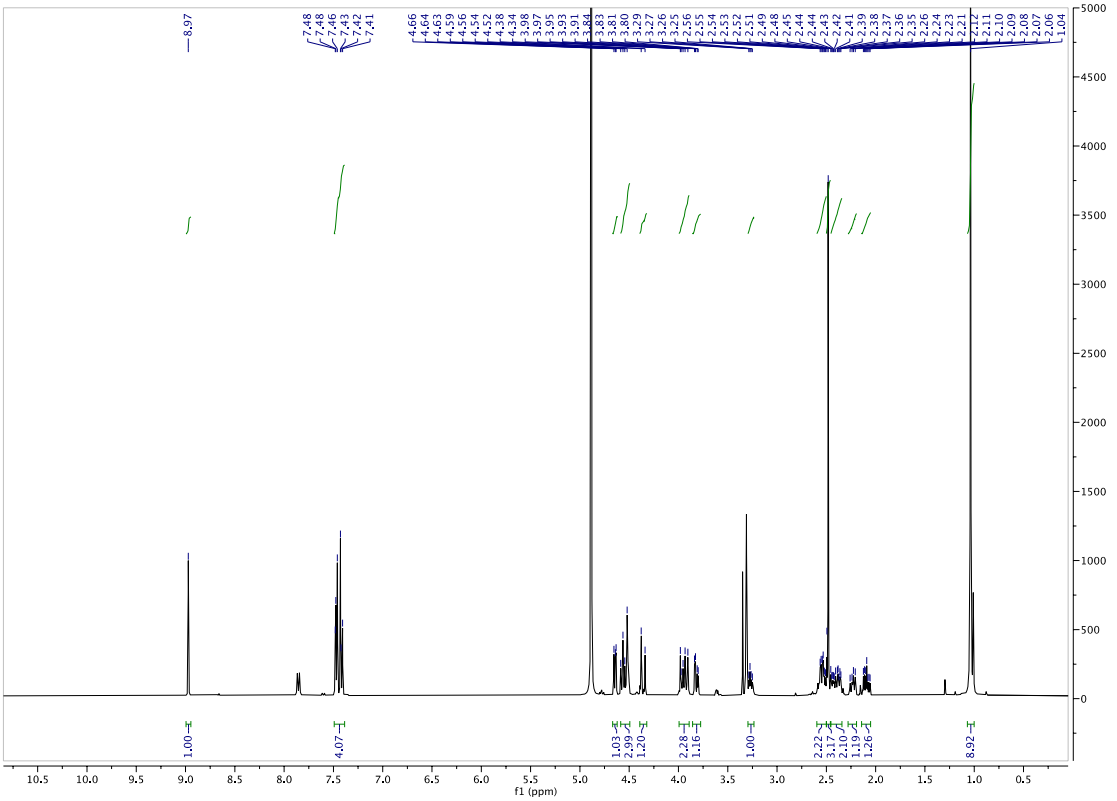


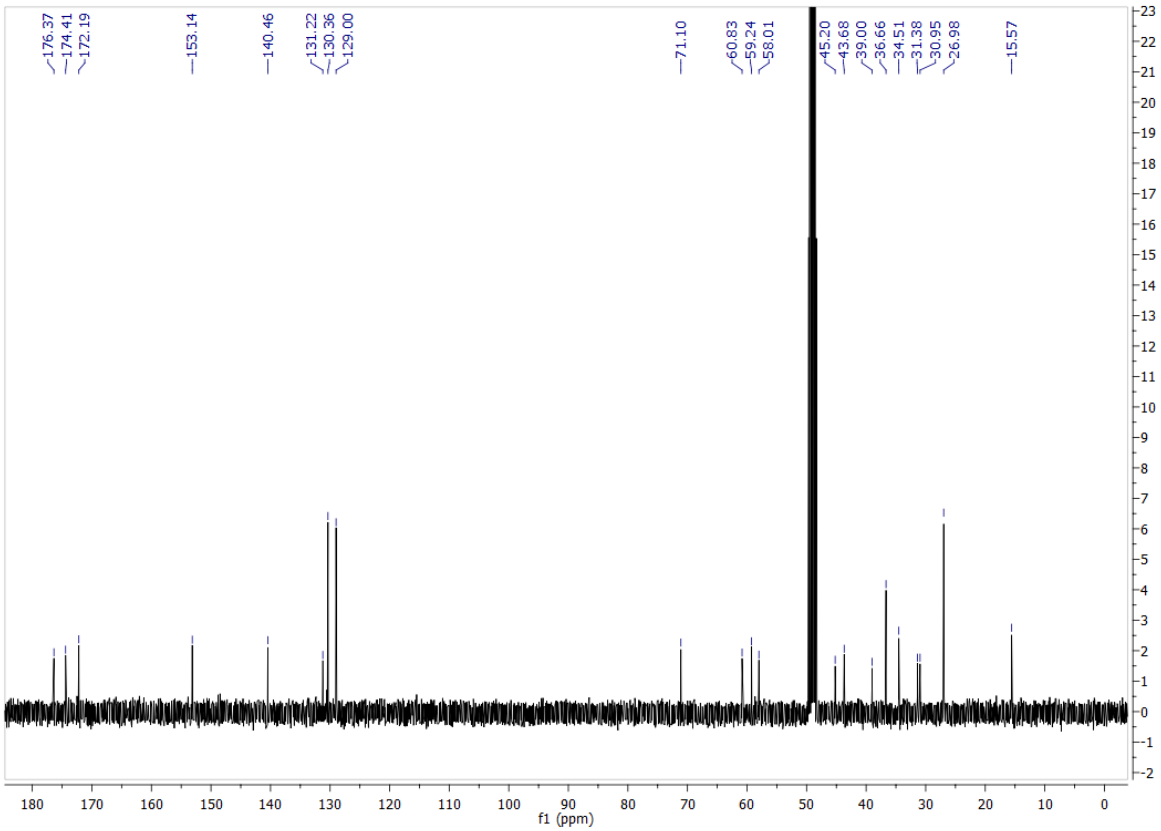
(2*S*,4*R*)-1-((*S*)-2-((1*R*,3*S*)-3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (3)

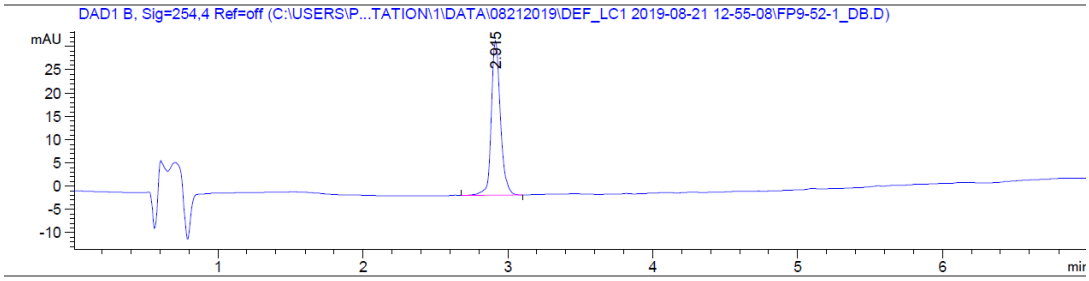


To a flask was added (1*R*,3*R*)-3-((*tert*-butoxycarbonyl)amino)cyclobutane-1-carboxylic acid (9.9 mg, 46 µmol, 1.0 equiv), DIPEA (26 µL, 0.15 mmol, 3.2 equiv), and TBTU (19 mg, 60 µmol, 1.3 equiv) in DMF (1.0 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (25 mg, 46 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by column chromatography (silica, 0-10% MeOH in CH_2Cl_2). The reaction was telescoped into the *N*-Boc deprotection with 20% TFA in CH_2Cl_2 (2 mL), concentrated *in vacuo* and purified by reverse phase column chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a clear oil (2*S*,4*R*)-1-((*S*)-2-((1*R*,3*S*)-3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**3**) (18 mg, 61 %) over two steps.

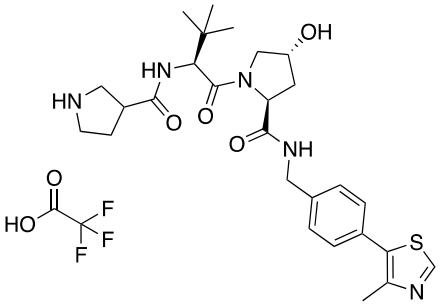
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 8.97 (s, 1H), 7.49 – 7.39 (m, 4H), 4.64 – 4.62 (m, 1H), 4.60 – 4.49 (m, 3H), 4.36 (d, *J* = 15.51 Hz, 1H), 3.98 – 3.90 (m, 2H), 3.82 (dd, *J* = 3.86, 10.96 Hz, 1H), 3.29 – 3.23 (m, 1H), 2.54 – 2.51 (m, 2H), 2.48 (s, 3H), 2.46 – 2.35 (m, 2H), 2.23 (dd, *J* = 7.65, 13.15 Hz, 1H), 2.12 – 2.06 (m, 1H), 1.04 (s, 9H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 176.37, 174.41, 172.19, 153.14, 140.46, 131.22, 130.36, 129.00, 71.10, 60.83, 59.24, 58.01, 45.20, 43.68, 39.00, 36.66, 34.51, 31.38, 30.95, 26.98, 15.57. LCMS: expected mass for $[M+H]^+$ ($C_{27}H_{38}N_5O_4S$) requires 528.26 m/z, found 528.20 m/z.


8.97
7.48
7.46
7.43
7.42
7.41
4.66
4.64
4.63
4.59
4.56
4.54
4.52
4.38
4.34
3.98
3.97
3.95
3.93
3.91
3.84
3.83
3.81
3.80
3.29
3.27
3.26
3.25
2.56
2.55
2.54
2.53
2.52
2.51
2.49
2.48
2.45
2.44
2.43
2.42
2.41
2.39
2.38
2.37
2.36
2.35
2.26
2.24
2.23
2.21
2.12
2.11
2.10
2.09
2.08
2.07
2.06
1.04
1.00
4.07
1.03
2.99
1.20
2.28
1.16
1.00
2.22
2.17
2.10
1.19
1.26
8.92
f1 (ppm)


176.37
174.41
172.19
153.14
140.46
131.22
130.36
129.00
71.10
60.83
59.24
58.01
45.20
43.68
39.00
36.66
34.51
31.38
30.95
26.98
15.57
f1 (ppm)

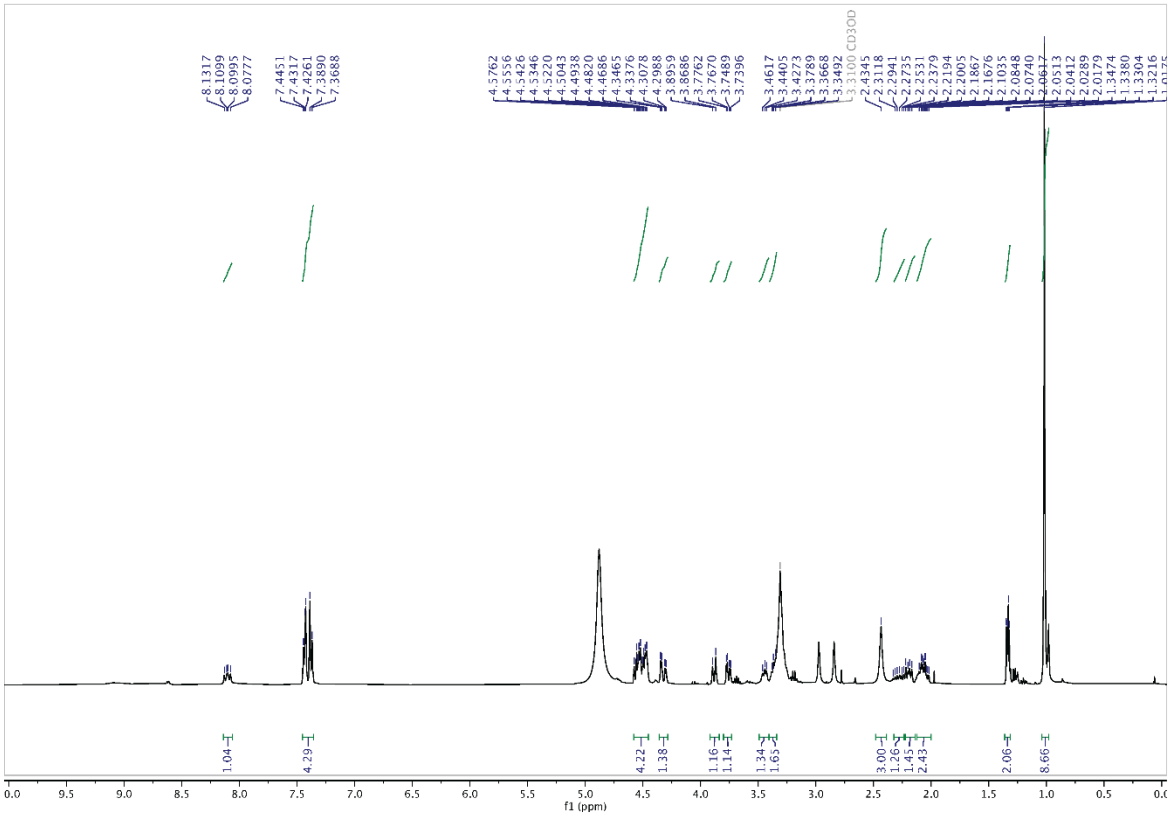


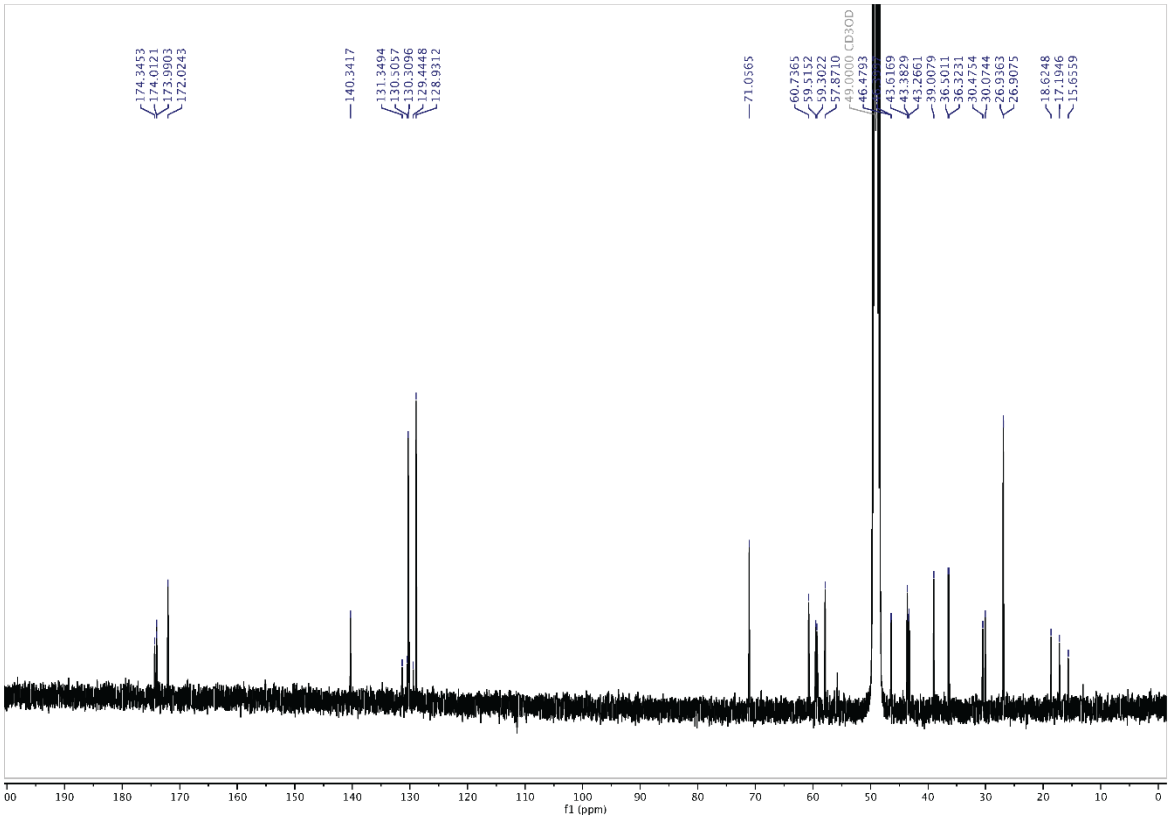
(2*S*,4*R*)-1-((2*S*)-3,3-dimethyl-2-(pyrrolidine-3-carboxamido)butanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate, (4)

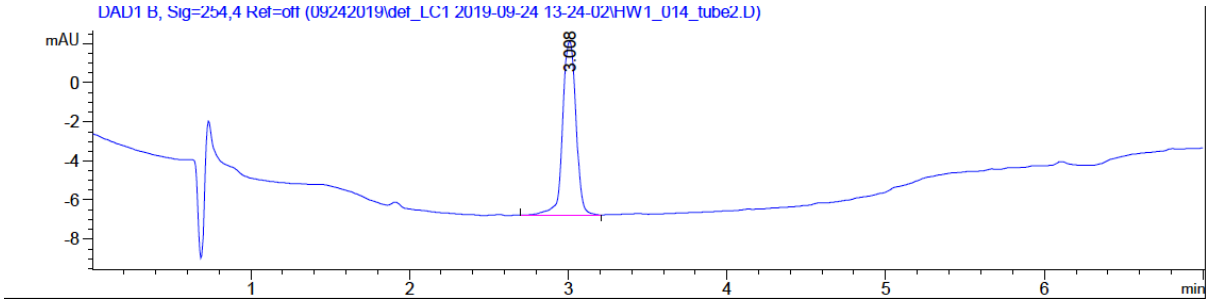


To a flask was added 1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid (20.7 mg, 96 µmol, 2.0 equiv), DIPEA (24 µL, 0.14 mmol, 2.9 equiv), TBTU (24 mg, 74 µmol, 1.6 equiv) in DMF (0.55 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (26 mg, 47 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by column chromatography (silica, 0-10% MeOH in CH_2Cl_2). The reaction was telescoped into the *N*-Boc deprotection with 20% TFA in CH_2Cl_2 (2 mL), concentrated *in vacuo* and purified by reverse phase column chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a clear oil (2*S*,4*R*)-1-((2*S*)-3,3-dimethyl-2-(pyrrolidine-3-carboxamido)butanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**4**) (12.1 mg, 40.3%) over two steps.

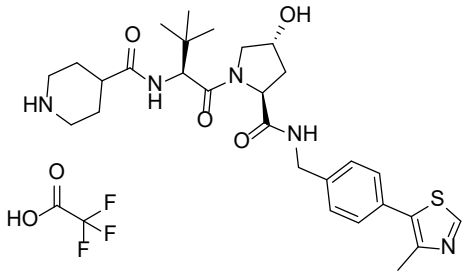
^{1}H NMR (CD_3OD-d_4, 400 MHz) δ 8.10 (dd, *J* = 12.9, 8.7 Hz, 1H), 7.47 – 7.35 (m, 4H), 4.60 – 4.43 (m, 4H), 4.32 (dd, *J* = 15.5, 3.6 Hz, 1H), 3.88 (d, *J* = 10.9 Hz, 1H), 3.76 (dd, *J* = 10.9, 3.7 Hz, 1H), 3.50 – 3.40 (m, 1H), 3.40 – 3.33 (m, 2H), 2.43 (s, 3H), 2.35 – 2.22 (m, 1H), 2.24 – 2/15 (m, 1H), 2.14 – 2.00 (m, 2H), 1.36 – 1.32 (m, 2H), 1.02 (s, 9H). ^{13}C NMR (CD_3OD-d_4, 101 MHz) δ 174.35, 174.01, 173.99, 172.02, 140.34, 131.35, 130.51, 130.31, 129.44, 128.93, 71.06, 60.74, 59.52, 59.30, 57.87, 46.48, 46.40, 43.62, 43.38, 43.27, 39.01, 36.50, 36.32, 30.48, 30.07, 26.94, 26.91, 18.62, 17.19, 15.66. Extra carbon peaks observed due to diasteromeric mixture. LCMS: expected mass for $[M+H]^+$ ($C_{27}H_{38}N_5O_4S$) requires 528.26 m/z, found 528.20 m/z.


8.1317
8.1099
8.0995
8.0777
7.4451
7.4317
7.4261
7.3890
7.3688
4.5762
4.5536
4.5426
4.5246
4.5220
4.5043
4.4938
4.4820
4.4686
4.3465
4.3376
4.3078
4.2988
3.8959
3.8686
3.7762
3.7670
3.7489
3.7396
3.4617
3.4405
3.4273
3.3789
3.3668
3.3492
3.3100 CD3OD
2.4345
2.3118
2.2941
2.2735
2.2531
2.2379
2.2194
2.2005
2.1867
2.1676
2.1035
2.0848
2.0740
2.0513
2.0412
2.0289
2.0179
1.3474
1.3380
1.3304
1.3216
1.04
4.29
4.22
1.38
1.16
1.14
1.34
1.65
3.00
1.26
1.45
2.43
2.06
8.66
f1 (ppm)


174.3453
174.0121
173.9903
172.0243
140.3417
131.3494
130.6057
130.3096
129.4448
128.9312
71.0565
60.7365
59.5152
59.3022
57.8710
49.0000 CD3OD
46.4793
43.6169
43.3829
43.2661
39.0079
36.5011
36.3231
30.4754
30.0744
26.9363
26.9075
18.6248
17.1946
15.6559
f1 (ppm)

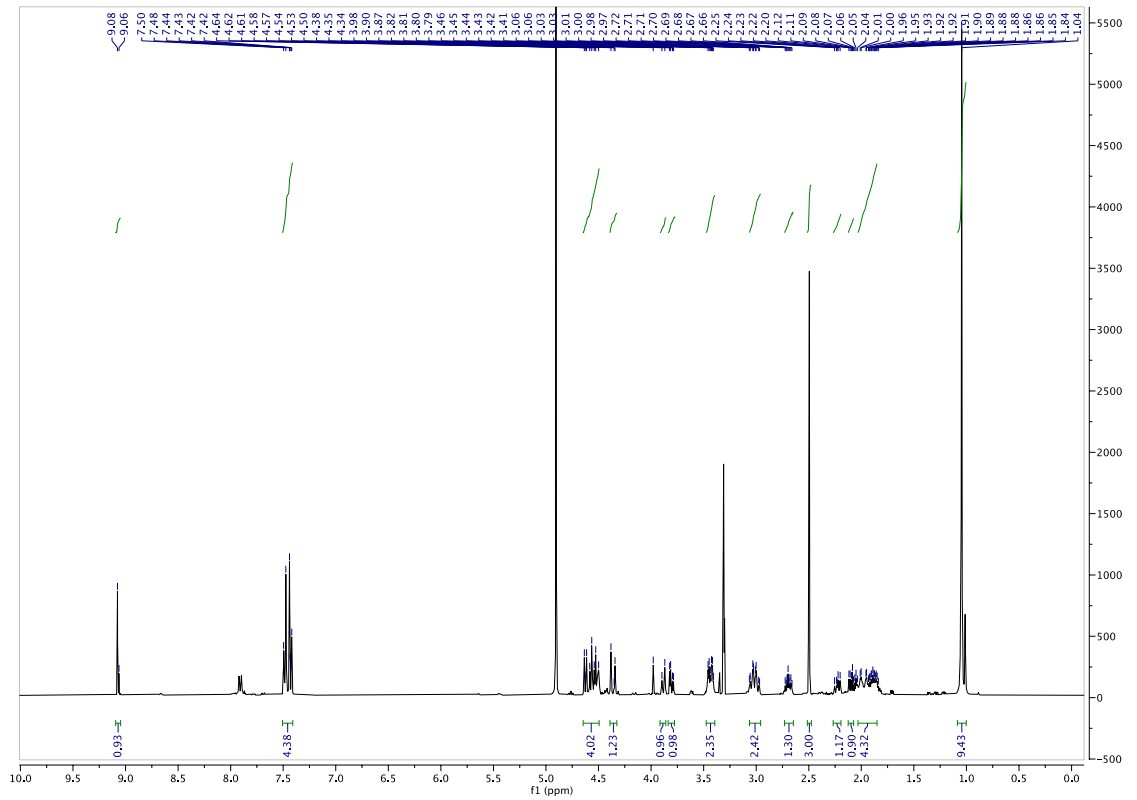


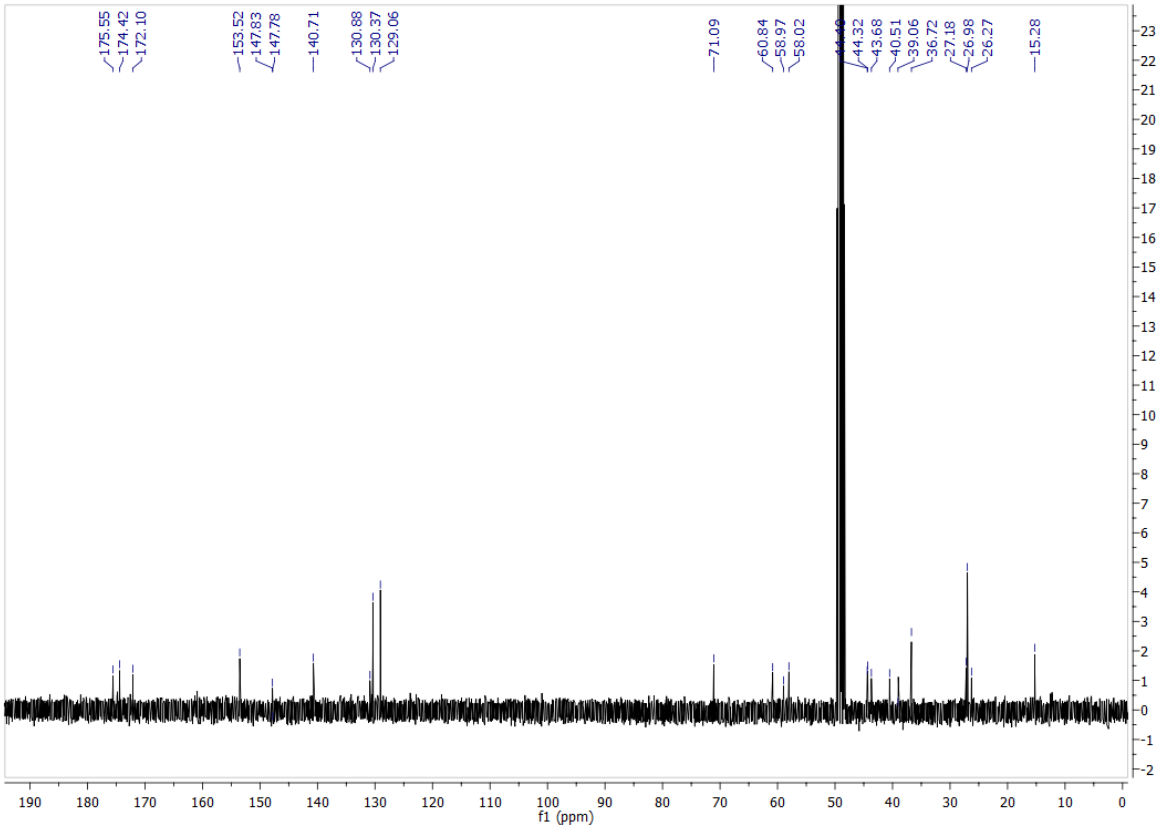
***N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide 2,2,2-trifluoroacetate (5)**

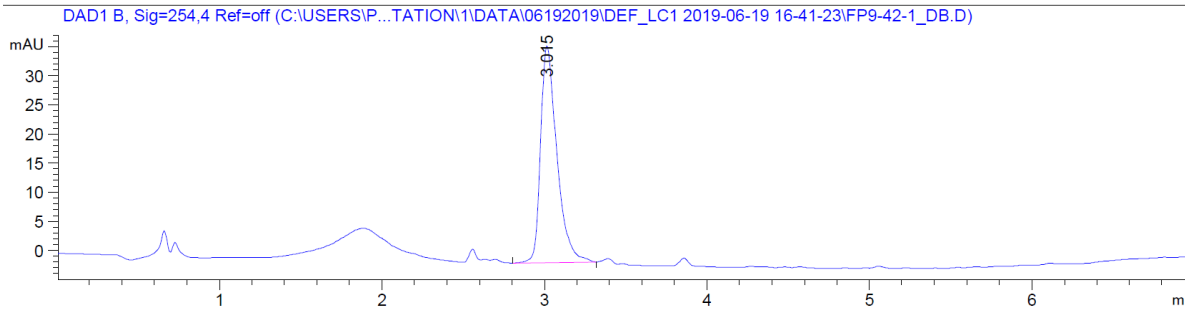


To a flask was added 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (21 mg, 92 µmol, 1.0 equiv), DIPEA (51 µL, 0.29 mmol, 3.2 equiv), and TBTU (38 mg, 0.12 mmol, 1.3 equiv) in DMF (1.0 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (50 mg, 92 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by column chromatography (silica, 0-10% MeOH in CH_2Cl_2). The reaction was telescoped into the *N*-Boc deprotection with 20% TFA in CH_2Cl_2 (2 mL), concentrated *in vacuo* and purified by reverse phase column chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a clear oil *N*-((S)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide 2,2,2-trifluoroacetate (**5**) (35 mg, 58 %) over two steps.

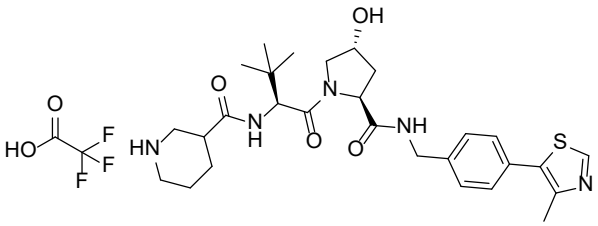
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.08 (s, 1H), 7.51 – 7.41 (m, 4H), 4.64 – 4.49 (m, 4H), 4.36 (d, *J* = 15.53 Hz, 1H), 3.88 (d, *J* = 11.08 Hz, 1H), 3.81 (dd, *J* = 3.82, 10.97 Hz, 1H), 3.47 – 3.40 (m, 2H), 3.02 (qd, *J* = 12.9, 3.3 Hz, 2H), 2.74 – 2.65 (m, 1H), 2.50 (s, 3H), 2.27 – 2.19 (m, 1H), 2.13 – 2.04 (m, 1H), 2.03 – 1.83 (m, 4H), 1.04 (s, 9H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 175.55, 174.42, 172.10, 153.52, 147.83, 147.78, 140.71, 130.88, 130.37, 129.06, 71.09, 60.84, 58.97, 58.02, 44.40, 44.32, 43.68, 40.51, 39.06, 36.72, 27.18, 26.98, 26.27, 15.28. LCMS: expected mass for $[M+H]^+$ ($C_{28}H_{40}N_5O_4S$) requires 542.27 m/z, found 542.20 m/z.


f1 (ppm)


f1 (ppm)

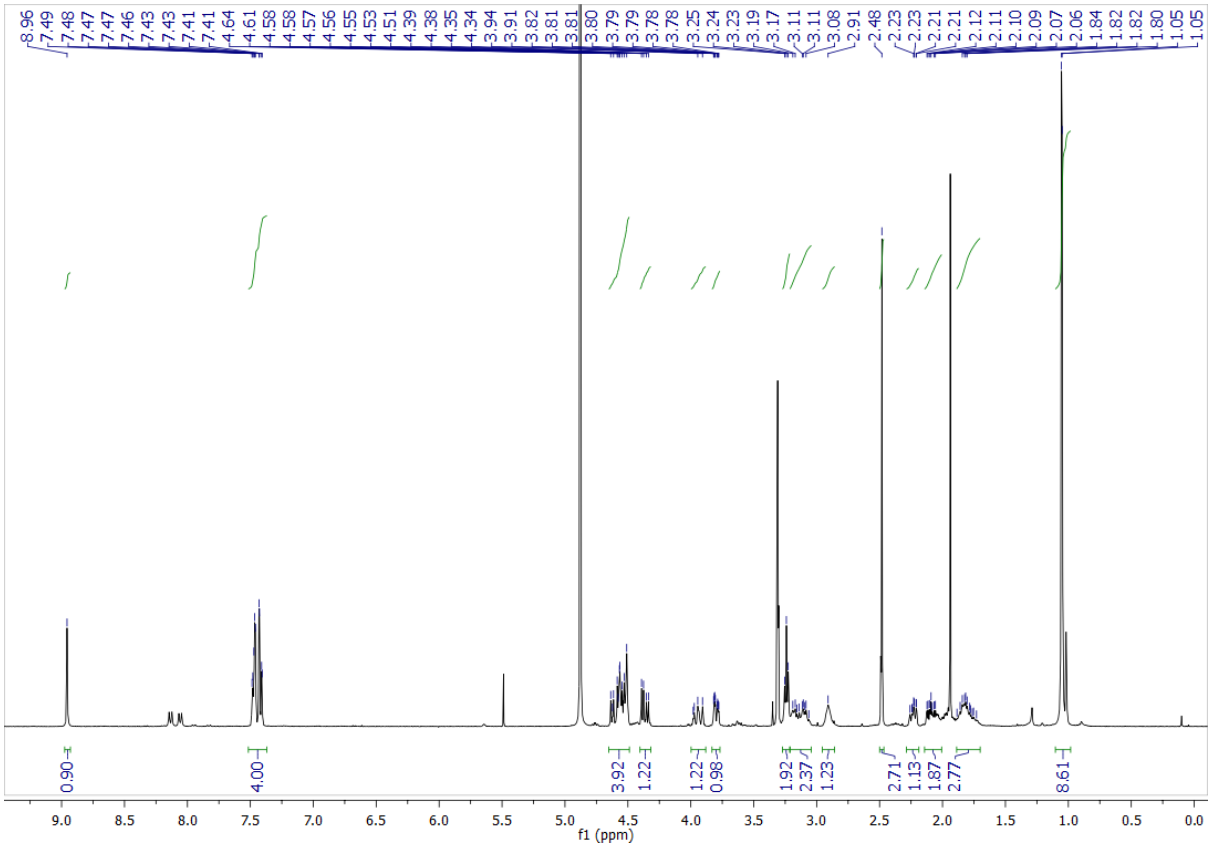


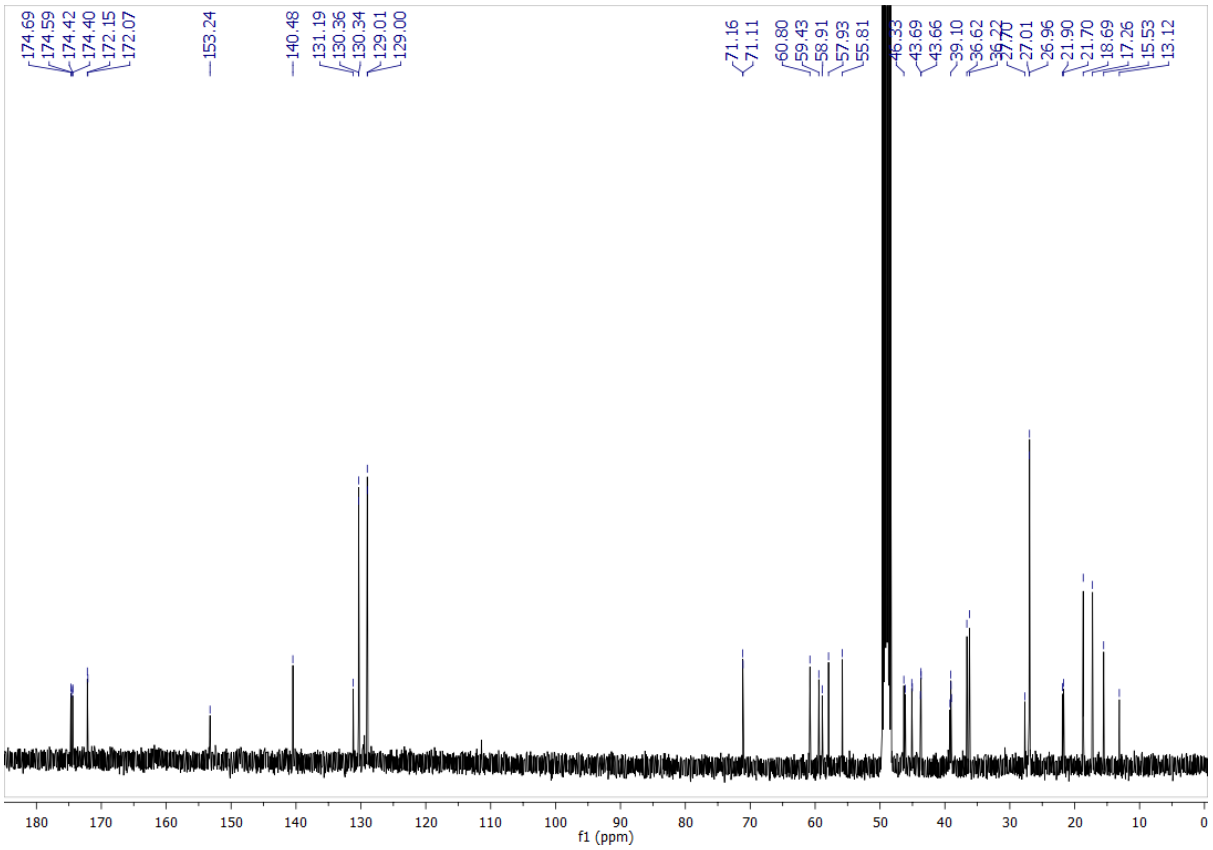
_N_-((_S_)-1-((2_S_,4_R_)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-3-carboxamide 2,2,2-trifluoroacetate (6)

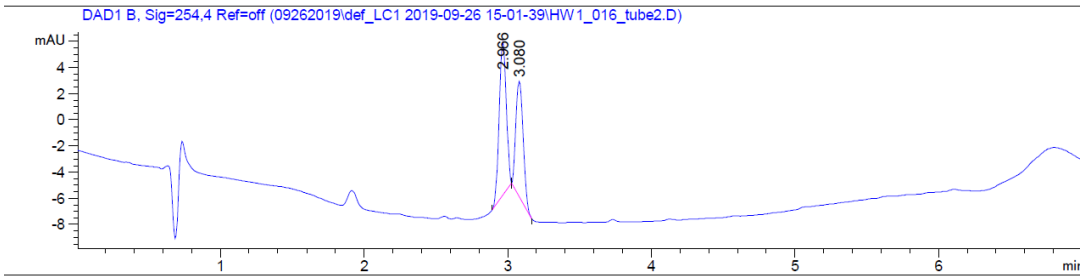


To a flask was added 1-(*tert*-butoxycarbonyl)piperidine-3-carboxylic acid (13.8 mg, 60 µmol, 1.0 equiv), DIPEA (24 µL, 0.14 mmol, 2.9 equiv), and TBTU (21.5 mg, 67 µmol, 1.42 equiv) in DMF (0.55 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (25.6 mg, 47 µmol, 1.0 equiv). The reaction was stirred at room temperature for 21 hr, concentrated *in vacuo* and purified by column chromatography (silica, 0-10% MeOH in CH_2Cl_2). The reaction was telescoped into the *N*-Boc deprotection with 20% TFA in CH_2Cl_2 (2 mL), concentrated *in vacuo* and purified by reverse phase column chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a clear oil *N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-3-carboxamide 2,2,2-trifluoroacetate (**6**) (14.2 mg, 46 %) over two steps.

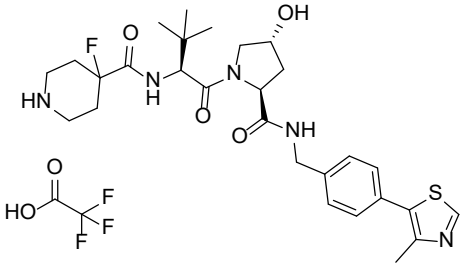
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 8.96 (s, 1H), 7.51 – 7.39 (m, 4H), 4.64 – 4.49 (m, 4H), 4.36 (dd, *J* = 6.51, 15.52 Hz, 1H), 3.99 – 3.90 (m, 1H), 3.24 (t, *J* = 5.69 Hz, 2H), 3.20 – 3.06 (m, 2H), 3.13 – 3.08 (m, 1H), 2.95 – 2.86 (m, 1H), 2.48 (s, 3H), 2.27 – 2.20 (m, 1H), 2.13 – 2.05 (m, 2H), 1.88 – 1.70 (m, 3H), 1.05 (app d, 9H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 174.69, 174.59, 174.42, 174.40, 172.15, 172.07, 153.24, 140.48, 131.19, 130.36, 130.34, 129.01, 129.00, 71.16, 71.11, 60.80, 59.43, 58.91, 57.93, 55.81, 46.33, 46.10, 45.11, 45.02, 43.76, 43.69, 43.66, 39.22, 39.10, 39.06, 39.00, 36.62, 36.22, 27.70, 27.01, 26.96, 21.90, 21.70, 18.69, 17.26, 15.53, 13.12. Extra carbon peaks observed due to diasteromeric mixture. LCMS: expected mass for $[M+H]^+$ ($C_{28}H_{40}N_5O_4S$) requires 542.27 m/z, found 542.20 m/z. Diasteroisomer A Retention time: 2.966 min; Diasteroisomer B Retention time: 3.080 min.


8.96
7.49
7.48
7.47
7.47
7.46
7.43
7.43
7.41
7.41
4.64
4.61
4.58
4.58
4.57
4.56
4.55
4.53
4.51
4.39
4.38
4.35
4.34
3.94
3.91
3.82
3.81
3.81
3.80
3.79
3.79
3.78
3.78
3.25
3.24
3.23
3.19
3.17
3.11
3.11
3.08
2.91
2.48
2.23
2.23
2.21
2.21
2.12
2.11
2.10
2.09
2.07
2.06
1.84
1.82
1.82
1.80
1.05
1.05
0.90
4.00
3.92
1.22
1.22
0.98
1.92
2.37
1.23
2.71
1.13
1.87
2.77
8.61
f1 (ppm)


174.69
174.59
174.42
174.40
172.15
172.07
153.24
140.48
131.19
130.36
130.34
129.01
129.00
71.16
71.11
60.80
59.43
58.91
57.93
55.81
46.33
43.69
43.66
39.10
36.62
36.22
27.70
27.01
26.96
21.90
21.70
18.69
17.26
15.53
13.12
f1 (ppm)

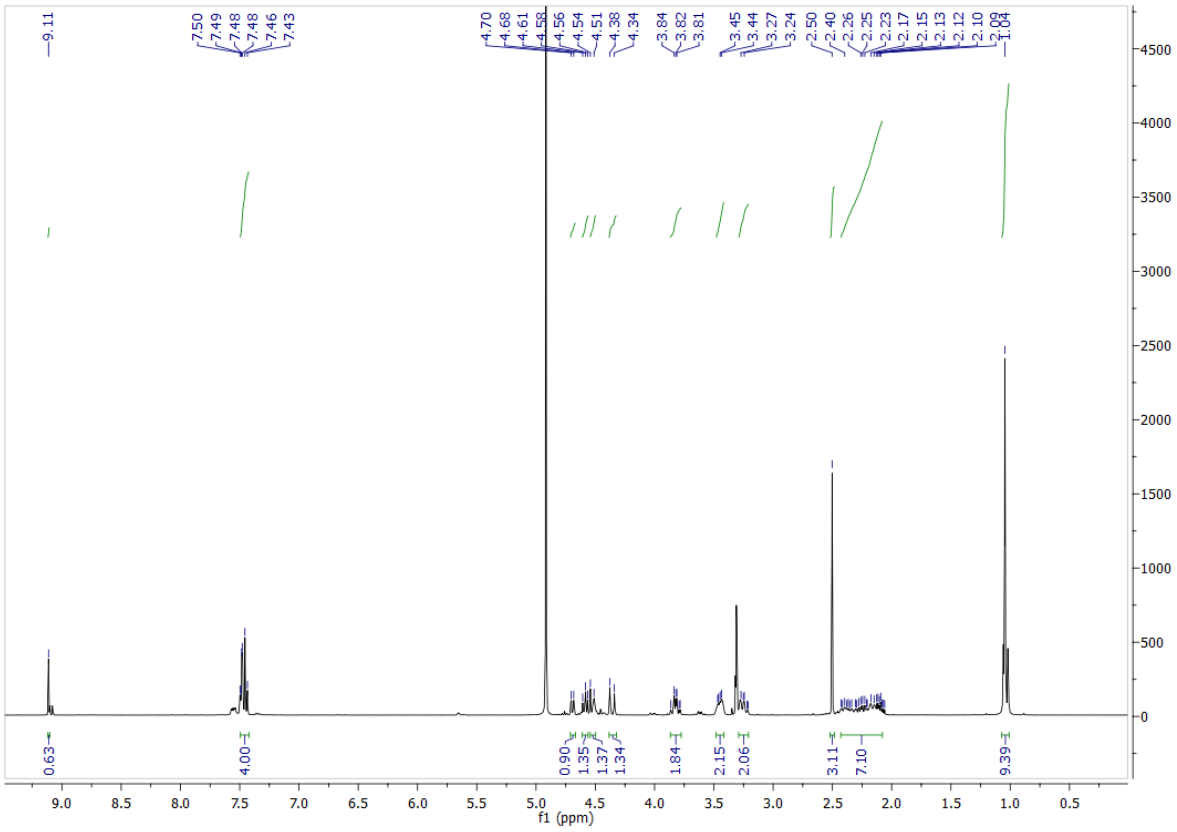


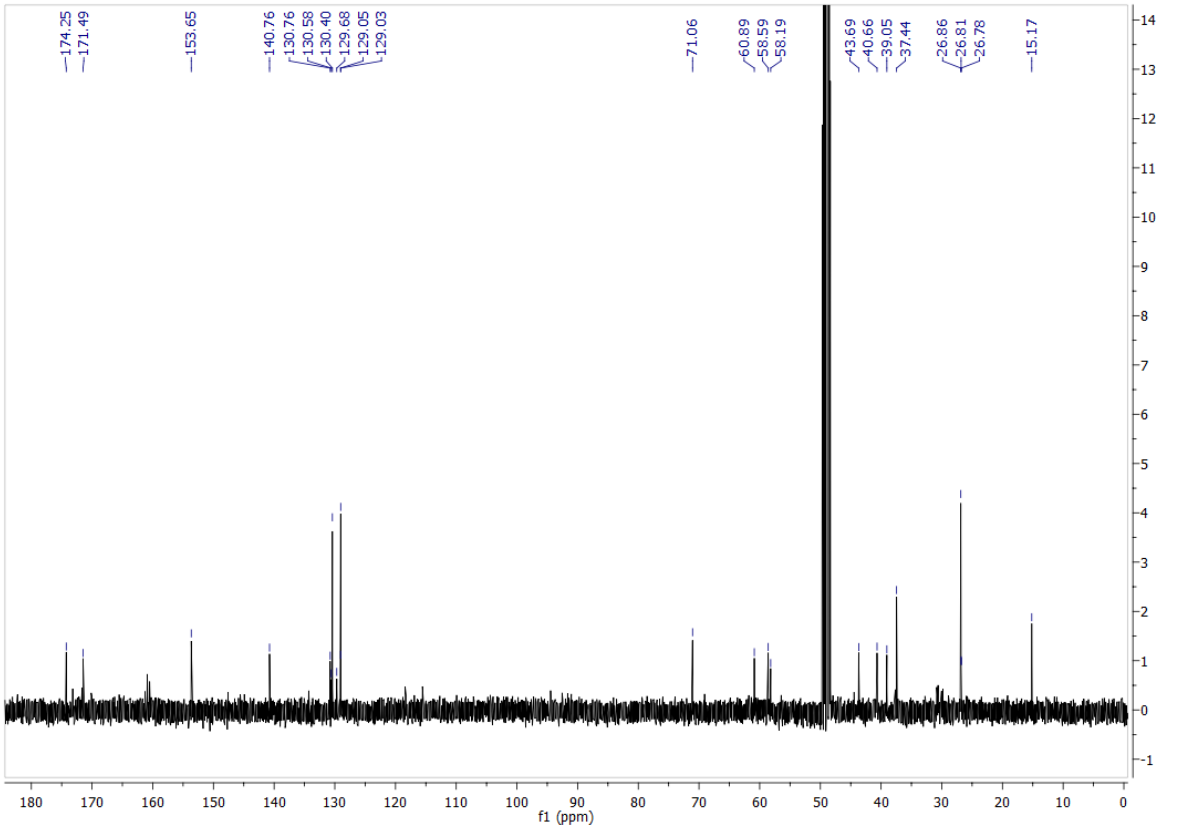
4-fluoro-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide 2,2,2-trifluoroacetate (7)

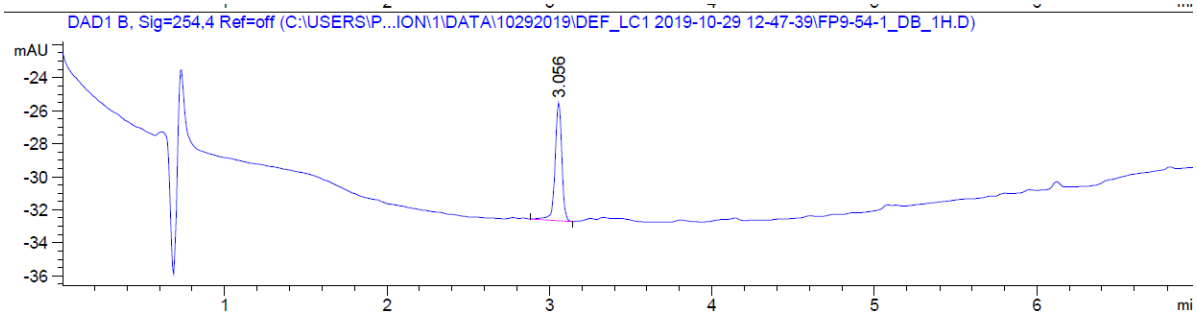


To a flask was added 1-(*tert*-butoxycarbonyl)-4-fluoropiperidine-4-carboxylic acid (12mg, 50 µmol, 1.1 equiv), DIPEA (26 µL, 0.15 mmol, 3.2 equiv), and TBTU (19 mg, 60 µmol, 1.3 equiv) in DMF (1 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (25 mg, 46 µmol, 1.0 equiv). The reaction was stirred at room temperature for 21 hr, concentrated *in vacuo* and purified by column chromatography (silica, 0-10% MeOH in CH_2Cl_2). The reaction was telescoped into the *N*-Boc deprotection with 20% TFA in CH_2Cl_2 (2 mL), concentrated *in vacuo* and purified by reverse phase column chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a clear oil 4-fluoro-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide 2,2,2-trifluoroacetate (**7**) (17.7 mg, 57 %) over two steps.

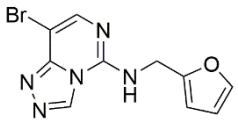
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.11 (s, 1H), 7.50 – 7.43 (m, 4H), 4.69 (d, *J* = 8.55 Hz, 1H), 4.62 – 4.55 (m, 1H), 4.56 – 4.50 (m, 1H), 4.36 (d, *J* = 15.50 Hz, 1H), 3.87 – 3.78 (m, 2H), 3.49 – 3.41 (m, 2H), 3.29 – 3.20 (m, 2H), 2.50 (s, 3H), 2.45 – 2.06 (m, 7H), 1.04 (s, 9H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 174.25, 171.49, 153.65, 140.76, 130.76, 130.58, 130.40, 129.68, 129.05, 129.03, 71.06, 60.89, 58.59, 58.19, 43.69, 40.66, 39.05, 37.44, 26.86, 26.81, 26.78, 15.17. LCMS: expected mass for $[M+H]^+$ ($C_{28}H_{39}N_5O_4S$) requires 560.26 m/z, found 560.20 m/z.


9.11
7.50
7.49
7.48
7.48
7.46
7.43
4.70
4.68
4.61
4.58
4.56
4.54
4.51
4.38
4.34
3.84
3.82
3.81
3.45
3.44
3.27
3.24
2.50
2.40
2.26
2.25
2.23
2.17
2.15
2.13
2.12
2.10
1.08
1.04
0.63
4.00
0.90
1.35
1.37
1.34
1.84
2.15
2.06
3.11
7.10
9.39
f1 (ppm)


174.25
171.49
153.65
140.76
130.76
130.58
130.40
129.68
129.05
129.03
71.06
60.89
58.59
58.19
43.69
40.66
39.05
37.44
26.86
26.81
26.78
15.17
f1 (ppm)

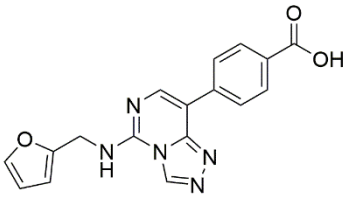


8-bromo-*N*-(furan-2-2-ylmethyl)-[1,2,4]triazolo[4,3-*c*]pyrimidin-5-amine, 8



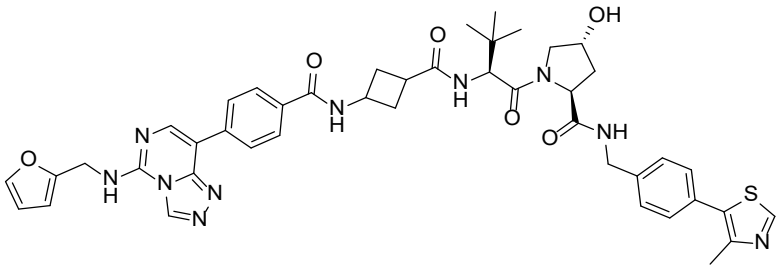
Synthesized as previously reported.[1]

4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoic acid, 9



Synthesized as previously reported.[1]

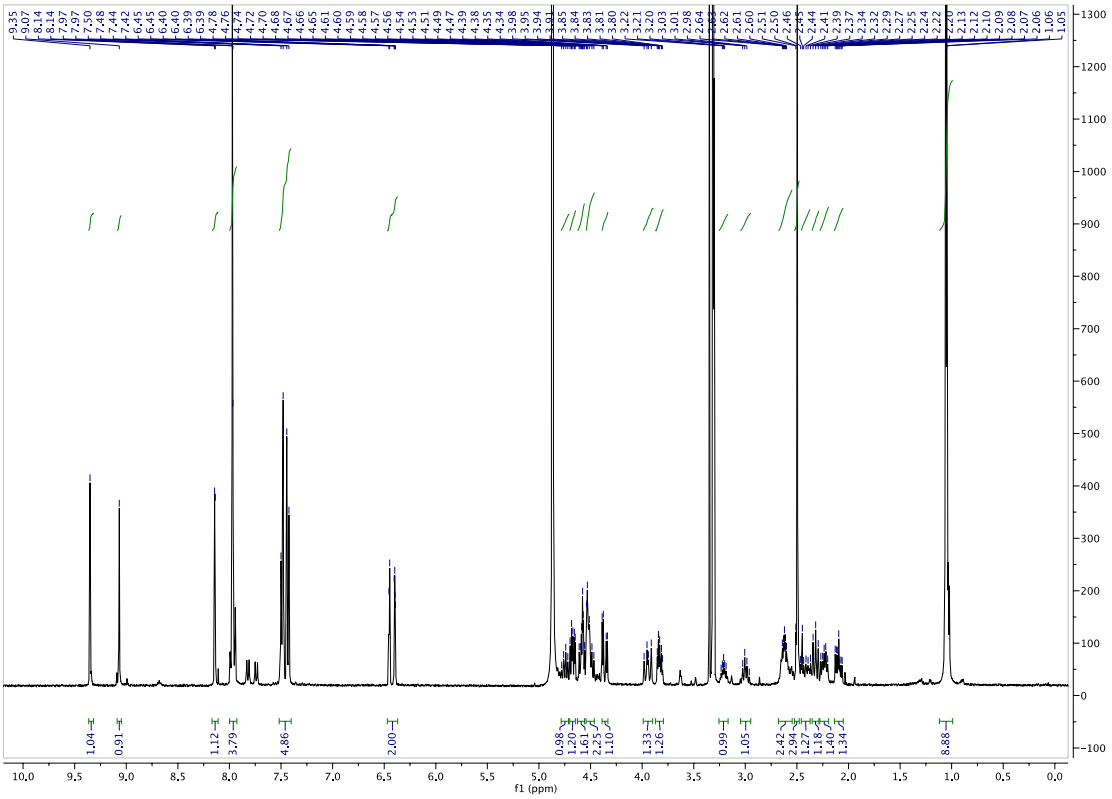
(2*S*,4*R*)-1-((*S*)-2-(3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, UNC7327

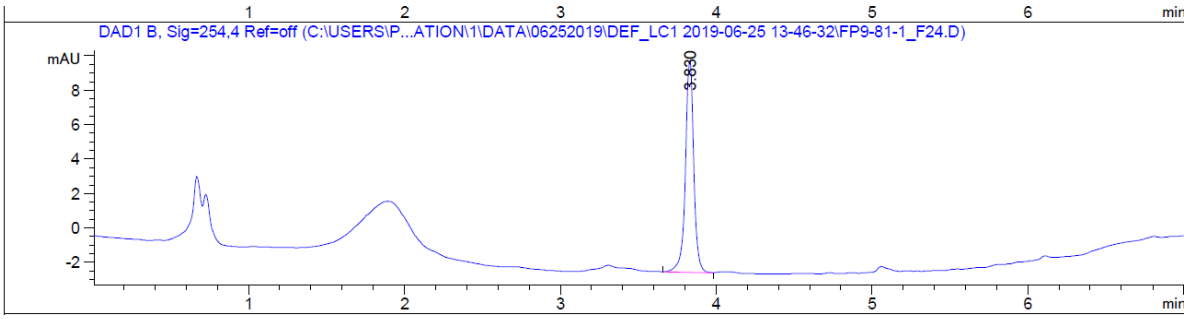


To a flask was added 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoic acid (**9**) (5.8 mg, 15 µmol, 1.0 equiv), DIPEA (7.8 µL, 45 µmol, 3.0 equiv), and TBTU (6.2 mg, 19 µmol, 1.3 equiv) in DMF (1 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-(3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (9.6 mg, 15 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a white

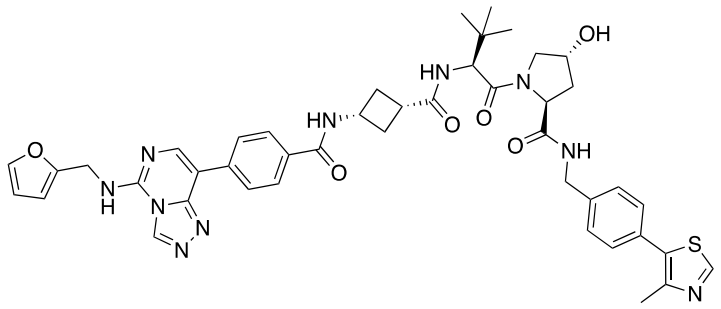
solid (2*S*,4*R*)-1-((*S*)-2-(3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (2.66 mg, 21 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.35 (s, 1H), 9.07 (s, 1H), 8.14 (d, *J* = 2.76 Hz, 1H), 8.00 – 7.93 (m, 4H), 7.53 – 7.41 (m, 5H), 6.46 – 6.39 (m, 2H), 4.79 – 4.71 (m, 1H), 4.71 – 4.64 (m, 1H), 4.62 – 4.55 (m, 2H), 4.55 – 4.46 (m, 2H), 4.36 (dd, *J* = 4.67, 15.59 Hz, 1H), 3.95 (dd, *J* = 11.27, 16.40 Hz, 1H), 3.86 – 3.79 (m, 1H), 3.25 – 3.17 (m, 1H), 3.03 – 2.95 (m, 1H), 2.67 – 2.54 (m, 2H), 2.50 (s, 3H), 2.46 – 2.37 (m, 1H), 2.35 – 2.28 (m, 1H), 2.27 – 2.19 (m, 1H), 2.14 – 2.05 (m, 1H), 1.05 (app d, *J* = 4.37 Hz, 9H). LCMS: expected mass for $[M+H]^+$ ($C_{44}H_{49}N_{10}O_6S$) requires 845.35 m/z, found 845.20 m/z.



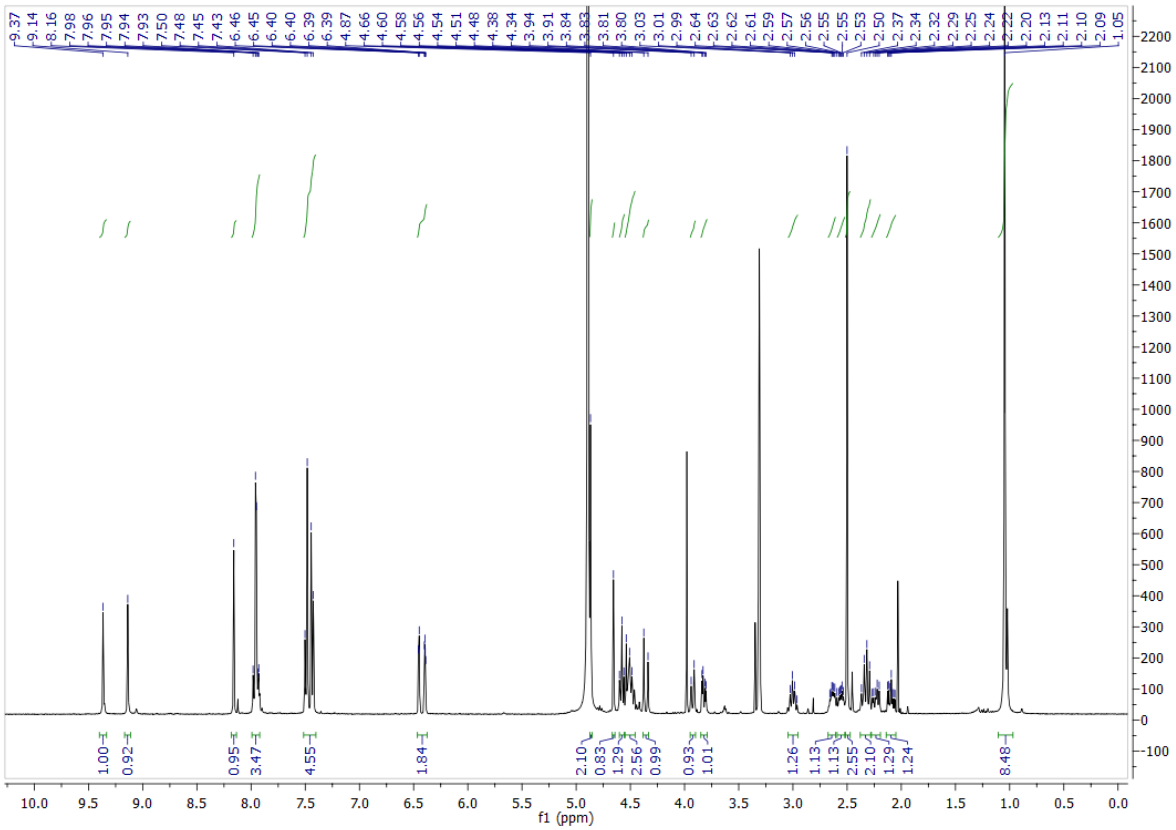


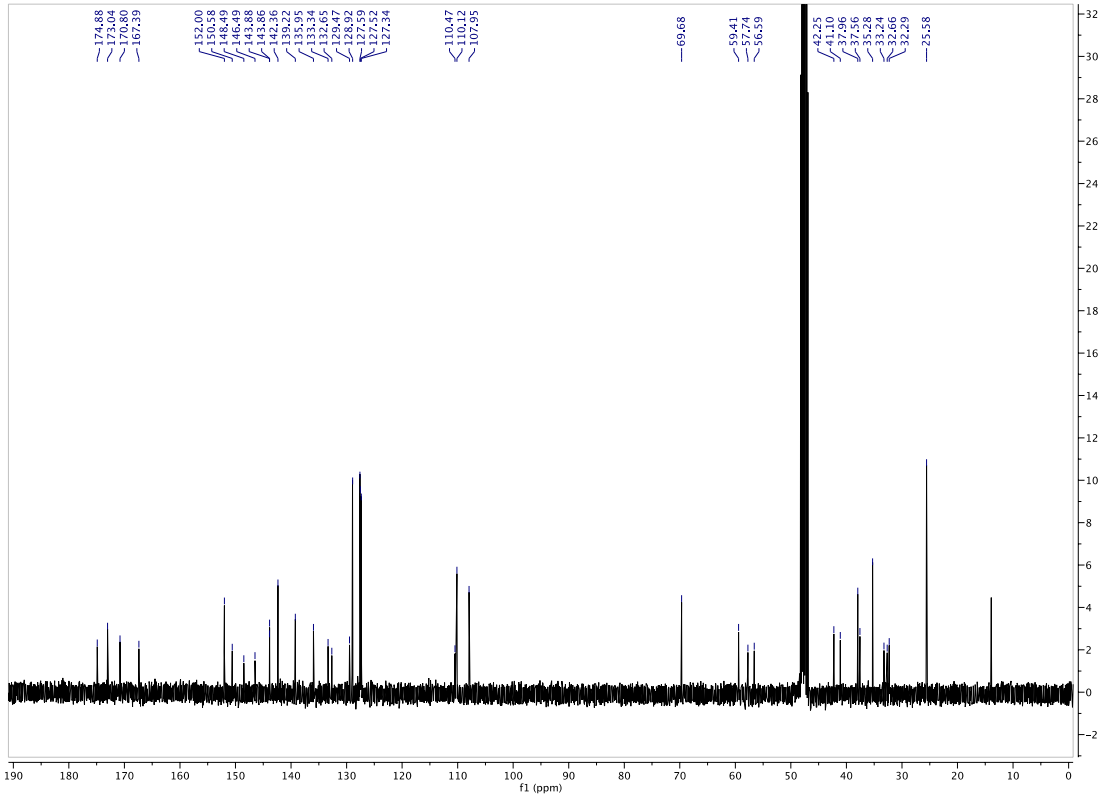
(2*S*,4*R*)-1-((*S*)-2-((1*S*,3*R*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, UNC7700

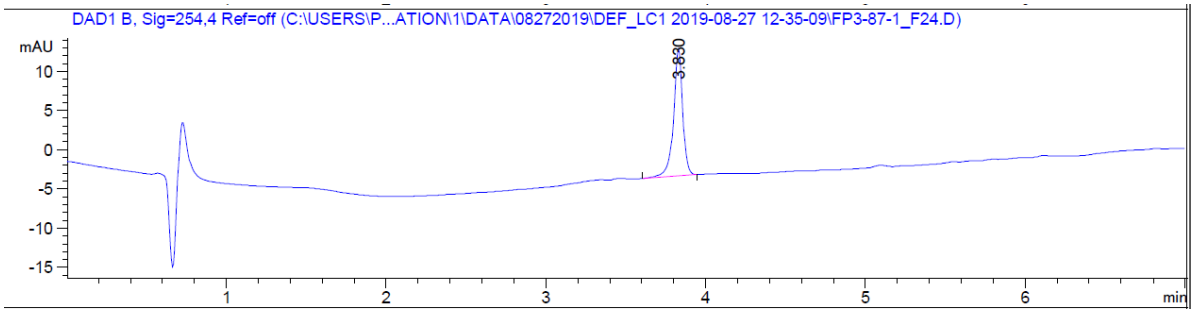


To a flask was added 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoic acid (**9**) (5 mg, 15 µmol, 1.0 equiv), DIPEA (7.8 µL, 45 µmol, 3.0 equiv), and TBTU (6.2 mg, 19 µmol, 1.3 equiv) in DMF (1 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-((1*S*,3*R*)-3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (9.6 mg, 15 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a white solid (2*S*,4*R*)-1-((*S*)-2-((1*S*,3*R*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (9.54 mg, 76 %).

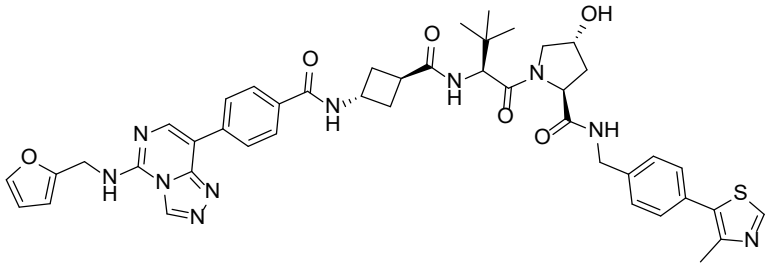
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.37 (s, 1H), 9.14 (s, 1H), 8.16 (s, 1H), 8.00 – 7.91 (m, 4H), 7.52 – 7.40 (m, 5H), 6.47 – 6.38 (m, 2H), 4.87 (s, 2H), 4.66 (s, 1H), 4.61 – 4.55 (m, 1H), 4.55 – 4.46 (m, 3H), 4.36 (d, *J* = 15.56 Hz, 1H), 3.93 (d, *J* = 11.00 Hz, 1H), 3.82 (dd, *J* = 3.86, 10.95 Hz, 1H), 3.05 – 2.95 (m, 1H), 2.67 – 2.61 (m, 1H), 2.58 – 2.53 (m, 1H), 2.50 (s, 3H), 2.36 – 2.28 (m, 2H), 2.27 – 2.19 (m, 1H), 2.15 – 2.04 (m, 1H), 1.05 (s, 9H). ^{13}C NMR (CD_3OD-d_4, 101 MHz) δ 174.88, 173.04, 170.80, 167.39, 152.00, 150.58, 148.49, 146.49, 143.88, 143.86, 142.36, 139.22, 135.95, 133.34, 132.65, 129.47, 128.92, 127.59, 127.52, 127.34, 110.47, 110.12, 107.95, 69.68, 59.41, 57.74, 56.59, 42.25, 41.10, 37.96, 37.56, 35.28, 33.24, 32.66, 32.29, 25.58. LCMS: expected mass for $[M+H]^+$ ($C_{44}H_{49}N_{10}O_6S$) requires 845.35 m/z, found 845.30 m/z.


9.37
9.14
8.16
7.98
7.96
7.95
7.94
7.93
7.50
7.48
7.45
7.43
6.46
6.45
6.40
6.40
6.39
6.39
4.87
4.66
4.60
4.58
4.56
4.54
4.51
4.48
4.38
4.34
3.94
3.91
3.84
3.83
3.81
3.80
3.03
3.01
2.99
2.64
2.63
2.62
2.61
2.59
2.57
2.56
2.55
2.55
2.53
2.50
2.37
2.34
2.32
2.29
2.25
2.24
2.22
2.20
2.13
2.11
2.10
2.09
1.05
1.00
0.92
0.95
3.47
4.55
1.84
2.10
0.83
1.29
2.56
0.99
0.93
1.01
1.26
1.13
1.13
2.55
2.10
1.29
1.24
8.48
f1 (ppm)


174.88
173.04
170.80
167.39
152.00
150.58
148.49
146.49
143.88
143.66
142.36
139.22
135.95
133.34
132.65
129.47
128.92
127.59
127.52
127.34
110.47
110.12
107.95
69.68
59.41
57.74
56.39
42.25
41.10
37.96
37.56
35.28
33.24
32.66
32.29
25.58
f1 (ppm)

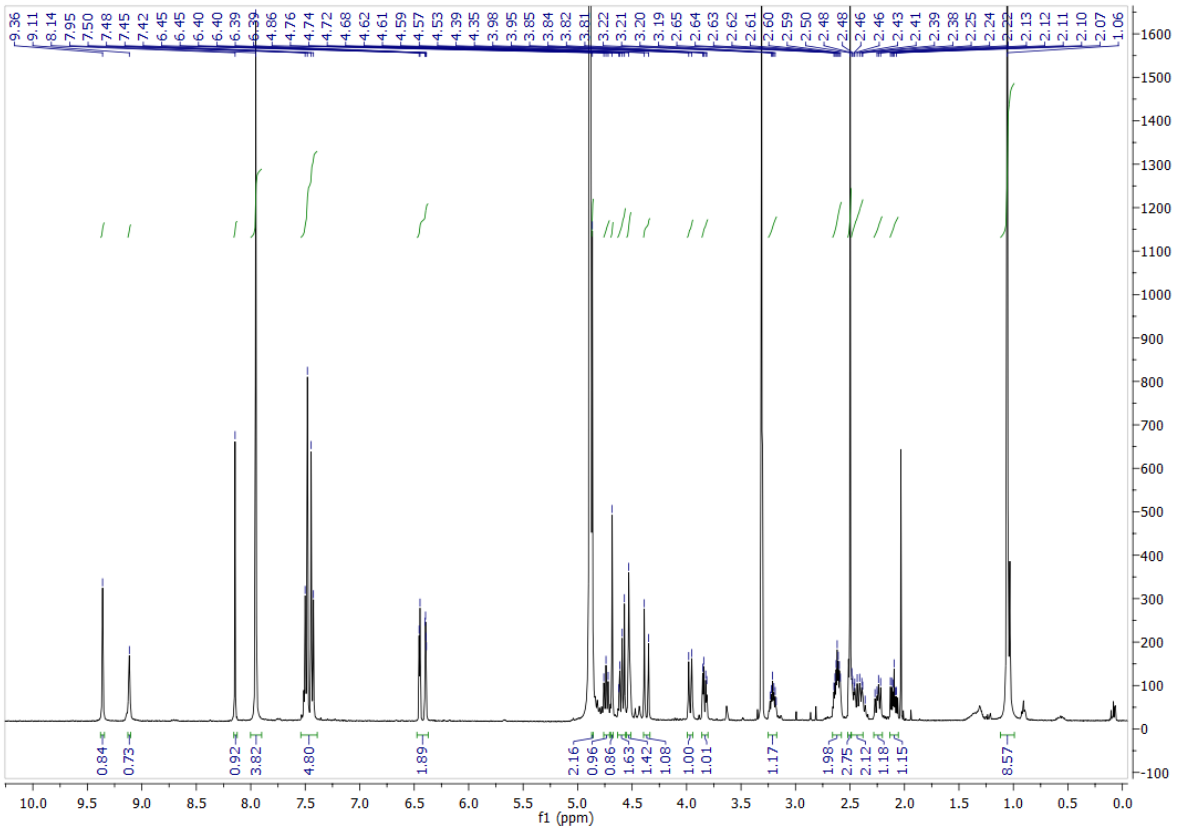


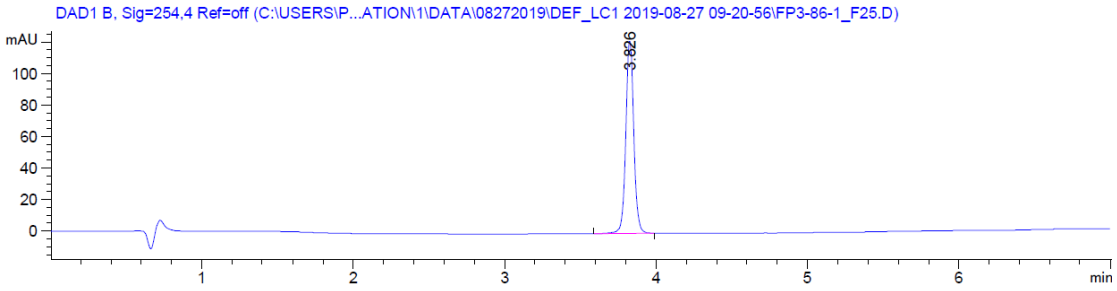
(2*S*,4*R*)-1-((*S*)-2-((1*R*,3*S*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, UNC7698



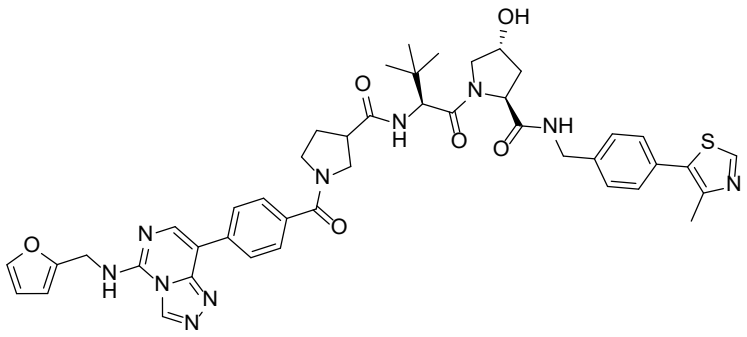
To a flask was added 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoic acid (**9**) (5 mg, 15 µmol, 1.0 equiv), DIPEA (7.8 µL, 45 µmol, 3.0 equiv), and TBTU (6.2 mg, 19 µmol, 1.3 equiv) in DMF (1 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-((1*R*,3*S*)-3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (9.6 mg, 15 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a white solid (2*S*,4*R*)-1-((*S*)-2-((1*R*,3*S*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (8.04 mg, 64 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.36 (s, 1H), 9.11 (s, 1H), 8.14 (s, 1H), 7.95 (ap s, 4H), 7.52 – 7.41 (m, 5H), 6.47 – 6.38 (m, 2H), 4.86 (s, 2H), 4.77 – 4.71 (m, 1H), 4.68 (s, 1H), 4.62 – 4.56 (m, 2H), 4.55 – 4.51 (m, 1H), 4.37 (d, *J* = 15.57 Hz, 1H), 3.97 (d, *J* = 11.24 Hz, 1H), 3.83 (dd, *J* = 3.81, 11.00 Hz, 1H), 3.20 (td, *J* = 4.26, 8.79, 8.51 Hz, 1H), 2.66 – 2.58 (m, 2H), 2.50 (s, 3H), 2.46 – 2.36 (m, 2H), 2.24 (dd, *J* = 7.61, 13.11 Hz, 1H), 2.10 (ddd, *J* = 4.46, 9.19, 13.31 Hz, 1H),1.05 (s, 9H). LCMS: expected mass for $[M+H]^+$ ($C_{44}H_{49}N_{10}O_6S$) requires 845.35 m/z, found 845.30 m/z.





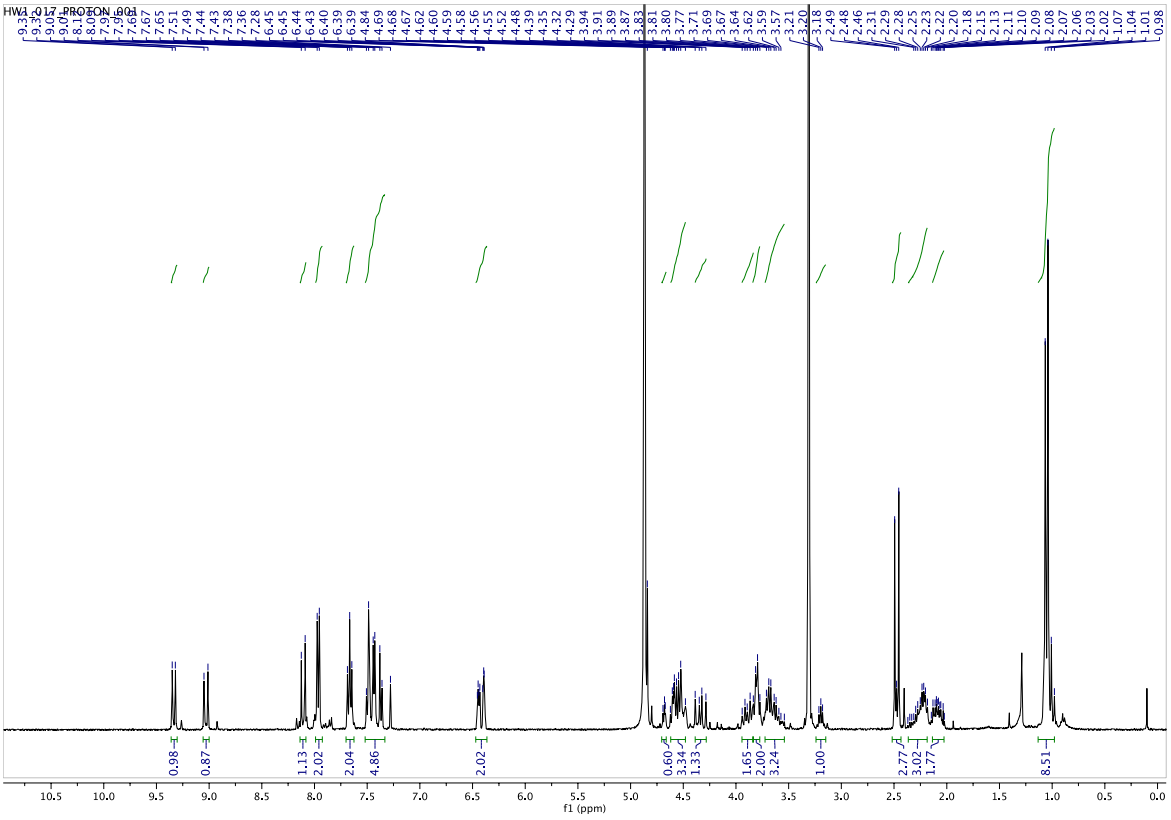
(2*S*,4*R*)-1-((2*S*)-2-(1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)pyrrolidine-3-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, UNC7701

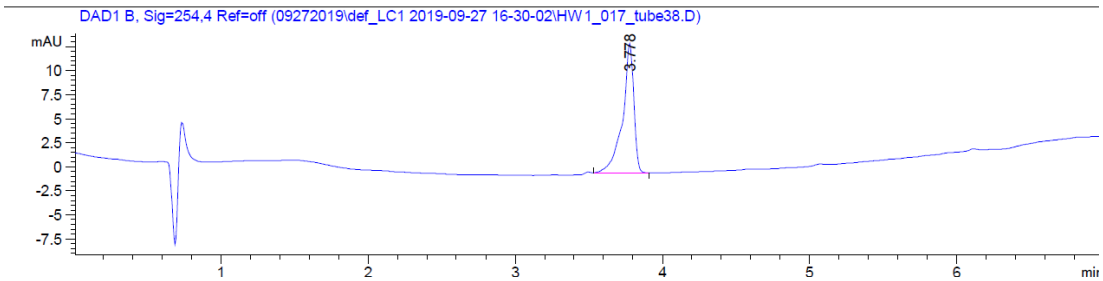


To a flask was added 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoic acid (**9**) (6.3 mg, 19 µmol, 1.0 equiv), DIPEA (9.9 µL, 57 µmol, 3.0 equiv), and TBTU (7.8 mg, 25 µmol, 1.3 equiv) in DMF (0.35 mL), followed by addition of 3-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamoyl)pyrrolidin-1-ium

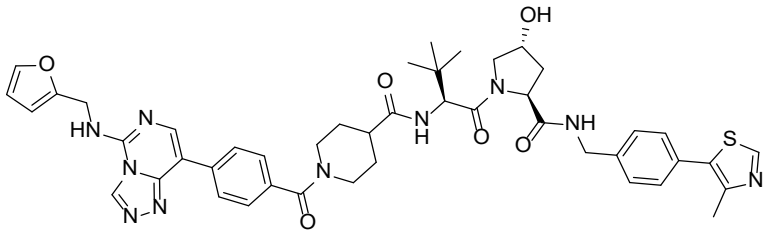
2,2,2-trifluoroacetate (12.1 mg, 19 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a white solid (2*S*,4*R*)-1-((2*S*)-2-(1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)benzoyl)pyrrolidine-3-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (3.28 mg, 21 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.33 (d, *J* = 11.77 Hz, 1H), 9.03 (d, *J* = 15.05 Hz, 1H), 8.11 (d, *J* = 14.32 Hz, 1H), 7.96 (d, *J* = 8.04 Hz, 2H), 7.70 – 7.63 (m, 2H), 7.52 – 7.35 (m, 5H), 6.47 – 6.36 (m, 2H), 4.63 – 4.46 (m, 3H), 4.40 – 4.27 (m, 2H), 3.95 – 3.89 (m, 2H), 3.87 – 3.76 (m, 2H), 3.74 – 3.53 (m, 3H), 3.24 – 3.15 (m, 1H), 2.47 (d, *J* = 15.49 Hz, 3H), 2.36 – 2.17 (m, 3H), 2.14 – 2.02 (m, 2H), 1.05 (app d, *J* = 10.20 Hz, 9H). LCMS: expected mass for $[M+H]^+$ ($C_{44}H_{49}N_{10}O_6S$) requires 845.35 m/z, found 845.25 m/z.



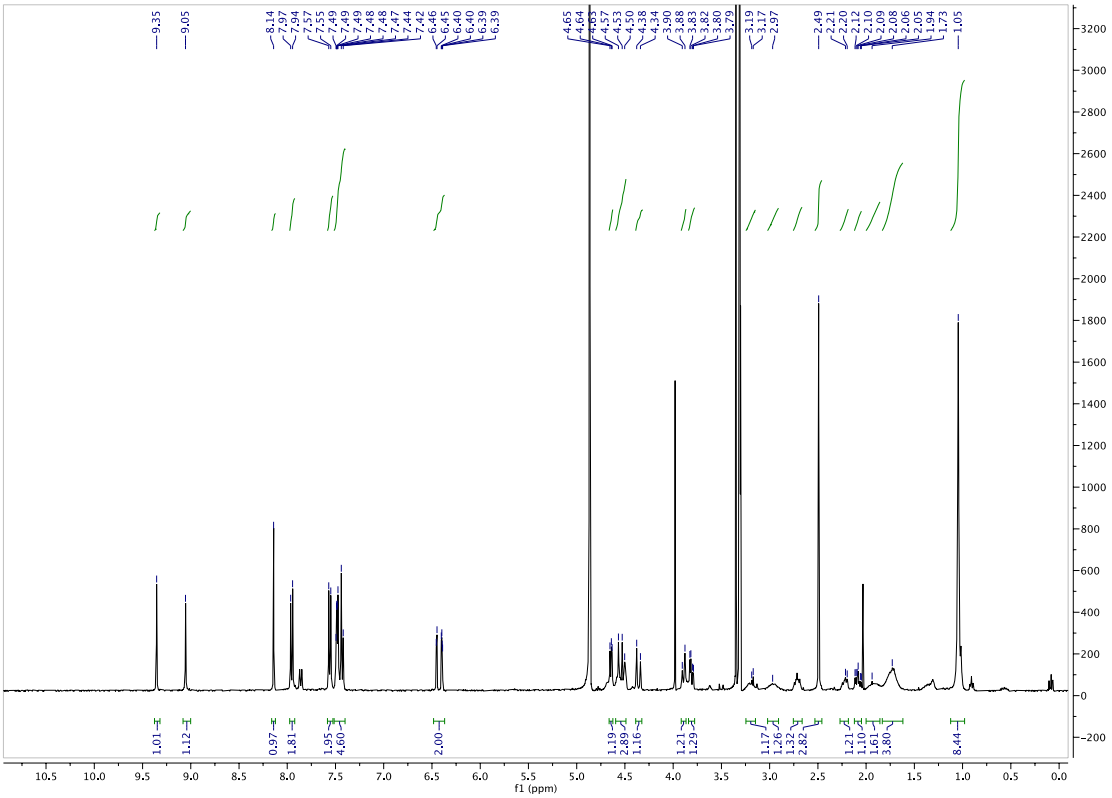


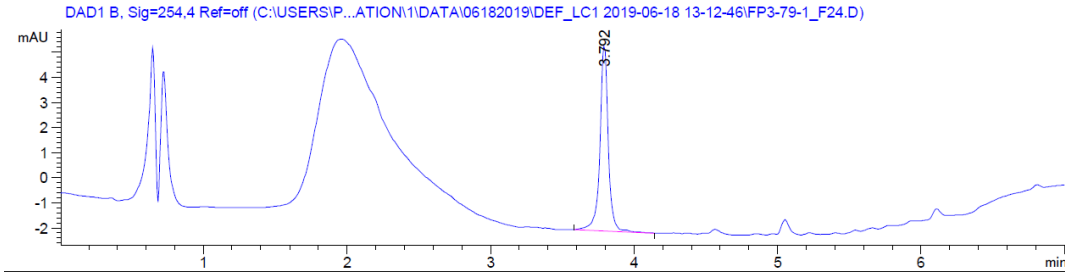
1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide, UNC7326



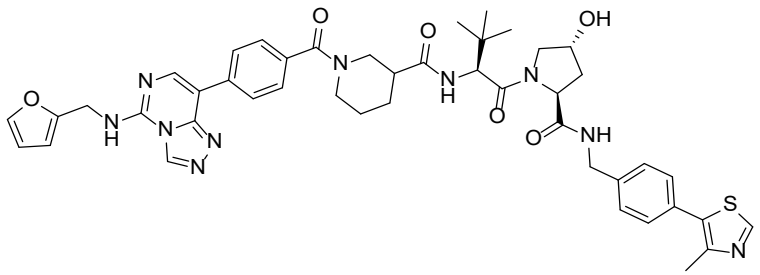
To a flask was added 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoic acid (**9**) (5.0 mg, 15 µmol, 1.0 equiv), DIPEA (7.8 µL, 45 µmol, 3.0 equiv), and TBTU (6.2 mg, 19 µmol, 1.3 equiv) in DMF (1 mL), followed by addition of *N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide 2,2,2-trifluoroacetate (9.0 mg, 15 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a white solid 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide (2.93 mg, 23 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.35 (s, 1H), 9.05 (s, 1H), 8.14 (s, 1H), 7.95 (d, *J* = 8.30 Hz, 2H), 7.56 (d, *J* = 8.30 Hz, 2H), 7.50 – 7.40 (m, 5H), 6.47 – 6.38 (m, 2H), 4.68 – 4.63 (m, 1H), 4.61 – 4.48 (m, 3H), 4.36 (d, *J* = 15.53 Hz, 1H), 3.89 (d, *J* = 10.90 Hz, 1H), 3.81 (dd, *J* = 3.78, 10.81 Hz, 1H), 3.24 – 3.15 (m, 1H), 3.02 – 2.91 (m, 1H), 2.77 – 2.66 (m, 1H), 2.49 (s, 3H), 2.26 – 2.19 (m, 1H), 2.13 – 2.04 (m, 1H), 1.97 – 1.85 (m, 2H), 1.80 – 1.66 (m, 4H), 1.05 (s, 9H). 2 × protons not observed. LCMS: expected mass for $[M+H]^+$ ($C_{45}H_{51}N_{10}O_6S$) requires 859.36 m/z, found 859.20 m/z.





1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-3-carboxamide Diasteroisomer A + B, UNC7702

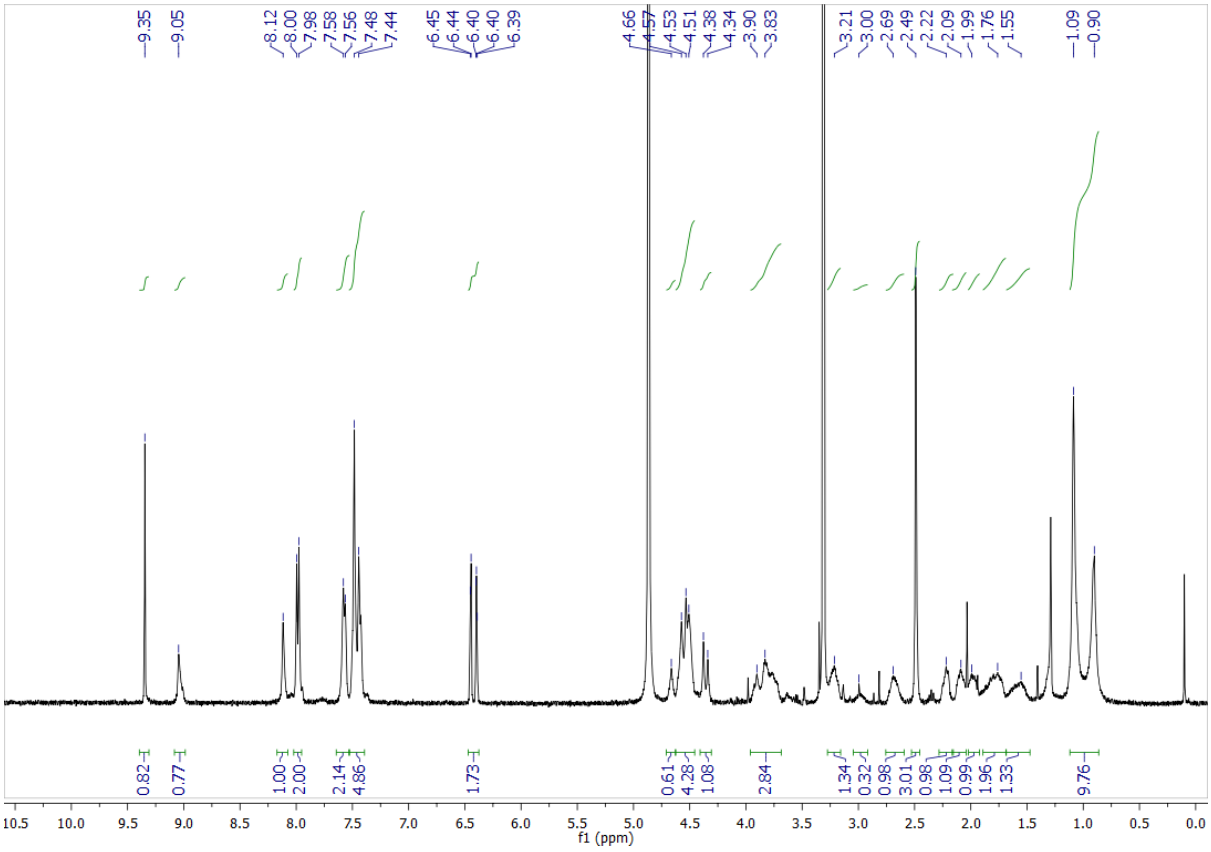


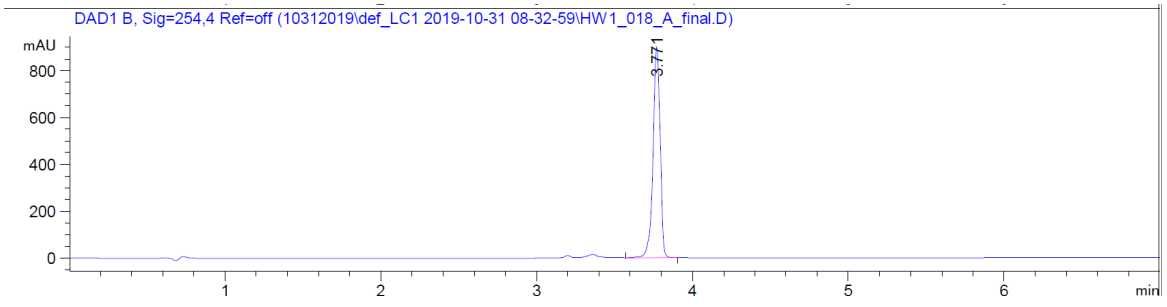
To a flask was added 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoic acid (**9**) (7.26 mg, 21 µmol, 1.0 equiv), DIPEA (11.3 µL, 65 µmol, 3.0 equiv), and TBTU (9.04 mg, 28 µmol, 1.3 equiv) in DMF (0.55 mL), followed by addition of 3-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamoyl)piperidin-1-ium 2,2,2-trifluoroacetatee (14.2 mg, 22 µmol, 1.0 equiv). The reaction was stirred at room temperature for 6 hr, concentrated *in vacuo* and purified by reverse phase high

performance liquid chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product containing separated diasteroisomers as a white solid 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-3-carboxamide (Diasteroisomer A: 1.93 mg, 10.4%; Diastereoisomer B: 1.56 mg, 8.4%). Diasteroisomeric mixture was tested at a 1:1 ratio in biological assays.

Diasteroisomer A Analysis

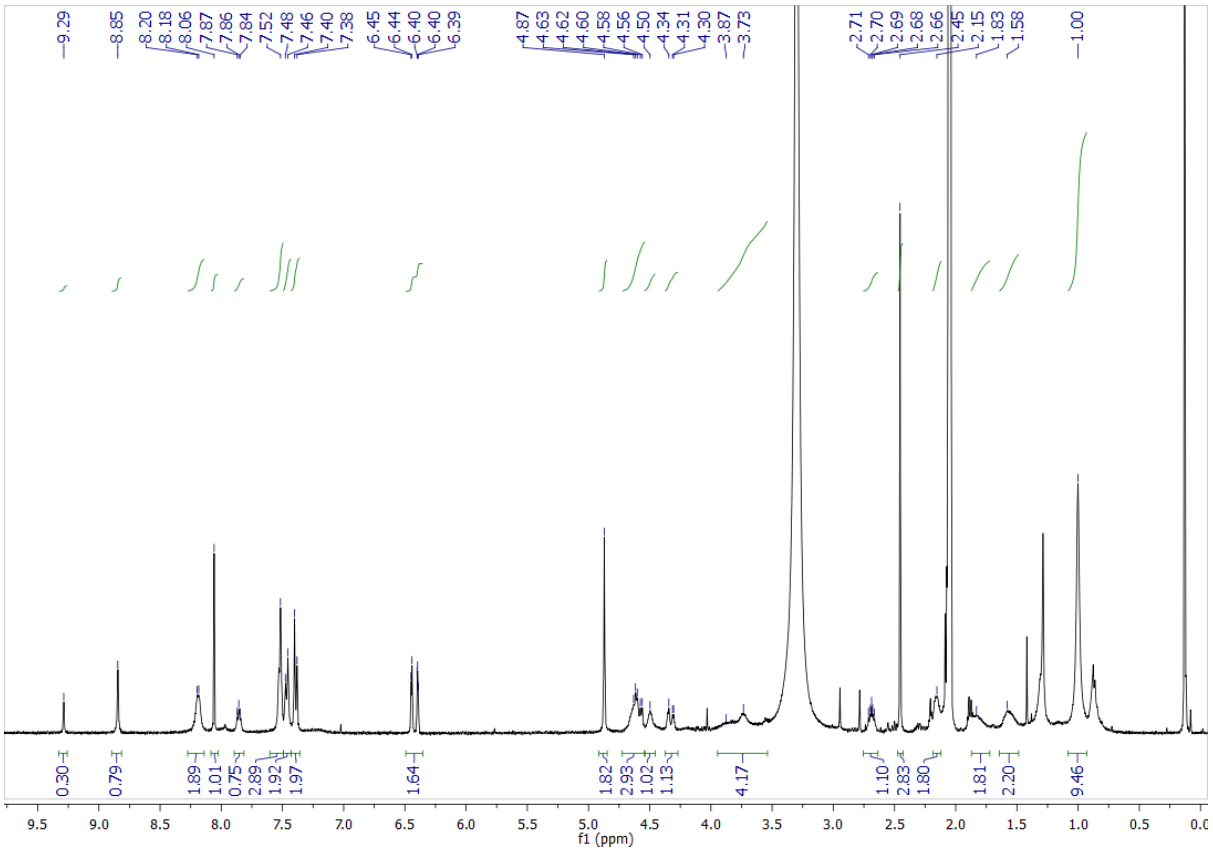
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.35 (s, 1H), 9.05 (s, 1H), 8.12 (s, 1H), 7.99 (d, *J* = 8.03 Hz, 2H), 7.57 (d, *J* = 6.70 Hz, 2H), 7.52 – 7.40 (m, 5H), 6.47 – 6.37 (m, 2H), 4.66 (s, 1H), 4.61 – 4.46 (m, 4H), 4.36 (d, *J* = 15.42 Hz, 1H), 3.94 – 3.71 (m, 3H), 3.27 – 3.16 (m, 1H), 3.03 – 2.93 (m, 1H), 2.75 – 2.61 (m, 1H), 2.49 (s, 3H), 2.26 – 2.19 (s, 1H), 2.14 – 2.05 (s, 1H), 2.02 – 1.95 (s, 1H), 1.88 – 1.71 (s, 2H), 1.66 – 1.48 (s, 1H), 0.99 (app m, 9H). (2 × 1H not observed – under H_2O peak). Exchangeable 1H not observed. LCMS: expected mass for $[M+H]^+$ ($C_{45}H_{51}N_{10}O_6S$) requires 859.36 m/z, found 859.30 m/z. Retention time = 3.771 mins.

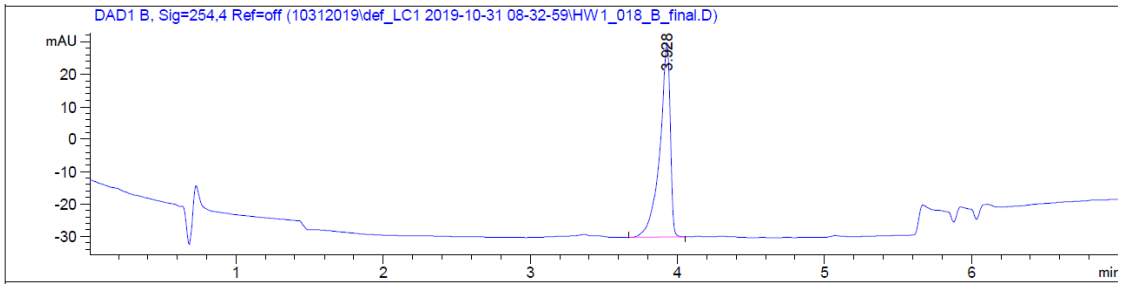




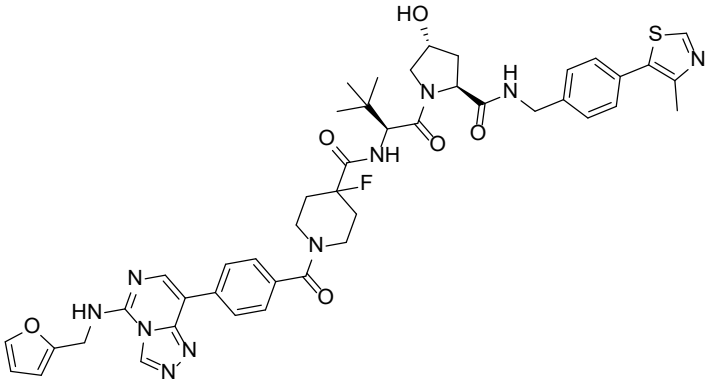
Diasteroisomer B Analysis

^{1}H NMR (Acetone-d_6, 400 MHz): δ 9.29 (s, 1H), 8.85 (s, 1H), 8.19 (d, *J* = 5.74 Hz, 2H), 8.06 (s, 1H), 7.86 (t, *J* = 5.21 Hz, 1H), 7.55 – 7.49 (m, 3H), 7.49 – 7.45 (m, 2H), 7.41 – 7.37 (m, 2H), 6.48 – 6.37 (m, 2H), 4.87(s, 2H), 4.69 – 4.55 (m, 3H), 4.53 – 4.46 (m, 1H), 4.33 (dd, *J* = 3.15, 15.28 Hz, 1H), 3.93 – 3.55 (m, 4H), 2.74 – 2.64 (m, 1H), 2.45 (s, 3H), 2.19 – 2.13 (m, 2H), 1.85 – 1.72 (m, 2H), 1.62 – 1.49 (m, 2H), 1.00 (s, 9H). LCMS: expected mass for $[M+H]^+$ ($C_{45}H_{51}N_{10}O_6S$) requires 859.36 m/z, found 859.25 m/z. Retention time = 3.928 mins.



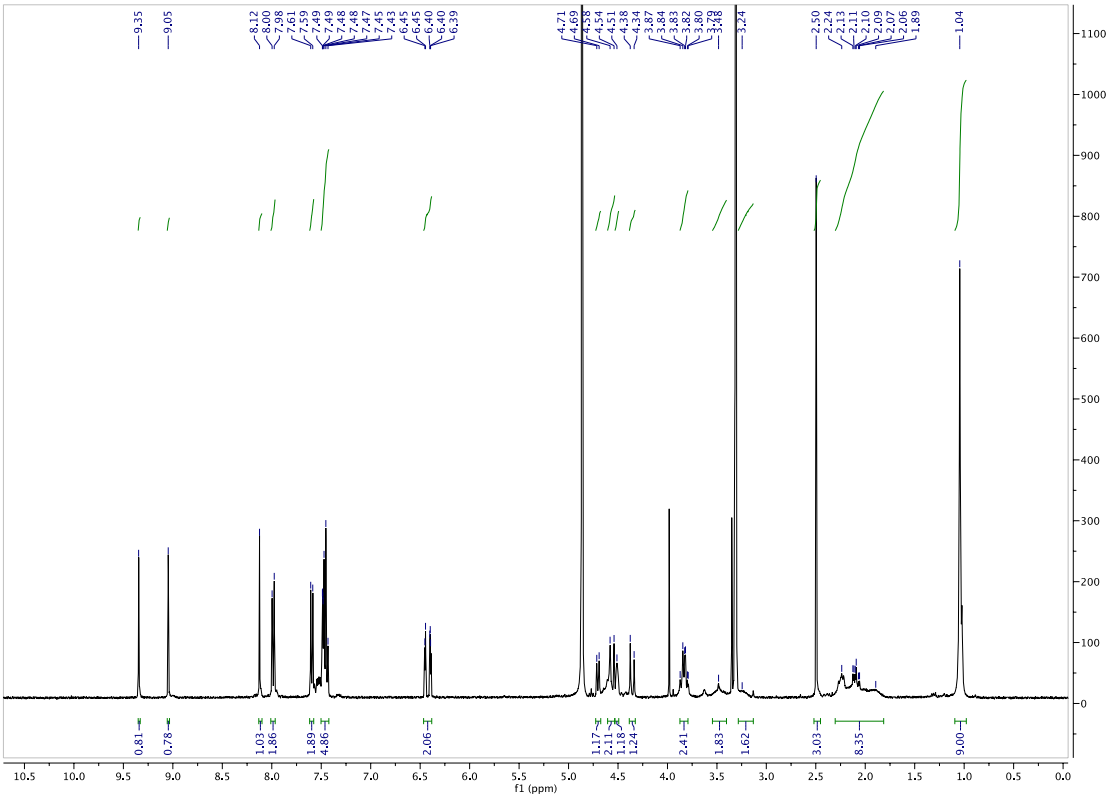


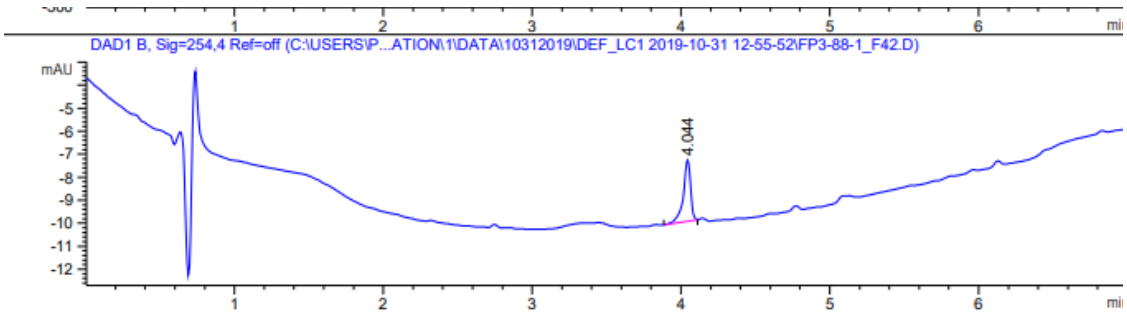
4-fluoro-1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide, UNC7703



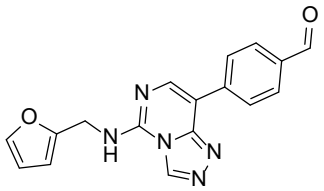
To a flask was added 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoic acid (**9**) (5.0 mg, 15 µmol, 1.0 equiv), DIPEA (7.8 µL, 45 µmol, 3.0 equiv), and TBTU (6.2 mg, 19 µmol, 1.3 equiv) in DMF (1 mL), followed by addition of 4-fluoro-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide 2,2,2-trifluoroacetate (10.0 mg, 15 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 0-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a white solid 4-fluoro-1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide (2.76 mg, 21 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.35 (s, 1H), 9.05 (s, 1H), 8.12 (s, 1H), 7.99 (d, *J* = 8.38 Hz, 2H), 7.60 (d, *J* = 8.34 Hz, 2H), 7.49 – 7.43 (m, 5H), 6.47 – 6.38 (m, 2H), 4.70 (d, *J* = 9.40 Hz, 1H), 4.62 – 4.56 (m, 2H), 4.55 – 4.48 (m, 2H), 4.36 (d, *J* = 15.60 Hz, 1H), 3.87 – 3.79 (m, 2H), 3.55 – 3.41 (m, 2H), 3.27 – 3.17 (s, 1H), 2.50 (s, 3H), 2.31 – 1.81 (m, 8H), 1.04 (s, 9H). LCMS: expected mass for $[M+H]^+$ ($C_{45}H_{49}FN_{10}O_6S$) requires 877.35 m/z, found 877.30 m/z.





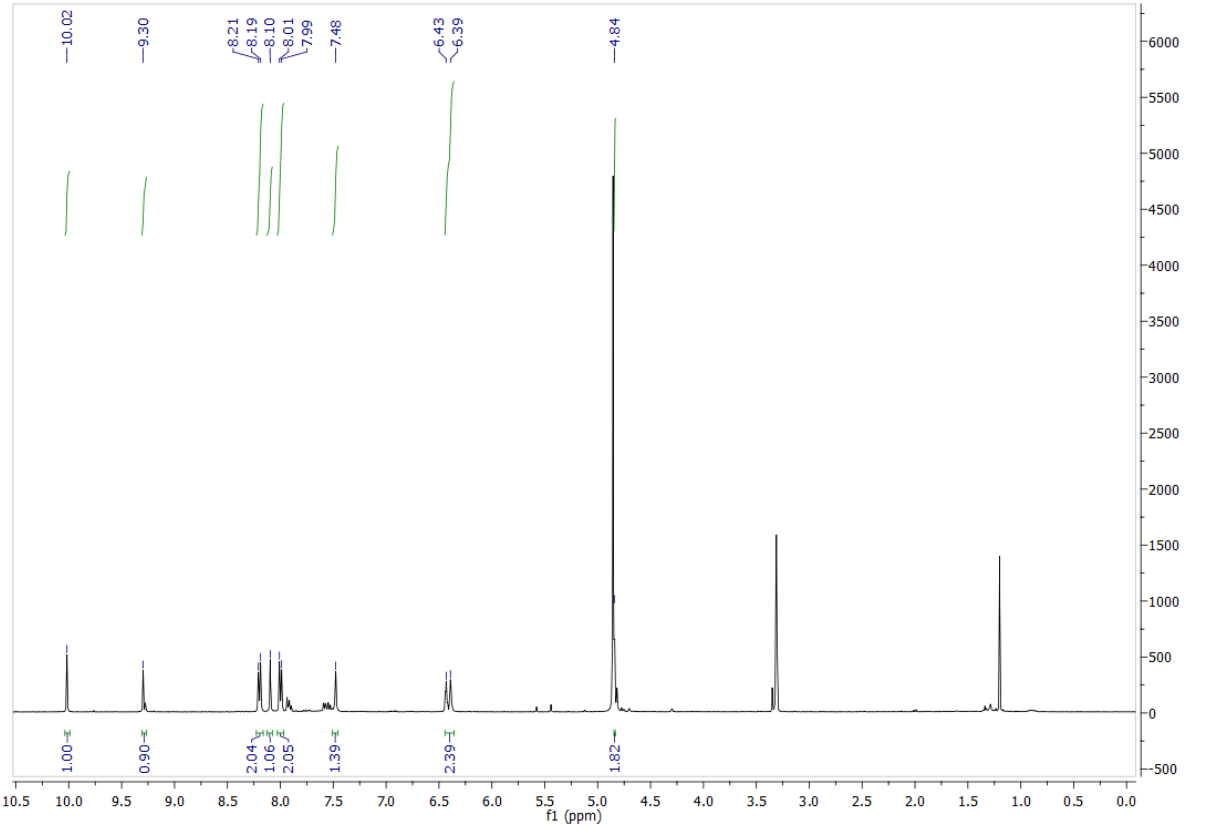
4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzaldehyde, 10



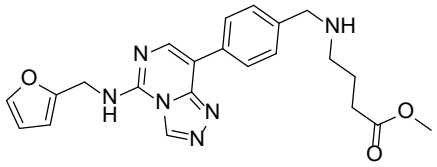
To a microwave vial was added 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (100 mg, 431 µmol, 1.5 equiv), 8-bromo-*N*-(furan-2-ylmethyl)-[1,2,4]triazolo[4,3-*c*]pyrimidin-5-amine (84.5 mg, 287 µmol, 1.0 equiv), Pd(dppf)Cl_2.CH_2Cl_2 (47 mg, 58 µmol, 0.2 equiv), sodium bicarbonate (60 mg, 718 µmol, 2.5 equiv) in THF (1 mL) and H_2O (0.5 mL). The vial was sealed at heated to 90 °C for 16 hr. The reaction was cooled and filtered through celite with EtOAc. The organics were washed with H_2O (2 × 200 mL), the organics dried with sodium sulfate and concentrated *in vacuo* to yield the crude product. Crude material was purified by column chromatography (0-100% EtOAc in hexane) to yield the desired

product as a yellow solid 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzaldehyde (**10**) (66 mg, 72%).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 10.02 (s, 1H), 9.30 (s, 1H), 8.20 (d, *J* = 8.19 Hz, 2H), 8.10 (s, 1H), 8.00 (d, *J* = 8.23 Hz, 2H), 7.48 (s, 1H), 6.46 – 6.36 (m, 2H), 4.84 (s, 2H).. LCMS: expected mass for $[M+H]^+$ ($C_{17}H_{14}N_5O_2$) requires 320.11 m/z, found 320.10 m/z.



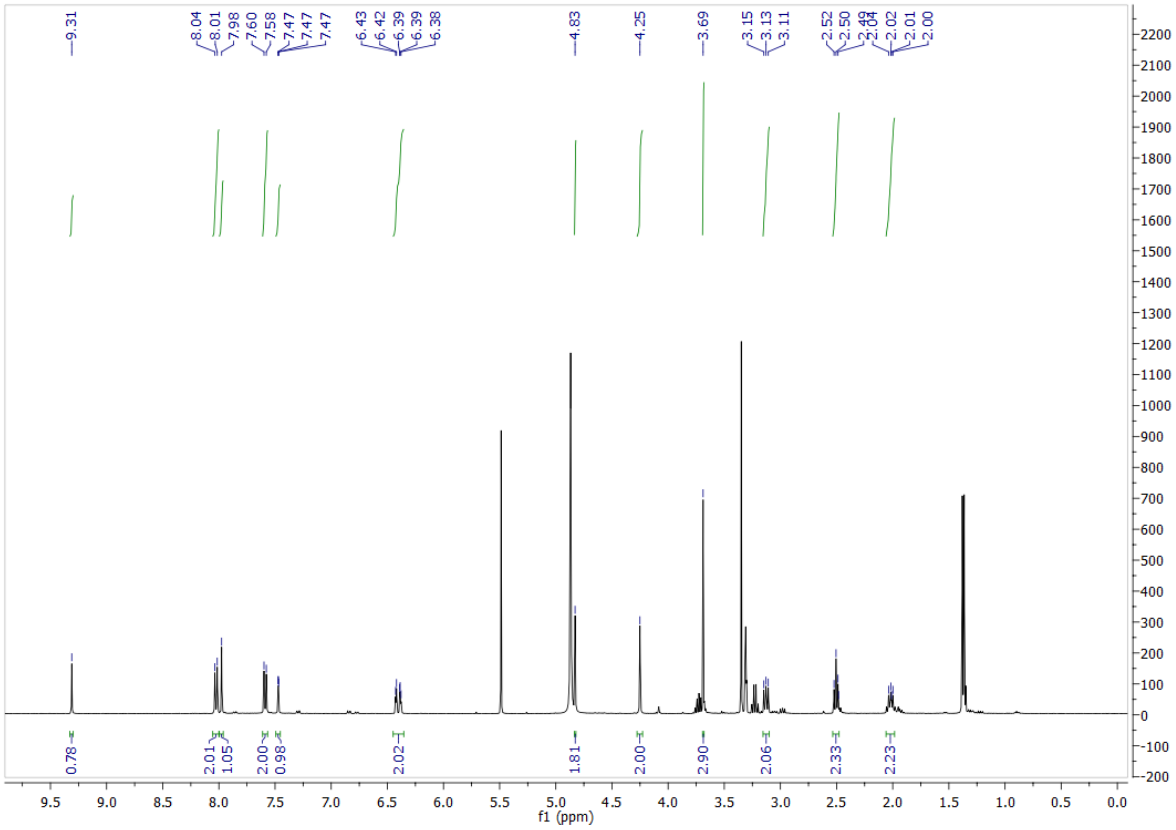
Methyl 4-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanoate, 11

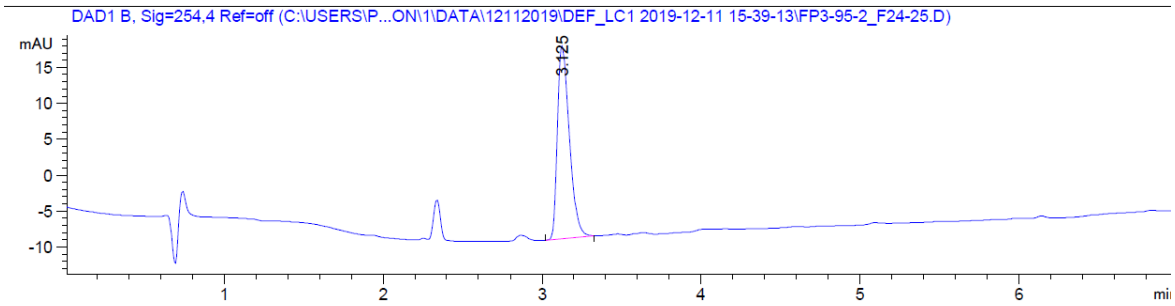


To a flask was added 4-aminobutanoate 2,2,2-trifluoroacetate (16 mg, 69 µmol, 1.1 equiv), 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzaldehyde (**10**) (20 mg, 63 µmol, 1.0 equiv), sodium sulfate (9.8 mg, 69 µmol, 1.1 equiv), and DIPEA (23 µL, 0.13 mmol, 2.1 equiv) in CH_2Cl_2

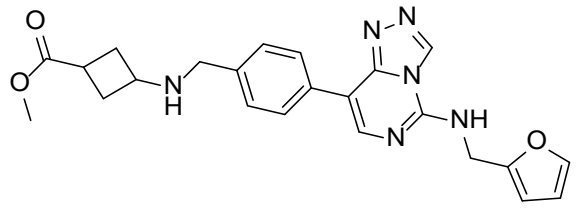
(0.5 mL). The reaction was refluxed at 40 °C for 1hr, concentrated *in vacuo*, resuspended in MeOH (0.5 mL) followed by addition of sodium tetrahydroborate (2.8 mg, 75 µmol, 1.2 equiv). The reaction was stirred at rt for 16 hr, concentrated *in vacuo* and purified by column chromatography (0-100% EtOAc in hexane) to yield the desired product as a brown gum methyl 4-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanoate (**11**) (9.8 mg, 37 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.31 (s, 1H), 8.03 (d, *J* = 8.32 Hz, 2H), 7.98 (s, 1H), 7.59 (d, *J* = 8.28 Hz, 2H), 7.49 – 7.45 (m, 1H), 6.44 – 6.37 (m, 2H), 4.83 (s, 2H), 4.25 (s, 2H), 3.69 (s, 3H), 3.16 – 3.10 (m, 2H), 2.50 (t, *J* = 7.07 Hz, 2H), 2.07 – 1.98 (m, 2H). LCMS: expected mass for $[M+H]^+$ ($C_{22}H_{25}N_6O_3$) requires 421.19 m/z, found 421.20 m/z.



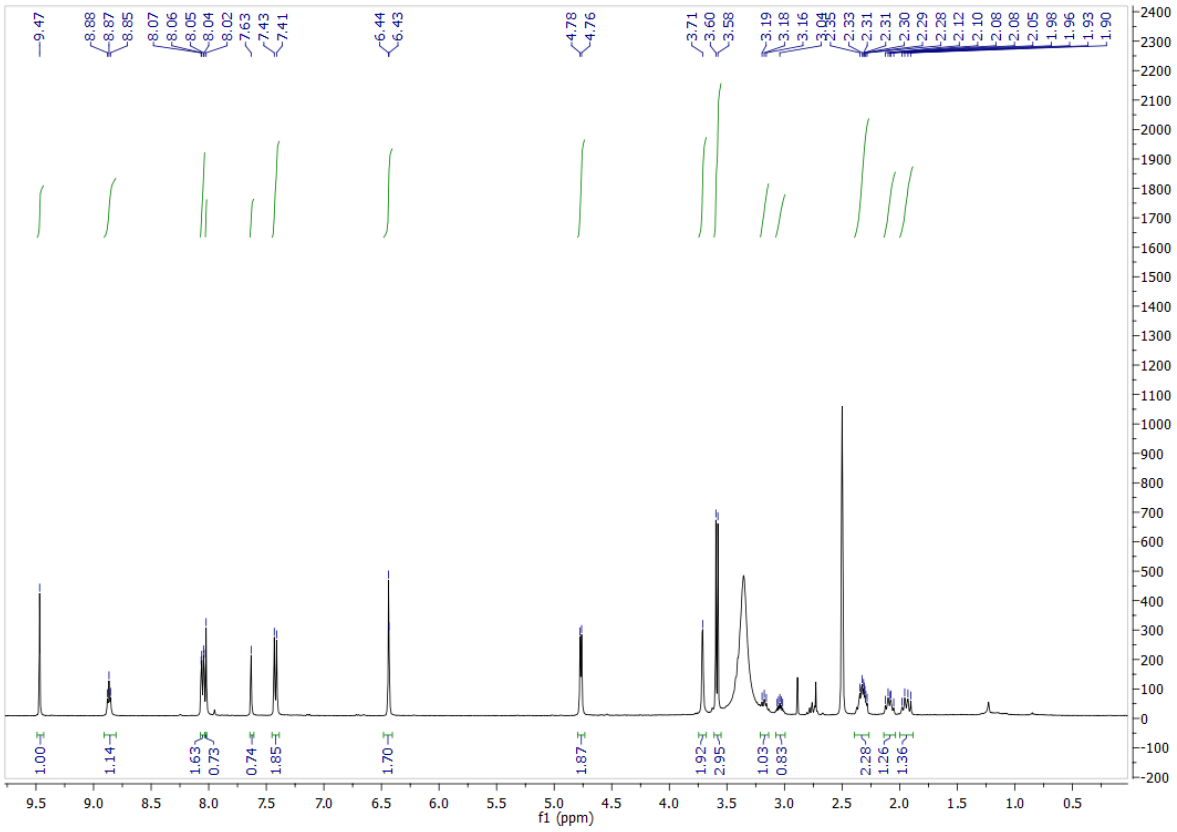


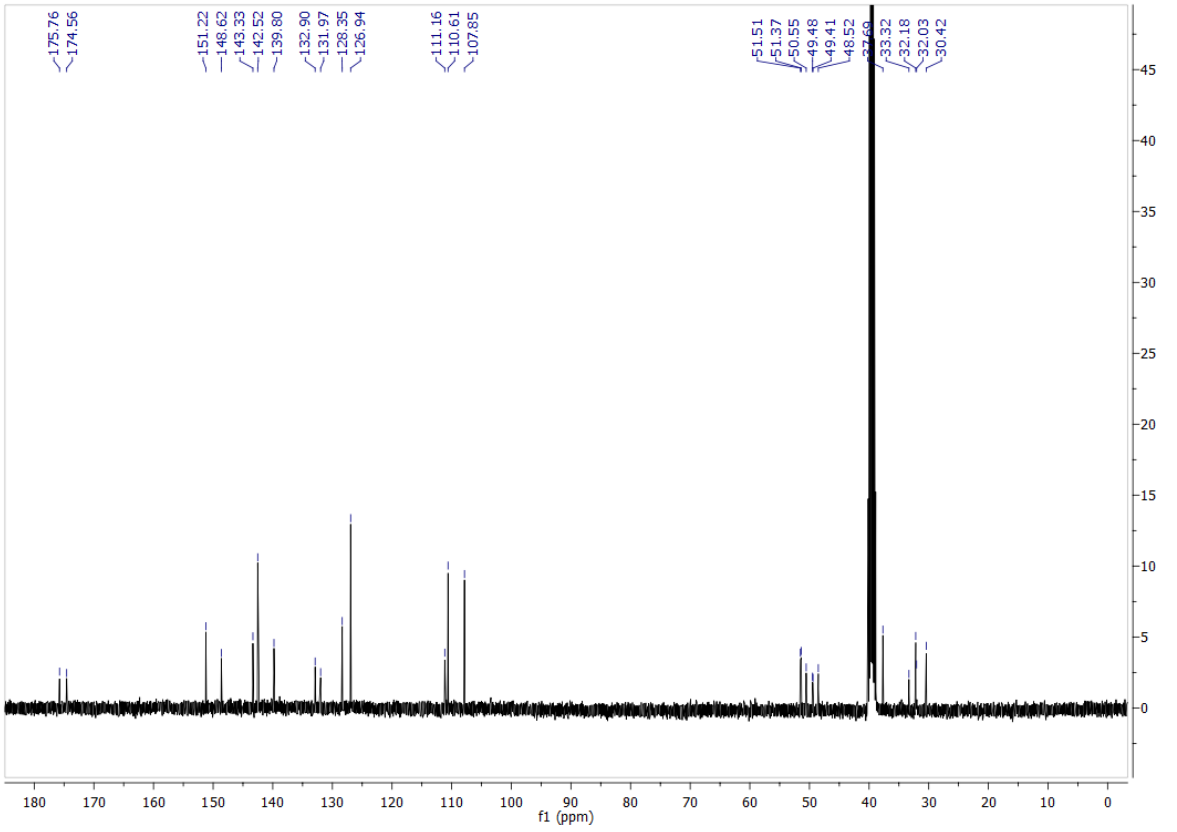
Methyl 3-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)cyclobutane-1-carboxylate, 12

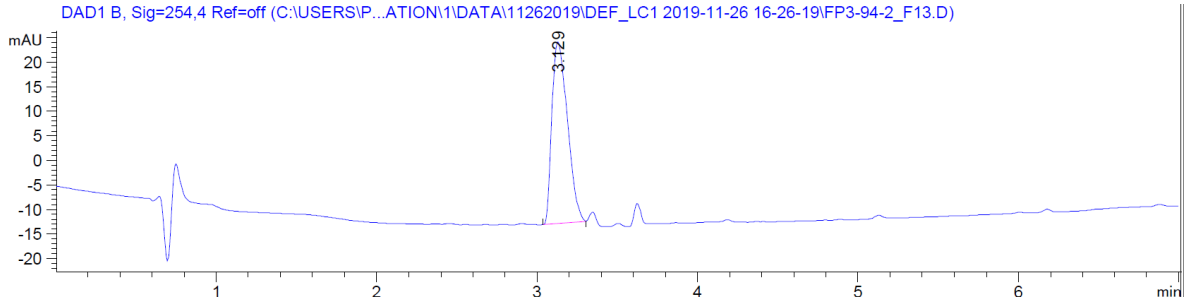


To a flask was added 3-aminocyclobutane-1-carboxylate 2,2,2-trifluoroacetate (8.4 mg, 34 µmol, 1.1 equiv), 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzaldehyde (**10**) (10 mg, 31 µmol, 1.0 equiv), sodium sulfate (4.9 mg, 34 µmol, 1.1 equiv), and DIPEA (11 µL, 66 µmol, 2.1 equiv) in CH_2Cl_2 (0.3 mL). The reaction was refluxed at 40 °C for 1hr, concentrated *in vacuo*, resuspended in MeOH (0.5 mL) followed by addition of sodium tetrahydroborate (1.4 mg, 38 µmol, 1.2 equiv). The reaction was stirred at rt for 16 hr, concentrated *in vacuo* and purified by column chromatography (0-100% EtOAc in hexane) to yield the desired product as a brown gum methyl 3-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)cyclobutane-1-carboxylate (**12**) (6.0 mg, 44 %).

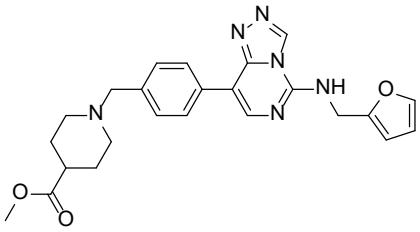
^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.47 (s, 1H), 8.87 (t, *J* = 5.28 Hz, 1H), 8.05 (dd, *J* = 2.09, 8.28 Hz, 2H), 8.03 – 8.02 (m, 1H), 7.63 (s, 1H), 7.42 (d, *J* = 8.19 Hz, 2H), 6.46 – 6.41 (m, 2H), 4.77 (d, *J* = 5.23 Hz, 2H), 3.71 (s, 2H), 3.59 (d, *J* = 6.87 Hz, 3H), 3.21 – 3.14 (m, 1H), 3.07 – 3.00 (m, 1H), 2.39 – 2.27 (m, 2H), 2.13 – 2.03 (m, 1H), 1.99 – 1.88 (m, 1H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 175.76, 174.56, 151.22, 148.62, 143.33, 142.52, 139.80, 132.90, 131.97, 128.35, 126.94, 111.16, 110.61, 107.85, 51.51, 51.37, 50.55, 49.48, 49.41, 48.52, 37.69, 33.32, 32.18, 32.03, 30.42. extra carbons peaks due to a diasteromeric mixture. LCMS: expected mass for $[M+H]^+$ ($C_{23}H_{25}N_6O_3$) requires 433.19 m/z, found 433.20 m/z.


9.47
8.88
8.87
8.85
8.07
8.06
8.05
8.04
8.02
7.63
7.43
7.41
6.44
6.43
4.78
4.76
3.71
3.60
3.58
3.19
3.18
3.16
3.04
2.33
2.31
2.31
2.30
2.29
2.28
2.12
2.10
2.08
2.08
2.05
1.98
1.96
1.93
1.90
1.00
1.14
1.63
0.73
0.74
1.85
1.70
1.87
1.92
2.95
1.03
0.83
2.28
1.26
1.36
f1 (ppm)


175.76
174.56
151.22
148.62
143.33
142.52
139.80
132.90
131.97
128.35
126.94
111.16
110.61
107.85
51.51
51.37
50.55
49.48
49.41
48.52
33.32
32.18
32.03
30.42
f1 (ppm)

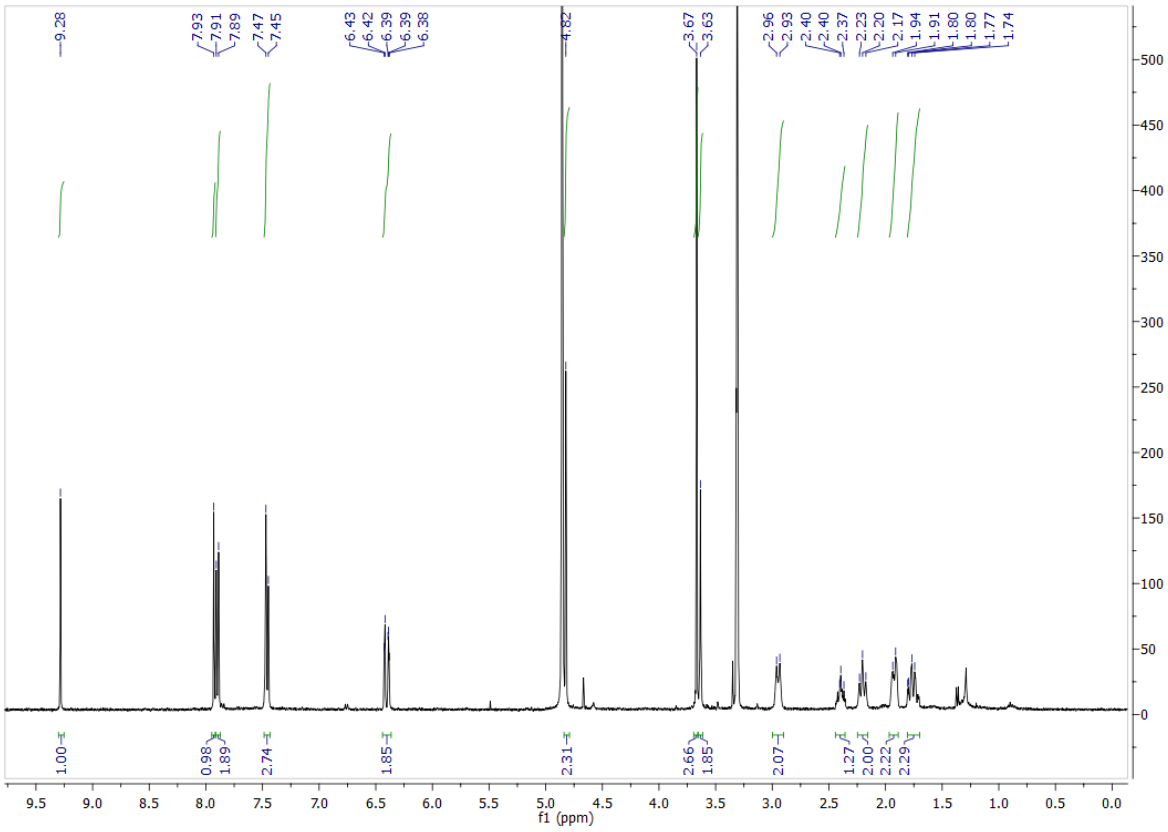


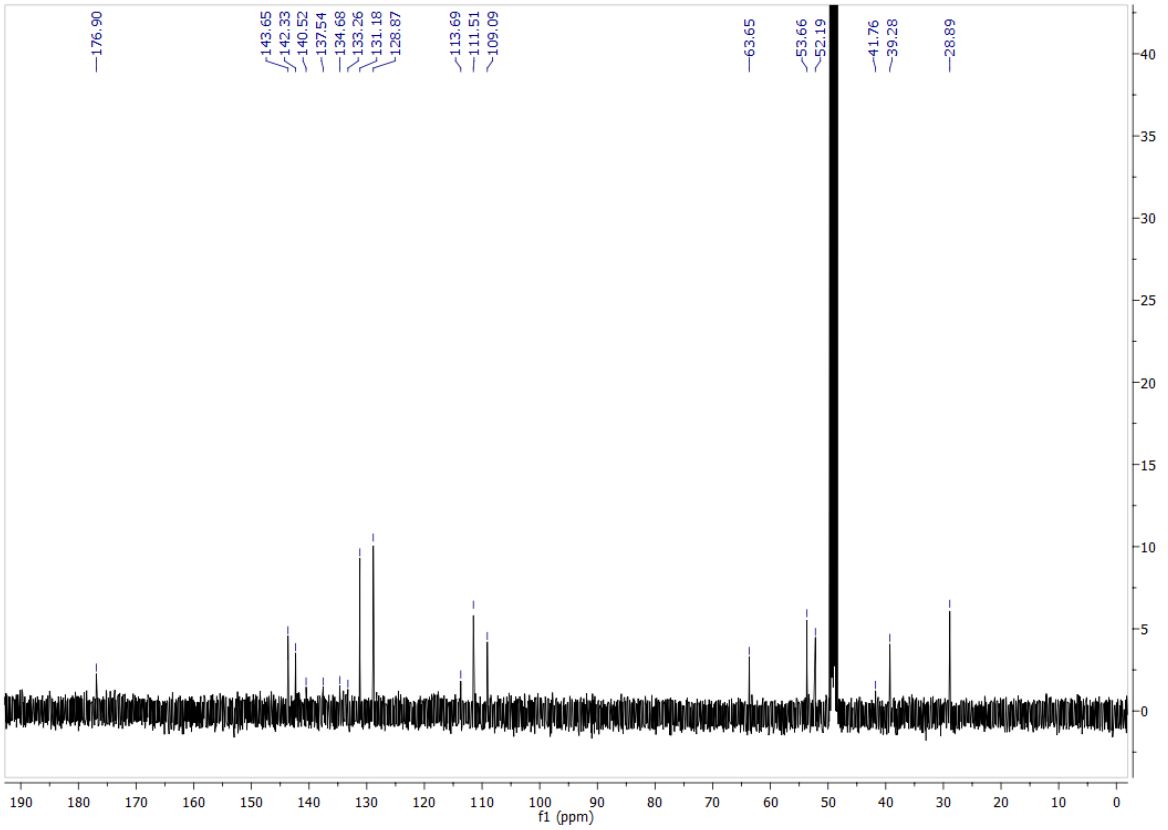
Methyl 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)benzyl)piperidine-4-carboxylate, 13

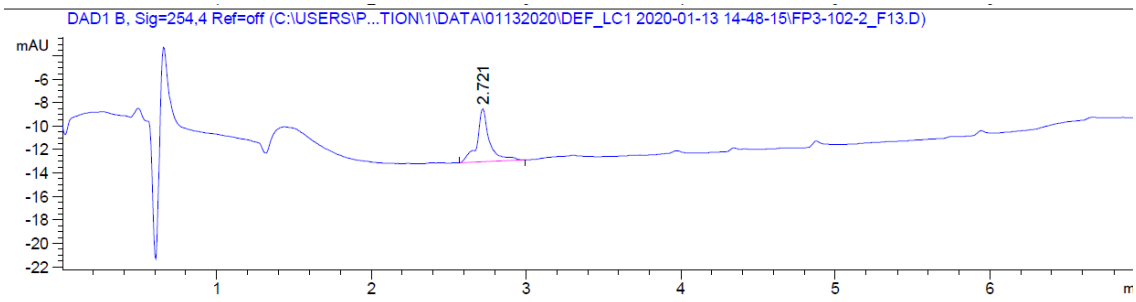


To a flask was added methyl piperidine-4-carboxylate 2,2,2-trifluoroacetate (8.9 mg, 34 µmol, 1.1 equiv), 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzaldehyde (**10**) (10 mg, 31 µmol, 1.0 equiv), sodium sulfate (4.9 mg, 34 µmol, 1.1 equiv), and DIPEA (11 µL, 66 µmol, 2.1 equiv) in CH_2Cl_2 (0.3 mL). The reaction was refluxed at 40 °C for 1hr, concentrated *in vacuo*, resuspended in MeOH (0.5 mL) followed by addition of sodium tetrahydroborate (1.4 mg, 38 µmol, 1.2 equiv). The reaction was stirred at rt for 16 hr, concentrated *in vacuo* and purified by column chromatography (0-100% EtOAc in hexane) to yield the desired product as a brown solid methyl 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)benzyl)piperidine-4-carboxylate (**13**) (4.7 mg, 34 %).

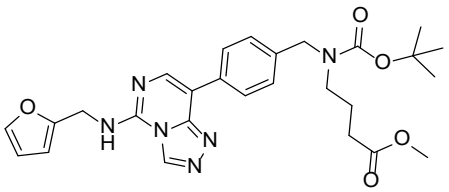
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.28 (s, 1H), 7.93 (s, 1H), 7.90 (d, *J* = 8.16 Hz, 2H), 7.49 – 7.44 (m, 3H), 6.44 – 6.37 (m, 2H), 4.82 (s, 2H), 3.67 (s, 3H), 3.63 (s, 2H), 2.95 (ap d, *J* = 11.53 Hz, 2H), 2.44 – 2.35 (m, 1H), 2.20 (t, *J* = 11.25 Hz, 2H), 1.98 – 1.88 (m, 2H), 1.82 – 1.70 (m, 2H). ^{13}C NMR (CD_3OD-d_4 CD_3OD-d_4, 101 MHz): δ 176.90, 143.65, 142.33, 140.52, 137.54, 134.68, 133.26, 131.18, 128.87, 113.69, 111.51, 109.09, 63.65, 53.66, 52.19, 41.76, 39.28, 28.89. 1 × aliphatic carbon not observed. LCMS: expected mass for $[M+H]^+$ ($C_{24}H_{27}N_6O_3$) requires 447.21 m/z, found 447.20 m/z.


9.28
7.93
7.91
7.89
7.47
7.45
6.43
6.42
6.39
6.39
6.38
4.82
3.67
3.63
2.96
2.93
2.40
2.40
2.37
2.23
2.20
2.17
1.94
1.91
1.80
1.80
1.77
1.74
1.00
0.98
1.89
2.74
1.85
2.31
2.66
1.85
2.07
1.27
2.00
2.22
2.29
f1 (ppm)


176.90
143.65
142.33
140.52
137.54
134.68
133.26
131.18
128.87
113.69
111.51
109.09
63.65
53.66
52.19
41.76
39.28
28.89
f1 (ppm)

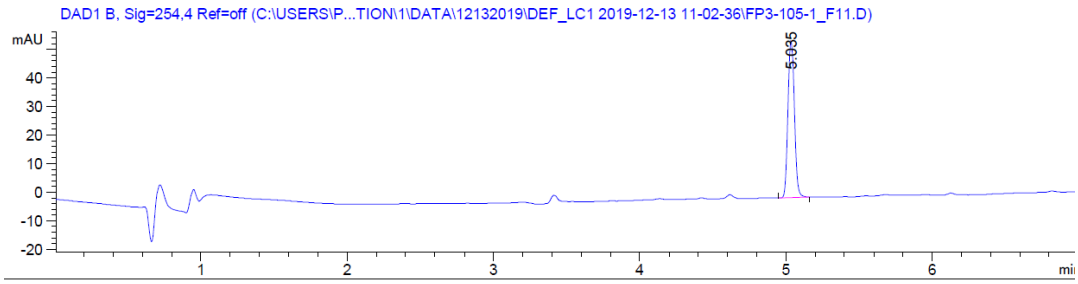


Methyl 4-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanoate, 14

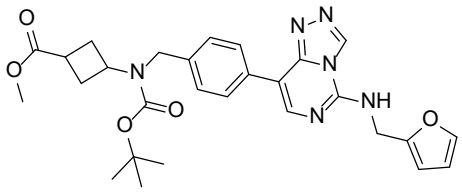


To a flask containing methyl 4-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)benzyl)amino)butanoate (**11**) (9.8 mg, 23 µmol, 1.0 equiv) in CH_2Cl_2 (93 µL) was added Boc anhydride (5.6 mg, 23 µmol, 1.1 equiv), NEt_3 (8.1 µL, 58 µmol, 2.5 equiv), and the reaction stirred at room temperature for 16 hr. The reaction was concentrated *in vacuo* and purified by column chromatography (0-10% MeOH in CH_2Cl_2) to yield the desired product as a yellow solid methyl 4-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanoate (**14**) (6.2 mg, 51%).

LCMS: expected mass for $[M+H]^+$ ($C_{27}H_{33}N_6O_5$) requires 521.24 m/z, found 521.30 m/z.



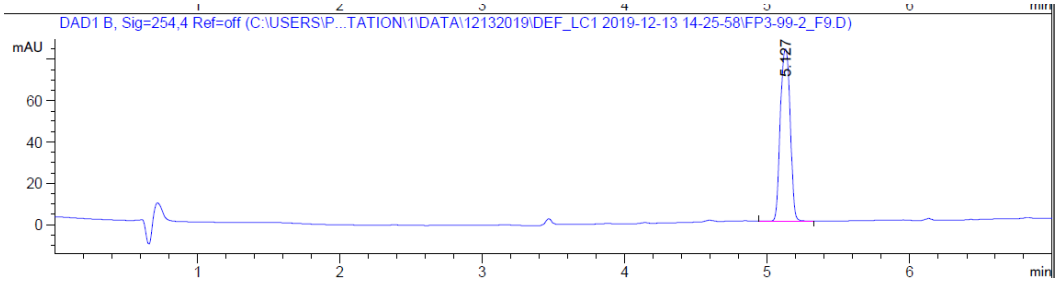
Methyl 3-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)cyclobutane-1-carboxylate, 15



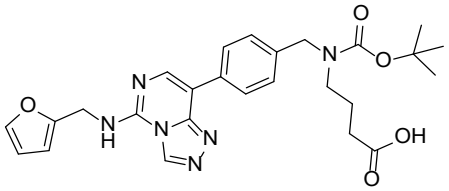
To a flask containing methyl 3-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)cyclobutane-1-carboxylate (**12**) (6.0 mg, 14 µmol, 1.0 equiv) in CH_2Cl_2 (55 µL) was added Boc anhydride (3.3 mg, 15 µmol, 1.1 equiv), NEt_3 (4.8 µL, 35 µmol, 2.5 equiv), and the reaction stirred at room temperature for 16 hr. The reaction was concentrated *in vacuo* and purified by column chromatography (0-10% MeOH in CH_2Cl_2) to yield the desired product as a yellow solid methyl 3-((*tert*-

butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)cyclobutane-1-carboxylate (**15**) (2.8 mg, 38%).

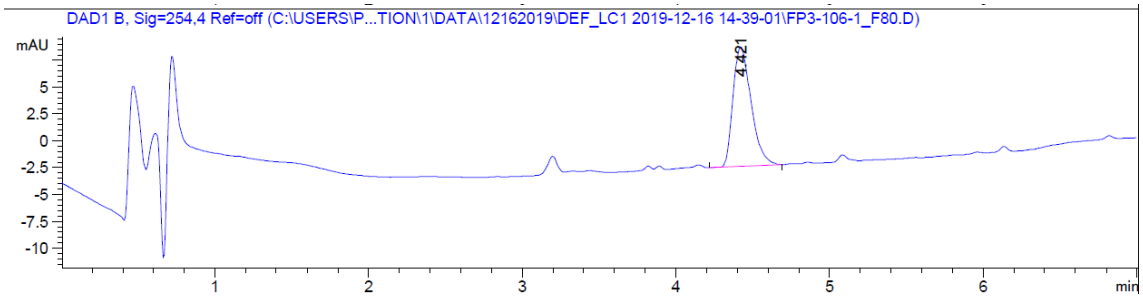
LCMS: expected mass for $[M+H]^+$ ($C_{28}H_{33}N_6O_5$) requires 533.24 m/z, found 533.20 m/z.



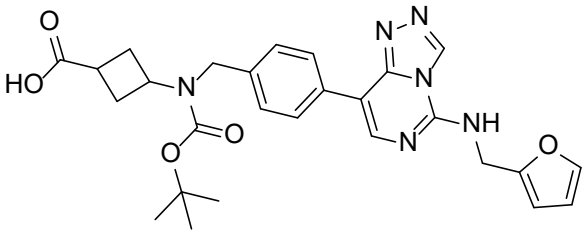
4-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanoic acid, 16



To a flask was added methyl 4-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanoate (**14**) (6.2 mg, 12 µmol, 1.0 equiv) and $LiOH.H_2O$ (1.5 mg, 36 µmol, 3.0 equiv) in H_2O (10 µL) and THF (0.12 mL), and the reaction was left to stir at room temperature for 16 hr. The reaction was concentrated *in vacuo* and purified by reverse phase column chromatography (10-100% MeCN in H_2O (+0.1% TFA)) to yield the desired product as a white gum 4-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanoic acid (**16**) (3.6 mg, 60%). LCMS: expected mass for $[M+H]^+$ ($C_{26}H_{31}N_6O_5$) requires 507.23 m/z, found 507.20 m/z

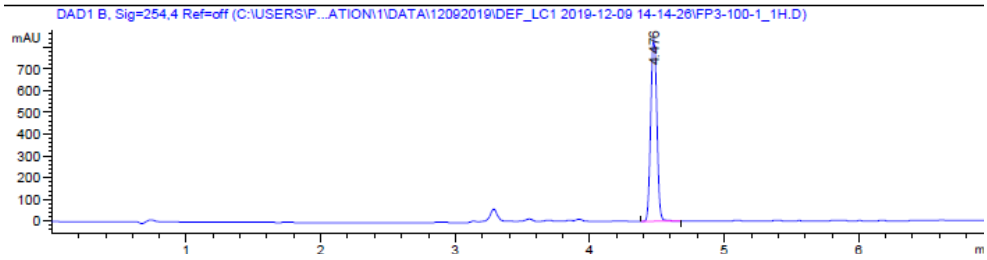


3-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)cyclobutane-1-carboxylic acid, 17

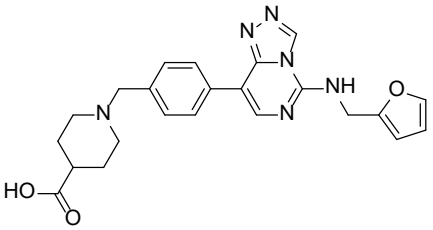


To a flask was added methyl 3-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)cyclobutane-1-carboxylate (**15**) (2.9 mg, 5.4 µmol, 1.0 equiv) and LiOH.H_2O (0.7 mg, 16 µmol, 3.0 equiv) in H_2O (5 µL) and THF (54 µL), and the reaction was left to stir at room temperature for 16 hr. The reaction was concentrated *in vacuo* and purified by reverse phase column chromatography (10-100% MeCN in H_2O (+0.1% TFA)) to yield the desired product as a white solid 3-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)cyclobutane-1-carboxylic acid (**17**) (2.70 mg, 96%).

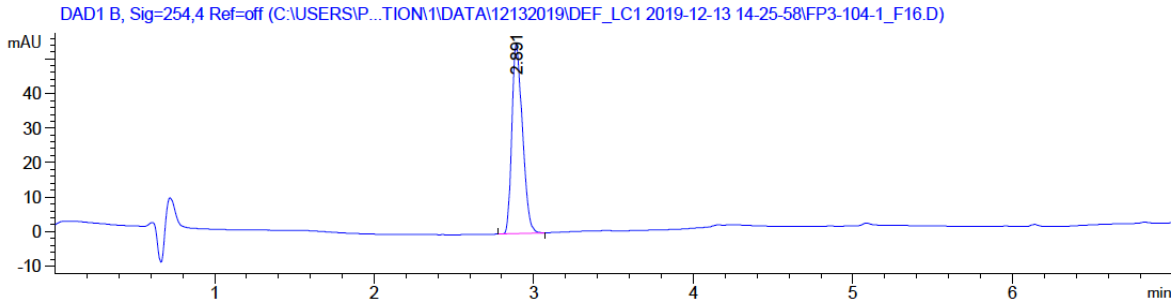
LCMS: expected mass for $[M+H]^+$ ($C_{27}H_{31}N_6O_5$) requires 519.23 m/z, found 519.20 m/z.



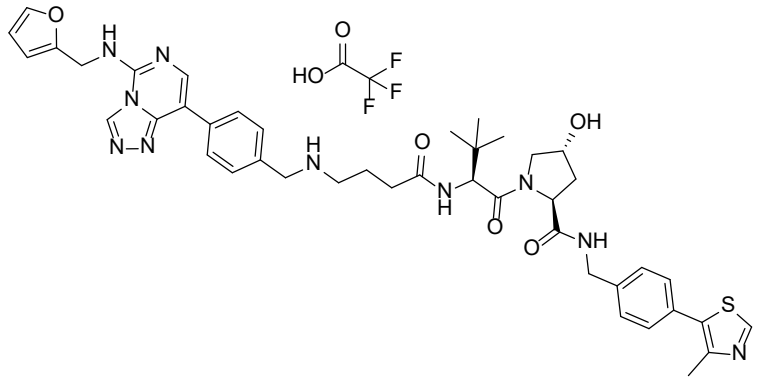
1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)piperidine-4-carboxylic acid, 18



To a flask was added methyl 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)piperidine-4-carboxylate (**13**) (2.2 mg, 4.9 µmol, 1.0 equiv) and LiOH.H_2O (0.6 mg, 15 µmol, 3.0 equiv) in H_2O (4 µL) and THF (50 µL), and the reaction was left to stir at room temperature for 16 hr. The reaction was concentrated *in vacuo* and purified by reverse phase column chromatography (10-100% MeCN in H_2O (+0.1% TFA)) to yield the desired product as a white solid 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)piperidine-4-carboxylic acid (**18**) (2.1 mg, 99%). LCMS: expected mass for $[M+H]^+$ ($C_{23}H_{25}N_6O_3$) requires 433.19 m/z, found 433.20 m/z.

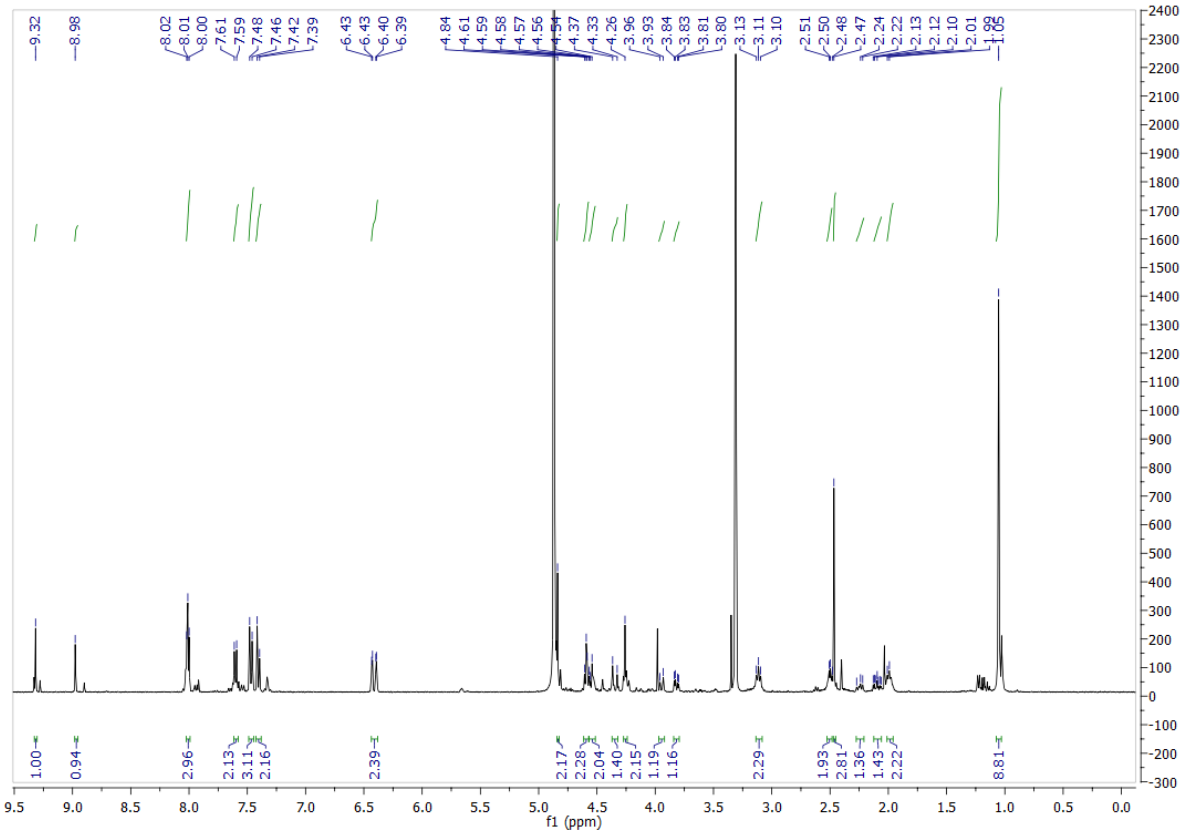


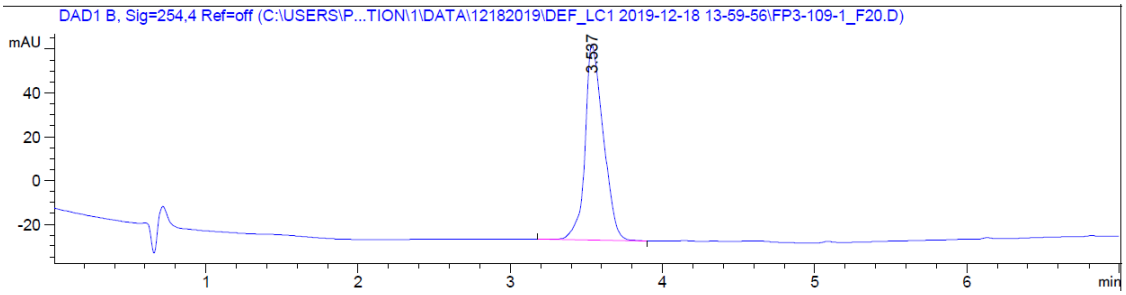
(2*S*,4*R*)-1-((*S*)-2-(4-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate, UNC7744



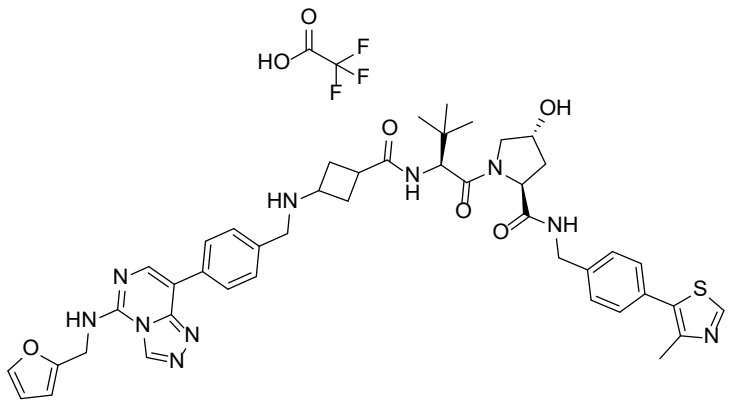
To a flask was added 4-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanoic acid (**16**) (2.1 mg, 4.1 µmol, 1.0 equiv), DIPEA (2.2 µL, 12 µmol, 3.0 equiv), and TBTU (1.7 mg, 5.4 µmol, 1.3 equiv) in DMF (1 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-((1*R*,3*S*)-3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (2.5 mg, 4.6 µmol, 1.1 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and deprotected with 20% TFA in CH_2Cl_2 (1 mL). The reaction was concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a white solid (2*S*,4*R*)-1-((*S*)-2-(4-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (2.61 mg, 67 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.32 (s, 1H), 8.98 (s, 1H), 8.02 – 7.99 (m, 3H), 7.60 (d, *J* = 8.21 Hz, 2H), 7.49 – 7.44 (m, 3H), 7.40 (d, *J* = 8.28 Hz, 2H), 6.41 (dd, *J* = 2.31, 13.92 Hz, 2H), 4.84 (s, 2H), 4.62 – 4.56 (m, 2H), 4.55 – 4.51 (m, 2H), 4.35 (d, *J* = 15.51 Hz, 1H), 4.28 – 4.23 (m, 2H), 3.95 (d, *J* = 11.25 Hz, 1H), 3.82 (dd, *J* = 4.03, 10.94 Hz, 1H), 3.14 – 3.08 (m, 2H), 2.53 – 2.48 (m, 2H), 2.47 (s, 3H), 2.28 – 2.20 (m, 1H), 2.15 – 2.06 (m, 1H), 2.02 – 1.95 (m, 2H), 1.05 (m, 9H). LCMS: expected mass for $[M+H]^+$ ($C_{43}H_{51}N_{10}O_5S$) requires 819.37 m/z, found 819.30 m/z.



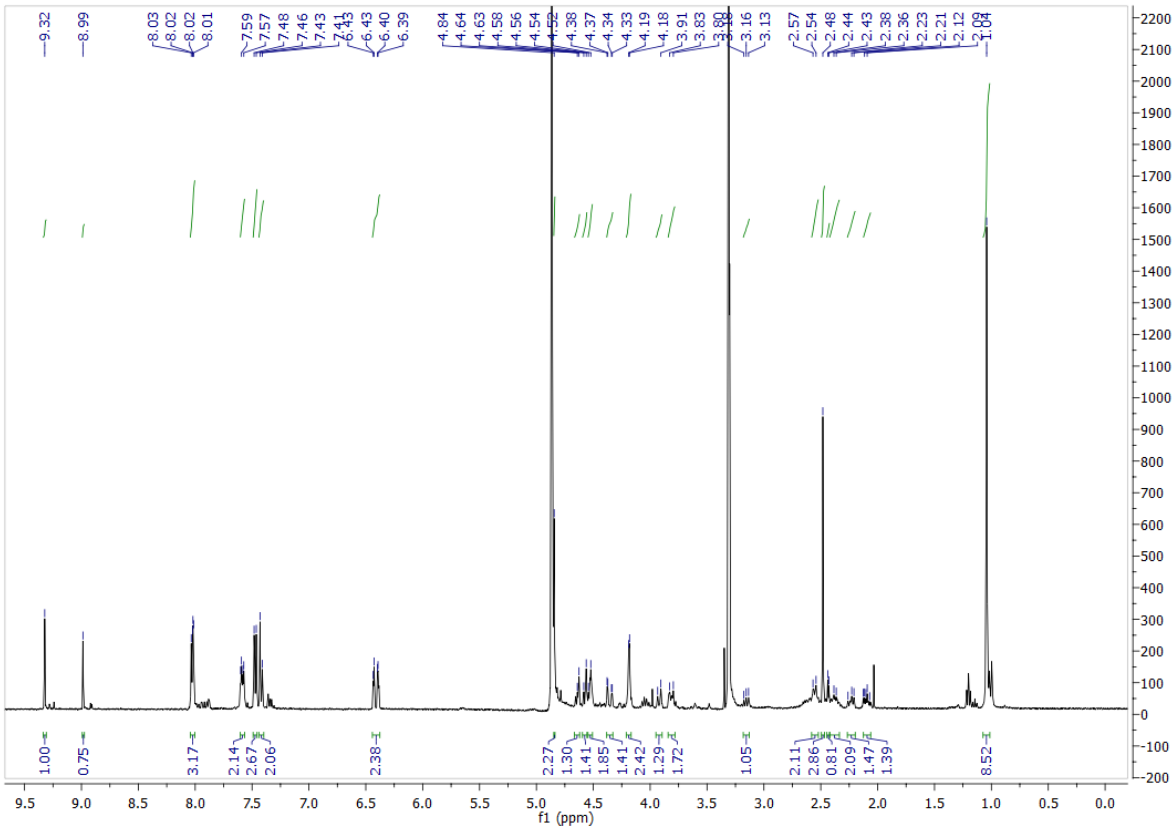


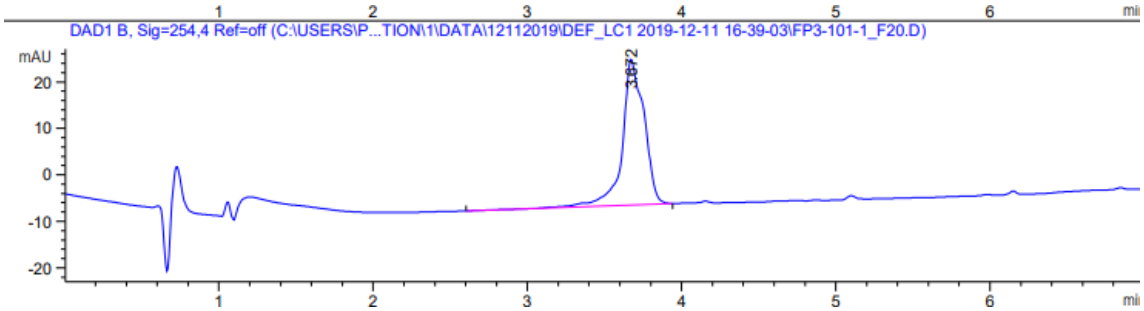
(2*S*,4*R*)-1-((*S*)-2-(3-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate, UNC7742



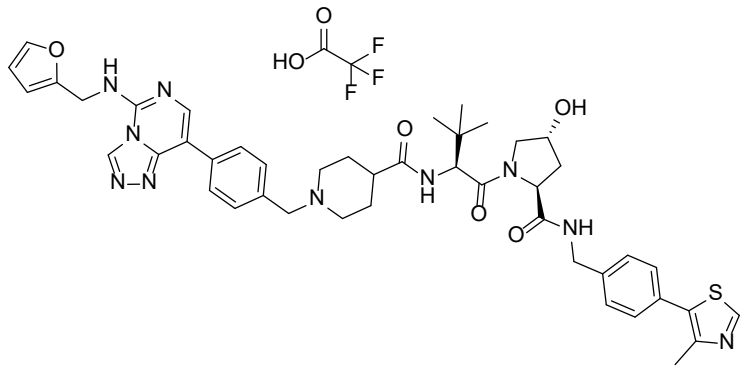
To a flask was added 3-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)cyclobutane-1-carboxylic acid (**17**) (2.7 mg, 5.2 µmol, 1.0 equiv), DIPEA (2.7 µL, 16 µmol, 3.0 equiv), and TBTU (2.2 mg, 6.8 µmol, 1.3 equiv) in DMF (1 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-((1*R*,3*S*)-3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (3.1 mg, 5.7 µmol, 1.1 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and deprotected with 20% TFA in CH_2Cl_2 (1 mL). The reaction was concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a white solid (2*S*,4*R*)-1-((*S*)-2-(3-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (2.2 mg, 46 %) over two steps.

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.32 (s, 1H), 8.99 (s, 1H), 8.05 – 8.00 (m, 3H), 7.59 (dd, *J* = 2.97, 8.17 Hz, 2H), 7.50 – 7.45 (m, 3H), 7.42 (d, *J* = 8.38 Hz, 2H), 6.41 (dd, *J* = 2.48, 14.30 Hz, 2H), 4.84 (s, 2H), 4.65 4.62 (m, 1H), 4.60 – 4.55 (m, 1H), 4.55 – 4.51 (m, 2H), 4.36 (dd, *J* = 2.72, 15.50 Hz, 1H), 4.19 (d, *J* = 2.98 Hz, 2H), 3.92 (d, *J* = 11.23 Hz, 1H), 3.84 – 3.78 (m, 2H), 3.18 – 3.12 (m, 1H), 2.58 – 2.53 (m, 2H), 2.48 (s, 3H), 2.43 (d, *J* = 3.97 Hz, 1H), 2.42 – 2.34 (m, 2H), 2.27 – 2.20 (m, 1H), 2.13 – 2.06 (m, 1H), 1.04 (s, 9H). LCMS: expected mass for $[M+H]^+$ ($C_{44}H_{51}N_{10}O_5S$) requires 831.37 m/z, found 831.30 m/z.



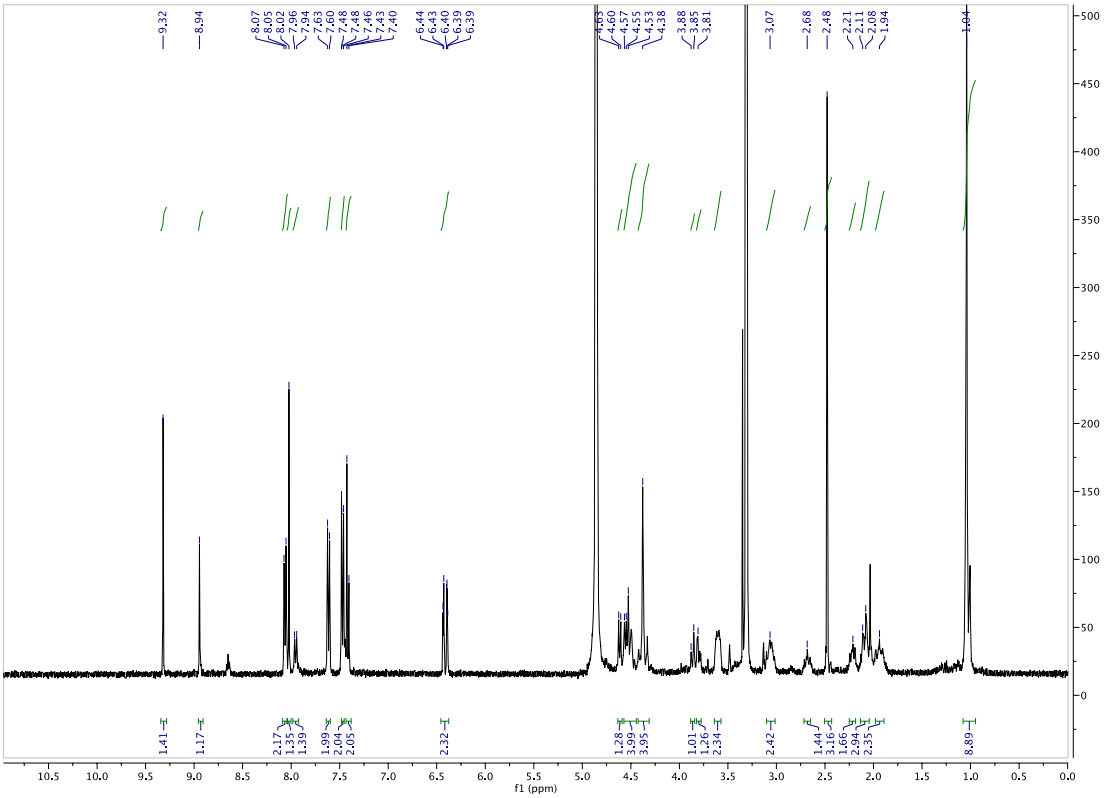


1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)-N-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide 2,2,2-trifluoroacetate, UNC7743

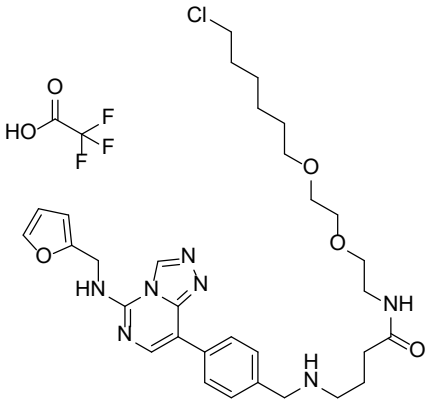


To a flask was added 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)piperidine-4-carboxylic acid (**18**) (2.1 mg, 4.9 µmol, 1.0 equiv), DIPEA (2.5 µL, 15 µmol, 3.0 equiv), and TBTU (2.0 mg, 6.3 µmol, 1.3 equiv) in DMF (1 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-((1*R*,3*S*)-3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (2.9 mg, 5.3 µmol, 1.1 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and deprotected with 20% TFA in CH_2Cl_2 (1 mL). The reaction was concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a white solid 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)-N-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide 2,2,2-trifluoroacetate (1.39 mg, 30 %) over two steps.

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.32 (s, 1H), 8.94 (s, 1H), 8.06 (d, *J* = 8.24 Hz, 2H), 8.02 (s, 1H), 7.95 (d, *J* = 8.80 Hz, 1H), 7.616 (d, *J* = 8.34 Hz, 2H), 7.50 – 7.45 (m, 2H), 7.43 – 7.39 (m, 2H), 6.45 – 6.38 (m, 2H), 4.64 – 4.60 (m, 1H), 4.58 – 4.47 (m, 4H), 4.44 – 4.31 (m, 4H), 3.87 (d, *J* = 10.78 Hz, 1H), 3.80 (dd, *J* = 3.81 – 11.20 Hz, 1H), 3.65 – 3.55 (m, 2H), 3.12 – 3.02 (m, 2H), 2.72 – 2.64 (m, 1H), 2.48 (s, 3H), 2.26 – 2.18 (m, 2H), 2.14 – 2.05 (m, 3H), 1.98 – 1.88 (m, 2H), 1.04 (s, 9H). LCMS: expected mass for $[M+H]^+$ ($C_{45}H_{53}N_{10}O_5S$) requires 845.38 m/z, found 845.30 m/z.



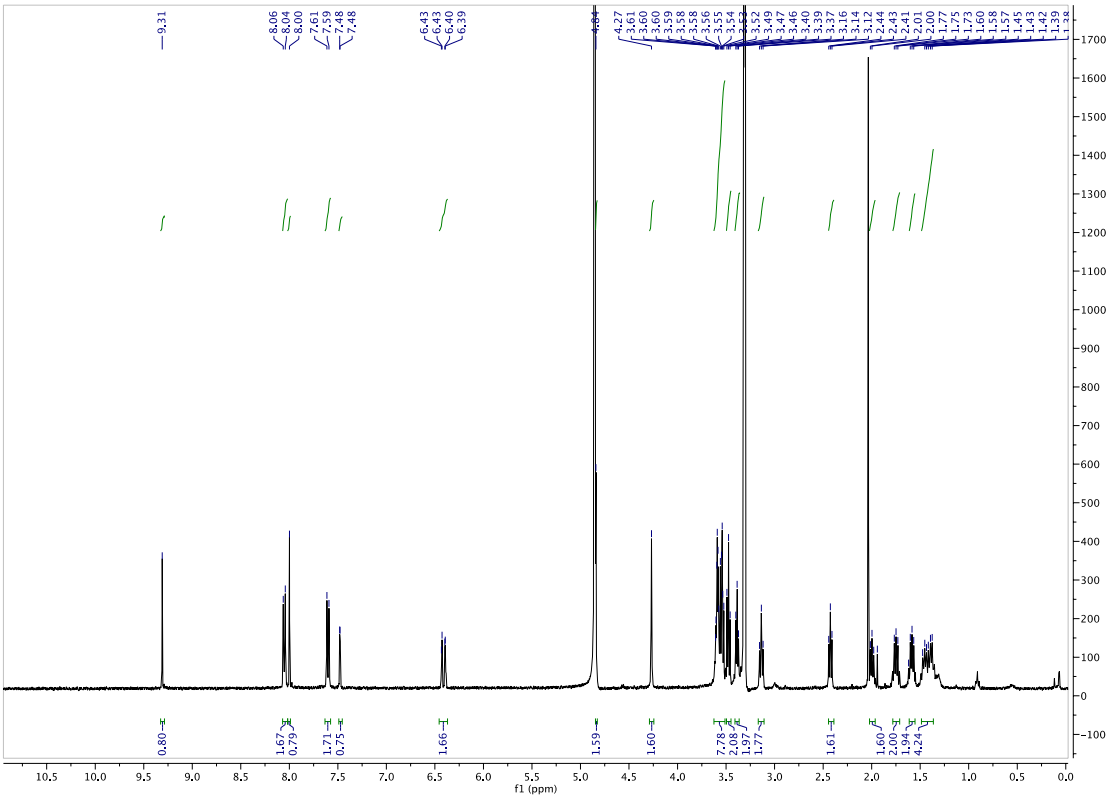
_N_-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-4-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanamide 2,2,2-trifluoroacetate, UNC7805

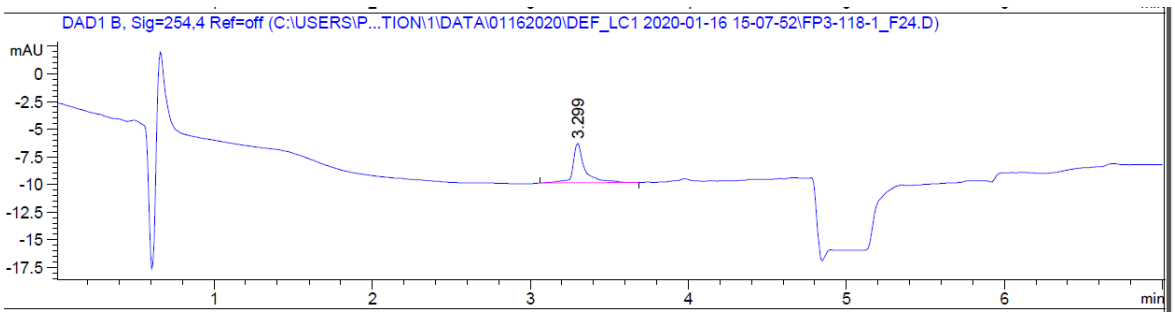


To a flask was added 4-((*tert*-butoxycarbonyl)(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanoic acid (**16**) (1.5 mg, 3.0 µmol, 1.0 equiv), DIPEA (1.5µL, 8.9 µmol,

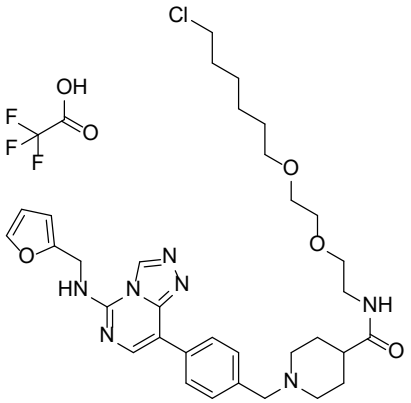
3.0 equiv), and TBTU (1.2 mg, 3.8 µmol, 1.3 equiv) in DMF (0.5 mL), followed by addition of 2-(2-((6-chlorohexyl)oxy)ethoxy)ethan-1-amine 2,2,2-trifluoroacetate (1.0 mg, 3.0 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and deprotected with 20% TFA in CH_2Cl_2 (0.5 mL). The reaction was concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a white solid *N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-4-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)amino)butanamide 2,2,2-trifluoroacetate (0.38 mg, 18 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.31 (s, 1H), 8.05 (d, *J* = 8.37 Hz, 2H), 8.00 (s, 1H), 7.60 (d, *J* = 8.36 Hz, 2H), 7.49 – 7.45 (m, 1H), 6.45 – 6.37 (m, 2H), 4.84 (s, 2H), 4.27 (s, 2H), 3.62 – 3.52 (m, 8H), 3.47 (t, *J* = 6.55 Hz, 2H), 3.39 (t, *J* = 5.44 Hz, 2H), 3.14 (t, *J* = 7.27 Hz, 2H), 2.43 (t, *J* = 6.69 Hz, 2H), 2.02 – 1.96 (m, 2H), 1.79 – 1.70 (m, 2H), 1.63 – 1.53 (m, 2H), 1.50 – 1.36 (m, 4H). LCMS: expected mass for $[M+H]^+$ ($C_{31}H_{43}ClN_7O_4$) requires 612.30 m/z, found 612.30 m/z.



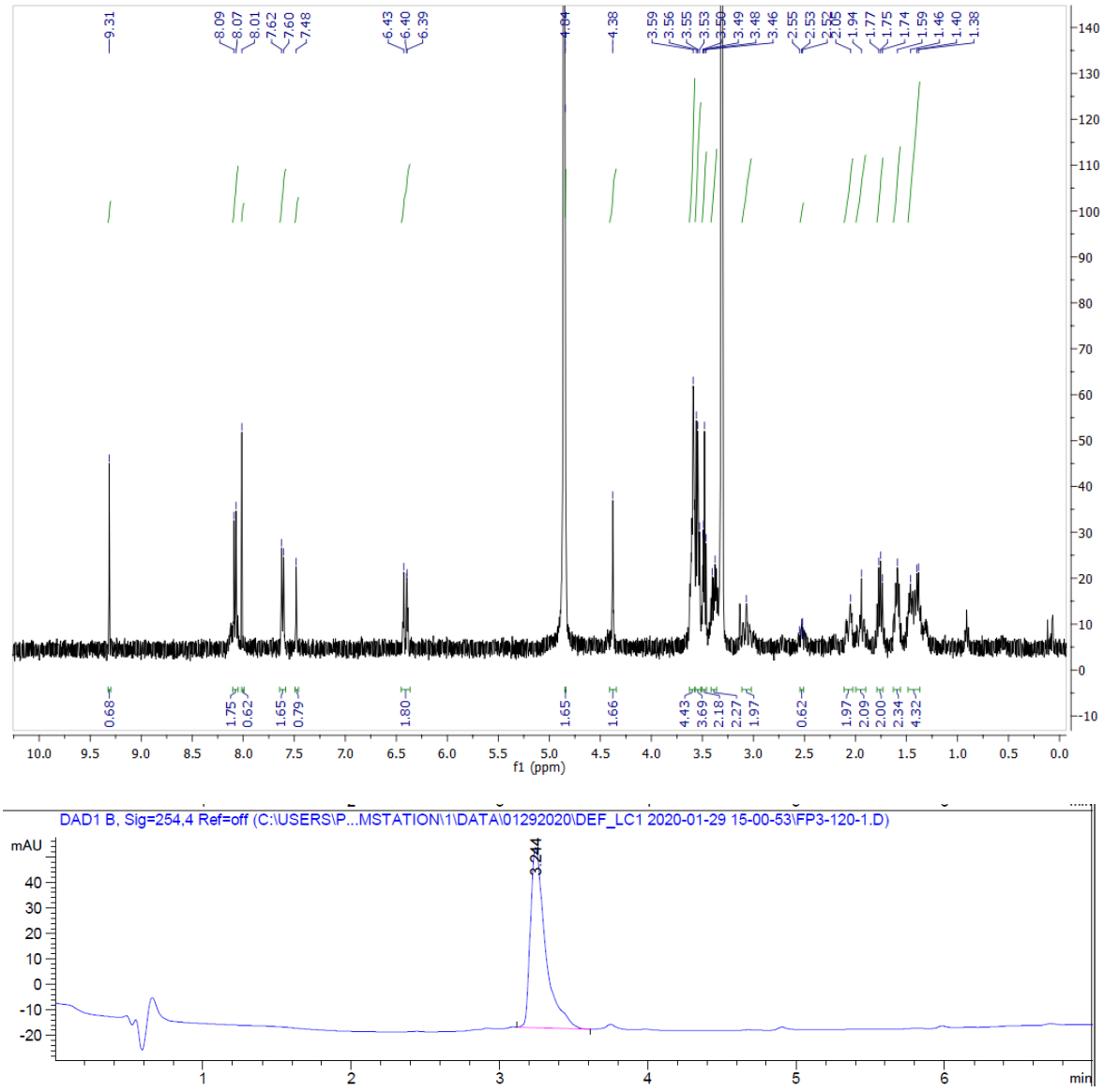


***N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)piperidine-4-carboxamide 2,2,2-trifluoroacetate, UNC7806**

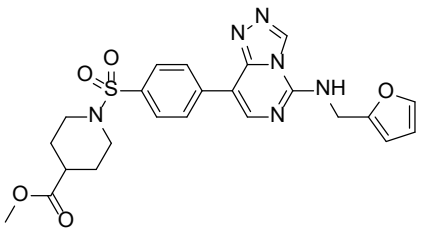


To a flask was added 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)piperidine-4-carboxylic acid (**18**) (0.9 mg, 2.1 µmol, 1.0 equiv), DIPEA (1.1 µL, 6.2 µmol, 3.0 equiv), and TBTU (0.9 mg, 2.7 µmol, 1.3 equiv) in DMF (0.5 mL), followed by addition of 2-(2-((6-chlorohexyl)oxy)ethoxy)ethan-1-amine 2,2,2-trifluoroacetate (0.8 mg, 2.3 µmol, 1.1 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA). The product was concentrated to yield the desired product as a white solid *N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzyl)piperidine-4-carboxamide 2,2,2-trifluoroacetate (0.49 mg, 31 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.31 (s, 1H), 8.08 (d, *J* = 8.38 Hz, 2H), 8.01 (s, 1H), 7.61 (d, *J* = 8.10 Hz, 2H), 7.49 – 7.47 (m, 1H), 6.44 – 6.37 (m, 2H), 4.84 (s, 2H), 4.38 (s, 2H), 3.64 – 3.58 (m, 4H), 3.57 – 3.52 (m, 4H), 3.50 – 3.45 (m, 2H), 3.42 – 3.34 (m, 2H), 3.07 (t, *J* = 12.11 Hz, 2H), 2.56 – 2.48 (m, 1H), 2.10 – 2.02 (m, 2H), 1.96 – 1.91 (m, 2H), 1.79 – 1.72 (m, 2H), 1.63 – 1.55 (m, 2H), 1.49 – 1.36 (m, 4H). LCMS: expected mass for $[M+H]^+$ ($C_{33}H_{45}ClN_7O_4$) requires 638.31 m/z, found 638.30 m/z.



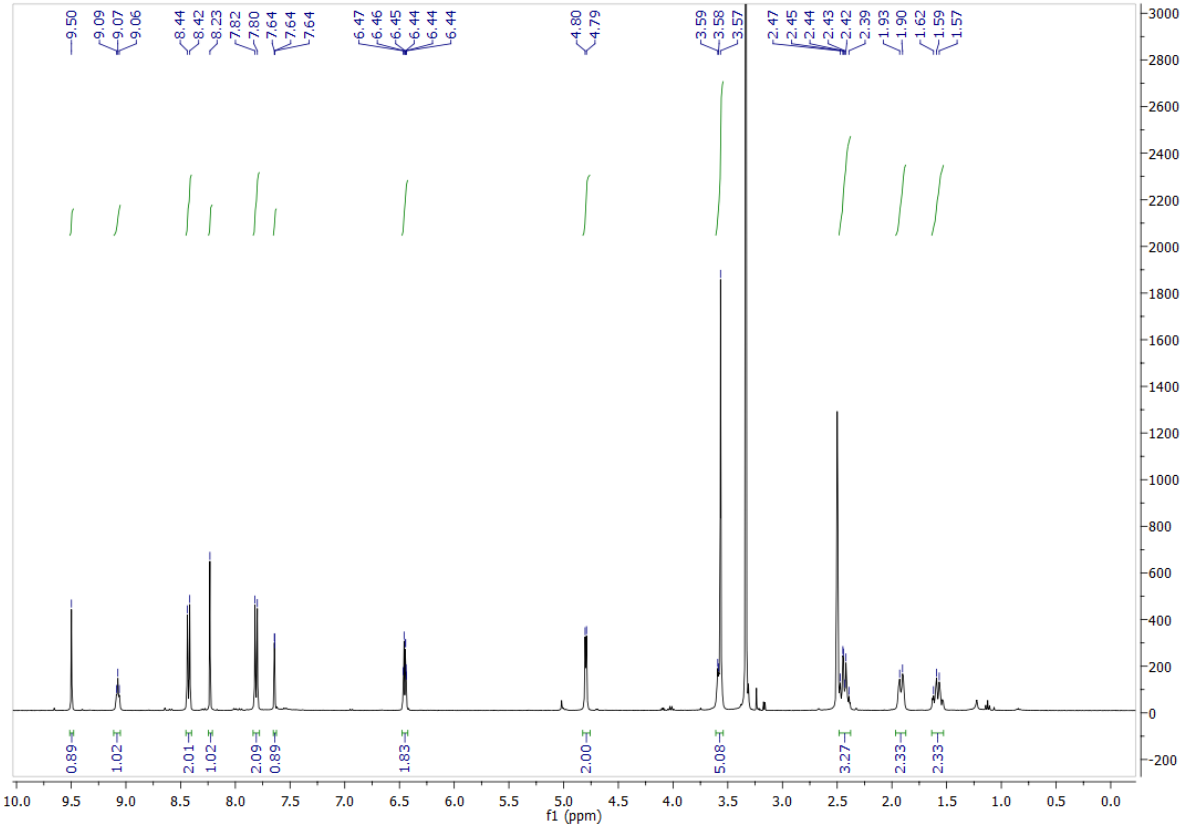
Methyl 1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxylate, 19

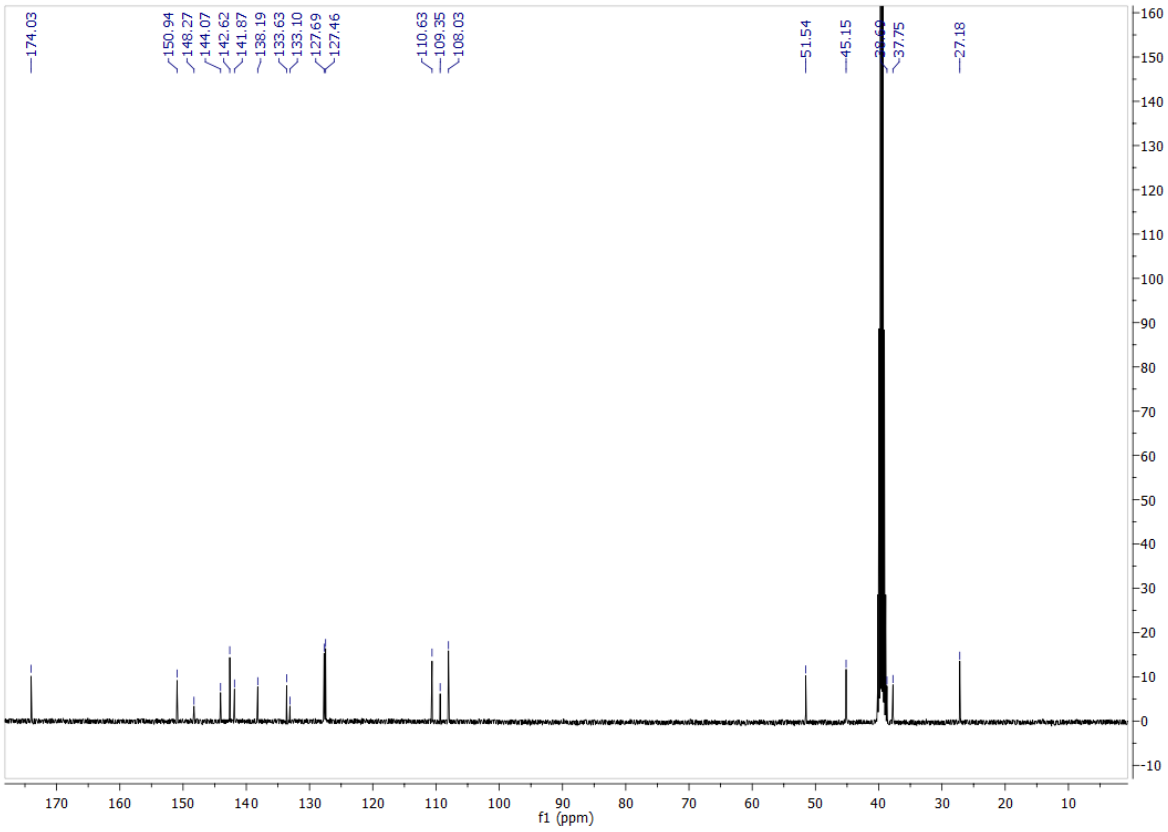


To a microwave vial was added methyl 1-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperidine-4-carboxylate (209 mg, 510 µmol, 1.5 equiv), 8-bromo-*N*-(furan-2-ylmethyl)-[1,2,4]triazolo[4,3-*c*]pyrimidin-5-amine (100 mg, 340 µmol, 1.0 equiv), $Pd(dppf)Cl_2.CH_2Cl_2$ (56 mg, 68 µmol, 0.2 equiv), sodium bicarbonate (71.4 mg, 850 µmol, 2.5 equiv) in THF (1 mL) and H_2O (0.5 mL). The vial was sealed at heated to 90 °C for 16 hr. The reaction was cooled and filtered through celite with EtOAc. The organics were concentrated *in vacuo* to yield the crude product. Crude material was purified by column chromatography (0-10% MeOH in CH_2Cl_2) to yield the desired product as a

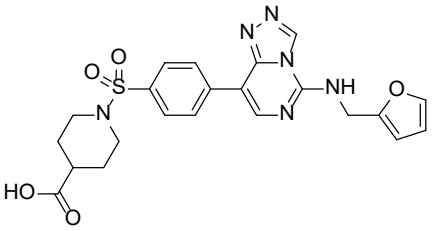
white solid methyl 1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxylate (**19**) (12.7 mg, 8%).

^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.50 (s, 1H), 9.07 (t, *J* = 5.19 Hz, 1H), 8.43 (d, *J* = 8.59 Hz, 2H), 8.23 (s, 1H), 7.81 (d, *J* = 8.60 Hz, 2H), 7.66 – 7.61 (m, 1H), 6.49 – 6.42 (m, 2H), 4.80 (d, *J* = 5.00 Hz, 2H), 3.61 – 3.54 (m, 5H), 2.48 – 2.37 (m, 3H), 1.95 – 1.88 (m, 2H), 1.57 (dt, *J* = 7.50, 14.86 Hz, 2H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 174.03, 150.94, 148.27, 144.07, 142.62, 141.87, 138.19, 133.63, 133.10, 127.69, 127.46, 110.63, 109.35, 108.03, 51.54, 45.15, 38.69, 37.75, 27.18. LCMS: expected mass for $[M+H]^+$ ($C_{23}H_{25}N_6O_5S$) requires 497.15 m/z, found 497.15 m/z.



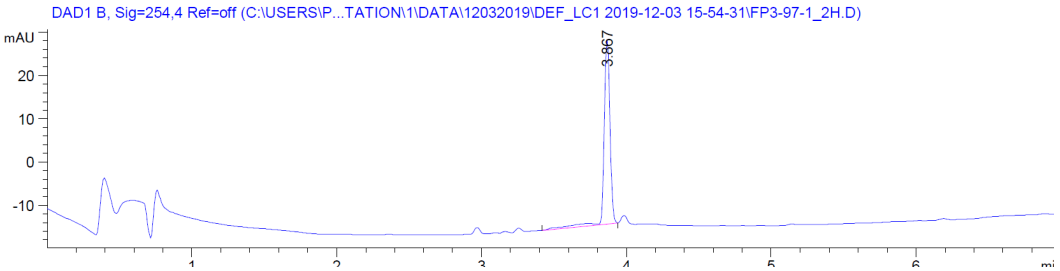


1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxylic acid, 20

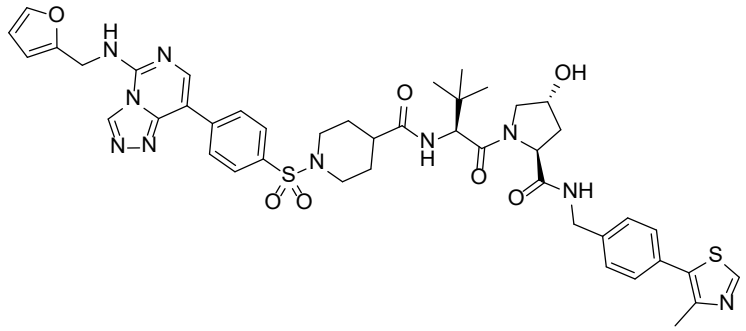


To a flask was added methyl 1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxylate (**19**) (12.7 mg, 26 µmol, 1.0 equiv) and $LiOH.H_2O$ (3.2 mg, 76.7 µmol, 3.0 equiv) in THF (256 µL) and H_2O (256 µL) and the reaction left to stir for 16 hr. The reaction was concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (10-100% MeCN in H_2O (+ 0.1% TFA)) to yield the desired product as a white solid 1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxylic acid (**20**) (5.8 mg, 47 %).

LCMS: expected mass for $[M+H]^+$ ($C_{22}H_{23}N_6O_5S$) requires 483.14 m/z, found 483.10 m/z.

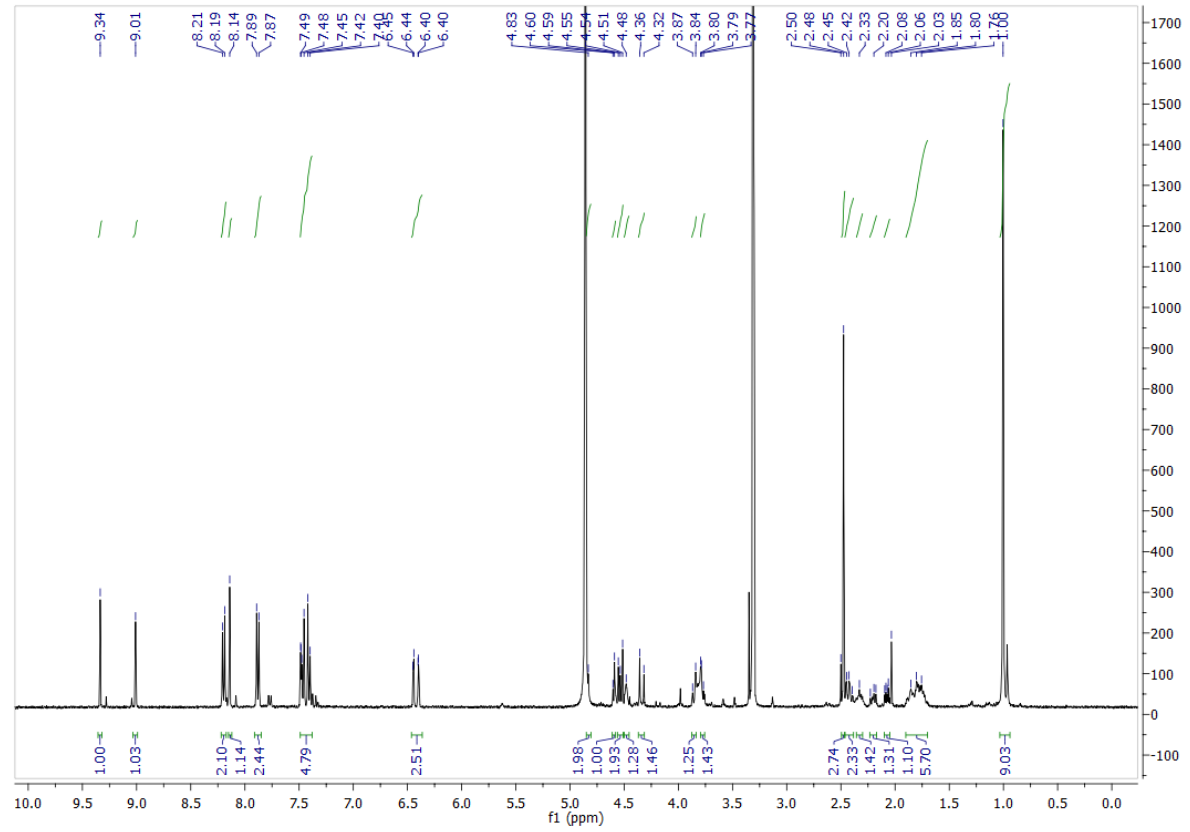


1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)phenyl)sulfonyl)-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide, UNC7741

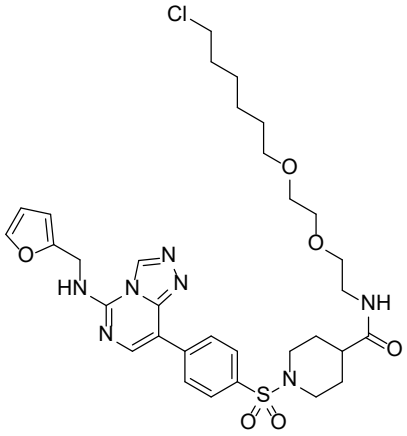


To a flask was added 1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxylic acid (**20**) (3.7 mg, 7.7 µmol, 1.0 equiv), DIPEA (4 µL, 23 µmol, 3.0 equiv), and TBTU (3.2 mg, 10 µmol, 1.3 equiv) in DMF (1 mL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-((1*R*,3*S*)-3-aminocyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (4.6 mg, 8.4 µmol, 1.1 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA) to yield the desired product as a white solid 1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)phenyl)sulfonyl)-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-4-carboxamide (0.97 mg, 14 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.34 (s, 1H), 9.01 (s, 1H), 8.20 (d, *J* = 8.51 Hz, 2H), 8.14 (s, 1H), 7.88 (d, *J* = 8.54 Hz, 2H), 7.49 – 7.39 (m, 5H), 6.46 – 6.38 (m, 2H), 4.83 (s, 2H), 4.60 – 4.57 (m, 1H), 4.56 – 4.51 (m, 2H), 4.50 – 4.45 (m, 1H), 4.34 (d, *J* = 15.61 Hz, 1H), 3.85 (d, *J* = 11.39 Hz, 1H), 3.78 (dd, *J* = 3.40, 10.81 Hz, 1H), 2.50 (s, 3H), 2.47 – 2.39 (m, 2H), 2.37 – 2.29 (m, 1H), 2.23 – 2.17 (m, 1H), 2.10 – 2.04 (m, 1H), 1.90 – 1.70 (m, 6H), 1.00 (s, 9H). LCMS: expected mass for $[M+H]^+$ ($C_{44}H_{51}N_{10}O_7S_2$) requires 895.33 m/z, found 895.20 m/z.



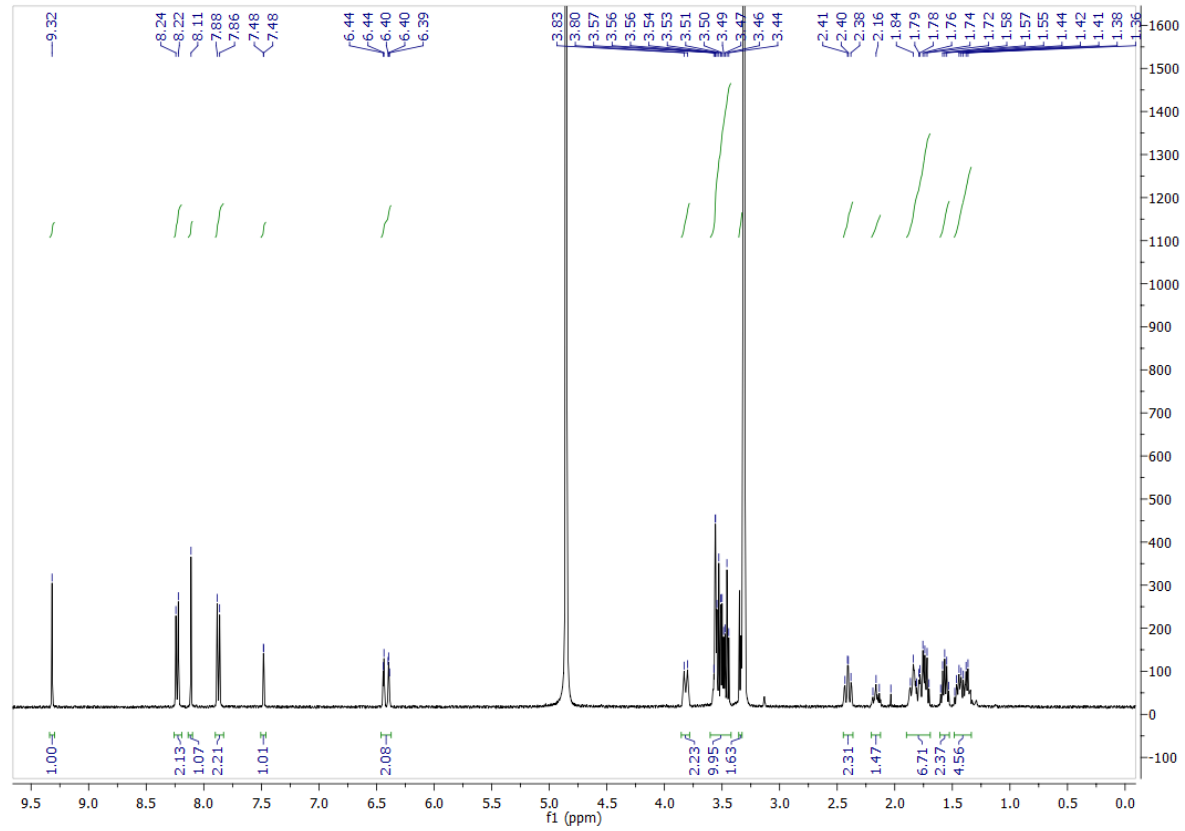
***N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxamide, UNC7793**

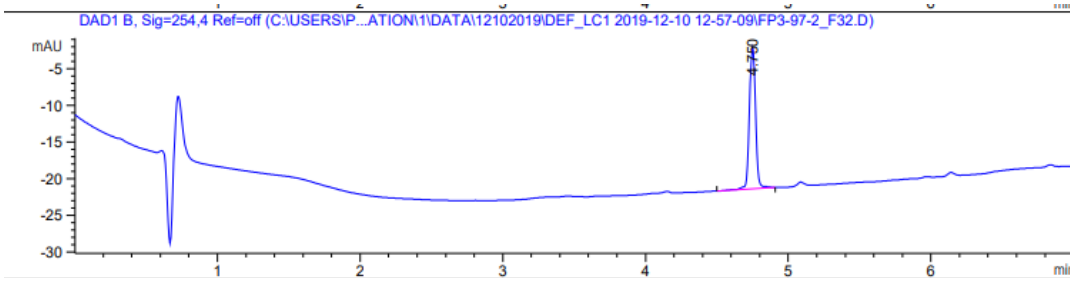


To a flask was added 1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxylic acid (**20**) (2.1 mg, 4.4 µmol, 1.0 equiv), DIPEA (2.3 µL, 13

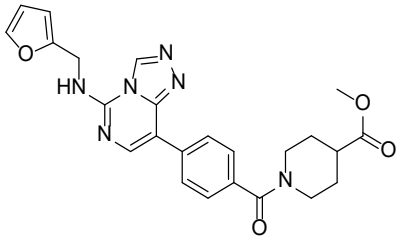
µmol, 3.0 equiv), and TBTU (1.8 mg, 5.7 µmol, 1.3 equiv) in DMF (0.5 mL), followed by addition of 2-(2-((6-chlorohexyl)oxy)ethoxy)ethan-1-amine 2,2,2-trifluoroacetate (1.6 mg, 4.8 µmol, 1.1 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA) to yield the desired product as a white solid *N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxamide (0.97 mg, 32 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.32 (s, 1H), 8.23 (d, *J* = 8.58 Hz, 2H), 8.11 (s, 1H), 7.87 (d, *J* = 8.56 Hz, 2H), 7.50 – 7.46 (m, 1H), 6.45 – 6.37 (m, 2H), 3.81 (m, 2H), 3.59 – 3.43 (m, 10H), 3.36 – 3.33 (m, 2H), 2.41 (td, *J* = 2.72, 11.66, 11.65 Hz, 2H), 2.21 – 2.10 (m, 2H), 1.89 – 1.69 (m, 7H), 1.57 (dt, *J* = 6.73, 13.79 Hz, 2H), 1.48 – 1.33 (m, 5H). LCMS: expected mass for $[M+H]^+$ ($C_{32}H_{43}ClN_7O_6S$) requires 688.26 m/z, found 688.15 m/z.



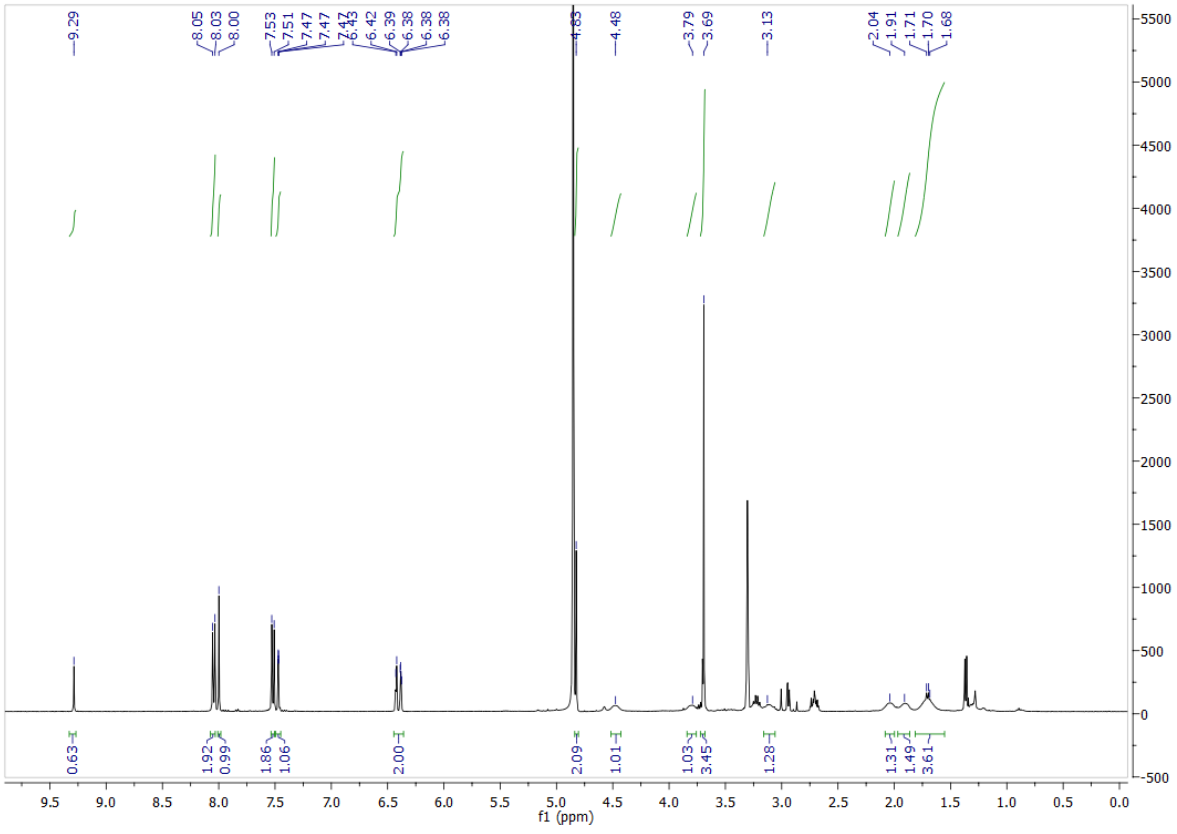


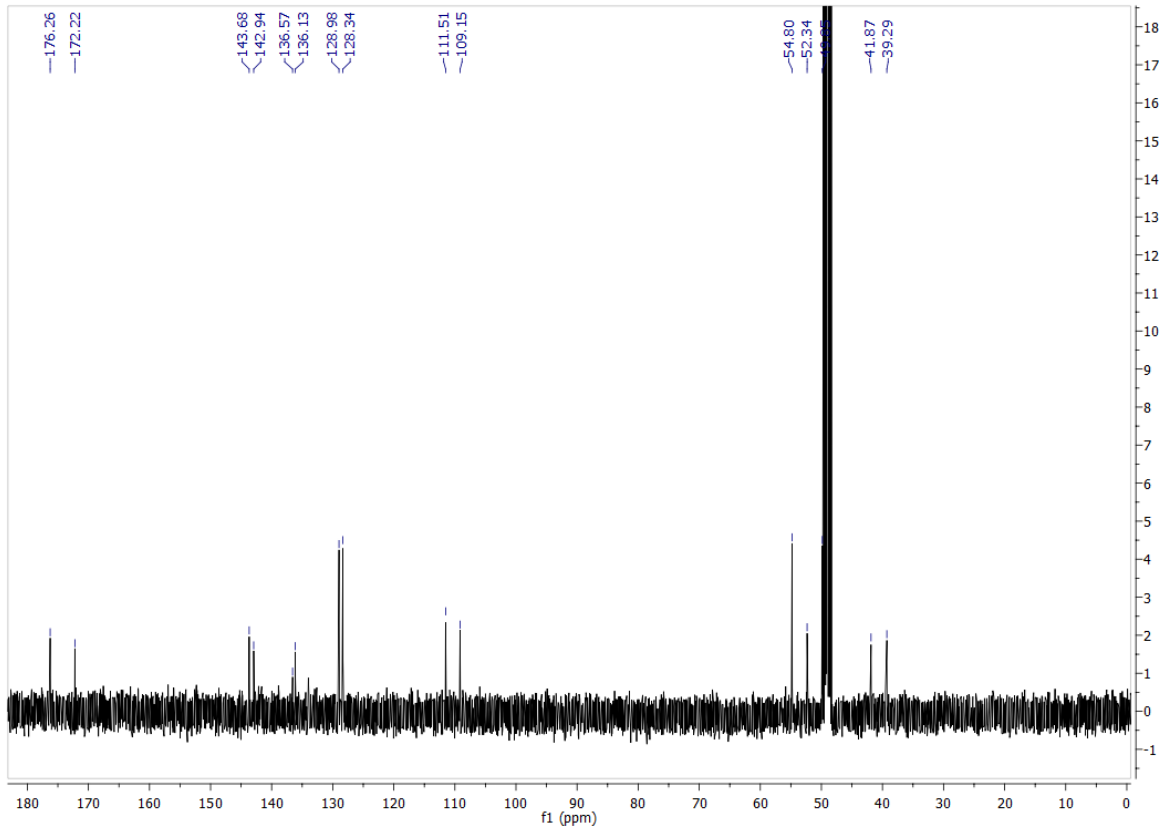
Methyl 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)piperidine-4-carboxylate, 22



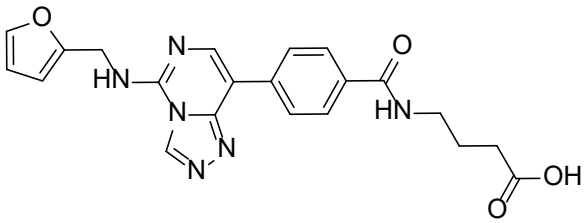
To a flask was added 1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxylic acid (**9**) (20 mg, 60 µmol, 1.0 equiv), DIPEA (31 µL, 180 µmol, 3.0 equiv), and TBTU (25 mg, 78 µmol, 1.3 equiv) in DMF (0.6 mL), followed by addition of methyl piperidine-4-carboxylate 2,2,2-trifluoroacetate (17 mg, 66 µmol, 1.1 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by normal phase column chromatography (0-10% MeOH in CH_2Cl_2) to yield the desired product as a white solid methyl 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)piperidine-4-carboxylate (**22**) (15.3 mg, 56 %).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.29 (s, 1H), 8.04 (d, *J* = 8.42 Hz, 2H), 8.00 (s, 1H), 7.52 (d, *J* = 8.41 Hz, 2H), 7.48 – 7.45 (m, 1H), 6.44 – 6.36 (m, 2H), 4.83 (s, 2H), 4.53 – 4.43 (m, 1H), 3.84 – 3.76 (m, 1H), 3.69 (s, 3H), 3.17 – 3.03 (m, 1H), 2.10 – 2.00 (m, 1H), 1.94 – 1.86 (m, 1H), 1.77 – 1.59 (m, 4H). ^{13}HC NMR (CD_3OD-d_4, 101 MHz): δ 176.26, 172.22, 143.68, 142.94, 136.57, 136.13, 128.98, 128.34, 111.51, 109.15, 54.80, 52.34, 49.85, 41.87, 39.29. LCMS: expected mass for $[M+H]^+$ ($C_{24}H_{25}N_6O_4$) requires 461.19 m/z, found 461.20 m/z.





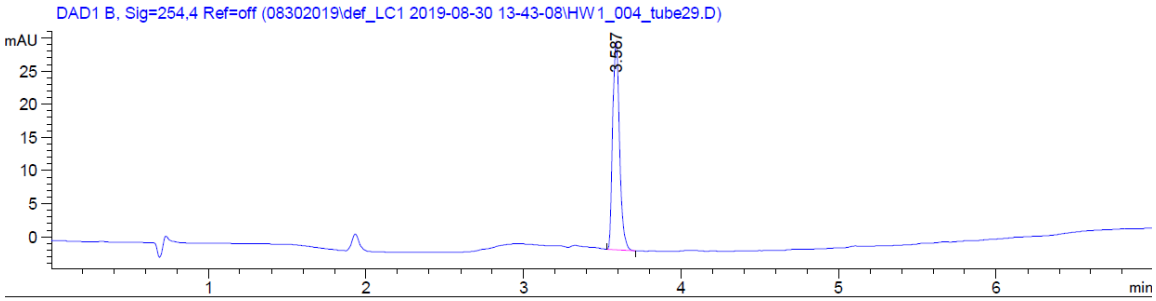
4-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)butanoic acid, 23



To a flask was added 1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxylic acid (**9**) (50.8 mg, 151 µmol, 1.0 equiv), DIPEA (80 µL, 453 µmol, 3.0 equiv), and TBTU (62.2 mg, 194 µmol, 1.3 equiv) in DMF (0.5 mL), followed by addition of methyl 4-aminobutanoate 2,2,2-trifluoroacetate (42.9 mg, 186 µmol, 1.22 equiv) in DMF (1.0 mL). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by column chromatography (0-10% MeOH in CH_2Cl_2). The product was concentrated to yield the desired product

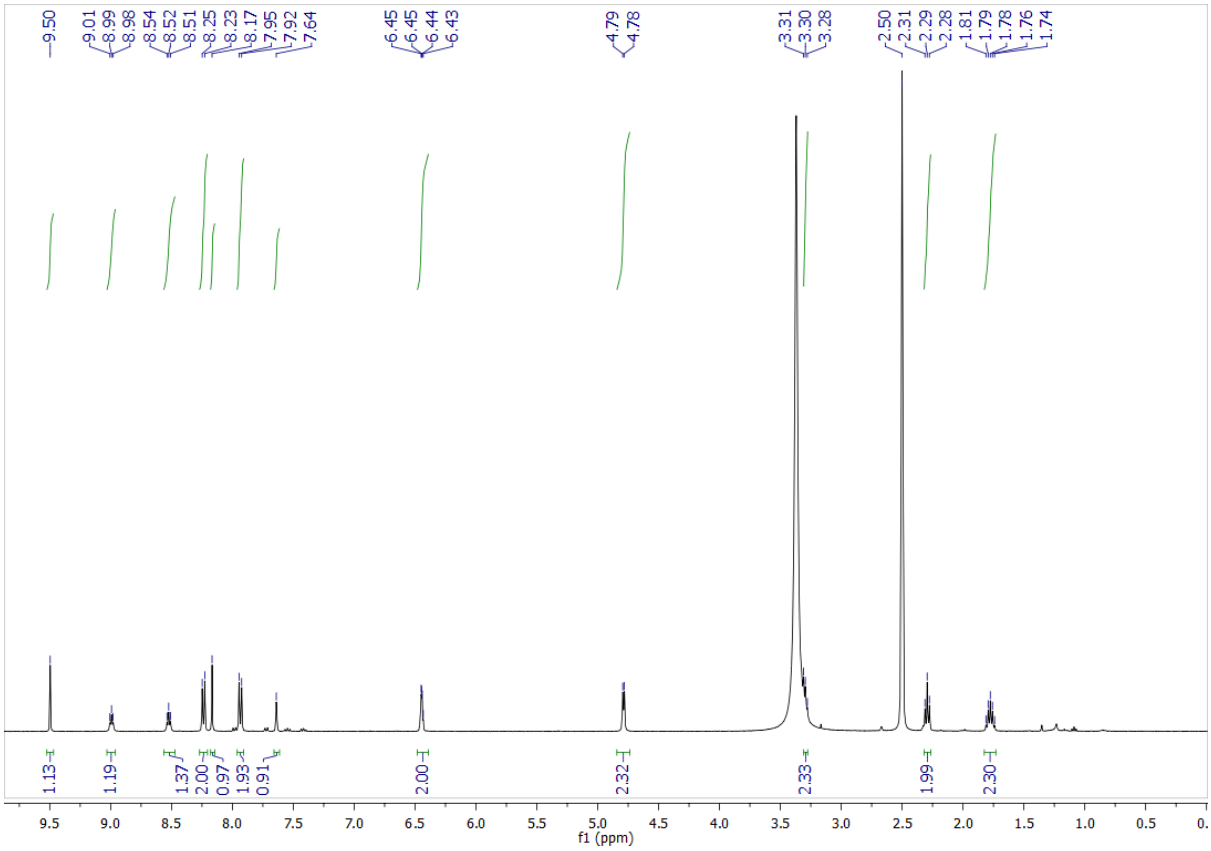
as a clear gum methyl 4-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)benzamido)butanoate (**21**).

LCMS: expected mass for $[M+H]^+$ ($C_{22}H_{23}N_6O_4$) requires 435.17 m/z, found 435.20 m/z.

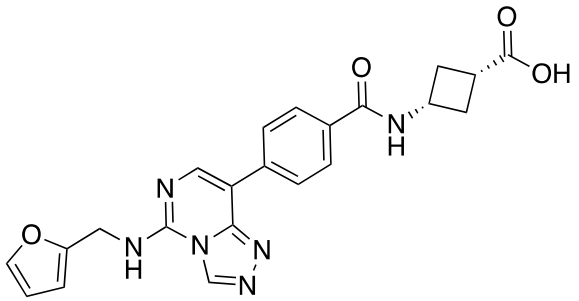


Intermediate methyl 4-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)benzamido)butanoate (**21**) was telescoped into the next step. To a flask was added methyl 4-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)benzamido)butanoate, $LiOH.H_2O$ (23.9 mg, 570 µmol, 3.8 equiv) in THF (1.2 mL) and H_2O (0.3 mL) and the reaction was stirred at 50 °C for 3 hr. THF was removed *in vacuo* and 1M HCl was added, the precipitate was filtered under vacuum and washed with H_2O then Et_2O to yield the desired product as a beige solid 4-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)butanoic acid (**22**) (44.5 mg, 70%) over two steps.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.50 (s, 1H), 8.99 (t, *J* = 5.27 Hz, 1H), 8.52 (t, *J* = 5.61 Hz, 1H), 8.24 (d, *J* = 8.49 Hz, 2H), 8.17 (s, 1H), 7.94 (d, *J* = 8.50 Hz, 2H), 7.65 – 7.62 (m, 1H), 6.44 (m, 2H), 4.79 (d, *J* = 5.18 Hz, 2H), 3.32 – 3.27 (m, 2H), 2.29 (t, *J* = 7.35 Hz, 2H), 1.82 – 1.74 (m, 2H).

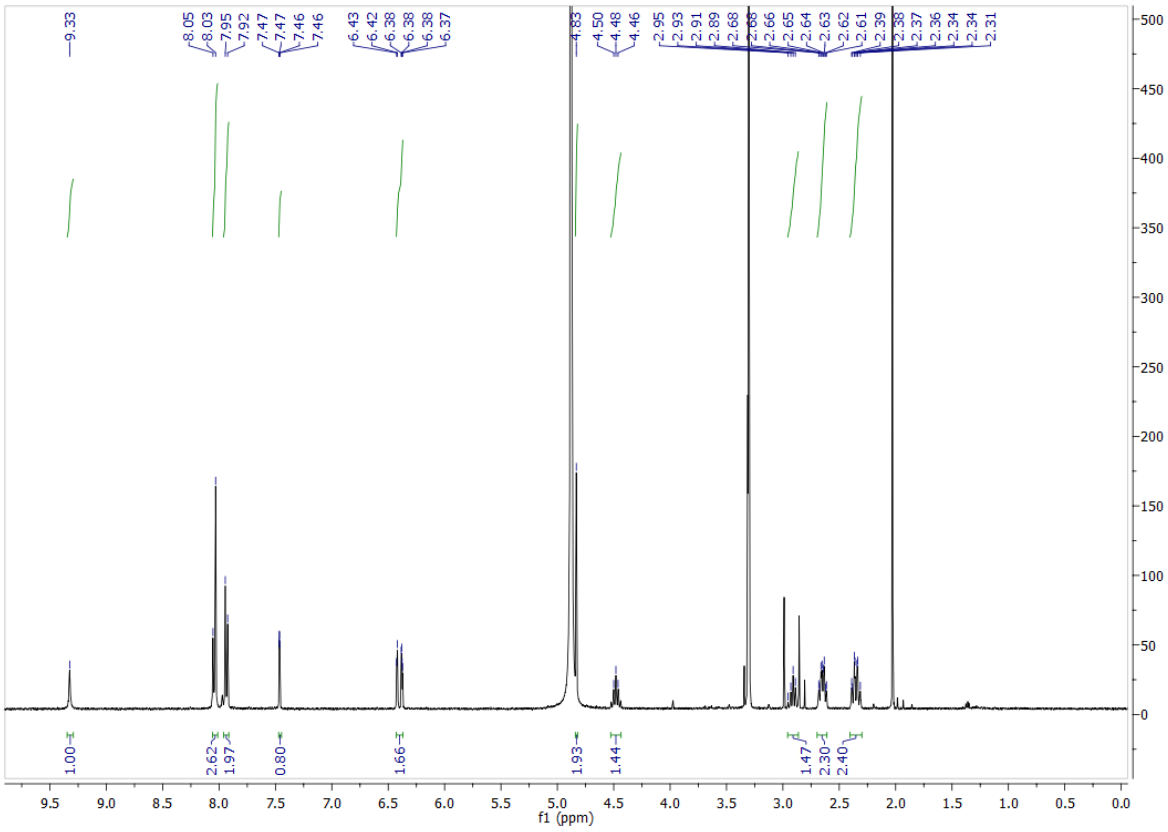


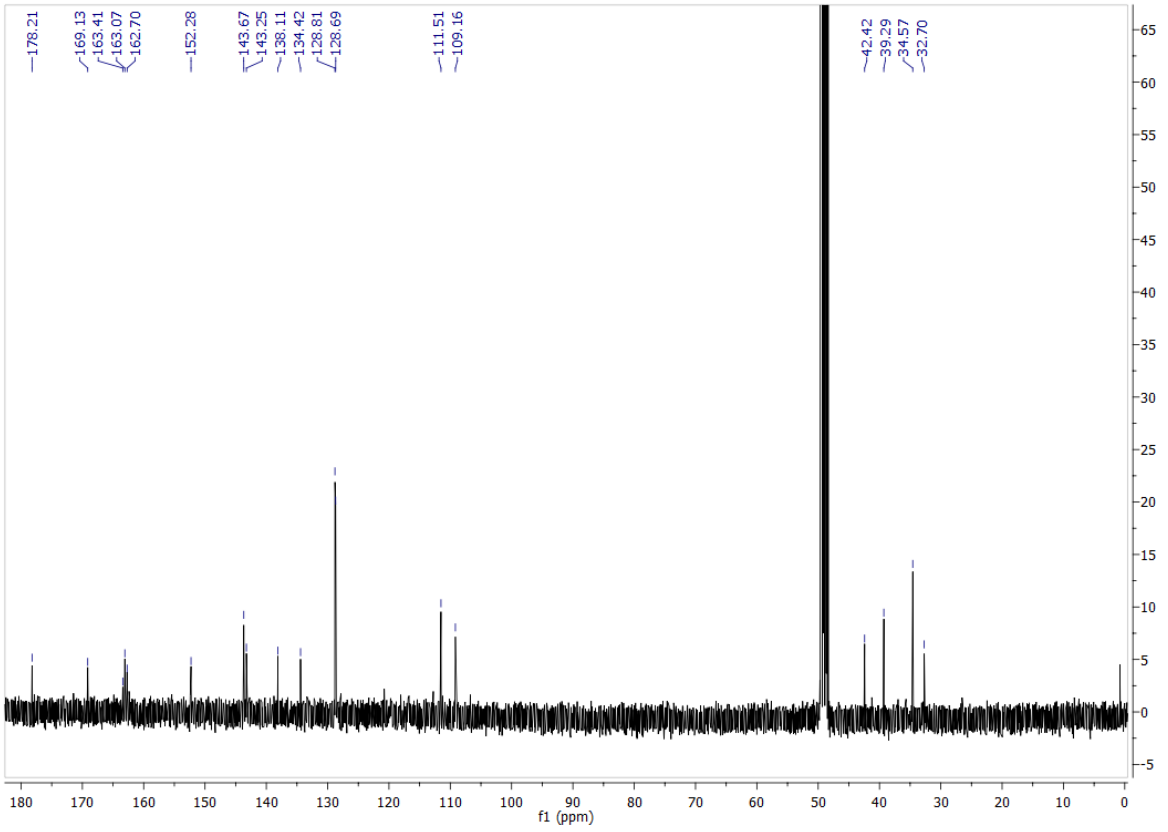
(1*S*,3*S*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxylic acid, 25

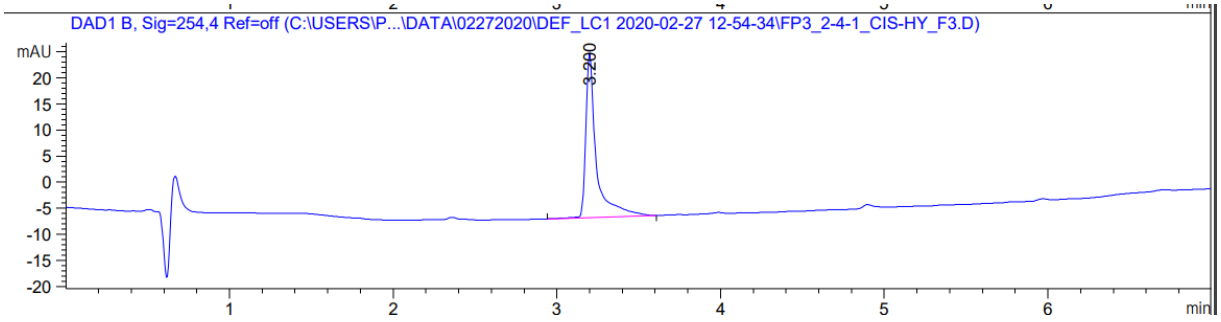


To a flask was added 1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxylic acid (**9**) (20 mg, 60 µmol, 1.0 equiv), DIPEA (33 µL, 192 µmol, 3.2 equiv), and TBTU (25 mg, 78 µmol, 1.3 equiv) in DMF (0.5 mL), followed by addition of methyl (1*S*,3*S*)-3-aminocyclobutane-1-carboxylate 2,2,2-trifluoroacetate (16 mg, 66 µmol, 1.1 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by column chromatography (0-10% MeOH in CH_2Cl_2). The product was concentrated to yield the desired product as a clear gum. Intermediate methyl (1*S*,3*S*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxylate was telescoped into the next step. To a flask was added methyl (1*S*,3*S*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxylate, $LiOH.H_2O$ (7.5 mg, 180 µmol, 3.0 equiv) in THF (0.24 mL) and H_2O (54 µL) and the reaction was stirred at 50 °C for 16 hr. THF was removed *in vacuo* and 1M HCl was added, the crude material concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA) to yield the desired product as a white solid (1*S*,3*S*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxylic acid (**25**) (9.4 mg, 36%) over two steps.

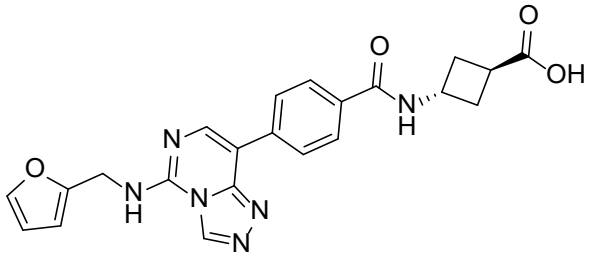
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.33 (s, 1H), 8.07 – 8.01 (m, 3H), 7.93 (d, *J* = 8.46, 2H), 7.49 – 7.45 (m, 1H), 6.44 – 6.36 (m, 2H), 4.83 (s, 2H), 4.53 – 4.43 (m, 1H), 2.95 – 2.86 (m, 1H), 2.65 (ddd, *J* = 2.64, 7.84, 15.65, 2H), 2.41 – 2.29 (m, 2H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 178.21, 169.13, 163.41, 163.07, 162.70, 152.28, 143.67, 143.25, 138.11, 134.42, 128.81, 128.69, 111.51, 109.16, 42.42, 39.29, 34.57, 32.70. LCMS: expected mass for $[M+H]^+$ ($C_{22}H_{21}N_6O_4$) requires 433.15 m/z, found 433.20 m/z.


9.33
8.05
8.03
7.95
7.92
7.47
7.47
7.46
7.46
6.43
6.42
6.38
6.38
6.37
4.83
4.50
4.48
4.46
2.95
2.93
2.91
2.89
2.68
2.66
2.65
2.64
2.63
2.62
2.61
2.39
2.38
2.37
2.36
2.34
2.34
2.31
1.00
2.62
1.97
0.80
1.66
1.93
1.44
1.47
2.30
2.40
f1 (ppm)


178.21
169.13
163.41
163.07
162.70
152.28
143.67
143.25
138.11
134.42
128.81
128.69
111.51
109.16
42.42
39.29
34.57
32.70
f1 (ppm)

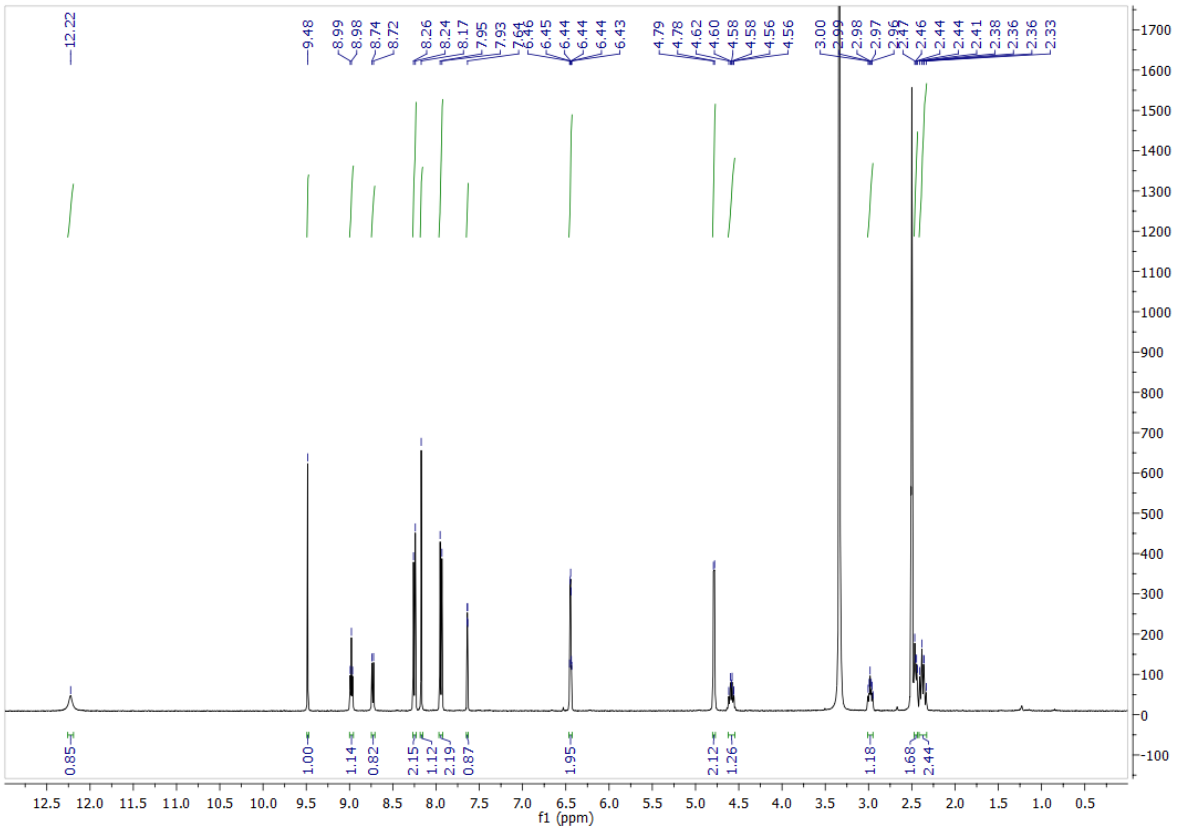


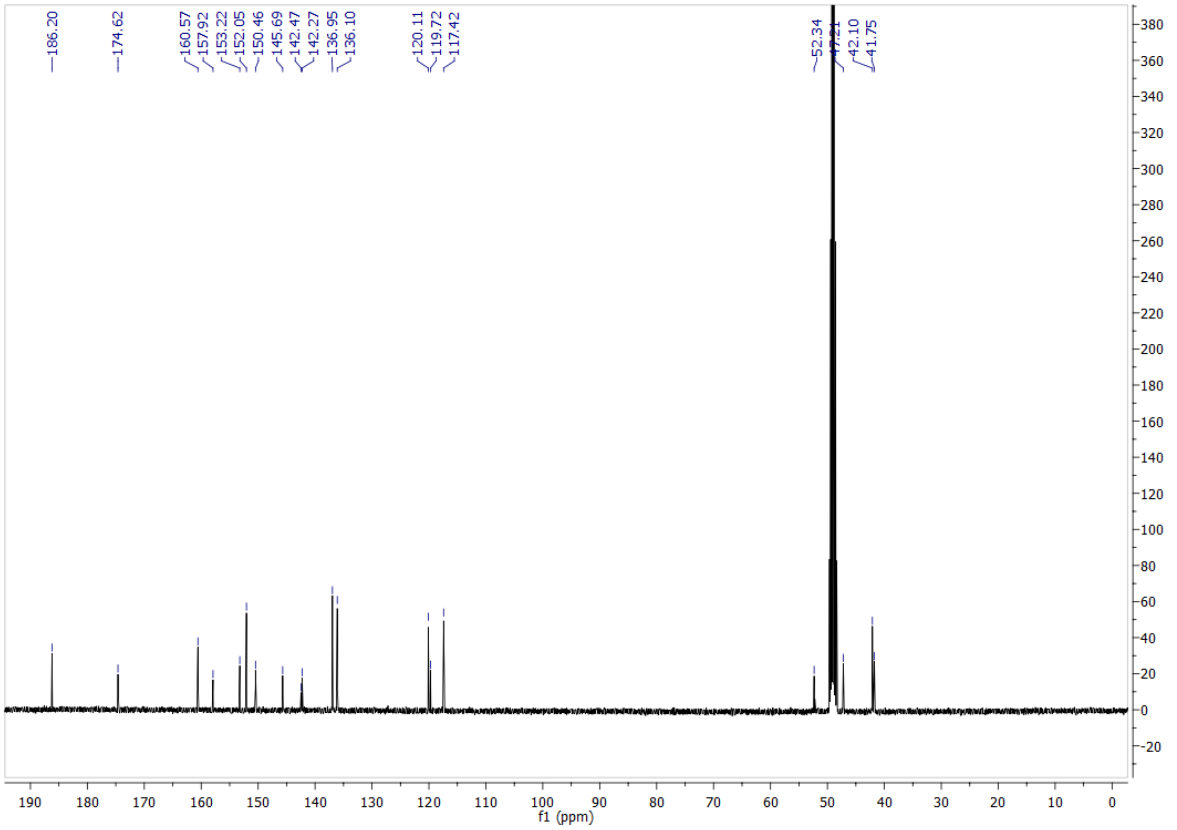
(1*R*,3*R*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxylic acid, 26

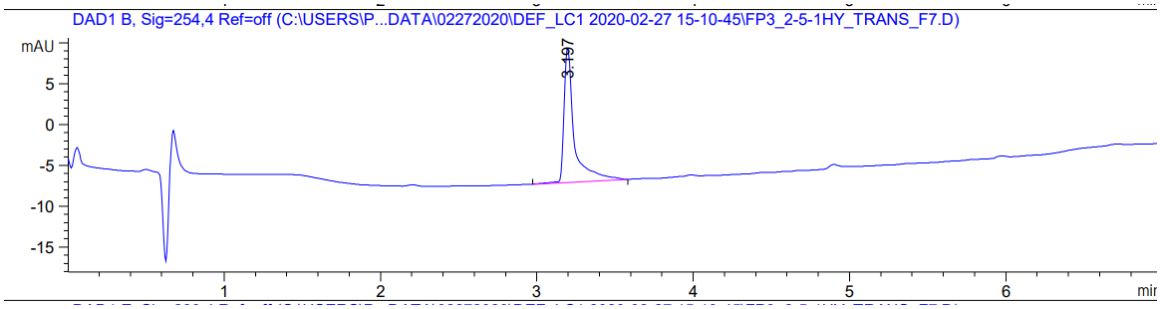


To a flask was added 1-((4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)phenyl)sulfonyl)piperidine-4-carboxylic acid (**9**) (10 mg, 30 µmol, 1.0 equiv), DIPEA (17 µL, 95 µmol, 3.2 equiv), and TBTU (12 mg, 39 µmol, 1.3 equiv) in DMF (0.3 mL), followed by addition of methyl (1*R*,3*R*)-3-aminocyclobutane-1-carboxylate 2,2,2-trifluoroacetate (8 mg, 33 µmol, 1.1 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by column chromatography (0-10% MeOH in CH_2Cl_2). The product was concentrated to yield the desired product as a clear gum. Intermediate methyl (1*R*,3*R*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxylate was telescoped into the next step. To a flask was added methyl (1*R*,3*R*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxylate, $LiOH.H_2O$ (3.8 mg, 89 µmol, 3.0 equiv) in THF (0.12 mL) and H_2O (27 µL) and the reaction was stirred at 50 °C for 16 hr. THF was removed *in vacuo* and 1M HCl was added, the crude material concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA) to yield the desired product as a white solid (1*R*,3*R*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxylic acid (**26**) (3 mg, 20%) over two steps.

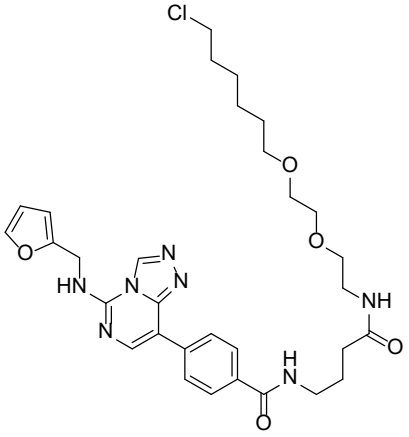
^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.22 (s, 1H), 9.48 (s, 1H), 8.98 (t, *J* = 5.44 Hz, 1H), 8.73 (d, *J* = 7.48 Hz, 1H), 8.25 (d, *J* = 8.54 Hz, 2H), 8.17 (s, 1H), 7.94 (d, *J* = 8.56 Hz, 2H), 7.66 – 7.62 (m, 1H), 6.47 – 6.41 (m, 2H), 4.79 (d, *J* = 5.34 Hz, 2H), 4.63 – 4.54 (m, 1H), 3.02 – 2.94 (m, 1H), 2.48 – 2.41 (m, 2H), 2.41 – 2.33 (m, 2H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 186.20, 174.62, 160.57, 157.92, 153.22, 152.05, 150.46, 145.69, 142.47, 142.27, 136.95, 136.10, 120.11, 119.72, 117.42, 52.34, 47.21, 42.10, 41.75. LCMS: expected mass for $[M+H]^+$ ($C_{22}H_{21}N_6O_4$) requires 433.15 m/z, found 433.20 m/z.


12.22
9.48
8.99
8.98
8.74
8.72
8.26
8.24
8.17
7.95
7.93
7.64
7.46
6.45
6.44
6.44
6.44
6.43
4.79
4.78
4.62
4.60
4.58
4.58
4.56
4.56
3.00
2.99
2.98
2.97
2.96
2.47
2.46
2.44
2.44
2.41
2.38
2.36
2.36
2.33
0.85
1.00
1.14
0.82
2.15
1.12
2.19
0.87
1.95
2.12
1.26
1.18
1.68
2.44
f1 (ppm)


186.20
174.62
160.57
157.92
153.22
152.05
150.46
145.69
142.47
142.27
136.95
136.10
120.11
119.72
117.42
52.34
47.21
42.10
41.75
f1 (ppm)

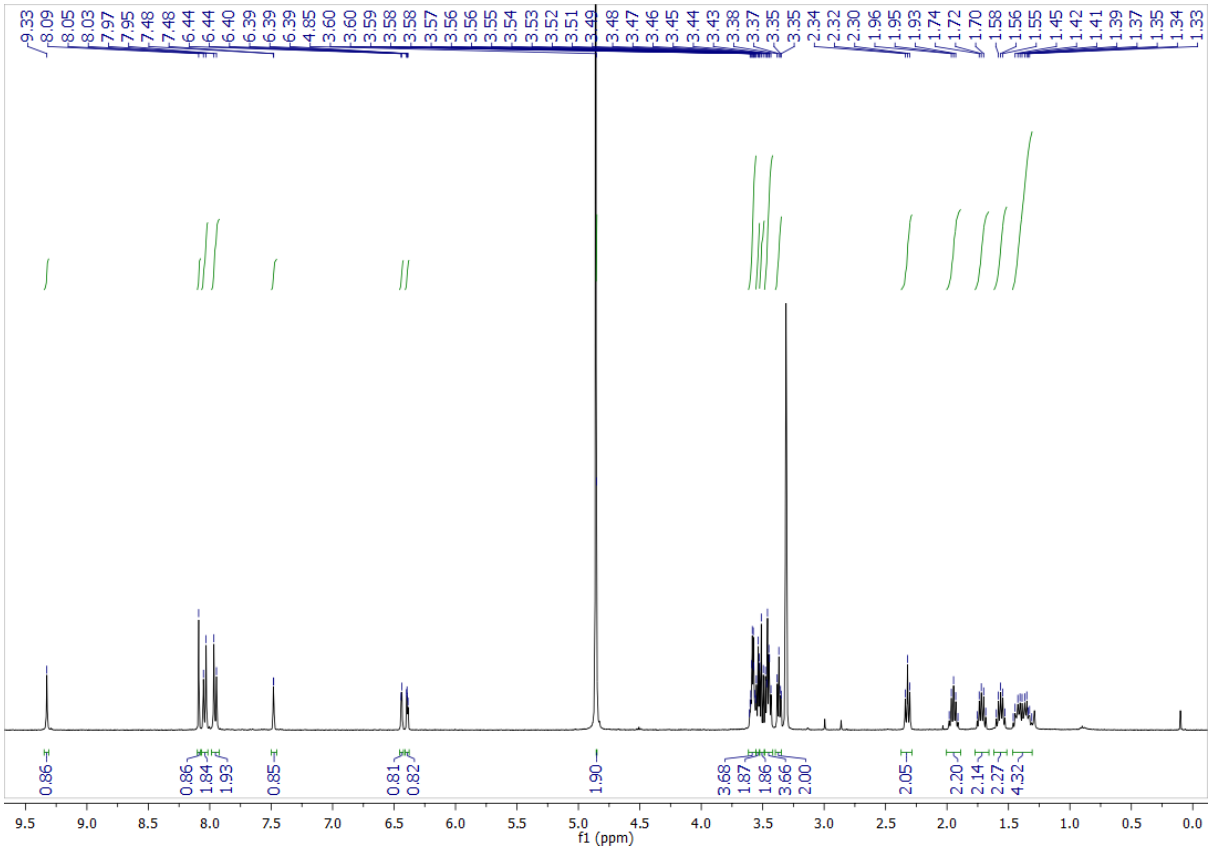


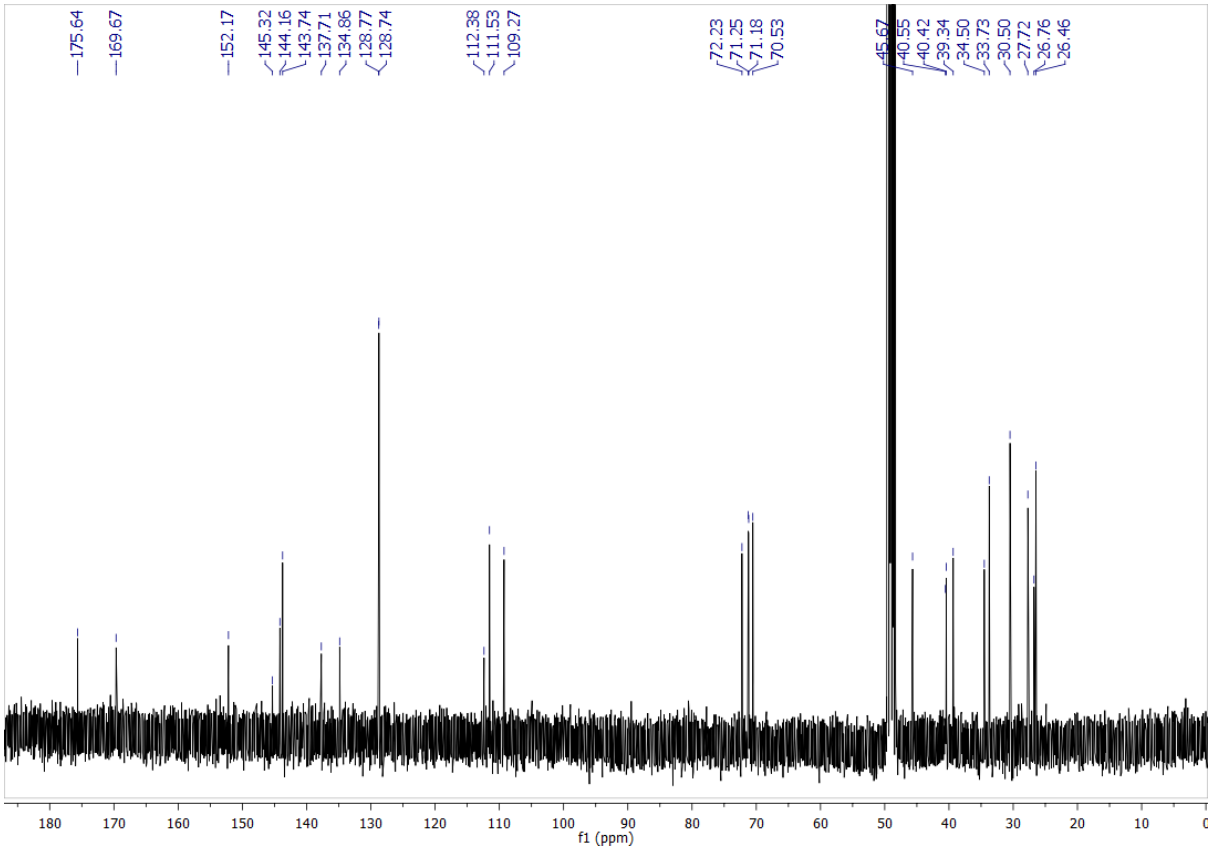
***N*-(4-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)amino)-4-oxobutyl)-4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamide, UNC7706**

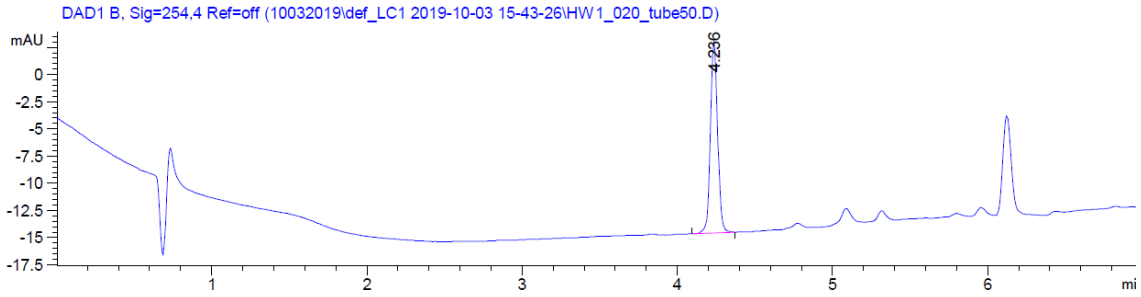


To a flask was added 4-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)butanoic acid (**23**) (5.6 mg, 13 µmol, 1.0 equiv), DIPEA (6.0 µL, 34 µmol, 2.6 equiv), and TBTU (6.4 mg, 20 µmol, 1.5 equiv) in DMF (0.2 mL), followed by addition of 2-(2-((6-chlorohexyl)oxy)ethoxy)ethan-1-amine 2,2,2-trifluoroacetate (6.0 mg, 18 µmol, 1.3 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA) to yield the desired product as a white solid *N*-(4-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)amino)-4-oxobutyl)-4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamide (2.3 mg, 27 %).

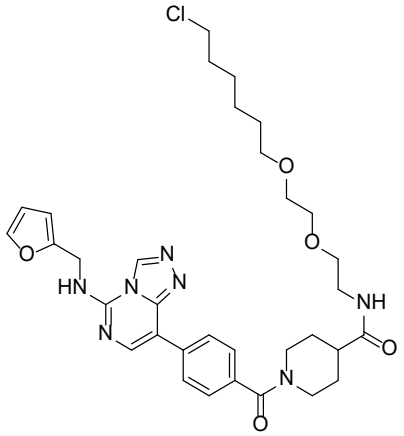
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.33 (s, 1H), 8.09 (s, 1H), 8.04 (d, *J* = 8.47 Hz, 2H), 7.96 (d, *J* = 8.50 Hz, 2H), 7.49 – 7.46 (m, 1H), 6.46 – 6.37 (m, 2H), 4.85 (s, 2H), 3.61 – 3.55 (m, 4H), 3.55 – 3.53 (m, 2H), 3.51 (t, *J* = 5.78 Hz, 2H), 3.48 – 3.41 (m, 4H), 3.37 (t, *J* = 5.46 Hz, 2H), 2.32 (t, *J* = 7.39 Hz, 2H), 1.99 – 1.91 (m, 2H), 1.76 – 1.68 (m, 2H), 1.60 – 1.52 (m, 2H), 1.46 – 1.32 (m, 4H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 175.64, 169.67, 152.17, 145.32, 144.16, 143.74, 137.71, 134.86, 128.77, 128.74, 112.38, 111.53, 109.27, 72.23, 71.25, 71.18, 70.53, 45.67, 40.55, 40.42, 39.34, 34.50, 33.73, 30.50, 27.72, 26.76, 26.46, 2 × aromatic C not observed. LCMS: expected mass for $[M+H]^+$ ($C_{31}H_{41}ClN_7O_5$) requires 626.28 m/z, found 626.20 m/z.


9.33
8.09
8.05
8.03
7.97
7.95
7.48
7.48
6.44
6.44
6.40
6.39
6.39
6.39
4.85
3.60
3.60
3.59
3.58
3.58
3.57
3.56
3.56
3.55
3.54
3.53
3.52
3.51
3.49
3.48
3.47
3.46
3.45
3.44
3.43
3.38
3.37
3.35
3.35
2.34
2.32
2.30
1.96
1.95
1.93
1.74
1.72
1.70
1.58
1.56
1.55
1.45
1.42
1.41
1.39
1.37
1.35
1.34
1.33
0.86
0.86
1.84
1.93
0.85
0.81
0.82
1.90
3.68
1.87
1.86
3.66
2.00
2.05
2.20
2.14
2.27
4.32
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

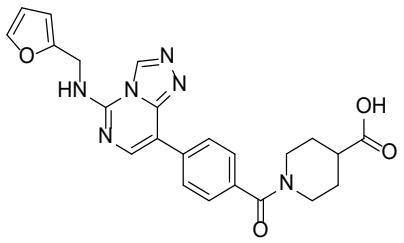

175.64
169.67
152.17
145.32
144.16
143.74
137.71
134.86
128.77
128.74
112.38
111.53
109.27
72.23
71.25
71.18
70.53
45.67
40.55
40.42
39.34
34.50
33.73
30.50
27.72
26.76
26.46
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)



***N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)piperidine-4-carboxamide, UNC7791**

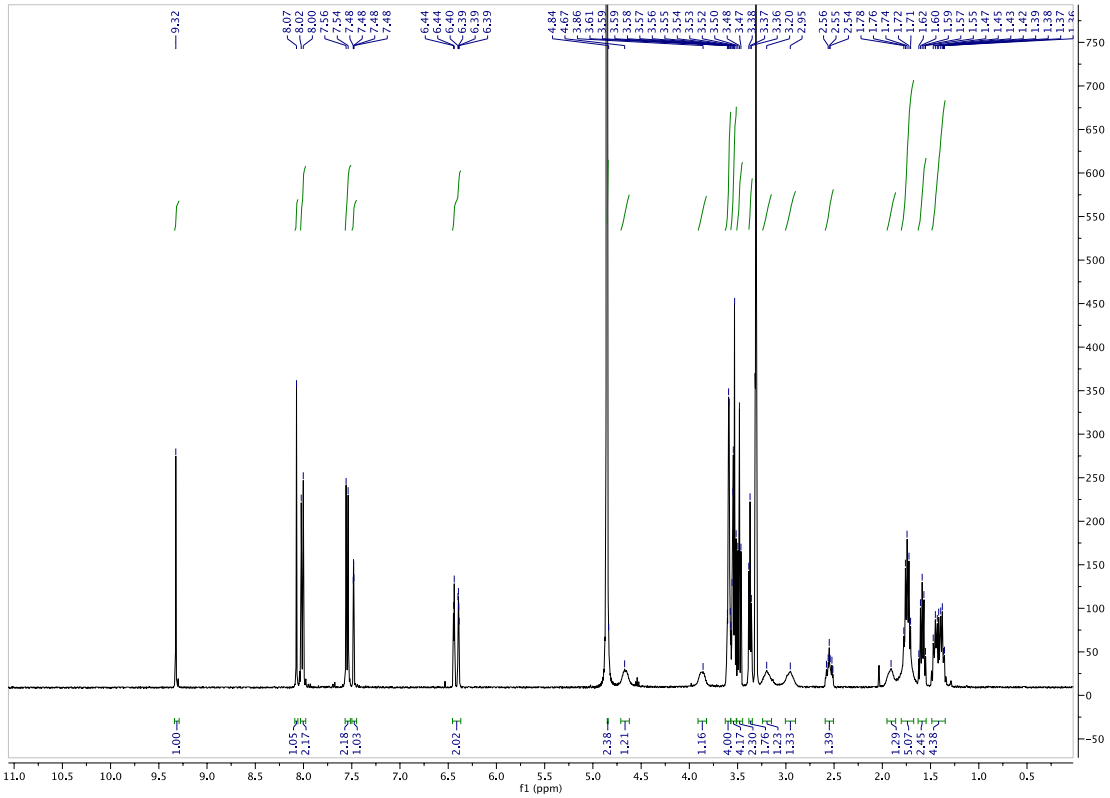


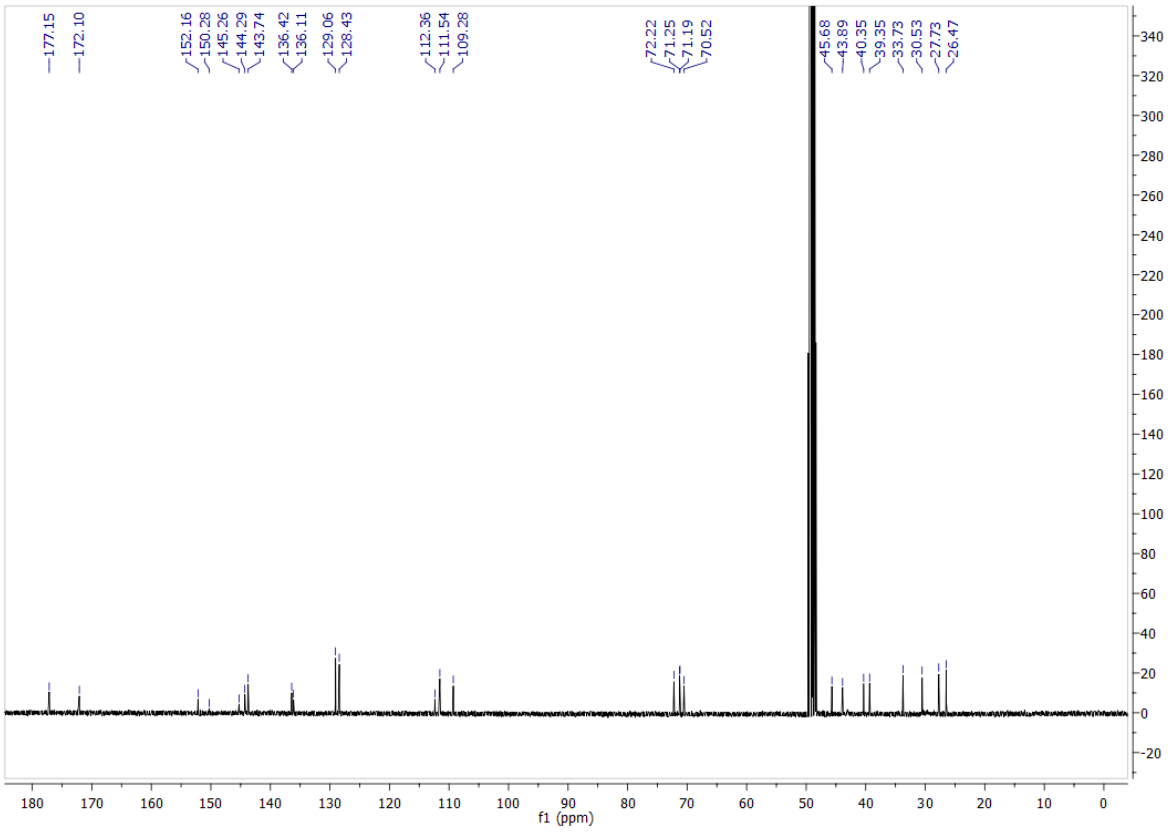
To a flask was added methyl 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)benzoyl)piperidine-4-carboxylate (**22**) (15.3 mg, 33.2 µmol, 1.0 equiv) and LiOH.H_2O (4.2 mg, 99.7 µmol, 3.0 equiv) in THF (133 µL) and H_2O (12 µL). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase column chromatography (10-100% MeCN in H_2O (+0.1% TFA)) to yield the desired product as a white solid 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)piperidine-4-carboxylic acid (**24**) (6.7 mg, 45 %).

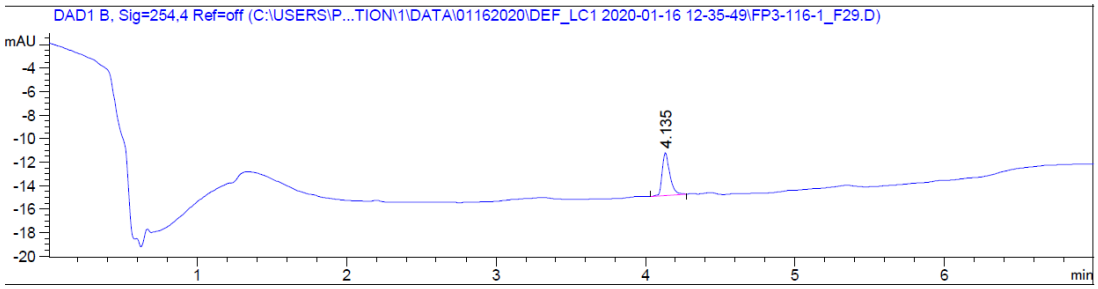


To a flask was added 1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)piperidine-4-carboxylic acid (**24**) (6.7 mg, 15 µmol, 1.1 equiv), DIPEA (7.1 µL, 41 µmol, 3.0 equiv), and TBTU (5.7 mg, 18 µmol, 1.3 equiv) in DMF (0.5 mL), followed by addition of 2-(2-((6-chlorohexyl)oxy)ethoxy)ethan-1-amine 2,2,2-trifluoroacetate (4.6 mg, 14 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA) to yield the desired product as a white solid *N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-1-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoyl)piperidine-4-carboxamide (3.5 mg, 39 %).

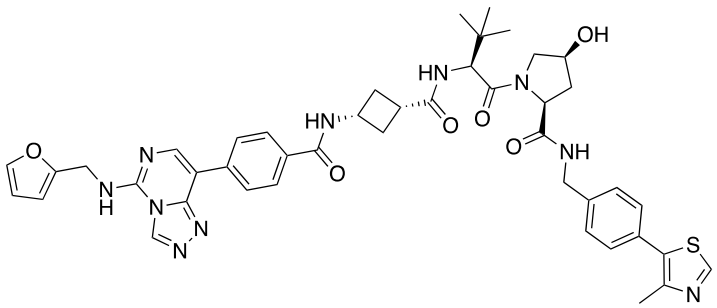
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.32 (s, 1H), 8.07 (s, 1H), 8.01 (d, *J* = 8.41 Hz, 2H), 7.55 (d, *J* = 8.39 Hz, 2H), 7.50 – 7.47 (m, 1H), 6.46 – 6.38 (m, 2H), 4.84 (s, 2H), 4.72 – 4.62 (m, 1H), 3.93 – 3.81 (m, 1H), 3.62 – 3.57 (m, 4H), 3.56 – 3.51 (m, 4H), 3.48 (t, *J* = 6.57 Hz, 2H), 3.37 (t, *J* = 5.43 Hz, 2H), 3.25 – 3.14 (m, 1H), 3.01 – 2.90 (m, 1H), 2.54 (m, 1H), 1.97 – 1.87 (m, 1H), 1.82 – 1.68 (m, 5H), 1.63 – 1.54 (m, 2H), 1.50 – 1.33 (m, 4H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 177.15, 172.10, 152.16, 150.28, 145.26, 144.29, 143.74, 136.42, 136.11, 129.06, 128.43, 112.36, 111.54, 109.28, 72.22, 71.25, 71.19, 70.52, 45.68, 43.89, 40.35, 39.35, 33.73, 30.53, 27.73, 26.47. LCMS: expected mass for $[M+H]^+$ ($C_{33}H_{43}ClN_7O_5$) requires 652.29 m/z, found 652.30 m/z.







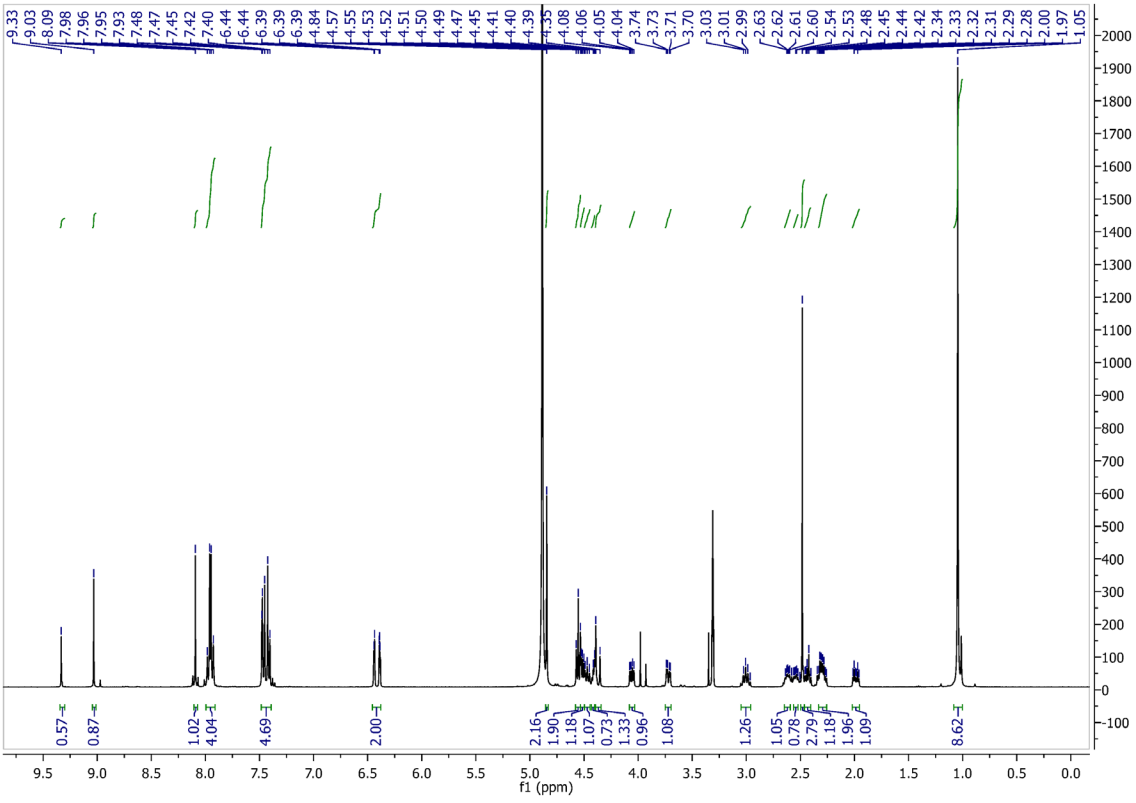
(2*S*,4*S*)-1-((*S*)-2-((1*S*,3*R*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, UNC7971

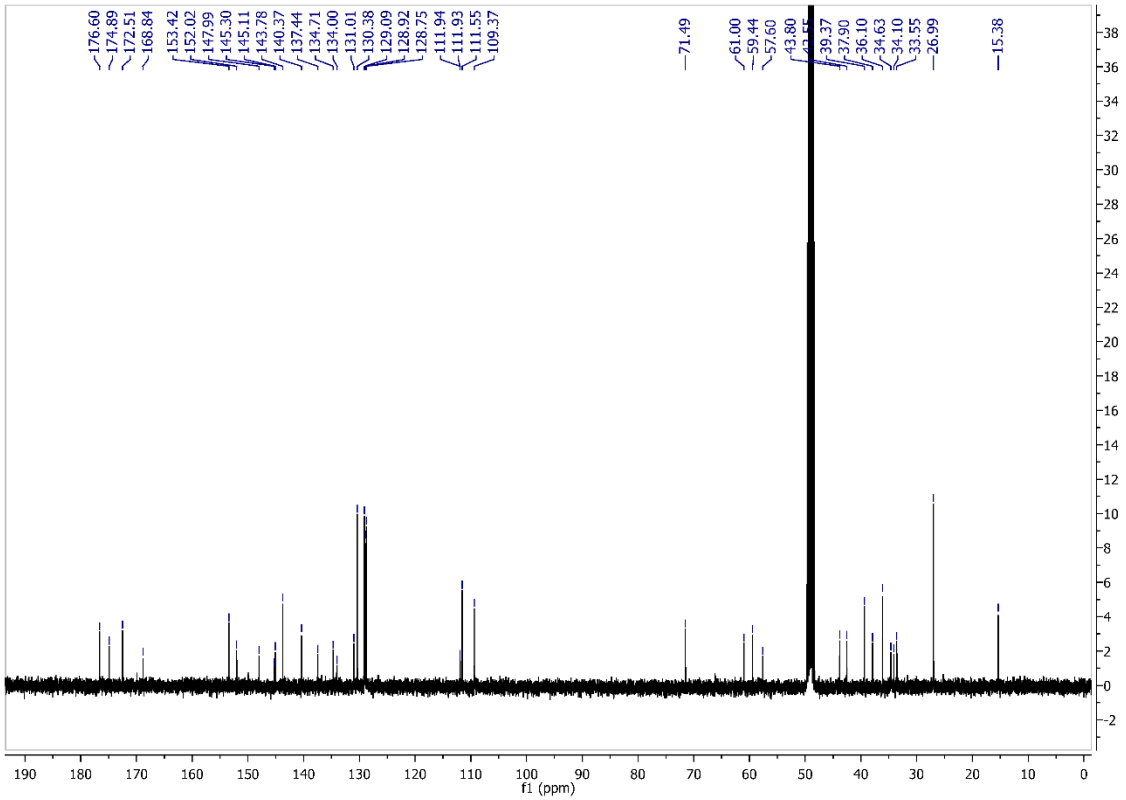


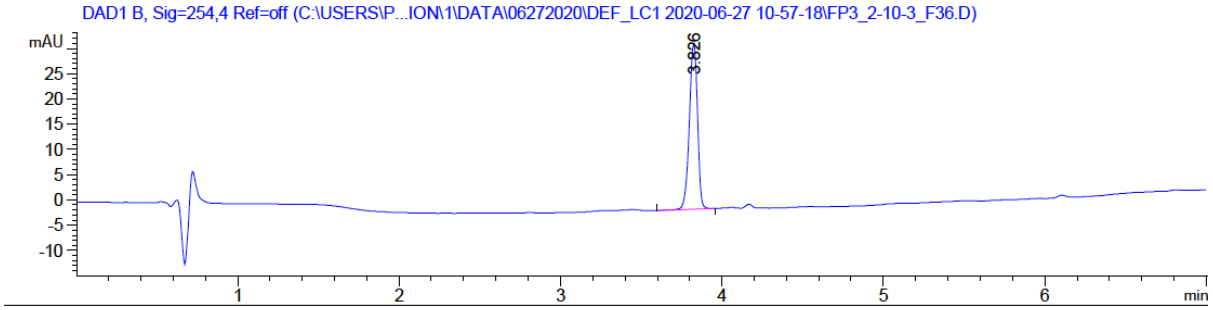
To a flask was added (1*S*,3*S*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxylic acid
(**25**) (3.3 mg, 8 µmol, 1.1 equiv), DIPEA (4 µL, 22 µmol, 3.2 equiv), and TBTU (3 mg, 22 µmol, 1.3 equiv) in DMF (0.5 mL), followed by addition of (2*S*,4*S*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (3.8 mg,

7 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by reverse phase high performance liquid chromatography (C18, 10-100% MeCN in H_2O, 0.1% TFA) to yield the desired product as a white solid (2*S*,4*S*)-1-((*S*)-2-((1*S*,3*R*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (1.8 mg, 31 %).

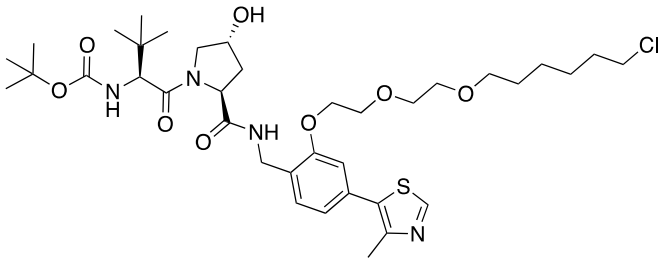
^{1}H NMR (CD_3OD-d_4, 400 MHz) δ 9.33 (s, 1H), 9.03 (s, 1H), 8.09 (s, 1H), 7.99 – 7.91 (m, 4H), 7.50 – 7.39 (m, 5H), 6.46 – 6.36 (m, 2H), 4.84 (s, 2H), 4.56 (d, *J* = 7.1 Hz, 2H), 4.54 – 4.49 (m, 1H), 4.46 (d, *J* = 8.1 Hz, 1H), 4.43 – 4.38 (m, 1H), 4.35 (s, 1H), 4.06 (dd, *J* = 10.6, 5.1 Hz, 1H), 3.72 (dd, *J* = 10.5, 3.7 Hz, 1H), 3.00 (q, *J* = 8.6 Hz, 1H), 2.66 – 2.58 (m, 1H), 2.57 – 2.50 (m, 1H), 2.48 (s, 3H), 2.43 (q, *J* = 6.2 Hz, 1H), 2.30 (ddt, *J* = 14.6, 9.8, 4.9 Hz, 2H), 1.99 (dt, *J* = 13.1, 4.4 Hz, 1H), 1.05 (s, 9H). ^{13}C NMR (CD_3OD-d_4, 101 MHz) δ 176.60, 174.89, 172.51, 168.84, 153.41, 152.02, 147.99, 145.30, 145.11, 143.78, 140.37, 137.44, 134.71, 134.00, 131.01, 130.38, 129.09, 128.92, 128.75, 111.94 (d, *J* = 1.2 Hz), 111.55, 109.37, 71.49, 61.00, 59.44, 57.60, 43.80, 42.55, 39.37, 37.90, 36.10, 34.63, 34.10, 33.55, 26.99, 15.38. LCMS: expected mass for $[M+H]^+$ ($C_{44}H_{49}N_{10}O_6S$) requires 845.35m/z, found 845.20 m/z.







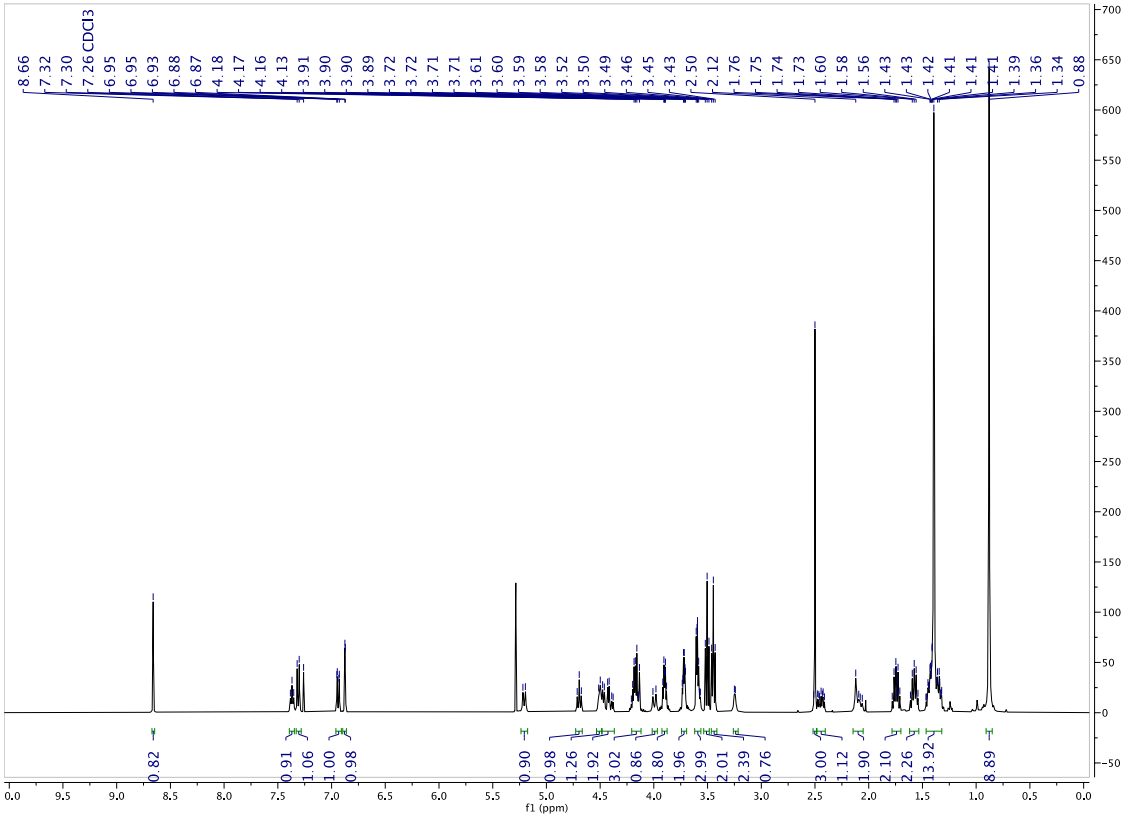
***Tert*-butyl ((*S*)-1-((2*S*,4*R*)-2-((2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)-4-hydroxypyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate, 27**

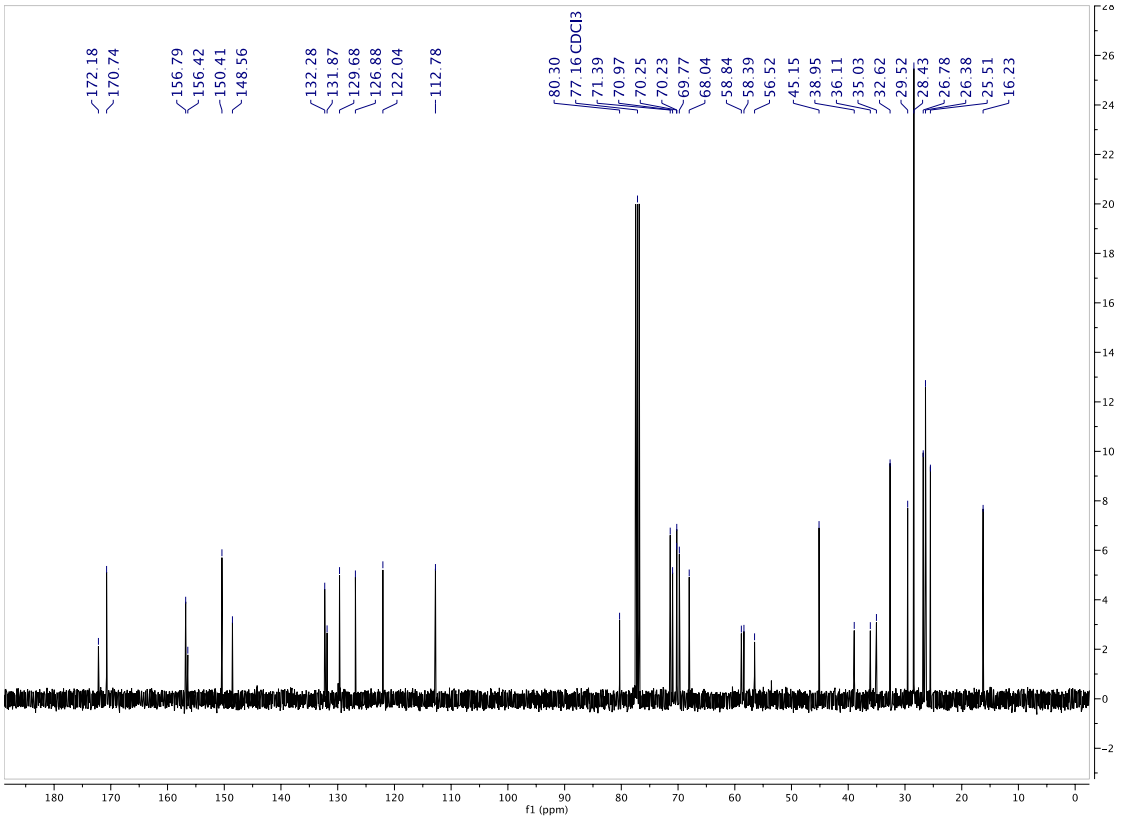


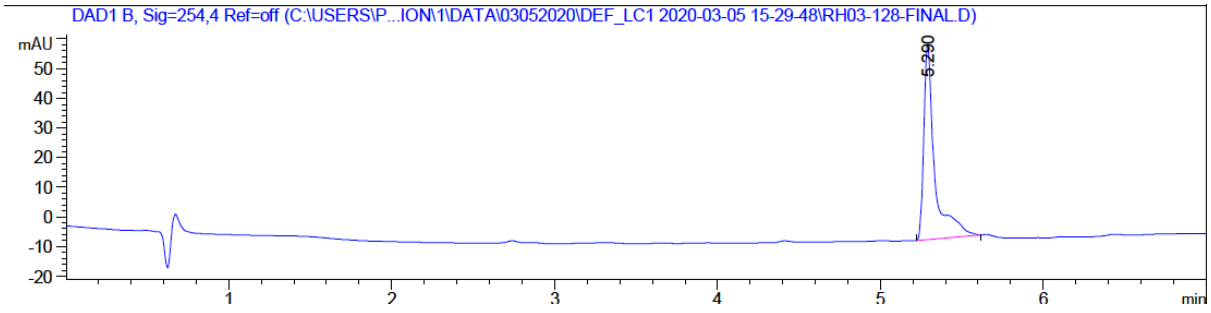
To a vial was added *tert*-butyl ((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (41 mg, 0.13 mmol, 1.2 equiv), Cs_2CO_3 (41 mg, 0.13 mmol, 1.2 equiv), followed by 2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl 4-methylbenzenesulfonate (41 mg, 0.11 mmol, 1.0 equiv) in DMF (1 mL) and the reaction stirred at room

temperature for 16 hr. The reaction was quenched with H_2O and extracted with EtOAc (x3). Organics were combined, washed with washed (×3) and brine, dried with a phase separator and concentrated *in vacuo* to yield a yellow residue. Crude material was purified by column chromatography (silica, 0-10% MeOH in CH_2Cl_2) to yield the desired product as a clear oil *tert*-butyl ((*S*)-1-((2*S*,4*R*)-2-((2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)-4-hydroxypyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (**27**) (46.2 mg, 73%).

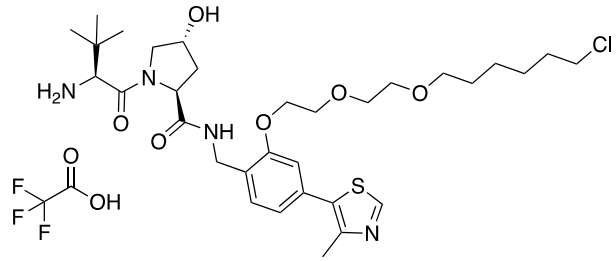
^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.66 (s, 1H), 7.37 (t, *J* = 6.0 Hz, 1H), 7.31 (d, *J* = 7.7 Hz, 1H), 6.94 (dd, *J* = 7.7, 1.6 Hz, 1H), 6.87 (d, *J* = 1.6 Hz, 1H), 5.21 (d, *J* = 9.2 Hz, 1H), 4.69 (t, *J* = 7.8 Hz, 1H), 4.54 – 4.44 (m, 1H), 4.40 (mz, 2H), 4.23 – 4.11 (m, 3H), 3.99 (d, *J* = 11.3 Hz, 1H), 3.90 (dt, *J* = 5.8, 4.3 Hz, 2H), 3.78 – 3.66 (m, 2H), 3.63 – 3.54 (m, 3H), 3.50 (t, *J* = 6.7 Hz, 2H), 3.45 (t, *J* = 6.7 Hz, 2H), 3.25 (s, 1H), 2.50 (s, 3H), 2.48 – 2.39 (m, 1H), 2.13 – 2.03 (m, 2H), 1.79 – 1.70 (m, 2H), 1.58 (p, *J* = 6.8 Hz, 2H) 1.52 – 1.28 (m, 14H), 0.88 (s, 9H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.18, 170.74, 156.79, 156.42, 150.41, 148.56, 132.28, 131.87, 129.68, 126.88, 122.04, 112.78, 80.30, 71.39, 70.97, 70.25, 70.23, 69.77, 68.04, 58.84, 58.39, 56.52, 45.15, 38.95, 36.11, 35.03, 32.62, 29.52, 28.43, 26.78, 26.38, 25.51, 16.23. LCMS: expected mass for $[M+H]^+$ ($C_{37}H_{58}ClN_4O_8S$) requires 753.36 m/z, found 753.30 m/z.







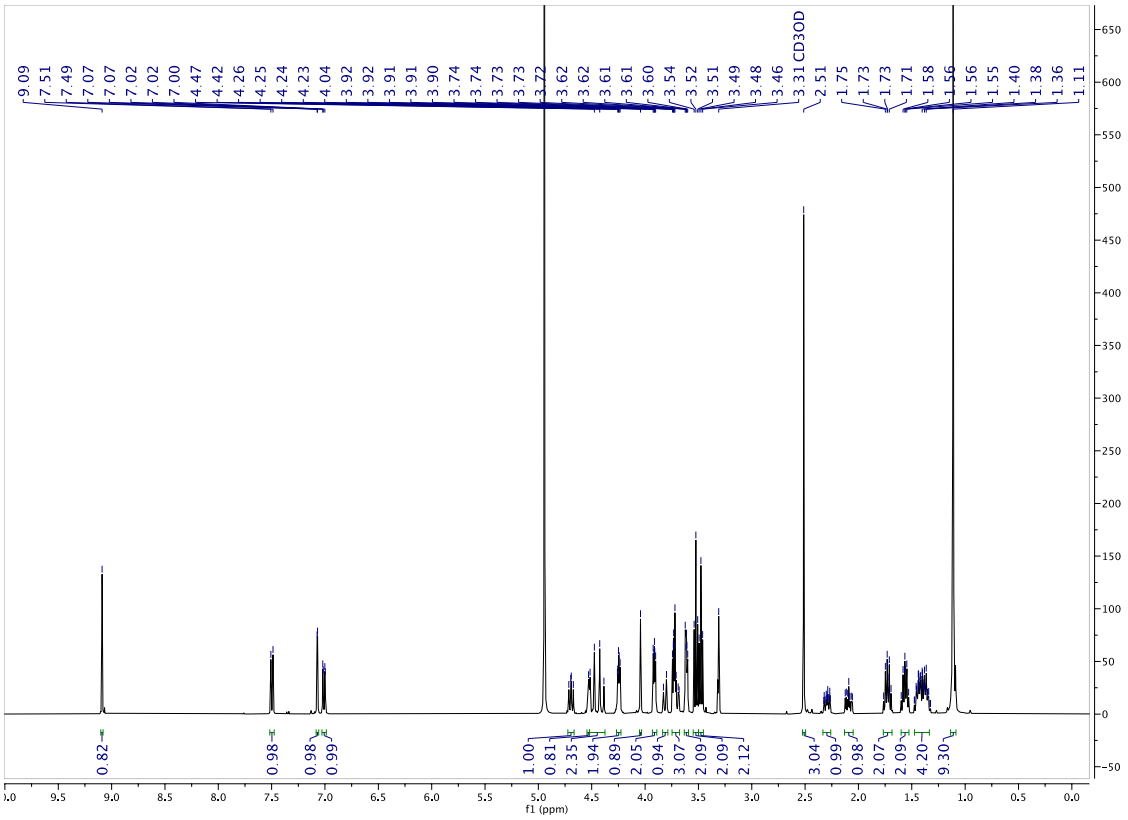
(2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-*N*-(2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-4-hydroxypyrrolidine-2-carboxamide 2,2,2-trifluoroacetate, 28

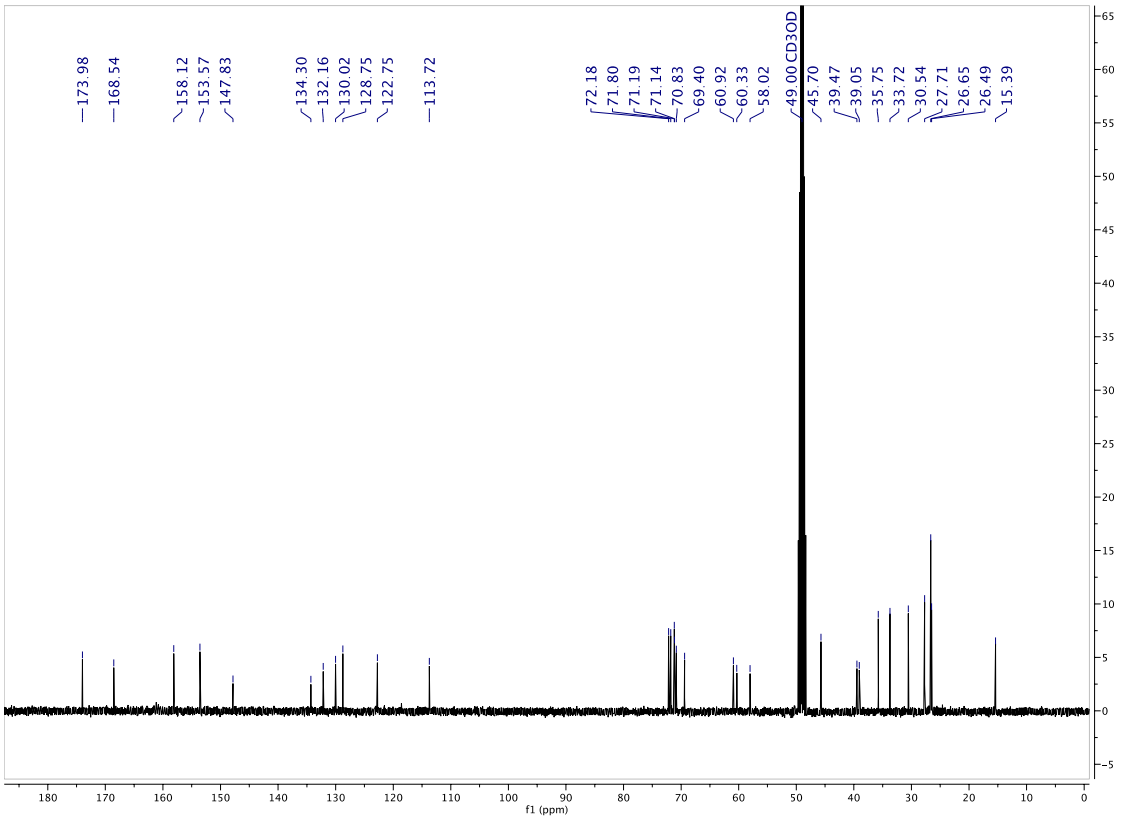


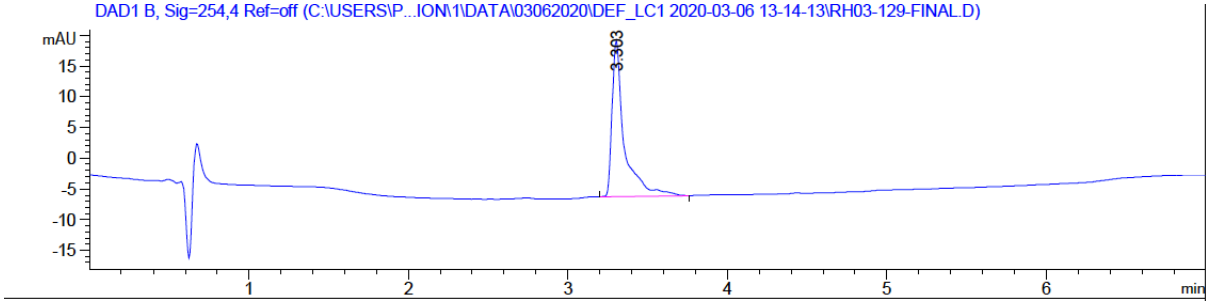
To a vial was added *tert*-butyl ((*S*)-1-((2*S*,4*R*)-2-((2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)-4-hydroxypyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (**27**) (45 mg, 61 µmol, 1.0 equiv) and 20% TFA in CH_2Cl_2 and the reaction stirred at room temperature. The reaction was concentrated *in vacuo* and purified by column chromatography (C18,

10-100% MeOH in H_2O + 0.1% TFA) to yield the desired product as a white solid (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-*N*-(2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-4-hydroxypyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**28**) (45 mg, 96%).

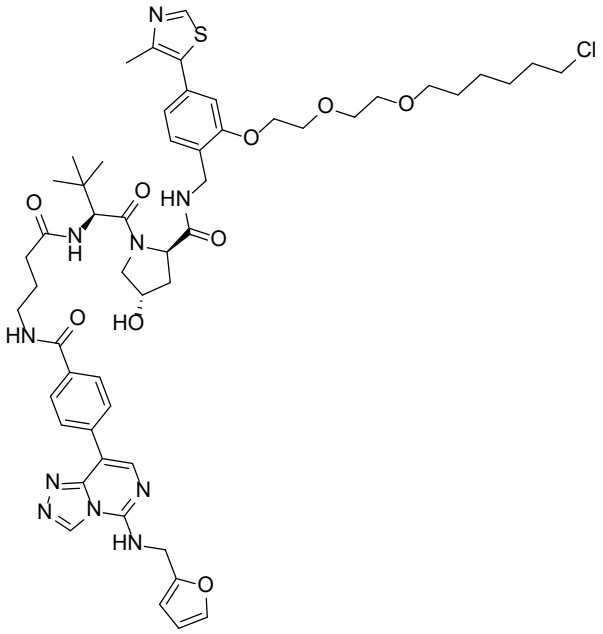
^{1}H NMR (CD_3OD-d_4, 400 MHz) δ 9.09 (s, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.07 (d, *J* = 1.7 Hz, 1H), 7.01 (dd, *J* = 7.8, 1.7 Hz, 1H), 4.69 (m, 1H), 4.53 (m, 1H), 4.54 – 4.36 (m, 2H), 4.28 – 4.21 (m, 2H), 4.04 (s, 1H), 3.95 – 3.88 (m, 2H), 3.81 (m, 1H), 3.77 – 3.66 (m, 3H), 3.65 – 3.58 (m, 2H), 3.52 (t, *J* = 6.6 Hz, 2H), 3.48 (t, *J* = 6.5 Hz, 2H), 2.51 (s, 3H), 2.35 – 2.24 (m, 1H), 2.09 (m, 1H), 1.79 – 1.66 (m, 2H), 1.56 (p, *J* = 6.7 Hz, 2H), 1.48 – 1.32 (m, 4H), 1.11 (s, 9H). ^{13}C NMR (CD_3OD-d_4, 400 MHz) δ 173.98, 168.54, 158.12, 153.57, 147.83, 134.30, 132.16, 130.02, 128.75, 122.75, 113.72, 72.18, 71.80, 71.19, 71.14, 70.83, 69.40, 60.92, 60.33, 58.02, 45.70, 39.47, 39.05, 35.75, 33.72, 30.54, 27.71, 26.65, 26.49, 15.39. LCMS: expected mass for $[M+H]^+$ ($C_{32}H_{50}ClN_4O_6S$) requires 653.31 m/z, found 653.30 m/z.




173.98
168.54
158.12
153.57
147.83
134.30
132.16
130.02
128.75
122.75
113.72
72.18
71.80
71.19
71.14
70.83
69.40
60.92
60.33
58.02
49.00 CD3OD
45.70
39.47
39.05
35.75
33.72
30.54
27.71
26.65
26.49
15.39
f1 (ppm)

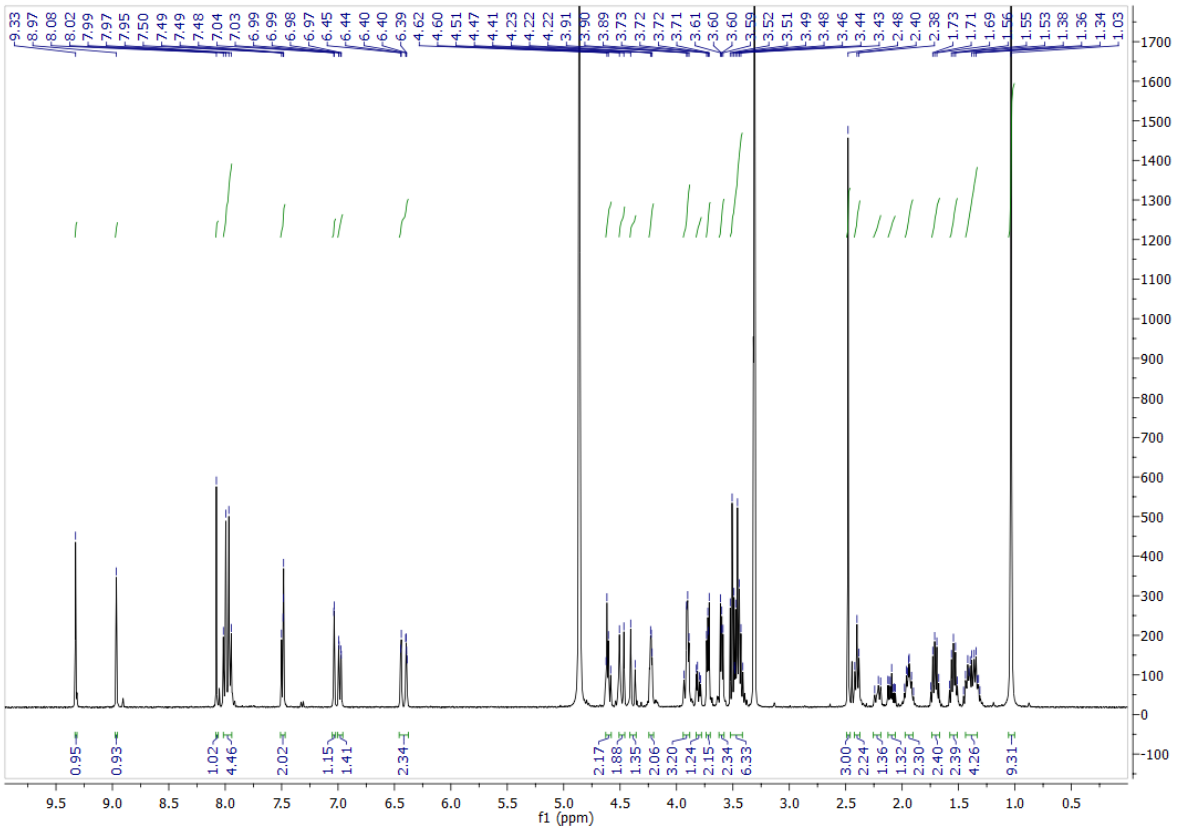

DAD1 B, Sig=254,4 Ref=off (C:\USERS\P...ION\1\DATA\03062020\DEF_LC1 2020-03-06 13-14-13\RH03-129-FINAL.D)
mAU
3.363
min

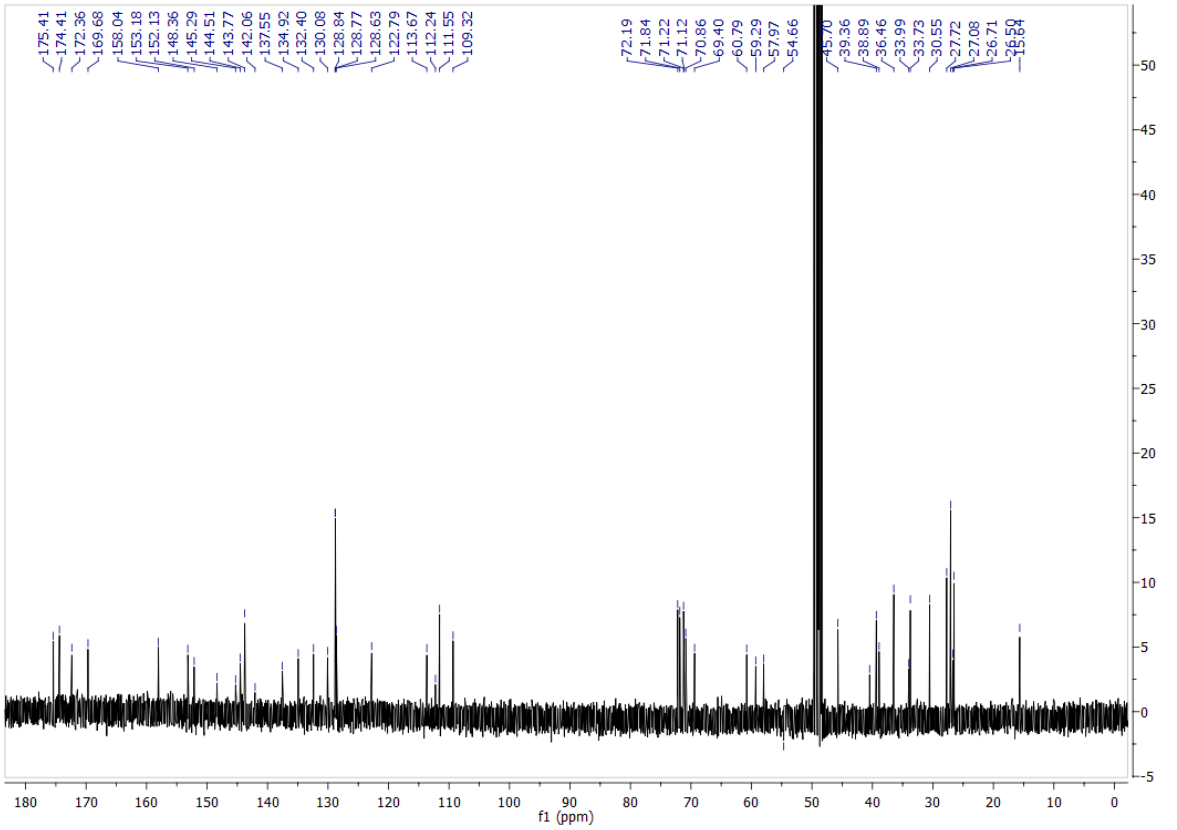
(2*R*,4*S*)-*N*-(2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-(4-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)butanamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide, UNC7962

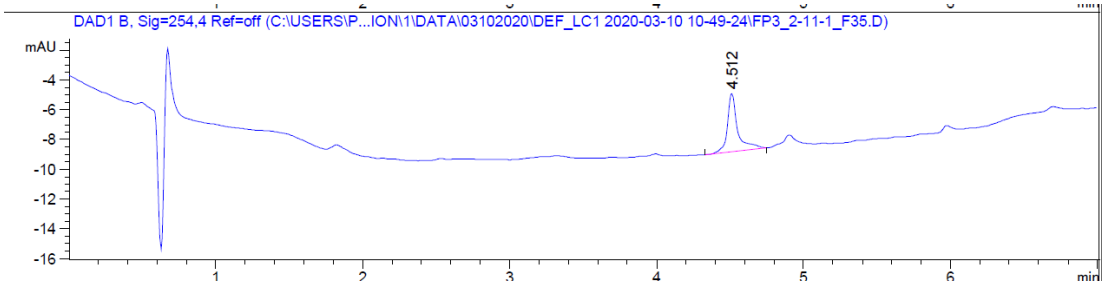


To a flask was added 4-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-c]pyrimidin-8-yl)benzamido)butanoic acid (**23**) (3.0 mg, 7 µmol, 1.1 equiv), DIPEA (4 µL, 20 µmol, 3.2 equiv), and TBTU (3.0 mg, 8 µmol, 1.3 equiv) in DMF (70 µL), followed by addition of (2S,4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-N-(2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-4-hydroxypyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**28**) (5.0 mg, 7 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by preparative high-performance liquid chromatography (10-100% MeCN in H_2O (+ 0.1% TFA)). The product was concentrated to yield the desired product as a white solid (2*R*,4*S*)-*N*-(2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-(4-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)butanamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (3.8 mg, 54%).

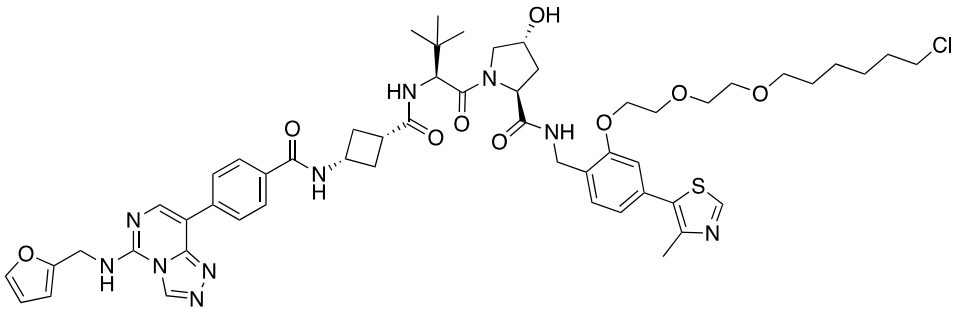
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.33 (s, 1H), 8.97 (s, 1H), 8.08 (s, 1H), 7.98 (m, 4H), 7.51 – 7.47 (m, 2H), 7.04(s, 1H), 6.98 (d, *J* = 1.51, 7.75 Hz, 1H), 6.46 – 6.38 (m, 2H), 4.64 – 4.58 (m, 2H), 4.53 – 4.46 (m, 2H), 4.39 (m, 1H), 4.25 – 4.20 (m, 2H), 3.94 – 3.88 (m, 3H), 3.80 (m, 1H), 3.74 – 3.70 (m, 2H), 3.62 – 3.57 (m, 2H), 3.53 – 3.40 (m, 6H), 2.48 (s, 3H), 2.40 (t, *J* = 7.43 Hz, 2H), 2.25 – 2.18 (m, 1H), 2.09 (m, 1H), 1.95 (m, 2H), 1.75 – 1.67 (m, 2H), 1.59 – 1.50 (m, 2H), 1.45 – 1.31 (m, 4H), 1.03 (s, 9H). 2 × ^{1}H not observed. ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 175.41, 174.41, 172.36, 169.68, 158.04, 153.18, 152.13, 148.36, 145.29, 144.51, 143.77, 142.06, 137.55, 134.92, 132.40, 130.08, 128.84, 128.77, 128.63, 122.79, 113.67, 112.24, 111.55, 109.32, 72.19, 71.84, 71.22, 71.12, 70.86, 69.40, 60.79, 59.29, 57.97, 54.66, 45.70, 40.47, 39.36, 38.89, 36.46, 33.99, 33.73, 30.55, 27.72, 27.08, 26.71, 26.50, 15.64. 2 × ^{13}C not observed. LCMS: expected mass for $[M+H]^+$ ($C_{53}H_{68}ClN_{10}O_9S$) requires 1055.45 m/z, found 1055.30 m/z.


9.33
8.97
8.08
8.02
7.99
7.97
7.95
7.50
7.49
7.49
7.48
7.04
7.03
6.99
6.99
6.98
6.97
6.45
6.44
6.40
6.40
6.39
4.62
4.60
4.51
4.47
4.41
4.23
4.22
4.22
3.91
3.90
3.89
3.73
3.72
3.72
3.71
3.61
3.60
3.60
3.59
3.52
3.51
3.49
3.48
3.46
3.44
3.43
2.48
2.40
2.38
1.73
1.71
1.69
1.56
1.55
1.53
1.38
1.36
1.34
1.03
0.95
0.93
1.02
4.46
2.02
1.15
1.41
2.34
2.17
1.88
1.35
2.06
3.20
1.24
2.15
2.34
6.33
3.00
2.24
1.36
1.32
2.30
2.40
2.39
4.26
9.31
f1 (ppm)


175.41
174.41
172.36
169.68
158.04
153.18
152.13
148.36
145.29
144.51
143.77
142.06
137.55
134.92
132.40
130.08
128.84
128.77
128.63
122.79
113.67
112.24
111.55
109.32
72.19
71.84
71.22
71.12
70.86
69.40
60.79
59.29
57.97
54.66
45.70
39.36
38.89
36.46
33.99
33.73
30.55
27.72
27.08
26.71
26.50
15.84
f1 (ppm)

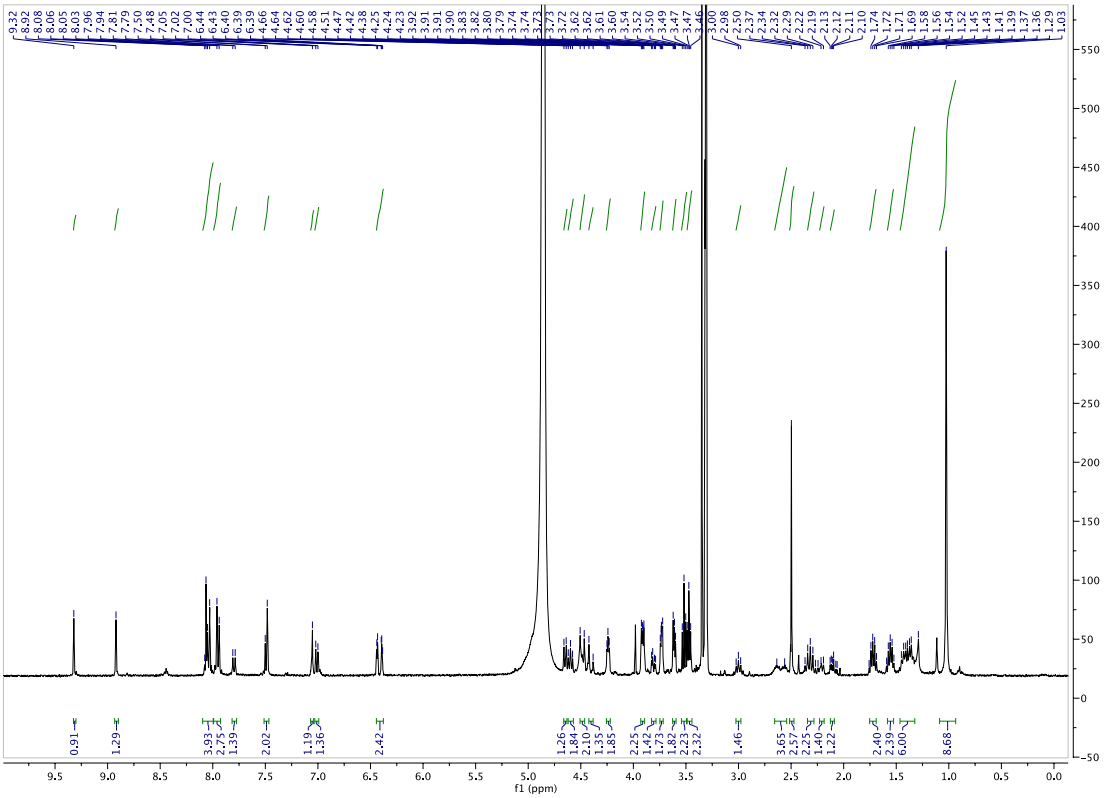


(2*S*,4*R*)-*N*-(2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-((1*S*,3*R*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide, UNC7960

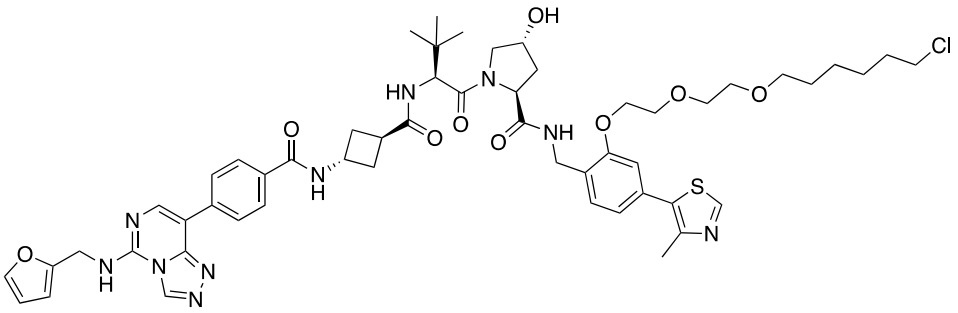


To a flask was added (1*S*,3*S*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxylic acid (**25**) (2.3 mg, 5.3 µmol, 1.1 equiv), DIPEA (2.7 µL, 15 µmol, 3.2 equiv), and TBTU (2.0 mg, 6,3 µmol, 1.3 equiv) in DMF (100 µL), followed by addition of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-*N*-(2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-4-hydroxypyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**28**) (3.7 mg, 4.8 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by preparative high-performance liquid chromatography (10-100% MeCN in H_2O (+ 0.1% TFA)). The product was concentrated to yield the desired product as a white solid (2*S*,4*R*)-*N*-(2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-((1*S*,3*R*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (1.4 mg, 28%).

^{1}H NMR (CD_3OD-d_4, 400 MHz) δ 9.32 (s, 1H), 8.92 (s, 1H), 8.10 – 8.00 (m, 4H), 7.95 (d, *J* = 8.5 Hz, 3H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.49 (m, 2H), 7.05 (m, 1H), 7.01 (m, 1H), 6.45 – 6.37 (m, 2H), 4.65 (m, 1H), 4.60 (t, *J* = 8.1 Hz, 2H), 4.53 – 4.45 (m, 3H), 4.44 – 4.38 (m, 2H), 4.27 – 4.21 (m, 2H), 3.95 – 3.88 (m, 2H), 3.81 (dd, *J* = 10.9, 3.9 Hz, 1H), 3.77 – 3.70 (m, 2H), 3.64 – 3.58 (m, 2H), 3.52 (t, *J* = 6.7 Hz, 2H), 3.47 (t, *J* = 6.5 Hz, 2H), 3.00 (t, *J* = 8.6 Hz, 1H), 2.66 – 2.54 (m, 4H), 2.50 (s, 3H), 2.34 – 2.28 (m, 2H), 2.20 (m, 1H), 2.15 – 2.01 (m, 1H), 1.72 (p, *J* = 6.8 Hz, 2H), 1.56 (p, *J* = 6.8 Hz, 2H), 1.47 – 1.33 (m, 6H), 1.03 (s, 9H). LCMS: expected mass for $[M+H]^+$ ($C_{54}H_{68}ClN_{10}O_9S$) requires 1067.45 m/z, $[M+H]^+/2$ requires 533.73 found m/z 533.75.



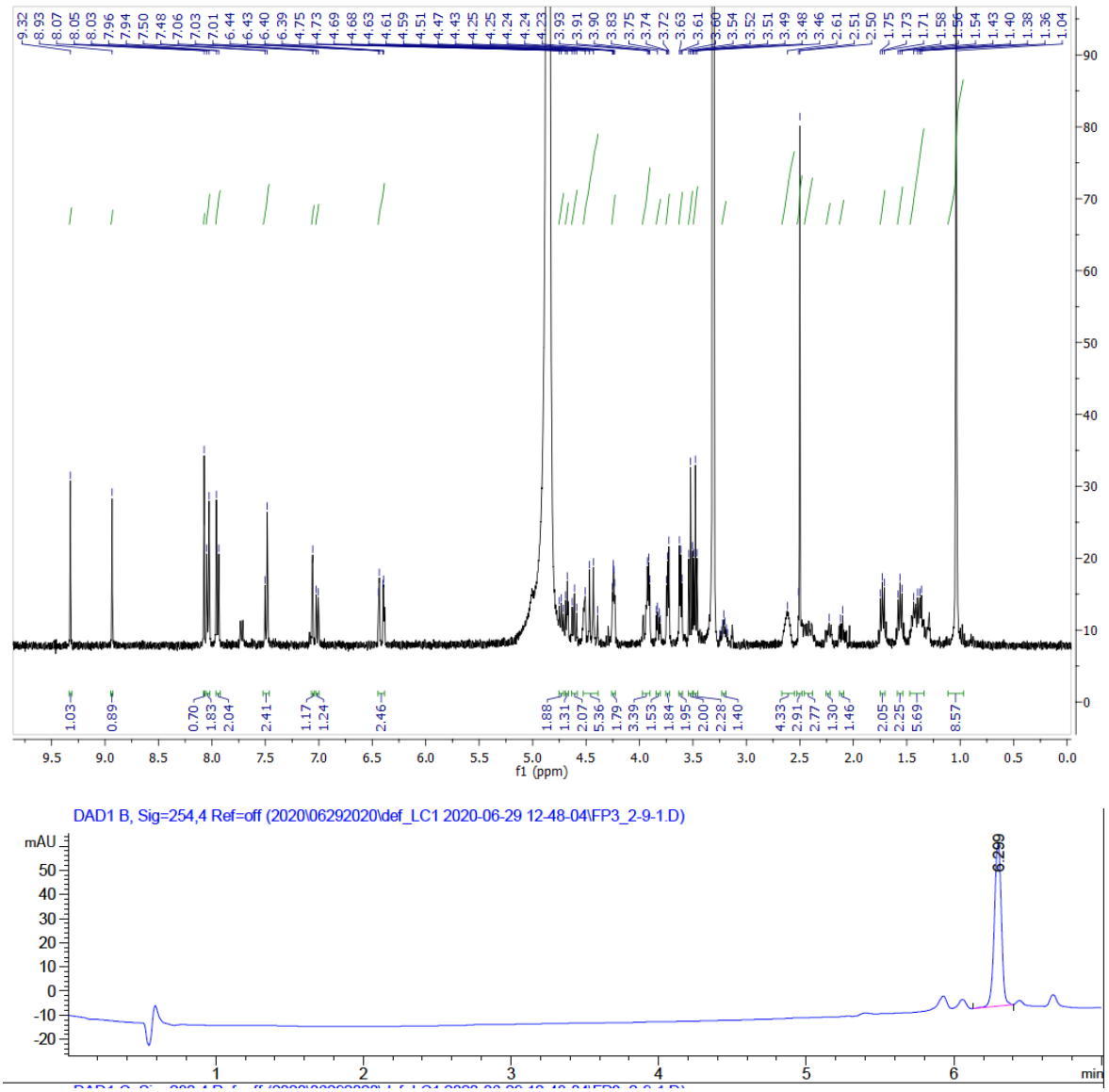
(2*S*,4*R*)-*N*-(2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-((1*R*,3*S*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide, UNC7961



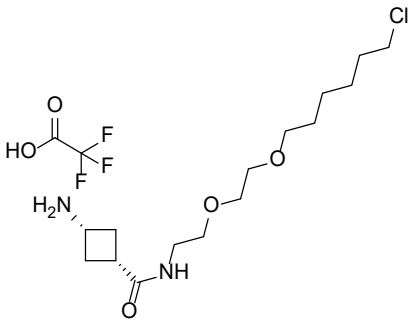
To a flask was added (1*R*,3*R*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxylic acid (**26**) (3.0 mg, 7 µmol, 1.1 equiv), DIPEA (4 µL, 20 µmol, 3.2 equiv), and TBTU (3.0 mg, 8 µmol, 1.3 equiv) in DMF (100 µL), followed by addition of (2*S*,4*R*)-1-((*S*)-

2-amino-3,3-dimethylbutanoyl)-*N*-(2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-4-hydroxypyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**28**) (5.0 mg, 7 µmol, 1.0 equiv). The reaction was stirred at room temperature for 16 hr, concentrated *in vacuo* and purified by preparative high-performance liquid chromatography (10-100% MeCN in H_2O (+ 0.1% TFA)). The product was concentrated to yield the desired product as a white solid (2*S*,4*R*)-*N*-(2-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-((1*R*,3*S*)-3-(4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamido)cyclobutane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (0.91 mg, 10%).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.32 (s, 1H), 8.93 (s, 1H), 8.07 (s, 1H), 8.04 (d, *J* = 8.59 Hz, 2H), 7.95 (d, *J* = 8.52 Hz, 2H), 7.49 (d, *J* = 7.80 Hz, 2H), 7.07 – 7.05 (m, 1H), 7.02 (m, 1H), 6.46 – 6.37 (m, 2H), 4.76 – 4.71 (m, 2H), 4.69 – 4.66 (m, 1H), 4.64 – 4.58 (m, 2H), 4.54 – 4.39 (m, 5H), 4.27 – 4.22 (m, 2H), 3.97 – 3.90 (m, 2H), 3.82 (dd, *J* = 3.87, 11.12 Hz, 1H), 3.76 – 3.71 (m, 2H), 3.64 – 3.59 (m, 2H), 3.52 (t, *J* = 6.65 Hz, 2H), 3.28 (t, *J* = 6.52 Hz, 2H), 3.25 – 3.18 (m, 1H), 2.66 – 2.57 (m, 4H), 2.50 (s, 3H), 2.45 – 2.37 (m, 3H), 2.27 – 2.20 (m, 1H), 2.14 – 2.06 (m, 1H), 1.76 – 1.69 (m, 2H), 1.60 – 1.52 (m, 2H), 1.46 – 1.32 (m, 6H), 1.04 (s, 9H). LCMS: expected mass for $[M+H]^+$ ($C_{54}H_{68}ClN_{10}O_9S$) requires 1067.45 m/z, found 1067.3 m/z.

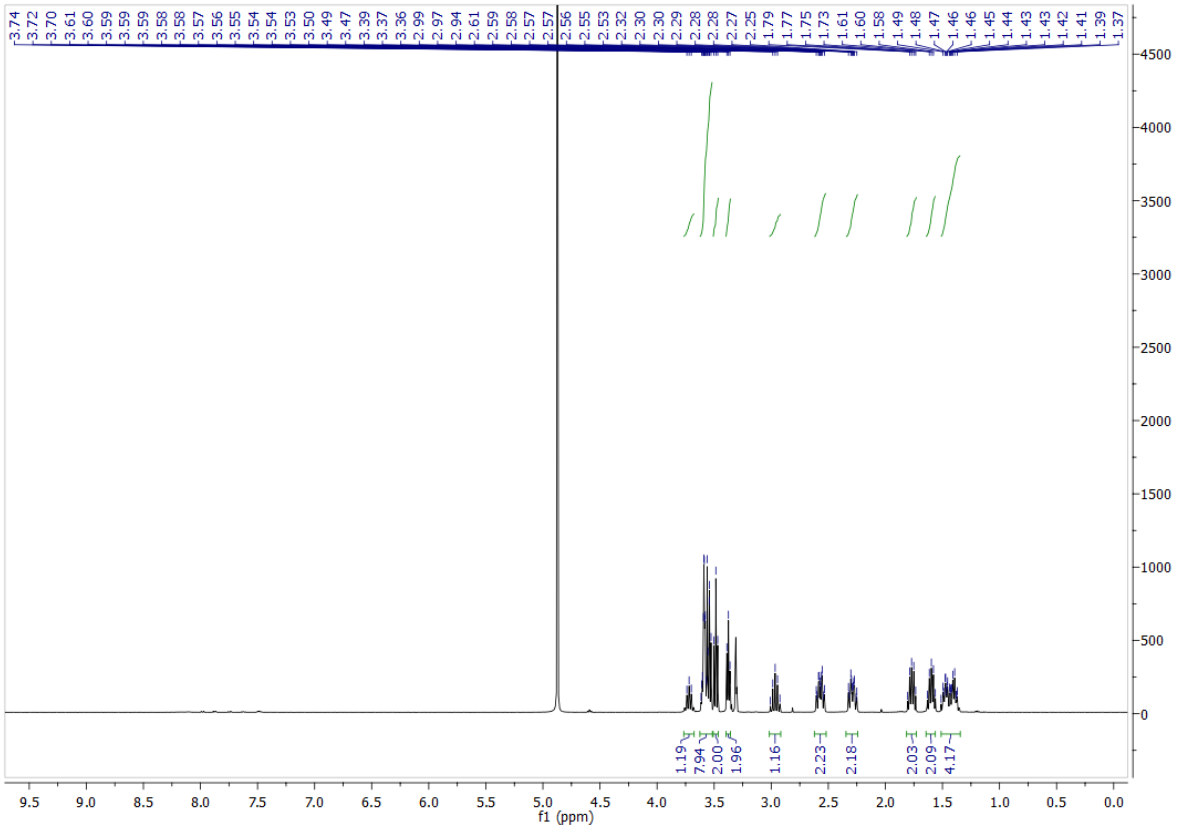


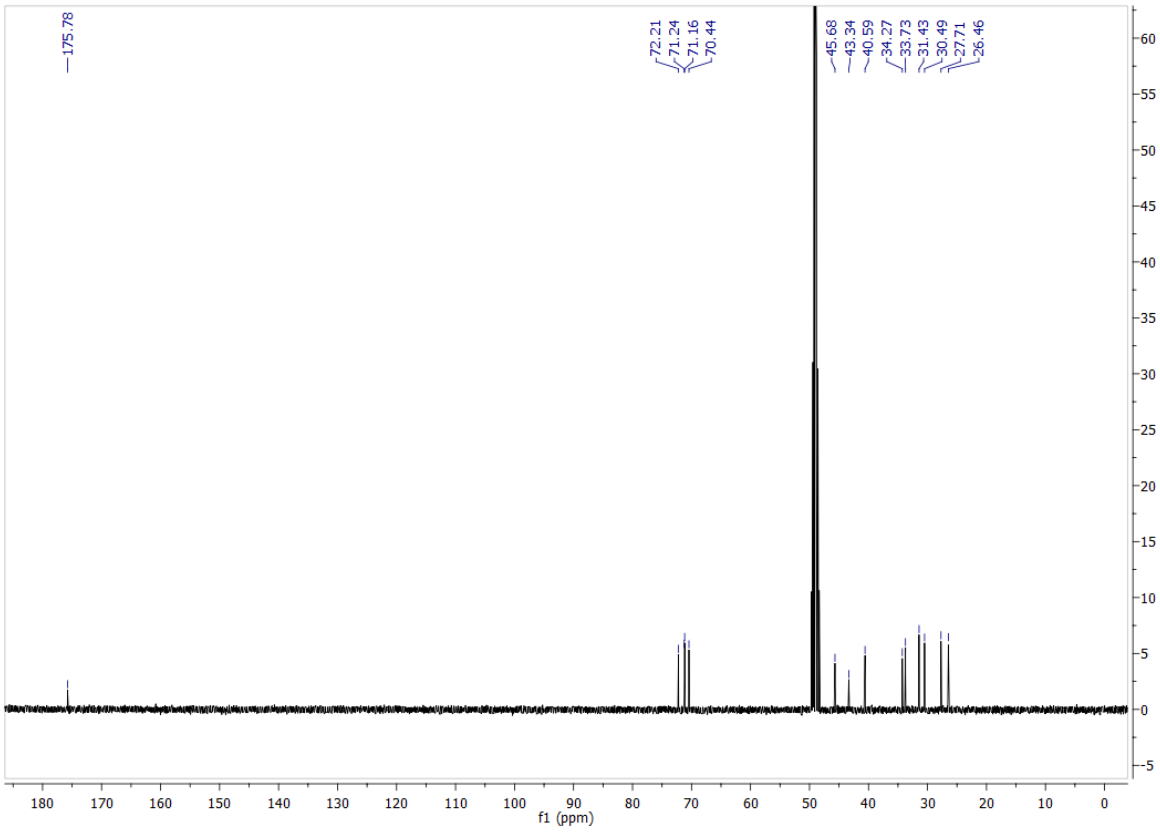
(1*S*,3*S*)-3-amino-*N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)cyclobutane-1-carboxamide 2,2,2-trifluoroacetate, 29



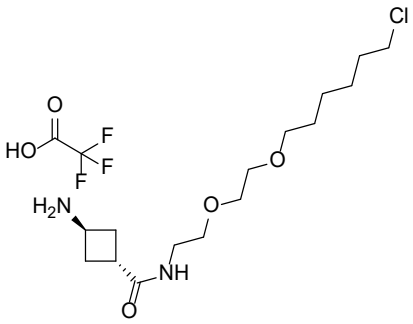
To a flask was added (1*S*,3*S*)-3-((*tert*-butoxycarbonyl)amino)cyclobutane-1-carboxylic acid (25 mg, 0.12 mmol, 1.0 equiv), DIPEA (61 µL, 0.35 mmol, 3.0 equiv), TBTU (48 mg, 0.15 mmol, 1.3 equiv), followed by 2-(2-((6-chlorohexyl)oxy)ethoxy)ethan-1-amine 2,2,2-trifluoroacetate (39 mg, 0.12 mmol, 1.0 equiv) in DMF (1 mL) and the reaction stirred at room temperature for 16 hr. The reaction was concentrated and purified by column chromatography (0-10% MeOH in CH_2Cl_2) to yield the intermediate as a clear oil. The *N*-Boc intermediate was deprotected with 20% TFA in CH_2Cl_2, concentrated *in vacuo* and purified by reverse phase column chromatography (MeOH in H_2O (+0.1% TFA)) to yield the desired product as a white solid (1*S*,3*S*)-3-amino-*N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)cyclobutane-1-carboxamide 2,2,2-trifluoroacetate (**29**) (15 mg, 30%).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 3.72 (p, *J* = 7.9 Hz, 1H), 3.62 – 3.52 (m, 8H), 3.49 (t, *J* = 6.56 Hz, 2H), 3.37 (t, *J* = 5.47 Hz, 2H), 2.97 (p, *J* = 8.4 Hz, 1H), 2.63 – 2.53 (m, 2H), 2.33 – 2.24 (m, 2H), 1.82 – 1.72 (m, 2H), 1.64 – 1.55 (m, 2H), 1.52 – 1.36 (m, 4H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 175.78, 72.21, 71.24, 71.16, 70.44, 45.68, 43.34, 40.59, 34.27, 33.73, 31.43, 30.49, 27.71, 26.46. LCMS: expected mass for $[M+H]^+$ ($C_{15}H_{30}ClN_2O_3$) requires 321.19 m/z, found 321.20 m/z.





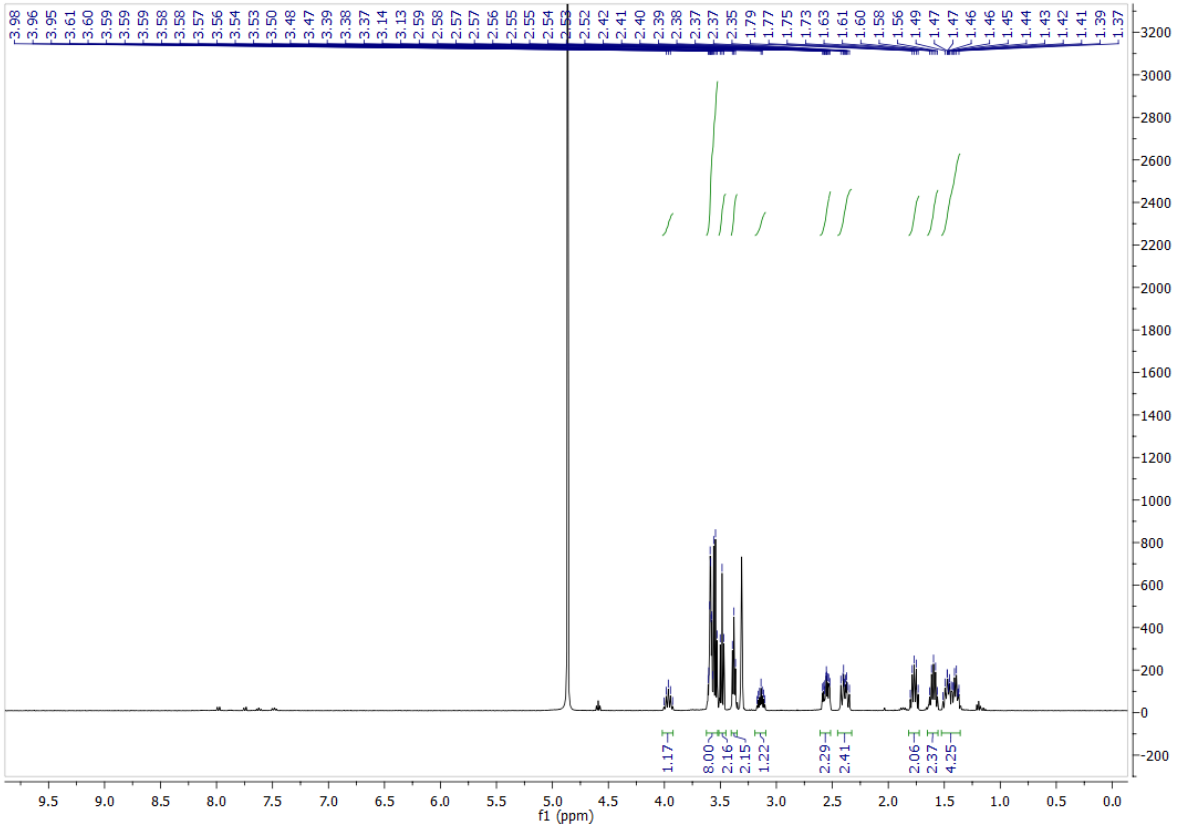
(1*R*,3*R*)-3-amino-*N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)cyclobutane-1-carboxamide 2,2,2-trifluoroacetate, 30

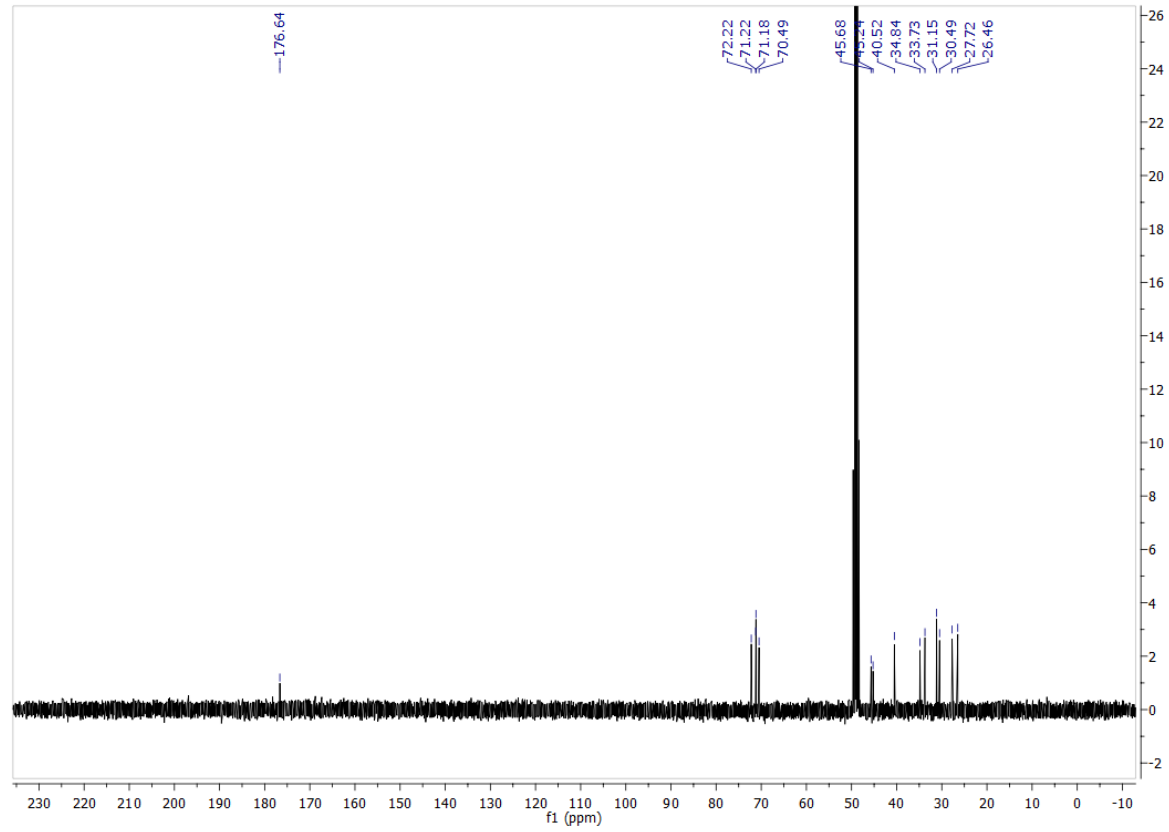


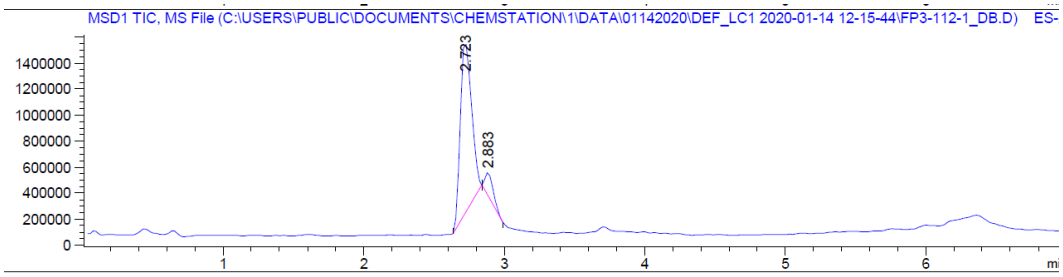
To a flask was added (1*R*,3*R*)-3-((*tert*-butoxycarbonyl)amino)cyclobutane-1-carboxylic acid (25 mg, 0.12 mmol, 1.0 equiv), DIPEA (61 µL, 0.35 mmol, 3.0 equiv), TBTU (48 mg, 0.15 mmol, 1.3 equiv), followed by 2-(2-((6-chlorohexyl)oxy)ethoxy)ethan-1-amine 2,2,2-trifluoroacetate (39 mg, 0.12 mmol, 1.0 equiv) in DMF (1 mL) and the reaction stirred at room temperature for 16 hr. The reaction was

concentrated and purified by column chromatography (0-10% MeOH in CH_2Cl_2) to yield the intermediate as a clear oil. The *N*-Boc was deprotected with 20% TFA in CH_2Cl_2, concentrated *in vacuo* and purified by reverse phase column chromatography (MeOH in H_2O (+0.1% TFA)) to yield the desired product as a white solid (1*R*,3*R*)-3-amino-*N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)cyclobutane-1-carboxamide 2,2,2-trifluoroacetate (**29**) (6 mg, 11%).

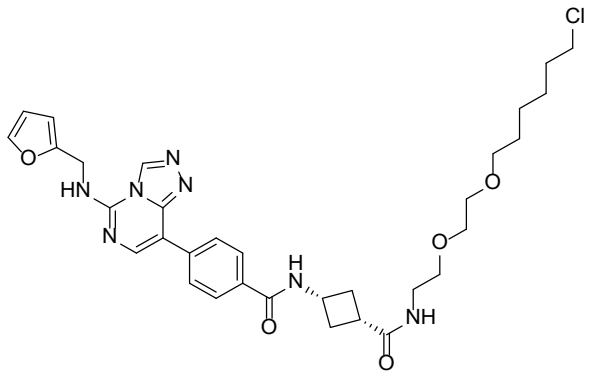
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 3.97 (p, *J* = 7.7 Hz, 1H), 3.61 – 3.52 (m, 8H), 3.48 (t, *J* = 6.57 Hz, 2H), 3.38 (t, *J* = 5.46 Hz, 2H), 3.18 – 3.09 (m, 1H), 2.61 – 2.50 (m, 2H), 2.44 – 2.33 (m, 2H), 1.81 – 1.72 (m, 2H), 1.64 – 1.56 (m, 2H), 1.51 – 1.36 (m, 4H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 176.64, 72.22, 71.22, 71.18, 70.49, 45.68, 45.24, 40.52, 34.84, 33.73, 31.15, 30.49, 27.72, 26.46. LCMS: expected mass for $[M+H]^+$ ($C_{15}H_{30}ClN_2O_3$) requires 321.19 m/z, found 321.20 m/z.







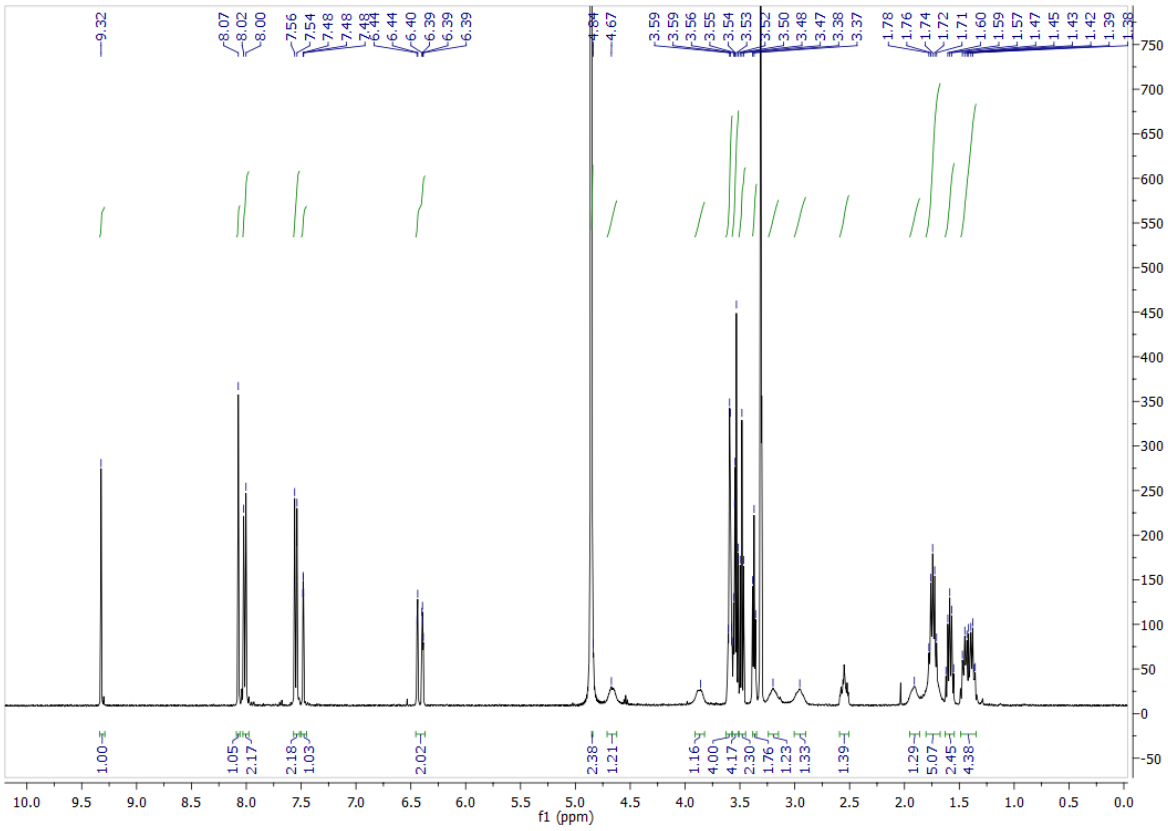
_N_-((1_S_,3_S_)-3-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)carbamoyl)cyclobutyl)-4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-_c_]pyrimidin-8-yl)benzamide, UNC7792

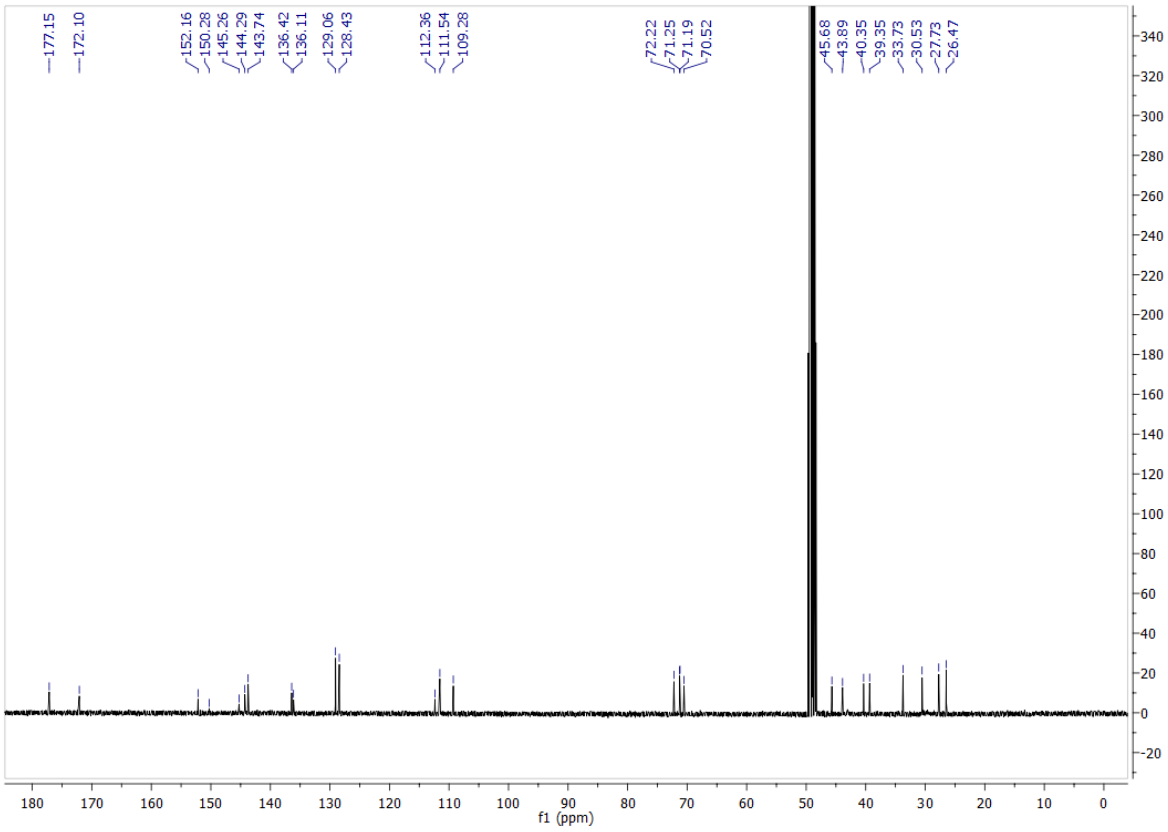


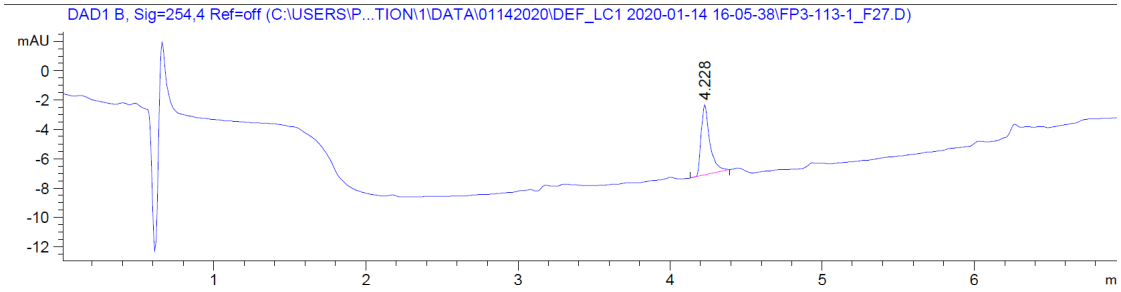
To a flask was added 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoic acid (**9**) (13 mg, 38 µmol, 1.1 equiv), DIPEA (18 µL, 102 µmol, 3.0 equiv), TBTU (14 mg, 45 µmol 1.3 equiv), followed by (1*S*,3*S*)-3-amino-*N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)cyclobutane-1-carboxamide

2,2,2-trifluoroacetate (**29**) (15 mg, 34 µmol, 1.0 equiv) in DMF (0.5 mL) and the reaction stirred at room temperature for 16 hr. The reaction was concentrated and purified by reverse phase column chromatography (MeOH in H_2O (+0.1% TFA)) to yield the desired product as a white solid *N*-((1*S*,3*S*)-3-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)carbamoyl)cyclobutyl)-4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamide (2 mg, 9%).

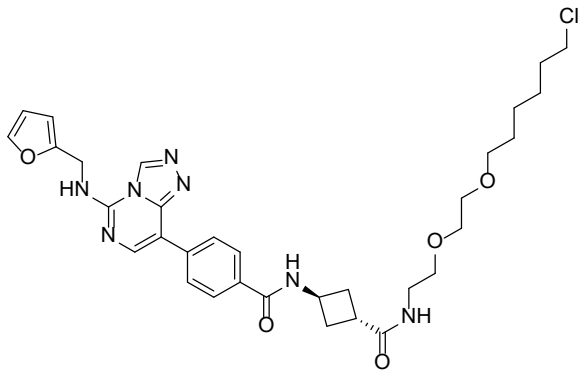
^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.32 (s, 1H), 8.08 (s, 1H), 8.04 (d, *J* = 8.55 Hz, 2H), 7.96 (d, *J* = 8.57 Hz, 2H), 7.50 – 7.46 (m, 1H), 6.45 – 6.38 (m, 2H), 4.55 – 4.44 (m, 1H), 3.63 – 3.58 (m, 4H), 3.57 – 3.52 (m, 4H), 3.49 (t, *J* = 6.56 Hz, 2H), 3.38 (t, *J* = 5.48 Hz, 2H), 2.91 – 2.81 (m, 1H), 2.59 (m, 2H), 2.37 – 2.27 (m, 2H), 1.80 – 1.71 (m, 2H), 1.64 – 1.56 (m, 2H), 1.51 – 1.35 (m, 4H). 2 × 1H not observed (under H_2O peak). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 176.70, 168.92, 152.19, 145.31, 144.04, 143.73, 137.84, 134.60, 128.86, 128.74, 112.46, 111.53, 109.25, 72.24, 71.28, 71.19, 70.56, 45.68, 42.37, 40.50, 39.34, 34.40, 34.04, 33.75, 30.54, 27.74, 26.49. 2 × C not observed. LCMS: expected mass for $[M+H]^+$ ($C_{32}H_{41}ClN_7O_5$) requires 638.28 m/z, found 638.30 m/z.







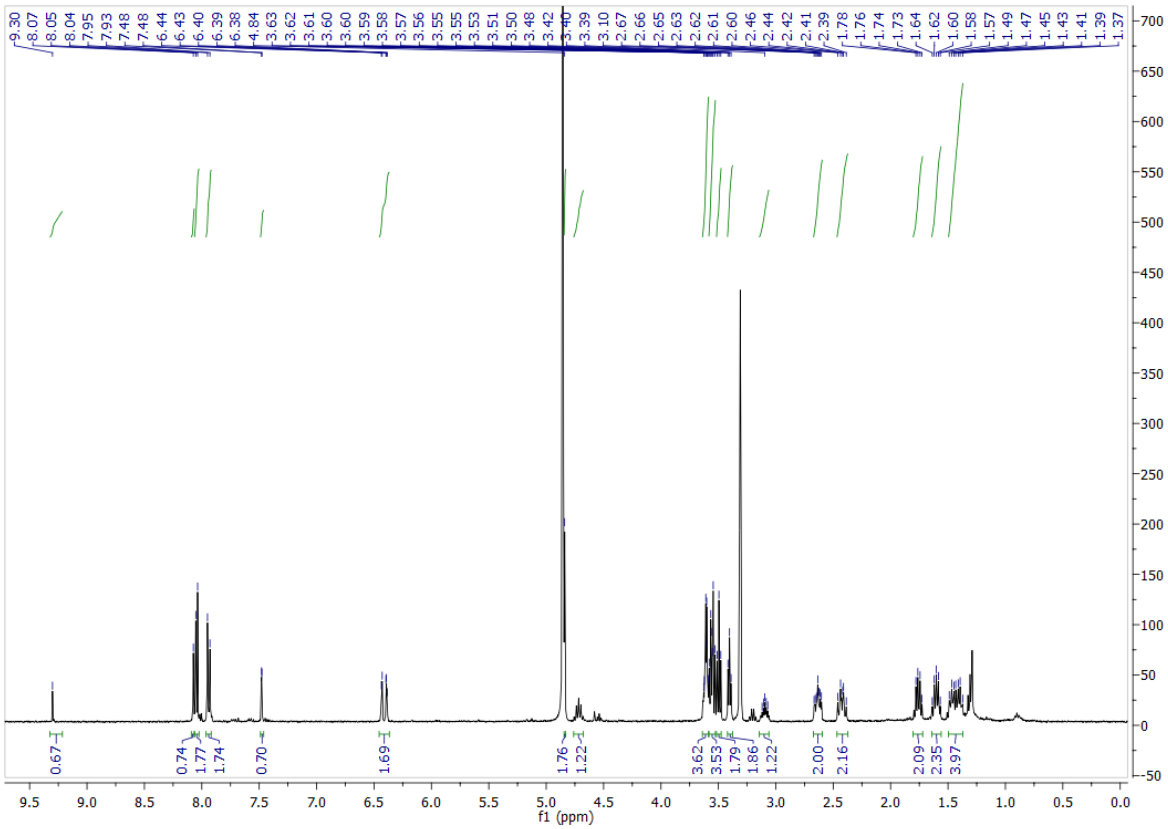
***N*-((1*R*,3*R*)-3-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)carbamoyl)cyclobutyl)-4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamide, UNC7794**

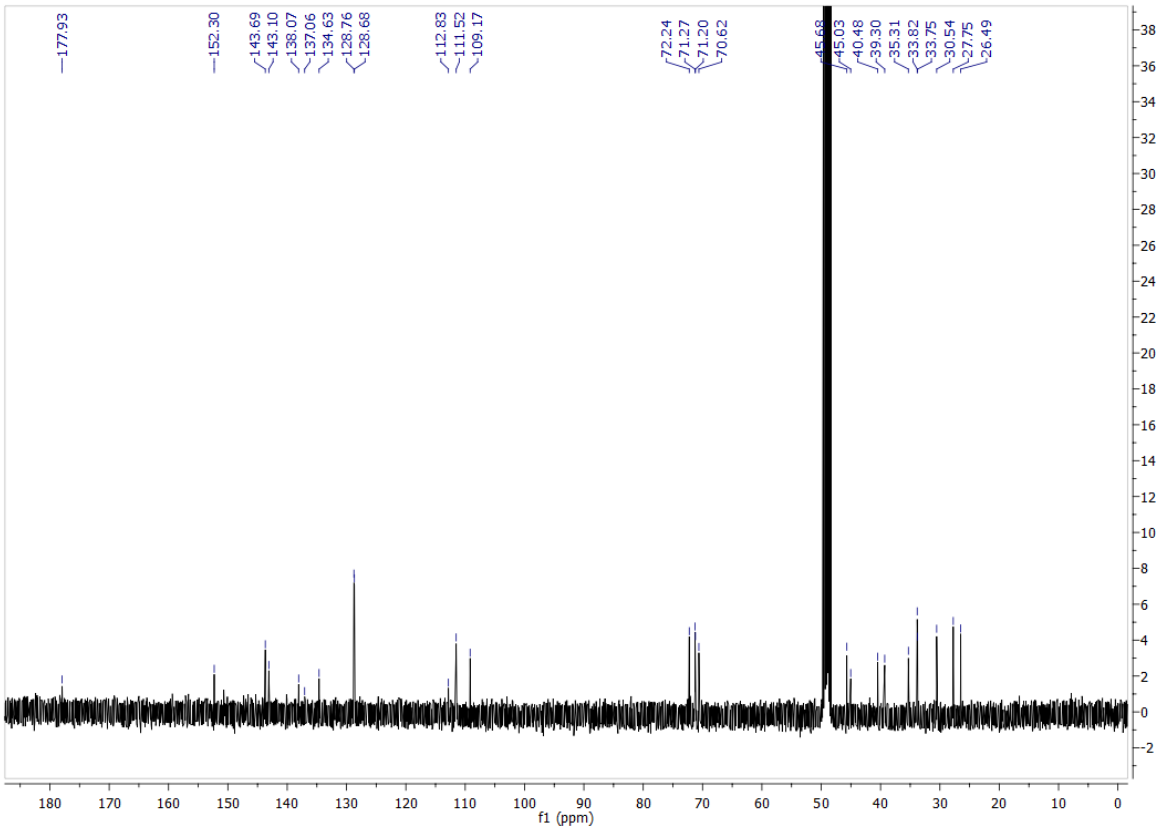


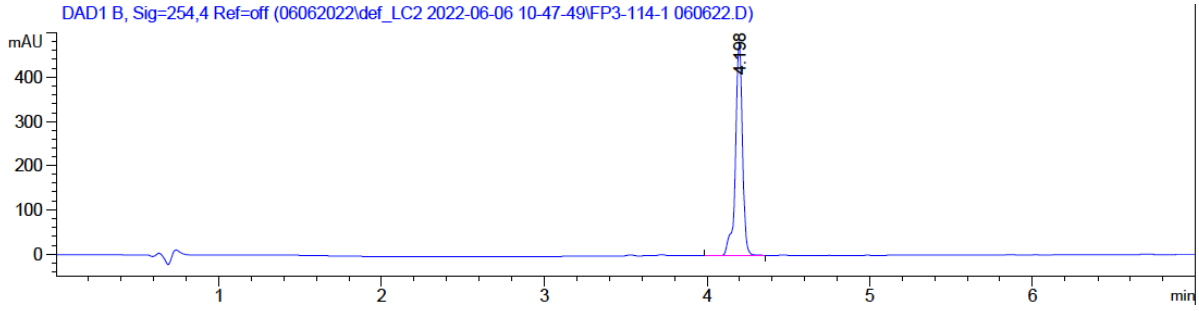
To a flask was added 4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzoic acid (**9**) (13 mg, 38 µmol, 1.1 equiv), DIPEA (18 µL, 102 µmol, 3.0 equiv), TBTU (14 mg, 45 µmol 1.3 equiv), followed by (1*R*,3*R*)-3-amino-*N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)cyclobutane-1-carboxamide

2,2,2-trifluoroacetate (**30**) (15 mg, 34 µmol, 1.0 equiv) in DMF (0.5 mL) and the reaction stirred at room temperature for 16 hr. The reaction was concentrated and purified by reverse phase column chromatography (MeOH in H_2O (+0.1% TFA)) to yield the desired product as a white solid *N*-((1*R*,3*R*)-3-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)carbamoyl)cyclobutyl)-4-(5-((furan-2-ylmethyl)amino)-[1,2,4]triazolo[4,3-*c*]pyrimidin-8-yl)benzamide (2 mg, 9%).

^{1}H NMR (CD_3OD-d_4, 400 MHz): δ 9.30 (s, 1H), 8.07 (s, 1H), 8.04 (d, *J* = 6.40 Hz, 2H), 7.94 (d, *J* = 8.55 Hz, 2H), 7.49 – 7.47 (m, 1H), 6.45 – 6.37 (m, 2H), 4.84 (s, 2H), 4.76 – 4.67 (m, 1H), 3.64 – 3.58 (m, 4H), 3.58 – 3.53 (m, 4H), 3.50 (t, *J* = 6.58 Hz, 2H), 3.40 (t, *J* = 5.45 Hz, 2H), 3.14 – 3.05 (m, 1H2.67 – 2.60 (m, 2H), 2.48 – 2.38 (m, 2H), 1.80 – 1.72 (m, 2H), 1.65 – 1.56 (m, 2H), 1.51 – 1.37 (m, 4H). ^{13}C NMR (CD_3OD-d_4, 101 MHz): δ 177.93, 152.30, 143.69, 143.10, 138.07, 137.06, 134.63, 128.76, 128.68, 112.83, 111.52, 109.17, 72.24, 71.27, 71.20, 70.62, 45.68, 45.03, 40.48, 39.30, 35.31, 33.82, 33.75, 30.54, 27.75, 26.49. 3 × C not observed. LCMS: expected mass for $[M+H]^+$ ($C_{32}H_{41}ClN_7O_5$) requires 638.28 m/z, found 638.3 m/z.







References

(1) Potjewyd, F.; Turner, A. M. W.; Beri, J.; Rectenwald, J. M.; Norris-Drouin, J. L.; Cholensky, S. H.; Margolis, D. M.; Pearce, K. H.; Herring, L. E.; James, L. I. Degradation of Polycomb Repressive Complex 2 with an EED-Targeted Bivalent Chemical Degrader. *Cell Chem Biol* 2020, *27* (1), 47-56.e15. https://doi.org/10.1016/j.chembiol.2019.11.006.