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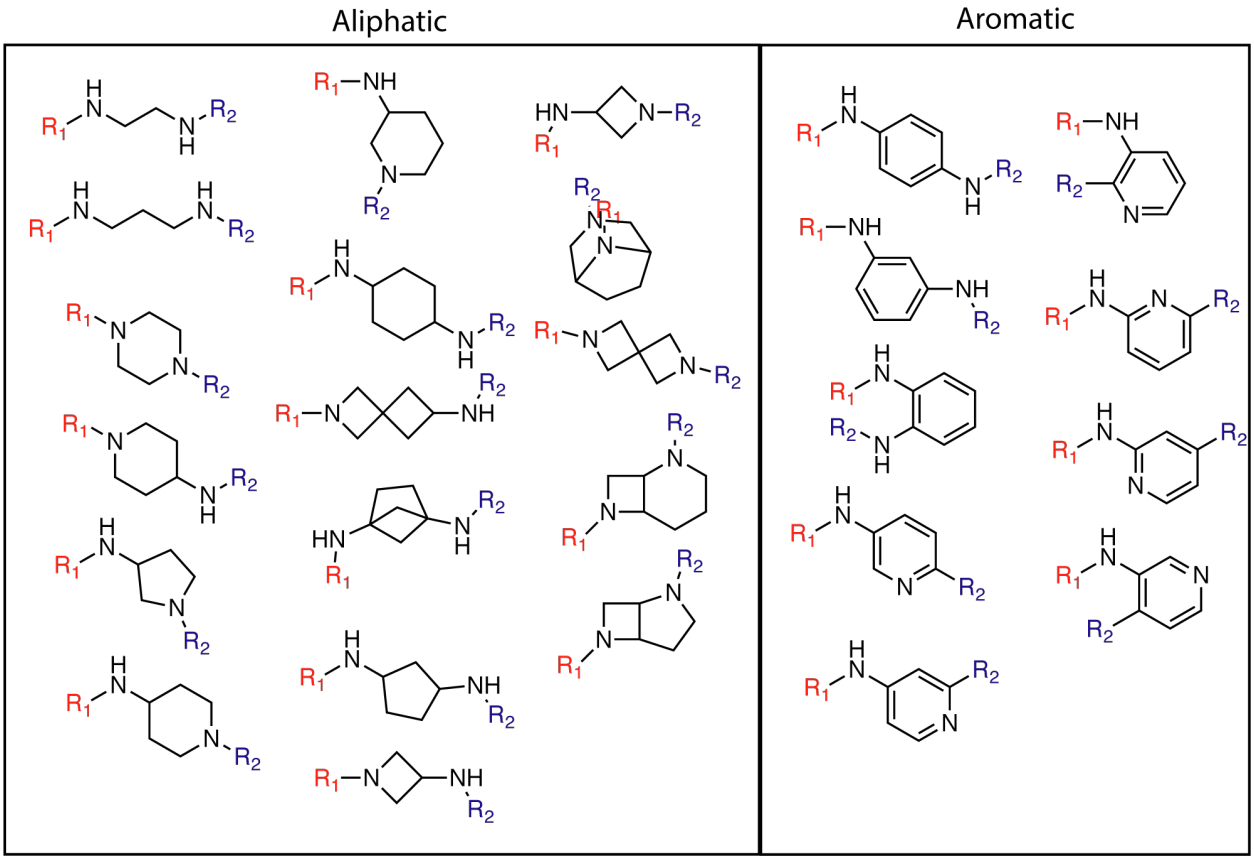
Supplemental information

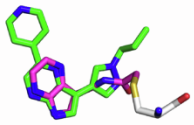
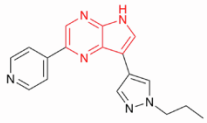
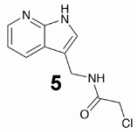
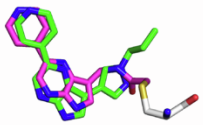
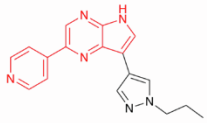
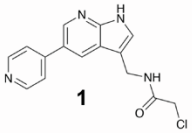
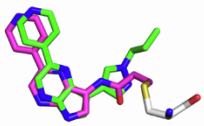
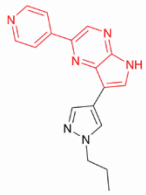
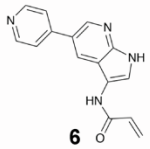
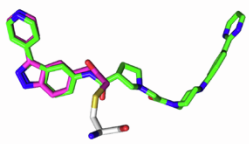
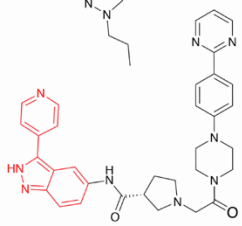
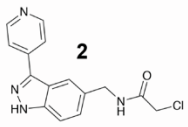
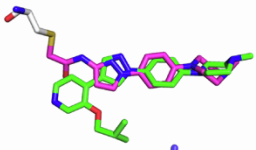
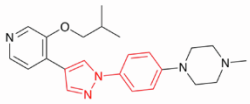
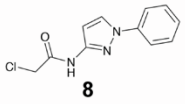
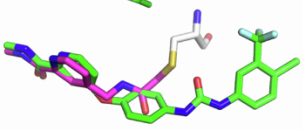
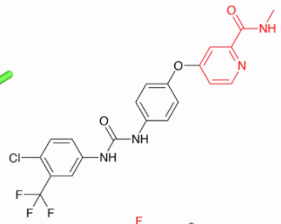
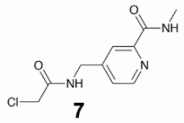
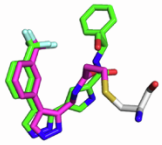
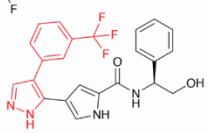
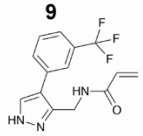
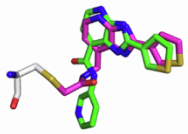
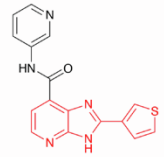
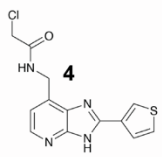
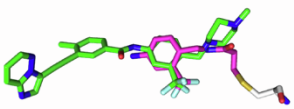
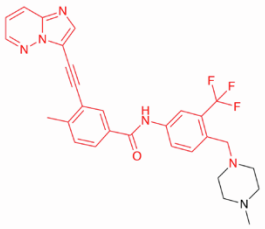
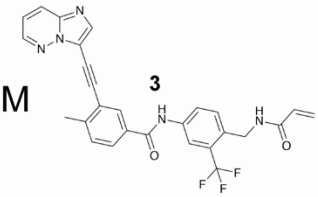
An automatic pipeline for the design of irreversible derivatives identifies a potent SARS-CoV-2 M^{pro} inhibitor

Daniel Zaidman, Paul Gehrtz, Mihajlo Filep, Daren Fearon, Ronen Gabizon, Alice Douangamath, Jaime Prilusky, Shirly Duberstein, Galit Cohen, C. David Owen, Efrat Resnick, Claire Strain-Damerell, Petra Lukacik, Covid-Moonshot Consortium, Haim Barr, Martin A. Walsh, Frank von Delft, and Nir London

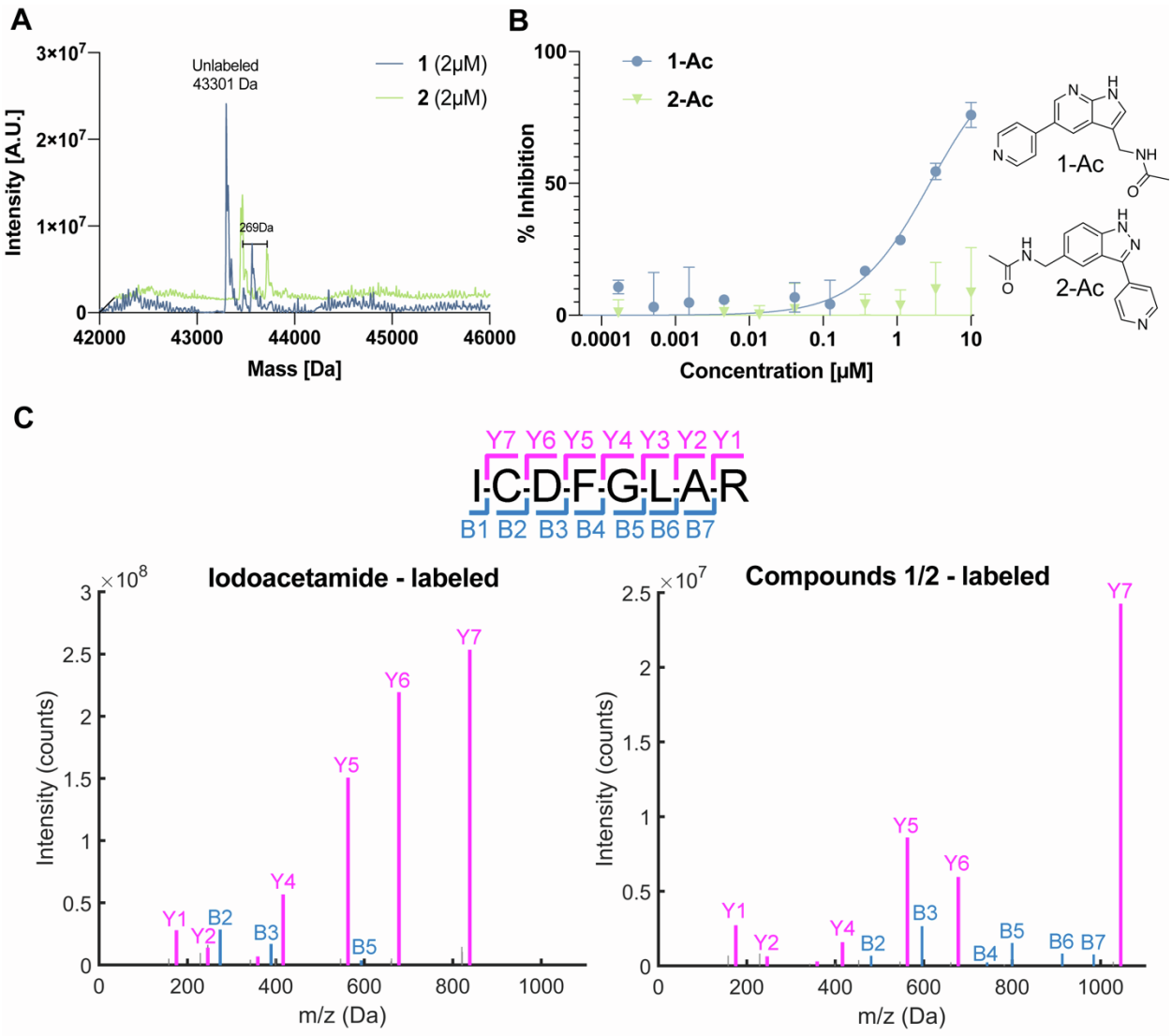
Supplementary Figures



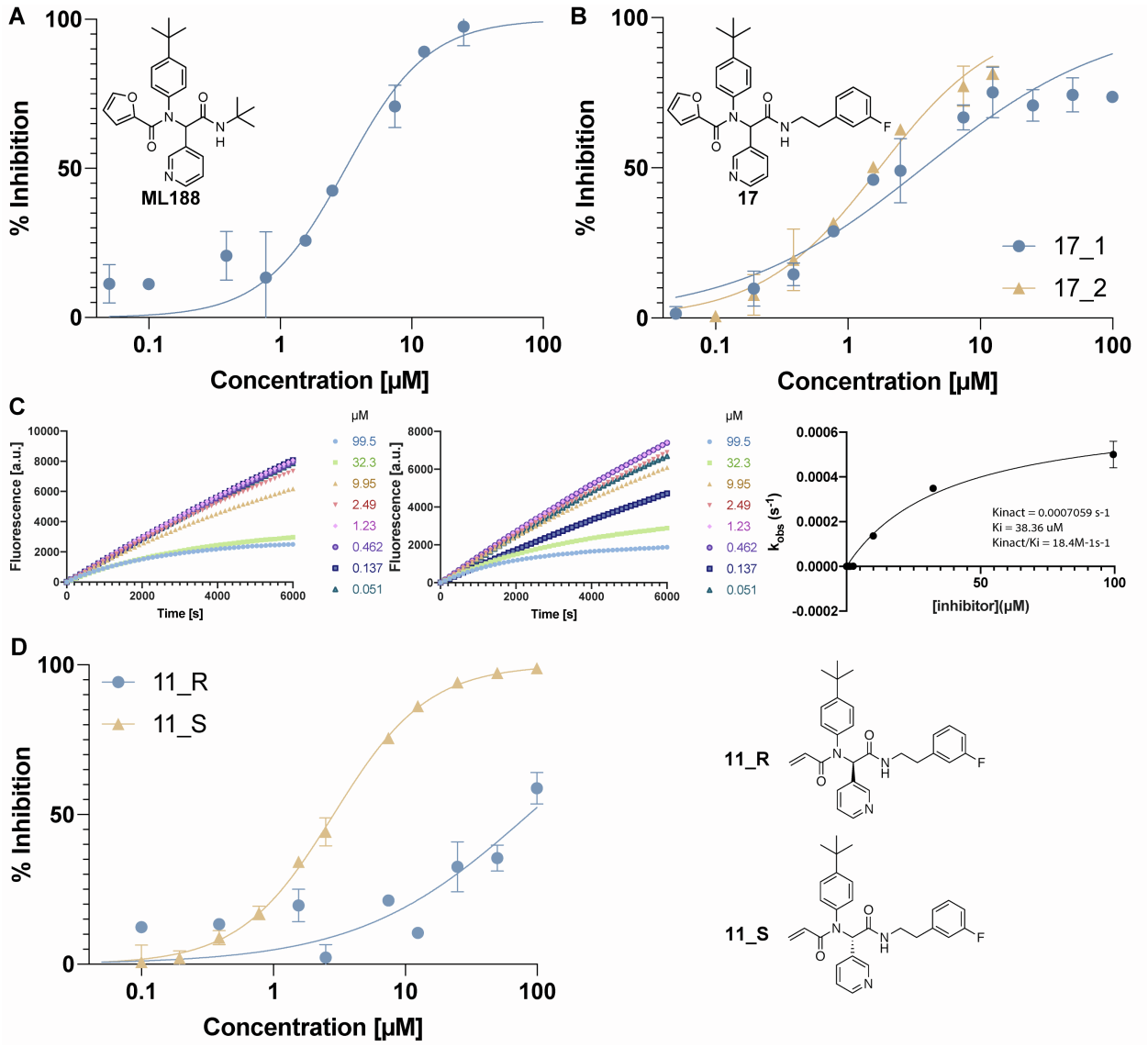
Supplementary Figure 1. Di-amine linkers for installation of electrophiles.
This figure is related to figure 1 in the main text. A list of di-amine linkers used in this manuscript to diversify the installation of electrophile unto reversible ligands. These were collected from the literature and were reported in the synthesis of covalent inhibitors (Shi et al. 2019; Caldwell et al. 2019; Liang et al. 2017; Engel et al. 2015). R1 - Electrophile; R2 - Fragment from the reversible binder. See also: WO201821765.

PDB RMSD	Model	Original ligand	IC50	Irreversible prediction tested	IC50
4QP9 0.42Å			71 nM		> 10 µM
4QP9 0.81Å			71 nM		3.1 µM
4QP9 0.8Å			71 nM		4.52 µM
4QTA 0.45Å			2.7 nM		2.9 µM
5IHA 0.96Å			9 nM		> 10 µM
3WZE 0.6Å			33 nM		> 10 µM
4FV1 0.94Å			83.2 nM*		> 10 µM
4DIT 0.9Å			13 nM		155 nM
4UXQ 0.76Å			16 nM		2.01 µM

Supplementary Figure 2. Prospective prediction of kinase covalent inhibitors.
This figure is related to figure 4 in the main text. For each of the nine prospective covalent kinase inhibitors we made and tested we show: the original PDB on which it was based including MCS RMSD. A structural alignment of the covalentizer candidate (magenta) and the original reversible ligand (green). The 2D structure of the original reversible ligand (red indicates the substructure the prediction was based on) and the 2D structure of the covalent candidate. Finally, we note the IC_{50} reported for the original ligand, and the IC_{50} we measured for the covalent analog. Note that for compounds derived from 4QP9 we used an azaindole scaffold for ease of synthesis. The choice of the initial pyrrolopyrazine scaffold was at least partially motivated by an intellectual property perspective, as hinted at in the main reference of 4QP9. * This value is based on BindingDB (Gilson et al. 2016).

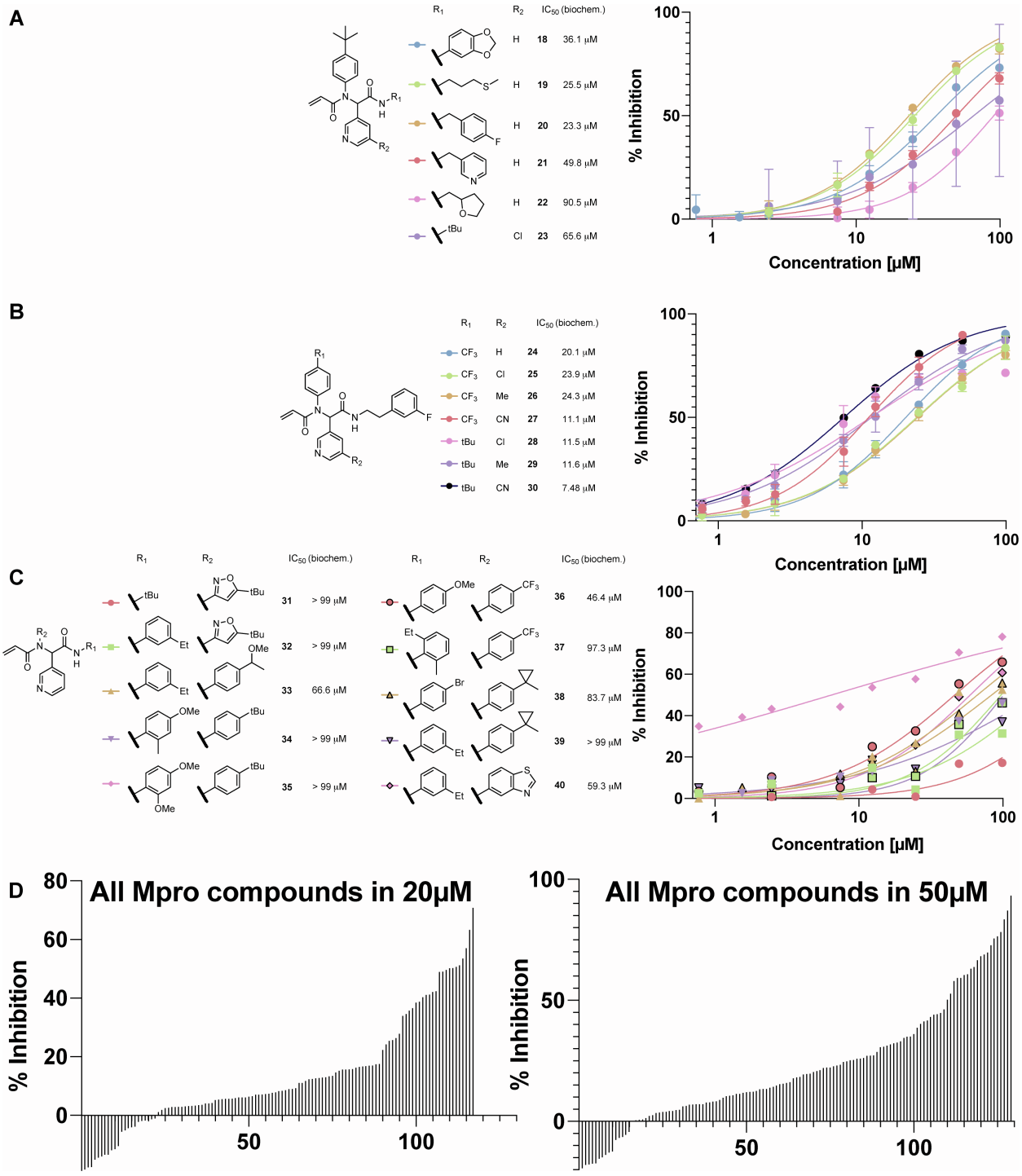


Supplementary Figure 3. Validation of covalent binding by ERK2 inhibitors.
This figure is related to figure 4 in the main text. **A.** Irreversible binding to ERK2 by intact protein mass spectrometry at low equimolar concentration (2 μM ERK2, 2 μM compound, 1 h incubation at room temperature). The expected protein-compound adducts were detected (25% and 33% labeling respectively; peak-to-peak Δm 265-270 Da for both compounds). **B.** IC_{50} curves of the acetyl versions **1-Ac** and **2-Ac** of **1** and **2** respectively. Each was tested in two repetitions. **1-Ac** had an IC_{50} of 2.79 μM while **2-Ac** was inactive with an IC_{50} of > 10 μM. **C.** MS/MS spectra of the +2 precursor ion from the tryptic peptide ERK2(165-172) modified by iodoacetamide (left) and labeled by compounds **1/2** (right). The modification by the molecule is evident by the increased difference between the Y6 and Y7 species as well as the larger mass of B ions.



Supplementary Figure 4. Covalent UGI based M^{pro} inhibitors.

This figure is related to figure 5 in the main text. M^{pro} biochemical inhibition of: **A.** Reversible racemic **ML188** (Jacobs et al. 2013) with an IC_{50} of 3.14 µM. **B.** Two independent repetition of reversible compound **17** with an IC_{50} of 3.71 µM (**17**-1) and 1.73 µM (**17**-2) and an average of 2.72 µM. **C.** K_i/K_{inact} of compound **11:** Two repetitions (left and middle) of the fluorescence inhibition assay in a kinetic mode (no pre-incubation) with a range of inhibitor concentrations. Curves were fitted to this rate equation to calculate k_{obs}: Y=(va*X)+(vb-va)*(1-EXP(-k_{obs}*X))/(k_{obs}). The right panel plots the k_{obs} as a function of inhibitor concentration. Each point is an average of the two values from the two repetitions on the left. K_i and K_{inact} were extracted by fitting Y=K_{inact}*X/(K_i+X). **D.** Pure enantiomers show very different inhibition, **11_R** was inactive with an IC_{50} of 86.32 µM, and **11_S** was slightly better than **11** with an IC_{50} of 2.86 µM. The structures of the two enantiomers are shown on the right side of the graph. For dose response curves in panels A,B,D,E,F,H: n=2, error bars represent standard deviation.



Supplementary Figure 5. Structure-activity relationship of Mpro inhibitors.

This figure is related to figure 5 in the main text. **A.** Biochemical IC_{50}'s and their associated curves for early inhibitors probing the S3 pocket. **B.** Biochemical IC_{50}'s and their associated curves for inhibitors with a 3'-fluorophenethylamide motif optimized for the S3 pocket combined with independently optimized substituents for the S1 and S2 sub-pockets showing non-synergistic effects. **C.** Biochemical IC_{50}'s and their associated curves for early combinatorial probing of the

S1 and S3 sub-pockets. **D.** Percent inhibition at two single doses: 20 μM (left) and 50 μM (right), for the majority of the 130 analogs of **10** which we made and tested. There is a large variability in inhibition over this large set of analogs, starting from inactive compounds, up to compounds that inhibit 60-80% in 20 μM. The data is available in Supp. Dataset 3.

Methods S1:

Organic Synthesis Methods – related to figures 4 and 5 in the main text

Unless otherwise noted, all reactions were performed in air with analytical grade solvents. All reagents and solvents used for the synthesis were used as acquired without further purification. Compounds **3, 4, 7, 9, 11, 12, 13, 14** and all other Ugi products were acquired from Enamine (custom synthesis). Flash chromatography was performed using a CombiFlash® System (Teledyne Isco, USA) with RediSep Rf Normal-phase Flash Columns. Reversed phase preparative HPLC was carried out using a Waters Prep 2545 Preparative Chromatography System, with UV/Vis detector 2489 at variable wavelengths (specified below), using XBridge® Prep C18 10µm 10x250 mm Column (PN: 186003891, SN:161I3608512502), 10 mL/min flow rate. Reaction progress and compounds' purity was monitored by Waters UPLC-MS system: Acquity UPLC® H class with PDA detector, and using Acquity UPLC® BEH C18 1.7 µm 2.1x50 mm Column (PN:186002350, SN 02703533825836). MS-system: Waters, SQ detector 2. TLC reaction progress analysis was carried out with aluminium backed SiO_2 plates with F254 indicator, and compounds were visualized either under UV light or with the typical staining methods.

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance III -300 MHz, 400 MHz spectrometer, equipped with a QNP probe. Chemical shifts are reported in ppm on the δ scale down field from TMS and are calibrated according to the deuterated solvents. All J values are given in Hertz. High resolution electron-spray mass spectrometry (HR-MS) of final compounds was performed by the Department of Chemical Research Support, Weizmann Institute.

N-Chloroacetamido-(5-pyridin-4-yl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)methanamine (1)

Step 1. *N*-Boc-(5-bromo-1*H*-pyrrolo[2,3-b]pyridin-3-yl)methanamine (**A1**)

A 25 mL RBF with stir bar was charged with 5-bromo-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (112.5 mg, 500 µmol, 1.00 equiv.) and glacial acetic acid (7 mL). The stirred solution was then cooled to 0 °C using an ice bath, followed by the slow addition of 25% aq. ammonia (4.18 mL, 54 mmol, 108 equiv.) and finally sodium cyanoborohydride (94.3 mg, 1.50 mmol, 3.00 equiv.) at the same temperature. The ice bath was allowed to warm overnight and the reaction mixture, containing a suspension, thoroughly stirred. After 16 h, the suspension had disappeared and the clear solution was basified to pH 12 using 4 M aq. NaOH at 0 °C. The aqueous layer was extracted with *i*PrOH:$CHCl_3$ 1:3 (v/v, 3 x 20 mL). The combined organic phases were dried over $MgSO_4$, filtered and the resultant solution concentrated (ca. 5 mL) using a rotary evaporator. The concentrate was transferred to a 20 mL vial, followed by the addition of Boc_2O (600 µL, ca. 2.5 mmol, 5 equiv. assuming complete conversion to the free amine). After 30 min, LC-MS analysis of the reaction mixture revealed a mixture of the product with some byproducts, including the corresponding free primary alcohol, 3-methyl-5-bromo-7-azaindole as well as the *N*-Boc protected secondary amine dimer. The solvent and excess reagent were removed by evaporation *in vacuo*. The crude product was purified by flash chromatography of a wet-loaded DCM extract of the crude solid (stationary phase: SiO_2, mobile phase: gradient from 0 to 8% MeOH in $CHCl_3$) to give the product as a fine white powder (18 mg, 80 µmol, 16%).

LC-MS: m/z 327 corresponding to $[M+H]^+$, m/z 329 corresponding to $[M+H]^+$ for the heavier bromine isotope.

Step 2. *N*-Boc-(5-pyrid-4-yl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)methanamine (**A2**)

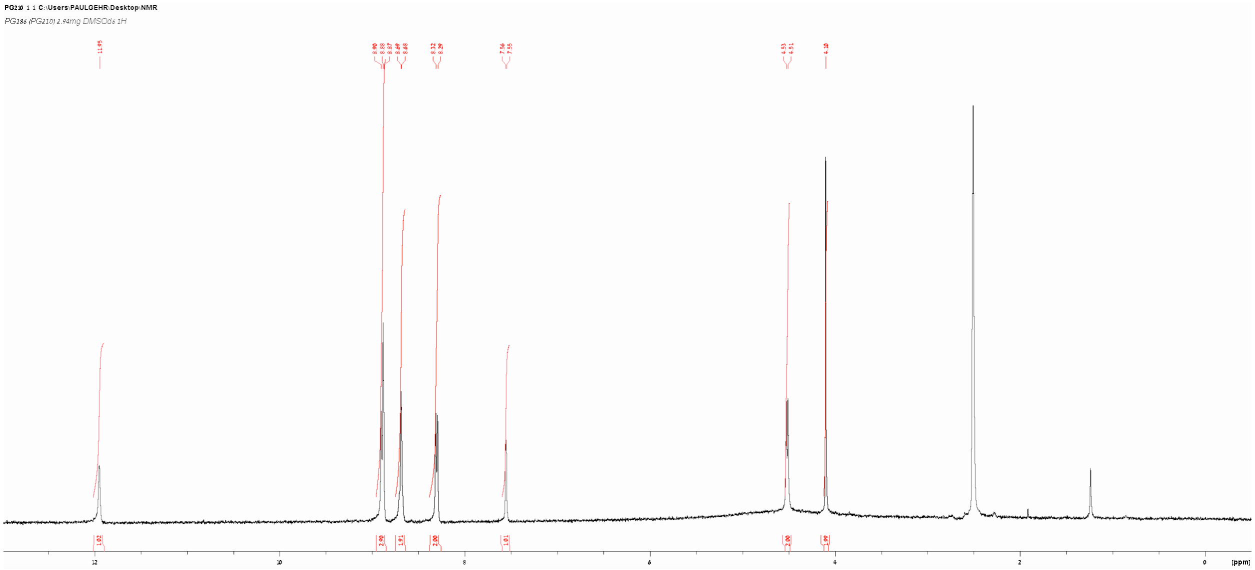
The heteroaryl bromide **A1** (18 mg, 55.2 µmol, 1 equiv.) was charged into a 2 dram vial containing a stir bar, pyrid-4-yl-boronic acid (8.2 mg, 66.2 µmol, 1.2 equiv.) and solid K_2CO_3 (22.9 mg, 165.55 µmol, 3.0 equiv.). The solids were dissolved in 1,4-dioxane (2 mL) and water (0.3 mL). The resultant stirred solution was thoroughly sparged with Ar gas for 15 min, followed by the addition of $Pd(dppf)Cl_2$ (4 mg, 5.52 µmol, 10 mol%) under an Ar stream. The solution was further sparged with Ar for 1 min. The vial was sealed, the solution stirred and heated to 85 °C using an oil bath. The initial yellow colored solution changed to a deep dark-red color. After 5 h, TLC and LC-MS analysis indicated complete conversion to the product. The reaction mixture was cooled to RT and diluted with water (1 mL) and EtOAc (2 mL). The phases were separated and the aq. layer extracted with EtOAc (2 x 1 mL). The combined organic phases were dried over Na_2SO_4 and then filtered in a Pasteur pipette filled with a plug of cotton. The plug was washed with a small amount of MeOH. The filtrate was concentrated to dryness by rotary evaporation, giving a brown solid residue as the product (13 mg, 40.1 µmol, 73%) which was directly employed in the next step without further purification.

LC-MS: m/z 325 corresponding to $[M+H]^+$.

Step 3. *N*-Chloroacetamido-(5-pyridin-4-yl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)methanamine (**1**)

N-Boc-amine **A2** (22 mg, 67.44 µmol, 1 equiv.) was suspended in DCM (1.5 mL) in a 2 dram vial under stirring, cooled to 0 °C using an ice bath, followed by the slow addition of TFA (0.5 mL) at the same temperature, which resulted in complete dissolution to give a cherry-red reaction mixture. After 10 min, the ice bath was removed and the reaction mixture allowed to stir at RT for 2 h. Excess solvent and TFA was removed by passing a stream of Ar over the reaction mixture. The residual oil was carefully treated with $NaHCO_3$ until basic and then extracted from the slurry using *i*PrOH:DCM 1:3 v/v (2 x 2 mL). The organic phase was dried over a small amount of $MgSO_4$ for 15 min, filtered through a cotton plug in a pasteur pipette and freed from solvent by rotary evaporation. The vial containing the dried free base was placed under an Ar atmosphere, followed by the dissolution of the oil in dry DMF. The dark solution was treated with DIPEA (12 µL, 67.44 µmol, 1.0 equiv.), cooled to 0 °C followed by the addition of chloroacetic acid NHS ester (13 mg, 80.9 µmol, 1.2 equiv) under a gentle Ar stream. After 1 h, the reaction was deemed complete by LC/MS and stopped by the addition of glacial AcOH (20 µL). Bulk DMF was removed by rotary evaporation under vacuum at 70 °C water bath temperature. The dark residue was partially dissolved in 50% aq. ACN (4 mL) and filtered through a 0.2 µm PVDF syringe tip filter. The filtrate was injected for purification by preparative RP-HPLC (C18 column, 5 to 95% MeCN in H_2O w. 0.1% TFA over 40 min, 10 mL/min, λ = 254 nm). After lyophilization of the target fractions, the product was obtained as a white solid (2.94 mg, 9.44 µmol, 14%).

^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 11.95 (s, 1H), 8.85 – 8.92 (m, 3H), 8.65 – 8.71 (m, 2H), 8.30 (d, J = 6.5 Hz, 2H), 7.55 (d, J = 2.1 Hz, 1H), 4.52 (d, J = 5.5 Hz, 2H), 4.10 (s, 2H). ^{13}C-NMR (101 MHz, DMSO-d_6): δ ppm 166.3, 158.7, 158.4, 153.3, 150.2, 144.7, 143.0, 127.3, 126.9, 123.0, 119.3, 112.8, 43.2, 34.8. LC-MS: m/z 301 corresponding to $[M+H]^+$, m/z 303 corresponding to $[M+H]^+$ for the heavier chlorine isotope. HRMS: m/z, $C_{15}H_{13}ClN_4O$, required 301.0856 $[M+H]^+$, found 301.0860 $[M+H]^+$, 1.3 ppm deviation.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7.55
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4.51
4.10
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2.00
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10
8
6
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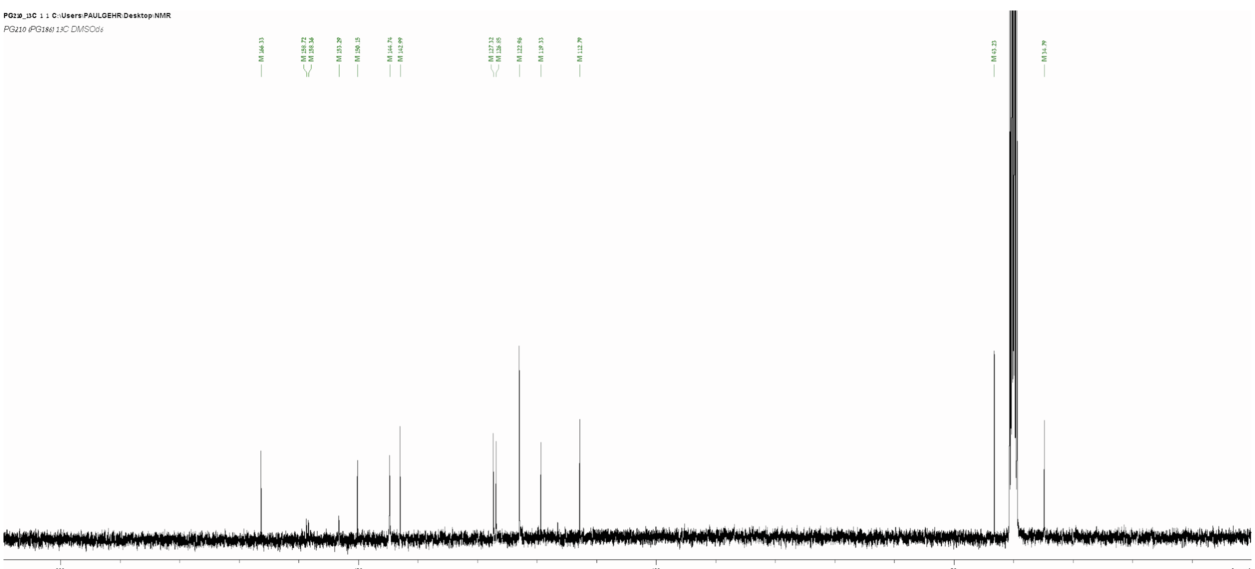
[ppm]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M 153.29
M 150.15
M 144.74
M 142.99
M 127.32
M 126.85
M 122.96
M 119.33
M 112.79
M 43.23
M 34.79
200
150
100
50
[ppm]

***N*-((3-(Pyridin-4-yl)-1H-indazol-5-yl)methyl)chloroacetamide (2)**

Step 1. 4-Methyl-N'-(pyridin-4-ylmethylene)benzenesulfonohydrazide (**B1**)

N-tolylhydrazine (1.98 g, 10.63 mmol, 1 equiv.) was dissolved in warm EtOH (8 mL), followed by the addition of isonicotinaldehyde (1 mL, 10.63 mmol, 1.0 equiv.) and the reaction mixture was cooled to 0 °C, reacted for 30 min and then vacuum filtered and washed with hexane and diethyl ether until the filter cake was nearly white. The yellow filtrate was discarded. The filter cake was dried to afford the product as an off-white solid (2.3 g, 7.76 mmol, 73%).

LC-MS: m/z 276 corresponding to $[M+H]^+$.

Step 2. 3-(Pyridin-4-yl)-1*H*-indazole-5-carbonitrile (**B2**)

The hydrazone **B1** (1.00 g, 3.63 mmol, 1 equiv.) and 4-nitrobenzonitrile (0.645 g, 4.36 mmol, 1.2 equiv.) were dissolved in dry DMF (24 mL) under Ar in a dried reaction vessel. NaH (522 mg of a 60% w/w oil dispersion, 13.08 mmol, 3.6 equiv.) was added to the mixture under a stream of Ar. The reaction vessel was stirred at 80 °C for 4 h under an Ar atmosphere, then cooled to RT, quenched with 1 M HCl until excess NaH was fully consumed. The reaction mixture was diluted with brine and then basified with 5% $NaHCO_3$. The solution was extracted with *i*PrOH:$CHCl_3$ 1:3 v/v (2 x 25 mL), then dried over $MgSO_4$, the volatiles removed by rotary evaporation. The crude product was thoroughly dried in vacuo, adsorbed on Celite by evaporation from a solution in EtOAc and MeOH, and then purified by flash chromatography (24 g SiO_2, 0 to 50% *i*PrOH in $CHCl_3$) to give the product as an orange solid (166 mg, 0.754 mmol, 21%).

LC-MS: m/z 221 corresponding to $[M+H]^+$.

Step 3. N^1-Boc-3-(Pyridin-4-yl)-1*H*-indazole-5-carbonitrile (**B3**)

B2 (110 mg, 495.4 µmol, 1.0 equiv.) was dissolved in MeCN (5 mL), followed by addition of DMAP (60.5 mg, 495.4 µmol, 1.0 equiv.) and then Boc_2O (171 µL, 743.1 µmol, 1.5 equiv.) and stirred for 3 d at RT. The crude reaction mixture was adsorbed on celite and purified by flash chromatography (4 g SiO_2, 0 to 50% EtOAc in Hexane) to give the product (93 mg, 0.288 µmol, 58%).

LC-MS: m/z 321 corresponding to $[M+H]^+$.

Step 4. N^1-Boc-3-(Pyridin-4-yl)-1*H*-indazole-5-carbonitrile (**B4**)

B3 (30 mg, 94 µmol, 1.0 equiv.) was dissolved in MeOH, cooled to 0 °C, followed by addition of $NiCl_2 \cdot 6\ H_2O$ (4.5 mg, 18.8 µmol, 20 mol%), Boc2O (43 µL, 187 µmol, 2.0 equiv.) and finally $NaBH_4$ (24.8 mg, 655.5 µmol, 7.0 equiv.) in several portions to avoid excessive bubbling. The reaction was allowed to warm to room temperature overnight. MeOH was removed by rotary

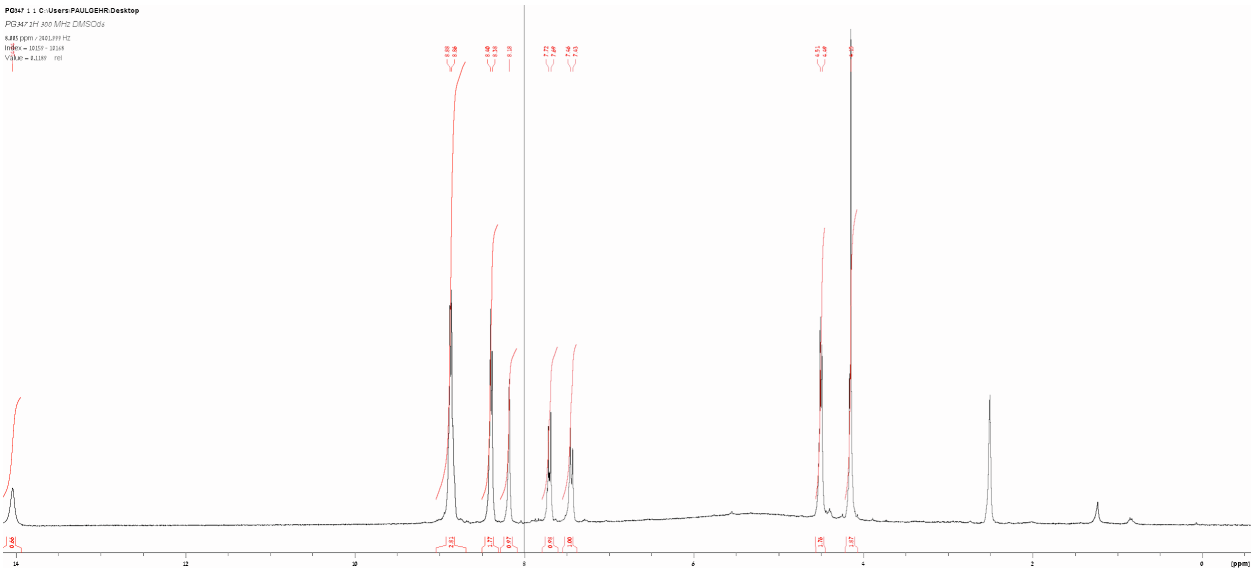
evaporation and the residue partitioned between aq. sat. $NaHCO_3$ and EtOAc. The organic phase was dried by filtration through a pipette plug of $MgSO_4$, adsorbed on celite and purified by flash chromatography (4 g SiO_2, 0 – 100% EtOAc in Hexane) to give the product (7 mg, 16.5 µmol, 18%).

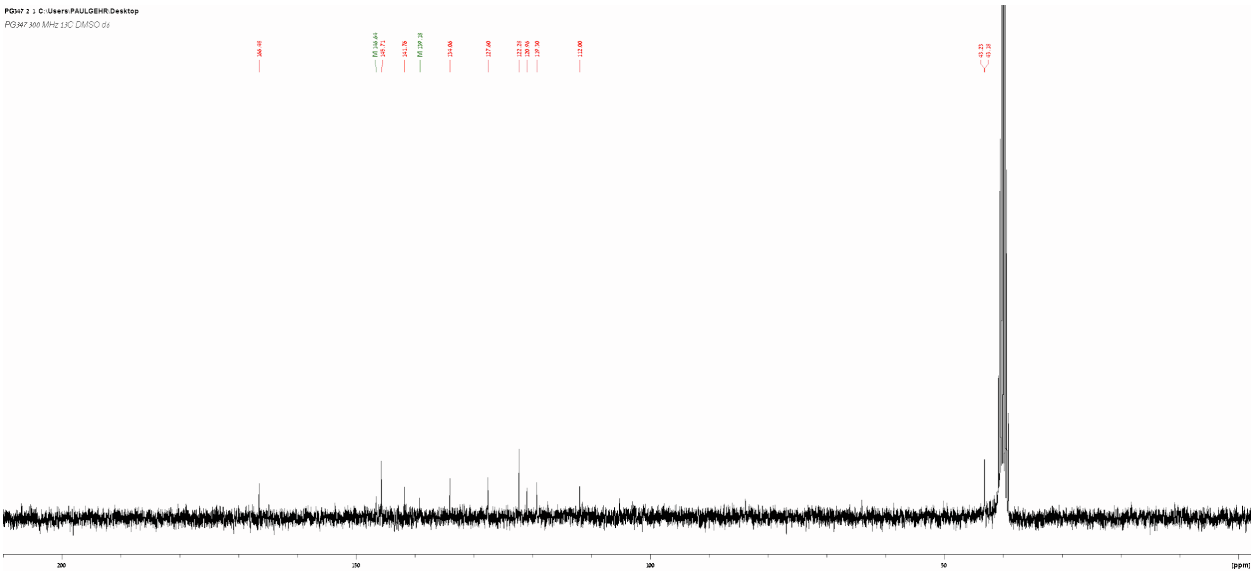
LC-MS: m/z 425 corresponding to $[M+H]^+$.

Step 5. *N*-((3-(Pyridin-4-yl)-1H-indazol-5-yl)methyl)chloroacetamide (**2**)

Intermediate **B4** (47 mg, 111.5 µmol. 1.0 equiv.) was dissolved in DCM (750 µL), followed by the addition of TFA (250 µL) at 0 °C under stirring. The reaction mixture was removed from the ice bath and stirred for 1 h at RT. Excess TFA and DCM was removed in a Ar stream. Under cooling, the mixture was basified with 2 M aq. NaOH, extracted with 20% *i*PrOH in $CHCl_3$ (3 x 2 mL), briefly dried over Na_2SO_4, filtered and the solvent removed by rotary evaporation. The resultant freebase (25 mg, 111.5 µmol, 1.0 equiv.) was immediately dissolved in THF (3 mL), cooled to 0 °C, followed by addition of Et_3N (31 µL, 223 µmol, 2.0 equiv.), then chloroacetic anhydride (19.1 mg, 111.5 µmol, 1.0 equiv.). The reaction was quenched by addition of TFA (1 equiv.), followed by rotary evaporation of all volatiles. The crude product was partially redissolved in 3 mL 5% aq. MeCN, 1 mL 50% aq. MeCN and 1 mL MeCN and then filtered over a 0.2 µm PVDF filter tip. The resultant clear solution was purified by injection into a preparative HPLC system (0 - 50% aq. MeCN over 30 min). The product was obtained as a white lyophilisate (7 mg, 23 µmol, 21%).

^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 14.1 (br s, 1H), 8.87 (d, J = 6.4 Hz, 3H), 8.39 (d, J = 6.4 Hz, 2H), 8.18 (s, 1H), 7.70 (d, J = 8.6 Hz, 2H), 7.46 (d, J = 8.6 Hz, 2H), 4.50 (d, J = 5.6 Hz, 2H), 4.15 (s, 2H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ ppm 166.5, 146.6, 145.7, 141.8, 139.2, 134.1, 127.6, 122.3, 121.0, 119.3, 112.0, 43.2, 43.2. LC-MS: m/z 301 corresponding to $[M+H]^+$, m/z 303 corresponding to $[M+H]^+$ for the heavier chloride isotope.





3-Acetamidomethyl-5-(pyrid-4-yl)-7-azaindole (1-Ac)

Step 1: 3-Azidomethyl-5-bromo-7-azaindole **(C1)**

5-Bromo-3-hydroxymethyl-7-azaindole (Fushimi et al. 2019) (250 mg, 1.1 mmol, 1.0 equiv.) was added into a dried 50 mL RBF under nitrogen, dissolved in dry THF (10 mL), followed by the addition of DBU (881 µmol, 0.8 equiv.) and diphenylphosphoryl azide (419 µL, 2.2 mmol, 2.0 equiv.). The resulting mixture was refluxed under nitrogen with stirring for 6 h. The reaction mixture was diluted with EtOAc, washed with brine and the crude material purified by flash chromatography to give the azide product as a white powder (157 mg, 0.623 mmol, 57%).

Step 2: N^1-Boc-3-Azidomethyl-5-bromo-7-azaindole **(C2)**

3-Azidomethyl-5-bromo-7-azaindole (157 mg, 623 µmol, 1.0 equiv) was dissolved in ice-cooled DCM (6 mL), followed by addition of DMAP (3.8 mg, 31 µmol, 5 mol%), Et_3N (87 µL, 623 µmol, 1.0 equiv) and finally melted Boc_2O (158 µL, 685 µmol, 1.1 equiv) at the same temperature. The reaction mixture was stirred at room temperature for 6 h, directly adsorbed on celite, followed by purification by flash chromatography (4 g SiO_2, 0 to 30% ethyl acetate in hexane over 15 min, 18 mL/min) to give the product (79 mg, 225 µmol, 36%)

Step 3: N^1-Boc-3-Acetamidomethyl-5-bromo-7-azaindole (**C3**)

N^1-Boc-3-Azidomethyl-5-bromo-7-azaindole (75 mg, 213 µmol, 1.0 equiv) was placed in a two-dram vial and dissolved in THF (p.A. grade, 1 mL), followed by addition of PPh_3 (56 mg, 213 µmol, 1.0 equiv) under stirring. After 1 h, the iminophosphorane was formed quantitatively by LC-MS analysis. To the reaction mixture was added water (100 µL, excess) and the reaction mixture stirred at 50 °C for 18 h. The reaction mixture was cooled to room temperature, basified by addition of Et_3N (70 µL, 500 µmol, x equiv.), followed by addition of Ac_2O (40 µL). The reaction mxiture was partitioned between EtOAc and water, the organic phase separated and dried over MgSO4, followed by flash chromatography to give the desired product.

Step 4: 3-Acetamidomethyl-5-(pyrid-4-yl)-7-azaindole (**1-Ac**)

N^1-Boc-3-Acetamidomethyl-5-bromo-7-azaindole (78 mg, 213 µmol) was placed in a two-dram vial, followed by dissolution in dioxane (1.5 mL) and water (0.5 mL), to which K_2CO_3 (31 mg, 256 µmol, 3.0 equiv.) and pyrid-4-ylboronic acid (31 mg, 256 µmol, 1.20 equiv.) was added. The vial was capped with a silicone septum, sparged with Ar for 15 min, followed by addition of $Pd(dppf)Cl_2$ under an Ar backflow. The vial was sealed with parafilm and heated at 80 °C for 18 h. The reaction mixture was diluted with EtOAc (2 mL) and water (1 mL). The aq. phase was separated, extracted with EtOAc (1 mL) and then 20 % ^{i}PrOH in $CHCl_3$ (1 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated by rotary evaporation. The crude residue was purified by preparative RP-HPLC to give the product as a white lyophilisate (7 mg, 26.3 µmol, 12%).

HRMS: m/z, calculated 267.1240 for $C_{15}H_{15}N_4O$ $[M+H]^+$, found 267.1246 $[M+H]^+$, 0.4 ppm deviation.

N-((3-(pyridine-4yl)-1H-indazol-5yl)methyl)acetamide (2-Ac)

Step 1. 3-(Pyridin-4-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazole-5-carbonitrile**(D1)**

3-(Pyridine-4-yl)-1H-indazole-carbonitrile (86.3 mg, 392 µmol, 1.0 equiv) was suspended in 1 mL of dry DMF. In a separate vial under inert conditions, sodium-hydride (23.51 mg, 60% w/w oil dispersion, 588 µmol, 1.5 equiv) was suspended in 0.75 mL of solvent containing 0.5 mL of dry DMF and 0.25 mL of dry DMSO and the suspension was chilled on ice. The indazole solution was added to NaH solution, on ice. After stirring for 15 minutes at room temperature, a deep red solution was obtained. This solution was cooled on ice and 2-(trimethylsilyl)ethoxymethyl chloride (104.5 mg, 111 µL, 627 µmol, 1.6 equiv) was added and the solution was warmed to room temperature and stirred overnight. The brown suspension was quenched with 5% NH_4Cl solution and the reaction products were extracted with chloroform (2x2 mL). The organic phases were combined, washed with brine and dried over anhydrous Na2SO4. The solids were filtered and the volatiles were evaporated under reduced pressure. The desired product was loaded on celite and purified using flash chromatography (4 g SiO2, 0 to 100% ethyl acetate in hexane, 18 mL/min) to give the product (57.6 mg, 164.3 µmol, 42%).

LC-MS: m/z 351 corresponding to $[M+H]^+$

Step 2. tert-butyl-((3-(pyridin-4-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazol-5-yl)-

-methyl)carbamate **(D2)**

3-(Pyridin-4-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazole-5-carbonitrile (32.5 mg, 93 µmol 1 equiv) was dissolved in 2 mL of dry methanol. To this solution NiCl2*6H2O (2.2 mg, 9.3 µmol, 0.1 equiv) was added, along with Boc_2O (24.3 mg, 20.4 µL, 111.3 µmol, 1.2 equiv) and the solution was chilled on ice. To minimize bubbling, NaBH4 (49 mg, 1.3 mmol, 14 equiv) was added in portions, which resulted in black solution. The solution was left to warm to room temperature and stirred. After 2h, an additional portion of reducing agent (25 mg, 0.65 mmol, 7 equiv) was added, in portions on ice. After 3h, the volatiles were evaporated under reduced pressure. To the crude, saturated NaHCO3 solution was added and the mixture was extracted with ethyl acetate (3x 4 mL). The organic layers were combined, dried over anhydrous MgSO4, filtered and the solvent was removed using a rotary evaporator. The product was used without further purification (21.5 mg, 47.3 µmol, 51%).

LC-MS: m/z 455 corresponding to $[M+H]^+$

Step 3. N-((3-(pyridin-4-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazol-5-yl)methyl)-

-acetamide **(D3)**

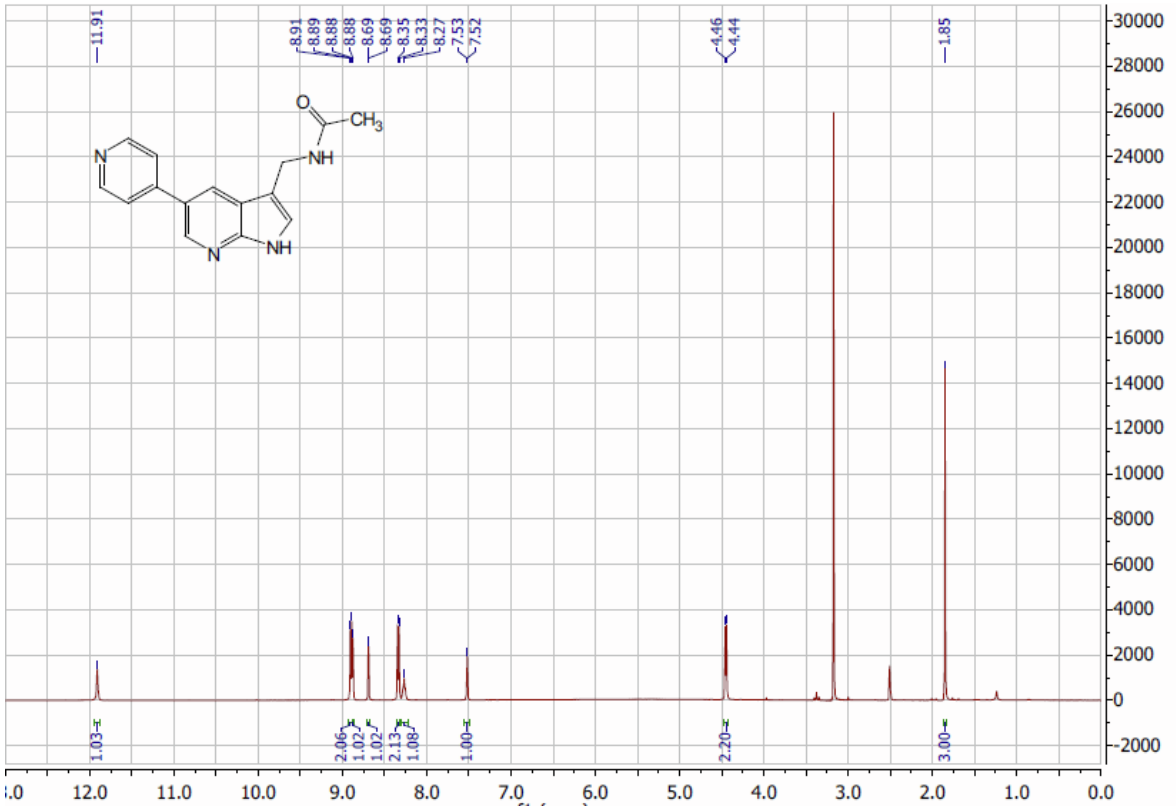
Tert-butyl((3-(pyridin-4-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazol-5-yl)methyl)-carbamate was deprotected using 15% TFA in DCM. The starting material (21.6 mg, 47.5 µmol, 1 equiv) was dissolved in DCM and cooled on ice. To chilled solution, TFA was added in portions, after which the solution was warmed to room temperature and stirred during 3h. When no starting material was detected, all the volatiles were removed under reduced pressure. The solid was treated with 1 mL of 2N NaOH. The solution was extracted with 20% propan-2-ol in chloroform (2x1 mL). The organic layers were combined, dried over anhydrous MgSO4, filtered and the solvent was removed using a rotary evaporator. The obtained free base was dissolved in 2 mL of dry THF containing triethylamine (5.71 mg, 7.8 µL, 56.4 µmol, 2 equiv). This solution was cooled on ice and acetic anhydride was added (3.46 mg, 3.2 µL, 33.8 µmol, 1.2 equiv) and the reaction mixture was warmed to room temperature and stirred overnight. The reaction was diluted with brine and extracted with 20% propan-2-ol in chloroform (2x2mL). The organic layers were combined, dried over anhydrous MgSO4, filtered and the solvent was removed using a rotary evaporator. The product was not further purified (20.05 mg 50.6 µmol, 82%).

LC-MS: m/z 397 corresponding to $[M+H]^+$

Step 4. N-((3-(pyridin-4-yl)-1H-indazol-5-yl)methyl)acetamide **(2-Ac)**

N-((3-(Pyridin-4-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazol-5-yl)methyl)acetamide (20.05 mg, 50.56 µmol) was dissolved in 2 mL of dry THF under Ar. To this solution 1M TBAF in THF was added (506 µL, 10 equiv) and the reaction mixture was heated to 70C overnight. All volatiles were removed under reduced pressure and the solid was dissolved in 50% MeCN/Water solution. The solution was filtered over a 0.2 µm PVDF filter tip. The obtained clear solution was purified by injection into a preparative HPLC system (0-100% aq. MeCN, over 30 minutes). The product was obtained after lyophilisation (9 mg, 33.7 µmol 67%).

^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 11.90 (s, 1H), 8.91 (d, *J* = 6.8 Hz, 2H), 8.88 (d, *J* = 2.3 Hz, 1H), 8.69 (d, J = 2.2 Hz, 1H), 8.34 (d, *J* = 6.8 Hz, 2H), 8.27 (t, *J* = 5.3 Hz, 1H), 7.52 (d, *J* = 2.3 Hz, 1H), 4.45 (d, J = 5.7 Hz, 2H), 1.85 (s, 1H). ^{13}C-NMR (101 MHz, DMSO-d_6): δ ppm 169.5, 150.2, 144.3, 143.0, 127.4, 126.6, 123.1, 122.7, 119.5, 113.6, 49.1, 34.3, 23.1. LC-MS: m/z 267 corresponding to $[M+H]^+$. HRMS: m/z, calculated 267.1246 for $C_{15}H_{15}N_4O$ $[M+H]^+$, found 267.1242 $[M+H]^+$, 1.5 ppm deviation.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4.44
1.85
O
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NH
N
NH
1.03
2.06
1.02
1.02
2.13
1.08
1.00
2.20
3.00
12.0
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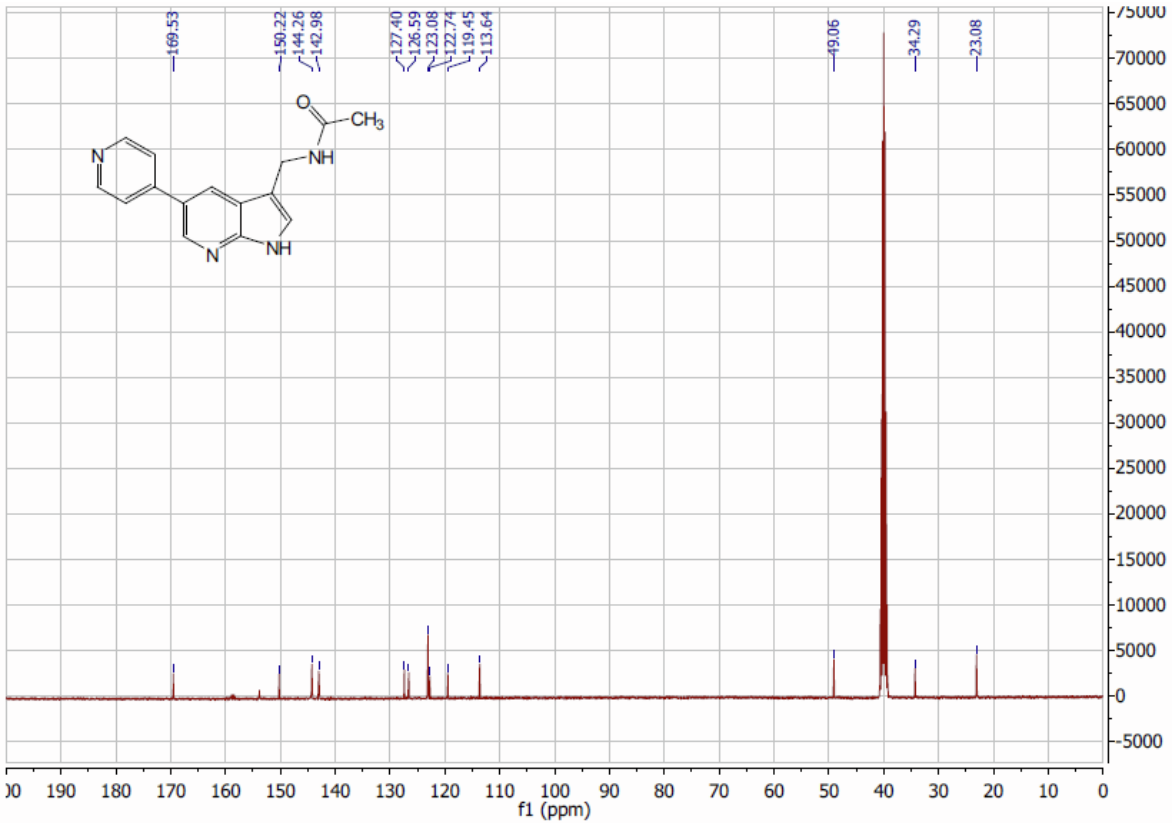
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NH
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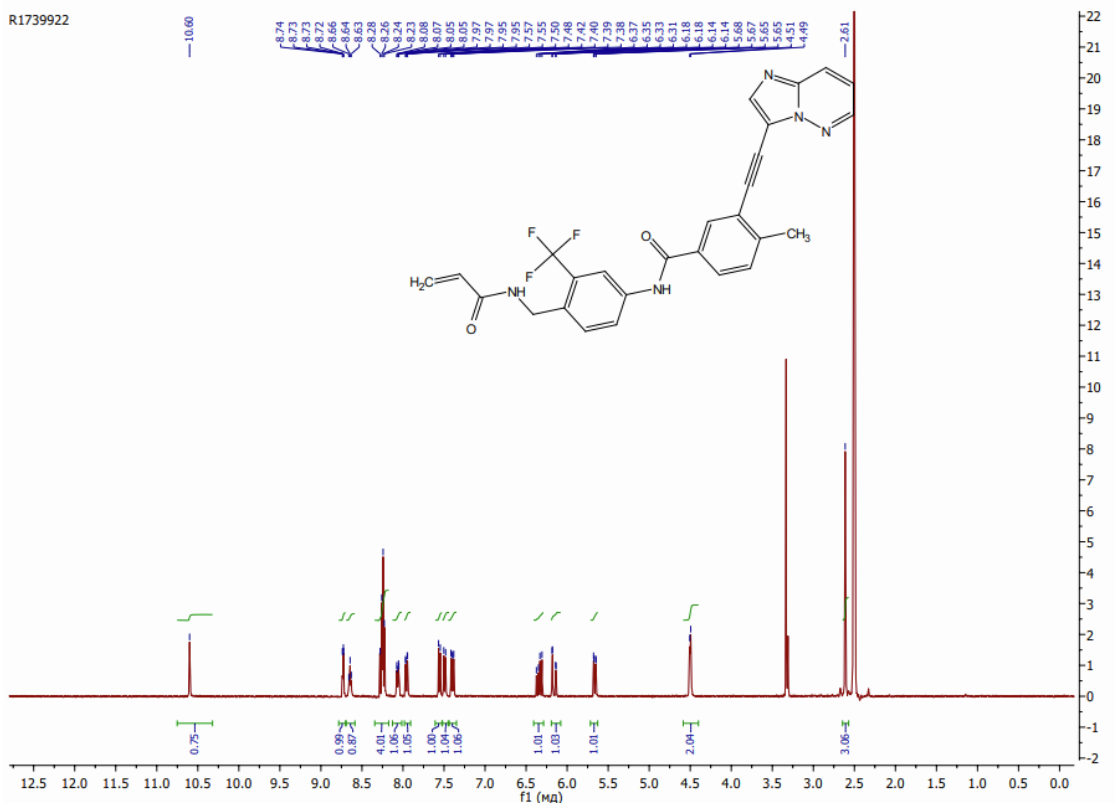
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

4-(acrylamidomethyl)-*N*-(3-(imidazo[1,2-b]pyridazin-3-ylethynyl)-4-methylphenyl)-3-(trifluoromethyl)benzamide (**3**)

This compound and the associated analytical data was provided by Enamine (Ukraine). HRMS: m/z, calculated 252.5858 $C_{27}H_{22}F_3N_5O_2$ $[M+2H]^{2+}$, found 252.5849 $[M+2H]^{2+}$, 3.92 ppm deviation.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6.18
6.18
6.14
6.14
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5.67
5.65
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4.49
2.61
0.75
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0.87
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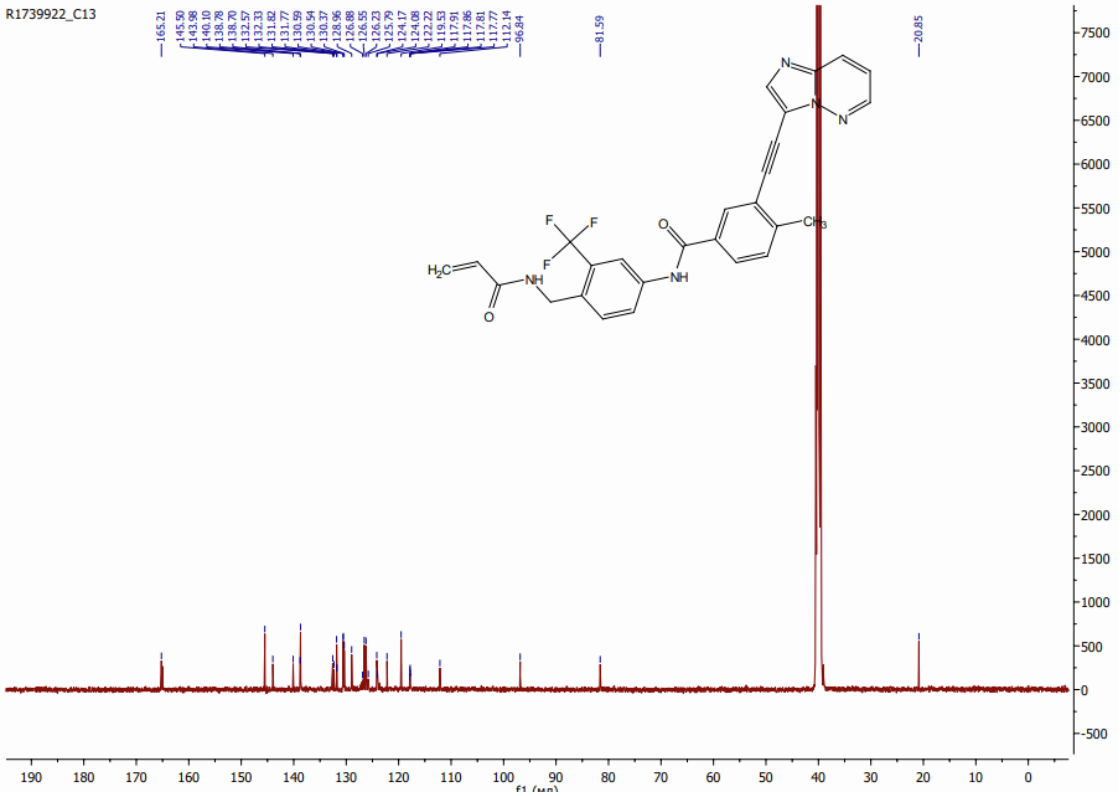
1.06
1.01
1.03
1.01
2.04
3.06
f1 (мд)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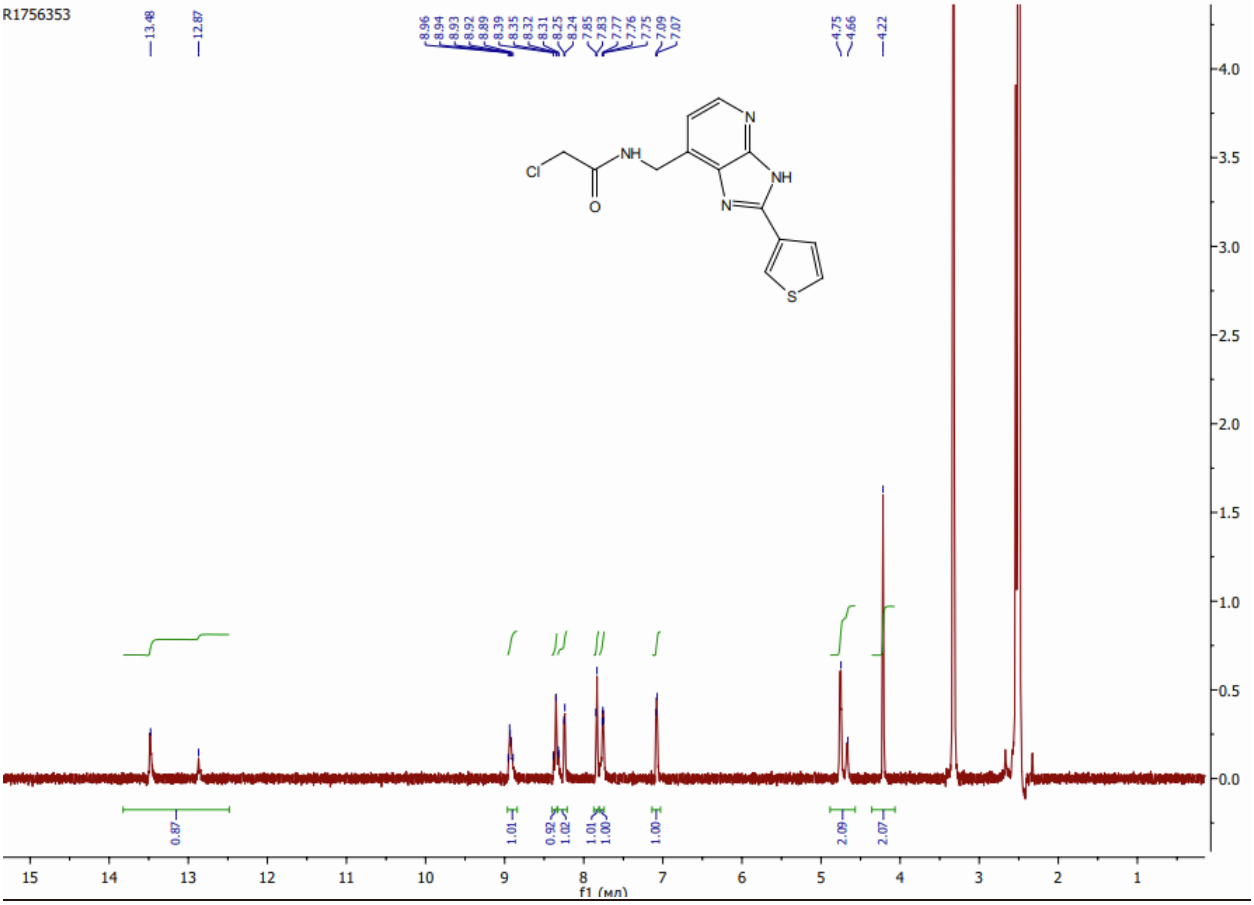
126.55
126.23
125.79
124.17
124.08
122.22
119.53
117.91
117.86
117.81
117.77
112.14
96.84
81.59
20.85
f1 (мд)

_N_-((2-(thiophen-3-yl)-3_H_-imidazo[4,5-b]pyridin-7-yl)methyl)chloroacetamide (4)

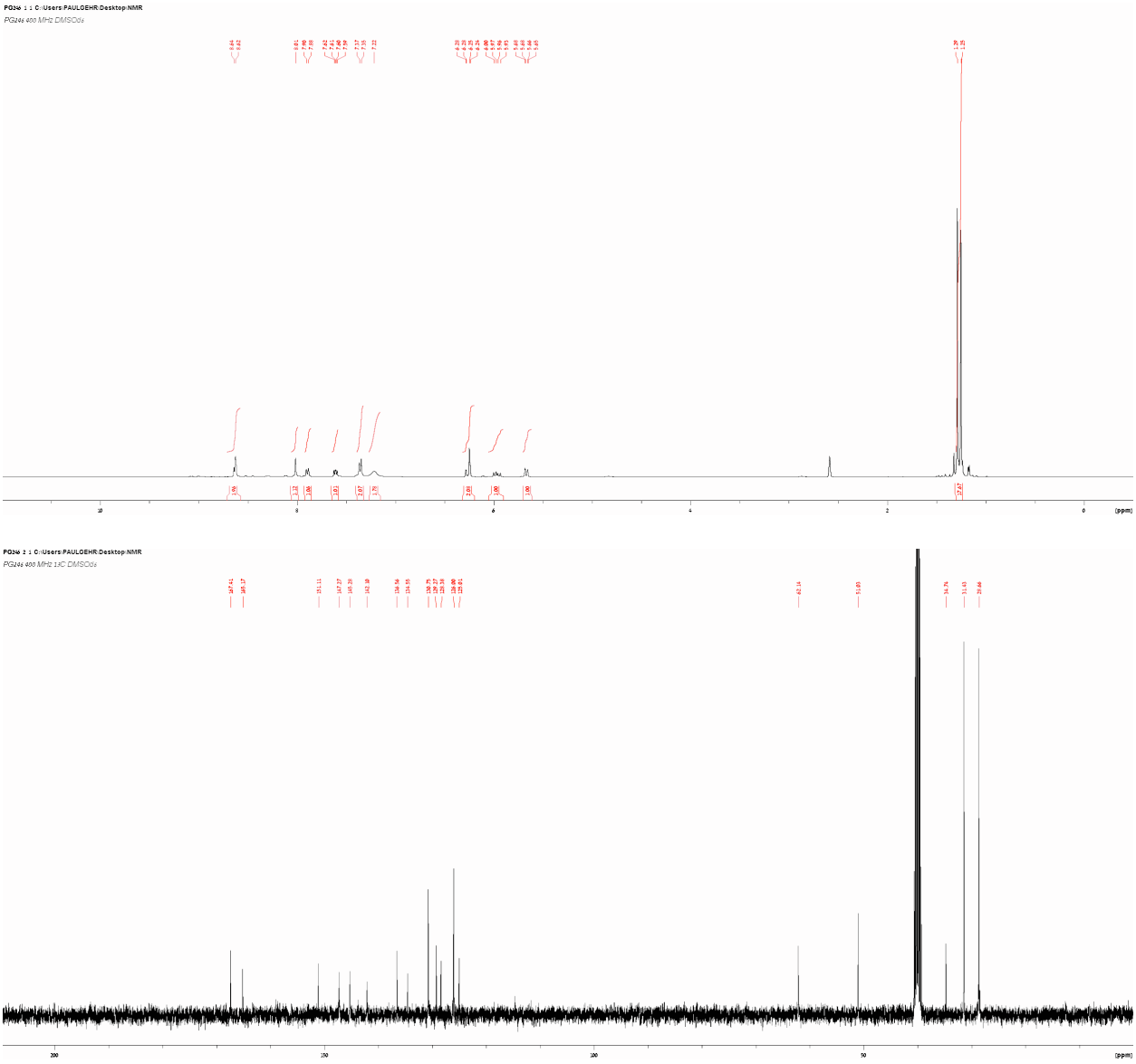
This compound and the associated analytical data was provided by Enamine (Ukraine). HRMS: m/z, $C_{13}H_{12}ClN_4OS$ calculated 307.0415 $[M+H]^+$, found 307.0419 $[M+H]^+$, 1.08 ppm deviation.



_N_-(4-(tert-butyl)phenyl)-_N_-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)acrylamide (10)

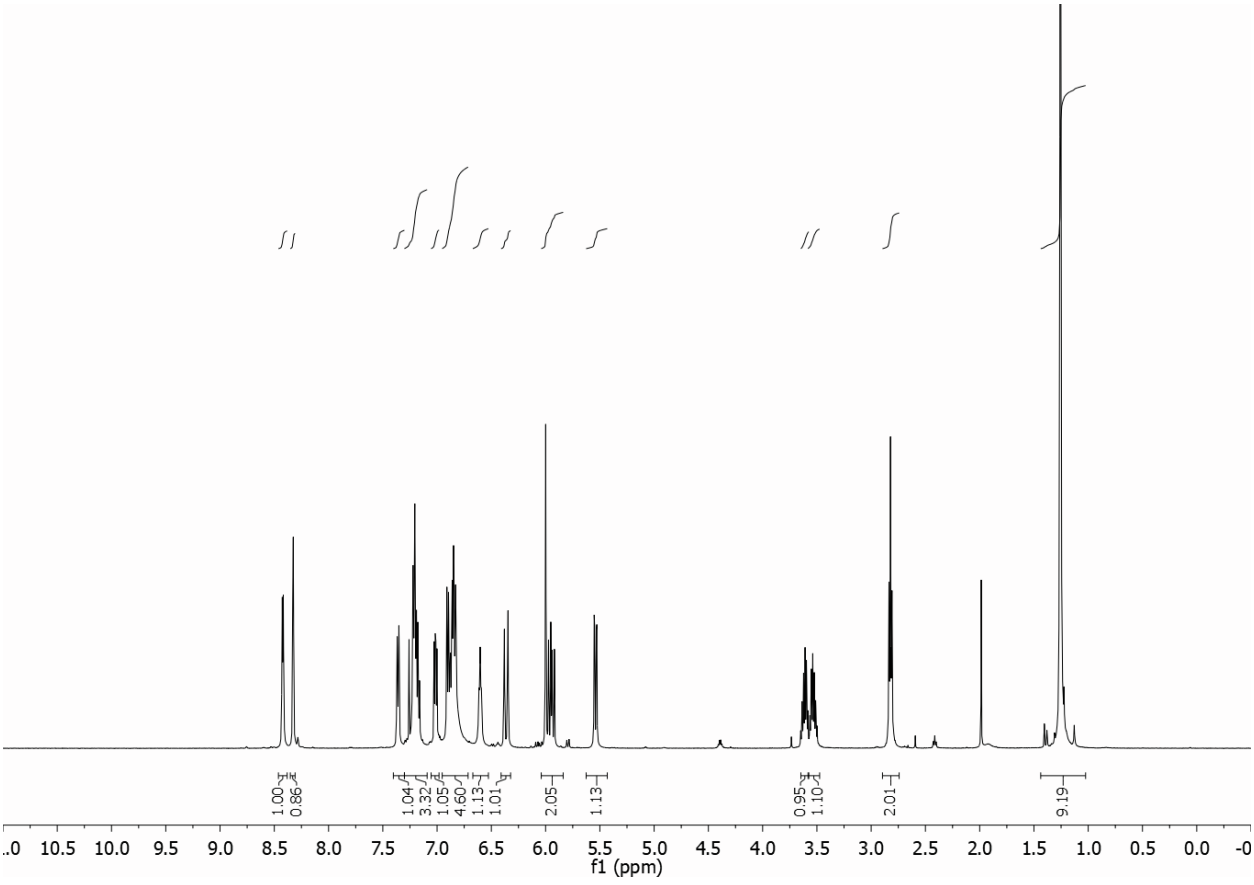
A two dram vial with Teflon stir bar was charged with 1.5 mL MeOH (AR grade, non-dried), followed by the addition of pyridine-3-carbaldehyde (10.9 µL, 100 µmol), 4-tert-butylaniline (15.9 µL, 100 µmol), acrylic acid (7 µL, 100 µmol) and tBuNC (11.3 µL, 100 µmol) exactly in this order. The vial was capped, protected from light and the reaction mixture stirred for 24 h at RT. After checking complete conversion by LC-MS, the solvent was removed by rotary evaporation inside a fumehood. Reactionware contaminated with tBuNC was washed with 10% conc. HCl in MeOH (v/v). The crude product was dissolved in 50% aq. ACN and 100 µL DMF, filtered over a 0.2 µm PVDF or PTFE syringe tip frit and subsequently purified by preparative RP-HPLC. The product was obtained as a white, hygroscopic lyophilisate (24 mg, 61 µmol, 61%).

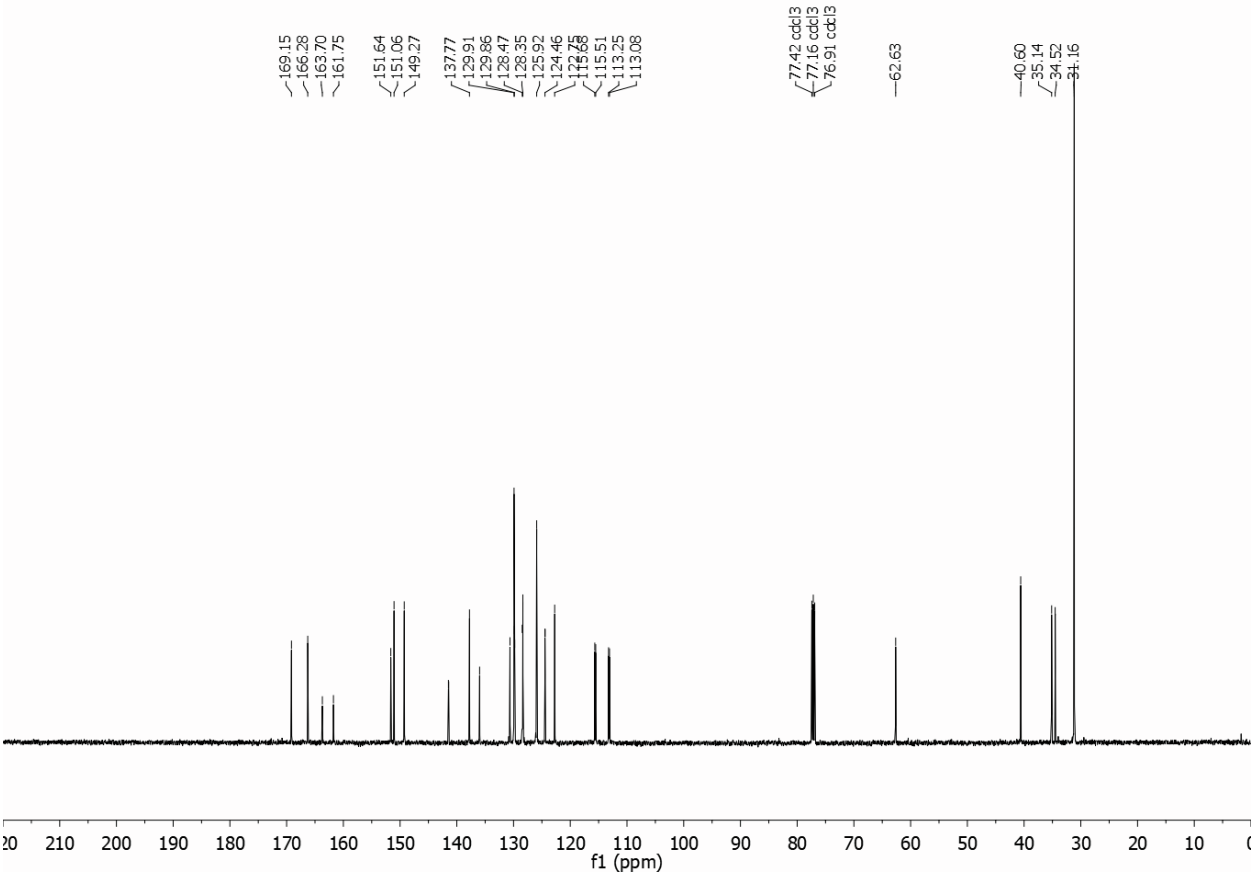
^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 8.65 - 8.60 (m, 2H), 8.01 (s, 1H), 7.89 (d, *J* = 8.1 Hz 1H), 7.60 (dd, *J* = 8.1 Hz, 5.3 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.22 (br s, 2H), 6.29 - 6.23 (m, 2H), 5.96 (dd, *J* = 16.8 Hz, 10.3 Hz, 1H), 5.66 (dd, *J* = 10.3 Hz, 2.0 Hz, 1H), 1.29 (s, 9H), 1.25 (s, 9H). ^{13}C-NMR (101 MHz, DMSO-d_6): δ ppm 167.4, 165.2, 151.1, 147.3, 145.3, 142.1, 136.6, 134.6, 130.8, 129.3, 128.4, 126.0, 125.0, 62.1, 51.0, 34.7, 31.4, 28.7. LC-MS: m/z 394 corresponding to $[M+H]^+$. HRMS: m/z, $C_{24}H_{32}N_3O_2$ calculated 394.2495 $[M+H]^+$, found 301.2492 $[M+H]^+$, 0.8 ppm deviation.



_N_-(4-(tert-butyl)phenyl)-_N_-(2-((3-fluorophenethyl)amino)-2-oxo-1-(pyridin-3-yl)ethyl)acrylamide (11)

This compound and the associated analytical data was provided by Enamine (Ukraine). ^{1}H NMR (500 MHz, Chloroform-d) δ 8.43 – 8.42 (m, 1H), 8.33 (d, J = 2.2 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.22 (d, J = 8.1 Hz, 2H), 7.18 (dd, J = 7.9, 6.2 Hz, 1H), 7.02 (dd, J = 8.0, 4.8 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H), 6.88 (d, J = 2.3 Hz, 1H), 6.85 (td, J = 7.7, 7.3, 2.1 Hz, 2H), 6.60 (t, J = 5.9 Hz, 1H), 6.37 (dd, J = 16.8, 2.0 Hz, 1H), 6.00 (s, 1H), 5.95 (dd, J = 16.8, 10.3 Hz, 1H), 5.54 (dd, J = 10.2, 1.9 Hz, 1H), 3.62 (dq, J = 13.0, 6.6 Hz, 1H), 3.53 (dq, J = 13.3, 6.7 Hz, 1H), 2.82 (t, J = 6.9 Hz, 2H), 1.26 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-d) δ 169.2, 166.3, 162.7 (d, J = 245.8 Hz), 151.6, 151.1, 149.3, 141.5 (d, J = 7.3 Hz), 137.8, 136.0, 130.6, 129.9, 129.8 (d, J = 8.4 Hz), 128.5, 128.4, 125.9, 124.5 (d, J = 2.8 Hz), 122.8, 115.6 (d, J = 20.9 Hz), 113.2 (d, J = 20.9 Hz), 62.6, 40.6, 35.2, 34.5, 31.2. HRMS (ESI+): m/z, $C_{28}H_{31}FN_3O_2^+$ calculated 460.2395 as $[M+H]^+$, found 460.2396 [M+H]+, 0.35 ppm deviation.





Resolution of the racemate by chiral SFC (Chiralcel OD-H column)

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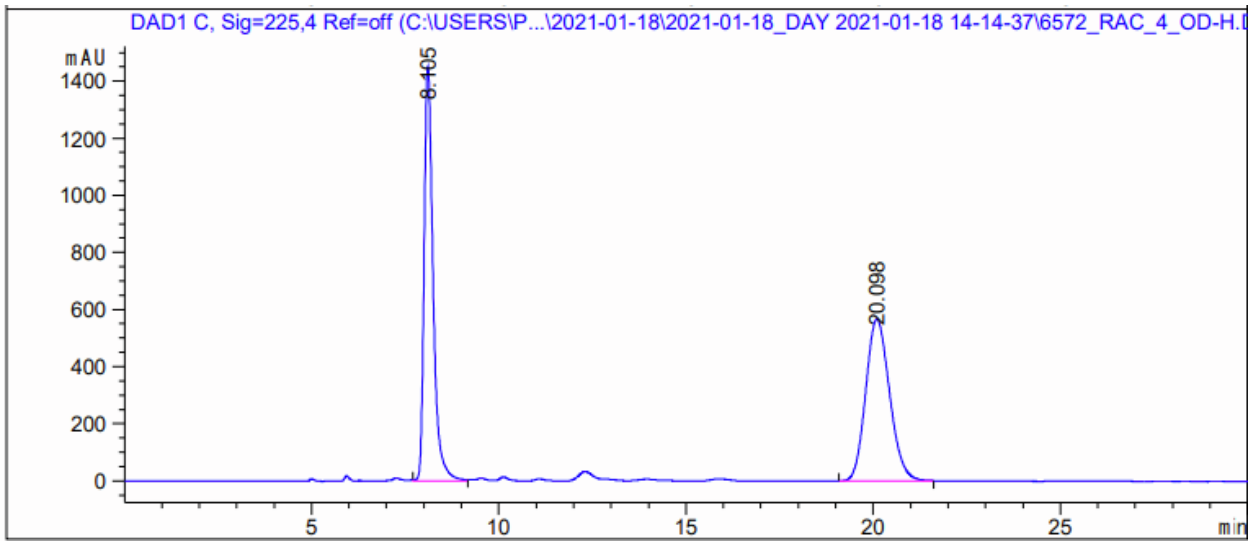
Injection Volume:   2  mkl
Sample Info: Chiralcel OD-H (250*4.6, 5mkm), IPA-MeOH-MeOH, 70-15-15, 0.6ml/
             min
================================================================================
```

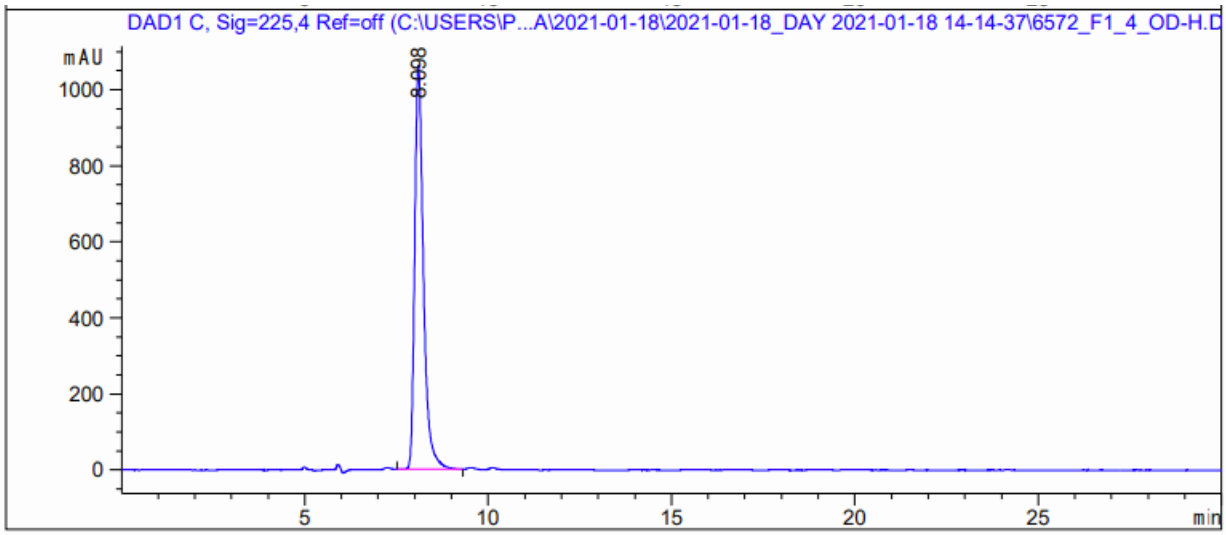


Signal: DAD1 C, Sig=225,4 Ref=off

RetTime(min)	Area,%	Symm.	Resolution	Selectivity
8.1052	49.06	0.663		
20.0982	50.94	0.837	15.16	2.48

After separation: First Enantiomer chiral SFC re-run

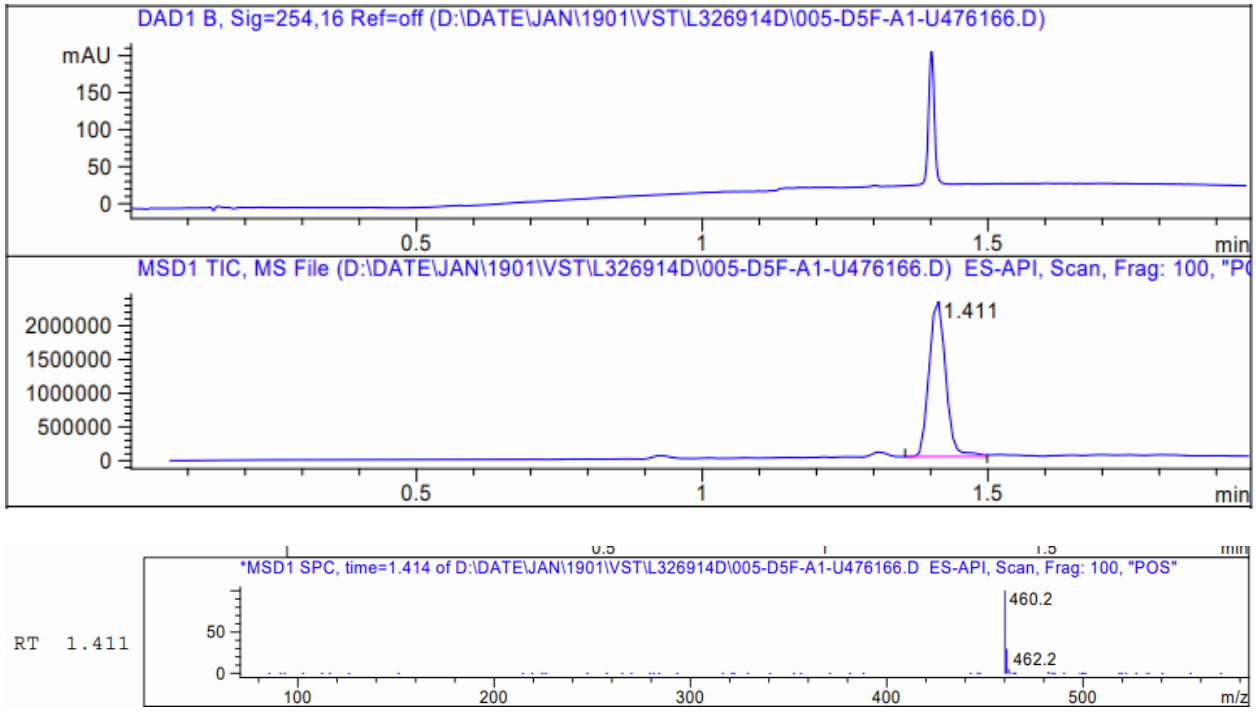
Injection Date : 3:08:04 PM 1/18/2021
Injection Volume: 2 mkl
Sample Info: Chiralcel OD-H (250*4.6, 5mkm), IPA-MeOH-MeOH, 70-15-15, 0.6ml/min



Signal: DAD1 C, Sig=225,4 Ref=off

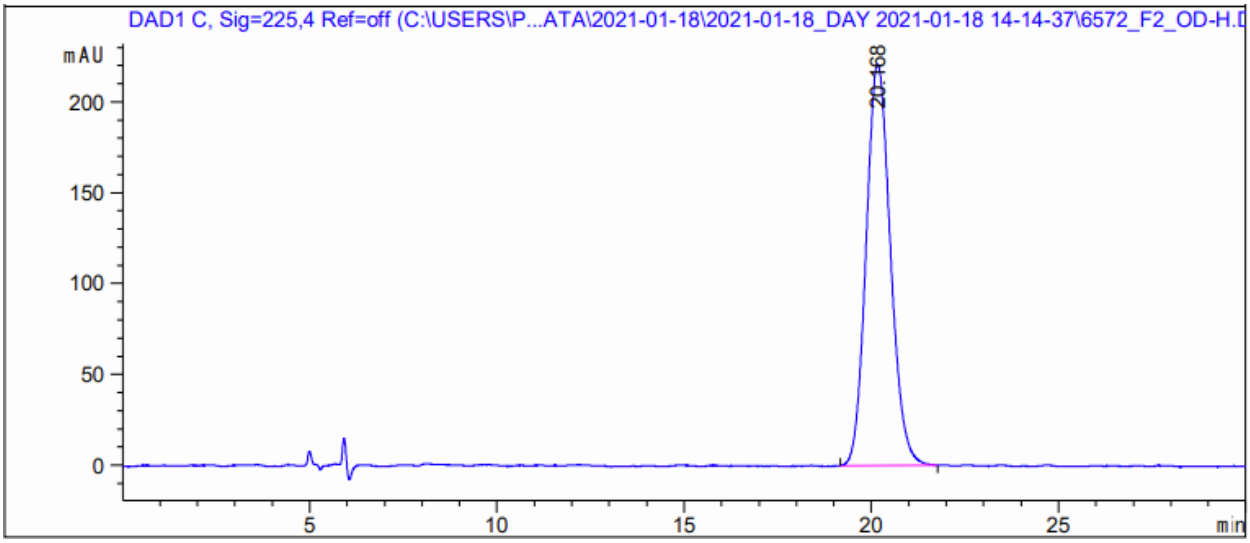
RetTime (min)	Area, %	Symm.	Resolution	Selectivity
8.0982	100.00	0.662		

Non-chiral LC-MS(ESI+) run first enantiomer



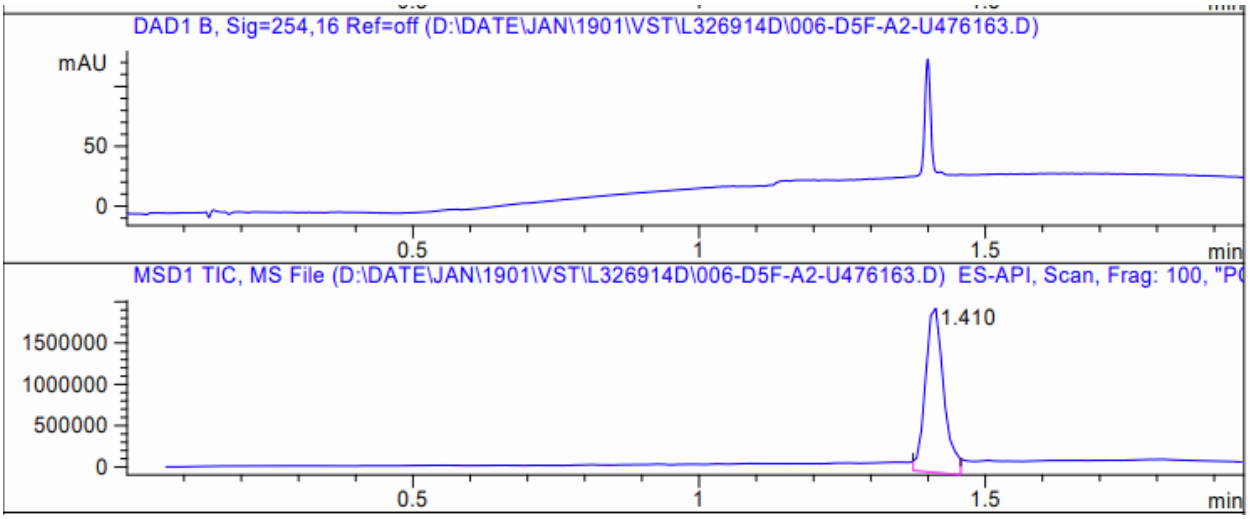
After separation: Second Enantiomer chiral SFC re-run

```
Injection Date  :   3:39:07 PM  1/18/2021
Injection Volume:   2  mkl
Sample Info: Chiralcel OD-H (250*4.6, 5mkm), IPA-MeOH-MeOH, 70-15-15, 0.6ml/
             min
================================================================================
```

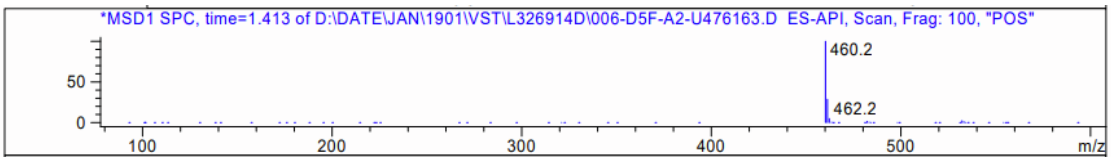



```
 Signal:  DAD1 C, Sig=225,4 Ref=off
================================================================================
 RetTime(min)   Area,%     Symm.    Resolution Selectivity
|------------|--------|----------|-----------|-----------|
 20.1682         100.00      0.852
================================================================================
```

Non-chiral LC-MS(ESI+) run second enantiomer



RT 1.410


*MSD1 SPC, time=1.413 of D:\DATE\JAN\1901\VST\L326914D\006-D5F-A2-U476163.D ES-API, Scan, Frag: 100, "POS"
460.2
462.2
50
0
100
200
300
400
500
m/z