Supporting Information

An approach to design reversible non-covalent inhibitors for human Granzyme B (hGrB)

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Scaffold hopping

According to some studies, a shape-based *ligand-centric* approach is often superior to a docking-based *protein-centric* approach. [1] To explore this avenue, the ROCS (Rapid Overlay of Chemical Structures)[2] method from OpenEye Scientific Software was used, a strategy designed to perform large scale 3D database searches by using shape-based superposition. Molecules are aligned by a solid-body optimization process that maximizes the overlap volume between them. In addition to a shape component, ROCS uses a "color force field" score based on defined atom types (cations, anions, hydrogen bond donors, hydrogen bond acceptors, hydrophobes, and rings) and a "TanimotoCombo (shape + color) scheme. Therefore, one can identify compounds that are similar in shape, charge properties and chemistry.

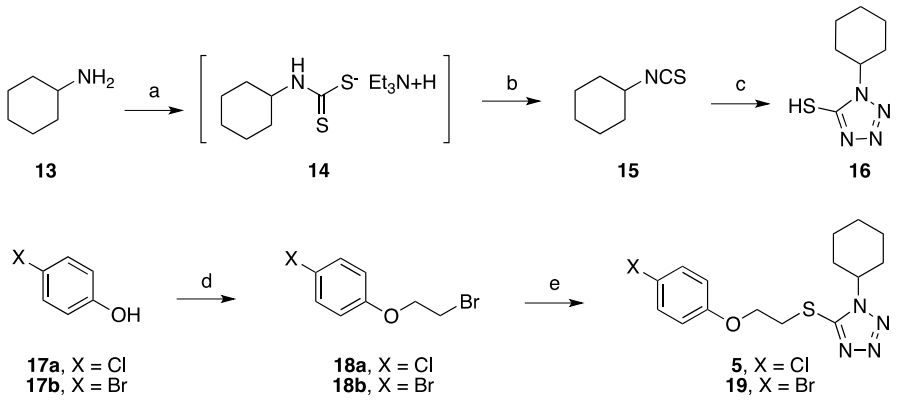
Several structures were selected as templates for scaffold-hopping. Compound **4** was included as were tricyclics **11** and **12** since they align well with triazole **4**. The low molecular weight tetrazole **5**, the various scaffolds with moderate activity identified by solvent mapping and virtual screening and IEPD-CHO completed the structural pool. As to available compound libraries, the protease and caspase-3 targeted libraries from Enamine[3] were screened in addition to the TimTec and ChemDiv libraries used for primary virtual screening.

Key steps of the extended virtual search procedure involved implementing a specific molecular query, performing a ROCS search for compounds in each library followed by Glide docking the ligand-centric hits into the GrB active site in compliance with the three constraints employed above. In order to reevaluate rejected structures from the ROCS search and to rescue false negatives, Induced Fit Docking (IFD)[4] was also performed, allowing the binding site to reorganize side chain

locations to more closely conform to the shape and binding mode of the various ligands.

From a total of 14,403 compounds from five libraries and three commercial sources screened using ROCS, Glide and IFD, 574 compounds were selected for estimation of molecular properties by QikProp[5] to focus on structures with the potential to combine both potency and reasonable ADME characteristics. QikProp not only predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, but also compares the computed property to those of 95% of known drugs. Of 44 possible descriptors, six important quantities (solubility, blockade of HERG K+ channels, Caco-2 cell permeability, brain/blood partition coefficient, human serum albumin binding and Lipinski's rule of five) were utilized in the selection of final hits to be acquired. Compound structures that fell outside of the 95% range were discarded. The remaining compounds were inspected by practicing chemists leading to a final choice of 28 compounds for bioassay. Unfortunately, the best compound proved to be a disappointingly weak blocker with an IC_{50} value of 73 μM.

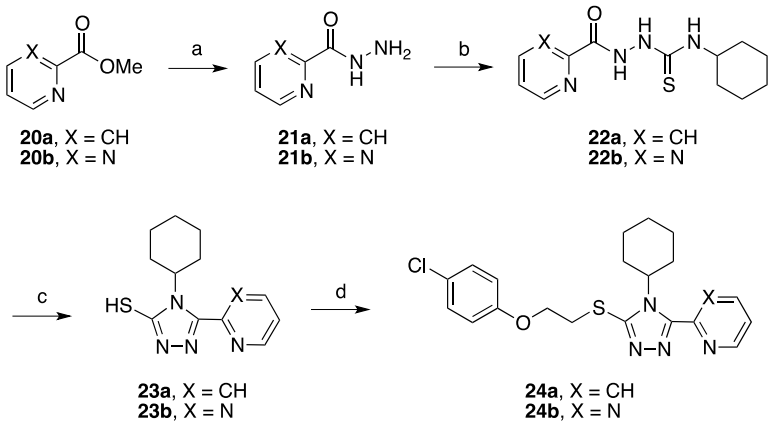
Scheme 1: Synthesis of tetrazoles **5** and **19**[a]



[a]Reagents and conditions: (a) CS_2, Et_3N, THF; (b) TsCl, THF, 80%; (c) NaN_3, *i*-PrOH:water (1:4), Δ; conc. HCl, 77%; (d) 1,2-dibromoethane, Cs_2CO_3, acetone, 49% (**18a**), 60% (**18b**); (e) **16**, (*iso*-Pr)$_2$NEt, THF, 65% (**5**), 70% (**19**).

Cyclohexylamine **13** was converted into the corresponding dithiocarbamate salt **14**, which was allowed to react *in situ* with 4-toluenesulfonyl chloride to afford isothiocyanate **15** in 80% yield.[6] Subsequent cycloaddition with sodium azide in a mixture of *iso*-propanol and water to overcome solubility issues gave tetrazolethiol **16** in 77% yield.[7] *S*-alkylation with 2-(4-chlorophenoxy)ethyl bromide **18a** gave the target tetrazole **5** (X = Cl). The corresponding bromide **19** (X = Br) was synthesized by an equivalent process.

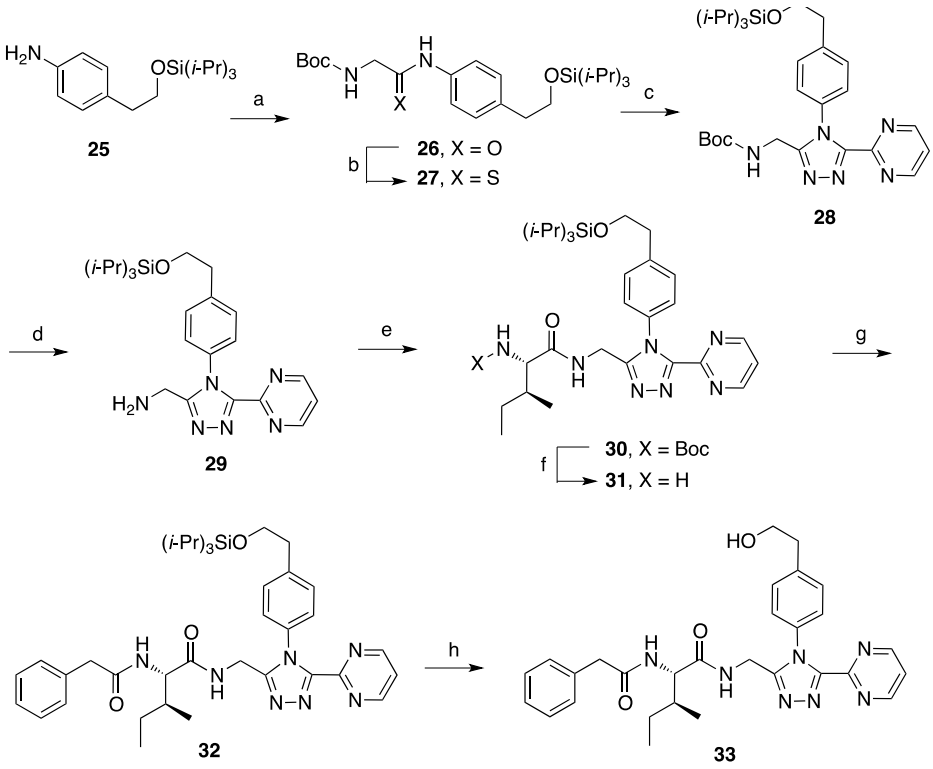
Scheme 2: Synthesis of 1,2,4-triazoles **24a** and **24b**[a]



[a]Reagents and conditions: (a) N_2H_4/H_2O, MeOH, Δ, 30 min, quant.; (b) **7**, EtOH, Δ, 75% (**22a**), 97% (**22b**); (c) 2M NaOH, Δ, 78% (**23a**), 50% (**23b**); (d) **18a**, (*iso*-Pr)$_2$NEt, THF, 85% (**24a**), 78% (**24b**).

Acyl hydrazides **21a**,**b**, which were readily synthesized from the corresponding methyl esters **20a**,**b** in quantitative yields, were allowed to react with isothiocyanate **15** to give thiosemicarbazides **22a**,**b** (**Scheme 2**). These were cyclodehydrated under basic conditions, affording 1,2,4-triazoles **23a**,**b**.[8] Finally, alkylation with bromoether **18a** gave the triazole analogs **24a** (X = CH) and **24b** (X = N).

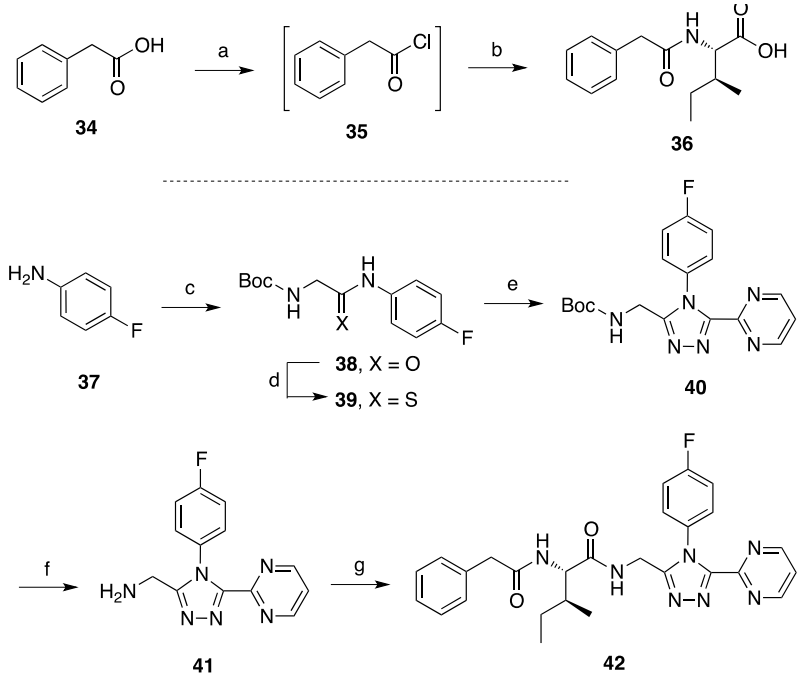
Scheme 3: Synthesis of analog **33**[a]



[a]Reagents and conditions: (a) *N*-Boc-glycine, EDCI, DMAP, CH_2Cl_2, 71%; (b) Lawesson's reagent, THF, Δ, 63%; (c) **21b**, pyridine, Δ, 47%; (d) Me_3SiOTf, 2,6-lutidine, CH_2Cl_2, 90%; (e) *N*-Boc-isoleucine, EDCI, DMAP, CH_2Cl_2, 81%; (f) Me_3SiOTf, 2,6-lutidine, CH_2Cl_2, 62%; (g) phenylacetic acid, EDCI, DMAP, CH_2Cl_2, 92%; (h) Bu_4NF, AcOH, THF, 0 ºC to rt, 83%.

EDCI mediated peptide coupling between aniline **25** and *N*-Boc-glycine gave amide **26**, which was thionated[9] to produce thioamide **27** using Lawesson's reagent, in moderate yield due to instability of the product at the necessary high temperature of reaction. Attempted condensation of thioamide **27** with acyl hydrazide **21b** in *n*-butanol, at reflux,[10] or in *t*-BuOH or 1,1,1,3,3,3-hexafluoro-2-propanol as a solvent, neat, with microwave activation, or via the intermediacy of the derived thioether or imidoyl chloride[11] from thionoamide **27** were all unsuccessful. However, it was found that condensation of thionamide **27** with freshly prepared acyl hydrazide **21b** in pyridine at reflux[12] gave the desired 1,2,4-triazole **28** with a yield of 47%. Selective deprotection of the Boc group of adduct **28** in the presence of the silyl ether was readily carried out using trimethylsilyl triflate and 2,6-lutidine[13] and gave amine **29** (90%), which was coupled to *N*-Boc-isoleucine to give amide **30**. A second selective Boc-deprotection gave amine **31**, which was coupled to phenylacetic acid, giving the peptoid **32**. Finally, deprotection of the TIPS group with tetrabutylammonium fluoride gave the target pyrimidine-triazole **33** in 7% overall yield.

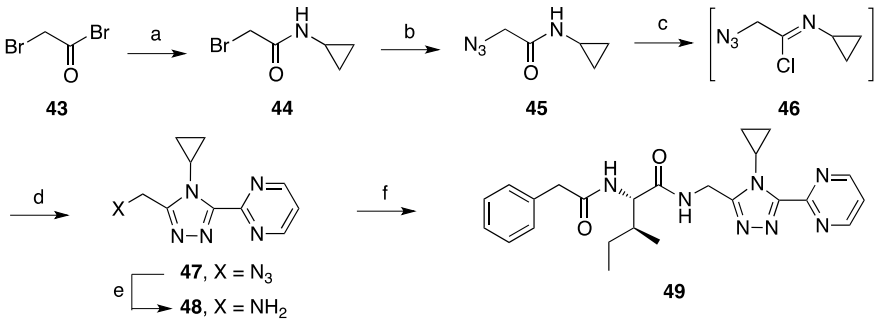
Scheme 4: Synthesis of fluoro analog **42**[a]



[a]Reagents and conditions: (a) $SOCl_2$, DMF (cat.), PhMe, 40 ºC; (b) isoleucine, 4M NaOH, 56% (2 steps); (c) *N*-Boc-glycine, EDCI, DMAP, CH_2Cl_2, 82%; (d) Lawesson's reagent, THF, Δ, 85%; (e) **21b**, pyridine, Δ, 57%; (f) 10% TFA, CH_2Cl_2, 32%; (g) **36**, EDCI, HOBt, Et_3N, CH_2Cl_2, 88%.

Phenylacetyl chloride **35**, derived from phenylacetic acid **34**, was allowed to react with isoleucine under Schotten-Baumann conditions to give amide **36**. Acylation of aniline **37** with *N*-Boc-glycine gave amide **38**, which was converted with Lawesson's reagent into the corresponding thioamide **39**. Cyclodehydration by heating in pyridine at reflux gave the desired 1,2,4-triazole **40** (57%). Amine **41** was obtained after trifluoroacetic acid mediated deprotection, and subsequent peptide coupling with carboxylic acid **36** gave the desired para-fluorophenyl analog **42** (11% overall).

Scheme 5: Synthesis of cyclopropyl analogs **49**[a]



[a]Reagents and conditions: (a) cyclopropylamine, $NaHCO_3$, THF, -78 ºC to 21 ºC, 87%; (b) NaN_3, MeCN, 80 ºC, 76%; (c) $(COCl)_2$, 2,6-lutidine, CH_2Cl_2, 0 ºC, 5 min; (d) **21b**, CH_2Cl_2; $NaHCO_3$, H_2O, Δ, 20% (2 steps); (e) H_2, Pd/C, MeOH, 99%; (f) **36**, EDCI, HOBt, Et_3N, CH_2Cl_2, 82%.

Sequential acylation of cyclopropylamine with bromoacetyl bromide **43** and bromide displacement using sodium azide gave azido-amide **45**. The amide moiety of **45** was converted to the corresponding imidoyl chloride **46** by reaction with oxalyl chloride and this was further allowed to react *in situ* with acyl chloride **21b**, followed by intermolecular condensation in aqueous sodium bicarbonate at reflux to give the desired 1,2,4-triazole **47**.[36] Finally, azide reduction by hydrogenolysis over palladium on carbon gave amine **48**, which was coupled with carboxylic acid **28**, to provide the desired amide **49** (10% overall).

Synthesis details.

General experimental. Cyclohexyl isothiocyanate (**15**) was prepared according to methods described by Wong[31] and phenylacetyl chloride (**35**) according to methods described by Huang.[14] Brine refers to saturated aqueous NaCl. Reaction solvents were distilled from CaH_2 (CH_2Cl_2, Et_3N), Na/Ph_2CO (THF, Et_2O), or obtained as dry or anhydrous from Aldrich Chemical Co. (DMF, MeCN, MeOH). Other solvents and all reagents were obtained from commercial suppliers and were used as obtained if purity was >98%. All manipulations of air or moisture sensitive materials were carried out in oven-dried glassware under an inert atmosphere of nitrogen or argon. Syringes, which were used to transfer reagents and solvents, were purged with nitrogen prior to use. All flash column chromatography was carried out on silica gel 60 particle size 0.040-0.063 mm, unless otherwise stated. Thin layer chromatography (TLC) was performed on pre-coated aluminum backed or glass backed plates and visualized under ultraviolet light (254 nm) or with potassium permanganate, vanillin, or phosphomolybdic acid (PMA) stains as deemed appropriate. ^{1}H NMR and ^{13}C NMR spectra were respectively recorded at 400 and 100 MHz. Chemical shifts (δ) are quoted in parts per million (ppm) downfield from tetramethylsilane and are referenced against a residual solvent peak, $CDCl_3$ (δ_H: 7.26, δ_C: 77.16), MeOD-d_6 (δ_H: 3.31, δ_C: 49.00), DMSO-d_6 (δ_H: 2.50, δ_C: 39.52). Coupling constants (J) are quoted in Hertz (Hz) to the nearest 0.1 Hz. The signal multiplicity is quoted as follow: singlet (s), doublet (d), triplet (t), quadruplet (q), broad singlet (br s), multiplet (m). Melting points were obtained on a hot stage apparatus and were not corrected. Infrared spectra were recorded on films on sodium chloride plates. LC-MS analysis was performed using a C18-reverse phase column of length 30 mm, inner diameter 2.1 mm and particle size 3 μm using a mobile phase of water (0.1% formic acid): acetonitrile,

monitoring at 254 nm. Compound purity was determined by NMR spectrometry or LC-MS analysis and was confirmed to be >95% for all compounds.

1-Cyclohexyl-1*H*-tetrazole-5-thiol (16). Following a literature procedure,[15] isothiocyanate **15** (1.42 g, 10.0 mmol, 1.00 equiv) in *iso*-propanol (2 mL) was added dropwise to NaN_3 (715 mg, 1.00 equiv) in water (8 mL) and the reaction mixture was heated to reflux at 100 °C for 1h. The solution was cooled to 0 °C and aqueous HCl solution (12 M; 3 mL) was added dropwise to pH 1. The resulting white crystals were collected by filtration, washed with cold water to pH 7 (3 × 10 mL), and dried overnight to afford the desired tetrazolethiol **16** (1.43 g, 77%) as a white solid: R_f 0.34 (CH_2Cl_2 : MeOH, 95 : 5); mp 88–90 °C (Et_2O); IR (film) 3054, 2771, 2748, 2678, 2555 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 12.69 (br s, 1H), 4.61 (tt, J = 4.0, 12.0 Hz, 1H), 2.14–2.04 (m, 2H), 2.00–1.90 (m, 2H), 1.89–1.79 (m, 2H), 1.80–1.74 (m, 1H), 1.48 (qt, J = 13.0, 3.3 Hz, 2H), 1.36–1.26 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.6, 57.3, 31.3, 25.1, 24.9; HRMS (NESI) calcd for $C_7H_{11}N_4S$ $[M-H]^+$ 183.0704, found 183.07045.

1-(2-Bromoethoxy)-4-chlorobenzene (18a). To 4-chlorophenol (**17a**) (1.73 g, 10.0 mmol, 1.00 equiv) in acetone (10 mL) was added sequentially cesium carbonate (4.98 g, 1.50 equiv) and 1,2-dibromoethane (3.46 mL, 4.00 equiv) and the reaction mixture was heated to reflux for 78h. The mixture was poured into water (50 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed (hexanes : AcOEt, 97 : 3) to give chloride **18a** (1.16 g, 49%) as a colorless oil: R_f 0.26 (hexanes : AcOEt, 99: 1); IR (film) 1489, 1238, 821 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, J = 9.1 Hz, 2H), 6.88 (d, J = 9.1 Hz, 2H), 4.29 (t, J = 6.3 Hz, 2H), 3.66 (t, J = 6.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7, 129.5, 126.3, 116.1,

68.1, 28.8; MS (EI^+) 234, 236, 238 $[M]^{+\bullet}$; HRMS (EI^+) calcd for $C_8H_8{}^{79}Br^{35}ClO$ $[M]^{+\bullet}$ 233.9447, found 233.9441.

1-Bromo-4-(2-bromoethoxy)benzene (18b). To 4-bromophenol (**17b**) (173 mg, 1.00 mmol, 1.00 equiv) in acetone (1 mL) was added sequentially cesium carbonate (498 mg, 1.50 equiv) and 1,2-dibromoethane (346 μL, 4.00 equiv) and the reaction mixture was heated to reflux for 78h. The mixture was poured into water (5 mL) and extracted with CH_2Cl_2 (3 × 3 mL). The combined organic layers were washed with brine (3 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed (hexanes : AcOEt, 97 : 3) to give bromide **18b** (168 mg, 60%) as a colorless oil: R_f 0.27 (hexanes : AcOEt, 99 : 1); IR (film) 1486, 1244, 815 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J = 8.9 Hz, 2H), 6.80 (d, J = 8.9 Hz, 2H), 4.26 (t, J = 6.3 Hz, 2H), 3.63 (t, J = 6.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.2, 132.4, 116.6, 113.7, 68.1, 28.8; HRMS (EI^+) calcd for $C_8H_8{}^{79}Br_2O$ $[M]^{+\bullet}$ 277.8942, found 277.8954.

5-(2-(4-Chlorophenoxy)ethylthio)-1-cyclohexyl-1*H*-tetrazole (5). *iso*-Pr_2NEt (81 μL, 1.10 equiv) was added with stirring to bromoether **18a** (100 mg, 0.42 mmol, 1.00 equiv) and thiol **16** (78 mg, 1.00 equiv) in THF (0.6 mL) at room temperature. After 7h, the mixture was partitioned between water (10 mL) and CH_2Cl_2 (10 mL). The layers were separated and the aqueous phase extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed (hexanes : AcOEt, 90 : 10) to give tetrazole **5** (93 mg, 65%) as a white solid: R_f 0.54 (hexanes : AcOEt, 70 : 30); mp 160–162 °C (hexanes : AcOEt, 70 : 30); IR (film) 2944, 2859, 1491, 1237 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, J = 9.1 Hz, 2H), 6.85 (d, J = 9.1 Hz, 2H), 4.34 (t, J = 6.0 Hz, 2H), 4.18–4.10 (m, 1H), 3.72 (t, J = 6.0 Hz, 2H), 2.06–2.00 (m, 2H), 2.00–1.89 (m, 4H), 1.79–1.72 (m, 1H), 1.47–1.25 (m, 3H); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 156.7, 152.2, 129.4, 126.2, 115.9, 66.2, 58.3, 32.2, 32.0, 25.1, 24.8; HRMS (ESI) calcd for $C_{15}H_{20}{}^{35}ClN_4OS$ $[M+H]^+$ 339.1046, found 330.1041.

5-(2-(4-Bromophenoxy)ethylthio)-1-cyclohexyl-1*H*-tetrazole (19). *iso*-Pr_2NEt (56 μL, 1.10 equiv) was added with stirring to bromoether **18b** (80 mg, 0.29 mmol, 1.00 equiv) and thiol **16** (54 mg, 1.00 equiv) in THF (0.5 mL) at room temperature. After 30h, the mixture was partitioned between water (10 mL) and CH_2Cl_2 (10 mL). The layers were separated and the aqueous phase extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed (hexanes : AcOEt, 90 : 10) to give tetrazole **19** (78 mg, 70%) as a white solid: R_f 0.42 (hexanes : AcOEt, 70 : 30); mp 90–92 °C (hexanes : AcOEt, 70 : 30); IR (film) 2940, 2856, 1489, 1236, 820 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, J = 8.9 Hz, 2H), 6.79 (d, J = 8.9 Hz, 2H), 4.33 (t, J = 6.0 Hz, 2H), 4.18–4.08 (m, 1H), 3.72 (t, J = 6.0 Hz, 2H), 2.08–1.87 (m, 6H), 1.79–1.70 (m, 1H), 1.48–1.22 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.3, 152.2, 132.4, 116.4, 113.6, 66.2, 58.4, 32.2, 32.0, 25.1, 24.8; HRMS (ESI) calcd for $C_{15}H_{20}{}^{79}Br\ N_4OS$ $[M+H]^+$ 383.0541, found 383.0546.

Picolinohydrazide (21a). Following a literature procedure,[16] hydrazine monohydrate (582 μL, 1.20 equiv) was added dropwise to methyl ester **20a** (1.20 mL, 10.0 mmol, 1.00 equiv) in MeOH (20 mL) and the solution was heated to reflux for 4h. The solvent was removed *in vacuo* and the residue was dissolved in MeOH (10 mL). The solvent was re-evaporated to afford hydrazide **21a** (1.36 g, 99%) as white needles. R_f 0.32 (CH_2Cl_2 : MeOH, 95 : 5); mp 78–80 °C (MeOH); IR (film) 3289, 3210, 1672 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.00 (br s, 1H), 8.56–8.52 (m, 1H), 8.17–8.13 (m, 1H), 7.85 (td, J = 7.6, 1.8 Hz, 1H), 7.44 (ddd, J = 7.6, 4.7, 1.2 Hz, 1H), 4.09 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7, 149.0, 148.4, 137.3, 126.5, 122.2; HRMS (ESI) calcd for $C_6H_8N_3O$ $[M+H]^+$ 138.0667, found 138.0662.

Pyrimidine-2-carbohydrazide (21b). Following a literature procedure,[45] hydrazine monohydrate (582 μL, 1.20 equiv) was added dropwise to methyl ester **20b** (1.38 g, 10.0 mmol, 1.00 equiv) in MeOH (20 mL) and the solution was heated to reflux for 4h. The solvent was removed *in vacuo* and the residue was dissolved in MeOH (10 mL). The solvent was re-evaporated again to afford hydrazide **21b** (1.38 g, 99%) as white needles: R_f 0.20 (CH_2Cl_2 : MeOH, 95 : 5); mp 136–138 °C (CH_2Cl_2); IR (film) 3329, 3292, 3209, 3065, 1671 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.03 (br s, 1H), 8.88 (d, J = 4.9 Hz, 2H), 7.46 (t, J = 4.9 Hz, 1H), 4.18 (br s, 2H); ^{13}C NMR (100 MHz, MeOD-d_6) δ 163.7, 159.2, 158.6, 124.7; HRMS (ESI) calcd for $C_5H_7N_4O$ $[M+H]^+$ 139.0620, found 139.0617.

***N*-Cyclohexyl-2-picolinoylhydrazinecarbothioamide (22a)**. Following a literature procedure,[33] isothiocyanate **15** (1.42 mL, 1.00 equiv) was added with stirring to freshly prepared acyl hydrazide **21a** (1.37 g, 10.0 mmol, 1.00 equiv) in EtOH (50 mL) and the mixture was heated to reflux for 6h. The mixture was allowed to cool and the white precipitate was collected by filtration, washed with cold EtOH (5 mL) and dried *in vacuo* to afford the desired product **22a** (2.09 g, 75%) as a white solid: R_f 0.63 (CH_2Cl_2 : MeOH : NH_4OH, 95 : 5 : 0.25); mp 140–142 °C (EtOH); IR (film) 3295, 3241, 3114, 1652 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.48 (br s, 1H), 9.31 (br s, 1H), 8.69–8.65 (m, 1H), 8.05–7.98 (m, 2H), 7.70–7.60 (m, 2H), 4.16–4.02 (m, 1H), 1.83–1.73 (m, 2H), 1.70–1.61 (m, 2H), 1.56–1.51 (m, 1H), 1.32–1.16 (m, 4H), 1.13–1.00 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 180.1, 162.9 (br), 149.2, 148.5, 137.7, 126.9, 122.4, 52.8, 31.8, 25.1, 24.8; HRMS (ESI) calcd for $C_{13}H_{19}N_4OS$ $[M+H]^+$ 279.1280, found 279.1274.

***N*-Cyclohexylaminothiocarbonyl-*N'*-2-(pyrimidine-2-carbonyl)hydrazine (22b)**. Following a literature procedure,[33] isothiocyanate **15** (773 μL, 1.00 equiv) was added with stirring to freshly prepared acyl

hydrazide **21b** (753 mg, 5.45 mmol, 1.00 equiv) in EtOH (25 mL) and the reaction mixture was heated to reflux for 6h. The mixture was allowed to cool and the white precipitate was collected by filtration, washed with cold EtOH (5 mL) and dried *in vacuo* to give semithiocarbazide **22b** (1.50 g, 97%) as a white solid: R_f 0.25 (CH_2Cl_2 : MeOH : NH_4OH, 95 : 5 : 0.25); mp 160–162 °C (EtOH); IR (film) 3276, 3114, 1668 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.61 (br s, 1H), 9.37 (br s, 1H), 8.99 (d, J = 4.8 Hz, 2H), 7.72 (t, J = 4.8 Hz, 1H), 7.66 (br s, 1H), 4.16–4.02 (m, 1H), 1.86–1.74 (m, 2H), 1.73–1.61 (m, 2H), 1.60–1.52 (m, 1H), 1.32–1.16 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.6, 161.0 (br), 157.3, 156.7, 123.0, 52.6, 31.5, 24.8, 24.5; HRMS (ESI) calcd for $C_{12}H_{18}N_5OS$ $[M+H]^+$ 280.1232, found 280.1227.

4-Cyclohexyl-5-(pyridin-2-yl)-4*H*-1,2,4-triazole-3-thiol (23a). Following a literature procedure,[33] thiosemicarbazide **22a** (2.00 g, 7.20 mmol, 1.00 equiv) was added portionwise with stirring to aqueous sodium hydroxide (2 M; 70 mL) at 80 °C, and the reaction mixture was heated to reflux for 16h. The mixture was allowed to cool to room temperature, and aqueous HCl solution (2 M, 100 mL) was added slowly to pH 1. The white precipitate was collected by filtration, washed with water and dried *in vacuo* to give triazolethiol **23a** (1.46 g, 78%) as a white solid: R_f 0.44 (CH_2Cl_2 : MeOH : NH_4OH, 90 : 10 : 0.1); mp 152–154 °C (Et_2O); IR (film) 3089, 2931, 2852 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 14.03 (s, 1H), 8.79–8.75 (m, 1H), 8.02 (td, J = 7.8, 1.9 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.61 (ddd, J = 7.6, 4.8, 1.0 Hz, 1H), 4.86–4.75 (m, 1H), 2.42–2.19 (m, 2H), 1.81–1.53 (m, 5H), 1.32–1.16 (m, 2H), 1.11–0.96 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.2, 150.1, 149.2, 146.5, 137.8, 125.3, 124.8, 56.9, 28.9, 25.5, 24.6; HRMS (ESI) calcd for $C_{13}H_{17}N_4S$ $[M+H]^+$ 261.1174, found 261.1184.

4-Cyclohexyl-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazole-3-thiol (23b). Following a literature procedure,[33]

thiosemicarbazide **22b** (1.22 g, 4.30 mmol, 1.00 equiv) was added portionwise with stirring to aqueous sodium hydroxide (2 M; 40 mL) at 80 °C, and the mixture was heated to reflux for 16h. The mixture was allowed to cool to room temperature, and aqueous HCl solution (2 M; 80 mL) was added slowly to pH 1. The white precipitate was collected by filtration, washed with water and dried *in vacuo* to give the desired product **23b** (563 mg, 50%) as a white solid: R_f 0.49 (CH_2Cl_2 : MeOH: NH_4OH, 90 : 10 : 0.1); mp 118–120 °C (Et_2O); IR (film) 3380, 3153, 2920, 2855 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 14.12 (s, 1H), 9.05 (d, J = 5.0 Hz, 2H), 7.74 (t, J = 5.0 Hz, 1H), 4.71–4.67 (m, 1H), 2.21–2.02 (m, 2H), 1.80–1.65 (m, 4H), 1.62–1.54 (m, 1H), 1.33–1.18 (m, 2H), 1.07–0.94 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.0, 158.0, 155.3, 149.0, 122.4, 57.0, 29.1, 25.4, 24.6; HRMS (ESI) calcd for $C_{12}H_{16}N_5S$ $[M+H]^+$ 262.1126, found 262.1115.

2-(5-(2-(4-Chlorophenoxy)ethylthio)-4-cyclohexyl-4*H*-1,2,4-triazol-3-yl) pyridine (24a). *iso*-Pr_2NEt (148 μL, 1.10 equiv) was added with stirring to bromoether **18a** (364 mg, 2.00 equiv) and thiol **23a** (200 mg, 0.77 mmol, 1.00 equiv) in THF (0.8 mL) at room temperature. After 96h, the mixture was partitioned between water (10 mL) and CH_2Cl_2 (10 mL). The layers were separated and the aqueous phase extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed (hexanes : AcOEt, 90 : 10) to give the desired product **24a** (170 mg, 85%) as a white solid: R_f 0.26 (hexanes : AcOEt, 50 : 50); mp 108–110 °C (hexanes : AcOEt, 50 : 50); IR (film) 2938, 2935, 2972, 1585 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.68–8.63 (m, 1H,), 8.11 (d, J = 8.0 Hz, 1H), 7.81 (td, J = 7.8, 1.8 Hz, 1H), 7.34 (ddd, J = 7.7, 4.9, 1.0 Hz, 1H), 7.28–7.20 (m, 2H), 6.92–6.86 (m, 2H), 5.22–5.12 (m, 1H), 4.38 (t, J = 6.2 Hz, 2H), 3.72 (t, J = 6.2 Hz, 2H), 2.30–5.17 (m, 2H), 1.92–1.82 (m, 4H), 1.73–1.75 (m, 1H), 1.41–1.17 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.1, 154.1, 151.2,

148.8, 148.3, 136.9, 129.3, 125.9, 124.6, 123.9, 116.0, 66.8, 57.0, 32.3, 30.8, 26.0, 25.0; HRMS (ESI) calcd for $C_{21}H_{24}{}^{35}ClN_4OS$ $[M+H]^+$ 415.1359, found 415.1338.

2-(5-(2-(4-Chlorophenoxy)ethylthio)-4-cyclohexyl-4*H*-1,2,4-triazol-3-yl) pyrimidine (24b). *iso*-Pr_2NEt (148 μL, 1.10 equiv) was added with stirring to bromoether **18a** (384 mg, 2.00 equiv) and thiol **23b** (200 mg, 0.77 mmol, 1.00 equiv) in THF (0.8 mL) at room temperature. After 96h, the mixture was partitioned between water (10 mL) and CH_2Cl_2 (10 mL). The layers were separated and the aqueous phase extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed (AcOEt : MeOH, 99 : 1 → 95 : 5) to give the desired product **24b** (251 mg, 78%) as a white solid: R_f 0.24 (AcOEt : MeOH, 99 : 1 → 95 : 5); mp 118–120 °C (AcOEt : MeOH, 95 : 5); IR (film) 2944, 2941, 2877, 1559 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.90 (d, J = 4.9 Hz, 2H), 7.35 (t, J = 4.9 Hz, 1H), 7.26–7.20 (m, 2H), 6.93–6.88 (m, 2H), 5.08–4.98 (m, 1H), 4.37 (t, J = 6.1 Hz, 2H), 3.72 (t, J = 6.1 Hz, 2H), 2.30–5.20 (m, 2H), 1.91–1.83 (m, 4H), 1.72–1.67 (m, 1H), 1.40–1.16 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.5, 157.0, 157.0, 153.1, 151.5, 129.3, 125.8, 120.7, 116.0, 66.6, 57.3, 32.2, 30.7, 26.0, 24.9; HRMS (ESI) calcd for $C_{20}H_{23}{}^{35}ClN_5OS$ $[M+H]^+$ 416.1312, found 416.1295.

***tert*-Butyl 2-oxo-2-(4-(2-(tri-isopropylsilyloxy)ethyl)phenylamino)ethylcarbamate (26)**. To a solution of *N*-Boc-glycine (90 mg, 0.50 mmol, 1.00 equiv), EDCI (115 mg, 1.20 equiv) and DMAP (6 mg, 0.05 equiv) in CH_2Cl_2 (6 mL) stirred at 0 °C for 20 min, was added amine **25** (147 mg. 1.00 equiv) in CH_2Cl_2 (4 mL) was added slowly with stirring. After 20h at room temperature, the mixture was diluted with CH_2Cl_2 and washed with cold aqueous HCl solution (1 M; 20 mL), cold water (20 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4) and evaporated. The residue was chromatographed (hexanes : AcOEt, 90

: 10 □ 80 : 20) to give carbamate **26** (160 mg, 71%) as a white solid: R_f 0.24 (hexanes : EtOAc, 80 : 20); mp 87–89 °C (hexanes : AcOEt, 70 : 30); IR (film) 3250–3460, 2941, 2865, 1671 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (br s, 1H), 7.41 (d, J = 8.3 Hz, 2H), 7.17 (d, J = 8.3 Hz, 2H), 5.24 (br s, 1H), 3.91 (d, J = 5.8 Hz, 2H), 3.84 (t, J = 6.5 Hz, 2H), 2.81 (t, J = 6.5 Hz, 2H), 1.48 (s, 9H), 1.04–1.00 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 156.5, 135.7, 135.1, 129.3, 119.8, 79.9, 64.6, 44.7, 39.0, 28.1, 17.8, 11.8; HRMS (ESI) calcd for $C_{24}H_{43}N_2O_4Si$ $[M+H]^+$ 451.2992, found 451.2990.

***tert*-Butyl 2-thioxo-2-(4-(2-(triisopropylsilyloxy)ethyl)phenylamino)-ethylcarbamate (27)**. Lawesson's reagent (10 mg, 0.50 equiv) was added with stirring to amide **26** (20 mg, 0.05 mmol, 1.00 equiv) in THF (1 mL) and the mixture was heated to reflux for 30h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with saturated aqueous $NaHCO_3$ solution (10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic layers were dried (Na_2SO_4) and evaporated. The residue was chromatographed (hexanes : AcOEt, 85 : 15) to afford carbamate **27** (15 mg, 63%) as a pale yellow oil: R_f 0.38 (hexanes : EtOAc, 80 : 20); IR (film) 2942, 2865, 1692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.11 (br s, 1H), 7.65 (d, J = 8.3 Hz, 2H), 7.25 (d, J = 8.3 Hz, 2H), 5.39 (br s, 1H), 4.28 (d, J = 6.4 Hz, 2H), 3.87 (t, J = 6.8 Hz, 2H), 2.85 (t, J = 6.8 Hz, 2H), 1.48 (s, 9H), 1.07–1.00 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.2, 156.9, 138.1, 136.6, 129.5, 123.2, 80.8, 64.5, 53.2, 39.2, 28.3, 18.0, 12.0; HRMS (ESI) calcd for $C_{24}H_{43}N_2O_3SSi$ $[M+H]^+$ 467.2764, found 467.2752.

***tert*-Butyl (5-(pyrimidin-2-yl)-4-(4-(2-(triisopropylsilyloxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methylcarbamate (28)**. Following a literature procedure,[35] freshly prepared acyl hydrazide **21b** (206 mg, 1.50 equiv) was added with stirring to thioamide **27** (466 mg, 1.00 mmol, 1.00

equiv) in pyridine (4 mL) and the resulting mixture was heated at 100 °C for 6h. The reaction mixture was cooled to 0 °C, diluted with CH_2Cl_2 (50 mL) and washed with aqueous HCl solution (1 M; 150 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were washed with brine (150 mL), dried (Na_2SO_4) and concentrated. The residue was chromatographed (CH_2Cl_2 : MeOH : NH_4OH, 95 : 5 : 0.25) to give carbamate **28** (262 mg, 47%) as a pale yellow oil. R_f 0.25 (CH_2Cl_2 : MeOH, 95 : 5); IR (film) 3409–3250, 2945, 2868, 1706 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, J = 4.9 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 7.22 (t, J = 4.9 Hz, 1H), 7.15 (d, J = 8.2 Hz, 2H), 5.54 (br s, 1H), 4.31 (d, J = 5.3 Hz, 2H), 3.93 (t, J = 6.8 Hz, 2H), 2.90 (t, J = 6.8 Hz, 2H), 1.41 (s, 9H), 1.06 (sept, J = 5.7 Hz, 3H), 1.03 (d, J = 5.7 Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.1, 155.8, 155.2, 154.0, 152.6, 141.1, 132.3, 130.1, 126.6, 120.6, 79.7, 64.0, 39.1, 36.2, 28.1, 17.9, 11.8; HRMS (ESI) calcd for $C_{29}H_{45}N_6O_3Si$ $[M+H]^+$ 553.3322, found 553.3320.

(5-(Pyrimidin-2-yl)-4-(4-(2-(tri-isopropylsilyloxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methanamine (29). Me_3SiOTf (0.7 mL, 9.00 equiv) was added dropwise with stirring to Boc-protected amine **28** (240 mg, 0.43 mmol, 1.00 equiv) and 2,6-lutidine (0.9 mL, 18.0 equiv) in CH_2Cl_2 (4.5 mL) at 0 °C and the resulting solution allowed to warm up to room temperature. After 6h, additional 2,6-lutidine (18.0 equiv) and Me_3SiOTf (9.00 equiv) were added at 0°C and, after 18h at room temperature, the solution was diluted with CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$ solution (100 mL), brine (100 mL), dried (Na_2SO_4) and concentrated. The residue was chromatographed (CH_2Cl_2 : MeOH : NH_4OH, 95 : 5 : 0.25) to give triazole **29** (180 mg, 90%) as a pale orange oil: R_f 0.14 (CH_2Cl_2 : MeOH : NH_4OH, 95 : 5 : 0.25); IR (film) 2942, 2864, 1562 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, J = 4.9 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.20 (t, J = 4.9 Hz, 1H), 7.16 (d, J = 8.4 Hz, 2H), 3.93 (t, J = 6.4 Hz, 2H), 3.84 (br s, 2H), 2.89 (t, J = 6.4 Hz, 2H), 1.70 (br s, 2H), 1.04 (sept, J =

5.9 Hz, 3H), 1.00 (d, *J* = 5.9 Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.0 (br), 157.3, 156.1, 152.6 (br), 141.4, 132.9, 130.3, 126.8, 120.6, 64.1, 39.2, 37.1 (br), 18.0, 11.9; HRMS (ESI) calcd for $C_{24}H_{37}N_6OSi$ $[M+H]^+$ 453.2798, found 453.2786.

***tert*-Butyl (2*S*,3*S*)-3-methyl-1-oxo-1-((5-(pyrimidin-2-yl)-4-(4-(2-(tri-isopropyl-silyloxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methylamino)pentan-2-ylcarbamate (30)**. Amine **29** (180 mg, 0.39 mmol, 1.00 equiv) in CH_2Cl_2 (2 mL) was added with stirring to an aged (20 min) solution of *N*-Boc-isoleucine (99 mg, 1.10 equiv), EDCI (90 mg, 1.20 equiv) and DMAP (5 mg, 0.10 equiv) in CH_2Cl_2 (6 mL) at 0 °C. After 25h at room temperature, the mixture was diluted with CH_2Cl_2 and washed with cold aqueous HCl solution (1 M; 20 mL), cold water (20 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4) and evaporated and the residue chromatographed (CH_2Cl_2 : MeOH, 95 : 5) to give triazole **30** (210 mg, 81%) as a colorless oil: R_f 0.34 (CH_2Cl_2 : MeOH, 95 : 5); $[\alpha]_D^{20}$ = -128 = 1.0, $CHCl_3$); IR (film) 3459–3135, 1743, 1673 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 4.9 Hz, 2H), 7.52 (br s, 1H), 7.29 (d, *J* = 8.3 Hz, 2H), 7.18 (t, *J* = 4.9 Hz, 1H), 7.17 (d, *J* = 8.3 Hz, 2H), 5.21 (d, *J* = 8.9 Hz, 1H), 4.46–4.32 (m, 2H), 4.14–4.01 (m, 1H), 3.90 (t, *J* = 6.8 Hz, 2H), 2.88 (t, *J* = 6.8 Hz, 2H), 1.97–1.79 (m, 1H), 1.49–1.40 (m, 1H), 1.38 (s, 9H), 1.11–0.97 (m, 4H), 1.00 (d, *J* = 5.7 Hz, 18H), 0.87 (d, *J* = 6.8 Hz, 3H), 0.84 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 156.9, 155.6, 155.4, 153.6, 152.3, 140.9, 132.1, 129.9, 126.6, 120.4, 79.2, 63.9, 58.9, 39.0, 37.3, 34.5, 28.1, 24.2, 17.7, 15.4, 11.6, 11.3; HRMS (ESI) calcd for $C_{35}H_{56}N_7O_4Si$ $[M+H]^+$ 666.4163, found 666.4171.

(2*S*,3*S*)-2-Amino-3-methyl-*N*-((5-(pyrimidin-2-yl)-4-(4-(2-(tri-isopropylsilyl-oxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methyl)pentanamide (31). Me_3SiOTf (480 µL, 9.00 equiv) was added with stirring to Boc-protected amine **30** (200 mg, 0.30 mmol, 1.00 equiv) and 2,6-lutidine (625 µL, 18.0 equiv) in CH_2Cl_2 (5 mL) at 0 °C. After 5 hours at room temperature, the

solution was diluted with CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$ solution (100 mL), brine (100 mL), dried (Na_2SO_4) and concentrated. The residue was chromatographed (CH_2Cl_2 : MeOH : NH_4OH, 95 : 5 : 0.25) to give traizole **31** (87 mg, 62%) as a yellow oil: R_f 0.08 (CH_2Cl_2 : MeOH : NH_4OH, 95 : 5 : 0.25); $[\alpha]_D^{20}$ = -101 (*c* = 1.0, $CHCl_3$); IR (film) 3453–3187, 2941, 2866, 1674 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 4.9 Hz, 2H), 8.19 (s, 1H), 7.34 (d, *J* = 8.2 Hz, 2H), 7.29 (t, *J* = 4.9 Hz, 1H), 7.19 (d, *J* = 8.3 Hz, 2H), 4.48 (dd, *J* = 16.6, 5.4 Hz, 1H), 4.44 (dd, *J* = 16.6, 5.1 Hz, 1H), 3.95 (t, *J* = 6.7 Hz, 2H), 3.33–3.29 (m, 1H), 2.93 (t, *J* = 6.7 Hz, 2H), 2.05–1.94 (m, 1H), 1.68 (br s, 2H), 1.45–1.31 (m, 1H), 1.17–1.02 (m, 4H), 1.05 (d, *J* = 5.8 Hz, 18H), 0.97 (d, *J* = 7.1 Hz, 3H), 0.91 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 157.2, 155.9, 153.9, 152.6, 141.3, 132.4, 130.2, 126.7, 120.7, 64.1, 59.9, 39.2, 38.0, 34.7, 23.7, 18.0, 16.1, 11.9, 11.8; HRMS (ESI) calcd for $C_{30}H_{48}N_7O_2Si$ $[M+H]^+$ 566.3639, found 566.3660.

(2*S*,3*S*)-3-Methyl-2-(2-phenylacetamido)-*N*-((5-(pyrimidin-2-yl)-4-(4-(2-(tri-iso-propylsilyloxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methyl)pentanamide (32). Amine **31** (20 mg, 0.04 mmol, 1.00 equiv) in CH_2Cl_2 (0.5 mL) was added with stirring to phenylacetic acid (7 mg, 1.10 equiv), EDCI (12 mg, 1.20 equiv) and DMAP (1 mg, 0.10 equiv) in CH_2Cl_2 (0.5 mL) at 0 °C. After 20 min at 0 °C and 16h at room temperature, the mixture was diluted with CH_2Cl_2 and washed with cold aqueous HCl solution (1 M; 20 mL), cold water (20 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4) and evaporated. The residue was chromatographed (CH_2Cl_2 : MeOH, 95 : 5) to give amide **32** (26 mg, 92%) as a yellow oil: R_f 0.29 (CH_2Cl_2 : MeOH, 95 : 5); $[\alpha]_D^{20}$ = -95 (*c* = 1.0, $CHCl_3$); IR (film) 3400–3133, 2939, 2865, 1640 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 4.9 Hz, 2H), 7.68 (br t, *J* = 5.0 Hz, 1H), 7.34-7.10 (m, 10H), 6.39 (d, *J* = 8.8 Hz, 1H), 4.42 (dd, *J* = 8.8, 6.1 Hz, 1H), 4.39–4.35 (m, 2H), 3.92 (t, *J* = 6.7 Hz, 2H), 3.58 (s, 2H), 2.89 (t, *J* = 6.7 Hz,

2H), 1.91–1.74 (m, 1H), 1.37–1.26 (m, 1H), 1.12–1.00 (m, 3H), 1.02 (d, J = 5.6 Hz, 18H), 0.98–0.84 (m, 1H), 0.79 (d, J = 6.9 Hz, 3H), 0.78 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 171.0, 157.2, 155.8, 153.7, 152.6, 141.3, 134.9, 132.1, 130.2, 129.3, 128.8, 127.1, 126.7, 120.7, 64.1, 57.6, 43.6, 39.2, 37.1, 34.8, 24.5, 17.9, 15.5, 11.9, 11.3; HRMS (ESI) calcd for $C_{38}H_{54}N_7O_3Si$ $[M+H]^+$ 684.4057, found 684.4084.

(2*S*,3*S*)-*N*-((4-(4-(2-Hydroxyethyl)phenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl)-3-methyl-2-(2-phenylacetamido)pentanamide (33). Bu_4NF in THF (1.0 M; 48 μL, 2.20 equiv) was added with stirring to silyl ether **32** (15 mg, 22 μmol, 1.00 equiv) and concentrated AcOH (6 μL, 4.00 equiv) in THF at 0 °C. After 4h at room temperature, further AcOH (8.00 equiv) and Bu_4NF in THF (4.40 equiv) were added in two portions at 0 °C and the mixture was stirred for 35h. The mixture was diluted with EtOAc (10 mL), washed with saturated aqueous $NaHCO_3$ solution (10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated. The residue was chromatographed (CH_2Cl_2 : Acetone : MeOH, 5 : 4 : 1), then further chromatographed a second time (CH_2Cl_2: MeOH, 92.5 : 7.5) to give amide **33** (10 mg, 83%) as a white solid: R_f 0.16 (CH_2Cl_2 : MeOH, 95 : 5); mp 192–194 °C (CH_2Cl_2 : PE, 50 : 50); $[\alpha]_D^{20}$ = -72 (c = 1.0, $CHCl_3$); IR (film) 3653–3106, 3058, 2962, 2929, 1646 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, J = 4.8 Hz, 2H), 8.05 (br s, 1H), 7.33 (d, J = 8.5 Hz, 2H), 7.25–7.19 (m, 5H), 7.19–7.08 (m, 3H), 6.82 (d, J = 9.1 Hz, 1H), 4.60 (dd, J = 15.8, 6.3 Hz, 1H), 4.43 (dd, J = 15.8, 4.8 Hz, 1H), 4.32 (dd, J = 9.1, 6.2 Hz, 1H), 3.91 (t, J = 6.0 Hz, 2H), 3.63–3.57 (m, 2H), 2.91 (t, J = 6.0 Hz, 2H), 2.71 (br s, 1H), 1.90–1.73 (m, 1H), 1.34–1.26 (m, 1H), 1.04–0.84 (m, 1H), 0.79 (d, J = 6.8 Hz, 3H), 0.75 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 171.2, 157.3, 155.6, 154.2, 152.4, 141.2, 135.2, 132.3, 130.5, 129.2, 128.7, 127.1, 127.0, 121.0, 63.2, 57.9, 43.5, 39.0, 37.1,

34.0, 24.6, 15.6, 11.3; HRMS (ESI) calcd for $C_{29}H_{34}N_7O_3$ $[M+H]^+$ 528.2723, found 528.2706.

(2*S*,3*S*)-3-Methyl-2-(2-phenylacetamido)pentanoic acid (36). Phenylacetyl chloride (**35**) (990 μL, 1.50 equiv) was added with stirring to L-isoleucine (650 mg, 7.50 mmol, 1.00 equiv) in aqueous NaOH (4 M; 4 mL). After 45 min at room temperature, the product was precipitated by addition of aqueous HCl solution (6 M; 3 mL). The resulting mixture was cooled to 0 °C and the white crystals were collected by filtration, washed with cold water (10 mL) and evaporated. The crystals were suspended in a mixture of dichloroethane and hexanes (1:1), stirred for 5 min, collected by filtration and dried *in vacuo*, to give carboxylic acid **36** (700 mg, 56%) as pure white solid: R_f 0.11 (CH_2Cl_2 : MeOH, 95 : 5); mp 110–112 °C (dichloroethane : hexanes, 50 : 50); $[\alpha]_D^{20}$ = -1.2 (*c* = 1.0, MeOH); IR (film) 3341, 1697, 1617 cm^{-1}; ^1H NMR (400 MHz, MeOD-*d*6) δ 7.34–7.12 (m, 5H), 4.38 (d, *J* = 5.7 Hz, 1H), 3.63–3.54 (m, 2H), 2.03–1.80 (m, 1H), 1.40–1.60 (m, 1H), 1.33–1.14 (m, 1H), 0.96–0.85 (m, 6H); ^{13}C NMR (100 MHz, MeOD-*d*6) δ 174.8, 174.1, 137.0, 130.2, 129.5, 127.9, 58.2, 43.4, 38.4, 26.2, 16.0, 11.8; HRMS (ESI) calcd for $C_{14}H_{20}NO_3$ [M+H]+ 250.1443, found 250.1432.

***tert*-Butyl 2-(4-fluorophenylamino)-2-oxoethylcarbamate (38)**. 4-Fluoroaniline (**37**) (189 μL, 1.00 equiv) in CH_2Cl_2 (5 mL) was added with stirring to an aged (15 min) solution of *N*-Boc-glycine (348 mg, 2.00 mmol, 1.00 equiv), EDCI (460 mg, 1.20 equiv) and DMAP (24 mg, 0.10 equiv) in CH_2Cl_2 (15 mL) at 0 °C. After 72h at room temperature, the mixture was diluted with CH_2Cl_2 and washed with cold aqueous HCl solution (1 M; 60 mL), cold water (60 mL) and brine (60 mL). The organic layer was dried (Na_2SO_4) and evaporated and the residue chromatographed (hexanes : AcOEt, 70 : 30) to give carbamate **38** (416 mg, 82%) as a white solid. R_f 0.16 (hexanes : AcOEt, 70 : 30); mp

140–143 °C (hexanes : AcOEt, 70 : 30); IR (film) 3452–3123, 1685 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.16 (br s, 1H), 7.47 (dd, J = 8.9, 4.8 Hz, 2H), 7.02 (dd, J = 8.9, 8.5 Hz, 2H), 5.22 (br s, 1H), 3.92 (d, J = 5.9 Hz, 2H), 1.48 (s, 9H); ^{13}C NMR (125 MHz, MeOD-d_6) δ 170.5, 160.7 (d, J = 246 Hz), 158.6, 135.7, 123.2 (d, J = 7 Hz), 116.3 (d, J = 22 Hz), 80.8, 45.0, 28.7; HRMS (CI^+) calcd for $C_{13}H_{18}FN_2O_3$ $[M+H]^+$ 269.1301, found 269.1305.

***tert*-Butyl 2-(4-fluorophenylamino)-2-thioxoethylcarbamate (39)**. Lawesson's reagent (247 mg, 0.50 equiv) was added with stirring to amide **38** (327 mg, 1.22 mmol, 1.00 equiv) in THF (12 mL) and the mixture was heated to reflux for 2h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ solution (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL) and the combined organic layers were dried (Na_2SO_4) and evaporated. The residue was chromatographed (hexanes : AcOEt, 70 : 30) to afford carbamate **39** (295 mg, 85%) as a white solid: R_f 0.24 (hexanes : AcOEt, 70 : 30); mp 91–95 °C (hexanes : AcOEt, 70 : 30); IR (film) 3340, 3160, 1698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.29 (br s, 1H), 7.67 (dd, J = 9.0, 4.8 Hz, 2H), 7.06 (dd, J = 9.0, 8.4 Hz, 2H), 5.69 (br s, 1H), 4.28 (d, J = 5.6 Hz, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.8, 160.5 (d, J = 243 Hz), 156.7, 134.2, 125.4 (d, J = 8 Hz), 115.5 (d, J = 24 Hz), 80.8, 52.8, 28.0; HRMS (ESI) calcd for $C_{13}H_{18}FN_2O_2S$ $[M+H]^+$ 285.1064, found 285.1073.

***tert*-Butyl (4-(4-fluorophenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl-carbamate (40)**. Following a literature procedure,[35] freshly prepared acyl hydrazide **21b** (210 mg, 1.50 equiv) was added with stirring to thioamide **39** (287 mg, 1.01 mmol, 1.00 equiv) in pyridine (10 mL) and the mixture was heated at 100 °C for 14h. Additional acyl hydrazide **21b** (140 mg, 1.00 eq) was added and the mixture was stirred at 100 °C for 25h. The mixture

was cooled to 0 °C, diluted with CH_2Cl_2 (50 mL) and washed with aqueous HCl solution (1 M; 150 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were washed with brine (150 mL), dried (Na_2SO_4) and concentrated. The residue was chromatographed (CH_2Cl_2 : MeOH, 95 : 5) to give carbamate **40** (230 mg, 57%) as a pale yellow solid: R_f 0.18 (CH_2Cl_2 : MeOH, 95 : 5); mp > 182 °C (decomp.) (CH_2Cl_2 : MeOH, 95 : 5); IR (film) 2968, 1673 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, J = 4.9 Hz, 2H), 7.29 (dd, J = 8.8, 4.7 Hz, 2H), 7.22 (t, J = 4.9 Hz, 1H), 7.14 (dd, J = 8.8, 8.4 Hz, 2H), 5.59 (br s, 1H), 4.34 (d, J = 5.5 Hz, 2H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.9 (d, J = 249 Hz), 157.2, 155.6, 155.2, 154.2, 152.6, 130.5, 129.1 (d, J = 8 Hz), 120.8, 116.5 (d, J = 21 Hz), 79.9, 35.9, 28.2; HRMS (ESI) calcd for $C_{18}H_{20}FN_6O_2$ $[M+H]^+$ 371.1632, found 371.1626.

(4-(4-Fluorophenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methanamine (41). Trifluoroacetic acid (500 μL, 12.0 equiv) was added dropwise with stirring to Boc-protected amine **40** (200 mg, 0.54 mmol, 1.00 equiv) in CH_2Cl_2 (5 mL) at 0 °C. After 1h at room temperature, the mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ solution (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL) and the combined organic layers were dried (Na_2SO_4) and evaporated. The residue was chromatographed (CH_2Cl_2 : MeOH, 95 : 5) to afford triazole **41** (48 mg, 32%) as a white solid: R_f 0.30 (CH_2Cl_2 : MeOH : NH_4OH, 90 : 10 : 0.25); mp 92–94 °C (CH_2Cl_2 : MeOH, 95 : 5); IR (film) 3502–3249 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, J = 4.9 Hz, 2H), 7.28 (dd, J = 8.9, 4.4 Hz, 2H), 7.21 (t, J = 4.9 Hz, 1H), 7.15 (dd, J = 8.9, 8.4 Hz, 2H), 3.83 (s, 2H), 1.83 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.8 (d, J = 250 Hz), 157.7, 157.2, 155.8, 152.4, 130.9, 129.2 (d, J = 9 Hz), 120.7, 116.4 (d, J = 23 Hz), 37.0; HRMS (ESI) calcd for $C_{13}H_{12}FN_6$ $[M+H]^+$ 271.1107, found 271.1102.

(2*S*,3*S*)-*N*-((4-(4-Fluorophenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl)-3-methyl-2-(2-phenylacetamido)pentanamide (42). Amine **41** (17 mg, 1.00 equiv) and Et_3N (12 μL, 1.20 equiv) were added with stirring to an aged (15 min) solution of carboxylic **36** (18 mg, 0.07 mmol, 1.00 equiv), EDCI (16 mg, 1.10 equiv) and HOBt (11 mg, 1.20 equiv) in CH_2Cl_2 (1 mL). After 24h at room temperature, the mixture was diluted with CH_2Cl_2 and washed with cold aqueous HCl solution (1 M; 60 mL), cold water (60 mL) and brine (60 mL). The organic layer was dried (Na_2SO_4) and evaporated and the residue chromatographed (CH_2Cl_2 : MeOH, 95 : 5) to give triazole **42** (33 mg, 88%) as a white solid: R_f 0.28 (CH_2Cl_2 : MeOH, 95 : 5); mp 172–175 °C (CH_2Cl_2); $[\alpha]_D^{20}$ = -12.7 (*c* = 1.00, MeOH); IR (film) 3227, 1636 cm^{-1}; ^1H NMR (400 MHz, MeOD-d_6) δ 8.70 (d, *J* = 4.5 Hz, 2H), 7.41 (t, *J* = 4.5 Hz, 1H), 7.40–7.32 (m, 2H), 7.32–7.24 (m, 4H), 7.22–7.13 (m, 3H), 4.50–4.42 (m, 1H), 4.42–4.32 (m, 1H), 4.14 (d, *J* = 7.6 Hz, 1H), 3.58–2.50 (m, 2H), 1.84–1.70 (m, 1H), 1.49–1.33 (m, 1H), 1.17–0.98 (m, 1H), 0.89–0.75 (m, 6H); ^{13}C NMR (125 MHz, MeOD-d_6) δ 173.8, 173.5, 164.6 (d, *J* = 248 Hz), 158.8, 156.4, 155.5, 153.9, 136.9, 131.7, 130.9 (d, *J* = 9 Hz), 130.2, 129.6, 127.9, 122.8, 117.4 (d, *J* = 23 Hz), 59.0, 43.7, 38.2, 35.0, 25.8, 15.9, 11.3; MS (ESI) 502 $[M+H]^+$; HRMS (ESI) calcd for $C_{27}H_{29}FN_7O_2$ $[M+H]^+$ 502.2367, found 502.2358.

2-Bromo-*N*-cyclopropylacetamide (44). Following a literature procedure,[17] cyclopropyl amine (2.08 mL, 1.20 equiv) was added with stirring to bromoacteyl bromide (**43**) (1.74 mL, 20.0 mmol, 1.00 equiv) and sodium bicarbonate (3.36 g, 2.00 equiv) in THF (150 mL) at -78 °C. After 10 min at -78 °C and 2h at room temperature, the mixture was diluted with AcOEt (200 mL) and washed with saturated aqueous $NaHCO_3$ solution (200 mL), brine (150 mL), dried (Na_2SO_4) and concentrated to give amide **44** (3.11 g, 87%) as a pale yellow solid, which was used directly without further purification: R_f 0.52 (CH_2Cl_2 : MeOH, 95 : 5); mp 48–50°C

(Et_2O); IR (film) 3269, 1683, 1642 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.53 (br s, 1H), 3.85 (s, 2H), 2.78–2.70 (m, 1H), 0.91–0.76 (m, 2H), 0.60–0.54 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.9, 28.9, 23.1, 6.4; HRMS (ESI) calcd for $C_5H_9{}^{79}BrNO$ $[M+H]^+$ 177.9868, found 177.9874.

2-Azido-*N*-cyclopropylacetamide (45). Following a literature procedure,[18] α-bromo amide **44** (3.00 g, 16.9 mmol, 1.00 equiv) and sodium azide (3.29 g, 3.00 equiv) in MeCN (150 mL) were heated to reflux at 80 °C for 10h. The solvent was removed, the residue partitioned between CH_2Cl_2 (150 mL) and water (150 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with brine (150 mL), dried (Na_2SO_4) and concentrated. The residue was chromatographed (CH_2Cl_2 : MeOH, 99 : 1) to give amide **45** (1.80 g, 76%) as a white solid: R_f 0.50 (CH_2Cl_2 : MeOH, 95 : 5); mp 28 °C (Et_2O); IR (film) 3253, 3063, 2098, 1682, 1649 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.37 (br s, 1H), 3.97 (s, 2H), 2.76–2.67 (m, 1H), 0.84–0.76 (m, 2H), 0.60–0.51 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.0, 52.6, 22.5, 6.4; HRMS (CI^+) calcd for $C_5H_9N_4O$ $[M+H]^+$ 141.0776, found 141.0772.

2-(5-(Azidomethyl)-4-cyclopropyl-4*H*-1,2,4-triazol-3-yl)pyrimidine (47). Following a literature procedure,[36] oxalyl chloride (124 μL, 1.00 equiv) was added dropwise with stirring to azide **45** (200 mg, 1.43 mmol, 1.00 equiv) and 2,6-lutidine (168 μL, 1.00 equiv) in CH_2Cl_2 (4 mL) at 0 °C. After 5 min at 0 °C, no further gas evolution was observed. Freshly prepared acyl hydrazide **21b** (200 mg, 1.00 equiv) was added with stirring at room temperature. After 12h, the solvent was evaporated and the residue was suspended in an ice-cold saturated aqueous $NaHCO_3$ solution (4 mL) and heated to reflux at 100 °C for 8h. The reaction mixture was diluted with water (50 mL), extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic layers were washed with brine (100 mL), dried

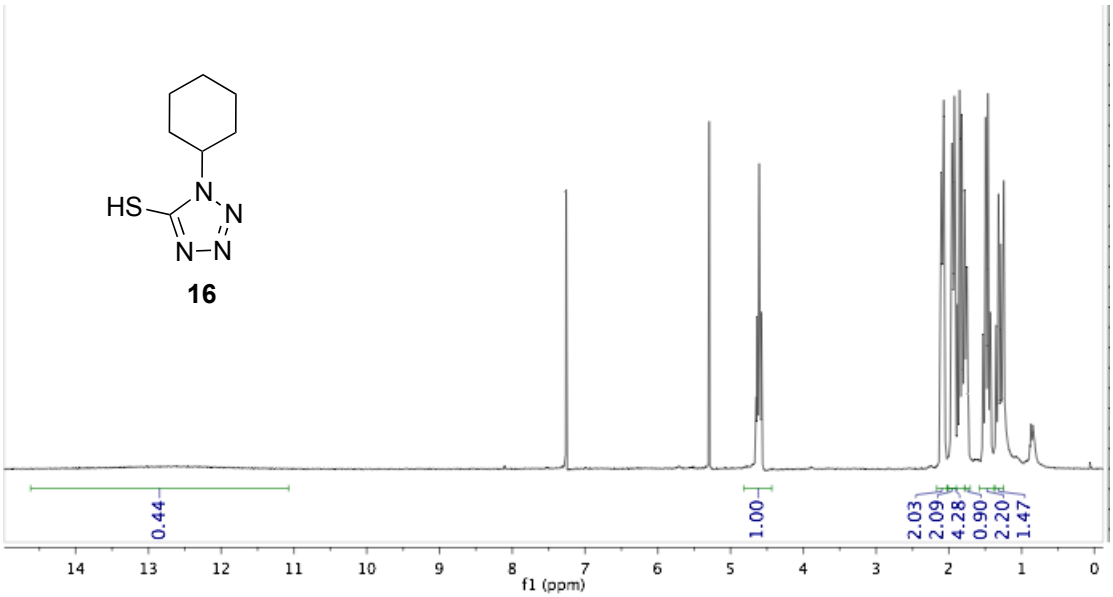
(Na_2SO_4) and concentrated. The residue was chromatographed (CH_2Cl_2 : MeOH, 99 : 1 □ 97 : 3) to give pyrimidine **47** (68 mg, 20%) as a white solid: R_f 0.30 (CH_2Cl_2 : MeOH, 95 : 5); mp 120–122°C (CH_2Cl_2); IR (film) 3062, 3014, 2091 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.92 (d, J = 4.9 Hz, 2H), 7.34 (t, J = 4.9 Hz, 1H), 4.69 (s, 2H), 3.62–3.54 (m, 1H), 1.15–1.08 (m, 2H), 0.82–0.74 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.4, 156.3, 154.5, 152.8, 120.9, 44.9, 27.4, 8.4; HRMS (ESI) calcd for $C_{10}H_{11}N_8$ $[M+H]^+$ 243.1107, found 243.1101.

(4-Cyclopropyl-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methanamine (48). Azide **47** (57 mg, 0.34 mmol, 1.00 equiv), 10% Pd/C (57 mg) and MeOH (2 mL) were stirred under 1 atm of H_2 at room temperature for 2h. The mixture was filtered through celite, washed through with MeOH (40 mL) and evaporated. The residue was chromatographed (CH_2Cl_2 : MeOH : NH_4OH, 95 : 5 : 0.25 □ 90 : 10 : 0.25) to give amine **48** (52 mg, 99%) as a colorless foam: R_f 0.20 (CH_2Cl_2 : MeOH : NH_4OH, 90 : 10 : 0.25); IR (film) 3671–3027 cm^{-1}; ^1H NMR (400 MHz, MeOD-d_6) δ 8.91 (d, J = 4.9 Hz, 2H), 7.37 (t, J = 4.9 Hz, 1H), 3.56–3.47 (m, 1H), 1.14–1.03 (m, 2H), 0.75–0.69 (m, 2H); ^{13}C NMR (125 MHz, MeOD-d_6) δ 158.7, 157.4, 156.6, 153.9, 120.6, 37.8, 29.2, 8.8; HRMS (ESI) calcd for $C_{10}H_{13}N_6$ $[M+H]^+$ 217.1202, found 217.1193.

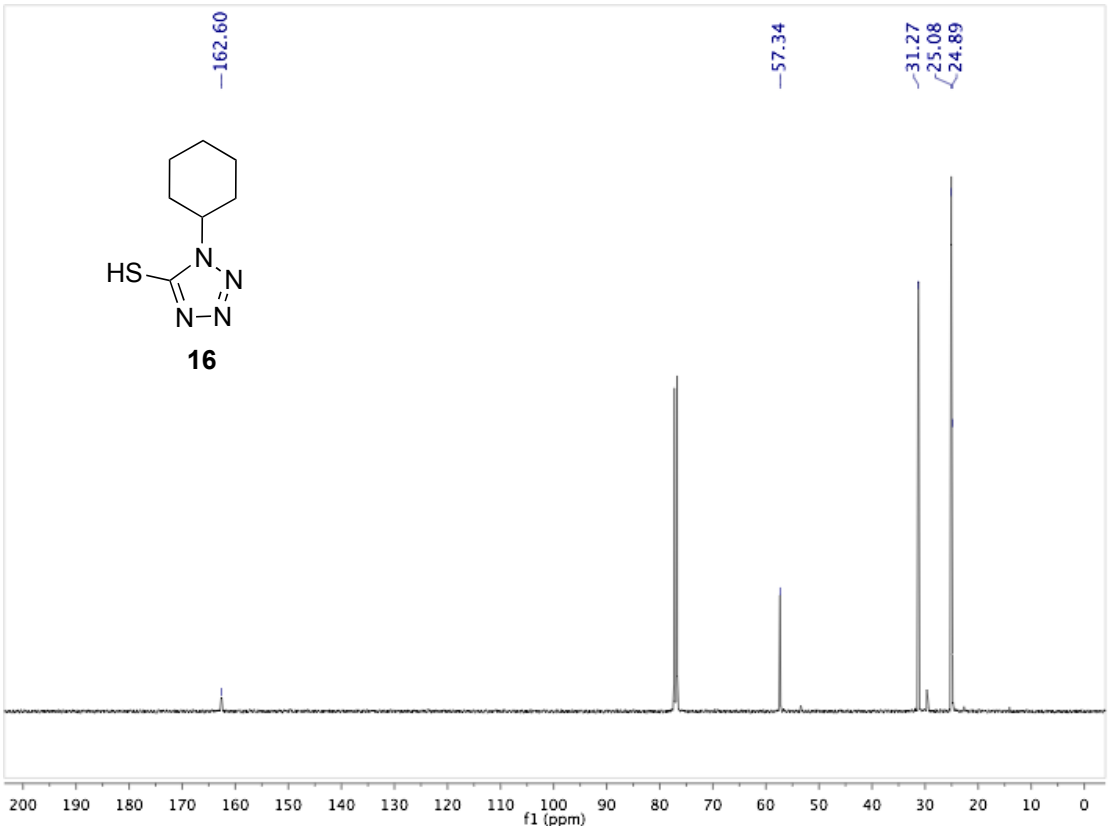
(2*S*,3*S*)-*N*-((4-Cyclopropyl-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl)-3-methyl-2-(2-phenylacetamido)pentanamide (**49**)**.** Amine 48 (47 mg, 1.00 equiv) and Et_3N (61 μL, 2.00 equiv) were added with stirring to an aged (15 min) solution of carboxylic acid 36 (55 mg, 0.22 mmol, 1.00 equiv), EDCI (46 mg, 1.10 equiv) and HOBt (35 mg, 1.20 equiv) in CH_2Cl_2 (6 mL). After 24h at room temperature, the mixture was diluted with CH_2Cl_2 and washed with cold aqueous HCl solution (1 M; 60 mL), cold water (60 mL) and brine (60 mL). The organic layer was dried (Na_2SO_4) and evaporated. The residue was chromatographed (CH_2Cl_2 : MeOH, 95 : 5) to give amide 49 (80 mg, 51%) as a white solid:

NMR analyses showed the presence of two inseparable diastereoisomers in an 11:9 ratio; R_f 0.32 (CH_2Cl_2 : MeOH, 95 : 5); mp 84–85 °C (CH_2Cl_2); $[\alpha]_D^{20}$ = 0.01 (*c* = 1.00, $CHCl_3$); IR (film) 3266, 2961, 2939, 1638 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.98 (d, *J* = 4.7 Hz, 2H, major + minor), 7.88 (br s, 1H, major + minor), 7.37 (t, *J* = 4.7 Hz, 1H, major + minor), 7.31–7.18 (m, 5H, major + minor), 6.69 (br s, 1H, major + minor), 4.80–4.67 (m, 2H, major + minor), 4.63 (dd, *J* = 4.8, 9.0 Hz, 1H, minor), 4.48 (dd, *J* = 4.8, 9.0 Hz, 1H, major), 3.58 (s, 2H, minor), 3.57 (s, 2H, major), 3.51–3.45 (m, 1H, major + minor), 2.02–1.93 (m, 1H, minor), 1.93–1.95 (2m, 1H, major), 1.43–1.33 (m, 1H, major), 1.33–1.23 (m, 1H, minor), 1.12–1.04 (m, 2H, major + minor), 1.06–1.02 (m, 1H, minor), 1.02–0.94 (m, 1H, major), 0.86–0.88 (m, 8H, major + minor); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0 and 171.7 (minor and major), 171.5 and 171.3 (minor and major), 157.4, 156.2, 154.8, 153.9, 135.0, 129.2, 128.8, 127.1, 120.8, 57.9 and 56.6 (major and minor), 43.5, 37.2 and 37.1 (minor and major), 34.8, 27.2, 26.4 and 24.7 (minor and major), 15.6 and 14.4 (major and minor), 11.6 and 11.2 (minor and major), 8.7 and 8.6 (minor and major); HRMS (ESI) calcd for $C_{24}H_{30}N_7O_2$ $[M+H]^+$ 448.2461, found 448.2460.

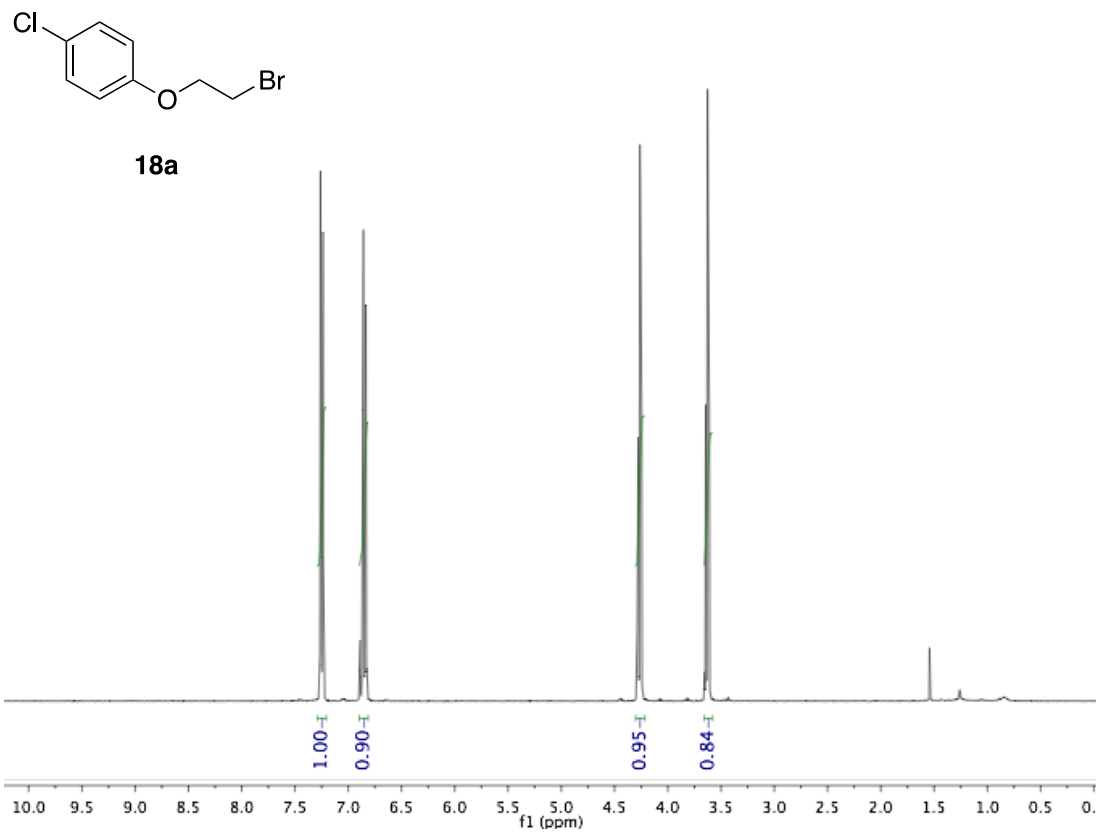
^{1}H NMR spectrum of 1-cyclohexyl-1*H*-tetrazole-5-thiol (**16**) (400 MHz, $CDCl_3$)



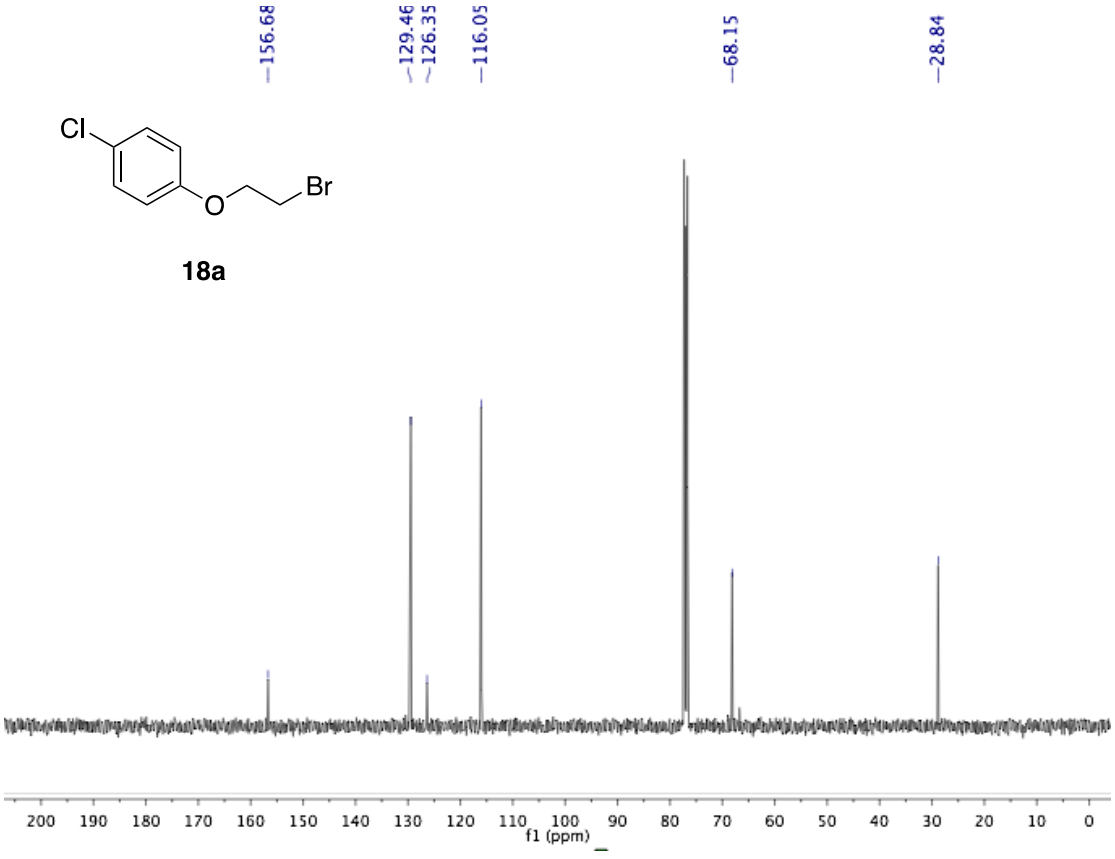
^{13}C NMR spectrum of 1-cyclohexyl-1*H*-tetrazole-5-thiol (**16**) (125 MHz, $CDCl_3$)



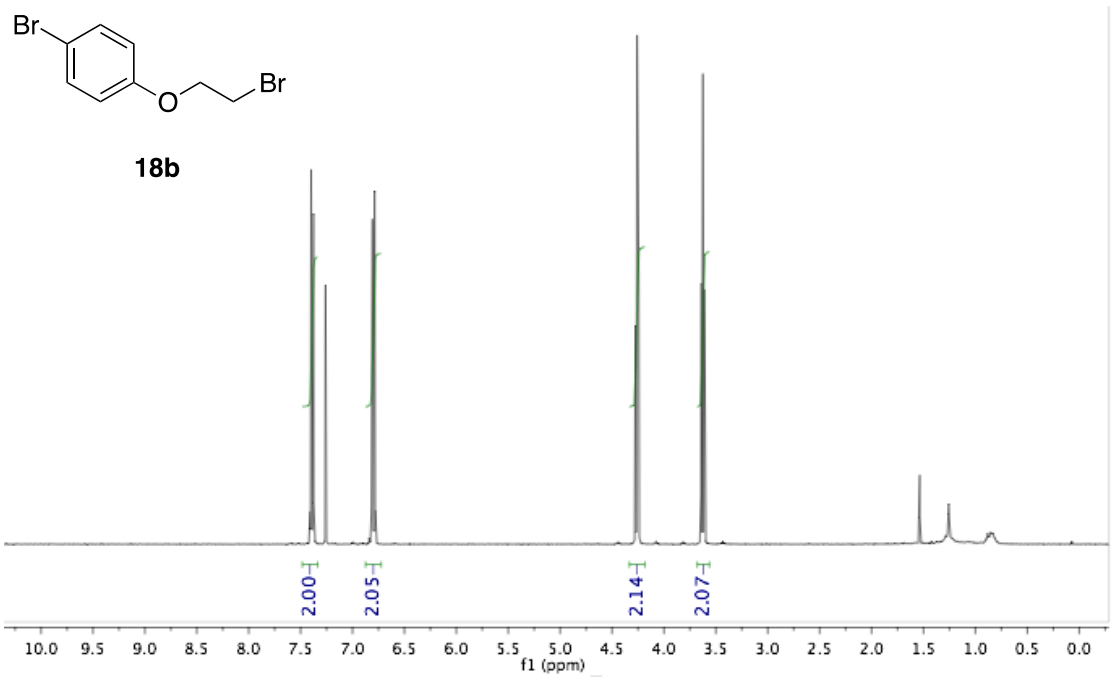
^{1}H NMR spectrum of 1-(2-bromoethoxy)-4-chlorobenzene (**18a**) (400 MHz, $CDCl_3$)



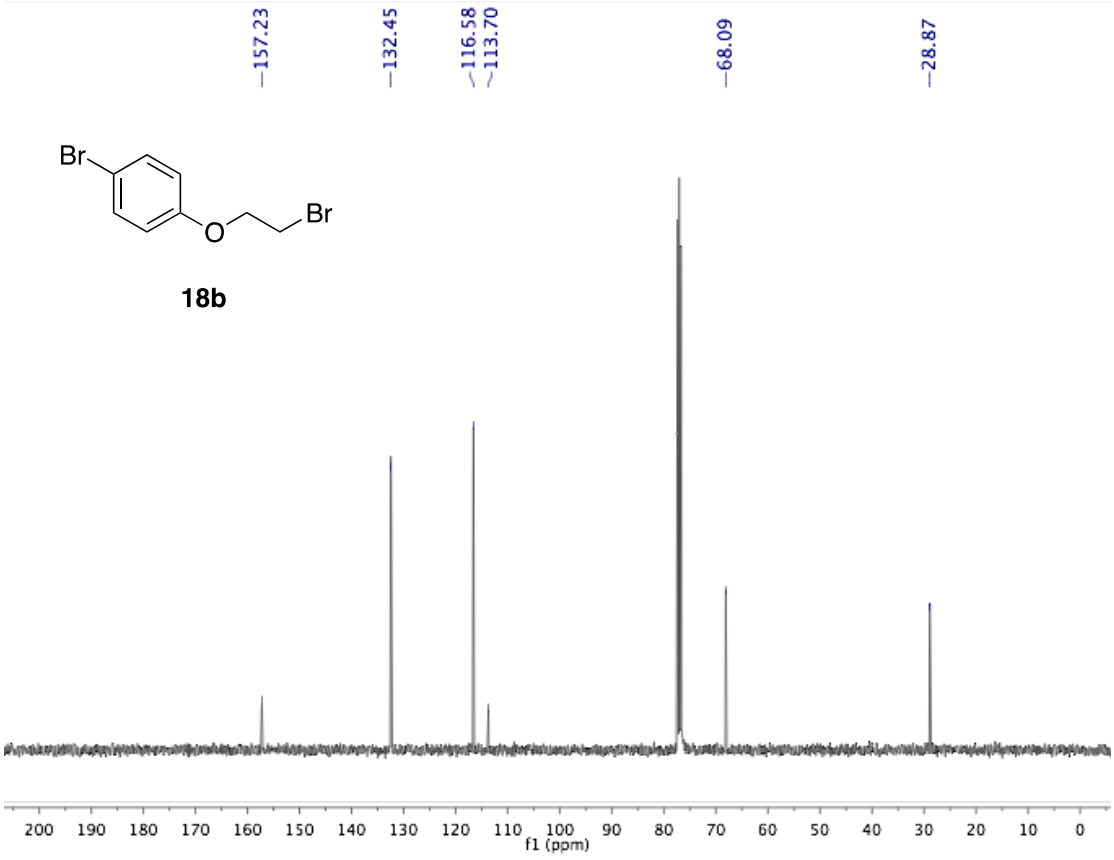
^{13}C NMR spectrum of 1-(2-bromoethoxy)-4-chlorobenzene (**18a**) (100 MHz, $CDCl_3$)



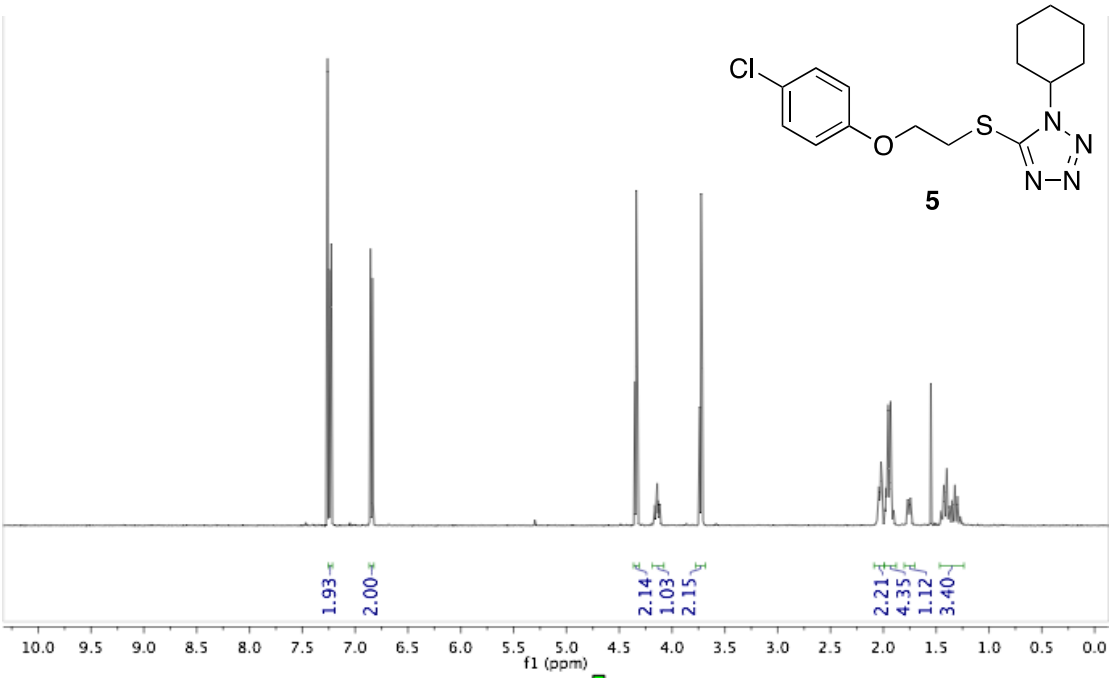
^{1}H NMR spectrum of 1-bromo-4-(2-bromoethoxy)benzene (**18b**) (400 MHz, $CDCl_3$)



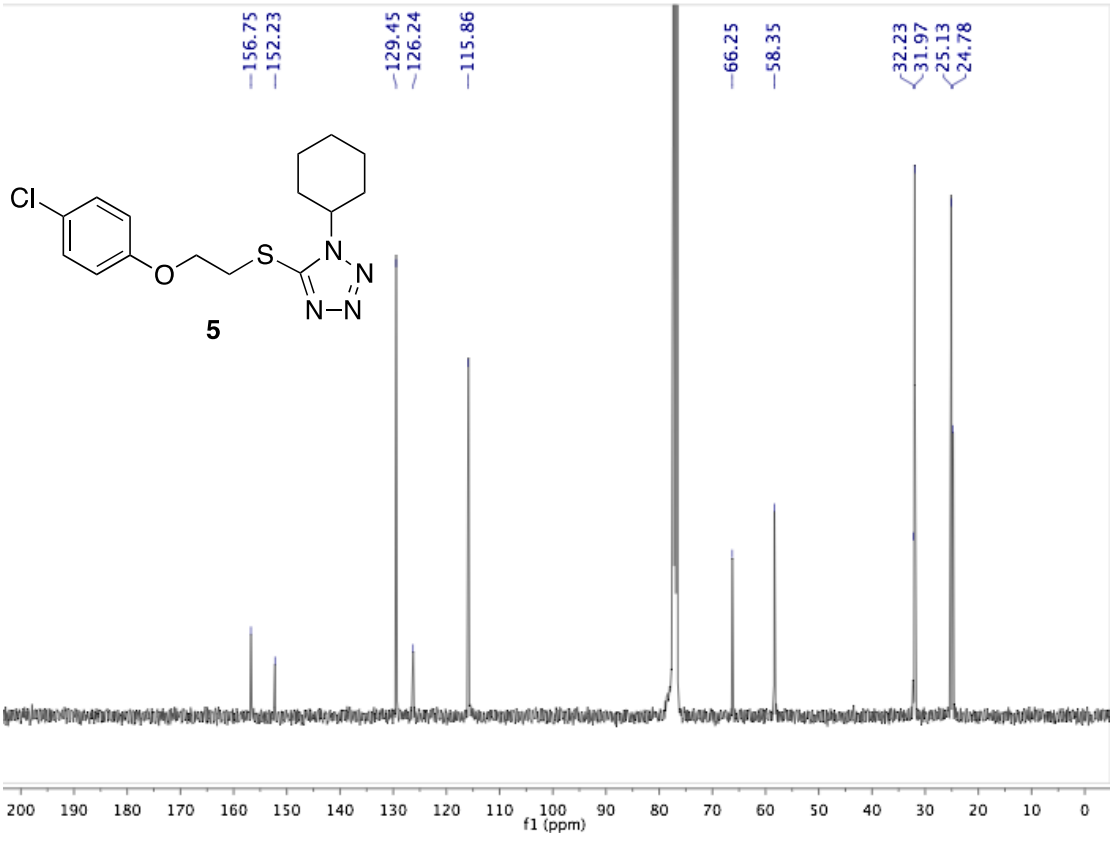
^{13}C NMR spectrum of 1-bromo-4-(2-bromoethoxy)benzene (**18b**) (100 MHz, $CDCl_3$)



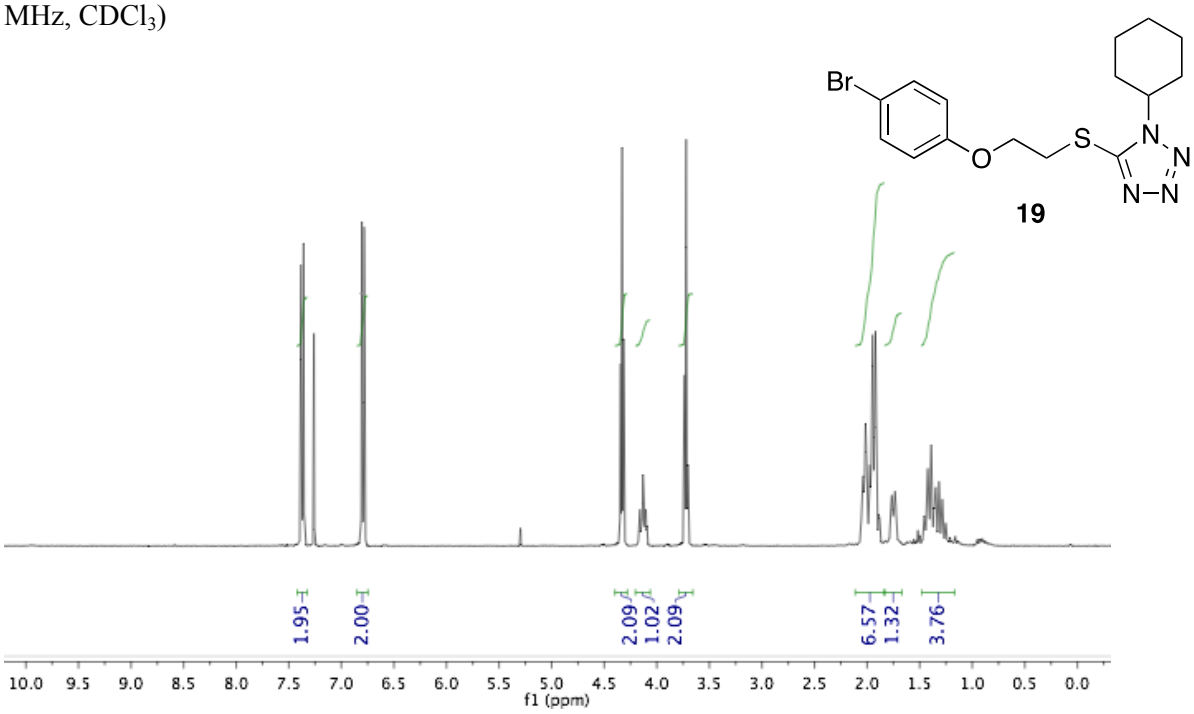
^{1}H NMR spectrum of 5-(2-(4-chlorophenoxy)ethylthio)-1-cyclohexyl-1*H*-tetrazole (**5**) (400 MHz, $CDCl_3$)



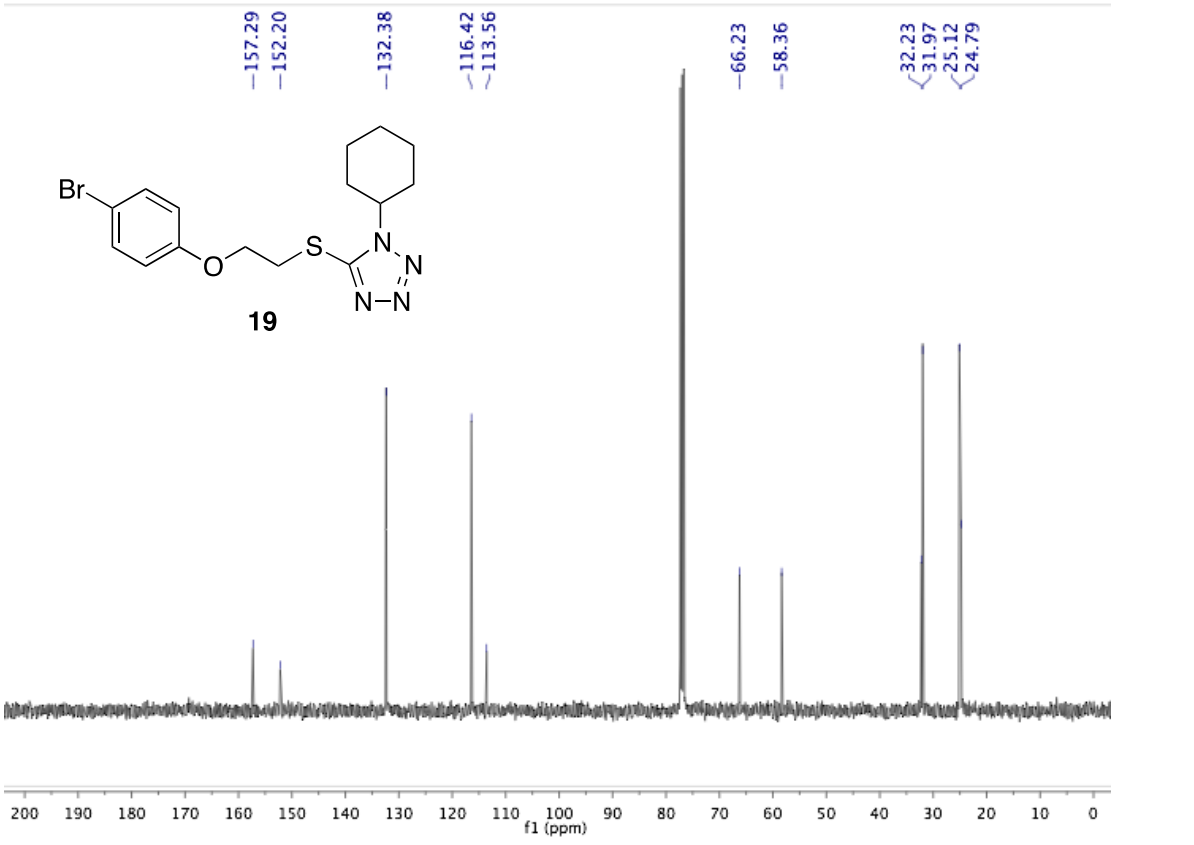
^{13}C NMR spectrum of 5-(2-(4-chlorophenoxy)ethylthio)-1-cyclohexyl-1*H*-tetrazole (**5**) (100 MHz, $CDCl_3$)



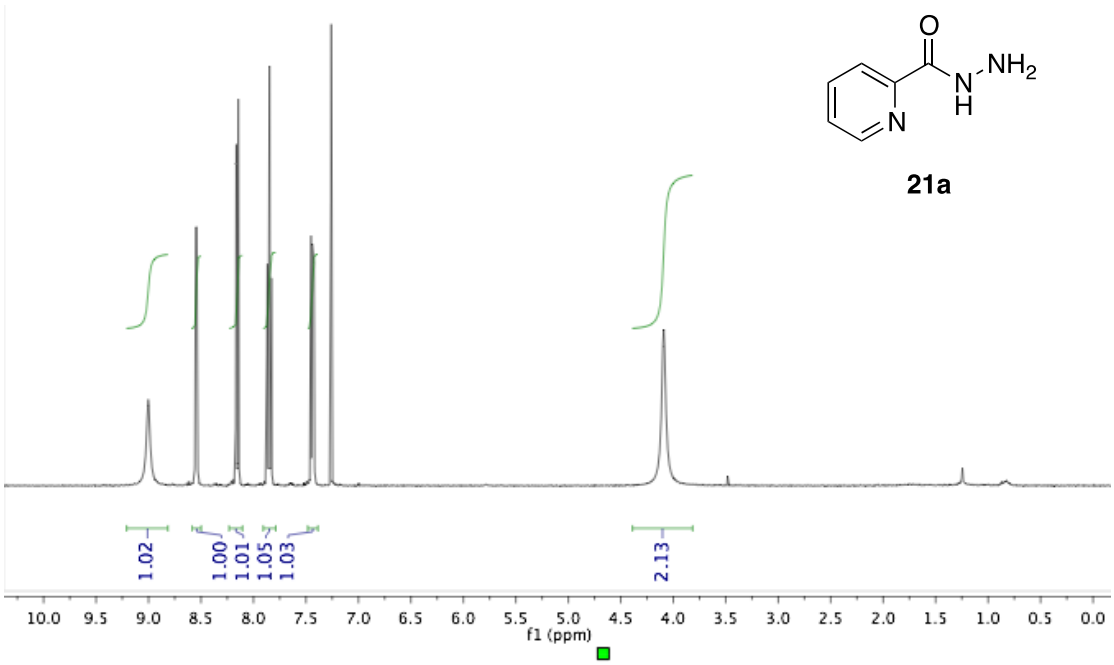
H NMR spectrum of 5-(2-(4-bromophenoxy)ethylthio)-1-cyclohexyl-1*H*-tetrazole (**19**) (400 MHz, $CDCl_3$)



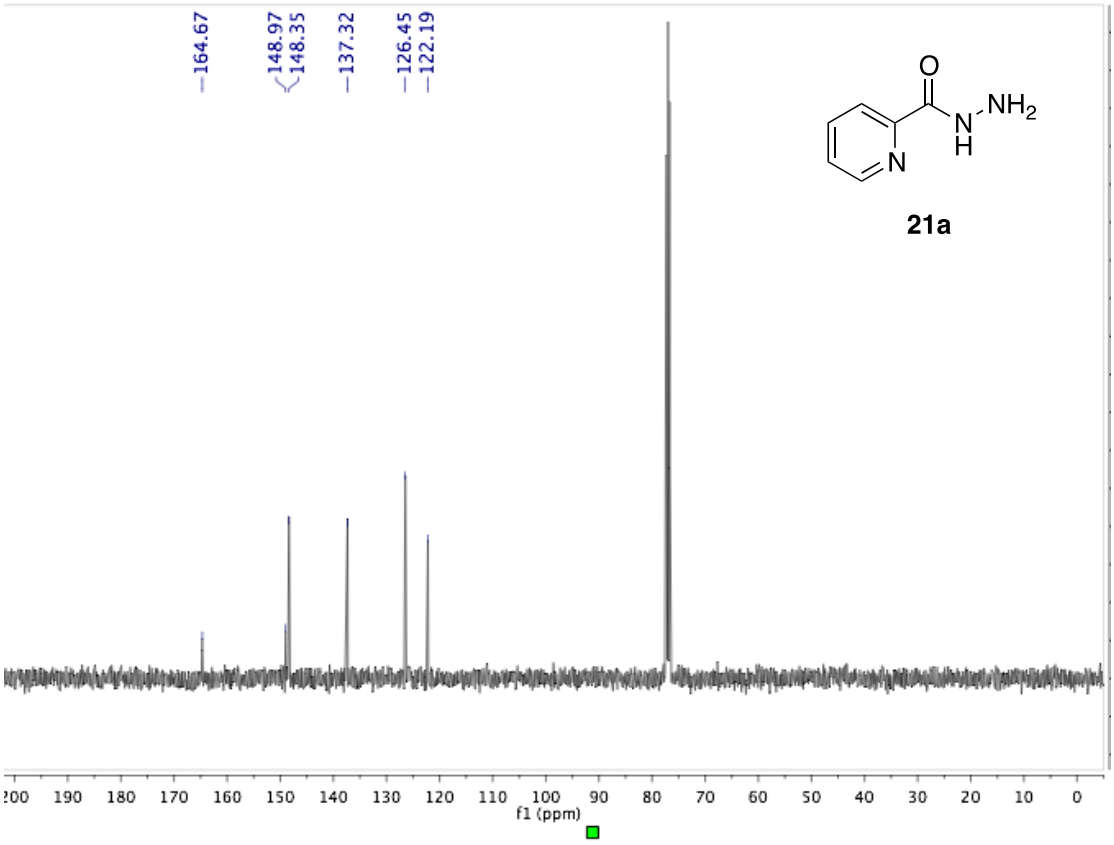
^{13}C NMR spectrum of 5-(2-(4-bromophenoxy)ethylthio)-1-cyclohexyl-1*H*-tetrazole (**19**) (100 MHz, $CDCl_3$)



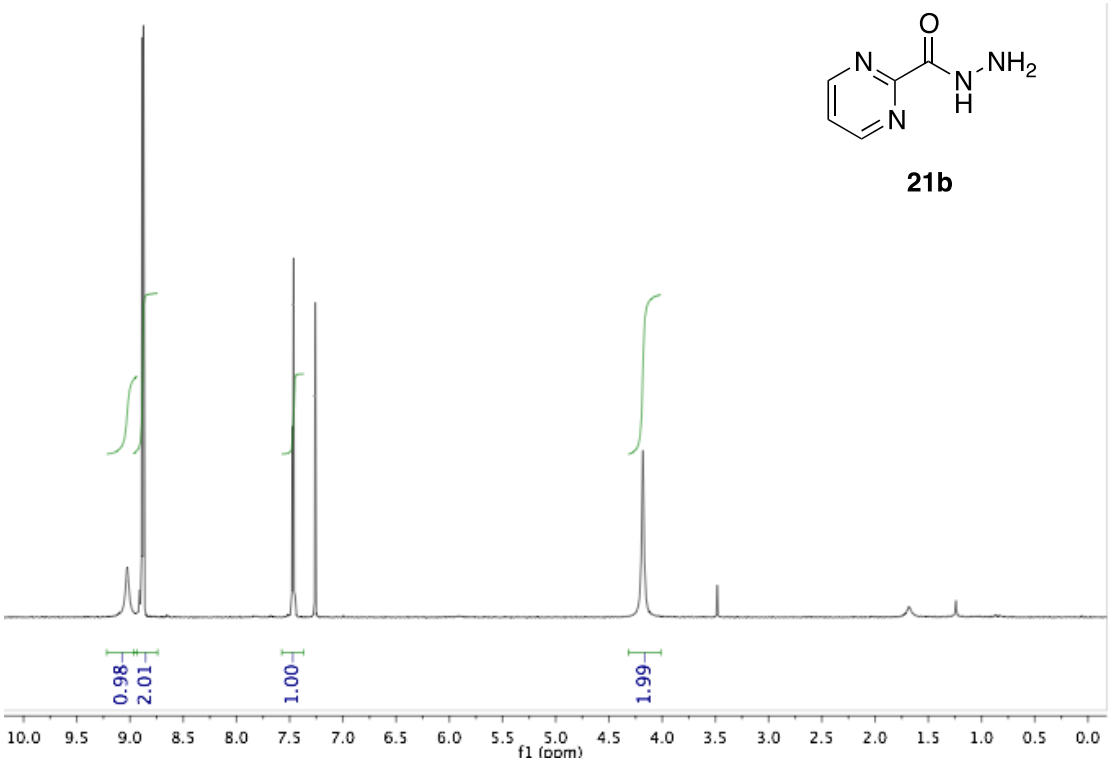
^{1}H NMR spectrum of picolinohydrazide (**21a**) (400 MHz, $CDCl_3$)



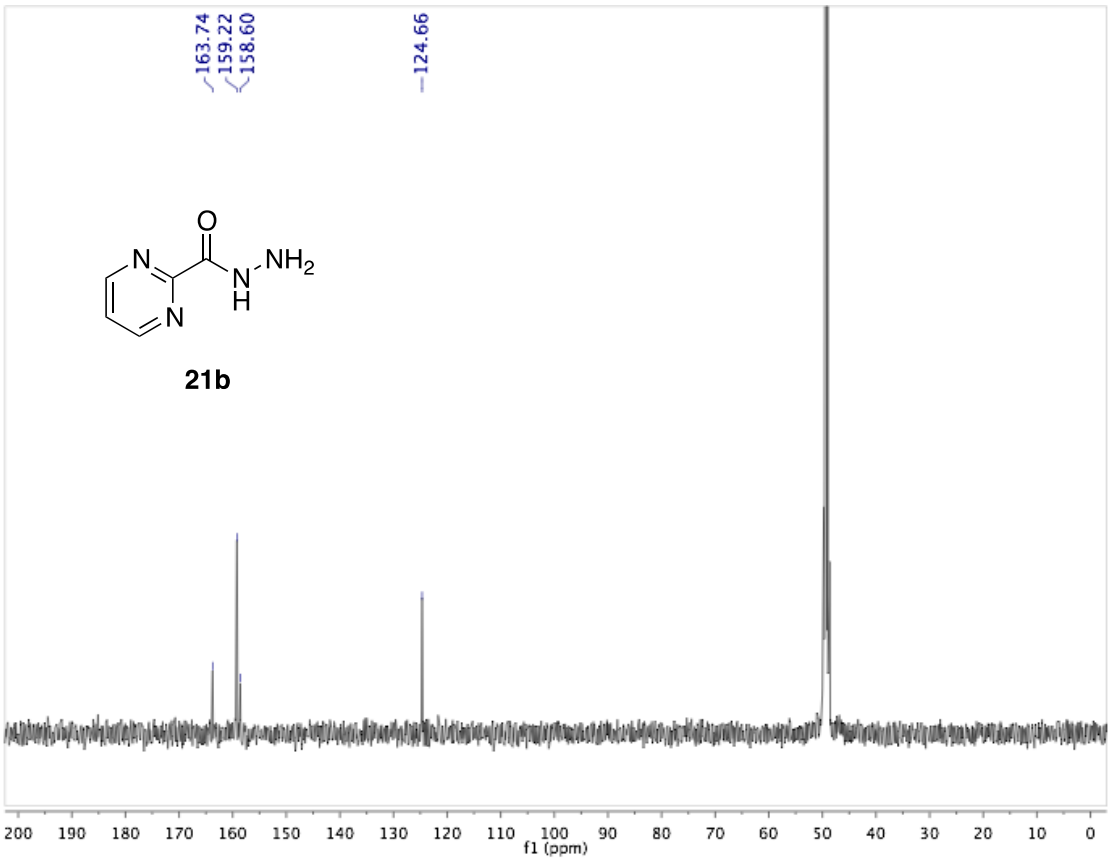
^{13}C NMR spectrum of picolinohydrazide (**21a**) (100 MHz, $CDCl_3$)



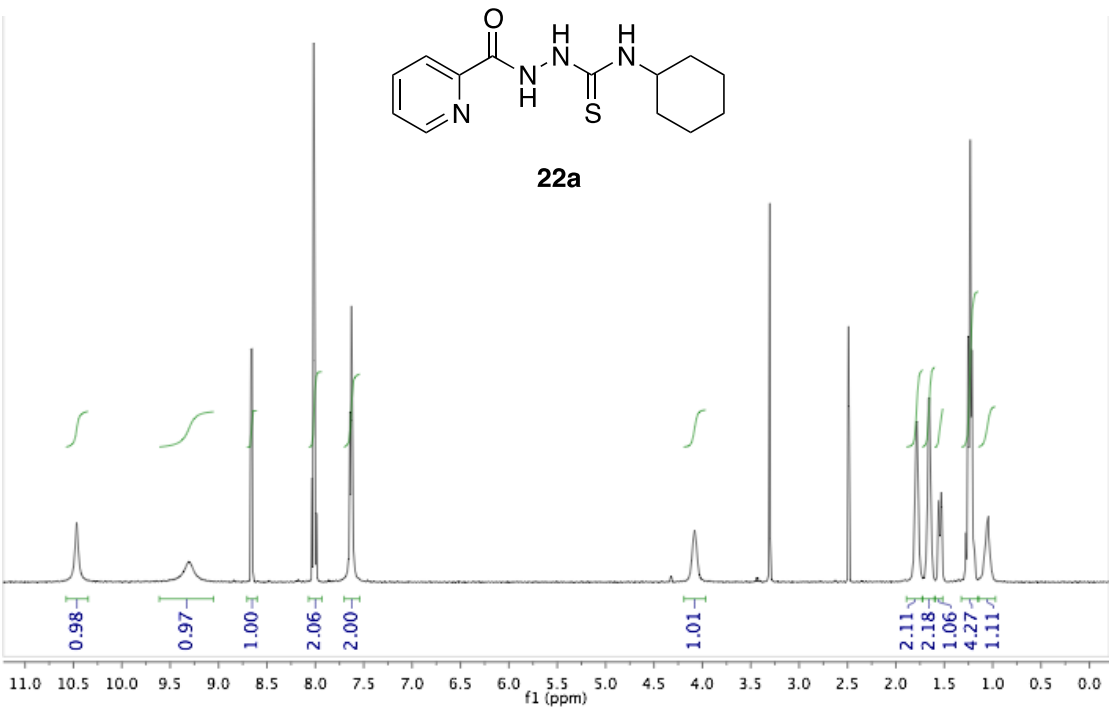
^{1}H NMR spectrum of pyrimidine-2-carbohydrazide (**21b**) (400 MHz, $CDCl_3$)



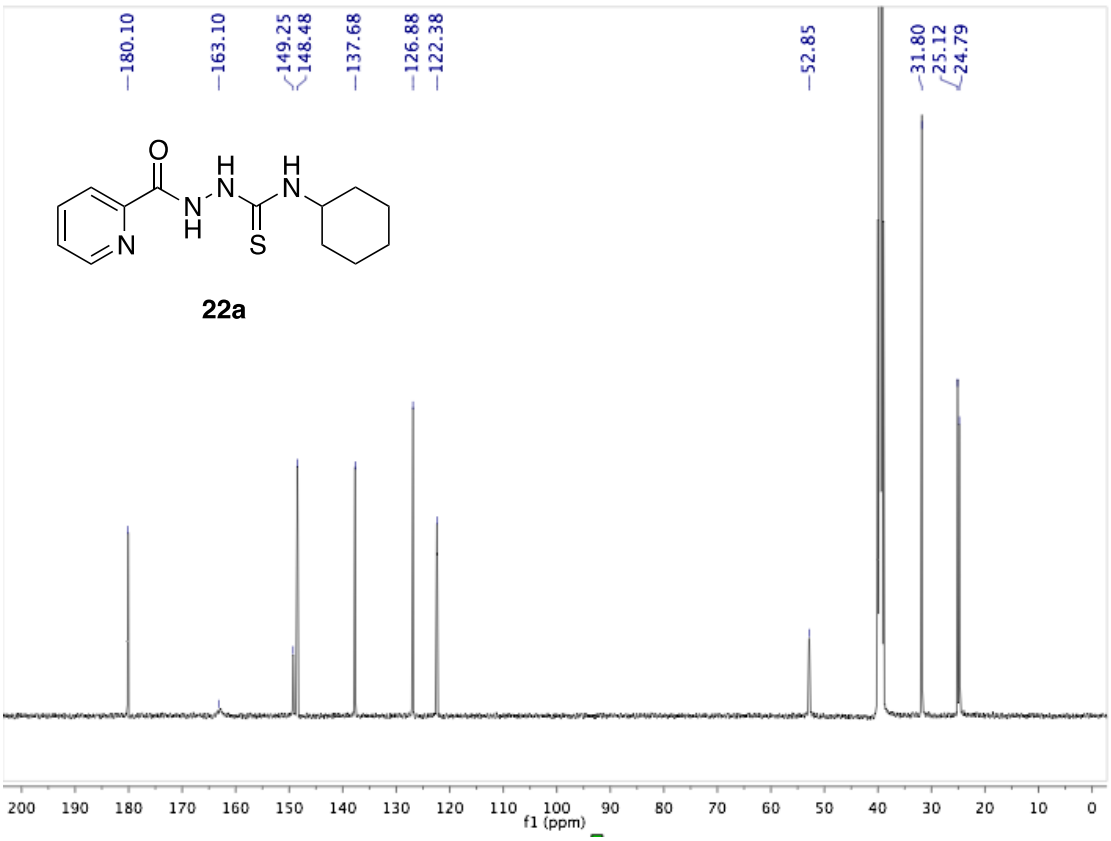
^{13}C NMR spectrum of pyrimidine-2-carbohydrazide (**21b**) (100 MHz, MeOD-d_6)



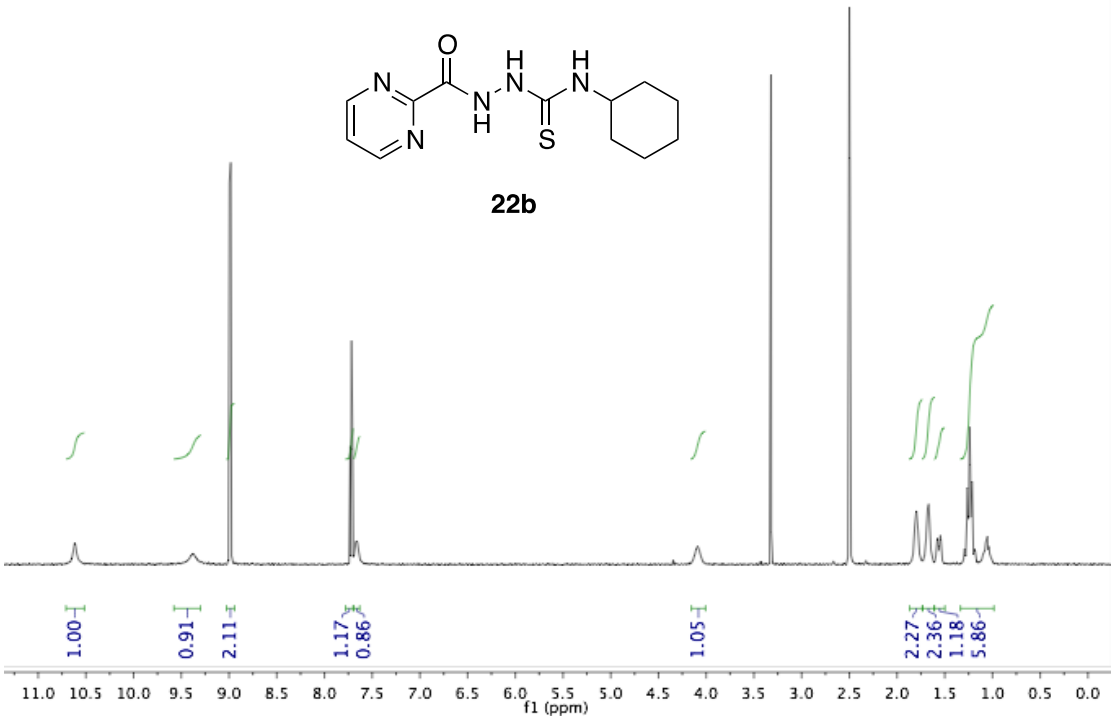
^{1}H NMR spectrum of *N*-cyclohexyl-2-picolinoylhydrazinecarbothioamide (**22a**) (400 MHz, DMSO-d_6)



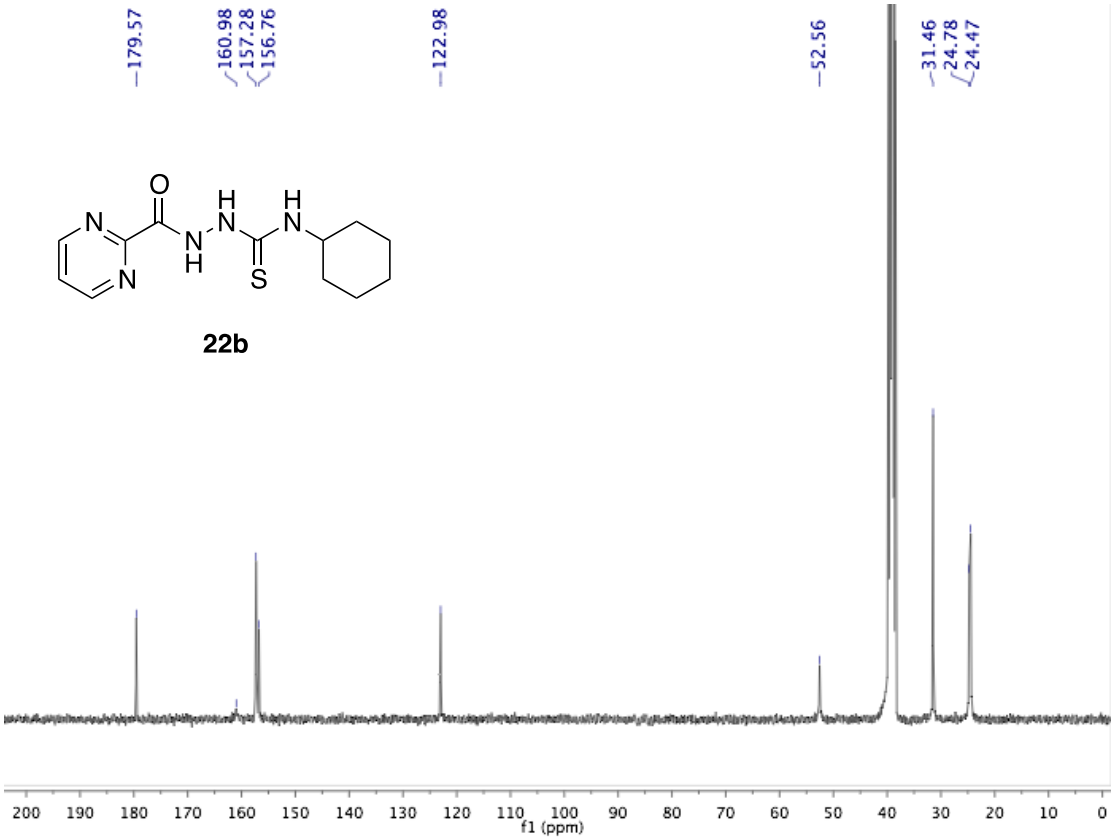
^{13}C NMR spectrum of *N*-cyclohexyl-2-picolinoylhydrazinecarbothioamide (**22a**) (125 MHz, DMSO-d_6)



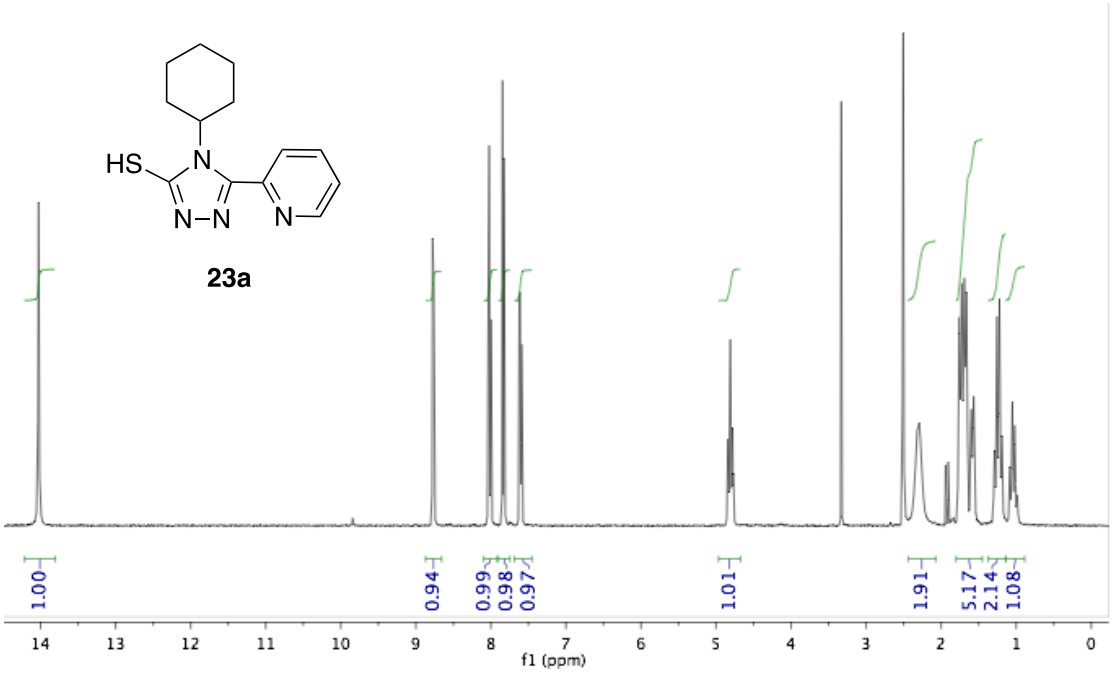
^{1}H NMR spectrum of *N*-cyclohexylaminothiocarbonyl-*N'*-2-(pyrimidine-2-carbonyl)hydrazine (**22b**) (400 MHz, DMSO-d_6)



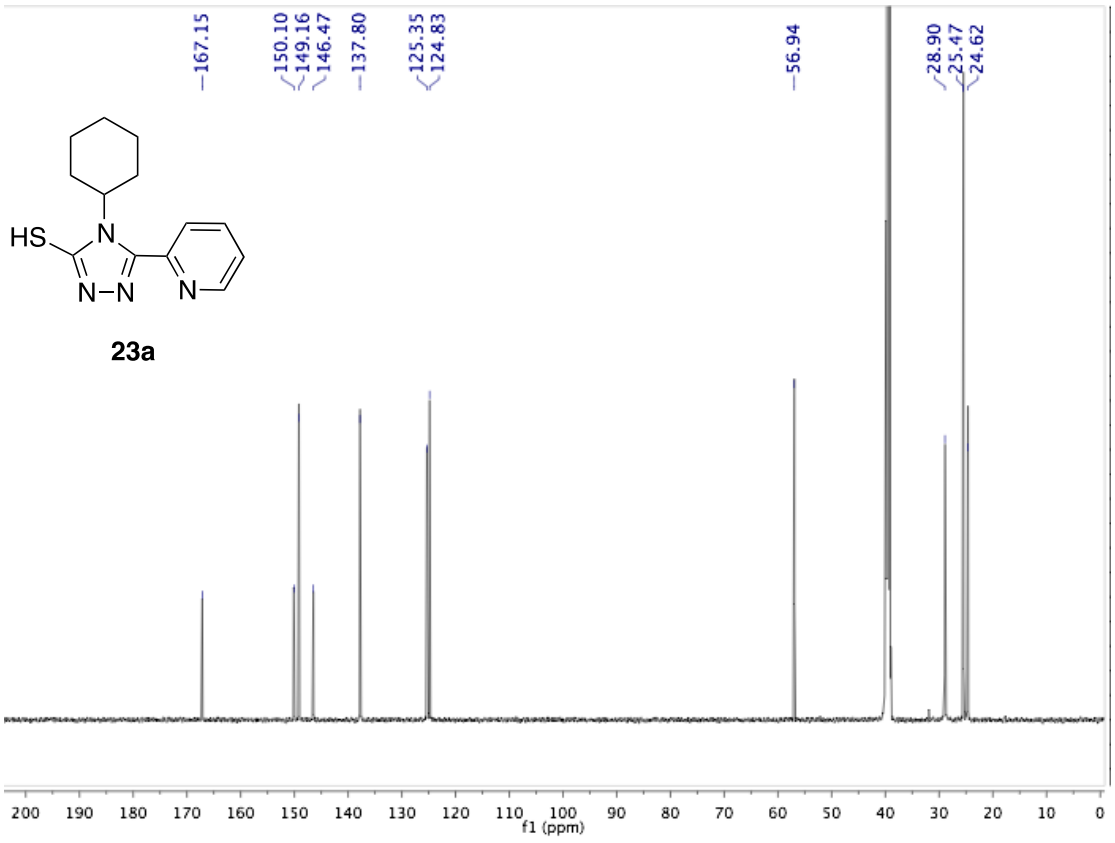
^{13}C NMR spectrum of *N*-cyclohexylaminothiocarbonyl-*N'*-2-(pyrimidine-2-carbonyl)hydrazine (**22b**) (100 MHz, DMSO-d_6)



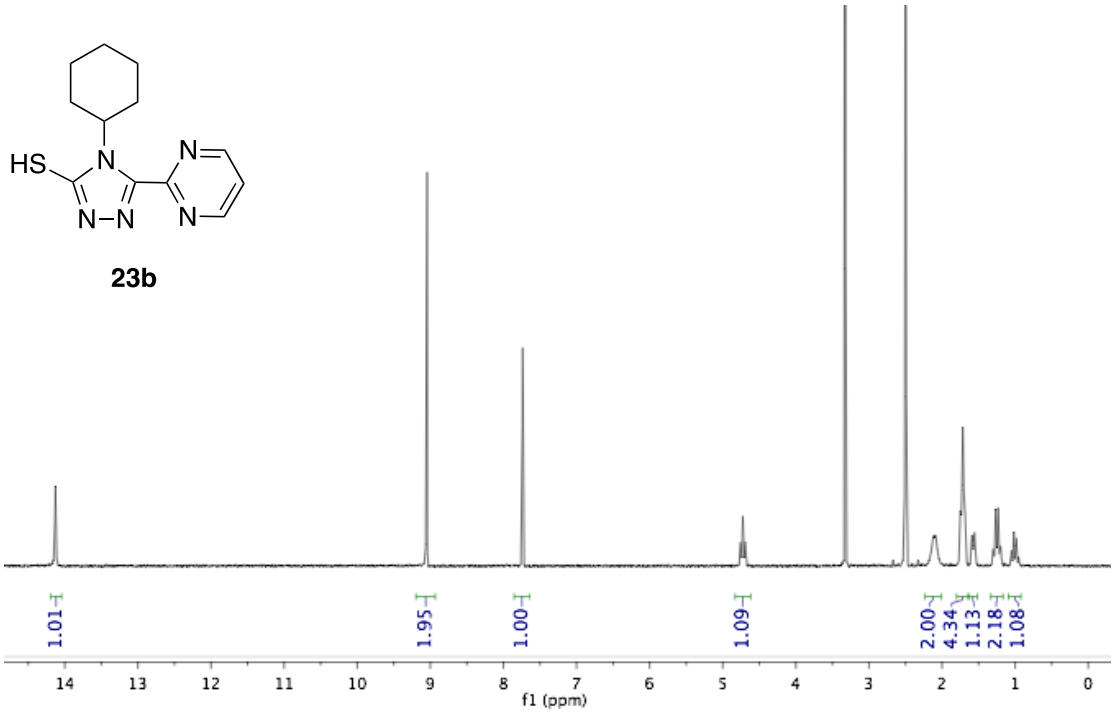
^{1}H NMR spectrum of 4-cyclohexyl-5-(pyridin-2-yl)-4*H*-1,2,4-triazole-3-thiol (**23a**) (400 MHz, DMSO-d_6)



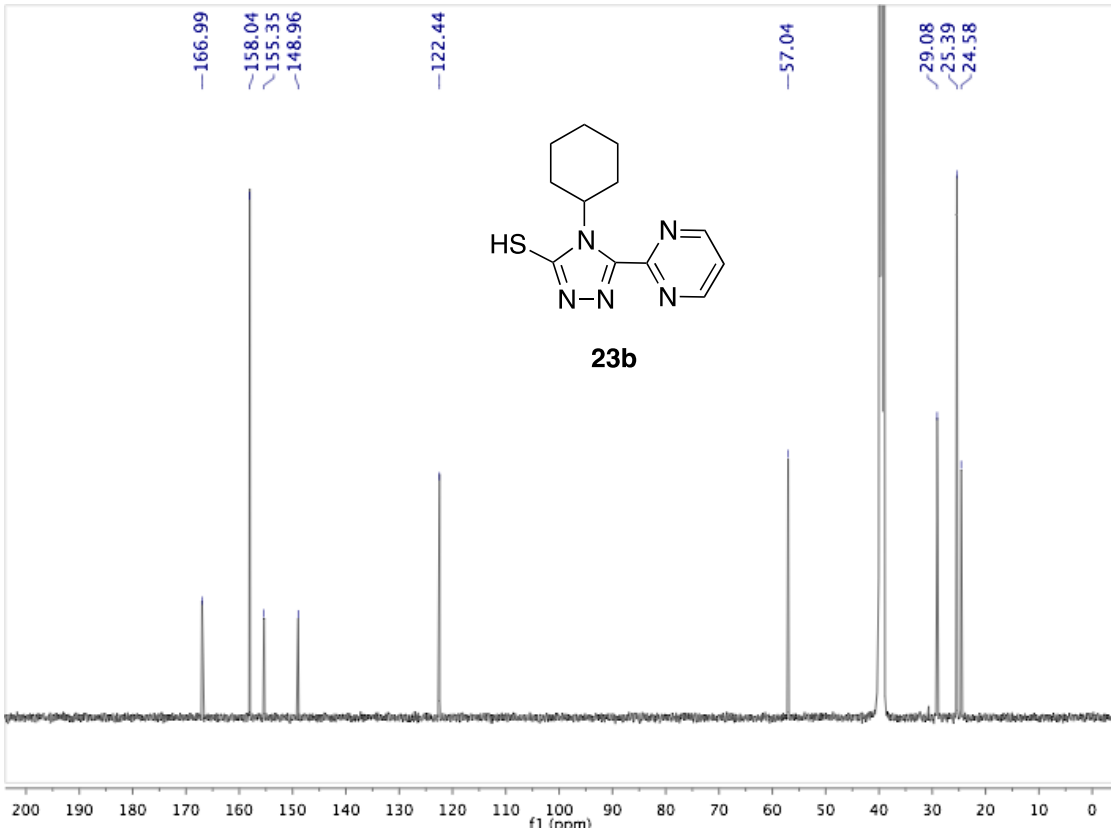
^{13}C NMR spectrum of 4-cyclohexyl-5-(pyridin-2-yl)-4*H*-1,2,4-triazole-3-thiol (**23a**) (125 MHz, DMSO-d_6)



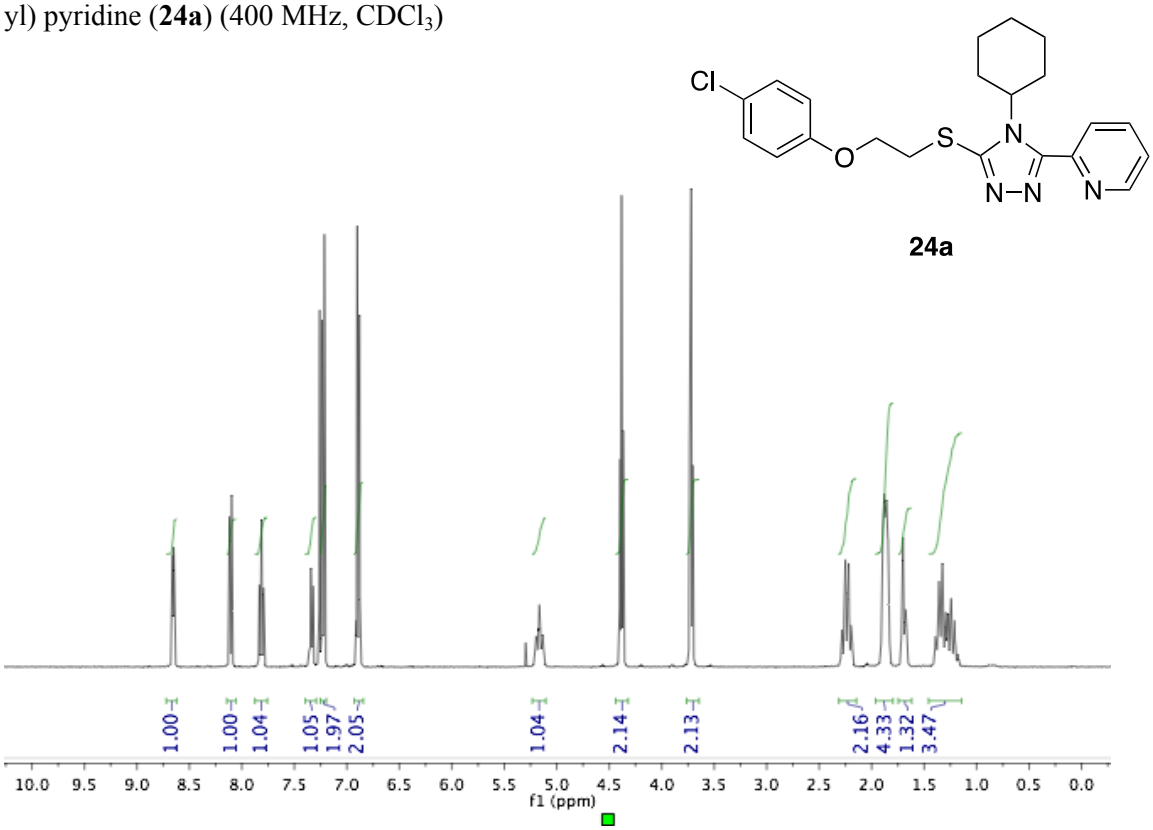
^{1}H NMR spectrum of 4-cyclohexyl-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazole-3-thiol (**23b**) (400 MHz, $CDCl_3$)



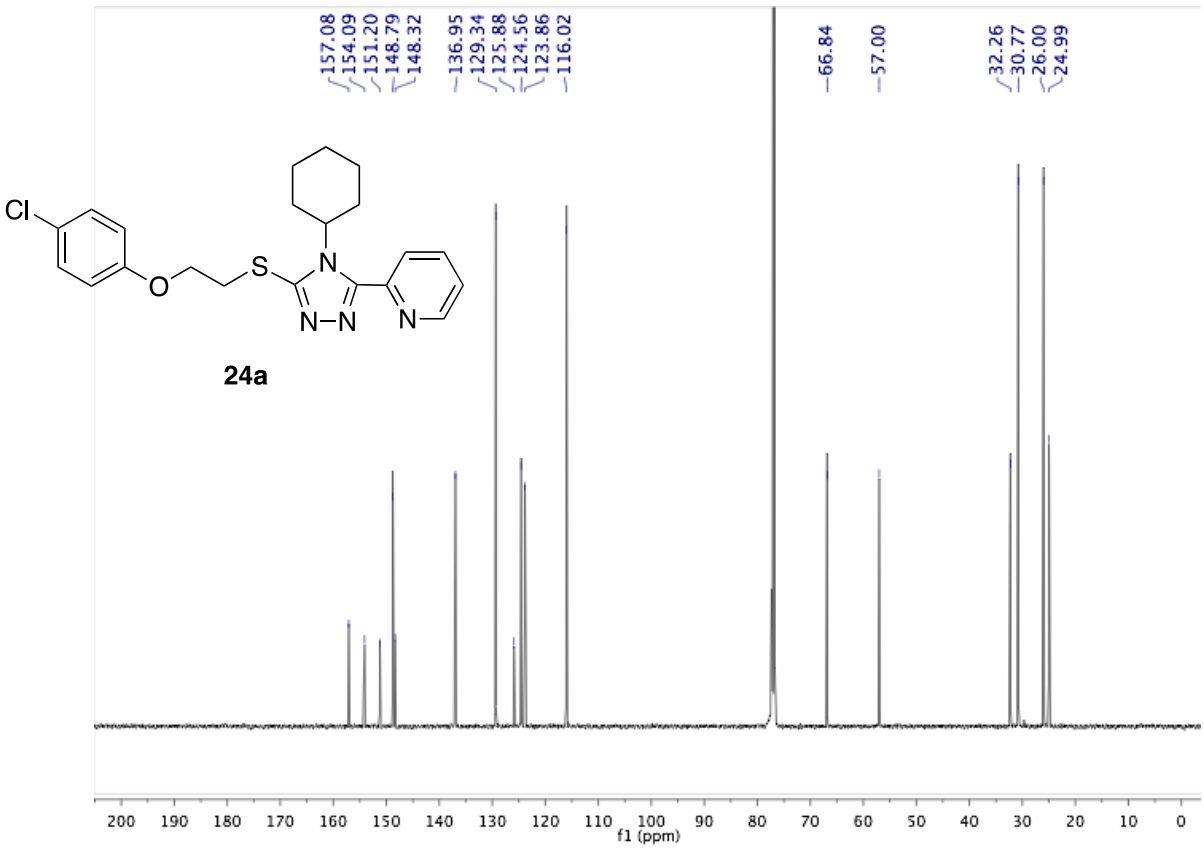
^{13}C NMR spectrum of 4-cyclohexyl-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazole-3-thiol (**23b**) (125 MHz, $CDCl_3$)



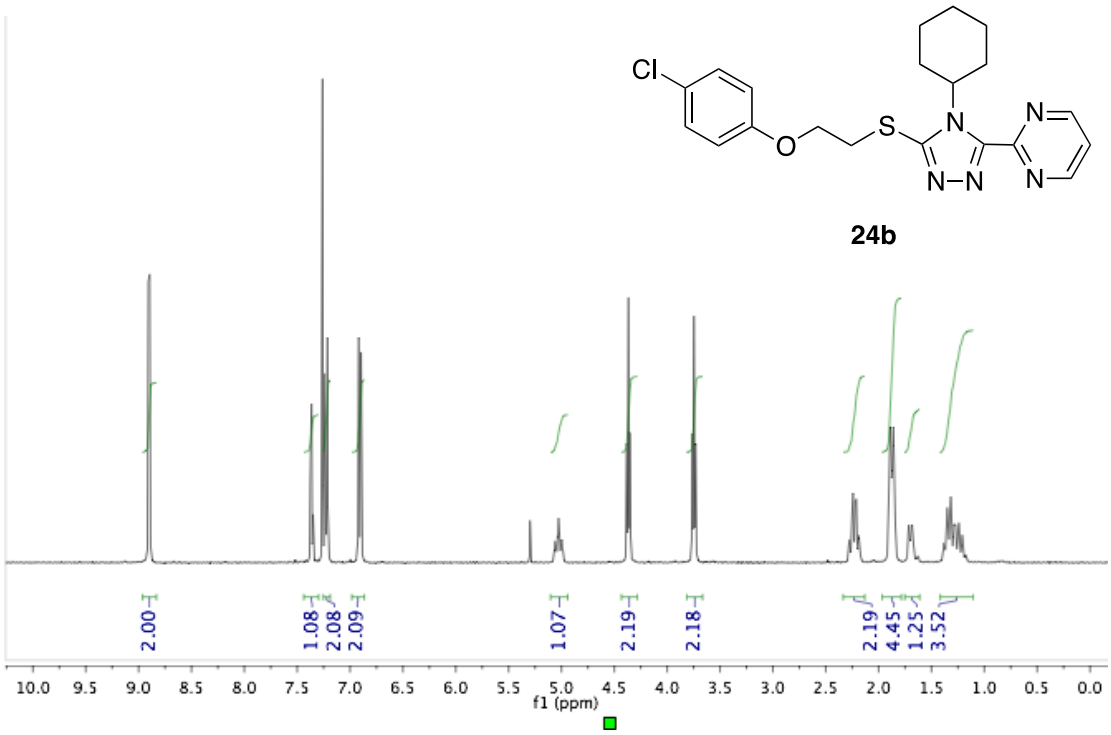
^{1}H NMR spectrum of 2-(5-(2-(4-chlorophenoxy)ethylthio)-4-cyclohexyl-4*H*-1,2,4-triazol-3-yl) pyridine (**24a**) (400 MHz, $CDCl_3$)



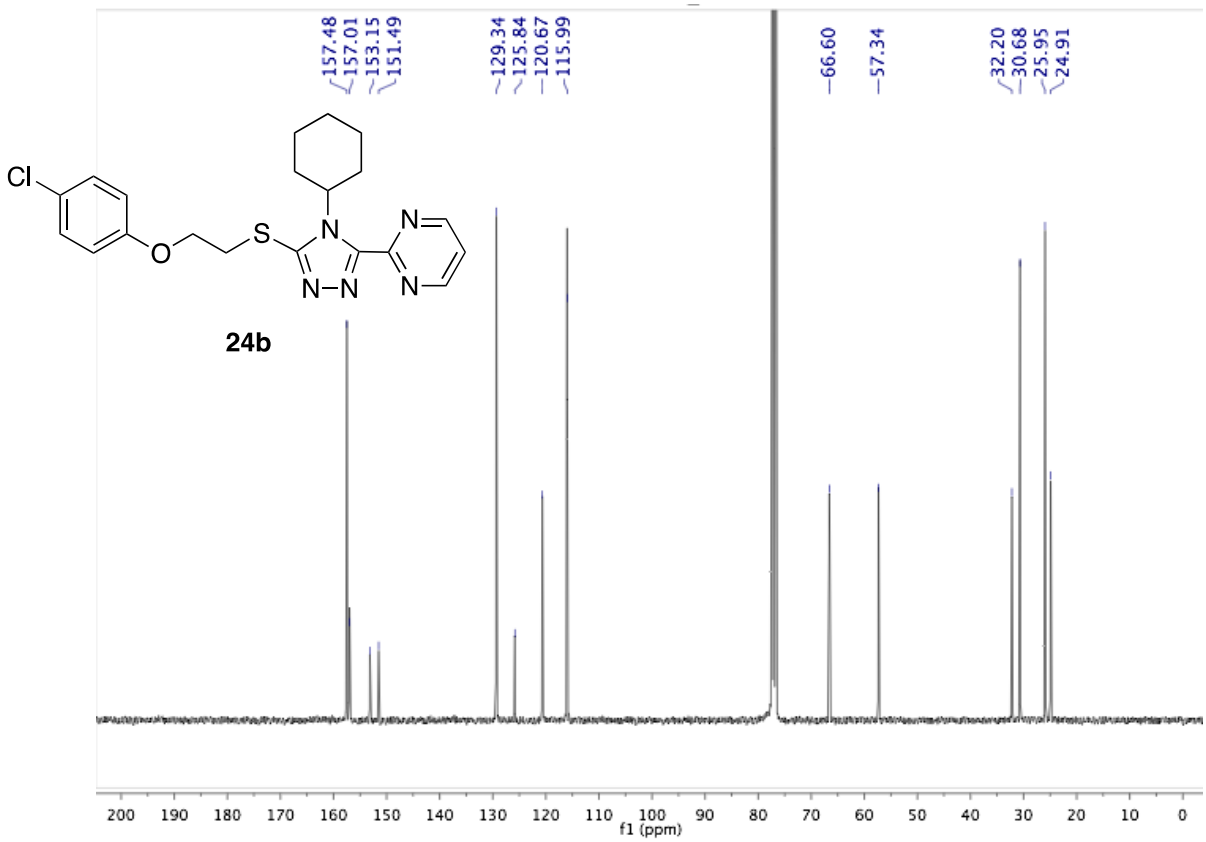
^{13}C NMR spectrum of 2-(5-(2-(4-chlorophenoxy)ethylthio)-4-cyclohexyl-4*H*-1,2,4-triazol-3-yl) pyridine (**24a**) (125 MHz, $CDCl_3$)



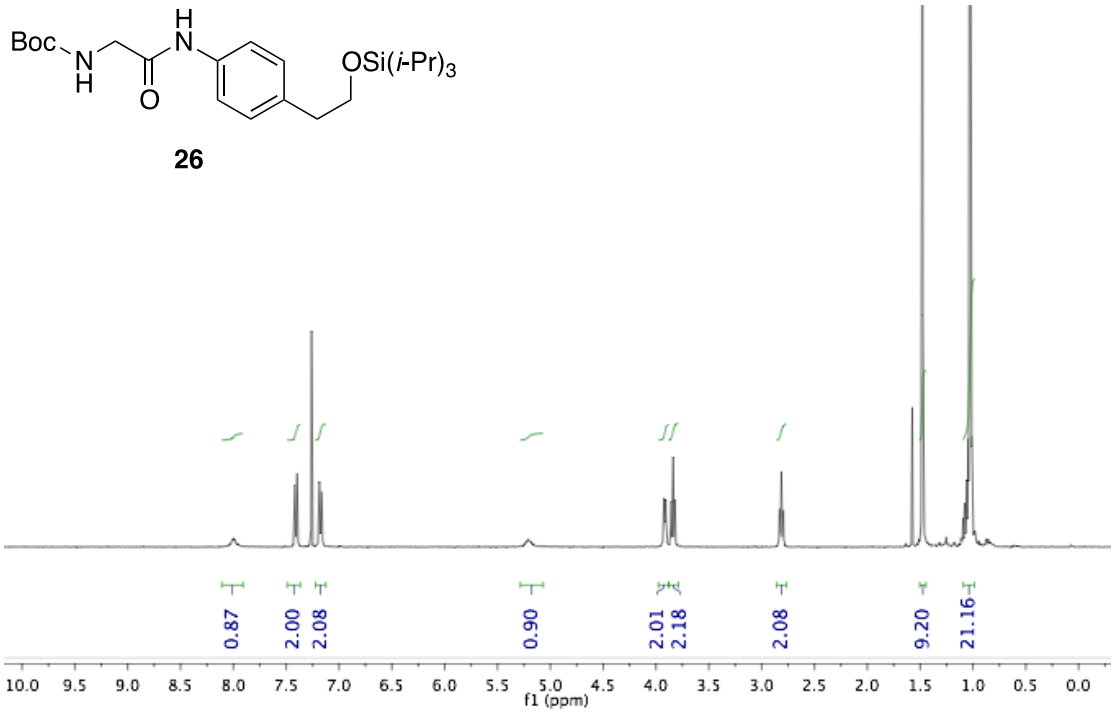
^{1}H NMR spectrum of 2-(5-(2-(4-chlorophenoxy)ethylthio)-4-cyclohexyl-4*H*-1,2,4-triazol-3-yl) pyrimidine (**24b**) (400 MHz, $CDCl_3$)



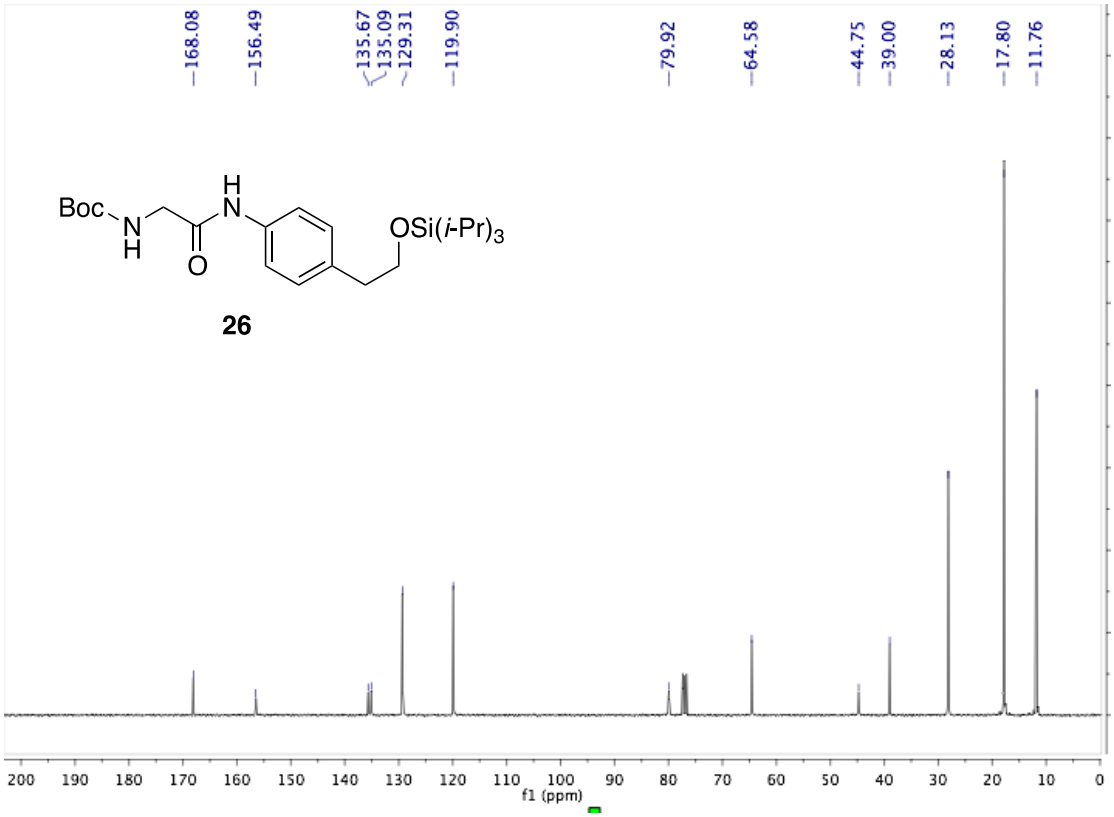
^{13}C NMR spectrum of 2-(5-(2-(4-chlorophenoxy)ethylthio)-4-cyclohexyl-4*H*-1,2,4-triazol-3-yl) pyrimidine (**24b**) (125 MHz, $CDCl_3$)



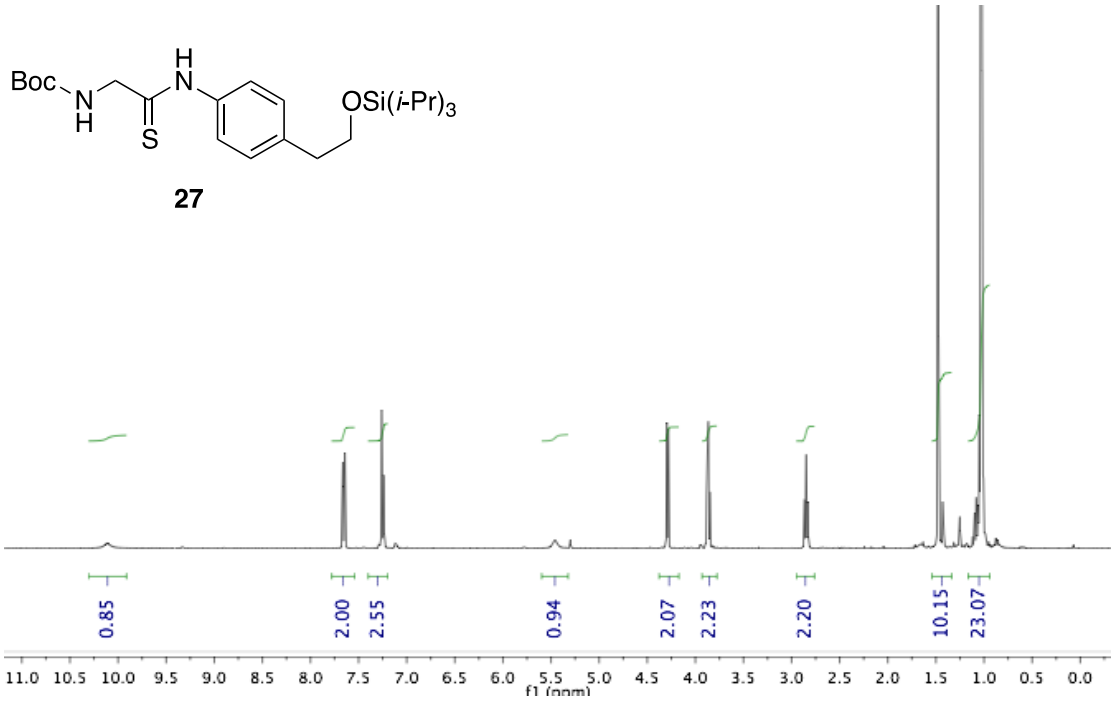
^{1}H NMR spectrum of *tert*-butyl 2-oxo-2-(4-(2-(tri-isopropylsilyloxy)ethyl)phenylamino)-ethylcarbamate (**26**) (400 MHz, $CDCl_3$)



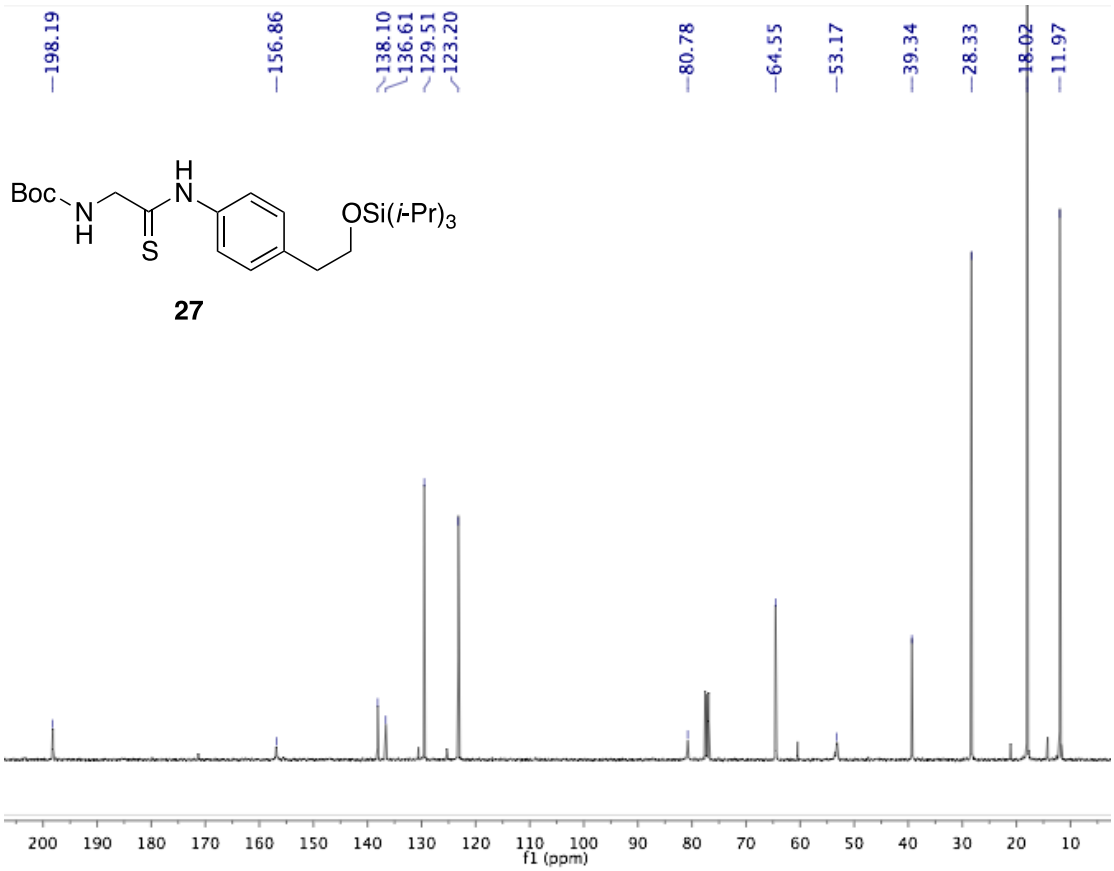
^{13}C NMR spectrum of *tert*-butyl 2-oxo-2-(4-(2-(tri-isopropylsilyloxy)ethyl)phenylamino)-ethylcarbamate (**26**) (100 MHz, $CDCl_3$)



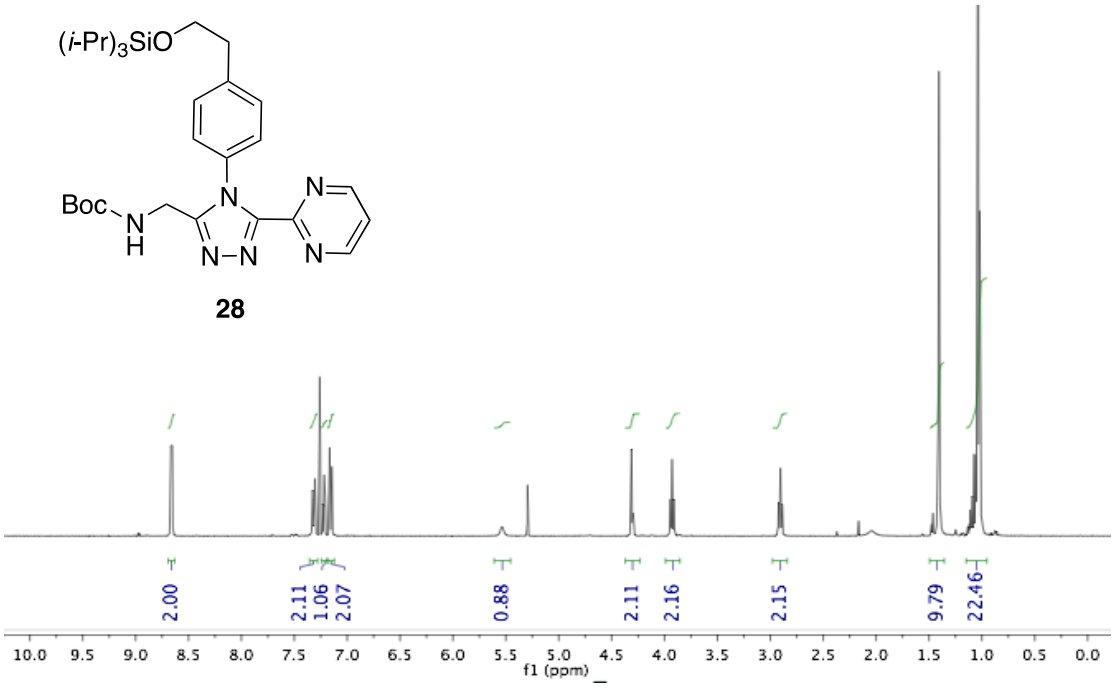
^{1}H NMR spectrum of *tert*-butyl 2-thioxo-2-(4-(2-(triisopropylsilyloxy)ethyl)phenylamino)-ethylcarbamate (**27**) (400 MHz, $CDCl_3$)



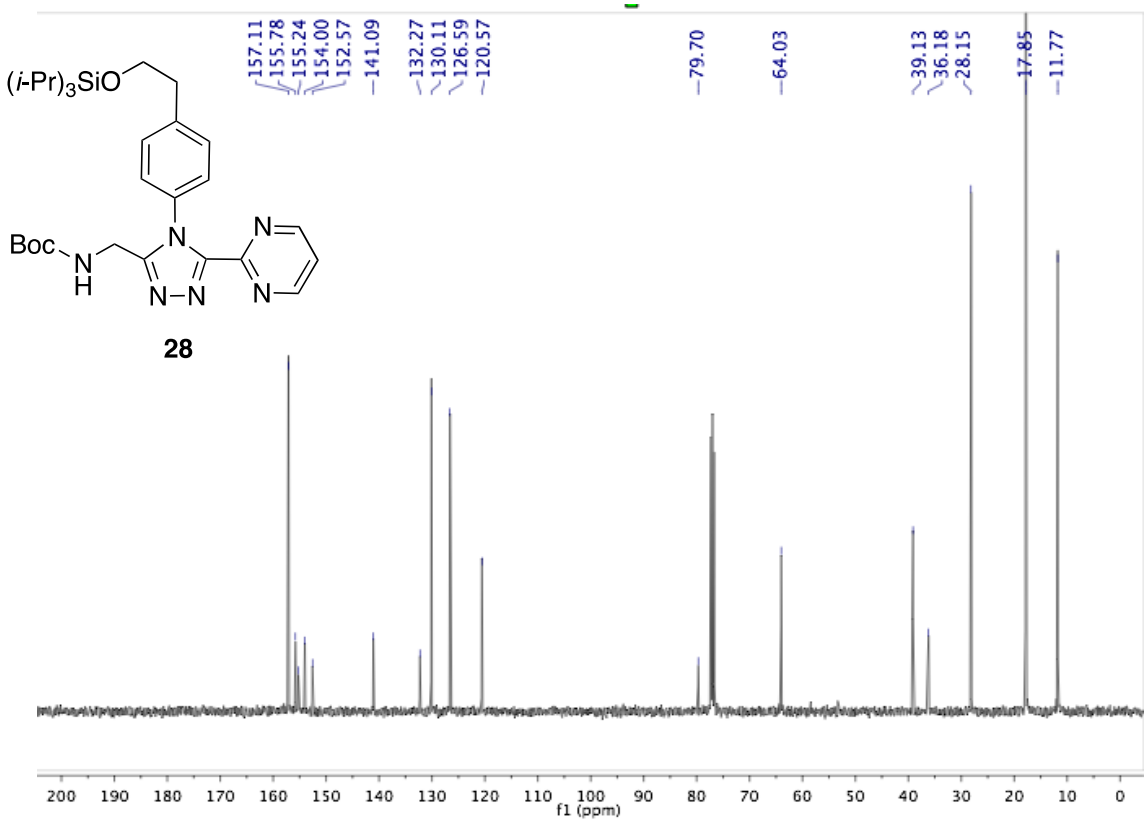
^{13}C NMR spectrum of *tert*-butyl 2-thioxo-2-(4-(2-(triisopropylsilyloxy)ethyl)phenylamino)-ethylcarbamate (**27**) (100 MHz, $CDCl_3$)



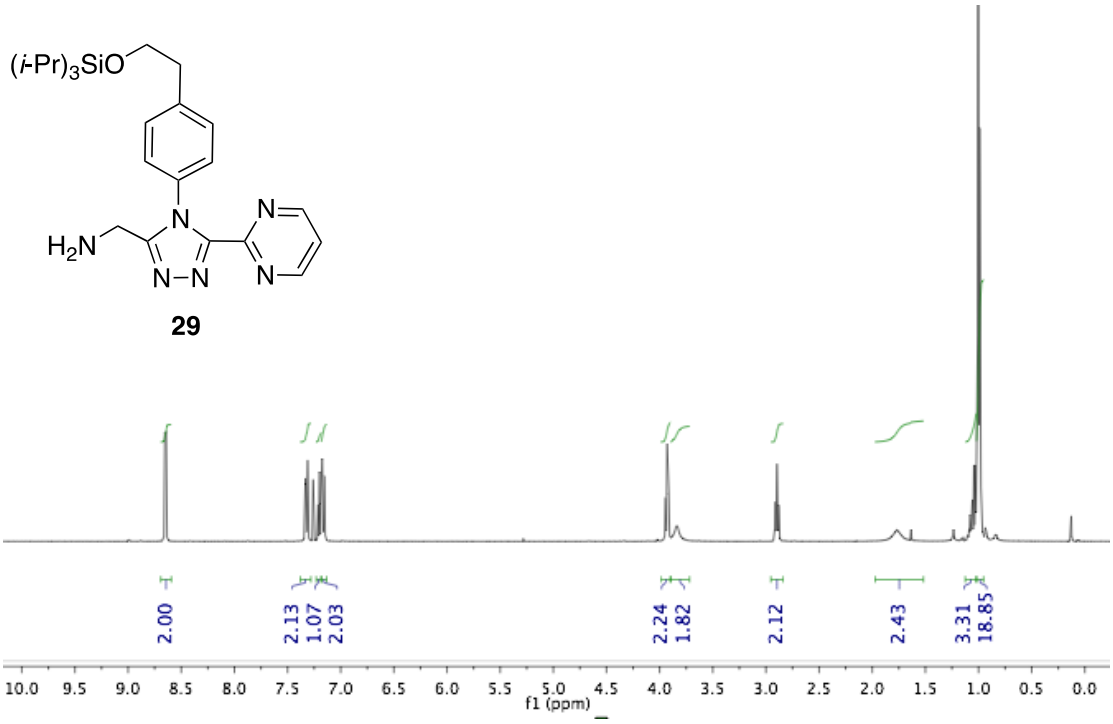
^{1}H NMR spectrum of *tert*-butyl (5-(pyrimidin-2-yl)-4-(4-(2-(triisopropylsilyloxy)ethyl)-phenyl)-4*H*-1,2,4-triazol-3-yl)methylcarbamate (**28**) (400 MHz, $CDCl_3$)



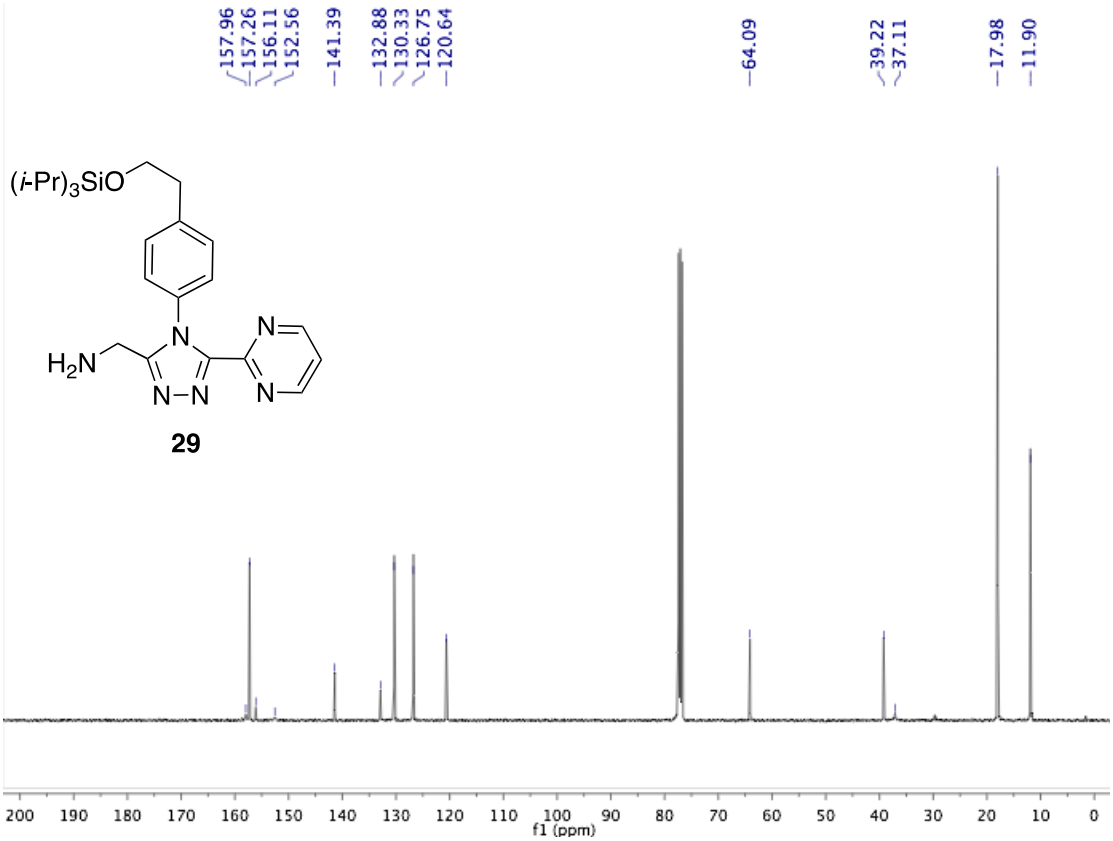
^{13}C NMR spectrum of *tert*-butyl (5-(pyrimidin-2-yl)-4-(4-(2-(triisopropylsilyloxy)ethyl)-phenyl)-4*H*-1,2,4-triazol-3-yl)methylcarbamate (**28**) (100 MHz, $CDCl_3$)



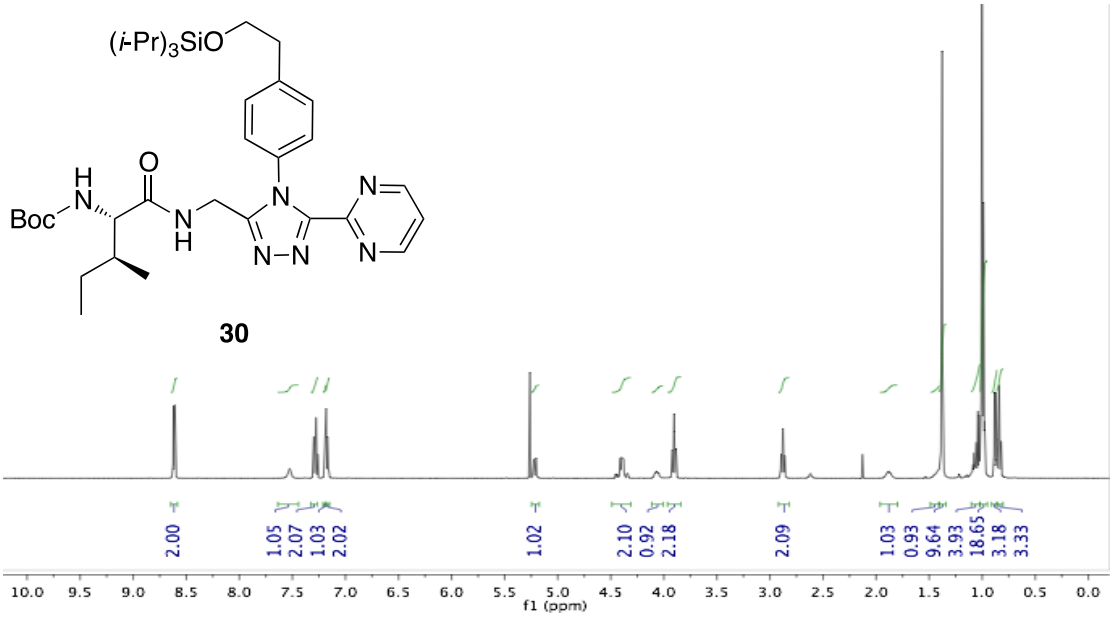
^{1}H NMR spectrum of (5-(pyrimidin-2-yl)-4-(4-(2-(tri-isopropylsilyloxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methanamine (**29**) (400 MHz, $CDCl_3$)



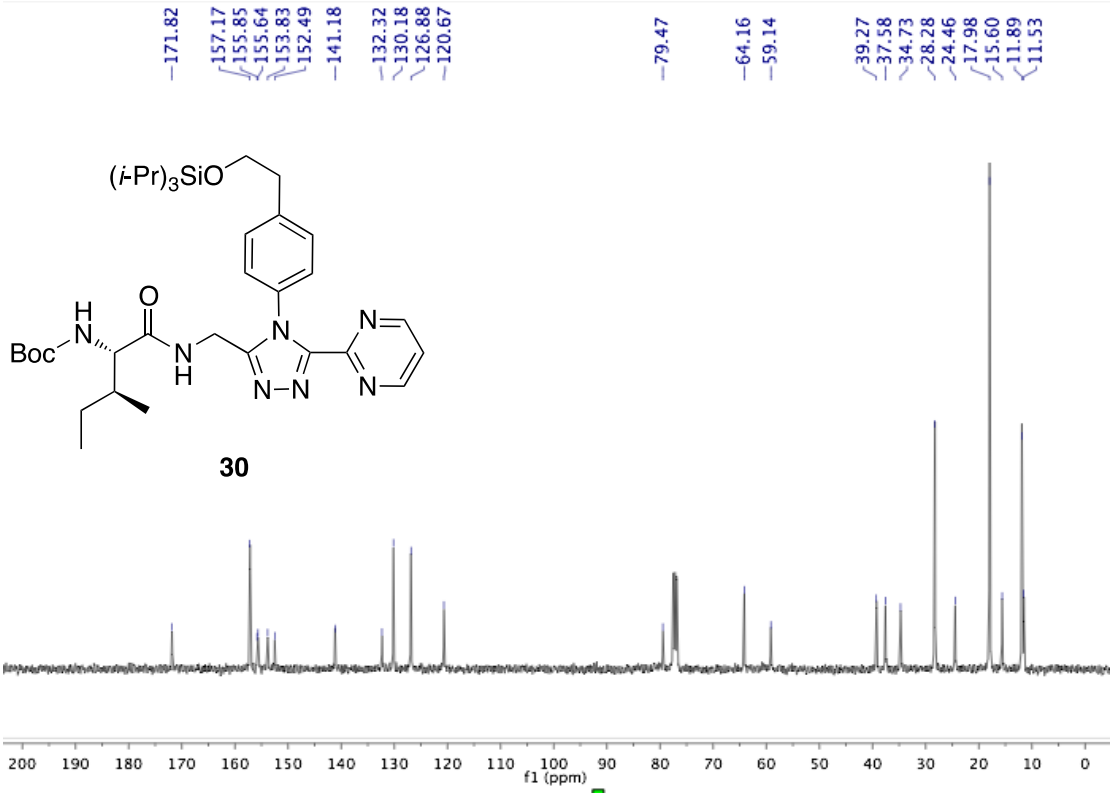
^{13}C NMR spectrum of (5-(pyrimidin-2-yl)-4-(4-(2-(tri-isopropylsilyloxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methanamine (**29**) (100 MHz, $CDCl_3$)



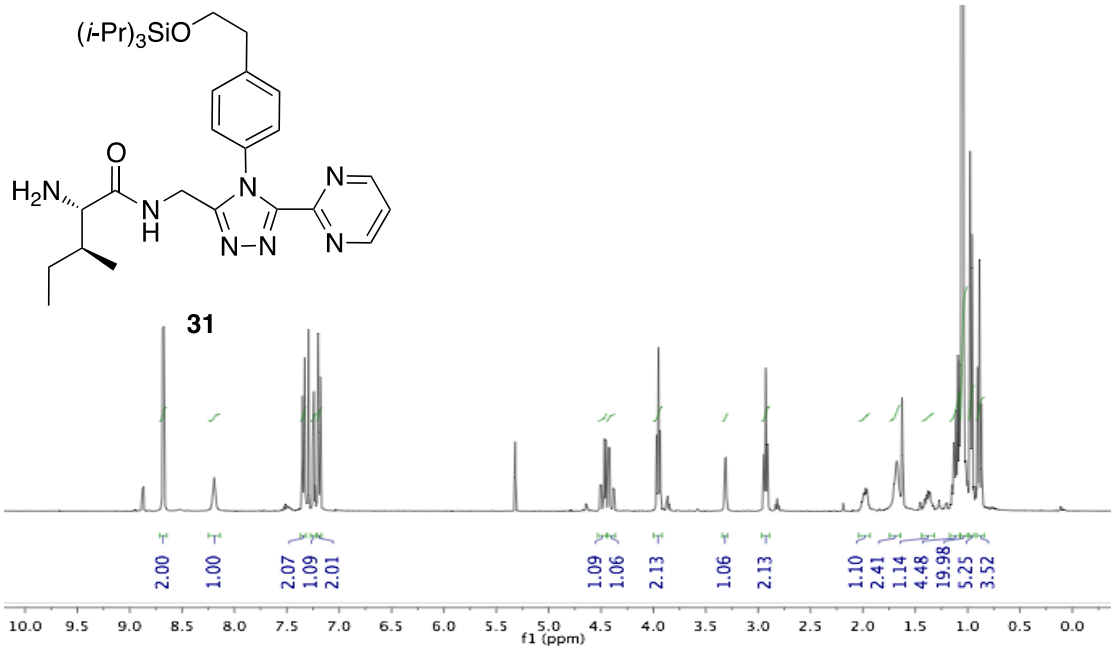
^{1}H NMR spectrum of *tert*-butyl (2*S*,3*S*)-3-methyl-1-oxo-1-((5-(pyrimidin-2-yl)-4-(4-(2-(tri-isopropyl-silyloxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methylamino)pentan-2-ylcarbamate (**30**) (400 MHz, $CDCl_3$)



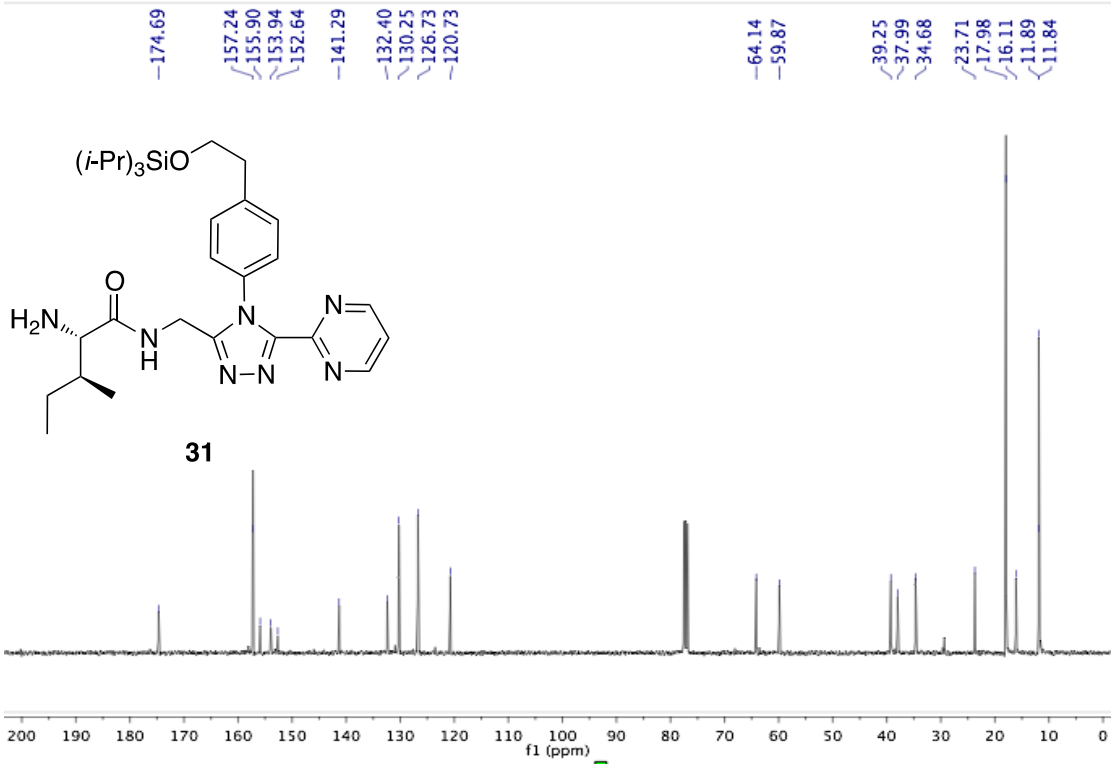
^{13}C NMR spectrum of *tert*-butyl (2*S*,3*S*)-3-methyl-1-oxo-1-((5-(pyrimidin-2-yl)-4-(4-(2-(tri-isopropyl-silyloxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methylamino)pentan-2-ylcarbamate (**30**) (100 MHz, $CDCl_3$)



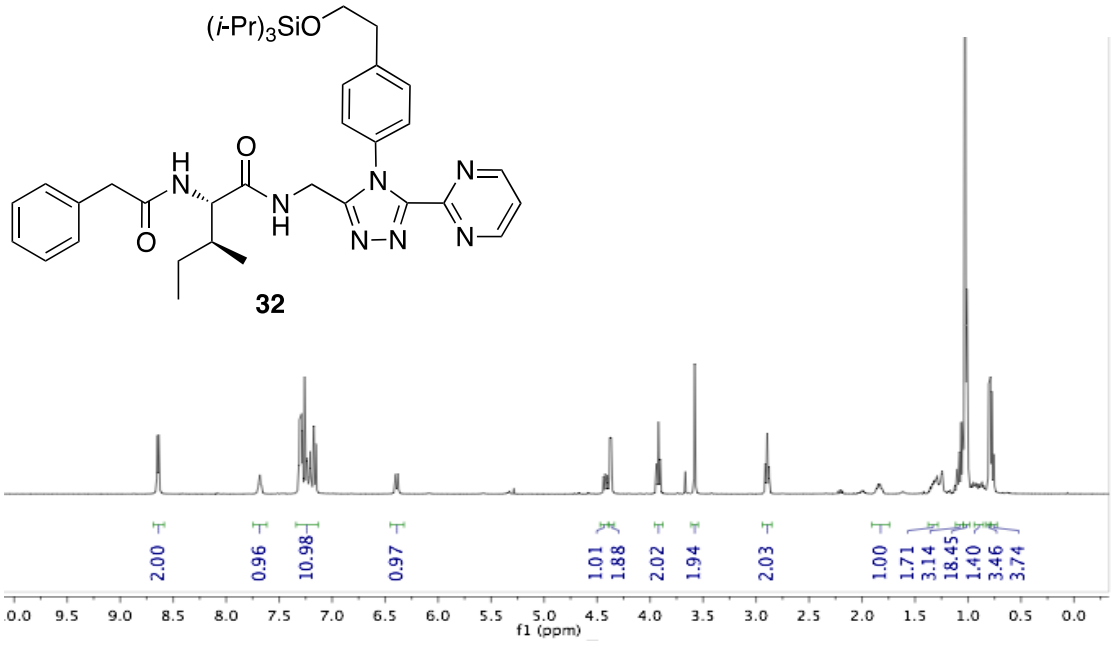
^{1}H NMR spectrum of (2*S*,3*S*)-2-amino-3-methyl-*N*-((5-(pyrimidin-2-yl)-4-(4-(2-(tri-isopropylsilyl-oxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methyl)pentanamide (**31**) (400 MHz, $CDCl_3$)



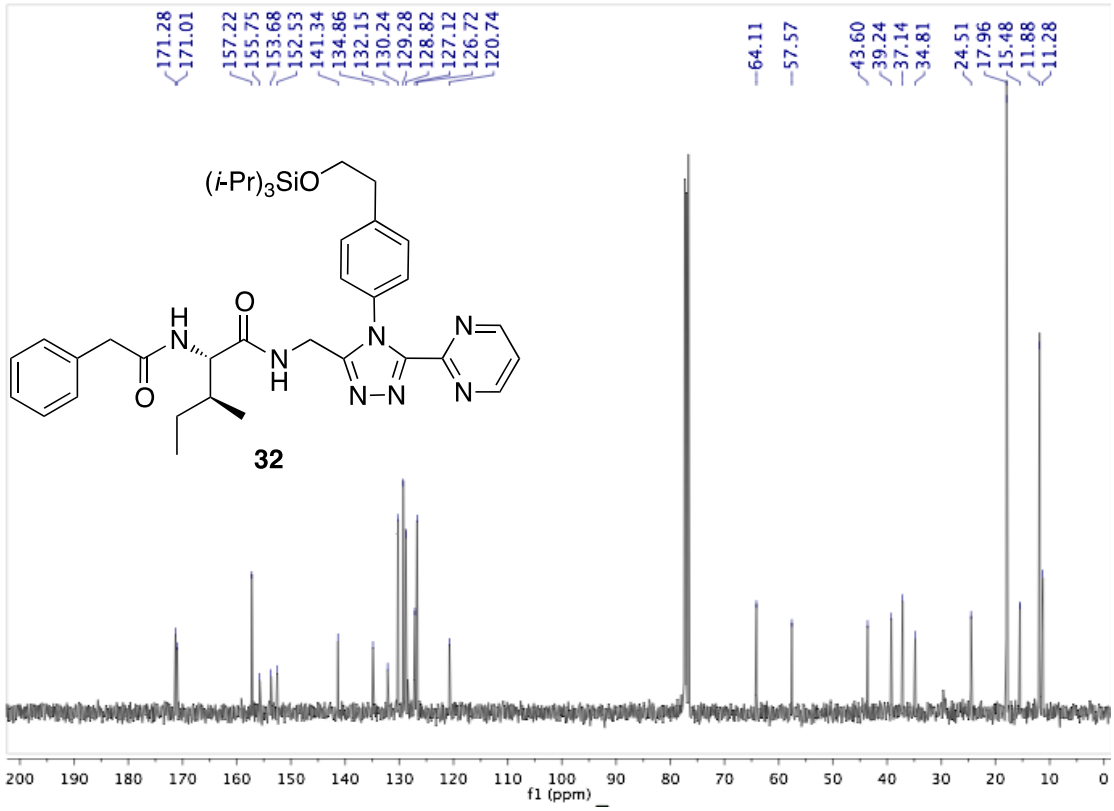
^{13}C NMR spectrum of (2*S*,3*S*)-2-amino-3-methyl-*N*-((5-(pyrimidin-2-yl)-4-(4-(2-(tri-isopropylsilyl-oxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methyl)pentanamide (**31**) (100 MHz, $CDCl_3$)



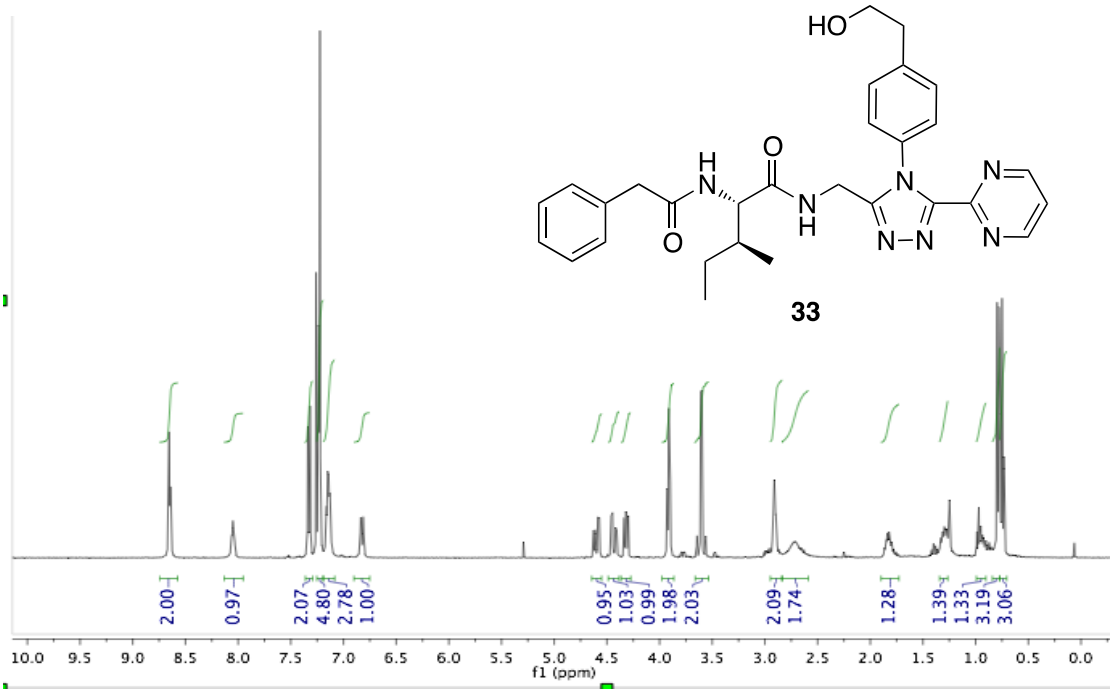
^{1}H NMR spectrum of (2*S*,3*S*)-3-methyl-2-(2-phenylacetamido)-*N*-((5-(pyrimidin-2-yl)-4-(4-(2-(tri-iso-propylsilyloxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methyl)pentanamide (**32**) (400 MHz, $CDCl_3$)



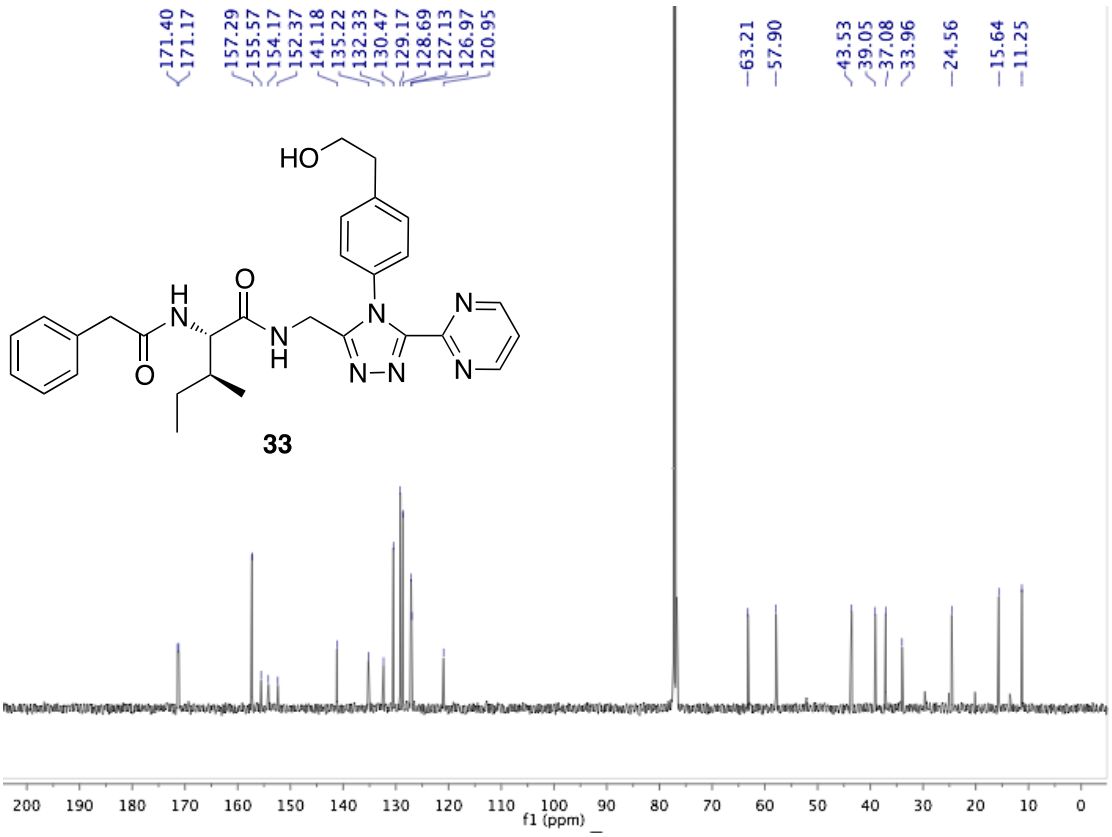
^{13}C NMR spectrum of (2*S*,3*S*)-3-methyl-2-(2-phenylacetamido)-*N*-((5-(pyrimidin-2-yl)-4-(4-(2-(tri-iso-propylsilyloxy)ethyl)phenyl)-4*H*-1,2,4-triazol-3-yl)methyl)pentanamide (**32**) (100 MHz, $CDCl_3$)



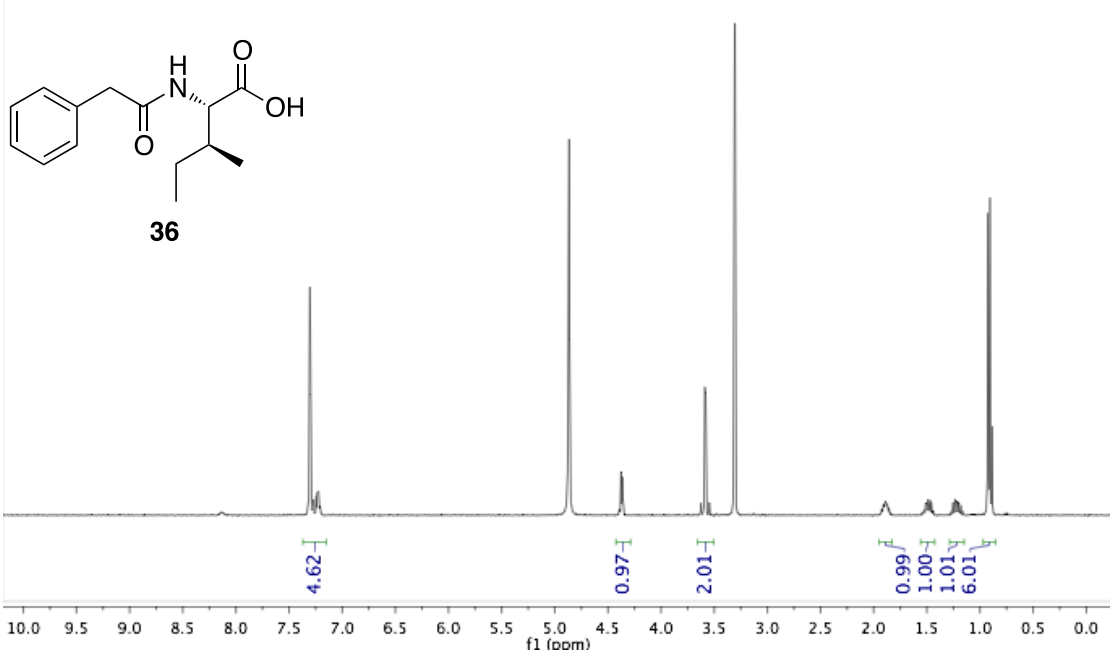
^{1}H NMR spectrum of (2*S*,3*S*)-*N*-((4-(4-(2-hydroxyethyl)phenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl)-3-methyl-2-(2-phenylacetamido)pentanamide (**33**) (400 MHz, $CDCl_3$)



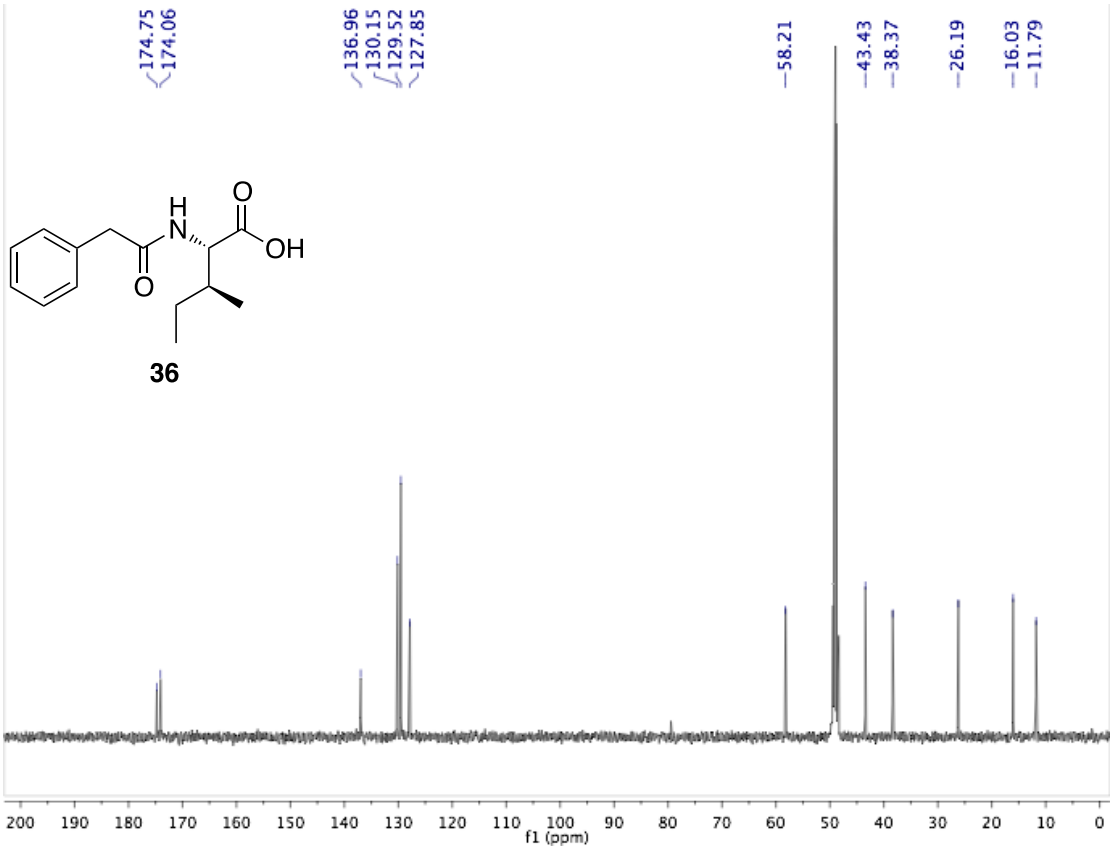
^{13}C NMR spectrum of (2*S*,3*S*)-*N*-((4-(4-(2-hydroxyethyl)phenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl)-3-methyl-2-(2-phenylacetamido)pentanamide (**33**) (125 MHz, $CDCl_3$)



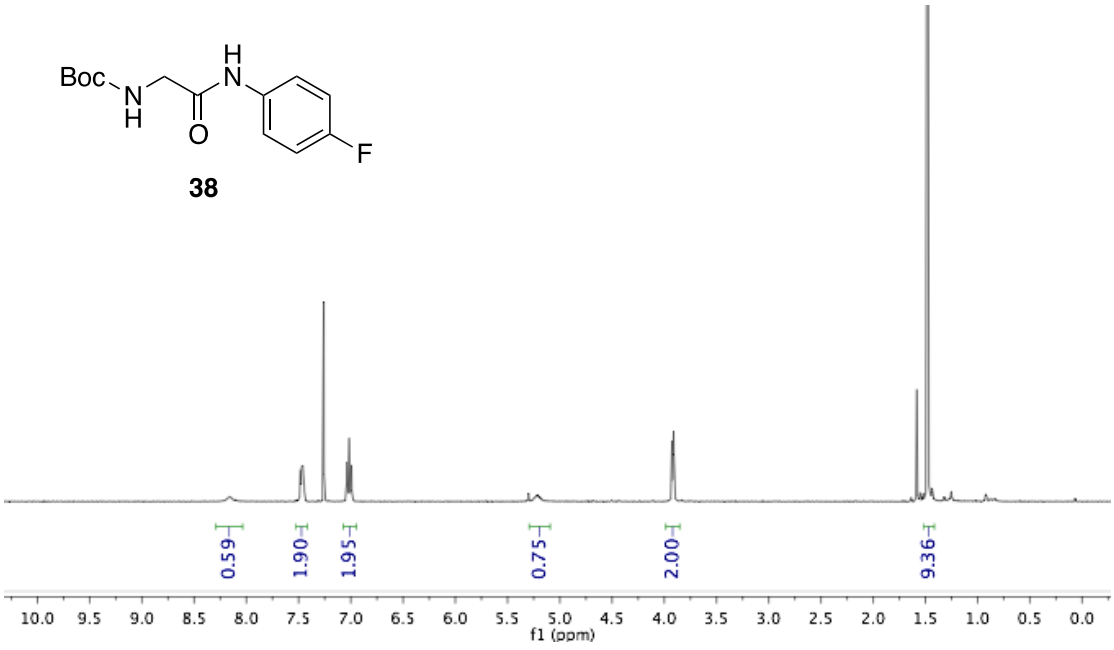
^{1}H NMR spectrum of (2*S*,3*S*)-3-methyl-2-(2-phenylacetamido)pentanoic acid (**36**) (400 MHz, MeOD-d_6)



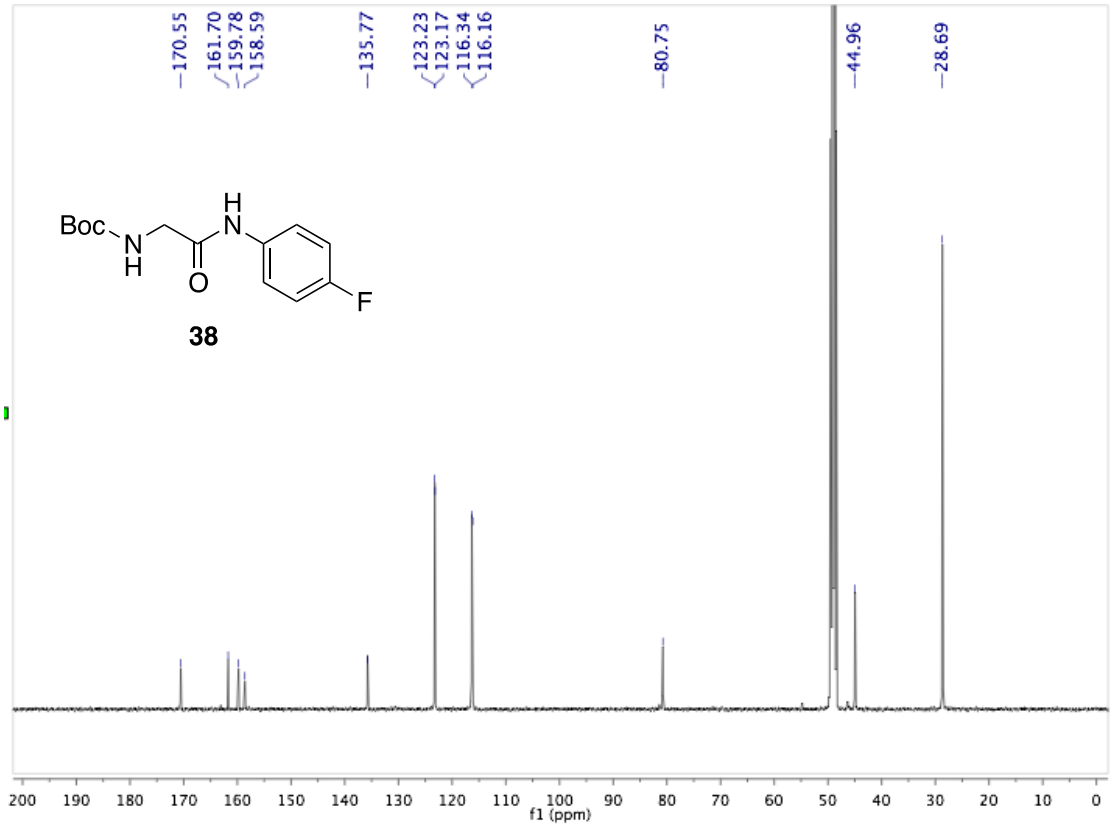
^{13}C NMR spectrum of (2*S*,3*S*)-3-methyl-2-(2-phenylacetamido)pentanoic acid (**36**) (100 MHz, MeOD-d_6)



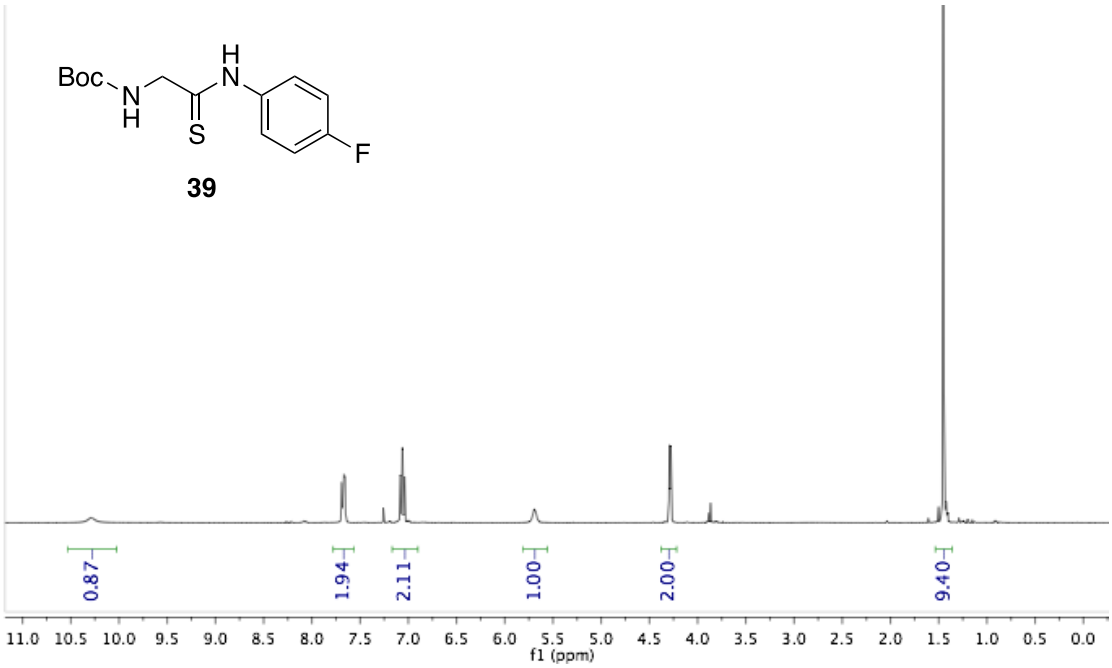
^{1}H NMR spectrum of *tert*-butyl 2-(4-fluorophenylamino)-2-oxoethylcarbamate (**38**) (400 MHz, $CDCl_3$)



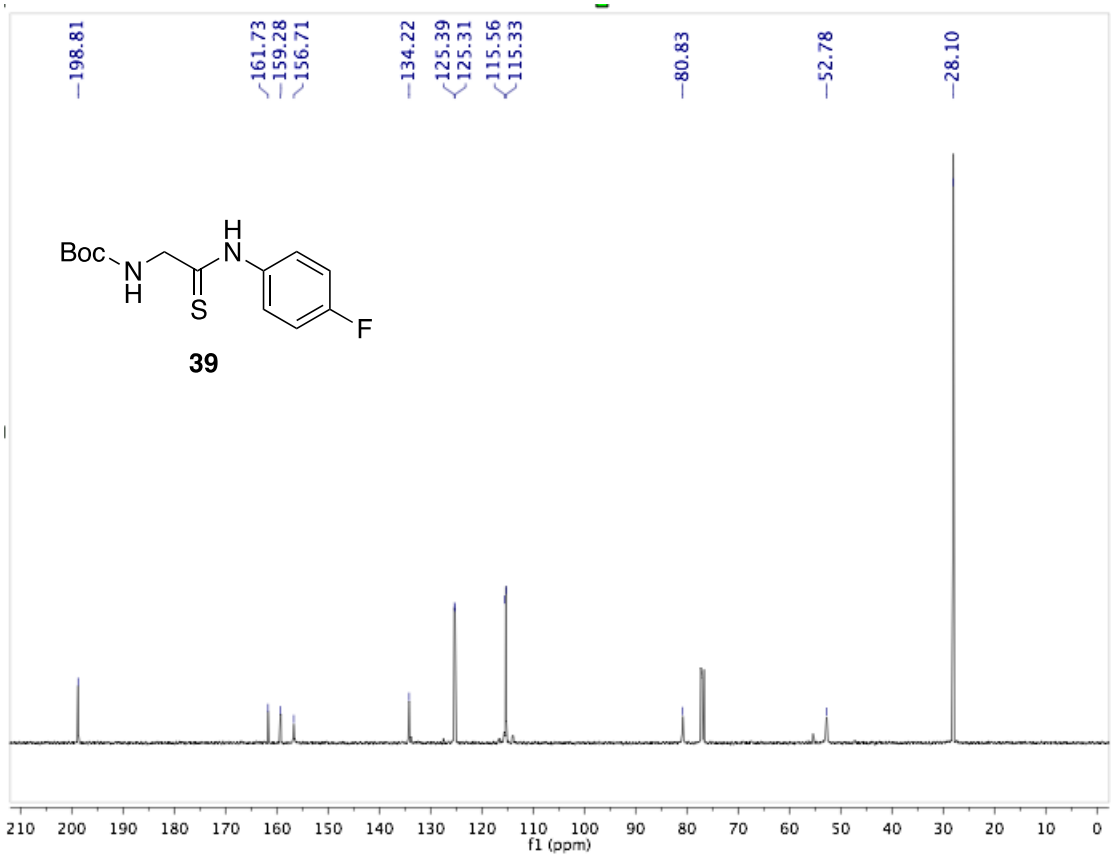
^{13}C NMR spectrum of *tert*-butyl 2-(4-fluorophenylamino)-2-oxoethylcarbamate (**38**) (125 MHz, MeOD-d_6)



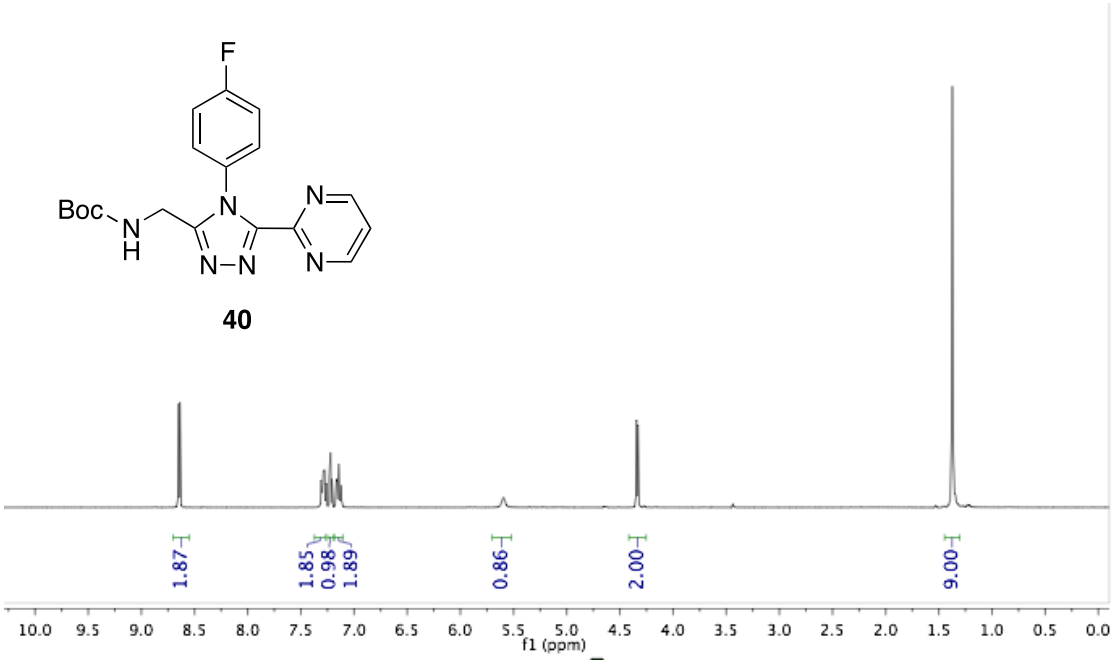
^{1}H NMR spectrum of *tert*-butyl 2-(4-fluorophenylamino)-2-thioxoethylcarbamate (**39**) (400 MHz, $CDCl_3$)



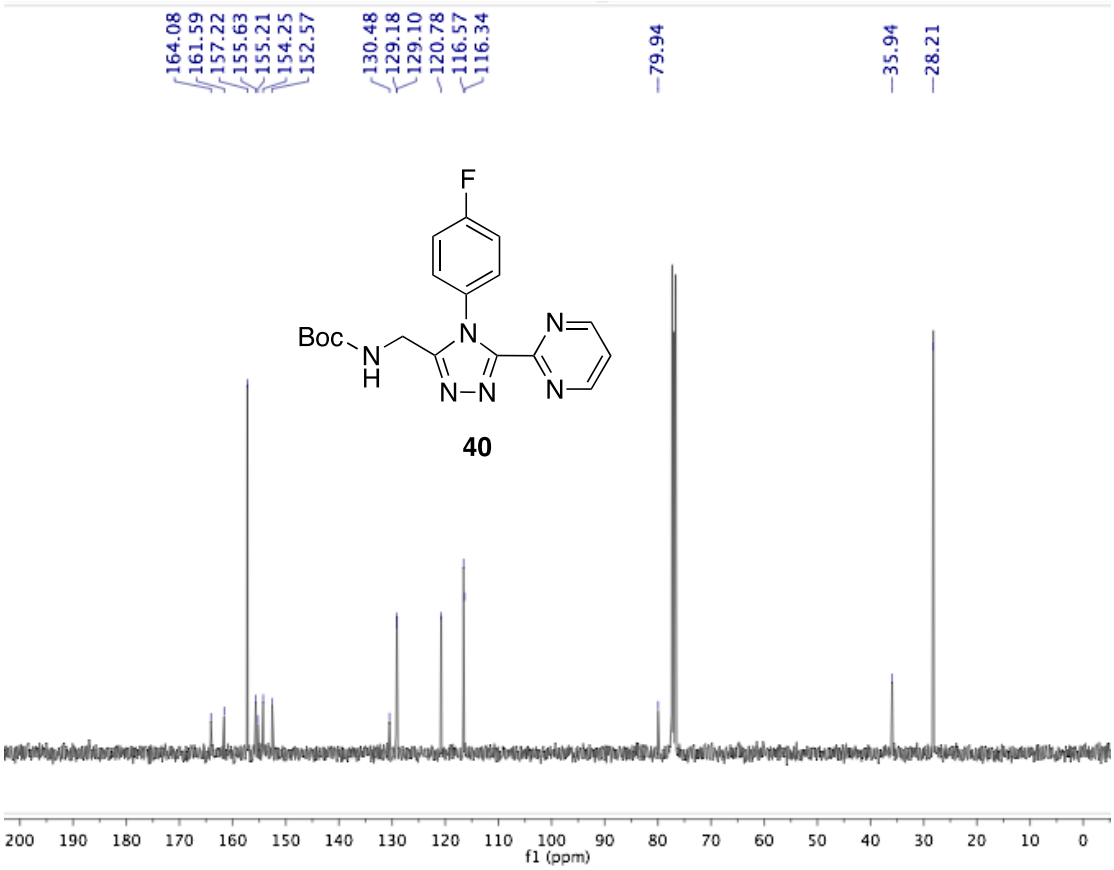
^{13}C NMR spectrum of *tert*-butyl 2-(4-fluorophenylamino)-2-thioxoethylcarbamate (**39**) (100 MHz, $CDCl_3$)



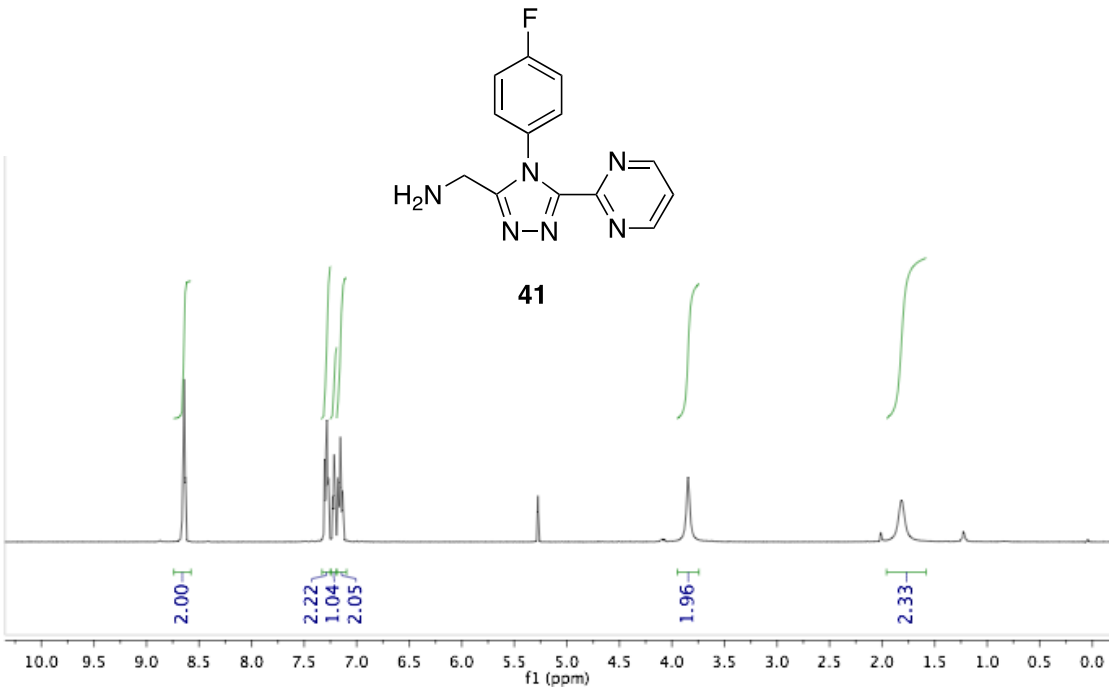
^{1}H NMR spectrum of *tert*-butyl (4-(4-fluorophenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl-carbamate (**40**) (400 MHz, $CDCl_3$)



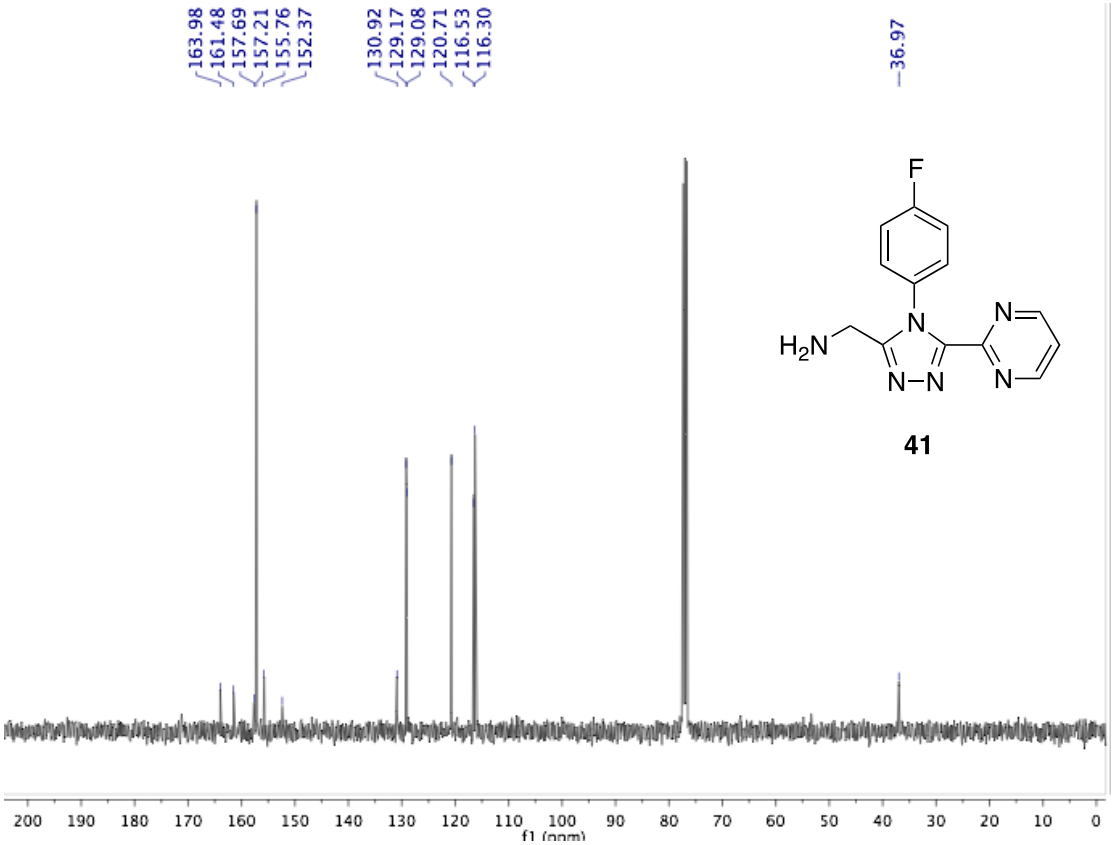
^{13}C NMR spectrum of *tert*-butyl (4-(4-fluorophenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl-carbamate (**40**) (100 MHz, $CDCl_3$)



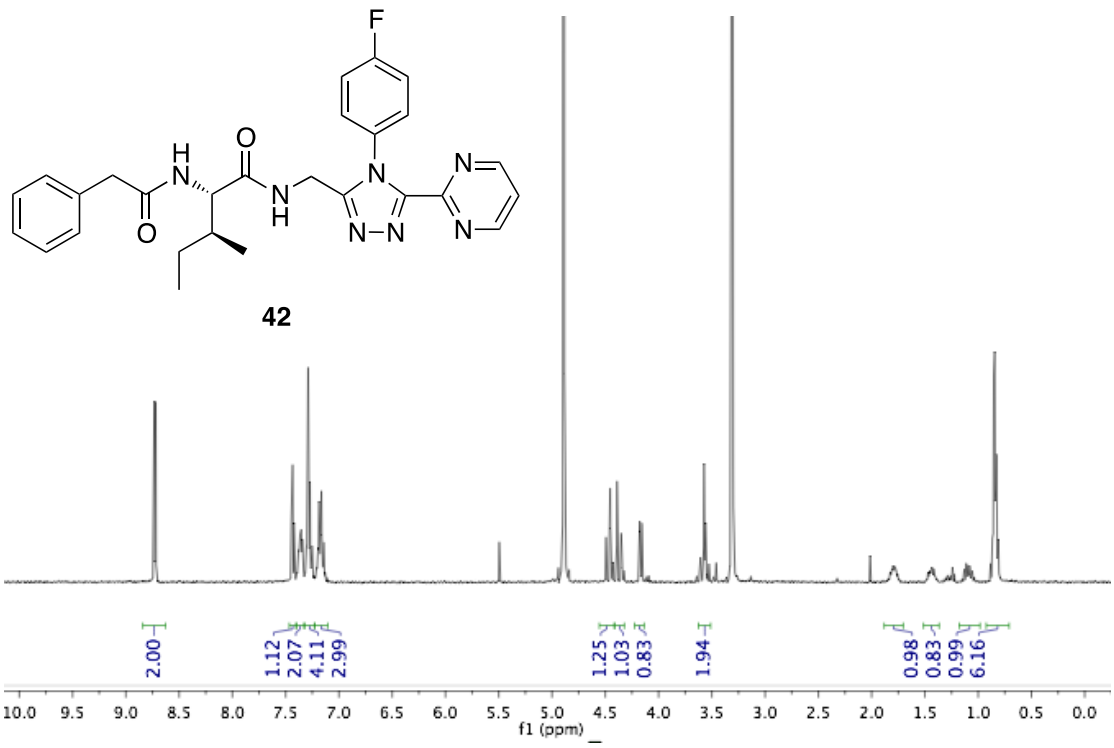
^{1}H NMR spectrum of (4-(4-fluorophenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methanamine (**41**) (400 MHz, $CDCl_3$)



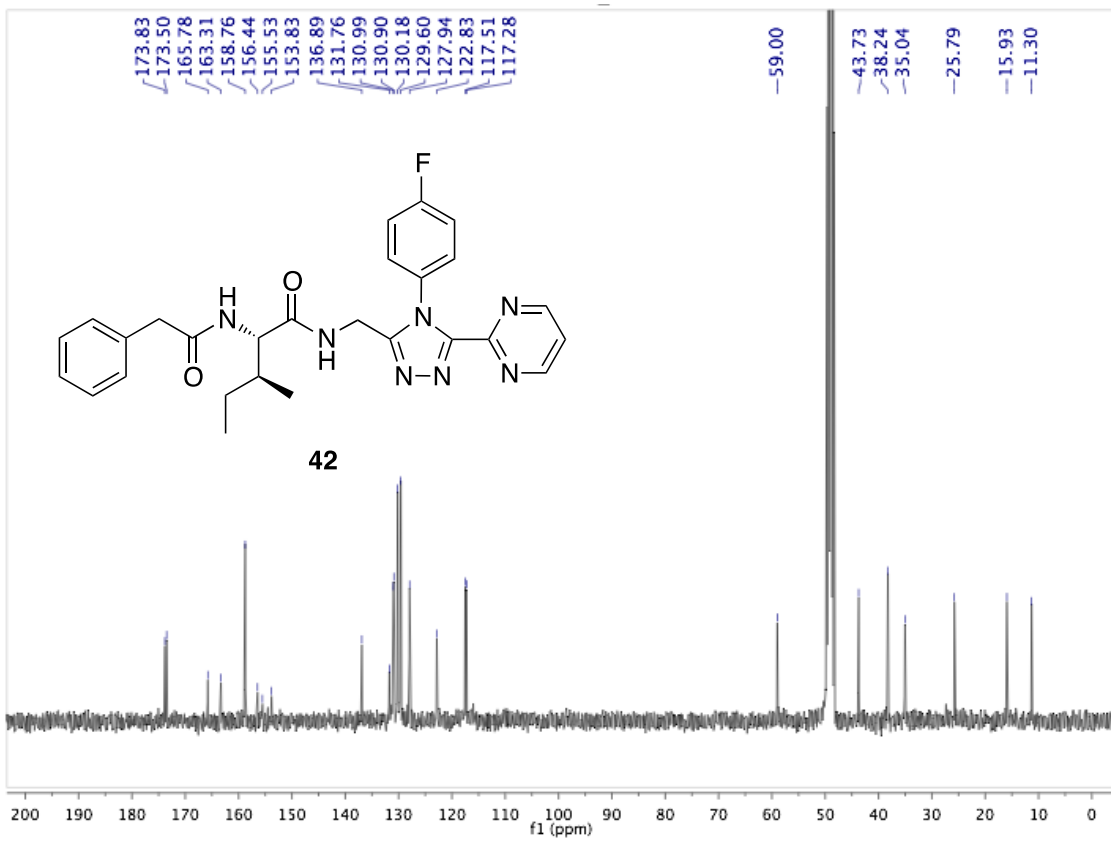
^{13}C NMR spectrum of (4-(4-fluorophenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methanamine (**41**) (100 MHz, $CDCl_3$)



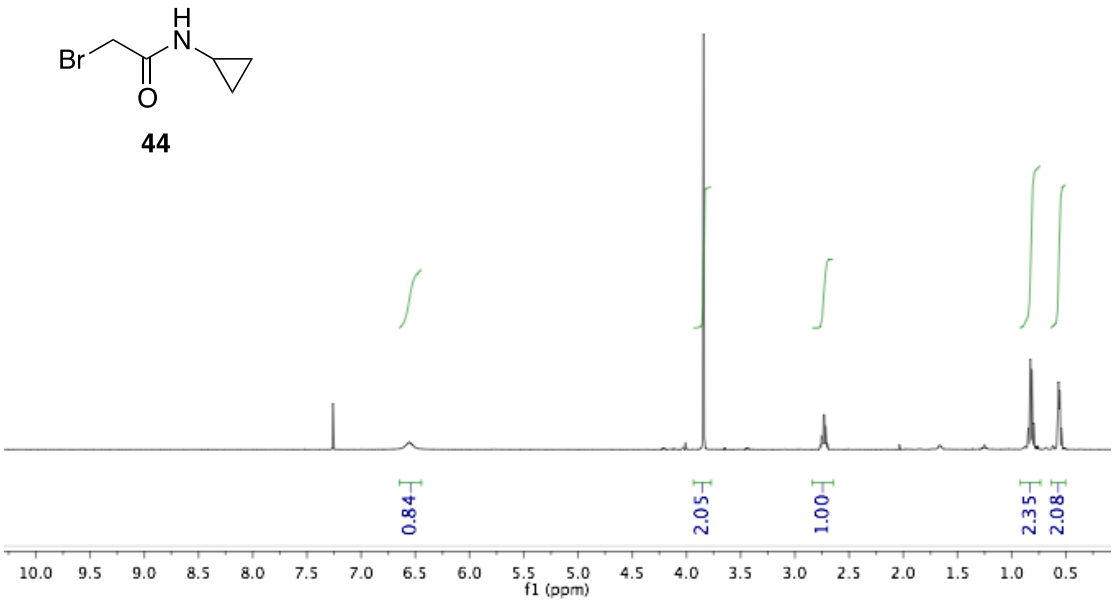
^{1}H NMR spectrum of (2*S*,3*S*)-*N*-((4-(4-fluorophenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl)-3-methyl-2-(2-phenylacetamido)pentanamide (**42**) (400 MHz, MeOD-d_6)



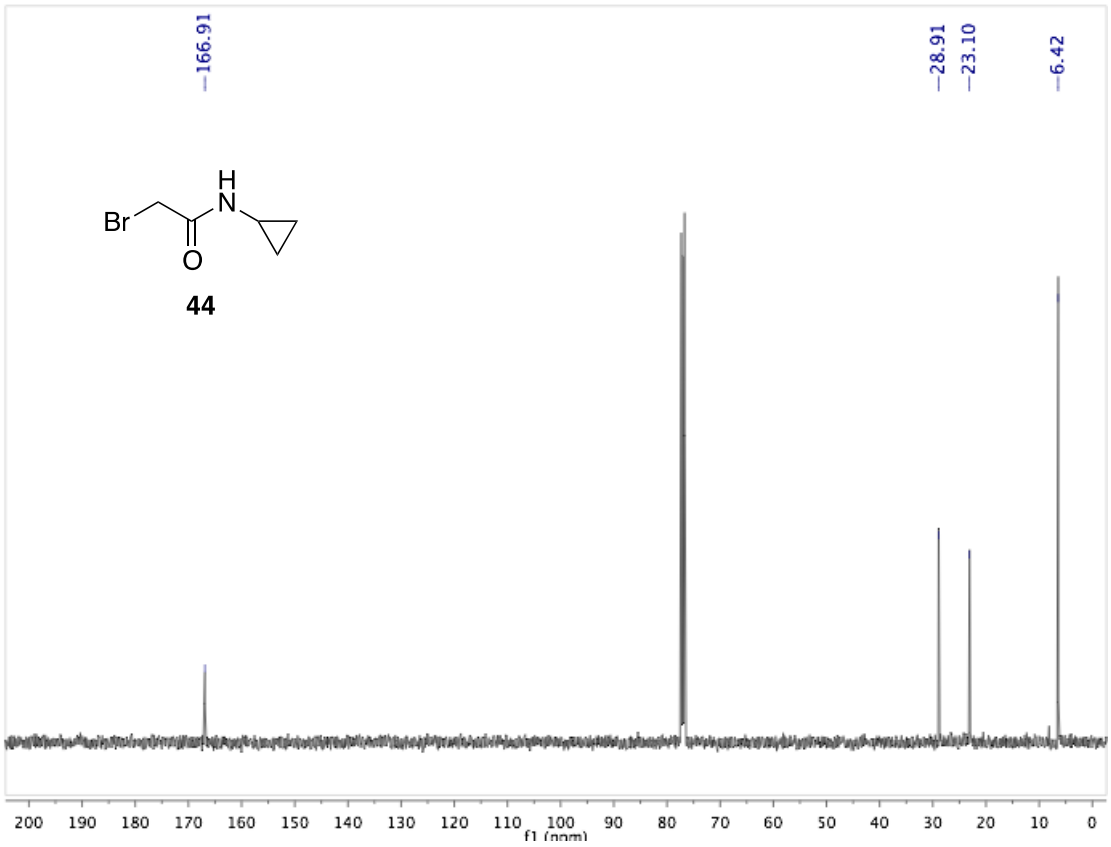
^{13}C NMR spectrum of (2*S*,3*S*)-*N*-((4-(4-fluorophenyl)-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl)-3-methyl-2-(2-phenylacetamido)pentanamide (**42**) (125 MHz, MeOD-d_6)



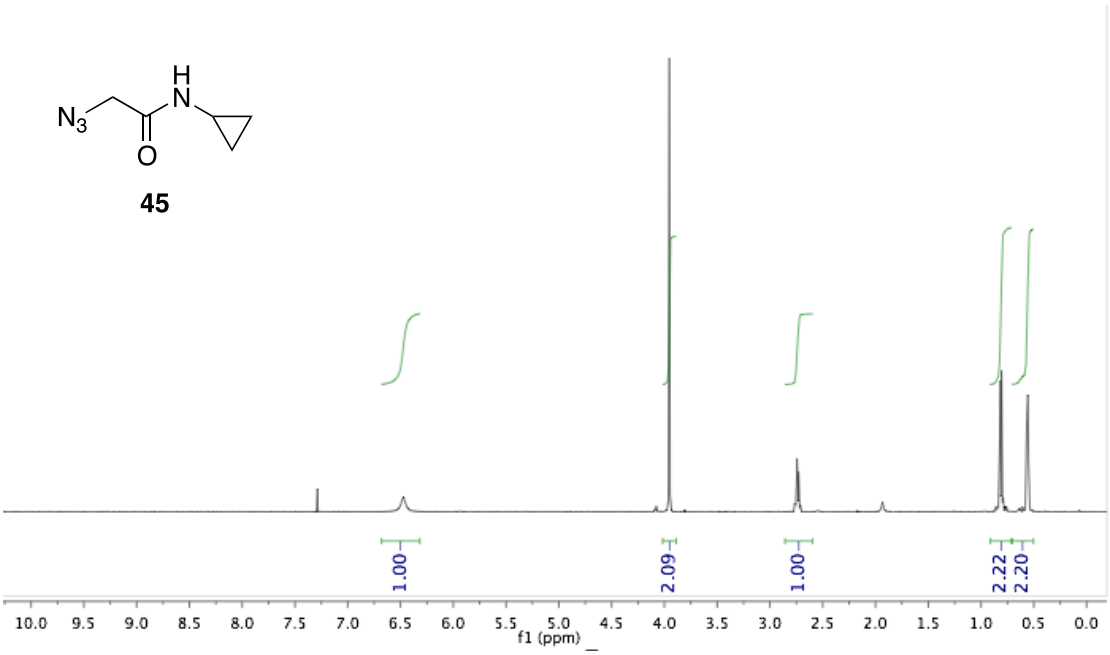
^{1}H NMR spectrum of 2-bromo-*N*-cyclopropylacetamide (**44**) (400 MHz, $CDCl_3$)



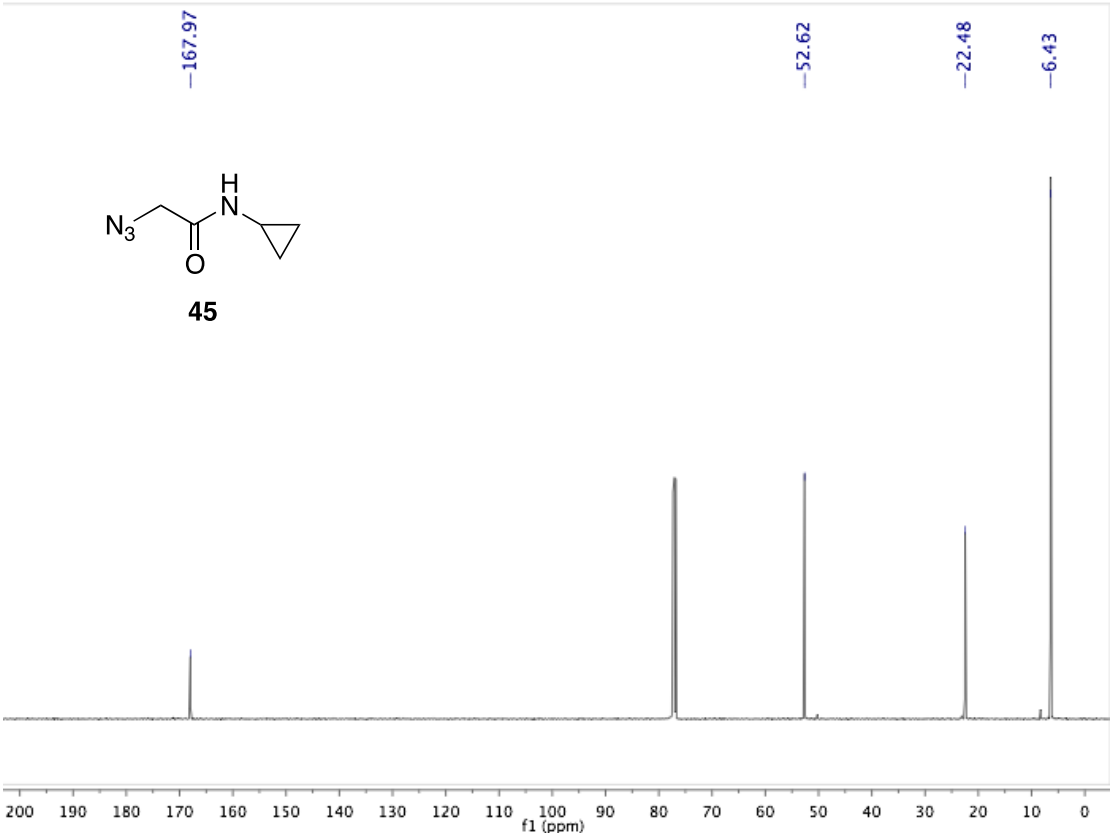
^{13}C NMR spectrum of 2-bromo-*N*-cyclopropylacetamide (**44**) (125 MHz, $CDCl_3$)



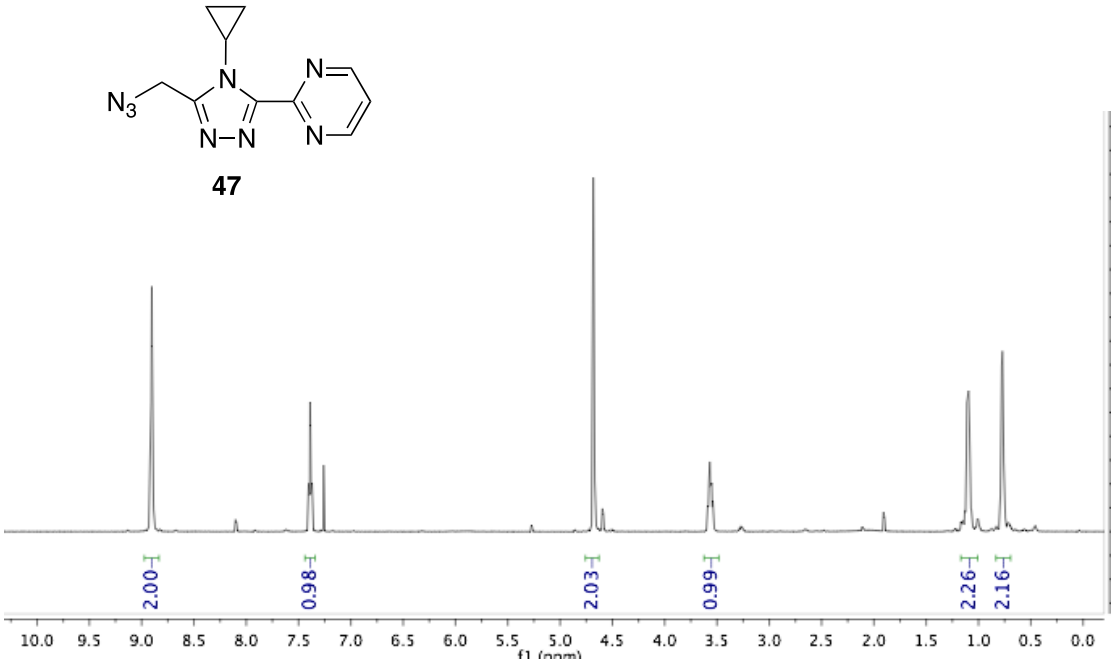
^{1}H NMR spectrum of 2-azido-*N*-cyclopropylacetamide (**45**) (400 MHz, $CDCl_3$)



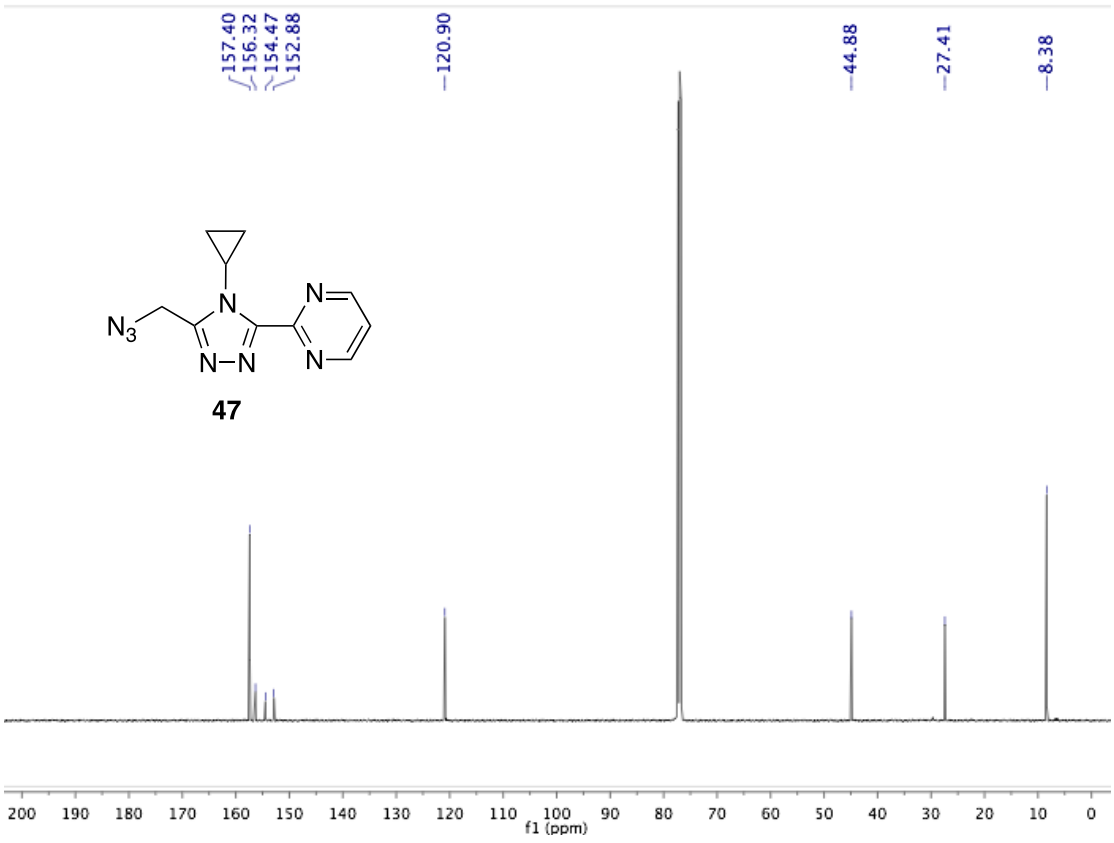
^{13}C NMR spectrum of 2-azido-*N*-cyclopropylacetamide (**45**) (125 MHz, $CDCl_3$)



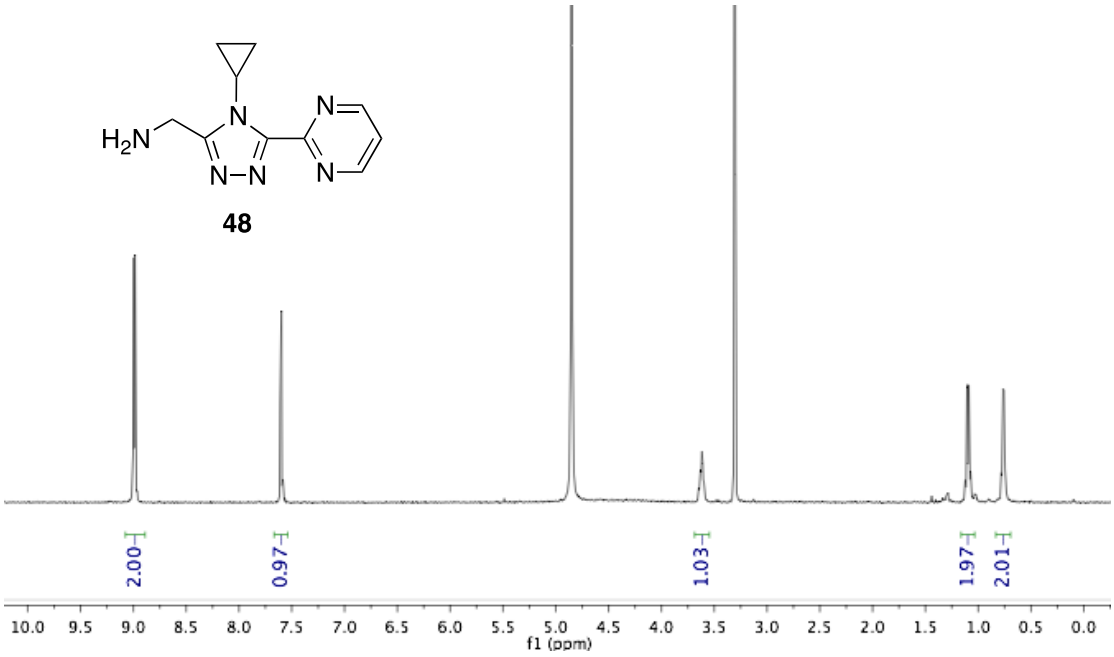
^{1}H NMR spectrum of 2-(5-(azidomethyl)-4-cyclopropyl-4*H*-1,2,4-triazol-3-yl)pyrimidine (**47**) (400 MHz, $CDCl_3$)



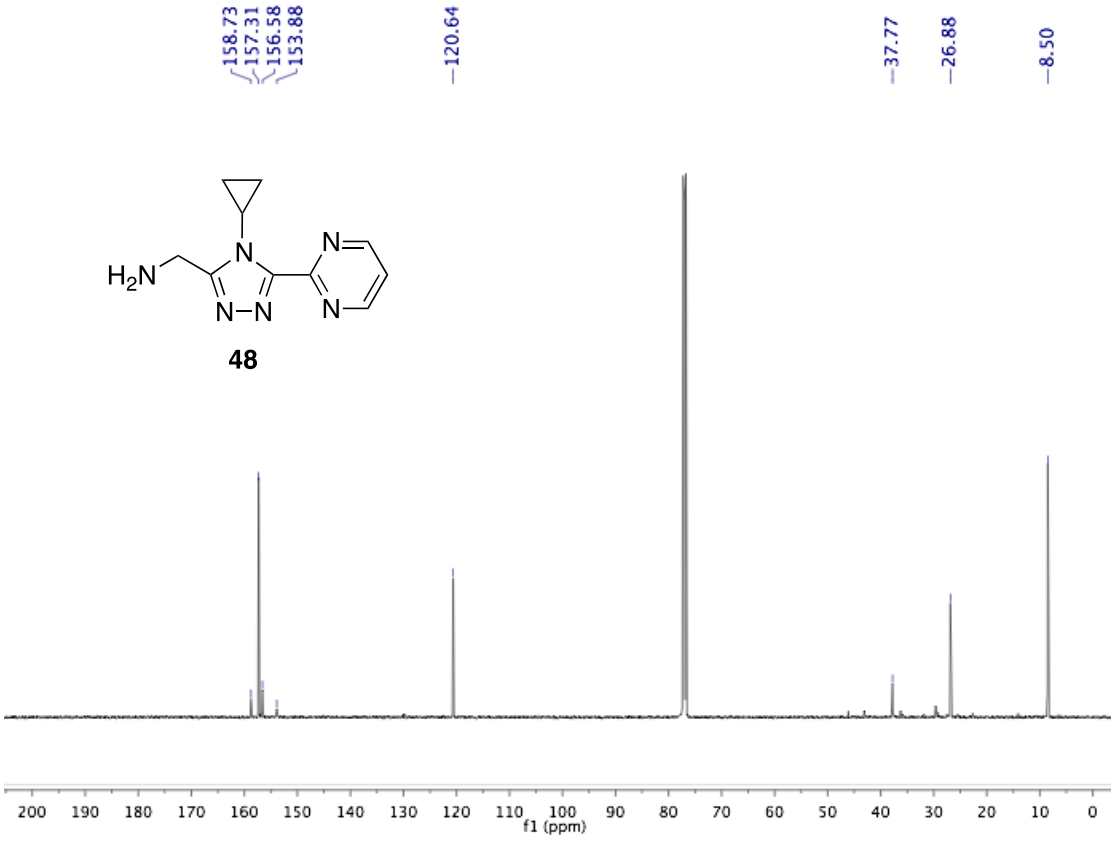
^{13}C NMR spectrum of 2-(5-(azidomethyl)-4-cyclopropyl-4*H*-1,2,4-triazol-3-yl)pyrimidine (**47**) (125 MHz, $CDCl_3$)



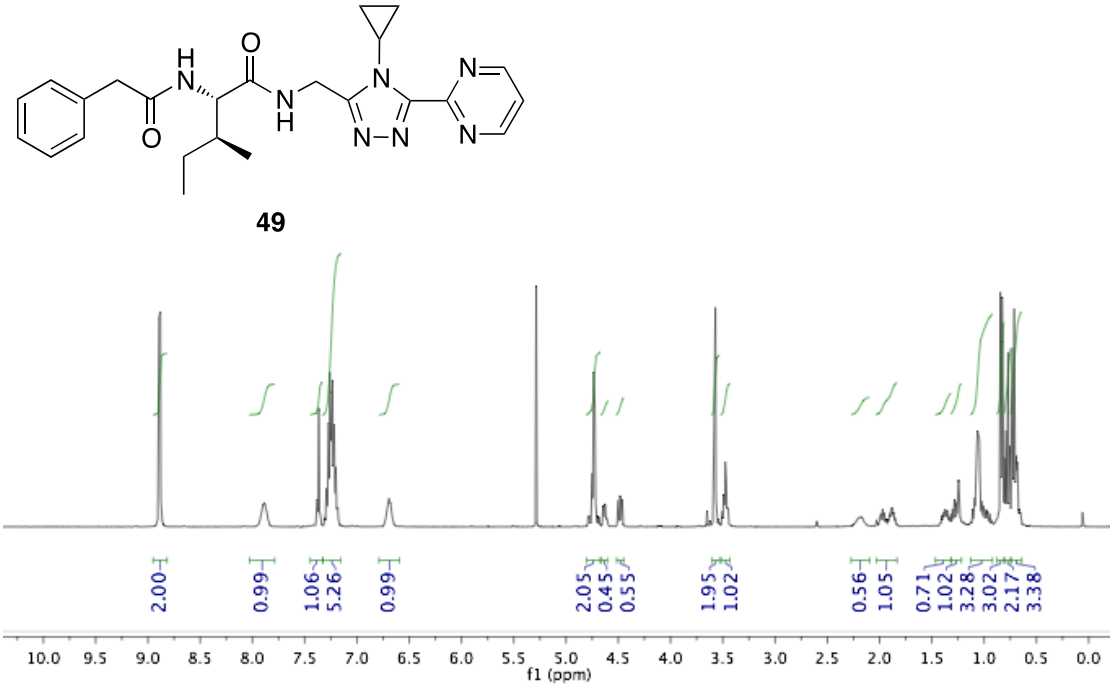
^{1}H NMR spectrum of (4-cyclopropyl-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methanamine (**48**) (400 MHz, MeOD-d_6)



^{13}C NMR spectrum of (4-cyclopropyl-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methanamine (**48**) (125 MHz, MeOD-d_6)



^{1}H NMR spectrum of (2*S*,3*S*)-*N*-((4-cyclopropyl-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl)-3-methyl-2-(2-phenylacetamido)pentanamide (**49**) (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of (2*S*,3*S*)-*N*-((4-cyclopropyl-5-(pyrimidin-2-yl)-4*H*-1,2,4-triazol-3-yl)methyl)-3-methyl-2-(2-phenylacetamido)pentanamide (**49**) (100 MHz, $CDCl_3$)

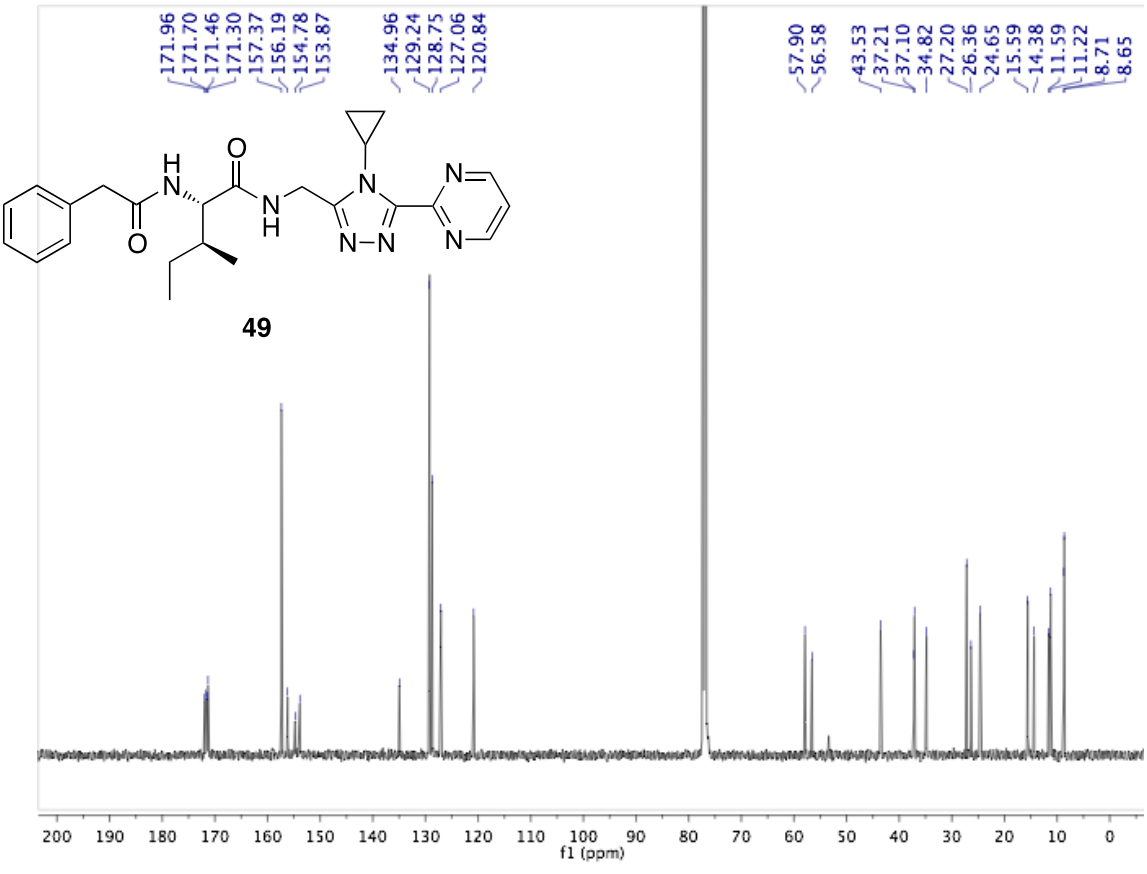
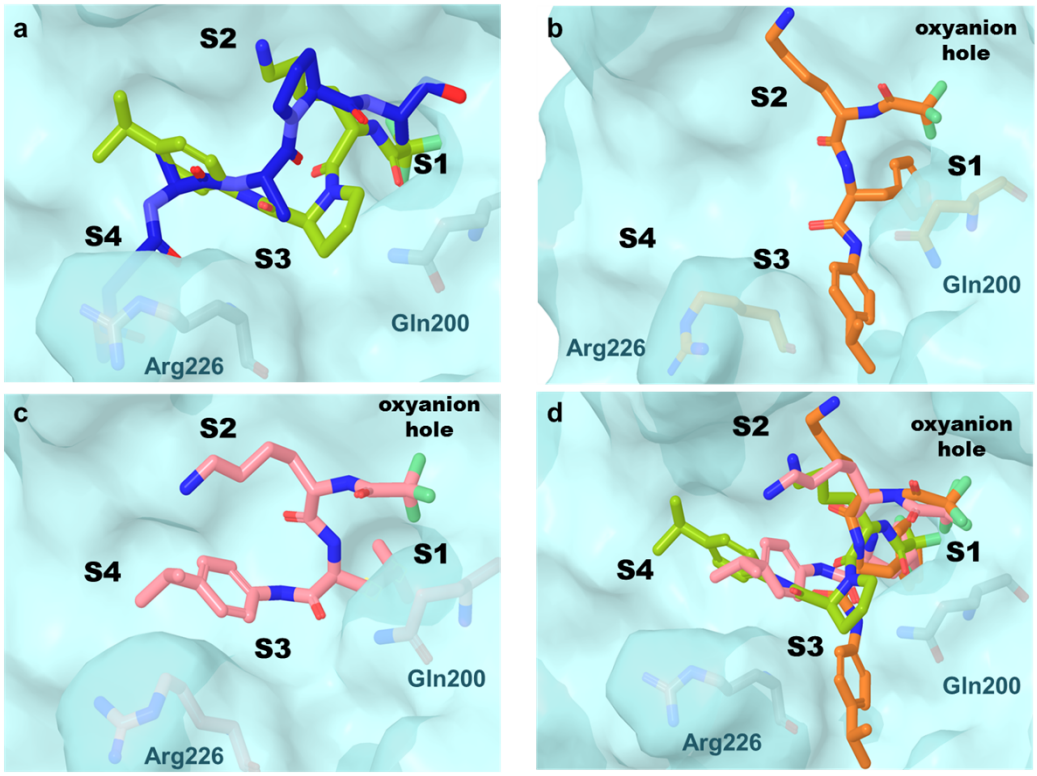


Figure 1S. Divergent crystal structures of elastase complexed with topologically similar ligands: N-blue, O-red, F-green. Arg226 and Gln200 which showed minor movements upon ligand binding were also depicted.[19] (a) **50** (lime green C) exhibits a pose identical to the Pro-to-Ala congener (not shown) and same way as covalent peptide inhibitor, MSACK (blue C), (b) **51** and (c) **52**, (d) Alignment of the three non-covalent inhibitors in the binding pocket.



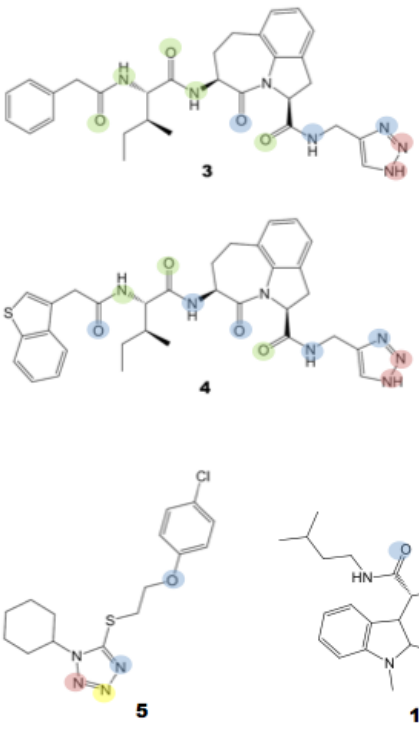
Non-covalently bound trifluoroacetyl (TFA)-Lys-Pro-isopropylanilide (ISO) (**50**) binds to elastase in the same fashion as covalent inhibitors oriented very similar to substrates that form acyl enzyme intermediates: TFA in the S1; Lys in the S2; Pro in the S3: anilide group in the S4 (**a**). However, replacing Pro with Phe or Leu (TFA-Lys-Phe-ISO (**51**) and TFA-Lys-Leu-ISO (**52**)) results in quite different non-covalent binding modes. As mentioned, the Pro-containing ligand locates the TFA moiety and the Lys residue in the S1 and the S2 pockets, respectively. In strong contrast, the Phe and Leu substitutions place TFA in the oxyanion hole and Phe and Leu side chains into S1 instead, while the anilide groups settle into new pockets (**b-c**). The striking lack of superposition is illustrated in **Figure 1d-S**. Thus, some non-covalent inhibitors

mimic the covalent binding pose, while other structurally similar compounds utilize the binding pocket in a quite different manner.

Superposition of elastase complexes populated by structurally analogous inhibitors illustrates that conformational reorganization of only a few residues at the serine protease binding site in combination with modest changes in ligand structure can strongly affect ligand binding poses. In the present case, the spatial organization of elastase side chains in three complexes are unchanged except for deceptively minor movements of Arg226 and Gln200. The observation that a Pro to Ala substitution in **50** leads to an identical binding pose eliminates the turn-forming Pro as the source of the pose differential. Arg226 side chains in S4 are essentially superimposed in the three structures, the cationic heads displaced by 2-4 Å from one another. This allows for insertion of a bridging water molecule between the arginine cationic head and the anilide ring for **50** (**a**), but not **52** (**c**). The latter displays a kink in the hydrophobic –$(CH_2)_3$– spacer and, thereby, enjoys a hydrophobic contact with the anilide ring. These observations suggest Arg226 is most likely not the cause of a covalent *vs.* non-covalent binding pose. Gln200, on the other hand, forms an intriguing partnership with the inhibitor amide groups of **51** and **52** (**b** and **c**, respectively) located between S1 and S3. The plane of the side chain is constant but occupied by terminal amide conformations rotated by exactly 180°. For **50**, the Pro nitrogen atom and an N-H of Gln200 form a weak H-bond near S1, while for **52** the same N-H engages the amide C=O near the S3 pocket. For ligand **51**, the Gln200 unit flips and makes a C=O---NH contact with the N-H attached to the anilide ring. These interactions assist and complement the variable occupation of S1 and the oxyanion hole, as well as the placement of the phenyl and leucine groups of **51** and **52** into the S1 site instead of into solvent.

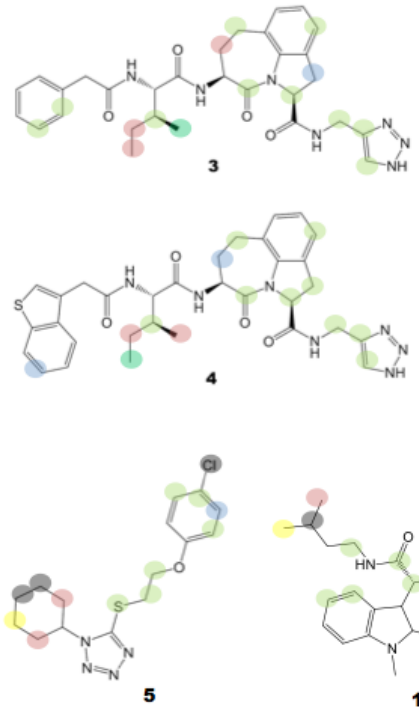
Ligand-Protein Contact (LPC) Analysis[20]

Hydrogen bond contacts



Compound	GrB IC_{50} (μM)	# of Atoms	# of H bonds	# of Hydrophobic interaction	# of Aromatic-aromatic interaction
IEPD-CHO	Ki = 80 nM	35	26	46	0
3	Ki = 13 nM	41	17	27	16
4	Ki = 7 nM	44	19	25	20
5	35	22	11	33	12
11	30	33	4	31	8
12	30	37	4	36	20

Hydrophobic contacts



Compound	GrB IC_{50} (μM)	# of Atoms	# of H bonds	# of Hydrophobic interaction	# of Aromatic-aromatic interaction
IEPD-CHO	Ki = 80 nM	35	26	46	0
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5	35	22	11	33	12
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12	30	37	4	36	20

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([2]) http://www.eyesopen.com/rocs.
([3]) http://www.enamine.net/index.php?option=com_content&task=view&id=23.
([4]) Schrödinger Suite 2010 Induced Fit Docking protocol, Glide, version 5.6, Schrödinger, LLC, New York, NY, 2010; Prime version 2.2, Schrödinger, LLC, New York, NY, 2010.
([5]) QikProp, version 3.3, Schrödinger, LLC, New York, NY, 2010.

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