

Supporting Information

Discovery of 2-[4-{{2-(2S, 5R)-2-cyano-5-ethynyl-1-pyrrolidinyl}-2-oxoethyl}amino]-4-methyl-1-piperidinyl]-4-pyridinecarboxylic acid (ABT-279); a very potent, selective, effective and well-tolerated inhibitor of dipeptidyl peptidase-IV, useful for the treatment of diabetes

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1. Partial Purification of Human DPPiV Caco-2 cells were obtained from American Type Culture Collection (P.O. Box 3605, Manassas, VA), cultured and maintained at 37 °C with 5% CO₂ in low glucose DMEM media supplemented with 10% Fetal Bovine Serum and antibiotic/antimycotic. In preparation for making an extract, cells were seeded at a density to achieve confluence within 7 days. The cells were cultured for an additional 14 days to allow for maximal DPPiV expression. On the day of harvest, cells were washed once with Dulbecco's PBS and solubilized in a 10 mM NaCl

containing 50 mM Tris HCl, 0.5% Nonidet P40 and 0.3 ug/mL aprotinin at pH 8.0. The extract was clarified by centrifugation at 35,000 g for 30 minutes at 4 °C.

2. Inhibition constant (K_i) measurement DPPIV activity was determined by measuring the rate of hydrolysis of a surrogate substrate Gly-Pro-7-amido-methylcoumarin (Gly-Pro-AMC, Sigma, St. Louis, MO). The assay was carried out at room temperature in black 96 well polypropylene or polyethylene plates in a total volume of 100 μ L per well. Appropriate dilutions of the compounds were made in DMSO and then diluted ten fold into water. 10 μ L of 5 concentrations of the inhibitor or 10% DMSO in water were added to individual wells containing 80 μ L of DPPIV diluted in assay buffer containing 25 mM HEPES (pH 7.5), 150 mM NaCl and 0.12 mg/mL BSA. After 10 minutes at room temperature, the reaction was initiated by adding 10 μ L of either 280, 700, 1750, or 3500 μ M Gly-Pro-AMC in water. The DPPIV activity results in the formation of the fluorescent product amido-methylcoumarin (AMC) which was continuously monitored by excitation at 350 nm and measurement of fluorescent emission at 460 nm every 112 seconds for 37 minutes using an appropriate plate reader. The fluorescence at 460 nm was converted to nanomoles of AMC using a standard curve and the initial rate of AMC formation was calculated. For each concentration of inhibitor or DMSO control, the initial rates were used to fit the rectangular hyperbola of Michaelis-Menten by non-linear regression analysis (GraphPad Software Prism 3.0). The ratio of the apparent K_m/V_{max} vs. inhibitor concentration was plotted and the competitive K_i was calculated by linear regression to be the negative x-intercept.

3. Chemical Stability Assay A solution of each DPPIV compound was prepared in an appropriate mobile phase. This was injected and analyzed by HPLC to identify the parent drug peak in the chromatograms. Stability solutions were prepared in 50 mM phosphate buffer pH 7.4 adjusted to $\mu=0.155$. Samples were weighed using a Mettler-Toledo microbalance and transferred into a 20 mL glass scintillation vial or a volumetric flask. The phosphate buffer pH 7.4 was added with an EDP Plus 10 mL electronic pipette. The samples were sonicated for approximately 2 minutes and filtered using a PTFE membrane syringe filter. The filtrate was transferred to amber HPLC vials and placed in an autosampler at 37 °C. The first sample was immediately injected and counted as the time zero sample. The solution concentration was measured at various timepoints using an appropriate HPLC method. After data acquisition was completed, the chromatograms were processed for each compound. The rate and half-life were determined from a plot of \log_{10} peak area remaining/peak area at zero time vs. exposure time @ 37 °C using Microsoft® Excel 2000. Rates of reaction and half-lives were calculated using a pseudo-first order kinetic model.

4. Pharmacokinetic Experiments (PK) Compounds were prepared as solutions in ethanol: propylene glycol: D5W vehicles (10/30/60 v/v) at concentrations appropriate for a 1 mL/kg dose volume for intravenous (IV) or oral administration. Sprague-Dawley rats (Charles River, Portage, MI), beagle dogs (Marshall Research Animals, North Rose, NY) or Cynomolgus monkeys were fasted overnight prior to dosing and three (monkey) or twelve (rat and dog) hours post dose, but were permitted free access to water. The IV dose (5 mg/kg in rat or 2.5 mg/kg in dog and monkey) was administered as a slow bolus while the oral dose was administered by gavage. Serial EDTA blood samples were

obtained from each animal at selected points following drug administration. Plasma was separated by centrifugation (16000 rpm x 4 minutes, ~4°C) and stored frozen (< -15°C) until analysis.

Compounds were separated from the plasma using protein precipitation with methanol and acetonitrile. A plasma aliquot (100 µL, sample or spiked standard) was combined with 150 µL of internal standard (prepared in methanol) and 150 µL acetonitrile. The samples were vortexed vigorously for one minute followed by centrifugation at 2000 rpm for 10 minutes (4°C). An aliquot of the supernatant (100 µL) was transferred to a clean 96-well plate and diluted with 500 µL 1% acetic acid in water. Samples were analyzed simultaneously with spiked plasma standards.

The parent compound and the internal standard were separated from each other and co-precipitated contaminants on a 100 x 3 mm Kromasil 3 µm column (Higgins) with an acetonitrile: 1% aqueous acetic acid mobile phase at a flow rate of 0.3 mL/min. Analysis was performed on a Sciex API4000™ Biomolecular Mass Analyzer with a turbo-ionspray interface. Analytes were ionized in the positive ion mode with a source temperature of approximately 400 °C. Detection was in the multiple reaction-monitoring (MRM) mode. Peak areas were determined using the Sciex Analyst™ software. The plasma drug concentration of each sample was calculated by least squares linear regression analysis (weighted as 1/concentration) of the peak area ratio (parent/internal standard) of the spiked plasma standards versus concentration.

5. Protein X-ray crystallography analysis methods Purified human DPPiV (39-766) was crystallized according to previously described conditions [1]. The complex was

prepared by soaking a crystal in the presence of compound and preserving it in liquid nitrogen for data collection at 100K. X-ray diffraction data were collected at the Advanced Photon Source of Argonne National Laboratory on the IMCA beamline 17-ID with an ADSC quantum 210 ccd detector, and processed using the program HKL2000 [2]. The structure was solved by molecular replacement with the program MOLREP using atomic coordinates with pdb accession code 1N1M [3]. The structure of the compound was built into the electron density maps that clearly revealed the protein-inhibitor complex, and the structure was refined against all data using the programs QUANTA (Accelrys) and REFMAC5 [4, 5].

Coordinates for the refined atomic structure will be submitted to the public RCSB protein data bank, and the accession code will be provided upon technical release.

Supplemental Table 1. X-ray data and structure refinement.

X-ray diffraction data	Complex ABT-279
Space Group	P2 ₁
Unit cell lengths (Å)	120.3, 127.0, 127.3
Unit cell angles (°)	90, 96.6, 90
Resolution (Å)	2.40 (2.49 – 2.40)
Observations	482,464
Unique	143,995
Completeness (%)	97 (96)
I / σ_I	5.1 (1.8)
R _{sym} (%)	11.6 (45)
Model Refinement	
Reflections (work / free)	136,246 / 7166
Rfactor (work / free %)	18.0 / 24.5

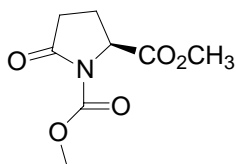
Protein molecules per ASU	4
Solvent molecules	2958
Mean B factor (Å ²)	51
RMSD ideal bond lengths (Å)	0.006
RMSD ideal bond angles (°)	1.35

$R_{\text{sym}} = \sum |I - \langle I \rangle| / \sum I$, where I is the integrated intensity for a reflection. $R_{\text{factor}} = \sum |F_{\text{p}} - F_{\text{c}}| / \sum F_{\text{p}}$, where F_{p} and F_{c} are the observed and calculated structure factor amplitudes, while R_{free} is calculated on 5% of the data excluded from refinement. Values in parenthesis are for the highest resolution shell.

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2. Otwinowski, Z. and W. Minor, *Processing of X-Ray Diffraction Data Collected in Oscillation Mode*. Methods in Enzymology, 1997. **276**: p. 307-326.
3. Vagin, A. and A. Teplyakov, *MOLREP: an Automated Program for Molecular Replacement*. Journal of Applied Crystallography, 1997. **30**: p. 1022-1025.
4. Murshudov, G.N., A.A. Vagin, and E.J. Dodson, *Refinement of Macromolecular Structures by the Maximum-Likelihood Method*. Acta Crystallographica, 1997. **D57**: p. 240-255.
5. CCP4, *The CCP4 Suite: Programs for Protein Crystallography*. Acta Crystallographica Section D, 1994. **D50**: p. 760-763.

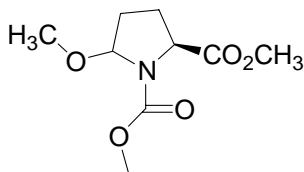
6. Compound Synthesis Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further drying or purification. All reactions were performed under nitrogen atmosphere unless otherwise noted. Normal-phase flash chromatography was done using Merck silica gel 60 (230-400 mesh) from E. M. Science or was performed on an Analogix IntelliFlash system with prepacked columns. Reverse-phase preparative chromatography was performed using a Gilson HPLC system equipped with a Gilson 215 liquid handler and a Waters Nova-Pak column 25x100 mm C18 column. Fraction collection was triggered by UV absorption at 214 nm. Final products were eluted with linear gradient 0-70% CH₃CN/H₂O with 0.1%

trifluoroacetic acid (TFA) in the aqueous phase as TFA salts. ^1H NMR spectra were recorded at 300 and 500 MHz spectrometers; all chemical shift (δ) values were referenced to tetramethylsilane as an internal standard and are reported as shift (multiplicity, proton count). Mass spectral analysis was accomplished using fast atom bombardment (FAB), electrospray (ESI), or direct chemical ionization (DCI) ionization techniques. All elemental analyses were done at QTI or Robertson and are consistent with theoretical values to within $\pm 0.4\%$ unless otherwise indicated.



dimethyl (2S)-5-oxopyrrolidine-1,2-dicarboxylate

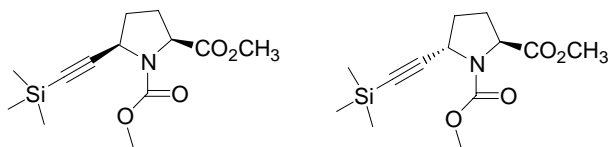
To a cold ($-78\text{ }^{\circ}\text{C}$) solution of methyl (S)-(+)-2-pyrrolidone-5-carboxylate (4.80 grams, 33.5 mmol) in tetrahydrofuran (90 mL) was added a solution of lithium bis(trimethylsilyl) amide (1 M solution in hexanes, 40.0 mL, 40.0 mmol) dropwise via syringe over 15 minutes; then methyl chloroformate (2.90 mL, 36.9 mmol) was added dropwise via syringe over 5 minutes. The resulting slurry was stirred at $-78\text{ }^{\circ}\text{C}$ for 1 hour after which the reaction was quenched with 1 M HCl (50 mL). The mixture was allowed to come to room temperature, concentrated under reduced pressure and the residue partitioned between ethyl acetate (200 mL) and 1 M HCl (200 mL). The aqueous layer was extracted with ethyl acetate (2 X 200 mL) and the combined organic layers were dried (sodium sulfate), filtered, and concentrated to provide the titled compound. MS (DCI/ NH_3) m/e 202 ($\text{M}+\text{H}$) $^+$; ^1H NMR (300 MHz, CDCl_3): δ ppm 4.70 (dd, 1H), 3.88 (s, 3H), 3.80 (s, 3H), 2.74-2.30 (m, 3H), 2.15-2.05 (m, 1H).



dimethyl (2S)-5-methoxypyrrolidine-1,2-dicarboxylate

To a cold solution (-78 °C) of dimethyl (2S)-5-oxopyrrolidine-1,2-dicarboxylate (5.80 g, 28.8 mmol) in tetrahydrofuran (100 mL) was added a solution of lithium triethylborohydride (1 M in THF, 35 mL, 35 mmol) dropwise via syringe over 10 minutes. The resulting solution was stirred at -78 °C for 30 minutes, quenched by the careful addition of saturated sodium bicarbonate solution (50 mL), allowed to warm to 0 °C and 30% hydrogen peroxide (6 mL) was carefully added dropwise. The mixture was stirred for 30 minutes at room temperature, reduced in volume under reduced pressure, and diluted with ethyl acetate (300 mL) and brine (200 mL). The milky aqueous layer was separated and further extracted with ethyl acetate (2 X 300 mL). The combined organic layers were dried (sodium sulfate), filtered, and concentrated to a light yellow oil. The yellow oil was taken up in methanol (50 mL) containing *para*-toluenesulfonic acid hydrate (487 mg, 2.6 mmol) and stirred at room temperature for 16 hours. The reaction was diluted with aqueous sodium bicarbonate solution (40 mL), the volatile solvents were removed under reduced pressure and the residue partitioned between ethyl acetate (200 mL) and brine (200 mL). The aqueous layer was further extracted with ethyl acetate (200 mL), the combined organic layers were dried (sodium sulfate), filtered, and concentrated to an oil which was chromatographed on a Biotage 40M with 60% hexane/40% ethyl acetate to provide the titled compound as a mixture of diastereomers. (mixture of amide bond rotomers) ¹H NMR (300 MHz, CDCl₃): δ ppm 5.37 (d, 1H), 5.33 (dd, 1H), 5.24

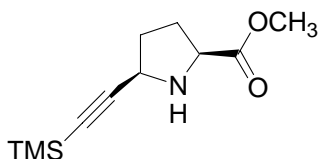
(d, 1H), 5.18 (dd, 1H), 4.44-4.31 (m, 2H), 3.76 (s, 3H), 3.73 (s, 3H), 3.72 (s, 3H), 3.42 (s, 3H), 3.34 (s, 3H).



dimethyl (2S,5R)-5-((trimethylsilyl)ethynyl)pyrrolidine-1,2-dicarboxylate (**5**) and dimethyl (2S,5S)-5-((trimethylsilyl)ethynyl)pyrrolidine-1,2-dicarboxylate (**6**)

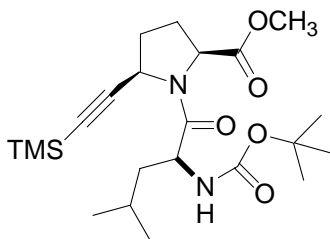
To a cold -45°C solution of dimethyl (2S)-5-methoxypyrrolidine-1,2-dicarboxylate (3.30 g, 15.20 mmol) and bistrimethylsilylacetylene (5.20 g, 30.4 mmol, 2.0 equiv) in methylene chloride (45 mL) was added a solution of tin (IV) chloride (1 M in methylene chloride, 20.0 mL, 20.0 mmol, 1.3 equiv) dropwise via syringe over 15 minutes. To the dark yellow solution was added solid aluminum chloride (2.77 g, 20.8 mmol, 1.4 equiv) in one portion. The resulting mixture was allowed to warm to room temperature and stirred at room temperature for 48 hours. The reaction mixture was carefully poured into aqueous sodium bicarbonate solution (100 mL) with ice cooling. A white precipitate forms and 1 M HCl (ca. 50 mL) was added until the solids dissolved. This mixture was extracted with ethyl acetate (2 X 200 mL). The combined organic layers were filtered, dried (sodium sulfate), filtered, and concentrated. The residue was chromatographed on a Biotage flash 40 M eluting with 70% hexane/30% ethyl acetate to afford (2S,5S)-5-((trimethylsilyl)ethynyl)pyrrolidine-1,2-dicarboxylate (trans compound R_f of 0.3 in 70% hexane/30% ethyl acetate) and dimethyl (2S,5R)-5-((trimethylsilyl)ethynyl)pyrrolidine-1,2-dicarboxylate (cis compound R_f of 0.2 in 70% hexane/30% ethyl acetate). Data for example **1D**: MS (DCI/NH₃) m/e 284 (M+H)⁺; The compound exists as a mixture of

rotomers. ^1H NMR (300 MHz, CDCl_3): δ ppm 4.60 (d, 1H), 4.51 (d, 1H), 4.30 (d, 1H), 4.24 (d, 1H), 3.62 (s, 3H), 3.59 (s, 3H), 3.57 (s, 3H), 3.54 (s, 3H), 2.40-2.28 (m, 2H), 2.11-2.04 (m, 2H), 1.90-1.81 (m, 4H), 0.0 (s, 18H). Data for example **1C**: MS (DCI/ NH_3) m/e 284 ($\text{M}+\text{H}$) $^+$; ^1H NMR (300 MHz, CDCl_3): δ ppm 4.55-4.40 (m, 1H), 4.20-4.15 (m, 1H), 3.59 (s, 6H), 2.15-1.89 (m, 4H), 0.00 (s, 9H).



methyl (5R)-5-((trimethylsilyl)ethynyl)-L-prolinate (**23**)

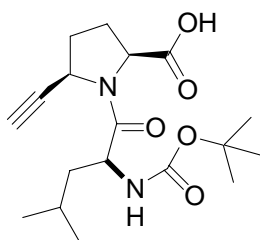
A solution of dimethyl (2S,5R)-5-((trimethylsilyl)ethynyl)pyrrolidine-1,2-dicarboxylate (5.43 g, 19.16 mmol) and iodotrimethylsilane (3 mL, 28.74 mmol) in chloroform (100 mL) was heated to 65 °C for 3 hours, was cooled to room temperature, concentrated under reduced pressure and flash chromatographed with 35% ethyl acetate/65% hexane to provide the titled compound. MS (DCI/ NH_3) m/e 226 ($\text{M}+\text{H}$) $^+$.



methyl *N*-(*tert*-butoxycarbonyl)-L-leucyl-(5R)-5-((trimethylsilyl)ethynyl)-L-prolinate

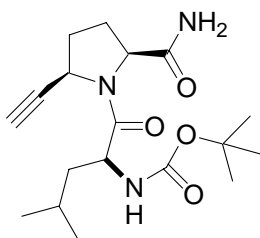
To a solution of methyl (5R)-5-((trimethylsilyl)ethynyl)-L-prolinate (1.6 grams, 7.48 mmol), dimethylaminopyridine (913 mg, 7.48 mmol), *N*-methylmorpholine (1.23 mL, 11.22 mmol), and *N*-(*tert*-butoxycarbonyl)-L-leucine monohydrate (2.24 g, 8.98 mmol) in dichloromethane (30 mL) at room temperature was added 1-(3-dimethylaminopropyl)-3-

ethylcarbodiimide hydrochloride (1.72 g, 8.98 mmol). The resulting mixture was stirred 16 hours at room temperature, and partitioned between ethyl acetate (200 mL) and 1 M HCl (200 mL). The aqueous layer was further extracted with ethyl acetate (200 mL). The combined organic layers were dried (sodium sulfate), filtered, and concentrated. The residue was chromatographed with a Biotage 40 M cartridge with 40% ethyl acetate/hexane provide the titled compound. MS (DCI/NH₃) m/e 439 (M+H)⁺.



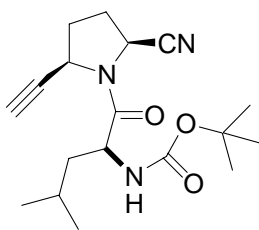
N-(*tert*-butoxycarbonyl)-L-leucyl-(5R)-5-ethynyl-L-proline

To a solution of methyl *N*-(*tert*-butoxycarbonyl)-L-leucyl-(5R)-5-((trimethylsilyl)ethynyl)-L-prolinate (1.24 g, 2.83 mmol) in dioxane (12 mL) at room temperature was added a solution of 2 M lithium hydroxide (3 mL, 6.0 mmol). The resulting mixture was stirred at room temperature for 6 hours. The reaction was diluted with 1 M HCl solution (50 mL), and the aqueous mixture was extracted with ethyl acetate (3 X 50 mL). The combined organic layers were dried (sodium sulfate), filtered, and concentrated to provide the titled compound which was used in the next experiment without further purification.



N-(*tert*-butoxycarbonyl)-L-leucyl-(5R)-5-ethynyl-L-prolinamide

To a cold (0 °C) solution of *N*-(*tert*-butoxycarbonyl)-L-leucyl-(5*R*)-5-ethynyl-L-proline (2.83 mmol) and *N*-methyl morpholine (0.39 mL, 3.50 mmol) in THF (15 mL) was added isobutyl chloroformate (0.42 mL, 3.25 mmol). The resulting cloudy white mixture was stirred at 0 °C for 30 minutes followed by the addition of a solution of ammonia (0.5 *M* in dioxane, 10.0 mL, 5.0 mmol). The solution was allowed to warm to room temperature and stirred for 16 hours. The reaction mixture was diluted by the addition of 1 *M* HCl (100 mL), and extracted with ethyl acetate (3 X 100 mL). The combined organic layers were dried (sodium sulfate), filtered, and concentrated to provide the titled compound which was used in the next experiment without further purification.



N-(*tert*-butoxycarbonyl)-L-leucyl-(5*R*)-5-ethynyl-L-pyrrolidine-2-carbonitrile

To a cold solution (-40 °C) of *N*-(*tert*-butoxycarbonyl)-L-leucyl-(5*R*)-5-ethynyl-L-prolinamide (1.07 g, 3.05 mmol) and imidazole (208 mg, 3.05 mmol) in pyridine (15 mL) was added POCl₃ (0.57 mL, 6.10 mmol) via syringe. The resulting mixture was stirred, maintaining the temperature below -20 °C, for 1 hour followed by the addition of 1 *M* HCl (100 mL). The aqueous mixture was extracted with ethyl acetate (3 X 100 mL), and the combined organic layers were dried (sodium sulfate), filtered, concentrated and chromatographed with 30% ethyl acetate/hexane to provide the titled compound. MS (DCI/NH₃) *m/e* 334 (M+H)⁺; ¹H NMR (300 MHz, CDCl₃): δ ppm 5.15 (t, 1H), 4.95 (d, 1H), 4.71 (t, 1H), 4.53 (ddd, 1H), 2.50 (d, 1H), 2.50-2.28 (m, 4H), 1.75-1.57 (m, 3H).

Compound **14** (2S,5R)-5-ethynyl-1-L-leucylpyrrolidine-2-carbonitrile hydrochloride

To a solution of *N*-(*tert*-butoxycarbonyl)-L-leucyl-(5R)-5-ethynyl-L-pyrrolidine-2-carbonitrile (250 mg) in ether (1 mL) was added 4 M HCl in dioxane (2 mL). The resulting mixture was stirred at room temperature for 5 hours and the solvents removed under reduced pressure. The white solid was triturated with ether to provide the titled compound. MS (DCI/NH₃) *m/e* 234 (M+H)⁺; ¹H NMR (300 MHz, MeOH-*d*₄): δ ppm 5.11 (d, 1H), 4.82 (m, 1H), 4.42 (dd, 1H), 3.19 (d, 1H), 2.58-2.56 (m, 1H), 2.49-2.24 (m, 3H), 2.05-1.95 (m, 1H), 1.87-1.81 (m, 2H), 1.08 (d, 3H), 1.04 (d, 3H).

(2S,5S)-1-L-leucyl-5-methylpyrrolidine-2-carbonitrile **15**

¹H NMR (400MHz, MeOH-*d*₄) δ 4.79 (dd, 1H, J = 8.1 Hz), 4.40 (m, 1H), 4.30 (dd, 1H, J = 4.1, 9.4 Hz), 2.37-2.50 (m, 2H), 2.08 (m, 1H), 1.75-1.92 (m, 3H), 1.66 (m, 1H), 1.36 (d, 3H, J = 6.5 Hz), 1.05 (d, 3H, J = 4.3 Hz), 1.04 (d, 3H, J = 4.3 Hz); ¹³C NMR (100MHz, MeOH-*d*₄) δ 119.9 for CN group; MS (ESI) *m/z* 224 (M+H)⁺.

methyl (5R)-1-{(2S)-2-((*tert*-butoxycarbonyl)amino)-2-cyclopentylethanoyl}-5-((trimethylsilyl)ethynyl)-L-prolinate

To a stirred solution of methyl (5R)-5-((trimethylsilyl)ethynyl)-L-prolinate (1.2 g, 5.32 mmol) in dichloromethane (30 mL) at ambient temperature under nitrogen was added 4-dimethylamino pyridine (0.65 g, 5.32 mmol), 4-methylmorpholine (0.9 mL, 7.98 mmol), 1-(3-(dimethylamino)propyl)-3-ethyl carbodiimide hydrochloride (1.22 g, 6.39 mmol), and (2S)-((*tert*-butoxycarbonyl)amino)(cyclopentyl)acetic acid dicyclohexylamine salt (1.55 g, 6.39 mmol). The reaction mixture was stirred at room temperature for 16 hours,

diluted with ethyl acetate and washed with 1 M HCl. The aqueous layer was further extracted with ethyl acetate (2X) and the combined ethyl acetate layers were dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography 30% ethyl acetate/hexane to provide the titled compound. MS (CI) m/e 451 ($\text{M}+\text{H}$)⁺.

To a stirred solution of methyl (5R)-1-{(2S)-2-((*tert*-butoxycarbonyl)amino)-2-cyclopentylethanoyl}-5-((trimethylsilyl)ethynyl)-L-prolinate (1.65 g, 3.66 mmol) in MeOH (10 mL) and H₂O (10 mL) at room temperature was added LiOH•H₂O (0.23 g, 5.49 mmol). The reaction mixture was stirred at room temperature for 16 hours and concentrated under reduced pressure. The residue was taken up in water and extracted with diethyl ether (2X). The aqueous layer was acidified to pH ~ 4 by adding 4% KHSO₄ dropwise. The clear solution was extracted with ethyl acetate (3X) and the combined ethyl acetate layers were washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to provide the titled compound. MS (CI) m/e 365 ($\text{M}+\text{H}$)⁺.

(5R)-1-{(2S)-2-((*tert*-butoxycarbonyl)amino)-2-cyclopentylethanoyl}-5-ethynyl-L-prolinamide

To a stirred solution of (5R)-1-{(2S)-2-((*tert*-butoxycarbonyl)amino)-2-cyclopentylethanoyl}-5-ethynyl-L-proline (1.28 g, 3.38 mmol) in THF (25 mL) at -15 °C under nitrogen, was added 4-methylmorpholine (0.44 mL, 4.05 mmol), and isobutylchloroformate (0.5 mL, 3.72 mmol) over 2 minutes. The reaction mixture was stirred at -15 °C under nitrogen for 30 minutes, and a solution of 0.5 M NH₃ in dioxane (34 mL, 16.90 mmol) was added. The reaction mixture diluted with water, the pH

adjusted to 4 by the addition of 4% KHSO₄ and extracted with ethyl acetate (3X). The combined extracts were washed with brine, dried (Na₂SO₄), filtered and evaporated. The residue was purified by flash column chromatography (5% MeOH/CH₂Cl₂) to provide the titled compound. MS (CI) m/e 364 (M+H)⁺.

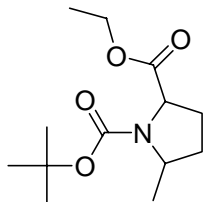
(5R)-1-{(2S)-2-((*tert*-butoxycarbonyl)amino)-2-cyclopentylethanoyl}-5-ethynyl-L-pyrrolidine-2-carbonitrile

Trifluoroacetic anhydride (0.086 mL, 0.605 mmol), was added dropwise to a stirred ice-cooled solution of (5R)-1-{(2S)-2-((*tert*-butoxycarbonyl)amino)-2-cyclopentylethanoyl}-5-ethynyl-L-prolinamide (200 mg, 0.55 mmol) in anhydrous dioxane (7 mL) and anhydrous pyridine (0.089 mL, 1.1 mmol) at such a rate that the temperature was kept below 5 °C. The reaction mixture was allowed to warm to room temperature and stirred for 16 hours. Ice was added to the residue, the solid product was collected by filtration and washed with water. Purification by flash chromatography (30% ethyl acetate/hexane) provided the titled compound. MS (CI) m/e 346 (M+H)⁺.

Compound **16** (2S,5R)-1-((2S)-2-amino-2-cyclopentylethanoyl)-5-ethynylpyrrolidine-2-carbonitrile hydrochloride

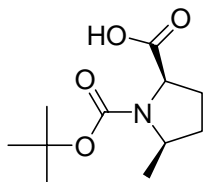
(5R)-1-{(2S)-2-((*tert*-butoxycarbonyl)amino)-2-cyclopentylethanoyl}-5-ethynyl-L-pyrrolidine-2-carbonitrile (0.03 mg, 0.087 mmol), and 4 M HCl in dioxane (0.15 mL, 0.6 mmol) were stirred at room temperature for 2 hours and evaporated under reduced

pressure. Diethyl ether was added to the residue, and the formed precipitate filtered. The solid was washed with diethyl ether (3x15 mL). The precipitate was dried *in vacuo* to provide the titled compound. MS (CI) m/z 246 (M+H)⁺; ¹H NMR (300 MHz, MeOH-*d*₄): δ ppm 5.05 (m, 1H) , 4.8 (t, 1H), 4.3 (d, 1H) 3.19 (d, 1H), 2.4-2.68 (m, 3H), 2.2-2.28 (m, 2H), 1.4-1.9 (m, 8H).



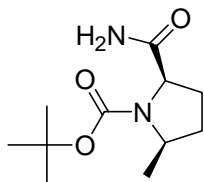
5-Methyl-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-ethyl ester

The ethyl (5S)-5-methyl-L-prolinate was prepared as the trifluoroacetic acid salt as described in Example 6 by substituting methyl magnesium bromide for ethyl magnesiumbromide. Ethyl (5S)-5-methyl-L-prolinate trifluoroacetic acid salt (18.08 mmol), triethyl amine (36.16 mmol) and DMAP (0.906 mmol) were mixed in 40 mL of dichloromethane and then cooled to 0 °C. Boc₂O (19.89 mmol) was added and the mixture was stirred overnight. The mixture was then diluted with dichloromethane, washed with 1N HCl and then with saturated NaHCO₃ solution. The organic layer was dried with Na₂SO₄, and then concentrated. The crude product was purified by chromatography (silica gel, 50% then 75-80% EtOAc/hexane) to give the desired titled compound. MS (CI) m/z +258 (M+H)⁺. (α)_D²⁰ = -35.9 (c 1.45, MeOH).



(2S,5S)-5-Methyl-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester

5-Methyl-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-ethyl ester (3.69 g, 14.34 mmol) in 15 mL of EtOH was treated with 14.3 mL of 1.7 N LiOH solution at room temperature. After 4 h, the mixture was concentrated, acidified with 1N HCl and then extracted with EtOAc (3X). The combined organic extracts were dried with Na₂SO₄, and then concentrated to give the crude acid. MS (ESI) m/z 228 (M-H)⁻.



(2S,5S)-2-Carbamoyl-5-methyl-pyrrolidine-1-carboxylic acid tert-butyl ester

(2S,5S)-5-Methyl-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester (2.055g, 8.96 mmol) and Et₃N (2.24 mL, 1.8 equiv.) were mixed in 15 mL of THF and then cooled to 0 °C. Isobutyl chloroformate (1.51 mL, 1.3 equiv.) was added. After stirring for 35 min, 0.5 M NH₃ in dioxane (35.8 mL, 2 equiv.) was added. After stirring at 0 °C for 3 h, the mixture was warmed to room temperature and stirred overnight. The volatiles were evaporated, and 1N HCl was added. The mixture was extracted with EtOAc (3X). The combined organic extracts were dried with Na₂SO₄ and then concentrated. The crude product was purified by chromatography (silica gel, 50% then 75-80% EtOAc/hexane) to give the desired amide. MS (ESI) m/z 229 (M+H)⁺.

(2S,5S)-5-Methyl-pyrrolidine-2-carboxylic acid amide trifluoroacetate

(2S,5S)-2-Carbamoyl-5-methyl-pyrrolidine-1-carboxylic acid tert-butyl ester (2.03 g, 8.89 mmol) in 4 mL of CH₂Cl₂ was treated with 6 mL of TFA at room temperature. After stirring for 5 h, toluene was added to azeotrope off all the volatiles to afford the crude amine. MS (CI) m/z 129 (M+H)⁺.

((1S)-2-((2S,5S)-2-Carbamoyl-5-methyl-pyrrolidin-1-yl)-1-cyclopentyl-2-oxo-ethyl)-
carbamic acid tert-butyl ester

(2S,5S)-5-Methyl-pyrrolidine-2-carboxylic acid amide trifluoroacetate (296 mg, 0.66 mmol), *L*-tert-butoxycarbonylamino-cyclopentyl-acetic acid dicyclohexylamine salt (308 mg, 0.726 mmol), and TBTU (275 mg, 0.858 mmol) were mixed in 2.5 mL of DMF. Then 0.275 mL of NEt₃ (1.98 mmol) was added. Approximately another 0.1 mL of NEt₃ was added until the pH of the mixture reached 6-7 (by wet pH paper). The mixture was stirred for 10 h, then purified by reverse-phase HPLC to give the desired amide (195 mg, 84%). (ESI) m/z 354 (M+H)⁺.

((1S)-2-((2S,5S)-2-Cyano-5-methyl-pyrrolidin-1-yl)-1-cyclopentyl-2-oxo-ethyl)-
carbamic acid tert-butyl ester

The dehydration of the above amide was performed in a similar manner as described for (5R)-1-{(2S)-2-((*tert*-butoxycarbonyl)amino)-2-cyclopentylethanoyl}-5-ethynyl-L-pyrrolidine-2-carbonitrile to provide the desired nitrile. MS (ESI) m/z 336 (M+H)⁺.

(2S,5S)-1-((2S)-2-amino-2-cyclopentylethanoyl)-5-methylpyrrolidine-2-carbonitrile **17**

The removal of Boc group was performed in a similar manner as described for (5R)-1-{(2S)-2-((*tert*-butoxycarbonyl)amino)-2-cyclopentylethanoyl}-5-ethynyl-L-pyrrolidine-2-carbonitrile to give **17**. ¹H NMR (400 MHz, MeOH-d₄) δ 1.37 (d, *J*=6.75 Hz, 3 H) 1.43 (m, 2 H) 1.6-1.8 (m, 6 H) 1.90 (m, 1 H) 2.13 (ddd, *J*=12.12, 7.36, 4.76 Hz, 3 H) 2.39 (m, 4 H) 4.11 (d, *J*=8.29 Hz, 1 H) 4.40 (m, 1 H) 4.78 (t, *J*=8.44 Hz, 1 H). MS (ESI) m/z 236 (M+H)⁺.

dimethyl (2S,5R)-5-propynyl-pyrrolidine-1,2-dicarboxylate

To a cold -45 °C solution of dimethyl (2S)-5-methoxypyrrolidine-1,2-dicarboxylate (10 g, 46.08 mmol) and trimethylsilylpropyne (14.24 mL, 92.16 mmol, 2.0 equiv) in methylene chloride (180 mL) was added a solution of tin (IV) chloride (1 M in methylene chloride, 60.0 mL, 60.0 mmol, 1.3 equiv) dropwise via an addition funnel over 30 minutes. To the dark yellow solution was added solid aluminum chloride (8.58 g, 64.52 mmol, 1.4 equiv) in one portion. The resulting mixture was allowed to warm to room temperature and stirred at room temperature for 48 hours. The reaction mixture was carefully quenched by saturated aqueous NH₄OH (100 mL) with ice cooling. A white precipitate formed and was removed by filtration. The crude product was obtained after concentration. The residue was chromatographed on a Biotage flash 40 M eluting with 70% hexane/30% ethyl acetate to afford the titled compound. MS (DCI/NH₃) m/e 226 (M+H)⁺.

methyl (5R)-5-propynyl-L-prolinate **9**

A solution of dimethyl (2S,5R)-5-propynyl-pyrrolidine-1,2-dicarboxylate (4.25 g, 18.90 mmol) and iodotrimethylsilane (3.23 mL, 22.7 mmol) in chloroform (60 mL) was heated to 65 °C for 3 hours, was cooled to room temperature, concentrated under reduced pressure and flash chromatographed with 35% ethyl acetate/ 65% hexane to provide the titled compound. MS (DCI/NH₃) m/e 168 (M+H)⁺.

Methyl-*N*-(*tert*-butoxycarbonyl)-cyclopentyl-L-glycyl-(5R)-5-propynyl-L-prolinate

To a solution of methyl (5R)-5-propynyl-L-prolinate (334 mg, 2 mmol), dimethylaminopyridine (244 mg, 2 mmol), *N*-methylmorpholine (0.33 mL, 3 mmol), and

Boc-cyclopentyl-L-glycine.dicyclohexylamine (1.02 g, 2.4 mmol) in dichloromethane (10 mL) at room temperature was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (383 g, 2.4 mmol). The resulting mixture was stirred 16 hours at room temperature, and partitioned between ethyl acetate (100 mL) and 1 M HCl (20 mL). The aqueous layer was further extracted with ethyl acetate (100 mL). The combined organic layers were dried (sodium sulfate), filtered, and concentrated. The residue was chromatographed with 70% ethyl acetate/hexane provide the titled compound (67%). MS (ESI) m/e 393 (M+H)⁺.

N-(*tert*-butoxycarbonyl)-cyclopentyl-L-glycyl-(5R)-5-propynyl-L-proline

To a solution of methyl *N*-(*tert*-butoxycarbonyl)-cyclopentyl-L-glycyl-(5R)-5-propynyl-L-prolinate (1.34 g, 3.42 mmol) in THF (10 mL)/water (5 mL) at room temperature was added lithium hydroxide (358 mg, 7.52 mmol). The resulting mixture was stirred at room temperature for 6 hours. The reaction was diluted with 1 M HCl solution, and the aqueous mixture was extracted with ethyl acetate (3 X 10 mL). The combined organic layers were dried (sodium sulfate), filtered, and concentrated to provide the titled compound. MS (ESI) m/e 379 (M+H)⁺.

N-(*tert*-butoxycarbonyl)-cyclopentyl-L-glycyl-(5R)-5-propynyl-L-prolinamide

To a cold (0 °C) solution of *N*-(*tert*-butoxycarbonyl)-cyclopentyl-L-glycyl-(5R)-5-propynyl-L-proline (3.42 mmol) and N-methyl morpholine (0.44 mL, 3.94 mmol) in THF (15 mL) was added isobutyl chloroformate (0.56 mL, 4.26 mmol). The resulting cloudy white mixture was stirred at 0 °C for 30 minutes followed by the addition of a solution of ammonia (0.5 M in dioxane, 19.7 mL, 9.84 mmol). The solution was allowed to warm to room temperature and stirred for 16 hours. The reaction mixture was diluted

by the addition of 1 M HCl (50mL), and extracted with ethyl acetate (3 X 100 mL). The combined organic layers were dried (sodium sulfate), filtered, and concentrated to provide the crude compound. The residue was chromatographed with 95% CH₂Cl₂/MeOH to provide the titled compound. MS (ESI) m/e 378 (M+H)⁺.

N-(*tert*-butoxycarbonyl)-cyclopentyl-L-glycyl-(5R)-5-propynyl-L-pyrrolidine-2-carbonitrile

To a cold solution (-35 °C) of *N*-(*tert*-butoxycarbonyl)-cyclopentyl-L-glycyl-(5R)-5-propynyl-L-prolinamide (280 mg, 0.743 mmol) and imidazole (51 mg, 0.743 mmol) in pyridine (6 mL) was added POCl₃ (0.14 mL, 1.49 mmol) via syringe. The resulting mixture was stirred, maintaining the temperature below -20 °C, for 1 hour followed by the addition of 1 M HCl (10 mL). The aqueous mixture was extracted with ethyl acetate (3 X 50 mL), and the combined organic layers were dried (sodium sulfate), filtered, concentrated and chromatographed with 30% ethyl acetate/hexane to provide the titled compound. MS (ESI) m/e 348 (M+H)⁺; ¹H NMR (300 MHz, CDCl₃) δ ppm 5.15 (t, 1H), 4.95 (d, 1H), 4.71 (t, 1H), 4.53 (ddd, 1H), 2.50 (d, 1H), 2.50-2.28 (m, 4H), 1.75-1.57 (m, 3H).

(2S,5R)-1-((2S)-2-amino-2-cyclopentylethanoyl)-5-prop-1-ynylpyrrolidine-2-carbonitrile **19**

To a solution of *N*-(*tert*-butoxycarbonyl)-cyclopentyl-L-glycyl-(5R)-5-propynyl-L-pyrrolidine-2-carbonitrile (490 mg) in ether (1 mL) was added 4 M HCl in dioxane (8mL). The resulting mixture was stirred at room temperature for 2 hours and the solvents removed under reduced pressure. The white solid was triturated with ether to provide the titled compound. MS (ESI) m/e 260 (M+H)⁺; ¹H NMR (DMSO) δ ppm 5.16

(m, 1H), 4.73 (m, 1H), 4.04 (m, 1H), 3.42 (m, 1H), 2.03-2.47 (m, 4H), 1.85 (d, 3H), 1.39-1.70 (8H,m).

(2S,5R)-1-((2S)-2-amino-2-cyclohexylethanoyl)-5-ethynylpyrrolidine-2-carbonitrile hydrochloride **20**

MS (CI) m/e 260 (M+H)⁺; ¹H NMR (300 MHz, MeOH-*d*₄): δ ppm 5.58 (m, 1H), 5.0 (t, 1H), 4.24 (d, 1H), 3.2 (d, 1H), 2.3-2.42 (m, 3H), 1.8-1.84 (m, 2H), 1.12-1.4 (m, 10H).

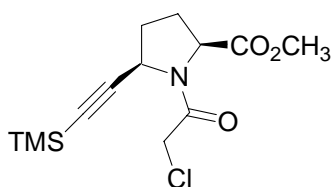
tert-butyl (1S)-2-((2S,5R)-2-cyano-5-vinylpyrrolidin-1-yl)-1-cyclopentyl-2-oxoethylcarbamate

(2S,5R)-1-((2S)-2-((*tert*-butoxycarbonyl)amino)-2-cyclopentylethanoyl)-5-ethynyl-L-pyrrolidine-2-carbonitrile (60 mg) was stirred under hydrogen (60 psi) at room temperature for 7 minutes in a mixture of quinoline (66 uL) and ethyl acetate (6 mL) using 5% Pd/BaSO₄ (24 mg). The mixture was filtered and the filtrate concentrated under reduced pressure. Purification by flash column chromatography with 20% ethyl acetate / hexane to provide the titled compound. MS (CI) m/e 348 (M+H)⁺.

(2S,5R)-1-((2S)-2-amino-2-cyclopentylethanoyl)-5-vinylpyrrolidine-2-carbonitrile hydrochloride **18**

The boc was removed in a similar manner that described for (2S,5R)-1-((2S)-2-amino-2-cyclopentylethanoyl)-5-prop-1-ynylpyrrolidine-2-carbonitrile **19**.

MS (CI) m/e 248 (M+H)⁺; ¹H NMR (300 MHz, MeOH-*d*₄): δ ppm 5.98-6.09 (m, 1H), 5.3-5.39 (m, 2H), 4.7-4.8 (m, 1H), 4.1 (d, 1H), 2.38-2.49 (m, 2H), 2.18-2.33 (m, 2H), 1.96-2.02 (m, 2H), 1.58-1.83 (m, 6H), 1.3-1.47 (m, 2H).

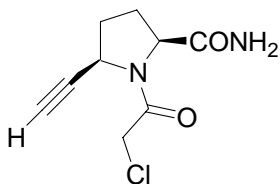


methyl (5R)-1-(chloroacetyl)-5-((trimethylsilyl)ethynyl)-L-prolinate

To a stirred solution of **23** (2.76 g, 12.24 mmol) and triethylamine (2.13 mL, 15.30 mmol) in dry THF (50 mL) at 0°C was gradually added a solution of chloroacetyl chloride (0.97 mL, 12.24 mmol) in dry THF (10 mL). After stirring at room temperature for 2 h, the triethylammonium chloride was filtered off. The solid cake was washed with THF and the filtrate and washings were combined, dried over sodium sulfate and evaporated. The residue was stripped from toluene and dried under reduced pressure to give methyl (5R)-1-(chloroacetyl)-5-((trimethylsilyl)ethynyl)-L-prolinate as a thick syrup, 3.69 g (100% yield). MS (CI+Q1) m/z 302 (M+1)⁺.

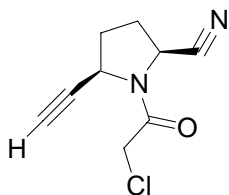
(5R)-1-(chloroacetyl)-5-ethynyl-L-proline

To a stirred solution of methyl (5R)-1-(chloroacetyl)-5-((trimethylsilyl)ethynyl)-L-prolinate (3.69 g, 12.2 mmol) in MeOH (24 mL) and H₂O (24 mL) at room temperature was added LiOH•H₂O (0.8 g, 18 mmol). The reaction mixture was stirred at ambient temperature overnight and concentrated under reduced pressure. The residue was taken up in water and extracted with diethyl ether (2X). The aqueous layer was acidified to pH ~ 3 by adding 4% KHSO₄ dropwise. The solution was extracted with ethyl acetate (3X). Combined ethyl acetate layers were washed with brine, dried (Na₂SO₄), filtered and concentrated under reduced pressure to provide the titled compound. MS (CI) m/z 216 (M+1)⁺.



(5R)-1-(chloroacetyl)-5-ethynyl-L-prolinamide

To a stirred solution of example 8C (1.89 g, 8.765 mmol) in THF (50 mL) at -15°C under nitrogen was added 4-methylmorpholine (1.16 mL, 10.52 mmol), and then isobutylchloroformate (1.25 mL, 9.64 mmol) over 3 min. A white precipitate was formed. The reaction mixture was stirred at -15°C under nitrogen for 30 min and a solution of NH_3 in dioxane (0.5M, 88 mL, 43.82 mmol) was added. The reaction mixture was stirred at -15°C for 30 min, warmed to rt and stirred at rt overnight. The reaction mixture was quenched by 4% KHSO_4 to $\sim \text{pH } 4$ and extracted with EtOAc (3X). The extracts were combined, washed with brine, dried (Na_2SO_4) and evaporated. Purification by flash column chromatography (60-75% EtOAc / hexane) gave the desired compound as a white solid, 1.26 g, 67 % yield. MS (CI+Q1) m/z 215 ($\text{M}+1$)⁺.



(2S,5R)-1-(chloroacetyl)-5-ethynylpyrrolidine-2-carbonitrile

To a stirred solution of example 8D (0.16 g, 0.745 mmol) and imidazole (0.05 g, 0.745 mmol) in dry pyridine (4 mL) at -35°C under nitrogen was added POCl_3 (0.15 mL, 1.49 mmol), dropwise. The reaction mixture was stirred between -35°C to -15°C for 1 h and evaporated. The residue was diluted with CH_2Cl_2 and washed with H_2O (2X), dried (Na_2SO_4) and evaporated. Purification by flash chromatography (10% EtOAc/hexane) gave the titled compound as a colorless oil, 60 mg, 41% yield. MS (CI+Q1) m/z 197

(M+1)⁺. ¹H NMR (300 MHz, DMSO) δ 4.74 (m, 2H), 4.36 (d, 1H), 4.18 (d, 1H), 2.55 (d, 1H), 2.46-2.34 (m, 4H).

(2S,5R)-1-(N-cyclopentylglycyl)-5-ethynylpyrrolidine-2-carbonitrile 28

To a stirred solution of (2S,5R)-1-(chloroacetyl)-5-ethynylpyrrolidine-2-carbonitrile (0.04 g, 0.203 mmol) in acetonitrile (3 mL) at ambient temperature under nitrogen was added cyclopentylamine (0.04 mL, 0.406 mmol). The reaction mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was flash chromatographed with 3% MeOH/CH₂Cl₂ to provide the desired compound as a pale yellow oil. MS (DCI) m/z 246 (M+H)⁺; ¹H NMR (300 MHz, DMSO) δ 1.5-2 (8H, m), 2.11-2.21 (2H, m), 2.45-2.48 (2H, m), 3.78 (1H, d), 3.8-4.5 (2H, m), 4.53-4.55 (1H, t), 5.01 (1H, m), 5.05 (1H, m).

(2S,5R)-1-(N-cyclohexylglycyl)-5-ethynylpyrrolidine-2-carbonitrile 29

To a stirred solution of (2S,5R)-1-(chloroacetyl)-5-ethynylpyrrolidine-2-carbonitrile (0.035 g, 0.178 mmol) in acetonitrile (3 mL) at room temperature under nitrogen was added cyclohexylamine (0.041 mL, 0.356 mmol). The reaction mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was flash chromatographed with 2-3% MeOH/CH₂Cl₂ to provide the desired compound as a pale yellow oil. MS (DCI) m/z 260 (M+H)⁺; ¹H NMR (300 MHz, DMSO) δ 1.5-2 (10H, m), 2.13-2.31 (2H, m), 2.41-2.48 (2H, m), 3.76 (1H, d), 3.8-4.5 (2H, m), 4.53-4.55 (1H, t), 4.9 (1H, m), 5.05 (1H, m).

(2S,5R)-5-ethynyl-1-(N-trans(4-hydroxycyclohexyl)glycyl)pyrrolidine-2-carbonitrile

To a stirred solution of (2S,5R)-1-(chloroacetyl)-5-ethynylpyrrolidine-2-carbonitrile (0.030 g, 0.15 mmol) in acetonitrile (1 mL) at room temperature was added trans-4-aminocyclohexanol (35 mg, 0.31 mmol). The reaction mixture was stirred at room temperature for 18 hours, concentrated under reduced pressure and purified by flash chromatography with 3% methanol:dichloromethane to provide the titled compound. MS (ESI) m/z 276 (M+H)⁺; ¹H NMR (CDCl₃) δ 4.73 (m, 1H), 4.60 (m, 1H), 3.62 (m, 2H), 3.60 (m, 1H), 2.52 (d, 1H), 2.48 (m, 1H), 2.38 (m, 2H), 1.98 (m, 4H), 1.25 (m, 4H).

6-(4- trans-aminocyclohexyloxy)-nicotinonitrile

To a stirred solution of trans-4-aminocyclohexanol (115mg, 1mmol) in DMF (6 mL) at 0 °C was added 60% NaH in mineral oil (120 mg, 3 mmol). The reaction mixture was stirred at 0 °C for ½ hour and then 2-chloro-5-cyanopyridine (151 mg, 1.25 mmol) was added. It was heated to 60 °C for 2 hours and stirred for 12 hours at room temperature. The reaction mixture was diluted with ethyl acetate and washed with water (3 times) and brine. The organic layer was dried (sodium sulfate), filtered, and concentrated under reduced pressure to provide the titled compound. MS (DCI) m/z 218 (M+H)⁺.

(2S,5R)-5-ethynyl-1-{N-(4-trans(5-cyano-pyridin-2-yloxy)cyclohexyl)glycyl}pyrrolidine-2-carbonitrile **31**

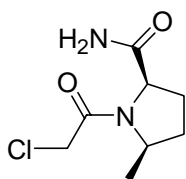
To a stirred solution of (2S,5R)-1-(chloroacetyl)-5-ethynylpyrrolidine-2-carbonitrile (0.03 g, 0.15 mmol) in acetonitrile (1 mL) at room temperature was added 6-(4-aminocyclohexyloxy)-nicotinonitrile (66 mg, 0.31 mmol). The reaction mixture was stirred at room temperature for 18 hours, concentrated under reduced pressure and purified by flash chromatography with 3% methanol:dichloromethane to provide the

titled compound. MS (ESI) m/z 377 (M+H)⁺; ¹H NMR (DMSO-*d*₆) δ 8.69 (m, 1H), 8.15 (m, 1H), 6.96 (m, 1H), 4.99 (m, 1H), 4.86 (m, 1H), 4.35 (m, 1H), 3.88 (m, 1H), 3.19 (m, 2H), 2.29 (m, 2H), 2.25 (d, 1H), 2.18 (m, 6H), 1.54 (m, 4H).

(2S,5R)-1-(N-(tert-butyl)glycyl)-5-ethynylpyrrolidine-2-carbonitrile **32**

To a stirred solution of (2S,5R)-1-(chloroacetyl)-5-ethynylpyrrolidine-2-carbonitrile (0.04 g, 0.203 mmol) in acetonitrile (3 mL) at room temperature under nitrogen was added tert-butylamine (0.043 mL, 0.406 mmol). The reaction mixture was stirred at ambient temperature overnight. The reaction mixture was concentrated under reduced pressure. The residue was flash chromatographed with 3% MeOH/CH₂Cl₂ to provide the desired compound as a pale yellow oil. MS (DCI) m/z 234 (M+H)⁺.

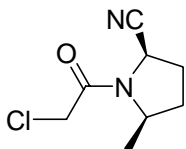
The free base and 1 M HCl in ether were stirred at room temperature for 2 h and then concentrated under reduced pressure. The residue was triturated with diethyl ether to provide the desired hydrochloride salt as a white powder. ¹H NMR (DMSO-*d*₆) (major rotamer) δ 5.20 (m, 1H), 4.86 (m, 1H), 4.33 (s, 1H), 3.78 (s, 1H), 3.76 (s, 1H), 2.45 (m, 1H), 2.26 (m, 1H), 2.31 (m, 1H), 2.13 (m, 1H), 1.33 (s, 1H).



(2S,5S)-1-(2-chloroacetyl)-5-methylpyrrolidine-2-carboxylic acid amide

(2S,5S)-5-methylpyrrolidine-2-carboxylic acid amide trifluoroacetate (2.0 g, 8.2 mmol) and triethylamine (1.9 mL, 24.6 mmol) were dissolved in CH₂Cl₂ (6 mL), and the mixture was cooled to 0 °C. Chloroacetyl chloride (0.39 mL, 9.0 mmol) was added

slowly via syringe. After 2 h, saturated NaHCO₃ was added, and the mixture was extracted with EtOAc (3X). The combined organic extracts were dried (Na₂SO₄), concentrated and purified by flash chromatography (10% MeOH/EtOAc) to give the title compound. MS (DCI) m/z 205 (M+H)⁺.



(2S,5S)-1-(2-Chloro-acetyl)-5-methyl-pyrrolidine-2-carbonitrile

(2S,5S)-1-(2-Chloro-acetyl)-5-methyl-pyrrolidine-2-carboxylic acid amide (0.25 g, 1.2 mmol) and imidazole (85 mg, 1.25 mmol) were mixed in pyridine (5 mL). The mixture was cooled to –35 °C and POCl₃ (0.18 mL, 1.8 mmol) was added slowly. The mixture was stirred at –35 °C for 1 h. Saturated NH₄Cl (20 mL) was added, and the mixture was extracted with EtOAc (3x). The combined organic layers were dried (Na₂SO₄), filtered and concentrated under reduced pressure. The oil was purified by flash chromatography (hexane: EtOAc, 2:1) to yield the title compound. MS (DCI) m/z 187 (M+H)⁺.

(2S,5S)-1-(N-cyclopentylglycyl)-5-methylpyrrolidine-2-carbonitrile

(2S,5S)-1-(2-Chloro-acetyl)-5-methyl-pyrrolidine-2-carbonitrile (50 mg, 0.30 mmol) and cyclopentylamine (0.059 mL, 0.6 mmol) were dissolved in acetonitrile (2 mL) and stirred overnight. The product was purified by reverse phase HPLC eluting with 0% to 70% acetonitrile/ 0.1% aqueous trifluoroacetic acid. ¹H NMR (300 MHz, methanol-*d*₄) δ ppm 1.36 (d, *J*=6.44 Hz, 3 H), 1.68 (m, 5 H), 1.85 (m, 3 H), 2.16 (m, 3 H), 2.38 (m, 3 H), 3.60 (m, 1 H), 4.20 (m, 1 H), 4.77 (m, 1 H). MS (ESI) m/z 236 (M+H)⁺.

methyl (5R)-1-(chloroacetyl)-5-propynyl-L-prolinate

To a stirred solution of methyl (5R)-5-propynyl-L-prolinate (1.5 g, 8.98 mmol, Example 85B) and triethylamine (1.87 mL, 13.47 mmol) in dry tetrahydrofuran (20 mL) at 0 °C was gradually added chloroacetyl chloride (0.86 mL, 10.78 mmol). After stirring at room temperature for 2 hours, the mixture was filtered. The solid cake was washed with THF, and the filtrate and washings were combined, dried over sodium sulfate and concentrated under reduced pressure. The residue taken up in toluene and concentrated to dryness under reduced pressure to provide the titled compound. MS (DCI) m/z 244 (M+H)⁺.

(5R)-1-(chloroacetyl)-5-propynyl-L-proline

To a stirred solution of methyl (5R)-1-(chloroacetyl)-5-propynyl-L-prolinate (1.26 g, 5.19 mmol) in THF (12 mL) and H₂O (6 mL) at room temperature was added LiOH•H₂O (326 mg, 7.78 mmol). The reaction mixture was stirred at ambient temperature overnight and concentrated under reduced pressure. The reaction mixture was acidified to pH ~ 3 by adding 1M HCl dropwise. The solution was extracted with ethyl acetate (3X). Combined ethyl acetate layers were washed with brine, dried (Na₂SO₄), filtered and concentrated under reduced pressure to provide the titled compound. MS (DCI) m/z 230 (M+H)⁺.

(5R)-1-(chloroacetyl)-5-propynyl-L-prolinamide

To a stirred solution of (5R)-1-(chloroacetyl)-5-propynyl-L-proline (1.10 g, 4.8 mmol) in CH₂Cl₂ (24 mL) at -15 °C under nitrogen was added 4-methylmorpholine (0.64 mL, 5.76 mmol), and then isobutyl chloroformate (0.81 mL, 6.24 mmol) over 10 minutes.

A white precipitate formed. The reaction mixture was stirred at $-15\text{ }^{\circ}\text{C}$ under nitrogen for 30 minutes, and a solution of NH_3 in dioxane (0.5 M , 29 mL, 14.4 mmol) was added. The reaction mixture was stirred at $-15\text{ }^{\circ}\text{C}$ for 30 minutes, warmed to room temperature, and stirred at that temperature for 16 hours. The reaction mixture was diluted with 1M HCl to $\sim\text{pH }4$ and extracted with ethyl acetate (3X). The extracts were combined, washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by flash column chromatography (60-75% ethyl acetate /hexane) provided the titled compound. MS (DCI) m/z 229 ($\text{M}+\text{H}$) $^{+}$.

(2S,5R)-1-(chloroacetyl)-5-propynylpyrrolidine-2-carbonitrile

To a stirred solution of (5R)-1-(chloroacetyl)-5-propynyl-L-prolinamide (0.28 g, 1.23 mmol) and imidazole (0.084 g, 1.23 mmol) in dry pyridine (6 mL) at $-35\text{ }^{\circ}\text{C}$ under nitrogen was added POCl_3 (0.23 mL, 2.46 mmol) dropwise. The reaction mixture was stirred between $-35\text{ }^{\circ}\text{C}$ to $-15\text{ }^{\circ}\text{C}$ for 1 hour and evaporated. The residue was diluted with dichloromethane and washed with H_2O (2X), dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by flash chromatography (10% ethyl acetate /hexane) provided the titled compound. MS (DCI) m/z 211 ($\text{M}+\text{H}$) $^{+}$.

(2S,5R)-1-(N-cyclopentylglycyl)-5-prop-1-ynylpyrrolidine-2-carbonitrile **34**

To a stirred solution of (2S,5R)-1-(chloroacetyl)-5-propynylpyrrolidine-2-carbonitrile (0.018 g, 0.086 mmol) in acetonitrile (1 mL) at room temperature was added cyclopentyl amine (0.017 mL, 0.17 mmol). The reaction mixture was stirred at room temperature for 18 hours, concentrated under reduced pressure and purified by flash chromatography with 3% methanol/ 97% dichloromethane to provide the titled compound. MS (ESI) m/z 260 ($\text{M}+\text{H}$) $^{+}$; ^1H NMR (DMSO) δ ppm 4.89 (m, 1H), 4.84

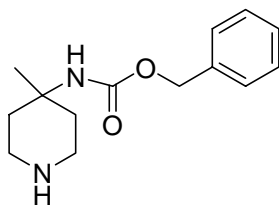
(m, 1H), 4.07-4.13 (m, 2H), 3.52 (m, 1H), 2.05-2.42 (m, 4H), 1.96 (m, 2H), 1.87 (d, 3H), 1.70 (m, 4H), 1.52 (m, 2H).

(2S,5R)-1-(N-1-adamantylglycyl)-5-ethynylpyrrolidine-2-carbonitrile 35

To a stirred solution of (2S,5R)-1-(chloroacetyl)-5-ethynylpyrrolidine-2-carbonitrile (0.03 g, 0.20 mmol) in acetonitrile (3 mL) at room temperature under nitrogen was added 1-adamantanamine (0.06 g, 0.408 mmol). The reaction mixture was stirred for two days and then concentrated under reduced pressure. The residue was flash chromatographed with 2% MeOH/CH₂Cl₂ to provide the titled compound. MS (DCI) *m/z* 312 (M+H)⁺.

-Benzyloxycarbonylamino-4-methyl-piperidine-1-carboxylic acid tert-butyl ester

To a solution of the 4-methyl-piperidine-1,4-dicarboxylic acid mono-tert-butyl ester (5 g, 20.6 mmol) in toluene (40 mL) at 23 °C was added triethylamine (4.3 mL, 31 mmol) and diphenylphosphoryl azide (6.1 mL, 28.0 mmol). The resulting mixture was stirred at 23 °C for 45 minutes after which benzyl alcohol (11.1 mL, 103 mmol) was added. The reaction mixture was then heated to 80 °C for 16 hours. The reaction mixture was allowed to cool to room temperature and the excess solvents were removed under reduced pressure. The crude residue was purified by chromatography (silica gel, eluting with 10% ethyl acetate/90% hexane to 30% ethyl acetate/70% hexane) to provide the titled compound (5.6 grams). MS (CI) *m/z* 249 (M-99)⁺; ¹H NMR (300 MHz, CDCl₃) δ ppm 7.38-7.31 (m, 5H), 5.05 (s, 2H), 4.57 (s, 1H), 3.67-3.60 (m, 2H), 3.18 (m, 2H), 1.98-1.90 (m, 2H), 1.44 (s, 9H), 1.37 (s, 3H).

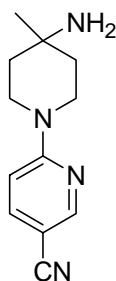


(4-Methyl-piperidin-4-yl)-carbamic acid benzyl ester

A mixture of Example 30A (4.7 g, 13.4 mmol) in 4 M HCl in dioxane (20 mL) was stirred at 23 °C for 12 hours. The dioxane was removed under reduced pressure and the crude solid was triturated several times with diethyl ether. The resulting white solid was dried in a vacuum oven overnight to afford the HCl salt of the titled compound (2.75 g). MS (CI) m/z 249 ($M+1$)⁺; ¹H NMR (300 MHz, methanol-*d*₄) δ ppm 7.37-7.29 (m, 5H), 5.05 (s, 2H), 3.19-3.07 (m, 4H), 1.76-1.70 (m, 4H), 1.37 (s, 3H).

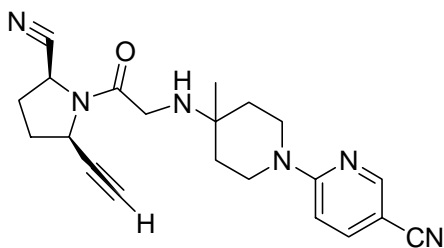
(5'-Cyano-4-methyl-3,4,5,6-tetrahydro-2H-(1,2')bipyridinyl-4-yl)-carbamic acid benzyl ester

A solution of (4-methyl-piperidin-4-yl)-carbamic acid benzyl ester hydrochloride salt (198 mg, 0.697 mmol), 2-chloro-5-cyanopyridine (90 mg, 0.65 mmol) and diisopropylethylamine (400 μ L) in dioxane (2 mL) in a sealed tube was heated to 170 °C under microwave conditions for 20 minutes. The reaction mixture was cooled, concentrated and then purified by chromatography (silica gel, eluting with 10% hexane/ethyl acetate to 60% ethyl acetate/hexane) to provide the titled compound (167 mg) as a white foam. MS (CI) m/z 380 ($M+1$)⁺; ¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 8.45 (s, 1H), 7.80 (dd, 1H), 7.37 (m, 5H), 7.18 (s, 1H), 6.93 (d, 1H), 5.00 (s, 2H), 4.00-3.95 (m, 2H), 3.35-3.26 (m, 2H), 2.19-2.07 (m, 2H), 1.49-1.43 (m, 2H), 1.27 (s, 3H).



4-Amino-4-methyl-3,4,5,6-tetrahydro-2H-(1,2')bipyridinyl-5'-carbonitrile

A solution of (5'-cyano-4-methyl-3,4,5,6-tetrahydro-2H-(1,2')bipyridinyl-4-yl)-carbamic acid benzyl ester (93 mg) and trimethylsilyliodide (62 μ L) in acetonitrile (1.5 mL) was heated to 45 $^{\circ}$ C for 20 minutes. The reaction mixture was cooled and concentrated under reduced pressure. The crude reaction mixture was purified by chromatography (silica gel, eluting with a gradient of 2% MeOH/ CH_2Cl_2 /0.1% NH_4OH to 6% MeOH/ CH_2Cl_2 /0.1% NH_4OH) to provide the titled compound (43 mg) as a yellow foam. MS (CI) m/z 217 ($\text{M}+1$) $^{+}$; ^1H NMR (300 MHz, DMSO) δ ppm 8.44 (s, 1H), 7.77 (d, 1H), 6.91 (d, 1H), 3.86-3.78 (m, 2H), 3.59-3.55 (m, 2H), 1.48-1.38 (m, 4H), 1.08 (s, 3H).



(2*S*,5*R*)-5-ethynyl-1-(*N*-(4-methyl-1-(3-cyano-pyridin-2-yl)piperidin-4-yl)glycyl)pyrrolidine-2-carbonitrile **40**

A mixture of 4-Amino-4-methyl-3,4,5,6-tetrahydro-2H-(1,2')bipyridinyl-5'-carbonitrile (60 mg, 0.31 mmol) and (2*S*,5*R*)-1-(chloroacetyl)-5-ethynylpyrrolidine-2-

carbonitrile (105 mg, 0.49 mmol) in acetonitrile (2 mL) was stirred at 23 °C for 72 hours. The reaction mixture was concentrated under reduced pressure, and the crude residue was purified by chromatography (silica gel, eluting with 96% dichloromethane/4% methanol/0.1% ammonium hydroxide) to provide the titled compound as a white foam. MS (CI) m/z 377 ($M+1$)⁺; ¹H NMR (300 MHz, CDCl₃) δ ppm 8.38 (s, 1H), 7.58 (dd, 1H), 6.59 (d, 1H), 4.74 (t, 1H), 4.63-4.57 (m, 1H), 3.78-3.68 (m, 6H), 2.53 (s, 1H), 2.43-2.34 (m, 2H), 1.69-1.50 (m, 4H), 1.19 (s, 3H).

6-Fluoro-nicotinic acid tert-butyl ester

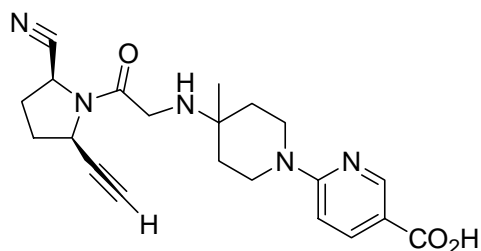
To a stirred and refluxed solution of 6-fluoro-nicotinic acid (0.092 g, 6.52 mmol) in benzene and 2-methyl-propan-2-ol (2:1, 15:7 mL) was added dropwise N,N-dimethylformamide di-*tert*-butyl acetal (8.2 mL, 29.6 mmol). The reaction mixture was refluxed for 3 hours, cooled to room temperature and partitioned between aqueous NaHCO₃ and dichloromethane. The organic layer was washed with water and brine, dried (MgSO₄), filtered, concentrated under reduced pressure and purified by flash chromatography with 15% to 30% ethyl acetate in hexane to provide the titled compound. MS (CI) m/z 197 ($M+1$)⁺; ¹H NMR (300 MHz, CDCl₃) δ ppm 8.82(d, 1H), 8.38(m, 1H), 6.98(d, 1H), 1.64 (s, 9H).

4-Amino-4-methyl-3, 4, 5, 6-tetrahydro-2H-(1,2')bipyridinyl-5'-carboxylic acid tert-butyl ester

To a stirred solution of (4-methyl-piperidin-4-yl)-carbamic acid benzyl ester (0.31 g, 1.28 mmol) in dioxane (5.0 mL) at room temperature was added 6-fluoro-nicotinic acid tert-butyl ester (0.21 g, 1.07 mmol). The reaction mixture was stirred at 80 °C for 18 hours, concentrated under reduced pressure and purified by flash chromatography with

10% to 30% ethyl acetate in hexane to provide 4-benzyloxycarbonylamino-4-methyl-3,4,5,6-tetrahydro-2H-(1,2')bipyridinyl-5'-carboxylic acid tert-butyl ester. MS (CI) m/z 426 ($M+1$)⁺; ¹H NMR (300 MHz, CDCl₃) δ ppm 8.74(d, 1H), 7.97 (dd, 1H), 7.35 (m, 5H), 7.26 (s, 1H), 5.07 (s, 2H), 4.68 (s, 1H), 3.97 (m, 2H), 3.37 (m, 2H), 2.08 (m, 2H), 1.66 (m, 2H), 1.56 (s, 9H), 1.42 (s, 3H).

To a stirred solution of 4-benzyloxycarbonylamino-4-methyl-3,4,5,6-tetrahydro-2H-(1,2')bipyridinyl-5'-carboxylic acid tert-butyl ester (0.29 g, 0.68 mmol) in isopropanol, methanol and ethyl acetate (1:1:1, 5.0 mL) at room temperature was added ammonium formate (0.25 g, 1.07 mmol) and 10% Pd/C (25 mg) under nitrogen. The reaction mixture was stirred at 80 °C for 30 minutes, cooled, filtered through Celite, and concentrated under reduced pressure to provide the titled compound. MS (CI) m/z 292 ($M+1$)⁺; ¹H NMR (300 MHz, methanol-*d*₄) δ ppm 8.61(d, 1H), 7.95 (d,d 1H), 6.79 (d, 1H), 4.80 (s, 2H), 3.85-3.79 (m, 2H), 3.66-3.60 (m, 2H), 1.67-1.59 (m, 4H), 1.58 (s, 9H), 1.23 (s, 3H).



(2S,5R)-5-ethynyl-1-(*N*-(4-methyl-1-(5-carboxy-pyridin-2-yl)piperidin-4-yl)glycyl)pyrrolidine-2-carbonitrile **41**

To a stirred solution of (*2S*, *5R*)-1-(chloroacetyl)-5-ethynylpyrrolidine-2-carbonitrile (0.058 g, 0.30 mmol, Example 8D) in dioxane (3.0 mL) and water (1.0mL) at room temperature was added 4-amino-4-methyl-3,4,5,6-tetrahydro-2H-(1,2')bipyridinyl-5'-carboxylic acid tert-butyl ester (0.170 g, 0.58 mmol). The reaction mixture was stirred

at room temperature for 48 hours, concentrated under reduced pressure and purified by flash chromatography with 5% methanol in dichloromethane. The product was mixed with TFA in dichloromethane(1:1, 6mL), and after 2 h, the volatiles were removed under reduced pressure. The residue was solidified by trituration with diethyl ether to provide the titled compound as the TFA salt. MS (CI) m/z 396 ($M+1$)⁺; ¹H NMR (300 MHz, methanol-*d*₄) δ ppm 8.68(d, 1H), 8.15 (d,d, 1H), 7.03 (d, 1H), 4.84 (m, 2H), 4.47 (d, 2H), 4.32-4.14 (q, 2H), 3.31-3.25 (m, 2H), 3.20 (d, 1H), 2.51-2.23 (m, 5H), 2.06-1.93 (m, 4H), 1.59 (m, 3H).

Compounds 1, 37, 3, 38 and 39 are known compounds and were prepared by the reported standard methods.

Appendix

Elemental Analysis for New Target Compounds

Compound	formula	calculated	found	Max. diff
14	C ₁₃ H ₂₀ ClN ₃ O•0.25HCl•0.05 H ₂ O	C 55.81 H 7.33 N 15.02	C 56.09 H 7.26 N 14.74	0.28
15	C ₁₄ H ₂₂ F ₃ N ₃ O ₃ •0.3 TFA•0.1 H ₂ O	C 46.97 H 6.07 N 11.25	C 47.1 H 6.21 N 11.28	0.14
16	C ₁₃ H ₂₀ ClN ₃ O•0.3 HCl•	C 57.44 H 6.99 N 14.35	C 57.34 H 6.80 N 14.22	0.19
17	C ₁₅ H ₂₂ F ₃ N ₃ O ₃ •0.25 TFA•0.6 H ₂ O	C 47.9 H 6.08 N 10.81	C 48.16 H 6.32 N 10.57	0.26
18	C ₁₄ H ₂₂ ClN ₃ O	C 59.25 H 7.14 N 14.63	C 59.53 H 7.14 N 14.63	0.67
19	C ₁₇ H ₂₂ F ₃ N ₃ O ₃ •0.25TFA•0.45 H ₂ O	C 51.27 H 5.69 N 10.25	C 51.3 H 5.9 N 10.44	0.21
20	C ₁₇ H ₂₀ F ₃ N ₃ O ₃ •0.4 TFA•0.5 H ₂ O	C 49.95 H 5.51 N 9.82	C 50.44 H 6.05 N 9.29	0.54
21	C ₁₅ H ₂₂ ClN ₃ O•0.25 C ₅ H ₉ O•0.05 H ₂ O	C 57.62 H 7.93 N 12.41	C 57.34 H 7.57 N 12.14	0.36
22	C ₁₇ H ₂₄ ClN ₃ O•0.2 HCl•0.05 H ₂ O	C 61.87 H 7.42 N 12.73	C 61.89 H 7.30 N 12.58	0.15
28	C ₁₄ H ₂₀ ClN ₃ O•0.05 H ₂ O	C 59.48 H 7.17	C 59.48 H 7.24	0.15

		N 14.86	N 14.71	
29	$C_{13}H_{20}ClN_3O \cdot 0.05HCl \cdot 0.05 H_2O$	C 60.35 H 7.48 N 14.08	C 60.42 H 7.42 N 13.90	0.18
30	$C_{15}H_{22}ClN_3O_2 \cdot 0.25 HCl \cdot 0.05 H_2O \cdot C_5H_9O$	C 52.94 H 7.14 N 11.76	C 52.96 H 7.14 N 11.63	0.13
31	$C_{21}H_{23}N_5O_2 \cdot 0.35 H_2O$	C 65.73 H 6.22 N 18.25	C 65.64 H 6.31 N 18.36	0.11
32	$C_{13}H_{20}ClN_3O \cdot 0.05HCl \cdot$	C 57.49 H 7.44 N 15.47	C 57.5 H 7.43 N 15.38	0.09
33	$C_{13}H_{22}ClN_3O \cdot 0.25HCl \cdot 0.05 H_2O$	C 52.94 H 7.14 N 11.76	C 52.96 H 7.14 N 11.63	0.13
34	$C_{13}H_{20}ClN_3O \cdot 0.05 HCl$	C 60.53 H 7.47 N 14.12	C 60.58 H 7.11 N 13.89	0.36
35	$C_{13}H_{20}ClN_3O \cdot 0.25HCl \cdot 0.05 H_2O$	C 55.81 H 7.33 N 15.02	C 56.09 H 7.26 N 14.74	0.28
40	$C_{21}H_{24}N_6O \cdot 0.4 CH_2Cl_2$	C 62.63 H 5.62 N 20.48	C 62.83 H 5.62 N 20.11	0.47
41	$C_{25}H_{27}F_6N_5O_7 \cdot 0.4 TFA \cdot 0.1 H_2O$	C 46.19 H 4.15 N 10.44	C 46.21 H 4.25 N 10.55	0.11